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trans-Platinum Complexes with Diclofenac, Aspirin, and 2,6-Di-*tert*-Butylphenol Fragment: Synthesis and Biological Activity

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Abstract—A series of σ-aryl platinum complexes with the sterically hindered phenol group of the general formula $RPt[PPh_3]_2X$ (R = 3,5-di-*tert*-butyl-4-hydroxyphenyl; X = Cl (**I**), diclofenac (**II**), aspirin (**III**), and OOCR (**IV**)) is synthesized and characterized by ^1H, ^{13}C, and ^{31}P NMR spectroscopy, IR spectroscopy, and elemental analysis. The molecular structure of compound **I** is determined by X-ray diffraction (XRD) (CIF file CCDC no. 2243100). The electron and hydrogen atom transfers are studied by spectrophotometry in the CUPRAC and DPPH tests. Complexes **I**, **II**, and **IV** are active reducing agents of Cu(II). The antioxidant activity is studied as the ability of the compounds to inhibit lipoxygenase (LOX-1B). Compound **I** is found to be an inhibitor of LOX-1B. The antiproliferative properties of the complexes are studied in vitro on the HCT-116, MCF-7, and A-549 cancer cells and WI-38 normal cells. The synthesized compounds have a lower antiproliferative activity than that of cisplatin.

Keywords: Pt(II) compounds, antioxidant activity, antiproliferative activity, aspirin, diclofenac

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INTRODUCTION

Cancer diseases represent an important social problem, since they worsen the life quality of the patients and are among the main causes of mortality. Surgery methods, chemotherapy, radiotherapy, hormonotherapy, immunotherapy, target therapy, and genetic engineering are used for the treatment of cancer diseases. Chemotherapy is one of the most popular methods for the treatment of malignant tumors of various types and, therefore, the search for new compounds used as cytostatic agents is a perspective task. The platinum compounds (cisplatin, oxaliplatin, and carboplatin) occupy the leading positions on the market of anticancer drugs. The mechanism of the action of cisplatin includes the binding of the platinum atom with DNA nitrogenous bases resulting in the transversal crosslinking of helices, cell cycle arrest, and apoptosis [1]. However, some cancer forms produced resistance to these compounds. The series of the Pt(IV) complexes with the *trans*-coordination geometry and the center at *trans*-ammineplatinum (cyclohexylaminodichlorodihydroxoplatinum) was studied in vitro in order to develop more efficient anticancer platinum-based drugs [2]. Cisplatin and transplatin were found to be capable of forming intra- and interchain crosslinkages with DNA, but there are substantial differences in the mechanisms that explain the opposite anticancer effects of these two agents. Transplatin is stereochemically incapable of forming 1,2-intrachain or cross bonds indicating differences in the anticancer activity because of different characters of violations induced in DNA by different intrachain cross bonds, which can depend on the nature of the ligand and its coordination.

The platinum compounds overcoming the cisplatin resistance of various cancer types due to the ligand environment in the platinum complexes are known. For instance, the introduction of the fragment of nonsteroid anti-inflammatory drug (NAID) aspirin into oxoplatin resulted in the formation of asplatin, which made it possible to overcome the resistance of cancer cells to the known platinum drugs [3]. Satraplatin (first perorally administrated Pt(IV) complex) showed no cross resistance with cisplatin on several human cancer cells in vitro [4].

The Pt(II) complexes containing NAID diclofenac in the ligands with different intracellularly cleaved linkers are powerful cytotoxic agents on several different cancer cell lines in vitro, and they are much more efficient against cisplatin-resistant tumor cells [5, 6].

Thus, the physiologically active platinum complexes can be modified by the transition to the trans complexes and by the introduction of various pharmacophoric or redox-active groups into the ligand environment.

Redox reactions are involved in various processes of cell survival. For example, the redox regulation of protein kinases plays a more general role in the regula-

tion of tyrosine kinases in the selective activation, duration, and enhancement of phosphorylation [7]. Protein oxidation is also a biologically necessary process. The mechanism of signal transmission and redox potential control includes, to a high extent, the oxidative modification of lateral amino acid chains in proteins (cysteine, methionine, proline, histidine, and tryptophan) by hydrogen peroxide. However, single errors related to the transfer of reactive oxygen metabolites can appear in these reactions, which leads to serious consequences for metabolism and important chemical processes in the cell. The full information transfer is impossible because of the oxidative damage of DNA and, as a consequence, valid cell division also becomes impossible [8]. 2,6-Dialkylphenols, which are mimetics of natural vitamin E and are widely used as antioxidants and stabilizers, are well known in the series of substances that control negative sequences of oxidative stress [9, 10].

