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Anticancer Potency of Platinum(II) Complexes Containing Both Chloride Anion and Chelated Carboxylate as Leaving Groups

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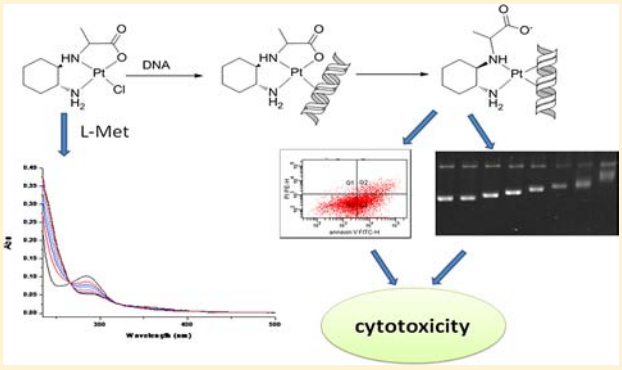
Supporting Information

ABSTRACT: Three platinum complexes with both a chloride anion and a chelated carboxylate as leaving groups were synthesized and spectrally characterized. In vitro cytotoxicity of complexes **1**−**3** was evaluated against human A549, HCT-116, MCF-7, and HepG-2 tumor cell lines. The results showed that all the compounds exhibited effective cytotoxicity against the tested cell lines, nearly comparable to those of cisplatin and oxaliplatin. Notably, the activity of complex **2** was about 2-fold better than that of oxaliplatin against the HCT-116 cell line. Flow cytometry analysis indicated that these complexes produced death of tumor cells through an apoptotic pathway. The DNA-binding properties of the platinum-based compounds were also studied by agarose gel electrophoresis. The kinetics study showed that the chloride anion departs from the Pt atom quickly, whereas the five and/or six-membered ring formed by coordination of N,O-donors and the metal ion is opened a little more slowly by the rupture of a Pt−O bond, which helps us to further understand the mechanism of action of the newly synthesized complexes with biomolecules. Furthermore, the reaction rate constants of complexes **1**−**3** were roughly the same.



INTRODUCTION

Cisplatin, one of the most effective anticancer drugs known, was first discovered by Rosenberg and his co-workers in the 1960s.[1] The successful application of cisplatin in clinical use has stimulated many researchers to search for more effective and less toxic platinum-based anticancer agents. The second- and third-generation platinum drugs, carboplatin and oxaliplatin, were successfully designed by replacing the leaving groups of chloride anions in cisplatin with 1,1-cyclobutanedicarboxylate and oxalate, respectively, effectively decreasing the side effects of cisplatin.[2] The chloride and carboxylate anions have different advantages as leaving groups. For example, chloride ions in cisplatin depart from the Pt atom more easily than do carboxylate ions, which can increase the DNA-binding ability of the Pt ion and improve the anticancer efficacy accordingly. The application of dicarboxylate as leaving groups in carboplatin or oxaliplatin can improve the aqueous solubility and stability of the platinum(II) complexes remarkably as compared with those of their corresponding chloride analogues.[3] Numerous anticancer platinum(II) complexes with different leaving groups have been designed and synthesized so far;[4] however, few of them bearing both a chloride anion and a carboxylate as leaving groups in a complex molecule have been reported.[5]

Oxaliplatin bearing 1*R*,2*R*-diaminocyclohexane (DACH) as a carrier ligand has been widely used in the chemotherapeutic regimen for the treatment of colorectal tumors.[6] The successful application of oxaliplatin was partly due to the skeleton of 1*R*,2*R*-diaminocyclohexane, which could form 1,2-{Pt-(DACH)}$^{2+}$-d(GpG) adducts to inhibit the replication of DNA.[7] On the basis of the above discussion, we attempted to design DACH derivatives with a carboxylic substituent at one of the nitrogen atoms to produce novel N,N,O-tridentate donors to chelate platinum(II) ions together with chloride ions. It is expected that the formation of five- and/or six-membered rings by the coordination of N,O-donors with a Pt atom may effectively reduce the interaction between the resulting platinum(II) complexes and sulfur-containing molecules, which may decrease the side effects and improve the anticancer effects of the platinum(II) complexes.

In this context, complexes **1**−**3** with both chelated carboxylate and chloride anions as leaving groups, shown in Figure 1, have been prepared for several purposes. First, the application of chloride anions as leaving groups may promote DNA-binding abilities of the newly synthesized complexes. Second, the carboxylate anions can improve the water solubility of the complexes, and the chelating rings formed by the N,O-donors and Pt atoms can increase the stability of the complexes.[8] According to our assumption, when these platinum(II) complexes enter the tumor cells, the chloride ion would leave the compounds fast because of the trans effect

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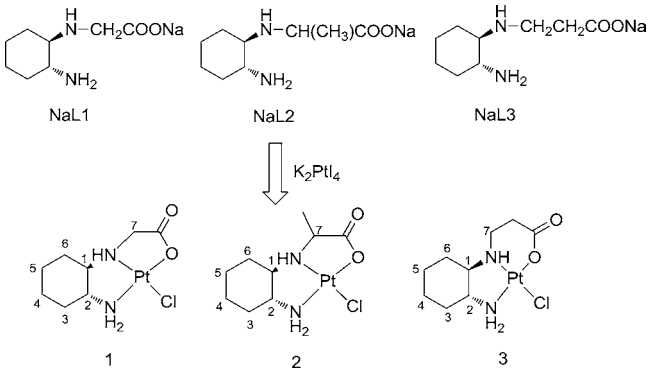

Figure 1. Chemical structures of the related ligands and platinum(II) complexes.

and the low concentration of chloride ion in the cells, and then the Pt ion could bind with the guanine of DNA easily. Meanwhile, the carboxylate anion would separate from the metal atom, which gives additional space for the interaction with DNA.

