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Biologically Active Palladium(II), Zinc(II), and Copper(II) Complexes with Terpene Ligands as Potential Pharmaceutical Drugs

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Abstract—A final review of the results of studies of versatile biological activities (in vitro) of chiral metal complexes with benzylamine and ethylenediamine derivatives of terpenes is presented. The cytotoxic profiles of palladacycles containing a Pd–C bond and palladium and zinc chelate complexes were determined. For a number of compounds, the possible mechanisms of potential anticancer action were analyzed, such as modulation of mitochondrial functioning and effect on the parameters of glycolytic function of tumor cells. The antibacterial and antifungal activities of palladium complexes of different types and copper chelate complexes were investigated. A correlation between high antimicrobial activity and antioxidant properties was found for a number of copper complexes. The material is supplemented by an extended analysis of publications in relevant subjects.

Keywords: palladium(II), zinc(II), and copper(II) complexes, terpene ligands, anticancer activity, antimicrobial activity, antioxidants

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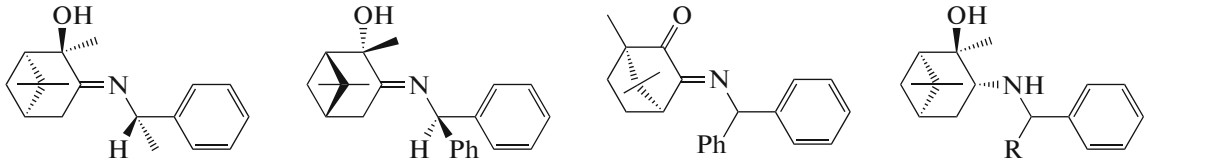
INTRODUCTION

Currently, hundreds of metal-containing pharmacological drugs are used in clinical practice [1–3]. They include both diagnostic compounds and therapeutic drugs. The interest in these compounds is constantly increasing. A special group is represented by metal complexes in which the organic ligand is coordinated to a metal ion. Metal ions play an important role in various biological processes [4]. The coordination to a metal can modify the properties of an organic ligand. This is confirmed by the fact that in almost 90% of the studied ligand–metal complex pairs, the biological activity of the original ligand is significantly lower [5–9]. In the therapy, there are numerous tasks that cannot be performed by traditional organic molecules because of increasing drug resistance. Reviews on recent advances and prospects for the use of metal complexes as pharmaceutical drugs are actively published [5–11].

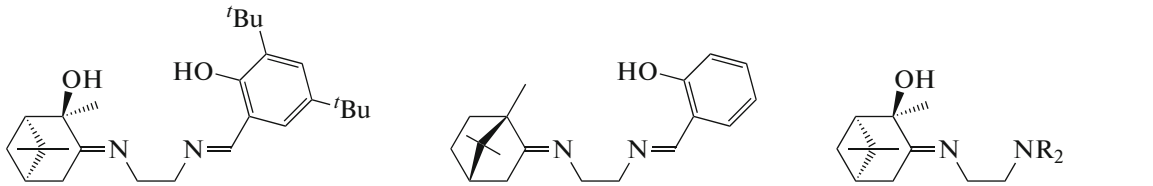
It was reliably established that chirality is an important factor for the efficiency of pharmaceuticals, as virtually all bimolecular targets are also chiral and exist, most often, as single stereoisomers. However, enantiomerically pure ligands are not always available; therefore, the search for these compounds is still a relevant task. Natural monoterpenoids such as camphor [12] and α-pinene [13] are inexpensive commercially available compounds; this enables successful use of their derivatives as precursors for the synthesis of ligands and metal complexes of different types. Versatile biological activities of synthetic derivatives of natural terpenoids were established reliably [14–18], in particular, for metal complexes noted in the review [19].

We prepared pinane and bornane derivatives of benzyamine HL^1–HL^5 (Scheme 1) and ethylenediamine HL^6–HL^{16} (Scheme 2) as N-donor ligands for the synthesis of various metal complexes. Camphor and α-pinene were chosen as the starting chiral compounds for this purpose; we used these compounds as different stereoisomers with high enantiomeric purity. Thus, we had two groups of ligands at our disposal: terpene derivatives of benzylamine are of interest for the possible preparation of palladacycles, while ethylenediamine ligands form metal chelates. The presented final report addresses the results of studies of versatile biological properties of palladium, zinc, and copper complexes with terpene ligands. Description of the methods of synthesis, structures, and characteristics of these metal complexes and free ligands can be found in our relevant publications. The ligands, benzylamine and ethylenediamine terpene derivatives, are depicted in Schemes 1 and 2, respectively:

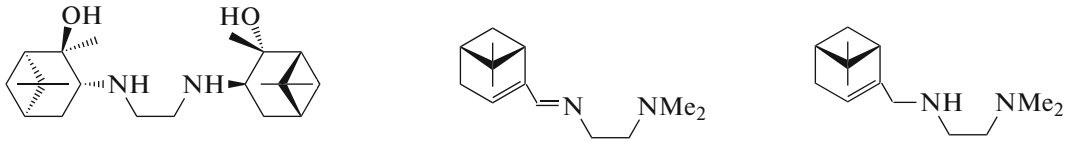


HL^1 [20] HL^2 [21] HL^3 [22] R = Me (HL^4), H (HL^5) [20]

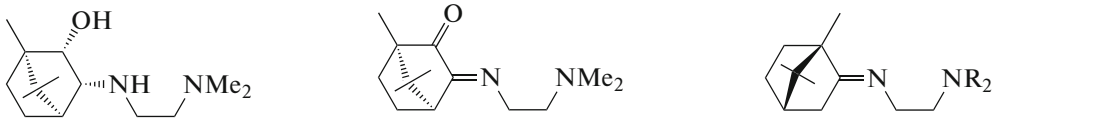
Scheme 1.



HL^6 [23] HL^7 [24] R = H (HL^8) [25], Me (HL^9) [26]



HL^{10} [25] HL^{11} [27] HL^{12} [27]



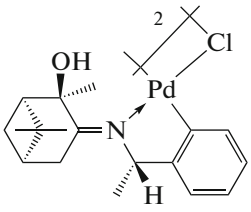
HL^{13} [28] HL^{14} [28] R = H (HL^{15}) [24], Me (HL^{16}) [26]

Scheme 2.

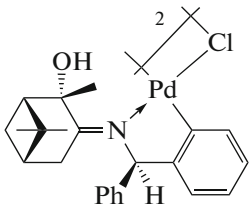
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF PALLADIUM(II) COMPLEXES OF VARIOUS TYPES

The growing number of multidrug-resistant microbes is a severe threat to existing antimicrobial therapy, and the lack of technical innovations hampers the development of new drugs to combat the growing resistance rates. This led to revival of non-traditional approaches to development of antimicrobial drugs that do not fit into the traditional paradigm of low-molecular-weight direct-acting drugs. Data on 906 metal-containing compounds evaluated for antimicrobial activity in the framework of the Community for Open Antimicrobial Drug Discovery (CO-ADD) international project are analyzed in published reviews [9, 29]. It was shown that 9.90% of the assayed metal compounds were active, while for organic molecules this value was 0.87%. The results suggest that the bacterial susceptibility to chemotherapeutic metal complexes is significantly influenced by both the nature of the metal ion and the connected coordination sphere.

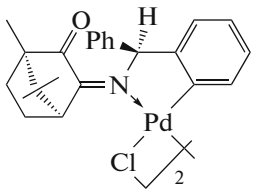
In order to establish the structure–biological activity relationships, we studied two types of chiral palladium(II) complexes. Complexes **Pd1**–**Pd5** (Scheme 3) are palladacycles containing a carbon–palladium bond. They were obtained by direct cyclopalladation of benzylamine terpene derivatives. The second group, compounds **Pd6**–**Pd12** (Scheme 4), are palladium chelates based on terpene derivatives of ethylenediamine.



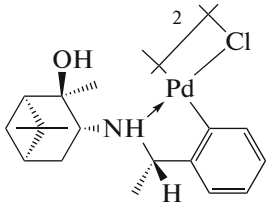
Pd1 [30]



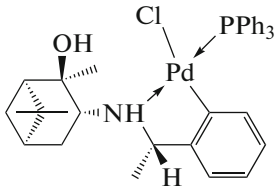
Pd2 [31]



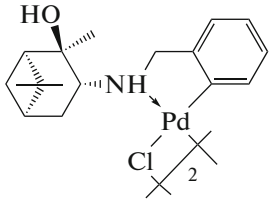
Pd3 [22]



Pd4 [30]

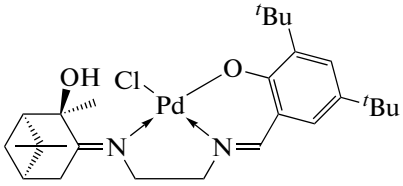


Pd4a[32]

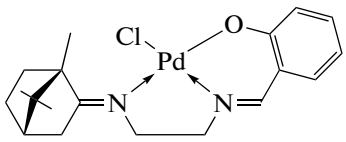


Pd5 [28]

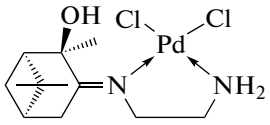
Scheme 3.



