Electronic Supporting Information

for

Long-acting response of COX-2-mediated metastasis inhibition by oxaliplatin-based CP-L-OHP

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Content

Experiment

1.1 Cell culture

The human cancer cells, including lung cancer cell line A549, epithelial cervical cancer cell line HeLa, colon cancer cell line LoVo, gastric cancer cell line BGC-823 and normal liver cell line LO2 were obtained from the American Type Culture Collection (ATCC). A549 and BGC-823 cells were maintained in RPMI 1640 medium containing 10% FBS at 5% CO_2 and 37 °C. The others were maintained in DMEM medium containing 10% FBS at 5% CO_2 and 37 °C.

1.2 MTT Assay

The *in vitro* cytotoxicity of compounds were evaluated by MTT assay. Cells (A549, HeLa, LoVo, BGC-823, and LO2) were seeded in 96-well plates at 3×10^3 cells/well. Overnight, the cells were treated with different compounds by half dilution method with an initial concentration of 100 μM. After 48 h, MTT (5 mg/mL) was added to 96-well plates and incubated for a further 4 h. Then, the medium was removed and 100 μM DMSO was added to dissolve the formazan. The absorbance of 96-well plates at 570 nm was measured by enzyme-linked immunosorbent assay (ELISA) reader.

1.3 Inductively coupled plasma massspectrometry (ICP-MS)

BGC-823 cells were seeded in 6-well plates at a density of 1×10^6 cells/well for overnight incubation. These cells were then treated with cisplatin, and complex **1** at the concentration of 10 μM at 37 °C for 3, 6 and 9 h, respectively. Then the cells were washed by cold PBS for three times and collected by centrifugation. Finally, the samples were freeze-dried and measured by quadrupole inductively-coupled plasma mass spectrometry (ICP-MS).

1.4 The stability and reduction of complex **1**

Complex **1** in PBS buffer (0.2 mM, 250 μL, pH = 7.4), in the presence or absence of AsA (50 μL, 10 mM), were shaked at 37 °C for 0, 3, 6, or 24 h, respectively. Carprofen in PBS was used as controls. 30 μL sample was diluted with chromatographic methanol to 200 μL, and then analysis was carried out on a

Shimadzu Prominence HPLC system equipped with a InertSustain C18 column (250 mm × 4.6 mm, 5 μm). HPLC profiles were recorded by UV detector at 254 nm using a gradient methanol-H_2O (containing 0.1% acetic acid) as mobile phase at a flow rate of 1 mL min^{-1}.

1.5 Immunofluorescence experiments

3 × 10^5 BGC-823 cells per well were seeded in glass bottom cell culture dishes with 2 mL of media overnight. Cells were incubated with L-OHP (10 μM), carprofen (20 μM), their mixture (1:2), and CP-L-OHP (10 μM), respectively, at 37 °C for 1, 5, or 24 h. Afterwards, cells were washed with PBS and fixed with 4% paraformaldehyde at room temperature for 30 min. The fixed cells were washed with PBS, treated with 0.3% Triton X-100 for 30 min and then blocked with 1% albumin bovine V solution (BSA) for 1 h. Then the samples were incubated with γH2AX and COX-2 primary antibody (1:200) at 4 °C overnight and incubated with Alexa Fluor 488 or 594 labeled secondary antibody (1:200) for 2 h. After washing with PBS, the cells were stained with nuclear by DAPI for 3 h and the samples were observed by confocal microscope.

1.6 Cell cycle

1 × 10^6 BGC-823 cells/per well were seed in 6-well plates for 24 h and then exposed to medium containing L-OHP (4 μM), carprofen (8 μM), their mixture (1:2), and CP-L-OHP (4 μM), respectively, for 48 h. Cells were collected by PBS and fixed in ice-cold 70% EtOH (200 μL) at –20 °C for 48 h. Cells were centrifuged and suspended with PBS. Then the samples were incubated with RNase A (2.5 μL) at 37 °C for 30 min, and then stained with PI (5 uL) for 15 min at room temperature in the dark. Finally, the samples were detected by BD FACS Calibur flow cytometry and analyzed with Cell Quest software.

1.7 Cell apoptosis

BGC-823 cells were plated in 6-well plate at a density of 1 × 10^6 cells/well. After 24 h, cells were treated with L-OHP (8 μM), carprofen (16 μM), their mixture (1:2), and CP-L-OHP (8 μM), respectively, for 48 h. Then cell samples were digested with

0.25% EDTA-free trypsin, harvested and washed with PBS. The samples were stained following the manufacturer's instructions of Annexin V-FITC/PI kit.

1.8 Wound healing

Briefly, BGC-823 cells were seeded into 6-well plates at a density of 1×10^6 cells/well. After 24 h, a sterilized 200 μL pipette tip was used to make wounds across the cells. Then the samples were washed by PBS to remove the fragments and incubated with L-OHP (4 μM), carprofen (8 μM), their mixture (1:2), and CP-L-OHP (4 μM), respectively. Finally, the wounded areas were observed at 0, 24, and 48 h under the microscope (Motic AE2000) and the images were captured with Mtico Images Advanced 3.2.

1.9 Cell adhesion assay

After treatments with L-OHP (4 μM), carprofen (8 μM), their mixture (1:2), and CP-L-OHP (4 μM), respectively, for 24 h, 3×10^3 BGC-823 cells per well were seeded into matrigel-coated 96-well-plate. 24 h later, cells were fixed with 4% paraformaldehyde for 30 min and then stained with 1% crystal violet solution for another 30 min. The images were observed on an inverted microscope (Leica DMI3000B) and processed with Image J.

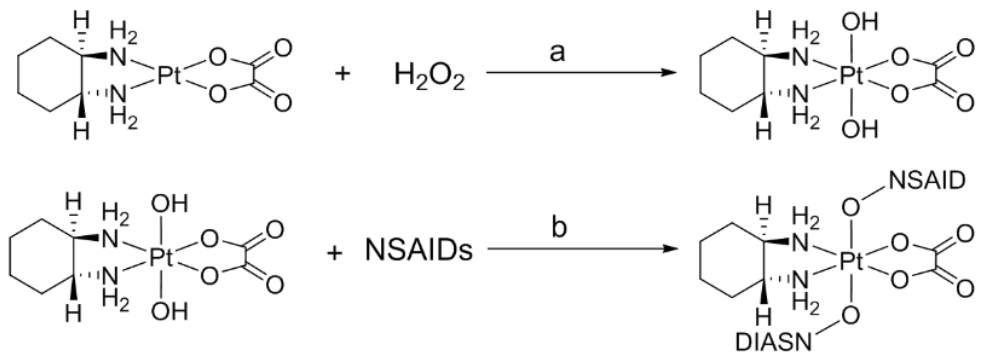

Fig. S1 Conditions and reagents: (a) H_2O, 70 °C, 5 h. (b) NSAIDs = carprofen, etodolac, ketoprofen, or sulindac; TBTU, TEA, DMF, 40 °C, 24 h.

