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A novel mitochondria-targeted ratiometric fluorescent probe for endogenous sulfur dioxide derivatives as a cancer-detecting tool

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1. Methods

1.1 Calculation of quantum yield

The UV-Vis spectra and the fluorescence spectra of the corresponding slit were determined, and the quantum yield of the probe in PBS (content 30% DMF v: v) was calculated according to the following equation:

$$\Phi = \Phi_s(IA_s/I_sA)(\eta^2/\eta_s^2) \qquad (1)$$

(A is the absorbance, I is the integrated fluorescence intensity, and η is the refractive index of the solvent. The standard used for the measurement of fluorescence quantum yield was quinine sulphate (Φ_s = 0.547 in 0.05 M H_2SO_4 aq.).

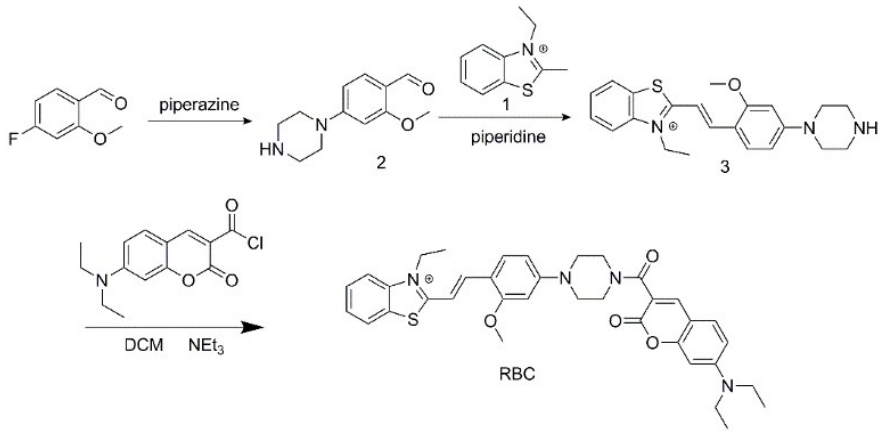
1.2 Calculation of the detection limit (LOD) and limit of quantitation (LOQ)

$$LOD = 3.3\sigma/k$$

$$LOQ = 10\sigma/k$$

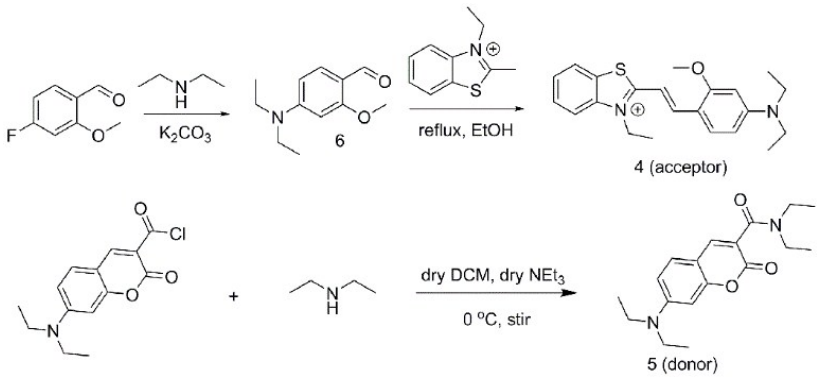
Where, σ is the standard deviation of the blank solution and k is the slope of the linear calibration plot between the fluorescence emission intensity and the concentration of HSO_3^-/SO_3^{2-}.

2. Synthesis



Scheme S1. Synthesis procedures of probe RBC.

2.1 Synthesis of compound **4** (acceptor) and **5** (donor)



Scheme S2. Synthesis procedures of the donor and acceptor.

Synthesis of Compound 4 (Acceptor)

4-Fluoro-2-methoxybenzaldehyde (380 mg, 2 mmol), K_2CO_3 (552 mg, 4 mmol) and $NHEt_2$ (549 mg, 7.5 mmol) was dissolved in ethanol (5 mL). The reaction mixture was refluxed and stirred for 4.5 h. After vacuum filtrated, the solvent was removed under reduced pressure to get light brown product. Subsequently, a crude product was purified by flash column chromatography (dichloromethane: methanol = 12: 1, v/v) to obtain compound **6** with 60% yield. Then compound **1** (306 mg) and compound **6** (207 mg) were dissolved in ethanol (15 mL) and catalytic amount piperidine were added to the mixture. The reaction mixture was

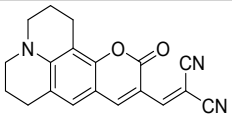
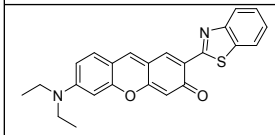
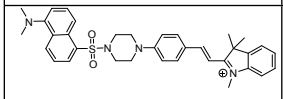
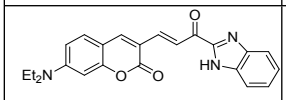
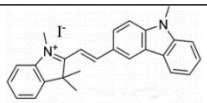
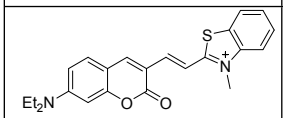
stirred and heated to reflux about 6 h (monitored by TLC). The solvent was removed under reduced pressure. Subsequently, the crude product was subjected to column chromatography using silica gel as the stationary phase and pure methanol as the mobile phase to obtain unmixed dark orange substance compound **4** (DCM: methanol = 20 : 1, v/v) with a yield of 65%, m. p.: 203-205 ℃.

