Synthesis and biological evaluation of mixed ammine/amine platinum(II) complexes with dicarboxylate containing organic nitrate as ligand

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\textbf{A B S T R A C T}

Two novel platinum(II) complexes cis-[Pt\{L\}'\{NH\}X] \hspace{0.5cm} (where L' = cyclopentylamine or cyclohexylamine, X = 3-(nitrooxy)cyclobutane-1,1-dicarboxylate) were synthesized and spectrally characterized in this study. The purity of complexes 1 and 2 were studied by HPLC–MS spectra, and the contents of complexes 1 and 2 were more than 98%. It was demonstrated that the newly synthesized compounds with dicarboxylate containing organic nitrate as ligand possessed DNA unwinding capability similar to cisplatin by the means of agarose gel electrophoresis. In addition, the antiproliferative study by WST-8 assay revealed that these platinum(II) complexes exhibited considerable cytotoxicity against tested cancer cell lines in vitro compared with positive agents (cisplatin, oxaliplatin and carboplatin), especially complex 1, showing higher in vitro antitumoractivity than oxaliplatin and carboplatin in SGC7901 and A549 cell lines.

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\textbf{1. Introduction}

The successful application of cisplatin as anticancer drugs in clinic has attracted much attention to designing new generation of platinum-based anticancer complexes that circumvent shortcomings of cisplatin \cite{1,2}. For several decades, numerous platinum(II) complexes have been synthesized and screened as potential anticancer antitumor agents. However, only two cisplatin analogues, carboplatin and oxaliplatin, were worldwide approved for malignant tumor chemotherapy \cite{3}. One of the reasons for this limited success in the anticancer research of platinum-based complexes is due to the relative lack in structural diversity \cite{4}. Most of the reported platinum(II) complexes are so called classical ones which have been designed based on the structure–activity relationships summarized by Cleare and Hoeschele \cite{5,6}, and these platinum(II) complexes often contain two identical amines, which may show cross-resistance with cisplatin, such as carboplatin \cite{4,7}.

Satriplatin [bis-acetato-ammine-dichlorido-cyclohexylamine-platinum(IV), JM216, Fig. 1] with two different amines in the equatorial plane showed promise against second-line hormone refractory prostate cancer in clinical trials \cite{8–10}. It exerted anticancer activity by losing its axial acetate groups to form a platinum(II) complex (JM118), an asymmetrical cisplatin analogue which binds to DNA via a similar mechanism to cisplatin \cite{11–13}. JM118 is considerably more active than cisplatin in numerous cisplatin sensitive and resistant human tumor cells \cite{14–17}. Hence, many mixed ammine/amine platinum(II) complexes analogous to JM118, have been synthesized and investigated for anticancer activity against various human solid tumor cell lines by other researchers. Zhang’s group has reported that mixed ammine/cyclohexylamine platinum(II) complexes with carboxylates/dicarboxylates as leaving groups show activity in vitro against EJ (human bladder carcinoma), HCT-8 (human colon carcinoma), BGC-823 (human gastric carcinoma), HL-60 (human immature granulocyte leukemia) and MCF-7 (human galactophore carcinoma) cell lines \cite{17,18}.

Carboplatin exhibits decreased side effects (nephrotoxicity and neurotoxicity) and higher aqueous solubility compared with cisplatin because of 1,1-cyclobutanedicarboxylate (CBDC) as ligand. Liu’s research group has reported a series of mixed ammine/cyclohexylamineplatinum(II) complexes with CBDC derivatives as leaving groups showing cytotoxicity against SKOV-3, SGC-7901, 22RV-1, A549 and Beas-2B cell lines \cite{19}. In our previous study, we have synthesized cisplatin and oxaliplatin derivatives with 3-(nitrooxy)cyclobutane-1,1-dicarboxylate (L2) as leaving group (GSH-1, GSH-2, Fig. 1), which showed considerable antitumor activity against the tested cancer cell lines \cite{20}. Organic nitrate, as one of the nitrogen oxide donors, is well known for...
its biological functions in vasodilation, neurotransmission, immune system, and cell apoptosis. Therefore, in this paper, 3-(nitrooxy)cyclobutane-1,1-dicarboxylate as a CBDC derivative, was introduced as a leaving group in the mixed ammine/amine platinum(II) complexes in order to adjust the antitumor activity (Scheme 1).

