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Supplementary information for

A red-light activatable and mitochondrion-targeting Pt^{IV} complex to overcome drug resistance

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Experimental section

Materials and instruments

All chemicals were obtained from commercial suppliers and used without further purification. All reaction were carried out under argon atmosphere using dry solvents. Cisplatin was bought from Shandong Boyuan Chemical Company. Succinic anhydride, N-hydroxy succinimide (NHS) and N,N'-dicyclohexylcarbodiimide (DDC) were purchased from Aladdin. IR-780 iodide was provided by J&K, Hoechst 33258 and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich. Mito-Tracker Green was purchased from KeyGen BioTECH. Bicinchoninic acid (BCA) protein assay kit, mitochondrial isolation kit, genomic DNA extraction kit and ATP assay kit were purchased from Beyotime Biotechnology. Mitochondrial membrane potential assay kit with JC-1 was bought from Solarbio Lifers Sciences.

^{1}H-NMR spectra were recorded at room temperature on a Bruker AVANCE DRX 500 spectrometer. Electrospray ionization mass spectrometry (ESI-MS) measurements were conducted on a Watera Quattro Premier XE system. UV-visible absorption spectra were measured on a Varian Cary 300 UV-visible spectrophotometer. X-ray photoelectron spectroscopy (XPS, Thermo ESCALAB 250) determined the oxidation state of Pt. In vitro cellular and histological fluorescence imaging were performed on confocal laser scanning microscope (CLSM) imaging system (Zeiss710, Japan). Cellular Pt content was detected by inductively coupled plasma mass spectrometry (ICP-MS, X Series II, Thermo Scientific). Cell viability assessments and cellular ATP contents were performed in Bio Tek Cytation^{TM}5. Cellular apoptosis was analyzed with flow cytometry analysis (Amnis). Photoirradiation experiments were carried out using CEAULIGHT CEL-MXL500 (λ = 650 nm, 10 mW/cm^2) and the intensity of LED source was measured by CEL-NP2000 optical power and energy meter.

Synthesis of PS-NH_2

0.21 mL triethylamine was added to IR-780 (133.4 mg, 0.2 mmol) in 15 mL CH_3OH, the mixture was stirred 5 min and then, ethylenediamine (120.1 mg, 2 mmol) was added. This reaction mixture was kept at room temperature for 6 h with vigorous stirring. After reaction, the solvent was evaporated to dryness. The residue was dissolved in CH_2Cl_2 and washed with water, and dried with anhydrous Na_2SO_4. After removing the solvent, the precipitate was further purified by silica gel column chromatography using CH_2Cl_2/CH_3OH = 50:1 as eluent to afford the target compound as blue powder (88.9 mg, yield: 65%). ^{1}HNMR (500 MHz, DMSO-d_6): δ 7.58 (m, 3H), 7.41 (d, 2H), 7.29 (t, 2H), 7.16 (d, 2H), 7.05 (t, 2H), 5.79 (d, 2H), 3.93 (t, 4H), 3.78 (t, 2H), 3.03 (t, 2H), 1,76-1.70 (m, 8H), 1.60 (s, 12H), 1.23 (s, 4H), 0.94 (t, 6H). ESI-MS (positive ion mode): m/z = 563.5 $[M-I]^+$.

Synthesized of PS-COOH

Succinic anhydride (12.0 mg, 0.12 mmol) and **PS-NH_2** (72.8 mg, 0.10 mmol) were added into DMF to stir at 60 ℃ for 24 h. After reaction, DMF was removed and precipitated in $(CH_3CH_2)_2O$. The obtained product was further purified by column silica gel column chromatography using CH_2Cl_2/CH_3OH = 50:1 (54.8 mg, yield: 75%). ^{1}HNMR (500 MHz, DMSO-d_6): δ 12.05 (s, 1H), 8.40 (s, 1H), 8.31 (s, 1H), 7.57 (d, 2H), 7.44 (d, 2H), 7.28 (t, 2H), 7.13 (d, 2H), 7.04 (t, 2H), 5.73 (d, 2H), 3.92 (t, 4H), 3.75 (s, 4H), 2.59 (s, 4H), 1.75-1.65 (m, 4H), 1.60 (s, 12H), 1.23 (s, 6H), 0.94 (t, 6H). ESI-MS (positive ion mode) m/z = 663.6 $[M-I]^+$.

Synthesized of Pt^{IV}-PS_2

$[Pt(NH_3)_2(Cl)_2(OH)_2]$ was synthesized according to reported procedures.[1] A mixture of **PS-COOH** (632 mg, 0.8 mmol), DDC (226.9 mg, 1.1 mmol) and NHS (126.6 mg, 1.1 mmol) in DMF was stirred for 1 h at 0 ℃. The reaction mixture was stirred at room temperature overnight. After reaction, reaction solution was filtered off and was added into ice-cold $(CH_3CH_2)_2O$, and then the resultant solution was putted in -20 ℃ for 12 h. The precipitate was collected by centrifugation and washed twice by ice-cold CH_2Cl_2. After lyophilization, the obtained precipitate (352 mg, 0.4 mmol) and c,c,t-$[Pt(NH_3)_2(Cl)_2(OH)_2]$ (50 mg, 0.15 mmol) were dissolved in DMSO to stir at room temperature for

48 h. The solvent was removed under vacuum, the crude product was purified by silica gel column chromatography using CH_2Cl_2/CH_3OH = 50:1 to 5:1 (297 mg, yield: 40%). ^1H NMR (500 MHz, 1:1 D_2O:methanol-d_4): δ 7.72 (d, 4H), 7.43 (d, 4H), 7.35 (t, 4H), 7.14 (d, 4H), 7.10 (t, 4H), 5.84 (d, 4H), 3.92 (t, 8H), 3.86 (t, 4H), 3.52 (t, 4H), 2.75 (s, 8H), 2.49 (s, 12H), 1.81 (m, 8H), 1.65 (s, 24H), 1.00 (t, 12H). ^{13}C NMR (500 MHz, DMSO-d_6) 173.65, 169.25, 166.94, 143.62, 140.25, 128.54, 122.67, 109.5, 94.62, 51.26, 47.52, 44.10, 31.77, 28.77, 25.63, 22.55, 21.52, 20.06, 11.73. HRMS (ESI): m/z = 813.4096 $[M-I]^{2+}$.

Cell culture

A549, A549cisR, MCF-7 and MCF-7cisR cells were obtained from Procell Life Science & Technology Co.,Ltd. A2780 and A2780cisR cells were purchased from Nangjing Cobioer Biosciences Co., Ltd.

A549, MCF-7, A2780, and A2780cisR cells were cultured in DMEM with 10% FBS 50 U/mL penicillin and streptomycin. MCF-7 and A549cisR cells were cultured in RPMI 1640 with 10% FBS, 2 mM L-glutamine, and 50 U/mL penicillin and streptomycin. All cisplatin resistant cells were incubated in culture medium containing 15 μM cisplatin to maintain the resistance. All cells were incubated in an atmosphere of 5% CO_2 at 37 ℃.

