Electronic Supporting Information

Design of Cyclometalated Iridium(III)-Metformin Complexes for Hypoxic Cancer Treatment

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Materials and measurements

Iridium chloride hydrate was purchased from Alfa Aesar; 2-(2,4-difluorophenyl) pyridine (dfppy), 2-phenylpyridine (ppy), 7,8-Benzoquinoline (bq), Potassium tert butyl alcohol, $AgSO_3CF_3$ and NH_4PF_6 were purchased from Innochem, Metformin and cisplatin were purchased from J&K; JC-1, H_2DCFDA (2',7'-dichloro dihydrofluorescein diacetate), MTDR (MitoTracker Deep Red), LTDR (LysoTracker Deep Red) and ERTR (endoplasmic reticulum - Tracker Red) were purchased from Life Technologies, USA; Hoechst 33342, MTT (3-(4,5-dimeth ylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), and Annexin-V/PI assay kit were purchased from Sigma Aldrich, USA; ATP Assay kit was purchased from Promega, USA; Primary rabbit polyclonal antibody for COX-2 and HIF-1 alpha, Alexa Fluor 488 goat anti-rabbit secondary antibody were purchased from Abcam (USA). Phospho-AMPK alpha (Thr172) antibody were also purchased from Abcam, the secondary antibody, goat anti-rabbit IgG (H&L) (HRP) were purchased from Beyotime Biotechonology.

All of the compounds tested were dissolved in DMSO immediately prior to the experiments, and the final concentration of DMSO was kept at 1% (v/v). NMR spectra were recorded on a Bruker Avance 400 spectrometer. Shifts were referenced relative to the internal solvent signals. Microanalysis (C, H, and N) was carried out using an Elemental Vario EL CHNS analyser (Germany). ESI-MS spectra were recorded on a Thermo Scientific LTQ XL spectrometer (USA). The quoted m/z values represent the major peaks in the isotopic distribution. UV–Vis spectra were recorded on a Varian Cary 300 spectrophotometer (USA). Emission spectra were recorded on a Edinburgh FLS-920 steady state fluorescence spectrometer (UK). Confocal images were captured by Conforcal Telescope (Germany, Carl Zeiss LSM 710). Flow cytometry: Becton Dickinson, USA; Multifunctional enzyme analyzer: Swiss infinite m200 Pro; Protein electrophoresis instrument: Bio rad, USA.

Synthesis and Characterization of Ir(III) complexes

Ir1: The dimeric iridium(III) precursors $Ir_2(dfppy)_4Cl_2$ was prepared according to the literature method.[1] A mixture of iridium(III) precursor (121.4 mg, 0.1 mmol) and Metformin (66.2 mg, 0.4 mmol) was resolved in CH_2Cl_2/ CH_3OH (2:1, v/v) solvent, then potassium tert butyl alcohol (89.8 mg, 0.8 mmol) was added and the solution was heated to 35 ~ 40 °C and reflux overnight under N_2 protection in the dark. The solution was cooled to room temperature,

evaporated to dryness, and the obtained solids were resolved in 2 ml of methanol followed by the addition of 10-fold excess of saturated NH_4PF_6 solution and 2 h stirring. Then the mixture was filtered and filtrate was evaporated to dryness with reduced pressure. The obtained solids were dissolved in CH_2Cl_2 and purified by column chromatography on silica gel eluted with CH_2Cl_2 and CH_3OH (10:1 v/v), followed by recrystallization from CH_2Cl_2/diethyl ether.

Light-yellow powder, yield: 62%. ESI-MS: m/z 702.44 $[M\text{-}PF_6]^+$. Elemental analysis: calcd (%) for $C_{26}H_{23}F_{10}IrN_7P\cdot CH_3OH\cdot H_2O$: C 36.16%, H 3.26%, N 10.93%; found: C 36.16%, H 3.14%, N 10.91%. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.77 (d, J = 5.4 Hz, 1H), 8.67 (d, J = 5.8 Hz, 1H), 8.25 (d, J = 4.4 Hz, 1H), 8.06 (q, J = 7.1 Hz, 3H), 7.51 (q, J = 6.2, 5.6 Hz, 3H), 6.79 – 6.61 (m, 2H), 6.40 (s, 2H), 6.35 (s, 1H), 5.98 (s, 1H), 5.60 (dd, J = 8.7, 2.2 Hz, 1H), 5.52 (dd, J = 8.6, 2.2 Hz, 1H), 2.87 (s, 6H). ^{13}C NMR (126 MHz,d_6-DMSO) δ 164.44, 159.51, 158.70, 152.45, 151.69, 149.74, 148.89, 138.66, 128.47, 128.37, 123.27, 123.12, 122.57, 122.42, 113.83, 113.71, 113.48, 113.35, 97.02, 96.81.

Ir2: The synthesis was similar to **Ir1** except using $(Ir_2(ppy)_4Cl_2$ as the Ir precursor. Light-yellow powder, yield: 70%. ESI-MS: m/z 630.50 $[M\text{-}PF_6]^+$. Elemental analysis: calcd (%) for $C_{26}H_{27}F_6IrN_7P\cdot 2CH_3OH$: 40.09%, H 4.21%, N 11.69%; found: C 40.34%, H 3.90%, N 11.62%. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.74 (d, J = 6.4 Hz, 1H), 8.64 (d, J = 5.7 Hz, 1H), 8.16 (d, J = 8.2 Hz, 2H), 7.98 – 7.89 (m, 2H), 7.79 – 7.71 (m, 2H), 7.40 (dddd, J = 11.1, 7.3, 5.8, 1.4 Hz, 2H), 6.81 (tdd, J = 7.5, 3.9, 1.3 Hz, 2H), 6.65 (t, J = 7.3 Hz, 2H), 6.37 (s, 2H), 6.22 (s, 1H), 6.17 (dd, J = 7.6, 1.2 Hz, 1H), 6.10 (dd, J = 7.5, 1.2 Hz, 1H), 5.57 (s, 1H), 2.84 (s, 6H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 168.20, 167.85, 154.41, 153.43, 152.19, 151.77, 149.10, 148.32, 144.77, 137.36, 132.09, 131.81, 128.70, 124.15, 124.07, 122.65, 122.58, 120.47, 120.34, 119.07, 118.87.

Ir3: The synthesis was similar to **Ir1** except using $(Ir_2(bq)_4Cl_2$ as the Ir precursor. Light-yellow powder, yield: 54%. ESI-MS: m/z 678.36 $[M\text{-}PF_6]^+$. Elemental analysis: calcd (%) for $C_{30}H_{27}F_6IrN_7P\cdot 2CH_3CH_2OH$: C 44.63%, H 4.30%, N 10.72% found: C 44.81%, H 4.05%, N 10.73%. ^{1}H NMR (400 MHz, DMSO-d6) δ 9.16 (dd, J = 5.4, 1.3 Hz, 1H), 9.04 (dd, J = 5.4, 1.3 Hz, 1H), 8.56 (ddd, J = 8.1, 3.0, 1.3 Hz, 2H), 7.93 – 7.78 (m, 6H), 7.33 (ddd, J = 7.9, 3.2, 0.9 Hz, 2H), 6.94 (td, J = 7.5, 4.5 Hz, 2H), 6.34 (d, J = 10.0 Hz, 3H), 6.22 (dd, J = 7.2, 0.9 Hz, 1H), 6.07 (dd, J = 7.2, 0.9 Hz, 1H), 5.80 (s, 1H), 2.84 (s, 6H). ^{13}C NMR (126 MHz, d_6-DMSO) δ 158.02, 157.68, 148.47, 147.72, 141.88, 141.69, 136.26, 136.20, 133.35, 133.31, 129.19, 129.09, 128.91, 128.77, 128.50, 126.23, 126.11, 123.76, 123.63, 121.89, 121.80, 118.33, 118.23.

IrCN: Iridium precursor $[Ir(dfppy)_2Cl]_2$ (121.4 mg, 0.1 mmol) and $AgSO_3CF_3$ (0.22 mmol) were mixed in acetonitrile solvent and refluxed under N2 protection for 24 h in the dark. After the reaction, it was filtered with diatomite, and the precipitate was washed with acetonitrile for 3 times. The filtrate and washing solution were collected to evaporate to 1 mL under reduced pressure, then ether was added to extract the solid, which was then washed with ether and n-hexane for several times. The obtained solids were further purified by recrystallization using acetonitrile/chloroform solvent.

Light-yellow powder, yield: 82%. ESI-MS: m/z 654.60 $[M\text{-}PF_6]^+$, 614.20 $[M\text{-}PF_6\text{-}CH_3CN]^+$，604.84 $[M\text{-}PF_6\text{-}CH_3CN\text{+}CH_3OH]^+$, 573.64 $[M\text{-}PF_6\text{-}2CH_3CN]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 9.10 (d, J = 5.7 Hz, 2H), 8.30 (d, J = 8.5 Hz, 2H), 8.01 – 7.94 (m, 2H), 7.46 (ddd, J = 7.4, 5.8, 1.4 Hz, 2H), 6.42 (ddd, J = 12.5, 9.1, 2.4 Hz, 2H), 5.48 (dd, J = 8.5, 2.4 Hz, 2H), 2.45 (s, 6H).

