Supporting Information

Isomeric Ir(III) complexes for tracking mitochondrial pH fluctuation and inducing mitochondria disfunction during photodynamic therapy

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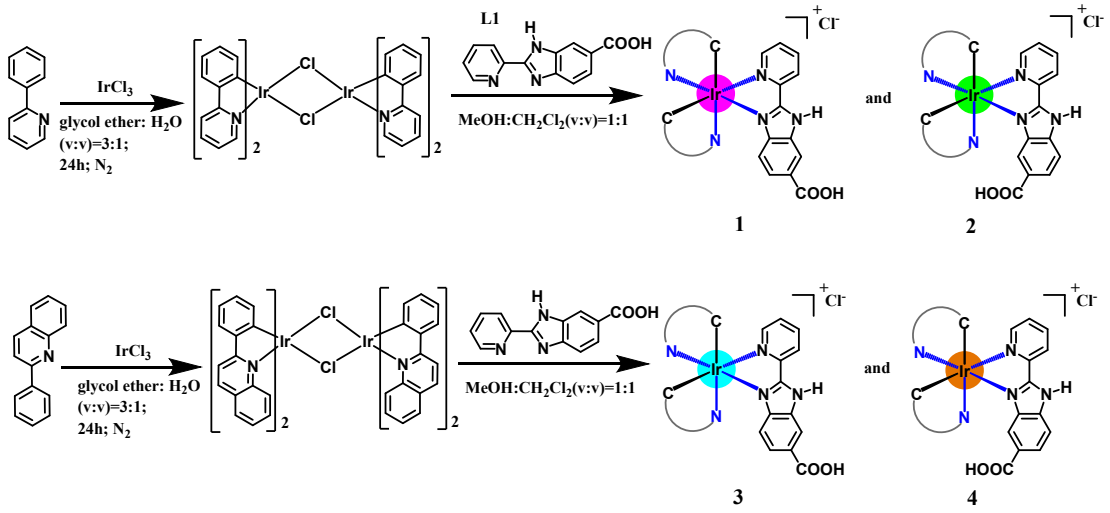
Contents



Scheme S1. The synthetic routes of the iridium complexes.

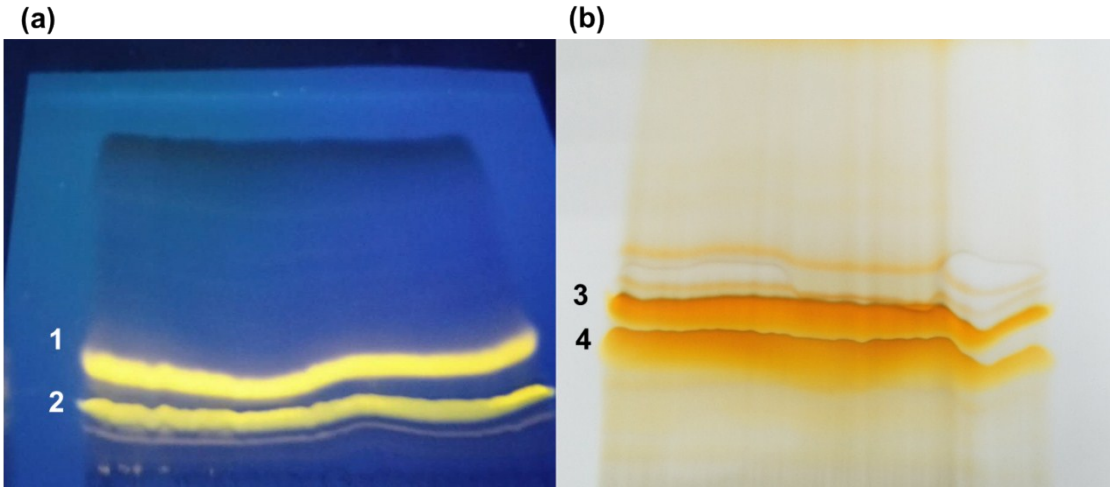


Fig. S1. The separation of **1-4** on preparative thin-layer silica gel plates with dichloromethane and methanol mixture solution (v:v = 10:1).

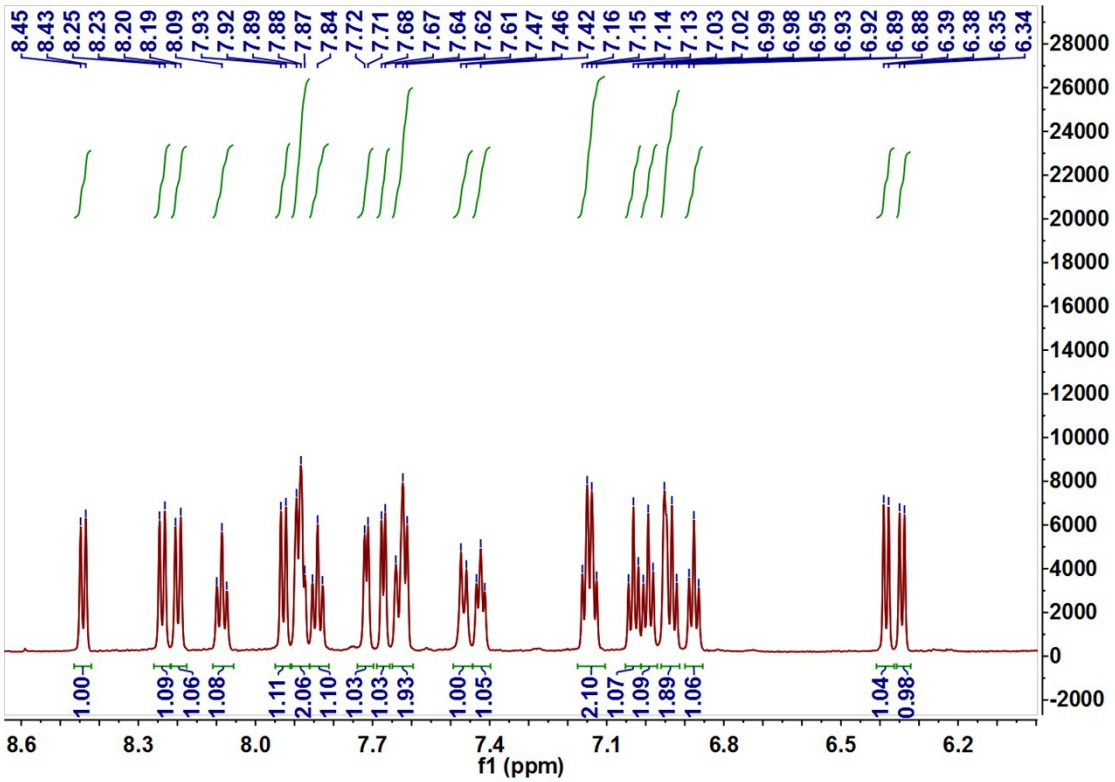


Fig. S2. The 500 MHz ^{1}H NMR spectrum of **1** in the DMSO-d^{6} solution.

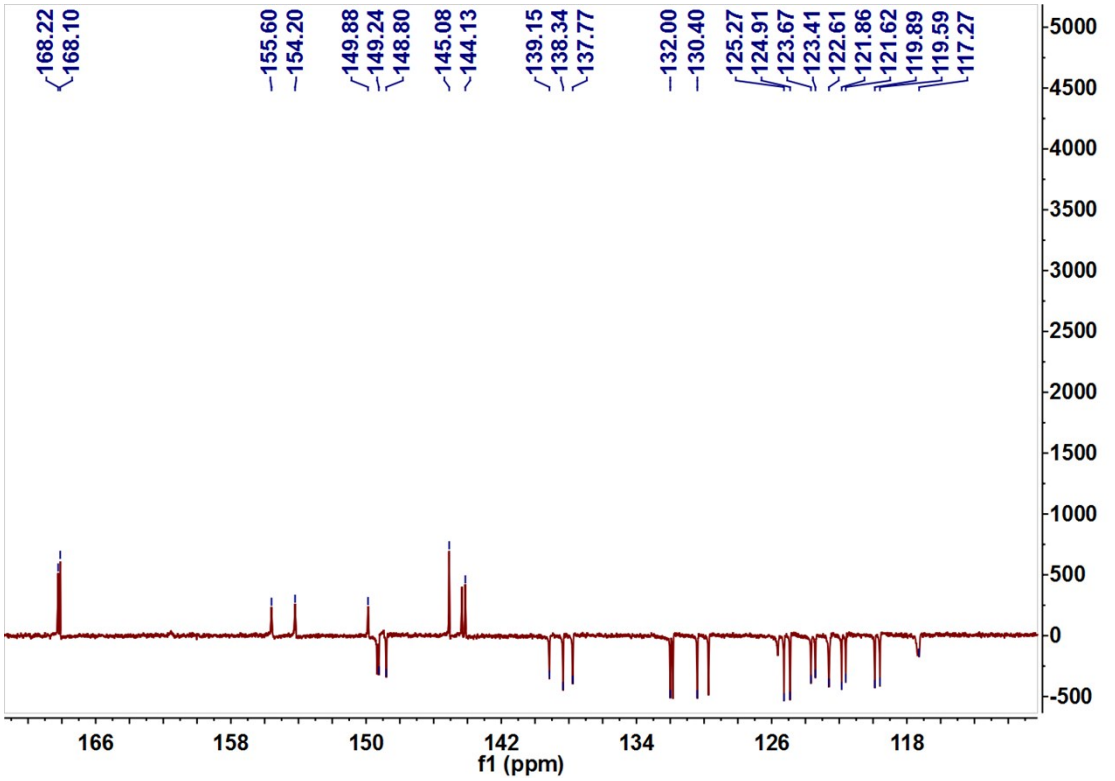


Fig. S3. The 500 MHz ^{13}C NMR spectrum of **1** in the DMSO-d^{6} solution.

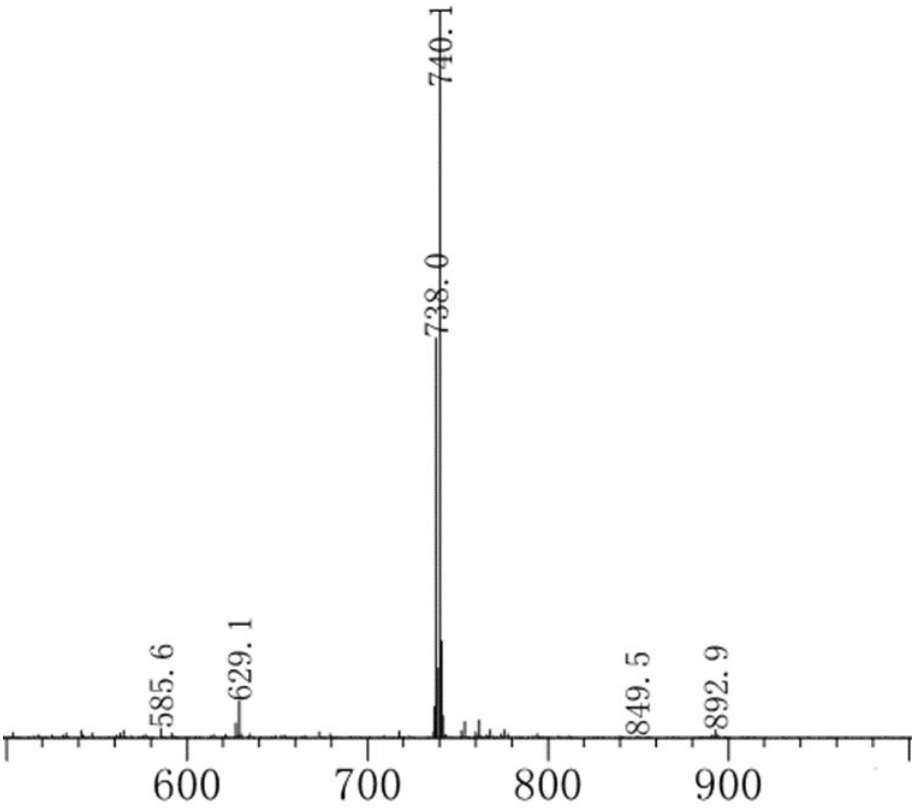


Fig. S4. The ESI-MS spectrum of **1** (CH_3OH, positive mode).

