SUPPORTING INFORMATION

Rapid and selective detection of cysteine over homocysteine and glutathione by a simple and effective coumarin-based fluorescent probe

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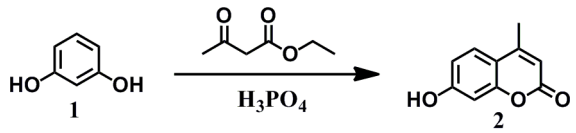
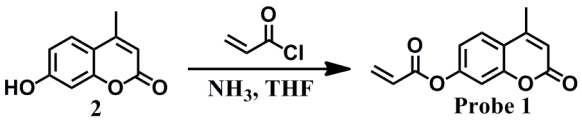
1. General information

Unless otherwise noted, materials were purchased from commercial suppliers and used without further purification. All the solvents were purified and dried according to general methods. ^{1}H NMR spectra were recorded on a Bruker AVIII-400 MHz spectrometer. Chemical shifts (in ppm) were determined by reference to the residual solvent peak (Acetone-d_6: 2.05 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constants (Hz) and integration. ^{13}C NMR spectra were recorded on the same NMR spectrometer. Chemical shifts (in ppm) were determined by reference to the residual solvent peak (Acetone-d_6: 30.92 ppm). High resolution mass spectra (HRMS) were measured with Thermo (orbitrap Elite). Absorption spectra were measured using a Thermo (BioMate 3S) UV/Vis spectrophotometer. Fluorescence measurements were carried out with a F97pro fluorospectrophotometer.

2. Synthesis of the probe 1



7-Hydroxy-4-methylcoumarin 2 To the mixture of 3-hydroxylphenol (2.2 g, 20 mmol) and ethyl acetoacetate (2.6 mL, 20 mmol) was added concentrated phosphoric acid (15 mL, 85%). After the reaction was stirred for 12 h at room temperature, the reaction mixture was poured into 30 mL water. The crude product was collected by filtration and further purified by recrystallization in ethanol to afford the 7-Hydroxy-4-methylcoumarin **2** as a white crystal (yield: 82%). **^{1}H-NMR** (400 MHz, acetone-d_6): δ = 2.41 (s, 3 H), 4.65 (s, 1 H), 6.07 (s, 1 H), 6.74 (d, *J* = 2.4 Hz, 1 H), 6.85 (dd, *J* = 8.8, 2.4 Hz, 1 H), 7.61 (d, *J* = 8.8, Hz, 1 H) ppm; **^{13}C-NMR** (100 MHz, acetone-d_6): δ = 17.6, 102.4, 110.9, 112.6, 112.8, 126.4, 152.9, 155.5, 160.1, 161.0 ppm.



7- Yl-acrylate-4-methylcoumarin Probe 1 To the solution of compound **2** (1.76 g, 10 mmol) in a anhydrous tetrahydrofuran (40 mL) was added dropwise triethylamine (2.1 mL, 15 mmol) at 0 ^{o}C. After the reaction was stirred for 0.5 h, then acryloyl chloride (1.2 mL, 15 mmol) was added dropwise to the above solution at 0 ^{o}C, then the mixture continues to react for 6 h. The solvent was evaporated and the residue was purified by column chromatography (silica gel; petroleum/ethyl acetate 15/1) to provide the 7-yl-acrylate-4-methylcoumarin Probe **1** as a white solid (yield: 60%). **^{1}H-NMR** (400 MHz, acetone-d_6): δ = 3.51 (s, 3 H), 6.15 (dd, *J* = 10.4, 1.2 Hz, 1 H),

6.31(s, 1 H), 6.41 (dd, *J* = 17.2, 10.4 Hz, 1 H), 6.61(dd, *J* = 17.2, 1.2 Hz, 1 H), 7.20 (d, *J* = 2.0 Hz, 1 H), 7.23(d, *J* = 2.0 Hz, 1 H), 7.84(d, *J* = 8.4 Hz, 1 H) ppm; **^{13}C-NMR** (100 MHz, acetone-d_6): δ = 17.7, 110.0, 114.1, 117.9, 118.0, 126.1, 127.5, 133.0, 152.4, 153.2, 154.4, 159.4, 163.5 ppm; **HRMS** (ESI) m/z calcd for $C_{13}H_{10}O_4$(M+H): 231.0652. Found: 231.0656, error: 1.7 ppm.

