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Dedicated to the 90th birthday of Academician Yu.A. Zolotov

Anticancer Properties of Au(III) Complexes

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Abstract—The reaction of a solution of $H[AuCl_4]$ with 1,10-phenanthroline (Phen) in acetonitrile gave the complex $(H_2Phen)[AuCl_4]Cl$ (**I**). According to X-ray diffraction data (CCDC no. 2165199), Phen exists in **I** as an unusual doubly protonated (cationic) form $(H_2Phen)^{2+}$. Binding of ionic structural units ($[AuCl_4]^-$, Cl^-, and $(H_2Phen)^{2+}$) by D–H···Cl hydrogen bonds (D = N, C) gives rise to supramolecular 2D pseudo-polymer layers. The biological activity of **I** was measured for human ovarian carcinoma cells (SKOV3). Using MTT assay results, the half-maximal inhibitory concentration was calculated, demonstrating high selectivity of **I** to cancer cells in combination with low toxicity towards normal fibroblasts.

Keywords: gold(III), 1,10-phenanthroline, molecular structure, biological activity, ovarian adenocarcinoma, cytotoxicity

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INTRODUCTION

It is known that metal-containing, first of all, platinum-containing drugs, demonstrate high anticancer properties and are widely used in medical practice. However, in the last two decades, chemists, biologists, medical specialists, and other researchers have directed their efforts towards the development of synthesis and biological studies of various types of "non-platinum" anticancer agents based on bio-essential (vital) metals such as Cu, Zn, Fe, Co, and so on [1–7]. This turn in the exploratory research is caused by several reasons: first, by the search for less expensive agents and, second, by the search for less toxic drugs. (Certainly, one should also bear in mind the engineering problems of industrial manufacture, methods for the choice and properties of organic ligands needed to prepare these compounds.) On the other hand, Ru, Ga, and Au compounds possessing good antiproliferative properties (suppression of the excessive cell multiplication) have now becoming relevant [8–10]. Gold, including the whole diversity of its species, has been used in medicine throughout the history of civilization, starting from archaic "Elixir Vitae, cinnabar" [11, 12] to real therapeutic agents, e.g., against rheumatoid arthritis (gold thiolates [13, 14]). Particularly in the 20th century, radioactive gold-198 started to be used in anticancer therapy: it is chemically inert, inhibits the formation of intracavitary fluid, and has a relatively short half-life (2–7 days) [15]. The isoelectronic structure of Au(III) and Pt(II) (d^8), which accounts for the formation of square planar complexes and relatively slow ligand exchange kinetics, provides prerequisites for the development and testing of gold complexes as potential antitumor agents. Previously [16–18], a study of gold(III) complexes with diverse N-donor ligands demonstrated that these compounds are stable under physiological conditions, show high cytotoxic activity against ovarian cancer cells (A2780), and can overcome drug resistance. Therefore, the purpose of the present study is to prepare gold(III) complex with 1,10-phenanthroline (Phen) $(H_2Phen)[AuCl_4]Cl$ (**I**), to establish its structure, and to elucidate the biological activity towards human ovarian carcinoma cells (SKOV3) for complex **I** and for previously described gold(III) dithiocarbamate chloride complexes $[Au(S_2CNR_2)Cl_2]$ (R = CH_3, $^iC_3H_7$) [19].

EXPERIMENTAL

The complex was synthesized in air using distilled water, 1,10-phenanthroline (98%, Acros), acetonitrile (special purity grade, Khimmed), hydrochloric acid (reagent grade, Khimmed), and nitric acid (65%, reagent grade, Khimmed).

Elemental analysis was carried out on a Carlo Erba EA 1108 automatic C,H,N-analyzer. IR spectra were recorded on a Perkin-Elmer Spectrum 65 FTIR spectrometer by the attenuated total reflectance (ATR) method in the 400–4000 cm^{-1} frequency range.

Synthesis of $(H_2Phen)[AuCl_4]Cl$ (I). A weighed amount of Phen (0.18 g, 1 mmol) was dissolved in MeCN (20 mL). A solution (2 mL) of $AuCl_3$ (in 2 M HCl) containing 22 mg of gold was added to the resulting solution, and the mixture was continuously stirred for 40 min (60°C). The solution was filtered and allowed to evaporate at room temperature. After 24 h, straw-colored crystals were formed; the crystals were separated from the mother liquor by decantation and dried in air. The yield of **I** was 0.25 g (76%).

