SUPPORTING INFORMATION

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

Yan-Fei Kang,*a Hai-Xia Qiao,a Ya-Li Meng,a Sai-Jin Cui,a Ya-Jun Han,a Zhi-Yong, Wu,b Jie Wu,a Xiao-Hui Jia,a Xiao-Lei Zhanga and Ming-Yan Daia

*aCollege of Laboratory Medicine, Hebei North University, 11 Diamond Street South, Zhangjiakou, Hebei, 075000, People’s Republic of China. Fax: +86-313-4029275;
Tel: +8618931319293. E-mail: kanyanfei172@163.com

bCollege of Science, Hebei North University, 11 Diamond Street South, Zhangjiakou, Hebei, 075000, China.
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-hydroxyphenol (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-$d_6$): $\delta$ = 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-$d_6$): $\delta$ = 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 °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 °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-yI-acrylate-4-methylcoumarin Probe 1 as a white solid (yield: 60%). $^1$H-NMR (400 MHz, acetone-$d_6$): $\delta$ = 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; \textsuperscript{13}C-NMR (100 MHz, acetone-d\textsubscript{6}): \delta = 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; \textbf{HRMS} (ESI) m/z calcd for C\textsubscript{13}H\textsubscript{10}O\textsubscript{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\textsuperscript{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 \textmu L medium with MTT solution was supplemented to each well and plates were incubated for 4 h at 37 °C in the dark. The culture medium was then removed followed by the addition of 100 \textmu L 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\textsuperscript{4}/mL) were seeded in 6-well flat microtiter plates for adherence for 24 h. Cells were incubated with probe 1 (10 \textmu M) 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

![Fluorescence spectra](image)

Fig. S1 Time-dependent fluorescence spectra of probe 1 (10 \mu M) with presence of Cys, Hys and GSH in aqueous solution (pH 7.4 PBS, containing 1% DMSO) at room temperature, \lambda_{ex} = 340 nm.
Fig. S2 The HRMS chart of probe 1 in the presence of Cys.

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

6.1. \textsuperscript{1}H NMR and \textsuperscript{13}C NMR of compound 2
6.2. $^1$H NMR, $^{13}$C NMR and HRMS of probe 1