3. Assay for cytotoxic activity of the probe 1

The cytotoxic activity was assessed by the well-known MTT assay, which is based on the formation of a purple formazan dye from the reduction of MTT mainly by the mitochondrial succinate dehydrogenase of active cells. HeLa cells (5×10^4/mL) were seeded in 96-well flat microtiter plates for adherence for 24 h, then the cells were incubated with assigned concentrations of probe **1** for another 24 h. Thereafter, 100 μL medium with MTT solution was supplemented to each well and plates were incubated for 4 h at 37 ℃ in the dark. The culture medium was then removed followed by the addition of 100 μL DMSO. The absorbance was read at 490 nm using a Thermo (Multiskan MK3) microplate reader. The percentage of cell viability was calculated relative to control wells designated as 100% viable cells.

4. Fluorescence image of probe 1 in HeLa cells

HeLa cells (5×10^4/mL) were seeded in 6-well flat microtiter plates for adherence for 24 h. Cells were incubated with probe **1** (10 μM) for 30 minutes, and then washed with PBS for 3 times. In the *N*-ethyl maleimide (NEM) experiment, cells were pre-incubated with NEM (1 mM) for 30 min. Cells were then washed with PBS before fluorescence images were acquired. Fluorescence images of the cells were obtained by a fluorescence microscope.

5. Figures S1 and S2

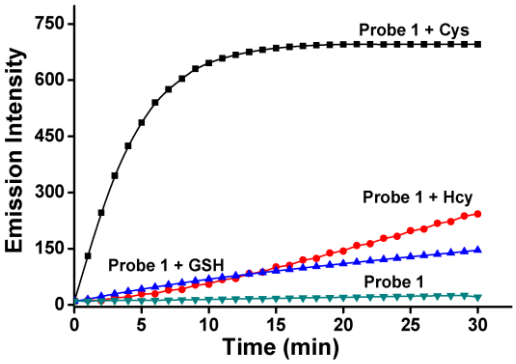


Fig. S1 Time-dependent fluorescence spectra of probe 1 (10 μM) with presence of Cys, Hys and GSH in aqueous solution (pH 7.4 PBS, containing 1% DMSO) at room temperature, λ_{ex} = 340 nm..

