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A Novel Mitochondria Targeting Fluorescent Probe as the Monitor of Hydrogen Sulfide in Living Cells

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Abstract:

A novel mitochondria targeting fluorescent probe compound **S-N_3** as the monitor of Hydrogen Sulfide (H_2S) in living cells has been designed and synthesized in this paper. This article contained the chemosynthesis and some studies on bioactivity of the target compound in living cells. Compound S-N_3 is easy to synthesize and can remain stable under the effect of pH, system and photo. In additional, it shows low cytotoxicity in cell imaging. And it can react with H_2S highly selective in PBS or FBS solution, which would cause the obvious increase of fluorescence intensity. Therefore, the low-cost detection method for H_2S is allowed for the monitoring the quantity of H_2S existed in the mitochondria.

Keywords: Fluorescent probe, Hydrogen sulfide, Mitochondria targeting, Selectively

Introduction

Hydrogen sulfide (H_2S) existing in agriculture, husbandry[1], organic manure fermentation[2], water treatment[3] and industrial production was recognized as the third well-known gas signal molecule stepping with NO and CO *in vivo*[4]. Some studies have suggested that excess H_2S *in vivo* can greatly harm the health of human and animals, leading to headaches, dizziness, fatigue, nausea and even death. However, recently it has been found that a balanced amount of H_2S plays an active role in the regulation of the nervous system[5], inflammatory stress[6] and the cardiovascular system[7], etc[8]. In addition, H_2S is also an important class of disease markers, such as Alzheimer's disease, hypertension and diabetes[9].

At present, there are many detection methods of H_2S, such as ultraviolet absorption method[10], electrochemical method[11] and chromatography[12]. However, compared to the complex, expensive equipment and reagents, the method of fluorescent probe which does not damage the biological samples is more suitable for the detection[13]. Recently, a number of fluorescent probes for the detecting and imaging of H_2S in living cells have been reported[14-16].

Furthermore, mitochondria, as a kind of important organelles, possesses its physiological-function closely related to the H_2S[17]. Selective tracking of H_2S in mitochondria is crucial to elucidate the complex contributions of H_2S for improving the activity of mitochondrial ATPase and the resistance to oxidation, etc[18]. Therefore, quantitative and accurate detection of the H_2S changes in mitochondrial with fluorescent probe is in strong need for elucidating the complex contributions of H_2S and studying some diseases and the damage of mitochondria. Up to now, several innovative fluorescent probes targeting to mitochondria for H_2S based on different reaction mechanisms have been revealed in the literature. Wu et al. have synthesized a fluorescent for a highly selective H_2S probe for mitochondria and lysosome, respectively[19]. Chen et al. and Wang et al. have reported mitochondrial targeting fluorescent probes based on coumarin−merocyanine and cyanine scaffold for H_2S detection[20,21]. Xu et al. have reviewed the progress of some small molecule fluorescent probes targeting different organelles[22].

To improve the flatness and solubility the of ring-fused 1,8-naphthalic anhydride and the medicinal properties, we designed and synthesized a new fluorescence probe targeting the mitochondria to detect H_2S in it, which emited at a different wavelength ($\lambda_{Ex/Em}$ = 460/545 nm), and then determined its activity under the condition of different effects [23, 24]. Results showed that the mitochondria targeting compound **S-N_3** had higher selectivity and lower cytotoxicity to H_2S. In addition, cells was valuable for further developing. The structure of the target compound was shown below (**Fig. 1**).

Results and Discussion

Design and Synthesis of S-N_3

By reacting, we got the target compound (**S-N_3**) prepared from 5-Nitroacenaphthene (**compound 1**) [25,26]. The synthetic route is shown in **Scheme 1** [27].

Fluorescence response between S-N_3 and H_2S

Firstly, 10μM **S-N_3** solution was added into the cuvette to detect the excitation wavelength and emission wavelength. Results showed that upon the addition of NaHS (50μM), **S-N_3** solution exhibited a robust increase in fluorescent intensity at 545 nm with the excitation at 460 nm testing by the Fluorescence Spectrophotometer and Perkin Elmer EnSpire™, respectively. And the relationship between the response time and Rel.Emission intensity of **S-N_3** (10μM) in NaHS (50 μM) solution at 37 ℃, as well as the detection of the most suitable excitation wavelength of S-N_3 (10μM) with emission at 545 nm were shown in **Fig. 2**, the fluorescence intensity of **S-N_3**（10μM）increased fast within 10 min after adding NaHS (50 μM), and obvious fluorescent yellow can be observed at 365 nm-UV compared to the **S-N_3** solution without NaHS. The fluorescence intensity was increasing and approximately trended to be stable at 10 min. Therefore, the equilibrium time between the probe and H_2S would be chosen as 10 min.