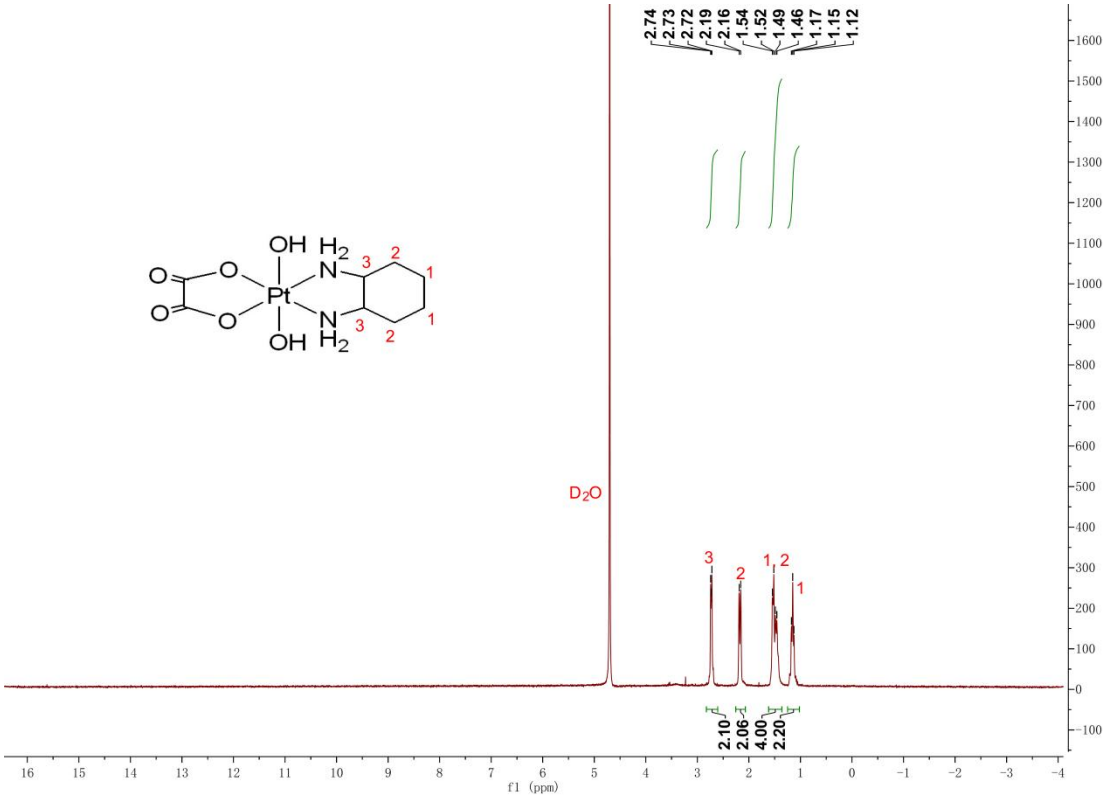

Fig. S2 ^{1}H NMR spectrum of OXO in D_2O.

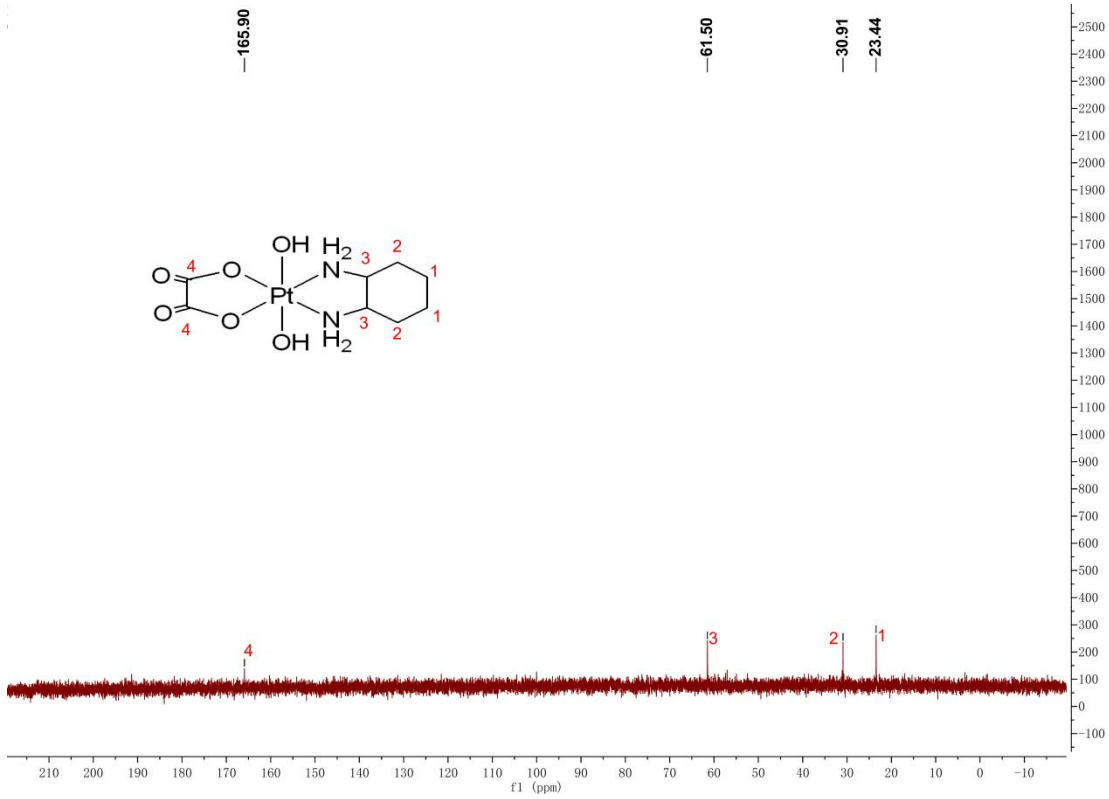

Fig. S3 ^{13}C NMR spectrum of OXO in D_2O.

