Supporting Information

for

A hetero-bimetallic Ru(II)-Ir(III) photosensitizer for effective cancer photodynamic therapy under hypoxia

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Content

Experimental Section

Materials and Instruments

$RuCl_3 \cdot nH_2O$ (Aladdin), $IrCl_3 \cdot nH_2O$ (Macklin), 2-phenylpyridine (ppy, Energy), 9,10-phenanthrenedione (Energy), 9,10-diphenyl-phenanthrene (dip, Energy), cisplatin (Merck), 4-amino-2,2,6,6-tetramethylpiperidine (TEMP, Merck), 1,3-diphenylisobenzofuran (DPBF, Merck), dihydroethidium (DHE, Merck), hydroxyphenyl fluorescein (HPF, Merck), 3,3',5,5'-tetramethyl-benzidine (TMB, Aladdin), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Beyotime), 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA, Beyotime), 5-tert-butoxycarbonyl-5-methyl-1-pyrroline-N-oxide (BMPO, Dojindo), 3-methyladenine (3-MA, Merck), necrostatin-1 (Nec-1, Merck), leupeptin (Merck), nucleus extraction kit (Sangon Biotech), mitochondria extraction kit (Sangon Biotech), lysosome extraction kit (GenMed Scientific), subcellular protein fractionation kit (for cytoplasm extraction, ThermoFisher), Annexin V-FITC apoptosis detection kit (AV/PI, BD), calcein/PI cell viability/cytotoxicity assay kit (Ca-AM/PI, Beyotime), Caspase Inhibitor Z-VAD-FMK (Promega), and Caspase-Glo® 3/7 assay systems (Promega) were used as received, without any further purification.

Electrospray ionization mass spectra (ESI-MS) were recorded on an LCQ system (Finnigan MAT, USA). ^1H NMR spectra were determined by using a Bruker AVANCE III 400 MHz NMR spectrometer at room temperature. The EPR measurements were carried out with a Bruker Model EMXPlus-10/12 spectrometer at 298 K. UV-vis spectra were obtained by using a Perkin Elmer Lambda 950 spectrophotometer. Emission spectra were recorded on a PerkinElmer LS 55 fluorescence spectrometer at room temperature. Inductively coupled plasma mass spectrometry (ICP-MS) measurements were recorded on an iCAP RQ (ThermoFisher) spectrometer. The absorption within 96-well plates was measured using a SpectraMax Absorbance reader CMax Plus (Molecular Devices). The nanoparticle size was measured using an EliteSizer. Confocal microscopy images were recorded on LSM 880 NLO (Zeiss) microscope. Flow cytometry was performed on a Beckman CytoFLEX flow cytometer.

Synthesis and characterization

The synthetic routes of **dpqq**, **Ru-Ru**, **Ir-Ir**, and **Ru-Ir** were presented in Scheme S1.

Synthesis of the ligand dqpp

5,6-diamino-1,10-phenanthroline and pyrene-4,5,9,10-tetraones were synthesized according to previous methods.[1-3]

A mixture of 5,6-diamino-1,10-phenanthroline (105 mg, 0.5 mmol) and pyrene-4,5,9,10-tetraones (60 mg, 0.23 mmol) was suspended in methanol and refluxed for 3.5 hours. After cooling to room temperature, the precipitate obtained was filtered off, washed

with diethyl ether, and dried in vacuo to afford pure **dqpp** as an orange-brown solid. Yield: 130 mg (92.7%). The ligand was then used directly for the subsequent reaction without purification

Synthesis of Ru-Ru

$[Ru(dip)_2Cl_2]\cdot 2H_2O$ was synthesized according to the literature.[4]

A mixture of **dqpp** (61 mg, 0.1 mmol) and $[Ru(dip)_2Cl_2]\cdot 2H_2O$ (174 mg, 0.2 mmol) was suspended in ethylene glycol (8 mL) and heated at 120 °C for 12 h in the dark under argon. After cooling to room temperature, 80 mL water was added, and the solution was filtered. KPF_6 was added to the filtrate and then precipitated into the product. The solid was filtered, washed with ether, and dried in vacuo. The crude product was purified on neutral alumina (CH_2Cl_2 : CH_3CN = 3 : 1) to yield a red solution. The solvent was evaporated to give a black solid. The product was purified by recrystallization using acetonitrile and toluene. Yield: 50 mg (18.4%). ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 10.03 (d, *J* = 8.2 Hz, 4H), 8.46 (d, *J* = 5.3 Hz, 4H), 8.39 (d, *J* = 5.5 Hz, 4H), 8.30 (d, *J* = 5.5 Hz, 4H), 8.23 (s, 8H), 8.00 (dd, *J* = 8.2, 5.4 Hz, 4H), 7.65 (d, *J* = 3.6 Hz, 28H), 7.61 (s, 26H). ESI-MS: m/z = 536.42 ($[M-4PF_6]^{4+}$).

Synthesis of Ir-Ir

$[Ir(ppy)_2Cl]_2$ was synthesized according to the literature.[5]

The synthesis of **Ir-Ir** was similar to **Ru-Ru**, except that $[Ru(dip)_2Cl_2]\cdot 2H_2O$ was replaced by $[Ir(ppy)_2Cl]_2$ (107.1 mg, 0.1 mmol). Finally, an orange-yellow powder was obtained. Yield: 32 mg (18.9%). ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 9.70 (s, 4H), 9.39 (s, 4H), 8.54 (d, *J* = 4.6 Hz, 2H), 8.50 (s, 2H), 8.22 (dd, *J* = 7.6, 4.6 Hz, 5H), 7.99 (d, *J* = 7.8 Hz, 5H), 7.95 – 7.90 (m, 6H), 7.80 (s, 4H), 7.23 (q, *J* = 7.1 Hz, 5H), 7.15 – 7.02 (m, 9H), 6.52 (d, *J* = 7.4 Hz, 4H). ESI-MS: m/z = 806.26 ($[M-2PF_6]^{2+}$).

Synthesis of Ru-Ir

$[Ru(dip)_2$(5,6-diamino-1,10-phenanthroline)$]Cl_2$ and $[Ir(ppy)_2$(5,6-diamino-1,10-phenanthroline)]Cl were synthesized according to the literature.[6-7]

Pyrene-4,5,9,10-tetraones (26.2 mg, 0.1 mmol) were added to a solution of ethanol, acetonitrile, and acetic acid (5 : 15 : 1, v/v). The reaction mixture was stirred at 80 ºC for dissolution. $[Ru(dip)_2$(5,6-diamino-1,10-phenanthroline)$]Cl_2$ (104.7 mg, 0.1 mmol) was dissolved in 50 mL EtOH and then added into the reaction mixture slowly. The obtained black solution was stirred for 3 hours in the dark under argon. Subsequently, an EtOH solution (20 mL) of $[Ir(ppy)_2$(5,6-diamino-1,10-phenanthroline)]Cl (74.6 mg, 0.1 mmol) was added and further refluxed for another 7 hours. After cooling to room temperature, adjust the pH value to 7.0 by adding ammonia. Evaporate solvent under vacuum. 30 mL H_2O containing 0.5 g KPF_6 was added to precipitate the product. The crude product was purified by column chromatography on neutral alumina (CH_3CN : CH_3CH_2OH = 10 : 1) to give an

orange solution, which is concentrated in a vacuum until orange-red powder is obtained. Yield: 50 mg (25.2%). ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 10.13 (s, 8H), 8.53 (s, 4H), 8.46 (d, *J* = 3.5 Hz, 2H), 8.42 (d, *J* = 4.5 Hz, 2H), 8.35 (d, *J* = 5.6 Hz, 2H), 8.28 (s, 4H), 8.16 (d, *J* = 8.0 Hz, 3H), 8.02 (s, 3H), 7.94 (d, *J* = 7.4 Hz, 3H), 7.90 – 7.84 (m, 3H), 7.75 – 7.67 (m, 12H), 7.64 (s, 9H), 7.21 – 7.14 (m, 3H), 7.10 – 7.03 (m, 3H), 6.97 (s, 3H), 6.48 (d, *J* = 7.1 Hz, 2H). ESI-MS: m/z = 626.39 ($[M-3PF_6]^{3+}$).