RESULTS AND DISCUSSION

Synthesis and Characterization. Ligands HL1−HL3 and their sodium salts (NaL1−NaL3) were synthesized according to our previous reports,[9] and complexes **1**−**3** were fully characterized by elemental analysis and IR, ^{1}H NMR, and ESI-MS spectra. Elemental analysis for each compound was in good agreement with the empirical formula proposed. In the IR spectra, the shifts of the C═O absorption from free carboxylic acid values of ∼1700 cm^{-1} to a 1642−1616 cm^{-1} band proved that the carboxylate anion was coordinated to a Pt ion in each case. All platinum compounds showed a peak of $[M + Na]^+$ or $[M + H]^+$ in their positive ESI mass spectra, which are in agreement with their molecular formula mass. For three isotopes of Pt [^{194}Pt(33%), ^{195}Pt(34%), and ^{196}Pt(25%)], all the mass spectra of platinum compounds were found to have three protonated ion isotopic peaks. The newly synthesized compounds were also proved by ^{1}H NMR spectra.

It is worth noting that a new stereogenic nitrogen center was formed in the prepared platinum(II) complexes. As described previously,[10] the chiral nitrogen atom of the ligand adopted an *S* configuration after coordination, which was further confirmed by recent crystal structure analysis of a platinum complex with a similar ligand.[11] Moreover, an asymmetric carbon atom (C7) was also produced in the preparation of ligand NaL2 and its complex **2**. However, no effort was made to isolate the pure enantiomers of complex **2**.

The solubilities of complexes **1**−**3** in water at 25 °C are 6.2, 3.1, and 4.6 mg/mL, respectively (Table 1), which are obviously higher than that of cisplatin (1 mg/mL), as expected, but lower than that of oxaliplatin (8 mg/mL). Optical rotations of the resulting platinum(II) complexes were tested by an automatic digital polarimeter. All compounds turned out to be optically active with values of specific rotations from +74.2° to +79.8° (Table 1), similar to that of oxaliplatin (+76.1°).

Table 1. Aqueous Solubility and Specific Rotations of Complexes 1−3

complex	aqueous solubility (mg/mL, 25 °C)	$[\alpha]_D^{25}$ (c = 1, MeOH)
1	6.2	+74.2
2	3.1	+79.8
3	4.6	+76.9

In Vitro Cytotoxicity Assay. The cytotoxicity of complexes **1**−**3** against the A549 (non-small cell lung cancer), MCF-7 (breast carcinoma), HepG-2 (hepatocellular carcinoma), and HCT-116 (colorectal cancer) cell lines was tested by MTT assay, and cisplatin, carboplatin, and oxaliplatin were used as positive agents. According to the IC_{50} values summarized in Table 2, all the complexes showed cytotoxicity superior to that of carboplatin and a little lower than that of cisplatin, with complex **2** being more potent than complex **1** or **3** against all tested cell lines. As for HCT-116 cell lines, complexes **1**−**3** showed cytotoxicity comparable to that of oxaliplatin, which is the first platinum-based complex effective in the treatment of colorectal cancer. It is indicated that HCT-116 is the most sensitive cell line to the newly synthesized complexes, which is probably due to the application of the DACH skeleton in these complexes. Notably, complex **2** was 2-fold more potent than oxaliplatin against the HCT-116 cell line, making it worth further study against the colorectal cancer cell line.

Table 2. In Vitro Cytotoxicity of Complexes 1−3 and Positive Agents against Human Tumor Cell Lines

	IC_{50} (μM)[a]			
compound	A549[b]	MCF-7[c]	HepG-2[d]	HCT-116[e]
1	18 ± 1	7.3 ± 0.5	4.6 ± 0.3	6.3 ± 0.2
2	5.3 ± 0.3	4.1 ± 0.3	2.3 ± 0.1	2.1 ± 0.1
3	8.7 ± 0.1	6.1 ± 0.4	3.6 ± 0.3	6.8 ± 0.2
carboplatin	12 ± 1	15 ± 1	9.7 ± 0.7	nd[f]
cisplatin	4.3 ± 0.2	3.6 ± 0.2	0.6 ± 0.02	nd
oxaliplatin	nd	nd	nd	4.3 ± 0.1

[a]Values represent the mean ± SD from three independent experiments; IC_{50} defined as the drug concentration required to inhibit 50% of cell growth as determined by MTT assay after a 48 h exposure (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). [b]Human non-small cell lung cancer cell line. [c]Human breast carcinoma cell line. [d]Human hepatocellular carcinoma cell line. [e]Human colorectal cancer cell line. [f]Not determined.

It is noted that complexes **1** and **2** possess similar structures except for the branched methyl group in the latter; however, the cytotoxicity of complex **2** is much better than that of complex **1** against all tested cell lines. It appears that the branched methyl group in the ligand ($L2^-$) plays an essential role in the anticancer activity of complex **2**.

Apoptosis Study. Apoptosis is a programmed cell death in multicellular organisms, which generally confers advantages during an organism's life cycle. Thus, the apoptotic analysis of complexes **1** and **2** against MCF-7 and HCT-116 was performed using an Annexin V-FITC/PI assay. The tested compounds were incubated with tumor cells (MCF-7 or HCT-116) for 24 h at 50 μM. Q1−Q4 are representatives of the unnatural death cells, necrotic cells, normal cells, and early apoptotic cells, respectively.

