Supporting Information

Improve emission performance of benzo[*a*]phenoxazine in aqueous solution through Host-Guest interaction between methyl-β-cyclodextrin and adamantyl group

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1. Experimental

1.1 Materials and apparatus

Starting materials were ordered from Aladdin Biochemical Technology Co. Ltd. (Shanghai, China), Leyan Chemistry Co., Ltd. (Shanghai, China), and Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All solvents (analytical grade) were purchased from Jiangsu Qiangsheng functional Chemistry Co., Ltd (Suzhou, China) without being purified before use. The reaction progress was detected using TLC silica gel plates. ^{1}H NMR (300, 600MHz) and ^{13}C NMR (151 MHz) spectra were recorded with Vnmrs and Bruker spectrometers. HRMS data were achieved with a Xevo G2-XS TOF mass spectrometer. Bruker VERTEX70 IR spectrometer was used to achieve infrared spectra. Melting points were determined on the X-4 microscope electron thermal apparatus (Taike, China). UV-Vis absorption spectra and fluorescence spectra were measured by a Shimadzu UV-1800 spectrometer and a Shimadzu RF-5301PC spectrometer, respectively. The fluorescence decay spectra were measured using an Edinburgh FLS1000 fluorescence spectrophotometer. Cell imaging experiments were conducted by using a Leica TCS SP5 II confocal laser scanning microscope. The pH values were measured with a Lei-Ci (pH-3C) digital pH-meter (Shanghai, China) using a combined glass-calomel electrode. All experiments were carried out at room temperature.

1.2 Preparation of test solutions

The test solution (10 μM) of probes **1a-1b** was prepared from 1 mM stock solution in DMSO. The preparation method was as follows: 100μL of stock solution was added into 10 mL volumetric flask, and the solvent was added to fix the volume.

Selective test: preparation of 1 mM stock solution for different biologically related analytes. Probes **1a-1b** (100 μL) was mixed with different biological analytes (cations (Ba^{2+}, Ca^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, K^{+}, Ni^{2+}), anions (Cl^{-}, NO_3^{-}, SO_4^{2-}) and amino acids (Cys, Gly, Phe)), then PBS solution (pH=7.4) was used to obtain a total volume of 10 mL test solution.

Preparation of pH test solution: PBS solution buffer solution was used in the pH experiments. Probes **1a-1b** solutions with different pH values were prepared by placing 1 mL stock solution (100 μM) and 1 mL DMSO to a 10 mL volumetric flask, and PBS solution with different pH were added to 10 mL. Host(M-β-CD)-guest (Probes **1a-1b**) solutions with different pH values were prepared by

placing 1 mL stock solution (100 μM) and 1 mL M-β-CD (2 mM, in DMSO) to a 10 mL volumetric flask, and adding PBS solution with different pH values to gain the final concentration of 10 mL.

1.3 Determination of the relative fluorescence quantum yield

The relative fluorescence quantum yields were measured using the following equation:

$$\Phi_x/\Phi_{st} = [A_{st}/A_x]\,[n_x/n_{st}]^2\,[D_x/D_{st}]$$

Where st is the standard; x is the sample; Φ is the quantum yield; A is the absorbance at the excitation wavelength; D is the area under the fluorescence spectra on an energy scale; and n is the refractive index of the solution. Oxazine 1 (Φ = 0.11 in ethanol) was used as the standard.

1.4 Photo-stability of probes 1a-1b

During the photostability testing, the acetonitrile solution of probes **1a-1b** was maintained at a concentration of 10 μM, while the acetonitrile concentration of M-β-CD was kept at 200 μM. The probes were continuously irradiated for 5 h under a 500 W Philips tungsten iodide lamp. The distance between the samples and the lamp was 25 cm. An 8 cm thick cold trap with $NaNO_2$ (60 g L^{-1}) was placed between samples and the lamp in order to reduce the interference of the heat and short wavelength light. The relative remaining absorption (%) at the maximum absorption wavelength before and after irradiation was calculated.

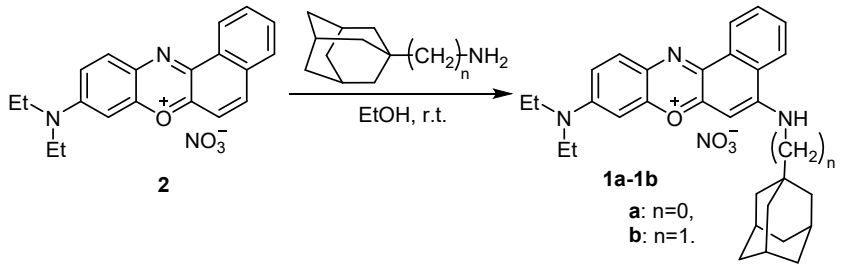
1.5 Cell culture and imaging methods

HeLa cells were cultivated in Roswell Park Memorial Institute culture medium (RPMI-1640) with L-glutamine (2.5×10^{-4} M), 10% calf serum, streptomycin (100 μg mL^{-1}) and penicillin (100 U mL^{-1}) in incubator containing 5: 95 CO_2 – air at 37 ℃. The HeLa cells were load on a glass-bottomed coverslip and adhered for two days before experiments. Cytotoxicity of probes **1a-1b** was determined by CCK-8 method.

In the colocalization experiments, HeLa cells were incubated with commercial lysosomal or mitochondrial markers (100 nM) (Lyso Tracker Green DND-26 or Mito Tracker Green FM) and probes **1a-1b** (2 μM) for 10 min, washed with PBS for 3 times, and then photographed. The confocal fluorescence imaging was performed on a microscope with 20X objective lens. The red channel images between 650 nm and 750 nm were obtained upon excitation at 561 nm, and the green channel images between 460 nm and 560 nm were collected upon excitation at 488 nm. All images were obtained in the same parameter and processed using supporting software of the Leica TCS SP5 II

confocal laser scanning microscope. The overlap between the red and green channels was obtained by ROIs (regions of interest) in the selected cells.

