Journal of Inorganic Biochemistry 175 (2017) 20–28

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Improve the anticancer potency of the platinum(II) complexes through functionalized leaving group

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ARTICLE INFO

Keywords:
Platinum(II) complexes
Functionalized leaving group
Kinetic study
Anticancer

ABSTRACT

Two platinum(II) complexes with 3,3-dimethoxycyclobutane-1,1-dicarboxylate as a leaving group were synthesized and spectrally characterized. In vitro cytotoxicity study on these complexes indicated that complex **2** showed considerable cytotoxicity against the tested cell lines. Notably, the higher antiproliferative activity of complex **2** relative to the corresponding parent compound [Pt(dach)(CBDCA)] demonstrated that the introduction of two methoxy groups in the 1,1-cyclobutanedicarboxylate (CBDCA) can improve the anticancer activity of the resulting platinum(II) complexes. Moreover, cellular accumulations of complexes **1** and **2** were slightly higher than those of their parent compounds carboplatin and Pt(dach)(CBDCA), respectively. Flow cytometry study revealed that complexes **1** and **2** produced death of tumor cells through an apoptotic pathway. Comparison of the chemical reactivity of Pt(dach)(CBDCA) and complex **2** with biologically relevant nucleophiles (L-Met and thiourea) via a kinetic method were studied by UV–Vis technique. The results showed that the reaction rates of complex **2** with nucleophiles were faster than that of Pt(dach)(CBDCA). DFT calculations showed that Pt(dach)(CBDCA) has slightly higher activation energies than complex **2** for the studied reactions. Overall, the introduction of two methoxy groups to the skeleton of 1,1-cyclobutanedicarboxylate can not only change the kinetic reactivity of the resulting platinum(II) complexes, but also enhance their anticancer efficacy.

1. Introduction

Platinum-based drugs as classical chemotherapeutic agents have been widely applied in treating with solid tumors, especially cisplatin, which is considered as the fundamental component of standard chemotherapy [1–3]. However, the severe side effects of cisplatin like nephrotoxicity, ototoxicity and neurotoxicity are inevitable, mainly owing to its high reactivity and limited solubility [4,5]. So carboplatin and oxaliplatin with bidentate dicarboxylates as leaving groups were developed to overcome the drawbacks of cisplatin [6]. Indeed, the application of dicarboxylates as leaving groups in carboplatin and oxaliplatin can remarkably improve the stability and aqueous solubility of platinum(II) complexes, resulting in a relative lower spectrum of side effects [7–11].

Nedaplatin, a carboplatin analogue, has been approved for clinical use in Japan [1,12]. The successful application of nedaplatin was partly due to the substitution of 1,1-cyclobutanedicarboxylate (CBDCA) by glycolate (Fig. 1), which can lead to more labile ring-opening in nedaplatin than in carboplatin towards the attack of nucleophiles, e.g. chloride anion [13]. However, platinum(II) complexes with easily labile leaving groups may hydrolyze so fast to interact with biomolecules like sulfur-containing amino acids in the blood, deactivating the anticancer platinum(II) complexes and inducing the severe side effects [14,15]. Hence, the suitable kinetic property of the antitumor platinum(II) complex is crucial for its anticancer activity, and a small discrepancy in kinetic performance between the platinum(II) complexes may lead to quite different biological activities. So far, most of the previous studies were focused on the impact of the carrier ligand of the platinum(II) complexes including electronic and steric effects [16,17], and few of them were concerned with the influence of the leaving groups. Thus, controlling the kinetic profile of the platinum(II) complex via leaving groups is meaningful and helpful [18–20].

According to our former reports [21–23], carboxylates with functional species such as hydroxyl, ether or ketone groups can be used to improve the anticancer potency of the resulting platinum(II) complexes by adjusting the balance between the lipophilicity and hydrophilicity, as well as the kinetic properties [21]. With the aim to extend our work, we herein report a 1,1-cyclobutanedicarboxylate derivative with two methoxy groups at the 3-position of the cyclobutyl ring as well as the biological activities and kinetic properties of the resulting platinum(II)

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http://dx.doi.org/10.1016/j.jinorgbio.2017.06.016
Received 23 April 2017; Received in revised form 26 June 2017; Accepted 28 June 2017
Available online 02 July 2017
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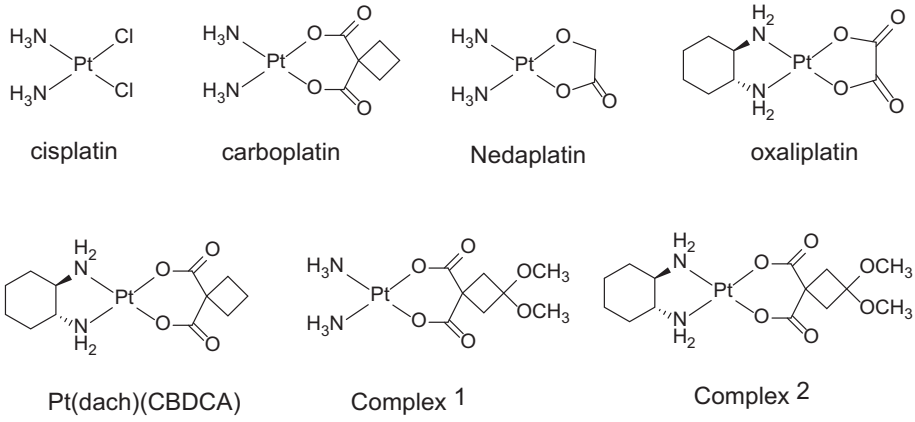


Fig. 1. Chemical structures of the platinum(II) complexes studied in this work.

complexes. In addition, a density functional theory computational analysis has also been used to elucidate the underlying mechanism, which may throw more light on the impact of functional species on the kinetic properties and biological activities of the resulting platinum(II) complexes.

