Supporting Experimental Methods

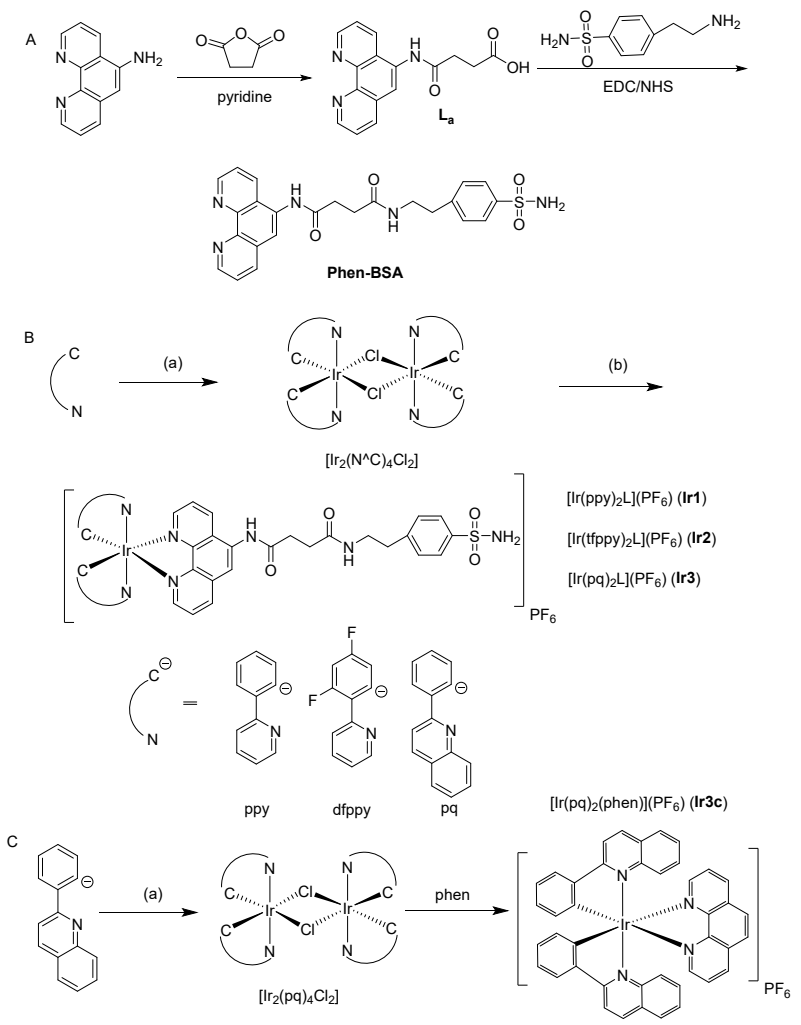
Materials and Measurements

Materials

Iridium chloride hydrate (Alfa Aesar, USA), 4-(2-Aminoethyl) Benzenesulfonamide (Macklin, China), ppy (2-Phenylpyridine, Sigma Aldrich, USA), pq (2-Phenylquinoline, Sigma Aldrich, USA), dfppy (2-(2,4-Difluorophenyl)pyridine ,Sigma Aldrich, USA), EDC•HCl (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, Sigma Aldrich, USA), ABDA (9,10-anthracenediyl-bis(methylene) dimalonic acid, Sigma Aldrich, USA), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Aldrich, USA), DMSO (dimethyl sulfoxide, Sigma Aldrich, USA), H_2DCFDA (2',7'-dichlorodihydrofluorescein diacetate, Sigma Aldrich, USA), Dihydroethidium (Sigma Aldrich, USA), Lipid Peroxidation MDA Assay Kit (Beyotime, China), BODIPY 581/591 C11 (Lipid Peroxidation Sensor, Invitrogen, USA) were used as received. All the compounds tested were dissolved in DMSO just before the experiments, and the final concentration of DMSO was kept at 1% (v/v).

Electrospray ionization mass spectrometry (ESI-MS) was recorded on a Thermo Finnigan LCQ DECA XP spectrometer (USA). The quoted m/z values represented the major peaks in the isotopic distribution. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 400 MHz spectrometer (Germany). Shifts were referenced relative to the internal solvent signals. Microanalysis of elements (C, H, and N) was carried out using an Elemental Vario EL CHNS analyzer (Germany). UV/Vis spectra were recorded on a Varian Cary 300 spectrophotometer (USA). Steady-state emission spectra and lifetime measurements were conducted on a combined fluorescence lifetime and steady state spectrometer (FLS 920, Edinburgh Instruments Ltd, England). Cell imaging experiments were carried out on a confocal microscope (LSM 880, Carl Zeiss, Göttingen, Germany). Flow cytometric analysis was done using a BD FACS Calibur™ flow cytometer (Becton Dickinson, USA). Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) was carried out using Bruker ultrafleXtreme (Germany). Antibody: calreticulin (CRT), high mobility group box 1 (HMGB1) and Alexa Fluor® 488 goat anti-rabbit secondary antibody were purchased from Abcam (USA). ATP Assay System Bioluminescence Detection Kit was purchased from Beyotime (China).

Synthesis of complexes Ir1, **Ir2**, **Ir3** and **Ir3c**



Scheme 1. Synthetic routes of L (A) and **Ir1**–**Ir3** (B). (a) $IrCl_3 \bullet 3H_2O$, ppy, tfppy or pq, toluene and 2-ethoxyethanol. (b) **L**, dichloromethane and methanol. (C) Synthetic routes of **Ir3c**.

Synthesis of Phen-BSA. $\mathbf{L_a}$ was synthesized according to a literature procedure.[1] $\mathbf{L_a}$ (295 mg, 1.0 mmol), 4-(2-Aminoethyl) Benzenesulfonamide (300 mg, 1.5 mmol), EDC•HCl (300 mg, 1.2 mmol) and NHS (138 mg, 1.2 mmol) were dissolved in N,N-Dimethylformamide (DMF, 15.0 mL). The mixture was stirred at room temperature for 24 h. The solvents were removed in vacuo. The crude product was purified using Al_2O_3 column chromatography (CH_2Cl_2/MeOH). ^{1}H NMR (400 MHz, DMSO) δ 10.20 (s, 1H), 9.13 (dd, *J* = 4.2, 1.4 Hz, 1H), 9.04 (dd, *J* = 4.2, 1.6 Hz, 1H), 8.67 (d, *J* = 8.3 Hz, 1H), 8.45 (dd, *J* = 8.1, 1.5 Hz, 1H), 8.16 (s, 1H), 8.07 (s, 1H), 7.81 (dd, *J* = 8.4, 4.3 Hz, 1H), 7.74 (dd, *J* = 8.0, 4.6 Hz, 3H), 7.42 (d, *J* = 8.2 Hz, 2H), 7.31 (s, 2H), 2.88–2.69 (m, 4H). MS (ESI): *m/z* 478.62 $[M + H]^+$.

Synthesis of complexes Ir1, **Ir2**, **Ir3** and **Ir3c**

The precursor $[Ir(ppy)_2Cl]_2$, $[Ir(dfppy)_2Cl]_2$ and $[Ir(pq)_2Cl]_2$ were synthesized by the literature method.[2] **Ir1**–**Ir3** were synthesized adopting a similar method. A mixture of $[Ir_2(N^C)_4Cl_2]$ (0.4 mmol, 1 eq) and **L** (0.8 mmol, 2 eq) in

CH_2Cl_2/CH_3OH (2:1, v/v) was heated to reflux under N_2 atmosphere in the dark. After 6 h, the solution was cooled to room temperature and then a six-fold excess of NH_4PF_6 was added. After being stirred for another 1 h, the mixture was filtered and the filtrate was evaporated to dryness under a vacuum. The solid obtained was purified by column chromatography on silica gel eluting with CH_2Cl_2/MeOH. **Ir3c** were synthesized by the literature method.[3]

$[Ir(ppy)_2(L)]PF_6$ (**Ir1**): Complex **Ir1** was obtained as a yellow powder. Yield: 78%.^{1}H NMR (400 MHz, CD_3CN) δ 9.84 (s, 1H), 8.91 (d, *J* = 8.6 Hz, 1H), 8.70 (s, 1H), 8.62 (d, *J* = 8.3 Hz, 1H), 8.35 (d, *J* = 4.9 Hz, 1H), 8.22 (d, *J* = 4.9 Hz, 1H), 8.08 (d, *J* = 8.1 Hz, 2H), 7.92 – 7.84 (m, 3H), 7.80 (dd, *J* = 13.6, 8.4 Hz, 3H), 7.75 (d, *J* = 7.4 Hz, 2H), 7.52 – 7.38 (m, 3H), 7.11 (t, *J* = 6.9 Hz, 2H), 6.99 (dd, *J* = 12.1, 7.0 Hz, 2H), 6.89 (dd, *J* = 11.8, 5.8 Hz, 2H), 6.71 (s, 1H), 6.41 (t, *J* = 7.5 Hz, 2H), 5.59 (s, 1H), 3.51 (d, *J* = 6.5 Hz, 2H), 2.92 – 2.75 (m, 3H), 2.64 – 2.56 (m, 2H). ^{13}C NMR (500 MHz, CD_3CN) δ 172.62, 172.29, 167.45, 151.26, 150.11, 149.99, 149.74, 149.55,149.44, 147.31, 144.56, 144.48, 144.28, 141.20, 138.44, 137.92, 134.39, 133.77, 131.73, 131.68, 131.46, 130.34, 129.56, 126.84, 126.29, 126.03, 124.86, 123.38, 122.62, 119.77, 118.23, 40.00, 35.13, 32.32, 30.43. ESI-MS (MeOH): m/z 978.67 $[M-PF_6]^+$.Purity (HPLC): 98.8%. Elemental Analysis: $C_{46}H_{39}F_6IrN_7O_4PS•H_2O$ Calcd.(%) C: 48.42; H: 3.62; N: 8.59 found (%) C: 48.64; H:3.92; N: 8.28.

