Supporting Information for:

Coumarin-Malonitrile Conjugate as a Fluorescence Turn-On Probe for Biothiols and its Cellular Expression

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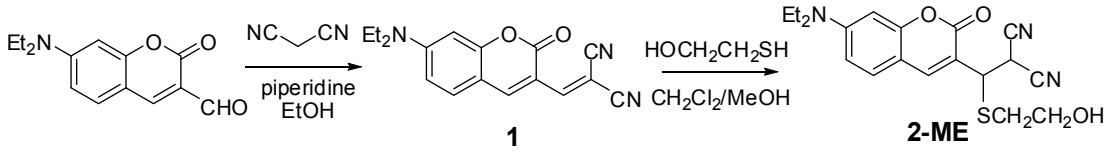
Experimental Section

General methods. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Chromatography was carried out on silica gel 60 (230-400 mesh ASTM; Merck). Thin layer chromatography (TLC) was carried out using Merck 60 F_{254} alumina plates with a thickness of 0.25 mm. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker 300 or Varian 200. Mass spectra were obtained using a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL). UV absorption spectra were obtained on Agilent 8453 Double Beam UV/VIS Spectrometer. Fluorescence emission spectra were obtained using JASCO FP-6500 Spectrofluorometer.

Synthesis



Compound 1 To a solution of coumarin aldehyde[1] (240 mg, 1.00 mmol) in EtOH (5 mL) was added 2 ~ 3 drops of piperidine. Resulting clear red solution was further stirred at rt for 5 h to afford red precipitates, which were filtered off, washed with EtOH, and dried in air to obtain the desired product in 75 % yield.
^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 8.62 (s, 1H), 8.02 (s, 1H), 7.63 (d, ^3J = 9.0 Hz, 1H), 6.87 (dd, ^3J = 9.0 Hz, ^4J = 2.1 Hz, 1H), 6.68 (d, ^4J = 2.1 Hz, 1H), 3.55 (q, ^3J = 6.0 Hz, 4H), 1.16 (t, ^3J = 6.0 Hz, 6H).
^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 159.57, 158.52, 154.76, 153.98, 146.16, 133.50, 115.84, 114.45, 111.76, 109.91, 108.79, 97.22, 75.66, 45.35, 12.93.

[1] Kim, T.-K.; Lee, D.-N.; Kim, H.- J. *Tetrahedron Lett.* 2008, **49**, 4879.

Compound 2-ME To a solution of **1** (45 mg, 0.15 mmol) in 2mL of CH_2Cl_2/MeOH (v/v 4:1) was added excess amount of 2-mercaptoethanol (43 mg, 0.55 mmol) and the reaction mixture was further stirred for 12 h at rt. After all the volatiles were evaporated under the reduced pressure, the residue was purified by column chromatography (EtOAc : Hexane = 2:1, v/v, R_f = 0.30) to afford the desired product in 50 yield.
^{1}H NMR (DMSO-d_6, 200 MHz) δ (ppm): 7.98 (s, 1H), 7.51 (d, ^3J = 9.0 Hz, 1H), 6.73 (dd, ^3J = 9.0 Hz, ^4J = 2.0 Hz, 1H), 6.58 (d, ^4J = 2.0 Hz, 1H), 5.47 (d, ^3J = 8.6 Hz, 1H), 4.76 (m, 1H), 3.54 (q, ^3J = 6.0 Hz, 4H), 3.50 (m, 2H), 2.77 (m, 2H), 1.11 (t, ^3J = 6.0 Hz, 6H).
MS (FAB$^+$, Glycerol): *m*/*z* obs'd 372 ([M+H]$^+$, 32 %).

Preparation for fluorescent study

A stock solution (10 mM) of **1** in DMSO was prepared and used by dilution in aqueous DMSO solution for *in vitro* and *in vivo* fluorescence experiments. In a typical experiment, test solutions were prepared by placing 2 μL of the probe stock solution into a test tube, adding an appropriate amount of each amino acid, and diluting the solution to 2 mL with buffered aqueous DMSO (0.10 M HEPES, pH 7.4). Normally, excitation was at 394 nm. Both the excitation and emission slit widths were 3 nm × 3 nm. Fluorescence spectra were monitored 1 h after addition of amino acids.

