Electronic Supplementary Material (ESI) for Chemical Science.
This journal is © The Royal Society of Chemistry 2024

Supporting Information

A *Λ*-Ir(III)-phenylquinazolinone Complex Enhances Ferroptosis by Selectively Inhibiting Metallothionein-1

Lu Zhu,[a] Xingyun Wang,[a] Tian Tian,[b] Yanyan Chen,[b] Wenjing Du,[b] Wei Wei,*[a,b,c] Jing Zhao,*[b,c] Zijian Guo,[b] and Xiuxiu Wang*[b]

[a] *School of Life Sciences, Nanjing University, Nanjing, 210023, China*

[b] *Chemistry and Biomedicine Innovation Center (ChemBIC), State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210023, China*

[c] *Nanchuang (Jiangsu) Institute of Chemistry and Health, Sino-Danish Ecolife Science Industrial Incubator, Jiangbei New Area, Nanjing, 210000, China*

*****Corresponding Authors: weiwei@nju.edu.cn; jingzhao@nju.edu.cn; wangxiuxiu@nju.edu.cn

Table of Contents

1. General procedures

Materials and methods.

All anhydrous solvents were bought from J&K Scientific and all reactants and reagents were obtained from Bide Pharm, Energy Chemical, Lajoo and Adamas, and used as supplied without further purification. ^{1}H NMR, ^{13}C NMR spectra were recorded on Bruker nuclear resonance (400 MHz) spectrometer. ^{1}H NMR chemical shifts are reported relative to residual solvent signals. Mass spectra were recorded on a high-resolution ion mobility mass spectrometer (HR-ESI MS, Thermo Fisher Q Exactive). UV-Vis absorption and fluorescence spectra were conducted on a Shimadzu UV-1800 and Shimadzu RF-6000 Spectrophotometer. Circular dichroism (CD) spectra were conducted on a Jasco J-810 spectropolarimeter. The intensity of light was maintained constant throughout the irradiations by measuring the output using an Oriel photodiode detection system (model 7072). The light source used in PDT measurement: Power consumption: 40 W, Voltage input: AC110/220 V, Wavelength: 380-390 nm, 30 mW/cm^2.

Preparation of compounds for biological test.

The complexes **PPQ**, ***Δ*-IrPPQ**, ***Λ*-IrPPQ** and ***rac*-IrPPQ** were found to be poorly soluble in PBS and required supplementation with DMSO (< 1%).

Cell culture.

HT-1080 human fibrosarcoma cells, PANC-1 human pancreatic cancer cells and SK-MEL-28 human malignant melanoma cells were purchased from the American Type Culture Collection (Manassas, VA). None of the cell lines were listed by the International Cell Line Authentication Committee as cross-contaminated or misidentified (v8.0, 2016). All cell lines were authenticated by using STR typing and confirmed to be mycoplasma-free by KeyGEN Biotech Co., Ltd. (Nanjing, China). HT-1080 human fibrosarcoma cells, PANC-1 human pancreatic cancer cells and SK-MEL-28 human malignant melanoma cells were cultured in DMEM or 1640 (Gibco) containing 10% FBS (Gibco), 100 units/mL penicillin, and 50 units/mL streptomycin at 37 °C in a CO_2 incubator (95% relative humidity, 5% CO_2).

Cytotoxicity assay.

Cells were seeded in 96-well tissue culture plates (5 × 10^3 cells/well) in medium at 37°C in a CO_2 incubator for 24 hours, then incubated with different concentrations of **PPQ, *Δ*-IrPPQ, *Λ*-IrPPQ** and ***rac*-IrPPQ** for a further 24 hours. For phototoxicity detection, cells were irradiated with 380-390 nm light (30 mW/cm^2) for 10 min and then incubated for another 24 h. Subsequently, 10 μL/well MTT solution was added and incubated for 4 h. After incubation, the culture medium was removed and replaced with 100 μL/well dimethyl sulfoxide (DMSO) and then measured at 570 nm using a microplate reader (Tecan Infinite M1000 PRO) to calculate cell viability. All experiments were performed in triplicate to ensure reproducibility of results.

Cell viability detection.

PANC-1 cells were seeded onto 35 mm glass bottom confocal culture dishes at 2.0 × 10^5 cells/well for 12 h. 5 μM ***Δ*-IrPPQ** and ***Λ*-IrPPQ** were added and incubated for 8 h. After 8 h incubation, cells were then irradiated with 380-390 nm light (30 mW/cm^2) for 10min and incubated for another 18 h. After washing for 3 times by PBS, the cells were then co-stained with calcein AM/ PI in 5% CO_2 at 37 °C for 40 min. And the cells were then imaged by confocal microscopy immediately upon excitation at 488 and 561 nm.

Cellular uptake.

Approximately 2× 10^6 PANC-1 cells were cultured in a 25 cm^2 culture flask with 5 mL DMEM medium. Then 10 μM ***Δ*-IrPPQ, *Λ*-IrPPQ** were added followed by incubation for 3, 7, 11 and 24 h. Then the medium was removed and washed 3 times with PBS. The cells were then harvested and counted, and the Ir concentration was determined by ICP-MS.

Cellular distribution.

Approximately 1× 10^7 PANC-1 cells were cultured in a 75 cm^2 culture flask with 15 mL DMEM medium. Then 10 μM ***Δ*-IrPPQ** and ***Λ*-IrPPQ** were added and incubated for another 24 h. Then the medium was removed and washed 3 times with PBS. The cells were then harvested, counted and the nuclei, mitochondria and cytoplasm were isolated using the Mitochondria/Nuclei Isolation Kit (KGA828, KeyGEN). The Ir content distributed in the nucleus, mitochondria and cytoplasm was determined by ICP-MS.

ROS detection.

ROS were detected using a reactive oxygen species assay kit (KeyGEN Biotech Co., Ltd, Nanjing, China) according to the manufacturer's protocol. Briefly, PANC-1 cells were seeded in 35 mm glass bottom confocal culture dishes at 2.0 × 10^5 cells/well for 24 h. Then 5 μM ***Δ*-IrPPQ** and ***Λ*-IrPPQ** were added and incubated for 8 h. The cells were then irradiated (Ex=380-390 nm, 30 mW/cm^2) for 10 min and incubated for 18 h. After 18 h incubation, the medium was removed and the cells were washed three times with serum-free DMEM. The cells were stained with DCFH-DA (5 μM) and incubated in 5% CO_2 at 37 °C for 20 minutes. The medium was then discarded and the cells carefully washed 3 times with PBS. The cells were then imaged by confocal microscopy immediately after excitation at 488 nm.

Singlet oxygen ($^1O_2$) detection.

PANC-1 cells were seeded on 35 mm glass bottom confocal culture dishes at 2.0 × 10^5 cells/well for 12 hours. 5 μM ***Δ*-IrPPQ** and ***Λ*-IrPPQ** were added and incubated for 8 hours. After 8 h incubation, the cells were then irradiated with 380-390 nm light (30 mW/cm^2) for 10 min and incubated for a further 18 h. After washing three times with PBS, the cells were stained with SOSG (5 μM) for 30 min in PBS buffer in the incubator. The cells were then imaged by confocal microscopy immediately after excitation at 488 nm.

Superoxide anion radical ($O_2^{\bullet-}$) detection.

PANC-1 cells were seeded on 35 mm glass bottom confocal culture dishes at 2.0 × 10^5 cells/well for 12 hours. 5 μM ***Δ*-IrPPQ** and ***Λ*-IrPPQ** were added and incubated for 8 hours. After 8 h incubation, the cells were then irradiated with 380-390 nm light (30 mW/cm^2) for 10 min and incubated for a further 18 h. After washing three times with PBS, the cells were then stained with MitoSOX (5 μM) for 30 min in PBS buffer in the incubator. The cells were then imaged by confocal microscopy immediately after excitation at 561 nm.

GSH detection.

PANC-1 cells were seeded on 6-well plates at 2.0 × 10^5 cells/well for 24 h. Then 10 μM ***Δ*-IrPPQ** and ***Λ*-IrPPQ** were added and incubated for 24 h. For the dark group, cells were further cultured for another 24 h. For the light group, cells were irradiated with 380-390 nm light (30 mW/cm^2) for 10 min and then incubated for another 24 h. After 24 h, the medium was removed and the cells were washed three times with PBS. The cells were harvested and the intracellular GSH level was determined using a GSH assay kit (S0052, Beyotime Biotechnology) according to the manufacturer's protocol.

Lipid ROS detection.

PANC-1 cells were seeded on 35 mm glass bottom confocal culture dishes at 2.0 × 10^5 cells/well for 12 hours. 5 μM ***Δ*-IrPPQ** and ***Λ*-IrPPQ** were added and incubated for 8 hours. After 8 h incubation, the cells were irradiated with 380-390 nm light (30 mW/cm^2) for 10 min and incubated for another 18 h. After washing three times with PBS, the cells were stained with BODIPY 581/591 C11 (5 μM) for 30 min in serum-free DMEM in an incubator. The cells were then imaged by confocal microscopy immediately after excitation at 488 nm.

Fe^{2+} detection.

