Electronic Supplementary Information (ESI)

Multifunctional platinum(IV) complex bearing HDAC inhibitor and biotin moiety exhibiting prominent cytotoxicity and tumor targeting ability

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EXPERIMENTAL SECTION

log P_{ow} determination

Excess M-4 and compound **1** were dissolved in KH_2PO_4/NaOH (0.1 M, pH 7.4) solution, which was presaturated with n-octanol for 48 h. The undissolved Pt(II) complexes were removed, and the concentrations of Pt content were measured by high performance liquid chromatography (HPLC) analysis. Then, an equal volume of n-octanol (presaturated with KH_2PO_4/NaOH buffer for 48 h) was added to Pt solution, and the heterogeneous mixture was shaken vigorously for 2 h before centrifuging for 15 min to achieve phase separation. At last, the final Pt content in aqueous phase was again measured by HPLC, and log P_{ow} was determined.

Stability Study

M-4 and compound **1** were dissolved in PBS solution containing 5% MeOH (pH 7.4) at the concentration of 0.1 mM, respectively, and the UV-vis spectra were recorded every two hours on a Shimadzu UV-2600 UV-vis spectrophotometer.

Cell culture

MCF-7 (human breast cancer), HepG-2 (human hepatocellular carcinoma), HCT-116 (human colorectal cancer) and LO2 (human normal liver) cell lines were cultured in a humidified, 5% CO_2 atmosphere at 37 °C, and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 mg/mL of streptomycin and 100 mg/mL of penicillin.

MTT assay

4-PBA, M-4 and compound **1** were tested for their cytotoxicity against MCF-7, HCT-116,

HepG-2, and LO2 cell lines by MTT assay. Cells were suspended in culture medium and seeded in 96-well plates (5000 cells/well) and then incubated for 24 h at 37 °C in a 5% CO_2 incubator. All tested compounds were predissolved in DMF and then diluted to the required gradient concentrations with culture medium, meanwhile, the final concentration of DMF was less than 0.3%. The solutions with gradient concentrations were added into 96-well plates and then incubated at 37 °C for 72 h. Cells were then stained with 10 μL of MTT (5 mg/mL) for 5 h incubation. The supernatant was removed following by adding 150 μL of DMSO to dissolve the resulting formazan. The absorbance at 490 nm was measured using a microplate ELISA reader. The IC_{50} values were calculated by SPSS software after three parallel experiments.

Cellular uptake

The intracellular platinum accumulations of complex **1** and M-4 in HepG-2 cells were measured by ICP-MS spectra. HepG-2 cells were seeded in a 6-well plate (10^5 cells/well) in 5% CO_2 at 37 °C and incubated overnight so as to be adherent. Cells were then treated with tested complexes (30 μM) in culture medium for 12 h cultivation. After that, the suspension was collected, centrifuged for 10 min and resuspended in PBS (1 mL). Adherent cells were washed twice with ice-cold PBS and collected by tyrisin, followed by centrifuged for 15 min and resuspended in PBS (1 mL). The resulting suspensions were combined and the number of cells was counted, followed by digested by HNO_3 (200 μL, 65%) at 65 °C for 15 min. The platinum contents were obtained by ICP-MS spectra after three parallel experiments.

Subcellular Distribution

HepG-2 cells were transferred into 6-well plates at a density of 10^5 cells/well, and cultured overnight at 37 °C. Then, complex **1** (30 μM) were added to the cells for 12 h cultivation. After that, the nuclei and cytoplasm were extracted by nuclei isolation kit Nuclei EZ Prep and cytoplasmic extraction reagents (ThermoFisher), respectively. After digestion, the Pt content of each fraction was analyzed by ICP-MS.

DNA-platination

HepG-2 cells were transferred into 6-well plates at a density of 10^5 cells/well, and cultured overnight at 37 °C. Then, complex **1** (30 μM) were added to the cells for 12 h cultivation. After that, cells were collected, and genomic DNA was extracted by GeneJET Genomic DNA Purification Kit (Thermofisher). The Pt content in the DNA was measured by ICP-MS, and the concentration of the DNA solution was measured using UV-vis spectrophotometer at OD 260 nm.

HDAC activity assay

The total HDAC activity was measured using the Fluor-de-Lys®HDAC fluorometric activity assay kit (Enzo Life Sciences). Briefly, HepG-2 cells (2×10^5 cells/well) were grown in a six-well plate, and treated with the tested compounds (10 μM) for 24 h. The following procedures was processed according to the manufacturer's protocol. Finally, the fluorescence of each reaction was determined with excitation at 360 nm and emission at 460 nm. The experiments were conducted in triplicate.

Hoechst 33258 staining

HepG-2 cells were seeded in 6-well plates (2×10^5 cells/well) and incubated overnight so as to be adherent. Cells were treated with the tested compounds (30 μM) for 24 h incubation and then washed

twice with PBS. Afterwards cells were dyed with Hoechst 33258 staining solution (5 mg/L) and incubated for 10 minutes protected from light at room temperature. HepG-2 cells were rinsed twice with PBS and then analyzed under fluorescence microscopy (Ex = 350 nm; Em = 460 nm).

Apoptosis analysis

According to the manufacturer's instructions, HepG-2 cells were seeded in 6-well plates (2×10^5 cells/well) and incubated overnight at 37 ℃. The tested compounds were dissolved and diluted to the final concentration of 30 μM with culture medium. Adherent cells were treated with tested compounds (30 μM) and incubated for 24 h following by digested and collected with trypsin. Then cells were washed twice with PBS and resuspended in binding buffer (10 mM Hepes/NaOH, 140 mM NaCl, 2.5 mM $CaCl_2$, pH = 7.4). 3 μL annexin V-FITC (100 ng/mL) and 3 μL PI (2 μg/mL) were used to stained the cells. Annexin V-FITC cell apoptosis detection kit (Roche) was applied to measure the fluorescence by a flow cytometer (FAC Scan, Becton Dickenson) and the apoptotic rates were analyzed by CellQuest software.

