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First Polynuclear Palladium Compounds $[(C_5H_{12}NO)(PdCl_3)]_n$ and $[(C_{10}H_{16}NO)_2(Pd_2Cl_6)]$ with High Antitumor and Radioprotective Activity

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Abstract—Polymeric palladium complexes $[(C_5H_{12}NO)(PdCl_3)]_n$ (**I**) and $[(C_{10}H_{16}NO)_2(Pd_2Cl_6)]$ (**II**) were synthesized for the first time and studied by X-ray diffraction. Complexes **I** and **II** were found to have moderate toxicity (3rd class of toxicity) and high antitumor and radioprotective activities. Treatment of Lewis lung carcinoma (LLC) with **I** and **II** induced statistically significant antitumor and antimetastatic effects exceeding those of cisplatin: the use of **I** and **II** retarded the growth of LLC by 60.0%, while cisplatin retarded it by 48.0%. The decrease in the LLC metastasis was 75% upon the action of **I**, 91.0% in the case of **II**, and 69.0% for cisplatin. It was shown that **II** has a clear-cut radioprotective action. On single exposure of mice to a dose of 8.2 Gy, their survival rate was 58.0% versus 4.5% for the control. Fractional exposure to increasing doses (2.5, 3, and 3.5 Gy) resulted in 75.0% survival rate versus 33.0% for the control.

Keywords: cation–anion polynuclear palladium compounds, antitumor activity, radioprotective activity
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INTRODUCTION

Currently, antitumor drugs based on platinum complexes (cisplatin, carboplatin, and oxaliplatin) are widely used in the clinical practice. However, quite a few malignant tumors are insensitive to these drugs; also drug resistance often develops during repeated courses of chemotherapy. In addition, the above-mentioned platinum-based antitumor drugs have high toxicity, which often precludes completion of chemotherapy because of the depression of immune system and hematopoietic function of bone marrow, disruption of vital functions of the cardiovascular and nervous systems, liver, kidneys, and other body systems [1–9].

Therefore, the search for new agents based on platinum metals is a relevant task, since this would provide cancer patients with more efficient and less toxic compounds and could solve the drug resistance problem.

Out of the large number of studied palladium compounds, a new class of compounds was revealed, namely, mononuclear cation–anion palladium complexes $[AmH]_n[PdCl_4]$, where Am are representatives of various groups of nitrogen-containing ligands (amines, amides, imidazoles, triazoles, etc.) [10–12]. The palladium complexes $[C_5H_{12}NO]_2[PdCl_4]$ and $[C_{10}H_{16}NO]_2[PdCl_4]$ containing protonated methylmorpholine and 2-methylamino-1-phenyl-1-propanol as the cations proved to be the most promising compounds of this class.

Our publications [13–15] and patents [16–19] present results of studies of the antitumor activity against some tumor strains. The possibility of preparing palladium compounds that can be more efficient and safe than cisplatin because of a different action mechanism was demonstrated [20–23]. It is noteworthy that the above-mentioned complexes also possess high radioprotective activity, and the results of relevant studies are given in the mentioned publications.

A decrease in the toxicity and and increase in the antitumor activity of the previously reported representatives of the new class of cation–anion palladium compounds with a high antitumor activity $(AmH_n)_m[PdCl_4]$ may be attained by: (1) replacement of chloride by carboxylate ions and (2) increase in the amount of the active principle (palladium) in the complex. According to the former approach, formation conditions for $(AmH)_2[Pd(RCOO)_4]$ were studied, where Am is morpholine (C_4H_9NO), R–CH_2Cl, $CHCl_2$, CCl_3, and CF_3. The choice of halo-substituted carboxylic acids with pK_a values in the 2.87–0.0 range is based on the known possibility of chloride replacement by acetate. The reaction of palladium

acetate with morpholine gave only the aminate complex *trans*-$(C_4H_9NO)_2Pd(CH_3COO)_2$ [24] with a low antitumor activity. Meanwhile, an acetate-bridged binuclear aminate complex with 2-phenylpyridine ligand exhibiting low toxicity and a higher antitumor activity than cisplatin was reported in the literature [25]. Our study demonstrated that the cation–anion complexes with a protonated morpholine molecule $(C_4H_{10}NO)_2[Pd(RCOO)_4]$ are formed only for R = CCl_3 and CF_3; in the case of R = CH_2Cl and $CHCl_2$, binuclear complexes $[(C_4H_9NO)_2Pd_2(\mu\text{-}RCOO)_2(RCOO)_2]$ with a coordinated morpholine molecule are formed [26].

The purpose of this study is to attain a higher antitumor activity of mononuclear palladium compounds by increasing the content of the active principle (palladium), i.e., by the synthesis of polynuclear compounds.

This paper describes the synthesis of the polynuclear complexes $[(C_5H_{12}NO)(PdCl_3)]_n$ (**I**) and $[(C_{10}H_{16}NO)_2(Pd_2Cl_6)]$ (**II**), the palladium content of which is more than 10% higher than that of mononuclear analogues. The results of studies of complexes **I** and **II** demonstrated not only high antitumor, in particular, antimetastatic, activity, but also high radioprotective activity, which opens up prospects for the combined treatment of cancer patients using a single drug. The prepared complexes **I** and **II** and their properties have not been described previously.

EXPERIMENTAL

Compounds **I** and **II** were prepared using high-purity grade $PdCl_2$ (Reakhim); 4-methylmorpholine $C_5H_{11}NO$ and *l*-2-methylamino-1-phenyl-1-propanol $C_{10}H_{15}NO$ (Fluka); benzonitrile $(C_6H_5CN)_2PdCl_2$ (Aldrich); acetone, dichloromethane, hydrochloric acid, and glacial acetic acid (Khimmed); cisplatin (EBEWE Pharma Ges.m.b.HHfg.KG., Austria); and normal saline (LLC Groteks and OJSC Dal'khimfarma).

