Electronic Supporting Information

For

Pt(IV) derivatives of cisplatin and oxaliplatin bearing EMT-related TMEM16A/COX-2-selective dual inhibitor against colorectal cancer cells HCT116

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Experimental Details.

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Fig. S12 Wound healing assay. HCT116 cells were treated with varied compound at equitoxic concentration (IC_{50} value) for 48 h. $^{*}P < 0.05$, compared with control group.

Cell culture

The cells were maintained in either DMEM medium (A549, HeLa, MCF-7, and HUVEC), or RPMI1640 medium (HCT116) containing 10% FBS in a humidified atmosphere containing 5% CO_2 at 37 ºC.

Cytotoxicity activity

Varied kinds of cells were seeded in 96-well microplates and incubated for 24 h, and then treated with graded amount of compounds, respectively. After 48 h, 10 μL MTT solution was added to each well, and incubated at 37 °C for an additional 4 h. Then, the supernatant was removed from each plate and 100 μL DMSO was added to dissolve the MTT formazan crystals. The absorbance was measured at 570 nm by an enzyme-linked immunosorbent assay (ELISA) reader.

Drug accumulation

In brief, 1×10^6 HCT116 cells were seeded in 6-well plates at 37 °C under 5% CO_2 and incubated overnight. The cells were incubated with oxaliplatin (10 μM), oxaPt(IV) (10 μM), complex **2** (10 μM), and mixture (oxaPt(IV) : NFA = 10 μM : 20 μM) for 3, 6, and 9 h, respectively. The medium was removed, and the cells was washed with ice-cold PBS, collected and centrifuged at 1500 rpm for 3 min. Then, the samples were lyophilized by a freeze dry system. Prior to analysis, HNO_3 was employed to digest the lyophilized cells. Eventually, ICP-MS was used to quantify the platinum content via three parallel experiments.

Intracellular drug release

1.5×10^7 HCT116 cells/well were cultured in 6-well plates, and then treated with 100

μM complex **2** for 4 or 10 h. The untreated cells serving as a control group. Then, the cells were washed with ice-cold PBS for three times and collected by centrifugation. The cells were re-suspended in a mixture of dichloromethane and methanol (1:1) for mechanical grinding, and centrifuged at 12000 rpm for 5 min to collect the supernatant. The supernatant was dried under N_2 flow, then dissolved in methanol, and ultimately analyzed by HPLC.

Cell cycle analysis

HCT116 cells were transferred into 6-well plates at the density of 1×10^6 cells mL^{-1} overnight at 37 °C, and then treated with complex **2** (10 μM), oxaliplatin (10 μM), oxaPt(IV) (10 μM), mixture (oxaPt(IV) : NFA = 10 μM : 20 μM), and NFA (20 μM) for 24 h. All cells, including floating and adherent cells, were harvested and rinsed twice with PBS and then fixed the pellets with 250 μL 70% cold ethanol at –20 °C over 24 h. Fixed cells were centrifuged to remove the ethanol and washed once with PBS. The cells were re-suspended by 250 μL PBS and added 2.5 μL RNase A at 37 °C to avoid RNA interference. After 0.5 h, the cells were stained with 50 μg/mL propidium iodide (PI) solution in the dark at 37 °C for another 0.5 h. Finally, the cell cycle distribution was measured immediately by a flow cytometer (BD FACS Verse). The data was carried out with ModFit 3.1 software.

Apoptosis analysis

HCT116 were incubated in 6-well plates at the density of 1×10^6 cells $well^{-1}$ for 24 h to attach. Then, the cells were induced apoptosis induced by complex **2** (10 μM), oxaliplatin (10 μM), oxaPt(IV) (10 μM), mixture (oxaPt(IV) : NFA = 10 μM : 20 μM), and NFA (20 μM) for 48 h. Next, the cells were washed twice with PBS, digested with non-EDTA trypsase, and collected by centrifugation. Cells were stained according to the instruction of AnnexinV-FITC Apoptosis Detection Kit (Solarbio), and detected using a flow cytometer (BD FACS Verse).

