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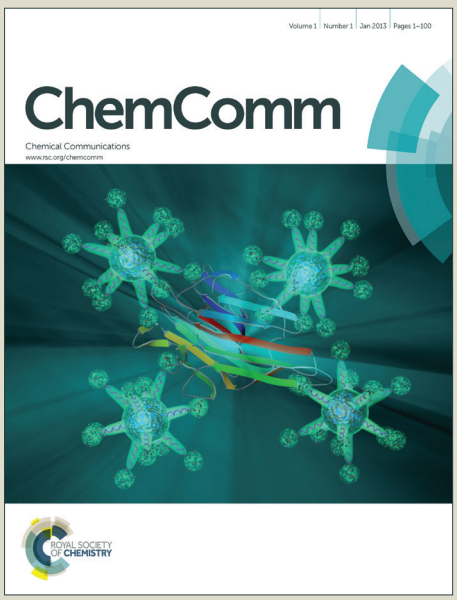

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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

A Twisted Intramolecular Charge Transfer Probe for Rapid and Specific Detection of Trace Biological SO_2 Derivatives and Bio-imaging Application

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

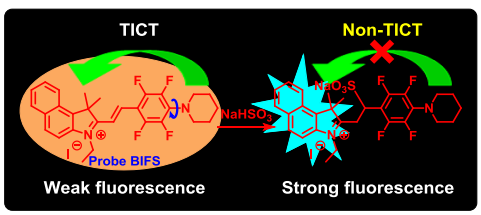
DOI: 10.1039/b000000x

We reported a reactive probe for HSO_3^-, which showed a colorimetric and ratiometric fluorescence response to HSO_3^- with fast response ($t_{1/2}$ = 20 s), good specificity and low detection limit (3.0 nM). The probe was cell membrane permeable and successfully used for visualizing trace SO_2 derivatives in living cells.

Sulfur dioxide (SO_2) is one of the major atmospheric pollutants. Long term exposure to SO_2 not only caused some respiratory responses[1] but also induced lung cancer, cardiovascular diseases[2] and neurological disorders including migraine headaches, stroke and brain cancer.[3] Inhaled SO_2 is generally hydrated to produce sulfurous acid in the respiratory tract and subsequently form its derivatives sulfite and bisulfite.[4] Sodium sulfite is a common food stuff that can preserve food and beverages from oxidation, however, excess amount of sulfite can cause asthma and other allergic reactions in some individual.[5] Thus, it is of great importance to develop an effective method for detection of trace SO_2 derivatives.

Fluorescent probe is a powerful tool for detection and visualization of some biological species including intracellular pH,[6] HClO[7] and H_2O_2[8] due to its non-invasiveness, high sensitivity, high temporal and spatial resolution.[9] Recently, a few fluorescent probes for the detection and visualization of sulfite in living cells have been developed based on nucleophilic reaction with the aldehyde,[10] the selective deprotection of levulinate,[11] Michael-type additions[12] and coordinative interactions.[13] However, most of these probes have some drawbacks such as unsatisfactory detection limit and long response time (5 min to 10 h). Besides, these probes usually suffer from the interference from biothiols, proteases or esterases, which limits their application for bio-imaging *in vitro* and *in vivo*.[14] Recently, some novel fluorescent probe for HSO_3^- has been developed.[15-17] Yu et al. reported a coumarin TCF-based probe for SO_2 derivatives, which displayed colorimetric and ratiometric fluorescence response to SO_2 with low detection limit.[15] Unfortunately, the fluorescence of the probe was highly dependent upon the pH value of solution. Guo et al judiciously designed a ratiometric fluorescent probe for SO_2 derivatives based on a coumarin-hemicynine dye.[16] However, this probe displayed unsatisfactory detection limit (0.38 μM). Thus, it is highly desired to develop a specific, sensitive, fast response and water soluble fluorescent probe for the sensing and bio-imaging of SO_2 derivatives.

In this work, a novel fluorescence probe (**BIFS**) for SO_2 derivatives was designed on the basis of nucleophilic addition reaction. Pentafluorobenzaldehyde, a strong electron-withdrawing group, was condensed with 1*H*-benzo[*e*]indolium in the presence of piperidine/HAc to extend the π-conjugation and decrease the electron density of alkenyl group, so that the alkenyl group was easily attacked by some nucleophilic reagents like HSO_3^-. 1*H*-benzo[*e*]indolium was used as a fluorophore to construct a ratiometric fluorescent probe and improve the water-solubility. We envisioned that the addition reaction of HSO_3^- toward alkenyl group would happen at ambient temperature, as was shown in Scheme 1. Consequently, significant changes in the UV-vis absorption spectra and fluorescence spectra were observed, allowing colorimetric and fluorogenic detection of HSO_3^-.