PANC-1 cells were seeded on 35 mm glass bottom confocal culture dishes at 2.0 × 10^5 cells/well for 12 hours. 5 μM ***Δ*-IrPPQ** and ***Λ*-IrPPQ** were added and incubated for 8 hours. After 8 h incubation, the cells were then irradiated with 380-390 nm light (30 mW/cm^2) for 10 min and incubated for a further 18 h. After washing three times with PBS, the cells were stained with FePhoNOX-1 (5 μM) for 30 min in PBS buffer in an incubator. The cells were then imaged by confocal microscopy immediately after excitation at 561 nm.

Mitochondrial morphology analysis

PANC-1 cells were seeded in a 75 cm^2 cell culture flask for 12 h. 10 μM ***Δ*-IrPPQ** and ***Λ*-IrPPQ** was added and incubated for 12 h. After 12 h, the cells were then irradiated with 380-390 nm light (30 mW/cm^2) for 10 min and incubated for another 24 h. After washing with PBS three times, cells were collected and transferred to fixative, sliced, and imagined by transmission electron microscopy (Hitachi HT7700).

Proteomic analysis.

PANC-1 cells (5 × 10^6) were incubated and treated with ***Δ*-IrPPQ** and ***Λ*-IrPPQ** (10 μM) for 12 h, then irradiated with 380-390 nm light (30 mW/cm^2) for 10 min and incubated for 24 h. Cells were treated with the same volume of PBS and used as control. After the cells were harvested, 300 μL of lysis buffer supplemented with 1 mM PMSF was added and the cells were further lysed by sonication. After sonication, samples were centrifuged at 15,000g for 15 minutes to remove insoluble particles and repeated once to further exclude precipitation. Protein concentration was determined by the BCA method and then enzymatically hydrolysed. 5 mM DTT was added to the above protein solution and incubated at 55°C for 60 minutes. Then, pre-cooled acetone was used in the above system to precipitate the protein and the precipitate was collected. According to the amount of protein, the corresponding volume of enzymolysis diluent (protein:enzyme = 50:1 (m/m), 100 ug protein added to 2 ug enzyme) was added to redissolve the protein precipitate, then the solutions were incubated for digestion at 37°C for 12 h. Finally, the samples were lyophilised or evaporated after enzymolysis. 100 mM TEAB buffer was added to the lyophilised sample, vortexed to mix, and the labelling reaction was carried out in a 1.5 mL Ep tube. TMT reagent was then added to the samples, vortexed, allowed to stand at room temperature for 1 h and 5% hydroxylamine was added to stop the reaction. RP separation was performed on an 1100 HPLC system (Agilent) using an Agilent Zorbax Extend RP column (5 μM, 150 mm × 2.1 mm). Mobile phases A (2% acetonitrile in HPLC water) and B (90% acetonitrile in HPLC water) were used for the RP gradient. Tryptic peptides were separated at a flow rate of 300 μL/min until the end of the gradient. The mass resolution of the primary MS was set to 70000 and the maximum injection time was 100 ms. The mass spectrometry scan was set to the full scan charge to mass ratio m/z range of 350-1750. MS/MS scans were performed on the 20 highest peaks. All MS/MS spectra were acquired using high energy collisional fragmentation in a data dependent positive ion mode with a collision energy of 35. The data were finally analysed using Proteome Discover 2.4 (Thermofisher Corporation).

***q*RT-PCR verification.**

PANC-1 cells were seeded in 6-well plates for 24 h and then treated with 10 μM ***Δ*-IrPPQ** or ***Λ*-IrPPQ** for 12 h. Cells were then irradiated with 380-390 nm light (30 mW/cm^2) for 10 min and incubated for 24 h. After three washes with PBS, cells were harvested and total RNA was purified using RNA Extraction Kits (OMEGA, USA) according to the manufacturer's instructions. The total RNA was then used in a reverse transcription reaction using the Fastking RT Kit with gDNA (TIANGEN, China). Primers for *q*PCR were designed using Primer Express. Quantitative PCR was performed on triplicate samples in 96-well format using Power SYBR Green Master Mix (Takara, China) on an Applied Biosystems Cycler set to absolute quantification. The change in expression of a gene between experimental and control conditions was calculated using the *ΔΔt* method with actin as an internal reference gene.

Western blot.

PANC-1 cells were seeded in a 25 cm^2 cell culture flask for 24 h. 10 μM ***Δ*-IrPPQ** and ***Λ*-IrPPQ** were added and incubated for 12 h, then irradiated with 380-390 nm light (30 mW/cm^2) for 10 min and incubated for 24 h. PANC-1 cells treated with PBS were used as control. Cells were washed

three times with PBS before collection. Whole cell proteins were prepared using the Whole Cell Lysis assay kit (KeyGEN Biotech Co., Ltd, Nanjing, China), and the protein concentration was determined using the Super-Bradford protein assay kit (CW Biotech, Inc., Beijing, China). The expression levels of GPX4 and FSP1 were then determined. Briefly, the extracts were first separated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Bio-Rad, CA, USA). The membrane was blocked with 5% BSA in PBS for 2 hours at 25°C and then incubated with antibodies overnight at 4°C. GAPDH expression was used as an internal standard. Primary antibodies against the following proteins were used GAPDH (A1978, 1:10,000 for WB) from Sigma; GPX4 (ab125066, 1:1000 for WB) from Abcam; FSP1 (aa114-163, 1:1000 for WB) from Gonxspan.

Molecular docking analysis.

The protein structure was optimised for energy minimisation, followed by the addition of polar hydrogen atoms and atomic charges. It was then opened using Autodock Tools and saved as a file in pdbqt format to be used as a receptor for molecular docking. The structure of the substrate polypeptide was mapped using pymol, adding a hydrogen atom and saving the file in mol2 format. The substrate structure was then opened using Autodock Tools and saved as a pdbqt file.

Autogrid 4 was used to calculate the lattice energy. The protein served as the receptor for molecular docking while ***Δ*-IrPPQ** and ***Λ*-IrPPQ** acted as the ligand. A lattice energy parameter file (.gPF) was constructed using Autodock Tools with XYZ cells set to 80 × 80 × 100 and cell spacing set to 0.375A. The coordinates of the centre of the box of receptor cavities obtained from Discovery Studio were set to (-5.279 0.204-4.167), which were then saved as the grid point calculation parameter file. Finally, the AutoGrid 4 module calculated various atom map files.

For molecular docking, the protein was again used as the receptor, while the substrate ***Δ*-IrPPQ** and ***Λ*-IrPPQ** were used as the ligand. The molecular docking parameter file (.dPF) was generated using Autodock Tools using the Lamarck genetic algorithm for conformation search during the molecular docking process, with the maximum iterations set at 27,000 and the maximum energy estimate (ga_num_evals) set at 2,500,000, along with 50 molecular docking runs (ga_run). Other parameters were left unchanged and stored in a separate molecular docking parameter file. Finally, AutoDock4 performed the molecular docking results.

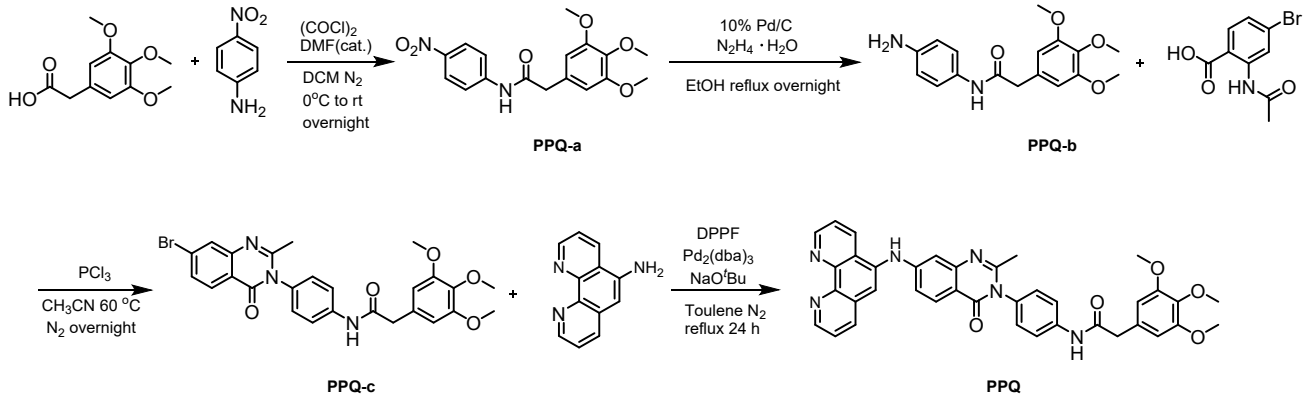
Cellular thermal shift assay (**CETSA).**

For the cell lysate CETSA experiments, PANC-1 cells were harvested and washed with PBS. The cell suspensions were freeze-thawed three times using liquid nitrogen. The soluble fraction (lysate) was separated from the cell debris by centrifugation at 20000 x g for 20 minutes at 4°C. The cell lysates were diluted with appropriate buffer and divided into three aliquots, two aliquots were treated with 100 μM ***Δ*-IrPPQ** and ***Λ*-IrPPQ** and one aliquot with DMSO as control. After 30 min incubation at room temperature, the respective lysates were divided into smaller (50μL) aliquots and individually heated at different temperatures for 3 minutes. The heated lysates were centrifuged at 20000 x g for 20 min at 4°C in order to separate the soluble fractions from precipitates. The supernatants were transferred to new microtubes and analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by western blot analysis.

