Supporting Information

S1. Syntheses and characterization

S1.1 General methods

Unless otherwise specified, all reagents and solvents were commercial and used without further purification. ^{1}H NMR, DOSY and COSY spectra were measured by Bruker AVANCE III 400 (400 MHz), applying TMS as the internal standard and using MestReNova software to analyze the spectra. Chemical shifts were quoted in parts permillion (ppm) referenced to the 0.0 ppm for TMS or appropriate solvent peak. The HR-MS spectra were recorded on Bruker maXis 4G ESI-Q-TOF equipment. The data of ESI-TOF mass spectrometry were analyzed by Bruker Data Analysis software and simulated by Bruker IsotopePattern software. The UV-vis absorption spectra were measured on SHIMADZU UV-3600. The photoluminescence spectra were recorded on EDINBURGH FLS980 fluorescence spectrophotometer. The quantum yields (QYs) were measured on a Hamamatsu C9920-02G absolute photoluminescence quantum yield (PLQY) measurement system.

The two-photon absorption (TPA) cross sections of Irqpy-2 metalloligand and MOC-53 were determined over a broad spectral region by the typical two-photon excited luminescence (TPEF) method using Rhodamine B in methanol as the standard. The TPA cross sections (δ_s) were calculated according to the following equation (1).

$$\delta_{\mathrm{s}} = \delta_{\mathrm{r}} \frac{\Phi_{\mathrm{r}} c_r I_s n_s}{\Phi_s c_s I_r n_r} \quad (1)$$

I is the integrated fluorescence intensity, c is the concentration, n is the refractive index, Φ is the quantum yield, subscript 'r' stands for reference samples, and 's' stands for the samples.

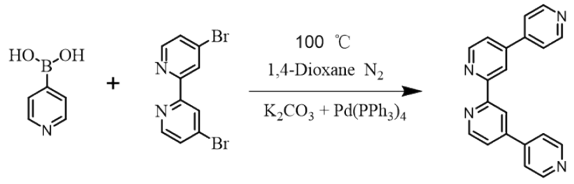
The quantum yields for $^1O_2$ production (Φ_Δ) of Irqpy-2 metalloligand and MOC-53 were evaluated using a steady-state method with ABDA as the $^1O_2$ indicator and $[Ru(bpy)_3]Cl_2$ as the standard (Φ_Δ = 0.18 in H_2O). The water containing the tested

samples and ABDA (100 μM) were prepared in the dark and irradiated with a 420 nm xenon lamp with filter. The absorption maxima of ABDA were recorded every 25 s. The absorbance at 425 nm of the samples and $[Ru(bpy)_3]Cl_2$ also was kept at 0.15. The Φ_Δ of the samples were calculated according to the following equation (2).

$$\Phi_{\Delta(x)} = \Phi_{\Delta(std)} \times \left(\frac{S_x}{S_{std}}\right) \times \left(\frac{F_{std}}{F_x}\right) \quad (2)$$

where subscripts *x* and *std* designate the sample and $[Ru(bpy)_3]Cl_2$, respectively, *S* stands for the slope of plot of the absorption maxima of ABDA against the irradiation time (s). *F* stands for the absorption correction factor, which is given by $F = 1 - 10^{-OD}$ (OD represents the optical density of sample and $[Ru(bpy)_3]Cl_2$ at 425 nm).

S2. ^{1}H NMR and MS Characterization



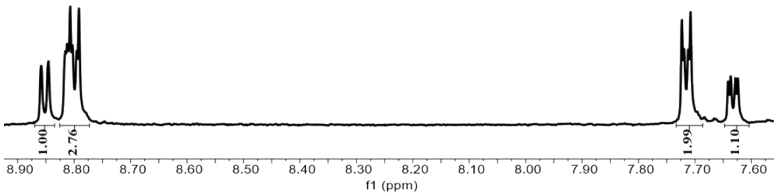
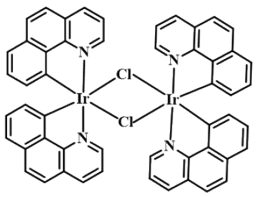


Fig. S1 ^{1}H NMR spectra of qpy measured in DMSO-*d*6 (400 MHz, 298 K).



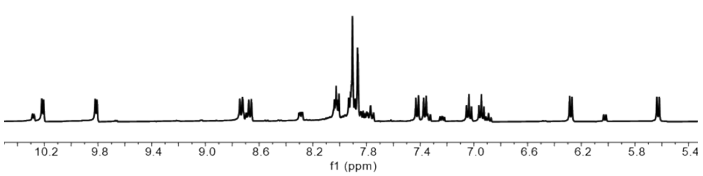


Fig. S2 ^{1}H NMR of $[Ir(bzq)_2(\mu\text{-}Cl)]_2$ measured in DMSO-*d*6 (400 MHz, 298 K).

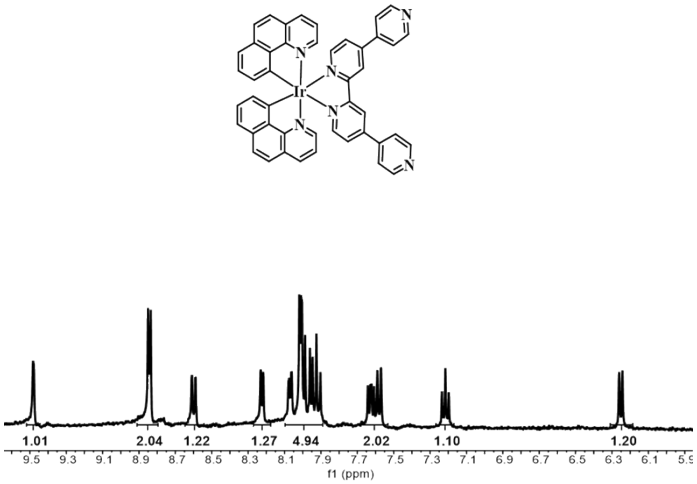


Fig. S3 ^{1}H NMR spectra of Irqpy-2 measured in DMSO-d_6 (400 MHz, 298 K).

