Electronic Supplementary Information

Synthesis of pH-responsive cyclometalated iridium(III) complex and its application in the selective killing of cancerous cells

Peng Yang, Shaoqing Zhang, Ke Wang and Honglan Qi*

Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an, 710062, P.R. China

*Corresponding author. Tel.: +86-29-81530726; Fax: +86-29-81530727.

E-mail: honglanqi@snnu.edu.cn.

Table of Contents

Experimental Section

Electron spin resonance (ESR)

Probe 1 (10 μM) was dissolved in disodium hydrogen phosphate/citric acid buffer solutions containing 20 mM TEMP (2,2,6,6–tetramethylpiperidine) as a $^1O_2$ scavenger or 20 mM DMPO (5,5-dimethyl-1-pyrroline N-oxide) as a •OOH or •OH radical scavenger. Capillary tubes were filled with the solution and sintered by fire. The samples were measured in exclusion from light and after irradiation (white light, 1 min, 20 mW cm^{-2}).

pH-dependent $^1O_2$ production quantum yields

The quantum yields of $^1O_2$ production ($\Phi_\triangle$) of probe 1 under irradiation in aerated disodium hydrogen phosphate/citric acid buffer solutions (pH 3.0, 5.0 and 7.4) were evaluated using a steady-state method with ABDA as the $^1O_2$ indicator and $[Ru(bpy)_3]Cl_2$ as the standard. Briefly, air-equilibrated buffer solutions containing the probe 1 (10 μM) and ABDA (100 μM) were prepared in the dark and exposed to white light irradiation (20 mW cm^{-2}). The absorbance of ABDA at 378, 379 and 380 nm in different pH solution, including pH 3.0, 5.0 and 7.4, respectively were recorded. The $\Phi_{\triangle(x)}$ of the probe 1 was calculated according to the following equation.

$$\Phi_{\triangle(x)} = \Phi_{\triangle(std)} \times \left(\frac{K_x}{K_{std}}\right) \times \left(\frac{A_{std}}{A_x}\right)$$

where K_x and K_{std} are the decomposition rate constants of ABDA by probe 1 and $[Ru(bpy)_3]Cl_2$, respectively, which are determined by the plot $\ln(Abs_0/Abs)$ versus irradiation time, where Abs_0 is the initial absorbance of ABDA and Abs is the ABDA absorbance at different irradiation times. A_x and A_{std} represent the light absorbed by probe 1 and $[Ru(bpy)_3]Cl_2$, respectively, and were determined by integrating of the areas under the absorption bands in the wavelength range of 400-600 nm. $\Phi_{\triangle(std)}$ is the $^1O_2$ quantum yield of $[Ru(bpy)_3]Cl_2$, which is 0.18 in water.

Cell lines and culture conditions

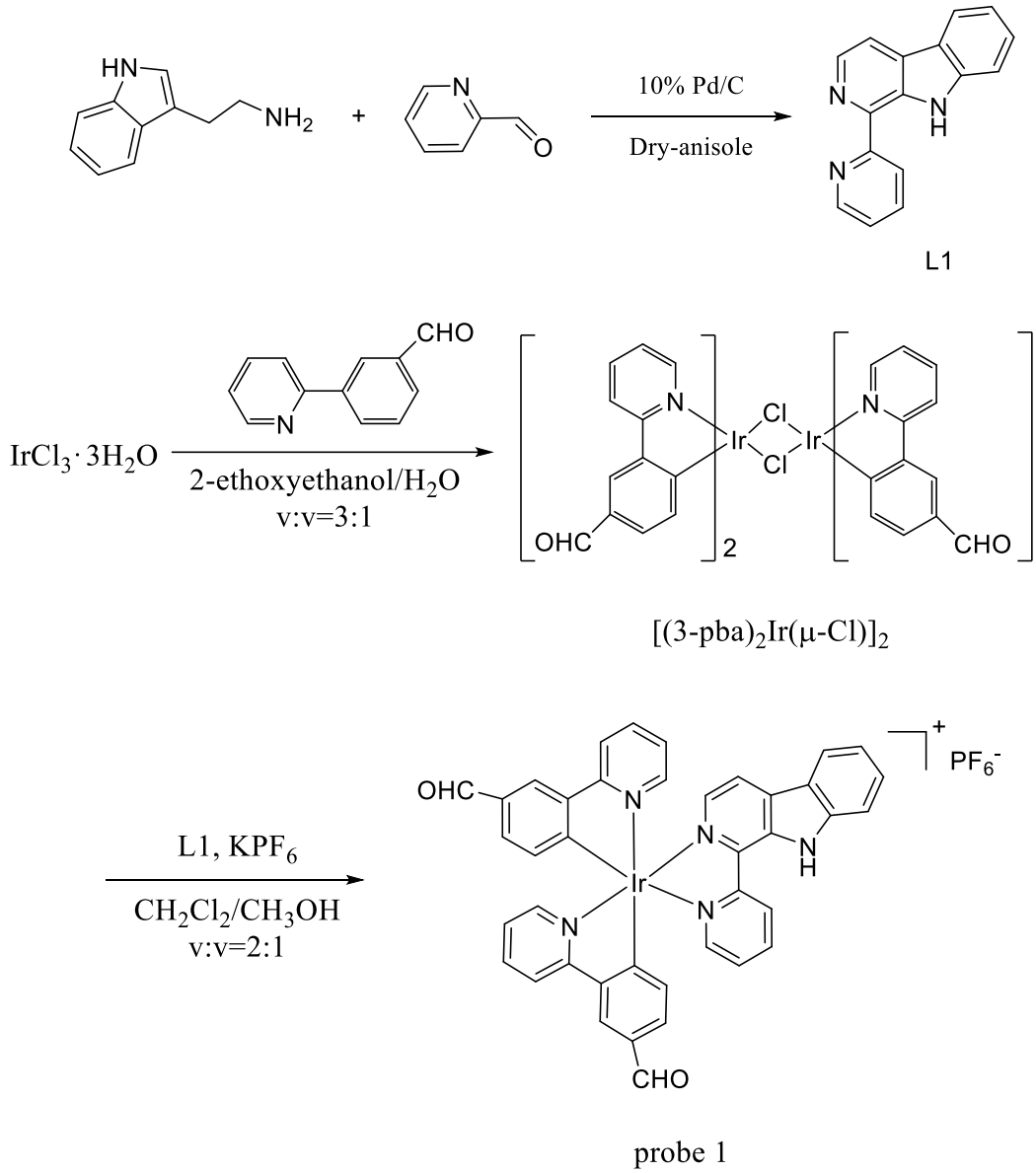
HeLa cells and MRC-5 cells were provided by Cell Bank, Chinese Academy of Sciences (China). Cells were seeded in cell culture medium (Dulbecco's modified Eagle's medium, DMEM, 4.5 g/L D-Glucose, L-Glutamine, 110 mg/L Sodium Pyruvate, Gibco, USA) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco, China) and 1% (v/v) antibiotics (penicillin/streptomycin, Gibco, China) at 37 ℃ with 5% CO_2. All cells were grown as adherent monolayers at 37 ℃ in a 5% CO_2 humidified incubator.

AO staining

HeLa cells were pre-incubated with different concentrations of probe 1 for 30 min at 37 ℃, then replaced by fresh cell culture medium and were exposed to white light irradiation (20 mW cm^{-2}) for 10 min or were incubated in the dark for 10 min. After that, the HeLa cells were incubated with AO (2 μg/mL) at 37 ℃ for 15 min. Cell imaging was carried out after washing the cells with PBS. Under CLSM, Emission was collected at range of 500-550 nm (green) and 600-650 nm (red) upon excitation at 488 nm.

Annexin V-FITC/propidium iodide staining

HeLa cells were pre-incubated with different concentrations of probe 1 for 30 min at 37 ℃, then replaced by fresh cell culture medium and were exposed to white light irradiation (20 mW cm^{-2}) for 10 min or were incubated in the dark for 10 min and further cultured for 20 min. After that, the cells were incubated with fresh cell culture medium without phenol red and further stained with Annexin V-FITC and propidium iodide following the protocols of the manufacturer (Beyotime Biotechnology) for 30 min. Under CLSM, emission was collected at range of 500-550 nm (Annexin V-FITC) upon excitation at 488 nm and 600-650 nm (propidium iodide) upon excitation at 559 nm.



Scheme S1. Synthetic procedures of 1-(2-pyridyl)-β-carboline (L1) and [(3-pba)$_2$Ir(1-Py-βC)]PF$_6$ (probe 1).