Immunofluorescence assay

After addition of complex **2** (10 μM), oxaliplatin (10 μM), oxaPt(IV) (10 μM), mixture (oxaPt(IV) : NFA = 10 μM : 20 μM), and NFA (20 μM) for 6 h, HCT116 cells were

fixed by 70% ethyl alcohol for 12 h. Then, cells were blocked in 0.5% albumin bovine V solution for 2 h. The rabbit polyclonal COX-2 were added, cells were co-incubated at 4 °C overnight. The 15 mm glass bottom cell culture dishes were washed and stained with Alexa Fluor 488-conjugated Goat Anti-Rabbit IgG(H+L) antibody for 2 h. After washed with PBS, the cells were mounted with DAPI for nucleus staining. The results were captured using a confocal laser scanning microscope (Olympus FV1000).

Wound healing assay

2×10^6 HCT116 cells/well were seeded in 6-well plates, grown to form confluent monolayers. Then the cells were wounded with 200 μL plastic pipette tips and washed with PBS. Cells were treated with complex **2** (5 μM), oxaliplatin (5 μM), oxaPt(IV) (5 μM), mixture (oxaPt(IV) : NFA = 5 μM : 10 μM) and NFA (10 μM), or varied compound at equitoxic concentration (IC_{50} value) for 48 h. The closure of wound was measured by the Motic AE2000 microscope and photographed with Mtico Images Advanced 3.2.

Cell adhesion assay

3×10^3 HCT116 cells/well were seeded into a matrigel-coated 96-well plate after 24 h treatments with complex **2** (5 μM), oxaliplatin (5 μM), oxaPt(IV) (5 μM), mixture (oxaPt(IV) : NFA = 5 μM : 10 μM), and NFA (10 μM), respectively. 24 h later, each well was washed twice by PBS. The plate was fixed in 4% paraformaldehyde and then stained with 1% crystal violet. After washed, the images were performed using an inverted microscope (Leica DMI3000B). And MTT (5 mg mL^{-1}) in 1640 medium was added into the other plate and the attached cells were incubated at 37 ºC for 4 h. Then, supernatant was removed and 100 μL DMSO was added to each well, and shaken for 10 min. The following processes were same as the measurement of cell viability.

Western blot analysis

HCT116 cells were seeded in 6-well plates and incubated until 90% confluent. The cells were exposed to complex **2** (10 μM), oxaliplatin (10 μM), oxaPt(IV) (10 μM), mixture (oxaPt(IV) : NFA = 10 μM : 20 μM), and NFA (20 μM) for 24 h. After treatments, the cells were harvested and washed twice with PBS. A solution of 200 μL RIPA buffer was added to each one. After 30 min, the cells were harvested and used as protein

extracts. After protein quantification, aliquots of 40 µg of proteins were denatured and departed in 10% SDS-PAGE. Then gels were transferred onto PVDF membrane. After blocking with 5% non-fat milk in tris-buffered saline–tween solution (TBST) for 4 h, the membrane was incubated overnight with primary polyclonal antibody at 1:2000 at 4 °C. After washing, the bands were added and incubated with secondary antibody conjugated to horseradish peroxidase (1:5000) for 1.5 h at room temperature. Finally, the bands were detected with Thermo Scientific Pierce ECL Western Blotting Substrate.

