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Electronic supplementary information

Oncosis-inducing cyclometalated iridium(III) complexes

Ruilin Guan,[a] Yu Chen,[a] Leli Zeng,[a,b] Thomas W. Rees,[a] Chengzhi Jin,[a] Juanjuan Huang,[a] Zhe-Sheng Chen,[b] Liangnian Ji,[a] and Hui Chao*[,a,c]

[a.] *MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, School of Chemistry, Sun Yat-Sen University, Guangzhou, 510275, P. R. China.*

[b.] *College of Pharmacy and Health Sciences, St. John's University, New York, NY 11439, USA.*

[c.] *MOE Key Laboratory of Theoretical Organic Chemistry and Functional Molecule, School of Chemistry and Chemical Engineering, Hunan University of Science and Technology, Xiangtan, 400201, P. R. China.*

Experimental Section

Materials

All reagents were used as received, unless noted otherwise. Double distilled (DD) water was used throughout all of the experiments. $IrCl_3 \cdot xH_2O$, cisplatin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), PBS, 2-phenylpyridine (ppy), 2-(2,4-difluorophenyl)pyridine (DFppy), 2-phenylquinoline (2pq), 4,4'-Dimethyl-2,2'-bipyridyl (dmb), 2',7'-dichlorofluorescin diacetate (DCFH-DA), 3-methyladenine, cycloheximide, leupeptin, necrostatin-1 were obtained from Sigma-Aldrich. MitoTracker® Green FM were purchased from invitrogen. Nucleoprotein Extraction Kit, Cytoplasmic and Mitochondrial Protein Extraction Kit were bought from Life Technology. Mitochondria Membrane Potential (JC-1) Kit, Actin-Tracker Green, Tubulin-Tracker Red were purchased from Biotime Biotechnology (China). Caspase-3/7 activity kit and CellTiter-Glo® Luminescent Cell Viability kit were purchased from Promega (USA). Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin were purchased from Gibco. Stock solutions of cisplatin (3 mM) were prepared in saline, stored in the dark and used within 1 week. Stock solutions of Ir(III) complexes (1 mM) were prepared in DMSO. Anti-bcl2 antibody, beta-actin rabbit antibody were purchased from Abcam. Anti-bax antibody, anti-cytochroome C antibody were purchased from Cell Signaling Technology (CST). Tubulin-α polyclonal antibody was purchased from Bioworld Technology.

Microanalysis (C, H, and N) was carried out using a Perkin-Elmer 240Q elemental analyzer. Electrospray mass spectra (ES-MS) were recorded on an LCQ system (Finnigan MAT, USA). ^1H NMR and ^{19}F spectra were recorded on Varian INOVA500NB. UV-Vis spectra were recorded on a Perkin-Elmer Lambda 850 spectrophotometer. Emission spectra were recorded by a Perkin-Elmer LS 55 spectrofluorophoto-meter at room temperature. Instruments for Western blot experiments were from Bio-Rad. TEM Images are recorded on from Transmission Electron Microscope (JEOL JEM-1400, Japan). Confocal cell imaging was conducted on a LSM 710 (Carl Zeiss, Germany) Laser Scanning Confocal Microscope. Flow cytometry experiments were conducted on BD FACSCanto II.

Synthesis

2-phenylbenzo[d]thiazole (pbt) was synthesized according to literature,[1] and the cyclometalated iridium(III) chloro-bridged dimers were synthesized according to a procedure described in the literature[2] by refluxing $IrCl_3 \cdot xH_2O$ with cyclometalated ligands in 2-ethoxythanol and water (3:1) for 24 h, and collecting the precipitate. 4,4'-bis(benzothiazol-2-yl)-2,2'-bipyridine (bbtb) was synthesized from 4,4'-Dicarboxy-2,2'-bipyridyl according to the literature.[3]

Synthesis of Ir(III) complexes OnIr1-OnIr4:

The synthesis of the Ir(III) complexes was achieved from the reaction of cyclometalated chloride-bridged dimers and the corresponding ancillary ligands. In a general procedure, 0.12 mmol Ir(III) dimer and 0.1 mmol bbtb were placed in a 50 mL three-necked flask with 20 mL of methanol and chloroform (1:1, v/v) and refluxed for 6 h in 65°C under argon. Then the solvent was removed under reduced pressure, the crude product was purified by column chromatography on aluminum oxide with CH_2Cl_2/CH_3CH_2OH (10:1, v/v) as the eluent. The obtained complex was then recrystallized with a mixture of CH_2Cl_2/toluene to obtain the Ir(III) complexes.

[Ir(ppy)$_2$(bbtb)]Cl (OnIr1): yield 51%. Anal. Calc. for $C_{46}H_{30}IrN_6S_2$ (%): C, 59.85; H, 3.28; N, 9.10. Found (%):C, 59.68; H, 3.42; N, 8.96. ES-MS (CH_3OH) m/z: 923.5 $[M\text{-}Cl]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.57 (s, 2H), 8.36 (dd, J = 5.8, 1.6 Hz, 2H), 8.34 – 8.24 (m, 6H), 8.07 (d, J = 5.8 Hz, 2H), 7.95 (t, J = 7.5 Hz, 4H), 7.82 (d, J = 5.5 Hz, 2H), 7.67 (t, J = 7.7 Hz, 2H), 7.61 (t, J = 7.1 Hz, 2H), 7.17 (t, J = 6.7 Hz, 2H), 7.05 (t, J = 7.9 Hz, 2H), 6.94 (t, J = 7.5 Hz, 2H), 6.20 (d, J = 7.4 Hz, 2H).

[Ir(DFppy)$_2$(bbtb)]Cl (OnIr2): yield 45%. Anal. Calc. for $C_{46}H_{26}F_4IrN_6S_2$ (%): C, 55.52; H, 2.63; N, 8.45. Found (%): C, 55.38; H, 2.80; N, 8.24. ES-MS (CH_3OH): m/z 995.3 $[M\text{-}Cl^-]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.61 (s, 2H), 8.37 – 8.30 (m, 6H), 8.27 (d, J = 8.0 Hz, 2H), 8.12 (d, J = 5.8 Hz, 2H), 8.05 (t, J = 8.2 Hz, 2H), 7.90 (d, J = 5.3 Hz, 2H), 7.68 (t, J = 7.2 Hz, 2H), 7.62 (t, J = 7.2 Hz, 2H), 7.25 (t, J = 6.6 Hz, 2H), 7.02 (t, J = 11.1 Hz, 2H), 5.63 (d, J = 6.1 Hz, 2H). ^{19}F NMR (470 MHz, DMSO) δ -106.35 (dd, *J* = 18.7, 9.6 Hz), -108.52 (t, *J* = 11.6 Hz).

[Ir(2pq)$_2$(bbtb)]Cl (OnIr3): yield 52%. Anal. Calc. for $C_{54}H_{34}IrN_6S_2$ (%): C, 63.39; H, 3.35; N, 8.21. Found (%): C, 63.26; H, 3.52; N, 8.06. ES-MS (CH_3OH): m/z 1023.1 $[M\text{-}Cl^-]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.18 (s, 2H), 8.61 (d, J = 8.9 Hz, 2H), 8.56 (d, J = 8.9 Hz, 2H), 8.37 – 8.29 (m, 6H), 8.27 (d, J = 7.9 Hz, 2H), 8.18 (d, J = 8.3 Hz, 2H), 7.92 (d, J = 7.6 Hz, 2H), 7.64 (t, J = 7.7 Hz, 2H), 7.58 (t, J = 7.5 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.32 (d, J = 8.9 Hz, 2H), 7.19 (dd, J = 11.8, 7.7 Hz, 4H), 6.85 (t, J = 7.5 Hz, 2H), 6.40 (d, J = 7.7 Hz, 2H).

[Ir(pbt)$_2$(bbtb)]Cl (OnIr4): yield 47%. Anal. Calc. for $C_{50}H_{30}IrN_6S_4$ (%): C, 58.01; H, 2.92; N, 8.12. Found (%): C, 57.87; H, 3.06; N, 8.03. ES-MS (CH_3OH): m/z 1035.0 $[M\text{-}Cl^-]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.55 (s, 2H), 8.44 (d, J = 4.2 Hz, 2H), 8.31 (d, J = 7.8 Hz, 2H), 8.23 (d, J = 7.3 Hz, 6H), 8.05 (d, J = 8.1 Hz, 2H), 7.66 (t, J = 7.1 Hz, 2H), 7.60 (t, J = 7.6 Hz, 2H), 7.40 (t, J = 7.7 Hz, 2H), 7.23 (t, J = 8.1 Hz, 2H), 7.16 (t, J = 7.9 Hz, 2H), 6.96 (t, J = 7.6 Hz, 2H), 6.35 (d, J = 8.5 Hz, 2H), 6.30 (d, J = 7.5 Hz, 2H).

Crystal structure analysis

Single crystals of **OnIr3** were obtained. The crystal structure was obtained on a Rigaku R-AXIS SPIDER IP diffractometer with graphite monochromated Mo Ka radiation (λ = 0.71073 Å). An absorption correction was applied with the SADABS program. The structure solution and full-matrix least-squares refinement, based on F^2 , was performed with the Shelxtl-2014 and OLEX2 program packages.[4, 5] The disordered solvent molecules were removed with the SQUEEZE S7 routine in the PLATON-2015 software.[6] All non-hydrogen atoms were refined anisotropically. CCDC 1578910 contain the supplementary crystallographic data for the present paper; these data can be obtained at http://www.ccdc.cam.ac.uk/conts/retrieving.html.

Cell culture

Cells were maintained in DMEM (high glucose, Gibco) media supplemented with fetal bovine serum (10%), penicillin (100 units/mL) and streptomycin (50 units/mL) at 37 °C in a CO_2 incubator (5% CO_2).

Cellular localization assay

A549R cells were incubated with **OnIr1-OnIr4** (2 μM) at 37 °C for 8 h and then co-incubated with MitoTracker® Green FM (150 nM,) or at 37 °C for 0.5 h, then washed by PBS 3 times and visualized by laser confocal microscopy with a 63× oil-immersion objective lens immediately. The excitation wavelengths for

OnIr1-OnIr4 were 405 nm, respectively, while the excitation wavelength of MTG is 488 nm. Emission filter: (peak wavelength of **OnIr1-OnIr4**) ± 20 nm respectively, and 520 ± 20 nm for MTG.

For ICP-MS study, exponentially growing HeLa cells were treated with **OnIr1-OnIr4** (2 μM) at 37 °C for 8 h, respectively. Upon completion of the incubation the cells were trypsinized, collected and counted. Each group of the cells were divided into two equal parts and processed with Nucleus Extraction Kit and Cytoplasmic and Mitochondrial Protein Extraction Kit by the manufacturer's protocols, respectively. The extractions were subsequently digested by 60% HNO_3 for over two days, and diluted by water to obtain 2% HNO_3 sample solutions for final determination. The Ir content was determined by standard curve method, and the content in nuclei, mitochondria and cytosol was calculated associated with cell numbers.

The mechanism of cellular uptake

A549R cells were stained with Ir(III) complexes under different conditions by varying the temperature (4°C and 37°C) as well as the addition of metabolic inhibitors (co-stain of 2-deoxy-Dglucose and oligomycin) and endocytic inhibitors (chloroquine and NH_4Cl)). The cells were observed under confocal microscope to determine the cellular uptake by their luminescence[7, 8]. Marked decrease in cellular uptake under low temperature, metabolic and endocytic inhibitors suggested that the compounds were taken up in an energy-dependent endocytic way.