Cell cycle measurement

HepG-2 cells were seeded in 6-well plates at a cell density of 2×10^5 cells/well and incubated overnight at 37 ℃. Adherent cells were treated with the tested compounds at the concentrations of 15 and 30 μM respectively and incubated for 24 h. Then cells were digested and collected with trypsin followed by washed twice with precooled PBS solution. To the collected cells was added precooled 70% ethanol and cells were fixed at 4 °C overnight. The supernatant was removed after centrifugation and cells were washed twice with precooled PBS solution following by stained with 400 μL of PI solution (50 μg/mL) containing 100 μg/mL RNase at 37 °C for 30 min. Flow cytometry (FAC Scan, Becton Dickenson) was applied to detect the samples. PI signals were recorded in the FL2 channel and the data of cell cycle distribution was analyzed by CellQuest software.

Western blot

HepG-2 cells were seeded in a 6-well plate (2×10^5 cells/well) and cultured overnight at 37 °C until the cell density reached 80%. Then cells were incubated with tested compounds (30 μM) for 12 h at 37 °C followed by lysed in a cell lysis buffer and collected by centrifugation. Proteins from cell lysates were separated with 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a poly(vinylidine difluoride) (PVDF) membrane (Amersham Biosciences). After that, the membrane was blocked with 5% nonfat dry milk in TBS buffer

containing 0.1% Tween-20 for 1 h. The membranes were incubated with primary antibodies overnight at 4 °C and then incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at 25 °C. The blot results were obtained on an Odyssey imaging system and analyzed by Image J software. β-actin was used as the internal control.

Comet assay

DNA damage in HepG-2 cells induced by compound **1** and M-4 was assessed using comet assay. 2 × 10^5 HepG-2 cells in culture medium were seeded in 6-well plates and incubated overnight at 37°C to attach followed by incubated with tested compounds (30 μM) for 12 h at 37 °C. After that, cells were collected and re-suspended in PBS, mixed with low melting point agarose (LMPA) and placed on a pre-coated slide with normal melting point agarose (NMPA) layer. A coverslip was placed over the gel and set at 4 °C for 10 min. The coverslip was then removed and the slide was immersed into cold lysis solution overnight and put in cold alkaline electrophoresis buffer. Electrophoresis was performed for 30 min at 25 V. Afterwards, the slides were rinsed twice with a neutralizing Tris buffer (pH 7.5). Each slide was stained with 100 μL PI (50 μg/mL) and visualized using a fluorescence microscopy.

Docking study

Molecular docking simulation was performed using Autodock 4.2. The crystal structure of streptavidin with a biotin molecule (PDB ID: 3RY2) was retrieved from the Protein Data Bank. The biotin and water molecules in 3RY2 were removed by software PyMOL before the simulation. The docking simulation was carried out based on the Lamarckian genetic algorithm (LGA). The number of docking runs was 150, and each run of the docking operation was terminated after a maximum of 25,000,000 energy evaluations. The protein was set as rigid structure, and rotation in the compound **1** was permitted about all single bonds during the simulation.

Fig. S1-S17:

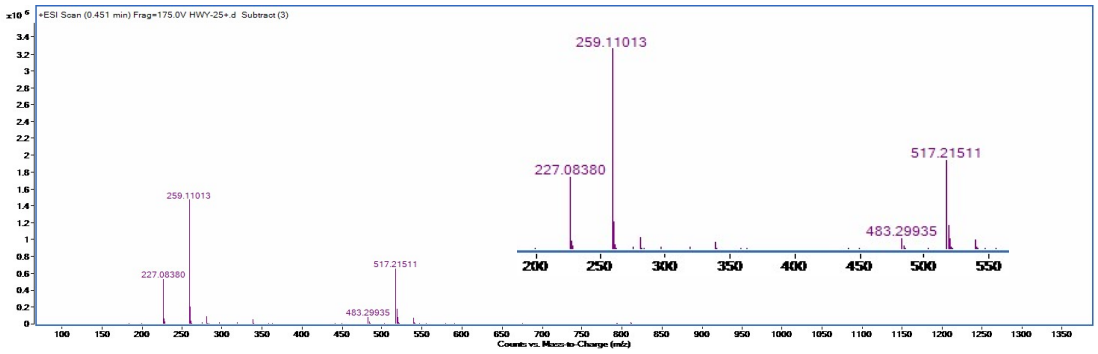

Fig. S1 HR-MS spectrum of compound M-1.

