Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information for

Highly photoactive Ir(III)–Pt(IV) heterometallic conjugates for anticancer therapy

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Reagents and instruments

All chemicals used in this study were obtained from commercial suppliers and used without any additional purification. Iridium complex **Ir** was bought from Bide Pharmatech (Shanghai, China). The reactions were conducted under atmospheric pressure and shielded from light using aluminum foil. ^{1}H and ^{13}C NMR spectra were acquired at room temperature using a Bruker AVANCE III HD 600 MHz NMR spectrometer. ^{195}Pt NMR spectra were measured at room temperature on a Bruker AVANCE III HD 500 MHz NMR spectrometer. The chemical shifts (δ) were reported in parts per million (ppm) and referenced to residual solvent peaks. High-resolution mass spectrometry (HRMS) was performed using a SCIEX X500R QTOF system. Purification of the synthesized complexes was accomplished using a Waters Alliance e2695 Separations Module equipped with a YMC-Pack Pro C18 RS column (5 µm, 80 Å, 250 x 10.0 mm, flow rate of 3 mL/min). LC-MS analysis was carried out on an Agilent 1260-6125 + LC-MS system equipped with either a YMC J'sphere ODS-H80 column (4 µm, 80 Å, 100 x 2.0 mm, flow rate of 0.3 mL/min) or a Phenomenex Kinetex C18 column (5 µm, 100 Å, 150 x 3.0 mm, flow rate of 0.4 mL/min). The detectors were set at wavelength of 254 nm.

Synthesis of IrPt

Ir (354 mg 0.4 mmol, 1.0 eq.), NHS (138 mg, 1.2 mmol, 3.0 eq.), and DCC (247 mg, 1.2 mmol, 3.0 eq.) were dissolved in 20 mL of anhydrous acetonitrile (ACN). The resulting mixture was stirred at room temperature for 6 h, during which time the byproduct DCU solid formed. The solution was then filtered through a 0.2 µm microporous membrane to collect the filtrate. Subsequently, 200 mL of diethyl ether (Et_2O) was added to the filtrate, resulting in the precipitation of a yellow solid. The solid was collected by filtration, followed by drying to obtain the desired product, **Ir-NHS**, as a yellow solid (260 mg 74% yield). This product was used without further characterization and purification.

Ir-NHS (200 mg, 0.23 mmol, 1.0 eq.) and oxo-oxaliplatin (296 mg, 0.69 mmol, 3.0 eq.) was dissolved in anhydrous DMSO (10 mL) and stirred at 40 °C overnight. The reaction was monitored by LC-MS, and stopped when the starting materials **Ir-NHS** were completely consumed. The reaction mixture was filtered to remove the unreacted oxo-oxaliplatin and the filtrate was lyophilized to yield a yellow light solid. The solid was re-dissolved in ACN followed with addition of Et_2O, which get a yellow solid. The solid was dried as raw product with a purity of 91%. The raw product was further purified by semi-preparative HPLC with 0.1% HCOOH in water/acetonitrile gradient and lyophilized to obtain the HCOOH salt of **IrPt** complex (110 mg, 43% yield) as an orange solid. RP-HPLC (t = 25 min, 0 – 3 – 18 – 20 min – 25 min, 20 – 20 – 70 – 90 – 100% acetonitrile with 0.05% HCOOH. 3 mL/min), R_t =12.8 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.00 (dd, *J* = 8.3, 5.3 Hz, 1H), 8.68 (s, 1H), 8.58 (d, *J* = 17.9 Hz, 1H), 8.45 (s, 1H), 8.28(overlap,1H), 8.26 (d, *J*=7.6 Hz, 2H), 8.17 (s, 1H), 8.11 (s, 1H), 7.95(t, *J* = 7.7 Hz, 2H), 7.93 (d, *J* = 7.9 Hz, 2H), 7.86 (d, *J* = 5.4 Hz, 1H), 7.70(d, *J* = 5.7 Hz, 1H), 7.69 (overlap, 1H), 7.61 (dd, *J* = 10.2, 4.5 Hz, 2H), 7.51 (d, *J* = 5.7 Hz, 1H), 7.43 (s, 1H), 7.17 (dd, *J* = 7.2, 7.0 Hz, 2H), 7.01 (dd, *J* = 7.4 Hz, 7.4 Hz 2H), 6.91 (dd, *J* = 7.4, 7.4, 1.7 Hz, 2H), 6.19 (d, *J* = 7.5 Hz, 2H), 2.67-2.75 (m, 2H), 2.55-2.64 (m, 2H), 2.32 – 2.20 (m, 2H), 2.09 (d, *J* = 11.8 Hz, 1H), 2.02 (d, *J* = 12.0 Hz, 1H), 1.87 – 1.76 (m, 2H), 1.50 (overlap, 3H), 1.31 (tt, *J* = 12.5, 7.2 Hz, 1H), 1.16 – 1.01 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 181.56, 181.54, 166.85, 166.83, 165.29, 164.17, 164.13, 164.11, 155.48, 155.15, 150.69, 150.55, 149.80, 149.23, 148.88, 143.86, 143.79, 139.60, 138.75, 131.06, 130.23, 128.61, 128.49, 125.08, 123.97, 122.23, 120.06, 120.03, 61.62, 36.35, 33.99, 30.74, 26.19, 26.17, 23.66, 23.64. ^{195}Pt NMR (125 MHz, DMSO-d_6) δ 1398.4. ESI-HRMS: calcd for $C_{44}H_{44}N_6O_7PtIr$ $[M]^+$: *m/z* 1156.2554; found: *m/z* 1156.2482.

Synthesis of IrPtC12

To a solution of raw **IrPt** (100 mg, 0.08 mmol 1 eq.) in 4 mL anhydrous DMF, 1-isocyanatododecane (122 µL, 0.48

mmol, 6.0 eq.) was added and stirred at RT. for 4 hours. The mixture was added to Et_2O to precipitate the raw product, which was further purified by semi-preparative HPLC with 0.1% HCOOH in water/acetonitrile gradient and lyophilized to obtain the HCOOH salt of **IrPtC12** (52 mg, 44% yield) as an orange solid. RP-HPLC (t = 25 min, 0 – 3 – 15 – 25 min, 20 – 20 – 100 – 100% acetonitrile with 0.05% HCOOH. 3 mL/min), R_t = 15.0 min. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.63(s, 1H), 8.98 (d, *J* = 8.2 Hz, 1H), 8.69 (s, 1H), 8.49 (s, 1H), 8.44 – 8.31 (s, 2H), 8.29 – 8.23 (overlap, 3H), 7.92 (overlap, 4H), 7.86 (dd, *J* = 5.7, 1.6 Hz, 1H), 7.71 (d, *J* = 5.7 Hz, 1H), 7.70 – 7.67 (m, 1H), 7.61 (dd, *J* = 5.5, 5.5 Hz, 2H), 7.52 (dd, *J* = 5.7, 1.6 Hz, 1H), 7.16 (ddd, *J* = 7.4, 5.5, 1.4 Hz, 2H), 7.01 (ddd, *J* = 7.5, 7.5, 1.4 Hz, 2H), 6.89 (ddd, *J* = 7.4, 7.4, 1.3 Hz, 2H), 6.77 (t, *J* = 5.8 Hz, 1H), 6.19 (dd, *J* = 7.6, 1.2 Hz, 2H), 2.89 (dhept, *J* = 26.2, 6.6 Hz, 2H), 2.77 – 2.69 (m, 2H), 2.60 – 2.55 (m, 2H), 2.40 – 2.28 (m, 2H), 2.13 (d, *J* = 11.7 Hz, 2H), 1.83 (p, *J* = 7.2 Hz, 2H), 1.48 (s, 2H), 1.44-1.35 (m, 2H), 1.33 (t, *J* = 7.0 Hz, 2H), 1.22 (s, 18H), 1.13-1.05 (m, 2H), 0.84 (t, *J* = 6.8 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 180.34, 166.86, 166.83, 165.24, 164.29, 163.63, 163.59, 155.47, 155.17, 155.03, 150.68, 150.54, 149.81, 149.25, 148.88, 143.86, 143.80, 139.60, 138.75, 131.06, 130.24, 128.62, 128.46, 125.08, 125.04, 124.97, 123.96, 122.24, 120.06, 120.04, 61.28, 35.34, 33.86, 31.30, 30.98, 30.82, 29.09, 29.05, 29.02, 28.84, 28.73, 26.30, 25.76, 23.63, 23.53, 22.10, 13.97. ^{195}Pt NMR (125 MHz, DMSO-d_6) δ 1593.4 (major isomer), 1588.5 (minor isomer). The configurational isomers of the carbamate group were also observered in orther platinium(IV) complexes containing carbamate groups.[1, 2] ESI-HRMS: calcd. for $C_{57}H_{69}N_7O_8PtIr$ $[M]^+$: *m/z* 1367.4492; found: *m/z* 1367.4469.

Stability Studies of the complexes in the dark.

The stability of the complexes in buffer was assessed using HPLC-MS. The complexes were prepared at a final concentration of 40 μM and incubated in a PB buffer (50 mM phosphate, pH 7.4) at 37 °C in the dark on an orbital shaker (100 rpm). At predetermined time points, aliquots of the solution were withdrawn and directly subjected to HPLC analysis. HPLC condition: 0 - 3 - 16 - 17 - 20 min, 5 - 5 - 80 - 100 -100% acetonitrile with 0.1% HCOOH. 0.3 mL/min.

To evaluate the stability of the complexes in the presence of reducing agents, a final concentration of 1 mM ascorbate or 5 mM glutathione was added to the solution. The solution was then incubated under the same conditions as mentioned above.

For stability measurement in Fetal Bovine Serum (FBS), the complexes (40 μM) were dissolved in FBS, and coumarin (250 μM) was added as an internal reference. At pre-determined time points, a 100 μL aliquot of the mixture was withdrawn and mixed with 300 μL acetonitrile. The resulting mixture was then centrifuged at 13,500 g for 5 minutes. The supernatant was collected and directly subjected for HPLC analysis. HPLC condition: 0 - 3 - 16 - 17 - 20 min, 5 - 5 - 80 - 100 -100% acetonitrile with 0.1% HCOOH. 0.3 mL/min.

Photoactivation Properties of the complexes. A PB buffer containing 40 μM complex was irradiated by visible light at 465 nm at a power density of 10 mW/cm^2 under stirring in a quartz cuvette. At pre-defined time points, Aliquot of the solution was directly used for HPLC-MS analysis. Ascorbate(1mM) or glutathione(5mM) were added into the solution to study the photoreduction in the presence of reducing agents. HPLC condition: 0 - 3 - 16 - 17 - 20 min, 5 - 5 - 80 - 100 - 100% acetonitrile with 0.1% HCOOH. 0.3 mL/min.

Cyclic voltammetry studies

Cyclic voltammetry experiment was carried on a CHI120 workstation using a three-electrode cell, which included a 2.0 mm-diameter glassy carbon disc working electrode, a platinum auxiliary electrode, and a Ag|Ag + reference

electrode containing 0.1 M $AgNO_3$. Samples were dissolved in dry DMF with (n-Bu_4N)PF_6 (0.1 mol/L) serving as a supporting electrolyte. The cell was purged with nitrogen for 10 minutes prior to the measurement. Data was collected at room temperature with a scan rate at 0.1 V/s. A trace amount of ferrocene ($E_{Fc+/Fc}$ = 0.72 V vs SHE) was added during the experiment as an internal reference.

The free energy electron transfer from reduced form of **Ir** to Pt(IV) was estimated using equation **(1)**

$$\Delta G_{ET} = F((E^{ox}_{\frac{1}{2}}(D/D^+) - E^{red}_{\frac{1}{2}}(A/A^-)) \quad \textbf{(1)}$$

Where $E^{ox}_{\frac{1}{2}}(D/D^+)$ is the half-wave oxidation potential of the donor (reduced form of **Ir**), $E^{red}_{\frac{1}{2}}(A/A^-)$ is the half-wave reduction potential of the acceptor (Pt(IV)), F is the Faraday constant (F = 1e for calculating the energy in eV).

The reduction of Pt(IV) is an irreversible reaction. Instead of the peak point, the inflection point was utilized as the half-wave reduction potential.