Stability in FBS and culture media

An diazepam (Sigma-Aldrich) solution was used as internal reference. For this experiment, **OnIr1-OnIr4** (10 μM, stock solution in ethanol) and 10 μM diazepam were added to the fetal bovine serum (FBS, 980 μL) to a total volume of 1000 μL. The solution was incubated for 0 h or 48 h at 37 °C with shaking (~300 rpm) respectively. 2 mL of acetonitrile was added, and the mixture was centrifuged for 45 min at 1000g at 4 °C. The supernate was evaporated, and the residue was suspended in 200 μL of ethanol. The suspension was filtered and analyzed by HPLC−UV. A C18 reverse phase column was used with a flow rate of 0.5 mL/min. The runs were performed with a linear gradient of A (ethanol, Sigma-Aldrich HPLC grade) in B (distilled water). **OnIr1-OnIr4** (10μM, from stock solution 1 mM in DMSO) were dissolved in DMEM (10% FBS) and incubated in 37°C for 0 h or 48 h, respectively. The absorption spectra of these mixtures was tested.

Caspase-3/7 activation detection assay

A549R cells were seeded in white-walled solid-bottomed 96-well plates at a density of 1.5×10^4 cells/well overnight to adhere. The cells were then treated with **OnIr1-OnIr4** and cisplatin in various concentrations for 24 h, respectively. Three sets of treatments were performed: 1) **OnIr1** and **OnIr2** were incubated in a concentration of 0.5 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 1 μM. Cisplatin was incubated in a concentration of 50 μM; 2) **OnIr1** and **OnIr2** were incubated in a concentration of 1 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 2 μM. Cisplatin was incubated in a concentration of 100 μM; 3) **OnIr1** and **OnIr2** were incubated in a concentration of 2 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 3 μM. Cisplatin was incubated in a concentration of 150 μM; Incubation time was 24 h. Subsequently, the cells were treated with Caspase-3/7 activity kit according to the manufacturer's protocol and the luminescence in RLUs was quantified by an Infinite M200 PRO (TECAN, Swiss).

Cell viability assay and IC50 value determination

The cell viability assay was performed by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) method. Cells were incubated with Ir(III) complexes in 96-well plates in the condition needed, along with the caspase inhibitors (Ac-DEVD-CHO and Z-VAD-fmk) for 24 h, then incubated with MTT for 4 h. The liquid was disposed of and 200 μL DMSO was added, the plate was shaken for 5 min and the absorbance intensity was determined by microplate reader (TecanInfiniteF200, Bio-rad). Similarly, IC_{50} values were calculated by cell viabilities under different incubation concentrations for 48 h without inhibitors.

Mitochondrial membrane potential (MMP) assay

MMP was assessed by JC-1 staining. A549R cells incubated with Ir(III) complexes (**OnIr1** and **OnIr2** in a concentration of 1 μM; **OnIr3** and **OnIr4** in a concentration of 2 μM) for various time (12 h and 24 h) were trypsinized and collected, then stained with JC-1 following the manufacture‘s protocol. The results were obtained by flow cytometry (BD FACS CantoII).

ROS generation assay

ROS generation assay was assessed by DCFH-DA staining. A549R cells were incubated with Ir(III) complexes (**OnIr1** and **OnIr2** in a concentration of 1 μM; **OnIr3** and **OnIr4** in a concentration of 2 μM) for various times (12 h and 24 h) were trypsinized and collected, then stained with DCFH-DA following the manufacturer‘s protocol. The results were obtained by flow cytometry (BD FACS CantoII).

Cell volume assay

A549R cells incubated with Ir(III) complexes (**OnIr1** and **OnIr2** in a concentration of 1 μM. **OnIr3** and **OnIr4** in a concentration of 2 μM) for various times (12 h and 24 h), trypsinized and collected, then directly measured by flow cytometry (BD FACS CantoII) in FCS-H channel.

LDH Leakage assay

A549R cells incubated with **OnIr1-OnIr4** in black-walled glass-bottomed 96-well plates. Three sets of treatments were performed: 1) **OnIr1** and **OnIr2** were incubated in a concentration of 0.5 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 1 μM. Incubation time was 24h; 2) **OnIr1** and **OnIr2** were incubated in a concentration of 1 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 2 μM. Incubation time was 24 h; 3) **OnIr1** and **OnIr2** were incubated in a concentration of 2 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 3 μM. Incubation time was 24 h. Subsequently, the cells were treated with CytoTox-ONE™ Homogeneous Membrane Integrity Assay Technical Bulletin (Promega) according to the manufacturer's protocol and the fluorescence in RLUs was quantified by an Infinite M200 PRO (TECAN, Swiss).

ATP depletion assay

A549R cells were seeded on white-walled non-trasparent bottomed 96-well plates at a density of 1.5×10^4 cells/well overnight to adhere. Three sets of treatments were performed: 1) **OnIr1** and **OnIr2** were incubated in a concentration of 0.5 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 1 μM. Incubation time was 24 h; 2) **OnIr1** and **OnIr2** were incubated in a concentration of 1μM. **OnIr3** and **OnIr4** were incubated in a

concentration of 2 μM. Incubation time was 24 h; 3) **OnIr1** and **OnIr2** were incubated in a concentration of 2 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 3 μM. Incubation time was 24 h. Upon completion, to each well was added an equal volume of CellTitler-Glo® Luminescent Cell 10 Viability kit (Promega), the plate was vigourously vibrated and then manufacturer's protocol was followed. The chemoluminescence was measured by a TECAN Infinite M200 PRO multifunctional reader. Luminescence intensity in control wells was set to be 100%. Relative ATP contents of other groups were presented by their luminescence intensity ratios to control group and calculated with the cell viability in each incubation condition.

Transmission Electron Microscope (TEM) observation

TEM expirements were performed according to the literature description with slight alterations.[9]

A549R cells were treated with **OnIr1** (1 μM), **OnIr2** (1 μM), **OnIr3** (2 μM), **OnIr4** (2 μM) and fixed overnight at 4 ºC in phosphate buffer (pH 7.4) containing 2.5% glutaraldehyde, respectively. The cells were then treated with osmium tetroxide, stained with uranyl acetate and lead citrate, and visualized with a transmission electron microscope (JEOL JEM-1400, Tokyo, Japan). Images were photographed and processed by Eversmart Jazz program (Scitex).

Western blotting

Western blotting was performed according to the literature description with slight changes.[9] GAPDH (36 kDa), VDAC1 (32 kDa), Cytochrome C (14 kDa), bcl-2 (25 kDa), bax (20 kDa), α-tubulin (52 kDa), β-actin (46 kDa), RIP3 (57kDa), LC3 (18kDa/16kDa), ERK/p-ERK (44kDa/42kDa), caspase 3 (32kDa),caspase 7 (36kDa), calpain 1 (80kDa/76kDa) and porimin (110kDa) were detected.

Cell lysate preparation

To determine the expression level of indicated proteins, Ir(III) complex treated cell lysates were prepared as described:

For cytochrome C:

A549R cells were incubated with **OnIr1** (2 μM), **OnIr2** (2 μM), **OnIr3** (3 μM), **OnIr4** (3 μM) or cisplatin (150 μM) for 24 h. Subsequently, the cells were collected and separated into two portions (cytosol and mitochondria) following the manufacturer's protocol of Cytoplasmic and Mitochondrial Protein Extraction Kit. SDS-PAGE Sample Loading Buffer (Biotime Biotechnology) was added and the samples were heated at 100 °C for 5 min, then stored at -20 °C.

For other proteins:

Two sets of treatments were performed: A549R cells were incubated with 1) 2 μM **OnIr1** for 12 h, 24 h, 36 h, 48 h, respectively; 2) **OnIr1** (2 μM), **OnIr2** (2 μM), **OnIr3** (3 μM), **OnIr4** (3 μM), for 48 h. Subsequently, the cells were collected and treated with RIPA-Lysis Buffer (Biotime Biotechnology) for 30 min on ice. The lysate was centrifuged (×20000g) and the precipitate was disposed of. SDS-PAGE Sample Loading Buffer (Biotime Biotechnology) was added to the lysate and the samples were heated at 100 °C for 5 min, then store at -20 °C.

Other procedure

20 μg of proteins were analyzed by Western blot (12% acrylamide gel for cytochrome C, 10% acrylamide gel for other proteins). The PVDF membranes (0.2 μm for cytochrome C, 0.45μm for other proteins，Millipore) was used to transfer proteins (250 mA, ~30 min). The PVDF membrane were blocked with skim milk (5% in

TBST) and then incubated with primary antibodies at 4 °C overnight and HRP-conjugated secondary antibodies at room temperature for 1.5 h. The signal was developed by enhanced chemiluminescence (ECL) reagent (Thermo Fisher Scientific) and visualized by X-ray films (FUJI, Japan).

Cytoskeleton observation on CLSM

Two sets of treatments are performed: A549R cells were incubated with 1) **OnIr1** in 0.5 μM, 1 μM and 2 μM for 24 h; 2) **OnIr1** (2 μM), **OnIr2** (2 μM), **OnIr3** (3 μM), **OnIr4** (3 μM), for 24 h. Subsequently, the cells were fixed and treated with Actin-Tracker Green and Tubulin-Tracker Red, respectively, following the manufacture's protocol. Briefly, the treated cells were fixed by methanol and washed with Triton X-100 solution. BSA solution containing Triton X-100 and Actin-Tracker Green or Tubulin-Tracker Red was added and incubated in the dark. The fixed cells were washed with Triton X-100 solution and observed on CLSM.

Cell morphology observation

Two sets of treatments were performed: A549R cells were incubated with 1) **OnIr1** (1 μM), **OnIr2** (1 μM), **OnIr3** (2 μM), **OnIr4** (2 μM) for 24 h; 2) **OnIr1** (2 μM), **OnIr2** (2 μM), **OnIr3** (3 μM), **OnIr4** (3 μM), for 24 h. Subsequently, the cells were photographed by CLSM and vacuolization in cytosol and blebbing on plasma membrane were observed.

Supporting Figures and Tables

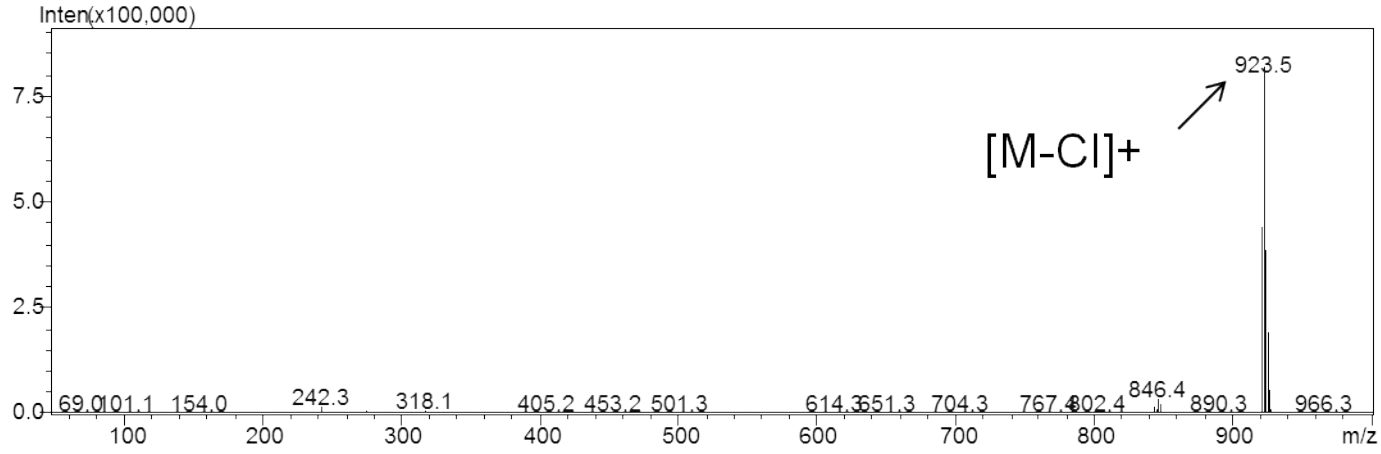


Figure S1. ES-MS spectrum (CH_3OH) of **OnIr1**.

