Nano Molar Detection of H$_2$S in aqueous medium: Application in Endogenous and Exogenous Imaging of HeLa cells and Zebrafish

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Abstract: The homeostasis of short-lived reactive species such as H$_2$S/HOCl in biological systems is essential for maintaining intercellular balance. An unchecked increase in biological H$_2$S concentrations impedes homeostasis. In this report, we present a molecular probe PBSH (Pyrene Based Sulfonyl Hydrazone) derived from pyrene for the selective detection of hydrogen sulfide endogenously as well exogenously through a “turn-off” response in water. The structure of the receptor is confirmed by FT-IR, $^1$H & $^{13}$C-NMR, ESI-MS, and single-crystal X-ray diffraction studies. The receptor has an excellent green emission both in the aqueous phase and solid-state. Quenching of green emission of the receptor is observed only when H$_2$S is present in water with a detection limit (LoD) of 18 nM. Other competing anions and cations do not have any influence on the receptor’s optical properties. The efficiency of H$_2$S detection is not negatively impacted by other RSS (Reactive Sulphur Species) too. The sensing mechanism of H$_2$S follows a chemodosimetric reductive elimination of SO$_2$ which is supported by product isolation. The receptor is found to be biocompatible, as evident by the MTT assay and its utility is extended to endogenous and exogenous fluorescence imaging of HeLa cells and Zebrafish.

Keywords: Hydrogen Sulphide, Endogenous Imaging, Water Soluble, Reactive Sulphur Species.

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### Table S1: Comparison table of recent publications.

| Ref. No. | Detector | LoD (nM) | Working Medium | Application |
|----------|----------|----------|----------------|-------------|
| 7.       | ![Detector 1](image1) | 1 µM in buffer/Tween and 5 µM in bovine serum | 20 mm sodium phosphate buffer (pH 7.5) with 0.5% Tween-20 (Buffer/Tween) | Blood Sample |
| 8        | ![Detector 2](image2) | 0.08 µM | 40 mM HEPES, pH 7.4, at 25°C | RAW 264.7 Cell Imaging |
| 15       | ![Detector 3](image3) | 0.15 µM | Tris buffer (50 mM, pH=8.0, 50% DMF) | HeLa Cells imaging |
| 19       | ![Detector 4](image4) | 0.09 µM | PBS buffers (pH 7.4, 10 mM) | HeLa Cells and Zebrafish Imaging |
| 20       | ![Detector 5](image5) | 205 nM | HEPES buffer; 5 mM, pH 7.4 | HeLa Cells and Zebrafish Imaging |

Cu²⁺ Complex
| 23b | Cu$^{2+}$ Complex | 1.9 μM | HEPES buffered (10 mM, 5 mM SDS, pH 7.4) water | NIL |
| 24a | | 6.9 nM | DMSO | gastric mucosa imaging |
| 24b | | 150 nM | PBS buffer (20 mM, pH 7.4) containing 1% DMSO (v:v) | Hela Cells imaging |
| 24c | | 0.024 μM | 50 mM HEPES buffer (pH 7.4) | Hela Cells imaging |
| 25a | | 0.40 μM (UV-vis measurement) and 0.23 μM (fluorescence measurement) | CH$_3$CN–H$_2$O (9/1, v/v) solution | Hela Cells imaging |
| 26a | | 158 nM | H$_2$O–DMSO (v/v = 20/80) | HeLa Cells and Zebrafish Imaging |
| 28a | ![Molecule](image1.png) | NIL | ethanol/PBS buffer solution (50/50, v/v, pH = 7.4) | T98G fixed cells imaging |
|-----|------------------------|-----|-----------------------------------------------|-------------------------|
| 28b | ![Molecule](image2.png) | 6 nM | DMSO | Cells imaging and animals |
| 35  | ![Molecule](image3.png) | 2.6 μM | Hepes buffer (10 mM, pH 7.4) | PC12 cells imaging |
| 36  | ![Molecule](image4.png) | 0.16 μM | PBS Buffer | NIL |
| 37a | ![Molecule](image5.png) | $1.92 \times 10^{-4}$ mol L$^{-1}$ | DMSO | NIL |
| 38  | ![Molecule](image6.png) | 0.13 nM | 50 mM HEPES buffer (containing 1% DMSO pH = 7.4) | HeLa Cells imaging |
| 39  | ![Molecule](image7.png) | $9.7 \times 10^{-7}$ M | EtOH/H$_2$O (v/v = 1:1) | PC12 cells imaging |
In this work

Table No. S2: Polarity index order of various solvents.

| Sl. No. | Solvent                        | Polarity Index (P) |
|--------|--------------------------------|--------------------|
| 1.     | Water                          | 9.0                |
| 2.     | Dimethylsulfoxide              | 7.2                |
| 3.     | N,N-dimethylformamide          | 6.4                |
| 4.     | Acetonitrile                   | 5.8                |
| 5.     | Ethanol                        | 5.2                |
| 6.     | Methanol                       | 5.1                |
| 7.     | Acetone                        | 5.1                |
| 8.     | Tetrahydrofuran                | 4.0                |
| 9.     | Dichloromethane                | 3.1                |
| 10.    | Toluene                        | 2.4                |
Figure S1: Unit cell packing of PBSH viewed along the (a) (100) direction; (b) (010) direction; (c) (001) direction.

