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Design, synthesis and anticancer activity of diam(m)ine platinum(II) complexes bearing a small-molecular cell apoptosis inducer dichloroacetate

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ABSTRACT

Four new diam(m)ine platinum complexes containing the dichloroacetate moiety in 3-dichoroacetoxylcyclobutane-1,1-dicarboxylate as the leaving group were synthesized, characterized by elemental analysis as well as by ESI^+-MS (electrospray ionization mass spectrometry in positive mode), FT-IR, ^1H- and ^{13}C-NMR, and evaluated for their *in vitro* anticancer activity against human lung cancer cell line (A549) and ovarian cancer cell lines (SK-OV-3, SK-OV-3/DDP). Diam(m)ines used in the present study belong to the carriers of six clinically approved platinum drugs. Among the complexes synthesized, complex **2**, *cis*-[Pt(II)(*1R,2R*-diaminocyclohexane)·(3-dichoroacetoxylcyclobutane-1,1-dicarboxylate)] is the most promising in terms of water solubility and potential of being totally devoid of cross-drug resistance with cisplatin. Therefore, complex **2** was selected for the dichloroacetate release test. The test shows dichloroacetate can be efficiently released from complex **2** under physiological conditions via the hydrolysis of an ester bond bridging the dichloroacetate moiety and platinum pharmacophores together. Our study supports the further evaluation of this complex as a drug candidate.

1. Introduction

In today's world malignant tumors have become one of the most common and serious diseases, and rank first in human disease-related lethality. Chemotherapy is a central component in the fight against malignant tumors, and it is based on different classes of anti-cancer drugs. Among them, platinum-based drugs represent an important class characterized by killing cancer cells primarily through cross-linking DNA and inhibiting transcription [1,2]. Platinum-based drugs now available for clinical options include cisplatin (DDP, *cis*-diamminedichloroplatinum(II)), carboplatin, oxaliplatin, nedaplatin, heptaplatin and lobaplatin, and they have been successfully used in the treatment of solid tumors [3–5]. However, like other chemotherapy agents, the clinical applications of platinum-based drugs are largely restricted by side-effects as well as by drug resistance [6–8]. Ovarian cancer is a typical example of drug resistance. Most women with ovarian cancer respond fully to the initial cisplatin-based chemotherapy, but as tumors recur, they develop resistance not only to cisplatin but also to other platinum drugs. Drug resistance leads to the failure of chemotherapy. As a result, ovarian cancer has the highest mortality among gynecological cancers, highlighting the need for the development of new strategies to overcome this drawback. One of the strategies involves the synthesis and evaluation of non-classical platinum compounds represented by picoplatin, polynuclear complexes, and trans-platinum complexes, however the outcomes of clinical trials remained below expectations and none of these compounds has been approved for clinical application [9].

Drug resistance can emerge from failure to execute apoptosis despite initiation of the apoptotic cascade caused by either the predominance of anti-apoptotic factors or defects in downstream effectors. It has been demonstrated that failure to achieve final cell death after the formation of platinum-DNA adduct might be an important factor contributing to the drug resistance mechanisms in platinum-based chemotherapy [10,11]. One innovative and efficient strategy for combating this drug resistance, as highlighted by Dhara, Xiao and Lippard [12,13], is to incorporate dichloroacetate groups into the existing platinum drugs to form a prodrug. Dichloroacetate is a small-molecular cell apoptosis inducer and can trigger apoptosis through selectively targeting the mitochondria of cancer cells resistant to the anticancer drugs [14–16]. The resulting complex molecules contain both the platinum pharmacophore and dichloroacetate moiety displaying a dual-functional profile. As a result, the enhanced drug sensitivity and decreased resistance of tumor cells via a synergistic effect between the two active components were verified. Furthermore, molecular hybridization is an important drug discovery strategy which involves the rational design of new chemical entities by the fusion of two drugs [17].

