Synthesis and antitumor properties of new platinum(IV) complexes with aminonitroxyl radicals

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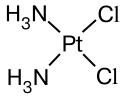
Acylation of *cis,trans,cis*-$Pt^{IV}(RNH_2)(NH_3)(OH)_2Cl_2$ with acetic anhydride afforded complexes *cis,trans,cis*-$Pt^{IV}(RNH_2)(NH_3)(OAc)_2Cl_2$, where R is 2,2,6,6-tetramethyl-1-oxylpiperidin-4-yl (**1b**) or 2,2,5,5-tetramethyl-1-oxylpyrrolidin-3-yl (**2b**). The complexes were characterized by elemental analysis, HPLC, and IR, UV, and ESR spectra. Complex **1b** exhibits high antitumor activity comparable with that of Cisplatin against leukemia P388 used as the experimental tumor. Simultaneous administration of low doses of **1b** and Cisplatin (1/20 of LD_{50} each) results in synergism of the antitumor activity and 100% cure of animals.

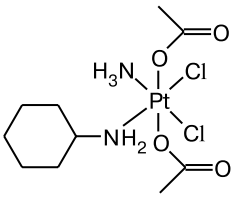
Key words: platinum(IV) complexes, nitroxyl radicals, antitumor activity, Cisplatin, synergism.

cis-Diamminedichloroplatinum(II) (Cisplatin) is the most known platinum complex with high activity in the treatment of a series of human tumors. Cisplatin finds wide clinical use but is highly toxic and its administration causes fast development of drug resistance in tumors. Platinum(IV) compounds, *e.g.*, drug JM216, being now under clinical testing, have attracted attention in the recent time as potential antitumor agents. These complexes are powerful inhibitors for the growth of tumor cells, including those resistant to Cisplatin, and possess moderate toxicities.[1]

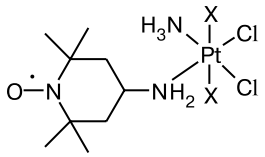
We have previously[2—5] studied di- and tetravalent platinum complexes with aminonitroxyl ligands. In the present work, we describe the synthesis, structure determination, and properties of new platinum(IV) aminonitroxyl complexes **1b** and **2b**. Their total toxicities and antitumor activities were studied both in the individual form and in combination with Cisplatin. The platinum aminonitroxyl complexes are of interest, because the nitroxyl radicals themselves manifest antitumor effect.[6—8] In addition, they are antioxidants and mimetics of superoxide dismutase enzyme,[9,10] can decrease the toxic effect of xenobiotics,[11] and make it possible to use ESR for studying the mechanism of biological activity.[12]



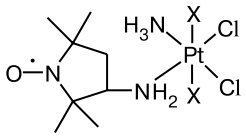
Cisplatin



JM216



1a,b



2a,b

X = OH (**a**), OAc (**b**)

Experimental

The platinum content was determined by atomic absorption spectroscopy on an AAS-3 spectrometer with an accuracy of determination of ±3 rel.%. A Milikhrom chromatograph was used for HPLC (column 2×64 mm, Separon C18 (5 μm), detection at 240 nm, 30% aqueous MeCN as eluent), the retention volumes (V_r) were 290 and 280 μL for **1b** and **2b**, respectively. IR spectra were recorded in the range of 400—4000 cm^{-1} on a Specord 75-IR spectrometer in Nujol. Electronic spectra were obtained in the interval from 200 to 800 nm on a Specord UV-VIS spectrophotometer. ESR spectra were measured at room temperature on an SE/X 2544 instrument at a UHF power of 2 mW and a modulation of 0.032 mT.

The starting platinum(IV) complexes, *viz.*, *e*-ammine-*d*-(4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl)-*a,f*-dihydroxo-*b,c*-dichloroplatinum(IV) (**1a**) and *e*-ammine-*d*-(3-amino-2,2,5,5-tetramethylpyrrolidine-1-oxyl)-*a,f*-dihydroxo-*b,c*-dichloroplatinum(IV) (**2a**) were synthesized according to a previously published procedure.[5]

***e*-Ammine-*d*-(4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl)-*a,f*-bis(acetato)-*b,c*-dichloroplatinum(IV) (1b).** Freshly dis-