$[Ir(tfppy)_2(L)]PF_6$ (**Ir2**): Complex **Ir2** was obtained as a light yellow powder. Yield: 80%.^{1}H NMR (400 MHz, CD_3CN) δ 9.88 (s, 1H), 8.95 (d, *J* = 8.5 Hz, 1H), 8.73 (s, 1H), 8.66 (d, *J* = 8.2 Hz, 1H), 8.40 (d, *J* = 4.9 Hz, 1H), 8.34 (d, *J* = 8.4 Hz, 2H), 8.27 (d, *J* = 4.9 Hz, 1H), 7.96 – 7.78 (m, 4H), 7.74 (d, *J* = 8.0 Hz, 2H), 7.53 – 7.44 (m, 2H), 7.42 (d, *J* = 7.9 Hz, 2H), 6.95 (dd, *J* = 11.7, 5.8 Hz, 2H), 6.81 – 6.67 (m, 3H), 5.86 (t, *J* = 8.4 Hz, 2H), 5.58 (s, 2H), 3.60 – 3.47 (m, 2H), 2.89 (t, *J* = 6.8 Hz, 2H), 2.83 – 2.75 (m, 2H), 2.65 – 2.56 (m, 2H). ^{13}C NMR (500 MHz, CD_3CN) δ 172.64, 172.30, 163.71, 162.47, 151.68, 150.36, 149.93, 149.84, 146.98, 144.56, 144.08, 141.18, 139.43, 138.48, 134.54, 134.34, 131.64, 129.57, 127.31, 126.99, 126.45, 126.03, 123.83, 123.72, 123.55, 118.16, 113.86, 110.01, 99.02, 98.81, 98.56, 40.00, 35.14, 32.34, 30.41. ESI-MS (MeOH): m/z 1050.56 $[M-PF_6]^+$. Purity (HPLC): 96.6%. Elemental Analysis: $C_{46}H_{35}F_{10}IrN_7O_4PS•6H_2O$ Calcd.(%) C: 42.40; H: 3.64; N: 7.52 found (%) C: 42.73; H: 3.37; N: 7.42.

$[Ir(pq)_2(L)]PF_6$ (**Ir3**): Complex **Ir3** was obtained as a dark yellow powder. Yield: 80%.^{1}H NMR (400 MHz, CD_3CN) δ 9.58 (s, 1H), 8.78 (d, *J* = 8.5 Hz, 1H), 8.61 (d, *J* = 5.0 Hz, 1H), 8.48 (d, *J* = 5.0 Hz, 1H), 8.43 (d, *J* = 8.2 Hz, 1H), 8.38 (d, *J* = 8.9 Hz, 3H), 8.35 – 8.30 (m, 2H), 8.22 (dd, *J* = 7.7, 3.0 Hz, 2H), 7.88 (dd, *J* = 8.3, 5.2 Hz, 1H), 7.77 (d, *J* = 7.3 Hz, 3H), 7.72 (d, *J* = 7.9 Hz, 2H), 7.38 (d, *J* = 7.8 Hz, 2H), 7.23 (dd, *J* = 17.6, 8.4 Hz, 6H), 6.93 – 6.75 (m, 4H), 6.66 (t, *J* = 7.2 Hz, 3H), 5.63 (s, 2H), 3.50 – 3.39 (m, 2H), 2.84 (t, *J* = 6.9 Hz, 2H), 2.73 – 2.63 (m, 2H), 2.56 – 2.47 (m, 2H). ^{13}C NMR (500 MHz, CD_3CN) δ 172.55, 172.10, 170.18, 150.90, 150.49, 148.87, 147.61, 147.52,146.85, 146.28, 146.23, 144.50, 144.08, 141.28, 140.01, 137.83, 134.65, 134.57, 133.84, 133.77, 130.62, 130.48, 129.52, 129.04, 127.67,

127.66, 127.50, 127.46, 126.74, 126.63, 126.31, 126.18, 126.04, 124.12, 123.04, 118.09, 117.85, 40.07, 35.02, 32.15, 30.33. ESI-MS (CH_2Cl_2): m/z 1078.67 $[M-PF_6]^+$. Purity (HPLC): 97.9%. Elemental Analysis:$C_{54}H_{43}F_6IrN_7O_4PS•2H_2O$ Calcd.(%) C: 51.51; H: 3.76; N: 7.79 found (%) C: 51.69; H: 3.70; N: 7.69.

$[Ir(pq)_2(phen)]PF_6$ (**Ir3c**)[3]: Complex **Ir3c** was obtained as a dark yellow powder. Yield: 80%.^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (dd, *J* = 8.3, 1.4 Hz, 2H), 8.59 (d, *J* = 8.9 Hz, 2H), 8.55 – 8.45 (m, 4H), 8.35 (dd, *J* = 8.1, 1.3 Hz, 2H), 8.08 (s, 2H), 8.04 (dd, *J* = 8.2, 5.1 Hz, 2H), 7.80 (dd, *J* = 8.2, 1.5 Hz, 2H), 7.22 (dt, *J* = 15.5, 7.7 Hz, 4H), 7.10 (d, *J* = 8.9 Hz, 2H), 6.93 – 6.76 (m, 4H), 6.56 – 6.50 (m, 2H).^{13}C NMR (500 MHz, DMSO-d_6) δ 170.26, 150.99, 148.71, 147.32, 146.45, 146.22, 140.80, 139.37, 134.60, 131.08, 131.05, 130.71, 129.69, 128.40, 128.22, 127.67, 127.47, 127.10, 123.90, 123.33, 118.76, 40.67, 40.62, 40.46, 40.41, 40.25, 40.20, 40.04, 40.00, 39.84, 39.79, 39.58, 39.46, 39.37.ESI-MS (CH_3OH): m/z 781.19 $[M-PF_6]^+$.

HPLC analysis of Ir1-Ir3. The procedure was performed as previously described.[4] **Ir1-Ir3** were firstly dissolved in MeOH at a concentration of 10 uM, and 5 μL was used for each HPLC analysis. Phase A is H_2O with 0.01% Trifluoroacetic acid (TFA), and phase B is methanol. 0–20 min: 10%B–100% B; 20–25 min: 100% B. The absorption wavelength was set to 254 nm. The percentage of **Ir1-Ir3** compounds remaining were calculated from the peak area ratio at 254 nm.

Optical properties

The UV-Vis absorption and emission spectra (λ_{ex} = 405 nm) of **Ir1**−**Ir3** in CH_2Cl_2, CH_3CN and PBS (10 μM) at 298 K were obtained on a Varian Cary 300 spectrophotometer (USA) and a FLS 920 combined fluorescence lifetime and steady state spectrometer (Japan), respectively.

Generation of singlet oxygen ($^1O_2$)

The quantum yields for $^1O_2$ production were measured according to the literature method.[5] **Ir1**−**Ir3** were incubated with ABDA (100 μM) in PBS (pH 7.4). Then the mixture was irradiated with a 425 nm light array for different time intervals. The absorption maxima of ABDA were recorded. $[Ru(bpy)_3]Cl_2$ was used as the standard (Φ_Δ = 0.18).

Cell lines and culture conditions

MDA-MB-231 and HT29 cells were obtained from Experimental Animal Center of Sun Yat-Sen University (Guangzhou, China). Cells were cultured in DMEM (Dulbecco's modified Eagle's medium, Gibco BRL) and McCoy's 5A (Gibco BRL) medium containing 10% FBS (fetal bovine serum, Gibco BRL), 100 μg mL^{-1} streptomycin, and 100 U mL^{-1} penicillin (Gibco BRL). The cells were cultured in tissue culture flasks in a humidified incubator at 37 °C, under an atmosphere of 5% CO_2 and 95% air. For hypoxia conditions, cells were cultured under an atmosphere of 5% CO_2, 1% O_2 and 94% N_2. In each experiment, cells treated with vehicle DMSO (1%, v/v) were used as the reference group.

MTT assay

The antiproliferative activities of **Ir1**−**Ir3** towards MBA-MD-231 and HT29 cell lines was determined by MTT assay. Cells cultured in 96-well plates were grown to confluence before incubated with **Ir1**−**Ir3** for 12 h. The medium containing the compelxes was replaced by fresh medium. Then the cells were irradiated with a 425 nm light array (12 J cm^{-2}) and further incubated for 32 h. 20 μL of MTT solution (5 mg mL^{-1}) was then added to each well. The plates were incubated for an additional 4 h before the media was carefully removed, and DMSO was added (150 μL per well). The plate was shaken for 3 min. The absorbance at 595 nm was measured using a microplate reader (Infinite M200 Pro, Tecan, Switzerland).

To investigate the impact of different cell death inhibitors on photocytotoxicity of **Ir3**, the cells were pretreated with cell-death inhibitors (Nec-1: 100 μM); NSA: 10 μM; Z-VAD-fmk: 50 μM) for 1 h before the addition of **Ir3** (1 or 2 μM). After a further incubation for 12 h, the cells were irradiated with a 425 nm laser (12 J cm^{-2}).

