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Development of a New Hydrogen Sulfide Fluorescent Probe Based on Coumarin–Chalcone Fluorescence Platform and Its Imaging Application

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Abstract: Hydrogen sulfide (H_2S), as one of the critical gaseous signaling molecules, has important physiological functions in the human body, and abnormal levels of hydrogen sulfide are closely related to tumors, Parkinson's disease, Alzheimer's disease, and other diseases. In order to enable the detection of H_2S in the physiological environment, herein, a new H_2S fluorescence probe, named **C-HS**, based on a coumarin–chalcone fluorescence platform was developed. The fluorescence probe provides specific recognition of H_2S within a wide pH detection range (5.5–8.5), a rapid recognition response (within 10 min) for H_2S molecules, and a high selectivity for competing species. The probe **C-HS** possesses low cytotoxicity and is used to achieve the detection of exogenous/ endogenous H_2S in living cells, indicating that the constructed probe **C-HS** has the ability to track changes in intracellular H_2S levels. Therefore, probe **C-HS** could be a potential tool for the early diagnosis of H_2S-related diseases.

Keywords: fluorescent probe; photoinduced electron transfer; cell imaging; hydrogen sulfide

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1. Introduction

Hydrogen sulfide (H_2S), which smells like rotten eggs, is an important endogenous signaling molecule in many physiological processes and has attracted a lot of attention. Endogenous H_2S is usually produced by three unique enzymatic reactions, including cystathionine-β-synthetase (CBS), cystathionine-γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST) [1–6]. Studies have shown that the catalytic enzymes of endogenous H_2S are distributed in various tissues of the body, and it has been shown that H_2S plays a crucial role in a lot of physiological and pathological processes, such as neurotransmission, vasodilation, cardiac protection, angiogenesis, apoptosis, islet secretion, and anti-inflammation [6–11]. However, abnormal H_2S levels could be very harmful to the body, causing diseases including Alzheimer's disease, Down's syndrome, diabetes, and cirrhosis [4,12–15]. Therefore, it is necessary to measure the content of endogenous H_2S, which is to the benefit of understanding how H_2S affects physiological activity and causes disease.

Up to now, there are some traditional detection methods, including colorimetry, potentiometric determination, polarographic analysis, and gas chromatography, have already been presented for H_2S detection [1,2,4,13]. Nevertheless, these methods are not applicable to H_2S detection at the cellular level or in vivo. Fluorescence techniques are increasingly popular because of excellent sensitivity and specificity, short detection time, low cost, simple operation, and high spatial and temporal resolution [16–19]. Therefore, fluorescence technology to detect H_2S is undoubtedly an excellent method. However, some of the H_2S fluorescence probes developed at this stage interfere with other biothiols, including cysteine (Cys), homocysteine (Hcy), and glutathione (GSH), which have similar reactivity with H_2S [18,20–26]. These biological thiols possess sulfhydryl groups that may not only

exhibit chemical properties similar to those of H_2S but also coexist with H_2S in complex biological environments, seriously interfering with the detection of H_2S by fluorescence probes. Therefore, developing fluorescence probes that could effectively distinguish H_2S from other biothiol molecules is essential for further studies on hydrogen sulfide's biological functions and pathological effects.

Many recent studies have shown that the use of small molecule fluorescent probes for H_2S detection is mainly based on (1) the reduction reaction of nitro compounds and azide [27,28]; (2) the Michael addition reaction with H_2S-specific response; (3) the disulfide bond cleavage reaction [29]; (4) the thiolysis reaction [30]; and (5) the induced metal displacement method and metal indicator displacement method to generate CuS precipitates [31], and so on [32,33]. Among them, the probe with 2,4-dinitrobenzenesulfonyl as the recognition site has higher specificity [34]. H_2S has strong nucleophilicity in physiological environments [35,36]. The 2,4-dinitrobenzenesulfonyl group, which has strong electron-withdrawing properties, easily triggers a thiolysis reaction after exposure to H_2S, thereby releasing the fluorophore. Compared with the traditional H_2S thiolysis functional group 2,4-dinitrophenyl, the 2,4-dinitrobenzenesulfonyl group has a stronger electron-withdrawing ability, and the introduction of it into a fluorophore can significantly accelerate the H_2S-mediated thiolysis reaction. Therefore, the H_2S probe based on the 2,4-dinitrobenzenesulfonyl group has a faster response ability. In addition, the 2,4-dinitrobenzenesulfonyl group is also a good fluorescence quencher group, so the fluorescent probes designed based on it have lower background signals, which can more accurately visualize H_2S in the living environment. As an unsubstituted thiol, H_2S also has unique double nucleophilic reactivity characteristics [37]. A nucleophilic reaction between H_2S and a 2,4-dinitrobenzenesulfonyl group (an electrophilic functional group) builds an intermediate containing a persulfide group. The H_2S probe constructed based on the 2,4-dinitrobenzenesulfonyl group has another electrophilic site at the oxygen atom, so the intermediate will be subjected to intramolecular nucleophilic attack to trigger fluorescence emission, thereby improving the selectivity for H_2S. Overall, the introduction of the 2,4-dinitrobenzenesulfonyl group can promote the probe to recognize H_2S selectively.

In this work, we designed and synthesized a H_2S fluorescence probe using a nucleophilic substitution reaction. The design strategy of the probe employs a 2,4-dinitrobenzenesulfonyl group as the H_2S recognition site, which exhibits a significant electron-withdrawing photoinduced electron transfer (PET) that reduces fluorescence emission. Upon interaction with H_2S, the probe **C-HS** underwent a nucleophilic reaction to release fluorophore, which was a coumarin–chalcone fluorescence platform, thus releasing a strong fluorescence signal and achieving a rapid response to H_2S. The probe **C-HS** exhibited excellent fluorescence emission signal-to-noise ratio, fast H_2S response time (<10 min), and significant H_2S selectivity. Furthermore, it is successful to use the probe **C-HS** to detect endogenous/exogenous H_2S in living cells. The probe **C-HS** showed potential application in biological analysis and detection.

