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for

Anticancer Profile of Coumarin 6-Based Ir(III) Photocatalysts under Normoxia and Hypoxia by ROS generation and NADH oxidation†

Ashish Kumar Yadav,[a] Aarti Upadhyay,[b] Arpan Bera,[b] Rajesh Kushwaha,[a] Arif Ali Mandal,[a] Sagar Acharjee,[a] Amit Kunwar,*[c,d] and Samya Banerjee*[a]

[a]*Department of Chemistry, Indian Institute of Technology (BHU) Varanasi, Uttar Pradesh 221005, India.* [b]*Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore, 560012, India.* [c]*Radiation and Photochemistry Division, Bhabha Atomic Research Center, Anushaktinagar, Mumbai 400085, India.* [d]*Homi Bhabha National Institute, Anushaktinagar, Mumbai – 400094, India*

* Corresponding authors' Emails: samya.chy@itbhu.ac.in; kamit@barc.gov.in

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Experimental Section

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Materials

$IrCl_3.xH_2O$ was purchased from Sigma Aldrich, and 1,10-phenanthroline was purchased from Finar Ltd. (India). Coumarin 6 was purchased from E. Merck India Pvt. Ltd. 1,2-phenylenediamine dihydrochloride, 9-anthracenecarboxaldehyde, ammonium acetate, methylene blue, glacial acetic acid and $Ru(bpy)_3Cl_2$ were purchased from Sigma Aldrich. Potassium bromide, tetrabutylammonium hexafluorophosphate, H_2SO_4, and HNO_3 were purchased from BLD Pharma. 1,3-diphenylisobenzofuran (DPBF), 2′,7′-Dichlorodihydrofluorescein diacetate (DCFDA), tetrabutylammonium hexafluorophosphate, Sulforhodamine B (SRB) and β-Nicotinamide adenine dinucleotide, reduced disodium salt (β-NADH) were purchased from Sigma Aldrich. Quantofix peroxide test sticks were purchased from Sigma Aldrich. 2-ethoxyethanol, trichloroacetic acid, methanol, dichloromethane (DCM), dimethyl sulfoxide (DMSO), and chloroform were purchased from Merck Life Science Pvt. Ltd., Dulbecco′s Phosphate Buffered Saline was purchased from Sigma Aldrich. A549, HeLa, and Beas-2B cell lines were procured from NCCS Pune India. Dulbecco's Modified Eagle Medium (DMEM) and 12 well cell culture plates were purchased from Genetix Pvt. Ltd. T-25 flask and 96 well plates were obtained from Eppendorf. 12 well cell culture plates were obtained from Genetix Biotech Asia Pvt. Ltd. FBS (Fetal Bovine Serum), Trypsin-EDTA, and Penicillin-streptomycin were procured from Gibco. JC-1 and Annexin V-FITC/PI Apoptosis Detection Kit were purchased from Genetix Biotech Asia Pvt. Caspase 3 activity assay kit (QIA 70) was procured from Calbiochem, USA, through local vendors. 1,10-phenanthroline-5,6-dione was prepared according to the reported literature method.[1] Dipyrido[3,2-a:2′,3′-c]phenazine (dppz) was prepared following a literature procedure using 1,10-phenanthroline-5,6-dione as a precursor.[1] 2-(9-Anthryl)-1H-imidazo[4,5-f][1,10]phenanthroline (aip) was prepared following a literature procedure using 1,10-phenanthroline-5,6-dione, 9-anthracenecarboxaldehyde, ammonium acetate, and glacial acetic acid.[2]

Instrumentation

Agilent Cary 60 UV-Vis spectrophotometer was used for recording the UV-Vis spectral data. MaXis impact 282001.00081 was used for the HR-MS. Fluorescence measurements were performed on a Techcomp FL970 fluorescence spectrophotometer. Cyclic voltammetric

measurements were done using a PAR model 273A electrochemistry system. In the 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) assay, the absorbance value of formazan was recorded using a TECAN microplate reader, and the graph was plotted using GraphPad Prism 6 software. In the SRB assay, Absorbance was measured at 564 nm using a microplate reader, and the IC_{50} values were calculated using GraphPad Prism 8 software. Flow cytometric experiments were performed using Becton Dickinson fluorescent-activated cell sorting (BD-FACS) Verse instrument (BD Biosciences). FACS analysis data acquisition and analysis were done using Windows 7 operating system and BD-FACS suite software. Confocal laser scanning microscopy (CLSM) was done using a Zeiss LSM 880 with Airyscan microscope containing an oil immersion lens. Confocal microscopic images of the JC-1 assay were taken at a magnification of 10x, and co-localization images were taken at a magnification of 63x.

NMR spectroscopy

NMR spectra were acquired at 293 K by Bruker DPX 500 (^{1}H = 500 MHz, ^{13}C = 125 MHz) spectroscopy. All data processing was carried out using MestReNova.

UV-Vis spectra

Agilent Cary 60 UV-Vis spectrophotometer was used with 1 cm path-length quartz cuvettes. Spectra were processed using Origin 2019b 64 Bits software. The UV-vis spectra of **Ir1** and **Ir2** in methanol were taken at 293 K from 250-700 nm.

Fluorescence spectra

The fluorescence spectra of complexes **Ir1** and **Ir2** (20 μM) in DMSO: water (1:9 v/v) were recorded with excitation at λ_{ex} = 460 nm in a 1 cm quartz cuvette at room temperature.

Photocatalytic reactions of complex Ir1 and Ir2 with NADH

Reactions between complexes **Ir1**/**Ir2** (2 μM), and NADH at different time intervals were monitored by UV-vis at 298 K on irradiation with green light (525 nm, 50.2 J cm^{-2}). Turnover number (TON) is defined as the number of moles of NADH that a mole of the complexes can convert within 2 min. Turnover frequency (TOF) was calculated from the difference in NADH concentration after 2 min irradiation divided by the concentration of complexes. The concentration of NADH was 240 μM.

$[NAD^{+}] = [Abs(340\ nm)_{initial} - Abs(340\ nm)_{final}]/Abs(340\ nm)_{initial} * [NADH]$

Turnover number (TON) = $[NAD^+]$/[Catalyst]

Turnover frequency (TOF) = Turnover number/time (h).

Cyclic Voltammetry

Cyclic voltammetric measurements were done using a PAR model 273A electrochemistry system. A glassy carbon working electrode, platinum wire auxiliary electrode, and a saturated calomel reference electrode (SCE) were used in a standard three-electrode configuration with tetrabutylammonium hexafluorophosphate as the supporting electrolyte (**Ir1, Ir2** concentration ≈ 1.0 mM; standard scan rate 100 mV s^{-1}). Cyclic voltammograms were scanned from −2.0 V to +2.0 V.

Detection of H_2O_2 generation

For the reaction of complex **Ir2** (2 μM) with NADH (240 μM) in 0.5% DMSO/99.5% PBS (v/v) at 293 K in the dark or after irradiation, H_2O_2 was detected by Quantofix peroxide test sticks.

Determination of singlet oxygen generation

The production of photo-induced singlet oxygen by the complexes was detected using the singlet oxygen sensor DPBF. Briefly, **Ir1/Ir2** (2 μM) in 0.5 % DMSO and 99.5% PBS (v/v) was mixed with DPBF (50 μM). The solution was then placed in quartz cuvettes followed by 525 nm (50.2 J cm^{-2}) light irradiation for different times at 298 K. The absorbance of the DPBF was then monitored by UV-vis spectroscopy at 298 K.

The $^1O_2$ generation quantum yield (Φ_Δ) of **Ir1** and **Ir2** was measured by recording UV-Vis absorption spectra of DPBF from 200-800 nm. **Ir1**, **Ir2** and $[Ru(bpy)_3]Cl_2$ were diluted in 0.5% DMSO and 99.5% PBS (v/v) solution to reach 0.1 absorbance at the irradiation wavelength, 525 nm. The solution was then irradiated in 1 cm path-length quartz cuvettes at intervals of 5 sec. The relative $^1O_2$ generation quantum yields were determined with $[Ru(bpy)_3]Cl_2$ ($\Phi_{\Delta s}$ = 0.22 in aq. DMSO) as the standard using the following equation: $\Phi_{\Delta x} = \Phi_{\Delta s} * (Fx/Fs) * (As/Ax)$ Where ΦΔ represents $^1O_2$ generation quantum yield; F stands for the integrated area under the corrected emission spectrum; A is the absorbance at 525 nm, the excitation wavelength; and the subscripts x and s refer to the complex sample and the standard, respectively.

Determination of hydroxyl radical generation

The production of photo-induced hydroxyl radical by the complexes was detected using the hydroxyl radical sensor methylene blue. Briefly, **Ir1/Ir2** (2 μM) in 0.5 % DMSO and 99.5% PBS (v/v) was mixed with methylene blue (15 μM). The solution was then placed in quartz cuvettes followed by 525 nm (50.2 J cm^{-2}) light irradiation for different times at 298 K. The absorbance of the methylene blue was then monitored by UV-vis spectroscopy at 298 K.

Photo-stability of complexes

UV-Vis spectroscopy was used to study the stability of complexes. The solution (containing 0.5 % DMSO and 99.5 % PBS (v/v)) of complexes **Ir1** and **Ir2** (2 μM) was prepared at ambient temperature. UV-Vis spectra of that solution were recorded at room temperature at different intervals of time after light irradiation with white light (400-700 nm, 10 J cm^{-2}). Mapping with the absorbance change of complexes *vs* irradiation time was done.

Cell culture and cell assays

MTT assay

A cytotoxicity assessment was conducted on A549 cells (human lung adenocarcinoma cell line), HeLa cells (a human cervical cancer cell line), and Beas-2B cells (non-tumorigenic human bronchial epithelial cell line) to evaluate the cytotoxic impact of complexes **Ir1** and **Ir2**. The cells were cultured in a mixture of 10% FBS and DMEM at 37°C in a 5% CO_2 environment. In 96-well plates, approximately 8000 cells of each type (A549, HeLa, and Beas-2B) were seeded. The cells were subjected to a variety of concentrations, commencing with an initial concentration of 50 μM, and subsequently, a serial dilution was performed, reducing the concentration to as low as 0.195 μM for both complexes **Ir1** and **Ir2**. This treatment was carried out in a medium consisting of 1% DMSO and DMEM for a duration of 4 h, all while being conducted in a light-restricted environment. Following the removal of the compound-containing medium, Dulbecco's phosphate-buffered saline (DPBS) was used to wash the cells. Subsequently, one set of cells was exposed to visible light for 1 h with a wavelength of 400-700 nm and a dose of 10 J cm^{-2}. After the light treatment, fresh DMEM media were added to the cells. The other set of cells was maintained in darkness with the fresh DMEM medium. Both sets of cells were then incubated in the dark for an additional 43 h, resulting in a total incubation period of 48 h. The IC_{50} values were determined using GraphPad Prism 8 software. MTT assay of cis-platin in Beas-2B cells was performed under similar conditions. For hypoxic

conditions (1% O_2), once the cells adhered, they were moved to a hypoxic incubator, where they were placed inside a biological safety cabinet within a glove box containing an atmosphere of 1% oxygen, 5% carbon dioxide, and 94% nitrogen for an additional 24 hours. This allowed the cells to adjust to low oxygen levels. Throughout the experiment, incubation occurred under consistent hypoxic conditions.

