Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Iridium Complex-Based Probe for Photoluminescence Lifetime Imaging of Human Carboxylesterase 2 in Living Cells

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Experimental Section

Reagents

Human serum albumin (HSA), Bovine albumin (BSA), recombinant human acetylcholinesterase (AChE), butyrylcholinesterase (BChE), amino acids including Cys, Glu, Gly, Tyr, Trp, Ser, Lys as well as GSH were obtained from Sigma-Aldrich (St. Louis, USA). Recombinant human carboxylesterases including hCE1 and hCE2 were obtained from RILD (Shanghai,China). Bisp-nitrophenyl phosphate (BNPP), loperamide (LPA) were purchased from TCI (Tokyo, Japan). Ethylene diamine tetraacetic acid (EDTA) and huperzine A (HA) were obtained from J&K Chemical Ltd. (Beijing, China). HepG2 cell lines were obtained from Biotechnology Co., Ltd. Shanghai enzyme research (Shanghai, China). Dulbecco's modified eagle media (DMEM), fetal bovine serum (FBS), and phosphate-buffered saline (PBS) solution and other materials used for cell culture were purchased from Gibco (Invitrogen, Carlsbad, CA). Other chemicals used were purchased from J&K Chemical Ltd. (Beijing, China) and Sigma-Aldrich (St. Louis, USA), unless otherwise specified.

Apparatus

^{1}H NMR and ^{13}C NMR spectra were measured with a JNM-ECA600 spectrometer (JEOL, Japan) in CDCl3. Mass spectra were obtained with a Thermo LTQ mass spectrometer (Thermo Scientific, San Jose CA). Ultrapure water (over 18 kΩ) were obtained from a Milli-Q water purification system (Millipore) in the experiments. The in-vitro photoluminescence lifetime of different solutions were recorded on a combined steady state and photoluminescence lifetime spectrometer (Edinburgh FLS 920, United Kingdom). The absorption spectra were recorded with UV visible spectrometer (JASCO V-550, Japan). The fluorescence spectra were recorded with fluorescence spectrometer (JASCO FP-8600, Japan). Photoluminescence imaging and photoluminescence lifetime imaging experiments were performed on a FV1200 confocal laser scanning microscope (Olympus, Japan) with a 60× objective lens.

Synthesis of the Probe Ir-TB

0.5 g of azidoethanol was dissolved in 30 ml of methylene chloride and placed in an ice-water bath. 2 ml of pyridine was added, and 1 ml of benzoyl chloride was dissolved in 10 ml of methylene chloride and slowly dropped into the reaction solution. The ice water bath was removed after half an hour of reaction and the reaction was allowed to proceed overnight at room temperature. The reaction solution was extracted with dichloromethane and spin-dryed to a colorless liquid 0.9 g. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (dt, *J* = 8.5, 1.6 Hz, 2H), 7.54 – 7.48 (m, 1H), 7.42 – 7.35 (m, 2H), 4.46 – 4.39 (m, 2H), 3.59 – 3.48 (m, 2H).

Under nitrogen protection, 0.6 g of compound 2 and 0.4 g of 4-ethynyl-2,2'-bipyridine were added and dissolved in 50 mL of acetonitrile, 80 mg of copper iodide and 1 ml of triethylamine were added, and the reaction was continued at 40 degrees for 24 hours. After washing once with EDTANa2, it was extracted three times with ethyl acetate (100 mL*3) and the organic phase was removed by column

chromatography to get 0.7 g of brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J* = 5.0 Hz, 1H), 8.62 (d, *J* = 4.4 Hz, 2H), 8.37 (d, *J* = 8.0 Hz, 1H), 8.10 (s, 1H), 7.96 – 7.91 (m, 2H), 7.86 (dd, *J* = 5.1, 1.6 Hz, 1H), 7.78 (td, *J* = 7.8, 1.7 Hz, 1H), 7.55 – 7.48 (m, 1H), 7.38 (dd, *J* = 10.6, 4.8 Hz, 2H), 7.31 – 7.24 (m, 1H), 4.79 (dd, *J* = 7.6, 3.1 Hz, 2H), 4.76 – 4.72 (m, 2H).

Cyclometalated Ir *μ*-chlorido-bridged dimer was synthesized based on the previous work reported[1]. Under nitrogen protection, 0.14 g of compound 3 and 0.24 g of cyclometalated Ir μ-chlorido-bridged dimer were added to a 100 ml round-bottomed flask, 25 mL of dichloromethane and 25 mL of methanol were added, and the mixture was stirred at 40 degrees for 24 hours. The disappearance of the starting material was detected by TLC, and dichloromethane was removed under reduced pressure, 0.5 g of NH_4PF_6 was dissolved in 40 ml of water and dropped into the reaction solution. The mixture was stirred at room temperature for half an hour. TLC showed that the starting material disappeared and was filtered to obtain 0.46 g of orange-red powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (s, 1H), 9.27 (d, *J* = 12.7 Hz, 2H), 8.88 (d, *J* = 4.1 Hz, 2H), 8.22 (d, *J* = 7.3 Hz, 2H), 8.12 (t, *J* = 7.6 Hz, 1H), 8.03 (d, *J* = 5.4 Hz, 1H), 7.96 (d, *J* = 7.4 Hz, 2H), 7.82 (dd, *J* = 9.2, 6.2 Hz, 2H), 7.76 – 7.69 (m, 3H), 7.66 (d, *J* = 5.6 Hz, 2H), 7.47 – 7.40 (m, 1H), 7.40 – 7.31 (m, 3H), 7.31 – 7.22 (m, 4H), 7.05 (dd, *J* = 7.2, 3.7 Hz, 2H), 7.05 (dd, *J* = 7.2, 3.7 Hz, 2H), 6.84 (t, *J* = 7.4 Hz, 2H), 6.84 (t, *J* = 7.4 Hz, 2H), 6.25 (dd, *J* = 15.7, 7.5 Hz, 2H), 4.82 (d, *J* = 4.5 Hz, 2H), 4.72 (d, *J* = 4.6 Hz, 2H).