Based on the above findings, we previously synthesized two mixed-NH_3/amine (amine = cyclopentylamine, cyclohexylamine) platinum(II) complexes featuring a dichloroacetate moiety tethered to the leaving group via an ester bond [18]. They exhibited markedly cytotoxicity

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toward cancer cells by selectively inducing the apoptosis of cancer cells, resulting in the decreased resistance of SK-OV-3 cancer cells to cisplatin. Unfortunately, the two complexes appeared very insoluble in water (≈0.034 mg/ml), a physico-chemical character unfavorable as a drug candidate. In the continuation of our interest to develop more effective platinum anticancer drugs, a series of new platinum complexes, as shown in Fig. 1, were designed based on the same strategy, synthesized and biologically evaluated in the present studies. Four diam(m)ines of clinically approved platinum drugs were used as the carriers and dichloroacetate-containing 3-dichoroacetoxylcyclobutane-1,1-dicarboxylate as the leaving group, with the intention of optimizing the structures and improving the solubility of the resulting complexes.

2. Experimental section

2.1. Materials and instrument

Potassium tetrachloroplatinate(II) and *1R,2R*-diaminocyclohexane were purchased from Alfa Aesar, and *trans*-1.2-bis(methylamine)cyclobutane was kindly provided by Hainan Changan International Pharmaceutical Co., Ltd., China. 3-Dichoroacetoxylcyclobutane-1,1-dicarboxylic acid and (*4R,5R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane were prepared as previously described [18,19]. All other chemicals obtained from commercial suppliers were of analytical grade and used as received. Water was distilled prior to use. Composition analyses for C, H and N were performed with a Carlo-Ebra instrument, whereas the content of platinum was analyzed according to the method in EP6.5. FT-IR spectra were measured in KBr pellets with a Perkin Elmer 880 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in DMSO on Brucker AV-400 MHz relative to TMS (tetramethylsilane) as an external standard. Electrospray ionization mass spectra (ESI-MS) were recorded on Agilent G6230 TOF MS equipped with an electrospray ion source. A Waters Associates system equipped with a 1525 pump, a 717 automated injector, and a Model 2998 photodiode array detector was employed to determine the release of dichloroacetate from the complexes.

2.2. General procedures for the synthesis of complexes 1–4

The synthetic procedures were carried out in light protected environment when platinum complexes were involved. $K_2[PtCl_4]$ (10 g, 28 mmol) was dissolved in water (100 ml) and treated with KI (20.9 g, 126 mmol). After standing for 40 min at room temperature, a solution of NH_3 (34 mmol in 50 ml water) or diamine (28 mmol in 50 ml water) was added dropwise while stirring, yielding the corresponding intermediate *cis*-$[PtA_2I_2]$ ($A_2 = 2NH_3$ or diamine) and the intermediate was filtered off, washed with water and ethanol and dried in vacuo at 55 °C. To a suspension of *cis*-$[PtA_2I_2]$ (6.00 mmol) in 40 ml distilled water, 2.039 g (12.00 mol) $AgNO_3$ in 10 ml distilled water was added, and the reaction mixture was stirred for 24 h at 35 °C. After the precipitated AgI was filtrated off, the resulting filtrate containing *cis*-$[PtA_2(H_2O)_2]^{2+}$ species was cooled down to 10 °C and then mixed with a freshly prepared aqueous solution of potassium 3-dichoroacetoxylcyclobutane-1,1-dicarboxylate (6.6 mmol). A white product precipitated. It was collected immediately by filtration, washed with distilled icy water and ethanol, dried under vacuum at 45 °C. Yield: 45% for complex **1**, 60% for complex **2**, 64% for complex **3**, 55% for complex **4**. The solubility of the resulting complexes in different solvents was determined by AAS (atomic absorption spectroscopy).