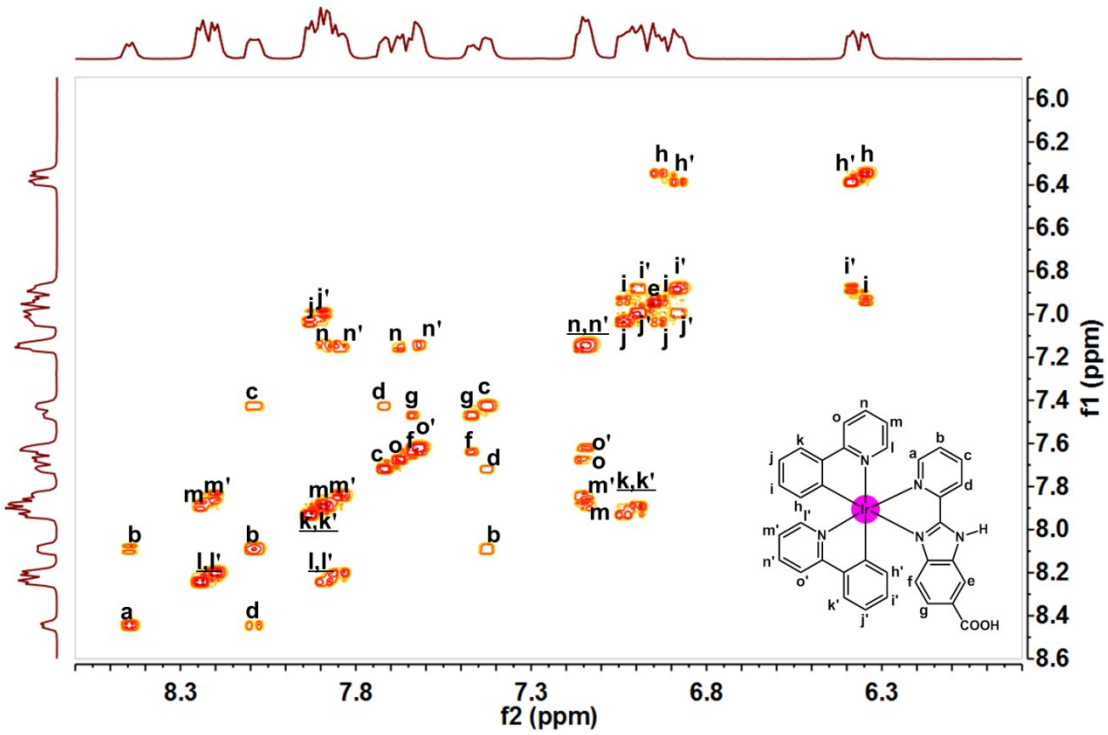


Fig. S5. The 500 MHz ^{1}H-^{1}H Cozy spectrum of **1** in the DMSO-d^6 solution.

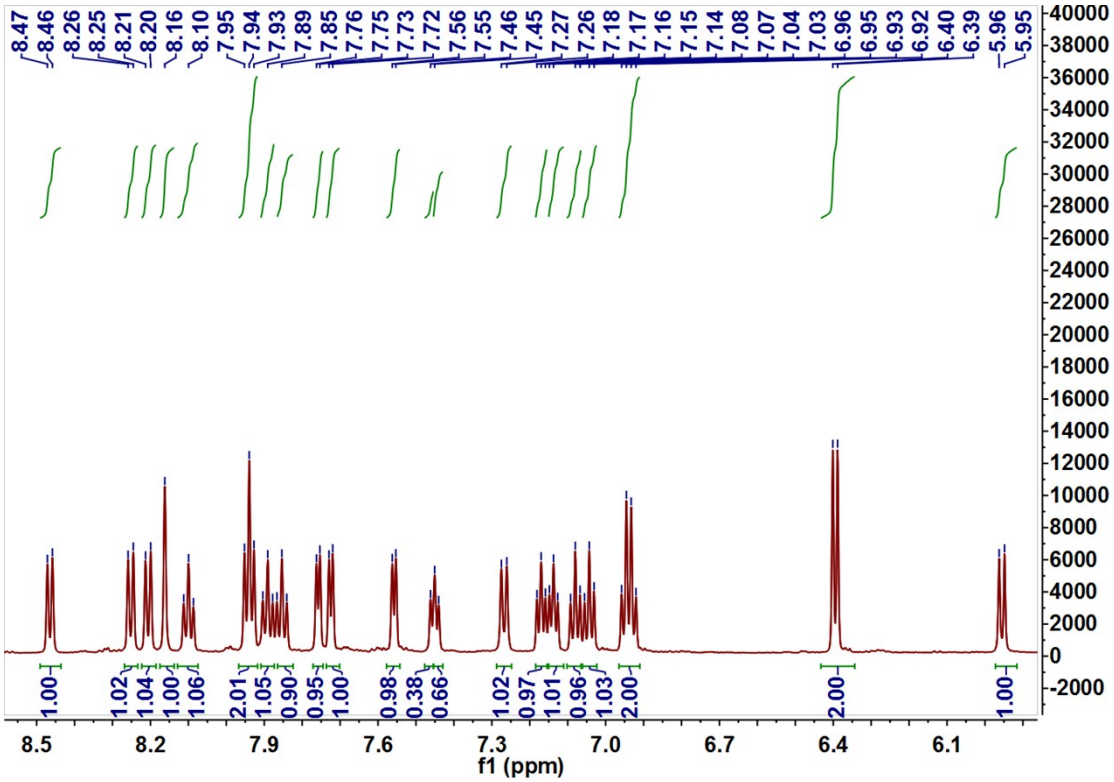


Fig. S6. The 500 MHz ^{1}H NMR spectrum of **2** in the DMSO-d^{6} solution.

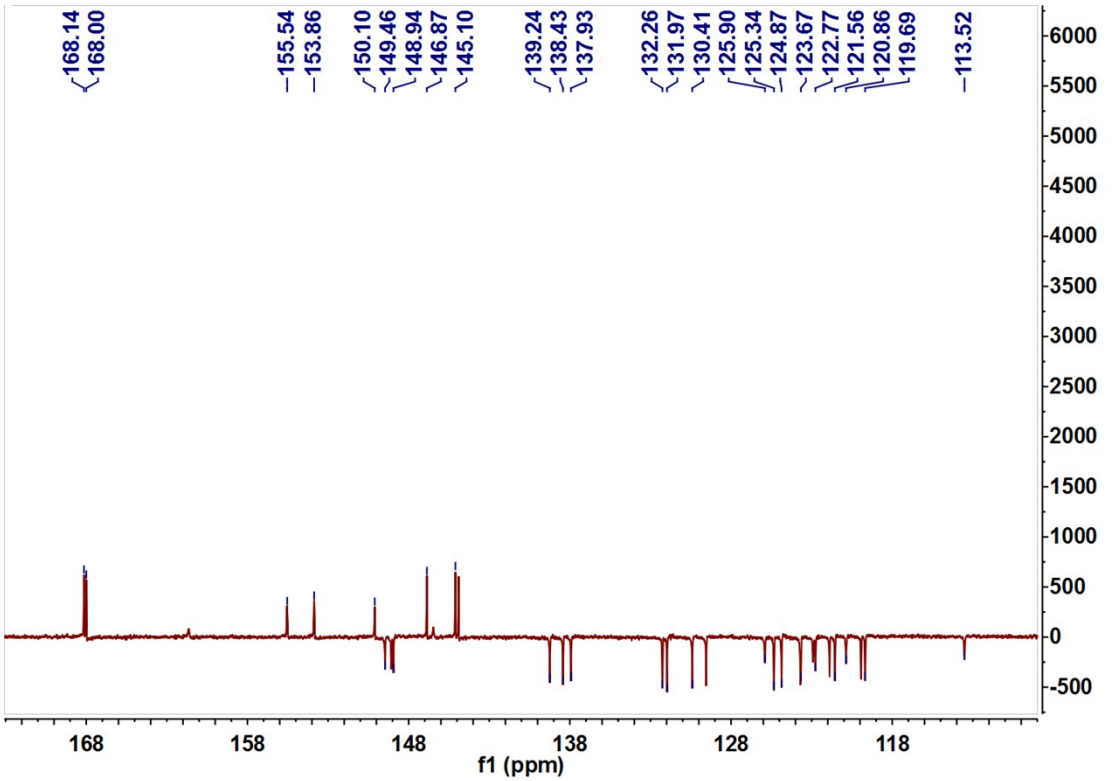


Fig. S7. The 500 MHz ^{13}C NMR spectrum of **2** in the DMSO-d^{6} solution.

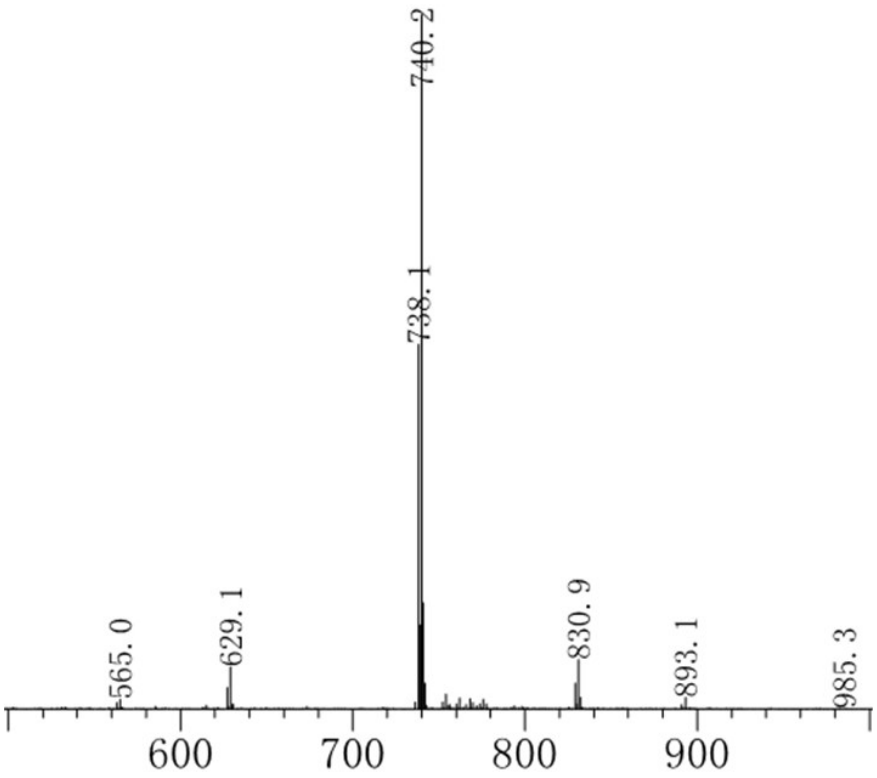


Fig. S8. The ESI-MS spectrum of **2** (CH_3OH, positive mode).

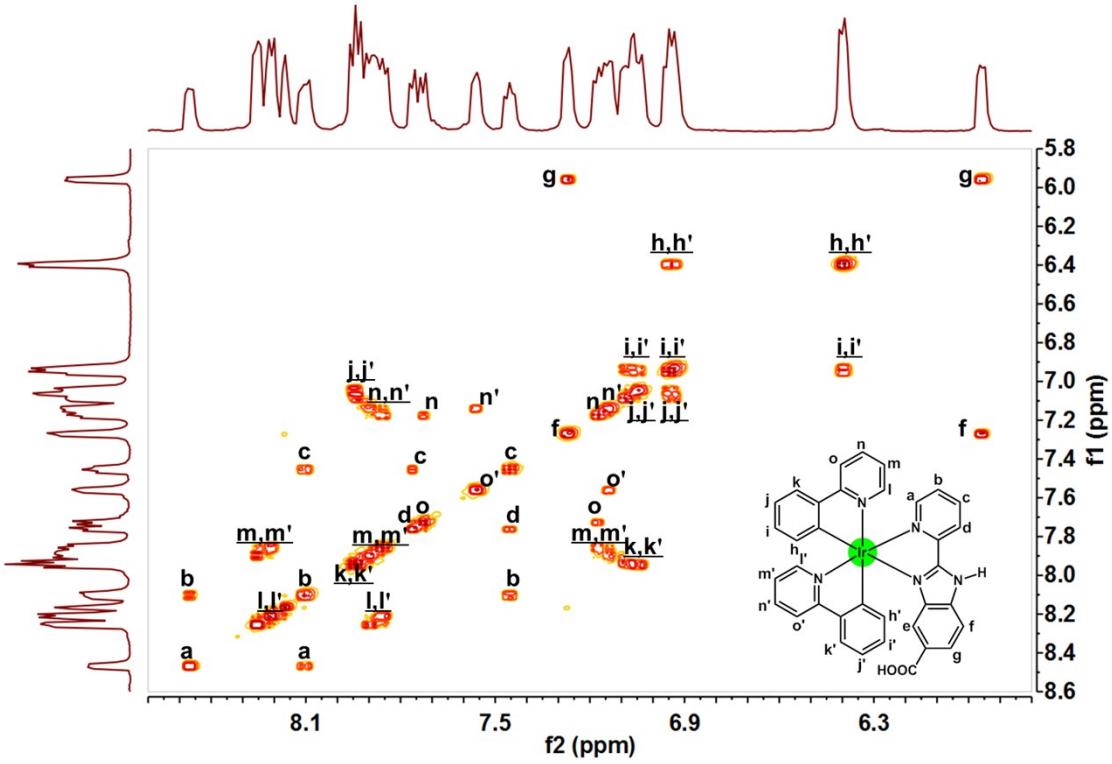


Fig. S9. The 500 MHz ^{1}H-^{1}H Cozy spectrum of **2** in the DMSO-d^6 solution.