The purpose of this work is the molecular design of the platinum complexes in which the Pt(II) atom, on the one hand, forms the σ bond with the sterically hindered phenol group and, on the other hand, is linked with the chelating fragment of the NAID. The antioxidant, antiproliferative, and anti-inflammatory activities of the complexes were also studied in vitro.

EXPERIMENTAL

3,5-Di-*tert*-butyl-4-hydroxybenzoic acid (99%, Sigma), triphenylphosphine (99%, Sigma), acetylsalicylic acid (Asp), and sodium diclofenac (NaDicl) (Moscow Pharmaceutical Plant) were used, and K_2PtCl_4 [11] and 3,5-di-*tert*-butyl-4-hydroxyphenylmercurium chloride [12] were synthesized using known procedures. Solvents $CHCl_3$, CH_2Cl_2, CH_3OH, C_2H_5OH, toluene, DMSO, and acetone (reagent grade); ethanol (96%); and petroleum ether (fraction 40–70°C) were used as received.

IR absorption spectra were recorded on an IR200 spectrophotometer (ThermoNicolet) with FT-IR in KBr pellets. NMR spectra were detected on a Bruker AMX-400 instrument in $CDCl_3$ (^{1}H, 400 MHz; ^{13}C, 100 MHz; ^{31}P, 162 MHz). Elemental analysis was conducted on a Vario Microcube C,H,N analyzer (Elementar). The melting point was determined by the capillary method on a Stuart SMP10 instrument (Bibby Scientific Limited Stone, UK) designed for the determination of the melting point (temperature).

The antioxidant activity of the compounds was determined using a MultiskanGo plate (96 wells) spectrophotometer (Thermo Fisher Sci., USA). The MTT test was conducted on a Zenyth200rt plate spectrophotometer (Anthos).

Tetrakis(triphenylphosphine)platinum $Pt[PPh_3]_4$ was synthesized according to a described procedure [13]. The yield was 0.81 g (72%). T_m = 148°C (T_m = 148–153°C [13]).

3,5-Di-*tert*-butyl-4-hydroxyphenylbis(triphenylphosphine)platinum chloride $RPt[PPh_3]_2Cl$ (I) was synthesized from tetrakis(triphenylphosphine)platinum and 3,5-di-*tert*-butyl-4-hydroxyphenylmercurium chloride [14]. The yield was 468 mg (56%). T_m = 247°C (T_m = 248°C [14]). The crystals of compound **I** suitable for XRD were isolated after the slow evaporation of a solution of the product from acetone at room temperature for 24 h.

^{1}H NMR ($CDCl_3$; δ, ppm): 0.97 (s, 18H, $2C(CH_3)_3$); 4.23 (s, 1H, –OH); 6.48 (s, 2H, 2CH (arom.), $^3J_{HPt}$ = 54 Hz); 7.16–7.53 (m, 30H, 2 PPh_3). ^{13}C NMR ($CDCl_3$; δ, ppm): 29.73 ($C(\underline{C}H_3)_3$); 32.83 ($\underline{C}(CH_3)_3$); 107.26, 124.51 (C–Pt); 127.28 (2C, $^3J_{CP}$ = 10 Hz); 129.42 (2C); 130.17 (C); 134.37 (2C, $^2J_{CP}$ = 12 Hz), 134.82, 153.12. ^{31}P NMR ($CDCl_3$; δ, ppm): 23.49 (t, 2P, $^1J_{P-Pt}$ = 3197 Hz).

Synthesis of 3,5-di-*tert*-butyl-4-hydroxyphenylbis(triphenylphosphine)platinum 2-[2-(2,6-dichloroaniline)phenyl]acetate $RPt[PPh_3]_2Dicl$ (II). A mixture of compound **I** (56 mg, 0.058 mmol) and sodium diclofenac (19 mg, 0.058 mmol) in acetone (3 mL) was stirred for 24 h. Then the solvent was distilled off in vacuo, and the residue was washed with *n*-hexane and water, and dried in air for 24 h. The yield was 34 mg (57%). T_m = 211°C.

For $C_{64}H_{61}O_3NP_2Cl_2Pt$

Anal. calcd., %:	C, 63.01	H, 5.04	N, 1.15
Found, %:	C, 62.64	H, 4.87	N, 1.03

IR (KBr; ν, cm^{-1}): 3625 ν(OH); 2869–3058 ν(CH); 3100–3300 ν(NH); 1611 ν(COO), 1452, 1435, 1358, 1098, 745, 692.

