Cyclometalated Iridium(III) N-Heterocyclic Carbene Complexes as Potential Mitochondrial Anticancer and Photodynamic Agents

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Table of Contents

Table S1 The lipophilicity and cellular uptake efficiency of complexes **Ir1-Ir4**.

Compounds	Lipophilicity (log $P_{o/w}$)	Amount of iridium (nmol per cell)[a]
Ir1	0.94	0.63 ± 0.084
Ir2	0.77	0.49 ± 0.054
Ir3	1.14	0.82 ± 0.13
Ir4	0.82	0.56 ± 0.066

[a]Data are presented as means ± standard deviation obtained in at least three independent experiments.

Table S2 IC_{50} (μM) values of the tested complexes towards HeLa, U87 and LO2 cell lines at dark and 450 nm.

Compounds	HeLa			U87			LO2		
	Dark [a]	Light [b]	PI [c]	Dark [a]	Light [b]	PI [c]	Dark [a]	Light [b]	PI [c]
Ir1	1.3 ± 0.1	0.069 ± 0.013	19	1.6 ± 0.3	0.029 ± 0.008	55	1.2 ± 0.1	0.10 ± 0.03	12
Ir2	1.0 ± 0.1	0.26 ± 0.06	4	1.8 ± 0.3	0.046 ± 0.01	39	2.0 ± 0.3	0.81 ± 0.05	2.5
Ir3	1.6 ± 0.4	0.11 ± 0.04	15	1.4 ± 0.2	0.069 ± 0.015	20	1.1 ± 0.1	0.17 ± 0.07	6.5
Ir4	1.8 ± 0.2	0.11 ± 0.06	4	1.6 ± 0.2	0.040 ± 0.009	40	2.1 ± 0.3	0.50 ± 0.04	4.2
cisplatin	14.1 ± 1.1	13.0 ± 1.2	1.1	33.4 ± 2.3	33.1 ± 2.6	1.0	11.5 ± 0.5	10.9 ± 0.8	1.0

[a]IC_{50} values are drug concentrations necessary for 50% inhibition of cell viability. Data are presented as means ± standard deviation obtained in at least three independent experiments. Cells are treated with complexes for 48 h.
[b] Phototoxicity index is the ratio of the IC_{50} value in dark to that obtained upon light irradiation. Cells were treated with the compounds for 12 h and then exposed to 450 nm LED light for 10 min.
[c] PI (Phototoxicity index) is the ratio of the IC_{50} value in the dark to that obtained upon light irradiation.

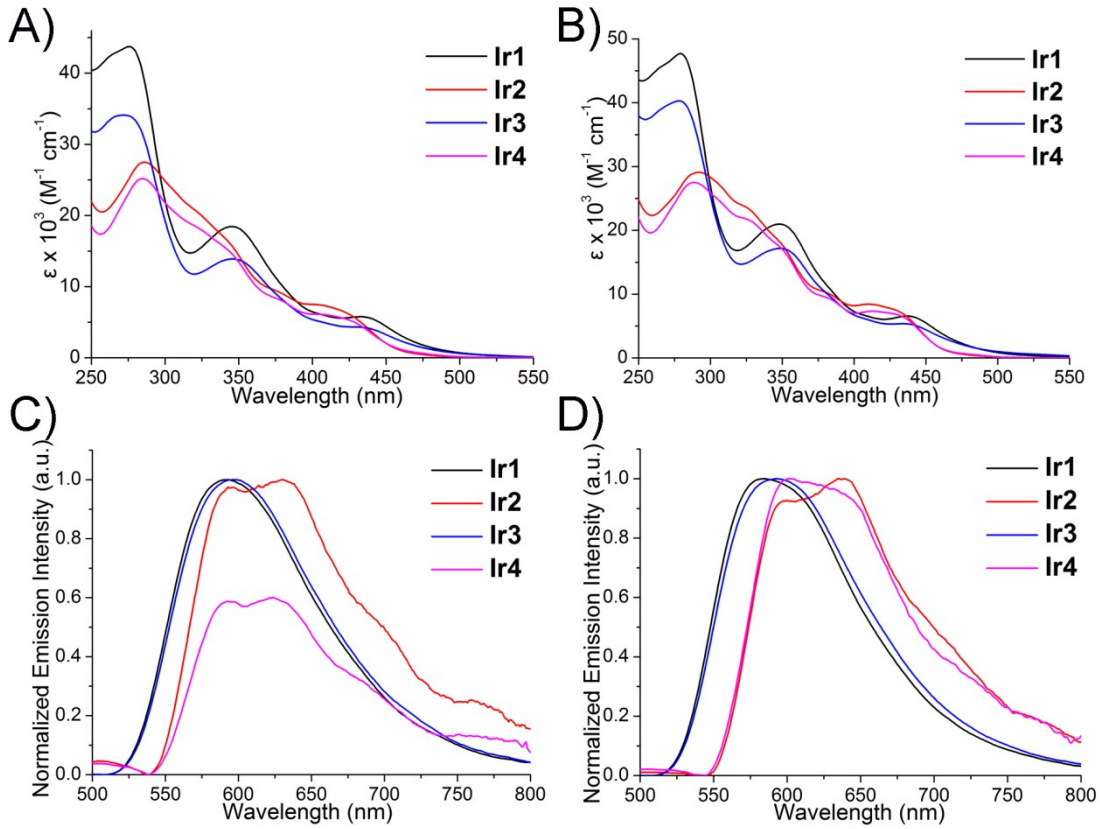

Fig. S1 A) UV/Vis spectra of **Ir1-Ir4** (20 μM) in CH_3CN at 298 K. B) UV/Vis spectra of **Ir1-Ir4** (20 μM) in CH_2Cl_2 at 298 K. C) Normalized emission spectra of **Ir1-Ir4** (20 μM) in CH_3CN at 298 K (λ_{ex} = 405 nm). D) Normalized emission spectra of **Ir1-Ir4** (20 μM) in CH_2Cl_2 at 298 K (λ_{ex} = 405 nm).