Scheme 1 The proposed sensing mechanism of probe for HSO_3^-.

As expected, **BIFS** has good solubility in PBS or glycerol. In 50 mM PBS solution, **BIFS** displayed two weak fluorescence bands centered at 465 nm and 592 nm, respectively. With the increase of solvent viscosity (performed in glycerol/MeOH mixture solvent), the band at 592 nm gradually increased, while the band located at 465 nm remarkably decreased (shown in Fig. S1, ESI†), which was due to strong twisted intramolecular charge transfer (TICT) effect in **BIFS**. The piperidinyl is an electron-donating group, while the 1*H*-benzo[*e*]indolium and tetrafluorobenzene are strong electron-withdrawing groups. In the exited state, very strong charge transfer from piperidinyl group to the conjugation of 1*H*-benzo[*e*]indolium occurred, and the piperidinyl group twisted out of the planar of the conjugation. Therefore, this chromorphore showed TICT effect. In 50 mM PBS solution containing 40% glycerol, the peak located at 465 nm completely diminished and the fluorescence intensity of **BIFS** at 592 nm was moderate. Hence, spectra titrations of **BIFS** with HSO_3^- were performed in glycerol/PBS solution (4/6, pH 7.40).

As shown in Fig. 1, free probe displayed a broad absorption band centered at 499 nm. Upon addition of increasing amount of HSO_3^-, the maximum absorption band centered at 499 nm decreased gradually and a new absorption band at 322 nm emerged with a well-defined isosbestic point at 340 nm, which indicated that a new compound has been formed upon treatment of the probe with HSO_3^-. By examining the UV-vis absorption spectra and fluorescence spectra, we found that the spectra of **BIFS**-$NaHSO_3$ were almost the same with that of 1*H*-benzo[*e*]indolium (shown in Fig. S2, ESI†). Thus, we speculated that HSO_3^- might add to the alkenyl by nucleophilic addition and produce a 1*H*-benzo[*e*]indolium group. When the concentration of HSO_3^- reached to 1.0 equiv. with respect to the probe, these changes in the absorption spectra were found to reach a plateau, and the color of **BIFS** solution changed from orange to colorless, allowing colorimetric detection of HSO_3^- by naked eyes (Fig. 1a. Insert). The absorbance at 499 nm decreased linearly (R = 0.9993) with concentration of HSO_3^- from 0 to 7 μM (see Fig. S3, ESI†).

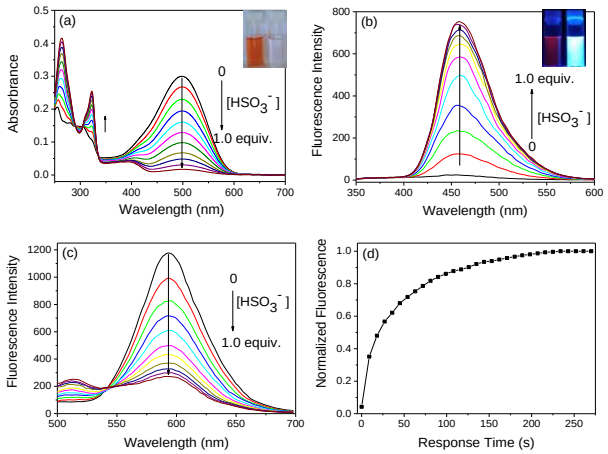


Fig. 1 UV-vis absorption spectra (a) and fluorescence spectra (b-c) changes of **BIFS** (10 μM in glycerol/PBS solution = 4/6, pH 7.40) upon addition of increasing amount of HSO_3^- (0 – 10 μM). Each spectrum was recorded after 3 min. Insert (a): The color of **BIFS** (10 μM) changed from orange to colorless. Insert (b): Fluorescence images of probe **BIFS** (10 μM) before and after addition of 1.0 equiv. of HSO_3^-. For (b) λ_{ex} = 322 nm, slit: 2.5/2.5 nm. For (c) λ_{ex} = 470 nm, slit: 5/10 nm. (d) Time course fluorescence responses of the probe (10 μM) to HSO_3^- (1.0 equiv.) in glycerol/PBS solution = 4/6 (pH 7.40). λ_{ex} = 322 nm, Slit: 2.5/2.5 nm.

