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ROYAL SOCIETY OF CHEMISTRY

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View Article Online
DOI: 10.1039/C5RA19081A

Journal Name

ARTICLE

A selective and sensitive phthalimide-based fluorescent probe for hydrogen sulfide with a large Stokes shift

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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A phthalimide-based fluorescent probe for hydrogen sulfide has been developed with high sensitivity and excellent selectivity. Upon the treatment with hydrogen sulfide, this probe displays a strong fluorescence enhancement (196-fold) with a large stokes shift (105 nm). Moreover, the potential application using this probe in biological systerm has been demesnstrated by imaging hydrogen sulfide in living cells.

Introduction

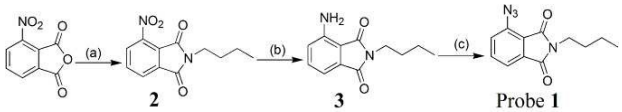
Hydrogen sulfide (H_2S) with rotten-egg odor is generally considered as a toxic gas to induce the toxication of central nervous system and the inhibition of the respiratory system.[1] H_2S can be endogenously produced through enzymatic actions by cystathionine-β-synthase,[2] cystathionine-γ-lyase[3] and 3-mercaptopyruvate sulfurtransferase[4] in mammalian system. Recently, it has been found that endogenous H_2S exerts vital functions in many physiological activities such as vasodilation,[5] neuromodulation,[6] anti-inflammation,[7] angiogenesis,[8] anti-apoptosis,[5] and inhibition of insulin signaling[9] and is therefore regarded as the third most important endogenous gasotransmitter alongside nitric oxide (NO) and carbon monoxide (CO). In addition, H_2S servers as an antioxidant or scavenger for endogenous reactive oxygen species (ROS) involving in the regulation of the cellular redox state.[10] Due to its important roles in physiological processes, abnormal levels of H_2S can cause cardiovascular dysfunction,[11] Alzheimer's Disease,[12] Down syndrome,[13] diabetes,[14] and liver cirrhosis.[15] As a result, it is extremely meaningful to explore an efficient method to detect H_2S in environmental and biological systems.

Owning to its high sensitivity, high spatiotemporal resolution and real-time imaging ability, fluorescent probes to detect and image H_2S in *vitro*/*vivo* have drawn considerable attention in the past decades.[16] Fluorescent probes with a large Stokes shift are more desirable because the large spectral separation between absorption and emission can minimize self-absorption and reduce the interference from auto-fluorescence.[17] So far there are only a few H_2S fluorecent probes with large Stokes shifts reported.[17,18] 3-Aminophthalimide derivatives, with an excited-state intramolecular proton transfer (ESIPT) characteristic, have an emission in green spectral region with a large Stokes shift and a relatively high fluorescent quantum yield.[19] In addition, 3-aminophthalimide derivatives can be easily prepared. Therefore, 3-aminophthalimide might be an ideal scaffold to construct fluorescent probes.

In this report, we designed and synthesized a derivative of 3-azidophthalimide as a turn-on fluorescent probe for the detection of H_2S with a large Stokes shift. We speculated that the azido group in this probe would efficiently quench the fluorescence and the treatment with H_2S would reduce the probe into 3-aminophthalimide which exhibits strong green fluorescence.[16a, 16d, 18b, 20]

Results and discussion

Synthesis



Scheme 1 Synthetic route of Probe **1**. (a) Butylamine, acetic acid, 120 °C, 2.5 h, yield 85.7%. (b) Palladium/C, H_2, CH_3OH, 65 °C, 12 h, 73.5%. (c) (1) $NaNO_2$, HCl, 0 °C, 30 min; (2) NaN_3, 0 °C, 40 min, yield 81.7%.

Probe **1** was readily prepared in three steps, as outlined in Scheme 1. First, 3-nitrophthalic anhydride reacted with butylamine to give compound **2** as a white solid in 85.7% yield. Next, compound **2** was hydrogenated with palladium/carbon catalyst in 73.5% yield. Finally, Probe **1** was obtained in 81.7% yield by the classical diazotization-azidation reaction from

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† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x
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DOI: 10.1039/C5RA19081A

compound **3**. All the reactions were neat with good yields and did not need column chromatography. The detailed synthetic procedures were described in experimental section and the characterization data for the investigated compounds was presented in supporting information.

