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# A FRET based ratiometric fluorescent probe for detection of sulfite in food†

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A new fluorophore pyrido[1,2-*a*]benzimidazole based ratiometric fluorescent probe for the selective detection of sulfite ions in water was investigated. It shows large (pseudo) Stokes shifts (260 nm), high FRET efficiency, high selectivity and sensitivity. A distinct color change from red to colorless was observed and importantly, it proves to be a convenient and efficient tool to detect the sulfite levels in sugar samples.



## Introduction

Sulfites are widely applied in the production of foods, pharmaceutical products and beverages as antimicrobial agents, antioxidants and enzyme inhibitors.[1] However, the levels of sulfites have been strictly controlled because of the harmful effects towards the human body, such as allergic reactions and food intolerance symptoms.[2–4] Therefore, for food quality control and quality assurance, it is important to develop a low-cost, selective and sensitive method for sulfite determination.

Compared with several traditional methods such as electrochemistry and chromatography, fluorescent probes are particularly attractive due to their simplicity, high selectivity and high sensitivity.[5] Many fluorescent probes are reported to detect sulfites based on the changes of emission intensity.[6] However, the signal output of these intensity-based fluorescent probes can be affected by instrumental factors, environmental effects and probe concentrations.[7] Therefore, ratiometric fluorescent probes which are independent of the environmental effects are more desirable.[8–11] Also, they have large Stokes shifts which can prevent serious self-quenching and fluorescence detection errors. So far, only a few well-behaved ratiometric probes for sulfite have been reported.[12–26] Herein, a new FRET based ratiometric fluorescent probe was designed (Scheme 1). A pyrido[1,2-*a*]benzimidazole fluorophore, due to its favorable photophysical properties, was selected as the donor. While a hemicyanine fluorophore of which the absorption band overlapped well the emission band of the donor was chosen as the acceptor (Fig. S1†).

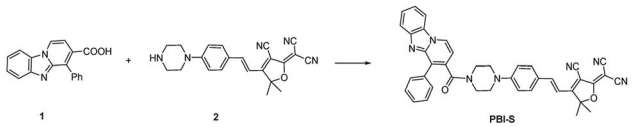
## Experimental

### Reagents and instrumentation

RF-5301PC luminescence spectrophotometer (Shimadzu) and UV-2600 spectrometer (Shimadzu) were used to measure the fluorescence and UV-vis absorption spectra, respectively. NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer (in $CDCl_3$, TMS as an internal standard). All commercial reagents and solvents were used without further purification.

### Preparation of the probe PBI-S

Compound **1** and compound **2** were synthesized according to the literature.[27,28] To a solution of compound **1** (580 mg, 2 mmol) in 20 mL $CH_2Cl_2$ was added DMAP (60 mg, 0.4 mmol) and EDC (576 mg, 3.0 mmol). Then, compound **2** (744 mg, 12 mmol) was added. The mixture was stirred for 12 h at room temperature. After removal of the solvent under reduced pressure, the crude probe was obtained, which was purified flash silica gel chromatography ($CH_3OH$ : $CH_2Cl_2$ = 1 : 100) to afford pure probe **PBI-S** (purity: 98.6%, 456 mg, yield: 71%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.55 (d, $J$ = 8.0 Hz, 1H), 8.00 (d, $J$ = 8.0 Hz, 1H), 7.96 (d, $J$ = 8.0 Hz, 1H), 7.87 (d, $J$ = 8.0 Hz, 2H), 7.54 (m, 6H), 7.44 (m, 2H), 6.95 (d, $J$ = 8.0 Hz, 1H), 6.79 (d, $J$ = 16.0 Hz, 1H), 6.74 (d, $J$ = 8.0 Hz, 2H), 3.92 (m, 1H), 3.52 (m, 2H), 3.24 (m,




Scheme 1 Synthesis of probe PBI-S.

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† Electronic supplementary information (ESI) available: $^1$H NMR, $^{13}$C NMR and MS spectra of probe, and additional cell images. See DOI: 10.1039/c8ra08967a

‡ Equal contribution.

