Supplementary information

A ratiometric fluorescent probe for rapid, sensitive and selective detection of sulfur dioxide with large Stokes shifts by single wavelength excitation

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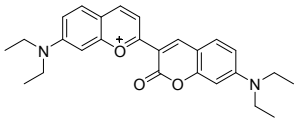
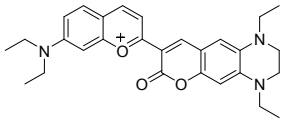
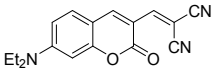
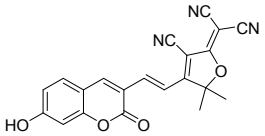
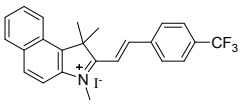
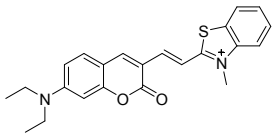
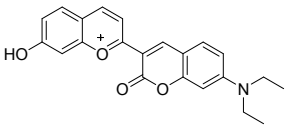
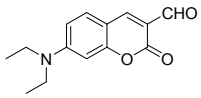
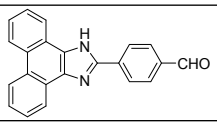
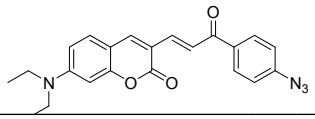
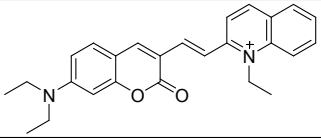
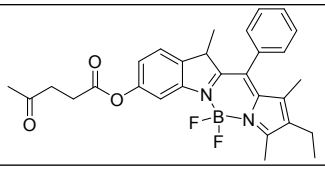
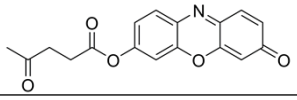
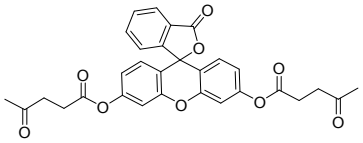
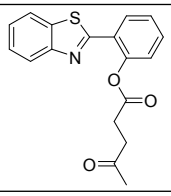
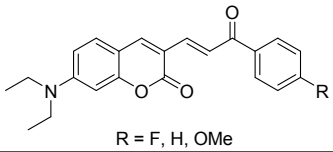
Contents

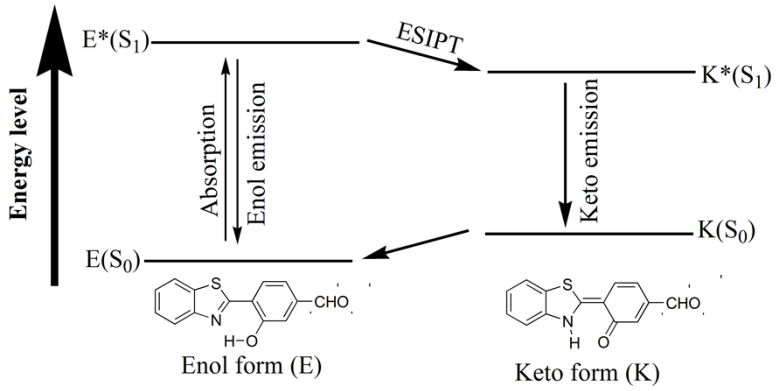
Table S1 The chemical structures and response times of the representative fluorescent probes for SO_3^{2-}/HSO_3^-.

Probe structure	Response time	Type	Reference
	< 10 s	Ratiometric	RSC Adv., 2015,5, 25409-25415
	< 15 s	Off-on	Anal. Chem., 2015, 87, 609-616
	30 s	Ratiometric	Analyst, 2013, 138, 3018-3025
	90 s	Ratiometric	Chem. Commun. 2014, 50, 183-185
	90 s	Ratiometric	J. Agric. Food Chem. 2014, 62, 3405-3409
	5 min	Ratiometric	Chem. Commun. 2013, 49, 2637-2639
	5 min	Ratiometric	Sensor. Actuat. B-Chem., 2015, 206, 268-275
	6 min	Off-on	J. Mater. Chem. B, 2013, 1, 4110-4114
	10 min	Ratiometric	Sensor. Actuat. B-Chem., 2013, 184, 274-280
	10 min	Ratiometric	Analyst, 2014, 139, 3373-3377
	15 min	Ratiometric	Org. Biomol. Chem., 2014, 12, 4637-4643

	20 min	Ratiometric	J. Agric. Food Chem., 2011, 59, 11935-11939
	20 min	Off-on	Org. Lett., 2010, 12, 5624-5627
	25 min	Off-on	Sensor. Actuat. B-Chem., 188 (2013) 1196-1200
	60 min	Ratiometric	RSC Adv., 2012, 2, 10869-10873
 R = F, H, OMe	60 min	Ratiometric	Anal. Chim. Acta., 2013, 788, 165-170

Fluorescence properties of Probe 1 and dye 2

We thoroughly studied the fluorescence properties of Probe **1** and its analogue dye **2**. Toluene, acetonitrile, dichloromethane and ethanol were used as solvents for the fluorescence spectrum measurements. As seen in Fig. S1, dye **2** exhibit the fluorescence at shorter wavelength in polar solvents (acetonitrile and ethanol) which is from local excited states whereas the Keto emission at longer wavelength from ESIPT state was observed in less polar solvents (dichloromethane and toluene). With regard to Probe **1**, the Keto emission dominated in all the solvents (Fig. S2), which is attributed to the electron-withdrawing effect by the aldehyde group.



Scheme S1 Schematic representation of the excitation and emission mechanism with the ESIPT photocycle.

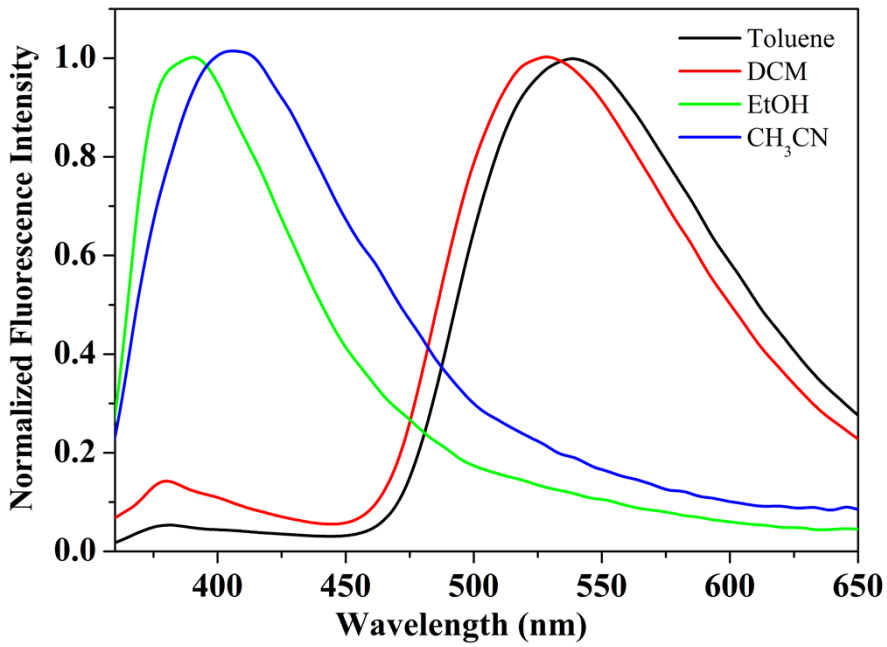


Fig. S1 Normalized fluorescence spectra of dye **2** in toluene, DCM, EtOH and CH_3CN.

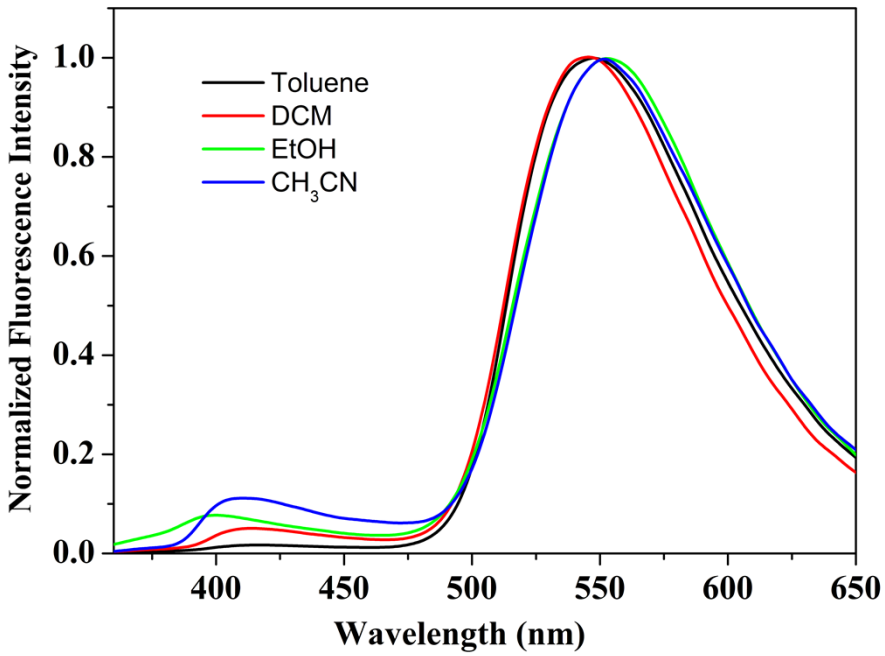


Fig. S2. Normalized fluorescence spectra of Probe **1** in toluene, DCM, EtOH and CH_3CN.

