Electronic Supplementary Materials for

Zwitterionic and Cationic Half-Sandwich Iridium(III) and Ruthenium(II) Complexes Bearing Sulfonate Groups: Synthesis, Characterization and Their Different Biological Activity

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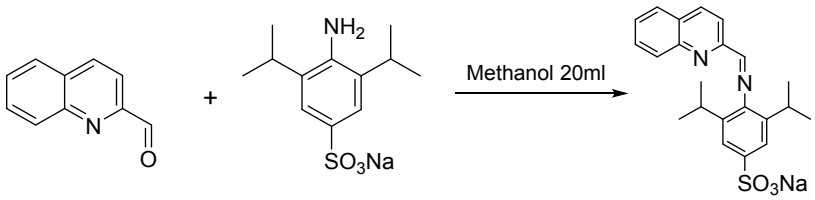
Supporting information includes the experimental details and data.

1. EXPERIMENTAL SECTION

1.1 Materials and instrumentation

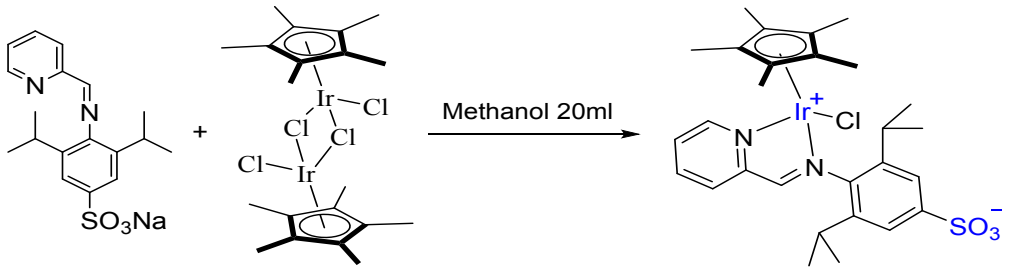
$[(\eta^5\text{-}C_5Me_5)IrCl_2]_2$, $[(\eta^6\text{-p-cym})RuCl_2]_2$ and Ligands **L1**, **L3** and **L4** were synthesized according to the reported methods.[1-3] All other reagents and solvents were purchased from commercial sources and used without purification. For the biological experiments, phosphate-buffered saline (PBS), penicillin/streptomycin mixture, fetal bovine serum, trypsin/EDTA and DMEM medium were purchased from Sangon Biotech.. Testing compounds was dissolved in DMSO and diluted with the tissue culture medium before use. ^{1}H NMR spectra were acquired in 5 mm NMR tubes at 298 K on Bruker DPX 500 and $CDCl_3$ or MeOD-d_4 or DMSO-d_6 or DMSO-d_6/D_2O as solvent. Mass spectra of ligand and complexes were recorded on a Atouflex Speed MALDI-TOF MS. X-ray Diffraction data were obtained on a Bruker Smart CCD area detector with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å).

1.2 Synthesis of the ligand.

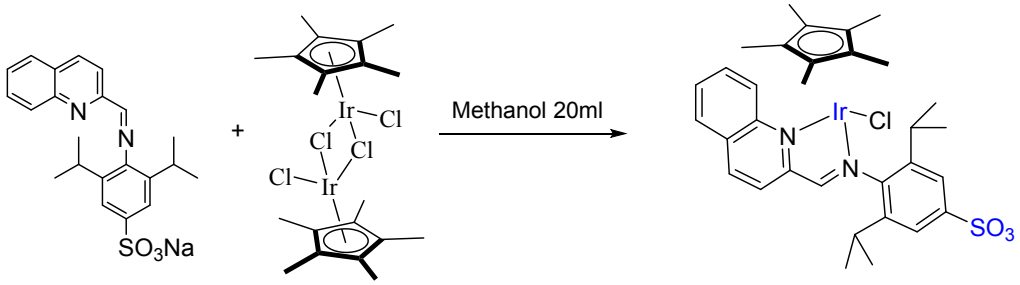


L2. 2-Quinolinaldehyde 0.066 g (0.42 mmol), sodium 4-sulfonate-2,6-diisopropylaniline 0.117 g (0.42 mmol) were dissolved in methanol (20 mL) in a dry round-bottom flask equipped with stirrer. A drop of formic acid was added at room temperature with constant stirring for 24 h. After complete conversion, most of the methanol was removed under reduced pressure and *n*-hexane was added. The solution was placed in a refrigerator overnight and the product was filtered through celite filtration funnel, washed with *n*-hexane and dried. Get yellowish green powder. Yield: 0.10 g (56.6 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (s, 1H), 8.43 (d, *J* = 8.5 Hz, 1H), 8.30 (d, *J* = 8.5 Hz, 1H), 8.20 (d, *J* = 8.2 Hz, 1H), 7.91 (d, *J* = 8.1 Hz, 1H), 7.79 (t, *J* = 7.7 Hz, 1H), 7.63 (t, *J* = 7.5 Hz, 1H), 7.20 (d, *J* = 6.8 Hz, 2H), 7.15 (dd, *J* = 8.7, 6.4 Hz, 1H), 3.01 (dt, *J* = 13.7, 6.8 Hz, 2H), 1.19 (d, *J* = 6.9 Hz, 12H). MALDI-TOF-MS (m/z): calcd for $C_{22}H_{23}N_2Na_2O_3S$ 441.1225, found: 441.1493 $[M+Na]^+$. Anal. calcd for $C_{22}H_{23}N_2NaO_3S$: C, 63.14; H, 5.54; N, 6.69 Found: C, 63.37; H, 5.42; N, 6.61.

1.3 Synthesis of the Complexes.

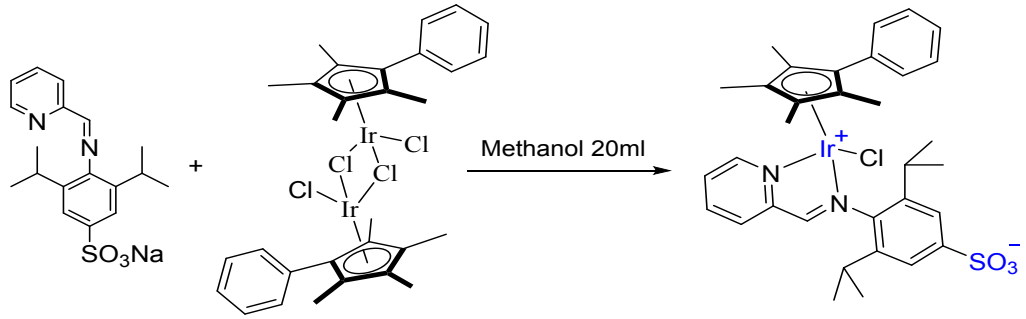


Ir1. $[(\eta^5\text{-}C_5Me_5)IrCl_2]_2$ 0.0241g (0.030 mmol) and **L1** 0.0233 g (0.060 mmol) were dissolved in methanol (20 mL) in a dry round-bottom flask equipped with stirrer. The reaction mixture was stirred for 24 h. After complete conversion, most of the methanol was removed under reduced pressure and diethyl ether was added. The precipitate was filtered through celite filtration funnel and recrystallized by slow diffusion of diethyl ether to a concentrated solution of the compound in methanol. Yield: 0.0243 g (54.8 %). ^{1}H NMR (500 MHz, CD_3OD): δ 9.56 (s, 1H), 9.07 (d, J = 5.4 Hz, 1H), 8.45 (d, J = 7.5 Hz, 1H), 8.33 (t, J = 7.7 Hz, 1H), 8.01 – 7.97 (t, 1H), 7.92 (d, J = 1.8 Hz, 1H), 7.89 (d, J = 1.9 Hz, 1H), 3.97 – 3.93 (m, 1H), 2.55 – 2.51 (m, 1H), 1.55 (s, 15H), 1.44 (d, J = 6.6 Hz, 3H), 1.38 (d, J = 6.7 Hz, 3H), 1.23 (d, J = 6.7 Hz, 3H), 0.99 (d, J = 6.6 Hz, 3H). MALDI-TOF-MS (m/z): calcd for $C_{28}H_{37}ClIrN_2O_3S$ 709.1843, found: 709.1799 $[M+H]^+$. Anal. calcd for $C_{28}H_{36}ClIrN_2O_3S$: C, 47.48; H, 5.12; N, 3.95 Found: C, 47.63; H, 5.18; N, 3.99.

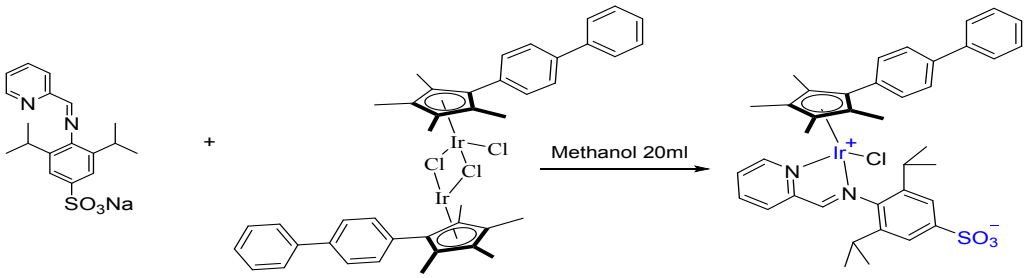


Ir2. $[(\eta^5\text{-}C_5Me_5)IrCl_2]_2$ 0.0239 g (0.030 mmol) and **L2** 0.0251 g (0.060 mmol) were dissolved in methanol (20 mL) in a dry round-bottom flask equipped with stirrer. The reaction mixture was stirred for 24 h. After complete conversion, most of the methanol was removed under reduced pressure and diethyl ether was added. The precipitate was filtered through celite filtration funnel and recrystallized by slow diffusion of ether to a concentrated solution of the compound in methanol. Yield: 0.0285 g (60.3 %). ^{1}H NMR (500 MHz, CD_3OD): δ 9.70 (s, 1H), 8.84 (d, J = 8.2 Hz, 1H), 8.43 (d, J = 8.3 Hz, 1H), 8.38 (d, J = 8.8 Hz, 1H), 8.27 (d, J = 8.1 Hz, 1H), 8.18 – 8.14 (t, 1H), 8.02 (t, J = 7.3 Hz, 1H), 7.97 (d, J = 1.8 Hz, 1H), 7.90 (d, J = 1.8 Hz, 1H), 4.18 – 4.13 (m, 1H), 2.73 – 2.69 (m, 1H), 1.52 (s, 15H), 1.49 (d, J = 6.6 Hz, 3H), 1.39 (t, J = 7.0 Hz, 6H), 0.92 (d,

J = 6.7 Hz, 3H). MALDI-TOF-MS (m/z): calcd for $C_{32}H_{39}ClIrN_2O_3S$ 759.1999, found: 759.1972 $[M+H]^+$. Anal. calcd for $C_{32}H_{38}ClIrN_2O_3S$: C, 50.68; H, 5.05; N, 3.69. Found: C, 50.70; H, 5.01; N, 3.66.

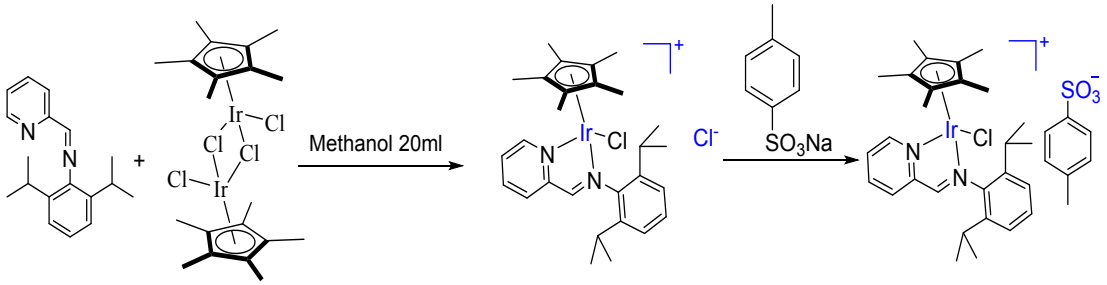


Ir3. $[(\eta^5\text{-}C_5Me_4C_6H_5)IrCl_2]_2$ 0.1392g (0.151 mmol) and **L1** 0.1345 g (0.365 mmol) were dissolved in methanol (20 mL) in a dry round-bottom flask equipped with stirrer. The reaction mixture was stirred for 24 h. After complete conversion, most of the methanol was removed under reduced pressure and diethyl ether was added. The precipitate was filtered through celite filtration funnel and recrystallized by slow diffusion of diethyl ether to a concentrated solution of the compound in methanol. Yield: 0.1633 g (70.2 %).1H NMR (500 MHz, CD_3OD)：δ 9.55 (s, 1H), 9.01 (d, J = 5.4 Hz, 1H), 8.51 (d, J = 7.5 Hz, 1H), 8.39 (t, J = 7.6 Hz), 7.99 (t, J = 6.2 Hz), 7.85 (d, J = 1.5 Hz, 1H), 7.72 (d, J = 1.5 Hz, 1H), 7.46 (t, J = 7.4 Hz, 1H), 7.39 (t, J = 7.6 Hz, 2H), 7.28 (d, J = 7.4 Hz, 2H), 3.99 (m, J = 13.5, 6.7 Hz, 1H), 2.19 (m, 1H), 1.74 (s, 3H), 1.68 (s, 3H), 1.62 (s, 3H), 1.36 (d, J = 6.7 Hz, 3H), 1.20 (s, 3H), 1.05 (d, J = 6.7 Hz, 3H), 0.99 (d, J = 6.6 Hz, 3H), 0.63 (d, J = 6.6 Hz, 3H). MALDI-TOF-MS (m/z): calcd for $C_{33}H_{39}ClIrN_2O_3S$: 771.1999, found: 771.2857, $[M+H]^+$. Anal. calcd for $C_{33}H_{38}ClIrN_2O_3S$: C, 51.45; H,4.97; N, 3.64. Found: C, 51.73; H, 5.00; N, 3.68.

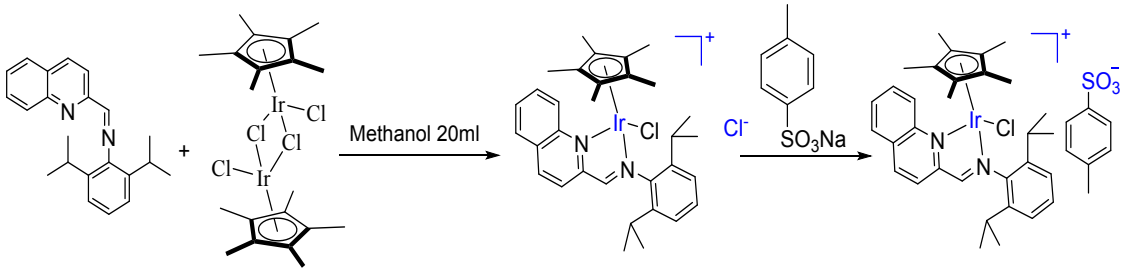


Ir4. $[(\eta^5\text{-}C_5Me_4C_{12}H_9)IrCl_2]_2$ 0.1852g (0.173 mmol) and **L1** 0.1272 g (0.345 mmol) were dissolved in methanol (20 mL) in a dry round-bottom flask equipped with stirrer. The reaction mixture was stirred for 24 h. After complete conversion, most of the methanol was removed under reduced pressure and diethyl ether was added. The precipitate was filtered through celite filtration funnel and recrystallized by slow diffusion of diethyl ether to a concentrated solution of the

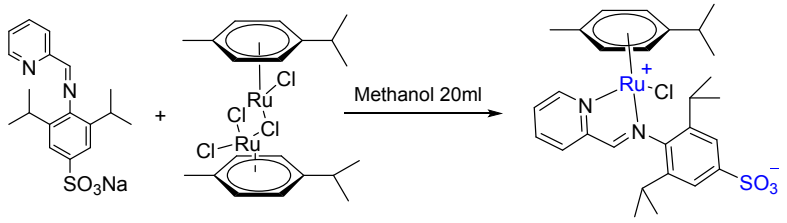
compound in methanol. Yield: 0.1199 g (41.1 %).1H NMR (500 MHz, CD_3OD)： δ 9.57 (s, 1H), 9.05 (d, J = 5.5 Hz, 1H), 8.52 (d, J = 7.6 Hz, 1H), 8.40 (t, J = 7.8 Hz, 1H), 8.01 (t, J = 6.7 Hz, 1H), 7.85 (d, J = 1.5 Hz, 1H), 7.73 (d, J = 1.6 Hz, 1H), 7.68 – 7.58 (m, 4H), 7.46 (t, J = 7.6 Hz, 2H), 7.38 (m, J = 11.7, 7.8 Hz, 3H), 4.00 (m, 1H), 2.23 (m, 1H), 1.80 (s, 3H), 1.70 (s, 3H), 1.64 (s, 3H), 1.36 (d, J = 6.7 Hz, 3H), 1.24 (s, 3H), 1.06 (d, J = 6.7 Hz, 3H), 1.00 (d, J = 6.6 Hz, 3H), 0.65 (d, J = 6.6 Hz, 3H). MALDI-TOF-MS (m/z): calcd for $C_{39}H_{43}ClIrN_2O_3S$: 847.2312, found: 847.2589, [M+H]+. Anal. calcd for $C_{39}H_{42}ClIrN_2O_3S$: C, 55.34; H,5.00; N, 3.31. Found: C, 55.47; H, 5.06; N, 3.48.



