Supporting information for

A Coumarin-quinolinium-Based Fluorescent Probe for Ratiometric Sensing of Sulfite in Living Cells

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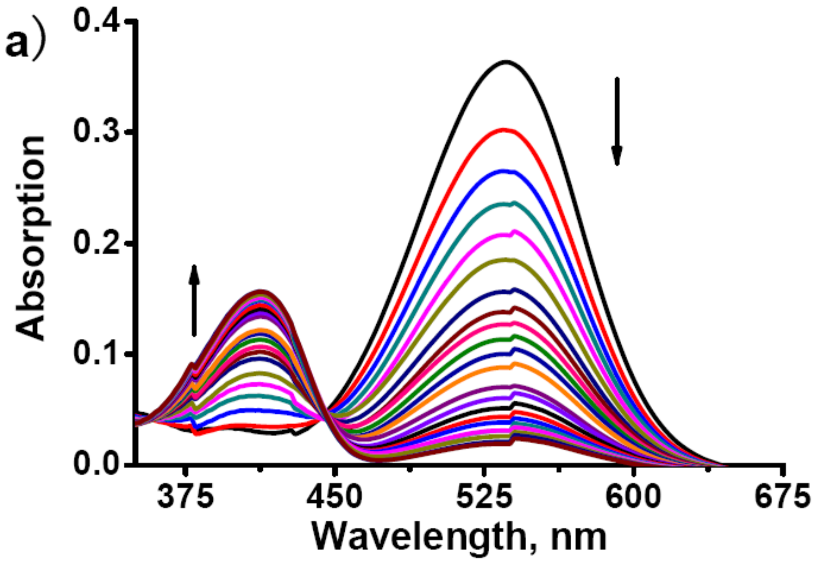


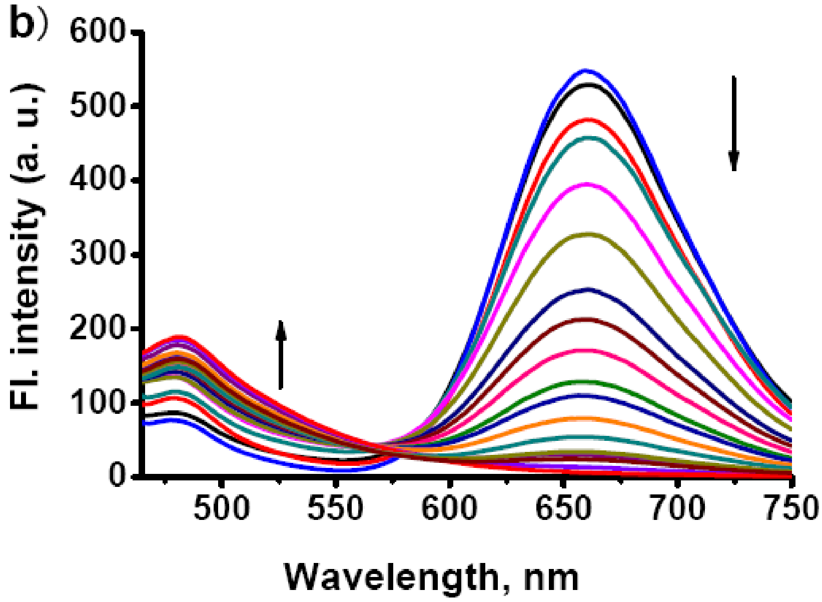


Fig. S1 Absorption (a) and Fluorescence (b) spectra of probe **2** (5 μM) in pH 7.4 PBS buffer/ethanol (7:3, v/v) in the presence of SO_3^{2-} (0-250 equiv.) with excitation at 410 nm..