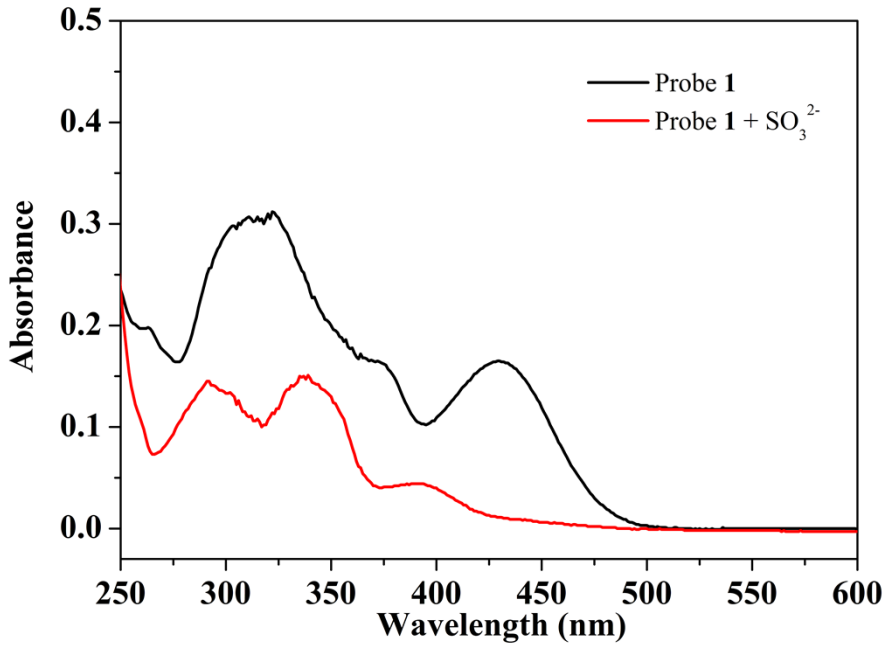
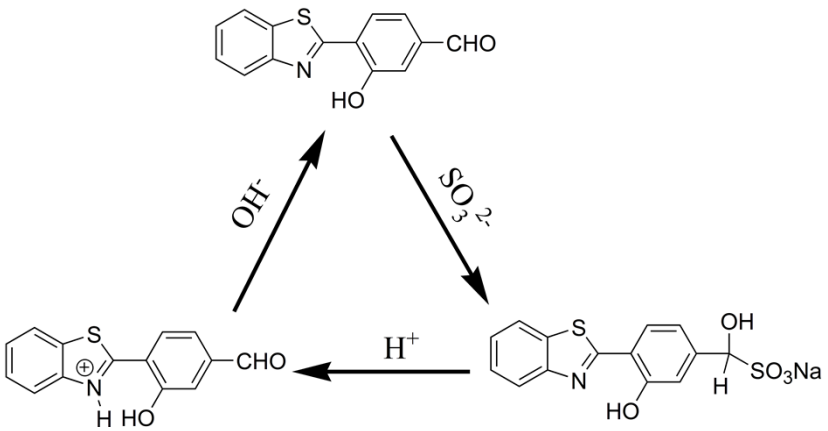


Fig. S3 Absorption spectral change of Probe **1** (5.0 μM) in the absence/presence of SO_3^{2-} (400.0 equiv.) in PBS buffer (pH 7.4, 10.0 mM, 1.0 mM CTAB).

Reaction reversibility study

It has been reported that the reaction of aldehyde group with SO_3^{2-}/HSO_3^{2-} is a reversible reaction， which is influenced by acid/alkali and is often used as an effective method to purify aldehydes by crystallization as their bisulfite adducts and then regeneration of the aldehyde.[1] The reversible process of the reaction between Probe **1** with SO_3^{2-} was shown in scheme S2. In order to further understand this reversible reaction, we investigate the fluorescence of this probe with SO_3^{2-} under acid and basic conditions. Upon the addition of hydrochloric acid to the solution of Probe **1** with SO_3^{2-}, the fluorescence at 467 nm gradually decreased. However, the fluorescence around 563 nm was not observed, which is probably ascribed the protonation of the nitrogen atom in benzothiazol moiety of the reaction product, **5**, in strong acidic condition. Then, the subsequent addition of NaOH aqueous solution to the foregoing solution, the emission peak around 563 nm corresponding to the fluorescence of Probe **1** appeared and the intensity was gradually enhanced. When the excess of base was added, the solution exhibit a fluorescence with a maximum at 467 nm which finally became the dominant signal, indicating the recovery

of the initial state (Probe **1** with SO_3^{2-}) (Fig. S13-S15). These above results clearly demonstrated the reversibility of the reaction of Probe **1** with SO_3^{2-}.



Scheme S2 The reversible process of the reaction of Probe **1** with SO_3^{2-}.

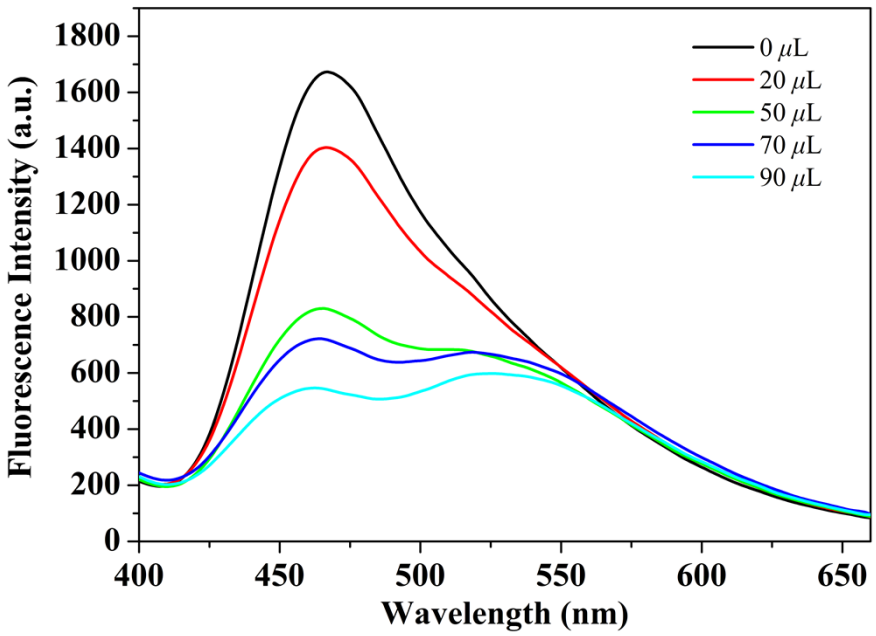


Fig. S4 Fluorescence spectra of Probe **1** (5.0 μM) with SO_3^{2-} (400.0 equiv.) upon the addition of hydrochloric acid (12 mol/L, 0.0 – 90.0 μL) in PBS buffer (pH 7.4, 10.0 mM) with 1.0 mM CTAB.

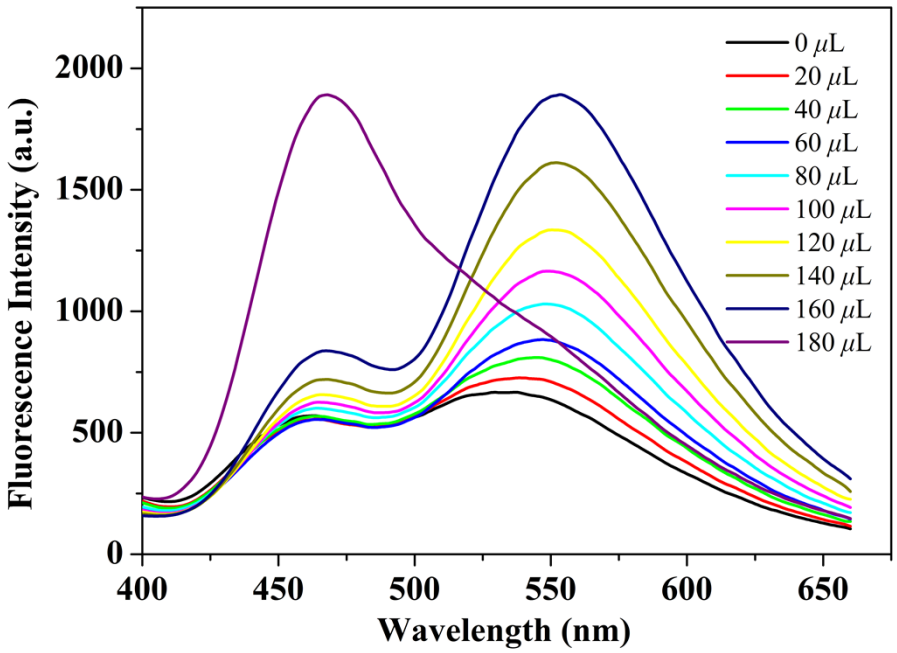


Fig. S5 Fluorescence spectra of Probe **1** (5.0 μM) with SO_3^{2-} (400.0 equiv.) and hydrochloric acid (12 mol/L, 70.0 μL) upon the addition of NaOH aqueous solution (6 mol/L, 0.0 – 180.0 μL) in PBS buffer (pH 7.4, 10.0 mM) with 1.0 mM CTAB.

