Synthesis, structure, and biological activity of mixed-ligand platinum(II) complexes with aminonitroxides

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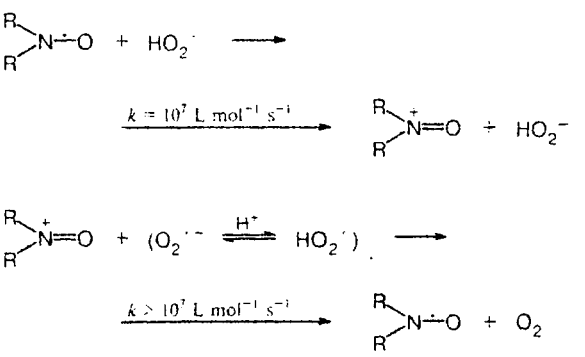
Mixed-ligand platinum complexes *cis*-$Pt^{II}(R^6NH_2)(NH_3)X_2$ and *cis*-$Pt^{II}(R^5NH_2)(NH_3)X_2$ (R^6 is 2,2,6,6-tetramethyl-4-piperidyl-1-oxyl and R^5 is 2,2,5,5-tetramethyl-3-pyrrolidinyl-1-oxyl) were synthesized by either the reaction of aminonitroxides RNH_2 with $Na[Pt^{II}(NH_3)Cl_2I]$ generated *in situ* (for X_2 = ClI) or by replacement of the iodo-chloro ligands in *cis*-$Pt^{II}(RNH_2)(NH_3)ClI$ by dichloro and oxalato ligands. The complexes obtained were characterized by elemental analysis and by IR, UV, and ESR spectra. For *cis*-$Pt^{II}(R^5NH_2)(NH_3)Cl_2$, crystal and molecular structures were determined by X-ray diffraction analysis. Cisplatin accelerates autooxidation of methyl linoleate and the platinum nitroxide complexes synthesized exhibit antioxidant properties. The rate of isolated DNA binding with the new complexes is almost as high as that for cisplatin. *cis*-$Pt^{II}(R^6NH_2)(NH_3)Cl_2$ exhibits the highest antitumor activity. The high antitumor activity of platinum nitroxide complexes shows that the possible "radical component" is not a crucial factor in the cytotoxic action of cisplatin.

Key words: platinum(II) complexes, nitroxides, structure, antioxidants, antitumor activity, cytotoxicity, cisplatin.

The antitumor activity of complexes of divalent platinum such as *cis*-diamminodichloroplatinum(II) (cisplatin) is attributed first of all to their ability to bind with DNA and thus deteriorate its structure and the replication process.[1] There exist indications that both cytotoxic action of cisplatin[2,3] and the main side effects of its action such as nephro-[4] and ototoxicity[5] and nausea[6] can be related to the oxidative damage of the tumor and normal tissues, respectively, due to intracellular induction by cisplatin of the formation of oxygen radicals such as $O_2^{\cdot -}$ and $^{\cdot}OH$. The toxic effect of cisplatin on some animal organs is markedly attenuated by simultaneous administration of antioxidants.[4-6] However, virtually no complexes with ligands possessing antioxidant properties have been described. Meanwhile, for example, nitroxides $R_2N{-}O^{\cdot}$ based on piperidine or pyrrolidine are able to catalyze disproportionation of $O_2^{\cdot -}$ and its protonated form (the $HO_2^{\cdot}$ radical); $R_2N{-}O^{\cdot}$ is oxidized by $HO_2^{\cdot}$ to give oxoammonium cations $R_2N{=}O^+$, while the latter readily oxidize superoxide radicals (Scheme 1),[7] *i.e.*, $R_2N{-}O^{\cdot}$ radicals can act as superoxide dismutase mimetics. The reaction of $^{\cdot}OH$ radicals with nitroxides has a diffusion-controlled rate constant $k = 10^{10}$ L mol^{-1} s^{-1}.[8]

Thus, if radical processes play an essential role in the cytotoxic action of platinum complexes, the introduction of nitroxides as ligands in these complexes should influence the chemotherapeutic properties of the new compounds. One successful example of nitroxide modification of an antitumor preparation is known to date. The nitroxide-containing analog of daunomycin, ruboxyl, showed encouraging results in clinical testing.[9]

Scheme 1



Recently, we prepared dinitroxide complexes **1**, which poorly platinate DNA due to steric reasons. Apparently, this accounts for the low toxicity and antitumor activity of these compounds.[10] In the present communication, we describe synthesis, structure, antioxidant properties, interaction with DNA, and cytotoxic and antitumor

