Supplementary Information

Trinal-site fluorescent probe for simultaneous sensing of hydrogen sulfide and glutathione and its bioimaging applications

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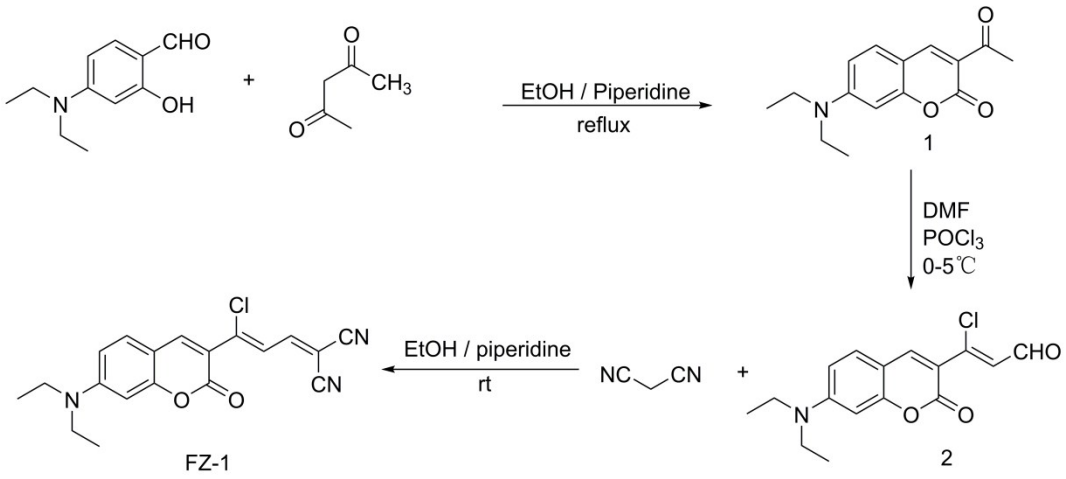
Contents