2. Experimental

2.1. Materials and instruments

All reagents and chemicals were of analytical reagent grade and used without further purification. Potassium tetrachloroplatinate(II), potassium iodide, cyclopentylamine, cyclohexylamine and silver nitrate were purchased from a local chemical company (Shandong Boyuan Chemical Co., Ltd., China). $^1$H NMR spectra were measured in D$_2$O with a Bruker 300 MHz spectrometer. Mass spectra and HPLC were tested on an Agilent 6224 TOF LC/MS instrument. Elemental analyses for C, H and N were performed on a Vario MICRO CHNOS Elemental Analyzer. Elemental analyses for platinum were carried out on a J-A1100 inductively coupled plasma (ICP) spectrometer. Infrared spectra were recorded in the range 400–4000 cm$^{-1}$ and measured in KBr pellets on a Nicolet IR200 FT-IR spectrometer.

2.2. Preparation of target complexes 1–2

A mixture of cis-[Pt(L)NH$_3$]$_2^2+$ (1 mmol) and silver nitrate (2 mmol) in distilled water (40 mL) was stirred for 24 h at 40 °C, and then the depositing AgI was filtered off. To the filtrate was added 3-(nitrooxy)cyclobutane-1,1-dicarboxylic acid (1 mmol) mixed with NaOH (2 mmol) in 10 mL water. After the mixture was stirred for 24 h at room temperature, it was concentrated to 10 mL and the precipitate was filtered, yielding white solid. The product was dried at 35 °C in vacuo and kept in the dark. Complex 1: Yield 0.31 g (62.3%). White solid $^1$H NMR (D$_2$O): $\delta$ 5.12 (quint, 1H, CH$_2$ONO$_2$), 3.06–3.34 (m, 5H, CH$_2$NH$_2$ and CH$_2$ of cyclobutyl), 1.96–2.78 (m, 4H, CH$_2$CH of cyclopentyl), 1.49–1.64 ppm (m, 4H, CH$_2$CH$_2$ of cyclopentyl); IR (KBr): 3222, 3125, 1635, 1384, 1283, 859 cm$^{-1}$; ESI-MS: $m/z$ (%): 561 (89), 562 (100), 563 (82) [M+NO$_3$]; Anal. Calc. for C$_{11}$H$_{19}$N$_3$O$_7$Pt: C, 26.40; H, 3.83; N, 8.40; Pt, 38.99. Found: C, 26.31; H, 3.75; N, 8.59; Pt, 38.45%. The preparation of complex 2 was similar to that of complex 1 described above by applying the corresponding intermediate. Complex 2: Yield 0.33 g (64.4%). White solid $^1$H NMR (D$_2$O): $\delta$ 5.11 (quint, 1H, CH$_2$ONO$_2$), 2.95–3.36 (m, 5H, CH$_2$NH$_2$ and CH$_2$ of cyclobutyl), 2.24–2.75 (m, 4H, CH$_2$CH of cyclopentyl), 1.04–1.68 ppm (m, 6H, CH$_2$CH$_2$ of cyclopentyl); IR (KBr): 3222, 3125, 1634, 1384, 1283, 860 cm$^{-1}$; ESI-MS: $m/z$ (%): 575 (85), 576 (100), 577 (78) [M+NO$_3$]; Anal. Calc. for C$_{12}$H$_{21}$N$_3$O$_7$Pt: C, 28.02; H, 4.11; N, 8.17; Pt, 38.94. Found: C, 26.31; H, 3.75; N, 8.59; Pt, 38.45%. The preparation of complex 2 was similar to that of complex 1 described above by applying the corresponding intermediate.