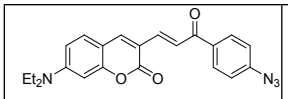
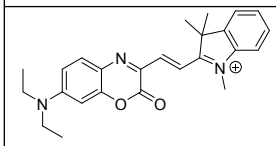
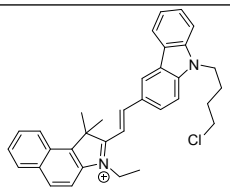
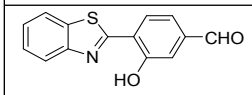
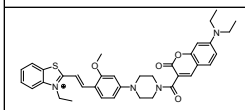
Synthesis of Compound 5 (Donor)

To a mixture of $NHEt_2$ (0.5 g, 6.8 mmol), dry Et_3N (1.0 mL) and dry CH_2Cl_2 (5 mL) in ice bath, 7-(diethylamino)-2-oxo-2H-chromene-3-carbonyl chloride (2.79 g) dissolved in dry CH_2Cl_2 (10 mL) was added dropwise over a period of 30 min. Then the mixture was stirred at room temperature for 1 h. The mixture was washed with water. The organic layer was concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (petroleum ether: ethyl acetate = 5: 1) to afford compound **5** (1.05 g, 93.5 %), a greenish yellow solid.

3. Table

Table S1 Summary of fluorescence probes for HSO_3^-/SO_3^{2-}

probe	Detection	λ_{ex} (nm)	λ_{em} (nm)	Detection system	Detection limit (M)	Cell imaging	Ref.
	HSO_3^-	440, 558	**494, 593**	HEPES buffer (10% DMSO)	**8.6×10^{-7}**	ratiometric	S1
	SO_3^{2-}	305	**453/578**	PBS buffer (40% DMF)	**2.2×10^{-7}**	ratiometric	S3
	HSO_3^-,SO_3^{2-}	410	**530/582**	PBS buffer (30% DMF)	**1×10^{-7}**	ratiometric	S4
	HSO_3^-	415, 800	**458/605 460/622**	PBS buffer (CTAB 1 mM)	**5.3×10^{-8}**	ratiometric	S5
	HSO_3^-	350	**490/590**	PBS buffer (30% DMF)	**1.5×10^{-7}**	ratiometric	S6
	HSO_3^-	445	**478/633**	PBS buffer (30% DMF)	**3.8×10^{-7}**	ratiometric	S7

	HSO_3^-, H_2S	410	**460/590**	PBS buffer (CTAB 1 mM)	**1×10^{-7}**	ratiometric	S8
	HSO_3^-, SO_3^{2-}	500	**560/717**	PBS buffer (10% DMF)	**8.7×10^{-8}**	ratiometric	S9
	HSO_3^-	322	**462/588**	PBS buffer (10% EtOH)	**1×10^{-8}**	ratiometric	S10
	HSO_3^-, SO_3^{2-}	340	**467/563**	PBS buffer (1mM CTAB)	**3.3×10^{-7}**	ratiometric	S12
	HSO_3^-, SO_3^{2-}	390	**545/637**	PBS buffer (30% DMF)	**6.6×10^{-8}**	ratiometric	This work