Drug affinity responsive target stability (DARTS) experiment

For the drug affinity responsive target stability (DARTS) experiment, PANC-1 cells were harvested and washed with PBS. The cell suspensions were freeze-thawed three times using liquid nitrogen. The soluble fraction (lysate) was separated from the cell debris by centrifugation at 20000 x g for 20 min at 4°C. The cell lysates were diluted with appropriate buffer and divided into three aliquots, two aliquots were treated with 100 μM ***Δ*-IrPPQ** and ***Λ*-IrPPQ** and one aliquot with DMSO as control. After 30 min incubation at room temperature, the respective lysates were divided into smaller (50μL) aliquots and added with gradient pronase E (0, 1: 1500, 1: 1000, 1: 600 and 1:300, w/w). After incubation at 37 °C for 30 min, the supernatants were transferred to new microtubes and analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by western blot analysis.

2. Synthesis of PPQ ligand



Scheme S1. Synthetic route of **PPQ** ligand.

Synthesis of PPQ-a. To a solution of 2-(3,4,5-trimethoxyphenyl)acetic acid (3.31 g, 24 mmol) in 30 mL anhydrous DCM, 5.08 mL oxalyl chloride dissolved in anhydrous DCM was added dropwise followed by two drops of DMF at room temperature under N_2 atmosphere. After stirring for 2 h, the reaction was concentrated on vacuum to remove the residual oxalyl chloride. The obtained solid was re-dissolved in 30 mL anhydrous DCM, and 4-nitroaniline in DCM solution was added dropwise followed by 1 mL pyridine at 0 °C under N_2 atmosphere. The final mixture was stirred for another 12 h at room temperature. After completion, the reaction was quenched with H_2O and extracted with DCM (30 mL × 3). The combined organic layer was washed with brine, dried by anhydrous Na_2SO_4 and concentrated on vacuum. The raw material was purified by silica gel flash chromatography (eluent: PE: EA = 25: 1, v/v) to afford the product **PPQ-a** as a white solid in 82% yield (19.68 mmol, 6.8 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, 1H), 8.22 (d, *J* = 9.3 Hz, 2H), 7.85 (d, *J* = 9.3 Hz, 2H), 6.65 (s, 2H), 3.76 (s, 6H), 3.65 (s, 2H), 3.63 (s, 3H).

Synthesis of PPQ-b. To a 100 mL round-bottom flask was added with **PPQ-a** (4.15 g, 12 mmol), 10% Pd/C (1.2 g), and 60 mL anhydrous EtOH. The mixture was bubbled with N_2 for 5 min, heated to 85 °C, and then 2 mL $N_2H_4{\cdot}H_2O$ dissolved in anhydrous EtOH was added dropwise. The final mixture was refluxed for another 12 h. After completion, the suspension was filtered and the filtrate was concentrated on vacuum. The raw material was purified by silica gel flash chromatography (eluent: PE: EA = 10: 1, v/v) to afford the product **PPQ-b** as a white solid in 90% yield (10.8 mmol, 3.42 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.65 (s, 1H), 7.20 (d, *J* = 8.8 Hz, 2H), 6.63 (s, 2H), 6.48 (d, *J* = 8.8 Hz, 2H), 4.83 (s, 2H), 3.76 (s, 6H), 3.63 (s, 3H), 3.46 (s, 2H).

Synthesis of PPQ-c. To a 100 mL round-bottom flask was added with **PPQ-b** (2.28 g, 7.2 mmol), 2-acetamido-4-bromobenzoic acid (1.55 g, 6 mmol), and 50 mL anhydrous CH_3CN. The mixture was bubbled with N_2 for 5 min, heated to 60 °C, and then 1.1 mL PCl_3 (12 mmol, 2 eq) dissolved in CH_3CN was added dropwise. The mixture was stirred at 60 °C for another 12 h. After completion, the reaction was quenched with $NaHCO_3$ saturated aqueous solution and extracted with EA (30 mL × 3). The combined organic layer was washed with brine, dried by anhydrous Na_2SO_4 and concentrated on vacuum. The raw material was purified by silica gel flash chromatography (eluent: PE: EA = 30: 1, v/v) to afford the product **PPQ-c** as a white solid in 74% yield (4.43 mmol, 2.38 g).^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 1H), 7.87 (d, *J* = 1.9 Hz, 1H), 7.76 (d, *J* = 8.8 Hz, 2H), 7.67 (dd, *J* = anhydrous 8.5, 1.9 Hz, 1H), 7.36 (d, *J* = 8.8 Hz, 2H), 6.67 (s, 2H), 3.78 (s, 6H), 3.64 (s, 3H), 3.61 (s, 2H), 2.13 (s, 3H).

Synthesis of PPQ. To a 100 mL schlenk tube was added with **PPQ-c** (2.26 g, 4.2 mmol), 1,10-phenanthrolin-5-amine (586 mg, 3 mmol), DPPF (167 mg, 0.3 mmol), $Pd_2(dba)_3$ (137 mg, 0.15 mmol), NaO^tBu (432 mg, 4.5 mmol) and 30 mL toluene. The mixture was bubbled with N_2 for 5 min and refluxed for 24 h. After completion, the reaction was quenched with H_2O and extracted with EA (30 mL × 3). The combined organic layer was washed with brine, dried by anhydrous Na_2SO_4 and concentrated on vacuum. The raw material was purified by silica gel flash chromatography (eluent: DCM: MeOH = 25: 1, v/v) to afford the product **PPQ** as a light yellow solid in 43% yield (1.3 mmol, 848 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 1H), 9.25 (s, 1H), 9.16 (dd, *J* = 4.2, 1.5 Hz, 1H), 9.01 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.64 (dd, *J* = 8.3, 1.3 Hz, 1H), 8.43 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.95 (d, *J* = 8.7 Hz, 1H), 7.93 (s, 1H), 7.83 (dd, *J* = 8.4, 4.3 Hz, 1H), 7.77 (d, *J* = 8.8 Hz, 2H), 7.73 (dd, *J* = 8.1, 4.3 Hz, 1H), 7.30 (d, *J* = 8.8 Hz, 2H), 7.26 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.00 (d, *J* = 2.1 Hz, 1H), 6.70 (s, 2H), 3.77 (s, 6H), 3.64 (s, 3H), 3.62 (s, 2H), 2.05 (s, 3H). ESI-MS: found m/z = 653.2498, calculated m/z = 653.2507 for $[M + H]^+$.

3. Synthesis of *Δ*-IrPPQ, *Λ*-IrPPQ and *rac*-IrPPQ

***Δ*- or *Λ*-Ir-R** (31 mg, 0.05 mmol) and **PPQ** (43 mg, 0.065 mmol) were dissolved in 10 mL anhydrous DCM, and the mixture was bubbled with N_2 for 5 min. Then 10 μL TFA dissolved in DCM was added and the reaction was stirred for 2 h. After completion, KPF_6 saturated aqueous solution was added to the mixture and stirred for another 1 h. The reaction was extracted with DCM (20 mL × 3), and the combined organic layer was washed with brine, dried by anhydrous Na_2SO_4 and concentrated on vacuum. The raw material was purified by silica gel flash chromatography (eluent: DCM: MeOH = 100: 1, v/v) to afford the product ***Δ*- or *Λ*-IrPPQ** as a yellow solid in a 75% yield.

***Δ*-IrPPQ**

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (s, 1H), 9.49 (s, 1H), 9.09 (dd, *J* = 8.5, 1.1 Hz, 1H), 8.72 (dd, *J* = 8.4, 1.2 Hz, 1H), 8.27 (t, *J* = 6.1 Hz, 3H), 8.18 (s, 1H), 8.12 – 8.05 (m, 2H), 8.01 (dd, *J* = 5.1, 1.3 Hz, 1H), 7.96 (d, *J* = 8.0 Hz, 2H), 7.92 (d, *J* = 4.7 Hz, 1H), 7.91 – 7.87 (m, 2H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.54 (d, *J* = 5.6 Hz, 1H), 7.50 (d, *J* = 5.8 Hz, 1H), 7.46 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.41 (d, *J* = 2.1 Hz, 1H), 7.33 (d, *J* = 8.6 Hz, 2H), 7.10 – 7.02 (m, 4H), 6.96 (td, *J* = 7.3, 1.2 Hz, 2H), 6.68 (s, 2H), 6.30 (dd, *J* = 13.8, 7.2 Hz, 2H), 3.78 (s, 6H), 3.64 (s, 3H), 3.62 (s, 2H), 2.10 (s, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.37, 166.92, 166.86, 160.72, 155.45, 152.73, 150.84, 150.28, 149.89, 149.25, 149.08, 148.94, 148.19, 147.35, 144.07, 144.01, 143.02, 139.59, 138.71, 138.58, 137.25, 136.33, 134.28, 132.48, 131.73, 131.35, 131.29, 131.20, 130.25, 130.21, 128.85, 128.19, 127.14, 127.05, 126.49, 125.06, 123.92, 122.39, 120.00, 119.86, 117.89, 113.87, 112.04, 106.55, 59.98, 55.86, 43.60, 23.96. (Some of signals were overlapped.)