Synthesis of $[(C_5H_{12}NO)(PdCl_3)]_n$ (I) in water. Methylmorpholine (0.89 g, 8.8 mmol) in a mixture of water (20 mL) and concentrated HCl (0.90 mL) was charged into a round-bottom flask. Then palladium dichloride (1.56 g, 8.8 mmol) pre-ground to a powder and water (25 mL) were added with stirring. The content of the flask was magnetically stirred at 40°C for 5 h until palladium dichloride completely dissolved. The brown solution was filtered and concentrated on a water bath at 35°C to a minimum volume. The precipitate formed upon cooling was collected on a filter and dried in vacuo at 93 Pa to a constant weight. The yield was 2.43 g (88% based on the palladium taken).

For $C_5H_{12}NOCl_3Pd$

Anal. calcd., %	C, 19.07	H, 3.84	N, 4.44	Cl, 33.77	Pd, 33.79
Found, %	C, 19.07	H, 4.34	N, 3.94	Cl, 33.97	Pd, 33.66

Synthesis of $[(C_5H_{12}NO)(PdCl_3)]_n$ (I) in nonaqueous medium. Methylmorpholinium hydrochloride (0.45 g, 3.32 mmol) and palladium chloride (0.59 g, 3.32 mmol) pre-ground to a powder were charged into a conical flask equipped with a reflux condenser, and dichloromethane (30 mL) was added. The mixture was stirred at 30°C for 15 h. The pink-beige precipitate that formed was collected on a filter, washed with dichloromethane, and dried in air at room temperature to a constant weight. Recrystallization from water gave cherry-colored single crystals suitable for X-ray diffraction. The product yield was 0.96 g (91% based on the palladium taken).

For $C_5H_{12}NOCl_3Pd$

Anal. calcd., %	C, 19.07	H, 3.84	N, 4.44	Cl, 33.77	Pd, 33.79
Found, %	C, 18.97	H, 4.47	N, 4.14	Cl, 33.52	Pd, 33.65

Synthesis of $[C_{10}H_{16}NO]_2[Pd_2Cl_6]$ (II) in nonaqueous medium. A hydrochloric acid solution of 2-amino-1-phenylpropanol (0.25 g, 1.24 mmol), acetone (10 mL), and glacial acetic acid (2 mL) were charged in a round-bottom flask. A solution of $(C_6H_5CN)_2PdCl_2$ (0.47 g, 1.25 mmol) in acetone (30 mL) was added. The reaction mixture was magnetically stirred at room temperature until the solid completely dissolved (4 h) and concentrated on a rotary evaporator to a volume of 10 mL. The solution was transferred into a porcelain cup, acetic acid (2 mL) was added, and the mixture was evaporated on a water bath at 45°C until a voluminous brown precipitate formed. After cooling of the mixture, the precipitate was collected on a glass filter and dried in vacuo (93 Pa) to a constant weight. The product yield was 0.34 g (93% based on the palladium taken). The light brown-colored single crystal was grown from the mother liquor after separation of the precipitate.

For $C_{20}H_{32}N_2O_2Cl_6Pd_2$

Anal. calcd., %	C, 31.69	H, 4.26	N, 3.70	Cl, 28.06	Pd, 28.08
Found, %	C, 32.23	H, 4.35	N, 4.02	Cl, 28.31	Pd, 28.43

Elemental analysis was carried out using a Carlo Erba Instruments CHNSOEA 1108 analyzer. Palladium was quantified by the gravimetric method.

X-ray diffraction study of I and II was carried out on a Bruker SMART APEX II (complex **I**) and Bruker SMART 1K (complex **II**) automated diffractometers (MoK_α radiation, $\lambda = 0.71073$ Å, graphite monochromator) in the ω-scan mode. The absorption corrections were applied by measuring equivalent reflection intensities [27]. The structures were solved by direct methods and refined by the full-matrix anisotropic least-squares method on F^2 for all non-hydrogen atoms (SHELXTL) [28]. In the structure of **I**, all hydrogen atoms were found from the difference series

Table 1. Crystallographic data and structure refinement parameters for **I** and **II**

Parameter	Value	
	I	**II**
M	314.91	757.98
Color, habit	Red, plates	Orange, plates
Crystal size, mm	0.16 × 0.10 × 0.01	0.28 × 0.10 × 0.02
System	Orthorhombic	Orthorhombic
Space group	*Pbca*	$P2_12_12_1$
Unit cell parameters:		
a, Å	12.9174(8)	9.0897(8)
b, Å	7.4348(5)	9.1997(8)
c, Å	19.8878(13)	33.717(3)
V, Å^3	1910.0(2)	2819.5(4)
Z	8	4
ρ_{calcd}, g cm^{-3}	2.190	1.786
μ, mm^{-1}	2.727	1.864
F(000)	1232	1504
Temperature, K	150	120
Range of θ, deg	2.58–26.00	2.27–27.00
Number of collected reflections	12051	17330
Number of unique reflections (R_{int})	1876 (0.0409)	6152 (0.0535)
Number of refinement parameters	136	293
Number of reflections $I > 2\sigma(I)$	1536	5279
GOOF	1.010	1.089
R-factors ($I > 2\sigma(I)$)	0.0211, 0.0450	0.0509, 0.1033
R-factors (for the whole data array)	$R_1 = 0.0293$, $wR_2 = 0.0484$	$R_1 = 0.0637$, $wR_2 = 0.1076$
Absolute structure parameter		0.01(5)
Residual electron density (max/min), e/Å^3	0.426/–0.384	1.361/–1.071