Octanol/water partition coefficients (log $P_{O/W}$) assay

The octanol/water partition coefficients (log $P_{O/W}$) of the complexes were accessed according to previous report.[8] A stock solution of **Ru-Ru**, **Ir-Ir**, and **Ru-Ir** in DMSO was added to a mixture (6 mL) containing equal volume of deionized water and octanol. The final content of DMSO is 0.1%. The final concentration of **Ru-Ru**, **Ir-Ir**, and **Ru-Ir** is 5 μM. The mixture was shaken overnight at 60 rpm to allow partitioning at 298 K. After the sample was centrifuged at 3000 rpm for 10 min, the aqueous layer was carefully separated from the octanol layer. The related metal concentration in the aqueous phase was determined by ICP-MS and used to calculate the log $P_{O/W}$ value.

Preparation of Ru-Ru@PEG, Ir-Ir@PEG and Ru-Ir@PEG

Ru-Ru (1 mg) was dissolved in anhydrous dichloromethane (1 mL), and then DSPE-$mPEG_{2000}$ (10 mg) was added to the solution. The solution was treated with ultrasonic pulses with a Scientz-II D ultrasonic homogenizer for 3 minutes. Transferred this solution to 10 mL double-distilled water and treated with ultrasonic pulses for 5 minutes. Subsequently, dichloromethane was removed by argon flow. A clear and transparent solution of **Ru-Ru@PEG** was obtained after dialysis for 24 hours. The amounts of encapsulated nanoparticles (based on metal) were determined by ICP-MS.

The preparation of **Ir-Ir@PEG** and **Ru-Ir@PEG** were similar to **Ru-Ru@PEG**.

Inductively coupled plasma mass spectrometry (ICP-MS) assay

The isotope of ruthenium and iridium tested in the experiment was chosen to be ^{101}Ru and ^{193}Ir, respectively. The calibration process was conducted by professional workers in charge of the ICP-MS machine. For quantitative analysis of ruthenium in **Ru-Ru@PEG** and **Ru-Ir@PEG**, 1 mL **Ru-Ru@PEG** and **Ru-Ir@PEG** was completely digested by 0.5 mL of concentrated HNO_3 and 1.0 mL of H_2O_2 at room temperature for 24 h and then were diluted to a total of 10 mL specimens with Milli-Q H_2O. Finally, samples were analyzed by ICP-MS. Quantification was calculated by the ruthenium standard curve. The quantitative analysis procedure of iridium in **Ir-Ir@PEG** was similar.

For cellular uptake determination, A549R cells were seeded in 10 cm^2 culture plates at 5 × 10^5 cells per plate and incubated for 24 h to adherent. Cells were incubated with **Ru-**

Ru@PEG (Ru amount, 10.0 μM), **Ir-Ir@PEG** (Ir amount, 10.0 μM), and **Ru-Ir@PEG** (Ru amount, 10.0 μM) for 6, 9, 12, and 24 hours, respectively. Cells were collected carefully after being washed with cold PBS three times. Next, the cells were counted and completely digested with 0.5 mL of concentrated HNO_3 and 1.0 mL of H_2O_2 at room temperature for two days. The samples were diluted with Milli-Q water to 10 mL with 3% of HNO_3. The concentrations of Ru or Ir were determined by ICP-MS. Data were reported as the mean ± standard deviation (n = 3).

For organelles distributions assay, A549R cells were cultured in 25 cm^2 culture plates at 1 × 10^6 cells per plate for 24 h and then incubated with **Ru-Ru@PEG** (Ru amount, 10.0 μM), **Ir-Ir@PEG** (Ir amount, 10.0 μM), and **Ru-Ir@PEG** (Ru amount, 10.0 μM) for 12 h. Cells were detached with trypsin and harvested carefully. Subsequently, cells were counted and equally divided into four portions for the nucleus, mitochondria, lysosome, and cytoplasm extraction according to the corresponding kit protocols, respectively.

ROS generation assay

Brief, unless special note, the light source of ROS generation experiments was 450 nm LED (Height-LED Instruments) for **Ru-Ru** and **Ru-Ir** and 405 nm LED (Height-LED Instruments) for **Ir-Ir**. The light dose was 20 mW/cm^2 for 5 min.

The $^1O_2$ yield measurement was performed according to the previous report.[9]

For the electron paramagnetic resonance (EPR) assay, TEMP was used for trapping $^1O_2$, while BMPO was used for trapping $^{\bullet}OH$ and $O_2^{\bullet-}$ as reference suggested.[10] The concentration of TEMP and BMPO was 25 mM and 100 mM, respectively. The hypoxic environment was achieved by bubbling with argon for 30 min and putting in an anaerobic workstation (LAI-3DT, Longyue Instruments) of 1% O_2 for another 30 min.

For *in vitro* ROS generation under hypoxic conditions, A549R cells were seeded in the density of 1 × 10^4 cells/cm^2 on the confocal microscope specialized cell culture dish and incubated under a 5% CO_2 atmosphere for 12 hours to allow adherence. Subsequently, cells were transferred to an anaerobic workstation (LAI-3DT, Longyue Instruments) with 1% O_2 and incubated for 24 hours, followed by the addition of **Ru-Ir@PEG** (Ru amount, 5.0 μM) for 12 hours of treatment. Cells were washed with PBS three times and incubated with the fluorescent probe DCFH-DA (5 μM), DHE (5 μM), or HPF (5 μM) for 30 min. The sample was irradiated with 450 nm LED (20 mW/cm^2, 5 min). Fluorescence imaging was performed by confocal laser scanning microscope (DCF: λ_{ex} = 488 nm, λ_{em} = 525 ± 10 nm; DHE: λ_{ex} = 510 nm, λ_{em} = 610 ± 10 nm; HPF: λ_{ex} = 488 nm, λ_{em} = 530 ± 10 nm).

Cell (photo)cytotoxicity assay

The (photo)cytotoxicity of **Ru-Ru@PEG**, **Ir-Ir@PEG**, and **Ru-Ir@PEG** toward cisplatin-resistant human non-small cell lung carcinoma cell lines A549R (incubated under

normoxic and hypoxic environments, respectively) was investigated by the classic MTT assay. A549R cells were seeded in 96 well plates at 5×10^3 cells per well.

For cytotoxicity assay under normoxic conditions, cells were incubated for 24 hours and then treated with different concentrations of **Ru-Ru@PEG**, **Ir-Ir@PEG**, and **Ru-Ir@PEG** for 48 hours. MTT solution (20 μL, 5 mg/mL) was added into each well for 4 hours. Finally, the supernatant medium was removed and added to each well with 150 μL of DMSO. The absorbance was measured after shaking for 180 s at 590 nm using a microplate reader (SpectraMax CMax Plus, Molecular Devices).

For photocytotoxicity assay under normoxic conditions, cells were incubated for 24 hours and then treated with different concentrations of **Ru-Ru@PEG**, **Ir-Ir@PEG**, and **Ru-Ir@PEG** for 24 hours. The medium was removed and replaced with a 200 μL fresh medium. The cells were irradiated by the corresponding wavelength (LED, 20 mW/cm^2) for 5 min. After they were incubated for another 24 h, the procedure was identical to cytotoxicity assay and was detected absorption at 595 nm by a microplate reader.

The (photo)cytotoxicity assay under hypoxic conditions was similar to that of normoxic conditions, except that after cell seeding, the cells were incubated for 12 hours and then transferred to an anaerobic workstation (LAI-3DT, Longyue Instruments) of 1% O_2 for another 12 hours, followed by the addition of drugs.