2. Synthesis and Characterization



2.1 General preparation of compound 2

Compound **2** was synthesized based on previous work.[1]

2.2 General preparation of compounds 1a-1b

***5-(Adamantan-1-ylamino)-9-(diethylamino)benzo[a]phenoxazin-7-ium nitrate* (1a).** The solution of 9-(diethylamino) benzo[*a*]phenoxazin-7-ium nitrate (**2**, 438.2 mg, 1.20 mmol), 1-adamantanamine (544.5 mg, 3.60 mmol) and ethanol (15 mL) were stirred for 24 h at room temperature. The solvent was removed under reduced pressure, the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH (15/1, v/v)) to afford **1a**. Blank solid, 63.2 mg, yield 10.2%, mp > 250 °C. IR ν (KBr, cm^{-1}): 3672, 3215, 2987, 2900, 1406, 1250, 1064, 895. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 8.84 (d, 1H, J = 7.8 Hz, Ar-*H*), 8.65 (d, 1H, J = 8.2 Hz, Ar-*H*), 8.46 (s, 1H, N*H*), 8.00 - 7.84 (m, 3H, 3×Ar-*H*), 7.36 (d, 1H, J = 9.3 Hz, Ar-*H*), 7.21 (s, 1H, Ar-*H*), 7.03 (s, 1H, Ar-*H*), 3.70 (q, 4H, J = 6.7 Hz, 2×CH_2), 2.30 (br, 6H, 3×CH_2), 2.22 (br, 3*H*, 3×C*H*), 1.85 - 1.72 (m, 6H, 3×CH_2), 1.25 (t, J = 6.4 Hz, 6H, 2×CH_3). ^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm) 156.0, 154.3, 150.9, 148.8, 133.3, 133.2, 132.2, 131.2, 131.0, 129.7, 124.7, 124.2, 124.1, 116.5, 96.3, 96.2, 56.9, 46.0, 41.0, 35.8, 29.5, 13.0. HRMS (TOF, ESI^+): m/z calcd $C_{30}H_{34}N_3O^+$ [M - NO_3^-]$^+$: 452.2697, found: 452.2695.

***5-((Adamantan-1-ylmethyl)amino)-9-(diethylamino)benzo[a]phenoxazin-7-ium nitrate* (1b).** Compound **1b** was prepared by **2** (182.7 mg, 0.50 mmol) and 1-adamantanemethylamine (247.9 mg, 1.50 mmol) using the identical procedure as **1a**. Cool black solid, 52.3 mg, yield 19.8%, mp > 250 °C. IR ν (KBr, cm^{-1}): 3238, 2899, 1582, 1433, 1325, 1275, 1163, 1124, 1006, 773, 705, 663. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 9.94 (s, 1H, N*H*), 8.85 (d, 1H, J = 7.8 Hz, Ar-*H*), 8.63 (d, J = 7.8 Hz, 1H, Ar-*H*), 8.00(t, 1H, J = 7.4 Hz, Ar-*H*), 7.91 (t, 2H, J = 9.0 Hz, 2×Ar-*H*), 7.29 (d,

2H *J* = 8.8 Hz, 2×Ar-*H*), 6.94 (s, 1H, Ar-*H*), 3.68 (q, 4H, *J* = 6.8 Hz, 2×C*H*$_2$), 3.52 (d, 2H, *J* = 5.4 Hz, C*H*$_2$), 1.97 (br, 3H, 3×C*H*), 1.66 (br, 12H, 6×C*H*$_2$), 1.24 (t, *J* = 6.2 Hz, 6H, 2×C*H*$_3$).^{13}C NMR (151 MHz, DMSO-d_6) δ (ppm) 159.2, 153.8, 152.2, 148.4, 134.2, 133.0, 132.5, 131.3, 130.2, 129.9, 124.5, 124.2, 123.7, 115.5, 96.1, 94.9, 55.4, 45.8, 40.4, 37.0, 36.7, 28.2, 13.0. HRMS (TOF, ESI$^+$): m/z calcd $C_{31}H_{36}N_3O^+$, [M - NO_3^-]$^+$: 466.2853, found: 466.2857.

3. Tables and Figures

Table S1. Optical properties of the protonated probes **1a-1b** in PBS before and after adding M-β-CD

Probe	$\lambda_{Abs,\ max}$(nm)	$\lambda_{Em,\ max}$(nm)	Stokes shift (nm)	ε^{a}	Φ(%)[b]
1a[c]	652	688	32	2.97	7.6
1a+M-β-CD [d]	658	690	32	4.28	11.5
1b[e]	649	680	31	1.38	7.9
1b+M-β-CD[f]	653	684	31	5.19	12.6

[a] $\times 10^4$ M^{-1} cm^{-1}; [b] Oxazine 1 (Φ = 0.11 in ethanol) was used as the reference compound; [c] pH = 7.0; [d] the concentration of M-β-CD is 50 μM (pH = 7.0); [e] pH = 5.0; [f] the concentration of M-β-CD is 20 μM (pH = 5.0).