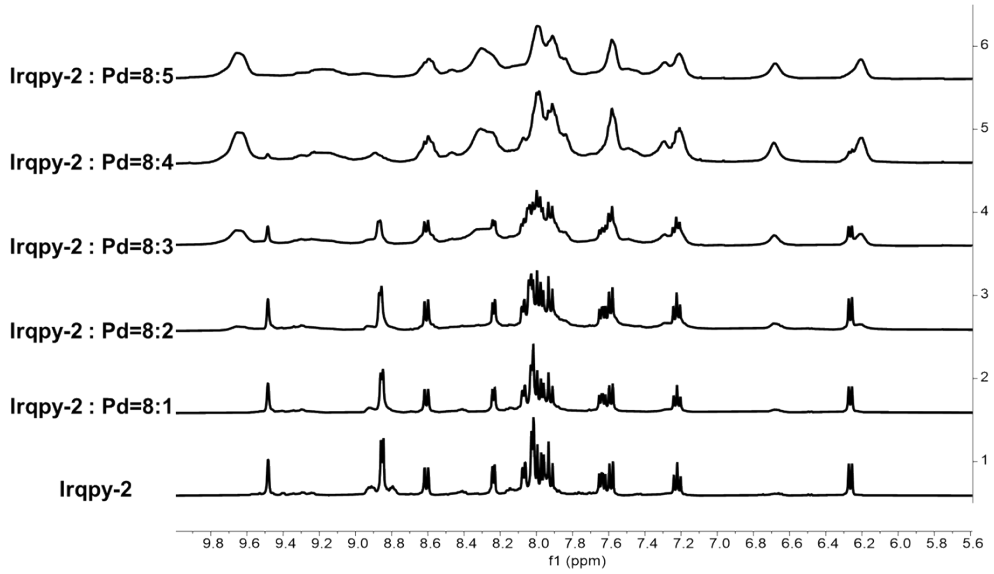
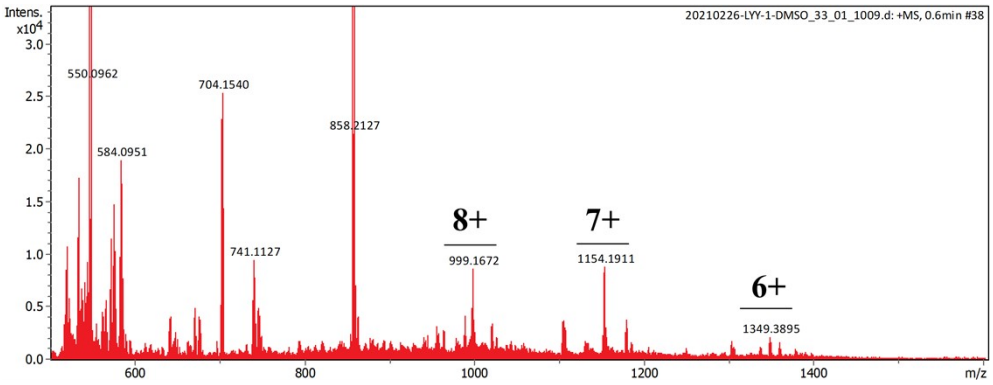
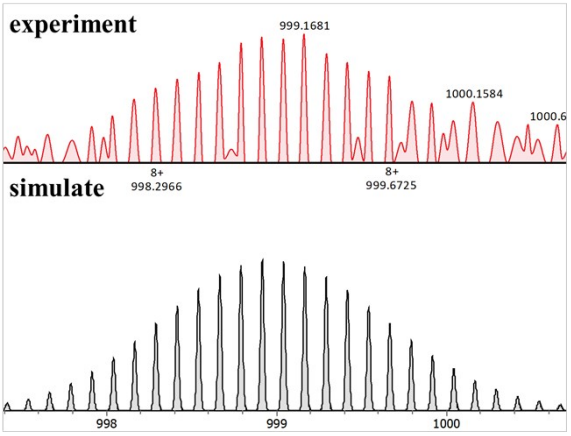


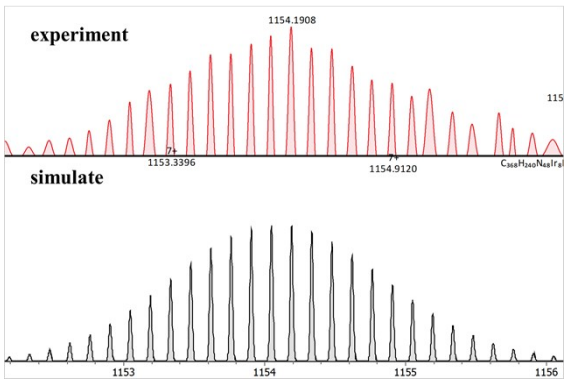
Fig. S4 ^{1}H NMR titration spectra of Irqpy-2 metalloligand (bottom) with the addition of $Pd(BF_4)_2(CH_3CN)_4$.



