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A new fluorescent "turn-on" chemodosimeter for the detection of hydrogen sulfide in water and living cells†

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A new fluorescent turn-on probe for the selective detection of hydrogen sulfide in water and living cells based on a 8-hydroxyquinoline fluorophore functionalized with a 2,6-dinitrophenyl ether moiety has been developed.

Hydrogen sulfide has traditionally been considered a highly toxic gas. However, recent studies have demonstrated that H_2S is an endogenously generated gaseous signaling molecule with very potent cytoprotective properties similar to those found for the two well-known endogenous nitric oxide (NO) and carbon monoxide (CO) gasotransmitters.[1] Hydrogen sulfide may interact with downstream proteins by the post-translational cysteine sulfhydration[2] and binding to iron centers,[3] which regulate various physiological processes, including ischemia reperfusion injury,[4] vasodilation,[5] apoptosis,[6] angiogenesis,[7] neuromodulation,[8] inflammation,[9] and insulin signaling.[10] The exact hydrogen sulfide mechanisms of action are still being actively investigated. Some chemical and biochemical catabolic reactions of H_2S have been reported; for example, hydrogen sulfide can react readily with methemoglobin to form sulfhemoglobin, which acts as the metabolic sink for sulfide. As a potential reductant, hydrogen sulfide is likely to be consumed by endogenous oxidant species, such as hydrogen peroxide, superoxide, peroxynitrite, *etc.* This process is potentially significant because it provides a possible mechanism by which sulfide changes the functions of a wide range of cellular proteins and enzymes. Moreover, abnormal levels of hydrogen sulfide are associated with various diseases, such as Alzheimer's[11] and Down syndrome.[12]

Based on these concepts and given the important roles that hydrogen sulfide plays in biological processes, growing interest has been shown in the development of reliable selective and sensitive detection systems for this chemical. Current techniques for sulfide detection, such as colorimetric,[13–15] electrochemical assays,[16] gas chromatography,[17] and metal-induced sulfide precipitation,[18] are often not suitable for monitoring sulfide levels in biological living environments. As an alternative to these methods, the design of fluorogenic probes has recently become an important focal point. In particular, reduction reactions of azide or nitro groups to amines coupled with emission changes,[19–22] and demetallation of macrocyclic Cu(II) complexes,[23,24] have been used for the development of highly sensitive probes for sulfide detection.

Another alternative to the above-mentioned protocols, the thiolysis of 2,4-dinitrophenyl ethers, has also been used as a mechanism in the preparation of HS^- turn-on fluorescent probes. In particular, Lin and co-workers synthesized a NIR fluorescent chemodosimeter for the detection of HS^- in solutions and living cells. This probe is based on a BODIPY dye functionalized with a 2,4-dinitrophenyl ether moiety that is non-fluorescent in PBS–ethanol 9 : 1 v/v mixtures. Addition of HS^- induces the hydrolysis of the ether with subsequent emission enhancement.[25] Very recently, Xu and co-workers synthesized a 1,8-naphthalimide probe also containing a 2,4-dinitrophenyl ether group which was utilized for the fluorescent detection of HS^- anion in PBS–acetonitrile 9 : 1 v/v solutions.[26]

Despite these interesting results, there is still room to improve the behavior of probes based on the HS^--induced hydrolysis of 2,4-dinitrophenyl ethers. For instance, the examples reported by Lin and Xu, displayed sensing features in media with a relatively large percentage (10%) of a non-aqueous solvent and displayed an interesting turn-on fluorescence

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‡ Deceased.

enhancement of *ca.* 42- and 18-fold (upon the addition of 10 eq. of HS^- anion) which could, however, still be improved.

Bearing in mind our interest in developing molecular chemosensors for anions of environmental and biological interest,[27] we report herein the synthesis and sensing features of fluorescent probe **1** (see Scheme 1) for hydrogen sulfide detection in water and living cells. Probe **1** was readily prepared, with a good yield and in a one-step procedure, by a nucleophilic aromatic substitution of 1-fluoro-2,4-dinitrobenzene with 8-hydroxyquinoline (see ESI† for synthetic details and characterization).

