New method for the synthesis of metal complexes based on Pt^{IV} and substituted nicotinamides and isonicotinamides and investigation of their antimetastatic activity

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Complexes of platinum tetrachloride with substituted nicotinamides and isonicotinamides were synthesized by the reactions of the corresponding amides with hexachloroplatinic acid. All these complexes are low-toxic. The strong antimetastatic activity of these complexes against Lewis lung carcinoma and B16 melanoma experimental tumors was found in an enzyme system and was then shown in experiments on animals.

Key words: hexachloroplatinic acid, substituted nicotinamides and isonicotinamides, metal complexes, overall toxicity, antimetastatic activity, calcium transport across biological membranes.

The synthesis of Pt^{IV} complexes with nicotinic and isonicotinic acid derivatives containing hydroxy, nitrate, and hydroxyamide groups as the ligands has been described earlier.[1—3] All complexes showed low acute toxicity (LD_{50} is 300—1000 mg kg^{-1}) and exhibited strong antimetastatic activity against B16 melanoma and Lewis lung carcinoma experimental metastases in animals.[3]

The aims of the present study were to investigate in detail the antimetastatic activity of the Pt^{IV} complexes synthesized earlier, to determine the structure-activity relationship, and to discuss the possibility of the synthesis of efficient antitumor agents based on biogenic compounds. To investigate the biological activity, it was necessary to synthesize considerable amounts of Pt^{IV} complexes. In addition, the goal of the present study was to develop a general preparative procedure for the one-pot synthesis of the above-mentioned metal complexes starting from substituted nicotinamides or isonicotinamides and hexachloroplatinic acid and to increase the yields and purity of the target products.

Results and Discussion

Earlier, these complexes have been synthesized by the reactions of substituted nicotinamides and isonicotinamides with K_2PtCl_6 in a water-alcohol medium.[1—3] However, the resulting metal complexes precipitate together with the starting K_2PtCl_6, and, consequently, the laborious purification of the target product by fractional crystallization is required, resulting in a substantial decrease in the yield. Preliminary experiments showed that the target complexes are stable in dilute hydrochloric acid. This allowed us to use hexachloroplatinic acid instead of dipotassium hexachloroplatinate as the complex-forming agent. The complexation reaction of substituted nicotinamides and isonicotinamides[4] (ligands **1**—**11**) with hexachloroplatinic acid is shown in Scheme 1. This reaction with hexachloroplatinic acid occurs extensively under mild conditions at 20—50 °C and affords complexes **12**—**22** in high yields (80—96%). The metal complexes precipitate as small crystals in the virtually pure form, and the isolation of the reaction products involves the filtration of the crystals followed by their washing with ice water. It was experimentally shown that the reaction is applicable to all pyridinecarboxylic acid derivatives.

It was found that *N*-nicotinoyltrimethylolaminomethane **5** reacts with hexachloroplatinic acid in water (the reagent ratio is 2 : 1) to form complex **16**, which crystallizes from the reaction mixture as shiny yellow crystals containing two moles of water of crystallization. The X-ray diffraction study showed that complex **16** has a *cis* structure (Fig. 1). The procedure developed in the present study allowed us to synthesize the target compounds in amounts necessary for performing biological activity assays.

The starting ligands (compounds **2** and **8**) and metal complexes **16**, **19**, and **21** were tested on animals at the Laboratory of Experimental Tumor Chemotherapy of the Institute of Problems of Chemical Physics of the Russian Academy of Sciences. Complexes **16**, **19**, and **21** showed

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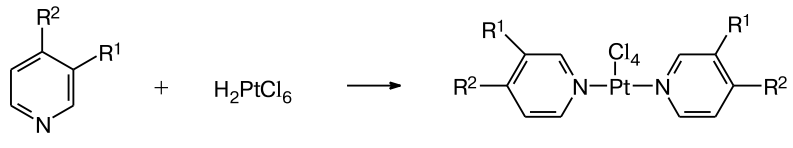
Scheme 1