2. Materials and Methods

2.1. Reagents and Chemicals

2,4-dinitrobenzenesulfonyl chloride and *p*-hydroxybenzaldehyde were obtained from Sun Chemical Technology Co., Ltd (Shanghai, China). Triethylamine, 4-(diethylamino)salicylaldehyde, and ethyl acetoacetate were purchased from Heowns Biochem Technologies, LLC (Tianjin, China). Acetonitrile was bought from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Chloroform and ethanol were obtained from Chengdu Kelong Chemical Co., Ltd (Chengdu, China). Reagents used were bought from commercial sources and used directly without further purification.

2.2. Apparatus

^{1}H NMR and ^{13}C NMR data were obtained on Bruker Avance III HD 500 MHz NMR spectrometer using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal reference.

Agilent 6520a Q-TOF LC/MS was used to obtain high-resolution mass spectrometry data (HRMS). The UV absorption spectra were measured by Shimadzu UV-2700 UV–visible spectrophotometer, and the fluorescence emission spectra were recorded by Hitachi F-4700 fluorescence spectrophotometer. Fluorescence imaging experiments were performed with a TCS-SP8 DIVE confocal fluorescence microscope in Leica, Germany. PHS-3e in China was used to measure pH values.

2.3. Synthesis of Probes

2.3.1. Synthesis of Compound **1**

4-(diethylamino)salicylaldehyde (6.3 mmol, 1.2 g) and ethyl acetate (9 mmol, 1.2 g) were dissolved in 15 mL ethanol, 0.5 mL piperidine was added, and then the mixture was refluxed for 2 h. Cool to room temperature and extract under reduced pressure to obtain yellow solid compound **1** (1.42 g, 97%). The crude product is directly used in the next reaction without further purification.

2.3.2. Synthesis of Compound **2**

Compound **1** (6 mmol, 1.55 g) and *p*-hydroxybenzaldehyde (6 mmol, 0.732 g) were dissolved in 30 mL acetonitrile, 200 µL piperidine was added, and the mixture was reflux for 11 h. After cooling to room temperature, the mixture was filtered, washed with 10 mL acetonitrile, and dried under pressure to obtain yellow powder compound **2**. (1.23 g, 56%, m. p. 264 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.06 (s, 1H), 8.57 (s, 1H), 7.79 (d, *J* = 15.7 Hz, 1H), 7.68 (d, *J* = 9.0 Hz, 1H), 7.63 (d, *J* = 15.7 Hz, 1H), 7.57 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 6.81 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.60 (d, *J* = 2.1 Hz, 1H), 3.50 (q, *J* = 7.0 Hz, 4H), 1.15 (t, *J* = 7.0 Hz, 6H). HRMS (ESI): m/z calculated for $[M+H]^+$ $C_{22}H_{22}NO_4^+$, 364.1543; found, 364.1544.

2.3.3. Synthesis of Compound **C-HS**

Compound **2** (0.363 g, 1 mmol) and triethylamine were dissolved in 20 mL trichloromethane. After refluxing for 5 min, 2,4-dinitrobenzenesulfonyl chloride (0.535 g, 2 mmol) was added and refluxed for 5 h. After cooling to room temperature, filtration, ethanol washing, and drying were performed to obtain crude products. The crude product was purified on a silica gel column ($V_{\text{ethyl acetate}}$:$V_{\text{petroleum ether}}$ = 1:1) to obtain a red solid probe **C-HS** with a yield of 40% (0.300 g, m. p. 225 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.12 (d, J = 2.3 Hz, 1H), 8.61 (m, 1H), 8.60 (s, 1H), 8.27 (d, *J* = 8.7 Hz, 1H), 7.94 (d, *J* = 15.8 Hz, 1H), 7.80 (d, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 9.1 Hz, 1H), 7.66 (d, *J* = 15.8 Hz, 1H), 7.27 (d, *J* = 8.7 Hz, 2H), 6.82 (dd, *J* = 9.1, 2.4 Hz, 1H), 6.61 (d, *J* = 2.3 Hz, 1H), 3.51 (q, *J* = 7.0 Hz, 4H), 1.15 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 185.33, 159.92, 158.26, 153.10, 151.54, 149.33, 148.54, 148.09, 139.68, 135.05, 133.63, 132.43, 130.62, 130.32, 127.50, 126.66, 122.60, 121.09, 115.19, 110.28, 107.91, 95.90, 44.47, 12.34. HRMS (ESI): m/z calculated for $[M+H]^+$ $C_{28}H_{24}N_3O_{10}S$, 594.1177; found, 594.1177.

2.4. Spectroscopic Test Method and Preparation of Test Solution

Dissolve the PBS (phosphate buffered saline) powder in ultrapure water and transfer it to a volumetric flask with a constant volume of 2 L. After standing, the pH was measured as 7.42 with a pH meter and sealed for use.

A 1.2 mg compound of **C-HS** was accurately weighed, dissolved in 2 mL DMSO, and evenly shaken to prepare 1 mM probe mother liquor, which was sealed for later use.

The test solution of probe concentration was 10 µM, pH = 7.4, V_{PBS}:V_{DMSO} = 8:2. In selective experiments, the analytes used DL-Hcy, L-Cys, GSH, L-Ala, L-Phe, L-Met, L-Gly, L-Arg, L-AsA, L-Lys, L-Leu, L-Pro, L-Trp, L-Ser, L-Asp, L-Val, L-Ile, His, GLu, methylglyoxal, sodium pyruvate, sodium thiocyanate, tert-butyl hydrogen peroxide, chloral hydrate, calcium chloride, magnesium chloride, sodium nitroferricyanide, sodium bisulfite, glyoxal, sodium hydrosulfide were all tested at a concentration of 50 µM.