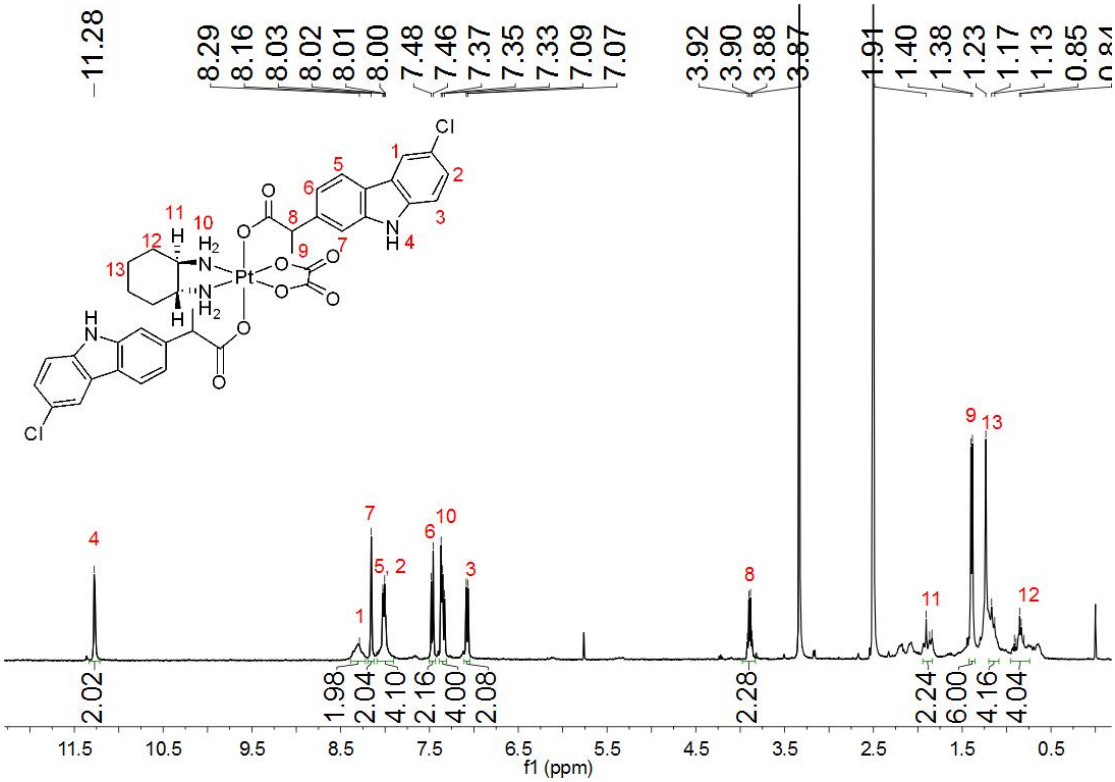

Fig. S4 ^{1}H NMR spectrum of complex **1** in DMSO-d_6.

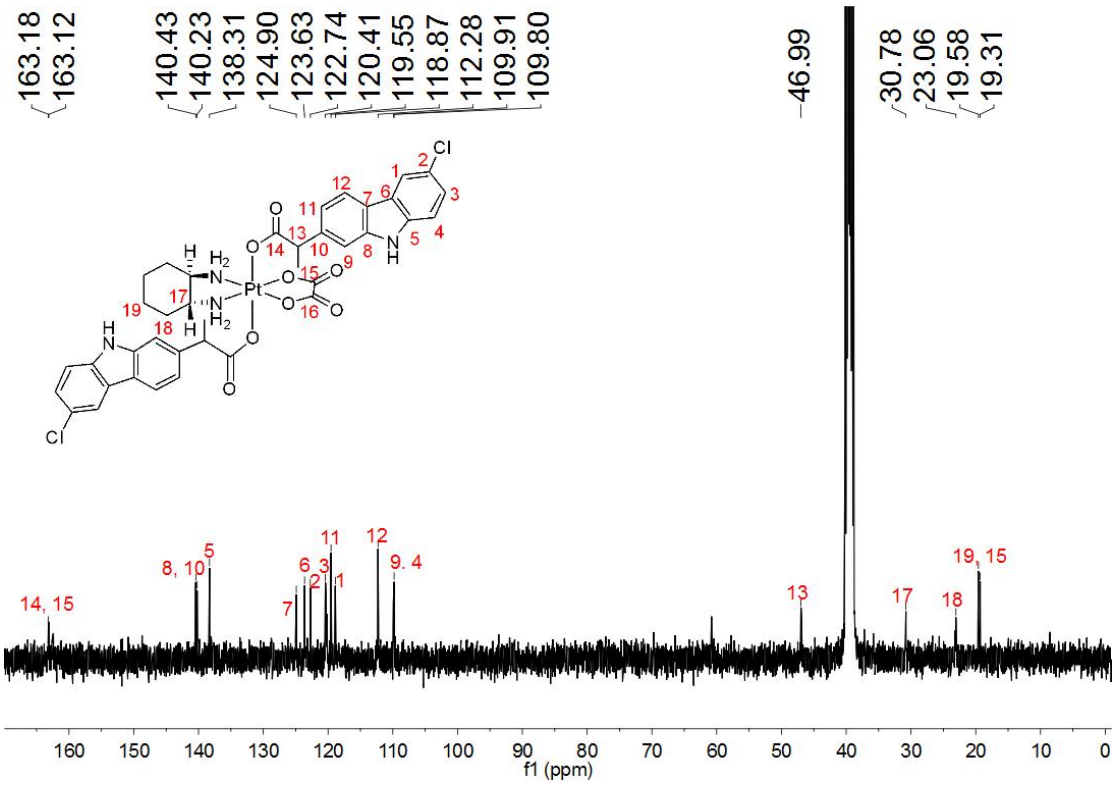

Fig. S5 ^{13}C NMR spectrum of complex **1** in DMSO-d_6.

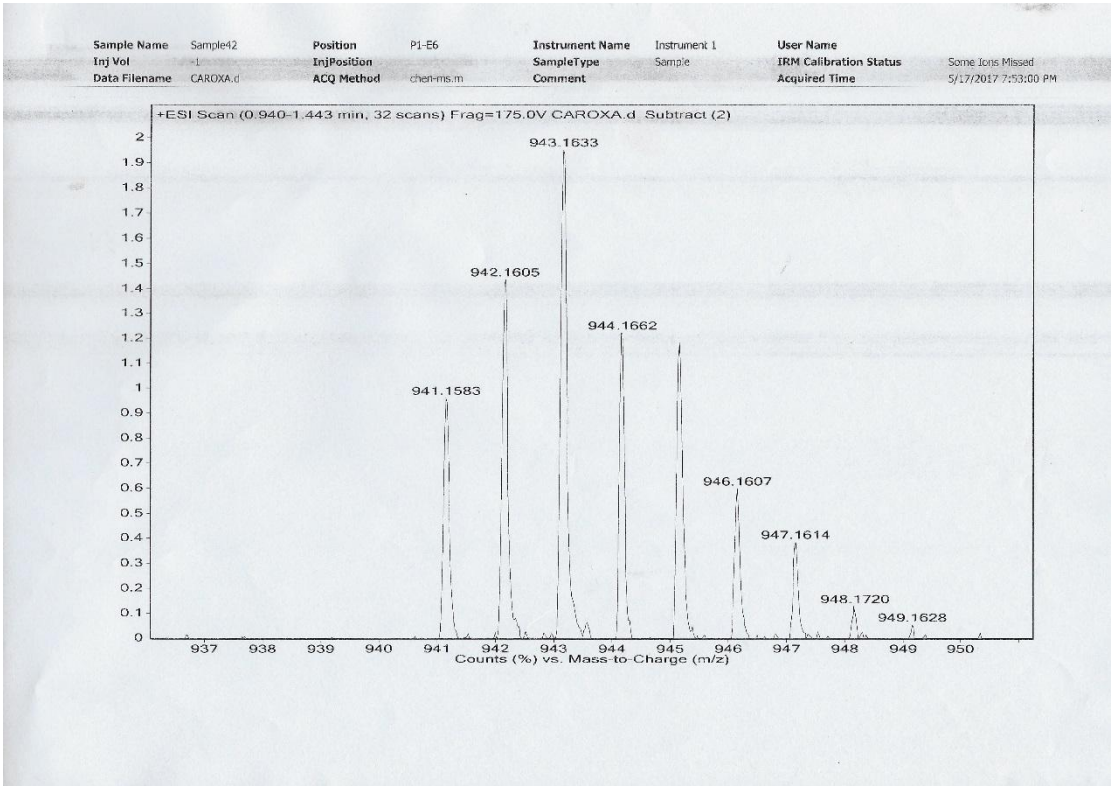

Fig. S6 HR-MS spectrum of complex **1**.

