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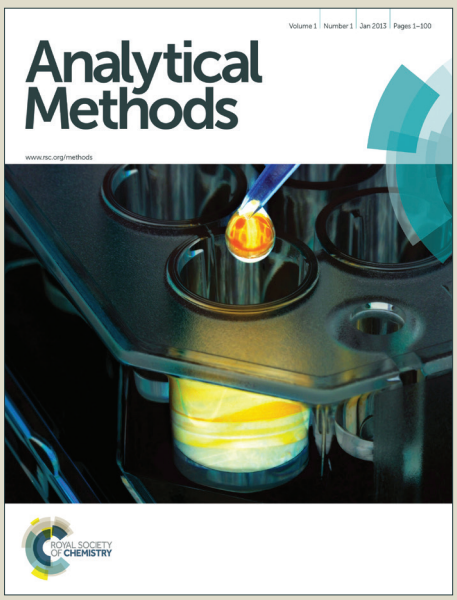

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PAPER

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

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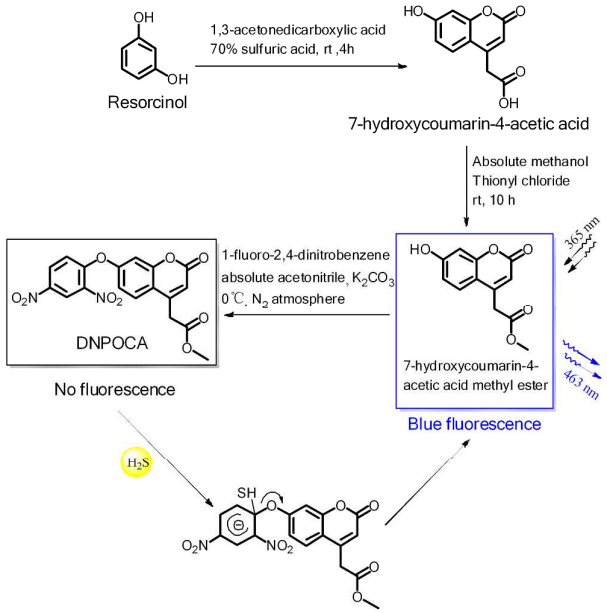
Hydrogen sulfide (H_2S) is the third endogenous gasotransmitter following nitric oxide (NO) and carbon monoxide (CO). Efficient methods for selective monitoring H_2S in biological systems are significant. In this study, we designed and synthesized a new coumarin-based probe DNPOCA for H_2S with colorimetric and fluorescent dual signaling. In this probe, 7-hydroxycoumarin-4-acetic acid methyl ester was employed as a fluorophore and dinitrophenyl (DNP) ether moiety was used as the recognition unit. The H_2S probe exhibited excellent selectivity and high sensitivity with the detection limit as low as 49.7 nM. The cytotoxicity assay showed that DNPOCA possessed low effect on cell viability. Furthermore, DNPOCA could be successfully applied for H_2S imaging in living cells.

Introduction

Hydrogen sulfide (H_2S), with unpleasant rotten egg smell, has been traditionally recognized as a noxious chemical species.[1,2] However, recent studies have challenged this view of H_2S as a toxin and demonstrate that H_2S is an important endogenous gasotransmitter following nitric oxide (NO) and carbon monoxide (CO).[3,4] In mammalian systems, H_2S is endogenously produced from cysteine or its derivatives through a series of reactions catalyzed by several enzymes such as cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) and 3-mercaptopyruvate sulfur transferase (3MST).[5-7] As a gasotransmitter, physiological level of endogenous H_2S has been known to be involved in a variety of physiological processes such as neuromodulation,[5] ischemia/reperfusion-induced injury,[8] angiogenesis,[9] vasodilation,[10] apoptosis,[11] insulin secretion[12] and inflammation regulation.[13] In addition, studies have demonstrated that abnormal levels of H_2S are closely linked with various diseases such as Alzheimer's disease,[14] Down's syndrome,[15] diabetes[16] and liver cirrhosis.[17] Although H_2S has been recognized to be connected to numerous physiological and pathological processes, many of its underlying molecular events remain unknown. Therefore, to better understand the origins, activities and biological functions of H_2S, it is very important to search for efficient methods and tools that can sensitively and selectively sense H_2S in biological systems.