General Procedure for hCE2 Detection

Unless otherwise specified, the photoluminescence and lifetime detection of hCE2 and other related experiments were carried out in 10 mM PBS buffer (pH=7.4) as follows. The stock solution of Ir-TB (1.0 mM) was prepared in DMSO. In a 600 μL total volume of centrifuge tube, corresponding amount of PBS buffer and diluted mentioned enzymes and were mixed together. Then different amount of Ir-TB was added to the mixed solution to start the reactions. After incubation on the shaker at 37 °C for different time according to the request of the experiments, 1 mM NaOH solution was added into the tube to terminate the reaction. Finally, 200 μL of the reaction solution was transferred to quartz cell to perform absorbance, fluorescence and in-vitro lifetime experiments.

Cell Culture

The HepG2 cells used in the experiments were grown in Dulbecco's modification of Eagle's medium Dulbecco (DMEM) supplemented with fetal bovine serum (FBS) of 10% and the cells were incubated in an atmosphere of 5% CO_2 and 95% air at 37℃.

Photoluminescence Imaging

One day before the experiment, the cells were transferred to a confocal cell culture dish (20 mm diameter). The adherent cells were washed and the medium was replaced by new DMEM with or without 200 μM BNPP. After 40 min pretreatment of BNPP, stock solution of 1 mM Ir-TB was diluted in fresh DMEM to obtain a 5 μM final concentration. The cells were then incubated with the fresh DMEM medium with or

without Ir-TB for another 30 min before the medium was replaced by fresh DMEM again. Finally, the cells used were directly performed photoluminescence imaging and photoluminescence lifetime imaging without washing steps. The photoluminescence imaging experiments were carried out under the same conditions using an Olympus FV1200 confocal laser scanning microscope. A 488-nm laser was used here to excite Ir-TB to collect the emission spectrum with a 560 to 660-nm band-pass filter, and the photoluminescence signal was collected in sequential mode with a 60× objective.

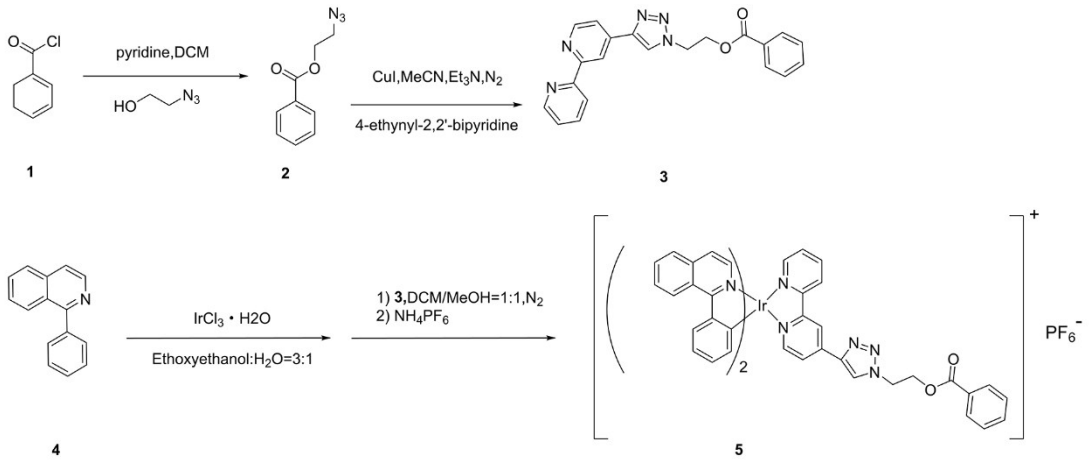
Photoluminescence Lifetime Imaging

An OLYMPUS FV1200 microscope with a 60x NA was exploited for photoluminescence lifetime imaging, and it is equipped with a Picoquant picoHarp300 (Germany) controller, time-correlated single photon counting (TCSPC) data sets were obtained using this. All of the experiment samples were excited picosecond 441 nm pulses generated by a 0.2 MHz laser. We collected the nondescanned emission using a 582/75 nm bandpass filter (PicoQuant, Germany) and then it is detected by a MPD SPAD detector (PicoQuant, Germany). PLIM images were acquired in 512 × 512 pixels. SymPhoTime64 mage software was used to analyze the photon data. The initial PLIM data obtained is analyzed by first grouping the time-dependent photon image by (3 pixels × 3 pixels) and allocating the minimum threshold counts of the 50 recorded photons used for modeling. Then, the lifetime values obtained from exponential fitting are displayed as a heat-map image.

Cytotoxicity Assay

The cytotoxicity of the probe Ir-TB was investigated by the standard MTT assay. The HepG2 cells were seeded in 96-well microtiter plates at a density of 9000 cells/well and cultured at 37℃ in a CO_2 incubator for 24 h. After 24 h incubation, the medium was removed and replaced with fresh DMEM containing 5 μM final concentration of Ir-TB. The cells were incubated with Ir-TB for 2 h, 4 h, and 6h respectively. Then 100 μL of the MTT solution (0.5 mg/mL) was added to each well. After 4 h, the MTT solution was removed and 100 μL of DMSO was added to each well to dissolve the formed formazan. The plates were shaken for 10 min, and the absorbance at 490 nm was measured with a microplate reader M3.