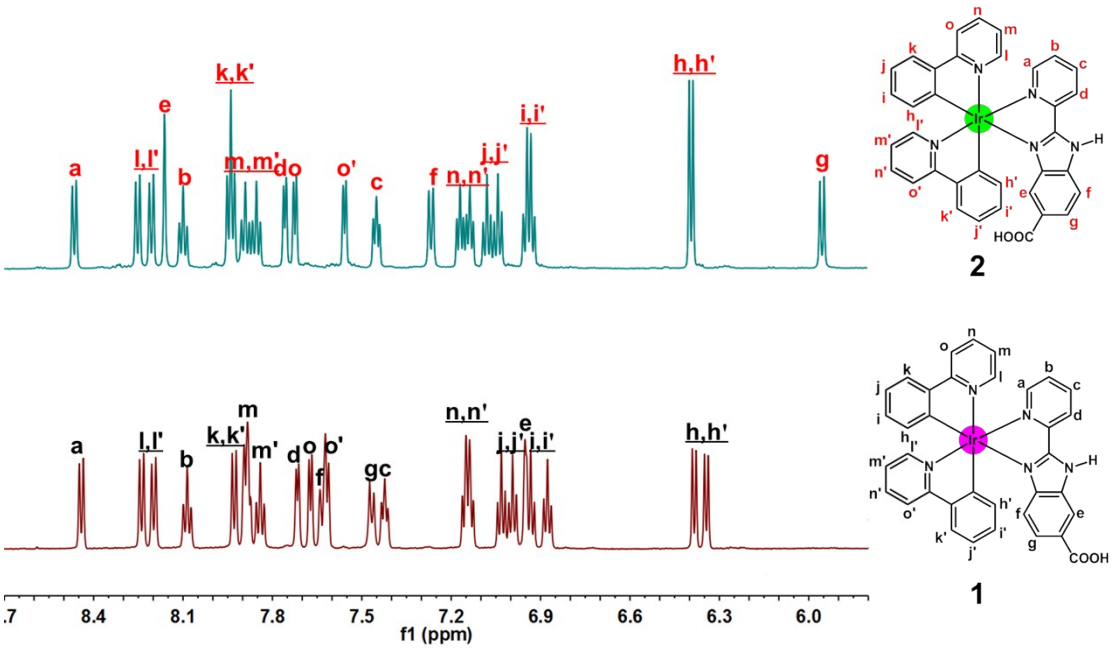


Fig. S10. The assignment of H atoms of the ^{1}H NMR spectra of **1** and **2**.

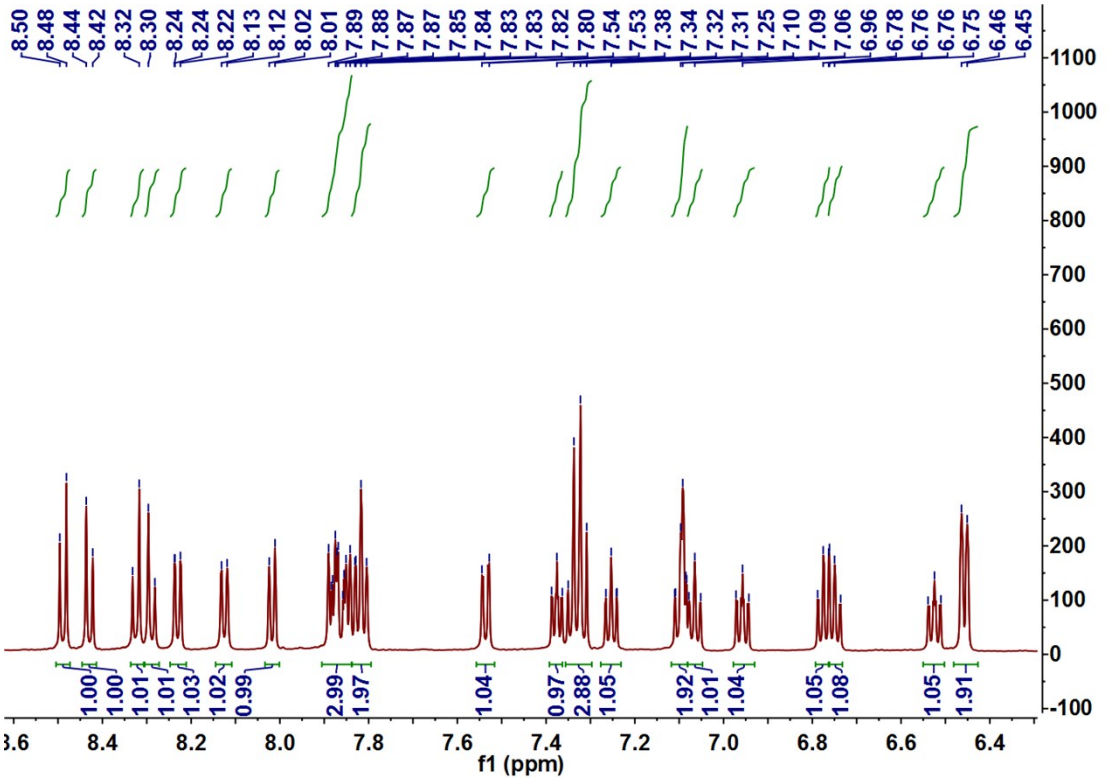


Fig. S11. The 500 MHz ^{1}H NMR spectrum of **3** in the DMSO-d^{6} solution.

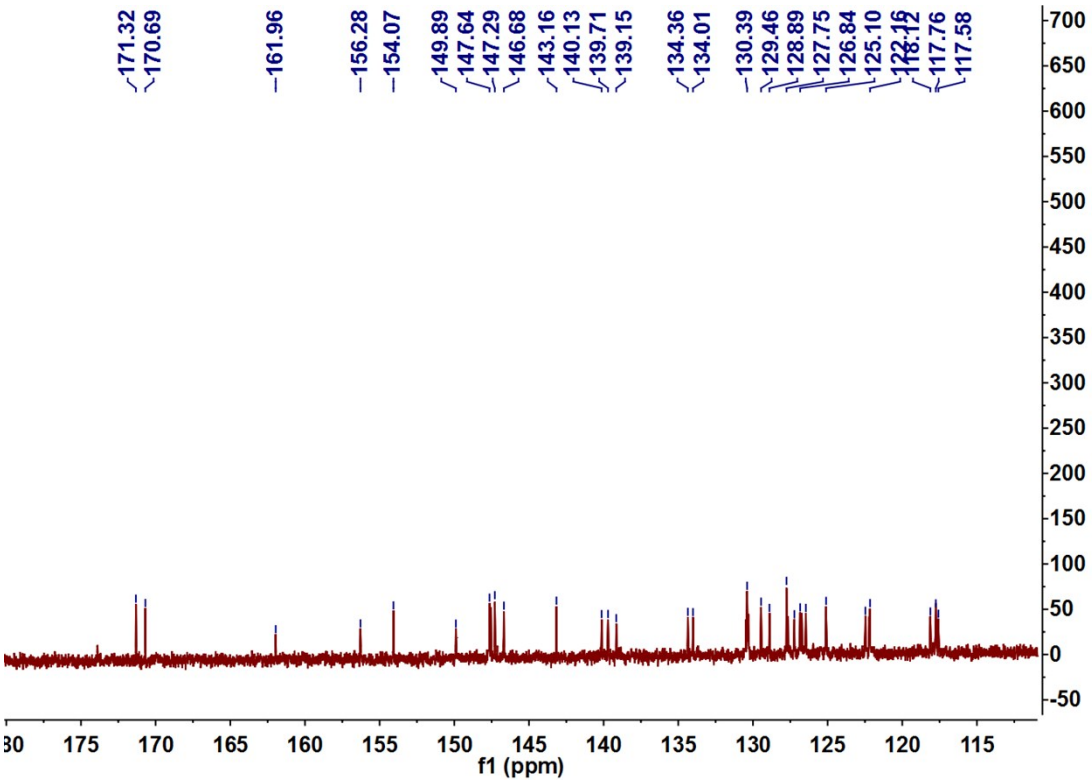


Fig. S12. The 500 MHz ^{13}C NMR spectrum of **3** in the DMSO-d^6 solution.

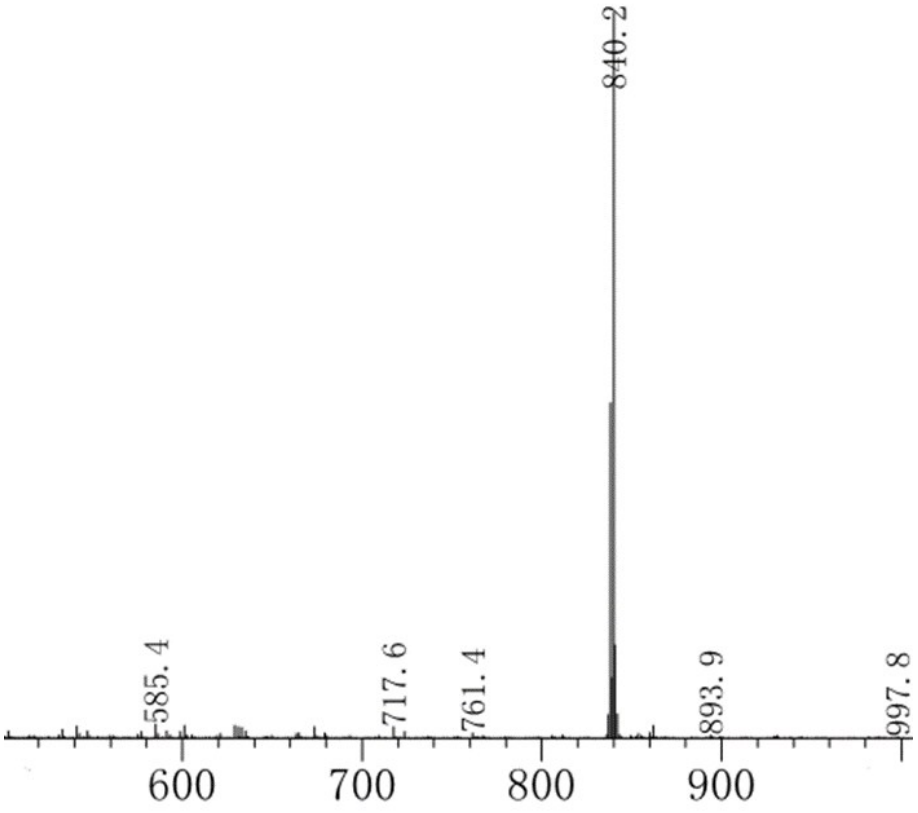


Fig. S13. The ESI-MS spectrum of **3** (CH_3OH, positive mode).

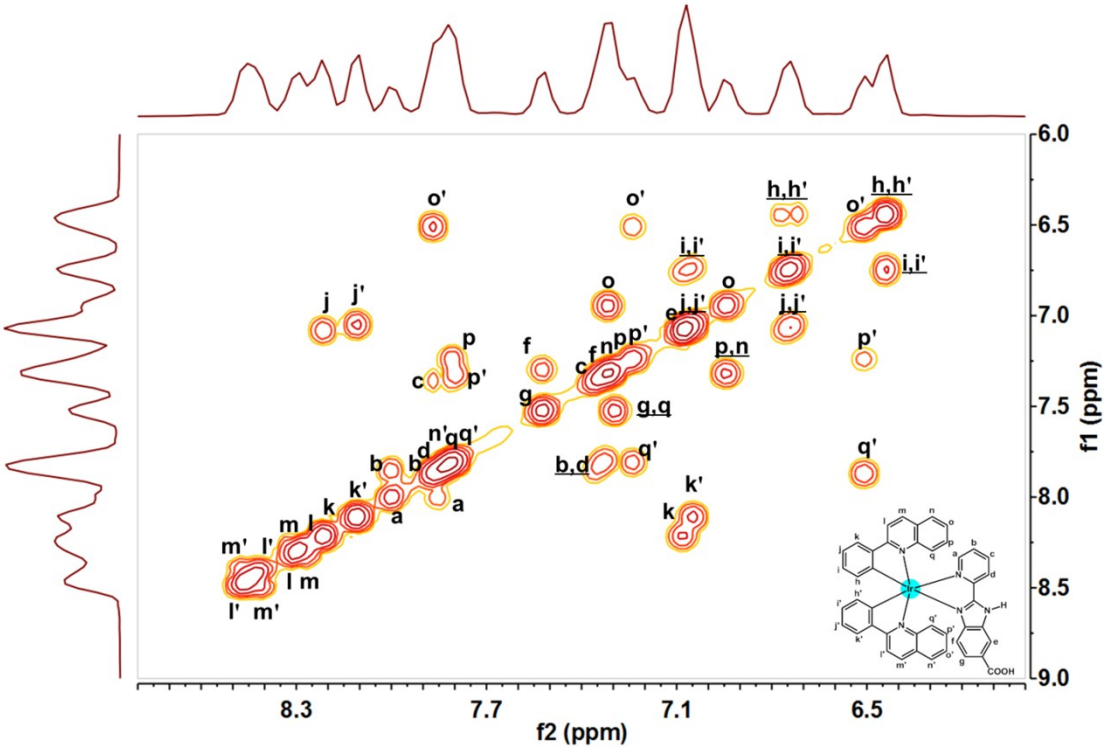


Fig. S14. The 500 MHz ^{1}H-^{1}H Cozy spectrum of **3** in the DMSO-d^{6} solution.