4. Spectra

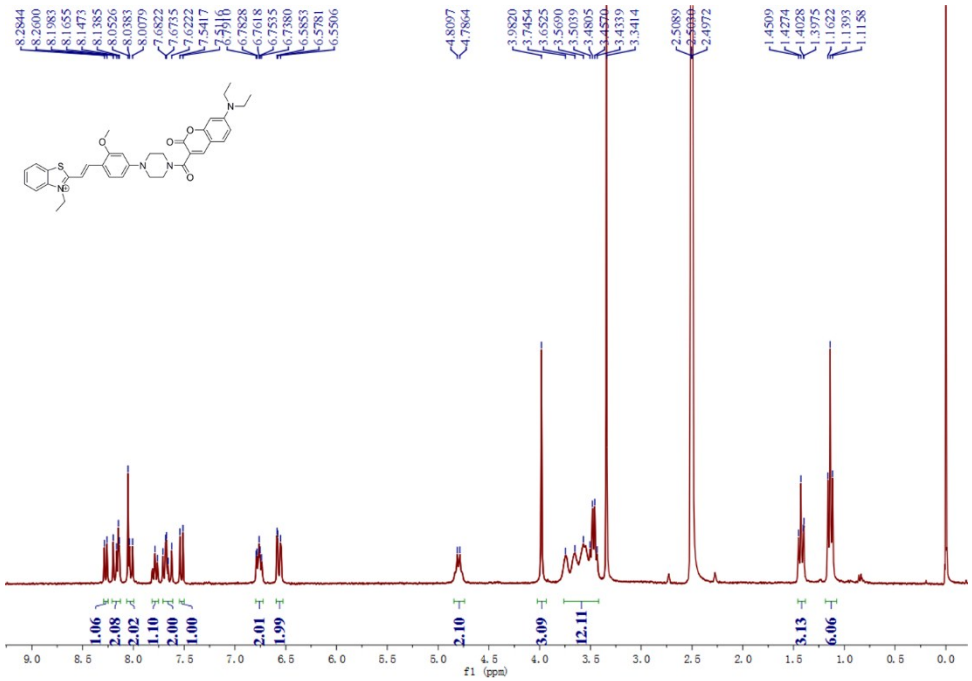


Fig. S1. ^{1}H NMR of RBC in DMSO-d_6

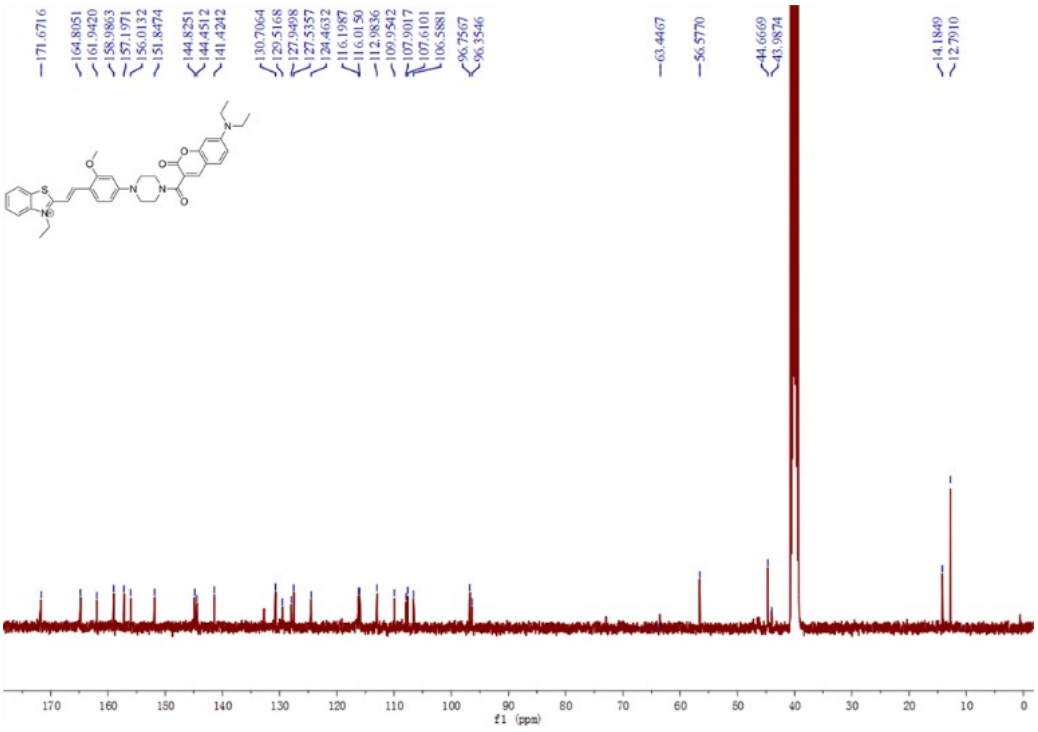


Fig. S2. ^{13}C NMR of RBC in DMSO-d_6

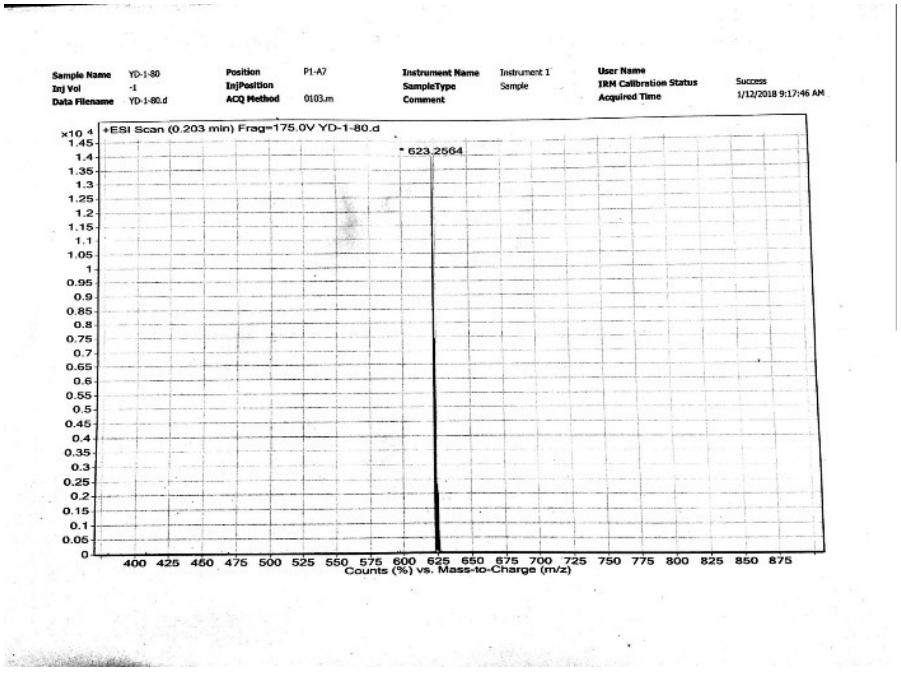


Fig. S3. MS of RBC

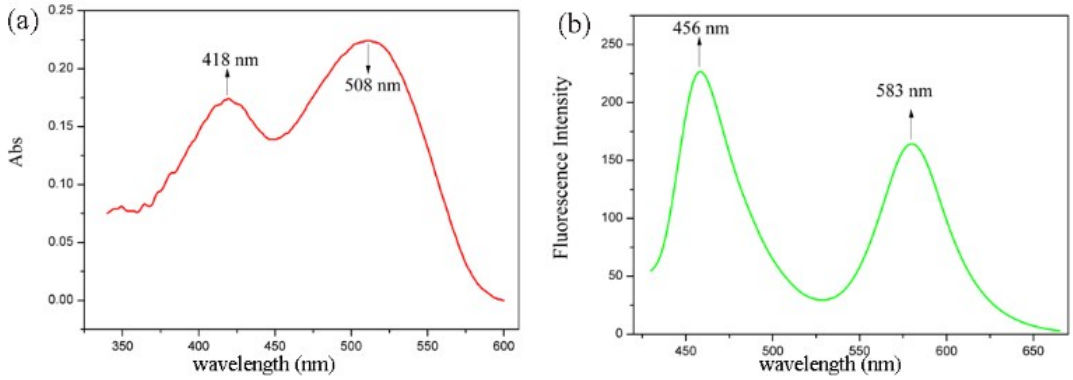


Fig. S4. (a) Absorption and (b) fluorescence spectrum of probe RBC (5 μM) in PBS buffer (pH = 7.38, 10 mM containing 30% DMF v: v). (λex = 380 nm, slit: 15 nm/9 nm).

