Supporting Information

To construct a novel fluorescent probe based on carbazole platform for rapid and specific detection of H_2S and its application in bioimaging and food detection

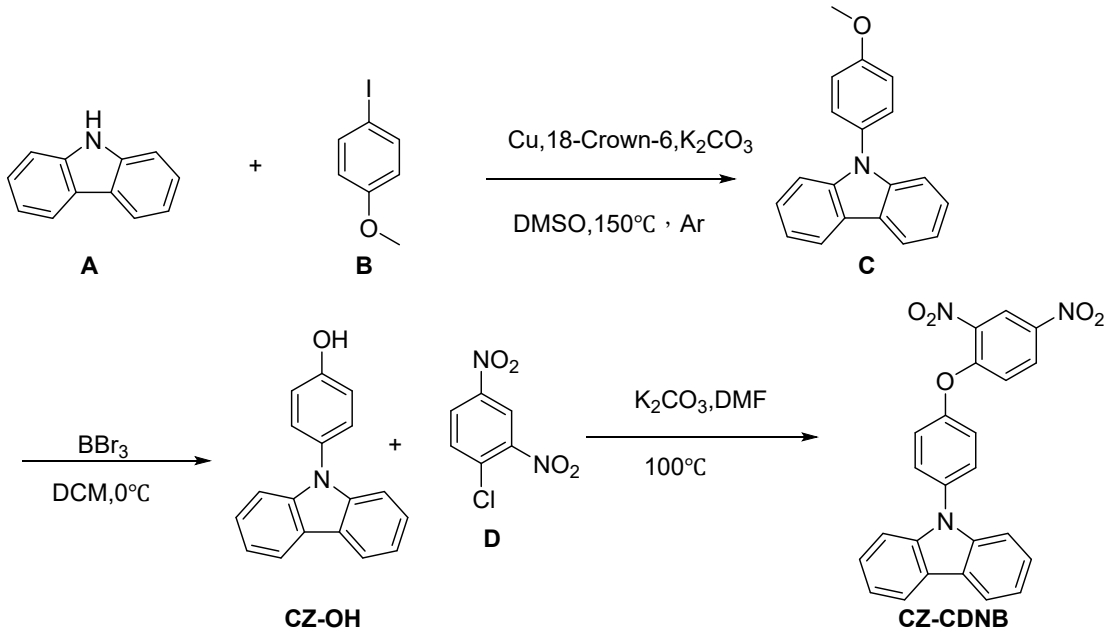
Shuntao Zhang, Xingyong Liu, Xiangjun Chen, Jiefeng Tang and Juan Wang*
College of Chemical Engineering, Sichuan University of Science & Engineering, Zigong 643000, China
*Corresponding Author: Juan Wang
Email: wangjuan2022@suse.edu.cn

Table of contents

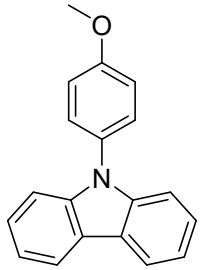
1. Experimental Section

1.1 Materials and Methods

All reagents and solvents were purchased from commercial suppliers and used without further purification. The water used in the experiments was deionized beforehand. Reactions were monitored by thin-layer chromatography (TLC), and reaction components were observed under UV light according to TLC. Fluorescence spectra and relative fluorescence intensity were measured using a Shimadzu RF-5301 fluorescence spectrometer, with an excitation wavelength of 295 nm, an excitation slit width of 1.0 nm, and an emission slit width of 5.0 nm for all fluorescence measurements. UV-visible spectra were measured using a Shimadzu UV-2700 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER600 spectrometer. pH was measured using a PHS-3C acidity meter. Electrospray ionization mass spectrometry (ESI-MS) was recorded on an Agilent 1100 series instrument. Cell images were obtained using a fluorescence microscope (Leica, Germany).

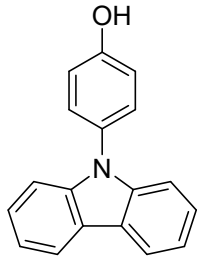


Preparation of compound **C**:



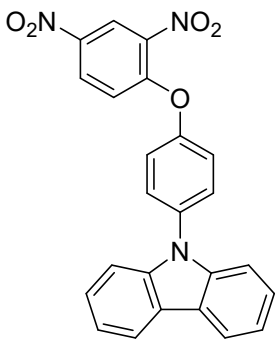
Copper powder (4.9g, 75mmol, 7.5eq), 16-Crown-6 (1.32g, 5mmol, 0.5eq), K_2CO_3 (10.37g, 75mmol, 7.5eq) were added to the mixture of compound **A** (1.67g, 10mmol, 1.5eq) and compound **B** (3.51g, 15mmol, 1.5eq), and refluxed at 150°C for 10 h under argon conditions. It was detected by TLC, and after the reaction was complete, it was extracted with ethyl acetate and recrystallized. Colorless crystal compound **C** (2.5g, 91.5%) was obtained. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.16 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.46 (d, *J* = 8.7 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.29 (t, *J* = 7.4 Hz, 1H), 7.15 – 7.10 (m, 1H).