$[MOC^{16+}+8BF_4]^{8+}$



$[MOC^{16+}+9BF_4]^{7+}$



$[MOC^{16+}+10BF_4]^{6+}$

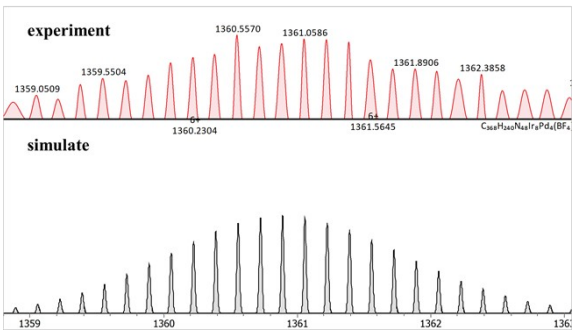


Fig. S5 HR-ESI-TOF-MS spectra of $[Pd_4(Irqpy\text{-}2)_8]^{16+}$ related species in CH_3CN.

S3. Photophysical spectra

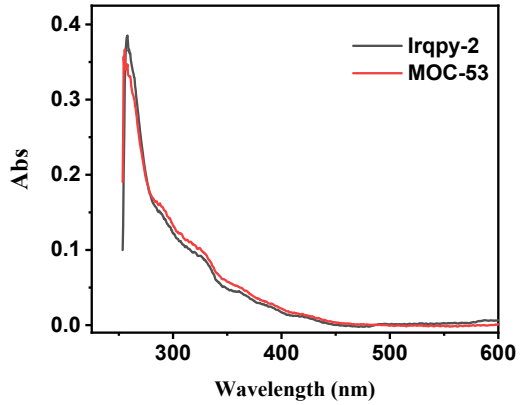


Fig. S6 UV-vis absorption spectra of MOC-53 (1.25×10^{-6} mol/L) and Irqpy-2 (1×10^{-5} mol/L) in DMSO solution

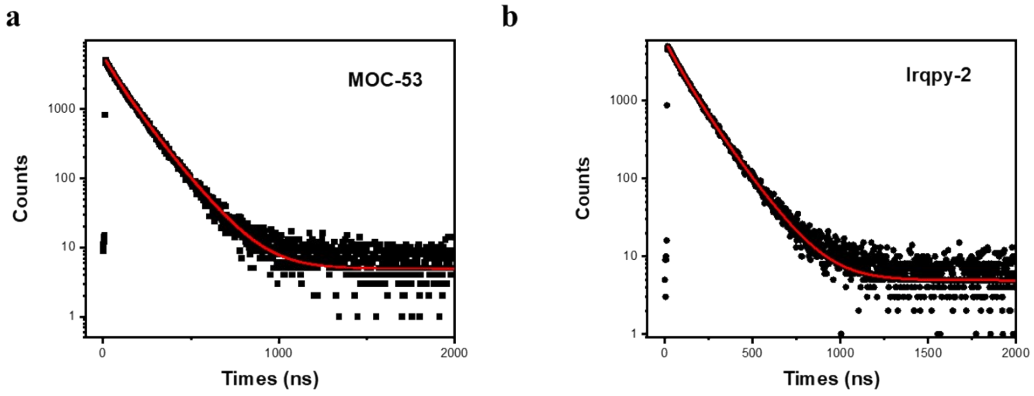


Fig. S7 Decay lifetimes of (a) MOC-53 and (b) Irqpy-2 in solid state at 298 K (λ_{ex} =405 nm).

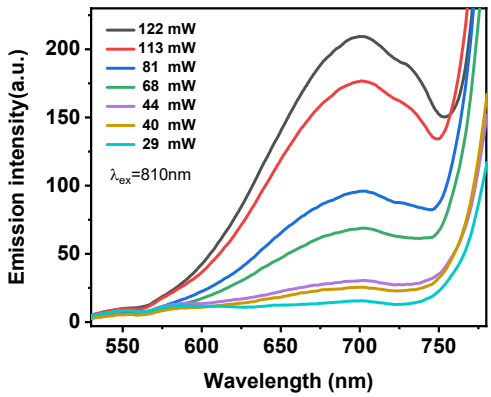


Fig. S8 Fluorescence spectra of two-photon excited of Irqpy-2 (1.25×10^{-6} mol/L) at different excitation power in DMSO (λ_{ex} = 810 nm).

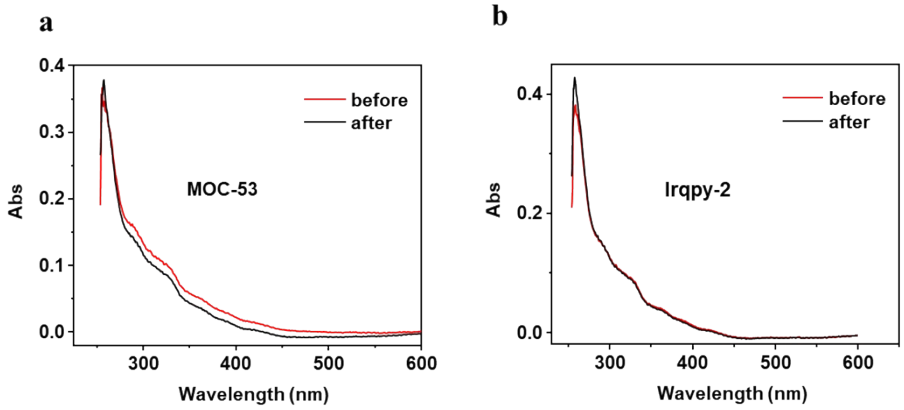


Fig. S9 UV-vis absorption spectra of (a) MOC-53 (1.25×10^{-6} mol/L) (b) Irqpy-2 (1×10^{-5} mol/L)in DMSO solution before and after laser irradiation for 1 h (425 nm 23 mW/cm^2).