Table 2. Geometric parameters in the structure of **II**

D–H···A	Distance, Å			DHA angle, deg.
	D–H	A···H	D···A	
O(1*A*)–H(1*C*)···Cl(2)	0.85	2.39	3.244(6)	179.9
O(2)–H(2)···Cl(2)	0.85	2.30	3.153(6)	179.9
N(1)–H(1*B*)···Cl(1)	0.92	2.33	3.204(7)	159.6
N(1*B*)–H(1*AB*)···Cl(6)	0.92	2.56	3.314(7)	139.9
N(2*A*)–H(2*AA*)···Cl(5)	0.92	2.65	3.214(7)	119.9
N(2*A*)–H(2*BA*)···Cl(6)	0.92	2.36	3.219(7)	154.5

and their positional parameters were refined. For the crystal of **II**, all hydrogen atoms were placed into calculated positions and refined using the riding model. The crystallographic data and X-ray diffraction experiment details for complexes **I** and **II** are summarized in Table 1 and the hydrogen bond parameters are given in Table 2.

The structural data for compounds **I** and **II** were deposited with the Cambridge Crystallographic Data Centre (CCDC nos. 1817357 (**I**), 1817358 (**II**); deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk/data_request/cif).

Study of the toxicity of compounds I and II. Acute toxicity was determined by a single intravenous injec-

tion of solutions of complexes over a range of doses to outbred mice (females). Prior to the injection, the complexes were dissolved in 0.9% normal saline solution to a concentration of 0.4%. The solutions were slowly injected into the mouse tail vein over a period of 15–20 s. Healthy adult outbred non-linear mice (females) weighing 18–20 g were used as the test system. Each experimental group included 6 mice. Each complex was tested on 36 mice. Two hours before the experiment, the animals were deprived of food, but not of water. The period of observation was 14 days. During the experiment, the death and clinical signs of intoxication were recorded.

Study of the antitumor activities of compounds I and II. The antitumor activity was evaluated using the transplantable mouse Lewis lung carcinoma (LLC). The LLC strain was received from the tumor bank of the Federal State Budgetary Institution Blokhin Russian Cancer Research Center and maintained on $C_{57}BL_{6j}$ male mice. The transplantation of LLC to male mice F_1 (CBA × $C_{57}BL_{6j}$) was performed by subcutaneous injection of 1.9×10^6 tumor cells in 0.1 mL of a suspension prepared on medium 199 (Paneko, Russia) into the lateral surface of the right thigh, from which the hair was removed before the tumor transplantation. The animals were included in the experiment on the 7th day after LLC transplantation when the tumor node has formed and has reached a measurable size in all mice. The mice were randomized into four groups, a control group and three test groups, 15 mice in each. The animals of the control group did not receive any treatment. The animals of the first test group were intravenously administered with the reference agent cisplatin in a 1.2 mg/kg dose as an 0.012% solution (0.1 mL per 10 g of the body weight) into the lateral tail vein on days 7, 8, 11, 12, and 13 of LLC growth. The mice of the second and third test groups received **I** and **II**, respectively, in a 22.0 mg/kg dose as a 0.22% solution (0.1 mL per 10 g of body weight), which was intravenously injected into the lateral tail vein at the same time points. All solutions were prepared *ex tempore* in normal saline and were slowly injected into the vein.

The tolerance of the test agents was evaluated by daily examination and monitoring of the animals, including their response to injections, the neurological status, considering the spontaneous motor activity, general excitability, response to tactile and sound stimuli, animal feeding activity and growth of the body weight.

The effect of the agents on the tumor process was studied by measuring the LLC growth dynamics and metastatic activity. For this purpose, every 3 or 4 days, the maximum (L) and minimum (W) tumor node diameters were measured with a caliper for all animals, and the tumor node volume was determined approximately as $V = \left(LW^2\right)\left(\pi/6\right)$, which was reliably correlated with MRI data [29].

The antitumor effect was evaluated by statistical comparison of the tumor node volumes in the control and test groups at different time points and by the tumor growth inhibition index (TGII): $\mathrm{TGII} = (V_C - V_T)/V_C \times 100\%$; where V_C and V_T are the average node volumes in the control and test groups [30]. On day 21 of the tumor growth, the animals were sacrificed by cervical dislocation under ether anesthesia, the lungs were separated and fixed in a Bouin solution for 24 h; the large and small lung metastases were counted under a CX21 microscope (Olympus Corp., Japan). The antimetastatic effect was evaluated by statistical comparison of the numbers of metastases in the groups and the metastasis inhibition index (MII) was calculated: $\mathrm{MII} = \left(f_C M_C - f_T M_T\right)/f_C M_C \times 100\%$; where f_C and f_T are the proportions of animals with metastases in the control and test groups, M_C and M_T are the average numbers of metastases in the control and test groups [30].

The statistical significance of the differences in the data obtained between the groups was evaluated by the Kruskal–Wallis rank sum test followed by application of the Dunn test. A difference was considered statistically significant at 0.05 confidence level. The calculations were performed using the Statistica 7.0 software package (StatSoft Inc., USA).

Study of the radioprotective activity of compounds I and II. The radioprotective properties of **I** and **II** were investigated by intravenous injection of the compounds in doses equal to 1/8 and 1/2 of LD_{16} to inbred $C_{57}B1$ male mice weighing 18–24 g. The injections were performed 15–20 min before a single exposure or 15–20 min after exposure to each fraction in the case of fractional irradiation three times a day.

