Supporting Information

Light Switchable Ir(III)-Based Photosensitizers: A Dual-State System for Non-Invasive, Reversible ROS Control in Tumor Therapy

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Experimental Section

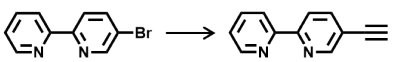
1. Materials and measurements

All reagents were commercially available and used without further purification unless specifically noted. Cuprous iodide (Heowns), Triethylamine (Heowns), 5-Bromo-2,2'-bipyridine (Leyan), TMSA (Trimethylsilylacetylene, Heowns), Bis(triphenylphosphine)palladium (II) chloride (Energy Chemical), DTE (1,2-bis (2'-methyl-5'-bromothien-3'-yl) perfluorocyclopentene, Alpha), chloride hydrate (J&K Scientific), cisplatin (Energy Chemical), DMSO (Dulbecco's modified Eagle's medium, KeyGEN BioTECH, China), MTT (3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Heoens), DMEM (Dulbecco's modified Eagle's medium, KeyGEN BioTECH, China) medium containing 10% FBS (fetal bovine serum, Procell, China), 100 μg/mL streptomycin, and 100 U/mL penicillin (Gibco BRL), RIPA lysis buffer (Beyotime Biotechnology, China), ROS Assay Kit (Beyotime Biotechnology, China), ATP Assay Kit (Beyotime Biotechnology, China), Annexin V-FITC/PI Apoptosis Detection Kit (KeyGEN BioTECH, China), JC-1 Apoptosis Detection Kit (KeyGEN BioTECH, China), Mitochondria/Nuclei Isolation Kit (Keygen Biotech, China), BCA Protein Quantitation Assay (Keygen Biotech, China), SDS-PAGE Sample Loading Buffer (6×) (Beyotime Biotechnology, China), LC3 Polyclonal Antibody (Proteintech, USA), PINK1 Rabbit mAb (Cell signalingUSA), p62 Polyclonal antibody (Proteintech, USA), γ-H2AX antibody (Cell Signaling Technology, USA), Caspase 9/p35/p10 Polyclonal antibody (Proteintech, USA), Cyclin B1(Abcam, UK), Fluorescein Isothiocyanate (FITC)-conjugated affinipure Goat Anti-Rabbit lgG(H+L) (Proteintech, USA). Deuterated solvents for NMR purposes were obtained from Merck and Cambridge Isotopes. Other organic reagents, which were of analytical grade, were obtained from domestic chemical corporations and used as received without any further purification.

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DXR-400 spectrometer at ambient temperature. Electrospray ionization mass spectra (ESI-MS) were obtained using an LCQ spectrometer (Thermo Scientific). UV-visible absorption spectra were

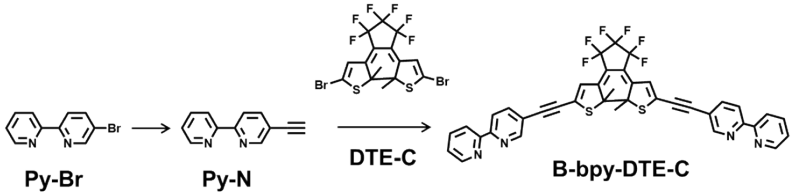
recorded on a PerkinElmer Lambda 365 UV-vis spectrophotometer. Emission spectra were measured using a FS5 Spectrofluorometer. Cells used in this work were incubated in a humidified incubator (BB 150 CO_2 incubator, Thermo Fisher Scientific, USA). MTT data were recorded on a microplate reader (LabServ K3, Part of Thermo Fisher Scientific, USA). Iridium contents in the samples were determined by ICP-MS (X Series 2, Thermo Fisher, USA). Fluorescence confocal imaging was performed with a laser scanning confocal microscope (A1, Nikon, Japan). Western blot experiments were conducted on the Mini-Protean Tetra System (BIO-RAD, Power PacTM HC, Singapore) and visualized by Tanon-5200 Multi. Flow cytometric analysis was done using a flow cytometer (BD Accuri C6 Plus).

2. Synthesis and characterization



Py-Br **Py-N**

Py-N: Py-Br (5 mmol), TMSA (6 mmol), CuI (0.15 mmol) and $Pd(PPh_3)_2CI_2$ (0.15 mmol) were dissolved in dry Et_3N (30 ml) and reacted at reflux temperature for 12 h under the protection of nitrogen atmosphere. It was concentrated under pressure and dissolved with petroleum ether and purified by PE/EA (20:1, v/v) eluted silica gel column chromatography. Yield:600 mg (86%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.77 (s, 1H), 8.69 (d, J = 4.9 Hz, 1H), 8.40 (dd, J = 8.2, 4.6 Hz, 2H), 7.90 (dd, J = 8.3, 1.9 Hz, 1H), 7.87 – 7.79 (m, 1H), 7.39 – 7.29 (m, 1H), 3.29 (s, 1H).



Py-Br Py-N B-bpy-DTE-C

Ligand **B-bpy-DTE-C**: Py-N (0.456 mmol), DTE-C (0.19 mmol), CuI (0.06 mmol) and $Pd(PPh_3)_2Cl_2$ (0.06 mmol) were dissolved in dry Et_3N (30 ml), and then reflow for 12 h under the protection of nitrogen atmosphere to reduce pressure and dissolve petroleum ether. PE/EA (25:1, v/v) eluted silica gel column chromatography purification. Yield:11mg (8%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.78 (d, J = 2.0

Hz, 2H), 8.73 – 8.68 (m, 2H), 8.45 (t, J = 8.7 Hz, 4H), 7.94 – 7.89 (m, 2H), 7.88 – 7.81 (m, 2H), 7.37 – 7.32 (m, 2H), 6.55 (s, 2H), 2.23 (s, 6H).

Ligand **B-bpy-DTE-O**: The 5 mg ligand **B-bpy-DTE-C** was dissolved in 0.5 ml of deuterium-chloroform solution and injected into a nuclear magnetic tube. After irradiating it under red light (630 nm, 0.6 $W \cdot cm^{-2}$) for about 30 minutes, the solution was observed to turn blue into a colorless transparent liquid, and **B-bpy-DTE-O** was obtained. ^1H NMR (400 MHz, Chloroform-*d*) δ 8.81 – 8.77 (m, 2H), 8.71 – 8.68 (m, 2H), 8.44 – 8.40 (m, 4H), 7.92 (dd, J = 8.3, 2.2 Hz, 2H), 7.86 – 7.81 (m, 2H), 7.34 (s, 4H), 1.98 (s, 6H).

M-Ir-DTE-C: The dimer $[Ir(ppy)_2Cl_2]_2$ was prepared according to the literature procedure. $[Ir(ppy)_2Cl_2]_2$ (0.0248 mmol) and the ligand **B-bpy-DTE-C** (0.0414 mmol) were reflux-heated in CH_2Cl_2 (25 ml) at the equivalent of 1.2:2 for 24 h. Yield:5 mg (10%). ^1H NMR (400 MHz, Chloroform-*d*) δ 9.77 (d, J = 8.7 Hz, 1H), 9.68 (d, J = 8.2 Hz, 1H), 8.77 (s, 1H), 8.70 (d, J = 4.6 Hz, 1H), 8.45 (t, J = 9.4 Hz, 2H), 8.31 (d, J = 8.8 Hz, 1H), 8.26 (t, J = 8.1 Hz, 1H), 7.98 – 7.88 (m, 5H), 7.84 (t, J = 8.3 Hz, 1H), 7.79 (d, J = 5.7 Hz, 2H), 7.70 (dd, J = 15.0, 7.8 Hz, 2H), 7.51 (d, J = 5.9 Hz, 1H), 7.45 (d, J = 5.6 Hz, 1H), 7.42 – 7.38 (m, 1H), 7.35 (d, J = 5.2 Hz, 1H), 7.11 – 7.00 (m, 4H), 6.98 – 6.88 (m, 2H), 6.51 (d, J = 21.2 Hz, 2H), 6.28 (dd, J = 13.6, 7.7 Hz, 2H), 2.18 (s, 6H).

M-Ir-DTE-O: The 5 mg ligand **M-Ir-DTE-C** was dissolved in 0.5 ml of deuterium chloroform solution and injected into the nuclear magnetic tube. After irradiation under red light (630 nm, 0.6 $W \cdot cm^{-2}$) for about 2 hours, the solution was observed to turn dark blue to a pale yellow transparent liquid, and **M-Ir-DTE-O** was obtained. ^1H NMR (400 MHz, Chloroform-*d*) δ 9.70 (d, J = 8.6 Hz, 1H), 9.62 (d, J = 8.1 Hz, 1H), 8.78 (s, 1H), 8. 69 (d, J = 3.9 Hz,, 1H), 8.42 (d, J = 8.2 Hz, 2H), 8.32 (d, J = 8.4 Hz, 1H), 8.25 (t, J = 7.8 Hz, 1H), 7.97 – 7.89 (m, 4H), 7.88 (d, J = 5.3 Hz, 1H), 7.86 – 7.81 (m, 1H), 7.80 – 7.75 (m, 2H), 7.70 (dd, J = 13.7, 7.7 Hz, 2H), 7.52 (d, J = 5.8 Hz, 1H), 7.46 (d, J = 5.8 Hz, 1H), 7.38 (t, J = 6.6 Hz, 1H), 7.33 (t, J = 6.3 Hz, 1H), 7.28 (d, J = 7.4 Hz, 2H), 7.09 – 6.99 (m, 4H), 6.97 – 6.89 (m, 2H), 6.28 (dd, J = 15.5, 7.5 Hz, 2H), 1.96 (d, J = 10.3 Hz, 6H).

B-Ir-DTE-C: $[Ir(ppy)_2Cl_2]_2$ (0.0207 mmol) and the ligand **B-bpy-DTE-C** (0.0207 mmol) were reflux-heated in CH_2Cl_2 (25 ml) at the equivalent of 1:1 for 24 h. Yield:7 mg (20%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.67 (d, J = 43.2 Hz, 4H), 8.29 (d, J = 25.7 Hz, 4H), 8.05 – 7.86 (m, 8H), 7.79 (s, 4H), 7.70 (dd, J = 13.8, 7.7 Hz, 4H), 7.51 (s, 2H), 7.43 (d, J = 11.4 Hz, 3H), 7.34 (d, J = 2.5 Hz, 1H), 7.13 – 6.99 (m, 8H), 6.98 – 6.87 (m, 4H), 6.47 (s, 2H), 6.27 (dd, J = 13.5, 7.5 Hz, 4H), 2.12 (s, 6H).

B-Ir-DTE-O: The 5 mg ligand **B-Ir-DTE-C** was dissolved in 0.5 ml deuterium chloroform solution and injected into a nuclear magnetic tube. After irradiation with red light (630 nm, 0.6 $W \cdot cm^{-2}$) for about 2 hours, the solution was observed to change from dark green to yellow transparent liquid, and **B-Ir-DTE-O** was obtained. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.59 (d, J = 47.0 Hz, 4H), 8.28 (d, J = 36.5 Hz, 4H), 7.97 – 7.84 (m, 8H), 7.77 (d, J = 8.2 Hz, 4H), 7.68 (d, J = 8.2 Hz, 3H), 7.55 – 7.31 (m, 8H), 7.13 (s, 2H), 7.09 – 6.95 (m, 8H), 6.91 (t, J = 7.0 Hz, 4H), 6.26 (d, J = 7.4 Hz, 3H), 2.02 (s, 6H).

Ir-0: $Ir_2(ppy)_4Cl_2$ (50 mg) and 2,2'- bipyridine (15 mg) were placed in a 50 mL three-neck flask, refluxed with CH_3OH and CH_2Cl_2 (1:2, 12 mL) at 65°C in argon overnight. Then the mixture was cooled to room temperature. After adding 10 equivalent ammonium fluorophosphate, the suspension was stirred for 1 h and then filtered to remove solids. The solvent was then removed under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (5% MeOH/CH_2Cl_2) to afford yellow solid Yield:28 mg (71%). ^{1}H NMR (400 MHz, Methylene Chloride-*d*2) δ 8.51 (d, J = 8.1 Hz, 2H), 8.17 – 8.10 (m, 2H), 8.03 (dd, J = 5.4, 1.5 Hz, 2H), 7.99 – 7.95 (m, 2H), 7.82 – 7.77 (m, 2H), 7.75 (dd, J = 7.8, 1.3 Hz, 2H), 7.52 – 7.45 (m, 4H), 7.11 – 7.05 (m, 2H), 7.02 – 6.98 (m, 2H), 6.97 – 6.92 (m, 2H), 6.32 (dd, J = 7.6, 1.1 Hz, 2H).