As shown in Figures 2 and 3, complexes **1** and **2** and carboplatin markedly improve the apoptotic rate of the MCF-7 cells as compared with that of the untreated cells (control). Moreover, the effects on tumor apoptosis shown for both complexes **1** and **2** are stronger than those shown for carboplatin, with apoptotic populations of 25.7% and 38.1%, respectively. With respect to HCT-116 cells, we can see that the apoptosis rate of negative control is very low (8.5%), while

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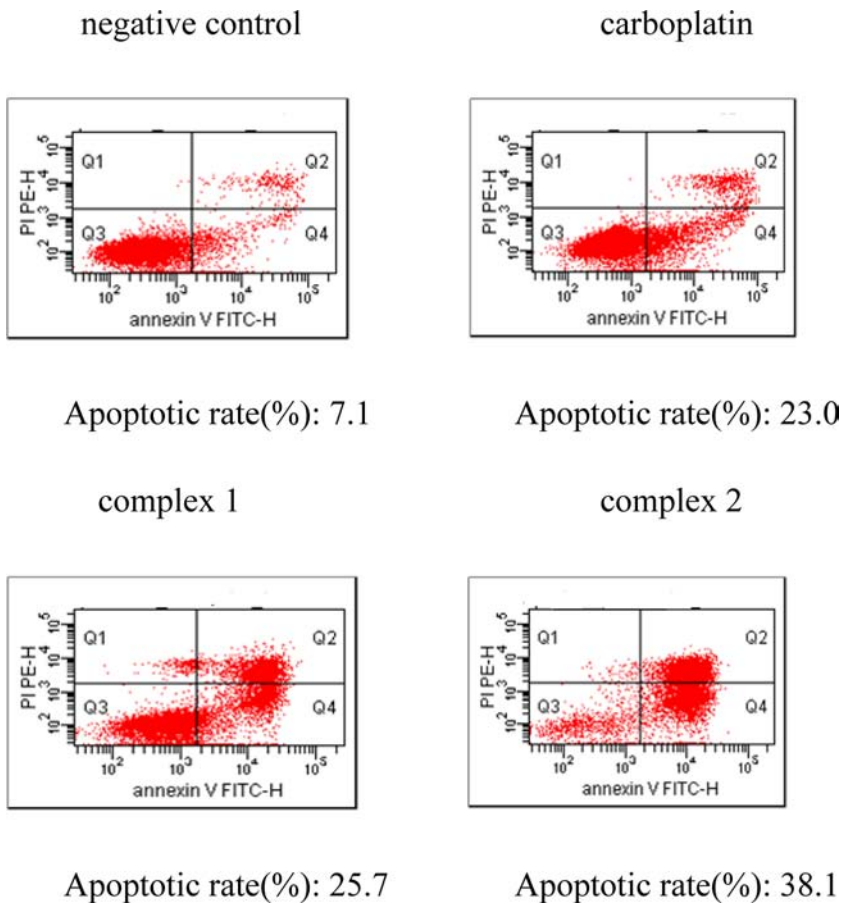

Figure 2. Flow cytometric analysis of the distribution of MCF-7 cells treated with complexes **1** and **2** and carboplatin at 50 μM.

complexes **1** and **2** and oxaliplatin apparently inhibited tumor cell growth by inducing apoptosis. Notably, the relative order of inducing apoptosis against HCT-116 cells is **2** (41.2%) > oxaliplatin (35.1%) > **1** (17.9%), which is consistent with the results of in vitro cytotoxicity assays, suggesting that the anticancer efficacy of complex **2** was higher than those of complex **1** and oxaliplatin. Overall, it can be concluded that these complexes produce tumor cell death through an apoptotic pathway, and the results of the apoptosis study were in line with the cytotoxicity data to some extent.

Interaction with pET28a Plasmid DNA. The DNA-binding ability of complexes **1** and **2** and cisplatin was examined by gel mobility electrophoresis, with pET28a plasmid DNA as the target. Each compound was incubated with DNA at different concentrations. At first, gels were dyed with ethidium bromide before being run. In the electrophoretogram (Figure 4), untreated pET28a plasmid DNA, which mainly consisted of covalently closed circular (form I) and a small amount of nicked (form II) bands, was used as negative control. When pET28a plasmid DNA was incubated with cisplatin increasing from 10 to 160 μM, an increase in the mobility of form I DNA was observed, probably because of the fact that the gel was run in the presence of ethidium bromide, and this intercalator in the gel ensured that the closed circular DNA is wound into a net positive superhelix. The result is consistent with previous literature reported by Lippard.[12] Besides, the intensity of the form I band (compared with that in the untreated DNA) decreased with the increase in the concentration of cisplatin. This can partially be attributed to the unwinding of supercoiled DNA to open circular DNA, demonstrating the binding between DNA and the platinum complexes. For complexes **1** and **3**, no migration of the form I and II bands of plasmid DNA was observed at the concentration range tested. As for complex **2**, the mobility of form I increased in the same pattern as that of cisplatin (Figure 4). Moreover, the decrease in intensity of the form I band of complex **2** was similar to that of cisplatin with the increased concentrations.