Sensing property of Probe 1 to H_2S

The sensing property of Probe **1** was studied in PBS buffer (pH 7.4) with 1.0 mM CTAB (cetyltrimethylammonium bromide) at 25 °C. The solution of Probe **1** was colorless (λ_{abs}max = 344 nm) and essentially non-fluorescent. Upon the addition of H_2S (Na_2S, a standard H_2S source), the solution of Probe **1** exhibited a significant fluorescence enhancement with λ_{em}max = 492 nm and a pale yellow color with λ_{abs}max = 387 nm, which were coincident with the hallmark of dye **3** (Fig. 1, Fig. S1 and S2). As expected, the reaction product of Probe **1** with H_2S exhibited a 105 nm Stokes shift. The fluorescence intensity of Probe **1** was increased with increasing the added amount of H_2S. The fluorescence enhancement was up to 196-fold when the solution of Probe **1** (10.0 μM) was treated with 12.0 equiv. of H_2S. As depicted in Fig. 2, a linear calibration curve (R = 0.99988) was obtained between the fluorescence intensity at 492 nm and the concentration of H_2S in the range of 0.0-40.0 μM. The detection limit was calculated to be 13.6 nM based on the signal-to-noise (S/N = 3), which was sufficiently low for the detection of H_2S in environmental and biological samples. These results indicated that Probe **1** was highly sensitive to H_2S.

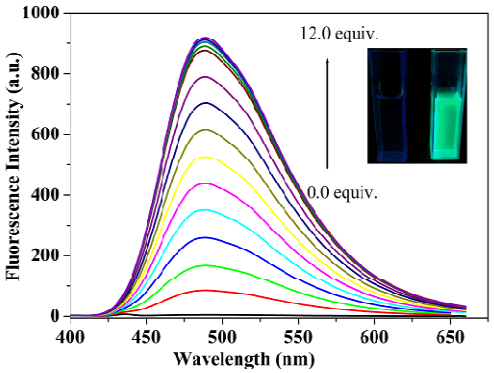


Fig. 1 Fluorescence spectra of Probe **1** (10.0 μM) upon the addition of H_2S (0.0-12.0 equiv.) in PBS buffer (10.0 mM, pH = 7.4, containing 1.0 mM CTAB). Inset: photographs of Probe **1** before (left) and after (right) the addition of H_2S. Spectra were recorded after incubation with H_2S for 40 min. λ_{ex} = 380 nm. Excitation and emission slits: 5.0 nm/2.5 nm.

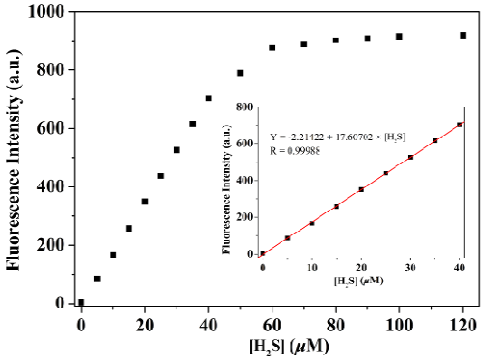
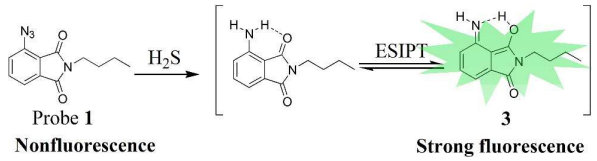


Fig. 2 Fluorescence intensity of Probe **1** (10.0 μM) at 492 nm as a function of H_2S concentration in PBS buffer (10.0 mM, pH = 7.4, containing 1.0 mM CTAB). Inset: the linear relationship between the fluorescence intensity at 492 nm and the concentration of H_2S (0.0 - 40.0 μM).