Sulforhodamine B (SRB) Assay

The cytotoxicity of the complexes was evaluated in vitro using the sulforhodamine B (SRB) assay. Human lung cancer cells (A549) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C under a 5% CO_2 atmosphere. Approximately 8000 cells were seeded into each well of a 96-well plate. The cells were then treated with various concentrations of the complexes, starting at 50 μM and serially diluted down to 0.195 μM. The treatment was conducted in a medium composed of 1% dimethyl sulfoxide (DMSO) and DMEM for 4 h in the absence of light. After treatment, the medium was removed, and the cells were washed with Dulbecco's phosphate-buffered saline (DPBS). One group of cells was then exposed to visible light (400-700 nm) with an intensity of 10 J cm^{-2} for 1 h. Following light exposure, the cells were replenished with fresh DMEM. The other group of cells was kept in the dark with a fresh DMEM medium. Both sets of cells were incubated for an additional 43 h in the dark, completing a total incubation period of 48 h. For the SRB assay, 50 μL of cold 1% trichloroacetic acid (TCA) was gently added to each well to achieve a final concentration of 10%. The plates were then incubated at 4 °C for 1 h to fix the cells. After fixation, the plates were washed four times with distilled water and allowed to air-dry. Subsequently, 100 μL of SRB dye solution (0.4% weight/volume in 1% acetic acid) was added to each well and incubated at room temperature for 1 h. The plates were then washed with 1% acetic acid to remove any unbound dye, air-dried again, and 100 μL of Tris buffer (10 mM, pH 10.5) was added to each well. The plates were gently shaken on a mechanical shaker for 15 min. Absorbance was measured at 564 nm using a microplate reader, and the IC_{50} values were calculated using GraphPad Prism 8 software.

Confocal microscopy

In aseptic conditions, approximately 1×10^5 A549 cells were seeded onto glass-bottomed petri dishes and allowed to adhere for 24 h in a CO_2 incubator. Subsequently, a solution of the complex at a concentration of 30 μM was introduced into the glass-bottom dishes, and the cells were incubated at 37 °C for 4 h. Following this incubation period, the cells were thoroughly

washed with DPBS. To label specific organelles, the cells were then exposed to staining agents: Hoechst dye at a concentration of 5 µg/ml, and MitoTracker red at 250 nM. Finally, triple rinsing with PBS was performed, and images were acquired using an oil immersion lens with a magnification of 63X. Image acquisition and processing were carried out utilizing ZEN 3.3 (blue edition software).

In-cell NADH oxidation

The detection of NAD^+ and NADH in the cell lysate was done by spectrophotometric assay described in the previous report.[3] Briefly, cells (1×10^6) cells were seeded into 100 mm culture disc and allowed to adhere for 24 h. Following this, cells were treated with 25 µM of complex **Ir2** and maintained under dark and light conditions as mentioned in previous section. After light exposure the cells were cultured for 24 h, harvested through trypsinization and cell lysate prepared using cellytic M® containing protease inhibitor cocktail. A portion of cell lysate was used for determination of protein content using Bradford assay as per manufacturer's instruction. The remainder of cell lysate was divided into two aliquots and extracted in two different solutions: the alkali extraction for NADH and the acid extraction for NAD^+. Both extractions were adjusted to neutral pH prior to performing the recycling assay to determine the concentration of NAD and NADH as mentioned in the previous reference.

DCFDA assay

2′,7′-Dichlorofluorescein diacetate (DCFDA) assay was used to detect intracellular ROS in which emission of green fluorescent oxidized DCFDA at 400-700 nm was compared for the irradiated and non-irradiated samples. A total of 1×10^5 cells were seeded onto glass-bottom dishes and permitted to adhere over a 24 h incubation period in a controlled incubator environment. Following this, the cells were subjected to treatment with complex **Ir2** at a concentration of 1 µM and were incubated in the dark at 37 °C with a 5% CO_2 atmosphere for a duration of 4 h. After the incubation period, the cells underwent a thorough washing with PBS buffer, repeated three times. The cells were detached using a 0.25% Trypsin-EDTA solution at 37 °C for 5 min, and the trypsin action was neutralized by adding media enriched with 10% FBS. Following this, the cells were treated with DCFDA (10 µM). A division was made: one batch of cells was exposed to photo-irradiation spanning from 400 to 700 nm at a dosage of 10 J cm^{-2} for 1 h, while the other batch was kept in darkness. Both sets of cells were then incubated in the dark for an additional 4 h, and a flow cytometric examination was conducted utilizing FACS VERSE equipment from BD Biosciences. The acquired data were

subsequently processed and analyzed through FCS Express 7 software, developed by DeNovo Software.

JC-1 assay

Approximately 1×10^5 A549 cells were seeded into 12-well plates and given a 24 h window to adhere. Subsequently, the cells were treated with the complex **Ir2** at a concentration of 1 μM and allowed to incubate in darkness at 37 °C for 4 h. Following this incubation period, the cells were subjected to a PBS buffer wash. After that, one group of cells was exposed to visible light with 10 J cm^{-2} light dose, while the other group remained in darkness. Cells were then incubated for 6 h post-irradiation. To visualize changes, the cells were then stained using JC-1 dye, and confocal images were captured at a 10X magnification. The acquired images were processed using ZEN 3.3 (blue edition) software.

Annexin V-FITC/Propidium Iodide assay

In 6-well plates, ~10^5 cells were initially seeded and allowed to incubate for 24 h. Following this, complex **Ir2** was introduced to the cells at a concentration of 2 μM, and they were left to incubate for 4 h in a light-restricted environment. Subsequently, two distinct groups were established: one group of cells was exposed to visible light with the light of wavelength 400-700 nm (10 J cm^{-2}) for 1 h, while the other group remained in the dark. An additional 43 h of incubation was provided to both sets of cells. Following this incubation period, the cells were trypsinized to detach them, and single-cell suspensions were prepared. To each sample, 500 μL of binding buffer was added. Then, 0.5 μl annexin and 0.5 μl propidium iodide were introduced into each sample containing the binding buffer, and a 10 min incubation followed. Finally, flow cytometry analysis was conducted.

Caspase 3 activity assay

The detection of caspase 3 activity was done in the cell lysate with a spectrofluorometric assay kit (QIA 70, Calbiochem, USA). Briefly, cells (2 x 10^6) cells were seeded into a 100 mm culture disc and allowed to adhere for 24 h. Following this, cells were treated with 25 μM of complex **Ir2** and maintained under dark and light conditions, as mentioned in the previous section. After light exposure, the cells were cultured for 48 h, harvested through trypsinization, and processed as per the manufacturer's instructions. The fluorescence emission was monitored using a multi-well plate reader (Bio Tek, Synergy Hybrid, USA).

Synthesis and Characterization

Synthesis of Ir(III) μ-chloro-bridged dimer

$[Ir(CO6)_2Cl]_2$ dimers were prepared using a previously reported method with slight modifications.[4] Under dark conditions, 0.28 mmol of $IrCl_3.xH_2O$ and 0.58 mmol of coumarin 6 were dissolved in a mixture of 2-ethoxyethanol and water (16 mL; 3:1 v/v). The reaction mixture was refluxed at 115 °C for 24 h. After cooling to room temperature, the precipitate was filtered off and washed with diethyl ether. The orange solid Ir(III) μ-chloro-bridged dimer was obtained.

Synthesis of Ir1-Ir2

To synthesize the complex **Ir1** and **Ir2**, 0.5 mmol of $[Ir(CO6)_2Cl]_2$ dimer and 1.1 mmol of N,N donor ligand were dissolved in 20 mL of $CHCl_3$/MeOH (3:1; v/v). The reaction mixture was refluxed at 60 °C for 12 h. After cooling to room temperature, the solvent was removed under reduced pressure. The resulting solids were extracted with DCM and water. Finally, Na_2SO_4 was added to the above extracted DCM solution to remove any water content. The solvent was then removed under a vacuum to afford the crude product. The crude product was purified by recrystallization from MeOH.

Complex **Ir1:** Yield = 75 %, $C_{58}H_{44}ClIrN_8O_4S_2$ (MW = 1208.83): calcd. C = 57.63, H =3.67, N = 9.27, exp. C = 57.92, H = 3.81, N = 9.16, UV−visible spectral data were recorded in MeOH where λ_{max} = 462 nm ($\varepsilon = 1.22 \times 10^4$ M^{-1} cm^{-1}), 494 nm ($\varepsilon = 1.42 \times 10^4$ M^{-1} cm^{-1}), λ_{em} = 502 nm. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.74 (d, J = 7.8 Hz, 2H), 9.38 (d, J = 5.0 Hz, 2H), 8.39 (dd, J = 7.9, 5.5 Hz, 2H), 8.30 (s, 2H), 8.09-8.00 (m, 4H), 7.09 (t, J = 7.6 Hz, 2H), 6.82 (t, J = 7.8 Hz, 2H), 6.50 (d, J = 13.6 Hz, 2H), 6.06 (dt, J = 11.3, 5.7 Hz, 4H), 5.84 (d, J = 8.5 Hz, 2H), 3.31 (s, 8H), 0.97 (t, J = 6.8 Hz, 12H). HR-MS (m/z): calcd = 1173.2556 $[M]^+$, exp. = 1173.2549.

Complex **Ir2:** Yield = 72%, $C_{67}H_{50}ClIrN_8O_4S_2$ (MW = 1322.98): calcd. C = 60.83, H = 3.81, N = 8.47, exp. C = 61.05, H = 3.78, N = 8.53, UV−visible spectral data were recorded in MeOH where λ_{max} = 346 ($\varepsilon = 8.39 \times 10^3$ M^{-1} cm^{-1}), λ_{max} = 365 ($\varepsilon = 9.38 \times 10^3$ M^{-1} cm^{-1}), λ_{max} = 385 ($\varepsilon = 1.00 \times 10^4$ M^{-1} cm^{-1}), λ_{max} = 456 nm ($\varepsilon = 2.47 \times 10^4$ M^{-1} cm^{-1}), 478 nm ($\varepsilon = 3.19 \times 10^4$ M^{-1} cm^{-1}), λ_{em} = 495 nm. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.23 (s, 4H), 9.01 (dd, *J* = 21.3, 14.2 Hz, 1H), 8.89 (s, 1H), 8.30 – 8.21 (m, 4H), 8.05 (d, *J* = 7.8 Hz, 2H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.56 (d, *J* = 7.0 Hz, 2H), 7.46 (s, 2H), 7.15 (t, *J* = 7.4 Hz, 2H), 6.84 (t, *J* = 7.5 Hz, 2H),

6.52 (s, 2H), 6.07 (t, J = 9.4 Hz, 4H), 5.78 (d, J = 8.4 Hz, 2H), 3.12 (d, J = 39.0 Hz, 8H), 0.97 (s, 12H). HR-MS (m/z): calcd = 1287.3026 $[M]^+$, exp.= 1287.2974.