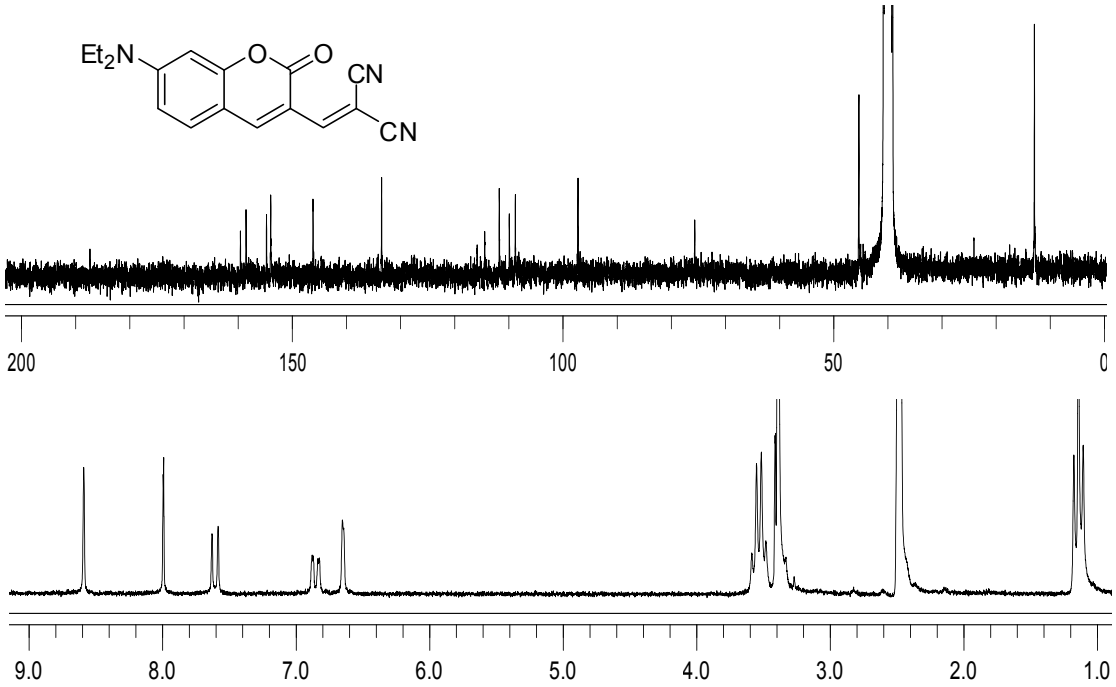


Fig. S1. ^{1}H and ^{13}C NMR spectra of **1** in DMSO-d_6.