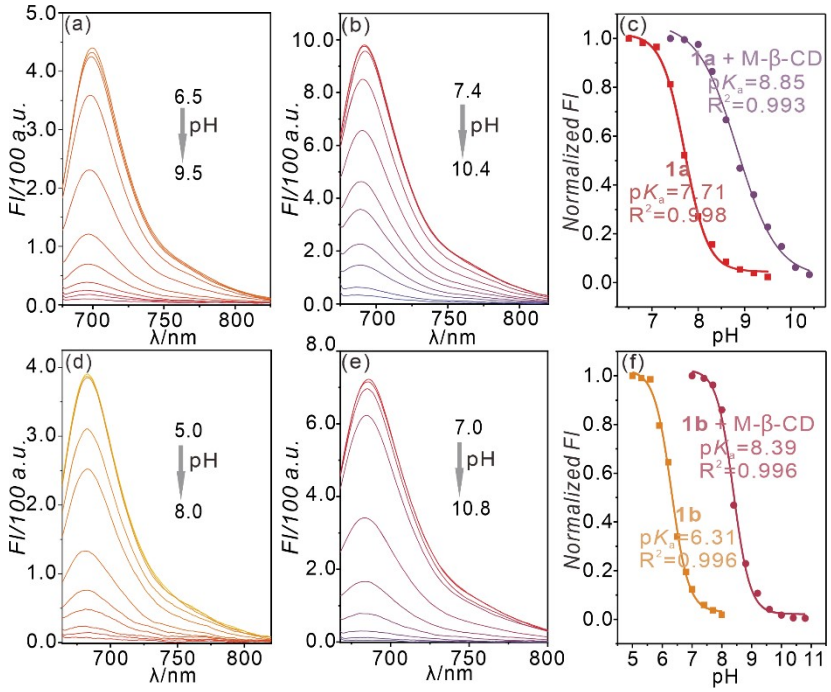


Figure S1. Fluorescent responses of probes **1a-1b** (10 μM) before and after adding M-β-CD toward different pH values in PBS buffer-DMSO (4/1, v/v) solutions. (a) probe **1a** (λ_{ex} = 650nm), (b) probe **1a** in the presence of M-β-CD (λ_{ex} = 650nm), (c) probe **1a** before and after adding M-β-CD in different pH at λ_{em} = 690 nm. (d) probe **1b** (λ_{ex} = 650nm), (e) probe **1b** in the presence of M-β-CD (λ_{ex} = 650nm), (f) probe **1b** before and after adding M-β-CD in different pH at λ_{em} = 684 nm.

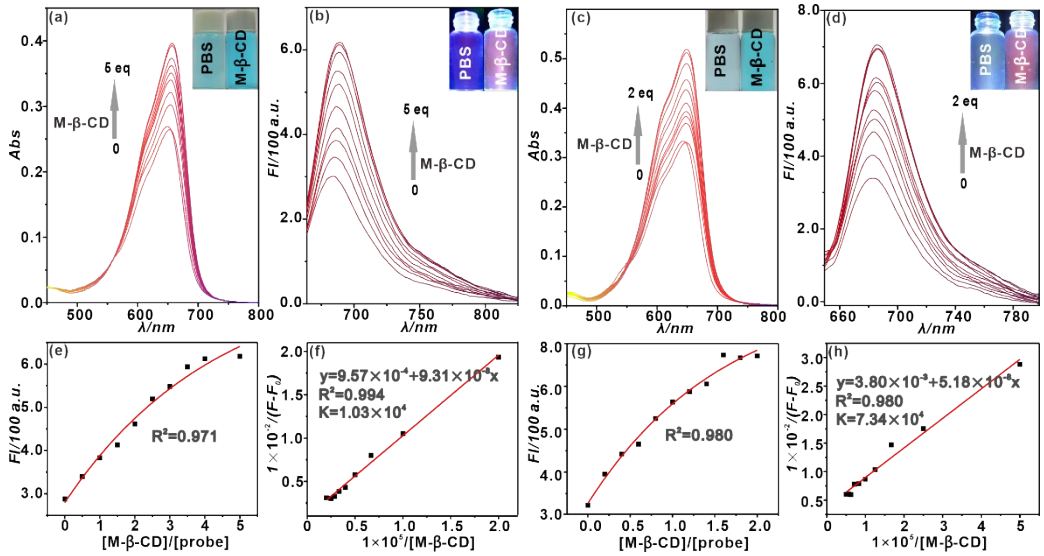


Figure S2. Optical responses of probes **1a-1b** (10 μM) in the protonated form to M-β-CD in PBS solution containing 5% DMSO. (a) Absorption spectra of probe **1a** (pH = 7.0); (b) emission spectra of probe **1a** (pH = 7.0, λ_{ex} = 650 nm, slit widths: 3 nm/3 nm); (c) Absorption spectra of probe **1b** (pH = 5.0); (d) emission spectra of probe **1b** (pH = 5.0, λ_{ex} =650 nm, slit widths: 5 nm/1.5 nm); (e) the relationship between F_{690nm} and [M-β-CD]/[probe] of probe **1a**; (f) double reciprocal diagram of fluorescence titration of probe **1a**; (g) the relationship between F_{688nm} and [M-β-CD]/[probe] of probe **1b**; (h) double reciprocal diagram of fluorescence titration of probe **1b**.