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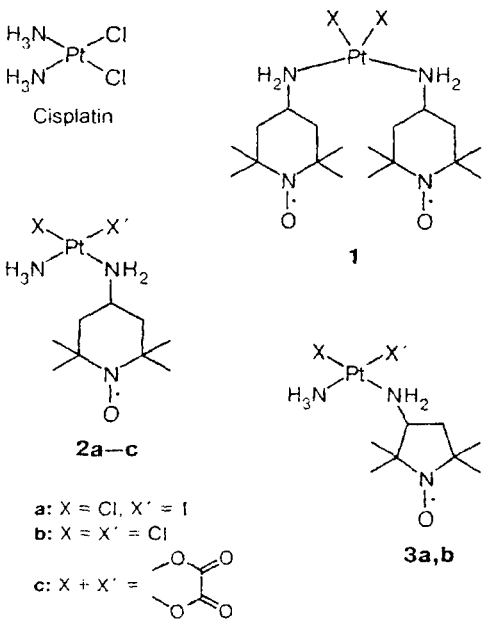


activity for new mononitroxide analogs of cisplatin, complexes **2** and **3**.

Experimental

Analysis for platinum was performed by atomic-absorption spectroscopy using an AAS-3 spectrometer; the accuracy of determination was ±3 rel.%. HPLC was carried out using a Milichrom chromatograph (a 2×64 mm column, Separon C18 (5 μm), detection at 240 nm). In the analysis of complexes **2** and **3**, a MeOH—0.02 *M* aqueous KCl (1 : 3) mixture was used as the eluent. IR spectra were measured in the 400—4000 cm^{-1} range on a Specord 75-IR spectrometer (mineral oil). Electronic spectra were recorded in the 200—800 nm range using a Specord UV—Vis spectrophotometer. ESR spectra were run at room temperature on an SE/X 2544 instrument at a microwave radiation power of 2 mW and modulation of 0.32 mT.

The starting 4-amino-2,2,6,6-tetramethylpiperidin-1-oxyl (R^6NH_2) and 3-amino-2,2,5,5-tetramethylpyrrolidin-1-oxyl (R^5NH_2) were prepared and purified as described above.[11]

***cis*-Ammine(4-amino-2,2,6,6-tetramethylpiperidin-1-oxyl)-iodochloroplatinum(II) (2a).** A solution of NaI (1.62 g, 8.7 mmol) in 1 mL of H_2O and a solution of R^6NH_2 (1.37 g, 8 mmol) in 1 mL of H_2O were added successively at ~20 °C to a stirred solution of $Na[Pt(NH_3)Cl_3]$ in 25 mL of H_2O, prepared from cisplatin (2.34 g, 7.8 mmol).[12] The resulting suspension was stirred for 2 h and the precipitate was filtered off, thoroughly washed with cold water (4×4 mL), and dried in air to give 2.77 g (65%) of complex **2a** (Table 1), which was used in the synthesis of **2b**. In order to prepare analytically pure samples of **2a**, the synthesis was carried out with $K[Pt(NH_3)Cl_3]\cdot 0.5H_2O$ isolated by a known procedure.[12] Compound **2a** consists of small yellow crystals, which get dark at temperatures of ≥200 °C and melt with decomposition at 261—263 °C. Recrystallization of **2a** from acetone gave a solvate (a band at 1708 cm^{-1} in the IR spectrum), which did not decompose during drying *in vacuo* ($3\cdot 10^{-2}$ Torr, 56 °C, 4 h). Recently,[13] complex **2a** was prepared by a different method; its structure was established relying only on NMR data.

***cis*-Ammine(4-amino-2,2,6,6-tetramethylpiperidin-1-oxyl)-dichloroplatinum(II) (2b).** Silver nitrate (830 mg, 4.88 mmol) was added to a suspension of **2a** (1.402 g, 2.57 mmol) in 25 mL of water and the mixture was stirred for 12 h with a magnetic stirrer away from light. The precipitated silver halides were thoroughly separated by centrifugation and filtration through a dense glass filter to give an orange solution of *cis*-$Pt(R^6NH_2)(NH_3)(NO_3)_2$ (**2d**). The completeness of the consumption of $AgNO_3$ was confirmed by the absence of immediate turbidity following the addition of one drop of 0.5 *M* KCl to three drops of the reaction solution. A solution of KCl (1.15 g) in 3 mL of water was added with stirring to the solution of **2d** and the mixture was allowed to stand for 12 h at ~20 °C. Then the reaction solution was concentrated to ~5 mL and the orange

Table 1. Data of elemental analysis and electronic and IR spectra of complexes **2** and **3**