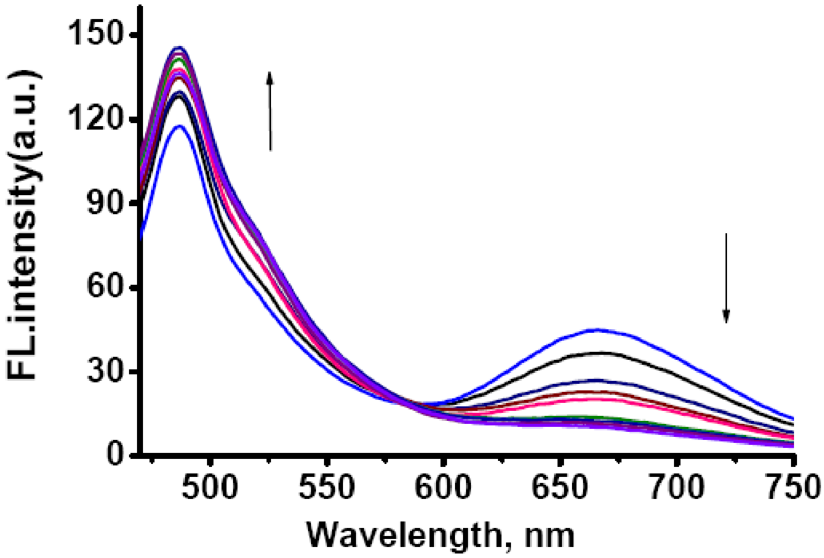


Fig. S2 Fluorescence spectra of the probe **2** (5 μM) in pH 7.4 PBS buffer in the presence of SO_3^{2-}(0-150 equiv.) with excitation at 410 nm..

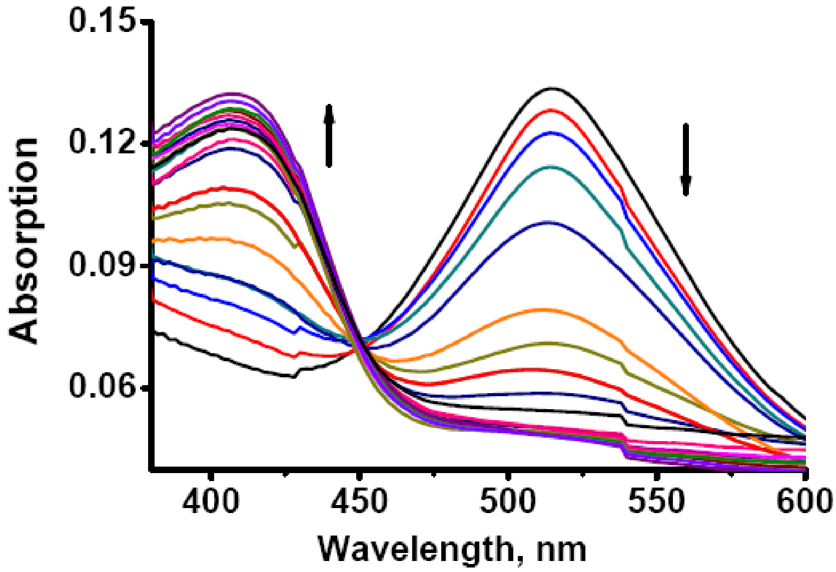


Fig. S3 Absoption spectra of probe **2** (5 μM) in PBS buffer (pH 7.4, containing 1 mg/mL BSA) in the presence of SO_3^{2-} (0-200 equiv.).

DFT calculation: [1]

