Electronic Supporting Information

Near-infrared Light-activatable Iridium(III) Complexes for Synergistic Photodynamic and Photochemotherapy

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Materials and Methods

All the chemicals and reagents were purchased from commercial sources and used without further purification. 2,3,3-trimethyl-3H-indole and indocyanine green (ICG) were purchased from BLD pharm (India). Iridium(III)chloride hydrate was purchased from Alfa Aesar (India) Chemical Co. Ltd. Different reagents and probes such as 1,3-diphenylisobenzofuran (DPBF), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT), Fluorescein diacetate (FDA) and propidium iodide (PI) were purchased from Sigma Aldrich. DAPI, JC-1, MitoTracker Green FM, Lysotracker green and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Thermo Fischer Scientific. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco. Human melanoma cell line (A375), human lung adenocarcinoma (A549), human colorectal carcinoma (HCT116), and normal human embryonic kidney cell line (HEK293) cells were purchased from the National Centre for Cell Science (NCCS) Pune, India.

Instruments

All the ^{1}H NMR and ^{13}C NMR were recorded on a JEOL-500 MHz spectrometer at 298 K. Mass spectra data were obtained from the Agilent ESI-MS mass spectrophotometer. UV-visible absorption spectra data were obtained from Shimadzu UV-2600 UV/visible and U-2900 UV/visible/NIR spectrophotometer. Photoluminescence was recorded using Horiba Fluoromax. HPLC analysis was performed on the UFLC Shimadzu system with autosampler and PDA detector with reverse phase C18 flowrosil ODS (4.6 × 250 mm × 5 µm). The MTT assay was performed using a SYNERGY H1 microplate reader. The irradiation process was conducted using an 808 nm laser and 637 nm LED by Luzchem EXPO-01 panel as a light source. Fluorescence microscopic images were captured with the help of Olympus fluorescent inverted microscope.

Synthetic procedure

Synthesis of compound 1: 2,3,3-trimethyl-3H-indole (1.0 g, 6.28 mmol), iodomethane (5.35 g, 37.7 mmol) in acetonitrile (10 mL) were stirred at 80 °C for 12 h under nitrogen atmosphere. After cooling to room temperature, the product was collected as purple needle-like crystals by filtration and washed with diethyl ether to afford compound 1. The crude product was used without further purification. Yield: 89.8%.

Synthesis of compound 2: Under ice bath conditions, phosphorus oxychloride (3.5 mL) in DCM was added slowly into dry N, N-dimethylformamide (5 mL) in DCM and stirred for 30 min at 0 °C. After this, cyclohexanone (1 mL) was added slowly, and the reaction mixture was heated to reflux at 80 °C for 5 h under nitrogen. After, the reaction solution was cooled to room temperature, and then the reaction mixture was poured into ice water and kept overnight at room temperature. The yellow crude product was collected by filtration and utilized without further purification. Yield: 85%.

Synthesis of compound 3 (hcy-Cl): Compound 2 (500 mg, 2.9 mmol), sodium acetate (1.47 g, 18.0 mmol), and compound 1 (1.75 g, 5.83 mmol) dissolved in 10 mL acetic anhydride and stirred at 130 °C for 2 h under nitrogen. The reaction mixture was cooled to room temperature, and diethyl ether was added to get a green solid precipitate. The residual sodium acetate was removed by dissolving the precipitate in DCM and filtered. The filtrate was then extracted with saturated sodium bicarbonate three times and dried with sodium sulfate overnight. Column chromatography was performed with dichloromethane and methanol (50:1, v/v) to give a leafy green solid product. Yield: 74.4%. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.32 (d, *J* = 14.2 Hz, 2H), 7.39 – 7.34 (m, 4H), 7.24 – 7.20 (m, 2H), 7.19 – 7.16 (m, 2H), 6.27 – 6.12 (m, 2H), 3.73 (s, 6H), 2.74 (dt, *J* = 21.9, 6.2 Hz, 4H), 1.94 (dt, *J* = 12.5, 6.4 Hz, 2H), 1.70 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.91, 154.23, 150.69, 144.41, 142.84, 140.97, 128.91, 127.84, 125.41, 122.22, 110.90, 101.77, 49.30, 32.73, 28.16, 26.85, 20.75. HRMS (ESI): *m/z* calculated for $C_{32}H_{36}ClN_2^+$: 483.2562 $[M\text{-}I^-]^+$; found: 483.2565.

Synthesis of compound 4: Piperazine (2 g, 23 mmol) was dissolved in water, and carbon disulfide (1.4 mL, 23 mmol) was added dropwise with vigorous stirring, leading to a pale green precipitate after 10 min. The product was vacuum filtered, washed with water, and vacuum dried. The crude product was utilized without further purification. Yield: 91.5%.

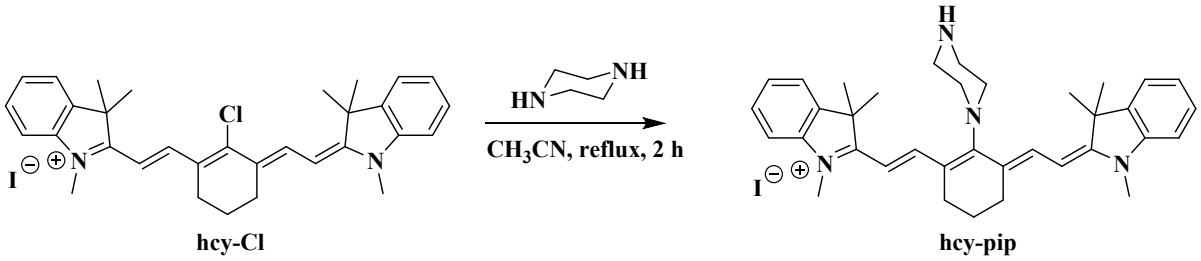
Synthesis of $[Ir(ppy)_2Cl]_2$ and $[Ir(thpy)_2Cl]_2$: Iridium dimers were synthesized according to the previous literature and used without further purification.[1]

Synthesis of compound 5: $[Ir(ppy)_2Cl]_2$ (200 mg, 0.1 mmol), compound 4 (151 mg, 1 mmol), and sodium carbonate (98.9 mg, 1 mmol) were dissolved in anhydrous DCM and stirred at 25°C for 12 h under nitrogen. The product was purified using column chromatography in DCM/methanol (50:1, v/v) to get a yellow solid compound. Yield: 68%. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.57 (ddd, *J* = 5.8, 1.5, 0.7 Hz, 2H), 7.87 – 7.84 (m, 2H), 7.73 (ddd, *J* = 8.1, 7.4, 1.6 Hz, 2H), 7.56 (dd, *J* = 7.7, 1.3 Hz, 2H), 7.20 (ddd, *J* = 7.3, 5.8, 1.5 Hz, 2H), 6.81 – 6.77 (m, 2H), 6.70 – 6.66 (m, 2H), 6.34 – 6.31 (m, 2H), 3.90 – 3.83 (m, 4H), 2.89 – 2.85 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 212.18, 168.72, 155.33, 151.53, 144.01, 136.03, 131.53, 128.91, 123.87, 122.19, 120.83, 118.49, 47.22, 45.47. HRMS (ESI): *m/z* calculated for $C_{27}H_{26}IrN_4S_2^+$: 663.1223 $[M]^+$; found: 663.1207.

Synthesis of compound 6: The synthetic procedure was similar to that of compound 5. The crude product was purified using column chromatography in DCM/methanol (50:1, v/v) to get an orange solid compound. Yield: 65%. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 9.35 (ddd, *J* = 5.9, 1.5, 0.8 Hz, 2H), 7.62 – 7.58 (m, 2H), 7.50 – 7.47 (m, 2H), 7.13 (dd, *J* = 4.7, 1.0 Hz, 2H), 6.99 (qd, *J* = 5.7, 2.8 Hz, 2H), 6.20 (dd, *J* = 4.5, 2.0 Hz, 2H), 3.91 – 3.81 (m, 4H), 2.91 – 2.87 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 212.05, 165.11, 156.65, 151.97, 136.47, 135.80, 131.08, 127.69, 118.97, 117.27, 46.75, 45.16. HRMS (ESI): *m/z* calculated for $C_{23}H_{22}IrN_4S_4^+$: 675.0351 $[M]^+$; found: 675.0351.

Synthesis of hcy-pip: Reference compound (hcy-pip) was synthesized by previous literature procedure.[2] Briefly, hcy-Cl (100 mg, 0.2 mmol) and piperazine (71.2 mg, 0.8 mmol) were dissolved in

acetonitrile and refluxed for 2 h under an inert atmosphere, and column chromatography was performed to get a blue solid compound. Yield: 72.8%.



Synthesis of Ir1@hcy: Compound 5 (200 mg, 0.3 mmol), compound 3 (146 mg, 0.3 mmol), and triethylamine (126 µL, 0.9 mmol) were dissolved in anhydrous DMF and stirred at 90°C for 12 h under inert atmosphere. The reaction mixture was vacuum-dried. The crude product was purified using DCM/methanol (50:2, v/v) column chromatography to obtain metallic blue-coloured solid compound. Yield: 29.4%. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 9.50 (dd, *J* = 5.8, 0.8 Hz, 2H), 8.19 (d, *J* = 8.3 Hz, 2H), 8.05 – 8.00 (m, 2H), 7.74 – 7.71 (m, 2H), 7.62 (d, *J* = 13.6 Hz, 2H), 7.52 (ddd, *J* = 7.3, 5.9, 1.4 Hz, 2H), 7.47 (d, *J* = 7.0 Hz, 2H), 7.37 – 7.33 (m, 2H), 7.26 (d, *J* = 7.9 Hz, 2H), 7.19 – 7.15 (m, 2H), 6.77 – 6.73 (m, 2H), 6.61 (td, *J* = 7.6, 1.2 Hz, 2H), 6.15 (dd, *J* = 7.7, 0.9 Hz, 2H), 5.94 (d, *J* = 13.7 Hz, 2H), 4.13 – 4.07 (m, 4H), 3.62 (t, *J* = 4.6 Hz, 4H), 3.51 (s, 6H), 2.57 – 2.51 (m, 2H), 2.44 – 2.38 (m, 2H), 1.72 (p, *J* = 6.5 Hz, 2H), 1.60 (s, 6H), 1.49 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 212.01, 170.63, 170.39, 168.28, 154.71, 151.44, 144.63, 143.74, 140.78, 137.66, 131.34, 129.10, 125.08, 124.74, 122.62, 121.29, 119.65, 110.70, 97.99, 63.32, 53.36, 48.34, 47.70, 32.06, 31.47, 29.96, 29.29, 28.75, 28.57, 25.16, 21.89. HRMS (ESI): *m/z* calculated for $C_{59}H_{60}IrN_6S_2^+$: 1109.3945 $[M\text{-}I^-]^+$; found: 1109.3946. %Purity (HPLC): 95.90%.

Synthesis of Ir2@hcy: The synthetic procedure was similar to compound Ir1@hcy. The product was purified using DCM/methanol (50:2, v/v) column chromatography to obtain metallic blue-coloured solid compound. Yield: 27.2%. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 9.30 (dd, *J* = 7.9, 4.5 Hz, 2H), 7.90 – 7.87 (m, 2H), 7.69 – 7.60 (m, 5H), 7.46 (d, *J* = 7.4 Hz, 2H), 7.34 (q, *J* = 3.0 Hz, 4H), 7.30 (dd, *J* = 7.0, 5.4

Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.17 (t, *J* = 7.4 Hz, 2H), 6.03 (d, *J* = 4.7 Hz, 2H), 5.94 (d, *J* = 13.7 Hz, 2H, 2H), 4.11 – 4.04 (m, 4H), 3.63 – 3.57 (m, 4H), 3.52 (s, 6H), 2.56 – 2.49 (m, 2H), 2.41 (dd, *J* = 9.3, 5.6 Hz, 2H), 1.71 (dd, *J* = 12.8, 6.3 Hz, 2H), 1.59 (s, 6H), 1.48 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) 211.44, 170.46, 164.53, 156.24, 152.08, 143.72, 141.30, 140.80, 138.14, 135.66, 131.05, 129.01, 125.25, 122.62, 117.96, 110.75, 98.14, 63.32, 53.18, 48.37, 47.51, 32.06, 31.48, 31.30, 29.53, 28.70, 28.54, 25.15, 21.85. HRMS (ESI): *m/z* calculated for $C_{55}H_{56}IrN_6S_4^+$: 1121.3073 $[M\text{-}I^-]^+$; found: 1121.3085. %Purity (HPLC): 96.98%.

Experimental section

Fluorescence quantum yield (ϕ_f)

The relative fluorescence quantum yield of synthesized complexes in DMSO was calculated based on the integrated area of emission spectra according to equation 1. ICG ($\phi_{(ICG)}$ = 14.3% in DMSO) was used as a reference.[3,4]

$$\phi_{f(complex)} = \phi_{f(ICG)}(m_{(complex)}/m_{(ICG)})(\eta^2{}_{(complex)}/\eta^2{}_{(ICG)}) \quad \text{.....(1)}$$

Where ϕ is the fluorescence quantum yield, η is a reflective index of the solvent, and m is the slope of integrated fluorescence vs absorbance of the standards (ICG) and samples at the wavelength of 720 nm. In this experiment, synthesized complexes and ICG were excited at 720 nm, and emission spectra were collected from 750 to 900 nm.

Measurement of log P value

Log P values or distribution coefficients between n-octanol and water ($K_{O/W}$) of the synthesized compounds (Ir1@hcy and Ir2@hcy) were calculated by the "shake-flask" method adapted from previous literature.[5] Solutions of the complexes (30 µM) in H_2O-saturated n-octanol (4 mL) were prepared from stock solution (10 mM) and sonicated for 5 min. 2 mL of this mixture was reserved, and an equal volume of n-octanol-saturated Milli-Q H_2O was added, and vigorously shaken in a vortex for 20 min and centrifuged for 5 min. The absorption of the organic phases, as well as those of the reserved aliquots, were measured using a microplate reader. Log P values were calculated according to the following equation 2:

$$\log P = \log\left(K_{O/W}\right) = log\left(\frac{A}{A_0 - A}\right) \quad \text{..... (2)}$$

where A_0 refers to the absorbance of the reserved aliquots at λ_{abs}= 720 nm, and A is the absorbance of the n-octanol phase at the same wavelengths. Data are expressed as mean ± SD from three independent experiments.

Table S1: Log P values of Ir1@hcy and Ir2@hcy in octanol/water represented as mean ± S.D.