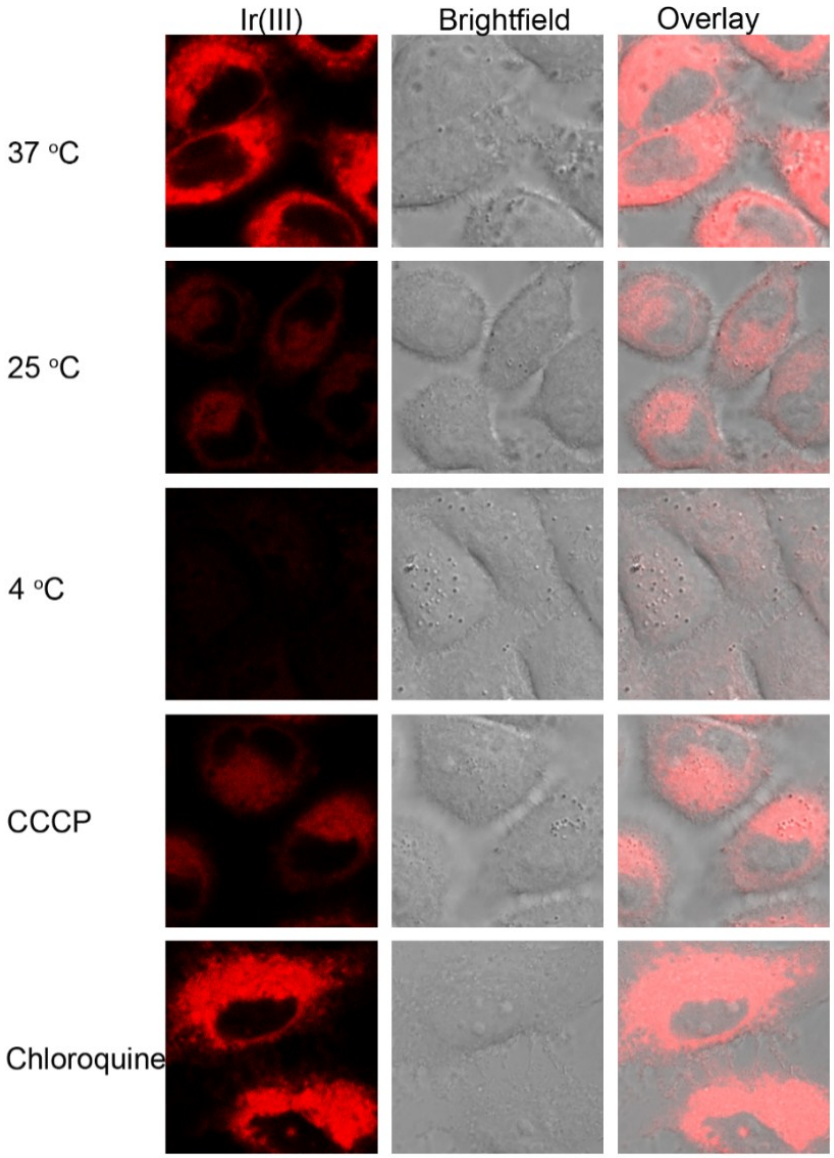

Fig. S2 Confocal images of A549 cells after incubation with **Ir1** (10 μM) under different conditions. (A) Cells were incubated with **Ir1** (10 μM) at 37 °C for 10 min. (B) Cells were incubated with **Ir1** (10 μM) at 4 °C for 10 min. (C) Cells were pre-incubated with CCCP (10 μM) for 1 h at 37 °C and then incubated with **Ir1** (10 μM) at 37 °C for 10 min. (D) Cells were pre-incubated with chloroquine (50 μM) for 1 h at 37 °C and then incubated with **Ir1** (10 μM) at 37 °C for 10 min. Complex **7** was excited at 405 nm and emission was collected at 600 ± 20 nm. Scale bar: 10 μm.