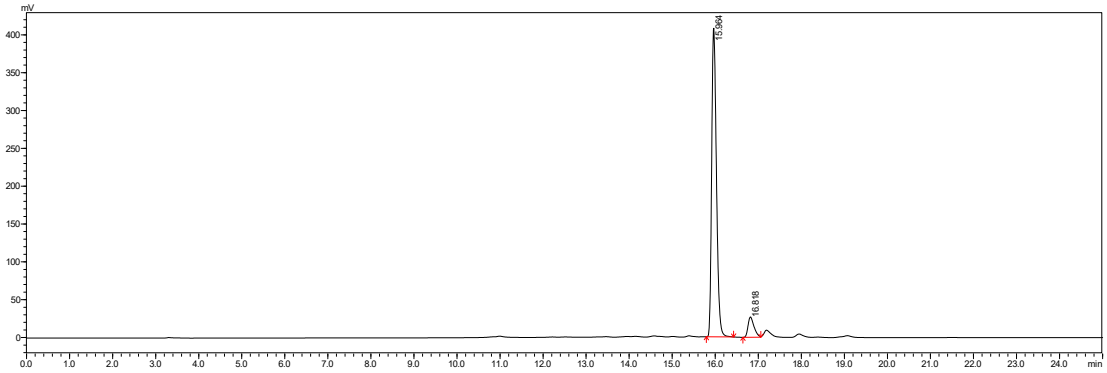

Fig. S1 HPLC characterization of **1**

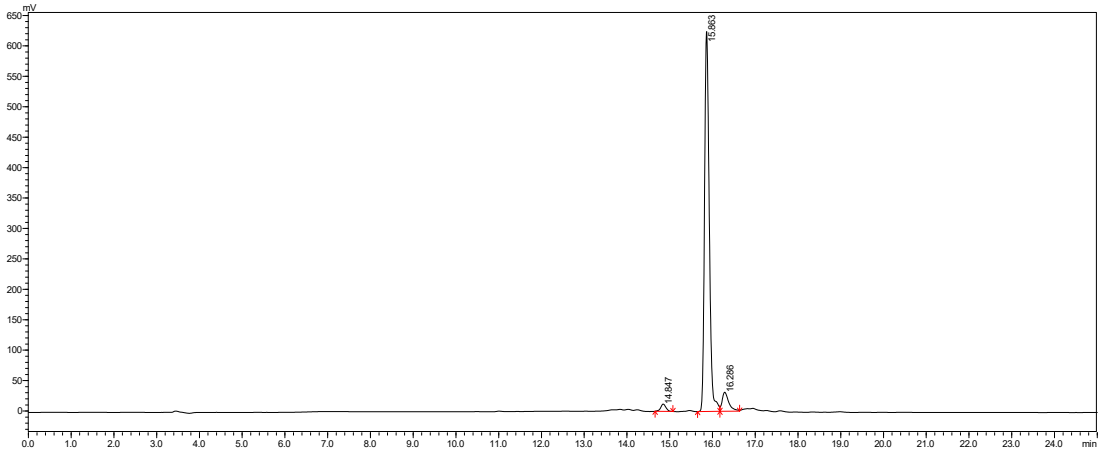

Fig. S2 HPLC characterization of **2**

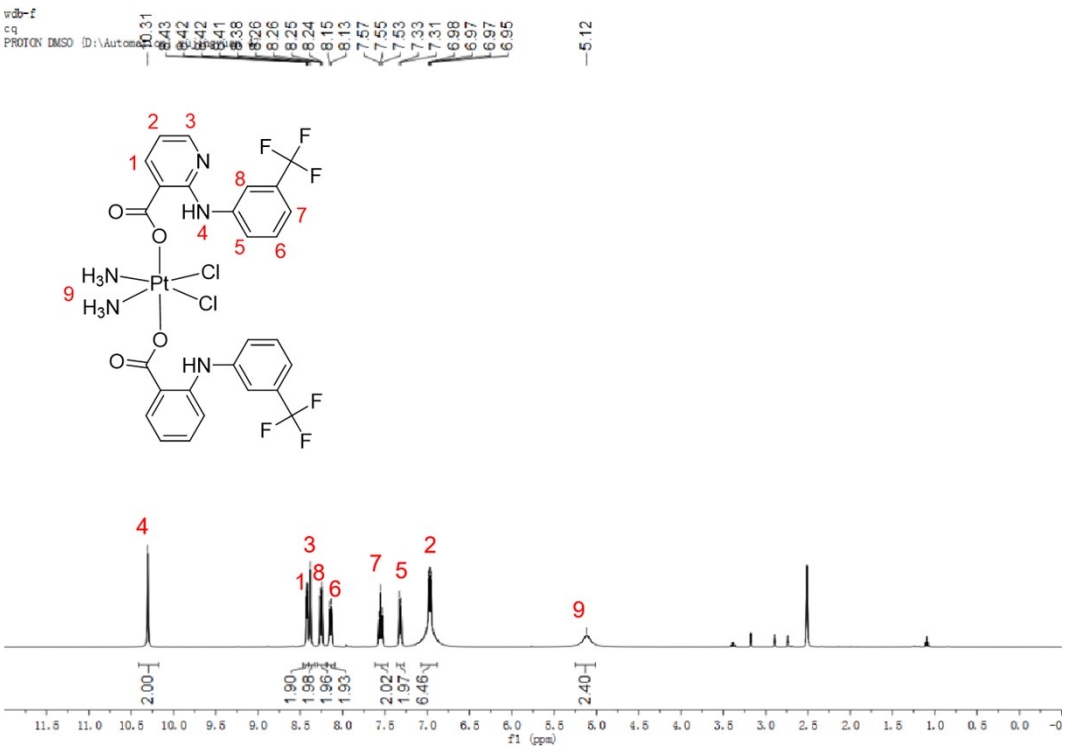

Fig. S3 ^{1}H NMR spectrum of **1** in DMSO-d_6