Supporting Figures



Scheme S1. Synthesis of Ir-TB

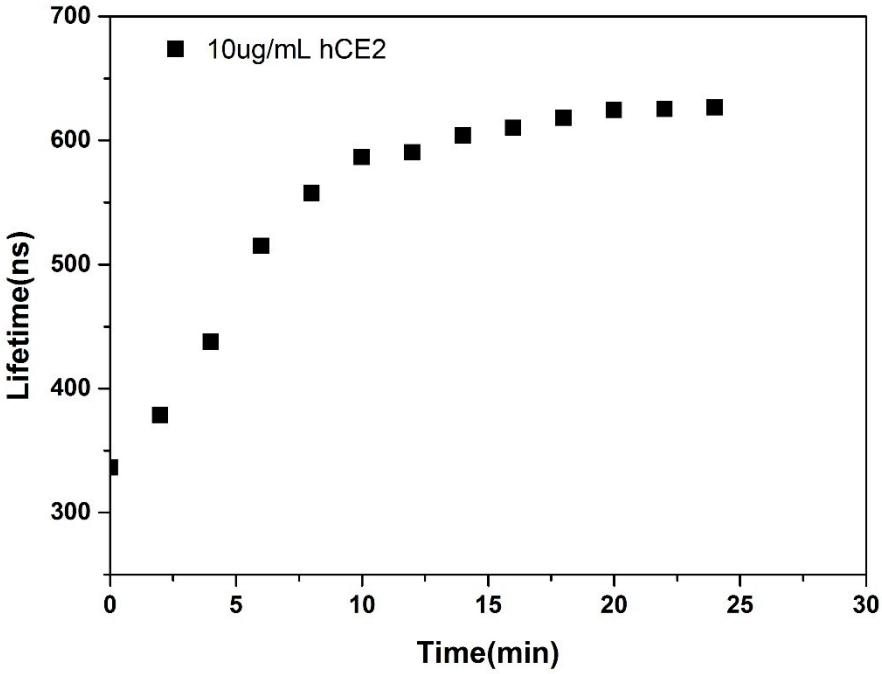


Figure S1. Time course of Ir-TB (10 μM) reacted with hCE2 (10 μg/mL) in PBS buffer.

Table S1. Kinetic Parameters for Ir-TB hydrolysis by hCE2

Enzyme	K_M(μM)	V_m(nmol/min/mg)
hCE2	2.21±0.35	43.9±1.6

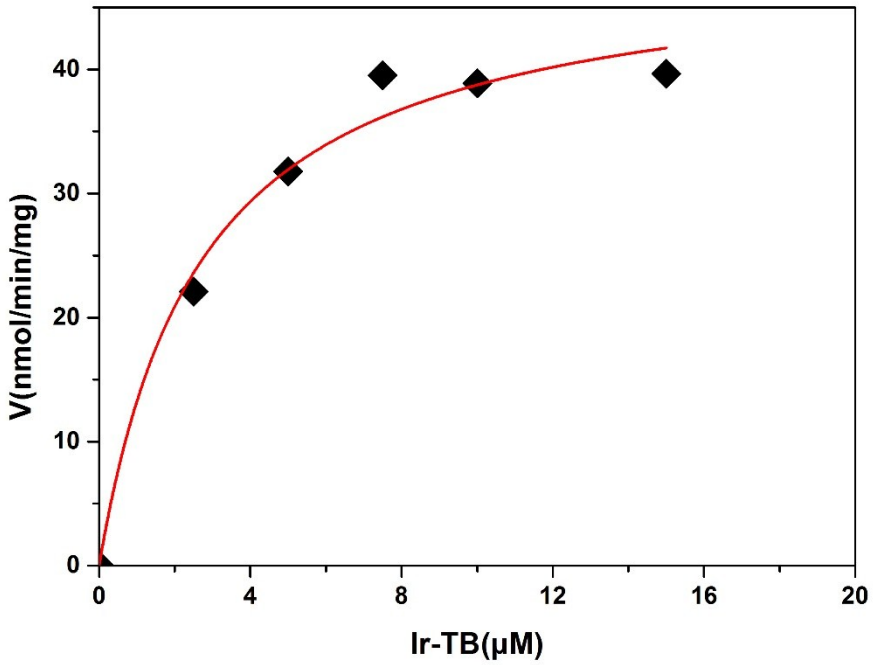


Figure S2. Hydrolysis kinetics of Ir-TB in hCE2 (10 μg/mL).

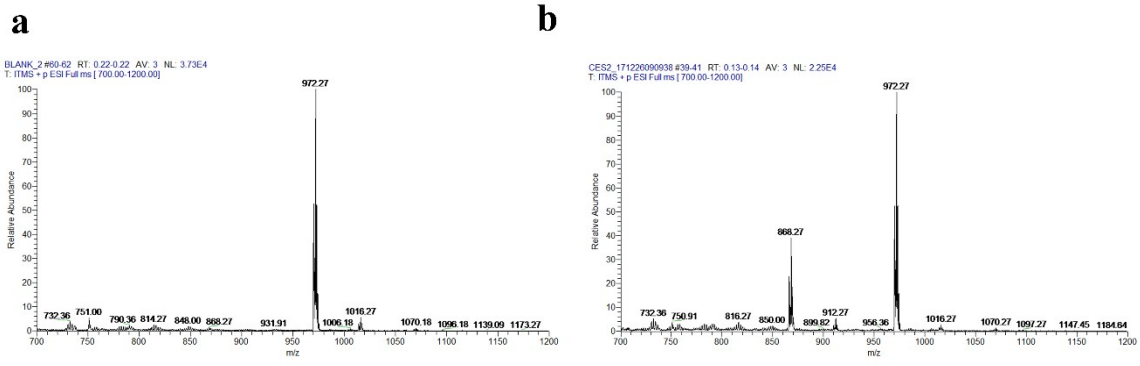


Figure S3. MS spectra of reaction solution before (a) and after (b) the addition of hCE2.

Table S2. T1 states before and after calculation from TDDFT approach.

Complex	States	Dominant excitations[a]	Character	Assignment	MLCT(%)
Ir-TB	T1	H → L (100%)	π(piq)/dπ(Ir) → π*(tzbpy)	MLCT/ LLCT	40.41
Ir-TO	T1	H → L (100%)	π(piq)/dπ(Ir) → π*(tzbpy)	MLCT/ LLCT	40.37

[a] H and L denote HOMO and LUMO, respectively; data in parentheses are the contributions of corresponding excitations.