X-ray crystallographic structure determination of Ir1

Crystals of **Ir1** qualified for X-ray analysis were obtained by the slow diffusion of diethyl ether into the acetonitrile solution of complexes. X-ray diffraction measurements were performed on a Bruker Smart 1000 CCD diffractometer with Mo Kα radiation (λ = 0.71073 Å) at 293(2) K. The crystal structures were solved by direct methods with program SHELXS[2] and refined using the full-matrix least-squares program Least Squares. The CCDC deposit number for **Ir1** is 1976433. Crystallographic data, details of data collection as well as selected bond distances and angles were listed in Table S1-S2.

Cell lines and culture conditions

HeLa, A549, MCF-7 and HLF cells were obtained from Experimental Animal Center of Sun Yat-sen University (Guangzhou, China). The Cells were maintained in DMEM (Dulbecco's modified Eagle'smedium, Gibco BRL) or RPMI 1640 (Roswell Park Memorial Institute 1640, Gibco BRL) medium containing 10% FBS (fetal bovine serum, Gibco BRL), 100 μg/mL streptomycin, and 100 U/mL penicillin (Gibco BRL). **Normoxia culture**: Cells were cultured in a humidified incubator at 37 °C under 5% CO_2. **Hypoxia culture**: Cells was maintained at 37 °C in a humidified atmosphere containing 1% oxygen, 5% carbon dioxide and 94% nitrogen.

Cellular uptake and distribution

MCF-7 cells were treated with **Ir1-Ir3** (10 μM) for 30 min at 37 °C, then washed three times with ice-cold PBS and visualised by confocal microscopy (LSM 710, Carl Zeiss, Göttingen, Germany). Emission was collected at 460 ± 20 nm, 525 ± 20 nm, 580 ± 20 nm for **Ir1-Ir3**, respectively, upon excitation at 405 nm.

MCF-7 cells were co-incubated with **Ir1** (10 μM) and various organelle-specific probe, *e.g.* MTDR (100 nM) or LTDR (150 nM) or ERTR (100 nM) probe, at 37 °C for 15 min. Cells were washed three times with PBS and visualized by confocal microscopy immediately (λ_{ex}=405 nm, λ_{em}= 460 ± 20 nm for **Ir1**; λ_{ex} = 633 nm, λ_{em} = 665±20 nm for MTDR and LTDR; λ_{ex} = 587 nm, λ_{em}= 615 ± 20 nm for ERTR.

MTT assay[3]

Cells cultured in 96-well plates were grown to confluence. The compounds were dissolved in DMSO (1%, v/v), and diluted with fresh media immediately. The cells were incubated with a series of concentrations of the tested compounds for 44 h at 37 °C. 20 μL MTT solution then

added to each well, and the plates were incubated for an additional 4 h. The media was removed, and DMSO was added (150 μL per well) and incubated for 10 min with shaking. The absorbance at 595 nm was measured using a microplate reader (Infinite M200 Pro, Tecan, Männedorf, Switzerland). To determine hypoxic toxicity, the cells were better be adapted to hypoxia 1 week in advance.

Detection of apoptosis

Annexin V/PI Assay: In flow cytometry, MCF-7 cells were cultured in 6-well tissue culture plates for 24 h and then treated with complex **Ir1** and **IrCN** at the indicated concentrations for 24 h. The cells were harvested and stained using an Annexin -V /PI apoptosis detection kit (Sigma Aldrich) according to the manufacturer's instructions. Data were collected by flow cytometer and analyzed with FlowJo 7.6 software.

Western blot of AMPKα and AIF expression

MCF-7 cells were incubated with **Ir1(**2.5 and 5.0 μM) and **IrCN** (2.5 and 5.0 μM) for 24 h before cell lysis (RIPA lysis buffer with 50 mM Tris pH 7.4, 150 mmol/L NaCl,1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, sodium orthovanadate, sodium fluoride, EDTA, leupeptin). Protein levels were quantified using the BCA protein assay (Applygen, Beijing,China) before loading into SDS gels for electrophoresis. Following transfer, polyvinylidene fluoride (PVDF) membranes were assessed for p-AMPKα and AIF, all Western blotting experiments were performed three times.

Immunofluorescence assay of HIF-1α, COX-2 and BAK

MCF-7 cells were incubated with **Ir1(**5.0 μM) and **IrCN** (5.0 μM) for 24 h. Then cells were then fixed with a 4% paraformaldehyde solution and permeabilized using 1% Triton X-100. After incubation with a blocking buffer (1×PBS, 0.1% goat serum, 0.075% glycin) for 1 h at room temperature. The primary rabbit polyclonal antibody for COX-2 (2.5 μg/mL in blocking buffer), or Anti-HIF-1 alpha (2 μg/mL in blocking buffer), or antibody for BAK (2 μg/mL in blocking buffer) was added and incubation was incubated at 4 ºC for 12 h. The cells were then incubated with the secondary Alexa Fluor 488 goat anti-rabbit antibody for 1 h at 37 ºC. After washed 5 times with PBST, the cells were visualized by confocal microscopy (LSM 710, Carl Zeiss, Germany). Emission was collected at 540 ± 20 nm upon excitation at 488 nm.

Measurement of Intracellular ROS levels

MCF-7 cells were cultured in 6-well tissue culture plates for 24 h and then treated with complex **Ir1** and **IrCN** at the indicated concentration for 6 h. Then cells were washed twice with serum-free DMEM, the fluorescence intensity of cells was measured immediately by flow cytometry with excitation at 488 nm and emission at 530 nm. Green mean fluorescence intensities

were analyzed using FlowJo 7.6 software.

MMP Assay

MCF-7 cells were cultured and treated with **Ir1** as described above. The cells were then collected and resuspended at 1 × 10^6/ml in pre-warmed PBS containing 5 μg/ml JC-1, and incubated for 30 min at 37 °C. Subsequently, the cells were washed twice with PBS and immediately analyzed in a flow cytometry. Fluorescence was monitored by measuring both the monomer and the aggregate forms of JC-1 following excitation at 488 nm. Red and green MFI were analyzed using Flow Jo 7.6 software (TreeStar, USA). 10, 000 events were acquired for each sample.

TMRM Assay

MCF-7 cells were cultured on a live cell imaging glass bottom dish at a density of 1x10^5 cells/mL and allowed to grow overnight. Cells were treated with complexes at the indicated concentration for 6 h at 37 °C. A solution of TMRM was added and incubation was carried out at 37 °C for 20 min. The cells were washed 3 times with PBS, and immediately visualized by confocal microscopy. (Ex = 549 nm, Em = 573 ± 20 nm for TMRM).

ATP Assay

ATP concentration of MCF-7 was conducted by the CellTiter-Glo luminescent Cell Viability Assay (Promega, USA) according to the manufacturer's instructions. Cell were cultured in 96 round black well plate for 24 h to welt and treated with complexes **Ir1** at indicated concentrations for 24 and 48 h, respectively. Cell was washed by PBS once and balanced in PBS for 30 min and 100 μl CellTiter-Glo luminescent Cell Viability reagent was added into each well. The mixture was lysed for 2 min by a shaken machine, then incubated at room temperature for 10 min. The luminescence was measured using a TECAN Infinite M200 station. On the same condition, standard curve was obtained by the known concentration of standard ATP sample, Ribonucleotide Triphosphates (rRTPs) (10mM), we can obtain the ATP concentration of cells.

Wound healing assay

The wound healing assay was carried out as previously described[4]. MCF-7 cells were seeded into the 6-well plate. We used the 200 μL sterile pipette tip to make the cross lines when the cells reached nearly 90% confluence. Wash each well carefully to remove the scratched cells using serum-free culture medium. The cells were maintained in serum free culture medium containing the tested compounds at indicated concentrations at 37 °C under hypoxia for 48 h. At the beginning and end of the expriments, the cross lines were photographed by an inverted microscope (Axio Observer Z1, Carl Zeiss, Göttingen, Germany).

Inhibition of angiogenesis

The assay was carried out as previously described[5]. The zebrafish used in this experiment was obtained from the National Zebrafish Resource Center. Fluorescein-labeled zebrafish Is5Tg/+ (AB) (CZRC catalog ID: CZ63) were plated on 24-well plate and treated with the 5 μM tested compounds for 48 h. Then the zebrafish were photographed using a confocal microscope (Zeiss LSM-710, ZEISS, Germany). The mCherry fluorescence protein was excited at 488 nm and recorded at 650 ± 50 nm.

Statistical analysis

All biological experiments were performed at least twice with triplicates in each experiment. Representative results were depicted in this report and data were presented as means ± standard deviations.

Live subject statement

All experiments were performed in strict compliance with the relevant laws and institutional guidelines of Sun Yat-Sen University, Guangzhou, China, and approved by the Ethics Committee of Sun Yat-Sen University. HeLa, A549, MCF-7 and HLF cells were also obtained from Experimental Animal Center of Sun Yat-sen University (Guangzhou, China).

References:

[1] C. Y. Li, M. X. Yu, Y. Sun, Y. Q. Wu, C. H. Huang and F. Y. Li, *J. Am. Chem. Soc.*, 2011, **133**, 11231-11239.

