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Synthesis and anticancer activity of diam(m)ine platinum(II) complexes with 3-oxo-cyclobutane-1,1-dicarboxylate as the leaving group

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Abstract Four water-soluble dia(m)mine platinum complexes with 3-oxo-cyclobutane- 1,1-dicarboxylate as the leaving group have been synthesized. These compounds were evaluated for their in vitro anticancer activity against three human A549, SK-OV-3, and HT-29 cancer cell lines. All the tested compounds showed potent activity in lung carcinoma (A549), ovarian carcinoma (SK-OV-3), and colon cancer cell (HT-29) lines. Complex **2**(IC_{50} value = 1.57 μM for A549 and IC_{50} value = 0.88 μM for HT-29 cells) and Complex **4** (IC_{50} value = 15.3 μM for SK-OV-3 cells) displayed the most potent anticancer activity of the series. Also, preliminary acute toxicity of the platinum complexes derivatives has shown the same level as carboplatin and lower toxicity than cisplatin in vivo. All the complexes were characterized by elemental analysis as well as by ESI^+-MS, FT-IR, ^{1}H- and ^{13}C-NMR, and have shown a satisfactory water solubility.

Keywords Synthesis · Platinum complexes · Anticancer activity · 3-Oxo-cyclobutane-1,1-dicarboxylate

Introduction

Malignant tumors have become one of the most common and serious diseases worldwide, 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

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classes of anticancer 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 (see Fig. 1) include cisplatin (DDP), carboplatin and oxaliplatin, nedaplatin, lobaplatin and heptaplatin, 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 and by drug resistance [6–8], highlighting the need for the development of new platinum anticancer drugs.

The past decade has witnessed a shift in focus toward nonclassical platinum compounds represented by picoplatin, polynuclear complexes, trans-platinum complexes, and Pt(IV) complexes [9]. Unfortunately, the outcomes of clinical trials of these complexes remained below expectations and none of these complexes has been approved for clinical application [10]. However, direct modification of the clinically established platinum drugs is still an effective way to find new derivatives exhibiting an improved toxicological profile [11, 12].

It has been demonstrated that cyclobutane-1,1-dicarboxylate and its 3-substituted-derivatives [11–16] are good leaving groups, which endow the platinum anticancer complexes not only with water solubility but also with an improved toxicological profile.

Based on the above findings, we successfully prepared a new derivative of cyclobutane-1,1-dicarboxylate, 3-oxo-cyclobutane-1,1-dicarboxylate and used it as the leaving group, resulting in four dia(m)mine platinum complexes (see Fig. 2). They were complex 1, complex 2 [17], complex 3, complex 4. Herein we report synthesis and in vitro anticancer activity.