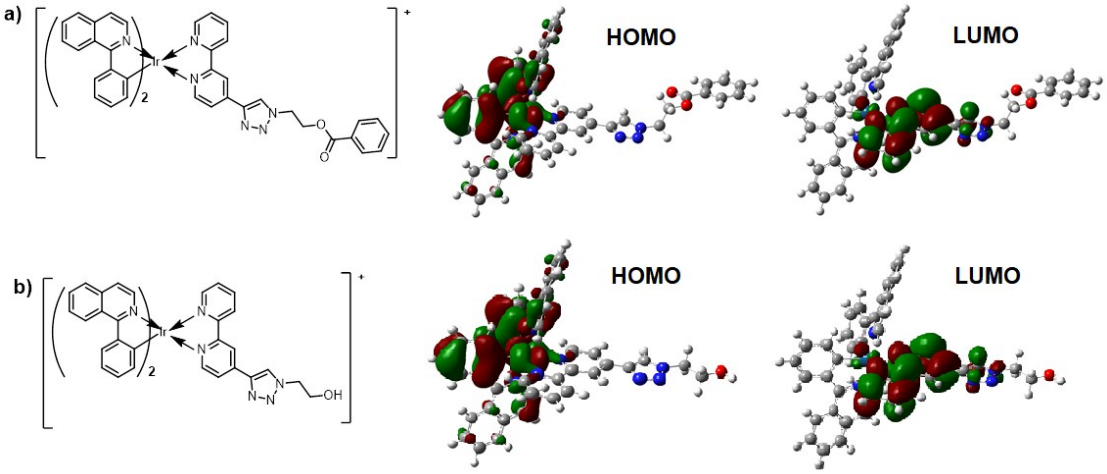


Figure S4. Molecular structures and the frontier molecular orbitals of Ir-TB (a) and Ir-TO (b) based on DFT-optimized geometry.

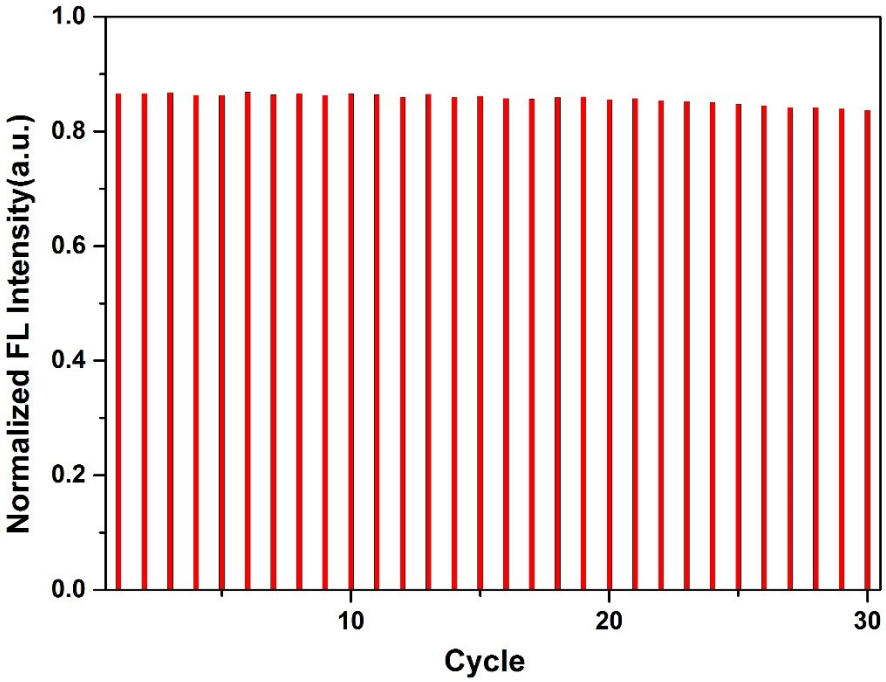


Figure S5. Fluorescence intensity of 10 μM Ir-TB after 10 min irradiation of ultraviolet lamp for 30 cycles.

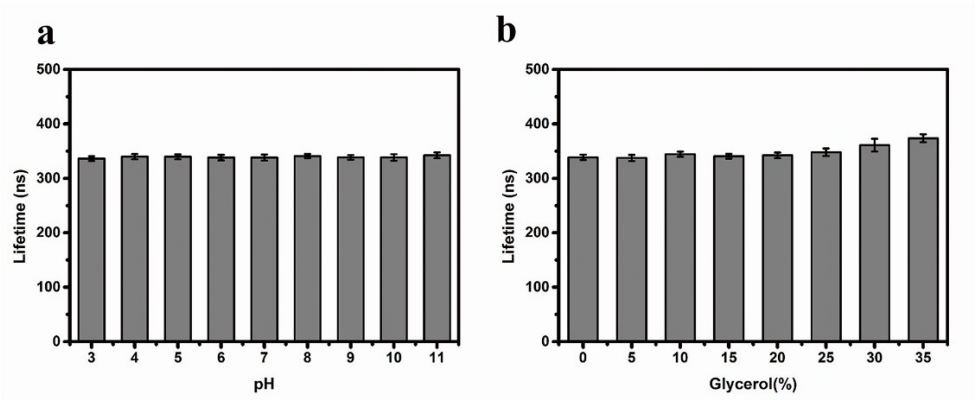


Figure S6. Effect of pH (a) and viscosity (b) on the photoluminescence lifetime of Ir-TB in PBS buffer.

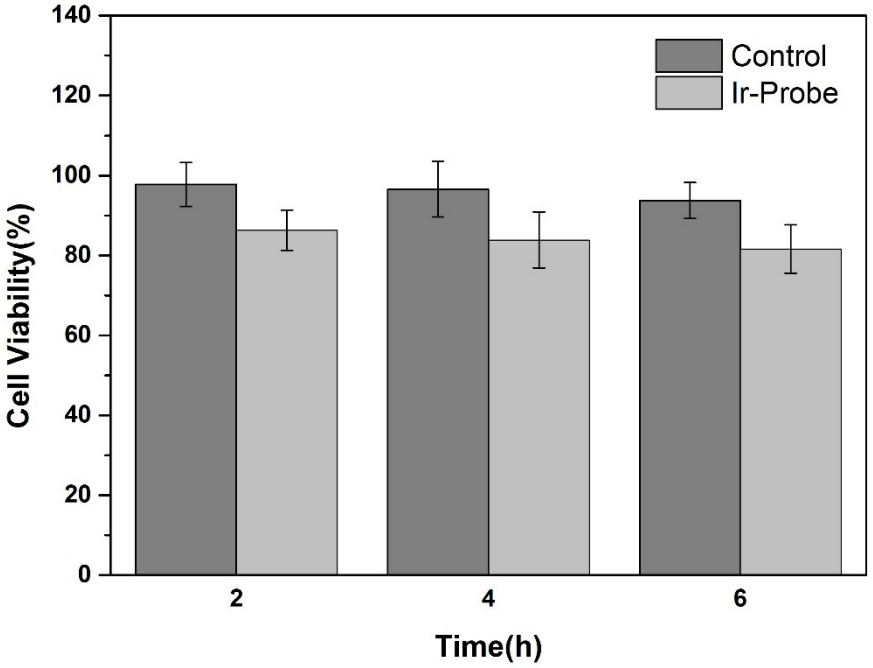


Figure S7. Cell viability assays.