3D Multicellular Tumor Spheroids (MCTSs)

Cell suspensions containing 5×10^3 cells were seeded in Nunclon™ Sphera™ 96 well plate and formed into MCTSs within one week. The MCTSs were cultivated in a cell culture incubator at 37 °C with 5% CO_2 atmosphere. The medium was replaced with fresh medium every two days. The integrity, diameter and volume of MCTSs were monitored with a LSM 880 (Carl Zeiss) laser scanning confocal microscope.

Cellular uptake levels by ICP-MS

The cellular uptake level of **Ir3** was determined by the intracellular iridium contents using a method reported in literature with slight modifications.[6, 7] Briefly, HT29 cells were seeded in 60 mm tissue culture dishes and incubated for 24 h. The medium was removed and replaced with medium/DMSO (v/v, 99:1) containing **Ir3** (5 μM). After 2 h or 4 h incubation, the cells were trypsinized, counted and harvested as cell pellet. HNO_3 (65%, 500 μL) was added to the pellet and the mixture was incubated at room temperature for 24 h to digest entirely. The solution was then diluted to a final volume of 10 mL with Milli-Q water (containing 10 ppb indium as internal standard). The concentration of iridium was measured using the XSERIES 2 ICP-MS.

Western blotting

HT29 cells were seeded in 6 cm dishes (Corning) and cultured for 24 h. the medium were replaced by fresh medium containing **Ir3** (1 μM). After 4 h, the cells were irradiated with a 425 nm laser (12 J cm^{-2}). After a further incubation for 4 h, the cells were collected and lysed. The intracellular proteins were extracted by differential centrifugation (Cell lysis

buffer, PMSF, 1 mM). The extracted protein was quantified with BCA protein detection kit, electrophoresed in 10 % polyacrylamide gel and transferred to PVDF membrane. PVDF membranes were blocked with QuickBlock™ and incubated with primary antibodies against CAIX (goat anti rabbit), HIF-1α (goat anti rabbit), VEGF (goat anti rabbit) or GAPDH (goat anti rabbit) overnight at 4 °C. After washing with PBST for 3 times, PVDF membranes were incubated with secondary antibody Goat Anti-Rabbit IgG H&L (HRP) for 2 h. Subsequent staining was performed with DAB horseradish peroxidase chromogenic kit, and chemiluminescence was detected with Fluochem M.

MALDI-TOF-MS Analysis

Molecular weight changes of the mixed solutions of **Ir3** and CA were determined using a Bruker ultrafleXtreme Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS). **Ir3** (50 μM) and CA (50 μM) were mixed in water for 10 min before irradiated with a 425 nm laser (12 J cm^{-2}).

Inhibition of CAIX

To test the inhibitory effects of **Ir3** on CAIX, 36 μL protein (11.1 μg mL^{-1}) was added to 96-well plate, then 4 μL stock solution of **Ir3** (1 mM) or AAZ (1 mM) was added. After shaking for 15 min, 40 μL of 4-NPA (4-nitrophenylacetate) solution was added. After an incubation at 37 °C for 5 h, the absorption at 400 nm was determined by microplate reader.

Cellular ROS Detection

HT29 cells were seeded in 6 cm dishes and incubated under hypoxia for 24 h. The cells were treated with **Ir3** at different concentrations for 5 h and then irradiated at 425 nm (12 J cm^{-2}). Then, the cells were harvested and incubated with DCFH-DA (10 μM) for 15 min at 37 °C in the dark. Cells treated with cisplatin (50 μM) for 6 h in dark were used as control samples. The samples were then analyzed by flow cytometry or confocal microscopy. λ_{ex} = 488 nm, λ_{em} = 530 ± 20 nm.

To investigte the imapct of different ROS scavengers on the ROS levels, the cells were preincubated with the scavengers (D-mannitol: 50 mM; Tirion: 5 mM; NaN_3: 5 mM; Ebselen: 50 μM) for 1 h under hypoxia. The cells were treated with **Ir3** (2 μM, 6 h) then irradiated with a 425 nm laser (12 J cm^{-2}). Then, the cells were harvested and incubated with DCFH-DA (10 μM) for 15 min at 37 °C in the dark. The samples were then analyzed by confocal microscopy. λ_{ex} = 488 nm, λ_{em} = 530 ± 20 nm.

Cellular Superoxide anion Detection

HT29 cells were seeded in 6 cm dishes and incubated under hypoxia for 24 h. After treated with **Ir3** for 5 h and irradiated at 425 nm(12 J cm^{-2}), the cells were stained with Dihydroethidium (DHE; 10 μM, 30 min). DHE: λ_{ex} = 561 nm, λ_{em} =

610 ± 20 nm.

Cellular lipid peroxidation Detection

HT29 cells were treated with **Ir3** (5 μM, 5 h) and irradiated at 425 nm (12 J cm^{-2}) under hypoxia. The cells were stained with CD11 BODIPY 581/591 (2 μM, 30 min). The cells were then observed by a confocal microscope. Reduced form: λ_{ex} = 561 nm, λ_{em} = 591 ± 20 nm. Oxidated form: λ_{ex} = 561 nm, λ_{em} = 510 ± 20 nm.

Cellular MDA Content Measurement

HT29 cells were treated with **Ir3** (5 μM, 5 h) and irradiated at 425 nm (12 J cm^{-2}) under hypoxia. The content of MDA in cell lysis was measured using the Lipid Peroxidation MDA Assay Kit (S0131S, Beyotime) following the manufacturer's protocol.

Transmission Electron Microscopy

HT29 cells were treated with **Ir3** (5 μM, 12 h) and irradiated at 425 nm (12 J cm^{-2}). The cells were further incubated for 4 h. After that, the cells were collected and fixed with electron microscopy fixative solution. The samples were visualized under a transmission electron microscope (JEM 100 CX, JEOL, Tokyo, Japan). Images were photographed using the Eversmart Jazz program (Scitex).

Flow cytometric analysis of CRT and HMGB1

HT29 cells were treated with **Ir3** at the indicated concentrations for 12 h and irradiated with a 425 nm laser (12 J cm^{-2}). The cells were harvested and fixed with 4 % paraformaldehyde for 30 min. For CRT measurement, the samples were incubated with Anti-Calreticulin (EPR3924, abcam) for 12 h in 4 °C. For HMGB1 measurement, the cells were incubated with Anti-HMGB1 (ab18256, abcam) for 12 h in 4 °C after permeabilization with 0.1% Triton 5 min. The samples were then incubated with the Goat polyclonal Secondary Antibody to Rabbit IgG H&L (Alexa Fluor® 488) and subjected to flow cytometry analysis. λ_{ex} = 488 nm; λ_{em} = 520 ± 20 nm.

Extracellular ATP detection assay

HT29 cells were treated with **Ir3** at the indicated concentrations for 12 h and irradiated with a 425 nm laser (12 J cm^{-2}). The supernatants were transferred into the white-walled non-transparent bottomed 96-well plates. The ATP content was measured using the ATP Assay Kit (S0026, Beyotime) following the manufacturer's protocol.

Supplementary Scheme, Figures and Tables

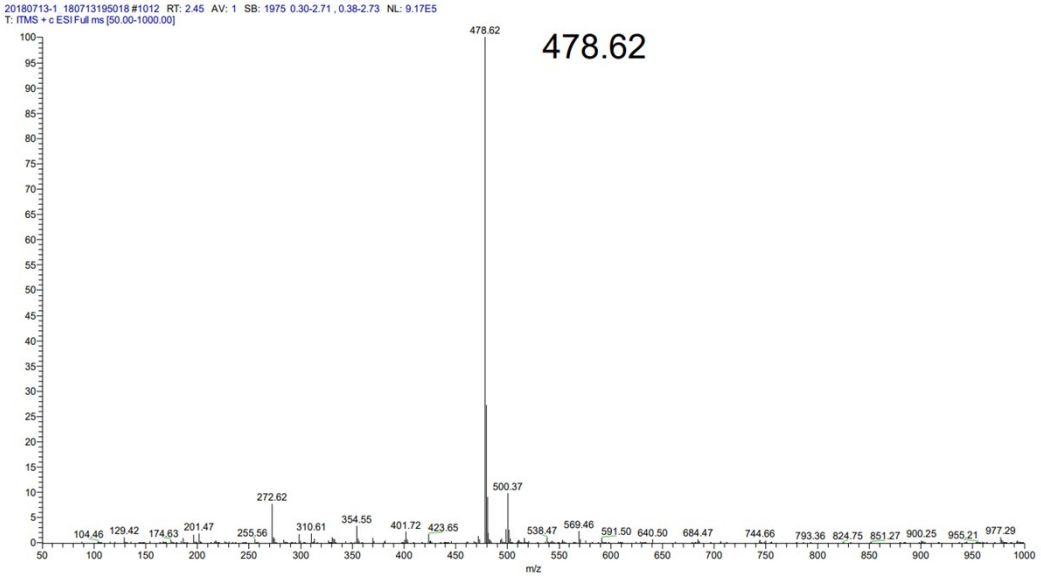


Figure S1. ESI-MS spectrum of **L**.