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tilled acetic anhydride (7 mL) was added with ice-cooling and stirring to thoroughly ground complex **1a** (1.20 g, 2.46 mmol). After the starting complex **1a** dissolved, the cooling bath was removed. The reaction course was monitored by TLC on Silufol plates using a MeCN—MeOH (2 : 1) solvent system, the R_f values being 0.35 for **1a** and 0.77 for **1b**. No starting complex **1a** was detected in the reaction mixture after ~0.5 h. The overall reaction time was 1 h. The reaction product was precipitated by the slow addition of an ether—hexane (1 : 1) mixture (20 mL). The suspension was stored for 20 min to complete crystallization. The reaction product was filtered off, washed with an ether—hexane (1 : 1) mixture (3 mL×3), and dried in air. Complex **1b** was obtained in a yield of 1.14 g (1.99 mmol, 81%) as orange crystals, m.p. 222—224 °C (decomp.) (from MeOH—ether). Found (%): C, 27.10; H, 4.82; Cl, 12.50; N, 7.24; Pt, 34.76. $C_{13}H_{28}Cl_2N_3O_5Pt$ (572.37). Calculated (%): C, 27.28; H, 4.93; Cl, 12.39; N, 7.34; Pt, 34.08. UV (H_2O), λ_{max}/nm (ε/L mol^{-1} cm^{-1}): 442 sh (19), 372 sh (120), 216 (33000). IR (Nujol), ν/cm^{-1}: 1573, 1670, 3108, 3178, 3242 (N—H, C=O). ESR (H_2O): three lines, *g*-factor 2.0056, a_N = 1.693 mT.

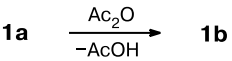
***e*-Ammine-*d*-(3-amino-2,2,5,5-tetramethylpyrrolidine-1-oxyl)-*a*,*f*-bis(acetato)-*b*,*c*-dichloroplatinum(IV) (2b)** was synthesized similarly from **2a** (1.35 g, 2.85 mmol) and acetic anhydride (8.1 mL). The yield was 1.43 g (2.57 mmol, 90%), m.p. 197—199 °C (decomp.) (from MeOH—ether). Found (%): C, 26.16; H, 4.63; Cl, 13.07; N, 7.70; Pt, 35.30. $C_{12}H_{26}Cl_2N_3O_5Pt$ (557.09). Calculated (%): C, 25.81; H, 4.69; Cl, 12.70; N, 7.53; Pt, 34.94. UV (H_2O), λ_{max}/nm (ε/L mol^{-1} cm^{-1}): 374 sh (170), 214 (32000). IR (Nujol), ν/cm^{-1}: 1572, 1630, 1648, 3208, 3250 (N—H, C=O). ESR (H_2O): three lines, *g*-factor 2.0053, a_N=1.575 mT.

Determination of toxicity and antitumor activity. The complexes were injected into animals intraperitoneally as aqueous solutions. The total toxicity (LD_{50}) of the compounds was tested in BDF_1 mice upon a single injection. The antitumor activity was examined in the experimental tumor, *viz.*, leukemia P388. The transplantation of tumors was carried out according to a standard procedure.[13] The activity of the complexes was estimated from an increase in the median life span (ILS) of treated animals compared to control animals: ILS (%) = $100(T_{m.t}/T_c - 1)$, where $T_{m.t}$ and T_c are the measured median life span (in days) of treated and control animals, respectively. The cured animals (remained alive for ≥60 days) were taken into account separately. The antitumor effect for the combined use of Cisplatin and the new complexes was studied at low doses of the drugs, being 1/20 of LD_{50}. The drugs were introduced simultaneously on the first, third, fifth, and seventh day after the tumor transplantation.

Results and Discussion

Synthesis and structure of the complexes. The purpose of acylation of the *trans*-hydroxy groups in complexes **1a** and **2a** is the synthesis of new complexes that are more lipophilic than the starting ones. In addition, the substitution of the OH groups by more electron-withdrawing OAc groups increases the reduction potential of Pt^{IV}/Pt^{II},[14] which can change metabolism and biological properties of the drugs. The reaction with weak carboxylic acid anhydrides proceeds smoothly and in high yields (Scheme 1).