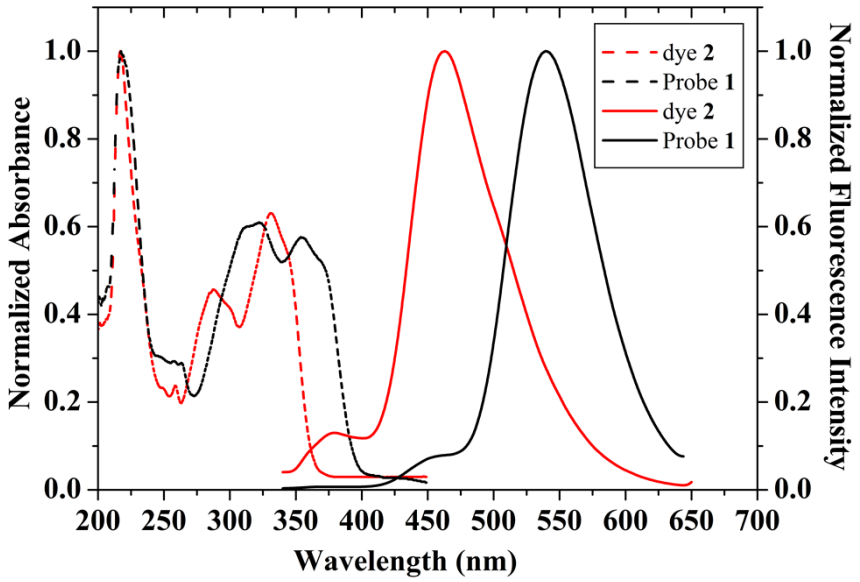


Fig. S6 The normalized absorption (dashed lines) and emission spectra (solid lines) of Probe **1** (black lines) and dye **2** (red lines) in HEPES buffer (pH 7.4, 20.0 mM, containing 30%CH_3CN).

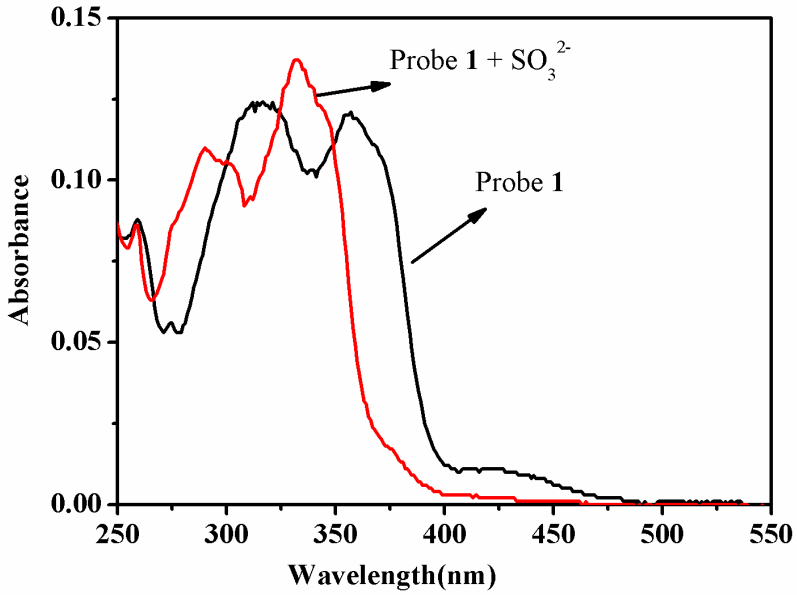


Fig. S7 Absorption spectral change of Probe **1** (5.0 μM) in the absence/presence of SO_3^{2-} (200.0 equiv.) in HEPES buffer (pH 7.4, 20.0 mM, containing 30% CH_3CN).

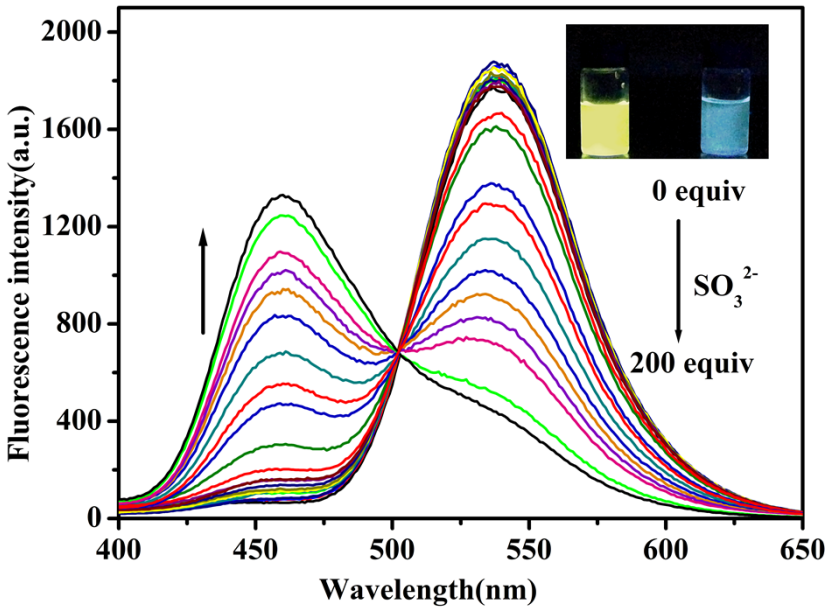


Fig. S8 Fluorescence spectra of Probe **1** (5.0 μM) upon the addition of SO_3^{2-} (0.0 − 200.0 equiv.) in HEPES buffer (pH 7.4, 20.0 mM, containing 30% CH_3CN). Inset: Photograph of Probe **1** under the irradiation at 365 nm (left: Probe **1** only, right: Probe **1** with SO_3^{2-}).

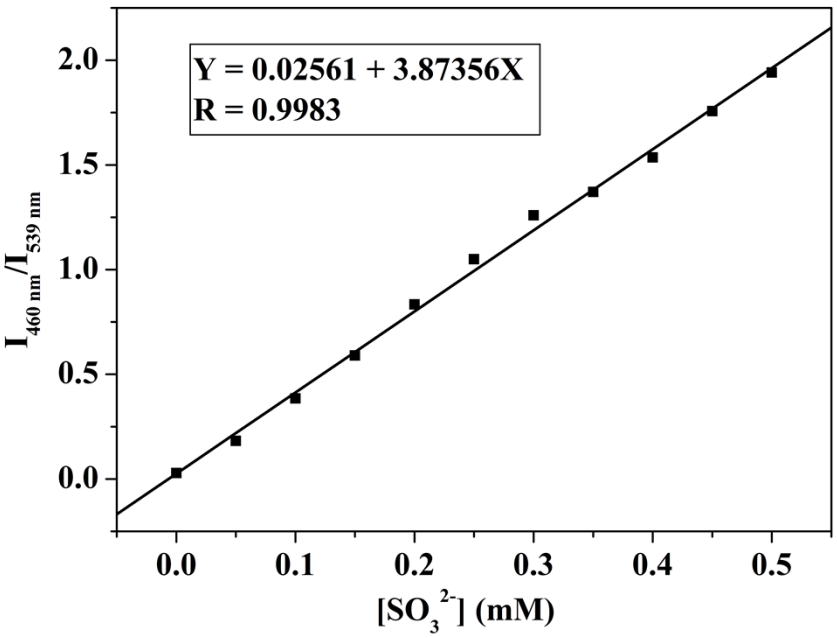


Fig. S9 The linear relationship between the ratio of $I_{460\ nm}/I_{539\ nm}$ for Probe **1** (5.0 μM) upon the addition of SO_3^{2-} (0.0 − 0.5 mM).

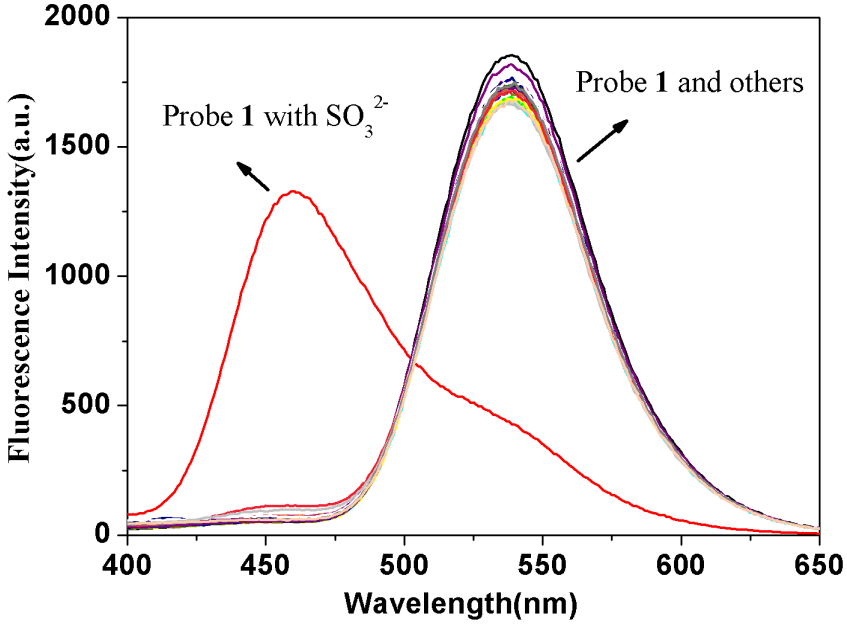


Fig. S10 Fluorescence spectra of Probe **1** (5.0 μM) upon the addition of various species in HEPES buffer (pH 7.4, 20.0 mM, containing 30% CH_3CN), including Na_2SO_3, NaF, NaCl, NaBr, NaI, $NaNO_3$, $NaNO_2$, CH_3COONa, NaN_3, Na_2SO_4, $Na_2S_2O_3$, NaSCN, Na_3PO_4, Na_2S, Na_2CO_3, Cys, Hcy, GSH, H_2O_2, NaClO (200.0 equiv. for each).