The irradiation was carried out with ^{137}Cs γ-rays (dose rate of 1.88–1.75 rad/s) using an IGUR setup. The single exposure was done using a 8.2 Gy dose, for which a protective effect of palladium-based compounds and aromatic amines was previously observed [16, 17]. In the case of fractional exposure three times a day, increasing doses of 2.5, 3.0, and 3.5 Gy were used.

The test animals received intravenous injections into the tail vein of a 0.35% solution of complexes in a normal saline in doses equal to 1/8 and 1/2 of LD_{16} (for **I**, LD_{16} = 71.0 mg/kg; for **II**, LD_{16} = 77.0 mg/kg). The irradiation control group mice were intravenously administered with normal saline (0.3 mL per mouse). The biological control group mice were not exposed to radiation, or administered with the test complexes, or the solvent. Each experimental group, including the irradiation and biological control groups, comprised 12 to 24 mice. The mice were monitored for 30 days. The condition and behavior of mice were followed, the

number of dead animals, and the time of death were recorded. The survival rates were determined on the 30th day of observation.

RESULTS AND DISCUSSION

The problem of increasing palladium content in the complexes was solved by development of synthesis of compounds **I** and **II**. The obtained complexes contain polynuclear anions. According to X-ray diffraction data, the polymeric chain-like $\left[PdCl_3^-\right]_n$, anion is present in **I**, while the binuclear $[Pd_2Cl_6]^{2-}$ anion occurs in **II**. The counter-ions are represented by protonated *N*-methylmorpholine (in **I**) and protonated 2-methylamino-1-phenyl-1-propanol (in **II**).

In the structure of **II**, the virtually planar (to within 0.145(2) Å) anion is located in a general position (Fig. 1). Both palladium atoms have slightly distorted square environments with ClPdCl *cis*-angles in the range of 85.70(6)°–94.33(7)°. As expected, the terminal Pd–Cl distances (2.273(2)–2.297(2) Å) are markedly shorter than the bridging ones (2.311(2)–2.339(2) Å). This is in agreement with the CCDC data (May 2019, version 5.40 [31]) in which these distances for 42 isolated $[Pd_2Cl_6]^{2-}$ anions are, on average, 2.277 and 2.338 Å, respectively.

The structure of **I** was found to contain polymeric chain $\left[PdCl_3^-\right]_n$ anions (Figs 2, 3). The $[PdCl_3]^-$ moieties occupy general positions, while the neighboring chain units are generated by 2_1 crystallographic axes stretched along the *a* direction. The palladium atom has a nearly perfect square environment with the ClPdCl *cis*-angles ranging from 89.44(2)° to 90.28(2)°. It is worth noting that, unlike the previous case, the terminal and bridging Pd–Cl distances in this anion are virtually equal: 2.2975(6), 2.3045(6) Å and 2.3082(6), 2.3137(7) Å, respectively. Furthermore, unlike the bridging dimeric $[Pd_2Cl_6]^{2-}$ anion in **II**, where the PdClPd angles at the bridging chlorine atoms are close to 90° (93.37(7)° and 94.33(7)°), in the $[PdCl_3^-]_n$ anion, this angle is 107.08(3)°. By now, the CCDB contains data about only one structure with a similar chain-like anion, namely, the dimethylammonium salt $[Me_2NH_2][PdCl_3]_n$ (**III**) [28, 31–33]. However, in **III**, the palladium atom is located at the crystallographic inversion center and the angle at the bridging chlorine atom is much smaller (101.2°). The bridging and terminal Pd–Cl distances in the crystal of **III**, like in **II**, are very similar (~2.31 Å).

In compound **I**, the methylmorpholinium cation has a boat conformation and the methyl group occupies an equatorial position. Compound **II** contains two crystallographically independent chiral cations with similar geometric parameters. It is of interest that they both have *gauche*-conformation relative to the Ph(HO)C–C(Me)NH_2Me bond, with Ph–C–C–N torsion angles being close to 180° (176.1(6)° and 172.8(6)°). Similarly, in both cations, the Me–C–N–Me bonds assume the *trans*-conformation with torsion angles of 158.5(7)° and 171.7(7)°, respectively.

In the crystal of **I**, the *N*-methylmorpholinium cations are connected by a weak bifurcated hydrogen bond N–H···Cl to the terminal chlorine atoms of the polymeric anion (N···Cl, 3.353(2) and 3.401(2) Å) (Fig. 3).

In the structure of **II**, the dimeric $[Pd_2Cl_6]^{2-}$ anions are connected into layers perpendicular to the crystallographic *a* axis through weak Pd(1)···Cl(1) (3.229 Å) and Pd(1)···Cl(6) (3.751 Å) interactions directed almost perpendicularly to the planes of anions. The interlayer spaces are occupied by cations, which are linked to anions by weak hydrogen bonds such as OH···Cl and NH···Cl, formed only via all four terminal chlorine atoms (Table 2, Figs. 4, 5).

The study of toxicity and biological activity of **I** and **II** with polynuclear anions showed that they significantly differ in these characteristics from their analogues with the mononuclear $[PdCl_4]^{2-}$ anion.

The study of acute toxicity of **I** and **II** upon intravenous injection showed that these compounds correspond to the 3rd class of toxicity and hazard, which means that they are moderately toxic [34]. The acute toxicity parameters LD_{16}, LD_{50}, and LD_{84} are 71.0, 95.0, and 119.0 mg/kg for **I** and 77.0, 100.0, and 124.0 mg/kg for **II**.

Primary analysis of LLC growth in various groups of animals showed that both cisplatin and the studied complexes inhibit the tumor growth (Table 3). However, the most clear-cut, statistically significant antitumor effect was observed in tumor-bearing animals that received **I** (Table 4). The characteristics of LLC growth in each animal were normalized to the volume of LLC in this animal on day 7 after transplantation.