Preparation of compound **CZ-OH**:



Under argon and ice bath, compound **C**(7.5g, 27mmol, 1eq) was dissolved in 100mL dichloromethane, and BBr_3(8.1g, 32.4mmol, 1.2eq) was slowly added to it. The reaction gradually increased to room temperature for 3h, and was detected by TLC. After the reaction was complete, dichloromethane was extracted and spin dried. The gray solid compound **CZ-OH** (6.9g, 98.67%) was obtained. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (s, 1H), 8.20 (d, *J* = 7.7 Hz, 2H), 7.43 – 7.33 (m, 4H), 7.30 – 7.21 (m, 4H), 7.07 – 7.01 (m, 2H).

Preparation of compound **CZ-CDNB**:



K_2CO_3 (336mg, 2.43mmol, 1.5eq) was added to the mixture of compound **CZ-OH** (420.5mg, 1.62mmol, 1eq) and compound **D** (394.5mg, 1.95mmol, 1.2eq) and

dissolved with 15mL DMF. Mix 8 h under 100 ℃, using TLC test, after the reaction with methanol and ethyl acetate recrystallization get a yellow solid compound **CZ-CDNB** (526 mg, 76.33%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.95 (d, J = 2.8 Hz, 1H), 8.51 (dd, J = 9.3, 2.8 Hz, 1H), 8.26 (d, J = 7.7 Hz, 2H), 7.78 (d, J = 8.7 Hz, 2H), 7.57 (d, J = 8.8 Hz, 2H), 7.50 – 7.42 (m, 5H), 7.34 – 7.29 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.98, 157.87, 146.92, 145.41, 144.81, 139.82, 134.38, 131.52, 127.97, 127.23, 127.17, 125.81, 125.43, 125.02, 114.85. HRMS (ESI): $C_{24}H_{16}N_3O_5$ for $[M+H]^+$, calculated 426.1090, found 426.1089.

1.2 Selectivity and Specificity

Prepare **CZ-CDNB** in dimethyl sulfoxide (DMSO) to make a 10 mM stock solution of H_2S. Prepare H_2S solutions by adding solid sodium hydrosulfide to aqueous solutions. The solutions were prepared using analytical grade reagents and deionized water. Solutions of Cu^{2+}, Ca^{2+}, Mn^{2+}, Al^{3+}, Ba^{2+}, Mg^{2+}, I^-, F^-, CO_3^{2-}, $S_2O_8^{2-}$, HSO_4^-, HSO_3^-, SCN^-, $S_2O_3^{2-}$, $S_2O_5^{2-}$, $S_2O_6^{2-}$, SO_3^{2-}, NO_2^-, CH_3COO^-, $(NaPO_3)n^-$, L-Cys, D-Cys, GSH were prepared at a concentration of 10.0 mM. These solutions were stored at room temperature (25 °C), and their fluorescence spectra were recorded.

1.3 Cell Viability Assay

Cell toxicity of probe **CZ-CDNB** was studied using A549 cells (lung tumor cells) and CCK-8 reagent. A549 cells were seeded in a 96-well plate and incubated for 24 hours at 37 °C and 5% CO2 prior to testing. After removing the old medium, various concentrations (0μM, 3μM, 5μM, 10μM, 20μM, 30μM) of **CZ-CDNB** were added and further incubated for 12 hours under the above conditions. After removing the old medium, cells were gently washed three times with PBS buffer. CCK solution (0.500 mg/mL, 100 μL) was then added to each well. After 4 hours of incubation, absorbance was measured at 371.4 nm using an ELISA reader to determine cell viability and calculate cell toxicity.

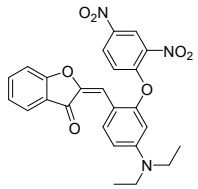
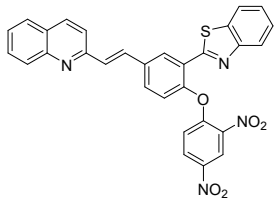
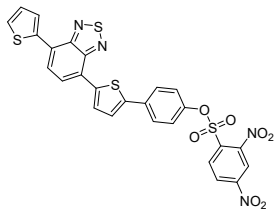
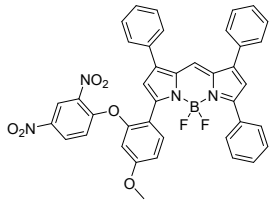
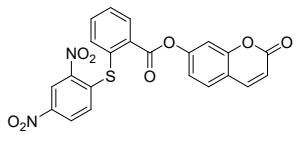
1.4 Cell Imaging

For exogenous imaging, A549 cells were incubated with **CZ-CDNB** (10μM) at 37°C for 30min, followed by cultivation with NaHS (50μM) for another 30min. As a blank control, A549 cells were incubated with **CZ-CDNB** (10μM) at 37°C for 60min. For endogenous imaging, A549 cells treated with **CZ-CDNB** (10μM) were divided into three plates after 30min of incubation at 37°C. The first plate was incubated with cysteine (Cys, 50μM) for 30min. The second plate was incubated with DL-propargylglycine (PPG, 100μM) for 30min. The third plate was incubated with PPG (100μM) and Cys (50μM) for 30min. Changes in fluorescence intensity among the test groups in the same field of view were observed under a fluorescence microscope.

