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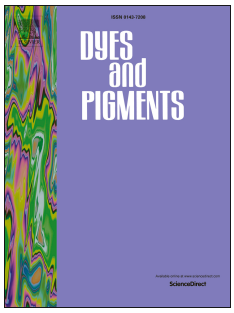

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A benzothiazole-based fluorescent probe for selective detection of H_2S in living cells and mouse hippocampal tissues

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†Electronic Supplementary Information (ESI) available: Synthesis, additional methods, additional plots of UV and fluorescence, NMR and HR-MS analysis data, additional other supporting data.

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Abstract

This study reports a benzothiazole-based fluorescent probe EPS-HS for real-time detection of H_2S. The probe is characterized in a large red-shift, good selectivity, high sensitivity and favorable biocompatibility. We measured the detection limit of EPS-HS in PBS buffer and fetal bovine serum. Moreover, in HT22 living cell imaging studies, EPS-HS showed good cell permeability and H_2S could be detected within the cells. Furthermore, *in situ* visualization of H_2S was performed on the hippocampal slices of normal mice with EPS-HS. We were also able to estimate the concentration of sulfide in mouse hippocampus tissues. The probe will be applied for better treatment of neurological deficits caused by damage to the hippocampus.

Key words: hydrogen sulfide, probe, fluorescence, hippocampus

1. Introduction

Hydrogen sulfide (H_2S), an colorless gas with the characteristic foul odor of rotten eggs, has been considered as a toxic pollutant for more than three hundred years[1]. Nevertheless, recent studies indicate H_2S as the third gaseous signal molecule which is almost as important as nitric oxide (NO) and carbon monoxide (CO) in biological systems[2,3,4]. H_2S may show either protective or toxic effects on cells at different concentrations[5-8]. Normally, in mammalian cells, H_2S is endogenously produced by enzymes such as cystathionine β-synthetase (CBS), cystathionine γ-lyase (CSE), 3-mercaptopyruvate sulfurtransferase (3-MST) and cysteine lyase (CL) in cysteine-related sources [9,10]. However, accumulating evidence has confirmed that abnormal H_2S concentrations are related to various diseases, including Alzheimer's disease[11], diabetes[12] and tumor[13], and found within many cell lines such as porcine oocytes[14] and MCF-7 cells[15].

Interestingly, several studies also provide evidence supporting the excellent role of H_2S in neuromodulation[16,17]. For example, H_2S can act as a neuroprotectant, protect neurons from oxidative stress, and produce potential therapeutic effects against neurodegenerative disorders[18-20]. Furthermore, researchers show that H_2S can inhibit the apoptosis of hippocampal neurons and reduce the damage of hippocampal neurons[21-24]. Hence, more attention have been drawn on the possible role that H_2S might play in neurological deficits caused by damage to the hippocampus. Thus, a highly selective and sensitive analytical measurement for better understanding how this gasotransmitter contributes to convoluted biological processes is directly

requested[25], which can accurately and reliably determine the biologically relevant concentrations of H_2S in the nervous system.

Traditional analytical techniques for H_2S detection include the methylene blue method, the monobromobimane method, gas chromatography and the sulfide ion selective electrode method[26-29]. In contrast, fluorescent probes have been widely applied for detecting the concentrations of H_2S in living biological systems due to their high selectivity and sensitivity, short response time, non-invasive detection, and real-time imaging[30,31]. Unfortunately, the short excitation wavelength light (ca. 350-550 nm) limits its application in deep-tissue imaging because of the shallow penetration depth. Recently, two-photon fluorescence probes, which can be excited by two-photon absorption, provided an opportunity to overcome the problems originated from the single-photon fluorescence technology compared with cellular imaging, the organic imaging also has iconic significance owning to its more complex structures and research value[32-35]. Most recently, Chen *et al.*[34] and Liu *et al.*[36] reported the detection and imaging of H_2S in cardiac and liver tissues. These remind us that histological-section researching is a breakthrough in *vivo* detection for H_2S.

In the current study, an easily synthesized benzothiazole-based fluorescent probe EPS-HS was designed. Its original group has stable chemical properties and fluorescence spectrum is not affected by environmental factors[37]. Therefore, first, density functional theory (DFT) calculations were performed to optimize the structure of EPS-HS at the B3LYP/6-31G* level using a suite of Gaussian 09 programs[38,39]. Then the structure of the target compound was fully characterized by the standard

^{1}H-NMR, ^{13}C-NMR, and high resolution mass spectrometry. Finally, the biological application of EPS-HS was discussed using HT22 living cells and the hippocampus of normal mice.

2. Experimental

2.1. Synthesis of EPS-HS

The synthesis process of EPS-HS is showed in Route 1. A mixture of Compound **1** which was synthesized by means of the reference paper[40,41] (0.227 g, 1.0 mmol), 2, 4-dinitrofluorobenzene (0.186 g, 1.0 mmol), triethylamine (0.303 g, 3.0 mmol) in 10 mL dry DMF (dimethyl formamide) were stirred at N_2 atmosphere, and the solution was stirred at 80°C for 6 - 8 h. The reaction mixture was cooled to room temperature and poured into a mixture of ice and water (100 mL) before a light yellow solid was precipitated. The raw product was filtered and recrystallized from EtOAc to provide a light yellow solid in 85 % yield. m.p. 128.2°C -129.3°C. TLC (silica, hexane : DCM, 2:1 v/v); R_f = 0.4; ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.93 (d, J = 2.8 Hz, 1H), 8.49 (dd, J_1 = 9.2 Hz, J_2 = 2.8 Hz, 1H), 8.22-8.24 (m, 2H), 8.17 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.55-7.59 (m, 1H), 7.40-7.51 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 121.1, 121.2, 122.4, 122.9, 123.4, 126.1, 127.2, 130.1, 130.2, 130.9, 135.1, 140.4, 142.6, 154.0, 154.4, 156.8, 166.5. HRMS (ESI$^+$): $(M + H)^+$ calcd. for EPS-HS ($C_{19}H_{12}N_3O_5S$) 394.0498; found 394.0489.

2.2 Characterization

Thin layer chromatography was performed on silica gel 60 F_{254} plates (250 μm) and column chromatography was conducted over silica gel (300-400 mesh).

Visualization of the developed chromatogram was accomplished by a UV lamp. UV-Vis absorption spectra were recorded on a Shanghai MAPADA UV-3100PC UV-Visible spectrophotometer. All fluorescence measurements were recorded on a Hitachi F4600 Fluorescence Spectrophotometer. The pH measurements were performed on a Mettler-Toledo Delta 320 pH meter. All fluorescence imaging experiments were conducted under a FV1000 confocal laser scanning microscope (Olympus, Japan). All the solvents were of analytic grade. The stock solution of $Na_2S{\cdot}9H_2O$ (≥98.0%) was prepared in doubly distilled water, which was freshly prepared each time before use. All fluorescence measurements were carried out at room temperature on a Hitachi Fluorescence Spectrophotometer F-4600. The samples were excited at 285 nm with the excitation and emission slit widths set at 5.0 nm. The emission spectrum was scanned from 350 nm to 560 nm at 1200 nm/min. The photomultiplier voltage was set at 900 V.

