Electronic Supplementary Information (ESI) for Analytical Methods

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Electronic Supplementary Information

Highly sensitive and selective coumarin probe for hydrogen sulfide imaging in living cells

Sheng Chen,[a] Chao Ma,[b] Dong-En Wang,[a] Xiang Han,[a] Longlong Zhang,[a] and Jinyi Wang*[a,b]

[a] *College of Science, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China*

[b] *College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China*

Abstract. This Supplementary data include all of the additional information as noted in the manuscript.

S1. ^{1}H, ^{13}C NMR and Mass Spectra of DNPOCA

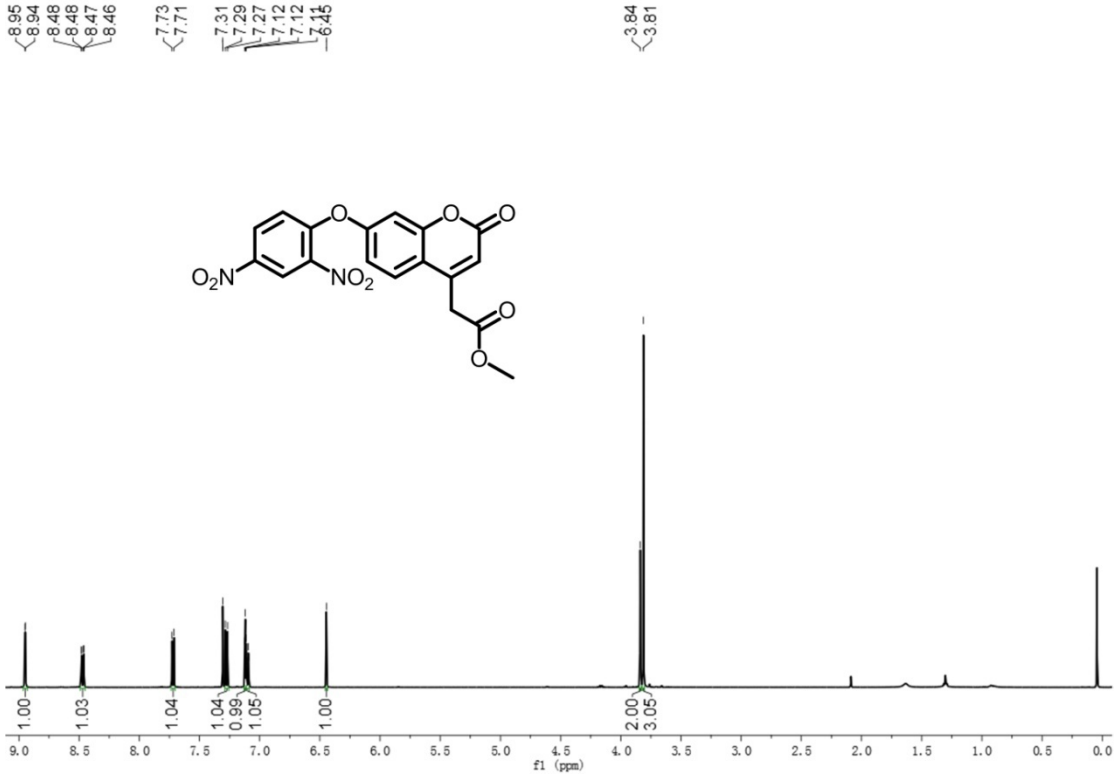


Figure S1. ^{1}H NMR spectrum of DNPOCA in $CDCl_3$.

