

## Supporting Information

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

Jing-Jing Liu, Xiang-Zhu Chen, Yuan-Yuan Zhang, Gui Gao, Xue-Yan Zhang, Shi-Cong Hou\*, Yuxia

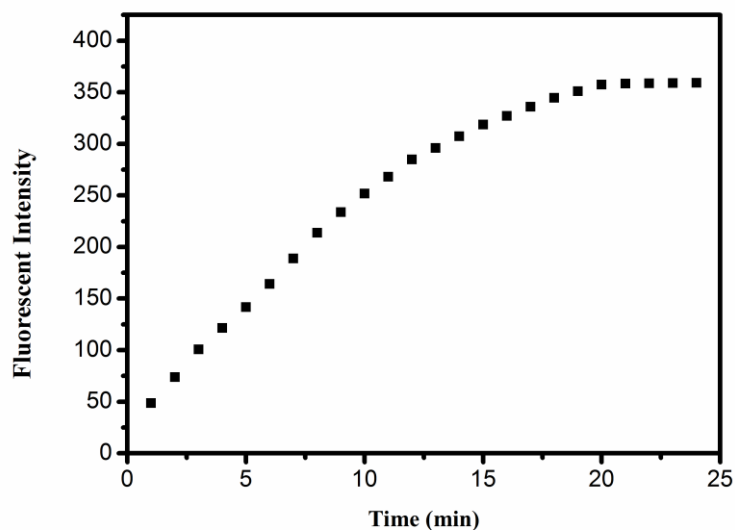
Hou\*

College of Science, China Agricultural University, Beijing, 100193, P.R. China.

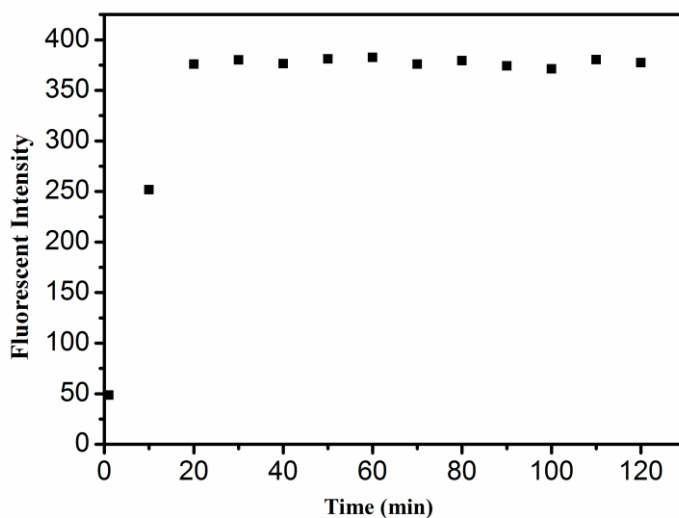
## **Contents**

- 1. Spectra**
- 2. Reaction mechanism**
- 3. Detection limit**
- 4. MTT assay**
- 5.  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and HRMS analyses**
- 6. Table S1**

