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Colorimetric and ratiometric fluorescent detection of bisulfite by a new HBT-hemicyanine hybrid

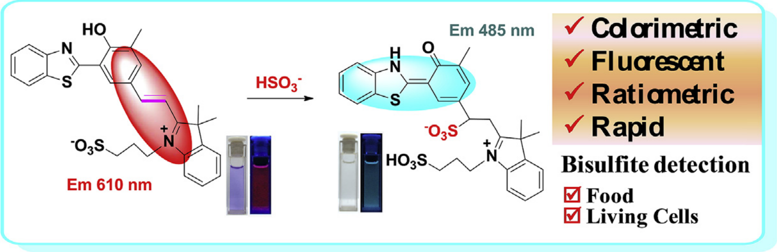
Haiyan Zhang [a, 1], Zijun Huang [b, 1], Guoqiang Feng [b, *]

[a] School of Chemical and Environmental Engineering, Wuhan Polytechnic University, 68 Xuefu South Road, Wuhan 430023, People's Republic of China
[b] Key Laboratory of Pesticide and Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, 152 Luoyu Road, Wuhan 430079, People's Republic of China

HIGHLIGHTS

- A new colorimetric and ratiometric fluorescent probe for bisulfite was reported.
- This probe is based on a novel HBT-hemicyanine hybrid and shows high selectivity and sensitivity for bisulfite.
- The probe can be used to quantitatively detect bisulfite in real food samples.
- Fluorescent imaging of bisulfite in living cells by this probe was also successfully applied.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel HBT-hemicyanine hybrid was prepared. This hybrid not only displays a large red-shifted ($\Delta\lambda = 125$ nm) emission compared to the well known ESIPT dye HBT, but also can be used as a new probe for rapid, colorimetric and ratiometric fluorescent detection of bisulfite with high selectivity and sensitivity in aqueous solution. The detection limit of this probe for HSO_3^- was calculated to be about 56 nM with a linear range of 0–25 μM. The potential application of this probe was exampled by detection of bisulfite in real food samples and living cells. Overall, this work not only provided a new ratiometric sensing platform, but also provided a new promising colorimetric and ratiometric fluorescent probe for bisulfite.

1. Introduction

The development of fluorescent probes for chemically and biologically important species has attracted continuing attention due to the simplicity, high sensitivity and non-invasiveness of fluorescence detection [1–3]. Among them, ratiometric fluorescent probes are more attractive over intensity-based probes because they allow the measurement of emission intensities at two different wavelengths, which provides a built-in correction for environmental effects and can also increase the dynamic range of fluorescence measurement [4]. Accordingly, much effort has been given to the development of ratiometric fluorescent probes in recent years. In general, ratiometric probes can be designed to function by the

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* Corresponding author.
E-mail address: gf256@mail.ccnu.edu.cn (G. Feng).
[1] These two authors contributed equally to this paper.

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following two mechanisms: fluorescence resonance energy transfer (FRET) and intramolecular charge transfer (ICT) [5–7].

The tautomerization of excited state intramolecular proton transfer (ESIPT) dyes has also been used for the development of ratiometric fluorescent probes, and such probes generally have the merit of large Stokes shift [4,7]. 2-(2′-Hydroxyphenyl)benzothiazole (HBT) and its derivatives are the most well-known ESIPT dyes, and variations in the relative intensities of their phototautomers (the keto and enol forms, Scheme 1) were often ingeniously applied for ratiometric fluorescent probes construction, for example, via a phenolic hydroxyl (–OH) protection/deprotection strategy [8–12]. However, the emission of HBT is generally limited in the blue and green region, and rare of the HBT-based probes can be found to have an emission exceed 600 nm in the literature, which may restrict their potential applications. Therefore, it is of great value to modify the structure of HBT and make the emission wavelength into the red or far-red region [13,14]. A straightforward method to access red-shifted emission is to extend the π-conjugation of the ESIPT fluorophore. However, so far, only a few fluorescent probes based on HBT derivatives with a large π-conjugation framework have been reported [15–19].

Due to the excellent spectroscopic properties, indolium derivatives including hybrid indolium dyes have been widely used as cyanine or hemicyanine dyes with long emission wavelengths [20–23]. For example, hybrid coumarin–hemicyanine dyes can show significant red-shifted emissions over coumarin, and these made them good platforms for construction of ratiometric fluorescent probes via the strategy of breaking the π-conjugation system [24–26]. However, to the best of our knowledge, the hybrid HBT-hemicyanine dyes remain largely unexploited, and such design concept has rarely been employed in the construction of ratiometric fluorescent probes.

