ANNOTATION

to the dissertation for the degree of Doctor of Philosophy (PhD)
in the specialty 6D070100 – "Biotechnology"
by **Sabina Tovekelkyzy Kenesheva**
on the topic "**The Effect of Iodine-Containing Semiorganic Compounds on Multidrug-Resistant Pathogenic Microorganisms**"

The dissertation is presented in the form of a series of articles published in journals included in the first and/or second quartile by impact factor according to the Journal Citation Reports (Clarivate Analytics), in accordance with clause 5-1 of the Rules for awarding academic degrees, approved by Order No. 127 of the Minister of Education and Science of the Republic of Kazakhstan dated March 31, 2011 (with amendments and additions as of January 6, 2025), regulating the activities of QACMSHE RK.

General characteristics of the dissertation research. The dissertation work is devoted to the study of original semiorganic iodine compounds with amino acids and their effect on multidrug-resistant microorganisms in *in vitro* experiments. The work presents data on the physicochemical characterization of the obtained complexes, assessment of their antimicrobial activity, their modulating effect on antibiotic susceptibility, as well as analysis of changes in differential gene expression profiles of multidrug-resistant strains under their influence.

Relevance of the research topic. Currently, the therapy of infectious diseases is complicated by the increasing number of resistant strains of microorganisms, which represents a serious medical and socio-economic problem. Despite the development of new antibiotics, their introduction is accompanied by the rapid formation of resistance, which necessitates the search for alternative anti-infective agents of non-antibiotic origin, as well as adjuvants or potentiators of antibiotics capable of modulating their effectiveness. The creation of iodine-containing complexes with various organic ligands expands the possibilities for their application and modification of biological properties. It is assumed that amino acids within the complexes may play an important role both in structural stabilization of the complex and in its biological activity. Due to amino acid ligands, such complexes may be capable of influencing intracellular processes, including gene expression and cellular metabolism. This substantiates the relevance of their detailed study in conjunction with the changes they induce in microbial cells.

Aim of the study. Comprehensive investigation of the effect of original semiorganic iodine compounds with amino acids on multidrug-resistant bacteria in *in vitro* experiments.

Objectives of the study:

1) synthesis and physicochemical characterization of original semiorganic iodine compounds with amino acids;

2) *in vitro* evaluation of the antimicrobial activity of semiorganic iodine compounds with amino acids against model strains of microorganisms;

3) investigation of the modulating effect of semiorganic iodine compounds with amino acids on the antibiotic susceptibility of model multidrug-resistant strains of microorganisms in *in vitro* experiments;

4) investigation of the effect of semiorganic iodine compounds with amino acids on differential gene expression profiles in model multidrug-resistant strains of microorganisms, using annotated reference genomes previously obtained by whole-genome sequencing.

Objects of the study. Clinically significant bacterial strains from the American Type Culture Collection (ATCC), recommended by the manufacturer for studies of antimicrobial resistance and drug development, were used as models of pathogenic microorganisms.

Subject of the study. Properties and biological effects of original semiorganic iodine compounds with amino acids (alanine, glycine, isoleucine) on multidrug-resistant bacteria.

Research methods.

For Objective 1: X-ray diffraction analysis (auto-refractometer CAD4 Enraf-Nonius); UV spectroscopy (spectrophotometer LAMBDA-35); IR spectroscopy (Fourier spectrometer NICOLET 6700); thermal analysis (simultaneous thermal analyzer STA 449 F1 Jupiter with thermogravimetry and differential scanning calorimetry); EDX (integrated with Quanta 3D 200i electron microscope); titration.

For Objective 2: twofold serial dilution method according to Clinical and Laboratory Standards Institute (CLSI) protocols.

For Objective 3: Kirby–Bauer disk diffusion method with final interpretation according to CLSI protocols; unpaired two-tailed Student's t-test ($p < 0.05$) (GraphPad Prism 6.0) for differences between

control and experimental groups; growth curve registration by optical density in microplate format (Multiskan Ascent).