Complex	**log P value**
Ir1@hcy	0.65 ± 0.03
Ir2@hcy	1.74 ± 0.02

Singlet oxygen ($^1O_2$) generation

The generation of $^1O_2$ by ICG, hcy-pip, and complexes (Ir1@hcy and Ir2@hcy) was monitored by a singlet oxygen probe, i.e., 1,3-diphenylisobenzofuran (DPBF) by measuring their singlet oxygen quantum yield (φ_Δ). In brief, the absorbance of DPBF (50 µM) at 417 nm was adjusted to 1, whereas the maximum absorbance of photosensitizers (5 µM) was adjusted to 0.1-0.2. The samples were irradiated (808 nm/637 nm, 2mW/cm^2) in an interval of 5 s, and absorbance changes in DPBF spectra were recorded. DPBF oxidized to form 1,2-dibenzoyl benzene (DBB) in the presence of ROS, which results in the decline of absorbance peak at 417 nm. The values of φ_Δ for all the complexes were calculated by the relative method using ICG as a reference with the formula (3).

$$\phi_{\Delta(PS)} = \phi_{\Delta(ref)} \times \left[\frac{m_{(PS)} \times F_{(ref)}}{m_{(ref)} \times F_{(PS)}}\right] \quad \text{..... (3)}$$

Where m stands for the slope of the plot between the absorbance change of DPBF with irradiation time, and F is the absorption correction factor (F = 1-10^{-OD}), where OD represents the optical density of PS and reference compound at 808 nm.

Electron paramagnetic resonance (EPR) studies

2,2,6,6-Tetramethylpiperidine (TEMP) and 5,5-dimethyl-1-pyroline-N-oxide (DMPO) were used as a trapping agent for $^1O_2$ and $^\bullet OH$, respectively. Sample containing Ir2@hcy (100 µM) and TEMP (1 mM) or DMPO (20 mM) were prepared in deaerated acetonitrile and irradiated with 700 nm laser for 5-20 min at RT. The EPR measurements were recorded at 298 K on a Bruker EMX spectrometer.

Cell viability assay

A375, A549, HCT116, and HEK293 cells were seeded onto 96 well plate at a cell density of 1×10^4 per well and grown for 24 h. For cell viability evaluation, cells were further treated with different concentrations (2.5, 5, 10, 20 µM) of complex and incubated for 4 h. For photocytotoxicity, the light plate was subjected to an LED source (637/808 nm, 2 or 100 mW/cm^2) for 20 min after replacing media with PBS. The dark plate was kept in the dark with PBS for the same time interval. Subsequently, media (w/FBS) is added in all wells after removing PBS and incubated for another 24 h at 37^{0}C (recovery period). Then, 10 µL (5mg/mL) MTT is added in each well and incubated for 4 h at 37^{0}C. After this, 150 µL of DMSO was added to each well, and absorbance was measured at 570 nm using a microplate reader. Cisplatin is used as a positive control. The cell viability was measured by the following formula.

$$Cell\ viability\ (\%) = \frac{OD(sample)}{OD(control)} \times 100 \qquad \text{.....(4)}$$

Where OD represents the absorbance value at 570 nm.

Intracellular ROS detection

2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) was used as a ROS probe to validate the generation of ROS in A375 cells. Cells were seeded into a 6-well plate with a density of 7×10^4 cells per well. Cells were categorized into five groups: Control (without any treatment), only treated with light, treated with Ir2@hcy (5 µM) without light, and Ir2@hcy+Light. After 1 h incubation, cells were irradiated with 637 nm light (2 mW/cm^2, 10 min). DCFH-DA (10 µM) was added to all the wells and incubated for 20 min. Then, the images were captured to observe the intracellular ROS level at an

exposure time of 43.5 ms. The quantification of ROS was done by a microplate reader with excitation (485 nm) and emission wavelength (535 nm), respectively.

Cellular uptake and Colocalization experiments

7×10^4 cells were seeded in 6 well plates and allowed to adhere for 24 h. Then, the cells were incubated with Ir2@hcy (5 μM) for 30 min at 37 °C and 5% CO_2, and uptake images at an exposure time of 3 s were taken using an inverted fluorescence microscope. For colocalization studies, 7×10^4 cells were seeded in 6 well plates and then incubated with Ir2@hcy (5 μM) for 30 min. After that, cells were incubated with Mito Tracker Green, lysotracker green (150 nM), and DAPI (1 μg/mL) at 37 °C for 30 min. Images were taken after washing the cells twice with PBS.

ICP-MS analysis

To examine the amount of the Ir complexes bound to the cytosol and mitochondria in A375 cells, the cells were seeded in 6-well tissue culture plates for 24 h and then treated with the complexes (20 μM). After 1 h incubation, the cells were washed with ice-cold PBS and collected by trypsinization followed by centrifugation. The cytosol and mitochondria were extracted using the Mitochondria Isolation Kit (TCI-India). Each sample was digested with concentrated HNO_3, and the resulting solutions were diluted with distilled water to a final concentration of 1.3% HNO_3. The metal content in the diluted solutions was determined by Agilent 8900 ICP-MS Triple Quad. The amount of Ir detected in the cell samples was converted from ppb to μg of metal. Data were normalized to the number of cells and expressed as μg of metal/number of cells.

Mitochondrial membrane potential (MMP) analysis

A375 cells were seeded in 6 well plates with a cell density of 7×10^4 and allowed to adhere for 24 h. Cells were incubated with Ir2@hcy (5 μM) for 2 h at 37 °C with 5% CO_2. After that, the cells were irradiated with 637 nm light (2 or 20 mW/cm^2) for 20 min. The cells were then treated with JC-1 (5 μg/mL) and incubated for 20 min in dark. Cells were viewed on fluorescence microscopy in a red channel of 281 ms for J-aggregates and a green channel at 72.5 ms for JC-1 monomers, respectively.

Dead/Live cell co-staining

A375 cells were seeded into 6 well plates with a cell density of 7×10^4 cells per well. After 24 h, cells were incubated with Ir2@hcy for 4 h, followed by 637 nm light (2 or 20 mW/cm^2, 20 min) irradiation. After washing the cells with PBS, the cells were stained with FDA (2 μg/mL) and PI (10 μg/mL) for 5 min. Finally, the cells were carefully washed two times with PBS. The images were captured by a fluorescence microscope in the green and red channels.

Spheroids Studies

A375 cells were seeded into low attachment 96 well plates with a cell density of 2×10^4 cells per well. After 48 h, cells were incubated with Ir2@hcy for 30 min, and uptake images were taken under a red channel in a fluorescence microscope. Live/dead assay was performed after irradiation with 637 nm light (2 or 20 mW/cm^2, 30 min). The spheroids were stained with FDA (2 μg/mL) and PI (10 μg/mL) for 10 min. Finally, the images were captured by a fluorescence microscope after two times washing with PBS.

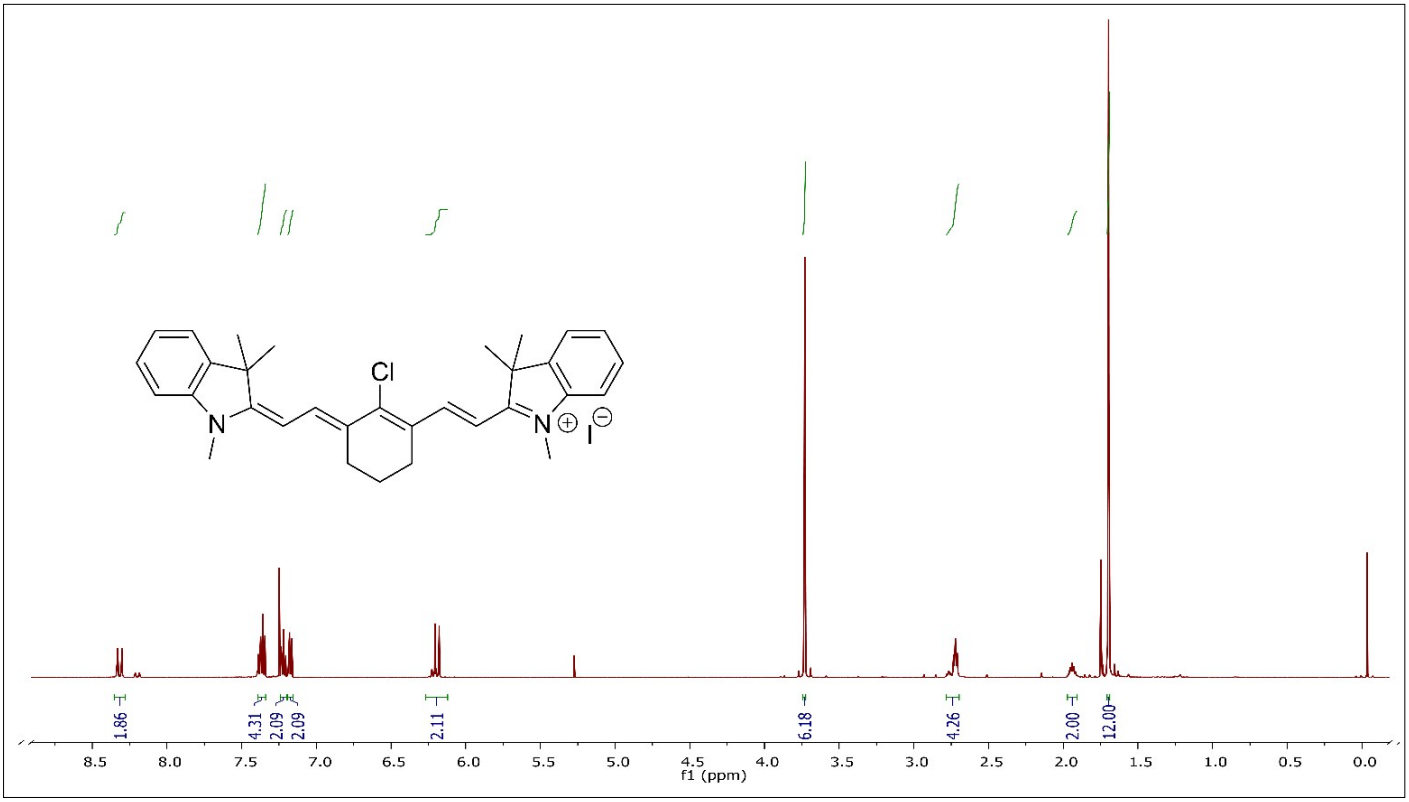


Fig. S1: ^{1}H NMR spectrum of compound 3 in $CDCl_3$.

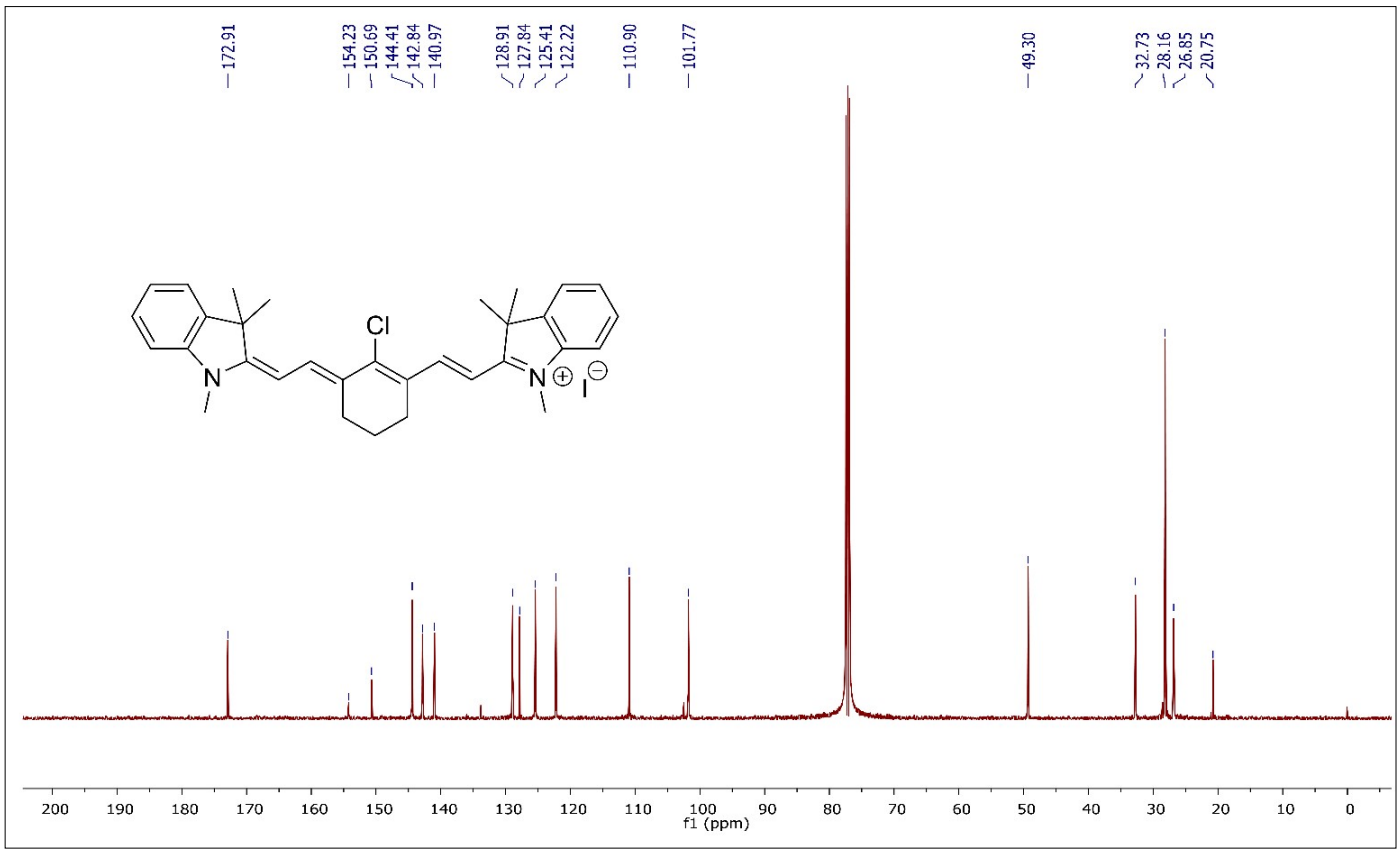


Fig. S2: ^{13}C NMR spectrum of compound 3 in $CDCl_3$.