For **IrPt** $\Delta G_{ET} =$ -1.84 - (-1.27) = -0.57 eV
For **IrPtC12** $\Delta G_{ET} =$ -1.85 - (-1.20) = -0.65 eV

Detection of ROS by DPBF assay

The freshly prepared DPBF solution (0.05 mg/mL ethanol solution, 100 µL) was added to 96-well plate. **Ir**, **IrPt** and **IrPtC12** at concentrations of 10 µM were added to different wells. The plate was irradiated at 465 nm (10 mW/cm^2), and the absorbance at 410 nm was measured using a microplate reader (Synergy H1, Biotek) at intervals of 10 seconds during the irradiation process.

Cell culture

A549, A549cisR, and MCF-7 cells were obtained from Procells (Wuhan, China). A549 and A549cisR cells were cultured in RPMI 1640 medium supplemented with 10% FBS and 100 units/mL penicillin. Additionally, the culture medium of A549cisR cells was supplemented with 1 µM cisplatin every two passages to maintain resistance. MCF-7 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FBS and 100 units/mL penicillin/streptomycin. All cell lines were incubated in a humidified incubator at 37 °C with 5% CO_2.

Cytotoxicity Studies

The cytotoxicity of the complexes was assessed using the Cell Counting Kit-8 (CCK8) assay. Cells were seeded into a 96-well plate at a density of 7000 cells per well and incubated until reaching 50% confluency. Subsequently, the medium was replaced with fresh medium containing various concentrations of the complexes and further incubated for 8 hours. Following this, the medium was removed, and the cells were washed with PBS. Then, 100 µL of PBS was added to each well, and the cells were either irradiated with visible light (465 nm, 10 mW/cm^2) for 15 minutes or kept in darkness. After irradiation, the culture medium was changed to complete medium and further incubated for an additional 40 h. Finally, CCK-8 (Dojindo, Japan) reagent was added to each well at a final concentration of 10% (v/v) and incubated for 30 minutes to 1 hour. The absorbance at 450 nm was measured using a microplate reader to determine the cell viability.

Cellular localization of IPtC12

A549 cells were seeded in confocal dishes at a cell density of 1.5 x 10^5 cells per dish and allowed to grow until the cell confluency reached 80%. Subsequently, the cells were incubated with **IrPtC12** at a concentration of 15 μM for 8 hours. During this incubation period, the cells were co-incubated with Lyso-Tracker Green, Mito-Tracker Green, or ER-Tracker green in combination with Hoechst33342 for a duration of 0.5 hours prior to imaging. After staining, the cells were washed with PBS 3 times and then visualized using laser confocal microscopy (ZEISS-LSM880). The excitation and emission wavelengths used for imaging were as follows: **IrPtC12** (λ_{ex} = 405 nm, λ_{em}= 600-700 nm), Mito-Tracker (λ_{ex} = 488 nm, λ_{em} = 500-531 nm), ER Tracker (λ_{ex} = 488 nm, λ_{em} = 493-531 nm), Lyso-Tracker (λ_{ex} = 488 nm, λ_{em} = 493-531 nm), Hoechest33342 (λ_{ex} = 405 nm, λ_{em} = 410-460 nm).

Cell uptake of platinum

A549 cells were cultured in 60 mm dishes at a density of 8.0 x 10^5 cells per dish and incubated for 24 hours. Following this, the complexes (10 μM) were added to the cells and incubated for 8 hours. Subsequently, the cells were collected through trypsinization, washed twice with cold PBS, and resuspended in 5 mL of PBS. The cell numbers were then counted using a Hemocytometer. After counting, the cells were centrifuged, and the resulting cell pellet was digested with 100 μL of hydrogen peroxide and 300 μL of concentrated nitric acid for 3 days at a temperature of 65 °C. The lysate was diluted with Milli-Q H_2O to achieve a final volume of 2 mL and then centrifuged at a speed of 13,500 g for a duration of 15 minutes. Finally, the platinum content in the supernatant was determined using inductively coupled plasma mass spectrometry (ICP-MS) on a PE Nexion 2000 instrument. The levels of platinum in the cells were expressed as ng Pt per million cells.

Platinum levels in genomic DNA

A549 cells were cultured in 100 mm dishes at a cell density of 2.5 x 10^6 cells per dish and incubated until reaching 70% confluency. The medium was then replaced with fresh medium containing **IrPtC12** at a concentration of 10 μM. After an incubation period of 8 hours, the cells were washed twice with PBS, and EBSS was added to each dish. The cells were either irradiated with visible light (465 nm, 10 mW/cm^2) for 15 min or kept in the dark. Subsequently, the EBSS was removed, and fresh complete medium was added to the dishes. The cells were further incubated for an additional 16 hours before being collected through trypsinization. The collected cells were washed twice with cold PBS and pelleted by centrifugation. Genomic DNA was obtained using a mammalian genomic DNA miniprep-kit from Sigma-Aldrich. The concentration of DNA was determined using a nanodrop spectrometer (ND-2000). The DNA solution was then dried using a spin vacuum and digested with 65% nitric acid (300 μL) for a period of 3 days at a temperature of 65 °C. The resulting lysate was diluted with Milli-Q water to achieve a final volume of 1 mL, and the platinum content was quantified using ICP-MS. The levels of platinum bound to DNA were expressed as ng Pt per μg DNA.

Intracellular ROS detection

A549 cells were cultured in 35 mm confocal dishes until reaching 50% confluency. The medium was then replaced with fresh medium containing 5 μM complexes and incubated for 8 hours. Following this, the medium was replaced with medium containing 5 μM 2′,7′-dichlorofluorescein diacetate (H_2DCFH-DA) dye and stained for 1 h. Subsequently, the staining solution was removed, and the cells were washed with PBS for 2 times before adding EBSS to each dish. The cells were then irradiated at a wavelength of 465 nm with a power density of 10 mW/cm^2 for 5 min. After irradiation, the cells were stained with Hoechst 33342 for 60 mins and immediately observed under a laser confocal microscope (ZEISS-LSM880). The excitation wavelength used for H_2DCFDA was 488 nm, while for Hoechst 33342, it was 405 nm. The emission filter applied ranged from 493 nm to 630 nm for H_2DCFDA and from 410 nm to 501 nm for Hoechst33342.

Apoptosis assays

A549 cells were cultured in a 6-well plate at a density of 2.0 x 10^5 cells per well. Upon the cells reaching 80% confluency, the medium was changed to fresh medium containing different complexes (**Ir**, 5 μM, **IrPt**, 5 μM, **IrPtC12**, 5 μM, Oxaliplatin, 5 or 200 μM). After an incubation period of 8 h, the medium was removed, and the cells were washed with PBS twice. Then EBSS was added to each well, and the cells were irradiated at 465 nm at a power density of 10 mW/ cm^2 for 15 mins. Following irradiation, the EBSS was removed and replaced with fresh complete cell culture medium. The cells were further incubated for an additional 16 h, before being collected by trypsinization. The collected cells were washed with ice-cold PBS and an annexin binding buffer. The cell density was determined and adjusted to 1 x 10^6 cells per mL. Annexin V-FITC conjugate (3 μL) and a 7-AAD (7-aminoactinomycin D) solution (1 μg/mL, 2 μL) were added to each 100 μL cell suspension to stain the cells at RT for 20 mins. Subsequently, 400 μL of annexin binding buffer was added to the samples and mixed gently. The prepared samples were kept on ice and immediately analyzed using a flow cytometer (Attune, Thermo Fisher) with excitation at wavelength of 488 nm and emission at wavelength of 530/30 nm and 695/40 nm for FITC and 7-AAD respectively.

Detection of CRT exposure by flow cytometry

A549 cells were seeded on a 6-well plate at a density of 3×10^5 cells per well and incubated for 24 hours. Then, the cells were treated with complexes (20 μM) for 8 hours. Following treatment, the cells were washed with PBS for 3 times, and irradiated under visible light (465 nm, 10mW/cm^2) for 15 mins, and incubated for another 16 hours. After that, the cells were collected by trypsinization, washed with PBS for 3 times and incubated with Alexa Fluor® 647 conjugated anti-Calreticulin antibody (ab196159, dilute in PBS with 2% goat serum) together with PI (1 μg/mL) for 30 min in the dark. Subsequently, the cells were centrifuged, and 500 μL of PBS was added to resuspend the cells, The samples were kept on ice and analyzed using a flow cytometer (Attune, Thermo Fisher) with excitation at wavelength of 488 nm and 637 nm, and emission at wavelength of 574/26 nm and 670/14 nm for PI and Alexa Fluor® 647 respectively.

Visualization of CRT exposure by confocal microscopy

A549 cells were seeded in confocal dishes at a density of 3×10^5 cells per well and incubated for 24 hours. Subsequently, the cells were treated with complexes at a concentration of 20 μM. After 8 hours of treatment, the cells were irradiated with visible light (465nm, 10mW/cm^2) for 15 minutes. Following an additional 16-hour incubation period, the cells were fixed with 4% paraformaldehyde (500 μL/dish) for 15 mins at R.T. The cells were then washed with cold PBS for 3 times and blocked with 5% goat serum for 1 hour. Then, the cells were treated with Alexa Fluor® 647 conjugated anti-Calreticulin antibody (ab196159, dilute in PBS with 2% goat serum) overnight at 4 °C. After that, the samples were washed twice with PBS and stained with DAPI for 20 minutes. Finally, the samples were rinsed with PBS and imaged under a laser confocal microscope (ZEISS-LSM880). The parameters used for imaging were as follows: Alexa Fluor® 647: λ_{ex} = 633 nm, λ_{em} = 638-755 nm; DAPI: λ_{ex} = 405 nm, λ_{em} = 410-585 nm.

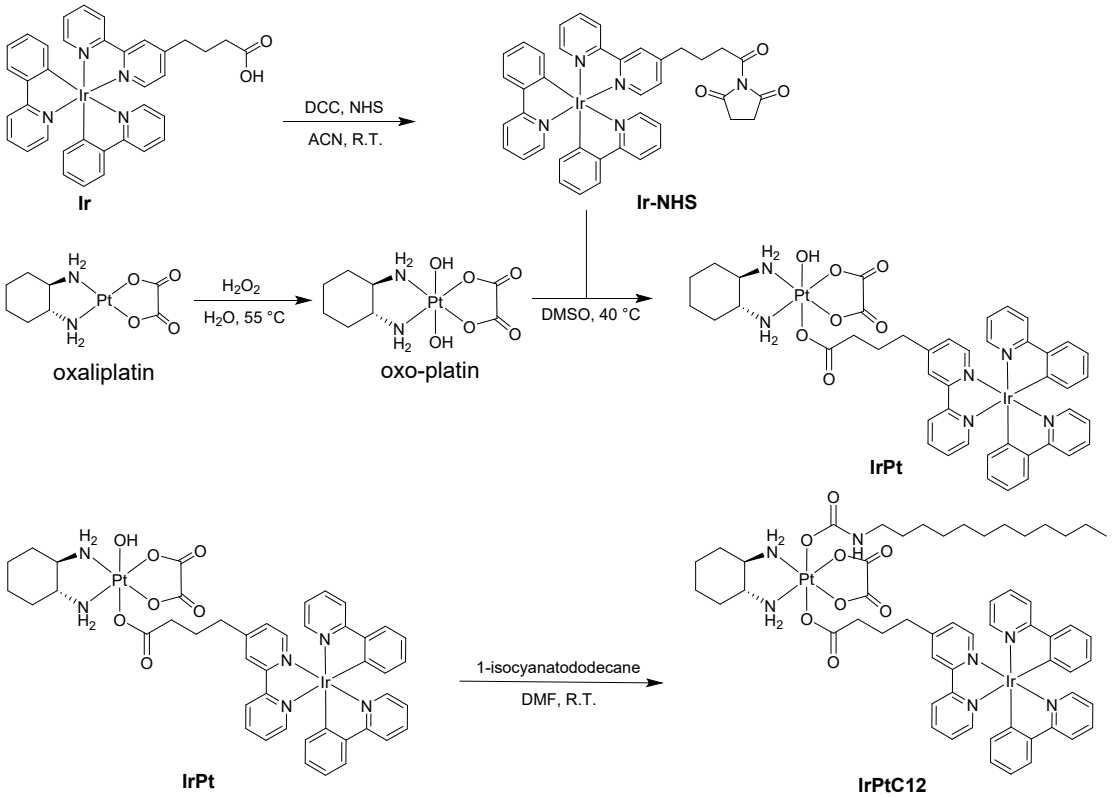
Measurement of extracellular ATP concentrations

A549 cells were seeded in 96-well plates at a density of 2 × 10^4 cells per well and incubated for 24 hours. The cells were then treated with different complexes at a concentration of 20 μM for 8 hours. After that, the medium was removed, and PBS was added to each well. Subsequently, the cells were irradiated (465 nm, 10 mW/cm^2) for 15 mins. Following irradiation, the medium was changed to fresh complete cell culture medium, and the cells were

further incubated for 16 hours. The ATP levels in the culture medium were measured by a commercial kit (Beyotime, S0027) following the manufacturer's instructions.