1.5 Detection of H_2S Content in Food Samples

Samples of shrimp, beef, and chicken were obtained from a local Walmart supermarket, washed with deionized water, and stored. Food samples and TLC aluminum foil plates containing the probe were placed in culture dishes at temperatures of 35 °C, 27°C, and -5 °C, and colorimetric and fluorescent photos were taken at 0 hours, 2 hours, and 4 hours.

2. Supplementary data

	Structure	Time	LOD	λ_{em}
1		**5min**	**48.9nM**	**570nm**
2		**3.3min**	**112nM**	**605nm**
3		**30min**	**62.1nM**	**652nm**
4		**20min**	**203nM**	**617nm**
5		**25min**	**300nM**	**465nm**

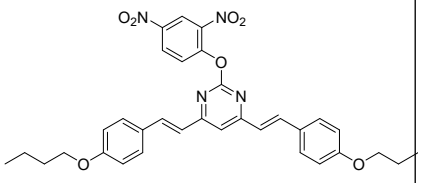
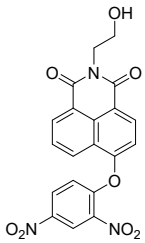
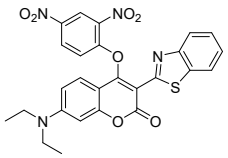
6		**100min**	**3.8uM**	**532nm**
7		**120min**	**1.4uM**	**548nm**
8		**150min**	**90nM**	**424nm**

Table S1. Comparison of other fluorescent probes based on thiolysis reactions

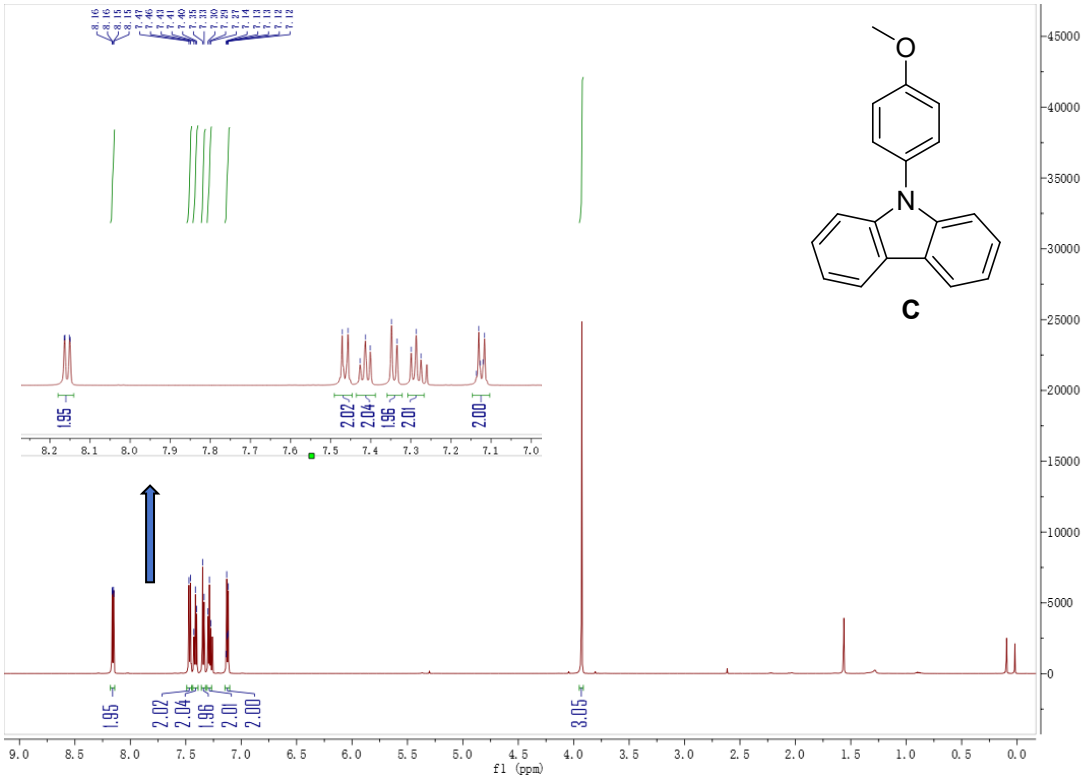


Fig. S1 ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **C**.

