Supporting Information

A novel 3-hydroxychromone fluorescence probe for hydrogen sulfide based on an excited-state intramolecular proton transfer mechanism

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Contents

1. Spectra

2. Reaction mechanism

3. Detection limit

4. MTT assay

5. $^1$H NMR, $^{13}$C NMR and HRMS analyses

6. Table S1
1. Spectra

**Fig. S1** Time-dependent fluorescent intensity changes of probe A (20μM) at 537nm upon addition of NaSH(200μM) in PBS buffer (20 mM, pH 7.4) with 20% DMSO and 3 mM CTAB at 37 °C.

**Fig. S2** The photo-stability of probe A. The fluorescent change at 537nm of probe A (20μM) upon addition of NaSH(200μM) in PBS buffer (20 mM, pH 7.4) with 20% DMSO and 3 mM CTAB at 37 °C.
2. Reaction mechanism

Fig. S3  HRMS spectrum (ESI negative ion mode) of probe A after treatment with NaHS.

3. Detection limit

The physiological relevant H$_2$S concentration is estimated ranging from nano- to millimolar levels.\textsuperscript{1} The detection limit of probe A for H$_2$S is 49 nM, which falls well within this range. The detection limit was calculated based on the method reported in the previous literature.\textsuperscript{2} The fluorescence emission spectrum of probe A was measured by three times and the standard deviation of blank measurement was achieved. The fluorescence intensity at 537 nm was plotted as a concentration of H$_2$S. The detection limit was calculated by using detection limit $3\sigma/k$: Where $\sigma$ is the standard deviation of blank measurement; $k$ is the slope between the fluorescence intensity versus H$_2$S concentration.

Reference


4. MTT assay

*In-vitro* cytotoxicity was measured using the colorimetric methyl thiazolyl tetrazolium (MTT) assay in MDBK cells. Cells were seeded in a 96-well plate and allowed to adhere for 24 h. Subsequently, the cells were incubated with different concentrations of probe A (0, 5, 10, 20, 40, or 80 µM, containing 1% DMSO) for 24 h. Finally, the viabilities of the MDBK cells in the presence of probe A were assessed using MTT cytotoxicity assays.

![Percentage of viable MDBK cells after incubation with different concentrations of probe A for 24 h.](image)

**Fig. S4** Percentage of viable MDBK cells after incubation with different concentrations of probe A for 24 h.

5. $^1$H NMR, $^{13}$C NMR and HRMS analyses
$^1$H NMR spectrum of compound 1 in CDCl$_3$.

$^{13}$C NMR spectrum of compound 1 in CDCl$_3$
$^1$H NMR spectrum of A-OH in DMSO-d6.

$^{13}$C NMR spectrum of A-OH in DMSO-d6.
$^1$H NMR spectrum of A in DMSO-d6.

$^{13}$C NMR spectrum of A in DMSO-d6.
High resolution mass spectra of probe A.

6. Table S1

<table>
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<th>Probe</th>
<th>Fluorophore</th>
<th>λ&lt;sub&gt;ex&lt;/sub&gt;/λ&lt;sub&gt;em&lt;/sub&gt; (nm)</th>
<th>Response time</th>
<th>Detection limit</th>
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<td>Sensors and Actuators, B.Chemical234( 201 6)231-238</td>
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Table S1 Summary of fluorescent probes for H<sub>2</sub>S