Synthesis and Antitumor Activity of (3-Hydroxyacrylato-O,O′) Diammineplatinum(II)

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Abstract

As an indispensable part of cancer chemotherapy, platinum drugs still play an important role in cancer treatment. In this study, two platinum(II) complexes with Michael acceptor 3-hydroxyacrylic acid as the leaving group were synthesized from *cis*-diamminediiodo platinum(II) and 3-ethoxyacrylic acid. The structures of complexes **1** and **2** were confirmed by elemental analysis, infrared, ^{1}H NMR, ^{13}C NMR, and HRMS (high-resolution mass spectrometry). Results from MTT assay showed that complexes **1** and **2** significantly inhibited the growth of the four human tumor cell lines (HCT-116, A549, CFPAC-1, and BxPC-3) with the IC_{50} values of the two compounds similar to that of the control drug (oxaliplatin) on HCT-116 and A549. Besides, results from an *in vivo* study in a mouse S180 sarcoma model showed that complex **1** had a higher antitumor activity in comparison to oxaliplatin. In conclusion, our article indicated that complex **1** deserved further research and development in cancer treatment.

Keywords
- platinum(II) complexes
- antitumor activity
- Michael acceptor

Introduction

Cisplatin, a platinum(II) complex, has made a major impact in the chemotherapeutic treatment of testicular and ovarian cancers since the accidental discovery of its biological activity, and is widely used in the treatment of these types of cancers.[1,2] However, it still has nonnegligible toxic and side effects such as nephrotoxicity, emetogenicity, and drug resistance, which cripple its overall effectiveness in cancer therapy.[3–6] For decades, thousands of platinum(II) complexes have been prepared in the hope of finding those with more tolerable toxicological profile and higher efficacy.[7] These efforts have brought several new drugs (carboplatin, oxaliplatin, nedaplatin, and lobaplatin) into market,[8–10] followed by several new complexes emerging in current clinical trials (►**Fig. 1**).[11]

With regard to most of the platinum(II) complexes, such as carboplatin and oxaliplatin, dicarboxylate plays a role as the leaving group in the mechanism of the interaction between platinum(II) complex and DNA, and in addition to platinum(II) complexes containing dicarboxylate as the leaving group, there are also platinum(II) complexes, such as nedaplatin and lobaplatin, containing α-hydroxylcarboxylate as the leaving group, which will have more stronger antitumor activity.[12] Thus, exploring a novel compound based on the structure of platinum(II) complexes containing α-hydroxylcarboxylate may represent an promising strategy to improve the antitumor activity of the platinum(II) complexes.[12]

To the best of our knowledge, the synthesis of platinum(II) complexes with Michael acceptor as leaving groups has not been reported. Michael acceptor is the functional group in which the olefins or acetylenes conjugated to electron-withdrawing groups. The compounds with Michael acceptor are considered as a class of biologically active molecules

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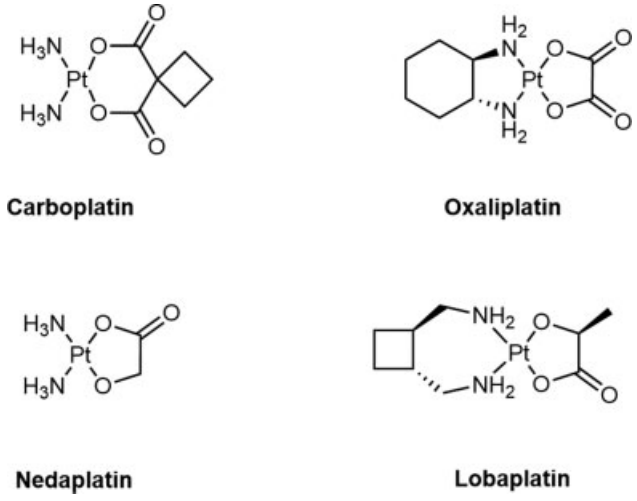