Biological selectivity and linearity assessment

In complex organisms, the selectivity of **S-N_3** is the major issues for the further monitor of H_2S over other molecules. At 10 min, by comparing with different influence factors, the investigation indicated that only HS^- (100μM) caused a dramatic enhancement at 545 nm, while other anions (F^-, Cl^-, Br^-, I^-, NO_2^-, NO_3^-, CO_3^{2-}, HCO_3^-, 1mM), inorganic reactive sulfur species (SO_4^{2-}, $S_2O_3^{2-}$, SO_3^{2-}, 1mM), and other biological thiols (GSH, Cys, 1mM) had just a weak increment of fluorescence intensity at 25℃ (**Fig. 3**). The results showed that **S-N_3** had potential research value in a biological environment for the detection of H_2S. A further study found that there was a great linearity between fluorescence intensity and concentrations of H_2S in the range of 5-40μM in PBS buffers at 10 min, 25□ (**Fig. 4**). In this linear range, the obvious spectral response can be got. And when the concentration of H_2S up to 50μM, the response mainly did not increase any more. Thus far, all the selectivity and linearity studies suggested this probe is very suitable for the detection of sulfide in biological samples. For a further test, the limit of detection (LOD) was as low as 1μM with a signal-to-noise ratio (S/N) of 3:1 and the limit of quantification (LOQ) was 4.2μM with S/N of 10:1.

pH and photo stability

Furthermore，pH and photo stability of **S-N_3** also had a tremendous influence on detection of H_2S in biological systems. Then we tested its stability with different pH values (pH= 1, 2, 3,……, 13) at 10 min, 25℃, 545 nm, and the fluorescence intensity for different time was shown in **Fig. 5(A)(B)**, respectively. The fluorescence intensity of **S-N_3** (100μM) had no detectable change in all the buffers at pH 3-11 primarily, which indicated that different pH values between 3 and 11 did not have any effect on the photometric characteristics of **S-N_3** without the adding of NaHS. In other word, this probe had a wide application scope of pH. Moreover, the photo stability of **S-N_3** was also estimated with a UV lamp at 365nm. **S-N_3** (100μM) was exposed to the irradiation and the fluorescence response was obtained at

different time within 10 min. Upon the addition of NaHS, there was a prominent enhancement to others which were stable without NaSH (**Fig. 5B**), which demonstrated that within 10 min the probe was stable to the photo (365 nm) itself, but it was sensitive to H_2S and there was a great response between them.

Stability in different systems

In order to get further research on the stability of **S-N_3**, we next determined the fluorescence intensity of the target compound in different environments, such as the systems of degassed PBS buffers and commercial fetal bovine serum (FBS). At different aging time within 30 min, the fluorescence intensity of **S-N_3** with concentration of 30μM and100μM at 25℃ was recorded in the two systems shown in **Fig. 6**. With the variation of the time, the same variation trend can be seen in both two systems. What's more, the increase of fluorescence intensity was relatively stable by increasing the aging time but turned stronger by increasing the concentration of **S-N_3**. While in FBS solutions, the response was relatively lower than that in the PBS buffers, which may be on account of various proteins in serum. The study focused on the stability of **S-N_3** explained this probe for detection of H_2S was a conclusive one in different testing environment.