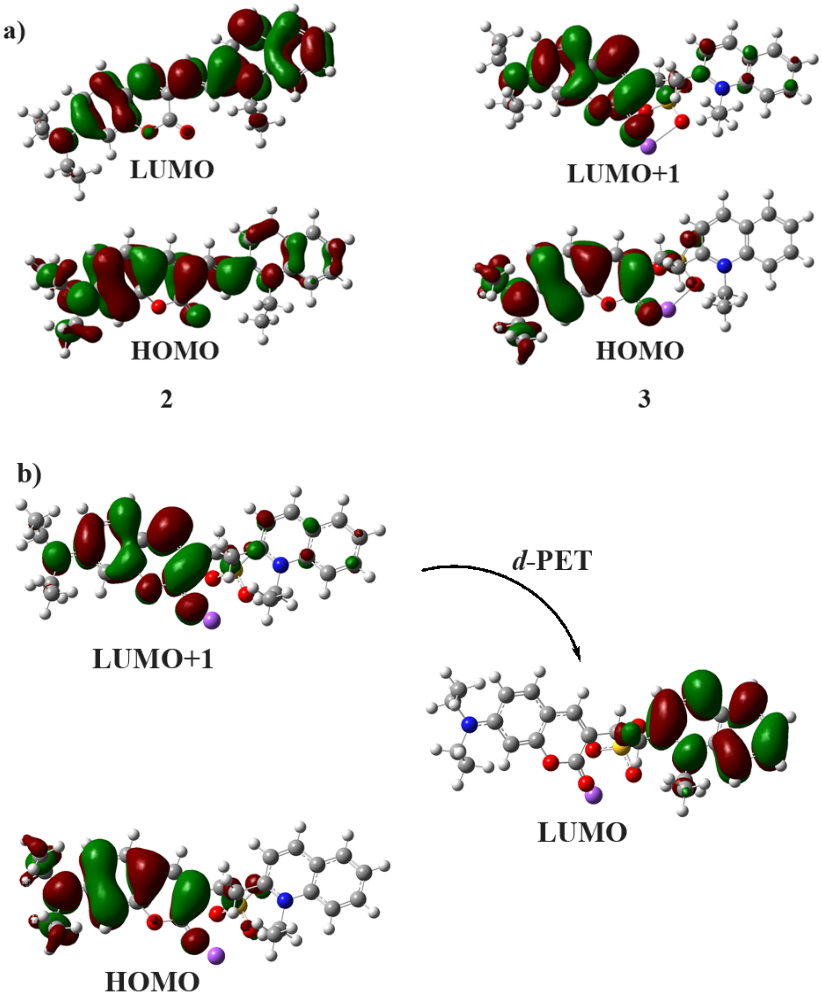


Fig. S4 (a) Frontier molecular orbital plots of compound **2** (left column) and **3** (right column) in water (CPCM model); (b) Frontier molecular orbital plots of **3** in water (CPCM model), the vertical excitation related calculations are based on the optimized geometry of the ground state (S_0), and the fluorescence emission of coumarin moieties is partially quenched by *d*-PET.

Kinetic Studies:

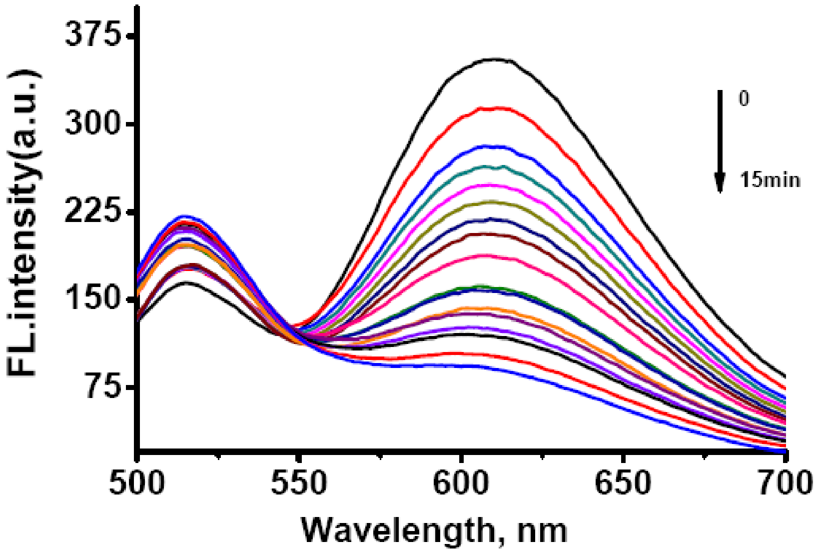


Fig. S5 Reaction-time profiles of 2 (5.0 μM) in the of Na_2SO_3 (20 equiv.). The fluorescence intensities at 508 and 610 nm were continuously monitored at time intervals in absoption spectra of probe **2** (5 μM) in PBS buffer (pH 7.4, containing 1 mg/mL BSA) in the presence of 20 equiv. SO_3^{2-} from 0 to 15 min with excitation at 450 nm.