Visualization of HMGB1 by confocal microscopy

A549 cells were planted on confocal dish at a density of 3×10^5 cells per well and incubated for 24 hours. Then, cells were treated with different complexes (20 μM) for 8 h followed with irradiation with visible light for 15 mins. (465 nm, 10 mW/cm²). The cells were further incubated for another 16 h, washed with PBS for 3 times, and fixed with 4% paraformaldehyde at R.T. for 15 min. After that, the cells were further washed with PBS for 3 times, and permeabilized with 0.1% Triton-X100 at 37 °C for 1 h. Subsequently, the cells were blocked with 5% goat serum at 37 °C for 1 h followed with incubation of HMGB1 primary (CST 3935, 1:100 dilute in PBS with 5% goat serum) antibody at 4 °C overnight. Then, the cells were washed with PBS and treated with Alexa Fluor® 647 conjugated secondary antibody (CST 4414, 1:100 dilute in PBS with 5% goat serum) at 37 °C for 3 hours. The nuclear was stained with DAPI for 20 mins before imaging. A confocal microscope (ZEISS-LSM880) was used for observing and capturing images of the samples. (Alexa Fluor 647：λ_{ex}= 633 nm, λ_{em}= 638-754 nm; DAPI: λ_{ex}= 405 nm, λ_{em}= 410-585 nm).



Scheme S1 Synthetic route of **IrPt** and **IrPtC12**. The anion was omitted for clarity.

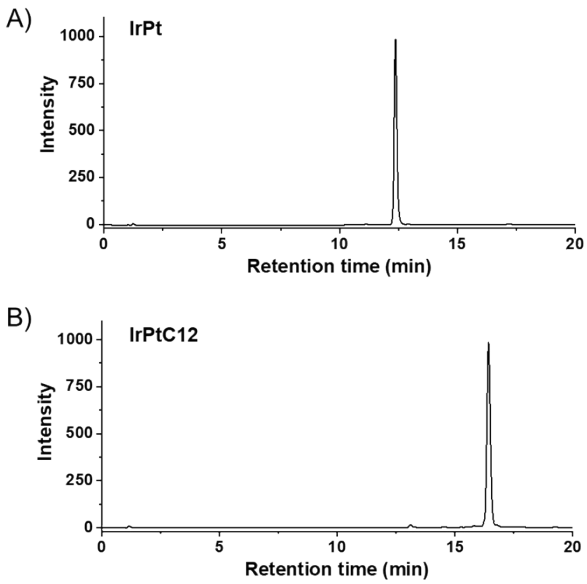


Figure S1 HPLC chromatograms of **IrPt** (A) and **IrPtC12** (B) respectively, showing the purity of the complexes.

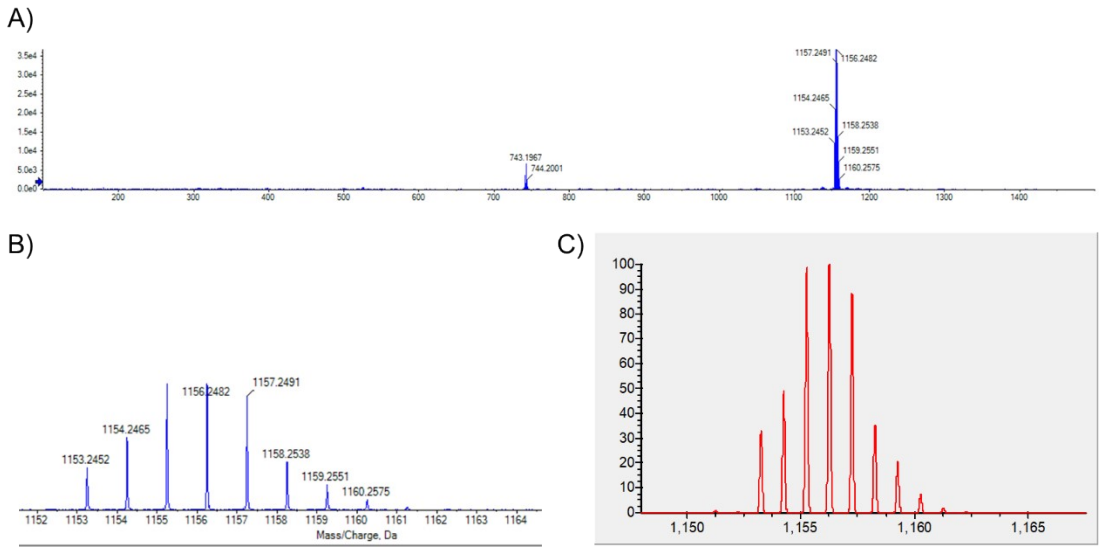


Figure S2 (A) ESI-HRMS (positive ion mode) spectrum of **IrPt**. (B) Enlarged area of the MS spectrum at m/z 1152-1164. (C) Simulated MS spectrum of **IrPt**. (calcd. for $C_{44}H_{44}N_6O_7PtIr$ $[M]^+$: *m/z* 1156.2554; found: *m/z* 1156.2482).

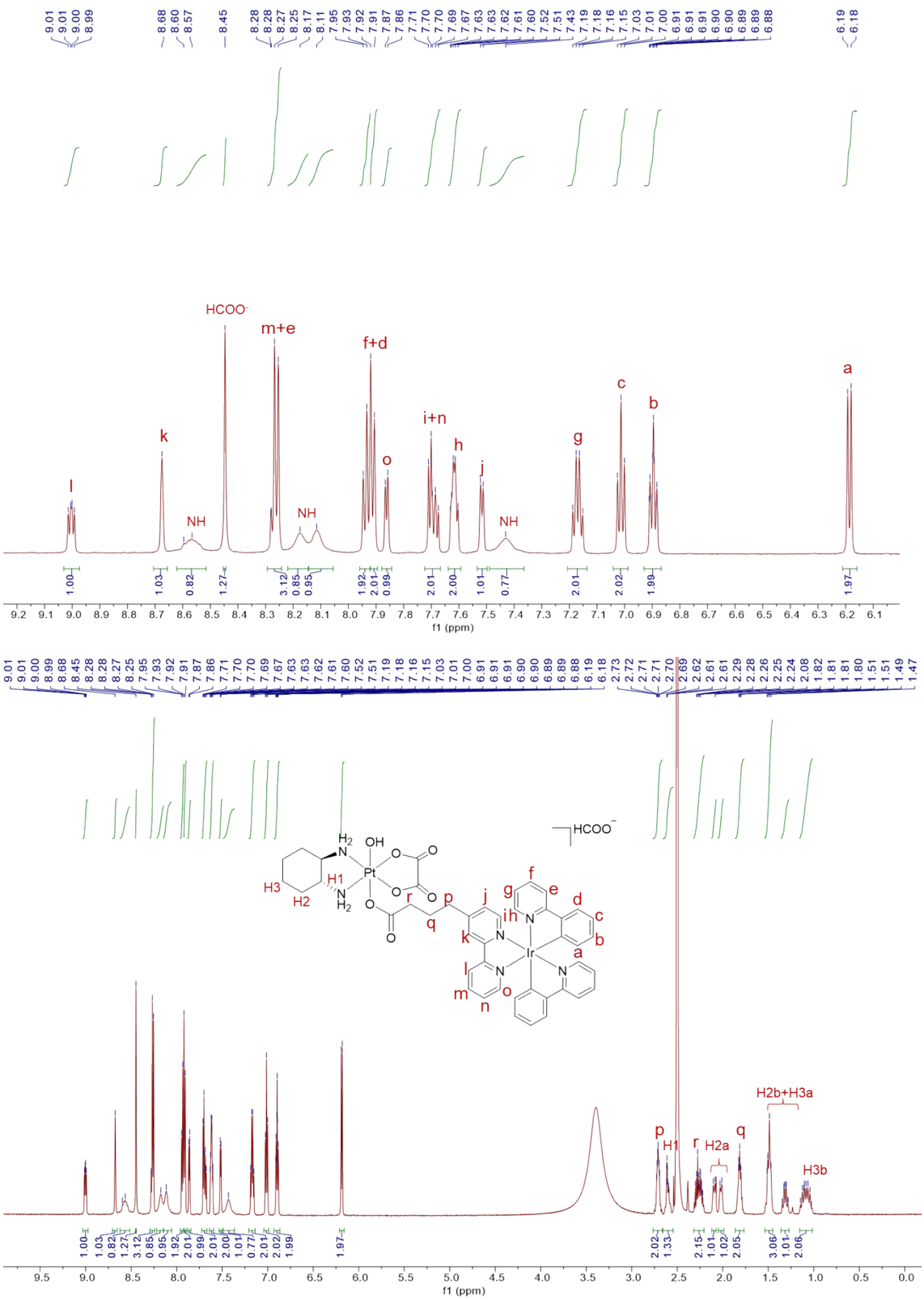


Figure S3 ^{1}H NMR spectrum of **IrPt** in DMSO-d_6 (upper, enlarged spectrum).

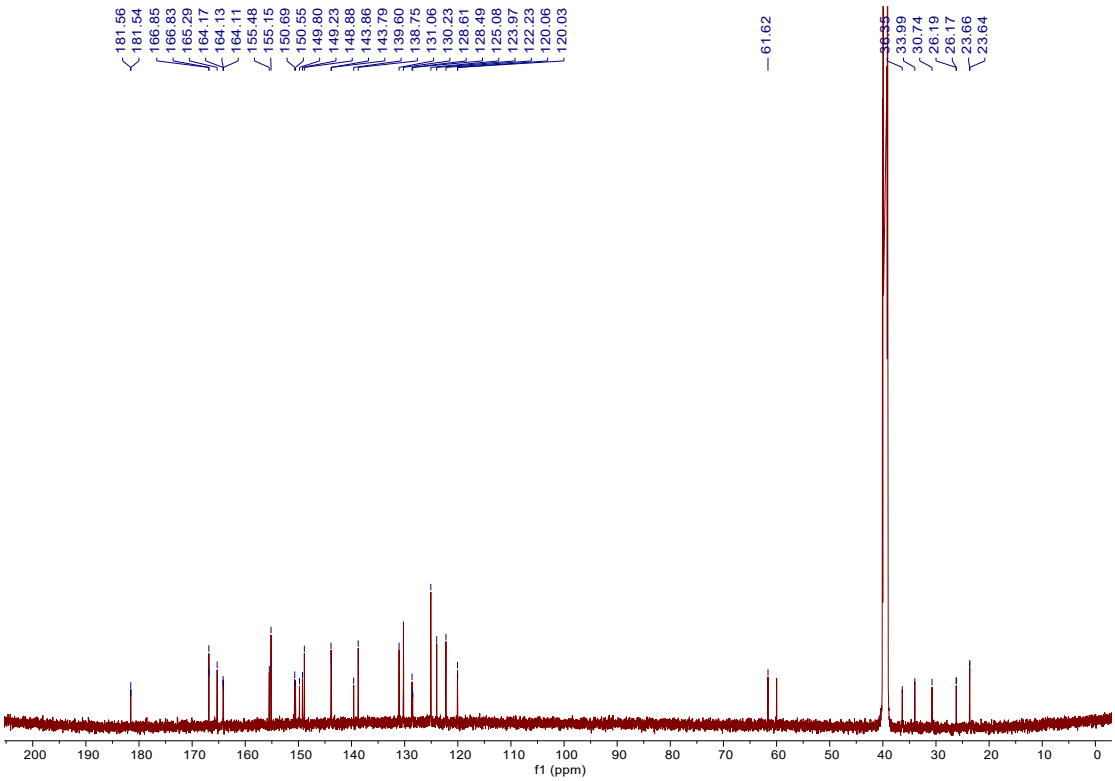


Figure S4 $^{13}C\{^{1}H\}$ NMR spectrum of **IrPt** in DMSO-d_6.