Cytotoxicity assay

In order to get the further confirmation for the application of **S-N_3**, the cytotoxicity of the targeted compound in living cells was determined firstly. Above all, the MTT assays for **S-N_3** and the reduced product **S-NH_2** were carried out, the inhibition rate of **S-N_3** and **S-NH_2** to MCF-7 cells at different concentrations (3.125, 6.25, 12.5, 25, 50, 100 μM) , 37℃ was demonstrated in **Table 1**, and the results showed that both the two compounds have scarcely any cytotoxicity and a high IC_{50} value (82.5 and 83.25μM) after 48 h by calculating. Thus, the probe at 20μM was selected for imaging in MCF-7 cells [24]

Mitochondria Visualization of H_2S in MCF-7

In this paper, the capability of mitochondria targeting of **S-N_3** was verificated with the visualization of MCF-7 cells [29]. As described in **Fig. 7**, cells incubated alone with Mito Tracker RED dye with red fluorescence were collected from the red channel (580 nm), and cells cultivated by S-N_3 and NaHS emited green fluorescence were collected from the green channel (460nm). The overlaid image was in yellow fluorescence. The significant fluorescence in green channel proved that probe **S-N_3** can be used for imaging H_2S in living cells. In addition, the result of colocalization of **S-N_3** and Mito Tracker RED dye at 37℃ was shown in **Fig. 8** which showed the high repetition between **S-N_3** and the dye for dyeing to the mitochondria. According to the results above, the target compound **S-N_3** had the same trend to mitochondria for dyeing. That is to say, the **S-N_3** showed the similar activity of targeting to mitochondria, which suggested probe **S-N_3** was mitochondria targeted as the famous Mito Tracker RED dye.

CONCLUSION

In summary, a novel mitochondria targeting fluorescence probe was developed for detection of H_2S. The probe was found to be obvious sensitive to H_2S among some anions tested with a detection limit of 1μM with a signal-to-noise ratio of 3:1 and the limit of quantification (LOQ) was 4.2μM with S/N of 10:1. And with the addition of H_2S, distinct fluorescence increments compared to that with no H_2S were obtained. What's more, this probe was easy to synthesize and can remain stable under the effect of pH, system and photo. In additional, it showed low cytotoxicity in cell imaging. Consequently, we feel that this mitochondria targeting, selective, efficient, and low-cost detection method for H_2S will be very useful in the booming research field.

EXPERIMENTAL SECTION

Reagents and Instruments

All reagents used for synthesis of compounds were bought from Aladdin, J&K, and were directly applied in the experiment without other purification. pH values of the buffers were recorded on the instrument Metter Toledo Five Easy pH FE20. The purity of each tested compounds (>95%) was determined on a Waters UPLC/MS instrument using a Waters C18 column (1.7 μm2.1×50 mm, with a flow rate of 0.2 mL/min and detection at 254 nm) employing a 10–90% acetonitrile/water/0.1% formic acid gradient. The spectra of ^1H NMR and ^{13}C NMR were analyzed by the Bruker model DPX-300 MHz NMR spectrometer (Bruker Instruments, Inc, Billerica, MA, USA). Elemental analysis was analyzed on CHN-O-Rapid instrument. The result of Mass spectra (MS) was obtained by Waters liquid chomatography-mass spectrometer system (ESI). Thin-layer chromatography (TLC) was visualized on GF/UV 254 plates, and the fluorescent points were shown under UV light with the wavelength 254 and 365 nm. Fluorescent data was presented by fluorescence spectrophotometer (RF-5301 PC, Shimadzu, Tokyo, Japan) and Perkin Elmer EnSpire™. Laser scanning confocal microscopy was taken on Olympus FluoView-300 confocal laser scanning system (Olympus, Tokyo, Japan).

Cell Lines

Human breast cancer cell lines MCF-7 were kindly provided by our laboratory. The cell lines were cultivated in hyclone DMEM medium with 10% fetal bovine serum (FBS) and incubated in the incubator with 5% CO_2 at 37 ℃ in air growth. All experiments were conducted with the cells we cultivated beforehand.

Fluoresence analysis, biological selectivity and stability test

PBS buffer used in this study was 10mM and pH 7.4, and in the study of pH stability, the pH value was adjusted with 1 N NaOH solution. 50mM stock solution of **S-NH_2 and S-N_3** were dissolved in DMSO firstly, and then diluted with PBS we prepared before to 100 and 30μM as the mother liquor. 10mM NaHS

was prepared in the PBS buffer as the donor of H_2S, and then diluted with PBS solution to different concentrations as shown below. In addition, the ions of F^-, Cl^-, Br^-, I^-, NO_2^-, NO_3^-, CO_3^{2-}, HCO_3^-, SO_4^{2-}, $S_2O_3^{2-}$, SO_3^{2-} and the biological mercaptan (such as GSH and Cys) were prepared with PBS buffer to the concentration of 1mM for the test of biological selectivity. These solutions above were then used for further study in fluoresence analysis, biological selectivity and stability test.

