Synthesis, reactions with DNA, and antitumor activity of platinum complexes with aminonitroxyl radicals

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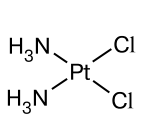
The work is a review of the data on the synthesis of mono- and biradical Pt^{II} complexes with mono- and diaminonitroxyl radicals, as well as of a binuclear complex with diaminonitroxyl radical. A "mild" method is considered for the synthesis of a number of Pt^{IV} nitroxyl complexes (**9**—**11**), whose lipophilicity varies within a wide range due to the *trans*-ligands, *i.e.*, the linear aliphatic acid moieties. Correlations between the structures of the complexes, efficiency of their binding to DNA, and the effect of this binding on the DNA stability were established. Cytotoxic properties of the complexes against the HeLa, H1299, and MCF7 tumor cells, the effect of the complexes on the cell cycle, and the p53 protein expression were studied. The data on the antitumor activity of the complexes in the animal tumor model, P388 leukemia, are given. The rate of the development of resistance to complex **10a** for P388 leukemia is 2.5 times lower than the corresponding value for cisplatin. It was found that a synergistic enhancement of antitumor activity is observed when low doses of cisplatin and complexes **9b** or **10b** are simultaneously administered. The specificities of biological activity of the platinum nitroxyl complexes are presumably due to the antioxidant properties of the nitroxyl pharmacophore and the ability of these complexes to cause the p53-independent tumor cell death.

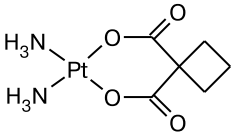
Key words: platinum(II) complexes, platinum(IV) complexes, nitroxyl radicals, cytotoxicity, antitumor activity, satraplatin (JM-216), cisplatin, synergy, apoptosis, p53 tumor suppressor.

Nitroxyl radicals (NR), which are sometimes called "organic nitrogen oxides", possess a wide range of biological activity including a hemodynamic effect, protection against ionizing radiation, suppression of oxidative stress in different types of pathology.[1] One of the directions initiated by Academician N. M. Emmanuel in the Institute of Chemical Physics of the Russian Academy of Sciences is the studies of antitumor activity of NR themselves, their combined application with known antitumor agents, and, finally, hybrid medicines, in which NR are covalently bound to the known antitumor pharmacophores. At the present time, such studies attract growing attention worldwide.[2] Antitumor activity of simplest NR was for the first time discovered in the mouse luekemia La tumor model.[3] Further works in the Institute of Chemical Physics and the Institute of Problems of Chemical Physics of the Russian Academy of Sciences led to the development and studies of nitroxyl derivatives of such known antitumor agents, as thiophosphoramides,[4] daunorubicin,[5] 5-fluorouracil,[6] and nitrosoureas.[7] Some representatives of the hybrid compounds under consideration showed significant improvement of chemotherapeutic properties as compared to the prototypes,[8] and under favorable circumstances could have used in practice.

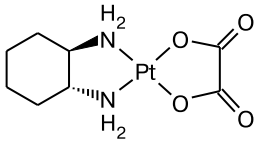
For the last 30 years, platinum complexes have taken one of the leading positions among cytostatics. Antitumor activity was discovered in cisplatin (CP), and then CP was



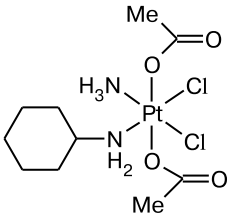
Cisplatin



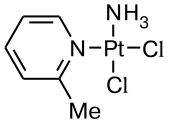
Carboplatin



Oxaliplatin



Satraplatin (JM-216)



Picoplatin

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approved for use in clinics.[9] Subsequent search for the improved analogs led to the introduction of carboplatin and oxaliplatin. Approximately 15 structurally different complexes were rejected for different reasons during clinical trials. At the present time, JM-216 (satraplatin) and picoplatin are in clinical trials.[10]

Cisplatin and other complexes of divalent platinum are effective against a number of human tumors and are used almost in the half of known combinations with other antitumor drugs.[10] The Pt^{II} complexes possess high reactivity and, therefore, are highly toxic agents, which require special regime of administration. For example, CP is administered by a prolonged infusion of a very dilute solution.[11] Another disadvantage of CP is a rapid development of tumor resistance to this drug.

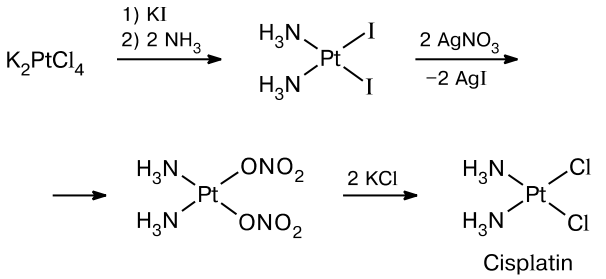
The Pt^{IV} complexes are chemically more inert than the Pt^{II} complexes, moderately toxic, and suitable for the oral administration. It was found that such complexes as satraplatin can pass the digestive tract, be absorbed into the blood, reach the target cells, and provide antitumor activity.[12] The Pt^{IV} complexes are prodrugs (drug precursors) and, after entering the cell or on the way to it, they are reduced to the corresponding active Pt^{II} analogs exhibiting cytotoxic activity. Simultaneously, the Pt^{IV} complexes are powerful tumor cell growth inhibitors, including those resistant to CP. Recent achievements in the studies of antitumor platinum amino complexes are summarized in the reviews.[9,10,13–15]

This review presents the data on the development in the Institute of Problems of Chemical Physics of the Russian Academy of Sciences of new highly active and low toxic antitumor agents, *viz.*, Pt^{II} and Pt^{IV} complexes with biologically active aminonitroxyl radicals. In addition to biological activity, these compounds have the advantage of being paramagnetic, which gives an opportunity to use them as spin labels, including in the study of the mechanism of antitumor action. The work was devoted to the synthesis of platinum nitroxyl complexes (PNC) and studies of their structures, physicochemical properties, and reactions with the main target, DNA. The cytotoxic properties of PNC were studied using cultures of the HeLa, H1299, and MCF7 tumor cells. An animal tumor model P388 leukemia was used in studies of antitumor activity, specificities of development of the tumor resistance to one of the obtained compounds as compared to CP, and synergistic antitumor effects when low doses of new complexes and CP were used in combination.