Currently, several techniques such as colorimetric methods,[18,19] electrochemical assays,[20,21] gas chromatography[22] and sulfide recipitation[23] have been developed for the detection of H_2S. However, these methods often require relatively high costs, time-consuming processes, complicated sample preparation and destruction of tissues or cells, which are not suitable for monitoring H_2S in the native biological environment. In recent years, fluorescence probes, with high selectivity and sensitivity, as well as real-time and nondestructive imaging properties, have been powerful tools for



Scheme 1. The synthesis of the fluorescent turn-on probe DNPOCA and the sensing mechanism of hydrogen sulfide with the probe DNPOCA.

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sensing various cations, anions and biomolecules in biological systems.[24,25] Several fluorescent probes have been designed for detecting H_2S by taking the advantage of some specific chemical reactions, including reduction of azides or nitro groups,[26-30] demetallation of macrocyclic Cu(II) complexes,[31-33] nucleophilic reaction[34-37] and thiolysis of dinitrophenyl ether.[38-40] Despite the great developments, some of these probes still have some drawbacks. For example, the probes based on the reduction of azides generally showed long response time to obtain maximum signal changes.[27,29] In addition, azido fluorogens are also known to be photolabile, which could generate undesired fluorescent products.[41] Although the probe based on the demetallation of macrocyclic Cu(II) complexes showed high selectivity and fast response, it had low sensitivity to H_2S.[31] Those probes based on the disulfide exchange or conjugate addition followed by intramolecular ester hydrolysis reactions may be hydrolysized by cellular ester hydrolysis enzymatic.[34,37,42] Therefore, the development of new fluorescence probes for H_2S is still highly demanded.

Herein, we design and synthesize a new 7-hydroxycoumarin-based fluorescent turn-on probe DNPOCA (Scheme 1) for H_2S. In the probe, 7-hydroxycoumarin-4-aceticacid methyl ester as fluorescent moiety and dinitrophenyl (DNP) ether moiety as recognition unit were combined to achieve its sensitivity and selectivity for H_2S. In addition, methyl ester group was introduced to the fluorescent coumarin moiety to enhance cell permeability.[43] The probe could detect H_2S by colorimetric and fluorescent signals with high sensitivity and selectivity. More importantly, DNPOCA could be successfully applied for imaging H_2S in living cells.

Experimental

Materials and general methods

1,3-Acetonedicarboxylic acid and 1-fluoro-2,4-dinitrobenzene were purchased from Xiya Reagent (Shandong, China). Resorcinol was obtained from Aladdin (Shanghai, China). All other reagents and solvents were of analytical grade and supplied by local commercial suppliers. Dry solvents used in the synthesis were purified using standard procedures. Ultrapurified water was supplied by a Milli-Q system (Millipore) for aqueous solution preparation. Reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 GF254 (Qingdao Haiyang Chem. Co., Ltd., Shangdong, China). Column chromatography was conducted using silica gel 60 (Qingdao Haiyang Chem. Co., Ltd.). ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR spectra were recorded using a BrukerAvance DMX 500 MHz/125 MHz spectrometer. Peaks were based on a tetramethylsilane (TMS) internal standard. Electrospray ionization mass spectroscopy (ESI-MS) data were obtained using a Thermo Scientific LCQ FLEET mass spectrometer equipped with an electrospray ion source and controlled by Xcalibur software (Thermo Fisher Scientific, Waltham, MA, USA).TV 615 polydimethylsiloxane (PDMS) prepolymer (RTV 615 A) and curing agent (RTV 615 B) were purchased from Momentive Performance Materials (Waterford, NY, USA).

Synthesis of 7-hydroxycoumarin-4-acetic acid

Resorcinol (1.10 g, 10 mmol) was dissolved in 70% sulfuric acid (10 mL) at 0 ^{o}C and 1,3-acetonedicarboxylic acid (1.46 g, 10 mmol) was then added in a few portions. The mixture was allowed to warm up to room temperature and further stirred for 4 h. The resulting solution was poured onto crushed ice and the white precipitate was collected by filtration. After washing with water, ethyl acetate and drying overnight under reduced pressure, 7-hydroxycoumarin-4-acetic acid[44] was afforded as a white solid (2.01 g, 91%). ^{1}H NMR (500 MHz, DMSO) δ (ppm): 7.53 (d, J = 8.7 Hz, 1H), 6.81 (dd, J = 8.7, 2.3 Hz, 1H), 6.73 (d, J = 2.3 Hz, 1H), 6.21 (s, 1H), 3.82 (s, 2H). ^{13}C NMR (125 MHz, DMSO) δ (ppm): 171.14, 161.63, 160.83, 155.47, 150.70, 127.17, 113.54, 112.43, 111.85, 102.77, 37.73.