Cell apoptosis detection

For calcein acetoxymethyl ester/propidium iodide (Ca-AM/PI) co-staining assay, A549R cells were seeded in the density of 1×10^4 cells/cm^2 on the confocal microscope specialized cell culture dishes and incubated under a 5% CO_2 atmosphere for 12 hours to allow adherence. Subsequently, cells were transferred to an anaerobic workstation (LAI-3DT, Longyue Instruments) with 1% O_2 and incubated for 12 hours, followed by the addition of **Ru-Ir@PEG** (Ru amount, 5.0 μM) for 24 hours of treatment. The medium was removed and replaced with a 1 mL fresh medium. After being irradiated with 450 nm LED (20 mW/cm^2, 5 min) and further incubated for 24 hours, cells were washed with PBS three times. Added 1 mL of working buffer containing Ca-AM (5 μM) and PI (50 μg/mL) to the dishes and incubated for 30 min, the samples were imaged by a confocal laser scanning microscope (Ca-AM: λ_{ex} = 490 nm, λ_{em} = 515 ± 5 nm; PI: λ_{ex} = 535 nm, λ_{em} = 620 ± 10 nm).

For Annexin V-FITC/PI double staining assay, A549R cells were seeded into 6-well plates at the density of 1×10^5 cells/well for 12 hours to adherent and then incubated in an anaerobic workstation (LAI-3DT, Longyue Instruments) with 1% O_2 for 12 hours, followed by the addition of **Ru-Ir@PEG** (Ru amount, 5.0 μM) for 24 hours of treatment. The medium was removed and replaced with a 1 mL fresh medium. After being irradiated with 450 nm LED (20 mW/cm^2, 5 min) and further incubated for 12 hours, cells were washed with PBS three times. Cells were collected and resuspended in 100 μL binding buffer. 5 μL Annexin

V-FITC and 5 μL PI were added and incubated for 15 min at room temperature in the dark. Added 400 μL binding buffer to each sample and then analyzed by flow cytometry.

For the caspase 3/7 activation assay, A549R cells were inoculated into 96-well white plates at the density of 1×10^5 cells/well for 12 hours to adherent and then incubated in an anaerobic workstation (LAI-3DT, Longyue Instruments) with 1% O_2 for 12 hours. Then, the cells were incubated with **Ru-Ir@PEG** (Ru amount, 5.0 μM) or cisplatin (50.0 μM) for 24 hours. After being irradiated with 450 nm LED (20 mW/cm^2, 5 min) and further incubated for 12 hours, caspase work solutions were added according to the manufacturer's protocol. The results were obtained by an Infinite M200 PRO (TECAN, Swiss). The experiments were performed in triplicates (n = 3).

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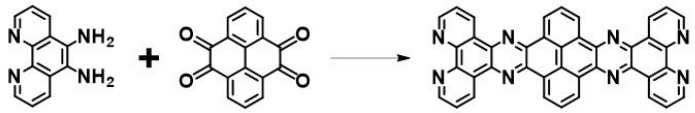
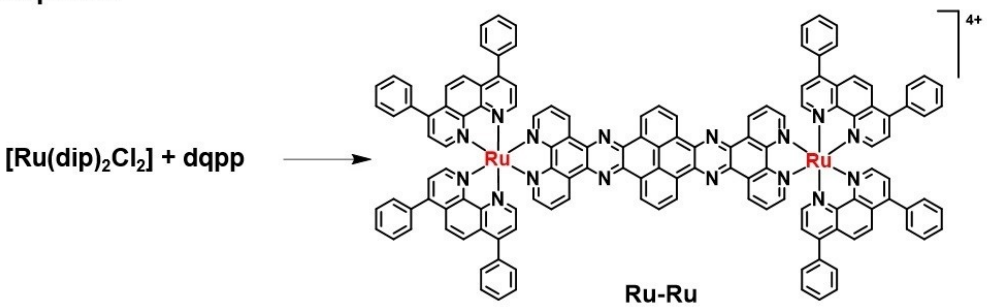
Supporting Figures and Tables

The ligand dqpp

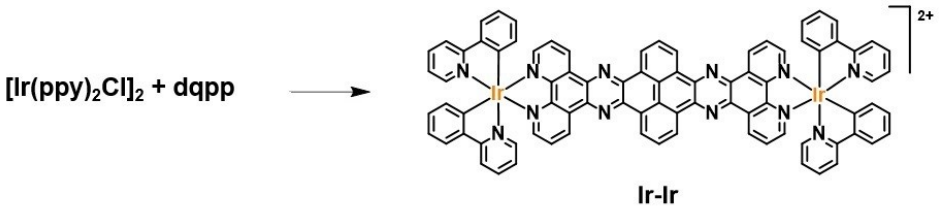


dqpp

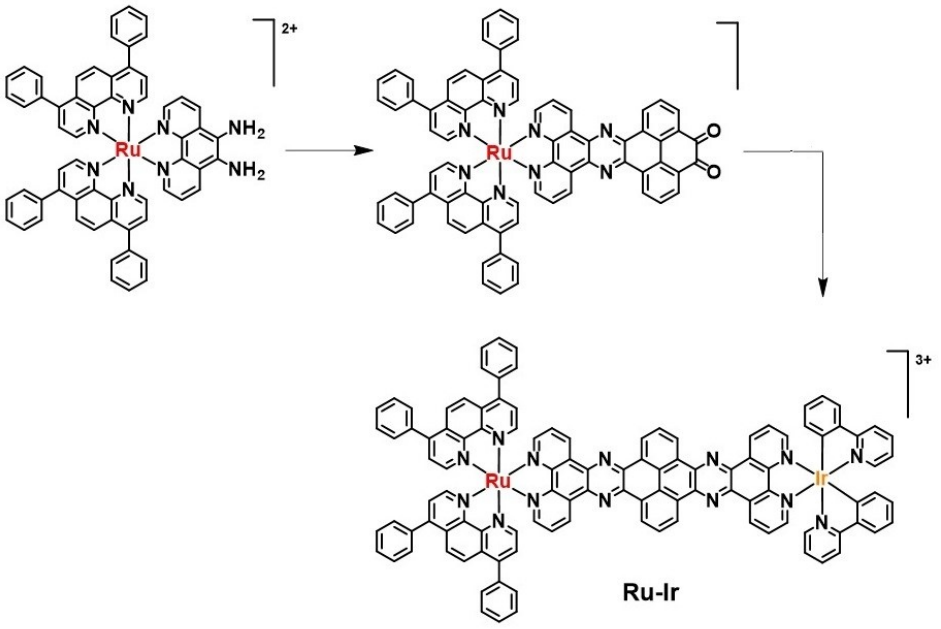
The complexes



Ru-Ru



Ir-Ir



Ru-Ir

Scheme S1 The synthetic routes of **dpqq**, **Ru-Ru**, **Ir-Ir**, and **Ru-Ir**.