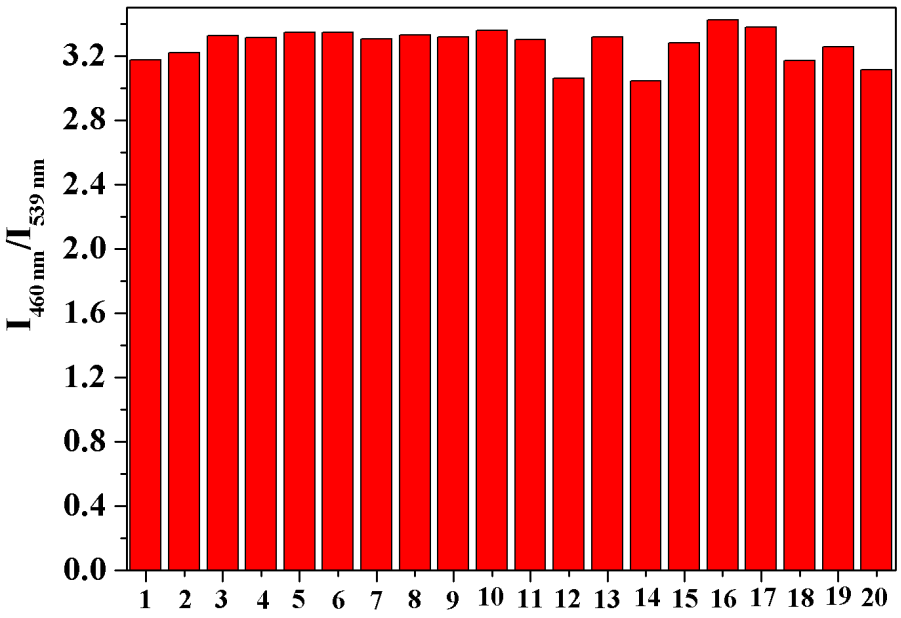


Fig. S11 Fluorescence intensity ratio ($I_{460\ nm}/I_{539\ nm}$) of Probe **1** (5.0 μM) with SO_3^{2-} (200.0 equiv.) in the coexistence of 200.0 equiv. of various species in HEPES buffer (pH 7.4, 20.0 mM, containing 30% CH_3CN). **1**, free; **2**, NaF; **3**, NaCl; **4**, NaBr; **5**, NaI; **6**, $NaNO_3$; **7**, $NaNO_2$; **8**, CH_3COONa; **9**, NaN_3; **10**, Na_2SO_4; **11**, $Na_2S_2O_3$; **12**, NaSCN ; **13**, Na_3PO_4; **14**, Na_2S; **15**, Na_2CO_3; **16**, Cys; **17**, Hcy; **18**, GSH; **19**, H_2O_2; **20**, NaClO.

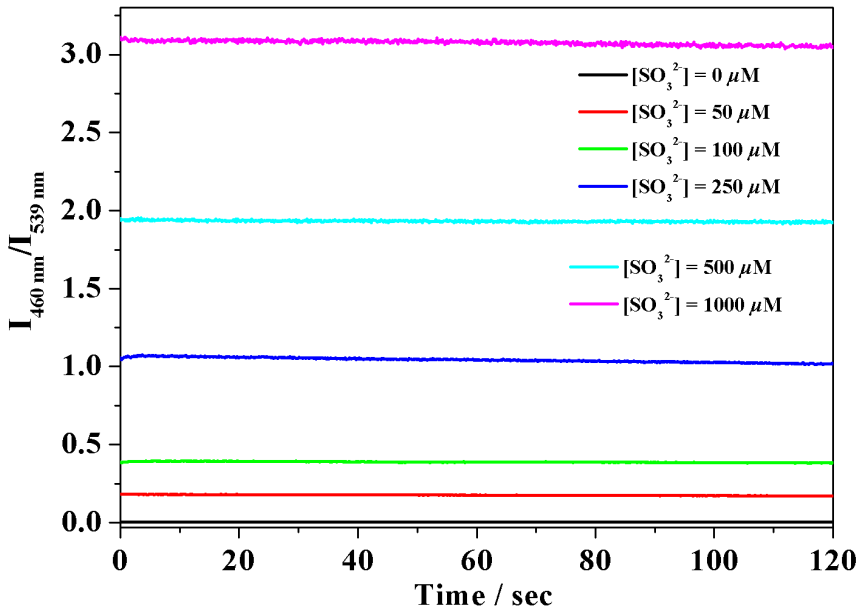


Fig. S12 Kinetics of $I_{460\ nm}/I_{539\ nm}$ for Probe **1** (5.0 μM) in the absence/presence of SO_3^{2-}.

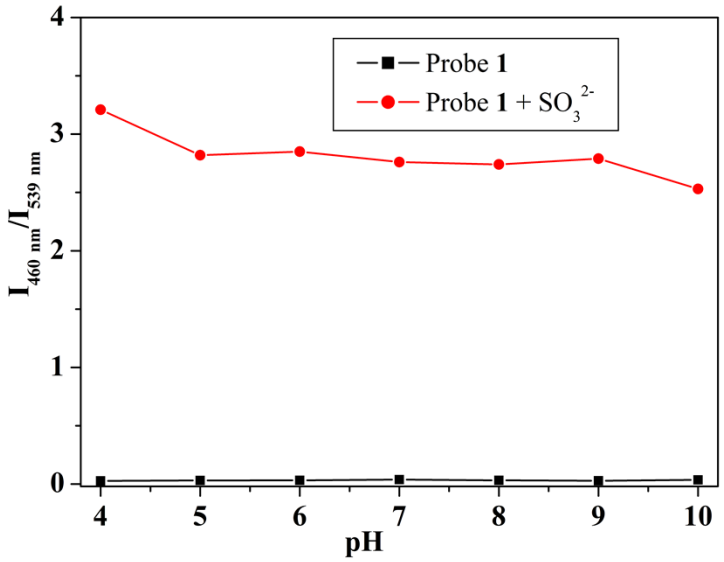


Fig. S13 The fluorescence intensity ratio $I_{460\ nm}/I_{539\ nm}$ of Probe **1** (5.0 μM) in the absence and presence of SO_3^{2-} (200.0 equiv.) at different pH values from 4.0 to 10.0.

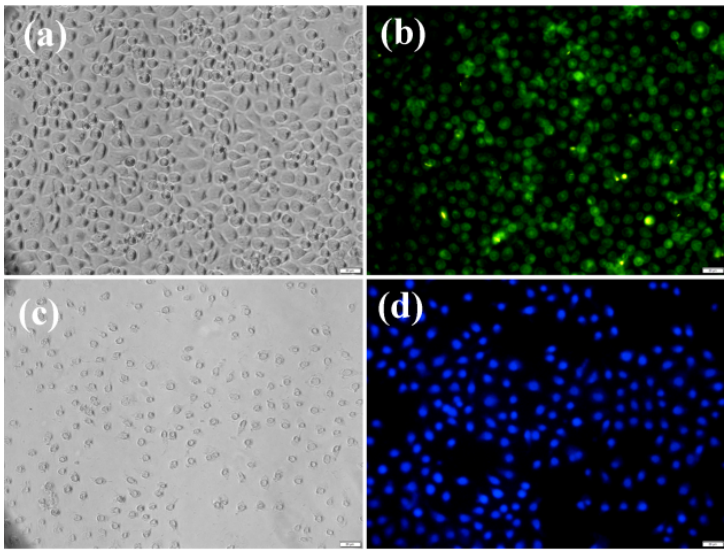


Fig. S14 (a) Bright-field and (b) fluorescence images of living HNE-2 cells incubated with Probe **1** for 30 min at 37 °C **(**5.0 μM) in PBS buffer (pH 7.4, 10.0 mM, containing 10% DMSO); (c) Bright-field and (d) fluorescence images of living HNE-2 cells firstly incubated with Probe **1** (5.0 μM**)** at 37 °C for 30 min in PBS buffer (pH 7.4, 10.0 mM, containing 10% DMSO) and then treated with SO_3^{2-} (1.0 mM) at 37 °C for another 30 min in PBS buffer (pH 7.4, 10.0 mM).