Complex **1** found (% calculated for $C_8H_{12}N_2O_6Cl_2Pt$): Pt 38.9 (39.2), C 19.1 (19.3), H 2.45 (2.40) and N 5.57 (5.62). MS-ESI^{+} m/z: 521 ($[M + Na]^{+}$, 19%). IR (KBr, cm^{-1}): 3430 (s, ν_{O-H}), 3281 (s, ν_{N-H}), 2953, 2857 (w, ν_{C-H}), 1749 (s, $\nu_{C=O}$), 1631 (vs, $\nu_{as(COO)}$), 1383 (vs, $\nu_{a(COO)}$), 1175 (m), 1023 (m), 894 (m), 819 (s) and 672 (m). ^{1}H NMR (dmso, δ): 2.10, 2.22 (≈4H, $2CH_2$, C-2, cyclobutane), 4.65 (≈1H, CH, cyclobutane), 5.57 (≈6H, $2NH_3$), and 6.84 (≈1H, $COCHCl_2$). ^{13}C NMR (dmso, δ): 42.1 (C-2, cyclobutane), 48.1 (C-1, cyclobutane), 60.5 (C-4, dichloroacetoxyl), 71.0(C-3, cyclobutane), 164.8 (C = O), 177.3 and 177.6 ($2COO^{-}$).

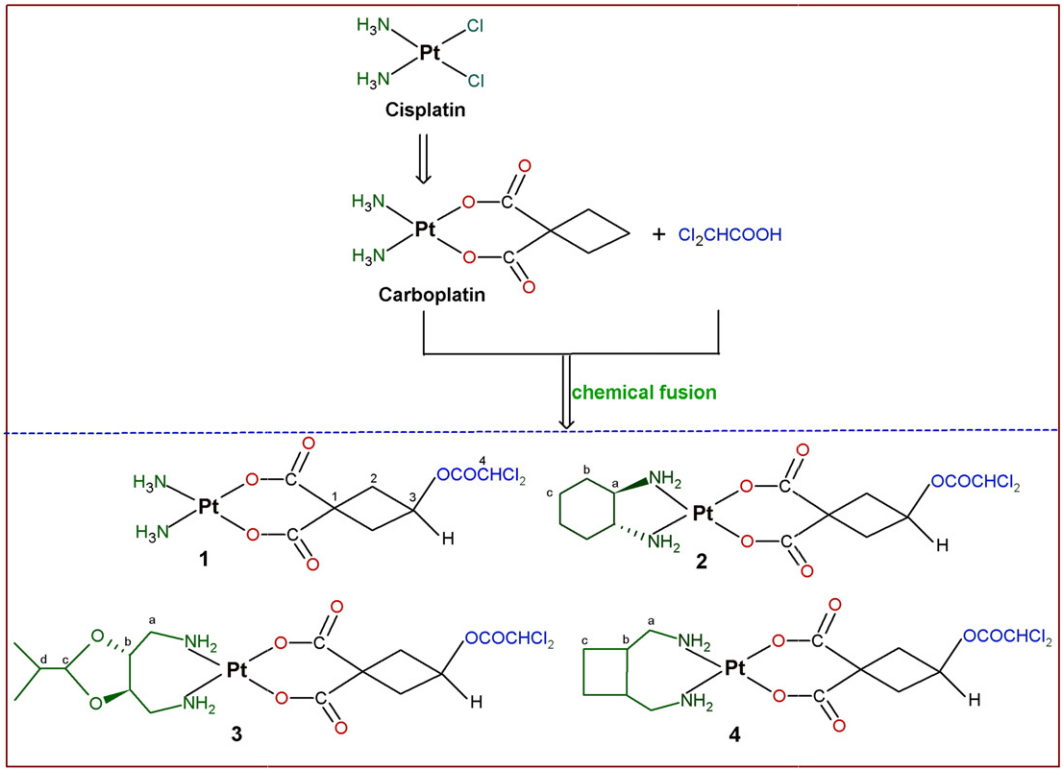

Fig. 1. Chemical structures of the designed platinum complexes.