
Supporting Information

Phosphorescent iridium(III) complexes as multicolour probes for imaging of hypochlorite ion in mitochondria

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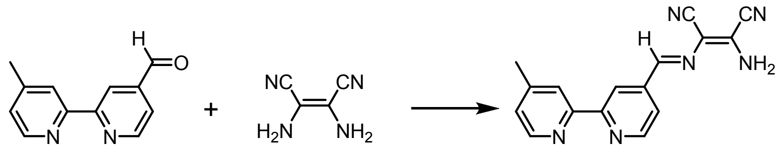
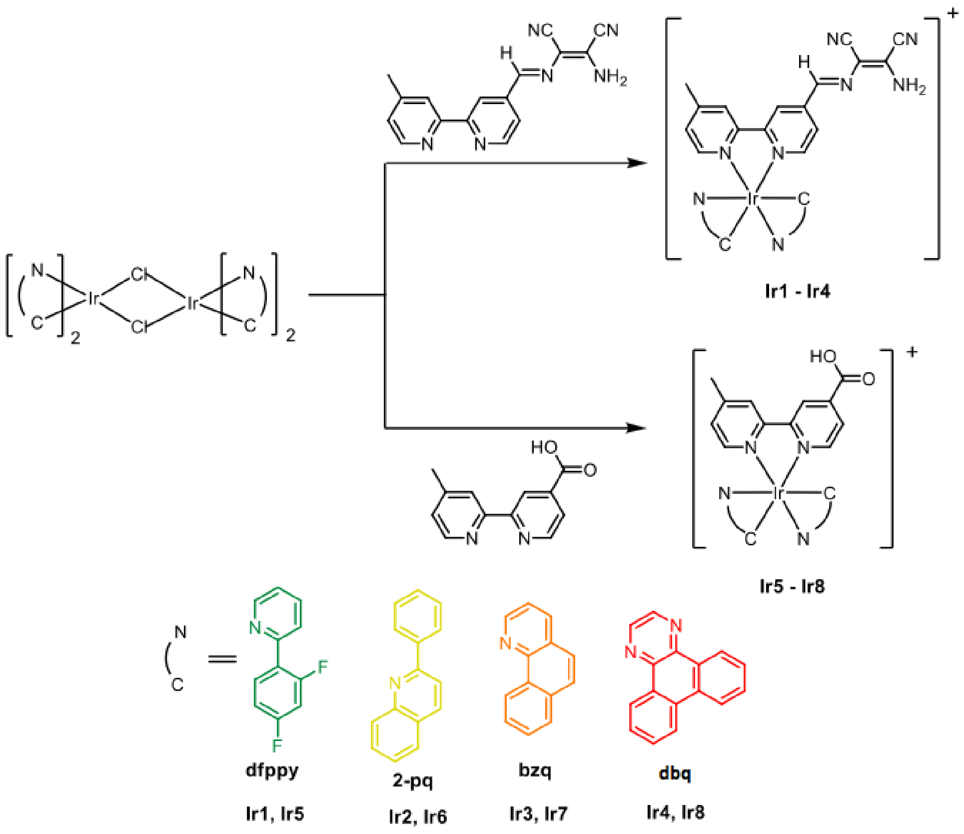


Scheme S1 Synthesis of ligand.

Synthesis of 4-methyl-2, 2'-bipyridine-4'-Diaminomaleonitrile (bpy-damn)

A mixture of 2,3-diaminomaleonitrile (1.2 mmol), **bpy-CHO** (1.0 mmol) in 20 mL of absolute ethanol containing two drops of acetate acid as a catalyst were stirred for 3 hours at room temperature. When the material disappeared, the reaction was terminated. The solution was poured into distilled water, and the resulting residue was filtered, further purified by chromatography on silica using CH_2Cl_2/CH_3OH (v/v, 5:1) as eluent to get the damn. Yield: 264.4 mg, 97.71%. ESI-MS (CH_3OH) m/z: 288.9 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d6) δ 8.71 (s, 2H), 8.54 (d, J = 4.8 Hz, 1H), 8.36 – 8.27 (m, 3H), 8.21 (s, 1H), 8.06 (d, J = 5.1 Hz, 1H), 7.29 (d, J = 4.5 Hz, 1H), 2.49 (s, 3H).



Scheme S2 Synthesis of iridium complexes.

Synthesis of iridium complexes (Ir1~Ir8)

The complexes **Ir1~Ir8** were synthesized in a similar manner. Briefly, a mixture of 2-ethoxyethanol and water (3:1, v/v) was added to a flask containing $IrCl_3$ (1.0 mmol) and the C^N ligands (C^N = **dfppy**, **2-pq**, **bzq**, **dbq**, 2.5 mmol). The mixture was refluxed for 24 h. After cooling, the precipitate was filtered to give crude cyclometalated Ir(III) chloro-bridged dimer, which can be utilized for the next step without further purification. The chloro-bridged dimer (0.10 mmol) and N^N ligand **bpy-damn** or **bpy-COOH** (0.2 mmol) were placed in a 50 mL round bottomed flask with 20 mL of methanol and trichloromethane (1:1, v/v). The mixture was heated at 65 ^{o}C for 6 h under argon. After 6 h reflux, the solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography on silica with CH_2Cl_2-MeOH (10:1, v/v) as eluent, and then recrystallized with CH_2Cl_2/hexane to get iridium complexes (yield=35~55%).

Ir1: Anal.Calc.for $C_{38}H_{24}F_4IrN_8Cl$ (%): C, 50.92; H, 2.71; N, 12.50. Found(%): C, 51.62; H, 2.24; N, 12.19. ESI-MS (CH_3OH): m/z 861.0 $[M-Cl^-]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.99 (s, 1H), 8.80 (s, 2H), 8.32 (s, 1H), 8.27 (d, *J* = 8.7 Hz, 2H), 8.16 (d, *J* = 5.7 Hz, 1H), 8.00 (t, *J* = 7.5 Hz, 2H), 7.86 (d, *J* = 5.7 Hz, 1H), 7.73 (t, *J* = 4.5 Hz, 2H), 7.69 (d, *J* = 5.7 Hz, 1H), 7.55 (d, *J* = 5.4 Hz, 1H), 7.22 (dd, *J* = 14.4 Hz, 2.4 Hz, 2H), 6.96 (t, *J* = 9.0 Hz, 2H), 5.60 (t, *J* = 5.7 Hz, 2H), 2.58 (s, 3H).

Ir2: Anal.Calc.for $C_{46}H_{32}IrN_8Cl$ (%): C, 59.76; H, 3.49; N, 12.12. Found(%): C, 60.34; H, 3.52; N, 12.15. ESI-MS (CH_3OH): m/z 889.1 $[M-Cl^-]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.67 (s, 2H), 8.54 (t, 4H), 8.27 (d, *J* = 7.8 Hz, 2H), 8.13 (t, 1H), 8.02 (s, 1H), 7.90 (t, 3H), 7.52 (d, *J* = 5.4 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 3H), 7.15 (m, 6H), 6.80 (t, *J* = 7.4, 3.2 Hz, 3H), 6.39 (d, *J* = 7.8 Hz, 2H), 2.43 (s, 3H).

Ir3: Anal.Calc.for $C_{42}H_{28}IrN_8Cl$ (%): C, 57.82; H, 3.24; N, 12.84. Found(%): C, 57.93; H, 3.47; N, 13.04. ESI-MS (CH_3OH): m/z 837.0 $[M-Cl^-]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 9.36 (s, 1H), 9.00 (s, 1H), 8.76 (s, 2H), 8.55 (d, *J* = 5.7 Hz, 2H), 8.27 (s, 1H), 8.12 - 8.02 (m, 3H), 7.95 (d, *J* = 8.7 Hz, 2H), 7.87 (d, *J* = 8.7 Hz, 2H), 7.76 (d, *J* = 5.7 Hz, 1H), 7.66 (d, *J* = 5.7Hz, 1H), 7.62 - 7.55 (m, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.43 (d, *J* = 5.7 Hz, 1H), 7.16 (t, *J* = 7.5 Hz, 2H), 6.20 (d, *J* = 7.2 Hz, 2H), 2.55 (s, 3H).

Ir4: Anal.Calc.for $C_{48}H_{30}IrN_{10}Cl$ (%): C, 61.39; H, 3.22; N, 14.92. Found(%): C, 61.97; H, 3.09; N, 15.21. ESI-MS (CH_3OH): m/z 939.0 $[M-Cl^-]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 9.37 (s, 1H), 9.14 (d, *J* = 1.8 Hz, 2H), 9.00 (s, 1H), 8.85 - 8.75 (m, 7H), 8.31 (d, *J* = 6.6 Hz, 3H), 8.24 (d, *J* = 3.0 Hz, 1H), 8.18 (d, *J* = 3.0 Hz, 1H), 8.09 (d, *J* = 3.6 Hz, 1H), 7.90 (t, *J* = 9.0 Hz, 3H), 7.85 (t, *J* = 6.0 Hz, 3H), 7.74 (d, *J* = 5.7 Hz, 1H), 7.47 (d, *J* = 5.7Hz, 1H), 7.29 (t, *J* = 7.8 Hz, 2H), 6.44 (d, *J* = 7.2 Hz, 2H), 2.56 (s, 3H).