In Table 3, one can see the statistically significant antitumor effect of **I** and **II**. A marked retardation of the LLC growth upon the cisplatin administration developed only by the end of treatment (TGII = 28.0%) and then increased till the end of observation (TGII = 48.0%). The retardation of the LLC growth by the test complexes developed more rapidly than with cisplatin: it was pronounced after the first two injections (TGII = 23–32%), increased to 42–55% by the end of treatment, and subsequently reached 60.0% (**I** and **II**). It is important that in this experiment, the clear-cut antitumor effect of **I** and **II** towards LLC persisted at least for 10 days after the end of treatment (11th day), which is a significant indication of the antitumor action.

Thus, according to the existing formal criteria for the selection of promising antitumor agents (TGII > 50%; duration of the effect after the end of treatment not less than 1 week [30]), **I** and **II** should be consid-

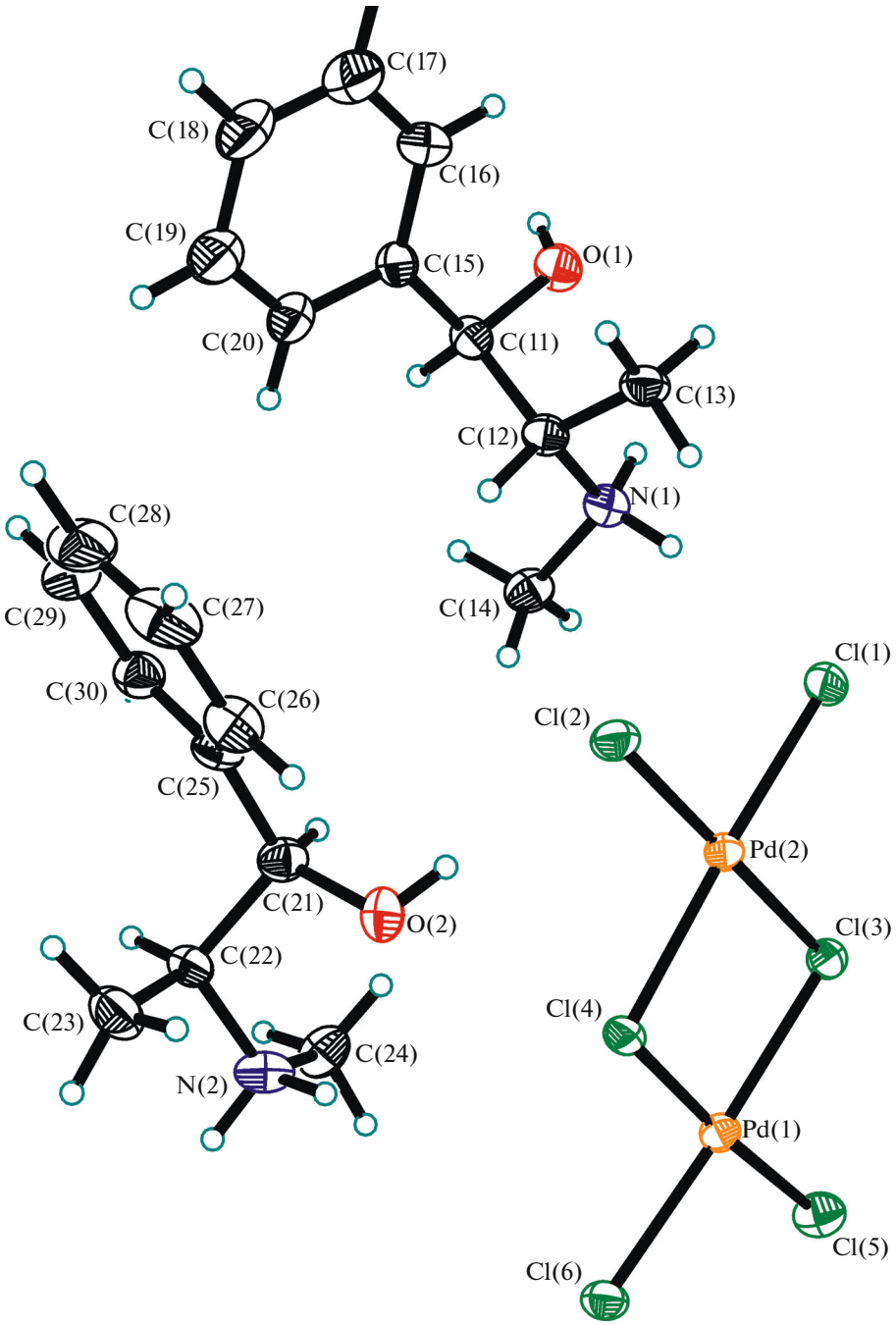

Fig. 1. Crystallographically independent area in the structure of salt **II**.

ered as promising compounds for the development of new antitumor agents.

The activity of LLC metastasis, like the LLC growth, was unusually highly variable in this experiment. Analysis of metastasis characteristics of the carcinoma demonstrated that the intensity of the antimetastatic effect of the complexes was generally correlated with their antitumor effect (Table 5). Cisplatin distinctly suppressed metastasis (MII = 69.0%); nevertheless, it caused only a statistical trend in this experiment (p = 0.08–0.17). A considerably more pronounced, statistically significant antimetastatic efficacy was found for complex **II** (MII = 91.0%). Complex **II** not only inhibited LLC metastasis, but also statistically significantly suppressed the growth of lung metastases: the number of large metastases decreased more than fourfold. The antimetastatic effect of complex **I** also exceeded that of cisplatin.