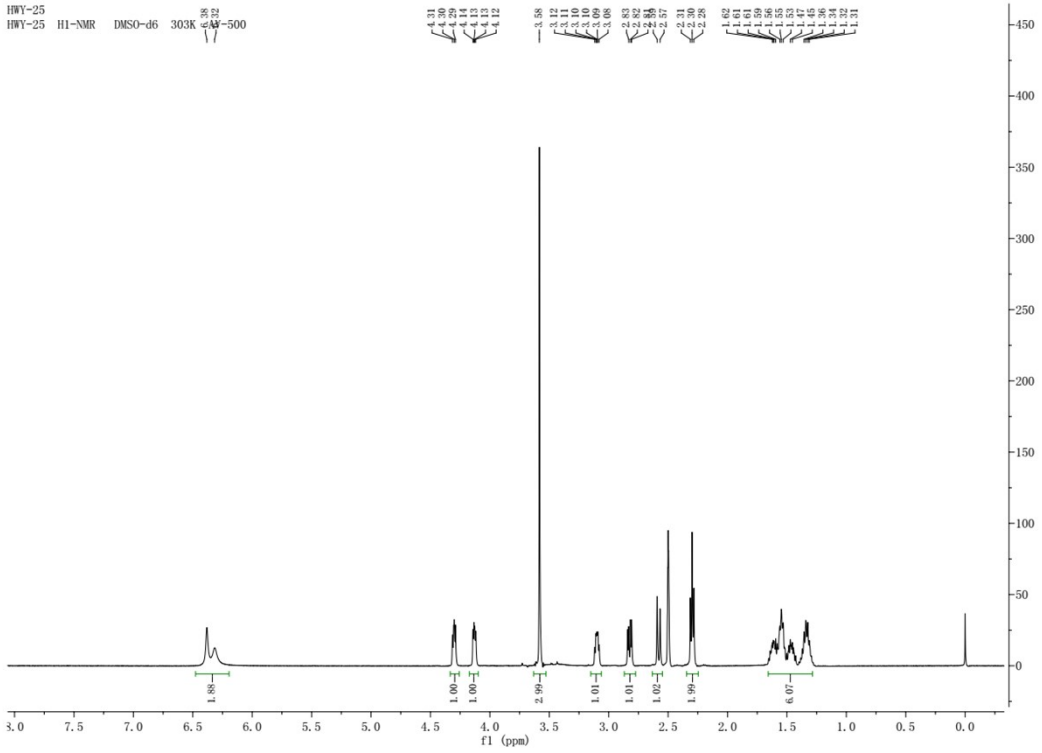

Fig. S2 ^{1}H NMR spectrum of compound M-1 (DMSO-d_6).

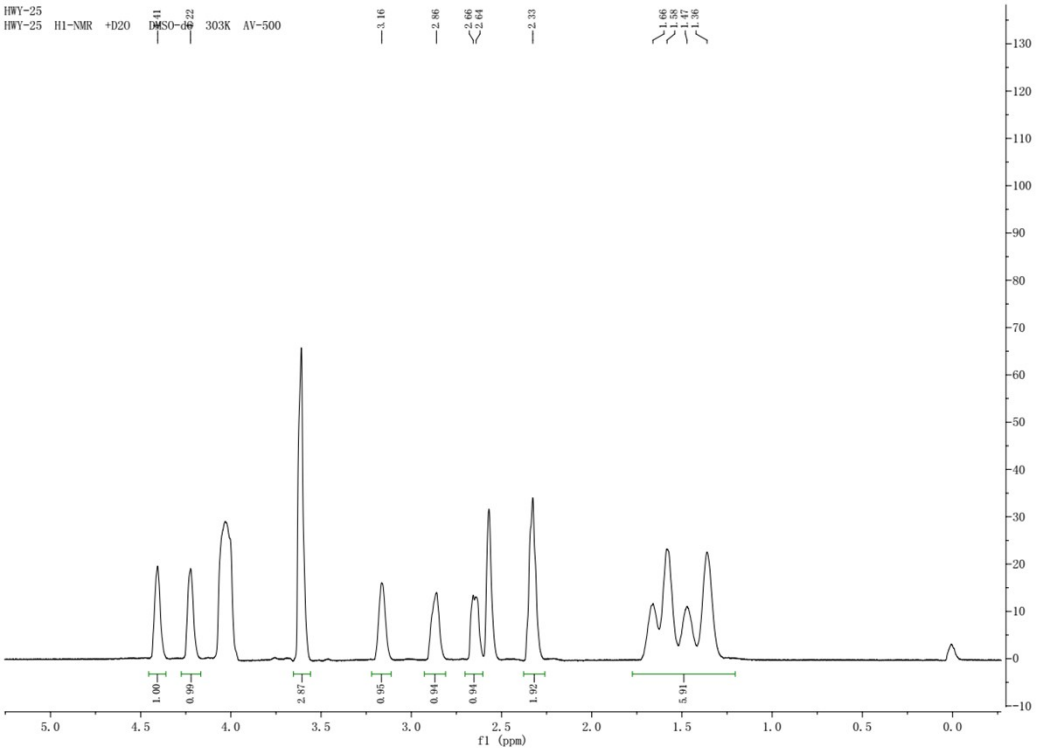

Fig. S3 ^{1}H NMR spectrum of compound M-1 (DMSO-d_6+D_2O).

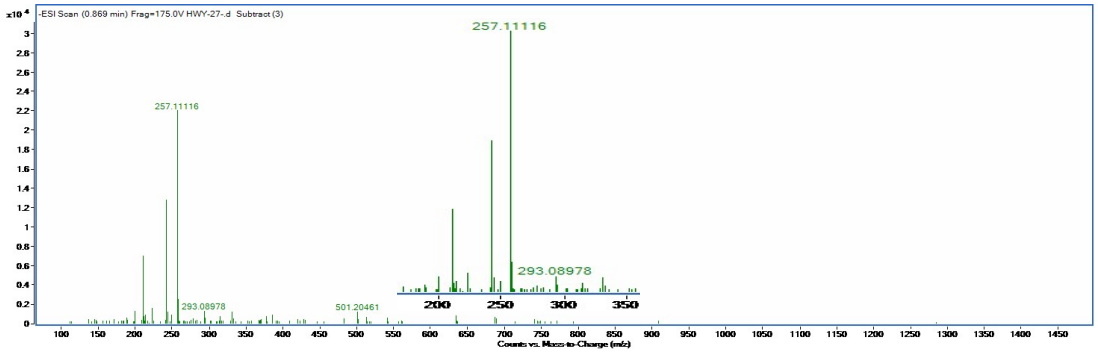

Fig. S4 HR-MS spectrum of compound M-2.