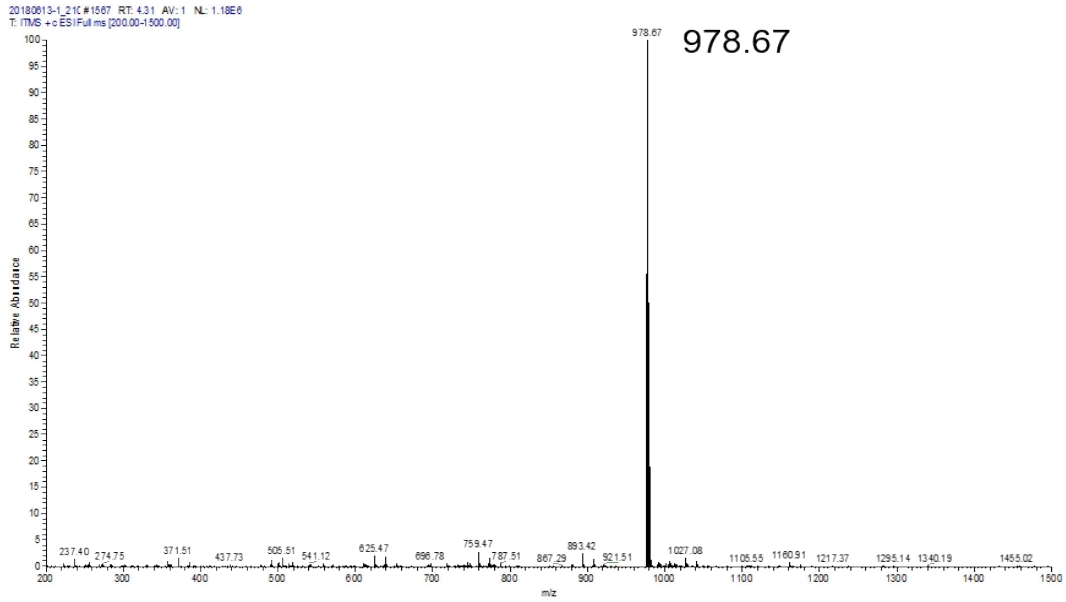


Figure S2. ESI-MS spectrum of **Ir1**.

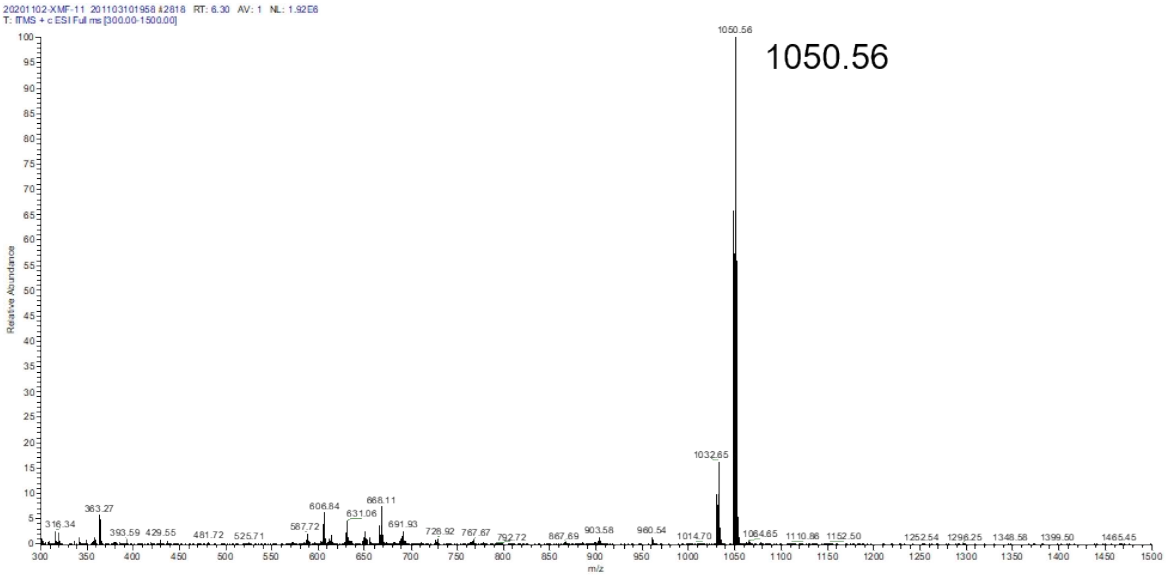


Figure S3. ESI-MS spectrum of **Ir2**.

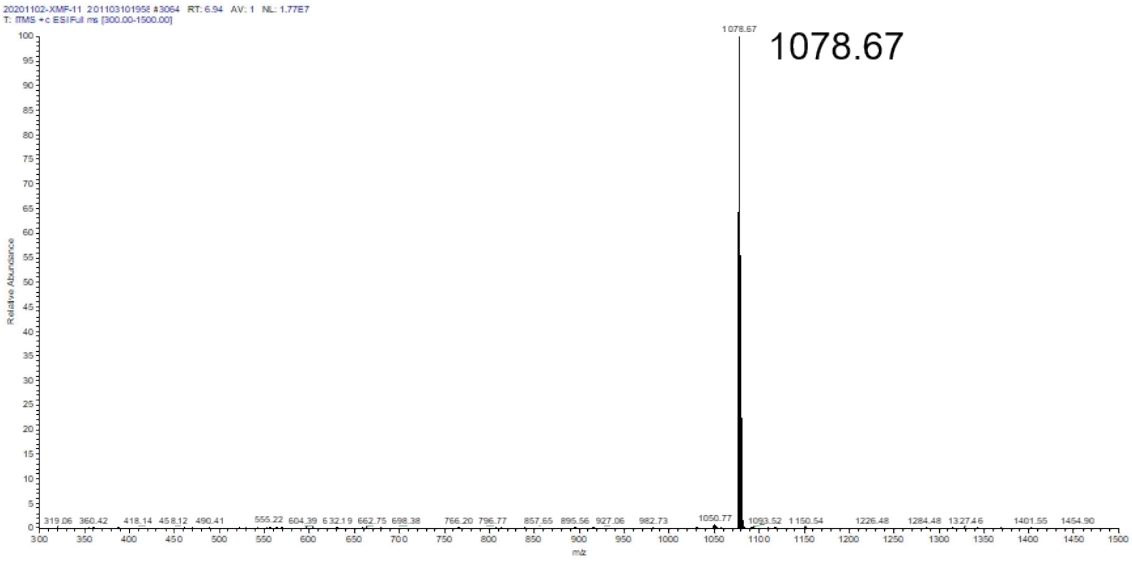


Figure S4. ESI-MS spectrum of **Ir3**.

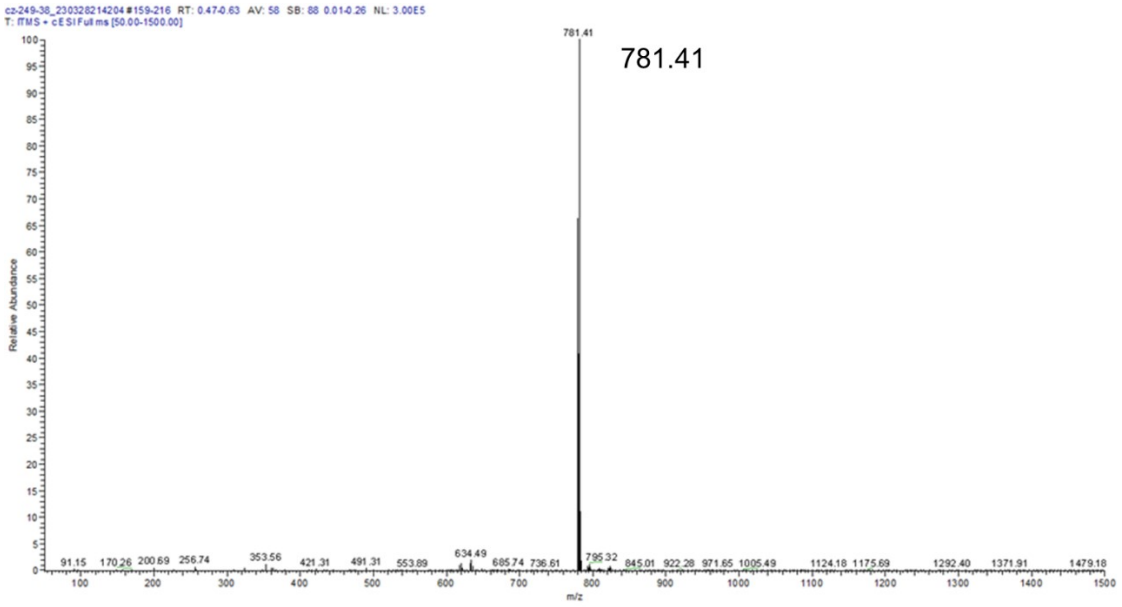


Figure S5. ESI-MS spectrum of **Ir3c**.