All measurements are made in accordance with the following procedure unless otherwise stated. The 100 μL probe **C-HS** reserve solution was diluted to 2 mL and then transferred to a 10 mL volumetric flask. The solution was diluted to 10 mL with PBS to prepare the test solution. Spectral measurements were recorded at room temperature with a 1 cm standard colorimeter. The excitation wavelength is 470 nm, the excitation slit width is 10 nm, and the emission slit width is 10 nm.

2.5. Cell Culture and MTT Analysis

HeLa cells were purchased from KeyGen Biotech (Nanjing, China). HeLa cells were cultured for 24 h in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 80 U/mL penicillin, and 0.08 mg/mL streptomycin in a 37 °C incubator with 5% CO_2 and 95% air.

Hela cells were inoculated into 96-well plates, 100 μL PBS was added to the periphery of 96-well plates, and 0–50 μM **C-HS** (99.9% DMEM and 0.1% DMSO) was added to the rest of the plates, respectively. Subsequently, the cells were cultured at 37 °C in 95% air and 5% CO_2 for 24 h. Then, the liquid was sucked up, and MTT and fresh culture medium were added into the well for further incubation for 4 h. Finally, the culture medium was removed. Add 100 μL DMSO to each well and shake well to dissolve the crystal completely. The absorbance of the solution was measured at 492 nm with a microplate reader. The formula for calculating the toxicity of **C-HS** is detailed in the Supporting Information.

2.6. Imaging Test

2.6.1. Imaging of Exogenous H_2S

The first group: cells were cultured with 10 μM **C-HS** for 30 min. The second group: cells were cultured with 10 μM **C-HS** for 30 min and then cultured with 20 μM NaHS for 30 min. The third group: cells were cultured with 10 μM **C-HS** for 30 min and then cultured with 50 μM NaHS for 30 min. The fourth group: cells were cultured with 10 μM **C-HS** for 30 min, then 100 μM NaHS for 30 min.

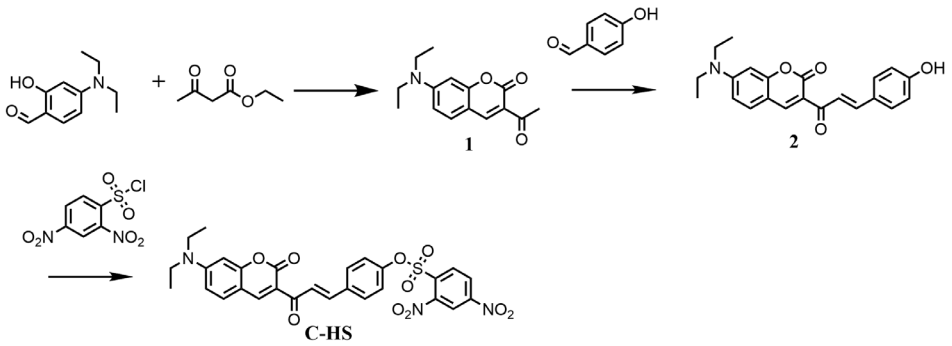
2.6.2. Imaging of Endogenous H_2S

The first group: cells were cultured with 10 μM **C-HS** for 30 min. The second group: cells were cultured with 100 μM $ZnCl_2$ for 30 min. The third group: cells were cultured with 100 μM $ZnCl_2$ for 30 min, then 10 μM **C-HS** for another 30 min. Group 4: Cells were co-cultured with 100 μM $ZnCl_2$, 10 μM **C-HS,** and 100 μM NaHS for 30 min.

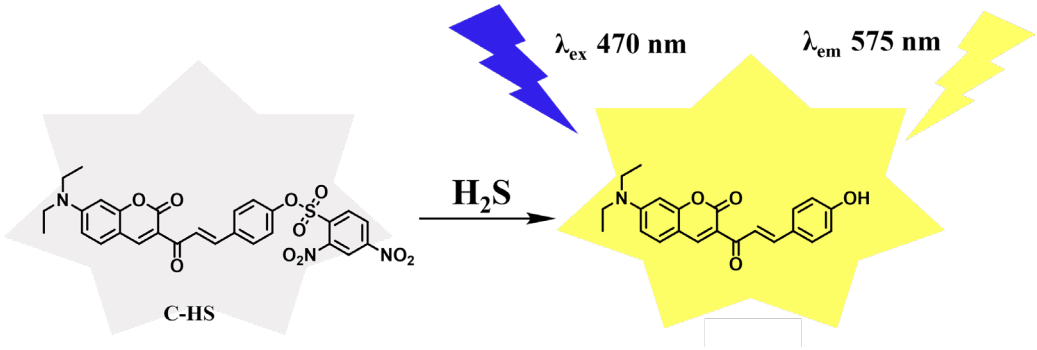
3. Results

*3.1. Designing Strategy of Probe **C-HS***

The synthetic rote is outlined as Scheme 1. The coumarin–chalcone fluorescence platform has been widely used in the design of fluorescence probes [38–44]. By introducing an electron-withdrawing group to the fluorescence platform, the fluorescence emission signal could be modulated. In this work, the H_2S fluorescence probe was constructed by using a coumarin–chalcone compound as the fluorescence platform and introducing a H_2S recognition site, the 2,4-dinitrobenzenesulfonyl group, on this platform. The introduction of the 2,4-dinitrobenzenesulfonyl group caused the fluorescence probe **C-HS** to form a PET effect, thereby causing the fluorescence probe **C-HS** to show a weak fluorescence emission signal. The 2,4-dinitrobenzenesulfonyl group would undergo a specific nucleophilic reaction with H_2S to form hydroxyl groups. As the hydroxyl group with strong electron-donating property, the electrical structure of the probe changed to "D-π-A", and the intermolecular charge transfer (ICT) effect was restored to the coumarin–chalcone fluorophore, producing a strong fluorescence emission signal. In this paper, the structure of the compound was characterized by NMR and HRMS, and the recognition mechanism of the probe and hydrogen sulfide was verified by HRMS. Scheme 2 outlines the design strategy of the fluorescence probes and the response mechanism of H_2S.