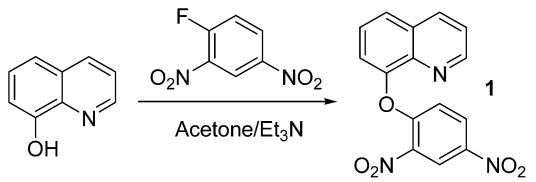
The emission behavior of probe **1** in the presence of 10 equivalents of selected anions (HS^-, OH^-, F^-, Cl^-, Br^-, I^-, N_3^-, CN^-, SCN^-, AcO^-, CO_3^{2-}, PO_4^{3-}), oxidants (H_2O_2), bio-thiols (Cys, Me-Cys, Hcy, GSH, lipoic acid) and reducing agents (SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$) was evaluated. HEPES (7 mM, pH 7.4)–DMSO 99 : 1 v/v solutions of **1** are essentially non fluorescent (upon excitation at 450 nm). Of all the chemicals tested, only the addition of HS^- induced the appearance of a strong emission band at 514 nm (λ_{ex} = 450 nm) with an impressive 345-fold enhancement upon the addition of 10 equivalents of the anion (see Fig. 1). Besides, time-dependent studies have shown that a maximum change in emission was observed after *ca.* 50 min upon the addition of HS^- (see ESI†). Moreover, fluorescence studies at different pHs in water–DMSO 99 : 1 v/v solutions of **1** in the absence and presence of 5 μM of HS^- anion have demonstrated that the probe can be used in a wide range of pH values (from 2 to 9) with no significant reduction in its sensing behavior (see ESI†).

After assessing the highly selective turn-on response of probe **1** to hydrogen sulfide, the sensitivity of the probe was assessed by studies on the emission modulation of **1** in HEPES–DMSO 99 : 1 v/v upon the addition of HS^-. Increasing the HS^- concentration (up to 100 eq.) resulted in the progressive growth of fluorescence intensity (see Fig. 2). From typical titration profiles (see ESI†), a remarkable limit of detection (LOD) of 60 nM was determined. **1** showed remarkably low sensitivity below the hydrogen sulfide concentration required to elicit physiological responses (10–1000 μM).[28]

The selectivity toward HS^- was ascribed to the HS^--induced hydrolysis of the 2,4-dinitrophenyl ether moiety, which yielded the highly fluorescent 8-hydroxyquinoline group.[29] This sensing mechanism was confirmed by isolating the reaction product between **1** and the HS^- anion, which was unequivocally characterized as 8-hydroxyquinoline.

The selective emission enhancement of **1** in the presence of HS^- and the poor response observed upon the addition of biothiols (GSH, Cys and Hcy) strongly suggest that the probe can be used for HS^- imaging in living cells. Based on these observations, the cytotoxicity of **1** was first evaluated. HeLa cells were treated with **1** at different concentrations over a 24-hour period and cell viability was determined by a WST-1 assay. The obtained results are shown in Fig. 3. As seen, probe **1** is essentially non-toxic in the range of concentrations tested (5–50 μM).

In a second step, and in order to verify the feasibility of the developed probe to detect HS^- in highly competitive environments, we prospectively used **1** for the fluorescence imaging of sulfide in living cells. In a typical experiment, HeLa cells were incubated in DMEM supplemented with 10% fetal bovine serum. To conduct fluorescence microscopy studies, HeLa cells were seeded in 24 mm glass coverslips in 6-well plates and were allowed to settle for 24 h. Cells were treated with **1** in DMSO (1%) at a final concentration of 30 μM. After 20 minutes, the medium was removed and solutions of different concentrations of NaHS in PBS were added (0, 100, 200 and 500 μM) and cells were incubated for another 10-minute period. The results are



Scheme 1 Synthesis of chemodosimeter **1**.

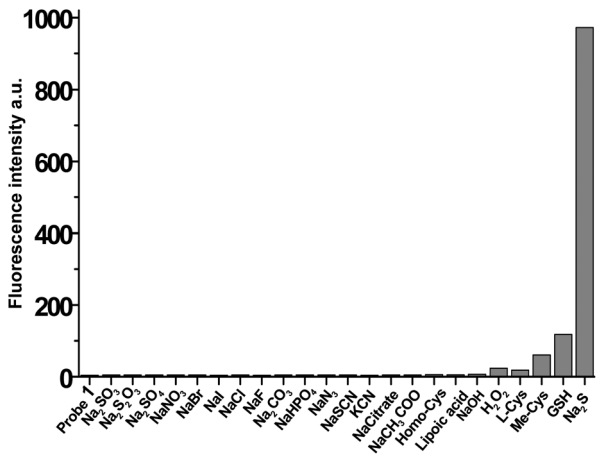


Fig. 1 Fluorescent intensity at 514 nm (excitation at 450 nm) of chemodosimeter **1** (5.0×10^{-5} M) in HEPES (7 mM, pH 7.4)–DMSO 99 : 1 v/v upon the addition of 10 equivalents of selected anions, biothiols, oxidants and reducing agents after 50 min of the addition.