Synthesis of 7-hydroxycoumarin-4-acetic acid methyl ester

The prepared 7-hydroxycoumarin-4-acetic acid (0.80 g, 3.64 mmol) was added to a mixture of absolute methanol (15 mL) and $SOCl_2$ (0.4 mL), and then stirred for a few minutes at room temperature until the solid was completely dissolved. The reaction was further stirred for 10 h at room temperature. The solvent was evaporated and the resulting residue was filtered and washed with ethyl acetate to afford 7-hydroxycoumarin-4-acetic acid methyl ester[44] as a white solid (0.72 g, 84%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.60 (s, 1 H), 7.52 (d, J = 8.7 Hz, 1 H), 6.81 (dd, J = 8.7, 2.4 Hz, 1 H), 6.74 (d, J = 2.4 Hz, 1 H), 6.25 (s, 1 H), 3.96 (s, 2 H), 3.66 (s, 3 H). ^{13}C NMR (125 MHz, DMSO) δ (ppm): 170.13, 161.76, 160.59, 155.52, 149.98, 127.79, 113.53, 112.64, 111.68, 102.82, 52.66, 37.11.

Synthesis of DNPOCA

7-Hydroxycoumarin-4-acetic acid methyl ester (88 mg, 0.5 mmol) and K_2CO_3 (76 mg, 0.55 mmol) were added to acetonitrile (2 mL) and stirred for 30 min at 0 ^{o}C under N_2 atmosphere. Then, 1-fluoro-2,4-dinitrobenzene (103 mg, 0.55mmol) dissolved in acetonitrile (2 mL) was add to the solution at 0 ^{o}C. The resulting mixture was stirred for 6 h at 0 ^{o}C. The solvent was evaporated and the resulting residue was finally purified by silica gel chromatography (petroleum ether: ethyl acetate = 2:1, v/v) to obtain DNPOCA as a white solid (62 mg, 31%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.95 (d, J = 2.7 Hz, 1H), 8.47 (dd, J = 9.1, 2.8 Hz, 1H), 7.72 (d, J = 8.6 Hz, 1H), 7.28 (d, J = 9.1 Hz, 1H), 7.12 (d, J = 2.3 Hz, 1H), 7.10 (dd, J = 8.6, 2.5 Hz, 1H), 6.45 (s, 1H), 3.84 (s, 1H), 3.81 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 168.83, 159.45, 157.02, 155.25, 154.00, 147.16, 142.95, 140.65, 129.05, 126.89, 122.31, 120.74, 116.91, 116.81, 115.78, 108.16, 52.91, 38.01.

Preparation of detection solution

The stock solution of the probe DNPOCA was prepared at 1 mM in CH_3CN. Stock solutions (10 mM) of various testing species in water were prepared from Na_2S, $CaCl_2$, $MgCl_2$, $ZnCl_2$, KCl, NaCl, NaF, NaBr, $NaNO_3$, $NaNO_2$, NaN_3, Na_2SO_4,

Na_2SO_3, $NaHSO_3$, $Na_2S_2O_3$•$5H_2O$, CH_3COONa, Na_2CO_3, H_2O_2, $NaHCO_3$, cysteine, and glutathione.

Spectrophotometric measurements

UV-vis absorption spectra were recorded on a Shimadzu UV1780 spectrometer (Shimadzu, Kyoto, Japan). Fluorescence emission spectra were obtained on a Shimadzu RF-5301 fluorescence spectrometer (Shimadzu, Japan). Both the fluorescence emission and UV−vis absorption measurements were conducted in phosphate buffer saline (PBS, 20 mM, pH 7.4) with 10% CH_3CN (v/v) and 3 mM cetyltrimethylammonium bromide (CTAB) at room temperature. The test solution of DNPOCA (5 μM) was prepared by adding 0.01 mL DNPOCA stock solution (1 mM) and 0.19 mL CH_3CN in 1.8 mL PBS (20 mM, pH 7.4). The resulting solution was shaken well and incubated with appropriate testing species for 40 min at room temperature before recording the spectra. Unless otherwise noted, for all measurements, the excitation wavelength was 365 nm,[45] the excitation slit widths were 3 nm, and emission slit widths were 3 nm.