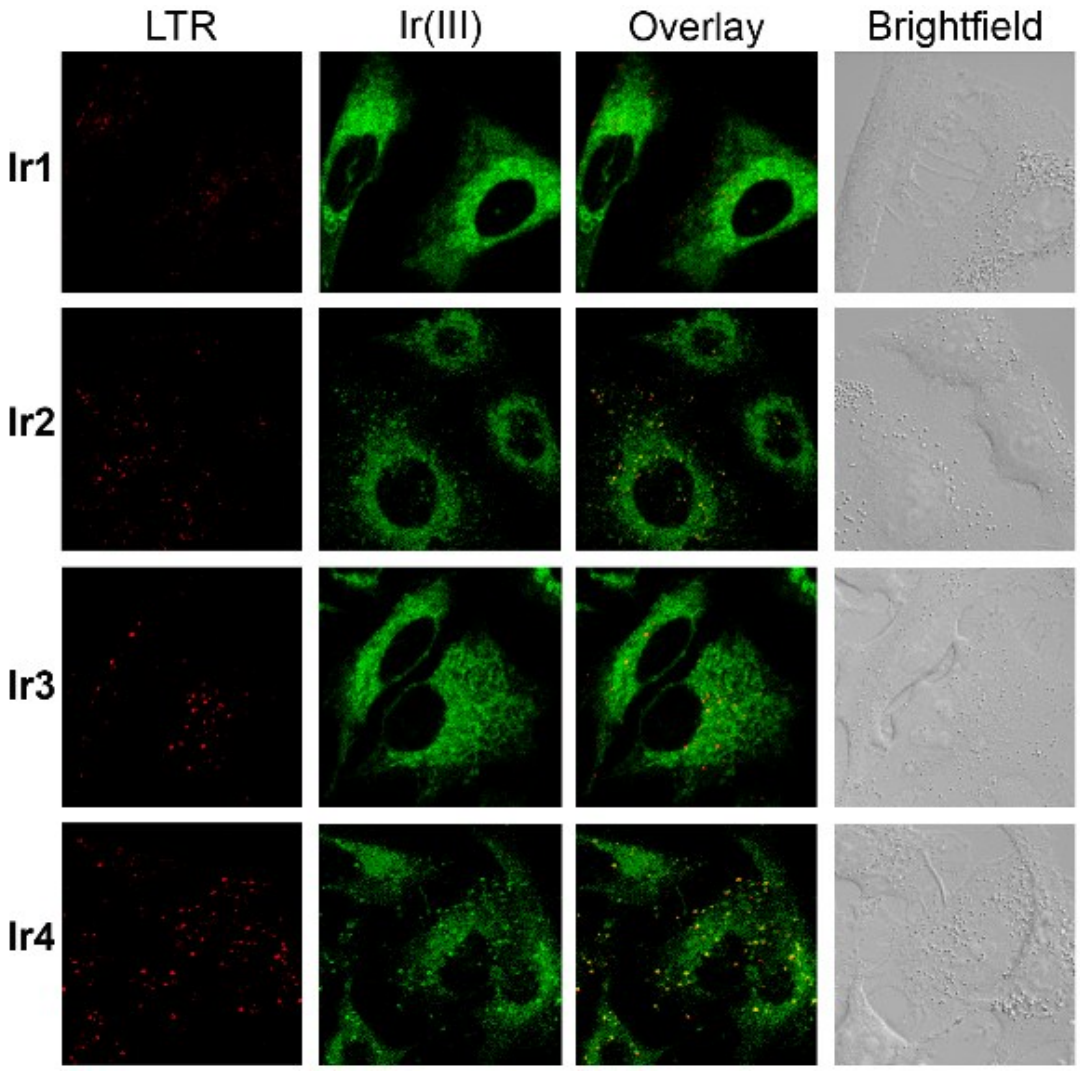

Fig. S3 Determination of intercellular localization of complexes **Ir1-Ir4** by confocal microscopy. A549 cells were incubated with LTDR (100 nM) for 20 min and then co-incubated with **Ir1-Ir4** (10 μM)) for another 10 min at 37 °C. The Ir(III) complexes were excited at 405 nm and the emission was collected at 600 ± 20 nm. LTDR was excited at 633 nm and the emission was collected at 660 ± 20 nm. Scale bar: 10 μm.

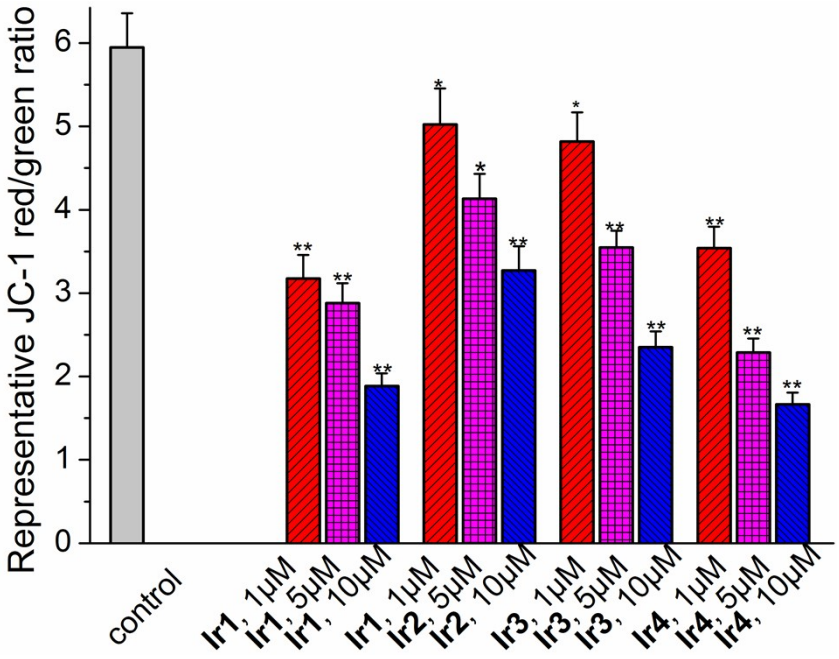

Fig. S4 Impact of complexes **Ir1-Ir4** on MMP. The fluorescent intensity ration of A549 cells treated with **Ir1-Ir4** at indicated concentrations for 6 h. Data shown are mean values ± standard deviations from three independent experiments. (*) $P < 0.01$, (**) $P < 0.005$, compared with the vehicle-treated cells.

