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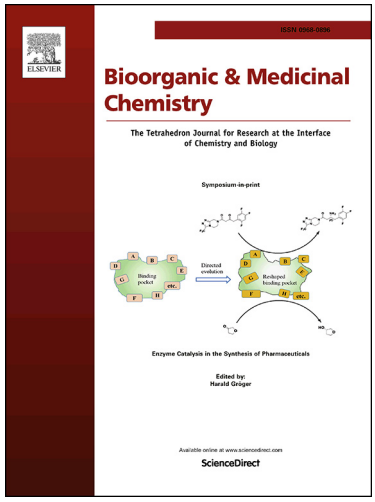


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# Synthesis and evaluation of bi-functional 7-hydroxycoumarin platinum(IV) complexes as antitumor agents

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**ABSTRACT**

A series of bi-functional 7-hydroxycoumarin platinum(IV) complexes were synthesized, characterized, and evaluated for antitumor activities. The 7-hydroxycoumarin platinum(IV) complexes display moderate to effective antitumor activities toward the tested cell lines and show much potential in overcoming drug resistance of platinum(II) drugs. In reducing microenvironment, the title compounds could be reduced to platinum(II) complex accompanied with two equivalents of coumarin units. By a unique mechanism, the 7-hydroxycoumarin platinum(IV) complex attacks DNA *via* the released platinum(II) compound, meanwhile it also inhibits the activities of cyclooxygenase by coumarin fragment. This action mechanism might be of much benefit for reducing tumor-related inflammation in the progress of inhibiting tumor proliferation and overcoming cisplatin resistance. The incorporation of 7-hydroxycoumarin leads to significantly enhanced platinum accumulation in both whole tumor cells and DNA. The HSA interaction investigation reveals that the tested coumarin platinum(IV) compound could effectively combine with HSA *via* van der Waals force and hydrogen bond.

**Keywords**: Antitumor; Platinum; DNA damage; Coumarin; Cyclooxygenase inhibition

## 1. Introduction

Cancer is ranked as the leading cause of death and the most important barrier to increase life expectancy worldwide.[1,2] The exploration of prominent chemotherapeutics has always been a hot topic in pharmaceutical field. Platinum drugs as the most attractive metal drugs represent one of the mainstays for clinical cancer chemotherapy and make up a large portion of the chemotherapeutic regimens.[3,4] Despite the remarkable achievements of platinum drugs in cancer treatment, their clinical success is rather limited by the severe toxic side effects and intrinsic/acquired resistance. Thereby, it is an urgent task to develop new prominent platinum drugs, especially the ones with new action modes.[5,6]

Platinum(IV) compounds are of great potential in improving pharmacological properties of the current clinical platinum(II) drugs.[7–13] Structurally, platinum(IV) with octahedral configuration is substitution inert in comparison with platinum(II), which enable the platinum(IV) compounds to keep stable in blood circulation, thus decreasing degradation and toxicity. Pharmaceutically, two axial ligands of platinum(IV) facilitate the modification of platinum drugs. It provides a convenient strategy to impart and fine-tune desired biological properties to the target complexes. The incorporation of various groups to platinum(IV) system to construct target complexes with bi- or multi-functional action modes has become a hot spot in new platinum drug exploration.[4,8]

Cancer-associated inflammation is tightly related to the occurrence, development and metastasis of malignancies. The control of inflammation is greatly beneficial for the cancer treatment in clinic by restraining tumor promotion, enhancing antitumor immunity and improving therapic effects of drugs, etc.[14,15] Cyclooxygenase (COX) which is the primary target for nonsteroidal anti-inflammatory drugs (NSAIDs) has become a promising target for antitumor drug design. Recently, great efforts have been devoted to the development of bi-functional platinum(IV) complexes with COX inhibitory properties. Complexes **I–III** (Figure 1) bearing ibuprofen, indomethacin or aspirin axial ligands have been reported as effective antitumor drugs in recent years,[16–18] which were superior to the corresponding combination therapy of platinum(II) drugs with NSAIDs. Consequently, the incorporation of COX inhibitory fragment to platinum(IV) system is a valid strategy to explore platinum complexes with new antitumor mechanism and improved bioactivities.

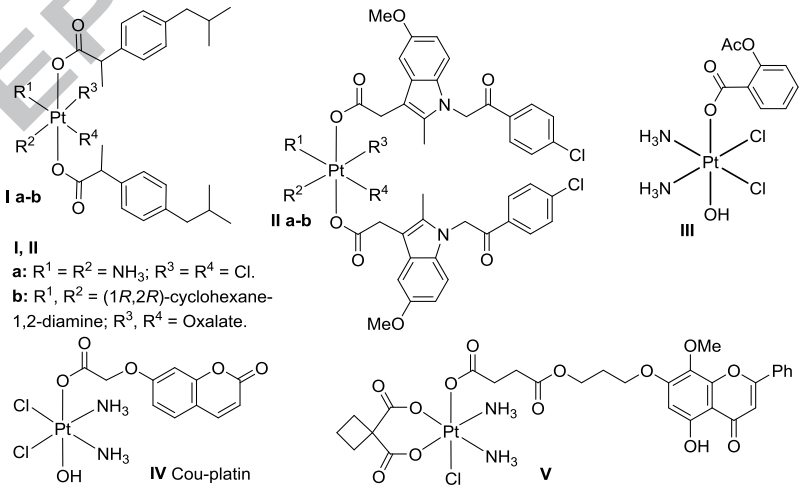



**Figure 1.** Platinum(IV) compounds with COX inhibitory features.

Coumarin compounds with a unique benzopyrene structure show great potential application in cancer treatment.[19–28] Moreover, some coumarin derivatives possess prominent anti-inflammatory activities *via* inhibiting COX. This unique structural backbone represents a rich resource for developing new NSAIDs.[29] Recently, several coumarin derived

platinum(IV) complexes were reported as bi-functional antitumor agents.[30–33] Cou-platin **IV** with mono 7-hydroxycoumarin ligand exhibited significant antitumor activities, especially to HCT116, HepG2, SGC7901 and SGC7901/cis which were over 19-fold more potent than cisplatin, and compound **V** with a wogonin ligand exerted satisfactory antitumor activities with prominent anti-inflammatory features.

Inspired by these observations and as an extension of our studies on novel potent antitumor agents,[34–36] a series of coumarin platinum(IV) complexes **1–4** with two 7-hydroxycoumarin ligands in axial position (Scheme 1) were prepared based on the following considerations: (1) It was extensively accepted that platinum(IV) complexes as prodrugs of platinum(II) drugs regain their activities after reduction to divalent form which would cause DNA damage to tumor cells.[37,38] The platinum cores deliver much influence on the antitumor activities. To this end, two prominent clinical platinum drugs cisplatin and oxaliplatin were employed to evaluate their impacts on the bioactivities of the platinum(IV) compounds. (2) The 7-hydroxycoumarin was introduced to platinum(IV) system as the axial ligand with the expectation that its integration would enable the target complexes to exhibit bi-functional action mode and further improve bioactivities. It was desired that the released platinum(II) complexes could cause DNA damage by forming intra- and interstrand cross-links with DNA helix, meanwhile the coumarin unit would endow the target compounds with COX inhibitory properties which will reduce the tumor-associated inflammation. (3) The linkage shows great influence on the bioactivities by modulating physicochemical properties. Thereby, the 7-hydroxycoumarin platinum(IV) complexes with one and three carbons alkyl linkers were designed to get the suitable linker for such serial target compounds. Based on these expectations, the target compounds were evaluated for antitumor activities. The structure-activity relationships (SAR) of the title compounds were primarily discussed and the likely action mechanism was also investigated.