Herein, we report a novel HBT-hemicyanine hybrid (**HH** in Scheme 1), which was designed to conjugate HBT with the indolium moiety via an ethylene bridge. This hybrid not only can be easily prepared, but also displays red emission at 610 nm after extending the π-conjugation framework of HBT by the hemicyanine moiety. More importantly, this hybrid can be used as a new ratiometric fluorescent probe for rapid, colorimetric and ratiometric fluorescent detection of bisulfite (HSO_3^-) with high selectivity and sensitivity in aqueous solution. Because of the chemical and biological importance of bisulfite, the development of ratiometric fluorescent probes for this anion species has attracted considerable attention in recently [27–42]. Therefore, this work not only showed that the HBT-hemicyanine hybrid can be a valuable ratiometric sensing platform, but also provided a new colorimetric and ratiometric fluorescent probe for bisulfite.

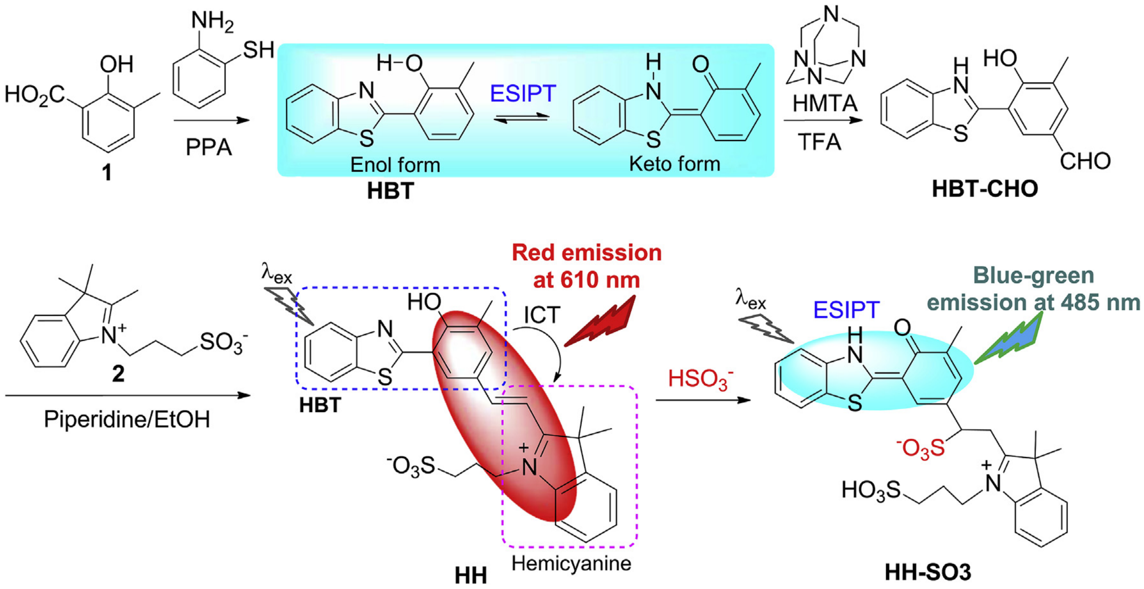
2. Materials and methods

2.1. Materials and instrumentation

All chemicals were purchased from commercial suppliers and used without further purification. 2,3,3-trimethyl-1-(propan-3-sulfonyl) indolenine (**2**) was synthesized by the published procedure [43]. Distilled water was used after passing through a water ultrapurification system. TLC analysis was performed using precoated silica plates. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 or 600 spectrometer. The low-resolution MS spectra were performed on an electron ionization mass spectrometer. HR-MS data were obtained with a LC-MS spectrometer. UV–Vis and fluorescence spectra were recorded on a Cary-100 UV–Vis spectrophotometer and a Cary-Eclipse fluorescence spectrophotometer with a temperature controller, respectively. Standard quartz cuvettes with a 10 mm lightpath were used for all optical spectra measurements. Cell imaging was performed in an OLYMPUS IX71 inverted fluorescence microscopy with a 40× objective lens.

2.2. Synthesis of HBT

HBT was prepared according to the literature method [44]. A mixture of 2-aminobenzenethiol (125 mg, 1 mmol) and 2-hydroxy-3-methylbenzoic acid (125 mg, 1 mmol) in polyphosphoric acid (5 mL) was heated in oil bath at 180 °C for 18 h under N_2 atmosphere, and then was cooled at room temperature. The mixture was then poured into ice, filtered, and the resulting solid product was washed with water, dried in air, and finally purified by column chromatography to yield a solid product (45% yield). Mp: 131–132 °C. TLC (silica plate): $R_f = 0.76$ (hexane:ethyl acetate 5:1, v/



Scheme 1. Preparation of the HBT-hemicyanine hybrid (**HH**) for ratiometric fluorescence sensing of bisulfite.