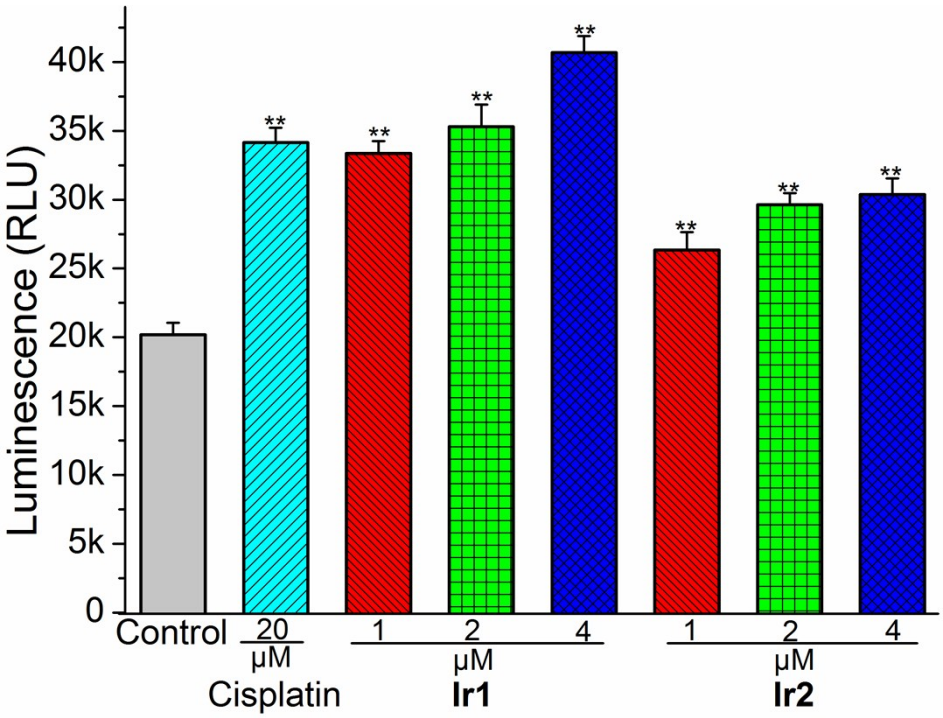

Fig. S5 Activation of caspases-3/7 by Ir(III) treatment. A549 cells were exposed to cisplatin, **Ir1** and **Ir2** at the indicated concentrations for 12 h. Data shown are mean values ± standard deviations from three independent experiments. (**) $P < 0.005$, compared with the vehicle-treated cells.

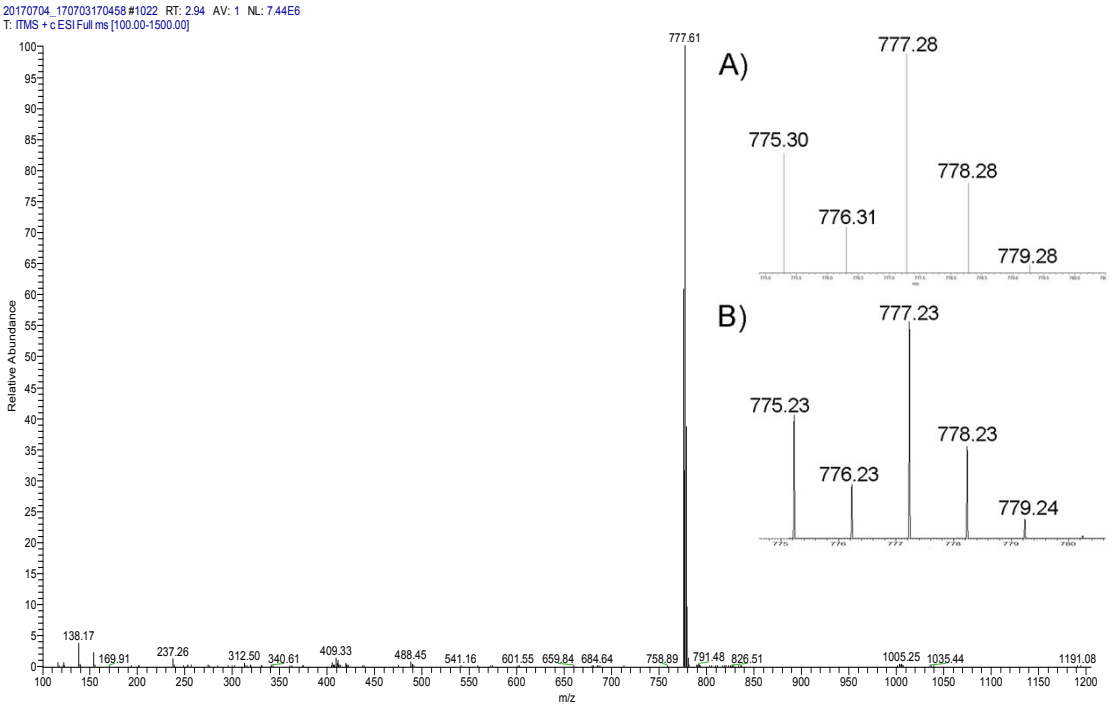

Figure S6 ESI-MS spectrum of complexes **Ir1**. A) Ion isotopes spectrum of complexes **Ir1**. B) Ion isotopes spectrum of computer simulation using formula $IrC_{39}H_{32}N_6$, corresponding to [**Ir1**-Cl]$^+$.