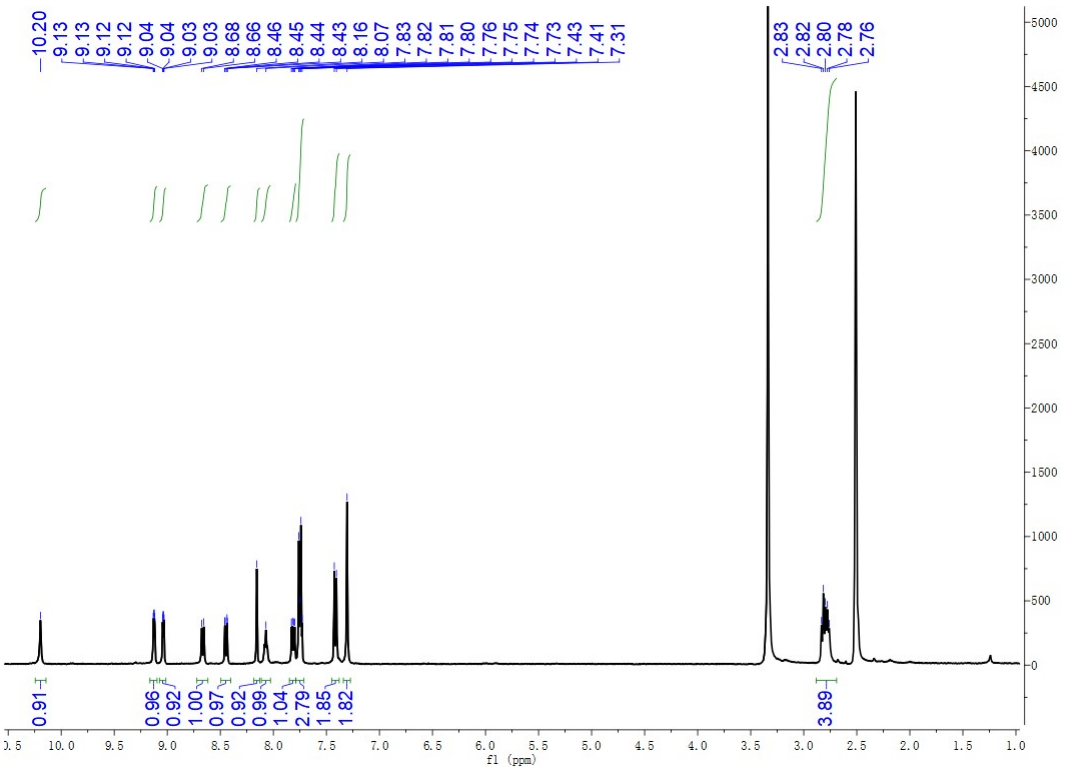


Figure S6. ^{1}H NMR of **L** in CD_3CN.

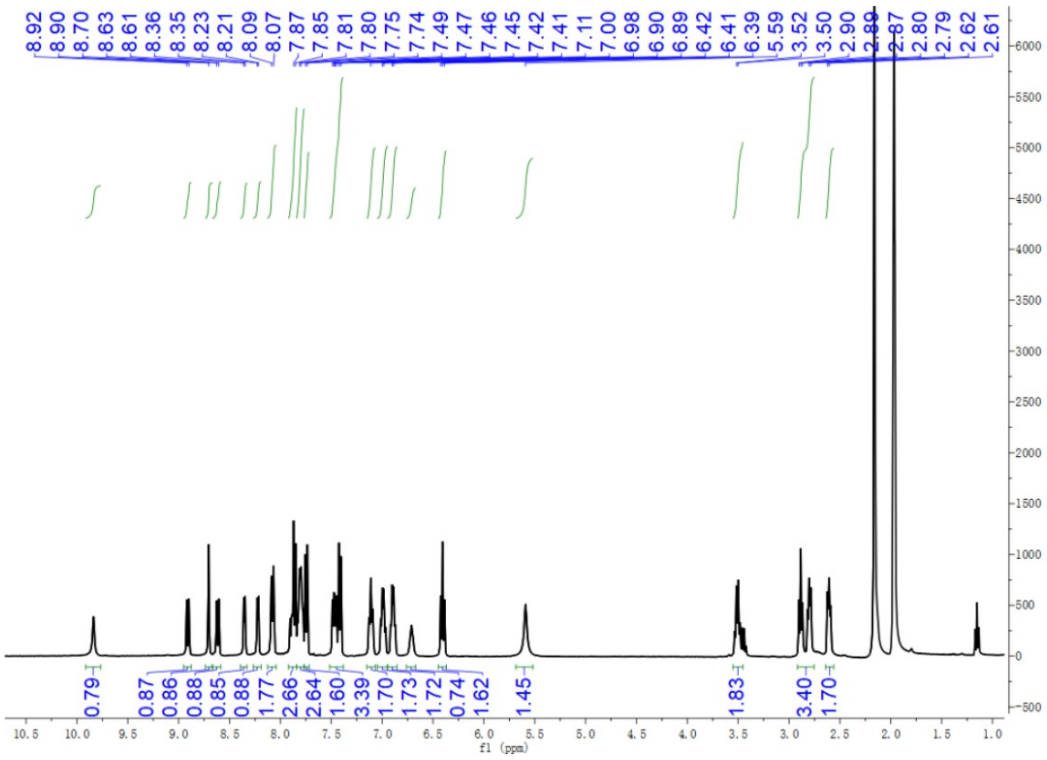


Figure S7. ^{1}H NMR of **Ir1** in CD_3CN.

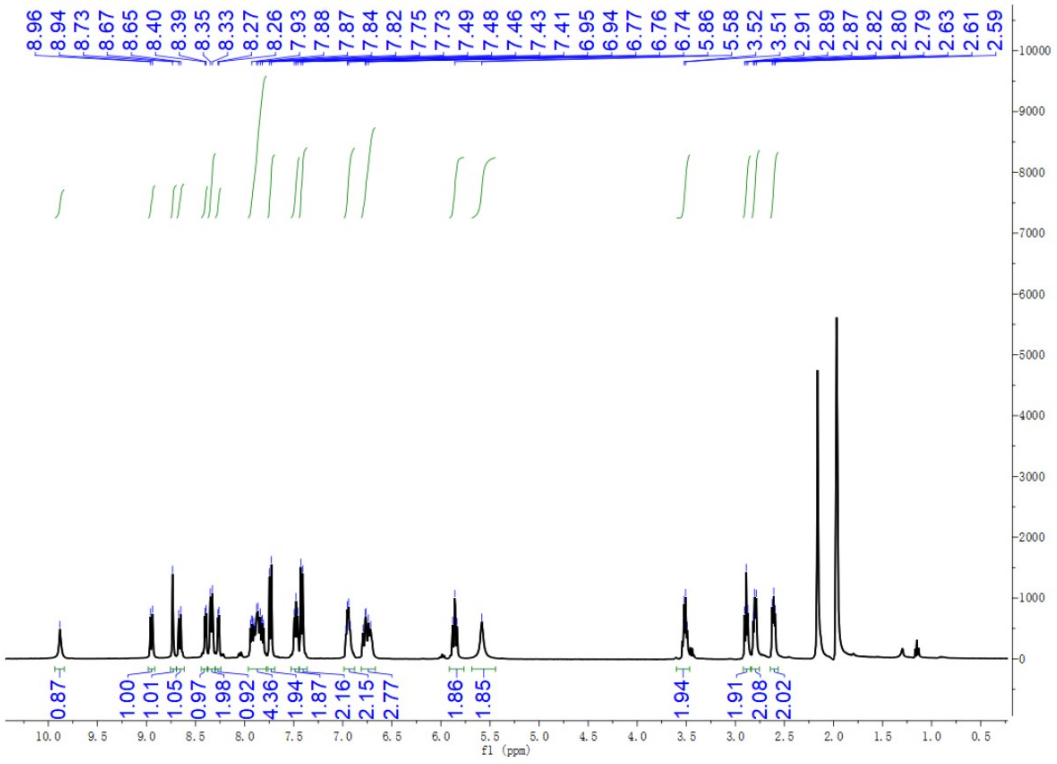


Figure S8. ^{1}H NMR of **Ir2** in CD_3CN.

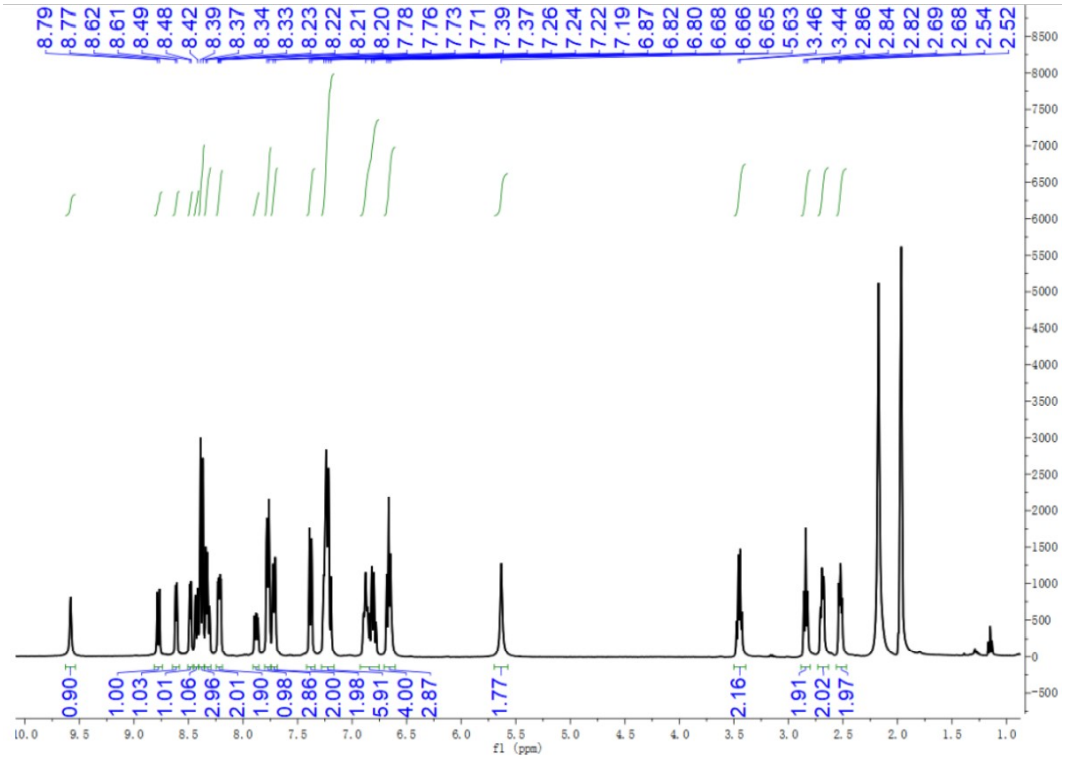


Figure S9. ^{1}H NMR of **Ir3** in CD_3CN.