Scheme 1. Synthesis of probe **C-HS**.



Scheme 2. Recognition mechanism of probe **C-HS**.

*3.2. Measurement of the Spectral Properties of Probe **C-HS***

3.2.1. Fluorescence Titration Test of the Interaction between Probe **C-HS** and H_2S

After synthesizing and determining the structure of the compound **C-HS** (Figures S1–S3), a series of optical experiments were performed to verify the optical properties of the probe. UV–Vis spectra indicated that **C-HS** has a visible absorption peak at 465 nm (Figure 1a), and the absorption peak began to increase with a weak redshift after adding H_2S. There were no obvious fluorescence emission peaks for the probe **C-HS** without H_2S addition (Figure 1b, fluorescence quantum yield: 0.01). After the addition of H_2S, there was a fluorescence emission peak at 575 nm under 470 nm excitation, and the fluorescence intensity increased with the increase in H_2S concentration. These spectral data are very close to those reported for the coumarin–chalcone fluorophore [43]. The reason for this phenomenon is that the probe releases a 2,4-dinitrobenzenesulfonyl group upon reaction with H_2S, making the PET effect disappear and thus releasing a strong fluorescence signal. When 60 μM H_2S was added, the fluorescence signal was enhanced 57 times (fluorescence quantum yield: 0.2). Obviously, **C-HS** has a significant recognition ability for H_2S, which is consistent with our design idea. In addition, the change in fluorescence intensity enhancement during the titration experiment has a linear relationship with H_2S concentration (0–50 μM), indicating the ability of quantitative detection of H_2S (Figure 1d). The detection limit of **C-HS** is 2.25 μM (3σ/K formula). Fluorescence titration experiments and detection limits indicated good sensitivity of the probe for H_2S detection, suggesting the potential application of the probe for detecting changes in H_2S concentration in living organisms.

3.2.2. Kinetic Test of the Interaction between Probe **C-HS** and H_2S

In order to detect the reaction rate of the probe **C-HS** with H_2S, the fluorescence kinetics tests of the interaction between **C-HS** and three groups of H_2S with different concentrations (10 μM, 20 μM, 50 μM) were carried out (Figure 2). When the probe **C-HS** (10 μM) reacted with different concentrations of H_2S, the fluorescence intensity of the probe **C-HS** increased gradually with time. When interacting with 50 μM of H_2S, the fluorescence intensity of the test system increased rapidly at first, and the rate of fluorescence intensity

change gradually decreased after 10 min of interaction. Even though the response time of 10 min was not short, it was acceptable for the detection of H_2S in biological samples. It is because the common incubation time settings in bioimaging are 10, 15, or 30 min [45–48].

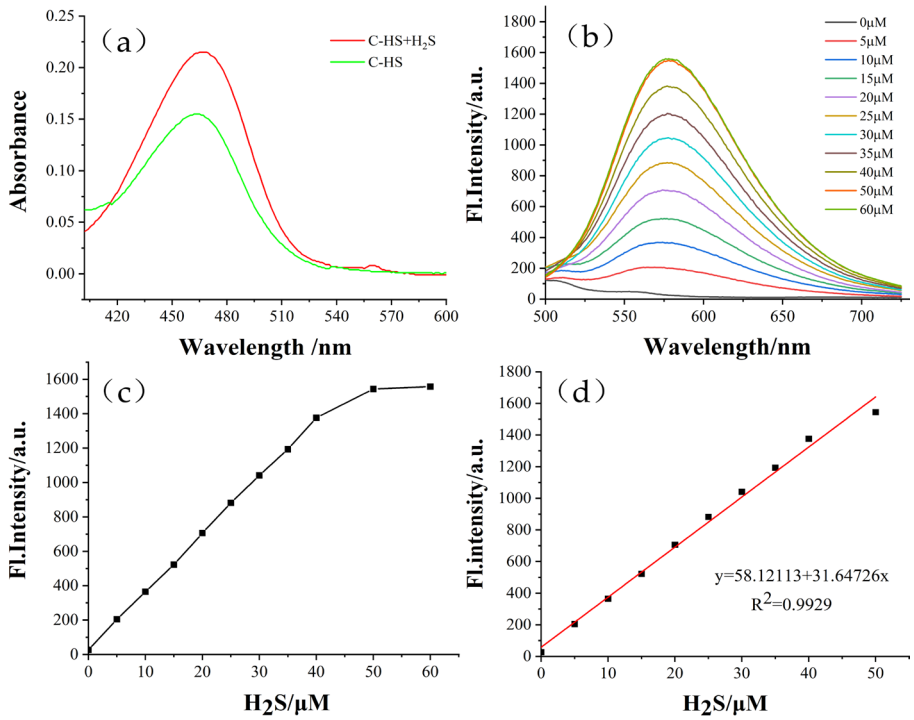


Figure 1. (**a**) UV absorption spectra of probe **C-HS** and recognizer H_2S. (**b**) The fluorescence titration spectra of **C-HS** (10 μM) probe for different concentrations of H_2S (0–60 μM) were studied. (**c**) Titration curve of probe **C-HS** and H_2S. (**d**) Linear relationship between the fluorescence intensity of **C-HS** (10 μM) and the concentration of H_2S (0–50 μM) at 575 nm (In the spectrum experiment, the incubation time of the probe and H_2S was 30 min).

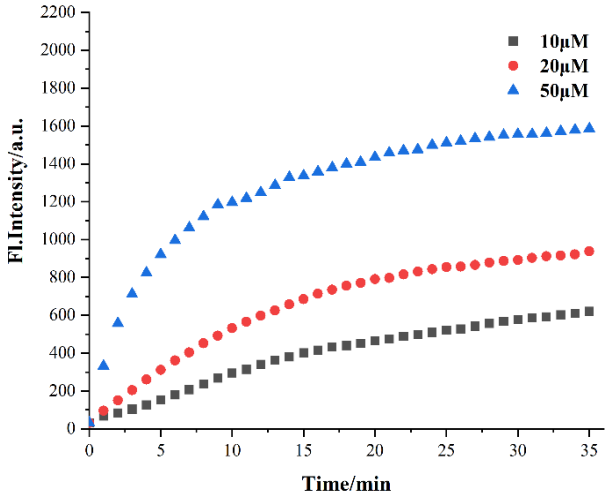


Figure 2. Time-dependent fluorescence intensities of **C-HS** (10 μM) treated with H_2S (10 μM, 20 μM, and 50 μM).