Experimental section

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

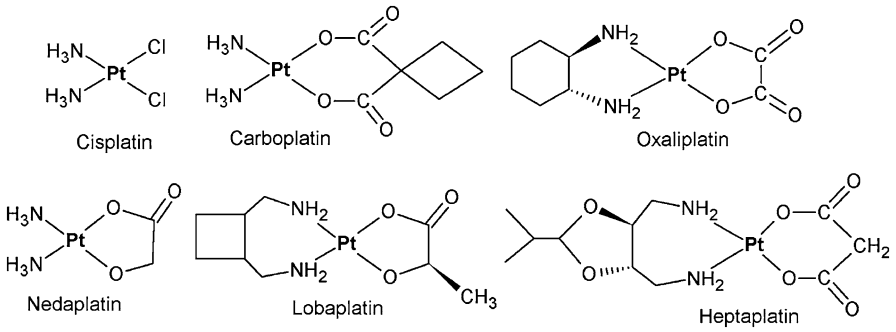


Fig. 1 Platinum-based drugs currently in clinical use

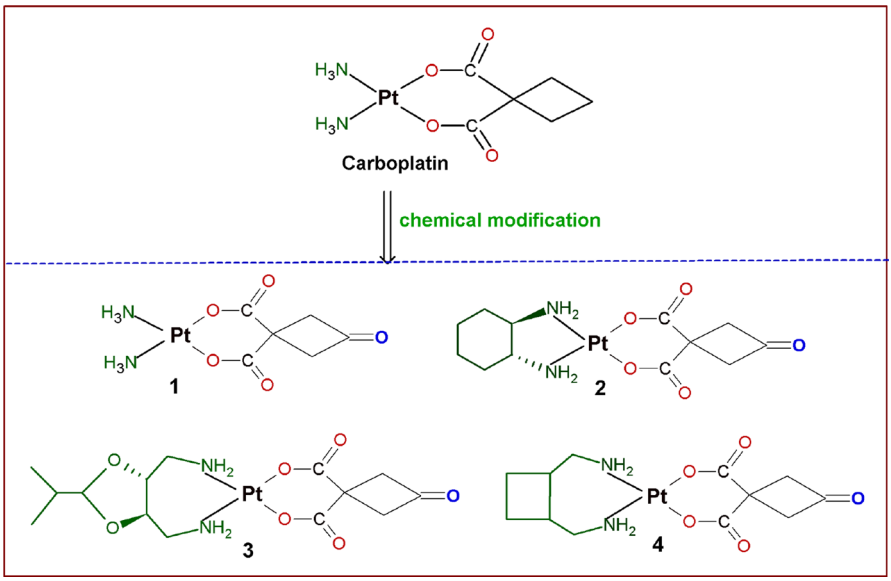


Fig. 2 Chemical structures of designed platinum complexes

by Hainan Changan International Pharmaceutical Co., Ltd., China. 3-Dichoro-acetoxyl-cyclobutane-1,1-dicarboxylic acid and (*4R,5R*)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane were prepared as previously described. 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-d_6 or $CDCl_3$ on Bruker AV-400 MHz relative to TMS (tetramethylsilane) as an external standard at room temperature. Electrospray ionization mass spectra (ESI–MS) were recorded on an Agilent G6230 TOF MS equipped with an electrospray ion source type.

The preparation of 3-oxo-cyclobutane-1,1-dicarboxylic acid

Synthesis of {[(1-bromo-3-chloropropan-2-yl)oxy]methyl}benzene

To a stirred mixture of benzyl bromide (129 g, 0.756 mol) and 0.8 g of mercuric chloride (notice: highly toxic product) was added epichlorohydrin (73.3 g, 0.792 mol). The reaction mixture was heated to 150 °C. The reaction was monitored by TLC. The product was collected under reduced pressure at 108–110 °C/(128 g, 63.5 % yield). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.58 (t, $J = 4.44$ Hz, 2H), 3.72 (d, $J = 5.2$ Hz, 2H), 3.80–3.86 (m, 1H), 4.68 (d, $J = 2.8$ Hz, 1H), 7.38–7.40 (m, 5H).

Synthesis of diethyl 3-(benzyloxy)cyclobutane-1,1-dicarboxylate

To a stirred suspension of anhydrous potassium carbonate (70 g, 0.5 mol) in 200 ml of dry DMF, was added diethyl malonate (64 g, 0.4 mol). Then the {[(1-bromo-3-chloropropan-2-yl)oxy]methyl}benzene (53 g, 0.2 mol) was added dropwise over 20 min. The mixture was heated to 50 °C, and GC analysis confirmed formation of product. After cooling to room temperature, 100 ml of toluene and 50 ml of water were added into the suspension, the product was extracted with toluene (2 × 50 ml), and then the organic layer was washed with brine (2 × 50 ml), dried over Na_2SO_4, and concentrated in vacuo to remove the diethyl malonate. Then to a stirred ethanol (100 ml) of sodium ethoxylate (0.22 mol) was added the residue (65 g). The reaction mixture was refluxed. GC analysis confirmed formation of product. The ethanol was removed under reduced pressure. Then, 100 ml of toluene and 50 ml of water were added into the suspension. The product was extracted with toluene (3 × 50 ml), then the organic layer was washed with brine (2 × 50 ml), dried over Na_2SO_4, and the extract was concentrated in vacuo to give product (53.5 g). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.23 (t, $J = 7$ Hz, 6H),2.52–2.58, 2.77–2.82 (2 m, 4H), 4.14–4.21 (m, 5H), 4.41 (s, 1H), 7.26–7.36 (m, 5H).