^{1}H NMR ($CDCl_3$, δ, ppm): 0.88 (s, 18H, $2C(CH_3)_3$); 4.35 (s, 1H, –OH); 6.48 (t, 2H, 2CH (arom.), $^3J_{HH}$ = 54 Hz); 6.15–6.54 (m, 3H, (arom.)); 6.75–6.94 (m, 4H, (arom.)); 7.16–7.53 (m, 30H, (arom.)). ^{31}P NMR ($CDCl_3$, δ, ppm): 21.97 (s, 2P, $^1J_{P-Pt}$ = 3967 Hz).

Synthesis of 3,5-di-*tert*-butyl-4-hydroxyphenylbis(triphenylphosphine)platinum acetylsalicylate $RPt[PPh_3]_2Asp$ (III). A 1 M solution of KOH (73 μL, 0.078 mmol) was added dropwise to a mixture of compound **I** (75 mg, 0.078 mmol) and acetylsalicylic acid (14 mg, 0.078 mmol) in acetone (5 mL), and the resulting mixture was stirred for 24 h. The solvent was distilled in vacuo, and the residue was filtered off,

washed with water and petroleum ether, and dried in air for 24 h. The yield was 72 mg (68%). T_m = 195°C.

For $C_{59}H_{58}O_5P_2Pt$

Anal. calcd., %:	C, 64.18	H, 5.29
Found, %:	C, 63.94	H, 5.39

IR (KBr; ν, cm^{-1}): 3627 ν(OH free), 2870–3055 ν(CH); 1607 ν(COO), 1593, 1558, 1456, 1385, 1220, 1197, 755.

^{1}H NMR ($CDCl_3$, δ, ppm): 0.97 (s, 18H, $2C(CH_3)_3$); 4.23 (s, 1H, –OH); 6.48 (t, 2H, 2CH (arom.), $^3J_{HH}$ = 54 Hz); 7.16–7.53 (m, 30H, (arom.)).

^{31}P NMR ($CDCl_3$, δ, ppm): 23.49 (s, 2P, $^1J_{P-Pt}$ = 3197 Hz).

Synthesis of 3,5-di-*tert*-butyl-4-hydroxyphenylcarboxylate-3,5-di-*tert*-butyl-4-hydroxyphenylbis(triphenylphosphine)platinum RPt[PPh_3]$_2$OOCR (IV). A 1 M solution of KOH (80 μL, 0.08 mmol) was added dropwise to a mixture of compound **I** (77 mg, 0.08 mmol) and 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (21 mg, 0.08 mmol) in acetone (5 mL), and the resulting mixture was stirred for 24 h. A yellow solution with a precipitate was formed. The solvent was distilled off in vacuo, and the colorless residue was filtered off, washed with water and petroleum ether, and dried in air for 24 h. The yield was 88 mg (75%). T_m = 201°C.

For $C_{66}H_{74}O_4P_4Pt$

Anal. calcd., %:	C, 66.71	H, 6.28
Found, %:	C, 66.48	H, 6.39

IR (KBr; ν, cm^{-1}): 3633 ν(OH free), 3200–3500 ν(OH bonded); 2873–3050 ν(CH); 1605 ν(COO), 1544, 1387, 1234, 693.

^{1}H NMR ($CDCl_3$, δ, ppm): 0.92 (s, 18H, $2C(CH_3)_3$); 1.31 (s, 18H, $2C(CH_3)_3$); 4.28 (s, 1H, –OH); 5.10 (s, 1H, –OH); 6.42 (s, 2H, 2CH (arom.)); 7.04 (s, 2H, 2CH (arom.)); 7.16–7.55 (m, 30H, (arom.)). ^{13}C NMR ($CDCl_3$, δ, ppm): 30.79 ($C(\underline{C}H_3)_3$); 31.21 ($C(\underline{C}H_3)_3$); 34.29 ($\underline{C}(CH_3)_3$); 34.94 ($\underline{C}(CH_3)_3$); 109.90; 111.73; 118.36; 124.64; 125.51; 126.14; 129.99; 135.96; 137.27; 150.18; 156.37; 181.00.

^{31}P NMR (δ, ppm, $CDCl_3$): 22.18 (s).

XRD was carried out on a BrukerQuest D8 diffractometer equipped with a Photon-III detector (ϕ and ω scan modes) using MoK_α radiation. An absorption correction was applied by a multiscan procedure implemented in SADABS (version 2016/2) [15]. The structure was solved by direct methods using the SHELXT program [16] and refined for F^2 using the SHELXL-2018 program [17]. The atoms were refined with individual anisotropic (non-hydrogen atoms) or isotropic (hydrogen atoms) displacement parameters.