Cytotoxicity assay

MCF-7 cells (cells per well) were seeded in 96-well plates. After 24 h incubation, cells were treated with various concentrations of **S-N$_3$** for 48 h in an atmosphere of 95% air with 5% CO_2 at 37 □. Then, MTT was added directly to the cells. After additional incubation for 4 h at 37 □, the absorbance at 490 nm was read on a microplate reader (Thermo Fisher Scientific). The IC_{50} values of the **S-N$_3$** for cytotoxicity were calculated by GraphPadPrism 5.0 software from the dose-response curves. Experiments were conducted in triplicates and repeated three times independently.

Mitochondria Visualization of H_2S in MCF-7

Firstly, the cells were pretreated with the target compound (20μM, 1 ml) and the Mito Tracker RED dye for mitochondria (250nM) for 1 h at 37 ℃, respectively**.** And then washed by the PBS buffers (1 ml ×3). Next the NaHS (20μM, 1 ml) was added to the cells in different systems mentioned above with reacting for 5 min. The cells then were set under the laser scanning confocal microscope with the two-channel fluorescent signal of 460 nm and 580 nm [30].

Synthesis of (5-((2-(6-Amino-1,3-dioxo-1*H*-benzo[de]isoquinolin-2(3*H*)-yl)ethyl)amino)-5 -oxopentyl)triphenylphosphonium bromide (S-NH$_2$)

The mixtures of compound 7 (9.2mmol), Zn (46.1mmol) and NH_4Cl (92.2mmol) were dissolved with solvent of EtOH and water (40ml, 3:1) and refluxed at 85 □ for 5 h. Then filtered with kieselguhr, and solution was extracted with DCM (40 mL×3). The combined organic layers were washed with saturated sodium bicarbonate solution and brine, dried over Na_2SO_4, and concentrated in vacuo. An orange powder (**S-NH$_2$**) was obtained. Yield: 64%; Orange powder; ^{1}H NMR (300 MHz, DMSO-d_6) δppm: 8.43 (d, J = 8.0 Hz, 2H), 8.05 (s, 1H), 7.92 (s, 5H), 7.76 (s, 12H), 5.78 (s, 1H), 4.10 (s, 2H), 3.74 (s, 2H), 3.35 (s, 2H), 2.42 (m, 4H), 1.64 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6)δ: 174.88, 162.53, 158.17, 135.25, 133.77, 132.63, 130.69, 130.33, 130.16, 125.94, 124.87, 115.97, 113.87, 111.70, 40.75, 38.66, 37.48, 25.34, 23.06, 22.34; ESI-MS *m/z*: 600.24 $[M+H]^+$. Anal. calcd. For $C_{37}H_{35}N_3O_3PBr$: C, 65.30; H, 5.18; N, 6.17; Found: C, 65.39; H, 5.52; N, 6.14.

Synthesis of (5-((2-(6-Azido-1,3-dioxo-1*H*-benzo[de]isoquinolin-2(3*H*)-yl)ethyl)amino)-5 -oxopentyl)triphenylphosphonium bromide (S-N_3).

S-NH_2 (4.4mmol) was dissolved in 60% Con.H_2SO_4 (20 ml) at -10 □, then adding $NaNO_2$ (8.8mmol) in batches while stirring for 30 min, and the temperature must be controlled within -5-0 □. Finally, NaN_3 (8.8mmol) was added into the system, and reacted for about 1 h. Then the reaction solution was diluted with water (20 ml), and extracted with DCM (25 mL×5). The combined organic layers were washed with saturated sodium bicarbonate solution and brine, dried over Na_2SO_4, and concentrated in vacuo. The MS spectrum of S-N_3 was shown in **Fig. 9**. The target **S-N_3** was obtained. Yield: 74%; orange powder; ^{1}H NMR (300 MHz, DMSO-d_6) δppm: ^{1}H NMR (300 MHz, DMSO) δ 8.45 (d, J = 7.9 Hz, 3H), 7.89 (s, 5H), 7.77 (s, 12H), 5.76 (s, 1H), 4.08 (s, 2H), 2.02 (s, 2H), 1.50 (s, 4H), 1.24 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6)δ: 174.88, 162.53, 149.12, 135.25, 133.77, 132.78, 131.10, 129.81, 129.15, 125.39, 120.84, 115.97, 40.75, 38.66, 37.48, 25.34, 23.06, 22.34; ESI-MS *m/z*: 626.3 $[M+H]^+$. Anal. calcd. For $C_{37}H_{33}N_5O_3PBr$: C, 62.90; H, 4.71; N, 9.91; Found: C, 62.86; H, 4.78; N, 9.83.