Ir5: Anal.Calc.for $C_{34}H_{22}F_4IrN_4O_2Cl$ (%): C, 49.67; H, 2.70; N, 6.81. Found(%): C, 50.04; H, 2.52; N, 7.15. ESI-MS (CH_3OH): m/z 786.6 $[M-Cl^-]^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.85 (s, 1H), 8.25 (d, *J* = 8.1 Hz, 2H), 7.99 (t, *J* = 10.2 Hz, 2H), 7.82 (t, *J* = 5.7 Hz, 2H), 7.67 (t, *J* = 6.1 Hz, 3H), 7.46 (d, *J* = 5.1 Hz, 1H), 7.20 (t, *J* = 5.7 Hz, 2H), 6.98 - 6.87 (t, *J* = 5.4 Hz, 2H), 5.59 (d, *J* = 10.4 Hz, 2H), 2.53 (s, 3H).

Ir6: Anal.Calc.for $C_{42}H_{30}IrN_4O_2Cl$ (%): C, 59.32; H, 3.56; N, 6.59. Found(%): C, 59.53; H, 3.75; N, 6.65. ESI-MS (CH_3OH): m/z 814.6 $[M-Cl^-]^+$. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.57-8.45 (m, 4H), 8.41 (s, 1H), 8.27 - 8.23 (m, 2H), 7. 99 (d, *J* = 6.0 Hz, 1H), 7.92 - 7.84 (m, 3H), 7. 75 (d, *J* = 6.0 Hz, 1H), 7.45 - 7.29 (m, 4H), 7.19 - 7.02 (m, 6H), 6.77 (t, *J* = 7.5 Hz, 2H), 6.36 (dd, *J* = 12.0 Hz, 9.0 Hz, 2H), 2.38 (s, 3H).

Ir7: Anal.Calc.for $C_{38}H_{26}IrN_4O_2Cl$ (%): C, 57.17; H, 3.28; N, 7.02. Found(%): C, 57.64; H, 3.32; N, 7.12; O, 4.36. ESI-MS (CH_3OH): m/z 762.8 $[M-Cl^-]^+$. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.96 (s, 1H), 8.86 (s, 1H), 8.53 (d, *J* = 8.1 Hz, 2H), 8.04 (d, *J* = 5.4 Hz, 2H), 7.89 (dd, *J* = 24.9, 9.3 Hz, 4H), 7.74 (q, *J* = 5.7 Hz, 2H), 7.61 - 7.55 (m, 3H), 7.50 (d, *J* = 6.0 Hz, 2H), 7.35 (d, *J* = 5.7 Hz, 1H), 7.13 (t, *J* = 7.5 Hz, 2H), 6.17 (t, *J* = 6.9 Hz, 2H), CH3 (~2.5 ppm), coverd by solvent peak.

Ir8: Anal.Calc.for $C_{44}H_{28}IrN_6O_2Cl$ (%): C, 58.69; H, 3.13; N, 9.33. Found(%): C, 58.64; H, 3.42; N, 9.37. ESI-MS (CH_3OH): m/z 864.8 $[M-Cl^-]^+$. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 9.12 (d, *J* = 7.5 Hz, 2H), 9.00 (s, 1H), 8.91 (s, 1H), 8.80 (dd, *J* = 9.0, 6.0 Hz, 3H), 8.28 (d, *J* = 8.1 Hz, 2H), 8.15 (dd, *J* = 7.2, 3.0 Hz, 2H), 7.95 - 7.75 (m, 7H), 7.67 (d, *J* = 5.4 Hz, 1H), 7.39 (d, *J* = 5.7 Hz, 1H), 7.25 (t, *J* = 7.6 Hz, 3H), 6.40 (t, *J* = 6.9 Hz, 2H), 2.51 (s, 3H).

Reaction of Ir1-Ir4 with ClO^- (Ir5’-Ir8’)

A mixture of the iridium(III) complexes **Ir1-Ir4** (0.01 mmol) and NaClO (1 mmol) were suspended in 10 mL CH_3CN-H_2O solution (9:1, v/v) at room temperature. The reaction quantitatively afforded one major product for 30 min. The crude product was successfully purified using chromatography on a silica gel column and the structure of the isolated product was confirmed using ^{1}H NMR spectroscopy and mass analysis.

Ir5': ES-MS (CH_3OH): m/z 786.6. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.36 (s, 1H), 9.14 (s, 1H), 8.26 (d, *J* = 8.7 Hz, 2H), 8.01 (t, *J* = 7.8 Hz, 2H), 7.83 (d, *J* = 5.7 Hz, 1H), 7.76 – 7.65 (m, 4H), 7.52 (d, *J* = 5.4 Hz, 1H), 7.22 (dd, *J* = 13.2, 6.3 Hz, 2H), 6.94 (t, *J* = 12.0 Hz, 2H), 5.59 (d, *J* = 8.1 Hz, 2H), 2.57 (s, 3H).

Ir6': ES-MS (CH_3OH): m/z 814.7. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.94 (s, 1H), 8.63 (s, 1H), 8.58 - 8.44 (m, 4H), 8.25 (dd, *J* = 7.5, 4.8 Hz, 2H), 8.12 (s, 1H), 7.99 (d, *J* = 5.4 Hz, 1H), 7.94 - 7.83 (m, 3H), 7.50 (d, *J* = 5.7 Hz, 1H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.26 - 7.03 (m, 6H), 6.77 (t, *J* = 6.0 Hz, 2H), 6.37 (d, *J* = 7.8 Hz, 2H), 2.42 (s, 3H).

Ir7': ES-MS (CH_3OH): m/z 762.6. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.34 (s, 1H), 9.02 (s, 1H), 8.53 (d, J=5.4, 2H), 8.08 (dd, *J* = 6.0, 2.4 Hz, 2H), 7.98 (d, *J* = 5.1 Hz, 1H), 7.90 (dd, *J* = 15.0, 7.8 Hz, 4H), 7.75 (d, *J* = 5.7 Hz, 1H), 7.65-7.50 (m, *J* = 24.0, 5H), 7.40 (d, *J* = 5.7 Hz, 1H), 7.14 (t, *J* = 7.5 Hz, 2H), 6.19 (d, *J* = 7.2 Hz, 2H), 2.53 (s, 3H).

Ir8': ES-MS (CH_3OH): m/z 864.8. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.41 (s, 1H), 9.14 (d, *J* = 8.1 Hz, 3H), 8.89 – 8.74 (m, 4H), 8.31 (d, *J* = 8.4 Hz, 2H), 8.25 (d, *J* = 3.3 Hz, 1H), 8.18 (d, *J* = 3.0 Hz, 1H), 8.03 (d, *J* = 6.0 Hz, 1H), 7.90 (dt, *J* = 15.0, 7.8 Hz, 5H), 7.73 (d, *J* = 5.4 Hz, 1H), 7.46 (d, *J* = 6.0 Hz, 1H), 7.29 (t, *J* = 7.5 Hz, 2H), 6.44 (d, *J* = 7.2 Hz, 2H), 2.57 (s, 3H).

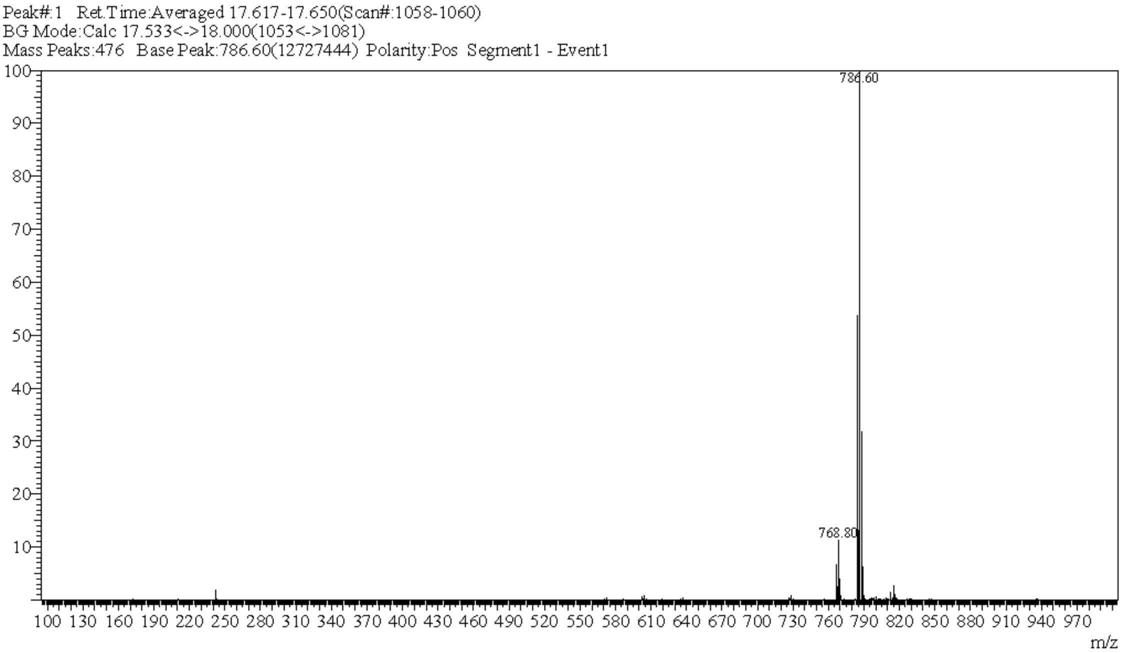


Fig. S1 ESI-MS spectra of **Ir1** +ClO$^-$ (**Ir5'**) in CH_3OH solutions.