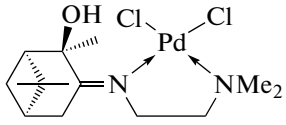
Pd6 [25]



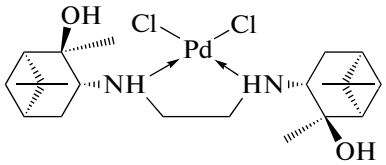
Pd7 [24]



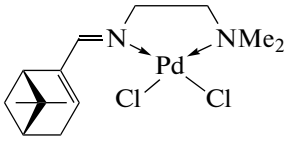
Pd8 [25]



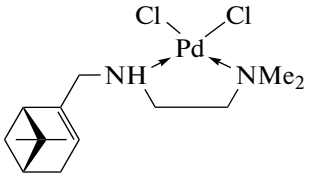
Pd9 [26]



Pd10 [25]



Pd11 [27]



Pd12 [27]

Scheme 4.

Table 1. MIC of palladium(II) complexes determined for *S. aureus* (MRSA), *C. albicans*, and *C. neoformans* var. *grubii H99* (μg/mL), CC_{50} (μg/mL), and HC_{10} (μg/mL)

Compound	*S. aureus* ATCC 43300	*C. albicans* ATCC 90028	*C. neoformans* ATCC 208821	CC_{50}	HC_{10}
Pd1	4	1	0.5	11.9	3.9
Pd2	1	0.5	≤0.25	12.4	1.8
Pd3	16	2	0.5	6.0	>32
Pd4	32	1	≤0.25	16.6	5.6
Pd4a	16	2	1	2.9	5.6
Pd6	8	8	4	4.5	7.8
Pd7	>32	16	4	>32	>32
Pd8	>32	8	4	>32	6.9
Pd9	>32	16	2	>32	>32
Pd10	>32	4	4	>32	4.6
Pd11	>32	≤0.25	≤0.25	10.8	>32
Pd12	>32	2	≤0.25	>32	>32
Vancomycin	1				
Fluconazole		0.125	8		
Tamoxifen				9	
Melittin					2.7

Biological assays were carried out in vitro in the framework of the CO-ADD international project (Australia) [32]. The activity was determined from the inhibition of the growth of five sorts of bacterial cells (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Escherichia coli*) and five sorts of fungi (*Candida albicans*, *Cryptococcus neoformans*). These sorts of widely abundant bacteria and fungi exhibit numerous resistance mechanisms against a number of clinical agents, which makes the search for new bactericidal and fungicidal drugs relevant.

The obtained results attest to high selectivity of the inhibitory action of structurally diverse palladium complexes against various types of bacterial strains. All of the compounds proved to be inactive against gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. High inhibitory activity against gram-positive *Staphylococcus aureus* was inherent in palladium complexes **Pd1**–**Pd4a** corresponding to the group of cyclometallated compounds (Table 1). The lead compound is the palladacycle **Pd2**, which has a minimum inhibitory concentration (MIC) of 1 μg/mL, being thus equivalent in activity to the antibiotic vancomycin. While comparing the inhibitory activities of close analogues, one can note that complexes **Pd1** and **Pd2** based on pinane imines are more active than the corresponding amine derivatives (e.g., **Pd4**). Transformation of binuclear palladacycle **Pd4** into mononuclear complex **Pd4a** containing an additional triphenylphosphine ligand leads to a 2-fold decrease in MIC. These facts indicate that the antimicrobial activity of palladium complexes significantly depends on the structural features of the ligands. It is noteworthy that the corresponding free ligands are inactive against *Staphylococcus aureus* (ATCC 29213, archival strain). Qualitative studies of the antimicrobial activity of the ligands were performed at the Institute of Fundamental Medicine and Biology of the Kazan Federal University.

According to assay results, all of the studied palladium complexes (**Pd1**–**Pd4a** and **Pd6**–**Pd12**) had high antifungal activities against *Candida albicans* and *Cryptococcus neoformans* (Table 1). The MIC values were not higher than 16 μg/mL, and in some cases, they were lower than 0.25 μg/mL. No clear-cut structure–property correlation was observed.

The toxicity of palladium complexes **Pd1**–**Pd4a** and **Pd6**–**Pd12** to cells was evaluated (Table 1). The cytotoxicity was determined using the HEK293 human embryonic kidney cells as the test system. The data are presented as the CC_{50} value, indicating the concentration (μg/mL) at which a 50% cell growth inhibition is attained. The hemolytic activity of the compounds against human red blood cells (RBC) was also examined. The data are presented as HC_{10} values, indicating the concentration of the agent providing a 10% hemolytic activity. All samples characterized by CC_{50} and HC_{10} values exceeding the highest tested concentration (32 μg/mL) are classified as nontoxic. This condition is met for palladium complexes **Pd7**, **Pd9**, and **Pd12**. This result implies that the toxicity of palladium coordination compounds depends on the ligand environment of the metal ion and can thus be deliberately reduced. Complex **Pd12** characterized by the selectivity index (SI = CC_{50}/MIC) above 128 was included in the group of compounds selected for studying the antifungal activity in vivo [9].

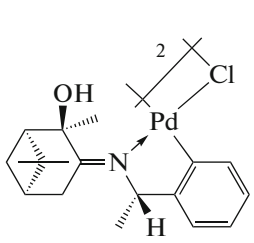
CYTOTOXIC ACTIVITY OF PALLADIUM COMPLEXES OF VARIOUS TYPES

Although metal-based drugs have been used in cancer chemotherapy for approximately 40 years, there is still a growing interest in the development of new metal-based anticancer agents to solve problems of drug resistance, presence of side effects, and improvement of efficacy and selectivity of drugs. The range of the studied metal complexes is being extended, as indicated by the reviews devoted to the latest achievements and prospects for the use of metal complex compounds as anticancer drugs [5, 7, 33–37]. Palladium complexes with various organic ligands are actively investigated in this respect [5, 7, 33–35]. It is necessary to note an enormous structural diversity of ligands, most important of which are N-donor polydentate molecules. A lot of information is given in a comprehensive review [7], devoted to palladium complexes (847 compounds!) that have been studied for anticancer activity in vivo or in vitro since the age of discovery of cisplatin up to 2015. Many palladium complexes exhibit significant anticancer activity, some of them being superior to cisplatin or other clinically used drugs. The authors performed analysis of the structure–property relationships, and the elucidated features are quite useful for the development of new palladium compounds with a higher cytotoxic activity.

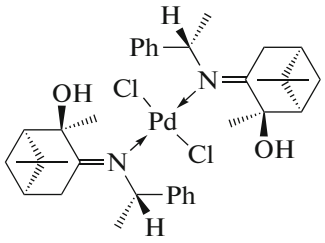
In many studies [5, 7, 33–35], it was noted that Pd(II) complexes exhibit a satisfactory cytotoxic activity against cancer cells, which moreover markedly exceeds the activity of the free ligands. However, low stability of these complexes is a concern. Palladium(II) complexes have a high susceptibility for ligand exchange, which is approximately 10^5 time higher than that of Pt(II) complexes [38]. This may account for rapid hydrolysis of palladium-based drugs. Ligand dissociation gives rise to very reactive types of species (ions, molecules), which can readily react with donors circulating in blood and in cell medium, thus preventing the drug from reaching the target. This process can be avoided by using bulky chelating ligands, which provide higher stability and optimal ligand exchange rate in order to preserve the structural integrity of the compound in vivo for a period sufficient for performing the therapeutic action [38]. A number of studies [7, 39–42] focus on cyclopalladated compounds in which the chelate ring contains a strong carbon–metal bond. These compounds have high stability and satisfactory cytotoxicity. In an analytical publication [7], C,N-palladacycles were noted among compounds with increased activity. From the standpoint of our interests, mention should be made of the studies of Spanish [39–42] and Iranian [43–45] scientists, which addressed cyclopalladated derivatives of benzylamines. The results indicate that these palladacycles have a high potential for clinical application, especially against chemotherapy-resistant cancer.

As noted above, one more object of our interest are palladium(II) chelate complexes with ethylenediamine derivatives. The cytotoxic activity of palladium complexes of this type was investigated by Serbian researchers [46–50]. In this group of metal complexes, no outstanding results were obtained, but subtle dependence of activity on minor changes in the ligand structure was clearly manifested.

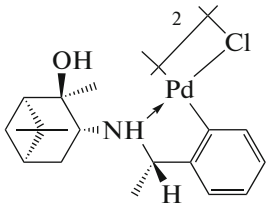
The biological activity of palladium complexes of various types that we synthesized was studied at the Institute of Physiologically Active Compounds, Federal Research Center of the Problems of Chemical Physics and Medicinal Chemistry, Russian Academy of Sciences [28]. In terms of the structure, two groups can be distinguished among the test compounds: palladium complexes **Pd1**,**1a**,**4**,**5**,**5a** with benzylamine-derived terpene ligands and chelate complexes **Pd6**,**8**,**9**,**13**,**14**,**15** with ethylenediamine derivatives. Diverse palladium(II) complexes studied for cytotoxicity are depicted in Scheme 5.