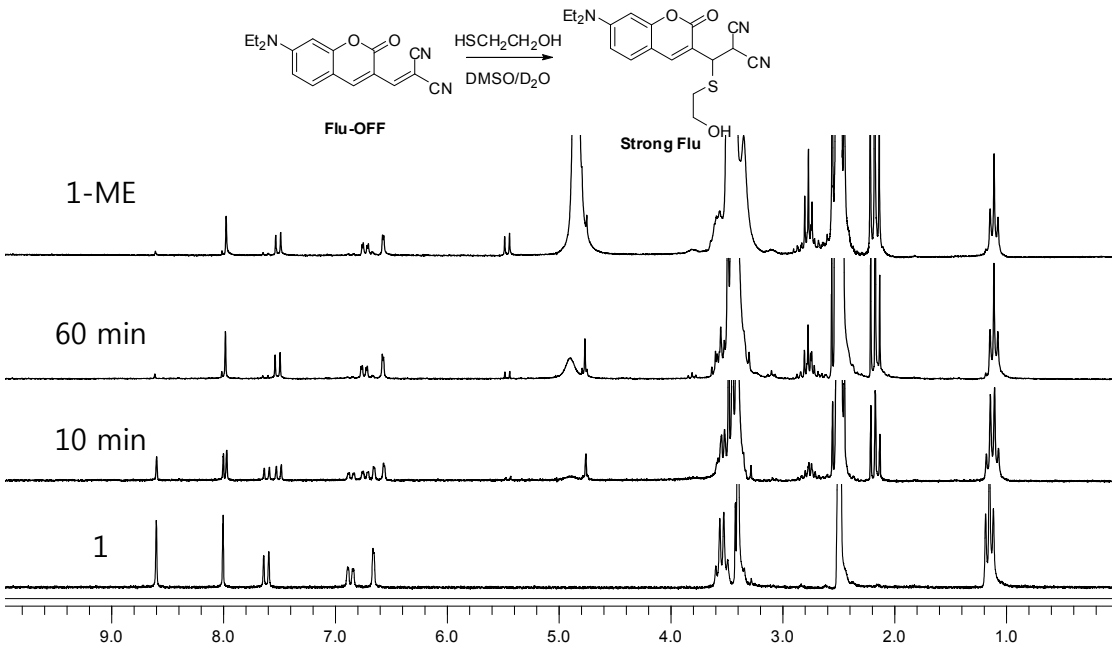


Fig. S2. Full ^{1}H NMR spectra of **1** (10 mM) upon addition of ME (1.8 equiv) in DMSO-d_6/D_2O (v/v, 10:1).

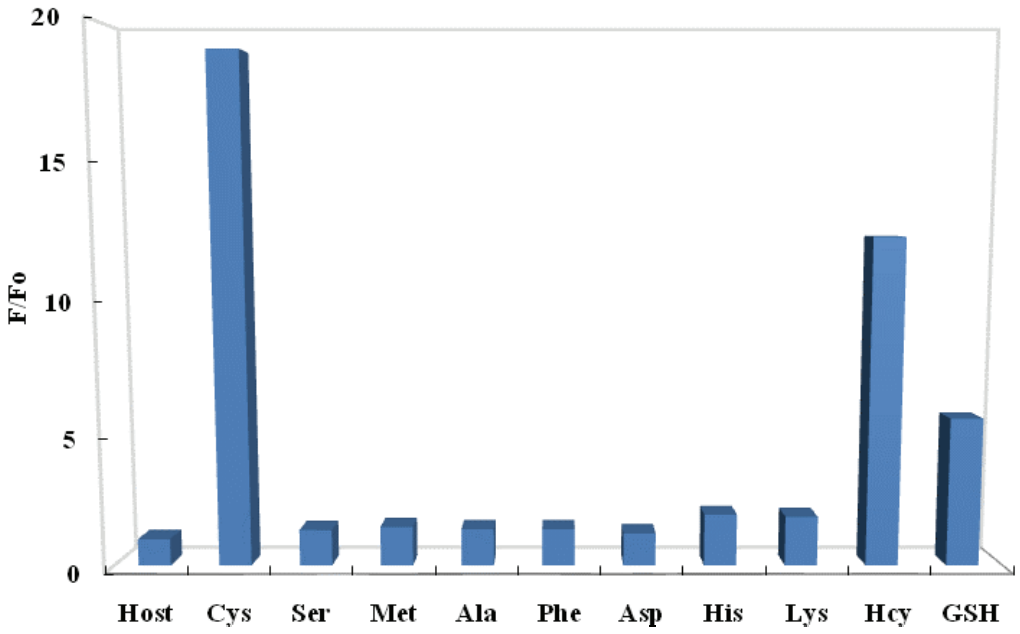


Fig. S3. Relative fluorescence intensities of **1** (20 μM) in DMSO-HEPES buffer (1:2, v/v; 0.10 M pH 7.4) upon addition of various amino acids and biothiols (Cys, Hcy, GSH). F_0 and F are intensities at λ_{em} 475 nm (λ_{ex} 394 nm) for **1** and **1** + AA, respectively.