Compound	Found / Calculated (%)				Molecular formula	IR spectrum (in mineral oil)		Electronic spectrum (in H_2O)	
	C	H	N	Pt		ν/cm^{-1}	Assignment	λ_{max}/nm	ε/L mol^{-1} cm^{-1}
2a	20.1	4.20	7.60	35.0	$C_9H_{22}ClIN_3OPt$	1582, 1590, 3106,	NH_2, NH_3	—	—
	19.8	4.06	7.70	35.7		3158, 3203, 3247			
2b	24.0	4.76	9.34	43.4	$C_9H_{22}Cl_2N_3OPt$	1585, 1648, 3180,	NH_2, NH_3	425 sh[a]	23
	23.8	4.88	9.25	42.9		3196, 3269, 3315		357	41
								240 sh	2310
2c	28.0	4.60	9.15	40.7	$C_{11}H_{22}N_3O_5Pt$	1576, 1594, 3135,	NH_2, NH_3	420 sh	31
	28.0	4.70	8.91	41.4		3214, 3270			
						1670, 1690	C=O	321 sh	320
								246	3660
3a	18.1	3.77	7.96	35.7	$C_8H_{20}ClIN_3OPt$	1582, 1593, 3112,	NH_2, NH_3	—	—
	18.1	3.79	7.90	36.7		3164, 3245			
3b[b]	22.0	4.69	9.54	43.0	$C_8H_{20}Cl_2N_3OPt$	1580, 1642, 3124,	NH_2, NH_3	365	40
	21.8	4.58	9.54	44.3		3188, 3258		304 sh	155
								234 sh	2930
								216 sh	5400

[a] Sh stands for shoulder.

[b] Found (%): Cl, 16.3. Calculated (%): Cl, 16.1.

crystals formed were filtered off, washed with cold water and ethanol, and dried *in vacuo* to give 830 mg (71%) of complex **2b**. Crystallization from ethanol gave analytically pure **2b**, which decomposed (got dark) without melting at a temperature of ≥205 °C.

***cis*-Ammine(4-amino-2,2,6,6-tetramethylpiperidin-1-oxyl)-oxalatoplatinum(II) (2c).** A solution of $K_2C_2O_4 \cdot H_2O$ (370 mg, 2 mmol) in 3 mL of water was added to a solution of complex **2d** (1 mmol), prepared as described above, in 5 mL of water and the mixture was allowed to stand for 12 h at 10 °C. The pale-pink crystals that precipitated were filtered off, washed with water and ethanol, and dried *in vacuo* to give 244 mg of **2c** (52%). Crystallization from water gave analytically pure compound **2c**, which decomposed (got dark) without melting at a temperature of ≥220 °C.

***cis*-Ammine(3-amino-2,2,5,5-tetramethylpyrrolidin-1-oxyl)-iodochloroplatinum(II) (3a)** was prepared in 63% yield similarly to **2a** from $Na[Pt(NH_3)Cl_3]$ and R^5NH_2. Compound **3a** consisted of yellow crystals, which decomposed (got dark) without melting at a temperature of ≥185 °C.

***cis*-Ammine(3-amino-2,2,5,5-tetramethylpyrrolidin-1-oxyl)-dichloroplatinum(II) (3b)** was prepared in 79% yield similarly to **2b**. Crystallization from an ethanol—0.05 *M* KCl (1 : 9) mixture gave analytically pure compound **3b**, which got dark at ≥215 °C and melted at 223—225 °C (with decomposition). The crystals of **3b** suitable for X-ray diffraction analysis were prepared by slow evaporation of its aqueous solution.

The main characteristics of complexes **2a—c** and **3a,b** are listed in Table 1.

X-ray diffraction analysis of complex 3b. An experimental set of 5715 independent reflections with $I > 3\sigma(I)$ was collected from a single crystal with dimensions 0.14×0.12×0.24 mm; a KM-4 diffractometer with kappa-geometry (Kuma—Diffraction, Poland), Mo-Kα-radiation. The main crystallographic data: $C_8H_{20}Cl_2N_3OPt$; space group $P2_1/n$, a = 6.676(1), b = 10.980(3), c = 18.351(3) Å, γ = 97.50(2)°, V = 1333.7(6) Å^3, Z = 4, M = 440.25, d_{calc} = 2.13 g cm^{-3}. The structure was solved by the direct method and refined by the least-squares method in the anisotropic approximation. The calculations were performed using the SHELX program package to R = 5.6%. Figure 1 shows the structure of molecule **3b** (hydrogen atoms are omitted). The main geometric parameters of complex **3b** are listed in Table 2.