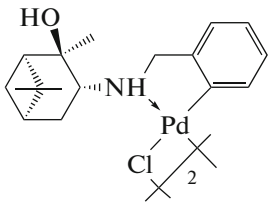
Pd1 [30]



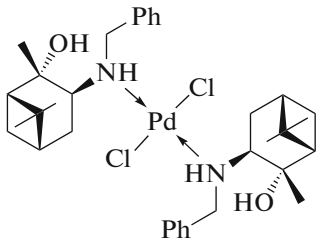
Pd1a [30]



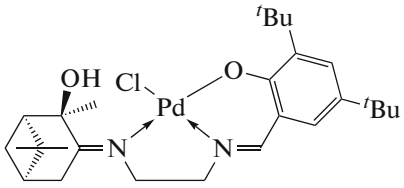
Pd4 [30]



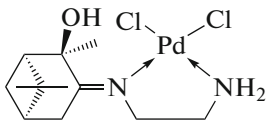
Pd5 [28]



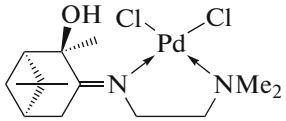
Pd5a [30]



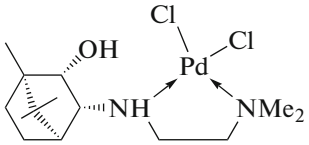
Pd6 [25]



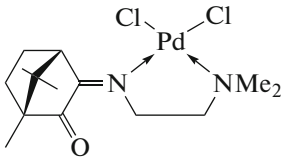
Pd8 [25]



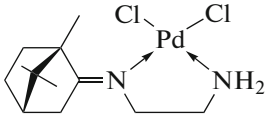
Pd9 [26]



Pd13 [28]



Pd14 [28]



Pd15 [24]

Scheme 5.

The cytotoxic profile was determined in relation to a panel of cancer cell lines: lung adenocarcinoma (A549), neuroblastoma (SH-SY5Y), laryngeal epidermoid carcinoma (Hep-2), and cervical carcinoma (HeLa). For determination of IC_{50} (concentration inducing 50% cell death) of palladium complexes, the cells were incubated for 24 h with the test compounds in concentrations of 0.1, 1, 10, 30, and 100 μM (three repetitions for each concentration). A clinically used anticancer drug, cisplatin (*cis*-diaminedichloroplatinum(II)), served as the positive control. The cell viability was determined by the MTT assay. The results are summarized in Table 2.

The ability to decrease the survival rate of cancer cells was detected for a group of palladium complexes (**Pd1**,**1a**,**4**,**5**,**5a**) containing a benzylamine moiety in the molecule. Palladium chelates **Pd8**,**9**,**13**,**14**,**15** with N,N-donor ligands (ethylenediamine terpene derivatives) in a maximum concentration of 100 μM were not effective against any of cell cultures. The most pronounced toxic action was demonstrated for salen type palladium chelate complex **Pd6**. This compound had the lowest IC_{50} value for the cytotoxic effect against all cell lines, especially SH-SY5Y ($IC_{50} < 0.1$ μM), which may be indicative of more preferable toxicity of **Pd6** against neuronal phenotype cells. It is noteworthy that all palladium complexes showed lower toxicity against

Table 2. Cytotoxicity of palladium(II) complexes against SH-SY5Y, HeLa, Hep-2, and A549 cancer cell lines

Compound	IC_{50}, μM			
	SH-SY5Y	HeLa	Hep-2	A549
Pd1	5.86 ± 0.31	6.79 ± 0.44	53.47 ± 1.56	31.62 ± 2.32
Pd1a	28.25 ± 0.70	28.44 ± 0.18	68.32 ± 0.28	41.22 ± 0.58
Pd4	6.27 ± 0.14	22.45 ± 2.65	53.94 ± 1.99	34.08 ± 0.71
Pd5	5.62 ± 0.46	9.43 ± 0.31	61.40 ± 0.97	43.35 ± 2.93
Pd5a	5.94 ± 0.30	4.28 ± 0.09	66.70 ± 0.57	9.45 ± 0.86
Pd6	<0.1	4.66 ± 0.84	20.41 ± 0.82	8.07 ± 0.19
Pd8	≥100	≥100	≥100	≥100
Pd9	≥100	≥100	≥100	≥100
Pd13	≥100	≥ 100	≥100	≥100
Pd14	≥100	≥100	≥100	≥100
Pd15	≥100	≥100	≥100	≥100
Cisplatin	10.08 ± 1.12	9.03 ± 0.74	5.41 ± 0.36	19.15 ± 2.31

Table 3. Biological activities of palladium complexes **Pd1**,**1a**,**4**,**5**,**5a**,**6**

Compound	Mitochondrial characteristics		Glycolysis
	Mito[a], SW	Mito[b], ψ	
Pd1	127.08 ± 11.68	85.89 ± 5.76	110.79 ± 12.77
Pd1a	**303.08 ± 14.30**	63.63 ± 5.02	63.51 ± 10.15
Pd4	41.01 ± 5.49	33.38 ± 2.59	
Pd5	32.18 ± 9.24	32.09 ± 9.79	61.74 ± 13.34
Pd5a	132.39 ± 5.78	30.61 ± 2.04	
Pd6	**307.23 ± 12.35**	83.75 ± 10.39	98.44 ± 15.47

[a] Mito, SW is V_{max} for the swelling of rat liver mitochondria after the addition of 100 μM of a compound, % of the control.
[b] Mito, ψ is the depolarization of rat liver mitochondria after the addition of 100 μM of a compound, % of the control.
[c] Glycolysis is inhibition of the maximum glycolysis in HeLa cells after the addition of 100 μM of a compound, % of the control.

Hep-2 cells, as indicated by higher IC_{50} values for the cytotoxic effect. Compounds **Pd1**,**1a**,**4**,**5**,**5a**,**6**, which can reduce the survival rate of cancer cells in low working concentrations down to the nanomolar range, were chosen for elucidation the possible mechanisms of cytotoxic action.

For compounds **Pd1**,**1a**,**4**,**5**,**5a**,**6**, some possible mechanisms of the potential anticancer action were analyzed, such as modulation of processes related to mitochondrial function and effect on the glycolytic function of cancer cells. The overall quantitative data from the studies of biological activity of palladium complexes are summarized in Table 3.

The abnormal mitochondrial functions are an important part in the pathogenesis of many human diseases, in particular cancer [51]; they cause disorder of cell bioenergetics and induce the metabolic reprogramming of cancer cells as a result of disorders in ATP production, Ca^{2+} homeostasis, and redox balance. It was shown that mitochondrial membrane hyperpolarization and impaired release of pro-apoptotic factors into the cytosol make cancer cells resistant to apoptosis [36]. This attests to direct relationship between the abnormal mitochondrial functions and cancer genesis. Therefore, the effect of complexes **Pd1**,**1a**,**4**,**5**,**5a**,**6** on the functional characteristics of isolated rat liver mitochondria used as the model system was studied in order to identify the potential pro-apoptotic activity of compounds. Analysis was carried out using two parameters: change in the mitochondrial swelling (determined from the light transmission of a suspension of the organelles) and in the transmembrane potential (found using the potential-dependent Safranin A as a tag).

It was shown that pre-incubation of isolated rat liver mitochondria with palladium(II) complexes leads to effective disruption of mitochondrial functioning. The most pronounced induction of swelling was observed for **Pd1a** and **Pd6**, as indicated by the substantially higher rate of mitochondrial permeability pore formation compared to the rate induced by Ca^{2+} ions, which are normally used for MPTP opening and served as the reference [52] (Table 3). In addition, these compounds were found to decrease the mitochondrial transmembrane potential, thus exerting a depolarizing action on the mitochondrial membrane (63.63% for **Pd1a** and 83.75% for **Pd6**). Evidently, this Ψ_m collapse induced by the test compounds attests to mitochondrial dysfunction, further permeabilization of their internal membrane by opening mitochondrial permeability transition pores, release of pro-apoptotic factors into the cytosol, and finally the death of cancer cells. Thus, the results suggest a pro-apoptotic mechanism of the anticancer action of the test compounds via modulation of mitochondrial functions in the cell.

The effect of compounds **Pd1**,**1a**,**4**,**5**,**5a**,**6** on the glycolysis of the HeLa cervical cancer cells was determined. Back in the mid-20th century Otto Warburg described for the first time the proliferative metabolic phenotype in which the tumor cells show a higher glycolytic activity and reduced mitochondrial respiration, unlike normal cells of the body. This phenomenon is known as the Warburg effect [53]. Indeed, tumors generate up to 90% of the cellular adenosine triphosphate (ATP) by glycolysis, and only the rest 10% come from oxidative phosphorylation. Meanwhile, in aerobic non-proliferative cells, mitochondria provide 95% of ATP formation [54]. In some studies, it is shown that compounds that inhibit glycolysis have a pronounced anticancer potential, leading to the death of tumor cells [55, 56]. Therefore, targeting the aberrant metabolism is a promising strategy to inhibit cancer cell growth and metastasis.