In kinetic studies of the probe for H_2S detection, the apparent rate constant k_{obs} for the reaction of DNPOCA with Na_2S was determined by fitting the fluorescence intensities to the pseudo-first-order equation:[46]

$$\ln[(F_{max} - F_t) / F_{max}] = k_{obs}t$$

where F_t and F_{max} are respectively the fluorescence intensities at 463 nm at a time t and the maximum value obtained after the reaction completed.

Cytotoxicity assay and cell imaging

The Human hepatocellular liver carcinoma (HepG2) cells were obtained from the Chinese Academy of Sciences (Shanghai, China) and cultured using high glucose Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Hyclone), 100 U mL^{-1} penicillin and 100 mg mL^{-1} streptomycin at 37°C in a humidified incubator containing 5% CO_2. The cytotoxicity of DNPOCA to HepG2 cells was measured by MTT assay.[47] Briefly, HepG2 cells were seeded at 1×10^4 cells per well in 96-well plates and cultured for 24 h, followed by exposure to probe DNPOCA (5 μM) for an additional 24 h. The cells treated with 0.1% acetonitrile (v/v, diluted with DMEM) were used as control experiments. The cells were then incubated with MTT solution (Sigma, 0.5 mg/mL MTT reagent in PBS) for 4 h. After removal of MTT solution, dimethyl sulfoxide was added to dissolve the formazan crystals. The absorbance was measured at 490 nm with a microplate reader (BioRad Model 680, USA). Each experiment was repeated at least three times. The cytotoxic effect of the probe to HepG2 cells was assessed by quantified the ratio of the absorbance of the probe treated cells versus the control cells.

For living cell imaging experiments, HepG2 cells were seeded on 12-well plates and cultured for 24 h. The cells were incubated with 5 μM DNPOCA for 10 min at 37 °C before being washed thrice with PBS. Then, the cells were treated with 100 μM Na_2S. After incubation for 30 min, cells were rinsed with PBS thrice and imaged by an inverted fluorescence microscopy (Olympus CKX41, Japan).

Results and discussion

Synthesis and sensing mechanism of DNPOCA

The synthetic route for DNPOCA is outlined in Scheme 1. Generally, DNPOCA was synthesized via a three-step reaction. 7-Hydroxycoumarin-4-acetic acid was first prepared by the reaction of resorcinol and 1,3-acetonedicarboxylic acid in 70% H_2SO_4 with a satisfied yield of 91%. 7-Hydroxycoumarin-4-acetic acid methyl ester was then synthesized though esterification reaction of 7-hydroxycoumarin-4-acetic acid in absolute methanol. Finally, the probe DNPOCA was directly obtained by a nucleophilic aromatic substitution of 1-fluoro-2,4-dinitrobenzene with 7-hydroxycoumarin-4-acetic acid methyl ester. The chemical structure of DNPOCA was well characterized by ^1H NMR, ^{13}C NMR and ESI-MS. The detailed synthetic procedures and relevant spectral data are given in the experimental section and supplementary material (Figs. S1-S3 ESI†).

In the study, the probe DNPOCA was constructed by connecting coumarin fluorophore and DNP group. It is