Tables

Table S1. Energy (eV) of the lowest vertical 10 singlet–singlet ($S_0 \rightarrow S_n$; n = 1 to 10) transitions for the complexes computed at the TD-B3LYP /LANL2DZ/6-31g* level of theory in water. The oscillator strengths are indicated in parentheses.

	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
Ir1	2.125 (0.0063)	2.147 (0.0047)	2.392 (0.0045)	2.411 (0.0016)	2.546 (0.1263)	2.559 (0.0748)	2.697 (0.4638)	2.712 (0.4994)	2.844 (0.5072)	2.845 (0.050)
Ir2	2.408 (0.0181)	2.428 (0.0070)	2.513 (0.0651)	2.575 (0.1135)	2.588 (0.0738)	2.712 (0.6292)	2.734 (0.777)	2.745 (0.0086)	2.905 (0.3110)	2.916 (0.0511)

Table S2. Energy (eV) of the lowest vertical 10 singlet–triplet ($S_0 \rightarrow T_n$; n = 1 to 10) transitions for the complexes computed at the TD-B3LYP /LANL2DZ/6-31g* level of theory in water.[a]

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
Ir1	2.025	2.030	2.143	2.156	2.212	2.383	2.405	2.558	2.563	2.574
Ir2	1.684	2.047	2.052	2.398	2.423	2.432	2.574	2.582	2.596	2.752

Table S3: IC_{50} values of **Ir1** and **Ir2** against A549 cancer cells determined by SRB assay.

Complex	Dark	Light
Ir1	45.9 ± 3.2	0.9 ± 0.3
Ir2	42.1 ± 2.7	0.7 ± 0.1

White light irradiation (400−700 nm, 10 J cm^{-2}). Light treatment: Incubation time: 4 h, Total irradiation = 10 J cm^{-2} over 1 h. Recovery time: 43 h. Dark treatment: Incubation time: 4 h, Recovery time: 43 h.

Table S4: IC_{50} (μM) values of **Ir1** and **Ir2** under hypoxic conditions.

Complex	A549		
	Dark	**Light**	**PI**
Ir1	54.58 ± 2.3	3.34 ± 0.09	16.3
Ir2	51.76 ± 1.1	1.71 ± 0.06	30.2

White light irradiation (400−700 nm, 10 J cm^{-2}). Light treatment: Incubation time: 4 h, Total irradiation = 10 J cm^{-2} over 1 h. Recovery time: 43 h. Dark treatment: Incubation time: 4 h, Recovery time: 44 h.

Figures

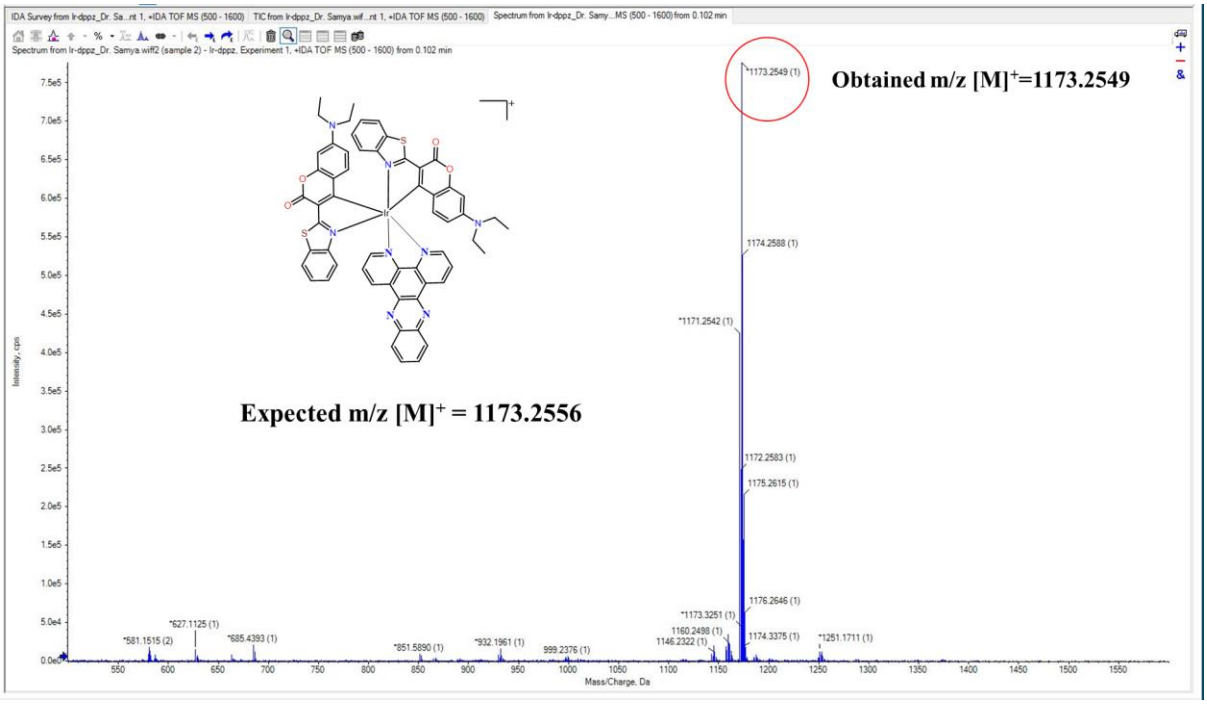

Fig. S1: HR-MS of complex **Ir1** in acetonitrile.

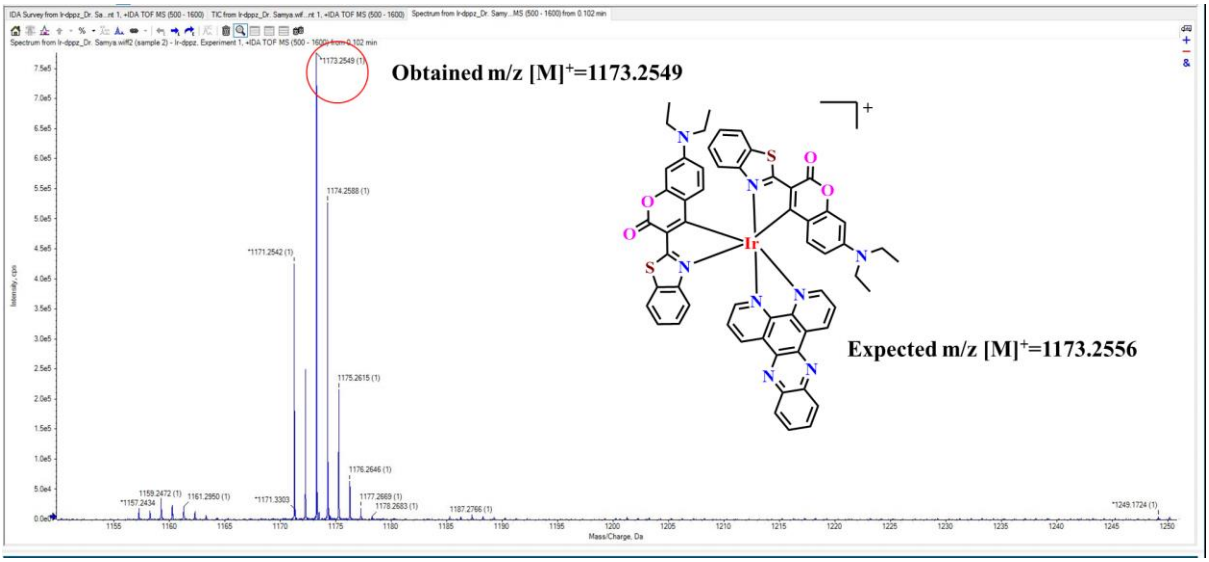

Fig. S2: - HR-MS of spectra of complex **Ir1** in acetonitrile (zoomed).

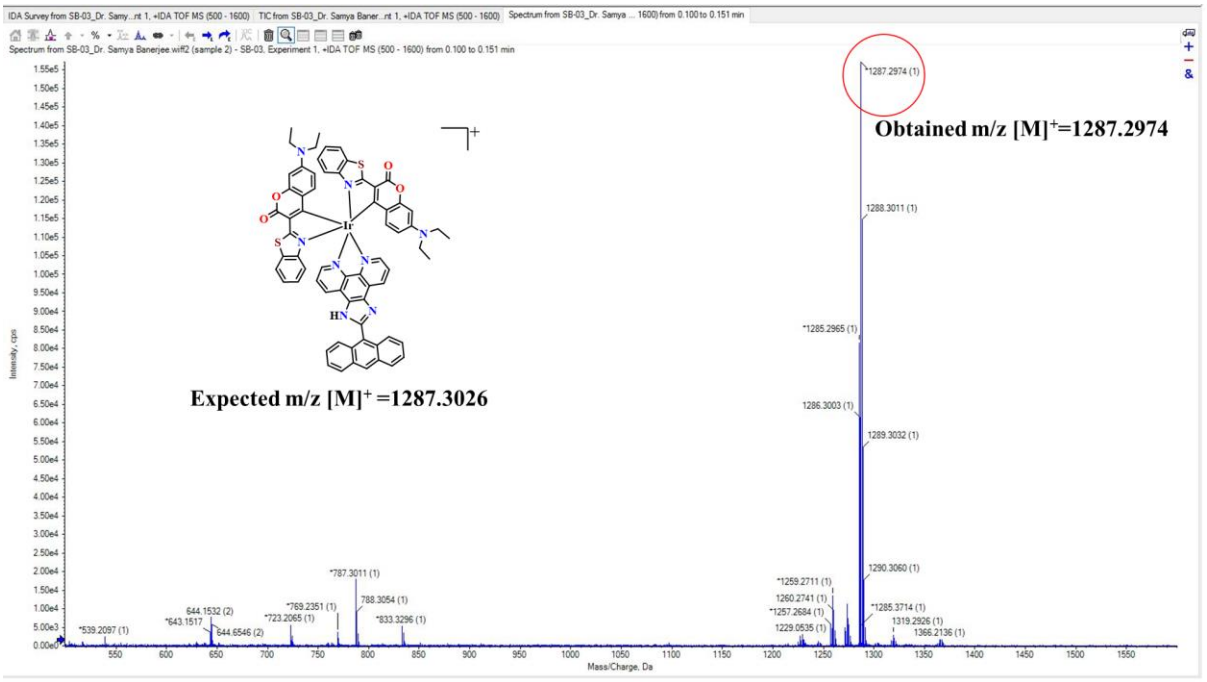

Fig. S3: HR-MS spectrum of complex **Ir2** in acetonitrile.