ESI-MS: found m/z =1153.3342, calculated m/z =1153.3371 for $[M\text{-}PF_6^-]^+$.

***Λ*-IrPPQ**

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H), 9.51 (s, 1H), 9.09 (dd, *J* = 8.6, 1.1 Hz, 1H), 8.72 (dd, *J* = 8.4, 1.2 Hz, 1H), 8.32 – 8.24 (m,3H), 8.18 (s, 1H), 8.11 – 8.05 (m, 2H), 8.01 (dd, *J* = 5.1, 1.3 Hz, 1H), 7.96 (d, *J* = 8.0 Hz, 2H), 7.92 (d, *J* = 4.8 Hz, 1H), 7.91 – 7.87 (m, 2H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.54 (d, *J* = 5.7 Hz, 1H), 7.50 (d, *J* = 5.9 Hz, 1H), 7.47 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.41 (d, *J* = 2.1 Hz, 1H), 7.33 (d, *J* = 8.7 Hz, 2H), 7.10 – 7.01 (m, 4H), 6.96 (td, *J* = 7.3, 1.1 Hz, 2H), 6.68 (s, 2H), 6.30 (dd, *J* = 13.7, 7.1 Hz, 2H), 3.78 (s, 6H), 3.64 (s, 3H), 3.62 (s, 2H), 2.11 (s, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.35, 166.92, 166.85, 160.77, 155.29, 152.72, 150.83, 150.28, 149.89, 149.23, 149.08, 148.87, 148.17, 147.34, 144.07, 144.00, 143.00, 139.56, 138.69, 138.61, 137.24, 136.32, 134.27, 132.54, 131.73, 131.35, 131.28, 131.19, 130.25, 130.20, 128.85, 128.16, 127.12, 127.04, 126.47, 125.04, 123.86, 122.34, 119.99, 119.85, 117.88, 113.94, 111.95, 106.55, 59.97, 55.86, 43.60, 24.02. (Some of signals were overlapped.)

ESI-MS: found m/z =1153.3366, calculated m/z =1153.3371 for $[M\text{-}PF_6^-]^+$.

***rac*-IrPPQ:**

rac-$[Ir(ppy)_2(\mu\text{-}Cl)]_2$ (27 mg, 0.025 mmol) and **PPQ** (49 mg, 0.075 mmol) were dissolved in 20 mL anhydrous DCM and MeOH (1:1, v/v), and the mixture was bubbled with N_2 for 5 min. The reaction was stirred at 65 °C for 12 h. After completion, KPF_6 saturated aqueous solution was added to the mixture and stirred for another 1 h. The reaction was extracted with DCM (20 mL × 3), and the combined organic layer was washed with brine, dried by anhydrous Na_2SO_4 and concentrated on vacuum. The raw material was purified by silica gel flash chromatography (eluent: DCM: MeOH = 100: 1, v/v) to afford the product ***rac*-IrPPQ** as a yellow solid in a 92% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (d, *J* = 8.4 Hz, 1H), 8.25 (t, *J* = 6.5 Hz, 2H), 8.10 (d, *J* = 4.0 Hz, 1H), 8.02 (d, *J* = 11.9 Hz, 3H), 7.91 (dd, *J* = 13.0, 8.2 Hz, 2H), 7.77 – 7.66 (m, 6H), 7.66 – 7.58 (m, 3H), 7.45 – 7.41 (m, 1H), 7.34 (dd, *J* = 14.8, 7.3 Hz, 2H), 7.23 (s, 1H), 7.07 (t, *J* = 7.4 Hz, 4H), 6.94 (ddd, *J* = 13.1, 10.9, 4.1 Hz, 4H), 6.60 (s, 2H), 6.39 (d, *J* = 7.6 Hz, 2H), 3.81 (d, *J* = 3.3 Hz, 8H), 3.62 (s, 3H), 2.08 (s, 3H).

ESI-MS: found m/z =1153.3336, calculated m/z =1153.3371 for $[M\text{-}PF_6^-]^+$.

4. ^{1}H NMR, ^{13}C NMR and HR-ESI-MS spectra of compounds

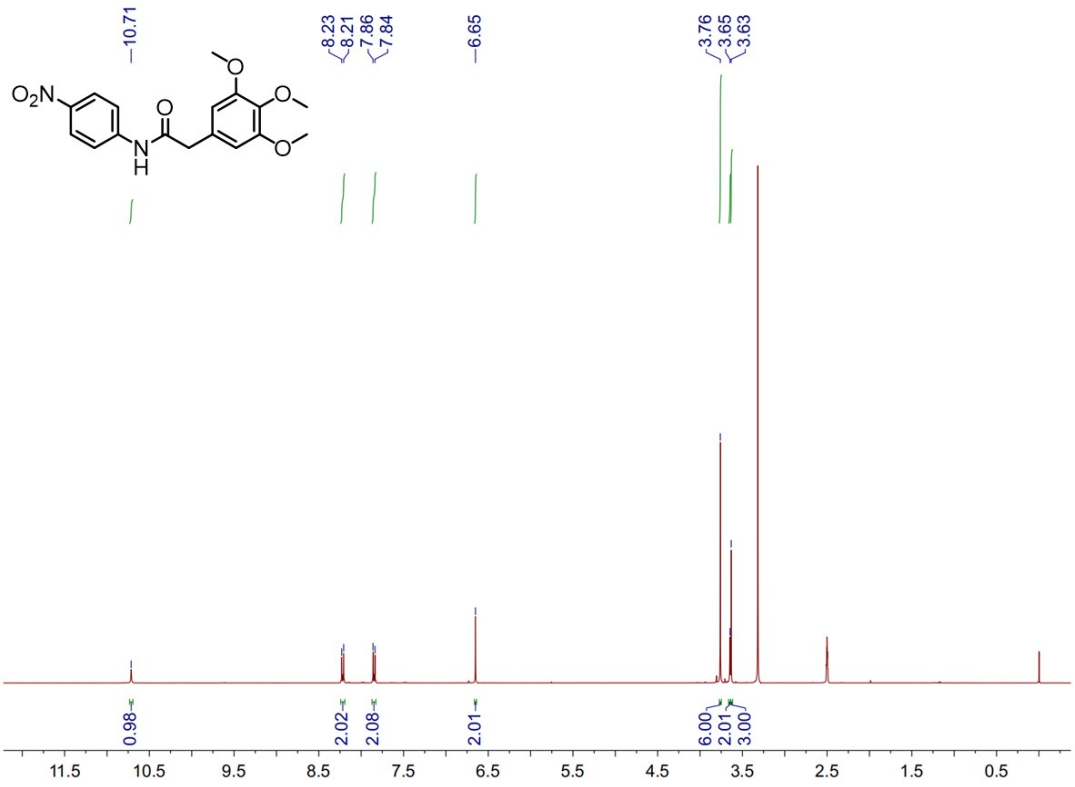


Figure S1. ^{1}H NMR spectra of **PPQ-a** in DMSO-d_6.

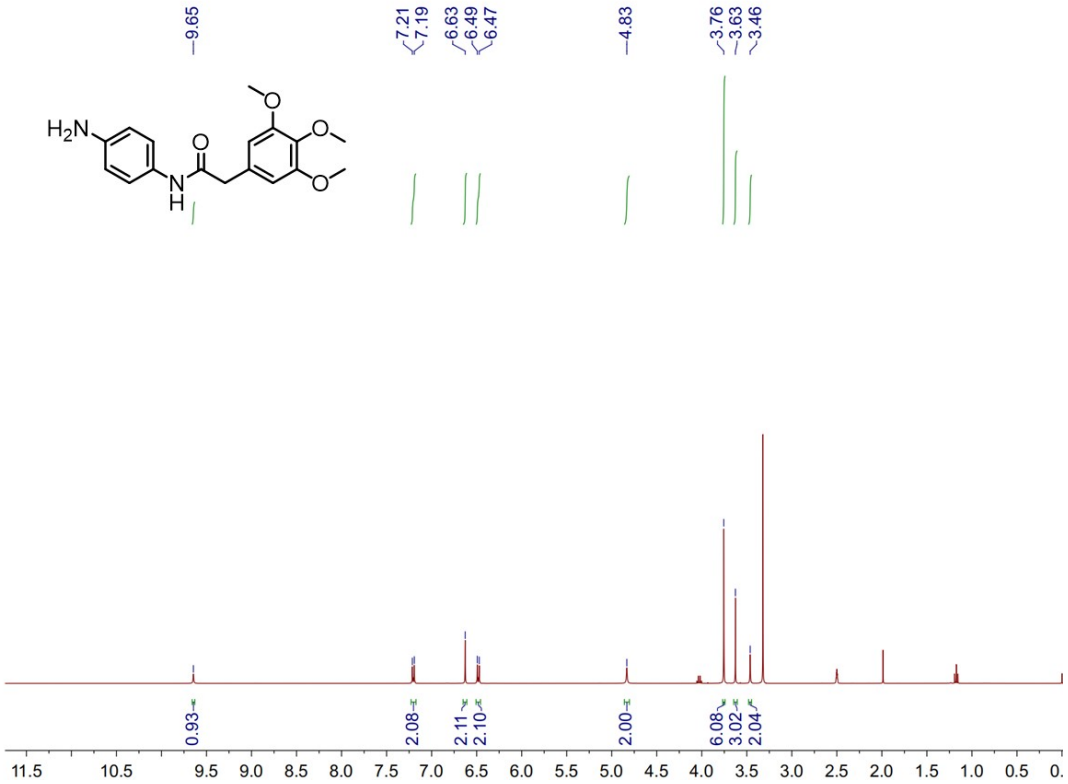


Figure S2. ^{1}H NMR spectra of **PPQ-b** in DMSO-d_6.