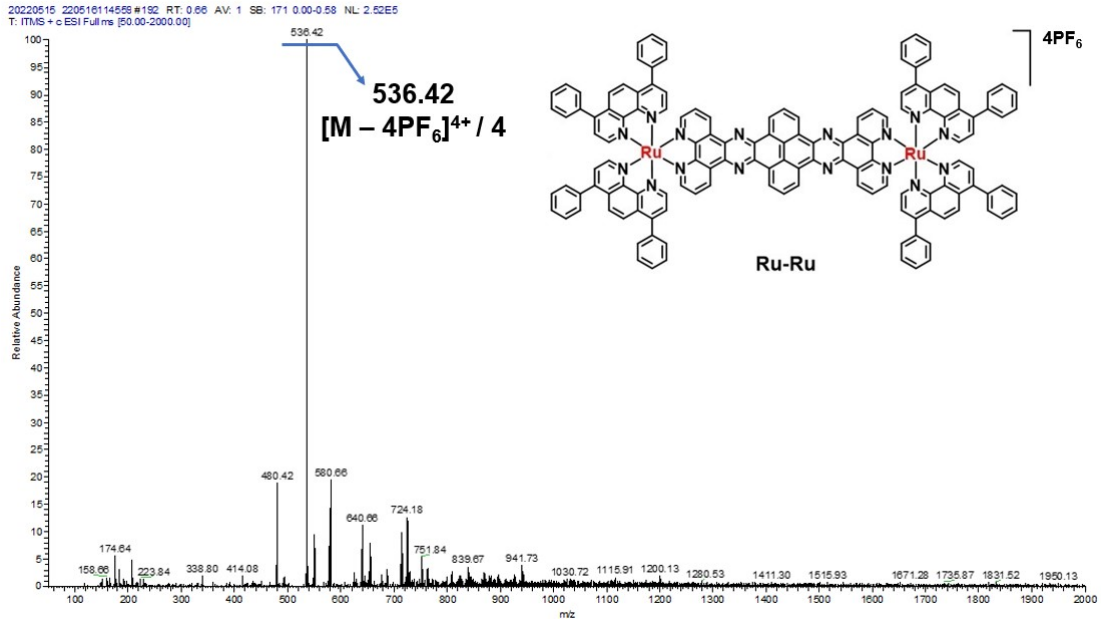


Fig. S1 ESI-MS spectrum of **Ru-Ru** in CH_3CN

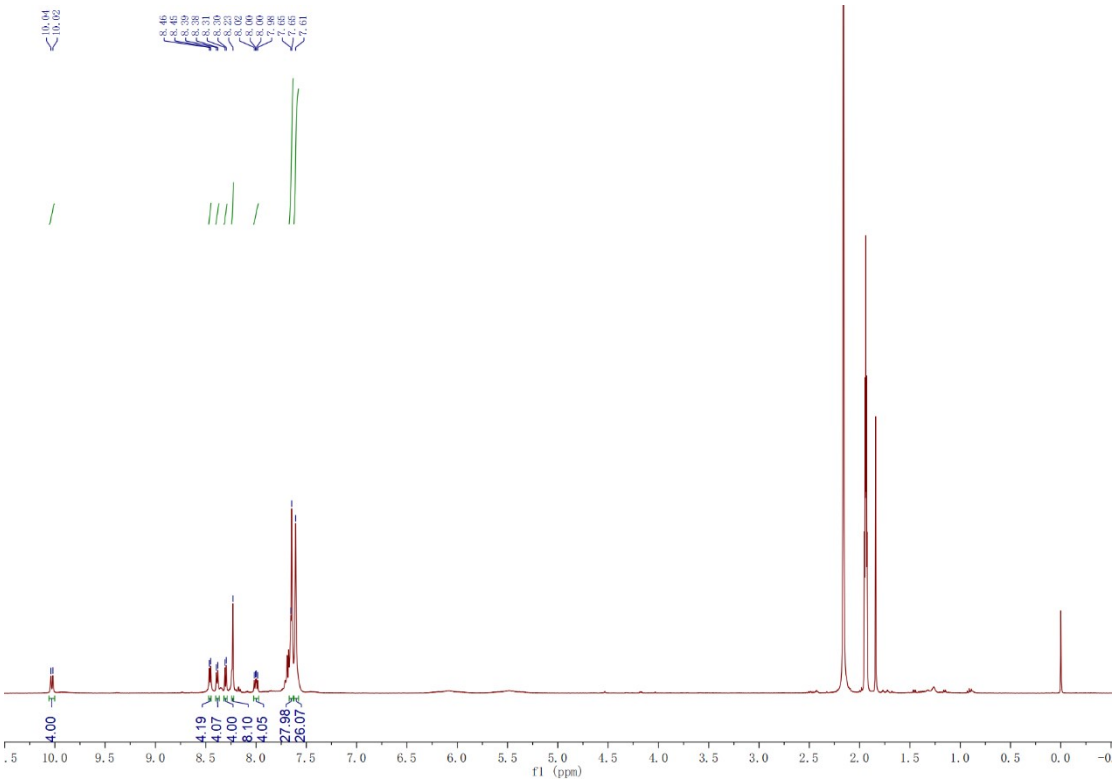


Fig. S2 ^{1}H NMR spectrum of **Ru-Ru** in acetonitrile-d_3

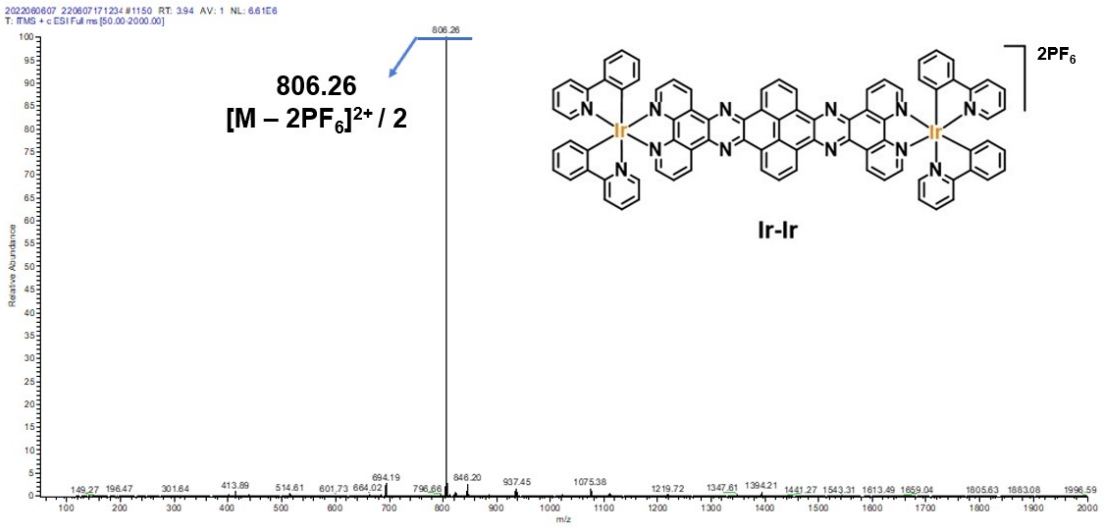


Fig. S3 ESI-MS spectrum of **Ir-Ir** in CH_3CN

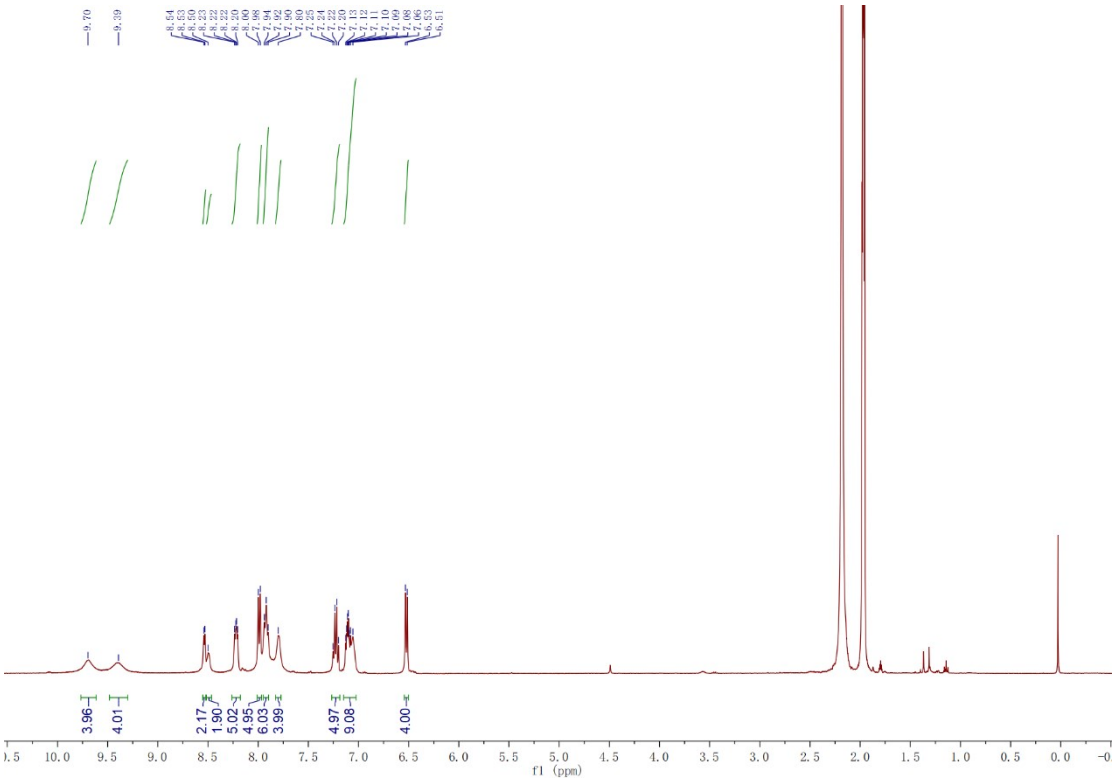


Fig. S4 ^{1}H NMR spectrum of **Ir-Ir** in Acetonitrile-d_3

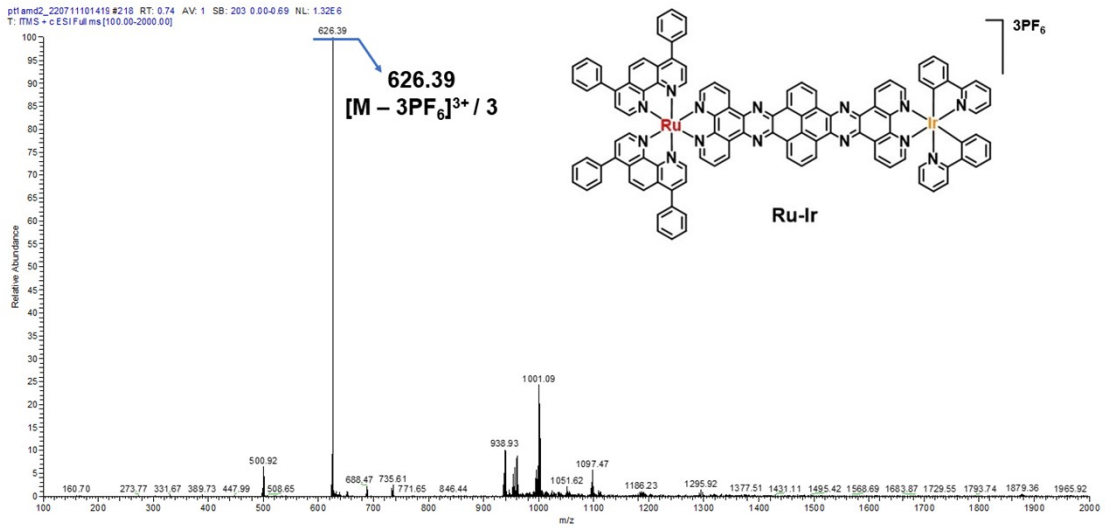