3. Density functional theory calculations

The ground state geometries of **M-Ir-DTE-O** and **M-Ir-DTE-C** were optimized using time-dependent (TD) density-functional theory (DFT) and the B3LYP function with a mixed basis set, where the LANL2DZ basis set was used for Ir and the 6-

31G(d,p) basis set was used for the rest of the atoms.[1, 2] The ground state energy is calculated based on geometrical optimization. The lowest singlet and triplet excited-state geometries were optimized based on the analytical gradient method within the TDDFT framework to obtain the emission energies. All the above calculations were carried out using Gaussian 09.[3]

4. UV-vis and Photoluminescence spectroscopic study

A Lambda 365 UV-vis spectrophotometer was used with 1 cm path-length quartz cuvettes (3 mL). **B-Ir-DTE-C**, **B-Ir-DTE-O**, **M-Ir-DTE-C** and **M-Ir-DTE-O** stock solution (10 mM) were prepared with DMSO and stored in a 298 K refrigerator. In THF, the absorption spectra of **B-Ir-DTE-C** (20 μM), **B-Ir-DTE-O** (20 μM), **M-Ir-DTE-C** (20 μM) and **M-Ir-DTE-O** (20 μM) were determined at room temperature.

An FS5 Spectrofluorometer was used with 1 cm path-length quartz cuvettes (3 mL). Stock solutions of **B-Ir-DTE-C** (20 μM), **B-Ir-DTE-O** (20 μM), **M-Ir-DTE-C** (20 μM) and **M-Ir-DTE-O** (20 μM) in DMSO were prepared before measurements and then diluted suitably with distilled water to the required concentration. Fluorescence spectra were obtained by recording the emission spectra (from 500 to 700 nm) at 25ºC (λ_{ex} = 510 nm).

5. Lipophilicity

The lipophilicity of **M-Ir-DTE-C**, **M-Ir-DTE-O**, **B-Ir-DTE-C** and **B-Ir-DTE-O** was determined by measuring the value of logPo/w, which was referred to as the octanol-water partition coefficients, following a reported procedure.

6. Cell culture

MDA-MB-231, MCF-7, and MCF-10A cells were obtained from the Experimental Animal Center of Sun Yat-Sen University (Guangzhou, China). Cells were routinely cultured in DMEM (Dulbecco's modified Eagle's medium, KeyGEN BioTECH, China) medium containing 10% FBS (fetal bovine serum, Procell, China), 100 μg/mL streptomycin, and 100 U/mL penicillin (Gibco BRL) Cells were cultured in tissue culture flasks in a humidified incubator (BB 150 CO_2 incubator, Thermo Fisher

Scientific, USA), which provided an atmosphere of 5% CO_2 and 95% air at a constant temperature of 37℃. In each experiment, cells treated with vehicle DMSO (2%, v/v) were used as the control group.

7. In vitro cytotoxicity assays

Before the 3-(4, 5-dimethylthiazole-2-yl) -2, 5-diphenyl tetrazole bromide (MTT) assay, cancer cells were inoculated in 96-well plates with a density of 5,000 cells per well and were allowed to adhere for 24 hours. The cells were incubated with different doses of **M-Ir-DTE-C**, **M-Ir-DTE-O**, **B-Ir-DTE-C** and **B-Ir-DTE-O** for 4 h, and then irradiated with light with a wavelength of 420 nm (20 $mW \cdot cm^{-2}$) for 40 minutes. Incubate 37℃ for 24 hours. The cells were incubated with different doses of **M-Ir-DTE-C** and **B-Ir-DTE-C** for 4 hours and then irradiated with 630 nm (0.6 $W \cdot cm^{-2}$) red light for 10 minutes to convert the Closed-loop drug into an open-loop drug. The treated cells were then exposed to 420 nm (20 $mW \cdot cm^{-2}$) for 40 minutes. Alternatively, the treated cells were left in the dark without 420 nm irradiation. After incubation at 310K for 24 h, 20 μL MTT (5 mg/mL) was added to each well and incubated for 4 h. The medium was carefully removed, 150 μL DMSO was added, and the absorbance of purple formaldehyde at 570 nm was measured by LabServ K3 (Thermo Scientific). After shaking the plate for 10 min, the cell survival rate was calculated according to the data of 3 parallel tests. The IC_{50} value is defined as the concentration of the compound required to inhibit cell viability by 50% compared to cells treated with a maximum amount of DMSO (2%), and cell viability is considered to be 100%. The IC_{50} values quoted are the mean ± standard deviation (SD).

8. ROS detection in solution

The total ROS generation was evaluated using a 2', 7′-dichlorodihy-drofluorescein (DCFH) indicator, intracellular ROS can oxidize non-fluorescent DCFH to generate fluorescent DCF. DCFH (2 mM) 15 μL was incorporated into 10 μM corresponding complexes solution in 3 mL PBS. The DCFH fluorescence spectra with the 488 nm excitation were monitored at 525 nm after irradiating by LED area light (420 nm, 20 $mW \cdot cm^{-2}$) at different times.

9. ICP-MS measurement

By measuring the iridium content, the uptake of **M-Ir-DTE-C**, **M-Ir-DTE-O**, **B-Ir-DTE-C** and **B-Ir-DTE-O** by MDA-MB-231 cells without irradiation was determined, and the localization of **M-Ir-DTE-O** by MDA-MB-231 cells with or without irradiation was determined. Simply put, the cells are seeded and incubated overnight under standard growth conditions. Remove the medium and replace it with fresh medium /DMSO (v/v, 98/2) containing the **M-Ir-DTE-O** (10 μM). In terms of lighting, after incubating in the dark for 4 hours, the cells were exposed to LED zone light irradiation (420 nm, 20 $mW \cdot cm^{-2}$, 40 min). After incubation for 6 hours, cells were collected with trypsin, washed with PBS, and counted. Cell microspheres were lysed in RIPA lysis buffer (Beyotime Biotechnology, China) and the nucleus and mitochondrial parts were extracted according to the manufacturer's instructions using a Mitochondria/Nuclei Isolation Kit (Keygen Biotech, China). A fully homogeneous buffer was obtained by digesting concentrated nitric acid (100 μL) at 95℃ for 2 h, hydrogen peroxide (30%, 50 μL) at 95℃ for 1.5 h, and concentrated hydrochloric acid (50 μL) at 95℃ for 1.5 h. Finally, the solution was diluted with MiliQ water to a final volume of 2 ml, and the iridium content of the sample was determined by ICP-MS (X Series 2, Thermo Fisher, USA). The average of the three parallel experimental data is taken as the final result.

10. ROS generation assay

MDA-MB-231 cells were inoculated in a 35 mm confocal culture dish (JET BIOFIL, Canada) and observed under a confocal microscope. The **M-Ir-DTE-O** (2.5,5 μM) and **M-Ir-DTE-C** (5 μM) were cultured at 37℃ in the dark for 4 h, and after 4 h, the cells were exposed to LED irradiation (420 nm, 20 $mW \cdot cm^{-2}$, 40 min). They were stained with 10 μM DCFH-DA, washed twice in serum-free medium, and immediately observed by confocal microscope (A1, Nikon, Japan). The excitation wavelength is 488 nm and the emission wavelength is 530 ± 20 nm.

To perform flow cytometry analysis of cell ROS, MDA-MB-231 cells were inoculated in 6-well plates for 24 h and cultured in the dark with **M-Ir-DTE-O** (2.5,5

μM) and **M-Ir-DTE-C** at 37℃ for 4 h, respectively. After incubation for 4 h, the cells were exposed to LED region light (420 nm, 20 $mW \cdot cm^{-2}$, 40 min). The cells were then collected and washed twice with PBS. Then, ROS probe (10 μM) DCFH-DA was added into the cell samples, incubated for 30 min, washed with culture medium without FBS, and analyzed by flow cytometry. The excitation wavelength is 488 nm and the emission wavelength is 530 ± 30 nm. FlowJo 7.6.1 software was used to analyze the data. Each sample obtained 10,000 cells.

11. Mitochondrial membrane potential (MMP) detection

The MDA-MB-231 cells were inoculated into a 35 mm confocal culture dish (JET BIOFIL, Canada) for confocal microscopic observation. After overnight culture, the cells were treated at 37℃ for 24 h with **M-Ir-DTE-O** (2.5, 5 μM) and **M-Ir-DTE-C** (5 μM), respectively. In the light group, after incubation for 4 h, the cells were exposed to LED region light irradiation (420 nm, 20 $mW \cdot cm^{-2}$, 40 min), and then cultured in the dark for another 20 h. After staining with JC-1 working solution for 30 minutes, the cells were washed with a double incubation buffer and immediately observed with confocal microscopy (A1, Nikon, Japan).

For flow cytometry analysis of MMP, MDA-MB-231 cells were inoculated in 6-well plates for 24 h and incubated with **M-Ir-DTE-O** (2.5, 5 μM) and **M-Ir-DTE-C** (5 μM) at 37℃ for 24 h, respectively. In the light group, after incubation for 4 h, the cells were exposed to LED region light irradiation (420 nm, 20 $mW \cdot cm^{-2}$, 40 min), and then cultured in the dark for another 20 h. The cells were then harvested with 0.25% trypsin and washed three times with PBS. After adding 0.5 mL JC-1 working solution, the solution was incubated at 37℃ for 30 min, then the staining solution was removed, and the cell samples were washed with a double incubation buffer. Flow cytometry analysis was performed using BD Accuri C6 Plus flow cytometry.

12. Detection of intracellular GSH level

Intracellular GSH levels were measured using the GSH and GSSG assay kit (Beyotime). First measure the standard curve of GSSG level and GSH level according

to the manufacturer's instructions. Then, the drug-treated MDA-MB-231 cells were collected and homogenized. The total glutathione level was determined by glutathione reductase and 5, 5-dithiobis (2-nitrobenzoic acid). The sulfhydryl group of GSH reacts with DTNB to produce yellow 5-thio-2-nitrobenzoic acid (TNB) with an absorbance of 405-414 nm. TNB (A405) can be detected by an enzyme-labeled assay and the reducing GSH level is calculated by subtracting the GSSG level from the total GSH (GSH = total GSH − 2 × GSSG).

13. Transmission electron microscopy assay

1×10^6 MDA-MB-231 cells were cultured in a 10 cm petri dish and treated separately at 37℃ for 24 h with **M-Ir-DTE-O** (10 μM) and **M-Ir-DTE-C** (10 μM), respectively. In the light group, after incubation for 4 h, the cells were exposed to LED region light irradiation (420 nm, 20 $mW\cdot cm^{-2}$, 40 min), and then cultured in the dark for another 20 h. Each sample was harvested in a 1.5 mL microcentrifuge tube. The cells were then washed twice with cold PBS and fixed overnight with a cold fixative (2.5% glutaraldehyde phosphate buffer) at 4°C. After that, the cell samples were dehydrated, fixed, embedded, sliced, and then observed by transmission electron microscopy (Hitachi, Japan).

14. Cell death mechanism

The inhibitors were z-VAD-fmk (50 μM), 3-methyladenine (200 μM), chloroquine (50 μM) or Necrostatin 1 (50 μM), and pre-incubated for 1 h. **M-Ir-DTE-O** (10 μM) was incubated directly for 4 h, respectively. After incubation for 4 h, the supernatant was replaced with the corresponding fresh medium, and the cells were irradiated at 420 nm, 20 $mW\cdot cm^{-2}$ for 40 min. The unirradiated cells are replaced with the corresponding fresh medium and stored in the dark. The survival rate of MDA-MB-231 cells was determined by the MTT method after 20 h co-culture with or without corresponding inhibitors in a fresh medium.

15. Cell cycle analysis

MDA-MB-231 cells were inoculated in 6-well cell plates, incubated overnight in 5% CO_2 and 95% air at 37°C, and the required concentrations of **M-Ir-DTE-O** and **M-Ir-DTE-C** were added, respectively. The cells were preincubated with **M-Ir-DTE-O** and **M-Ir-DTE-C** at 37°C for 24 h. In the light group, after incubation for 4 h, the cells were exposed to LED region light irradiation (420 nm, 20 $mW \cdot cm^{-2}$, 40 min), and then cultured in the dark for another 20 h. The cells were collected by centrifuge and washed twice with PBS. The cells were then immobilized overnight with 70% ethanol at 4°C. The fixed mixture was washed with cold PBS twice, pre-treated with RNase A (100 μg/mL) for 10 min, incubated with 500 μL propyl iodide (PI) for 30 min, washed with PBS twice, and cell samples were detected by flow cytometry and analyzed by BD Accuri C6 Plus flow cytometry.