Thus, according to the existing criteria for the primary selection of potential antimetastatic agents

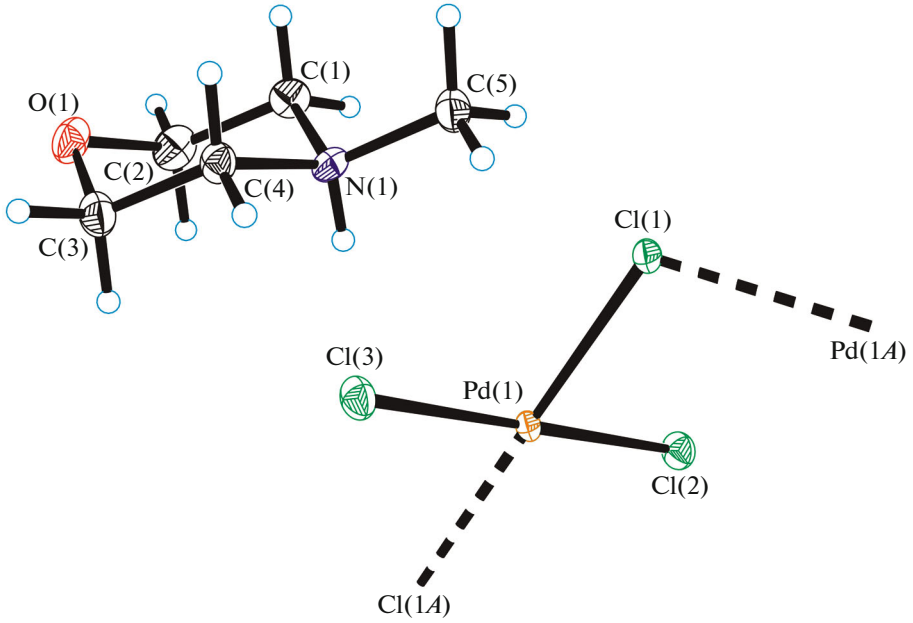

Fig. 2. Fragment of the structure of compound **I**.

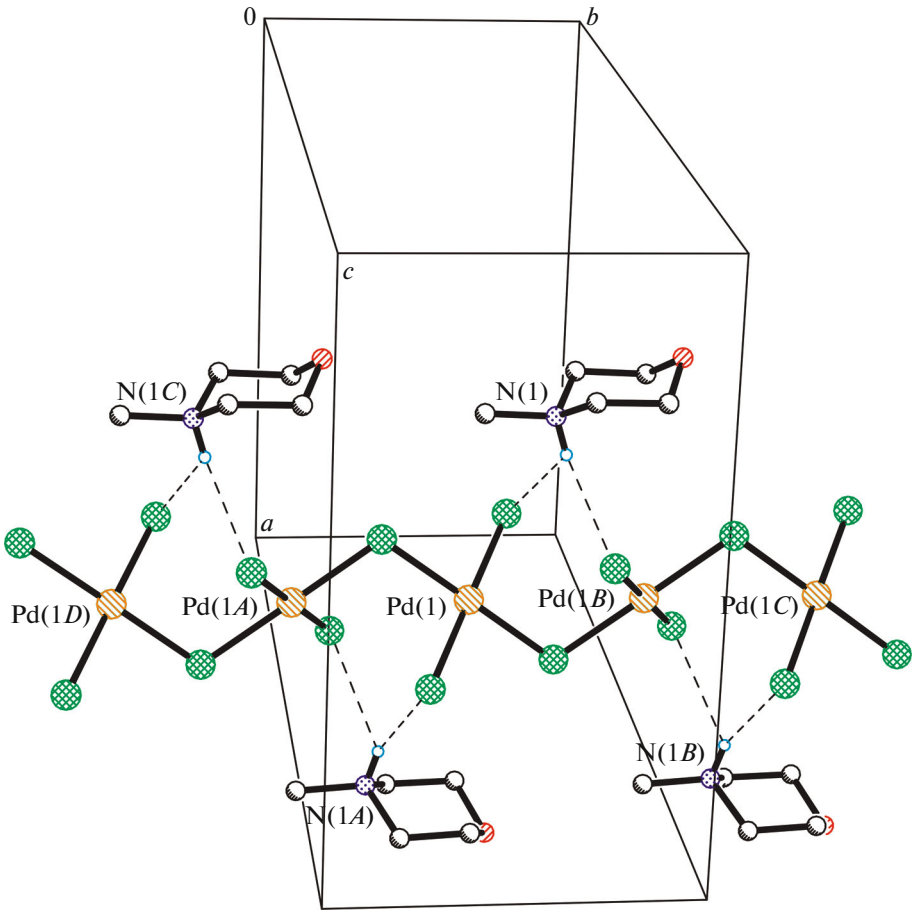

Fig. 3. Anionic chains in the structure of **I**.