## 2. Materials and methods

### *2.1. Chemistry*

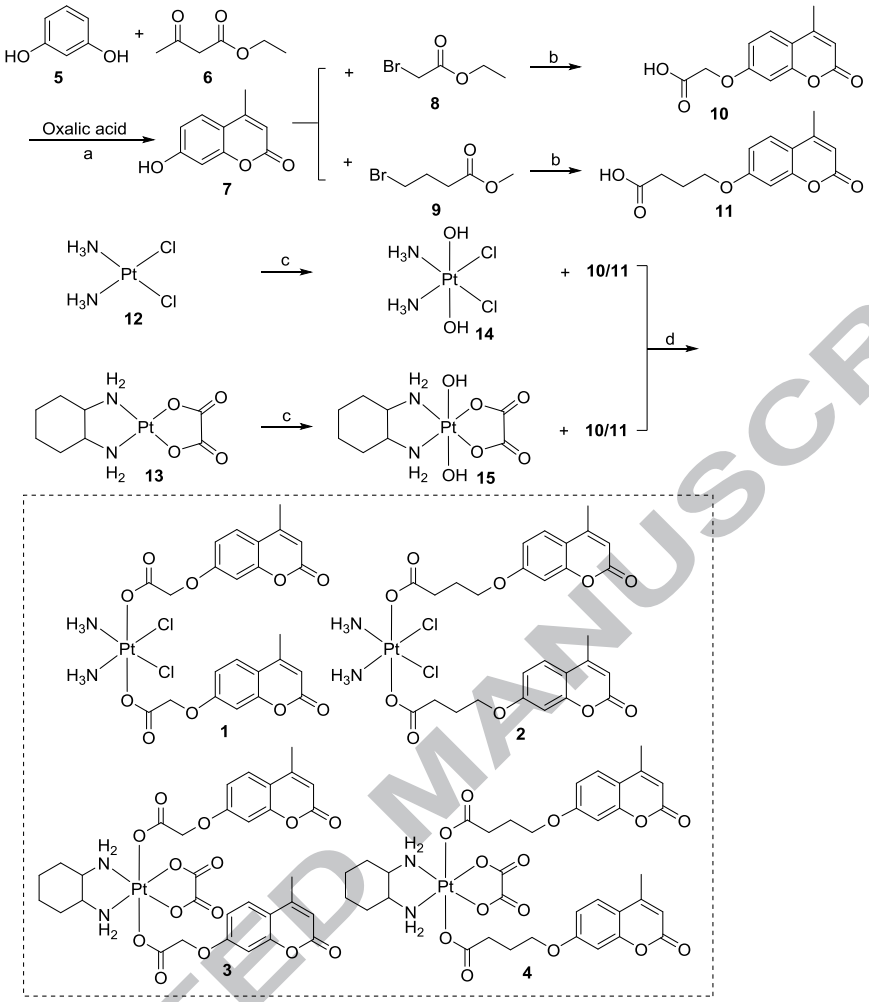
#### *2.1.1. General*

All reactions were carried out under an atmosphere of nitrogen in flame-dried glassware with magnetic stirring unless otherwise indicated. Cisplatin and oxaliplatin were purchased from Yurui chemical Co. Ltd. (Shanghai, China). Reagents were purchased from Alfa Aesar, Sigma-Aldrich, and J&K Scientific and were used without further purification. The HSA was purchased from Sigma. The cyclooxygenase 2 inhibitor screening kit and Genomic DNA mini preparation kit were purchased from Beyotime, China. The annexin V-FITC/PI apoptosis detection kit was purchased from KeyGen BioTech, China. All cells were kindly donated by Prof. Peng George Wang (College of Pharmacy, Nankai University, Tianjin, China).

$^{1}$H NMR and $^{13}$C NMR spectra were recorded on a Varian (400 MHz) and all NMR chemical shifts were referenced to residual solvent peaks or to TMS as an internal standard. All coupling constants *J* were quoted in Hz. High resolution mass spectra (HRMS) were obtained on an IonSpec QFT mass spectrometer with ESI ionization. The HPLC analyses were performed on a Thermo Ultimate 3000 RS equipped with an Agilent Eclipse XDB-C18 column (250×4.6 mm, 5 μm). UV-vis spectra were measured on a Scinco S-3100 UV-vis spectrophotometer. Fluorescence spectroscopic data were recorded on a Hitachi F-7000 spectrofluorometer. CD spectra were recorded on a Jasco J-810 spectropolarimeter.

#### *2.1.2. Synthetic procedures*

The synthetic route for title compounds **1–4** are established in Scheme 1. The detail methods for the preparation of the coumarin acids **10**, **11** and the oxoplatin **14**, **15** are supplied in supporting information based on the literature procedures.




**Scheme 1**. Synthetic route for 7-hydroxycoumarin platinum(IV) compounds **1–4**. Conditions and reagents: (a) 80 °C, 8 h; (b) i: TBAI, $K_2CO_3$, DMF, 40 °C, 24 h; ii: EtOH, 5% NaOH/$H_2O$, room temperature, 12 h; (c) $H_2O_2/H_2O$, 60 °C, 4 h; (d) TBTU, TEA, DMF, 50 °C, 48 h.

2.1.2.1. Preparation of compound **1**

Coumarin acid **10** (176 mg, 0.75 mmol) and TBTU (241 mg, 0.75 mmol) was added to a 50 mL flask. Then, dry DMF 5 mL was added as solution. The mixture was allowed to stir for 10 min after injection of TEA (104 μL, 0.75 mmol). Subsequently, oxoplatin **14** (100 mg, 0.30 mmol) was added and the reaction system was stirred for 48 h at 50 °C. After the reaction completed, the solvent was removed under reduced pressure. The residue was purified by column chromatography. The product was obtained as white solid (78 mg, 34%).

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.65 (d, $J$ = 8.8 Hz, 2H, coumarin 5-*H*), 7.03 – 6.92 (m, 4H, coumarin 6,8-*H*), 6.59 (br, 6H, N$H_3$), 6.22 (d, $J$ = 1.0 Hz, 2H, coumarin 3-*H*), 4.79 (s, 4H, OC$H_2$), 2.40 (s, 6H, coumarin-C$H_3$). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 175.3 (O=*C*), 161.6 (coumarin 7-*C*), 160.6 (coumarin 2-*C*), 155.0 (coumarin 8a-*C*), 153.8 (coumarin 4-*C*), 126.7 (coumarin 5-*C*), 113.7 (coumarin 3-*C*), 113.1 (coumarin 4a-*C*), 111.6 (coumarin 6-*C*), 102.1 (coumarin 8-*C*), 65.0 (*C*$H_2$), 18.6 (*C*$H_3$). IR (KBr) ν: 1697 (C=O), 1614, 1558, 1512, 1427 (aromatic frame), 1394, 1330, 1268, 1155, 1079, 987, 854, 815 cm$^{-1}$. HRMS: Calcd. for $C_{24}H_{24}Cl_2N_2O_{10}Pt$ $(M+H)^+$:766.0529, found: 766.0651. Mp: 215–217 °C. 98.5% purity determined using HPLC: $t_R$ = 25.6 min.

2.1.2.2. Preparation of compound **2**

Coumarin acid **11** (197 mg, 0.75 mmol) and TBTU (241 mg, 0.75 mmol) was added to a 50 mL flask. Then, dry DMF 5 mL was added as solution. The mixture was allowed to stir for 10 min after injection of TEA (104 μL, 0.75 mmol). Subsequently, oxoplatin **14** (100 mg, 0.30 mmol) was added and the reaction system was stirred for another 48 h at 50 °C. After the reaction completed, the solvent was removed under reduced pressure. The residue was purified by column chromatography. The product was obtained as white solid (54 mg, 22%).