16. Western Blot Analysis

MDA-MB-231 cells were cultured in a 10 cm petri dish and treated separately with **M-Ir-DTE-O** (5,10 μM) and **M-Ir-DTE-C** (10 μM) at 37°C for 24 h. In the light group, after incubation for 4 h, the cells were exposed to LED region light irradiation (420 nm, 20 $mW \cdot cm^{-2}$, 40 min), and then cultured in the dark for another 20 h. The cells were harvested in a 1.5 mL microcentrifuge tube for each sample. Then the proteins were extracted in ice-cold lysis buffer (Kegan Biotech), and the cell lysates were incubated on ice for 20 min and centrifuged at 12,000 g for 20 min at 4°C. Protein concentrations were measured using protein assay reagents, and equal amounts of protein per lane were separated on SDS-PAGE gel and transferred to a PVDF membrane (Millipore). The membranes were incubated with specific antibodies at 4°C overnight and then incubated with a horseradish peroxidase coupled secondary antibody for 1 h at room temperature. After washing with PBST, the signal was visualized by Tanon High-sig ECL Western Blotting Substrate and Tanon 5200 Multi.

17. Immunofluorescence assay

MDA-MB-231 cells were seeded into 35 mm confocal dishes (JET BIOFIL, Canada) for confocal microscopy. After cultured overnight, the cells were treated with compound **M-Ir-DTE-O** (5 μM) and compound **M-Ir-DTE-C** (5 μM) for 24 h at 37°C.

In the lighting group, after 4 h incubation, cells were exposed to LED area light irradiation (420 nm, 20 $mW \cdot cm^{-2}$, 40 min) and then cultured for an additional 20 h in the dark. Then MDA-MB-231 cells were fixed with 4% paraformaldehyde /PBS for 30 min, washed three times with PBS, stained with anti-LC3B antibody (1:100) and γ-H2AX antibody (1:500) at room temperature for 1 h, and coupled with (fluorescein (FITC) to pure goat anti-rabbit lgG (h +L). 1:50) for detection. Cells were reverted with 300 nM DAPI for 10 minutes and immediately observed with confocal microscopy (A1, Nikon, Japan)

18. Intracellular ATP detection test

MDA-MB-231 cells were inoculated in 6-well petri dishes with a density of 10^5 cells /mL and cultured until the cells grew to 60-70%. Then replace the media with one containing **M-Ir-DTE-O** and **M-Ir-DTE-C**, respectively. ATP concentration was measured after incubation for 24 h. In the light group, after incubation for 4 h, the cells were exposed to LED region light irradiation (420 nm, 20 $mW \cdot cm^{-2}$, 40 min), and then cultured in the dark for another 20 h. Wash with PBS. The cells were added three times, and then 100 μL lysis reagent was added. The cell lysate was centrifuged at 12000 g and 4℃ for 5 min. Cell culture was performed according to the instructions on a 96-well whiteboard (beyotime Biotechnology). The cell lysate supernatant was added to the ATP working solution (100 μL, Beyotime Biotechnology). The luminescence of each hole is immediately detected by a multifunctional microplate reader (Thermo Scientific Varioskan flash).

19. Apoptosis detection

The ability of **M-Ir-DTE-O** and **M-Ir-DTE-C** to induce apoptosis of MDA-MB-231 cell line was determined by flow cytometry. The cells were seeded overnight in 6-well plates, and then the **M-Ir-DTE-O** and **M-Ir-DTE-C** complexes were added at specified concentrations, respectively. After incubation for 24 h, the cells were harvested, washed twice with PBS, and re-suspended in 500 μL binding buffer. The suspended cells were stained with 5 μL annexin V-FITC and 10 μL PI in the dark at room temperature for 20 min. The cells were analyzed by flow cytometry (BD Accuri

C6 Plus). At 488 nm excitation, emission spectra were collected at 530 ± 20 nm (annexin V) and 620 ± 20 nm (PI), respectively.

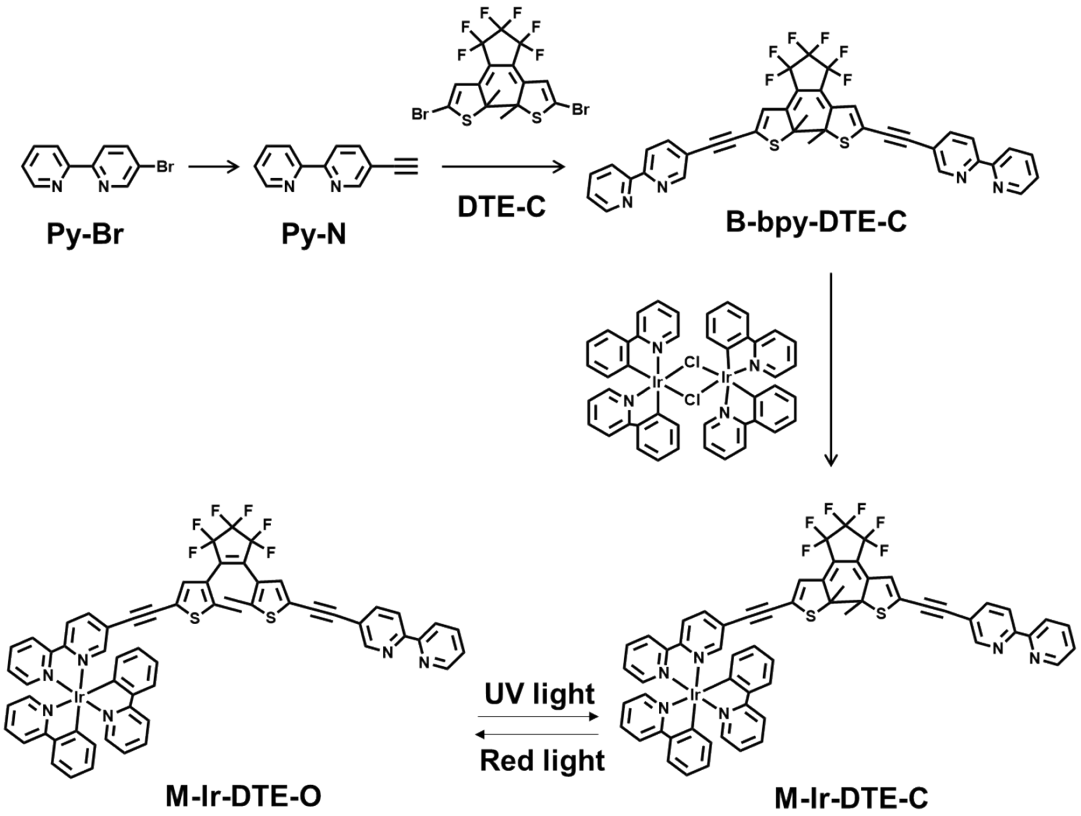
20. Three-dimensional multicellular tumor spheroids (3D MCTSs) culture and related study

MDA-MB-231 cells were divided into the prepared and ultra-low adhesion 96-well round bottom microporous plates (Corning) with 2500 cells per well. The MDA-MB-231 MCTSs with a diameter of 500 μM were formed after 24 h. For 3D models, the MDA-MB-231 MCTSs were divided into different groups and cultured in media containing **M-Ir-DTE-O** (40 μM) and **M-Ir-DTE-C** (40 μM) respectively. MCTSs were irradiated with a laser of (420 nm, 20 $mW \cdot cm^{-2}$, 40 min) after 4 h incubation every two days. The growth medium was changed every two days. Three rounds of irradiation were performed in this experiment.

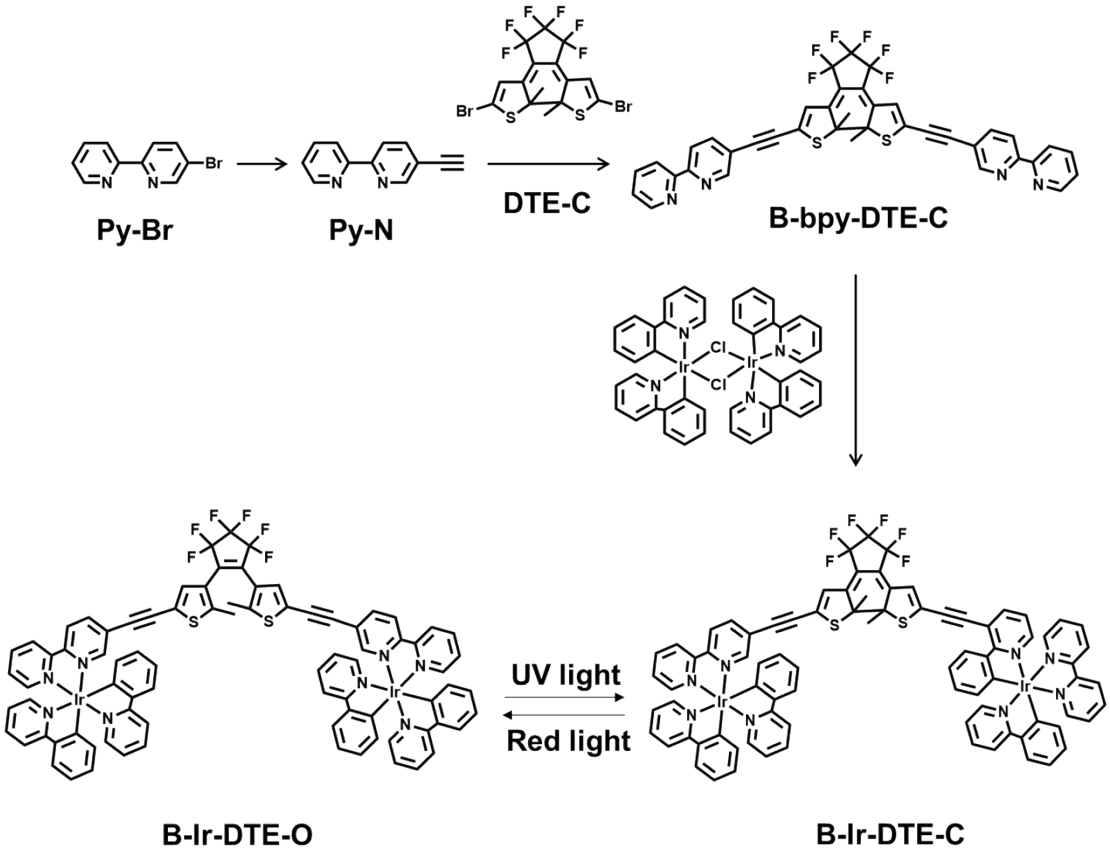
The spheroid growth was monitored using a live cell phase contrast microscope (Axio Observer, Zeiss). Then spheroids were washed twice with PBS, stained with Calcein AM/PI following the manufacturer's instructions (Beyotime, China), and fixed in 4% paraformaldehyde. Spheroids were placed in a glass-bottom dish and imaged at different depths (z-stacking) with a confocal scanning microscopy system (Calcein AM: λ_{ex} = 488 nm and λ_{em} range 500-550 nm, PI: λ_{ex} = 561 nm and λ_{em} range 570-620 nm).

21. Statistical analysis

Data are given as Mean ± SD. Statistical significance was performed using a two-tailed Student's t-test. Statistical significance was set at $*p < 0.05$, and extreme significance was set at $**p < 0.01$, and $***p < 0.001$, $****p < 0.0001$.



Scheme S1 Synthetic routine of complex **M-Ir-DTE-O** and **M-Ir-DTE-C**.



Scheme S2 Synthetic routine of complex **B-Ir-DTE-O** and **B-Ir-DTE-C**.