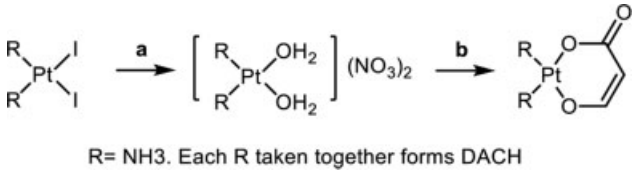
Fig. 1 Chemical structures of platinum drugs.

which directly or indirectly involved in the life processes. A series of studies suggested that the Michael acceptor moiety especially α,β-unsaturated carbonyl fragment is the essential active group with cytotoxicity among various anticancer compounds.[13–16] Michael acceptor is a fragment for covalent binding and might improve the nonselective cytotoxicity of platinum(II) complexes. In this study, two 3-hydroxyacrylatoplatinum(II) complexes containing Michael acceptors as leaving groups (complexes **1** and **2**) were synthesized and characterized (►**Fig. 2**). Our data suggested the potential use of these two compounds in cancer treatment in the future.

Results and Discussion

Successful Synthesis of Complexes 1 and 2

Scheme 1 shows the synthesis of complexes **1** and **2** following a general method.[17] Starting from *cis*-$[PtR_2I_2]$ (commercially available), the first step was performed in water with $AgNO_3$ to form $[PtR_2(H_2O)_2](NO_3)_2$. Furthermore, $[PtR_2(H_2O)_2](NO_3)_2$ was mixed with sodium 3-ethoxyacrylate to produce a yellow solution. The reaction mixture was concentrated in vacuum and purified by silica gel chromatography to obtain the target complex. The products were then characterized by elemental analysis, infrared (IR), ^{1}H NMR, ^{13}C NMR, mass spectrometry, and high-resolution mass spectrometry, respectively. The elemental analysis data for each compound were in good agreement with the designed structure formula. The binding of the 3-hydroxyacrylate to platinum atoms as a bidentate ligand was confirmed by the shift of $\nu_{C=O}$ to lower frequencies and the absence of $\nu_{O\text{-}H}$ absorption in IR spectra in the resulting



Scheme 1 Synthetic route for complexes **1** and **2**. *Reagents and conditions:* a) $AgNO_3$, 60°C, 4 hours; b) 3-ethoxyacrylic acid, NaOH, 60°C, 3 hours. DACH, *trans*-1,2-Diaminocyclohexane.

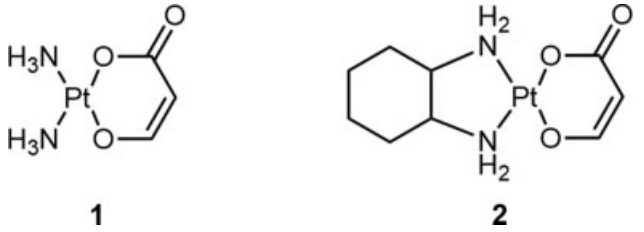


Fig. 2 Chemical structures of platinum(II) complexes **1** and **2**.

complexes.[4] All complexes showed $[M+H]^+$ peaks, corresponding to their molecular weights, and had three typical protonated molecular ion peaks reflecting the platinum isotopes: ^{194}Pt(33%),^{195}Pt (34%), and ^{196}Pt (25%). ^{1}H and ^{13}C NMR spectral peaks matched the chemical structures given in ►**Fig. 2**. At room temperature, the solubility values of complexes **1** and **2** in phosphate buffered saline (pH = 7.4) are 36.5 and 1.5 mg/mL, respectively. Complex **1** possesses sufficient water solubility.

An *In Vitro* Study Showing Cytotoxic Activities of Complexes 1 and 2 on Cancer Cells

In this study, the *in vitro* cytotoxicity of the platinum(II) complexes was tested by measuring the effect of the complexes on the proliferation of the four cancer cells (the colorectal cancer cell line HCT-116, the lung carcinoma cell line A549, as well as the pancreatic cancer cell line CFPAC-1 and BxPC-3). These four cell lines were continuously exposed to different concentrations of oxaliplatin (serving as a control drug), as well as complexes **1** and **2** for 48 hours, and then IC_{50} of the three drugs was assessed using MTT assay according to a reported study.[18] ►**Table 1** shows that both complexes **1** and **2** showed cytotoxic activity on the four cell lines with the significant effect being seen in HCT-116 and A549 cells. Interestingly, the cytotoxicity of complex **1** was less and closer to that of the control drug when compared with complex **2**. Thus, we chose complex **1** for the following study.