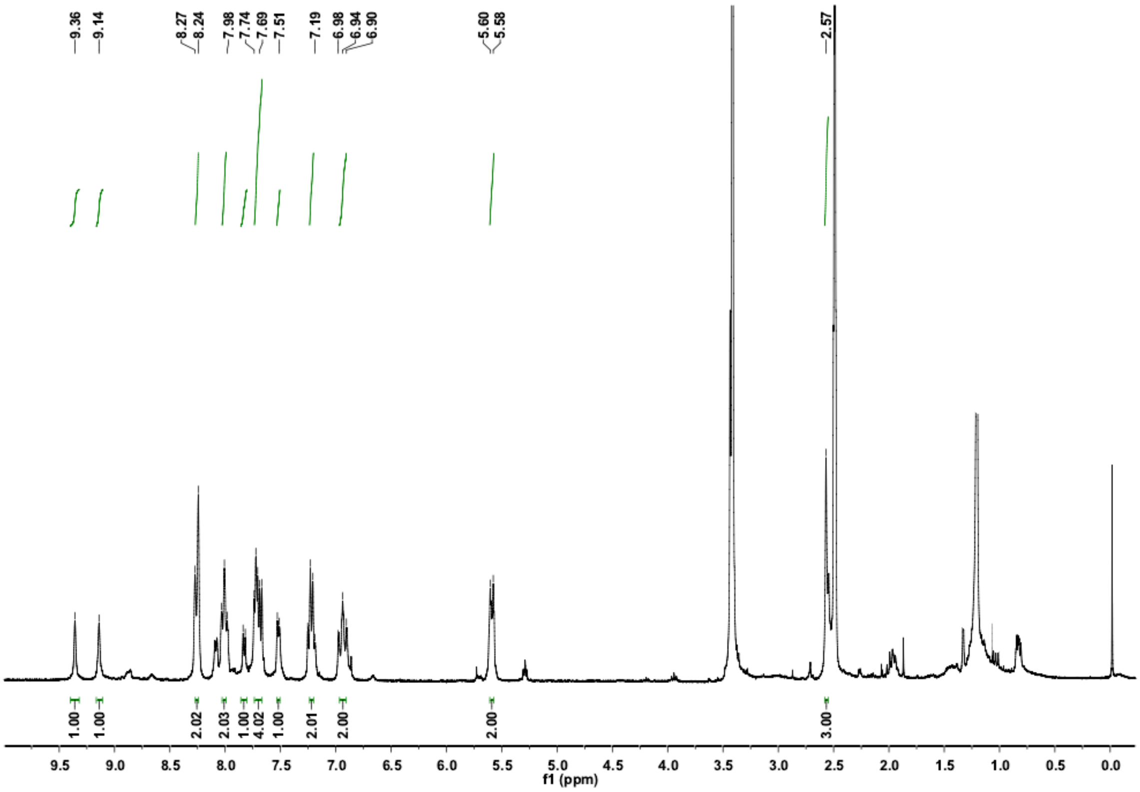


Fig. S2 ^{1}H NMR spectra of **Ir1** +ClO$^-$ (**Ir5'**) in d_6-DMSO, 300 MHz.

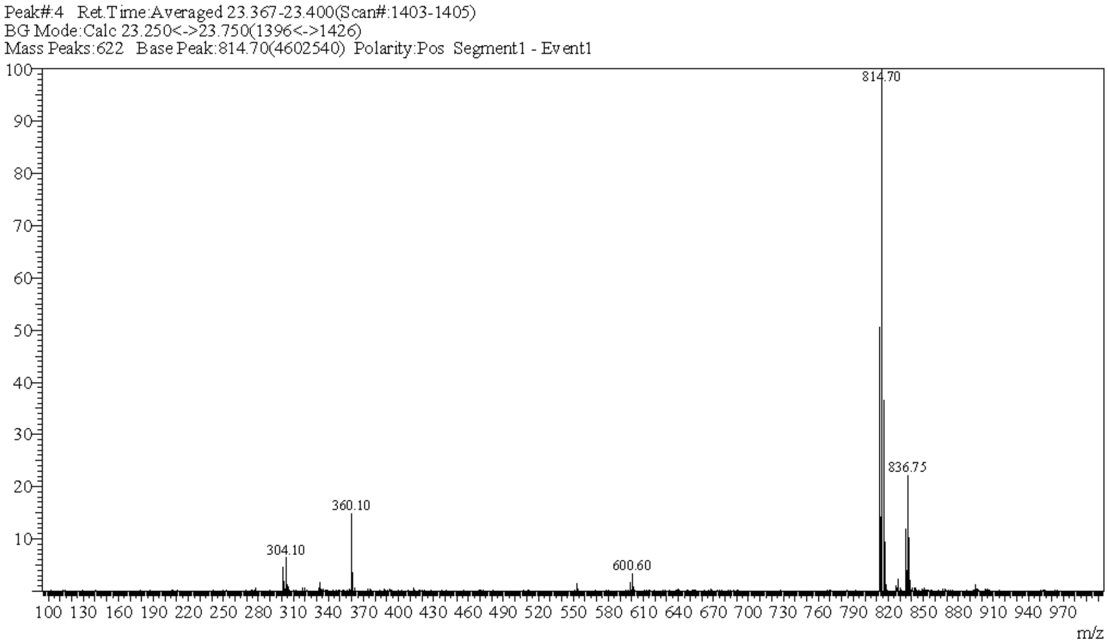


Fig. S3 ESI-MS spectra of **Ir2** +ClO$^-$ (**Ir6'**) in CH_3OH solutions.

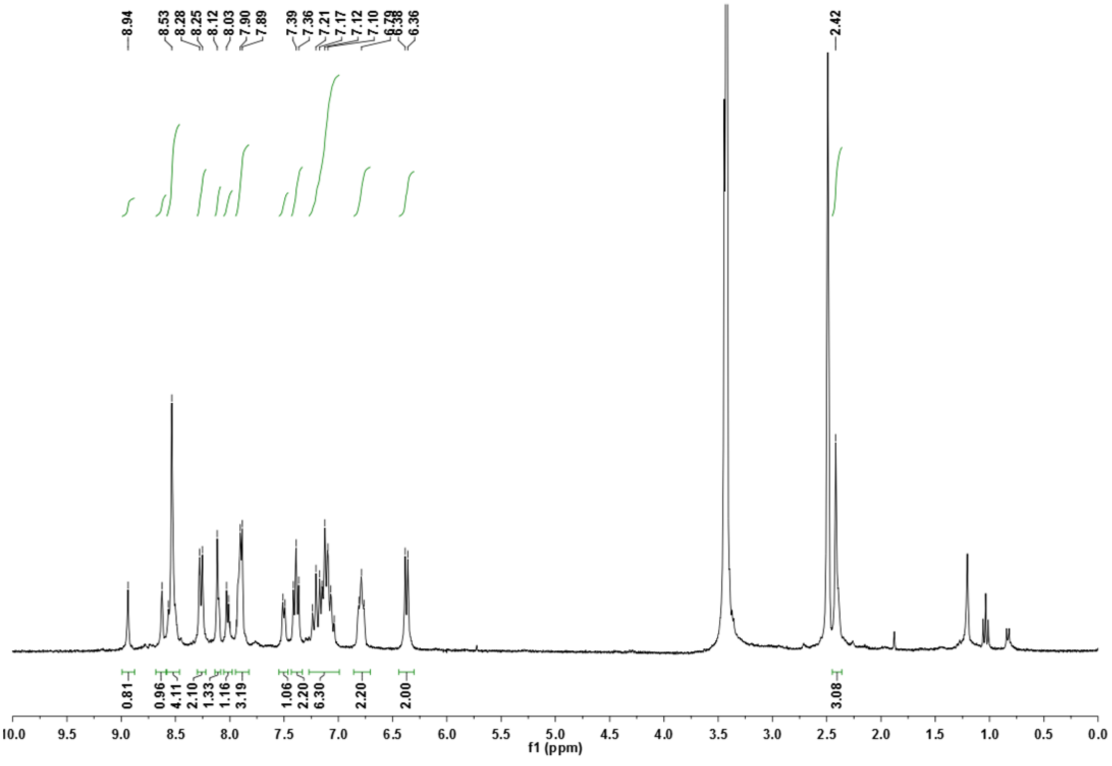


Fig. S4 ^{1}H NMR spectra of **Ir2** +ClO$^-$ (**Ir6'**) in d_6-DMSO, 300 MHz.