For $C_{24}H_{18}O_{12}N_2Cu_2$ (**I**)

Anal. calcd., %	C, 44.11;	H, 2.88;	N, 4.29
Found, %	C, 44.23;	H, 3.06;	N, 4.41

IR (ATR; ν, cm^{-1}): 3141 w, 3130 vw, 3061 vw, 2656 n.w, 2579 n.w, 2043 vw, 1672 vs, 1582 m, 1468 vs, 1426 m, 1383 m, 1365 w, 1297 vs, 1235 m, 1191 vs, 1143 vw, 1125 m, 1104 w, 1258 w, 1076 m, 1017 vs, 992 m, 930 s, 885 w, 851 w, 803 vw, 753 vs, 698 m, 592 m, 547 m.

X-ray diffraction study of complex **I** was carried out at 150 K on a Bruker Apex II diffractometer (CCD detector, MoK_α, λ = 0.71073 Å, graphite monochromator). The absorption corrections were applied semiempirically using the SADABS program package [20]. The structure was solved using the ShelXT program package [21] and refined by the full-matrix least squares method using the SHELXL-2018/3 program [22] in the anisotropic approximation for non-hydrogen atoms. The hydrogen atom at nitrogen was located from the difference Fourier maps, the positions of hydrogen atoms at carbons were calculated geometrically. All of them were refined in the isotropic approximation in the riding model. The main crystallographic data and structure refinement details were as follows: $C_{12}H_{10}AuCl_5N_2$, M = 556.44 g/mol, monoclinic space group $C2/c$, a = 22.1986(13), b = 9.7043(5), c = 7.1404(5) Å, β = 94.404(2)°, V = 1533.66(16) $Å^3$, ρ(calcd.) = 2.410 g/cm^3, Z = 4, scanning angle 2.29° < θ < 30.61°, μ(Mo) = 10.452 mm^{-1}; 9145 reflections were measured, of which 2057 reflections had $I \geq 2\sigma$; R_{int} = 0.0386, R_1 = 0.0244, and wR_2 = 0.0717 for observable reflections with $F > 2\sigma(F^2)$; and R_1 = 0.0285 and wR_2 = 0.0754 for all reflections; 96 refined parameters.

The full set of X-ray diffraction parameters was deposited with the Cambridge Crystallographic Data Centre (CCDC no. 2165199; deposit@ccdc.cam.uk).

MTT assay. The cytotoxic effects of various concentrations of complex **I** and the complex $[Au(S_2CNR_2)Cl_2]$ were measured for human ovarian adenocarcinoma cell line (SKOV3) and for primary human dermal fibroblast (HDF) cells by the MTT assay. The assay is based on measurement of the activity of mitochondrial succinate dehydrogenase enzyme and is widely used for *in vitro* evaluation of the antitumor activity of potential therapeutic agents. The results of the MTT assay were used to calculate the half-maximal inhibitory concentrations (IC50) for both compounds. The SKOV3 cells were received from the ATCC collection, the primary HDF culture was previously obtained from a healthy donor. The SKOV3 and HDF cells were cultured in DMEM (10% FBS, 2 mM glutamine, 1% gentamicin). The cells were cultured in plastic vials under sterile conditions and incubated at 37°C with 5% CO_2.

The stock solutions (50 mM) of compounds **I**, $[Au\{S_2CN(CH_3)_2\}Cl_2]$, and $[Au\{S_2CN(^iC_3H_7)_2\}Cl_2]$ [19] were prepared in DMSO as described previously [6]. The final volume of the medium in the wells was 100 μL. The cell viability was measured 48 h after addition of the test compounds using the MTT reagent (Sigma). Working solution of MTT (10 μL; 7 mg/mL) was added into wells containing cells (to 100 μL of the medium), the mixtures were incubated for 3 h, and the medium was replaced by a DMSO solution. The absorbance was determined for each well at 570 nm using a plate reader (TECAN Infinite M Plex), and the background absorption was subtracted. The concentration causing 50% inhibition of cell population growth (IC50) was determined from dose dependence curves.