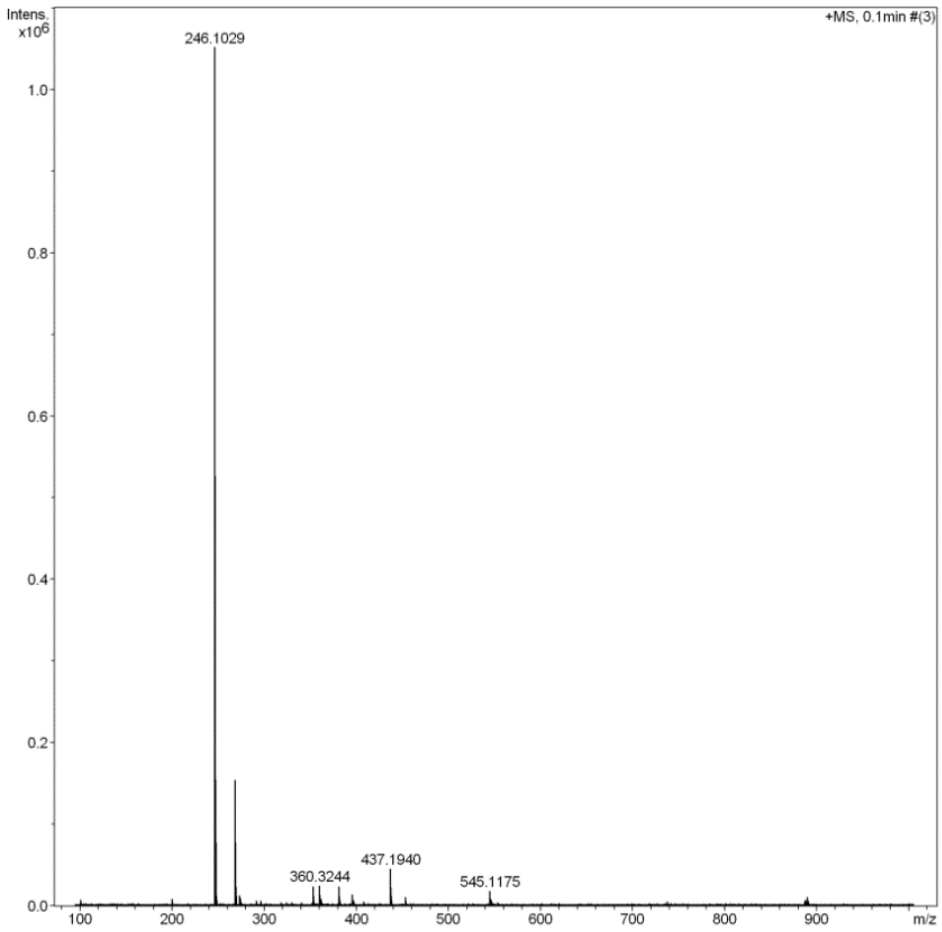


Fig. S1 ESI-MS spectrum of L1.

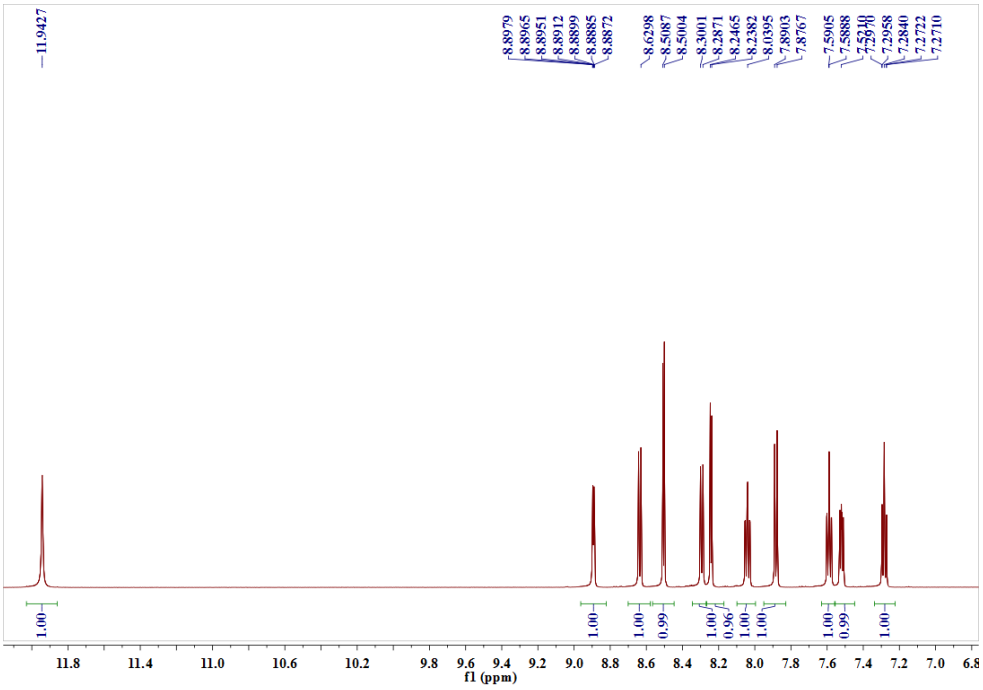


Fig. S2 ^{1}H NMR spectrum of L1 in DMSO-d_6.

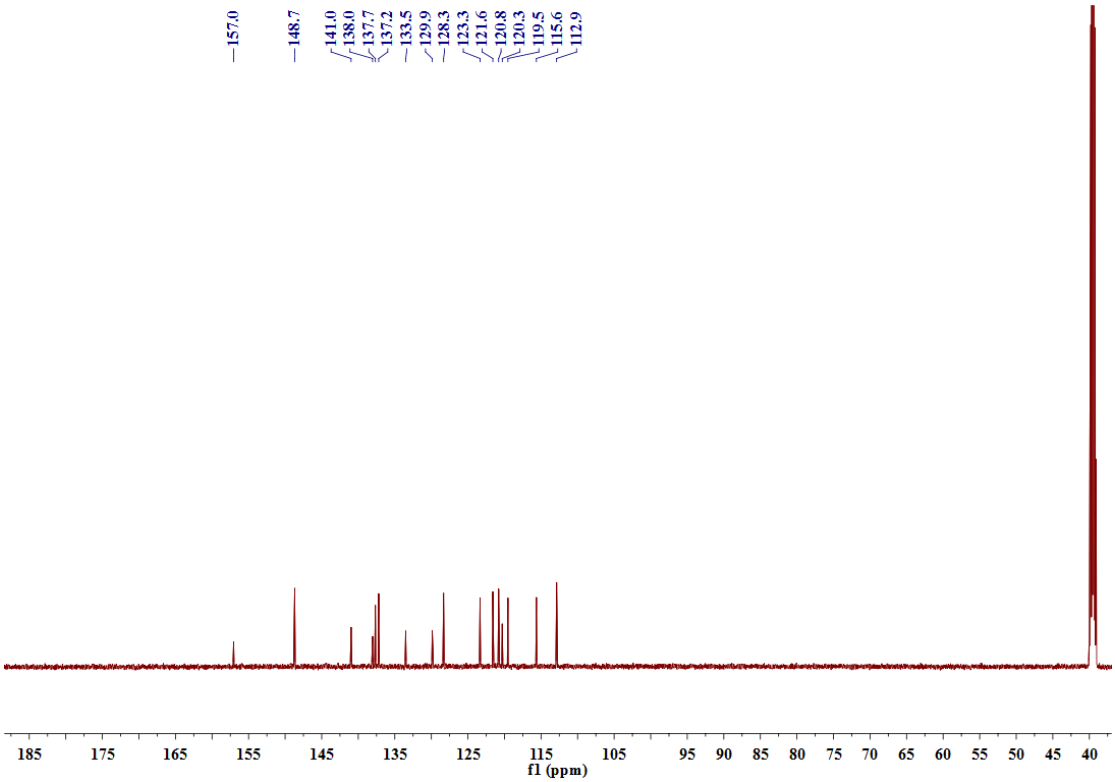


Fig. S3 ^{13}C NMR spectrum of L1 in DMSO-d_6.