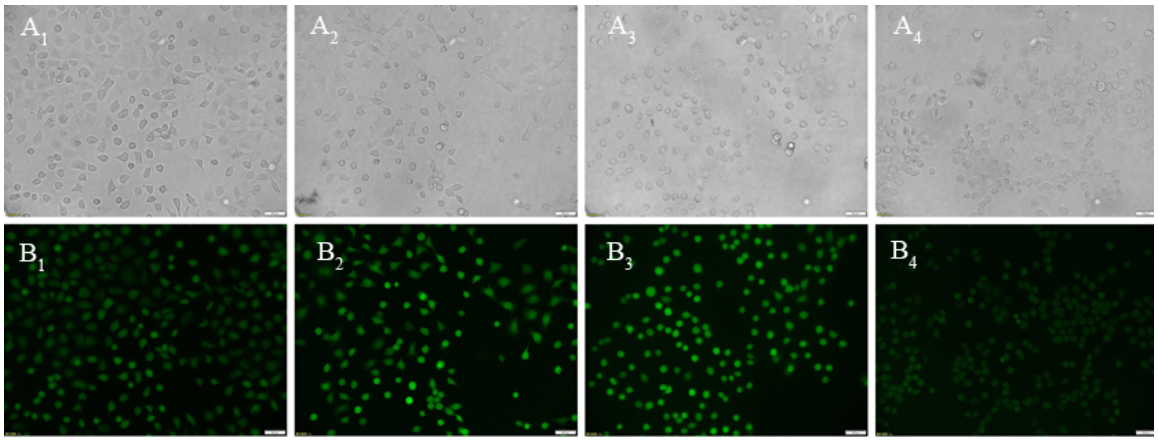


Fig. S15 Fluorescence images of living HNE-2 cells. Bright-field (A1-A4) and fluorescence images (B1-B4) of living HNE-2 cells firstly incubated with PBS buffer (pH 7.4, 10.0 mM) containing different concentration of DMSO (from left to right: 0%(1), 2%(2), 5%(3), 10%(4)) at 37 C for 1 h and then treated with fluorescein diacetate (100 μg/mL) at 37 °C for another 5 min.

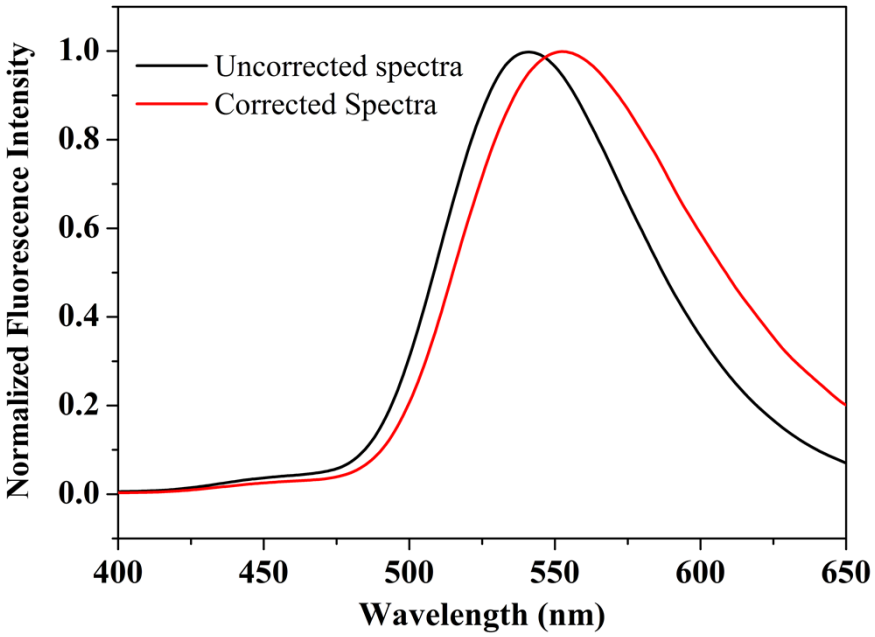


Fig. S16 Normalized corrected and uncorrected fluorescence spectra of Probe **1** in HEPES buffer (pH 7.4, 20.0 mM, containing 30% CH_3CN).

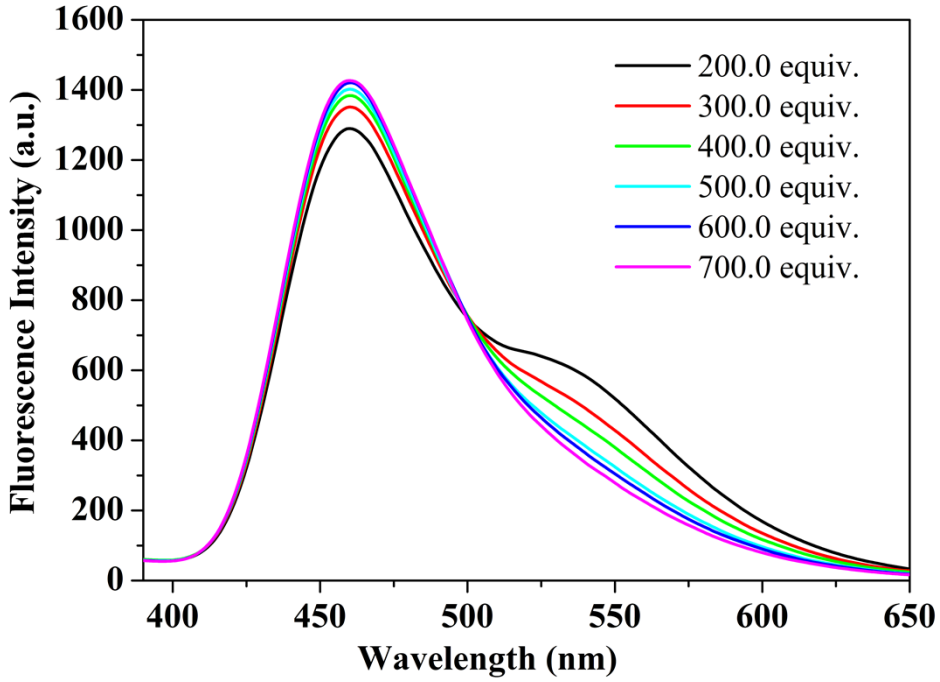


Fig. S17 Fluorescence spectra of Probe **1** (5.0 μM) upon the addition of SO_3^{2-} (200.0 − 700.0 equiv.) in HEPES buffer (pH 7.4, 20.0 mM, containing 30% CH_3CN).

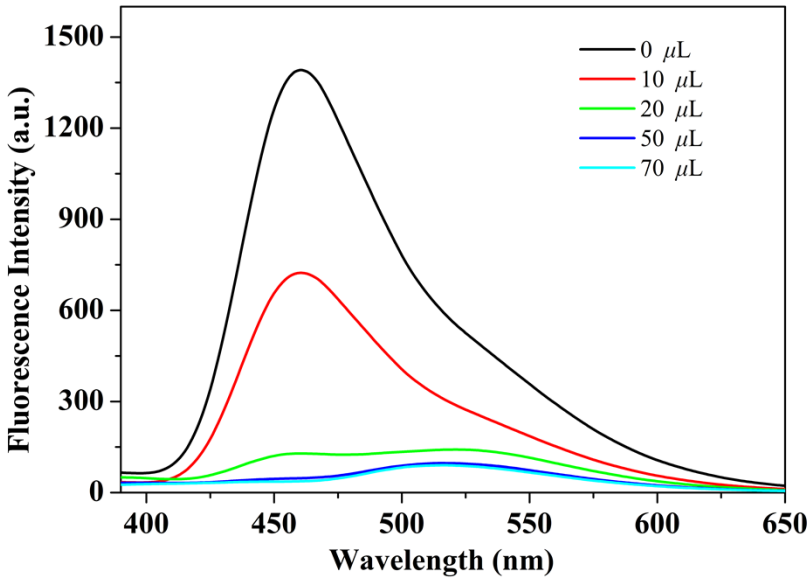


Fig. S18 Fluorescence spectra of Probe **1** (5.0 μM) with SO_3^{2-} (700.0 equiv.) upon addition of hydrochloric acid (12 mol/L, 0.0 – 70.0 μL) in HEPES buffer (pH 7.4, 20.0 mM, containing 30% CH_3CN).

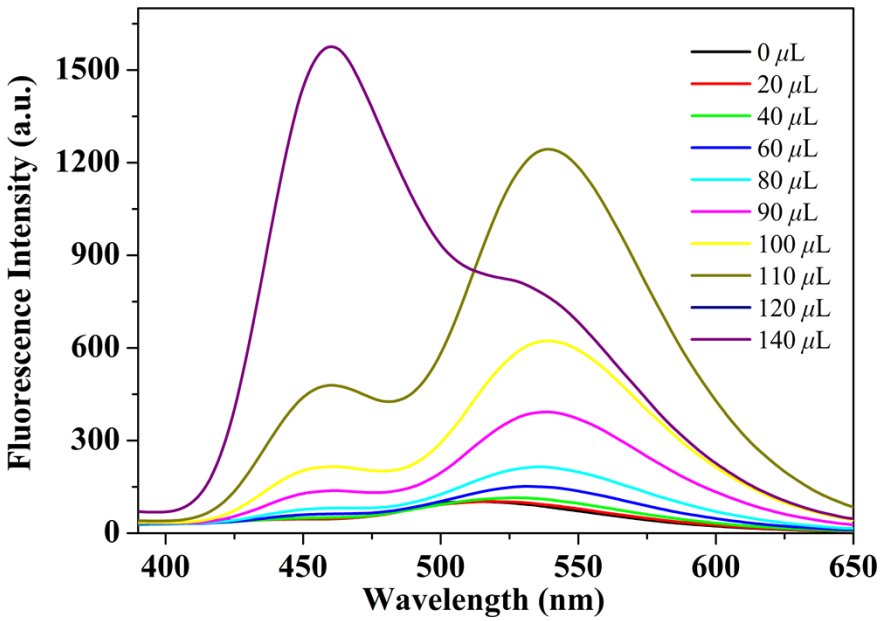


Fig. S19 Fluorescence spectra of Probe **1** (5.0 μM) with SO_3^{2-} (700.0 equiv.) and hydrochloric acid (12 mol/L, 70.0 μL) upon addition of NaOH aqueous solution (6 mol/L, 0.0 – 140.0 μL) in HEPES buffer (pH 7.4, 20.0 mM, containing 30% CH_3CN).