RESULTS AND DISCUSSION

According to X-ray diffraction data, ionic compound **I**, containing the square planar $[AuCl_4]^-$ anion (Au–Cl, 2.2800(11), 2.2875(6) Å; ClAuCl, 89.63(3)°, 90.37(3)°, 180°), chloride anion, and doubly protonated phenanthroline $(H_2Phen)^{2+}$, crystallizes in the monoclinic space group $C2/c$. In the crystal, the Au atom is located at the inversion center; a twofold axis passes through the discrete Cl(3) atom and the $(H_2Phen)^{2+}$ cation (between the C(5) and C(5A), C(6) and C(6A) pairs of atoms). The H atoms at the nitrogen N(1) and carbon C(6) atoms of the $(H_2Phen)^{2+}$ cation are involved in H-bonding to the Cl(3) atom, thus forming an infinite supramolecular ribbon along the 0b axis (Fig. 1a; main parameters of the H-bond are given in Table 1). The neighboring ribbons are

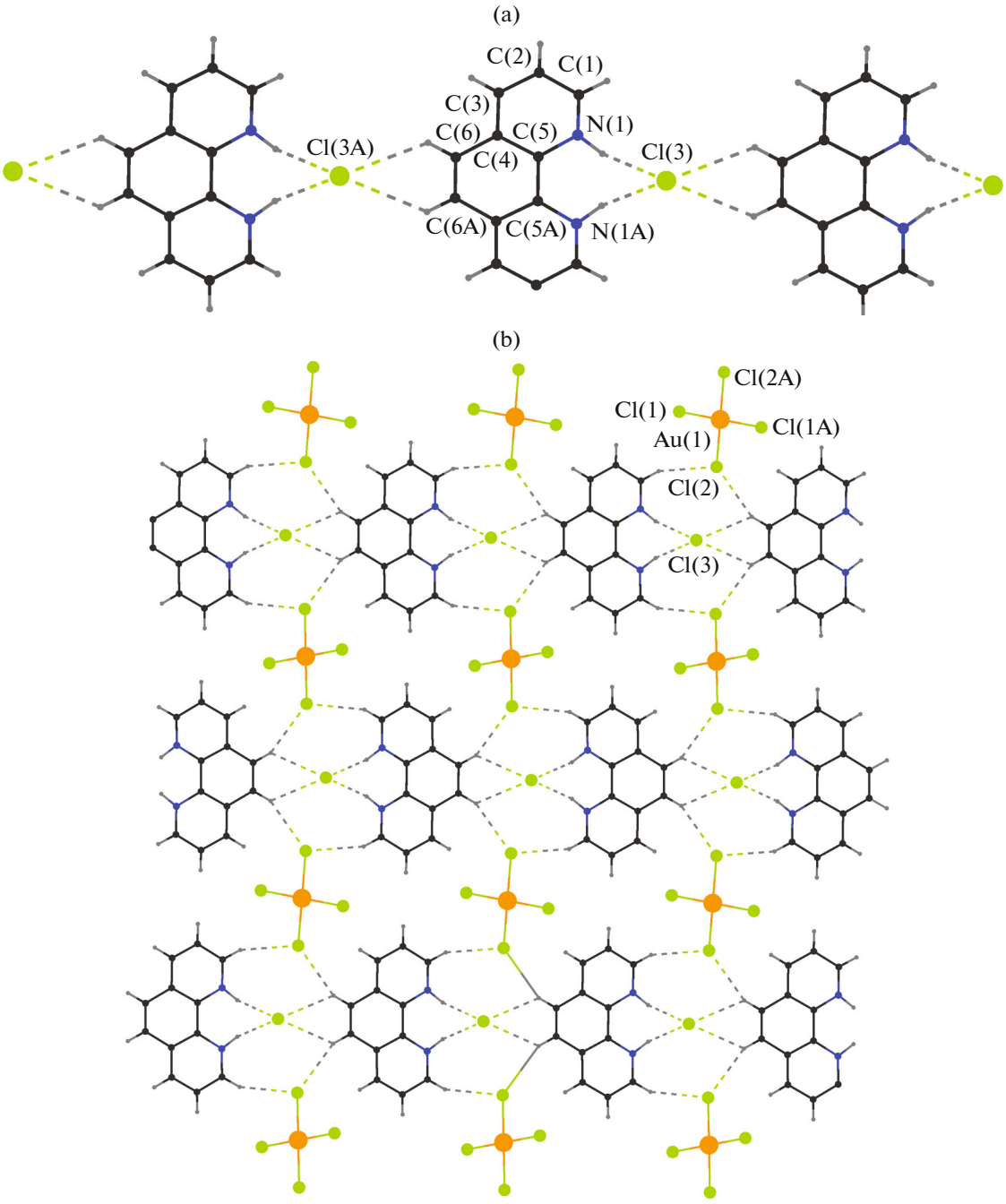

Fig. 1. Fragments of (a) a supramolecular ribbon and (b) a 2D pseudo-polymer layer in the crystal of **I**.

linked into a pseudo-polymer layer (*hkl* = 1, 0, −1) by the C−H...Cl contacts formed by two diagonally arranged chlorine Cl(2) atoms of the $[AuCl_4]^-$ anion and four H atoms at the carbon atoms of four nearest $(H_2Phen)^{2+}$ cations (Fig. 1a, Table 1).

Biological assays were carried out against SKOV3 ovarian carcinoma cells for **I** and for previously structurally characterized dithiocarbamate chloride complexes $[Au\{S_2CN(CH_3)_2\}Cl_2]$ and $[Au\{S_2CN(^iC_3H_7)_2\}Cl_2]$ [19]. The cytotoxicity was determined against human ovarian adenocarcinoma SKOV3 cells and a primary culture of HDF human fibroblasts as a non-tumor control. The test compounds that cause the tumor cell death when present in minor concentrations, while disrupting the viability of normal cells to a lower extent, are considered to be promising. Complex **I** is more toxic for tumor cells than for normal fibroblasts (Fig. 2). The IC50 values for SKOV3 and HDF are 27 μM and >150 μM, respectively.