Mechanism studies

The optical changes of Probe **1** in response to H_2S suggested that H_2S reduced the azido group into amino group in Probe **1** to generate dye **3**, which displayed a strong green fluorescence with a large Stokes shift due to the ESIPT process. The proposed mechanism was illustrated in Scheme 2. In order to further confirm this sensing mechanism, we performed the ^{1}H NMR and mass spectral analysis on the reaction product of Probe **1** with H_2S. As shown in Fig. S4, the ^{1}H NMR spectrum of the reaction product was in good agreement with that of reference dye **3**. The mass spectrum of the reaction product exhibited a peak at m/z = 219.1131 (Fig. S12) which is nearly identical to the exact molecular weight of dye **3** ([M+1] = 219.1134). Furthermore, we conducted HPLC analysis on Probe **1**, dye **3** and the reaction solution of Probe **1** with H_2S (shown in Fig. 3). Probe **1** showed a single peak with a retention time at 9.7 min (Fig. 3(a)) and dye **3** produced a single peak at 5.7 min (Fig. 3(d)). When treated the solution of Probe **1** with 1.0 equiv. of H_2S, the peak assigned to Probe **1** decreased with the appearance of a new peak at 5.7 min. When the solution of



Scheme 2 The proposed sensing mechanism of Probe **1** with H_2S.

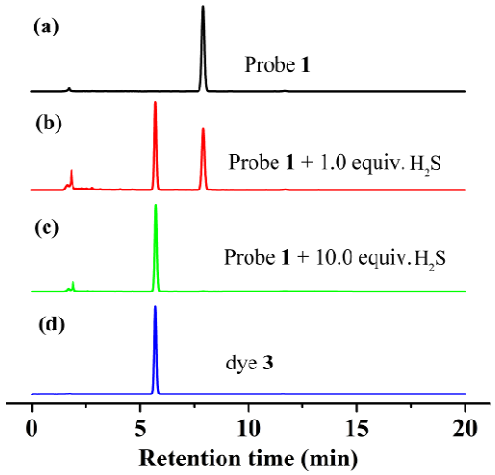


Fig. 3 The HPLC chromatograms: (a) Probe **1** (100.0 μM); (b) Probe **1** (100.0 μM) with 1.0 equiv. of H_2S incubated for 1 h in CH_3CN/H_2O (v/v, 2/1); (c) Probe **1** (100.0 μM) with 10.0 equiv. of H_2S incubated for 1 h in CH_3CN/H_2O (v/v, 2/1); (d) reference dye **3** (100.0 μM). Condition: eluent, H_2O/CH_3CN (v/v, 3/7), flow rate, 1.0 mL/min; temperature, 25 °C; detection wavelength, 360 nm; injection volume, 20.0 μL.

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of Probe **1** was treated with 10.0 equiv. of H_2S, the peak at 9.7 min, assigned to Probe **1**, completely disappeared whereas only the peak at 5.7 min, assigned to dye **3**, was left. These results strongly supported the proposed sensing mechanism in Scheme 2.

Selectivity and competition studies

In order to evaluate the selectivity of Probe **1** for H_2S, we investigated the fluorescence change of Probe **1** in response to various relevant species. As shown in Fig. 4, no fluorescence

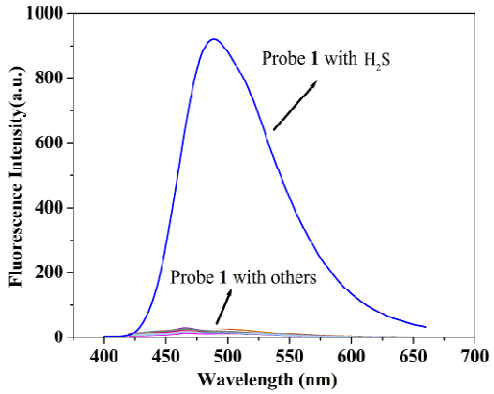

Fig. 4 Fluorescence spectra of Probe **1** (10.0 μM) upon the addition of various species in PBS buffer (10.0 mM, pH = 7.4, 1.0 mM CTAB). These species included H_2S, F^-, Cl^-, Br^-, I^-, NO_3^-, NO_2^-, CH_3COO^-, N_3^-, SO_4^{2-}, $S_2O_3^{2-}$, $S_2O_5^{2-}$, CN^-, SO_3^{2-}, PO_4^{3-}, CO_3^{2-}, H_2O_2, ClO^-, Cys, Hcy and GSH.