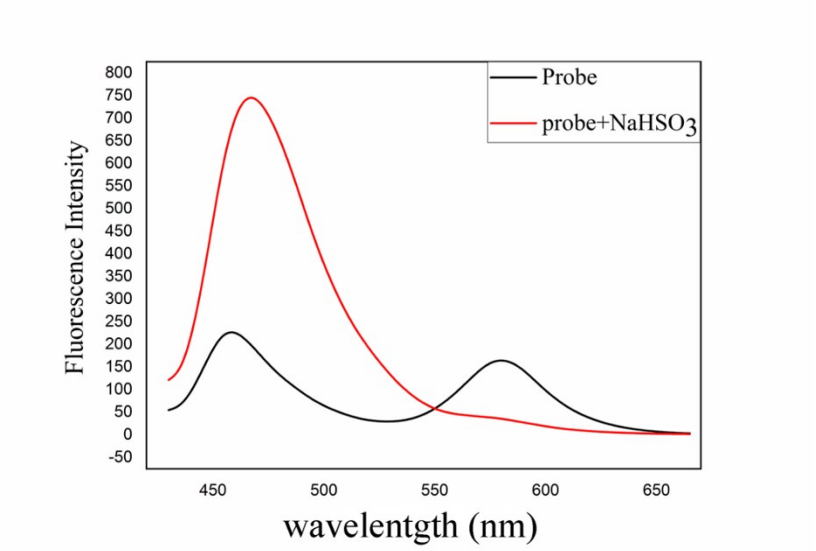


Fig. S5. Fluorescence of probe RBC (5 μM) in the absence (black line) or presence (red line) of bisulfite (6 equiv.).

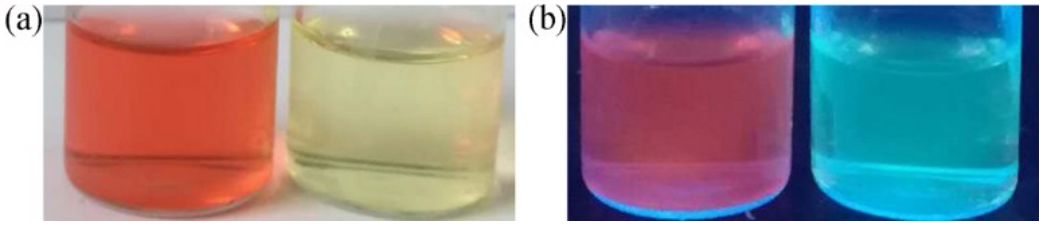


Fig. S6. A visual fluorescence change photograph of probe RBC (5 μM) in the absence or presence of bisulfite (6 equiv.). (a) under light, (b) under illumination using a 365 nm UV lamp.