With the purpose of observing the unwinding of the supercoiled DNA induced by complexes **1** and **3**, high concentrations (320 and 640 μM) of the platinum(II) compounds were applied and the gels were dyed with ethidium bromide after being run (Figure 5). Cisplatin was used as a positive control. Notably, a decrease in the rate of migration for closed circular DNA (form I) was observed for complex **2** and cisplatin, which demonstrated the unwinding of the closed circular DNA. A coalescence of the closed circular DNA (form I) and open circular DNA (form II) was observed for cisplatin, which is indicative of a strong unwinding of the supercoiled DNA. As for complexes **1** and **3**, an obvious decrease in the rate of migration for closed circular DNA (form I) was observed at 320 and 640 μM, indicating that complexes **1** and **3** did not unwind the DNA significantly. According to the study above, the strong unwinding of the supercoiled DNA induced by complex **2** and cisplatin may lead to high cytotoxic activity against cancer cells, although the unwinding of DNA is not directly correlated to the cytotoxic activity of these compounds.

Kinetics Study. It is well-known that sulfur-containing molecules (e.g., glutathione, metallothionine, methionine) are a double-edged sword. On one hand, platinum(II) ions can interact with these biomolecules before binding with DNA,

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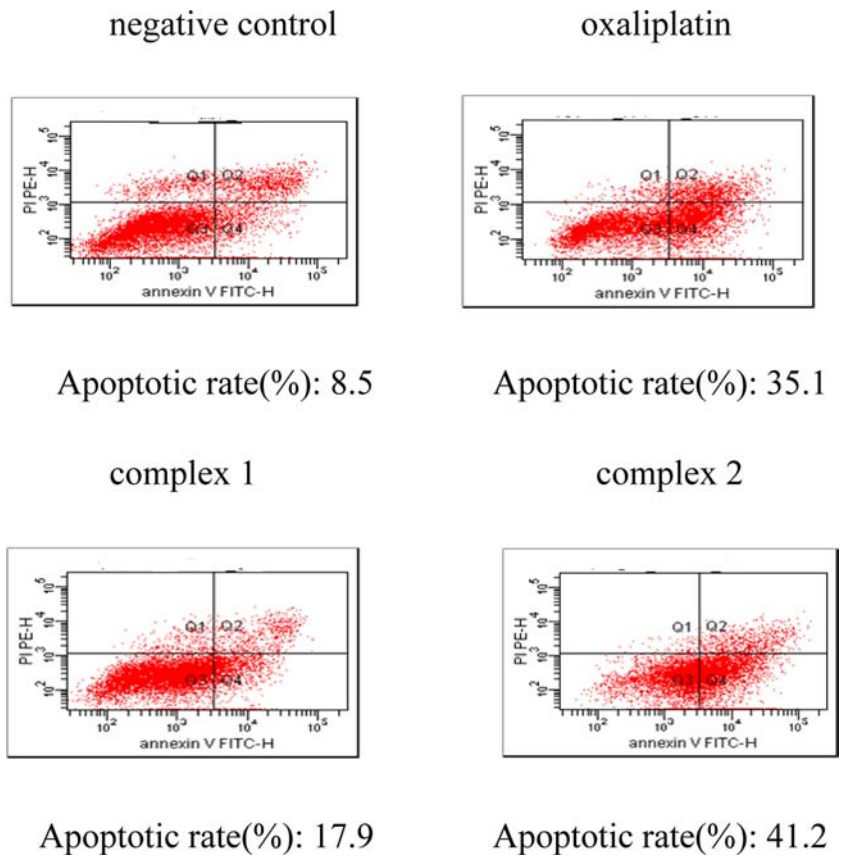

Figure 3. Flow cytometric analysis of the distribution of HCT-116 cells treated with complexes **1**and **2** and oxaliplatin at 50 μM.

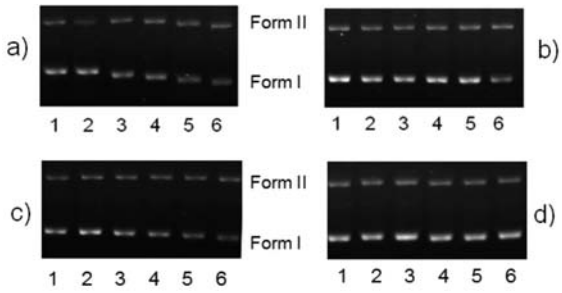

Figure 4. Gel electrophoretic mobility pattern of pET28a plasmid DNA incubated with various concentrations of platinum(II) complexes. The dye gels contained ethidium bromide. Lanes 1−6 (0, 10, 20, 40, 80, and 160 μM, respectively) with DNA. (a) Cisplatin, (b) complex **1**, (c) complex **2**, and (d) complex **3**.

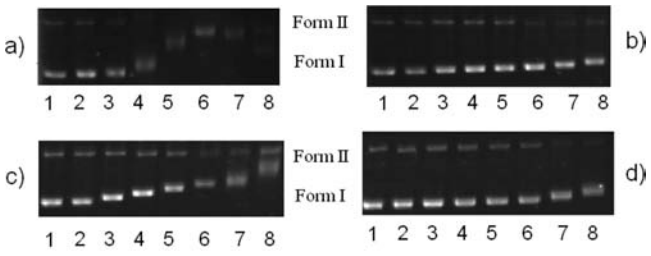

Figure 5. Gel electrophoretic mobility pattern of pET28a plasmid DNA incubated with various concentrations of platinum(II) complexes. Lanes 1−8 (0, 10, 20, 40, 80, 160, 320, and 640 μM, respectively) with DNA. (a) Cisplatin, (b) complex **1**, (c) complex **2**, and (d) complex **3**.

which may deactivate the anticancer ability of platinum(II) complexes.[13] In addition, the binding of platinum(II) ions with biomolecules may be associated with the side effects and cross-resistance of the platinum complexes.[14] On the other hand, many studies have shown that Pt-sulfur (thiothers) adducts could be substituted by DNA to form effective intrastrand Pt-(N7)DNA adducts, and Pt-sulfur adducts were postulated as a drug reservoir for platinum complexes.[3a,15] Thus, the kinetics study of the resulting compounds and sulfur-containing molecules was very important for understanding how the platinum(II) ion attains the DNA.