Ir5. $[(\eta^5\text{-}C_5Me_5)IrCl_2]_2$ 0.0837 g (0.105 mmol) and **L3** 0.0580 g (0.218 mmol) were dissolved in methanol (20 mL) in a dry round-bottom flask equipped with stirrer. The reaction mixture was stirred for 24 h. After complete conversion, most of the methanol was removed under reduced pressure and 0.0521 g (0.217 mmol) of sodium p-toluenesulfonate was added at room temperature with constant stirring for 1 h. Diethyl ether was added and the precipitate was filtered through celite filtration funnel and recrystallized by slow diffusion of ether to a concentrated solution of the compound in methanol. Yield: 0.1067 g (56.3 %). ^{1}H NMR (500 MHz, CD_3OD): δ 9.49 (s, 1H), 9.05 (d, *J* = 5.5 Hz, 1H), 8.42 (d, *J* = 7.7 Hz, 1H), 8.31 (t, *J* = 7.7 Hz, 1H), 7.97 (t, *J* = 7.4 Hz, 1H), 7.71 (d, *J* = 8.1 Hz, 2H), 7.46 (t, *J* = 10.7 Hz, 3H), 7.23 (d, *J* = 7.9 Hz, 2H), 3.90 (m, *J* = 13.4, 6.7 Hz, 1H), 2.49 (m, *J* = 13.3, 6.7 Hz, 1H), 2.37 (s, 3H), 1.52 (s, 15H), 1.40 (d, *J* = 6.7 Hz, 3H), 1.33 (d, *J* = 6.8 Hz, 3H), 1.20 (d, *J* = 6.7 Hz, 3H), 0.96 (d, *J* = 6.6 Hz, 3H). MALDI-TOF-MS (m/z): calcd for $C_{28}H_{37}ClIrN_2$ 629.2275, found: 629.4719 $[(\eta^5\text{-}C_5Me_5)Ir(N\text{^}N)Cl]^+$. Anal. calcd for $C_{35}H_{44}ClIrN_2O_3S$: C, 52.52; H, 5.54; N, 3.50. Found: C, 52.56; H, 5.52; N, 3.54.

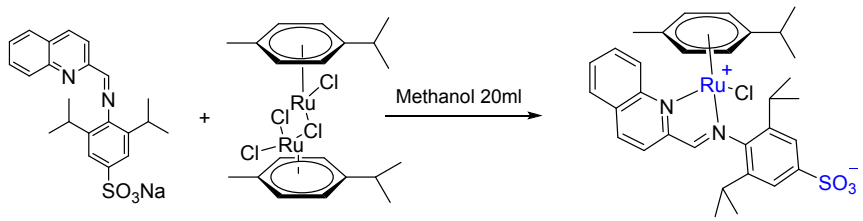


Ir6. $[(\eta^5\text{-}C_5Me_5)IrCl_2]_2$ 0.0321 g (0.040 mmol) and **L4** 0.0255 g (0.080 mmol) were dissolved in methanol (20 mL) in a dry round-bottom flask equipped with stirrer. The reaction mixture was stirred for 24 h. After complete conversion, most of methanol was removed under reduced pressure and 0.0155 g (0.080 mmol) of sodium p-toluenesulfonate was added at room temperature with constant stirring for 1 h. Diethyl ether was added and the precipitate was filtered through celite filtration funnel and recrystallized by slow diffusion of ether to a concentrated solution of the compound in methanol. Yield: 0.0412 g (55.8%). ^{1}H NMR (500 MHz, CD_3OD): δ 9.65 (s, 1H), 8.82 (d, *J* = 8.2 Hz, 1H), 8.40 (d, *J* = 8.3 Hz, 1H), 8.37 (d, *J* = 8.9 Hz, 1H), 8.26 (d, *J* = 8.0 Hz, 1H), 8.15 (t, *J* = 7.9 Hz, 1H), 8.00 (t, *J* = 7.2 Hz, 1H), 7.70 (d, *J* = 8.2 Hz, 2H), 7.56 – 7.49 (m, 2H), 7.45 (m, *J* = 6.5, 2.7 Hz, 1H), 7.22 (d, *J* = 7.9 Hz, 2H), 4.12 (dt, *J* = 13.4, 6.7 Hz, 1H), 2.68 (dt, *J* = 13.0, 6.5 Hz, 1H), 2.36 (s, 3H), 1.51 (s, 15H), 1.46 (d, *J* = 6.6 Hz, 3H), 1.39 (d, *J* = 6.7 Hz, 3H), 1.35 (d, *J* = 6.7 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H). MALDI-TOF-MS (m/z): calcd for $C_{32}H_{39}ClIrN_2$ 679.2431, found: 679.2202 $[(\eta^5\text{-}C_5Me_5)Ir(N\text{^}N)Cl]^+$. Anal. calcd for $C_{39}H_{46}ClIrN_2O_3S$: C, 55.07; H, 5.45; N, 3.29. Found: C, 55.11; H, 5.47; N, 3.21.

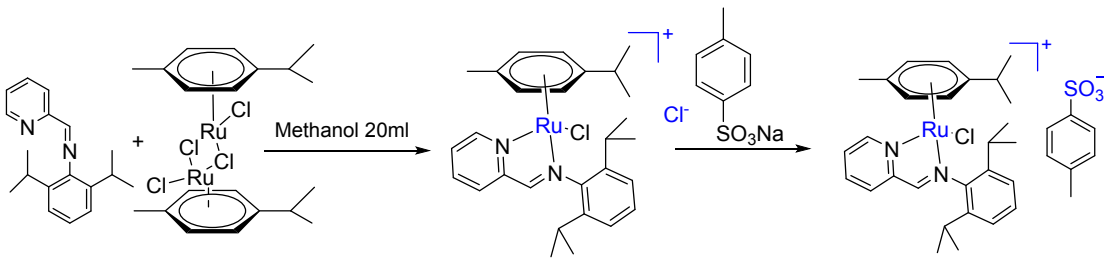


Ru1. $[(\eta^6\text{-p-cym})RuCl_2]_2$ 0.0218 g (0.035 mmol) and **L1** 0.0262 g (0.071 mmol) were dissolved in methanol (20 mL) in a dry round-bottom flask equipped with stirrer. The reaction mixture was stirred for 24 h. After complete conversion, most of the methanol was removed under reduced pressure and diethyl ether was added. The precipitate was filtered through celite filtration funnel and recrystallized by slow diffusion of diethyl ether to a concentrated solution of the compound in methanol. Yield: 0.0274 g (61.0 %). ^{1}H NMR (500 MHz, CD_3OD): δ 9.60 (d, *J* = 5.4 Hz, 1H), 8.93 (s, 1H), 8.32 – 8.26 (m, 2H), 7.94 – 7.88 (m, 3H), 5.98 (d, *J* = 6.3 Hz, 1H), 5.65 (d, *J* = 6.4

Hz, 1H), 5.48 (d, *J* = 5.9 Hz, 1H), 5.28 (d, *J* = 5.9 Hz, 1H), 3.86 (m, *J* = 13.5, 6.7 Hz, 1H), 2.77 – 2.68 (m, 2H), 2.23 (s, 3H), 1.56 (d, *J* = 6.8 Hz, 3H), 1.37 (d, *J* = 6.7 Hz, 3H), 1.23 (d, *J* = 6.7 Hz, 3H), 1.17 (d, *J* = 6.9 Hz, 3H), 1.14 (d, *J* = 6.9 Hz, 3H), 0.93 (d, *J* = 6.7 Hz, 3H). MALDI-TOF-MS (m/z): calcd for $C_{28}H_{36}ClN_2O_3RuS$ 617.1179, found: 617.1383 $[M+H]^+$. Anal. calcd for $C_{28}H_{35}ClN_2O_3RuS$: C, 54.58; H, 5.73 N, 4.55. Found: C, 54.60; H, 5.78; N, 4.59.

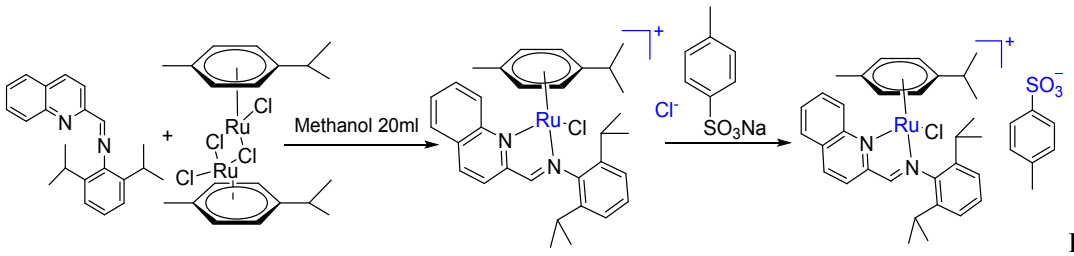


Ru2. A solution of $[(\eta^6\text{-p-cym})RuCl_2]_2$ 0.0254 g (0.041 mmol) and **L2** 0.0343 g (0.082 mmol) were dissolved in methanol (20 mL) in a dry round-bottom flask equipped with stirrer. The reaction mixture was stirred for 24 h. After complete conversion, most of the methanol was removed under reduced pressure and diethyl ether was added. The precipitate was filtered through celite filtration funnel and recrystallized by slow diffusion of diethyl ether to a concentrated solution of the compound in methanol. Yield: 0.0331 g (59.4%). ^{1}H NMR (500 MHz, CD_3OD): δ 9.14 (s, 1H), 8.86 (d, *J* = 8.2 Hz, 1H), 8.81 (d, *J* = 8.9 Hz, 1H), 8.33 (d, *J* = 8.2 Hz, 1H), 8.27 (d, *J* = 8.5 Hz,1H), 8.24 – 8.19 (m, 1H), 8.06 – 8.01 (m, 1H), 8.00 (d, *J* = 1.9 Hz, 0.6H), 7.93 (d, *J* = 1.8 Hz, 1H), 7.90 (s, 0.4H), 6.34 (d, *J* = 6.5 Hz, 1H), 6.01 (d, *J* = 6.7 Hz, 1H), 5.52 (d, *J* = 6.2 Hz, 1H), 5.20 (d, *J* = 5.9 Hz, 1H), 4.06 – 4.02 (m, 1H), 2.73 (m, *J* = 13.5, 6.8 Hz, 1H), 2.43 – 2.38 (m, 1H), 2.28 (s, 3H), 1.59 (d, *J* = 6.7 Hz, 6H), 1.44 – 1.29 (m, 12H), 1.06 (d, *J* = 6.9 Hz, 6H), 0.89 (d, *J* = 6.7 Hz, 6H), 0.67 (d, *J* = 6.9 Hz, 6H). MALDI-TOF-MS (m/z): calcd for $C_{32}H_{38}ClN_2O_3RuS$ 667.1335, found: 667.1496 $[M+H]^+$. Anal. calcd for $C_{32}H_{37}ClN_2O_3RuS$: C, 57.69; H, 5.60; N, 4.20. Found: C, 57.63; H, 5.65; N, 4.23.



Ru3. $[(\eta^6\text{-p-cym})RuCl_2]_2$ 0.0612 g (0.100 mmol) and **L3** 0.0533 g (0.200 mmol) were dissolved in methanol (20 mL) in a dry round-bottom flask equipped with stirrer. The reaction mixture was

stirred for 24 h. After complete conversion, most methanol was removed under reduced pressure and 0.0583 g(0.300 mmol) of sodium p-toluenesulfonate was added at room temperature with constant stirring for 1 h. Diethyl ether was added and the precipitate was filtered through celite filtration funnel and recrystallized by slow diffusion of ether to a concentrated solution of the compound in methanol. Yield: 0.0727 g (51.3%). ^{1}H NMR (500 MHz, CD_3OD): δ 9.60 (d, J = 5.3 Hz, 1H), 8.87 (s, 1H), 8.32 – 8.23 (m, 2H), 7.90 (t, J = 6.0 Hz, 1H), 7.71 (d, J = 7.9 Hz, 2H), 7.51 (d, J = 6.2 Hz, 2H), 7.44 (m, 1H), 7.23 (d, J = 7.8 Hz, 2H), 5.95 (d, J = 6.3 Hz, 1H), 5.64 (d, J = 6.2 Hz, 1H), 5.48 (d, J = 6.7 Hz, 1H), 5.23 (d, J = 5.9 Hz, 1H), 3.81 (m, 1H), 2.77 – 2.67 (m, 2H), 2.37 (s, 2H), 2.22 (s, 3H), 1.53 (d, J = 6.7 Hz, 3H), 1.33 (d, J = 6.7 Hz, 3H), 1.22 (d, J = 6.7 Hz, 3H), 1.17 – 1.12 (m, 6H), 0.91 (d, J = 6.6 Hz, 3H). MALDI-TOF-MS (m/z): calcd for $C_{28}H_{36}ClN_2Ru$ 537.1611, found: 537.1231 $[(\eta^6\text{-p-cym})Ru(N\text{^}N)Cl]^+$. Anal. calcd for $C_{35}H_{43}ClN_2O_3RuS$: C, 59.35; H, 6.12; N, 3.95. Found: C, 59.56; H, 6.37; N, 4.03.



Ru

4. $[(\eta^6\text{-p-cym})RuCl_2]_2$ 0.0476 g (0.078 mmol) and **L4** 0.0492 g (0.155 mmol) were dissolved in methanol (20 mL) in a dry round-bottom flask equipped with stirrer. The reaction mixture was stirred for 24 h. After complete conversion, most methanol was removed under reduced pressure and 0.0301 g(0.155 mmol) of sodium p-toluenesulfonate was added at room temperature with constant stirring for 1 h. Diethyl ether was added and the precipitate was filtered through celite filtration funnel and recrystallized by slow diffusion of ether to a concentrated solution of the compound in methanol. Yield: 0.0677 g (53.5%). ^{1}H NMR (500 MHz, CD_3OD) δ 9.08 (d, *J* = 13.5 Hz, 1H), 8.84 (t, *J* = 7.9 Hz, 2H), 8.32 (d, *J* = 8.2 Hz, 1H), 8.27 (d, *J* = 8.0 Hz, 1H), 8.23 (t, *J* = 7.9 Hz, 1H), 8.03 (t, *J* = 7.5 Hz, 1H), 7.71 (d, *J* = 8.2 Hz, 1H), 7.65 – 7.54 (m, 2H), 7.54 – 7.48 (m, 1H), 7.23 (d, *J* = 8.0 Hz, 1H), 6.32 (d, *J* = 6.1 Hz, 1H), 6.02 (d, *J* = 6.3 Hz, 1H), 5.56 – 5.49 (d, 1H), 5.16 (d, *J* = 5.8 Hz, 1H), 4.01 (m, *J* = 13.4, 6.7 Hz, 1H), 2.75 – 2.66 (m, 1H), 2.41 (m, *J* = 13.1, 6.5 Hz, 1H), 2.42 – 2.29 (m, 3H), 2.29 (d, *J* = 15.7 Hz, 3H), 1.58 (d, *J* = 6.7 Hz, 3H), 1.40

(dd, J = 13.4, 6.7 Hz, 6H), 1.05 (d, J = 11.2 Hz, 3H), 0.90 (d, J = 8.0 Hz, 3H), 0.65 (dd, J = 34.1, 6.9 Hz, 3H). MALDI-TOF-MS (m/z): calcd for $C_{32}H_{38}ClN_2Ru$ 587.1762, found: 587.1800 [(η^6-p-cym)Ru(N^N)Cl]$^+$. Anal. calcd for $C_{39}H_{45}ClN_2O_3RuS$: C, 61.77; H, 5.98; N, 3.69. Found: C, 61.76; H, 5.93; N, 3.62.