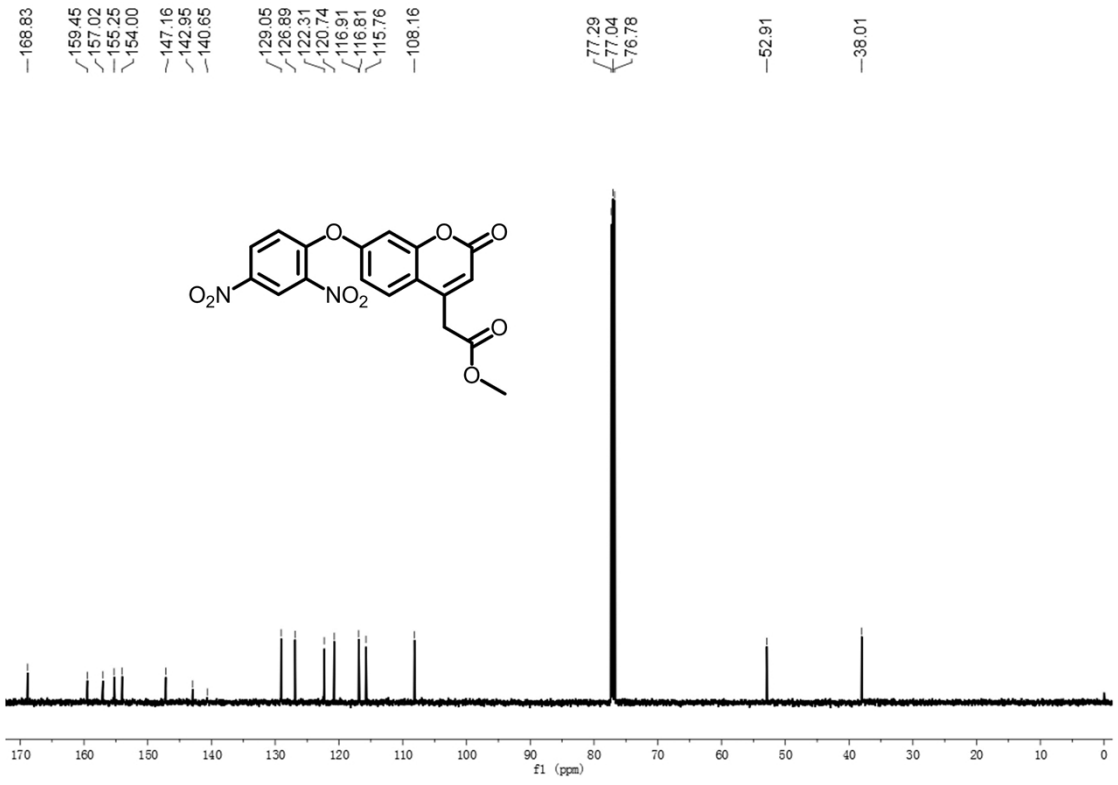


Figure S2. ^{13}C NMR spectrum of DNPOCA in $CDCl_3$.