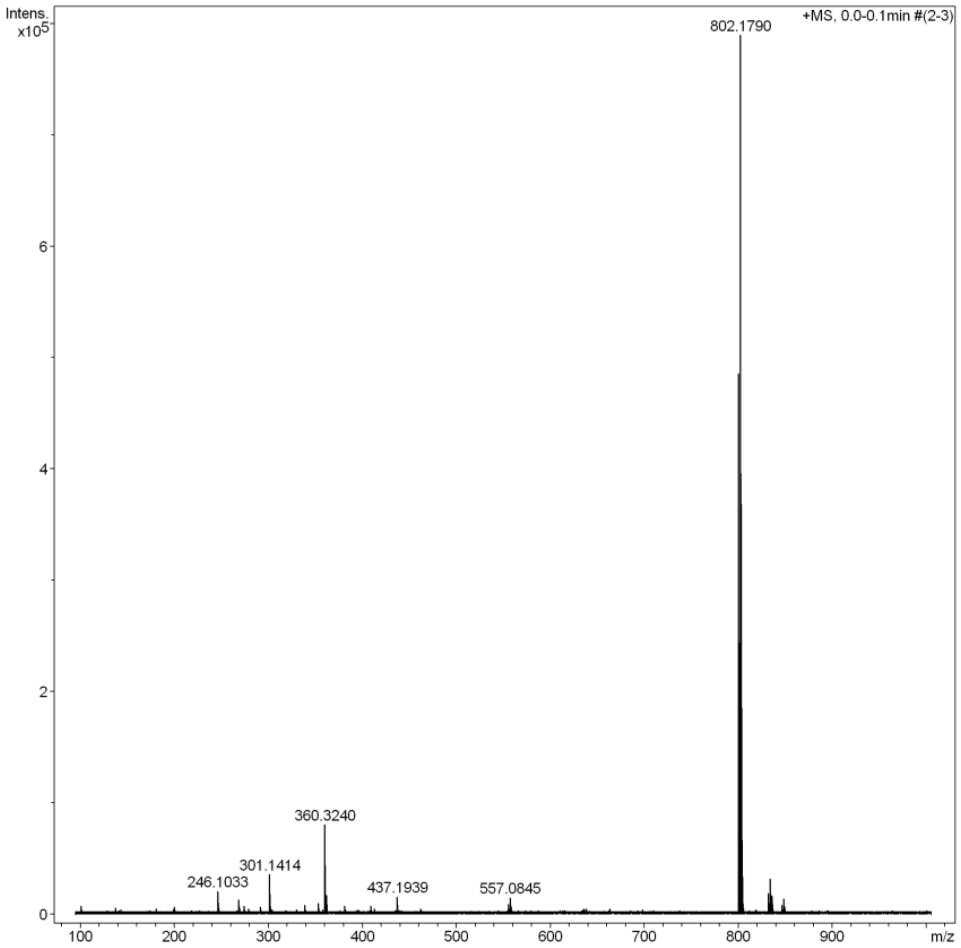


Fig. S4 ESI-MS spectrum of probe 1.

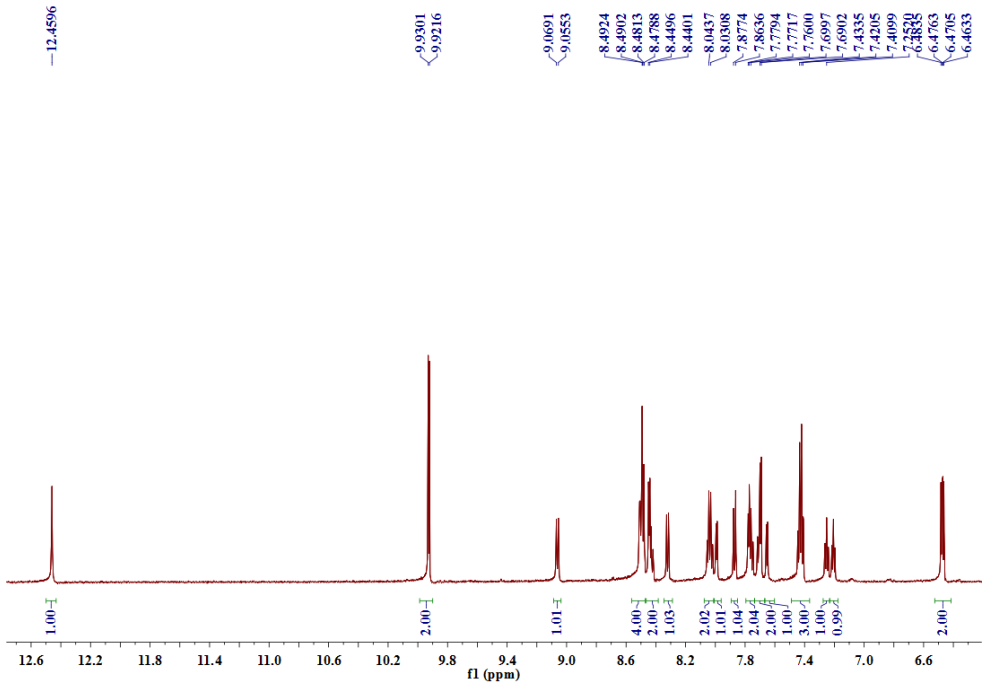


Fig. S5 ^{1}H NMR spectrum of probe 1 in DMSO-d_6.

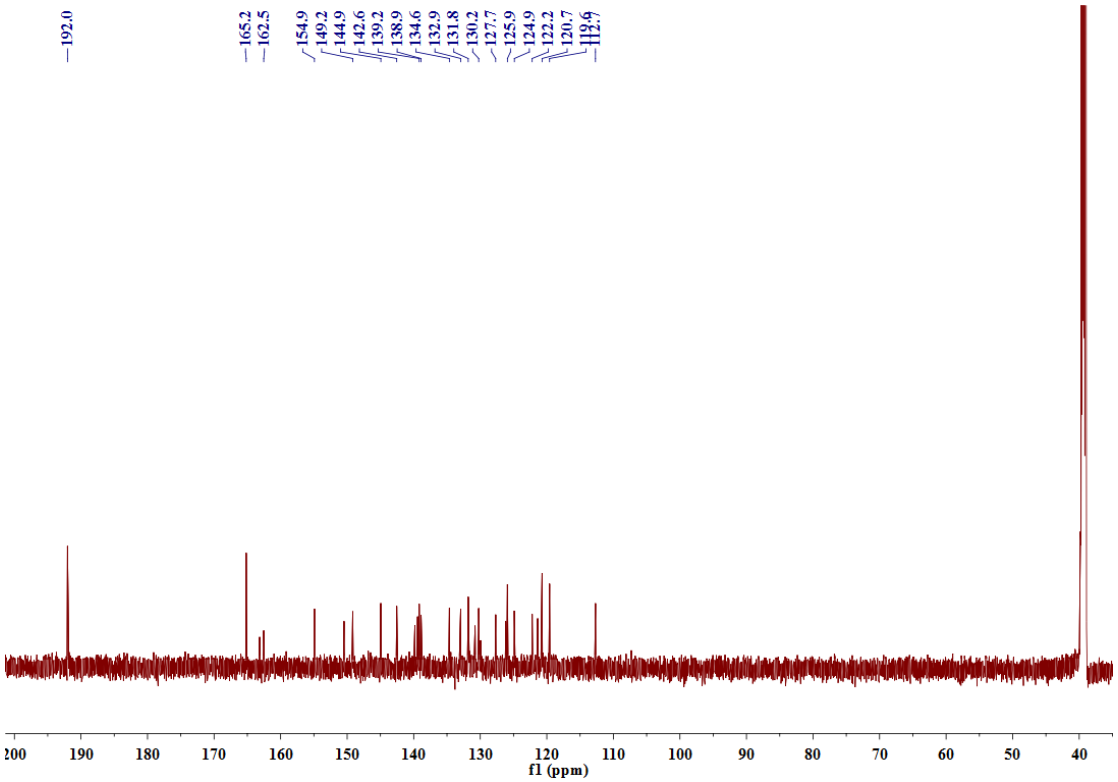


Fig. S6 ^{13}C NMR spectrum of probe 1 in DMSO-d_6.