Synthesis of PNC

Pt^{II} complexes. The formulas given above show that the high antitumor activity is displayed by the neutral complexes with *cis*-oriented amino ligands. The synthesis of complexes with the same amino ligands or one diamino ligand is similar to the synthesis of CP and is generally outlined in Scheme 1.

Scheme 1



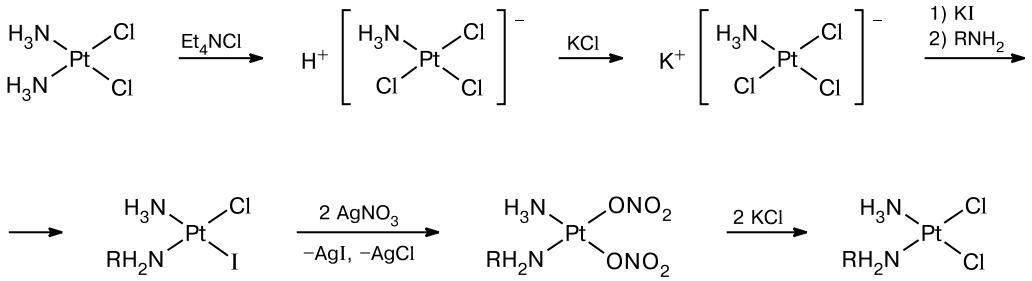
The exchange reaction through the water-soluble dinitrate complex (see Scheme 1) leads to the analogs, in which the I-ligands are replaced by the moieties of various organic and inorganic acids. During studies, it was found that the complexes with two bulky amino ligands, such as compounds **1** (see below), have poor binding to the target, which is a DNA, and, therefore, possess weak antitumor activity. This gave impetus to search for and successful development of an approach to the synthesis of *cis*-diamino complexes containing one bulky amino ligand.[16] We modified the method[16] for the preparation of PNC of general formulas **2** and **3** (Scheme 2).

The formulas of obtained[17–19] Pt^{II} complexes with aminonitroxyl radicals are given below. To synthesize compounds of general formula **4**, which are the structural analogs of oxaliplatin, we for the first time accomplished the synthesis of NR with two vicinal amino groups, *viz.*, *trans*-3,4-diamino-2,2,6,6-tetramethylpiperidine-1-oxyl.[20] Binuclear complex **5b** was also obtained based on this radical.

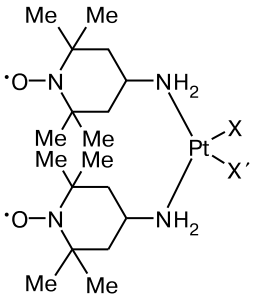
Pt^{IV} complexes. The Pt^{IV} complexes with the mixed amino ligands can be obtained only by oxidation of the corresponding Pt^{II} precursors. According to the method described earlier,[16] the starting Pt^{II} complexes **6** are oxidized with an excess of H_2O_2 under relatively harsh conditions (70 °C, ≥2 h). Under such conditions, the oxidation of platinum(II) nitroxyl complexes leads to the formation of considerable amounts of by-products, possibly, because of oxidation of NR with platinum(IV) at elevated temperature. We found that catalytic amounts of tungstic acid salts strongly accelerate the reaction, and the preparative oxidation under mild conditions (0—20 °C) is limited only by the rate of dissolution of the starting complex and takes from 0.5 to 2.5 h. This significantly increases the selectivity of the reaction and the yields of the target products. *Trans*-dihydroxo complexes **7** resulted from the oxidation are of independent interest. Their acylation with organic acid anhydrides affords to *trans*-dicarboxylate derivatives **8** (Scheme 3).[21,22]

An approach to the synthesis of such complexes starting from CP (see Schemes 2 and 3) is very flexible,

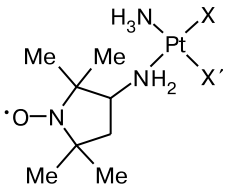
Scheme 2



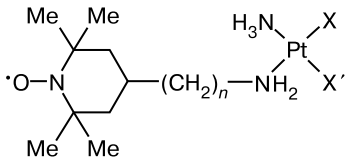
Note. Here and in Scheme 3 R is the nitroxyl radical.



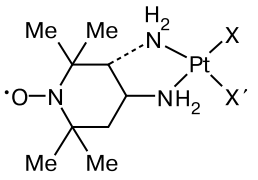
1b—d



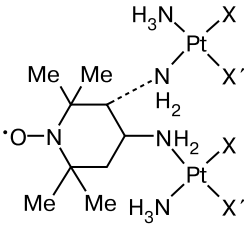
2a,b



3$_n$a—c

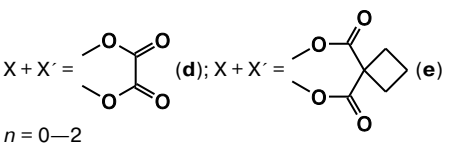


4b—e



5b

X = Cl, X´ = I (**a**), X = X´ = Cl (**b**); X = X´ = NO_3 (**c**);



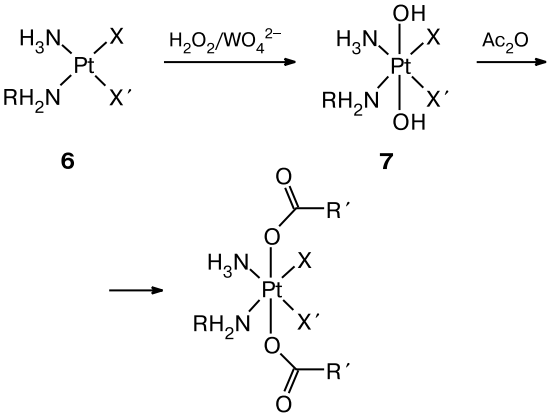
(**d**); X + X´ =

(**e**)

n = 0—2

it enables the introduction of different amines and exchange of the so-called leaving X ligands in the step of obtaining Pt^{II} complexes and incorporate various carboxylate ligands with the alkyl part R´ of different length in the final step (see Scheme 3). Such an approach can lead

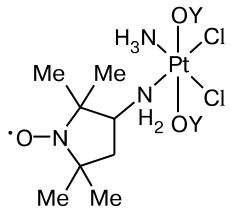
Scheme 3



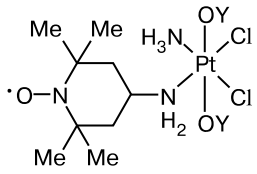
6 **7** **8**

to Pt^{IV} amino complexes **9**—**11** differing in chemical activity, solubility in water, and water-lipid distribution.