The reaction of the probe **2** (5 μM) with SO_3^{2-} (20 equiv.) in PBS buffer (pH 7.4, containing 1mg/ mL BSA) was monitored using the fluorescence intensity at 610 nm. The reaction was carried out at room temperature. The *pseudo*-first-order rate constant for the reaction was determined by fitting the fluorescence intensities of the samples to the *pseudo* first-order equation:

$$\mathrm{Ln}\ [(I_{max} - I_t) / I_{max}] = - k't$$

Where F_t and F_{max} are the fluorescence intensities at 610 nm at time t and the maximum value obtained after the reaction was complete. k' is the *pseudo*-first-order rate constant.

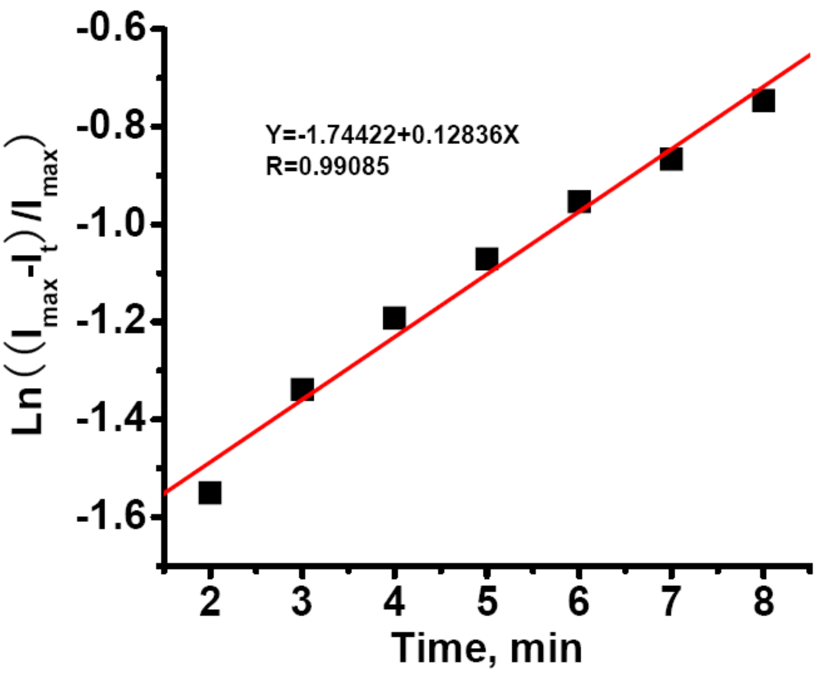
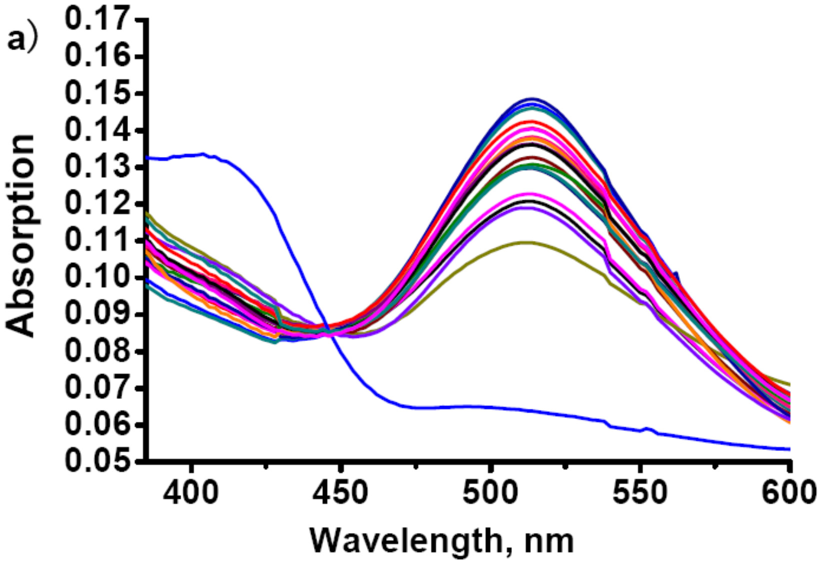


Fig. S6 *Pseudo* first-order kinetic plot of the reaction of **2** (5 μM) with sulfite (20 equiv.) in PBS buffer (pH 7.4, containing 1mg/mL BSA) with excitation at 450 nm.