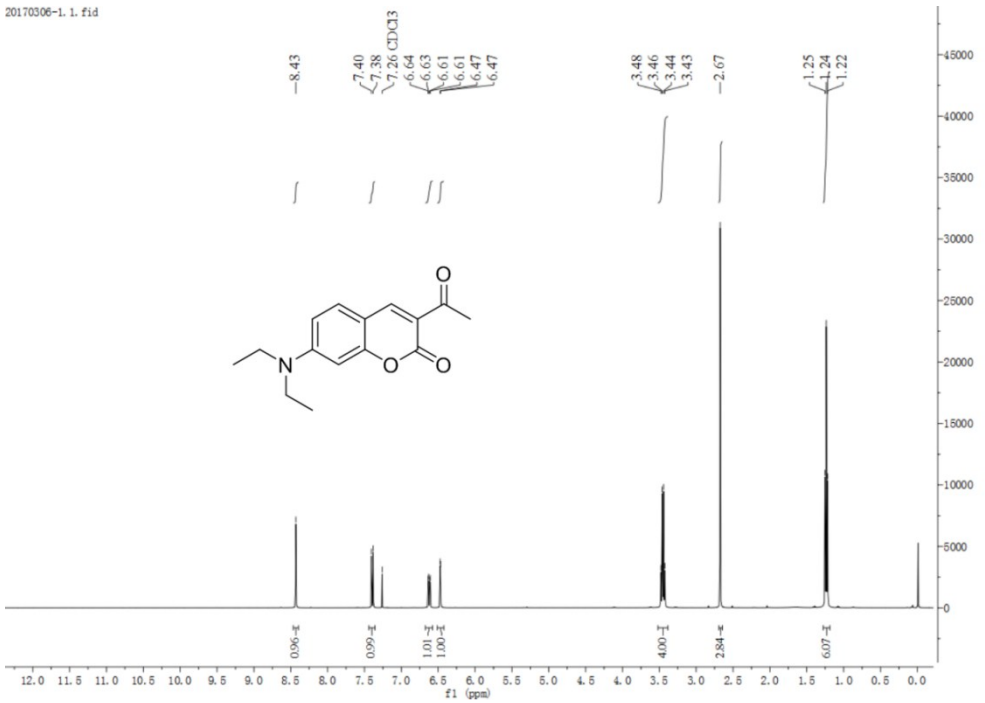
Synthesis



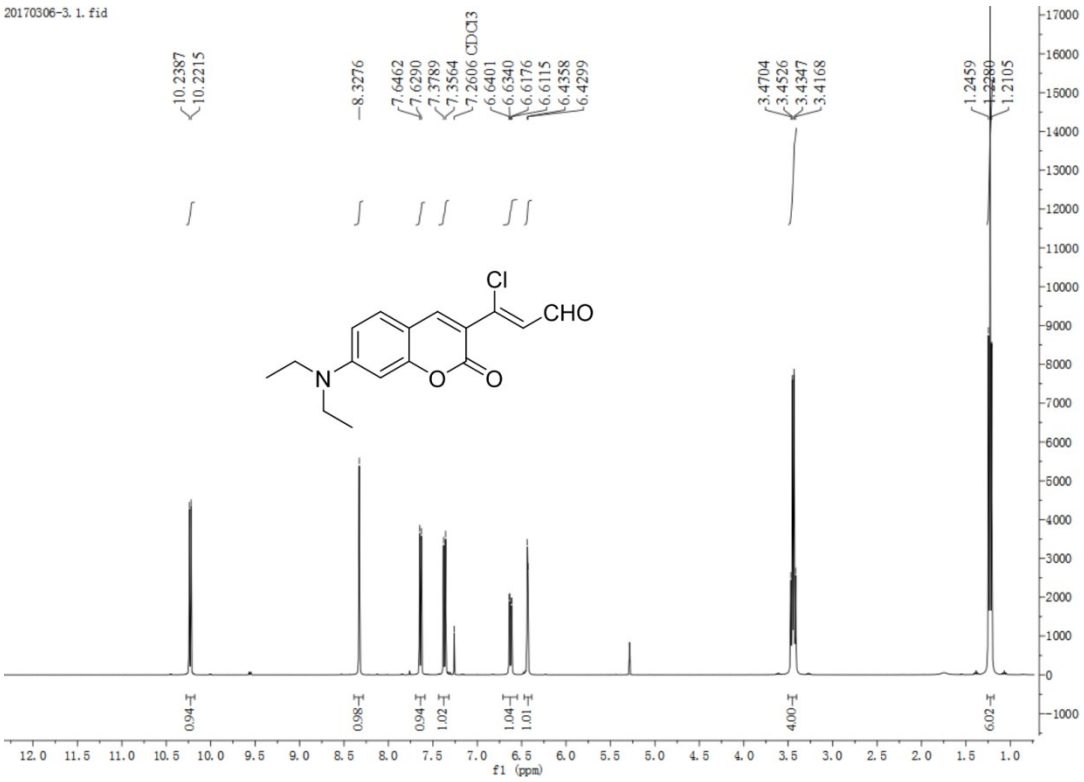
Synthesis of probe **FZ-1**

Characterization

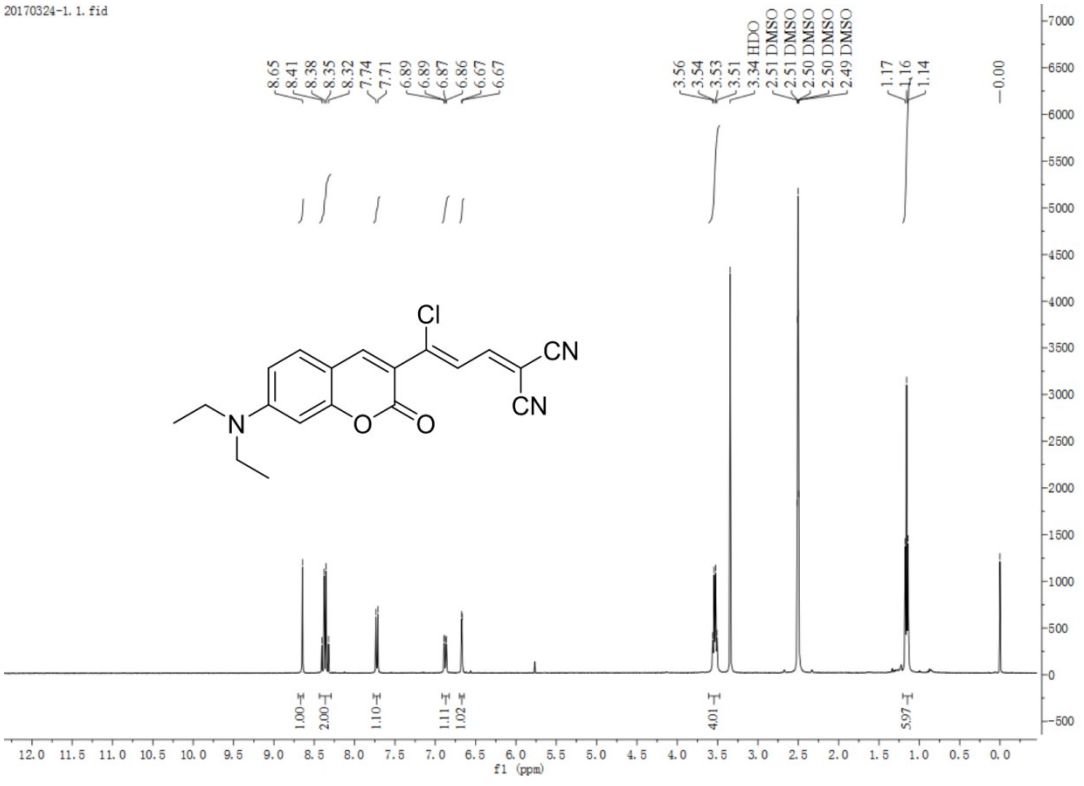
^{1}H/ ^{13}C NMR and HRMS spectra of compound 1, compound1 and probe FZ-1



