Supplementary Materials

for

Adjusting lipid-water distribution coefficient of Iridium (III) complexes for enhancing its cellular penetration and treatment efficacy to antagonize cisplatin resistance in cervical cancer

Biological experimental section

Lipophilicity

Take the same volume of n-octanol and water (20 mL), in a constant temperature oscillator and shake for 24 h, so that the two phases can be saturated with each other. Static liquid separation. Then, the two-phase distribution is carried out. The Ir-Se, Ir-S, Ir-CHO were added to 2 mL water-saturated n-octanol, and the final concentration of the solution was 100 μM. Then add the same amount of aqueous solution saturated with n-octanol, mix the solution and vibrate in a constant temperature oscillator for 6 hours. After stationary, the two-phase solutions were dispersed in 2 mL ethanol, and the complexes were quantified by the absorbance of the maximum absorption wavelength at MLCT. The calculation formula is that the concentration of logP= complex in oil phase / the concentration of complex in water phase = c (oil phase) / c (water phase).

Cell lines, cell culture and MTT assay

Three human cervical cancer cell lines including HeLa cells and Siha cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). And two normal cell lines including human cervical epithelial cells and Human embryonic lung fibroblasts were brought from ATCC. These cells were grown in DMEM media containing 10% FBS, streptomycin (50 units/mL), and penicillin (100 units/mL) in a humidified incubator with a 5% CO_2 atmosphere. In order to culture cisplatin resistance subline, R-HeLa cells, HeLa cells in logarithmic growth phase were cultured in DMEM medium containing 0.125 μg/mL cisplatin. After 24 h, the culture medium containing cisplatin was poured out and replaced with normal DMEM. When the density of HeLa cells reached 80%, the cells were subcultured, followed by addition of cisplatin (the concentration was twice as high as that of the previous one). After this repeated dressing change, the cells were induced by gradually increasing the concentration of cisplatin until the concentration of cisplatin reached 2 μg/mL. The HeLa cell line induced by this method was named R-HeLa. R-HeLa cells were cultured in a medium containing 2 μg/mL cisplatin to maintain their drug resistance. Cytotoxicity induced by Ir-Se, Ir-S, Ir-CHO, Se-O-NH_2 and Cisplatin were evaluated by MTT assay. Briefly, different concentrations of complex were added to 96-well plate for 72 h. Next, 30 μL MTT (5 mg/mL) solution was added to 96-well plate and then incubated for 3 h The solution was replaced by DMSO of 150 mL, and the absorption value was measured at 570 nm by Microplate Reader.

Colony Formation Assay

R-HeLa cells and HeLa cells were seeded in 6-well plate at the density of 2500 cells/well and incubated for 24 h. These complexes of different concentrations were co-cultured with the cells followed by incubation for 8 days. Finally, these cells were fixed in Paraformaldehyde and then stained with crystal purple solution.

Intracellular localization of complexes

R-HeLa cells with a density of 80000/mL were inoculated into a glass dish of 2 cm and allowed to attach for 24 h. Then these cells were stained with lyso-tracker staining for 2 h, and then stained with Hoechst staining for 15 minutes. Next, the cells were exposed to these complexes including Ir-S, Ir-Se and Ir-CHO and cultured for 2 h, and then photographed by laser confocal microscope. The colocalization of complexes and lysosome was evaluated by Image J software.

***In vitro* cellular uptake of complexes**

The difference of cytotoxicity of complexes in R-HeLa cells should be attributed to its different uptake levels in cells. Hence, in order to demonstrate this possibility, we measured the cellular uptake levels of complex in R-HeLa cells at different times. Briefly, R-HeLa cells were inoculated into 6-well plate at the density of 20000/mL and incubated for 24 h. Ir-CHO, Ir-S and Ir-Se at a concentration of 4 μM were added to each well, and cultured for 0.5 h, 1.5 h, 3 h and 4 h. The cells were collected by centrifugation and detected by flow cytometry.

Cellular retention of complexes in R-HeLa cells

Cellular retention of complexes in R-HeLa cells also plays a critical role in inducing cytotoxicity. Hence, we compared the drugs retention levels of Ir-CHO, Ir-S and Ir-Se in different time points. In brief, equivalent doses of complexes were added to 6-well plate containing 40000/well attached cells and incubated for 4 hours. Then, the medium was extracted and added to the fresh medium. Subsequently, the cells were collected at 0 h, 1 h, 3 h and 4 h respectively, and then detected by using flow cytometry.

Penetrating and inhibitory effects to R-HeLa multi-cellular tumor spheroids

Multi-cellular tumor spheroids as an effective tumor platform *in vitro* have been widely used in the field of biochemistry for detecting drug penetration and activity. We established R-HeLa tumor spheres at a density of 2500 cells / well in an ultra-low adsorption six-well plate to gradually aggregate.Then the complexes including Ir-S, Ir-Se and Ir-CHO were added to the well. After 8 hours of culture, the treated R-HeLa spheres were scanned by confocal laser scanning fluorescent microscope (Zeiss LSM 700) from from the top to the bottom of the spheroid.

Effect of complexes on cell cycle distribution

The R-HeLa and HeLa cells were inoculated to 6-well plate with the density of 20000/mL. After attachment, the complexes of different concentrations were added to the 6-well plate and incubated for 24 h. Next, the cells were collected and fixed in70% ethanol solution for 12 h according to the description of the existing literature. Finally, the effect of complexes on cell cycle distribution was analyzed by flow cytometry.

Annexin V-FITC/PI double-staining for apoptosis detection

Flow cytometry was used to detect the apoptosis of R-HeLa cells caused by complexes by using Annexin V-FITC/PI double-staining kits. R-HeLa cells, at a density of 40000/mL in logarithmic growth phase, were laid in 6-well plate and cultured for 24 h in the medium supplied with different concentrations of Ir-S and Ir-Se complexes. Next, the treated cells were resuspended in a buffer solution provided by a 200 μL kit containing 5 μL Annexin V-FITC and 10 μL PI and then stained for 30 min at room temperature, as previously described.

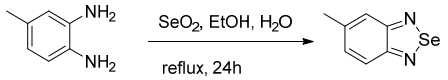
Detection of mitochondrial membrane potential

JC-1, an ideal fluorescent probe, was widely used to detect mitochondrial membrane potential for reflecting the mitochondrial function. After treatment of Ir-S, Ir-Se and Ir-CHO complexes for 24 h, the cells were collected, resuspended in PBS solution and then staining with JC-1 (10 μg/mL) for 30 min, followed by flow cytometry analysis. Meanwhile, the images of R-HeLa cells treated with different complexes for 24 h and then stained by JC-1 probes were recorded by a fluorescence microscopy (EVOS FL auto, Life Technologies).

ROS Generation induced by complexes

The singlet oxygen production in R-HeLa and HeLa cells caused by different complexes within 2 h was measured by using DPBF probe. In short, R-HeLa and HeLa cells were seeded at 96-well plate at a density of 100000/mL for overnight and then incubated with 10 μM DPBF at 37 °C for 30 min. After that, different concentrations of complexes were added to treat the cells. Finally, the level of intracellular singlet oxygen was measured every 5 minutes by measuring the fluorescence intensity conducted by microplate reader.

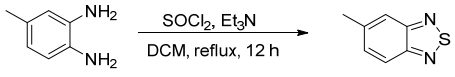
Synthetic section of various compounds



1a 1b

1a (23.73 mmol) and SeO_2 (36.01 mmol) were dissolved in 50 mL (9:1, v/v) of ethanol and water, and reacted at 70 °C for 24 h. After the reaction was completed, the solvent was removed under reduced pressure. The product was purified on a silica gel column eluting with petroleum ether and ethyl acetate. Yield 90%.

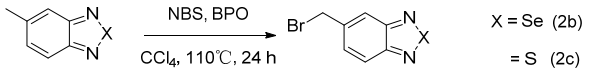
Compound 1b : NMR Spectroscopy: ^{1}H NMR (300 MHz, $(CD_3)_2SO$, 25 ºC, δ): 7.77 (d, J = 9.15 Hz, 1H), 7.6 (s, 1H), 7.37-7.33 (dd, J = 1.62, 9.15 Hz, 1H) , 2.4 (s, 3H).



1a 1c

1a (30 mmol) and triethylamine (150 mmol) were dissolved in dry DCM, then $SOCl_2$ (60 mmol) was slowly added dropwise in an ice bath, and refluxed for 12 h. After the reaction was completed, the system was evaporated under reduced pressure. Solvent, a large amount of a brown-brown solid was obtained, and the solid was dissolved in deionized water, and concentrated hydrochloric acid (2 mL) was evaporated. Purification by column chromatography on silica gel eluting with EtOAc. Yield 75%.

Compound 1c : NMR Spectroscopy: ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC, δ): 7.82-7,79 (d, *J* = 8.82 Hz, 1H), 7.68 (s, 1H), 7.36-7.33 (d, *J* = 8.85 Hz, 1H), 2.47 (s, 3H).