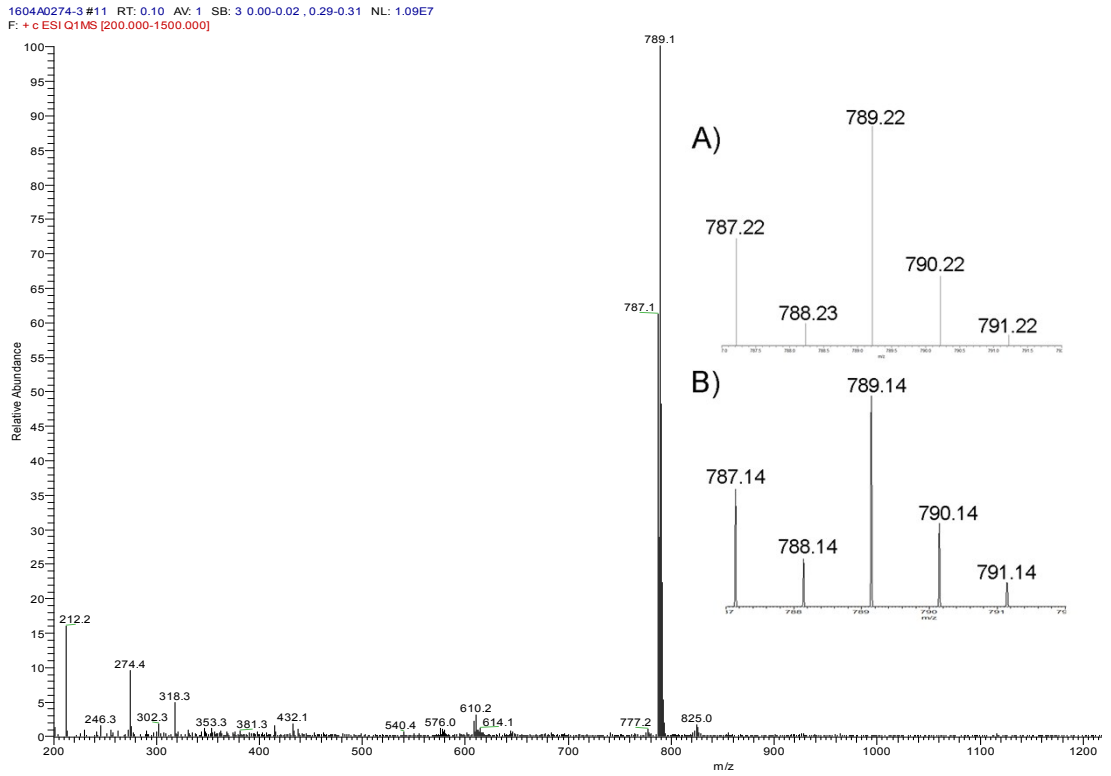

Figure S7 ESI-MS spectrum of complexes **Ir2**. A) Ion isotopes spectrum of complexes **Ir2**. B) Ion isotopes spectrum of computer simulation using formula $IrC_{35}H_{28}N_6S_2$, corresponding to [**Ir2**-Cl]$^+$.