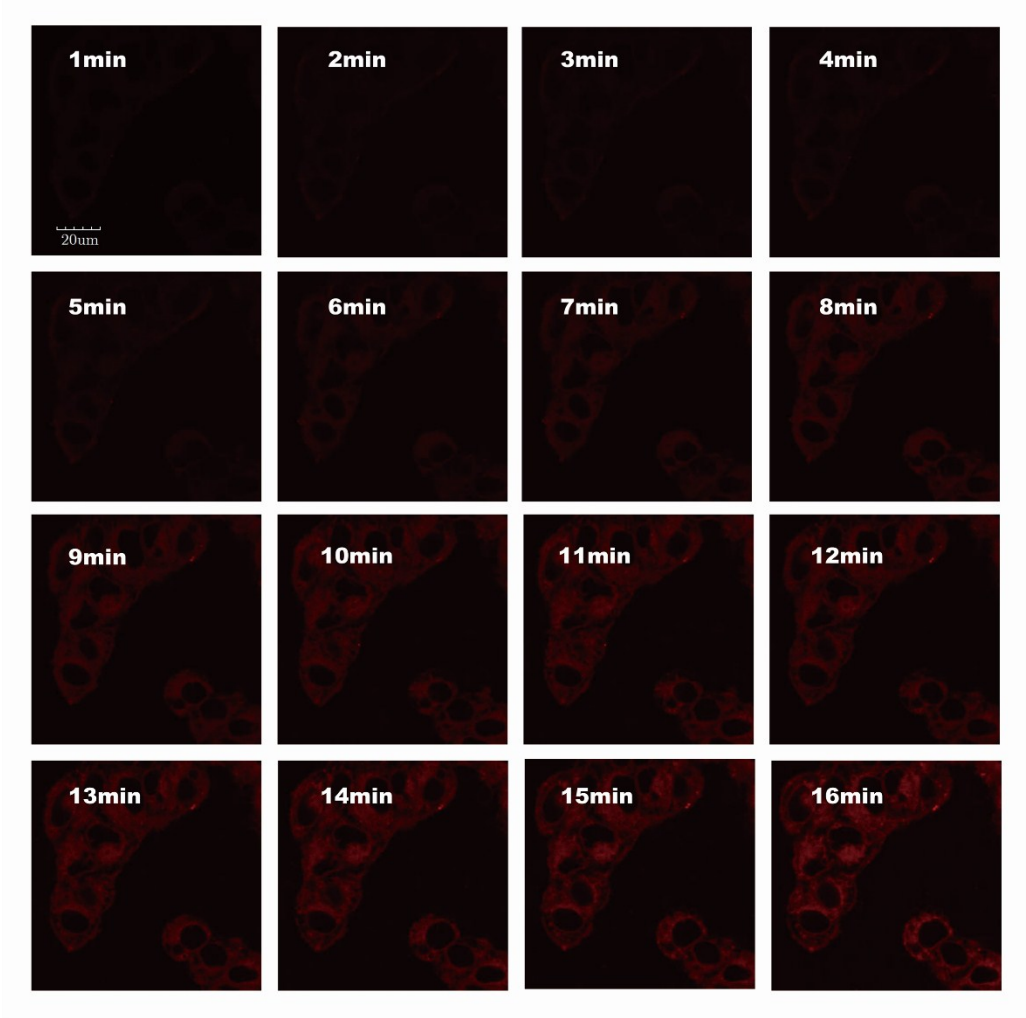


Figure S8. The dynamic photoluminescence images of HepG2 cells incubated with Ir-TB (2 μM), the images were collected after adding Ir-TB for 1 min to 16 min. λ_{ex} = 488 nm, these images were collected at 560−660 nm. Scale bars: 20 μm.

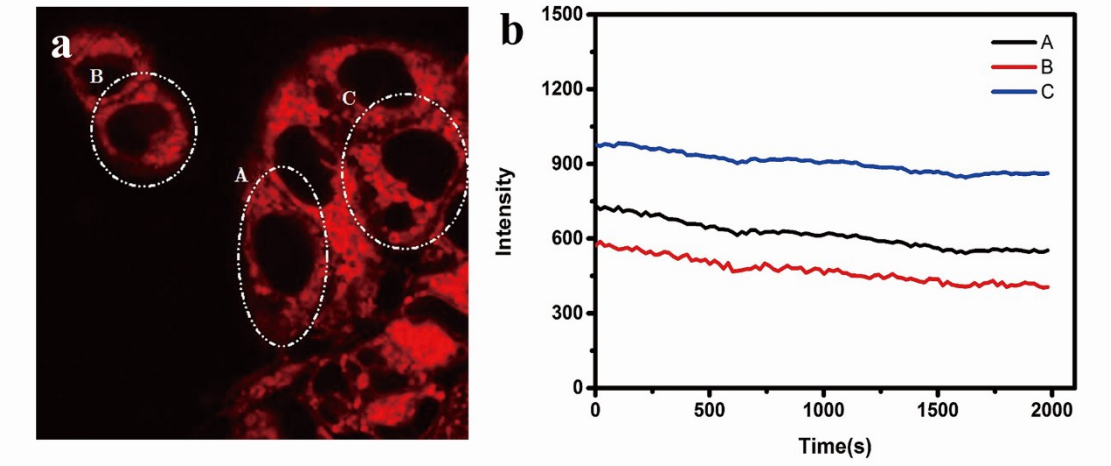


Figure S9. (a) Photoluminescence images of HepG2 cells labeled with Ir-TB. (b) The relative photoluminescence intensity from A–C in (a) as a function of time.