Fig. S5 ESI-MS spectrum of **Ru-Ir** in CH_3CN

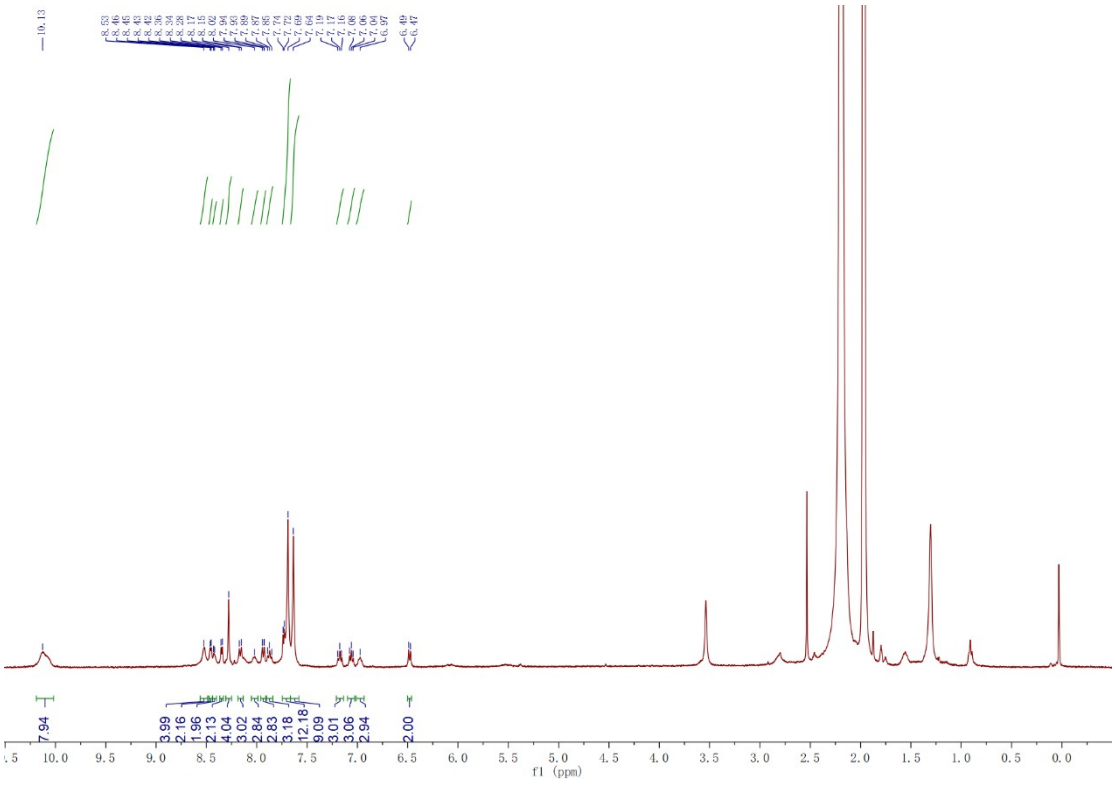


Fig. S6 ^{1}H NMR spectrum of **Ru-Ir** in Acetonitrile-d_3

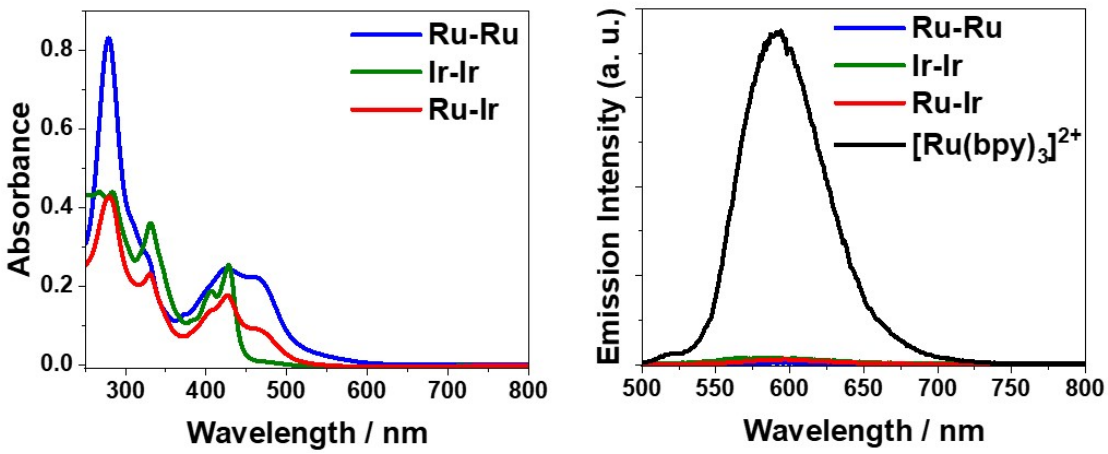


Fig. S7 (Left) the UV-vis spectra of **Ru-Ru**, **Ir-Ir**, and **Ru-Ir** (10 μM) in methanol; (Right) the emission spectra of **Ru-Ru**, **Ir-Ir**, **Ru-Ir**, and $[Ru(bpy)_3]^{2+}$ (10 μM) in methanol.