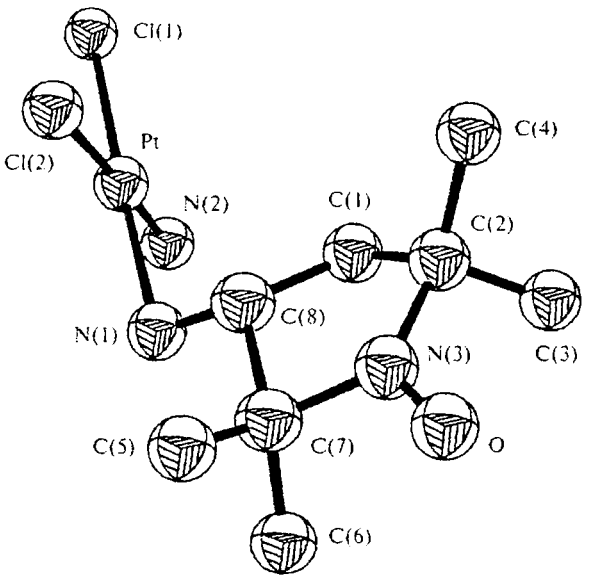


Fig. 1. Crystal structure of complex **3b**.

Table 2. Bond lengths (d) and bond angles (ω) for complex **3b**

Bond	d/Å	Bond	d/Å
Pt—N(1)	2.075(9)	C(1)—C(2)	1.50(2)
Pt—N(2)	2.03(1)	C(1)—C(8)	1.51(2)
Pt—Cl(1)	2.307(3)	C(2)—C(3)	1.61(2)
Pt—Cl(2)	2.302(3)	C(2)—C(4)	1.55(2)
N(1)—C(8)	1.50(2)	C(5)—C(7)	1.55(2)
N(3)—O	1.26(1)	C(6)—C(7)	1.52(2)
N(3)—C(2)	1.51(2)	C(7)—C(8)	1.53(2)
N(3)—C(7)	1.48(2)		

Angle	ω/deg	Angle	ω/deg
N(1)—Pt—N(2)	92.5(4)	C(1)—C(2)—C(3)	111.8(13)
N(1)—Pt—Cl(1)	178.0(3)	N(3)—C(2)—C(3)	107.4(11)
N(1)—Pt—Cl(2)	87.9(3)	N(3)—C(2)—C(4)	107.8(14)
N(2)—Pt—Cl(1)	88.1(3)	N(3)—C(7)—C(5)	108.4(10)
N(2)—Pt—Cl(2)	176.9(4)	N(3)—C(7)—C(6)	109.1(9)
Cl(2)—Pt—Cl(1)	91.57(12)	N(3)—C(7)—C(8)	98.7(10)
C(8)—N(1)—Pt	113.1(7)	C(4)—C(2)—C(3)	111(2)
O—N(3)—C(2)	124.3(11)	C(5)—C(7)—C(6)	112.6(11)
O—N(3)—C(7)	122.1(11)	C(5)—C(7)—C(8)	112.5(10)
C(2)—N(3)—C(7)	113.3(10)	C(6)—C(7)—C(8)	114.4(10)
C(1)—C(2)—N(3)	102.8(10)	C(1)—C(8)—C(7)	106.0(11)
C(1)—C(2)—C(4)	115.8(14)	N(1)—C(8)—C(1)	113.6(11)
C(2)—C(1)—C(8)	103.5(11)	N(1)—C(8)—C(7)	114.7(10)

Determination of antioxidant activity. The influence of cisplatin and complexes **2b** and **3b** on the autooxidation of methyl linoleate was studied at 60 °C. The rate of oxidation of methyl linoleate was monitored based on the absorption of oxygen using a high-sensitivity differential manometric setup.[14] The complexes are insoluble in pure methyl linoleate; therefore, they were introduced into the pre-heated substrate being oxidized as solutions in dimethylacetamide (DMAA) in such a way that the content of DMAA was 10% (v/v) in all cases. This gave transparent solutions, the rate of whose oxidation depended on the concentration of the complexes. It was established in a separate experiment that DMAA is inert in methyl linoleate autooxidation; the introduction of 10% (v/v) DMAA decreased the rate of O_2 absorption by ~10% with respect to that observed for pure methyl linoleate. The results are presented in Fig. 2.

Platination of DNA. The DNA of calf thymus (Serva) (0.1 mg mL^{-1} in a 6.3 m*M* potassium phosphate buffer containing 0.15 m*M* NaCl, pH 7.2) was incubated at 50 °C with cisplatin or with **3b** for periods of time shown in Fig. 3. The preparations were taken in equitoxic doses, 0.35 m*M* for cisplatin and 0.875 m*M* for **3b**. The cross-links in the DNA were determined by spectrofluorometry with ethidium bromide.[15] DNA (20 μg) dissolved preliminarily in 200 μL of the potassium phosphate buffer with pH 7.2 was added to 3 mL of a solution containing 5 μg mL^{-1} of ethidium bromide and 26 m*M* of K_2HPO_4. The solution was alkalified by KOH to pH 12.0. Fluorescence was measured in a 1-cm cell using an Elumin spectrophotometer designed and manufactured at the central design department of the Russian Academy of Medical Sciences.