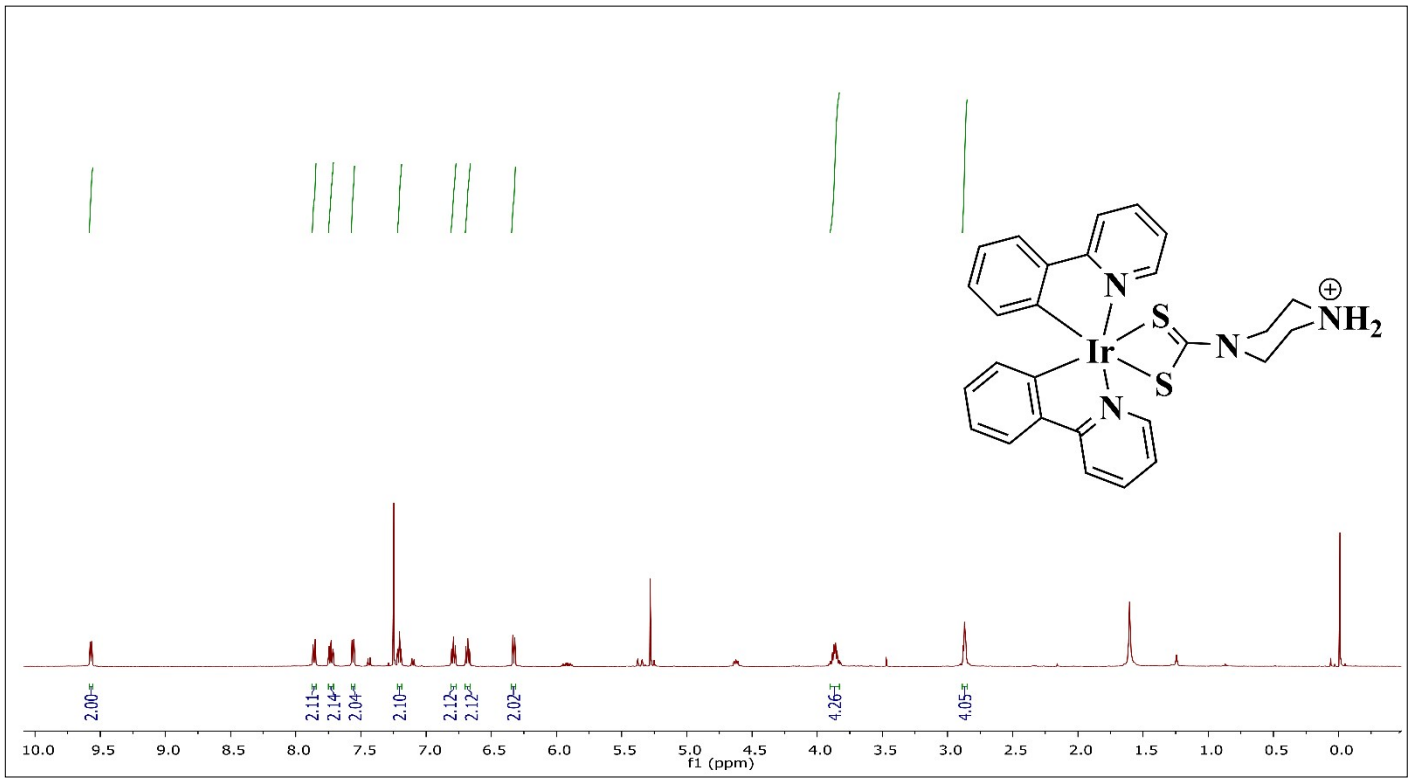


Fig. S3: ^{1}H NMR spectrum of Compound 5 in $CDCl_3$.

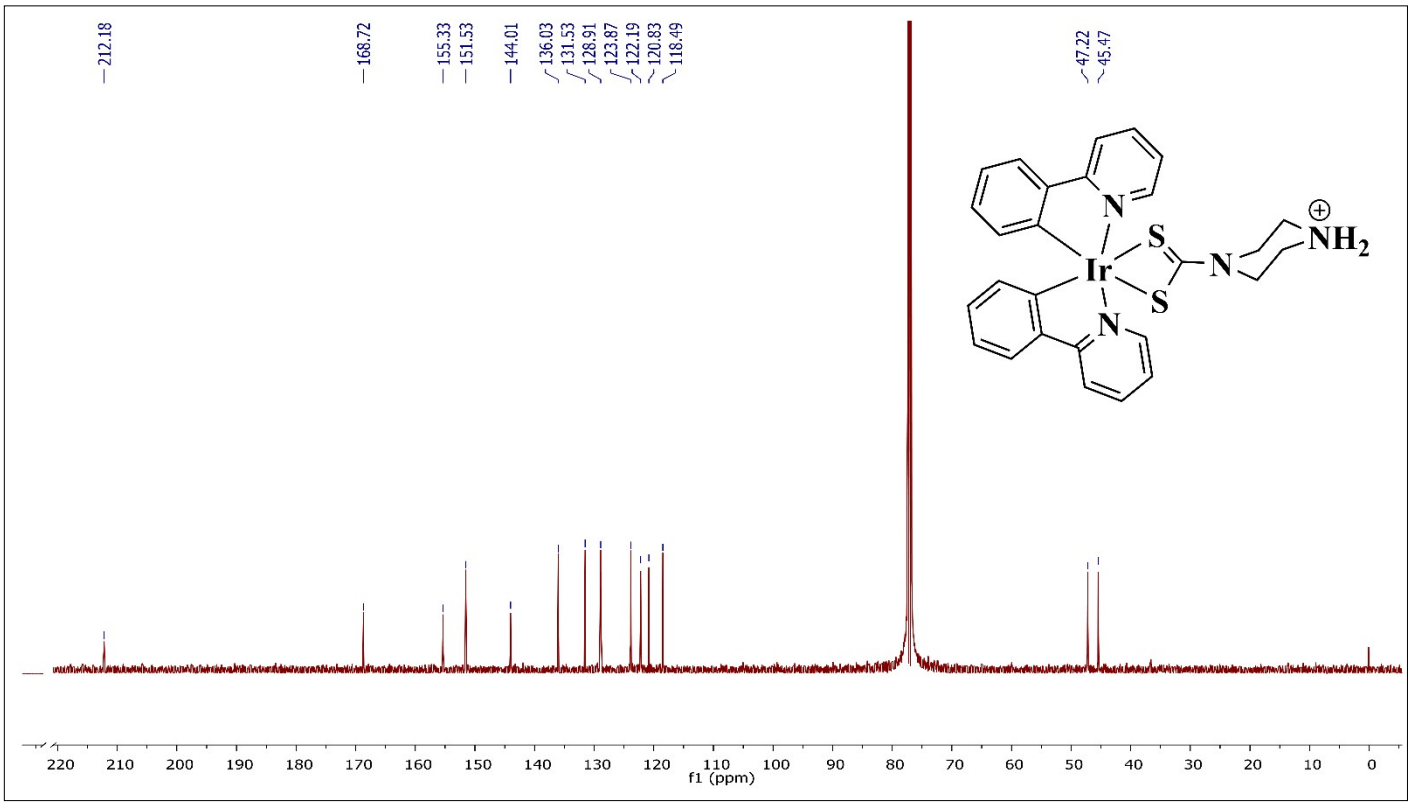


Fig. S4: ^{13}C NMR spectrum of Compound 5 in $CDCl_3$.

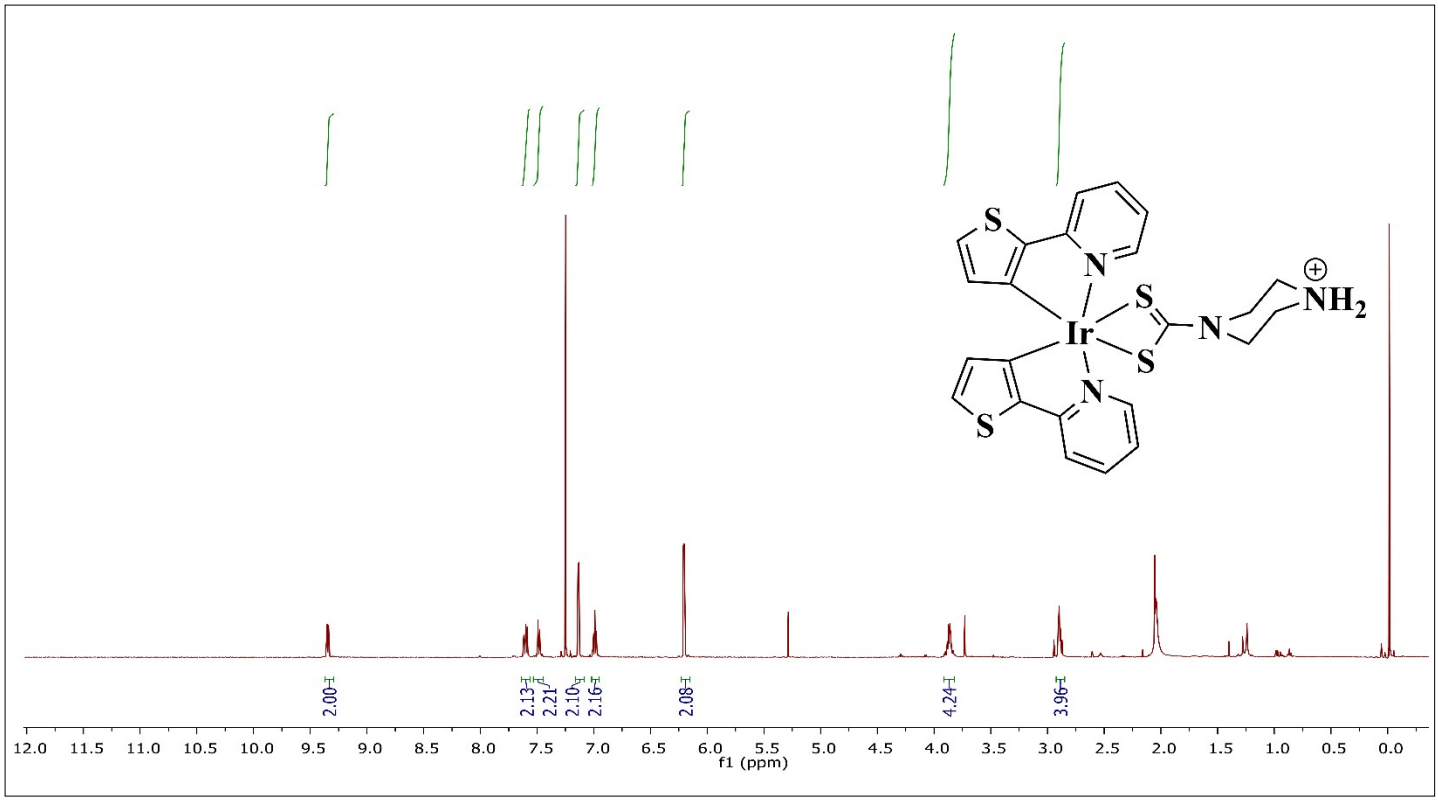


Fig. S5: ^{1}H NMR spectrum of Compound 6 in $CDCl_3$.

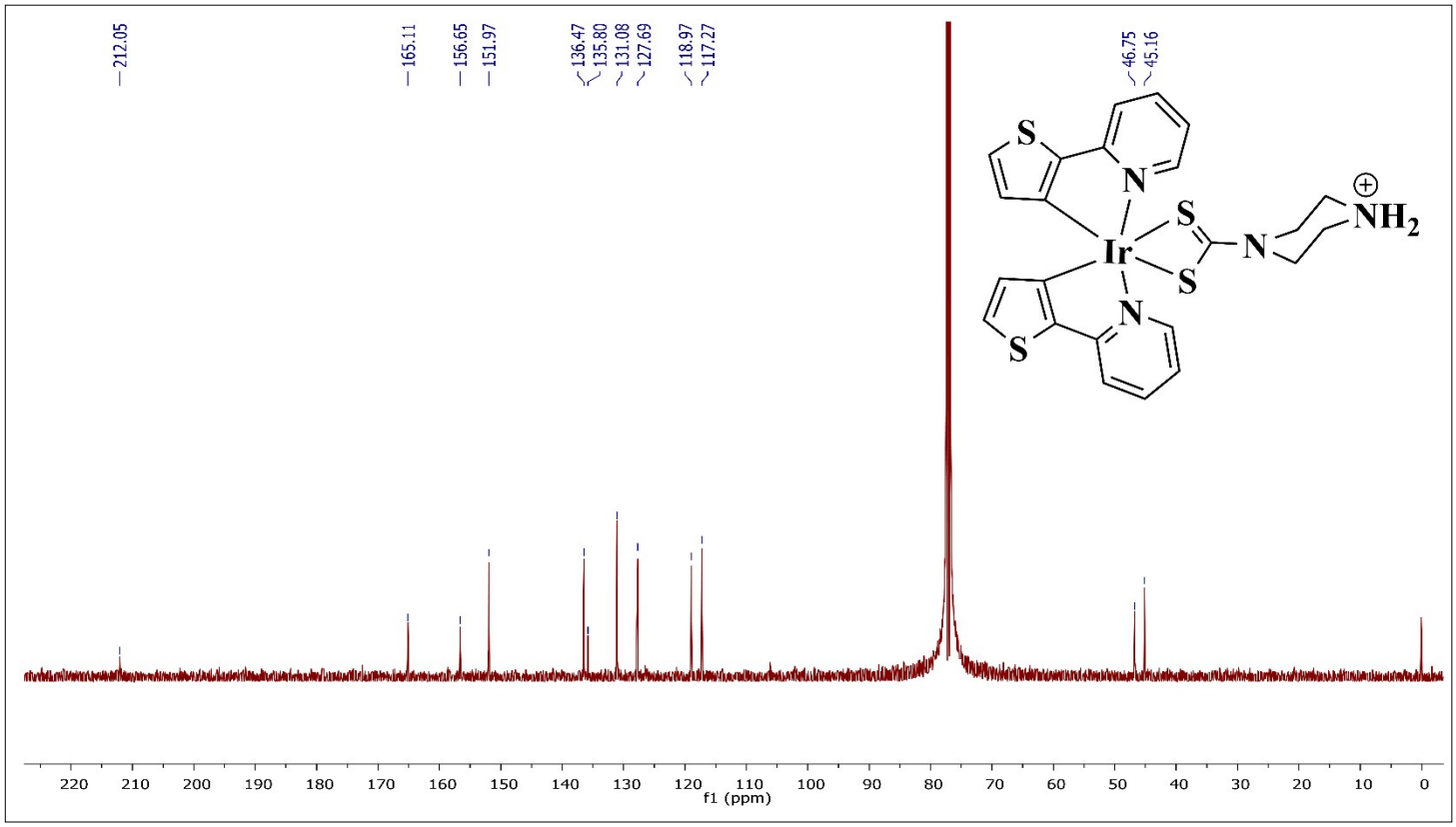


Fig. S6: ^{13}C NMR spectrum of Compound 6 in $CDCl_3$.