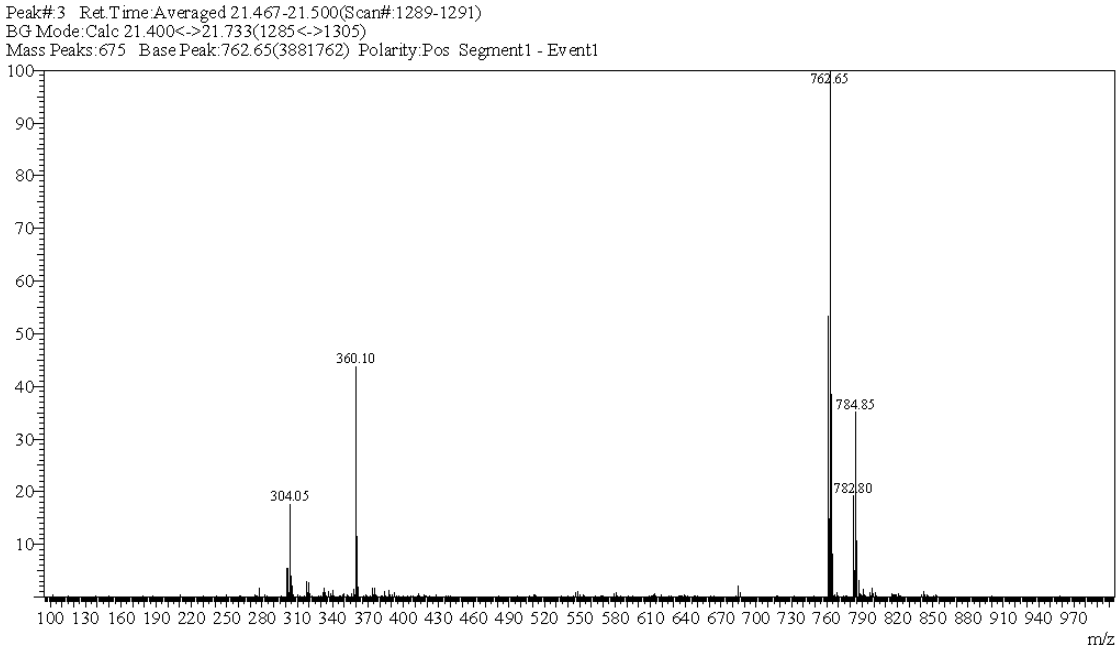


Fig. S5 ESI-MS spectra of **Ir3** +ClO$^-$ (**Ir7'**) in CH_3OH solutions.

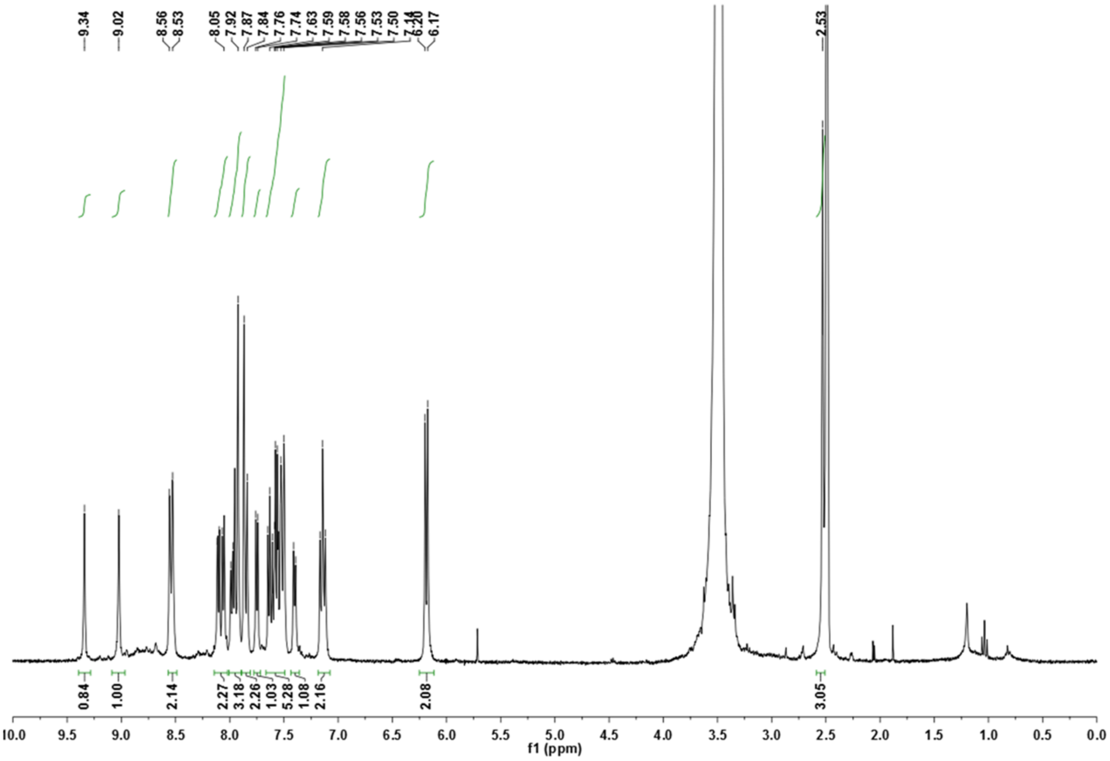


Fig. S6 ^1H NMR spectra of **Ir3** +ClO$^-$ (**Ir7'**) in d_6-DMSO, 300 MHz.

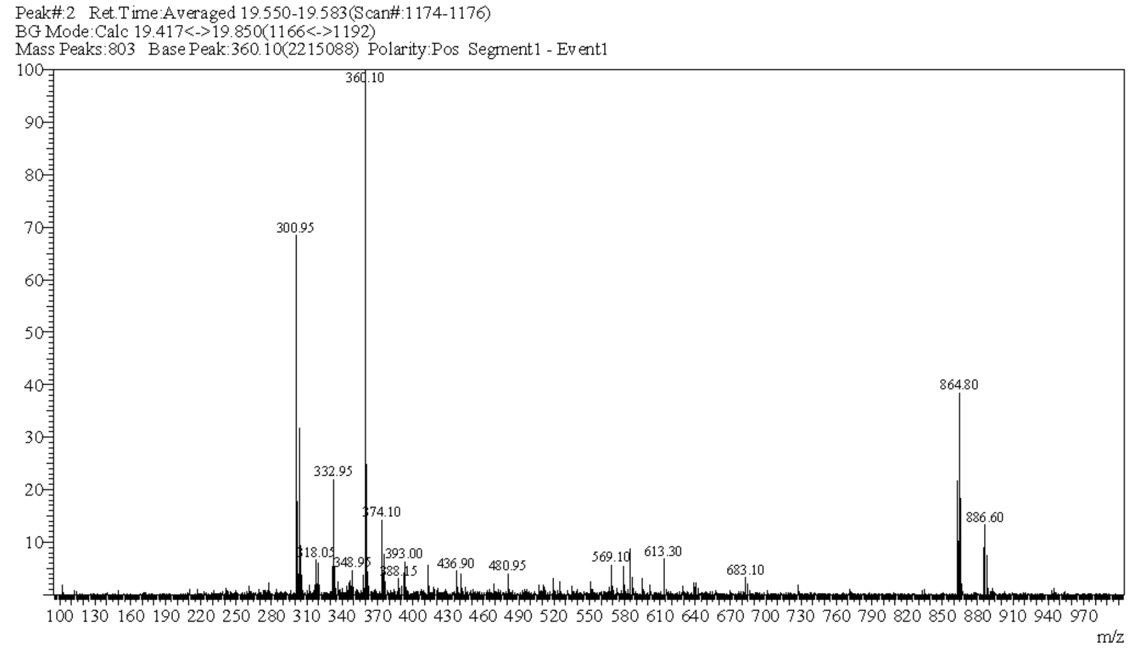


Fig. S7 ESI-MS spectra of **Ir4**+ClO$^-$ (**Ir8'**) in CH$_3$OH solutions.