3.2.3. Selective Testing of Probe **C-HS**

It is an important criterion to evaluate the application potential of an optical probe and whether it can detect the object specifically. In order to explore whether probe **C-HS**

could specifically recognize H_2S, we selected representative analytes to conduct further selective experiments on probe **C-HS**. After reacting with the analyte for 30 min, the test system was subjected to fluorescence emission spectroscopy. As can be seen from Figure 3a, probe **C-HS** showed good selectivity for H_2S. After adding H_2S, the fluorescence intensity of the solution was significantly enhanced. However, there was no significant change in fluorescent intensity after the addition of the interfering analyte. In order to investigate whether biothiol molecules such as GSH and Cys can interfere with probe **C-HS**, the probe (10 μM) was tested on three groups of GSH, Cys, and H_2S with a concentration of 50 μM (Figure 3b). The results showed that the fluorescence intensity changes slightly after adding GSH and Cys, while the fluorescence intensity increases significantly after adding H_2S to probe **C-HS**. The selective fluorescence emission spectroscopy experiments demonstrated that the probe **C-HS** has promising applications for the detection of H_2S in complex biological environments (in coexistence with biological thiols).

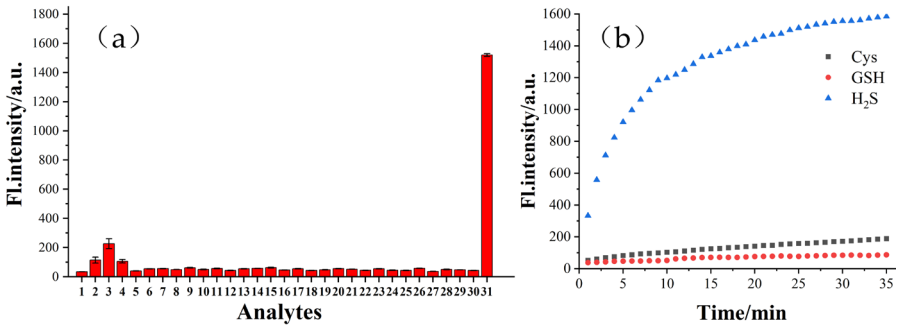


Figure 3. (**a**) The fluorescence intensity (I_{575}) of probe **C-HS** (10 μM) changed in the presence of different biorelated analytes (50 μM). The numbers represent the following: 1. PBS, 2. DL-Hcy, 3. L-Cys, 4. GSH, 5. L-Ala, 6. L- Phe, 7. L-Met, 8. L-Gly, 9. L-Arg, 10. L-ascorbic acid, 11. L-Lys, 12. L-Leu, 13. L-Pro, 14. L-Try, 15. L-Ser, 16. L-Asp, 17. L-Val, 18. L-Iso, 19. L-Chloral, 20. glucose, 21. acetone aldehyde, 22. sodium pyruvate, 23. sodium thiocyanate, 24. tert-butyl hydroperoxide, 25. chloral hydrate, 26. $CaCl_2$, 27. $MgCl_2$, 28. sodium nitroferricyanide, 29. sodium bisulfite, 30. glyoxal, 31. sodium hydrosulfide. (**b**) The fluorescence intensity changes in **C-HS** (10 μM) treated with 50 μM Cys, GSH, H_2S.

3.2.4. Fluorescence Response Spectra of Probe **C-HS** to Different pH

The pH of the test system may affect the fluorescence emission intensity of the fluorescence probe. Subsequently, we tested the response of the probe **C-HS** to H_2S at different pH conditions (3–10). The fluorescence intensity of probe **C-HS** hardly changes under different pH environments (Figure 4). This indicated that pH change had no significant effect on probe **C-HS**. When the probe **C-HS** reacted with H_2S (30 μM), the fluorescence intensity changed at different pH. The fluorescence inhibition of the probe **C-HS** + H_2S was obvious in the ultra-acidic (pH = 3) or alkaline (pH = 10) environment. The fluorescence intensity was obviously enhanced at pH 6–8. The lower fluorescence at pH < 3 may be due to the fact that the generated product compound 2 is difficult to deprotonate under this condition, resulting in a very weak fluorescence. When pH > 10, NaHS is easy to undergo acid–base neutralization reaction with the solution, which makes it difficult for the probe to react with H_2S, so it has very weak fluorescence. This indicates that the probe **C-HS** can effectively monitor H_2S under a physiological pH environment.

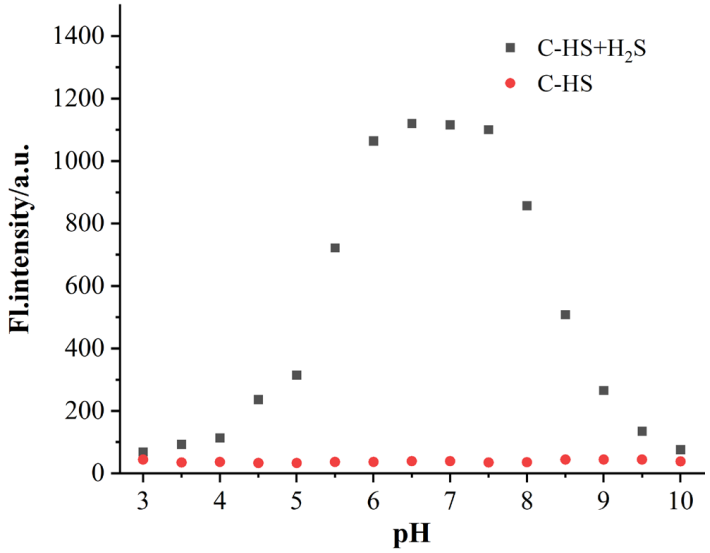


Figure 4. The fluorescence intensity changes in **C-HS** (10 μM) treated or untreated with H_2S (30 μM) at different pH solutions.