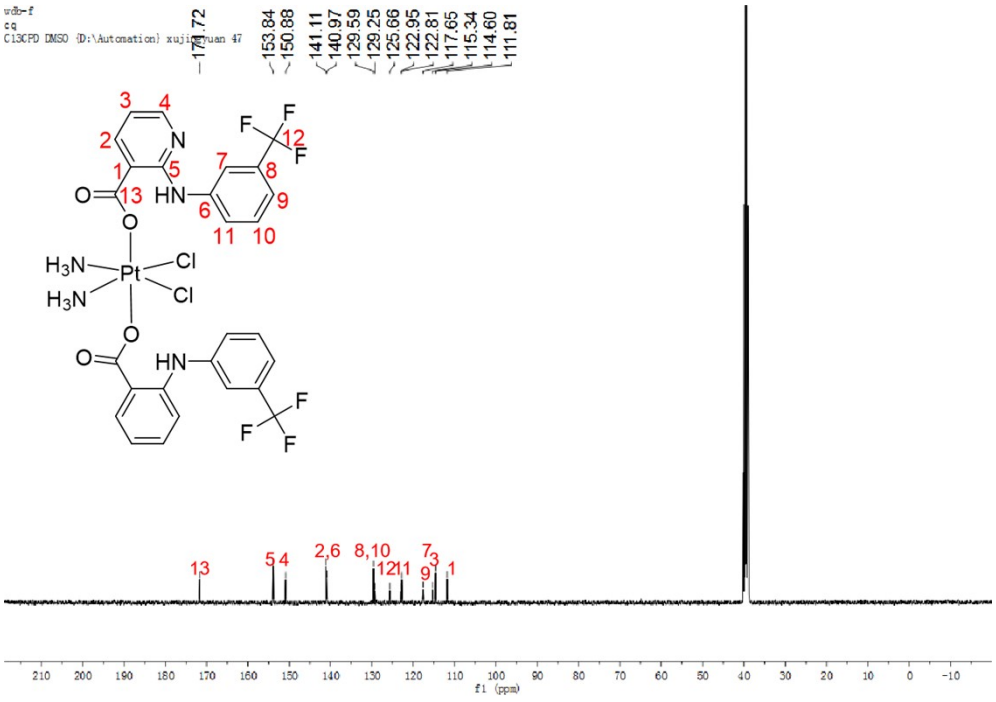

Fig. S4 ^{13}C NMR spectrum of **1** in DMSO-d_6

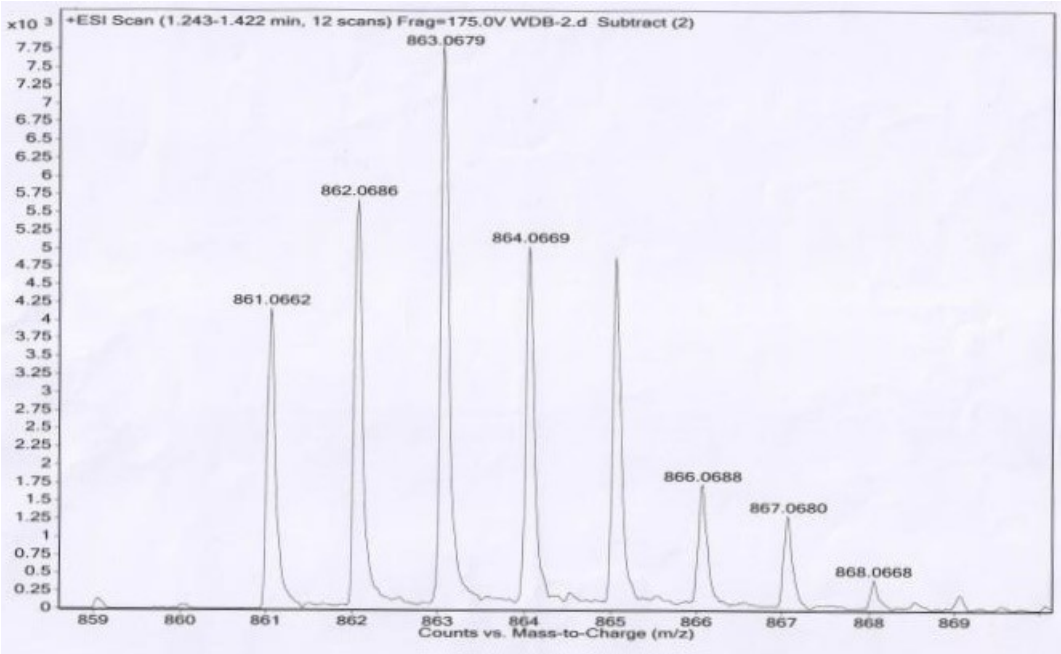

Fig. S5 ESI-HRMS spectrum of **1**

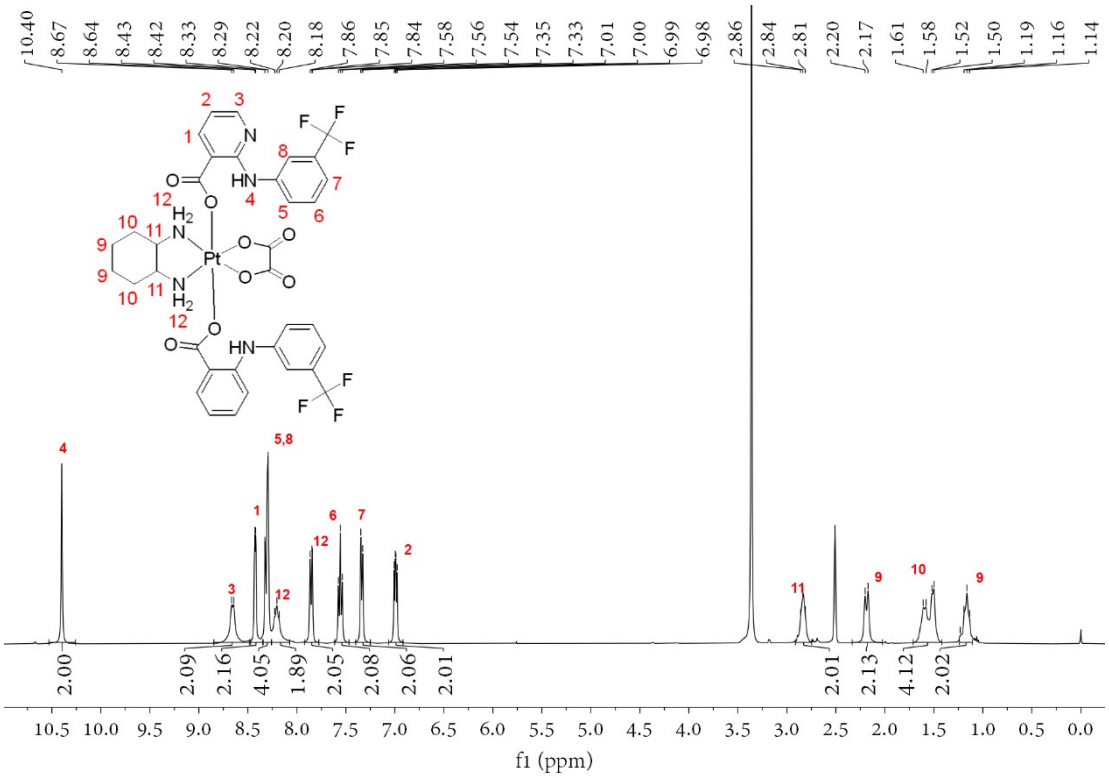