1H), 3.12 (m, 2H), 2.98 (m, 1H), 2.18 (m, 1H), 1.75 (s, 6H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 175.9, 174.2, 167.8, 153.2, 147.5, 146.7, 145.0, 131.7, 130.1, 129.4, 128.7, 126.1, 125.0, 124.2, 122.0, 120.6, 114.4, 111.5, 111.0, 110.7, 110.6, 109.9, 97.2, 96.3, 46.5, 46.2, 45.0, 41.0, 26.7. HRMS: 642.2590 ([M + H]$^+$); calcd for $C_{40}H_{32}N_7O_2$: 642.2617.

## Results and discussion

**PBI-S** was synthesized by the classical condensation of compound **1** with compound **2** in one step in good yield (Scheme 1).

The solvent and effect of water content on fluorescence spectra of the probe was investigated firstly (Fig. S2 and S3†). Considering its better peak shapes, we chose the solvent ratio (DMF/PBS = 3 : 7) as the *vitro* test solvent system.

The absorption band of **PBI-S**, attributing to hemicyanine and pyrido[1,2-*a*]benzimidazole moiety, centered at 556 nm and 334 nm. When sodium sulfite was added gradually to the solution of **PBI-S** (DMF/PBS = 3 : 7), the absorption peak at 334 nm increased while the peak at 556 nm decreased (Fig. 1). Concomitantly, the solution turned from red to colorless in color, which proves that **PBI-S** could act as a "naked-eye" probe for $HSO_3^-$. It was calculated that the ratio of the absorbance at 334 and 556 nm displayed an over 300-fold enhancement, which indicates that **PBI-S** is capable for detecting sulfite by UV-vis absorption.

Next, the fluorescence titration was conducted. The free **PBI-S** displayed two obvious fluorescence bands at 465 nm and 640 nm, which attributed to pyrido[1,2-*a*]benzimidazole and hemicyanine unit, respectively. Upon addition of $HSO_3^-$ incrementally, fluorescence emission band at 465 nm increased gradually while the fluorescence at 640 nm decreased (Fig. 2), which implies that the acceptor moiety was destroyed by the reaction of the probe with $HSO_3^-$ and the FRET between the donor and the acceptor was switched off.

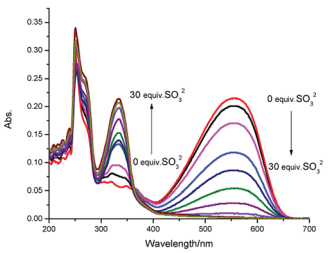



Fig. 1 Absorption spectra of **PBI-S** (5 μM) upon addition of $HSO_3^-$ (150 μM) in DMF–PBS buffer solution (3/7, v/v, pH = 7.20).

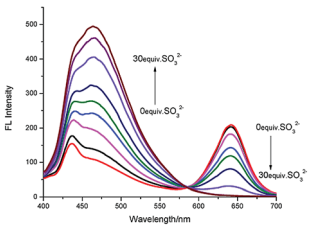



Fig. 2 Fluorescence spectra of **PBI-S** (5 μM) upon addition of $HSO_3^-$ (150 μM) in DMF–PBS buffer solution (3/7, v/v, pH = 7.20), ($\lambda_{ex}$ = 380 nm, slit = 10 nm/10 nm).

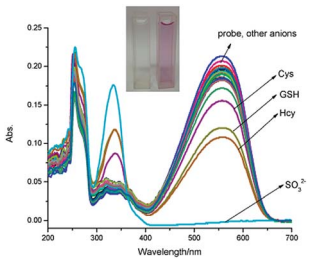



Fig. 3 UV-vis spectral changes of probe **PBI-S** (5 μM) in the presence of various analytes (150 μM).

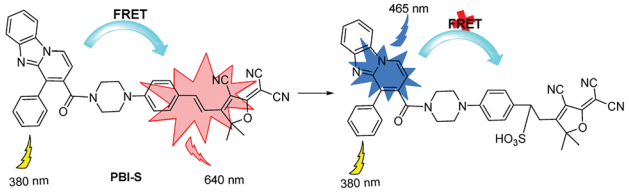
The ratio of emission intensities at 465 and 640 nm ($I_{465}/I_{640}$) changed from 0.52 in the absence of $HSO_3^-$ to 123.75, a 238-fold variation in the ratios. Furthermore, the emission ratio ($I_{465}/I_{640}$) was found to increase linearly with the $HSO_3^-$ concentration changed from 3 to 9 μM (Fig. S4†). The detection limit was determined to be 62 nM which is calculated by $3\sigma/k$ ("$\sigma$" is the standard deviation of 15 blank measurements, "$k$" is the slope of the fitting line).