1.4 NMR Spectroscopy.

^{1}H NMR spectra were captured in 5 mm NMR tubes at 298 K on Bruker DPX 500 (^{1}H = 500.13 MHz) spectrometers. ^{1}HNMR chemical shifts were internally referenced to $(CHD_2)(CD_3)SO$ (2.50 ppm) for DMSO-d_6, $CHCl_3$ (7.26 ppm) for chloroform-d_1, CHD_2OD (3.33 ppm) for methanol-d_4. All data processing was done using XWIN-NMR version 3.6 (Bruker UK Ltd.).

1.5 UV-Vis Spectroscopy.

The properties of the compounds were determined by TU-1901 UV spectrophotometer. Quartz cuvette path length is 1 cm (3 mL). Spectra were processed using UV Winlab software. If there is no particular requirement, the experiment will be carried out at room temperature.

1.6 Stability Studies.

Solutions of **Ir1** and **Ir5** in DMSO-d_6 were prepared by dissolution of the complexes, respectively. ^{1}H NMR spectra were recorded at 310 K. Two samples maintained at 310 K for 24 hours and their ^{1}H NMR spectra were measured.

Solutions of **Ir1** and **Ir5** in MeOH-d_4 were prepared by dissolution of the complexes, respectively. ^{1}H NMR spectra were recorded at 310 K. Two samples maintained at 310 K for 24 hours and their ^{1}H NMR spectra were measured.

Solutions of complexes **Ir1** and **Ir5** with final concentrations of 1 mM in 80% DMSO-d_6/20% D_2O (v/v) were prepared by dissolution of the complexes in DMSO-d_6 followed by rapid dilution with D_2O, respectively. ^{1}H NMR spectra were recorded at 310 K. Two samples maintained at 310 K for 24 hours and their ^{1}H NMR spectra were measured.

1.7 Measurement of Lipophilicity.

The octanol-water partition coefficients (P) of **Ir1** or **Ir5** were determined using a shake-flask method. Water (50 mL, distilled after milli-Q purification) and 1-octanol (50 mL, vacuum distilled) were shaken together using a laboratory shaker, for 24 h to allow saturation of both phases. Stock solutions of the three complexes (50 μM) were prepared in the aqueous phase and aliquots (5 mL) of each of these stock solutions were then added to an equal volume of the 1-octanol phase. The resultant biphasic solutions were mixed for 2 h and then centrifuged (3000 × g, 5 min) to separate

the phases. The concentrations of the complex in the organic and aqueous phases were then determined using UV-vis (260 nm). Log P was defined as the logarithm of the ratio of the concentrations of the complex in the organic and aqueous phases (Log P = Log; values reported are the means of three separate determinations).

1.8 Cellular-metal Accumulation.

A549 cells were seeded in 35 mm dishes for 24 h. The media was removed and replaced with fresh media containing the tested complexes (5 μM) for 12 h. After the removal of the culture media and rinse with 1 mL of PBS buffer (1×), the cells were treated with 500 μl of 0.25% trypsin and centrifuged at 1000 rpm. The cells were counted, and digested with concentrated nitric acid (65%, 225 μL) at 353 k overnight. The solution was then diluted to a final volume of 3 mL with Milli-Q water. The concentration of iridium was determined directly by the inductively coupled plasma mass spectrometer (ICP-MS; VG Elemental). The experiment was performed in triplicate, and the average of the data was obtained.

1.9 Viability Assay (MTT assay)

After plating 5000 cells per well in 96-well plates, the cells were preincubated in drug-free media at 310 K for 24 h before adding different concentrations of the compounds to be tested. In order to prepare the stock solution of the drug, the solid complex was dissolved in DMSO. This stock was further diluted using cell culture medium until working concentrations were achieved. The drug exposure period was 48 h. Subsequently, 15 μL of 5 mg mL^{-1} MTT solution was added to form a purple formazan. Afterwards, 100 μL of dimethyl sulfoxide (DMSO) was transferred into each well to dissolve the purple formazan, and results were measured using a microplate reader (DNM-9606, Perlong Medical, Beijing, China) at an absorbance of 570 nm. Each well was triplicated and each experiment repeated at least three times. IC_{50} values quoted are mean ± SEM.

1.10 Cell Cycle Analysis.

The A549 cancer cells at 1.5 × 10^6 per well were seeded in a six-well plate.[4] Cells were preincubated in drug-free media at 310 K for 24 h, after which **Ir1** or **Ir5** was added at concentrations of 5.1, 10.3, 20.5 and 41.0 μM of **Ir1** or **Ir5** against A549 cancer cells. After 48 h of drug exposure, supernatants were removed by suction and cells were washed with PBS. Finally, cells were harvested using trypsin-EDTA and fixed for 24 h using cold 70 % ethanol. DNA staining was achieved by resuspending the cell pellets in PBS containing propidium iodide (PI) and RNAse. Cell pellets were washed and resuspended in PBS before being analyzed in a flow cytometer (ACEA NovoCyte, Hangzhou, China) using excitation of DNA-bound PI at 488 nm, with emission at 585 nm. Data were processed using NovoExpress™ software. The cell cycle distribution is shown as the percentage of cells containing G_0/G_1, S and G_2/M DNA as identified

by propidium iodide staining.

1.11 Induction of Apoptosis.

Flow cytometry analysis of apoptotic populations of the cells caused by exposure to ruthenium complexes was carried out using the Annexin V-FITC Apoptosis Detection Kit (Beyotime Institute of Biotechnology, China) according to the supplier's instructions. Briefly, A549 cancer cells (1.5×10^6 / 2 mL per well) were seeded in a six-well plate. Cells were preincubated in drug-free media at 310 K for 24 h, after which **Ir1** and **Ir5** were added at concentrations of 10.3, 20.5, 41.0 and 61.5 μM against A549 cancer cells. After 48 h of drug exposure, cells were collected, washed once with PBS, and resuspended in 195 μl of annexin V-FITC binding buffer which was then added to 5 μl of annexin V-FITC and 10 μl of PI, and then incubated at room temperature in the dark for 15 min. Subsequently, the buffer placed in an ice bath in the dark. The samples were analyzed by a flow cytometer (ACEA NovoCyte, Hangzhou, China).

1.12 Mitochondrial Membrane Assay.

Analysis of the changes of mitochondrial potential in cells after exposure to ruthenium complexes was carried out using the Mitochondrial membrane potential assay kit with JC-1 (Beyotime Institute of Biotechnology, Shanghai, China) according to the manufacturer's instructions. Briefly, 1.5×10^6 A549 cancer cells were seeded in six-well plates left to incubate for 24 h in drug-free medium at 310 K in a humidified atmosphere. Drug solutions, at concentrations of 5.1, 10.3, 20.5 and 41.0 μM of **Ir1** or **Ir5** against A549 cancer cells, were added in triplicate, and the cells were left to incubate for a further 48 h under similar conditions. Supernatants were removed by suction, and each well was washed with PBS before detaching the cells using trypsin-EDTA. Staining of the samples was done in flow cytometry tubes protected from light, incubating for 30 min at ambient temperature. The samples were immediately analyzed by a flow cytometer (ACEA NovoCyte, Hangzhou, China). For positive controls, the cells were exposed to carbonyl cyanide 3-chlorophenylhydrazone, CCCP (5 μM), for 20 min. Data were processed using NovoExpress™ software.

1.13 ROS Determination.

Flow cytometry analysis of ROS generation in the cells caused by exposure to ruthenium complexes was carried out using the CellROX™ Deep Red Flow Cytometry Assay Kit (Invitrogen) according to the supplier's instructions. Briefly, 1.5×10^6 A549 cancer cells per well were seeded in a six-well plate. Cells were preincubated in drug-free media at 310 K for 24 h in a 5 % CO_2 humidified atmosphere, and then Ir4 was added at concentrations of 5.1 and 10.3 μM of **Ir1** or **Ir5** against A549 cancer cells. After 48 h of drug exposure, Oxidative stress was evaluated with CellRox Deep Red and flow cytometry according to the manufacturer's instructions.

Notes and References

1. Z. Liu, A. Habtemariam, A. M. Pizarro, S. A. Fletcher, A. Kisova, O. Vrana, L. Salassa, P. C. A. Bruijnincx, G. J. Clarkson, V. Brabec and P. J. Sadler, *J. Med. Chem.*, 2011, **54**, 3011-3026.
2. S. B. Jensen, S. J. Rodger and M. D. Spicer, *J. Organomet. Chem.*, 1998, **556**, 151-158.
3. M. E. Hanhan and Y. Senemoglu, *Transit. Metal Chem.*, 2012, **37**, 109-116.
4. J. Li, L. Guo, Z. Tian, M. Tian, S. Zhang, K. Xu, Y. Qian and Z. Liu, *Dalton Trans.*, 2017, **46**, 15520-15534.

2. [1]HNMR SPECTURE DATA

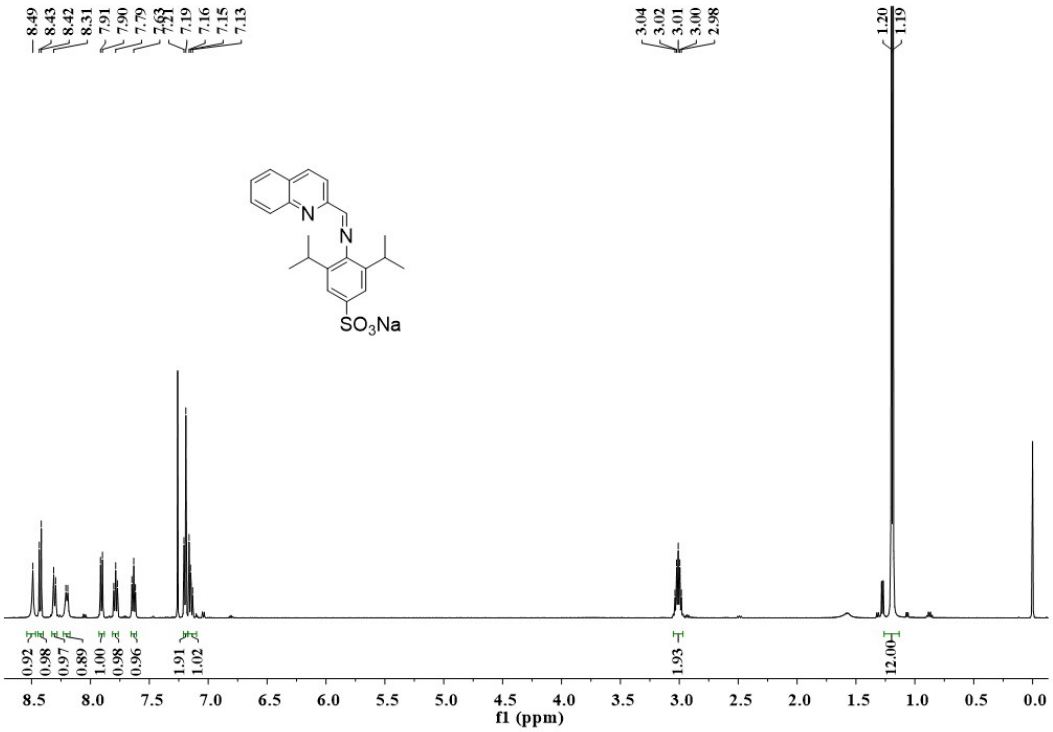


Figure S1 The [1]H NMR (500.13 MHz, $CDCl_3$) spectra of **L2**.

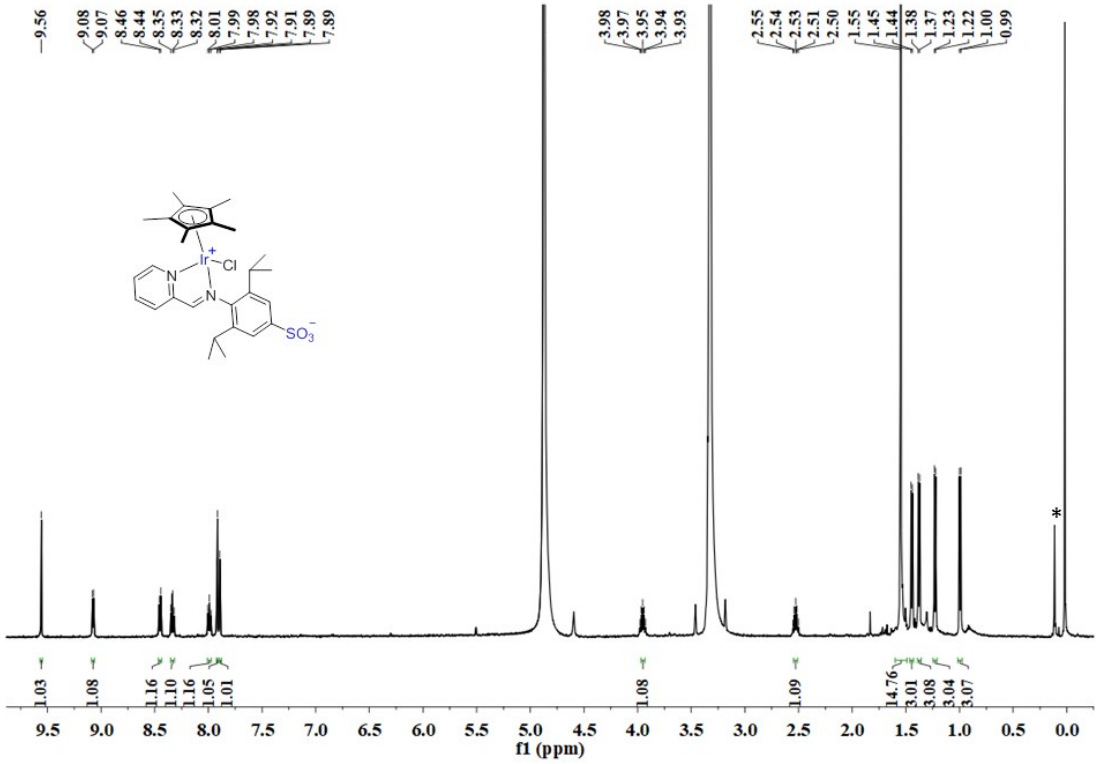


Figure S2 The [1]H NMR (500.13 MHz, MeOD-d_4) spectra of **Ir1**. *silicone grease.

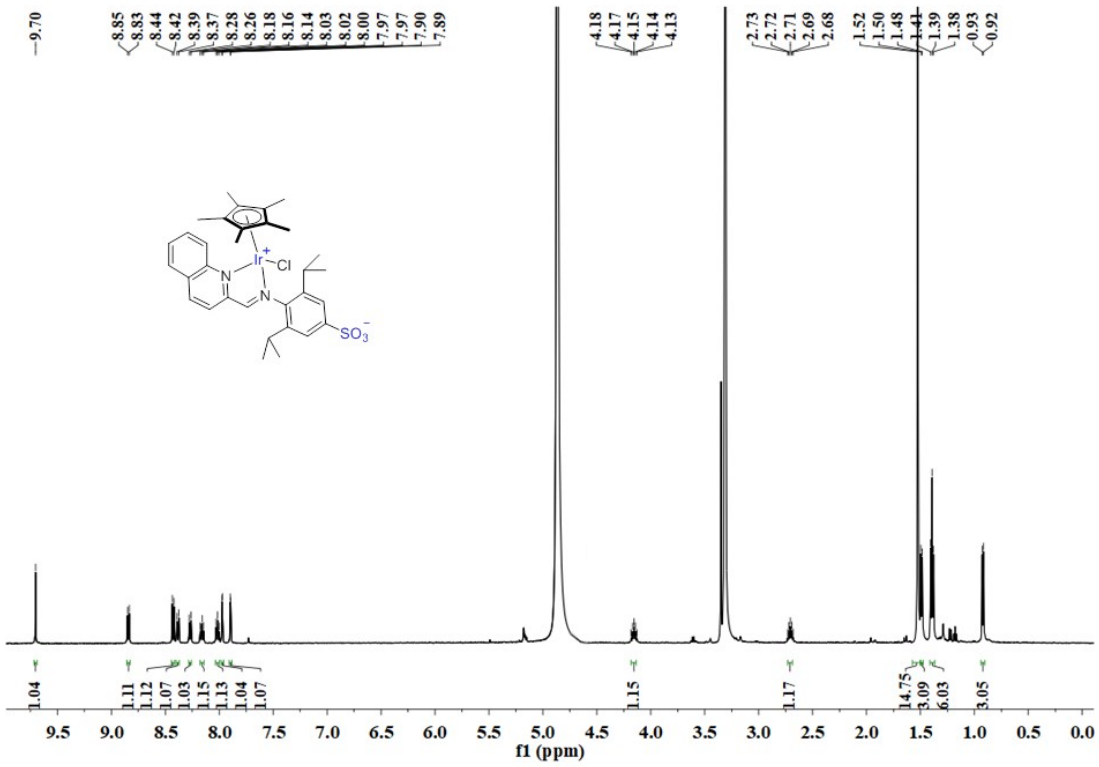


Figure S3 The ^{1}H NMR (500.13 MHz, MeOD-d_4) spectra of **Ir2**.