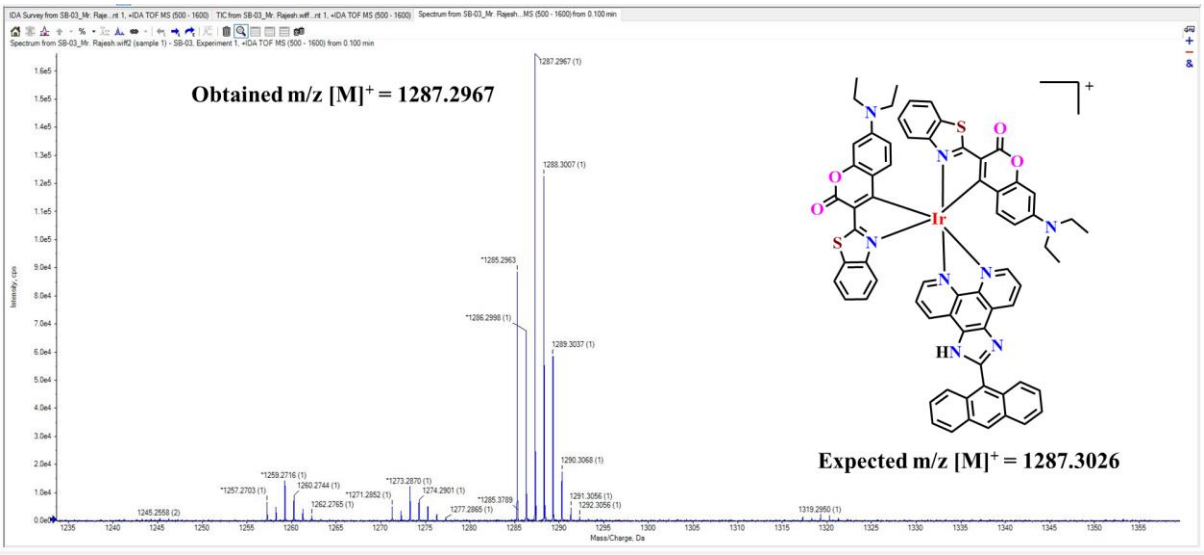

Fig. S4: HR-MS of complex **Ir2** in acetonitrile (zoomed).

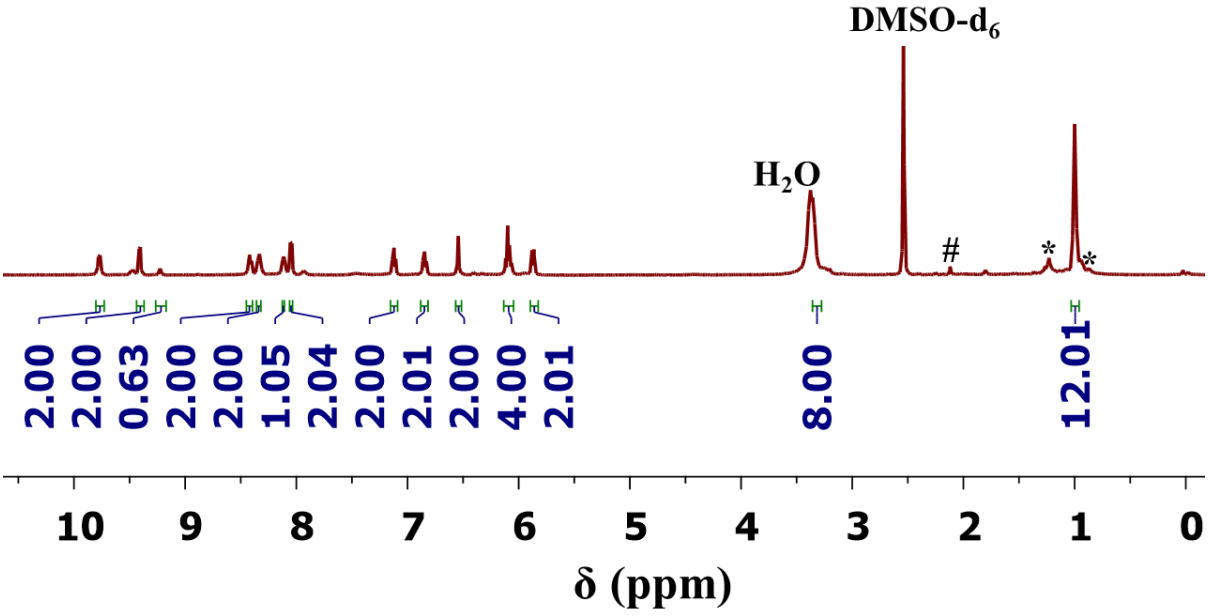

Fig. S5: ^{1}H NMR spectra of complex **Ir1** in DMSO-d_6 (500 MHz). (Residual solvent peaks: *-n-Hexane, #-Acetone).

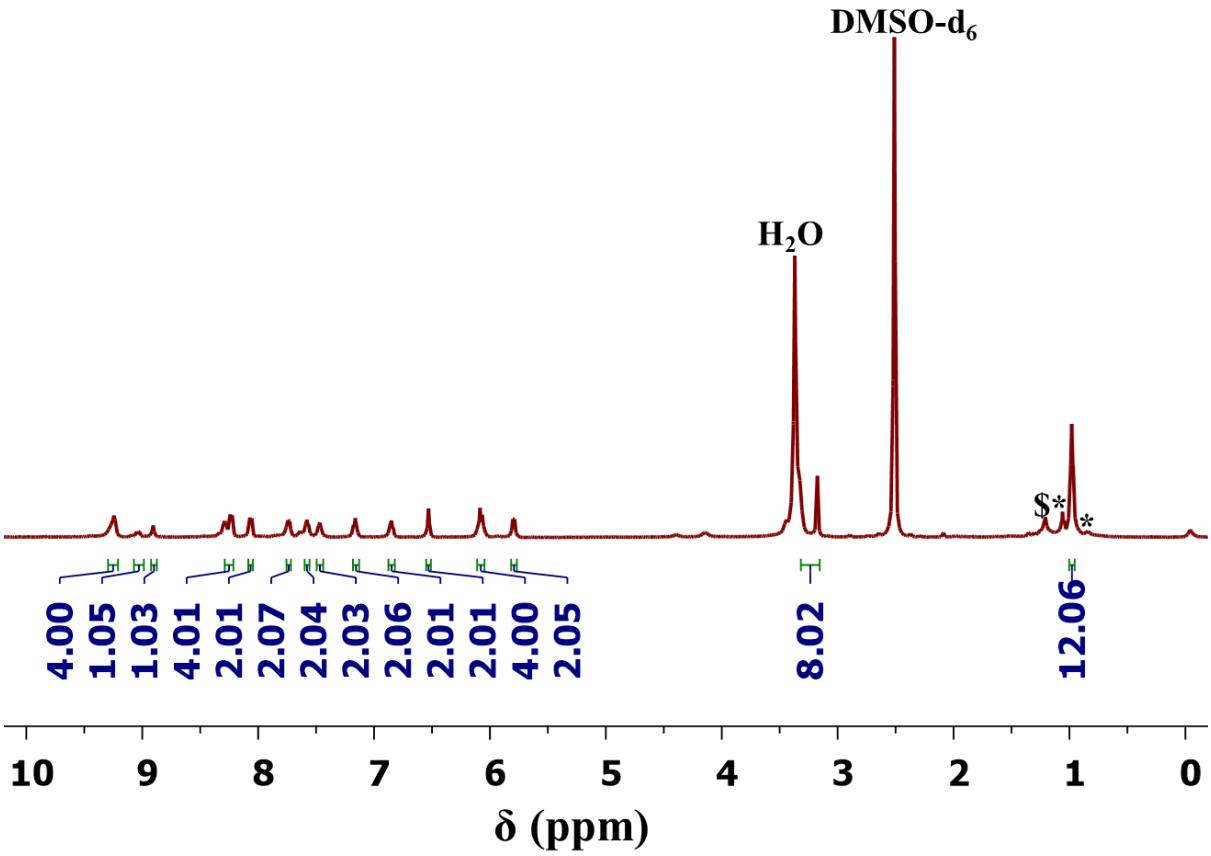

Fig. S6: ^{1}H NMR spectra of complex **Ir2** in DMSO-d_6 (500 MHz). (Residual solvent peaks: *- n-Hexane, $- Diethyl ether).

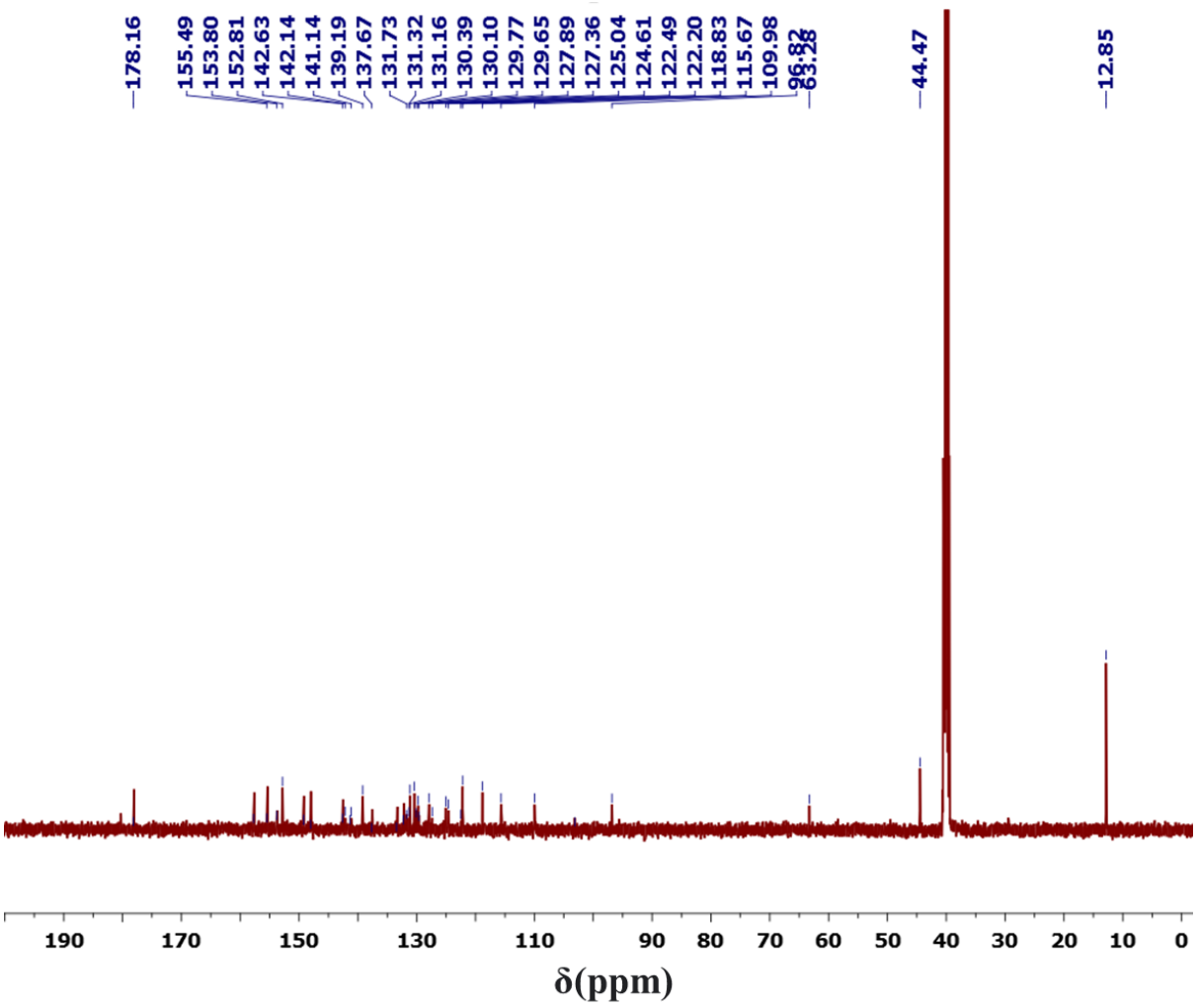

Fig. S7: $^{13}C\{^{1}H\}$ NMR spectra of complex **Ir1** in DMSO-d_6 (125 MHz).