## 1. Spectra

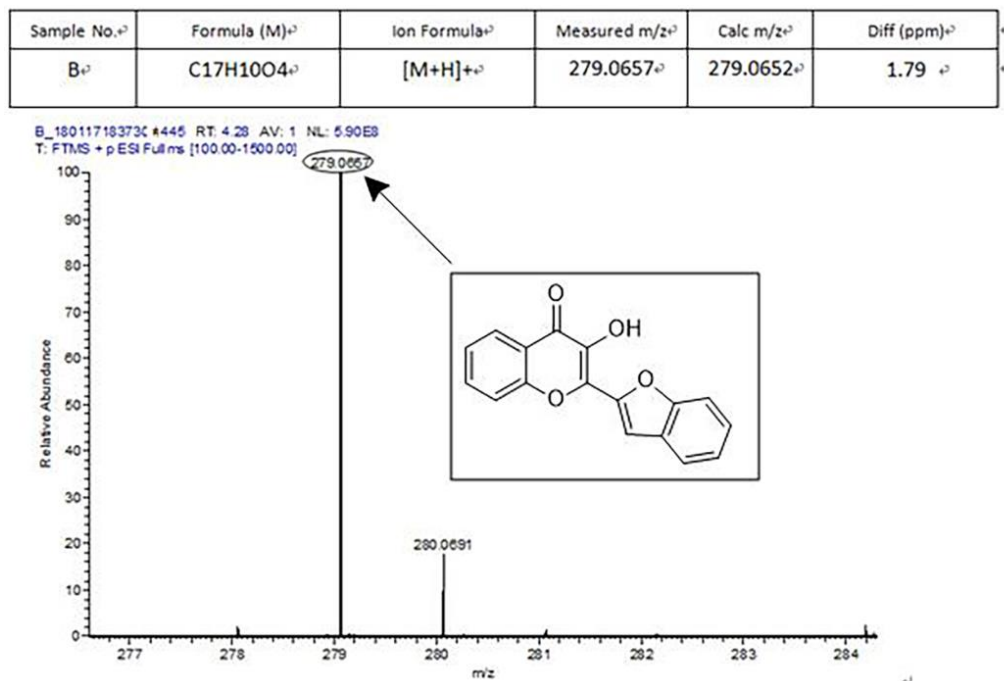


**Fig. S1** Time-dependent fluorescent intensity changes of probe A ( $20\mu\text{M}$ ) at 537nm upon addition of NaSH( $200\mu\text{M}$ ) in PBS buffer (20 mM, pH 7.4) with 20% DMSO and 3 mM CTAB at 37 °C.



**Fig. S2** The photo-stability of probe A. The fluorescent change at 537nm of probe A ( $20\mu\text{M}$ ) upon addition of NaSH( $200\mu\text{M}$ ) 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

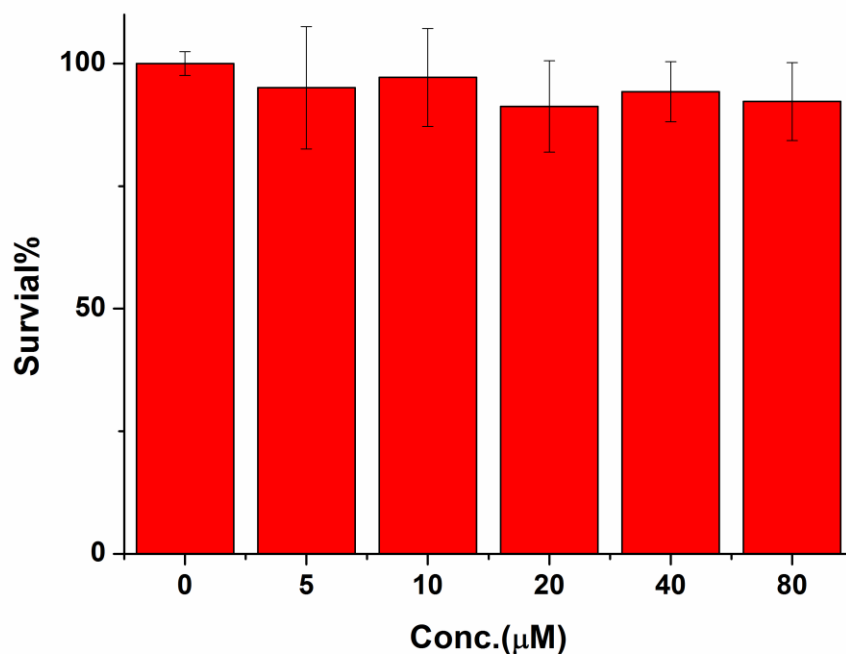
The physiological relevant H<sub>2</sub>S concentration is estimated ranging from nano- to millimolar levels.<sup>1</sup> The detection limit of probe **A** for H<sub>2</sub>S is 49 nM, which falls well within this range. The detection limit was calculated based on the method reported in the previous literature.<sup>2</sup> 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<sub>2</sub>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<sub>2</sub>S concentration.

## Reference

- [1] (a) J. Furne, A. Saeed, and M. D. Levitt, *Am. J. Physiol.*, **2008**, 295, R1479; (b) Y. Han, J. Qin, X. Chang, Z. Yang, and J. Du, *Cell. Mol. Neurobiol.*, **2006**, 26, 101.
- [2] B. Zhu, C. Gao, Y. Zhao, C. Liu, Y. Li, Q. Wei, Z. Ma, B. Du, X. Zhang, *Chem. Commun.* **2011**, 47, 8656.