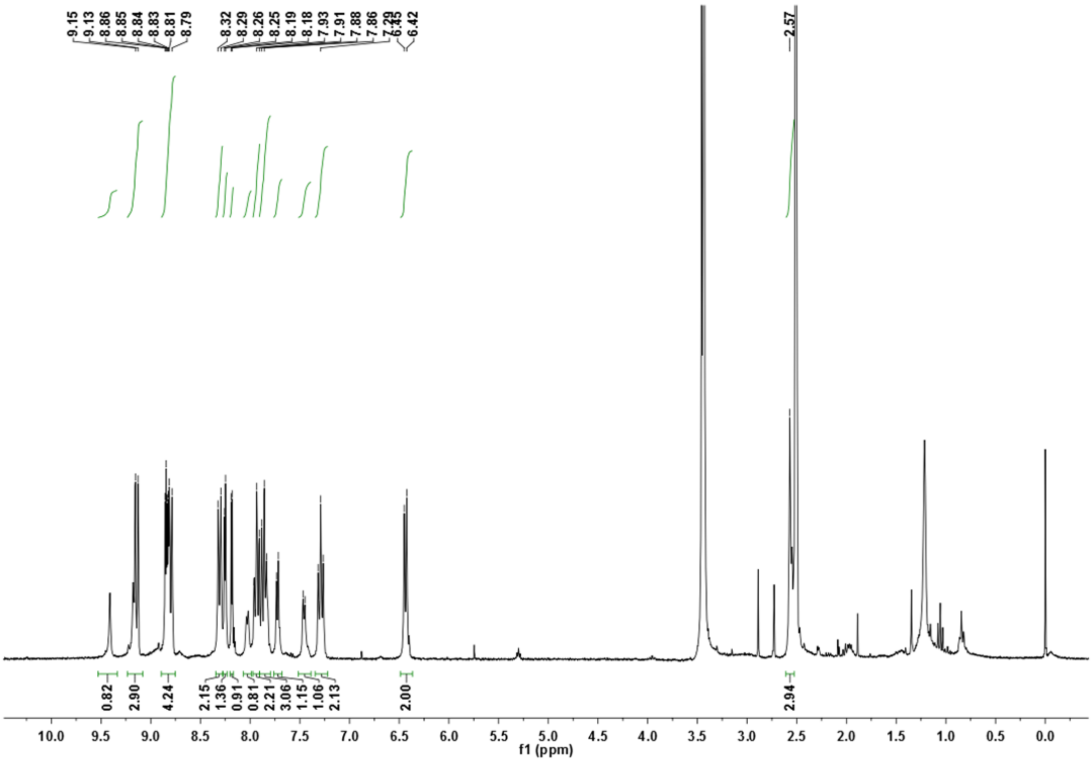


Fig. S8 ^{1}H NMR spectra of **Ir4**+ClO$^-$ (**Ir8'**) in d_6-DMSO, 300 MHz.

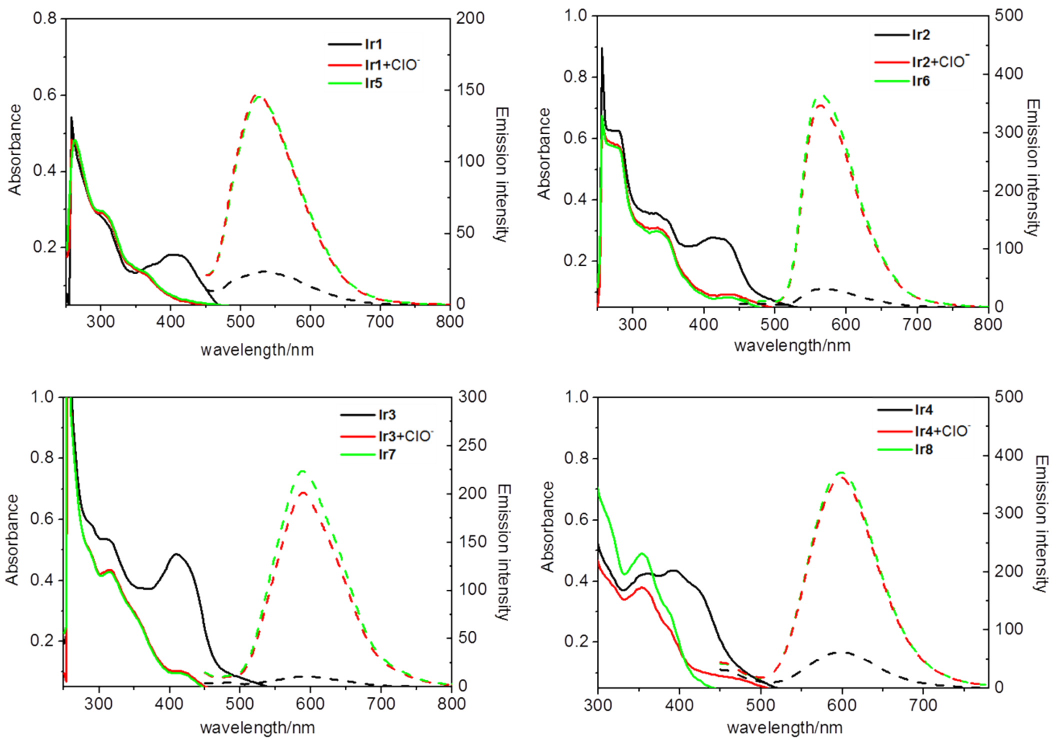


Fig. S9 The absorption (solid line: λ=300-450 nm) and emission (dash line: λ=450-800 nm) spectra of **Ir1**~**Ir4** (black), **Ir1**~**Ir4** + ClO$^-$ (**Ir5'**~**Ir8'**, red) and **Ir5**~**Ir8** (green) in DMF-PBS (10 mM, pH 7.40, v/v, 3:7) solutions.

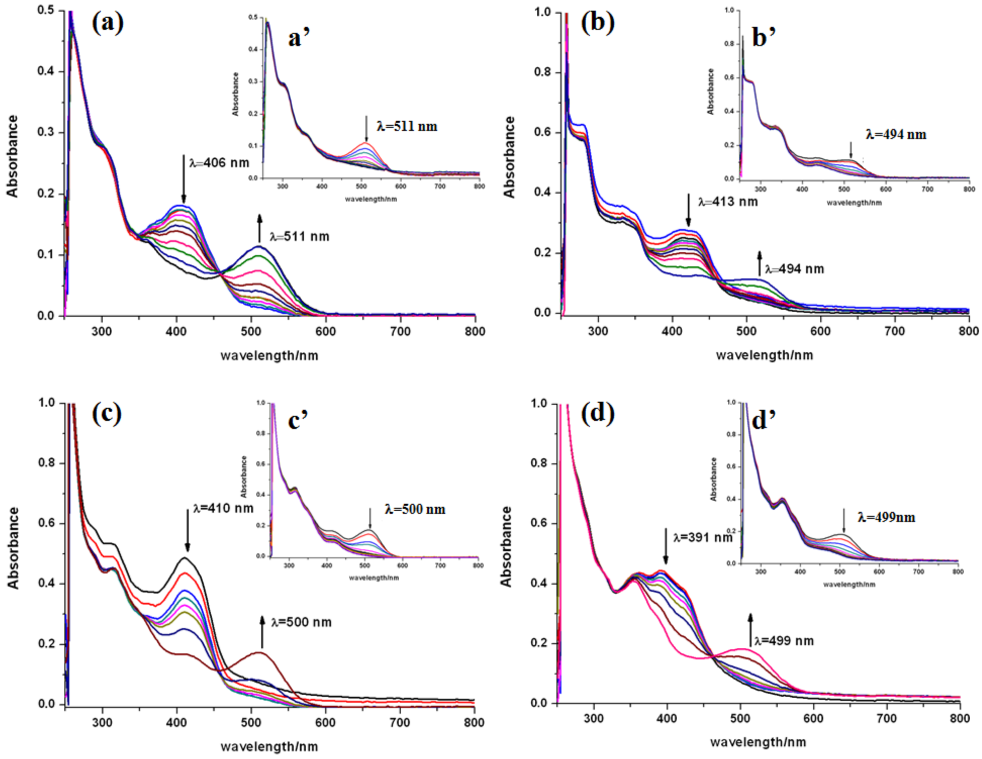


Fig. S10 UV-Vis spectral changes of **Ir1~Ir4** (10 μM) in DMF-PBS (10 mM, pH 7.4, v/v, 3:7) by gradual addition of NaClO.