Cellular localization of Pt^{IV}-PS_2

Cells (2 × 10^4 cells/well) were seeded in 2 mm confocal dishes and incubated overnight. They were treated with **Pt^{IV}-PS_2** (20 μM) for 1 h, 12 h, and 24 h, then the medium was carefully removed and all wells were washed twice with PBS. Cells were treated with Mito-Tracker Green for another 2 h. After incubation, cells were washed twice with PBS and fixed with 4% aqueous paraformaldehyde for 20 min. Each well was washed twice PBS and fluorescence images were observed by CLSM.

A549cisR cells (2 × 10^4 cells/well) were seeded in 6-well plates overnight. Cells were treated with **Pt^{IV}-PS_2** (20 μM) for 1 h. After treatment, cells were wash with PBS twice and collected by trypsin. Then mitochondria isolation kit was used to obtain nuclei, cytosol and mitochondria. The concentration of Pt in proteins of different fractions was detected by ICP-MS.

Pt levels in genomic DNA

A549cisR cells (3 × 10^4 cells/well) were cultured in 6-well plates for 24 h before treated with **Pt^{IV}-PS_2** (20 μM) for 1 h. After incubation, cells were washed twice with PBS, fresh culture medium was added, and cells were irradiated with or without red light for 30 min and cultured for another 12 h. Then, cells were carefully washed with PBS and digested by trypsin. Genomic DNA was collected from the collected cell using DNA extraction kit, then Pt levels in DNA solution was quantified by ICP-MS.

Determination of uptake pathway

A549cisR cells (1 × 10^4 cells/well) were seeded in 12-well plates for 24 h. **Pt^{IV}-PS_2** (20 μM) was added into cells and incubated for 1 h at 37 ℃ or 4 ℃ in dark wherein the energy-dependent endocytosis was blocked. In addition, cells were respectively pre-incubated with medium with 10 μg/mL wortmannin, 10 μg/mL chlorpromazine, 5 mM mβCD, or100 μg/mL genistein for 30 min at 37 ℃, and then cells were continued to incubate with **Pt^{IV}-PS_2** (20 μM) for another 1 h at 37 ℃. Cells were washed with PBS twice and imaged by with confocal microscopy.

Cytotoxicity

The cytotoxicity of the complexes was assessed by MTT. Briefly, cells (3 × 10^3 cells/well) were planted in 96-well plates and cultured for 24 h. Various concentrations of **PS-COOH**, cisplatin, **PS-COOH** + cisplatin (1:1) or **Pt^{IV}-PS_2** containing 0.5% DMSO were added into cells and incubated 1 h in dark. The medium was removed and cells were carefully washed twice, and then fresh medium was added. For irradiation groups, cells were irradiated by red light (10 mW/cm^2) for 30 min and continued to be incubated for another 48 h in dark. Meanwhile, cells of dark groups were incubated for 48 h in dark. Finally, MTT (5 mg/mL) was added and incubated for 4 h, then the medium was removed and 150 μL DMSO was added into each plate. Absorbance at 490 nm was measured by microplate reader.

Live/dead cell staining assay

A549cisR cells (2×10^4 cells/well) were cultured in 2 mm confocal dishes for 24 h, then **PS-COOH** (10 μM), cisplatin (5 μM) and **Pt^{IV}-PS_2** (5 μM) were added into each well for 1 h. After incubation, each well was washed with PBS three times and fresh culture medium was added. Cells were treated with or without irradiation for 30 min before incubation for another 2 h. Then, culture medium was replaced with medium containing calcein-AM (10 μg/mL) and PI (5 μg/mL), and cells were incubated for 20 min. After that, cells were carefully washed twice with PBS and imaged by CLSM.

Apoptosis assay

The apoptosis of A549cisR cells were determined by Annexin V-FITC apoptosis detection kit. Early apoptotic cells were selectively internalized of Annexin V-FITC dye, late apoptotic/necrotic cells were permeable to both FITC and PI, and dead cells were marked by PI. Hence, apoptotic modes of cells were analyzed by the flow cytometer. A549cisR cells (3×10^4 cells/well) were cultured in 6-well plates for 24 h before treated with **PS-COOH** (40 μM), cisplatin (20 μM) or **Pt^{IV}-PS_2** (20 μM) for 1 h in dark. Cells were treated with or without irradiation for 30 min, and then incubated in dark for another 2 h. After that, cells were collected by trypsinization and subsequent centrifugation. Cells were resuspended in Annexin-binding buffer, and then FITC and PI were stained cells for 15 min in dark. The percentage of early and late apoptotic cells were quantified by flow cytometer.

Cell cycle analysis

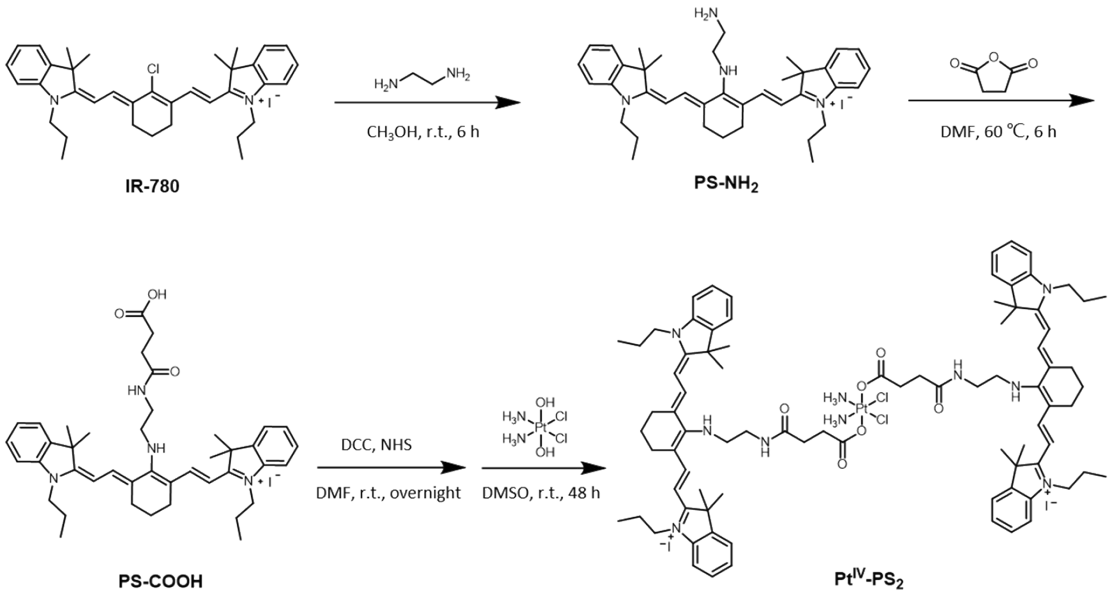
Cell cycle and apoptosis analysis kit was used to study the effects of **Pt^{IV}-PS_2** on cell cycle of A549cisR cells. Intracellular DNAs were stained by PI and quantified with flow cytometer. Subsequently, the cell cycle was analyzed according to the distribution of DNA content. Cells (3×10^4 cells/well) were cultured in 6-well cell plates for 24 h. Cells were treated with **PS-COOH** (10 μM), cisplatin (5 μM) or **Pt^{IV}-PS_2** (5 μM) for 1 h in dark. Then, cells were washed with PBS twice and fresh culture medium was added. Cells were irradiated for 30 min, and then incubated in dark for 12 h. The cells were digested by trypsin and centrifuged at 1000 g for 5 min. The supernatants were discarded and the resulting pellet was resuspended in 1 mL of pre-chilled PBS. This was repeatedly centrifuged and the supernatant was discarded. Pre-chilled 70% ethanol was added into the pellet to fix 4 ℃ for 2 h. After that, this was centrifuged again and the pellet was stained by PI at 37 ℃ for 30 min. The impact of **Pt^{IV}-PS_2** on cell cycle arrest was evaluated by flow cytometer.