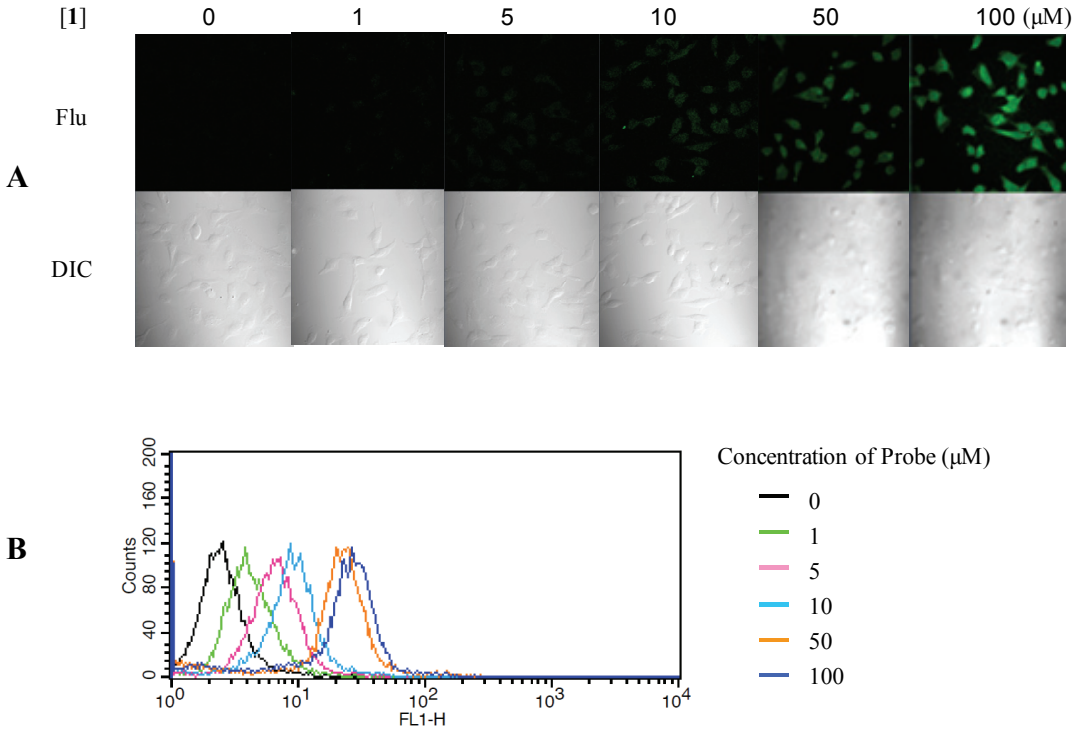


Fig. S4. Cellular expression of **1** in HeLa cells. (A) Concentration-dependent cellular images of **1**, (B) their FACS analyses.

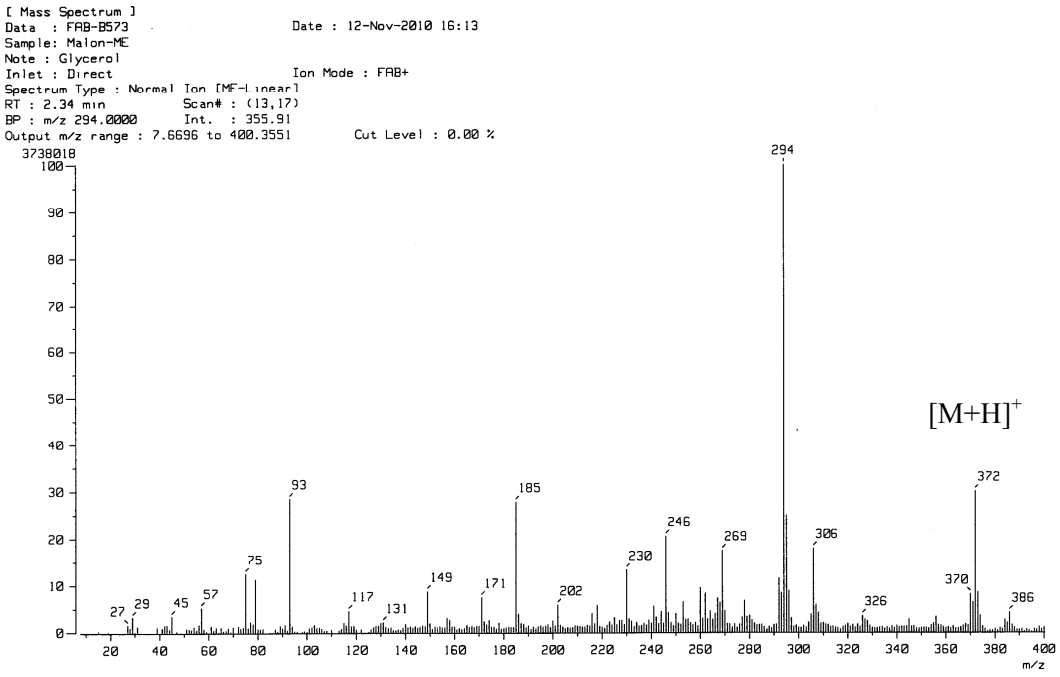


Fig. S5. Mass spectral data for **2**-ME.