Table 1. Selected bond lengths (Å) and angles (deg) for compound **I**

Bond *d*, Å	
Pt(1)–C(1)	2.004(5)
Pt(1)–P(1)	2.2981(16)
Pt(1)–P(2)	2.2971(16)
Pt(1)–Cl(1)	2.4116(17)
Angle ω, deg	
C(1)Pt(1)P(1)	89.39(17)
C(1)Pt(1)P(2)	90.64(17)
P(1)Pt(1)P(2)	178.44(7)
C(1)Pt(1)Cl(1)	176.88(18)
P(1)Pt(1)Cl(1)	92.89(6)
P(2)Pt(1)Cl(1)	87.02(6)

Selected bond lengths and angles in the structure of compound **I** are listed in Table 1.

The full set of XRD parameters was deposited with the Cambridge Crystallographic Data Centre (CIF file CCDC no. 2243100; deposit@ccdc.cam.ac.uk; http://www.ccdc.cam.ac.uk).

Determination of the activity of the compounds in the electron transfer reaction (CUPRAC test). Trolox (Sigma-Aldrich) was used without additional purification. The method was named according to the abbreviation CUPRAC (Copper Reducing Antioxidant Capacity) that implies the ability of an antioxidant to reduce the Cu(II) ion [18]. This method is based on the reduction of copper in a complex with neocuproine (2,9-dimethyl-1,10-phenanthroline) and demonstrates the ability of the studied substance to act as an electron donor. The experiment was carried out on the basis of an increase in the absorbance of a solution of the complex at the wavelength λ_{max} = 450 nm using a Thermo Scientific Multiskan plate (96 wells) spectrophotometer. The reaction mixture (V = 0.2 mL) contained an acetate buffer (0.05 mL), a 10^{-2} M solution of $CuCl_2$ (0.05 mL), a 7.5 M solution of neocuproine (0.05 mL), and a 2×10^{-3} M solution of the studied compounds in methanol (0.05 mL).

The results were presented in Trolox equivalents (TEAC), the values of which were determined graphically from the absorbance using the calibrated plot: the concentration dependence of the amount of the reduced Cu(II) complex on the Trolox content.

Investigation of the radical-binding activity (DPPH test). The procedure is based on the spectrophotometric measurement of a decrease in the absorbance at 517 nm due to the reaction of the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical with the studied compounds [19]. The reaction occurred in wells of a Thermo Scientific MultiskanGo plate (96 wells). A series of solutions with a specified concentration was

prepared, and the reaction mixture contained DPPH (0.1 mL) and studied substances (0.1 mL) in different concentrations (0.02, 0.04, 0.08, 0.12, 0.16, and 0.2 mM). The measurements were carried out at 20°C for 30 min.

The antioxidant activity in percentage (I, %) was calculated from the obtained data using the equation

$$I = (A_0 - A_1)/A_0 \times 100,$$

where A_0 is the absorbance of a control solution of DPPH, and A_1 is the absorbance of a solution of the reaction mixture.

The values of EC_{50} (effective concentration) were determined by the approximation of the concentration dependence of the logistic curve in the general form

$$I = 1/(1 + [c]/EC_{50}) \times 100,$$

where c is the concentration of the studied substance.

Inhibition of lipoxygenase enzyme. The activity of lipoxygenase (LOX-1B) was determined by spectrophotometric measurements. The content of the products of linoleic acid oxidation (the corresponding isomeric hydroperoxides) was measured at $\lambda_{max} = 234$ nm [20]. The analyzed solution contained 2 mL of a solution of linoleic acid (0.3 mM), 0.89 mL of a borate buffer (pH 9.0), and 0.01 mL of a solution of the compound in DMSO. The reaction was initiated by the addition of 0.1 mL of a solution of LOX-1B enzyme (500 units), and the measurements were carried out at 2°C for 10 min. The experiments for each substance were carried out at five concentrations and in three repetitions.