Mitochondrial membrane potential detection

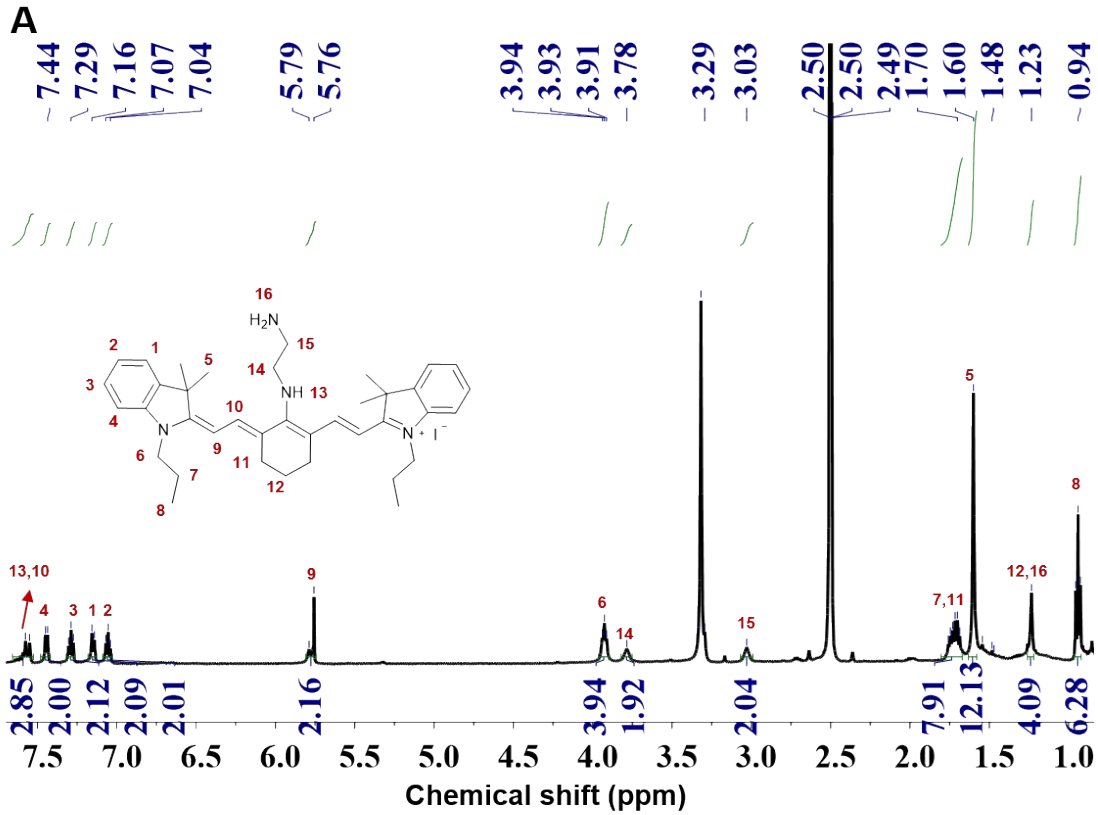
The mitochondrial membrane potential of A549cisR cells were detected by JC-1 assay kit. Cells (2×10^4 cells/well) were seeded in 2 mm confocal dishes and incubated for 24 h before treated with **PS-COOH** (10 μM), cisplatin (5 μM) and **Pt^{IV}-PS_2** (5 μM) for 1 h. After incubation, the medium of each well was removed and was washed twice with PBS, and fresh culture medium was added. The cells were incubated with or without irradiation for 30 min, then JC-1 dye was treated at 37 ℃ for 20 min. After multiple rinse with PBS, the cells were imaged by confocal microscopy.

Cellular ATP levels determination

The ATP of A549cisR cells were measured using ATP assay kit. Cells (3×10^4 cells/well) were cultured in 6-well plates for 24 h before treated with **Pt^{IV}-PS_2** (5 μM) for 1 h. After incubation, each plate was washed twice with PBS and added fresh culture medium. Then the cells were treated with or without irradiation for 30 min, followed by another culture for 12 h. Cells were isolated by trypsinization and washed with PBS. Cell lysates were added and incubated on ice for 20 min, and then cells were centrifuged (12000 g at 4 ℃ for 5 min). Following this, the resulting supernatant was carefully collected and the ATP content was quantified.



Scheme S1. Synthetic procedure of $\mathbf{Pt^{IV}\text{-}PS_2}$.