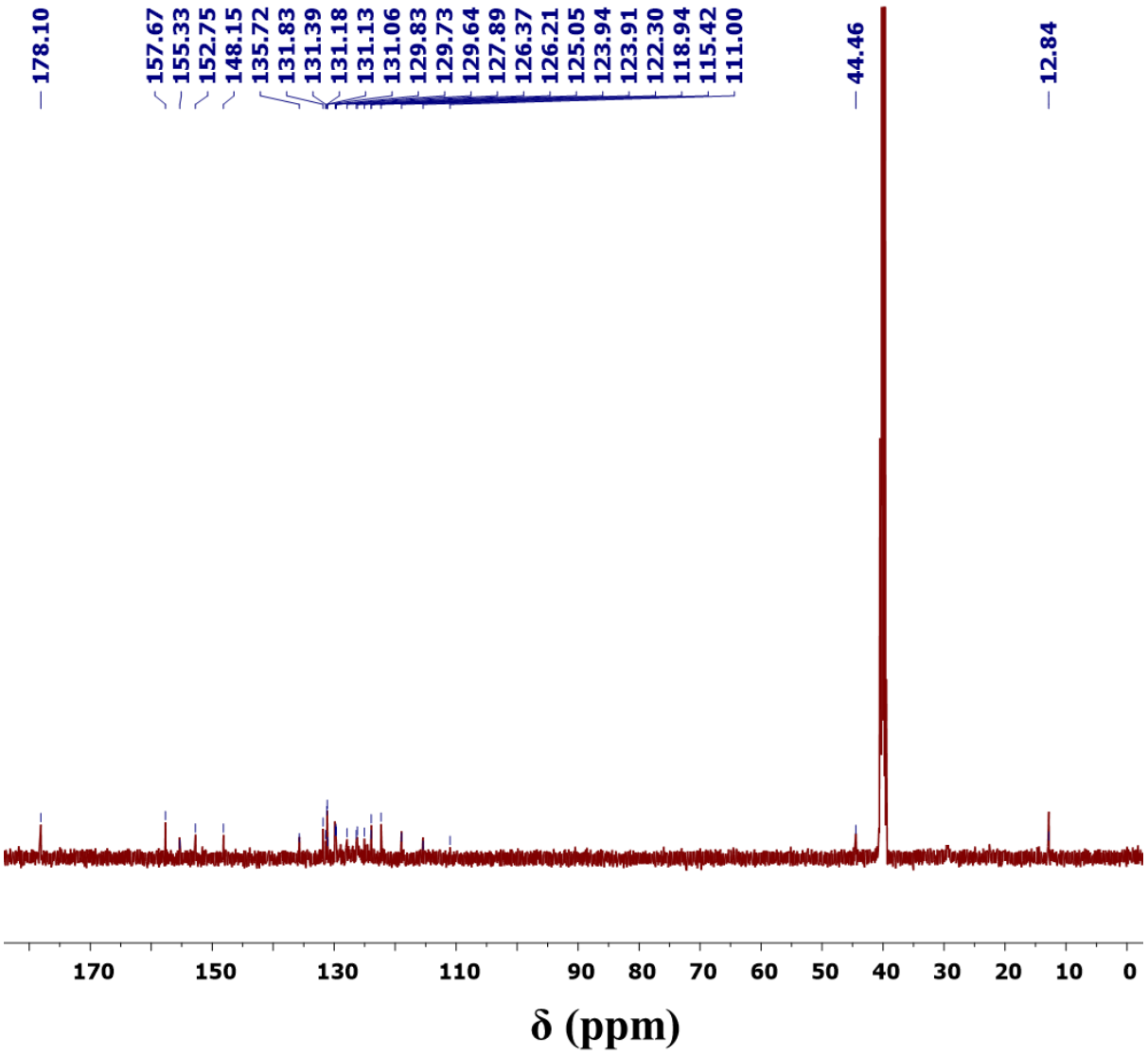

Fig. S8: $^{13}C\{^{1}H\}$ NMR spectra of complex **Ir2** in DMSO-d_6 (125 MHz).

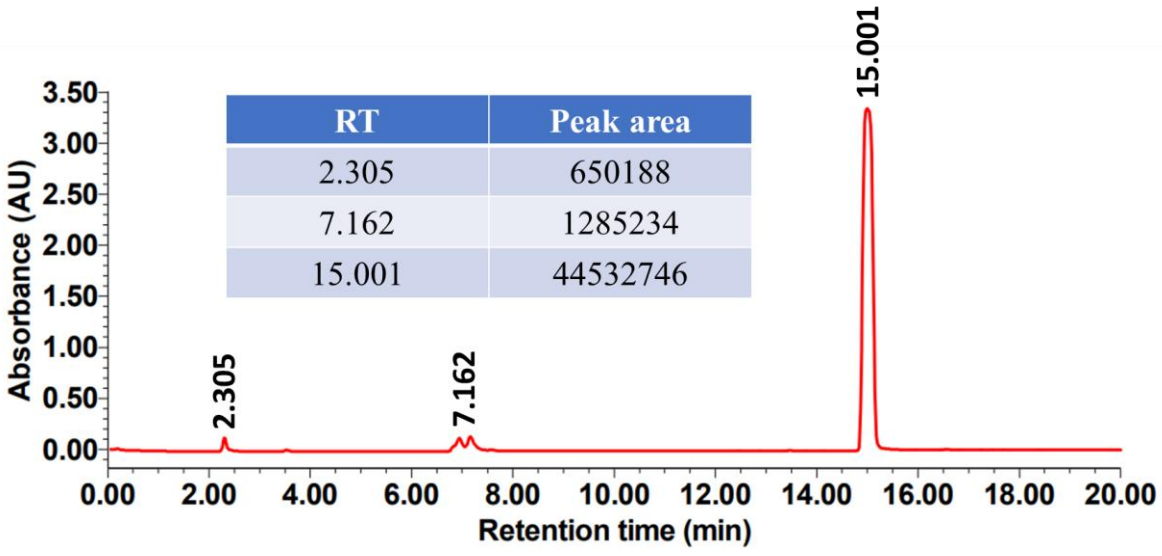

Fig. S9: High-performance liquid chromatography (HPLC) spectra for analysis of complex **Ir1** using HPLC column: Chromasol ONYX C18, (250 mm x 4.0 mm), 5 micron, 100Å; Mobile Phase: ACN/Water; Flow: 0.8 mL/min; Injection volume: 15 µL; Detector: UV (254 nm); Instrument: Waters 2489.

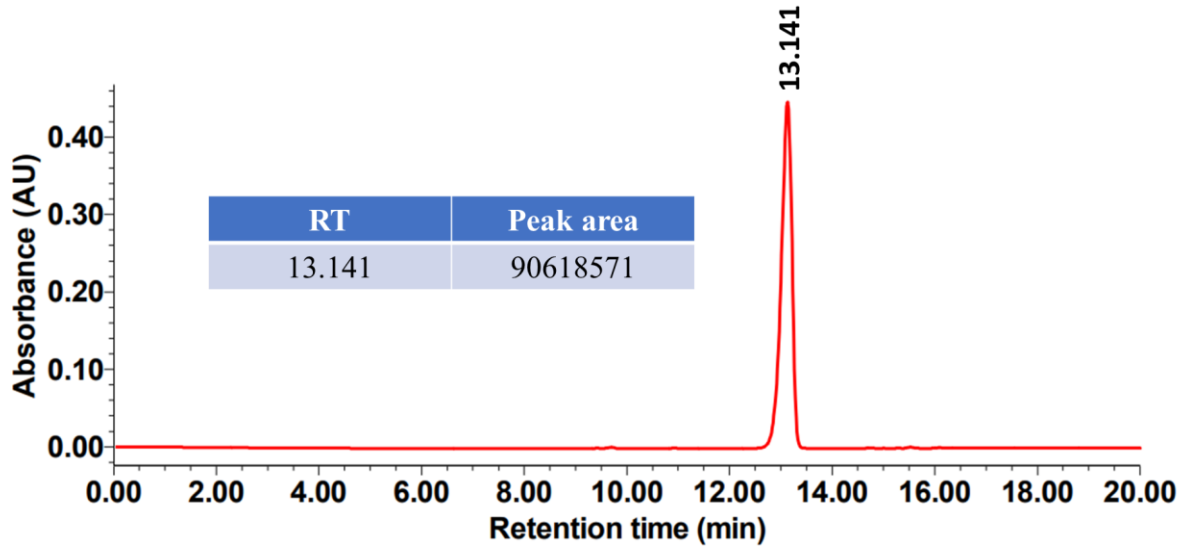

Fig. S10: High-performance liquid chromatography (HPLC) spectra for analysis of complex **Ir2** using HPLC column: Chromasol ONYX C18, (250 mm x 4.0 mm), 5 micron, 100Å; Mobile Phase: ACN/Water; Flow: 0.8 mL/min; Injection volume: 15 µL; Detector: UV (254 nm); Instrument: Waters 2489.

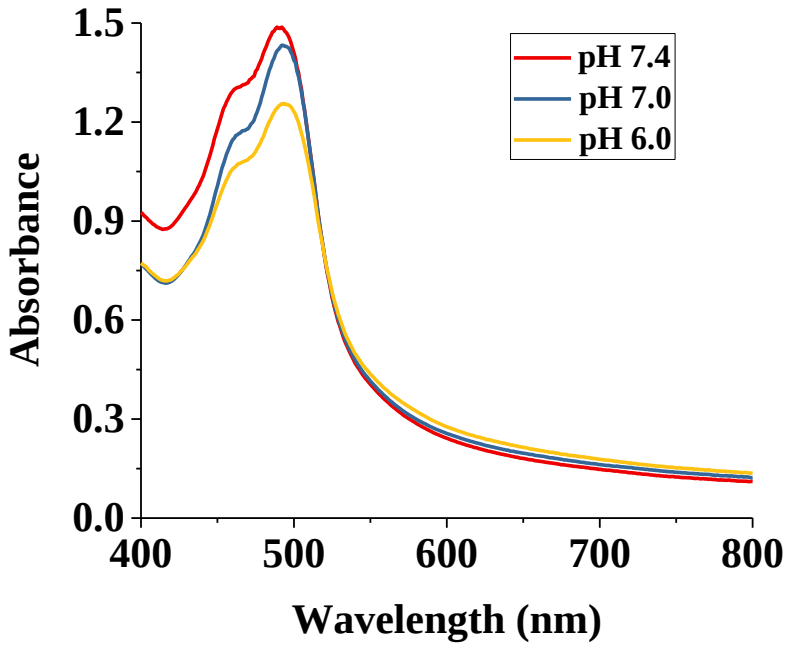

Fig. S11: Absorption spectra of **Ir2** in PBS solution of different pH.