The degree of inhibition of lipoxygenase (I, %) was determined by the equation

$$I = V_o(\text{solution of substance})/V_o(\text{DMSO}) \times 100.$$

Procedure of operating with the cell cultures. The HCT-116, MCF-7, and A-549 cells and WI-38 fibroblasts were cultured in the DMEM complete cultural medium under standard conditions reseeding them two times a week depending on the seeding density. For reseeding, the cells were washed down from the vial walls with 2 mL of a versene solution (0.02% EDTA), which chelates calcium ions necessary for cell adhesion due to which the cells are detached from the support. For a more complete detachment of the cell mass, the vial was placed in a CO_2 incubator for 15–20 min (the CO_2 content was 5%, T = 37°C, Galaxy 170S incubator, New Brunswick an eppendorf company, USA). A portion of the cell mass (~100 μL) was left in the cultural vial, suspended in a DMEM medium (7–8 mL), and placed in the incubator for the further growth. The remained portion of the cell mass (1.5–2 mL) was neutralized by the addition of a DMEM cultural medium (5 mL), and the cells were precipitated by centrifugation (2 min, 2000 rpm, Universal 320R centrifuge, Germany). After centrifugation, the supernatant was removed and a precipitate of the cells was suspended in the cultural medium (5 mL). Then the cell mass (50 μL) was transferred to the eppendorf and dissolved in DMEM (450 μL). The number of cells was counted in a Goryaev chamber using a Magnus inversion biological microscope (Germany).

The cells were counted by the equation

$$(n \times 25)/100 \times [10]^5 \text{ cells/mL}.$$

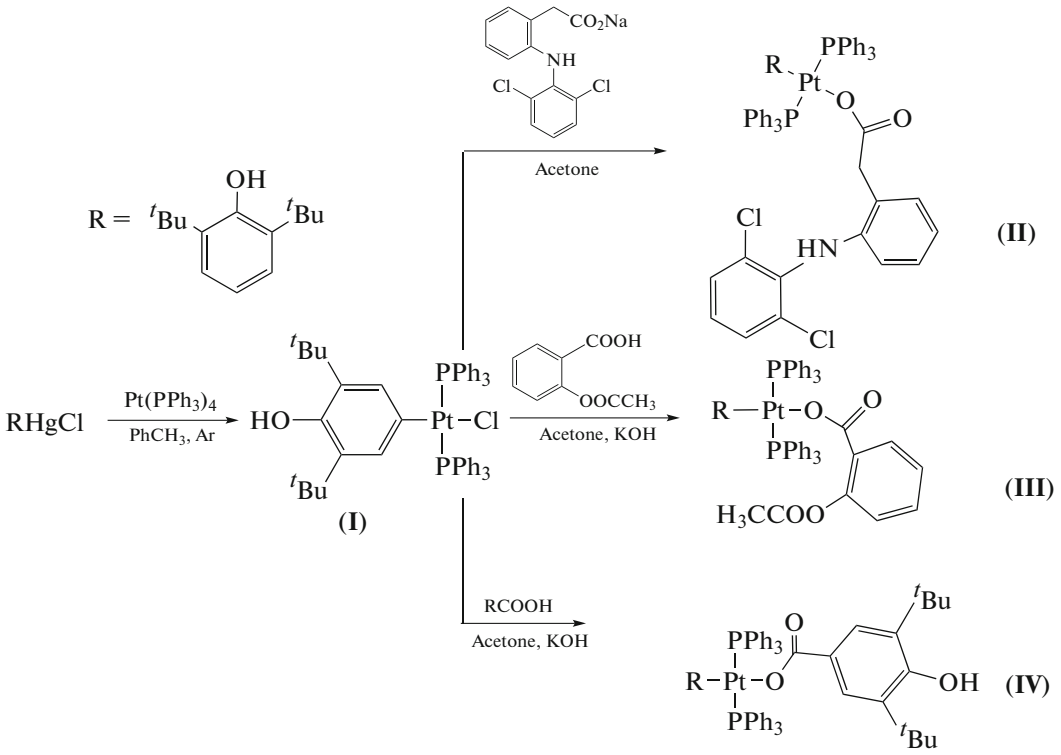
For the further operation with the cell cultures, a necessary dilution was attained using a DMEM medium to reach a cell density of 5×10^4 cells/mL. Then, a portion of the cell mass (190 μL) was introduced into each well of a sterile 96-well plate followed by cell cultivation for 24 h in a CO_2 incubator.