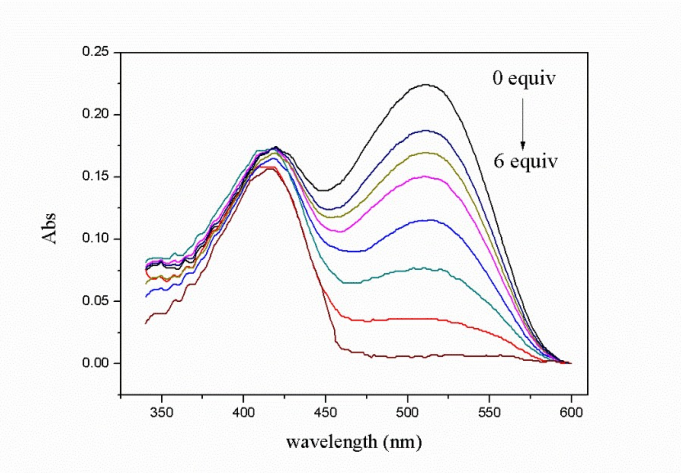


Fig. S7. UV-Vis absorption spectra of RBC (5 μM) in the presence of different amounts of $NaHSO_3$ (0-6 equiv.) in PBS (pH = 7.38, 10 mM, containing 30% DMF) in 5 min.

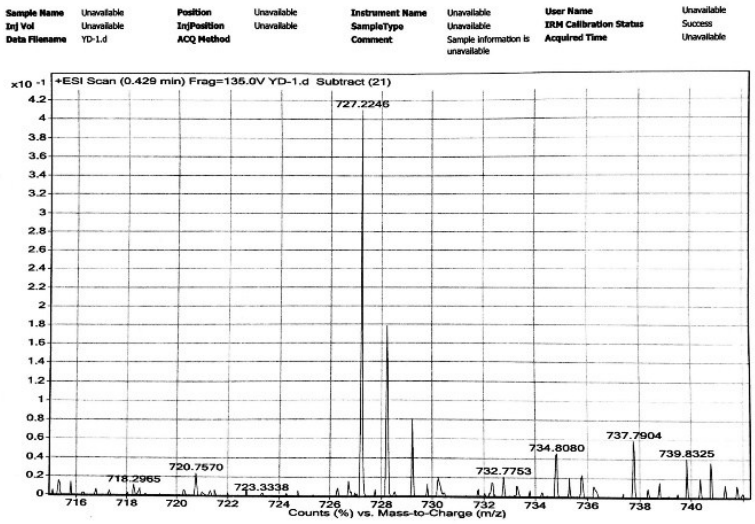


Fig. S8. MS spectrum of probe RBC (5 μM) in the presence of $NaHSO_3$ (6 equiv.) in PBS (pH = 7.38, containing 30% DMF v: v).

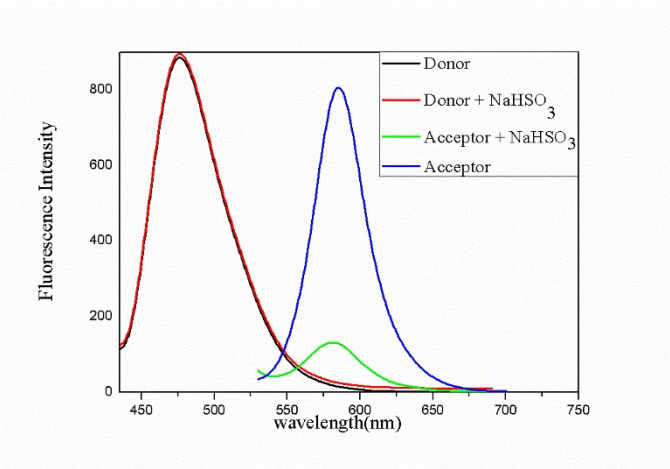


Fig. S9. Fluorescence emission of Donor (5 μM) and Acceptor (5 μM) in the absence and presence of $NaHSO_3$ (6 equiv.) in PBS (pH = 7.38, 10 mM, containing 30% DMF v: v). λex = 380 nm, slit: 15 nm/4 nm (donor), 15 nm/15 nm (acceptor).

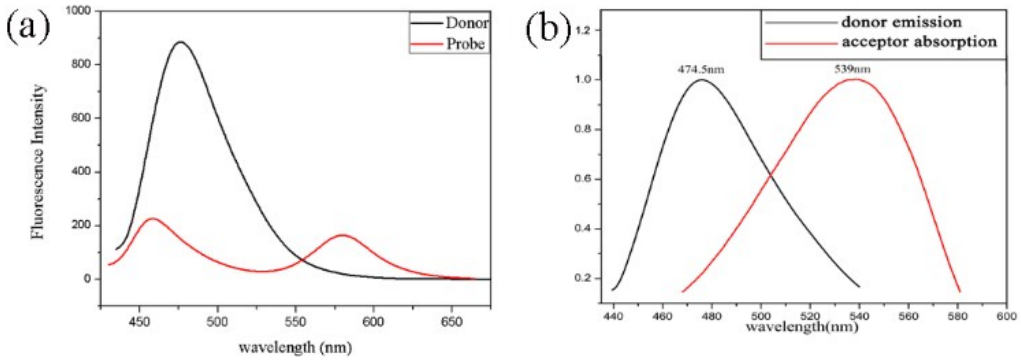


Fig. S10. (a) The fluorescence emission spectra of probe RBC (5 μM) (the red line) and the donor (5 μM) (the black line) in PBS (pH = 7.38, 10 mM, containing 30% DMF, v: v); λex = 380 nm (slit widths: 15 nm/9 nm); (b) The normalized absorption spectra of the acceptor (5 μM) in PBS (pH = 7.38, 10 mM, containing 30% DMF, v: v); and the normalized fluorescence spectra of the donor (5 μM) in PBS (pH = 7.38, 10 mM, containing 30% DMF, v: v);, λex = 380 nm (slit widths: 15 nm/6 nm).