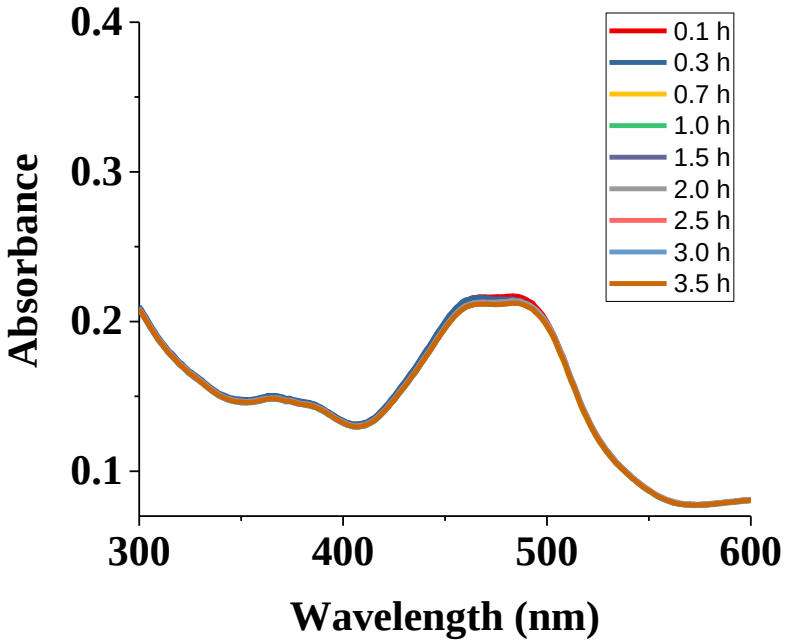

Fig. S12: Dark stability of complex **Ir1** at various time intervals in 0.5 % DMSO and 99.5 % PBS.

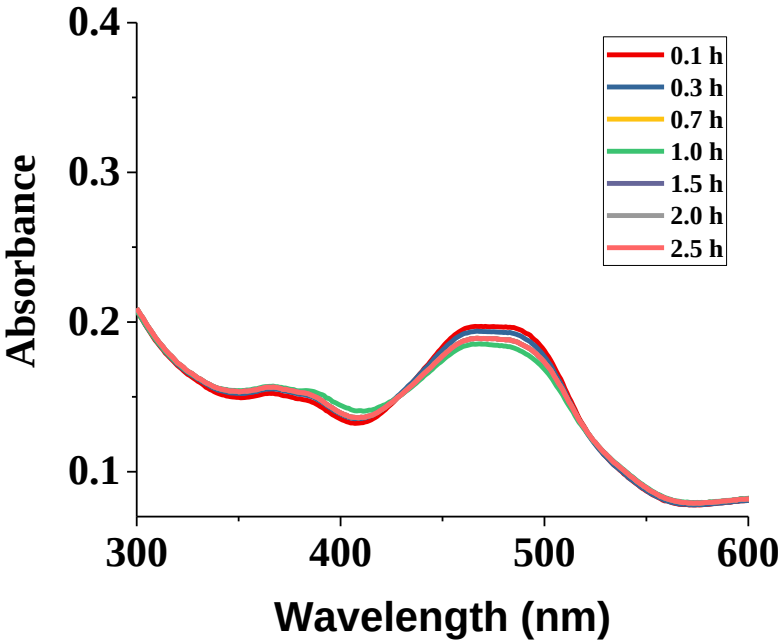

Fig. S13: Photo-stability of complex **Ir1** under exposure to white light (400-700 nm, 10 J cm^{-2}) at various time intervals in 0.5 % DMSO and 99.5 % PBS.

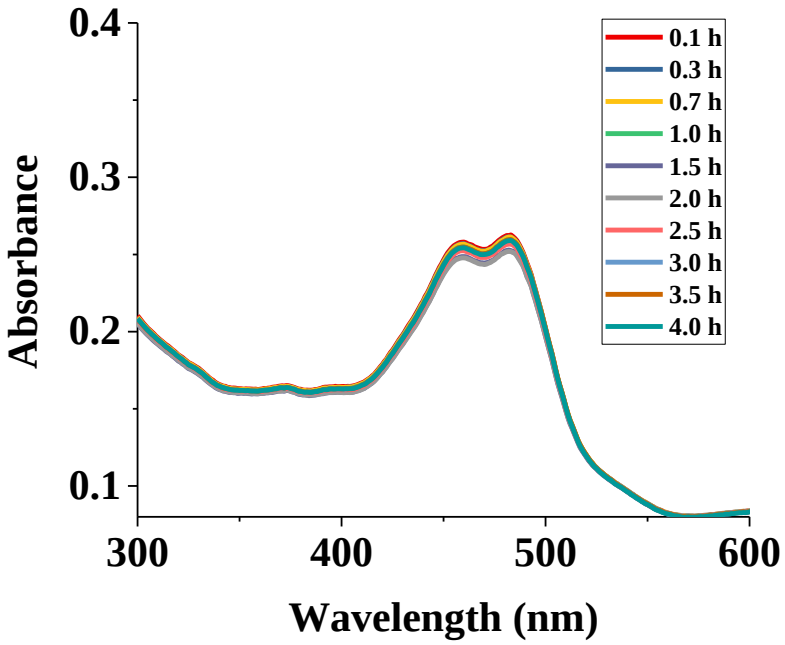

Fig. S14: Dark-stability of complex **Ir2** at various time intervals in 0.5 % DMSO and 99.5 % PBS.

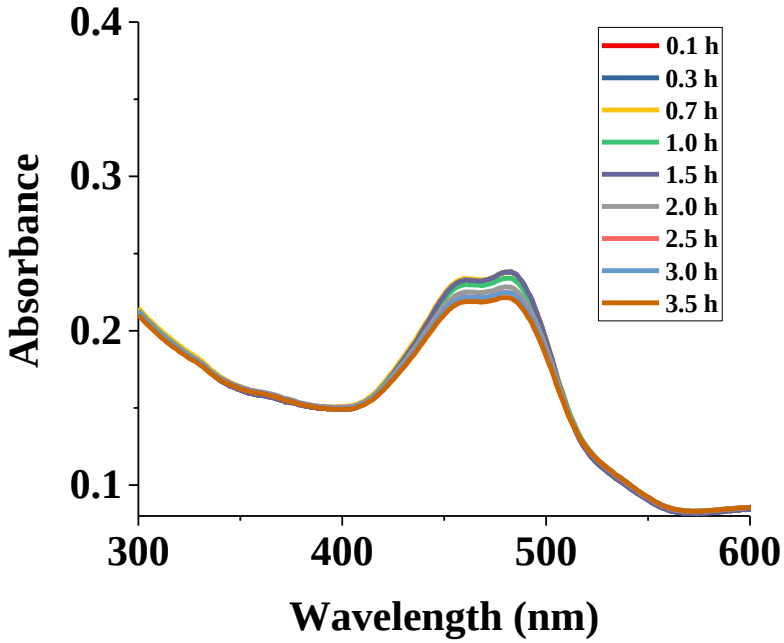

Fig. S15: Photo-stability of complex **Ir2** under exposure to white light (400-700 nm, 10 J cm^{-2}) at various time intervals in 0.5 % DMSO and 99.5 % PBS(v/v).

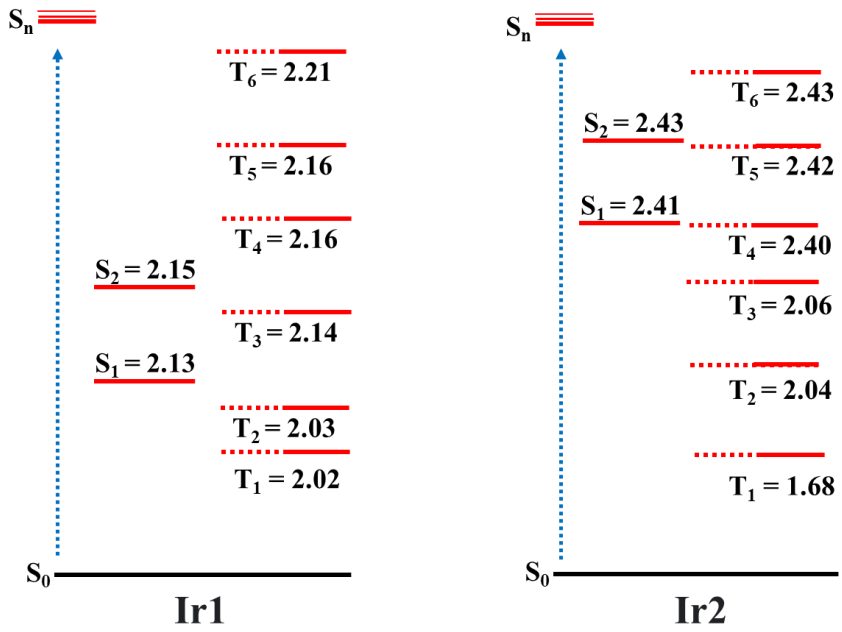

Fig. S16: Vertical energy levels of the different electronic states of **Ir1** and **Ir2** obtained from TD-B3LYP/LANL2DZ/6-31g* in water. All energy values are in eV.

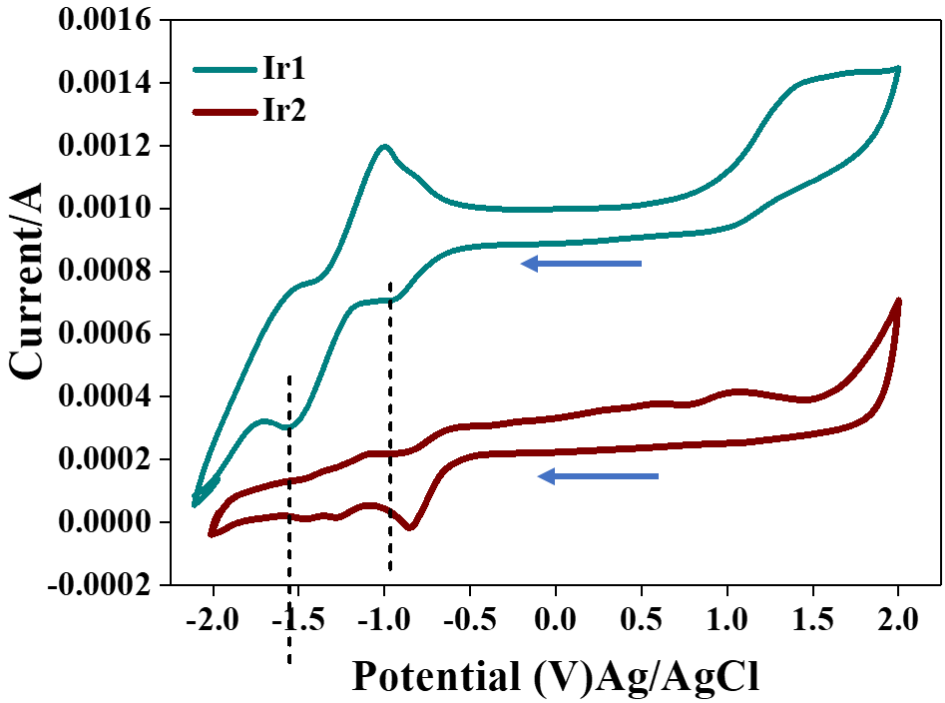

Fig. S17: The CV profiles of the **Ir1** and **Ir2** in acetonitrile using a three-electrode system.