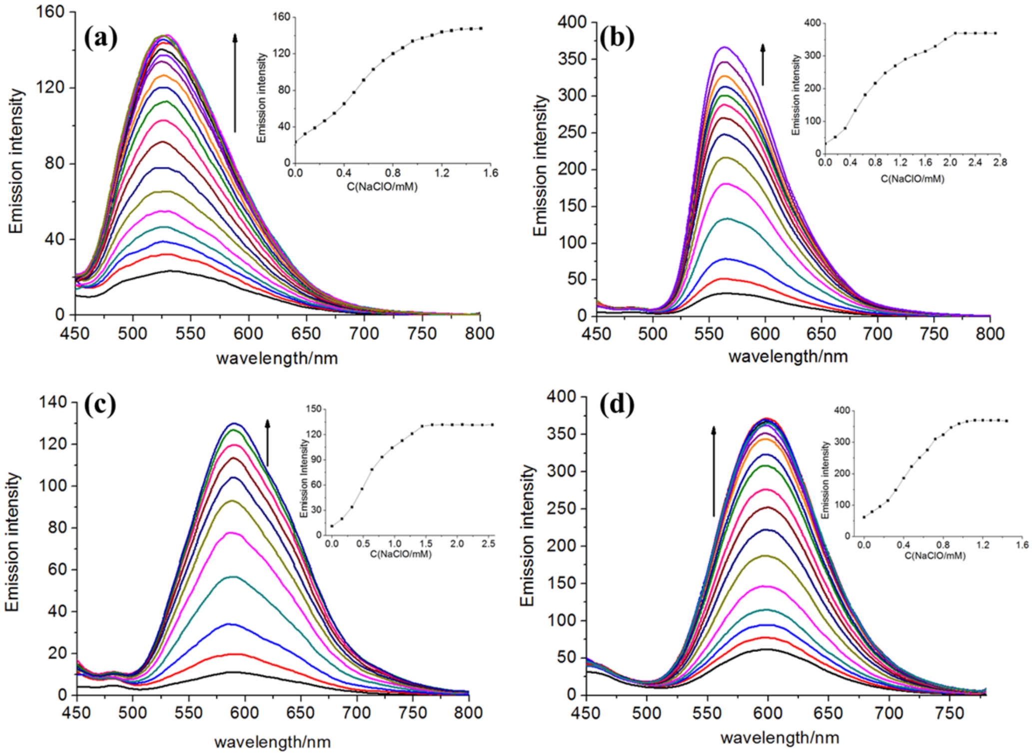


Fig. S11 Emission spectral changes of **Ir1~Ir4** (10 μM) in DMF-PBS (10 mM, pH 7.4, v/v, 3:7) by gradual addition of NaClO.

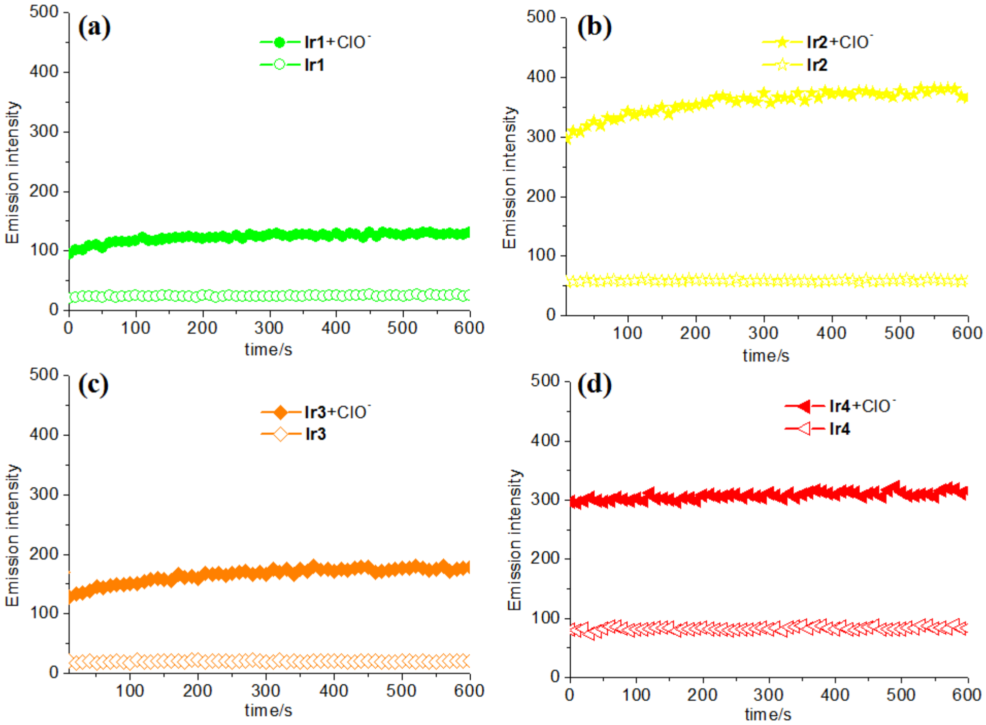


Fig. S12 The time-dependent emission changes acquired from reaction of **Ir1~Ir4** (a~b) (10.0 μM) in the absence (hollow) and presence (solid) of ClO^- in 10 mM PBS buffer solution with pH 7.4 at room temperature.

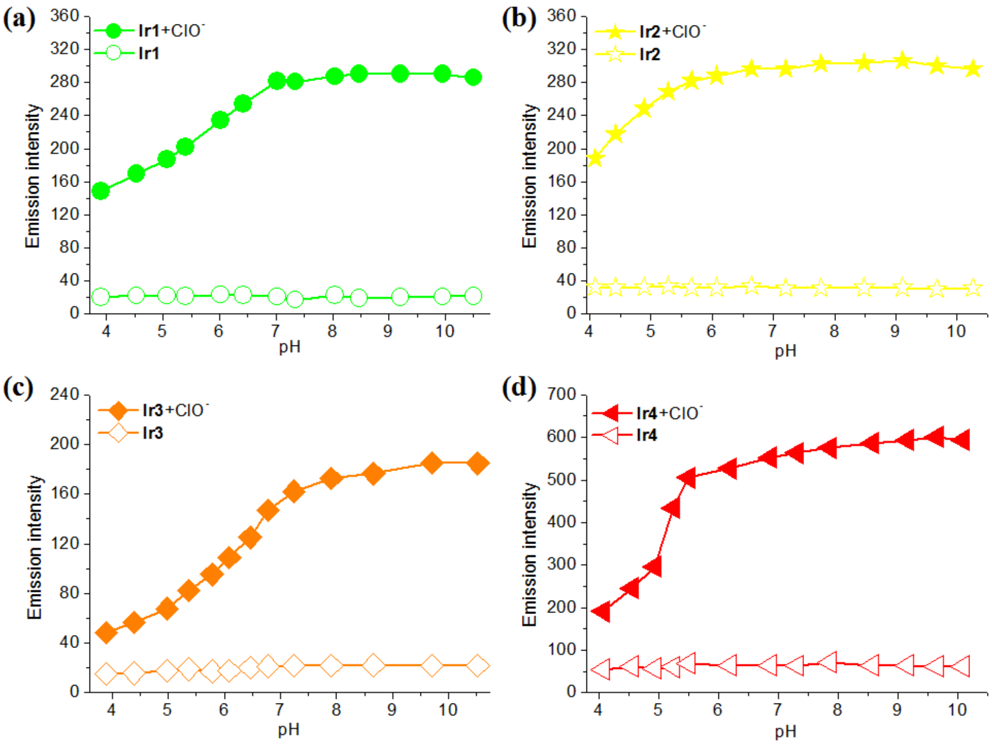


Fig. S13 The emission intensity of **Ir1~Ir4** (a~d) (10 μM) in the absence (hollow) and presence (solid) of ClO^- at various pH values in PBS buffer.

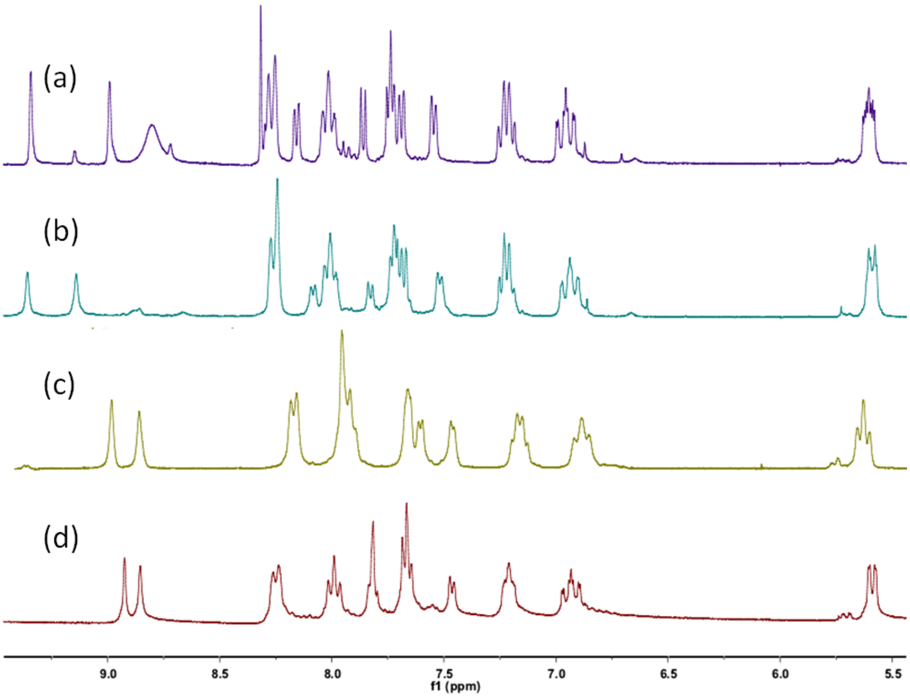


Fig. S14 ^{1}H NMR spectra **Ir1** (a), **Ir1** + ClO$^-$ (b), **Ir1** + ClO$^-$ +CF$_3$SO$_3$H (c) and **Ir5** (d) in *d*$_6$-DMSO solution, 300 MHz.