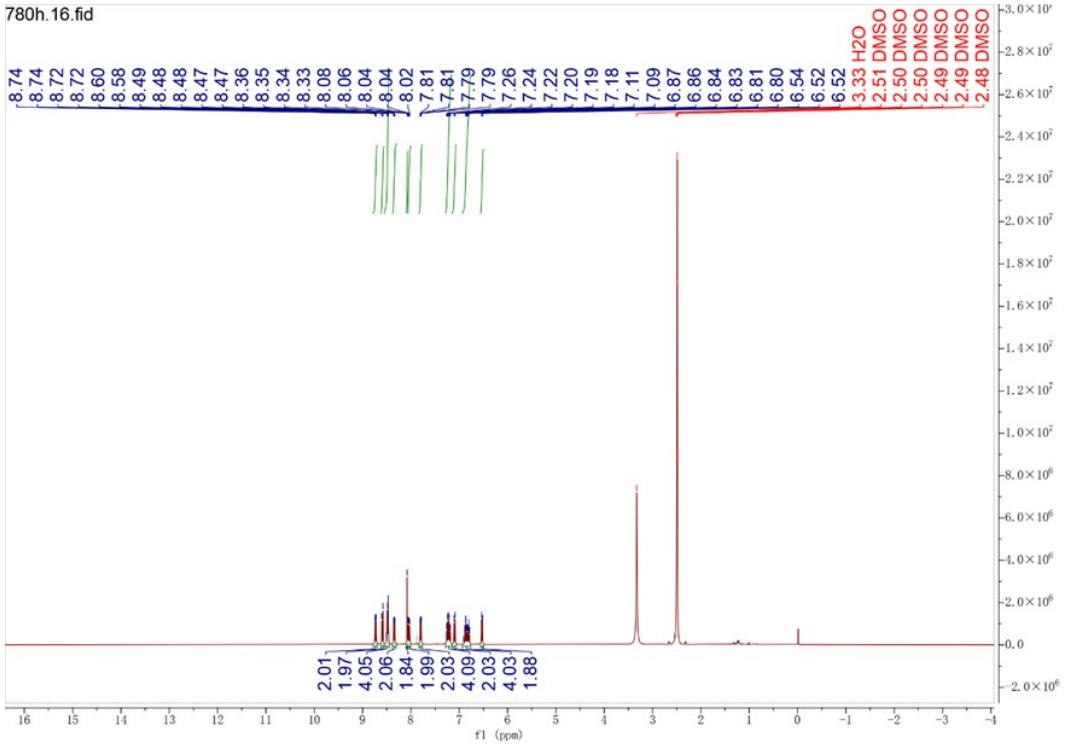


Figure S10. ^{1}H NMR of **Ir3c** in DMSO-d_6

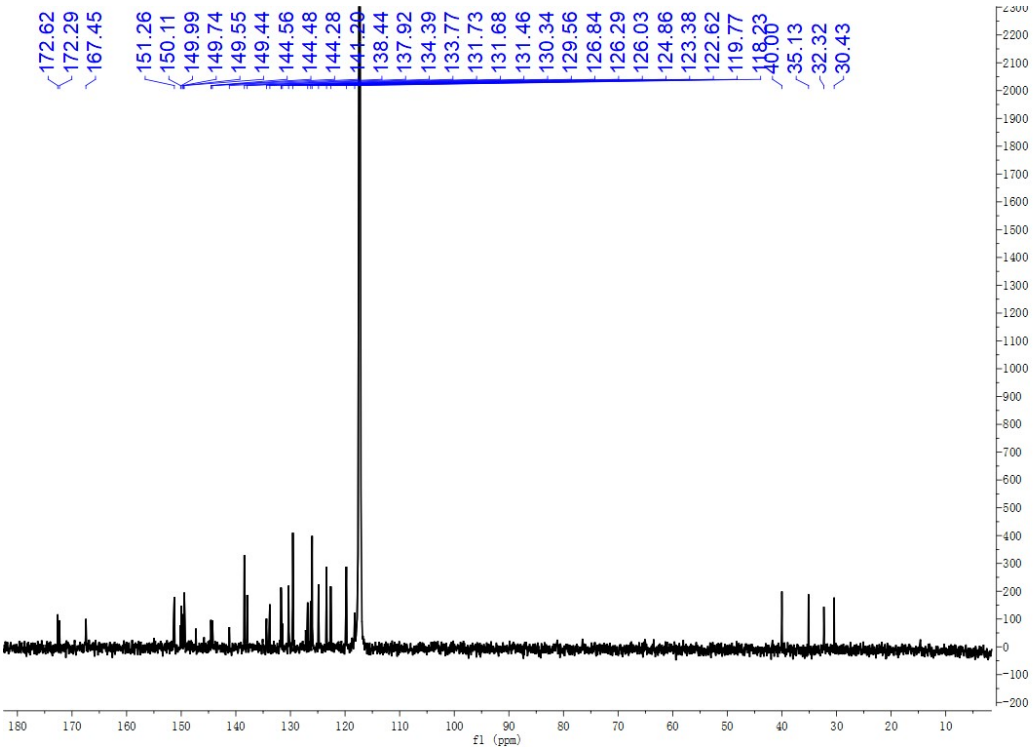


Figure S11. ^{13}C NMR spectrum of **Ir1** in CD_3CN.

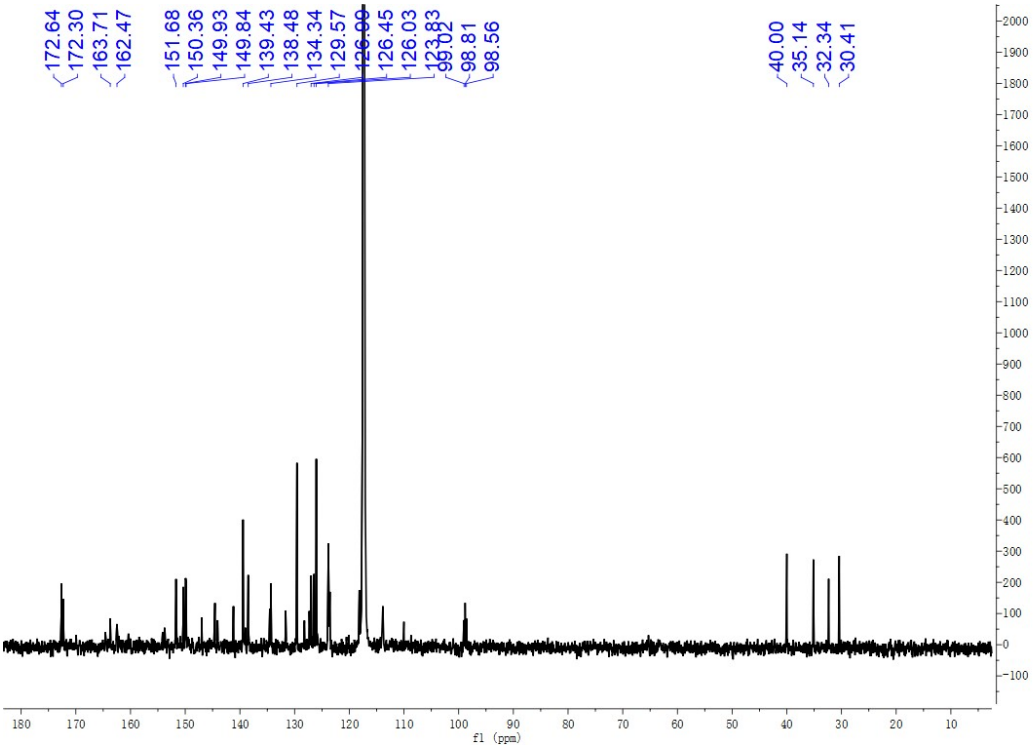


Figure S12. ^{13}C NMR spectrum of **Ir2** in CD_3CN.