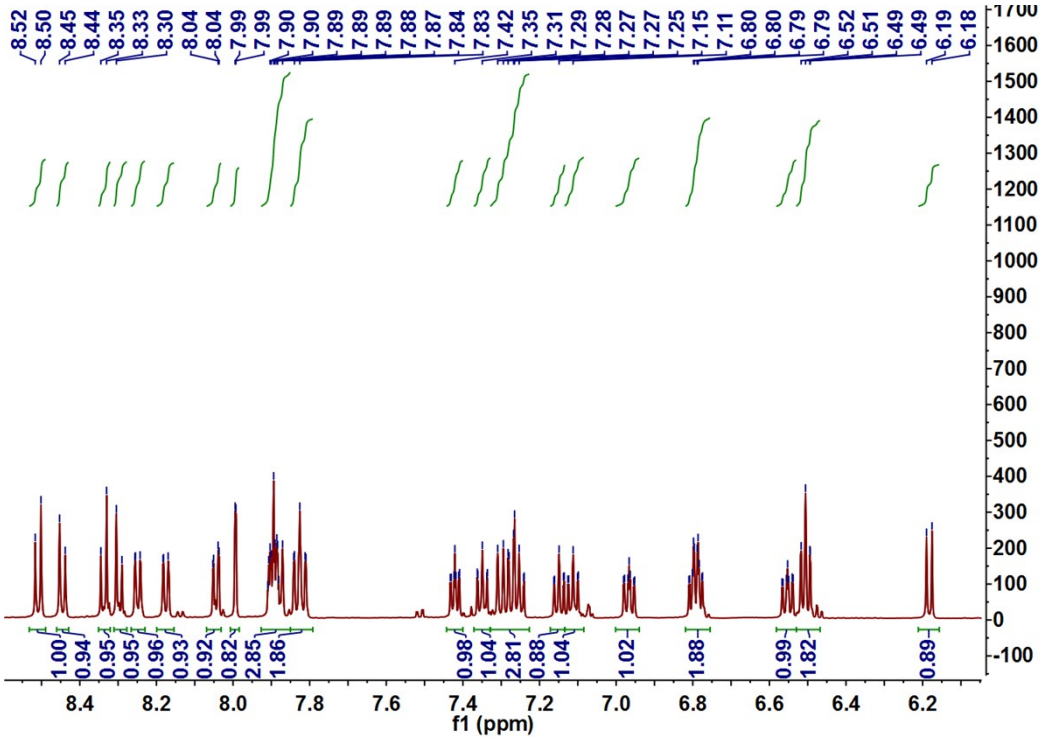


Fig. S15. The 500 MHz ^{1}H NMR spectrum of **4** in the DMSO-d^{6} solution.

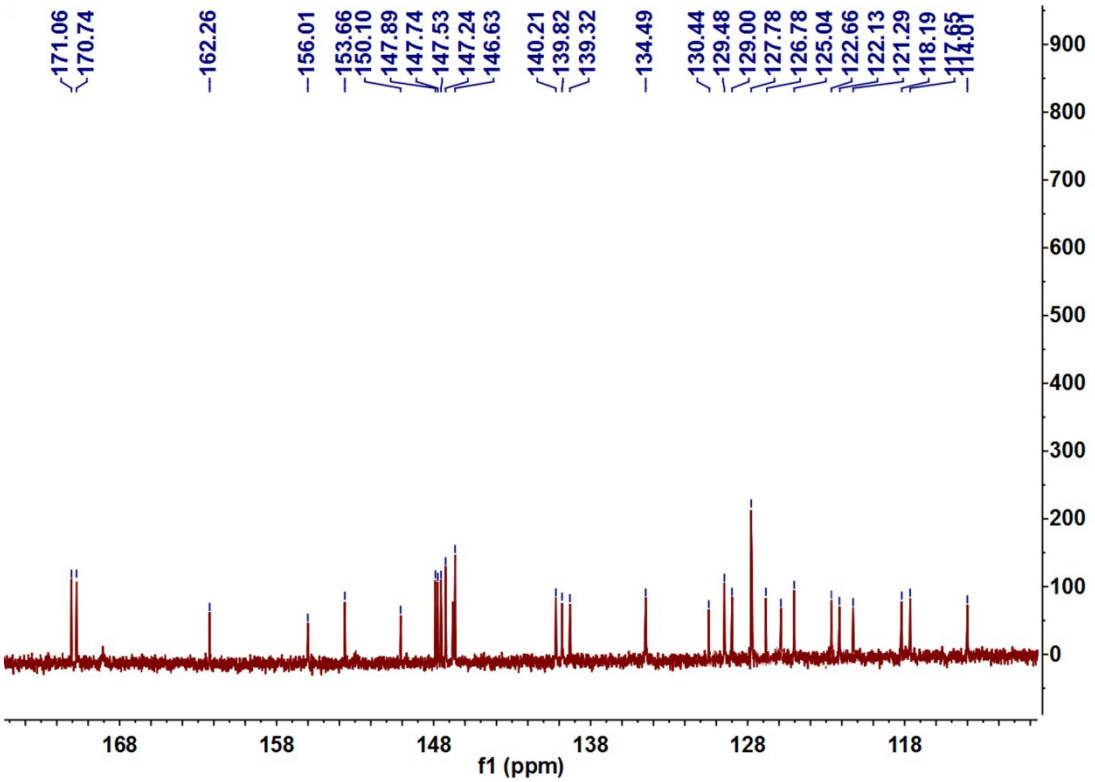


Fig. S16. The 500 MHz ^{13}C NMR spectrum of **4** in the DMSO-d^6 solution.

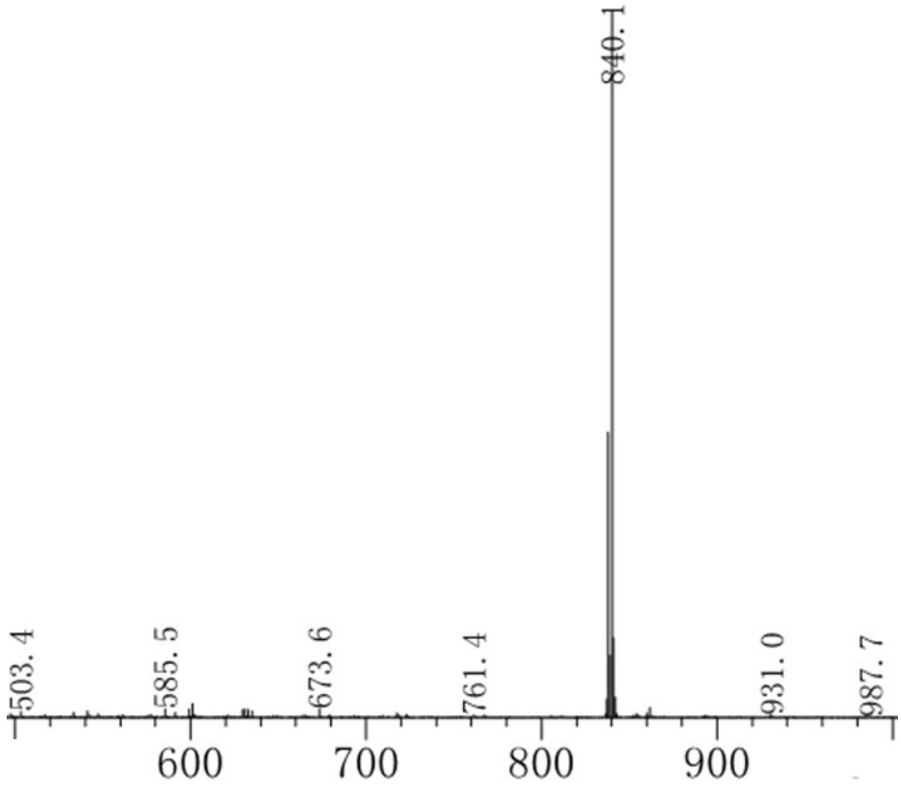


Fig. S17. The ESI-MS spectrum of **4** (CH_3OH, positive mode).

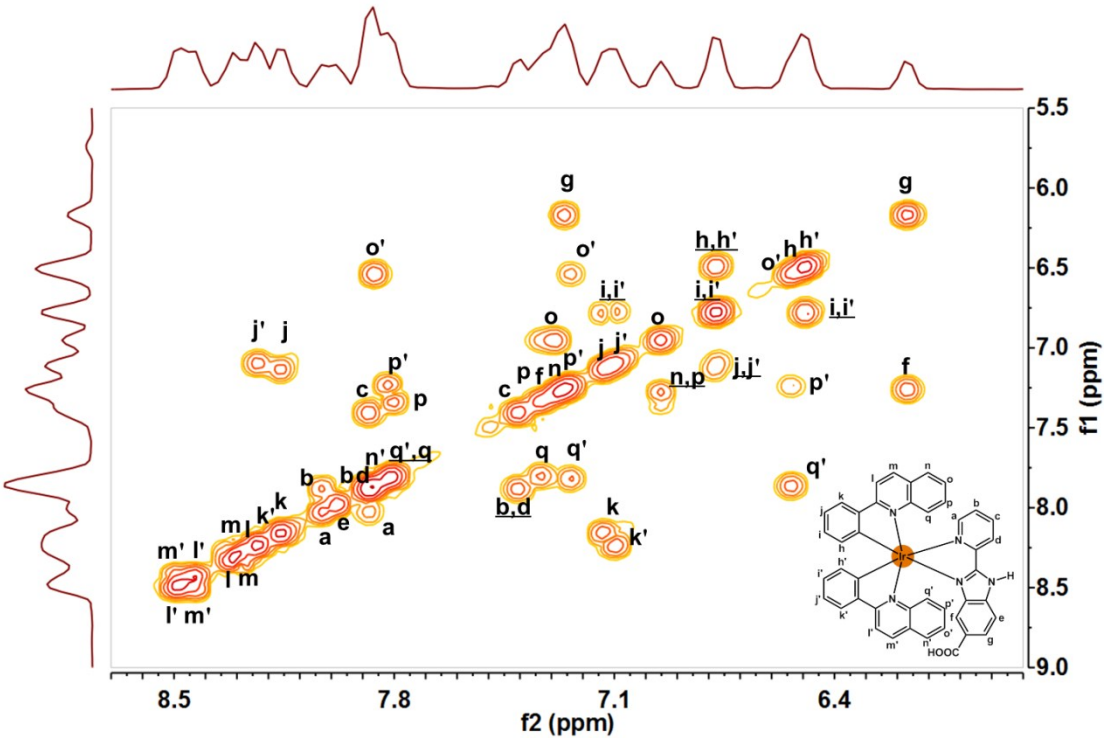


Fig. S18. The 500 MHz ^{1}H-^{1}H Cozy spectrum of **4** in the DMSO-d^{6} solution.

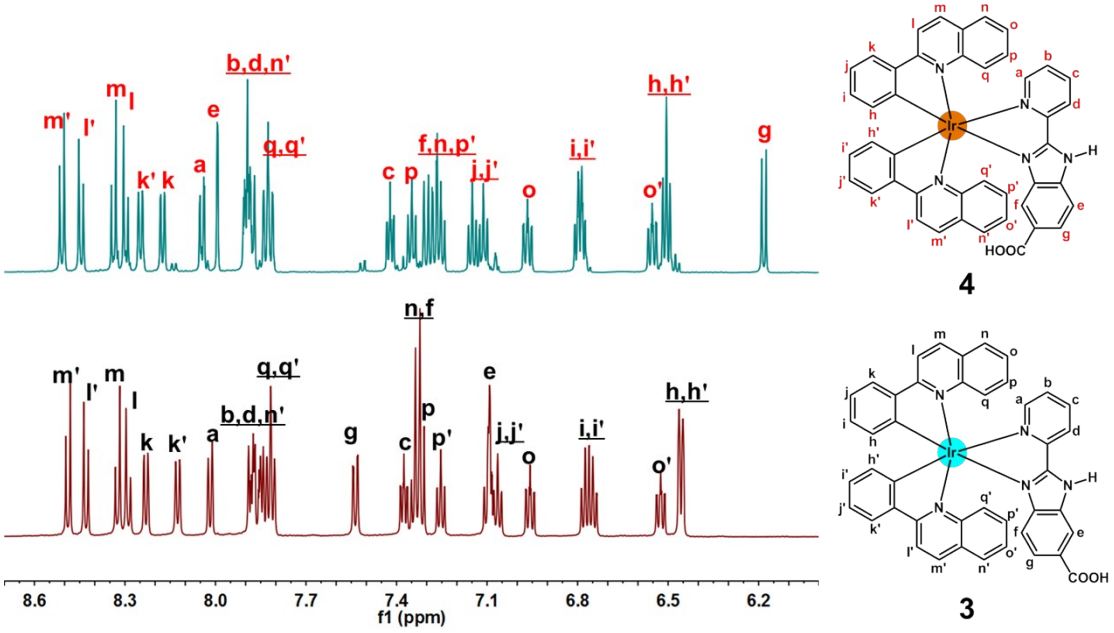


Fig. S19. The assignment of H atoms of the ^{1}H NMR spectra of **3** and **4**.