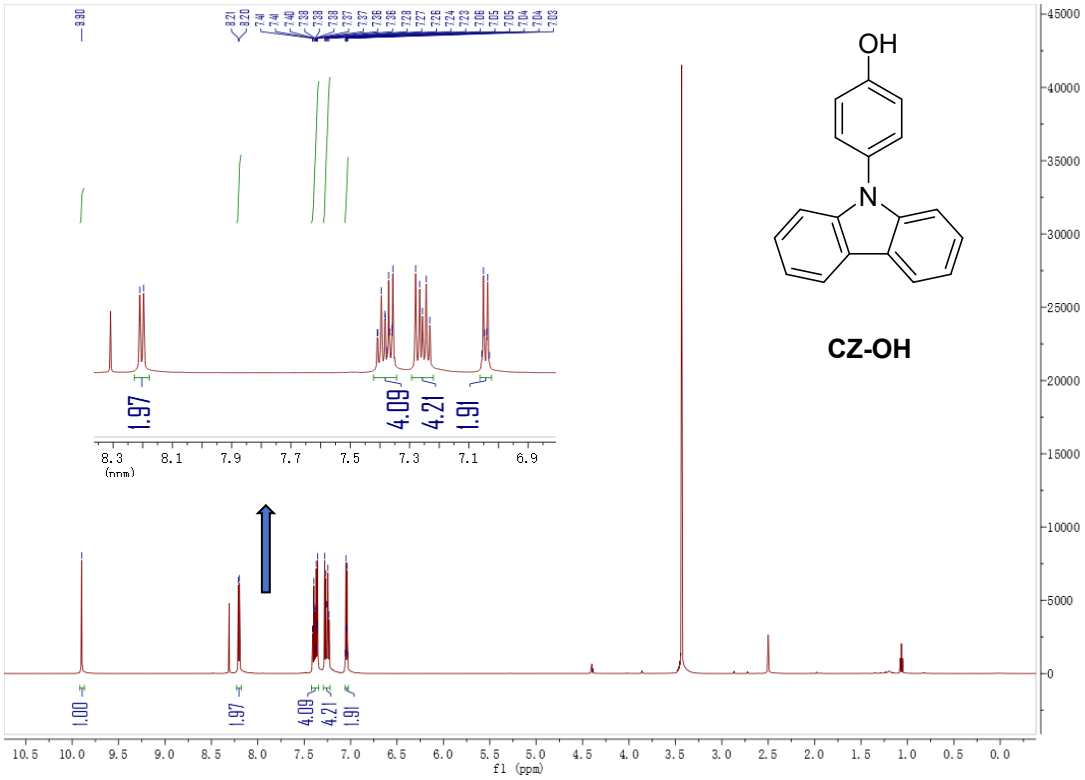


Fig. S2 ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of CZ-OH.

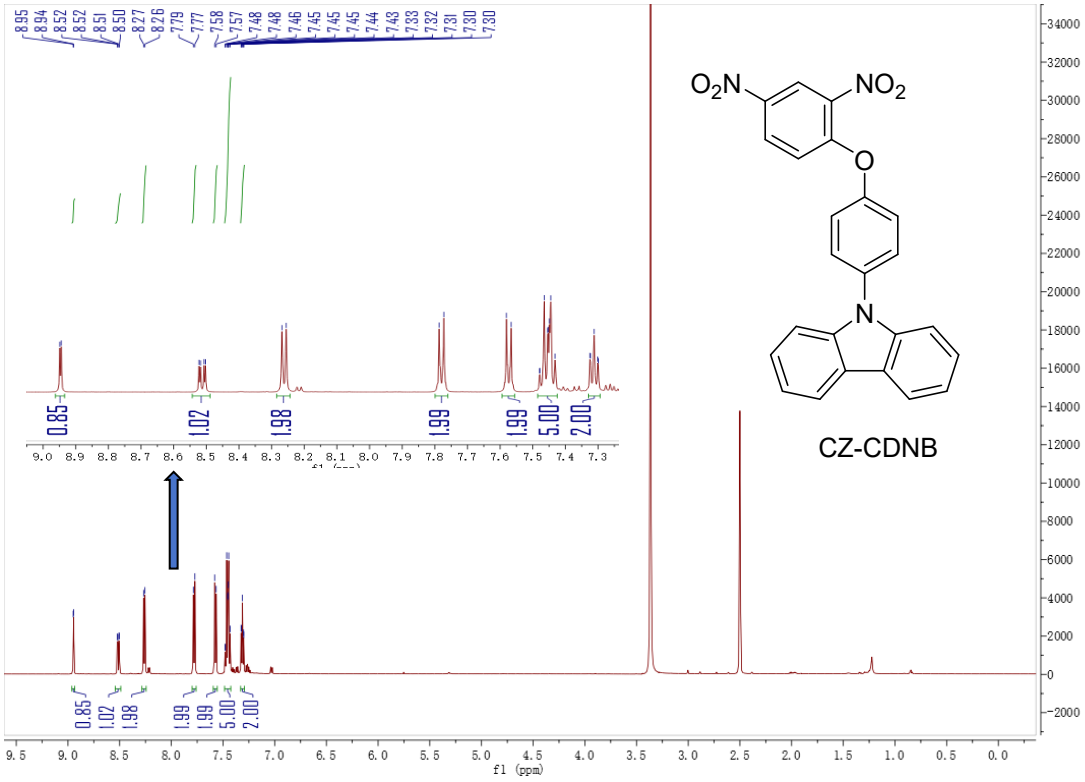


Fig. S3 ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of **CZ-CDNB**.