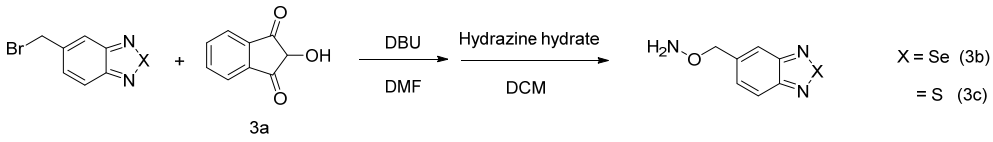


1b (13.33 mmol), NBS (13.99 mmol) and BPO (0.66 mmol) were added to CCl_4, heated and refluxed for 24 h. At the end of the reaction, the filtrate was filtered and the solvent was removed by decompressing the filtrate to obtain yellow solid. The crude product was purified on a silica gel column with petroleum ether and ethyl acetate to obtain brown solid. Yield 76.52%.

Compound 2b : NMR Spectroscopy: ^{1}H NMR (300 MHz, $(CD_3)_2SO$, 25 ºC, δ): 7.94 (s, 1H), 7.86-7.84 (d, *J* = 7.41 Hz, 1H), 7.59-7.55 (dd, *J* = 1.71, 9.21 Hz, 1H), 4.87 (s, 2H).

The synthesis method of 2c refers to the synthesis of 2b.

Compound 2c : NMR Spectroscopy: ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC, δ): 8.02-7.99 (m, 2H), 7.66-7.63 (m, 1H), 4.64 (s, 2H).



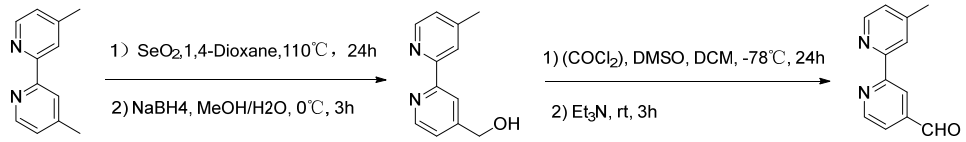
3a

2b (0.72 mmol) and 3a (0.66mmol) were dissolved in a small amount of DMF, DBU (0.8mmol) was added, 30min was reacted with 10 mL 1 N HCl, filtration, white precipitation was obtained, and drying was directly used in the next step. The dry white solid (0.56 mmol) was dissolved in anhydrous DCM, and hydrazine hydrate (1.2 mmol),) was added to the reaction for 3 hours. After the reaction, the white solid was filtered and extracted with 2 mol/L NaOH solution and brine in turn. The organic layer is dried with anhydrous magnesium sulfate. Filter, spin dry, that is, the product. Yield:70%.

Compound 3b : NMR Spectroscopy: ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC, δ): 7.81-7.76 (t, *J* = 9.24, 6.51 Hz, 2H), 7.48-7.45 (dd, *J* = 1.44, 9.21 Hz, 1H), 5.55 (s, 2H), 4.79 (s, 2H).

The synthesis method of 3c refers to the synthesis of 3b.

Compound 3c : NMR Spectroscopy: 1H NMR (300 MHz, $CDCl_3$, 25 ºC, δ): 7.99-7.95 (t, *J* = 9.09, 4.23 Hz, 2H), 7.61-7.58 (d, *J* = 8.94 Hz, 1H), 5.55 (s, 2H), 4.85 (s, 2H).

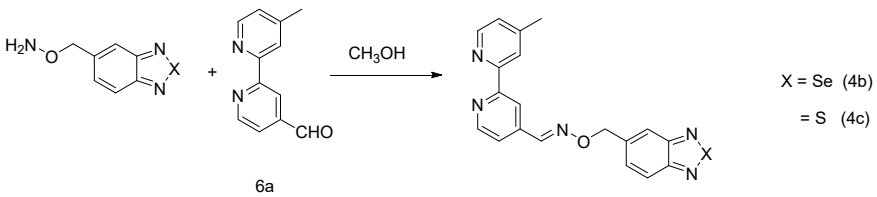


4a 5a 6a

4a (80 mmol) and selenium dioxide (72.7 mmol) were dispersed with 1,4-dioxane of 350 mL, heated and refluxed at 130 ℃ for 24 h under the protection of nitrogen. After the reaction, filter paper was used to filter while it was hot, and methanol was used to wash the filter cake. The solvent was removed by vacuum evaporation and a large number of yellow and pink solids were obtained. The solid was dispersed in 400 mL ethyl acetate, heated and refluxed for 3 hours, filtered while heat, evaporated to remove the solvent, and the yellow and pink solid was obtained. The yellow and pink solids were dispersed in 200 mL methanol, and the aqueous solution of sodium borohydride (40.5mmol) was added in batches under the ice bath, stirred overnight. After the reaction was finished, the filtrate was filtered and the filtrate was rotated and dried. Then the solid was dispersed in saturated sodium carbonate solution, stirred for 30 min, extracted with chloroform for 3 times, the organic layer was extracted, dried with anhydrous magnesium sulfate, filtered, reduced pressure evaporation to remove the solvent. The crude product was purified by dichloromethane and methanol on silica gel column chromatography, and the white solid 5a was obtained. Yield: 30%.

Oxalyl chloride (30 mmol) was dispersed in 100 mL dry DCM, precooled at -78 ℃ for 30 min, and then dripped with dry DMSO (450 mmol),) to produce a large amount of gas. 30min was stirred at-78 ℃. 5a (7.5 mmol) dissolved in a small amount of dry DCM, droplets was added to the reaction system and stirred for 1 h at -78 ℃. After the reaction, triethylamine (90 mmol) was added to stir for the night. Then add 15 mL saturated ammonium chloride aqueous solution, stir 30 min, and extract with dichloromethane for three times. The organic phase was collected, dried with anhydrous magnesium sulfate, filtered, reduced pressure evaporation to remove the solvent, and the crude product was purified on silica gel column with dichloromethane and methanol. To get an orange solid. Yield 70.1%.

Compound **6a** : NMR Spectroscopy: ^{1}H NMR (300 MHz, $(CD_3)_2SO$, 25 ºC, δ): 10.18 (s, 1H), 8.94-8.93 (d, J = 4.8 Hz, 1H), 8.77 (s, 1H), 8.58-8.57 (d, J = 4.89 Hz, 1H), 8.25 (s, 1H), 7.84-7.82 (dd, J = 1.41, 4.8 Hz, 1H), 7.34-7.32 (d, J = 4.11 Hz, 1H), 2.42 (s, 3H).



6a

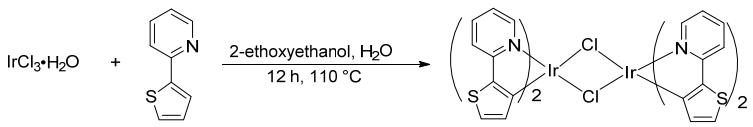
3b (0.44 mmol) and 6a (0.44 mmol) were dissolved in methanol and reacted for 3 h. A large amount of white precipitate was formed in the system, which was filtered, and the white solid was washed twice with a small amount of methanol and water and dried. The product was obtained. Yield: 51%.

Compound **4b** : NMR Spectroscopy: ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC, δ): 8.67-8.66 (d, J = 4.98 Hz, 1H), 8.55-8.53 (d, J = 4.74 Hz, 1H), 8.5 (s, 1H), 8.24-8.23 (d, J = 4.14 Hz, 2H), 7.83-7.81 (d,

J = 6.15 Hz, 2H), 7.54-7.50 (d, *J* = 9.57 Hz, 2H), 7.16-7.15 (d, *J* = 3.93 Hz, 1H), 5.37 (s, 2H), 2.44 (s, 3H).

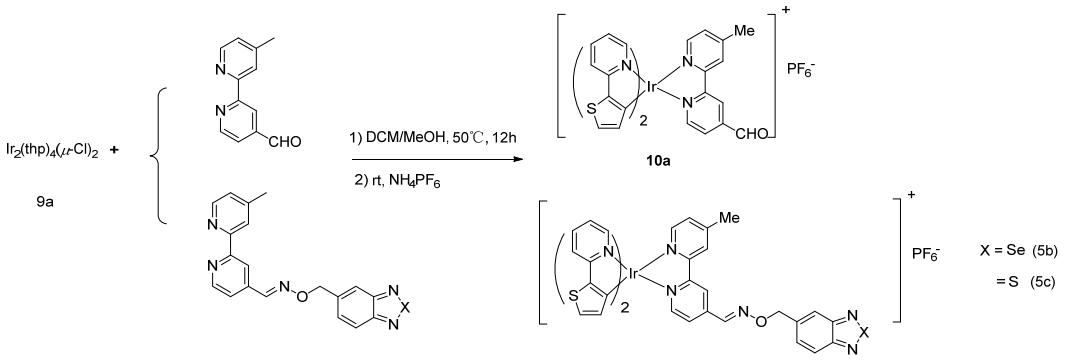
The synthesis method of 4c refers to the synthesis of **4b**.

Compound **4c** : NMR Spectroscopy: ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC, δ): 8.68-8.66 (d, *J* = 5.04 Hz, 1H), 8.55-8.54 (d, *J* = 4.8 Hz, 1H), 8.5 (s, 1H), 8.24-8.23 (d, *J* = 3.45 Hz, 2H), 8.03-8.00 (d, *J* = 8.67 Hz, 2H), 7.67-7.64 (dd, *J* = 1.32, 9.39 Hz, 1H), 7.54-7.52 (dd, *J* = 1.5, 5.07 Hz, 1H), 7.17-7.15 (d, *J* = 5.04 Hz, 1H), 5.44 (s, 2H), 2.44 (s, 3H).