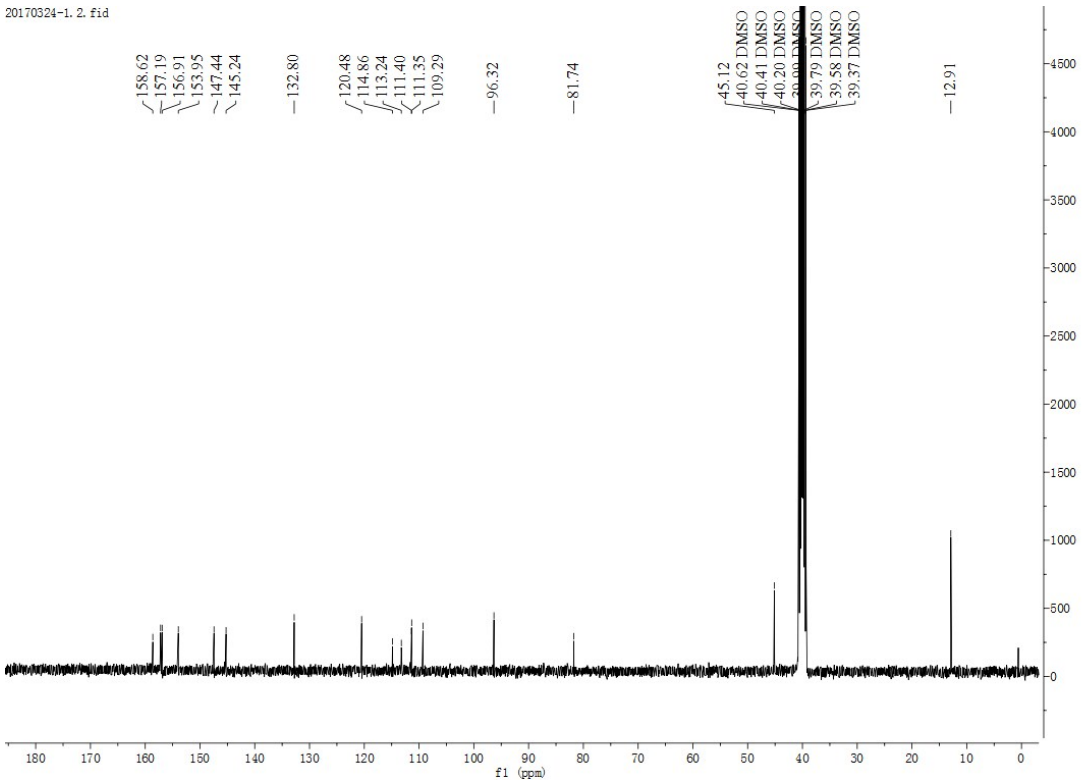
^{1}H-NMR spectrum of compound **1** in $CDCl_3$



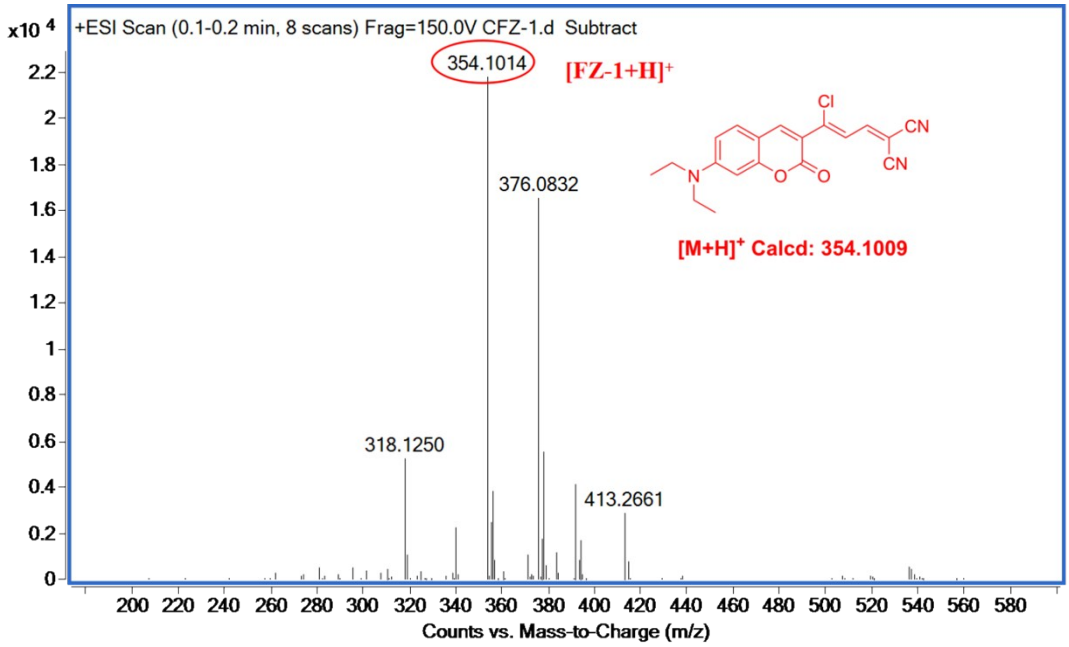
^{1}H-NMR spectrum of compound **2** in $CDCl_3$



^{1}H-NMR spectrum of probe **FZ-1** in DMSO-d_6



^{13}C-NMR spectrum of probe **FZ-1** in DMSO-d_6



HRMS spectrum of probe **FZ-1**

Optimization of the buffer system

It is a common phenomenon that the solvation effects have significant effect on some reactive fluorescent probes. Meixing Li et al.[1] have presented a ratiometric fluorescent probe that sense SO_3^{2-} / HSO_3^- and the probe also can detect hydrazine simultaneously in different buffer solutions according to the latest work of Yangyang He et al.[2]. In this work, the influence of solvent polarity on the probe **FZ-1** is particularly obvious. We had optimized the solvent-buffer of the reaction system before other spectra were tested, and the results as shown in Fig. 1 and Fig. 2 as follows: the probe **FZ-1** could response well to H_2S in weak polar organic solvent-buffer such as THF/PBS buffer (v/v=1:1, 10 mM, pH=7.4) and acetonitrile/PBS buffer (v/v=1:1, 10 mM, pH=7.4). However, the probe **FZ-1** hardly responds to H_2S in strong polar solvent-buffer such as DMSO/PBS buffer (v/v=1:1, 10 mM, pH=7.4). On the contrary, the probe **FZ-1** could response well to GSH in strong polar solvent-buffer of DMSO/PBS (v/v=1:1, 10 mM, pH=7.4) but hardly responds to GSH in weak polar organic solvent-buffer of acetonitrile/PBS (v/v=1:1, 10 mM, pH=7.4). Considering these results, we chose the optimal solvent buffer for subsequent spectral tests which can detect the H_2S in the weak polar organic solvent-buffer of acetonitrile/PBS (v/v=1:1, 10 mM, pH=7.4) and sense the GSH in the strong polar organic solvent-buffer of DMSO/PBS (v/v=1:1, 10 mM, pH=7.4).