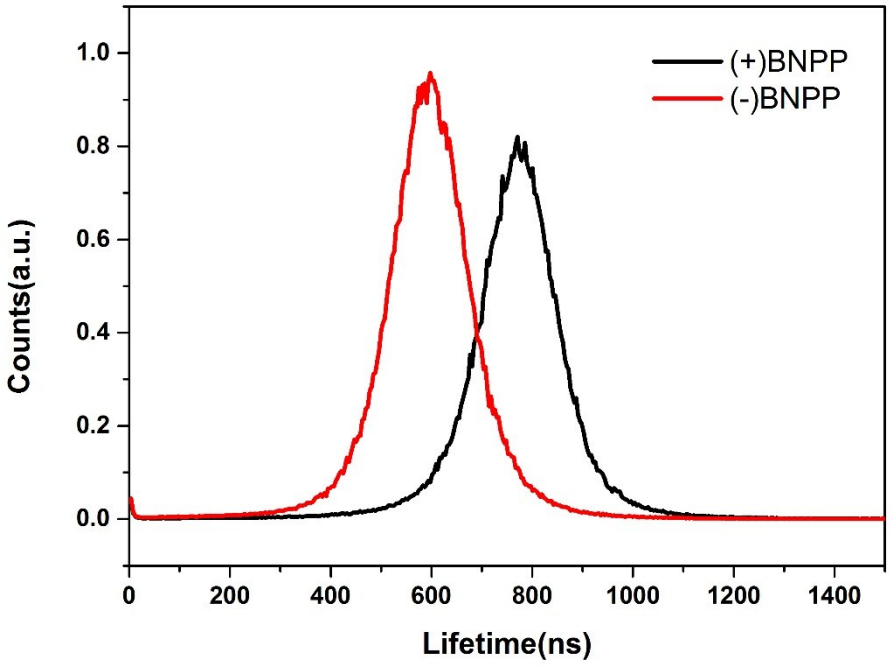
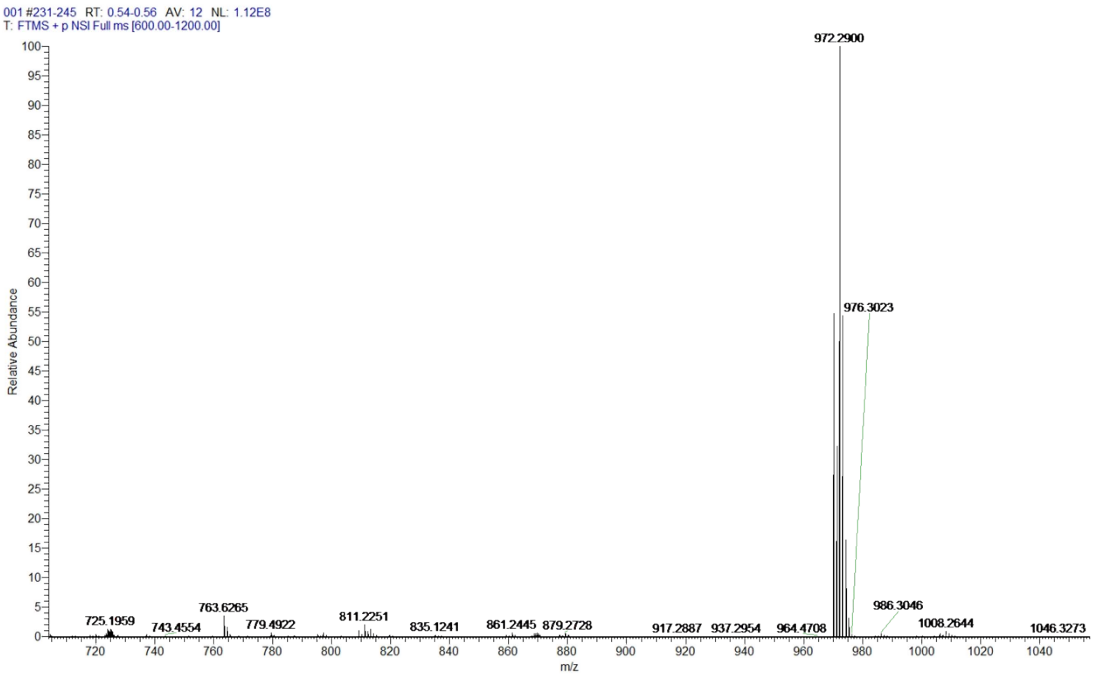


Figure S10. The statistical distribution chart of lifetimes in groups of HepG2 cells with Ir-TB incubated with and without BNPP.



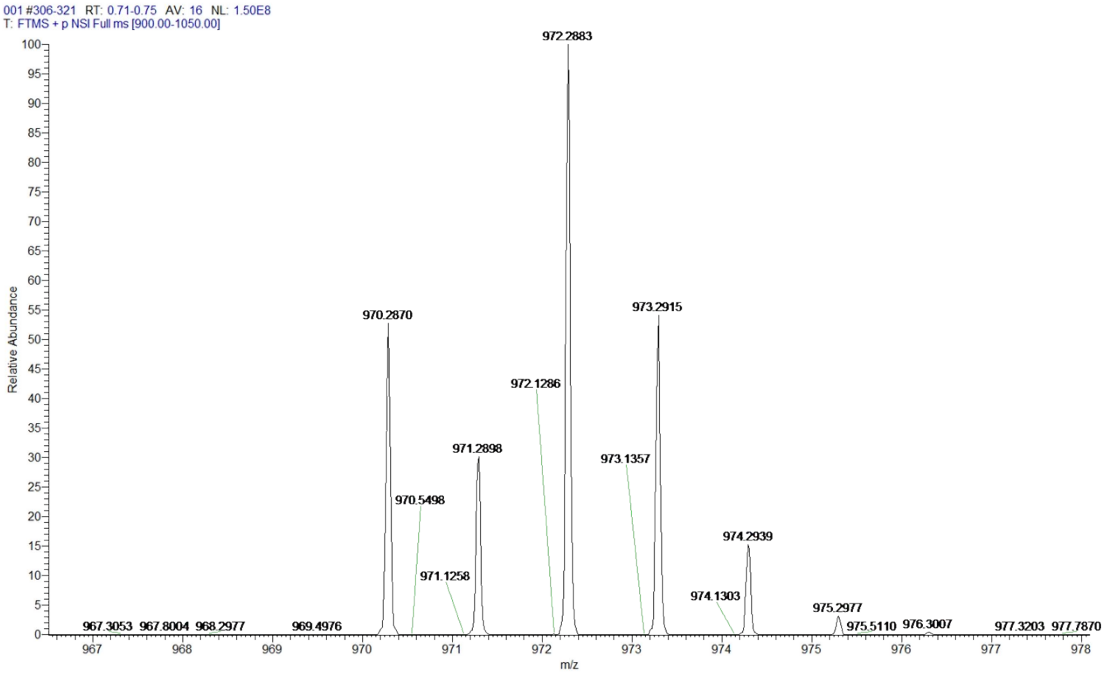


Figure S11. HRMS spectrum of Ir-TB.