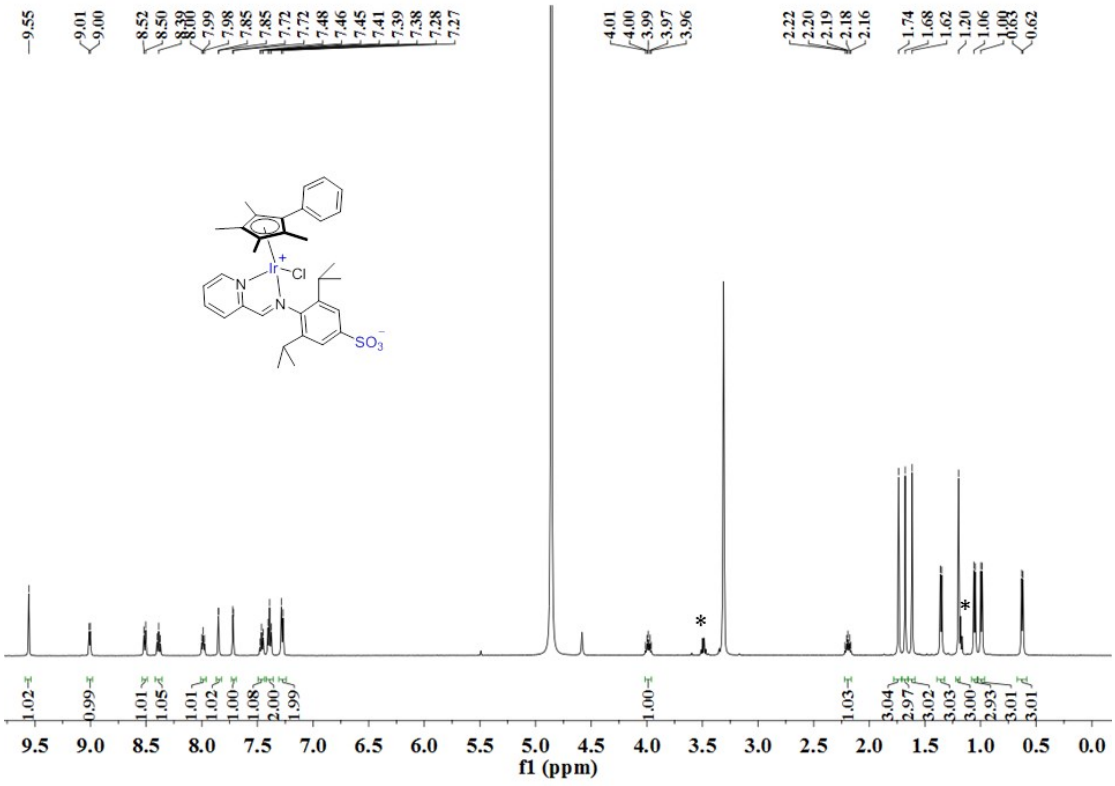


Figure S4 The ^{1}H NMR (500.13 MHz, MeOD-d_4) spectra of **Ir3**. *Ether

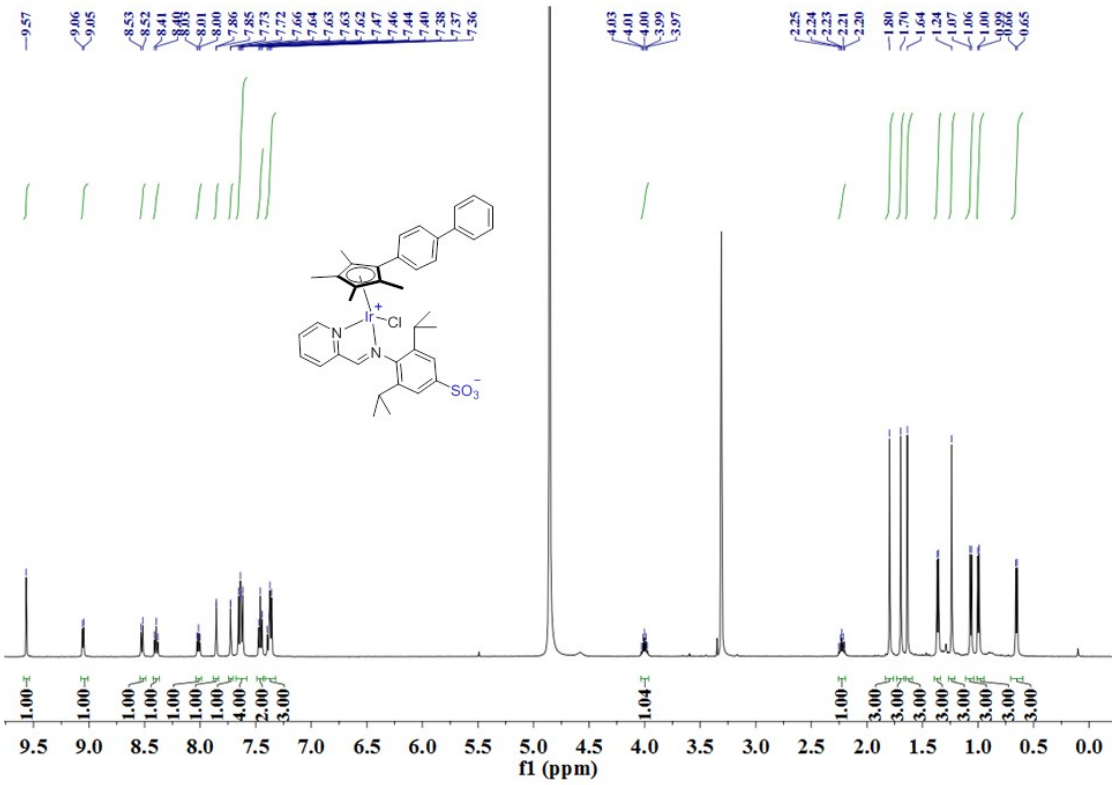


Figure S5 The ^{1}H NMR (500.13 MHz, MeOD-d_4) spectra of **Ir4**.

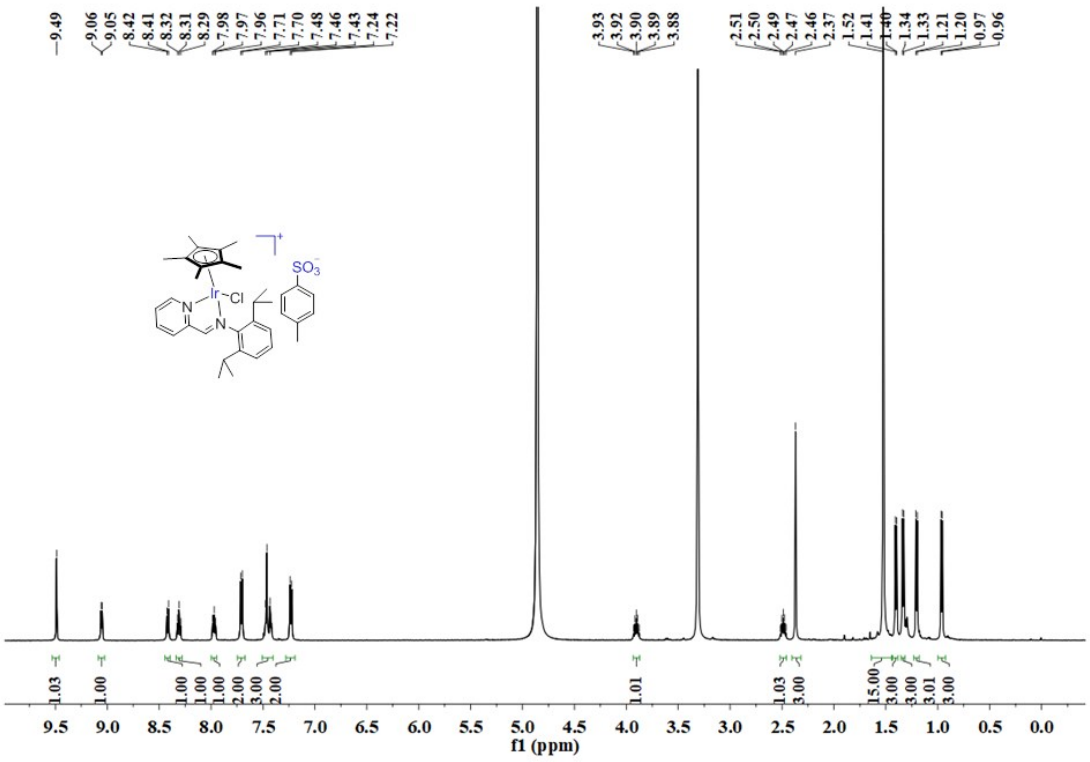


Figure S6 The ^{1}H NMR (500.13 MHz, MeOD-d_4) spectra of **Ir5**.

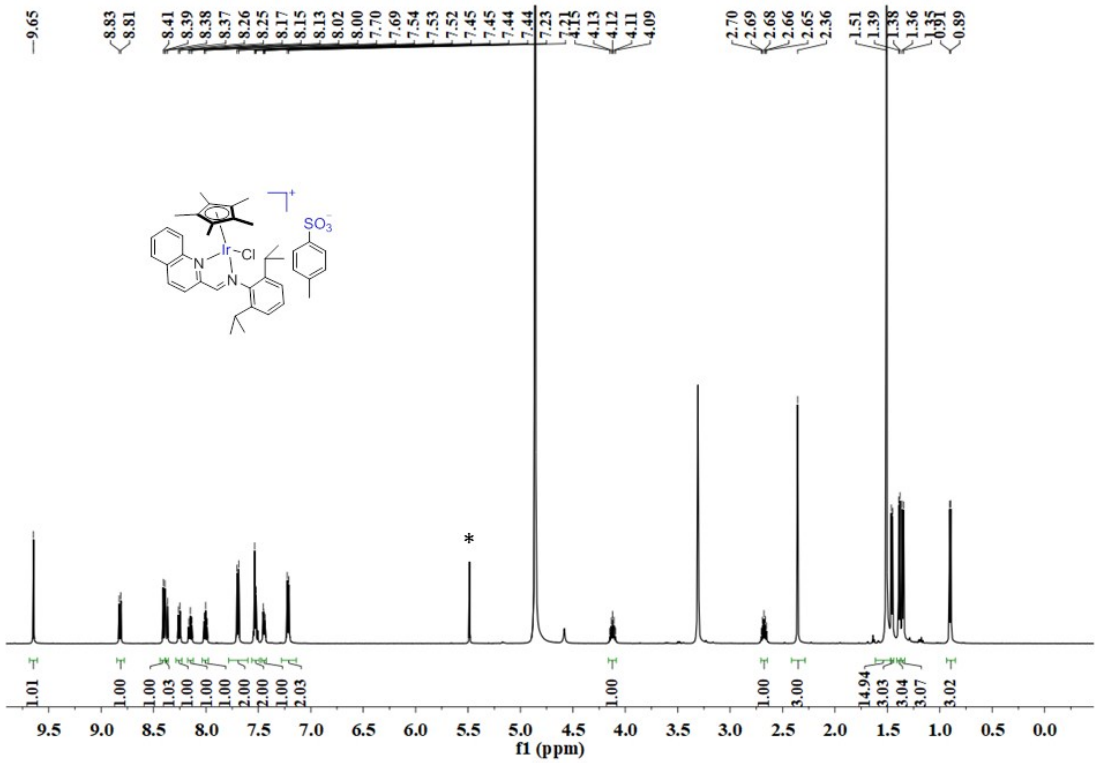


Figure S7 The ^{1}H NMR (500.13 MHz, MeOD-d_4) spectra of **Ir6**. *CH_2Cl_2.

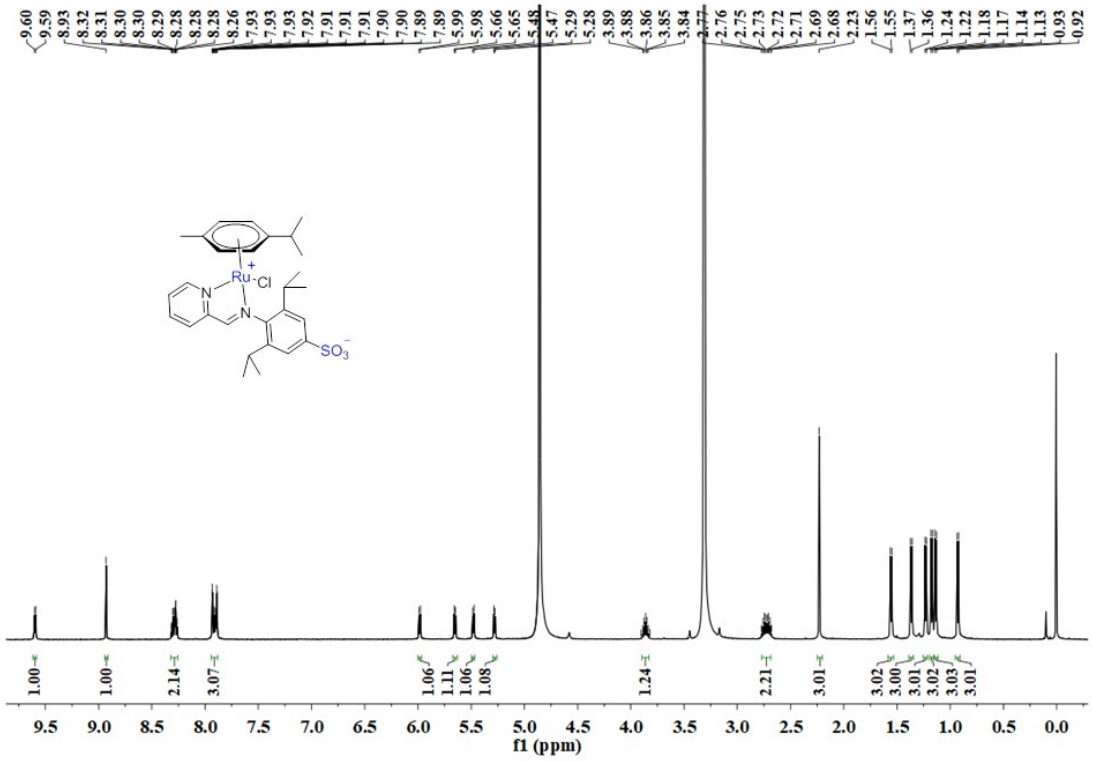


Figure S8 The ^{1}H NMR (500.13 MHz, MeOD-d_4) spectra of **Ru1**.