MTT test is based on the ability of living cell dehydrogenases, in particular, succinate dehydrogenase, to reduce uncolored forms of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazole (MTT) to blue formazan soluble in DMSO. The MTT test was conducted via a published procedure [21] with minor modifications. Solutions with concentrations of 1, 0.25, 0.0625, 0.015, and 0.00375 mM in DMEM were prepared from the studied substances. If necessary, the substances were preliminarily dissolved in DMSO (the DMSO concentration was not higher than 0.5% of the final solution volume). The prepared solutions of the studied substances were introduced into a sterile planar-bottom 96-well plate containing cell cultures with 5- and 10-μL micropipettes in such a way that the final concentrations of the substance in the wells would be 50, 25, 12.5, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, and 0.1 μM. The plate with the cells and studied substances was placed in a CO_2 incubator for 72 h. Then 10 μL of a solution of MTT (5 μg/mL) were introduced into each well of the plate with the primary culture and studied substance, and incubation was conducted at 37°C for 2 h in a wet atmosphere with 5% CO_2. After 2 h of exposure, the living cells reduce yellow MTT to dark violet granules of formazan. The formazan granules were dissolved in DMSO (150 μL), and the amount of the reduced product was measured by spectrophotometry on a Zenyth 2000rt plate reader at a wavelength of 570 nm. The test results were presented as a plot of the dependence of % survived cells on the concentration of the studied substances. Cisplatin was applied as the standard. The experiments with the tested compounds were carried out in three repetitions.

RESULTS AND DISCUSSION

The initial σ-aryl platinum complex (**I**) was synthesized by the transmetallation reaction [14] of tetrakis(triphenylphosphine)platinum with 3,5-di-*tert*-butyl-4-hydroxyphenylmercurium chloride. The compounds of the general formula $RPt[PPh_3]_2X$ were synthesized using the nucleophilic substitution of Cl in the initial compound **I** (Scheme 1). Diclofenac is a

sodium salt and a convenient nucleophile for the introduction into the reaction with compound **I** to give complex **II**. To synthesize complex **III**, an equimolar amount of alkali should be introduced into the reaction of compound **I** with acetylsalicylic acid, since an excess results in the hydrolysis of the ligand at the acyl group (Scheme 1). The reaction of compound **I** with 3,5-di-*tert*-butyl-4-hydroxybenzoic acid was conducted in acetone with the addition of an equimolar amount of KOH for the deprotonation of the carboxy group and formation of the nucleophilic center. The initial complex **I** was completely dissolved in acetone after the addition of the acid and base, which indicated that the reaction occurred to afford complex **IV** (Scheme 1).



Scheme 1.

The composition and purity of the organoplatinum compounds were confirmed by ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy, IR spectroscopy, and elemental analysis. The yields of the compounds were 56–75%. The IR spectra of compounds **I**–**IV** exhibit narrow absorption bands in a range of 3592–3639 cm^{-1} corresponding to stretching vibrations of the O–H bond of the sterically hindered unassociated phenol group, and stretching vibrations of the C–H bond are observed in a range of ~2800–3060 cm^{-1}.

The ^{1}H NMR spectrum of compound **I** in a range of 6.42–6.55 ppm shows the spin-spin interaction H–Pt with the constant $^{3}J_{H-Pt}$ = 52 Hz. The low-field shift of signals of the aromatic ring protons compared to the initial ligand confirms the coordination with the metal. The ^{31}P NMR spectrum contains the spin-spin interaction P–Pt with the constant $^{1}J_{P-Pt}$ = 3197 Hz.

The ^{1}H NMR spectrum of compound **II** demonstrates the spin-spin interaction H–Pt in a range of 6.65–6.73 ppm with the constant $^{3}J_{H-Pt}$ = 32 Hz. The signal of protons of the *tert*-butyl substituent exhibits the chemical shift relative to the initial compound **I**, which confirms the formation of a new compound. The ^{1}H NMR spectrum exhibits signals of aromatic protons at 6.30–6.90 ppm related to the diclofenac fragment. The ^{31}P NMR spectrum contains the signal at 21.97 ppm shifted relative to the initial compound **I** (23.49 ppm).

Compound **IV** contains two nonequivalent phenol fragments and, hence, the ^{1}H NMR spectrum exhibits two groups of signals of the corresponding substituents. For instance, the signal from the *tert*-butyl groups of the phenol fragment σ-bonded with Pt in compound **I** is observed in a range of 0.92 ppm. The singlet at 1.31 ppm corresponds to protons of the *tert*-butyl groups of 3,5-di-*tert*-butyl-4-hydroxybenzoate. The protons of the phenol groups of the platinum-containing fragment and hydroxybenzoate appear at 4.28 and 5.10 ppm, respectively.