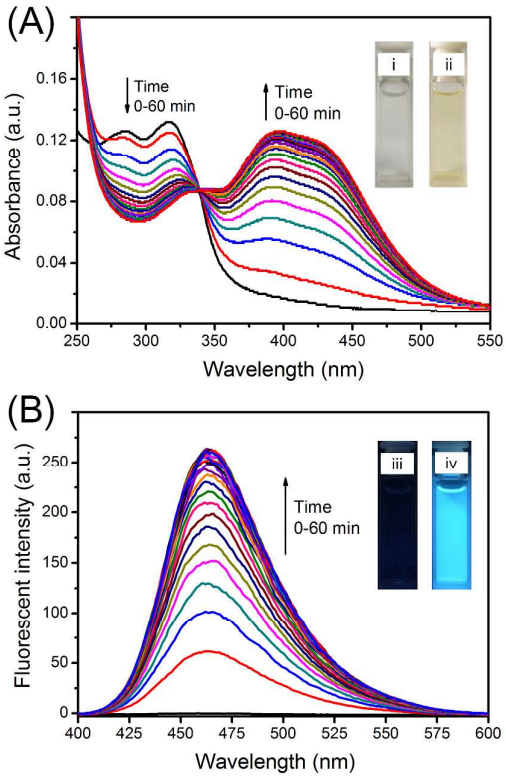

Fig. 1 Time-dependent absorption (A) and fluorescence (B) spectra of the probe DNPOCA (5.0 μM) upon the addition of Na_2S (100 μM, a standard source of H_2S) for 60 min in aqueous solution (20 mM, pH = 7.4, CH_3CN/PBS = 1:9, 3 mM CTAB) at room temperature. Inset: the color (A, under visible light) and fluorescence (B, under a 365 nm UV light) changes of DNPOCA (5.0 μM) before (i and iii) and after (ii and iv) the addition of Na_2S (100 μM).

expected that the introduction of electron-withdrawing DNP group that has the notorious fluorescence quenching effect[48] into the coumarin fluorophore could protect the HO- group and result in no fluorescence emission of DNPOCA. While the dinitrophenyl ether moiety was specifically removed by thiolysis reaction,[49,50] it could release 7-hydroxycoumarin-4-acetic acid methyl ester and recover the fluorescence emission of the coumarin moiety (Scheme 1). To demonstrate the mechanism of the reaction of H_2S with DNPOCA, DNPOCA was incubated with Na_2S (a standard source of H_2S). As we expected, the maximal emission of the thiolysis product appeared at 463 nm, which was identical to the emission of the authentic 7-hydroxycoumarin-4-acetic acid methyl ester (Fig. S4 ESI†). ESI-MS analysis showed that the fluorescence enhancement was indeed due to the formation of 7-hydroxycoumarin-4-acetic acid methyl ester (Fig. S5 ESI†). All the results demonstrated that the sensing mechanism of DNPOCA for H_2S was due to the release of 7-hydroxycoumarin-4-acetic acid methyl ester from DNPOCA via thiolysis reaction.

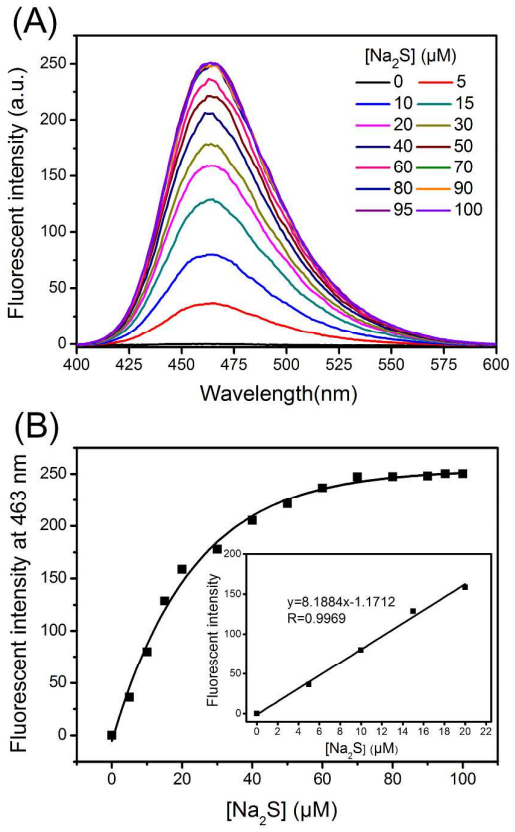

Fig. 2 (A) The fluorescence spectra of the probe DNPOCA (5.0 μM) upon the addition of different concentrations of Na_2S (0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 95, 100 μM) in aqueous solution (20 mM, pH = 7.4, CH_3CN/PBS = 1:9, 3 mM CTAB) at room temperature. (B) The fluorescence intensity changes of the probe DNPOCA (5.0 μM) at 463 nm against concentration of Na_2S in aqueous solution (20 mM, pH = 7.4, CH_3CN/PBS = 1:9, 3 mM CTAB) at room temperature. Inset: the linear relationship between fluorescence intensity at 463 nm and the concentration of Na_2S in the range of 0 to 20 μM. Each spectrum was recorded at 40 min after Na_2S addition.