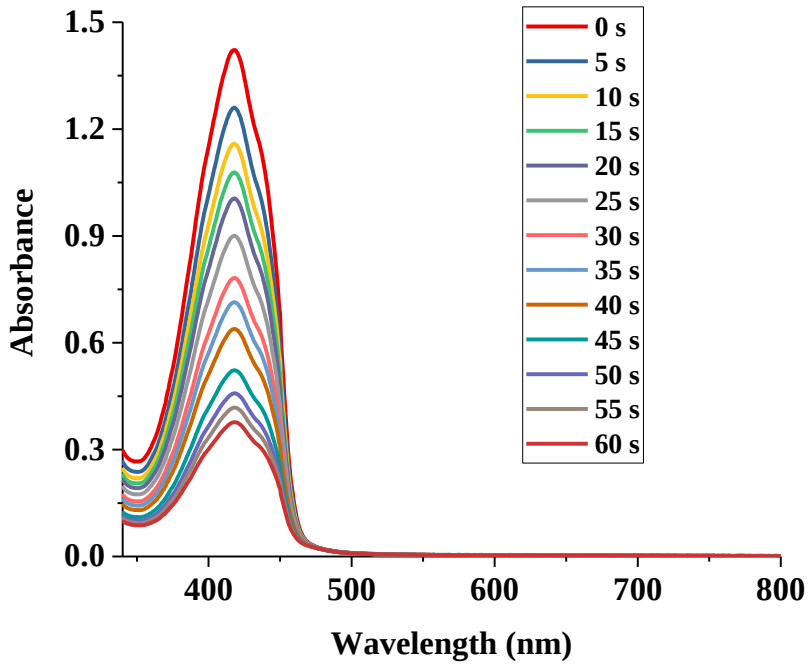

Fig. S18: $^1O_2$ generation by $[Ru(bpy)_3]Cl_2$ (2 μM) in a solution containing 0.5% DMSO and 99.5% PBS as was detected with DPBF (50 μM) and monitored by UV-Vis spectroscopy upon irradiation with 525 nm light (50.2 J cm^{-2}) at different time intervals.

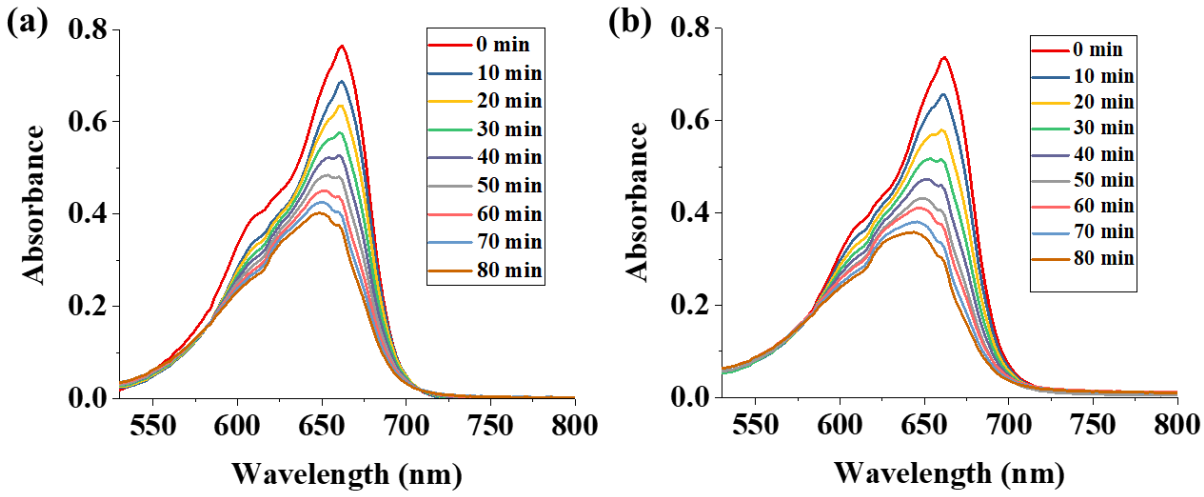

Fig. S19: (a) and (b) represent ˙OH generation by complexes **Ir1** and **Ir2** (2 μM each) in a solution containing 0.5% DMSO/99.5% PBS (v/v) solution as was detected with methylene blue (15 μM) and monitored by UV-Vis spectroscopy upon irradiation with 525 nm light (50.2 J cm^{-2}) at different time intervals.

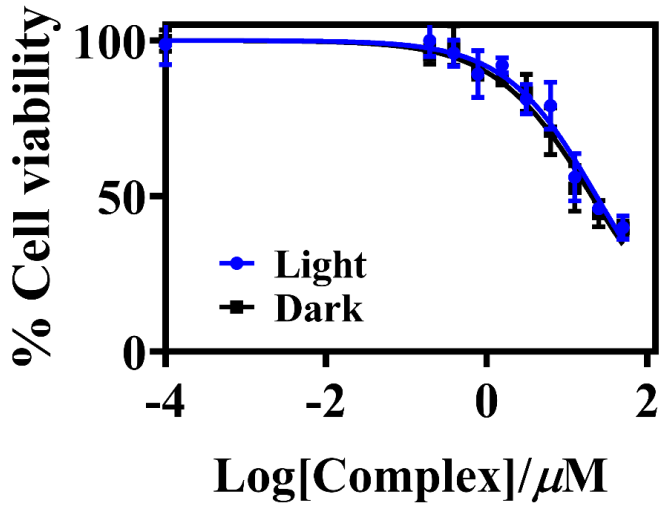

Fig. S20: Cell viability plots for Cisplatin in normal human bronchial epithelial BEAS-2B cell after 4 h incubation. One set of cells was exposed to visible light (λ = 400-700 nm, 10 J cm^{-2}) for 1 h while the other set was kept in the dark. Blue and black plots represent light-exposed and dark, respectively.

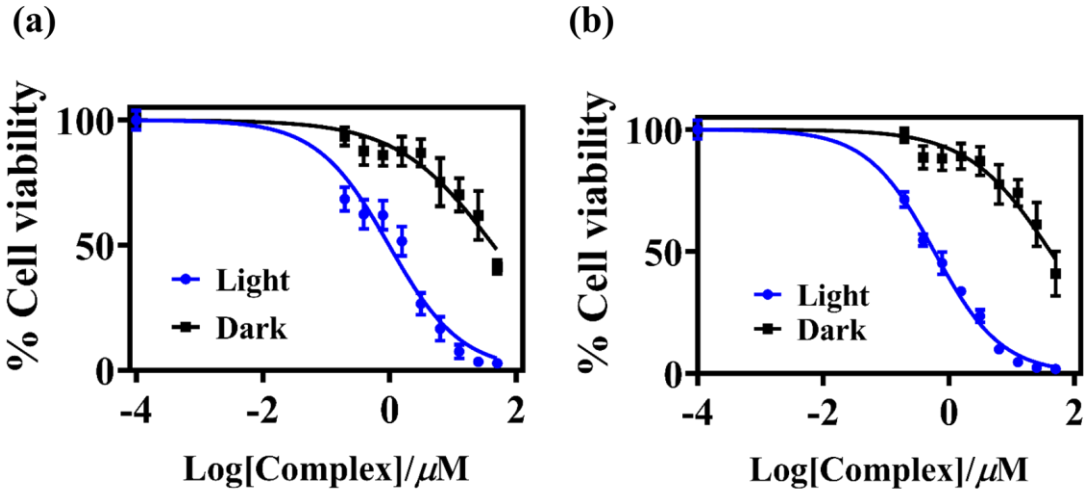

Fig. S21: Cell viability plots for complexes **Ir1** (a) and **Ir2** (b) in A549 cells after 4 h incubation. One set of cells was exposed to visible light (λ = 400-700 nm, 10 J cm^{-2}) for 1 h while the other set was kept in the dark. Blue and black plots represent light-exposed and dark, respectively.

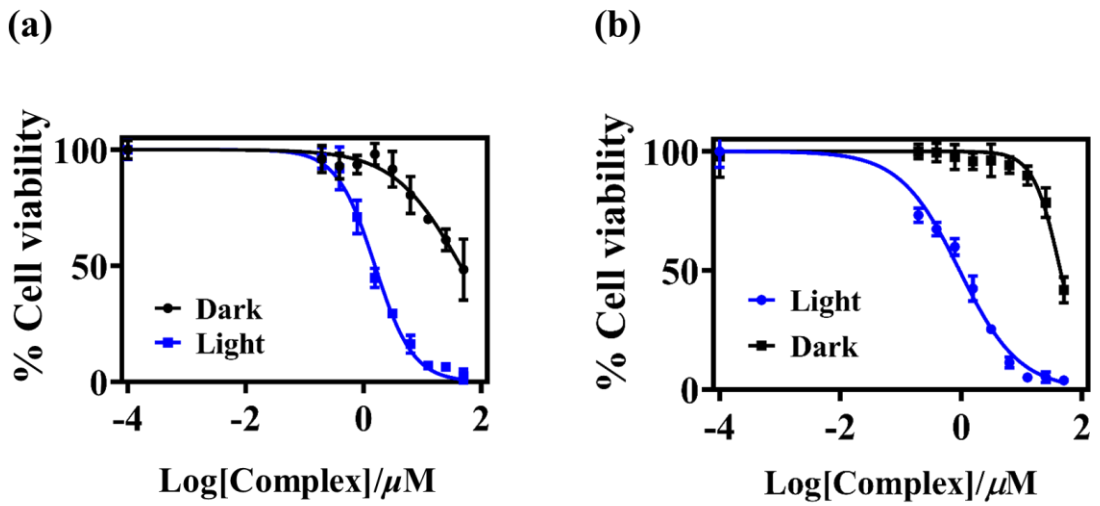

Fig. S22: Cell viability plots for complexes **Ir1** (a) and **Ir2** (b) in HeLa cells after 4 h incubation. One set of cells was exposed to visible light (λ = 400-700 nm, 10 J cm^{-2}) for 1 h while the other set was kept in the dark. Blue and black plots represent light-exposed and dark, respectively.