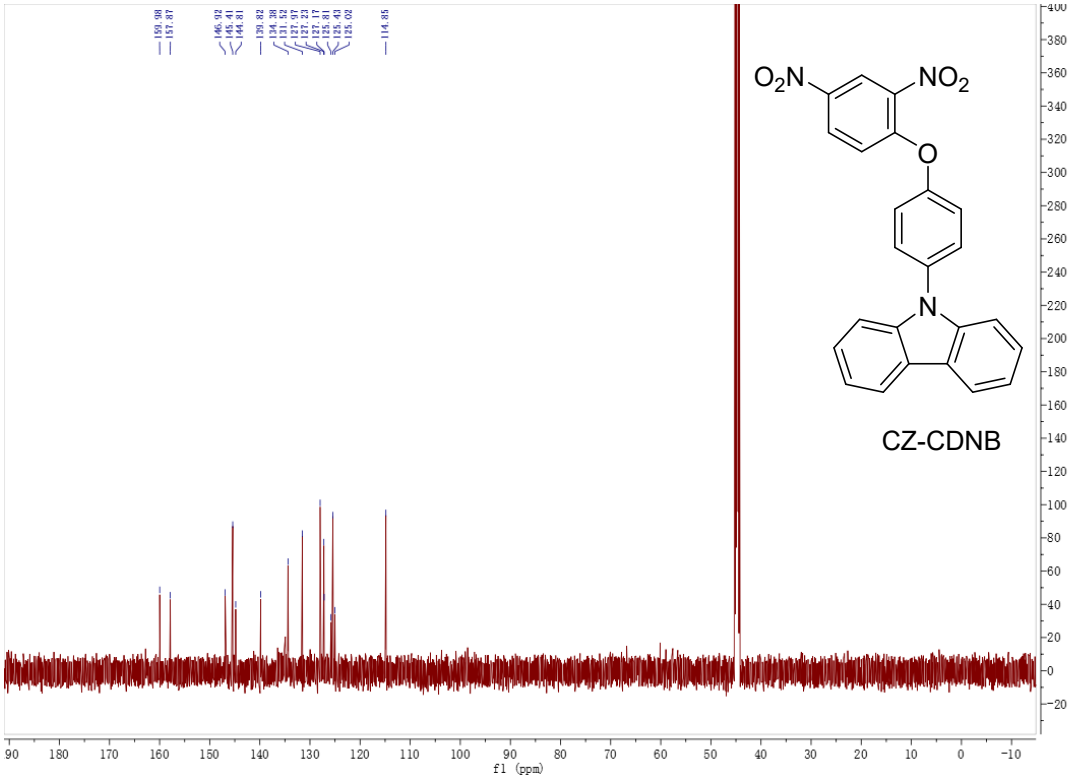
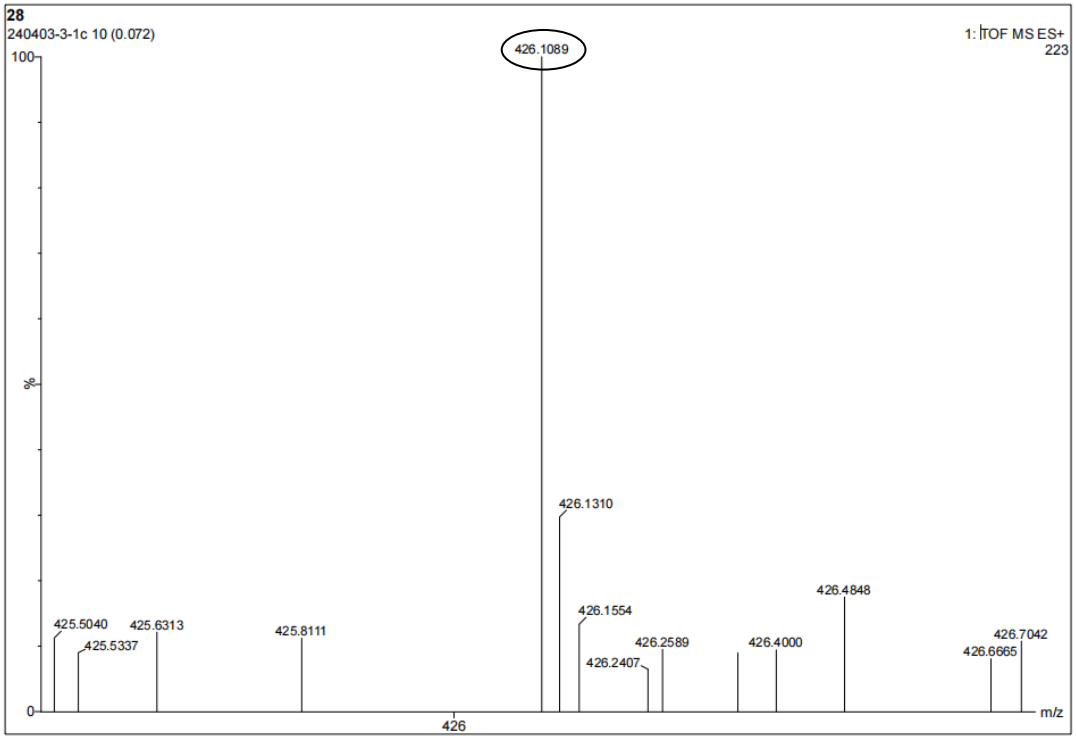


Fig. S4 ^{13}C NMR spectrum (150 MHz, DMSO-d_6) of **CZ-CDNB**.