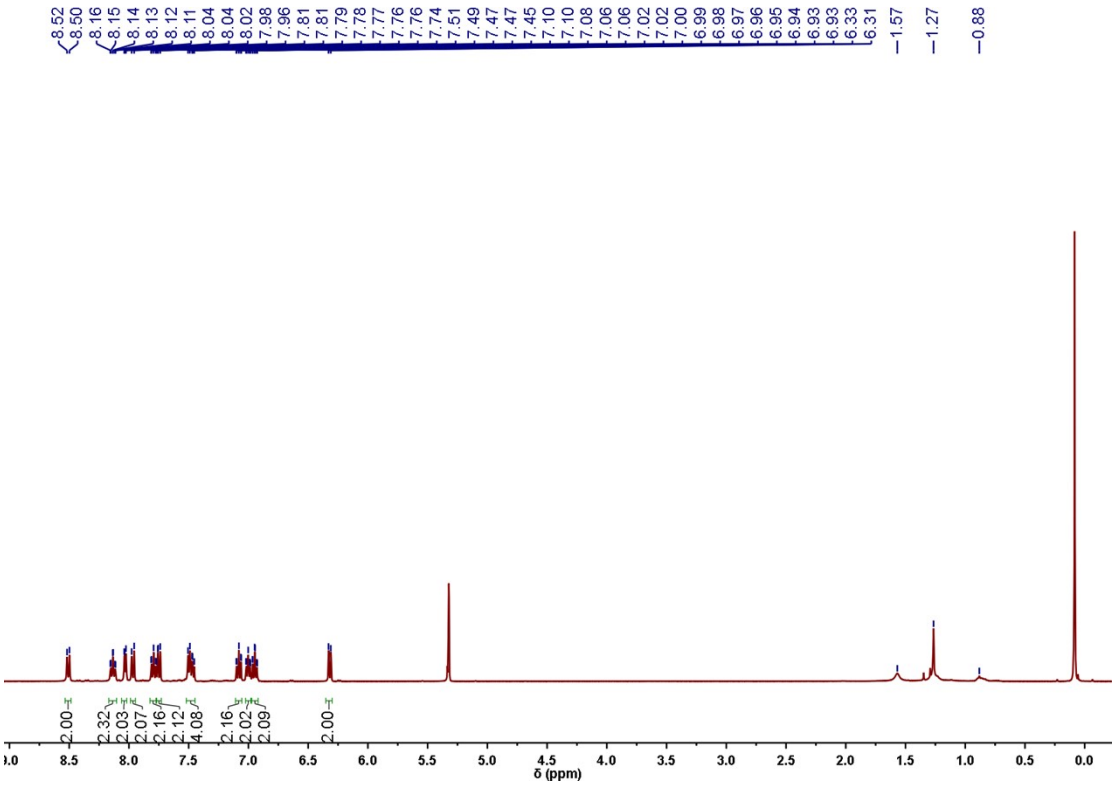


Fig. S1 ^{1}H NMR spectrum (400 MHz, d_2- CD_2Cl_2) of complex **Ir-0**.

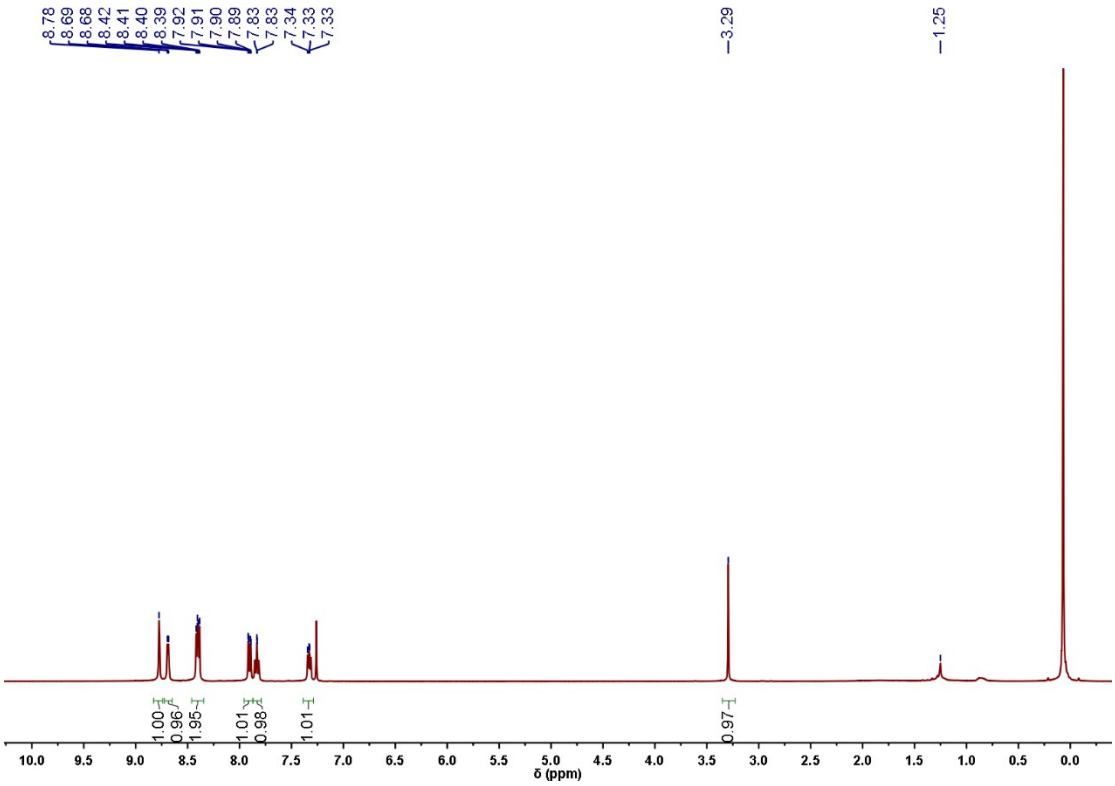


Fig. S2 ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of ligand **Py-N**.

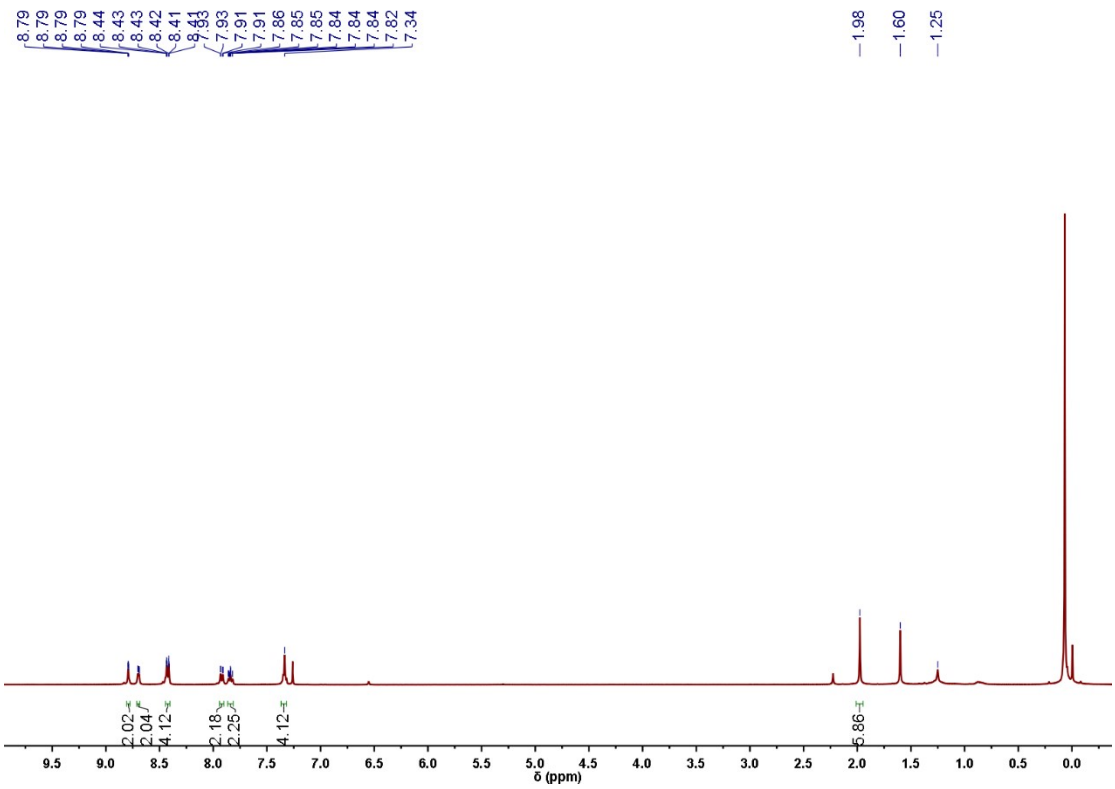


Fig. S3 ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of Ligand **B-pby-DTE-O**.

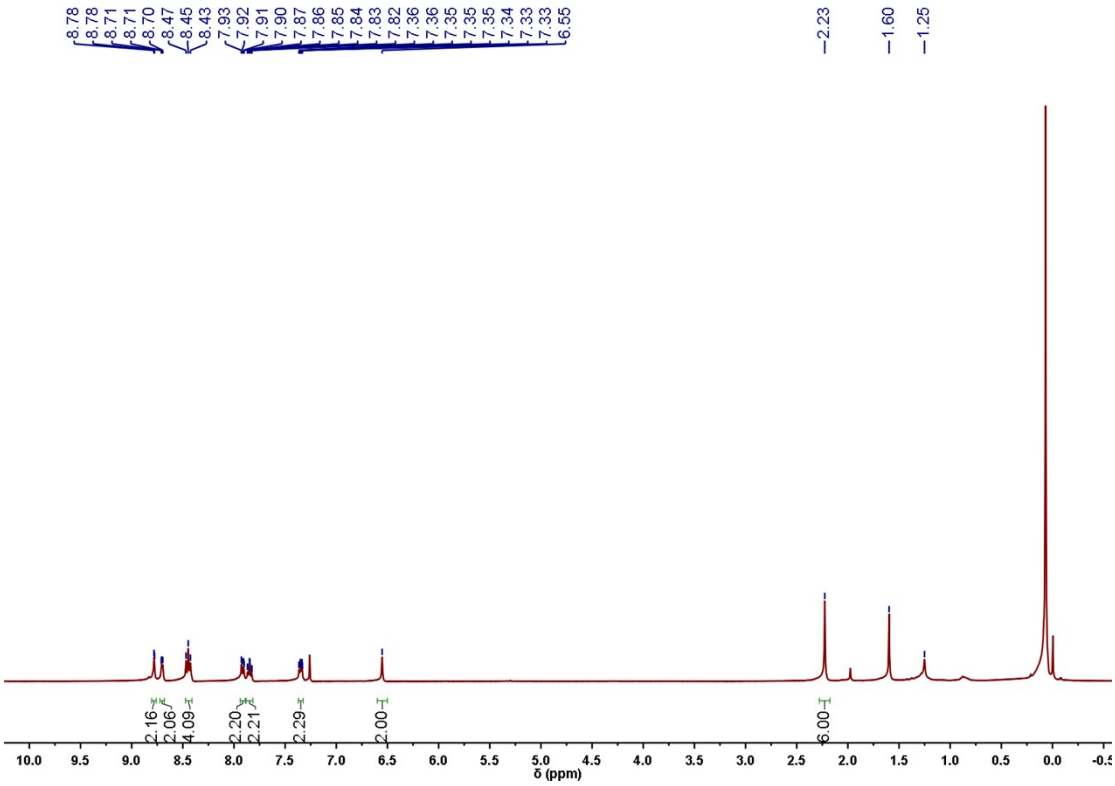


Fig. S4 ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of Ligand **B-bpy-DTE-C**.

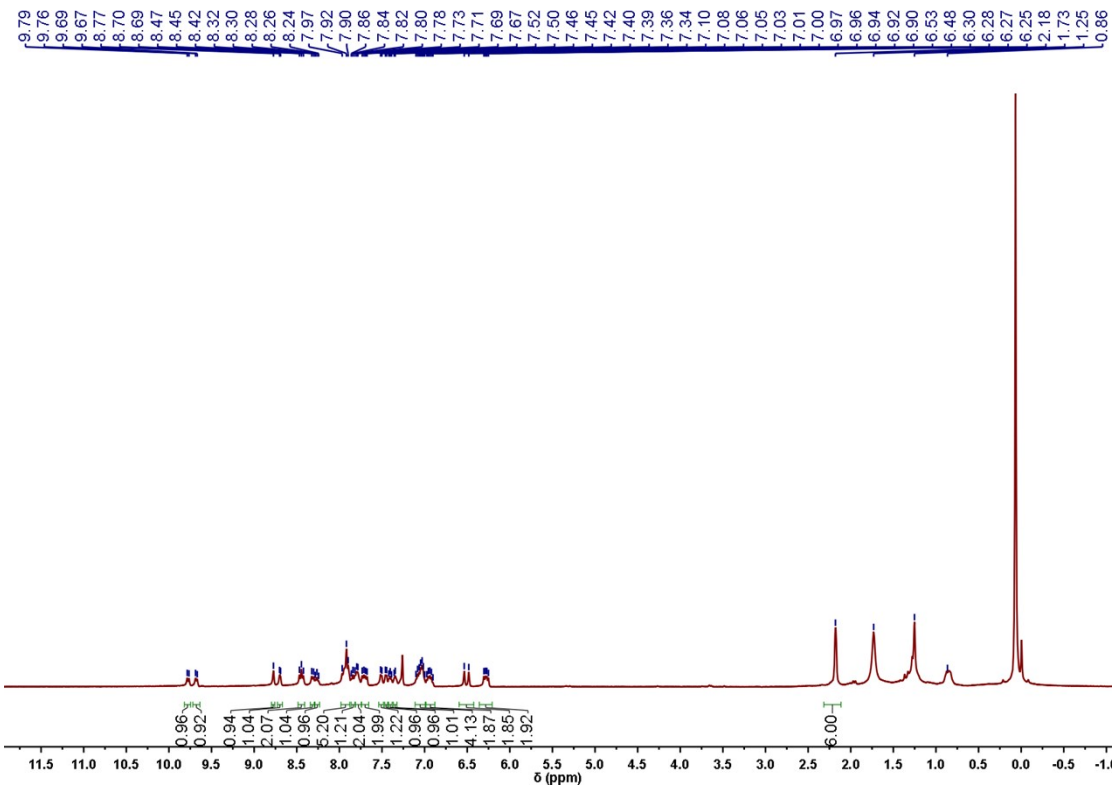


Fig. S5 ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of complex **M-Ir-DTE-C**.