Fig. S6 ^{1}H NMR spectrum of **2** in DMSO-d_6

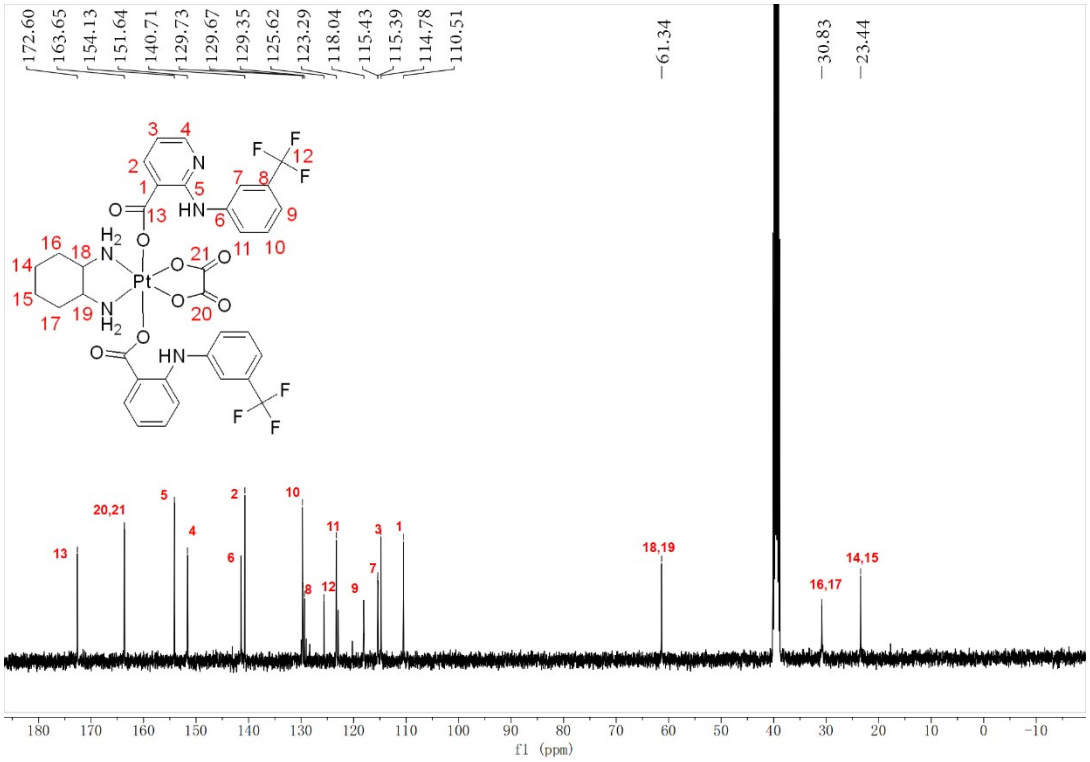

Fig. S7 ^{13}C NMR spectrum of **2** in DMSO-d_6

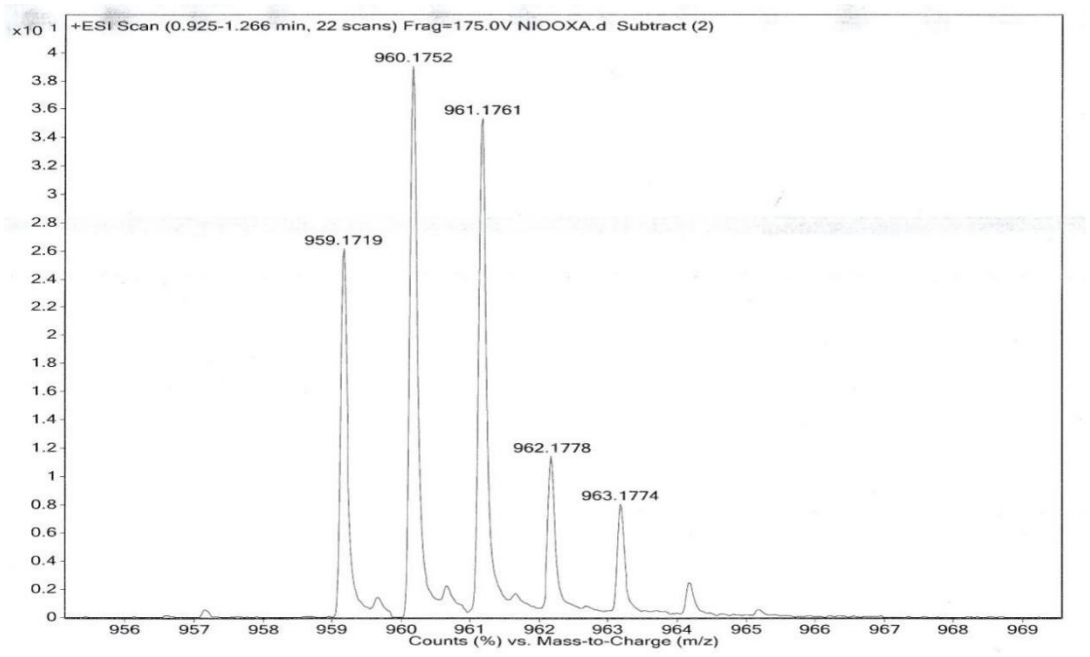

Fig. S8 HR-MS spectrum of **2**

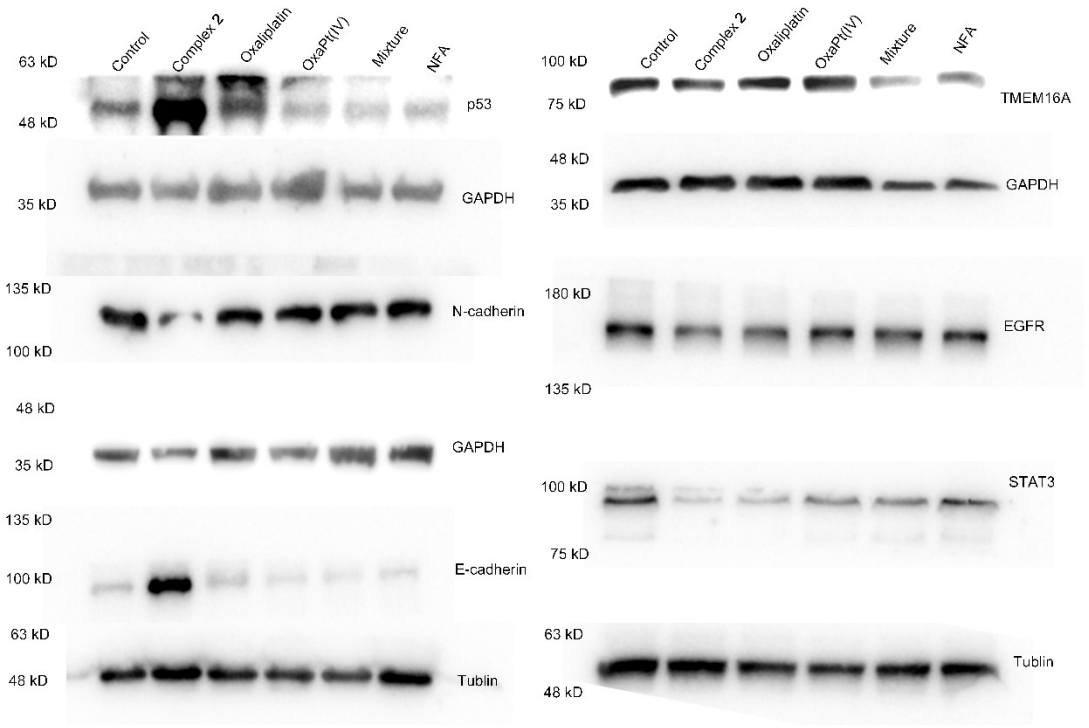