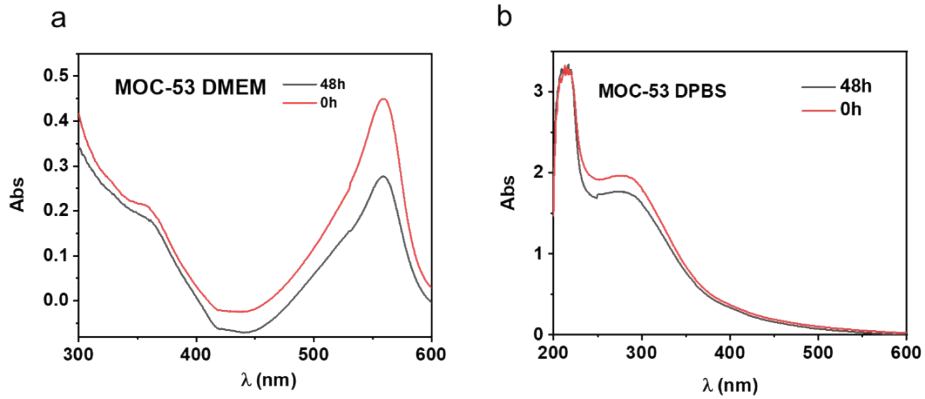


Fig. S10 UV-vis absorption spectra of MOC-53 (1.25×10^{-6} mol/L)in (a) DMEM and (b) DPBS solution before and after 24 h at room temperature.

Table S1 Photophysical properties of Irqpy-2 and MOC-53 (r.t.).

Complex	λ_{ex}/nm	λ_{em}/nm	τ/ns	ϕ_{em}
Irqpy-2	330	660	122.7	7%
MOC-53	330	660	120.9	5%

S4. Quantification of singlet oxygen generation

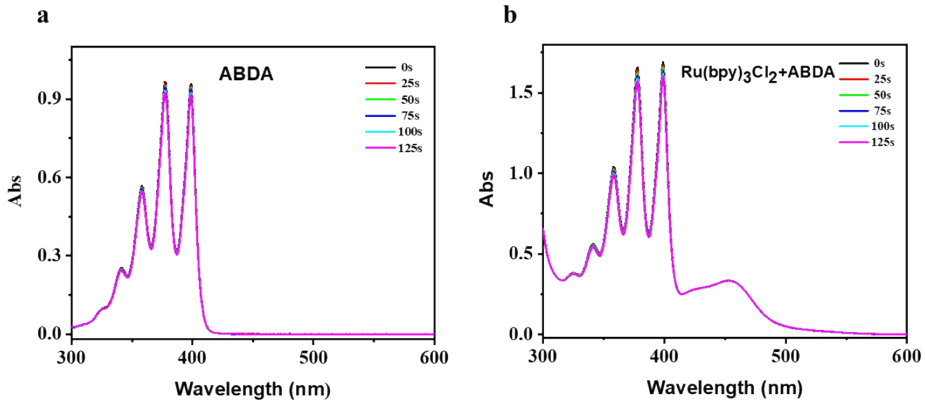


Fig. S11 The quantum yields for $^1O_2$ production (Φ_Δ) by detecting time-evoluted UV-vis absorption spectra of (a) ABDA, (b) $[Ru(bpy)_3]Cl_2$ + ABDA. Condition: 425 nm xenon lamp with filter, 23 mW cm^{-2}.

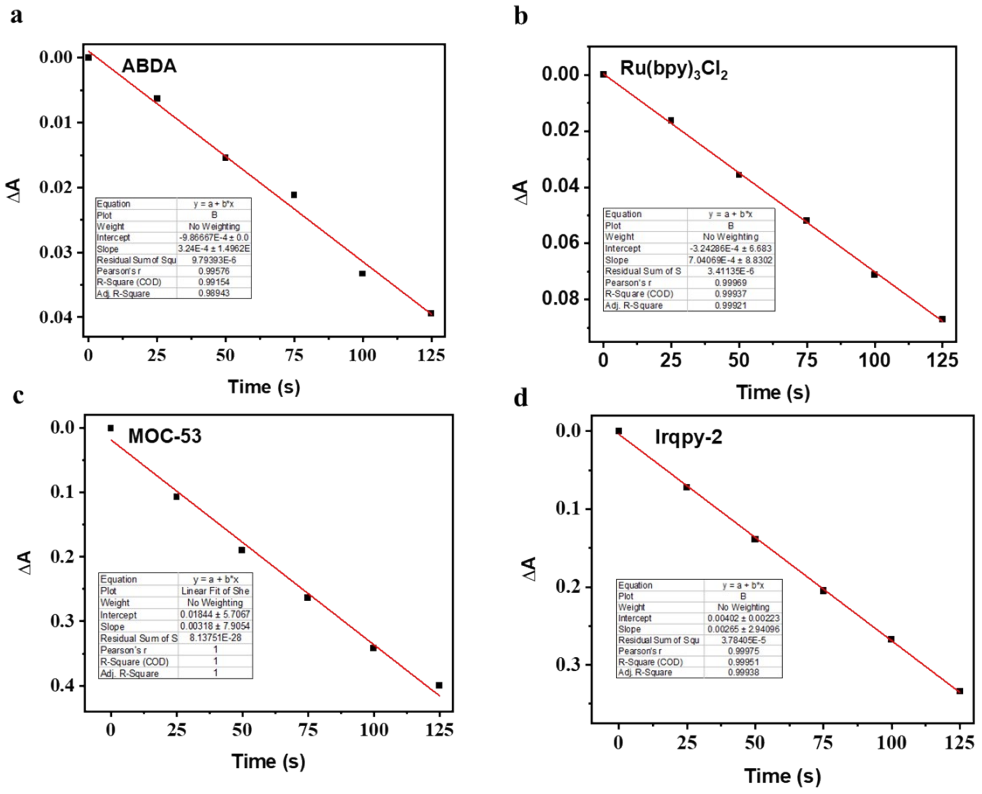


Fig. S12 The slope of plot of the absorption maxima of ABDA against the irradiation time (s). (a) ABDA; (b) $[Ru(bpy)_3]Cl_2$; (c) MOC-53; (d) Irqpy-2. Condition: 425 nm xenon lamp with filter, 23 mW cm^{-2}.