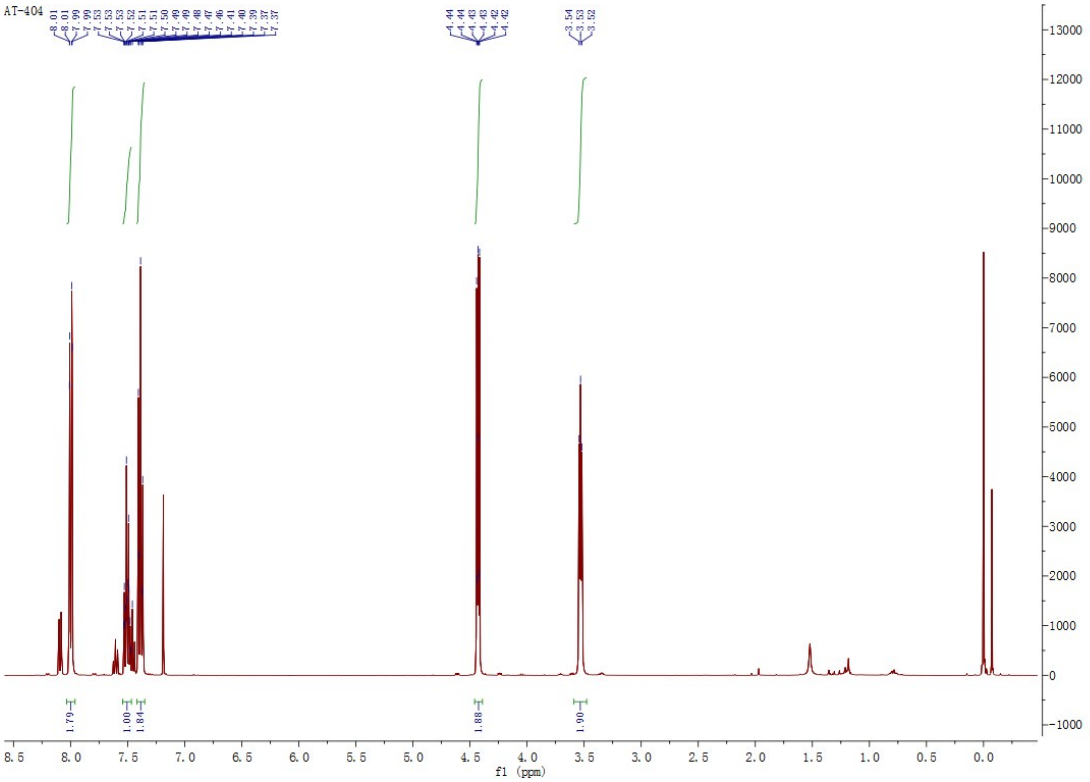


Figure S12. ^{1}H-NMR spectrum (400 MHz) of Compound 2

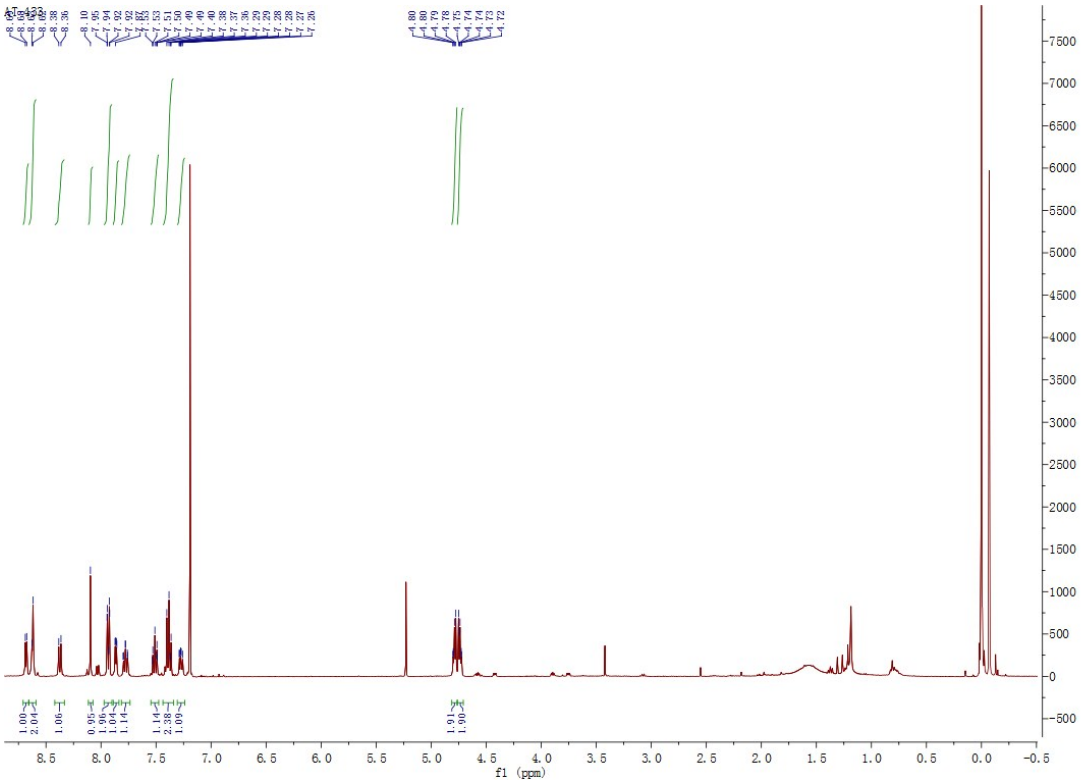


Figure S13. ^{1}H-NMR spectrum (400 MHz) of Compound 3

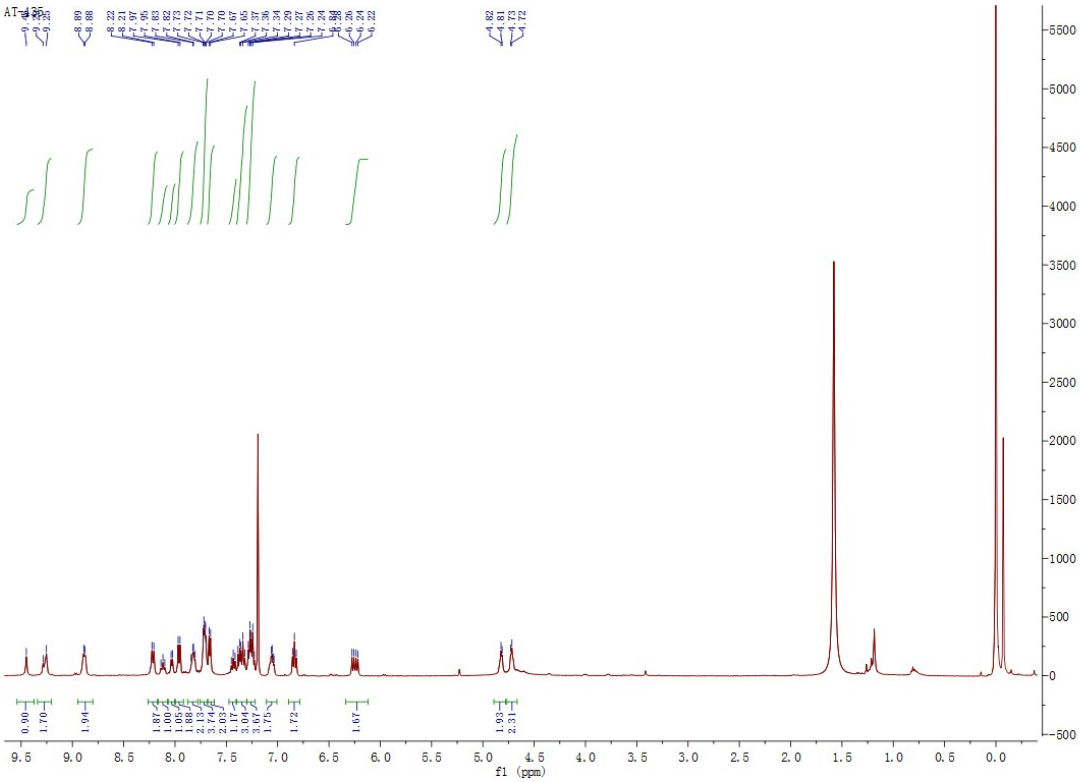


