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Short Note

Cis-(3-benzyloxy-1,1-cyclobutanedicarboxylato κ^{2}O,O′)bis(1-methyl-1*H*-pyrazole)platinum(II)

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Abstract: A huge variety of types of cancer makes it necessary to search for new effective drugs with a defined molecular target. Modification of substituents in ligands based on 3-hydroxy-1,1-cyclobutanedicarboxylic acid are one of the effective directions to design a better version of carboplatin. In the present study, we combined in one molecule a derivative of 3-hydroxycyclobutane-1,1-dicarboxylic acid and N-methylpyrazole as a carrier ligand. The antiproliferative of the novel complex Pt(II) was established for cell lines HCT116, MCF7, A549, and WI38 by means of a standard MTT colorimetric assay.

Keywords: platinum (II) complexes; 1-methyl-1*H*-pyrazole ligand; 3-(benzyloxy)cyclobutane-1,1-dicarboxylic acid; antiproliferative activity

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1. Introduction

Today, in the design of antitumor platinum (II) complexes, active research is being conducted in the search for the defined structure–activity relationship; however, to date, no strict rules have been formulated. It seems impossible or very difficult to design a platinum-based antitumor active complex that would be effective, possesses low general toxicity, and has good water solubility and stability [1–3]. Both the nature of the leaving groups and the structure of the carrier ligands affect drug toxicity and possible side effects. Complexes with more labile ligands, such as chloride ions, in general, have a more pronounced general toxicity [4]. In addition to cisplatin, two compounds are approved for worldwide use: carboplatin and oxaliplatin (Figure 1).

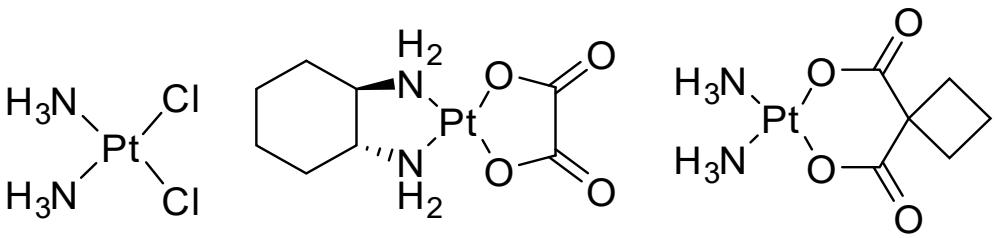


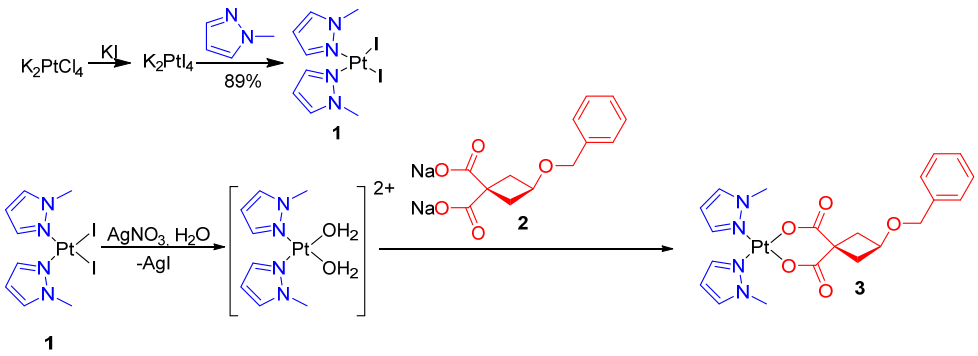
Figure 1. The structures of cisplatin (**left**), oxaliplatin (**middle**), and carboplatin (**right**).

The leaving groups in these complexes are anions of dicarboxylic acids. Bidentate dicarboxylates are a good alternative to chloride ions. Such ligands increase both the water solubility and lipophilicity of the complexes, and the chelating effect increases the stability of compounds in the bloodstream and reduces the number of side effects. In order to change the activity profile and the lipophilicity and reduce systemic toxicity,