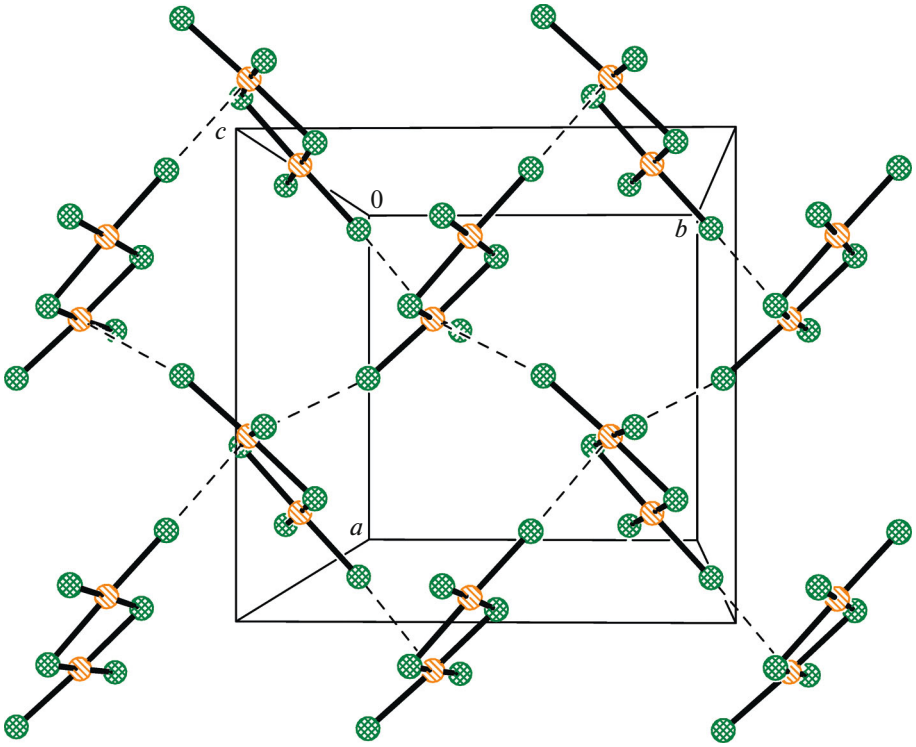

Fig. 4. Anionic layers in the structure of **II** formed via weak Pd···Cl interactions.

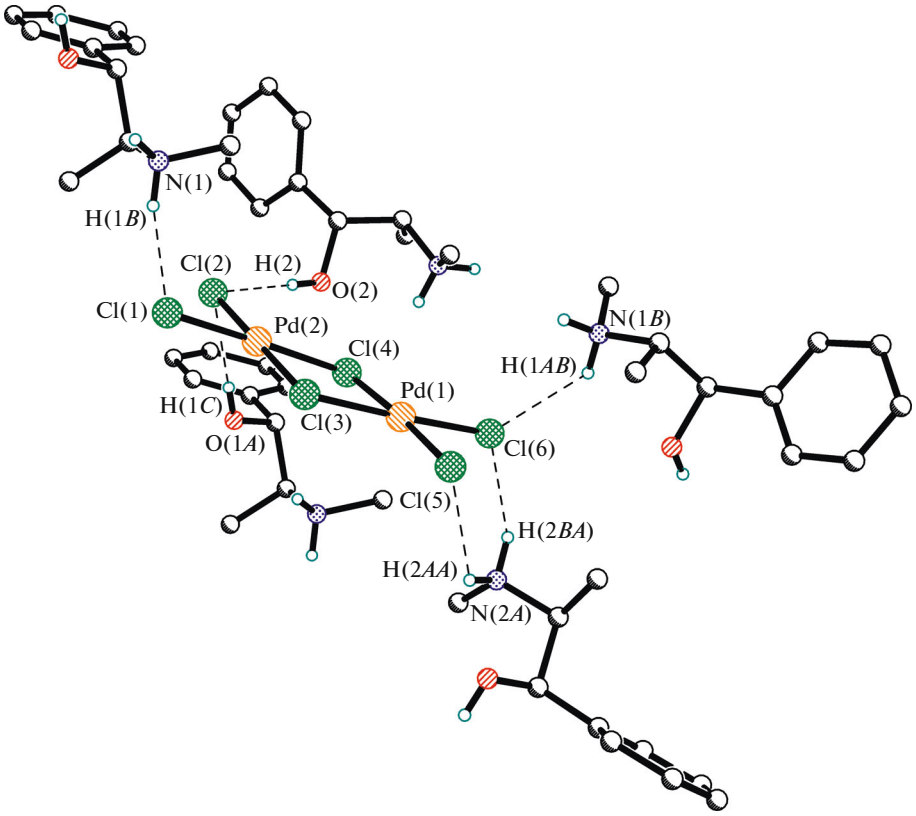

Fig. 5. Hydrogen bonds in the structure of **II**.

Table 3. Growth dynamics of Lewis lung carcinoma in experimental groups

Group	Mean tumor volume, mm^3 ($M \pm S.D.$)				
	7 days	10 days	13 days	17 days	21 days
Control	34.9 ± 21.0 (n = 15)*	76.2 ± 48.9 (n = 15)	195.4 ± 130.7 (n = 15)	504.7 ± 348.5 (n = 15)	731.7 ± 531.3 (n = 15)
Cisplatin	31.6 ± 21.1 (n = 15)	76.1 ± 73.7 (n = 15)	163.3 ± 168.1 (n = 15)	420.5 ± 450.7 (n = 15)	518.2 ± 564.0 (n = 15)
I	28.3 ± 12.8 (n = 15)	44.5 ± 27.1 (n = 13)	87.6 ± 89.9** (n = 13)	248.1 ± 320.2** (n = 13)	352.9 ±459.4** (n = 13)
II	51.9 ± 41.4 (n = 15)	88.4 ± 78.6 (n = 14)	184.6 ± 164.1 (n = 14)	407.9 ± 307.8 (n = 11)	586.9 ± 458.2 (n = 11)

* n is the number of animals in the group.
** Statistically significant ($p < 0.05$) according to Dunn test.

Table 4. Relative growth dynamics of Lewis lung carcinoma in experimental groups

Group	Mean relative tumor volume, rel.u. ($M \pm S.D.$)				
	7 days	10 days	13 days	17 days	21 days
Control	1.0 (n = 15)*	2.2 ± 1.1 (n = 15)	6.5 ± 4.4 (n = 15)	19.6 ± 17.0 (n = 15)	27.8 ± 23.0 (n = 15)
Cisplatin	1.0 (n = 15)	2.1 ± 1.5 (n = 15)	4.7 ± 5.5 (n = 15)	11.9 ± 13.8 (n = 15)	14.4 ± 14.7** (n = 15)
I	1.0 (n = 15)	1.6 ± 0.7 (n = 13)	2.9 ± 2.1** (n = 13)	7.7 ± 8.2** (n = 13)	10.9 ± 12.1** (n = 13)
II	1.0 (n = 15)	1.5 ± 0.7 (n = 14)	3.5 ± 2.2 (n = 14)	7.8 ± 6.6** (n = 11)	11.2 ± 10.4 (n = 11)

* n is the number of animals in the group.
** Statistically significant ($p < 0.05$) according to Dunn test.