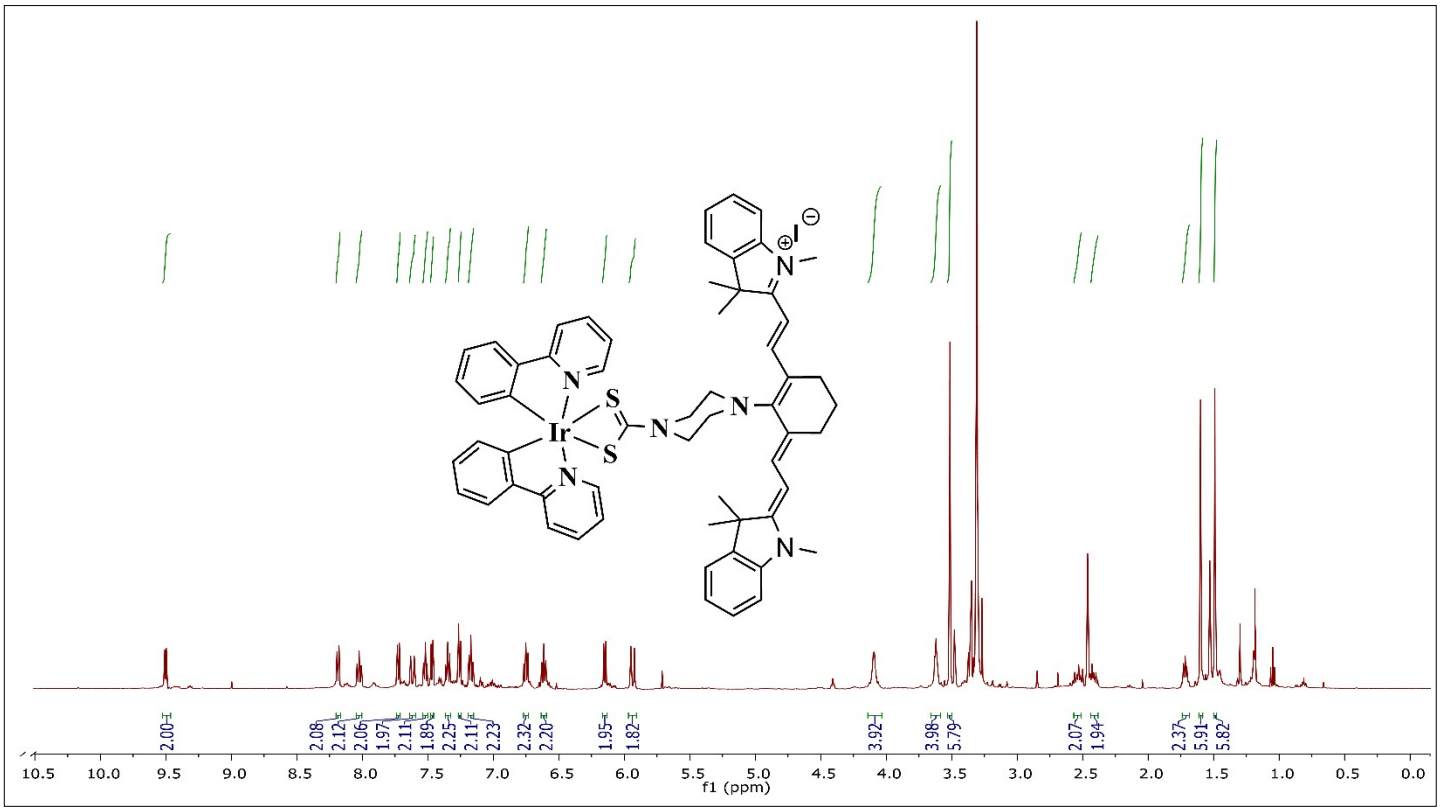


Fig. S7: ^{1}H NMR spectrum of Ir1@hcy in DMSO-d_6.

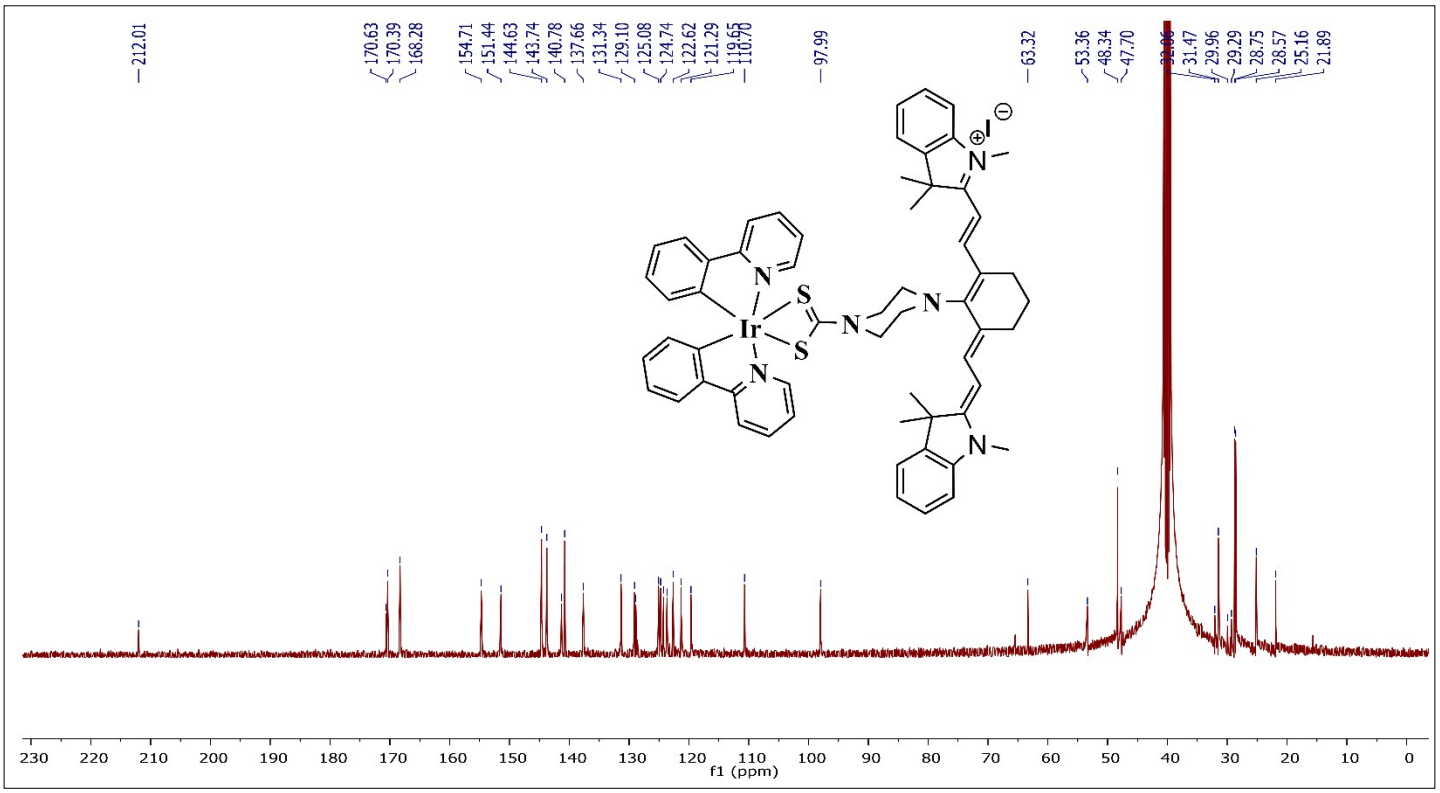


Fig. S8: ^{13}CNMR spectrum of Ir1@hcy in DMSO-d_6.

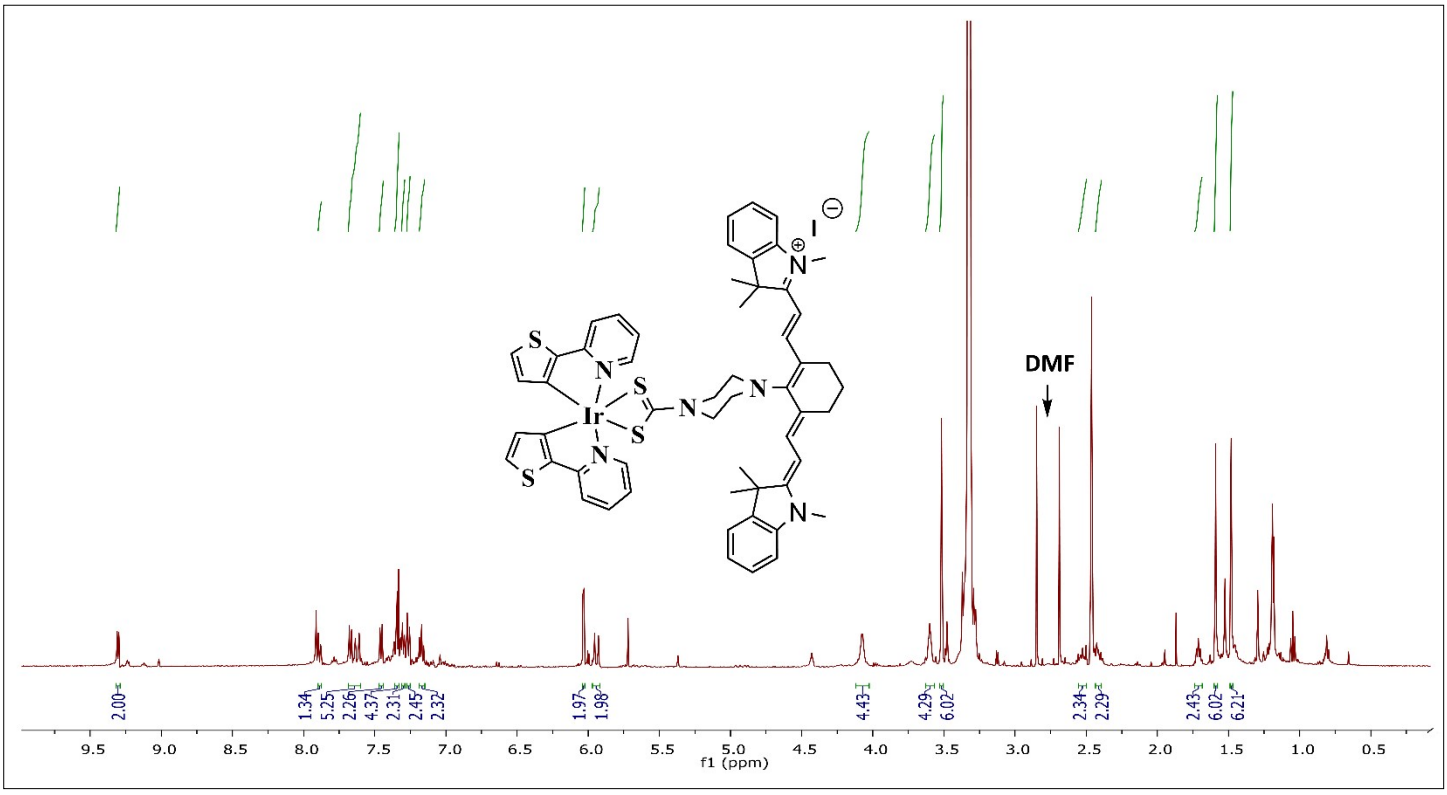


Fig. S9: ^{1}H NMR spectrum of Ir2@hcy in DMSO-d_6.