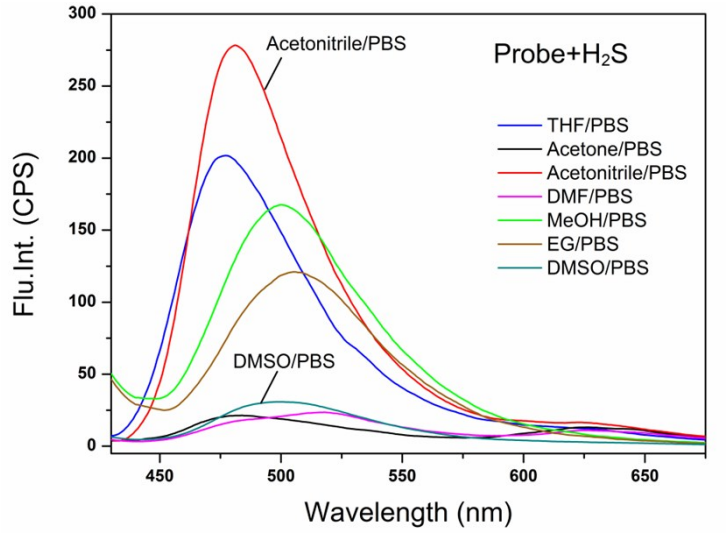


Fig. 1 Fluorescent spectra response of probe **FZ-1** (10 μM) for H_2S (50 μM) in the various organic solvent-buffer (solvent/PBS, v/v=1:1, 10 mM, pH=7.4), E_x = 420 nm, Slits: 5/5 nm.

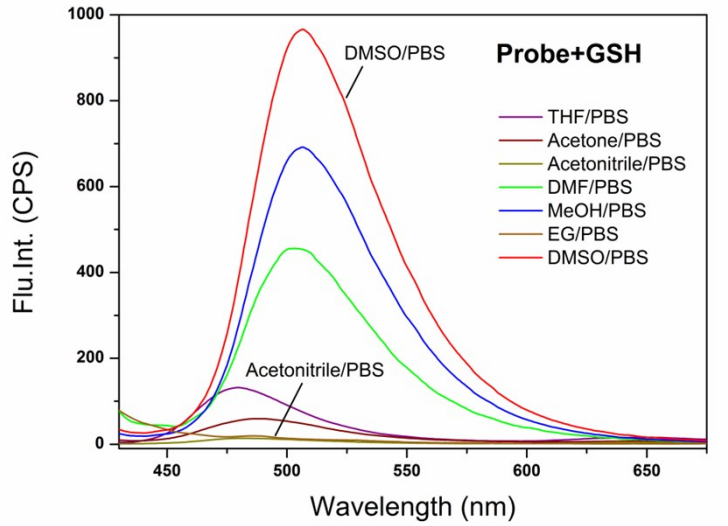


Fig. 2 Fluorescent spectra response of probe **FZ-1** (10 μM) for GSH (30 μM) in the various organic solvent-buffer (solvent/PBS, v/v=1:1, 10 mM, pH=7.4), E_x = 420 nm, Slits: 5/5 nm.

