Supplementary Information

A real-time colorimetric and ratiometric fluorescent probe for rapid detection of SO_2 derivatives in living cells based on a near-infrared benzopyrylium dye

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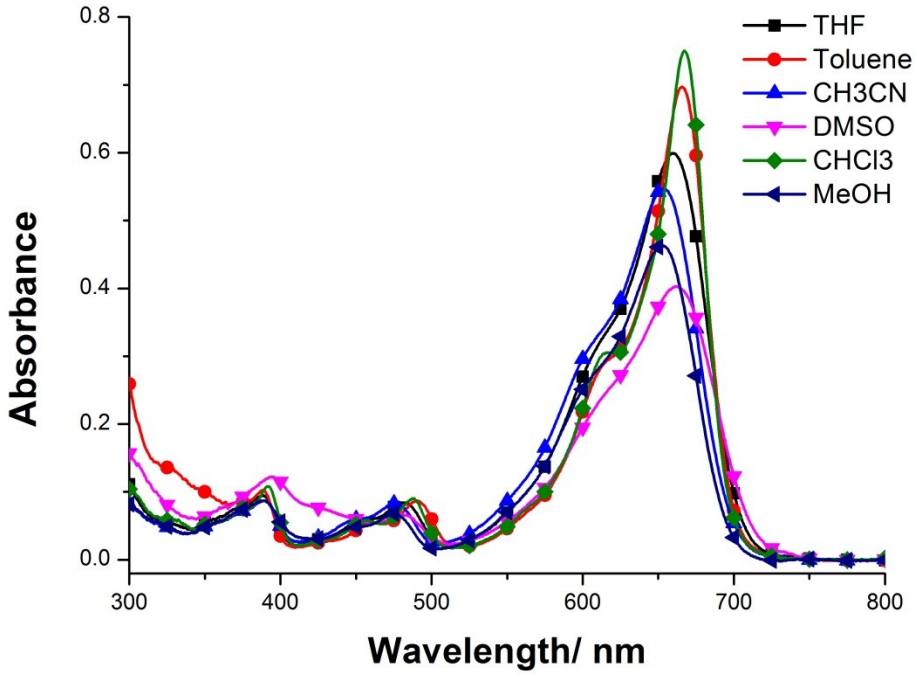

Figure S1. Uv-vis absorption spectra of Probe **1** in different solvents.

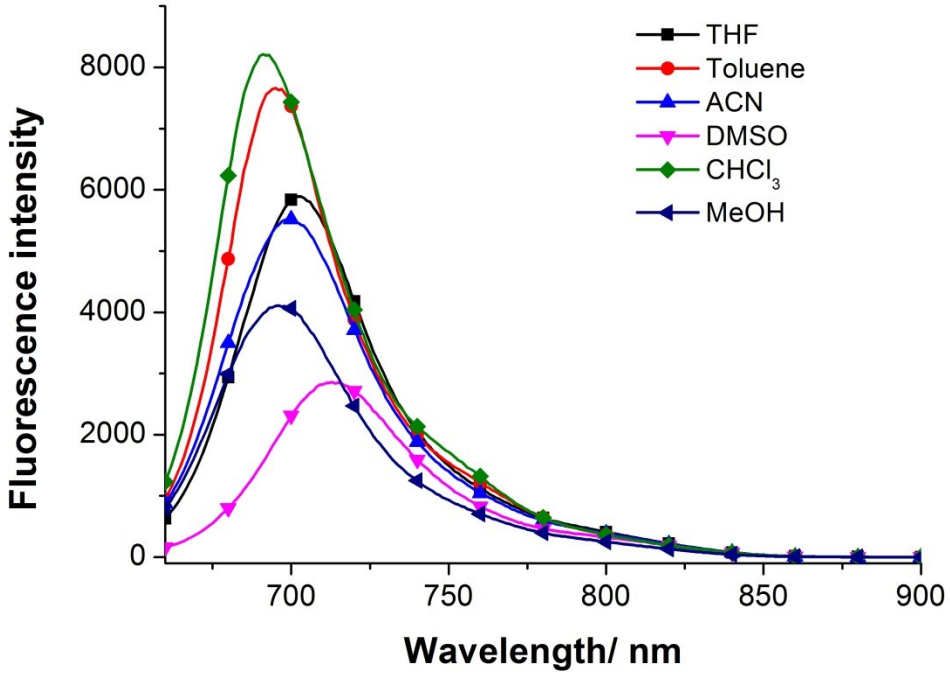

Figure S2. Fluorescence spectra of Probe **1** in different solvents.

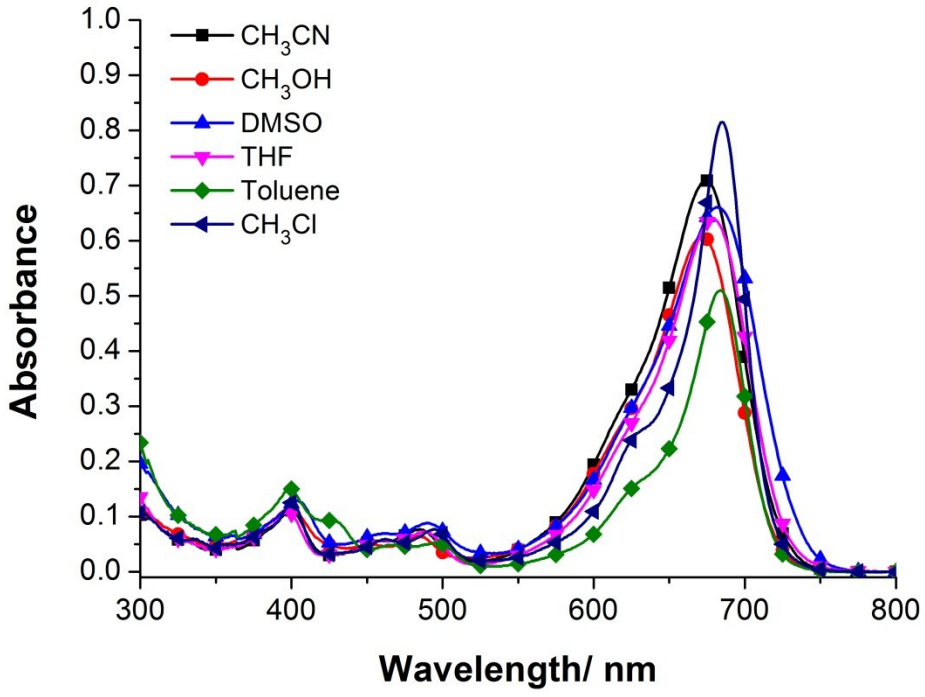

Figure S3. Uv-vis absorption spectra of Probe **2** in different solvents.

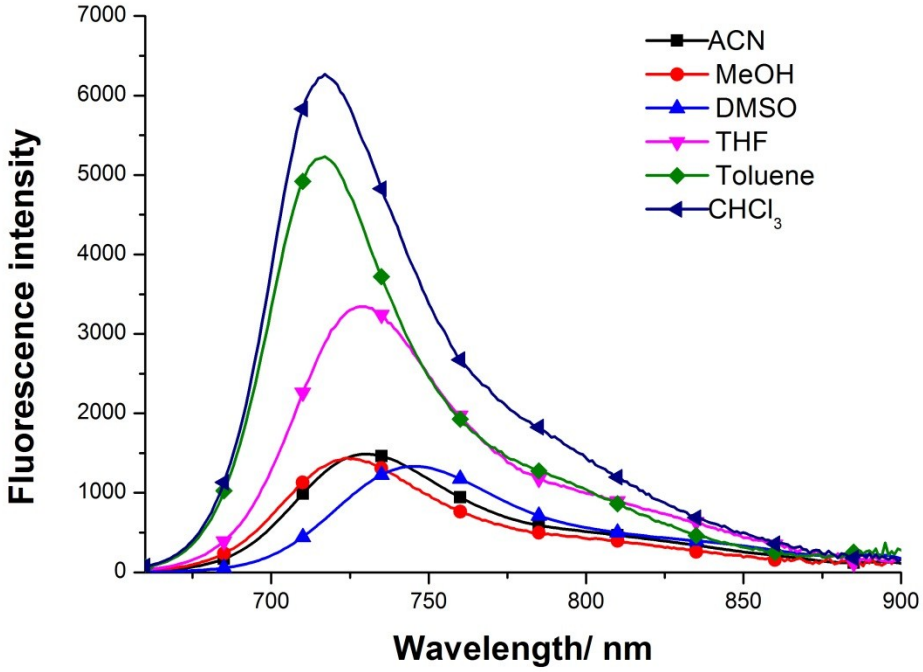

Figure S4. Fluorescence spectra of Probe **2** in different solvents.