Scheme 1



An attempt to use trifluoroacetic anhydride was unsuccessful because of the formation of a mixture of products. This was caused, most likely, by the partial disproportionation of nitroxyl radicals under the action of stronger trifluoroacetic acid that formed in the reaction.[15] Complexes **1b** and **2b** are yellow or orange stable crystalline compounds with high solubility in water (≥10 mg mL^{-1} at ~20 °C). Their characteristics remain unchanged on storage in a refrigerator (5 °C) for more than 1 year.

The proposed structures of the complexes are completely consistent with the elemental analysis and electronic, ESR, and IR spectroscopic data (see Experimental). The ESR spectra of **1b** and **2b** consist of three lines of approximately equal intensity, and the values of the hyperfine splitting constants a_N and *g*-factors are characteristic of piperidine and pyrrolidine nitroxyls. The bands in the electronic spectra are caused by the superimposition of the absorption from the nitroxyl and Pt^{IV} chromophores. The IR spectra contain the expected bands of stretching and bending vibrations of the N—H bonds at 3100—3250 and 1570—1600 cm^{-1}, respectively. The bands at 1630—1670 cm^{-1} in the spectra of **1b** and **2b** belong to stretching vibrations of the carbonyl groups of the *trans*-carboxylate ligands. The proposed structures of the complexes are confirmed by the previously obtained X-ray diffraction data[5] for the starting complex **1a**.

Toxicity and antitumor activity of the complexes. The data on the total toxicity and antileukemic activity of the complexes are presented in Table 1. The toxicity of complexes **1b** and **2b** with the *trans*-acyloxy ligands (X = OAc) is approximately half of that of their *trans*-hydroxy analogs **1a** and **2a** (X = OH).[5] The Pt^{IV} complexes in an animal organism are known[14,16] to be reduced rapidly to the chemically more active and more cytotoxic Pt^{II} complexes. For instance, for the JM-216 drug, the half-period of transformation in blood is only 6.3 min (see Ref. 16). For the same amino ligands, the reduction rate and cytotoxicity *in vitro* increase in the series OH < OAc (see Ref. 14). In our case, the OAc-substituted complexes possess, on the contrary, lower toxicity, which is probably caused by specific features of their pharmacokinetics. The toxic properties of complexes **1** and **2** are modulated by the influence of the aminonitroxyl ligands. The latter, unlike inert alkylamino ligands in complexes of the JM-216 type, exhibit the antioxidant properties. The na-

Table 1. Toxicity and antileukemic (P388) activity of complexes **1b** and **2b**

Complex	LD_{50}[a]/mg kg^{-1} (mmol kg^{-1})	Single dose[b] /mg kg^{-1}	ILS[c] (%)
1b	46 (0.080)	7.5	220 (4/8)
2b	100 (0.180)	34	247 (0/8)
Cisplatin	12 (0.040)	4	373 (6/8)

[a] The dose that caused the death of 50% of mice.
[b] The compounds were injected into animals intraperitoneally on the first, third, fifth, and seventh day after the tumor transplantation.
[c] The median life span of animals in the control group was 11.2 days. The number of cured animals (their life span was more than 60 days) and the total number of animals in the group are given in the numerator and denominator, respectively.

ture of nitroxyl substituents also exerts a substantial effect, and piperidine-1-oxyl complexes **1a,b** are more toxic than their pyrrolidine-1-oxyl analogs **2a,b**. This can be due to differences in the redox properties of piperidine- and pyrrolidine-1-oxyls.[4]

Complex **1b** demonstrated high antitumor activity for the experimental tumor, *viz.*, leukemia P388. In the test group consisting of eight animals, this complex cured four animals (the animals that remained alive for ≥60 days considered as cured animals), and for the rest four animals it increases the life span by 220% on the average. In this test, the activity of complex **1b** exceeds substantially that of complex **2b** and approaches the activity of Cisplatin (see Table 1).

Interesting results were obtained in the study of the combined effect of Ciplatin and complexes **1b** and **2b** in the treatment of leukemia P388. Very low doses of the drugs (1/20 of LD_{50}) were used in these experiments. The separate administration of the drugs in these doses results in low ILS values and no animals remained alive. However, their combined use manifests 100% cure of animals for the **1b**+Cisplatin combination and five of eight cured animals (~60%) for the **2b**+Cisplatin combination (Fig. 1). A low difference in structures of the nitroxyl radicals in the composition of the synthesized complexes exerts a substantial effect on their antitumor activity. Complex **1b** containing the piperidine-1-oxyl fragment is more active for both separate use (see above) and in combination with Cisplatin.