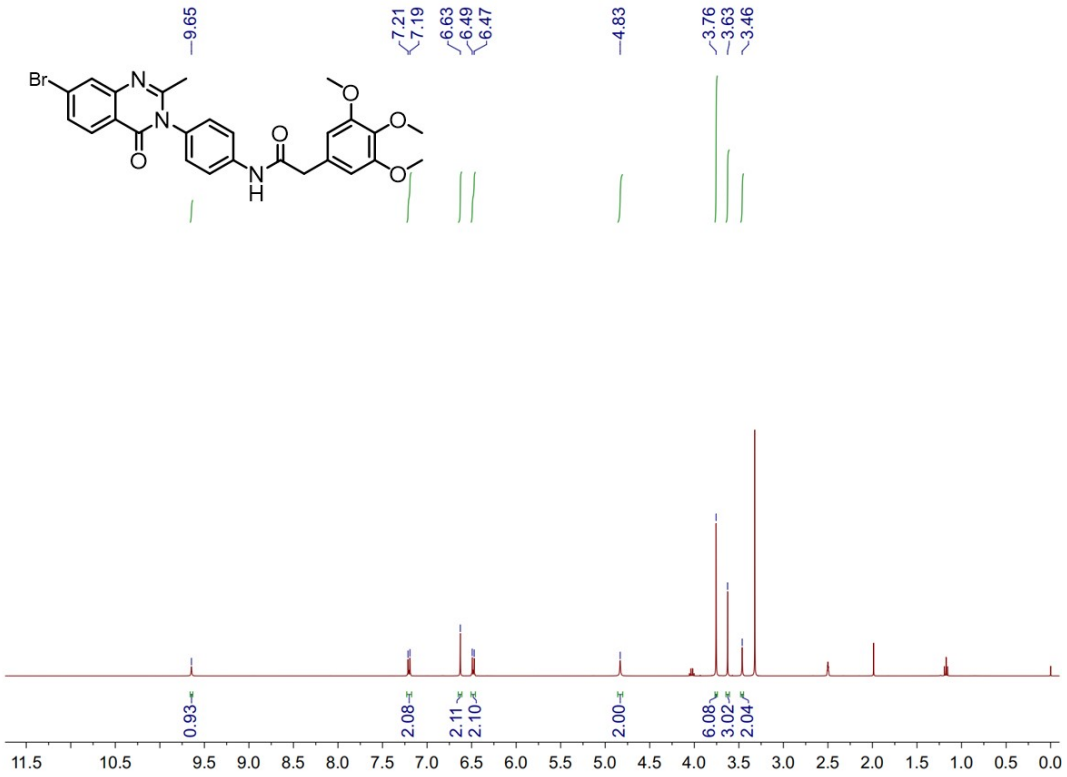


Figure S3. ^{1}H NMR spectra of **PPQ-c** in DMSO-d_6.

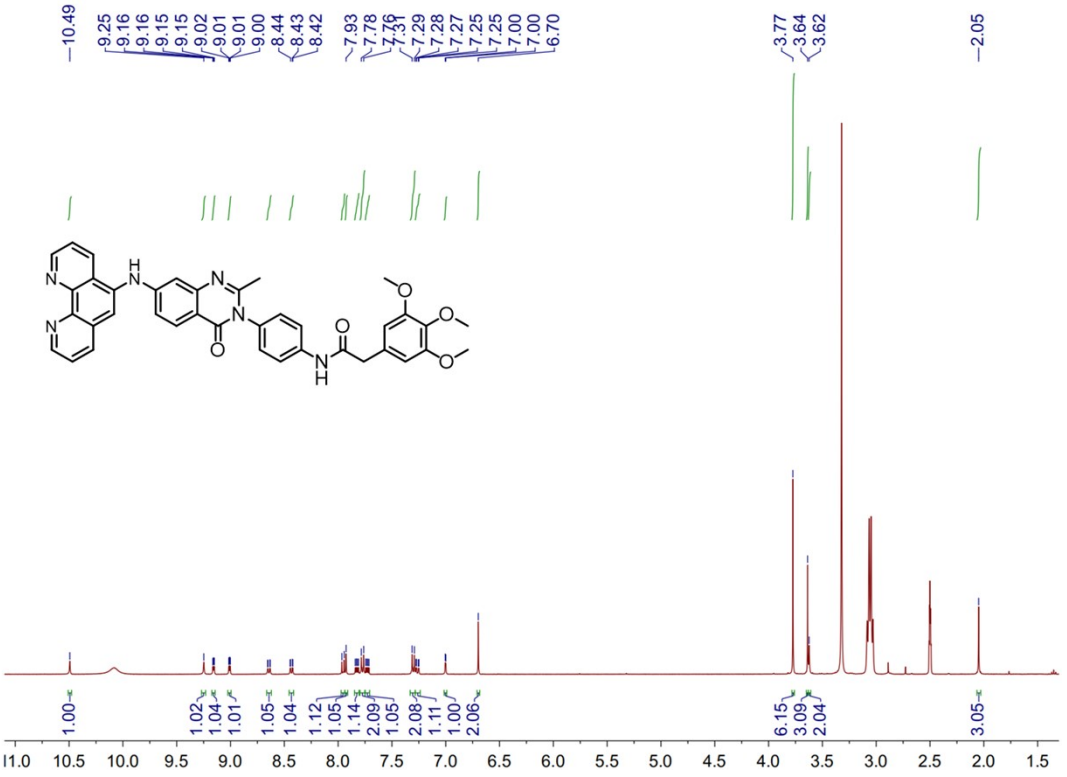


Figure S4. ^{1}H NMR spectra of **PPQ** in DMSO-d_6.

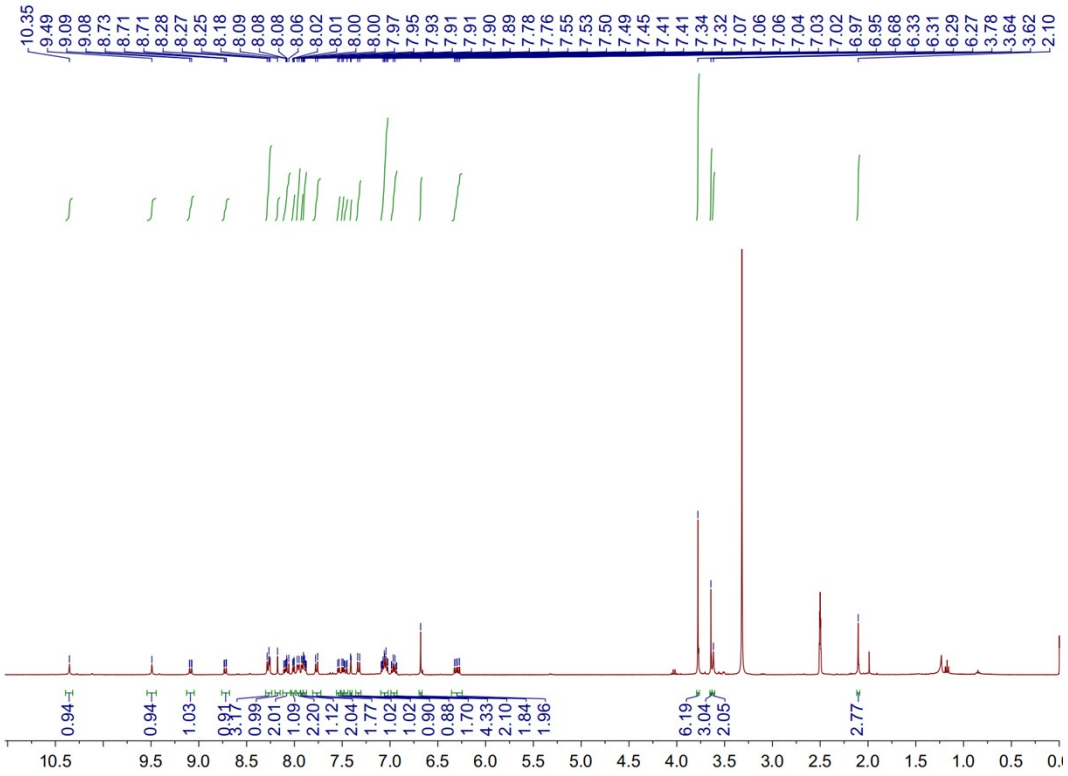


Figure S5. ^{1}H NMR spectra of ***Δ*-IrPPQ** in DMSO-d_6.