response was observed when the solution of Probe **1** was treated with the common anions (F^-, Cl^-, Br^-, I^-, NO_3^-, NO_2^-, CH_3COO^-, N_3^-, SO_4^{2-}, $S_2O_3^{2-}$, $S_2O_5^{2-}$, CN^-, SO_3^{2-}, PO_4^{3-} and CO_3^{2-}, 0.4 mM for each) and reactive oxygen species (H_2O_2 and ClO^-, 0.4 mM for each). The counter ion for all the anions was Na^+ except $(n\text{-}Bu)_4N^+CN^-$ was used for CN^-. To our delight, none of the biothiols (Cys and Hcy, 0.5 mM for each; 1.0 mM GSH), the main competitive species in biological systems, induced obvious fluorescence. Moreover, the co-existence of all these above mentioned species had little impact on the ability of

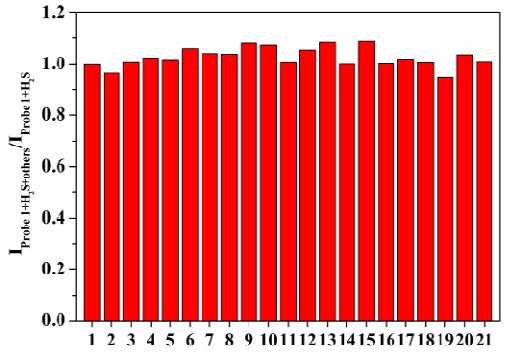

Fig. 5 The fluorescence responses of Probe **1** (10 μM) to H_2S (12.0 equiv.) in the presence of various relevant species in PBS buffer (10.0 mM, pH = 7.4, 1 mM CTAB). (1) H_2S (120.0 μM); (2-18) F^-, Cl^-, Br^-, I^-, NO_3^-, NO_2^-, CH_3COO^-, N_3^-, SO_4^{2-}, $S_2O_3^{2-}$, $S_2O_5^{2-}$, CN^-, SO_3^{2-}, PO_4^{3-}, CO_3^{2-}, H_2O_2 and ClO^- (0.4 mM for each); (19, 20) Cys and Hcy (0.5 mM for each); (21) GSH (1.0 mM).

Probe **1** in the detection of H_2S (shown in Fig. 5). Thus, it could be concluded that Probe **1** exhibited an excellent selectivity toward H_2S.

Kinetic studies

Time-dependent fluorescence experiments were performed on Probe **1** (10.0 μm) with 12.0 equiv. of H_2S by monitoring the fluorescence intensity at 492 nm. After the addition of H_2S to the solution of Probe **1**, the fluorescence enhancement was 21-fold within 1 min and reached a plateau (196-fold) within 40 min (shown in Fig. 6). In contrast, no fluorescence enhancement occurred when the solution of Probe **1** was in the absence of H_2S within 40 min. The observed first order rate constant, k_{obs}, was determined to be 0.064 min^{-1} (shown in Fig. S4).

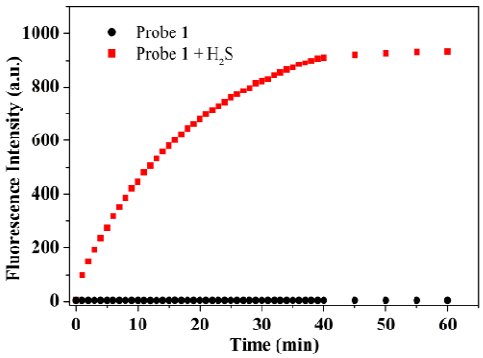

Fig. 6 Time-dependent fluorescence behavior of Probe **1** (10.0 μM) (492 nm) in the presence of H_2S (12.0 equiv.) in PBS buffer (10.0 mM, pH = 7.4, containing 1.0 mM CTAB).

pH effect studies

Moreover, we sought to study pH influence on the fluorescence behavior of Probe **1** in the detection of H_2S. As shown in Fig. 7. In the pH range of 2.0 to 12.0, the fluorescence spectra of Probe **1** remained unchanged, indicating that this probe was stable in wide pH range. When

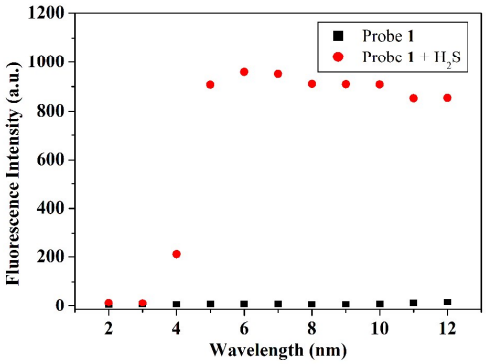

Fig. 7 Fluorescence intensity of Probe **1** (10.0 μM) at 492 nm under different pH values in the absence/presence of H_2S (12.0 equiv.).