1—11 **12—22**

Compound	R^1	R^2
1,12	$C(O)NHCH_2CH_2OH$	H
2,13	H	$C(O)NHCH_2CH_2OH$
3,14	$C(O)N(CH_2CH_2OH)_2$	H
4,15	H	$C(O)N(CH_2CH_2OH)_2$
5,16	$C(O)NHC(CH_2OH)_3$	H
6,17	H	$C(O)NHC(CH_2OH)_3$
7,18	$C(O)NHCH_2CH_2ONO_2$	H
8,19	H	$C(O)NHCH_2CH_2ONO_2$
9,20	H	$C(O)NHCH_2C(O)NHCH_2CH_2ONO_2$
10,21	H	C(O)NHOH
11,22	$C(O)NHCH_2CH_2C(O)NHCH_2CH_2ONO_2$	H

high indices of metastasis inhibition (IMI) of B16 melanoma and Lewis lung carcinoma experimental tumors both upon intraperitoneal (in the case of compound **16**) and peroral injection. Upon peroral injection of complex **19**, the index of metastasis inhibition was 99% both in B16 melanoma and Lewis lung carcinoma experimental metastasis models (at a dose of 300 mg kg^{-1} after five injections). The antimetastatic activity of complex **21** substantially increases with increasing dose of the agent (Fig. 2) and reaches 92% at a dose of 150 mg kg^{-1}. Taking into account the low overall toxicity of compound **21** (LD_{50} is larger than 1000 mg kg^{-1}), one might expect a positive effect when using high doses, as opposed to cytostatics having high toxicity. It should be noted that compounds **19** and **21** exhibiting high activity contain groups capable of generating NO through biotransformation.

In the present study, we compared the activity of complex **19** synthesized according to the new scheme with that of cisplatin currently used in oncology. We also investigated the antimetastatic activity of two ligands, *viz.*, *N*-(2-hydroxyethyl)isonicotinamide (**2**) and *N*-(2-nitroxyethyl)isonicotinamide (**8**). One of these ligands (compound **8**) contains the nitrate group (exogenous NO donor), whereas this group is absent in another ligand (compound **2**). In addition, we studied the inhibition of active transport of Ca^{2+} ions across biological membranes by Ca^{2+}-Mg^{2+}-dependent ATPase of the sarcoplasmic reticulum isolated from white muscles of rabbit hind limbs.[5] The procedure for the determination of the active transport of Ca^{2+} ions across biological membranes has been described in detail in our earlier study.[6]

As can be seen from Table 1, the data on the inhibition of active transport of Ca^{2+} ions correlates well with the indices of metastasis inhibition in mice. Table 1 also shows

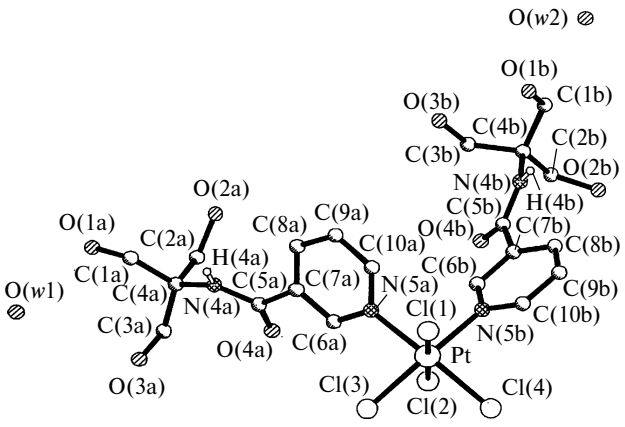


Fig. 1. X-ray diffraction structure of complex **16**.

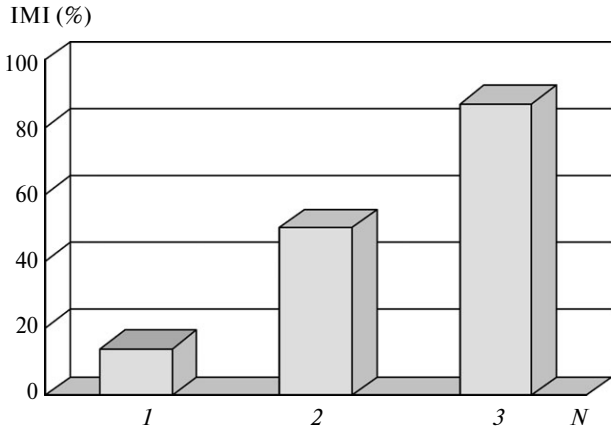


Fig. 2. Dependence of the index of metastasis inhibition (IMI) of B16 melanoma experimental metastasis on the dose of complex **21**: 37 (*1*), 75 (*2*), and 150 mg kg^{-1} (*3*). The administration of the agent was performed from two to nine days after the tumor transplantation.