. Slope = 0.128 min^{-1}.



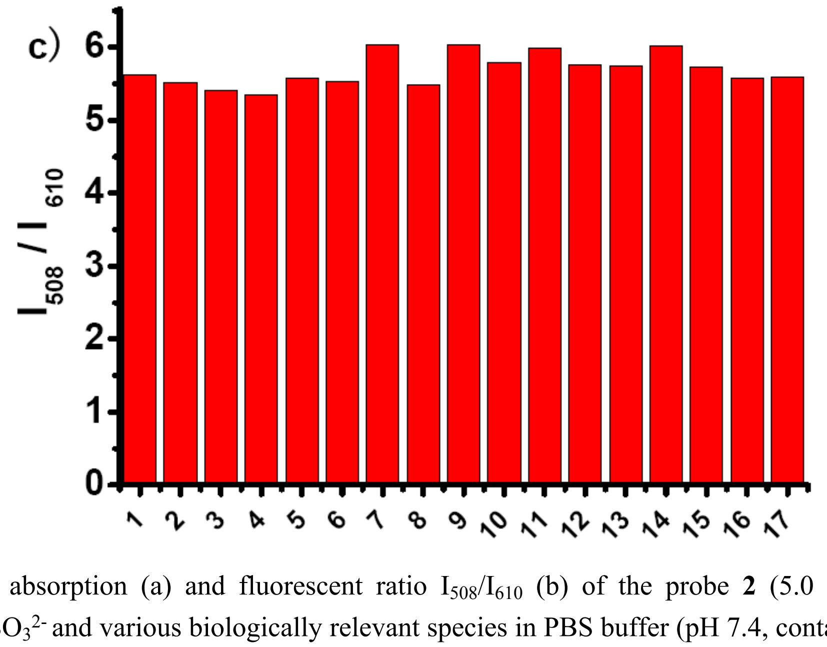


Fig. S7 The absorption (a) and fluorescent ratio I_{508}/I_{610} (b) of the probe **2** (5.0 μM) in the presence of SO_3^{2-} and various biologically relevant species in PBS buffer (pH 7.4, containing 1mg/ mL BSA). Red bars represent the addition of the excess of representative species and SO_3^{2-}. 1. CH_3COO^-，2. I^-，3. Br^-，4. Cl^-，5. cys, 6. N_3^-，7. NO_2^-，8. $H_2PO_4^-$，9. F^-, 10. NO_3^-, 11. SO_4^{2-}, 12. SCN^-，13. $S_2O_3^{2-}$，14. S^{2-}, 15. Vc，16. CO_3^{2-}, 17. GSH.

Cytotoxicity assays:

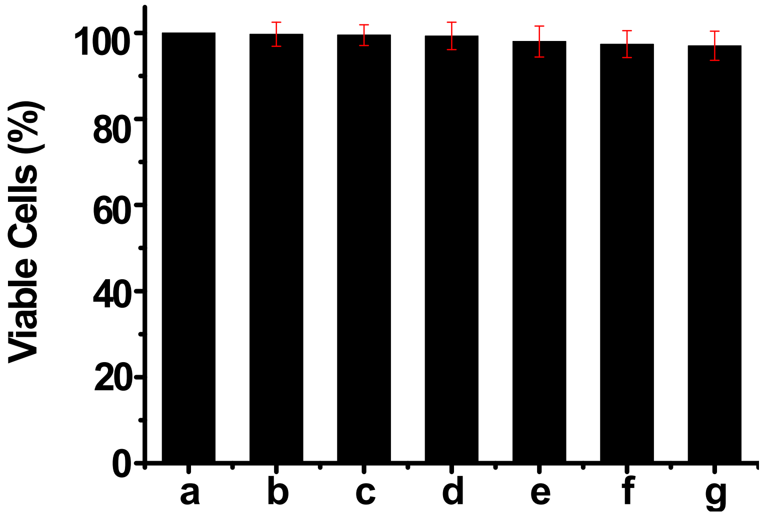


Fig. S8 Cytotoxicity assay of probe **2** at different concentrations (a: 0 μM; b: 0.5 μM; c: 1 μM; d: 3 μM; e: 5 μM; f: 7 μM; g: 10 μM;) for RAW 264.7 macrophage cells.