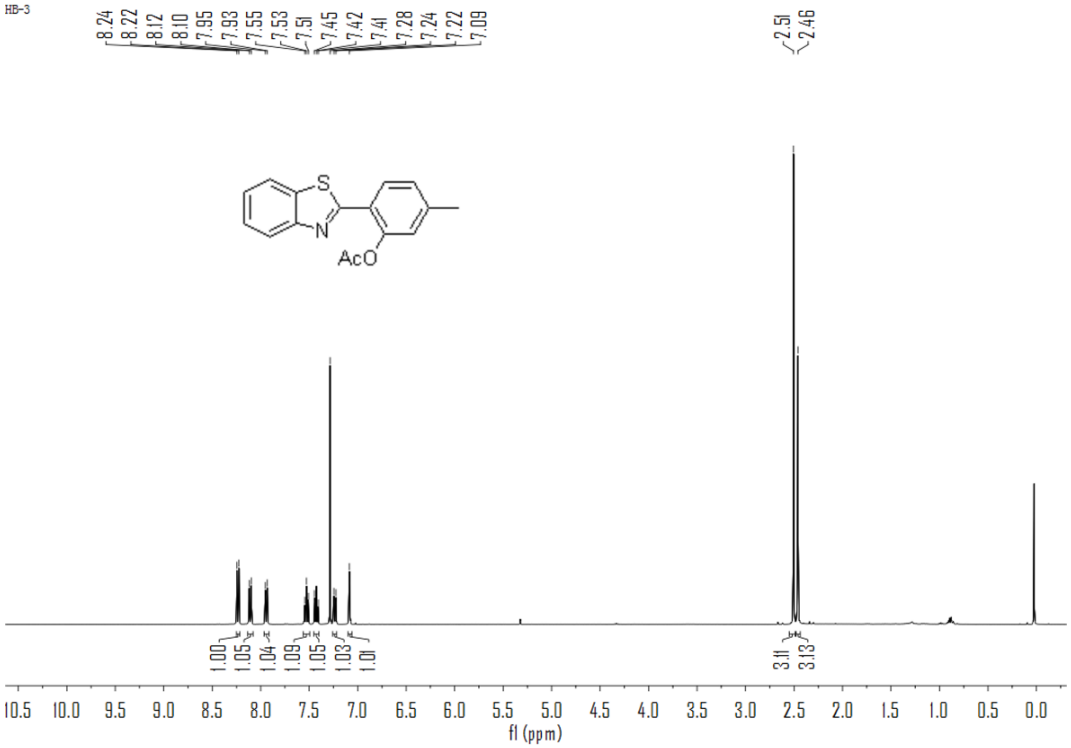


Fig. S20 ^{1}H NMR spectrum of compound **3**.

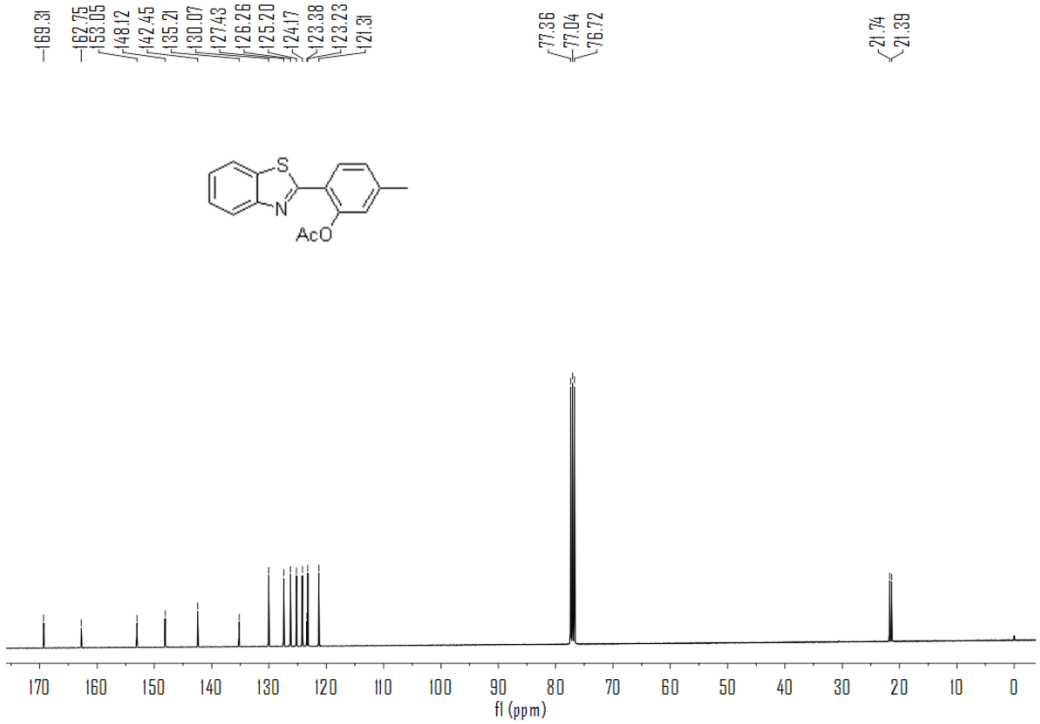


Fig. S21 ^{13}C NMR spectrum of compound **3**.

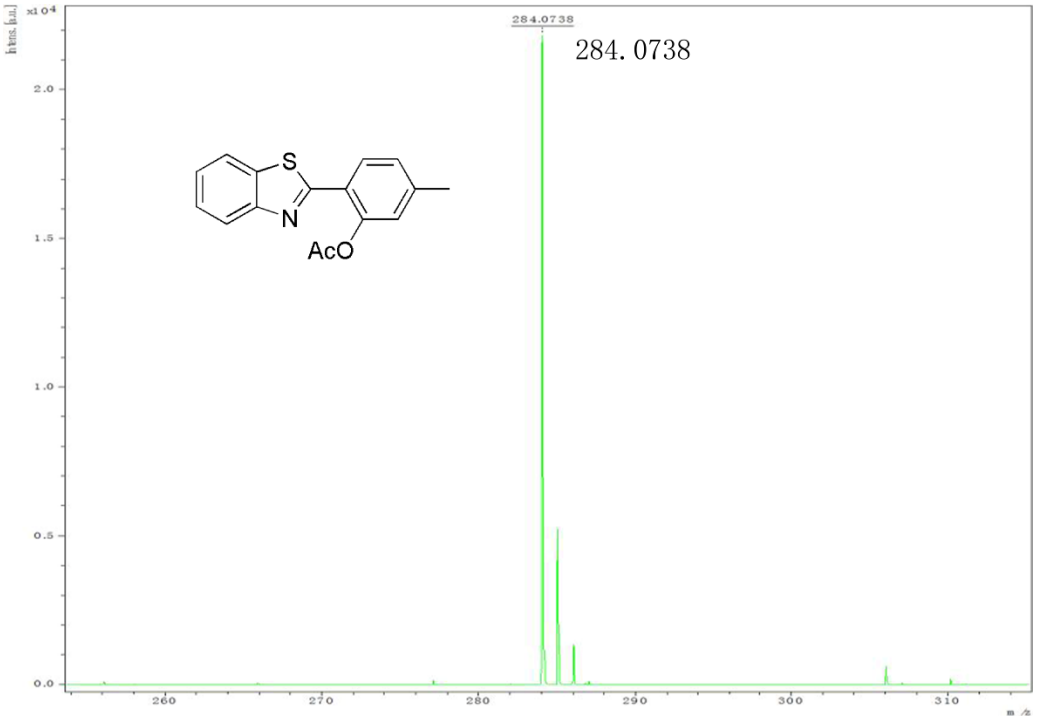


Fig. S22 HRMS spectrum of compound **3**.