3. Results and discussion

3.1 Computational calculations

DFT calculations were conducted to achieve the preferential conformation of EPS-HS and its parent nucleus (Compound 1) (Figures 1A and 1B) and to investigate the mechanism by which EPS-HS reacted with H_2S (Figure 1C). Results showed that the photoexcitation of EPS-HS from S0 to S1 states mainly involved electron transitions from the highest occupied molecular orbital (HOMO) to the lowest occupied molecular orbital (LUMO). The HOMO of EPS-HS was mainly located at

the benzothiazolyl part, but the LUMO were mostly located at the nitrobenzene part. It can be seen in the frontier molecular orbital diagram that the HOMO did not overlap with the LUMO.

Based on the frontier molecular orbital theory, the computational results of EPS-HS indicate that the increased electric density area is mainly within the nitrobenzene part. The ether linkage becomes the target of nucleophilic reagent (H_2S). There was no obvious difference as to average energy between the LUMO and the HOMO.

3.2 Fluorescence and absorption spectroscopy

The changes in the fluorescence and absorption spectroscopy of EPS-HS (10 μM) was tested at 37°C in 20 mM PBS buffer (pH 7.4) using Na_2S (100 μM) as a sulfide source. Results showed that the maximum absorption peak of EPS-HS was shifted which was similar to Compound 1 (Figure 2A). Meanwhile, a robust increase in fluorescence intensity (> 40-fold) was found with the maximum emission peak at 470 nm when excited at 285 nm, which was completed within 20 min (Figure 2B). These data indicated that our experimental results were consistent with the frontier orbital theory.

Next, we examined the sensitivity of EPS-HS for H_2S using various concentrations of Na_2S (0 - 300 μM) (Figure 3A). Upon exposure to H_2S, the probe was cleaved to release fluorophores. The fluorescence intensity was increased about 0 - 40 folds. Then, EPS-HS was further reacted with different concentrations of Na_2S (0 - 60 μM), showing a linear relationship of emission intensity versus sulfide

concentration in PBS buffer and fetal bovine serum (Figure S1, ESI†).

The fluorescence intensity of EPS-HS (10 μM) in PBS and fetal bovine serum was assessed with the presence of different concentrations of Na_2S for 20 min (Figure S1, Supporting Information). But, the fluorescence response in serum was lower than that observed in PBS buffer, which may attribute to the fast metabolism of sulfide in plasma. The fast responses and excellent linear relationship provided a real-time quantitative method for detecting sulfide in biological samples. The detection limit was calculated to be 108 nM in PBS butter and 154 nM in fetal bovine serum. After the addition of 100 μM Na_2S buffered solution, a significant fluorescence increase was observed between 0 to 45 min after mixture, and the reaction was completed at 37°C within 20 min (Figure 3B). Further studies indicated that the emission intensity reached the peak from pH 7.0 to 9.0 (Figure S3, Supporting Information), which meets the requirement of real-time detection of H_2S in the living body.

3.3 Selectivity of EPS-HS

Moreover, according to turn-on fluorescence responses, EPS-HS was found to be more selective for sulfide than other biologically relevant thiols such as GSH, Cys and Hcy *et al.*(Figure 4A), amino acids (Figure 4B) and other species (Figure S2, Supporting Information) in PBS buffer. This may because that H_2S is a small gas molecule, its pKa_1 is about 6.9, while pKa value of other thiol (such as GSH, Cys) in cell is higher (about 8.5). The design principle of this type probe is based on thiol pKa values to distinguish each other. The recognizing groups of probe EPS-HS we designed is m-dinitrobenzene. The m-dinitrobenzene ether is used to protect tyrosine

in synthesis of peptide chain. In weak alkali conditions, using thiol as sulfur agent can remove the protection group. Therefore under the physiological conditions, the probe EPS-HS could detect hydrogen sulfide selectively rather than thiols like GSH and Hcy.

3.4 Application of EPS-HS in live-cell imaging

Then, the potential ability of EPS-HS to detect H_2S was tested in HT22 cells using confocal microscopy imaging (Figure 5). First of all, the cytotoxicity of EPS-HS toward HT22 cells was examined by MTT assay. EPS-HS exhibited an IC_{50} of 58.99 ± 1.7 μM, which demonstrated that EPS-HS was of low toxicity toward cultured cell lines (Figure 5). Then, to assess the ability of EPS-HS for H_2S fluorescence imaging, HT22 cells were incubated with 10 μM EPS-HS alone at 37°C for 20 min (Figure 6, 1A) which is equivalent to the intracellular basal level of endogenous H_2S. As expected, incrementally stronger yellowish-green fluorescence was detected in HT22 cells treated with 10 μM EPS-HS and 10 μM or 100 μM Na_2S (Figure 6, 2B and 3B). These results indicate that EPS-HS is readily internalized into living cells and act as a fluorescent probe to detect the concentration of H_2S in living cells (Figure 6C). Subsequently, cells were pretreated with $ZnCl_2$ (an effcient eliminator of H_2S) and then incubated with EPS-HS (10 μM) for 10 min. With the addition of Na_2S (100 μM) and GSH (100 μM) to the $ZnCl_2$-pretreated cells, no fluorescence intensity increases were observed (Figure 6, 4B). The results indicated that the fluorescence change of EPS-HS in the cells arises from H_2S.

3.5 Application of EPS-HS in mouse hippocampus imaging

We further evaluated the visualization of H_2S in KM mice hippocampal tissues using EPS-HS. The mouse brains were rapidly taken and fixed in 4% paraformaldehyde for 30 min. Then the tissues were embedded in ornithine carbamoyltransferase (OCT), and serially sectioned at 7 μm for fluorescent detection analysis (Figure 7). As shown in Figure 7A, there was no background fluorescence emission for hippocampal tissue slices after incubation with PBS working fluid. When the slices pretreated with Na_2S (10 μM) (Figure 7B) and Na_2S (100 μM) (Figure 7C) were incubated with EPS-HS (10 μM) working fluid at 37°C for 20 min, yellowish-green fluorescence signals were obviously observed. When the slices pretreated with GSH (100 μM) (Figure 7D) were incubated with EPS-HS (10 μM) working fluid at 37°C for 20 min, yellowish-green fluorescence signals were hardly observed. These findings demonstrated that EPS-HS is capable of detecting H_2S in the tissues.

3.6 Detection of sulfide in mouse hippocampus

Finally, EPS-HS was added to measure sulfide concentrations in mouse hippocampal tissues, where the spiked Na_2S (X, X+0.2, X+0.4, X+0.6, X+0.8 μM) were used as internal standard. The spiked homogenate samples were subsequently precipitated by DMSO to remove proteins. To the supernatant of the spiked hippocampus homogenates, EPS-HS (10 μM) was added. The mixture was incubated in PBS buffer at 37°C for 20 min before analysis. We found that the average sulfide concentration in fresh mouse hippocampus was 1.267 ± 0.020 μmol/g protein (Table 1). Overall, these findings demonstrated that EPS-HS is suitable to detect sulfide in

real biological samples in a rapid manner. Importantly, hydrogen sulfide is recognized as a neuromodulator as well as neuroprotectant in the brain. Then, we repeated the measurement with probe NAP-1[42]. The concentration of sulfide was determined to be 1.242 ± 0.047 μmol g^{-1} protein, which was consistent with the results from EPS-HS .