3.2.5. Photostability Test of Probe **C-HS**

Optical stability is an important factor for judging the excellent performance of fluorescent probes. In order to test the optical stability of the probe, the probe **C-HS** was continuously irradiated at 470 nm excitation wavelength for fluorescence scanning. As shown in Figure 5, within 60 min, the probe **C-HS** with and without H_2S addition showed high chemical stability.

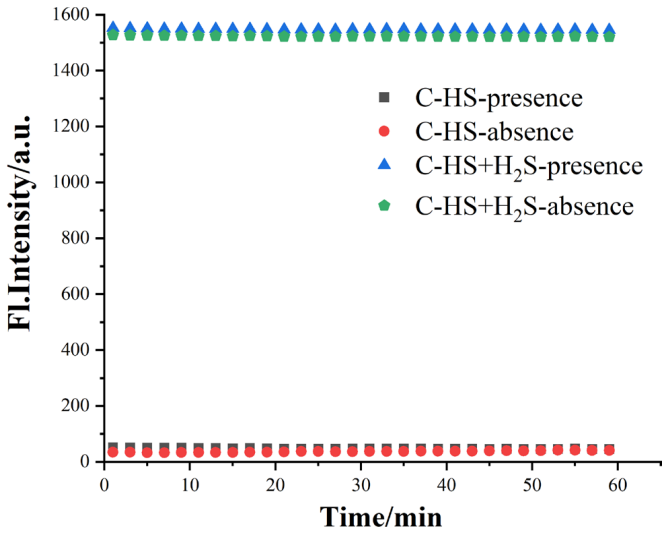


Figure 5. Plots of fluorescence intensity changes in 10 μM probe **C-HS** in the absence or presence of light irradiation and the addition of 50 μM H_2S in the absence and presence of light irradiation.

3.2.6. Verification of Fluorescence Detection Mechanism of Probe **C-HS**

The mechanism of the response of the probe to recognize H_2S was investigated using high-resolution mass spectrometry. 2,4-dinitrobenzenesulfonyl group could quench the fluorescence signal by selecting an electron-donating group to block the ICT process and produce a d-PET effect [49]. After the reaction between the probe and H_2S, a new mass peak was found at an m/z of 364.1543 (Figures S3 and S4), which is identical to the dye compound **2**. It indicates that the probe **C-HS** reacted with H_2S recognition as we expected.

*3.3. Application of Probe **C-HS** in Biology*

3.3.1. Cell Toxicity Test of Probe **C-HS**

As a fluorescence tracer, the fluorescence probe **C-HS** should provide good biocompatibility and not have toxic effects on biological samples. Therefore, before biological imaging applications, the survival of cells in the presence of the probe **C-HS** was tested using standard MTT methods to ensure that the probe **C-HS** would not cause unnecessary damage to the biological sample. The HeLa cells maintained more than 95% viability after 24 h incubation with the probe (0–50 μM). As shown in Figure 6, the results showed that the probe **C-HS** had low toxicity to cells and could be applied to biological samples.

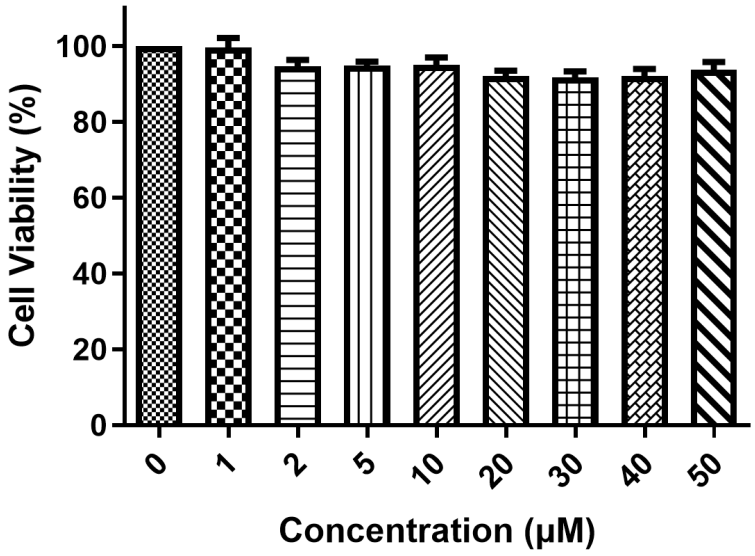


Figure 6. HeLa cells cytotoxicity of probe **C-HS**.

3.3.2. Cell Imaging Experiments

Exogenous Experiments

After verifying that probe **C-HS** has good cytocompatibility, the ability of probe **C-HS** to detect intracellular H_2S concentration changes was verified by exogenous H_2S imaging experiments of cells. The HeLa cells incubated with the 10 μM probe **C-HS** alone exhibited a faint yellow fluorescence signal, indicating that the probe could sensitively recognize the sparse intracellular H_2S (Figure 7a). Subsequently, after pretreatment with H_2S and the addition of probe **C-HS**, the fluorescence signals of HeLa cells were enhanced. And the fluorescence cells released by HeLa cells gradually increased as the concentration of extracellularly added H_2S increased (Figure 7b–d). The results of cellular exogenous H_2S fluorescence imaging experiments (Figure 7e) showed that the probe **C-HS** was able to effectively detect intracellular H_2S content change and re-validated the design strategy of the probe **C-HS** to recognize H_2S.