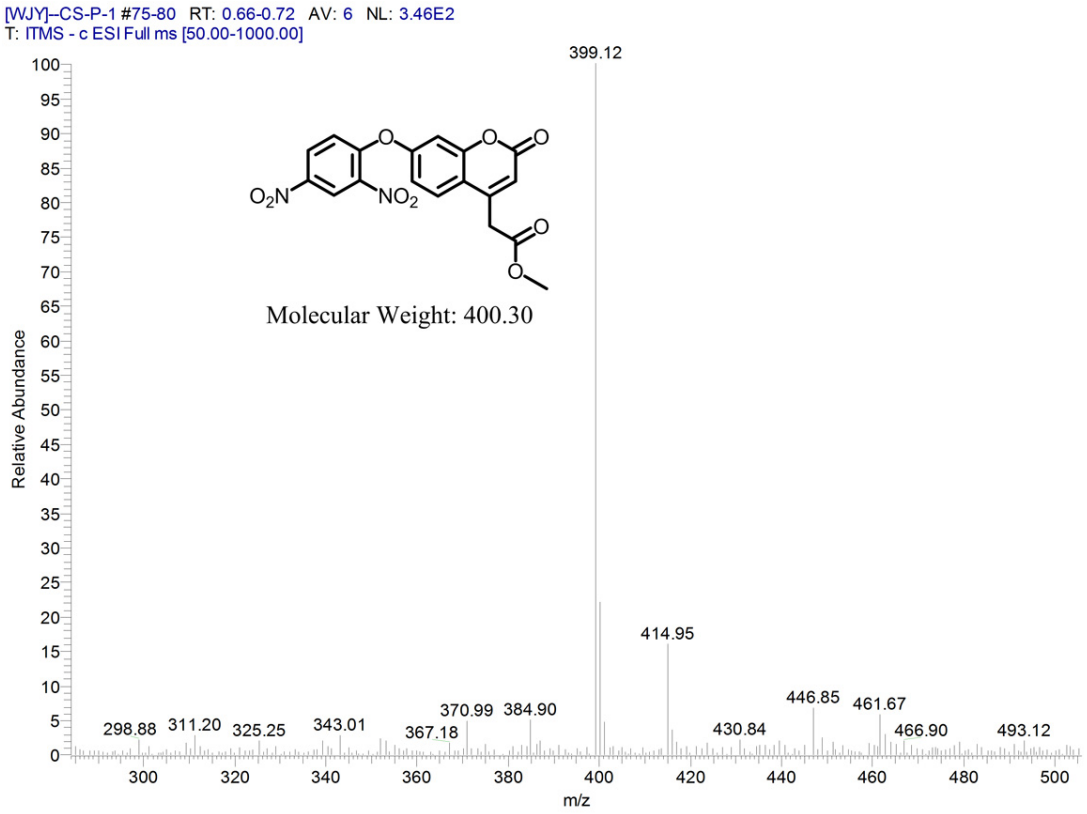


Figure S3. ESI-MS of DNPOCA

S2. The acquisition and analysis of thiolysis product of DNPOCA after incubated with Na_2S

DNPOCA (20 mg, 0.05 mmol) was dissolved in CH_3CN (10 mL), followed by the addition of $Na_2S•9H_2O$ (199.35 mg, 0.83 mmol). The resultant mixture was stirred for 5 hours at room temperature. Subsequently, the solvent was evaporated. The fluorescent product was purified by column chromatography and analyzed by mass spectrometry.

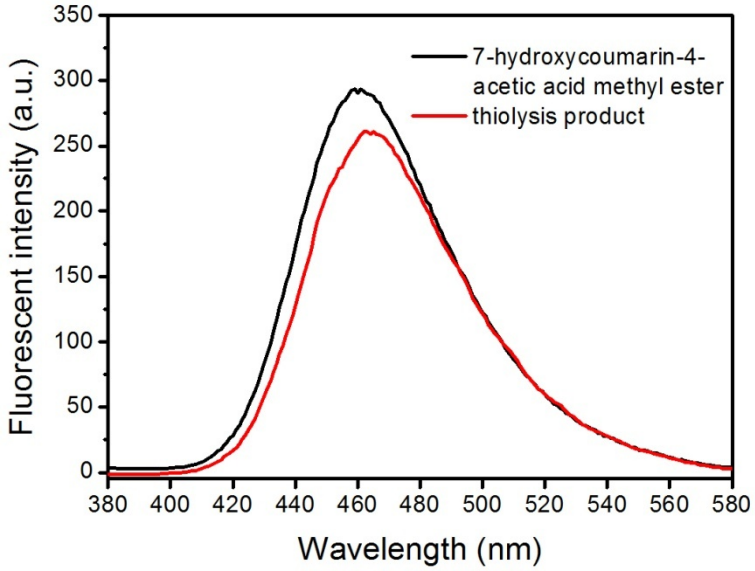


Figure S4. Fluorescence spectra of 7-hydroxycoumarin-4-acetic acid methyl ester and thiolysis product in aqueous solution (20 mM, pH = 7.4, CH_3CN/PBS = 1:9, 3 mM CTAB) at room temperature.