Table 1. Indices of metastasis inhibition (IMI) of B16 melanoma experimental metastasis and the inhibition of active calcium transport by Ca^{2+}-Mg^{2+}-dependent ATPase of the sarcoplasmic reticulum with cisplatin and compounds **2**, **8**, and **19**

Compound	IMI* (%)	Inhibition of active Ca^{2+} transport (%)
Cisplatin	92	87±9
2	26	23±2
8	78	80±8
19	99	95±9

* $\text{IMI} = \frac{(A_C B_C) - (AB)}{A_C B_C} \cdot 100\%$ where A_C and A are the frequencies of metastasis in the control and test groups, respectively; B_C and B are the average numbers of metastases in the control and test groups, respectively.

that the index of metastasis inhibition by metal complex **19** is comparable with that of cisplatin (99 and 92%, respectively). The overall toxicity LD_{50} of complex **19** was 1000 mg kg^{-1} *versus* 12.5 mg kg^{-1} for cisplatin. Hence, we synthesized the virtually nontoxic complex exhibiting the activity comparable with that of cisplatin. This therapeutic effect is due to a combination of the following three fragments in the molecule: (1) an antimetabolite capable of participating in the metabolism; 2) groups providing the generation of nitric oxide as an activator of a cyclic nucleotide system; 3) the transition metal Pt^{IV} capable of covalently binding to the target molecule in a tumor cell.

A comparison of the antimetastatic activity of *N*-(2-hydroxyethyl)isonicotinamide (**2**) and *N*-(2-nitroxyethyl)-isonicotinamide (**8**) with that of metal complex **19** shows that the maximum therapeutic characteristics are not achieved in the absence of transition metal atoms (tetravalent platinum) serving as the complex-forming agent (see Table 1). A considerable difference in the activity of *N*-(2-hydroxyethyl)isonicotinamide (**2**) and *N*-(2-nitroxyethyl)isonicotinamide (**8**) (26 and 78%, respectively) is, apparently, attributed to the presence of the nitrate group capable of generating nitric oxide through biotransformation. The results of the present study confirm our earlier concept of the synthesis of the highly selective antitumor agent.[7—11] The main idea is to use metabolically active compounds as carriers of functional groups.

The correlation between the inhibition of active transport of Ca^{2+} ions across biological membranes and the indices of metastasis inhibition confirms the mechanism of action of the complexes based on Pt^{IV} and $PdCl_2$, which we have proposed earlier.[6] This mechanism involves the inhibition of calcium transport across biological membranes. Complexes **12—22** synthesized in the present study act on Ca^{2+}-Mg^{2+}-dependent ATPase of the sarcoplasmic reticulum, thus inhibiting the calcium transport across biological membranes. This leads to the disturbance of the normal ratio of calcium ions on the extra- and intracellular membrane surfaces followed by the disturbance of the aggregation of thrombocytes and their binding to metastatic cells, which results in the loss of the cell adhesion to blood-vessel walls.

The results of the present study give a different insight into the problem of the synthesis of antitumor agents. For many years, the prevailing opinion is that synthetic antitumor agents are necessarily highly toxic. This opinion is based on the data on the use of cisplatin and carboplatin in oncology, because their action is directly related to cytotoxicity, *i.e.*, to the active suppression of the DNA synthesis. However, it is also well known that these drugs are characterized by low selectivity.