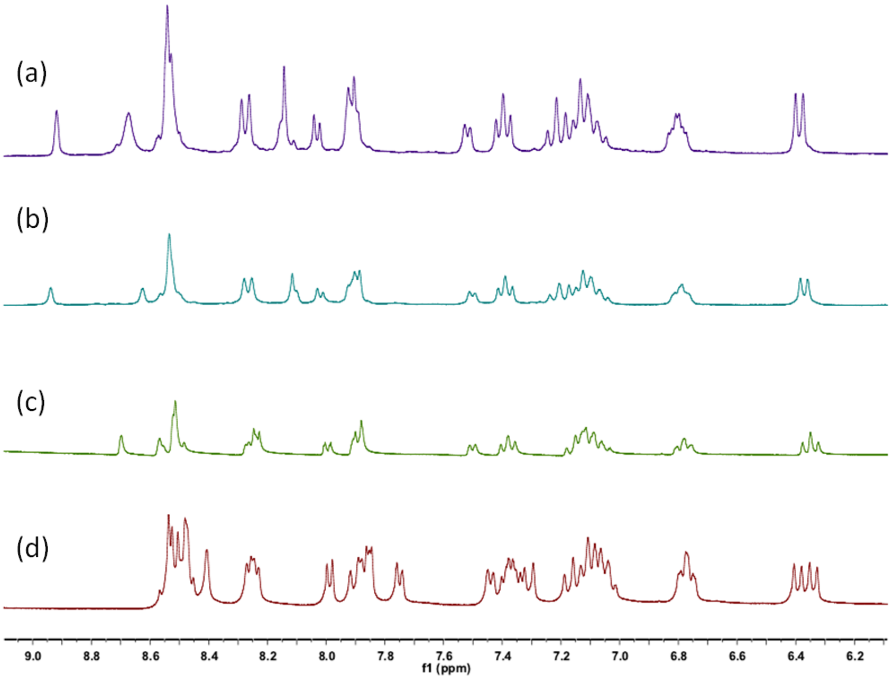


Fig. S15 ^{1}H NMR spectra **Ir2** (a), **Ir2** + ClO$^-$ (b), **Ir2** + ClO$^-$ +CF$_3$SO$_3$H (c) and **Ir6** (d) in *d*$_6$-DMSO solution, 300 MHz.

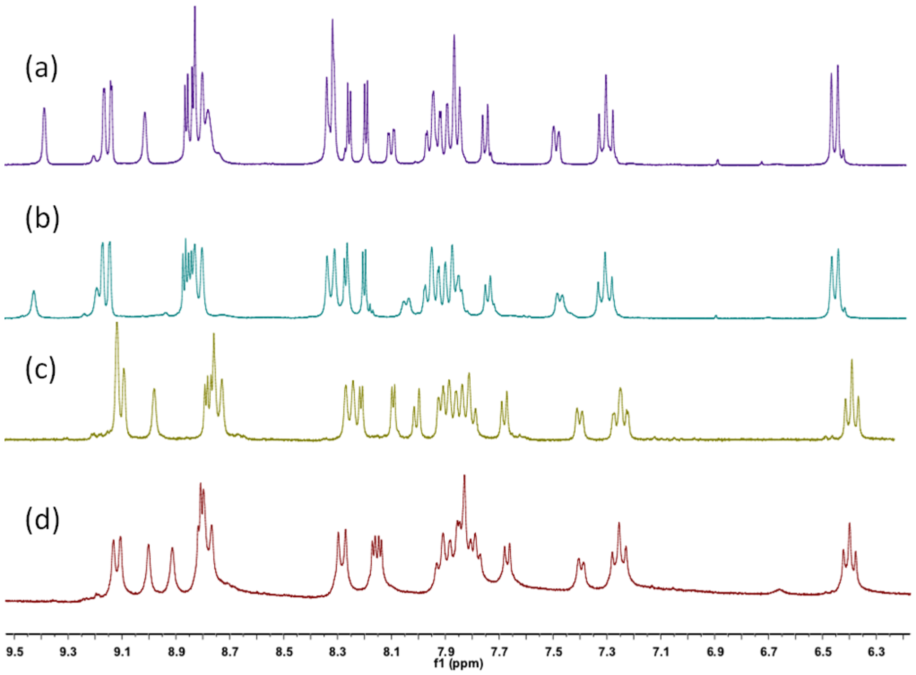


Fig. S16 ^{1}H NMR spectra **Ir4** (a), **Ir4** + ClO$^-$ (b), **Ir4** + ClO$^-$ +CF$_3$SO$_3$H (c) and **Ir8** (d) in d_6-DMSO solution, 300 MHz.

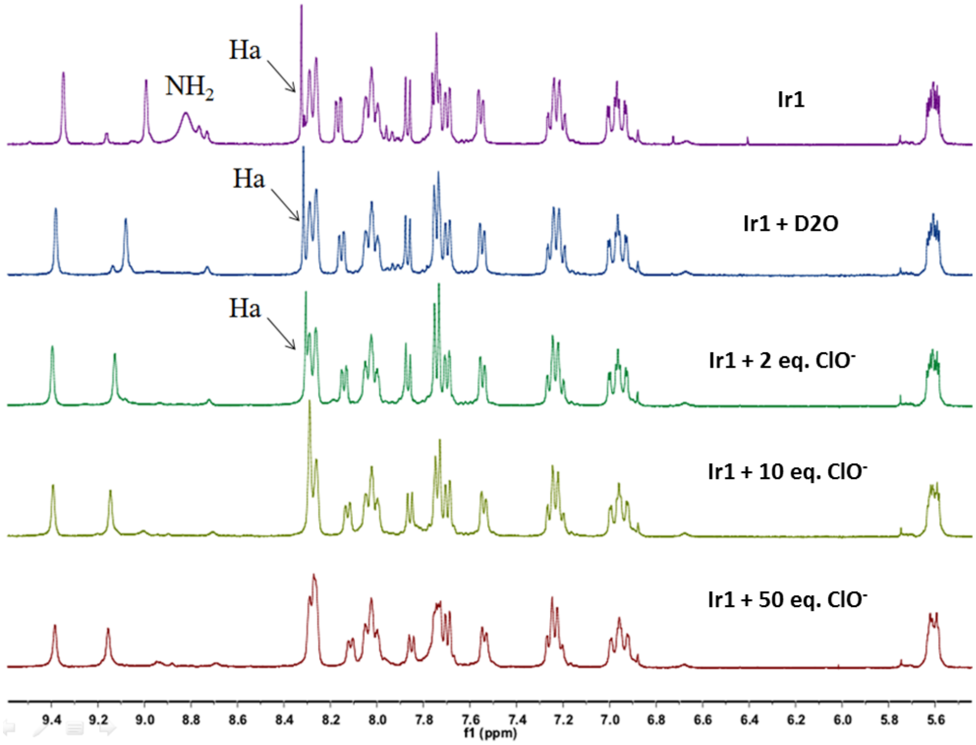


Fig. S17 ^{1}H NMR spectral change of **Ir1** reacted with different amount of ClO-.