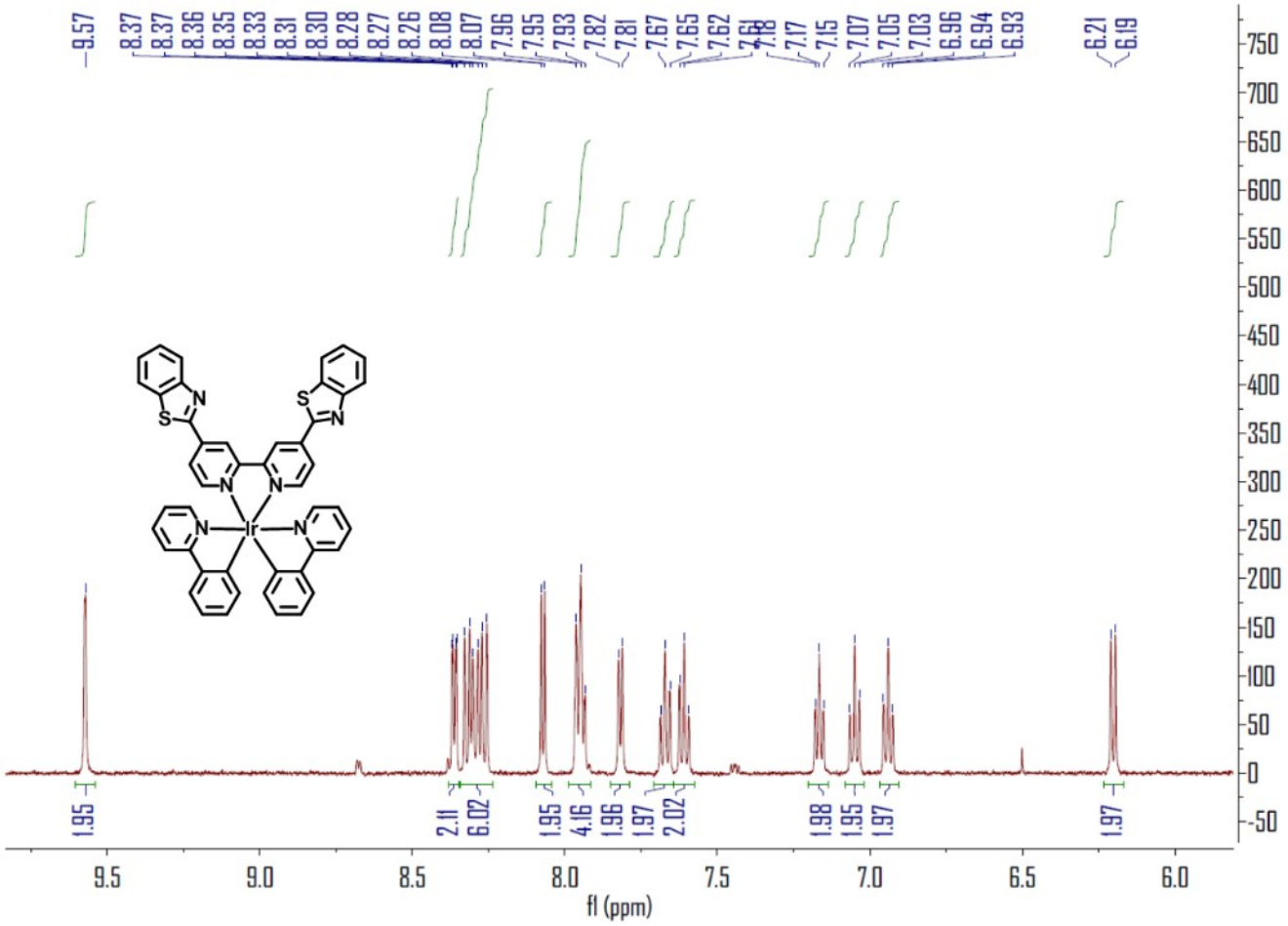


Figure S2. ^1H NMR spectrum (500 MHz, DMSO-d_6) of **OnIr1**.

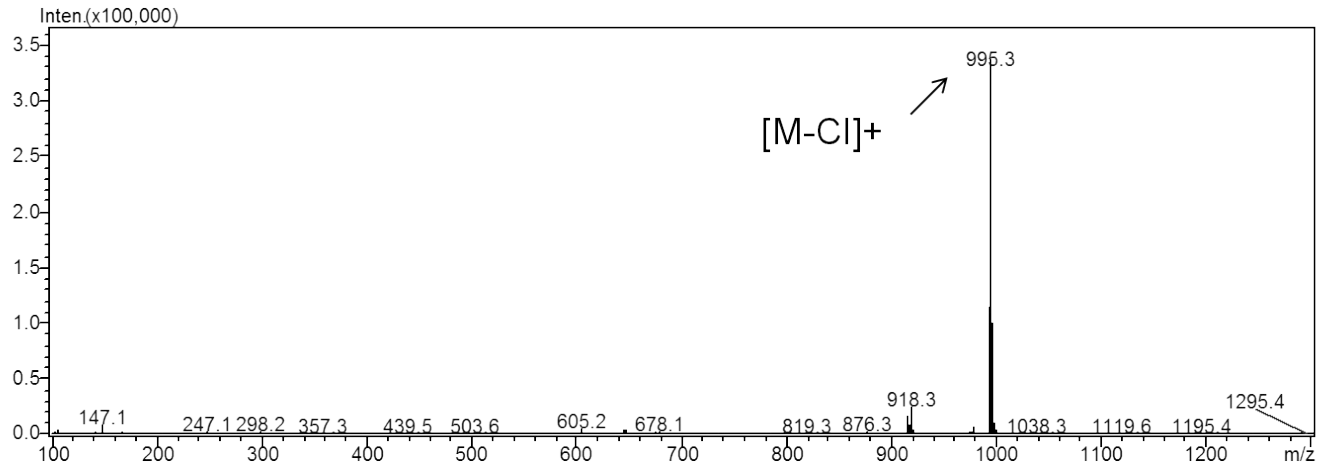


Figure S3. ES-MS spectrum (CH_3OH) of **OnIr2**.

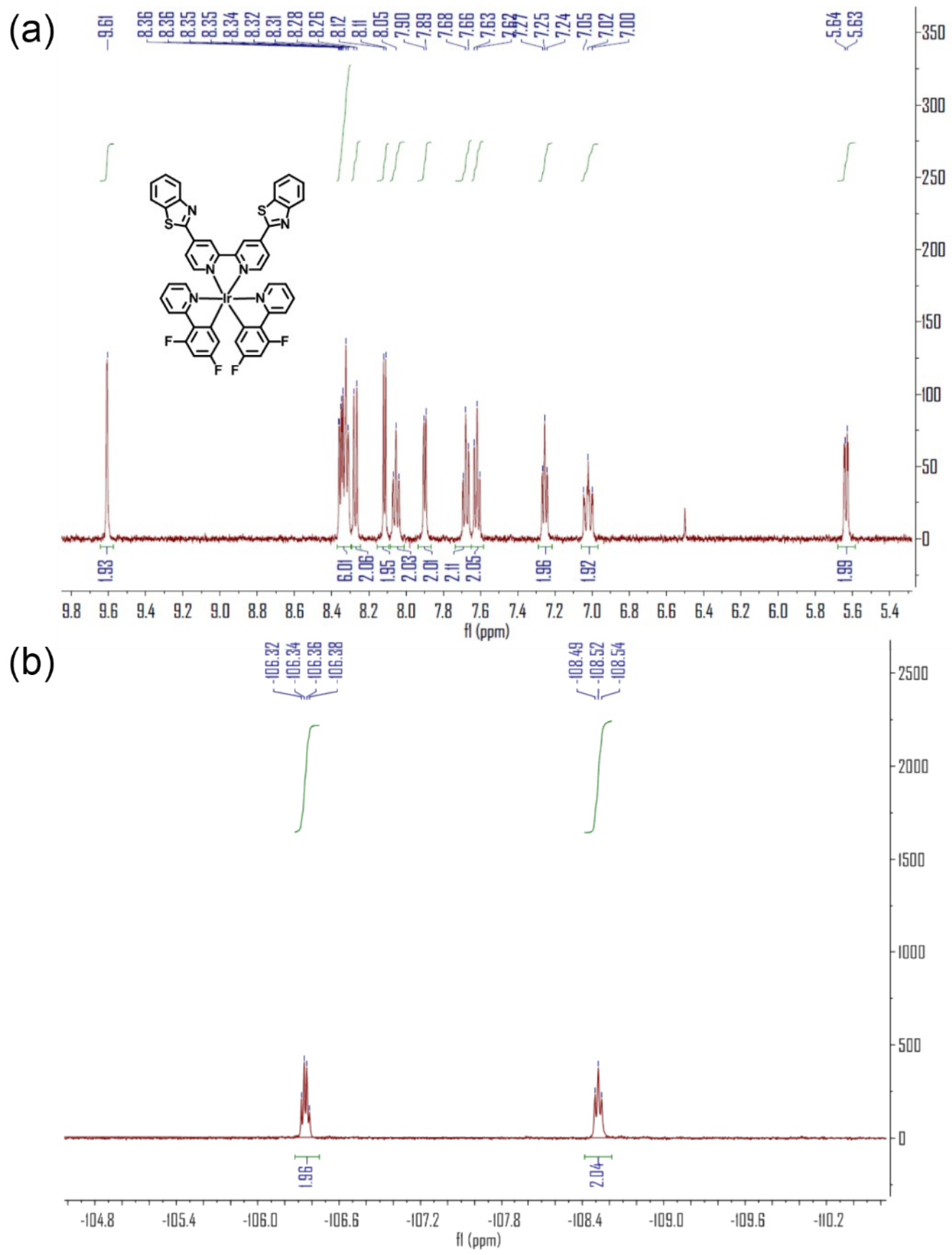


Figure S4. (a) ^{1}H NMR spectrum (500 MHz, DMSO-d_6) of **OnIr2**; (b) ^{19}F NMR spectrum (470 MHz, DMSO-d_6) of **OnIr2**.