2.3. Cell culture

Five human solid tumor cell lines including HCT-116 (human colorectal carcinoma), HepG-2 (human hepatocellular carcinoma), A549 (human non-small cell lung cancer), SGC7901 (human gastric cancer) and COC1 (human ovarian carcinoma) were used in the cytotoxicity test for the platinum complexes. They were cultured...
in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 µg mL\(^{-1}\) of penicillin and 100 µg mL\(^{-1}\) of streptomycin in an atmosphere of 5% CO\(_2\) at 37 °C.

2.4. In vitro cytotoxicity assay

Cytotoxicity of all the complexes against tested cells was determined by WST-8 assay (WST-8 = sodium2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium) with Cell Counting Kit-8 (CCK-8). The cells were plated in 96-well culture plates at density of 5000 cells per well and incubated for 24 h at 37 °C in a water-atmosphere (5% CO\(_2\)). The tested compounds with desired concentrations were obtained by dissolving in DMSO and diluting with culture medium (DMSO final concentration <0.4%). Then the diluted solution of complexes was treated with the cells for 48 h at 37 °C in a CO\(_2\) incubator. After that, 10 µL of a freshly diluted CCK-8 solution (5 mg/mL in PBS) were added to each well for 1 h. The cell survival was evaluated by measuring the absorbance at 540 nm. IC\(_{50}\) values were calculated from the chart of cell viability (%) against compound concentration (µM). All experiments were carried out in triplicate.

2.5. Gel electrophoresis experiment

DNA binding produced by complexes 1–2 and cisplatin was investigated by agarose gel electrophoresis. pET28a plasmid DNA (50 ng/µL) was used as the target. Appropriate dilutions of tested compounds were made, and the required volumes of solutions were added to achieve a set of concentrations in the range of 0–240 µM; pET28a DNA (5 µL, 0.20 µg) was added to each tube, and the mixtures of platinum complexes and pET28a plasmid DNA were then incubated at 37 °C for 24 h. Afterward, the agarose gel (made up to 1% w/v) containing ethidium bromide was prepared with TA buffer (50 mM Tris-acetate, pH 7.5). The mixtures with loading buffer (1 µL) underwent electrophoresis in agarose gel in TA buffer at 100 V for 60 min. Bands were imaged using a Molecular Imager (Bio-Rad, USA) under UV light.

3. Results and discussion

3.1. Synthesis and characterization

Platinum(II) complexes 1 and 2 were prepared in five steps following the procedures shown in Scheme 1. Ligand H\(_2\)L was synthesized according to the method described in our previous work [20]. The intermediate cis-[Pt(L′)](NH\(_3\))L\(_2\) (L′ = cyclopentylamino cyclohexylamine) were synthesized according to the reported procedure [13]. The synthesized compounds were characterized by elemental analysis, \(^1\)H NMR, IR, and ESI-MS spectroscopy. In the IR spectra of the platinum complexes, the N–H stretching vibrations appeared between 3125 and 3226 cm\(^{-1}\), red shifting compared with the single amino group, due to the amino group coordinating with the metal ion. Because of the coordination of the carboxylate groups, \(\nu_{\text{as}}(C=O)\) vibration of the complexes shifted near 1700 cm\(^{-1}\) from free acids to lower frequencies, while \(\nu_{\text{as}}(-NO_2)\), \(\nu_s(-NO_2)\) and \(\nu(C=O)\), characteristic of organic nitrate groups, were found near 1640, 1280 and 860 cm\(^{-1}\), respectively. All the \(^1\)H NMR spectral data are reasonably attributed to the molecular structures of the synthesized compounds. In the ESI-MS spectra, a negative peak of [M+NO\(_3\)^−] \(^−\) for complexes 1 and 2 was observed, in agreement with the molecular structure of the complexes shown in Scheme 1. The purity of complexes 1 and 2 were ascertained by HPLC–MS spectra, and the contents of complexes 1 and 2 were more than 98% (Fig. 2).