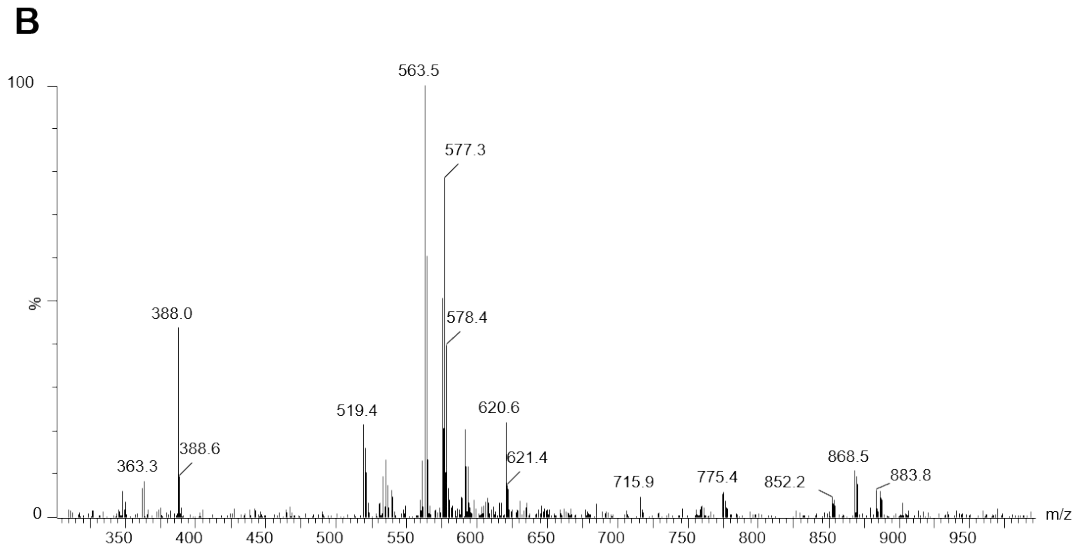
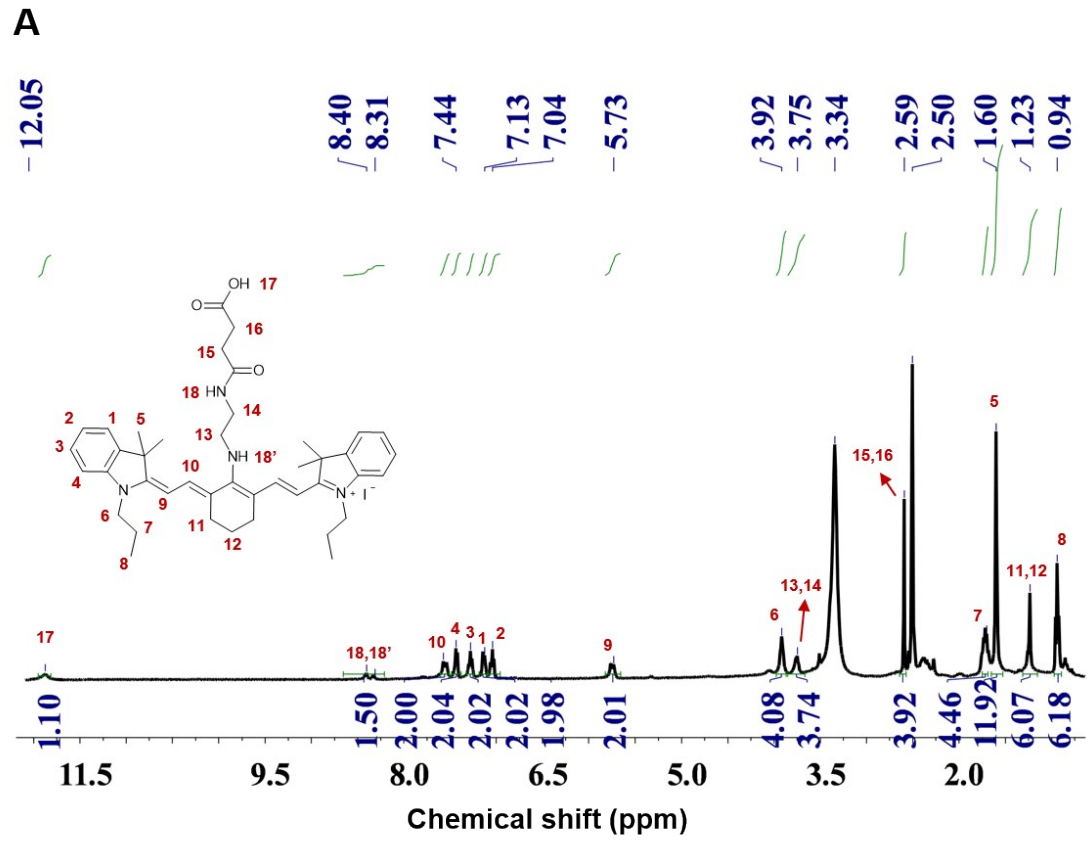


Fig. S1 The (A) ^{1}H NMR and (B) ESI-MS spectra of **PS-NH$_2$**.



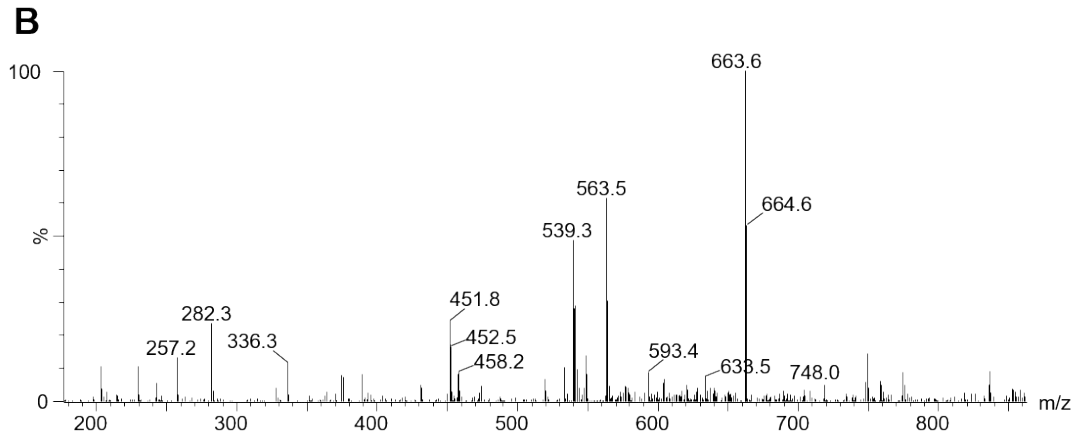
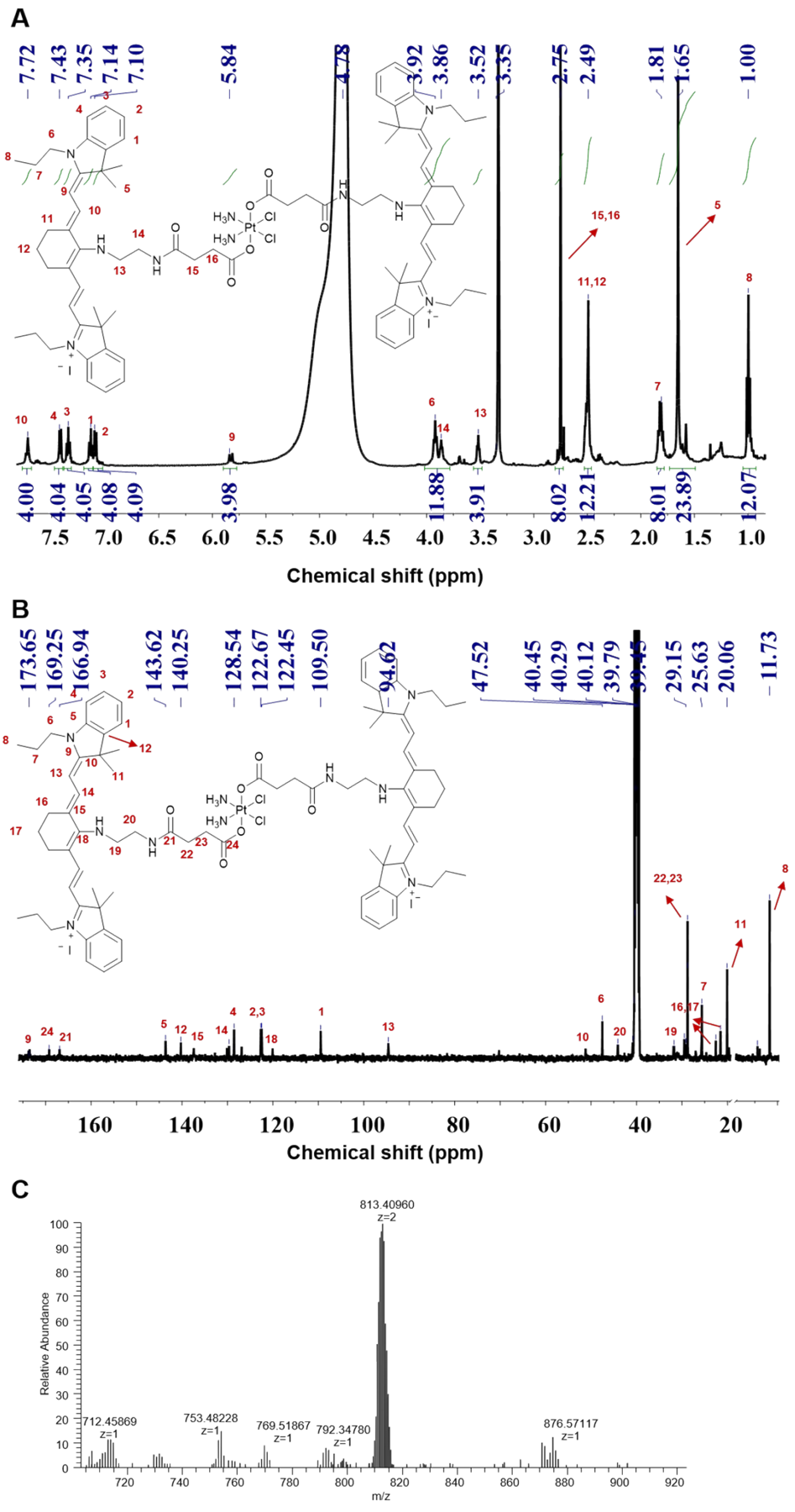