The ^{13}C NMR spectra of compound **IV** exhibit the chemical shifts of the carbon signals indicating com-

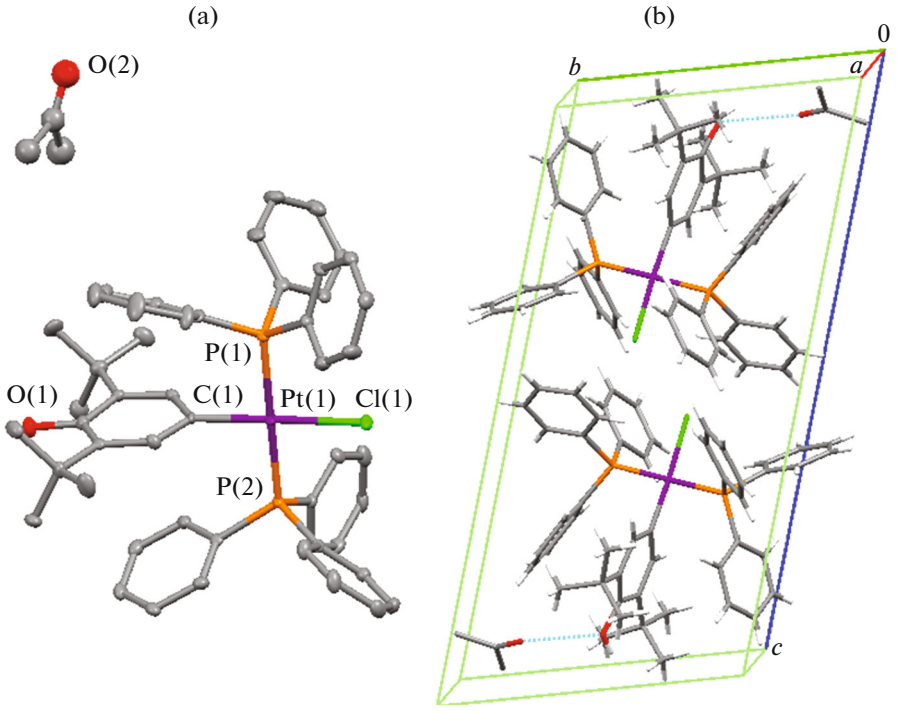


Fig. 1. (a) Molecular structure of compound **I** (hydrogen atoms are omitted) and (b) hydrogen bonds in the crystal packing of compound **I**.

plex formation. The sequence of the signals resembles that for the initial compound **I**, but not all signals are distinctly observed because of a low solubility of compound **IV** in $CDCl_3$. The ^{31}P NMR spectrum of compound **IV** also demonstrates the formation of the complex due to the chemical shift (22.18 ppm) compared to 23.5 ppm for compound **I**.

The molecular structure of compound **I** was studied by XRD. The recrystallization of compound **I** from acetone gave light yellow single crystals used for XRD.

The compound was found to crystallize in the triclinic system with the space group $P\bar{1}$; $a = 10.4531(18)$, $b = 11.828(2)$, $c = 22.049(4)$ Å; $\alpha = 75.160(4)°$, $\beta = 86.396(5)°$, $\gamma = 64.287(3)°$; $V = 2370.6(7)$ Å^3; $Z = 2$; $\Delta\rho_{max}/\Delta\rho_{min} = 2.155/1.178$ e/Å^3; MoK_α radiation; $\mu = 3.122$ mm^{-1}; R_1/wR_2 ($I \geq 2\sigma(I)$) = 0.0499/0.1105; GOOF = 1.020; angle range $\theta = 1.964°–25.998°$; crystal sizes 0.1 × 0.2 × 0.2 mm. It is found that compound **I** in the crystalline state is solvate with the outer-sphere acetone molecule. The coordination number of Pt is 4, and the coordination polyhedron is a distorted square (Fig. 1a).

Intermolecular hydrogen bonds between the OH group of the 2,6-di-*tert*-butylphenol fragment and the oxygen atom of the acetone molecule were found in the complex: O(1)–H(1)···O(2) (H(1)···O(2) 2.37 Å, O(1)–H(1)···O(2) 4.06 Å, angle O(1)H(1)O(2) 66.43°) (Fig. 1b).

The antioxidant activity of the platinum compounds was studied by spectrophotometry according to their ability to the one-electron reduction of the Cu^{2+} ion to Cu^+ using the CUPRAC test [18, 22]. Neocuproine (2,9-dimethyl-1,10-phenanthroline) is known to form the Cu(I) complex in the presence of antioxidants with the absorption maximum at 450 nm. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, TEAC = 1.00 ± 0.03) served as the standard. The results are given in Trolox equivalents TEAC (Table 2).

Complexes **I**, **II**, and **IV** were shown to be most active. The platinum complex with diclofenac has a high activity exceeding by 1.5 times the activity of the known antioxidant ionol (BHT).

The ability of the synthesized compounds to reduce the 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radical was estimated by the hydrogen atom transfer as another method for studying the antioxidant activity [19].