$^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 7.68 (d, *J* = 8.3 Hz, 2H, coumarin 5-*H*), 6.98 (dd, *J* = 7.0, 3.7 Hz, 4H, coumarin 6,8-*H*), 6.56 (br, 6H, N$H_3$), 6.20 (s, 2H, coumarin 3-*H*), 4.19 – 4.03 (m, 4H, OC$H_2$), 2.47 – 2.32 (m, 10H, COC$H_2$, C$H_3$), 2.02 – 1.88 (m, 4H, OCH$_2$C$H_2$). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 180.0 (O=*C*), 161.7 (coumarin 7-*C*), 160.2 (coumarin 2-*C*), 154.8 (coumarin 8a-*C*), 153.4 (coumarin 4-*C*), 126.5 (coumarin 5-*C*), 113.1 (coumarin 3-*C*), 112.4 (coumarin 4a-*C*), 111.1 (coumarin 6-*C*), 101.3 (coumarin 8-*C*), 67.6 (OC$H_2$), 31.8 (COC$H_2$), 25.0 (OCH$_2$C$H_2$), 18.1 (C$H_3$). IR (KBr) ν: 3249, 3080 (Ar-H), 2939 (C$H_2$, N$H_3$), 1699, 1678 (C=O), 1612, 1566, 1510, 1444 (aromatic frame), 1392, 1288, 1271, 1205 1151, 1076, 839, 804 cm$^{-1}$. Mp: 201–203 °C. 98.9% purity determined using HPLC: $t_R$ = 29.0 min.

2.1.2.3. Preparation of compound **3**

Coumarin acid **10** (136 mg, 0.58 mmol) and TBTU (187 mg, 0.58 mmol) was added to a 50 mL flask. Then, dry DMF 5 mL was added as solution. The mixture was allowed to stir for 10 min after injection of TEA (80 μL, 0.58 mmol). Subsequently, oxoplatin **15** (100 mg, 0.23 mmol) was added and the reaction system was stirred for another 48 h at 50 °C. After the reaction completed, the solvent was removed under reduced pressure. The residue was purified by column chromatography. The product was obtained as white solid (54 mg, 27%).

$^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 8.38 – 8.28 (m, 2H, N$H_2$), 7.86 – 7.75 (m, 2H, N$H_2$), 7.64 (d, *J* = 9.4 Hz, 2H, coumarin 5-*H*), 6.94 – 6.88 (m, 4H, coumarin 6,8-*H*), 6.20 (s, 2H, coumarin 3-*H*), 4.81 (s, 4H, OC$H_2$), 2.42 – 2.32 (m, 8H, cyclohexyl C*H*, coumarin-C$H_3$), 2.07 – 2.01 (m, 2H, cyclohexyl C$H_2$), 1.45 – 1.37 (m, 4H, cyclohexyl C$H_2$), 1.03 – 0.91 (m, 2H, cyclohexyl C$H_2$). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 174.6 (coumarin O=*C*), 163.9 (oxaliplatin O=*C*), 161.3 (coumarin 7-*C*), 160.5 (coumarin 2-*C*) (coumarin 2-*C*), 155.0 (coumarin 8a-*C*), 153.8 (coumarin 4-*C*), 126.9 (coumarin 5-*C*), 113.8 (coumarin 3-*C*), 112.8 (coumarin 4a-*C*), 111.7 (coumarin 6-*C*), 101.7 (coumarin 8-*C*), 65.3 (OC$H_2$), 61.4 (cyclohexyl C*H*), 31.2 (cyclohexyl C$H_2$), 23.9 (cyclohexyl C$H_2$), 18.6 (C$H_3$). IR (KBr) ν: 1737 (C=O), 1619, 1558, 1510, 1427 (aromatic frame), 1392, 1367, 1248, 1153, 1080, 850, 812, 708 cm$^{-1}$. HRMS: Calcd. for $C_{32}H_{32}N_2O_{14}Pt$ $(M+H)^+$: 864.1574, found: 864.1537. Mp: 199–201 °C. 97.9% purity determined using HPLC: $t_R$ = 25.0 min.

2.1.2.4. Preparation of compound **4**

Coumarin acid **10** (152 mg, 0.58 mmol) and TBTU (187 mg, 0.58 mmol) was added to a 50 mL flask. Then, dry DMF 5 mL was added as solution. The mixture was allowed to stir for 10 min after injection of TEA (80 μL, 0.58 mmol). Subsequently, oxoplatin **15** (100 mg, 0.23 mmol) was added and the reaction system was stirred for another 48 h at 50 °C. After the reaction completed, the solvent was removed under reduced pressure. The residue was purified by column chromatography. The product was obtained as white solid (65 mg, 31%).

$^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 8.56 – 8.33 (m, 2H, N$H_2$), 8.28 – 8.05 (m, 2H, N$H_2$), 7.74 – 7.59 (m, 2H, coumarin 5-*H*), 7.00 – 6.86 (m, 4H, coumarin 6,8-*H*), 6.20 (d, *J* = 1.1 Hz, 2H, coumarin 3-*H*), 4.13 – 4.01 (m, 4H, OC$H_2$), 2.62 – 2.51 (m, 2H, cyclohexyl C*H*), 2.46 (t, *J* = 7.3 Hz, 4H, COC$H_2$), 2.39 (s, 6H, coumarin-C$H_3$), 2.15 – 2.03 (m, 2H, cyclohexyl C$H_2$), 1.99 – 1.86 (m, 4H, OCH$_2$C$H_2$), 1.46 – 1.27 (m, 4H, cyclohexyl C$H_2$), 1.11 – 0.96 (m, 2H, cyclohexyl C$H_2$). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 180.5 (coumarin O=*C*), 163.9 (oxaliplatin O=*C*), 162.1 (coumarin 7-*C*), 160.6 (coumarin 2-*C*), 155.2 (coumarin

8a-*C*), 153.8 (coumarin 4-*C*), 126.9 (coumarin 5-*C*), 113.5 (coumarin 3-*C*), 112.8 (coumarin 4a-*C*), 111.5 (coumarin 6-*C*), 101.7 (coumarin 8-*C*), 67.9 (O*C*$H_2$), 61.5 (cyclohexyl C*H*), 32.5 (CO*C*$H_2$), 31.4 (cyclohexyl C*H*$_2$), 25.2 (OCH$_2$*C*H$_2$), 23.9 (cyclohexyl C*H*$_2$), 18.5 (*C*$H_3$). IR (KBr) ν: 3190, 3070 (Ar-H), 2950, 2870 ($CH_2$, $NH_2$), 1730, 1660 (C=O), 1621, 1557, 1510, 1450 (aromatic frame), 1368, 1291, 1204, 1150, 1074, 1029, 847, 806 $cm^{-1}$. Mp: 180–182 °C. 98.3% purity determined using HPLC: $t_R$ = 28.1 min.

*2.2. Biological evaluation*

*2.2.1. In vitro cellular cytotoxicity assay*

RPMI1640, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), 0.25% trypsin/EDTA solutions and penicillin-streptomycin solutions were purchased from Gibco. MTT was purchased from Sigma. The cells were maintained in RPMI1640 (for SKOV-3, A549, A549R cells) or DMEM (for HeLa, HeLa/DDP cells) medium containing 10% FBS in a humidified atmosphere containing 5% $CO_2$ at 37 °C. The A549R and HeLa/DDP cells were maintained with 2 μg/mL cisplatin. Phosphate buffered saline (PBS) contained 137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$ and 2 mM $KH_2PO_4$ (pH = 7.4). MTT solution (5 mg·$mL^{-1}$) for MTT assays was prepared before used.

The tumor cells were seeded in 96-well plates at 5000 cells per well in 100 μL of complete medium, and incubated in a 5% $CO_2$ atmosphere at 37 °C for 24 h. Then 100 μL of freshly prepared culture medium containing drugs at different concentrations were added. The cells were further incubated for 48 h. After that, the freshly prepared MTT solution (5 mg·$mL^{-1}$) 20 μL was added and the mixtures were incubated for another 4 h. After removal of the medium, the residue was dissolved in DMSO (150 μL) and quantified by a microplate reader (570 nm). The $IC_{50}$ values were calculated using GraphPad Prism 6 based on three parallel experiments.