Fig. S2 The (A) ^{1}H NMR and (B) ESI-MS spectra of **PS-COOH**.



dFig. S3 The (A) ^{1}H NMR, ^{13}C NMR and (C) HRMS spectra of **Pt^{IV}-PS_2**.

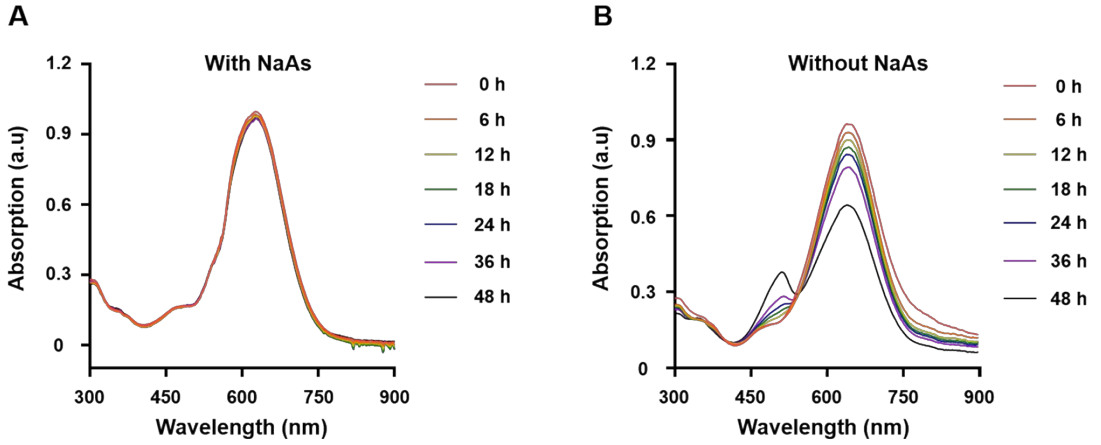


Fig. S4 The UV-Vis spectra of **Pt^{IV}-PS_2** in PBS buffer (pH = 7.4) with or without sodium ascorbate.

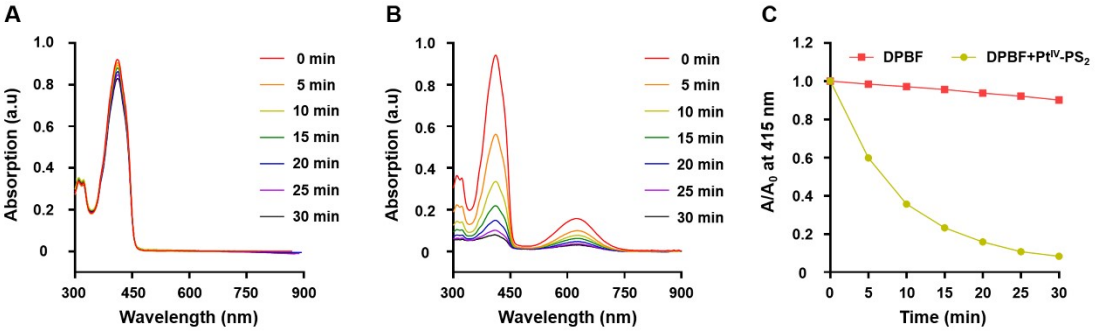


Fig. S5 Singlet oxygen detection of **Pt^{IV}-PS_2** by DPBF. The UV-Vis spectra of (A)DPBF and (B) **Pt^{IV}-PS_2** with DPBF in DMF upon irradiation. (C) The change in the DPBF absorption at 415nm over time under irradiation (650 nm, 10 mW/cm^2).

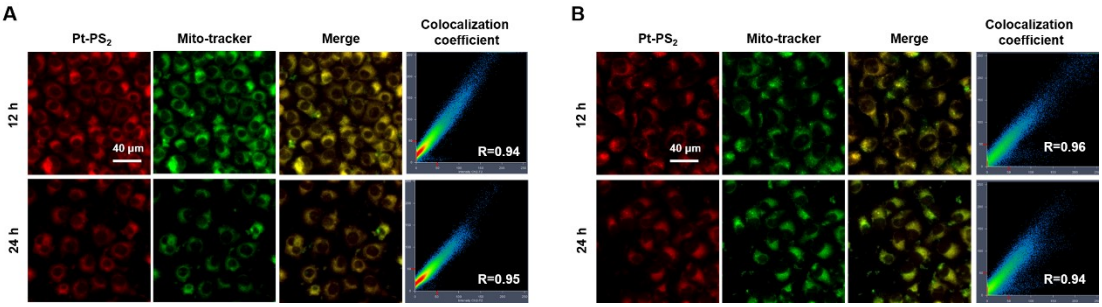


Fig. S6 Fluorescent images of mitochondrial tracker-stained (A) A549 cells and (B) A549cisR cells were treated with **Pt^{IV}-PS_2** for 12 h or 24 h in the dark.