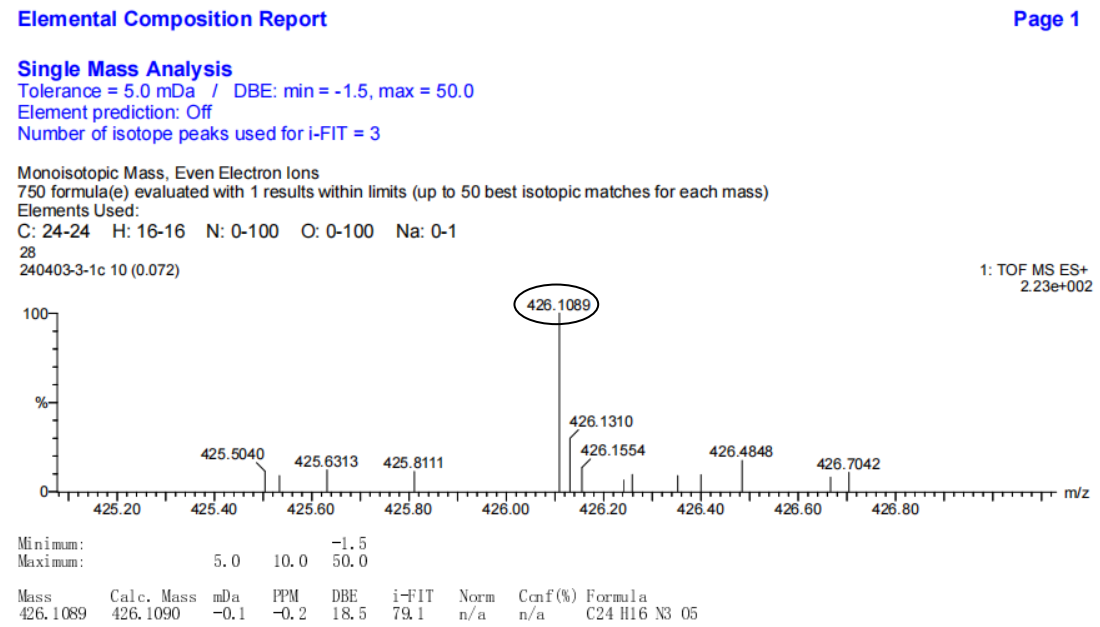


Fig. S5 Mass spectrum of probe-**CZ-CDNB**.

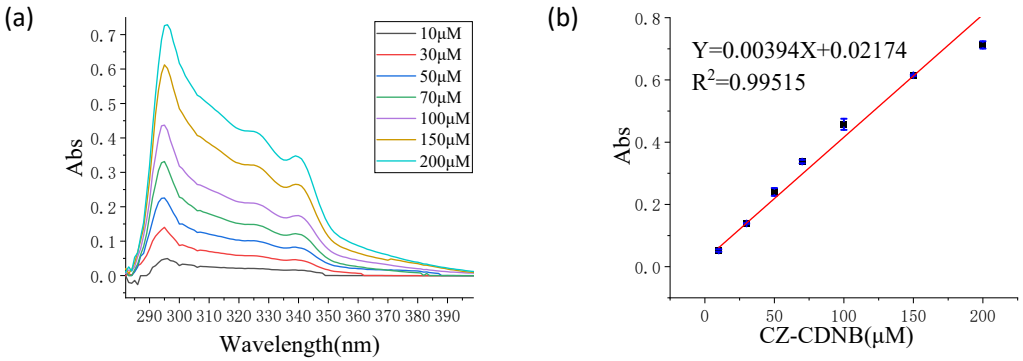


Fig. S6. (a) UV-Vis spectrum of **CZ-CDNB** fluorescent probe in the range of 10μM to 200μM.

(b) Linear relationship between UV absorbance at 295 nm and the concentration gradient of the **CZ-CDNB** fluorescent probe.

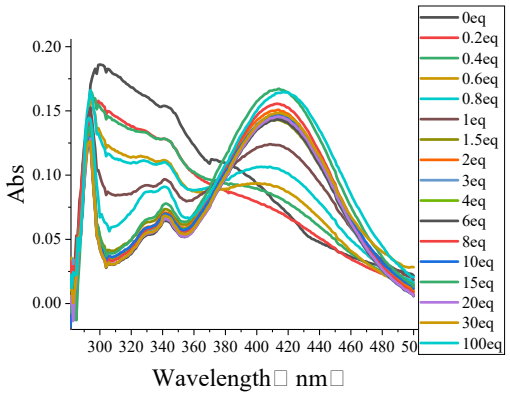


Fig. S7. UV absorption spectrum of probe **CZ-CDNB** (50μM) after addition of NaHS concentration (0-30eq).