Complex 1 Inhibited Tumor Growth in a Mouse S180 Sarcoma Model

The antitumor activities of complex **1** and oxaliplatin were further compared in a mouse S180 sarcoma model.[19] Based on preliminary studies, tumor-bearing mice were administered with intraperitoneal injection of complex **1** (25 mg/kg) once-daily. ►**Table 2** shows that complex **1** displayed a strong antitumor effect (tumor growth inhibition: 69.78%). However, administration of complex **1** also led to severe mouse weight loss, suggesting that the mice could not tolerate once-a-day

Table 1 *In vitro* cytotoxicity of complexes 1 and 2 against tumor cell lines

	IC_{50} (μmol/L)			
	HCT-116	A549	CFPAC-1	BxPC-3
Oxaliplatin	39.22	45.28	43.97	31.81
Complex **1**	35.34	43.46	92.39	69.05
Complex **2**	15.05	28.94	66.18	94.50

Table 2 Antitumor activity of complex 1 in mouse S180 sarcoma models[a]

Group	Dose (mg/kg)	Dosing regimen	Mean body weight (g)		Tumor weight (g)	TGI (%)
			D1	D7		
Control	Vehicle	once-daily	18.88 ± 0.97	21.45 ± 1.83	3.21 ± 0.33	/
Complex **1**	25	once-daily	19.21 ± 0.94	15.44 ± 0.90	0.97 ± 0.35	69.78**

Abbreviation: TGI, tumor growth inhibition.
[a]Tumor-bearing mice were treated by intraperitoneal (ip) injection of complex **1** for 7 days. Data are presented as mean ± SD. The comparison between the two groups was conducted using *t*-test with statistically significant at $^{**}p < 0.01$ versus control.

Table 3 Different dose and dosing regimens on antitumor effect of complex 1 in mouse S180 sarcoma models[a]

Group	Dose (mg/kg)	Dosing regimen	Mean body weight (g)		Tumor weight (g)	TGI (%)
			D1	D11		
Control	Vehicle	Once-daily	21.18 ± 0.91	24.08 ± 3.32	2.95 ± 0.38	/
Complex **1**	15	Once-daily	20.94 ± 0.64	18.82 ± 1.08	1.66 ± 0.39	43.58**
Complex **1**	30	Once in 2 days	20.86 ± 1.13	20.21 ± 3.22	1.60 ± 0.35	45.87**
Oxaliplatin	9	Once in 2 days	21.14 ± 1.04	17.46 ± 1.85	1.18 ± 0.24	59.85**

Abbreviation: TGI, tumor growth inhibition.
[a]Tumor-bearing mice were treated by intraperitoneal (ip) injection of complex **1** for 11 days. Data are presented as mean ± SD. The comparison between the two groups was conducted using *t*-test with statistically significant at $^{**}p < 0.01$ versus control.

administration of complex **1**, and the safe and effective dosage regimen should be explored.

Increased Drug Given Dose and Extended Drug Given Interval May Enhance Antitumor Effect While Reducing Toxicity of Complex 1 in Mouse Xenograft Models

Based on the results obtained from ►**Fig. 2**, we further assessed whether decreasing complex **1** dose (15 mg/kg, once-daily) or prolonging its dosing interval (30 mg/kg, once in 2 days) will improve the effect of complex **1** on mouse body weight and tumor weight. ►**Table 3** shows the antitumor effect of complex **1** when administered at 30 mg/kg; once in 2 days was more effective than daily dose at 15 mg/kg, while the mouse weight loss of the complex was less severe. Interestingly, although the antitumor effect of complex **1** (30 mg/kg, once in 2 days) was weaker than the control drug (oxaliplatin, 9 mg/kg) at a same dose interval (once in 2 days), the toxicity of compound **1** was significantly reduced.