7a 8a 9a

7a (1.7 mmol) and **8a** (3.57 mmol) were taken into the reaction bottle. Under the protection of nitrogen, the mixed solvent of ethylene glycol ether and water (3:1) was injected into the reaction bottle at 110 ℃ for 12 h. After the reaction was finished, the mixed solvent of ethylene glycol ether and water was injected into the reaction bottle at 110 ℃ for 12 h, and the mixture of ethylene glycol ether and water was injected into the reaction bottle at 110 ℃ for 12 h. When the temperature of the system drops to room temperature, the dark orange solid is obtained by filtration, and the filter cake is further washed with water and methanol. Then dissolve the filter cake with DCM, wash it with saturated salt water for three times, dry the organic layer with anhydrous magnesium sulfate, collect the filtrate after filtration, reduce pressure and dry to get brown solid. Yield: 46.61%.



9a 10a

9a (0.18 mmol) and 6a (0.4 mmol) were added to the reaction bottle. Under the protection of nitrogen, the mixed solvent of dichloromethane and methanol was injected into the reaction system at 60 mL (1:1, v / v). The temperature was raised to 50 ℃ and reacted for 12 h. After the reaction, wait until the system temperature drops to room temperature, add ammonium hexafluorophosphate (3.65 mmol) to stir for 3 hours, then filter, take the filtrate, remove the solvent by vacuum evaporation, and get the yellow crude product. The crude product was purified on silica gel column with dichloromethane and methanol. Bright orange-red solids are obtained by evaporation of solvents. Yield 46.5%.

The synthesis method of 5b, 5c refers to the synthesis of **10a**.

Compound **10a** : NMR Spectroscopy: ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC, δ): 10.2 (s, 1H), 9.04 (s, 1H), 8.60 (s, 1H), 8.1-8.08 (d, *J* = 5.55 Hz, 1H), 7.8-7.78 (dd, *J* = 1.23, 5.58 Hz, 1H), 7.75-7.73 (d, *J* = 5.58 Hz, 1H), 7.65-7.60 (t, *J* = 7.98, 7.47 Hz, 2H), 7.53-7.51 (d, *J* = 8.31 Hz, 2H), 7.44-7.37 (m, 4H), 7.31-7.3 (d, *J* = 5.22 Hz, 1H), 6.89-6.81 (m, 2H), 6.29-6.24 (q, *J* = 4.71 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, 25 ºC, δ): 190.3, 164.0, 163.7, 158.2, 154.8, 153.0, 152.1, 152.0, 151.8, 150.3, 149.4, 149.1, 143.3, 138.9, 138.8, 137.1, 136.8, 130.8, 130.7, 130.6, 130.3, 129.7, 126.6, 125.3, 125.1, 121.0, 120.8, 118.5, 118.4, 21.5.

Compound **5b** : NMR Spectroscopy: ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC, δ): 8.68 (s, 1H), 8.51 (s, 1H), 8.4 (s, 1H), 7.78-7.6 (m, 7H), 7.53-7.47 (m, 3H), 7.4-7.36 (m, 4H), 7.28 (s, 1H), 6.84-6.82 (m, 2H), 6.27-6.26 (d, *J* = 3.66 Hz, 2H), 5.33 (s, 2H), 2.60 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, 25 ºC, δ): 164.0, 160.5, 160.3, 157.0, 155.1, 152.9, 152.5, 150.7, 150.2, 149.1, 146.4, 143.0, 139.2, 138.7, 136.8, 130.8, 130.4, 130.0, 129.5, 126.3, 124.1, 123.6, 123.1, 121.8, 120.6, 118.4, 21.4. Mass Spectrometry: HRMS (ESI-TOF) (m/z): calcd for $C_{37}H_{27}IrN_7OS_2Se$ $(M\text{-}PF_6)^+$, 922.0513, found, 922.0088

Compound **5c** : NMR Spectroscopy: ^{1}H NMR (300 MHz, $CDCl_3$, 25 ºC, δ): 8.72 (s, 1H), 8.58 (s, 1H), 8.43 (s, 1H), 7.99-7.95 (m, 2H), 7.80-7.78 (d, *J* = 5.67 Hz, 1H), 7.70-7.59 (m, 5H), 7.53-7.49 (m, 2H), 7.39-7.36 (m, 4H), 7.23 (s, 1H), 6.82-6.8 (m, 2H), 6.26-6.24 (m, 2H), 5.4 (s, 2H), 2.62 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, 25 ºC, δ): 164.1, 157.2, 155.2, 153.0, 152.7, 150.6, 150.1, 149.1, 146.6, 143.2, 139.3, 138.7, 136.8, 130.8, 130.7, 130.5, 130.4, 130.0, 129.4, 126.7, 124.0, 123.7, 121.8, 120.7, 120.6, 120.3, 21.6. Mass Spectrometry: HRMS (ESI-TOF) (m/z): calcd for $C_{37}H_{27}IrN_7OS_3$ $(M\text{-}PF_6)^+$, 874.1068, found, 874.1063

Result Section

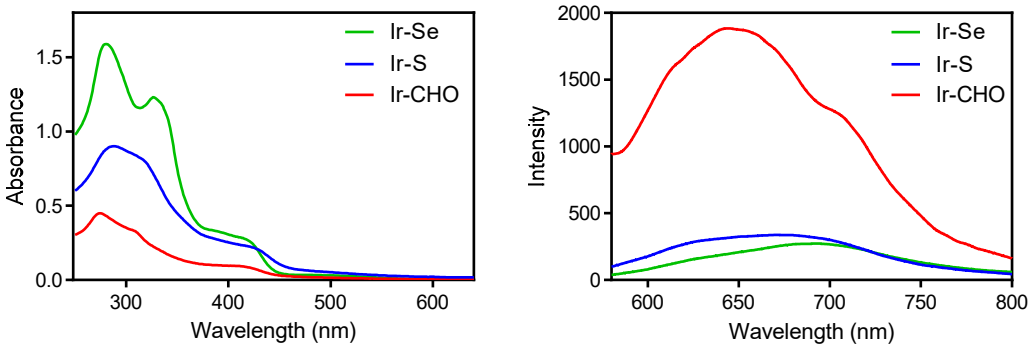


Figure S1. Ultraviolet absorption and fluorescence (Ex = 420 nm) emission spectrum of Ir (III) (30 μM).

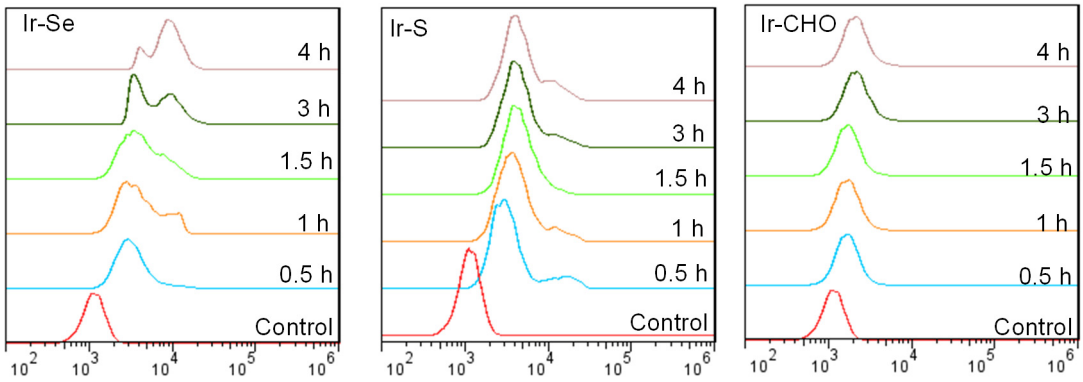


Figure S2. Uptake efficiency of R-HeLa cells after incubation for Ir (III) complexes at different time points by using flow cytomery.

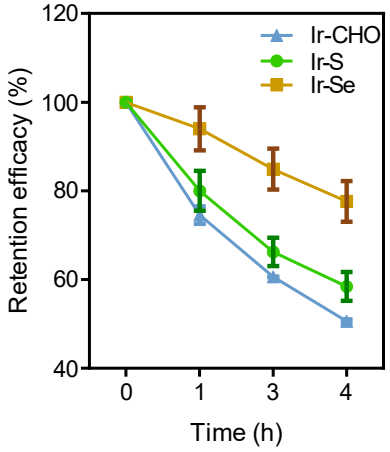


Figure S3. The retention efficacy of Ir (III) complexes in R-HeLa cells with an observation period of 4 h. The R-HeLa cells were initially treated with 4 μM Ir (III) complexes respectively.

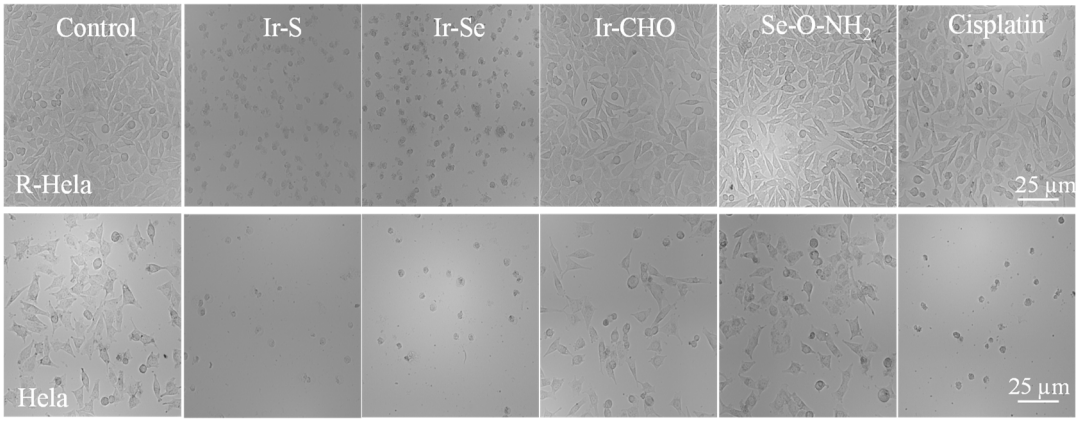


Figure S4. Pictures of R-HeLa and HeLa cells treated with Ir (III) complexes (10 μM) for 72 h.