Time-dependent absorption and fluorescence spectra of detecting H_2S

The time-dependent absorption and fluorescence spectra of DNPOCA upon the addition of Na_2S were recorded in PBS (20 mM, pH 7.4) with 10% CH_3CN (v/v) and 3 mM CTAB at room temperature (Fig. 1), in which CH_3CN was chosen as a co-solvent to increase the solubility of DNPOCA, and CTAB was employed as catalyst to enhance the reaction rates.[37,39] Prior to reaction with Na_2S, the DNPOCA solution was colorless with a maximum absorption at 318 nm and displayed no fluorescence emission with λ_{ex} 463 nm because of the quenching effect of 2,4-dinitrobenzene group in DNPOCA.[48] Upon the addition of Na_2S (100 μM, 20 equiv) to DNPOCA solution (5 μM), DNPOCA showed a rapid response of which the absorption at 318 nm decreased rapidly and a new absorption band centered at 395 nm increased, accompanied by the color changing from colorless to yellow (Fig. 1A, inset). Simultaneously, the fluorescence emission intensity gradually increased at 463 nm with a bright blue fluorescence (Fig. 1B, inset). These results indicated that the protection of 7-hydroxy group by the electron-withdrawing dinitrophenyl ether group, which could be specifically cleaved by H_2S-induced thiolysis, provided a switch for colorimetric and fluorescent detection of H_2S.

Owing to the rapid catabolism of H_2S under physiological conditions, the reaction kinetics of a probe is an important parameter for its biological applicability.[26-28,38] To obtain the reaction kinetics parameter of DNPOCA, the fluorescence signal at 463 nm was plotted as a function of time for data analysis (Fig. S6 ESI†), which indicated that the fluorescence emission intensity reached the maximum after around 30 min at room temperature. The observed pseudo-first-order rate, k_{obs}, was determined to be about 1.82×10^{-3} s^{-1} (Fig. S7 ESI†), which is comparable with other H_2S probes.[26-28,38]

Sensitivity of DNPOCA for H_2S

To investigate the sensitivity of DNPOCA for H_2S, a series of emission spectra of DNPOCA (5 μM) with 0 to 100 μM Na_2S were recorded (Fig. 2A). Upon the addition of increasing concentrations of Na_2S, a characteristic fluorescence emission at 463 nm was observed; its fluorescent intensity gradually increased and reached saturation when the amount of Na_2S was more than 70 μM (Fig. 2B). More than 2000-fold fluorescence enhancement was observed when the reaction completed, indicating the high sensitivity of DNPOCA for H_2S. Further study showed that the fluorescent intensity at 463 nm increased linearly with the increasing concentration of Na_2S in the range of 0-20 μM (R = 0.9969) (Fig. 2B, inset). The limit of detection (LOD) of DNPOCA for H_2S was experimentally determined to be as low as 49.7 nM based on 3σ/S (ESI†), which was much lower than those obtained by most of the existing small-molecule fluorescent probes for H_2S (Table S1 ESI†). All the results demonstrated that DNPOCA could react with H_2S sensitively and be able to quantitatively detect H_2S.

To further verify the sensitivity of DNPOCA for H_2S, the concentration-dependent absorption spectra of the DNPOCA solution with various concentrations of Na_2S were also

investigated in PBS (Fig. S8 ESI†). When Na_2S was added progressively from 0 to 100 μM to DNPOCA solution (5 μM), the absorption intensity at 318 nm gradually decreased, accompanied by increase of the absorption intensity at 395 nm. The DNPOCA solution could be used for visualizing different low concentrations of H_2S without using any expensive instruments (Fig. S9 ESI†). Even for 10 μM Na_2S, a light yellow color could be clearly observed.

