Supporting Information

A selective fluorescent probe for thiols based on α , β unsaturated acyl sulfonamide

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Contents

1. Material and Methods	1
1.1 Reagents and instruments	1
1.2 Synthesis of 1 and 2	2
1.2.1 Compound 2	
1.2.2 Compound 1	
1.3 Procedure for fluorescence measurement	
1.4 Fluorescence microscope experiment	
2. Supplementary Figures	
3. References	

1. Material and Methods

1.1 Reagents and instruments

All chemicals and solvents were purchased from commercial suppliers and applied directly in the experiment without further purification. The progress of the reaction was monitored by TLC on pre-coated silica plates (Merck 60 F254, 250 μ m thickness), and spots were visualized by KMnO₄, UV light or iodine. Merck silica gel 60 (70-200 mesh) was used for column chromatography purification. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker model DPX-400 MHz NMR spectrometer. Chemical shifts are reported in parts per million relative to internal standard tetramethylsilane (Si(CH₃)₄ = 0.00 ppm) or residual solvent peaks (CHCl₃ = 7.26 ppm). ¹H NMR coupling constants (*J*) are reported in Hertz (Hz), and multiplicity is indicated as the following: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublet). Mass spectra were obtained on PC Sciex API 150 EX ESI-MS system. High resolution mass spectrum was carried out on ABI Qstar Elite Q-TOF. Fluorescence signal was recorded with a FluoroMax-

4 fluorescence photometer. Fluorescence images were acquired using a Leica TCS SPE Confocal Scanning Microscope.

1.2 Synthesis of 1 and 2

1.2.1 Compound 2



Dansyl chloride (114 mg, 0.42 mmol) was added in a round bottom flask containing ammonium hydroxide (20 ml). The reaction mixture was stirred and refluxed for 2 hours. After the reaction mixture cooled down, the aqueous solution was extracted by DCM twice. The combined DCM layer was subsequently washed with deionized water and brine, and dried with anhydrous MgSO₄. The solvent was then evaporated under reduced pressure, and the crude product was purified by column chromatography with DCM-hexane (9:1) to afford a yellow solid (72 mg, 68% yield). ¹H NMR (400 MHz, CDCl₃) : 8.54 (d, J = 8.4 Hz, 1H), 8.31-8.24 (m, 2H), 7.63-7.51 (m, 2H), 7.21 (d, J = 7.6 Hz, 1H), 4.90 (br, 2H), 2.88 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) : 152.2, 143.4, 130.6, 129.3, 128.7, 128.1, 123.3, 120.0, 118.4, 115.2, 45.4; ESI-MS: m/z 251.3 [M+H]⁺.

1.2.2 Compound 1



Dansyl amide (72 mg, 0.29 mmol) was dissolved in 8 ml DCM in a round bottom flask. Acryloyl chloride (93 µl, 1.15 mmol) in 3 ml DCM and 10 eq triethylamine (401 µl, 2.9 mmol) were added to the solution at 4 °C in dropwise manner. The reaction mixture was stirred for 10 min at 4 °C and then for 4 hours at room temperature. The solvent was removed under reduced pressure. The crude product was dissolved in DCM and washed with H₂O and brine. The DCM layer was dried over anhydrous MgSO₄. The resulting crude reaction mixture was evaporated and purified by column chromatography with DCM/methanol (9:1) to give 1 as a pale yellow solid (77 mg, 88% yield). ¹H NMR (400 MHz, CDCl₃) : 8.60 (d, J = 8.4 Hz, 1H), 8.53 (d, J = 7.2 Hz, 1H), 8.28 (d, J = 8.4 Hz, 1H), 7.60-7.52 (m, 2H), 7.16 (d, J = 7.6 Hz, 1H), 6.31 (d, J = 17.2 Hz, 1H), 6.15-6.08 (dd, J = 17.2, 10.4 Hz, 1H), 5.70 (d, J = 10.4 Hz, 1H), 2.88 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) : 162.9, 152.2, 133.1, 132.3, 132.0, 131.4, 129.8, 129.6, 128.9, 128.4, 123.3, 117.8, 115.2, 45.4; ESI-MS: m/z 305.6 [M+H]⁺.