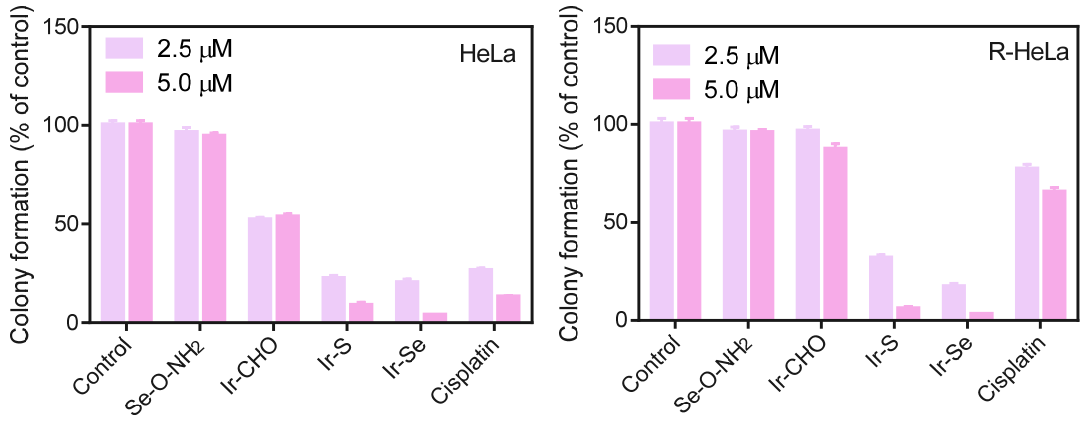


Figure S5. Quantitative analysis of colony formation of HeLa and R-HeLa cells treated with different concentrations of Ir (III) complexes.

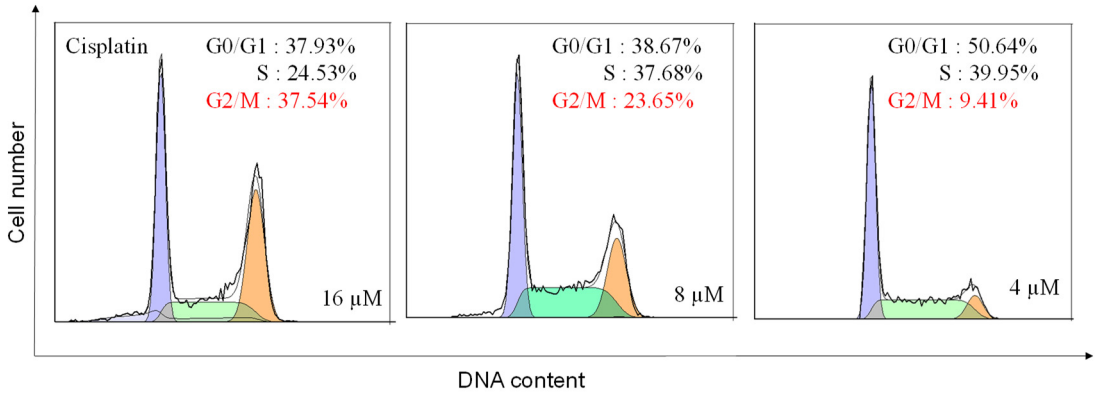


Figure S6. Flow cytometry analysis of R-HeLa cells after treated with different concentrations of Cisplatin for 24 h.

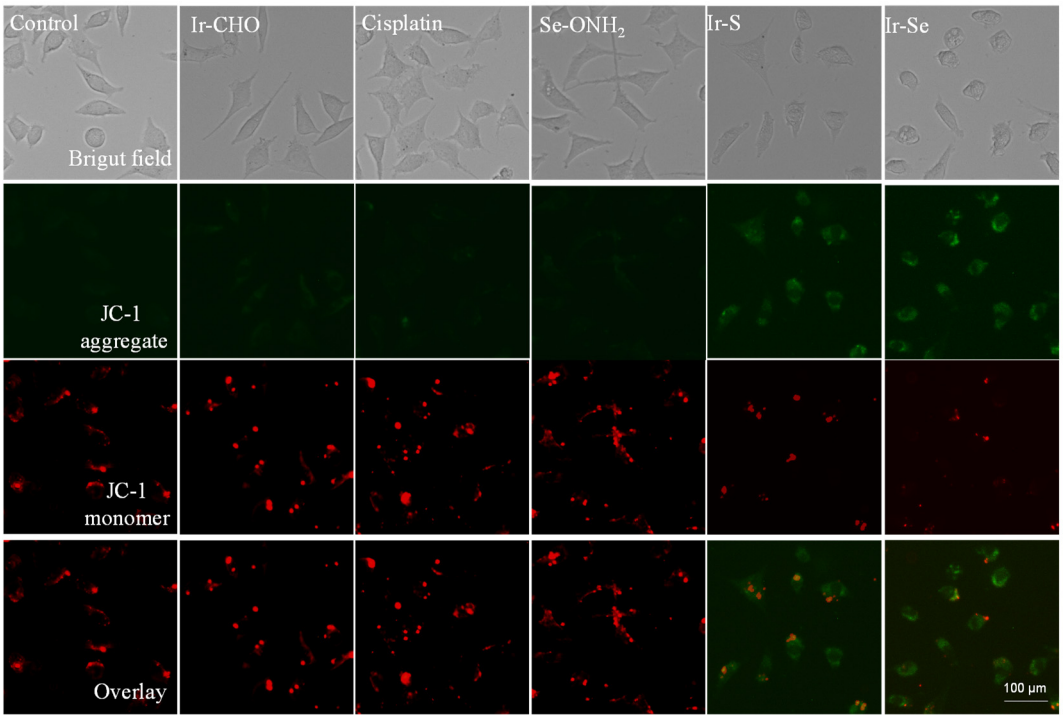


Figure S7. Fluorescence images of R-HeLa cells exposed to Ir complexes for 4 h and then stained with JC-1 probes.

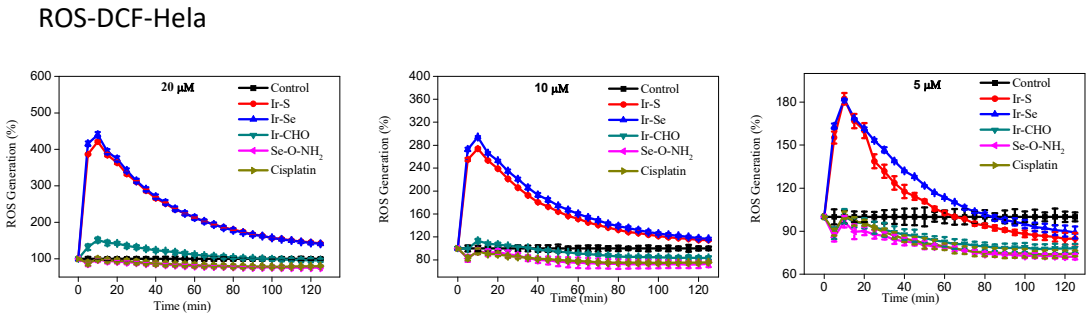


Figure S8. The ROS levels induced by Ir complexes were quantified by the fluorescent intensity of DCFH-DA.