[2] G. M. Sheldrick, *Acta. Crystallogr. Sect. A*, 2008, **64**, 112-122.

[3] T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55-63.

[4] X. W. Wu, Y. Zheng, F. X. Wang, J. J. Cao, H. Zhang, D. Y. Zhang, C. P. Tan, L. N. Ji, Z. W. Mao, *Chem. Eur. J.* **2019**, *25*, 7012-7022.

[5] F. X. Wang, M. H. Chen, Y. N. Lin, H. Zhang, C. P. Tan, L. N. Ji, Z. W. Mao, *ACS Appl. Mater. Interfaces* **2017**, *9*, 42471-42481.

Supporting Figures and Tables

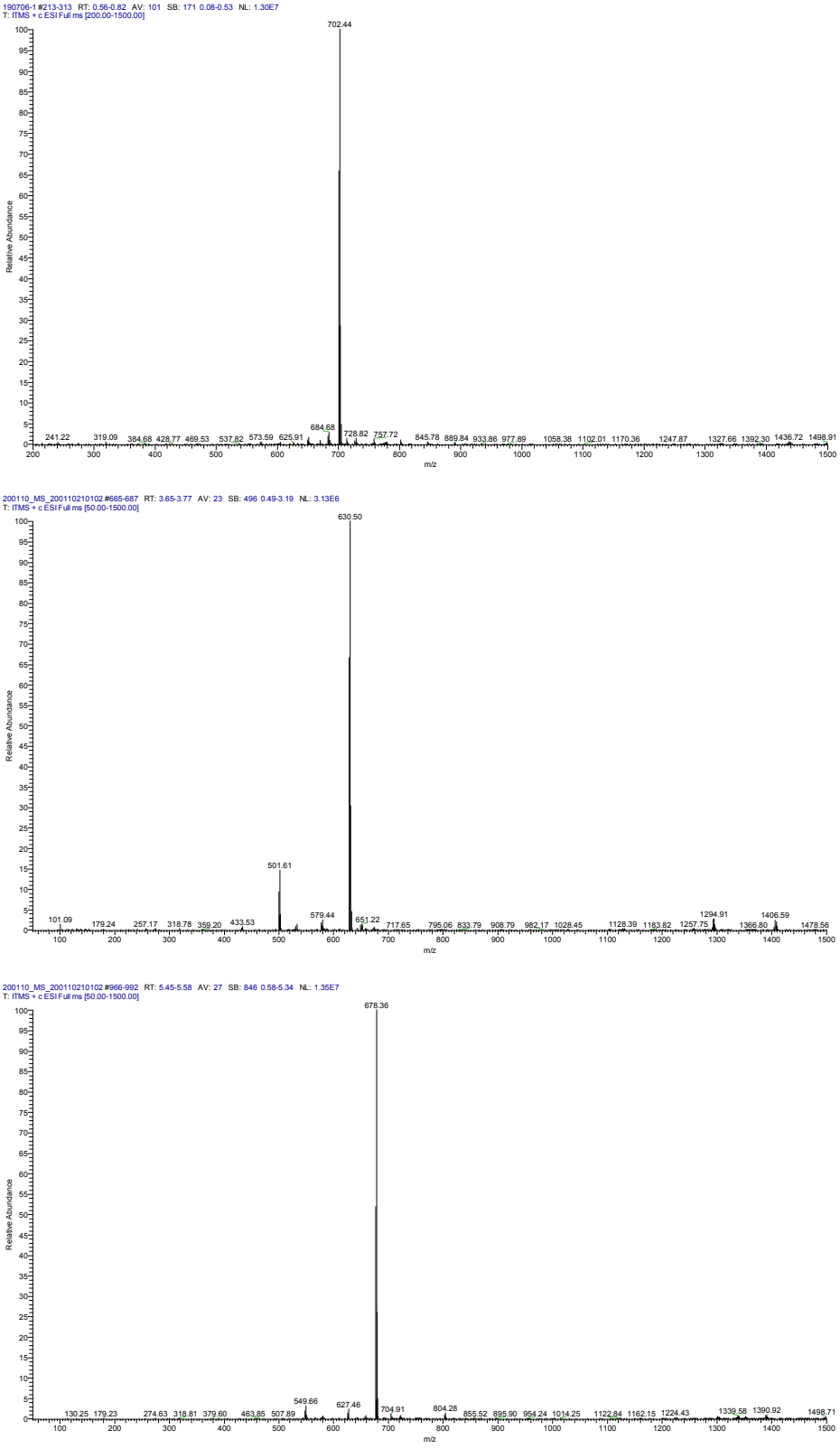

Fig. S1 ESI-MS characterization of (top) **Ir1**, 702.27 $[M\text{-}PF_6]^+$; (middle) **Ir2**, 630.50 $[M\text{-}PF_6]^+$; (bottom) **Ir3**, 678.36 $[M\text{-}PF_6]^+$.

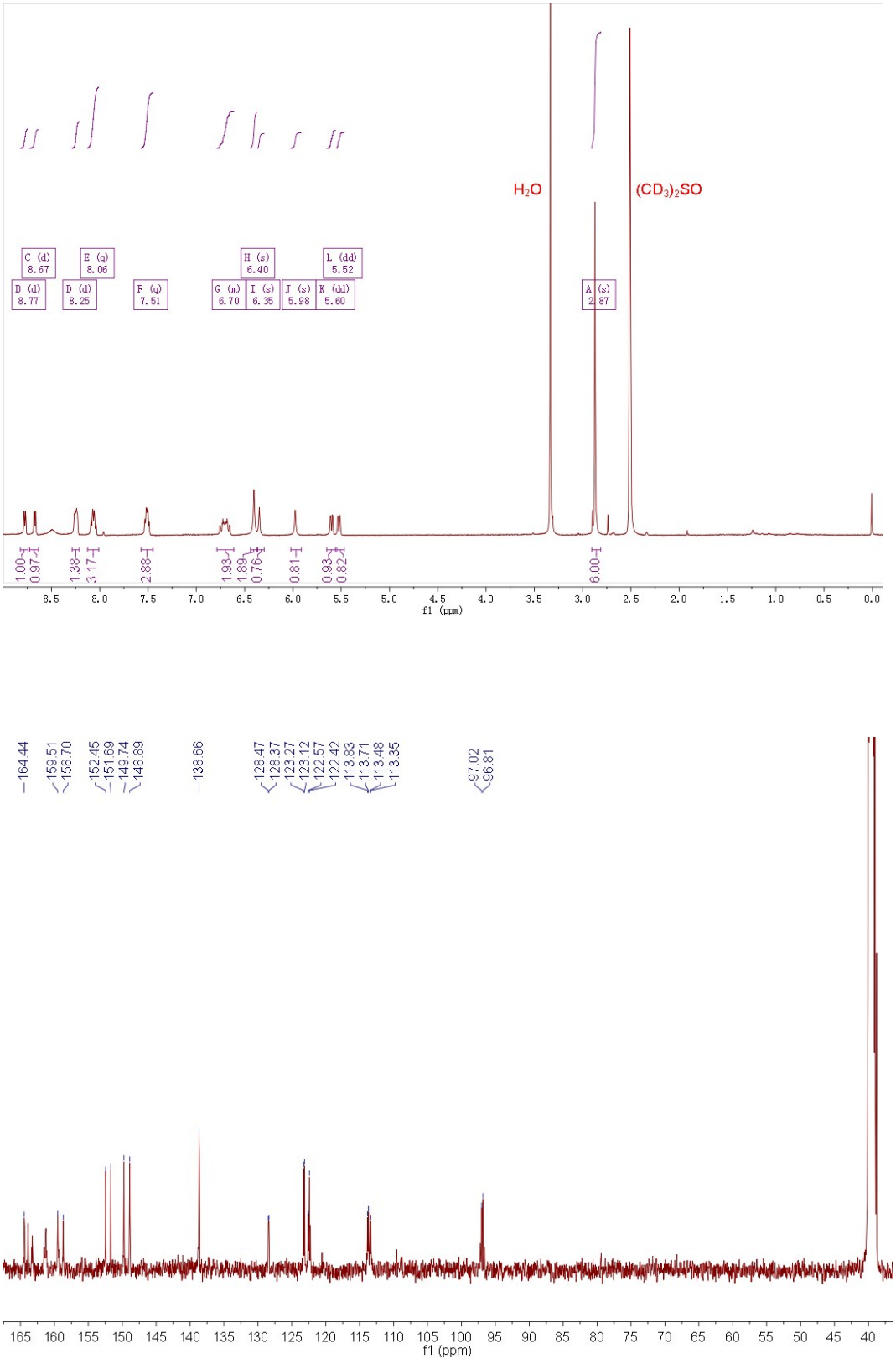

Fig. S2 ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **Ir1** in d6-DMSO solution.