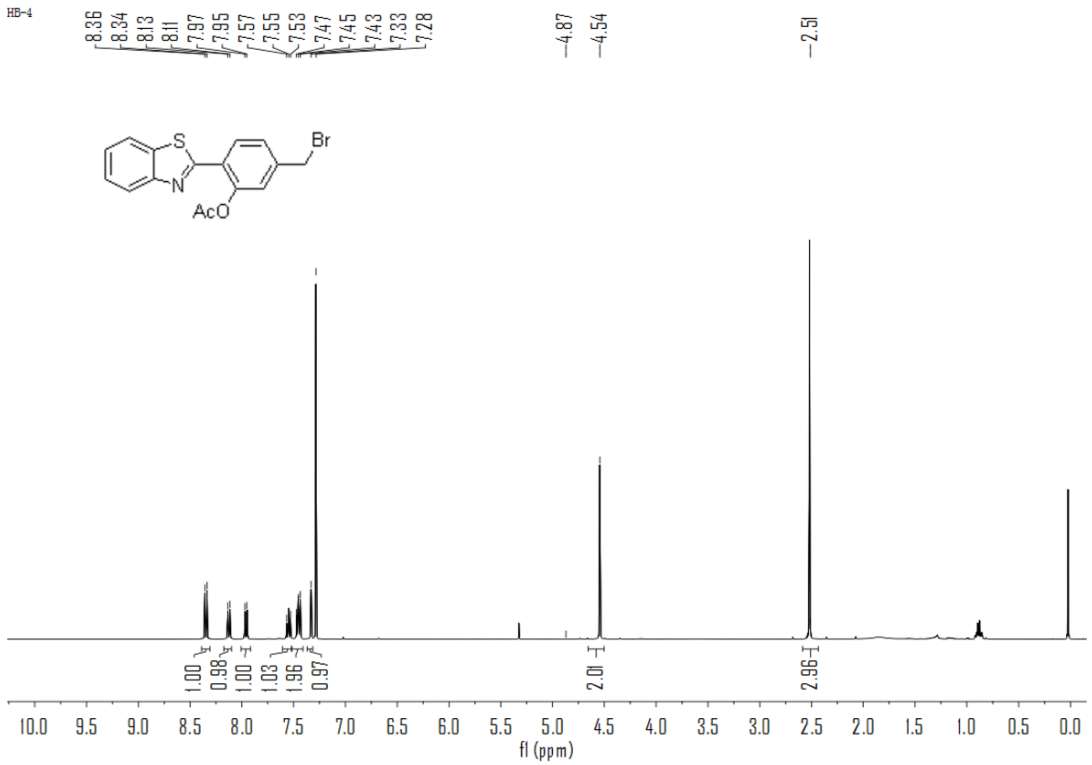


Fig. S23 ^{1}H NMR spectrum of compound **4**.

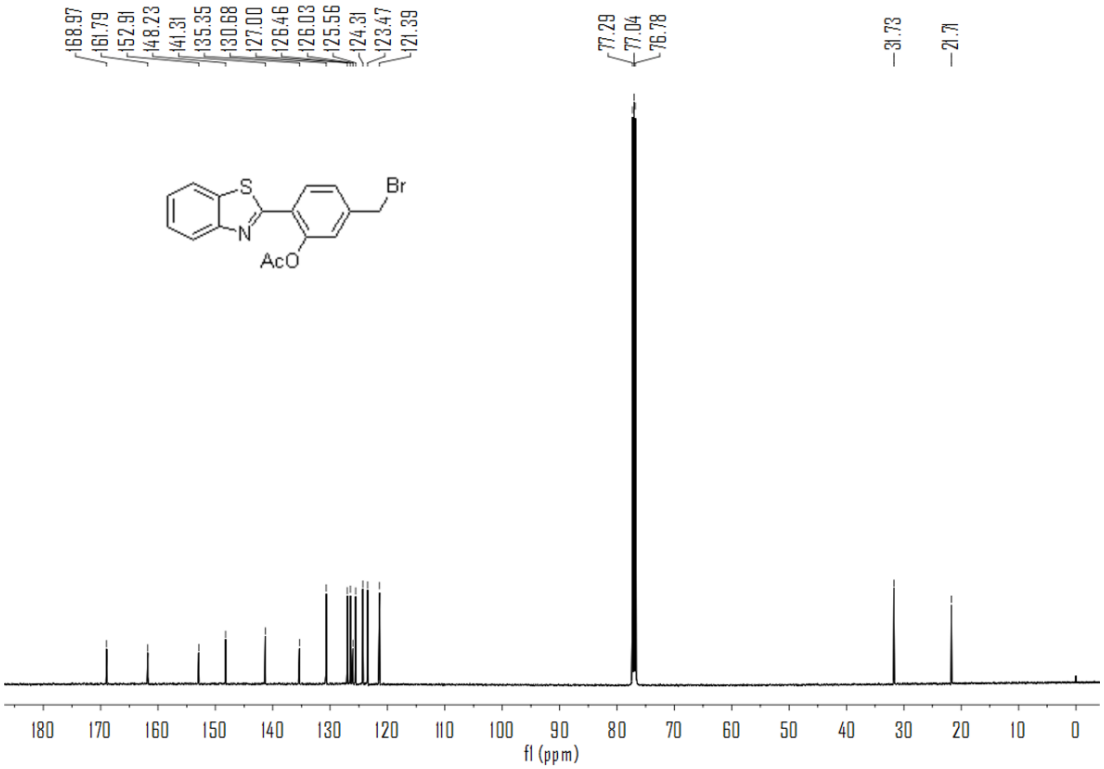


Fig. S24 ^{13}C NMR spectrum of compound **4**.

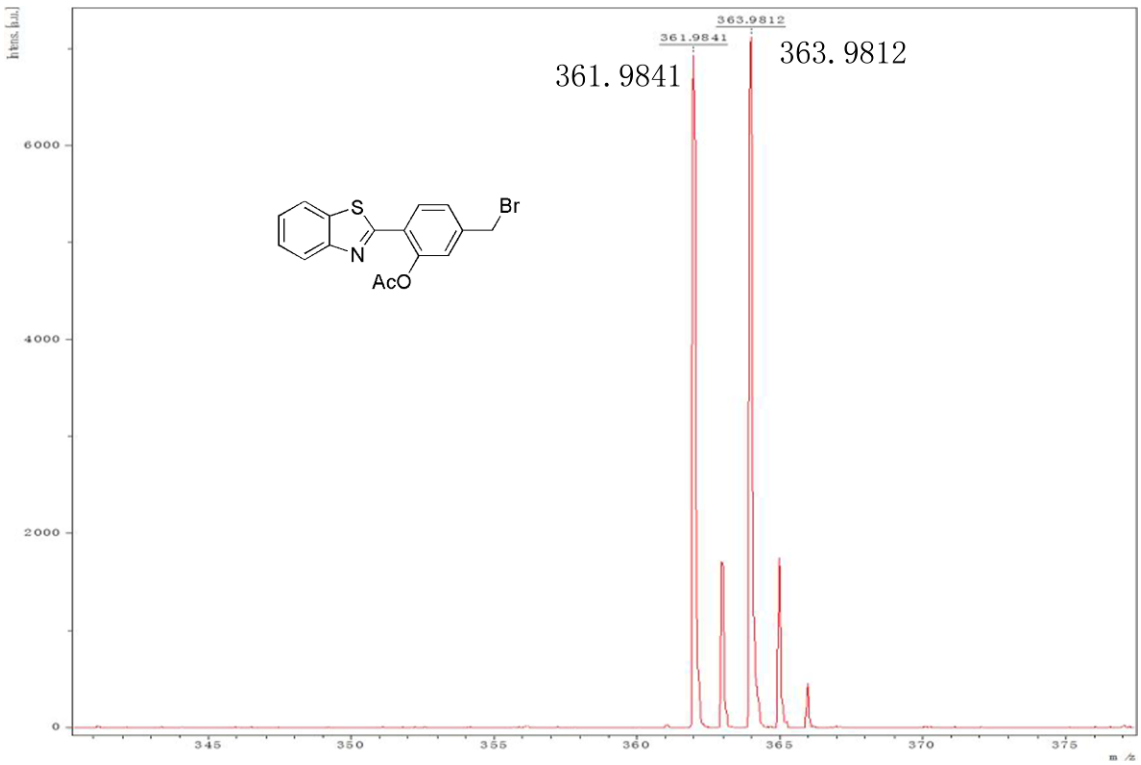


Fig. S25 HRMS spectrum of compound **4**.

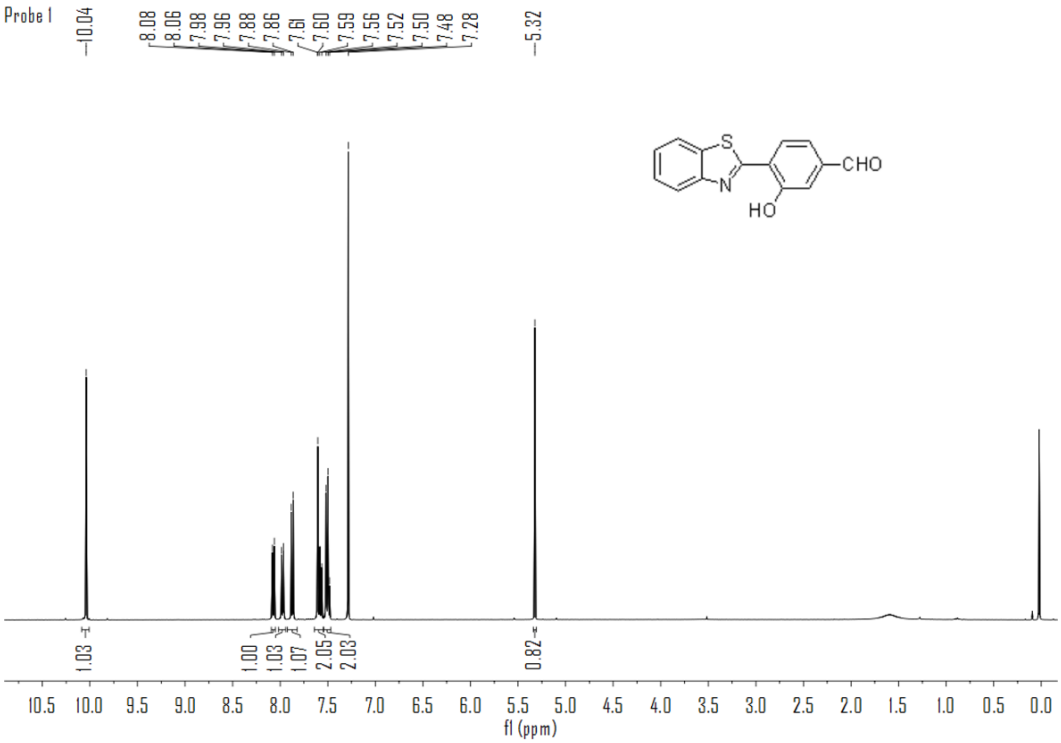


Fig. S26 ^{1}H NMR spectrum of Probe **1**.