Synthesis of diethyl 3-hydroxycyclobutane-1,1-dicarboxylate

Compound diethyl 3-benzyloxy-cyclobutane-1,1-dicarboxylate(2 g) was dissolved in EtOH (100 ml), and 10 % Pd/C(400 mg, 20 % eq.) was added. The suspension was hydrogenated at room temperature overnight. The product was evaporated under reduced pressure (1.3 g, 91.5 % yield). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 2.44–2.49, 2.85–2.90 (2 m, 4H), 4.35–4.37 (m, 1H), 4.21 (q, $J = 7.2$ Hz, 4H), 1.26 (t, $J = 10.8$ Hz, 6H).

Synthesis of diethyl 3-oxo-cyclobutane-1,1-dicarboxylate

To a suspension of pyridinium chlorochromate (1.94 g, 9.02 mmol) in CH_2Cl_2 (60 ml) was added diethyl 3-hydroxycyclobutane-1,1-dicarboxylate (1.5 g, 6.94 mmol), whereupon the mixture became a dark homogeneous solution. The reaction was stirred overnight. Diethyl ether (3 × 300 ml) was added, and the product was passed through a short column of Florisil to remove the chromium salts. Removal of the solvent in vacuo to yield an oil that was further purified by flash chromatography (petroleum ether/ethyl acetate 3:1) to give diethyl 3-oxo-cyclobutane-1,1-dicarboxylate as an oil (1.262 g, 85.6 % yield). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.63 (s, 4H), 4.30 (q, $J = 6.8$ Hz, 4H), 1.31 (t, $J = 7.2$ Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 13.8 (2C), 35.0 (2C), 56.4, 62.8, 71.0, 168.0 (2C), 173.2. IR (KBr, cm^{-1}): 3,744, 3,646, 3,563, 3,472, 2,987, 1,794, 1,738, 1,467, 1,298, 1,266, 1,093, 1,030, 680, 538, 452.

Synthesis of 3-oxo-cyclobutane-1,1-dicarboxylic acid

To the solution of NaOH (0.949 g, 23.73 mmol) in water (20 ml) were added dropwise the solution of diethyl 3-oxo-cyclobutane-1,1-dicarboxylate (1.953 g, 9.12 mmol) in

MeOH (20 ml) over 10 min. The reaction mixture was stirred at 50 °C. TLC analysis confirmed formation of product. The methanol was distilled under reduced pressure. The aqueous layer was extracted with 2 × 25 ml of ethyl acetate to remove organic impurities. Then the aqueous phase was adjusted to pH = 1 with diluted hydrochloric acid and extracted with ethyl acetate (3 × 30 ml). The combined organic extracts were washed with brine (3 × 30 ml), dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was recrystallized from water (5 ml) to give 3-oxo-1,1-cyclobutane dicarboxylic acid (1.1 g, 76.29 % yield) as a pale yellow solid. Mp: 142–146 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.75 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 33.4 (2C), 48.3, 170.4(2C), 172.6. IR (KBr, cm^{-1}): 3,943, 3,865, 3,742, 3,102, 2,961, 1,731, 1,411, 1,285, 1,189, 861, 446. MS–ESI$^+$ *m/z*: 157([M–H]$^-$, 100 %).

General procedures for the synthesis of complexes ***1–4***

The synthetic procedures in the presence of platinum complexes were carried out in a light-tight environment. $K_2[PtCl_4]$ (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 filtrated 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 150 ml of distilled water, freshly prepared disilver 3-oxo-cyclobutane-1,1-dicarboxylate (12 mmol) was added and mixed for 36 h with stirring at 37 °C. After AgI was filtrated off, the solution was concentrated at 45 °C in vacuo to 20 ml to give a white crystalline product. It was collected, washed successively with icy water and ethanol, and dried under reduced pressure at 45 °C.