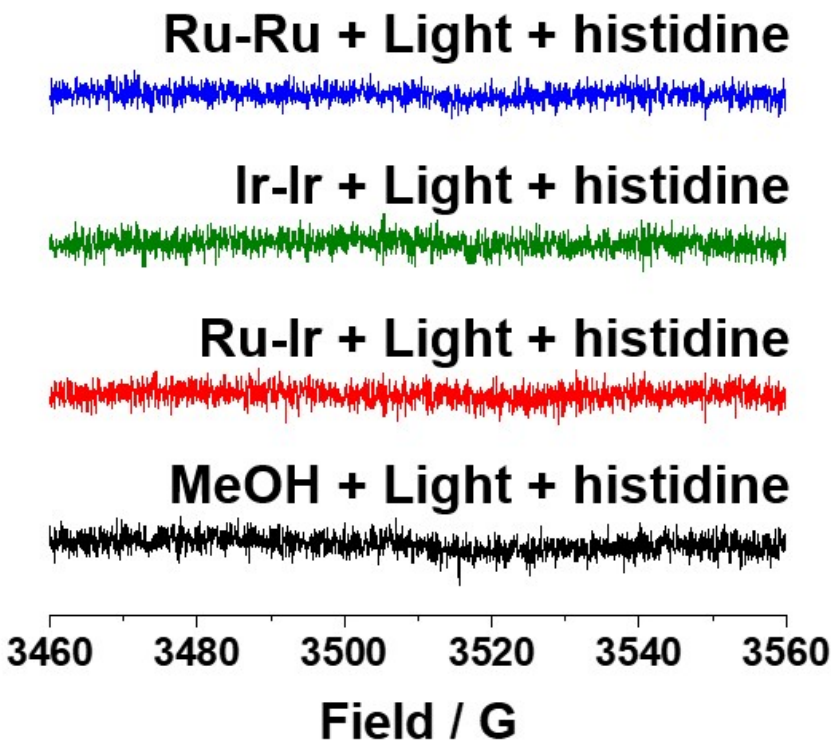


Fig. S8 The EPR signals of singlet oxygen for **Ru-Ru**, **Ir-Ir**, and **Ru-Ir** (10 μM) in the presence of histidine (20 mM) under normoxia. Light: 450 nm LED for **Ru-Ru**, **Ru-Ir**, and MeOH; 405 nm LED for **Ir-Ir**. Light dose: 20 mW/cm^2 for 5 min.

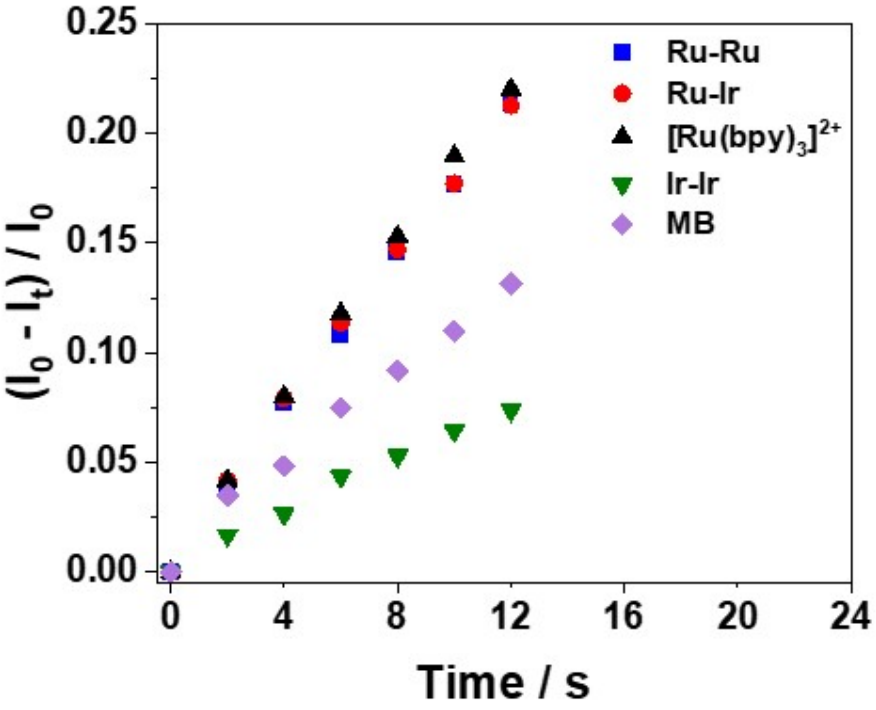


Fig. S9 Plots of DPBF fluorescence attenuation vs. irradiation time in the presence of **Ru-Ru**, **Ir-Ir**, **Ru-Ir**, methylene blue (MB), and $Ru(bpy)_3^{2+}$. Light: 450 nm LED for **Ru-Ru**, **Ru-Ir**, MB, and $Ru(bpy)_3^{2+}$; 405 nm LED for **Ir-Ir**

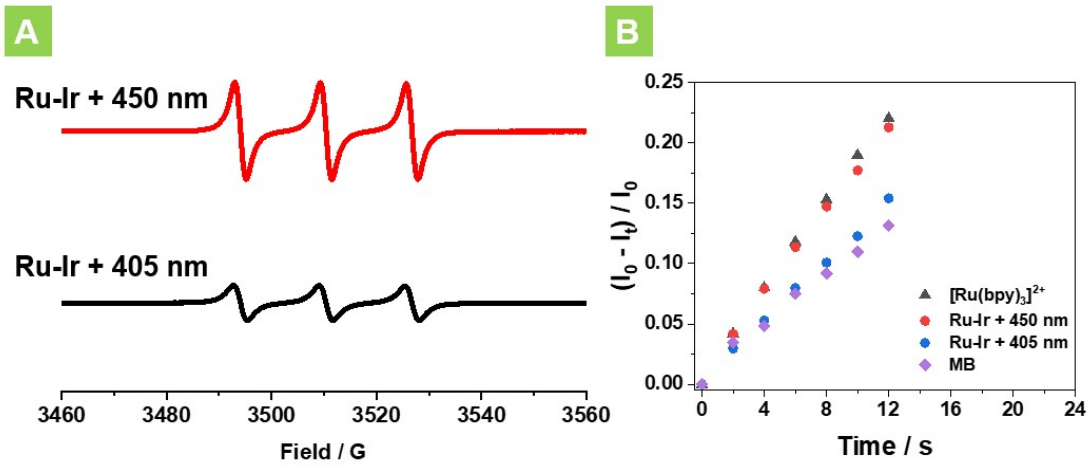


Fig. S10 (A) The EPR signals of singlet oxygen for **Ru-Ir** (10 μM) upon 405 and 450 nm irradiation (20 mW/cm^2 for 5 min) under normoxia. (B) Plots of DPBF fluorescence attenuation vs. irradiation time in the presence of **Ru-Ir**, methylene blue (MB), and $Ru(bpy)_3^{2+}$. Light: 450 nm LED for **Ru-Ir**, MB, and $Ru(bpy)_3^{2+}$; 405 nm LED for **Ru-Ir**

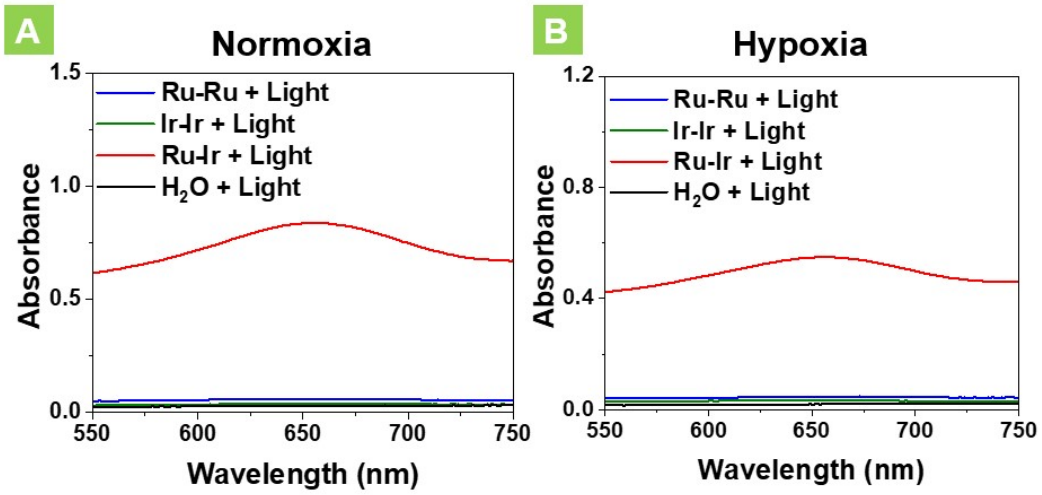


Fig. S11 UV–vis absorption spectra of TMB oxidized by •OH generated by **Ru-Ir** upon irradiation under (A) normoxic and (B) hypoxic conditions.