The data obtained in the present study suggest that the mechanism of action of the metal complexes, which were synthesized based on tetravalent platinum and substituted nicotinamides or isonicotinamides, differs from that of cisplatin and carboplatin. Thus, the antitumor activity of cisplatin and carboplatin is associated with the suppression of the DNA synthesis, whereas the newly synthesized complexes influence predominantly the enzymatic activity. The results of the present study allow us to formulate the radically new concept of the synthesis of antitumor agents and make it possible to work out the synthesis of biogenic compounds, which can, on the one hand, partially block the nutrition of tumor cells (no nutrition, no life) and, on the other hand, restore the mitochondrial functioning. It is known that the transformation of normal cells into malignant cells is accompanied by the disturbance of the Krebs cycle, resulting in that mitochondria suffer from ATP deficiency. Under hypoxic conditions typical of malignant cells, the ATP biosynthesis occurs *via* glycolysis. From this it is evident that the selective suppression of glycolysis may be one of the ways of the tumor growth retardation. In turn, the restoration of the mitochondrial functioning will result in that mitochondria will again fulfill one of their main functions, *e.g.*, will promote apoptosis (programmed cell death as a mechanism by which unwanted or useless cells, including cancer cells, are eliminated). As an example, let us refer to capsaicin (8-methyl-*N*-vanillyl-*trans*-6-nonenamide) isolated from chili peppers. It is known[12] that this alkaloid causes the mass death of malignant cells due to the action on mitochondria, which are organelles providing cells with the energy.

Our experience in the work with biogenic compounds, which were used as carriers of functional groups, including groups capable of generating nitric oxide through biotransformation, allowed us to synthesize an antitumor agent based on aminoacetic acid (the simplest amino acid). This compound has the overall toxicity LD_{50} of 300 mg kg^{-1} and causes the retardation of the growth of carcinoma 755 (breast cancer) by 54%.[13] In other words,

if malignant tumors are accompanied by the disturbance of the metabolism, metabolically active antitumor compounds should be used for the treatment.

To sum up, we developed a simple and facile method suitable for the synthesis of complexes in the pure form and in high yields based on substituted nicotinamides or isonicotinamides and platinum tetrachloride. It was shown that the resulting complexes exhibit strong antimetastatic activity in B16 melanoma and Lewis lung carcinoma experimental metastasis models.

Experimental

***cis*-Bis-*N*-{3-[1,3-dihydroxy-2-(hydroxymethyl)prop-2-yl]-carbamoylpyridine}tetrachloroplatinum(IV) dihydrate (16).** A solution of *N*-nicotinoyltri(hydroxymethyl)aminomethane (**5**) (3.5 g, 15.5 mmol) in water (150 mL) was added with stirring to a solution of hexachloroplatinic acid (3 g, 7.3 mmol) in water (60 mL) at room temperature. After mixing, the reaction mixture was kept at room temperature for 3 h and then kept in a refrigeration chamber for 72 h. The yellow crystalline precipitate that formed was filtered off, washed with cold water and ethanol, and dried in air. The yield of complex **16** was 5.4 g (92.3%), m.p. 135 °C (with decomp.). Found (%): C, 29.20; H, 3.76; N, 6.77; Cl, 17.29; Pt, 23.76. $C_{20}H_{32}N_4O_{10}PtCl_4$. Calculated (%): C, 29.01; H, 3.87; N, 6.77; Cl, 17.41; Pt, 23.58. IR (ν/cm^{-1}): 344 s, 348 s, 564 w, 624 w, 684 m, 740 m, 820 w, 892 m, 924 w, 1052 s, 1116 w, 1200 m, 1284 s, 1320 m, 1432 m, 1528 m, 1652 s, 3400 s.

Complexes **12—15** and **17—22** were synthesized according to the analogous procedure. The physicochemical parameters and the ^{1}H NMR spectroscopic data are consistent with the data published earlier.[3]

X-ray diffraction study of complex 16. The unit cell parameters were measured and the three-dimensional X-ray diffraction data set was collected on an Enraf-Nonius CAD-4 automated X-ray diffractometer (MoKα radiation, graphite monochromator). Pale-yellow transparent crystals of **16** are monoclinic, $C_{20}H_{32}Cl_4N_4O_{10}Pt$, $M = 827.1$; $a = 16.812(6)$ Å, $b = 13.83(4)$ Å, $c = 12.768(5)$ Å, $\beta = 104.59(3)°$, $V = 2873(2)$ Å^3, $Z = 4$, $d_{calc} = 1.908$ g cm^{-3}, $\mu(MoK\alpha) = 53.13$ cm^{-1}, space group $P2_1/c$.

The structure of complex **16** was solved by direct methods using the SHELXS-97 program package and refined by the full-matrix least-squares method with anisotropic displacement parameters for nonhydrogen atoms against F^2 with the use of the SHELX-97 program package to $R = 0.041$ based on 3150 reflections with $I \geq 2\sigma(I)$.