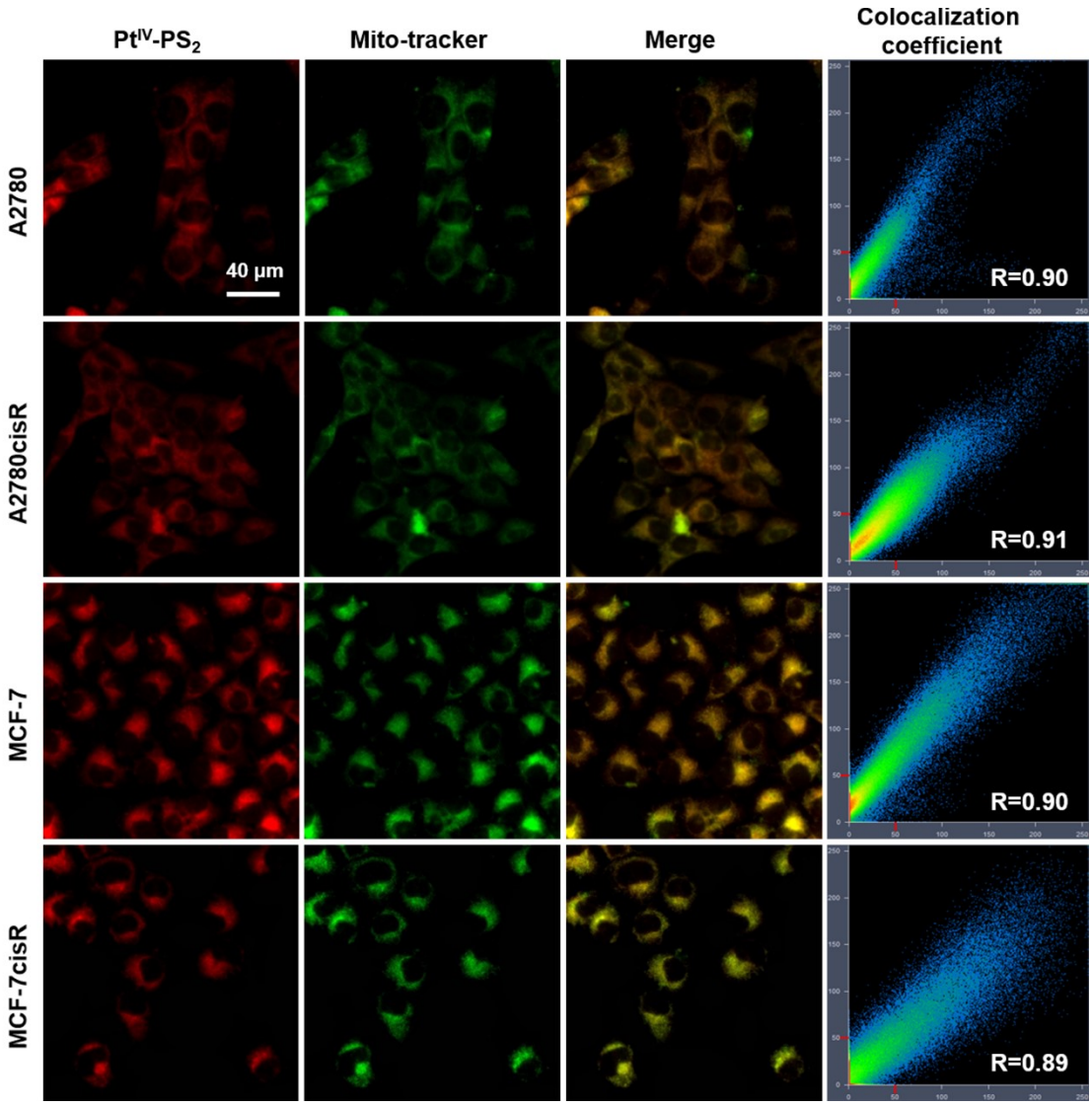


Fig. S7 Fluorescent images of mitochondrial tracker-stained virous cancer cells were treated with **Pt^{IV}-PS_2** for 1 h in the dark.

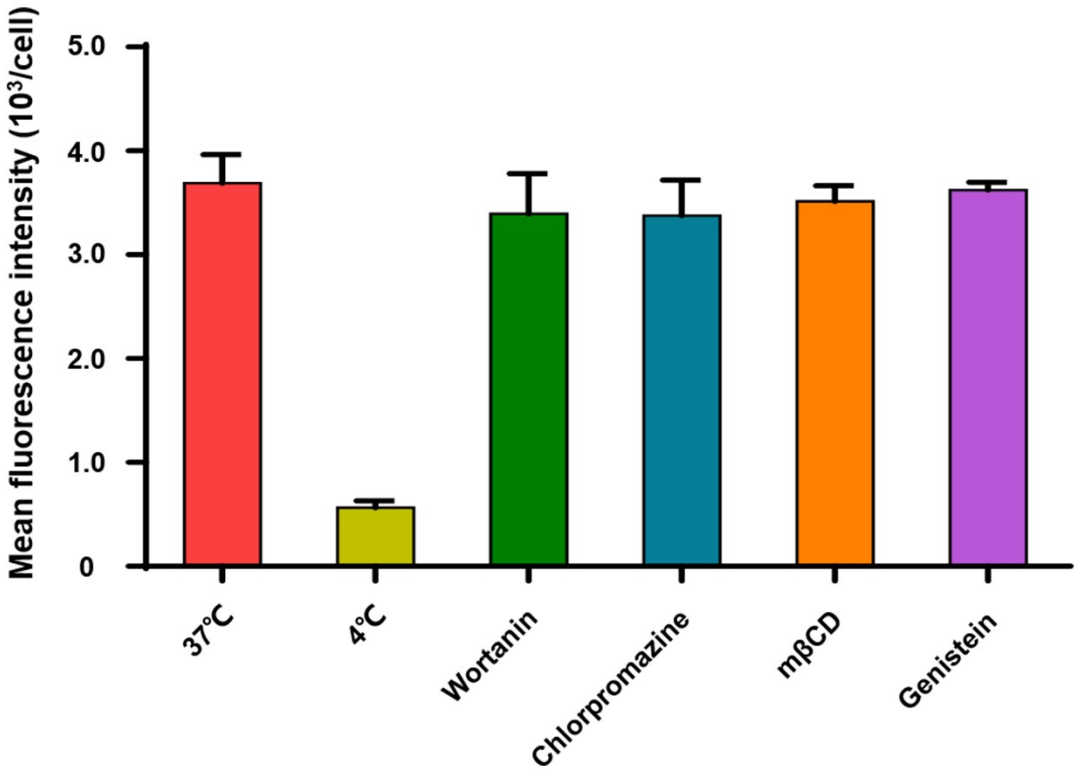


Fig. S8 The mean fluorescence intensities of $\mathbf{Pt^{IV}}$-$\mathbf{PS_2}$ at pear A549cisR cell. A549cisR cells were treated with $\mathbf{Pt^{IV}}$-$\mathbf{PS_2}$ (10 μM) at low temperature or in presence of various endocytic inhibitors for 1 h.