Assay of cytotoxic activity *in vitro*. The activity of the complexes *in vitro* was assayed using the human breast cancer

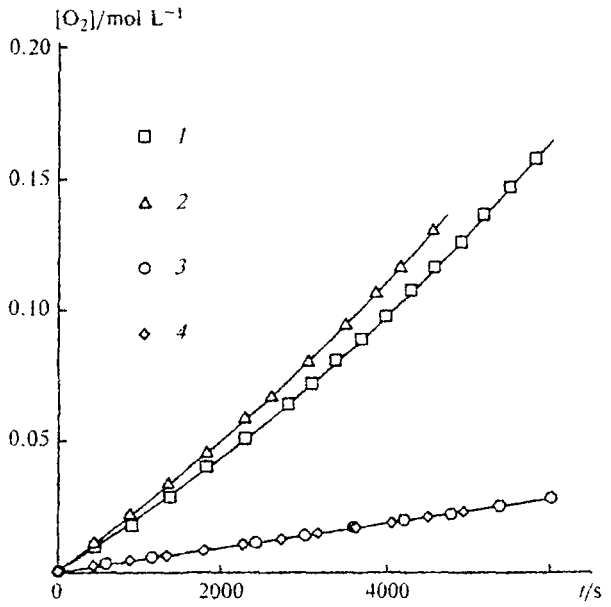


Fig. 2. Effect of platinum complexes ($2.3 \cdot 10^{-4}$ mol L^{-1}) on the kinetics of autooxidation of a methyl linoleate–DMAA mixture (9 : 1) at 60 °C: without a complex (*1*), with cisplatin (*2*), complex **2b** (*3*), and complex **3b** (*4*).

cell culture MCF-7 (Fig. 4). The cells were spread over 24-well plates (10^5 cells in each); after 24 h, the preparations in various concentrations were added to the wells (at least three wells for each concentration were used) and the material was incubated for 1 h. Then the cells were washed with fresh medium, cultivated in the conditioned medium for 24 h, and plated in Petri dishes, 100—300 cells in each, to form colonies. Appropriate amounts of a solvent were added to the control wells. After 10 days, the cell colonies were fixed by methanol, colored by azur-eosin, and counted. Colonies containing more than 50 cells were regarded as viable. The survival fraction was determined as

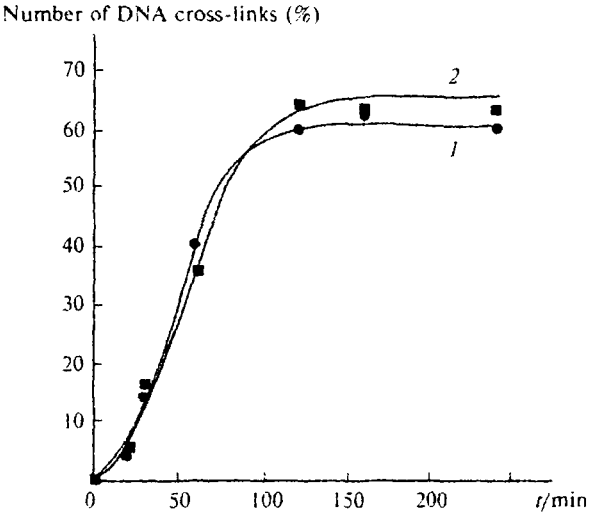


Fig. 3. Kinetics of cross-linking of calf thymus DNA during incubation with $3.5 \cdot 10^{-4}$ mol L^{-1} of cisplatin (*1*) and $8.75 \cdot 10^{-4}$ mol L^{-1} of complex **3b** (*2*).

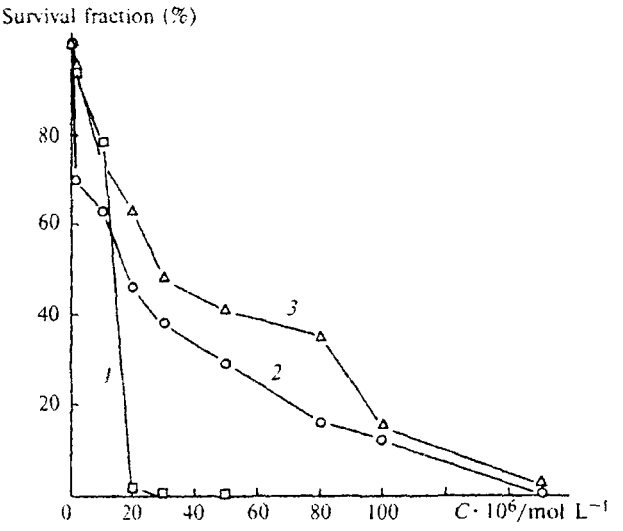


Fig. 4. Viability of the MCF-7 cell culture *vs.* concentration of complexes: cisplatin (*1*), **2b** (*2*), **3b** (*3*).

the plating efficiency in the experiment corrected for the plating efficiency in the control; the cytotoxicity IC_{50} was measured as the concentration of the complex corresponding to a 50% survival fraction.