For Objective 4: DNA isolation (PureLink Genomic DNA Kit); library preparation (SMRTbell); DNA sequencing (PacBio RS II, SMRT Cell 8Pac v3); read quality control and trimming (UGENE v1.32.0); de novo genome assembly (SMRT Link v5.0.1); genome annotation (RAST); identification of orthologous genes (OrthoFinder); detection of horizontally transferred genomic islands (SeqWord Sniffer); RNA isolation (RiboPure Bacteria Kit); removal of ribosomal RNA (MICROBExpress Bacterial mRNA Purification Kit); RNA quantity and quality assessment (NanoDrop 2000c, Bioanalyzer 2100 with RNA 6000 Nano LabChip Kit); library preparation (enzymatic fragmentation – Ion Total RNA Seq kit V2, barcoding – Ion Xpress RNA-Seq Barcode 01–16 Kit); RNA sequencing (Ion Torrent PGM, Ion 318 Chip Kit V2); read quality control and adapter trimming (Trimmomatic, RNA QC in UGENE v.36); alignment of reads to the reference genome (Rsubread); counting reads overlapping gene regions (featureCounts); normalization and statistical analysis of differential gene expression (DESeq2, GenomicFeatures); metabolic pathway analysis (Pathway Tools v.26.0, KEGG); enrichment analysis by gene ontology (GO) terms and metabolic pathways (SmartTable Pathway Tools); identification of homologous genes (GET_HOMOLOGUES); evaluation of gene expression changes was performed under conditions of statistical significance ($|\text{fold change}| \geq 2$ and $p \leq 0.05$); Pearson correlation coefficients (Cp) were calculated to assess gene co-regulation.

Main provisions submitted for defense:

1) Semiorganic complexes KS-25, KS-33, KS-51, KS-270, KS-271 represent structurally organized coordination systems possessing antimicrobial activity against model microorganisms, including MDR strains [1–3].

2) Semiorganic complexes KC-270, KC-271 with glycine modulate antibiotic susceptibility in MDR strains of *Staphylococcus aureus* BAA-39 and *Escherichia coli* BAA-196. The most pronounced effects are observed when the complexes are introduced in the lag phase of growth and are accompanied by a statistically significant increase in the diameters of growth inhibition zones, as well as transitions in susceptibility categories for individual antibiotics. The observed effects indicate a phase-dependent pattern. [1].

3) Semiorganic complexes KS-25, KS-33, KS-51 with alanine, glycine, and isoleucine significantly affect differential gene expression in MDR strains of *Escherichia coli* ATCC BAA-196 and *Staphylococcus aureus* ATCC BAA-39. The action of the complexes leads to statistically significant changes in differential gene expression profiles, including both common conserved response patterns (in *S. aureus* and *E. coli*) and specific features. The most pronounced changes affect genes associated with redox processes, central metabolism, protein synthesis, respiration, biosynthesis of amino acids, fatty acids, cell wall components, stress response, and carbohydrate metabolism, including glycolysis, gluconeogenesis, the TCA cycle, and alternative energy-generating pathways. The nature of the transcriptomic response depends on the amino acid ligand and the growth phase of the bacterial culture at the time of exposure [3].

Main results of the study.

Within Objective 1, original semiorganic iodine compounds with amino acids were obtained and characterized: KS-25 (with alanine), KS-51 (with isoleucine), KS-33, KS-270, and KS-271 (with glycine). It was shown that KS-25 represents an alanine dimer stabilized by triiodide; KS-33 is a glycine trimer involving Li^+ and I^-; KS-51 is an isoleucine complex involving iodide ions and water; KS-270 and KS-271 are triiodide complexes of two glycine molecules with cesium chloride. Physicochemical and spectral data confirm the formation of coordination structures involving functional groups of amino acids and the presence of different iodine forms (I_2, I^-, I_3^-) in solutions.

The results of these studies are presented in publications [1–3].