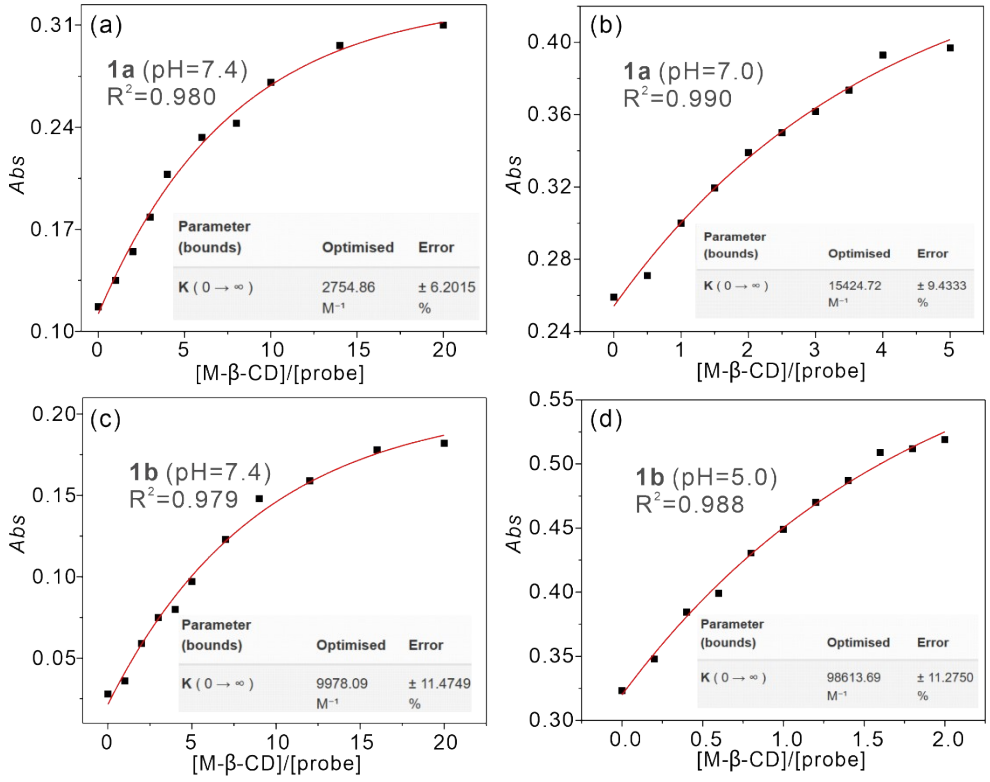


Figure S3. The maximum absorption peak of probes **1a-1b** changes with the concentration of M-β-

CD. Inset: The value of the complexation constant K obtained by fitting the data using Bindfit software.[2]

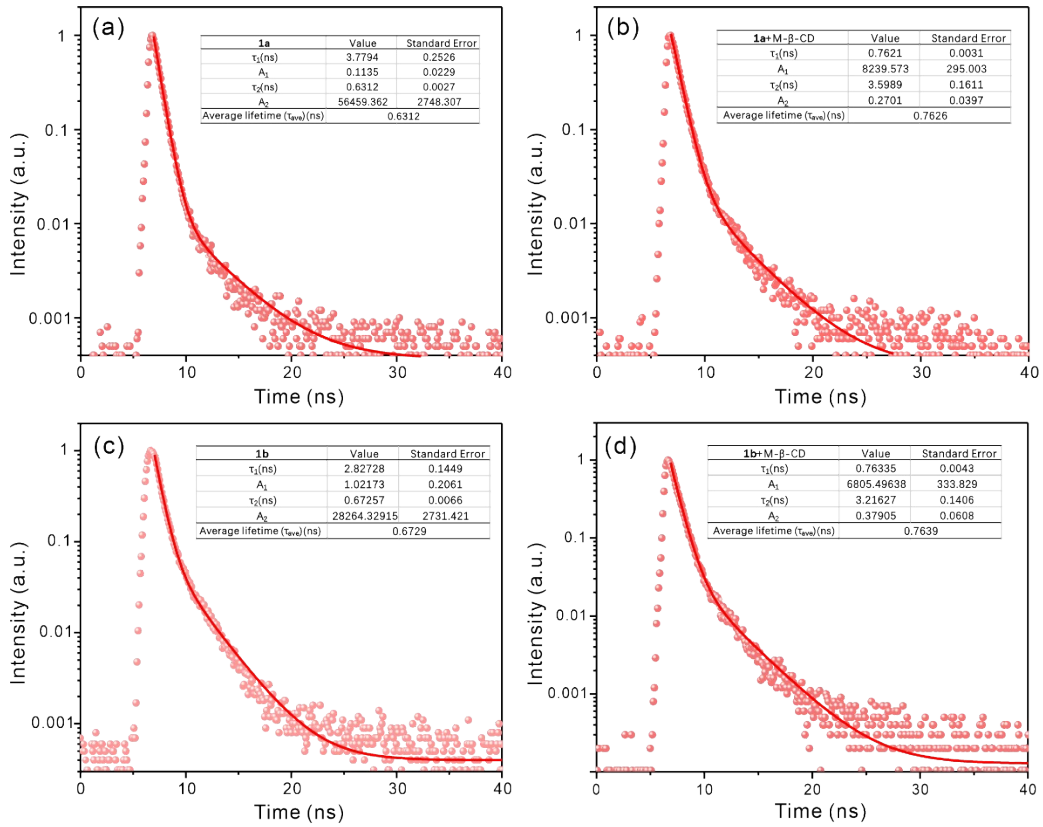


Figure S4. Fluorescence lifetime decay of probes **1a-1b** before and after adding M-β-CD in PBS solution (pH = 7.4).

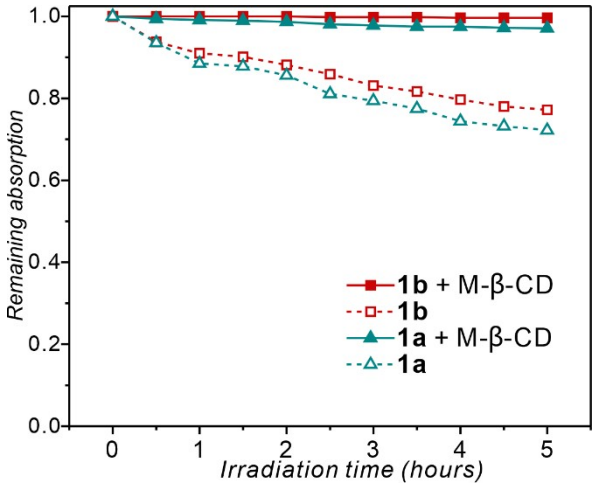


Figure S5. Photofading behaviors of probes **1a-1b** before and after adding M-β-CD in acetonitrile.