Selectivity of DNPOCA for H_2S

To explore the selectivity of DNPOCA for H_2S, various biologically relevant species and DNPOCA were respectively spiked into PBS to test their fluorescence response. These biologically relevant species (100 μM, 20 equiv.) included common anions and cations (Cl^-, F^-, Br^-, N_3^-, CH_3COO^-, CO_3^{2-}, HCO_3^-, SO_4^{2-}, Ca^{2+}, Mg^{2+}, Zn^{2+}, K^+, Na^+), reactive sulfur species (SO_3^{2-}, HSO_3^-, $S_2O_3^{2-}$, cysteine (Cys), glutathione (GSH)), reactive oxygen species (H_2O_2), and reactive nitrogen species (NO_3^-, NO_2^-). As shown in Fig. 3A, the responses of DNPOCA were found to be highly selective for H_2S over the anions, cations, as well as the reactive oxygen, sulfur and nitrogen species. Among the tested species, only glutathione showed limited fluorescence response (Fig. S10 ESI†). However, the probe DNPOCA displayed ~17-fold greater response for H_2S than those by GSH. Thus, the probe DNPOCA could detect H_2S with good selectivity over biothiols (i.e. GSH, Cys) at physiological pH, which could be attributed to the differences of molecular size and pKa between H_2S and biothiols. First, due to the steric effect, the thiolysis of the dinitrophenyl ether by H_2S could be more effectively than biothiols owing to its smaller size.[51] Second, the pKa of H_2S is around 6.9, while the typical free thiols have high pKa values about 8.5.[51-53] Thus, the thiolysis of the dinitrophenyl ether reaction may be selective for H_2S at physiological pH.

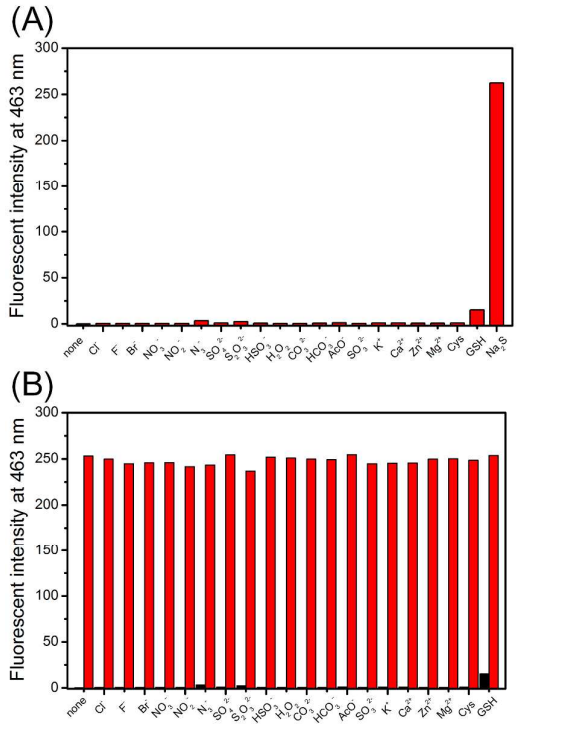

Fig. 3 (A) Fluorescent intensity changes of the probe DNPOCA (5.0 μM) at 463 nm in the presence of various species in aqueous solution (20 mM, pH = 7.4, CH_3CN/PBS = 1:9, 3 mM CTAB) at room temperature. (B) Fluorescence intensity changes of the probe DNPOCA (5.0 μM) at 463 nm upon the addition of various species (100 μM) (black column), and upon subsequent addition of Na_2S (100 μM) (red column) in aqueous solution (20 mM, pH = 7.4, CH_3CN/PBS = 1:9, 3 mM CTAB) at room temperature. Each spectrum was obtained at 40 min after the addition of various species.

To further verify the selectivity of DNPOCA for H_2S, a competition experiment was also performed by adding Na_2S to DNPOCA solution containing other biologically relevant species. The results (Fig. 3B) showed that before the addition of Na_2S, there were almost no fluorescence signals at 463 nm in the presence of other species, except GSH (a relative weak fluorescence intensity). When Na_2S (20 equiv.) were introduced to the above solutions, remarkable enhancement in fluorescence intensities at 463 nm was observed. These interesting features clearly demonstrated that the probe DNPOCA possessed high selectivity toward H_2S when other analytes presented.