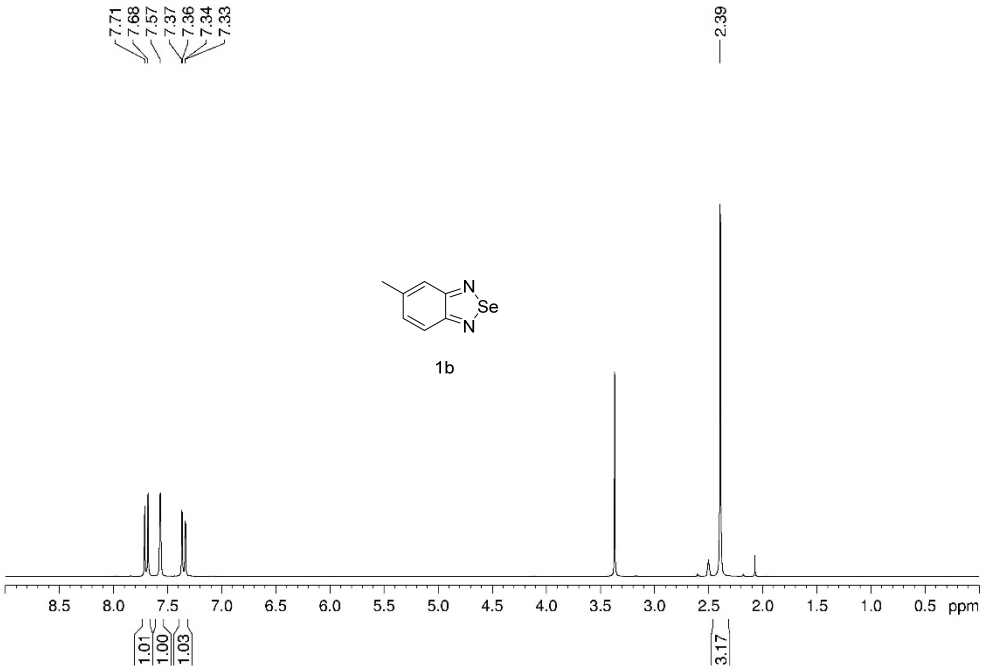


Figure S9. ^{1}H NMR spectrum of **1b**

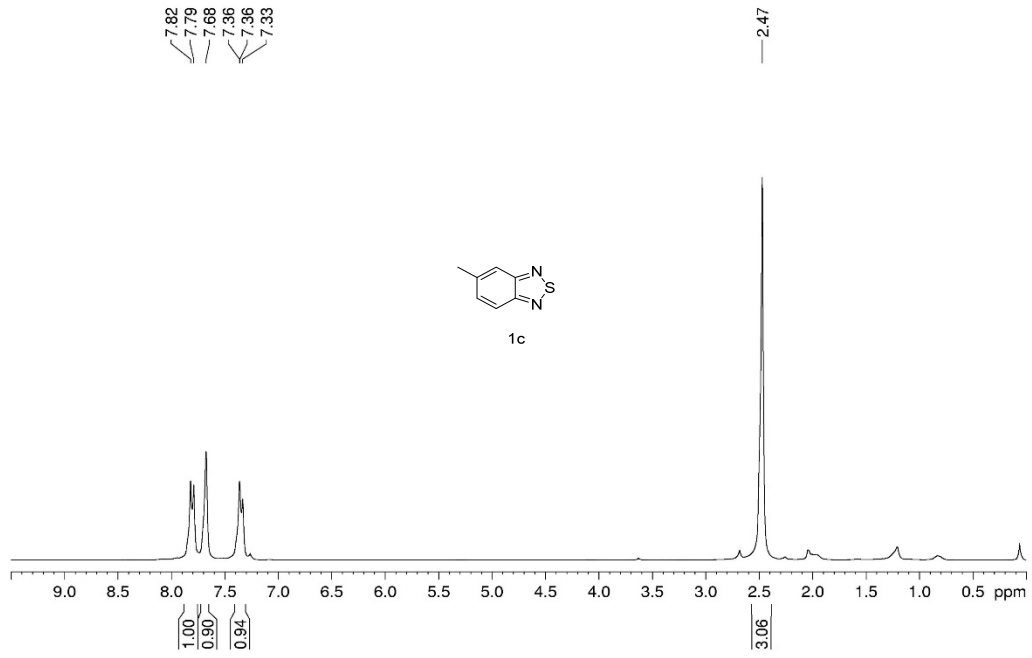


Figure S10. ^{1}H NMR spectrum of **1c**

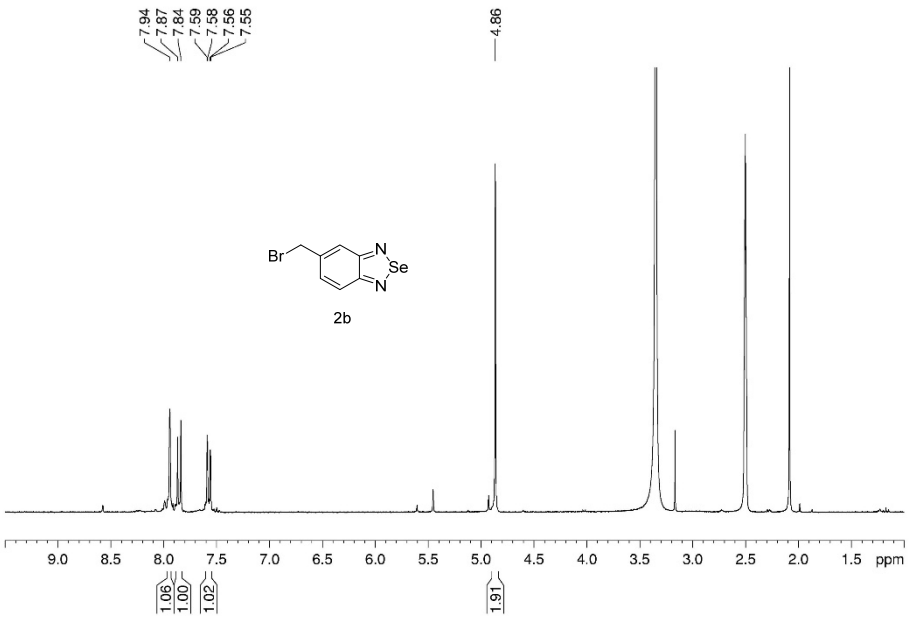


Figure S11. ^{1}H NMR spectrum of **2b**

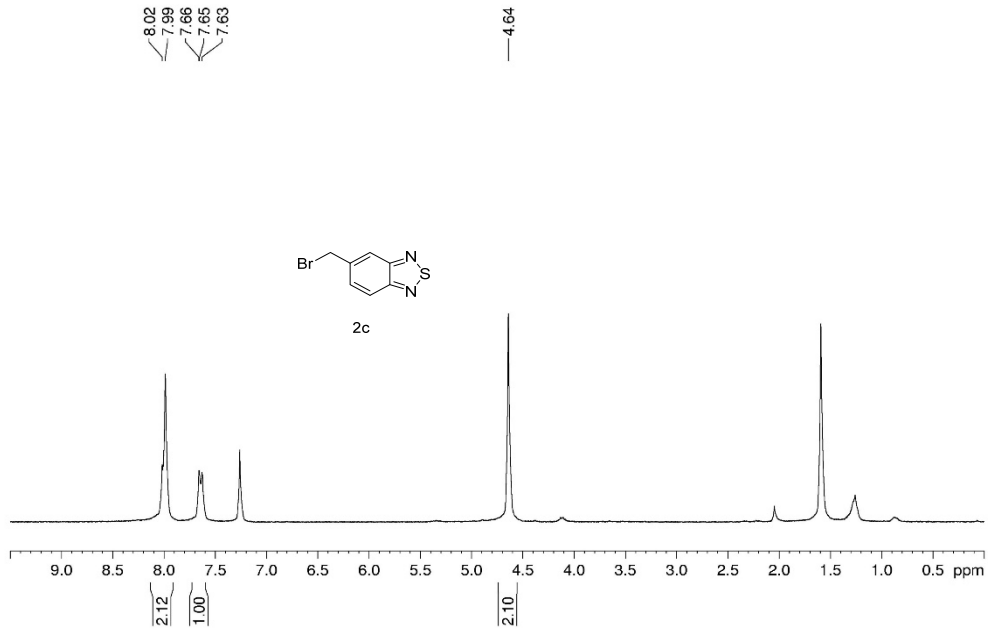


Figure S12. ^{1}H NMR spectrum of **2c**

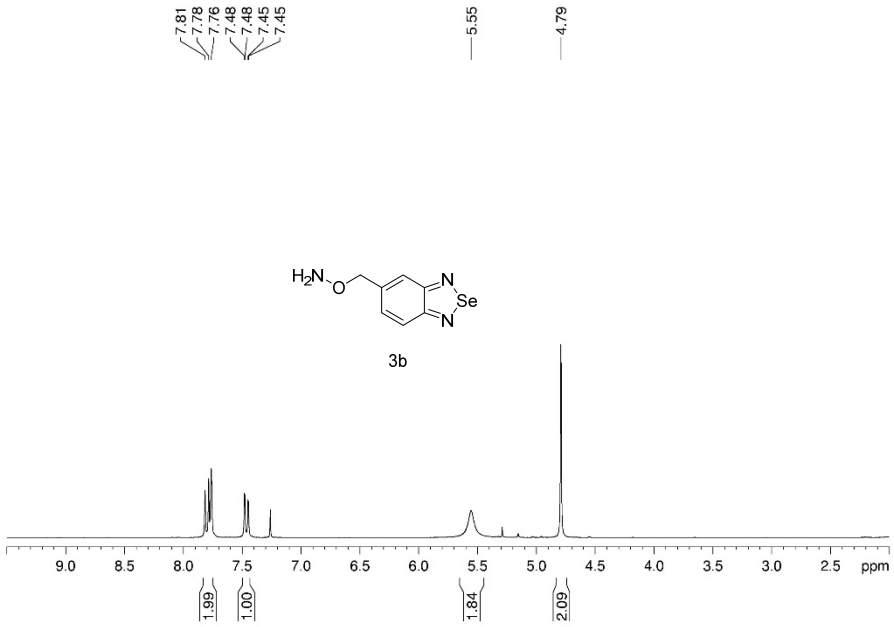