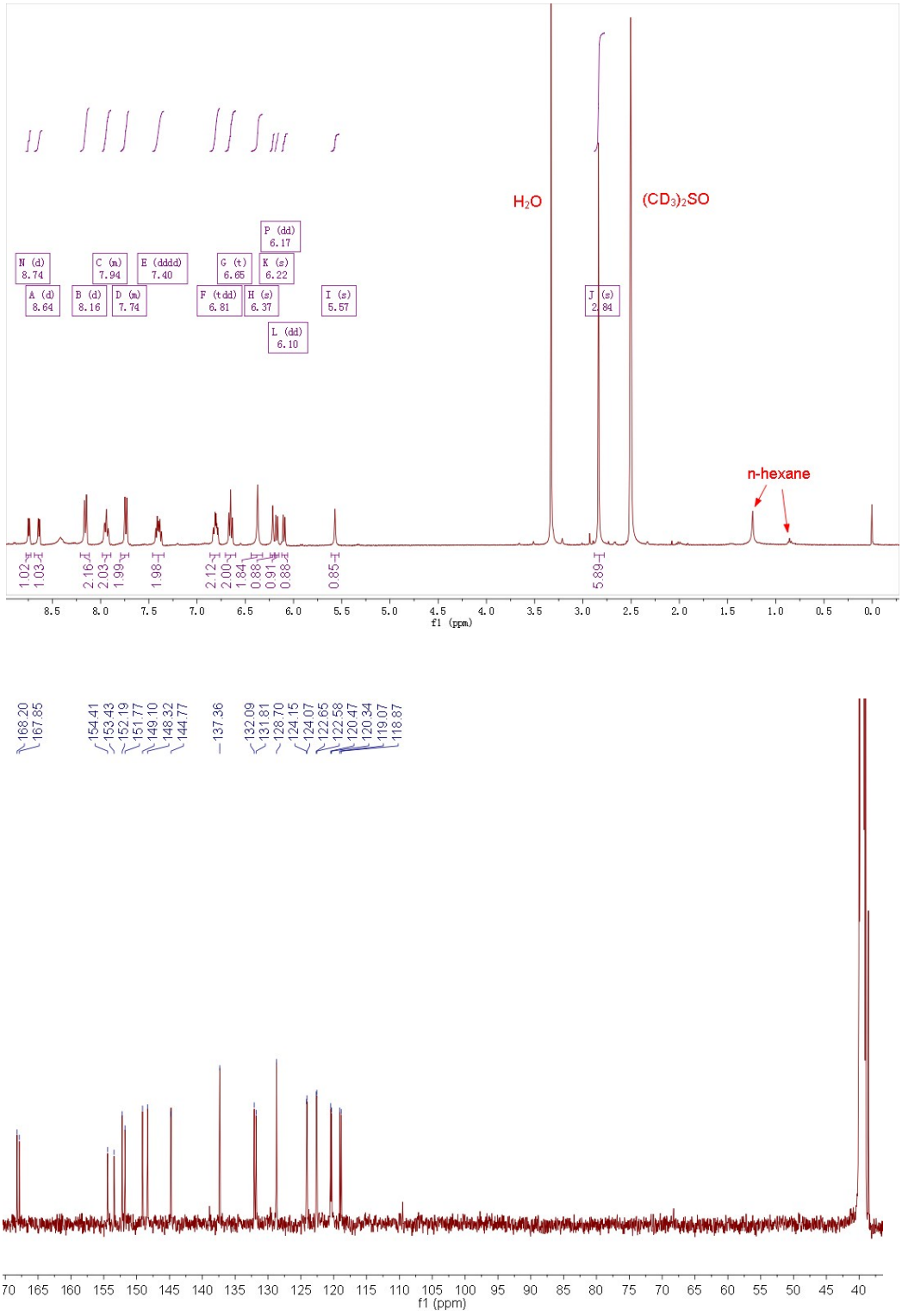

Fig. S3 ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **Ir2** in d6-DMSO solution.

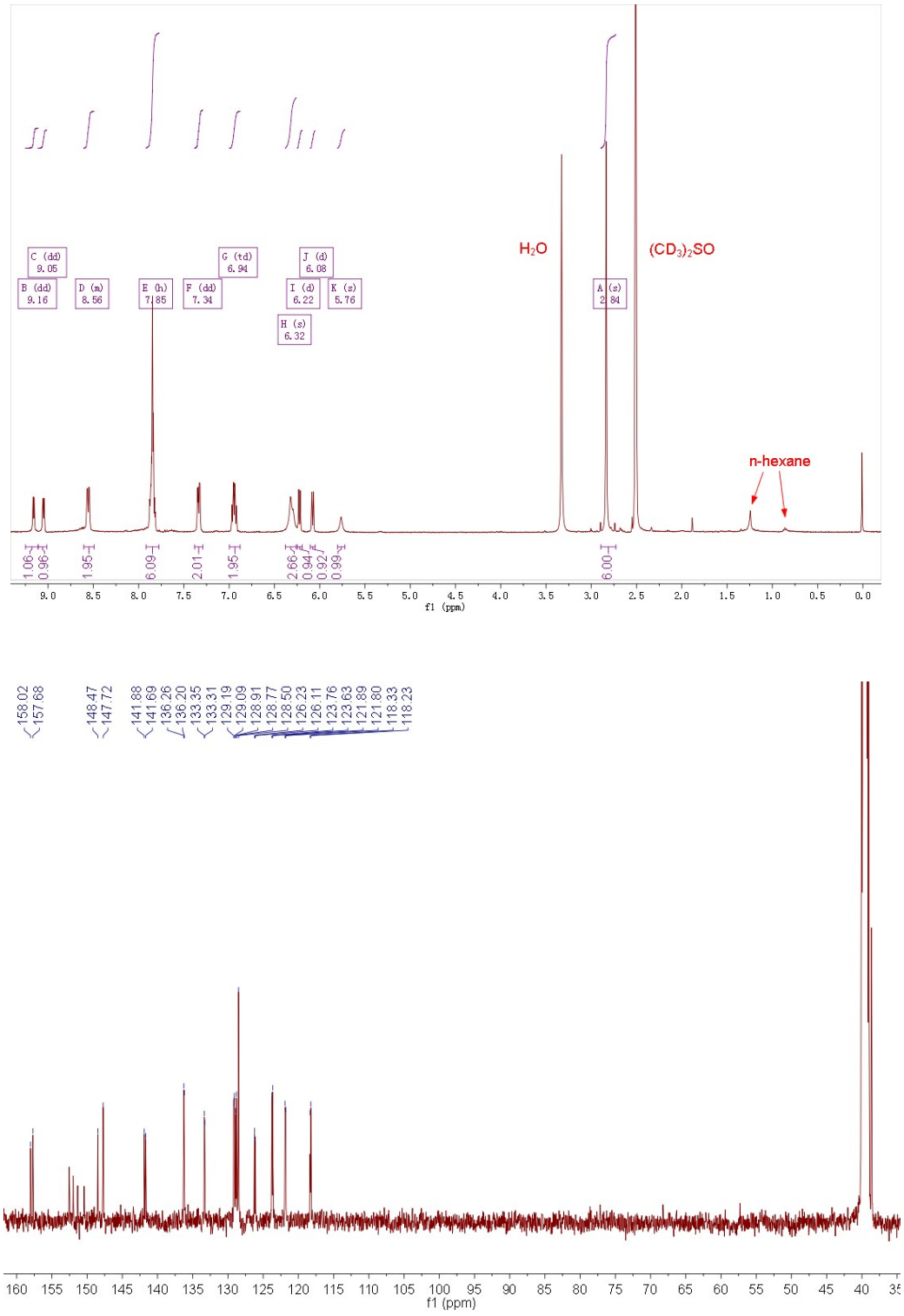

Fig. S4 ^{1}H NMR (top) and ^{13}C NMR (bottom) spectra of **Ir3** in d6-DMSO solution.