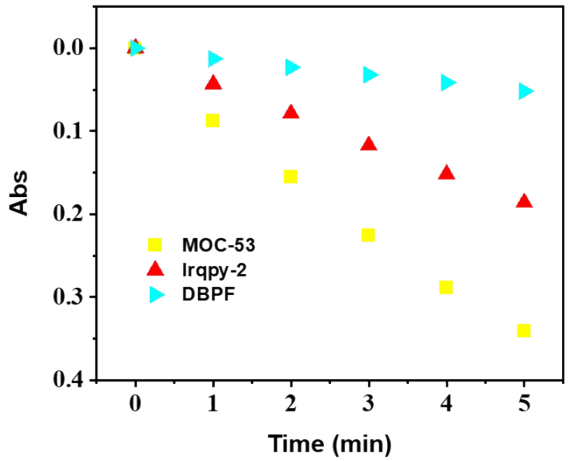
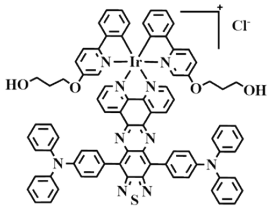
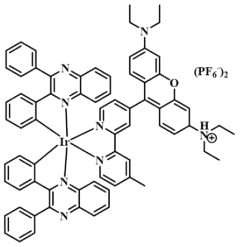
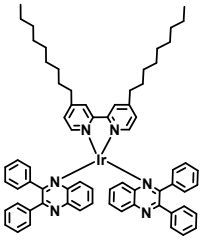
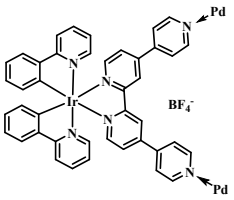
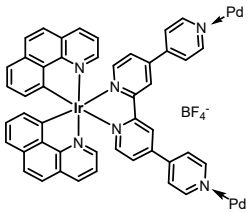


Fig. S13 Changes in the absorption spectra of DPBF at 410 nm upon irradiation of 425 nm light (23 mW cm^{-2}) in the presence of Irqpy-2 and MOC-53 were recorded.

Table S2 The singlet oxygen ($^1O_2$) quantum yield (Φ_Δ) of some Iridium complexes.

Complexes	Φ_Δ
	14.6 % [1]
	72.6 % [2]
	64 % [3]
 **(MOC-51)**	46 % [4]
 **（MOC-53, Present work）**	84 %

S5. Biological section

Materials and Equipment

Dulbecco's Modified Eagle Medium (Hyclone, USA), Penicillin/Streptomycin (Life Techonologies, USA), 0.25% Trypsin-EDTA (Life Techonologies, USA), Fetal Bovine Serum (Life Techonologies, USA), MitoTracker Green (Beyotime, China), LysoTracker Green (Beyotime, China), 4',6-diamidino-2-phenylindole (Shanghai yuanye, China), Dulbecco's Phosphate Buffered Saline (Life Techonologies, USA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoli-um bromide (Sigma Aldrich, USA), 2',7'-dichlorofluorescein diacetate (Solarbio, China), JC-1 assay (Beyotime, China), Annexin V-FITC/PI assay (KeyGEN, China), Caspase -3/7 activity kit (Promega, USA). The cell viability was measured with a Tecan Infinite M200 Microplate Reader (Switzerland). All confocal images were taken with a Carl Zeiss LSM 710 laser scanning confocal microscope (Germany). All compounds tested were dissolved in DMSO just before the experiments, and the final concentration of DMSO was kept at 1% (v/v).

Cell culture

The human cervical carcinoma (HeLa) cells were provided from Experimental Animal Center, Sun Yat-Sen University. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Hyclone) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 ^{o}C in a humidified atmosphere containing 5% CO_2.

Cell viability

For the cell viability assay, the cytotoxicity of Hela cells treated with different concentrations of **MOC-53** was determined by MTT assay. Hela cells were seeded at 10^5 cells per well in a 96-well plate and incubated overnight. The **MOC-53** with a series of different concentrations (0.5, 1, 2 μmol/L) were dissolved in DMSO, followed by diluting with fresh cell medium immediately with 1% (v/v) DMSO. After removing the

cell medium, the cells were then incubated with fresh medium containing different concentrations of **MOC-53** for 12 h. Then the medium was replaced by fresh medium, and then incubated for another 32 h. And 20 μL of MTT (5 mg/mL) solution were added into each well and cultured for additional 4 h. The media of each well were carefully removed, and 150 μL of DMSO for each well were added. The plate was gently shaken for 10 min before measurement. The absorptions were measured with a microplate reader at wavelength of 570 nm. Cells treated with 1% DMSO were kept as the blank control group. Other materials such as Irqpy-2, and $Ru(bpy)_3Cl_2$ were operated under the same procedure.

For phototoxicity, the cells were exposed with various concentrations of **MOC-53** for 12 h and irradiated with a 425 nm LED light (23 mW cm^{-2}). After additional 32 h incubation, 20 μL of MTT was added. The light dose was measured by an optical power meter (CEAULIGHT CEL-NP2000-2) and the viability of the cells preserved in the dark are almost the same as those of the irradiated cells. Other materials such as Irqpy-2, and $Ru(bpy)_3Cl_2$ were operated under the same procedure.