3.2. In vitro antitumor activity

The cytotoxicity of complexes 1, 2 and positive controls were tested by means of CCK8 assay against five human cancer cell lines: HepG-2 (human hepatocellular carcinoma cell line), SGC7901 (human gastric carcinoma cell line), A549 (human non-small cell lung cancer cell line), COC1 (human ovarian cancer cell line), HCT116 (human colorectal cancer cell line). Cisplatin, carboplatin and oxaliplatin were used as positive controls. The results of cytotoxicity against tumor cells in vitro are expressed as IC\(_{50}\) values (the compound concentration effective in inhibiting 50% of the cell growth as compared with control untreated cells) which are presented in Table 1.

According to the IC\(_{50}\) values of the platinum(II) complexes in Table 1, complexes 1 and 2 showed higher cytotoxicity than carboplatin, and displayed comparable cytotoxicity to oxaliplatin. It is noted that complex 1 presents better cytotoxic activity than oxaliplatin against selected tumor cell lines except HepG-2 cells, while complex 2 exhibits low IC\(_{50}\) values against COC1 and HCT116 cell lines as compared with oxaliplatin. Comparing complex 1 and KK-01, both compounds possess similar structures except that hydroxyl group of KK-01 was replaced by organic nitrate group. The cytotoxicity of the precursor compound KK-01 with mixed amine/cyclopentylamine and 3-(hydroxy)cyclobutane-1,1-dicarboxylate, which was reported by Liu et al. was about 2.2-fold higher than carboplatin against SGC7901 and A549 cell lines.
However, it is noted from Tables 1 and 2 that complex 1 was more effective in vitro than its precursor KK-01, suggesting that the introduction of organic nitrate in the metal complex may improve its in vitro antitumor activity.

Overall, the newly synthesized Pt(l) complexes showed considerable cytotoxicity against the tested cell lines. Notably, compound 1 was more potent than oxaliplatin in most of the tested tumor cells, which makes it an ideal anticancer agent for further research. Moreover, the presence of organic nitrate in compound 1 improved its cytotoxicity, indicating the positive synergic action of NO in oncology [21].

3.3. Interaction with pET22b plasmid DNA

The DNA unwinding ability of complexes 1 and 2 was tested by agarose gel electrophoresis, and the pET28a plasmid DNA was used as the target. The gel electrophoretic mobility pattern of plasmid DNA treated with different concentrations of cisplatin, complexes 1 and 2 was shown in Fig. 3. In the electrophoretogram, the untreated plasmid DNA (lane 1), which mainly consisted of covalently closed circular (form I, ccc) and a small amount of open circular (form II, oc) bands, was used as negative control. Lanes 1–8 correspond to the DNA distast pattern produced by the tested compounds with concentrations of 0, 10, 20, 40, 80, 160, 320 and 640 μM, respectively. It can be seen in Fig. 3 that the mobility of the plasmid DNA have different changes for the compounds tested compared with negative control after pET28a plasmid DNA was incubated with the compounds at 37°C for 4 h and 24 h, respectively.

As for cisplatin after 4 and 24 h incubation with plasmid DNA, it was observed that the electrophoretic mobility of the covalently closed circular DNA (form I) decreased with increased concentration of complex from 10 to 640 μM, suggesting that cisplatin bound with the supercoil DNA at high concentration after 4 or 24 h incubation. With regard to complexes 1 and 2, it can be observed that the electrophoretic mobility of form I had a significant decrease with increased concentration of the complex after 24 h incubation, indicating that complexes 1 and 2 showed capability to distort plasmid DNA after long-time incubation.

References