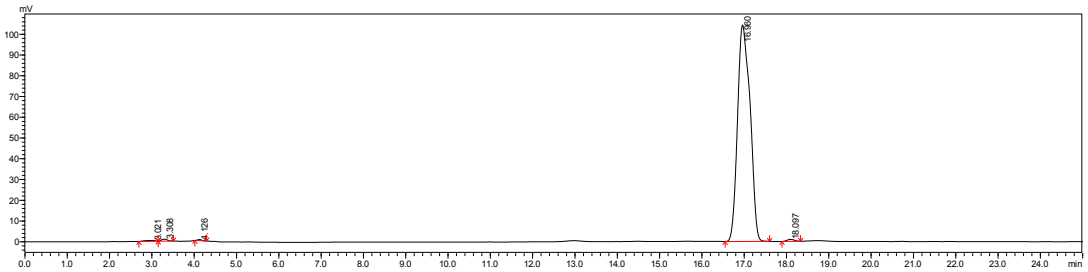

Fig. S7 HPLC traces of complex **1**.

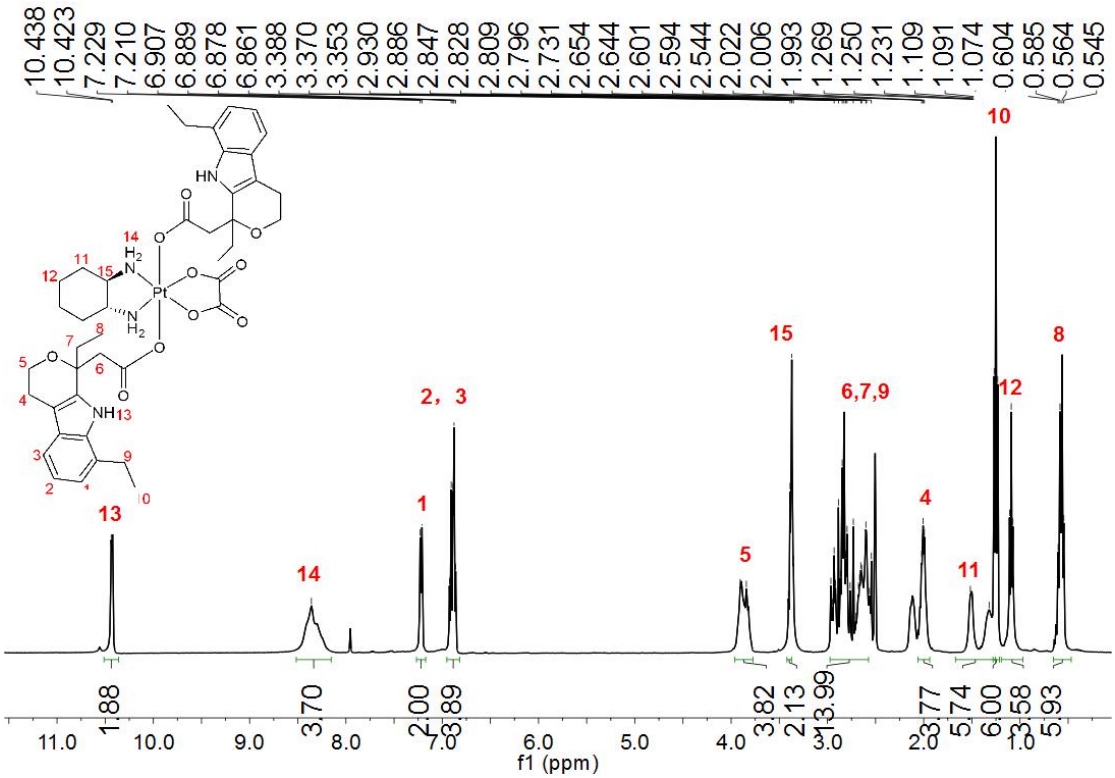

Fig. S8 ^{1}H NMR spectrum of complex **2** in DMSO-d_6.

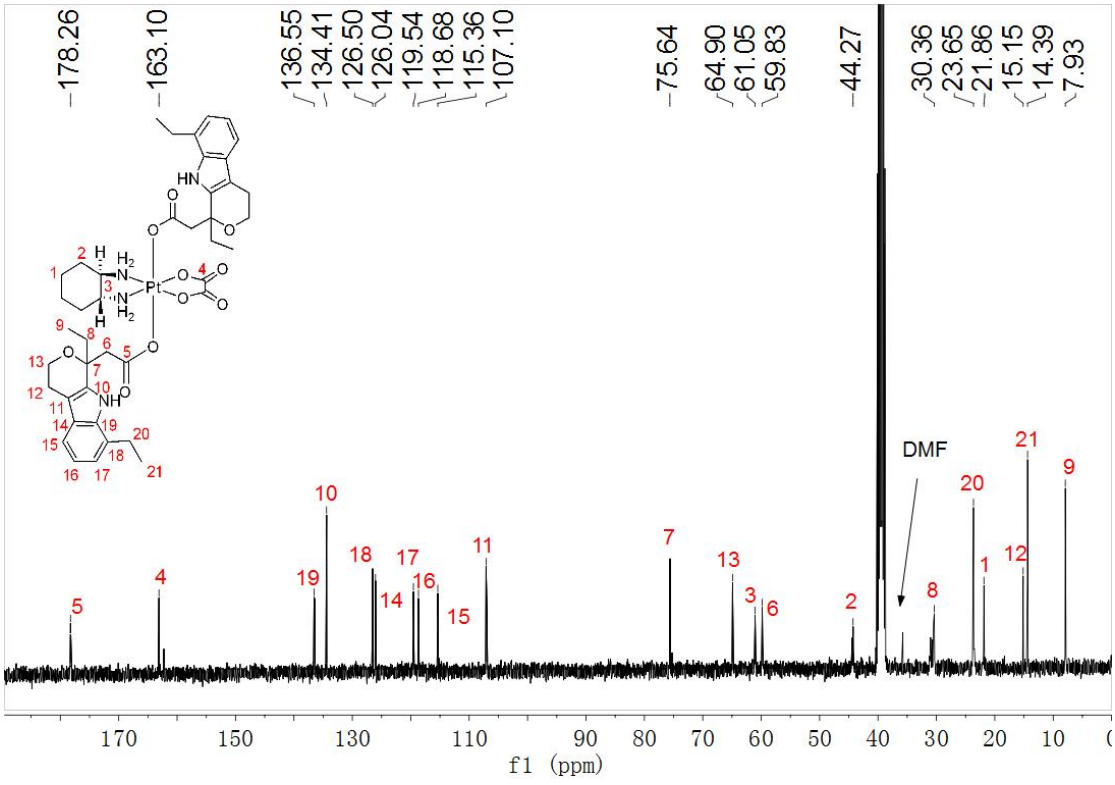

Fig. S9 ^{13}C NMR spectrum of complex **2** in DMSO-d_6.