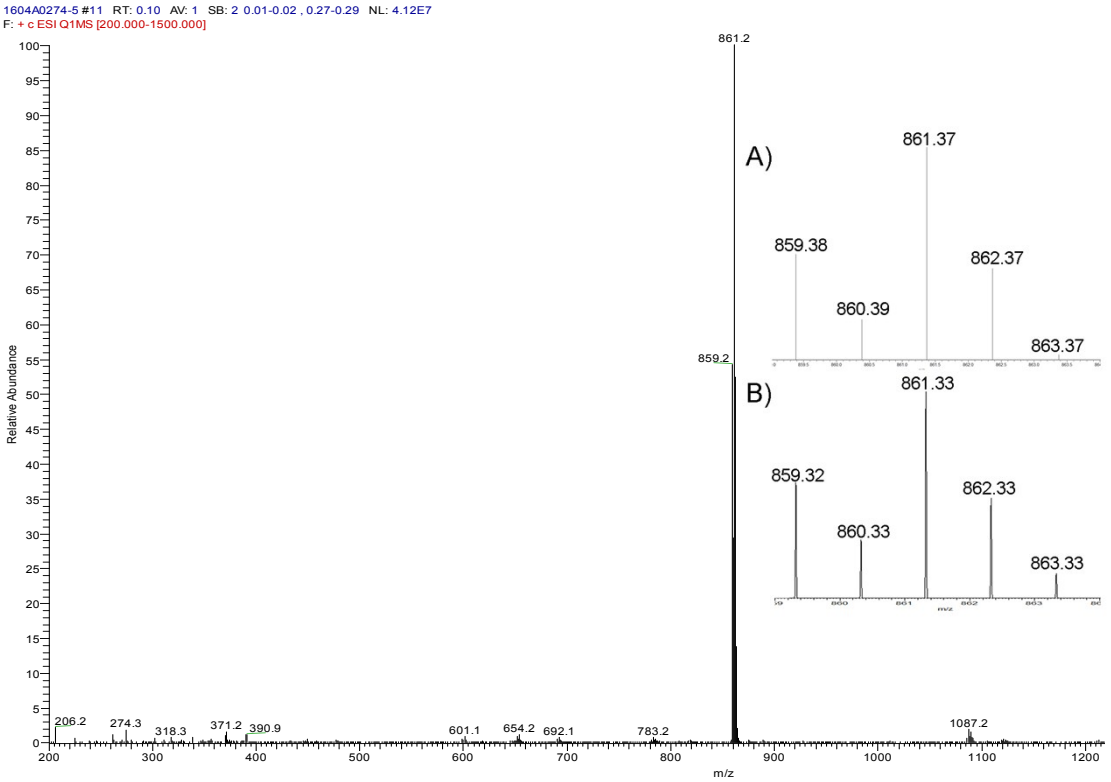

Figure S8 ESI-MS spectrum of complexes **Ir3**. A) Ion isotopes spectrum of complexes **Ir3**. B) Ion isotopes spectrum of computer simulation using formula $IrC_{45}H_{44}N_6$, corresponding to [**Ir3**-Cl]$^+$.

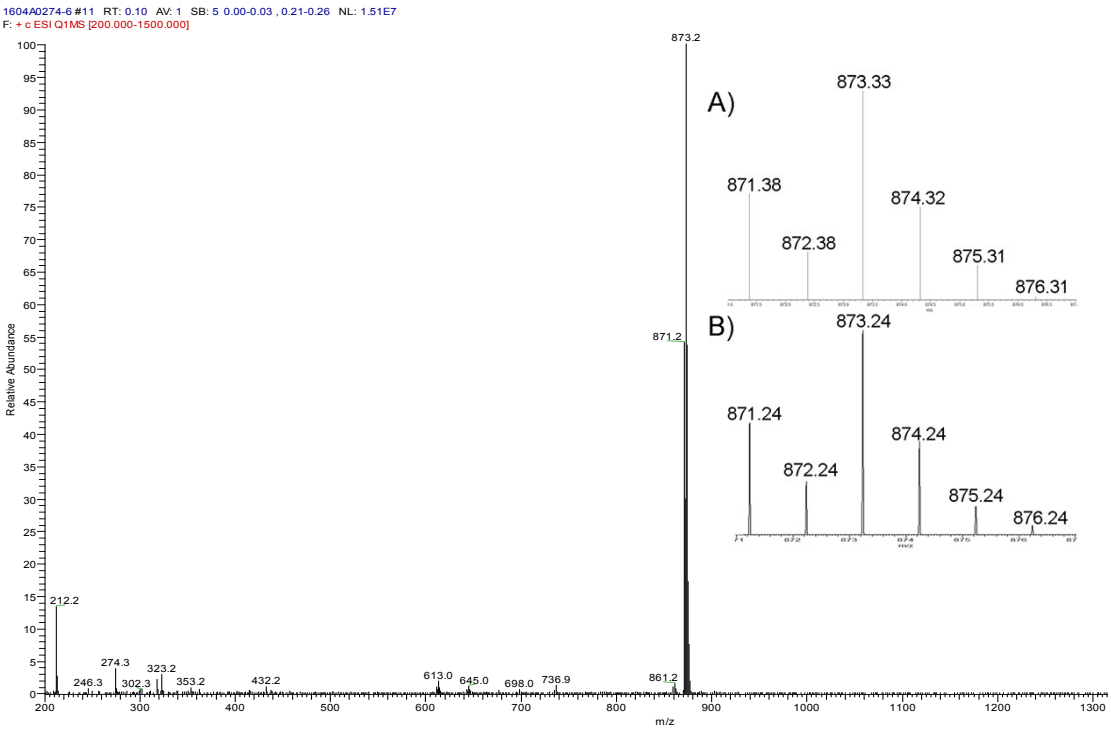

Figure S9 ESI-MS spectrum of complexes **Ir4**. A) Ion isotopes spectrum of complexes **Ir4**. B) Ion isotopes spectrum of computer simulation using formula $IrC_{41}H_{40}N_6S_2$, corresponding to [**Ir4**-Cl]$^+$.