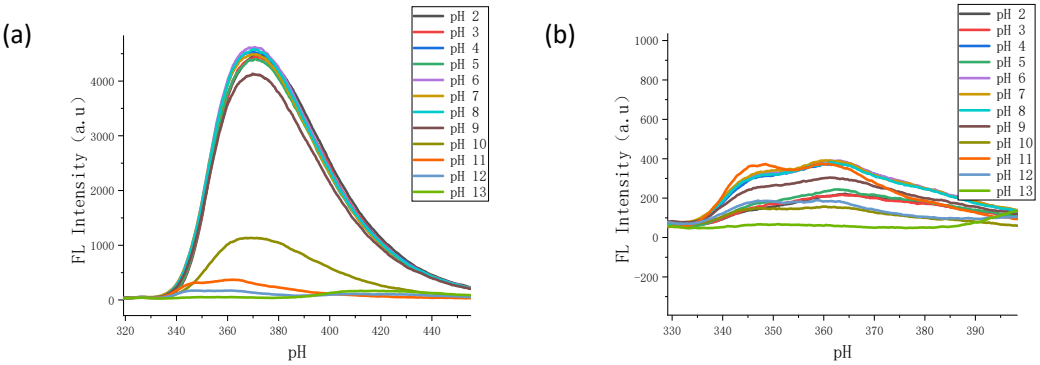


Fig. S8. Fluorescence intensity of probe **CZ-CDNB** (10μM) in PBS/DMSO (V/V=4/1, pH=7.4) buffer solution at different pH values, normalized to 1.0 using NaOH and HCl to adjust the pH. (a) Fluorescence intensity of probe **CZ-CDNB** at different pH values (pH 2-13). (b) Fluorescence intensity of probe **CZ-CDNB** + H_2S at different pH values (pH 2-13).

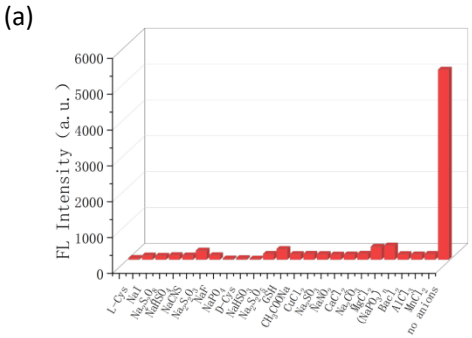


Fig. S9. (a) The bar chart shows the selectivity of the probe for detecting H_2S against other analytes. The measurements were conducted in PBS/DMSO (V/V=4/1, pH=7.4) buffer solution, with an excitation wavelength of 295 nm.

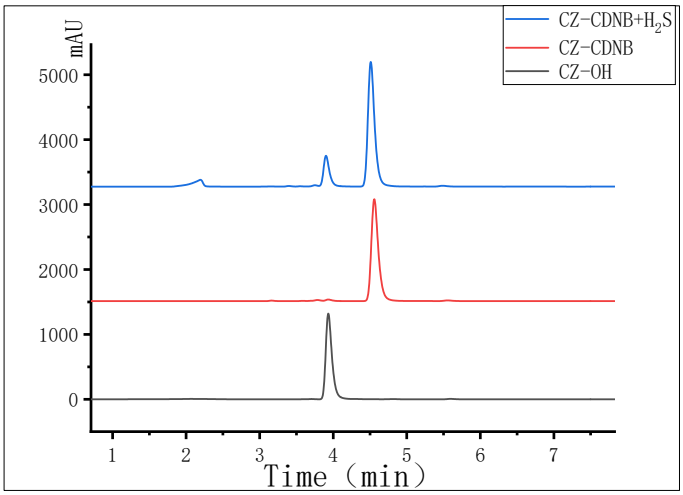


Fig. S10. HPLC titration experiment results of probe **CZ-CDNB.** (a) **CZ-CDNB**, (b) **CZ-CDNB** + NaHS, (c) CZ-OH. The mobile phase was CH_3CN/H_2O with gradient elution: 0 ~ 10 min, 90/10; flow rate was 0.8 mL/min, temperature was 30°C. Detection wavelength was 254 nm, injection volume was 5.0 μL.