Figure S13. ^{1}H NMR spectrum of **3b**

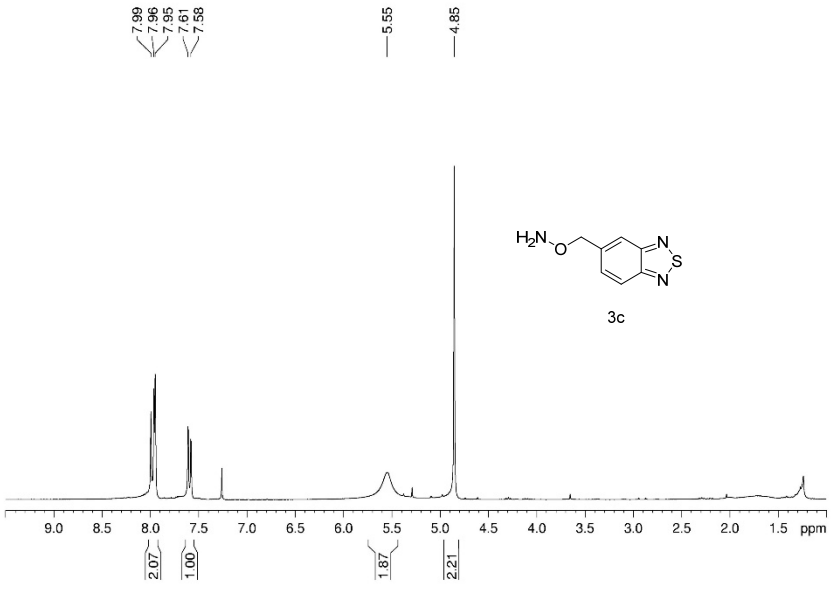


Figure S14. ^{1}H NMR spectrum of **3c**

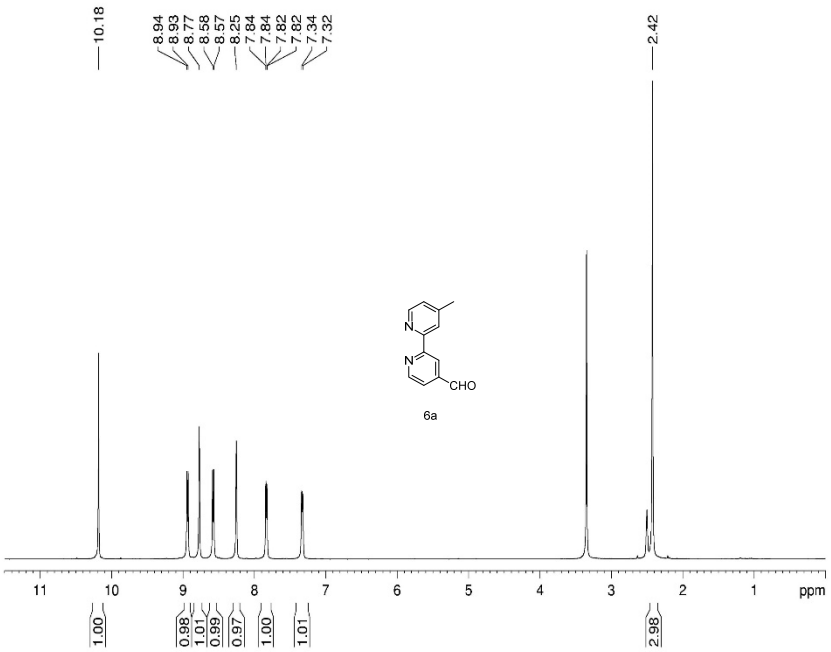


Figure S15. ^{1}H NMR spectrum of **6a**

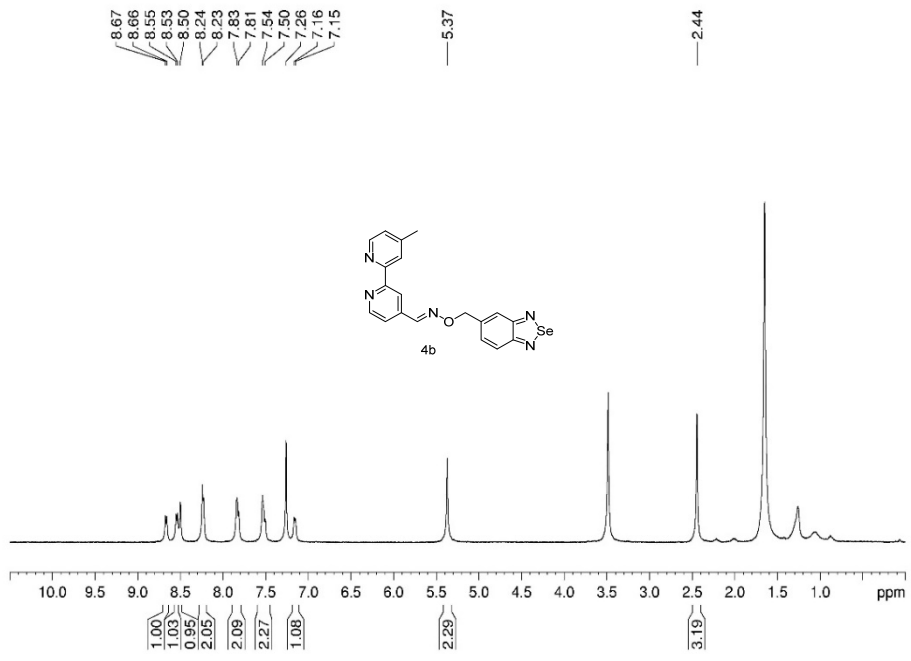


Figure S16. ^{1}H NMR spectrum of **4b**

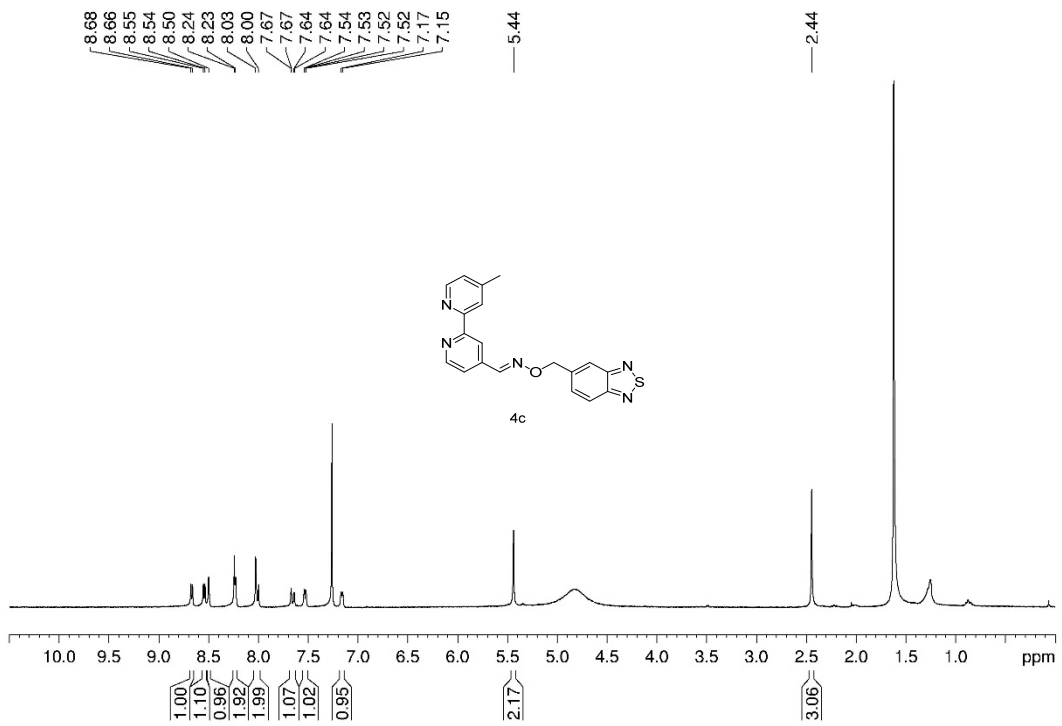


Figure S17. ^{1}H NMR spectrum of **4c**