4. Conclusions

In summary, EPS-HS was designed as a benzothiazole - based H_2S fluorescent probe, which shows a significant emission increase in the fast response to sulfide within the biologically relevant pH range. EPS-HS produces a turn-on fluorescence signal for responding H_2S. It can be applied for parallel measurement of sulfide concentrations in HT22 cells and mouse hippocampus.

Acknowledgements

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Article---Figure Legends

Figure 1. Optimized, low-energy conformations of the benzothiazole rings using DFT (B3LYP/6-31G*) calculations: (A) Compound 1. (B) EPS-HS. (C) the structure of EPS-HS on the HOMO and LUMO.

Route 1. The synthetic route of EPS-HS.

Figure 2. Fluorescence spectra (A) and absorption spectra (B) of EPS-HS (10 μM), Compound 1 (10 μM) and EPS-HS (10 μM) + Na_2S (100 μM) in PBS buffer (20 mM, pH = 7.4, 5% DMSO).

Figure 3. Fluorescence spectra of EPS-HS (10 μM) in PBS buffer (20 mM, pH 7.4, 5% DMSO) at 37°C for 20 min. Excitation: 285 nm, emission: 350 - 560 nm. (A) Incubated with different concentrations of Na_2S (0, 1.0, 2.0, 5.0, 10, 20, 30, 40, 50, 60, 80, 100, 150, 200 and 300 μM) for 20 min. (B) Incubated with 100 μM Na_2S after 0, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 45 min. Excitation: 285 nm, emission: 350 - 560 nm. Data are presented as the mean ± SD (n = 3).

Figure 4. Fluorescence responses of EPS-HS (10 μM) towards Na_2S (100 μM) and various biothiols after 20 min of treatment. (A) (1) Na_2S (0 μM); (2) Na_2S (100 μM); (3) Hcy (100 μM); (4) GSH (100 μM); (5) Cys (100 μM); (6) Hcy (1 mM); (7) GSH (1 mM); (8) Cys (1 mM); (9) Na_2S (100 μM)+Hcy (100 μM); (10) Na_2S (100 μM) +Hcy (1 mM); (11) Na_2S (100 μM) + GSH (100 μM); (12) Na_2S (100 μM) + GSH (1 mM); (13) Na_2S (100 μM) + Cys (100 μM); and (14) Na_2S (100 μM) + Cys (1 mM). (B) Fluorescence responses of EPS-HS (10 μM) towards Na_2S (100 μM) and amino acids. Data are presented as the mean ± SD (n = 3).

Figure 5. (A) The inhibitory effect of EPS-HS on the cell growth of HT22 cells after treatment for 24 h. (B) The viability of HT22 cells after exposure to EPS-HS (10.0 μM) for different times. Data are presented as the mean ± SD (n = 3).

Figure 6. Confocal fluorescence images in living cells. HT22 cells were incubated with EPS-HS alone (10 μM) for 20 min (1A and 1B). The cells were exposed to EPS-HS (10 μM) followed by Na_2S (10 μM) at 37°C for 20 min (2A). The cells were exposed to EPS-HS (10 μM) followed by Na_2S (100 μM) at 37°C for 20 min (3A). Cells were pretreated with 1 mM $ZnCl_2$ for 10 min, then incubated with EPS-HS (10 μM) for 10 min and then incubated with Na_2S (100μM) and GSH (100 μM) at 37°C for 20 min (4A); Overlay of the bright field image and the green channel (2B, 3B and 4B). Fluorescence intensity per one cell. Fluorescence images were acquired by confocal microscopy (C). Scale bars = 10 μm. Data are presented as the mean ± SD (n = 3).

Figure 7. In *situ* visualization of hippocampal tissues of the brains with EPS-HS. An image of frozen mouse hippocampal slices incubated with PBS working fluid (A); an image of frozen mouse hippocampal slices incubated with EPS-HS (10 μM) working fluid after pretreatment with Na_2S (10 μM) (B) and Na_2S (100 μM) (C) at 37°C for 20 min; Slices pretreated with GSH (100 μM) were incubated with EPS-HS (10 μM) working fluid at 37°C for 20 min (D). Scale bars = 20 μm. The mean fluorescence intensity was obtained with 6-8 slices from different mice.

Table 1. Measurement of sulfide concentrations in fresh mouse hippocampus tissues.

Electronic Supplementary Information for

A benzothiazole-based fluorescent probe for selective detection of H_2S in living cells and mouse hippocampal tissues

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1. Preparation of the test solutions

1.1 EPS-HS stock solution

EPS-HS (3.93 mg, 1 mmol) was dissolved into DMSO (10 mL) to get 1.0 mM stock solution for general use.

1.2 Na_2S stock solution

5 mg EDTA was dissolved in 10 mL DI H_2O in a 25 mL volumetric flask. The solution was purged vigorously with nitrogen gas for 15 min. Then 48 mg sodium sulfide ($Na_2S \cdot 9H_2O$) was dissolved in the solution under nitrogen gas. The resulting solution was 20 mM Na_2S, which was then diluted to 1.0 - 2.0 mM stock solution for general use.

2. Route and characterization of Compound 1

2-Aminothiophenol (0.625 g, 5 mmol) and 4-hydroxybenzaldehyde (0.611 g, 5 mmol) which was dissolved in 20 mL dry ethanol were added to a 250 mL eggplant-shape flask equipped with a magnetic stirrer. Aq 37% HCl (15.0 mmol) and aq 30% H_2O_2 (30 mmol) were added and the mixture was stirred for 1.5 h at room temperature. A light yellow crystal was got by filtration. Then, the crude product was purified by flash chromatography on silica gel obtaining at a yield of 92%. m.p. 188°C - 189.2°C. TLC (silica, hexane : EtOAc, 3:1 v/v): R_f = 0.3.

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.54 (s, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.72 (dd, J_1= 8.0 Hz, J_2= 1.6 Hz, 1H), 7.51-7.55 (m, 1H), 7.39-7.46 (m, 2H), 7.13-7.15 (m, 1H), 6.97-7.01 (m, 1H). ESI-MS (m/z): 228.1 [M +

H]$^+$, calcd. for $C_{13}H_9NOS$ = 227.04.

3. Evidence of mechanism detection

EPS-HS (78.6 mg, 0.2 mmol) was dissolved in DMSO (15 mL), followed by the addition of Na_2S - $9H_2O$ (240 mg, 1.0 mmol) in PBS buffer (15mL, 20 mM, pH = 7.4). The resultant mixture was stirred at room temperature for 3 h. Subsequently, EtOAc (3 × 10 mL) was added into the solution for extraction. The thiolysis product was characterized by HRMS and ^{1}H NMR.

4. UV-Vis absorption spectrogram

Na_2S is widely recognized as H_2S donor. EPS-HS (10.0 μM) was dissolved in DMSO, which was then diluted in PBS buffer (2:3, v/v, 20 mM, pH 7.4) with 5% DMSO. Before the addition of Na_2S, EPS-HS (10 μM) displayed an absorption peak at 285 nm. After incubated with Na_2S (100 μM) in doubly distilled water for 20 min, a new absorption peak was presented at 386 nm.