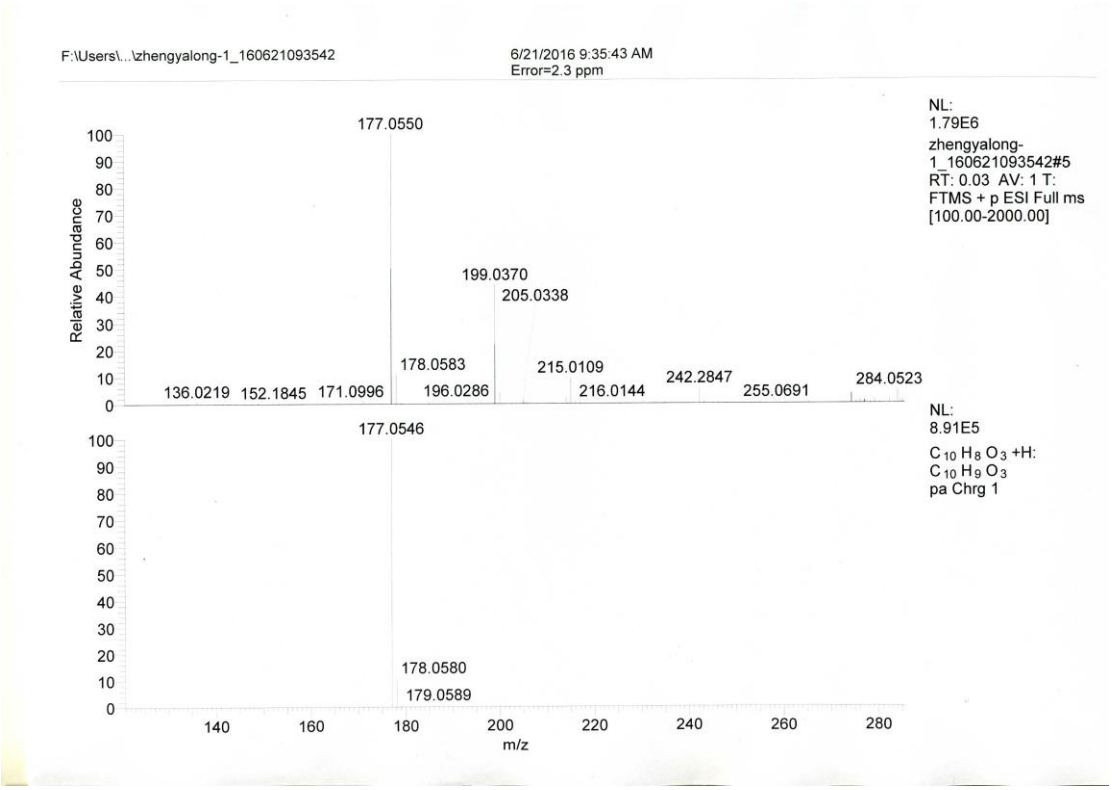
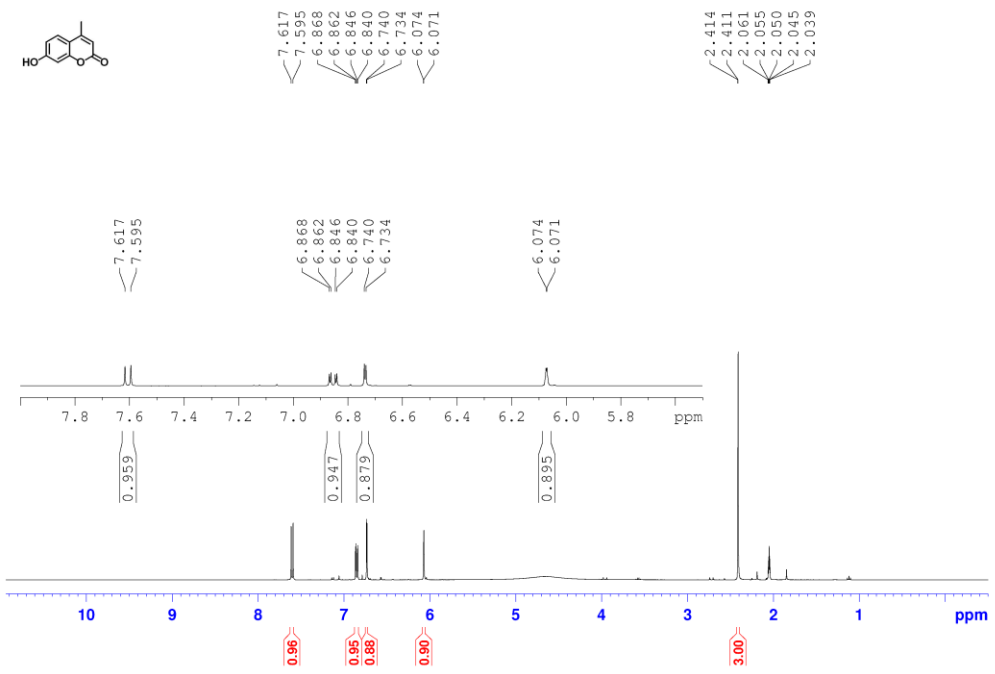
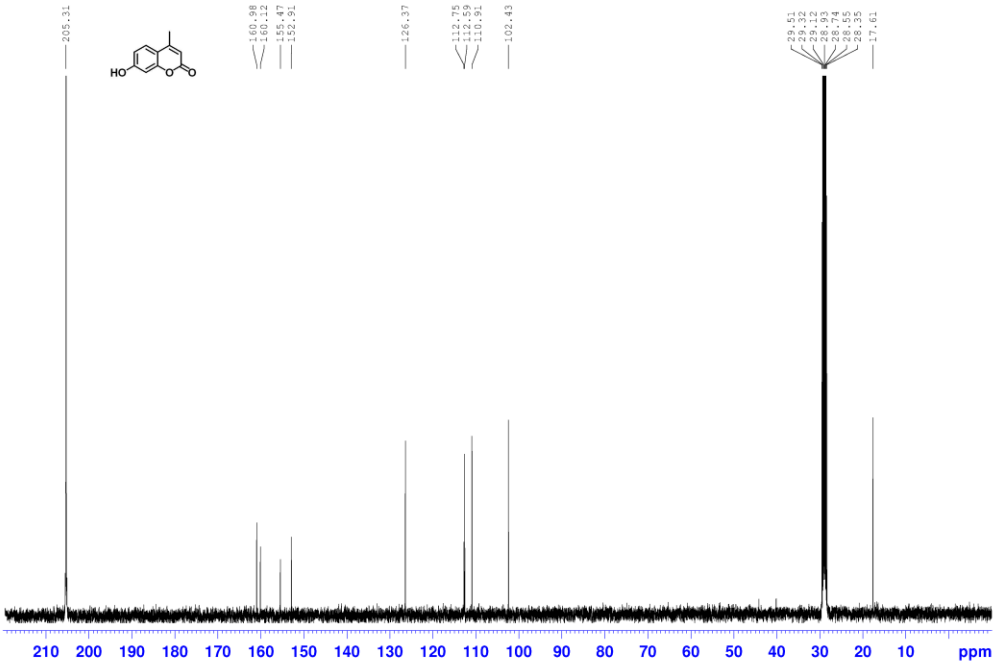