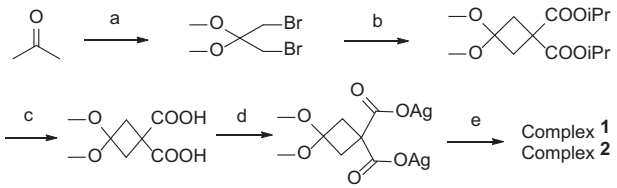
2. Results and discussion

2.1. Synthesis and characterization

Complexes **1** and **2** were prepared by following the procedure shown in Scheme 1. 3,3-Dimethoxycyclobutane-1,1-dicarboxylic acid was synthesized by using the reported method with some modifications and improvements [24]. Interaction of $[Pt(NH_3)_2I_2]$ and $[Pt(dach)Cl_2]$ with silver 3,3-dimethoxycyclobutane-1,1-dicarboxylate generated complexes **1** and **2**, respectively, which was characterized by elemental analysis, ^{1}H, ^{13}C and ^{195}Pt NMR spectra along with ESI-MS spectrometry. The spectral data are in good agreement with the corresponding structure of the platinum(II) complexes. Moreover, X-ray crystallographic data proved that complex **1** has the expected molecular structure (Fig. 2).

2.2. In vitro cytotoxicity

The cytotoxic activities of complexes **1** and **2** against HepG-2 (hepatocellular carcinoma) and A549 (non-small cell lung cancer) cell lines were investigated by MTT assay. Cisplatin, carboplatin, oxaliplatin and Pt(dach)(CBDCA) were used as positive agents. The IC_{50} values (dose required to inhibit 50% cellular growth) were calculated from the plot of cell viability against compound concentrations (Table 1). Based on the IC_{50} values, complexes **1** and **2** showed considerable cytotoxicity against the tested cell lines, especially, complex **2** showed potent in vitro anticancer activity comparable to cisplatin. Notably, comparing Pt(dach)(CBDCA) and complex **2**, both compounds possess similar structures except that the latter has two extra methoxy groups, but the IC_{50} values of Pt(dach)(CBDCA) against the tested cancer cell lines are much higher than those of complex **2**, indicating that the introduction of two methoxy groups in the leaving ligand has an important influence



Scheme 1. Preparation of complexes **1** and **2**: a) Br_2, CH_3OH, rt; b) anhydrous DMF, NaH, Diisopropyl malonate, 140 °C, 48 h; c) NaOH, rt, 12 h; HCl; d) NaOH, $AgNO_3$; e) $[Pt(NH_3)_2I_2]$ or $[Pt(dach)Cl_2]$, 50 °C, 24 h.

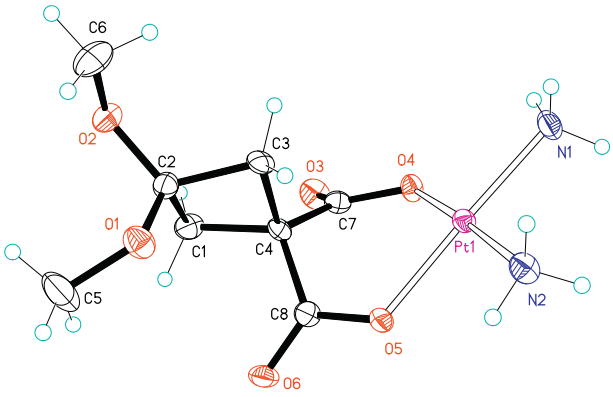


Fig. 2. Molecular structure of complex **1**. Crystal data, selected bond lengths and angles were shown in Table S1 and Table S2.

Table 1
In vitro cytotoxicity (IC_{50} μM) of the platinum(II) complexes against human cancer cell lines.

Compd	IC_{50} (μM)	
	HepG-2[a]	A549[b]
Cisplatin	4.8 ± 0.3	4.9 ± 0.3
Carboplatin	25.6 ± 1.7	28.4 ± 1.6
Oxaliplatin	13.1 ± 0.8	11.3 ± 0.8
Pt(dach)(CBDCA)	17.2 ± 0.6	20.1 ± 0.5
Complex **1**	20.2 ± 0.9	18.7 ± 0.7
Complex **2**	4.2 ± 0.3	6.7 ± 0.4

[a] Human hepatocellular carcinoma cell line.
[b] Human non-small-cell lung cancer cell line.

on the anticancer activity of complex **2**. A similar picture was observed by comparing complex **1** and carboplatin, demonstrating that the introduction of two methoxy groups in the leaving ligand can improve the anticancer activity of the resulting platinum(II) complexes. The reason for the different cytotoxicity of complex **2** and Pt(dach)(CBDCA) may be ascribed to the nature of the leaving ligands, which may affect the balance between hydrophilicity and lipophilicity of the resulting platinum(II) complexes as well as the kinetic properties of the complexes.

2.3. Cellular uptake

To compare the cellular accumulations of complexes **1-2** and their parent Pt complexes carboplatin and Pt(dach)(CBDCA) in HepG-2 cells, the cellular levels of Pt were examined by using the inductively coupled plasma mass spectrometry (ICP-MS). As shown in Table 2, the Pt accumulations of complex **1** (240 ± 23 ng/10^6 cells) and complex **2** (487 ± 28 ng/10^6 cells) in HepG-2 cells were slightly higher than