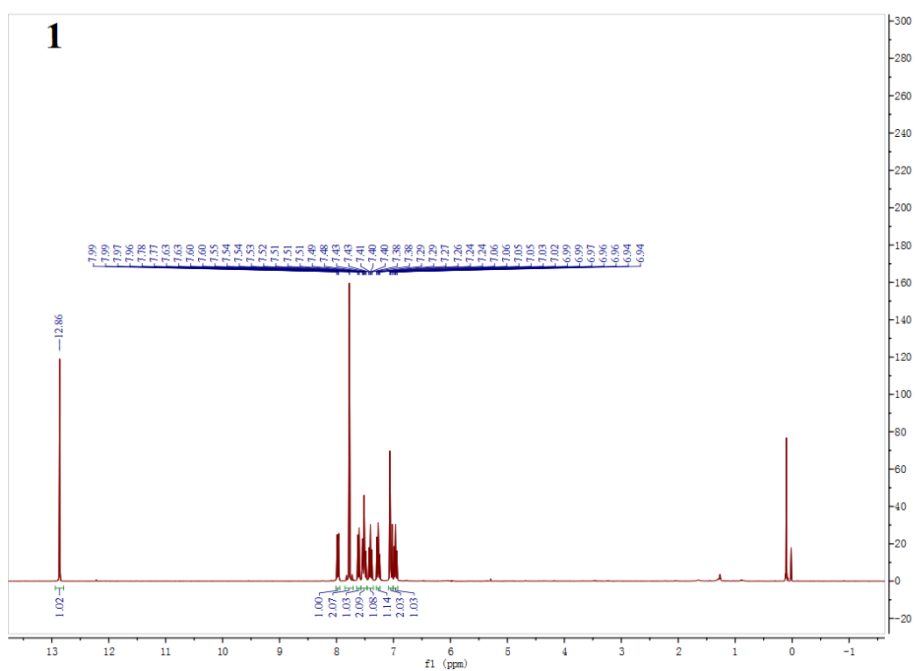
#### 4. MTT assay

*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  $\mu\text{M}$ , 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.