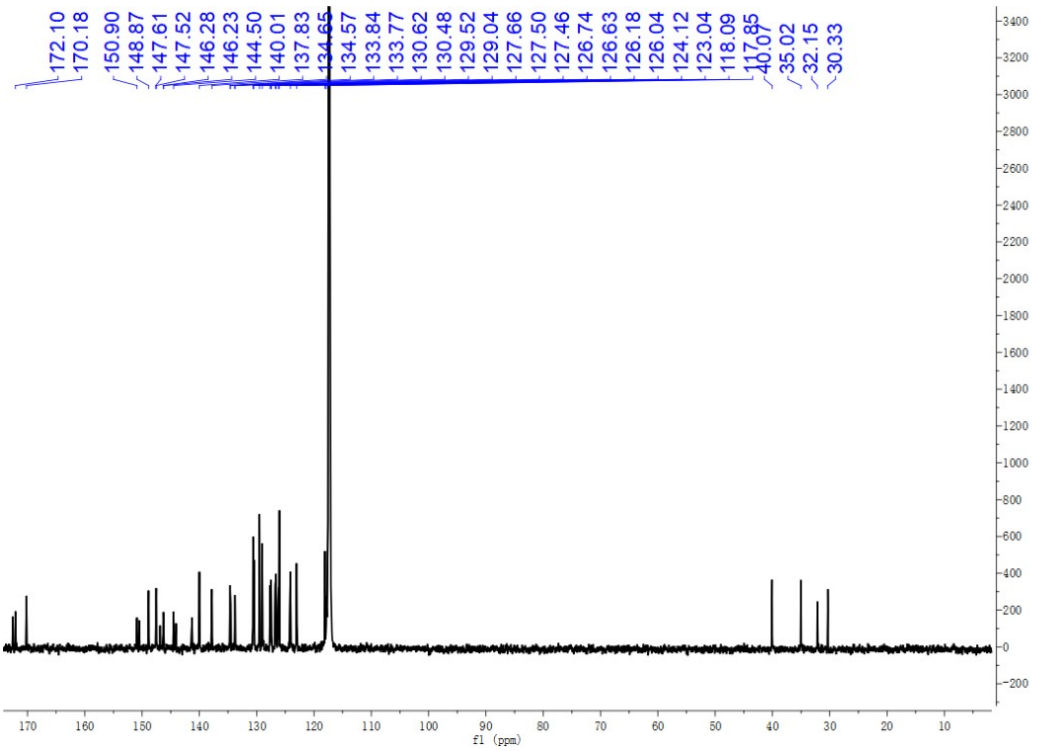


Figure S13. ^{13}C NMR spectrum of **Ir3** in CD_3CN.

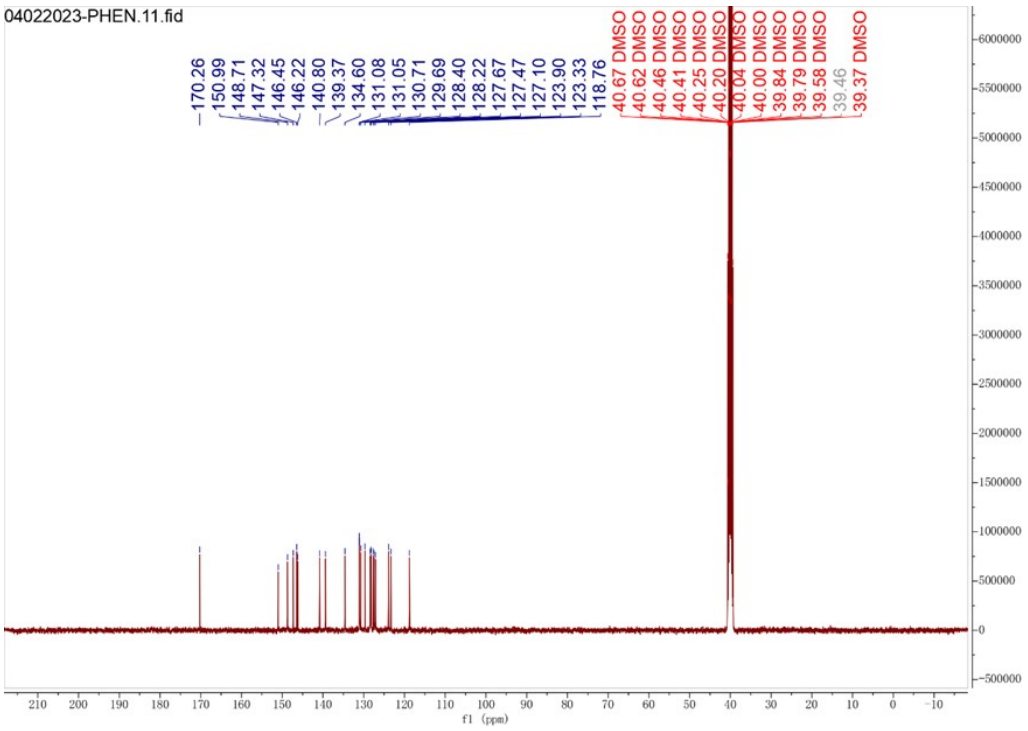


Figure S14. ^{13}C NMR spectrum of **Ir3c** in DMSO-d_6.

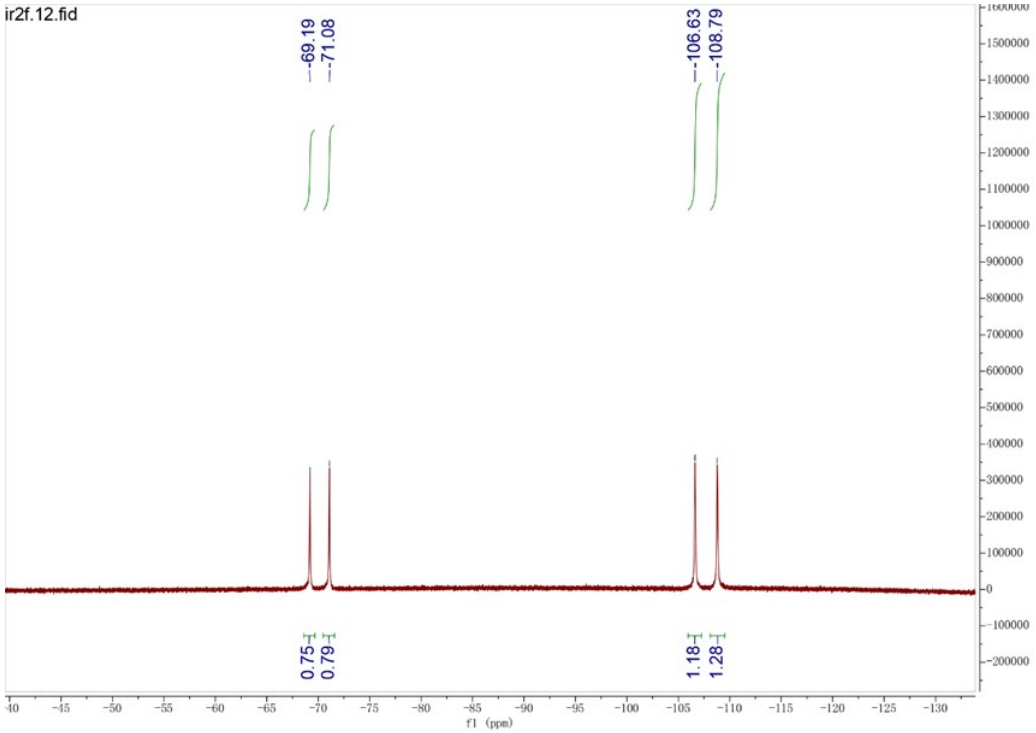


Figure S15. ^{19}F NMR of **Ir2** measured in DMSO-d_6.

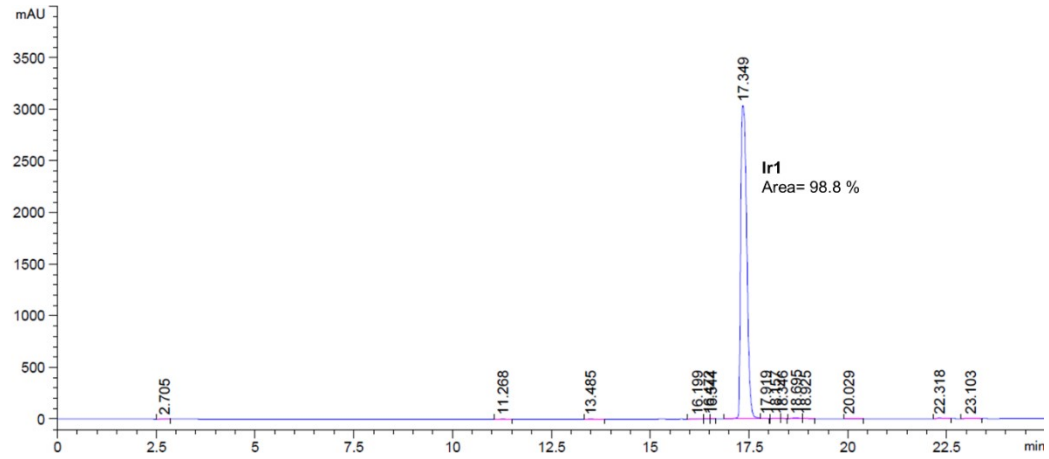


Figure S16. The purity of **Ir1** analyzed by HPLC.

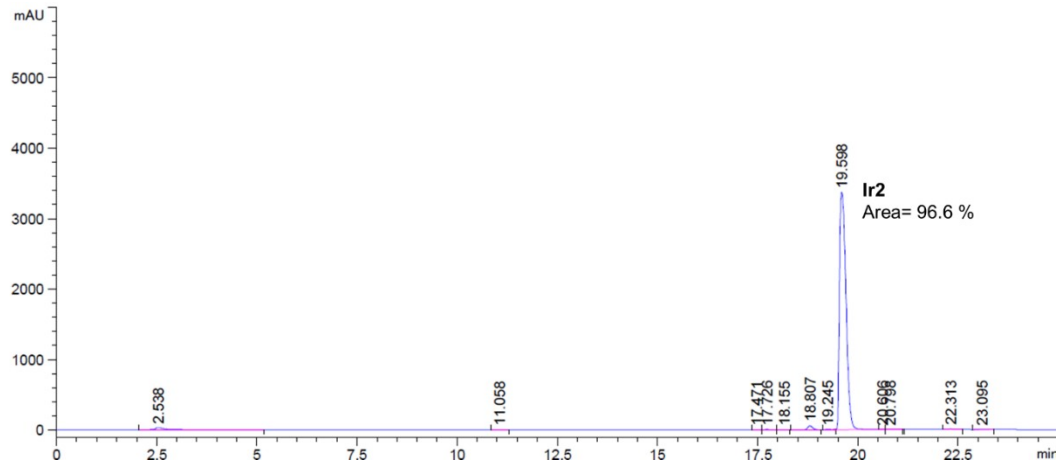


Figure S17. The purity of **Ir2** analyzed by HPLC.