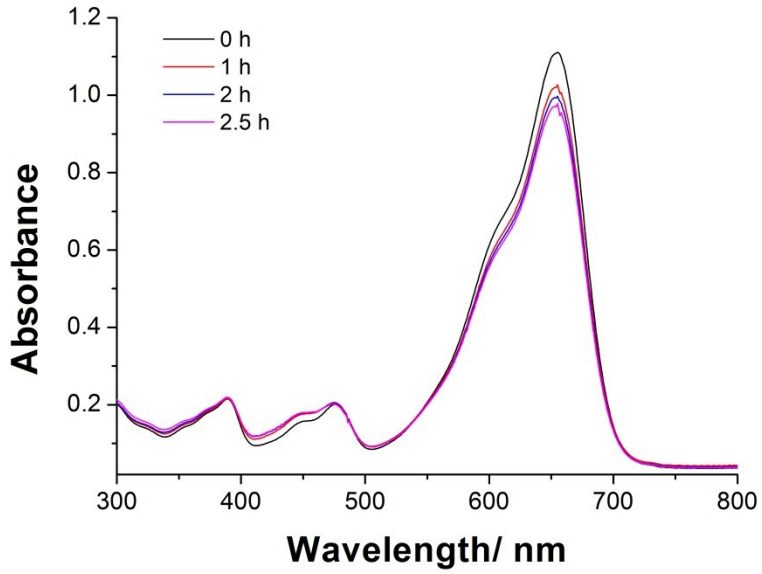

Figure S5. Photodegradation of Probe **1** in CH_3CN under the continuous irradiation with a 300 mW, 635 nm continuous wave laser. The distance between the light source and the sample is 10 cm.

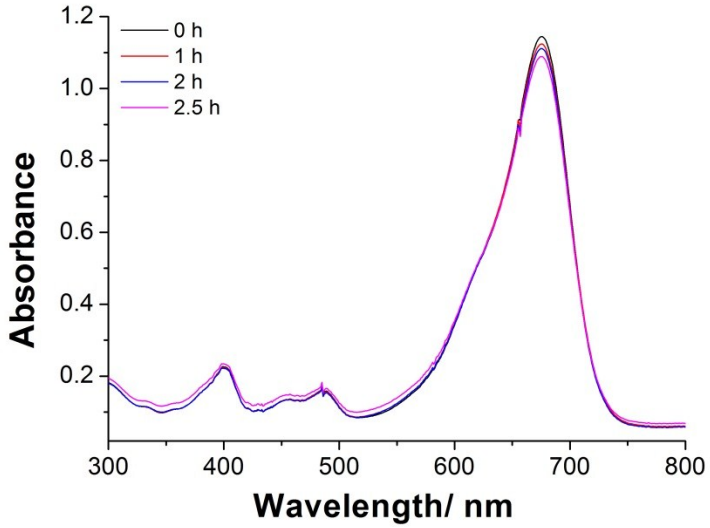

Figure S6. Photodegradation of Probe **2** in CH_3CN under the continuous irradiation with a 300 mW, 635 nm continuous wave laser. The distance between the light source and the sample is 10 cm.

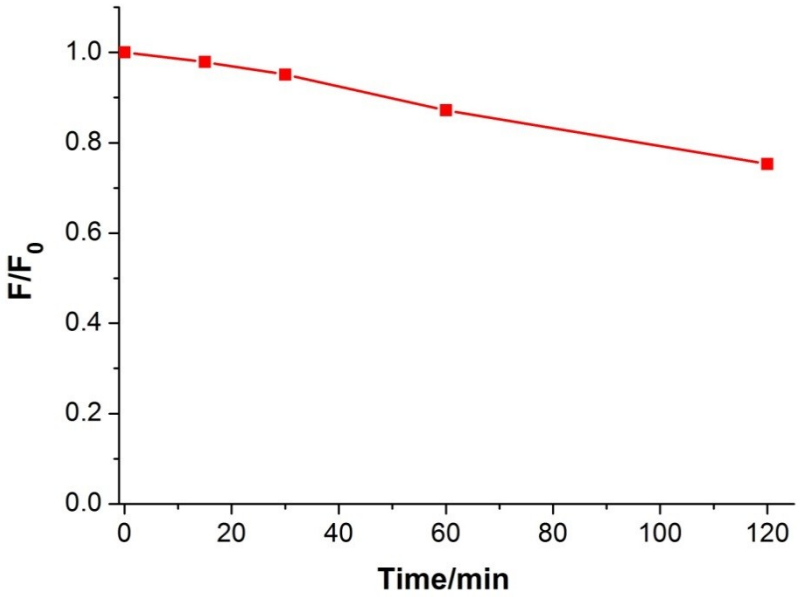

Figure S7. Photodegradation of Probe **1** in CH_3CN under the continuous irradiation with a 300 mW, 635 nm CW laser. F and F_0 are the fluorescent intensities of the sample and reference, respectively. The distance between the light source and the sample is 10 cm. The optical density of the sample is 0.3.

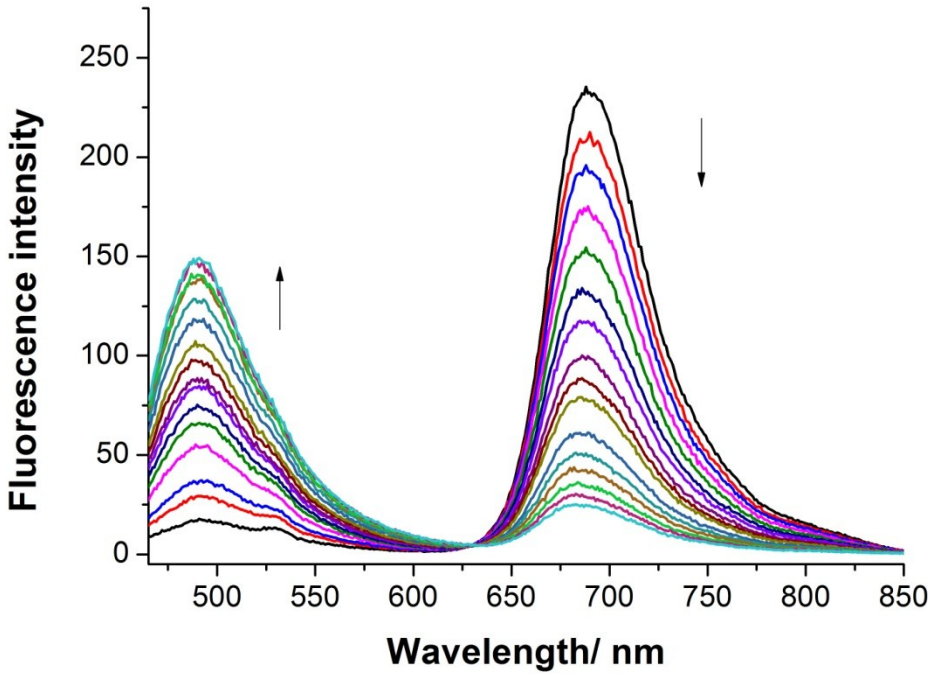

Figure S8. Fluorescence spectra changes of the Probe **1** (5.0 μM) in presence of Na_2SO_3 (0.0-30.0 μM) in HEPES buffer (20.0 mM, pH =7.4).