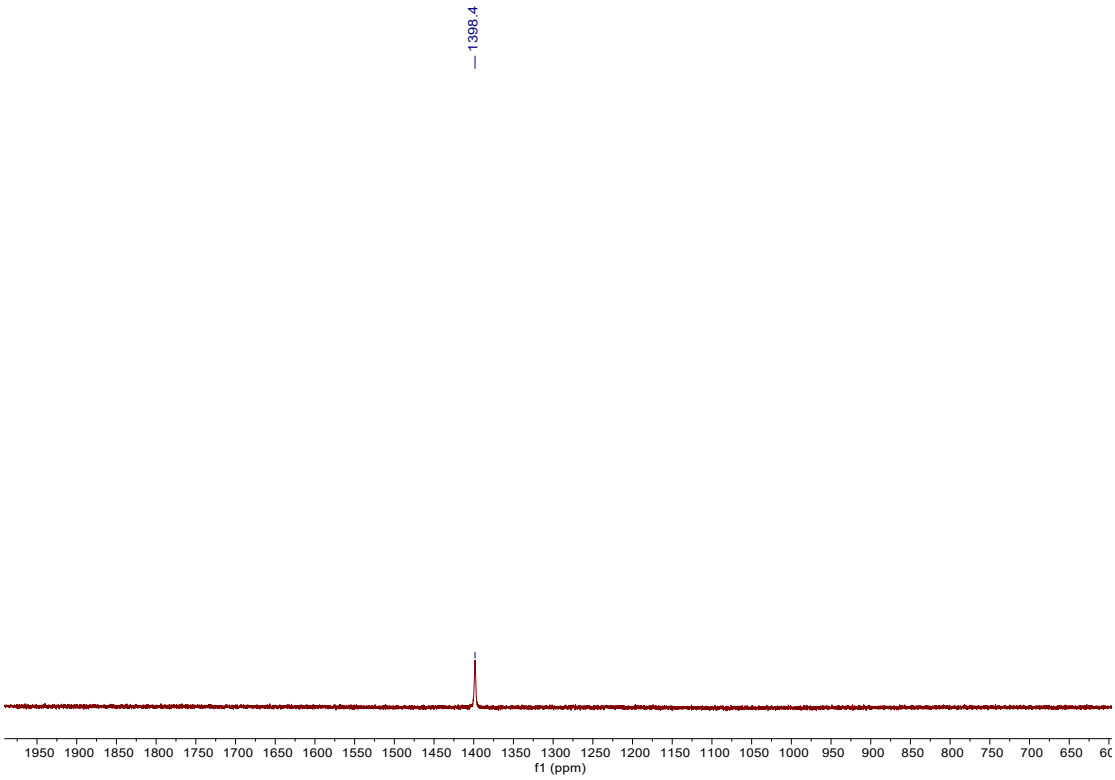


Figure S5 ^{195}Pt NMR spectrum of **IrPt** in DMSO-d_6.

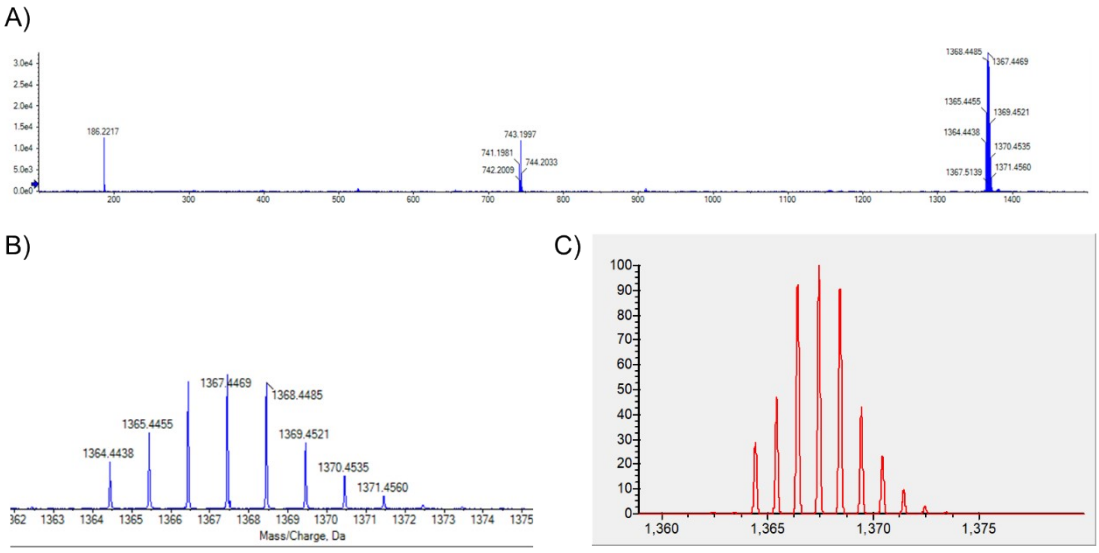


Figure S6 (A) ESI-HRMS (positive ion mode) spectrum of **IrPtC12**. B) Enlarged area of the MS spectrum at m/z 1362-1375. (C) Simulated MS spectrum of **IrPtC12**. (calcd. for $C_{57}H_{69}N_7O_8PtIr$ $[M]^+$: m/z 1367.4492; found: m/z 1367.4469).

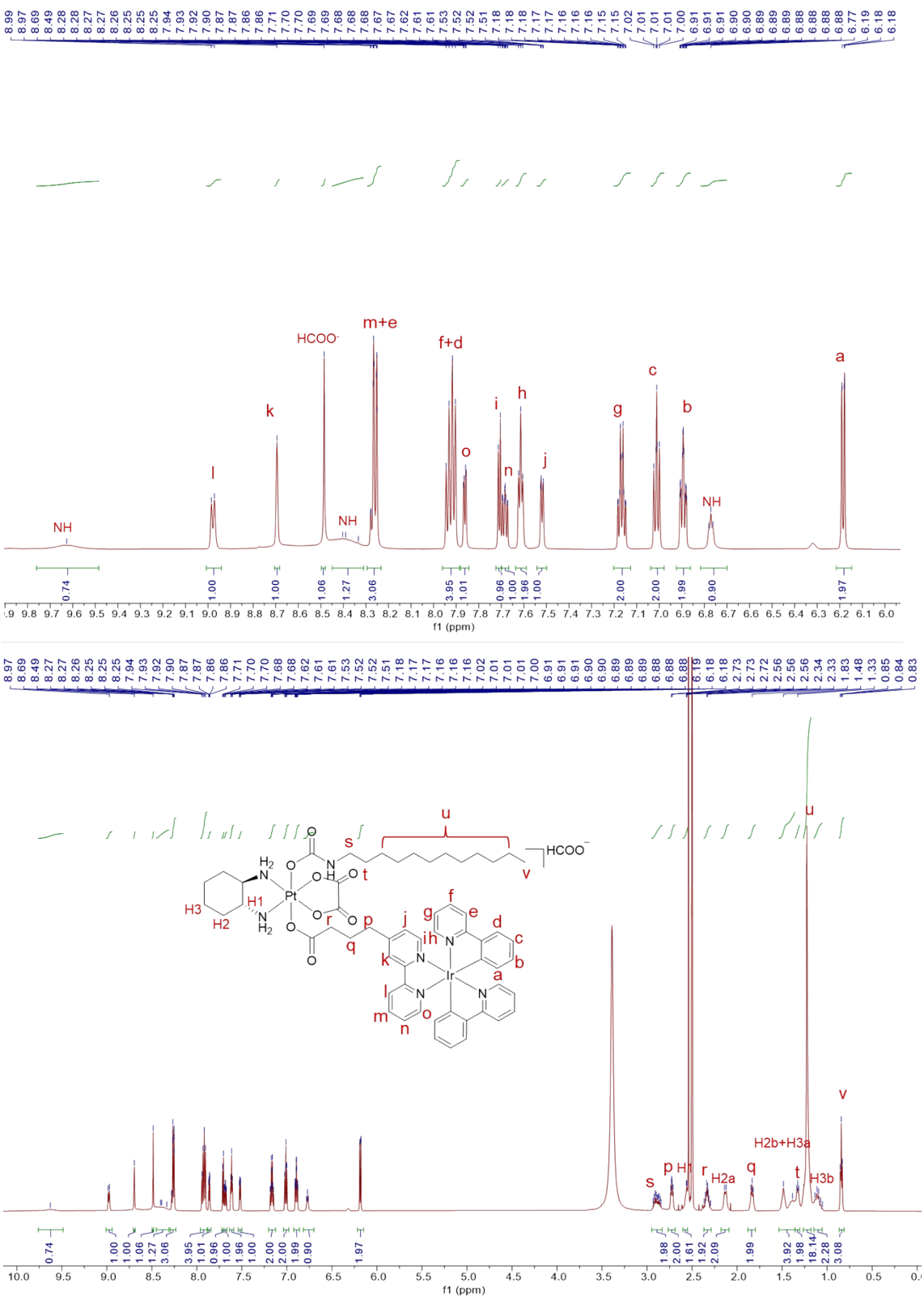


Figure S7 ^{1}H NMR spectrum of **IrPtC12** in DMSO-d_6 (upper, enlarged spectrum).

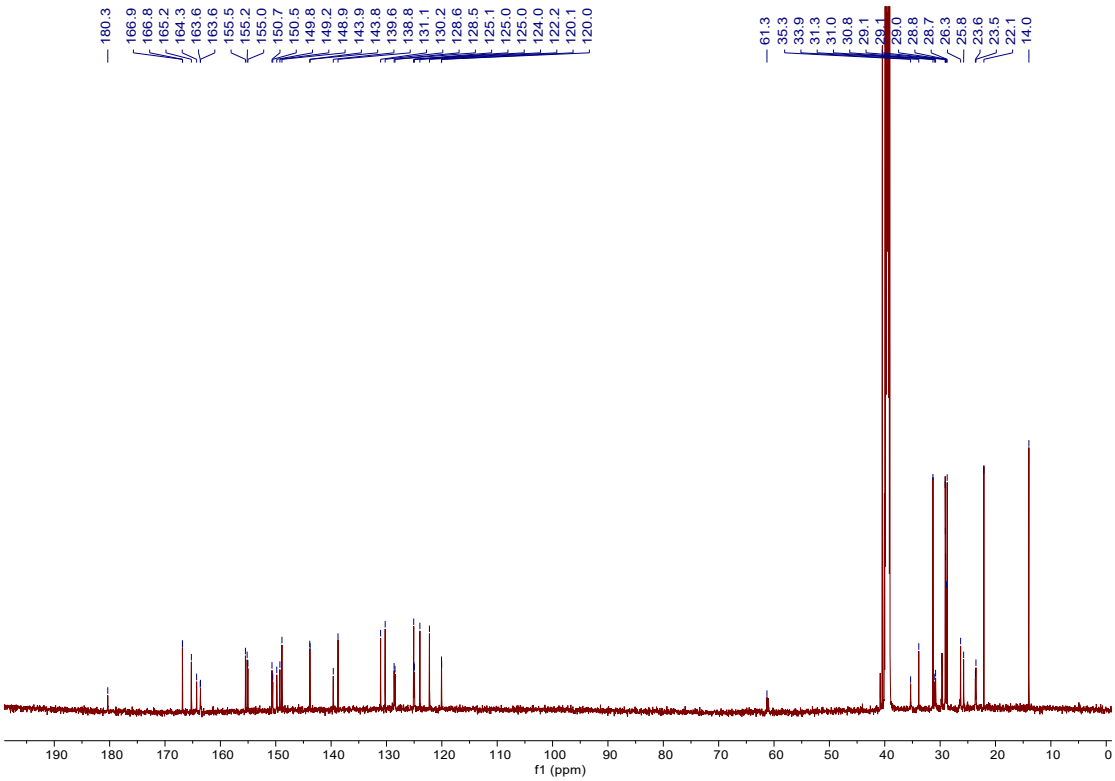


Figure S8 $^{13}C\{1H\}$ NMR spectrum of **IrPtC12** in DMSO-d_6.