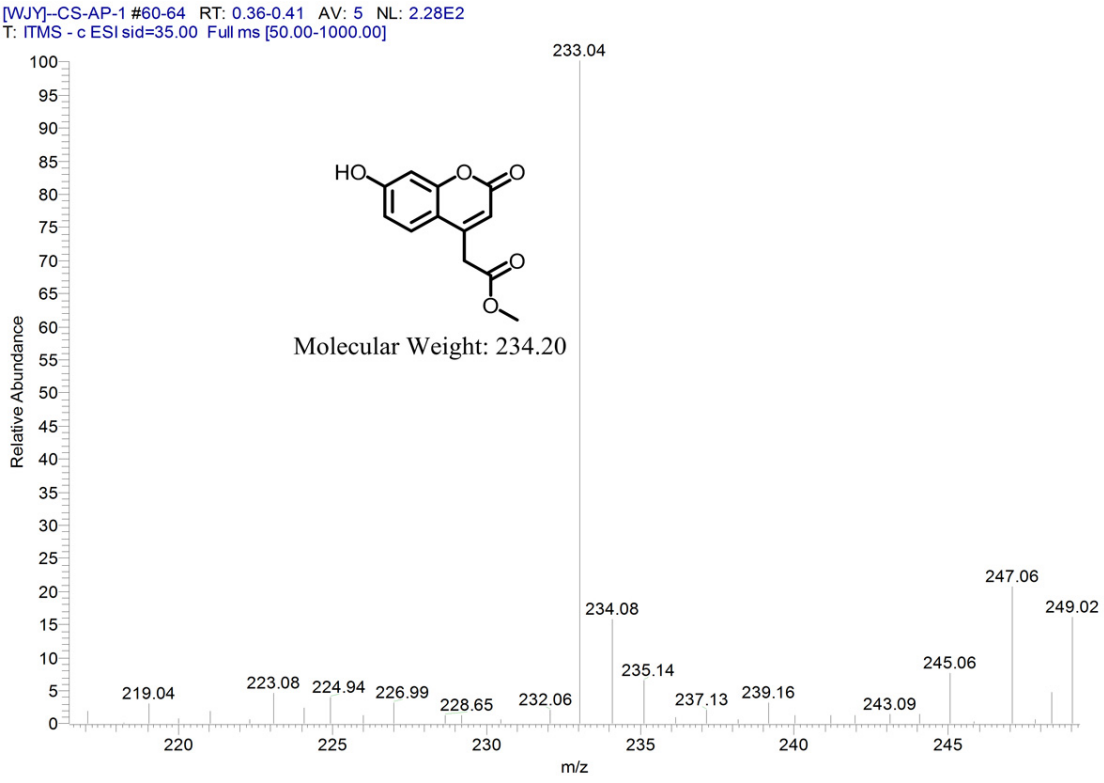


Figure S5. ESI-MS confirmed the formation of 7-hydroxycoumarin-4-acetic acid methyl ester in the reaction of DNPOCA with Na_2S.