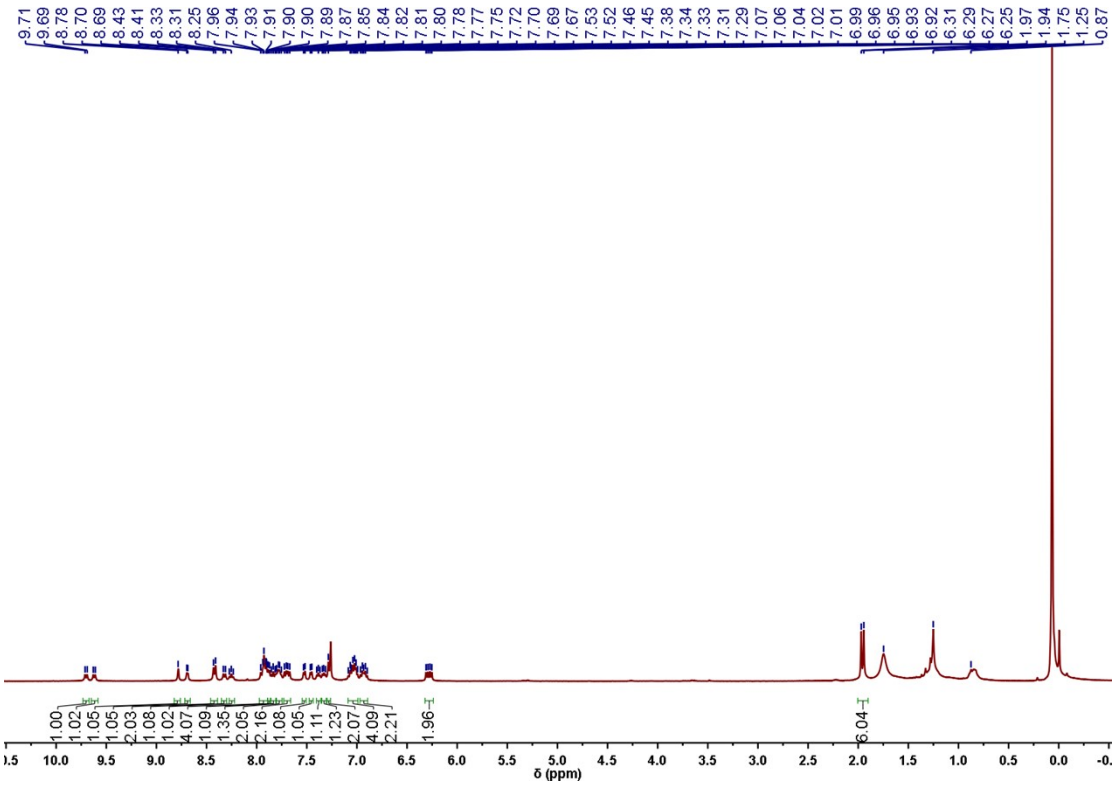


Fig. S6 ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of complex **M-Ir-DTE-O**.

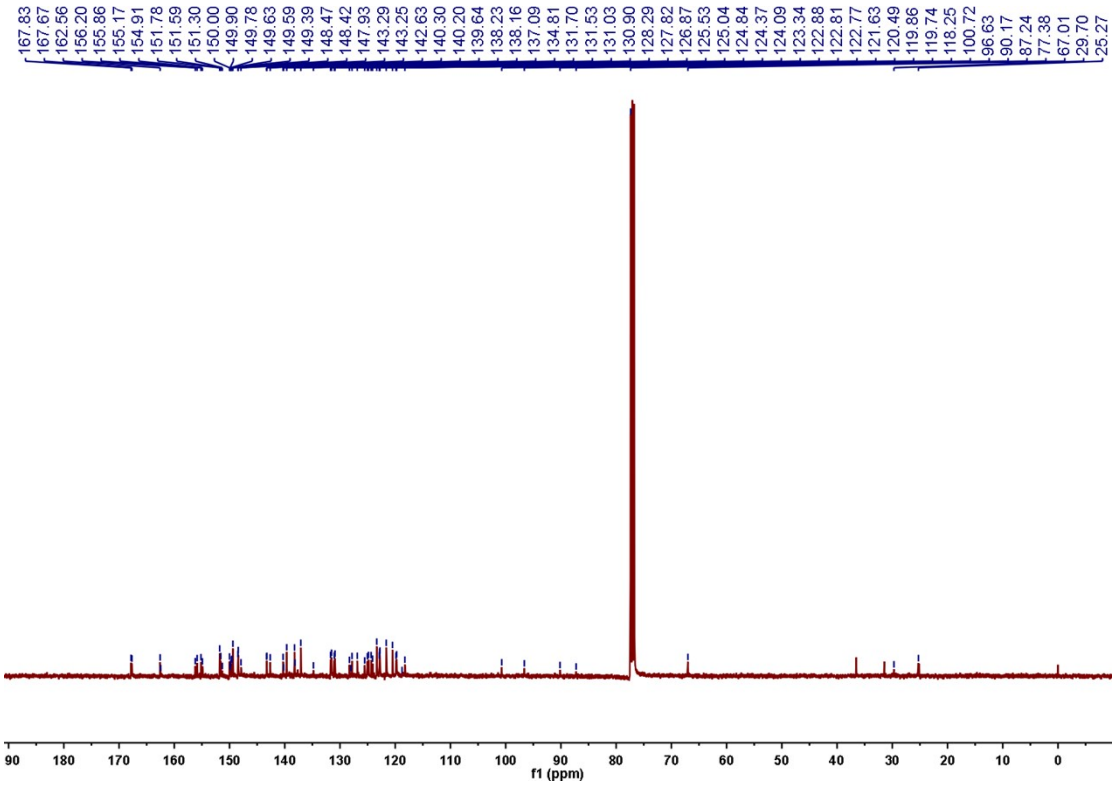


Fig. S7 ^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **M-Ir-DTE-C**.

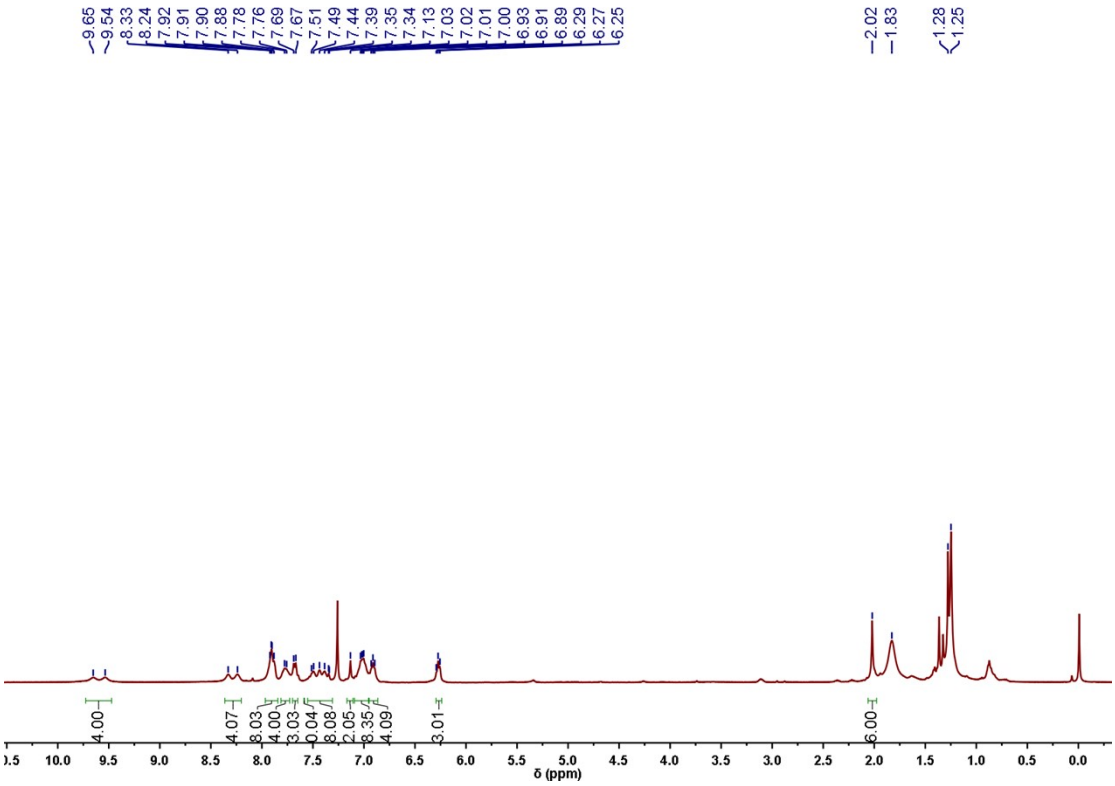


Fig. S8 ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of complex **B-Ir-DTE-O**.

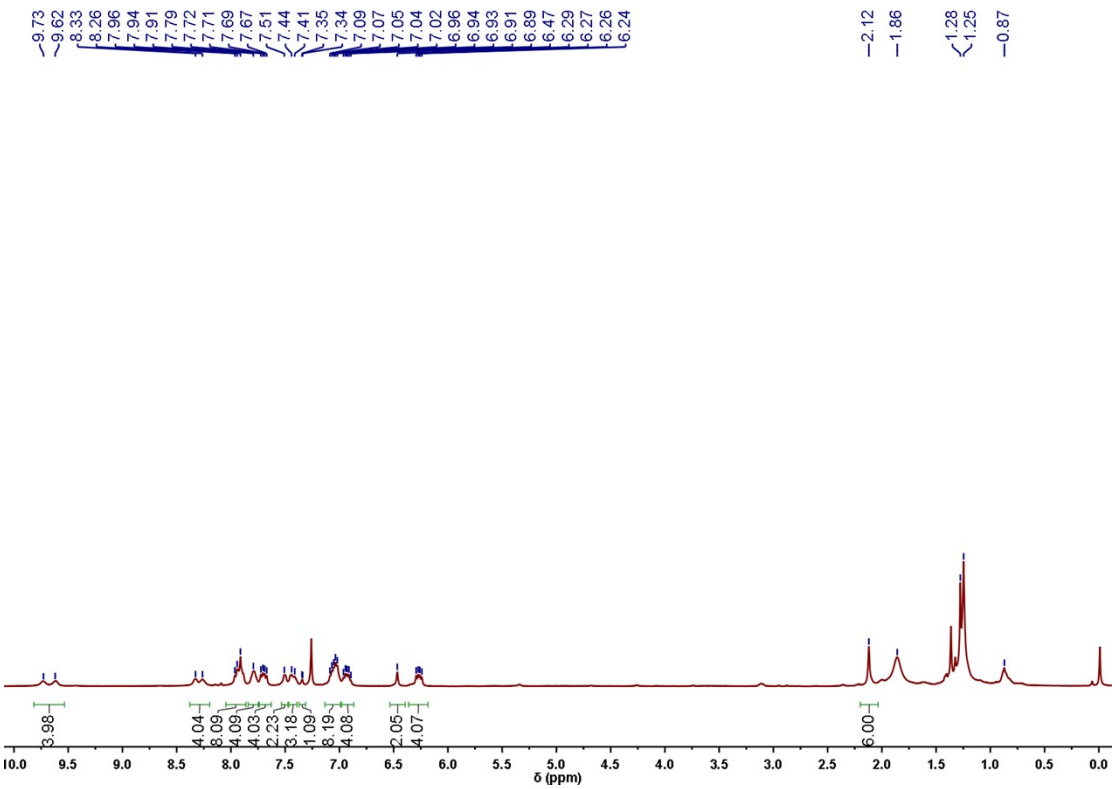


Fig. S9 ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of complex **B-Ir-DTE-C**.