Table 1. Parameters of H-contacts in the crystal of **I**

Type of contact	Distance, Å			D–H...A, deg	Symmetry code
	D–H	H...A	D...A		
N(1)–H...Cl(3)	0.93(4)	2.11(4)	3.015(3)	166(3)	
C(1)–H...Cl(2)	0.95	2.75	3.634(4)	156	$x, 1-y, 1/2+z$
C(6)–H...Cl(2)	0.95	2.876	3.759(4)	155	$x, 1-y, 1/2+z$
C(6)–H...Cl(3)	0.95	2.849	3.505(4)	127	$x, -1+y, z$

Table 2. Concentrations (IC_{50}) of complex **I** and cisplatin (CP) acting on SKOV3 and HDF

Complex	IC_{50}, μmol/L		Ref.
	SKOV3	HDF	
I	27	>150	This study
$[Au\{S_2CN(CH_3)_2\}Cl_2]$	0.7	1.42	[19]
$[Au\{S_2CN(^iC_3H_7)_2\}Cl_2]$	1.56	0.45	[19]
CP	6.5	22	

Complex **I** is less toxic to ovarian adenocarcinoma cells than cisplatin (CP), but it is much safer for normal fibroblasts (the toxicity is almost 7 times lower than that of CP) (Table 2). Thus, **I** can be considered for further study as a potential antitumor agent.

In [18], gold complex $[Au(Phen)Cl_2]Cl$ with coordinated phenanthroline was investigated for the cytotoxicity against several cancer cell lines, CCRF-CEM (leukemia) and A2780 (ovarian cancer). Analysis of the resulting cytotoxicity profiles shows similarity with those of CP; however, it overcomes the CP resistance and is effective against resistant cells. For comparison of the antitumor efficacy of Au(III) complexes, we studied dithiocarbamate chloride complexes, $[Au\{S_2CN(CH_3)_2\}Cl_2]$ and $[Au\{S_2CN(^iC_3H_7)_2\}Cl_2]$, structurally characterized earlier [19]. Unlike **I**, these complexes have enhanced toxicity against not only tumor cells, but also against healthy cells (Fig. 3; Table 2), which significantly complicates (but does not rule out) the possibility of their use for therapeutic purposes.