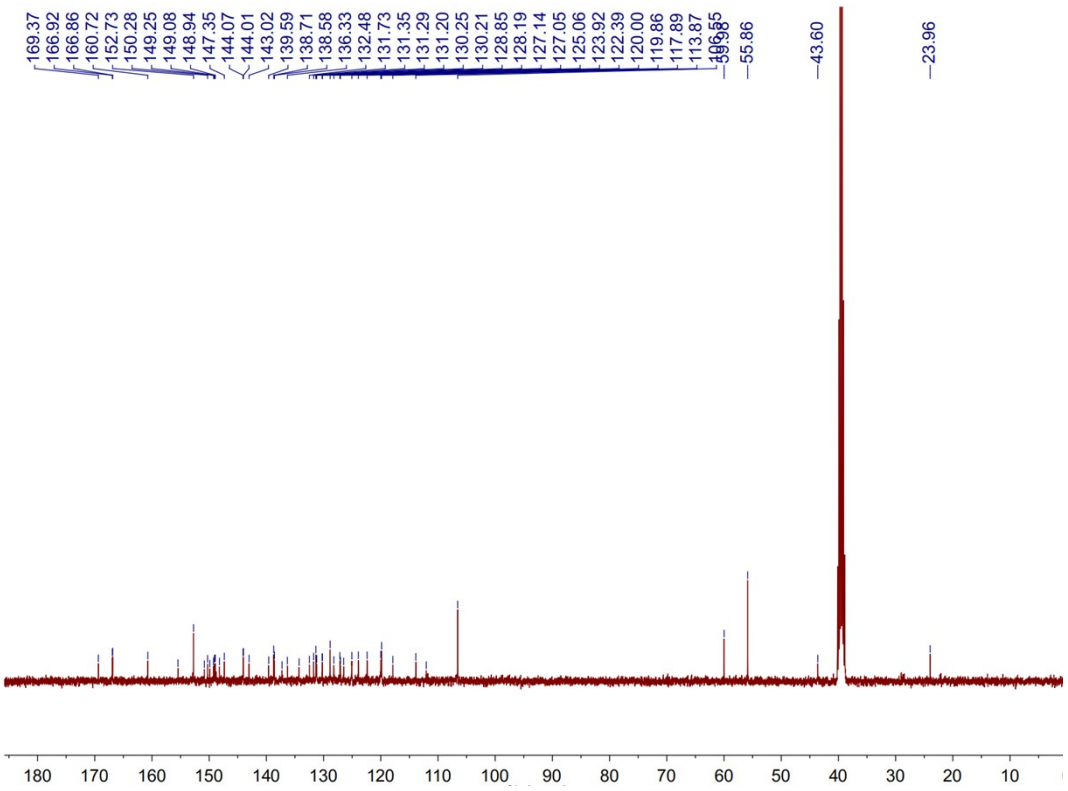


Figure S6. ^{13}C NMR spectra of ***Δ*-IrPPQ** in DMSO-d_6.

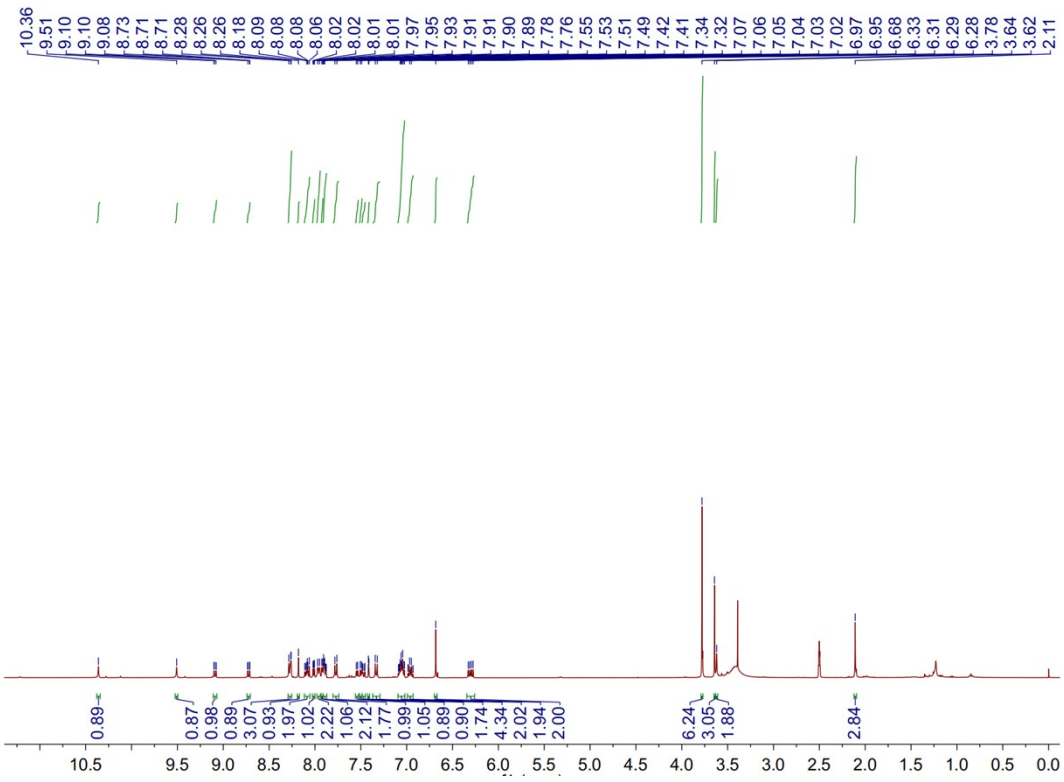


Figure S7. ^{1}H NMR spectra of ***Λ*-IrPPQ** in DMSO-d_6.

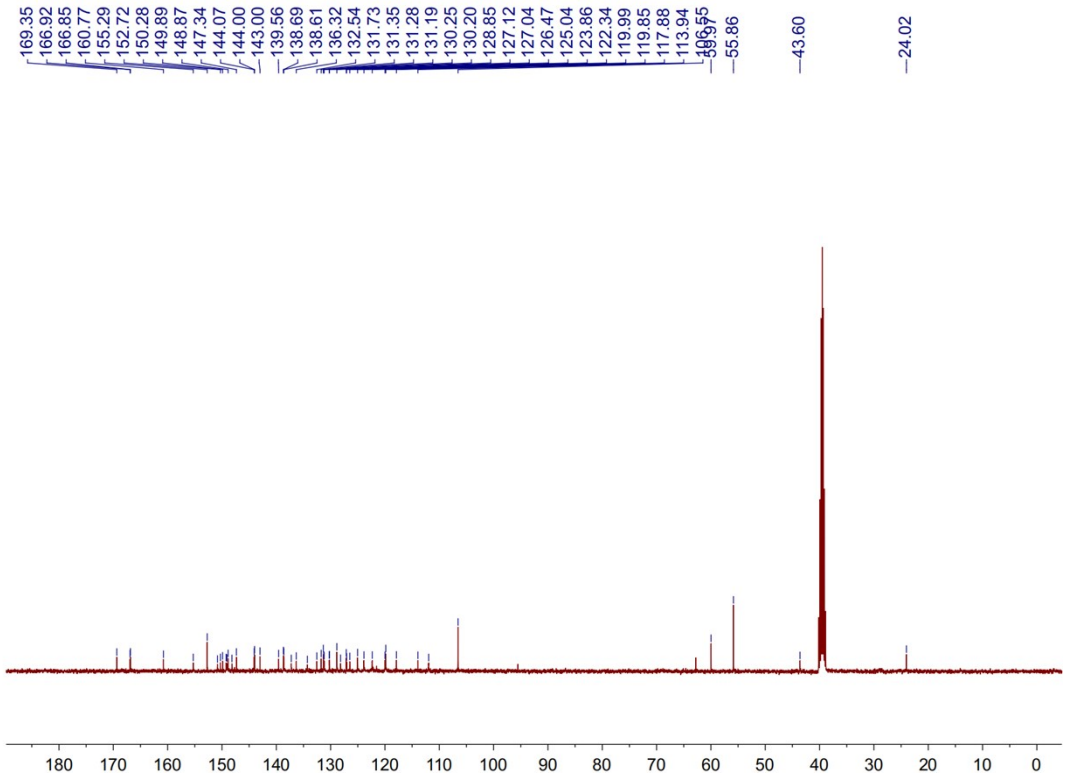


Figure S8. ^{13}C NMR spectra of ***Λ*-IrPPQ** in DMSO-d_6.