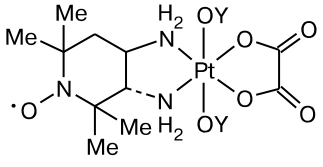
The structures of PNC were inferred from the elemental analysis and spectroscopic data;[17—19,21—23] for com-



9a—f



10a—f



11a—f

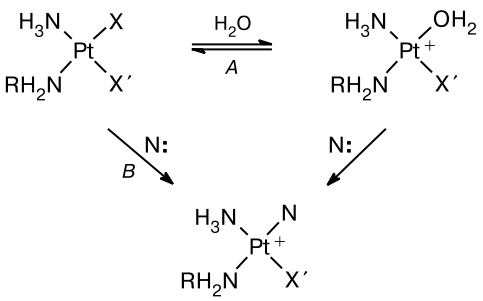
Y = H (**a**), C(O)Me (**b**), $C(O)(CH_2)_2Me$ (**c**), $C(O)(CH_2)_3Me$ (**d**), $C(O)(CH_2)_4Me$ (**e**), $C(O)(CH_2)_6Me$ (**f**)

plexes **2b**, **4d**, and **10a**, the structures were determined by X-ray diffraction.[19,21,24]

Reactions with DNA

Reactivity of Pt^{II} diamino complexes strongly depends on the nature of leaving X ligands. For example, the pseudomonomolecular rate constants of hydrolysis of the X ligands for **4c**, **4d**, CP, and **4e** at 25 °C in 0.08 *M* NaOH are $\geq 10^{-2}$, $1.2 \cdot 10^{-4}$, $1.9 \cdot 10^{-5}$, and $2.9 \cdot 10^{-7}$ s^{-1}, respectively, *i.e.*, differ by five orders of magnitude.[25] Therefore, the reaction of the complexes with S- or N-donor groups can proceed either through the step of preliminary hydrolysis with the formation of an active intermediate aqua complex (Scheme 4, path *A*) or by the direct substitution for the X ligands (path *B*).

Scheme 4



N is the nucleophilic nitrogen atom of the target molecule

The path *A* works for the comparably easily hydrolyzed complexes, including CP,[26] but confirmations of the direct substitution for the X ligand by the N-donor group were obtained for the complexes of the type **4e** (X + X′ = = cyclobutanedicarboxylate).[27]

Analysis of the ESR spectra of the modified DNA in combination with hydrolytic determination of platinated DNA bases showed[25] that complexes $\mathbf{3_0b}$ and **4d** form with DNA predominantly (≥95%) bidentate intrastrand adducts. Rotation of NR is equally slow in both adducts (Fig. 1) (the correlation time, $\tau \sim 10^{-8}$ s^{-1}). Such a result can be explained by immobilization of the radical fragment of the adduct from complex $\mathbf{3_0b}$ in the major groove of DNA and immobilization and/or rigid structure of the radical fragment doubly bound to the Pt atom in the adduct from complex **4d**. The adducts formed by complexes $\mathbf{3_1b}$ and $\mathbf{3_2c}$, whose radical fragment is separated from the Pt atom by the methylene or ethylene bridge, are characterized by approximately an order of magnitude smaller parameter τ. Presumably, this is due to the fact that the NR partially comes out of the comparatively shallow major groove of DNA, that increases its rotational mobility.[23]

The ability of complexes **1**—**5** to bind to the isolated DNA was determined under standard conditions and characterized by the parameter *r*, which is equal to the number of bound labels per nucleotide. In the series of complexes with the same amino ligand **4c**—**e**, the parameter *r* grows with an increase in the rate of hydrolysis of X ligands. Platination activity of compounds with different amino ligands depends on the total volume of these ligands and/or their linear sizes. Biradical complex **1c** and complexes $\mathbf{3_1b}$ and $\mathbf{3_2c}$, whose sizes are enlarged due to the methylene or ethylene bridges, are bound to DNA 5—10 times less strongly than CP or complexes **4c**,**d** (Fig. 2). Coordinates of the points in Fig. 2 have the values of specific destabilization δT_m of the DNA duplex, which corresponds to the reduction of the "melting" point (decomposition of the duplex at elevated temperature) of DNA caused by the formation of one adduct per 100 nucleotides and was calculated by the following formula

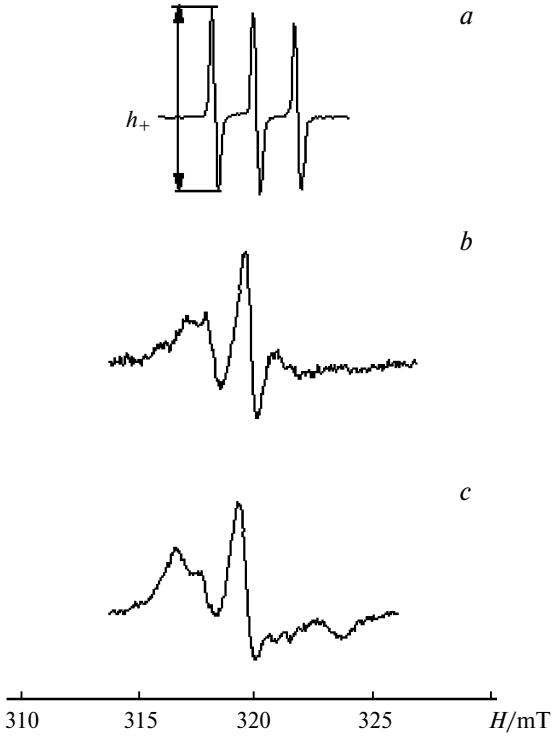


Fig. 1. The ESR spectra of solutions of complex **4d** (*a*) and DNA modified with complexes $\mathbf{3_0b}$ (*b*) and **4d** (*c*) (the degree of modification label/nucleotide $r = 0.16$) in water at 25 °C.

$$\delta T_m = (T_m' - T_m)/(100r),$$

where T_m and T_m' are the "melting" points for the starting and platinated DNA, respectively.