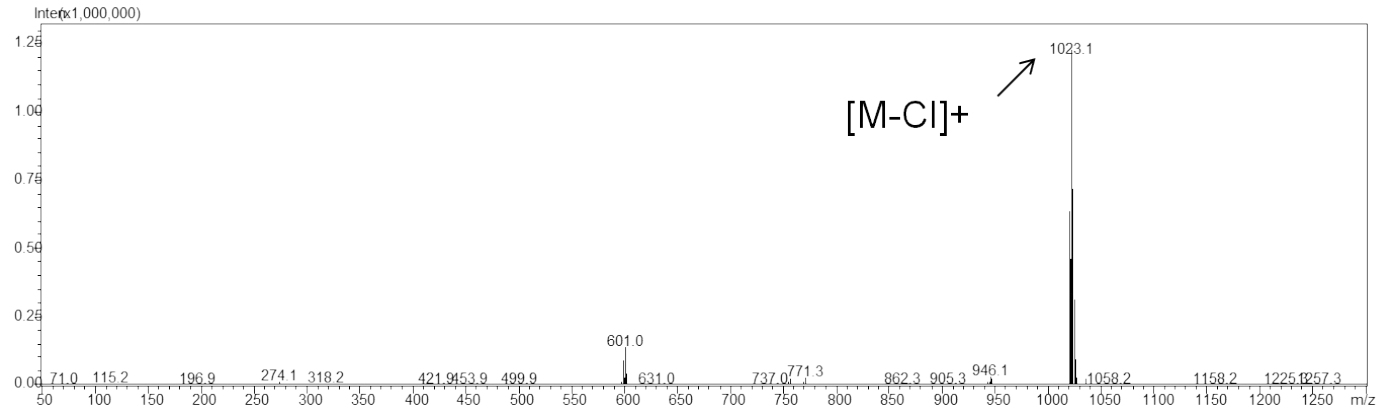


Figure S5. ES-MS spectrum (CH_3OH) of **OnIr3**.

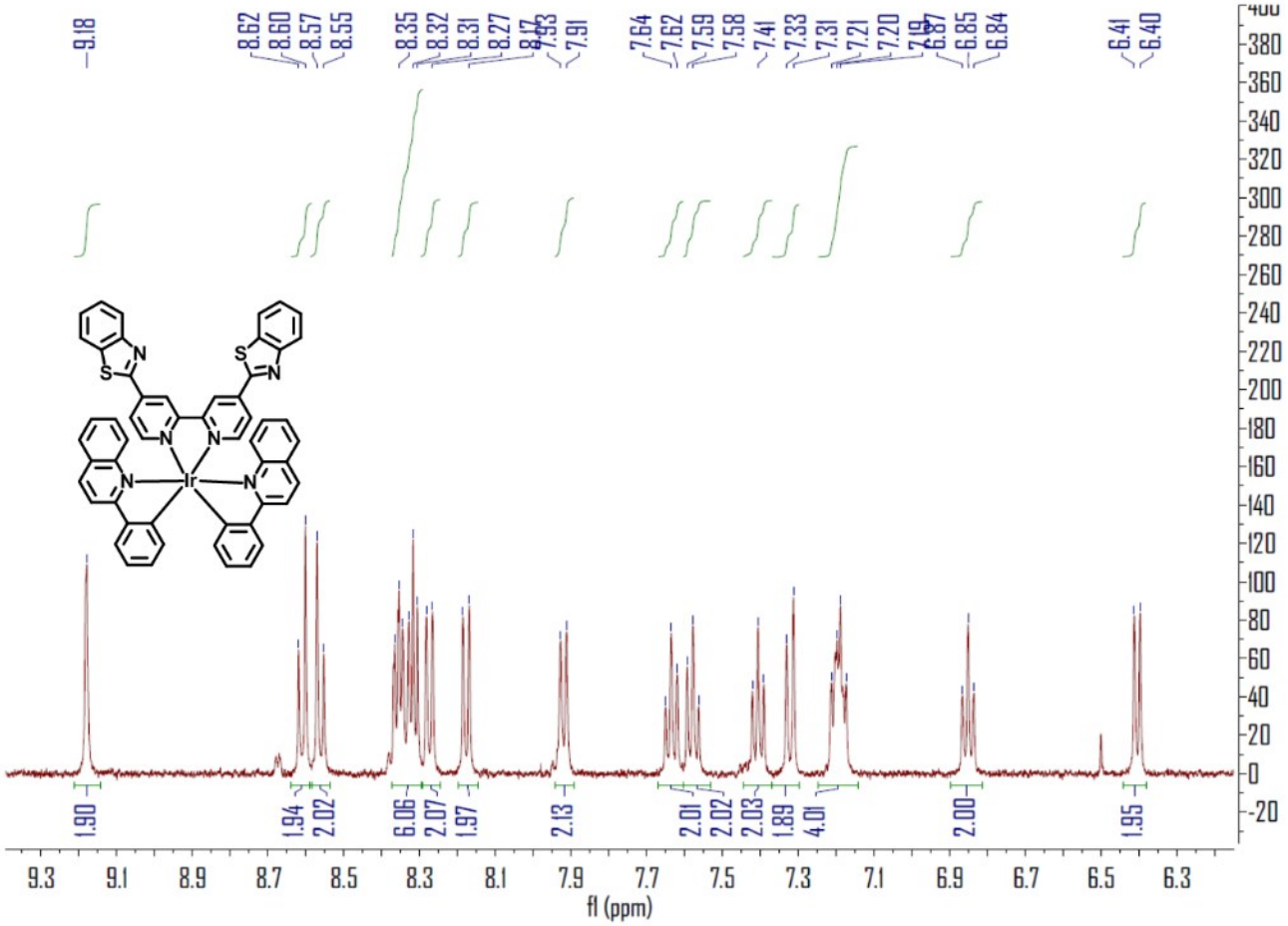


Figure S6. ^1H NMR spectrum (500 MHz, DMSO-d_6) of **OnIr3**.

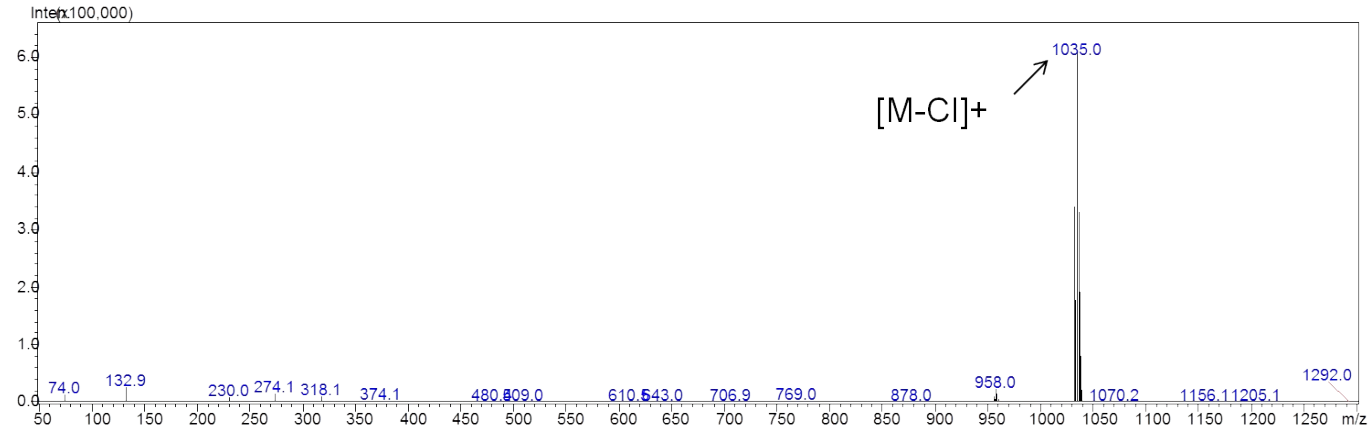


Figure S7. ES-MS spectrum (CH_3OH) of **OnIr4**.

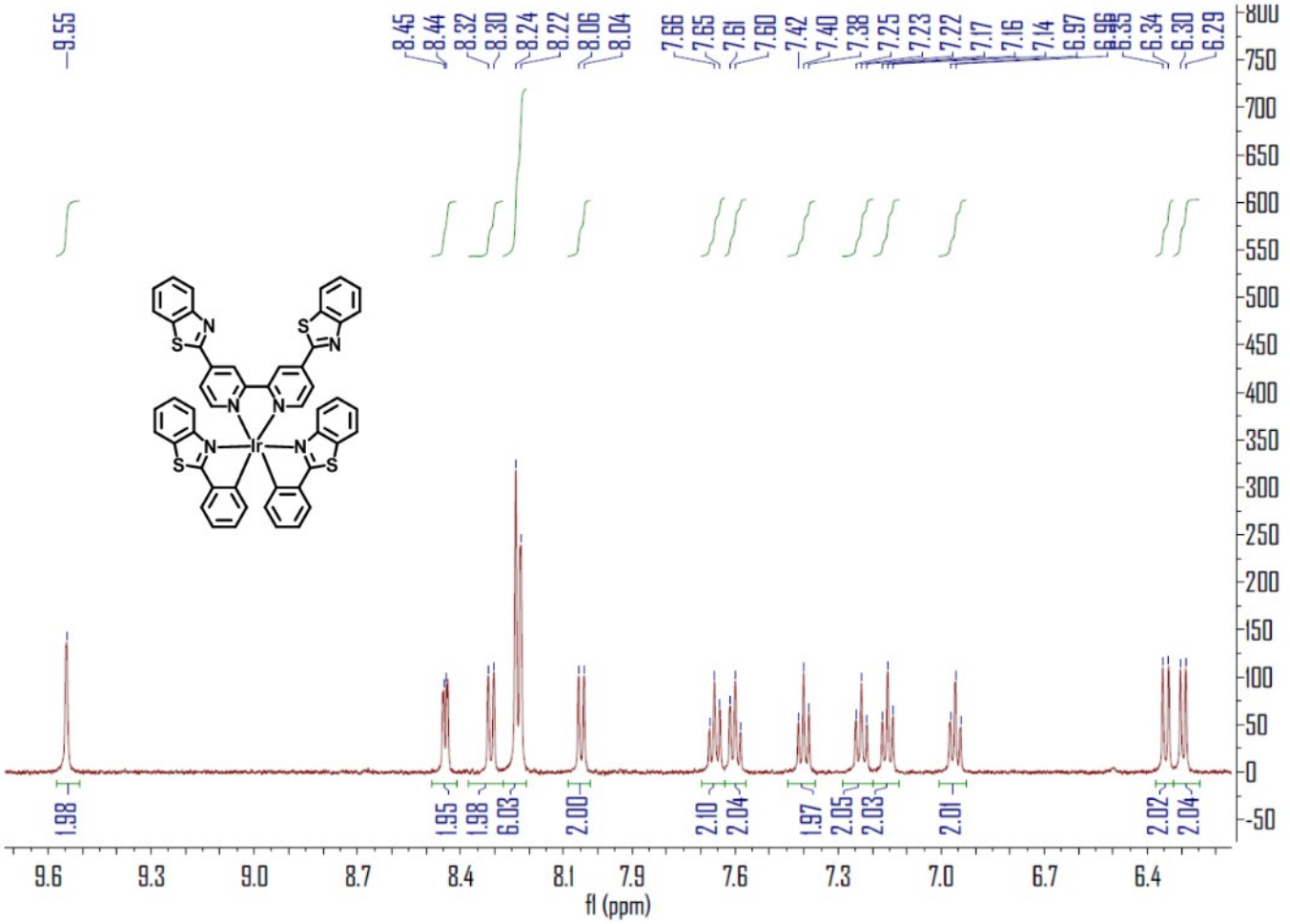


Figure S8. ^1H NMR spectrum (500 MHz, DMSO-d_6) of **OnIr4**.