*2.2.2. HSA binding experiments*

2.2.2.1. UV-vis spectra assay

To detect the combination of the title platinum(IV) compounds with HSA, the UV-vis spectroscopy was used. The experiments were carried out in Tris-HCl buffer (10 mM Tris-HCl/10 mM NaCl, pH = 7.4) at room temperature using a Scinco S-3100 UV-vis spectrophotometer in the range of 200 nm to 500 nm with a scan rate of 600 nm·$min^{-1}$. The final spectra were background-corrected by subtracting the buffer spectra. The UV-vis of HSA (10.0 μM) in buffer was recorded as a negative experiment (spectrum **A**). The solution containing compound **3** (15.0 μM) and HSA (10.0 μM) was measured (spectrum **B)**. Then, the solution of free compound **3** (15.0 μM) in buffer was tested (spectrum **C**). To judge the interaction of compound **3** with HSA, the subtraction spectrum **D** of the mixed solution compound **3**-HSA (spectrum **B)** and free compound **3** (spectrum **C)** was calculated and drawn to compare with HSA (spectrum **A)**.

2.2.2.2. Fluorescence spectra assay

The fluorescence experiments were performed in Tris-HCl buffer (10 mM Tris-HCl/10 mM NaCl, pH = 7.4). The quenching spectra of HSA solution (4.0 μM) by compounds **1–4**, **14, 15**, cisplatin and oxaliplatin (0.0–12.5 μM, at increments of 1.25 μM) were recorded at 298K. Then the quenching spectra of HSA solution (4.0 μM) by compound **3** (0.0–12.5 μM, at increments of 1.25 μM) at 295 K and 301 K were also tested. A 1.00 cm quartz cell was used for the measurements. The well-mixed solutions were incubated in water bath for 20 min for equilibrium, and the fluorescence emission spectra were then tested from 285 to 500 nm ($\lambda$ex = 280 nm) at scan rate of 1200 nm·$min^{-1}$. The widths of both the excitation and emission slits were 5.0 nm.

2.2.2.3. CD spectrum assay

The CD spectra were recorded on a Jasco J-810 spectropolarimeter under nitrogen stream with two channels, CD and HT. The CD spectrum was recorded as an average of three scans at room temperature in the range of 190 to 350 nm with a scan speed of 100 nm·min$^{-1}$ and 1 s response time. The final spectrum was background-corrected by subtracting the corresponding buffer spectra. The HSA-Compd. solution containing HSA (2 μM) and platinum(IV) complex **3** (2 μM) in buffer solution (10 mM Tris-HCl/10 mM NaCl, pH 7.4) was incubated for 2 h in dark at room temperature. As negative experiment, the CD spectrum of HSA (2 μM) solution without tested complex was also tested to compare with the HSA-Compd. spectrum to detect if complex **3** interact with HSA.

*2.2.3. Cell uptake and DNA platination*

The SKOV-3 cells were seeded in 6-well cell culture plate and incubated for 3 h at 37 °C. Then the cells were treated with platinum compounds (100 μM) including compounds **2**, **3** and reference drugs cisplatin, oxaliplatin for 10 h in incubator. Then all the cells were harvested, collected and washed for three times with PBS. *Ca.* 1 million cells were mineralized with 70% $HNO_3$ (LC), and the platinum in cells was determined with ICP-MS. Results were presented as the mean of 3 determinations for each data point.

To test the DNA platination of the tumor cells, the DNA of *ca.* 1 million cells was isolated with a Genomic DNA Mini Preparation Kit. Then the DNA was mineralized with 70% $HNO_3$ and measured by ICP-MS. The results were calculated based on 3 determinations for each data point.

*2.2.4. The antitumor mechanism detection*

2.2.4.1. Reduction of platinum(IV) complexes by AsA

The reduction of platinum(IV) complexes and the further DNA binding properties of the reduced platinum(II) compounds were detected by HPLC. HPLC analyses were performed on Thermo Ultimate 3000 RS equipped with an Agilent Eclipse XDB-C18 column (250×4.6 mm, 5 μm) with a flow rate of 1 mL/min.

The linear gradient was given in Table 1.

**Table 1.** The linear gradient for HPLC.

| Time (min) | A (0.1% aqueous TFA) | B (Methanol) |
|---|---|---|
| 0 | 90 | 10 |
| 5 | 90 | 10 |
| 35 | 0 | 100 |
| 45 | 0 | 100 |

To confirm the stability of platinum(IV) complexes, the solution of compound **3** (250 μM) in PBS was evaluated for 88 h. The reduction potential was confirmed by the evaluation of compound **3** (250 μM) in the presence of ascorbic acid (AsA) (1 mM, similar concentration as in tumor cells). The DNA interaction abilities were testified with the addition of guanosine-5'-monophosphate (5'-GMP) (3 mM) which was often applied as a model of DNA base. With the aim of confirming if the reduction of platinum(IV) compound was essential for DNA combination, the solution containing compound **3** (250 μM) and 5'-GMP (3 mM) without AsA was also detected as negative experiment.

2.2.4.2. The cyclooxygenase inhibitory experiment

The cyclooxygenase inhibition experiment was carried out according to the manufacturer's protocol (Cyclooxygenase 2 inhibitor screening kit, Beyotime, China). The rhCOX-2 was applied in the experiments. The coumarin platinum(IV) compound **3** was evaluated at concentrations of 5 μM, 62 μM, 125 μM, 250 μM as rhCOX-2 inhibitor, meanwhile the precursor coumarin **7** and acid **10** at the corresponding concentrations 10 μM, 125 μM, 250 μM, 500 μM were also tested.

Then, the mixtures of coumarin **7** with oxaliplatin **13** and oxoplatin **15** (**13/15** + **7**) (1 equivalent of **13/15** mixed with 2 equivalents of **7**) were also evaluated at concentrations of 62 μM, 125 μM and 250 μM. The sample with no rhCOX-2 inhibitor was evaluated as blank, and the celecoxib was used at the advised concentration (100 nM) in the protocol as positive reference sample.

*2.2.5. Apoptosis experiments*

The apoptosis experiment was carried out according to the manufacturer's protocol (Annexin V-FITC/PI Apoptosis Detection Kit, KeyGEN, China). SKOV-3 cells were incubated for 24 h in incubator with or without the test compounds (blank: no compound; cisplatin, oxaliplatin, compound **3**: 20 μM). Then the cells were harvested and strained by annexin V-FITC and PI for 15 min at room temperature. The apoptosis-inducing properties were measured using a flow cytometric assay.

## 3. Results and discussion

### *3.1. Chemistry*

The precursor 7-hydroxycoumarin **7** was conveniently prepared starting from resorcinol **5**, ethyl acetoacetate **6** and oxalic acid (Scheme 1). The reaction of coumarin **7** with compounds **8** and **9** afforded the ester derivatives, and the following hydrolysis with sodium hydroxide yielded the coumarin acids **10** and **11**. The oxoplatin **14** and **15** were obtained by the oxidation of cisplatin **12** and oxaliplatin **13** in the presence of hydrogen peroxide. The coupling reaction of oxoplatin with coumarin acids in dry DMF in the presence of TBTU and TEA offered the title compounds **1–4** in yields of 22%−34%.