Fig. 1d showed the time-dependent fluorescence intensity changes of **BIFS** in the presence of 1.0 equiv. of HSO_3^-. The fluorescence intensity has a great enhancement and nearly reached to a plateau within 3 min, suggesting that **BIFS** could serve as a "fast response" fluorescent probe for HSO_3^-. The pH-dependent fluorescence responses of the probe to HSO_3^- were also investigated, as was shown in Fig. S5 (ESI†). The fluorescence intensity of the probe kept constant from pH 2.5 to 8.5, suggesting this probe was very stable at a wide range of pH values. Upon addition of 1.0 equiv. of HSO_3^-, the fluorescence of **BIFS** increased drastically at the pH value ranging from 6.5 to 8.5, which was compatible with the pK_{a2} of HSO_3^- (pK_{a2} = 7.20).

The fluorescence spectra titrations were performed in glycerol/PBS solution (4/6, pH 7.40), as was shown in Fig. 1b and Fig. 1c. Upon addition of increasing amount of HSO_3^-, fluorescence band at 592 nm decreased remarkably, while the fluorescence intensity of **BIFS** at 465 nm increased progressively until the concentration of HSO_3^- reached to 1.0 equiv. with respect to the probe. As a result, the fluorescence changed from dark red to strong blue (Fig. 1b insert). It was noteworthy that a maximal fluorescence change was obtained within 3 min in the presence of 1.0 equiv. of HSO_3^-, which was impressive compared with those of the reported HSO_3^- probes (sometimes required 200 equiv.). The ratios of fluorescence intensity at 465 nm and 592 nm (I_{465}/I_{592}) showed a good linear relationship (R = 0.9914) with the concentration of HSO_3^- ranging from 0 to 8 μM (Fig. S4, ESI†), and the detection limit for HSO_3^- was calculated to be 3.0 nM based on signal-to-noise ratio (S/N = 3), which was much lower than the threshold levels of HSO_3^- in food and medicine (< 30 mg kg^{-1}).

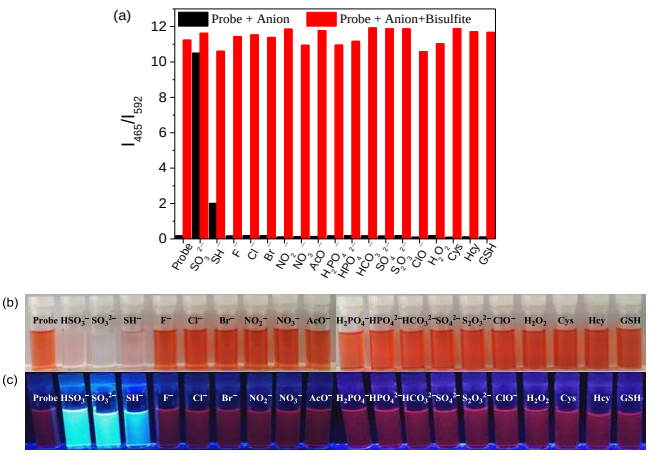


Fig. 2 (a) Fluorescence intensity changes of **BIFS** (10 μM) in the presence of various anions (HSO_3^- or SO_3^{2-}: 1.0 equiv., others: 5.0 equiv.) in glycerol/PBS solution = 4/6 (pH 7.40), λ_{ex} = 322 nm, Slit: 2.5/2.5 nm, λ_{ex} = 470 nm, slit: 5/10 nm; (b) Color changes and (c) Fluorescence photographs of probe **BIFS** (10 μM) in the presence of HSO_3^- (1.0 equiv.) and other anions (5.0 equiv.) in glycerol/PBS solution = 4/6 (pH 7.40).

The selectivity of **BIFS** for HSO_3^- was also examined in glycerol/PBS solution = 4/6 (pH 7.40). As shown in Fig. 2, the addition of 5.0 equiv. of representative anions (F^-, Cl^-, Br^-, NO_2^-, NO_3^-, CH_3COO^-, $H_2PO_4^-$, HPO_4^{2-}, HCO_3^-, SO_4^{2-} and $S_2O_3^{2-}$) and reactive oxygen species (ClO^- and H_2O_2) did not induce any changes in color and fluorescence of the probe. Beside, this probe exhibited no response to some nucleophilic reagents like Cys, Hcy and GSH. By contrast, a significant color change and a great fluorescence enhancement of the probe were observed in the presence of 1.0 equiv. of HSO_3^- or SO_3^{2-}, which could be observed by the naked eyes (shown in Fig. 2b and Fig. 2c). Although the probe also displayed fluorescence response to HS^- due to the nucleophilic addition to C=N bond, the fluorescence intensity was much weaker than that of HSO_3^-. These results suggested that the probe has excellent selectivity for HSO_3^- or SO_3^{2-} over other anions and biological species. Therefore, the probe has potential applications for HSO_3^- detection in complex biological environments.