Conflict of interest

Declare none.

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References

1. Liu, Z., Powers, W., Murphy, J., & Maghirang, R. (2014). Ammonia and hydrogen sulfide emissions from swine production facilities in North America: A meta-analysis. *Journal of animal science*, *92*(4), 1656-1665.
2. Wang, K., Huang, D., Ying, H., & Luo, H. (2014). Effects of acidification during storage on emissions of methane, ammonia, and hydrogen sulfide from digested pig slurry. *Biosystems Engineering*, *122*, 23-30.
3. Ankersmit, H. A., Tennent, N. H., & Watts, S. F. (2005). Hydrogen sulfide and carbonyl sulfide in the museum environment—Part 1. *Atmospheric Environment*, *39*(4), 695-707.
4. Wang, R., Li, Z., Zhang, C., Li, Y., Xu, G., Zhang, Q. Z., ... & Xi, Z. (2016). Fast‐Response Turn‐on Fluorescent Probes Based on Thiolysis of NBD Amine for H2S Bioimaging. *ChemBioChem*, *17*(10), 962-968.
5. Kimura, H. (2002). Hydrogen sulfide as a neuromodulator. *Molecular neurobiology*, *26*(1), 13-19.
6. Dufton, N., Natividad, J., Verdu, E. F., & Wallace, J. L. (2012). Hydrogen sulfide and resolution of acute inflammation: A comparative study utilizing a novel fluorescent probe. *Scientific reports*, *2*.
7. Wang, R. (2009). Hydrogen sulfide: a new EDRF. *Kidney international*, *76*(7), 700-704.
8. Gadalla, M. M., & Snyder, S. H. (2010). Hydrogen sulfide as a gasotransmitter. *Journal of*

neurochemistry, *113*(1), 14-26.