The data in Fig. 2 show that the complexes characterized by the low *r* values cause the largest disorder of the DNA duplex, which is apparently easily determined by the DNA repair mechanism. This is in agreement with the data on the low antitumor activity of such complexes (see below). Binuclear complex **5b** stabilizes DNA due to a predominant (~70%) formation of the interstrand crosslinks interfering

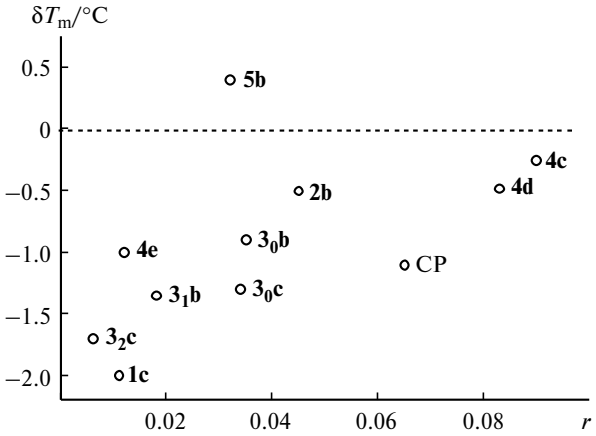


Fig. 2. Specific destabilization of the DNA duplex δT_m caused by adducts of Pt^{II} complexes *versus* their platination activity *r*; CP is cisplatin.

with the thermal decomposition of the DNA duplex. Bi- and trinuclear platinum amino complexes differ from the mononuclear ones in their cytotoxic properties, in particular, they are active against the cells resistant to CP.[28]

Impressive opportunities of the instrumental use of PNC are shown in the work.[29] An adduct of complex **3_0a** with the synthetic DNA fragment containing 11 pairs of nucleotides was obtained. The structure of the modified DNA fragment in solution was determined by NMR from the dependence of the paramagnetic broadening of the proton signals of the DNA bases unequally distant from the NR. The formation of the adduct results in the bending of the macromolecule with respect to the major groove, forming the angle ~80°, whereas the minor groove strongly broadens.

Cytotoxic activity of the complexes in tumor cell cultures

A simplified mechanism of cytotoxic action of CP and its analogs includes the transport of the complexes into the cell, their activation by the hydrolysis of leaving ligands (Cl^-, carboxylates), penetration into the nucleus, and formation of adducts with DNA.[9,10] The modified DNA is either repaired or initiates a complex process of the programmed cell death, *i.e.*, apoptosis. In addition, it is known that CP directly or indirectly causes generation of active oxygen radicals, and this process is important for the initiation of apoptosis,[30,31] as well as for the side effects, for example, nephrotoxicity.[32]

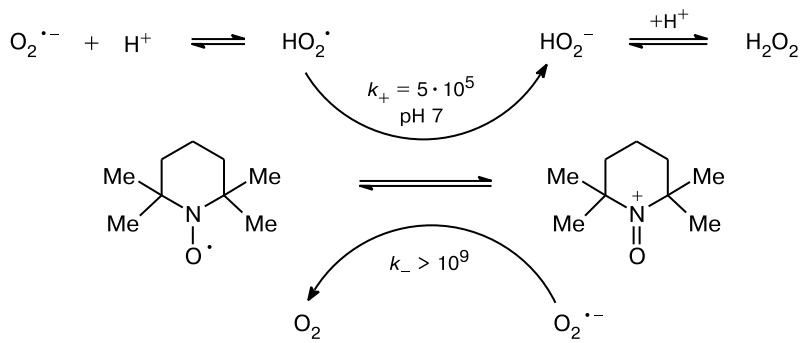
At the same time, NR are antioxidants capable not only of stoichiometrical reacting with active radicals but also serving as catalysts for the redox reactions and mimetics of enzymatic systems, for example, superoxide dismutase[33,34] (Scheme 5).

Likewise other antioxidants, NR can exhibit pro-oxidant activity under certain conditions. The structures of nitroxyls, properties of the medium, as well as other factors, which are difficult to make allowance for, determine anti- or pro-oxidant effects of NR. Nitroxyl radicals in submillimolar concentrations, as a rule, exhibit antioxidant properties and protect cells from apoptosis.[1] In millimolar concentrations, they possess cytotoxicity in the tumor cell cultures[35,36] and are active against the animal tumor models.[3,36] Nitroxyl radicals cause the cell death in cells possessing both intact and mutant p53 genes.[36]

The study of how the interaction of platinum and nitroxyl pharmacophores bound in one molecule affects biological activity of the complexes is of interest also in respect of the effect of antioxidants on the tumor chemotherapy.[37]

Studies of a combined action of CP and NR 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl on the cells revealed a pronounced antagonism of their cytotoxic effects. The experimental data were analyzed in accordance with the combination index theorem.[38] The combination index (CI) reflects the contribution of each component of the combination to the total cytotoxicity (the fractional effect f_a). The log(CI) values close to 0 characterize the additivity of cytotoxic action, when log(CI) < 0, the synergy of compounds is observed, whereas log(CI) > 0 is

Scheme 5



Note. The k_+ and k_- values are given in L mol^{-1} s^{-1}.

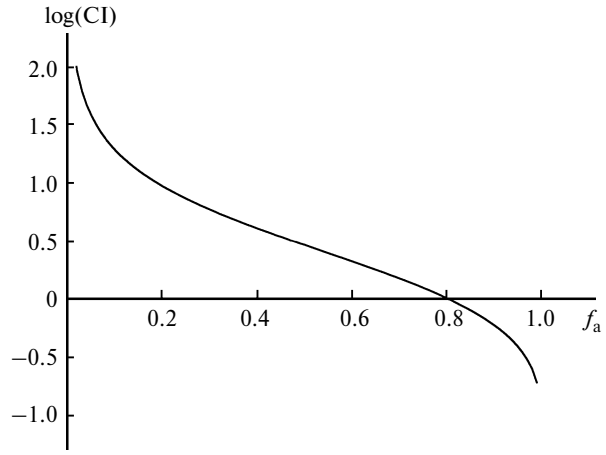


Fig. 3. The combination index (CI) of cytotoxic effect of CP in combination with 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl *versus* the fractional effect (f_a).

indicative of the antagonism. Figure 3 shows the curve of the change in the combination index for the combination of CP and 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl. It is seen that log(CI) is considerably higher than 0 in a wide range of cytotoxic effect, and only for very high values of cytotoxicity, synergistic action of two compounds is observed. These results agree with the indicated above antioxidant properties of NR at their low concentrations and the pro-oxidant properties at high concentrations.