Then, we further increased drug dose and extended drug dosing interval, and investigated whether a more effective and safer use of compound **1** would be achieved when compared with the control drug (oxaliplatin, 9 mg/kg, once in 2 days). Thus, the drug dosing interval was increased to once in 3 days (60 mg/kg) or once in 6 days (120 mg/kg) in the mouse xenograft models. As shown in ►**Table 4**, bolus application of compound **1**, both 60 mg/kg, once in 3 days, and 120 mg/kg, once in 6 days, exhibited stronger antitumor activity than oxaliplatin with mouse weight being preserved even on 11th day, suggesting better safety profile of a larger dose at a longer interval of complex **1** at the two dosage regimens. Interestingly, a single bolus of complex **1** at 120 mg/kg once in 6 days gave the best result.

Conclusion

In summary, two 3-hydroxyacrylatoplatinum(II) complexes with novel six-membered ring structures containing Michael acceptors as leaving groups were synthesized and characterized. Both complexes **1** and **2** were evaluated for cytotoxicity against four human cancer cell lines and complex **1** was evaluated for antitumor activity in a mouse S180 xenograft

Table 4 *In vivo* antitumor activity of complex 1 in mouse S180 sarcoma models with the improved drug administration[a]

Group	Dose (mg/kg)	Dosing regimen	Mean body weight (g)		Tumor weight (g)	TGI (%)
			D1	D11		
Control	Vehicle	Once in 3 days	22.81 ± 1.17	24.56 ± 3.67	3.08 ± 0.32	/
Complex **1**	60	Once in 3 days	22.21 ± 0.73	22.46 ± 2.28	1.10 ± 0.56	64.39**
Complex **1**	120	Once in 6 days	22.78 ± 0.75	20.34 ± 2.44	1.02 ± 0.36	66.83**
Oxaliplatin	9	Once in 2 days	23.18 ± 0.62	19.92 ± 2.50	1.16 ± 0.20	62.42**

Abbreviation: TGI, tumor growth inhibition.
[a]Tumor-bearing mice were treated by intraperitoneal (ip) injection of complex **1** for 11 days. Data are presented as mean ± SD. The comparison between the two groups was conducted using *t*-test with statistically significant at $^{**}p < 0.01$ versus control.

model. The results showed that the anticancer effects of complexes **1** and **2** were similar to that of oxaliplatin in two human cancer cell lines. Furthermore, we explored the different dosing regimens of complex **1** in an *in vivo* study. Our data showed that administration of complex **1** at 120 mg/kg once in 6 days was more efficacious and safer than the control drug (oxaliplatin). In conclusion, 3-hydroxyacrylato-platinum(II) complexes with novel six-membered ring structures containing Michael acceptors (complexes **1** and **2**) at a higher dose and a longer interval may serve as promising drug candidates in cancer therapy.

Experimental Section

(3-Hydroxyacrylato-O,O′) diammineplatinum(II) (complex 1): To a solution of 3-ethoxylacrylic acid (1.0 g) in water (100 mL) was added NaOH (340 mg). The solution was then shaken ultrasonically, adjusted to pH = 7 by NaOH, and concentrated in vacuum. The residue was washed with water and EtOH, respectively, to give the sodium 3-ethoxylacrylate (yellow solid, 1.15 g, 97.1% yield). *cis*-Diammenediiodo platinum(II) (4.16 g) was dissolved in water (300 mL). $AgNO_3$ (2.92 g, in 50 mL water) was added to the solution. The mixture was stirred for 4 hours at 50°C under darkness and filtered to remove the precipitate. To the filtrate was added sodium 3-ethoxylacrylate (1.15 g, in 100 mL water). The mixture was stirred for 4 hours at 65°C under darkness and filtered. The filtrate was concentrated in vacuum to remove most of the solvent. The residual solution (~15 mL) was cooled to room temperature and filtered. The precipitate was respectively washed with water and EtOH twice and dried at 60°C to give complex **1** (1.1 g, white solid, 40.5% yield). Melting point: 185°C (decomp). Found (calcd. for $C_3H_8N_2O_3Pt$) C 11.12 (11.43), H 2.78 (2.56), N 8.54 (8.89). IR (KBr, v, cm^{-1}): 3284 (s), 1584 (s), 1521 (s), 1437 (s), 1347 (m), 1287 (vs). ^1H NMR (DMSO, 400 MHz): $\delta_H = 4.14$ (d, $J = 6$ Hz, 1H, OCH), 6.55 (d, $J = 6$ Hz, 1H, CCH), 3.92 (brs, 3H, NH_3), 3.82 (brs, 3H, NH_3). ^{13}C NMR (100 MHz, CD_3OD): $\delta_C = 165.8$ (C = O), 164.7 (CH), 95.7 (CH). MS(ESI): *m/z* $[M + H]^+ = 316.11$. HR MS (ESI): calcd. $C_3H_8N_2O_3Pt$ $[M + H]^+$ 316.0261, found 316.0263.