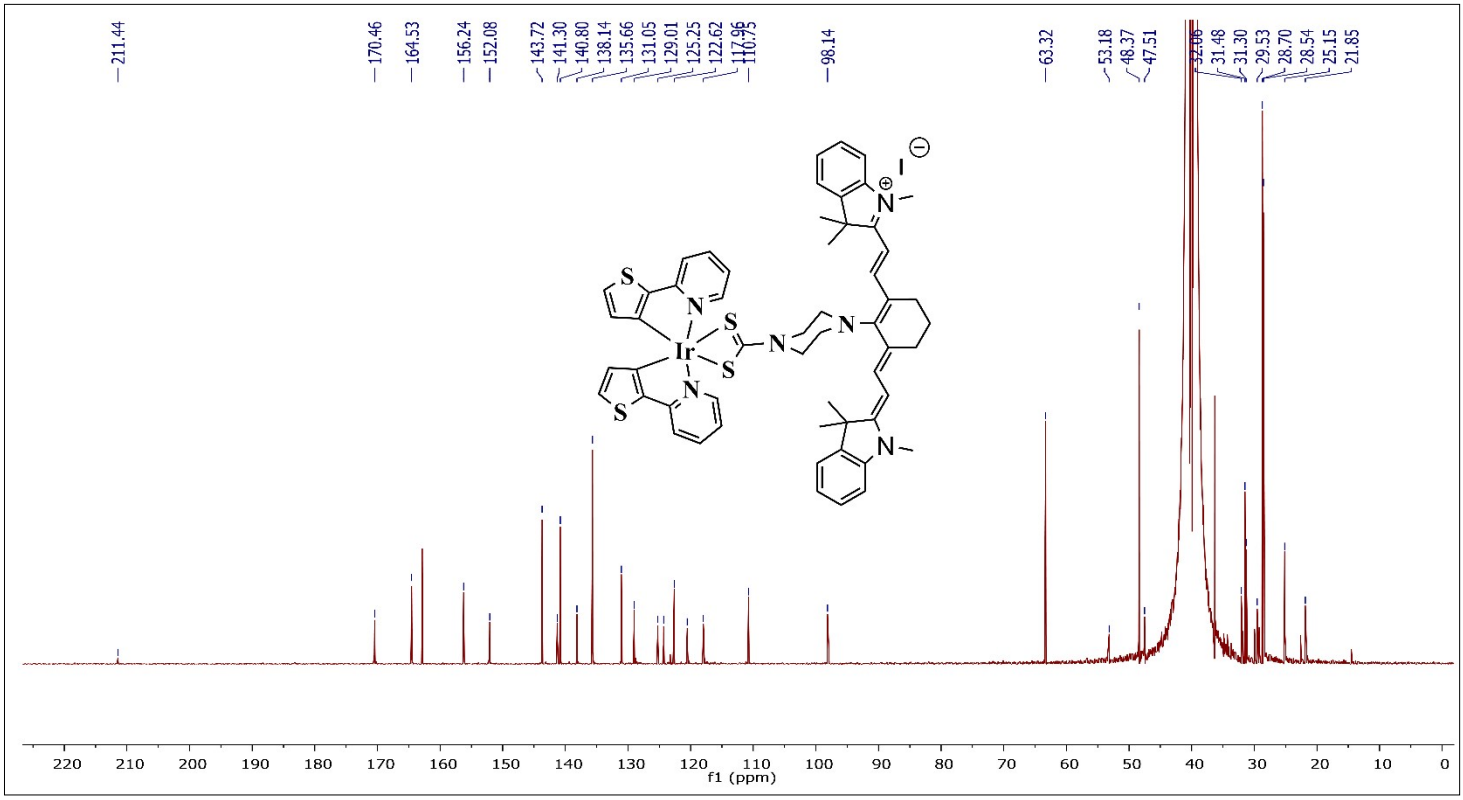


Fig. S10: ^{13}C NMR spectrum of Ir2@hcy in DMSO-d_6

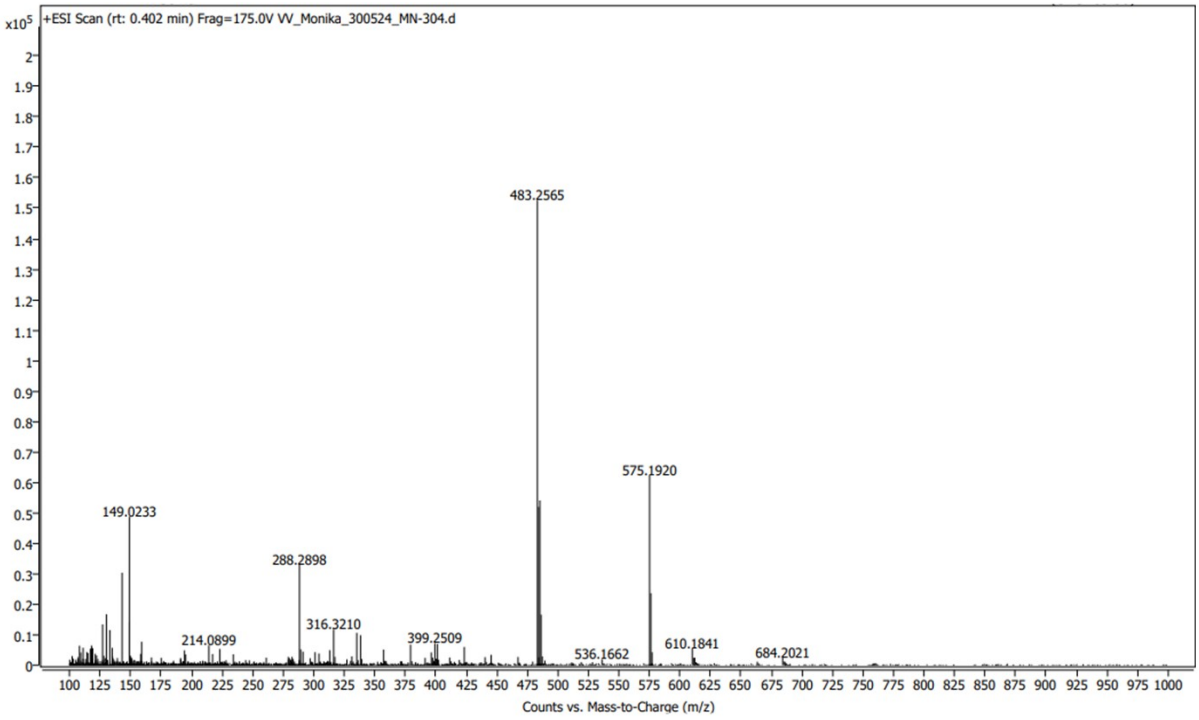


Fig. S11: ESI-HRMS spectrum of hcy-Cl (compound 3).

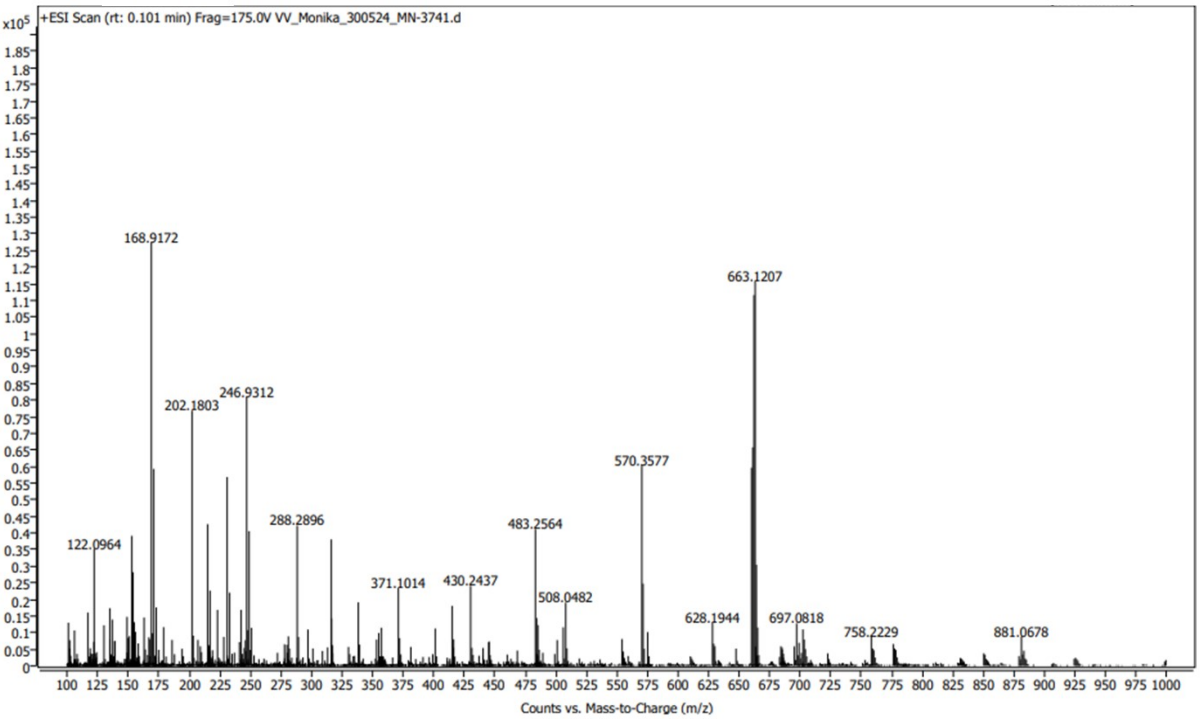


Fig. S12: ESI-HRMS spectrum of Compound 5.

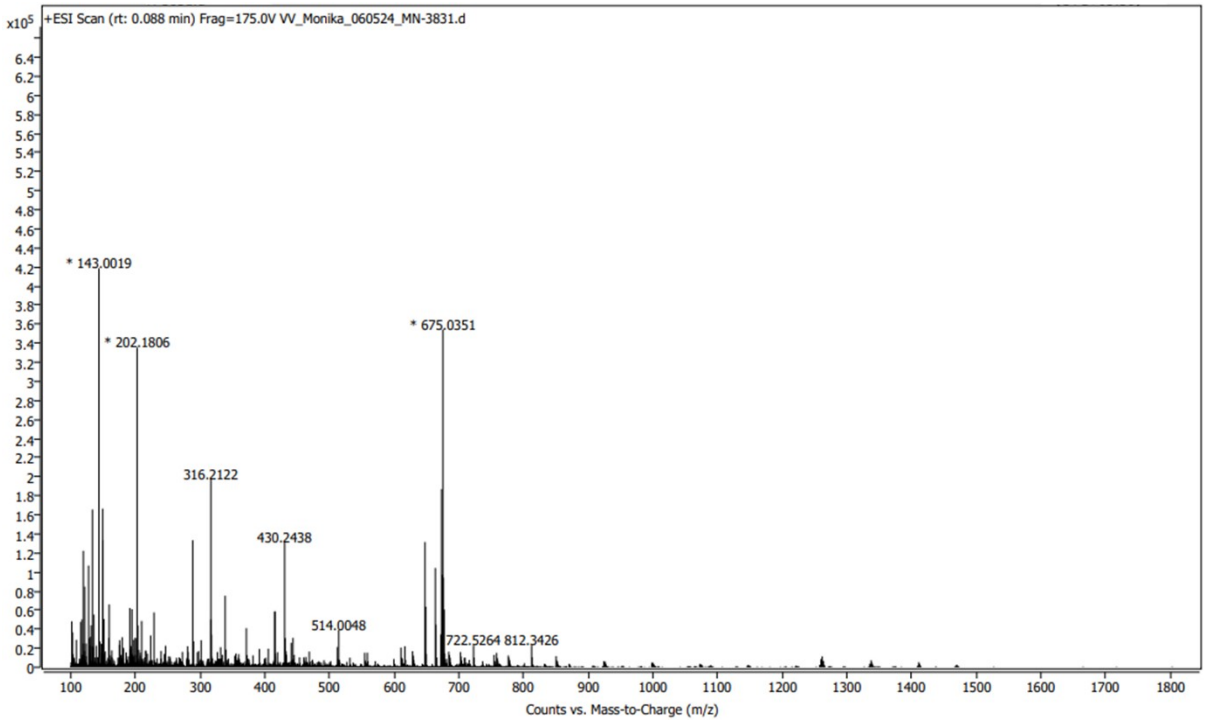


Fig. S13: ESI-HRMS spectrum of Compound 6.

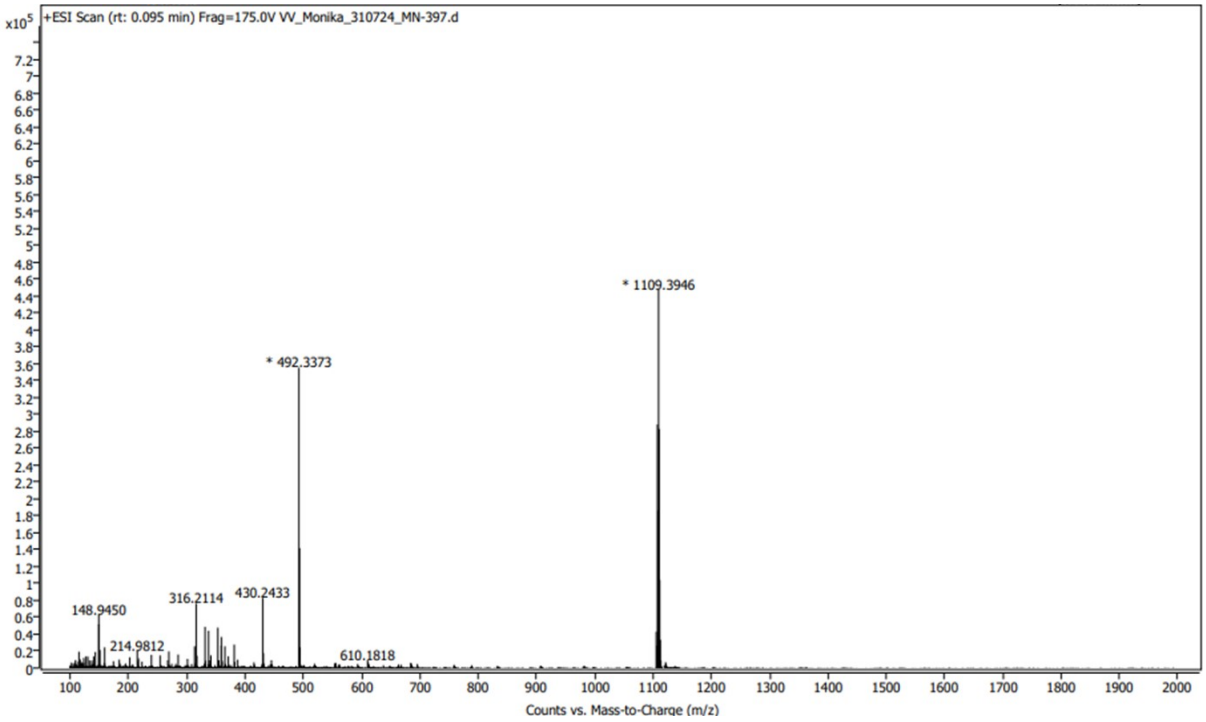


Fig. S14: ESI-HRMS spectrum of Compound Ir1@hcy.

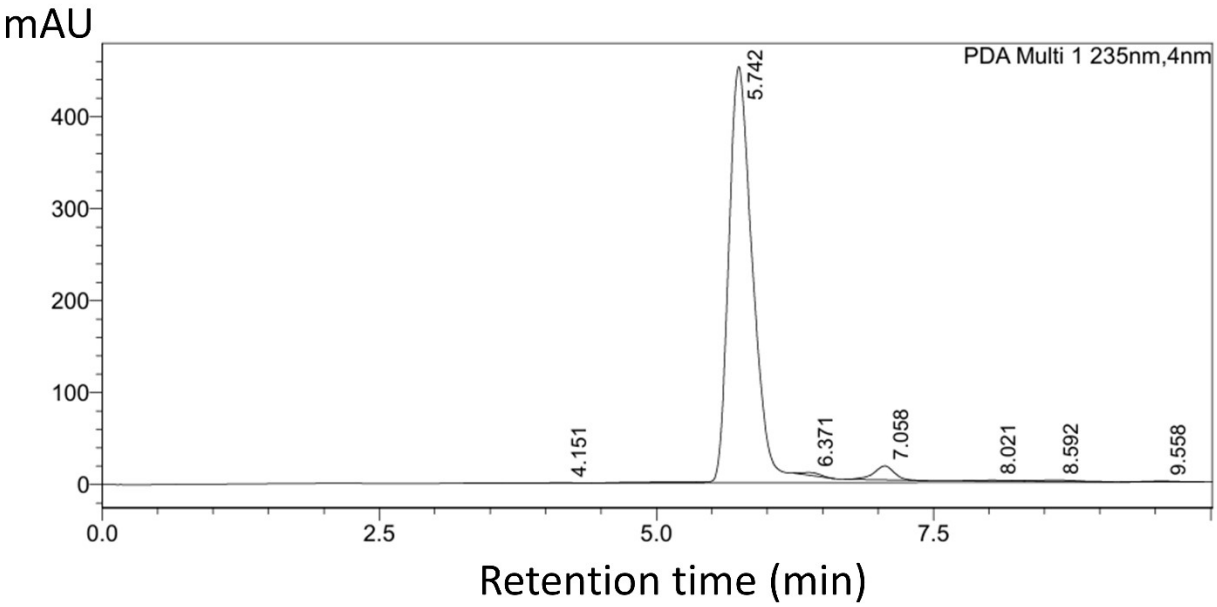


Fig. S15: HPLC chromatogram of Compound Ir1@hcy.