S3. Study on the reaction kinetics of DNPOCA

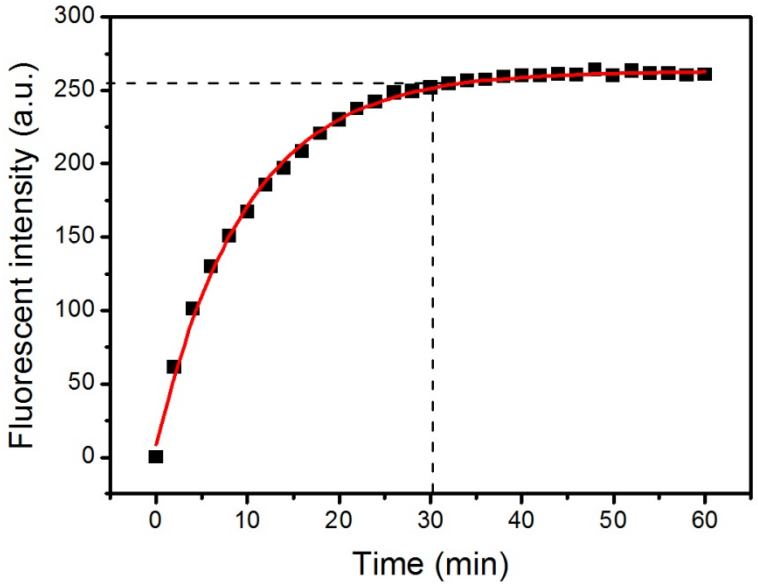


Figure S6. Time-dependent fluorescence intensity changes at 463 nm of the probe DNPOCA (5.0 μM) in the absence of Na_2S (100 μM) in aqueous solution (20 mM, pH = 7.4, CH_3CN/PBS = 1:9, 3 mM CTAB) at room temperature.

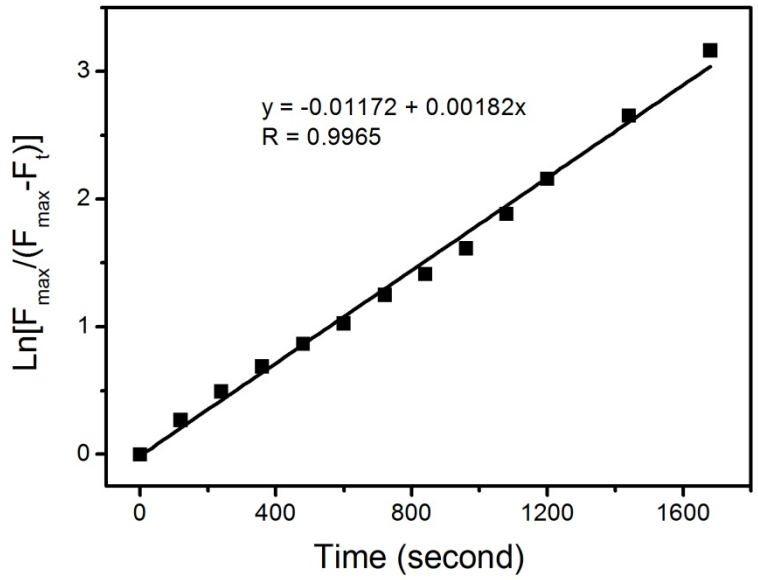


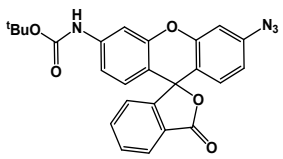
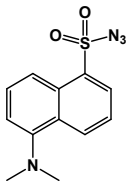
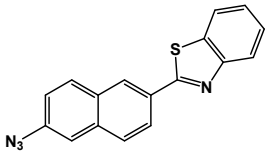
Figure S7. The pseudo-first-order kinetic plot of the reaction of the probe DNPOCA (5.0 μM) with Na_2S (100 μM) in aqueous solution (20 mM, pH = 7.4, CH_3CN/PBS = 1:9, 3 mM CTAB) at room temperature.