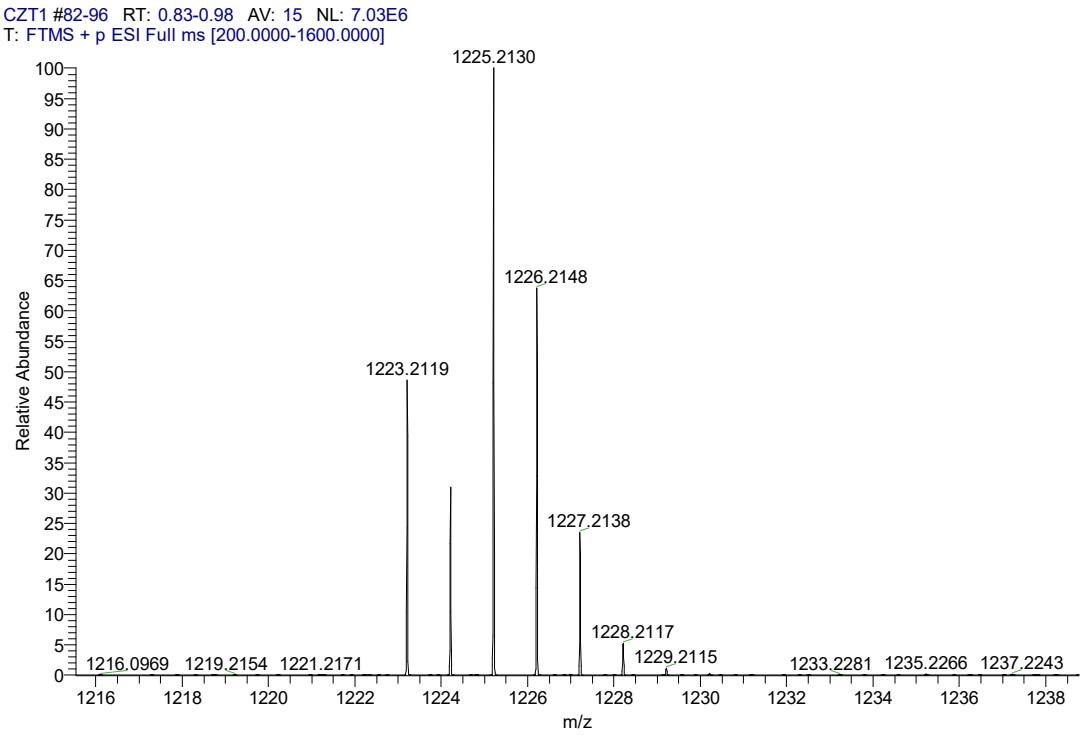


Fig. S10 HR-ESI-MS spectrum (CH_3OH) of **M-Ir-DTE-C/O**.

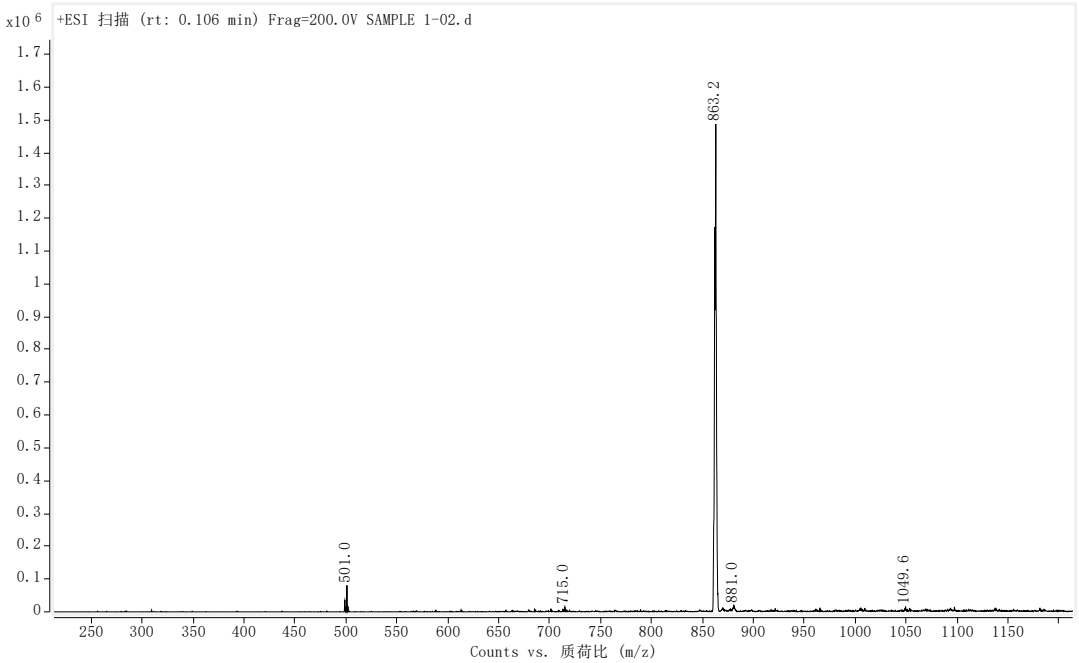


Fig. S11 ESI-MS spectrum (CH_3OH) of **B-Ir-DTE-C/O**.

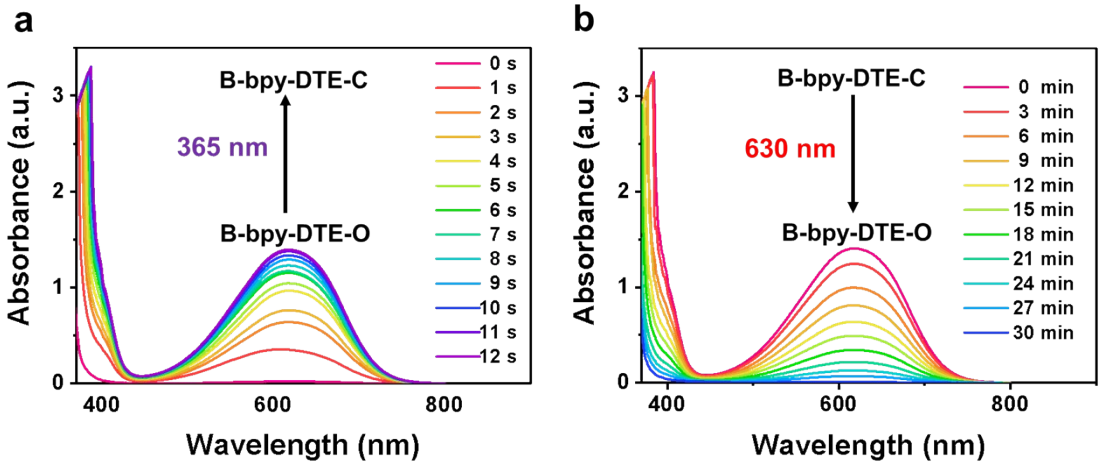


Fig. S12 Absorption spectra of complex **B-bpy-DTE-O** (a) in THF at different times under UV light (365 nm, 0.1 W·cm^{-2}) irradiation and of complex **B-bpy-DTE-C** (b) at different times in THF under red light (630 nm, 0.6 W·cm^{-2}) irradiation.

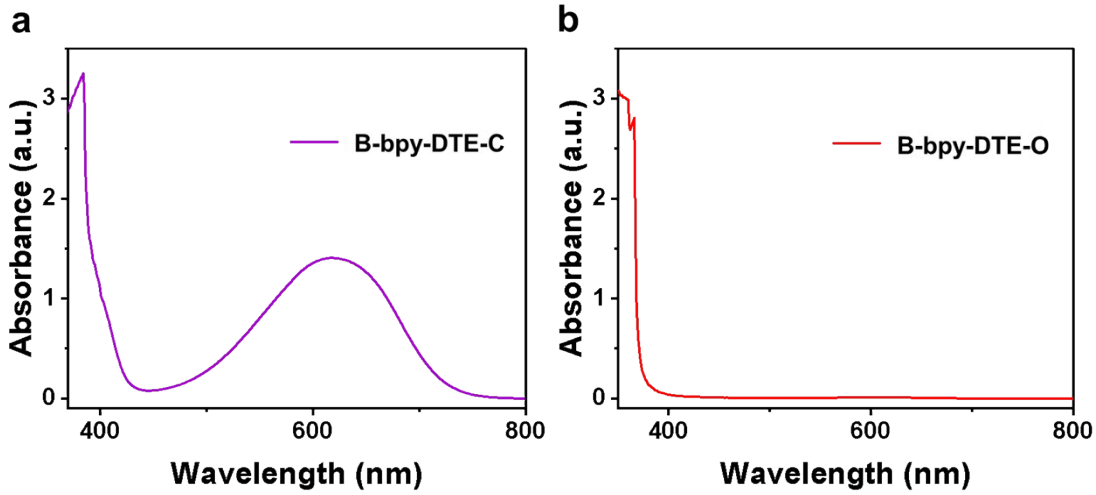


Fig. S13 UV-vis spectra of complexes **B-bpy-DTE-C** (a) and **B-bpy-DTE-O** (b) in THF solution.

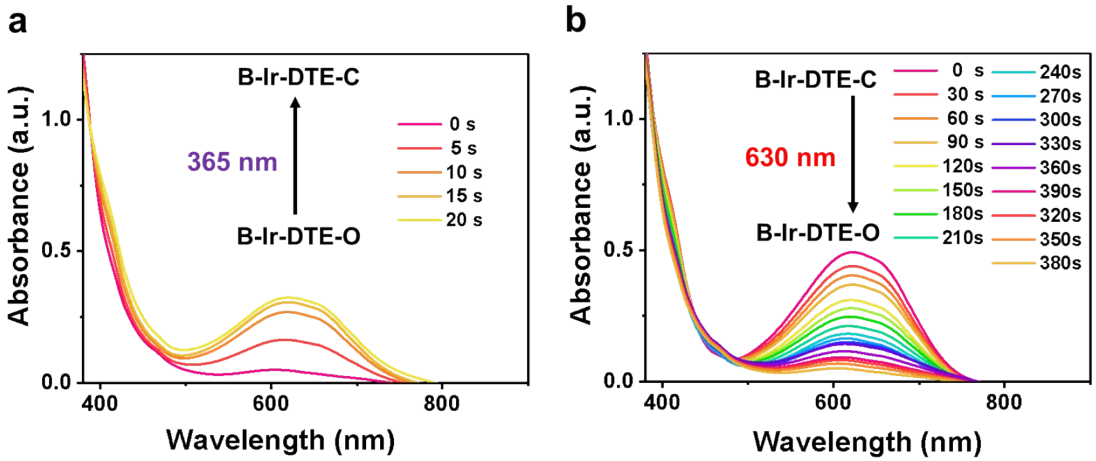


Fig. S14 Absorption spectra of complex **B-Ir-DTE-O** (a) in THF at different times under UV light (365 nm, 0.1 W·cm^{-2}) irradiation and of complex **B-Ir-DTE-C** (b) at different times in THF under red light (630 nm, 0.6 W·cm^{-2}) irradiation.

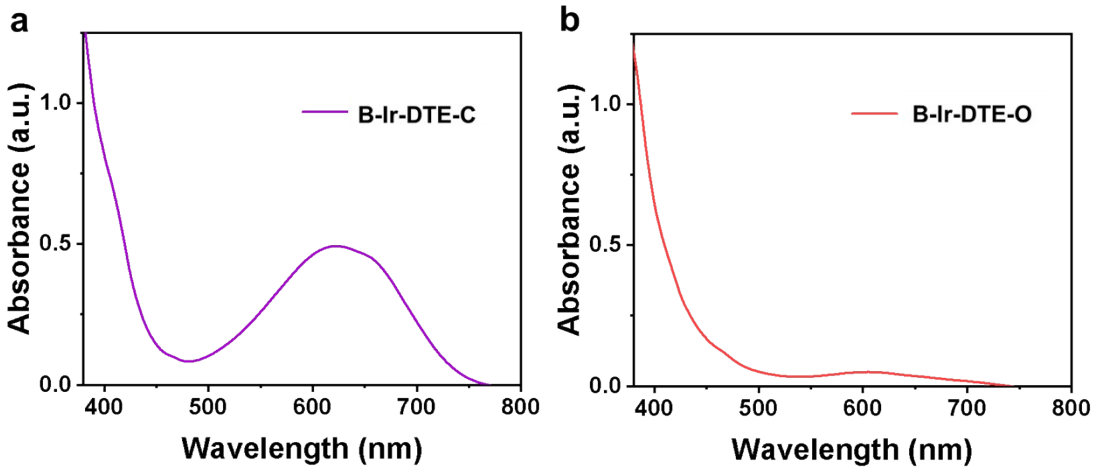


Fig. S15 UV-vis spectra of complexes **B-Ir-DTE-C** (a) and **B-Ir-DTE-O** (b) in THF solution.