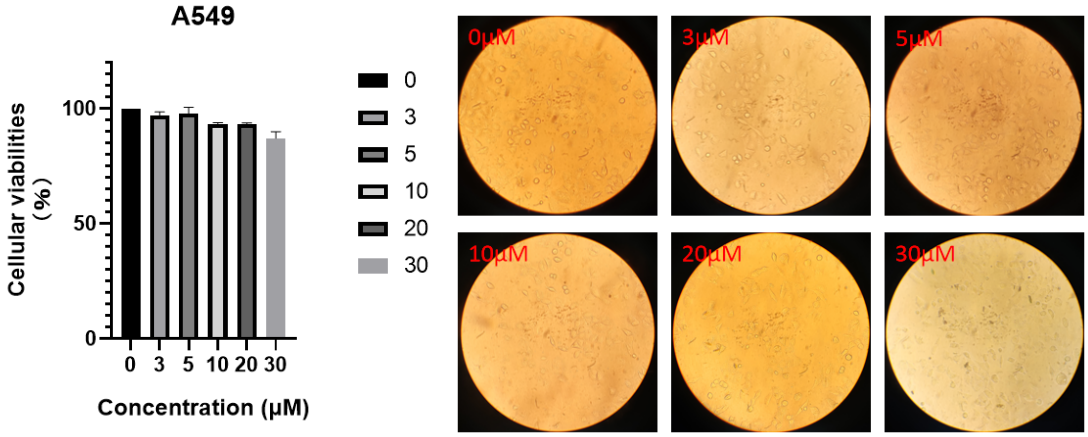


Fig. S11. Survival rates of A549 cells incubated with different concentrations of probe **CZ-CDNB** (0μM, 3μM, 5μM, 10μM, 20μM, 30μM) for 12h.

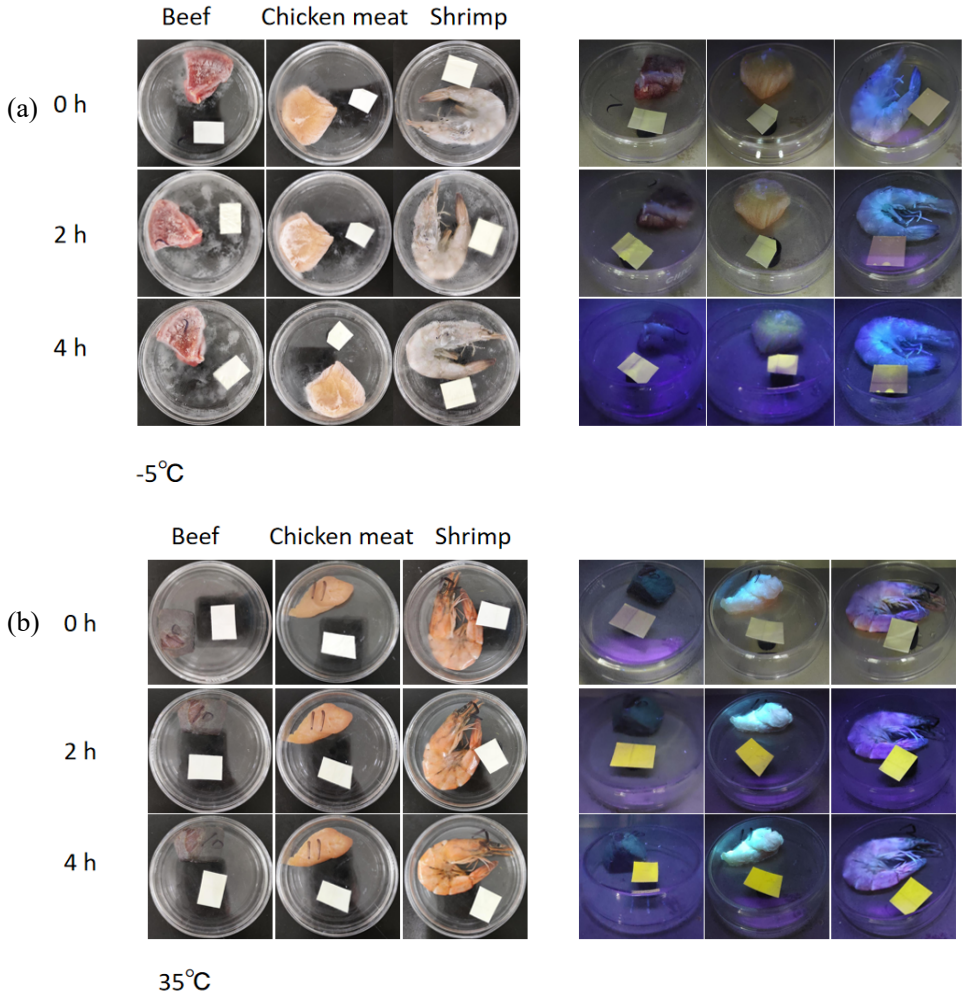
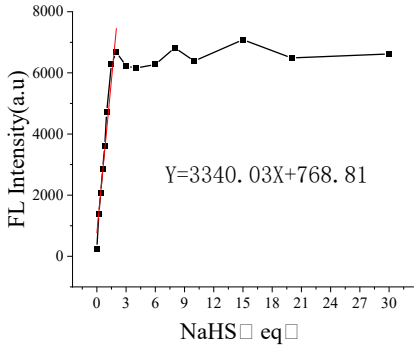


Fig. S12. Natural light and fluorescence images of aluminum foil sheets loaded with **CZ-CDNB** after exposure to raw meat (including beef, chicken, and shrimp) at 0h, 2h, and 4h. (a) At -5℃. (b) At 35℃.



261.828	261.828	264.318	263.826
266.097	266.896	271.773	265.89

Standard deviation:σ=3.062171449

Slope of slope：k =3340.03

LOD =3σ/k

The detection limit of the fluorescent probe was $2.7x10^{-8}$

Fig. S13. Detection limits of fluorescent probes