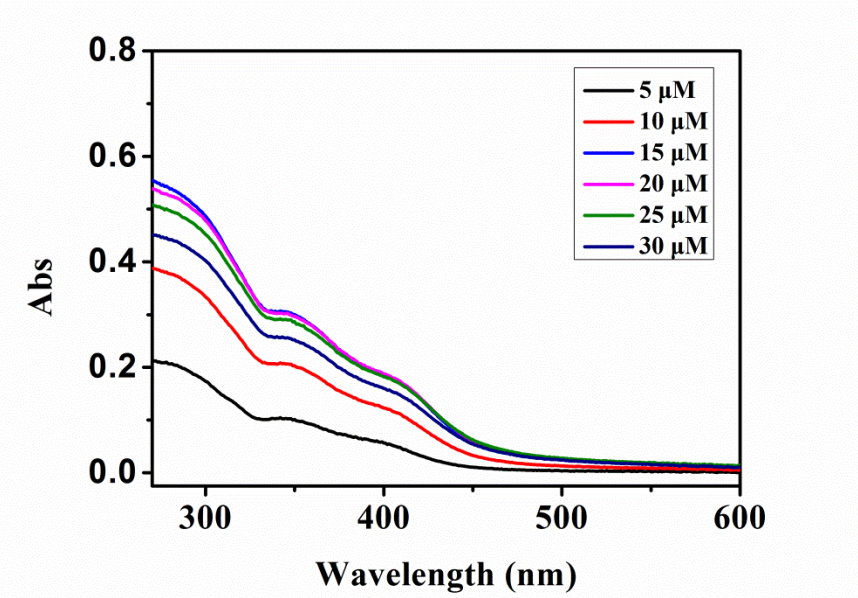


Fig. S7 UV-Vis absorption spectra of probe 1 with different concentrations in disodium hydrogen phosphate/citric acid buffer solutions at pH 5.0.

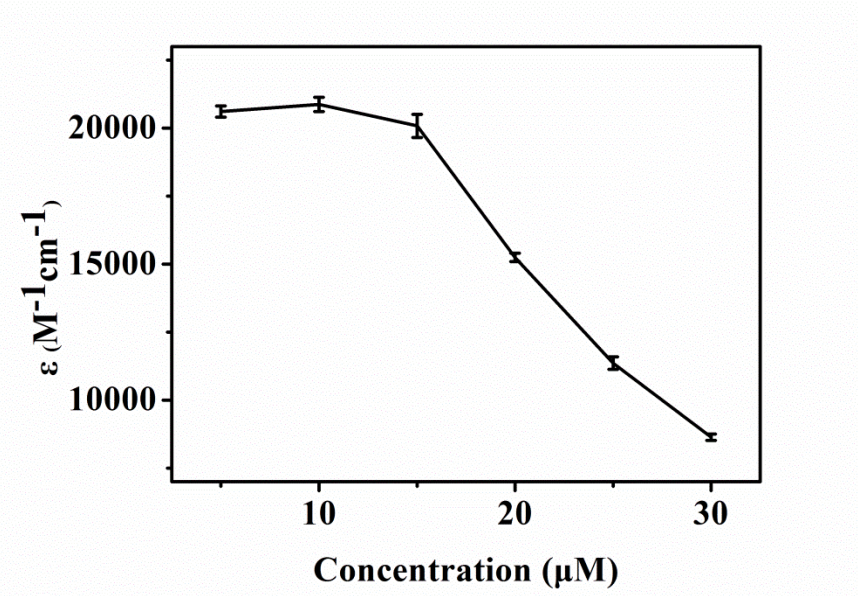


Fig. S8 Effect of molar absorption coefficient on the concentration of probe 1 at 345 nm at pH 5.0.

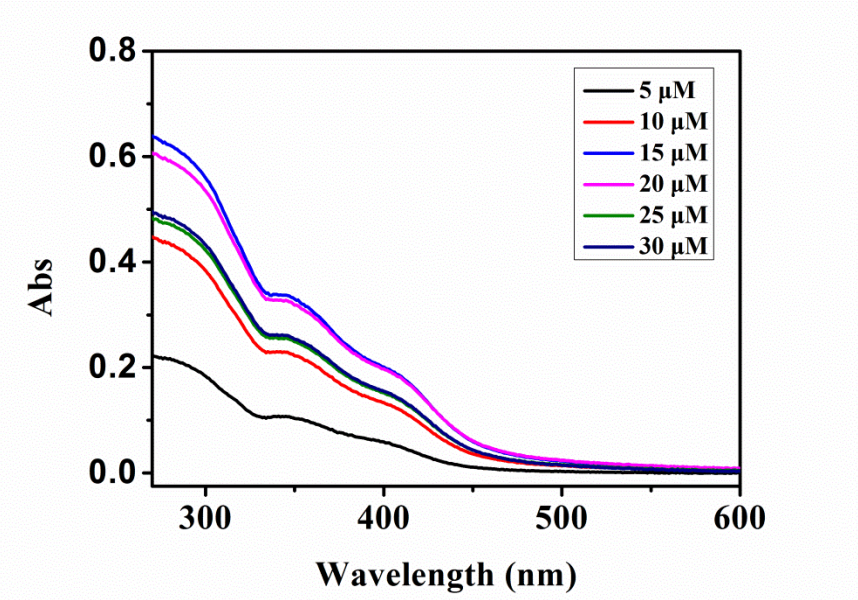


Fig. S9 UV-Vis absorption spectra of probe 1 with different concentrations in disodium hydrogen phosphate/citric acid buffer solutions at pH 7.4.

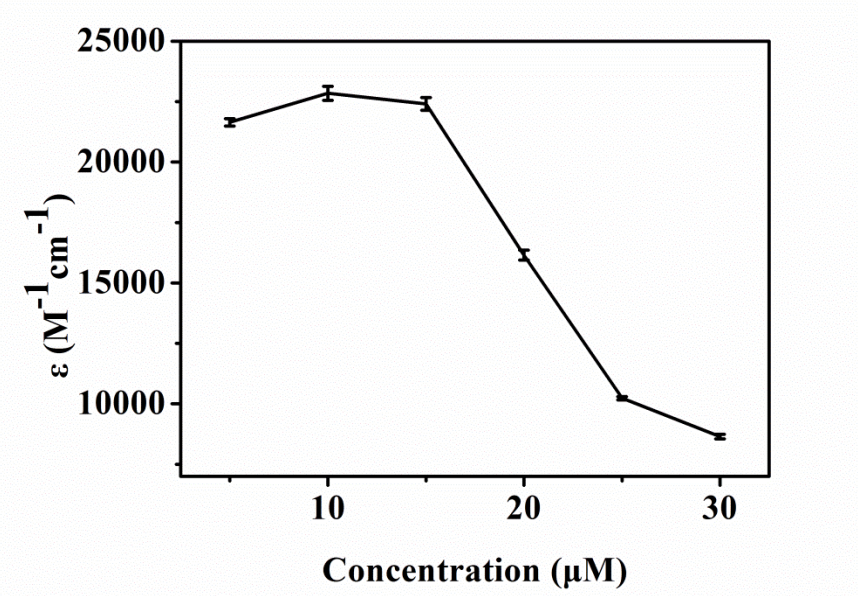


Fig. S10 Effect of molar absorption coefficient on the concentration of probe 1 at 345 nm at pH 7.4.

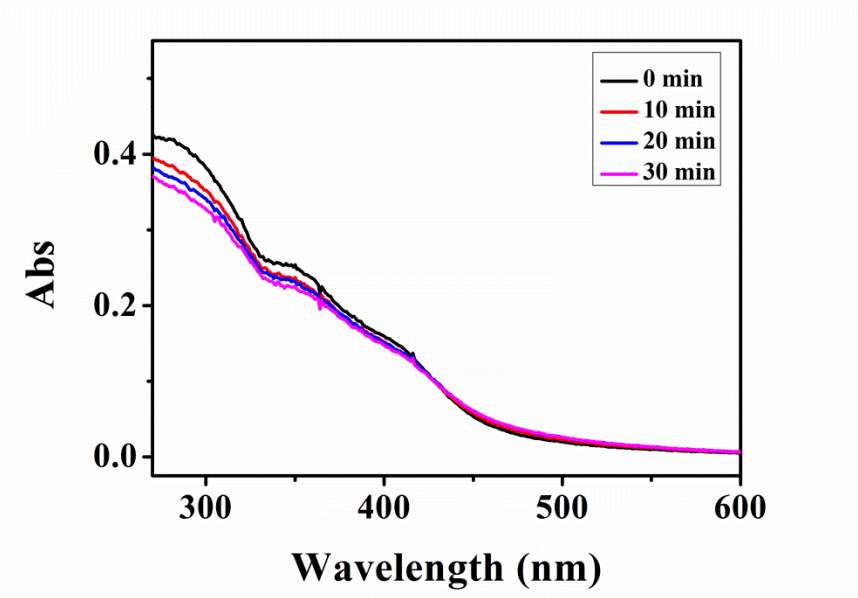


Fig. S11 UV-Vis absorption spectra of probe 1 (10 μM) by irradiation under white light irradiation (20 mW·cm^{-2}) in disodium hydrogen phosphate/citric acid buffer solutions at pH 5.0.