Within Objective 2, an in vitro assessment of the antimicrobial activity of the obtained semiorganic complexes KS-25, KS-33, KS-51, KS-270, and KS-271 was carried out against model strains of microorganisms, including MDR strains. It was shown that complexes KS-25, KS-33, and KS-51 exhibit comparable antimicrobial activity in the range of 0.32–0.57 mg/mL against MDR strains of *E. coli* ATCC BAA-196 and *S. aureus* ATCC BAA-39; for complexes KS-270 and KS-271, this value is 0.76–1.31 mg/mL. Tests with short-term exposure to KS-25 demonstrated pronounced bactericidal activity of the complex in the range of 0.24–0.98 μg/mL against susceptible and MDR strains: *E. coli* ATCC 8739, *E. coli* ATCC BAA-2523, *P. aeruginosa* ATCC 9027, *P. aeruginosa* TA2, *S. aureus* ATCC 6538-P, *S. aureus* ATCC 33591, and *A. baumannii* ATCC BAA-1790.

The results of these studies are presented in publications [1–3].

Within Objective 3, a study was conducted to investigate the modulating effect of semiorganic complexes on the antibiotic susceptibility of MDR strains in in vitro experiments. It was shown that complexes KS-270 and KS-271 with glycine modulate antibiotic susceptibility in *S. aureus* ATCC BAA-39 and *E. coli* ATCC BAA-196. In *S. aureus*, KS-270 in the lag phase modulated sensitivity to amikacin, oxacillin, and ceftriaxone; increased inhibition zones for β-lactams (ampicillin, amoxicillin, carbenicillin) and gentamicin; resistance to cefepime and tobramycin decreased to intermediate. In the ½ log phase, modulation was observed for amoxicillin, carbenicillin, and cefazolin. KS-271 showed similar but less pronounced effects, including changes in sensitivity to amikacin and modulation toward cephalosporins and aminoglycosides. In *E. coli*, KS-270 in the lag phase modulated sensitivity to ampicillin, gentamicin, and tobramycin; increased inhibition zones for amoxicillin, carbenicillin, chloramphenicol, and erythromycin; in the ½ log phase, modulation persisted for carbenicillin, chloramphenicol, and tobramycin. KS-271 exerted a less pronounced effect, limited to increased inhibition zones for amoxicillin, tobramycin (in the lag phase), and chloramphenicol in the ½ log phase. It was established that the complexes are capable of modulating antibiotic susceptibility in MDR *S. aureus* and *E. coli*, with KC-270 being the most effective in the lag phase.

The results of these studies are presented in publication [1].

Within Objective 4, studies were conducted to assess the effect of semiorganic complexes KS-25, KS-33, KS-51 with alanine, glycine, and isoleucine on differential gene expression profiles in MDR strains of *S. aureus* ATCC BAA-39 and *E. coli* ATCC BAA-196. To ensure correct transcriptomic analysis, whole-genome sequencing of these strains was preliminarily performed with subsequent de novo genome assembly, gene annotation, and deposition of the obtained sequences in the NCBI database. It was determined that the genome of *S. aureus* BAA-39 is represented by a single chromosome of 2,791,218 bp (GC – 32.9%) and contains a chromosomal cassette element SCCmec with the mecA gene associated with methicillin resistance. The genome of *E. coli* BAA-196 has a size of 4,682,561 bp (GC – 51%) and includes a plasmid of 266,396 bp (GC – 47%) containing genes for resistance to various classes of antibiotics and virulence determinants. The genomic sequences are deposited in the NCBI database under ID numbers CP033505 for *S. aureus* ATCC BAA-39 and CP042865 (chromosome) and CP042866 (plasmid) for *E. coli* ATCC BAA-196, respectively. The obtained sequences were used as references in the analysis of differential gene expression. This stage is auxiliary within the framework of the present study, and its results are presented in additional publications related to the dissertation topic.