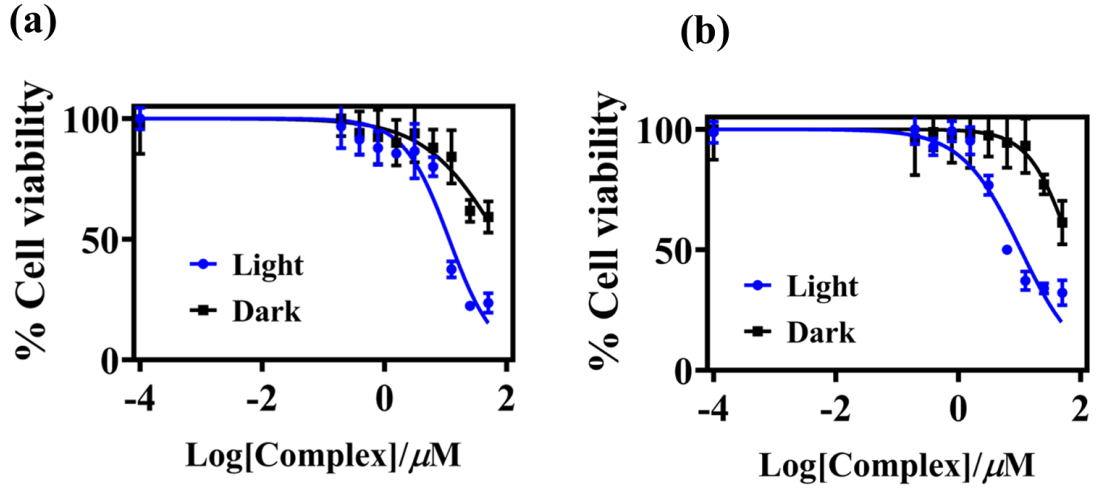

Fig. S23: Cell viability plots for complexes **Ir1** (a) and **Ir2** (b) in normal human bronchial epithelial BEAS-2B cell after 4 h incubation. One set of cells was exposed to visible light (λ = 400-700 nm, 10 J cm^{-2}) for 1 h while the other set was kept in the dark. Blue and black plots represent light-exposed and dark, respectively.

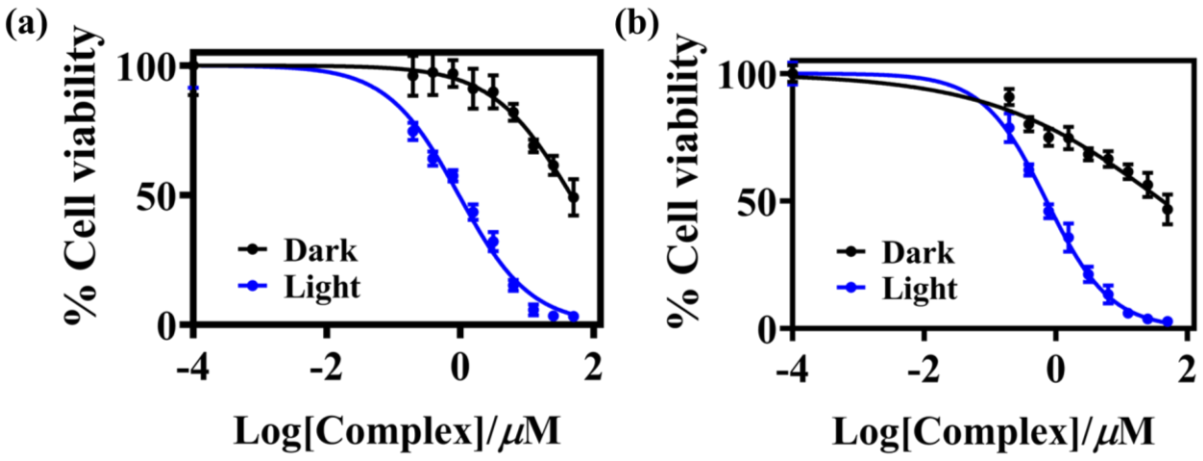

Fig. S24: Cell viability plots using sulforhodamine B (SRB) assay for complexes **Ir1** (a), and **Ir2** (b) in A549 cells after 4 h incubation. One set of cells was exposed to visible light (λ = 400-700 nm, 10 J cm^{-2}) for 1 h while the other set was kept in the dark. blue and black plots represent light-exposed and dark, respectively.

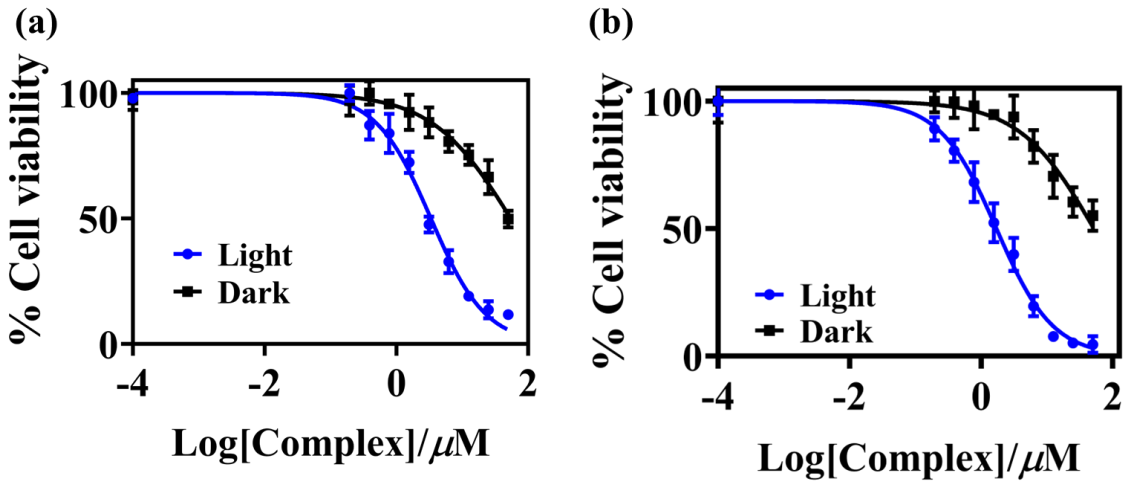

Fig. S25: Cell viability plots for complexes **Ir1** (a) and **Ir2** (b) under hypoxic conditions in A549 cells after 4 h incubation. One set of cells was exposed to visible light (λ= 400-700 nm, 10 J cm^{-2}) for 1 h while the other set was kept in the dark. Blue and black plots represent light-exposed and dark, respectively.

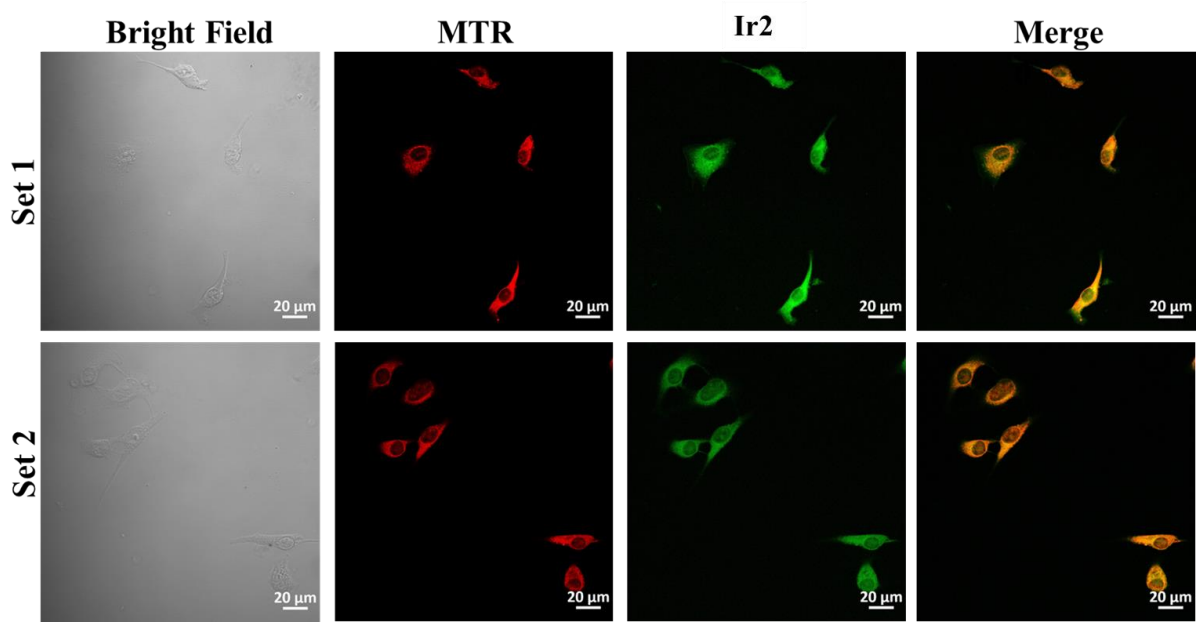

Fig. S26: Cellular localization study of complex **Ir2** in A549 cancer cells. A549 cells were incubated with 30 µM complex **Ir2** for 4 h, followed by co-staining with MitoTracker dye (250 nM). The merged panel of MitoTracker dye and complex **Ir2** (MTR + **Ir2**) clearly indicates the mitochondrial localization of complex **Ir2**. Under the following conditions, confocal images were recorded: blue emission of nucleus staining dye Hoechst (λ_{ex}~405 nm, λ_{em}~460-475 nm), red emission of MTR (λ_{ex}~630 nm, λ_{em}~650-665 nm), green emission of **Ir2** (λ_{ex}~488 nm, λ_{em}~500-520 nm).

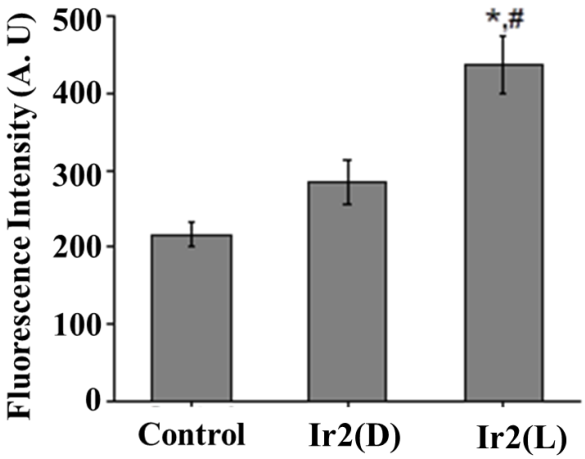

Fig. S27: The plot shows the activity levels of caspase 3 in A549 cells treated with complex **Ir2** (25 µM) through spectrofluorometric analysis. One set of cells was exposed to visible light (λ = 400-700 nm, 10 J cm^{-2}) after 4 h of incubation with complex while the other set was kept

continuously in the dark. The total incubation period following the addition of the complex was 48 h. The results are presented as mean ± SD. * $p < 0.05$ as compared to control group by student T test. # $p<0.05$ as compared to dark condition by student T test.

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4. Xie, Z. F, J.; Sadhukhan, T.; Liang, C.; Huang, C.; Li, W.; Li, T.; Zhang, P.; Banerjee, S.; Raghavachari, K.; Huang, H. Highly Efficient Ir (III)-Coumarin Photo-Redox Catalyst for Synergetic Multi-Mode Cancer Photo-Therapy. *Chem. Eur. J.* **2022**, *28*, e202103346.