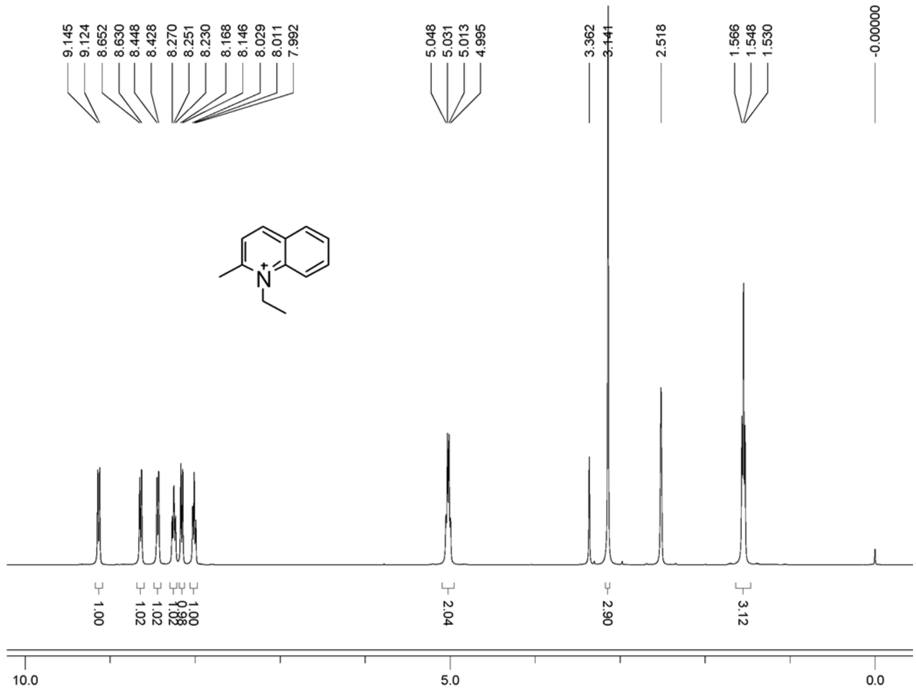


Fig. S9 [1]H NMR spectrum of compound **1**.

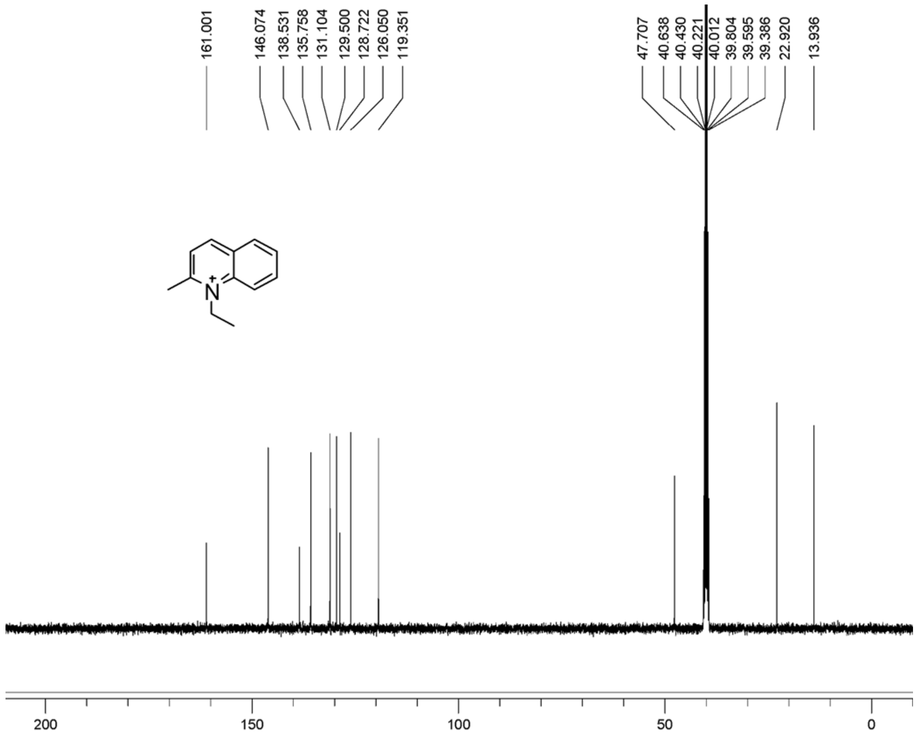


Fig. S10 [13]C NMR spectrum of compound **1**.