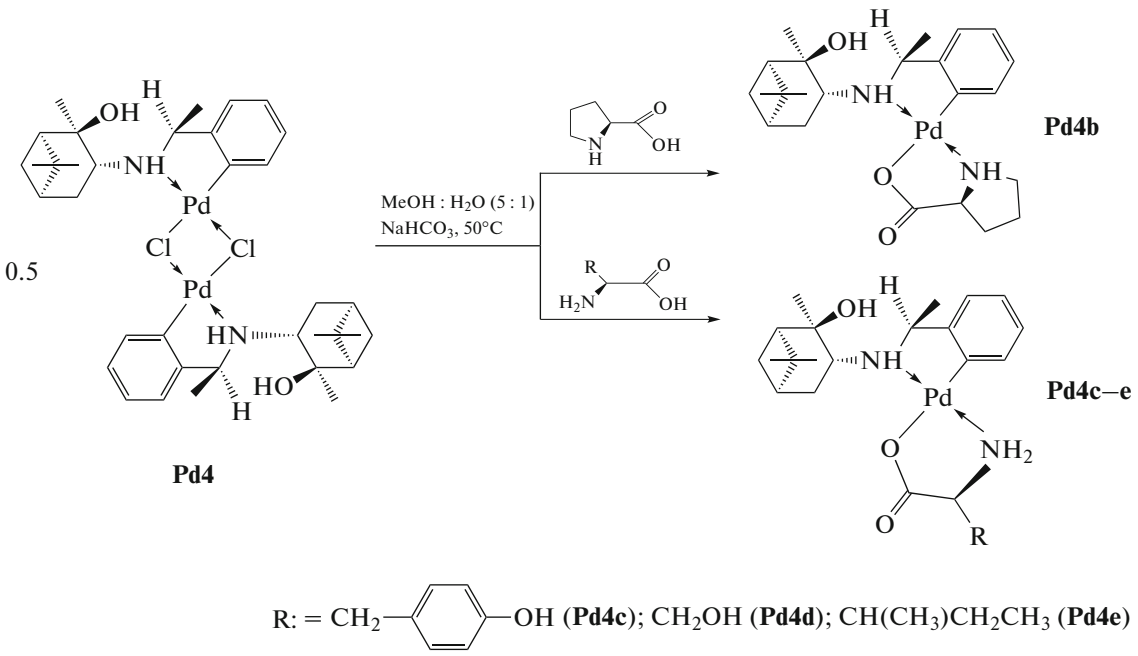
The effect of metal complexes with terpene ligands on anaerobic glycolysis was studied using the Agilent Seahorse XF96e analyzer (Seahorse Bioscience, USA) by measuring the proton generation in tumor cells by the glycolysis stress test [57]. The extracellular acidification rate was measured on a real time basis with alternating addition of modulating agents, which made it possible to evaluate the intensity of glycolysis in the cells by recording the key parameters of the glycolytic function: glycolysis, glycolytic capacity, and glycolytic reserve.

It was shown that complexes **Pd1**,**1a**,**5**,**6** possess clear-cut glycolysis-inhibiting properties. These compounds provided a statistically significant decrease in the glycolytic capacity of the HeLa human cervical cancer cell culture by more than 50%: **Pd5** (glycolysis by 83.86 ± 4.96%; glycolytic capacity by 61.94 ± 3.90%; and glycolytic reserve by 97.79 ± 3.78%), **Pd1** (glycolysis by 91.37 ± 3.61%; glycolytic capacity by 95.63 ± 3.90%; and glycolytic reserve by 97.19 ± 3.78%), and **Pd1a** (glycolysis by 53.52 ± 1.98%; glycolytic capacity by 64.10 ± 2.95%; and glycolytic reserve by 79.59 ± 0.92%), salen type **Pd6** palladium complex (glycolytic capacity by 93.40 ± 3.21% and glycolytic reserve by 97.79 ± 0.43%). The results correlate with the results of studying the cytotoxic profile, namely, the above complexes were found to have a high toxic action against most cell cultures. Evidently, while initiating the glycolysis inhibition cascade, these compounds are able to disrupt the energy metabolism and thus lead to the death of cancer cells.

The obtained results confirm the good prospects of our synthetic approach to the development of efficacious anticancer compounds based on various palladium complexes with terpene ligands. Lead compounds possessing clear-cut cytotoxic properties against various cancer cell lines were identified. First of all, this is the palladium complex **Pd6** with a N,N,O-ligand and palladium compounds **Pd1**,**1a**,**4**,**5**,**5a** containing benzylamine terpene derivatives as the ligands. In the latter group, no correlation between the cytotoxic activity of the palladium complex and the coordination fashion of the ligand was identified. The mechanism of the cytotoxic action of these compounds may be associated with both their ability to damage mitochondria and their influence on the energetic function of cancer cells (glycolysis).

CYTOTOXIC ACTIVITY OF PALLADACYCLES CONTAININGAN AMINO ACID AS A CO-LIGAND

We chose binuclear complex **Pd4** as the starting compound for the synthesis of a series of mixed-ligand mononuclear derivatives **Pd4b**–**e**, which contain, together with the C,N-palladacycle, an amino acid as a second N,O-donor chelating ligand (Scheme 6). Binuclear palladacycles (PCs) readily react with additional ligands (both monodentate and bidentate ones) via chloride (or acetate) bridge cleavage to give the corresponding mononuclear complexes [31, 58]. As the co-ligands, we used proteinogenic *L*-amino acids, including proline (Pro), tyrosine (Tyr), serine (Ser), and isoleucine (Ile). The biological activity of these compounds, which occur as parts of proteins, is quite evident. Iranian researchers [58, 59] described the synthesis of phosphorus-containing PCs with additional amino acid ligands. A clear correlation between the PC activity and the nature of the amino acid moiety was established. Unfortunately, the authors did not provide data for the original binuclear PCs that would allow one to evaluate the contribution of the amino acid moiety. It is noteworthy that high anticancer activity of PCs is combined with their low toxicity to normal human cells [58, 59].



Scheme 6.

The biological activity of the mixed-ligand mononuclear palladium complexes with amino acid ligands that we synthesized was assayed at the Institute of Physiologically Active Compounds, Federal Research Center of the Problems of Chemical Physics and Medicinal Chemistry, Russian Academy of Sciences [60]. Study of the anticancer potential of new C,N-PCs containing *L*-amino acids included evaluation of the effect of test compounds on the survival rate of the neuroblastoma (SH-SY5Y), lung adenocarcinoma (A549), laryngeal epidermoid carcinoma (Hep-2), and cervical cancer (HeLa) cell lines. The cell viability was determined using the MTT assay, which is based on the ability of mitochondrial dehydrogenases of living cells to reduce MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to insoluble formazan crystals. Under our conditions, the well-known anticancer drug cisplatin was used as the reference to validate the method. The corresponding free ligands, terpene amine (HL^4) and amino acids (Pro, Tyr, Ser, and Ile), were used as the reference agents.

The data on the cytotoxic activity are summarized in Table 4. It was found that all mixed-ligand metal complexes **Pd4b**–**e** had a moderate effect on the survival rate of the used cell lines. The most pronounced cytotoxic effect was inherent in **Pd4c** and **Pd4e** when tested against the SH-SY5Y cell line; IC_{50} values were 28.65 ± 0.36 and 23.66 ± 0.41 μmol L^{-1}, respectively. However, these values proved to be higher than IC_{50} of the initial binuclear complex **Pd4** (6.27 ± 0.14 μmol L^{-1}). Only for **Pd4d** against Hep-2, was the toxic effect more than three times higher than that of the pristine **Pd4**. For the initial ligands (HL^4 and Pro, Tyr, Ser, and Ile), IC_{50} values were above 100 μmol L^{-1} for all cancer cell lines.

The obtained results provide two important conclusions. The first one is that in most cases, the introduction of amino acid ligand does not lead to a higher cytotoxic effect for the resulting mononuclear palladium complexes **Pd4b**–**e** compared to the initial binuclear complex **Pd4** containing only terpene ligands. The second conclusion is that the coordinated terpene ligand is generally responsible for the cytotoxic properties, as the uncoordinated amine HL^4 showed no activity.

The effect of cytotoxic metal complexes **Pd4** and **Pd4b**–**e** on the mitochondrial transmembrane potentials was analyzed using isolated rat liver mitochondria as the model system by recording fluorescence of potential-dependent Safranin A. It was shown that pre-incubation of isolated rat liver mitochondria with complexes **Pd4** and **Pd4b**–**e** caused depolarization of the mitochondrial membrane increasing with time (Fig. 1a). The level of observed mitochondrial membrane depolarization after 10 min was lower for compounds **Pd4b**–**e** than for the pristine metal complex **Pd4** (Fig. 1b). Apparently, this accounts for their lower cytotoxicity against cancer cell lines. The obtained results indicate that the introduction of amino acid ligands into the molecule decreases the effect of mononuclear complexes **Pd4b**–**e** on the membrane potential.

It was found that the cytotoxic action of palladacycles may be attributable to the ability of these compounds to induce destruction of mitochondria upon mitochondrial membrane depolarization, which, in

Table 4. Cytotoxicity (in vitro) of palladium(II) complexes and free ligands against cancer cell lines

Compound	IC_{50}, μmol L^{-1}			
	SH-SY5Y	HeLa	A549	Hep-2
Cisplatin	11.31 ± 0.92	9.56 ± 1.01	17.65 ± 1.28	6.07 ± 0.04
Pd4	6.27 ± 0.14	22.45 ± 2.65	34.08 ± 0.71	53.94 ± 1.99
Pd4b	53.36 ± 0.31	47.32 ± 0.29	95.99 ± 1.91	77.20 ± 0.97
Pd4c	28.65 ± 0.36	60.03 ± 0.42	73.83 ± 0.02	70.01 ± 1.65
Pd4d	58.08 ± 0.27	52.97 ± 0.05	58.74 ± 0.01	15.80 ± 0.71
Pd4e	23.66 ± 0.41	57.09 ± 0.29	61.13 ± 0.78	65.20 ± 0.04
HL^4	≥100	≥100	≥100	≥100
Pro	≥100	≥100	≥100	≥100
Tyr	≥100	≥100	≥100	≥100
Ser	≥100	≥100	≥100	≥100
Ile	≥100	≥100	≥100	≥100

turn, promotes triggering of apoptosis processes through the escape of pro-apoptotic factors into the cytoplasmic space of the cell. Analysis of the identified structure–property relationships provides the conclusion that the palladium-coordinated terpene ligand is generally responsible for the cytotoxic activity, while the introduction of amino acid ligand most often does not enhance the cytotoxic effect of the resulting mononuclear palladium complexes **Pd4b–e** in comparison with the pristine binuclear complex **Pd4**.