The interaction rate of *cis*- or *trans*-platinum(II) complexes and DNA can be acceralated by L-methionine.[16] Hence, L-methionine (L-Met) was used as a nucleophile to react with complexes **1**−**3**, and the study was carried out at 37 °C under pseudo-first-order conditions by using at least a 10-fold excess of nucleophile. Hepes buffer was used to control the pH at 7.2. The suitable wavelength for studying the reaction was determined by recording the spectral changes of the mixture over the wavelength range 200−600 nm, and 245 nm was selected for all the kinetic measurements (Figure 6).

The absorbance−time trace observed was fitted well to a two-exponential function (Figure 7 and Supporting Information, Figures S1 and S2), which means that the substitution reactions we studied probably occur in two steps (Scheme 1) with the constants k_{obs1} and k_{obs2}. To ensure the reliability of the data, the measurement was also studied at 240 nm, and the results were basically similar as those obtained at 245 nm (Figure 8). k_{obs1} and k_{obs2} are presented in Supporting Information, Table S1. In the case of k_{obs1}, the observed rate constants were plotted against the concentration of L-Met to

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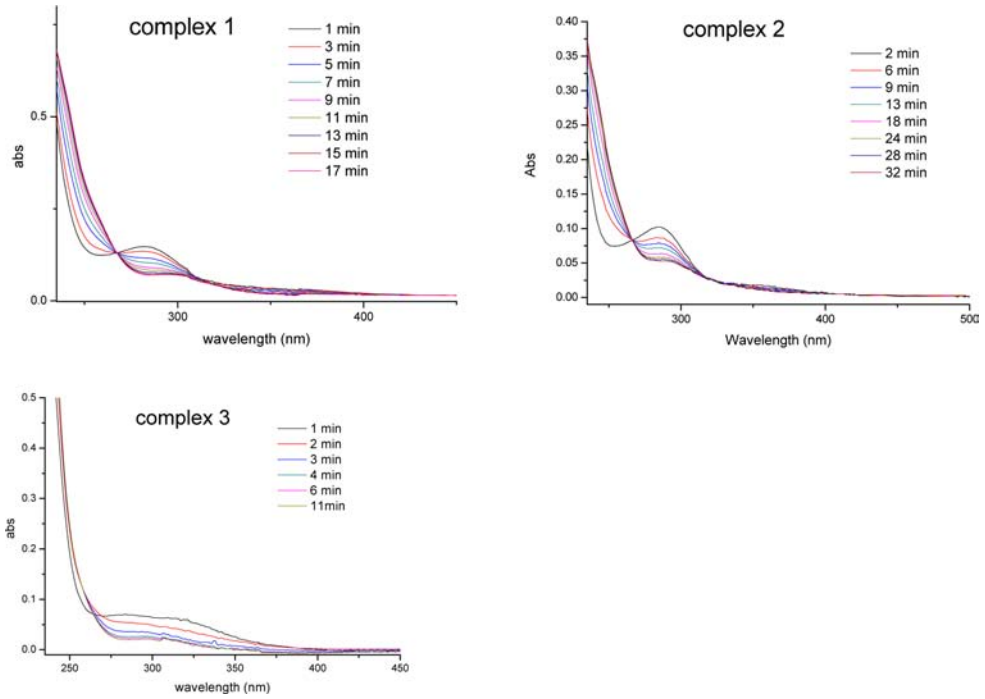


Figure 6. UV−vis spectra recorded for the reaction of 0.25 mM complexes **1**−**3** with 25 mM L-Met at different times. T = 310 K, pH = 7.2 (Hepes 0.25 M).

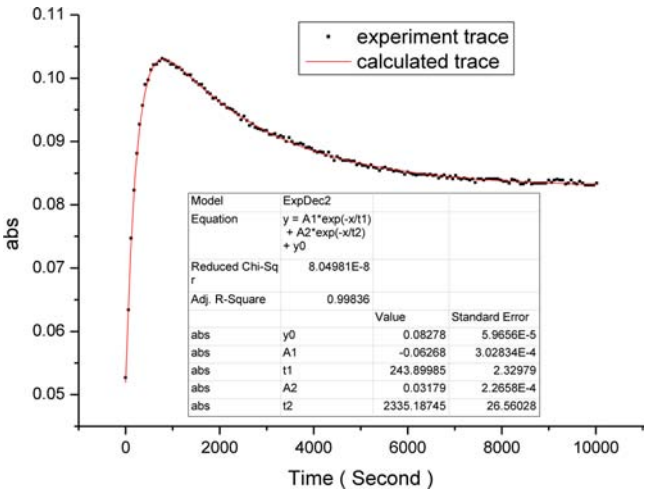


Figure 7. absorbance−time trace recorded for the reaction of complex **2** (0.25 mM) with L-Met (18.75 mM) at 37 °C, pH = 7.2 (Hepes 0.25M).