The activity of the platinum compounds was determined by electronic absorption spectroscopy measuring a decrease in the absorbance of DPPH at the wavelength 517 nm for 30 min. The effective concentration of the compound necessary for decreasing the DPPH radical concentration by 50% (EC_{50}) was determined graphically from the dependence of the remained DPPH (in %) on the primary concentration of the

Table 2. Values of TEAC and EC_{50} (μM) for platinum compounds **I**–**IV**, initial ligands, and ionol (R = 3,5-di-*tert*-butyl-4-hydroxyphenyl)

Compound	TEAC (CUPRAC)	EC_{50}, μM (DPPH)
I	1.84 ± 0.05	84 ± 5
II	1.57 ± 0.24	n/a*
III	0.66 ± 0.01	142 ± 62
IV	>3	104 ± 6
Diclofenac	n/a	n/a
Aspirin	n/a	n/a
RCOOH	1.56 ± 0.07	79 ± 3
R-CH_3	1.10 ± 0.03	67 ± 4

* n/a means that the compound is not active.

compounds (0.01–0.1 mM). The values of EC_{50} are listed in Table 2.

The antioxidant/anti-inflammatory properties of platinum compounds **I**–**IV** were studied in the model enzymatic oxidation of linoleic acid under the action of lipoxygenase LOX-1B in vitro. The by-products of the oxidation are reactive oxygen metabolites, whose accumulation leads to the oxidative stress. Therefore, the capability of inhibiting lipoxygenase can indicate potential antioxidant and anti-inflammatory properties of the studied substances. The activity of lipoxygenase was determined by spectrophotometry measuring the content of the product of linoleic acid oxidation (the corresponding hydroperoxides) at λ_{max} = 234 nm [20]. Only compound **I** was found to be a moderate inhibitor of lipoxygenase (EC_{50} = 182.8 ± 81 μM). It should be mentioned specially that other compounds promote the oxidation of linoleic acid.

The antiproliferative activity of the synthesized platinum complexes and initial diclofenac and aspirin was estimated by the MTT test [21] on the human colon cancer cells (HCT-116), human breast adenocarcinoma (MCF-7), human lung carcinoma (A-549), and human diploid cell line consisting of fibroblasts (WI-38), and IC_{50} were determined in comparison with those of cisplatin (Table 3).

Unlike cisplatin, the synthesized compounds have a lower antiproliferative activity, which is caused, most likely, by both the *trans*-coordination environment of the platinum atom and the presence of the cytoprotector phenol group of 2,6-di-*tert*-butylphenol in the molecule. This provides a possibility of the further study of these platinum compounds as potential anticancer drugs with a milder effect on the organism. The selectivity of the effect of compound **I** on the colon cancer cell line HCT-116 (IC_{50} = 59.6 μM) and normal cells WI-38 (diploid cell line of human fibroblasts) (IC_{50} = 169.5 μM) was observed. An almost threefold exceeding IC_{50} can serve as a basis for the search for similar trans complexes to achieve a high selectivity.

Thus, the *trans*-Pt(II) complexes containing the 2,6-di-*tert*-butylphenol fragments and nonsteroid anti-inflammatory drugs aspirin and diclofenac were synthesized and characterized. The introduction of the organoplatinum fragment into the 2,6-di-*tert*-butylphenol derivatives leads to an increase in the antioxidant activity. Compound **I** was found to be a lipoxygenase inhibitor, which possibly indicates its potential anti-inflammatory properties. The antiproliferative activity of the platinum complexes was studied in vitro on the human cell lines. Unlike cisplatin, the synthesized compounds are characterized by a substantially lower activity.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

Table 3. Values of IC_{50} (μM) for complexes **I**–**IV**, diclofenac, aspirin, and cisplatin against the HCT-116, MCF-7, A-549, and WI-38 cell lines

Compound	IC_{50}, μM			
	HCT-116	MCF-7	A-549	WI-38
I	59.6 ± 6	101.5 ± 15	105.6 ± 12	169.5 ± 52
II	93.7 ± 15	146.5 ± 35	>200	51.8 ± 5
III	69.4 ± 11	118.2 ± 50	106.5 ± 62	65.3 ± 24
IV	187.6 ± 100	>200	>200	61.9 ± 10
Diclofenac	n/a*	>200	>200	81.7 ± 61
Aspirin	n/a*	n/a*	n/a*	111 ± 34
Cisplatin	9.04 ± 0.7	11 ± 1	16.7 ± 3	4.8 ± 0.5

* n/a means that the compound is not active.

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