Fig. S2 The HRMS chart of probe **1** in the presence of Cys.

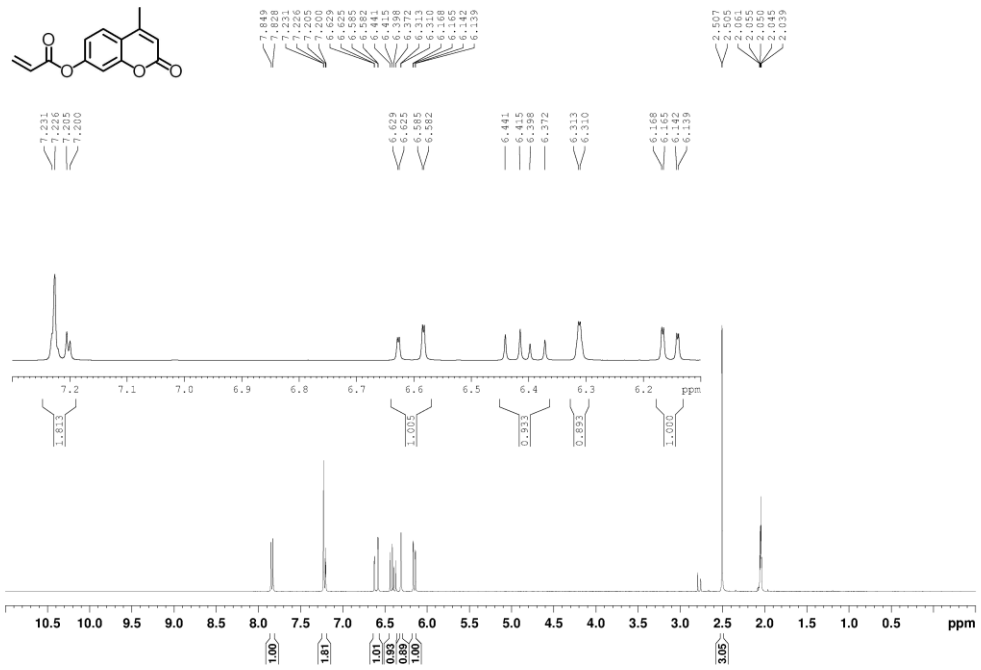
6. ^{1}H NMR, ^{13}C NMR and HRMS of compounds

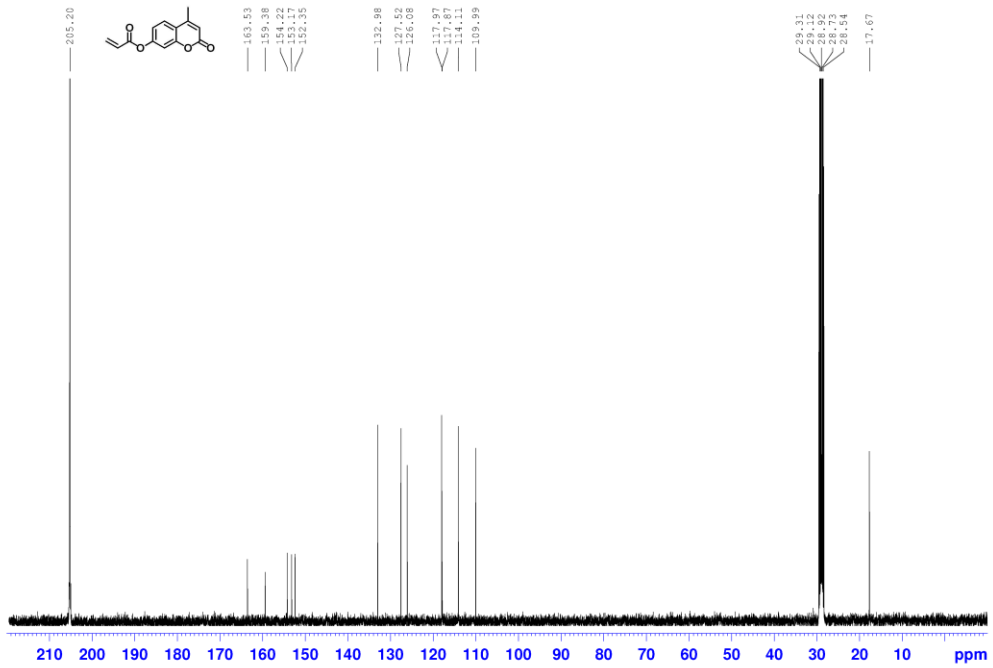
6.1. ^{1}H NMR and ^{13}C NMR of compound 2