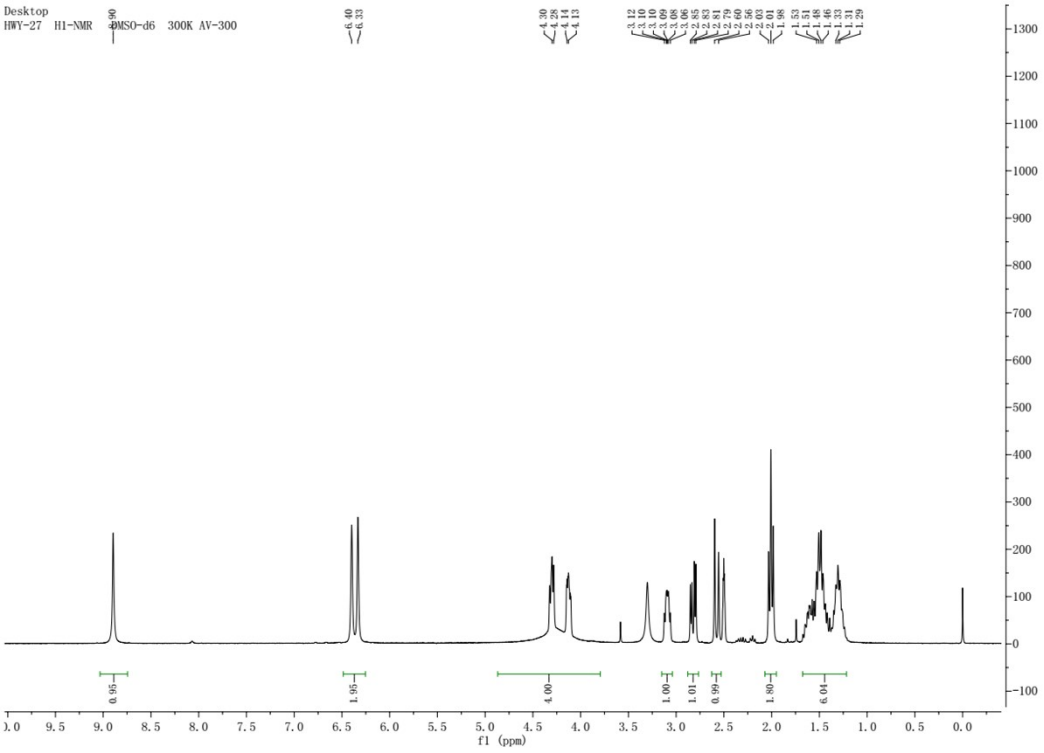

Fig. S5 ^{1}H NMR spectrum of compound M-2 (DMSO-d_6).

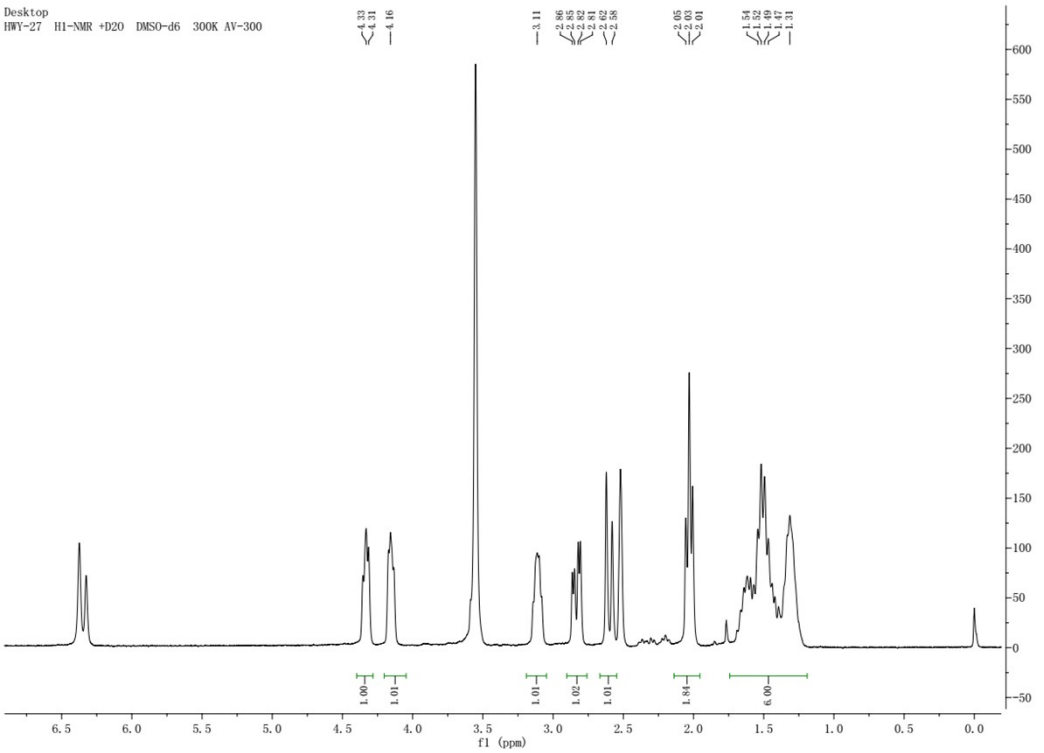

Fig. S6 ^{1}H NMR spectrum of compound M-2 (DMSO-d_6+D_2O).

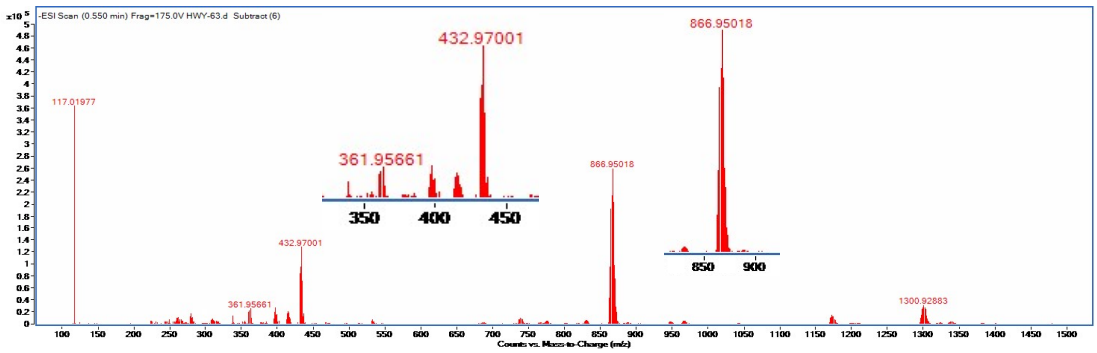

Fig. S7 HR-MS spectrum of compound M-3.