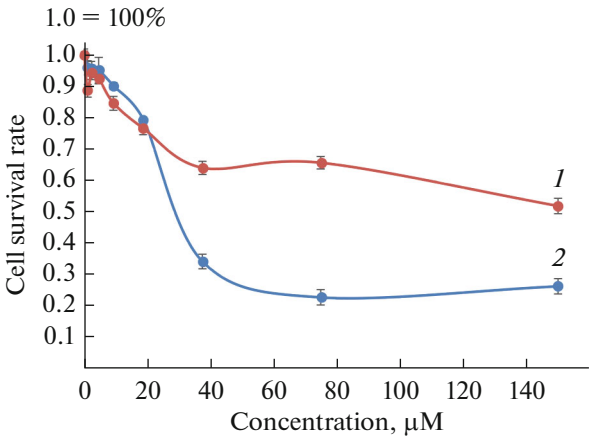

Fig. 2. Cytotoxicity data for **I**. Survival rates of (*1*) HDF and (*2*) SKOV3 cells incubated with various concentrations of **I** or DMSO as the control (the value is the mean MTT index ± standard deviation calculated from three measurements).

Thus, we prepared and structurally characterized the ionic gold(III) complex $(H_2Phen)[AuCl_4]Cl$ (**I**), which has the square planar $[AuCl_4]^-$ anion and a rare doubly protonated phenanthroline cation $(H_2Phen)^{2+}$. The system of D–H···Cl hydrogen bonds (D = N, C) combines the alternating outer-sphere $(H_2Phen)^{2+}$ cations and the Cl^- anions into pseudo-polymer ribbons, which are combined by gold(III) anions into 2D supramolecular layers. Compound **I** was experimentally found to have high antitumor activity against human ovarian adenocarcinoma (SKOV3) cells, in combination with low toxicity to normal cells of primary human dermal fibroblast culture.

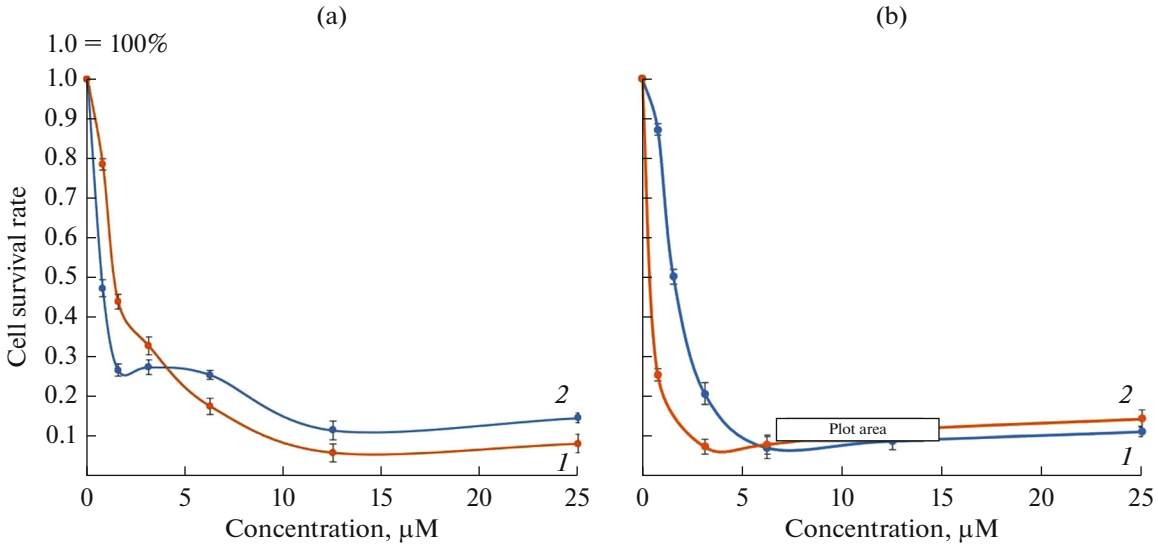

Fig. 3. Cytotoxicity data for (a) $[Au\{S_2CN(CH_3)_2\}Cl_2]$ and (b) $[Au\{S_2CN(^iC_3H_7)_2\}Cl_2]$. Survival rates of (*1*) HDF and (*2*) SKOV3 cells incubated with various concentrations of **I** or DMSO as the control (the value is the mean MTT index ± standard deviation calculated from three measurements).

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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