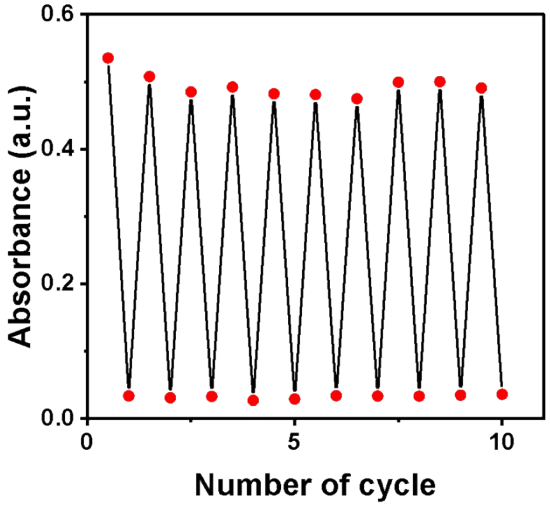


Fig. S16 The absorption intensity at 630 nm of the **B-Ir-DTE** during ten circles of red light (630 nm, 0.6 W·cm^{-2})/UV light (365 nm, 0.1 W·cm^{-2}) irradiation processes.

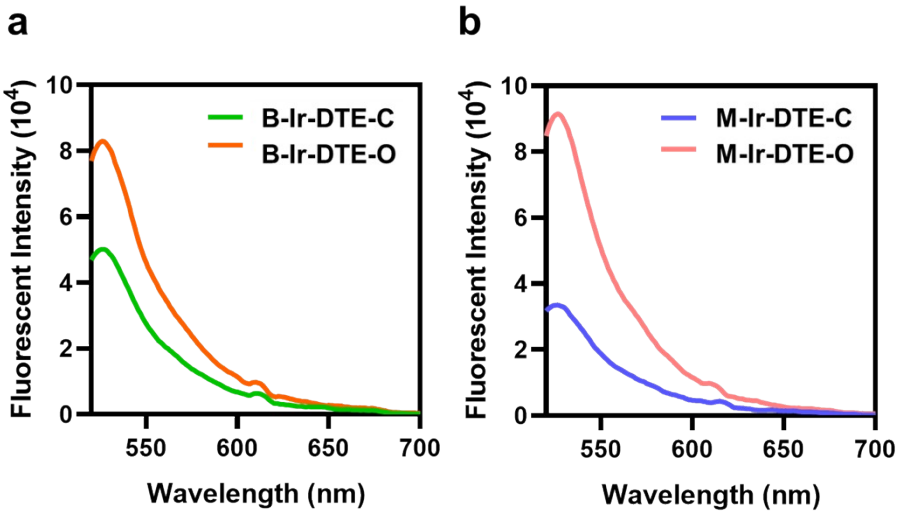


Fig. S17 Fluorescence spectra (λ_{ex} = 500 nm) of complexes **B-Ir-DTE-C** (20 μM) (a), **B-Ir-DTE-O** (20 μM) (a), **M-Ir-DTE-C** (20 μM) (b), **M-Ir-DTE-O** (20 μM) (b).

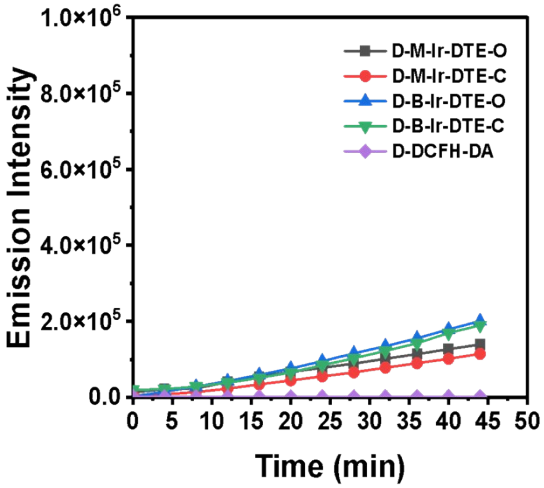


Fig. S18 Time-course plots of DCF fluorescence enhancement in the presence of different compounds under light irradiation (420 nm, 20 $mW \cdot cm^{-2}$).

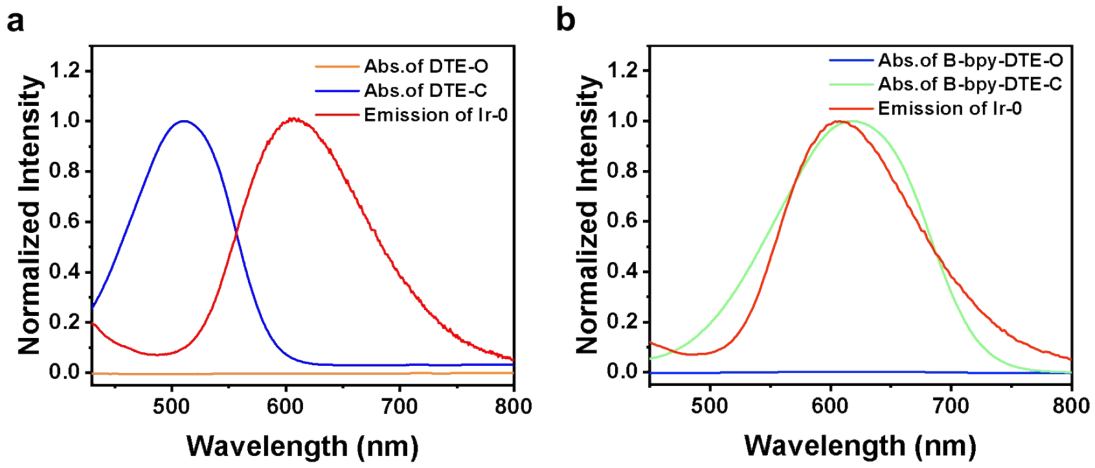


Fig. S19 Normalized absorption spectra of complexes **DTE-O** and **DTE-C** and emission spectra of **Ir-0** (a), Normalized absorption spectra of complexes **B-bpy-DTE-O** and **B-bpy-DTE-C** and emission spectra of **Ir-0** (b). (λ_{ex} = 350 nm)

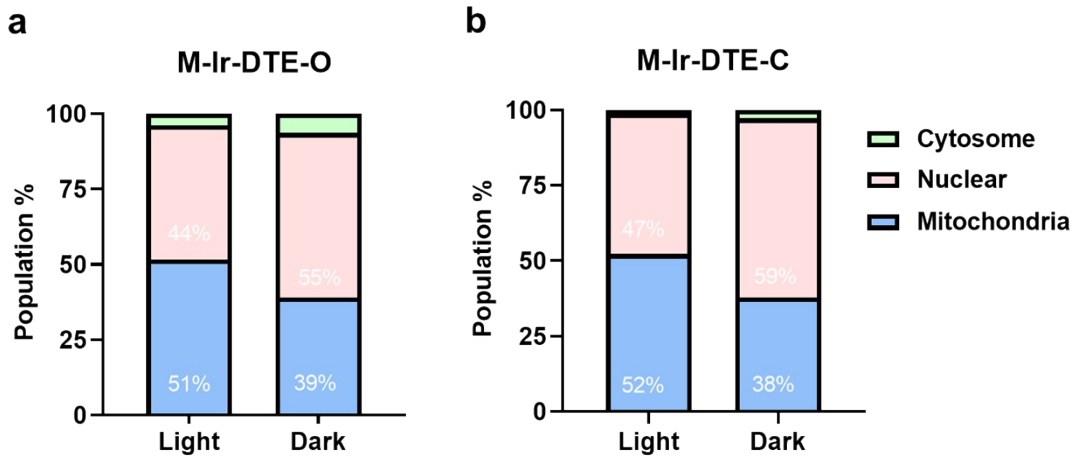


Fig. S20 Intracellular distribution of Ir (ng/10^6 cells) in MDA-MB-231 cells after incubation with **M-Ir-DTE-O** (a) and **M-Ir-DTE-C** (b) (10 μM) for 6 h at 37°C. Mitochondria and nuclei were isolated using the Mitochondria/Nuclei Isolation Kit.

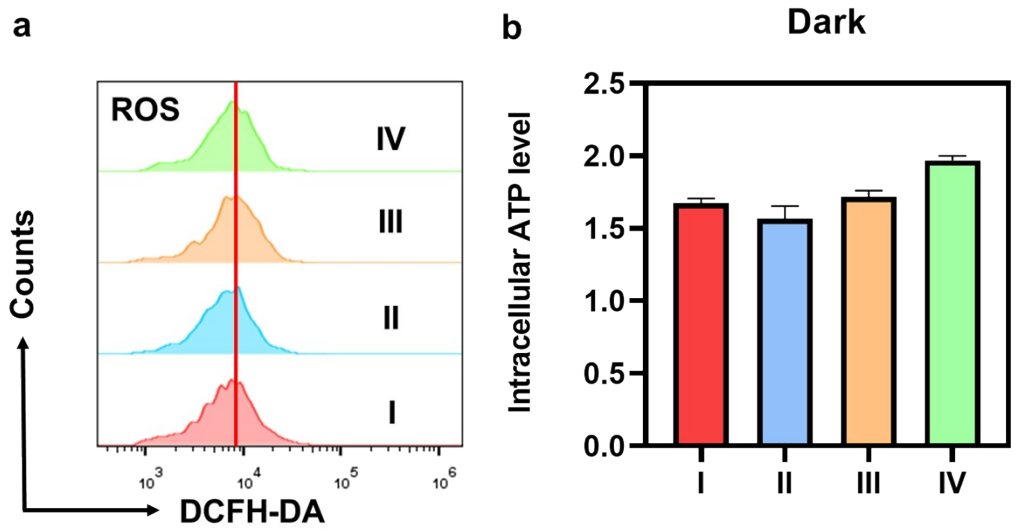


Fig. S21 (a) ROS production determined by flow cytometry after treating MDA-MB-231 cells with different conditions under dark. (b) Intracellular ATP level in MDA-MB-231 cells under dark. I: Ctrl, II: **M-Ir-DTE-O** 5 μM, III: **M-Ir-DTE-O** 10 μM, IV: **M-Ir-DTE-C** 10 μM.

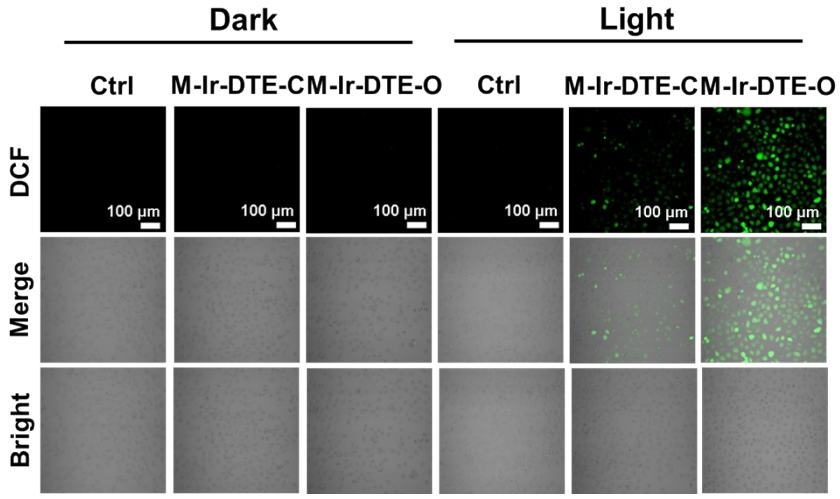


Fig. S22 ROS production in MDA-MB-231 cells was detected by CLSM.

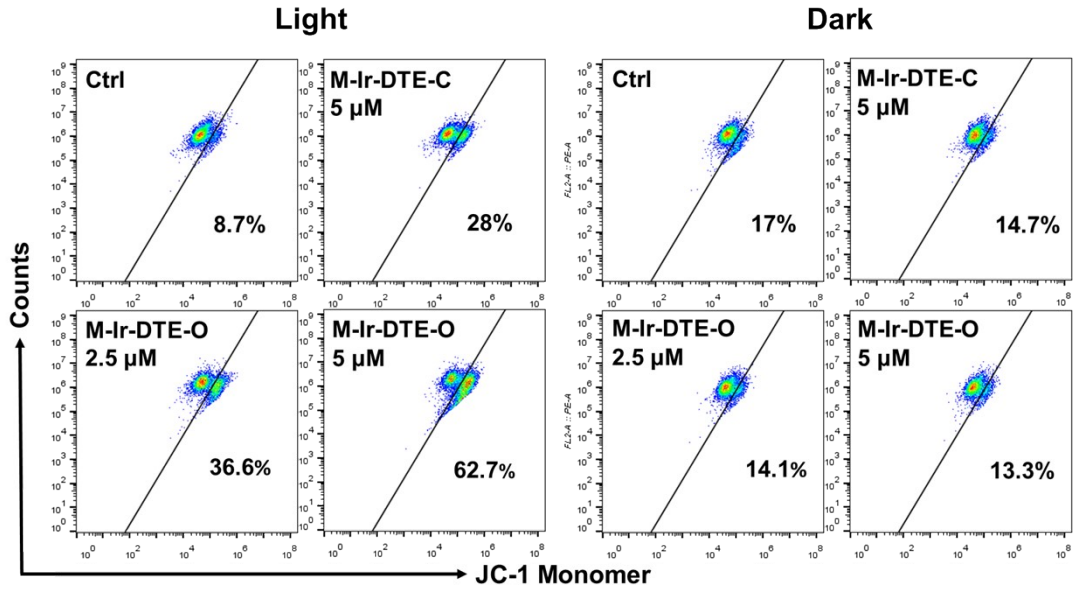


Fig. S23 The changes of MMP in MDA-MB-231 cells treated with different complexes were detected by flow cytometry.