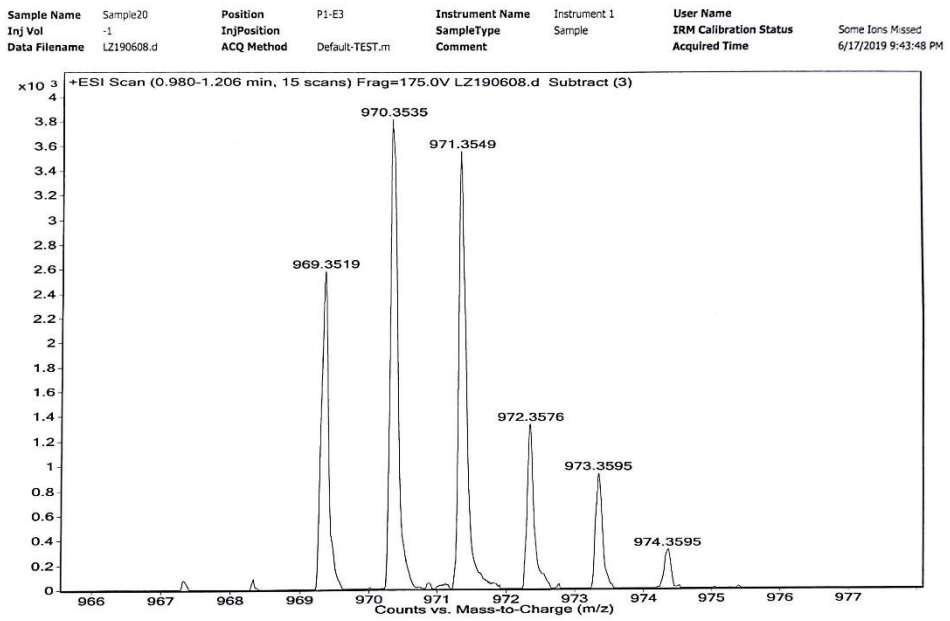

Fig. S10 HR-MS spectrum of complex **2**.

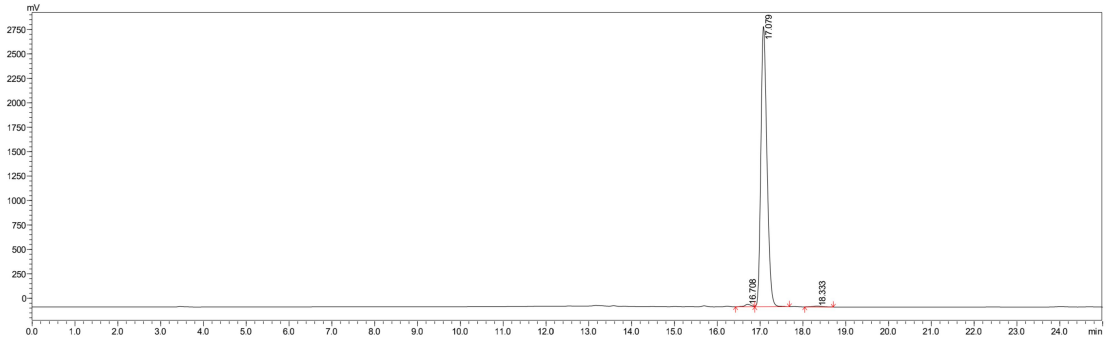

Fig. S11 HPLC traces of complex **2**.

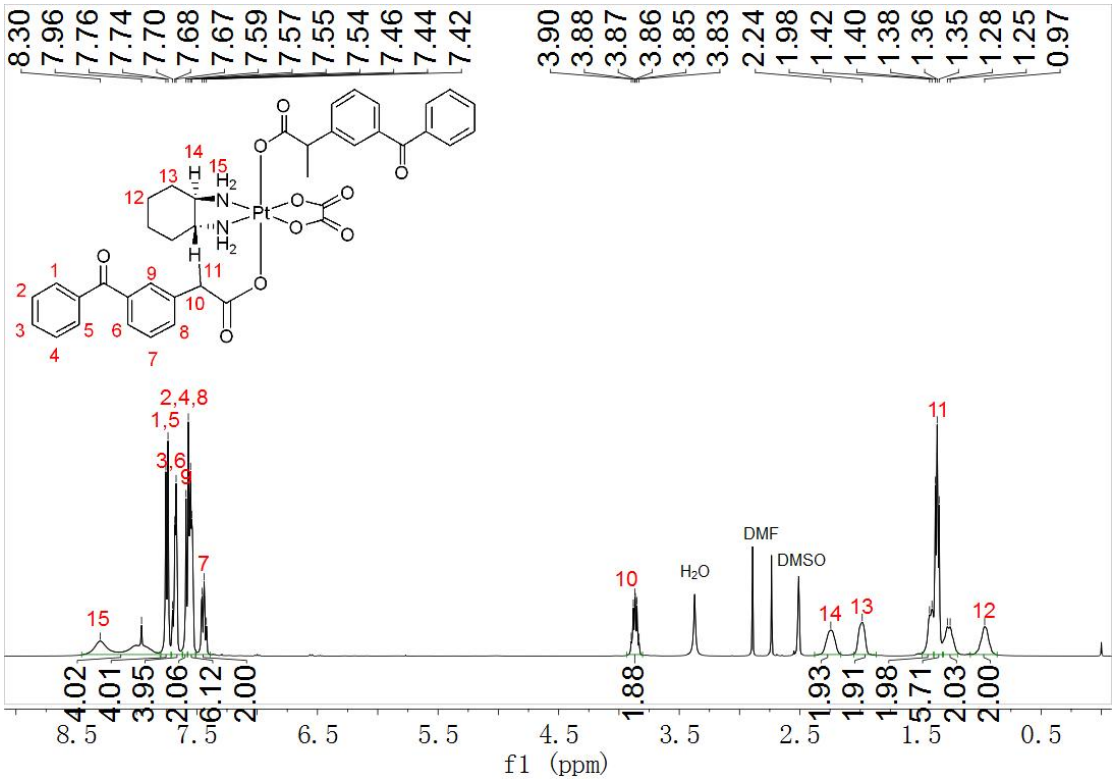

Fig. S12 ^{1}H NMR spectrum of complex **3** in DMSO-d_6.