Fluorescence Analysis

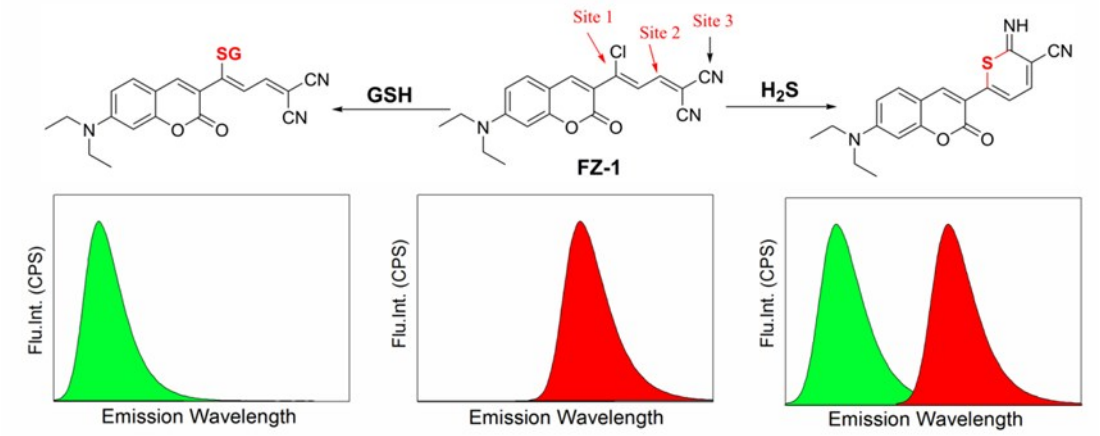


Fig. 3 The spectral sensing properties of the probe **FZ-1** for H_2S and GSH

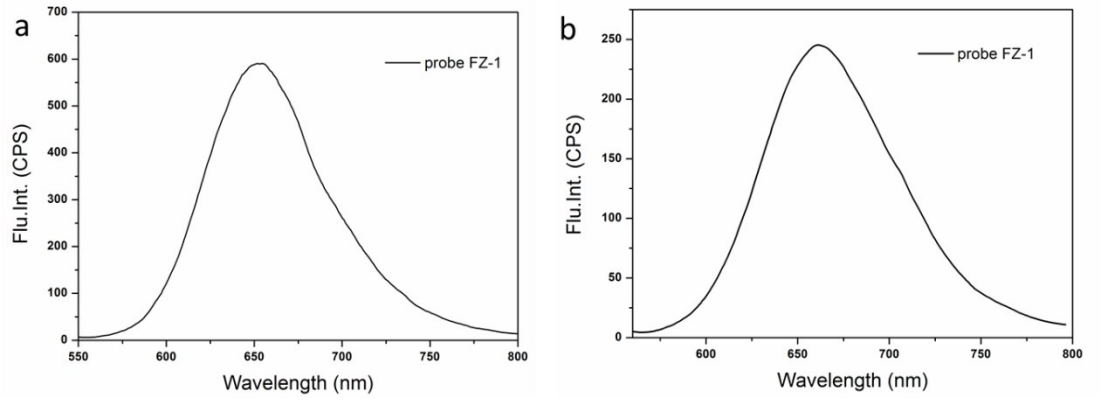


Fig. 4 The fluorescence spectrum of free probe **FZ-1**, (a) in the CH_3CN/PBS buffer solution (v/v 1:1, 10 mM, pH=7.4); (b) the in DMSO/PBS buffer solution (v/v=1:1, 10 mM, pH=7.4). E_x = 530 nm, Slits: 5/5 nm.

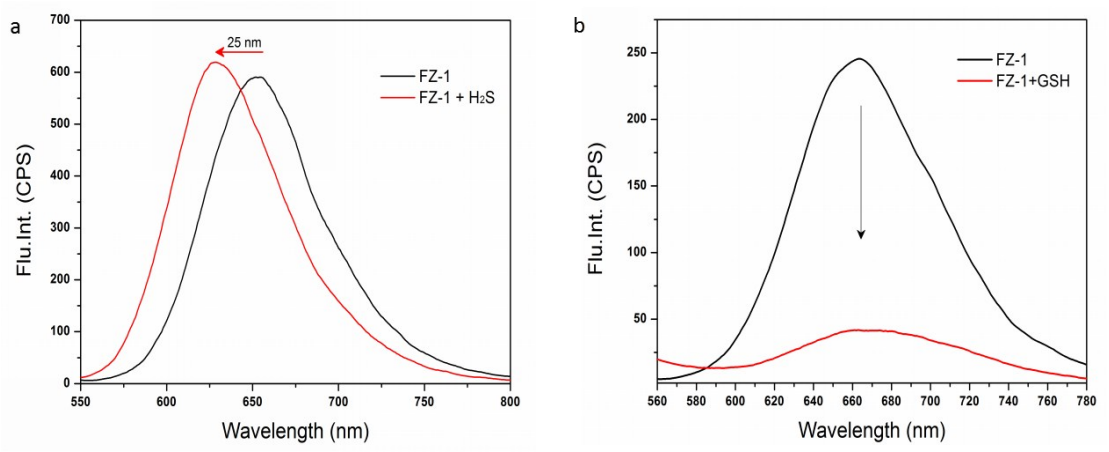


Fig. 5 The fluorescence spectrum of probe **FZ-1** react with H_2S and GSH respectively. (a) probe **FZ-1** react with H_2S in the CH_3CN/PBS buffer solution (v/v 1:1, 10 mM, pH=7.4); (b) probe **FZ-1** react with GSH in the DMSO/PBS buffer solution (v/v 1:1, 10 mM, pH=7.4). E_x = 530 nm, Slits: 5/5 nm.

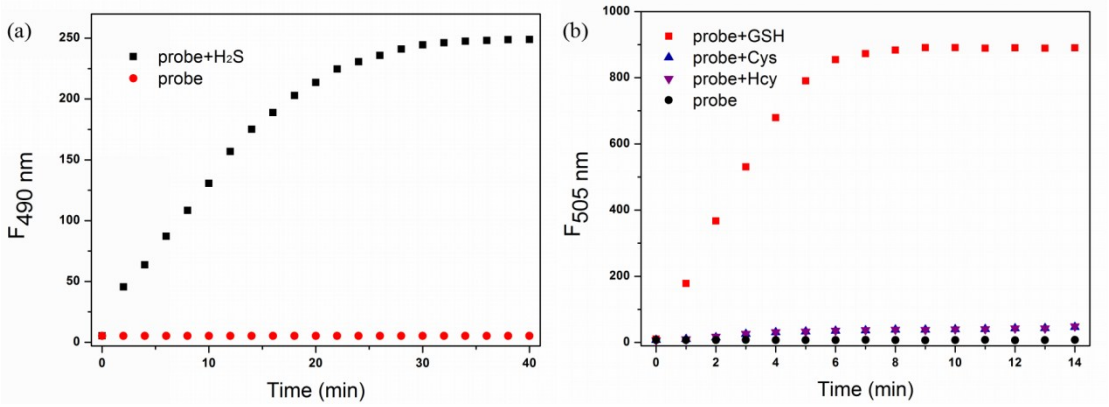


Fig. 6 (a) The fluorescence intensity changes of probe **FZ-1** (10 μM) treated with H_2S (50 μM) at 490 nm in the CH_3CN/PBS buffer solution, (b) The fluorescence intensity changes of probe **FZ-1** (10 μM) treated with GSH, Cys and Hcy (50 μM) at 505 nm in the DMSO/PBS buffer solution. E_x = 530 nm, Slits: 5/5 nm.