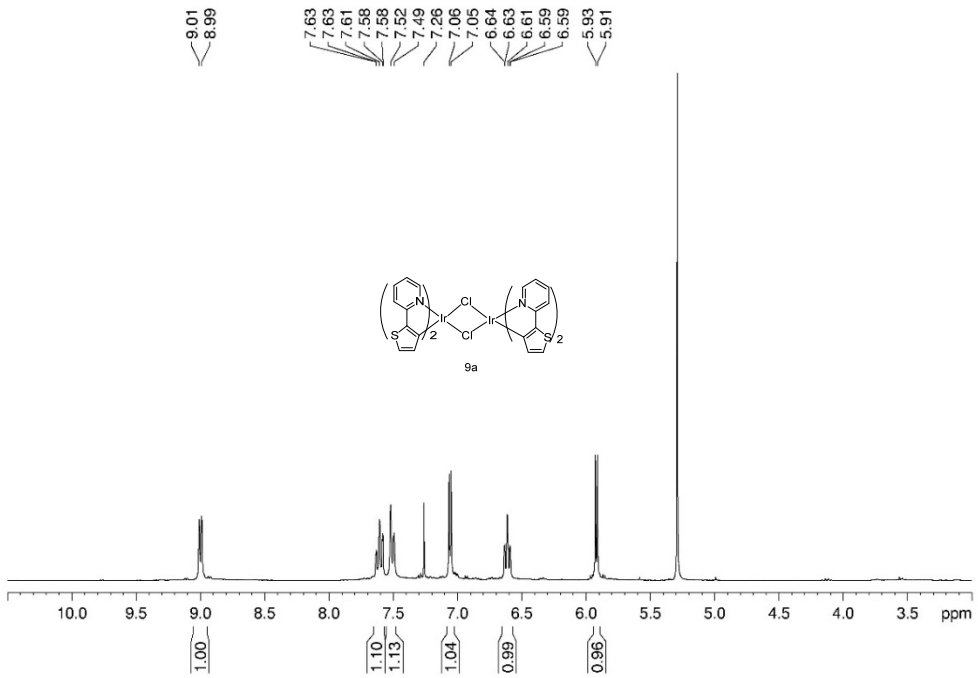


Figure S18. ^{1}H NMR spectrum of **9a**

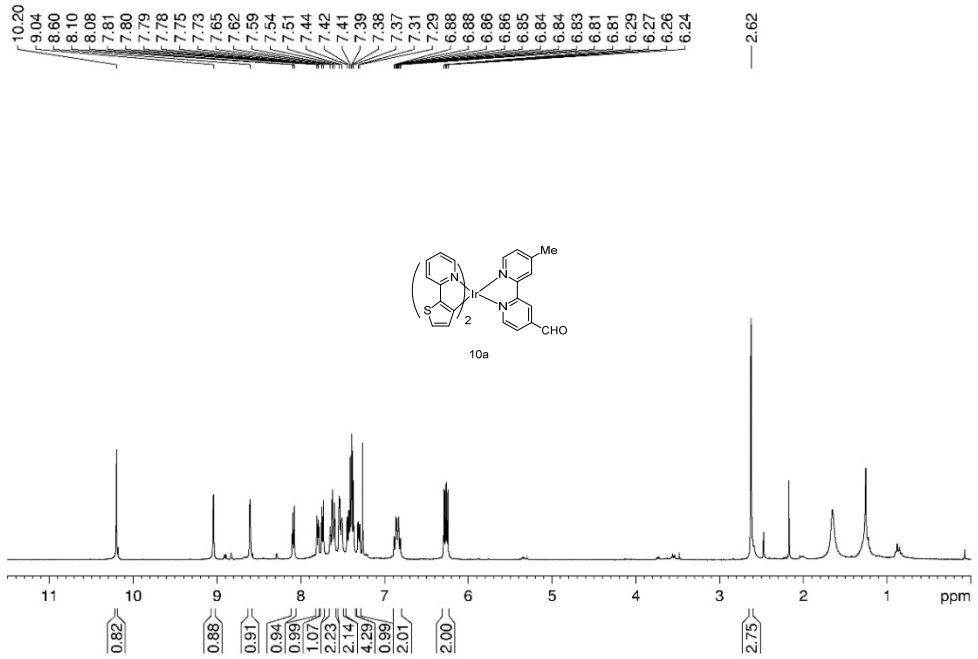


Figure S19. ^{1}H NMR spectrum of **10a**

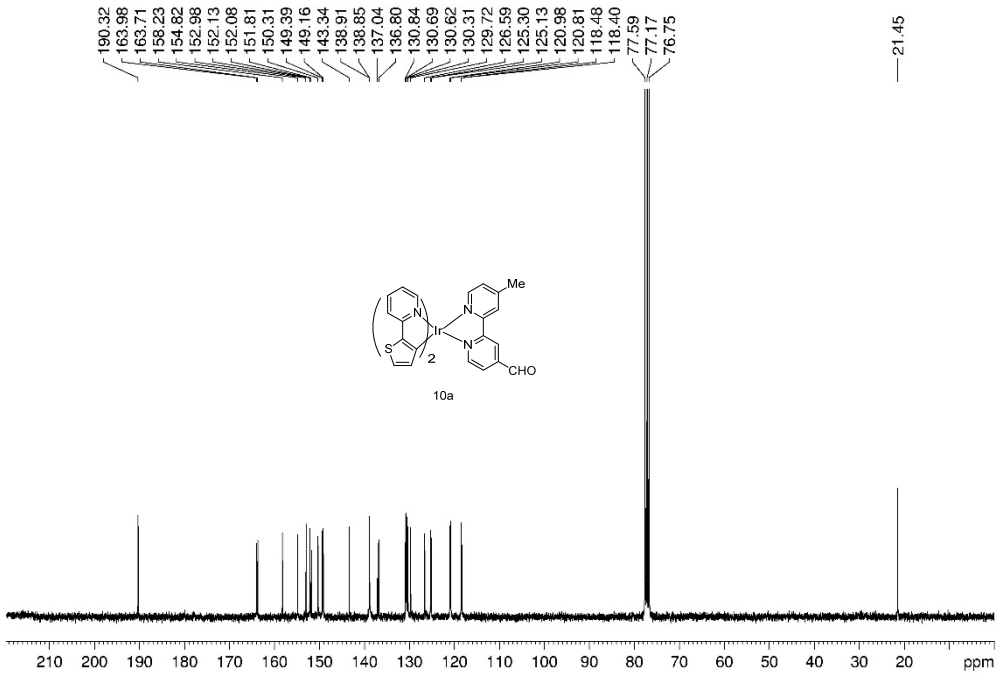


Figure S20. ^{13}C NMR spectrum of **10a**

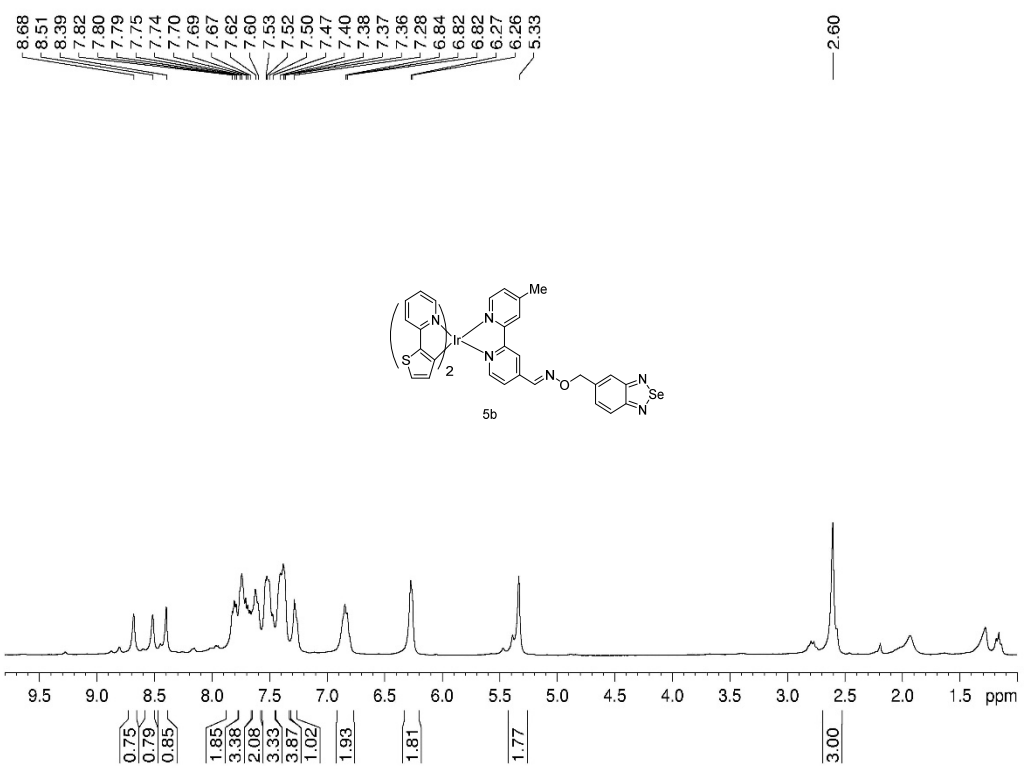


Figure S21. ^{1}H NMR spectrum of **5b**