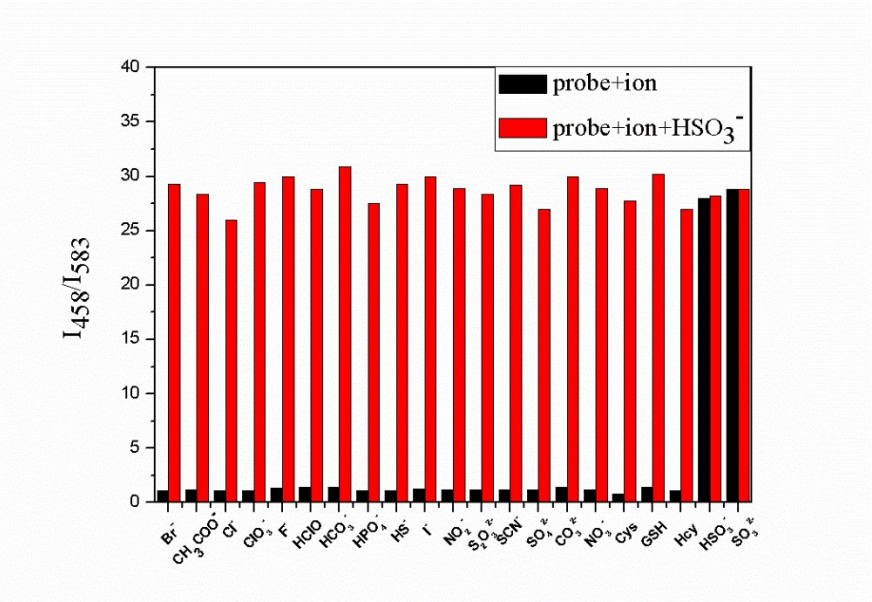


Fig. S11. Ratiometric responses (I_{456}/ I_{583}) of RBC (5 μM) with or without HSO_3^- (100 μM) in the presence of various analytes (100 μM) in PBS (pH = 7.38, 10 mM, containing 30% DMF, v: v). λ_{ex} = 380 nm, slit: 15.0/8.0 nm).

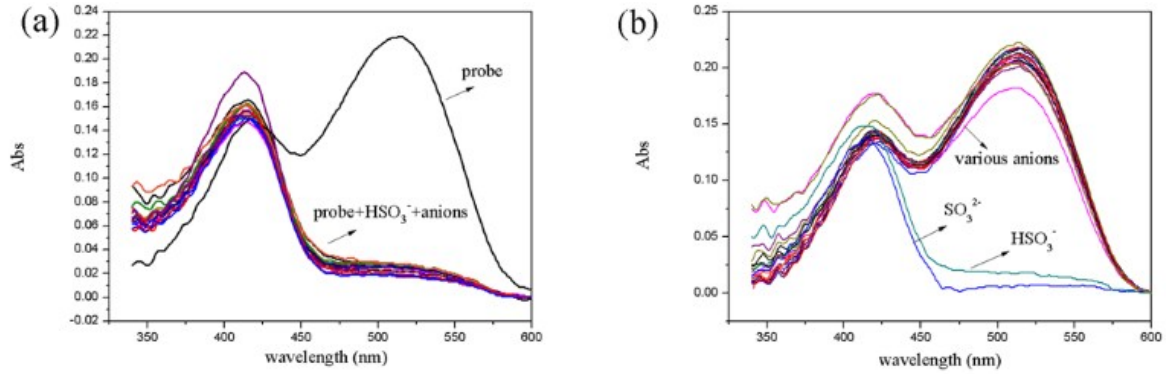


Fig. S12. UV-Vis absorption spectra of probe RBC (5 μM) with or without HSO_3^- (100 μM) and various anions (100 μM) in PBS (pH = 7.38, 10 mM, containing 30% DMF, v: v).