Determination of the lipophilicity

The lipophilicity of MOC-53 was determined by a shake-flask ultraviolet spectrophotometry method as previously reported. The lipophilicity was presented as octanol/water partition coefficients Log Po/w valves, which was defined as the logarithmic ratio of the MOC-53 concentration in octanol phase to that in the aqueous phase. Step 1: 50 mL of n-octanol and 50 mL of water was mixed, and the mixture solution was shaken at room temperature for two days to obtain fully saturated octanol phase and water phase. Step 2: 5 mg of MOC-53 was dispersed in 6 mL saturated octanol phase and shaken at 150 rpm for 24 h, then the octanol solution containing MOC-53 was centrifuged to remove the insoluble samples. 5 mL of supernatant in octanol layer was gently taken out and mixed with 5 mL of saturated water phase, followed by another 24 h shaking at room temperature with 150 rpm of speed. Step 3: the water layer and octanol layer were carefully separated for the next analysis. The concentration of samples in the octanol phase and water phase were measured by using UV-Vis spectrophotometry.

Cellular localization

Hela cells were seeded at a density of 10^5 cells per dish in cell culture dishes (Nest) for overnight. After incubated with **MOC-53** (2 μmol/L) at 37 °C for 2 h, the cells were gently washed with DPBS for three times. Then the adherent cells were visualized by Carl Zeiss LSM 710 confocal microscopy by using 63X oil immersion objective. The excitation wavelength and emission wavelength of **MOC-53** were 405 nm and 670 ± 20 nm, respectively. For the subcellular localization, Hela cells were exposed with **MOC-53** for 2 h at 37 °C. And then co-incubated with the Mito-Tracker Green (150 nmol/L, MTG) or the Lyso-Tracker Green (150 nmol/L, LTG) at 37 °C for another 30 min. Then the cells were washed with DPBS for three times and visualized by confocal microscopy immediately. The excitation wavelength of MOC-53 and LTG were 405 nm. And the excitation wavelengths of MTG was 488 nm, respectively. Emission filter: 670 ± 20 nm (MOC-53), 516 ± 20 nm (MTG), 550 ± 20 nm (LTG).

Detection of mitochondrial membrane potential

Mitochondrial membrane potential was measured by JC-1 assay. Briefly, Hela cells were seeded at a density of 10^5 cells per dish in cell culture dishes (Nest) for overnight. The cells were exposed to the MOC-53 with a concentration of 2 μmol/L in cell medium for 12 h, and then irradiated by 425 nm LED light (23 mW cm^{-2}). After another 8 h incubation in the dark at 37 °C, the JC-1 was added and then incubated for 20 min at 37 °C. Then cells were washed for 2~3 times with ice-cold assay buffer, followed by the imaging by a confocal microscopy. J-monomer: λ_{ex} = 488 nm, λ_{em} = 530 ± 20 nm; J-aggregates: λ_{ex} = 545 nm, λ_{em} = 590 ± 20 nm.

Time-dependent cellular uptake of MOC-53

Hela cells were seeded at a density of 10^5 cells per dish in cell culture dishes (Nest) for overnight. Briefly, the cells were incubated with fresh medium containing 2 μmol/L of **MOC-53** for different incubation time (30, 60, 120 and 150 min). Then the medium was removed, cells were washed with DPBS for 3 times. Followed by the imaging by a confocal microscopy (λ_{ex} = 405 nm, λ_{em} = 670 ± 20 nm).

Detection of cellular uptake mechanism

Hela cells were seeded at a density of 10^5 cells per dish in cell culture dishes (Nest) for overnight. Briefly, the cells were incubated with fresh medium containing 2 μmol/L of **MOC-53** for different incubation time (4 ^{o}C, 25 ^{o}C, 37 ^{o}C). Then the medium was removed, cells were washed with DPBS for 3 times. Followed by the imaging by a confocal microscopy. λ_{ex} = 405 nm, λ_{em} = 670 ± 20 nm.

The detection of cellular reactive oxygen species (ROS)

The ROS assay kit was employed to detect the cellular ROS level. Once the 2,7-dichlorofluorescein diacetate (DCFH-DA) was passively diffused in the cells, the intracellular esterase could cleave the acetate groups thus remained the DCFH. Then the non-fluorescent DCFH could be oxidized by the intracellular ROS to form the fluorescent DCF. Hela cells were seeded in cell culture dishes (Nest) for 24 h. The cells were incubated with **MOC-53** (2 μmol l^{-1}) for 12 h, followed by the exposure to the 425 nm LED light (23 mW cm^{-2}). Then the cell culture medium containing **MOC-53** were removed, and the cells were further treated with DCFH-DA (10 μmol l^{-1}) in the serum-free DMEM at 37 °C for another 20 min. After washed with DPBS for 3 times, the cells were immediately imaged by the confocal microscopy. The excitation and emission wavelength for the DCF was 488 nm and 525 ± 20 nm, respectively.

Annexin V/PI staining assay

The assay was performed according to the manufacturer's (KeyGEN, China) protocol. Cells treated with **MOC-53** for 24 h and harvested in tube stained with annexin V reagent at room temperature for 15 min in the dark. The cells were immediately imaged by the confocal microscopy. The excitation and emission wavelength for Annexin V was 488 nm and 525 ± 20 nm, respectively. The excitation and emission wavelength for PI was 561 nm and 570-670 nm.

Caspase 3/7 activity assay

Caspase 3/7 activity was measured using the Caspase-Glo®Assay kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Cells cultured in 96 well plates were treated with MOC-53 for 24 h. After incubation, 100 μL of Caspase Glo® 3/7 reagent was added to each well containing 100 μL culture medium. The mixture was incubated at room temperature for 1 h and then luminescence was measured using a micro-plate reader (Infinite M200 Pro, Tecan, Switzerland).