BIOLOGICAL ACTIVITY OF ZINC COMPLEXES WITH TERPENE DERIVATIVES OF ETHYLENEDIAMINE

Zinc complexes with organic ligands hold great promise as a base for the development of potential therapeutic drugs. A comparison of the biological activities of zinc complexes with those of other metal-based pharmaceutical drugs showed that they have a lower general systemic toxicity, less pronounced side effects, but simultaneously a comparable efficiency

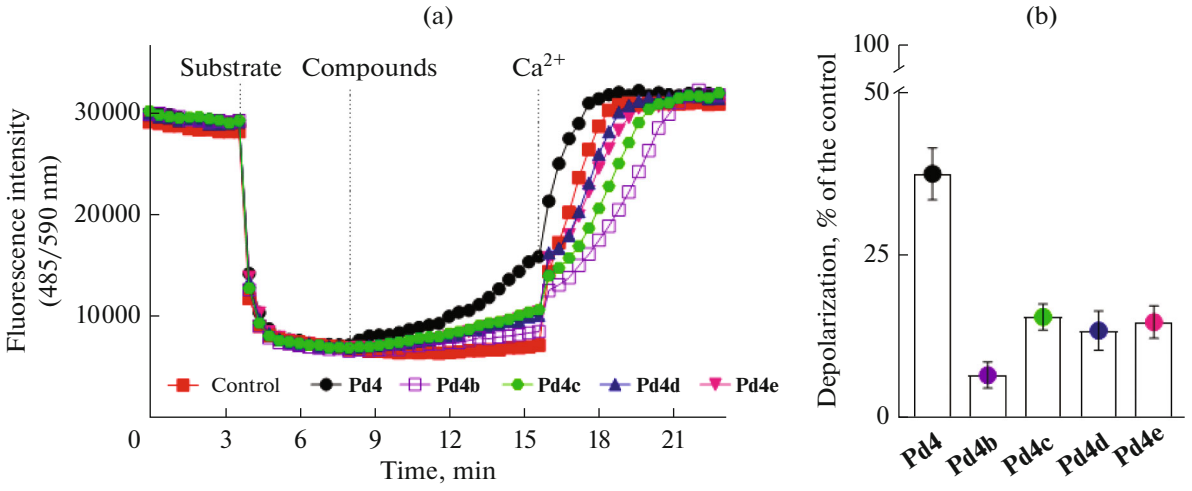
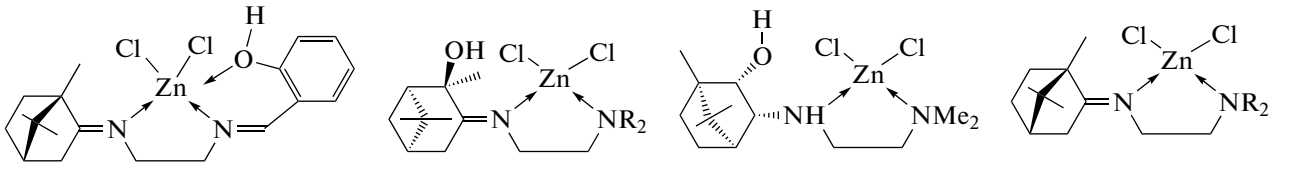


Fig. 1. Effect of palladium complexes **Pd4** and **Pd4b–e** on the membrane potential of rat liver mitochondria (0.5 mg mL): (a) kinetic curves for the variation of membrane potential, (b) histograms demonstrating the quantitative results after 10 min of incubation with the test compounds at λ_{excit} = 485 nm, λ_{emis} = 590 nm. Concentrations of the compounds: 100 μmol L^{-1}; Ca^{2+} concentration: 25 μmol L^{-1}. An equivalent volume of the solvent (DMSO) was used as the control; mitochondria were energized by potassium succinate (5 μmol L^{-1}) and rotenone (1 μmol L^{-1}).

[61]. Zinc-containing metal complexes exhibit a broad range of biological activities, including antibacterial [62–64], antifungal [65–68], anticancer [60, 69–73], antidiabetic [74, 75], and antiparasitic [76] activities.

The cytotoxicity of zinc complexes **Zn7**,**8**,**9**,**13**,**15**,**16** with terpene derivatives of ethylenediamine (Scheme 7) was studied at the Institute of Physiologically Active Compounds, Federal Research Center of the Problems of Chemical Physics and Medicinal Chemistry, Russian Academy of Sciences [77]. Their activity was markedly lower than the activity of structurally similar palladium complexes. Nevertheless, it appeared of interest to study their modulating effect on the functional state of isolated rat mitochondria. As noted above, mitochondria play a crucial role in the development and progression of a wide range of diseases, being the cell metabolism centers and the main regulators of redox balance. Therefore, the vital activity of cells can be modulated by affecting functional characteristics of mitochondria, in order to attain the desired pharmacological action.



Zn7 R = H (**Zn8**), CH_3 (**Zn9**) **Zn13** R = H (**Zn15**), CH_3 (**Zn16**)

Scheme 7.

The action of complexes **Zn7**,**8**,**9**,**13**,**15**,**16** and reference drugs on the functioning of mitochondria was studied by analyzing the influence of these compounds on the following characteristics: transmembrane potential, swelling, and operation of electron transport chain complexes. The mitochondrial membrane potential ($\delta\Psi$), supported by the proton driving force of the respiratory chain, is used for ATP generation [78], and the strong membrane depolarization results, first of all, in opening of mitochondrial permeability transition pores and induction of apoptosis. Therefore, the influence on the swelling and $\delta\Psi$ is one of the mechanisms for inhibition of tumor cell proliferation [79] and for the action of some therapeutic agents such as antiprotozoal, antifungal, and other.

Chiral zinc(II) complexes **Zn7**,**8**,**9**,**13**,**15**,**16** cause pronounced depolarization of rat liver mitochondria and induce their swelling (Fig. 2). Zinc chloride and cisplatin, a well-known cytostatic, served as reference compounds. Depolarization of the mitochondrial membrane and triggering of mitochondrial swelling by the test compounds were comparable with the action of cisplatin, while zinc chloride and ethylenediamine derivatives L^8, L^{16}, and L^{13} showed no activity in these assays.

It is well known that the mitochondrial respiratory chain makes an important contribution to the physiological and pathological production of reactive oxygen species, key mediators of cell death [80]. To date, a considerable progress has been made in the study of functioning of complexes (I, II, III, IV) of the electron-transport chain as sensors of apoptotic cell death and the role of their dysfunction in various pathologies. For example, it was shown that inhibition of respiratory chain complex II upon binding of the TRAP1 (tumor necrosis factor receptor associated protein 1) mitochondrial chaperone protein to succinate dehydrogenase induces oncogenesis through the formation of a pseudohypoxic state [81, 82], while a decrease in the enzymatic activity of this complex accompanies tumor growth in the case of paraganglioma [83], feocromocitoma [84], and other types of malignant growth [85, 86]. In turn, targeting the electron transport chain and, as a consequence, stimulation of cell death in a specific way was found for compounds of various chemical structure and therapeutic purpose. One more promising trend is development of therapeutic strategies for the treatment of malaria in which the electron transport chain is considered as a key target for the drug pharmacological action [87]. On the basis of the large body of experimental data, it becomes evident that attaining a pharmaceutical action on the electron transport respiratory chain for the targeted cell elimination is a highly promising strategy.

The addition of rotenone (a specific inhibitor of electron transport chain complex I) reduced the consumption of oxygen by energized rat liver mitochondria (Fig. 3). Upon the injection of succinate (substrate of complex II), all of the test metal complexes **Zn7**,**8**,**9**,**13**,**15**,**16** were able to stimulate the succinate dehydrogenase complex and enhance the intensity of electron flux, while for the initial ethylenediamine terpene derivatives L^8, L^{16}, and L^{13} no statistically significant differences from the control were detected (Fig. 3). After the addition of antimycin, a decrease in oxygen consumption is observed in the control as in the norm, but zinc complexes **Zn7**,**8**,**9**,**13**,**15**,**16** show impaired electron transport.