We also studied the combined effect of low doses of another known drug Carboplatin (*cis*-diammine(cyclobutane-1,1-dicarboxylato)platinum(II)) and complex **1b** (Fig. 2). In this case, the synergistic effect is less pronounced and no animals were cured. Therefore, the criterion of estimation was an increase in the median life span of the cured animals compared to control animals. The ILS parameter for the combined use of Carboplatin and complex **1b** is approximately twice as large as the sum of

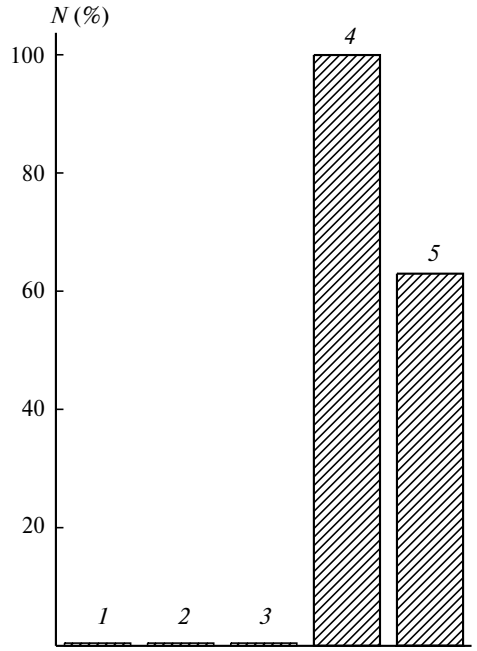


Fig. 1. Synergism of the antitumor effect for the combined use of Cisplatin and complexes **1b** and **2b** in the treatment of leukemia P388: *1*, Cisplatin; *2*, **1b**; *3*, **2b**; *4*, Cisplatin + **1b**; and *5*, Cisplatin + **2b**. Doses of drugs/mg kg^{-1}: 0.6 (Cisplatin), 2.3 (**1b**), and 5.0 (**2b**). The drugs were injected simultaneously on the first, third, fifth, and seventh day after the tumor transplantation. *N* (%) designates animals remained alive.

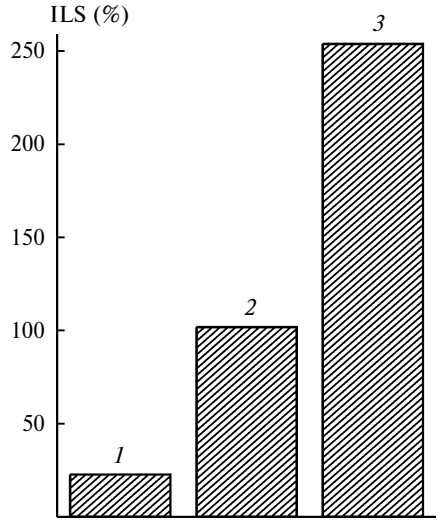


Fig. 2. Enhancement of the antitumor effect for the combined use of Carboplatin and complex **1b** in the treatment of leukemia P388: *1*, Carboplatin; *2*, **1b**; *3*, Carboplatin + **1b**. Doses of drugs/mg kg^{-1}: 1.8 (Carboplatin) and 2.3 (**1b**). The drugs were injected simultaneously during 1—7 days after the tumor transplantation. ILS is the life span increase.

effects caused by separate injection of these drugs (see Fig. 2). There are few published data[17—19] on enhance-

ment of the antitumor effect for the use of two different bivalent platinum complexes. As far as we know, synergism for the use of a combination of the Pt^{II} and Pt^{IV} complexes was not described. The synergism observed can be caused by differences in targets and/or mechanisms of action of the Pt^{II} and Pt^{IV} complexes. Since chemotherapy of tumors by the Platinum Group drugs causes strong side effects, a possibility to decrease the applied doses and retain high antitumor activity seems promising in the practical aspect. Another serious problem for therapy by platinum compounds is fast development of drug resistance. Since it was found[5] that the resistance of leukemia P388 to complex **1a** develops much more slowly than that to Cisplatin, we can hope that this problem can be solved partially by using complexes of the types of **1a,b** and **2a,b**.

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