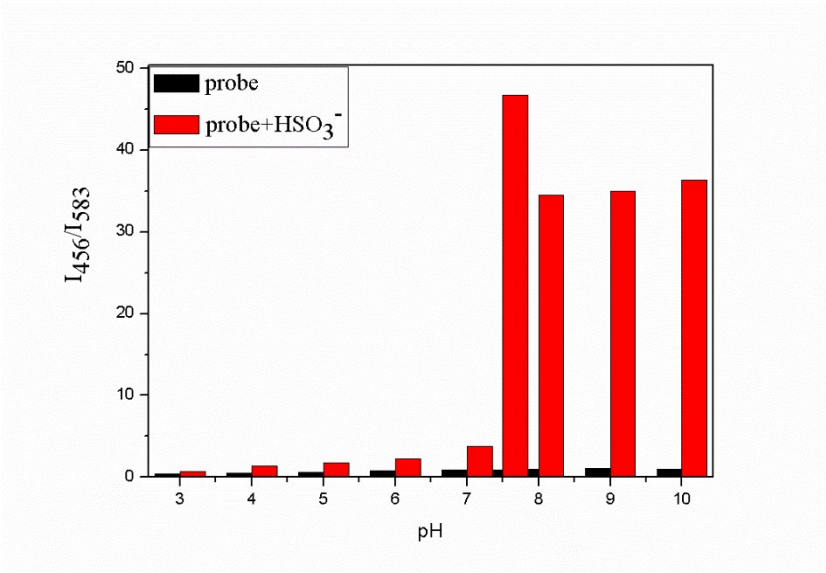


Fig. S13. Effect of pH values on RBC (5 μM) in absence (black column) or presence (red column) of bisulfite (6 equiv.). All data were acquired in PBS containing 30% DMF in 5 min. λex = 380 nm, slits: 15 nm/9 nm.

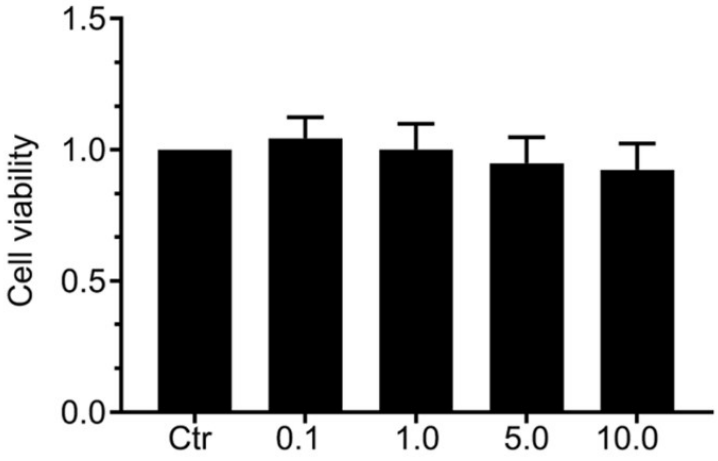


Fig. S14. Cells viability by a standard SRB assay. HeLa cells were incubated with RBC (0, 0.1, 1, 5 and 10 μM) for 6 h.

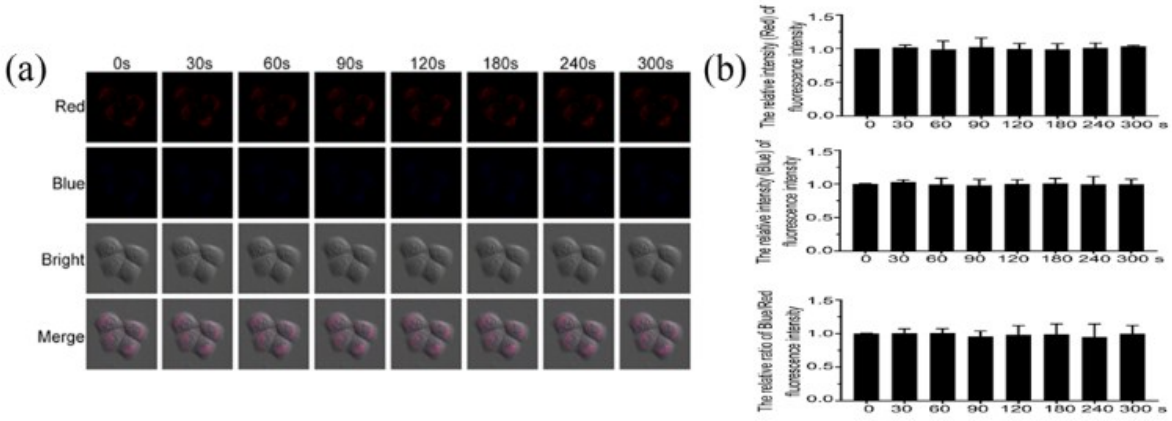


Fig. S15. (a) Photostability of RBC for fluorescence images of HeLa cells. The cells were incubated with RBC (1 μM) for 1 h beforehand. First line: fluorescence images from the red channel (610-700 nm); second line: fluorescence images at the blue channel (450-610 nm); third line: bright field images; fourth line: overlay images of the first, second and third lines. λ_{ex} = 405 nm. (b) The relative ratio of blue/red fluorescence intensity in correspondence with (a).

