A chalcone-based fluorescence probe for H2S detecting utilizing ESIPT coupled ICT mechanism

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Abstract

The accurate and effective identification of hydrogen sulfide holds great significance for environmental monitoring. Azide-binding fluorescent probes are powerful tools for hydrogen sulfide detection. We combined the 2'-Hydroxychalcone scaffold with azide moiety to construct probe Chal-N$_3$, the electron-donating azide moiety was utilized to block the ESIPT process of 2'-Hydroxychalcone and quenches the fluorescence. The fluorescent probe was triggered with the addition of hydrogen sulfide, accompanied by great fluorescence intensity enhancement with a large Stokes shift. With excellent fluorescence properties including high sensitivity, specificity selectivity, and wider pH range tolerance, the probe was successfully applied to natural water samples.

1. Introduction

Hydrogen sulfide (H$_2$S), a colorless and foul-smelling toxic gas, is produced by industrial processes and exists in multiple hydrocarbon sources such as exhaust emission, anaerobic digestion, and oil production [1–3]. Due to well solubility, H$_2$S can easily pollute water systems, causing significant corrosion and operating issues for industrial equipment and affecting water quality, so far as to break biological diversity [4]. Usually, it transfers into acid rain or toxic sulde-bearing wastewater by reaction with water molecules. On the other hand, H$_2$S belongs to the group of organisms' inherent signaling molecules after CO and NO, constituting an integral part of a diverse array of physiological functions. These processes encompass vasodilation, neuromodulation, apoptosis, and various others [5]. Nonetheless, abnormal H$_2$S in the human body leads to miserable physical problems, including ischemic disease, atherosclerosis, tumor, diabetes, and cardiac hypertension [6–9]. One of the pivotal aspects of water quality detection is monitoring the H$_2$S concentration fluctuation in water.

Nowadays, various methods have been applied for H$_2$S quantitative detection, ranging from electrochemical analysis [10] and chromatography to colorimetry [11]. When contrasted with the methods above, fluorescence analysis has garnered substantial attention due to its notable benefits, including but not limited to higher sensitivity, superior selectivity, outstanding compatibility and in situ imaging capability [12–16]. Numerous fluorescent probes have been obtained through diverse design strategies, encompass a range of chemical processes such as azide reduction [17] and nitro compound reduction [18–19], nucleophilic reactions [20–22], hydroxylamine reduction [23], disulfide exchange [24–25], Cu$^{2+}$ replacement approach [26–27], thiolysis of 2,4-dinitrophenyl ethers [28–29] and so on. The utilization of fluorescent probes, which rely on azide reduction, has been acknowledged as a powerful approach for identifying H$_2$S, thanks to the extraordinary selectivity between H$_2$S and biological thiols. Such selectivity effectively prevents any potential mutual interference. In recent years, significant advancements have been made toward constructing azide-based fluorescent probes that differentiate between H$_2$S and biological thiols, specifically GSH, Cys, and Hcy [30–32]. Nonetheless, most of the fluorescent probes used for H$_2$S detection have been noted to possess various inadequacies such as intricate synthesis, minimal Stokes shifts, and restricted applicability. Amounts of probes that have been reported recently
more or less seem imperfect (Table S2). Therefore, improving H₂S fluorescence probes with simple synthesis, significant Stokes shifts and multiple applications is still challenging.

Chalcones are a category of organic substances recognized for their various pharmacological traits, including but not restricted to antiphlogistic, antiviral, antitumor, and antioxidant activities. In addition to such remarkable biological properties, chalcones also exhibit remarkable optical characteristics [33–35]. Due to the facile synthetic methodology, the chalcone scaffolds have gained widespread utilization in the advancement of fluorescent probes employing fluorescence resonance energy transfer (FRET) or intramolecular charge transfer (ICT). Numerous probes have been constructed based on these principles. However, an investigation has yet to be conducted on the utility of azide-based chalcone in detecting H₂S [36–38]. In this study, a novel fluorescent probe denoted as Cha-N₃ has been synthesized. The probe has been designed to detect H₂S by incorporating azide into chalcone fluorophore, with aryl azide serving as the particular recognition site for identifying H₂S. Due to azide reduction, the probe Cha-N₃ can transfer into an excellent fluorescent fluorophore, greatly enhancing fluorescence intensity. This investigation exhibits high selectivity and sensitivity to H₂S, expressing notable variations in fluorescent turn-on signals with significant Stokes shifts. Additionally, it exhibits significant potential in accurately detecting hydrogen sulfide in diverse environmental water samples.