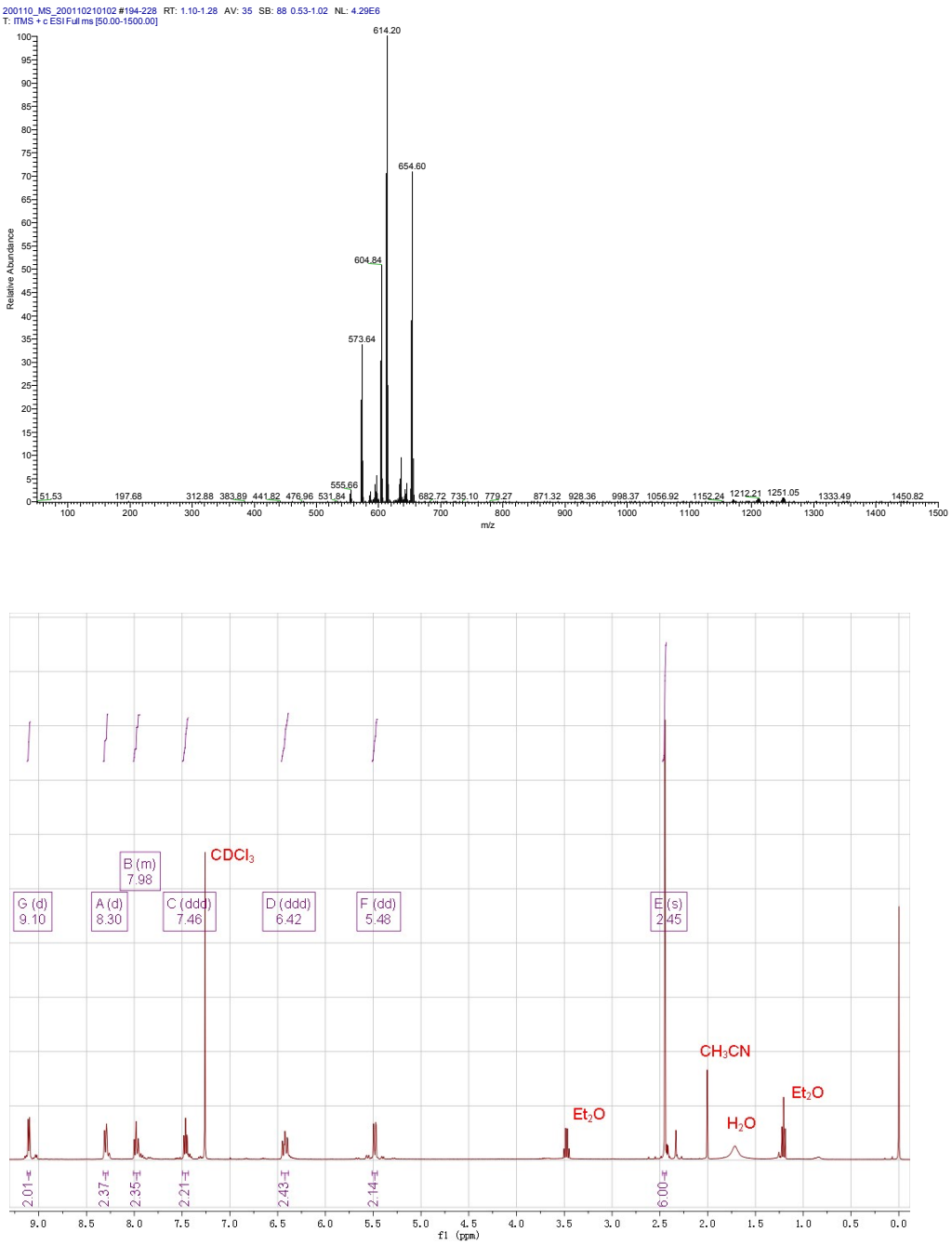

Fig. S5 ESI-MS characterization (top) of **IrCN**, 654.60 $[M\text{-}PF_6]^+$, 614.20 $[M\text{-}PF_6\text{-}CH_3CN]^+$, 604.84 $[M\text{-}PF_6\text{-}CH_3CN\text{+}CH_3OH]^+$, 573.64 $[M\text{-}PF_6\text{-}2CH_3CN]^+$; ^1H NMR spectrum (bottom) of **IrCN** in $CDCl_3$ solution.

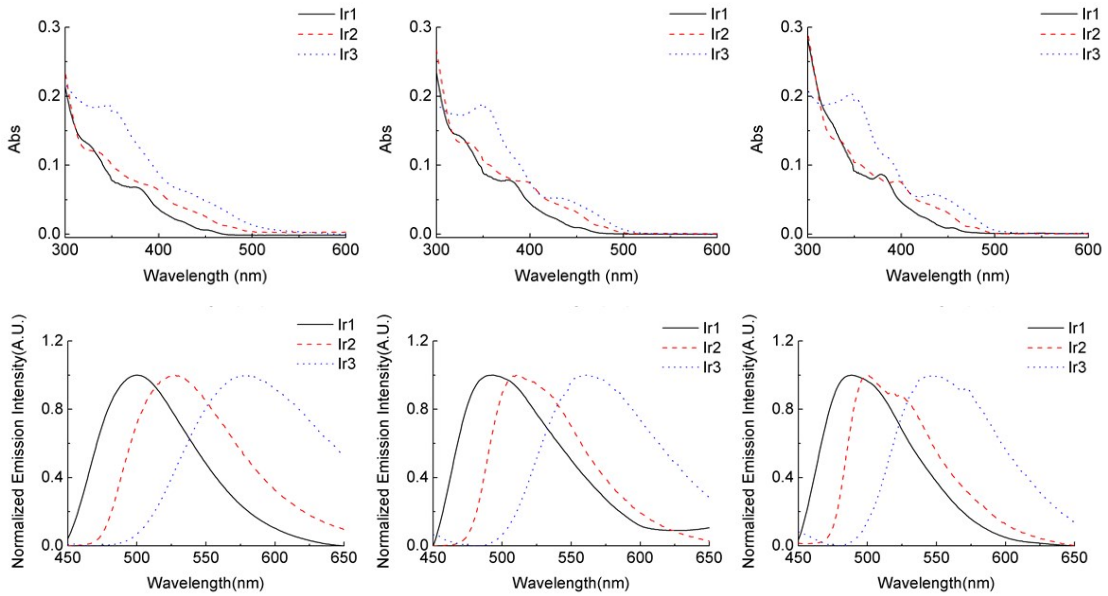

Fig. S6 UV/Vis absorption (top) and emission spectra (bottom) of **Ir1**-**Ir3** (20 μM) measured in degassed PBS, degassed CH_3CN and degassed CH_2Cl_2 at 298 K; λ_{ex}=365 nm.

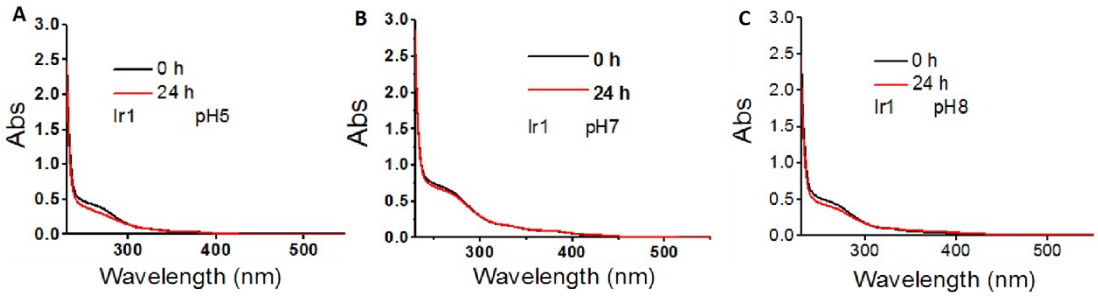

Fig. S7 UV/Vis absorption spectra of **Ir1** (20 μM) measured at 0 h and 24 hours in aqueous solution at different pH values.

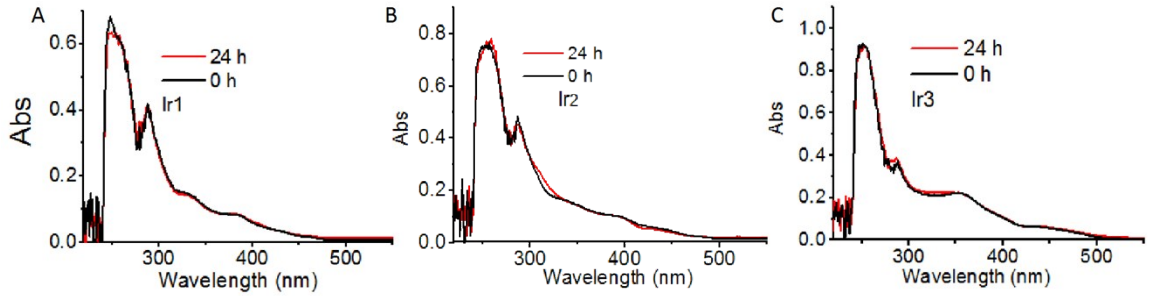

Fig. S8 UV/Vis absorption spectra of **Ir1**-**Ir3** (20 μM) measured at 0 h and 24 hours in PBS containing 10% FBS.

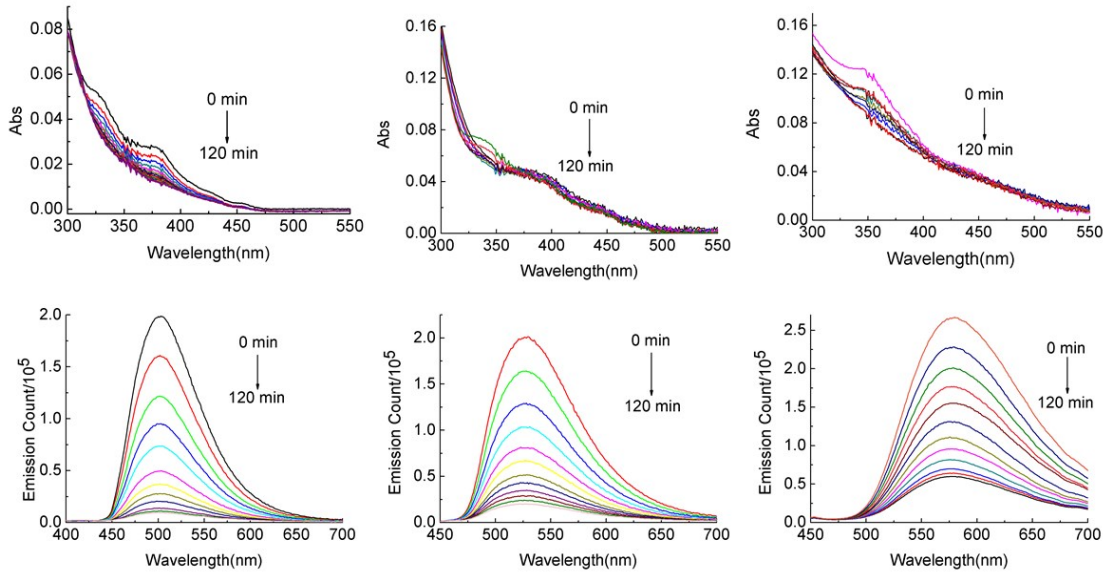

Fig. S9 Changes in the UV/Vis absorption (top) and emission spectra (bottom) of **Ir1** (left)**, Ir2** (middle) and **Ir3** (right) in PBS after adding 10 mM GSH. Complex concentration =10 μM, λ_{ex}=365 nm.