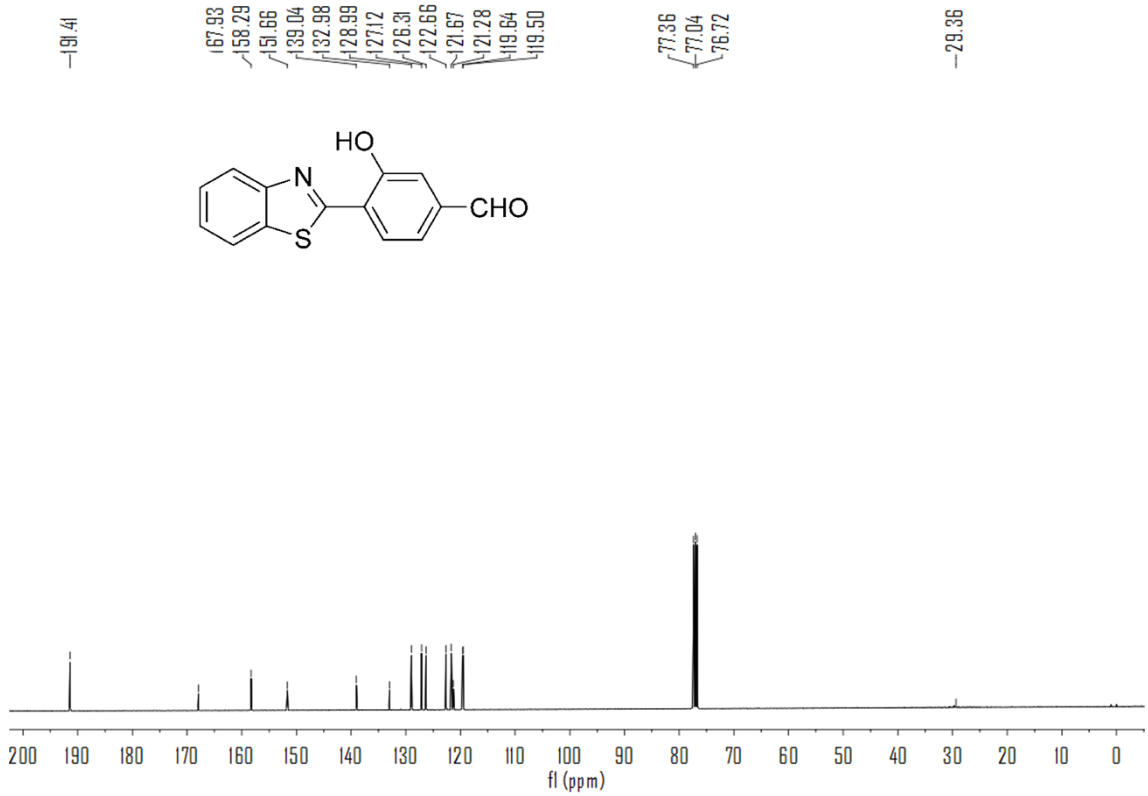


Fig. S27 ^{13}C NMR spectrum of Probe **1**.

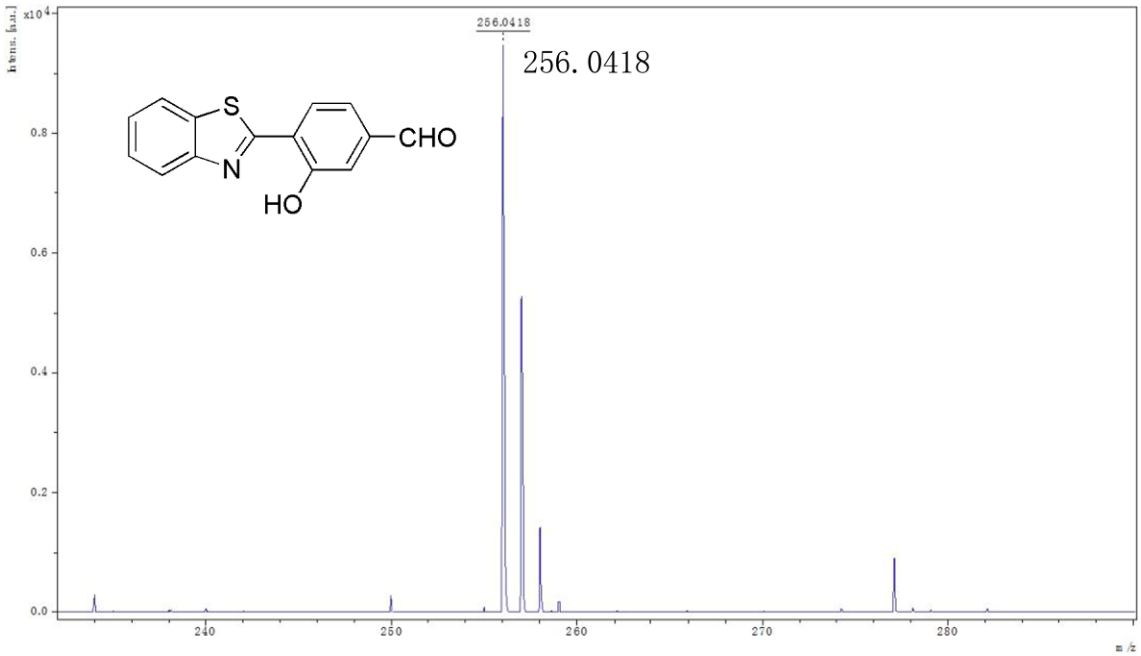


Fig. S28 HRMS spectrum of Probe **1**.

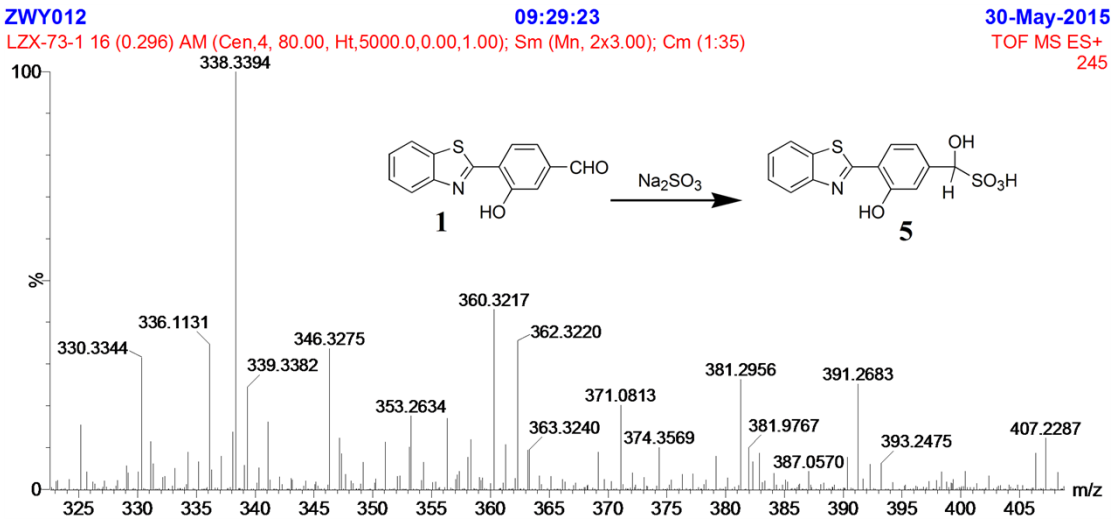


Fig. S29 HRMS spectrum of the reaction product of Probe **1** with SO_3^{2-}, dye **5**.

Reference:

1. (a) E. A. Betterton, Y. Erel and M. R. Hoffmann, *Environ. Sci. Technol.,* **1988**, *22*, 92-9; (b) L. C. de Azevedo, M. M. Reis, L. F. Motta, G. O. da Rocha, L. A. Silva and J. B. de Andrade, *J. Agr. Food. Chem.,* **2007**, *55*, 8670-8680; (c) D. P. Kjell, B. J. Slattery and H. J. Semo, *J. Org. Chem.,* **1999**, *64*, 5722-5724.