9. Szabó, C. (2007). Hydrogen sulphide and its therapeutic potential. *Nature reviews Drug discovery*, *6*(11), 917-935.
10. Hughes, M. N., Centelles, M. N., & Moore, K. P. (2009). Making and working with hydrogen sulfide: the chemistry and generation of hydrogen sulfide in vitro and its measurement in vivo: a review. *Free Radical Biology and Medicine*, *47*(10), 1346-1353.
11. Kolluru, G. K., Shen, X., Bir, S. C., & Kevil, C. G. (2013). Hydrogen sulfide chemical biology: pathophysiological roles and detection. *Nitric oxide*, *35*, 5-20.
12. Lawrence, N. S., Deo, R. P., & Wang, J. (2004). Electrochemical determination of hydrogen sulfide at carbon nanotube modified electrodes.*Analytica Chimica Acta*, *517*(1), 131-137.
13. Cui, J., Zhang, T., Sun, Y. Q., Li, D. P., Liu, J. T., & Zhao, B. X. (2016). A highly sensitive and selective fluorescent probe for H 2 S detection with large fluorescence enhancement. *Sensors and Actuators B: Chemical*, *232*, 705-711.
14. Zhang, Y. C., Yao, L., Zhang, G., Dionysiou, D. D., Li, J., & Du, X. (2014). One-step hydrothermal synthesis of high-performance visible-light-driven SnS 2/SnO 2 nanoheterojunction photocatalyst for the reduction of aqueous Cr (VI). *Applied Catalysis B: Environmental*, *144*, 730-738.
15. Yu, F., Han, X., & Chen, L. (2014). Fluorescent probes for hydrogen sulfide detection and bioimaging. *Chemical Communications*, *50*(82), 12234-12249.
16. Wang, K., Peng, H., & Wang, B. (2014). Recent advances in thiol and sulfide reactive probes. *Journal of cellular biochemistry*, *115*(6), 1007-1022.
17. Ishigami, M., Hiraki, K., Umemura, K., Ogasawara, Y., Ishii, K., & Kimura, H. (2009). A source of hydrogen sulfide and a mechanism of its release in the brain. *Antioxidants & redox signaling*, *11*(2), 205-214.
18. Hildebrandt, T. M., & Grieshaber, M. K. (2008). Three enzymatic activities catalyze the oxidation of sulfide to thiosulfate in mammalian and invertebrate mitochondria. *Febs Journal*, *275*(13), 3352-3361.
19. Wu, Z., Liang, D., & Tang, X. (2016). Visualizing Hydrogen Sulfide in Mitochondria and Lysosome of Living Cells and in Tumors of Living Mice with Positively Charged Fluorescent Chemosensors. *Analytical Chemistry*, *88*(18), 9213-9218.
20. Chen, Y., Zhu, C., Yang, Z., Chen, J., He, Y., Jiao, Y., ... & Guo, Z. (2013). A ratiometric fluorescent probe for rapid detection of hydrogen sulfide in mitochondria. *Angewandte Chemie*, *125*(6), 1732-1735.
21. Wang, X., Sun, J., Zhang, W., Ma, X., Lv, J., & Tang, B. (2013). A near-infrared ratiometric fluorescent probe for rapid and highly sensitive imaging of endogenous hydrogen sulfide in living cells. *Chemical Science*, *4*(6), 2551-2556.
22. Xu, W., Zeng, Z., Jiang, J. H., Chang, Y. T., & Yuan, L. (2016). Discerning the Chemistry in Individual Organelles with Small - Molecule Fluorescent Probes. *Angewandte Chemie International Edition*, *55*(44), 13658-13699.
23. Yang, Y., Lei, Y., Zhang, X., & Zhang, S. (2016). A ratiometric strategy to detect hydrogen sulfide with a gold nanoclusters based fluorescent probe.*Talanta*, *154*, 190-196.
24. Hou, Y., Yang, X. F., Zhong, Y., & Li, Z. (2016). Development of fluorescent probes for hydrogen polysulfides by using cinnamate ester as the recognition unit. *Sensors and Actuators B: Chemical*, *232*, 531-537.
25. Veale, E. B., & Gunnlaugsson, T. (2010). Synthesis, photophysical, and DNA binding studies of

fluorescent Troger's base derived 4-amino-1, 8-naphthalimide supramolecular clefts. *The Journal of organic chemistry*,*75*(16), 5513-5525.

26. Lee, M. H., Park, N., Yi, C., Han, J. H., Hong, J. H., Kim, K. P., ... & Kim, J. S. (2014). Mitochondria-immobilized pH-sensitive off–on fluorescent probe.*Journal of the American Chemical Society*, *136*(40), 14136-14142.
27. Liu, X. L., Du, X. J., Dai, C. G., & Song, Q. H. (2014). Ratiometric two-photon fluorescent probes for mitochondrial hydrogen sulfide in living cells. *The Journal of organic chemistry*, *79*(20), 9481-9489.
28. Qiu, Q., Deng, X., Jiao, L., Zhao, T., Meng, F., Huang, W., & Qian, H. (2015). A High‐Sensitivity Coumarin‐Based Fluorescent Probe for Monitoring Hydrogen Sulfide in Living Cells. *Chemical biology & drug design*,*86*(2), 173-179.
29. Kim, C. Y., Kang, H. J., Chung, S. J., Kim, H. K., Na, S. Y., & Kim, H. J. (2016). Mitochondria-targeting chromogenic and fluorescence turn-on probe for the selective detection of cysteine by caged oxazolidinoindocyanine.*Analytical Chemistry*, *88*(14), 7178-7182.
30. Kaur, A., Brigden, K. W., Cashman, T. F., Fraser, S. T., & New, E. J. (2015). Mitochondrially targeted redox probe reveals the variations in oxidative capacity of the haematopoietic cells. *Organic & biomolecular chemistry*,*13*(24), 6686-6689.

Figure Legends

Fig. 1 New fluorescent probe (**S-N_3**) for the detection of H_2S.

Scheme 1: Synthesis of target compound **S-N_3**.