5. Determination of the detection limit

The detection limit was calculated based on the method reported in previous literature. The fluorescence emission spectrum of EPS-HS without Na_2S was measured by 10 times and the standard deviation of blank measurement was obtained. Then, the solution was treated with Na_2S at concentrations from 0 to 60 μM. A linear regression curve was then achieved. The detection limit was calculated with the following equation: Detection limit = $3\sigma/k$, where σ is the standard deviation of blank

measurements, and *k* is the slope between the fluorescence intensity ratios and Na_2S concentrations. The detection limit was 108 nM in PBS buffer and 154 nM in fetal bovine serum, respectively.

6. MTT assay

The effect of EPS-HS on cell growth was measured using a colorimetric MTT assay kit (Sigma-Aldrich). HT22 cells were seeded in 96-well plates at a density of 50,000 cells/well and then maintained in DMEM medium with 10% fetal bovine serum/penicillin/streptomycin in a 5 % CO_2 atmosphere at 37°C. The cells were incubated with different concentrations of EPS-HS for 24 h, respectively. HT22 cells in culture medium without EPS-HS were used as control. Then, 20 μL of MTT dye (3-[4, 5-dimethylthiazol-2-yl]- 2, 5-diphenyl tetrazolium bromide, 5 mg/mL in phosphate buffered saline), was added to each well, and the plates were incubated at 37 °C for 4 h. Then, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. The plate was shaken for 10 min and the absorbance was measured at 570 nm on a microplate reader (ELX808IU, Bio-tek Instruments Inc, USA). Each sample was performed in triplicate, and the entire experiment was repeated three times. Calculation of IC_{50} values was done according to Huber and Koella[1].

7. Fluorescence imaging in living HT22 cells

HT22 cells were seeded in a 6-well plate in DMEM supplemented with 10 %

fetal bovine serum in an environment of 5 % CO_2 at 37°C for 24 h. For a control experiment, HT22 cells were incubated with EPS-HS (10 μM) at 37°C for 60 min. After washing with PBS three times to remove the remaining dyes, the cells were then incubated with Na_2S (10 μM) or Na_2S (100 μM) for another 20 min. The fluorescence imaging was carried out after washing with PBS buffer for three times.

8. In *situ* fluorescent detection of H_2S in the hippocampal tissues of the brains

The mouse brains were rapidly taken and fixed in 4 % paraformaldehyde. Then the tissues were embedded in ornithine carbamoyltransferase (OCT), and serially sectioned at 7 μm using a Leica CM1950 cryostat (Leica Microsystems, Wetzlar, Germany) for fluorescence detection. After dried for 1 h, the frozen sections were washed for 3 times with 10 mM PBS for 3 min. The slices treated with Na_2S (100 μM) were incubated with EPS-HS working fluid (10 μM) at 37°C for 20 min and subsequently rinsed in PBS for 3 times for 3 min. The slices were mounted with 50 % buffered glycerol before observation under a fluorescence microscope in time. The mean fluorescence intensity was obtained using 6 - 8 slices from different mice.

Figure Legends

Figure S1. EPS-HS probe (10 μM) incubated with 0, 1.0, 2.0, 5.0, 10, 20, 30, 40, 50, and 60 μM Na_2S at 37°C for 20 min in PBS buffer (A) and fetal bovine serum (B) (5 % DMSO). The insert: A graph of the fluorescence intensity trend of EPS-HS (10 μM) with the presence of different concentrations of Na_2S for 20 min in PBS buffer (20 mM, pH = 7.4, 5 % DMSO) and fetal bovine serum (5 % DMSO), respectively. Data are presented as the mean ± SD (n = 3).

Figure S2. (A) Fluorescence spectra of EPS-HS (10 μM) with Na_2S (100 μM) and biologically relevant species in 20 mM PBS (pH 7.4) at 37°C for 20 min. (B) Fluorescence responses of EPS-HS (10 μM) towards Na_2S (100 μM) and biologically relevant species. (1) Blank, (2) Na_2S, (3) H_2O_2, (4) OCl^-, (5) O^{2-}, (6) -OH, (7) ^tBuOOH, (8) NO_2^-, (9) NO, (10) S-nitroso glutathione, (11) KCl, (12) $ZnSO_4$, (13) $CaCl_2$, (14) $MgCl_2$, (15) $FeCl_3$, (16) NaCl, (17) NaH_2PO_4, (18) NADH, (19) Glucose, (20) $S_2O_3^{2-}$, (21)$S_2O_5^{2-}$,(22) $S_2O_4^{2-}$, (23) SO_3^{2-}, (24) SO_4^{2-}, and (25) SCN^-.

Figure S3. (A) Fluorescence spectra of EPS-HS (10 μM) with Na_2S (100 μM) in buffer solutions at different pH vlues (20 mM, pH 5.8, 6.2, 6.6, 7.0, 7.4, 7.8, 8.2, 8.6, and 9.0, 5 % DMSO) at 37°C for 20 min. (B) Fluorescence responses of EPS-HS (10 μM) with Na_2S (100 μM) in buffer solutions at different pH values (20 mM, pH 5.8, 6.2, 6.6, 7.0, 7.4, 7.8, 8.2, 8.6, and 9.0, 5 % DMSO) at 37°C for 20 min.

Figure S4. ^1H NMR spectrum of Compound 1.

Figure S5. HR-MS spectrum of EPS-HS.

Figure S6. ^1H NMR spectrum of EPS-HS.

Figure S7. ^{13}C NMR spectrum of EPS-HS.

1 Huber, W. & Koella, J. C. A comparison of three methods of estimating EC 50 in studies of drug resistance of malaria parasites. *Acta Tropica* **55**, 257-261 (1993).

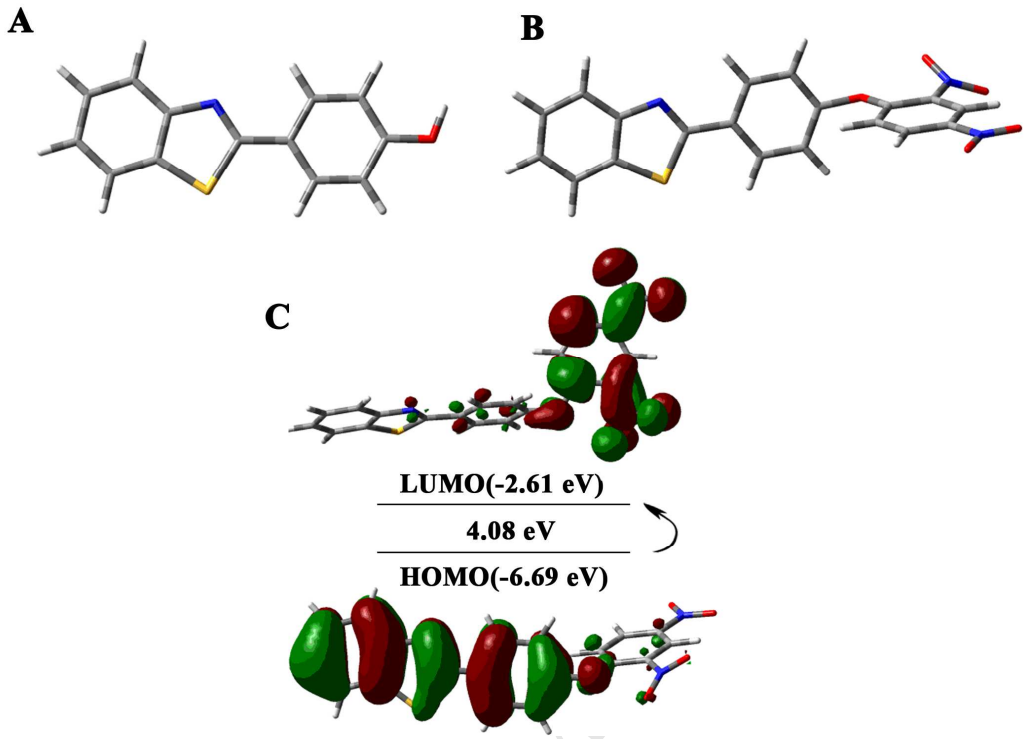
Table 1.