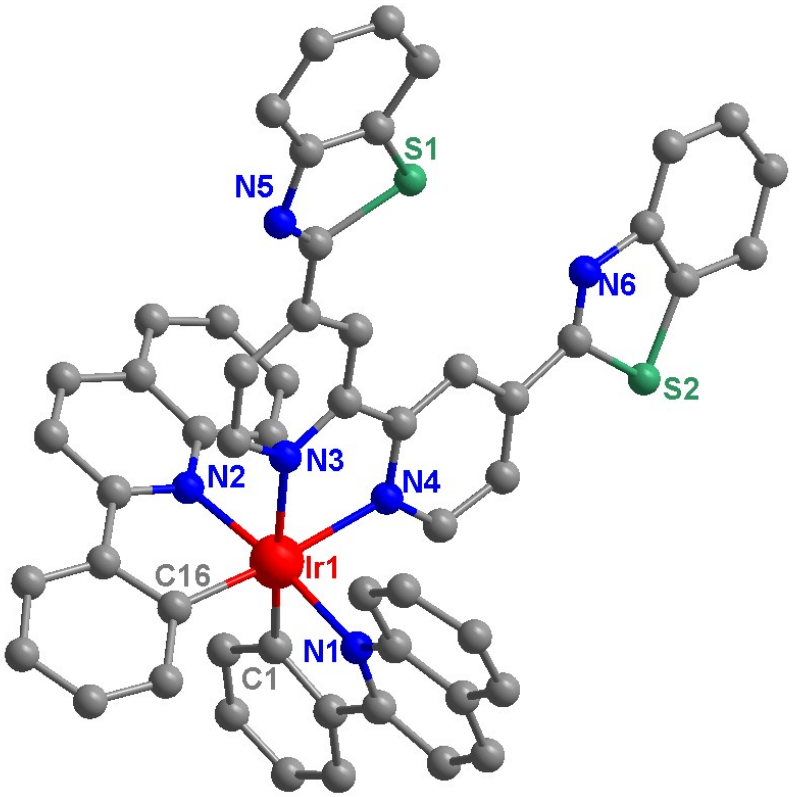
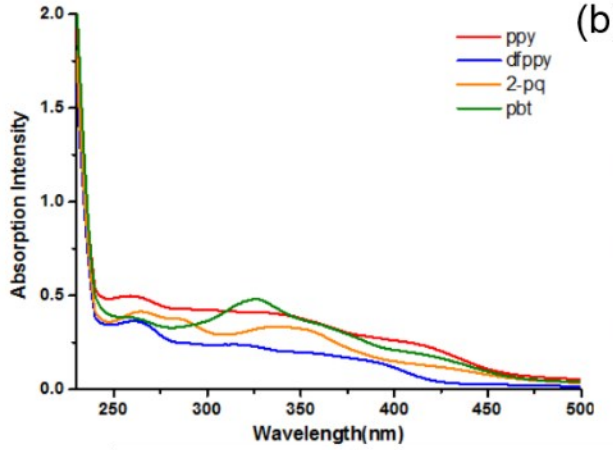
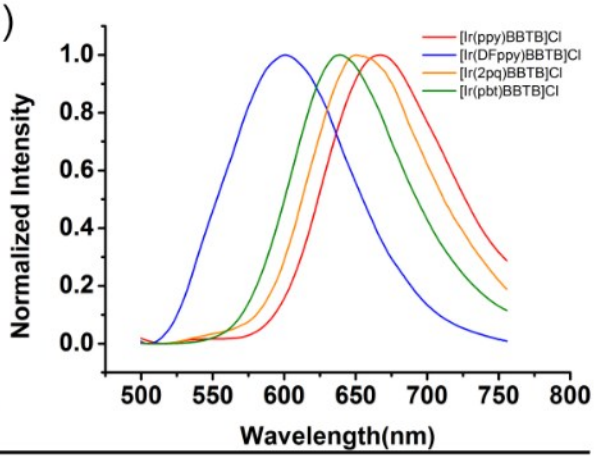


Figure S9. Crystal structure of **OnIr3** (The H atoms, counter anion and solvent have been omitted for clarity).

(a)



(b)



(c)

Compound	λ_{abs}/nm	λ_{em}/nm	Φ_u
OnIr1	258, 289, 380	671	3.9 %
OnIr2	259, 379	605	11.3%
OnIr3	265, 286, 380	657	7.5%
OnIr4	256, 281, 379	641	16.6%

Figure S10. (a) UV/Vis and (b) luminescent spectra of Ir(III) complexes. (c) Table of photophysical data of Ir(III) complexes. All results were obtained in PBS buffer in a concentration of 10 μM.

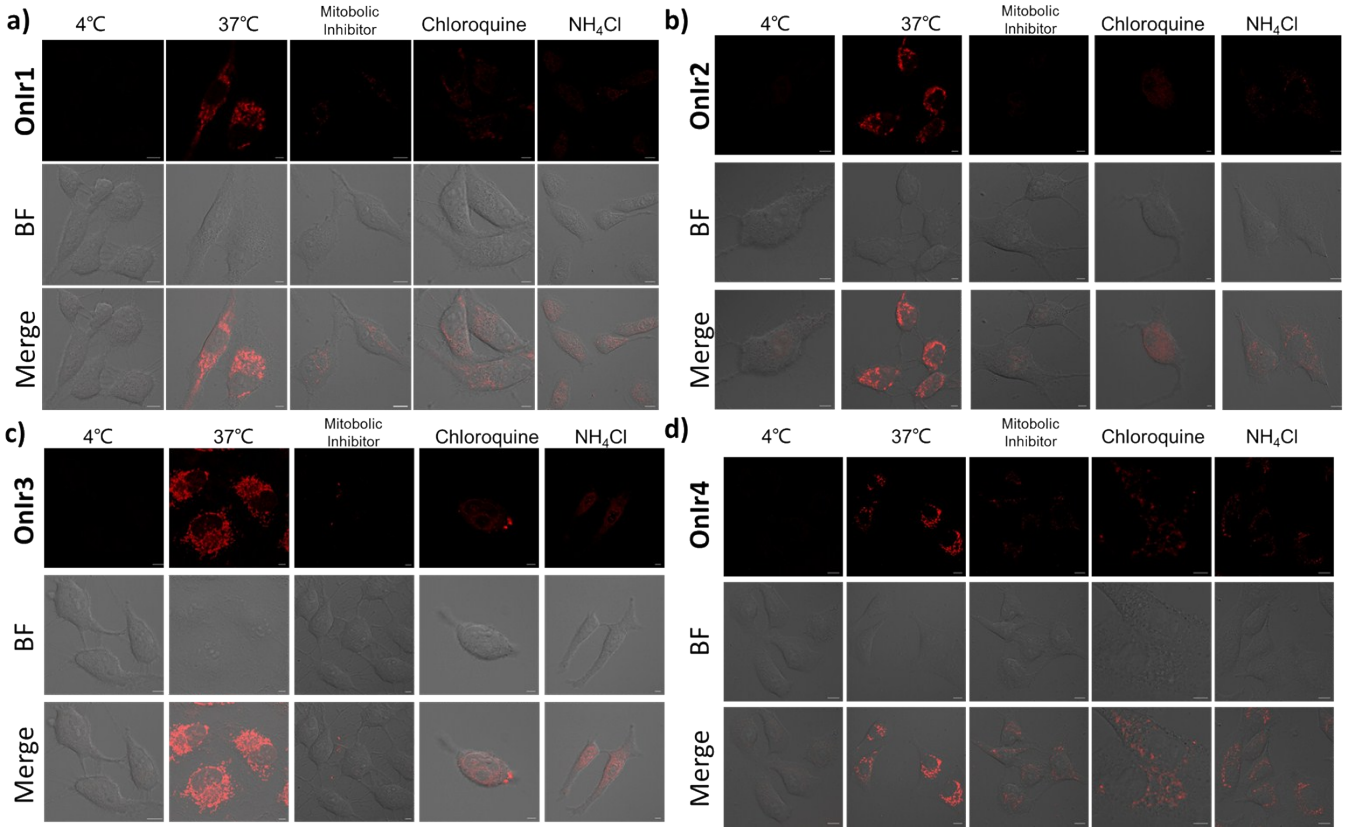


Figure S11. The Cellular uptake mechanisms of **OnIr1**-**OnIr4**. Scale bar: 5 μm.

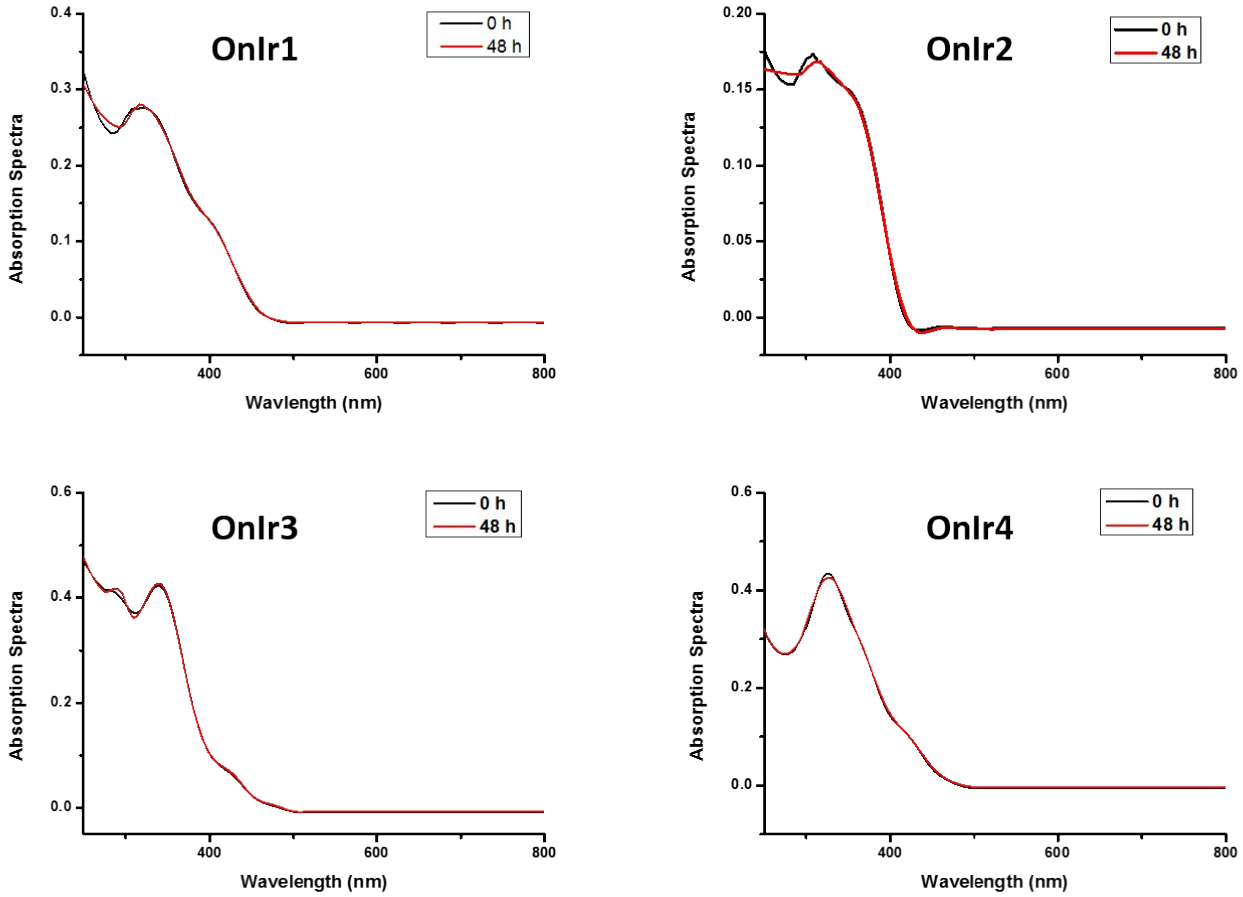


Figure S12. Absorption spectra of **OnIr1-OnIr4** (10 μM) incubated in DMEM (10% FBS) for 0 h or 48 h.