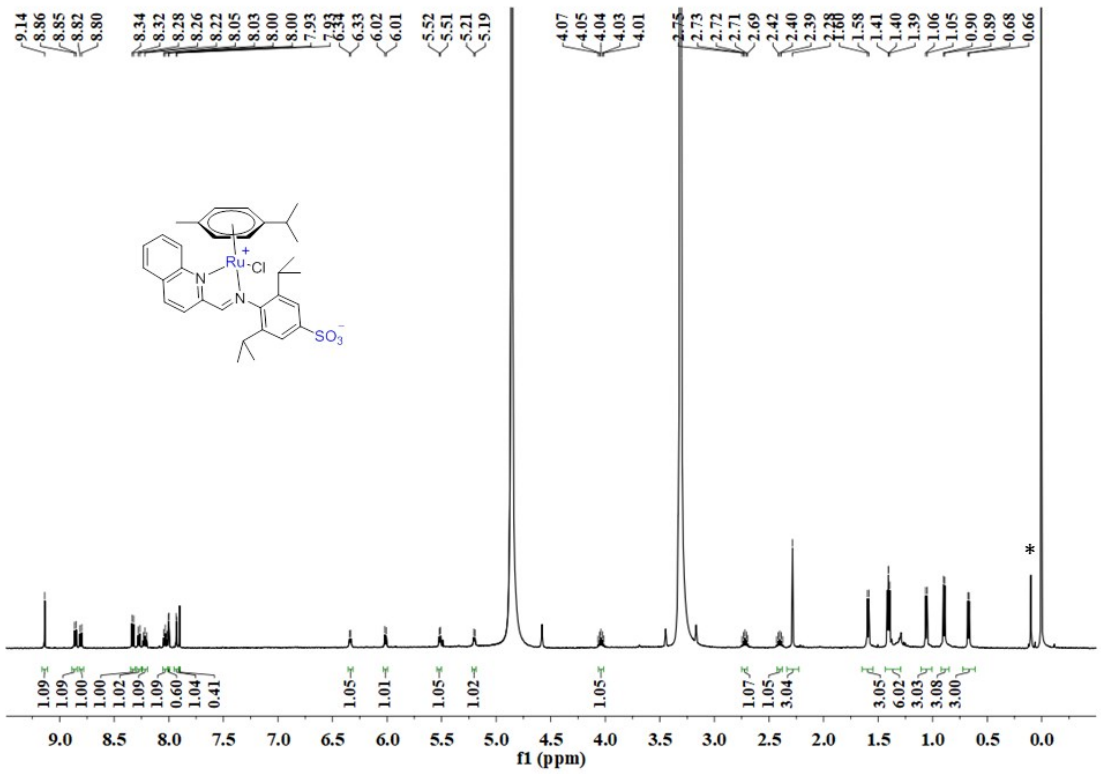


Figure S9 The ^{1}H NMR (500.13 MHz, MeOD-d_4) spectra of **Ru2**. *silicone grease

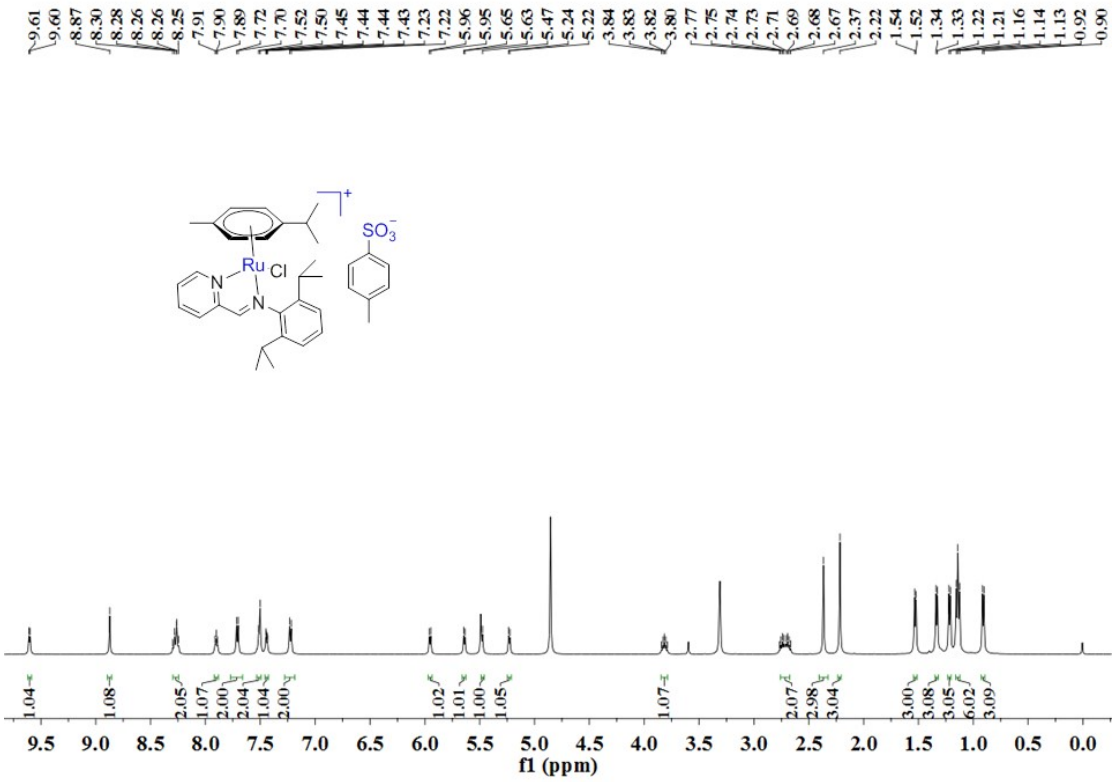


Figure S10 The ^{1}H NMR (500.13 MHz, MeOD-d_4) spectra of **Ru3**.

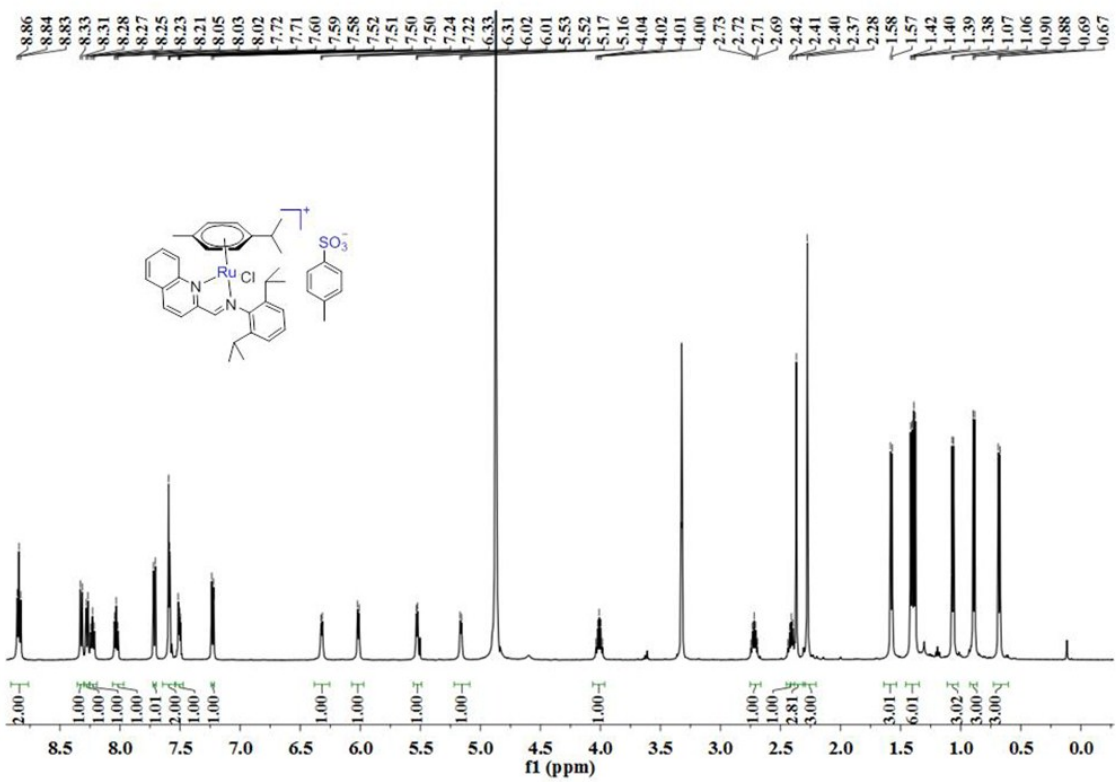


Figure S11 The ^{1}H NMR (500.13 MHz, MeOD-d_4) spectra of **Ru4**.

3. MALDI-TOF-MS DATA

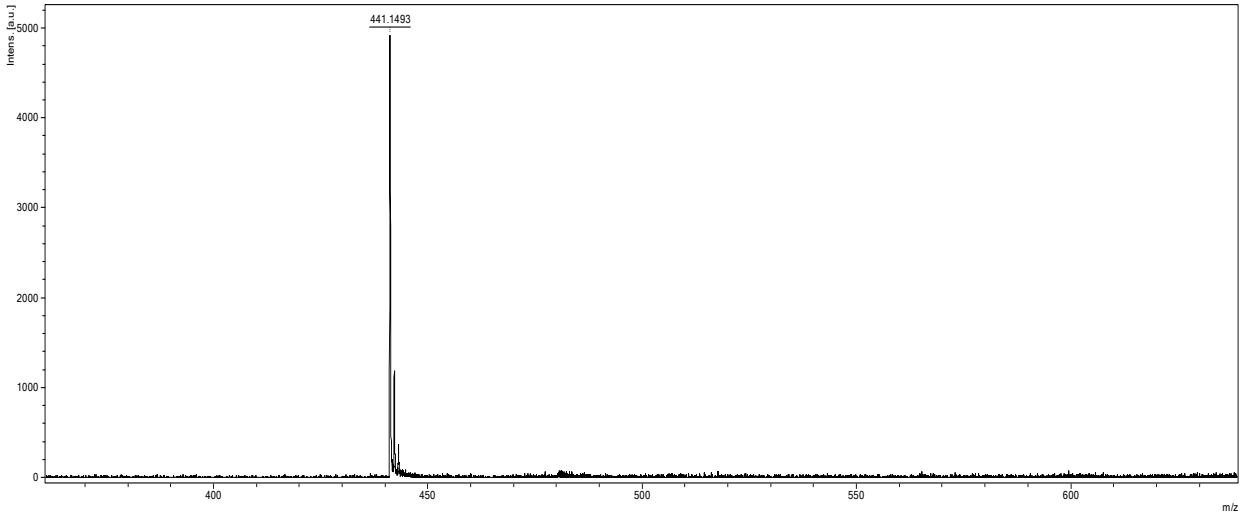


Figure S12 MALDI-TOF-MS of **L2**.

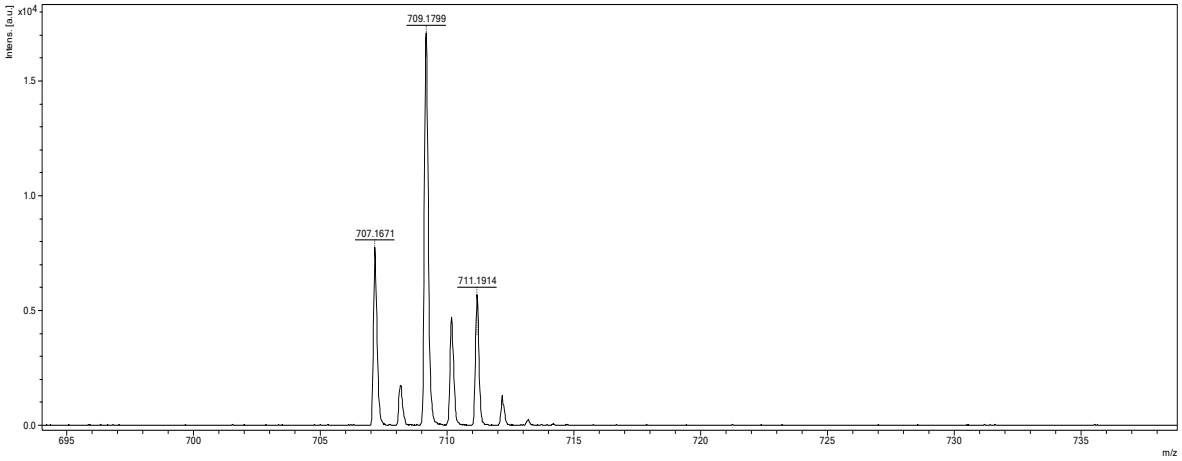


Figure S13 MALDI-TOF-MS of **Ir1**.

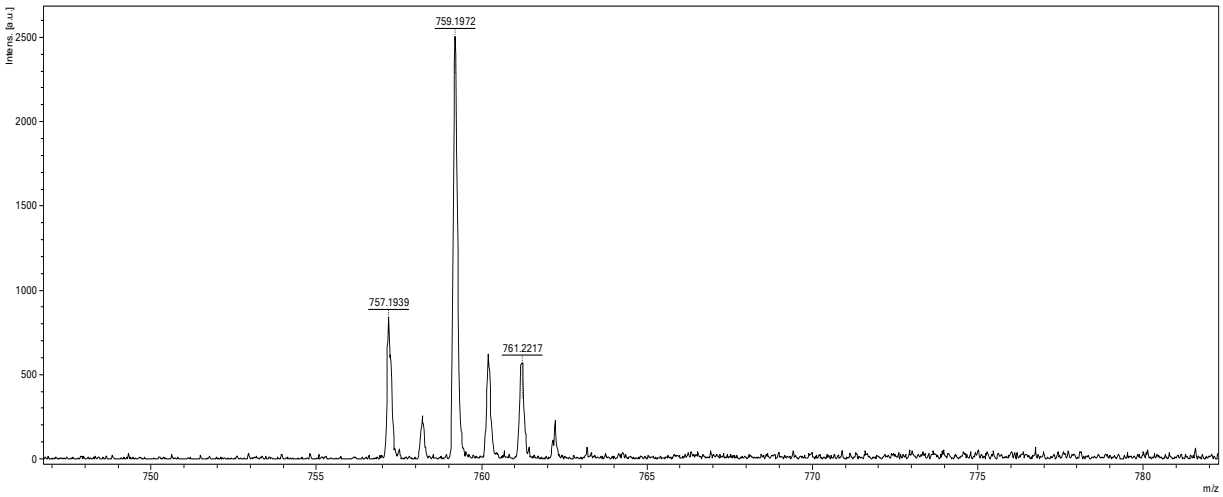


Figure S14 MALDI-TOF-MS of **Ir2**.

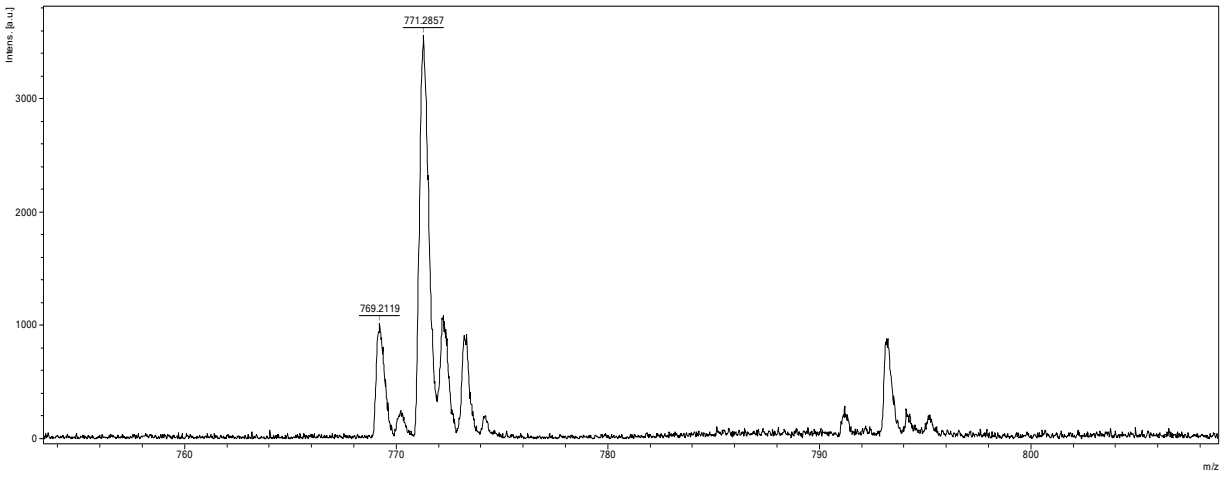


Figure S15 MALDI-TOF-MS of **Ir3**.