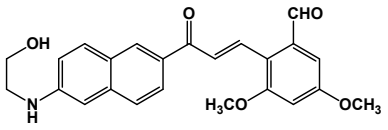
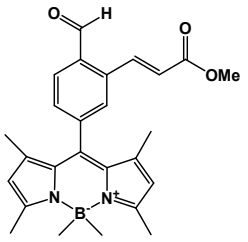
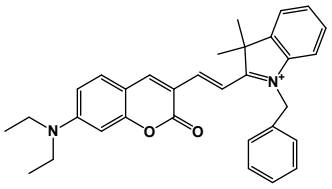
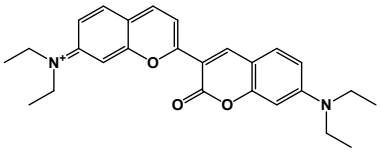
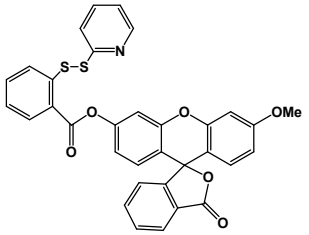
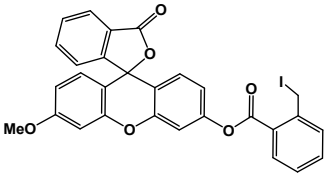
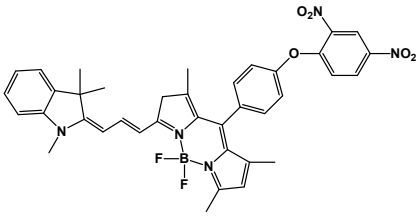
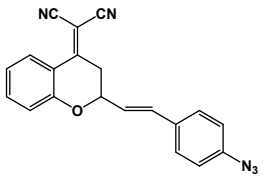
S4. The calculation method for the detection limit.

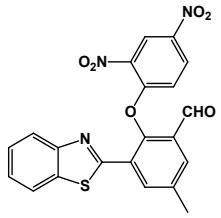
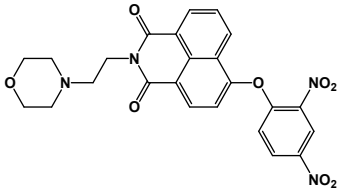
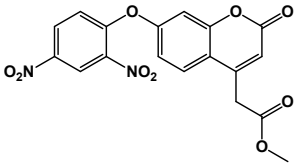
The limit of detection (LOD) for DNPOCA was calculated based on the fluorescence titration. To determine the S/N ratio, the fluorescence emission intensity of DNPOCA without Na_2S was measured by 10 times and the standard deviation of blank measurements was determined. Then, the solution was treated with Na_2S from 0 to 100 μM. A linear regression curve was then achieved according to the fluorescence emission intensity in the range of Na_2S from 0 to 20 μM. The detection limit was calculated with the equation (Eq. 1) at S/N=3, in which σ is the standard deviation of the background and *s* is the sensitivity.

$$LOD = 3 \times \frac{\sigma}{S} \qquad \text{(Eq. 1)}$$

Table S1. Comparison of fluorescent probes for hydrogen sulfide

Probe	$\lambda_{ex}/\lambda_{em}$ (nm)	Detection medium	Detection limit	Ref.
	490/525	HEPES (pH 7.4), 0.2% DMF	5-10 μM	1
	340/535	PBS (pH 7.4), 0.5% Tween-20	1 μM	2
	360/480	HEPES (pH 7.4), 80% DMF	0.1 μM	3

	360/480	HEPES (pH 7.4), 1% CH_3CN	50 nM	4
	465/510	PBS (pH7.0), 1% DMSO	5 μM	5
	475/510 475/652	PBS (pH7.4), 2% DMSO	1 μM	6
	450/485 475/690	PBS (pH7.4), 30% CH_3CN	0.14μM	7
	465/515	PBS (pH 7.4), 10% CH_3CN	1-10 μM	8
	455/517	PBS (pH 7.4), 20% CTAB	0.1 μM	9
	650/708	PBS (pH 7.0), 20% ethanol, 3mM CTAB	0.1 μM	10
	520/670	PBS (pH 7.4), 50% DMSO	3.05 μM	11

	440/545	PBS (pH 7.4), 25% CH_3CN	48 nM	12
	450/555	PBS (pH 7.4), 10% CH_3CN	0.48 μM	13
	365/463	PBS (pH 7.4), 10% CH_3CN 3mM CTAB	49.7 nM	This work

S5. Study on the sensitivity of DNPOCA by UV-visible absorption spectra.