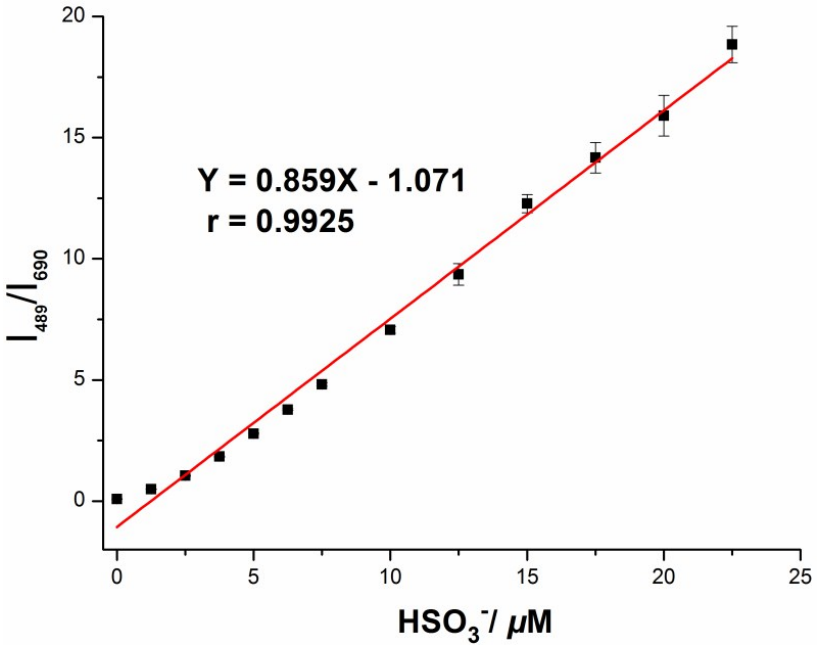

Figure S9. The line relationship between the fluorescence ratio (I_{489}/ I_{690}) of Probe **1** (5.0 μM) and the concentration of HSO_3^- (0.0–22.5 μM) in HEPES buffer (20.0 mM, pH = 7.4).

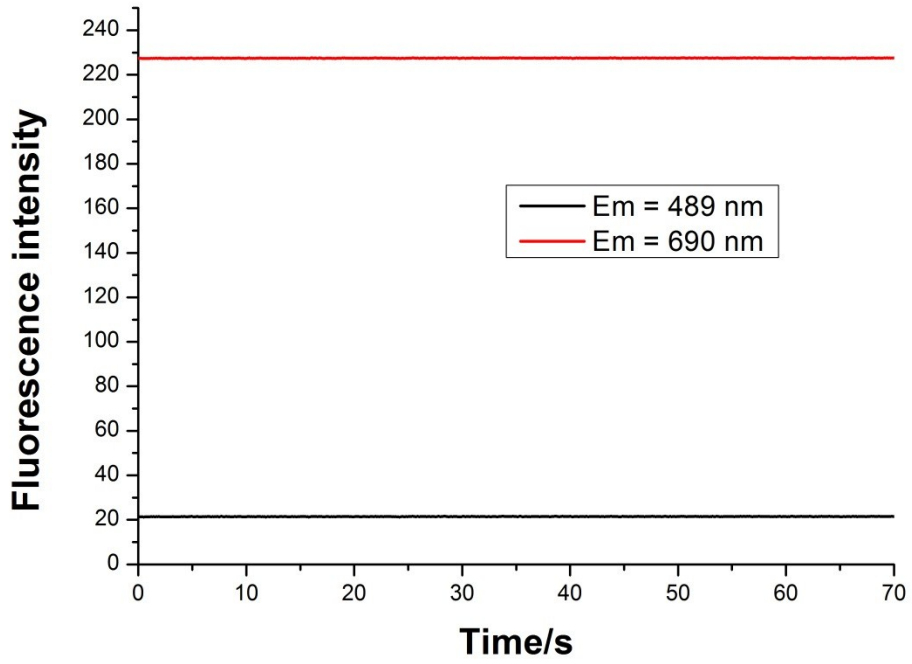

Figure S10. Time-dependent Fluorescence spectral changes of Probe **1** (5.0 μM) in HEPES buffer (20.0 mM, pH =7.4).

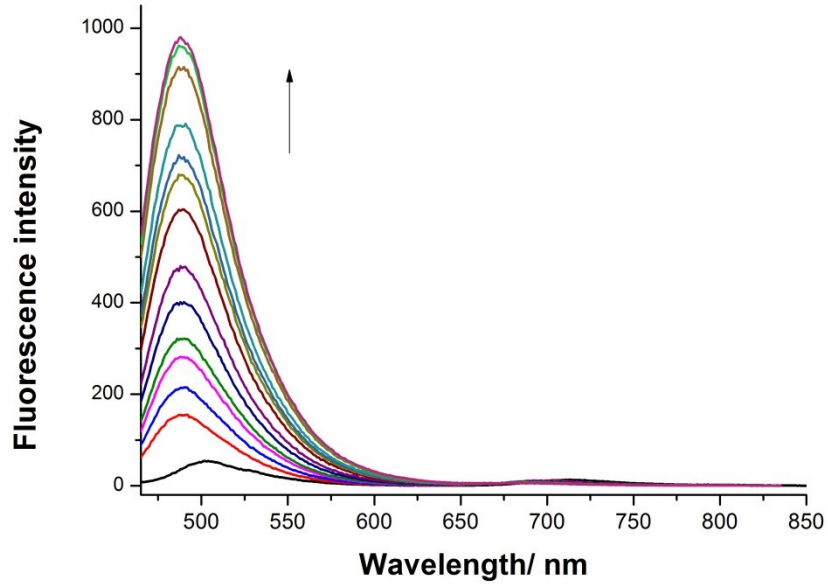

Figure S11. Emission spectra of Probe **2**(5.0 μM) upon the addition of different

concentrations of $NaHSO_3$ (0.0-30.0 μM) in HEPES buffer.