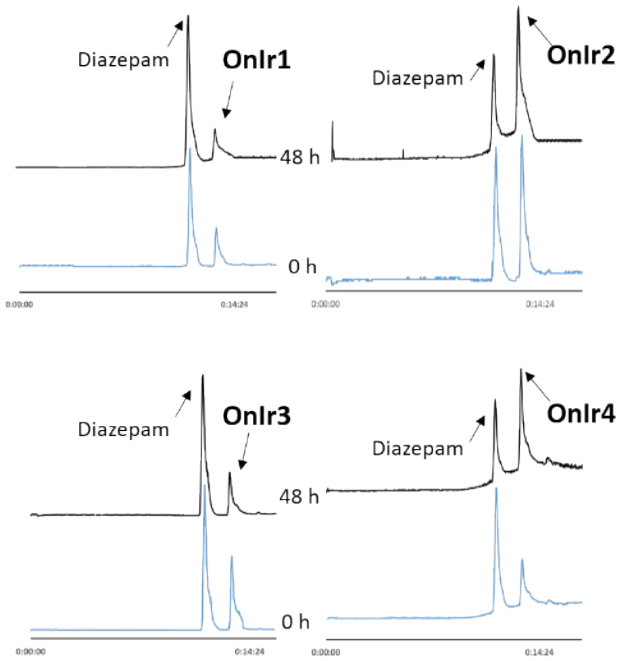


Figure S13. HPLC analysis of **OnIr1**-**OnIr4** incubated in FBS for 0 h or 48 h.

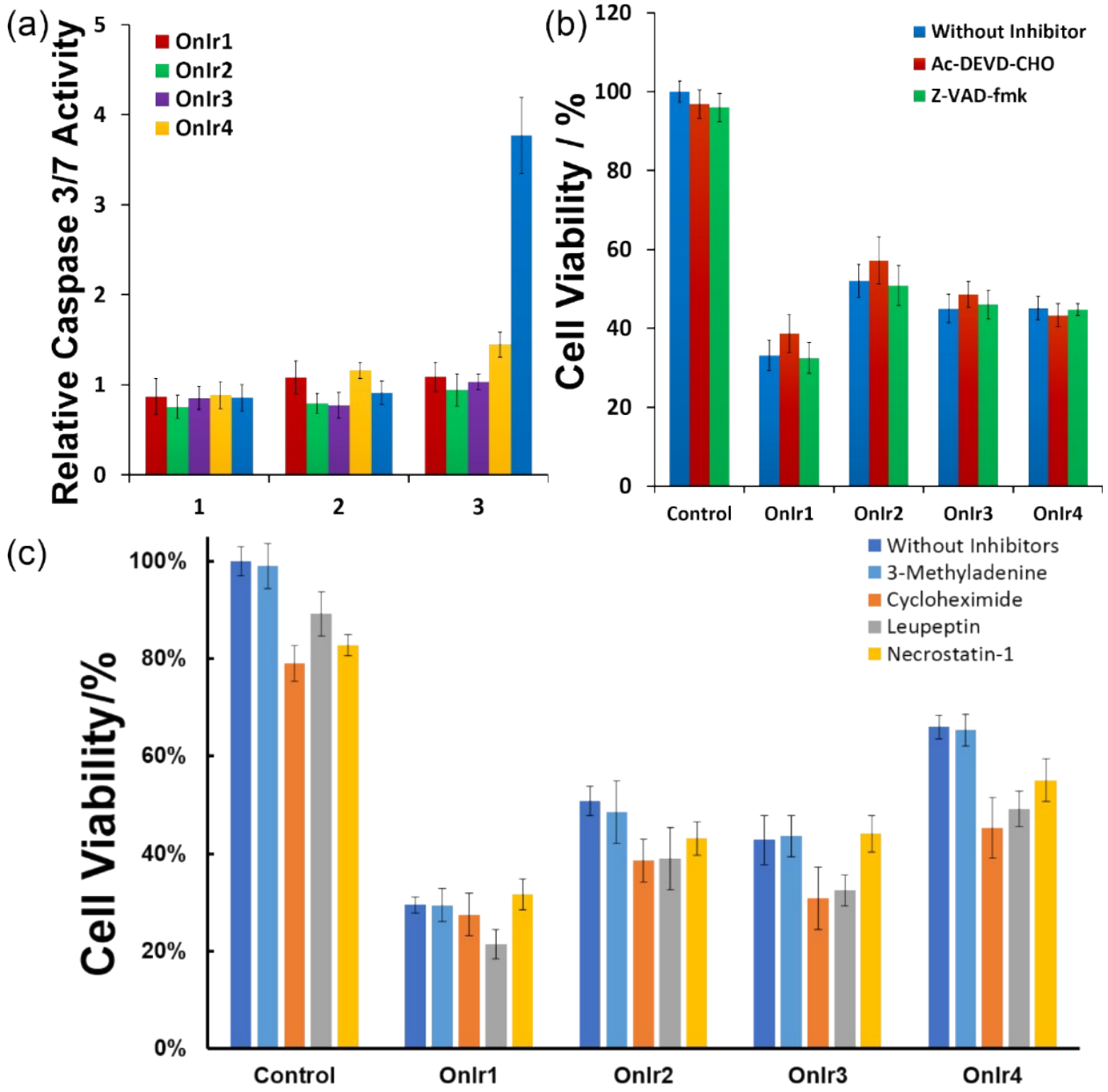


Figure S14. Caspase activation study. (a) Caspase 3/7 activity study. 1: **OnIr1** and **OnIr2** were incubated in a concentration of 0.5 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 1 μM. Cisplatin was incubated in a concentration of 50 μM; 2: **OnIr1** and **OnIr2** were incubated in a concentration of 1 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 2 μM. Cisplatin was incubated in a concentration of 100 μM; 3: **OnIr1** and **OnIr2** were incubated in a concentration of 2 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 3 μM. Cisplatin was incubated in a concentration of 150 μM; Incubation time was 24 h. (b and c) Cell viabilities of A549R with the co-incubation of various inhibitors and Ir(III) complexes. Ac-DEVD-CHO (10 μM) and Z-VAD-fmk (10 μM), 3-Methyladenine (100 μM), Cycloheximide (0.1 μM), Leupeptin (100 μM), Necrostatin-1 (60 μM) were pre-incubated for 30 min. **OnIr1** and **OnIr2** were then incubated in a concentration of 1 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 2 μM. Incubation time was 24 h.

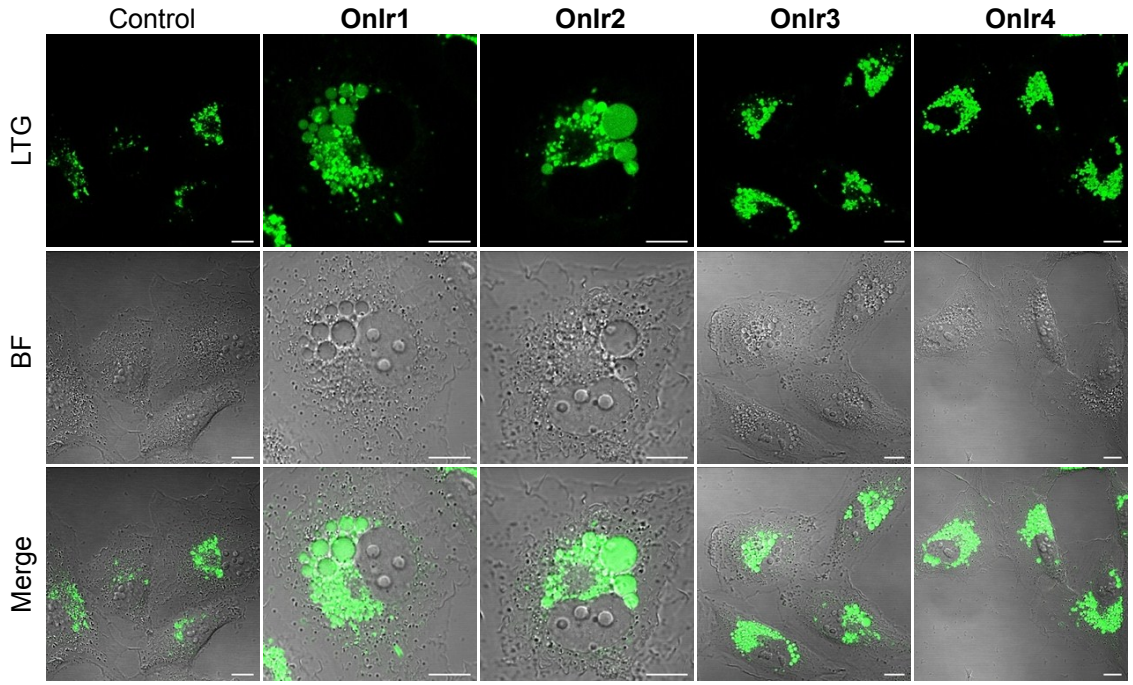


Figure S15. LysoTracker® Green (LTG) staining with cytoplasma vacuolization. **OnIr1** and **OnIr2** were incubated in a concentration of 1 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 2 μM. Incubation time was 24 h. The vacuoles were stained by LTG, suggesting a strong link between lysosomes and the vacuoles. Scale bar: 10 μm

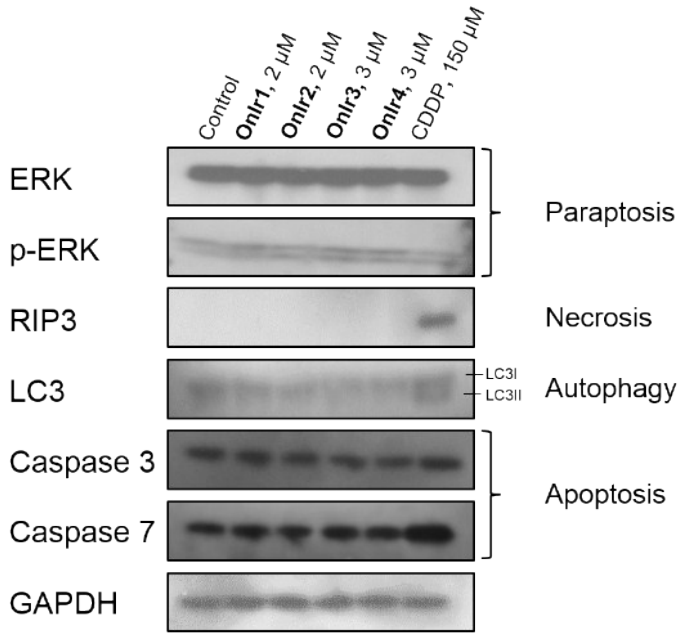


Figure S16. Expression and activation of ERK/p-ERK, RIP3, LC3, caspase 3 and caspase 7. A549R cells were treated with **OnIr1**-**OnIr4** and CDDP for 48 h.