Probe **1** (10.0 μM) was incubated with H_2S (12.0 equiv.), negligible fluorescence was observed at pH 2.0 and 3.0, and

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week fluorescence signal was obtained at pH 4.0. It suggested that H_2S could not reduce N_3 group to NH_2 in strong acid solution (shown in Scheme S1). However, a strong fluorescence signal was observed in the solution of Probe **1** with H_2S in the pH range (5.0 to 12.0). The wide pH range for Probe **1** in the detection of H_2S implied its potential application in environmental and biological studies (pH = 7.4 under physiological condition).

Fluorescence imaging of H_2S in living cells

Encouraged by the above merits of Probe **1** in aqueous solution, we sought to evaluate its ability to visualize intracellular H_2S in living cells. When HNE-2 cells, nasopharygeal carcinoma cell lines, were treated with H_2S (120.0 μM) for 30 min at 37 °C, washed with PBS buffer three times and then incubated with Probe **1** (10.0 μM) for another 30 min, a strong green fluorescence was observed inside the cells (Fig. 8b). However, the cells only incubated with Probe **1** (10.0 μM) for 30 min at 37 °C (Fig. 8d) exhibited negligible fluorescence. These fluorescence imaging experiments demonstrated that Probe **1** had a potential for imaging H_2S in living cells.

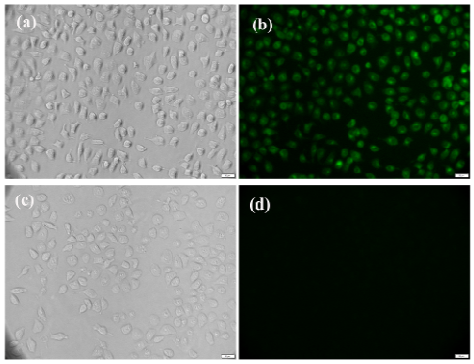

Fig. 8 (a, c) Bright-field and (b, d) fluorescence images of living HNE-2 cells. Top row: cells pretreated with H_2S (120.0 μM) for 30 min at 37 °C, washed with PBS buffer and then incubated with Probe **1** (10.0 μM) for 30 min at 37 °C (a, b). Bottom row: cells incubated with Probe **1** (10.0 μM) for 30 min at 37 °C. The cells were magnified 20 times.

Experimental section

Instruments and Materials

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 500 NMR spectrometer using tetramethylsilane (TMS) as the internal standard for chemical shifts. Mass spectra were obtained on a high resolution mass spectrometer (IonSpec4.7 T FTMS-MALDI/DHB). UV-vis absorption spectroscopy was measured with a UV-2450 spectrophotometer and fluorescence spectra was recorded on a Hitachi F-7000 spectrometer. Fluorescence imaging experiments were performed on an Olympus IX83 inverted microscope. pH measurement was carried out on a Leici PHS-3C meter. TLC silica gel plates and silica gel (mesh 200-300) for column chromatography were purchased from Qingdao Ocean Chemicals, China. Unless otherwise noted, all reagents and starting materials were obtained from commercial suppliers and used as received. Double distilled water was used throughout all the experiments. Nasopharygeal carcinoma cell lines (HNE-2 cells) were provided by Xiangya Third People's Hospital of Central South University (China).

Synthesis of compound 2

To a solution of 3-nitrophthalic anhydride (1.9361 g, 10.0 mmol) in acetic acid (20.0 mL) was slowly added butylamine (1.0951 g, 15.0 mmol) within 5 min. After stirring at room temperature for 10 min, the resulting mixture was refluxed at 120 °C for 2.5 h. Next, the reaction mixture was cooled to room temperature and then poured into 50.0 mL cold water to afford a precipitate. The solid was collected and washed with water (3.0 mL × 3) to give pure compound **2** as a white solid (2.1252 g, 85.7% yield). ^{1}H NMR (500 MHz, DMSO-d_6, TMS) δ_H 8.27 (d, J = 8.1 Hz, 1H), 8.16 (d, J = 7.5 Hz, 1H), 8.05 (t, J = 7.8 Hz, 1H), 3.57 (t, 2H), 1.63-1.51 (m, 2H), 1.38-1.24 (m, 2H), 0.90 (t, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 166.5, 163.8, 144.6, 136.5, 134.1, 128.6, 127.2, 123.5, 38.1, 30.2, 19.9, 13.9.