(MII > 70% [30]), **I** and **II** should be considered as promising compounds for the development of antitumor agents with clear-cut antimetastatic properties.

The experimental results indicate that Lewis lung carcinoma, a transplantable tumor of mice, which is on the list of obligatory tumors for the preclinical studies of antitumor agents, is sensitive to **I** and **II** [30].

The results of a study of radioprotective activity of **I** and **II** attest to the presence of radioprotective properties, which are more pronounced for **II** in a dose of 10.0 mg/kg (1/8 LD_{16}) for both single and fractional (three-time) exposures to radiation. In the case of single exposure, the preventive administration of **II** in a dose of 10.0 mg/kg (1/8 LD_{16}) markedly mitigated the

Table 5. Metastasis characteristics of LLC in experimental groups

Group	Average number of metastases on the 21st day ($M \pm S.D.$)			MII, %
	large	small	total	
Control	17.1 ± 20.2 (n = 15)*	28.9 ± 30.2 (n = 15)	45.9 ± 49.0 (n = 15)	
Cisplatin	5.1 ± 7.0 (n = 15)	13.5 ± 21.5 (n = 15)	18.7 ± 27.4 (n = 15)	68.7
I	4.4 ± 7.2 (n = 11)	12.4 ± 15.2 (n = 11)	16.8 ± 23.6 (n = 11)	74.4
II	3.7 ± 6.8** (n = 13)	8.5 ± 13.7** (n = 13)	12.2 ± 20.1** (n = 13)	90.6

* n is the number of animals in the group.
** Statistically significant ($p < 0.05$) according to Dunn test.

Table 6. Results of studying radioprotective properties of **I** and **II** upon single exposure

Group	Dose of the test compound	Dose of the test compound, mg/kg	Number of animals in the group	Survival rate		Average life span of the mice that died, days
				abs.	%	
I	1/8 LD_{16}	9	24	5	21	13.0 ± 1.6
I	1/2 LD_{16}	36	24	3	12.5	12.8 ± 0.9
II	1/8 LD_{16}	10	24	14	58*	14.2 ± 1.5
II	1/2 LD_{16}	39	24	8	33	15.7 ± 1.1
Irradiation control	Normal saline		22	1	4.5	15.0 ± 0.8
Biological control			12	12	100	

* Statistically significant ($p < 0.01$) according to the Wilcoxon–Mann–Whitney test.

Table 7. Results of studying radioprotective properties of **I** and **II** upon fractional three-time exposure

Group	Dose of the test compound	Dose of the test compound, mg/kg	Number of animals in the group	Survival rate		Average life span of the mice that died, days
				abs.	%	
I	1/8 LD_{16}	9	12	5	42	14.1 ± 1.9
I	1/2 LD_{16}	36	12	3	25	12.9 ± 2.6
II	1/8 LD_{16}	10	12	9	75*	21.3 ± 5.5
II	1/2 LD_{16}	39	12	6	50	18.7 ± 3.9
Irradiation control	Normal saline		12	4	33.3	13.6 ± 2.4
Biological control			12	10	100	

* Statistically significant ($p < 0.01$) according to the Wilcoxon–Mann–Whitney test.

radiation injury and provided a statistically significant increase in the survival rate of animals to 58.0% versus 4.5% observed for the irradiation control ($p < 0.05$). The injection of **II** in a dose of 39.0 mg/kg (1/2 LD_{16}) prior to the single exposure to radiation also resulted in a higher survival rate (33.0%). Complex **I** also exhibited a radioprotective effect: the survival rate was 21.0% for the 1/8 LD_{16} dose and 12.5% for 1/2 LD_{16} dose, which attests to an additional toxic action of **I** in the 1/2 LD_{16} dose. Analysis of the mortality in the irradiated control groups and the groups administered with **I** and **II** in 1/8 LD_{16} and 1/2 LD_{16} doses showed no differences: the average life spans of animals that died were 13.0 ± 1.6 to 15.0 ± 0.8 days in these groups. It is known that acute arrest of hematopoiesis and development of acute radiation injury take place particularly on days 10 to 20 after irradiation (Table 6) [35].

In the experiment with three-time fractional exposure to increasing doses of 2.5, 3.0, and 3.5 Gy, compound **II** was also most efficient. The use of 1/8 LD_{16} (10.0 mg/kg) of **II** resulted in 75.0% surviving animals versus 33.3% surviving in irradiated control and ensured the maximum protection at the peak of development of acute radiation injury (10–20 days after irradiation), thus extending the life span of mice to 21.3 ± 5.5 days, which is 8 days longer than observed in the irradiated control group (13.6 ± 2.4 days). The efficiency of **I** in the fractional exposure was 42.0% survival at a dose of 1/8 LD_{16} (9.0 mg/kg) (Table 7).

Thus, the communication describes the methods of synthesis and properties of the first cation–anion palladium complexes with polymeric $[PdCl_3]_n^-$ and binuclear $[Pd_2Cl_6]^{2-}$ anions, characterized by high antitumor and radioprotective activities, which, therefore, can be considered as a base for efficient agents for cancer therapy and radiology. The results were protected by a patent [36].

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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