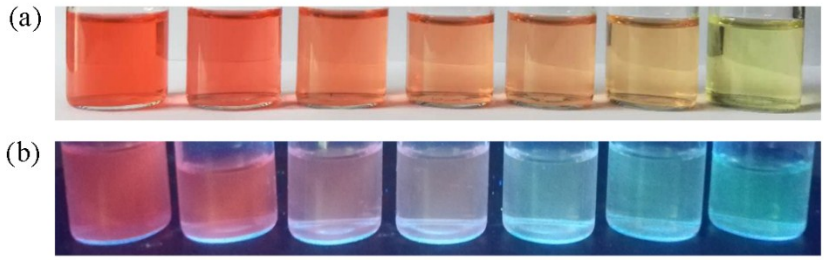


Fig. S16. (a) Photograph shows RBC in different concentrations of $NaHSO_3$ under light. (b) Photograph shows RBC in different concentrations of $NaHSO_3$ under 365 nm UV lamp.

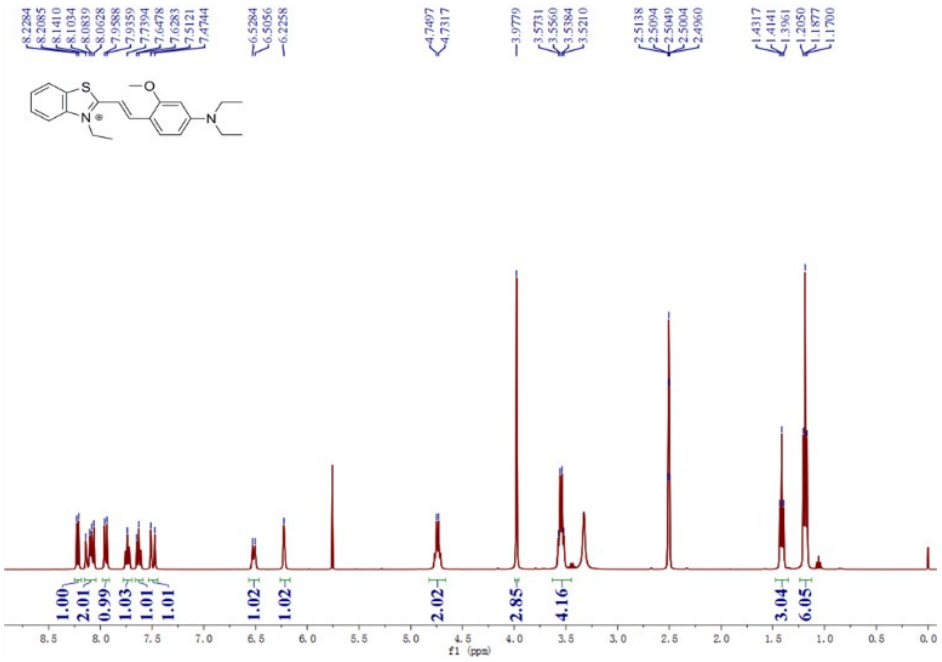


Fig. S17. ^{1}H NMR of Compound **4** in DMSO-d$_{6}$

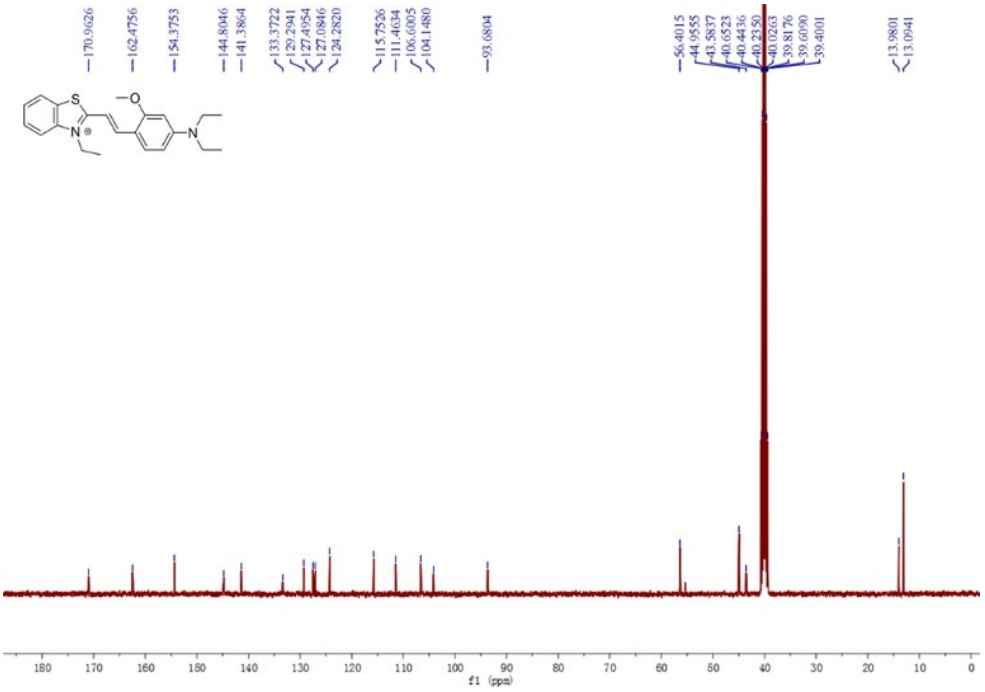


Fig. S18. ^{13}C NMR of Compound **4** in DMSO-d$_{6}$