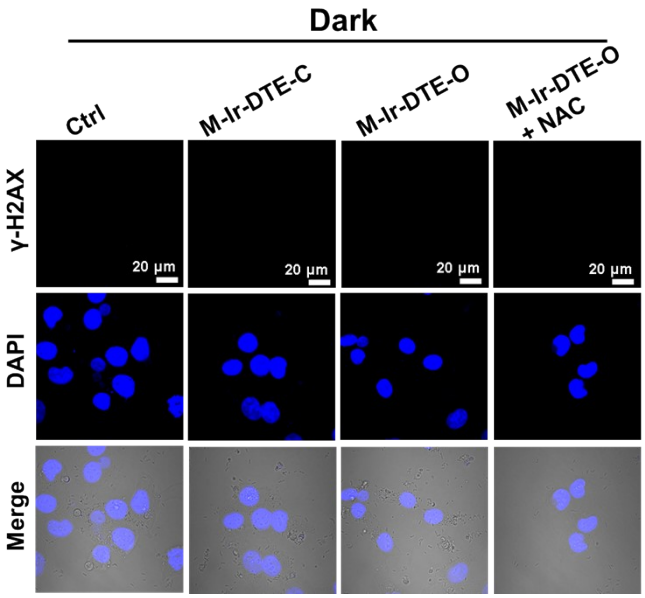


Fig. S24 The changes in the nucleus were observed by CLSM.

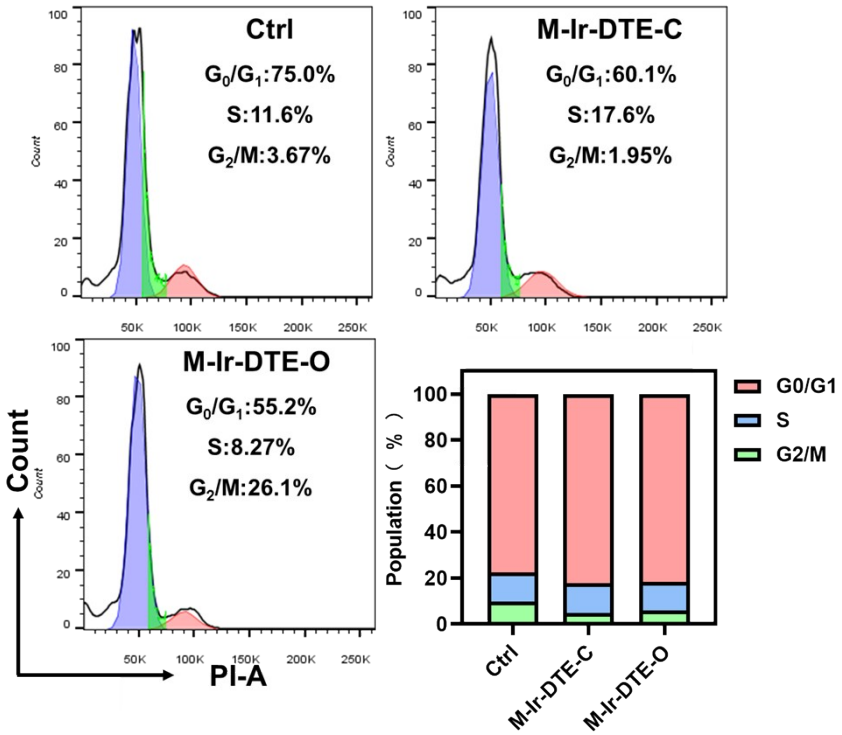


Fig. S25 The cell arrest distribution and histogram of cell arrest statistical results of different complexes on MDA-MB-231 cells by flow cytometry under dark.

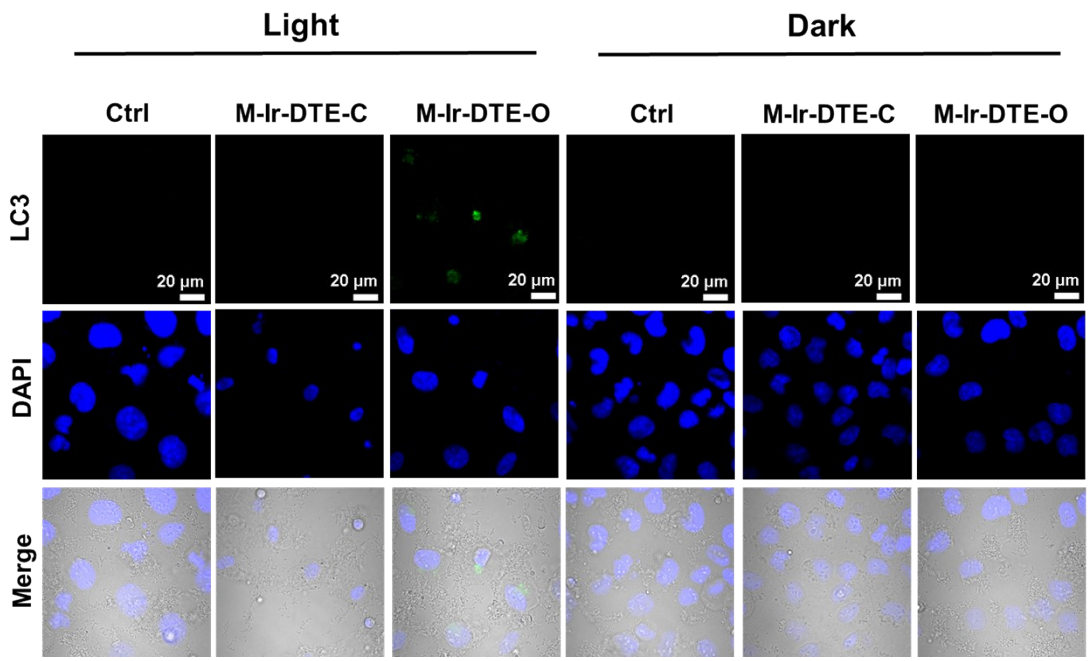


Fig. S26 Immunofluorescence assay of LC3 expression in MDA-MB-231 cells with different complexes.

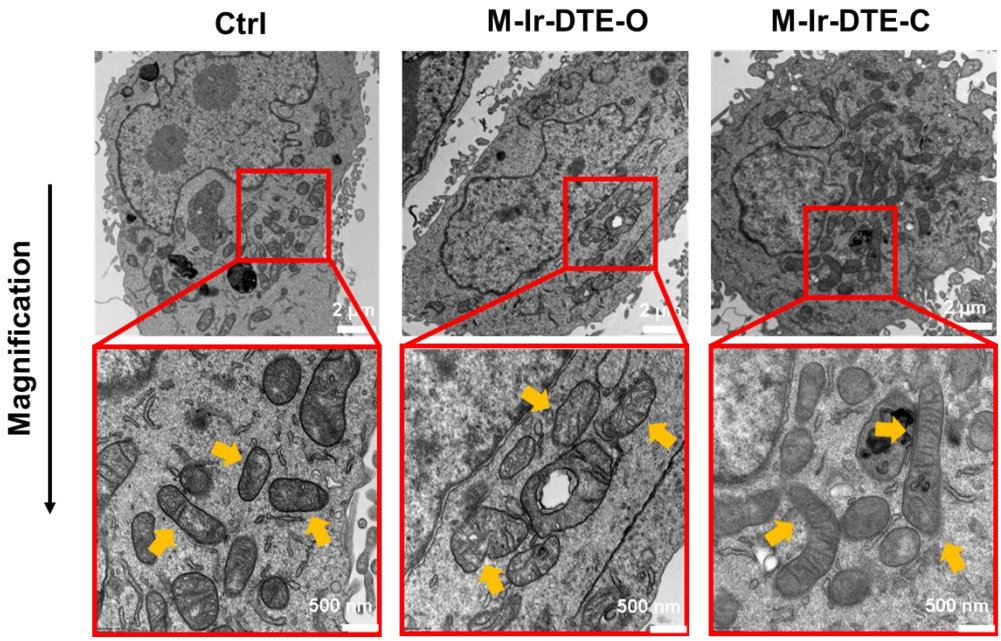


Fig. S27 Transmission electron microscopy (TEM) imaging of MDA-MB-231 cells incubated with different complexes under dark for 24 h. Orange arrow: Normal mitochondria.

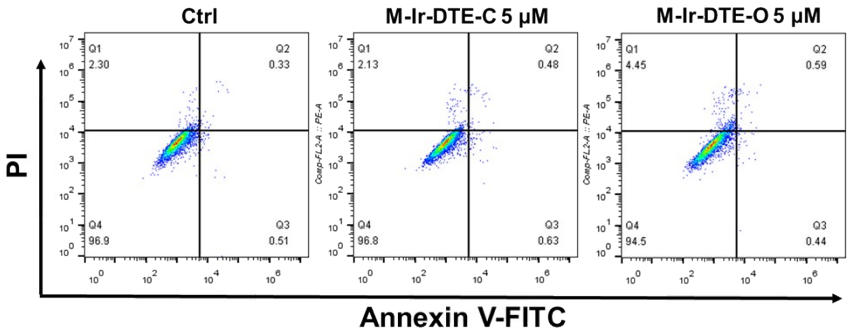


Fig. S28 Detection of apoptosis and necrosis in MDA-MB-231 cells induced by different complexes using flow cytometry under dark.

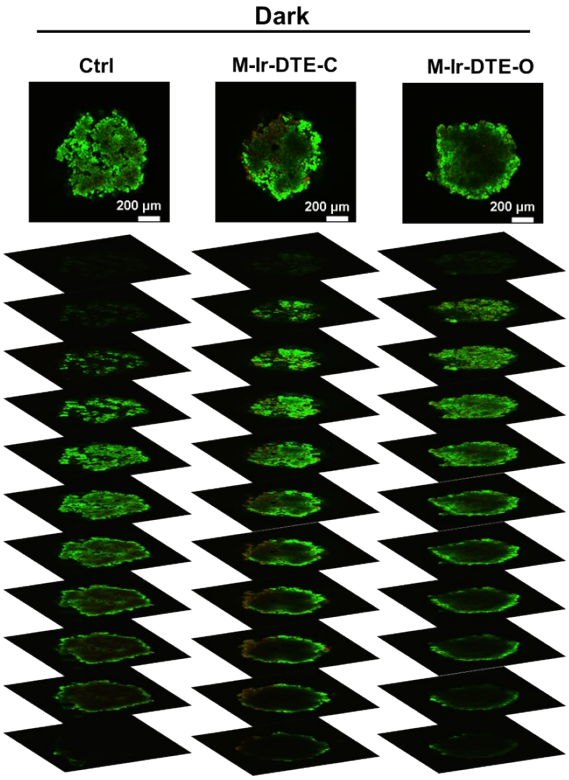


Fig. S29 Confocal images of living/dead cells after 5 days of tumor spheres treated by **M-Ir-DTE-C** (50 μM) and **M-Ir-DTE-O** (50 μM) under dark were stained by Calcein AM/PI and taken under confocal microscope.

Table S1 IC_{50} values (μM) of indicated complexes towards MCF-7, MDA-MB-231 and MCF-10A cell lines after 24 h treatment. Data are shown as mean ± standard deviation (S.D.; n=3).

Complexes	MCF-7		MDA-MB-231		MCF-10A
	Light	Dark	Light	Dark	Dark
M-Ir-DTE-C	20.1±0.1	>40	34.6±0.4	>40	>40
M-Ir-DTE-O	9.3±0.1	>40	5.4±0.2	>40	>40
Convert M-Ir-DTE-O	11.1±0.6	>40	6.0±0.5	>40	>40
CDDP	22.7±0.6	23.7±0.4	38.9±0.4	>40	>40
B-Ir-DTE-O	16.3±0.1	>40	14.6±0.3	>40	>40
B-Ir-DTE-C	>40	>40	>40	>40	>40

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2. T. Clark, J. Chandrasekhar, G. W. Spitznagel and P. V. R. Schleyer, *J. Compu. Chem.*, 1983, **4**, 294-301.

3. M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery, Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, O. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision A.01, Wallingford CT: Gaussian Inc.; 2009.