Effect of pH on H_2S detection

The stability of the probe DNPOCA in acidic or basic condition is very important for practical applications. In the current study, the pH effect on DNPOCA sensing property was investigated (Fig. S11 ESI†) to confirm the practical applicability of DNPOCA for H_2S detection in biological system. The probe DNPOCA solutions don't show fluorescence response between pH 6.59 and 9.69, which suggests that the probe DNPOCA is stable over this range. However, a remarkable fluorescent signal enhancement was observed at around physiological pH 7.4 when the probe DNPOCA was treated with Na_2S (20 equiv.), indicating that the probe DNPOCA could function properly at physiological pH.

Cell imaging

The results above demonstrated that DNPOCA could be used as a rapid, colorimetric and fluorescent sensor for H_2S in aqueous solution with high sensitivity and selectivity, which also showed the potential utility of DNPOCA in biological samples. Accordingly, to evaluate the bio-imaging capability of the probe DNPOCA in living systems, the fluorescence imaging of H_2S in HepG2 cells was carried out. First, the cytotoxicity of DNPOCA to HepG2 cells was primarily evaluated using standard cell viability protocols (MTT assay) before bio-imaging experiments (Fig. S12 ESI†). The obtained results showed that >90% cells survived after 24 h with a low concentration (5 μM) of DNPOCA incubation, demonstrating DNPOCA to be only minimal cytotoxicity toward cultured cell lines. Thus, the probe at 5 μM was selected to image H_2S in living cells. HepG2 cells were incubated with 5 μM DNPOCA in culture medium for 10 min at 37 °C and then the cells were incubated with 100 μM Na_2S for 30 min. Owing to a low level of H_2S in cells, an exogenous H_2S (Na_2S) was added to increase the intracellular H_2S level.[27,54] As displayed in Fig. 4B, no fluorescence was observed when HepG2 cells were incubated

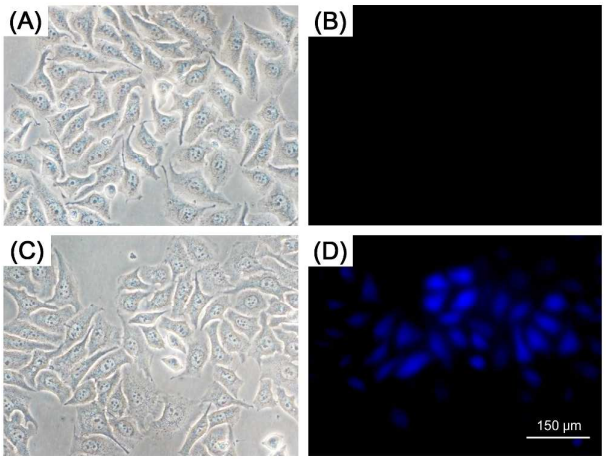

Fig. 4 Fluorescent imaging of H_2S in HepG2 cells incubated with 5.0 μM probe DNPOCA. (A) and (B) show that cells were incubated with DNPOCA only for 10 min. (C) and (D) show that cells were pre-incubated with DNPOCA for 10 min, and then incubated with 100 μM Na_2S for 30 min. (A) and (C) are bright field images. (B) and (D) are fluorescence images of (A) and (C), respectively.

with DNPOCA only, indicating that biological species did not cause interference. In contrast, addition of Na_2S to the cells pre-incubated with DNPOCA produced strong blue fluorescence (Fig. 4D). Previous studies have shown that H_2S could be rapidly metabolized under physiological conditions, which resulted in the difficulties to analyze this important molecule.[27,28] However, in the current study, the intracellular H_2S by exogenous addition can be directly captured by the probe DNPOCA to produce strong blue fluorescence emission, indicating the possibility of the probe DNPOCA to visualize H_2S in living cells.

Conclusions

In summary, we have developed a rapid and simple method for recognition and quantification of H_2S by using a coumarin-based fluorescence probe. In the probe, 7-hydroxycoumarin-4-acetic acid methyl ester was employed as the fluorophore and dinitrophenyl ether moiety was used as the recognition unit. The probe for H_2S exhibited a dual-mode of optical signal output. Meanwhile, the probe exhibited high sensitivity with the detection limit as low as 49.7 nM and excellent selectivity toward H_2S in aqueous solution. Furthermore, DNPOCA was successfully applied for fluorescent imaging H_2S in HepG2 cells, showing its practical utility in biological systems.

Acknowledgements

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