2. Experimental section

2.1. Reagents and instruments

All materials of analytical reagent grade were procured from commercial suppliers and utilized in their original state. The NMR spectra were recorded on Bruker 400 MHz spectrometers. The crystals were coated with an inert oil and subsequently placed under a continuous flow of inert nitrogen within a Bruker Apex II CCD diffractometer (Cu Kα radiation, λ = 0.71073 Å). IR spectra were acquired on Thermo Fisher Nicolet iS10 FT-IR. The pH was recorded by INESA pHS-3C. HRMS were measured with a Mass Spectrometer of Agilent 1290-6545XT. UV spectra were measured with Shimadzu UV-2450. The fluorescence spectra were acquired utilizing a Hitachi F-4700 fluorescence spectrometer.

2.2. Synthesis of Cha-N₃

Synthetic route of Cha-N₃ was shown in Scheme 1. 4-azido benzaldehyde was synthesized according to the reported literature, and the comprehensive synthesis procedure is available in the Supplementary Materials [39]. In a 250 mL flask, the 4-azido benzaldehyde (2.94 g, 20 mmol) and 1-(2-Hydroxy-4-methoxyphenyl) ethanone (3.32 g, 20 mmol) were added and dissolved in 50 mL of EtOH. Then, the NaOH (2.0 g, 50 mmol) was added. The reaction was stirred at room temperature for 12 h. After that, the solvent was removed through rotary evaporation and purify the product using silica gel column chromatography (eluent: EA/PE = 1/20, v/v) to obtain orange solid Cha-N₃ (2.12 g, yield 36%). The dichloromethane solution of Cha-N₃ was stored at 0°C to provide dark orange crystals. The crystal
configuration of probe Cha-N$_3$ was shown in Fig. 1 (CCDC 2250386). The structure was characterized by detail (Fig. S2-5, Table S1). IR (KBr, cm$^{-1}$) 3556.65, 3477.27, 3414.73, 2149.40, 1635.82, 1577.29, 1222.09, 825.56, 608.28. $^1$H NMR (500 MHz, DMSO-d$_6$) δ 13.49 (s, 1H), 8.30 (d, J = 9.1 Hz, 1H), 8.01–7.97 (m, 3H), 7.21 (d, J = 8.6 Hz, 2H), 6.58 (d, J = 9.0 Hz, 1H), 6.52 (d, J = 2.5 Hz, 1H), 3.86 (s, 3H) $^{13}$C NMR (126 MHz, DMSO) δ 192.27, 166.52, 166.25, 143.69, 142.22, 133.19, 131.98, 131.42, 121.12, 120.12, 114.34, 107.93 101.40, 56.27. HRMS (ESI): m/z found 295.1179, calculated for [M]$^+$: 295.0951.

2.3. Preparation of testing solution

The stock solution of Cha-N$_3$ at a concentration of 1 mM in HPLC grade DMSO, and the H$_2$S stock solution was prepared with a concentration of 10 mM by dissolving Na$_2$S•9H$_2$O in two-distilled water. Stock solutions of the analytes (10 mM), including salts (NaF, NaCl, KBr, KI, NaHSO$_3$, Na$_2$SO$_4$, NaSCN, Na$_2$CO$_3$, NaHCO$_3$, NaNO$_2$, Na$_2$SO$_4$, CH$_3$COONa, Na$_2$S$_2$O$_3$, NaClO, H$_2$O$_2$, MgSO$_4$, CaCl$_2$, PhSNa) and biothiols (Cys, Hcy, GSH) were prepared in twice-distilled water and used freshly. We carried out the spectrophotometric experiments of the probe Cha-N$_3$ (5 µM) with DMSO-PBS buffer (4:1, v/v, pH 7.4) in a 10 mm light path quartz cell. For fluorescence measurements, emission spectra were performed on excitation at 430nm over the scan range of 450–700 nm at room temperature. The widths of both the excitation and emission slits were 5 nm.

2.4. Analysis of the water specimens

Samples of tap water, mineral water, river water, and lake water were procured from our laboratory, supermarket, Zhujiang River, and Tianhe Lake correspondingly. The river and lake water were filtered to remove the sand before use. The methodology of standard addition was utilized to detect the concentration of H$_2$S that existed within the provided specimen. Several concentrations of H$_2$S solutions were added into the water specimens, after which the fluorescence spectra were examined.