The structure of the title compounds were confirmed by $^{1}$H-NMR, $^{13}$C-NMR, HRMS and IR. The purity was confirmed by HPLC, and the melting points were also tested. Final purity of all title compounds exceeds 97.9%. The spectral data are in accordance with the assigned structures. In $^{1}$H-NMR spectra, 7-hydroxycoumarin groups in title compounds display signal peaks in down field of 6.0−7.7 ppm. The -$NH_3$ in cisplatin core of compounds **1**, **2** display broad peaks at about 6.6 ppm, meanwhile the -$NH_2$ in oxaliplatin core of complexes **3**, **4** exhibit two peaks in area of 7.8−8.3 ppm. The cyclohexyl moieties on oxaliplatin cores give typical absorption signals below 3.0 ppm. In the $^{13}$C-NMR spectra, all carbons display proper $^{13}$C-NMR peaks in accordance with the assigned structures. As for the IR spectra, the strong absorption peaks in the range of 1600−1800 $cm^{-1}$ are ascribed to the stretching vibration of C=O in the coumarin and oxaliplatin moieties, and the aromatic frame of coumarin scaffold gives typical absorption bands in 1400−1650 $cm^{-1}$. The HRMS of the tested compounds further confirms the formation of coumarin platinum(IV) complexes. Accordingly, the spectral data were in accordance with the assigned structures.

### *3.2. Antitumor activities in vitro*

The 7-hydroxycoumarin platinum(IV) complexes **1–4**, free 7-hydroxycoumarin acid **11**, oxoplatin **14**, **15** and the mixtures of **11** with **14** and **15** (**11&14/15**) were evaluated for antitumor activities using a MTT assay against five tumor cell lines including ovarian cancer (SKOV-3), lung cancer (A549), cervical cancer (HeLa) and two cisplatin resistant cells A549R and Hela/DDP taking cisplatin and oxaliplatin as reference drugs. The results were shown in Table 2 expressed as $IC_{50}$ values. The resistant factors (RF) of the cisplatin resistant cells to the tested compounds were also calculated based on the $IC_{50}$ values.

It was observed that the conjunction of coumarin fragment to platinum(IV) system is a useful strategy to improve antitumor activities. The title compounds **1–4** exhibit moderate to significant activities to all tested cell lines, which are remarkably more effective than the precursor coumarin acid **11** and oxoplatins **14** and **15**. Then, the combined mixtures **11&14/15** give no remarkable enhanced bioactivities in comparison with the free compounds.

The SAR analysis manifests that the platinum core and the linkage in the structure of the title compounds exert crucial influence on the bioactivities simultaneously. Compound **3** displays the most effective antitumor abilities among the tested compounds which are comparable to cisplatin and better than oxaliplatin especially against SKOV-3, A549R and HeLa/DDP. Complex **3** with oxaliplatin core possesses larger volume than the corresponding cisplatin derived one **1**, and exhibits superior activities than complex **1** especially to SKOV-3 and HeLa/DDP which exceed 2-fold more potent. The steric hindrance of cyclohexanediamine ligand in oxaliplatin core is favorable for inducing fatal damage to DNA and reducing the DNA self-repair of the tumor cells. When the linkage is converted to three carbons one, compounds **2** and **4** with increased volumes exhibit rather reduced activities in comparison with complexes **1** and **3**. Subsequently, it seems that larger steric hindrance of platinum core and shorter linker in the title 7-hydroxyxoumarin platinum(IV) complexes are more suitable for tumor proliferation inhibition.

It was generally accepted that the drug resistances of platinum drugs were induced by the reduced drug uptake, degradation and deactivation by intracellular thiols in cells, etc.[9] The bioactivities to cisplatin resistant cells A549R and HeLa/DDP demonstrate that this series of compounds are of much potential in overcoming drug resistance of platinum(II) drugs. Especially, compound **3** reduces the RF values toward A549R and HeLa/DDP to 1.1 and 1.9 respectively, which are lower than cisplatin (2.6 and 4.9) and oxaliplatin (1.6 and 2.4). These facts are probably because of that the stable configuration of platinum(IV) complexes **1–4** effectively reduces the intracellular deactivation of platinum drugs by thiols. Moreover, construction of coumarin platinum(IV) complexes enhances the uptake in tumor cells and DNA (see 3.4). Additionally, the COX inhibition of the title compounds also could improve the bioactivities to some extent besides the DNA damage of the reduced platinum(II) complex (see 3.5). Subsequently, the abilities of the title compounds to overcome cisplatin resistance are probably a synergistic functional procedure. Accordingly, the 7-hydroxycoumarin platinum(IV) complexes are of much value for further development as antitumor agents, and the most prominent complex **3** was selected for the following investigations.

In comparison to cou-platin **IV** with mono 7-hydroxycoumarin in platinum(IV) system[32], the incorporation of the second coumarin group into platinum(IV) system to yield the title complexes shows no remarkable positive effects on the bioactivities, which is probably due to the limited solubility. These results provide new reference for future design of coumarin platinum(IV) drugs.

**Table 2.** Cytotoxicity profiles of coumarin platinum(IV) complexes toward five human carcinoma cell lines expressed as $IC_{50}$ (μM).

| Compd. | Connolly Surface: Volume/ Area | SKOV-3 | A549 | A549R | RF(A549R)[a] | HeLa | HeLa/DDP | RF(HeLa/DDP)[a] |
|---|---|---|---|---|---|---|---|---|
| **1** | 488.61 Å³/ 478.55 Å² | 2.5±0.6 | 7.3±1.2 | 27.6±3.8 | 3.8 | 8.2±3.2 | 30.9±7.6 | 3.8 |
| **2** | 544.87 Å³/ 543.89 Å² | 3.8±0.9 | 25.9±3.5 | 23.2±4.7 | 0.9 | 51.7±9.5 | 39.9±8.6 | 0.8 |
| **3** | 582.55 Å³/ 526.44 Å² | 1.1±0.5 | 14.8±3.0 | 16.1±5.5 | 1.1 | 5.5±1.2 | 10.3±1.7 | 1.9 |
| **4** | 635.18 Å³/ 602.22 Å² | 31.2±2.0 | 77.7±6.8 | 70.2±12.8 | NC[c] | 85.1±15.6 | 63.7±4.6 | NC |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **11** | NC | >100 | 86.6±13.5 | >100 | NC | >100 | >100 | NC |
| **11&14** [b] | NC | 32.1±4.7 | 35.7±2.2 | 64.6±3.4 | NC | 34.8±4.2 | 29.6±5.5 | NC |
| **11&15** [b] | NC | 56.3±5.4 | 75.2±10.8 | 85.4±12.7 | NC | 75.4±8.6 | 54.3±9.3 | NC |
| **14** | NC | 32.6±7.1 | 45.8±18.5 | 59.8±7.6 | NC | 42.8±15.8 | 31.5±5.9 | NC |
| **15** | NC | 61.5±15.8 | >100 | 68.5±24.2 | NC | 77.6±18.7 | 54.6±15.7 | NC |
| **Cisplatin** | 85.71 Å³/ 109.81 Å² | 1.6±0.3 | 10.5±1.7 | 27.4±5.4 | 2.6 | 3.2±0.5 | 15.8±2.9 | 4.9 |
| **Oxaliplatin** | 183.06 Å³/ 196.08 Å² | 3.8±0.8 | 15.7±2.6 | 24.6±4.8 | 1.6 | 9.8±2.5 | 23.6±4.9 | 2.4 |

[a] RF: Resistant factor. RF(A549) = $IC_{50}$(A549R)/$IC_{50}$(A549); RF(HeLa) = $IC_{50}$(HeLa/DDP)/$IC_{50}$(HeLa).

[b] **11&14/15**: 2 equivalents of acid **11** mixed with 1 equivalent of oxoplatin **14** or **15**.

[c] NC: Not calculated.

*3.3. HSA binding studies*

It was widely accepted that the protein binding of small molecular drugs in blood assumes great importance in their pharmacokinetic and pharmacodynamic properties such as the stability, bioactivity and toxicity, etc. The human serum albumin (HSA), as the most abundant protein constituent of blood plasma, is considered as an important protein influencing the delivery, distribution, free concentration and metabolism of exogenous drugs. Moreover, the formation of HSA-drug complexes is an effective strategy to defeat the poor tumor targeting drawbacks of platinum drugs owing to the enhanced permeability and retention (EPR) effect.[39] Herein, the HSA binding properties of 7-hydroxycoumarin platinum(IV) complex were investigated by fluorescence, circular dichroism (CD) and UV-vis absorption techniques taking compound **3** as a typical example.