The data on antagonism of CP and NR also agree with the data on cytotoxicity of CP nitroxyl derivatives containing NR in the structure of the platinum complex itself. Platinum(II) complexes **2b** and **3_0b** containing NR of different structures are less toxic to cells than CP (Table 1). The platinum nitroxyl complexes **9b** and **10b** structurally similar to their analog JM-216 are considerably inferior to the latter in cytotoxicity. This indicates the antioxidant effect of NR when their concentration is low (antagonism of platinum and nitroxyl pharmacophores) and/or slower penetration into the cells by such complexes. When the axial ligands Y are lengthened, lipophilicity of the complexes increases and their cytotoxicity magnifies (complexes **9c** and **10c—f**), that, obviously, is due to the higher accumulation of the complexes in the cells. A small difference in cytotoxicity of analogous complexes **10c** and **9c** with piperidine and pyrrolidine oxyls reflects the same small difference in the structures of radicals forming them. The H1299 cells are less sensitive to all the platinum complexes, since, unlike the HeLa cells, they do not contain the p53 protein that plays a key role in the apoptosis process.[39]

The cytofluorimetric data showed that CP and complex **10d** exhibit cytotoxic effect on the HeLa (accumulation of the cells in the subG1 region) and H1299 cells (accumulation of the cells in the early S-phase without enrichment of the subG1 region) (Fig. 4). The cytotoxic action of platinum complexes on the HeLa cells is mediated *via* apoptosis. Studies of the morphology of cell nuclei and DNA fragmentation of the HeLa cells upon treatment with CP and complex **10d** showed that both complexes cause in cells nuclei fragmentation and DNA internucleosomal degradation characteristic of apoptosis (Fig. 5, *a—d*). It was found that CP and its analogs form adducts with DNA, that, when unrepaired, initiates activation of the tumor suppressor protein p53.[9,10,26] Comparative studies of the effects of CP and complex **10d** unexpectedly showed that, unlike CP, complex **10d** does not cause the p53 protein expression (Fig. 5, *e*) in the MCF7 cells containing the wild-type p53. Cytotoxicity of many compounds was demonstrated in cells possessing both the wild-type and mutant p53 gene. In particular, this is known for both the NR (see Ref. 36) and platinum complexes.[40] However, p53-independent tumor cell death with the intact p53 gene, to the best of our knowledge, was established for the first time.

Antitumor activity in animal tumor models

Pt^{II} complexes. Toxicity and biological activity of the Pt^{II} diamino complexes are affected, first of all, by the nature of the carrier amino ligands and leaving ligands which are replaced in the processes of metabolism and binding to the targets. Biradical complexes **1** containing two bulky amino ligands poorly bind to the isolated DNA (see above), possess low toxicity, and weakly suppress P388 tumor growth (Table 2). Derivatives **2** and **3** with one bulky substituent have properties similar to CP. Their LD_{50} (mmol kg^{-1}) are only 1.5 times lower than that for CP. They efficiently platinate DNA and possess antitumor activity comparable with CP. The effect of the radical nature can be observed when comparing complexes **2b** and **3_0b**, differing only in the size of NR ring. Compound **3_0b** is more toxic and significantly more active against P388 leukemia.

Table 1. Doses IC_{50} for the platinum complexes under study

Cell line	IC_{50}/μmol L^{-1}										
	CP	**2b**	**3_0b**	JM-216	**9b**	**9c**	**10b**	**10c**	**10d**	**10e**	**10f**
HeLa	14.8	125.3	112.7	14.1	>200	13.4	>200	5.5	2.1	0.4	0.2
H1299	66.7	>150	>150	33.8	>200	25.4	>200	9.5	3.5	1.5	1.1