numerous analogs of platinum drugs have been obtained [5–7]. For example, a series of 3-monosubstituted derivatives containing fluorine, chlorine, bromine, a hydroxyl group, as well as a 3,3-difluoro derivative was obtained [8]. The presence of a hydroxyl group in the 3-hydroxycyclobutane-1,1-dicarboxylate molecule provides some opportunities for further modification [9]. Attempts to create dual drugs based on a carboplatin analog and dichloroacetate have been made [10]. The ester bond in this molecule undergoes hydrolysis in the cell. This leads to the release of dichloroacetate, which can induce apoptosis in cancer cells, showing a synergistic effect [11,12]. In the design of antitumor complexes of platinum (II), the direction of introducing carriers of azaheterocycles as ligands are currently being actively developed. One of the first successful examples of such a design was the AMD473 molecule, later named picoplatin [13]. In general, it is less active on cell lines sensitive to cisplatin, but much more active against the cisplatin-resistant [14]. The use of heterocycles is justified for several reasons. First, heterocycles have a large area, which allows more efficient and stronger interaction with nitrogenous bases in DNA using π-π-stacking. Second, sterically hindered complexes interact more slowly with S-containing amino acid residues in blood proteins and other tissues, which prevents the inactivation of the therapeutic agent [4]. Several examples of the use of pyrazoles for the design of antitumor platinum (II) complexes with a classical structure are reported in the literature [15,16]. In the present study, we applied the idea of combining, in one molecule, a derivative of 3-hydroxycyclobutane-1,1-dicarboxylic acid and N-methylpyrazole as a carrier ligand.

2. Results

A new platinum (II) complex was synthesized using standard procedure (method Dhara) starting from 3-(benzyloxy)cyclobutane-1,1-dicarboxylic acid as the leaving group ligand and 1-methyl-1H-pyrazole as amine moiety. The synthetic route is presented in Scheme 1.



Scheme 1. Synthesys of Pt(II) complex.

At first, K_2PtCl_4 was converted to cis-$[PtI_2(Py)_2]$ **1** [16] (Py=1-methyl-1*H*-pyrazole). The suspension of light yellow cis-$[PtI_2(Py)_2]$ **1** in deionized water was treated with silver nitrate, filtrated, and the aqua moiety was reacted with sodium 3-(benzyloxy)cyclobutane-1,1-dicarboxylate **2** to give the resulting platinum complex **3**. The structure of novel platinum complex **3** was proved with ^{1}H, $^{13}C\{^{1}H\}$, and ^{195}Pt NMR spectroscopy, and HRMS-ESI and purity were confirmed by the elemental analysis (full spectra available in Supplementary Materials). In the NMR spectrum, two groups of signals corresponding to the atoms of the pyrazole rings were observed. This fact can be explained by spatially non-equivalent pyrazole rings due to the hindered rotation around the Pt-N bond. ESI-mass spectra were recorded without the addition of any acid or base in positive mode; hence, the ions corresponding to complex **3** appeared mainly due to the gain of the proton or sodium ($[M + H]^+$, $[M + Na]^+$ ions). Complexes were stable in DMF and DMSO solutions at least for 24 h.

The antiproliferative activity of compound **3** against the human HCT116 colorectal carcinoma, MCF7 breast adenocarcinoma, A549 non-small cell lung carcinoma, and WI38 nonmalignant lung fibroblast cell lines was evaluated by means of a standard MTT colorimetric assay as the IC_{50} value after 72 h of incubation [17]. Compound **3** was found to be not active up to 200 μM and can be considered as almost nontoxic against cancerous and nonmalignant cells, especially compared to the standard Pt anticancer drug cisplatin.

3. Materials and Methods

3.1. General

All commercial reagents were used without further purification. All solvents were purified and degassed before use. ^{1}H NMR, ^{13}C NMR, and ^{195}Pt spectroscopy were performed at 298 K on Bruker Avance 600 (Brucker, Karlsruhe, German). ^{1}H and ^{13}C NMR spectra were calibrated against the residual solvent: $CDCl_3$. ^{195}Pt spectra were calibrated by external reference (K_2PtCl_4 in H_2O, δ = 0 ppm). The splitting of the proton resonances in the reported ^{1}H spectra is defined as s = singlet, d = doublet, t = triplet, and m = multiplet. High-resolution mass spectra (HRMS) were recorded on a Shimadzu IT-TOF instrument (Shimadzu Europa GmbH, Duisburg, Germany) with a Shimadzu LC-20AD pump with atmospheric pressure electrospray ionization (AP-ESI) in the positive ion registration mode (an ion trap type mass analyzer). Recording conditions: the mobile phase rate was 0.3 mL min^{-1}, and the solvent was acetonitrile.

3.2. Synthesis

Cis-(3-benzyloxy-1,1-cyclobutanedicarboxylato κ^2O,O′)bis(1-methyl-1*H*-pyrazole)platinum(II)