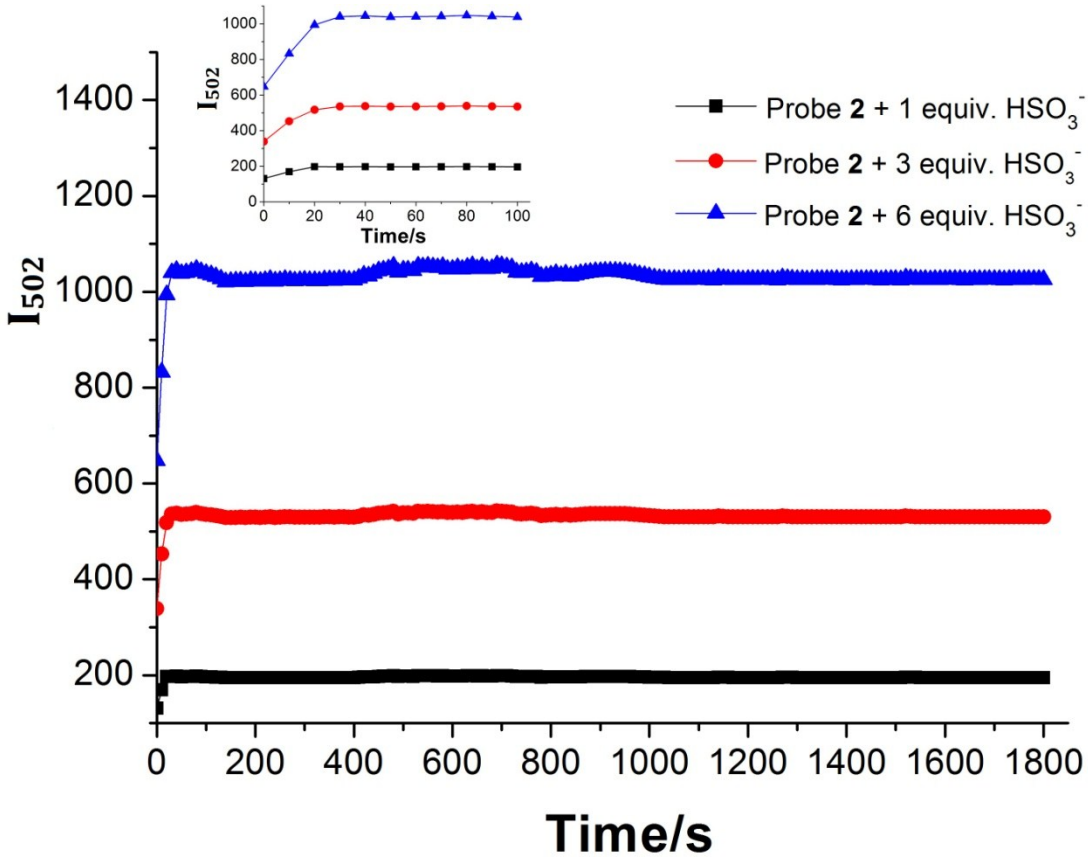

Figure S12. Time-dependent fluorescence intensity at 502 nm of Probe **2** (5.0 μM) upon the addition of different concentrations of $NaHSO_3$ (5.0, 15.0, and 30.0 μM) in HEPES buffer (20.0 mM, pH = 7.4).

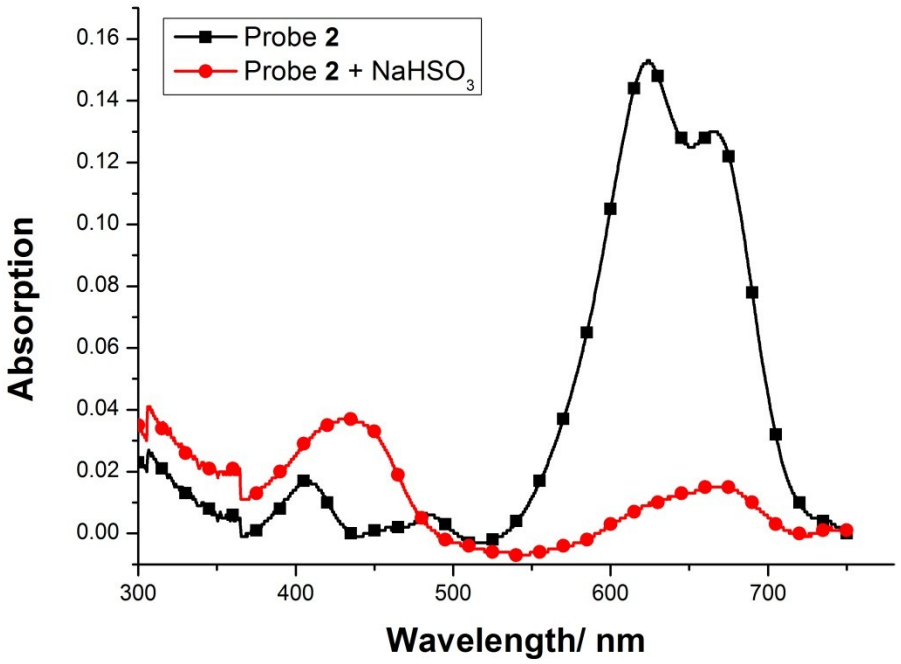

Figure S13. The Uv-vis absorption spectra of Probe **2** (5.0 μM) in the absence/presence of $NaHSO_3$ (30.0 μM).

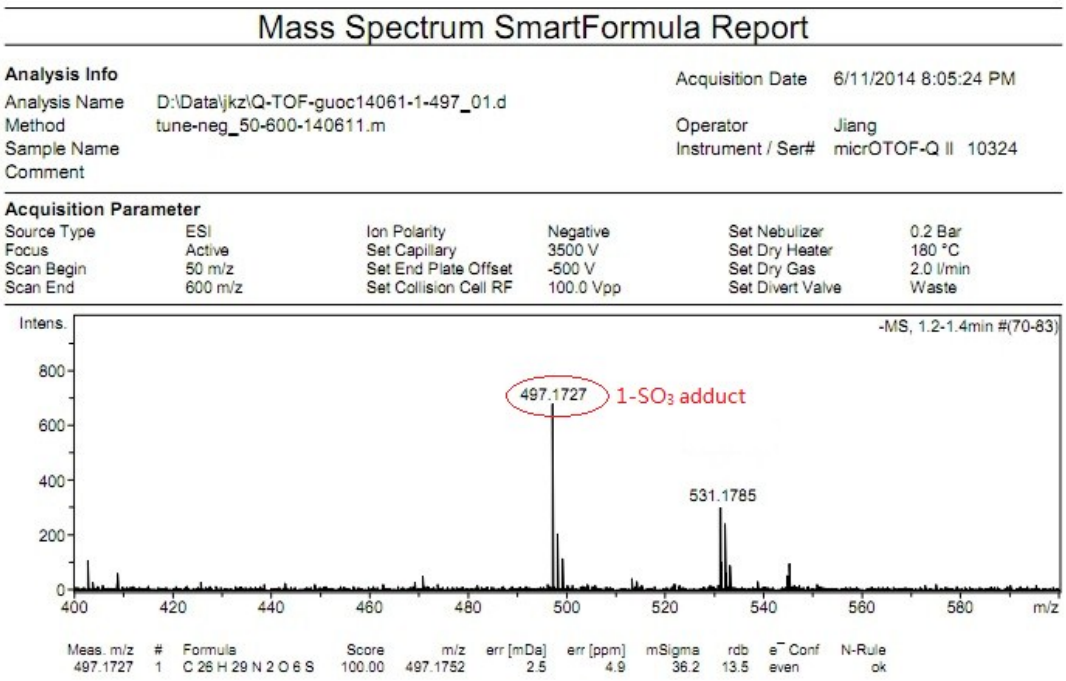

Figure S14. Mass spectrum of the Probe **1** (20.0 μM) with $NaHSO_3$ (100.0 μM).