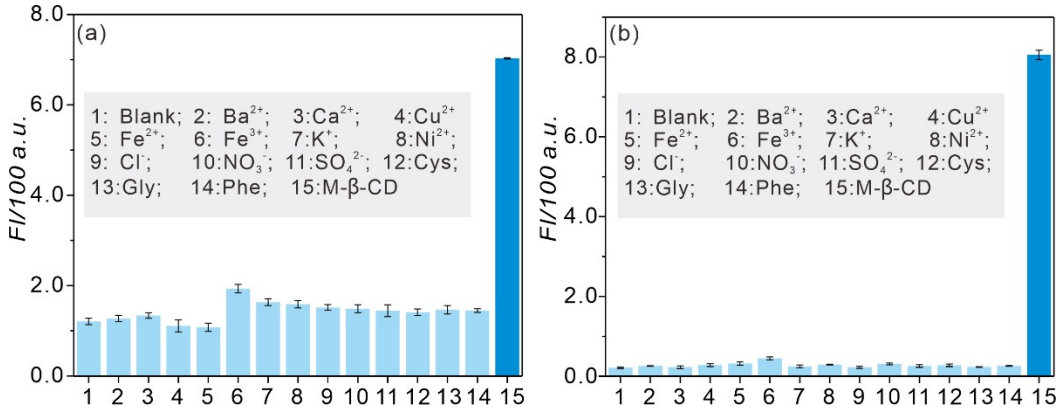


Figure S6. Fluorescence responses of probes **1a-1b** (10 μM) in the presence of 1 mM of different biologically relevant analytes. (a) probe **1a**; (b) probe **1b.**

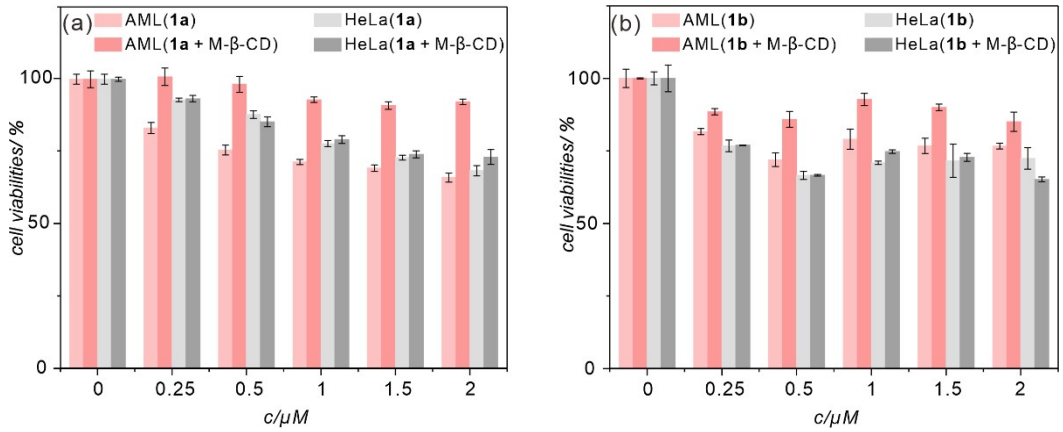


Figure S7. Percentages of HeLa cell and AML cell viabilities remaining after treatment with probes **1a-1b** before and after adding M-β-CD (5 μM) for 6h. (a) probe **1a**; (b) probe **1b**. The data were shown as mean ± SD (n = 3).

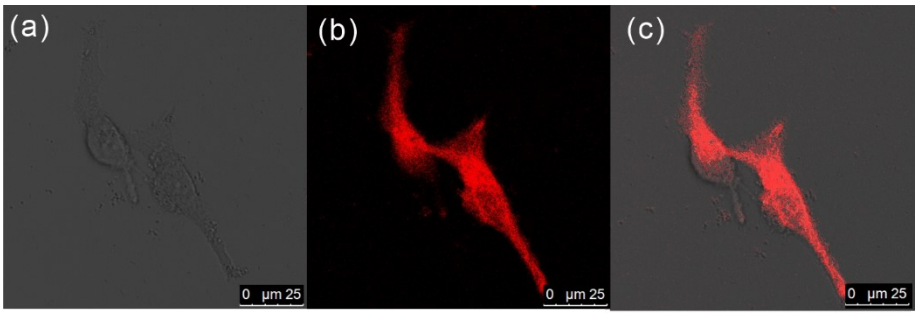


Figure S8. Fluorescence images of HeLa cells with Meldola's Blue (4 μM). (a) Brightfields images of HeLa cells. (b) Cells with Meldola's Blue in red channel. (c) Merged images of (a) and (b).

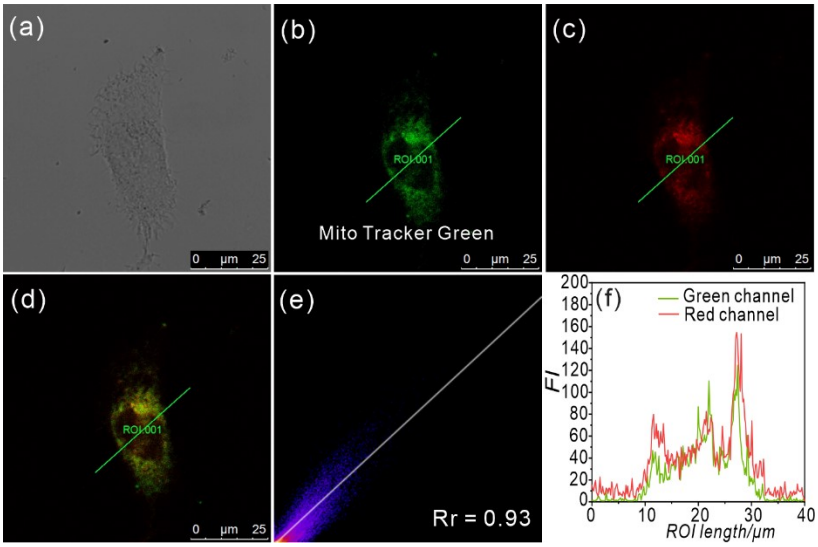


Figure S9. Fluorescence images of HeLa cells containing with probe **1a** (0.25 μM) and Mito Tracker Green FM (100 nM). (a) Bright field image; (b) confocal image (green channel) of Mito Tracker Green FM; (c) confocal image (red channel) with probe **1a** (0.25 μM) after adding M-β-CD (5 μM); (d) merged image of (b) and (c); (e) fluorescence intensity correlation plot from the red channel and green channel; (f) fluorescence intensity of the regions of interest (ROI) across the HeLa cells.