Complex 1 ^{1}H NMR (400 MHz, DMSO-d_6, δ): 3.75 (s, 4H), 4.22 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 46.5 (2C), 55.9, 175.7 (2C), 205.8. IR (KBr, cm^{-1}): 3,291 (s, $\nu_{N–H}$), 1,782 (s, $\nu_{C=O}$), 1,630 (vs, $\nu_{as\ (COO)}$), 1,368 (vs, $\nu_{a(COO)}$), 454 (w, $\nu_{Pt–N}$). MS–ESI$^+$ *m/z*: 408 ([M + Na]$^+$, 100 %). Anal. calcd for $C_6H_{10}N_2O_5Pt$: C, 18.7; H, 2.60; N, 7.27; Pt, 50.7 %. Found C, 18.5; H, 2.62; N, 7.28; Pt, 50.4 %.

Complex 2 ^{1}H NMR (400 MHz, DMSO-d_6, δ): 0.93, 1.20 (m, 4H, cyclohexane), 1.44, 1.78 (m, 4H, cyclohexane), 2.03 (2H, cyclohexane), 3.71 (4H, cyclobutane), 5.27, 5.97 (4H, $2NH_3$). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 24.1(2C), 31.5(2C), 46.4(2C), 55.9(2C), 62.1, 175.6(2C), 206.6. IR (KBr, cm^{-1}): 3,229 (s, $\nu_{N–H}$), 2,941(w, $\nu_{C–H}$), 1,797 (s, $\nu_{C=O}$), 1,635 (vs, $\nu_{as\ (COO)}$), 1,363 (vs, $\nu_{a(COO)}$), 454 (w, ν_{Pt-N}). MS–ESI$^+$ *m/z*: 488 ([M + Na]$^+$, 100 %). Anal. calcd for $C_{12}H_{18}N_2O_5Pt$: C, 31.0; H, 3.87; N, 6.02; Pt, 41.9. Found C, 31.3; H, 3.91; N, 6.08; Pt, 41.4 %.

Complex 3 ^{13}C NMR (100 MHz, DMSO-d_6, δ): 16.6, 16.7, 31.4, 46.5, 47.9, 48.2, 55.6, 56.2, 77.9, 79.6, 107.0, 175.8(2C), 206. IR (KBr, cm^{-1}): 3,253(s, $\nu_{N–H}$), 2,967(w, $\nu_{C–H}$), 1,783(s, $\nu_{C=O}$), 1,628 (vs, $\nu_{as\ (COO)}$), 1,383 (vs, $\nu_{a(COO)}$), 456 (w, $\nu_{Pt–N}$). **MS–ESI$^+$** *m/z*: 548.0967 ([M + Na]$^+$, 42 %). Anal. calcd for $C_{14}H_{22}N_2O_7Pt$: C, 32.0; H, 4.19; N, 5.33; Pt, 37.2. Found C, 30.8; H, 3.90; N, 6.00; Pt, 41.6 %.

Complex 4 ^{13}C NMR (100 MHz, DMSO-d_6, δ): 22.4 (2C), 46.6 (2C), 47.8, 50.4 (2C), 56.0 (2C), 175.9 (2C), 205.7. IR (KBr, cm^{-1}): 3,237 (s, $\nu_{N\text{-}H}$), 2,940(w, $\nu_{C\text{-}H}$), 1,785 (s, $\nu_{C=O}$), 1,624 (vs, $\nu_{as(COO)}$), 1,372 (vs, $\nu_{a(COO)}$), 454 (w, $\nu_{Pt\text{-}N}$). MS–ESI$^+$ *m/z*: 488 ([M + Na]$^+$, 60 %). Anal. calcd for $C_{12}H_{18}N_2O_5Pt$): C, 31.0; H, 3.87; N, 6.02; Pt, 41.9. Found C, 30.8; H, 3.90; N, 6.00; Pt, 41.6 %.