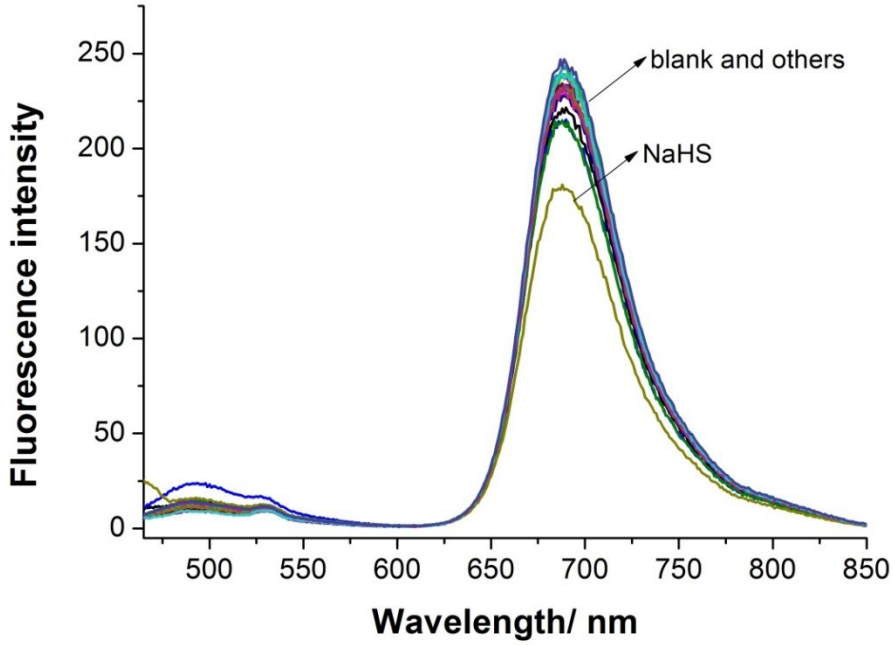

Figure S15. Fluorescence spectra of Probe **1** (5.0 μM) in the presence of various analytes (25.0 μM for F^-, Cl^-, Br^-, I^-, N_3^-, NO_2^-, NO_3^-, SO_4^{2-}, $S_2O_3^{2-}$, AcO^- , HS^-, Mg^{2+}, Zn^{2+}, Ca^{2+} and K^+; 50.0 μM for H_2O_2 and NaClO; 0.5 mM for Cys and Hcy; 4.0 mM for GSH) in HEPES buffer (20.0 mM, pH =7.4).

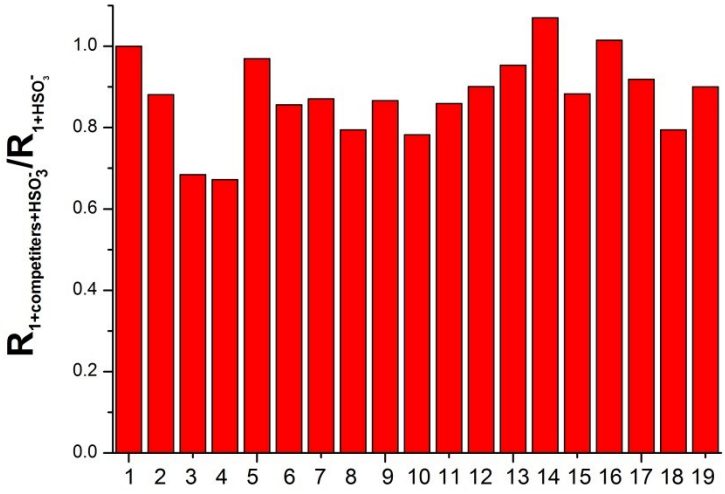

Figure S16. Interfering effect of various tested analytes on the fluorescence intensity of Probe **1** (5.0 μM) in response to HSO_3^- (25.0 μM) in HEPES buffer (20.0 mM, pH = 7.4). λ_{ex} = 450 nm. R= I_{489}/I_{690}. Bars: 25.0 μM for (1)blank, (2) Br^-, (3) F^-, (4) I^-, (5) Cl^-, (6) $S_2O_3^{2-}$, (7) AcO^-, (8) SO_4^{2-}, (9) NO_2^-, (10) NO_3^-, (11) N_3^-, (12) HS^-, (13)

Mg^{2+}, (14) Zn^{2+}; 50.0 μM for (15) H_2O_2, (16) NaClO; 0.5 mM for (17) Cys, (18) Hcy; 4.0 mM for (19) GSH.

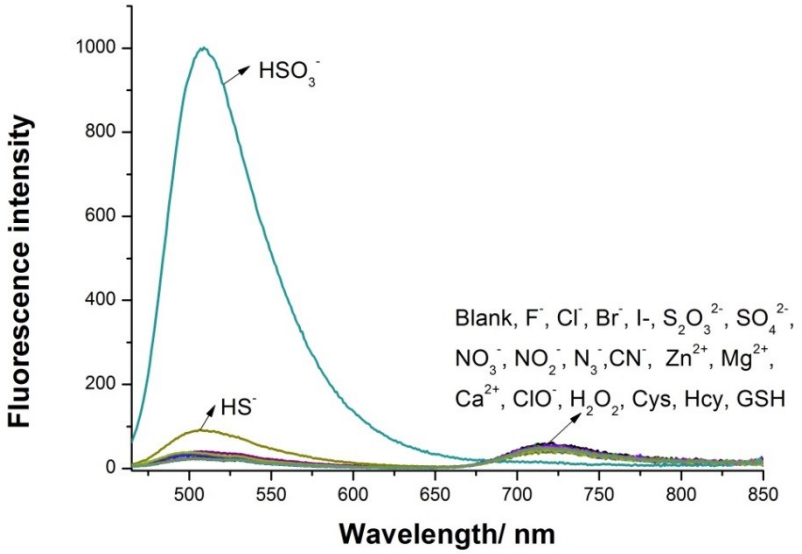

Figure S17. Fluorescence spectra of Probe **2** in the presence of various analytes (30.0 μM for F^-, Cl^-, Br^-, I^-, N_3^-, CN^-, NO_2^-, NO_3^-, SO_4^{2-}, $S_2O_3^{2-}$, HS^-, Mg^{2+}, Zn^{2+} and Ca^{2+}; 50.0 μM for H_2O_2 and NaClO; 0.5 mM for Cys and Hcy; 4.0 mM for GSH) in HEPES buffer (20.0 mM, pH =7.4).

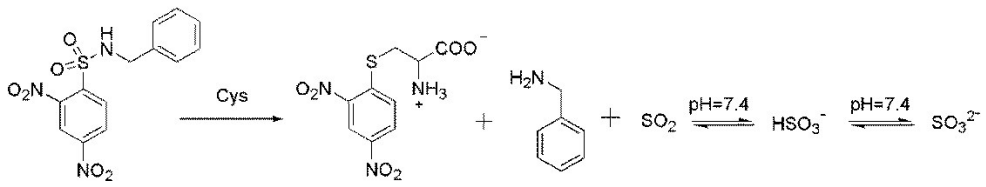

Figure S18. The reaction of SO_2 donor in the presence of Cys in pH 7.4 buffered solution.