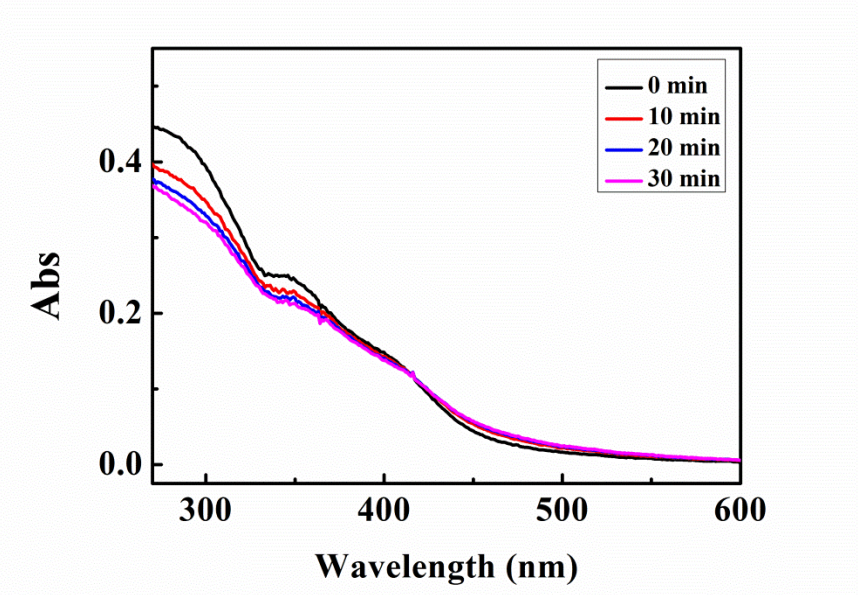


Fig. S12 UV-Vis absorption spectra of probe 1 (10 μM) by irradiation under white light irradiation (20 mW·cm^{-2}) in disodium hydrogen phosphate/citric acid buffer solutions at pH 7.4.

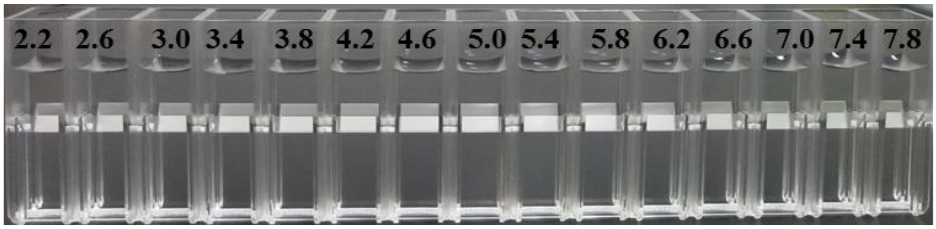


Fig. S13 The photographs of probe 1 (10 μM) in disodium hydrogen phosphate/citric acid buffer solutions at different pH values. (Without excitation).

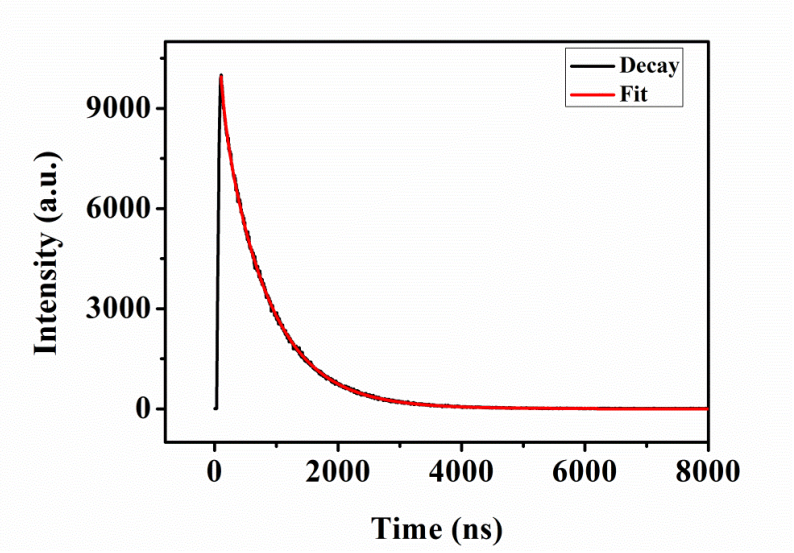


Fig. S14 Lifetime spectra of the probe 1 (10 μM) in aerated disodium hydrogen phosphate/citric acid buffer solutions at pH 3.0.
698 ns (0.87%: 73 ns; 99.13%: 755 ns).

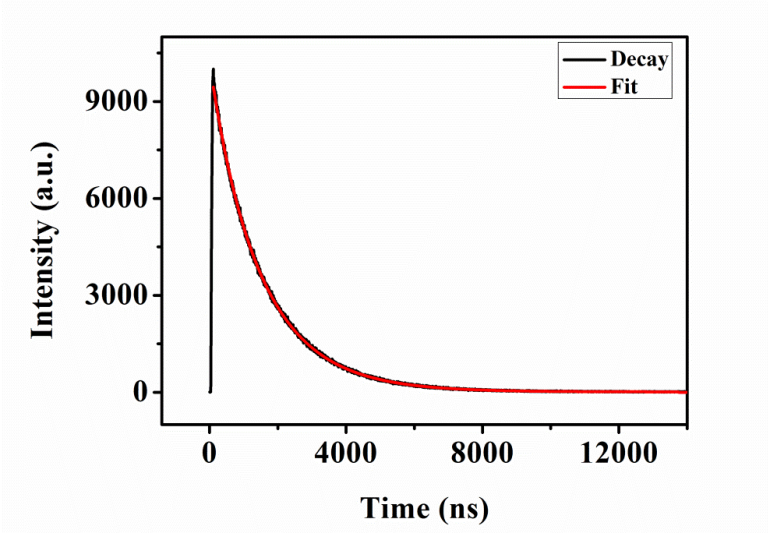


Fig. S15 Lifetime spectra of the probe 1 (10 μM) in degassed disodium hydrogen phosphate/citric acid buffer solutions at pH 3.0.
1487 ns (6.27%: 781 ns; 93.73%: 1583 ns).

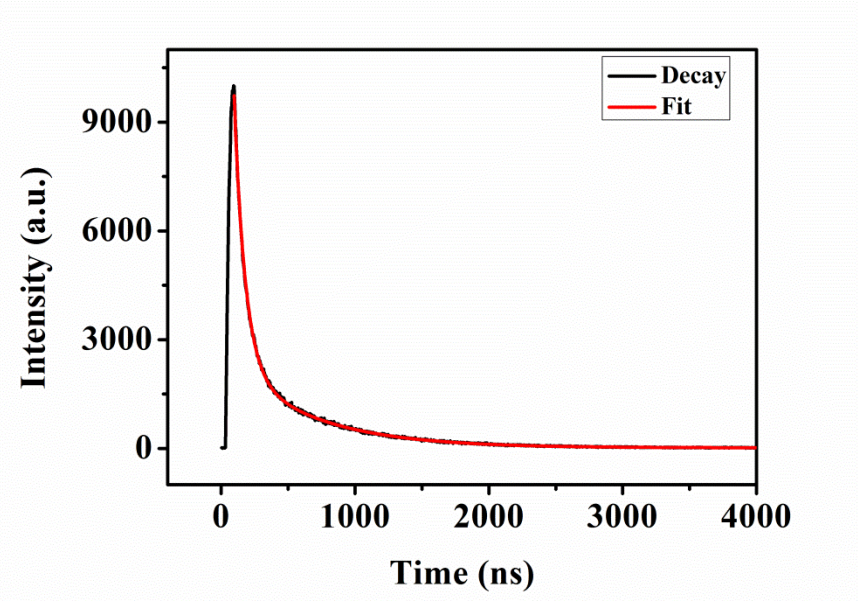


Fig. S16 Lifetime spectra of the probe 1 (10 μM) in aerated disodium hydrogen phosphate/citric acid buffer solutions at pH 5.0.
203 ns (31.01%: 82 ns; 68.99%: 610 ns).