Figure S2: Supercell (3X3) packing of PBSH viewed along the (a) (100) direction; (b) (010) direction; (c) (001) direction.
Figure S3: Supercell (3X3) packing of PBSH viewed along the (a) (110) direction; (b) (101) direction; (c) (111) direction.

Figure S4: Effect of solvent polarity on emission behavior of receptor PBSH. (a) The absorption spectrum; (b) emission spectrum of PBSH in various solvents.
**Figure S5:** Effect of water content in DMSO solvent on emission behavior of receptor PBSH. (a) The absorption spectrum and (b) emission spectrum of PBSH in various ratios of (Water: DMSO) solvents.

**Figure S6:** Anion selectivity experiment of receptor PBSH. (a) The naked eye image of PBSH+ various anions, (b) The absorption spectrum and (c) emission spectrum in 100% water. Photograph courtesy of ‘Sanay Naha’. Copyright 2020.
Figure S7: Cation selectivity experiment of receptor PBSH. (a) The naked eye image of PBSH+ various anions, (b) emission spectrum of PBSH in 100% water and (c) The absorption spectrum. Photograph courtesy of ‘Sanay Naha’. Copyright 2020.

Figure S8: Quantum mechanical calculation for PBSH ground state, excited state, and most stable optimized structural conformation.
**Theoretical Calculation:**

From the AIE experiment, it was seen that the receptor in water showed a 20 nm red shift in the electronic spectrum and a 100 nm red shift in the emission spectrum in comparison to the spectrum resulted in DMSO. To support these experimental observations, DFT and TD-DFT calculations were performed to determine the energy of molecular orbitals of PBSH and its excited states in water.

Elucidation of conformational optimization, ground state energy states, and excited-state energy states was performed using Gaussian 09 software and obtained results were envisaged using Gauss View 5 GUI. Geometry optimizations were conducted using (B3LYP) functional with basic set 6-31G and the water solvation factor was incorporated. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) for PBSH were generated from these optimized structures (Fig. 8).

From the calculated results, the energy gap between HOMO and LUMO of PBSH had decreased in the excited state in comparison to the ground state from wavelength 371 nm and 435 nm. As the AIE is a ground state phenomenon, the calculated energy gap between HOMO and LUMO of PBSH in the ground state is 371 nm which is well coincided with the observed absorbed wavelength 382 (±11 nm) nm.

![pH: 4 5 6 7 8 9 10 11 12](image)

**Figure S9:** The naked eye image of PBSH under UV light in various pH solutions. Photograph courtesy of ‘Sanay Naha’. Copyright 2020.
**Figure S10:** Quantification of H$_2$S and photochemical yield calculation. (a) The linear fit line of the corresponding normalized fluorescence intensity from incremental addition, (b) emission spectrum of PBSH, PBSH+H$_2$S and rhodamine B in water for calculating area of emission plot and (c) table of calculated quantum yield for the sensing process.

**Calculation of LoD and LoQ:**
The limit of detection and limit of quantification of the analyte by the receptor is calculated using formula (1) and (2) respectively.

\[
y = 3 \frac{S_d}{m} \quad \ldots \ldots \ldots \ldots (1)
\]

\[
y = 10 \frac{S_d}{m} \quad \ldots \ldots \ldots \ldots (2)
\]

Where \( S_d \) is the standard deviation of blank/ or only receptor’s absorption or emission intensity, \( y \) = Limit of detection and ‘\( m \)’ is the slope obtained from the plot of A (absorbance) in y-axis against [G] (concentration of guest during titration) in the x-axis of the incremental titration.

**Lifetime Measurement for H\(_2\)S Detection:**

The emission lifetime decay experiment at (\( \lambda_{em} = 522 \) nm) of ‘PBSH’ was experimented using a 550 nano-LED source, which was used for providing excitation energy. After excitation at 522 nm, the average lifetime of the excited state of ‘PBSH’ showed \( 10.6 \times 10^{-11} \) S. On the subsequent introduction of 10 µM of H\(_2\)S to ‘PBSH’ solution, the average lifetime of the excited state of ‘PBSH’+H\(_2\)S system decreased to \( 6.67 \times 10^{-12} \) S. The fluorescence decay curves of ‘PBSH’ and ‘PBSH’+H\(_2\)S were evaluated using triple-exponential decay theoretical calculation. Surplus decrement of an average life after the addition of H\(_2\)S implies that the excited state of ‘PBSH’ is stable enough to show the emissive property, whereas H\(_2\)S treated ‘PBSH’ can not. Hence, an enormous improvement in the decay rate takes place when H\(_2\)S disturbs ‘PBSH’. There is a platform for the sharp quenching of fluorescence of ‘PBSH’+H\(_2\)S (Fig. 6).

![Lifetime experimental plot for only PBSH and PBSH + H\(_2\)S.](image)

**Figure S11:** Lifetime experimental plot for only PBSH and PBSH + H\(_2\)S.
Figure S12: $^1$H-NMR spectrum in DMSO-d$_6$ of the product obtained after reaction between PBSH and H$_2$S.
Figure S13: $^{13}$C-NMR spectrum of the product obtained after reaction between PBSH and H$_2$S in ACN medium.

Figure S14: Experimental data of MTT assay of PBSH for HeLa cells. The plot shows that even at the concentration of 25 µM of PBSH, cell viability is around (85-88)%.
Figure S15: IR spectrum of PBSH.
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