Then the selectivity of **PBI-S** toward sulfite over other relevant anions was conducted and it was observed that only by the addition of sulfite could the absorption and fluorescence change. While with other anions, no change in the UV and FL spectra was noted (Fig. 3 and S5†). However, some spectral changes in GSH, Hcy and Cys were observed due to nucleophilic property of the SH group in them.

Furthermore, we studied the responses of **PBI-S** to the sulfite in the presence of the other competitive anions (including GSH, Hcy and Cys). It was observed that when the other anions were added subsequently, the fluorescence emission ratios ($I_{465}/I_{640}$) were not influenced (Fig. S6†).

As shown in Fig. S7,† the probe was stable and can function well over a wide range of pH (6.0–10.0). Time-dependent fluorescence response to $SO_3^{2-}$ was also carried out (Fig. S8†). The reaction could be completed within 2 min (50 eq. $SO_3^{2-}$). Such a rapid response is appealing for real-time detection.

The supposed mechanism is shown in Scheme 2. For the probe alone, FRET between the pyrido[1,2-*a*]benzimidazole and hemicyanine moiety resulted in red emission from hemicyanine. Upon the addition of $HSO_3^-$, the energy transfer from pyrido[1,2-*a*]benzimidazole donor to hemicyanine acceptor was




Scheme 2 Proposed mechanism of probe.

Table 1 Concentration of the sulfite in sugar samples[a]

| Sample | $HSO_3^-$ level ± SD (μmol $L^{-1}$) | Added (μmol $L^{-1}$) | Found ± SD (μmol $L^{-1}$) | Recovery ± SD (%) |
|---|---|---|---|---|
| Crystal sugar | 0.63 ± 0.048 | 0.5 | 1.07 ± 0.031 | 94.69 ± 2.6% |
| | | 1 | 1.58 ± 0.036 | 96.93 ± 2.3% |
| Granulated sugar | 1.21 ± 0.049 | 0.5 | 1.80 ± 0.026 | 105.26 ± 0.51% |
| | | 1 | 2.04 ± 0.030 | 92.31 ± 0.03% |
| Soft sugar | 0.76 ± 0.045 | 0.5 | 1.28 ± 0.0140 | 101.16 ± 3.3% |
| | | 1 | 1.70 ± 0.0127 | 96.59 ± 3.5% |

[a] The results were presented as means ± SE with replicates $n = 5$.

interrupted because of the breakage of C=C bond by the addition reaction with $HSO_3^-$. The MS spectra of the reaction product also supported the deduction (calcd for $C_{40}H_{34}N_7O_5S$: 723.2342, found 723.2334, Fig. S9 and S10†).

Finally, **PBI-S** was applied to detect sulfite in sample analysis of commercially purchased granulated sugar, crystal sugar, and soft sugar. The sugar (5.0 g) was dissolved in deionized water and then was diluted to 10 mL. To the solution of the probe **PBI-S** (5 μM) in the DMF/PBS buffer (DMF/PBS = 3 : 7, pH 7.20, 10 mM), aliquots of the sugar solution were added. As shown in Table 1, the $HSO_3^-$ concentration in sugar was successfully determined by the probe **PBI-S** with good recovery.

It was calculated that the $HSO_3^-$ concentration in the three sugar samples were 0.79, 1.52, and 0.96 mg $kg^{-1}$, respectively. A traditional titration method was carried out to test the accuracy of this method and Table S1† showed that results of the two methods were equivalent.

## Conclusions

In summary, we have developed a new fluorophore pyrido[1,2-*a*] benzimidazole based ratiometric fluorescent probe for selective detection of sulfite ion in water. It shows large (pseudo) Stokes shifts (260 nm), high FRET efficiency, high selectivity and sensitivity. A distinct color change from red to colorless was observed and importantly, it proves to be a convenient and efficient tool to detect the sulfite levels in sugar samples.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

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