In vivo PDT experiment

BALB/c-female nude mice of the age of six weeks were purchased and bred following the protocols of the laboratory animal center. All experimental protocols were approved by the Sun Yat-sen University Animal Care and Use Committee. Each mouse was subcutaneously injected with 100 mL (1-2*107/ml) CT-26 single-cell suspension in a mixture of PBS, and the CT-26 xenografted tumor models were established in 1-2 weeks. The tumor size was tracked by measuring the length and width of the tumor. When the volume of the tumor reached *ca.* 100 mm3, the mice were randomly allocated into six groups (5 mice per group). The PDT process spaining 20 days was divided into five courses, and each course took four days (i.e., the mice received PDT every four days). The volume of the tumors and the weight of the mice were recorded every four days. The tumor volume was evaluated by the following formula

$$V = \frac{1}{2} \times Width^2 \times Length$$

We randomly divided mice into six groups (five mice per group): 1) PBS, 2) PBS + Light, 3) Irqpy-2, 4) Irqpy-2 + Light, 5) MOC-53, 6) MOC-53 + Light (termed as two-photon PDT). Upon completion of the 20-day PDT therapeutics, all mice were sacrificed. One mouse from each group was randomly chosen and its tumor was carefully carved out and washed with 4% paraformaldehyde. A digital color camera was used to photograph the mice and tumors.

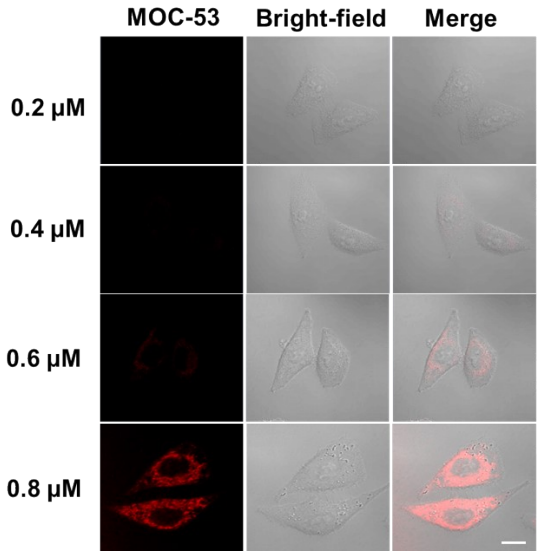


Fig. S14 Confocal fluorescence images of MOC-53 uptake in Hela cells at 37 ℃ for 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM, respectively. Scale bar: 20 mm

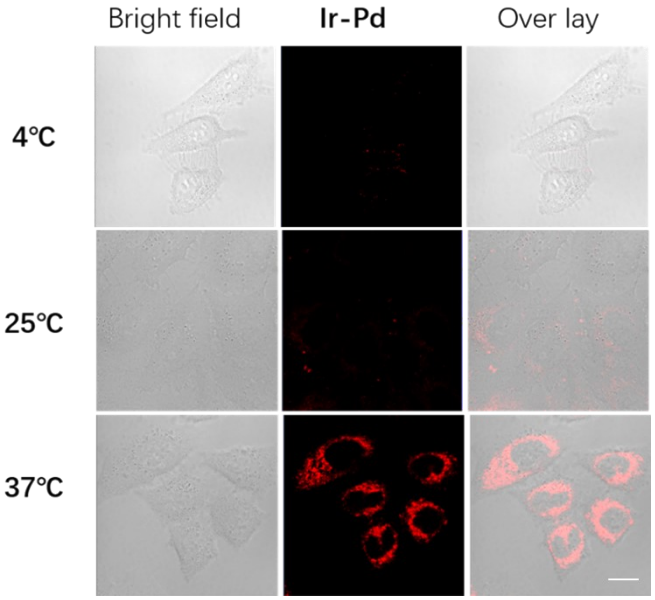


Fig. S15 Confocal fluorescence images of Hela cells cultured with MOC-53 (2 μM) under different experimental temperature. Scale bar: 20 mm

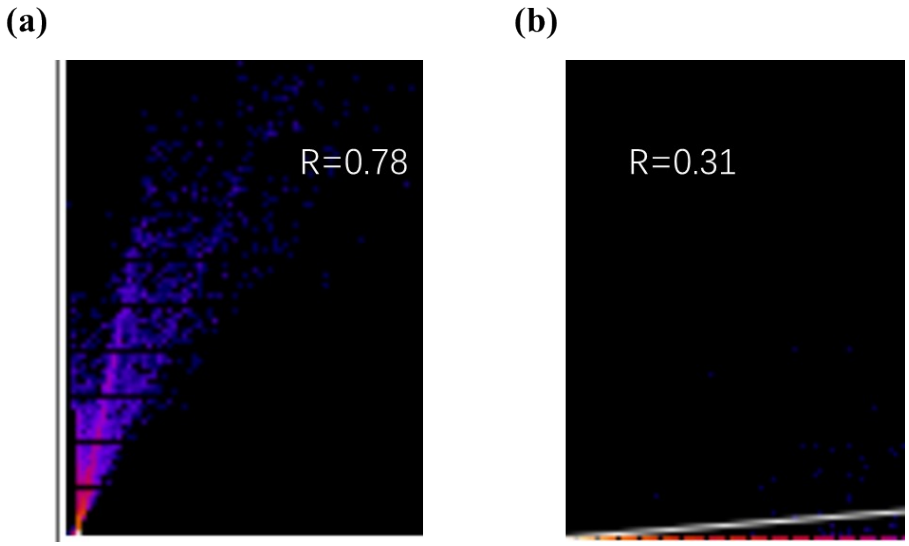


Fig. S16 Corresponding correlation coefficients of a) MOC-53/MTG channels, b) MOC-53/LTG channels