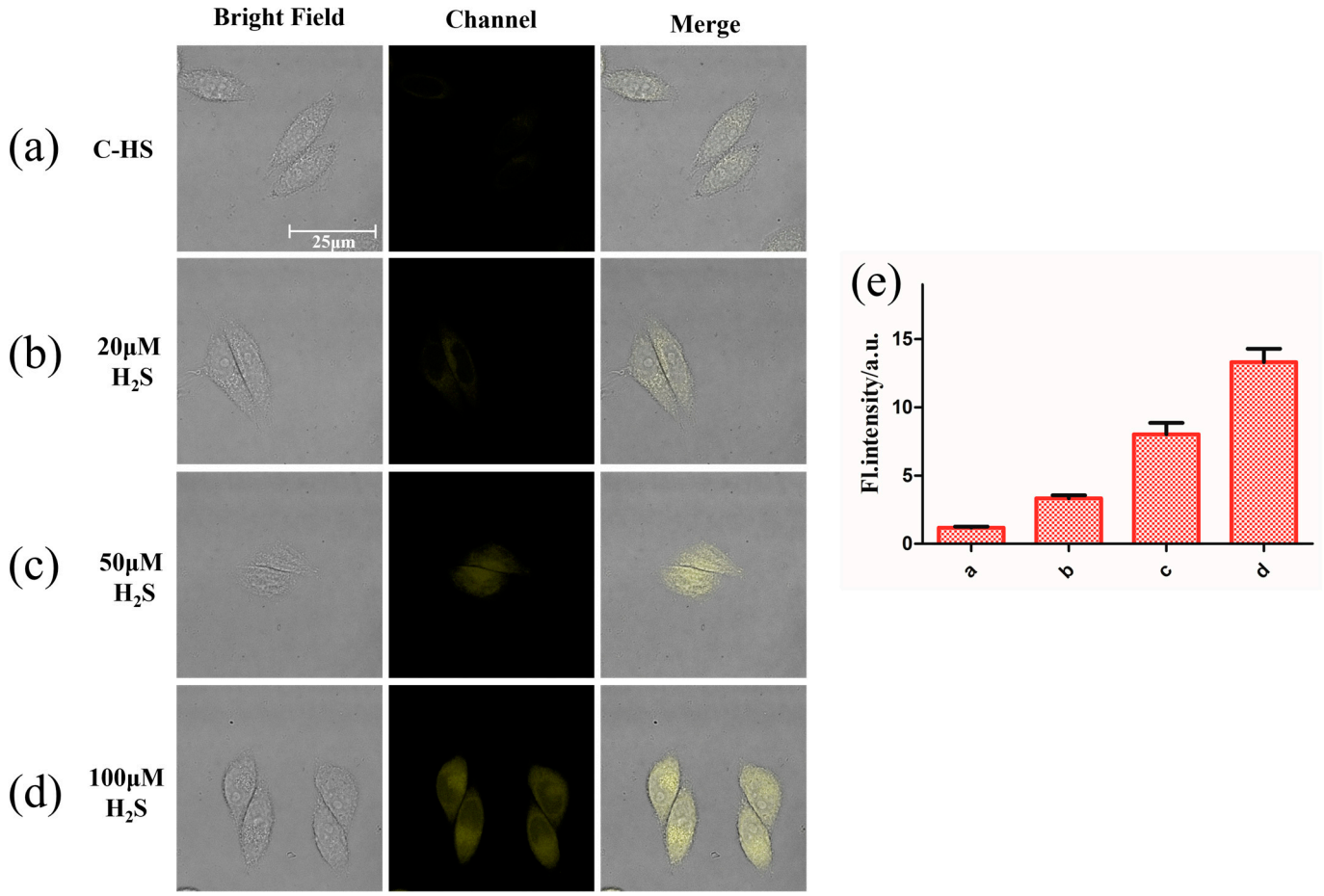


Figure 7. Imaging of endogenous H_2S in HeLa cells by a 10 μM probe **C-HS**. (**a**) Cells were cultured with 10 μM **C-HS**; (**b**) cells were pre-cultured with 20 μM NaHS, then cultured with 10 μM **C-HS**; (**c**) cells were pre-cultured with 50 μM NaHS, then cultured with 10 μM **C-HS**; (**d**) cells were pre-cultured with 100 μM NaHS, then cultured with 10 μM **C-HS**. (**e**) Quantified mean fluorescence intensities of cells in the channel. The horizontal coordinates a, b, c and d indicate the fluorescence intensity of the corresponding fluorescence channels, respectively. fluorescence intensity of the corresponding fluorescence channel. λ_{em} = 500–600 nm, λ_{ex} = 470 nm, scale bar = 25 μm. The error bars represent the standard deviation (±SD), n = 5.

Endogenous Experiments

Motivated by the promising results of exogenous cell imaging, endogenous cellular H_2S imaging tests were subsequently performed. Like the results shown in Figure 8a, HeLa cells incubated by the probe exhibited a faint yellow fluorescence signal. To verify whether this weak yellow fluorescence signal originated from the detection of endogenous traces of H_2S in the cells by the probe, we introduced zinc chloride ($ZnCl_2$), a H_2S scavenger. To ensure that $ZnCl_2$ would not have toxic effects on HeLa cells, we performed imaging experiments of $ZnCl_2$ stimulated cells. The imaging results showed that the HeLa cells stimulated by $ZnCl_2$ maintained their normal cells shape, indicating that $ZnCl_2$ did not affect the cell activity (Figure 8b). HeLa cells first incubated with 100 μM $ZnCl_2$ and then further treated with the probe released a reduced fluorescence signal, indicating that the probe could effectively detect intracellular traces of H_2S (Figure 8c). In sharp contrast, the HeLa cells stimulated by $ZnCl_2$ and then incubated with additional H_2S exhibited an intense yellow fluorescent signal (Figure 8d). The results of cellular endogenous H_2S fluorescence imaging experiments demonstrated that the probe possesses good sensitivity in living cells and enables monitoring of the changing state of endogenous H_2S content in living cells.