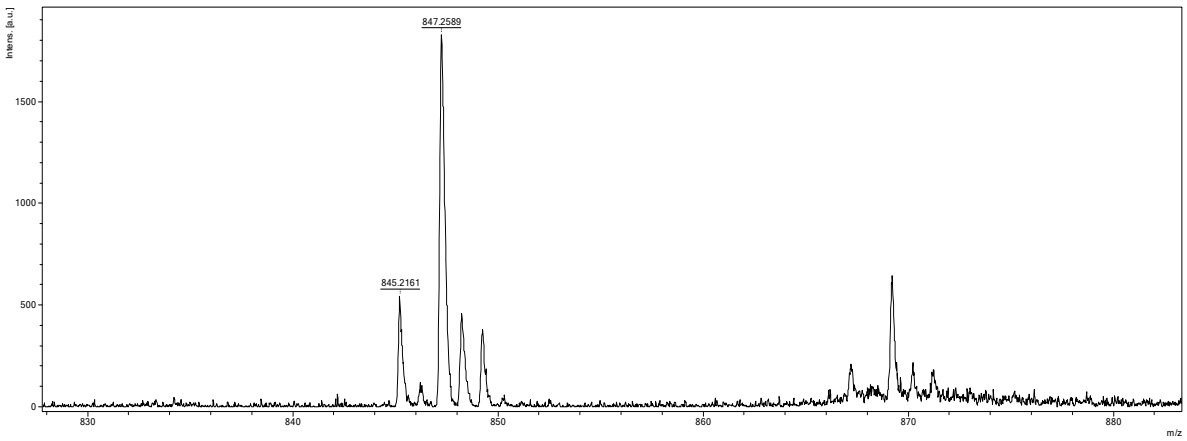


Figure S16 MALDI-TOF-MS of **Ir4**.

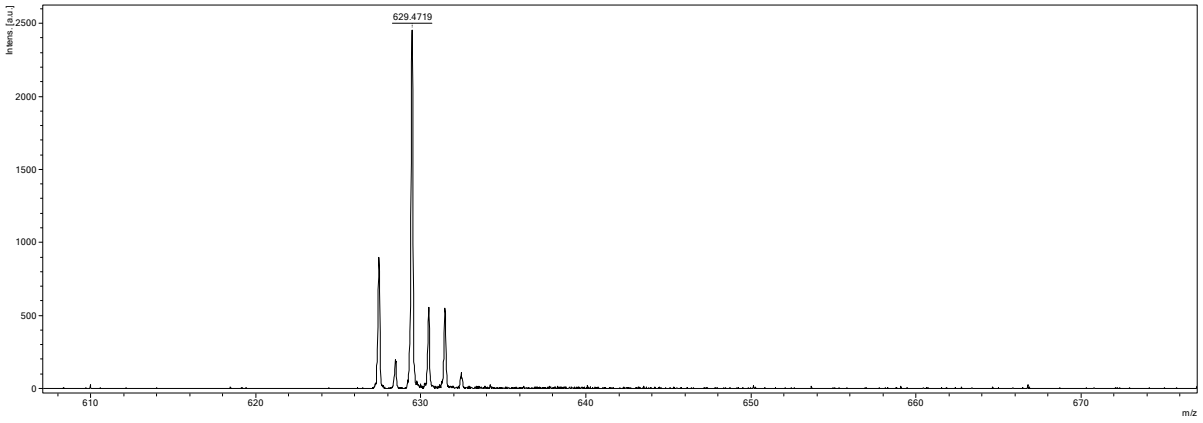


Figure S17 MALDI-TOF-MS of **Ir5**.

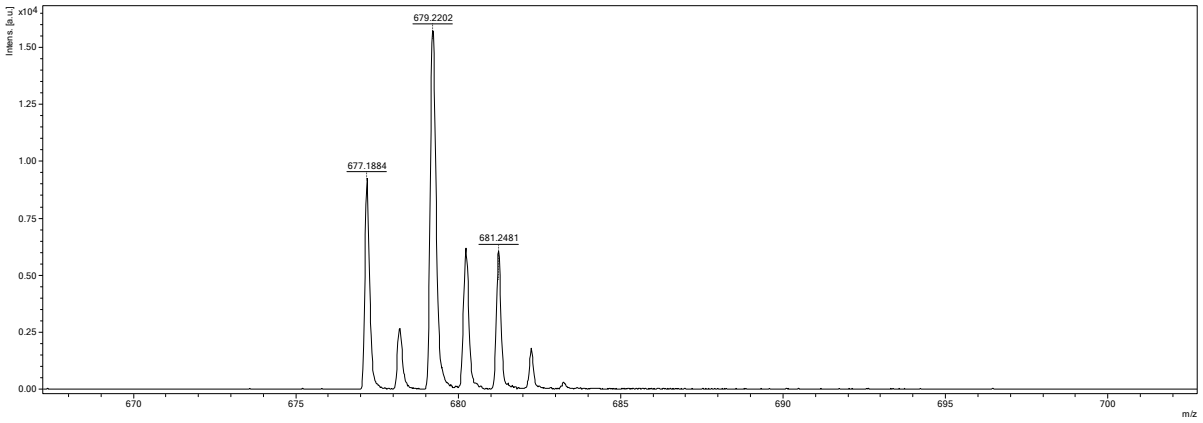


Figure S18 MALDI-TOF-MS of **Ir6**.

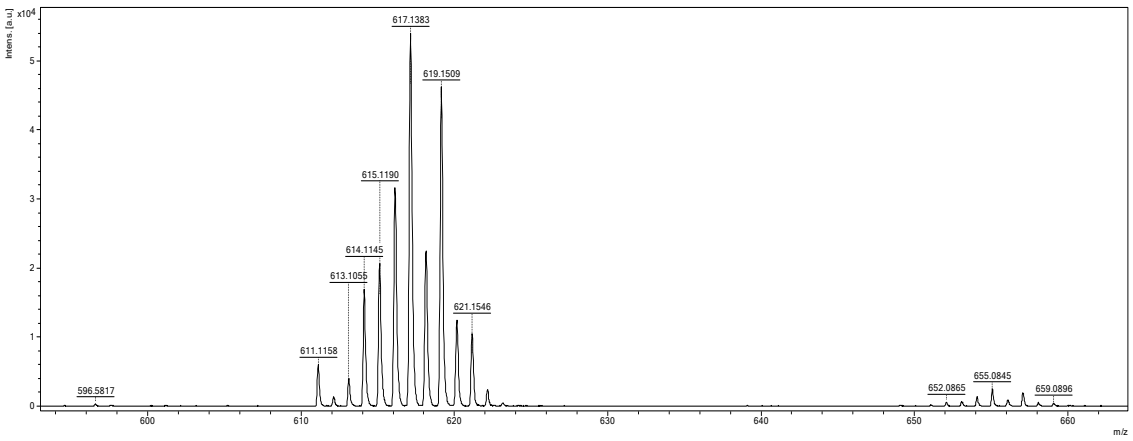


Figure S19 MALDI-TOF-MS of **Ru1**.

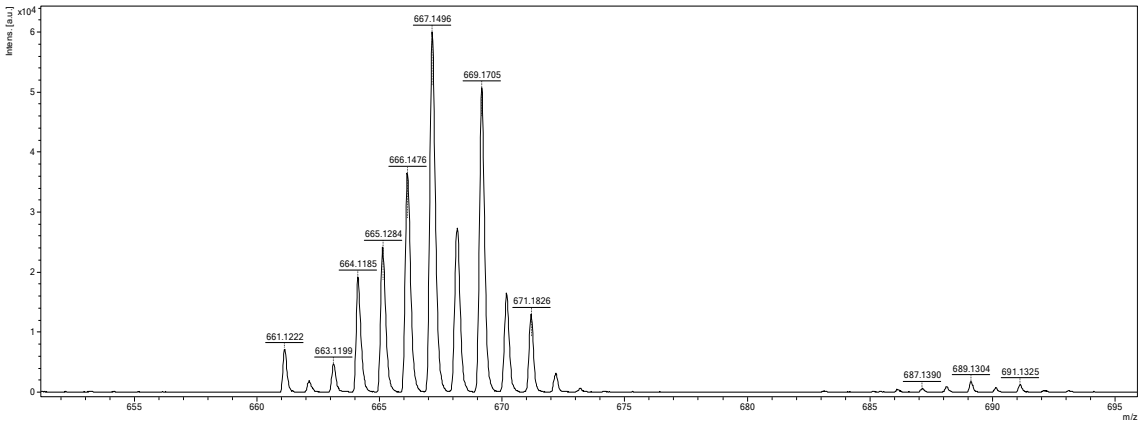


Figure S20 MALDI-TOF-MS of **Ru2**.

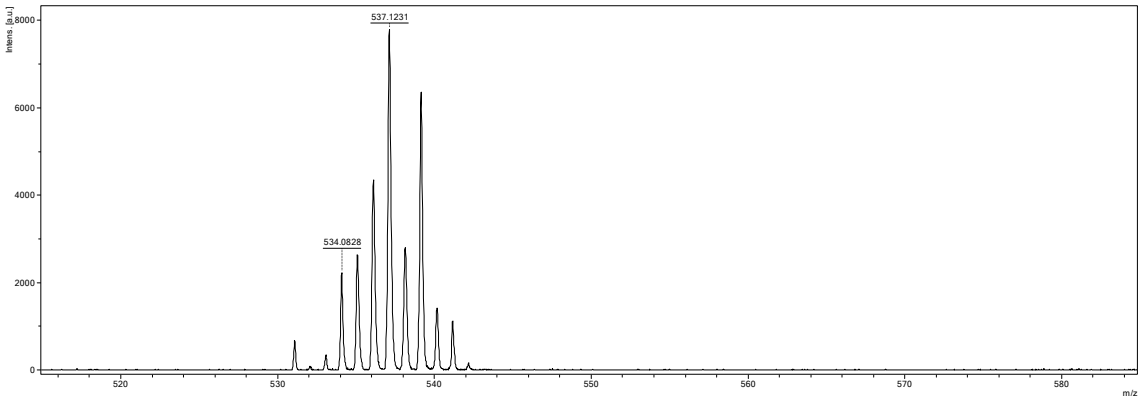


Figure S21 MALDI-TOF-MS of **Ru3**.

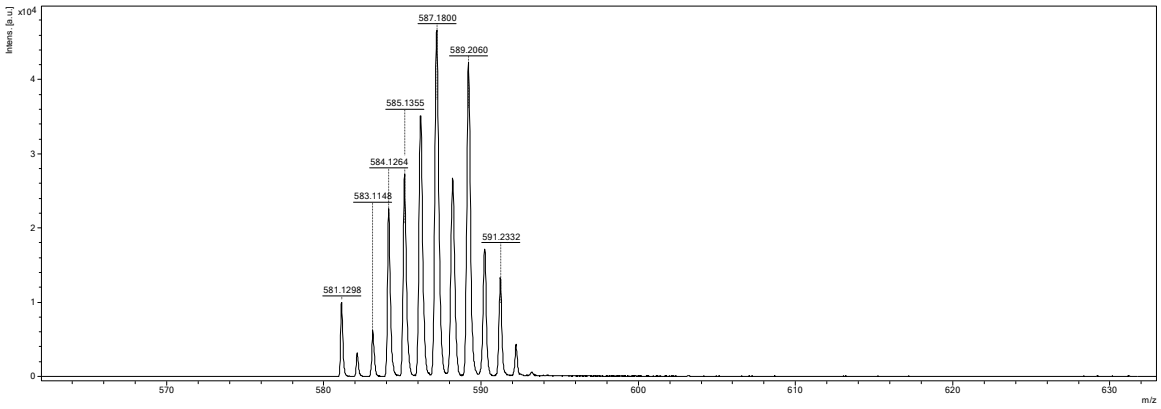


Figure S22 MALDI-TOF-MS of **Ru4**.

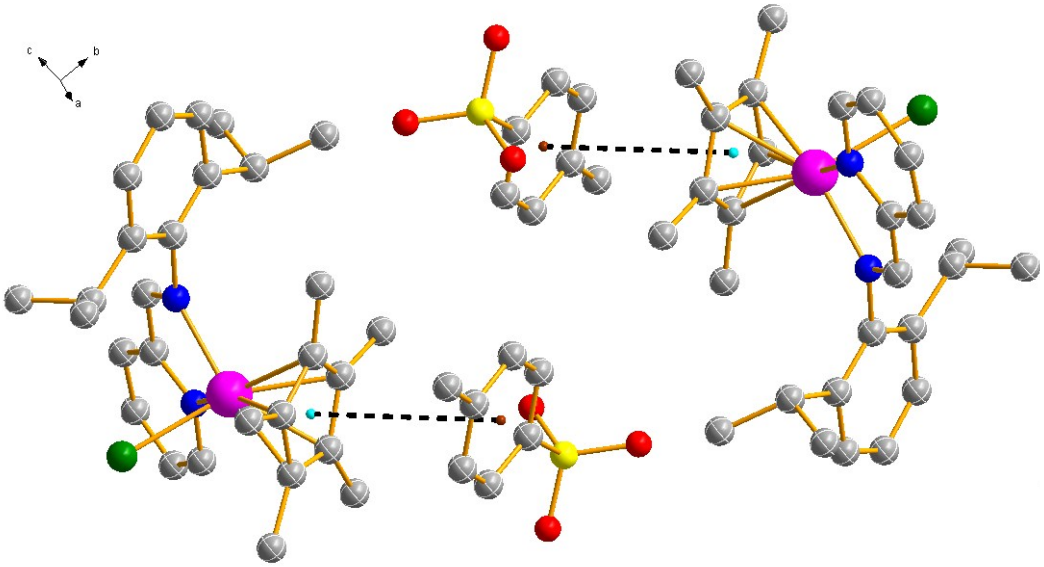


Figure S23 Molecular packing of **Ir5** with the aromatic rings involved in the π-π interactions. All the hydrogen atoms are omitted for clarity.

4. FIGURE AND TABLE.

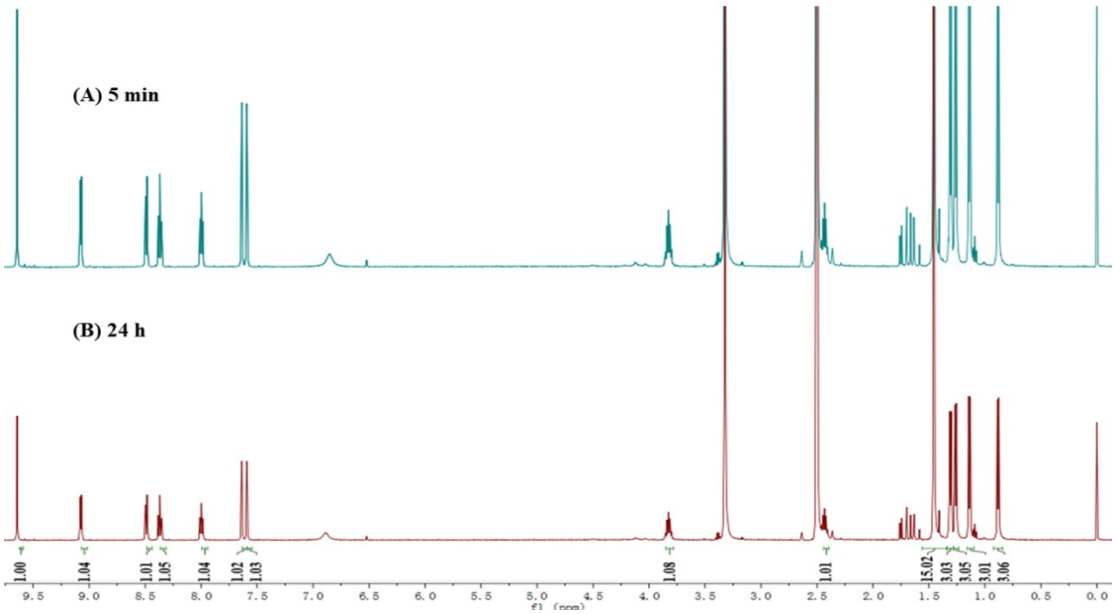


Figure S24 The ^{1}H NMR spectra showing the stability of **Ir1** (1 mM) in DMSO-d_6 at 310 K. (A) after 5 min; (B) after 24 h.