Sample	H_2S^{α} (μ mol g^{-1} protein)	H_2S^{b} (μ mol g^{-1} protein)
1	1.275	1.201
2	1.245	1.231
3	1.282	1.294
Mean ± SD	1.267 ± 0.020	1.242 ± 0.047

$^{\alpha}$ Measurement of H_2S concentrations in mouse hippocampus with EPS-HS; ^{b} Measurement of H_2S concentrations in mouse hippocampus with NAP-1; data are presented as mean ± SD.

Figures

Figure 1.



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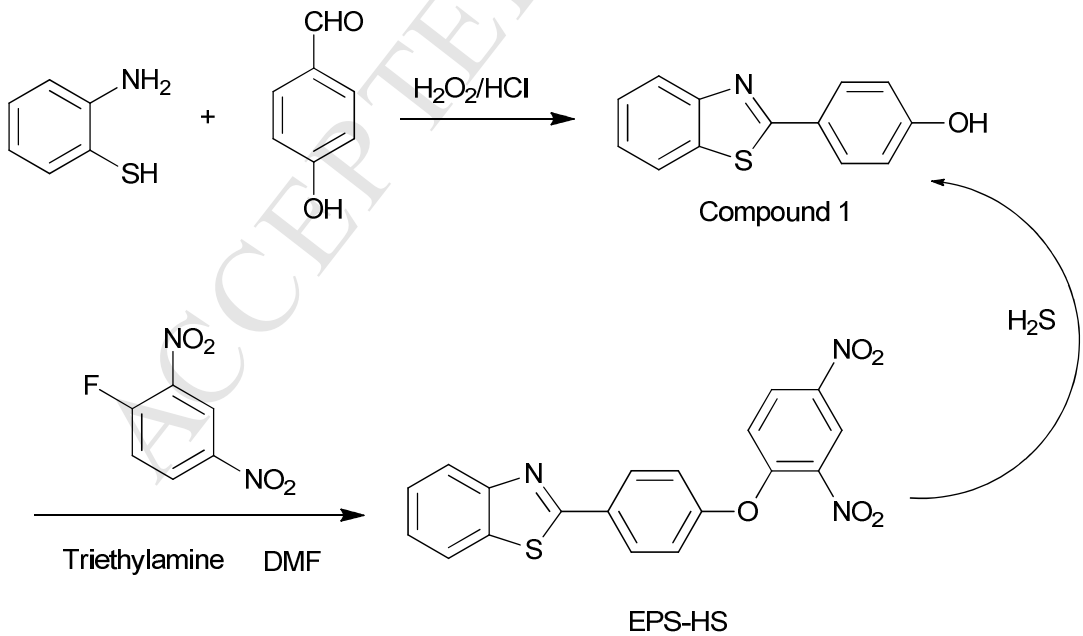
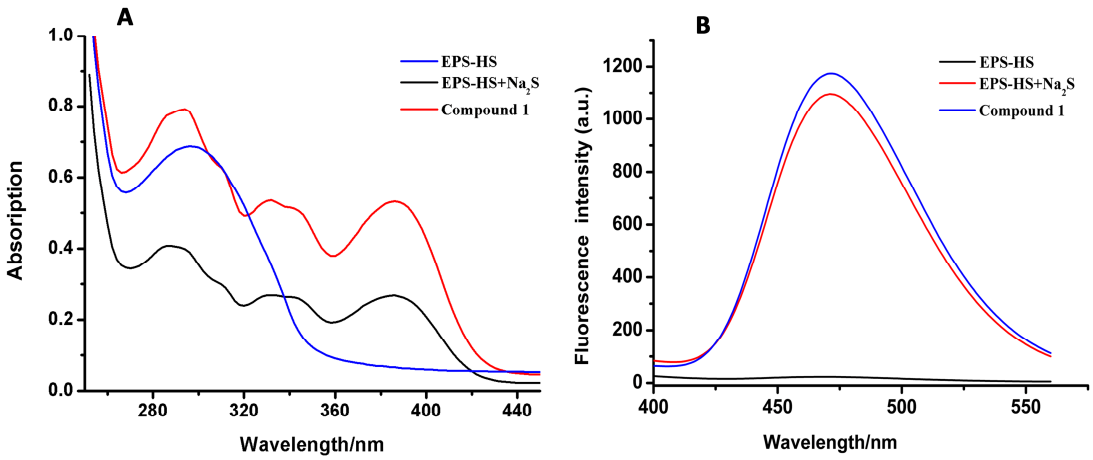


Figure 2.



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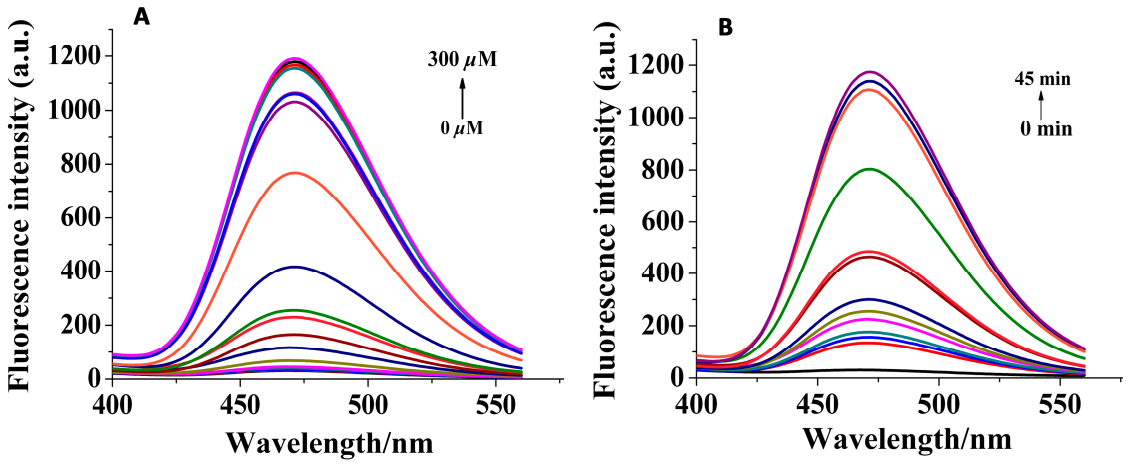