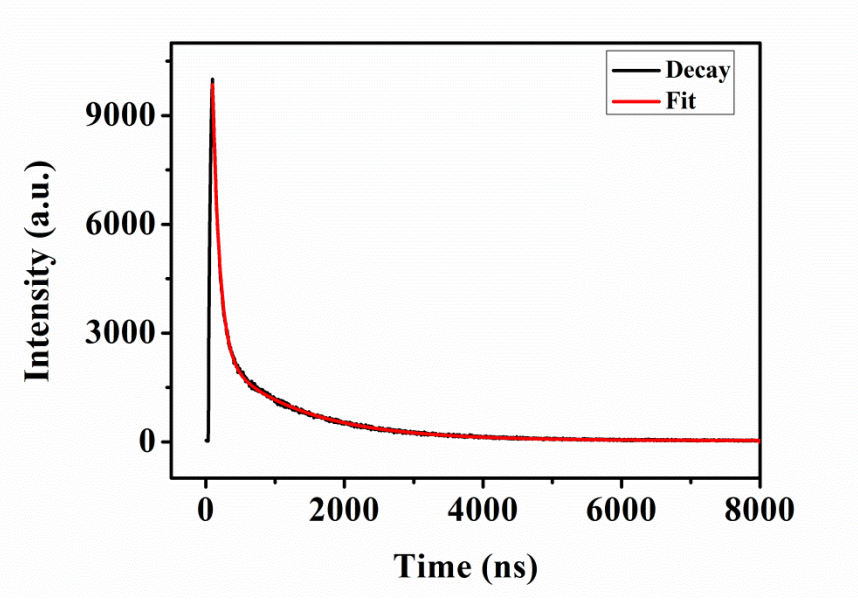


Fig. S17 Lifetime spectra of the probe 1 (10 μM) in degassed disodium hydrogen phosphate/citric acid buffer solutions at pH 5.0.
370 ns (21.05%: 103 ns; 78.95%: 1206 ns).

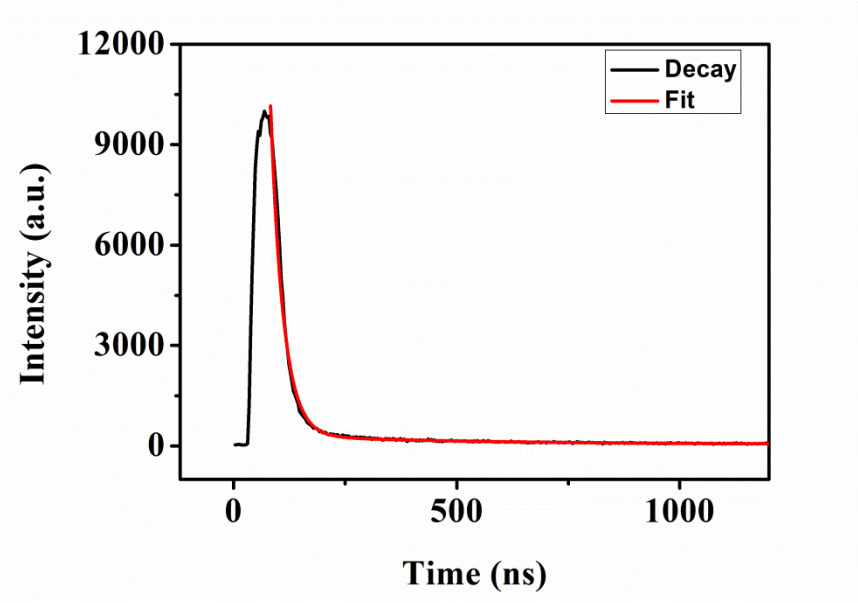


Fig. S18 Lifetime spectra of the probe 1 (10 μM) in aerated disodium hydrogen phosphate/citric acid buffer solutions at pH 7.4.
38 ns (72.18%: 28 ns; 27.82%: 445 ns).

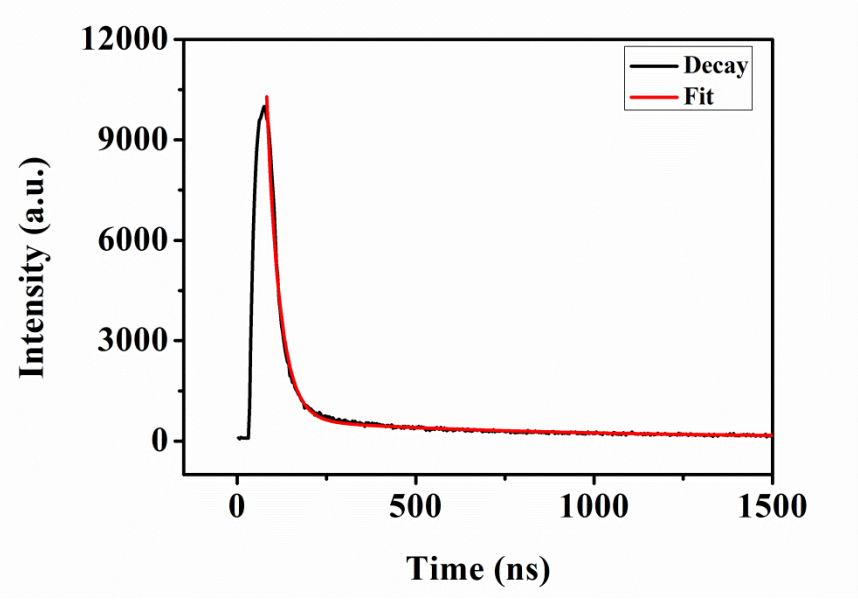


Fig. S19 Lifetime spectra of the probe 1 (10 μM) in degassed disodium hydrogen phosphate/citric acid buffer solutions at pH 7.4.
70 ns (50.51%: 702 ns; 49.49%: 37 ns).

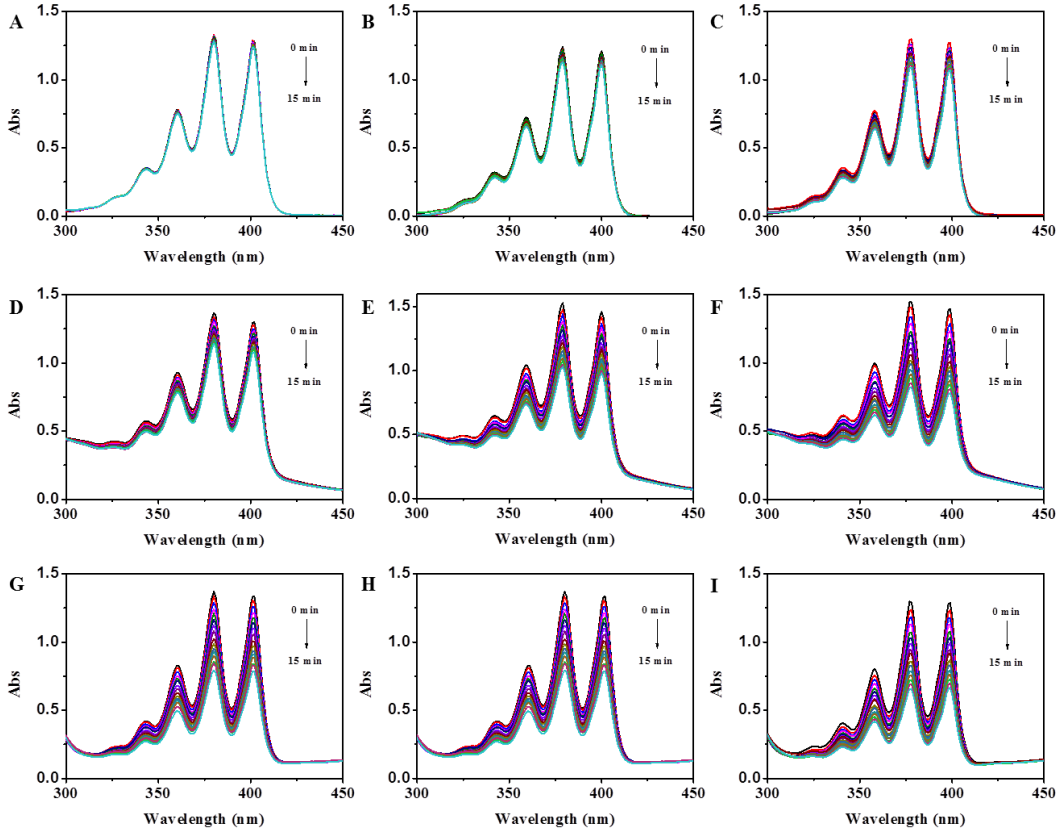


Fig. S20 UV-Vis absorption spectra of ABDA (100 μM) sensitized by blank (A, B and C), probe 1 (D, E and F), $[Ru(bpy)_3]Cl_2$ (G, H and I) in aerated disodium hydrogen phosphate/citric acid buffer solutions (pH=7.4: A, D and G; pH=5.0: B, E and H; pH=3.0: C, F and I) under white light irradiation (20 $mW.cm^{-2}$) at different times.