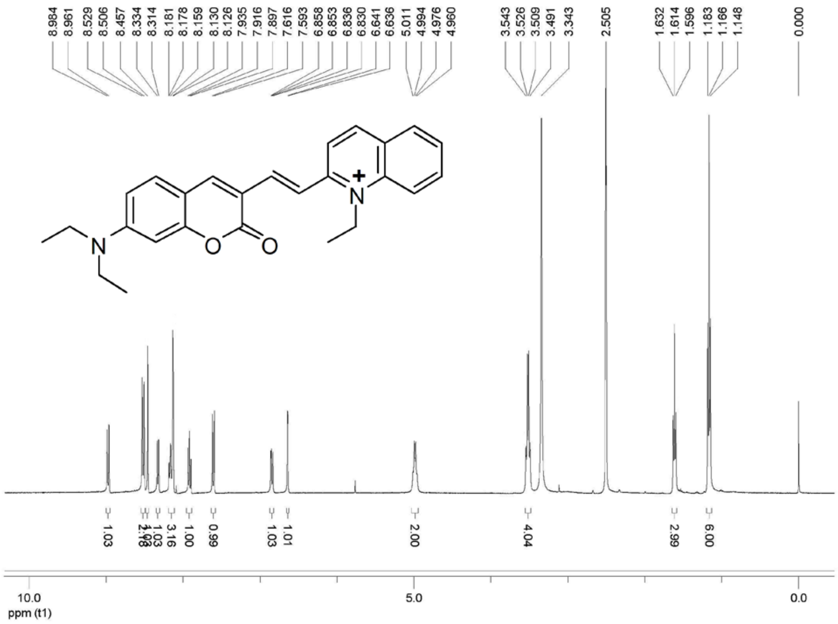


Fig. S11 [1]H NMR spectrum of the probe **2**.

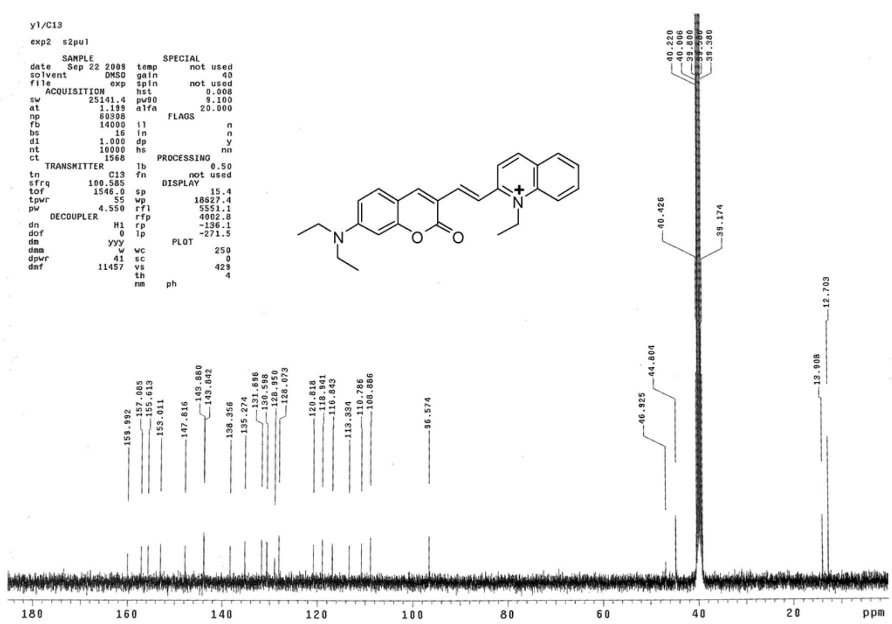


Fig. S12 [13]C NMR spectrum of the probe **2**.