Analysis of differential gene expression showed that complexes KS-25 and KS-33 form similar patterns of transcriptomic response in microbial cells, whereas KS-51 is characterized by a distinct profile. In *E. coli*, exposure to KS-25 and KS-33 is characterized by increased expression of genes involved in energy and carbohydrate metabolism (including the TCA cycle, gluconeogenesis, and alternative catabolic pathways), while exposure to KS-51 shows the opposite trend. In *S. aureus*, the transcriptomic response differs from *E. coli* and is characterized by predominant suppression of transport processes and restructuring of energy metabolism; KS-51 produces the most distinct gene expression profile. Exposure of both MDR strains to KS-25, KS-33, and KS-51 is accompanied by statistically significant changes in differential expression of genes associated with redox processes, central metabolism (glycolytic and gluconeogenic pathways, TCA cycle, and alternative energy pathways), translation, transmembrane transport, functioning of respiratory systems, biosynthesis of amino acids, fatty acids, cell wall components, and cellular stress responses. The results indicate the contribution of the organic ligand to the formation of transcriptomic changes and features of the biological action of the studied complexes. The analysis demonstrates that the similarity or difference in the effects of complexes KS-25 and KS-33 (glycine and alanine) and KS-51 (isoleucine) directly depends on the amino acid in their composition. The magnitude and direction of the effects of the complexes are determined by the amino acid ligand in their composition, the ratio of iodine forms (I^-, I_2, I_3^-) present in the structure, and the growth phase of the bacteria.

The results of these studies are presented in publication [3].

Main conclusions of the study. Based on a comprehensive analysis of experimental data, a relationship between the structure of semiorganic iodine-containing complexes and the nature of their biological action has been established, which confirms the role of the amino acid ligand as a key factor determining the efficiency and direction of antimicrobial and modulating effects. It was shown that:

1) Complexes KS-25, KS-33, KS-51, KS-270, and KS-271 represent structurally organized coordination systems differing in physicochemical characteristics;

2) Complexes KS-25, KS-33, KS-51, KS-270, and KS-271 possess antimicrobial activity against model microorganisms, including MDR strains, which confirms their potential as prospective antimicrobial agents;

3) Glycine-containing complexes KS-270 and KS-271 exert a phase-dependent modulating effect on the antibiotic susceptibility of MDR strains of *E. coli* ATCC BAA-196 and *S. aureus* ATCC BAA-39, leading to increased effectiveness of a number of clinically significant antibiotics – ampicillin, amoxicillin, carbenicillin, gentamicin, and tobramycin, which allows these compounds to be considered as potential adjuvants of antibacterial therapy;

4) Complexes KS-25, KS-33, KS-51 with alanine, glycine, and isoleucine, respectively, alter differential gene expression profiles in MDR strains of *E. coli* ATCC BAA-196 and *S. aureus* ATCC BAA-39, associated with translation, functioning of respiratory systems, biosynthesis of fatty acids and amino acids, cell wall components, cellular stress response, and central metabolism, while exposure to KS-25 and KS-33 results in similar patterns of gene expression changes, differing from those observed for KS-51.

Scientific novelty.

1) For the first time, a series of semiorganic iodine compounds with amino acids (alanine, glycine, isoleucine) has been obtained and characterized: their structure and physicochemical properties have been established;

2) For the first time, a comparative assessment of the antimicrobial activity of the obtained complexes against model strains of microorganisms has been carried out: the presence of antimicrobial activity has been established;

3) For the first time, the modulating effect of semiorganic iodine-containing complexes on the antibiotic susceptibility of MDR strains of *Staphylococcus aureus* ATCC BAA-39 and *Escherichia coli* ATCC BAA-196 has been identified: it has been shown that subinhibitory concentrations modulate susceptibility to a number of antibiotics, and the effects are phase-dependent;

4) For the first time, whole-genome sequencing of MDR strains of *Staphylococcus aureus* ATCC BAA-39 and *Escherichia coli* ATCC BAA-196 has been performed, followed by de novo genome assembly, annotation, and deposition of the obtained sequences in the NCBI database;

5) For the first time, an assessment of the effect of these complexes on differential gene expression in MDR strains of *Staphylococcus aureus* ATCC BAA-39 and *Escherichia coli* ATCC BAA-196 has been carried out: it has been established that the changes affect genes associated with redox processes, translation, metabolism, functioning of respiratory systems, and stress response, including key elements of central metabolism; differences in transcriptomic responses between Gram-positive and Gram-negative bacteria, phase dependence, and dependence of the effect on the ligand within the complex have been demonstrated.