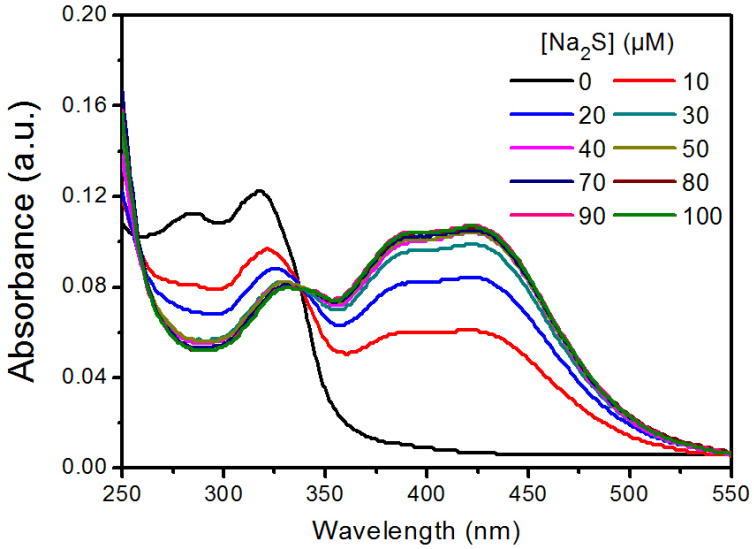


Figure S8. UV-vis absorption spectra changes of the probe DNPOCA (5.0 μM) in the presence of Na_2S (0-20 equiv.) in aqueous solution (20 mM, pH = 7.4, CH_3CN/PBS = 1:9, 3 mM CTAB) at room temperature.

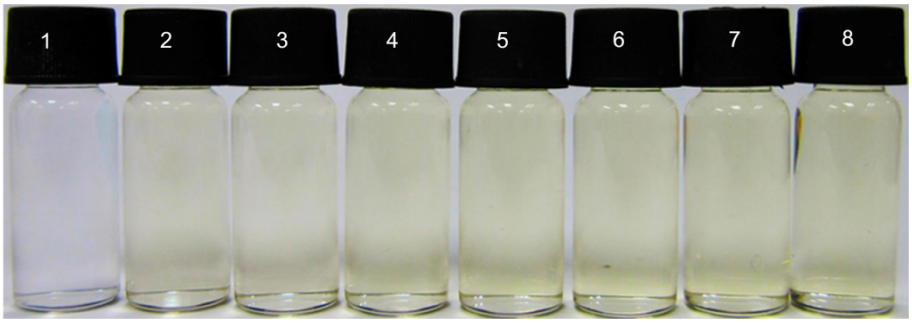


Figure S9. Color changes of the probe DNPOCA (5.0 μM) with different concentrations of Na_2S for 40 min at room temperature (From 1 to 8: 0, 10, 30, 50, 70, 80, 90, 100 μM Na_2S, respectively).

S6. Study on the selectivity of DNPOCA for H_2S

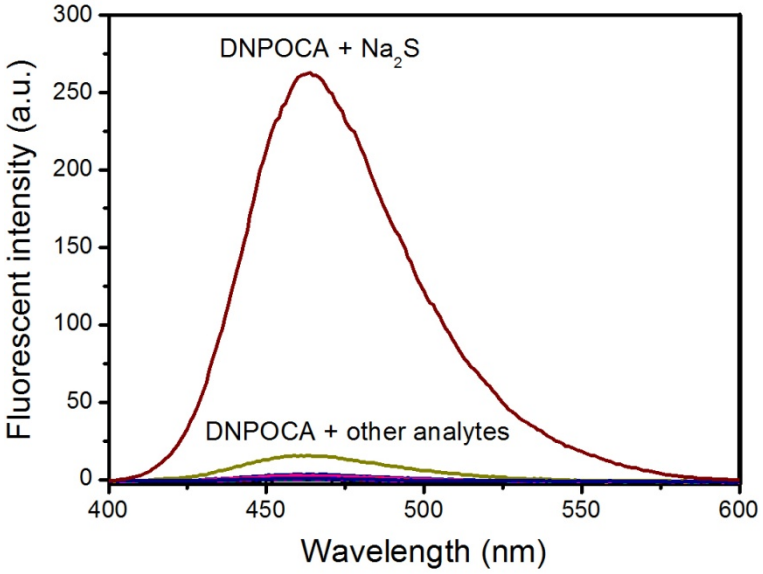


Figure S10. Fluorescent spectra changes of the probe DNPOCA (5.0 μM) in the presence of various species (20 equiv.) in aqueous solution (20 mM, pH = 7.4, CH_3CN/PBS = 1:9, 3 mM CTAB) at room temperature.