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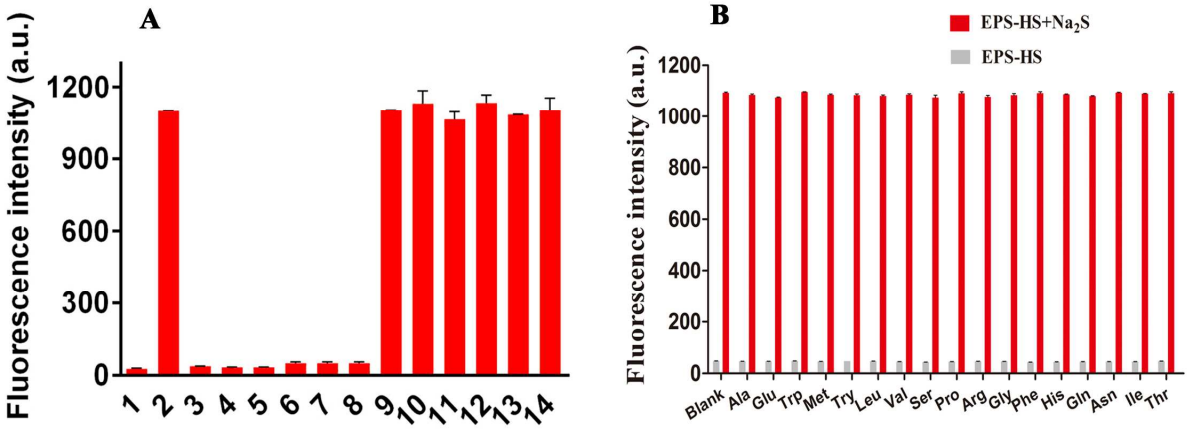

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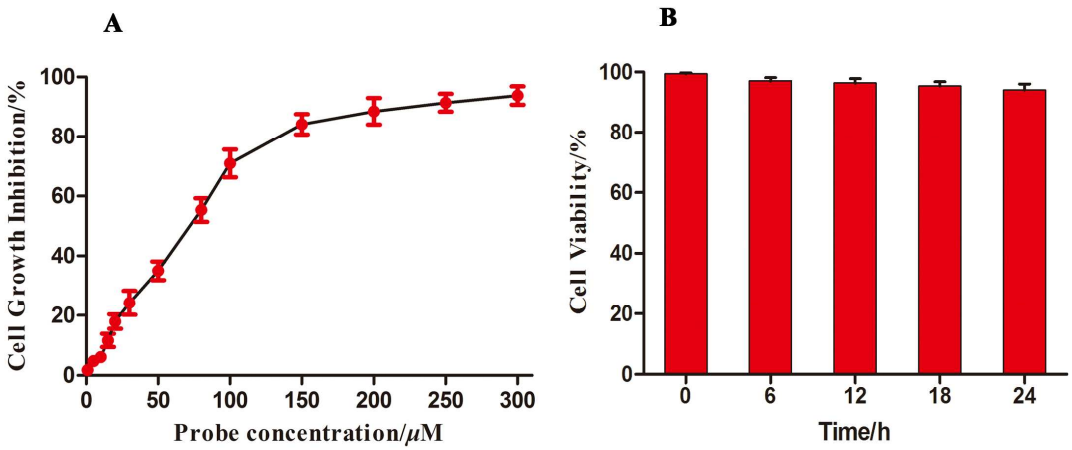

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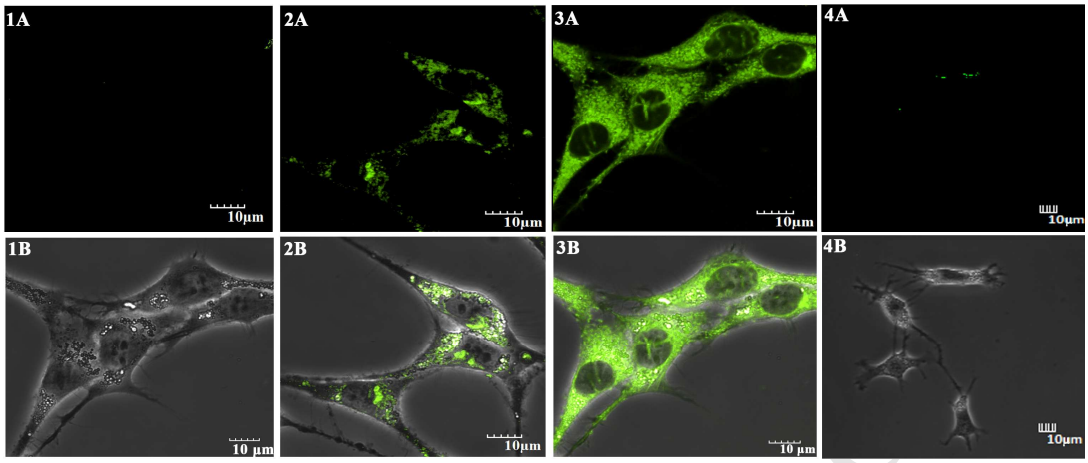

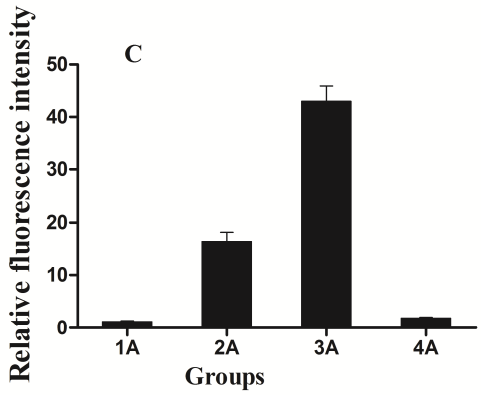

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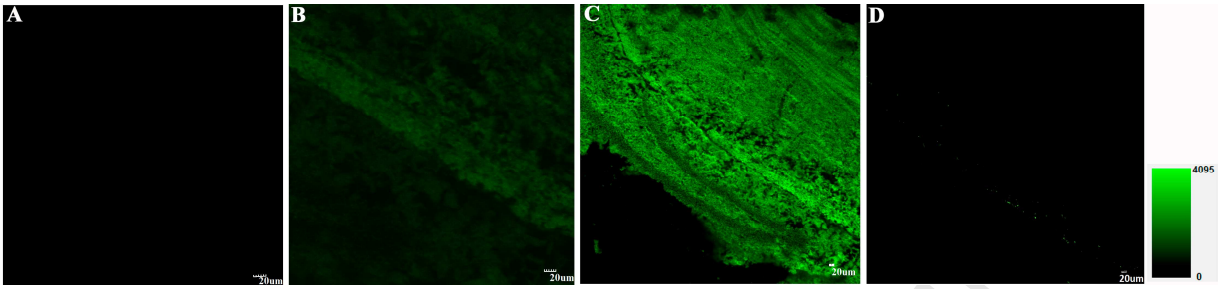


ESI-Figures

Figure S1.