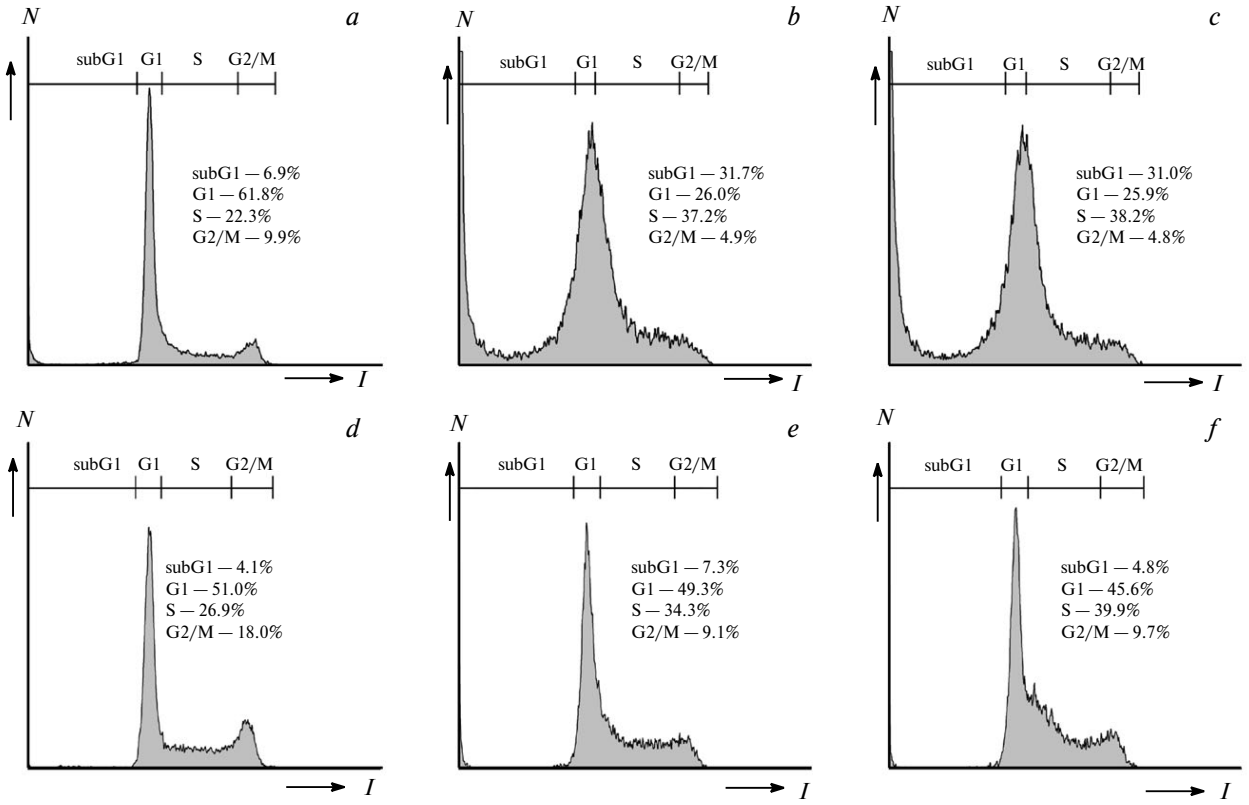


Fig. 4. The histograms of DNA staining with propidium iodide in the HeLa (*a*—*c*) and H1299 cells (*d*—*f*) in the control experiment (*a*, *d*) and after administration of CP (*b*, *e*) and complex **10d** (*c*, *f*); *I* is the fluorescence intensity, *N* is the number of cells.

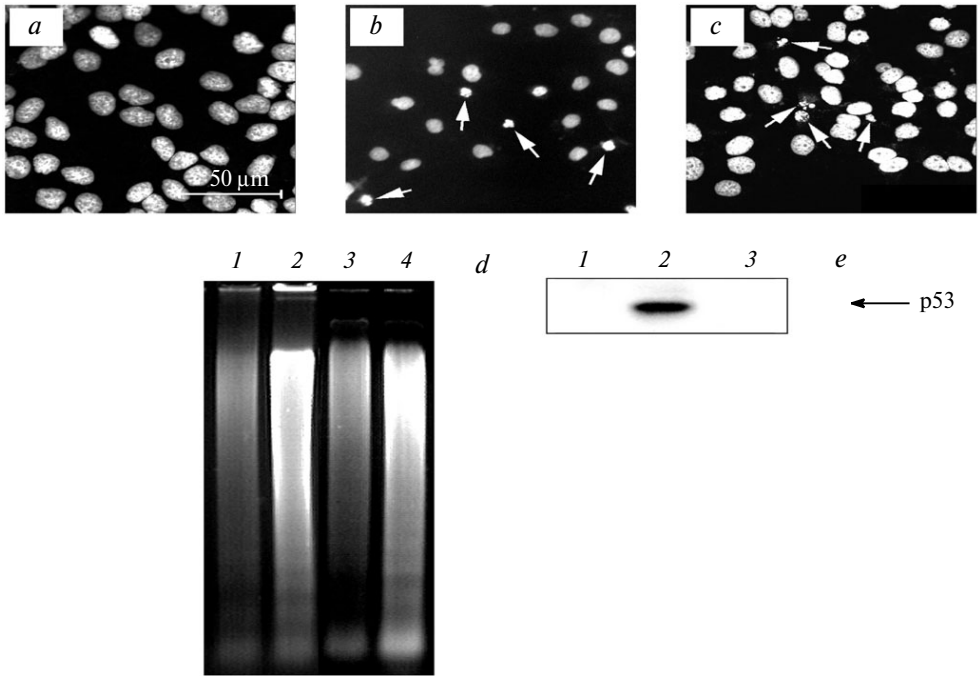


Fig. 5. The mechanism of cytotoxic action of platinum complexes: *a*—*c*, the detection of apoptosis by DAPI staining of HeLa cell DNA in the control experiment (*a*) and after administration of CP (*b*) and complex **10d** (*c*), the arrows show the fragmented nuclei of the apoptotic cells; *d*, the detection of apoptosis by analysis of fragmentation of HeLa cell DNA after administration of CP (*1*, *2*) and complex **10d** (*3*, *4*): after 12 (*1*, *3*) and 24 h (*2*, *4*); *e*, the immunoblotting of MCF7 cell proteins with antibodies against p53 in the control experiment (*1*) and 6 h after administration of CP (*2*) and complex **10d** (*3*).

Table 2. Toxicity and antileukemic (P388) activity of PNC

Complex	LD_{50}[a]/mg kg^{-1} (mmol kg^{-1})	Single dose /mg kg^{-1}	ILS[b] (%)
1b	570 (0.94)	190	106 (0)
1c · H_2O	500 (0.74)	166	79 (1)
1d	380 (0.61)	127	76 (0)
2b	27 (0.061)	6.8	237 (1)
3_0b	15 (0.033)	3.8	292 (2)
4b	80 (0.18)	16	189 (0)
4c	11 (0.022)	—	—
4d · $2H_2O$	50 (0.10)	11	132 (0)
4e	500 (0.95)	133	202 (2)
9a	45 (0.095)	15	133 (0)
9b	100 (0.180)	34	247 (0)
10a · $2H_2O$	27 (0.052)	9	270 (4)
10b	46 (0.080)	7.5	220 (4)
Cisplatin	12 (0.040)	3.0	245 (1)

[a] The dose causing the death of 50% of healthy mice.
[b] Increase in the life span ILS = [100(T/C − 1)], where T and C are the median life span (in days) of treated and control animals, respectively. The number of survived animals (remained alive for more than 60 days) in the group of six animals is given in parentheses.

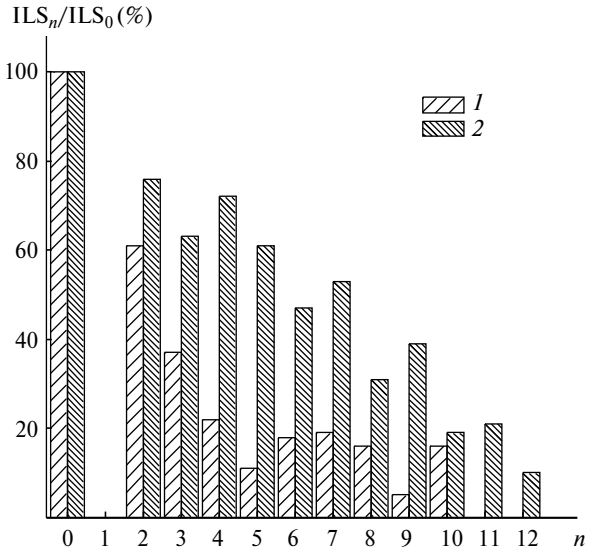


Fig. 6. The reduction of sensitivity of P388 leukemia to CP (*1*) and complex **10a** (*2*) in the serial transplantation generations *n*. For the zero generation, the sensitivity of P388 leukemia to the agents is taken as 100%.