*3.3.1. The fluorescence analysis*

The HSA shows a strong intrinsic fluorescence emission at about 343 nm under the excitation of 280 nm, and the fluorescence intensity would change upon the interaction with small molecules. The effects of the platinum(IV) complexes on the fluorescence intensity of HSA at 298 K in Figure 2 and S5 reveal that complexes **1–4** could induce fluorescence quenching of HSA. With the gradually addition of platinum(IV) compounds from 0.0 to 12.5 μM, the fluorescence emission of HSA decreases steadily with a significant red shift, which evidences the interaction of platinum(IV) compounds with HSA in solution. The quenching procedures are in agreement with the Stern-Volmer equation, and the Stern-Volmer quenching constants $K_{SV}$, quenching rate constants $K_q$ and binding constants $K_b$ are calculated and offered in Table S3. It is noticed that the $K_q$ of all compounds **1–4** are much greater than the maximum scatter collision quenching constant of the biomolecule ($2.0 \times 10^{10}$ $M^{-1}S^{-1}$) which indicate the probable static quenching procedures. Moreover, the oxaliplatin derivatives **3**, **4** could more easily bind to HSA and exert larger $K_b$ values than the cisplatin compounds **1**, **2**. Then, the reference drugs including platinum(II) drugs cisplatin, oxaliplatin and the platinum(IV) precursors **14**, **15** were also evaluated. It is displayed that cisplatin, oxaliplatin and compounds **14**, **15** without coumarin ligand induce negligible fluorescence quenching of HSA. These facts demonstrate that the incorporation of coumarin moiety dramatically promotes the title platinum(IV) complexes to interact with HSA.

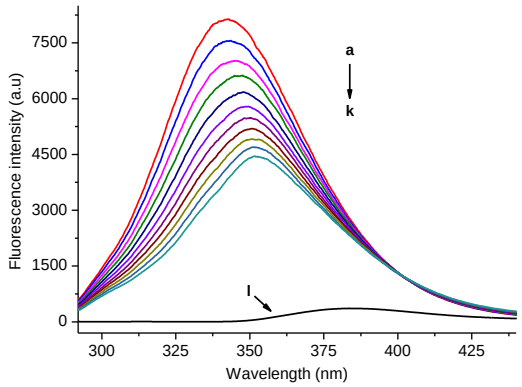



**Figure 2.** Fluorescence spectra of HSA in the absence and presence of platinum(IV) complex **3** (λex= 280nm, T= 298K). a–k: *c*(HSA) = 4.0 μM, *c*(complex **3**) = 0.0–12.5 μM, at increments of 1.25 μM; l: *c*(complex **3**) = 12.5 μM.

The quenching procedures of HSA are principally classified as static quenching or dynamic quenching. To identify the probable quenching mode, the fluorescence spectra of HSA in the absence and presence of platinum(IV) complex **3** at other two temperatures 295 K and 301 K (Figure S1) were also recorded besides 298 K. Three quenching trends are all in agreement with the Stern-Volmer equation. The $K_{SV}$ (Table 3) are inversely correlated with temperature. It has been proved that the quenching constants in dynamic quenching procedures would improve with the increasing temperature; meanwhile the static quenching procedures would exert slightly lower quenching constants in higher temperatures. The $K_q$ (6.33–8.50 × $10^{12}$ $M^{-1}S^{-1}$) in Table 3 decreases with the increasing temperature and are higher than the maximum scatter collision quenching constant of the biomolecule (2.0 × $10^{10}$ $M^{-1}S^{-1}$). These facts prove that 7-hydroxycoumarin platinum(IV) complex **3** could quench the fluorescence emission of HSA in a static quenching procedure.

**Table 3.** Stern-Volmer quenching constants and quenching constants for the interaction of compound **3** with HSA at three temperatures.

| T (K) | $10^{-4}K_{SV}$ ($M^{-1}$) | $10^{-12}K_q$ ($M^{-1}S^{-1}$) |
|---|---|---|
| 295 | 8.50 ± 0.23 | 8.50 ± 0.23 |
| 298 | 8.14 ± 0.19 | 8.14 ± 0.19 |
| 301 | 6.33 ± 0.12 | 6.33 ± 0.12 |

**Table 4.** The binding constants and binding sites of compound **3**-HSA interaction.

| T (K) | $10^{-5}K_b$ ($M^{-1}$) | *n* |
|---|---|---|
| 295 | 7.612 ± 0.075 | 1.19 |
| 298 | 4.205 ± 0.092 | 1.14 |
| 301 | 2.167 ± 0.099 | 1.11 |

To further understand the interaction mode of compound **3** with HSA, the binding constants $K_b$ and binding sites *n* were calculated (Table 4). The $K_b$ reduces slightly with the rise of temperature from 295 K to 301 K which is in line with $K_{SV}$. The calculation of binding sites *n* manifests that one high affinity binding site is associated with the HSA combination of complex **3**. Then the thermodynamic parameters were obtained (Table 5) to conclude the type of interaction forces in this process (The detail calculation procedure was provided in ESI). The negative Gibbs free energy change $\Delta G$ indicates the spontaneous combination of 7-hydroxycoumarin platinum(IV) complex with HSA in solution, and the process is primarily driven by enthalpy ($\Delta H < 0$, $\Delta S < 0$). Moreover, the negative enthalpy change and entropy change disclose that the above HSA conjunction is probably attributed to hydrogen bond and van der Waals force.

**Table 5.** The thermodynamic parameters of compound **3**-HSA interaction.

| T (K) | $\Delta H$ (kJ/mol) | $\Delta G$ (kJ/mol) | $\Delta S$ (J/mol K) |
|---|---|---|---|
| 295 | -154.54 ± 0.70 | -33.25 ± 1.39 | -411.16 ± 2.35 |
| 298 | | -32.01 ± 1.40 | |
| 301 | | -30.78 ± 1.41 | |

*3.3.2. CD spectroscopy*

The CD spectroscopy is widely applied in the monitor of the interaction of protein with small molecular compounds. To further validate the HSA combination of the platinum(IV) complex, the CD spectra of HSA were measured with and without complex **3** (Figure 3). The HSA (2 μM) exhibits two absorption bands at 208 and 220 nm, which are typical characters of the α-helix structure of classic proteins. The addition of complex **3** (2 μM) induces a decrease at 208 nm, revealing the secondary structural changes of protein, which is probably ascribed to the combination of HSA with platinum(IV) compound. The further quantitative calculation shows that complex **3** reduces the α-helix content from 47.1% of free HSA to 46.0% of complex **3**-HSA system (at ratio of 1:1). Accordingly, the CD spectroscopy demonstrates that the 7-hydroxycoumarin platinum(IV) could interact with HSA and induce secondary structural changes of the protein, which are coincident with the results of fluorescence analysis.

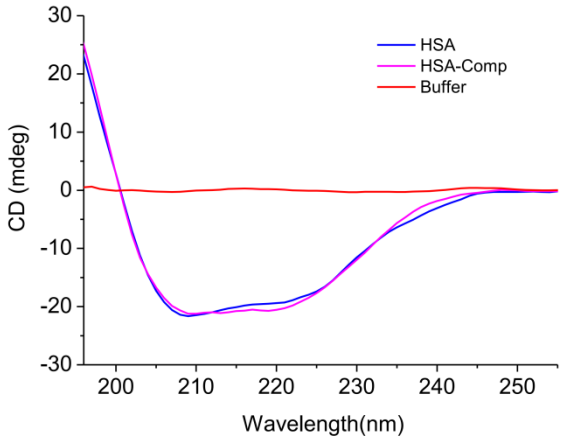



**Figure 3.** CD spectra of HSA in the absence and presence of the platinum(IV) compound **3**. Buffer: 10 mM Tris-HCl/10 mM NaCl, pH 7.4; HSA: *c*(HSA) = 2 μM, HSA-Compd.: *c*(HSA) = 2 μM, *c*(compound **3**) = 2 μM.