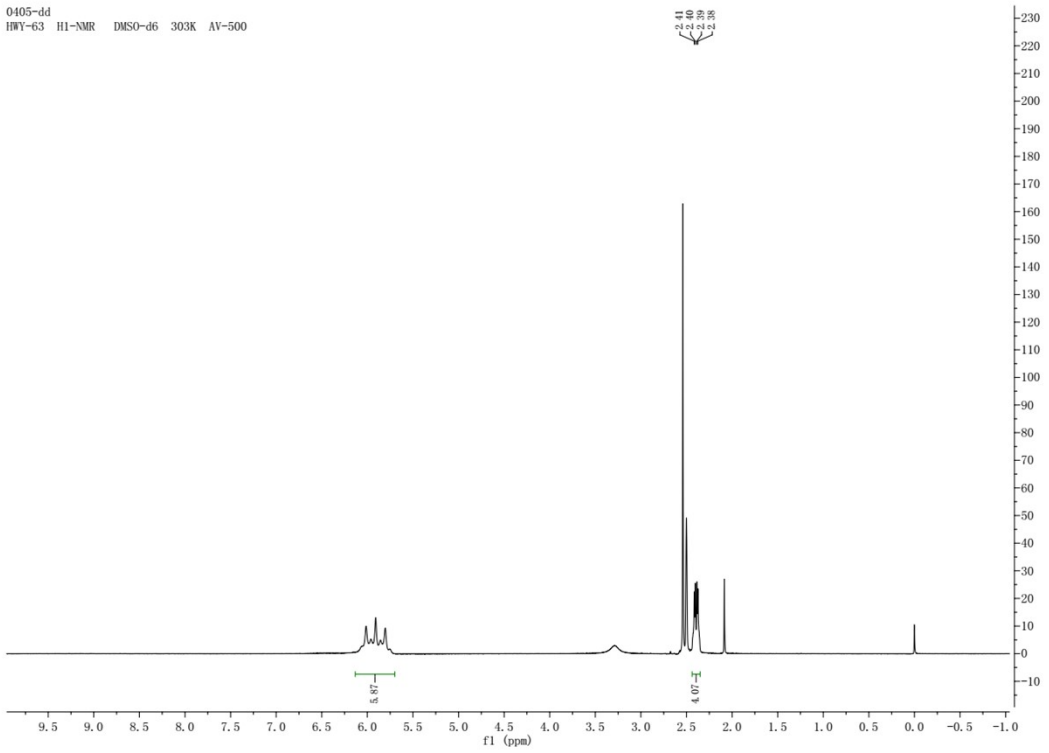

Fig. S8 ^{1}H NMR spectrum of compound M-3 (DMSO-d_6).

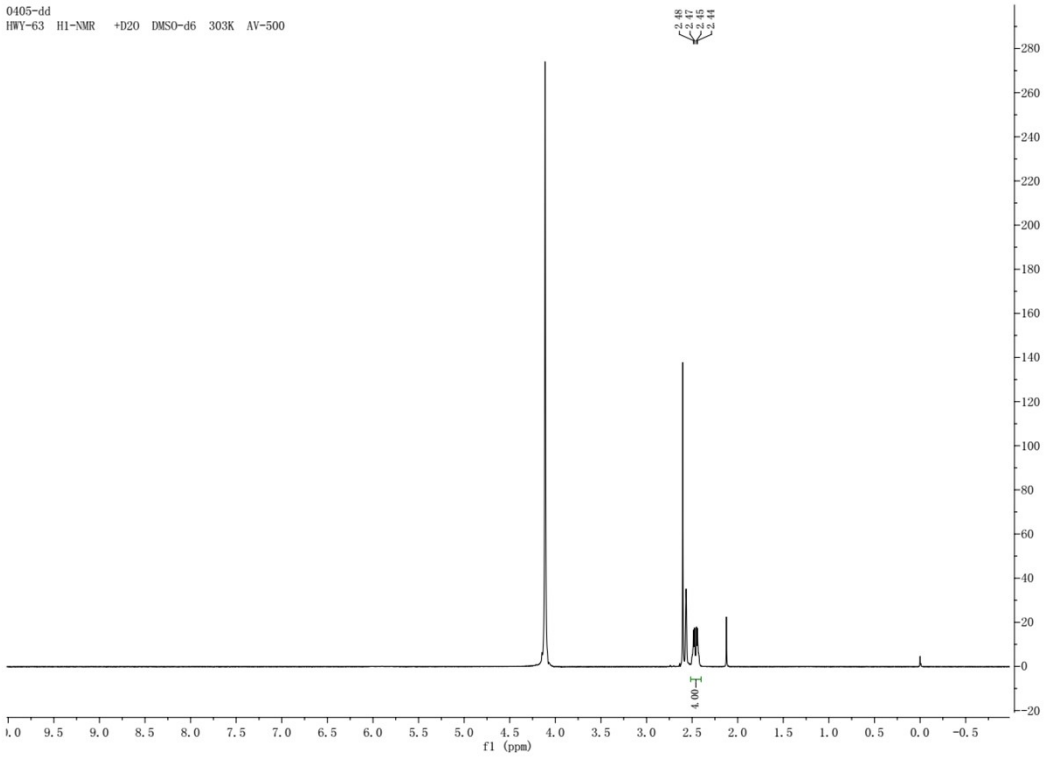

Fig. S9 ^{1}H NMR spectrum of compound M-3 (DMSO-d_6+D_2O).

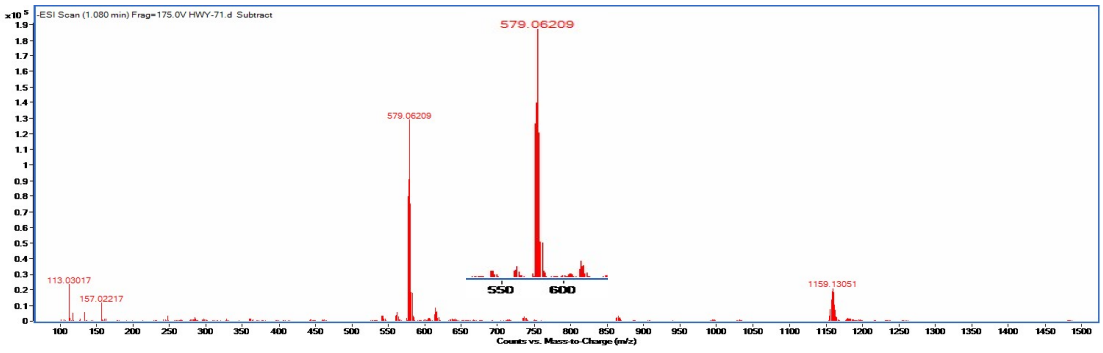

Fig. S10 HR-MS spectrum of compound M-4.