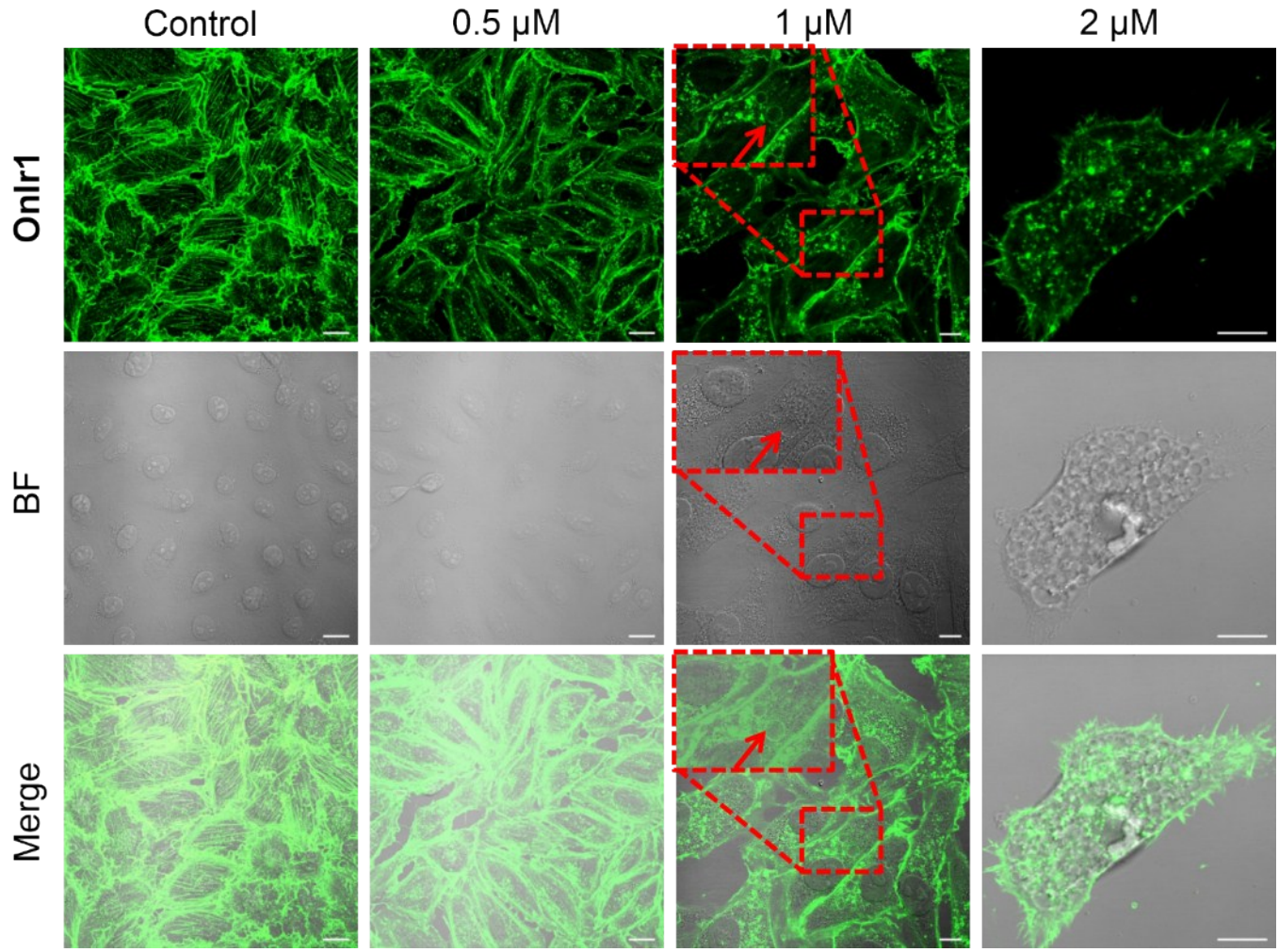


Figure S17. The images of Actin-Tracker Green distribution in a dose-dependent manner. **OnIr1** was incubated in various concentration for 24 h. Scale bar: 10 μm

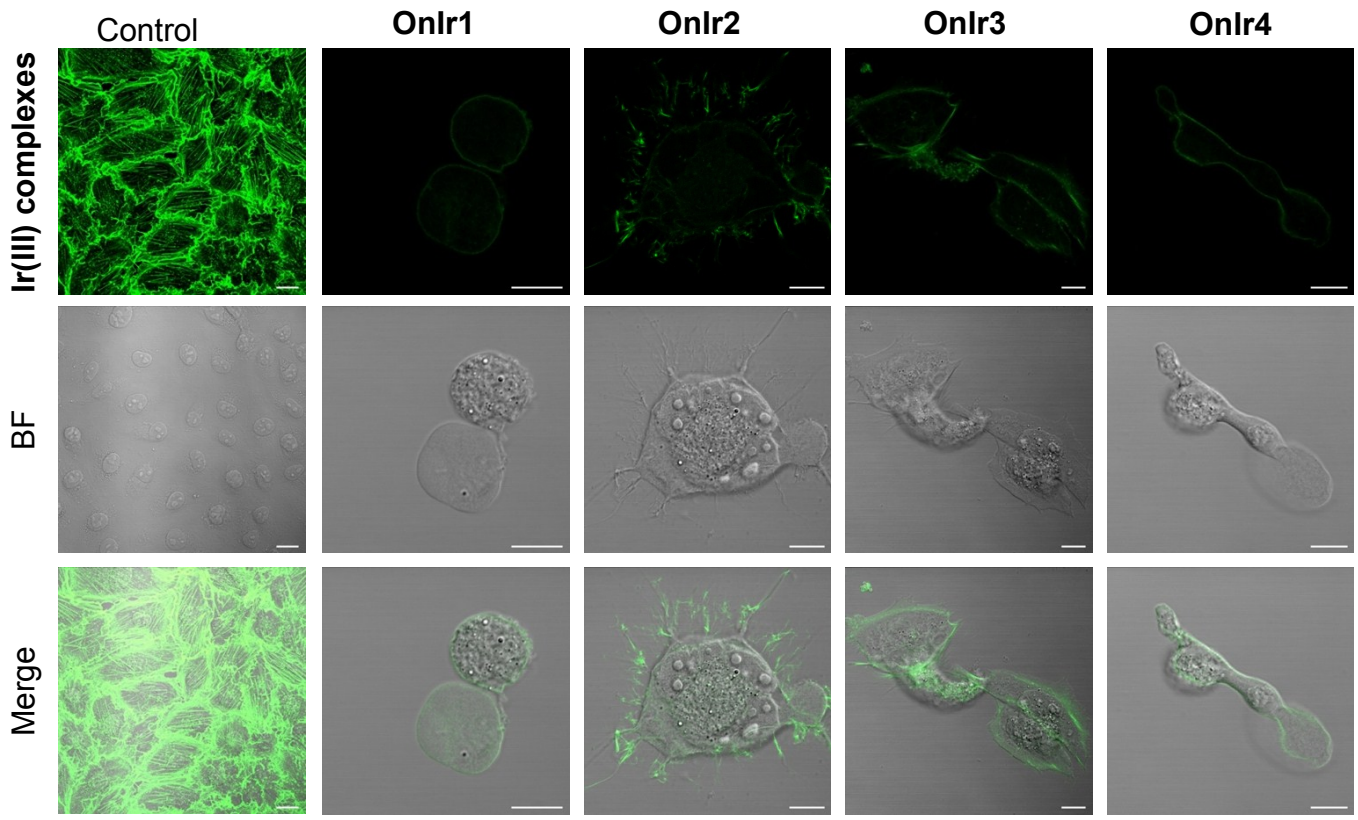


Figure S18. Cellular distribution of actin with the different complexes. **OnIr1** and **OnIr2** were incubated in a concentration of 2 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 3 μM. Incubation time was 24 h. Scale bar: 10 μm

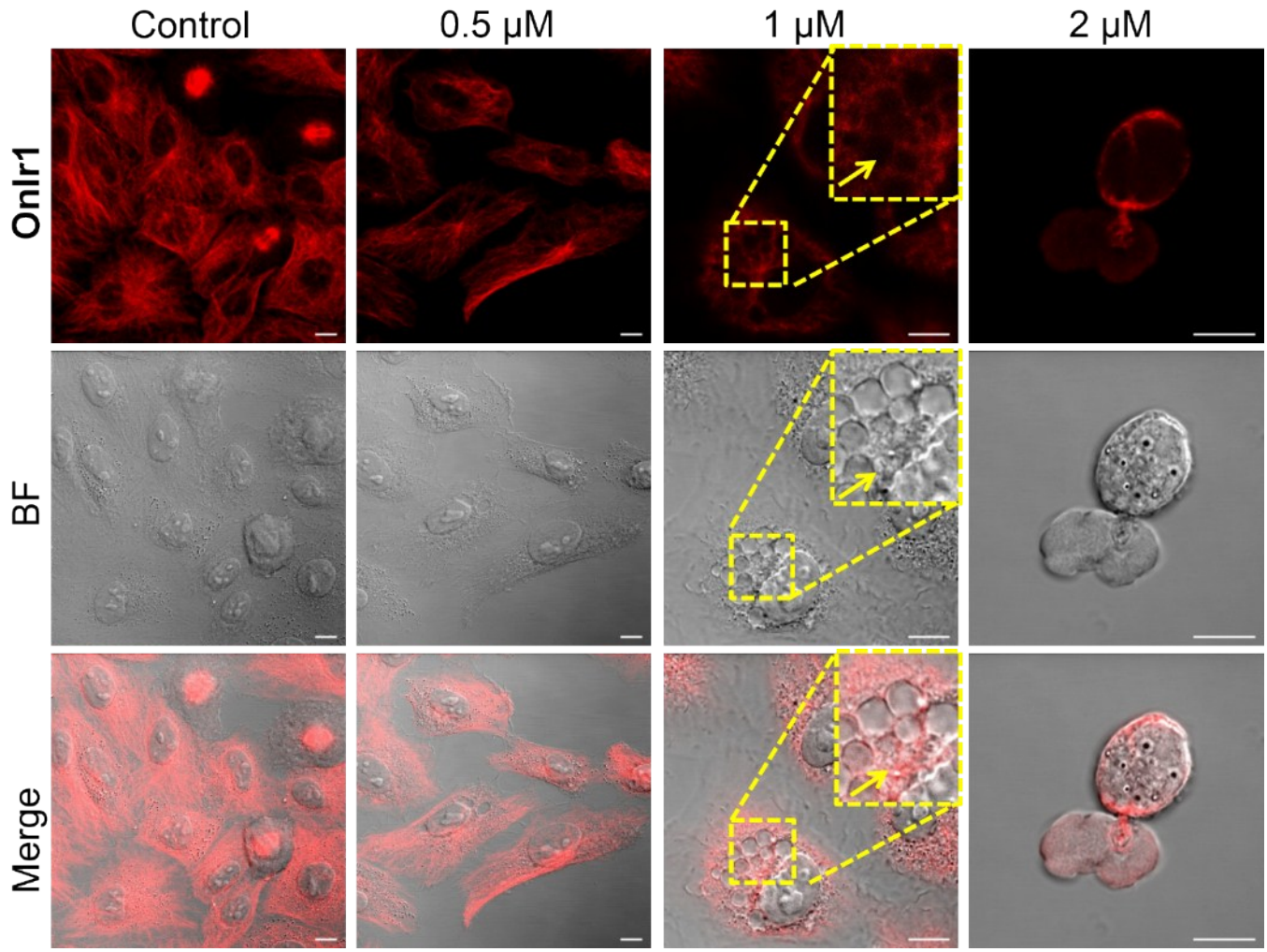


Figure S19. The images of Tubulin-Tracker Red distribution in a dose-dependent manner. **OnIr1** was incubated in various concentrations for 24 h. Scale bar: 10 μm