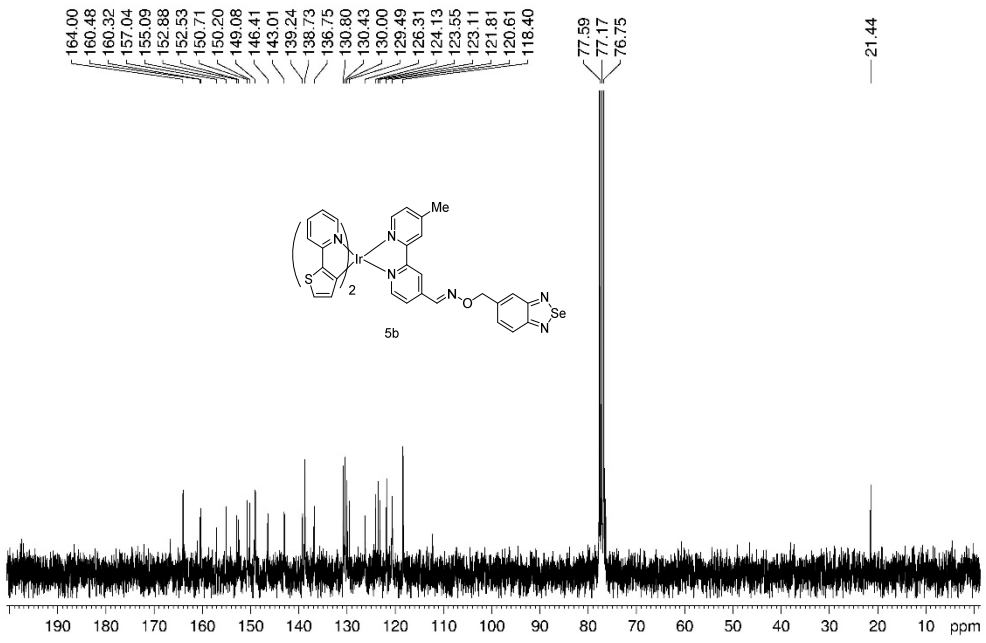


Figure S22. ^{13}C NMR spectrum of **5b**

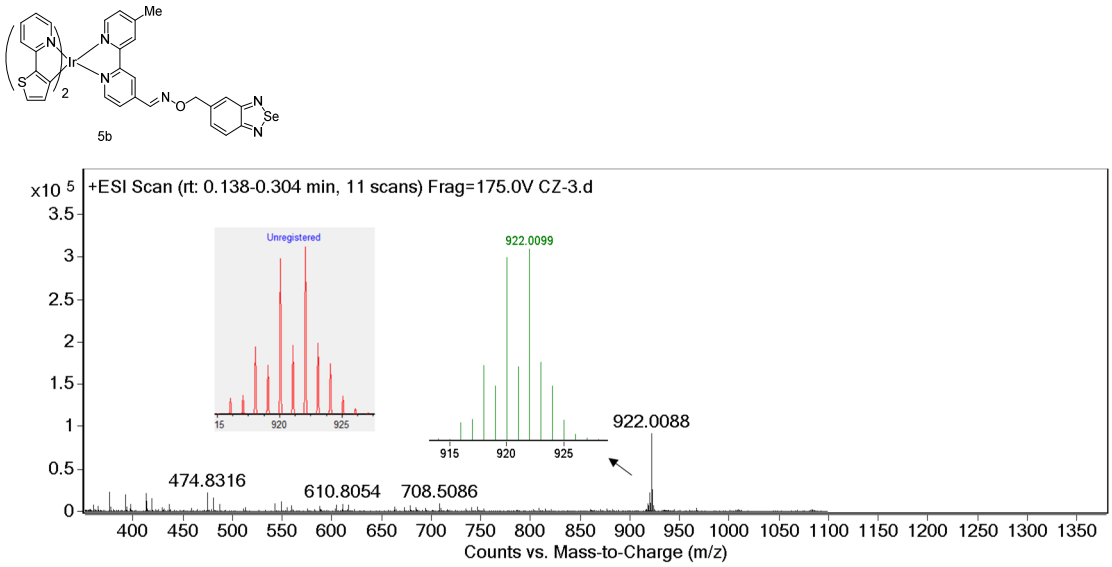


Figure S23. HRMS of **5b**

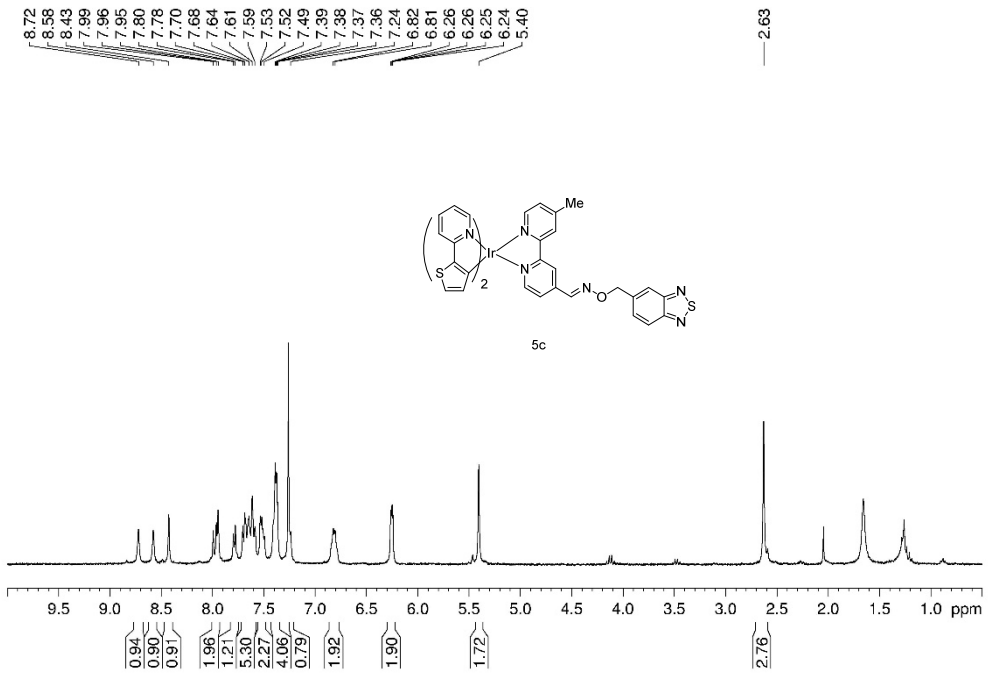


Figure S24. ^{1}H NMR spectrum of **5c**

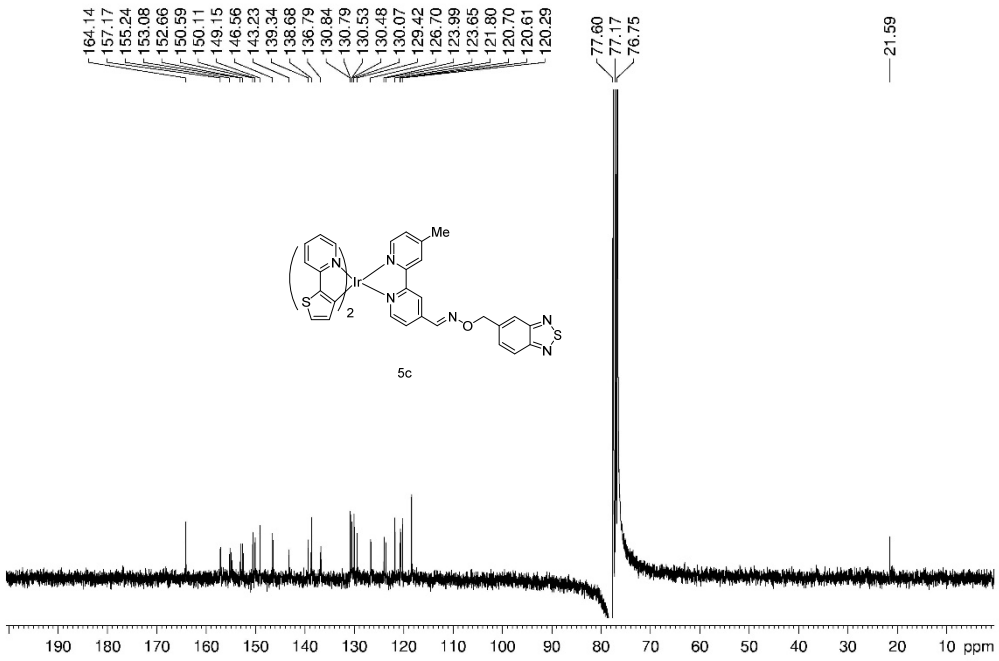


Figure S25. ^{13}C NMR spectrum of **5c**

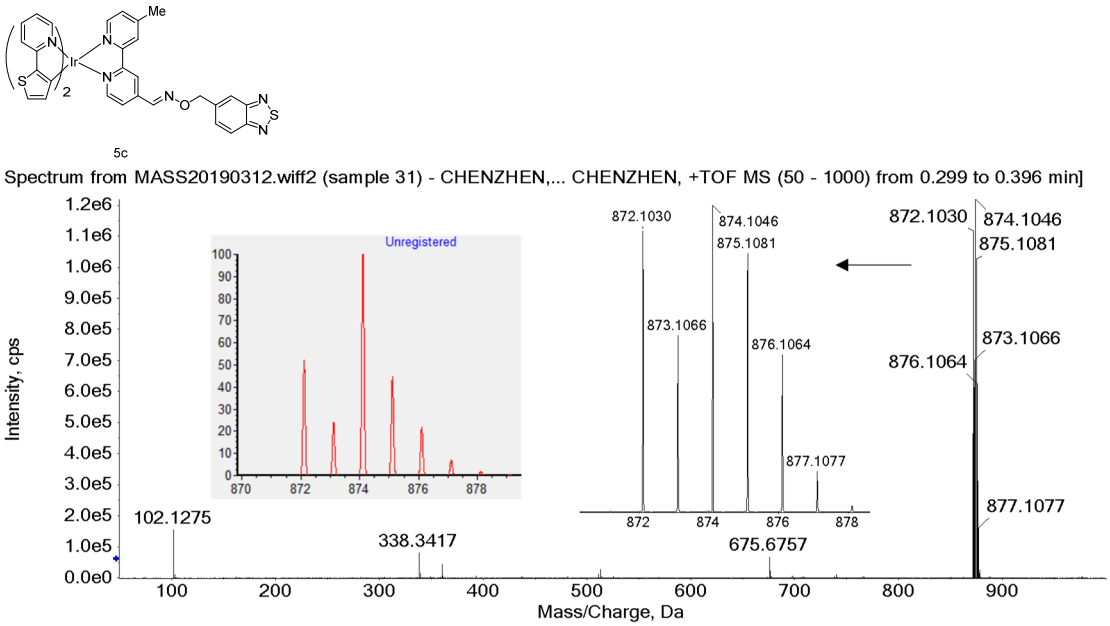


Figure S26. HRMS of **5c**