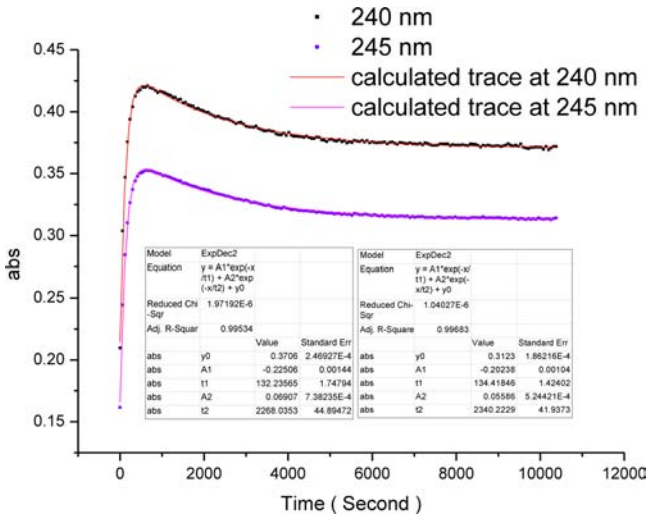
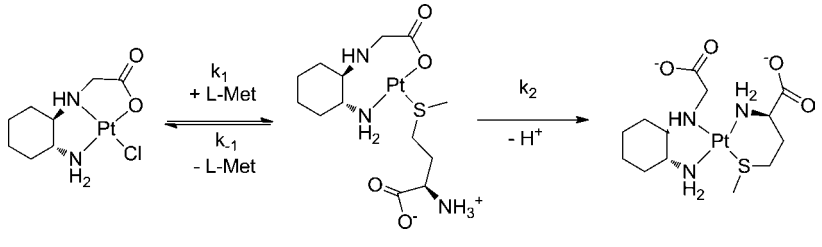


Figure 8. Measured and calculated absorbance data at two wavelengths (240 and 245 nm) for the reaction between complex **2** and L-Met (31.25 mM) at 37 °C, pH = 7.2 (Hepes 0.25M).

obtain the second-order rate constants k_1 (Figure 9), which was expressed by eq 1 ($k_{obs} = k_1[\text{L-Met}] + k_{-1}$), while k_{obs2} was found to be independent of the L-Met concentration, which was reported by Bugarcic and van Eldik previously,[17] demonstrating a chelation process (Figure 9). The values of the intercepts for

Scheme 1. Proposed Pathway of the Reaction for Complex 1 with L-Met



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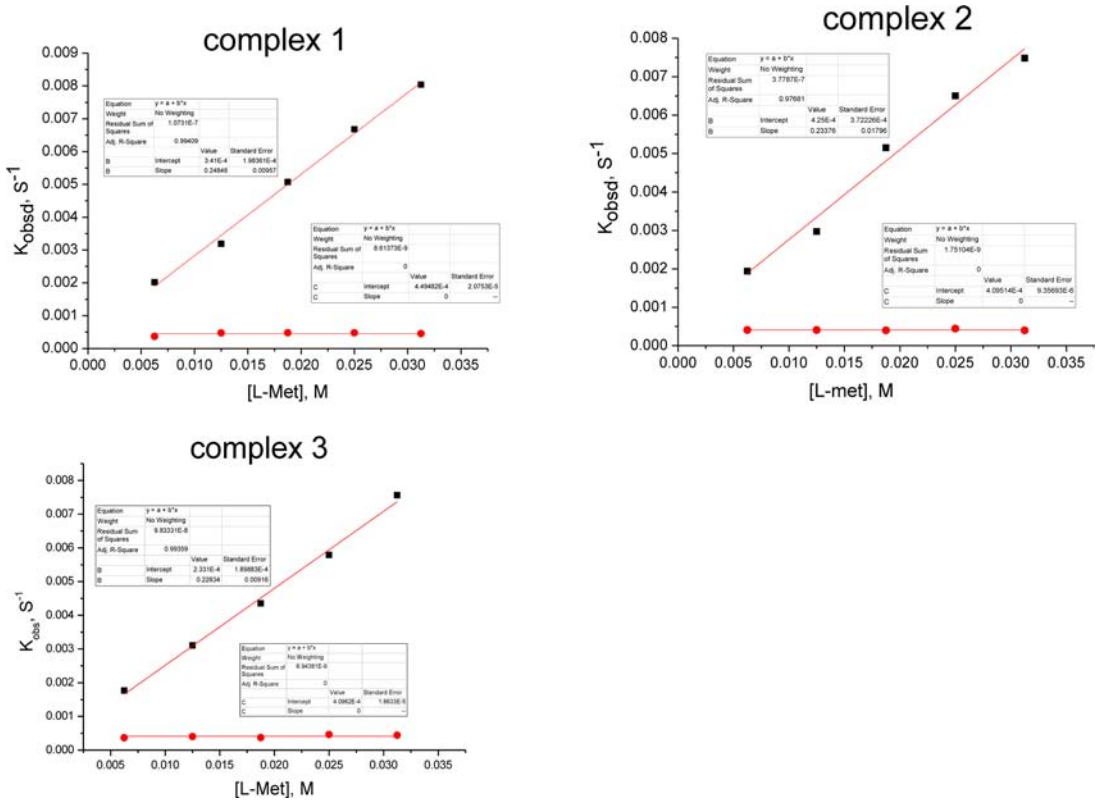

Figure 9. Plots of k_{obs} vs L-Met concentration for complexes **1**–**3** (0.25 mM). T = 37 °C, pH = 7.2.

the first-step reactions were low, so they are not discussed here. As shown in Table 3, both the first- and second-step rate constants for the reactions between complexes **1**–**3** and L-Met were almost similar, which is reasonable because of their analogous structures. Moreover, the results are in line with our assumption that the chloride anion leaves the compounds fast, giving space for the Pt ion to react with the nucleophile; meanwhile, the chelated carboxylate anion separates from the metal atom to offer extra space for the nucleophile. Overall, the kinetics study helps us further understand the mechanism of action of the newly synthesized complexes with the biomolecules.