*3.3.3. UV-vis spectroscopy*

The UV-vis spectroscopy is used to investigate the interaction of 7-hydroxycoumarin platinum(IV) complex **3** with HSA. The results in Figure 4 prove that the UV-vis absorption of HSA (Curve A) in the range of 250–300 nm is not superposed to the subtraction spectrum (Curve D) of the mixed solution compound **3**-HSA (Curve B) and compound **3** (Curve C). These results supply new proof to confirm the combination of compound **3** with HSA.

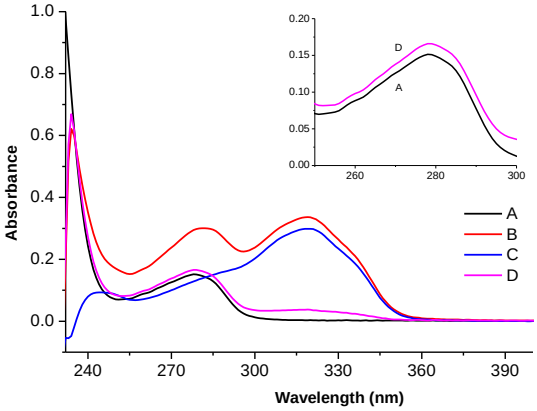



**Figure 4.** UV-vis spectra of HSA with and without compound **3**. (A) absorption spectrum of HSA, *c*(HSA) = 10 μM; (B) absorption spectrum of compound **3**-HSA system, *c*(HSA) = 10 μM, *c*(compound **3**) = 15 μM; (C) absorption spectrum of compound **3**, *c*(compound **3**) = 15 μM; (D) subtracting spectrum of (B) and (C). Inset: the curves (A) and (D) for the wavelength ranging from 250 to 300 nm.

*3.4. Cellular uptakes and DNA platination of compounds **1** and **3***

The cellular uptakes make a great deal of sense for the bioactivities of drugs. Moreover, the accumulation of platinum drugs in DNA influences their antitumor efficacy directly. To explore the mechanism of the title compounds, the cellular uptakes and DNA platination of compounds **1** and **3** were measured using an ICP-MS method. After 10 h incubation with different platinum compounds (100 μM), the platinum content in SKOV-3 cells were determined (Figure 6). The accumulations of complexes **1** and **3** are 2.6–2.9 times higher than that of cisplatin and oxaliplatin, meanwhile, the coumarin platinum(IV) complexes also generate high DNA platination in comparison with cisplatin and oxoplatin (4.3–2.8 folds higher). Thereby, the ligation of coumarin fragment leads pronounced positive effects on the cellular uptakes in both whole tumor cells and DNA. These results are probably ascribed to the enhanced lipophilicity of the title compounds in comparison with the platinum(II) drugs, which would facilitate their membrane permeability.[40-42] Accordingly, cellular responses differ from that of the traditional platinum drugs might exist in the tested coumarin platinum(IV) complexes.

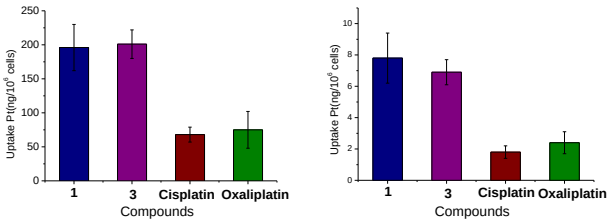



**Figure 6.** Cellular uptake and DNA platination of complexes **1**, **3**, cisplatin and oxaliplatin in SKOV-3 cells. (A) Platinum in whole cells; (B) Platinum in DNA.

*3.5. The antitumor mechanism detection*

The coumarin platinum(IV) complex was expected to possess DNA injury *via* the liberated platinum fragment, and exert COX inhibition properties from the coumarin unit. To detect the bi-functional mechanism of the target compound, the DNA damage activities were monitored by HPLC, and the COX inhibitory properties were also evaluated.

*3.5.1. Reduction and DNA damage properties of compound **3***

The reduction is an essential step in the activation of platinum(IV) compounds. To determine the reduction potential of the 7-hydroxycoumarin platinum(IV) complexes, compound **3** was measured using a HPLC method in the absence and presence of AsA as reductant. It is disclosed that compound **3** keeps stable for at least 88 h in solution (Figure S5). The addition of 5'-GMP as a model of DNA leads to no reduction of the platinum(IV) compounds (Figure S6). Then compound **3** undergoes a gradual reduction in the presence of AsA with the quenching of the UV absorption peak (r.t. = 25 min), accompanied by the emergence of the peak of acid **10** (Figure 7). Besides that, the generation of platinum(II) complex is also verified by the observation of platined GMP peak which indicates the DNA damage competence of coumarin platinum(IV) complex.[43] These facts demonstrate that the coumarin platinum(IV) complexes could be reduced in reducing environment, and further cause DNA injury to tumor cells by the liberated platinum(II) complexes.

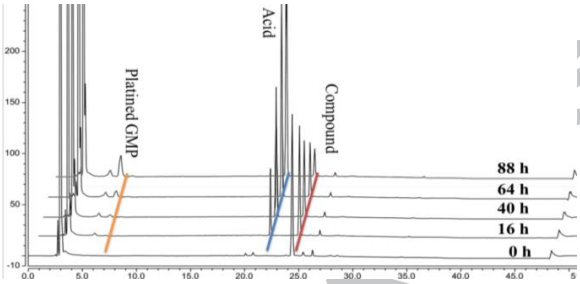



**Figure 7.** HPLC spectra of compound **3** in the presence of AsA and 5'-GMP.

*3.5.2. The cyclooxygenase inhibition*

To evaluate whether the incorporation of coumarin moiety endows the platinum(IV) complexes with COX inhibitory properties, compound **3** was evaluated with recombinant human COX-2 (rhCOX-2) as target enzyme. As reference, celecoxib, coumarin **7** and acid **10** as well as the mixtures of coumarin **7** with oxaliplatin **13** and oxoplatin **15** (**13/15** + **7**) were also evaluated. The results in Figure 8 display that the coumarin **7** shows weak inhibitory abilities to COX-2, meanwhile the coumarin acid **10** displays moderate COX-2 inhibition at high concentrations. Then, the mixture of coumarin **7** with oxaliplatin **13** and oxoplatin **15** (**13/15** + **7**) exhibit no remarkable enhanced bioactivities. Notably, coumarin platinum(IV) complex **3** could inhibit the activities of rhCOX-2 in a dose dependent manner. With the increasing concentration of compound **3** from 5 μM to 250 μM, the inhibited proportion of rhCOX-2 raises from 20.1% to 65.8%, which is coincide to the trend of coumarin acid **10** (from 16.6% to 47.3%). Accordingly, the coumarin platinum(IV) complex in tetravalent form displays COX inhibitory properties, meanwhile the released coumarin acid after reduction also inhibits the activities of COX to some extent. Summarily, it is reasonable to conclude that the COX inhibitory competence of the coumarin platinum(IV) complex are of great potential in reducing tumor-associated inflamation in the tumor proliferate inhibition progress.