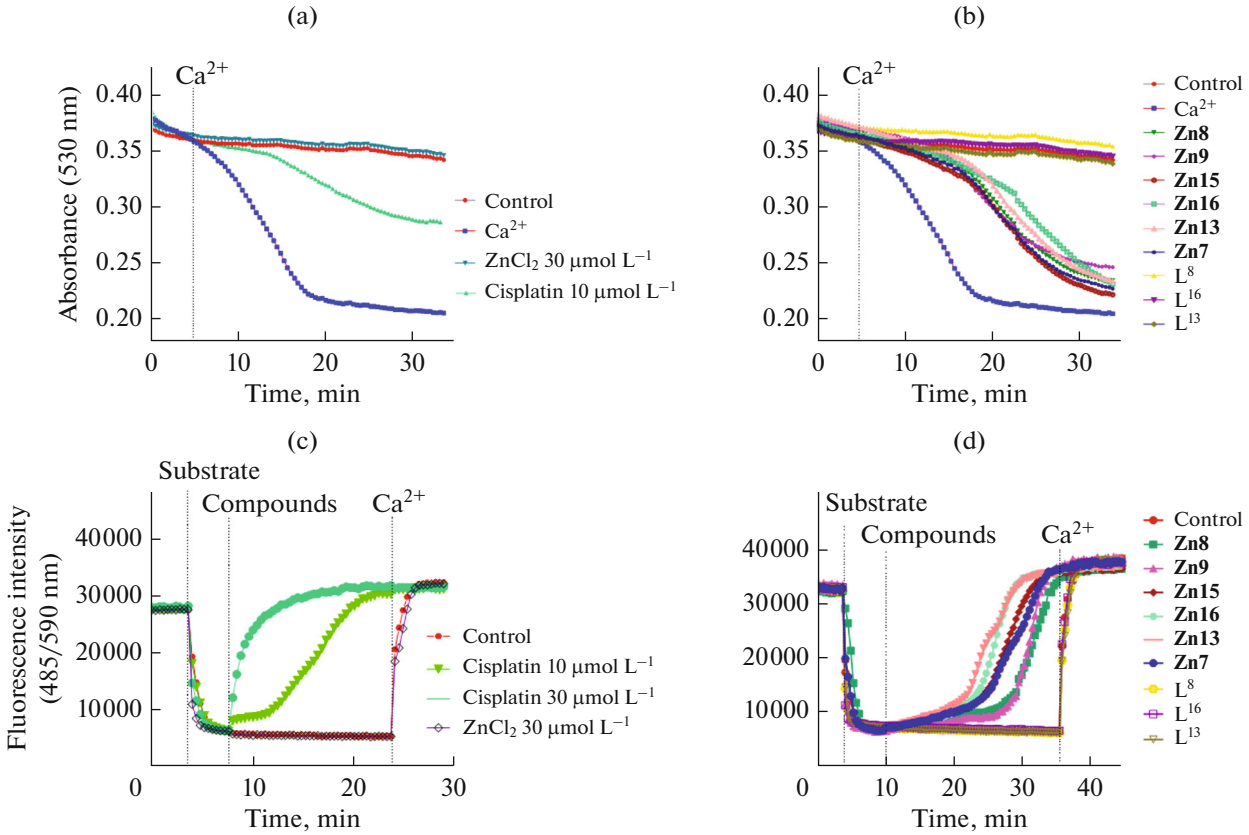


Fig. 2. (a, b) Effect of the addition of test compounds **Zn7**,**8**,**9**,**13**,**15**,**16**, L^8, L^{16}, and L^{13} on the membrane potential; and (c, d) swelling of isolated rat liver mitochondria. Concentrations: test compounds, 30; rotenone, 0.5; potassium succinate, 5; $ZnCl_2$, 30; Ca^{2+}, (a, b) 50 and (c, d) 25; cisplatin, (c) 10 and 30 and (a) 30 μmol L^{-1}. C is control containing an equivalent amount of the solvent. The data are presented as mean values ($n = 3$).

The results indicate that the chiral complexes of zinc with ethylenediamine terpene derivatives have a destabilizing effect on mitochondria via mitochondrial membrane depolarization, induction of mitochondrial permeability transition, and modulation of the operation of respiratory chain complexes. These properties may be important for the search for potential drugs with possible antitumor, antiprotozoal, or antifungal action.

BIOLOGICAL ACTIVITY OF COPPER(II) COMPLEXES WITH TERPENE DERIVATIVES OF ETHYLENEDIAMINE

Copper(II) complexes have a high potential for biomedical applications, which is confirmed by numerous studies. Some publications report the antioxidant activity of copper complexes [88–97]. In recent years, considerable attention has been paid to the development and applications of low-molecular-weight copper complexes that possess properties similar to those of superoxide dismutase (SOD) [93, 98–103]. Superoxide dismutase is one of the most important antioxidant enzymes, which catalyzes the dismutation reaction of superoxide radical anions by converting them into hydrogen peroxide and oxygen. It was shown [104] that metal complexes efficiently catalyze the dismutation of superoxide radicals and thus can be a good alternative to SOD. The interest of researchers in copper(II) complexes is due to the fact that copper is a vital metal, present in many vitamins, hormones, enzymes, and respiratory pigments and is involved in metabolic processes, tissue respiration, and other biochemical processes [4]. Both copper excess and deficiency may significantly impair the viability of organisms and cause diseases [105, 106]. Analysis of published data provides the conclusion that the synthesis and biological activity assays of copper(II) complexes are promising for the preparation of new pharmaceutical agents. Nevertheless, the molecular mechanisms of action of copper complexes have not yet been adequately investigated [107, 108].

The antibacterial and antifungal activities of copper(II) chelates **Cu7**,**8**,**9**,**13** (Scheme 8) with ethylenediamine terpene derivatives have been studied [109]. The efficacy was determined by cell growth inhibition for five bacterial species (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycobacterium vaccae*, *Bacillus*

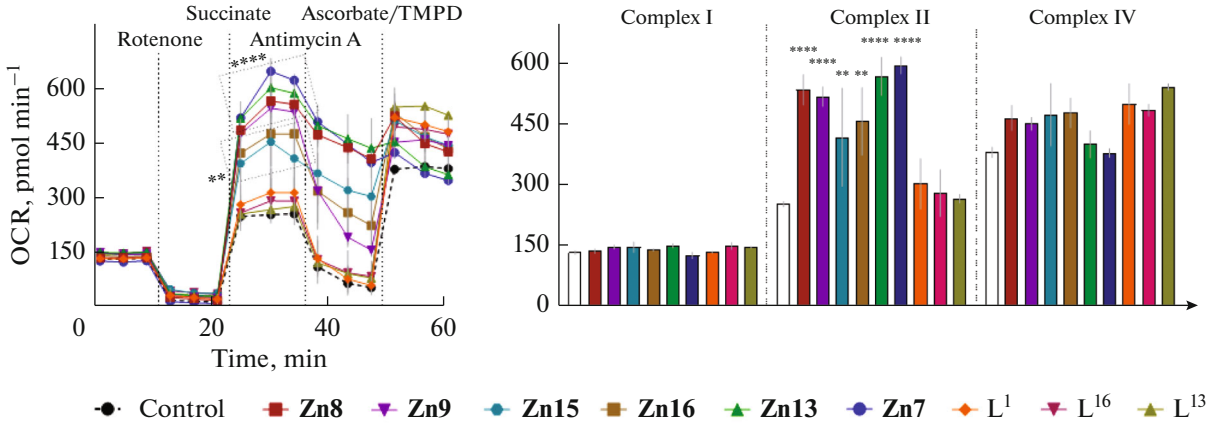
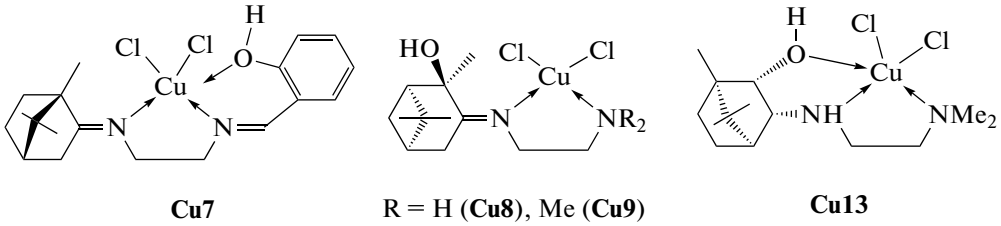


Fig. 3. Effect of compounds **Zn7**,**8**,**9**,**13**,**15**,**16**; L^8, L^{16}, and L^{13} on the oxygen consumption rate (OCR) by isolated rat liver mitochondria (10 μg per well) for evaluation of the operation of respiratory chain complexes. Concentrations: test compounds, 30; rotenone, 2; succinate, 2; antimycin A, 4; ascorbate/tetramethylphenylenediamine (TMPD), 0.5 μmol L^{-1}. The data are presented as the mean value ± error in mean ($n = 3$). ** $p \le 0.01$, **** $p \le 0.0001$ in comparison with the control (one-way analysis of variance, ANOVA).

subtilis, and *Escherichia coli*) and three fungal species (*Candida albicans*, *Sporobolomyces salmonicolat*, and *Penicillium notatum*). These widely occurring bacterial and fungal species have multiple drug resistance mechanisms to quite a number of clinical drugs, which makes the search for new bactericidal and fungicidal agents a relevant task. The investigations were carried out at the Bach Institute of Biochemistry, Federal Research Center of Biotechnology, Russian Academy of Sciences.



Scheme 8.

Generally, copper complexes **Cu7**,**8**,**9**,**13** showed a high antibacterial and antifungal activities (Figs. 4 and 5). The antibacterial and antifungal activities were determined by disk-diffusion method [110, 111] by measuring the inhibition of cell growth for five bacterial species (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycobacterium vaccae*, *Bacillus subtilis*, and *Escherichia coli*) and three fungal species (*Candida albicans*, *Sporobolomyces salmonicolor*, and *Penicillium notatum*). In particular, high antibacterial activity was found for copper complexes **Cu7**,**8**,**9**,**13** against the multiply resistant *S. aureus* (MRSA) strain, which is resistant, in particular, to ciprofloxacin used as the reference antibiotic. The activity of these complexes against *S. aureus* (511 B3) was comparable with the activity of ciprofloxacin (Fig. 5).

All of copper complexes **Cu7**,**8**,**9**,**13** showed a markedly higher antifungal activity against *Candida albicans*, *Sporobolomyces salmonicolor*, and *Penicillium notatum* than amphotericin, a clinically used antifungal agent (Fig. 5).

For comparison, the antimicrobial activity of free ligands was estimated. They turned out to be inactive against the same group of pathogenic strains. The obtained results confirm the regularity established in many publications, namely, metal complexes exhibit a high antimicrobial activity, while the free ligands have a zero activity [91, 99, 112, 113]. The aurthors attributed this result to increase in the lipophilicity of the Cu(II) ion upon coordination to the organic ligand [112].