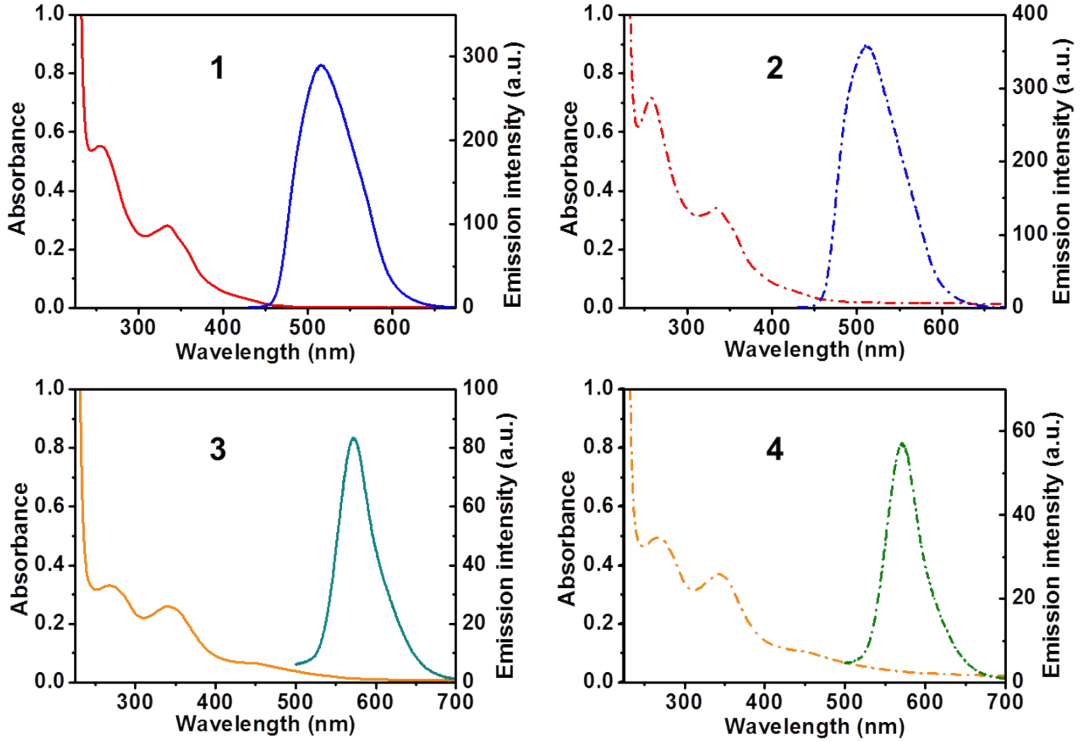


Fig. S20. The UV-vis spectra and emission spectra of **1**-**4** (5 μM) in PBS solution (pH=7.4). The excitation wavelength of the emission spectra was 405 nm.

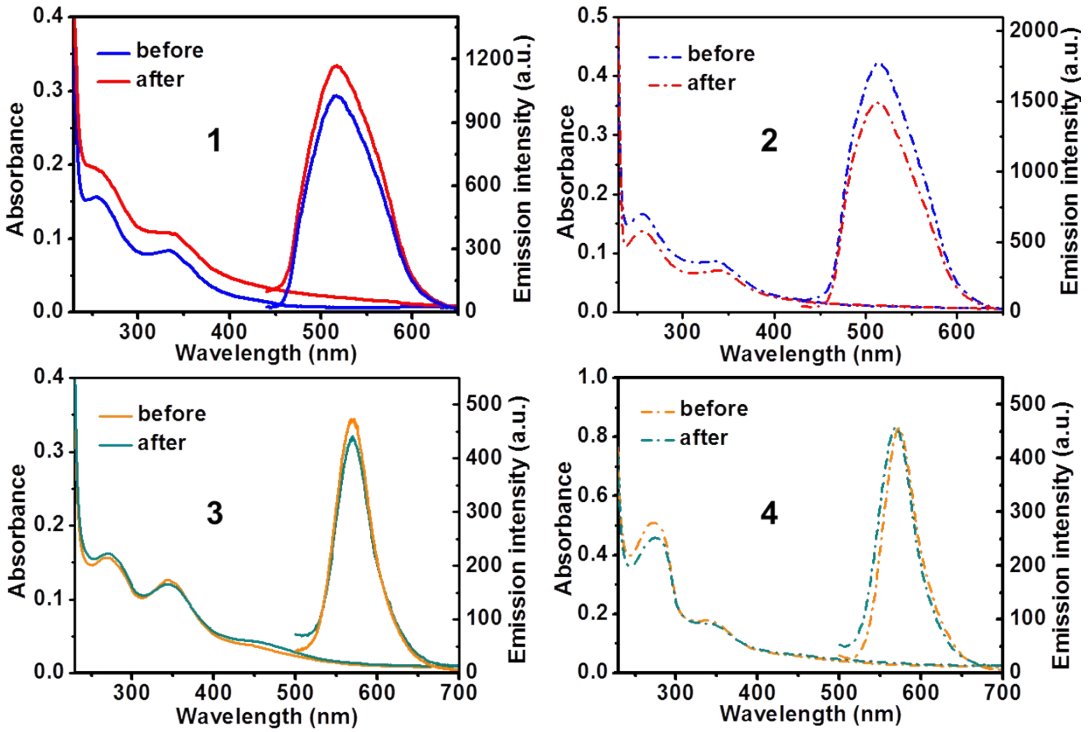


Fig. S21. The UV-vis spectra and emission spectra of **1**-**4** (5 μM) in PBS solution before and after laser irradiation for 1 h (465 nm, 6.5 mW/cm^2). The excitation wavelength of the emission spectra was 405 nm.

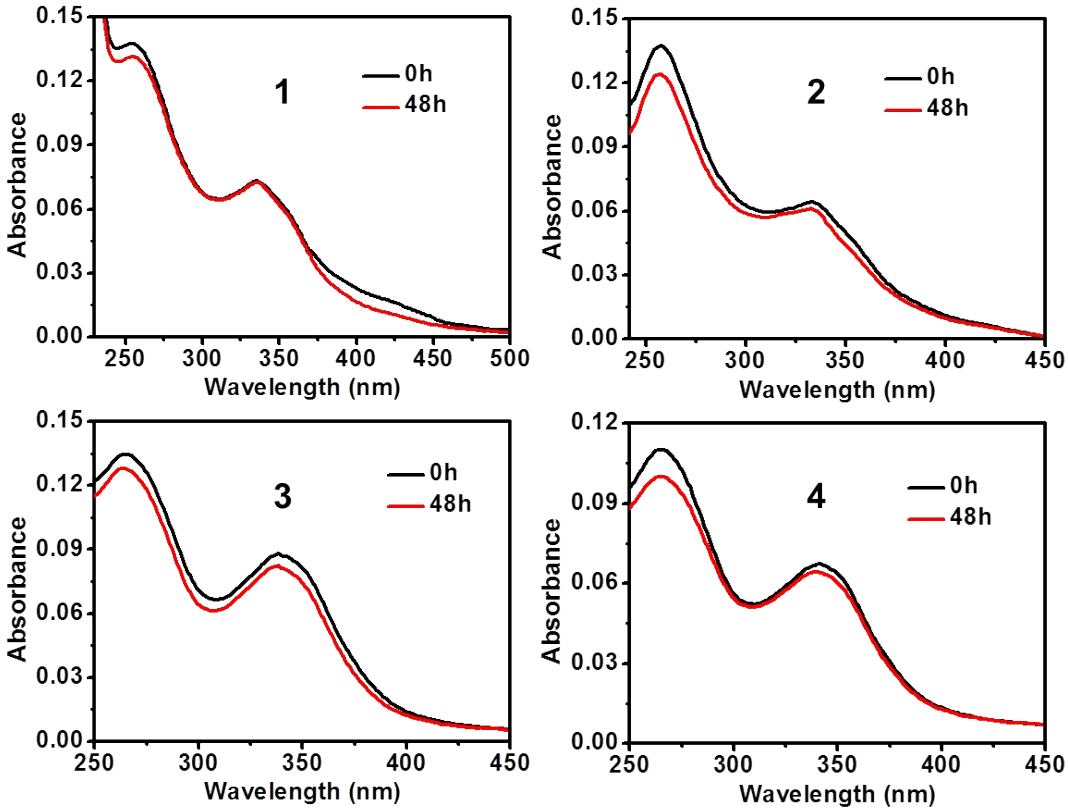


Fig. S22. The UV-vis spectra of **1**-**4** (5 μM) in PBS solution for 48 h at room temperature.

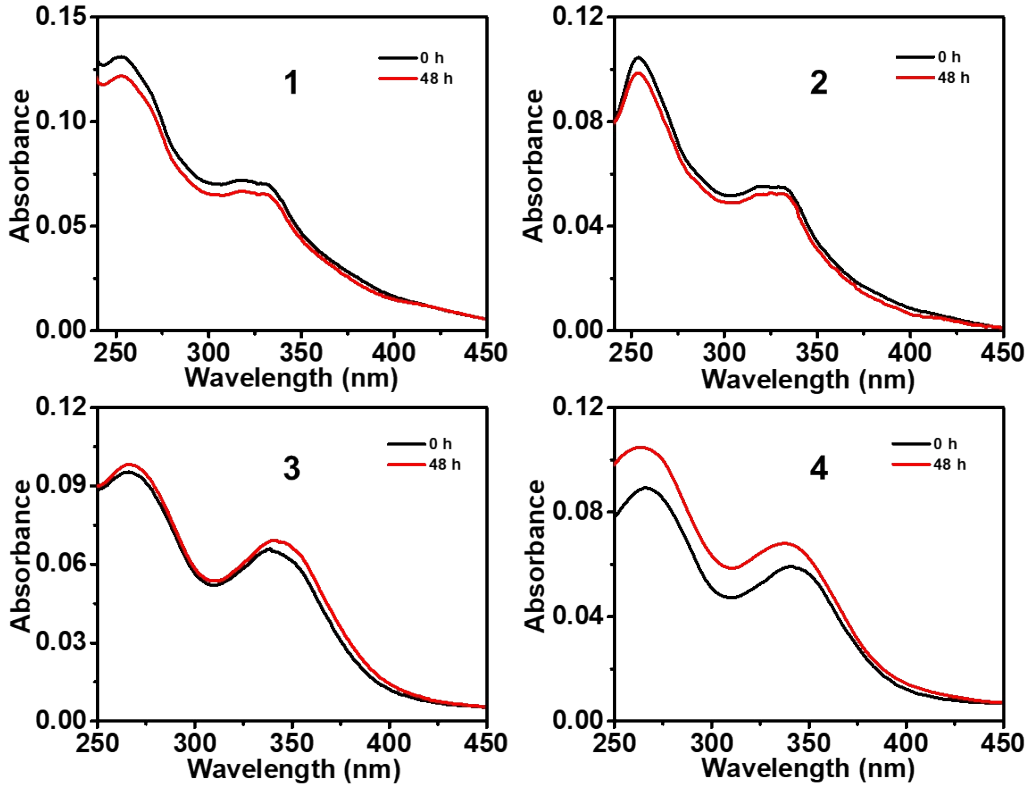


Fig. S23. The UV-vis spectra of **1**-**4** (5 μM) in cell culture medium (DMEM) for 48 h at room temperature.