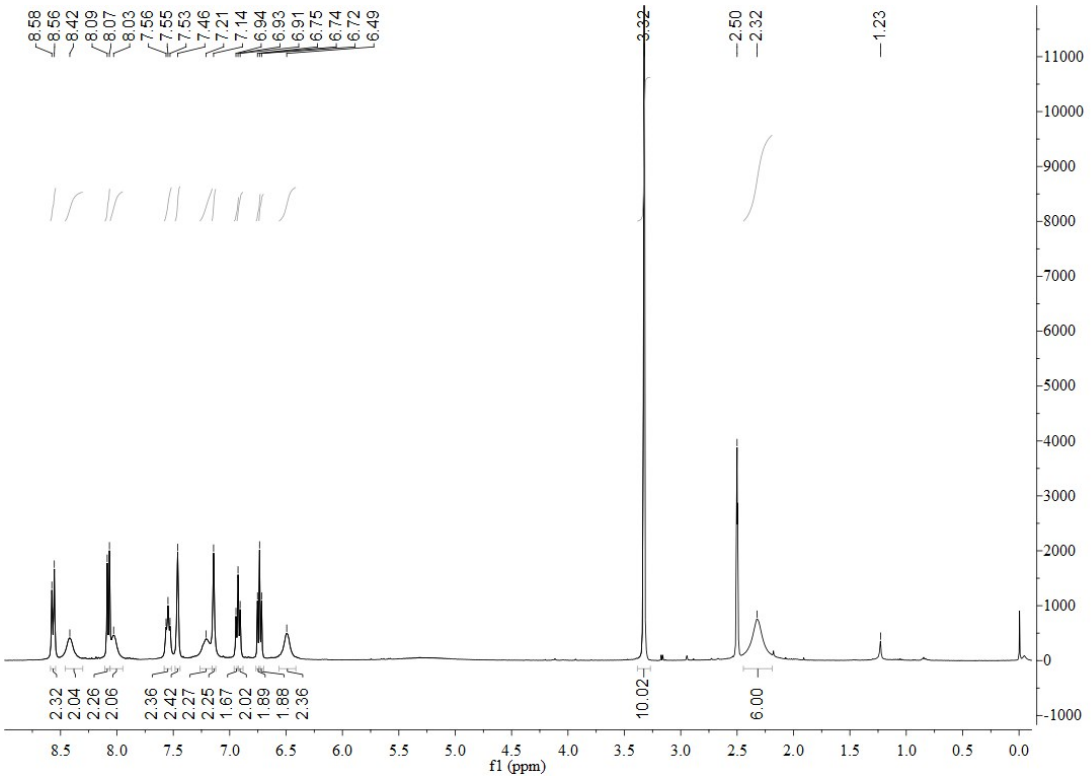

Figure S10 [1]H NMR spectrum of complexes **Ir1.**

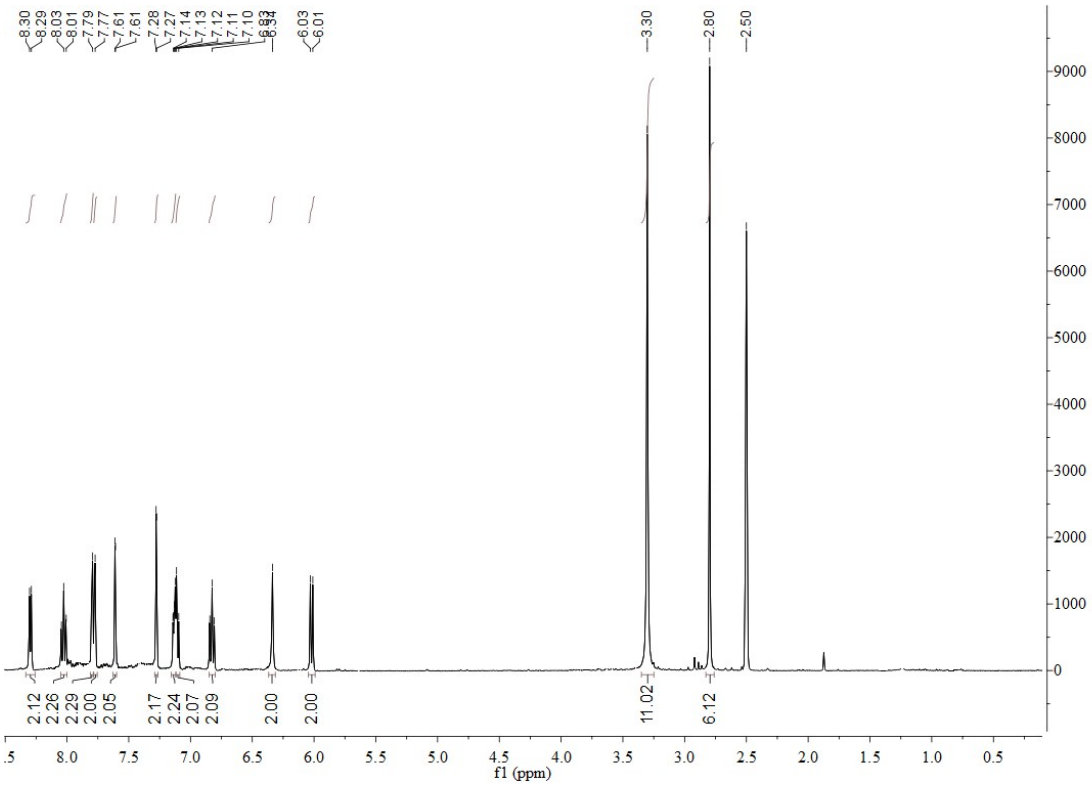

Figure S11 [1]H NMR spectrum of complexes **Ir2.**