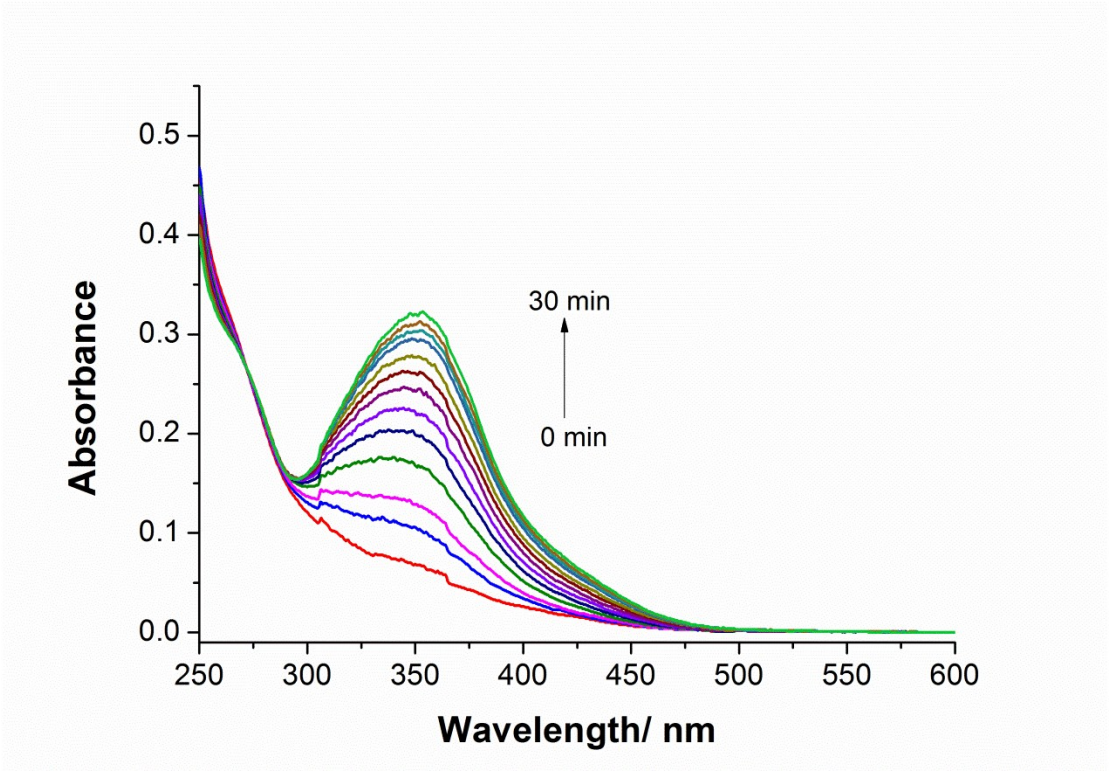

Figure S19. Time-dependent Uv-vis absorption spectra of SO_2 donor (40.0 μM) in the presence of Cys (400.0 μM) in HEPES buffer (20.0 mM, pH = 7.4).

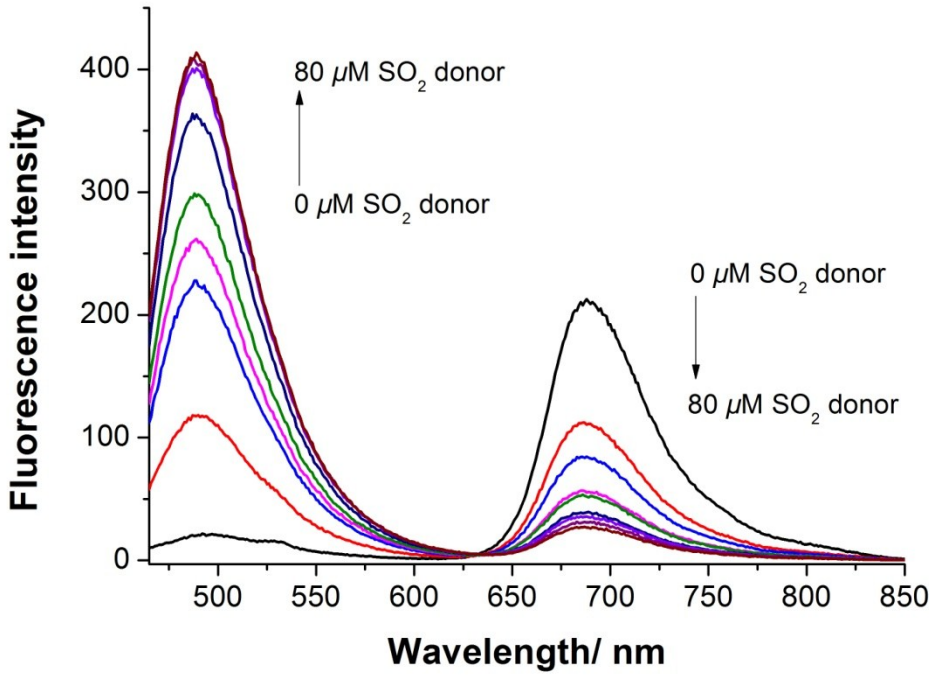

Figure S20. Fluorescence spectra of Probe **1** (5 μM) in the presence of SO_2 donor (0.0-80.0 μM) in HEPES solution (20 mM, pH = 7.4, containing 400.0 μM Cys). Each spectrum was recorded after incubation for 30 min at room temperature.

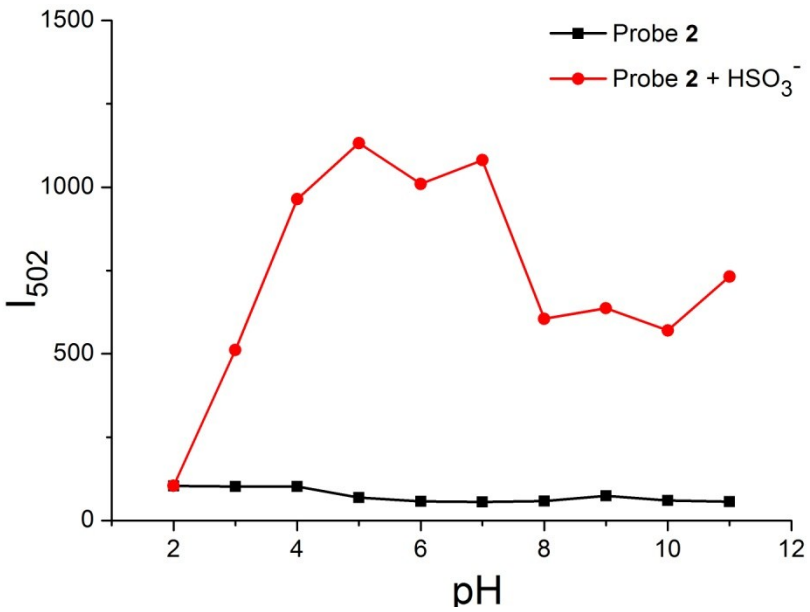

Figure S21. The fluorescence intensity at 502 nm of Probe **2** (5.0 μM) in the absence and presence of HSO_3^- (25.0 μM) at varied pH values.

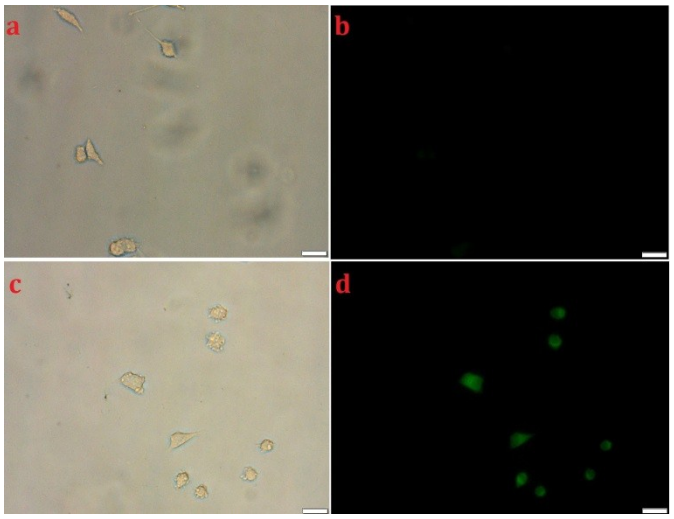

Figure S22. Images of living A431 cells. Top row: cells incubated with Probe 2 (5.0 μM) for 30 min. Bottom row: cells pretreated with $NaHSO_3$ (50.0 μM) for 30 min, then washed with PBS buffer (20 mM, pH = 7.4) and further incubated with Probe 2 (5.0 μM) for 30 min. (a), (c) Bright field images; (b), (d) Fluorescence images (excited with blue light). Scale bar = 50 μm.

MTT assays

MTT assays were performed to evaluate the cytotoxicity of Probe **1** and **2**. HepG2 cells were grown in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% FBS (Fetal Bovine Serum) and 1% antibiotics at 37 ºC in a humidified environment containing 5% CO_2. Before the experiment, the cells were placed in 96-well plates, followed by the addition of different concentrations of Probe **1** or **2** (0.0 to 20.0 μM). The cells were then incubated at 37 ºC in an atmosphere of 5% CO_2 and 95% air for 48 h, followed by MTT assays (n = 10). Untreated assay with Minimum Essential medium (n = 10) was also conducted under the same conditions.