Synthesis of compound 3

Compound **2** (0.4964 g, 2.0 mmol) was hydrogenated in methanol (15.0 mL) under reflux at 65 °C for 12 h with 10% Pd/C (0.0496 g) as a catalyst. Then, the reaction mixture was filtered through celite to remove the catalyst. Next, the filtrate was concentrated in vacuum to give a solid. The solid was collected and washed with cold ethanol to give pure compound **3** (0.3210 g, 73.5% yield) as a pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$, TMS) δ_H 7.42 (dd, J = 8.3, 7.1 Hz, 1H), 7.16 (d, J = 7.6 Hz, 1H), 6.86 (d, J = 8.8 Hz, 1H), 5.13 (s, 2H), 3.65 (t, 2H), 1.63-1.70 (m, 2H), 1.35-1.42 (m , 2H). 0.95 (t, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.4, 168.8, 145.2, 135.02, 132.9, 121, 112.6, 111.4, 37.4, 30.8, 20.1, 13.7.

Synthesis of Probe 1

The mixture of compound **3** (0.2183 g, 1.0 mmol) and concentrated hydrochloric acid (4.0 mL) was stirred at 0 °C for 10 min. Then, the solution of sodium nitrite (0.1383g, 2.0 mmol in 1.0 mL water) was added dropwise and the reaction mixture was stirred for 30 min to afford a clear solution. Next, the solution of sodium azide (0.1301 g, 2.0 mmol in 1.0 mL water) was added dropwise to the above reaction mixture. After stirring for 40 min at 0 °C, the precipitated solid was collected and washed with water (3.0 mL × 3) to give pure Probe **1** as a white solid (0.1993 g, 81.7% yield). HRMS (EI) m/z: calcd for $C_{12}H_{12}N_4NaO_2$ $[M + Na]^+$, 267.0858; found, 267.0860. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.71 (t, J = 7.5, 1H), 7.63 (d, J = 7.0 Hz, 1H), 7.41 (d, J = 8.1 Hz, 1H), 3.69 (t, J = 7.2 Hz, 2H), 1.75 - 1.58 (m, 2H), 1.48 - 1.27 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 167.5, 166.4, 138.1, 135.2, 134.3, 124.9, 120.9, 119.4, 37.9, 30.5, 20.0, 13.6.

Synthesis of the reaction product of Probe 1 with H_2S

To a solution of Probe **1** (0.0244 g, 0.1 mmol) in a mixture of acetonitrile (15.0 mL) and PBS buffer (10.0 mL) was added a

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solution of Na_2S (0.0782 g, 1.0 mmol in water 5.0 mL) at room temperature. Next, the reaction mixture was stirred at room temperature for 2 h and then extracted with dichloromethane (20.0 mL × 3). The organic layer was separated, combined and dried over anhydrous Na_2SO_4. After removal of the solvent, the solid was purified by silica gel flash chromatography using petroleum ether/dichloromethane (v/v, 1:1) as eluent to afford the target product (0.0148 g, 67.7% yield). HRMS (EI) m/z: calcd for $C_{12}H_{15}N_2O_2$ $[M + 1]^+$, 219.1134; found, 219.1131. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (dd, J = 8.2, 7.2 Hz, 1H), 7.14 (d, J = 7.1 Hz, 1H), 6.85 (d, J = 8.3 Hz, 1H), 5.25 (s, 2H), 3.64 (t, J = 7.3 Hz, 2H), 1.71 - 1.59 (m, 2H), 1.44 - 1.30 (m, 2H), 0.95 (t, 3H).

Imaging of HNE-2 cells

HNE-2 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Hyclone) as well as 1% penicillin, and incubated at 37 °C in 5% CO_2 and 95% air. One day before imaging, cells were passaged and plated on 9-well plate. Before used, the growth medium was removed and the cells were washed three times with PBS buffer. The cells were incubated with H_2S (120.0 μM) for 30 min at 37 °C, washed with PBS buffer and then incubated with Probe **1** (10.0 μM) for another 30 min. After washing with PBS buffer, cell imaging experiments were performed. For a control experiment, HNE-2 cells were only incubated with Probe **1** (10.0 μM) for 30 min at 37 °C.