Biological assays. Experiments were carried out on BDF hybrid mice with a weight of 22—24 g. Each group included 8—10 animals. The inoculum size for B16 melanoma and Lewis lung carcinoma experimental metastasis models was $5 \cdot 10^6$ and 10^6 tumor cells, respectively. The Lewis lung carcinoma and B16 melanoma cells were injected subcutaneously. The B16 melanoma- and Lewis lung carcinoma-bearing mice were sacrificed 24 and 28 days, respectively, after the transplantation of tumor cells. The number of lung metastases was determined, and then the index of metastasis inhibition, which is an indicator of the antimetastatic activity, was calculated.

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References

1. RF Pat. 2241713; *Byul. Izobr.* [*Inventor Bull.*], 2004, No. 34 (in Russian).
2. RF Pat. 2245328; *Byul. Izobr.* [*Inventor Bull.*], 2005, No. 33 (in Russian).
3. B. S. Fedorov, M. A. Fadeev, G. I. Kozub, S. M. Aldoshin, Z. G. Aliev, L. O. Atovmyan, N. P. Konovalova, T. E. Sashenkova, T. A. Kondrat´eva, S. V. Blokhina, *Khim.-farm. Zh.*, 2009, **43**, № 3, 12 [*Pharm. Chem. J.* (*Engl. Transl.*), 2009, **43**, 134].
4. RF Pat. 2147301; *Byul. Izobr.* [*Inventor Bull.*], 2000, No. 10 (in Russian).
5. B. V. Rigov, V. M. Mel´gunov, P. G. Komarov, *Dokl. Akad. Nauk SSSR*, 1977, **233**, 730 [*Dokl. Chem.* (*Engl. Transl.*), 1977].
6. L. V. Tat´yanenko, N. P. Konovalova, G. N. Bogdanov, O. V. Dobrokhotova, B. S. Fedorov, *Biomed. Khim.*, 2006, **52**, 52 [*Biomed. Chem.* (*Moscow*) (*Engl. Transl.*), 2006].
7. B. S. Fedorov, N. I. Golovina, M. A. Fadeev, A. B. Eremeev, V. V. Arakcheeva, G. V. Strukov, V. V. Kedrov, G. V. Shilov, R. F. Trofimova, L. O. Atovmyan, *Izv. Akad. Nauk, Ser. Khim.*, 1998, 527 [*Rus. Chem. Bull.* (*Engl. Transl.*), 1998, **48**, 527].
8. B. S. Fedorov, N. I. Golovina, M. A. Fadeev, G. V. Strukov, V. V. Kedrov, G. V. Shilov, G. N. Boiko, L. O. Atovmyan, *Izv. Akad. Nauk, Ser. Khim.*, 2001, 499 [*Rus. Chem. Bull., Int. Ed.*, 2001, **50**, 520].
9. B. S. Fedorov, *Tez. dokl. III S´ezda biofizikov Rossii* [*Abstrs, III Congress of Biophysicists of Russia*], *(June 24—29, 2004, Voronezh)*, Voronezh, 2004, **2**, p. 593 (in Russian).
10. N. P. Konovalova, L. M. Volkova, L. V. Tat´yanenko, *Vopr. Onkol.*, 1997, **43**, 309 [*Probl.* Oncol. (*in Russian*), 1997, **43**, 309].
11. N. P. Konovalova, L. M. Volkova, L. V. Tatyanenko, R. A. Kotelnikova, T. N. Yakushchenko, T. V. Kagiya, *Neoplasma*, 1997, **44**, 361.
12. A. Athanasiou, P. A. Smith, S. Vakilpour, N. M. Kumaran, A. E. Turner, D. Bagiokou, R. Layfield, D. E. Ray, A. D. Westwell, S. P.H. Alexander, D. A. Kendall, D. N. Lobo, S. A. Watson, A. Lophatanon, K. A. Muir, De-an Guo, T. E. Bates, *Biochem. Biophys. Res. Commun.*, 2007, **354**, 50.
13. Pat. RF 2429224, *Byul. Izobret.* [*Invention's Bulletin*], 2010, No. 26 (in Russian).

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