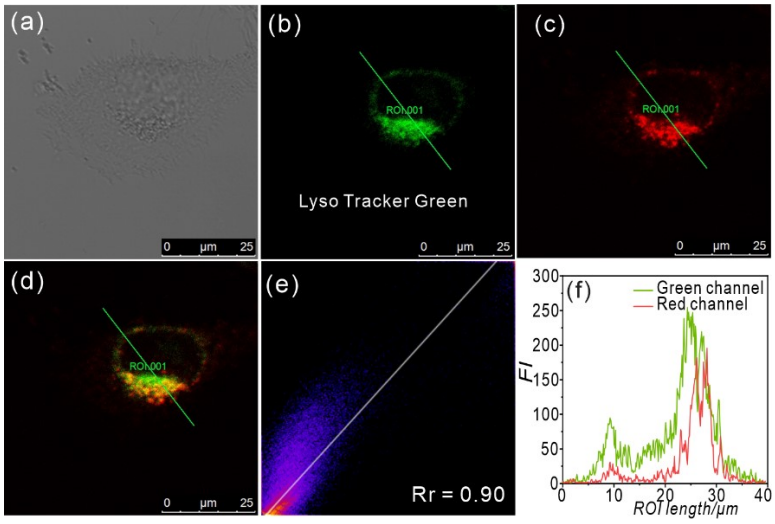


Figure S10. Fluorescence images of HeLa cells containing with probe **1b** (0.25 μM) and Lyso Tracker Green DND-26 (100 nM). (a) Bright field image; (b) confocal image (green channel) of Lyso Tracker Green DND-26; (c) confocal image (red channel) with probe **1b** (0.25 μM) after adding M-β-CD (5 μM); (d) merged image of (b) and (c); (e) fluorescence intensity correlation plot from the red channel and green channel; (f) fluorescence intensity of the regions of interest (ROI) across the HeLa cells.

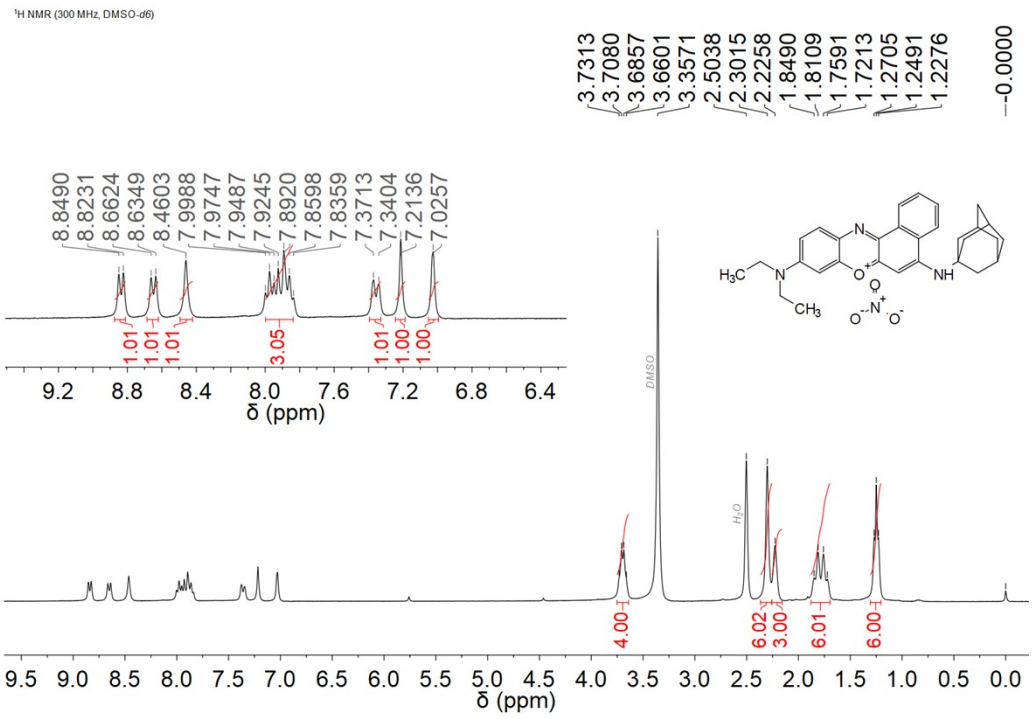


Figure S11. ^{1}H NMR spectrum of probe **1a**.