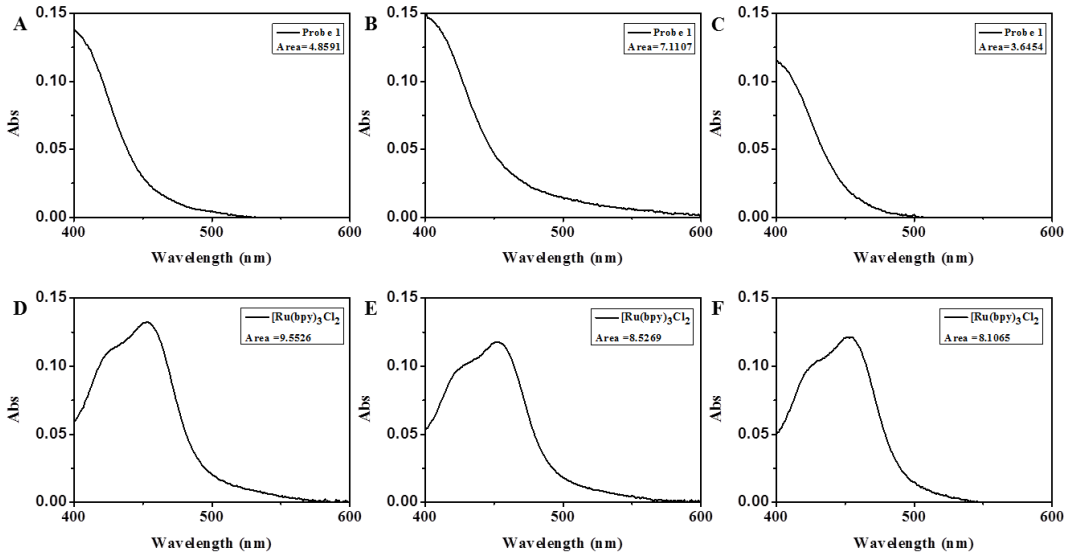


Fig. S21 The absorption peak area of probe 1 and $[Ru(bpy)_3]Cl_2$ measured in aerated disodium hydrogen phosphate/citric acid buffer solutions at pH 7.4 (A and D), 5.0 (B and E) and 3.0 (C and F).

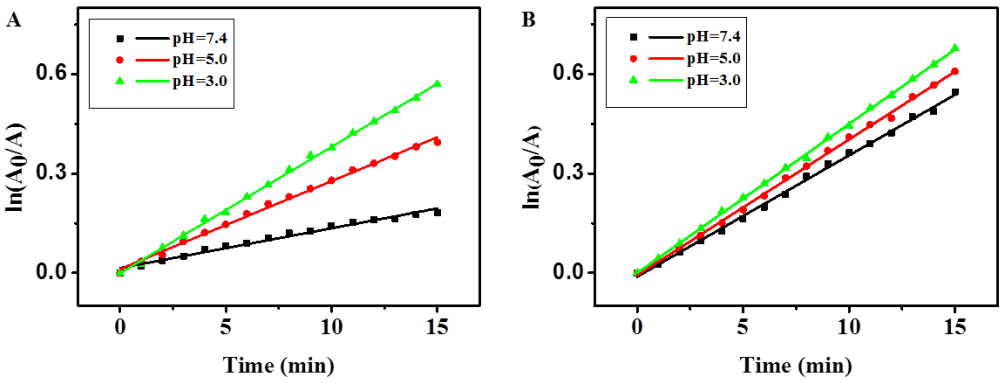


Fig. S22 $\ln(A_0/A)$ vs time profiles of ABDA sensitized by probe 1 (A) and $[Ru(bpy)_3]Cl_2$ (B) in aerated disodium hydrogen phosphate/citric acid buffer solutions at 378, 379, 380 nm for pH 3.0, 5.0 and 7.4, respectively.

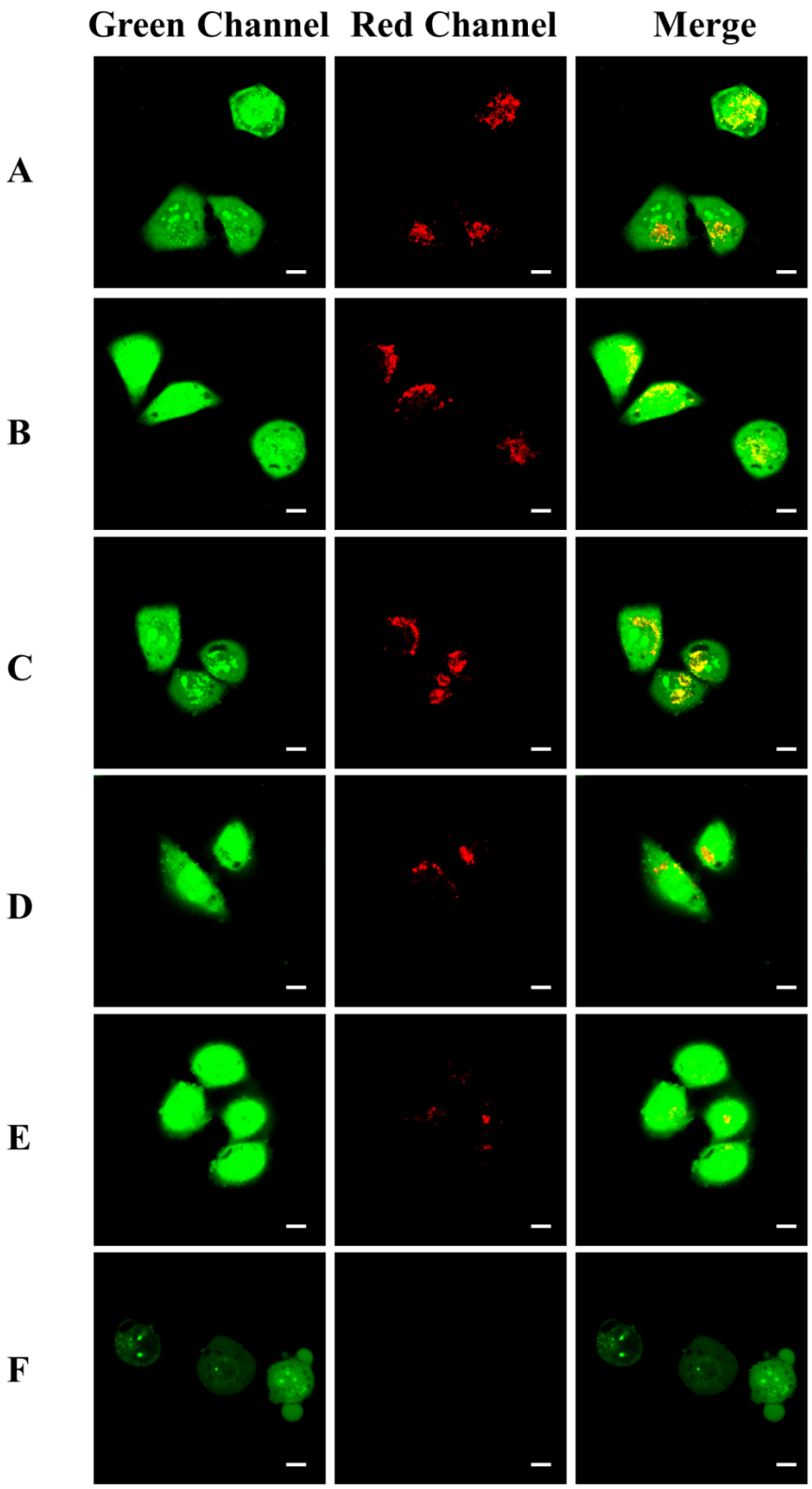


Fig. S23 CLSM images of HeLa cells in different conditions. (A) HeLa cells under dark, (B) HeLa cells under light alone, (C) HeLa cells treated with probe 1 (10 μM) under dark, (D-F) HeLa cells treated with probe 1 (D, 2.5 μM; E, 5 μM; F, 10 μM) under light irradiation. Scale bar: 10 μm.