Figure S14. ^{1}H-NMR spectrum (400 MHz) of Ir-TB

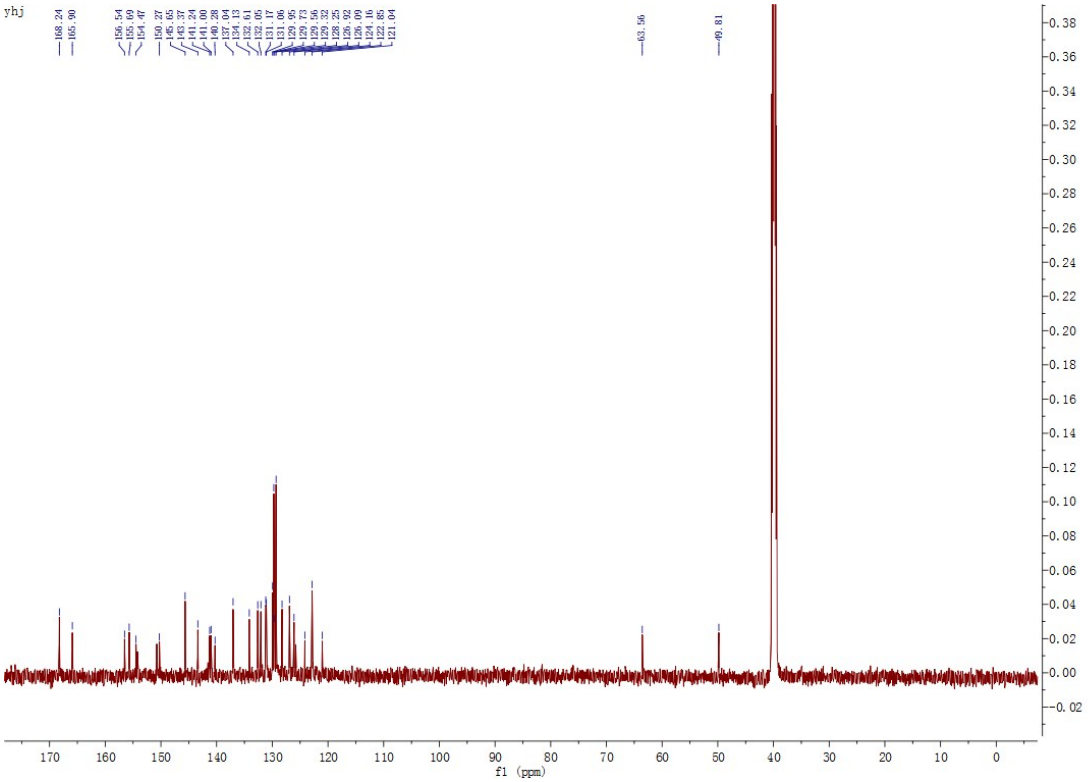


Figure S15. ^{13}C-NMR spectrum (100 MHz) of Ir-TB

References

1. J. Wang, J. Xue, Z. Yan, S. Zhang, J. Qiao and X. Zhang, *Angew Chem Int Ed Engl*, 2017, **56**, 14928-14932.