Fig. S9 Original images of the bands in western blot analysis

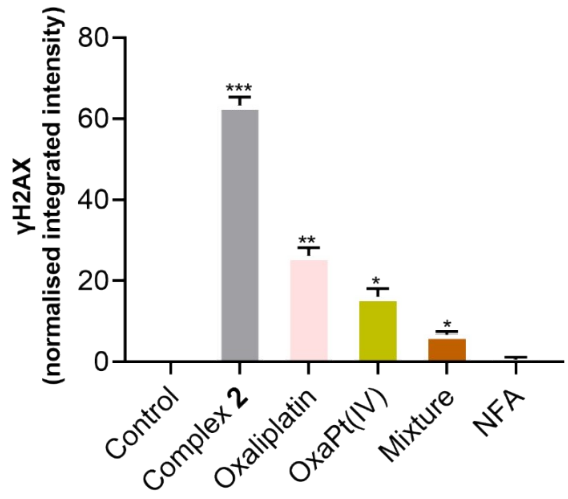

Fig. S10 Statistical analysis of the expression of γH2AX in HCT116 cells after treatment with varied compounds for 6 h. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with control group.

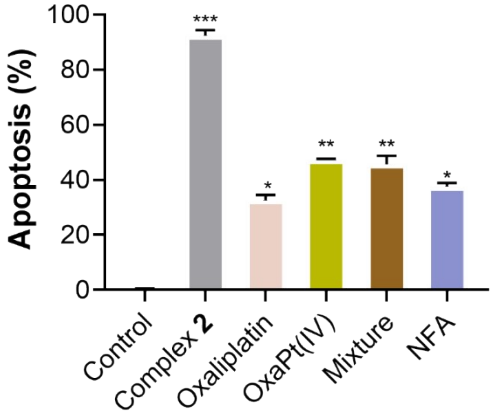

Fig. S11 Statistical analysis of the apoptosis in HCT116 cells after treatment with varied compounds for 48 h. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with control group.