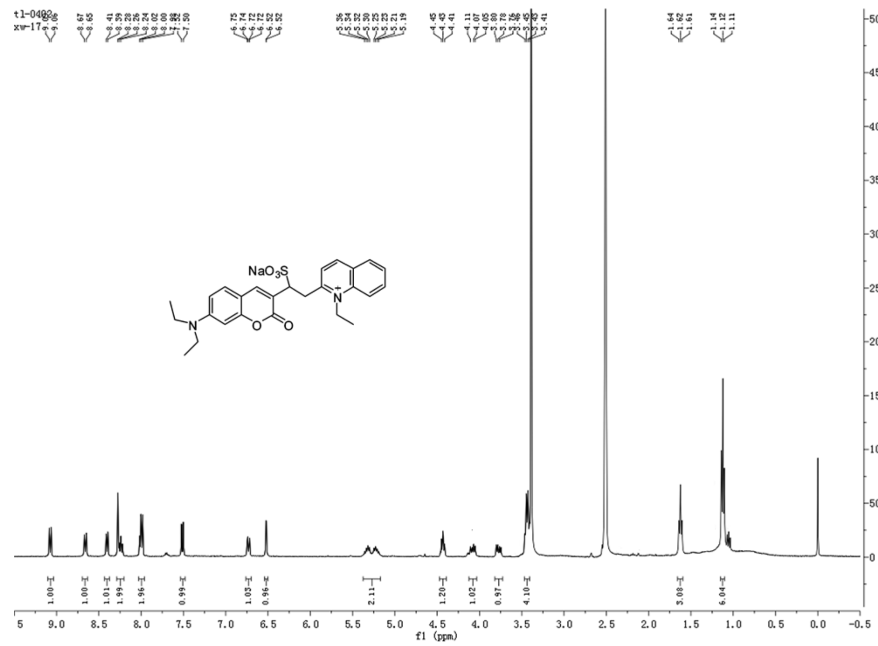


Fig. S13 ^{1}H NMR spectrum of the compound **3**.

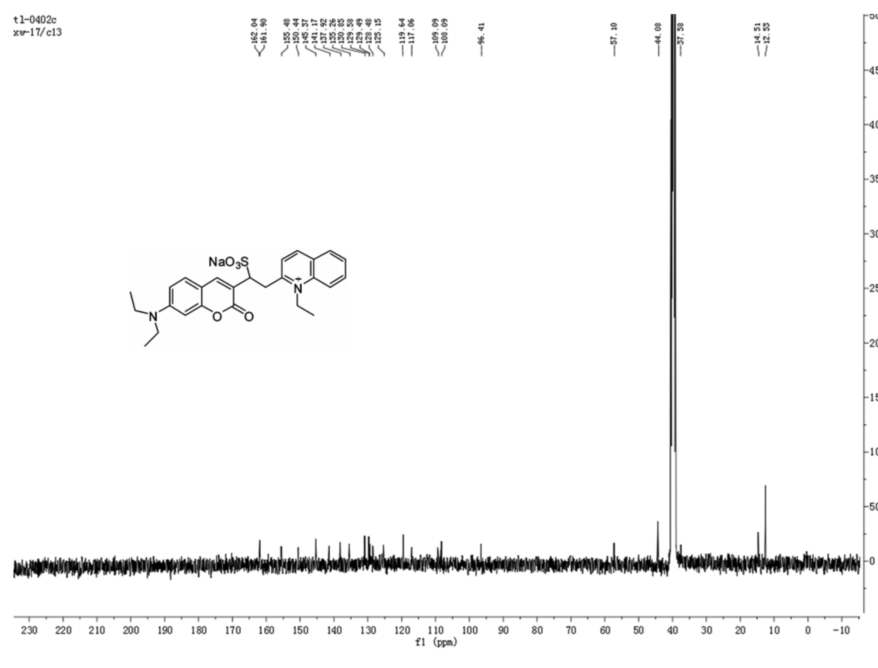


Fig. S14 ^{13}C NMR spectrum of the compound **3**.

References:

1. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian, Inc., Wallingford CT, GAUSSIAN 09 (Revision A.02), Gaussian, Inc., Pittsburgh, PA, 2009.