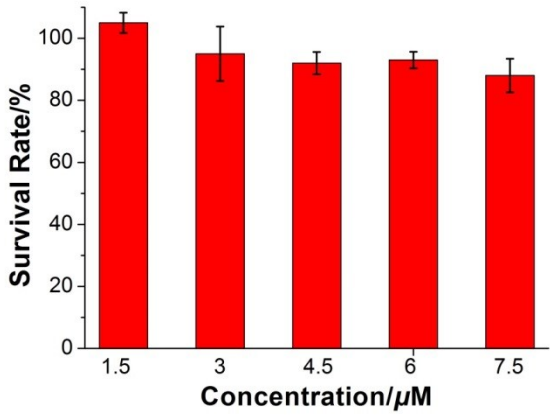

Figure S23. Percentage of viable HepG2 cells after treatment with various concentrations of Probe **1** for 48 hours.

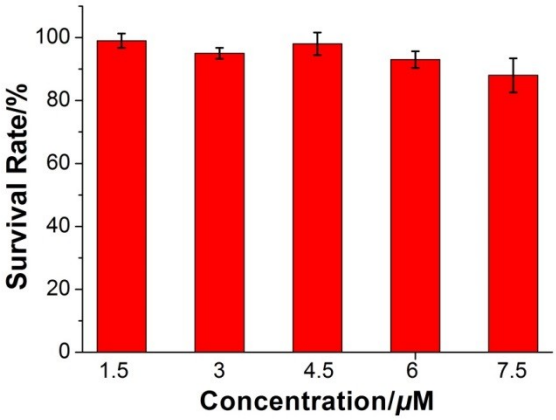

Figure S24. Percentage of viable HepG2 cells after treatment with various concentrations of Probe **2** for 48 hours.

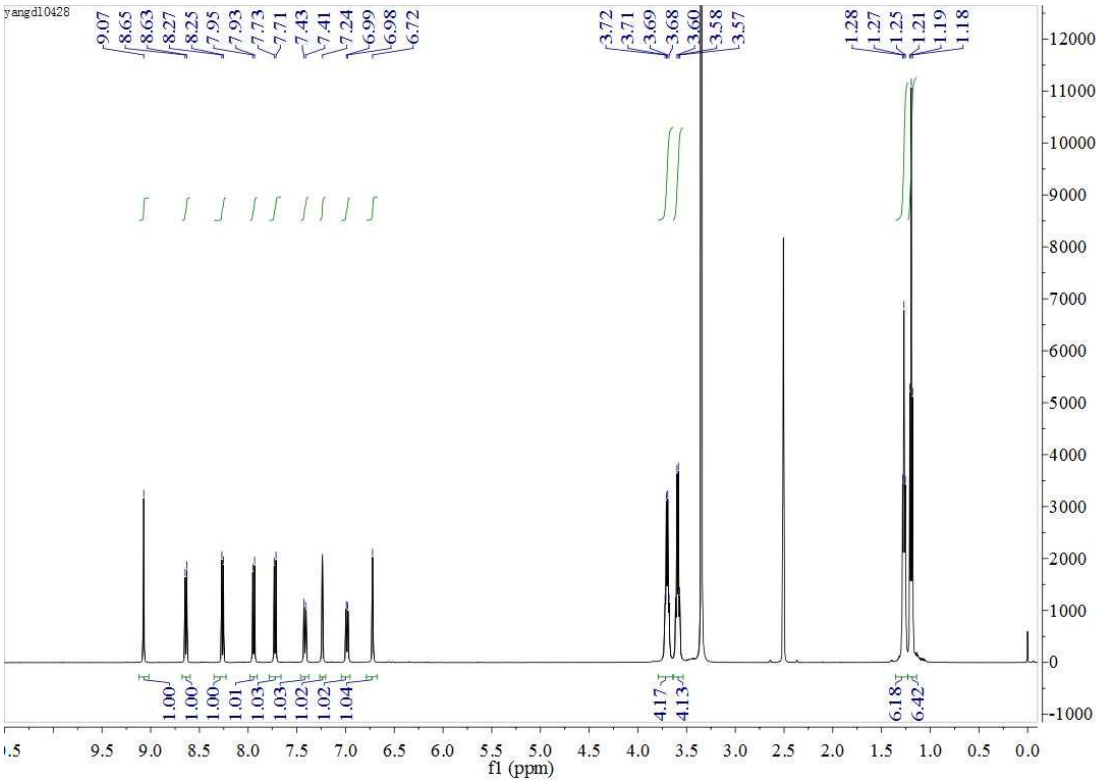

Figure S25. ^{1}H NMR spectrum of Probe **1**.

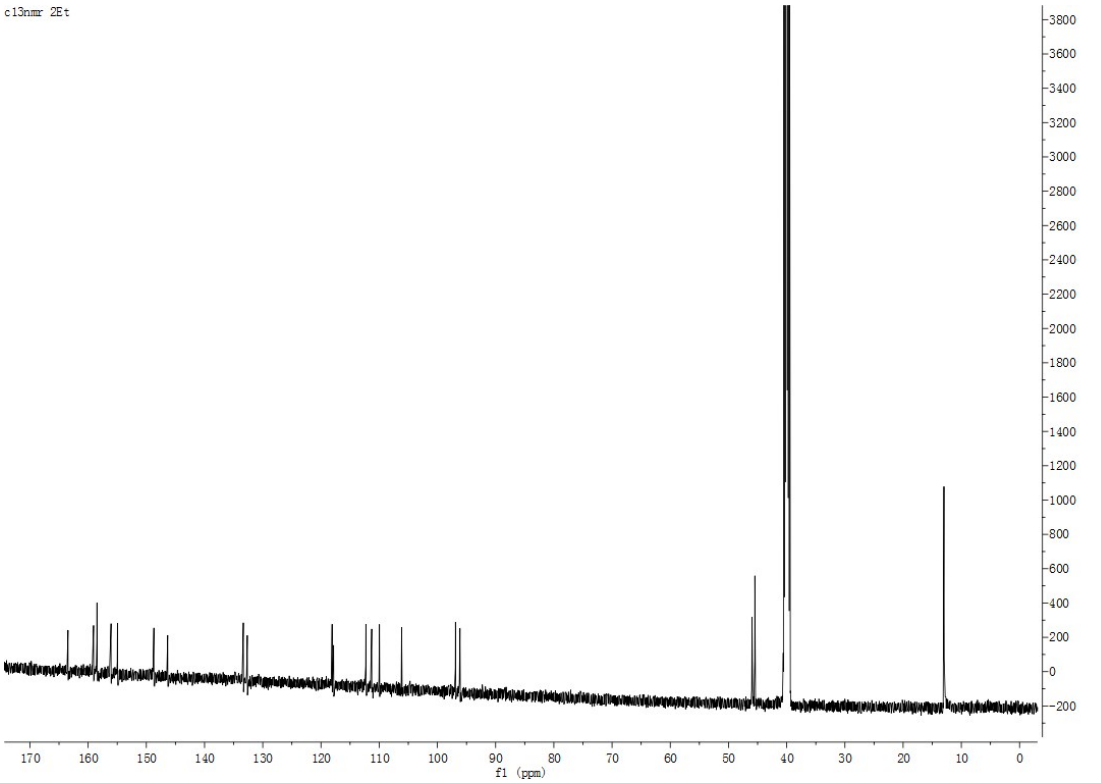

Figure S26. ^{13}C NMR spectrum of Probe **1**.