Table 3. Summary of the Rate Constants for the Reactions between Complexes 1–3 and L-Met at 310 K

complex	$k_1 \times 10$ (M^{-1} s^{-1})[a]	$k_2 \times 10^4$ (s^{-1})[a]
1	2.49	4.49
2	2.34	4.10
3	2.28	4.03

[a]Values represent the mean ± SD (<10% of the mean value) from three separate experiments.

CONCLUSIONS

Platinum(II) complexes containing both a chloride anion and a chelated carboxylate as leaving groups were synthesized and spectrally characterized. The results of in vitro cytotoxicity assays indicated that all the compounds exhibited considerable cytotoxicity against the tested cell lines, comparable to those of cisplatin and oxaliplatin. Complex **2** showed the best antiproliferative activity against HCT-116 cells among the tested compounds. It is noted that complexes **1** and **2** with similar structures possess different anticancer activity, in which the latter showed much better cytotoxicity than the former. It is believed that the branched methyl group in ligand ($L2^-$) plays an essential role in the anticancer activity of complex **2**. Flow cytometry studies demonstrated apoptosis as the mechanism of cell death induced by the tested compounds. Moreover, the DNA interactions with the platinum-based compounds were studied by agarose gel electrophoresis, which is indicative of the DNA unwinding produced by complex **1** and especially by complex **2**. Finally, the reactions between complexes **1**–**3** and L-Met were studied by kinetics methods. The results support our assumption that the chloride ion departs from the Pt atom quickly; meanwhile, the chelated carboxylate anion separates more slowly from the metal atom. The kinetics study helps us further understand the mechanism of action of the newly synthesized complexes with the biomolecules.

The newly synthesized compounds probably have the following advantages. First, the application of chloride anions as leaving groups can promote DNA-binding abilities of the newly synthesized complexes with cytotoxicity comparable to that of cisplatin and oxaliplatin. Second, as expected, the introduction of carboxylate can improve the aqueous solubility of the complexes. Moreover, the chelating rings formed between the N,O-donors and Pt atoms can increase the stability of the complexes. Consequently, these platinum(II) complexes containing both chloride and chelated carboxylate anions as leaving groups deserve further investigation as leading compounds, especially complex **2**, and represent a new view for the design of anticancer drugs.

EXPERIMENTAL SECTION

Materials and Instruments. All chemicals and reagents were of analytical reagent grade and were used without further purification.

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Potassium tetrachloroplatinate(II) and 1*R*,2*R*-diaminocyclohexane were obtained from a local chemical company (Shandong Boyuan Chemical Company Ltd.). Elemental analyses for C, H, and N were done on a Vario MICRO CHNOS Elemental Analyzer (Elementar). IR spectra were measured on KBr pellets with a Nicolet IR200 FTIR spectrometer in the range of 4000−400 cm^{-1}, and ^{1}HNMR spectra were recorded in D_2O with a Bruker 300 MHz spectrometer. Mass spectra were measured on an Agillant ESI-MS instrument. UV−vis spectra and kinetic traces were recorded on a Shimadzu UV1700 instrument equipped with a thermostatically controlled cell holder.

General Synthesis of Platinum(II) Complexes 1−3. *Synthesis of Complex [PtLI].* To a stirring aqueous solution (50 mL) of KI (120 mmol) was added K_2PtCl_4 (15 mmol) in water (50 mL). The blending solution was stirred at 40 °C for 30 min under a nitrogen atmosphere to afford a black solution of K_2PtI_4. Then an aqueous solution (50 mL) of NaL1, NaL2, or NaL3 (15 mmol) was added dropwise under stirring in the dark at 25 °C. After 12 h, a dark yellow precipitate was filtered, washed sequentially with water, ethanol, and ether, and dried in vacuum.

A suspension of the corresponding $AgNO_3$ (20 mmol) and [PtLI] (10 mmol) in 100 mL of water was stirred at 60 °C under a nitrogen atmosphere in the dark for 24 h, and the resulting AgI deposit was filtered off. NaCl (10 mmol) was added to the filtrate. The blending solution was stirred at 50 °C for 12 h, and then the solution was evaporated nearly to dryness. Some white solids precipitated, which were washed with water and ethanol several times and dried in vacuum.

Complex ***1***. Yield: 1.7 g (43%). White powder. Anal. Calcd for $C_8H_{15}ClN_2O_2Pt$: C, 23.92; H, 3.76; N, 6.97. Found: C, 23.84; H, 3.81; N, 7.08. IR (KBr, cm^{-1}): 3141 s(br), 2934 m, 2859 m, 1642 vs, 1447 m, 1335 s, 1293 m, 1078 w, 1010 m, 912 w, 649 w, 459 m. ^{1}H NMR (D_2O, δ): 1.10−1.56 (m, 6H, $3CH_2$ of DACH), 1.95−2.18 (m, 2H, CH_2 of DACH), 2.49−2.75 (m, 2H, 2CH of DACH), 3.58−3.76 (m, 2H, $NHCH_2COO$). ESI-MS *m*/*z*: $[M + H]^+$ = 402 (35%). $[\alpha]_D^{25}$ + 74.2 (*c* 1.0, MeOH).