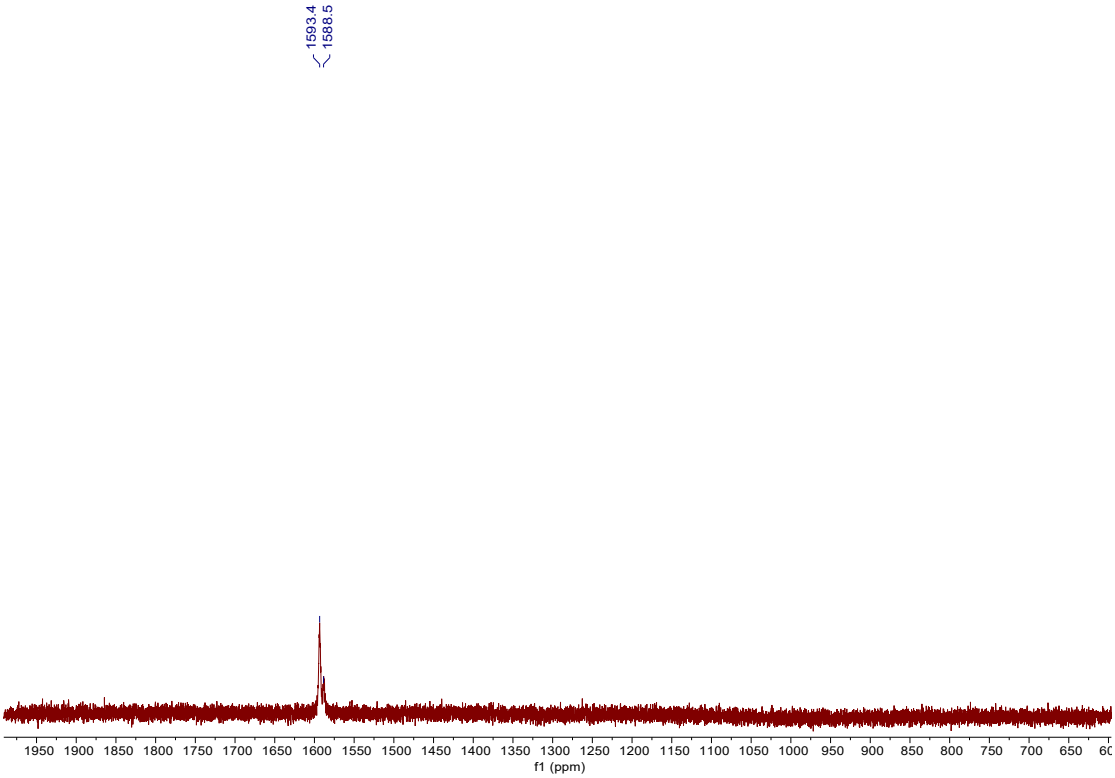


Figure S9 ^{195}Pt NMR spectrum of **IrPtC12** in DMSO-d_6.

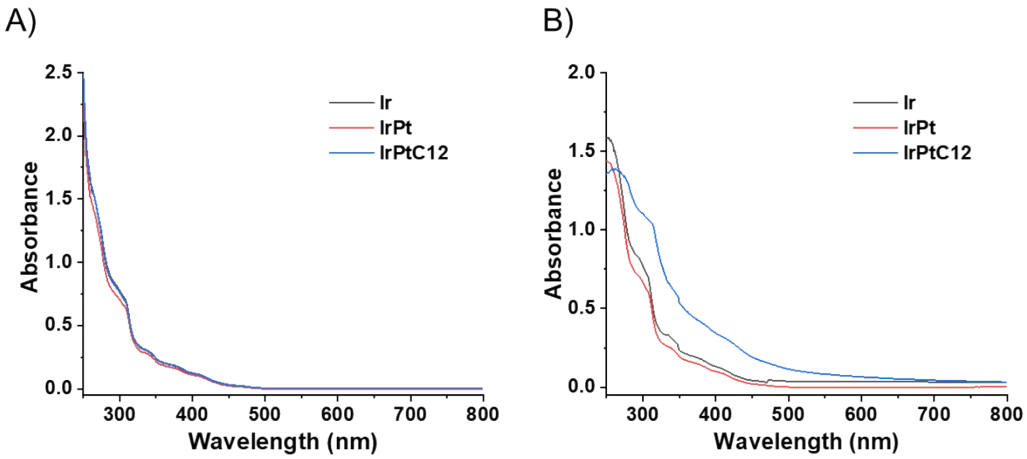


Figure S10 UV-Vis absorption spectra of **Ir**, **IrPt**, and **IrPt12** in MeCN (A) or PBS (B) at a concentration of 30 µM.

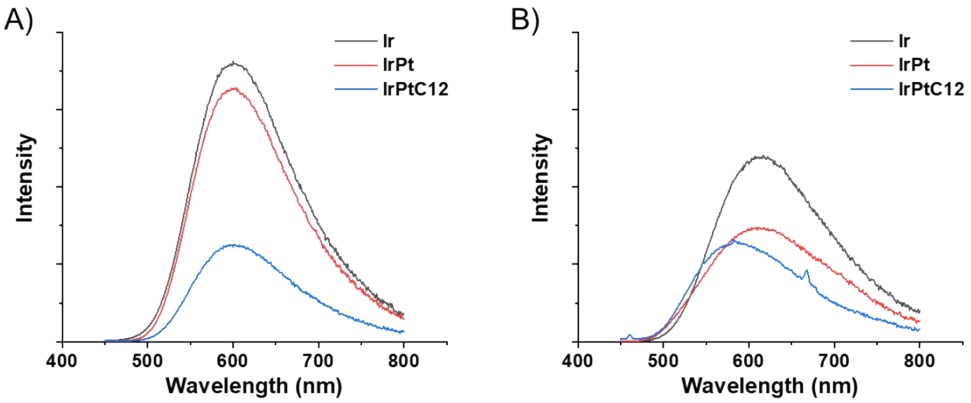


Figure S11 Fluorescence spectra of **Ir**, **IrPt**, and **IrPtC12** in MeCN (A) or PBS (B). at a concentration of 30 µM. Excitation wavelength = 375 nm.

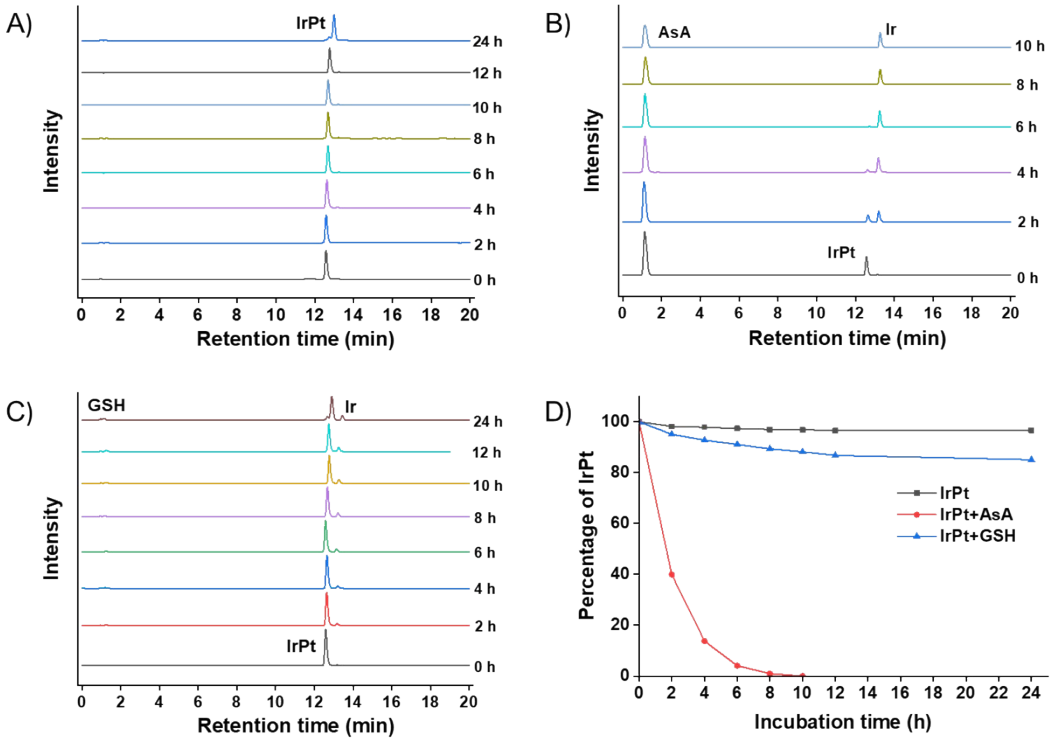


Figure S12 HPLC chromatograms of **IrPt** (40 μM) in a PB buffer (50 mM phosphate, pH 7.4) in the dark. A) **IrPt**. B) **IrPt** with AsA (1mM). C) **IrPt** with GSH (5 mM). D) Percentage of remained **IrPt** in the dark.

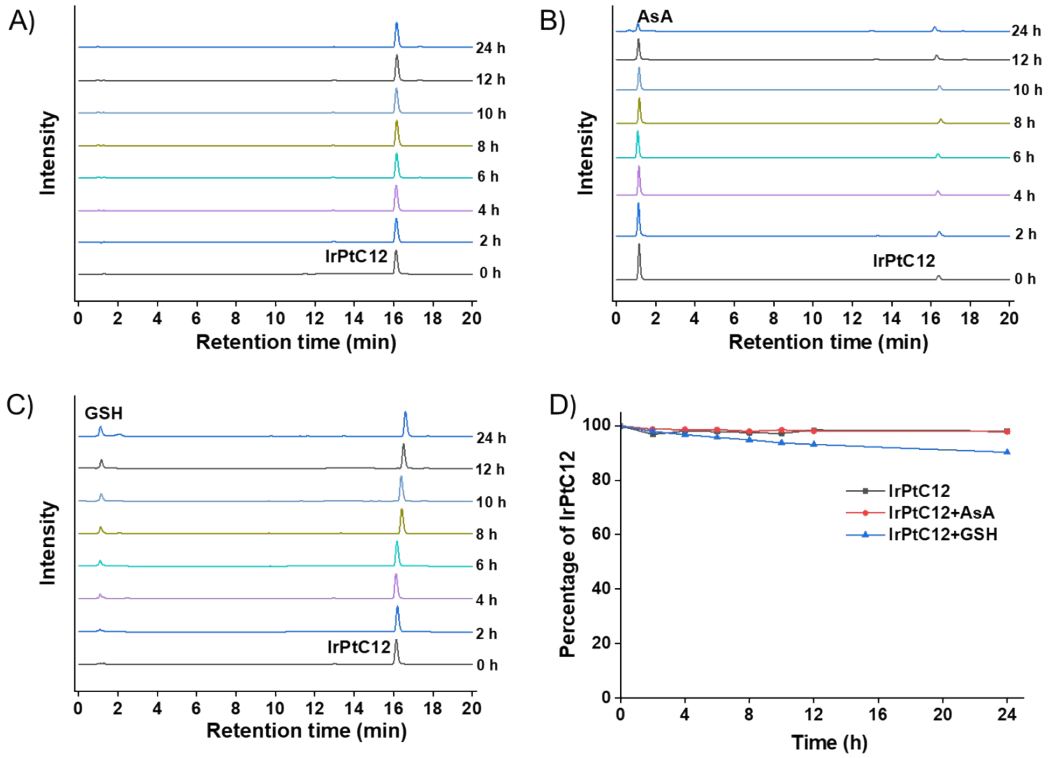


Figure S13 HPLC chromatograms of **IrPtC12** (40 μM) in a PB buffer (50 mM phosphate, pH 7.4) in the dark. A) **IrPtC12**. B) **IrPtC12** with AsA (1mM). C) **IrPtC12** with GSH (5 mM). D) Percentage of remained **IrPtC12** in the dark.