The antioxidant activity (AOA) of copper complexes **Cu7**,**8**,**9**,**13** was studied using various assay systems (in vitro). The AOA was evaluated by inhibition of the lipid peroxidation (LPO) in a substrate containing brain lipids of laboratory mice. Lipid peroxidation was initiated in two ways: using either Fe^{2+}/ascorbate or H_2O_2. The content of secondary LPO products reacting with 2-thiobarbituric acid (TBA-RS) was

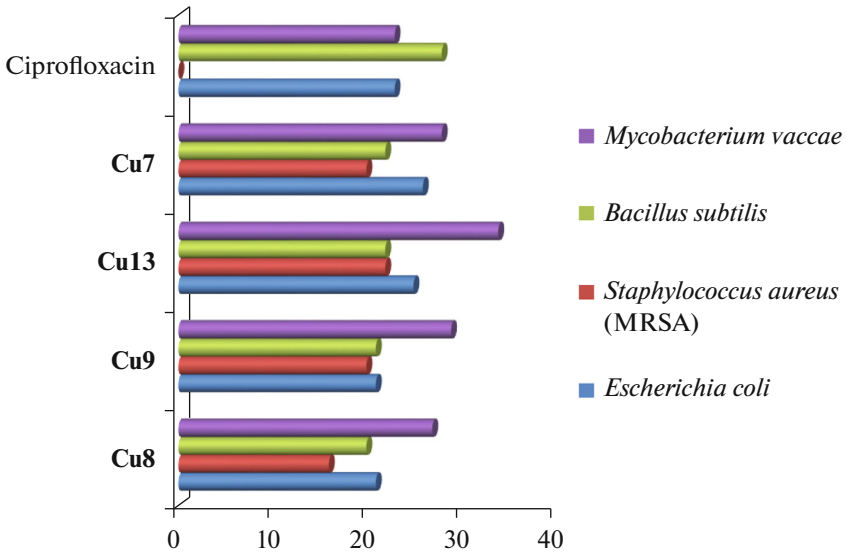


Fig. 4. Antibacterial activity of copper complexes **Cu7**,**8**,**9**,**13** in comparison with the antibiotic ciprofloxacin.

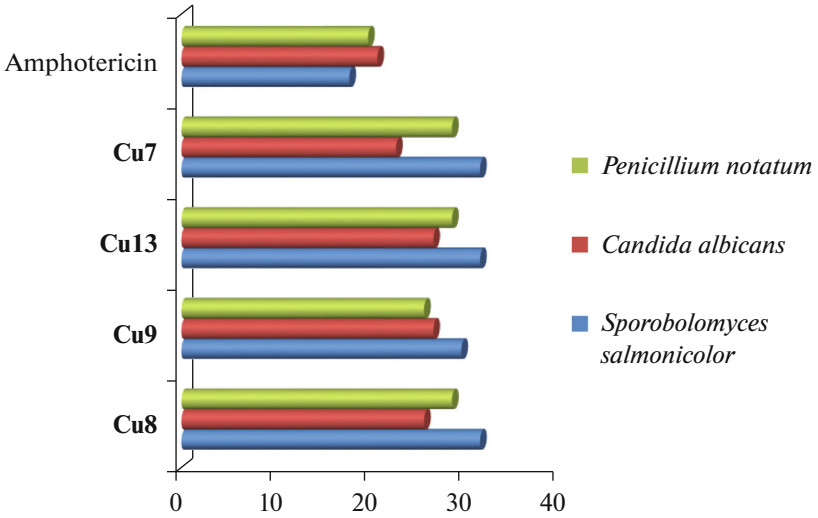


Fig. 5. Antifungal activity of copper complexes **Cu7**,**8**,**9**,**13** in comparison with the fungicidal agent amphotericin.

determined by spectrophotometry (Fig. 6). From analysis of the data obtained for 500 μM concentration of compounds, it follows that irrespective of the way of LPO initiation, a higher inhibitory activity in the ligand–complex pair was found for the complexes. High AOA at the indicated concentrations were also found for copper complexes **Cu13** and **Cu7**. Note that the results obtained upon LPO initiation by different methods are closely correlated with each other (Spearman rank correlation coefficient $R_s = 0.75$, $p = 0.05$, $n = 7$).

As the compound concentration decreases to 100 μM, complex **Cu7** virtually does not lose the ability to inhibit LPO and is still the lead compound (Fig. 6). This compound is comparable in activity with the standard antioxidant, 2,6-di-*tert*-butyl-4-methylphenol (BHT), for both ways of LPO initiation (Fe^{2+}/ascorbate and H_2O_2). In the structural aspect, copper complex **Cu7** differs considerably from **Cu8**,**9**,**13**, as it contains a salen type N,N,O-donor tridentate ligand. Presumably, high AOA of **Cu7** is provided by the presence of the phenol moiety. We define particularly this type of complexes as most promising for further studies.

Before carrying out a comparative study of the membrane-protective activity (MPA) of compounds **Cu7**,**8**,**9**,**13**, L^8, and L^9 in the model oxidative hemolysis of erythrocytes, we studied the compound toxic-

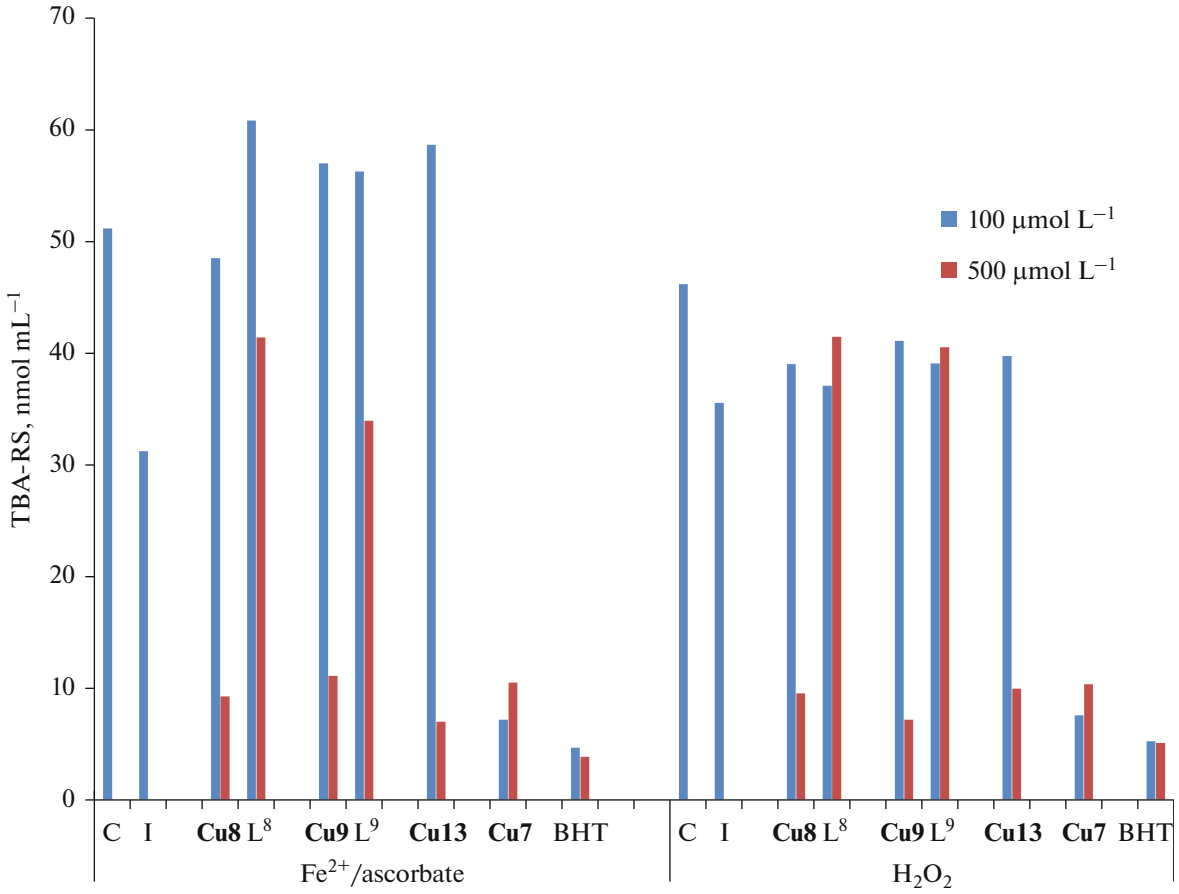


Fig. 6. Comparative evaluation of AOA for the test compounds (copper complexes **Cu7**,**8**,**9**,**13**; ligands L^8 and L^9) in concentrations of 100 and 500 μmol L^{-1}. C is control without test compounds. I is intact sample (without initiated oxidation). BHT is 2,6-di-*tert*-butyl-4-methylphenol, a standard antioxidant.

ity by considering the degree of erythrocyte hemolysis. It was found (Fig. 7) that virtually all of the test compounds in 10 μM concentration have a low hemolytic efficiency: the erythrocyte death in the presence of these compounds exceeds only slightly the spontaneous level. Only in the presence of **Cu9**, did the hemolysis of erythrocytes exceed the control level by a factor of ~2. The subsequent biological activity assays using blood cells were carried out at for concentration of 1 μmol L^{-1}.