Complex ***2***. Yield: 2.0 g (49%). White powder. Anal. Calcd for $C_9H_{17}ClN_2O_2Pt$: C, 26.00; H, 4.12; N, 6.74. Found: C, 26.11; H, 4.08; N, 6.81. IR (KBr, cm^{-1}): 3210 s, 3094 s, 2934 s, 2860 m, 1639 vs, 1570 m, 1449 m, 1376 m, 1327 m, 1269 w, 1252 w, 1163 w, 1102 m, 1028 m, 869 w, 735 w, 644 w, 597 w, 459 m. ^{1}H NMR (D_2O, δ): 1.17 (s, 3H, CH_3), 1.48−2.39 (m, 8H, $4CH_2$ of DACH), 2.58−3.01 (d, 2H, 2CH of DACH), 3.92 (s, 1H, $NHCH(CH_3)COO$). ESI-MS *m*/*z*: $[M + Na]^+$ = 438 (100%). $[\alpha]_D^{25}$ + 79.8 (*c* 1.0, MeOH).

Complex ***3***. Yield: 2.2 g (56%). White powder. Anal. Calcd for $C_9H_{17}ClN_2O_2Pt$: C, 26.00; H, 4.12; N, 6.74. Found: C, 26.09; H, 4.14; N, 6.85. IR (KBr, cm^{-1}): 3254 s, 3111 s, 2946 m, 2868 m, 1616 vs, 1459 m, 1350 m, 1270 m, 1212 m, 1078 w, 1011 m, 976 w, 914 w, 817 w, 699 w, 473 m. ^{1}H NMR (D_2O, δ): 1.15−1.62 (m, 6H, $3CH_2$ of DACH), 2.05−2.66 (m, 6H, 2CH of DACH and CH_2 of DACH and $NHCH_2CH_2COO$), 3.21−3.41 (m, 2H, $NHCH_2CH_2OO$). ESI-MS *m*/*z*: $[M + H]^+$ = 416 (80%), $[M + Na]^+$ = 438 (100%). $[\alpha]_D^{25}$ + 76.9 (*c* 1.0, MeOH).

Cell Culture. Four human solid tumor cell lines, HepG-2 (human hepatocellular carcinoma), MCF-7 (human breast carcinoma), A549 (human non-small cell lung cancer), and HCT-116 (human colorectal cancer) were used in testing the in vitro antitumor activities of all platinum complexes. They were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 μg/mL penicillin, and 100 μg/mL streptomycin under an atmosphere containing 5% CO_2 at 37 °C.

In Vitro Cytotoxicity Assay. The IC_{50} values of all complexes were determined by MTT assay. This assay is based on the cleavage of the yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; MTT, Sigma), forming purple formazan crystals by viable cells. The cells were plated in 96-well culture plates at a density of 3000 cells per well and incubated for 24 h at 37 °C in a 5% CO_2 incubator. All compounds were dissolved in water and then diluted to the required concentration with culture medium. The diluted complexes were then added to the wells, and cells were incubated at 37 °C under 5% CO_2 for 48 h. Afterward, the cells were treated with 20 μL of MTT dye solution (5 mg/mL) for a 4 h cultivation. The media with MTT were removed and replaced with DMSO (100 μL). The absorbance was measured at λ = 540 nm. IC_{50} values were determined by plotting cell viability (%) against dose of complex added (μM).

Cell Apoptosis Study by Flow Cytometry. Apoptosis induced by platinum complexes was determined by flow cytometry using an AnnexinV-FITC Apoptosis Detection Kit (Roche) according to the manufacturer's instructions. After induced apoptosis of selected cell lines (HCT-116 and MCF-7) by the addition of 50 μM selected compounds and positive controls including cisplatin (50 μM) and oxaliplatin (50 μM) for 24 h, cells were collected by centrifugation (5 min, 25 °C, 2000 rpm). The cells were then washed twice with cold water and resuspended in AnnexinV-FITC binding buffer at a concentration of 1 × 10^6 cells/mL. Cells were stained with 5 μL of AnnexinV-FITC and incubated in the dark at 25 °C for 10 min. The cell suspension was centrifuged for 5 min (25 °C, 2000 rpm), and cells were resuspended in AnnexinV-FITC binding buffer. Propidium iodide (10 μL) was added, and the tubes were placed on ice away from light. The fluorescence was measured using a flow cytometer (FACScan, Becton Dickinson). The results were analyzed by FCSExpress software and are expressed as the percentage of normal and apoptotic cells at various stages.

Gel Electrophoresis. DNA binding produced by complexes **1**−**3** and cisplatin was investigated by agarose gel electrophoresis. pET28a plasmid DNA (50 ng/μL) was used as the target. Appropriate dilutions of the tested compounds were made, and the required volumes of solutions were added to achieve a set of concentrations in the range of 0−160 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) under UV light. For the other method in which agarose gels were dyed with ethidium bromide after being run, the same procedure was used except that the 1.0% agarose gel was stained with ethidium bromide (0.5 mg/L) for 20 min after the gels had been run and the concentrations were increased to 640 μM.

Kinetics Study. The ligand substitution reactions were studied by observing the change in absorbance at suitable wavelengths under pseudo-first-order conditions (at least 10-fold excess of L-Met over the complexes). A suitable wavelength for kinetic trace was determined by recording spectra of the reaction mixture over the wavelength range 200−600 nm, and 245 nm was selected for all the kinetics measurements. All the reactions were studied at 37 ± 0.1 °C and pH 7.2 (0.25 M Hepes) in the presence of 0.1 M $NaClO_4$. The reactions were initiated by mixing an aqueous solution (1 mL) of the platinum(II) complex with L-Met in water (1 mL).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The absorbance−time trace observed for complexes **1** and **3** and table of k_{obs1} and k_{obs2} values. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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