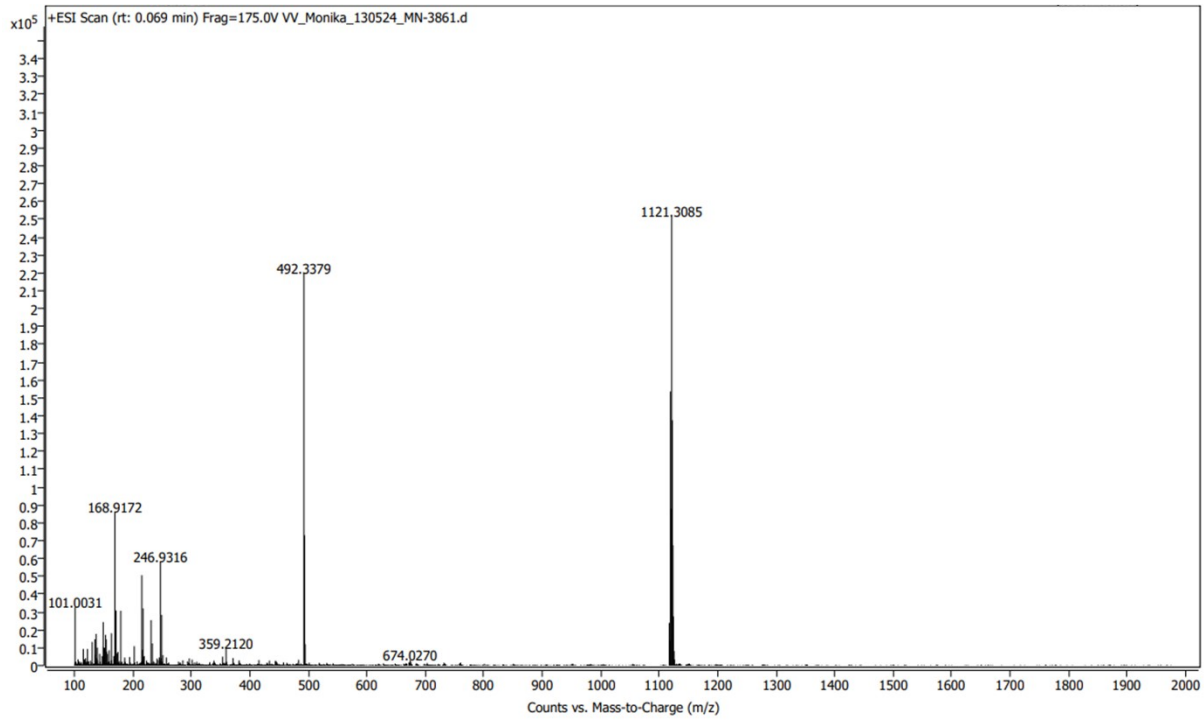


Fig. S16: ESI-HRMS spectrum of Compound Ir2@hcy.

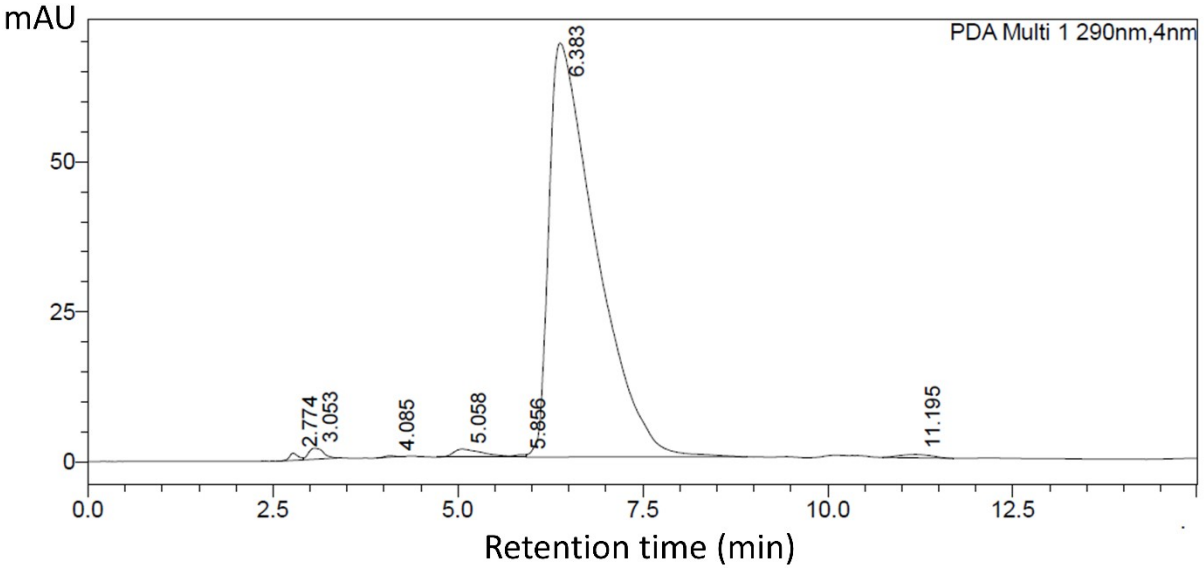


Fig. S17: HPLC chromatogram of Compound Ir2@hcy.

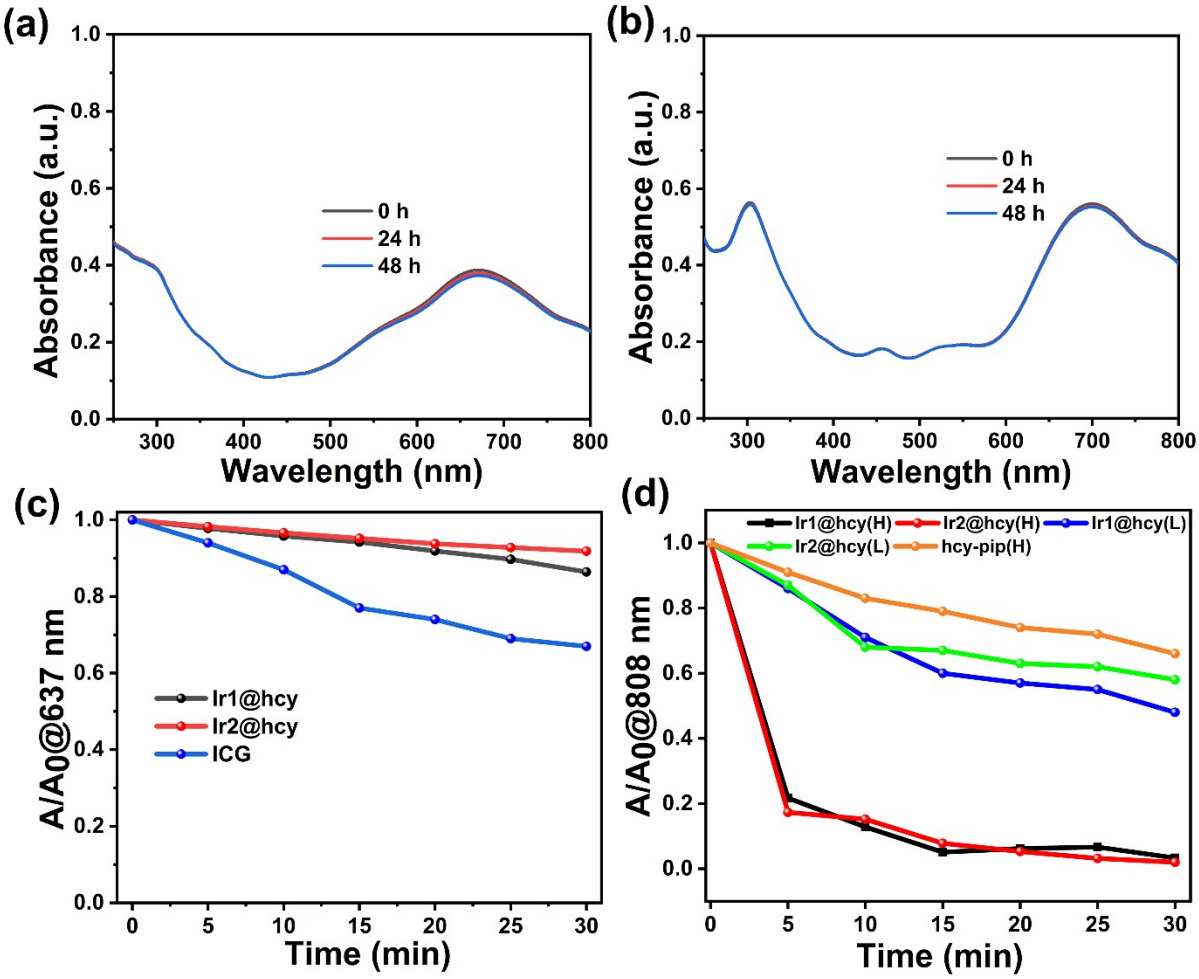


Fig. S18: Absorption spectra of Ir(III) complexes (a) Ir1@hcy, (b) Ir2@hcy in DMSO/PBS (0.4%) buffer under dark conditions. Photostability of Ir1@hcy, Ir2@hcy, and ICG under (c) light (637 nm, 2 mW/cm^2, 30 min), (d) light (808 nm, 30 min) in DMSO/PBS (0.4%) buffer. H represents high-density power (100 mW/ cm^2), and L represents low-density power source (2 mW/ cm^2).

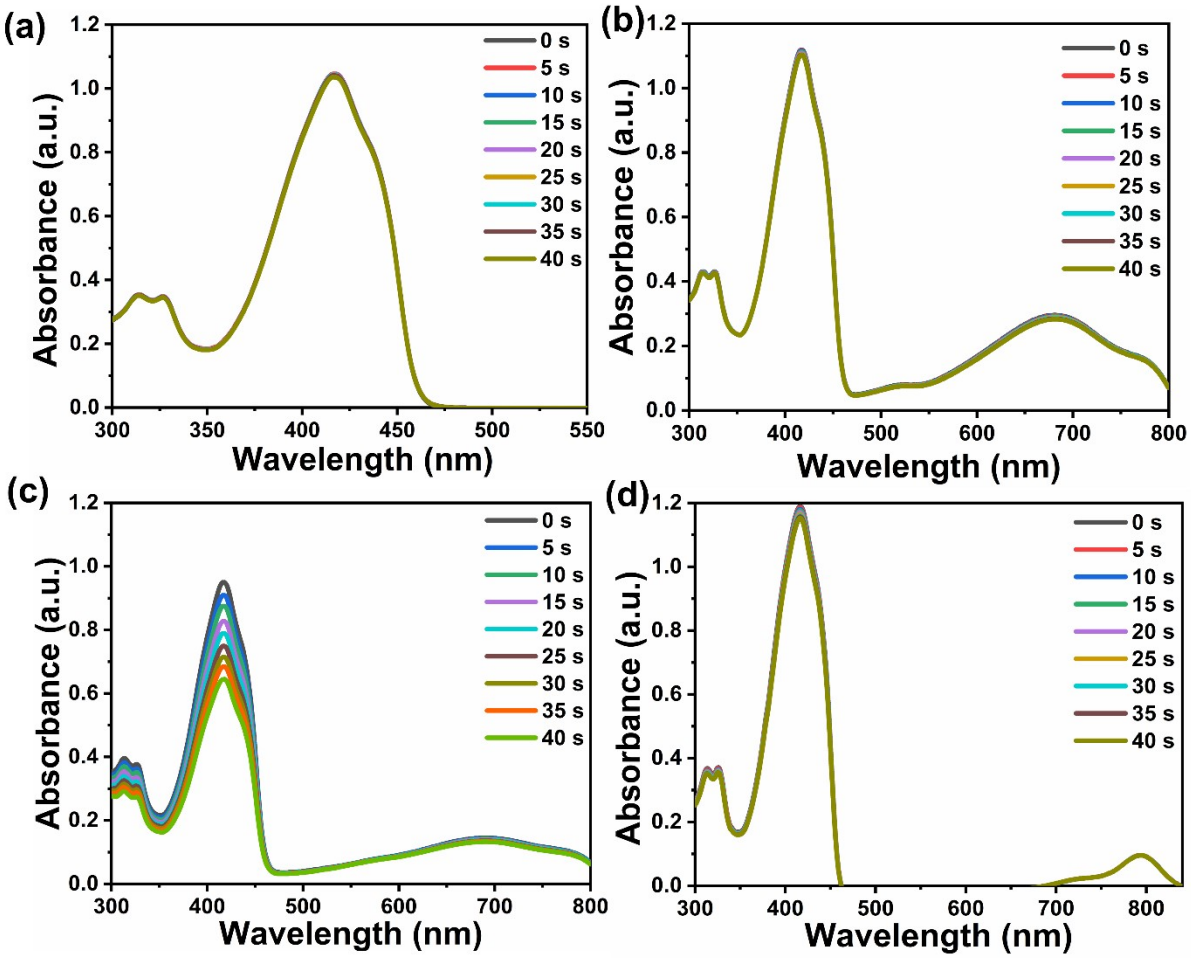


Fig. S19: Absorption spectra of DBPF with light irradiation (0-40 s) (a) DPBF alone (b) hcy-pip (c) Ir1@hcy (d) ICG.