6.2. ^{1}H NMR, ^{13}C NMR and HRMS of probe 1

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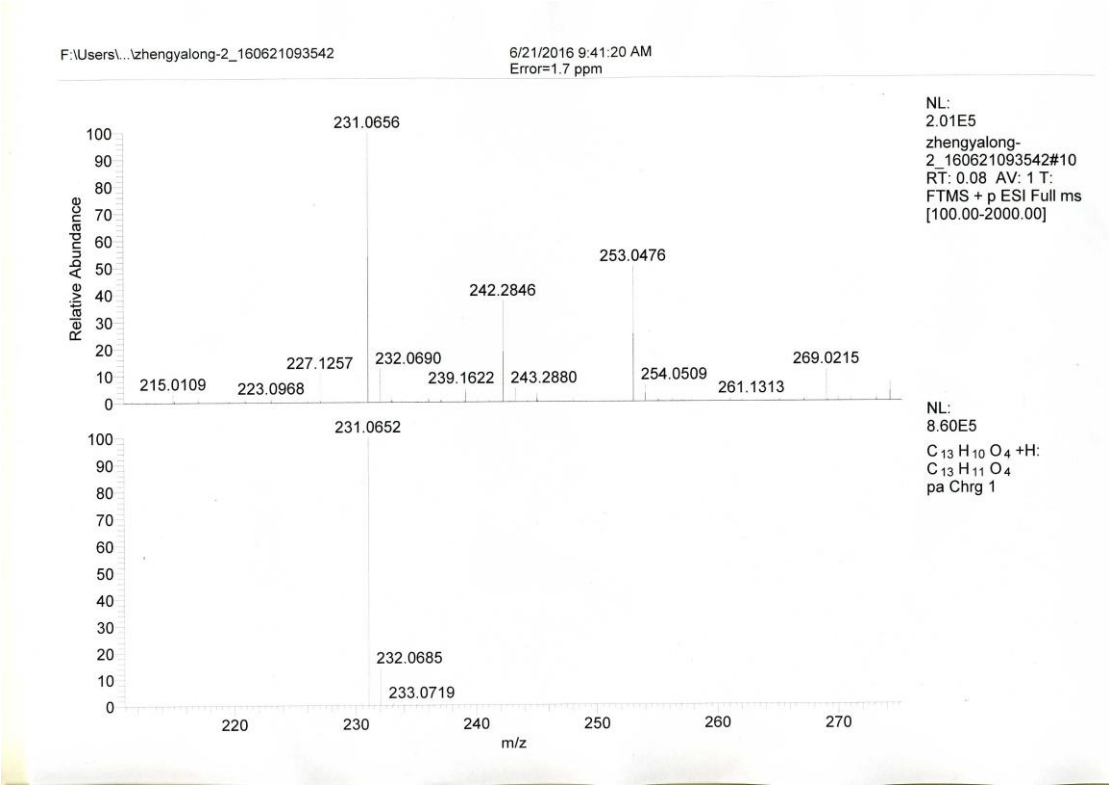
159.38
154.22
153.17
152.35
132.98
127.52
126.08
117.97
117.87
114.11
109.99
29.31
29.12
28.92
28.73
28.54
17.67
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm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227.1257
232.0690
269.0215
215.0109
223.0968
239.1622
243.2880
254.0509
261.1313
NL:
8.60E5
C13 H10 O4 +H:
C13 H11 O4
pa Chrg 1
231.0652
232.0685
233.0719
Relative Abundance
m/z
220 230 240 250 260 270