Reagents and conditions: (a) $K_2Cr_2O_7$, CH_3COOH, reflux, 110℃, 4 h, 62%; (b) K_2CO_3, Di-tert-butyl pyrocarbonate, CH_3OH, r.t., 24 h, 59%; (c) Anhydrous EtOH, N_2, reflux, 80℃, 12 h, 65%; (d) Hydrogen chloride saturated ethyl acetate, 3 h, 92%; (e) (4-Caboxybutyl)- triphenylphosphonium bromide, $(COCl)_2$, DCM, 24 h, 55%; (f) Zn, NH_4Cl, ethanol, water, 85℃, 5 h, 81%; (g) $NaNO_2$, NaN_3, 60% H_2SO_4, -5-0℃, 1 h, 74%

Fig. 2 (A) The relationship of **S-N_3**（10μM）to NaHS (50μM) between Rel.Emission intensity and the time at 37 □ with emission at 545 nm **(B)** The detection of the most suitable excitation wavelength of S-N_3 (10μM) with emission at 545 nm, 37℃

Fig. 3 Biological selectivity of **S-N_3** at 10 min at 25℃

Fig. 4 Linearity between fluorescence intensity and concentrations of H_2S at 25□

Fig. 5(A) pH stability of 100μM **S-N_3** in PBS buffers at 10 min, 25℃ without the adding of NaHS **(B)** Photo stability of 100μM **S-N_3** in PBS buffers with UV light irradiation at different time and the addition of 50μM NaHS for 10 min at 25℃ (the last column)

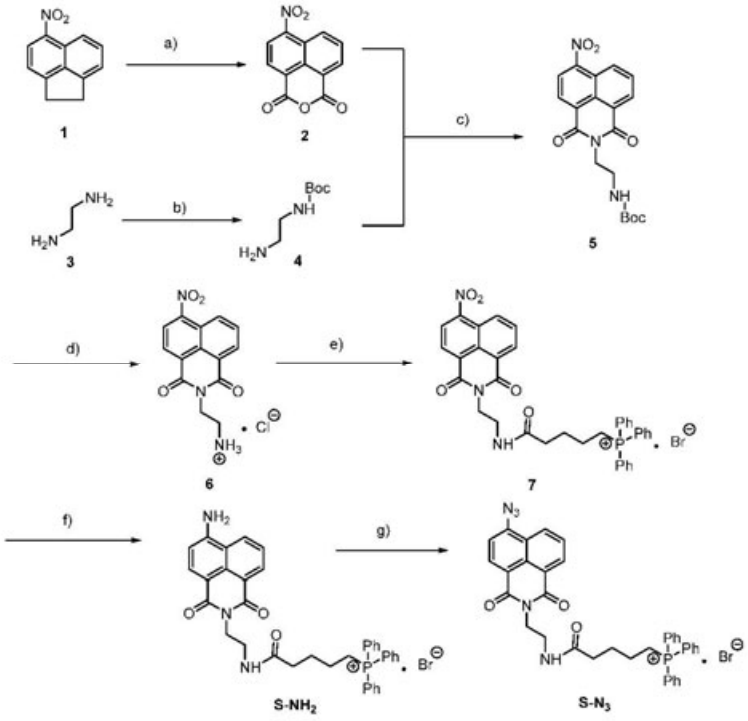
Fig. 6 Stability of 30μM and 100μM **S-N_3** in PBS and FBS systems at different time 25℃ within 30 min

Table 1 Inhibition rate of S-N_3 and S-NH_2 to MCF-7 cells at different concentrations, 37℃

Fig. 7 Visualization of MCF-7 cells by dyeing with **S-N_3** and Mito Tracker RED dye at 37℃. (A) image of MCF-7 cells dyeing by probe **S-N_3**; (B) image of MCF-7 cells dyeing by Mito Tracker RED dye; (C) the overlaid image of (A) and (B); (D) image of MCF-7 cells in dark field

Fig. 8 Colocalization of **S-N_3** and Mito Tracker RED dye

Fig. 9 The MS profile of S-N_3



Concentration(μM)	Inhibition rate (%)	
	$S\text{-}N_3$	$S\text{-}NH_2$
3.125	4.23	3.94
6.25	5.32	5.01
12.5	7.49	8.12
25	23.40	30.50
50	32.13	36.13
100	72.64	84.64