3. Result and discussion

3.1. Design and synthesis of Cha-N$_3$

The probe Cha-N$_3$ was synthesized through a simple route with two steps. As illustrated in Scheme 1, the chalcone fluorescent probe for H$_2$S detection was successfully synthesized. Probes designed to detect H$_2$S using chalcone as a strong fluorophore, and linked to the azide group as a recognition site, have been synthesized through Claisen-Schmidt condensation based on the reported procedures [40]. Several effective fluorescent probes based on an H$_2$S-mediated azide reduction strategy have been investigated recently. [41–44]. As shown in Scheme. 2, utilizing the azide group to construct probe structure, forming a “pull-pull” system by directly binding the azide group to the 4-position of chalcone. Electron-withdrawing azide group was used to hinder the intramolecular charge transfer (ICT) mechanism, resulting in
quenching the fluorescence of the chalcone efficiently. The 2-(2’-Hydroxyphenyl)benzazole derivatives are commonly presented as the prototypical depiction of molecules exhibiting Solvent-Induced Emission (SLE) characteristics that arise from the combined effects of Intramolecular Charge Transfer (ICT) and Excited-State Intramolecular Proton Transfer (ESIPT) [45–46]. Reacting 4-amino benzaldehyde with sodium azide to yield 4-azido benzaldehyde, then efficiently transferred into probe Cha-N₃ through treatment with 2’-Hydroxychalcone under the mild condition in EtOH.

### 3.2. Concentration-dependent behavior of H₂S with probe Cha-N₃

The fluorescent properties of probe Cha-N₃ were investigated comprehensively. To determine the detection capacity and limit of detection of probe Cha-N₃, titration experiments were conducted by adding increasing concentrations of H₂S (ranging from 0 to 80 µM) into 5 µM probe Cha-N₃ solution (PBS buffer/DMSO, 1:4, v/v) As shown in Fig. 2a, no notable emission peak was detected in the fluorescence spectrum of the in the free probe Cha-N₃ solution. After incubating for 30 min, the fluorescent spectra of the specimens were assessed on excitation at 430 nm. By encountering H₂S, the probe Cha-N₃ exhibited a gradual increase in fluorescence maximum at 570 nm due to the aryl azide reduction stimulated by H₂S. Meanwhile, the absorption band at 381 nm increased obviously also (Fig. S6). Compared with Cha-N₃, a significant emission enhancement with a maximum 258-fold increment in fluorescence at 570 nm was observed. The colorless solution turn to a yellowish-orange solution corresponding with the fluorescence enhancement. The correlation between the fluorescence intensity measurements obtained from the Cha-N₃ probe and the concentrations of H₂S within the range of 0–50 µM demonstrated an exceptional degree of linearity (Fig. 2b) with the limit of detection of 122 nM (LOD = 3σ/k, where k is the slope and σ is the standard deviation of a blank measurement). The results suggest that probe Cha-N₃ exhibits a remarkable sensitivity to H₂S, making it a promising tool for detecting trace amounts of this compound in natural water systems.

### 3.3. The fluorescence intensity of Cha-N₃ with different pH and time parameters

To better investigate the pH dependence of Cha-N₃, the fluorescence signals were examined with and without the presence of H₂S under varying pH values. As illustrated in Fig. 3a, within the pH range from 4 to 8, the probe Cha-N₃ showed a low level of fluorescence emission at 570 nm, thus signifying the probe’s exceptional stability across a wider pH range. Moreover, a small portion of the probe degraded when the pH was around 8.5–10. Then probe reacted with the H₂S, and fluorescence emission at 570 nm enhanced sharply within the pH range of 4.0 to 9.0, while a decline was evident in the pH of 10. The pH-dependent investigation implies that Cha-N₃ can increase emission intensity in the main physiological pH range, illustrating that Cha-N₃ proceeds difficulty with H₂S in a robust base environment. Thus, Cha-N₃ is suitable for H₂S detection in the natural environment.
The time-dependent fluorescence dynamics of probe Cha-N₃ encounter with two equivalents of H₂S were determined by closely observing changes in fluorescence signals at a wavelength of 570 nm over a specific duration of time. As demonstrated in Fig. 3b, the fluorescence peak rapidly increments in a short time under low H₂S concentration. After 30 min, the signal gradually reached a plateau at room temperature. The kinetic process of Cha-N₃ with H₂S displayed the fast response of the probe under mild conditions.