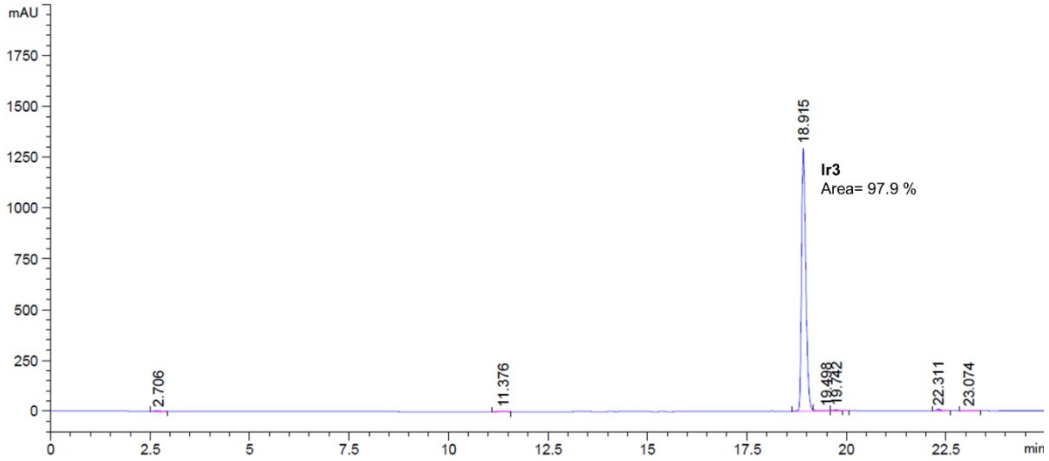


Figure S18. The purity of **Ir3** analyzed by HPLC.

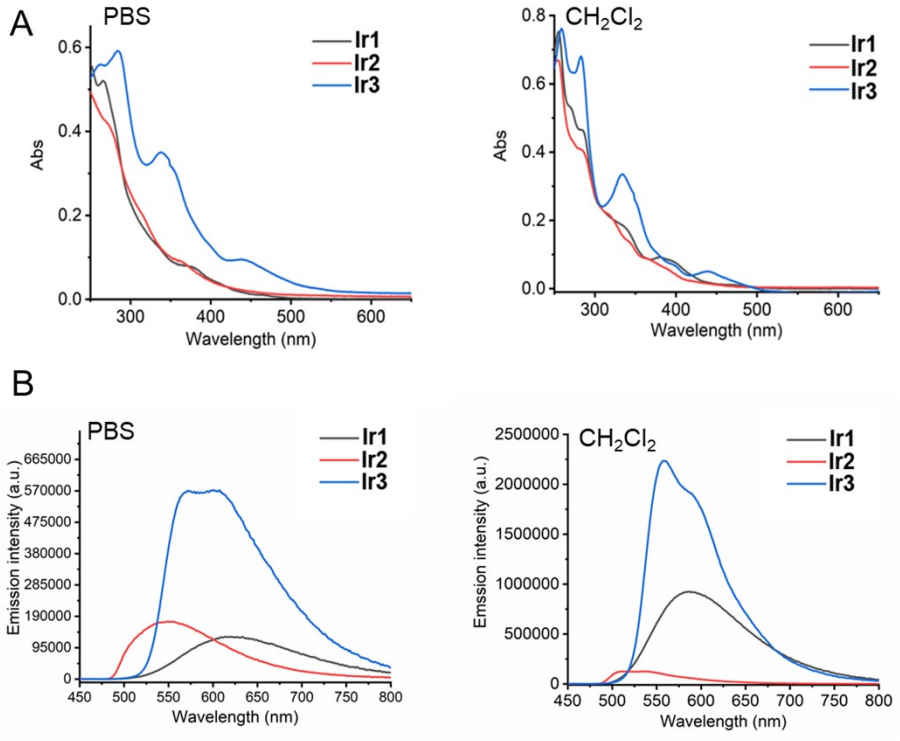


Figure S19. (A) and emission (B) spectra of **Ir1**–**Ir3** (2×10^{-5} M) measured in PBS and CH_2Cl_2 at 298 K.

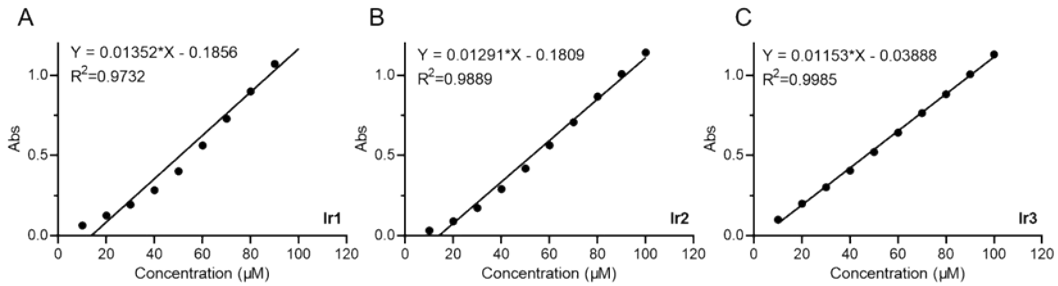


Figure S20. Fitting curves of the absorbance of **Ir1** (A), **Ir2** (B) and **Ir3** (C) at 385 nm verus concentration. **Ir1**–**Ir3** were dissolved in cell culture medium and 1% DMSO (v/v) was used for solubilization.

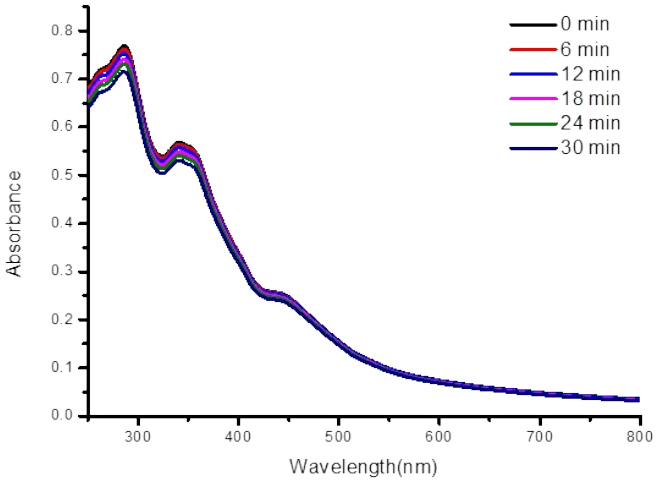


Figure S21. The photo-stability of **Ir3** in the cell culture medium measured by UV/vis spectroscopy.

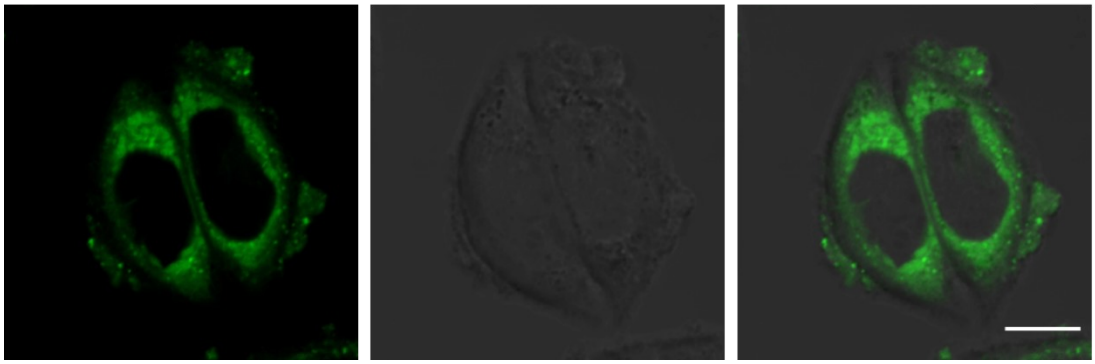


Figure S22. Cellular localization of **Ir3** in HT29 cells. Cells were treated with **Ir3** (5 μM, 2 h) and observed under a confocal microscope immediately. λ_{ex} = 405 nm; λ_{em} = 550 ± 50 nm. Scale bar: 10 μm.

Table S1. Photophysical data of complexes **Ir1**–**Ir3**[a]

Compounds	Medium	$\lambda_{abs,\ max}$ (nm)	$\lambda_{em,\ max}$ (nm)	τ_{av} [b]/(ns)
Ir1	PBS	370	621	141.4
	CH_3CN	380	595	162.4
	CH_2Cl_2	381	587	346.1
Ir2	PBS	360	540	1224.3
	CH_3CN	364	530	477.2
	CH_2Cl_2	348	534	1275.8
Ir3	PBS	439	607	654.2
	CH_3CN	436	562	330.8
	CH_2Cl_2	438	557	991.4

[a] Samples were 2 × 10^{-5} M in concentration. [b] All curves were fitted into a two exponential formula $F(t) = A + B_1\,exp(-t/\tau_1) + B_2\,exp(-t/\tau_2)$; $\tau_{av} = \frac{B_{1\tau1}{}^2 + B_{2\tau2}{}^2}{B_{1\tau1} + B_{2\tau2}}$

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