The relationship between the rate of hydrolysis of X ligands and toxicity of the compounds was determined for the series of complexes **4** with *trans*-3,4-diamino-2,2,6,6-tetramethylpiperidine-1-oxyl.[17,25] Easily hydrolyzable compound **4c** possesses the highest toxicity. The most difficult to hydrolyze compound **4e** is characterized by the lowest toxicity but, like carboplatin, possesses good antitumor activity only at high doses. Compound **4d**, structurally closest to oxaliplatin, is ~2 times less toxic than the prototype. Such a decrease in toxicity can be due to the effect of the nitroxyl group. The above data allow us to conclude that among Pt^{II} complexes with aminonitroxyl radicals, high antitumor activity is exhibited by the complexes containing no more than one bulky amino ligand; they efficiently platinate an isolated DNA and cause only moderate destabilization of its duplex.

Pt^{IV} complexes. Toxicity of Pt^{IV} complexes is 1.6—3 times lower than that of the corresponding Pt^{II} analogs (see Table 2), and, as in the case of divalent metal complexes, piperidine oxyl derivatives **10** are more toxic and more active than pyrrolidine oxyl derivatives **9**.

We compared the rates of development of resistance in P388 leukemia to one of the new compounds (**10a**) and CP (Fig. 6). The resistance was induced by the serial transplantation of tumor cells from animals treated with equitoxic doses of agents.[21] The tumor acquired resistance (sensitivity ≤20% of the sensitivity of the parent tumor) for CP in the fourth, whereas for **10a** in the tenth tumor generation. This means that the resistance to **10a** is developed 2.5 times slower than to CP.

Interesting results were obtained when low doses (1/20 of LD_{50}) of Pt^{IV} complexes and CP were used together in leukemia P388.[21,22] A separate use of the drugs in these doses results in a small increase in the life span and the absence of survived animals, whereas the synergistic effect is observed when they are used in combination: 65% of animals survived for the combination **9b** + CP and 100% of animals survived for the combination **10a** or **10b** + CP (Fig. 7). A small difference in the structures of NR ligands of complexes **9b** and **10b** significantly affects their antitumor activity. Complex **10b** containing the piperidine oxyl fragment is more active when it is used either separately or in combination with CP.

In conclusion, Pt^{II} and Pt^{IV} complexes with biologically active aminonitroxyl radicals are characterized by features which distinguish them among complexes with common alkylamines. Platinum nitroxyl complexes **9b** and **10b** structurally close to JM-216 possess lower cytotoxicity in the tumor cell cultures. It can be due to the moderate suppression of the p53-dependent apoptosis because of the antioxidant properties of NR. The antagonism of platinum and nitroxyl pharmacophores observed in the cell cultures is not so pronounced in the animal tumor models, and the antitumor activity of some PNC is comparable to CP. It can be expected that PNC would have lower side toxic effects characteristic of CP, such as nephro- and neurotoxicity. For example, the daunorubicin nitroxyl derivative, ruboxyl, has virtually no cardiotoxicity, a property which limits the dosage of the parent compound.[8] In addition, PNC can cause the p53-independent tumor cell death, that can be a reason for the lower (as compared to

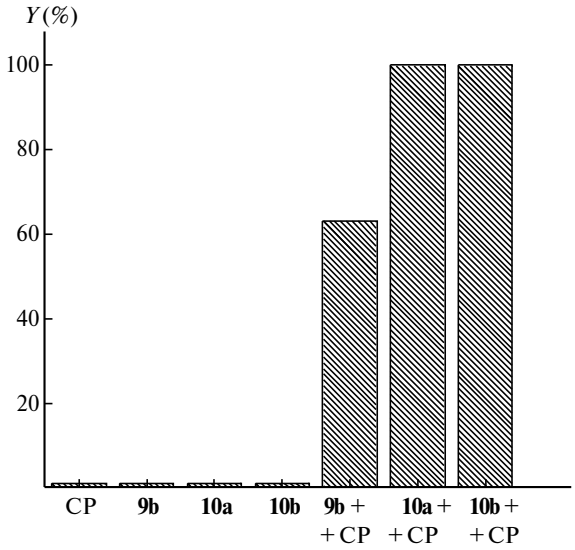


Fig. 7. The synergistic antitumor effects of the combined use of CP and complexes **9b** and **10a,b** against P388 leukemia. The doses of agents/mg kg^{-1}: 0.6 (CP), 5.0 (**9b**), 1.4 (**10a**), 2.3 (**10b**). The agents were administered simultaneously in the 1st, 3d, 5th, and 7th days after the tumor transplantation; *Y* is the number of survived animals.

CP) rate of development of resistance and the synergistic interaction in low dose combinations of CP and PNC.