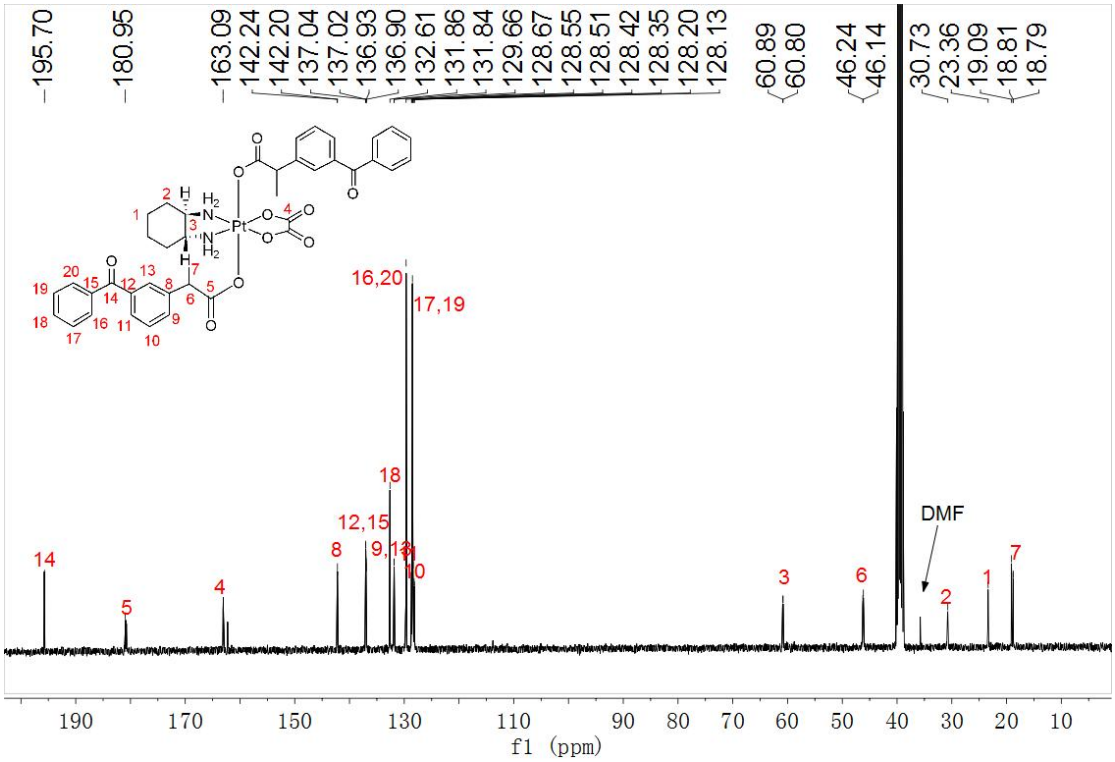

Fig. S13 ^{13}C NMR spectrum of complex **3** in DMSO-d_6.

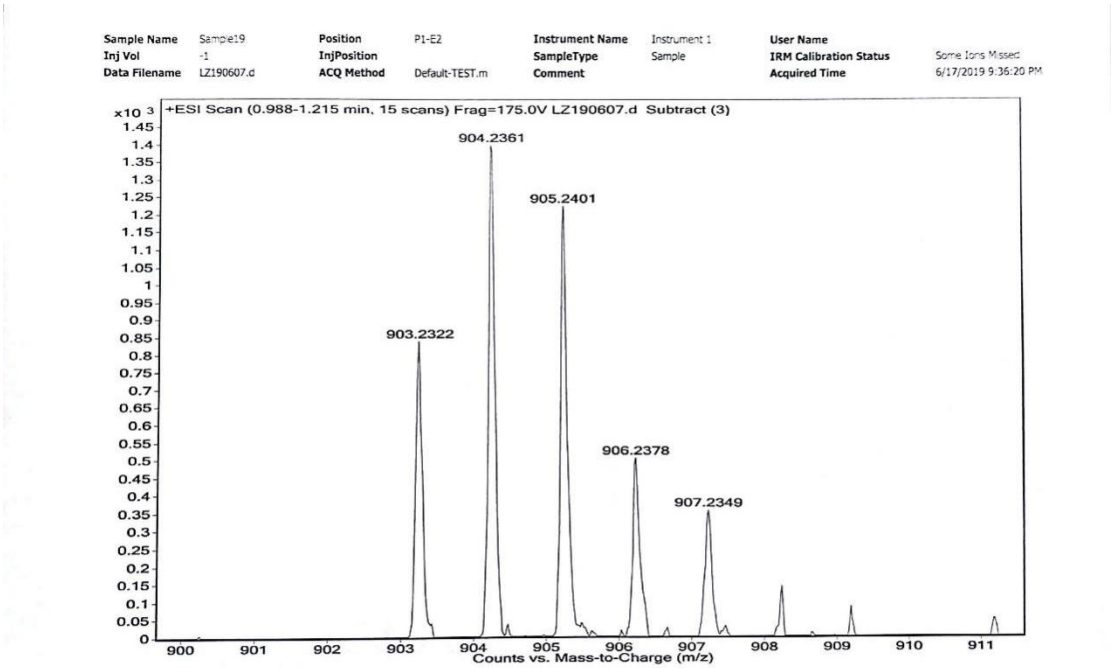

Fig. S14 HR-MS spectrum of complex **3**.

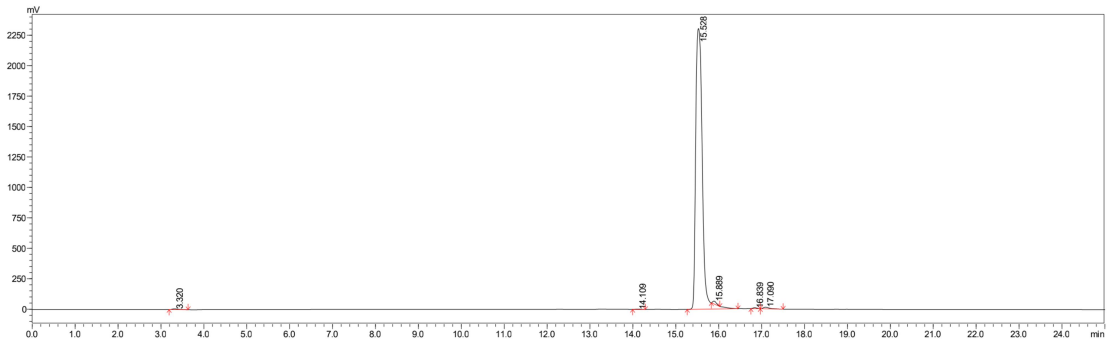

Fig. S15 HPLC traces of complex **3**.

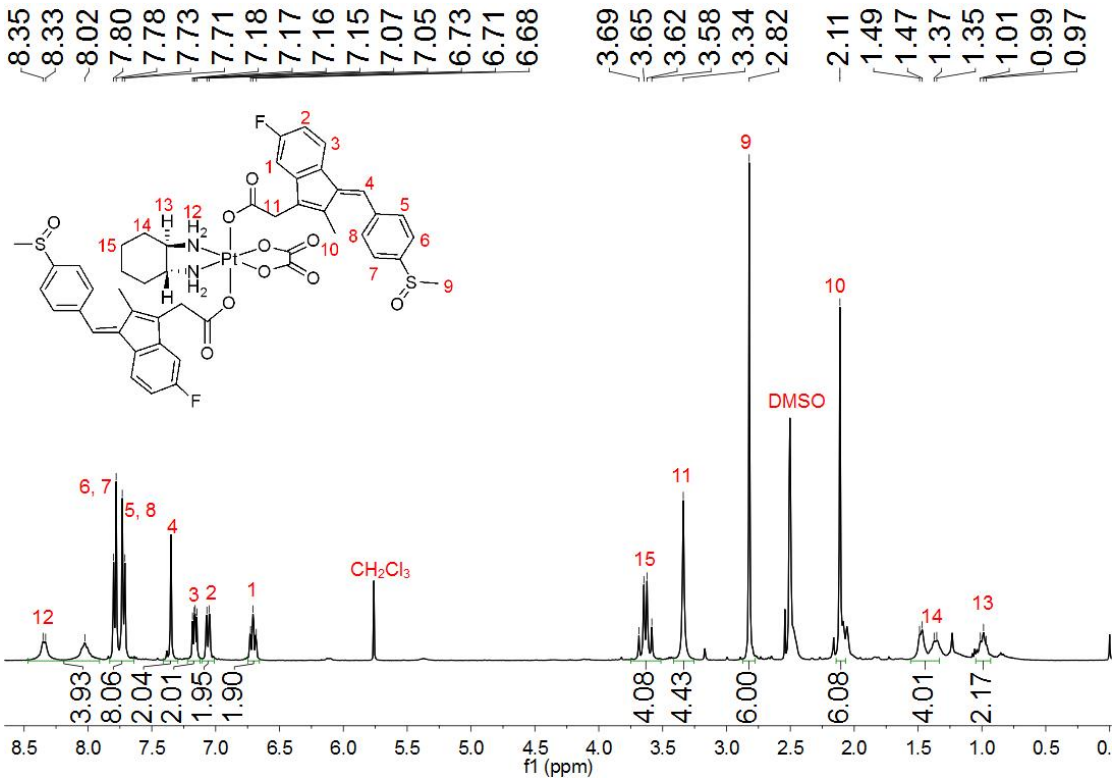

Fig. S16 ^{1}H NMR spectrum of complex **4** in DMSO-d_6.