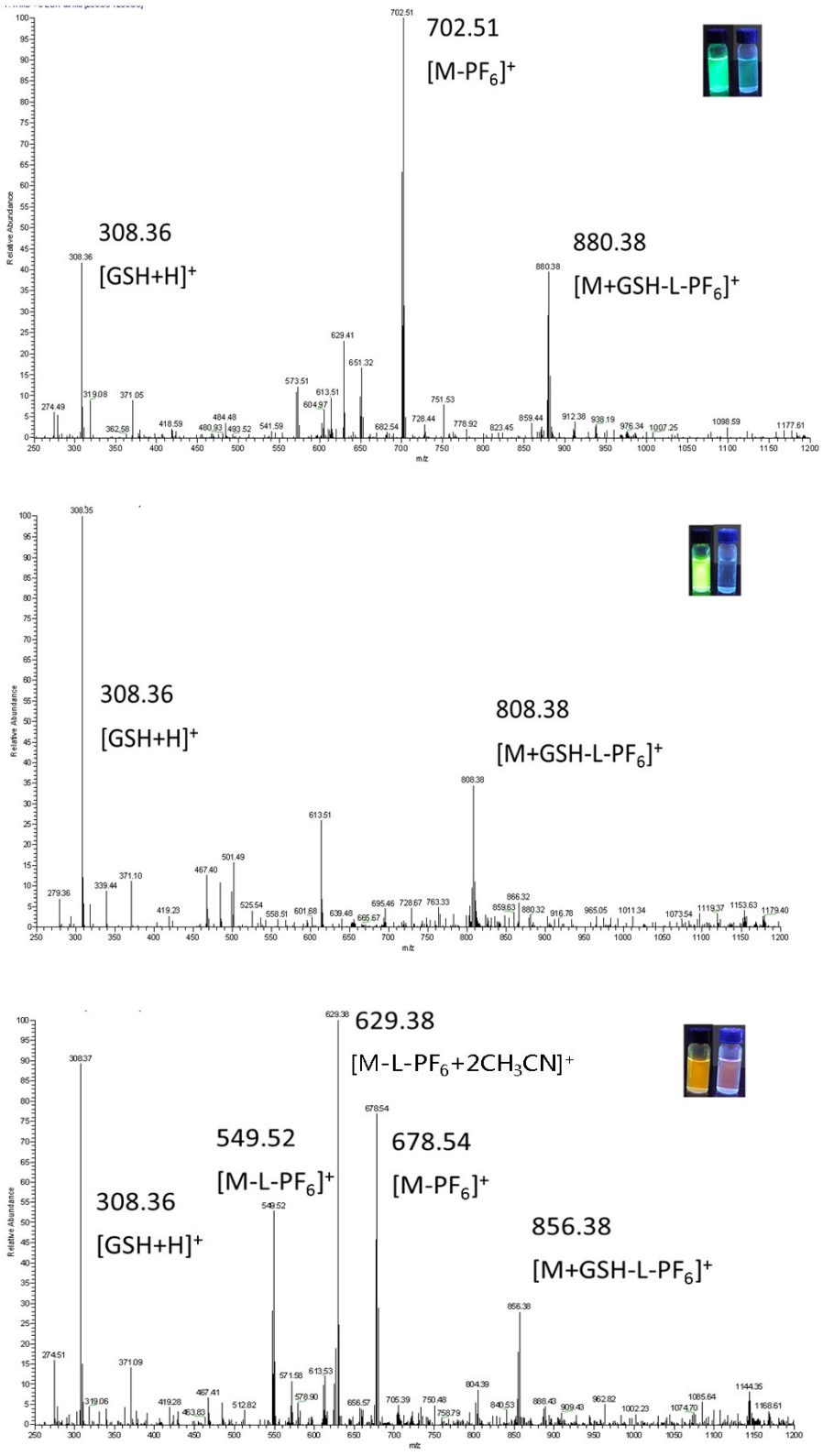

Fig. S10 ESI-MS spectrum collected 24 h after the incubation of **Ir1**-**Ir3** (10 μM) in PBS containing GSH (10 mM), which showed the appearance of $[Ir(C^N)_2(GSH)]^+$ peaks (L = Metformin). **Ir1**, top; **Ir2**, middle, **Ir3**, bottom. Inset pictures are **Ir1**-**Ir3** in PBS before(left) and 24 h after (right) the addition of GSH.

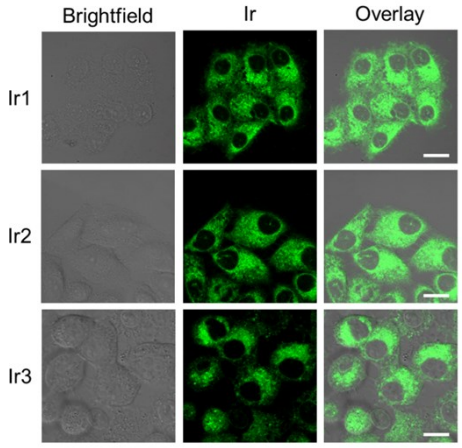

Fig. S11 Cellular uptake of complex **Ir1**-**Ir3** (10 μM, 10 min) in MCF-7 cells measured by confocal microscopy. Scale bar: 20 μm.

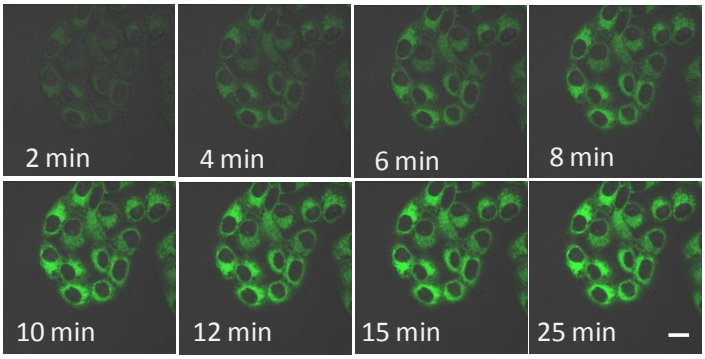

Fig. S12 Time-dependent cellular uptake of **Ir1** (10 μM) in MCF-7 cells. Scale bar: 20 μm.

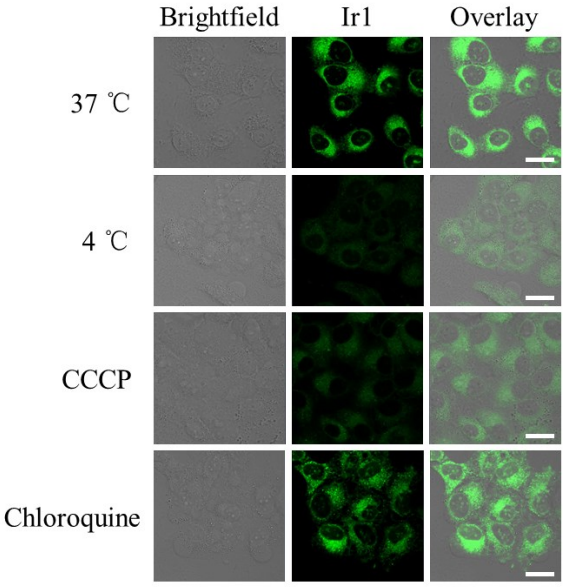

Fig. S13 Effect of incubation temperature (37 ºC and 4 ºC), metabolic inhibitor (CCCP, 30 μM) and chloroquine (50 μM) on cellular uptake of **Ir1** (10 μM, 30 min). Complex **Ir1** was excited at 405 nm and emission was collected at 500 ± 20 nm. Scale bar: 20 μm.