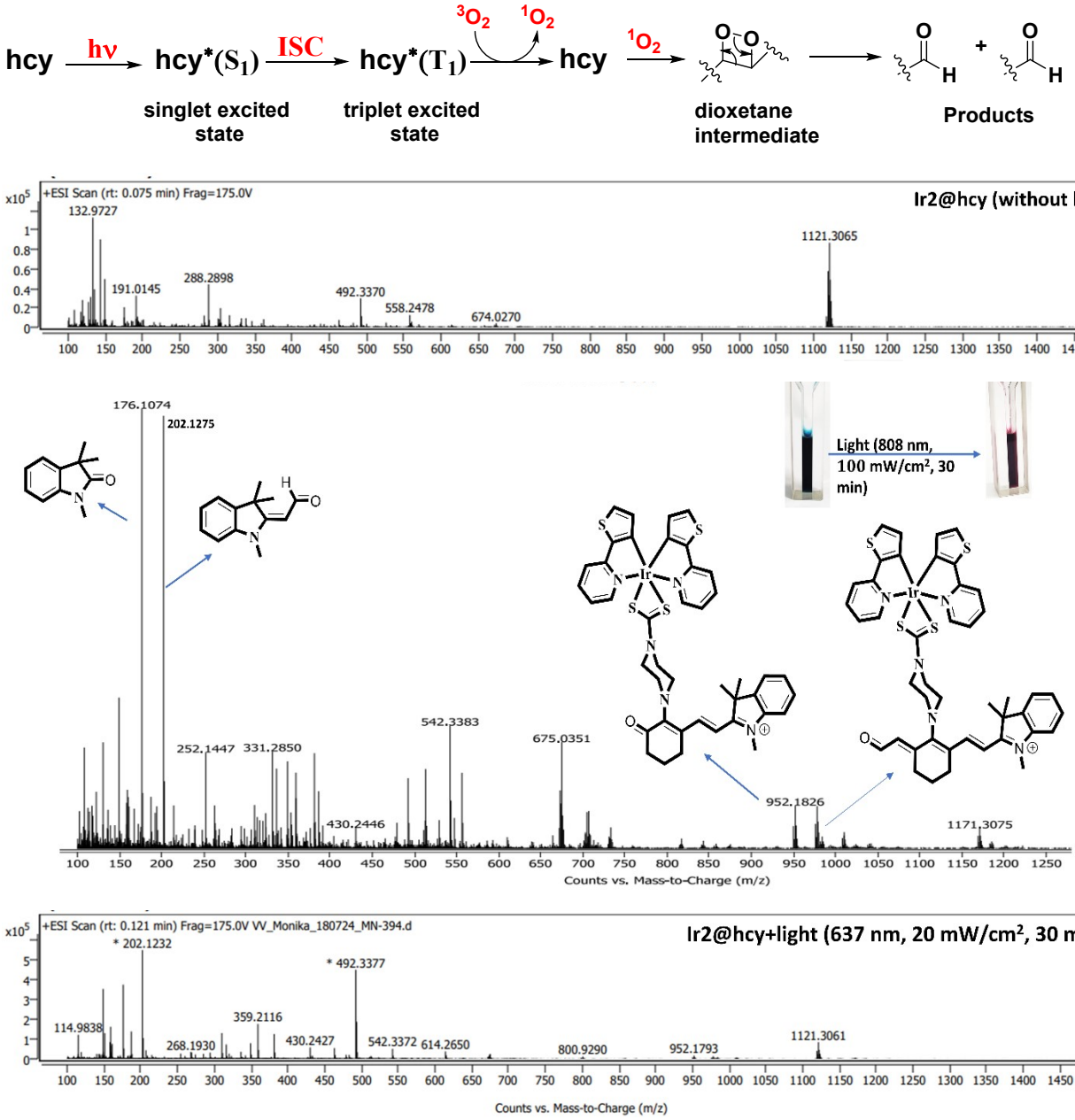


Fig. S20: Self-degradation studies by ESI-HRMS spectrum of Ir2@hcy with and without light irradiation (808 nm, 100 mW/cm², 30 min) and (637 nm, 20 mW/cm², 30 min).

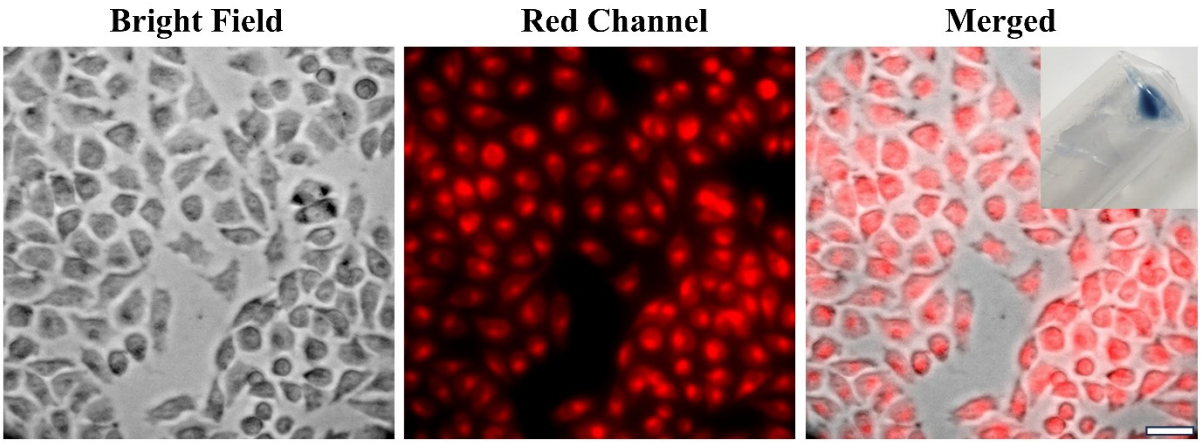
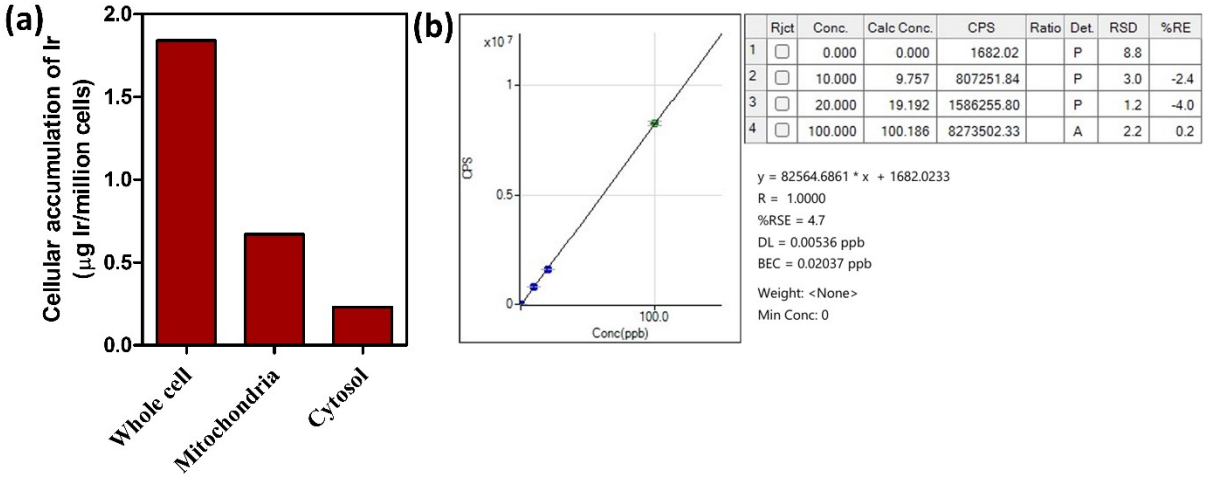


Fig. S21: Uptake images of A375 after incubation with Ir2@hcy after 30 min. Magnification: 40x, scale bar: 20 µm. (inset: photograph of cell pellet after uptake of Ir2@hcy by the cells in 30 min).



	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD	%RE
1	☐	0.000	0.000	1682.02		P	8.8	
2	☐	10.000	9.757	807251.84		P	3.0	-2.4
3	☐	20.000	19.192	1586255.80		P	1.2	-4.0
4	☐	100.000	100.186	8273502.33		A	2.2	0.2

Fig. S22: (a) Cellular distribution of Ir2@hcy in whole cell, mitochondria, and cytoplasmic matrices after 1 h of exposure to 20 µM complexes by ICP-MS. (b) Calibration curve for ICP-MS.

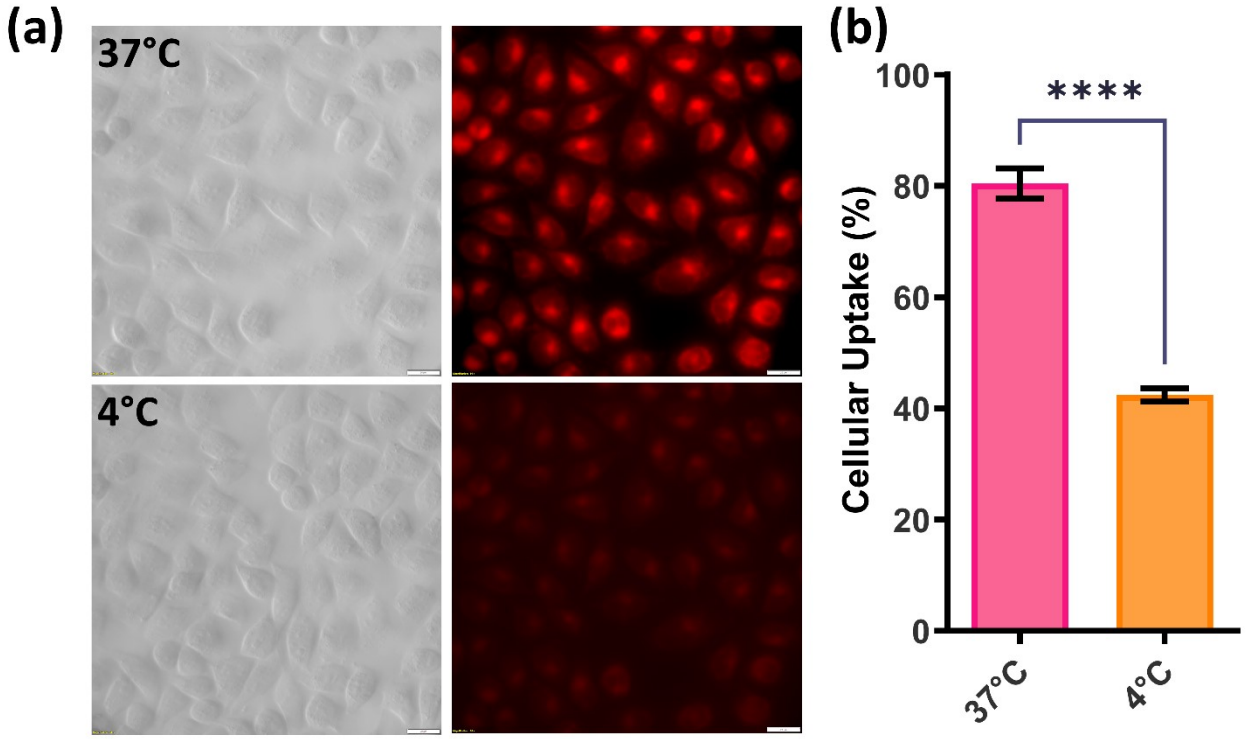


Fig. S23: (a) Fluorescence microscopic imaging for cellular uptake of Ir2@hcy at 4 °C and 37 °C after 4 h incubation. Magnification 60x. Scale bar: 20 µm. (b) Percentage cellular uptake of Ir2@hcy at 4 °C and 37 °C after 4 h incubation.

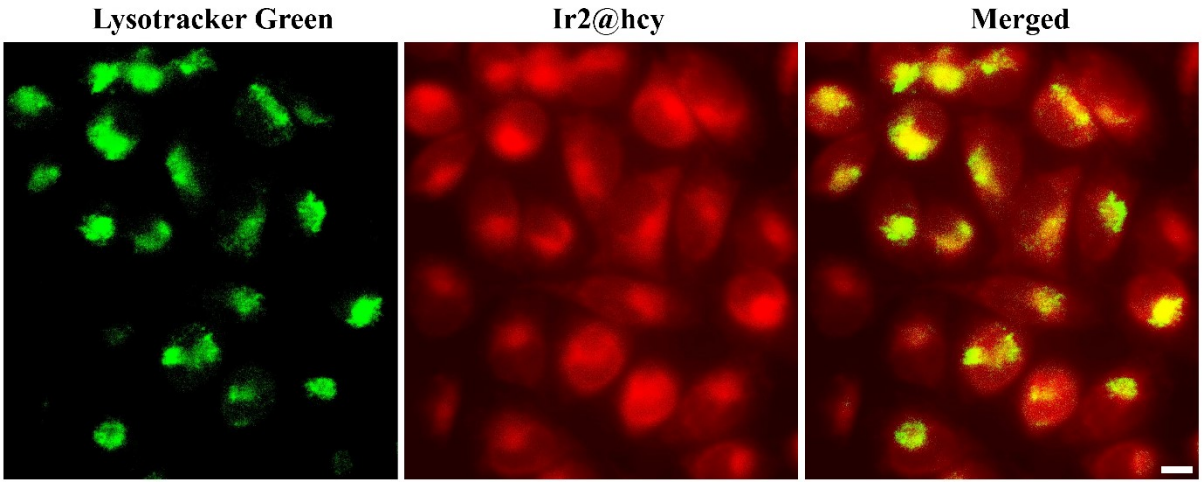


Fig. S24: Colocalization study of Ir2@hcy with lysotracker green (LTG) in A375 cells (P= 0.62). Magnification: 60x, scale bar: 20 µm.

Table S2: Photo-cytotoxicity (IC_{50}) of complexes (μM) against A375, HCT116, and A549 cell lines. Data represented as mean ± standard error with photocytotoxicity index (PI = $IC_{50(dark)}$/ $IC_{50(light)}$). (Light: 637 nm, 2 mW/cm^2, 20 min).

	A375			HCT116			A549		
Compound	**Dark**	**Light**	**PI**	**Dark**	**Light**	**PI**	**Dark**	**Light**	**PI**
Ir1@hcy	20.49±5.8	0.175±2.7	117	>20	0.62±1.9	>32.3	>20	0.98±4.6	>20.4
Ir2@hcy	13.02±6.1	0.069±2.2	188	>20	0.35±1.5	>57.2	>20	0.84±3.8	>23.8
Cis-platin	>20	>20	~1	>20	>20	~1	>20	>20	~1

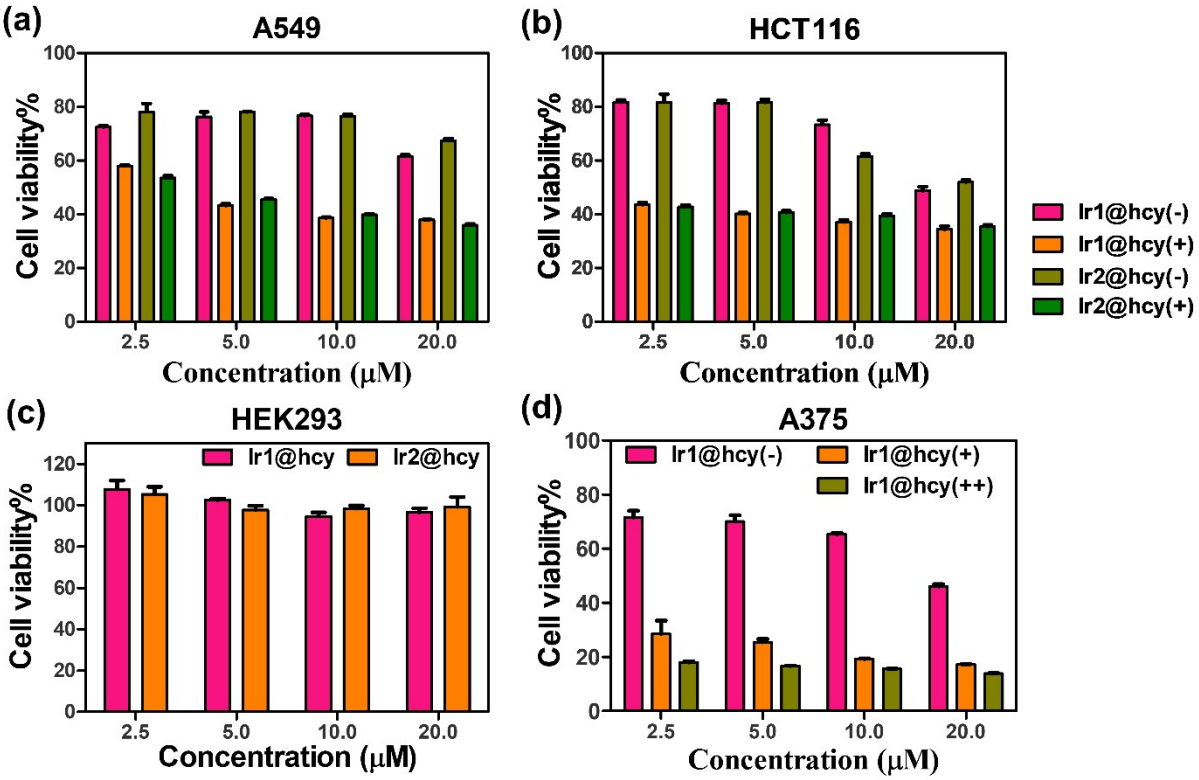


Fig. S25: Cell viability analysis in (a) A549, (b) HCT116 cells after incubation with Ir1@hcy, Ir2@hcy in dark and light (637 nm, 2 mW/cm^2, 20 min) conditions. (c) Cell viability studies in HEK293 cells after 24h incubation with complex. (d) Cell viability studies in A375 cells treated with Ir1@hcy followed by light irradiation. ("++" represents high power intensity light i.e. 20 mW/cm^2).