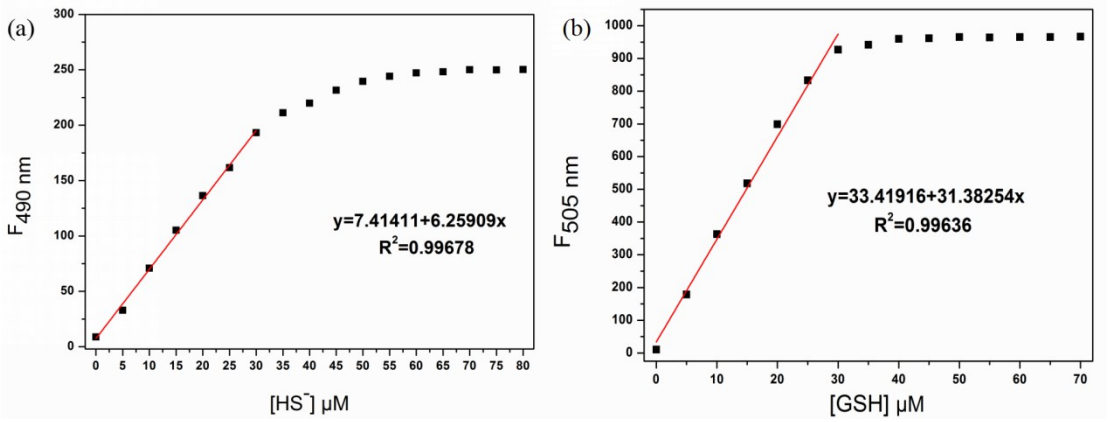


Fig. 7 (a) The working curve of probe **FZ-1** at 490 nm with H_2S, (b) The working curve of probe **FZ-1** at 505 nm with GSH. E_x = 530 nm, Slits: 5/5 nm.

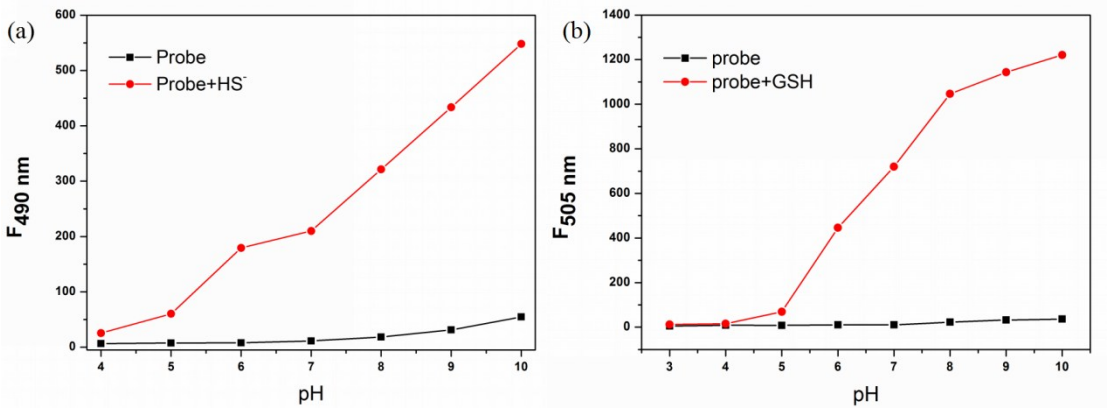


Fig. 8 (a) Effect of pH on the fluorescence increment of probe **FZ-1** (10 μM) treatment with H_2S (50 μM) in the CH_3CN/PBS buffer (v/v=1:1) solution. (b) effect of pH on the fluorescence increment of probe **FZ-1** (10 μM) treatment with 50 μM of GSH, Cys and Hcy in the DMSO/PBS buffer (v/v=1:1) solution.