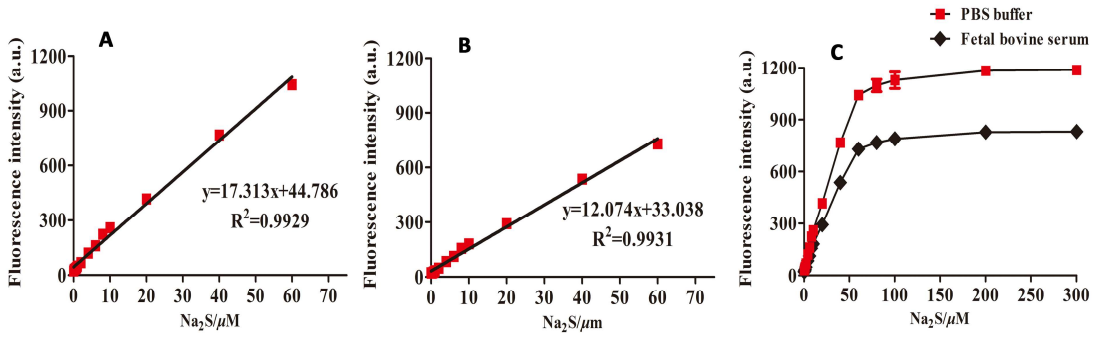


Figure S2.

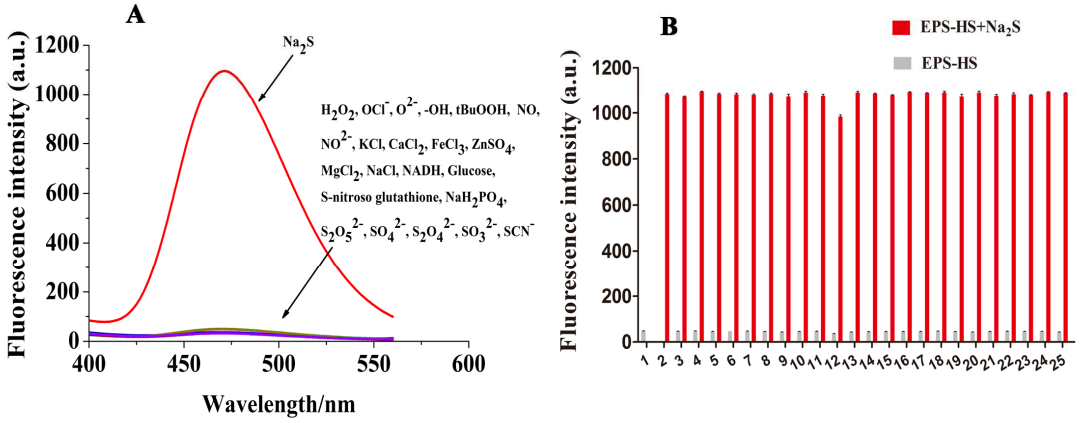
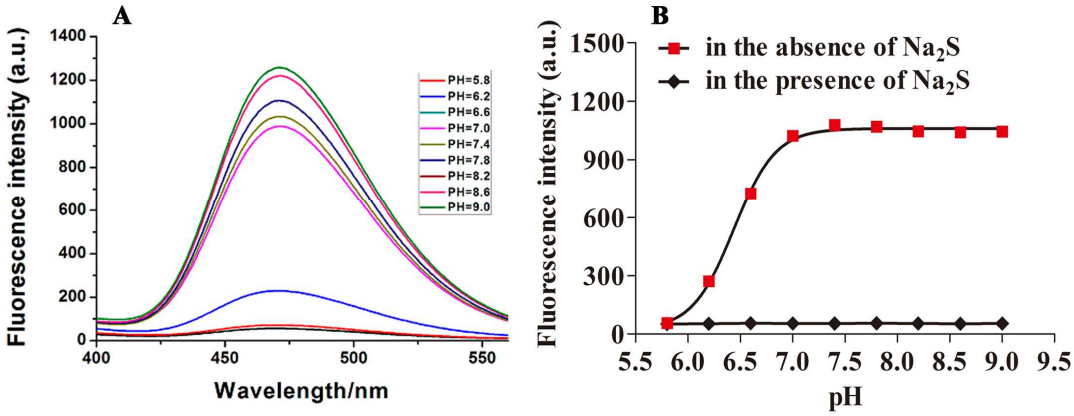


Figure S3.



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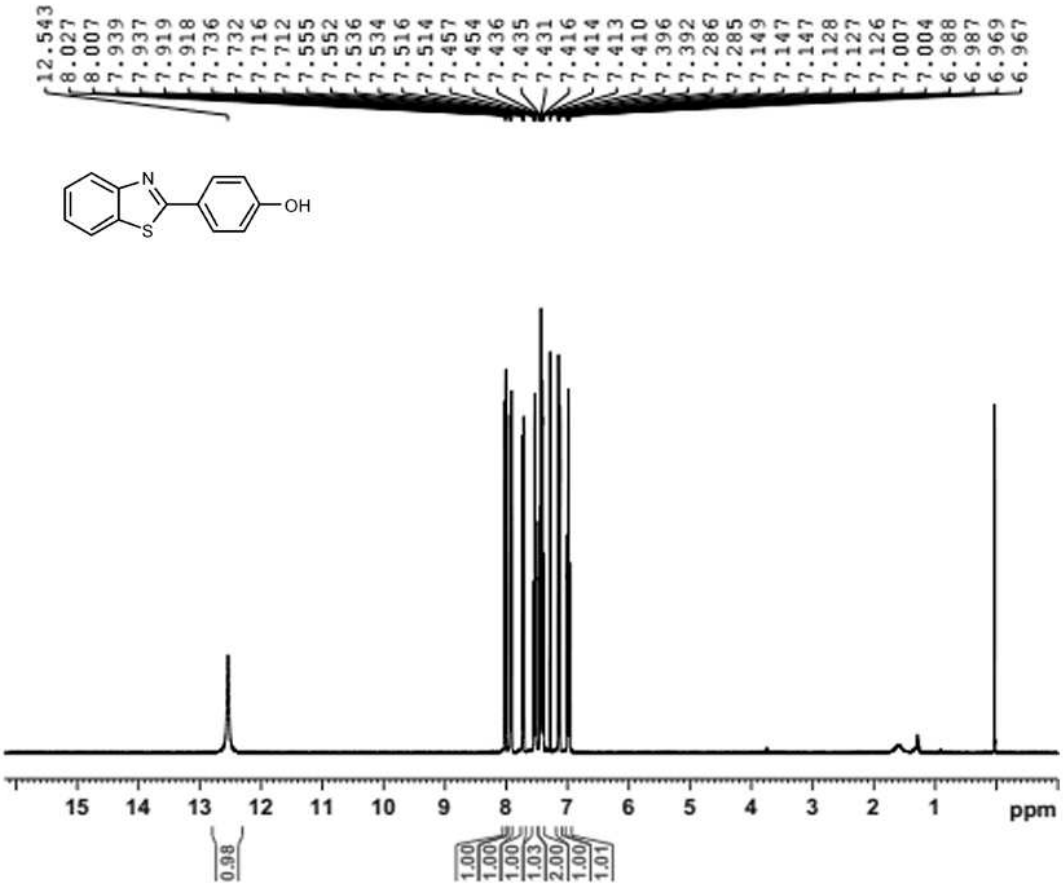