Assay of toxicity and antitumor activity. The complexes were injected to animals intraperitoneally as aqueous solutions. The total toxicity (LD_{50}) of compounds was determined in BDF_1 mice after single administration. The antitumor activity was studied in relation to leukemia P388. The BDF_1 mice were inoculated for leukemia intraperitoneally using an inoculum containing 10^6 cells. Response to therapy was evaluated using generally accepted index ILS (the increase in median life span of treated mice as compared with control mice), ILS (%) = $100(T/C - 1)$, where T and C are the median life span (in days) of the treated and control animals, respectively. The animals that survived throughout the whole experiment (60 days) were counted separately (Table 3). The adenocarcinoma-755 was introduced subcutaneously using tumor cells in 1 : 2 dilution (0.3 mL); the average tumor diameters in the control and test groups were measured at regular intervals and kinetic curves were constructed (Fig. 5).

Table 3. Toxicity and antileukemic (P388) activity of complexes **2b** and **3b**[a]

Complex	LD_{50}[b]/mg kg^{-1} (mmol kg^{-1})	Single dose /mg kg^{-1}	ILS[c] (%)
2b	15 (0.033)	3.75	292 (2/6)
3b	27 (0.061)	6.75	237 (1/6)
Cisplatin	12 (0.040)	3.0	245 (1/6)

[a] The compounds were administered in animals intraperitoneally on the 1st, 4-, 7-, 10-, and 13th day after inoculation with the tumor.

[b] The dose inducing the death of 50% of healthy mice.

[c] The median life span of animals in the control group was 11.2 days. In parentheses: the number of cured mice (surviving for more than 60 days)/the total number of animals in the group.

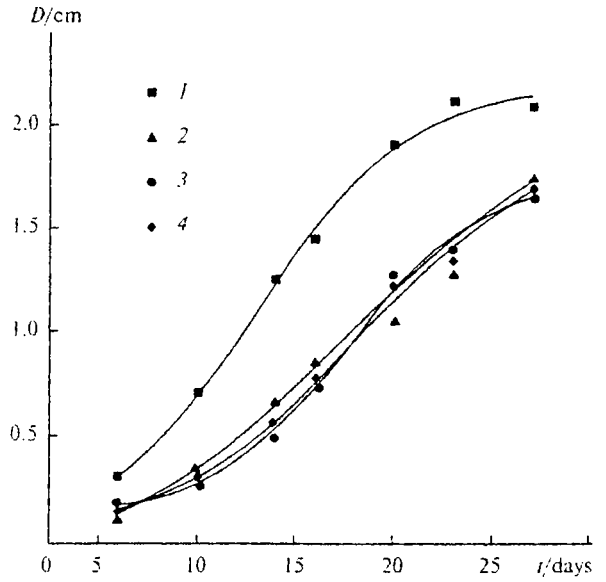


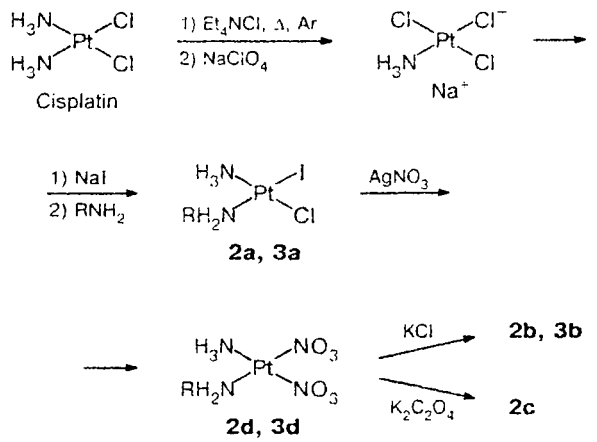
Fig. 5. Kinetic curves for the growth of adenocarcinoma-755 in the control (*1*), after introduction of cisplatin in a dose of 2.4 mg kg^{-1} (*2*), complex **2b** in a dose of 2.5 mg kg^{-1} (*3*), and complex **3b** in a dose of 5.4 mg kg^{-1} (*4*).