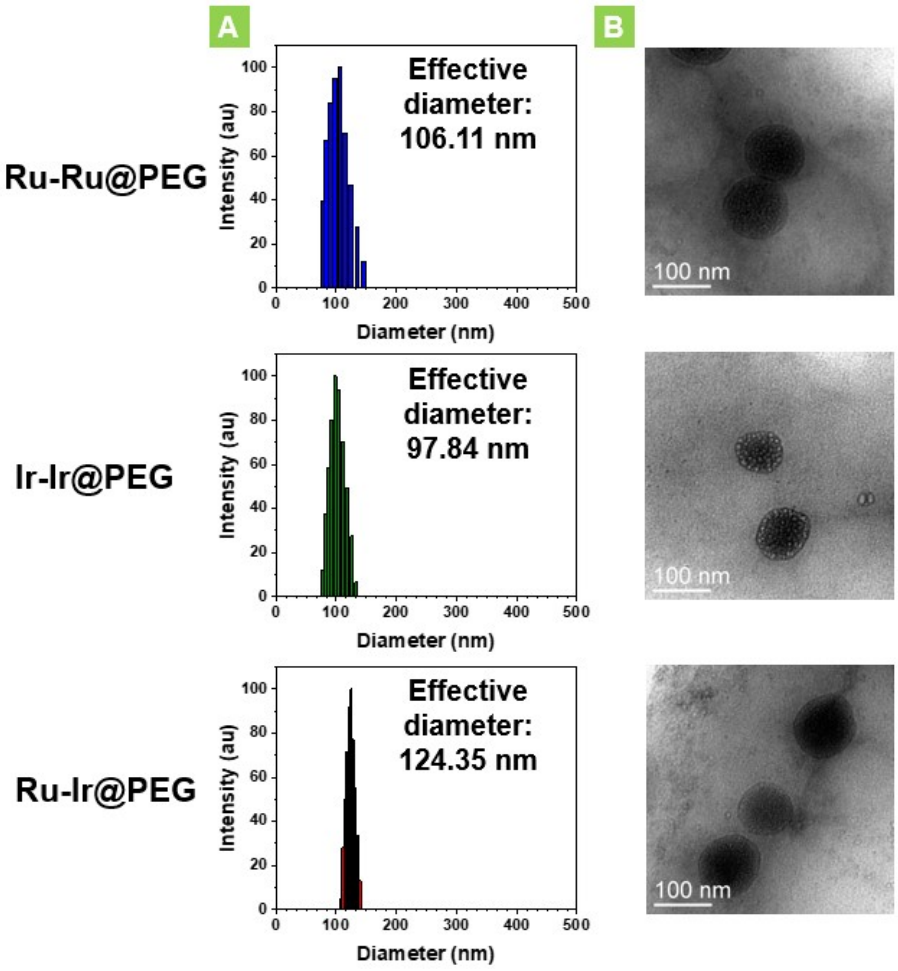


Fig. S12 The nanodrugs **Ru-Ru@PEG**, **Ir-Ir@PEG**, and **Ru-Ir@PEG** characterized by (A) DLS and (B) TEM.

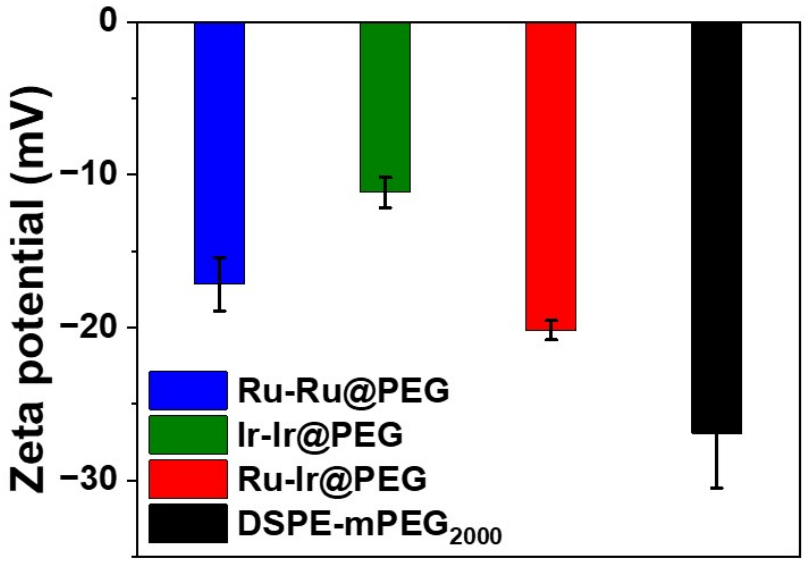


Fig. S13 The Zeta potential of DSPE-mPEG2000, **Ru-Ru@PEG**, **Ir-Ir@PEG**, and **Ru-Ir@PEG**.

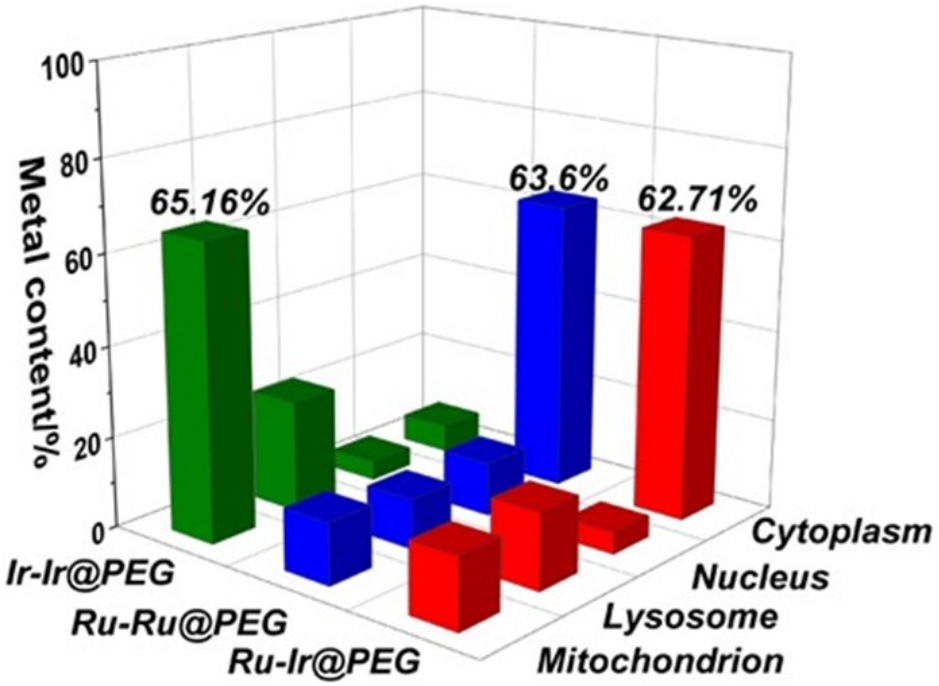


Fig. S14 The organelle distribution of **Ru-Ru@PEG**, **Ir-Ir@PEG**, and **Ru-Ir@PEG** in A549R cancer cells.