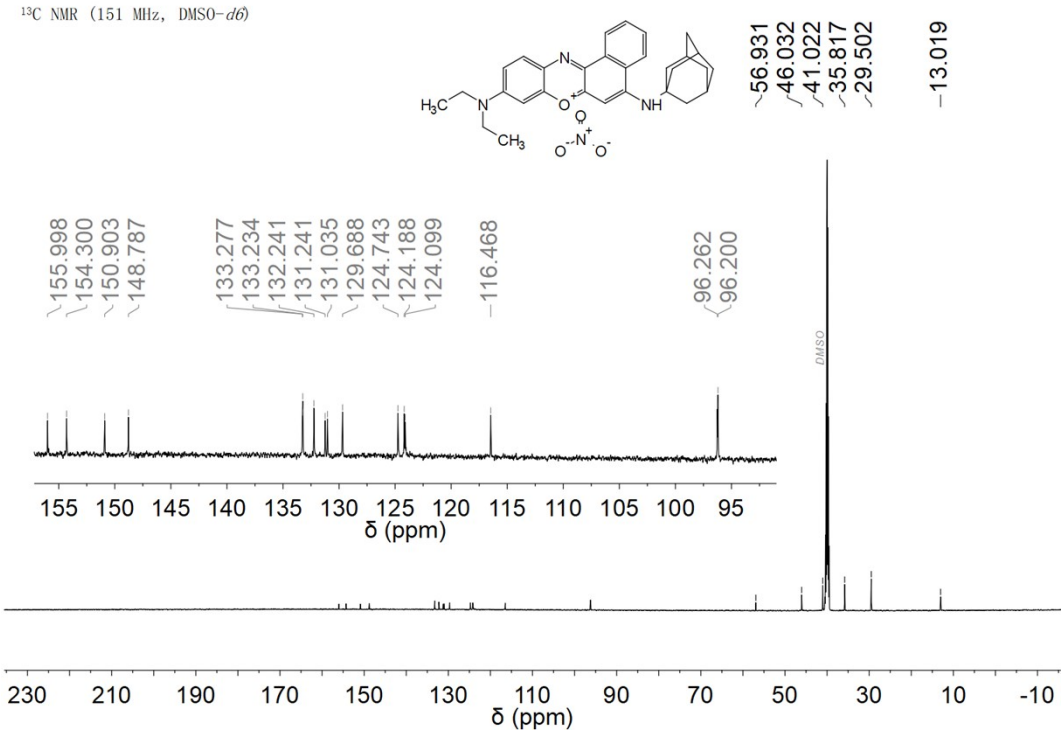


Figure S12. ^{13}C NMR spectrum of probe **1a**.

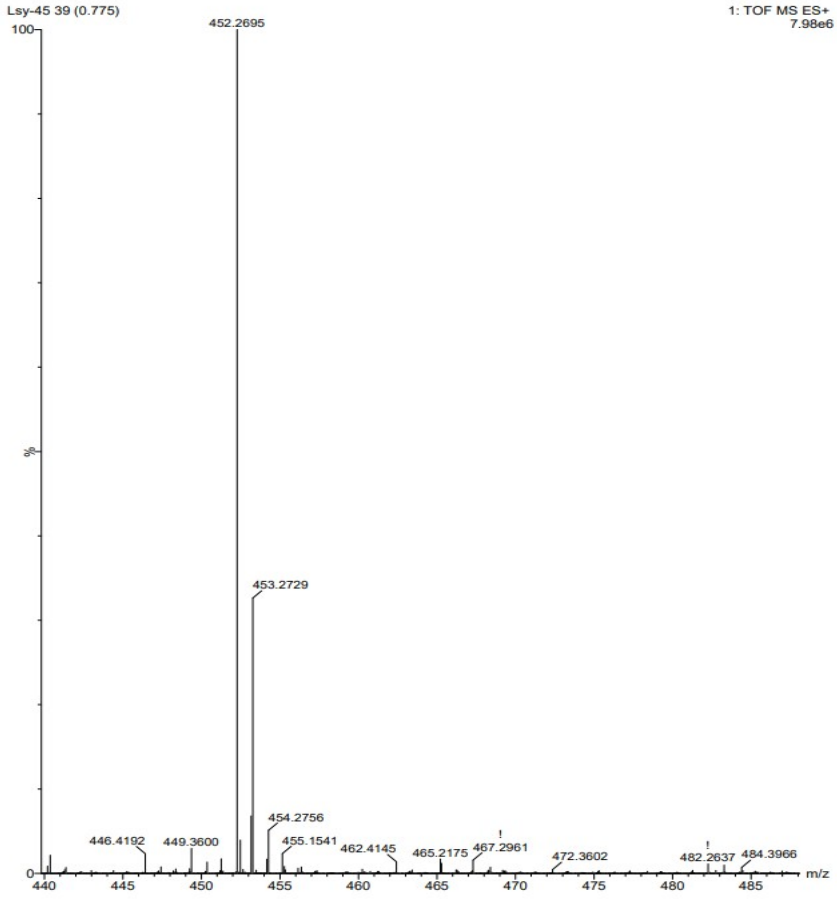


Figure S13. HRMS (ESI$^+$, TOF) spectrum of probe **1a**.

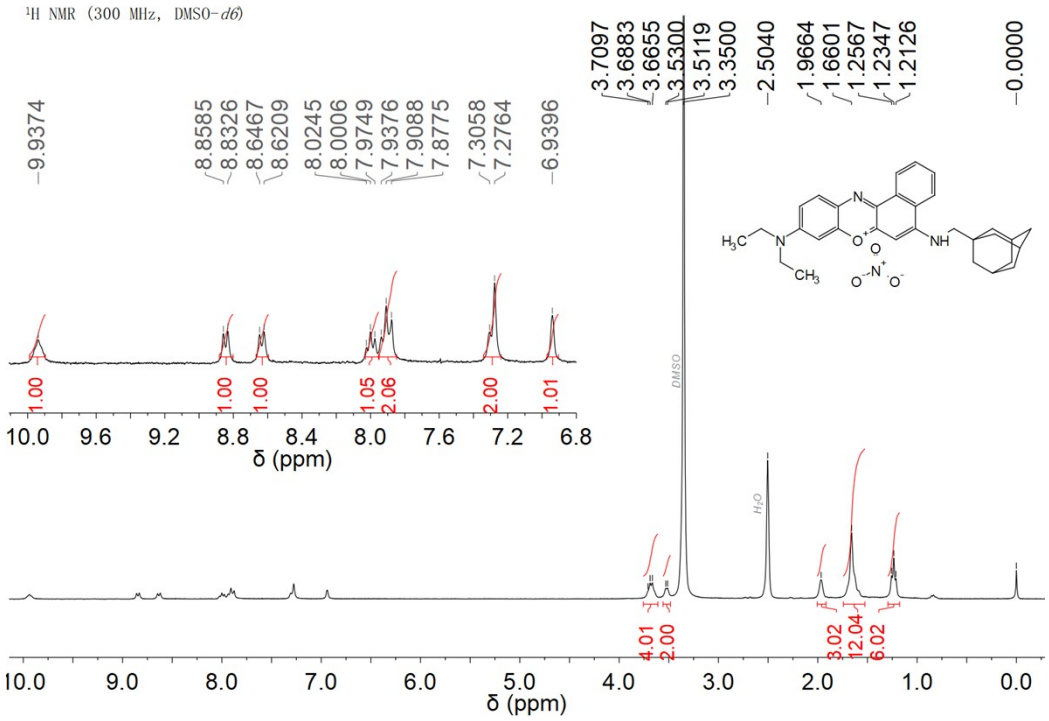


Figure S14. ^{1}H NMR spectrum of probe **1b**.