Conclusions

To close, we have developed a phthalimide-based fluorescent probe for H_2S in both aqueous solution and living cells. This turn-on ESIPT-based probe features a significant fluorescence enhancements (196-fold) and low detection limit (13.6 nM) in response to H_2S. Importantly, this probe shows a large Stokes shift (105 nm) which can minimize the self-absorption and reduce the interference from auto-fluorescence. Preliminary biological experiments demonstrates that this probe is cell permeable and capable of detecting H_2S in living cells.

Acknowledgements

This research was supported by the Fundamental Research Funds for the Central South University and State Key Laboratory of Fine Chemicals (KF1202). We thank Modern Analysis and Testing Center of Central South University for NMR spectral data of all the compounds.

Notes and references

1. T. L. Guidotti, *Int. J. Toxicol.,* **2010**, *29*, 569-581.
2. (a) M. H. Stipanuk and P. W. Beck, *Biochem. J.,* **1982**, *206*, 267-277; (b) M. Swaroop, K. Bradley, T. Ohura, T. Tahara, M. D. Roper, L. Rosenberg and J. Kraus, *J. Biol. Chem.,* **1992**, *267*, 11455-11461.
3. S. Singh, D. Padovani, R. A. Leslie, T. Chiku and R. Banerjee, *J. Biol. Chem.,* **2009**, *284*, 22457-22466.
4. N. Shibuya, M. Tanaka, M. Yoshida, Y. Ogasawara, T. Togawa, K. Ishii and H. Kimura, *Antioxid. Redox Signal.,* **2009**, *11*, 703-714.
5. W. Zhao and R. Wang, *Am. J. Physiol. Heart Circ. Physiol.,* **2002**, *283*, H474-H480.
6. K. Abe and H. Kimura, *J. Neurosci.,* **1996**, *16*, 1066-1071.
7. R. C. Zanardo, V. Brancaleone, E. Distrutti, S. Fiorucci, G. Cirino and J. L. Wallace, *FASEB J.,* **2006**, *20*, 2118-2120.
8. R. Baskar and J. Bian, *Eur. J. Pharmacol.,* **2011**, *656*, 5-9.
9. Y. Kaneko, Y. Kimura, H. Kimura and I. Niki, *Diabetes,* **2006**, *55*, 1391-1397.
10. O. Kabil and R. Banerjee, *J. Biol. Chem.,* **2010**, *285*, 21903-21907.
11. Y.-H. Liu, M. Lu, L.-F. Hu, P. T.-H. Wong, G. D. Webb and J.-S. Bian, *Antioxid. Redox Signal.,* **2012**, *17*, 141-185.
12. K. Eto, T. Asada, K. Arima, T. Makifuchi and H. Kimura, *Biochem. Biophys. Res. Commun.,* **2002**, *293*, 1485-1488.
13. P. Kamoun, M. C. Belardinelli, A. Chabli, K. Lallouchi and C. K. Bernadette, *Am. J. Med. Genet. Part,* **2003**, *116*, 310-311.
14. W. Yang, G. Yang, X. Jia, L. Wu and R. Wang, *J. Physiol.,* **2005**, *569*, 519-531.
15. S. Fiorucci, E. Antonelli, A. Mencarelli, S. Orlandi, B. Renga, G. Rizzo, E. Distrutti, V. Shah and A. Morelli, *Hepatology,* **2005**, *42*, 539-548.
16. (a) H. Peng, Y. Cheng, C. Dai, A. L. King, B. L. Predmore, D. J. Lefer and B. Wang, *Angew. Chem. Int. Ed.,* **2011**, *50*, 9672-9675; (b) C. Liu, J. Pan, S. Li, Y. Zhao, L. Y. Wu, C. E. Berkman, A. R. Whorton and M. Xian, *Angew. Chem. Int. Ed.,* **2011**, *123*, 10511-10513; (c) Y. Chen, C. Zhu, Z. Yang, J. Chen, Y. He, Y. Jiao, W. He, L. Qiu, J. Cen and Z. Guo, *Angew. Chem. Int. Ed.,* **2013**, *125*, 1732-1735; (d) A. R. Lippert, E. J. New and C. J. Chang, *J. Am. Chem. Soc.,* **2011**, *133*, 10078-10080; (e) S. Yang, Y. Qi, C. Liu, Y. Wang, Y. Zhao, L. Wang, J. Li, W. Tan and R. Yang, *Anal. Chem.,* **2014**, *86*, 7508-7515; (f) Y. Cai, L. Li, Z. Wang, J. Z. Sun, A. Qin and B. Z. Tang, *Chem. Commun.,* **2014**, *50*, 8892-8895; (g) K. Huang, L. Yu, P. Xu, X. Zhang and W. Zeng, *RSC Adv.,* **2015**, *5*, 17797-17801; (h) T. Liu, Z. Xu, D. R. Spring and J. Cui, *Org. Lett.,* **2013**, *15*, 2310-2313; (i) X. Li, S. Zhang, J. Cao, N. Xie, T. Liu, B. Yang, Q. He and Y. Hu, *Chem. Commun.,* **2013**, *49*, 8656-8658; (j) Y. Qian, L. Zhang, S. Ding, X. Deng, C. He, X. E. Zheng, H.-L. Zhu and J. Zhao, *Chem. Sci.,* **2012**, *3*, 2920-2923.
17. (a) Z. Xu, L. Xu, J. Zhou, Y. Xu, W. Zhu and X. Qian, *Chem. Commun.,* **2012**, *48*, 10871-10873; (b) Z. Huang, S. Ding, D. Yu, F. Huang and G. Feng, *Chem. Commun.,* **2014**, *50*, 9185-9187; (c) Q. Huang, X.-F. Yang and H. Li, *Dyes Pigm.,* **2013**, *99*, 871-877.
18. (a) Y. Liu and G. Feng, *Org. Biomol. Chem.,* **2014**, *12*, 438-445; (b) B. Gu, N. Mi, Y. Zhang, P. Yin, H. Li and S. Yao, *Anal. Chim. Acta.,* **2015**, *879*, 85-90.
19. (a) M. Nakazono, K. Saita, C. Kurihara, S. Nanbu and K. Zaitsu, *J. Photochem. Photobiol. A-Chem.,* **2009**, *208*, 21-26; (b) R. Perez-Ruiz, R. Fichtler, Y. D. Miara, M. Nicoul, D. Schaniel, H. Neumann, M. Beller, D. Blunk, A. G. Griesbeck