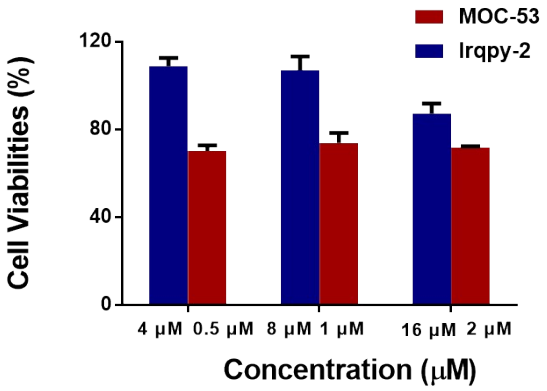


Fig. S17 The dark cell viabilities and phototoxicity (425 nm, 23 mW cm^{-2}) of Hela cells treated with MOC-53 and Irqpy-2, respectively.

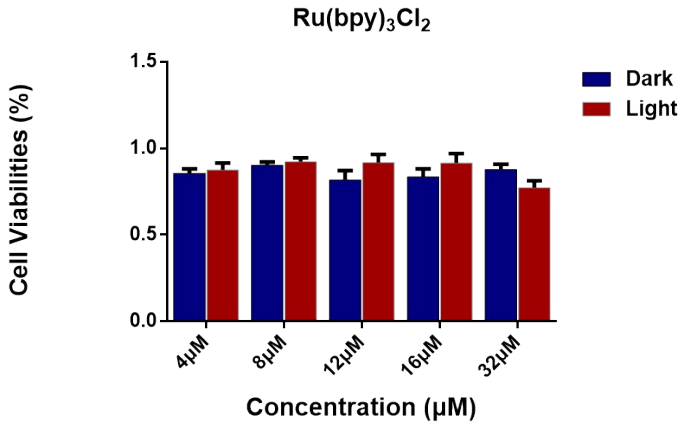


Fig. S18 The dark cell viabilities and phototoxicity (425 nm, 23 mW cm^{-2}) of Hela cells treated with $Ru(bpy)_3Cl_2$.

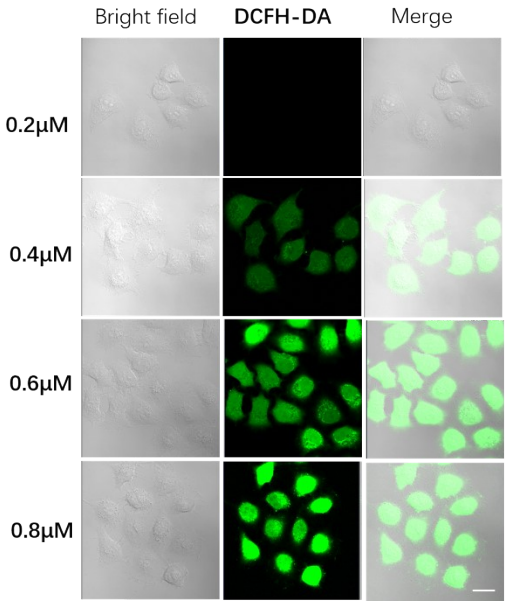


Fig. S19 Images of ROS generation in HeLa cells incubated with different concentrations of MOC-53. Scale bar: 20 mm.

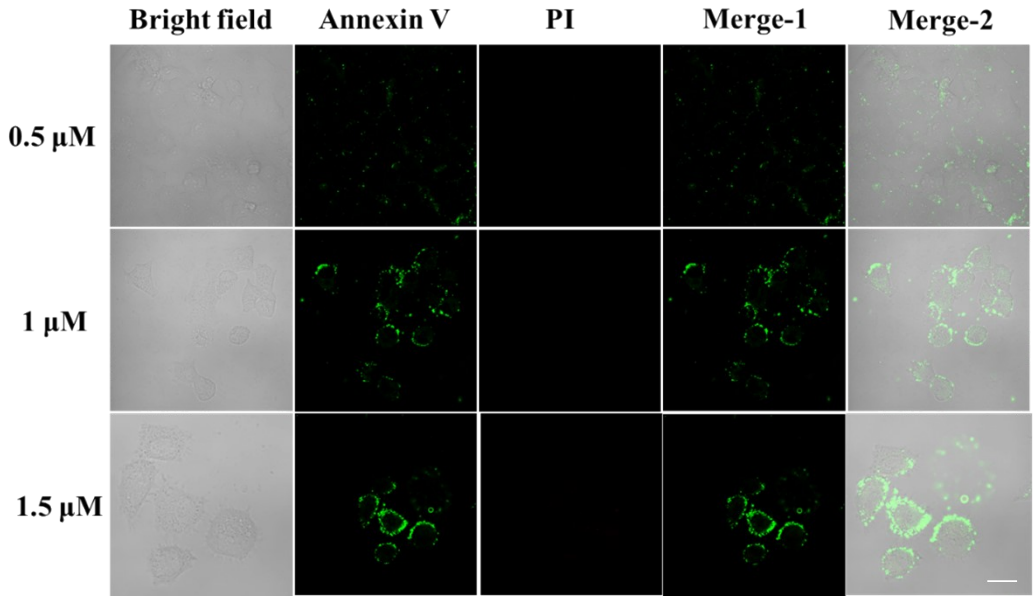


Fig. S20 Confocal fluorescence images of Hela cells incubated with different concentrations of MOC-53 and Annexin V-FITC/PI (10 min) before and after irradiation (425 nm, 23 mW cm^{-2}). Scale bar: 20 mm.

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