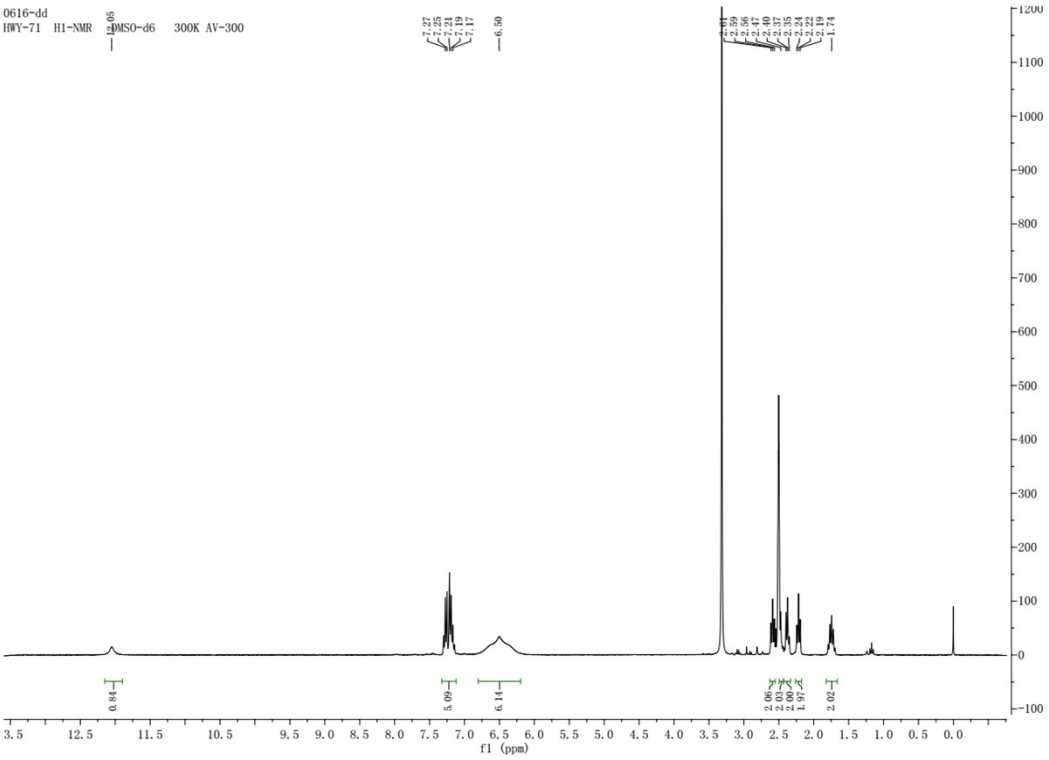

Fig. S11 ^{1}H NMR spectrum of compound M-4 (DMSO-d_6).

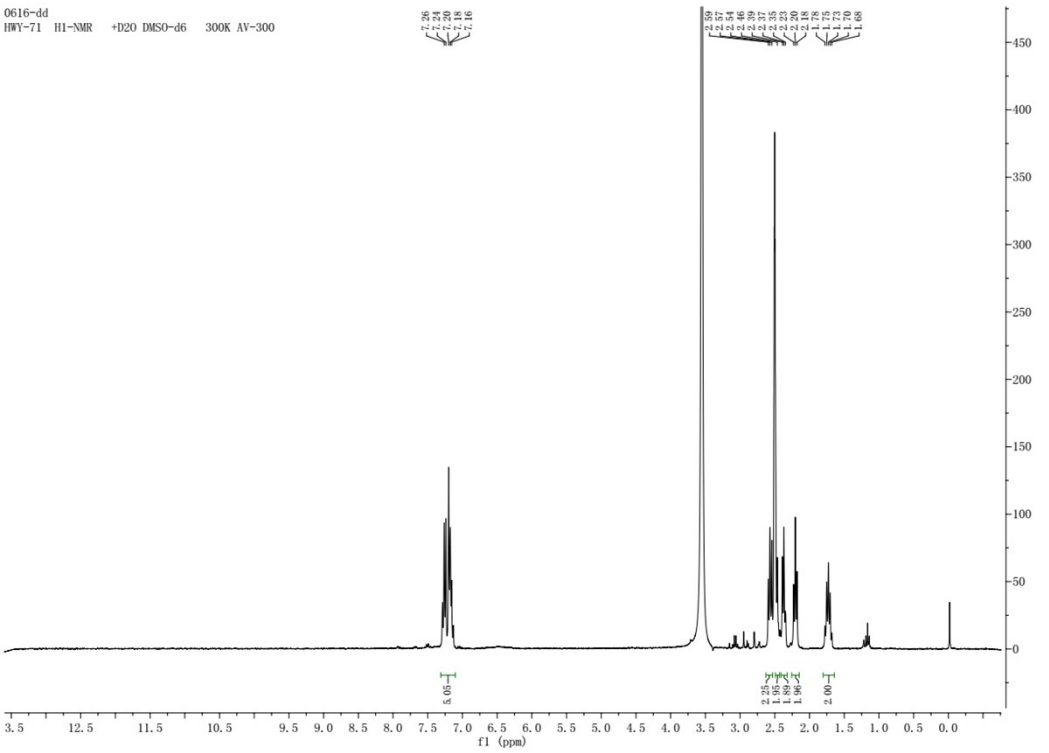

Fig. S12 ^{1}H NMR spectrum of compound M-4 (DMSO-d_6+D_2O).