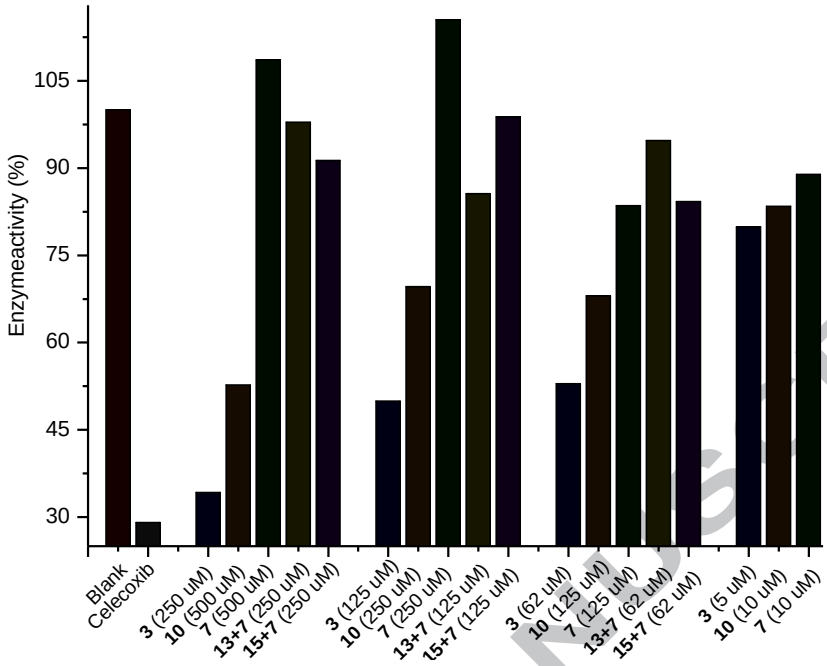


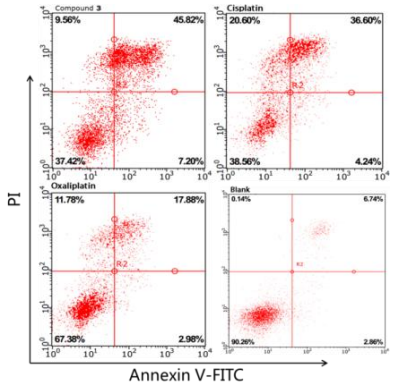

**Figure 8.** The inhibition of the selected compounds at different concentrations to rhCOX-2. Compound **3**: 5 μM, 62 μM, 125 μM, 250 μM; Compounds **7** and **10**: 10 μM, 125 μM, 250 μM, 500 μM; Compound **13/15** + **7** (1 equivalent of **13/15** mixed with 2 equivalents of **7**): 62 μM, 125 μM, 250 μM. Celecoxib: 100 nM.

Thereby, it is concluded that the 7-hydroxycoumarin platinum(IV) compounds exhibit a synergistic bi-functional action mechanism. On one hand, the HPLC results manifest that the platinum(IV) compounds would be reduced in tumor tissues and release equivalent platinum(II) complexes which would exert DNA damage to tumor cells. On the other hand, the COX inhibitory assay demonstrates that the title compounds could inhibit the COX activities, which would be helpful in deducing the tumor-related inflammation. This bi-functional action mode may play key roles in improving the biological activities and overcoming drug-resistance of cisplatin.

*3.6. Apoptosis experiments*

The apoptosis of SKOV-3 cells by compound **3** were evaluated using an annexin V-FITC/propidium iodide (PI) staining assay with cisplatin and oxaliplatin as reference drugs. As displayed in Table 6, complex **3** causes 62.58% cells undergoing apoptosis at concentration of 20 μM with early apoptosis of 7.20% and late apoptosis of 45.82% respectively. The apoptosis inducing competence of complex **3** is comparable to that of cisplatin and better than oxaliplatin at the same concentration. Meanwhile, it is noticed that compound **3** induces only 9.56% of necrosis, which is lower than the references cisplatin and oxaliplatin (20.60% and 11.78%). Generally, the inflammation is related with the necrotic mechanism of cancer cells in the anticancer therapy, which liberates a huge amount of proteins and other metabolic fragments with important antigenic properties. The low necrosis portion induced by complex **3** is probably ascribed to the inflammation inhibition of the coumarin platinum(IV) complex. Accordingly, the results above suggest that the tumor proliferation inhibition of the coumarin platinum(IV) compound correlates with apoptosis.

**Table 6.** Quantification of apoptosis in SKOV-3 cells using an annexin V-FITC/PI staining assay.




| Compd. | Early apoptosis | Late apoptosis | Necrosis | Sum |
|---|---|---|---|---|
| **3** | 7.20 | 45.82 | 9.56 | 62.58 |
| Cisplatin | 4.24 | 36.60 | 20.64 | 61.48 |
| Oxaliplatin | 2.98 | 17.88 | 11.74 | 32.6 |
| Untreated | 2.86 | 6.74 | 0.14 | 9.74 |

## 4. Conclusions

In summary, this work presents the synthesis, characterization, and anticancer properties of a series of bi-functional platinum(IV) complexes in which two 7-hydroxycoumarin units are appended to the axial positions of cisplatin or oxaliplatin center. The antitumor evaluations *in vitro* indicate that all the 7-hydroxycoumarin platinum(IV) compounds exhibit moderate to potent antitumor activities to the tested tumor cell lines. The SAR study evidences the significant impacts of platinum core and linkage on the activities. Particularly, oxaliplatin derived complex **3** with one carbon liner exhibits the most prominent antitumor efficacy. Moreover, the bioactivities of the title compounds to A549R and Hela/DDP indicate that these compounds exert much potential in overcoming drug resistance of cisplatin. The action mechanism investigation displays that the coumarin platinum(IV) compounds in reducing microenvironment could be reduced to platinum(II) complexes and release coumarin acids. They display DNA damage to tumor cells by the liberated platinum(II) compounds. Then the coumarin units endow the title compounds with COX inhibitory activities. The synergistic bi-functional action mechanism is favour for reducing tumor-related inflammation and further influencing tumor proliferation. The conversion of platinum(II) complex to platinum(IV) compound *via* the incorporation of 7-hydroxycoumarin fragment leads to higher platinum accumulation in both the whole tumor cells and DNA. Moreover, the HSA interaction investigation manifests that the tested coumarin platinum(IV) compound could combine with HSA *via* hydrogen bond and van der Waals force. Eventually, the attractive biological performance of bi-functional coumarin platinum(IV) complexes mentioned above could be of prime importance for further endeavours at developing novel platinum drugs based on platinum(IV) backbone in future.

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**Declaration of interest**

None.

**Abbreviations**

AsA ascorbic acid
COX cyclooxygenase
DMF *N*,*N*-dimethylformamide
DMSO dimethyl sulfoxide
5'-GMP guanosine-5'-monophosphate
HPLC high performance liquid chromatography
HSA human serum albumin
$IC_{50}$ half maximal inhibitory concentration
ICP-MS inductively coupled plasma mass spectrometry
MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NSAIDs nonsteroidal anti-inflammatory drugs
PBS phosphate buffer saline
RF resistant factor
TBTU *N*,*N*,*N'*,*N'*-tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate
TEA *N*,*N*,*N*-triethylamine
TFA trifluoroacetic acid

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Graphical Abstract

# Synthesis and evaluation of bi-functional 7-hydroxycoumarin platinum(IV) complexes as antitumor agents

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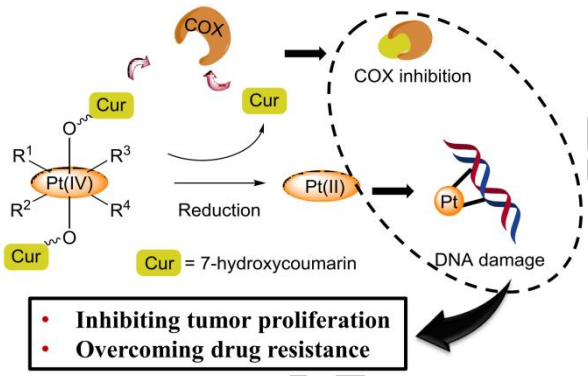

**Highlights**

- Bifunctional 7-hydroxycoumarin platinum(IV) complexes as antitumor agents
- Have much potential in overcoming cisplatin drug-resistance
- Antitumor mechanism was investigated
- Enhanced accumulation in whole cells and DNA
- Combination with HSA by noncovalent forces

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