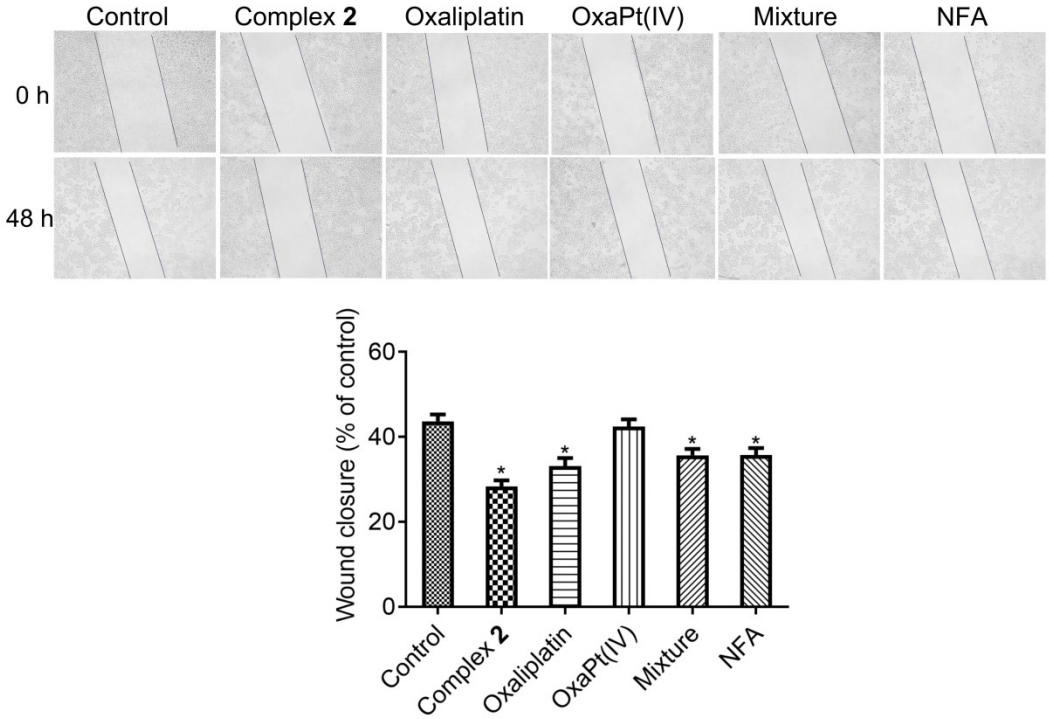

Fig. S12 Wound healing assay. HCT116 cells were treated with varied compounds at equitoxic concentration (IC_{50} value) for 48 h. $^{*}P < 0.05$, compared with control group.