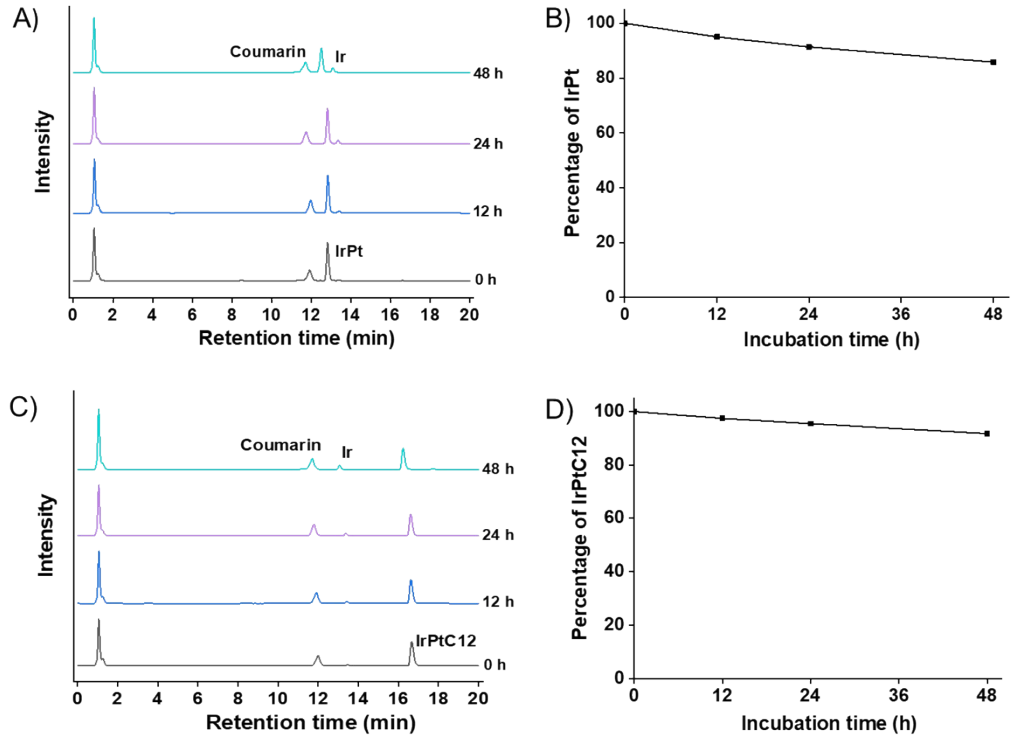


Figure S14 HPLC analysis of the stability of heterometallic complexes in FBS in the dark. A) **IrPt**. B) Percentage of remained **IrPt** in the dark. C) **IrPtC12.** D) Percentage of remained **IrPtC12** in the dark. Heterometallic complexes (40 μM) were dissolved in Fetal Bovine Serum (FBS), and coumarin (250 μM) was added as an internal reference.

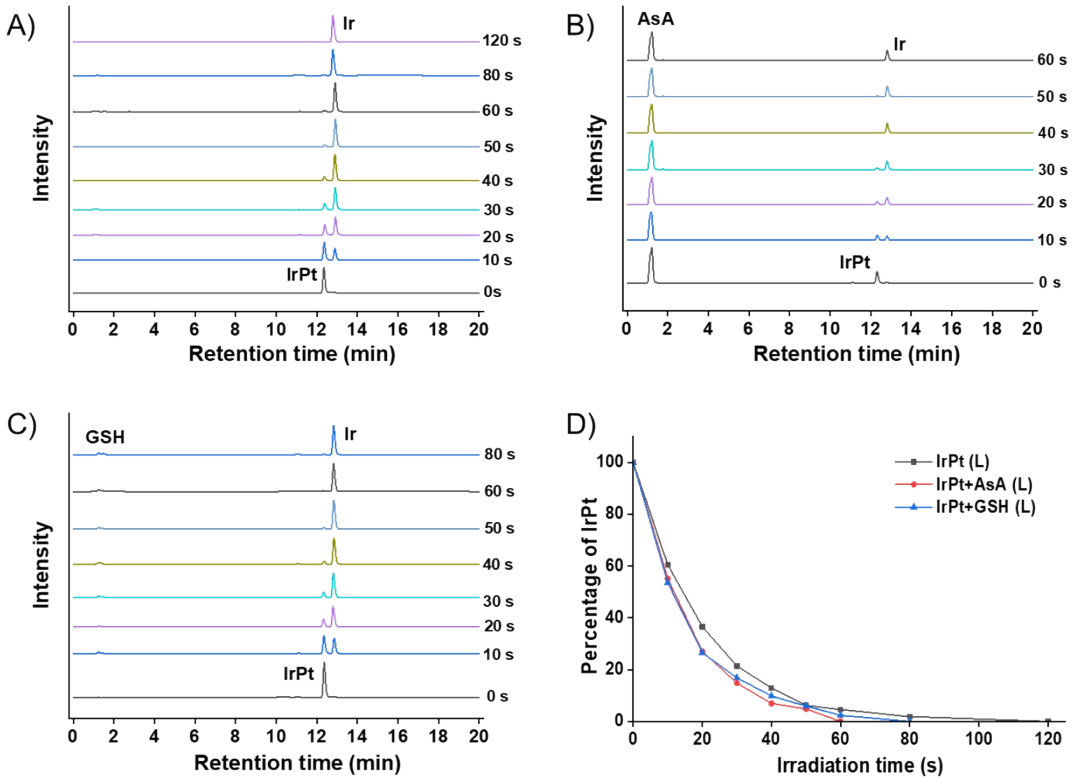


Figure S15 HPLC chromatograms of **IrPt** (40 μM) in a PB buffer (50 mM phosphate, pH 7.4) under irradiation at 465 nm (10 mW/cm^2) for different time. A) **IrPt**. B) **IrPt** with AsA (1mM). C) **IrPt** with GSH (5 mM). D) Percentage of remained **IrPt** under irradiation.

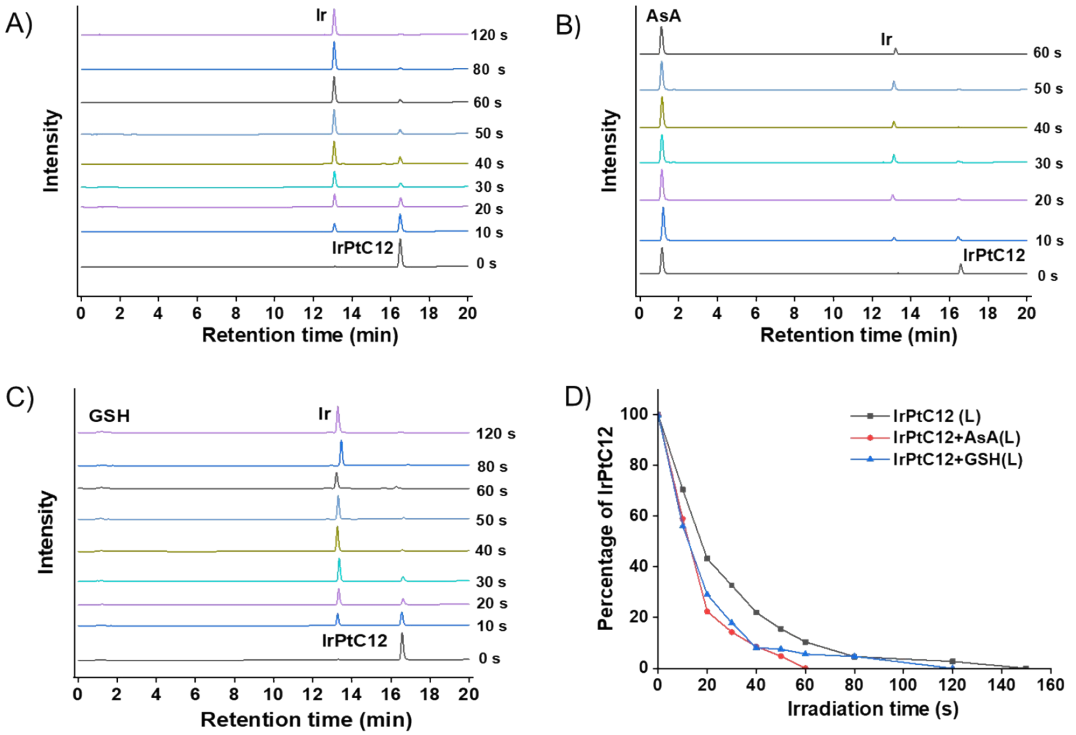


Figure S16 HPLC chromatograms of **IrPtC12** (40 μM) in a PB buffer (50 mM phosphate, pH 7.4) under irradiation at 465 nm (10 mW/cm^2) for different time. A) **IrPtC12**. B) **IrPtC12** with AsA (1mM). C) **IrPtC12** with GSH (5 mM). D) Percentage of remained **IrPtC12** under irradiation.

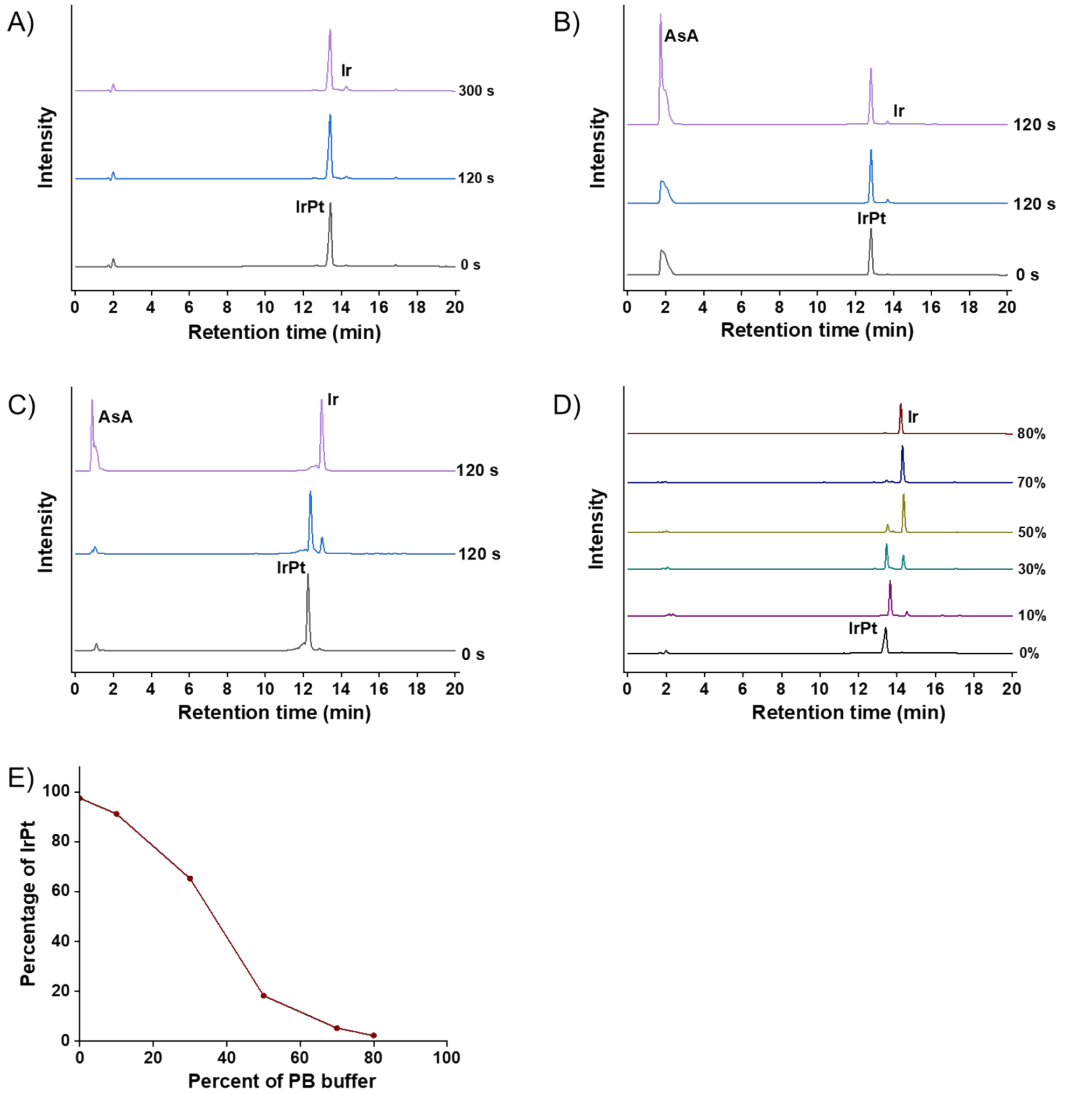


Figure S17 A) HPLC chromatograms of **IrPt** (40 μM) in ACN under irradiation at 465 nm (10 mW/cm^2) for different time. B) HPLC chromatograms of **IrPt** (40 μM) in DMF under irradiation at 465 nm (10 mW/cm^2) with or without AsA (1mM). C) HPLC chromatograms of **IrPt** (40 μM) in MeOH under irradiation at 465 nm (10 mW/cm^2) with or without AsA (1mM). D) HPLC chromatograms of **IrPt** (40 μM) in ACN containing different percent of PB buffer (v/v) under irradiation at 465 nm (10 mW/cm^2) for 120 s. D) The Percentage of remained **IrPt** calculated from Figure D. Note: The observed photoactivation of **IrPt** after 300 seconds of irradiation in ACN is similar to that after 120 seconds of irradiation. Therefore, we chose a 120-second irradiation time for further experiments. Ascorbic acid is not soluble in ACN, so we did not study the photoactivation of **IrPt** in ACN with ascorbic acid.