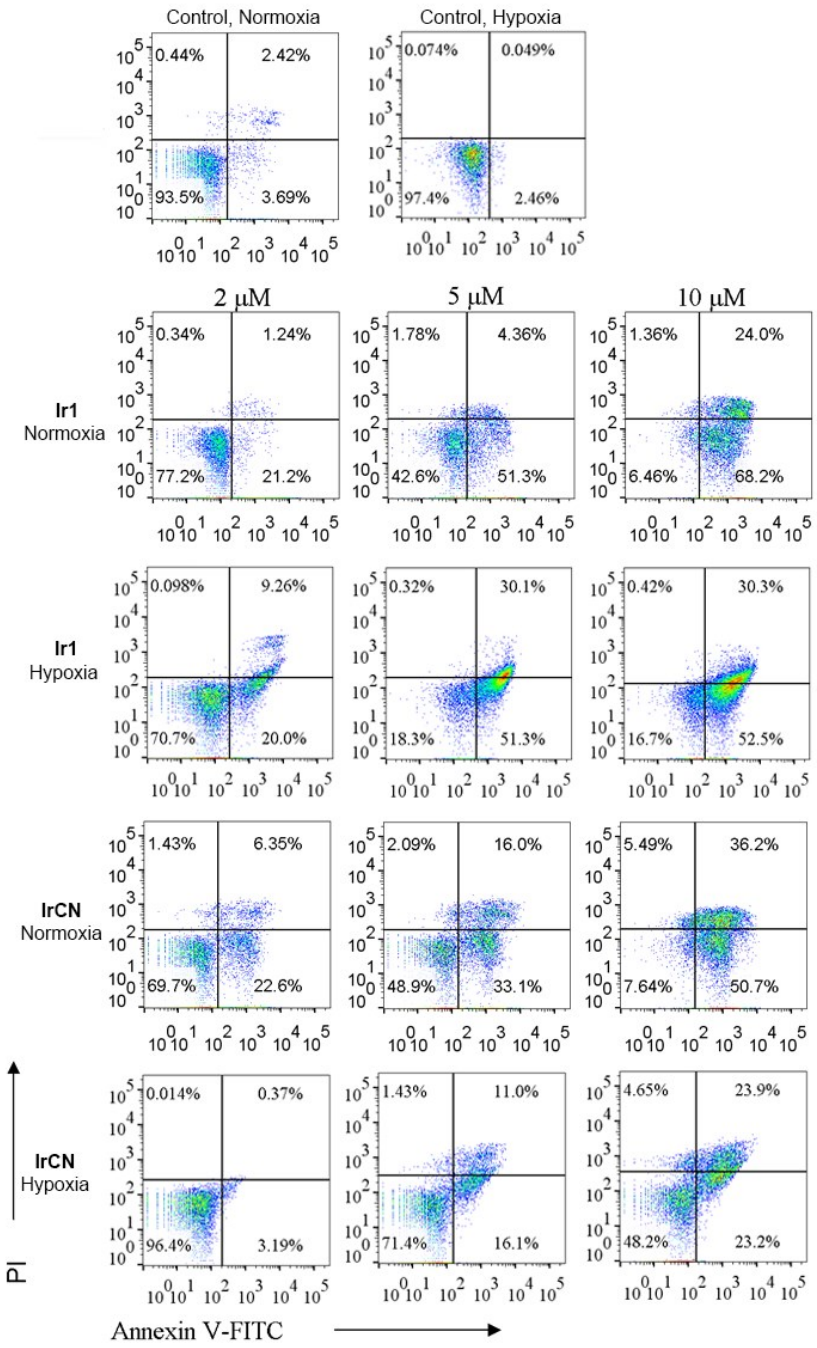

Fig. S14 Flow cytometric quantification of annexin V-PI double labeled MCF-7 cells after treatment with **Ir1** and **IrCN** for 24 h at the indicated concentrations under normoxia (21 % O_2) and hypoxia (1% O_2), respectively.

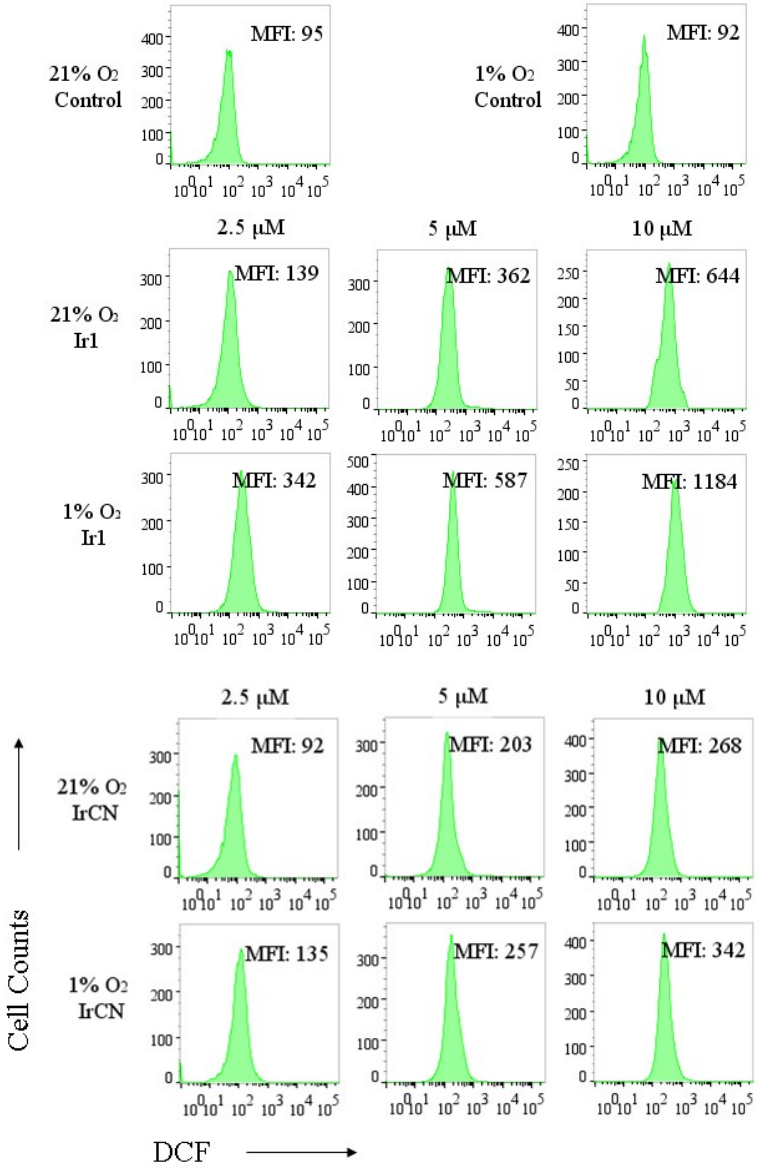

Fig. S15 Flow cytometric quantification of intracellular ROS levels in MCF-7 cells after treatment with **Ir1** and **IrCN** for 6 h at the indicated concentrations under normoxia (21 % O_2) and hypoxia (1% O_2), respectively.

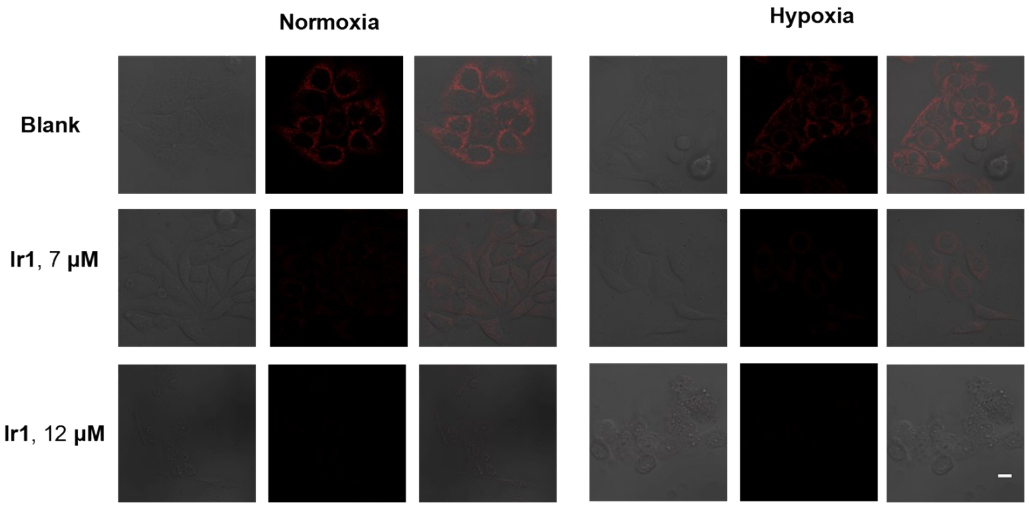

Fig. S16 TMRM assay of the mitochondrial permeability transition (MPT) of MCF-7 cells after treatment with **Ir1** for 6 h at the indicated concentrations under normoxia (21% O_2) and hypoxia (1% O_2), respectively. TMRM: excitation = 549 nm; emission = 573 ± 20 nm. Scale bar: 10 μm.