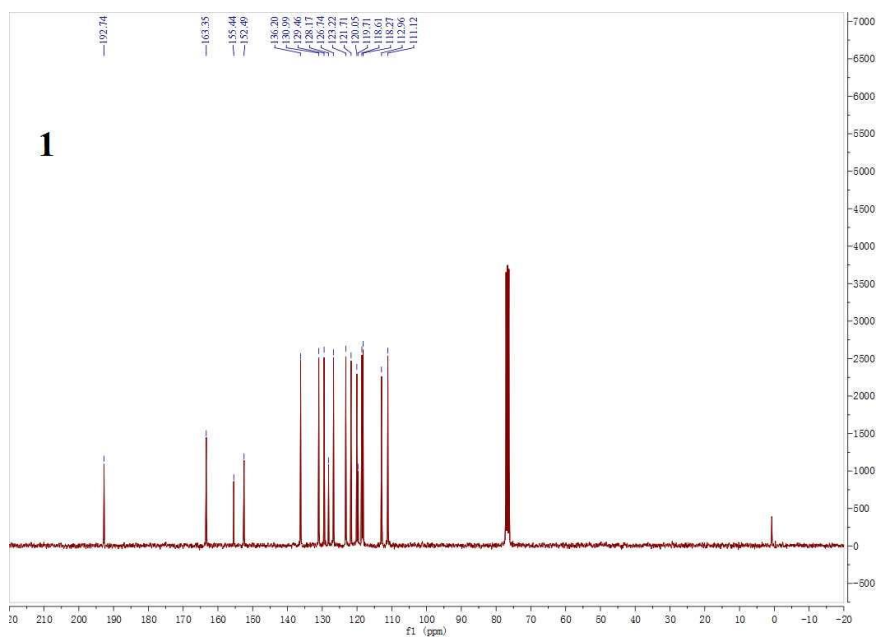


**Fig. S4** Percentage of viable MDBK cells after incubation with different concentrations of probe A for 24 h.

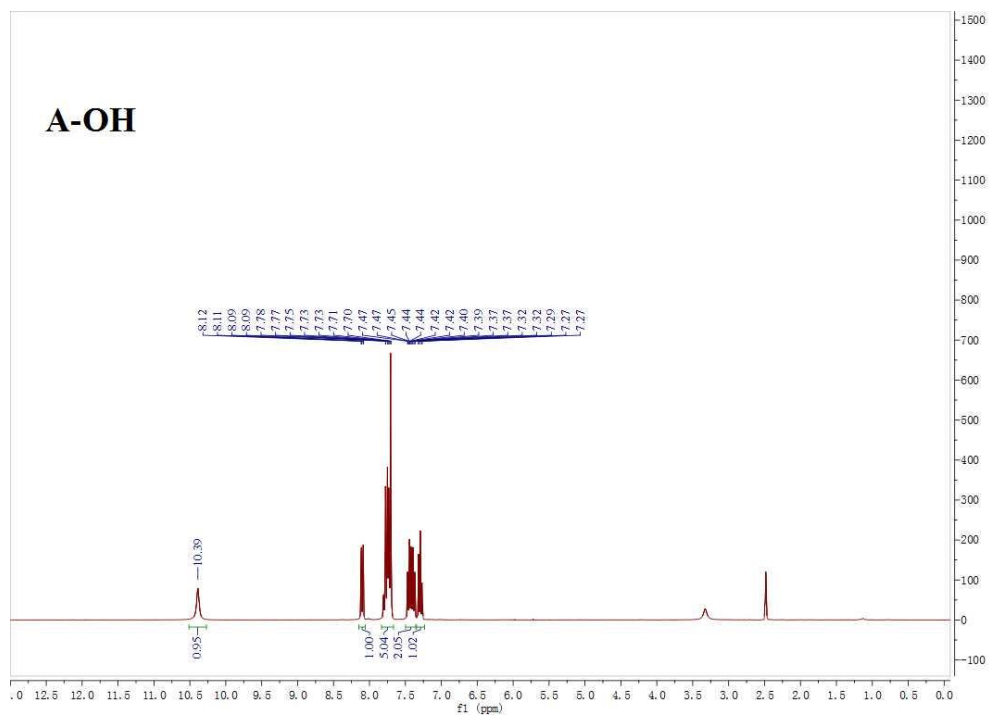
#### 5. $^1\text{H}$ NMR, $^{13}\text{C}$ NMR and HRMS analyses



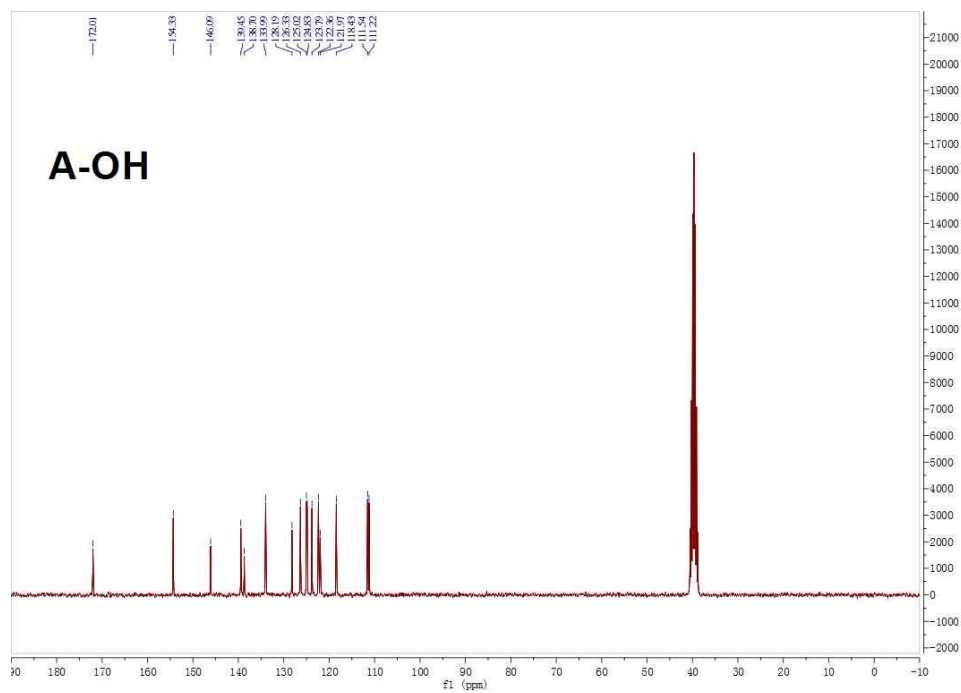
$^1\text{H}$  NMR spectrum of compound 1 in  $\text{CDCl}_3$ .



