Supporting information

A reversible fluorescent probe for circulatory detecting of sulfites through a redox-based tandem reaction

Zhixue Liu, Shujing Guo, Jingyu Piao, Xin Zhou* and Xue Wu*^a

^a Department of Chemistry, Faculty of Science, Yanbian University, Yanji, Jilin, 133002 (P.R. China). E-mail: wuxue@ybu.edu.cn. Fax: +86-433-2732456; Tel: +86-433-2732299.

^b Research Center for Chemical Biology, Yanbian University, Yanji, Jilin, 133002 (P.R. China). E-mail: Hsinzh@yahoo.com.
Experimental Section

Materials and Methods

All chemicals and solvents were of analytic grade and bought from commercial sources without further purification. The solutions of anions were prepared from their sodium salts. The 1 was dissolved into DMSO to prepare the stock solutions with a concentration of 1 mM and the iodine solutions was also prepared stock in DMSO. All spectroscopic measurements were performed in PBS (10 mM, pH 7.4) buffer. UV-vis spectra were recorded on Perkin Elmer Lambda 3500 UV-vis spectra with a 1.0 cm quartz cell. PL spectra were conducted on Fluorescence Spectrophotometer (RF-540). MALDI-TOF mass spectra were recorded on a Shimadzu MALDI AXIMA-CFR+ Spectrometer. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AV-300 (300 MHz) spectrometer with TMS as the reference.

Detection limit

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of 1 without HSO$_3^-$ was measured by 10 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and the HSO$_3^-$ concentration could be obtained in the 0-60 µM ($R = 0.99191$). The detection limit is then calculated with the equation: detection limit = $3\sigma_b/m$, where $\sigma_b$ is the standard deviation of blank measurements; $m$ is the slope between intensity versus
sample concentration. The detection limit was measured to be 21 nM at S/N = 3.

**Chromatographic conditions and sample preparation**

The HPLC column was C18, 250 × 4.6 mm ID, with 5 μm particles (Thermo Scientific, USA) with a suitable guard-column. Mobile phase A was comprised of a mixture of methanol and water (70:30 [v/v]) and mobile phase B was comprised of a mixture of methanol and water (40:60 [v/v]). The elution programme was employed with a mobile phase flow-rate of 1 ml/ min. The column-oven and auto-sampler temperatures were set at 30 °C and 25 °C, respectively. The detection wavelength was 518 nm for A and 410 nm for B. Samples were prepared as used for detection of UV-Vis spectra and fluorescence spectra.

**Cell culture and fluorescence imaging experiment**

Hela cells were grown in SPP medium (1% proteose peptone, 0.2% glucose, 0.1% yeast extract, 0.003% EDTA ferric sodium salt) at 37 °C in 5% CO₂. Cells were plated on 6-well plate and allowed to adhere for 12 hours. Fluorescence imaging was performed with a Fluorescence Microscope with a 40×oil-immersion objective lens. For fluorescence microscopy experiments, cells were washed with PBS and then incubated with 1 (5 μM) in PBS for 30 min at 37 °C. Experiments to assess HSO₃⁻ uptake were performed in the same media supplemented with 100 μM NaHSO₃ for 30 min and H₂O₂ for 30 min at 37 °C, respectively. Cell imaging was investigated after washing cells with physiological saline. Emission was collected at 400~440 nm
(excited at 410 nm) for green channel and at 530∼550 nm (excited at 518 nm) for red channel.

**Synthesis**

![Chemical structure](image)

**Compound 1**: diethylaminocoumarin-3-aldehyde 3 (100 mg, 0.4 mmol) and compound 2 (46 mg, 0.4 mmol) were dissolved in ethanol (3 mL) and refluxed for 10 h. After being cooled to room temperature, the solid was filtered off and washed with ethanol to afford compound 1 (95 mg, yield: 70%). $^1$H NMR (300 MHz, CDCl₃) δ 8.89 (s, 1H), 8.56 (s, 1H), 7.42 (d, $J = 9.0$ Hz, 1H), 6.66 (d, $J = 9.0$ Hz, 1H), 4.36 (q, $J = 7.1$ Hz, 2H), 3.49 (q, $J = 7.1$ Hz, 4H), 1.38 (t, $J = 7.1$ Hz, 3H), 1.27 (t, $J = 7.1$ Hz, 6H).

MS: [M$^+$] at 340.5.

**Fig S1.** Fluorescent intensity changes of 1 (10 µM) as a function of the concentration of HSO$_3^-$: Each spectrum was recorded after 5 min. ($\lambda_{ex} = 446$ nm. Slits: 3/5 nm)
**Fig S2.** Time-course fluorescence response spectra of 1 (10 µM) towards HSO$_3^-$ (5 equiv.) in PBS buffer (pH 7.4, 10 mM, containing 30% DMSO). ($\lambda_{ex}$ = 446 nm. Slits: 3/5 nm)

**Fig S3.** (Left): Fluorescence spectra at 575 nm of 1 (10 µM) at varied pH values. (Right): The ratio of fluorescent intensities at 477 nm and 575 nm for probe 1 (10 µM) in the presence of NaHSO$_3$ (5 equiv.) at varied pH values. Each spectrum was recorded after 5 min. ($\lambda_{ex}$ = 446 nm. Slits: 3/5 nm)
**Fig S4.** The line relationship between the fluorescent intensity ratio of the 1 (10 µM at 477 nm and 575 nm) and the concentration of the HSO$_3^-$ in PBS buffer (pH 7.4, 10 mM, containing 30% DMSO). Each spectrum was recorded after 5 min. ($\lambda_{ex} = 446$ nm. Slits: 3/5 nm)

**Fig S5.** Fluorescence ratio of 1 (10 µM) with NaHSO$_3$ (50 µM) upon addition I$_2$ (0–60 µM) in PBS buffer (pH 7.4, 10 mM, containing 30% DMSO). Each spectrum was recorded after 5 min. ($\lambda_{ex} = 446$ nm. Slits: 3/5 nm)
**Figure S6.** Fluorescence spectra of 1/HSO$_3^-$ mixtures ([1] = 10 µM, [HSO$_3^-$] = 50 µM) upon addition of H$_2$O$_2$ (3mM) in PBS buffer (pH 7.4, 10 mM, containing 30% DMSO). Each spectrum was recorded after 5 min. ($\lambda_{ex}$ = 446 nm. Slits: 3/5 nm).

**Figure S7.** $^1$H NMR spectra of compound 1.
Figure S8. $^{13}$C NMR spectra of compound 1.

Figure S9. TOF-MS spectra of compound 1.