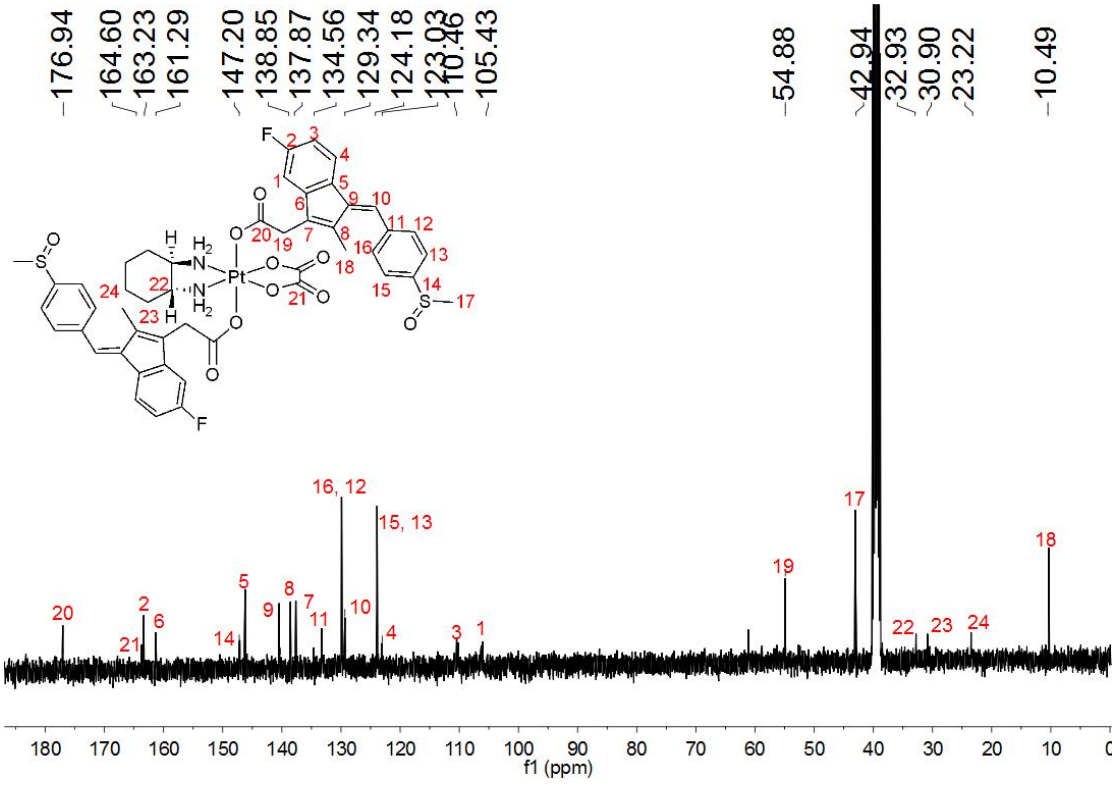

Fig. S17 ^{13}C NMR spectrum of complex **4** in DMSO-d_6.

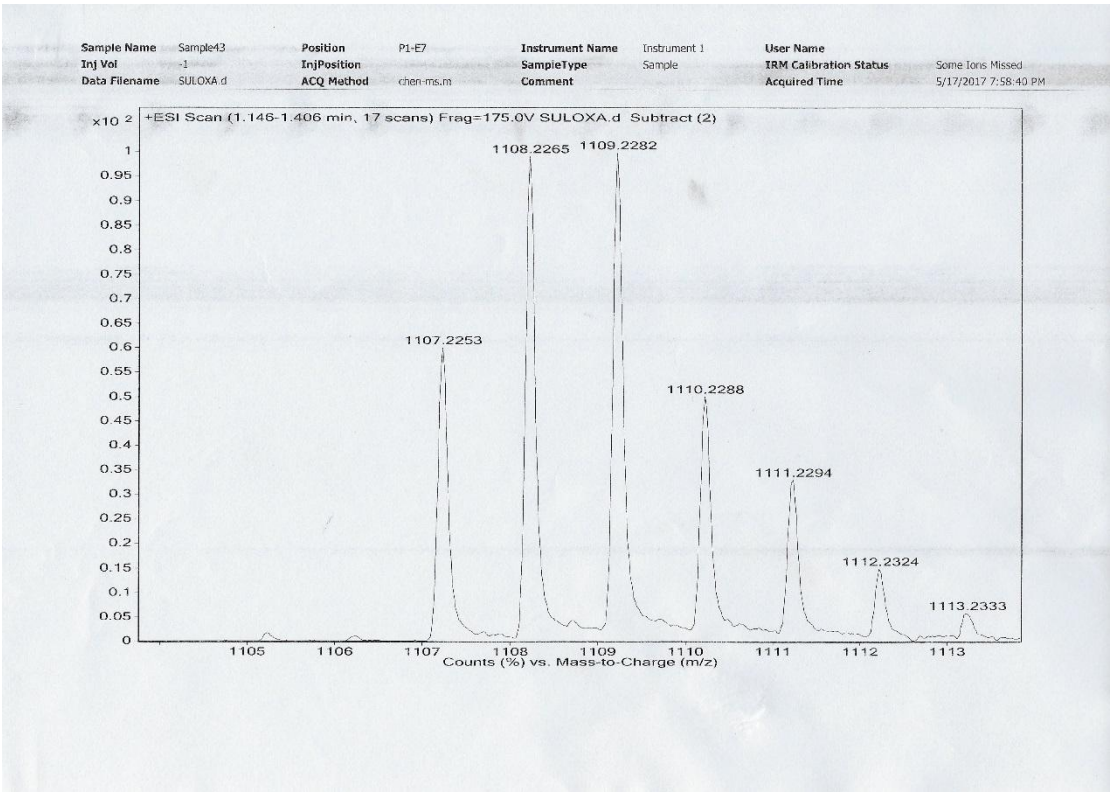

Fig. S18 HR-MS spectrum of complex **4**.