HRMS Spectrum of the Reaction Mixture

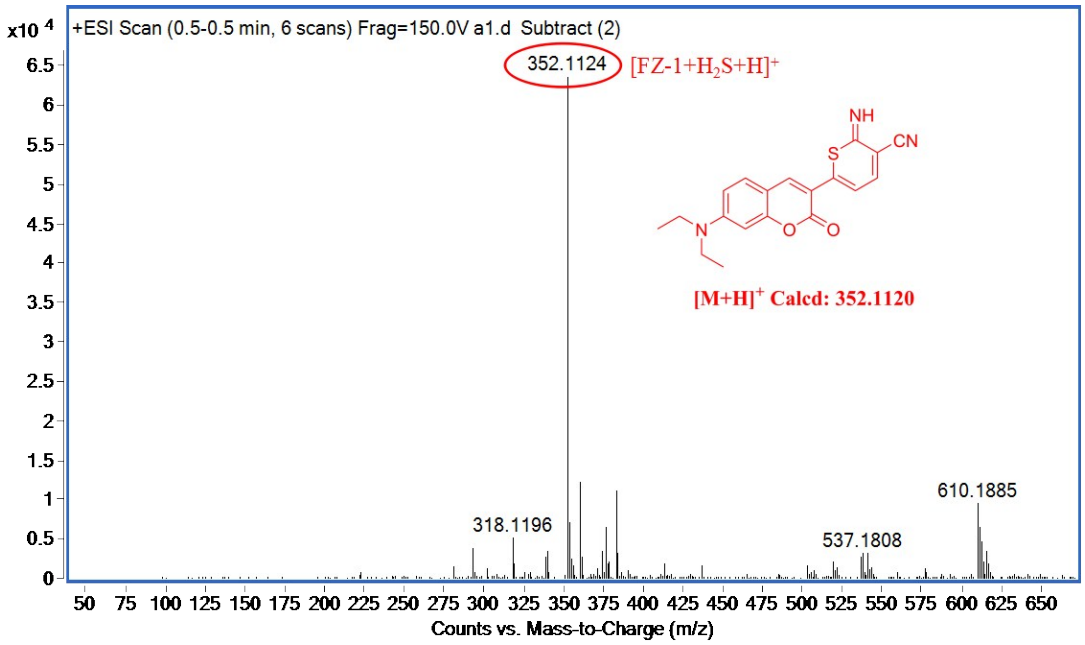


Fig. 9 HRMS spectrum of [**FZ-1**+ H_2S+H]$^+$

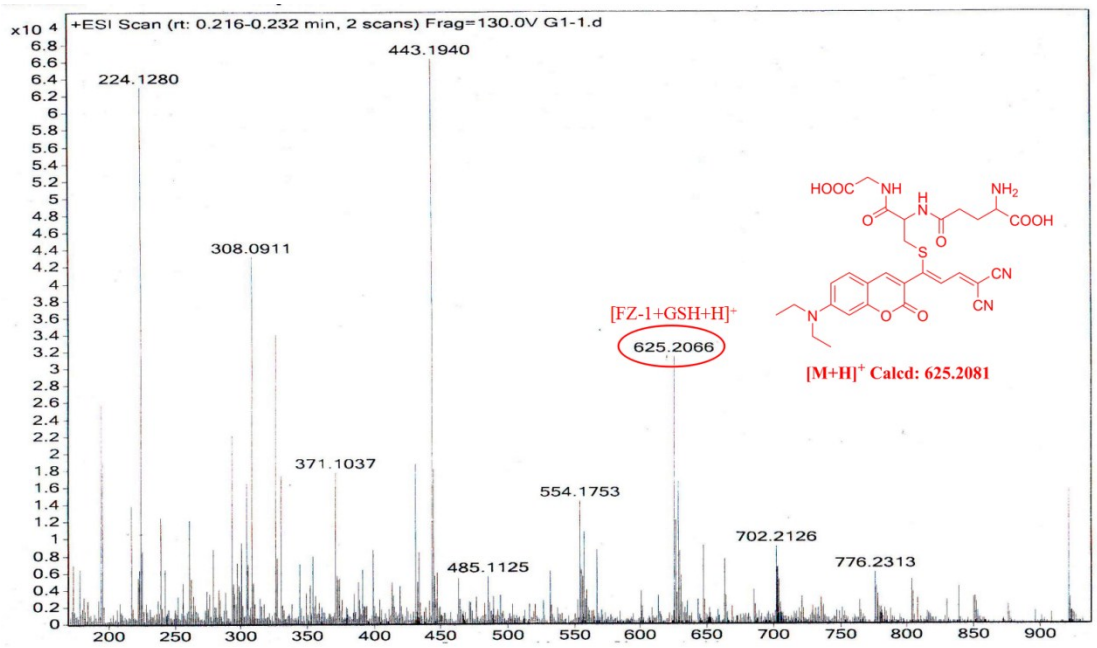


Fig. 10 HRMS spectrum of [**FZ-1**+ GSH+H]$^+$

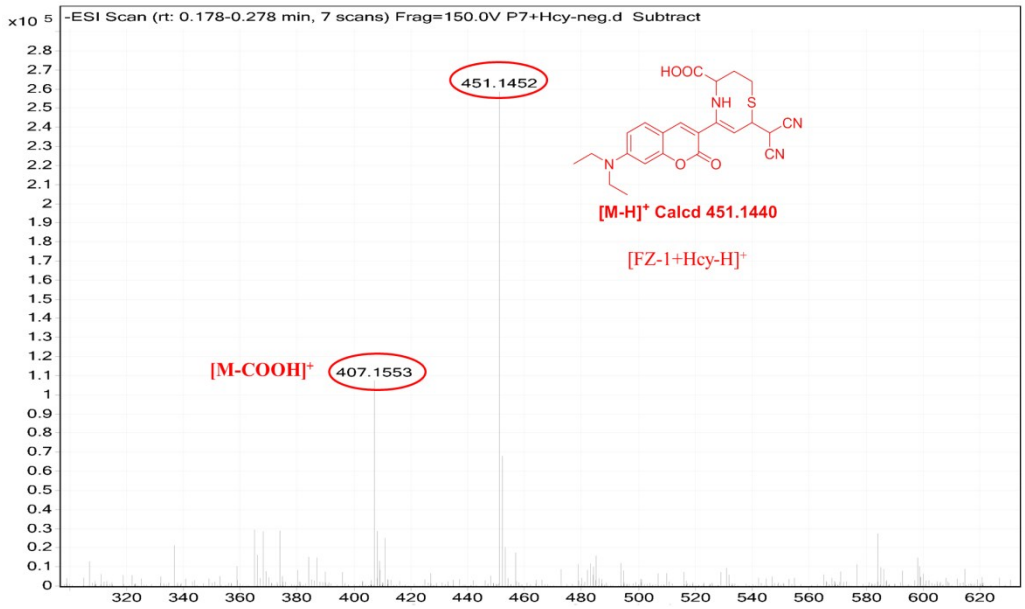


Fig. 11 HRMS spectrum of [**FZ-1**+ Hcy-H]$^+$

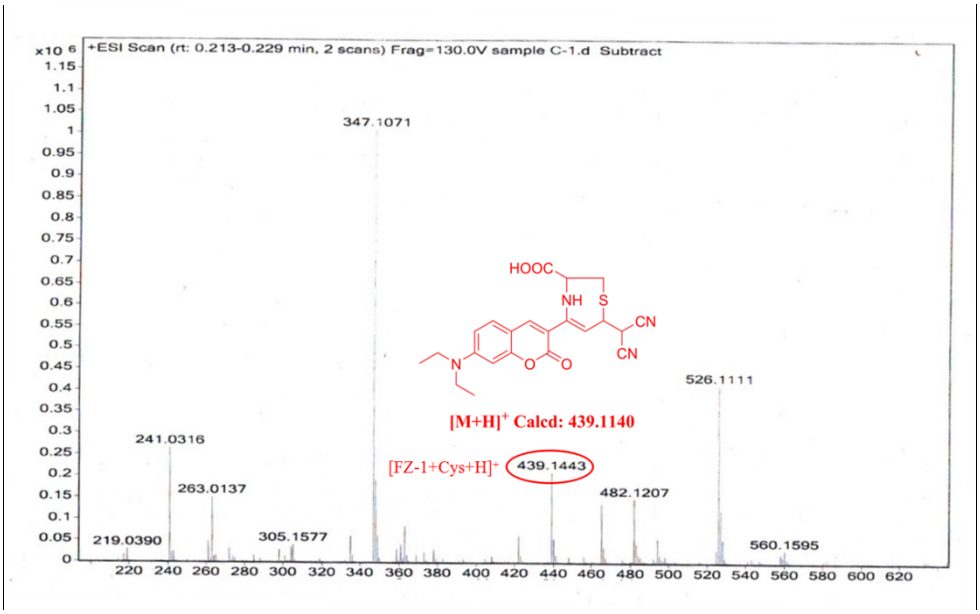


Fig. 12 HRMS spectrum of [**FZ-1**+ Cys+H]$^+$

Results from Cell Viability Assays

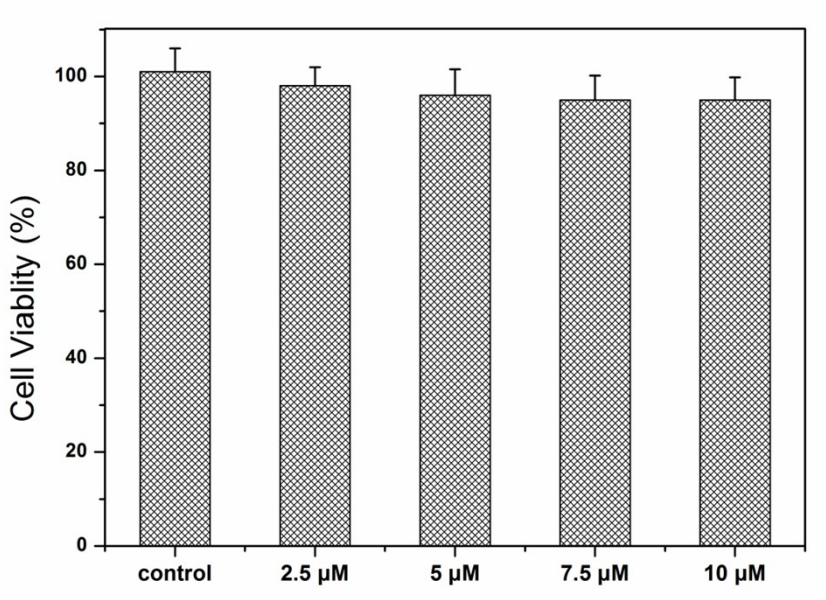
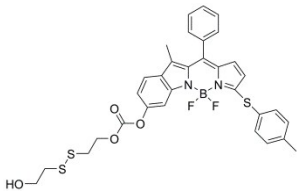
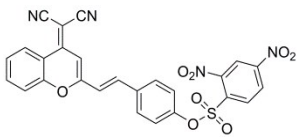
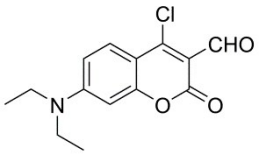
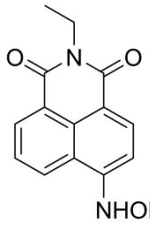
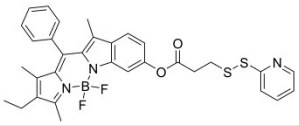
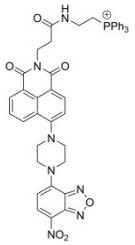
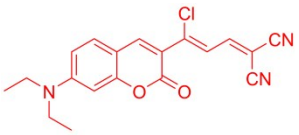


Fig. 13 Cell viability of MCF-7 cells treated with different concentrations of probe **FZ-1** (0, 2.5, 5.0, 7.5 and 10.0 μM) for 12 h. The cell viability was observed via CCK-8 assay.

Table S1. Comparison of fluorescent probe for GSH and H2S