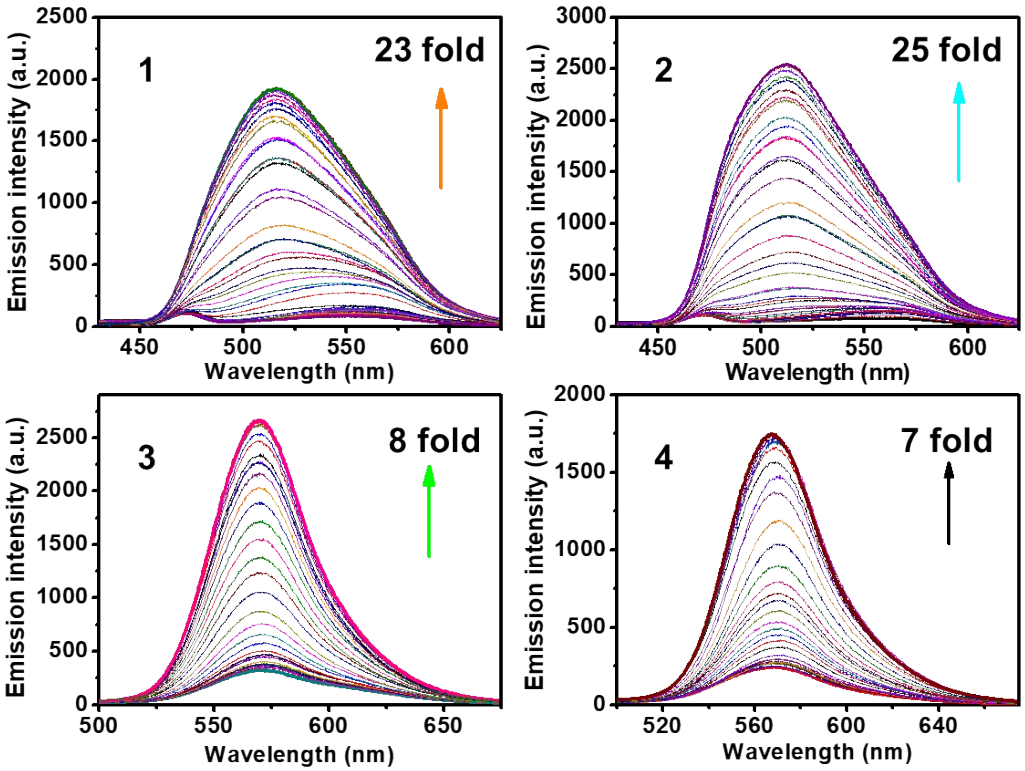


Fig. S24. Change of the phosphorescence spectra of 5 μM **1-4** in PBS solution with varying pH values from 3.0 to 12.1.

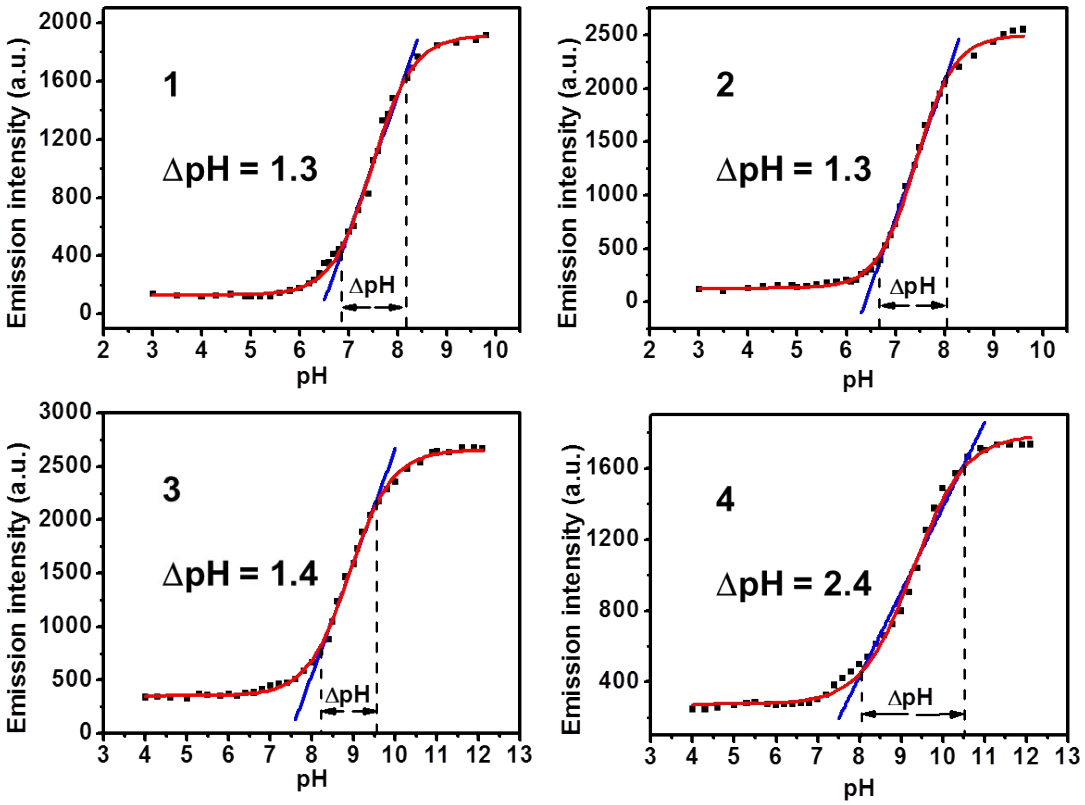


Fig. S25. Linear fitting was used in titration jump discontinuity region of luminescence titration curve in **Fig. 2** and the acid/base transition widths were calculated.

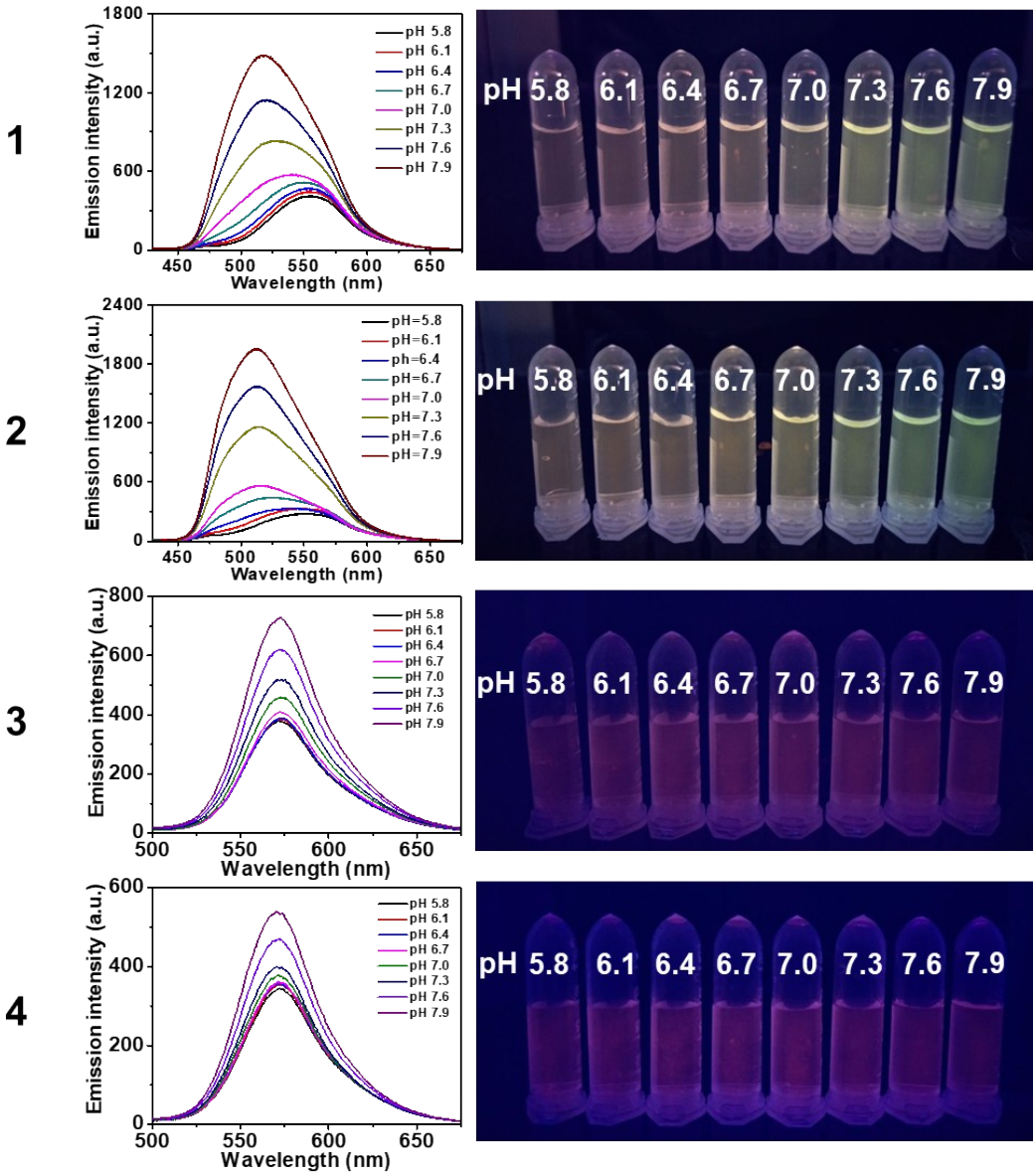


Fig. S26. The phosphorescence emission spectra and photographs of **1-4** with varying pH values from 5.8 to 7.9 (λ_{ex} = 405 nm).

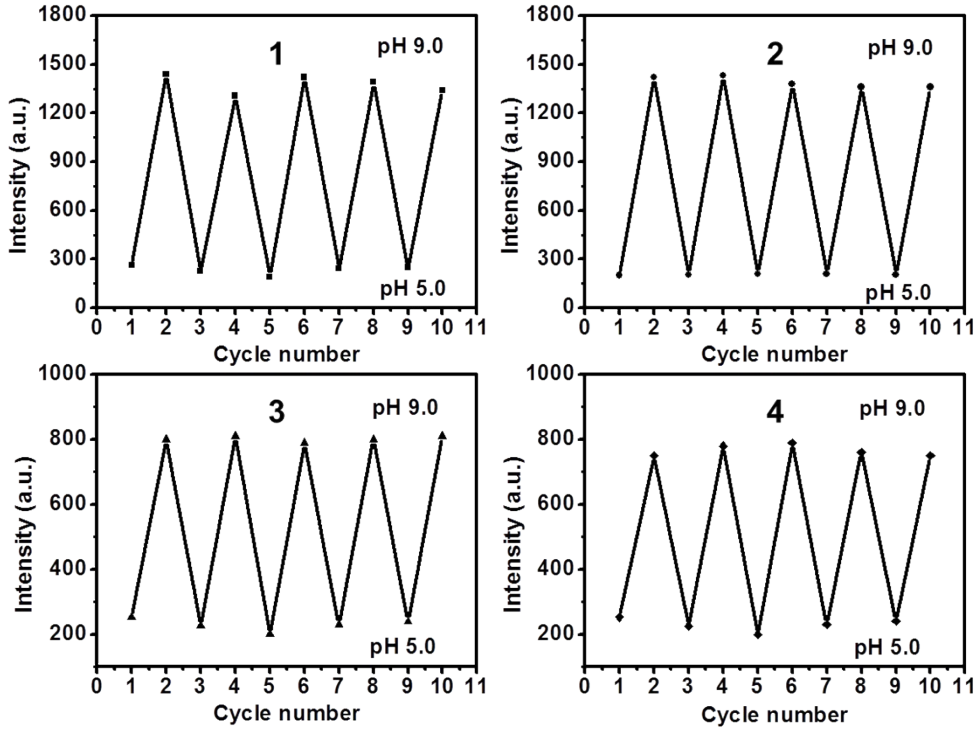


Fig. S27. The pH reversibility of the phosphorescence intensity of **1**-**4** between pH 5.0 and 9.0 in PBS solution.

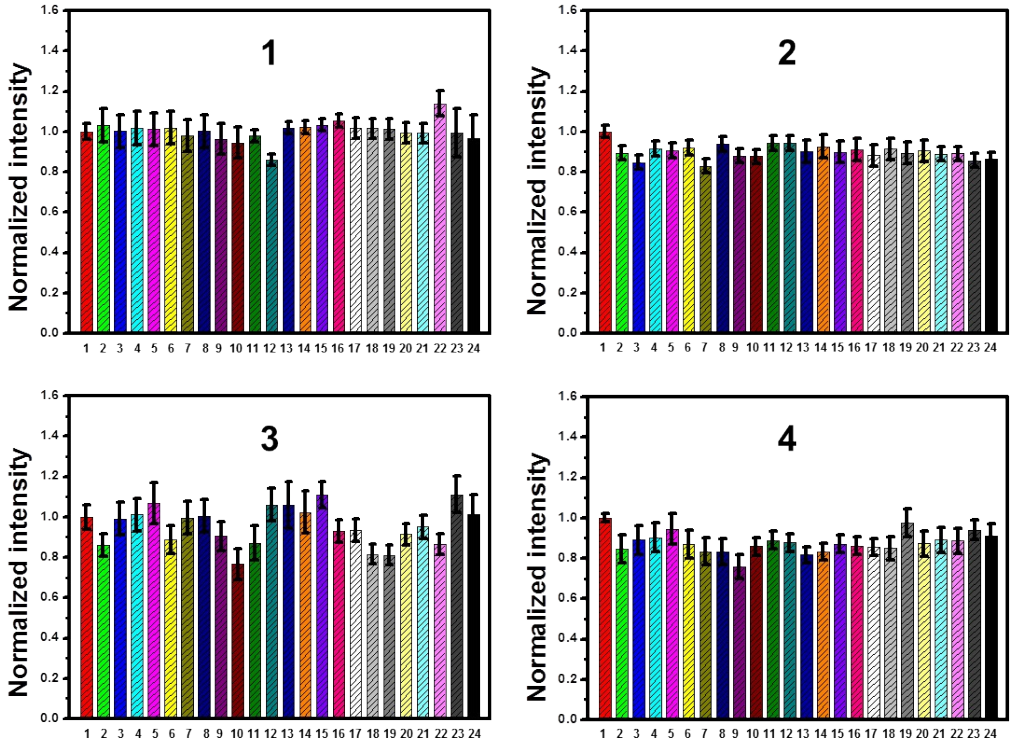


Fig. S28. The emission intensities of **1**-**4** (5 μM) in the presence of 50 μM amino acids or 1 equiv. DNA and RNA. 1-control, 2-Ala, 3-Arg, 4-Asn, 5-Cys, 6-Gln, 7-Glu, 8-Gly, 9-GSH, 10-GSSH, 11-His, 12-Ile , 13-Leu, 14-Lys, 15-Met, 16-Phe, 17-Pro, 18-Ser, 19-Thr, 20-Trp, 21-Tyr, 22-Val, 23-RNA, 24-DNA.