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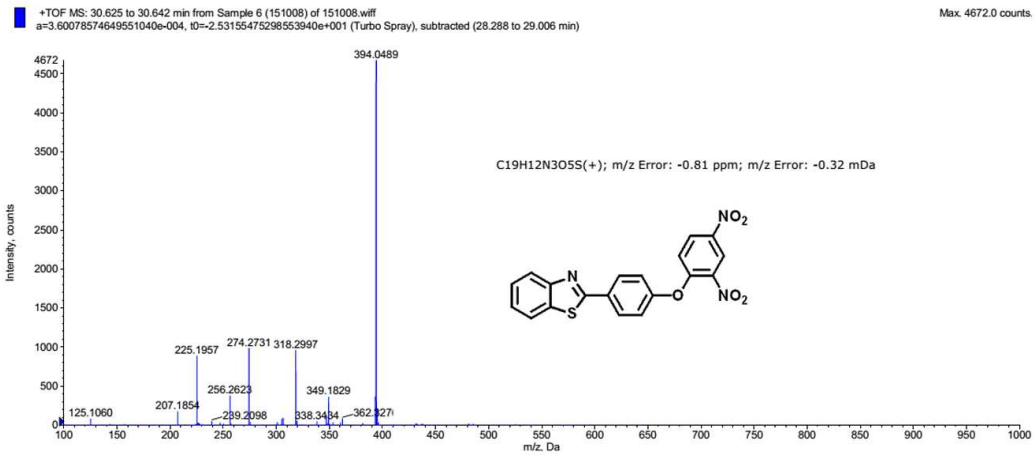

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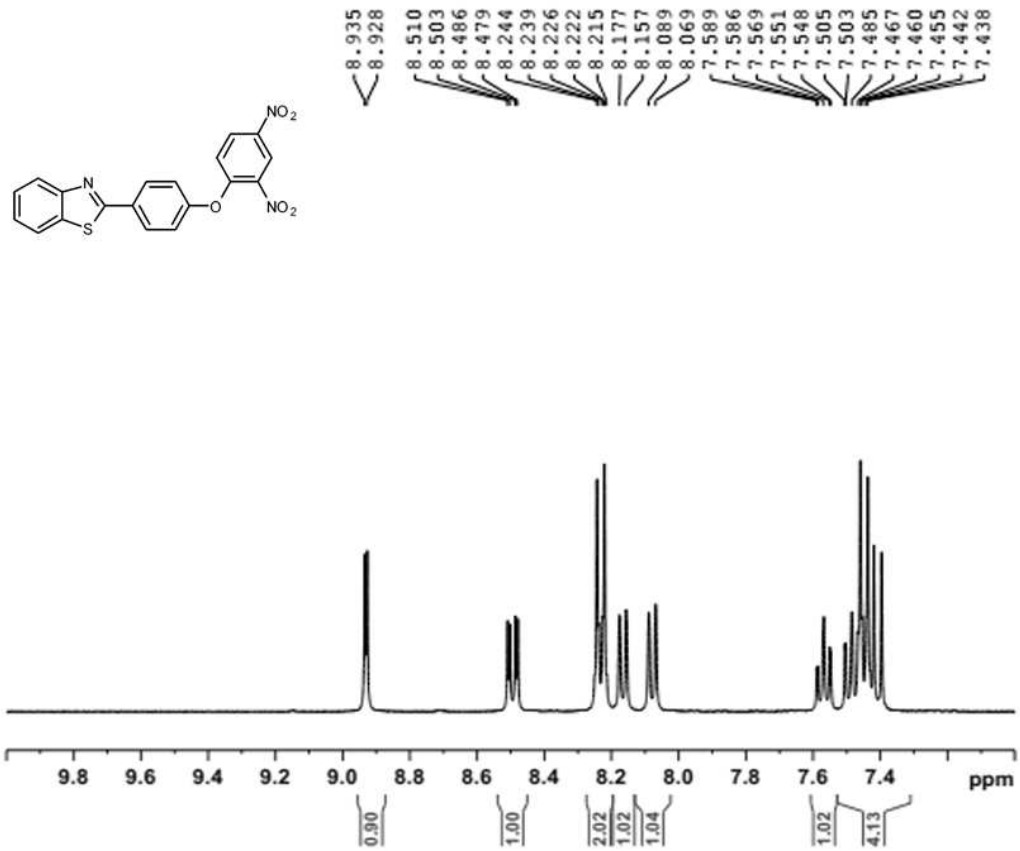

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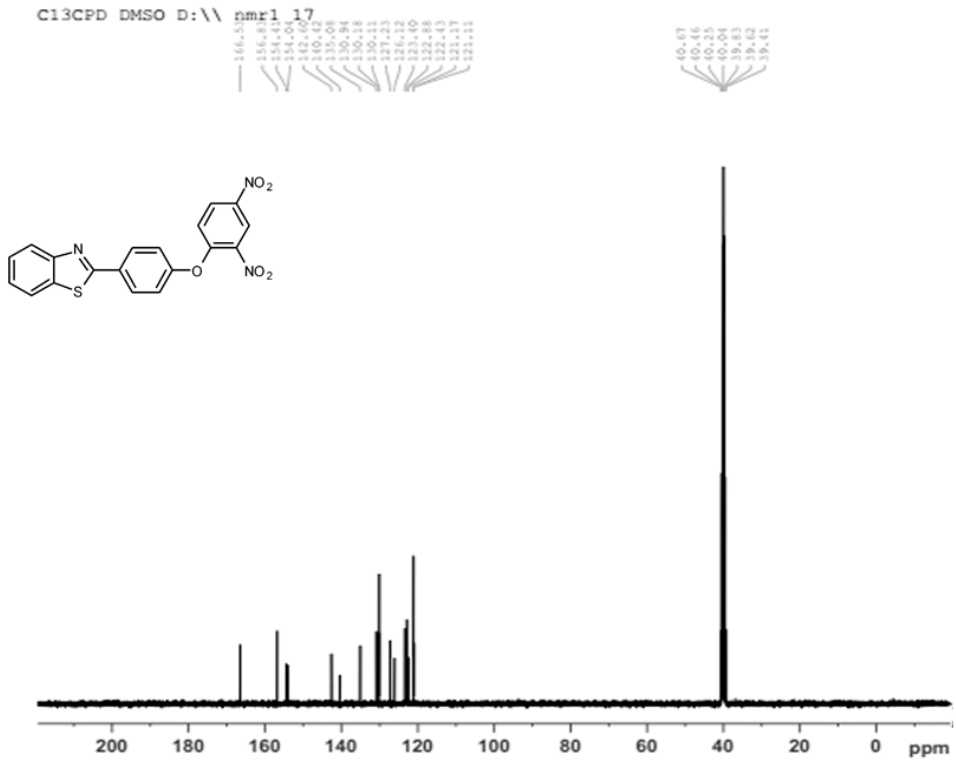

Research highlights

1. We designed and synthesized a benzothiazole-based fluorescent probe EPS-HS for selective detection of H_2S.

2. The probe EPS-HS showed good cell permeability and H2S could be detected within the cells

3. Iin *situ* visualization of H_2S was performed on the hippocampal slices of normal mice with probe EPS-HS.

4. The probe will be applied for better treatment of neurological deficits caused by damage to the hippocampus.