Results and Discussion

We found that reactions of $Na[Pt(NH_3)Cl_3]$ [12] treated successively with NaI and RNH_2 (R = 2,2,6,6-tetramethyl-4-piperidyl-1-oxyl (R^6) or 2,2,5,5-tetramethyl-3-pyrrolidinyl-1-oxyl (R^5)) gives the complexes *cis*-$Pt(RNH_2)(NH_3)Cl$ (R = R^6 (**2a**), R^5 (**3a**)) (Scheme 2). Treatment of **2a** and **3a** with $AgNO_3$ in water afforded *cis*-$Pt^{II}(RNH_2)(NH_3)(NO_3)_2$. The completion of the reaction was checked by TLC based on the consumption of the starting **2a** and **3a** and on the negative result of testing the reaction solution for residual $AgNO_3$. After thorough separation of the precipitated AgI and AgCl, complexes **2d** and **3d** were converted without isolation into **2b** or **2c** and **3b** by reactions with KCl or $K_2C_2O_4 \cdot H_2O$, respectively. The preparation of **2a** and **3a** from isolated $K[Pt(NH_3)Cl_3] \cdot 0.5H_2O$ gives purer samples; however, perfoming the reaction *in situ via* the intermediate $Na[Pt(NH_3)Cl_3]$ is simpler and provides higher yields of target **2b,c** and **3b** in relation to the starting cisplatin.

Complexes **2a—c** and **3a,b** are yellow crystalline materials. The solubilities of complexes **2a** and **3a** in water at ~20 °C are ~0.1 mg mL^{-1}, those of complexes **2b** and **3b** are ~1 mg mL^{-1}, and the solubility of compound **2c** is ~0.4 mg mL^{-1}. The chromatogram of each water-soluble complex has one peak; the retention volumes are 330, 300, and 270 μL for **2b**, **2c**, and **3b**, respectively. The results of elemental analysis (see Table 1) show good agreement between the found and calculated values. The *cis*-structure of the complexes was suggested in view of the stronger *trans*-effect of halo ligands compared to N-ligands[12] and confirmed by X-ray

Scheme 2



diffraction data for **3b** (see Fig. 1). In molecule **3b**, the Pt^{II} has square-planar surroundings consisting of two Cl atoms (Pt—Cl 2.302(3), 2.307(3) Å) and two N atoms (Pt—N 2.075(9), 2.03(1) Å) contained in the R^5 and NH_3 ligands (the deviation of atoms from the plane is ≤0.05 Å). Note that the complex geometry is close to that found for other mixed-ligand platinum complexes[16] (see Table 2). The pyrrolidine ring has a distorted envelope conformation (the C(1) atom deviates from the C(2)—N(3)—C(7) plane by 0.07 Å and the C(8) atom deviates from it by 0.51 Å; the angle between the C(2)—N(3)—C(7) plane and the N(3)—O line is 5°).

The ESR spectra of dilute solutions of complexes consist of three lines, which is in agreement with the monoradical structure. The hyperfine splitting constant at the N atom (a_N) and the *g*-factor in aqueous solutions for **2a—c** are 1.69 mT and 2.0056, respectively; these values for **3a,b** are 1.55 mT and 2.0054. The bands at 3106—3315 cm^{-1} in the IR spectra of **2a—c** and **3a,b** are due to stretching vibrations of the NH_2 groups and the bands at 1576—1648 cm^{-1} correspond to deformation vibrations. The IR spectrum of **2c** contains the expected stretching bands for the carbonyl groups in the oxalate ligand at 1670 and 1690 cm^{-1}.

The electronic spectra of water-soluble complexes (XX′ = Cl_2 or XX′ = Ox) in the range of 200—800 nm exhibit bands for the nitroxide and Pt^{II} chromophores (see Table 1). The $n \rightarrow \pi^*$ band, which is usually observed for piperidinoxyls in the visible region of the spectrum, at ~440 nm, in the case of **2b,c**, is shifted by ~20 nm to shorter wavelengths and is manifested as a shoulder due to the wing of the broad Pt^{II} band at 320—360 nm. In the spectra of pyrrolidinoxyls, the $n \rightarrow \pi^*$ band has an ~2 times lower ε than that for piperidinoxyls; in the case of **3b**, it cannot be detected at the wing of the Pt^{II} band. In the UV region of the spectrum, the absorption due to the $\pi \rightarrow \pi^*$ transition in the >N—O• group (for piperidin- and pyrrolidinoxyls, $\lambda_{max} \approx 240$ nm, $\varepsilon \approx 2000$ L mol^{-1} cm^{-1}) is overlapped by the absorption of

the Pt^{II} chromophore, which depends on the nature of the X ligand in a complex manner.[17]

The relationship between the structure and antitumor activity of platinum diammino complexes is complicated and depends on a large number of factors.[18] However, it is clear that, in order to exhibit activity, a complex having got into a cell should bind efficiently to DNA. It is also interesting to evaluate the possible influence of new complexes containing a redox-active nitroxide fragment on the oxidative processes.

A comparative study of the reactions of **3b** and cisplatin with isolated DNA was carried out. It can be seen in Fig. 3 that these compounds taken in equitoxic doses induce the formation of nearly equal amounts of cross-links in DNA. When the duration of incubation is >2 h, the number of cross-links (~65%) stops increasing, which can be explained by virtually complete platination of reactive guanine and adenine in the DNA.[19] Thus, unlike the two bulky ligands in complex **1**,[10] one pyrrolidinoxyl fragment creates no steric restrictions to platination; complex **3b** binds to DNA at a rate close to that of binding of cisplatin.