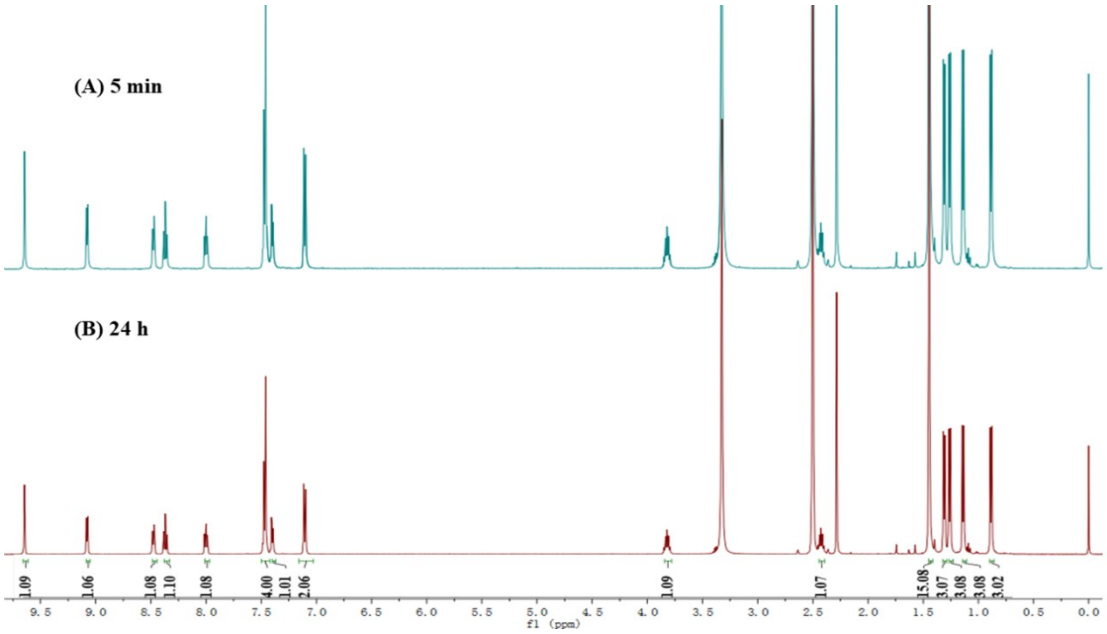


Figure S25 The ^{1}H NMR spectra showing the stability of **Ir5** (1 mM) in DMSO-d_6 at 310 K. (A) after 5 min; (B) after 24 h.

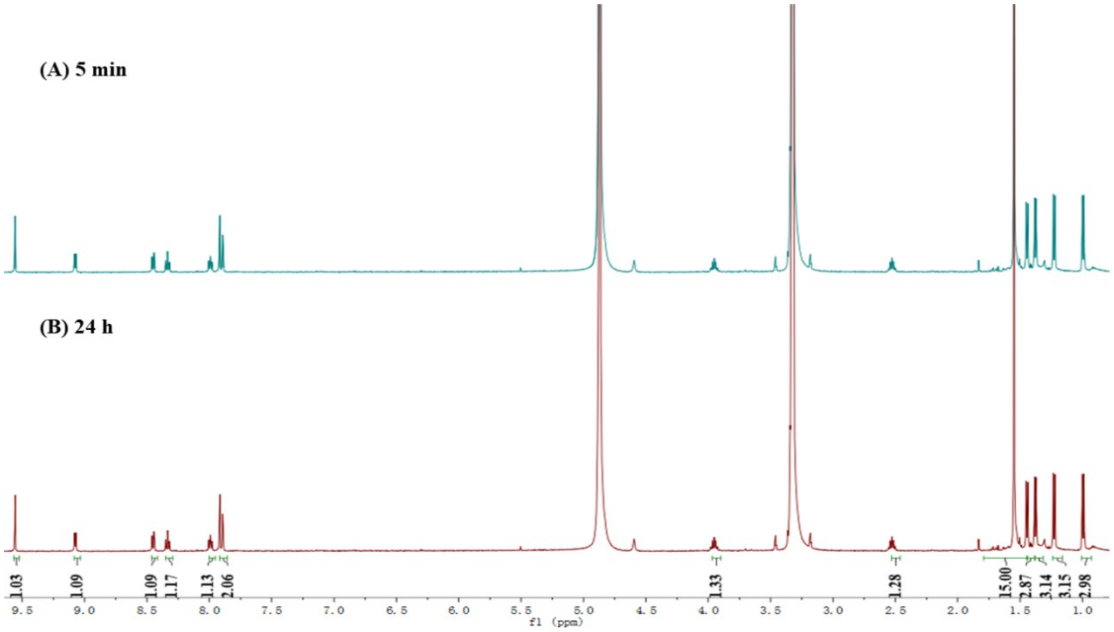


Figure S26 The ^{1}H NMR spectra showing the stability of **Ir1** (1 mM) in MeOD-d_4 at 310 K. (A) after 5 min; (B) after 24 h.

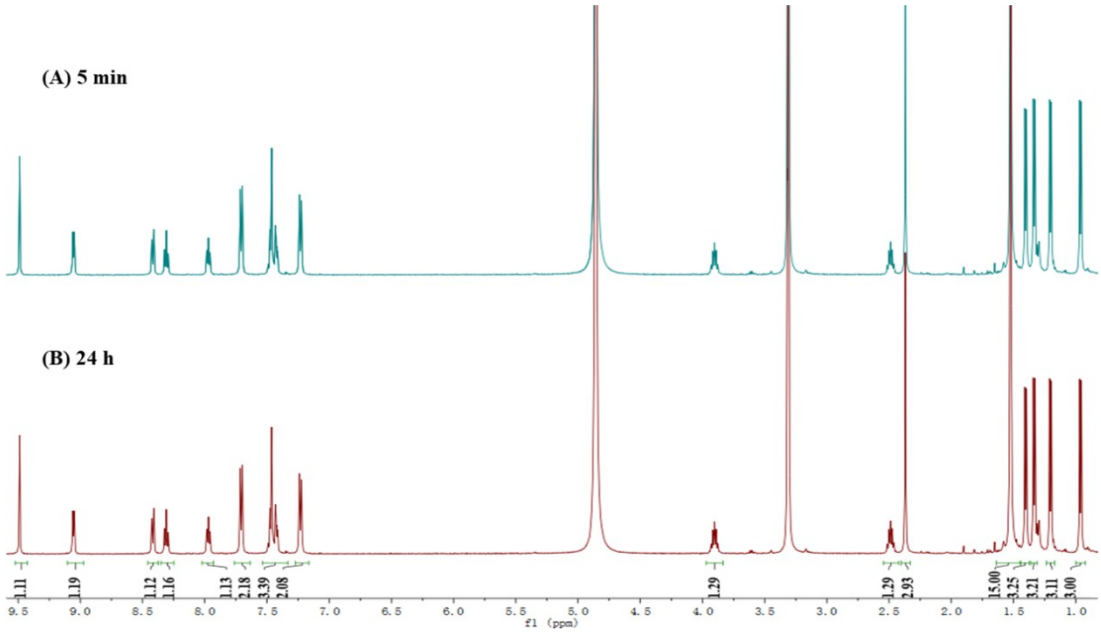


Figure S27 The ^{1}H NMR spectra showing the stability of **Ir5** (1 mM) in MeOD-d_4 at 310 K. (A) after 5 min; (B) after 24 h.

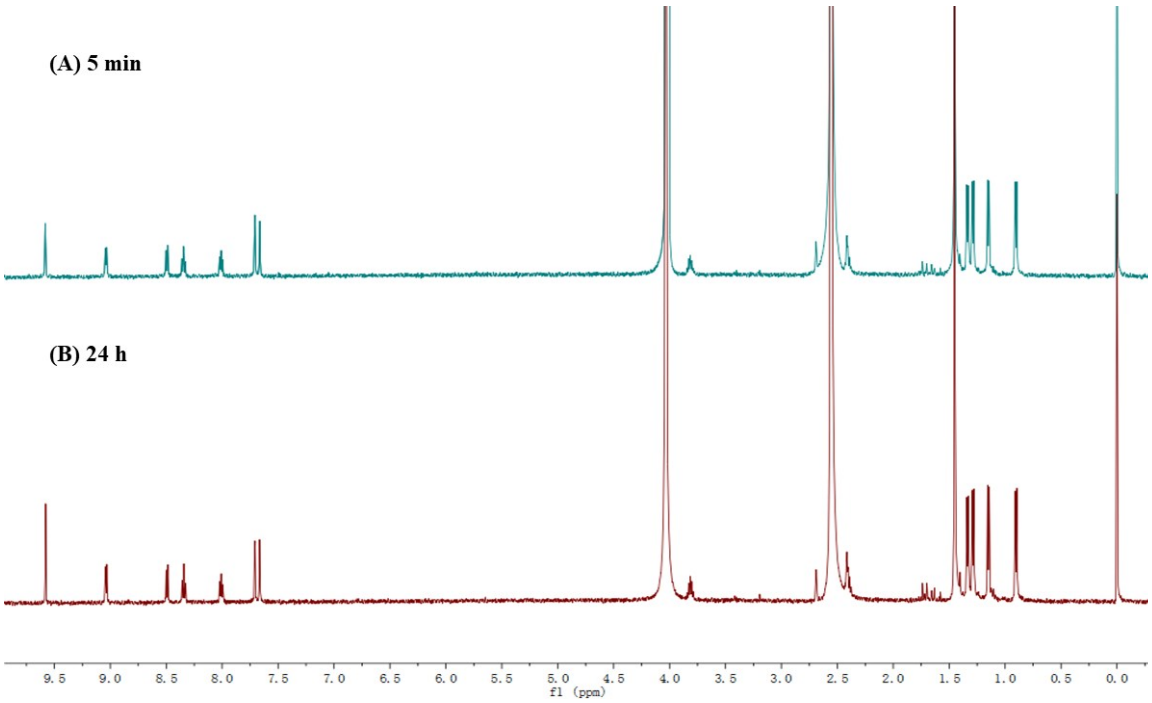


Figure S28 The ^{1}H NMR spectra showing the stability of **Ir1** (1 mM) in 80% DMSO-d_6/20% D_2O (v/v) at 310 K. (A) after 5 min; (B) after 24 h.

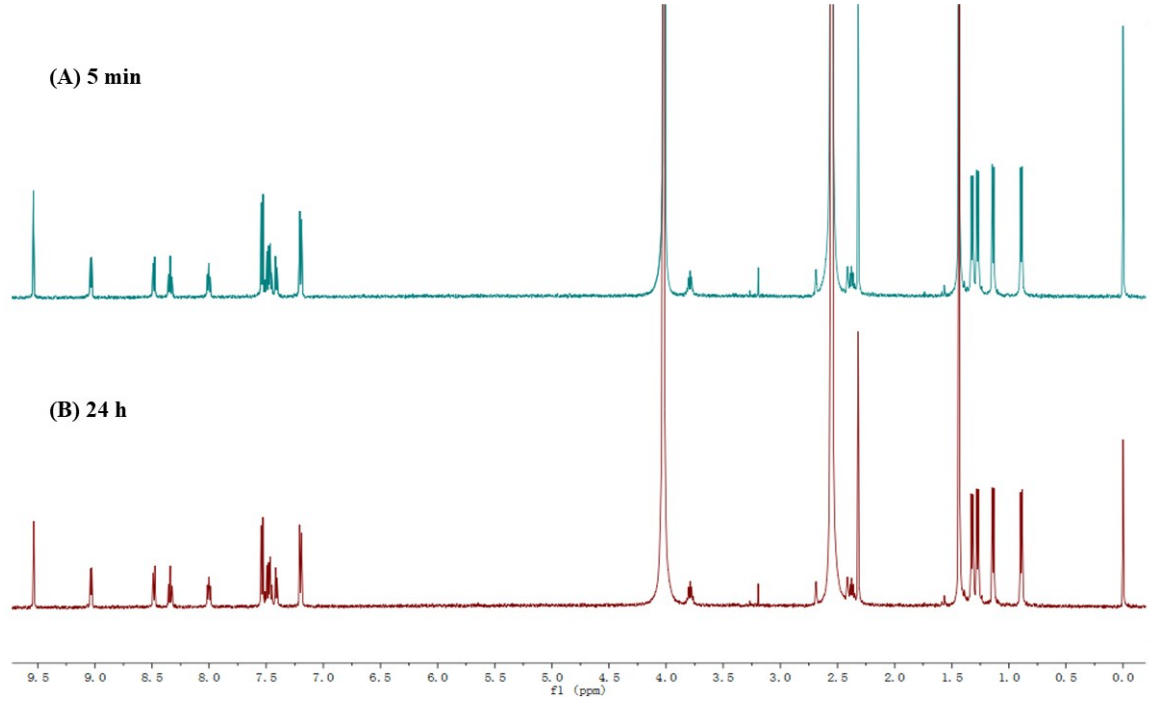


Figure S29 The ^{1}H NMR spectra showing the stability of **Ir5** (1 mM) in 80% DMSO-d_6/20% D_2O (v/v) at 310 K. (A) after 5 min; (B) after 24 h.

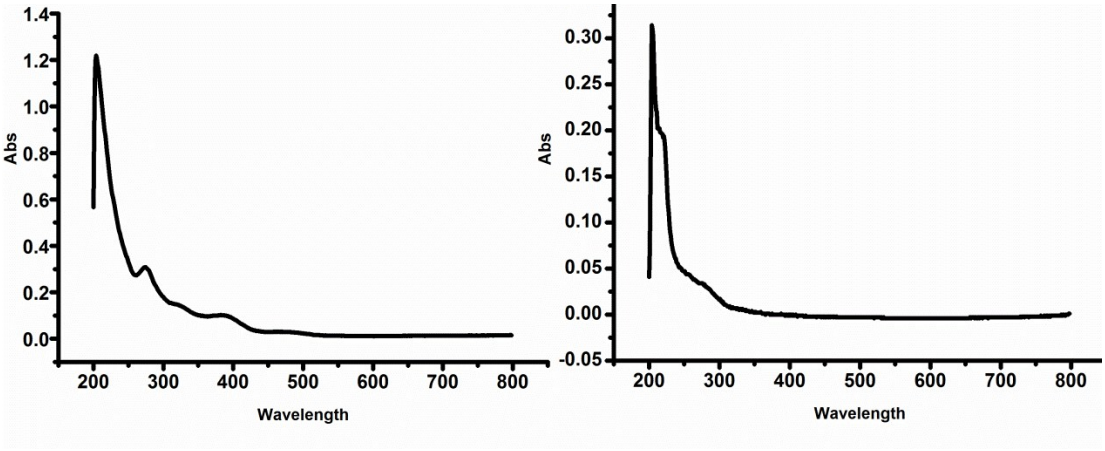


Figure S30. UV-Vis spectrum for a 50 μM solution of **Ir1** in water (left) and 1-octanol (right) recorded at 298 K (log P).

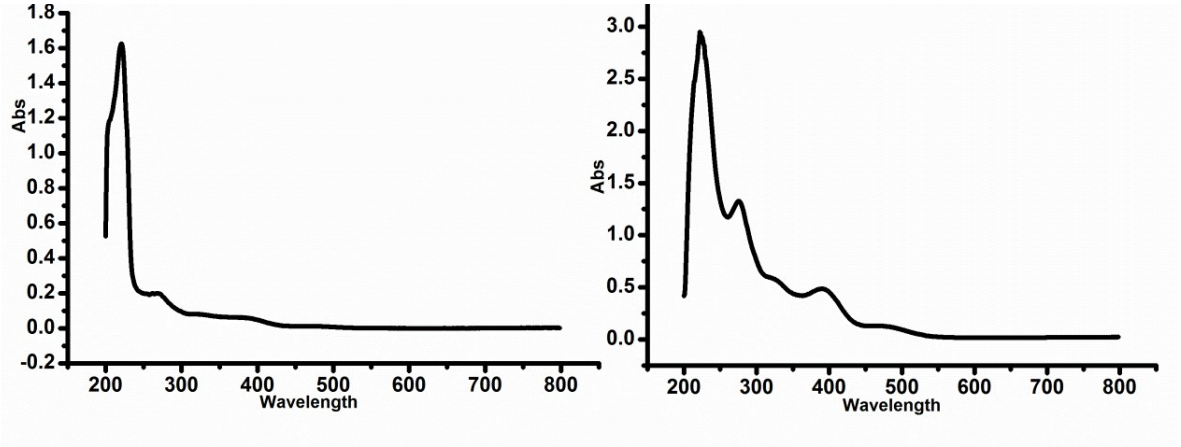


Figure S31. UV-Vis spectrum for a 50 μM solution of **Ir5** in water (left) and 1-octanol (right) recorded at 298 K (log P).