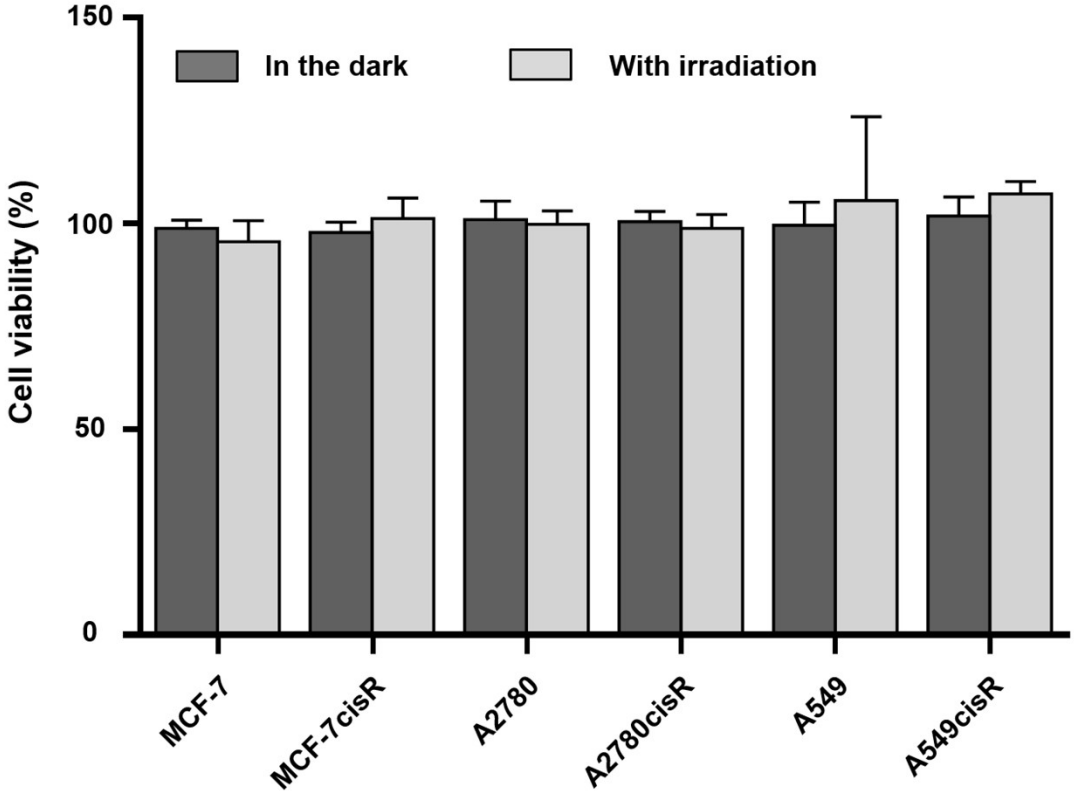


Fig. S9 Cell viability of virous tumor cells treated with or without irradiation for 30 min. After that, cells were cultured for another 48 h, and then their viabilities were detected by MTT assay.

Table. S1 IC_{50} values of virous cell lines treated with different complexes.

Cell line	IC_{50} (μM)									
	Pt^{IV}		PS-COOH		Cisplatin		PS-COOH + Cisplatin		Pt^{IV}-PS_2	
	-	+	-	+	-	+	-	+	-	+
MCF-7	160.3	154.2	＞50	29.3	35.3	32.6	25.0	17.1	35.3	7.7
MCF-7cisR	＞200	＞200	＞50	44.3	＞50	＞50	＞50	31.0	44.9	8.9
A2780	148.6	132.3	＞50	27.4	29.9	25.2	16.4	9.7	38.0	12.0
A2780cisR	＞200	192.3	＞50	33.2	＞50	＞50	＞50	29.6	44.6	12.5
A549	178.8	163.8	＞50	27.7	49.7	43.3	35.5	19.7	38.6	11.1
A549cisR	＞200	＞200	＞50	33.8	＞50	＞50	＞50	29.1	＞50	13.8

(-) In the dark (+) With irradiation

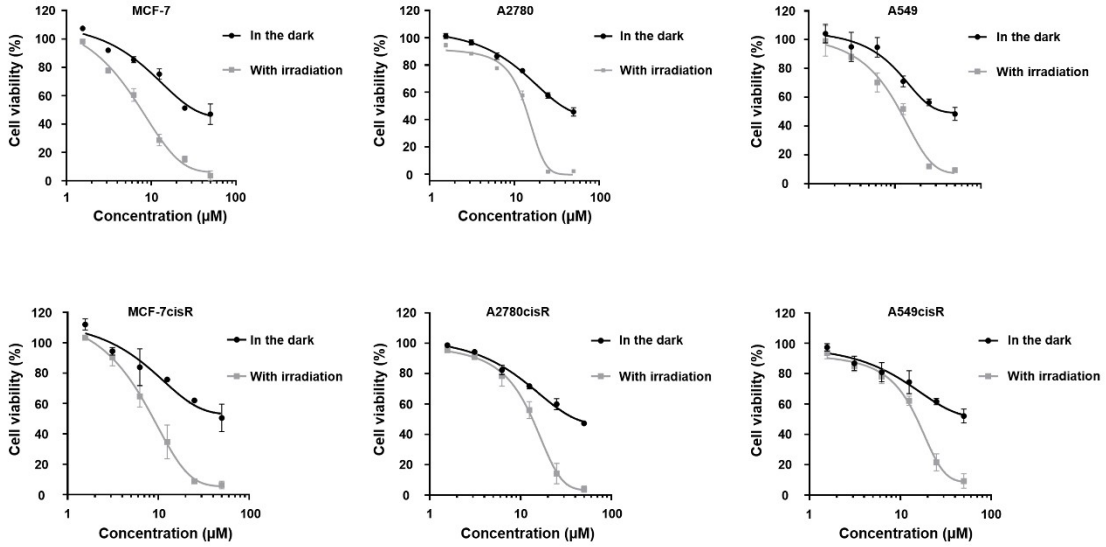


Fig. S10 Cell viability of **Pt^{IV}-PS_2** on virous tumor cells. Cells were treated with this ptodrug for 1 h; then the medium was replaced with fresh culture medium, cells were treated with or without irradiation for 30 min and cultured for another 48 h. Cell viability was examined by MTT asasy.

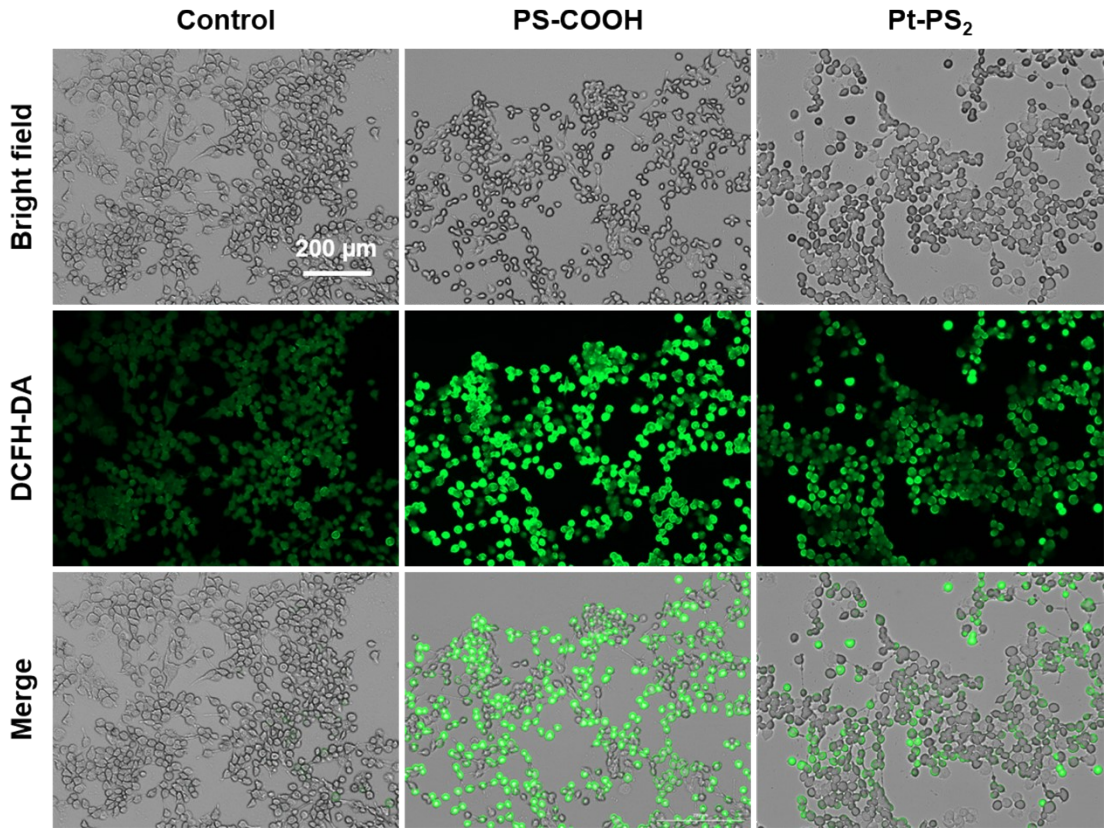


Fig. S11 Images of H_2DCFDA-stained A549cisR cells were treated with **PS-COOH** or **Pt^{IV}-PS_2** for 1 h followed by irradiation for 30 min.