S7. Effect of pH for H_2S detection

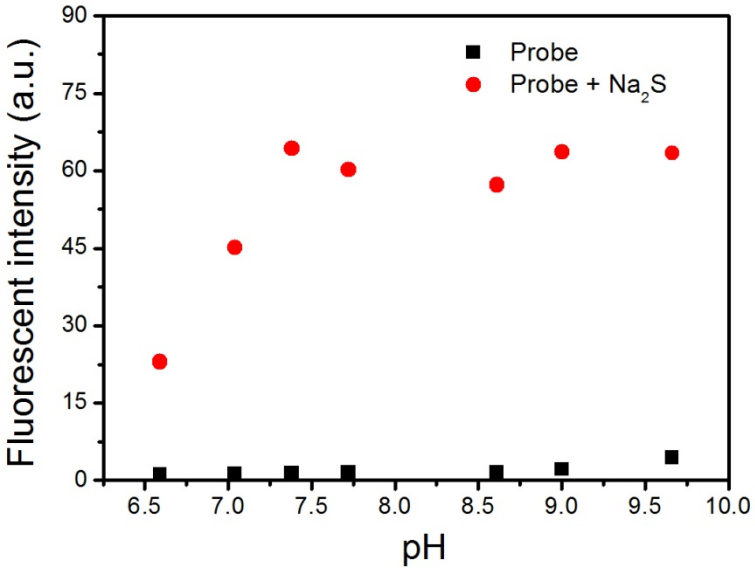


Figure S11. Fluorescence intensity changes of the probe DNPOCA (5 μM) at different pH values in the absence (■) or presence (●) of Na_2S (100 μM) in aqueous solution (20 mM, CH_3CN/PBS = 1:9, 3 mM CTAB) at room temperature. λ_{ex} = 365 nm, d_{ex} =1.5, d_{em} = 3 nm.

S8. Cytotoxicity assay

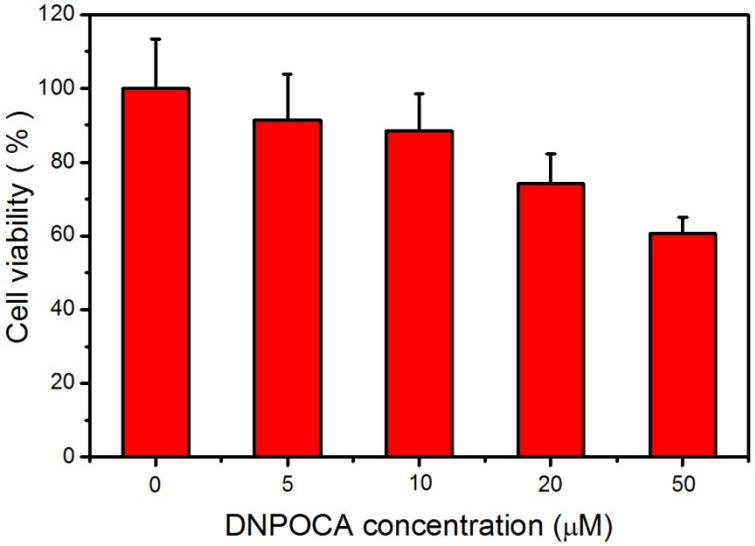


Figure S12. Cytotoxicity assay of the probe DNPOCA for HepG2 cells after 24-h culture.

References

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