To verify the proposed sensing mechanism depicted in Scheme 1, ^1H NMR titration of **BIFS** with $NaHSO_3$ was performed. As shown in Fig. 3, the proton signals of 1*H*-benzo[*e*] indolium were clearly identified, and the chemical shifts at 8.12

ppm and 7.60 ppm were assigned to Ha and Hb in **BIFS**, respectively. Upon addition of 1.0 equiv. of $NaHSO_3$, the protons signals of Ha and Hb disappeared and two peaks located at 4.72 and 4.60 ppm emerged. For H7, no significant change in the chemical shift was observed, indicating that bisulfite did not attack C=N bond of the 1*H*-benzo[*e*]indolium. Moreover, the formation of **BIFS**-$NaHSO_3$ adduct was confirmed by high-resolution mass spectroscopy (Fig. S6, ESI†), where a dominant peak at m/z 585.1859 (calcd 585.1856) was corresponding to [**BIFS** + $NaHSO_3$]$^+$. Based on these facts, we concluded that the alkenyl group underwent nucleophilic addition by $NaHSO_3^-$.

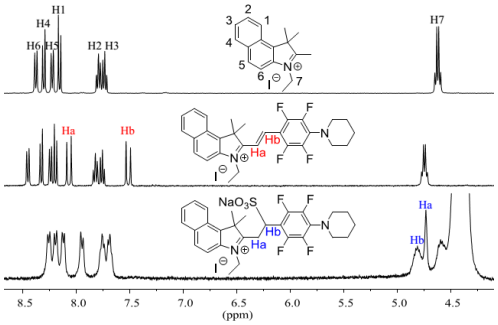


Fig. 3 Partial ^{1}H NMR spectra of 1*H*-benzo[*e*]indolium in DMSO-d_6, **BIFS** in DMSO-d_6 and **BIFS**-$NaHSO_3$ in DMSO-d_6-D_2O.

Furthermore, we explored the potential application of **BIFS** for sensing HSO_3^- in living cells. A549 cells were co-incubated with **BIFS** (10 μM) and nucleus-specific staining probe SYTO 9 for 30 min, and then they were washed with PBS for three times. As shown in Fig. 4a, A 549 cells were still alive and only showed green fluorescence in the nucleus (shown in Fig. 4b). When the cells were further treated with 10 μM $NaHSO_3$ for 10 min, a clear cell profile with strong blue fluorescence was observed (shown in Fig. 4c), which indicated that **BIFS** could penetrated into the cells and been consumed by HSO_3^- to produce some products with blue fluorescence. Cell staining results indicated that the probe was cell membrane permeable and could be used for the detection of trace bisulfite in living cells.

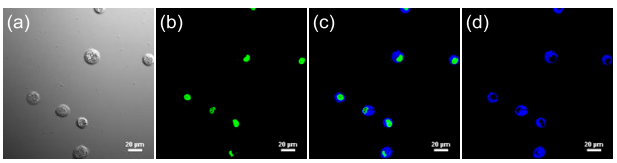


Fig. 4 (a) Bright field image of A549 cells; (b) Fluorescence imaging of A549 cells incubated with probe **BIFS** (10 μM) and SYTO 9 (1 μM) for 30 min; (c) Fluorescence imaging of A 549 cells incubated with **BIFS** (10 μM) and SYTO 9 (1 μM) for 30 min, and further treated with 10 μM $NaHSO_3$ for 10 min; (d) Fluorescence images of (c) from blue channel. Scale bar: 20 μm.

In summary, we have successfully developed a novel fluorescent probe based on 1*H*-benzo[*e*]indolium, which displayed a noticeable colorimetric and ratiometric fluorescence response to HSO_3^- on the basis of nucleophilic addition reaction. The probe exhibited some advantages such as quantitative reaction with HSO_3^-, fast response, high specificity, and extremely low detection limit (3.0 nM), which were impressive compared with those of the reported HSO_3^- probes. Cell staining results indicated that the probe could be used for visualizing trace bisulfite in living cells.

This work was financially supported by NSFC (No.21203138, 31371750, 21103123), the Natural Science Foundation of Tianjin (13JCQNJC05300), and Hubei Provincial Educational Department (D20132702).

Notes and references

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† Electronic Supplementary Information (ESI) available: [Synthetic procedure and characterization data, spectroscopic properties]. See DOI: 10.1039/b000000x/

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