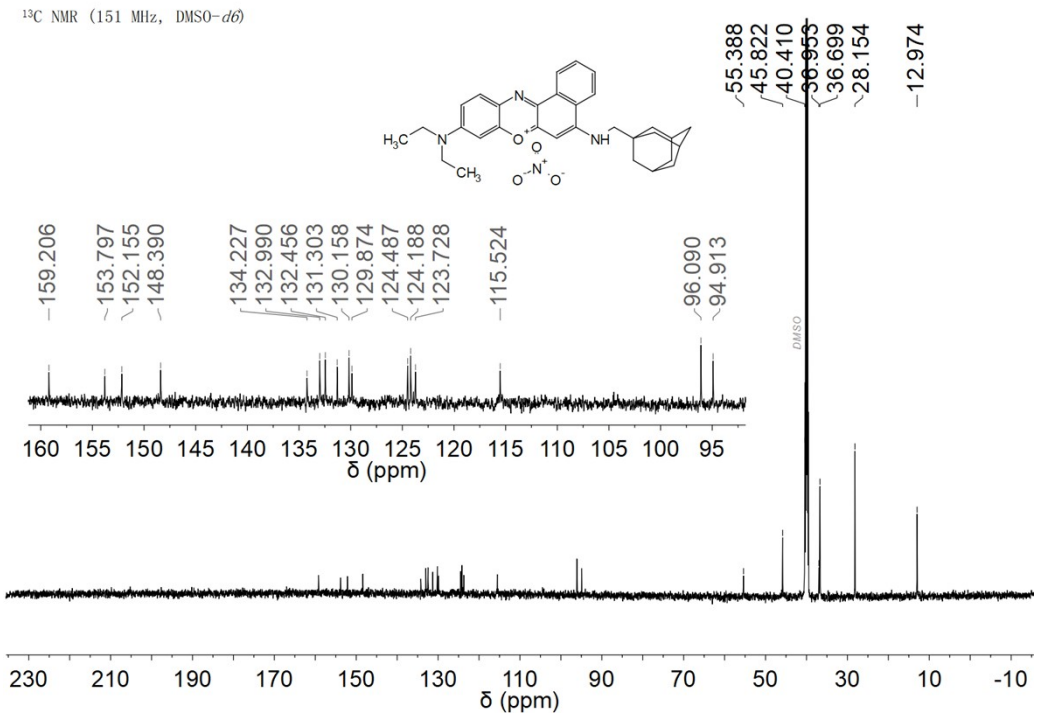


Figure S15. ^{13}C NMR spectrum of probe **1b**.

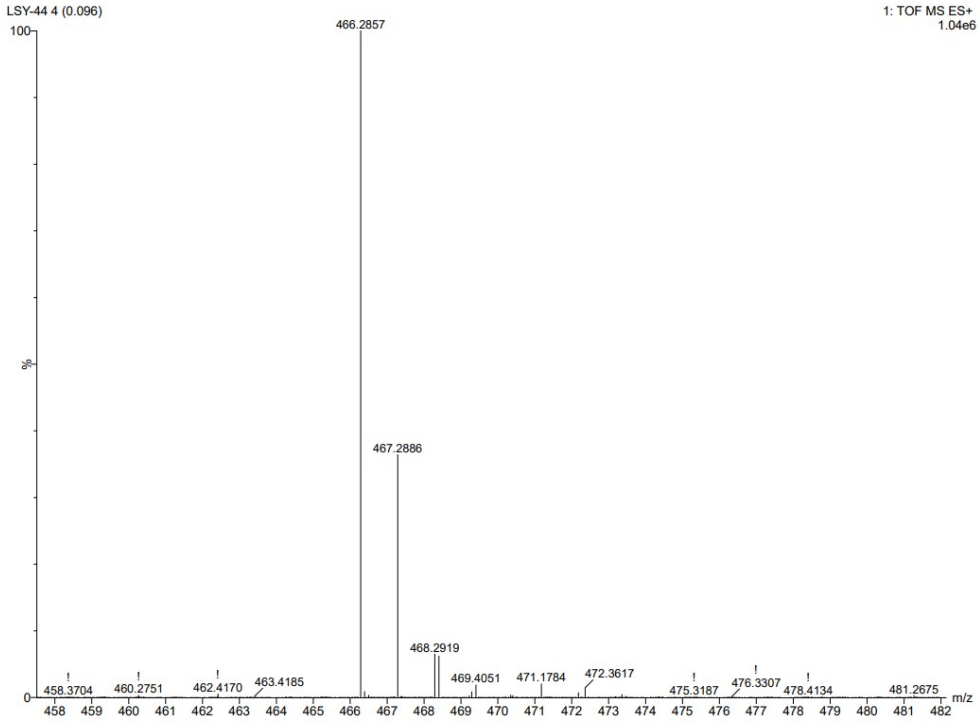


Figure S16. HRMS (ESI$^+$, TOF) spectrum of probe **1b**.

4. Reference

1 X. L. Shi, J. F. Ge, B. Q. Liu, M. Kaiser, S. Wittlin, R. Brun and M. Ihara, *Bioorg Med Chem Lett*, 2011, **21**, 5804-5807.

2 P. Thordarson, Chem. *Soc. Rev.*, 2011, **40**, 1305-1323.