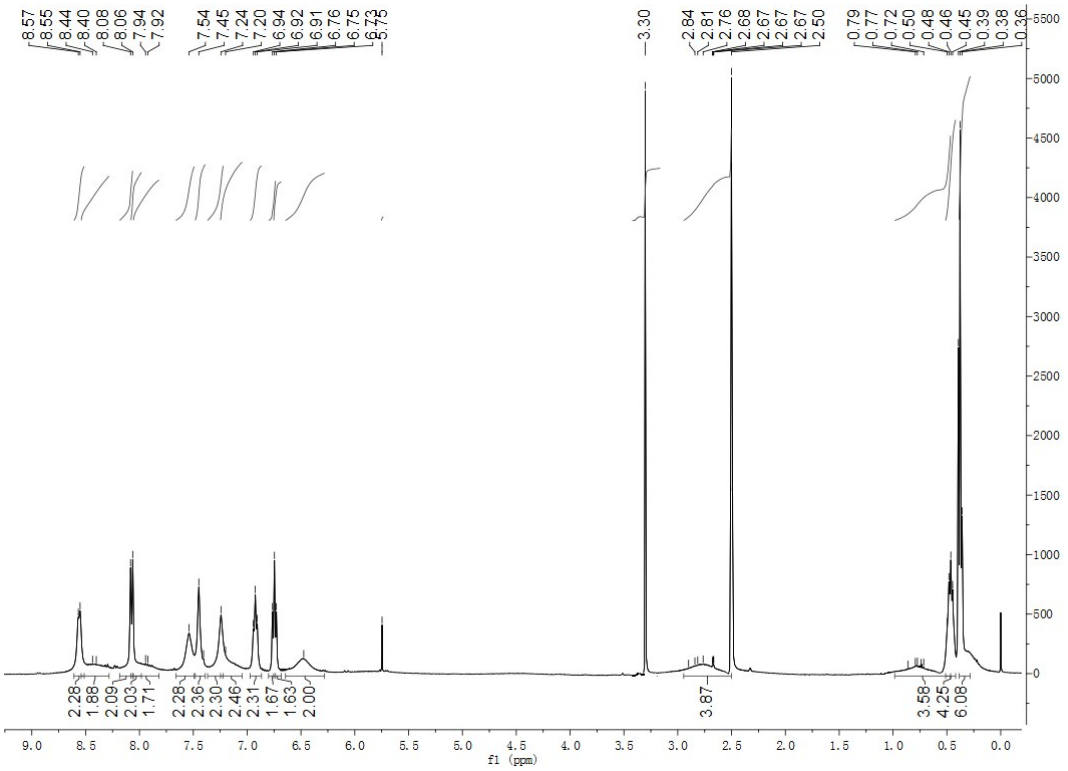

Figure S12 ^{1}H NMR spectrum of complexes **Ir3.**

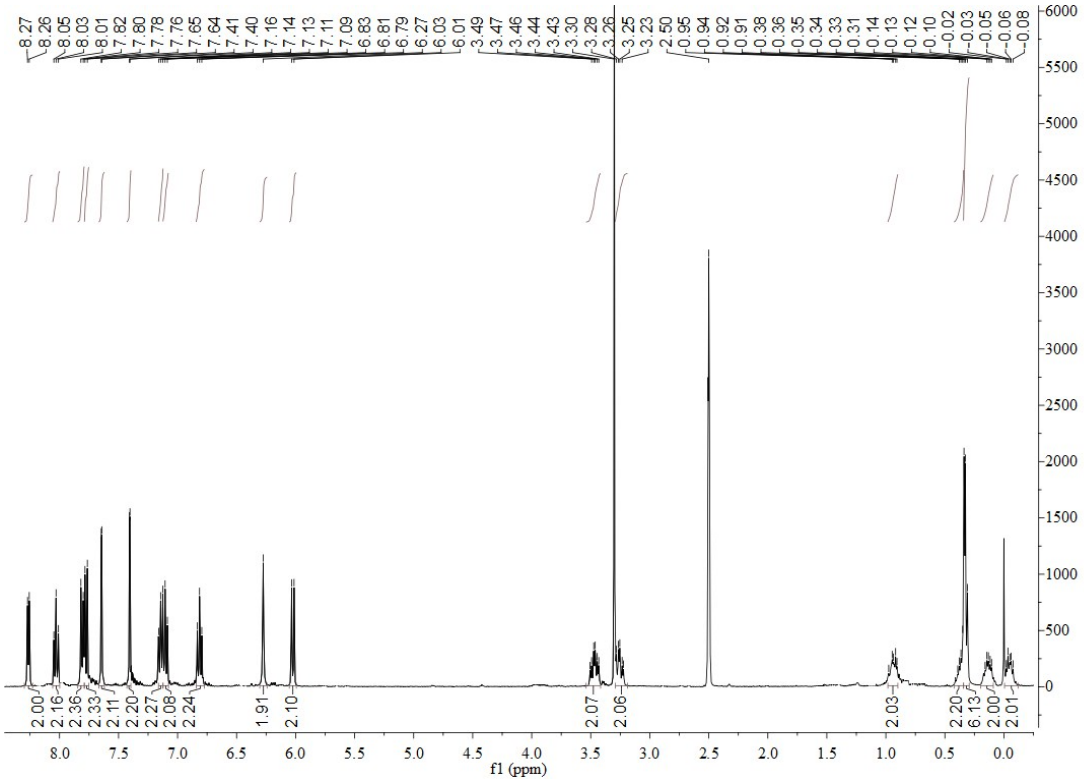

Figure S13 ^{1}H NMR spectrum of complexes **Ir4.**