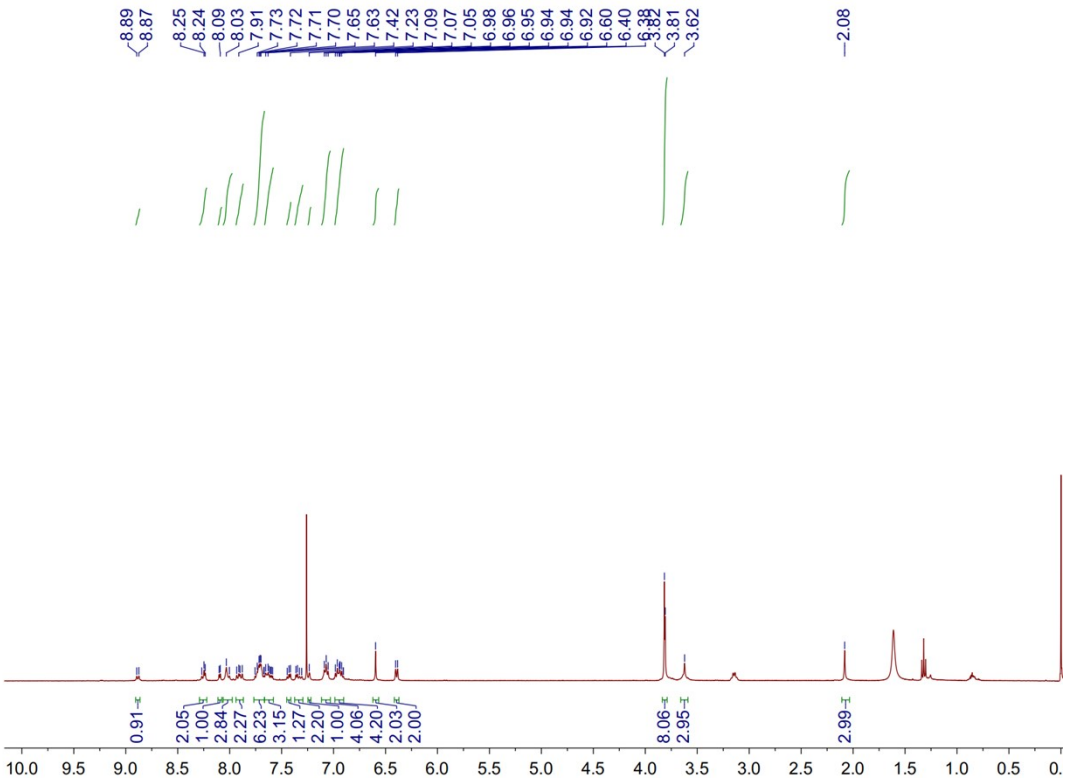


Figure S9. ^{1}H NMR spectra of ***rac*-IrPPQ** in $CDCl_3$.

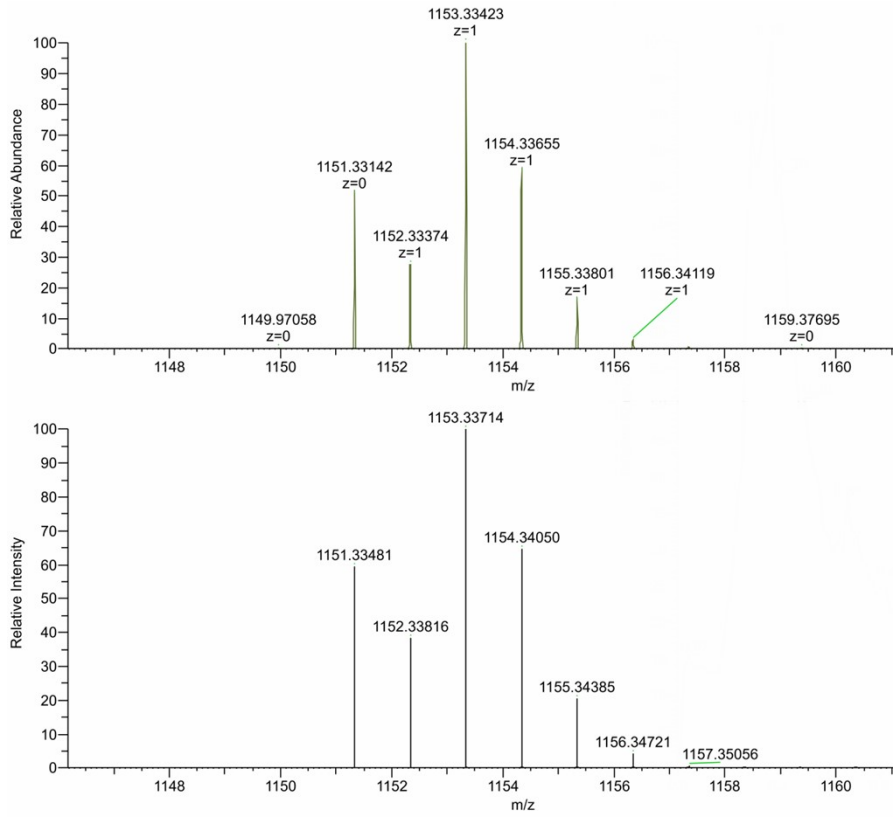


Figure S10. HR-ESI-MS spectra of ***Δ*-IrPPQ** (green: experimental, black: theoretical).

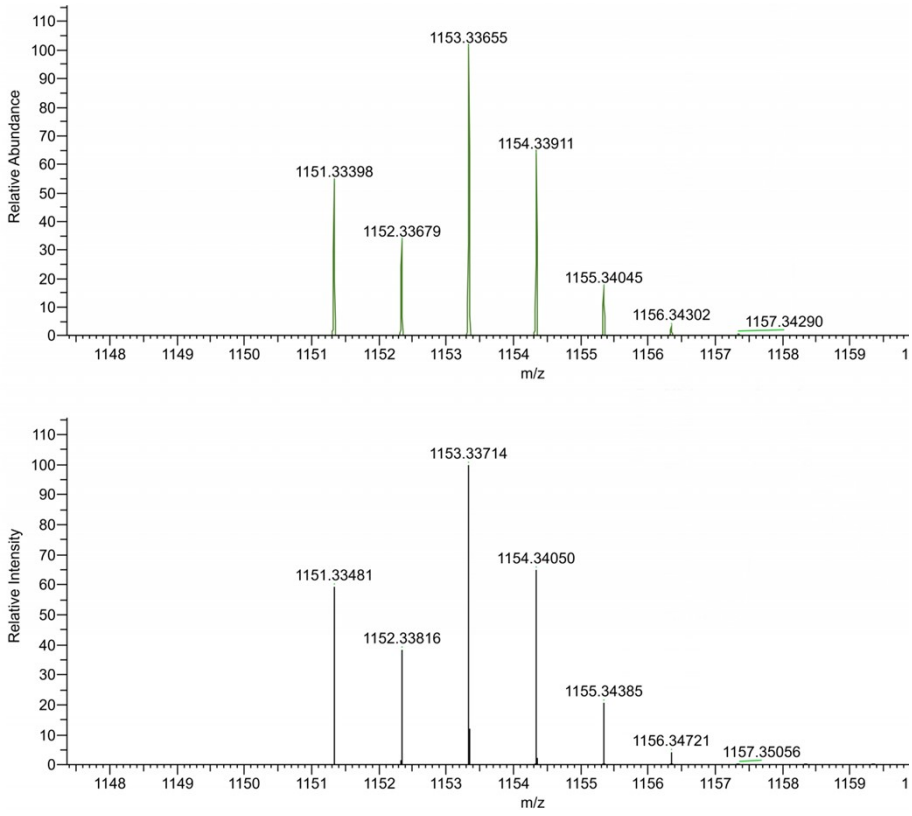


Figure S11. HR-ESI-MS spectra of ***Λ*-IrPPQ** (green: experimental, black: theoretical).

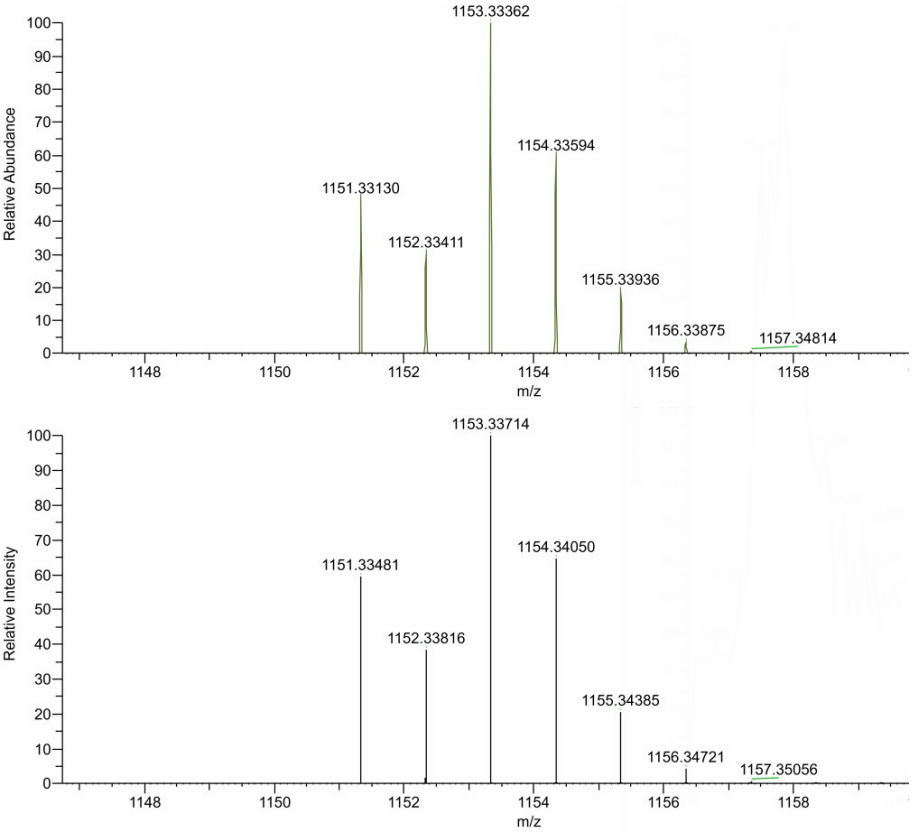


Figure S12. HR-ESI-MS spectra of ***rac*-IrPPQ** (green: experimental, black: theoretical).