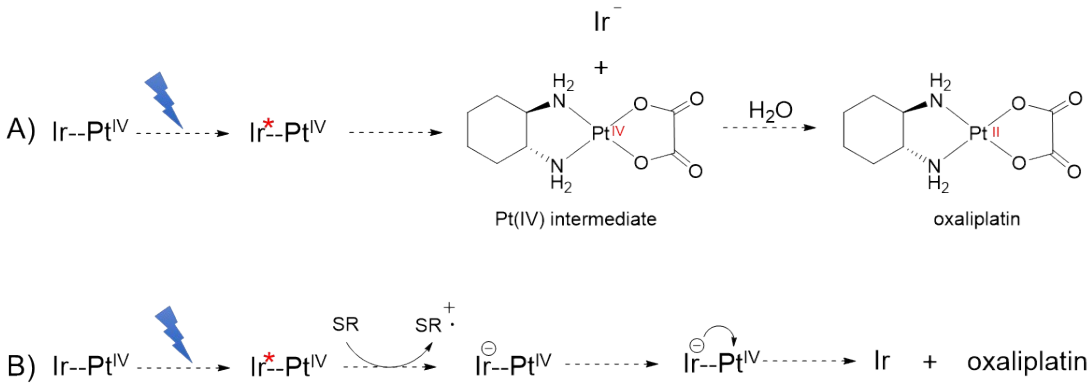


Figure S18 Purposed mechanism of photoactivation of **IrPt**. (A). **Ir** is excited under irradiation and transfer energy to the Pt(IV) center, the Pt-O bonds are broken, and the Pt(IV) intermediate reduced to Pt(II) in the presence of water.(B) **Ir** is excited and reduced under irradiation in the presence of scarified reducing (SR) agent, the reduced form of **Ir** transfer electron to Pt(IV) center, followed with reduction of Pt(IV) to Pt(II).

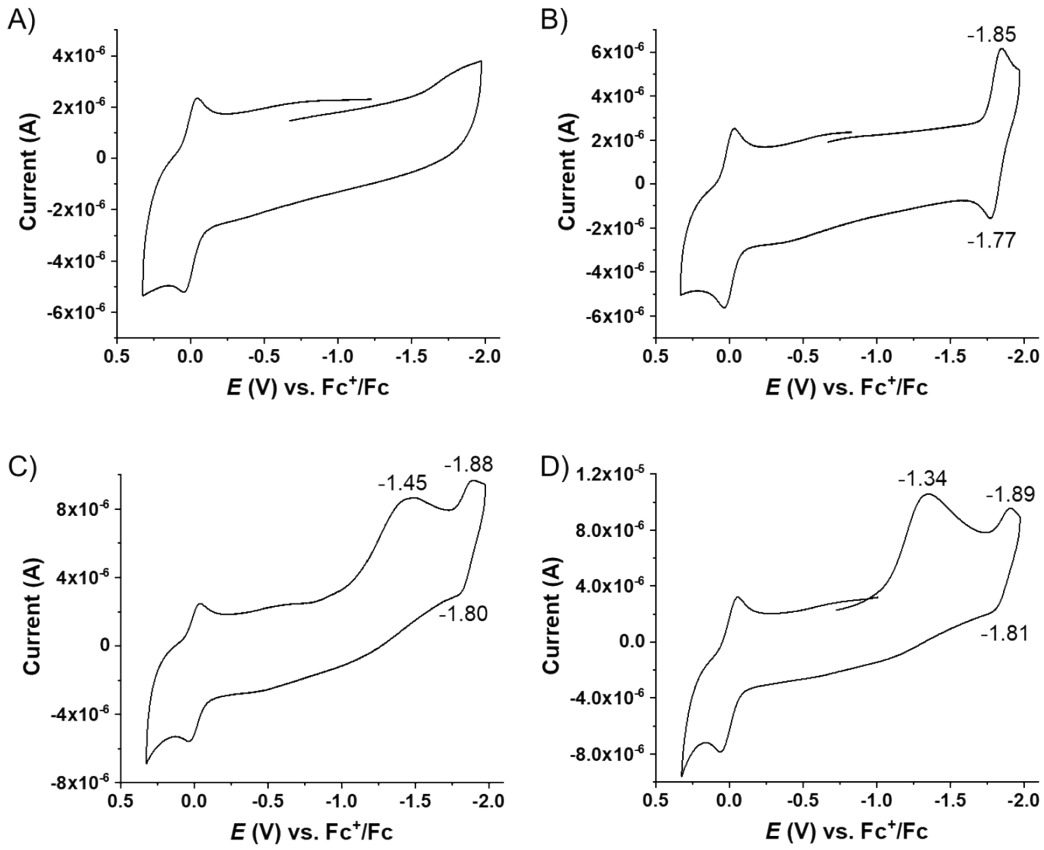


Figure S19 Cyclic voltammogram of blank (A), **Ir** (B), **IrPt** (C), **IrPtC12** (D) in DMF containing 0.1M (n-Bu_4N)PF_6. A trace amount of ferrocene was added as internal reference, and the data was reported versus the $E_{1/2}$ of Fc^+/Fc.

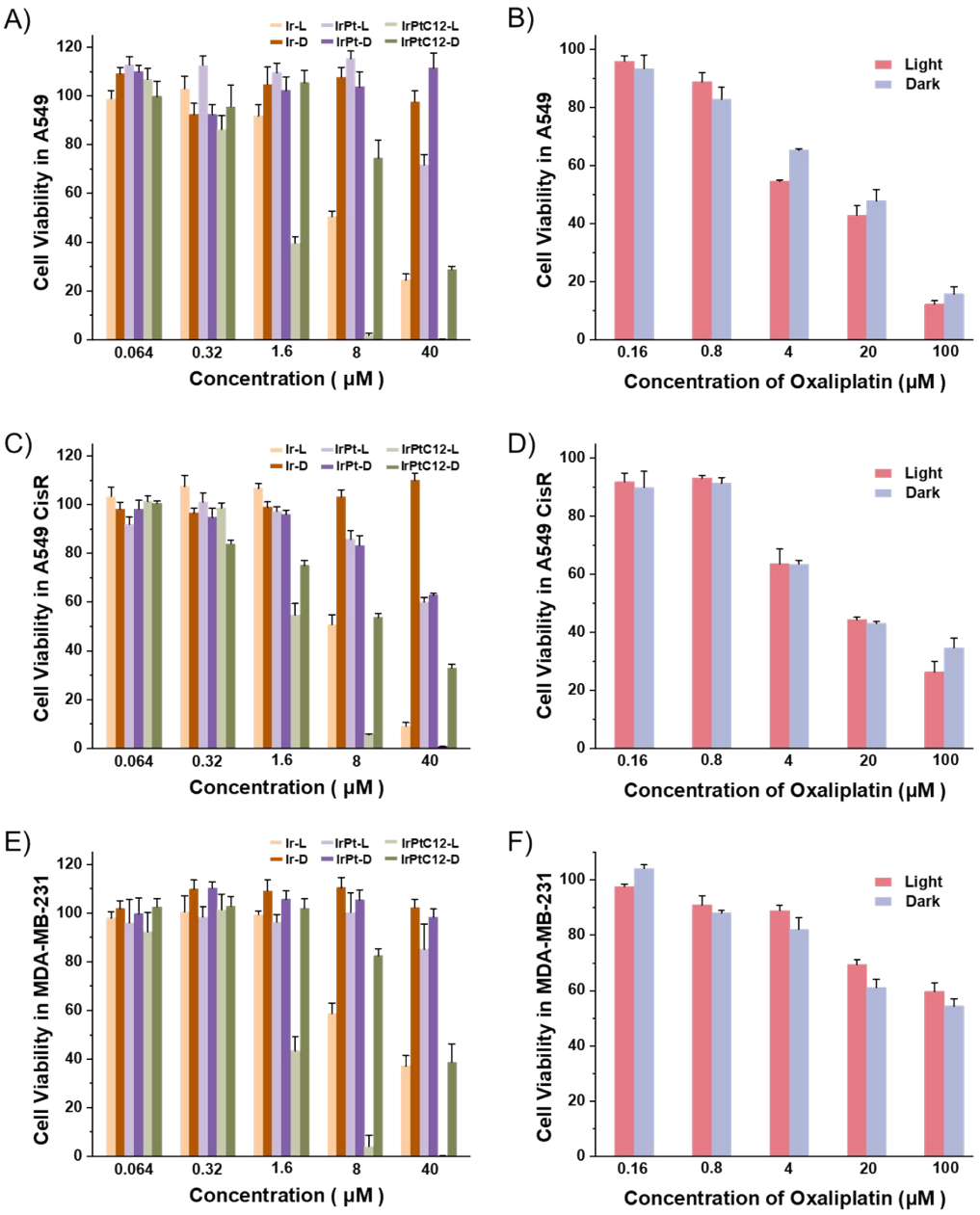


Figure S20 Cell viability of A549 (A and B), A549cisR (C and D) and MDA-MB-231 (E and F) cells after treatment of various concentrations of complexes. Cells were treated with complexes for 8 h, followed with irradiation at 465 nm (10 mW/cm^2) or kept in dark for 15 min, and further incubated for 40 h before CCK8 measurement.

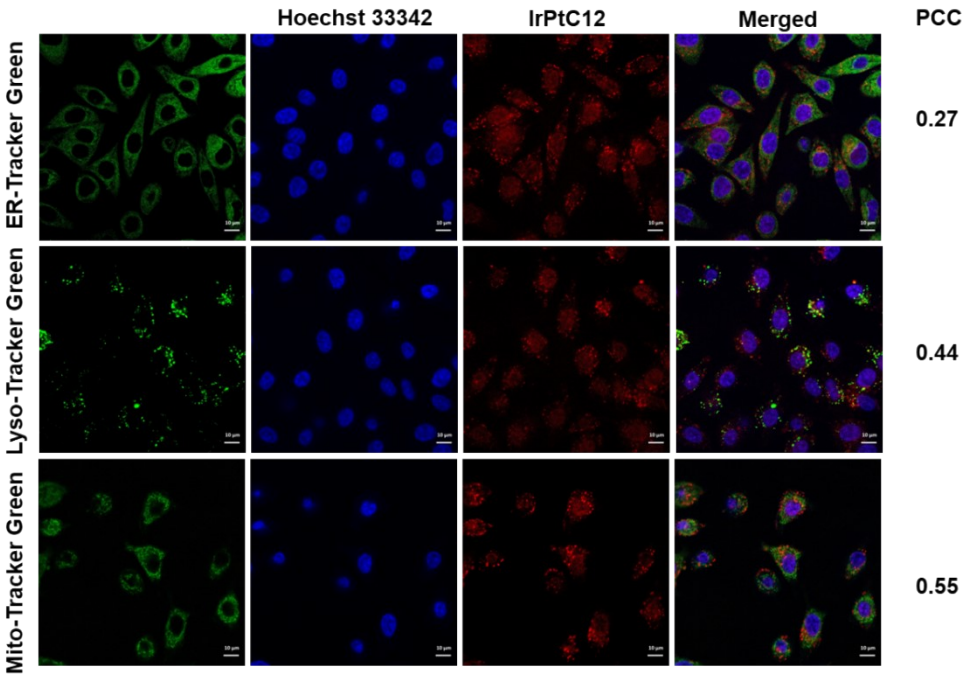


Figure S21 Confocal images of A549 cells treated with 15 μM **IrPtC12** for 8 h. The blue color corresponds to nuclei stained by Hoechst 33342 (λ_{ex} = 405 nm, λ_{em} = 410-460 nm), and the red color is the fluorescence of **IrPtC12** (λ_{ex} = 405 nm, λ_{em} = 600-700 nm) (scale bar,10 μm). The green color is the fluorescence of organelle specific dye. ER Tracker (λ_{ex} = 488 nm, λ_{em} = 493-531 nm), Lyso-Tracker (λ_{ex} = 488 nm, λ_{em} = 493-531 nm), Mito-Tracker (λ_{ex} = 488 nm, λ_{em} = 500-531 nm). PCC means Pearson's correlation coefficient. (scale bar,10 μm).