3.4. Selectivity and anti-interference of probe Cha-N₃

Subsequently, an examination was conducted to explore the specificity of Cha-N₃ under conditions both with and without the presence of H₂S. A variety of analytes, including inorganic salts and biothiols were added. The fluorescence at 570 nm yielded a dramatic increment as Cha-N₃ encountered H₂S, but only a negligible modification was observed upon adding other species, whether inorganic salts or biothiols. Compared with other analytes, the reaction between Cha-N₃ and H₂S leads to apparent fluorescence emission color changes which can be observed with the naked eye, which further indicates that H₂S can cause the reduction process of the azido group, producing the fluorescent specie Cha-NH₂ (Scheme. 2). Furthermore, an assessment was conducted on the potential interference of other analytes in the presence of H₂S and one interfering analyte. The results in Fig. 4b showed that these interferents did not cause noticeable alterations in fluorescence with probe Cha-N₃. The high selectivity for H₂S among many interfering species ensures that probe Cha-N₃ has immense prospective for H₂S detection in complicated water systems.

3.5. The utilization of probes with samples from natural water

To evaluate the further applicability of probe Cha-N₃, we collected river water from Zhujiang River, lake water from Tianhe Lake, tap water from our lab, and Ganten mineral spring from the mall, respectively. The experiments were carried out with the standard method upon adding different H₂S concentrations (10, 20, 30 µM) in the DMSO/PBS solution. The recoveries range was 88–121%, indicating that the probe Cha-N₃ exhibits heightened sensitivity and selectivity as a fluorescent for H₂S measurement in intricate water samples.
Table 1
Detection results of \( \text{H}_2\text{S} \) in real water samples.

<table>
<thead>
<tr>
<th>Water sample (^a)</th>
<th>( \text{H}_2\text{S} ) added (µM)</th>
<th>( \text{H}_2\text{S} ) found (µM) (^b)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>10</td>
<td>9.20 ± 0.62</td>
<td>92.07</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.47 ± 0.50</td>
<td>97.35</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>26.34 ± 0.47</td>
<td>87.79</td>
</tr>
<tr>
<td>Tianhe lake</td>
<td>10</td>
<td>11.09 ± 0.42</td>
<td>110.93</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.62 ± 0.31</td>
<td>103.10</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>29.96 ± 0.54</td>
<td>99.87</td>
</tr>
<tr>
<td>Ganten spring</td>
<td>10</td>
<td>9.31 ± 1.57</td>
<td>93.07</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.11 ± 2.00</td>
<td>100.53</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>28.56 ± 1.91</td>
<td>95.20</td>
</tr>
</tbody>
</table>

\(^a\) Probe \( \text{Cha-N}_3 \) in water samples (PBS buffer/DMSO, 1/4, v/v, pH 7.4). \(^b\) Average of three measurements (± S.D.).

3.6. Proposed sensing mechanism of the \( \text{Cha-N}_3 \) to \( \text{H}_2\text{S} \)

The fluorescent product was characterized with the HRMS spectrum to gain mechanistic insight into the reaction of \( \text{Cha-N}_3 \) with \( \text{H}_2\text{S} \). The noteworthy peak detected at \( m/z = 270.1121 \) can be accurately attributed to the fluorophore \( \text{Cha-NH}_2 \), providing solid evidence for reducing the 4-azide moiety on the chalcone to the amino group (Fig. S7). Based on the evidence at hand, the 4-azide group's electron-withdrawing ability in chalcone derivatives possesses the ability to quench the fluorescence of the chalcone fluorophore. This result is contingent on the intramolecular charge transfer (ICT) mechanism [47–49]. Besides, the crystallographic analysis of probe \( \text{Cha-N}_3 \) shows a solid intramolecular hydrogen bonding between the hydroxyl group's hydrogen atom and the carbonyl oxygen atom. As shown in Fig. 1, the length of H-bond O3-H2 in the probe is 1.76 Å, close to 1.70 Å observed for 2'-hydroxychalcone derivatives. Compared to single C-O bond, the C6-O2 distance of 1.347 Å is shorter than the average value by 0.083 Å. The C8-O3 length of 1.252 Å is slightly longer than the average value of the C = O bond by 0.052 Å, indicating that the oxygen atom of C8 = O3 is partly protonation by the hydrogen bond. The crystallographic structure of probe \( \text{Cha-N}_3 \) suggests the involvement of the ESIPT process, consistent with the 2'-hydroxychalcone derivatives, confirmed to undergo excited-state intramolecular proton transfer (ESIPT) in the solution [50–51]