5. Additional Biological Experimental Data

Table S1. IC_{50} values of **PPQ**, ***Δ*-IrPPQ**, ***Λ*-IrPPQ** and ***rac*-IrPPQ** toward PANC-1, HT-1080 and SK-MEL-28 cells in dark and light condition (380-390 nm, 30 mW/cm^2, 10 min).

IC_{50} (μM)		PANC-1	HT-1080	SK-MEL-28
PPQ	Dark	> 500	> 500	> 500
***Δ*-IrPPQ**	Dark	> 500	> 500	> 500
	Light	> 50	16.72	5.55
***Λ*-IrPPQ**	Dark	> 500	> 500	> 500
	Light	2.70	4.74	1.78
***rac*-IrPPQ**	Dark	> 500	> 500	> 500
	Light	66.67	> 50	12.72

Table S2. Comparison of *Λ*-IrPPQ with other metallodrugs toward cancer cells.

Metallodrug	Cell line	IC_{50} (μM) / Incubation Time	Ref.
***Λ*-IrPPQ**	PANC-1	2.70 ± 0.11 (PI = 185.2) / 24 h after irradiation	This work
IrFN	PANC-1	6.60 ± 0.19 / dark, 48 h	This work
MitoIrL2	PANC-1	2.45 ± 0.24 / 24 h after irradiation	[20]
Ir1	PANC-1	9.19 / dark, 48 h	[21]
Ir-pbt-Bpa	B16F10	0.42 ± 0.05 (PI = 33.7)/ 40 h after irradiation	[22]
Ir-PEG-Fn	Hela	12.66 ± 0.25 μM / 24 h after irradiation	[23]
Ir2	MCF-7	0.78 (PI = 128.2) / 40 h after irradiation	[24]
Ir1	MCF-7	0.7 ± 0.1 / dark, 72 h	[25]
IrFc1	MDA-MB-231	0.05 ± 0.03 (PI = 1509) / 48 h after irradiation	[26]
1	RD	1.3 ± 0.1 / dark, 72 h	[32]
4	Caco-2CR	12.8 ± 2.1 / dark, 48 h	[33]
Ga-1	Hela	1.2 ± 0.1 / dark, 48 h	[34]
TTC-Pt	Hela	0.34 ± 0.05 (PI = 188.2) / 24 h after irradiation	[35]

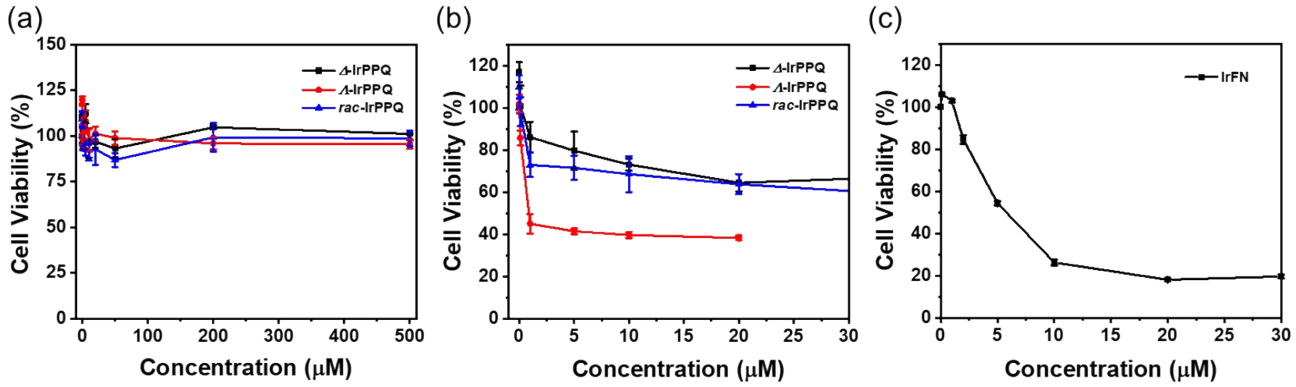


Figure S13. Cell viability of PANC-1 cells treated with different concentrations of ***Δ*-IrPPQ**, ***Λ*-IrPPQ** and ***rac*-IrPPQ** under dark condition (a) or after light irradiation (b). (c) Cell viability of PANC-1 cells treated with IrFN under dark condition.

Table S3. Molecular docking results of binding energies and interaction sites.

Compound	Target	Binding Energies	H-Bond	π-π interactions
***Δ*-IrPPQ**	FSP1	9.13	-	LYS293
***Λ*-IrPPQ**	FSP1	10.93	LEU329	GLY24, PHE360
***Δ*-IrPPQ**	MT1X	8.25	LYS22, LYS31	-
***Λ*-IrPPQ**	MT1X	9.09	LYS22, GLY52, LYS31	-

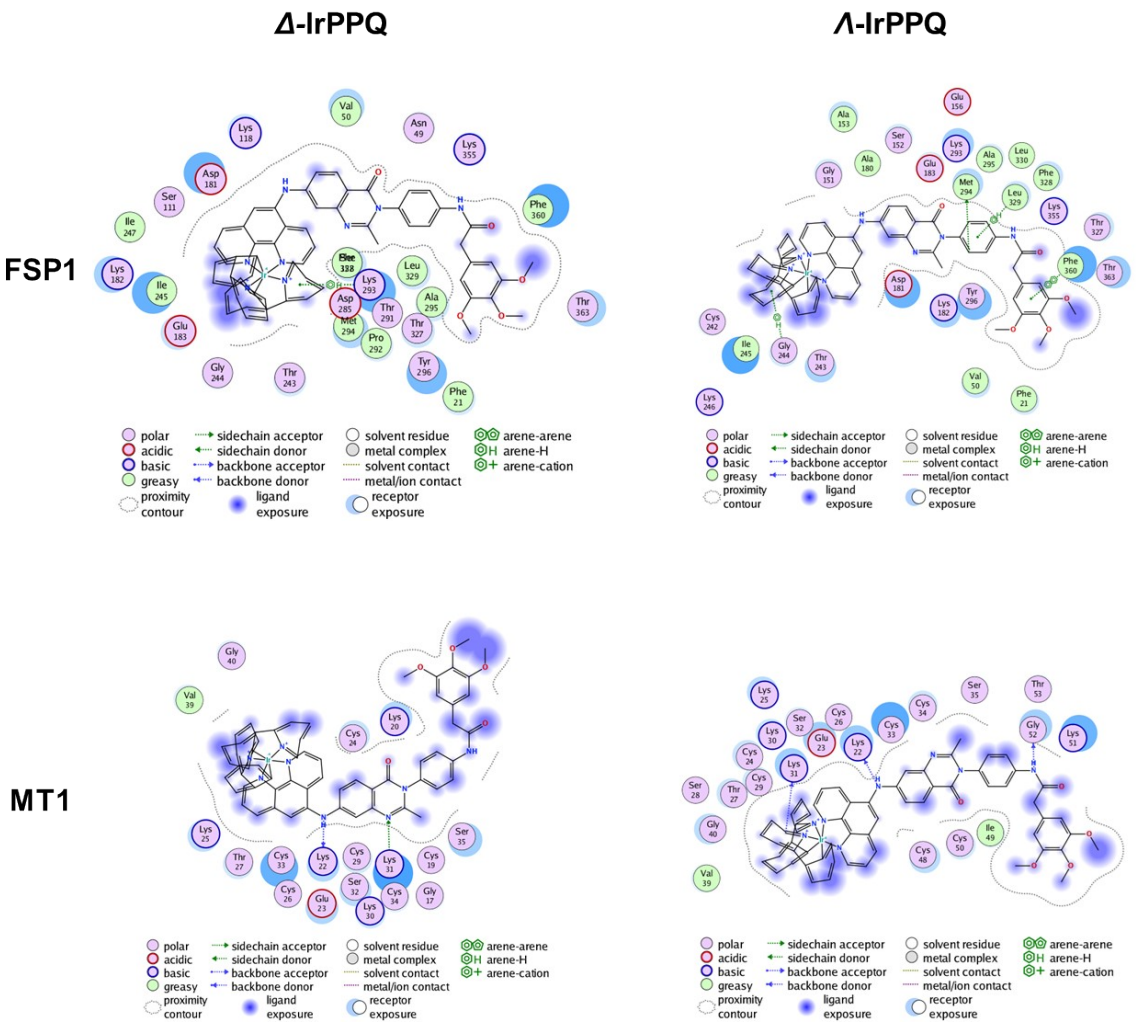


Figure S14. 2D interaction of ***Δ*-IrPPQ** and ***Λ*-IrPPQ** with FSP1 and MT.