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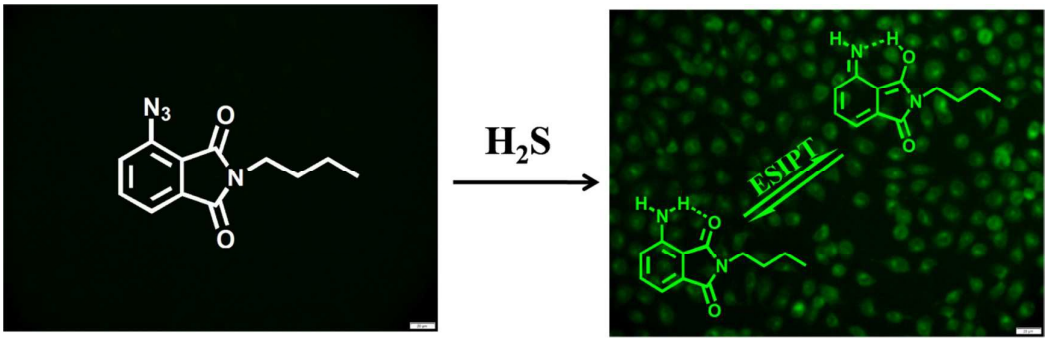
DOI: 10.1039/C5RA19081A

and A. Jacobi von Wangelin, *J. Fluoresc.,* **2010**, *20*, 657-664; (c) Y. G. Siretskii and O. E. Kalinovskaya, *Russ. J. Gen. Chem.,* **2000**, *70*, 832-836; (d) A. Tan, E. Bozkurt, N. Kishali and Y. Kara, *Helv. Chim. Acta,* **2014**, *97*, 1107-1114.

20. J. Zhang and W. Guo, *Chem. Commun.,* **2014**, *50*, 4214-4217.

View Article Online
DOI: 10.1039/C5RA19081A

Graphical Abstract



A phthalimide-based fluorescent probe for H_2S with a large Stokes shift has been developed. This probe displayed good selectivity and high sensitivity toward H_2S. Imaging intracellular H_2S by using this probe was successfully achieved in living cells.