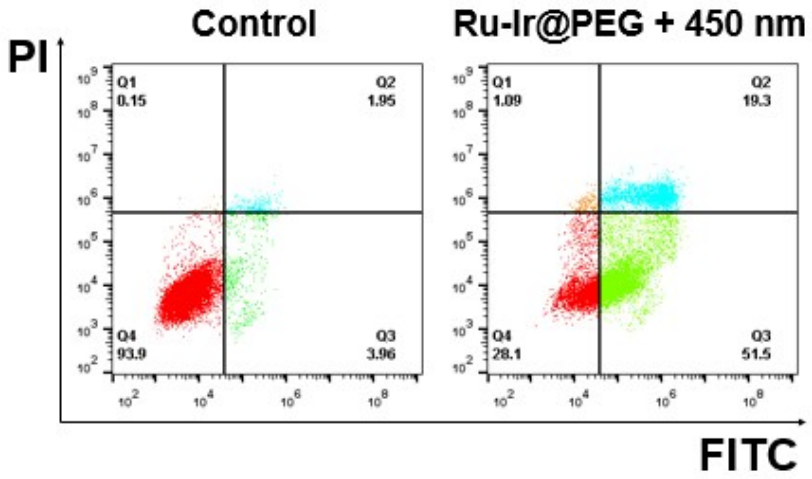


Fig. S15 Annexin V-FITC/PI dual staining of A549R cells upon PDT process of **Ru-Ir@PEG** (Ru amount, 5.0 μM) under hypoxic conditions.

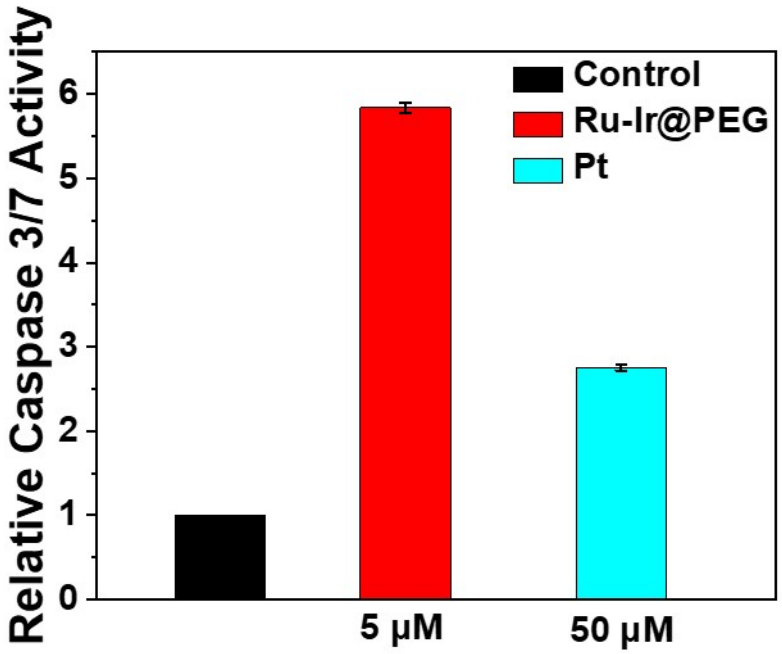


Fig. S16 Caspase 3/7 activation of A549R cells treated with cisplatin (50 μM) or upon PDT process of **Ru-Ir@PEG** (Ru amount, 5.0 μM) under hypoxic conditions.

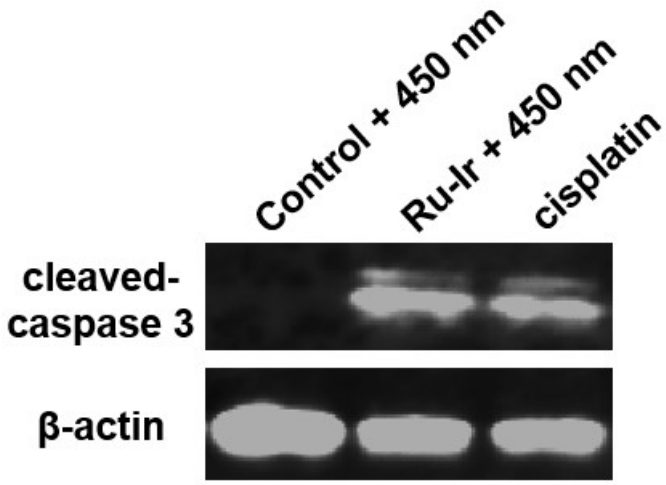


Fig. S17 The express level of cleaved-caspase 3 in A549R cells upon PDT process of **Ru-Ir@PEG** (Ru amount, 5.0 μM) and cisplatin (50 μM) under hypoxic conditions.

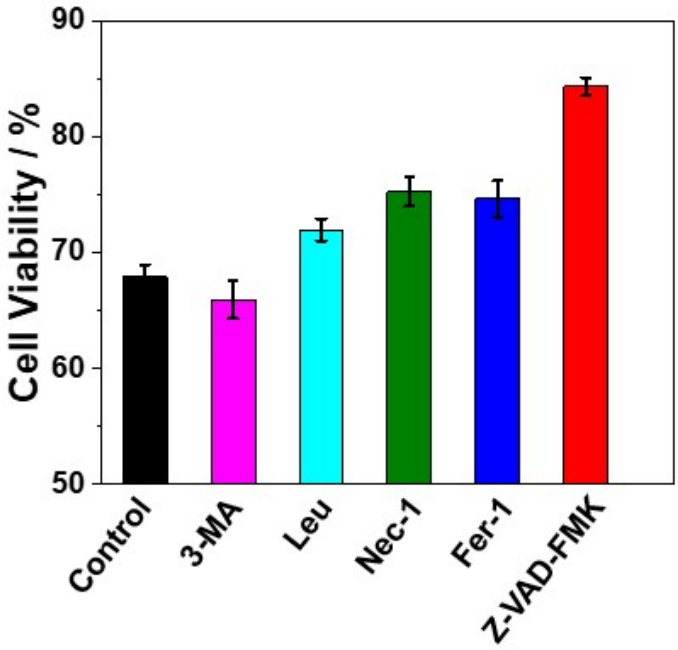


Fig S18. Cell viability of A549R cells upon preincubation with autophagic inhibitor 3-metheyladenine (3-MA), lysosomal protease-mediated cell death inhibitor leupeptin (Leu), necrosis/necroptosis inhibitor necrostatin-1 (Nec-1), ferroptosis inhibitor ferrostatin-1 (Fer-1), and apoptosis inhibitor Z-VAD-FMK, followed by treatment with **Ru-Ir@PEG** upon irradiation (450 nm, 20 mW/cm^2 for 5 min) under hypoxic conditions.

Table S1. (Photo)cytotoxicity of **Ru-Ru@PEG, Ir-Ir@PEG, and Ru-Ir@PEG** against A954R cells

		IC_{50} (μM)			
		Ru-Ru@PEG	**Ir-Ir@PEG**	**Ru-Ir@PEG**	Cisplatin
Normoxia	Dark [a]	50.79 ± 3.21	16.90 ± 1.28	30.72 ± 2.62	64.98 ± 1.32
	Light [b]	2.49 ± 1.30	4.89 ± 1.50	1.40 ± 0.98	61.85 ± 3.96
	PI [c]	20.40	3.46	21.94	1.05
Hypoxia	Dark [a]	64.07 ± 1.55	23.61 ± 2.13	35.19 ± 1.71	68.23 ± 4.88
	Light [b]	62.16 ± 3.27	20.03 ± 0.40	3.48 ± 0.66	63.27 ± 3.41
	PI [c]	1.03	1.18	10.11	1.08

[a] Cells were incubated with the indicated compounds for 48 h.

[b] Irradiations (20 mW/cm^2 for 5 min) were given after 24 h incubation, and the photocytotoxicity was measured 24 h after PDT treatment.

[c] PI (photocytotoxicity index) is the ratio of dark-to-light toxicity and reflects the effective PDT range of the compounds.

Table S2. The cell uptake of **Ru-Ru@PEG**, **Ir-Ir@PEG**, and **Ru-Ir@PEG** in A954R cells for different incubation times.

	Cellular uptake (ng/10^6 cell)			
	6 h	9 h	12 h	24 h
Ru-Ru@PEG	6.48 ± 1.36	8.52 ± 2.57	12.20 ± 1.78	12.21 ± 2.13
Ir-Ir@PEG	4.39 ± 0.58	4.98 ± 0.69	6.40 ± 2.54	6.37 ± 2.69
Ru-Ir@PEG	5.06 ± 1.48	7.91 ± 1.99	14.31 ± 3.05	14.55 ± 2.94