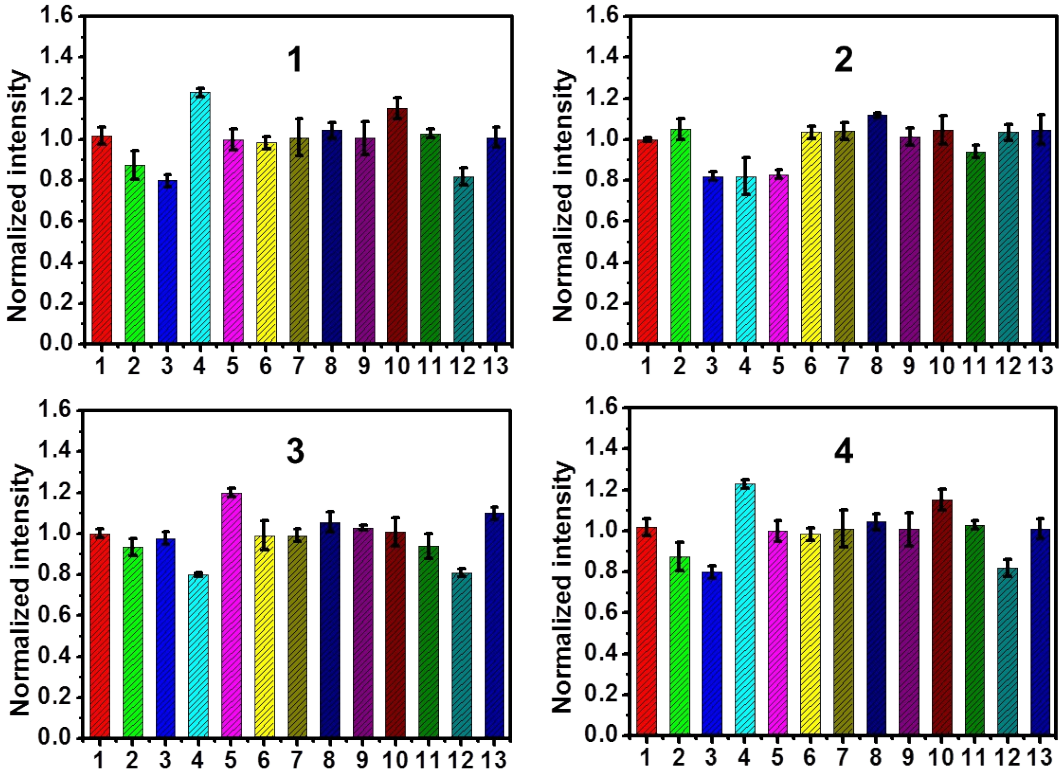


Fig. S29. The emission intensities of **1**-**4** in the presence of 50 μM ions. 1-control, 2-Ca^{2+}, 3-Co^{2+}, 4-Cu^{2+}, 5-Fe^{3+}, 6-K^{+}, 7-Li^{2+}, 8-Mg^{2+}, 9-Mn^{2+}, 10-Cl^{-}, 11-SO_4^{2-}, 12-Br^{-}, 13-NO_3^{-}.

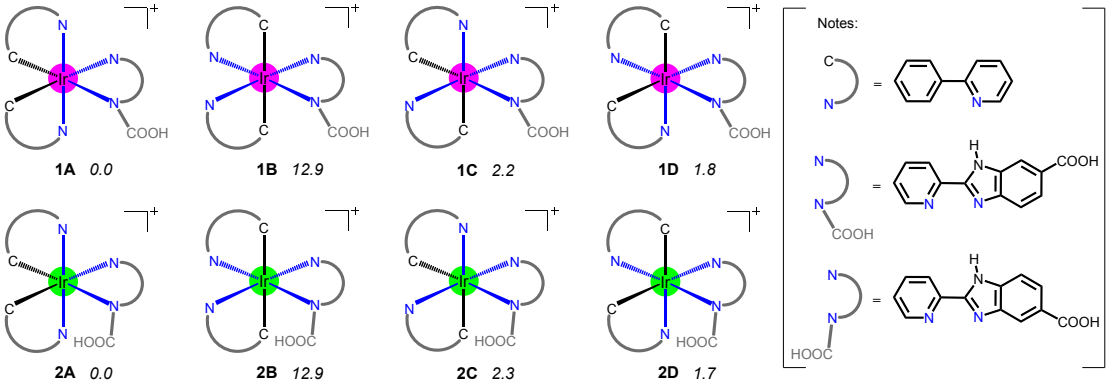


Fig. S30. The schematic isomers of **1** and **2** and their respective relative free energies (in kcal/mol).

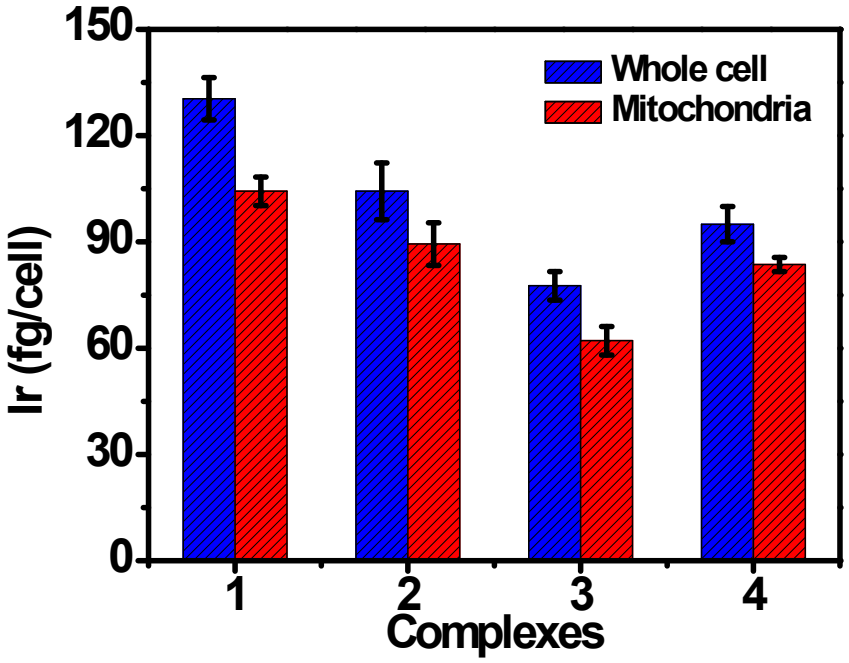


Fig. S31. The iridium concentrations were determined in mitochondria of Hep-G2 cells with exposure to **1**-**4** (5 μM) for 1 h by ICP-MS.

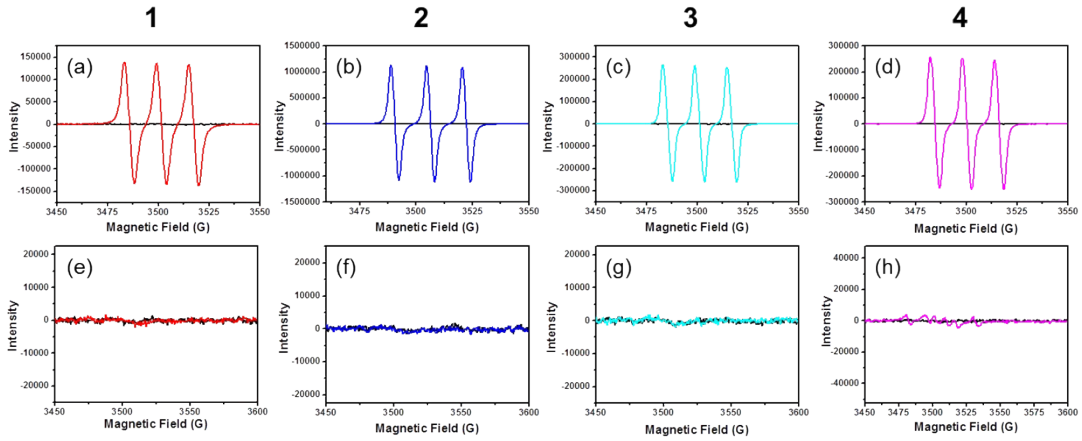


Fig. S32. (a-d) EPR signals of $^1O_2$ trapped by TEMP with producing by **1-4** in the dark or upon light irradiation. (e-h) EPR signals of $^{\bullet}O_2^-$ or $^{\bullet}OH$ trapped by DMPO with producing by **1-4** in the dark or upon light irradiation. Black lines represent dark and color lines represent 465 nm light irradiation for 5 min.

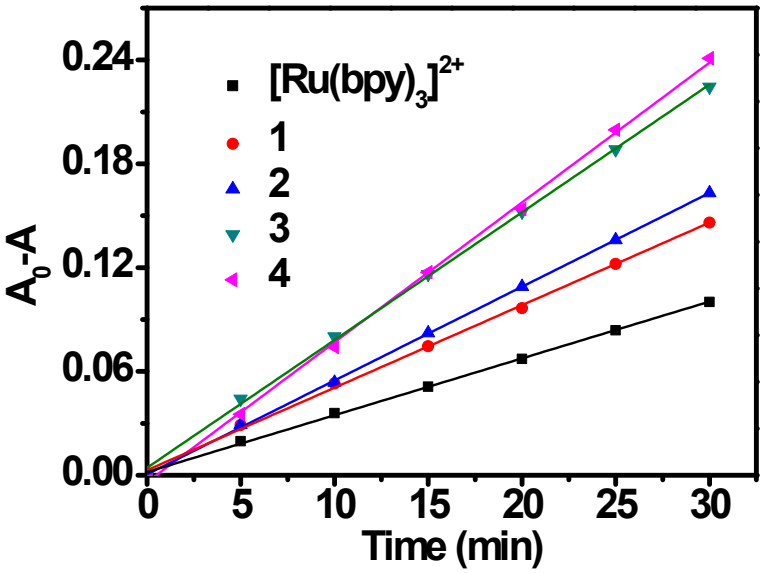


Fig. S33. Measurement of $^1O_2$ quantum yields. Changes in absorbance of RNO at 440 nm against irradiation time in the presence of $Ru(bpy)_3^{2+}$ or **1**-**4** in histidine-PBS solution. The irradiation time interval was 5 min. The light source was a 465 nm LED- lamp (6.5 mW/cm^2).