1.3 Procedure for fluorescence measurement

Probe 1 was diluted in NaH₂PO₄-NaHPO₄ (PBS, pH = 7.4) buffer to afford the final concentration of 20 μ M. The amino acids were prepared as stock solutions of 100 mM in Milli Q water. Appropriate amount of cysteine and other amino acids were added to separate portions of the solution and mixed thoroughly. The reaction mixture was shaken uniformly before emission spectra were measured. Measurement of the fluorescence emission spectra was conducted using a FluoroMax-4 fluorescence photometer with a 10 mm quartz cuvette. The excitation wavelength was set at 350 nm. The emission wavelength was set in the range of 400nm to 650nm. The slit widths of excitation and emission wavelength were both set at 5 nm.

1.4 Fluorescence microscope experiment

A549 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and appropriate amount of antibiotic (penicillin and streptomycin). Approximately 10^5 cells were seeded in a confocal dish (35 mm) with the medium at 37 °C. To prepare the experiment setup, the cells to be tested were allowed to adhere to the dish for 24 hrs. They were then incubated with probe $1(5 \times 10^{-5} \text{ M})$ for different time intervals at 37 °C. Fluorescence images were taken by a Leica TCS SPE Confocal Scanning Microscope equipped with a UV filter.

2. Supplementary Figures



Fig. S1. Photograph of probe 1 reacting with cysteine in PBS buffer (10 mM, pH = 7.4). (a) probe 1 with cysteine; (b) Probe 1 only.



Fig. S2. ¹H NMR spectral comparison between probe 1 and its β -mercaptoethanol adduct. a) probe **1** only; b) β -mercaptoetahnol adduct.



Fig. S3. Plot of the normalized fluorescence intensity $(I_{min} - I)/(I_{min} - I_{max})$ at emission wavelength of 520 nm as a function of cysteine concentration. An excellent linear relationship is derived (R = 0.9945). The detection limit is $3\sigma/m$ as reported in previous literature.^{S1} σ is the standard deviation of blank measurements; m is the slope between fluorescence intensity and cysteine concentration. The detection limit was calculated as 1.67×10^{-6} M when S/N = 3.



Fig. S4. Fluorescence response of different concentrations of probe 1 with the addition of excess cysteine (200 μ M) in PBS buffer. From left to right: 1. 0.625 μ M, 2. 1.25 μ M, 3. 2.5 μ M, 4. 5 μ M, 5. 10 μ M.



Fig. S5. Fluorescence intensity changes at 520 nm during the reaction of probe **1** and 10 eq cysteine in PBS buffer (pH = 7.4). The k_{obs} (pseudo-first-order rate) was determined by fitting the fluorescence intensity data into the following equation: Ln ($F_{max} - F_t$) = Ln $F_{max} - k_{obs} \times t$.^{S2}



Fig. S6. Fluorescence response of probe 1 with Na₂S and NaHS (400 μ M) after incubation for 12 hours in PBS buffer (pH = 7.4).



Fig. S7. pH dependent experiments of probe **1**. The reaction of probe **1** and cysteine was performed with buffers at different pH values (pH 2-10).



Fig. S8. Fluorescence microscopy experiments to detect thiol with A549 cells. (a) brightfield image of cells incubated with probe **1** (b) fluorescence image of cells incubated with probe **1** (c)) brightfield image of cells preincubated with NEM (1 mM) for 1h followed by addition of probe **1** (d) fluorescence image of cells preincubated with NEM (1 mM) for 1h followed by addition of probe **1**.



Figure S9. HPLC profile of Probe **1**. The gradient program is set as the following: 20-100% ACN over 10 min. The compound is eluted at around 8 min.



Figure S10. HR-MS spectrum of probe 1.

3. References

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