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References

1. C. S. Wilcox, *Pharmacol. Ther.*, 2010, **126**, 119.
2. B. P. Soule, F. Hyodo, K. Matsumoto, N. L. Simone, J. A. Cook, M. C. Krishna, J. B. Mitchell, *Free Rad. Biol. Med.*, 2007, **42**, 1632.
3. N. P. Konovalova, G. N. Bogdanov, V. B. Miller, E. G. Rozantsev, M. B. Neiman, N. M. Emanuel, *Dokl. Akad. Nauk SSSR*, 1964, **157**, 707 [*Dokl. Chem.* (*Engl. Transl.*), 1964].
4. A. B. Shapiro, A. A. Kropacheva, V. I. Suskina, B. V. Rozynov, E. G. Rozantsev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1971, 864 [*Bull. Acad. Sci. USSR, Div. Chem. Sci.* (*Engl. Transl.*), 1971, **20**, 780].
5. N. M. Emanuel, N. P. Konovalova, L. S. Povarov, A. B. Shapiro, R. F. Dyachkovskaya, V. I. Suskina, L. K. Denisova, US Pat. 4332934, 1982; http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&adjacent=
6. N. M. Emanuel, A. N. Rosenberg, V. A. Golubev, G. N. Bogdanov, L. S. Vasilieva, N. P. Konovalova, US Pat. 4558127, 1985; http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&adjacent=
7. N. M. Emanuel, V. D. Sen´, V. A. Golubev, G. N. Bogdanov, L. V. Vasil´eva, N. P. Konovalova, USSR Inventor´s Certificate No. 1259650, 1986; http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&adjacent=.
8. N. M. Emanuel, N. P. Konovalova, in *Bioactive Spin Labels*, Ed. R. I. Zhdanov, Springer, Berlin, 1992, 439.
9. L. R. Kelland, *Nature Rev.*, 2007, **7**, 573.
10. N. J. Wheate, S. Walker, G. E. Craig, R. Oun, *Dalton Trans.*, 2010, **39**, 8113.
11. N. N. Blokhin, N. I. Perevodchikova, *Khimioterapiya opukholevykh zabolevanii* [*Chemotherapy of Tumor Diseases*], Moscow, Meditsina, 1984, 95 (in Russian).
12. L. R. Kelland, *Uses of Inorganic Chemistry in Medicine*, Ed. N. P. Farrell, Springer, Berlin, 1999, 109.
13. A. V. Klein, T. W. Hambley, *Chem. Rev.*, 2009, **109**, 4911.
14. B. Koeberle, M. T. Tomicic, S. Usanova, B. Kaina, *Biochim. Biophys. Acta*, 2010, **1806**, 172.
15. *Platinum and Other Heavy Metal Compounds in Cancer Chemotherapy*, Eds A. Bonetti, R. Leone, F. M. Muggia, S. B. Howell, Humana Press, New York, 2009, 383 pp.
16. C. M. Giandomenico, M. J. Abrams, B. A. Murrer, J. F. Vollano, M. I. Rheinheimer, S. B. Wyer, G. E. Bossard, J. D. Higgins, *Inorg. Chem.*, 1995, **34**, 1015.
17. V. D. Sen´, V. A. Golubev, L. M. Volkova, N. P. Konovalova, *J. Inorg. Biochem.*, 1996, **64**, 69.
18. V. D. Sen´, A. V. Kulikov, A. V. Shugalii, N. P. Konovalova, *Izv. Akad. Nauk, Ser. Khim.*, 1998, 1640 [*Russ. Chem. Bull.* (*Engl. Transl.*), 1998, **47**, 1598].
19. V. D. Sen´, N. A. Rukina, V. V. Tkachev, A. V. Pis´menskii, L. M. Volkova, S. A. Goncharova, T. A. Raevskaya, A. G. Tikhomirov, L. B. Gorbacheva, N. P. Konovalova, *Izv. Akad. Nauk, Ser. Khim.*, 2000, 1624 [*Russ. Chem. Bull., Int. Ed.*, 2000, **49**, 1613].
20. V. D. Sen´, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1989, 2094 [*Bull. Acad. Sci. USSR, Div. Chem. Sci.* (*Engl. Transl.*), 1989, **38**, 1928].
21. V. D. Sen´, V. V. Tkachev, L. M. Volkova, S. A. Goncharova, T. A. Raevskaya, N. P. Konovalova, *Izv. Akad. Nauk, Ser. Khim.*, 2003, 403 [*Russ. Chem. Bull., Int. Ed.*, 2003, **52**, 421].
22. V. D. Sen´, V. A. Golubev, N. Yu. Lugovskaya, T. E. Sashenkova, N. P. Konovalova, *Izv. Akad. Nauk, Ser. Khim.*, 2006, 60 [*Russ. Chem. Bull., Int. Ed.*, 2006, **55**, 62].
23. V. D. Sen´, A. V. Shugalii, A. V. Kulikov, *Izv. Akad. Nauk, Ser. Khim.*, 2002, 972 [*Russ. Chem. Bull., Int. Ed.*, 2002, **51**, 1058].
24. A. N. Chekhlov, *Koord. Khim.*, 2005, **31**, 179 [*Russ. J. Coord. Chem.* (*Engl. Transl.*), 2005, **31**].
25. A. V. Shugalii, A. V. Kulikov, V. A. Golubev, M. V. Lichina, V. D. Sen´, *J. Inorg. Biochem.*, 1998, **69**, 67.
26. R. A. Alderden, M. D. Hall, T. W. Hambley, *J. Chem. Education*, 2006, **83**, 728.
27. U. Frey, J. D. Ranford, P. J. Sadler, *Inorg. Chem.*, 1993, **32**, 1333.
28. N. Farrell, Y. Qu, U. Bierbach, M. Valsecchi, E. Menta, *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drugs*, Ed. B. Lippert, Verlag Helvetica Chimica Acta, Zurich, 1999, 479.
29. S. U. Dunham, C. J. Turner, S. J. Lippard, *J. Am. Chem. Soc.*, 1998, **120**, 5395.
30. A. Miyajima, J. Nakashima, K.Yoshioka, M. Tashibana, H.Tazaki, M. Murai, *British J. Cancer*, 1997, **76**, 206.
31. P. Bravado, A. Armesilla, A. Silva, A. Porras, *Apoptosis*, 2007, **12**, 1733.

32. Y. Tsutsumishita, T. Onda, K. Okada, M. Takeda, H. Endou, S. Futaki, M. Niwa, *Biochem. Biophys. Res. Comm.,* 1998, **242**, 310.
33. V. D. Sen´, V. A. Golubev, I. V. Kulyk, E. G. Rozantsev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1976, 1745 [*Bull. Acad. Sci. USSR, Div. Chem. Sci.* (*Engl. Transl.*), 1976, **25**, 1647].
34. S. Goldstein, G. Merenyj, A. Russo, A. Samuni, *J. Am. Chem. Soc.*, 2003, **125**, 789.
35. M. B. Gariboldi, V. Rimoldi, R. Supino, E. Favini, E. Monti, *Free Radic. Biol. Med.*, 2000, **29**, 633.
36. S. Suy, J. B. Mitchell, A. Samuni, S. Mueller, U. Kasid, *Cancer*, 2005, **103**, 1302.
37. H. E. Seifried, S. S. McDonald, D. E. Anderson, P. Greenwald, J. A. Milner, *Cancer Res.*, 2003, **63**, 4295.
38. H. R. Sanders, M. Albitar, *Cancer Gen. Cytogen.*, 2010, **203**, 7.
39. T.-C. Chou, *Pharm. Rev.*, 2006, **58**, 621.
40. W. Gorczyca, J. Gong, B. Ardelt, F. Traganos, Z. Darzynkiewicz, *Cancer Res.*, 1993, **53**, 3186.

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