$^{13}\text{C}$  NMR spectrum of compound 1 in  $\text{CDCl}_3$ .



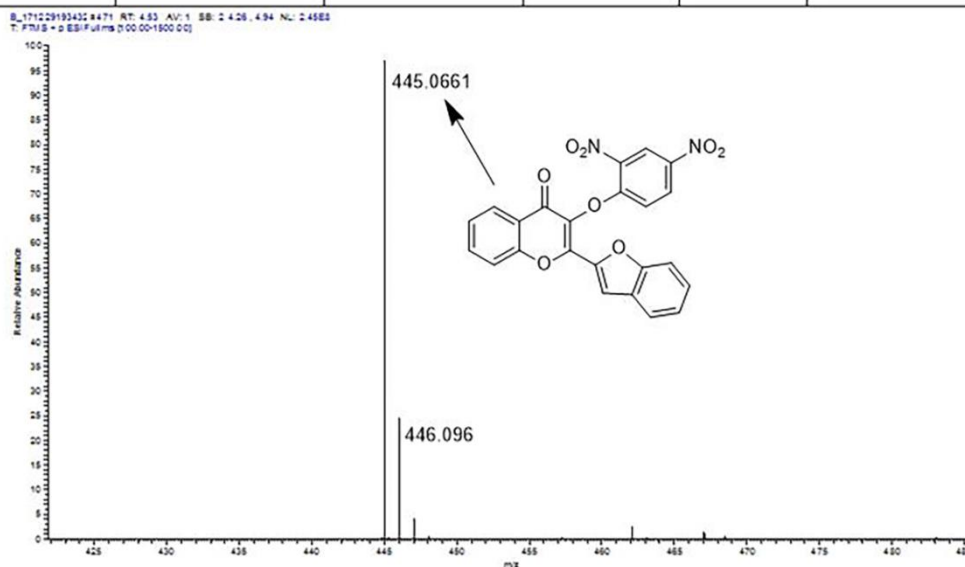
$^1\text{H}$  NMR spectrum of **A-OH** in  $\text{DMSO-d}_6$ .



$^{13}\text{C}$  NMR spectrum of **A-OH** in  $\text{DMSO-d}_6$ .



Sample No. <sup>Ⓢ</sup>	Formula (M) <sup>Ⓢ</sup>	Ion Formula <sup>Ⓢ</sup>	Measured m/z <sup>Ⓢ</sup>	Calc m/z <sup>Ⓢ</sup>	Diff (ppm) <sup>Ⓢ</sup>
Probe A <sup>Ⓢ</sup>	C <sub>23</sub> H <sub>12</sub> N <sub>2</sub> O <sub>8</sub> <sup>Ⓢ</sup>	[M+H] <sup>+</sup>	445.0661 <sup>Ⓢ</sup>	445.0666 <sup>Ⓢ</sup>	-1.12 <sup>Ⓢ</sup>



High resolution mass spectra of probe A.

## 6. Table S1

Probe	Fluorophore	$\lambda_{\text{ex}}/\lambda_{\text{em}}(\text{nm})$	Response time	Detection limit	Reference
P-N <sub>3</sub>	tetraphenylimidazole	300/436	80min	0.19 $\mu\text{M}$	Anal. Chim. Acta 879 (2015) 85–90
FD-NO <sub>2</sub>	rhodol	480/518	50min	1 $\mu\text{M}$	Org. Chem. Front. 1 (2014) 501–505
Probe-1	Fluorescein	465/515	60min	1–10 $\mu\text{M}$	Angew. Chem., Int. Ed., 2011,50, 10327–10329
GCTPOC-H <sub>2</sub> S	GCTPOC	410/514	40min	3.02 $\mu\text{M}$	Anal. Chim. Acta 853 (2015) 548–554.
HF-PBA	3-Hydroxyflavone	345/520	30min	0.075 $\mu\text{M}$	Sensors and Actuators, B:Chemical234( 2016)231-238
This work	2-(benzofuran-2-yl)-3-hydroxy-4H-chromen-4-one	440/537	20min	0.049 $\mu\text{M}$	The present work

Table S1 Summary of fluorescent probes for H<sub>2</sub>S