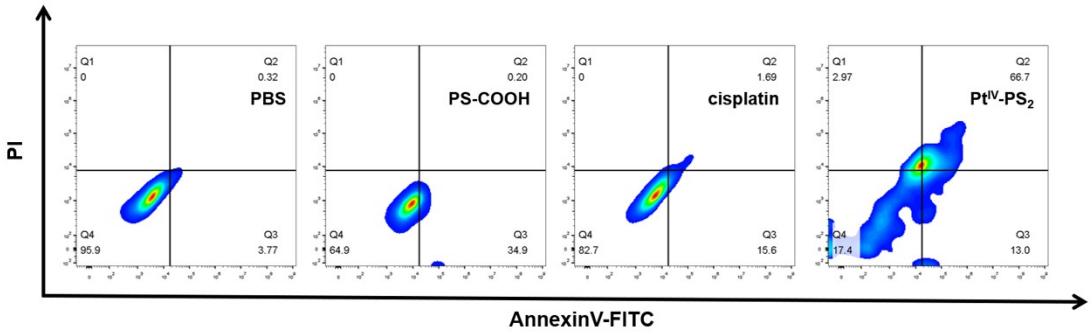


Fig. S12 The apoptosis of A549cisR cells with treated with different complexes. After incubation for 1 h, the medium was changed with fresh culture medium, and then cells were irradiated for 30 min. The apoptosis assay was detected by flow cytometry.

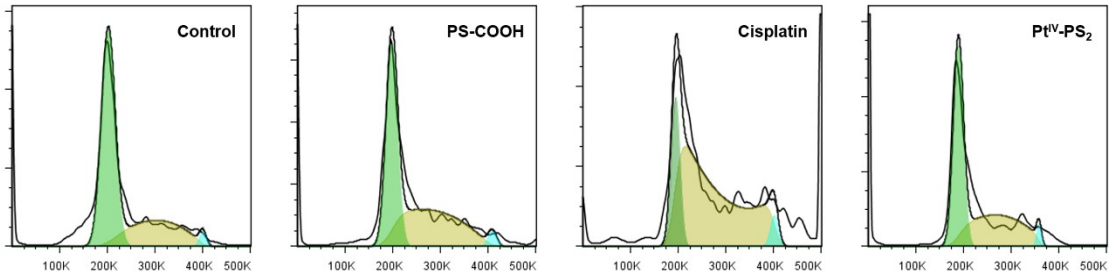


Fig. S13 Cell cycle after different treatments. A549cisR cells were treated with **PS-COOH**, cisplatin or **Pt^{IV}-PS_2** for 1 h; then the medium was replaced with fresh culture medium, and cells were irradiated for 30 min. The cell cycle progression was analyzed by flow cytometry.

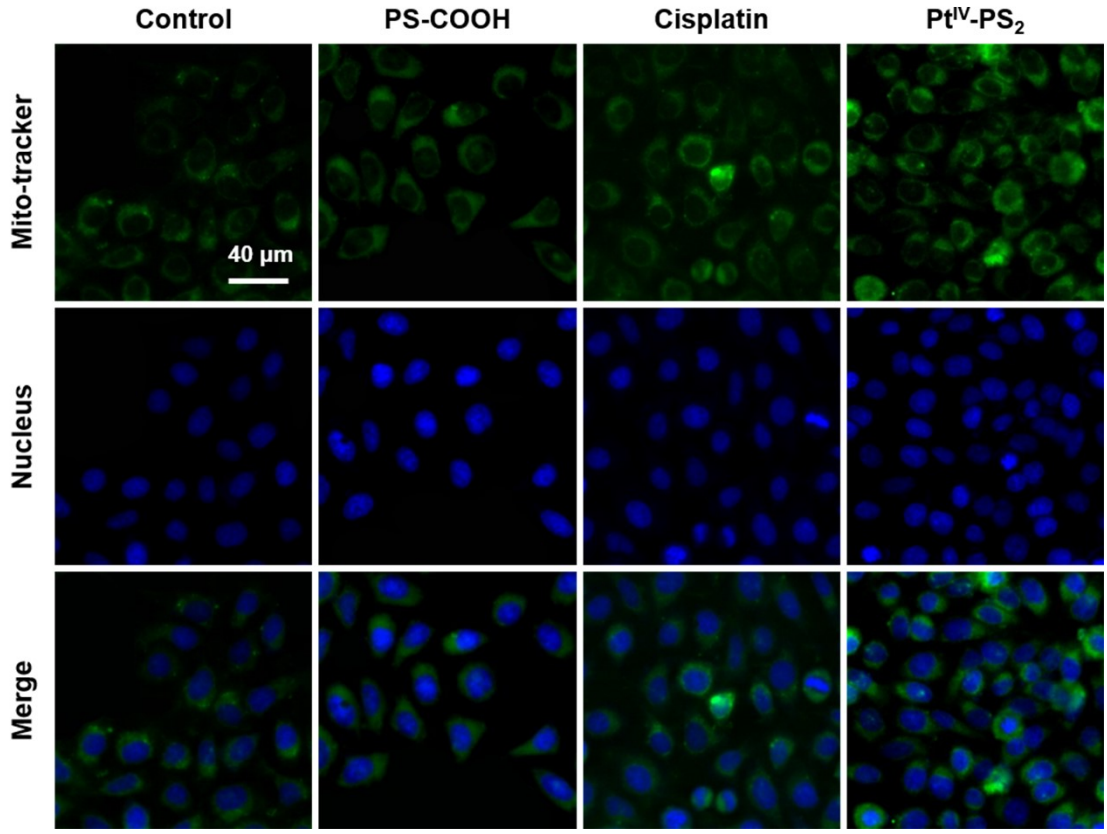


Fig. S14 The apoptosis of A549cisR cells with treated with different complexes. After incubation for 1 h, the medium was changed with fresh culture medium, and then cells were irradiated for 30 min. The apoptosis assay was detected by flow cytometry.

Reference

1. Z. Deng, C. Li, S. Chen, Q. Zhou, Z. Xu, Z. Wang, H. Yao, H. Hirao and G. Zhu, *Chem. Sci*, 2021, **12**, 6536-6542.