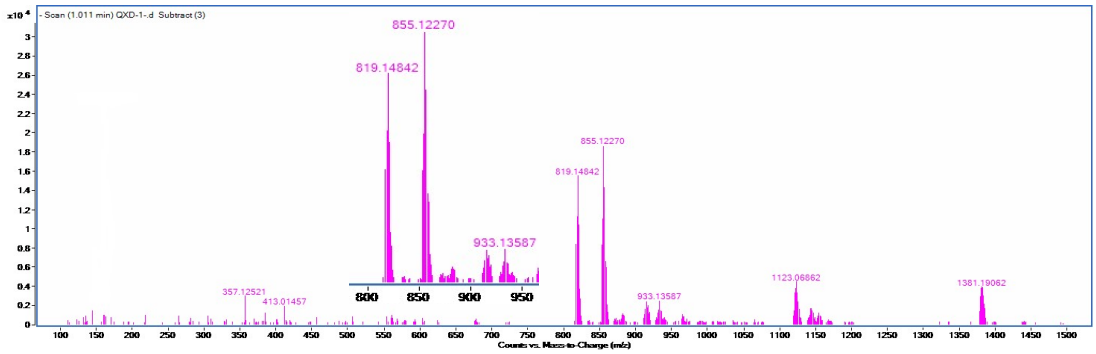

Fig. S13 HR-MS spectrum of compound **1**.

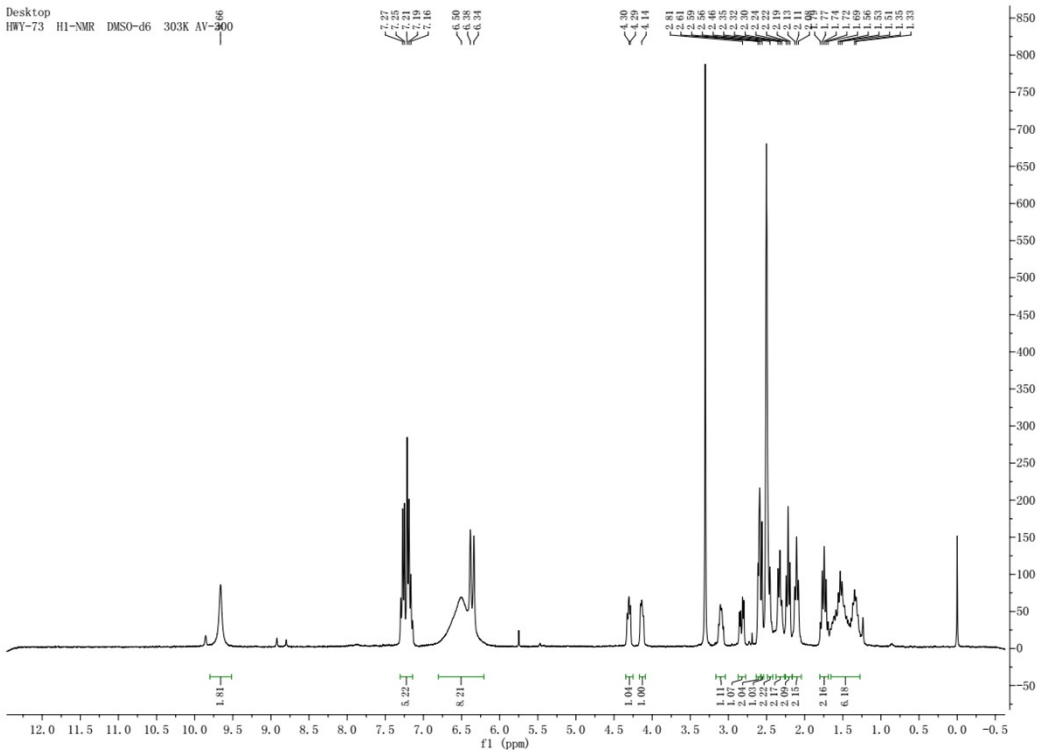

Fig. S14 ^{1}H NMR spectrum of compound **1** (DMSO-d_6).

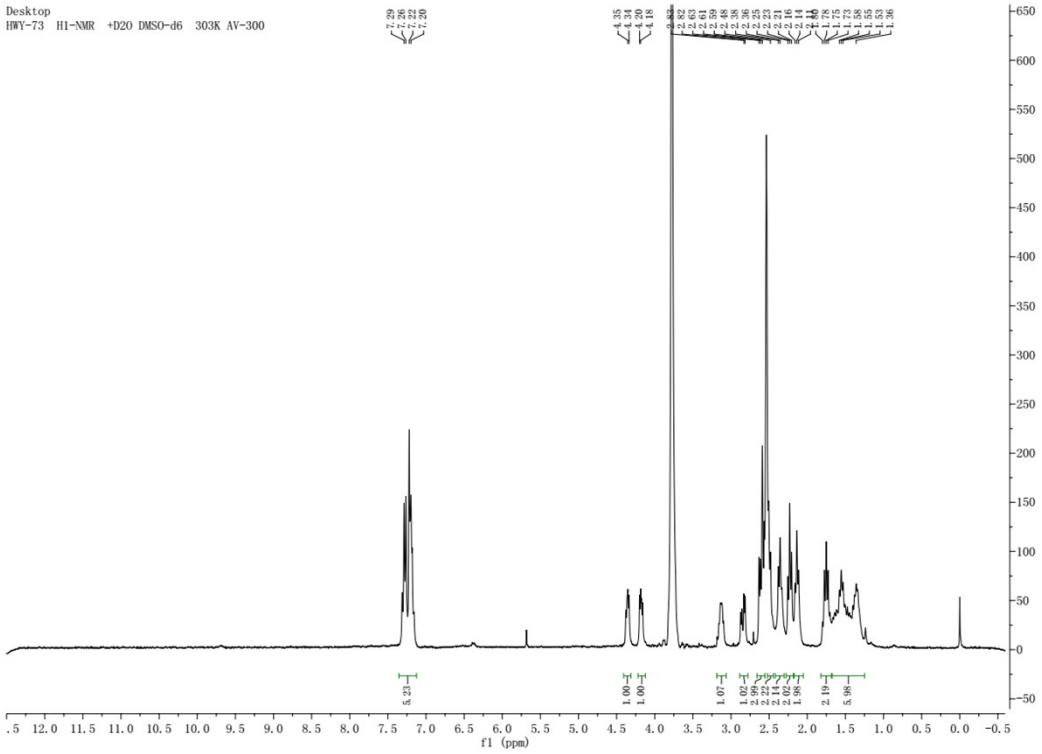

Fig. S15 ^{1}H NMR spectrum of compound **1** (DMSO-d_6+D_2O).