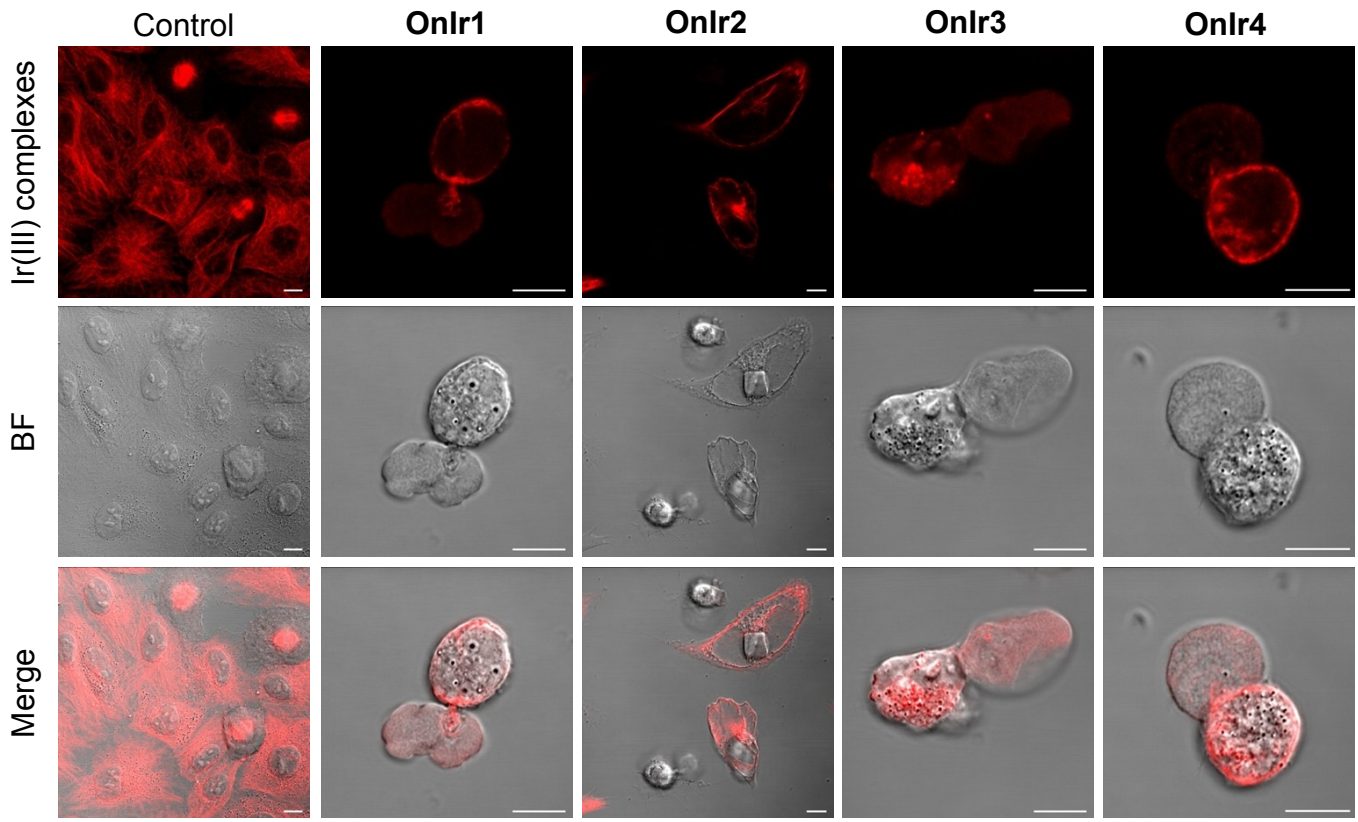


Figure S20. Cellular distribution of actin with **OnIr1-OnIr4**. **OnIr1** and **OnIr2** were incubated in a concentration of 2 μM. **OnIr3** and **OnIr4** were incubated in a concentration of 3 μM. Incubation time was 24 h. Scale bar: 10 μm

Table S1. Crystal data and structure refinement for **OnIr3**

Empirical formula	$C_{54}H_{33}ClIrN_6S_2$
Formula weight	1057.63
Temperature/K	153(2)
Crystal system	monoclinic
Space group	P21/n
a/Å	14.6864(4)
b/Å	15.1284(5)
c/Å	29.6362(7)
α/°	90
β/°	99.0370(10)
γ/°	90
Volume/Å^3	6502.9(3)
Z	4
ρ_{calc}g/cm3	1.08
μ/mm^{-1}	2.19
F(000)	2100
Crystal size/mm^3	0.277 × 0.270 × 0.208
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	6.022 to 54.94
Index ranges	$-18 \leq h \leq 19$, $-19 \leq k \leq 18$, $-38 \leq l \leq 38$
Reflections collected	50417
Independent reflections	14741 [R_{int} = 0.0427, R_{sigma} = 0.0419]
Data/restraints/parameters	14741/69/607
Goodness-of-fit on F^2	1.055
Final R indexes [I>=2σ (I)]	R_1 = 0.0533, wR_2 = 0.1492

Table S2. Bond angles and lengths for **OnIr3**

Atoms	Angle/Length (° /Å)	Atoms	Angle/Length (° /Å)
C16 Ir1 N3	96.40(19)	N1 Ir1 N2	172.72(17)
C16 Ir1 N1	96.7(2)	N4 Ir1 N3	75.32(18)
C16 Ir1 N4	171.69(19)	N2 Ir1 N3	82.21(18)
C16 Ir1 N2	79.2(2)	N2 Ir1 N4	100.01(19)
C1 Ir1 C16	90.0(2)	C21 C16 Ir1	116.3(4)
C1 Ir1 N3	172.25(18)	C17 C16 Ir1	127.8(4)
C1 Ir1 N1	79.3(2)	C35 N3 Ir1	116.1(4)
C1 Ir1 N4	98.4(2)	C35 N3 C31	118.4(5)
C1 Ir1 N2	94.7(2)	C31 N3 Ir1	125.4(4)
N1 Ir1 N3	104.31(17)	C2 C1 Ir1	127.5(5)
C36 N4 Ir1	116.4(4)	C6 C1 Ir1	116.6(4)
C22 N2 Ir1	113.3(4)	C7 N1 Ir1	113.4(4)
C30 N2 Ir1	127.3(4)	C1AA N1 Ir1	128.1(4)
N1 Ir1 N4	84.97(19)	C40 N4 Ir1	125.1(4)
Ir1 C16	1.993(5)	Ir1 N1	2.088(5)
Ir1 N3	2.170(4)	Ir1 N4	2.167(5)
Ir1 C1	1.979(6)	Ir1 N2	2.103(5)

Table S3. IC_{50} values of Ir(III) complexes (48 h, μM)

Cell lines	OnIr1	OnIr2	OnIr3	OnIr4	CDDP	Mitoxantrone	Doxorubisin	As_2O_3/ATO
7404	0.84 ± 0.07	1.57 ± 0.04	1.82 ± 0.04	1.97 ± 0.08	12.12 ± 0.21	-	-	-
7404/CP20 [a]	1.24 ± 0.08	2.23 ± 0.08	2.01 ± 0.09	2.06 ± 0.04	73.45 ± 1.24	-	-	-
H460	0.62 ± 0.06	1.3 ± 0.09	1.71 ± 0.16	2.12 ± 0.11	16.67 ± 0.67	0.167 ± 0.05	-	-
H460/MX20 [b]	0.76 ± 0.08	1.45 ± 0.09	1.78 ± 0.09	2.01 ± 0.08	15.6 ± 0.51	3.56 ± 0.18	-	-
SW620	1.87 ± 0.05	1.75 ± 0.06	2.12 ± 0.09	3.24 ± 0.11	9.58 ± 0.28	-	1.54 ± 0.06	-
SW620/AD300 [c]	5.27 ± 0.11	7.38 ± 0.11	3.86 ± 0.09	3.16 ± 0.08	10.17 ± 0.25	-	33.46 ± 0.52	-
SKOV3	1.83 ± 0.05	2.54 ± 0.05	2.37 ± 0.09	2.91 ± 0.06	7.64 ± 0.14	-	-	-
SKOV/Pt [a]	1.97 ± 0.06	2.86 ± 0.06	2.84 ± 0.21	3.16 ± 0.20	41.57 ± 0.85	-	-	-
KB-3-1	1.14 ± 0.06	1.35 ± 0.04	1.41 ± 0.05	2.03 ± 0.09	8.56 ± 0.08	-	0.33 ± 0.05	2.75 ± 0.08
KCP4 [a]	1.31 ± 0.05	1.74 ± 0.07	1.21 ± 0.06	1.57 ± 0.05	34.42 ± 0.35	-	-	-
KBC-2 [c]	1.16 ± 0.02	1.76 ± 0.04	1.52 ± 0.05	1.66 ± 0.06	8.74 ± 0.19	-	7.62 ± 0.26	-
KB-CV60 [c]	1.05 ± 0.05	1.56 ± 0.06	1.68 ± 0.07	2.21 ± 0.13	12.5 ± 0.25	-	4.53 ± 0.20	-
KB/ATO [d]	1.43 ± 0.05	1.88 ± 0.03	1.97 ± 0.08	2.21 ± 0.08	-	-	-	30.47 ± 0.75
MCF-7	1.57 ± 0.07	1.88 ± 0.06	1.14 ± 0.06	1.42 ± 0.05	9.56 ± 0.27	-	0.03 ± 0.01	-
MCF-7/ADR [a]	1.62 ± 0.05	1.92 ± 0.04	1.33 ± 0.03	1.52 ± 0.01	16.63 ± 0.27	-	6.85 ± 0.30	-
A549	1.50 ± 0.02	2.85 ± 0.09	2.15 ± 0.11	2.44 ± 0.10	15.05 ± 0.17	-	-	-
A549R [a]	0.48 ± 0.02	0.75 ± 0.05	0.64 ± 0.05	0.82 ± 0.07	97.79 ± 0.43	-	-	-
HeLa	0.95 ± 0.05	0.75 ± 0.06	1.54 ± 0.02	1.55 ± 0.07	13.55 ± 0.22	-	-	-
HCT-116	1.68 ± 0.09	1.27 ± 0.10	1.60 ± 0.09	1.85 ± 0.08	17.93 ± 0.17	-	-	-
HepG2	1.55 ± 0.08	1.78 ± 0.08	4.92 ± 0.12	4.38 ± 0.12	15.99 ± 0.28	-	-	-
Bel-7402	1.79 ± 0.08	2.35 ± 0.11	2.30 ± 0.12	2.00 ± 0.06	15.63 ± 0.20	-	-	-
HL-7702	16.40 ± 0.32	8.88 ± 0.22	10.01 ± 0.20	15.63 ± 0.28	9.49 ± 0.13	-	-	-
HEK293	3.11 ± 0.11	3.42 ± 0.15	3.03 ± 0.08	4.12 ± 0.20	18.34 ± 0.65	0.26 ± 0.02	-	-

[a] Anti-Cisplatin(CDDP) cell line
[b] Anti-Mitoxantrone cell line
[c] Anti-Doxorubisin cell line
[d] Anti-As_2O_3/ATO cell line

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