4. Conclusions
We have described a readily and efficient ICT coupled ESIPT fluorescent probe Cha-N$_3$ for H$_2$S detection based on 2’-hydroxychalcone. The probes demonstrated significant specificity for H$_2$S in DMSO/PBS (4/1, v/v, 20 mM, pH = 7.4), responding excellent selectively for these substances despite the presence of biological thiols or other potentially interfering substances. The maximum fluorescence intensity of Cha-N$_3$ enhanced 258-fold with a significant Stokes shift (140 nm) after encounter with the H$_2$S. Application of the probe Cha-N$_3$ was investigated, involving the detection of natural water samples. Concentrations of H$_2$S shall be precisely measured with the range of 0 ~ 50 µM.

**Declarations**

**Supplementary Information** This contains experimental details, characterization of the products, crystallographic data and cif files. This information is available free of charge on the Springer Nature website.

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**Ethical Approval** Not applicable.

**Competing interests** The authors declare no competing financial interest.

**Authors' contributions** Xiaochun Zhang synthesis and test spectral property of the probe, Xingshang Weng writing-original draft, Zongmei Yang synthesis of the probe, Peng Zhao was responsible for supervision, Weijian Chen in charge of X-ray single crystal structural analyses of the probe, Zhengxu Wu conducted NMR and X-ray single crystal structural test, Xuewen Zhuang in charge of the design of the probe, writing-review & editing and supervision.

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**Data Availability** The data that support the findings of this study are available from the corresponding author upon reasonable requests.

**References**


Schemes

Schemes 1 and 2 are available in the Supplementary Files section

Figures

![Figure 1](image.png)

Solid-state structure of showing 50% probability ellipsoids. Selected bond distances (Å): O2-H2, 0.840; O3-H2, 1.761; C6-O2, 1.347; C8-O3, 1.252; C2-O1, 1.359; C14-N1, 1.429; N1-N2, 1.241; N2-N3, 1.123.
Figure 2

(a) Fluorescence spectral changes of probe Cha-N$_3$ (5 μM) upon addition of Na$_2$S (0-80 μM). $\lambda_{ex} = 430$ nm, slits 5/5 nm. (b) The linear relationship of the fluorescence intensity of probe Cha-N$_3$ at 570 nm with the concentration of H$_2$S.

Figure 3

(a) The fluorescence intensity at 570 nm of probe Cha-N$_3$ in the absence (light blue column) and the presence (red column) of H$_2$S at different pH values. (b) The time-dependent fluorescence intensity of probe (5 μM) toward H$_2$S (10 μM) in DMSO/PBS (4:1, v/v, pH=7.4).
Figure 4

(a) The fluorescent spectral changes of probe Cha-N$_3$ upon addition of Na$_2$S and interfering analytes. (b) The fluorescence intensity changes of Cha-N$_3$ (5 μM) at 570 nm toward the tested analytes including: (1) NaF, (2) NaCl, (3) KBr, (4) KI, (5) NaHSO$_3$, (6) Na$_2$SO$_3$, (7) NaSCN, (8) Na$_2$CO$_3$, (9) NaHCO$_3$, (10) NaNO$_2$, (11) Na$_2$SO$_4$, (12) CH$_3$COONa, (13) Na$_2$S$_2$O$_3$, (14) NaClO, (15) H$_2$O$_2$, (16) MgSO$_4$, (17) CaCl$_2$, (18) PhSNa, (19) GSH, (20) Cys, (21) Hcy, (22) None. The concentration of each analyte is 200 μM. All experiments were carried out in solvent DMSO/PBS (4:1, v/v, pH=7.4) at room temperature. Each emission spectrum was performed on excitation at room temperature for 30 min after addition of an analyte. $\lambda_{ex}$ = 430 nm, slits 5/5 nm.

Supplementary Files

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- Onlinescheme1.png
- scheme2.jpg
- ESI.docx