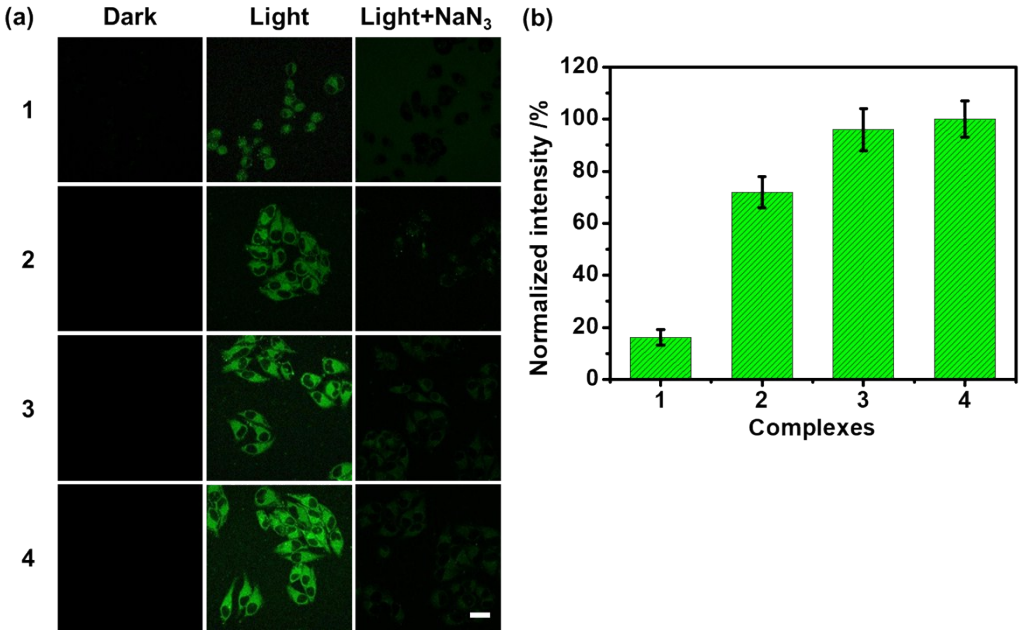


Fig. S34. (a) Confocal microscopy imaging of the Hep-G2 cells colabeled with 5 μM **1**-**4** for 1 h and singlet oxygen sensor green (SOSG, 5 μM, 30 min) in the absence or presence of NaN_3 (5 mM, 1 h) in the dark and under light irradiation for 5 min at 465nm. The power of LED-lamp was 6.5 mW/cm^2. (b) Normalized emission intensity of SOSG after light irradiation for 5 min from (a). The normalized intensity was collected from average per-cell intensity. λ_{ex} = 488 nm, λ_{em} = 525 ± 30 nm. Scale bar: 40 μm.

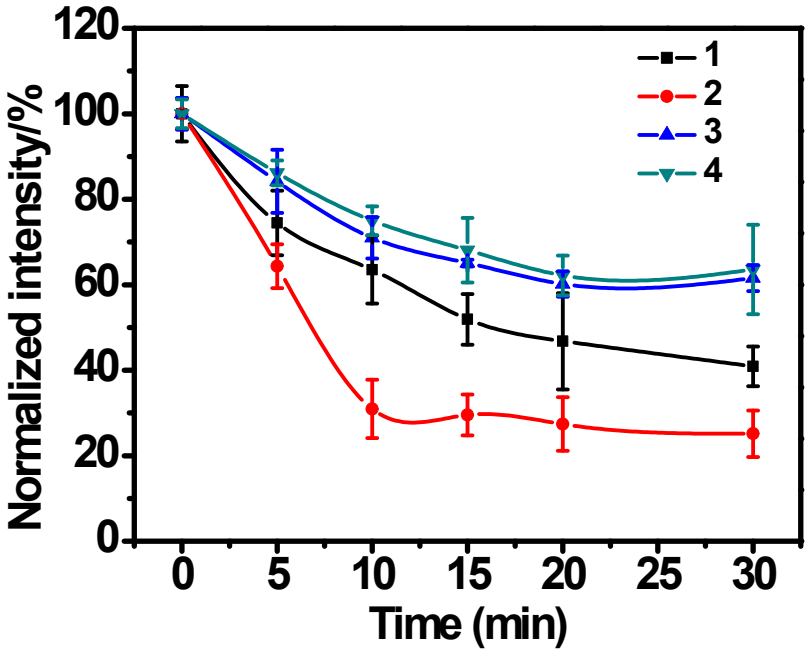


Fig. S35. The normalized emission intensity in Hep-G2 cells of **1**-**4** after irradiation according to **Fig. 6**.

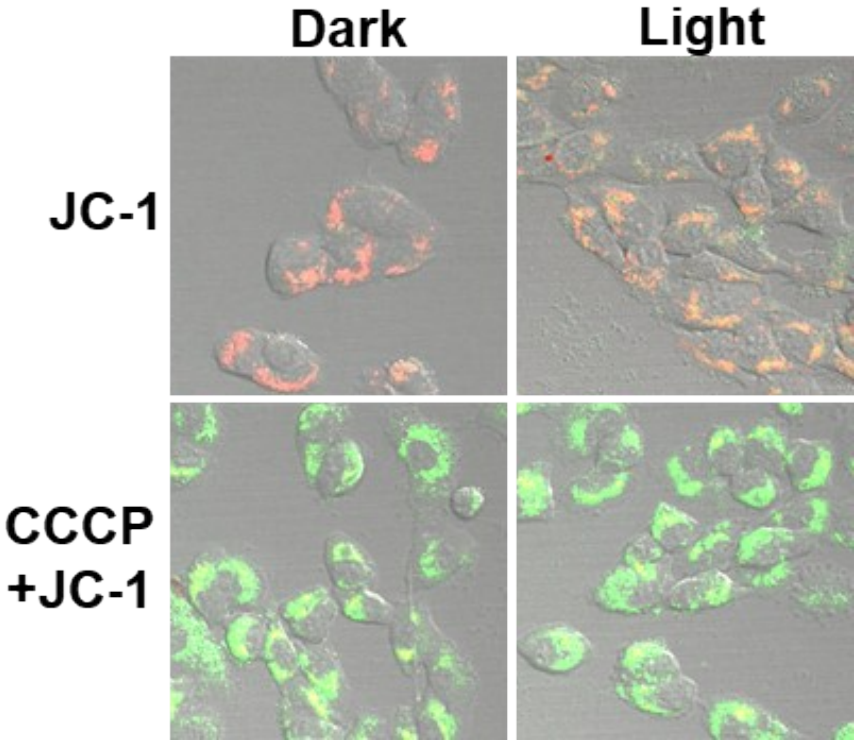


Fig. S36. Confocal microscopy images of Hep-G2 cells incubated with JC-1 (100 nM, 30 min) in the presence or absence of CCCP (10 μM, 20 min), then the cells were located in the dark or upon light irradiation, respectively. The lighting group was irradiated under 465 nm light (6.5 mW/cm^2, 30 min). JC-1 aggregates (red channel): λ_{ex} = 514 nm, λ_{em} = 595 ± 20 nm; JC-1 monomers (green channel): λ_{ex} = 488 nm, λ_{em} = 530 ± 20 nm.

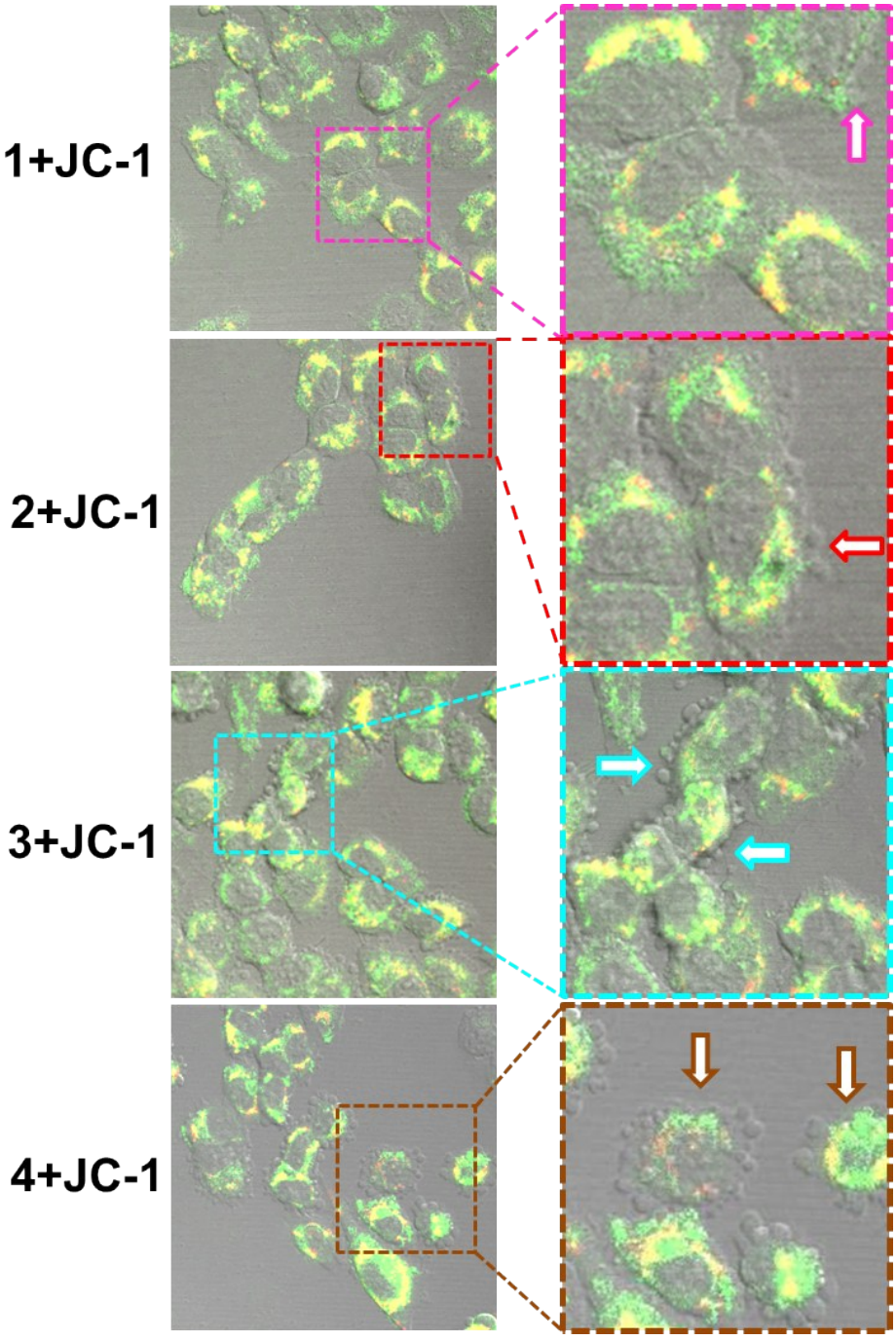


Fig. S37. The enlarged images of irradiation treatment in **Fig. 7**.

Table S1. Blue shifts of the complexes at various pH values from pH 6.0 to pH 8.0.

	Wavelength/nm					
Complexes	[a]pH 6.0	pH 7.0	pH 8.0	[b]ΔW_1	[c]ΔW_2	$\Delta W_1/\Delta W_2$
1	557.0	537.6	515.6	19.4	22.0	~0.9
2	551.0	516.2	510.4	34.8	5.8	~6.0
3	574.8	575.2	575.4	0.4	0.2	2
4	570.6	570.2	569.6	0.4	0.6	~0.7

[a]The maximum emission intensity at the pH value. [b]$\Delta W_1 = W_{pH\ 7.0} - W_{pH\ 6.0}$; [c]$\Delta W_2 = W_{pH\ 8.0} - W_{pH\ 7.0}$.

Table S2. The phosphorescence quantum yields of **1-4** at different pH values.

Complexes	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0
1	0.0081	0.0071	0.0073	0.0098	0.0138	0.0229
2	0.0016	0.0025	0.0032	0.0075	0.0119	0.0324
3	0.0020	0.0034	0.0036	0.0042	0.0057	0.0064
4	0.0020	0.0021	0.0022	0.0027	0.0030	0.0092

Table S3. Determination of the $^1O_2$ quantum yields.

Complexes	$\Phi(^1O_2)$
$[Ru(bpy)_3]^{2+}$	0.22
1	0.29
2	0.32
3	0.44
4	0.57

Table S4. The dark cytotoxicity and phototoxicity (IC_{50}, μM) of the complexes towards Hep-G2 cell line upon different irradiation time.

Complex	Dark	Light 10 min (PI)	Light 20 min (PI)	Light 40 min (PI)
1	>100	48.76 (>2.1)	48.95 (>2.0)	35.26 (>2.84)
2	>100	3.56 (>28.1)	2.91 (>34.4)	2.76 (>36.2)
3	>100	0.55 (>182)	0.54 (>185.2)	0.27 (>370.4)
4	99.72±0.78	0.25 (398.9)	0.13 (767.1)	0.072 (1385)
cisplatin	>100	>100	>100	>100

The cells incubated with the compounds for 1 h, medium replaced with fresh non-drug medium in both 'dark' and 'light' plates. The 'light' plates were irradiated with blue LED (465 nm, 6.5 mW/cm^2) for different irradiation time. PI = IC_{50}(dark)/IC_{50}(light).

Table S5. The dark cytotoxicity and phototoxicity (IC_{50}, μM) of the complexes towards human normal lung (MRC-5) and liver (LO2) cell lines.

Complexes	MRC-5		LO2	
	Light	Dark	Light	Dark
1	36.23±0.83	87.67±1.01	40.30±0.88	95.56±0.97
2	4.65±0.11	70.24±1.14	3.88±0.07	90.86±0.84
3	0.12±0.03	>100	0.11±0.04	>100
4	0.07±0.02	>100	0.09±0.02	>100
cisplatin	>100	>100	>100	>100

The cells incubated with the compounds for 1 h, medium replaced with fresh non-drug medium in both 'dark' and 'light' plates, 'light' plate irradiated with blue (465 nm, 6.5 mW/cm^2, 1 h). All plates incubated for another 46 h. Data are presented as the means ± standard deviations.