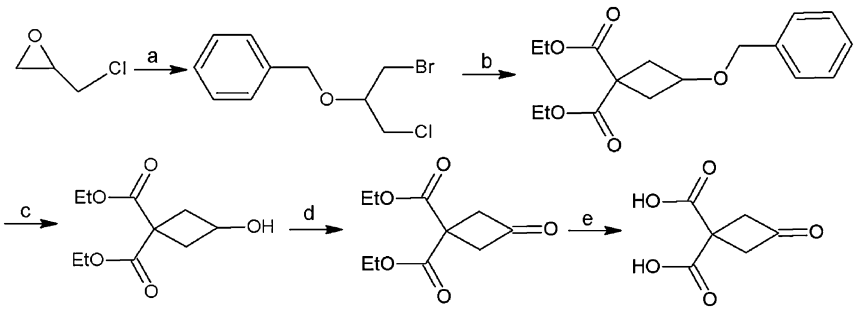
In vitro anticancer activity

Human cancer cell lines A549, SK-OV-3, HT-29 were purchased from the American Type Culture Collection (Manassas, VA, USA), Cells were grown in DMEM or RPMI-1640 medium containing 10 % fetal bovine serum and supplemented with 100 U/ml of penicillin and 100 μg/ml of streptomycin. Cells were maintained at 37 °C in a humidified incubator with an atmosphere of 5 % CO_2.

Cytotoxicity was determined by MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H- tetrazolium bromide] assay. The tested compounds were dissolved in water and diluted in culture media at the indicated concentrations. A 100-μl cell suspension was seeded in 96-well cell culture plates and allowed to adhere overnight. The cells were treated with drugs for 72 h, and then 20 μl of CellTiter 96 AQ_{ueous} One Solution Reagent (Promega, Madison, WI, USA) was added and the cells were further incubated at 37 °C for 1–2 h. Cell viability was measured by reading the absorbance at a wavelength of 490 nm. Concentrations of 50 % inhibition of growth (IC_{50}) were calculated on the basis of the relative survival curve.

Results and discussion

As the leaving group, 3-oxo-1,1-cyclobutanedicarboxylic acid was prepared according to Scheme 1 with epichlorohydrin as the starting material. Treatment of epichlorohydrin with benzyl bromide in the presence of $HgCl_2$ gave benzylated alcohol in 63.5 % yield, which condensed with diethylmalonate in alkaline condition yielded cyclobutyl derivative. Subsequently, the benzyl ether was



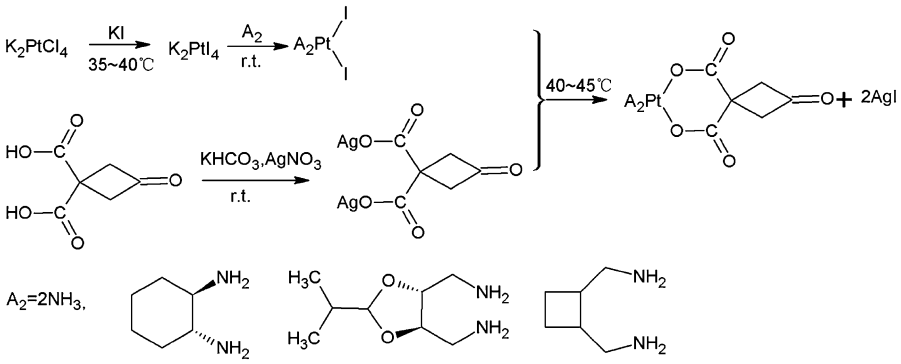
Scheme 1 Preparation of 3-oxocyclobutane-1,1-dicarboxylic acid. **a** $PhCH_2Br$, $HgCl_2$,160 °C; **b** $CH_2(COOC_2H_5)_2$, DMF, K_2CO_3, NaOEt, N_2, reflux; **c** Pd/C, H_2; **d** PCC; **e** NaOH, 50 °C

hydrogenated using Pd/C as catalyst and the resulting alcohol was further oxidized to ketone using PCC as an oxidant, and then the 3-oxo-1,1-cyclobutanedicarboxylic acid was obtained from corresponding ester hydrolysis. The product was confirmed by ESI^+-MS, FT-IR, ^1H-, and ^{13}C-NMR (See Supplementary Fig.S1–S3).