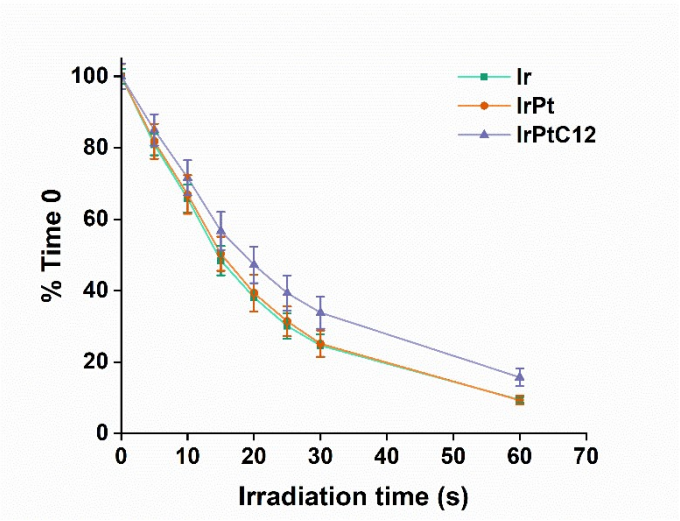


Figure S22 ROS generation ability of **Ir**, **IrPt** or **IrPtC12**. (10 μM） upon irradiation at 465 nm (10 mW/cm^2) for different times. The ROS was determined by measuring the absorbance of DPBF at 410 nm.

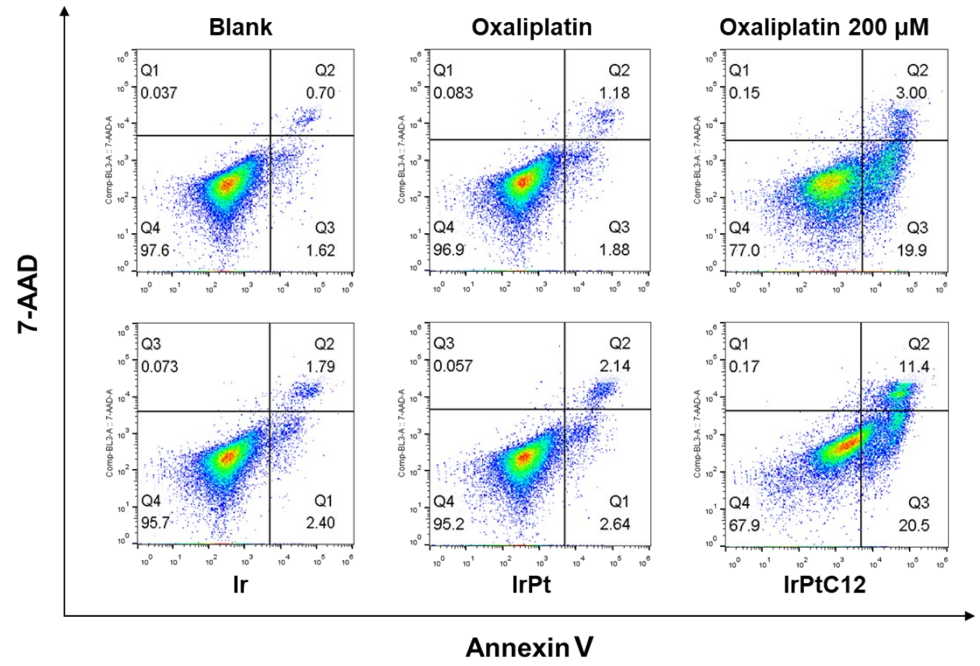


Figure S23 Flow cytometric analysis of FITC Annexin-V / 7-aminoactinomycin D (7-AAD) double-stained A549 cells treated with different complexes (**Ir**, 5 μM, **IrPt**, 5 μM, **IrPtC12**, 5 μM, Oxaliplatin, 5 or 200 μM) for 8 h, followed with irradiation at 465 nm (10 mW/cm^2) for 15 min and further incubation for 16 h.

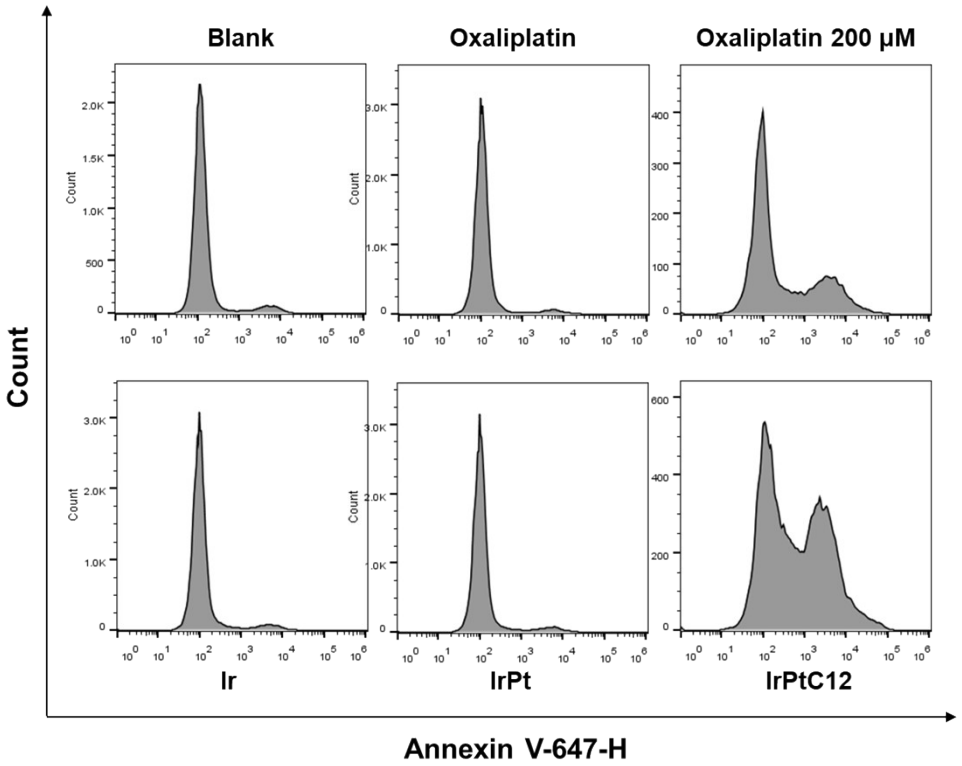


Figure S24 Flow cytometric analysis of PI and Alexa Fluor® 647 conjugated calreticulin antibody double stained A549 cells treated with different complexes (20 μM) for 8 h, followed by irradiation at 465 nm (10 mW/cm^2) for 15 min and further incubation for 16 h. PI: λ_{ex}= 488 nm, λ_{em}= 574/26 nm; Alexa Fluor® 647: λ_{ex}= 637 nm, λ_{em}= 670/14 nm.

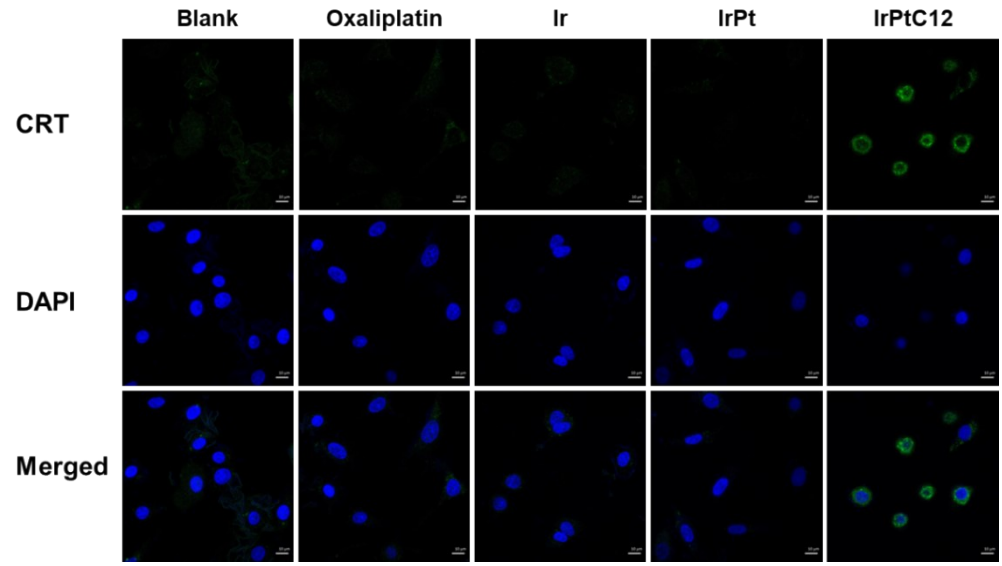


Figure S25 Confocal images of A549 cells treated with 20 μM complexes for 8 h followed by irradiation at 465 nm (10 mW/cm^2) for 15 min. The blue color corresponds to nuclei stained by DAPI: λ_{ex}= 405 nm, λ_{em}= 410-585 nm; and the green color is the fluorescence of Alexa Fluor® 647 conjugated calreticulin antibody, λ_{ex}= 633 nm, λ_{em}= 638-755 nm; (scale bar,10 μm).

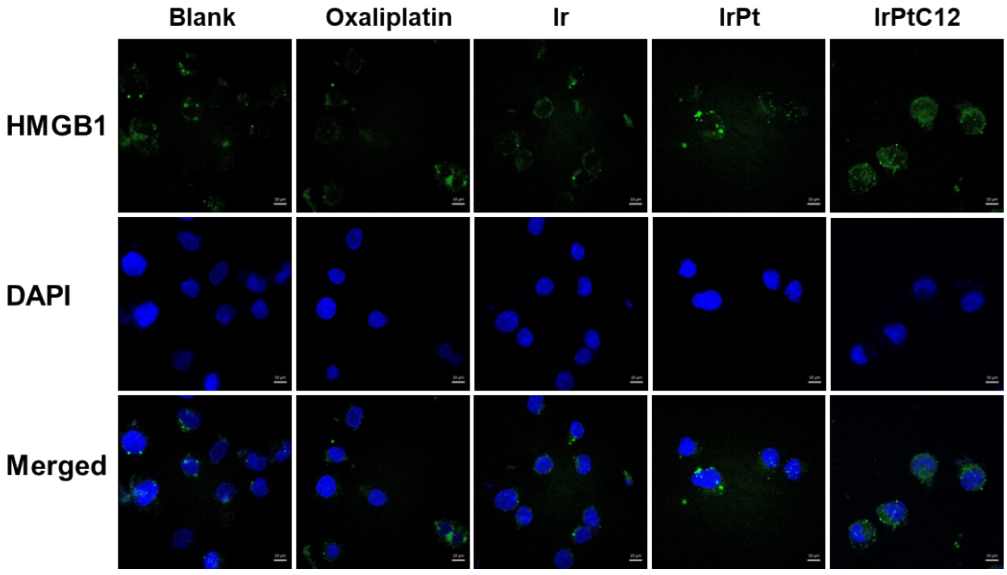


Figure S26 Confocal images of A549 cells treated with 20 μM complexes for 8 h followed by irradiation at 465 nm (10 mW/cm^2) for 15 min. A) The blue color corresponds to nuclei stained by DAPI: λ_{ex}= 405 nm, λ_{em} = 410-585 nm; and the green color is the fluorescence of Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 647 Conjugate), showing the intensity of HMGB1, λ_{ex}= 633 nm, λ_{em}= 638-754 nm; (scale bar,10 μm).

Table S1 IC_{50} values (μM) and photocytotoxicity index (PI) of complexes determined by CCK-8 assay in cancer cells with 8 h incubation in dark or 15 mins of irradiation by 465 nm light (10 mW/cm^2) and further incubation for 40 h. PI is the dark IC_{50} value divided by the light IC_{50} value. NA means not applicable.

Complex	A549			A549cisR			MDA-MB-231		
	dark	irradiation	PI	dark	irradiation	PI	dark	irradiation	PI
Ir	> 40	8.7 ± 2.5	>5	> 40	8.6 ± 2.9	>4.6	> 40	19.3 ± 8.6	>2.1
IrPt	> 40	> 40	NA	> 40	> 40	NA	> 40	> 40	NA
IrPtC12	25.1 ± 3.6	1.3 ± 0.1	19.2	13.6 ± 2.3	2.2 ± 0.6	6.2	31.7 ± 4.7	1.4 ± 0.3	22.6
Oxaliplatin	18.0 ± 3.3	10.2 ± 2.4	1.8	14.5 ± 0.6	15.3 ± 1.8	1	> 100	> 100	1

References

1. J. J. Wilson and S. J. Lippard, *Inorg. Chem.*, 2011, **50**, 3103-3115.
2. S. Chen, H. Yao, Q. Zhou, M.-K. Tse, Y. F. Gunawan and G. Zhu, *Inorg. Chem.*, 2020, **59**, 11676-11687.