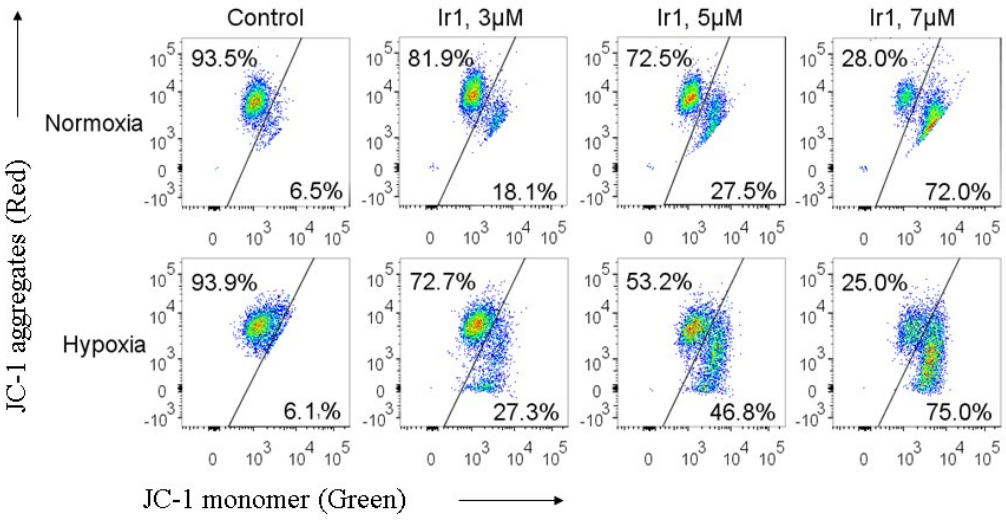

Fig. S17 Changes of mitochondrial membrane potentials (MMP, $\Delta\Psi_m$) of MCF-7 cells after treatment with **Ir1** for 6 h at the indicated concentrations under normoxia (21% O_2) and hypoxia (1% O_2), respectively. JC-1: excitation = 488 nm; emission = 515~545 nm (Green) and 575~605 nm (Red).

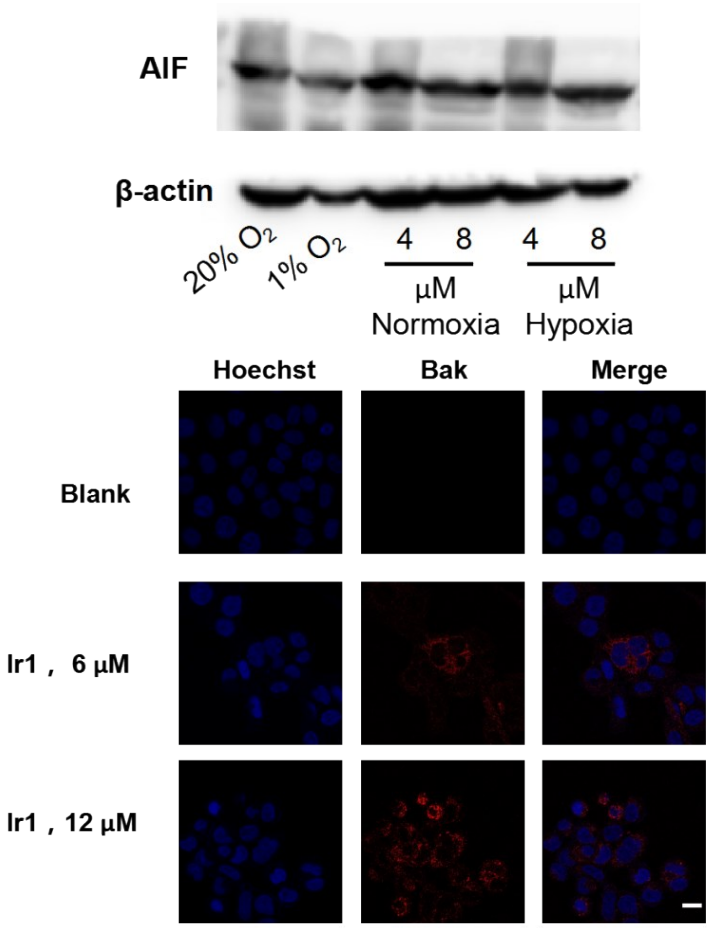

Fig. S18 (Top) Western blot of AIF expression level in hypoxic MCF-7 cells (1% O_2) after treatment with **Ir1** (4 and 8 μM) for 24 h; (Bottom) Immunofluorescence of Bak expression in hypoxic MCF-7 cells (1% O_2) after treatment with **Ir1** (6 and 12 μM) for 12 h; Hoechst: excitation = 350 nm; emission = 460 ± 20 nm; Bak: excitation = 488 nm; emission = 540 ± 20 nm. Scale bar: 20 μm.

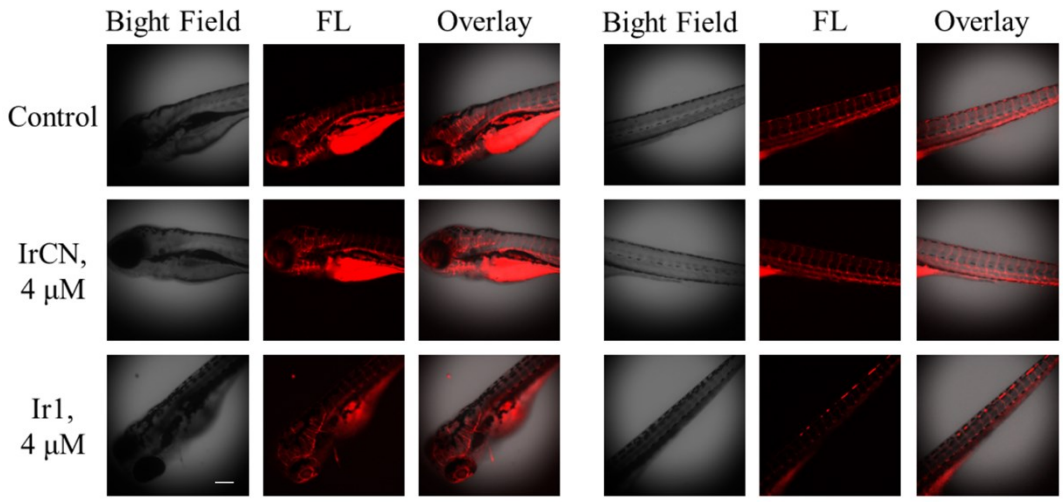

Fig. S19 Confocal images of zebrafish after incubation with Ir compound (5 μM) for 48 h. The mCherry fluorescence protein was excited at 488 nm and recorded at 650 ± 50 nm. Scale bar: 1 mm.

Table S1 Crystallographic data of complex **Ir1**

Compound	**Ir1**
CDCC N.O.	1976433
Empirical formula	C26 H23 F10 Ir N7 P [+ solvent]
Molecular weight	845.70
Description	Block, yellow
Temperature(K)	150
Crystal system	hexagonal
Space group	R-3
a/Å	42.2108(9)
b/Å	42.2108(9)
c/Å	9.0380(3)
a/deg	90
β/deg	90
γ/deg	120
V/Å3	13946.0(9)
Z	18
D_{calcd}/g·cm^{-3}	1.813
μ/mm^{-1}	4.450
θ_{max}(deg)	26.000
Data completeness	0.990
h, k, l_{max}	52,52,11
R1, wR2	0.0654(4688), 0.1640(6036)

Table S2 Bond lengths (Å) and bond angles (deg) of **Ir1**

Compound	**Ir1**	
Bond Length(Å)	Ir1-N1	2.022(6)
	Ir1-N2	2.022(5)
	Ir1-N6	2.132(5)
	Ir1-N3	2.113(5)
	Ir1-C5	1.990(6)
	Ir1-C16	2.001(5)
Bond Angles (deg)	C5-Ir1-C16	89.9(2)
	C5-Ir1-N1	81.6(3)
	C5-Ir1-N2	93.9(2)
	C5-Ir1-N6	176.8(2)
	C5-Ir1-N3	92.5(2)
	C16-Ir1-N1	95.4(2)
	C16-Ir1-N2	80.3(2)
	C16-Ir1-N6	93.4(2)
	C16-Ir1-N3	175.7(2)
	N1-Ir1-N2	173.8(2)
	N1-Ir1-N6	98.0(2)
	N1-Ir1-N3	88.4(2)
	N2-Ir1-N6	86.8(2)
	N2-Ir1-N3	96.0(2)
	N3-Ir1-N6	84.22(19)

Table S3 Photophysical data of complexes **Ir1**~**Ir3** in degassed medium

Compounds	Medium	$\lambda_{abs,\ max}$(nm)	$\lambda_{em,\ max}$ (nm)
Ir1	CH_2Cl_2	379	488
	CH_3CN	377	492
	PBS	376	500
Ir2	CH_2Cl_2	332	500
	CH_3CN	330	510
	PBS	331	527
Ir3	CH_2Cl_2	346	548
	CH_3CN	347	562
	PBS	345	579

Table S4 Measurement of Water-Octanol Partition Coefficient ($P_{o/w}$)

Compound	logPo/w
Ir1	1.35
Ir2	0.69
Ir3	1.22

Table S5 IC_{50} (μM) values of metformin towards different cell lines.[a]

Metformin IC_{50} (μM)				
Normoxia (21% O_2)	A549	HeLa	MCF-7	HLF
	> 200	> 200	> 200	> 200
Hypoxia (1% O_2)	A549	HeLa	MCF-7	/
	> 200	> 200	> 200	/

[a] Data are presented as the means ± standard deviations (SD) of three repeated measurements.