(3-Hydroxyacrylato-O,O′)*trans*-cyclohexane-1,2-diammineplatinum(II) (complex 2): To a solution of 3-ethoxyl acrylic acid (1.0 g) in water (100 mL) was added NaOH (340 mg). The solution was shaken ultrasonically, adjusted to pH = 7 by NaOH, and concentrated in vacuum. The residue was washed with water and EtOH respectively to give the sodium 3-ethoxylacrylate (yellow solid, 1.16 g, 97.5% yield). *cis*-Diiodo-(*trans*-(-)-1,2-diaminocyclohexane)platinum(II) (3.40 g) was dissolved in water (300 mL). $AgNO_3$ (1.46 g in 50 mL water) was added to the solution. The mixture was stirred for 5 hours at 55°C under darkness and filtered to remove the precipitate. To the filtrate was added sodium 3-ethoxylacrylate (1.16 g in 100 mL water). The mixture was stirred for 5 hours at 60°C under darkness and filtered. The filtrate was concentrated in vacuum to remove most of the solvent. The residual solution (~15mL) was purified by a reversed-phase silica gel (eluted by a mixture of MeOH: water = 1:9). The eluent was collected and concentrated in vacuum to give a white solid. The white solid was dried at 60° C under darkness to give the complex **2** (0.73 g, white solid, 30.5% yield). Melting point: 208°C (decomp). Found (calcd. for $C_9H_{16}N_2O_3Pt$) C 26.95 (27.34), H 4.05 (4.08), N 6.97(7.09). IR (KBr, v, cm^{-1}): 3221 (m), 2939(m), 1588 (vs), 1426(m), 1340 (m), 1297 (m). ^1H NMR (DMSO, 400 MHz): $\delta_H = 4.13$ (d, $J = 6$ Hz, 1H, OCH), 6.64 (d, $J = 6$ Hz, 1H, CCH), 0.98–1.22 (m, 4H, $CH_2CH_2CH_2CH_2$ of DACH), 1.45–1.47 (m, 2H, $CH_2CH_2CH_2CH_2$ of DACH), 1.80–1.83(m, 2H, $CH_2CH_2CH_2CH_2$ of DACH), 2.11 (s, 2H, $2 \times CHNH_2$), 5.00 (brs, 2H, NH_2), 5.56 (brs, 2H, NH_2). ^{13}C NMR (100 MHz, CD_3OD): $\delta_C = 165.7$ (C = O), 164.9 (CH), 95.7 (CH), 62.1 (CH), 61.8 (CH), 31.9 ($2 \times CH_2$), 24.2 ($2 \times CH_2$). MS(ESI): *m/z* $[M + H]^+ = 396.09$. HR MS (ESI): calcd. $C_9H_{12}N_2O_3PtNa$ $[M + Na]^+$ 417.0685, found 417.0676.

Ethical Approval

In this study, the use of mice was approved by Animal Care and Use Committee of Shanghai Institute of Pharmaceutical Industry.

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Conflict of Interest

None.

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