Theoretical significance is determined by the contribution to the development of understanding of the role of organic ligands in shaping the biological effect of iodine-containing compounds, which opens new perspectives for the rational design of compounds with predictable properties. The obtained transcriptomic data make it possible to move from phenotypic observations to the molecular level of interpretation of possible mechanisms of action, indicating the probable involvement of key genes and metabolic pathways.

Practical significance. Semiorganic iodine compounds with amino acids exhibit antimicrobial activity against multidrug-resistant strains and may be considered as objects for further applied research. It has been shown that these compounds are capable of influencing the antibiotic susceptibility profile, which indicates their potential applicability as auxiliary components for increasing the effectiveness of antibacterial drugs. The obtained results may be used in planning preclinical studies, as well as in the development of combined approaches to the therapy of infections and strategies for the rational use of antimicrobial agents.

Connection with priority areas of science development and state programs. The studies were carried out within the framework of research programs BR24992760, BR218004/0223, O.0776, and BR09458960 of the Ministry of Science and Higher Education of the Republic of Kazakhstan, the Ministry of Health of the Republic of Kazakhstan, and the Ministry of Industry and Infrastructure Development of the Republic of Kazakhstan.

Publications and personal contribution of the author to the research. The dissertation is presented in the form of a series of scientific articles published in journals included in Q1/Q2 according to JCR (Clarivate Analytics): *Biomedicines* – Q1 (WoS, Scopus); *BMC Research Notes* – Q2 (WoS, Scopus);

Microorganisms – Q2 (WoS), Q1 (Scopus). Additionally, scientific works have been published and patents have been obtained on the research topic that were not included in the dissertation report: 1 article in *Engineered Science* (Q1, Scopus), 2 in *Microbiology Resource Announcements* (Q3, Scopus), 2 in journals recommended by the QACMSHE RK, as well as 2 utility model patents.

[1] Kenesheva, S. T., Turganbay, S., Jumagaziyeva, A. B., Askhatkyzy, G., Askarova, D. A., Azembayev, A. A., Ilin, A. I., Reva, O. N., & Karpenyuk, T. A. (2025). The Effect of Semiorganic Iodine-Containing Compounds on the Antibiotic Susceptibility of Pathogenic Microorganisms. *Biomedicines*, 13(8), 1790. https://doi.org/10.3390/biomedicines13081790.

Q1 (JCR, Web of Science), Q1 (CiteScore, Scopus, 84th percentile)

The author's contribution included development of the research methodology, validation of the obtained results, performance of formal data analysis, execution of the experimental part of the work, data processing and systematization, preparation of the initial draft of the manuscript, as well as visualization of the obtained results. In this publication, the doctoral candidate is the first and corresponding author.

[2] Turganbay, S., Kenesheva, S. T., Jumagaziyeva, A. B., et al. (2024). Synthesis, physicochemical properties and antimicrobial activity of a di-aminopropionic acid hydrogen tri-iodide coordination compound. *BMC Research Notes*, 17, 384. https://doi.org/10.1186/s13104-024-07052-8.

Q2 (JCR, Web of Science), Q2 (CiteScore, Scopus, 70th percentile)

The author's contribution included analysis and interpretation of the obtained data, as well as preparation of the manuscript text. In this publication, the doctoral candidate is the second and corresponding author.

[3] Kenesheva, S. T., Taukobong, S., Shilov, S. V., Kuznetsova, T. V., Jumagaziyeva, A. B., Karpenyuk, T. A., Reva, O. N., & Ilin, A. I. (2023). The Effect of Three Complexes of Iodine with Amino Acids on Gene Expression of Model Antibiotic Resistant Microorganisms *Escherichia coli* ATCC BAA-196 and *Staphylococcus aureus* ATCC BAA-39. *Microorganisms*, 11(7), 1705. https://doi.org/10.3390/microorganisms11071705.

Q2 (JCR, Web of Science), Q1 (CiteScore, Scopus, 78th percentile)

The author's contribution included performing the experimental part of the work, data processing and systematization, preparation of the manuscript text, as well as reviewing and editing of the manuscript. In this publication, the doctoral candidate is the first author.