Four designed complexes **1**–**4** with $2NH_3$ or diamines as the carriers were synthesized as white precipitates in aqueous solution by the general procedures (see Scheme 2) owing to their great water solubility. Shortly, K_2PtCl_4 was first converted to K_2PtI_4 in situ by the treatment with KI, followed by addition of $2NH_3$ or diamines, forming an insoluble diam(m)inediiodoplatinum(II) intermediate. The quantitative reaction of the intermediate with silver 3-oxo-1,1-cyclobutanedicarboxylate in water offered a solution of desired complexes after AgI formed was filtrated out. The solution was condensed at 45 °C under reduced pressure to 20 ml to precipitate a white crystalline product. The resulting platinum complexes were characterized by elemental analysis as well as by ESI^+-MS, FT-IR, ^1H-, and ^{13}C-NMR (Supplementary Fig.S4-S21), and gave satisfactory analytical and spectroscopic data, which are in accordance with their proposed structures (Fig. 2). The introduction of the oxo group is verified both by the development of a strong C=O vibration band approximately at 1,780 cm^{-1} and by appearance of a distinct carbon resonance at 204 ppm belonging to C=O.

The solubility of four complexes in water was found by AAS (atomic absorption spectroscopy) to be 19, 5, 8, 100 mg/ml at room temperature, respectively, for complex **1**, **2, 3**, and **4**, soluble enough as a compound candidate. No close relationship for these complexes could be deducted between the structure and solubility.

In vitro anticancer activity of the newly synthesized complexes **1**–**4** was determined by means of MTT assay in the present work against three human cancer cell lines representing three tumor entities: lung carcinoma (A549), ovarian carcinoma (SK-OV-3) and colon cancer cell (HT-29) in comparison to carboplatin and oxaliplatin. The IC_{50} values, defined as the concentrations corresponding to 50 % growth inhibition, are presented in Table 1. Four complexes yielded IC_{50} values in the micro-molar range, displaying considerably anticancer activity.



Scheme 2 Synthesis of four platinum(II) complexes

Table 1 In vitro anticancer activity of tested compounds ($t = 72$ h, $n = 3$)

Compounds	IC_{50} (mean ± SD, μM)		
	A549	SK-OV-3	HT-29
Complex **1**	18.0 ± 2.52	20.7 ± 3.03	19.0 ± 2.86
Complex **2**	1.57 ± 0.18	36.5 ± 4.92	0.88 ± 0.12
Complex **3**	17.0 ± 1.80	18.9 ± 1.95	4.50 ± 0.57
Complex **4**	7.39 ± 0.81	15.3 ± 1.47	4.68 ± 0.50
Carboplatin	>100	84.1 ± 10.8	84.6 ± 7.57
Oxaliplatin	3.28 ± 0.28	45.3 ± 4.11	1.55 ± 0.17

Among them, complex **2** is the most potent activity against A549 cells and HT-29 cells, even more active than oxaliplatin. The overall activity order are complex **2**> oxaliplatin> **4**> **3**> **1**> carboplatin for A549 cells and complex 2> oxaliplatin> 3> 4> 1> carboplatin for HT-29 cells. Meanwhile, complex 4 showed the best activity against SK-OV-3 cells with the potency of the complexes follow the order complex 4> 3> 1> 2> oxaliplatin> carboplatin. These results reveal that for the complexes of 3-oxo-1,1-cyclobutanedicarboxylate as the leaving group, the carriers have also an obvious influence on anticancer activity.

Conclusions

In this study, four diam(m)ine complexes have been synthesized and evaluated for their anticancer activity against human A549 and SK-OV-3 and HT-29 cancer cell lines. All these derivatives have satisfactory water solubility and were active against human cancer cells. Preliminary acute toxicity test of complex 3 and 4 have shown lower toxicity than cisplatin and the same level acute toxicity as carboplatin in vivo. 3-oxo-cyclobutane-1,1-dicarboxylate as a new leaving group can offer platinum complexes good water solubility and greater in vitro anticancer activity. In vitro anticancer activity of the compounds showed that complex **2** was more active than oxaliplatin and carboplatin. It suggested that complex **2** and 4 can be used further in vivo evaluation of these complexes as an anticancer agent's candidate. Further investigation into the pharmacological activity of these compounds is underway.

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