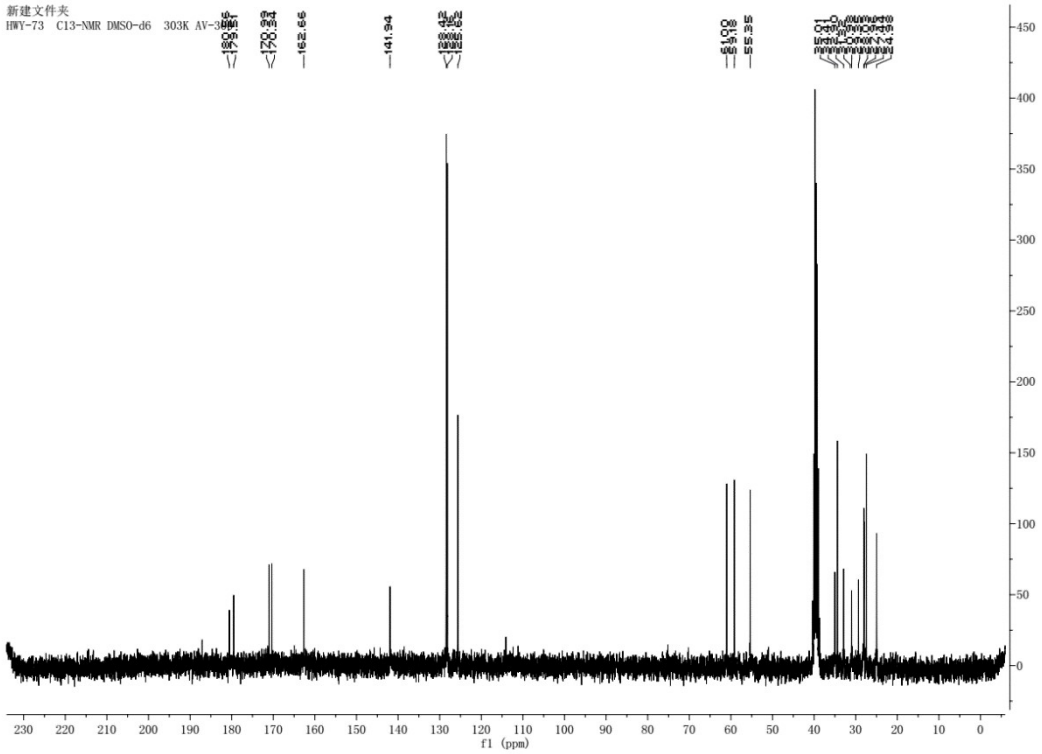

Fig. S16 ^{13}C NMR spectrum of compound **1** (DMSO-d_6+D_2O).

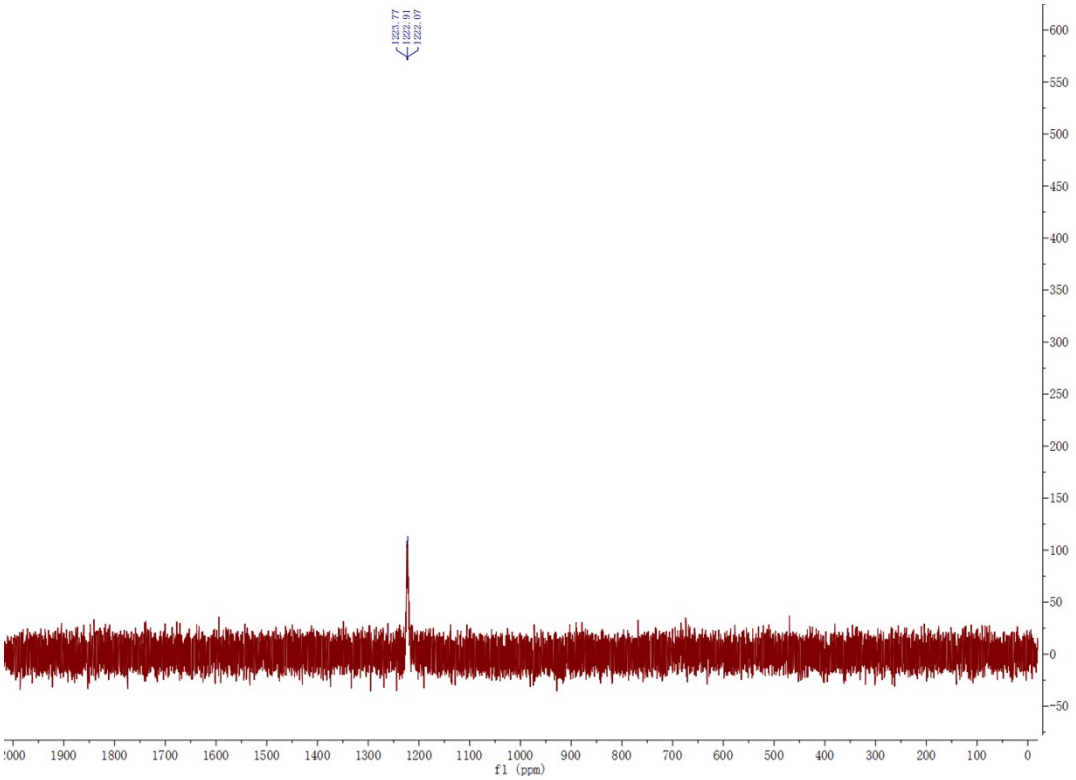

Fig. S17 ^{195}Pt NMR spectrum of compound **1** (DMSO-d_6).