The membrane-protective activity of complexes **Cu7**,**8**,**9**,**13** and ligands L^8 and L^9 was determined by measuring the degree of inhibition of the oxidative hemolysis of erythrocytes of laboratory mice. The oxidative hemolysis was initiated by 2,2'-azo-bis(2-amidinopropane) hydrochloride (AAPH) or by H_2O_2. In water at physiological temperatures, AAPH undergoes unimolecular thermal decomposition at a constant rate to generate peroxyl radicals that are unable to penetrate inside the cell and affect the membrane from the outside [114]. The AAPH-initiated oxidative hemolysis is widely used to measure the antioxidant and membrane-protective activities of various compounds and plant extracts [114–117]. It was shown that all of the test compounds (complexes **Cu7**,**8**,**9**,**13** and ligands L^8 and L^9) have statistically significant MPA, as follows from the decrease in the AAPH-induced cell death rate in the presence of these compounds (Table 5). Two hours after the induction of oxidative stress, the level of hemolysis was 23.2% in the control, only 6.2–10.9% in the presence of complexes **Cu7**,**8**,**9**,**13**, and 12.1–15.0% in the presence of L^8 or L^9. The highest MPA among the test compounds was found for **Cu9**, which exceeded in activity not only L^9, but also BHT and retarded the oxidative hemolysis throughout the whole experiment (5 h).

Apart from AAPH, hydrogen peroxide is also widely used to initiate the oxidative hemolysis in the studies of antioxidant and membrane-protective properties of various compounds [117–119]. Unlike AAPH, hydrogen peroxide easily penetrates into a red blood cell, mainly targeting hemoglobin [120]. Table 2 presents the results of a comparative determination of MPA for the test compounds under H_2O_2-induced hemolysis. In this experiment, the highest activity among complexes **Cu7**,**8**,**9**,**13** was found for salen type

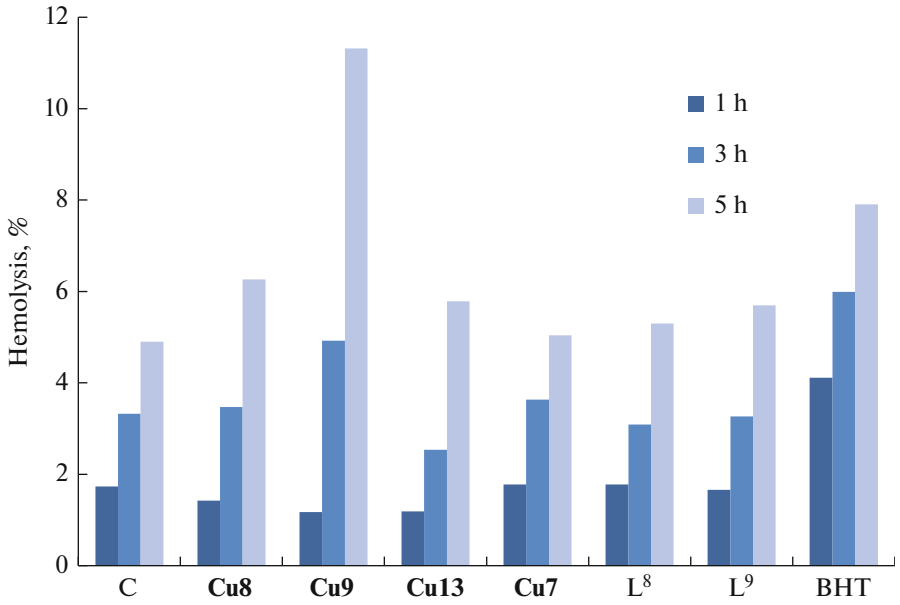


Fig. 7. Comparative evaluation of the hemolytic activity for the test compounds (copper complexes **Cu7**,**8**,**9**,**13**, ligands L^8, L^9) in 10 μmol L^{-1} concentration after 1, 3, and 5 h of incubation. C is control without test compounds. BHT is 2,6-di-*tert*-butyl-4-methylphenol, a standard antioxidant.

Cu7 containing a phenol moiety and characterized by the highest AOA (found in the initiated oxidation model of a substrate containing animal lipids). Complex **Cu7** showed the most pronounced inhibition of H_2O_2-induced cell death throughout the whole experiment (5 h).

Thus, the results of determination of AOA for terpene ligands and copper complexes provide the following key conclusions: irrespective of the LPO initiation method (Fe^{2+}/ascorbate or H_2O_2), in both ligand–complex pairs, a higher inhibitory activity was found for the copper complexes. Salen type complex **Cu7**, characterized by the highest AOA in the initiated oxidation model of a substrate containing animal lipids and showing no hemolytic activity even in 10 μM concentration, surpassed other copper complexes in the ability to protect erythrocytes from the H_2O_2-induced hemolysis.

CONCLUSIONS

The versatile biological activities of palladium, copper, and zinc complexes with terpene ligands of various types were studied. The obtained results enable one to consider these complexes as potential pharmacological agents and attest to high promise of the search for efficacious compounds among this group of metal complexes.

An important conclusion is that the free ligands are inactive, unlike the corresponding metal complexes (or much less active than the complexes) in the performed biological assays, which confirms the important role of metal ions.

Palladium(II) complexes of various structures showed high selectivity to various bacterial strains. All of the compounds proved to be inactive against gram-negative bacteria. Regarding gram-positive *Staphylococcus aureus*, high inhibitory activity was found for palladium(II) complexes corresponding to the group of cyclometallated compounds. The lead compound had MIC of 1 μg/mL, which is equivalent to the activity of antibiotic vancomycin. All of the palladium complexes showed high antifungal activity against *Candida albicans* and *Cryptococcus neoformans*. Determination of the hemolytic activity and cytotoxicity against human embryonic kidney cells (HEK293) revealed non-toxic compounds (with selectivity index higher than 128) and defined the avenue for further studies.

Estimation of the cytotoxic activity of palladium complexes with various types of terpene ligands against a panel of cancer cell lines revealed lead compounds, for which the cytotoxic effect IC_{50} (the concentration at which 50% of cells are killed) is comparable with that of the clinically used cisplatin. These are, first of all, cyclopalladated terpene derivatives of benzylamine. A study of the possible mechanisms of the antineoplastic action of efficacious palladium complexes demonstrated that these compounds can modulate the functional characteristics of mitochondria by triggering the mitochondrial swelling and

Table 5. Comparative estimation of MPA of copper complexes **Cu7**,**8**,**9**,**13** and ligands L^8, L^9 in 1 μM concentration under AAPH- and H_2O_2-induced hemolysis (1–5 h of incubation)

Compound	Hemolysis, %		
	1 h	3 h	5 h
AAPH-induced hemolysis			
C*	2.7 ± 0.0	55.9 ± 0.7	81.8 ± 0.4
Cu7	2.5 ± 0.0	45.5 ± 0.6	82.9 ± 0.4
Cu8	2.8 ± 0.0	42.4 ± 0.6	81.3 ± 0.6
Cu9	**2.4 ± 0.1**	**31.6 ± 0.1**	**75.5 ± 0.7**
Cu13	2.8 ± 0.0	47.1 ± 1.4	81.2 ± 0.8
HL^8	2.7 ± 0.0	42.7 ± 0.6	79.0 ± 0.7
HL^9	2.9 ± 0.2	47.5 ± 1.2	80.7 ± 1.7
BHT	3.0 ± 0.1	46.2 ± 0.8	81.0 ± 0.3
H_2O_2-induced hemolysis			
K	11.5 ± 0.7	30.7 ± 0.7	39.9 ± 0.4
Cu7	**10.9 ± 0.6**	**20.9 ± 0.5**	**28.0 ± 0.7**
Cu8	7.1 ± 0.4	24.8 ± 0.9	34.5 ± 0.8
Cu9	6.9 ± 0.5	25.5 ± 0.3	33.5 ± 0.4
Cu13	11.0 ± 0.5	29.1 ± 0.8	40.5 ± 0.8
HL^8	8.0 ± 0.5	29.8 ± 1.5	36.3 ± 1.0
HL^9	10.3 ± 0.6	26.7 ± 0.5	31.2 ± 0.3
BHT	5.6 ± 0.4	11.9 ± 0.5	22.3 ± 1.1

* C is control without test compounds; BHT is a standard antioxidant, 2,6-di-*tert*-butyl-4-methylphenol.

depolarizing the mitochondrial membrane, and can inhibit glycolysis in HeLa cancer cells.

Binuclear palladacycles, for which the cytotoxic and antimicrobial activities have been confirmed, form a good basis for the preparation of multimodal structures with the goal to find more efficient agents with high selectivity indices. By cleavage of chloride bridges, additional biogenic ligands can be introduced. The results of studies of palladacycles containing an amino acid as a co-ligand confirmed the fact that the terpene moiety of the coordinated ligand is the main structural factor determining the cytotoxic activity.

Zinc chelate complexes with terpene derivatives of ethylenediamine have a much lower cytotoxic activity than palladium complexes of the same type. Nevertheless, they were found to have a destabilizing effect on isolated rat mitochondria, and this, according to published data, may be important in the search for potential drugs with antiprotozoal or antifungal effects.

Copper(II) chelate complexes with terpene derivatives of ethylenediamine showed a high antibacterial activity against the multidrug resistant *S. aureus* strain (MRSA), which is resistant, in particular, to ciprofloxacin used as the reference antibiotic. All of the tested copper(II) complexes exhibited a higher antifungal activity against *Candida albicans*, *Sporobolomyces salmonicolor*, and *Penicillium notatum* than the clinically used antifungal drug amphotericin. It is important to note that copper complexes possessing low hemolytic activity simultaneously proved to be efficient antioxidants in in vitro assays based on inhibition of lipid peroxidation.

Thus, the obtained data on the estimation of the biological activity of the metal complexes we synthe-

sized with terpene ligands of various types make it possible to regard this class of compounds fairly promising for the search for new pharmaceutical agents and to identify the growth points for further research.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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