$K_2[PtCl_4]$ (100 mg, 0.241 mmol) was dissolved in 3 mL of deionized water at room temperature, and KI (400 mg, 2.41 mmol, 10 eq.) was added to the solution. The reaction mixture was stirred for 30 min at room temperature in the dark. 1-methyl-1*H*-pyrazole (39 mg, 0.475 mmol) was added to the solution, and the reaction mixture was stirred overnight at room temperature in the dark. A precipitated product, cis-$[PtI_2(Py)_2]$, was separated by centrifugation; washed with deionized water (3 × 4 mL), ethanol (3 × 3 mL), and diethyl ether (3 × 3 mL); and was dried under a vacuum. The solution of $AgNO_3$ (70 mg, 0.412 mmol) in 1 mL of deionized water was added to the suspension of cis-$[PtI_2(Py)_2]$ (131 mg, 0.214 mmol). In 3 mL of deionized water the reaction mixture was stirred for 24 h at room temperature in the dark. A precipitated AgI was filtered off, and sodium 3-(benzyloxy)cyclobutane-1,1-dicarboxylate (62 mg, 0.212 mmol) in 2 mL of deionized water was added to the filtrate. The reaction mixture was stirred for 1 h at room temperature. The precipitated white solid product was separated by centrifugation, washed with ethanol (2 × 3 mL) and diethyl ether (2 × 3 mL), and was dried under a vacuum. Yield 88 mg, (60%). Mp 217 °C (decomp).

^{1}H NMR (600.13 MHz, DMF-d7): δ (ppm) 8.17 (t, 2H, *J* = 2.2 Hz, Py), 8.06 (d, 1H, *J* = 2.0 Hz, Py), 8.01 (d, 1H, *J* = 1.9 Hz, Py), 7.31–7.40 (m, 5H, Ph), 6.50 (t, 1H, *J* = 2.6 Hz, Py), 6.49 (t, 1H, *J* = 2.6 Hz, Py), 4.47 (s, 2H, O$\underline{CH_2}$Ph), 4.29 (s, 6H, $2CH_3$), 3.99 (q, 1H, *J* = 7.2 Hz, CH of cyclobutyl), 3.32–3.35 (m, 2H, $\overline{CH_2}$ of cyclobutyl), 2.65–2.68 (m, 2H, CH_2 of cyclobutyl). ^{13}C{^{1}H} NMR (150.92 MHz, DMF-d7): δ (ppm) 177.3 (CO), 177.0 (CO), 143.2 (C_{Py}), 143.09 (C_{Py}), 139.2 (C_{arene}), 135.7 (C_{Py}), 135.7 (C_{Py}), 128.5 (C_{arene}), 128.1 (C_{arene}), 127.7 (C_{arene}), 107.6 (C_{Py}), 69.8 (O$\underline{CH_2}$Ph), 67.9 (CH of cyclobutyl), 49.2 ($\underline{C}(COO)_2$), 39.6 ($2CH_3$), 39.1 (CH_2 of cyclobutyl), 33.0 (CH_2 of cyclobutyl). ^{195}Pt (129.01 MHz, DMF-d7): δ (ppm) −1623. HRMS-ESI: *m*/*z* calcd. for $[C_{21}H_{24}N_4O_5Pt + H]^+$ 607.1446, found 607.1439. Elemental analysis calculated for $C_{21}H_{24}N_4O_5Pt$: C 41.52, H 3.98, N 9.22, found: C 41.59, H 3.67, N 9.12.

3.3. Cells and In Vitro Antiproliferative Assays

The human HCT116 colorectal carcinoma, A549 non-small cell lung carcinoma, MCF7 breast adenocarcinoma, and WI38 nonmalignant lung fibroblast cell lines were obtained

from the European collection of authenticated cell cultures (ECACC; Salisbury, UK). All cells were grown in a DMEM medium (Gibco™, Dublin, Ireland) supplemented with 10% fetal bovine serum (Gibco™, Brazil). The cells were cultured in an incubator at 37 °C in a humidified 5% CO_2 atmosphere and were sub-cultured 2 times a week. The effect of the investigated compounds on cell proliferation was evaluated using a common MTT assay. The cells were seeded in 96-well tissue culture plates («TPP», Switzerland) at a 1×10^4 cells/well in 100 μL of the medium. After overnight incubation at 37 °C, the cells were treated with the solution of tested compounds in DMEM (a nontoxic concentration of max 0.5% DMSO was used for solubilization) in the concentration range of 0 to 200 μM. Cisplatin was used as a standard. After 72 h of treatment, the solution was removed, a freshly diluted MTT solution (100 μL, 0.5 mg/mL in cell medium) was added to the wells, and the plates were further incubated for 50 min. Subsequently, the medium was removed, and the formazan product was dissolved in 100 μL of DMSO. The number of living cells in each well was evaluated by measuring the absorbance at 570 nm using the «Zenith 200 rt» microplate reader (Biochrom, Cambridge, UK).

4. Conclusions

The synthesis and characterization of analogs of carboplatin with 3-hydroxycyclobutane-1,1-dicarboxylic acid and N-methylpyrazole ligands have been reported here for the first time. Unfortunately, the complex showed a lack of antiproliferative activity; however, future modification of dicarboxylic acid moiety might improve the activity and selectivity of the compounds.

Supplementary Materials: The following supporting information can be downloaded: copies of ^{1}H, ^{13}C, ^{195}Pt NMR, and mass-spectra.

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