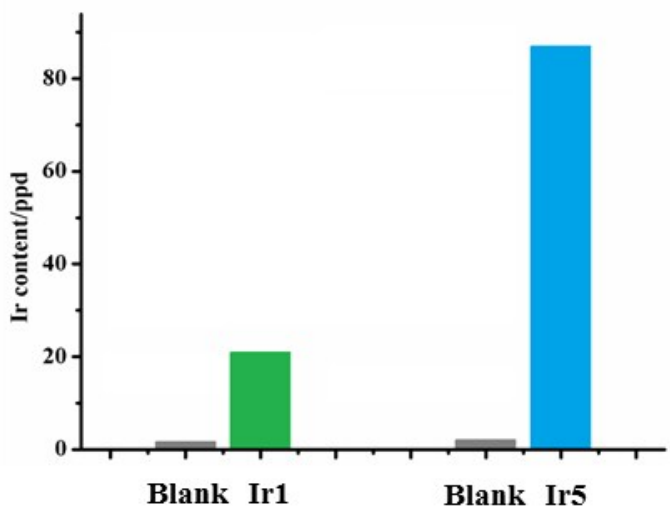


Figure S32. Ir content in 1×10^5 cells determined by ICP-MS measurement for the digestion solutions of HeLa cells incubated, with **Ir1** and **Ir5** (5 μM), respectively. The incubated cells were digested for ICP-MS determination using a standard procedure after 12 h of incubation. Blank, Ir contents for the cells without any Ir complexes incubation.

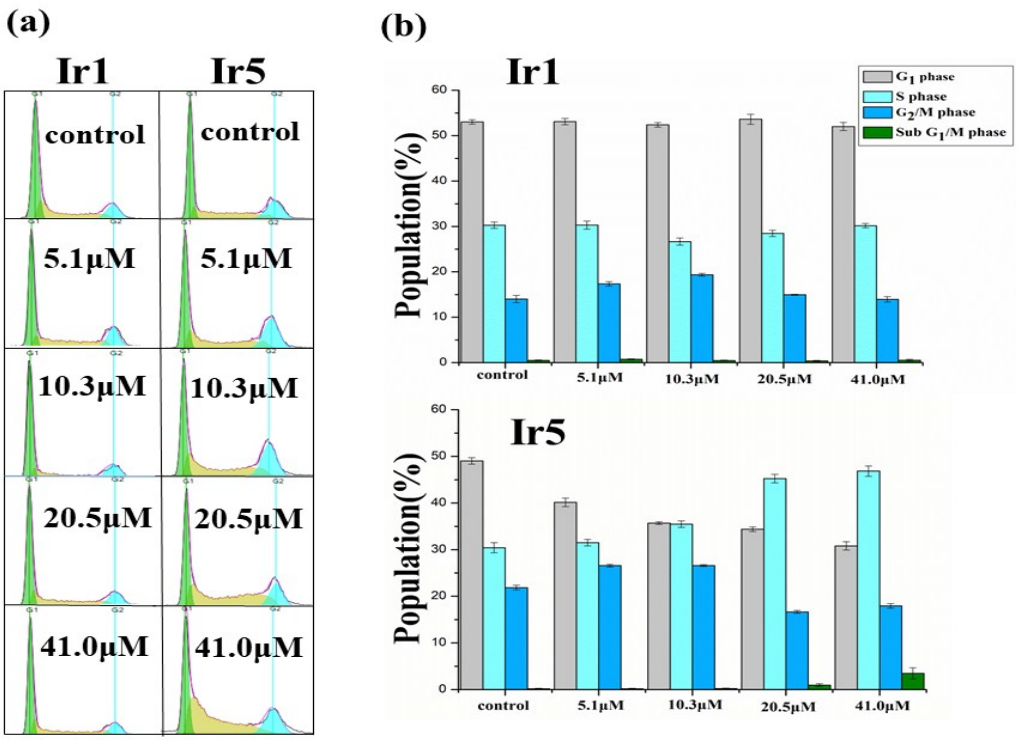


Figure S33. Flow cytometry data for cell cycle distribution of A549 cancer cells exposed to **Ir1** and **Ir5** for 48 h. Concentrations used were 0.25, 0.5, 1 and 2 × IC_{50} equipotent concentrations of IC_{50}. Cell staining for flow cytometry was carried out using PI/RNase.

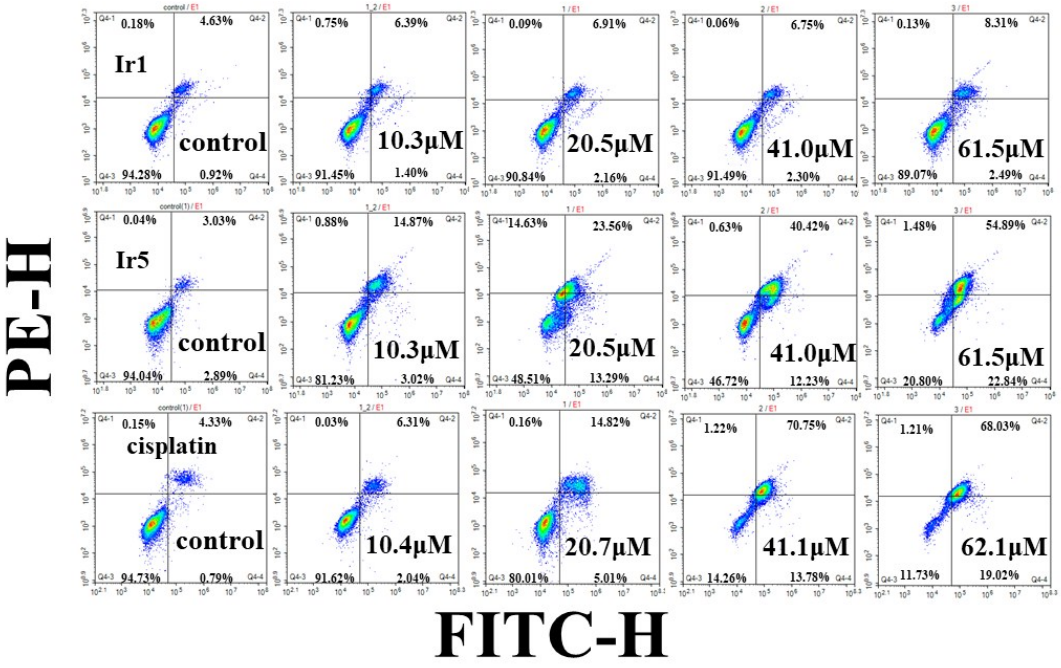


Figure S34. Apoptosis analysis of A549 cells after 48h of exposure to **Ir1**,**Ir5** and cisplatin at 310K determined by fiow cytometry using annexin V-FITC vs PI stainning. A549 cells left untreated (control) or treated with different concentrations of **Ir1**,**Ir5** and cisplatin for 48h.

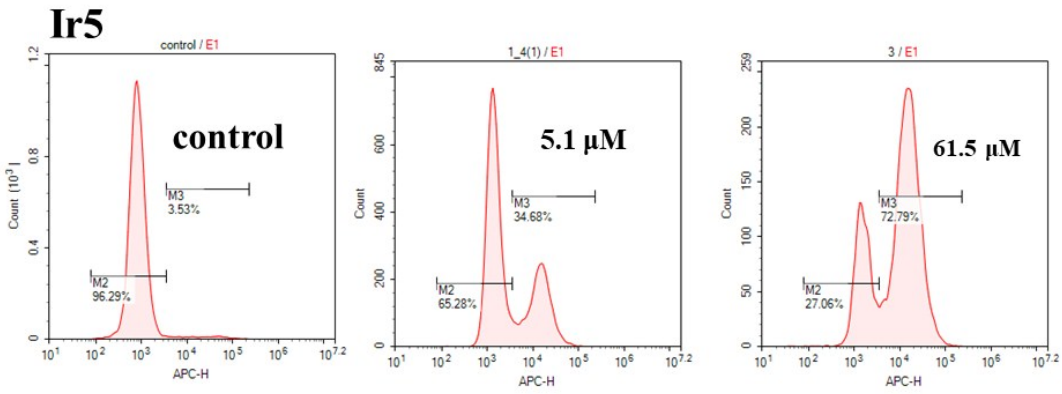


Figure S35. Caspase 3 activation in A549 cells after treatment with **Ir5** at the 0.25 and 3 equipotent concentrations of IC_{50} for 48 h.

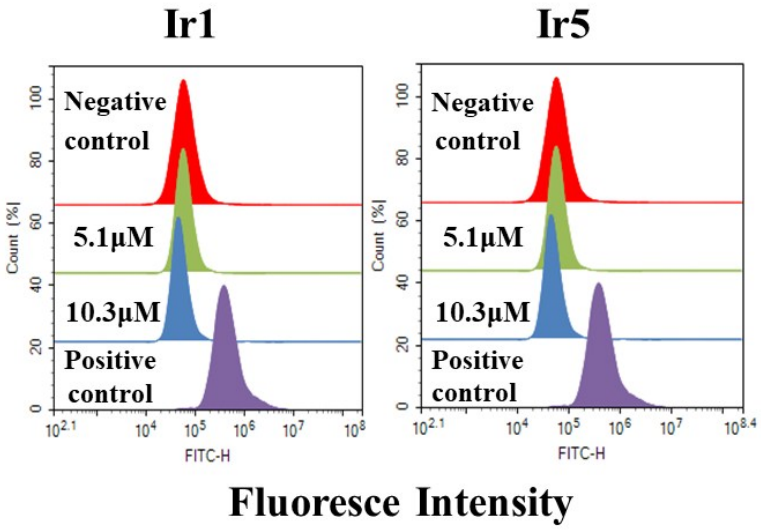


Figure S36. Analysis of ROS level by flow cytometry after A549 cells were treated with **Ir1** and **Ir5** at the concentrations of 5.1 and 10.3 μM for 24 h.

Table S1. Crystallographic data for **Ir2**(CCDC number: **1864243**) and **Ir5** (CCDC number: **1864242**).

	Ir2	**Ir5**
formula	$C_{32}H_{38}ClIrN_2O_3S$	$C_{35}H_{44}ClIrN_2O_3S$
MW	758.35	800.43
cryst size (mm)	0.35 x 0.14 x 0.08	0.48 x 0.45 x 0.40
λ(Å)	0.71073	0.71073
temp (K)	298	298
cryst syst	Monoclinic	Monoclinic
space group	P2(1)/n	P2(1)/n
a (Å)	13.0071(12)	13.6458(12)
b (Å)	18.2846(16)	17.4382(15)
c (Å)	15.6503(13)	14.3952(14)
α (°)	90	90
β (°)	111.658(4)	105.352(3)
γ (°)	90	90
vol ($Å^3$)	3459.3(5)	3303.2(5)
Z	4	4
density (calc) ($Mg \cdot m^{-3}$)	1.456	1.610
abs coeff (mm^{-1})	4.028	4.223
F(000)	1512	1608
θ range (deg)	2.63 to 25.02	2.40 to 25.02
index ranges	$-15 \le h \le 15$, $-21 \le k \le 17$, $-18 \le l \le 16$	$-16 \le h \le 16$, $-20 \le k \le 19$, $-13 \le l \le 17$
reflns collected	17320	16189
indep reflns	6090 [R (int) = 0.047]	5812 [R (int) = 0.0468]
data / restraints / params	6090 / 20 / 370	5812 / 0 / 426
final R indices [$I > 2\sigma(I)$]	R1 = 0.0476, wR2 = 0.1584	R1 = 0.0349, wR2 = 0.0752
GOF	1.886	1.014
largest diff peak and hole ($e Å^{-3}$)	1.49 and -2.68	1.930 and -0.966

Table S2. Cell cycle analysis carried out by flow cytometry using PI staining after exposing A549 cells to **Ir1**.

		Population(%)			
Complex	**Ir concentration**	**G_0/G phase**	**S phase**	**G_2/M phase**	**Sub G_1/M phase**
	5.1 μM	53.10±0.7	30.30±0.9	17.34±0.5	0.74±0.1
	10.3 μM	52.38±0.5	26.68±0.8	19.36±0.3	0.48±0.1
Ir1	20.5 μM	53.61±1.1	28.49±0.7	14.97±0.1	0.39±0.1
	41.0 μM	52.02±0.9	30.17±0.5	13.94±0.6	0.53±0.2
control		53.02±0.5	30.29±0.7	14.01±0.8	0.50±0.1

Table S3. Cell cycle analysis carried out by flow cytometry using PI staining after exposing A549 cells to **Ir5.**

		Population(%)			
Complex	**Ir concentration**	**G_0/G phase**	**S phase**	**G_2/M phase**	**Sub G_1/M phase**
	5.1 μM	40.14±0.9	31.52±0.7	26.58±0.3	0.19±0.1
	10.3 μM	35.69±0.3	35.49±0.7	26.61±0.2	0.23±0.1
Ir5	20.5 μM	34.39±0.5	45.23±0.9	16.65±0.3	0.97±0.3
	41.0 μM	30.82±0.9	46.84±1.1	17.95±0.5	3.49±1.2
control		49.03±0.7	30.42±1.1	21.85±0.5	0.18±0.1

Table S4. Flow cytometry analysis to determine the percentages of apoptotic cells, using Annexin V-FITC vs PI staining, after exposing A549 cells to **Ir1**.

		Population (%)			
Complex	**Ir concentration**	**Viable**	**Early apoptosis**	**Late apoptosis**	**Non-viable**
	10.3 μM	91.45±0.3	1.40±0.5	6.39±0.2	0.75±0.6
	20.5 μM	90.84±0.2	2.16±0.3	6.91±0.4	0.09±0.4
Ir1	41.0 μM	91.49±0.7	2.30±0.6	6.75±0.1	0.06±0.6
	61.5 μM	89.07±0.5	2.49±1.1	8.31±1.1	0.13±0.4
control		94.28±0.6	0.92±0.3	4.63±0.5	0.18±0.1

Table S5. Flow cytometry analysis to determine the percentages of apoptotic cells, using Annexin V-FITC vs PI staining, after exposing A549 cells to **Ir5**.

		Population (%)			
Complex	**Ir concentration**	**Viable**	**Early apoptosis**	**Late apoptosis**	**Non-viable**
	10.3 μM	81.23±2.9	3.02±0.5	14.87±0.9	0.88±0.3
	20.5 μM	48.51±1.5	13.29±1.1	23.56±1.3	14.63±1.2
Ir5	41.0 μM	46.72±0.9	12.23±1.7	40.42±1.7	0.63±0.2
	61.5 μM	20.80±0.3	22.84±1.3	54.89±2.3	1.48±0.5
control		94.04 ±3.7	2.89±0.3	3.03±0.2	0.04±0.01

Table S6. Flow cytometry analysis to determine the percentages of apoptotic cells, using Annexin V-FITC vs PI staining, after exposing A549 cells to cisplatin.

		Population (%)			
Complex	**Ir concentration**	**Viable**	**Early apoptosis**	**Late apoptosis**	**Non-viable**
	10.4 μM	91.62±3.7	2.04±0.7	6.31±0.9	0.03±0.0
	20.7 μM	80.01±2.3	5.01±0.5	14.82±0.3	0.16±0.1
cisplatin	41.4 μM	14.26±1.2	13.78±0.9	70.75±3.5	1.22±0.2
	62.1 μM	11.73±0.8	19.02±0.5	68.03±2.8	1.21±0.1
control		94.73±4.1	0.79±0.2	4.33±1.1	0.15±0.1

Table S7. The mitochondrial membrane polarization of A549 cells induced by **Ir1**

		Population (%)	
Complex	**Ir concentration**	**JC-1 Aggregates**	**JC-1 Monomers**
	5.1 μM	94.0±0.1	5.9±0.1
	10.3 μM	94.1±0.6	5.9±0.2
Ir1	20.5 μM	92.6±1.8	7.4±0.5
	41.0 μM	92.1±0.1	7.9±0.1
Negative Control		96.4±0.1	3.6±0.1
Positive Control		19.8±0.1	80.2±0.1

Table S8. The mitochondrial membrane polarization of A549 cells induced by **Ir5**

		Population (%)	
Complex	**Ir concentration**	**JC-1 Aggregates**	**JC-1 Monomers**
	5.1 μM	83.96±0.1	16.04±0.1
	10.3 μM	75.72±0.6	24.27±0.2
Ir5	20.5 μM	48.38±1.8	51.62±0.5
	41.0 μM	34.11±0.1	65.88±0.1
Negative Control		94.1±0.1	5.9±0.1
Positive Control		19.8±0.1	80.2±0.1