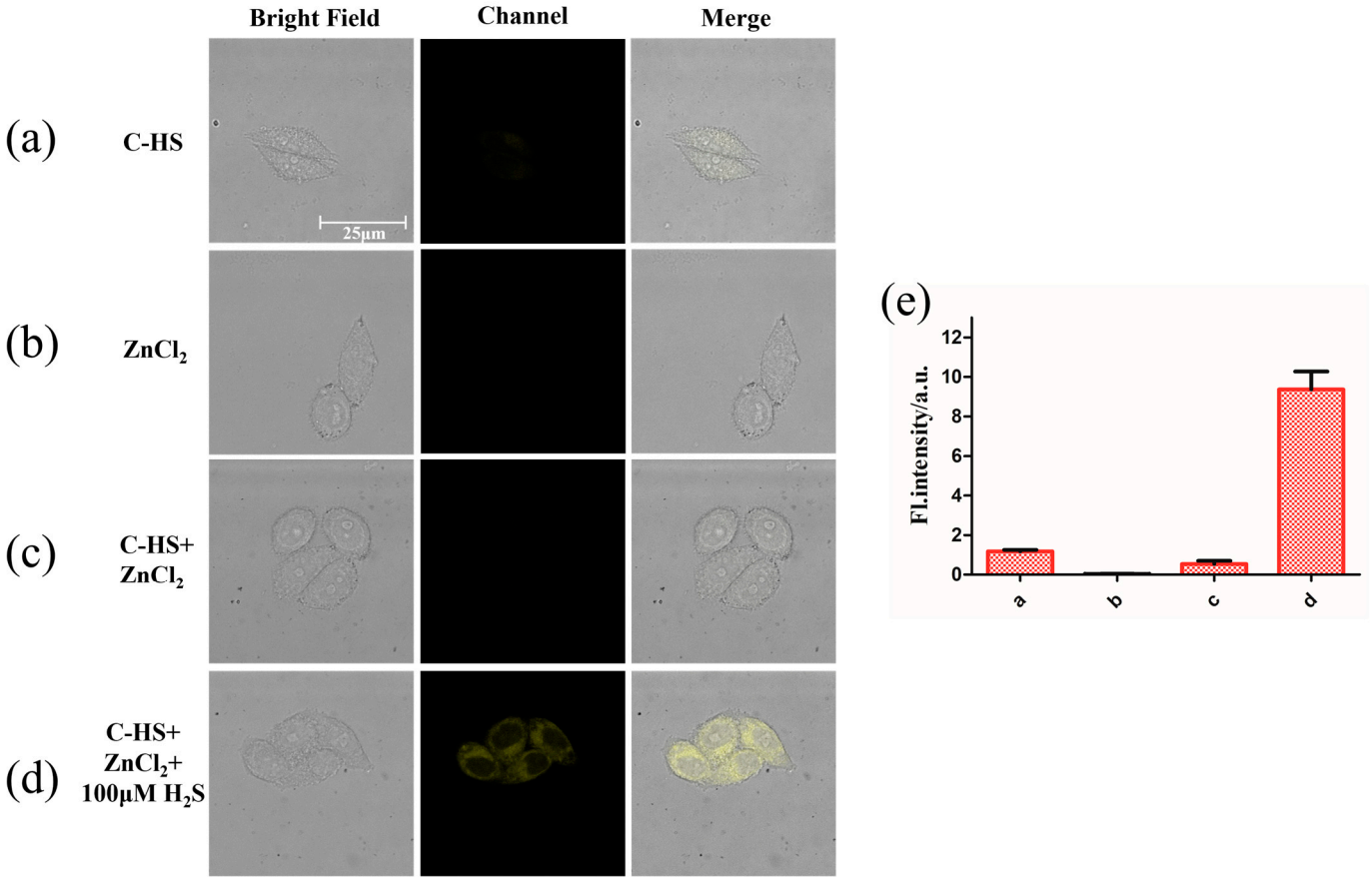


Figure 8. Fluorescent images of probe **C-HS** in living HeLa cells for detecting endogenous H_2S. (**a**) Cells were cultured with 10 μM **C-HS**; (**b**) cells were cultured with 100 μM $ZnCl_2$; (**c**) cells were pre-cultured with 100 μM $ZnCl_2$, then cultured with 10 μM **C-HS**; (**d**) cells were cultured with 100 μM $ZnCl_2$, 10 μM **C-HS**, and 100 μM NaHS in turn. (**e**) Quantified mean fluorescence intensities of cells in the green channel. The horizontal coordinates a, b, c and d indicate the fluorescence intensity of the corresponding fluorescence channels, respectively. fluorescence intensity of the corresponding fluorescence channel. λ_{em} = 500–600 nm, λ_{ex} = 470 nm, scale bar = 25 μm. The error bars represent the standard deviation (±SD), n = 5.

4. Conclusions

In summary, a new H_2S fluorescence probe based on the coumarin–chalcone fluorescent platform and combined with the 2,4-dinitrobenzenesulfonyl group as the H_2S recognition site was developed. The probe exhibits a significant PET effect that reduces fluorescence emission. Upon interaction with H_2S, the probe undergoes a nucleophilic reaction to release the fluorophore, which was the coumarin–chalcone fluorescence platform, thus releasing a strong fluorescence signal and achieving a rapid response to H_2S. The probe has excellent optical properties and excellent biocompatibility and can be used for the detection of H_2S in complex biological samples (and the detection is not interfered with by biothiols). What's more, **C-HS** also can detect exogenous and endogenous H_2S in living cells. We believe that **C-HS** can be used as a bioactive molecular tracer for H_2S imaging to further explore the physiological and pathological functions of H_2S.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemosensors11080428/s1, Figure S1: The 1H NMR of **C-HS**. Figure S2: The ^{13}C NMR of **C-HS**. Figure S3: ESI-MS spectrum of **C-HS**. Figure S4: HRMS (ESI) spectra of **C-HS** and H_2S after reaction.

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