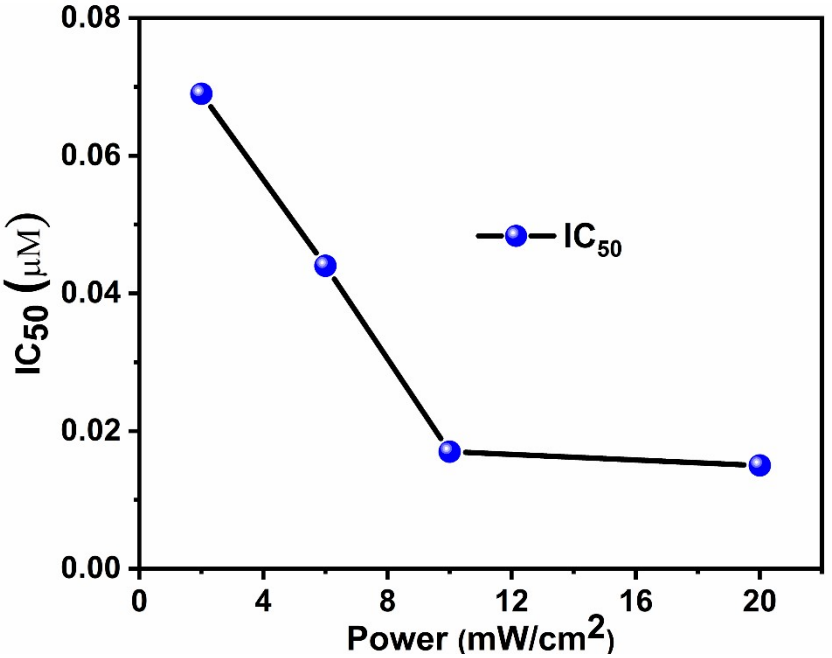


Fig. S26: Change in IC_{50} values of Ir2@hcy in A375 cell line with different power light irradiation.

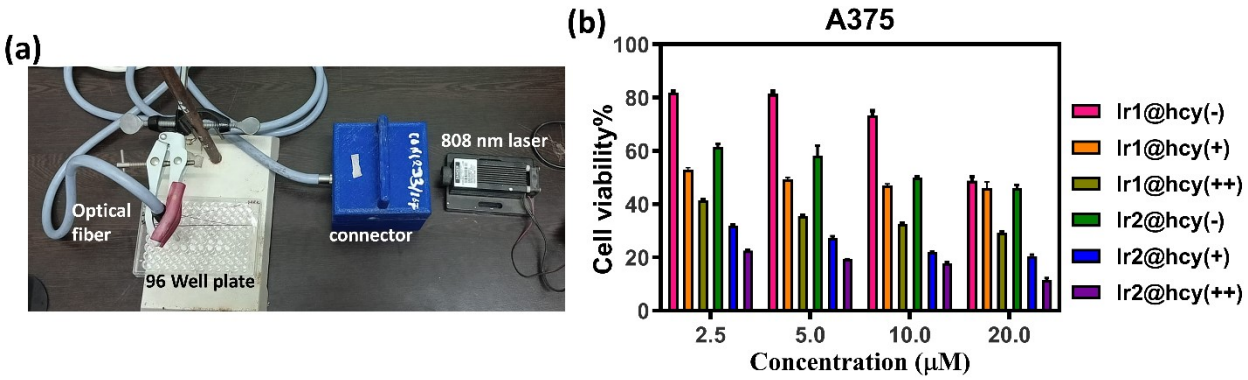


Fig. S27: (a) 808 nm laser setup for cell-viability studies. (b) Cell viability studies in A375 cells treated with complexes followed by light irradiation (808 nm, 5 min). Where "+" represents a low-density power source (2 mW/ cm^2) and "++" represents high-density power (100 mW/ cm^2).

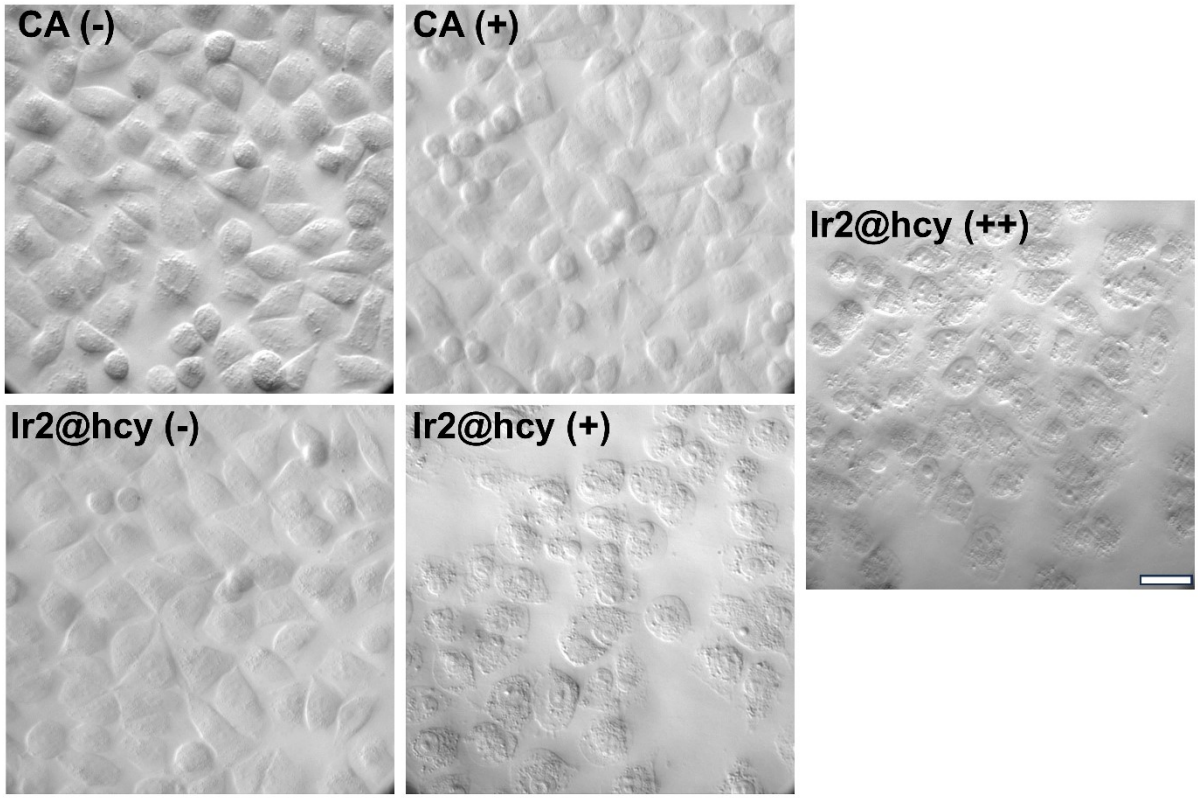


Fig. S28: Morphology changes in A375 cells with and without incubation with Ir2@hcy in dark and light conditions (637 nm, "+" and "++" represents 2 and 20 mW/cm^2 respectively). Magnification: 60x, scale bar: 20 µm.

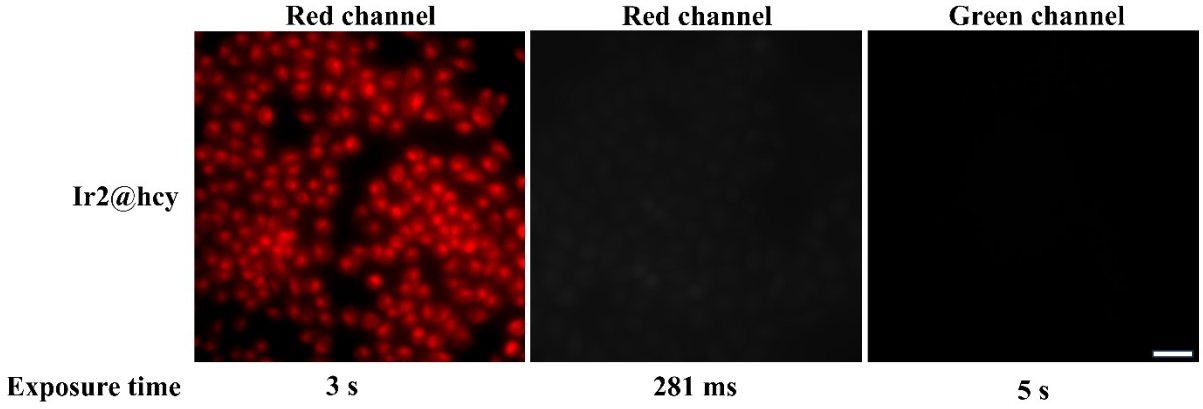


Fig. S29: Fluorescence microscopic images of A375 cells after incubation with Ir2@hcy in different channels at exposure time for complex in uptake images, JC-1 aggregates, and the green channel. Magnification: 40x, scale bar: 20 µm.

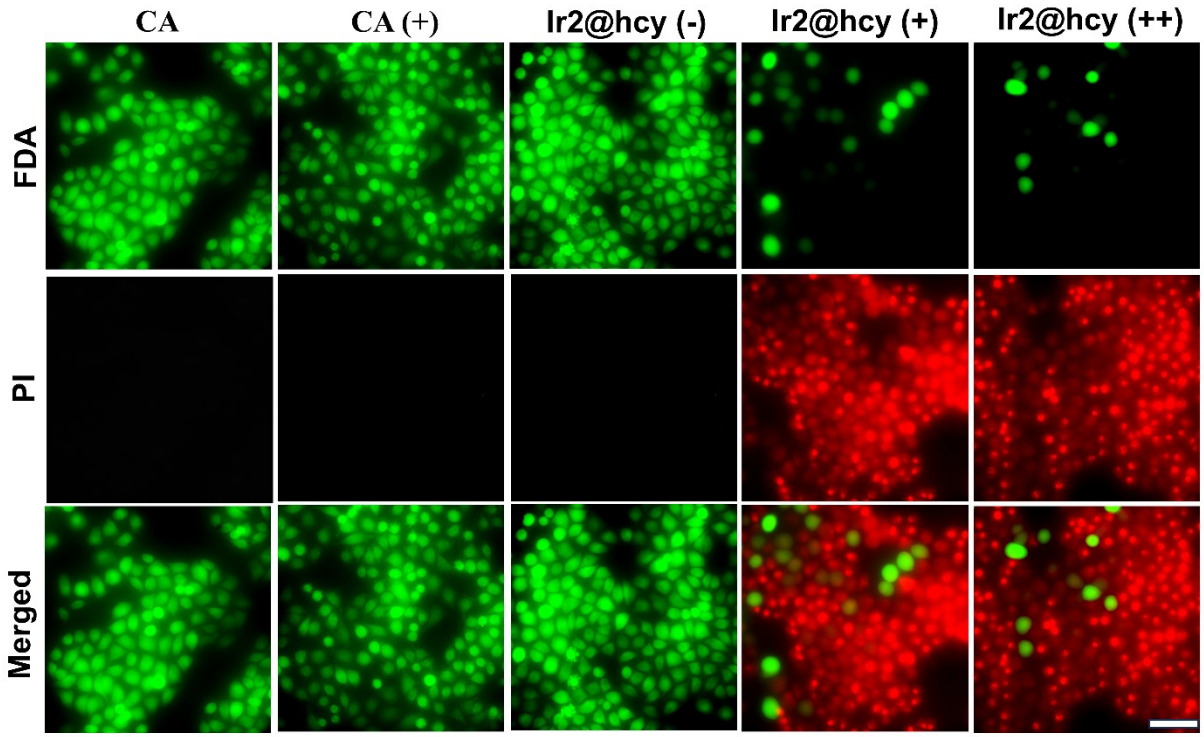


Fig. S30: Live/dead assay of A375 cells with and without incubation with Ir2@hcy in dark and light (637 nm, 2 and 20 mW/cm^2, 20 min) conditions. Magnification: 40x, scale bar: 20 µm.

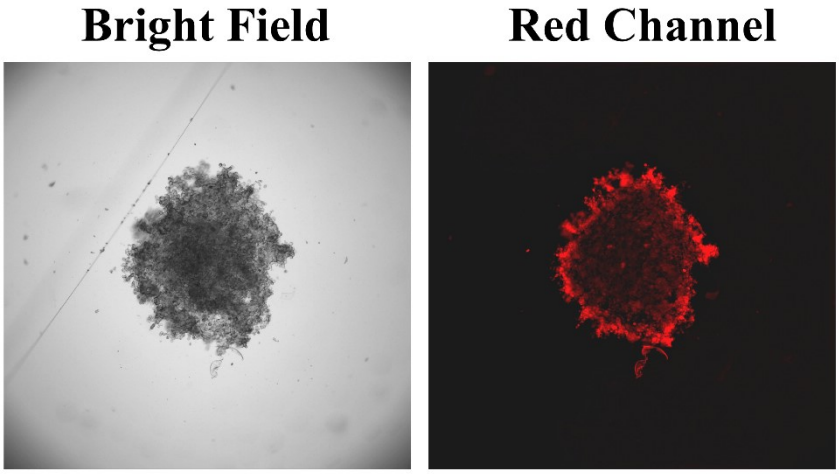


Fig. S31: Uptake of Ir2@hcy in A375 MCTSs after 30 min of incubation. Magnification: 4x, scale bar: 200 µm.

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