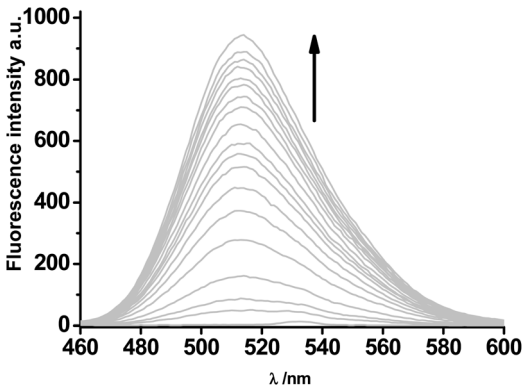


Fig. 2 Emission spectra of probe **1** (5.0×10^{-5} M) in HEPES (7 mM, pH 7.4)–DMSO 99 : 1 v/v (excitation at 450 nm) upon the addition of increasing quantities of HS^- anion (from 0 to 10 equivalents) and after 50 minutes of the addition.

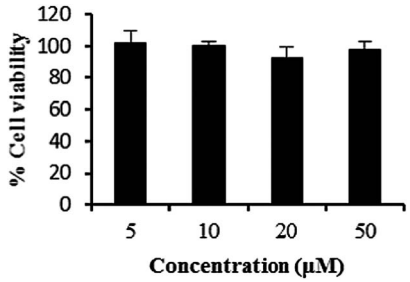


Fig. 3 Cell viability test of different concentrations of probe **1** at 24 h in HeLa cells by a WST-1 assay.

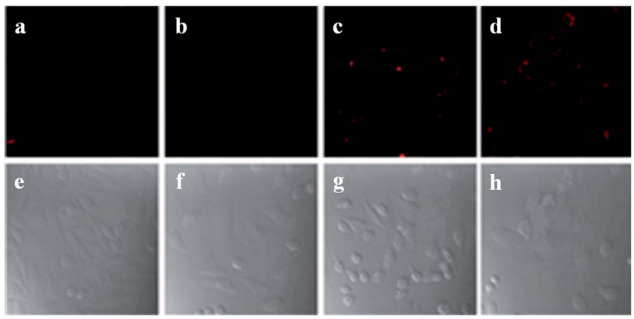


Fig. 4 Detection of HS^- levels in living cells using probe **1**. HeLa cells were incubated with **1** (30 μM) for 30 min at 37 °C in DMEM. Transmitted light and fluorescence images were captured for (a) Hela cells, (b) Hela cells incubated with **1**, and Hela cells incubated with **1** in the presence of HS^- at concentrations of (c) 200 μM and (d) 500 μM. The range of excitation was 330–380 nm and emission was monitored for wavelengths exceeding 420 nm.

shown in Fig. 4. The control experiment (HeLa cells without probe **1**) and the cells incubated with **1** showed no fluorescence, whereas a marked enhancement in intracellular emission was observed in the HS^--treated cells, clearly indicating the possible use of **1** to detect hydrogen sulfide in complex media.

In summary, here we report the synthesis and sensing features of new fluorescent probe **1**. Probe **1** is easy to prepare and is able to selectively detect the HS^- anion in HEPES–DMSO 99 : 1 v/v solutions *via* remarkable turn-on emission at 514 nm. The observed fluorescence enhancement is ascribed to a selective HS^--induced hydrolysis of a 2,4-dinitrophenyl moiety in **1**, which gives a free 8-hydroxyquinoline fluorophore. The probe can selectively and sensitively detect HS^- anion in water over other anions, biothiols and common oxidants. Moreover, real-time fluorescence imaging measurements have confirmed that probe **1** can be used to detect intracellular HS^- at micromolar concentrations. Similar designs using other available dyes for the straightforward signaling of HS^- in cells are currently being investigated in our laboratory.

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