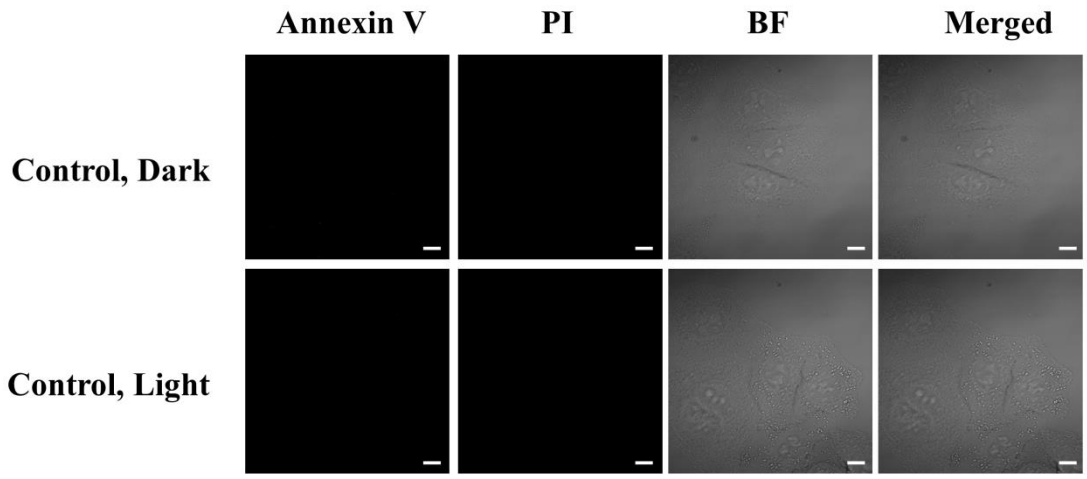


Fig. S24 CLSM images of HeLa cells co-stained with Annexin V-FITC and PI in different conditions. HeLa cells were incubated in the dark or irradiated with white light (20 mW·cm^{-2}) for 10 min. Scale bar: 10 μm.

Table S1 Elements for calculation of singlet oxygen quantum yields

pH	A_x	K_x	A_{std}	K_{std}	Φ_Δ
3.0	3.6454	0.03812	8.1065	0.04497	0.3393
5.0	7.1107	0.02652	8.5269	0.0410	0.1396
7.4	4.8591	0.01201	9.5526	0.0366	0.1161

Table S2 IC_{50} and PI values of tested compounds towards different cell lines

Complexes	Cancerous cells			Normal cells			Ref
	Light	Dark	PI	Light	Dark	PI	
Probe 1 [(3-pba)$_2$Ir(1-Py-βC)]PF_6	3.8 ± 0.6	31.3 ± 2.8	8.24 (HeLa)	50.7 ± 5.8	>100	1.97 (MRC-5)	This work
Cisplatin	>100	>100	- (HeLa)	>100	>100	- (MRC-5)	This work
Ir 1 [Ir(2Fppy)$_2$L]PF_6	0.16 ± 0.01	9.54 ± 0.35	59.6 (HeLa)	-	-	-	S1
Ir 2 [Ir(ppy)$_2$L]PF_6	0.24 ± 0.02	10.63 ± 0.42	44.3 (HeLa)	-	-		S1
Ir 3	53.3 ± 4.5	89.6 ± 3.7	1.68 (A549)	76.9 ± 1.6	90.6 ± 1.7	1.18 (MRC-5)	S2
Ir 4	1.1 ± 0.3	62.3 ± 2.6	56.64 (A549)	78.7 ± 2.3	96.4 ± 6.1	1.22 (MRC-5)	S2
Ir 5	32.7 ± 4.9	95.2 ± 6.4	2.9 47 (HeLa)	-	-	-	S3

	(Green) 2.02 ± 0.24 (Blue)						
Ir 6	2.51 ± 0.32 (Green) 1.32 ± 0.09 (Blue)	213 ± 14	85 161 (HeLa)	-	-	-	S3
Ir 7 $[Ir(L_1)(bpy)Cl](PF_6)_2$	>200	>200	- (HeLa)	>200	>200	- (LO2)	S4
Ir 8 $[Ir(L_1)(ppy)Cl]PF_6$	9.5 ± 1.3	120.4 ± 5.4	12.7 (HeLa)	14.9 ± 2.5	>200	>13.4 (LO2)	S4
Ir 9 $[Ir(L_2)(bpy)Cl](PF_6)_2$	>200	>200	- (HeLa)	190.5 ± 7.4	>200	>1.0 (LO2)	S4
Ir 10 $[Ir(L_2)(ppy)Cl]PF_6$	16.5 ± 0.1	49.0 ± 1.2	3.0 (HeLa)	28.2 ± 1.4	56.2 ± 4.0	2.0 (LO2)	S4
Ir 11 $[Ir(ppy)_2L1]PF_6$	1.39 ± 0.05	11.69 ± 1.27	8.4 (HeLa)	-	-		S5
Ir 12 $[Ir(ppy)_2L2]PF_6$	0.24 ± 0.01	15.13 ± 0.59	63 (HeLa)	-	-	-	S5
Ir 13 $[Ir(ppy)_2L3]PF_6$	0.50 ± 0.04	>100	>200 (HeLa)	-	-		S5
Ir 14 $[Ir(ppy)_2L4]PF_6$	0.21 ± 0.01	>100	>476 (HeLa)	-	-	-	S5
Ir 17 $[Ir(tpy)(pbpq)Cl]PF_6$	15.9 ± 0.4	82.6 ± 0.7	5.2 (HeLa)	45.6 ± 0.3	>100	>2.2 (LO2)	S6
Ir 18 $[Ir(tpy)(pbpz)Cl]PF_6$	0.45 ± 0.1	32.8 ± 0.6	72.9 (HeLa)	2.4 ± 0.5	>100	>41.7 (LO2)	S6
Ir 19 $[Ir(tpy)(pbpn)Cl]PF_6$	0.26 ± 0.1	9.5 ± 0.6	59.4 (HeLa)	1.8 ± 0.6	85.3 ± 0.1	47.4 (LO2)	S6

PI = IC_{50}(dark)/IC_{50}(light). 3-pba=3-(2-pyridyl)benzaldehyde, 1-Py-βC=1-(2-pyridyl)-β-carboline). S1: 2Fppy=2-(2,4-difluorophenyl)pyridine, ppy= 2-phenylpyridine, L=1-phenyl-2-(pyridin-2-yl)-1H-phenanthro[9,10-d]- imidazole. S4: L1 = 2,6-bis(2-benzimidazolyl)pyridine, bpy = 2,2'-bipyridine, L2 = 2,6-bis(1-methyl-benzimidazol-2-yl)pyridine. S5: L1 = 2-(2-pyridyl)benzimidazole, L2=2-(2-pyridyl)naphtha[b]imidazole, L3=benzimidazol-2-yl-quinoline, L4= 2-(naphtha[3,4]imidazole-2-yl)-quinoline.

Supporting references

S1 K. Qiu, M. Ouyang, Y. Liu, H. Huang, C. Liu, Y. Chen, L. Ji and H. Chao, *J. Mater. Chem. B*, 2017, **5**, 5488−5498.

S2 P. Zhang, H. Huang, S. Banerjee, G. J. Clarkson, C. Ge, C. Imberti and P. J. Sadler, *Angew. Chem. Int. Ed.*, 2019, **58**, 2350–2354.

S3 V. Novohradsky, A. Rovira, C. Hally, A. Galindo, G. Vigueras, A. Gandioso, M. Svitelova, R. B. Obach, H. Kostrhunova, L. Markova, J. Kasparkova, S. Nonell, J. Ruiz, V. Brabec and V. Marchán, *Angew. Chem. Int. Ed.*, 2019, **58**, 6311–6315.

S4 Y. Zheng, L. He, D. Y. Zhang, C. P. Tan, L. N. Ji and Z. W. Mao, *Dalton Trans.*, 2017, **46**, 11395–11407.

S5 F. X. Wang, M. H. Chen, Y. N. Lin, H. Zhang, C. P. Tan, L. N. Ji and Z. W. Mao, *ACS Appl. Mater. Interface*, 2017, **9**, 42471–42481.

S6 B. Yuan, J. Liu, R. Guan, C. Jin, L. Jia and H. Chao, *Dalton Trans.*, 2019, **48**, 6408-6415.