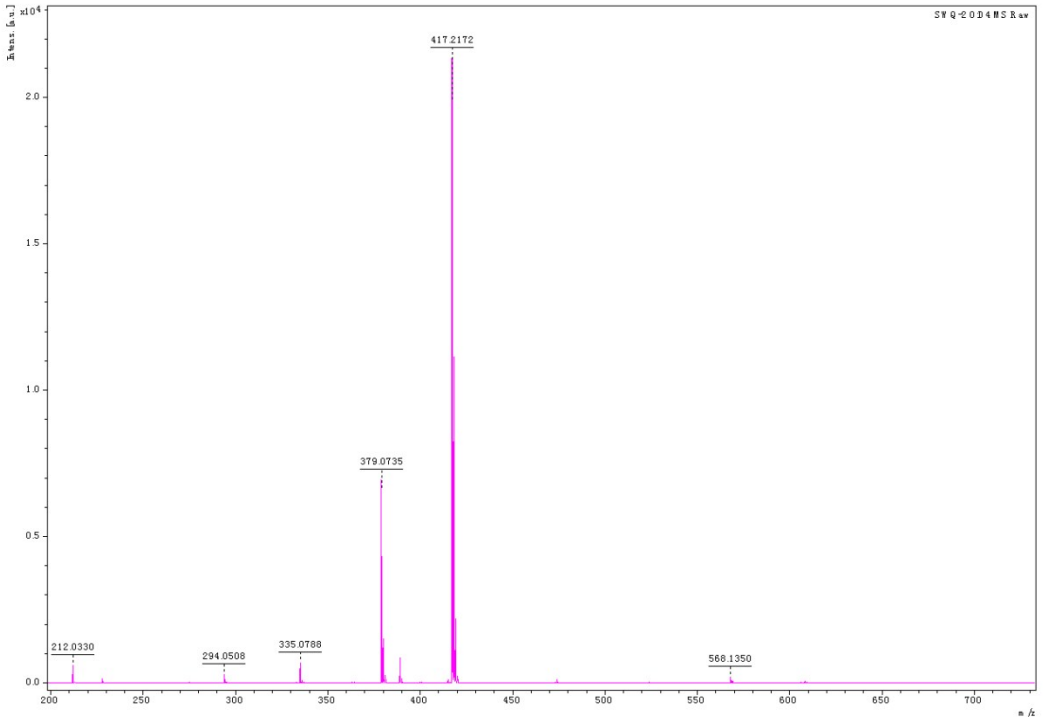

Figure S27. Mass spectrum of Probe **1**.

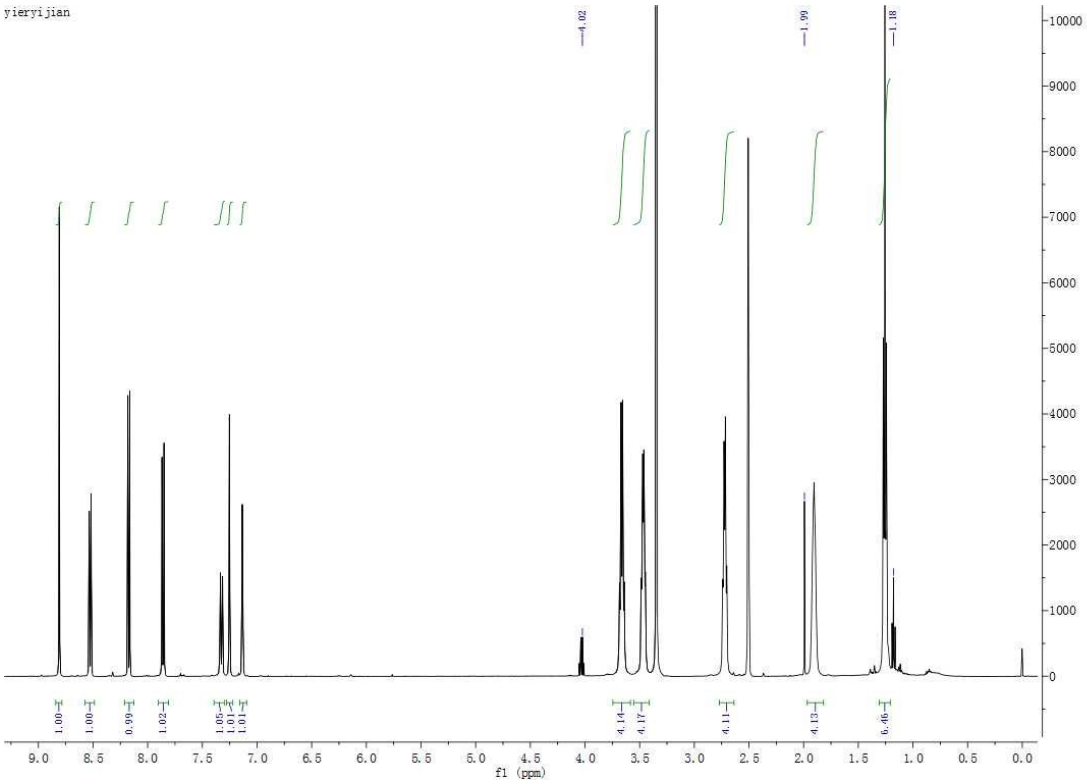

Figure S28. ^{1}H NMR spectrum of Probe **2**.

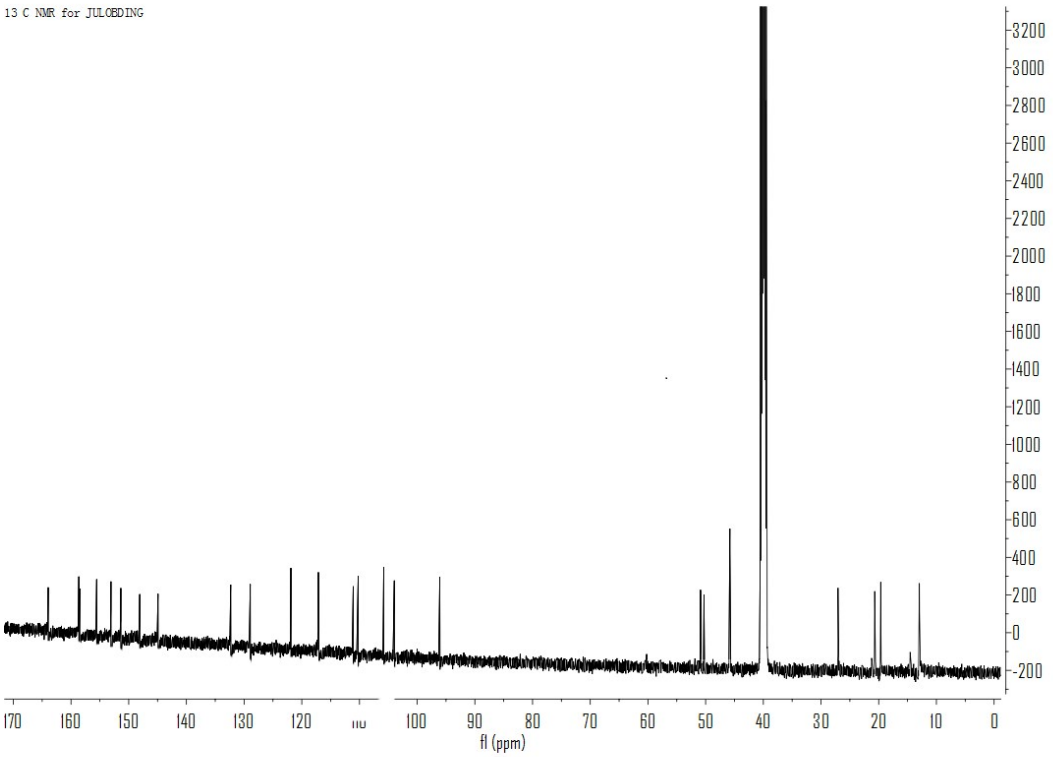

Figure S29. ^{13}C NMR spectrum of Probe **2**.