In order to compare the possible influence of normal and nitroxide-containing ammino complexes of platinum on the oxidative processes in the living cell, we studied the action of these complexes in relation to a readily accessible and well studied test reaction, namely, autooxidation of methyl linoleate. It can be seen in Fig. 2 that cisplatin and platinum nitroxide complexes present in equal concentrations have opposite effects on the rate of methyl linoleate oxidation. Whereas cisplatin accelerates the oxidation (curve *2*), complexes **2b** and **3b** in the concentrations studied here decelerate methyl linoleate autooxidation almost 4-fold (curves *3* and *4*). This outcome confirms the published data[2–6] that cisplatin can catalyze radical oxidation.

Biological testing of the complexes included determination of the activity *in vitro* in the cell culture MCF-7 of human breast cancer and primary screening in experimental models of tumors, *viz.*, leukemia P388 and adenocarcinoma-755. Study of the cytotoxic activity of the complexes *in vitro* using the MCF-7 cells showed that the concentrations inducing 50% inhibition of the cell viability (IC_{50}) for **2b** and cisplatin are close (18 and 13 μM, respectively). In the case of **3b**, this value is 29 μM, which is ~2 times as high as that of cisplatin (see Fig. 4). Attention is drawn by the fact that the concentrations of **2b** and **3b** that cause 90% inhibition of the cell viability (IC_{90}) are nearly equal and are ~6 times as high as IC_{90} for cisplatin.

Data on the overall toxicity and antitumor activity of the complexes *in vivo* are presented in Table 3 and in Fig. 5. The characteristics of toxicity (see Table 3) for **2b** and cisplatin nearly coincide. Complex **3b** exhibits a markedly lower (by ~1.5—2 times) toxicity. Study of the antileukemic activity of the complexes showed that, when the complexes are administered five times in doses corresponding to $0.25 \cdot LD_{50}$, their activity is equal to or even somewhat higher than that of cisplatin (see Table 3). Figure 5 shows the kinetic curves for the variation of the average diameter of the adenocarcinoma-755 grown hypodermically either in the control or under conditions of the therapy with cisplatin or its nitroxide derivatives. Complexes **2b** and **3b**, like cisplatin, moderately inhibit the growth of adenocarcinoma-755.

Thus, the results of our studies show that the formal replacement of one NH_3 ligand in cisplatin by aminonitroxide gives rise to complexes, which are not inferior to the starting cisplatin in efficiency of binding to DNA. Unlike cisplatin, platinum nitroxide complexes exhibit antioxidant properties. The biological properties of **2b** and **3b** not only differ from those of cisplatin but also differ markedly from each other. In the experiments with cell culture MCF-7, the cytotoxicity of the complexes decreases in the sequence cisplatin > **2b** > **3b**, which can be interpreted as being due to the inhibition of the platinum-initiated radical reactions by the nitroxide, resulting in a decrease in the radical component of cytotoxicity. However, in the experiments with mice, **2b** and cisplatin exhibit virtually equal toxicities, while **3b** is less toxic. A decrease in the general toxicity of antitumor cytostatics by introduction of nitroxides as ligands in their structure or by simultaneous administration of these components has also been demonstrated previously.[20–22] The structural distinctions between **2b** and **3b** are too minor for expecting that they would influence significantly the platinum surroundings and chemical activity in the complexes. The difference between **2b** and **3b** is more likely to be due to different redox properties of the piperidin-1-oxyl and pyrrolidin-1-oxyl. For example, it is known[23] that piperidin-1-oxyl and pyrrolidin-1-oxyl are reduced in fresh blood to hydroxylamines, the corresponding half-reduction times being ~1 and ~10 h, respectively. Although both nitroxides and hydroxylamines, resulting from their reduction *in vivo*, are antioxidants, their antioxidation properties are nonequivalent. In addition, the different degrees of reduction of the complexes *in vivo* in combination with the differences in the basicity and, correspondingly, the degree of protonation of the hydroxylamines formed can appreciably differentiate the metabolism routes of **2b** and **3b** and thus affect the biological properties studied here.

The high antitumor activity of platinum nitroxide complexes indicates that the possible "radical component" does not play a crucial role in the cytotoxic action of cisplatin in *in vivo* experiments. It has been found[24] that the combined use of cisplatin and antioxidants that do not bind to it provides the possibility of decreasing the damage of healthy organs without substantial loss of the antitumor activity. In this connection, the new complexes **2b** and **3b** can be practically promising provided that they would display a considerable decrease in the specific toxic effects typical of cisplatin.

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