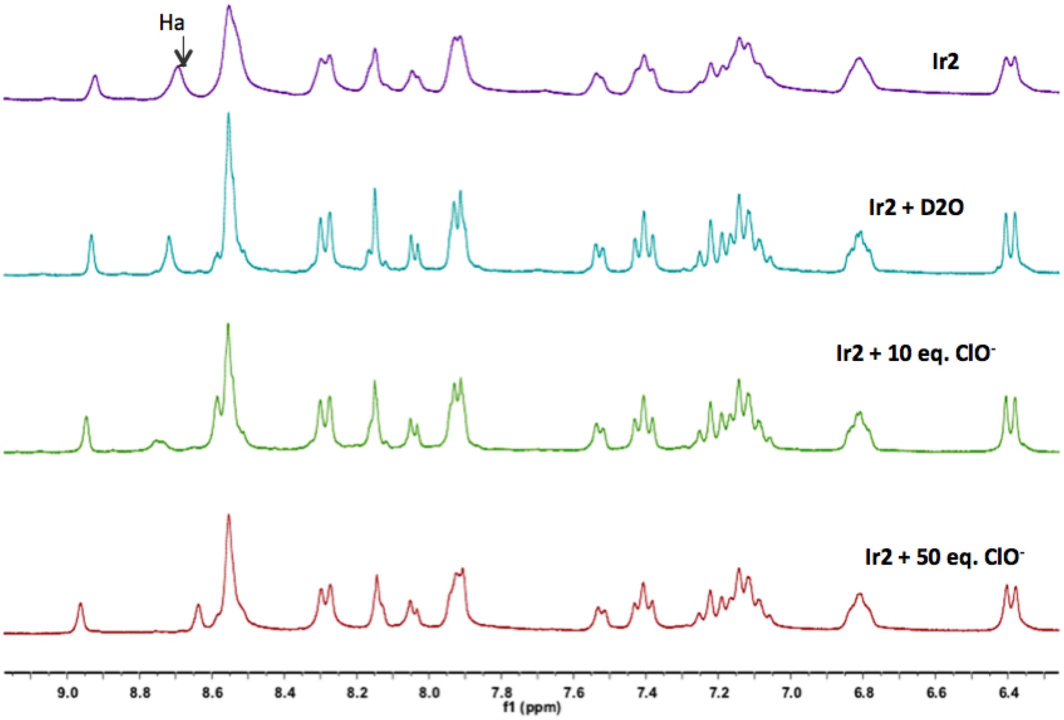


Fig. S18 ^{1}H NMR spectral change of **Ir2** reacted with different amount of ClO$^-$.

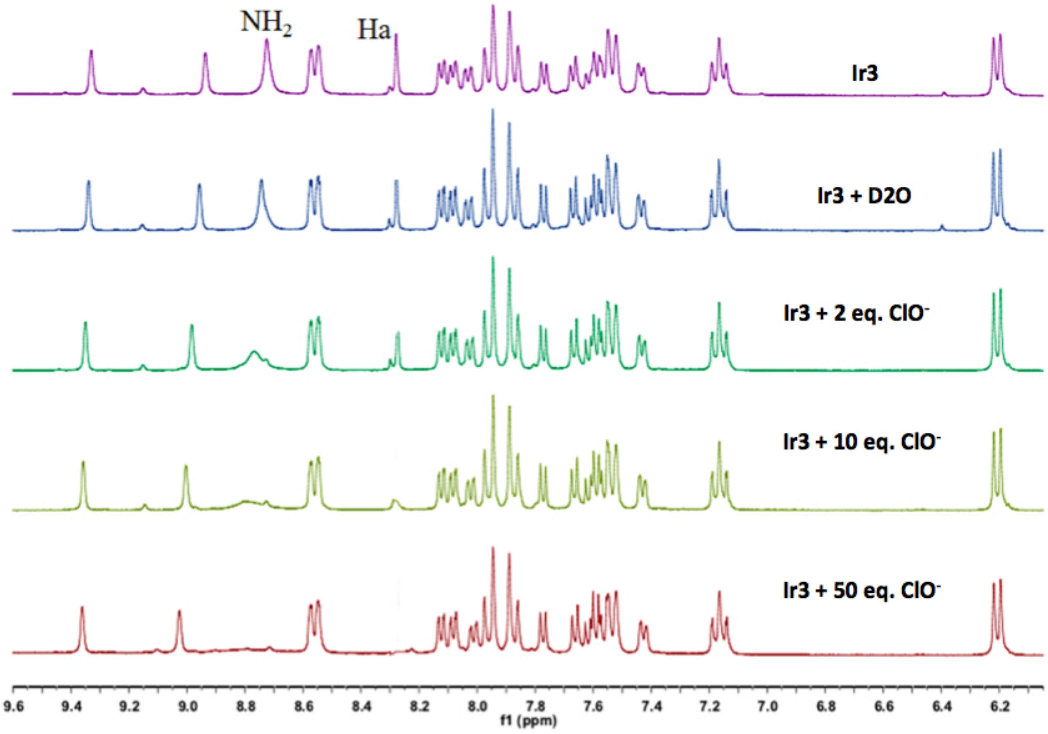


Fig. S19 ^{1}H NMR spectral change of **Ir3** reacted with different amount of ClO$^-$.

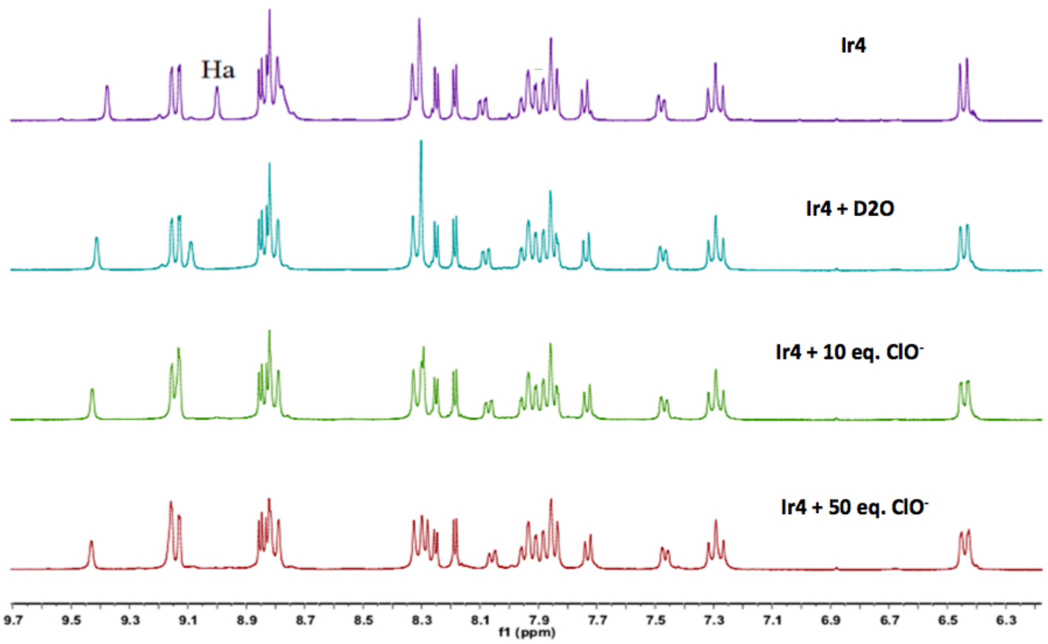


Fig. S20 ^{1}H NMR spectral change of **Ir4** reacted with different amount of ClO^-.

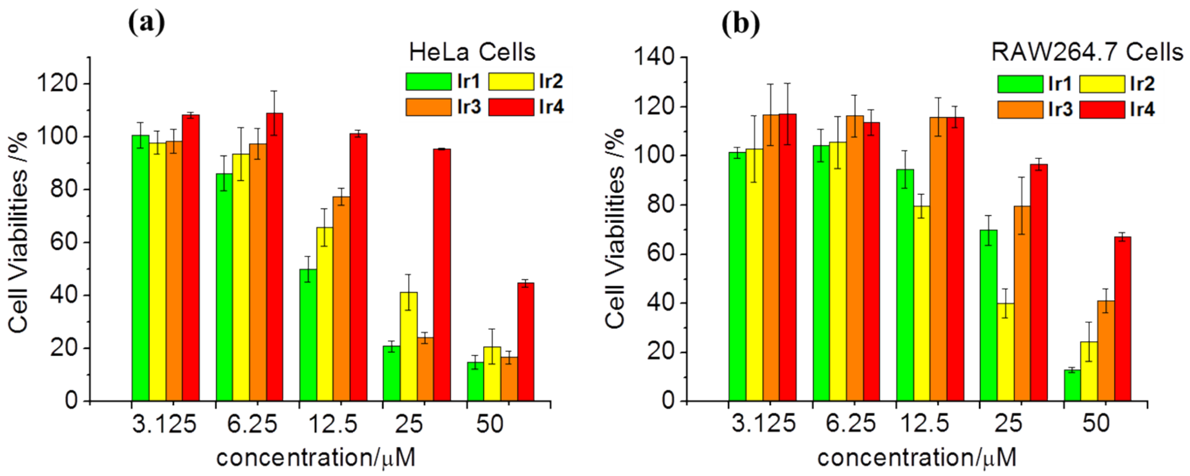


Fig. S21 Cellular viabilities of HeLa cells (a) and RAW264.7 cells (b) after incubation with different concentrations of iridium(III) complexes for 12 h.