SUPPORTING INFORMATION

Selective detection of cysteine over homocysteine and glutathione by a simple and effective probe

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1. General information

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 (CCl$_3$D: 7.27 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 (CCl$_3$D: 77.05 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 probe 1

3-Hydroxy-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (2) 1,3-Dihydroxybenzene (0.55 g, 5 mmol) was dissolved in dioxane (15 mL), then, the ethyl 2-cyclohexanonecarboxylate (0.94 g, 5.5 mmol) was added to the above solution. Soon afterwards, the concentrated sulfuric acid (8 drips) was dropwise added. Next, the mixture was stirred for 4 h at 60 °C. Lastly, the solvent was evaporated and the residue was washed with water to provide the crude product 2, which was directly used for the next step reaction.

6-Oxo-7,8,9,10-tetrahydro-6H-benzo[c]chromen-3-yl acrylate (Probe 1) Potassium carbonate (0.28 g, 2.04 mmol), compound 2 (0.37 g, 1.7 mmol) were added to dichloromethane (50 mL). After the reaction was stirred for 0.5 h, acryloyl chloride (0.18 g, 2.04 mmol) was added dropwise to the above solution at 0 °C, and the mixture continues to react for 12 h. The reaction was quenched with water and the mixture was extracted by ethyl acetate and dried by sodium sulfate. The solvent was evaporated and the residue was purified by column chromatography (silica gel; petroleum ether / ethyl acetate 5/1) to provide the 6-oxo-7,8,9,10-tetrahydro-6H-benzo[c]chromen-3-yl acrylate probe 1 (yield: 50%). $^1$H-NMR (400 MHz, CCl$_3$D): $\delta$ = 1.85 (m, 4 H), 2.59 (t, $J$ = 6.0 Hz, 2 H), 2.78 (t, $J$ = 6.0 Hz, 2 H), 6.07 (dd, $J$ = 10.4, 1.2 Hz, 1 H), 6.34 (dd, $J$ = 17.2, 10.4 Hz, 1 H), 6.65 (dd, $J$ = 17.2, 1.2 Hz, 1 H), 7.09
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(dd, J = 8.4, 2.4 Hz, 1 H), 7.14 (d, J = 2.4 Hz, 1 H), 7.58 (d, J = 8.4 Hz, 1 H) ppm;
$^{13}$C-NMR (100 MHz, CCl$_4$D): $\delta$ = 21.3, 21.5, 24.0, 25.3, 110.1, 117.8, 118.2, 123.4,
124.0, 127.4, 133.5, 146.7, 151.7, 152.5, 161.5, 164.0 ppm; HRMS (ESI) m/z calcd for C$_{16}$H$_{14}$O$_4$(M+H): 271.0965. Found: 271.0959, error: 2.2 ppm.

3. Rate constant analysis

The pseudo-first-order rate constants was detected in view of the large excess of Cys, Hcy, or GSH (150 equiv.) over probe 1 (10 µM) in an aqueous PBS buffer (pH 7.4, containing 20% acetonitrile) at room temperature. The pseudo-first-order rate constant $k'$ was calculated, according to below equation: $\ln[(F_{\text{max}} - F_t)/F_{\text{max}}] = -k't$
Where $F_t$ and $F_{\text{max}}$ denote the fluorescence intensities at 454 nm at time t and the maximum value obtained with the reaction saturation, respectively, and $k'$ is the pseudo-first-order rate constant.

4. Cytotoxicity evaluation of probe 1

The cytotoxicity of probe 1 was evaluated by the well-known MTT method in Hela 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 with 24 h. Thereafter, 100 µL mixture of medium and MTT(10:1 v/v) was supplemented to each well and plates were incubated for 4 h at 37 °C in the dark. The culture medium was then removed and the addition of 100 µL DMSO was added. The absorbance was read at 490 nm by Thermo (Multiskan MK3) microplate reader. The percentage of cell viability was calculated relative to control wells designated as 100% viable cells.

5. Bioimaging 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 1 h, and then washed with PBS for 3 times. In addition, in the N-ethyl maleimide (NEM) experiment, cells were pre-incubated with NEM (1 mM) for 30 min. Bioimaging were acquired after cells were then washed with PBS. Fluorescence images of probe 1 were got by a fluorescence microscope.

6. Figures and scheme
Fig. S1 The emission intensity response for the acetonitrile content, λex = 370 nm. Grey bar: probe 1 (10 μM); Black bar: probe 1 (10 μM) + Cys (100 μM).

Fig. S2 Time-dependent Uv-vis absorbance Change of probe 1 for Cys in an aqueous PBS buffer (pH 7.4, containing 20% acetonitrile) at room temperature. The absorbance at 370 nm was gradually increased with the time extension.

Fig. S3 Concentration-dependent fluorescent intensity response of probe 1 for Cys in
an aqueous PBS buffer (pH 7.4, containing 20% acetonitrile) at room temperature, \( \lambda_{\text{ex}} = 370 \) nm. 3s/m = 70 nM, s represents the standard deviation of 20 times blank; m = 1.2596.

**Fig. S4** pH-Dependent fluorescent intensity changes of probe 1 and mixture of probe 1 and Cys at room temperature, \( \lambda_{\text{ex}} = 370 \) nm.

**Fig. S5** High resolution mass spectrometry analysis for reaction between probe 1 and Cys in an aqueous PBS buffer (pH 7.4, containing 20% acetonitrile) at room temperature.

**Fig. S6** The measurement of *pseudo*-first-order rate constant \( k' \) with a large excess of Cys, Hcy, or GSH (150 equiv.) over probe 1 (10 \( \mu \)M) in an aqueous PBS buffer (pH 7.4, containing 20% acetonitrile) at room temperature.
**Fig. S7** The cell viability analysis with incubation of appointed concentration.

**Scheme S1** Proposed reaction mechanism of probe 1 with Cys.

**7.** $^1$H NMR, $^{13}$C NMR and HRMS of probe 1