Probes	Detection system	Target	Response time	Detection limit	Reference
	CH_3CN/HEPES buffer (1 : 1, v/v, 20 mM, pH 7.4)	GSH	200 min	0.85 μM	Chem. Sci., 2015, 6, 2584
	DMSO/H_2O(1 : 1, v/v) with a PBS buffer solution (10 mM, pH = 7.4).	GSH	5 min	0.018 μM	Chem. Commun., 2014, 50, 1751
	PBS buffer (pH 7.4) containing 10% DMSO	GSH	20 min	0.38 μM	Biosens. Bioelectron. 2015, 72, 275

	PBS buffer (pH 7.4) containing 1% DMSO	H_2S	120 min	0.5 μM	Chem. Commun., 2012, 48, 10669.
	PBS buffer (pH 7.4) containing 30% CH_3CN	H_2S	9 min	0.15 μM	Chem. Commun., 2015, 51, 13135.
	PBS buffer (pH 7.4) containing 10% CH_3CN	H_2S	40 min	2.46 μM	Anal. Chem., 2016, 88 (10), 5476.
	PBS buffer (pH 7.4) containing 50% CH_3CN or DMSO	H_2S and GSH	10 min	H_2S: 0.075 μM GSH: 0.28 μM	This work

References

1. M. Li, W. Feng, H. Zhang and G. Feng, *Sens. Actuators. B*, 2017, **243**, 51-58.

2. Y. He, Z. Li, B. Shi, Z. An, M. Yu, L. Wei and Z. Ni, *Rsc Adv.*, 2017, **7**, 25634-25639.