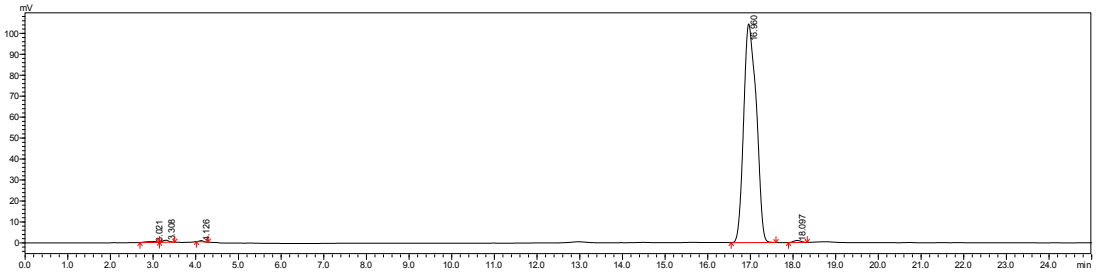

Fig. S19 HPLC traces of complex **4**.

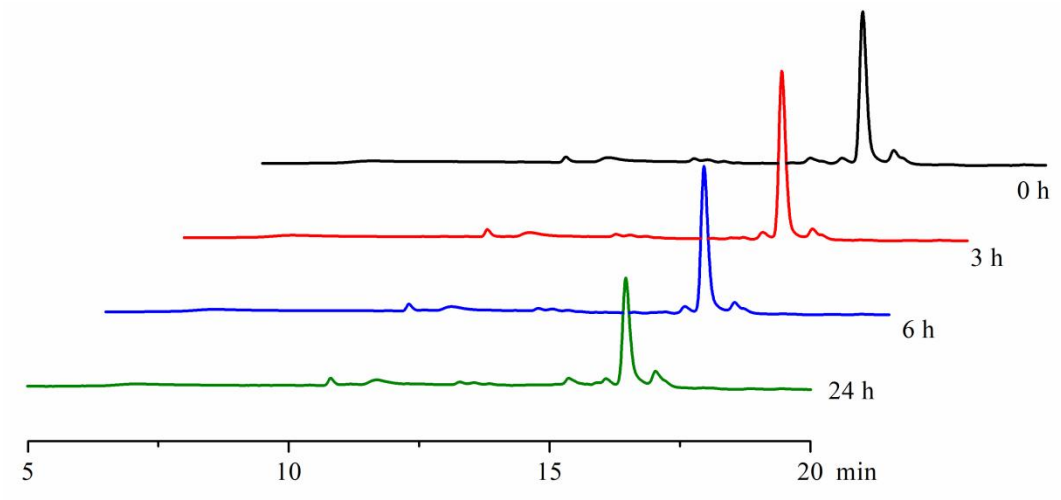

Fig. S20 HPLC profiles of complex **1** in PBS at 37 °C for 0, 3, 6 and 24 h.

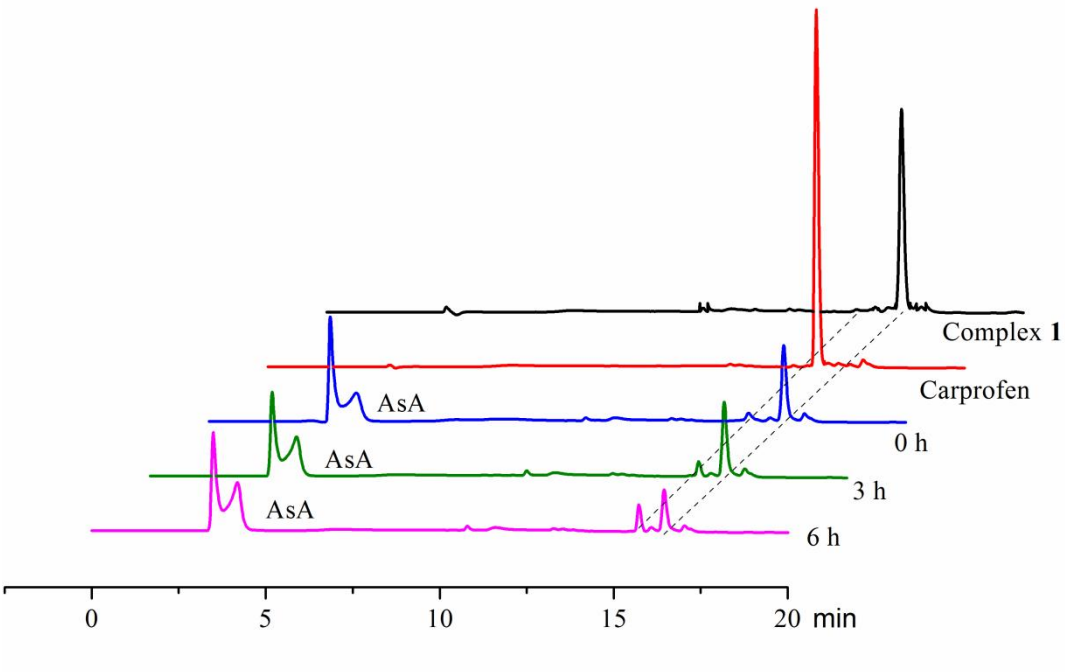

Fig. S21 HPLC profiles of complex **1** in PBS with AsA at 37 °C for 0, 3, and 6 h. The standard samples (complex **1** and carprofen) in the absence of AsA were treated as controls.

Table S1. HPLC traces of complex **1**–**4**.

Complex	R.T.	Purity(%)
1	16.960	98.542
2	17.079	98.552
3	15.528	98.340
4	12.246	97.031

Table S2. Cell viability (IC_{50} in μM) of compounds for 48 h.

Cell Lines	A549	HeLa	LoVo	BGC-823	LO2
L-OHP	16.45 ± 4.90	10.65 ± 0.49	3.74 ± 1.04	6.39 ± 0.17	2.38 ± 0.28
1	10.87 ± 1.14	7.37 ± 0.55	8.49 ± 2.35	1.93 ± 0.34	2.72 ± 0.27
2	16.28 ± 0.35	18.91 ± 2.42	7.33 ± 0.71	4.86 ± 0.42	1.88 ± 0.19
3	27.60 ± 1.00	30.91 ± 0.26	7.73 ± 1.21	3.33 ± 0.39	2.36 ± 0.43
4	11.89 ± 1.14	48.69 ± 12.51	13.31 ± 3.23	13.81 ± 1.49	15.07 ± 1.52
FI[a]	1.51	1.45	0.44	3.31	0.88
FI[b]	1.01	0.56	0.51	1.31	1.27
FI[c]	0.59	0.34	0.48	1.92	1.01
FI[d]	1.38	0.22	0.28	0.46	0.16
L-OHP+carprofen (1 : 2)	10.05 ± 0.37	11.41 ± 1.23	4.29 ± 0.37	6.07 ± 1.42	3.49 ± 0.29
L-OHP+etodolac (1 : 2)	9.70 ± 1.02	10.51 ± 0.95	4.89 ± 0.38	6.57 ± 0.34	3.05 ± 0.51
L-OHP+ketoprofen (1 : 2)	12.49 ± 2.95	10.13 ± 1.13	5.00 ± 0.14	7.50 ± 0.08	4.30 ± 0.97
L-OHP+sulindac (1 : 2)	11.38 ± 1.18	18.75 ± 0.20	4.04 ± 0.45	5.21 ± 0.25	3.39 ± 0.35

[a]IC_{50}(L-OHP)/IC_{50}(Complex **1**)

[b]IC_{50}(L-OHP)/IC_{50}(Complex **2**)

[c]IC_{50}(L-OHP)/IC_{50}(Complex **3**)

[d]IC_{50}(L-OHP)/IC_{50}(Complex **4**)