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"Quinone–phenol" transduction activated excited-state intramolecular proton transfer: A new strategy toward ratiometric fluorescent probe for sulfite in living cells

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ABSTRACT

2-(2′-Hydroxyphenyl) benzothiazoles (HBT) are well-known excited-state intramolecular proton transfer (ESIPT) molecules and have been ingeniously elaborated for fluorescent probes construction. However, most of HBT-based ratiometric fluorescent probes are based on the phenolic hydroxyl (-OH) protection/deprotection strategy. In this work, we integrate the π-conjugation interruption reaction and the "quinone–phenol" transduction-activated ESIPT within a benzothiazole-containing rhodol derivative (**1**), and develop a ratiometric fluorescent probe for sulfite. Upon treating with sulfite, the π-conjugation system of **1** was interrupted and the "quinone–phenol" transduction occurred simultaneously, which leads to the ESIPT process between the phenolic OH and benzothiazole unit upon photoexcitation. The above conversions result in the decrease of the emission band at 578 nm along with a concomitant increase of a new fluorescence peak at 453 nm. The fluorescent intensity (I_{453}/I_{578}) increases linearly with sulfite concentration up to 8 μM with a detection of 0.22 μM (3δ). The proposed probe shows excellent selectivity toward sulfite over other common anions and nucleophiles. Moreover, the cellular imaging experiment indicated probe **1** possesses low cytotoxicity and desirable cell permeability for biological applications.

1. Introduction

Fluorescent probes have attracted continuing attention due to the simplicity and high sensitivity of fluorescence detection. With the development of related scientific research, many fluorescent probes have been developed based on different photophysical processes such as photo-induced electron transfer [1,2], intramolecular charge transfer [3–5], fluorescence resonance energy transfer [6], and through-bond energy transfer [7–9]. In recent years, a new sensing mechanism known as excited-state intramolecular proton transfer (ESIPT) is emerging as a suitable optical response for designing molecular probes for different species [10–16]. ESIPT compounds exhibit interesting photophysical properties such as large Stokes shifts, a desired optical response from any fluorescent probe as it minimizes artifacts such as self-absorption and an inner-filter effect [17]. In addition, dual emission of ESIPT compounds are ideal scaffolds for developing ratiometric probes as they become strategically more advantageous over normal probes by minimizing the error arising from physical or chemical fluctuations in the sample and experimental conditions.

2-(2'- hydroxyphenyl) benzothiazoles (HBT) are well-known ESIPT molecules and have been ingeniously elaborated for fluorescent probes construction [18,19]. The phenolic OH-caged HBT derivatives exhibit exclusively enol-like emission due to the blockage of ESIPT. However, when the O-functionalized compounds are converted to the starting HBT by analyte-triggered reactions, the ESIPT process is retrieved and the corresponding phototautomers (the keto form) are generated upon irradiation, which give rise to a more strong fluorescence emission at longer wavelength compared to their enol forms (Scheme S1 Supporting Information). The above conversions provide a new design strategy for ratiometric fluorescent probes development as HBT and its

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O-functionalized derivatives exhibit keto- and enol-like emissions, respectively. This signal transduction mechanism has yielded ratiometric fluorescent probes for a variety species [20–28]. While several fluorescent probes have been developed by taking advantage of HBT, most of them are based on the phenolic hydroxyl (-OH) protection/deprotection strategy and ESIPT units are rarely combined with external stimuli-responsive chromophores to attain switching effect on the spectral properties. Therefore, much room is still left for integrating the elusive photophysics of ESIPT chromophores with a large π-conjugation framework to develop fluorescence probes.

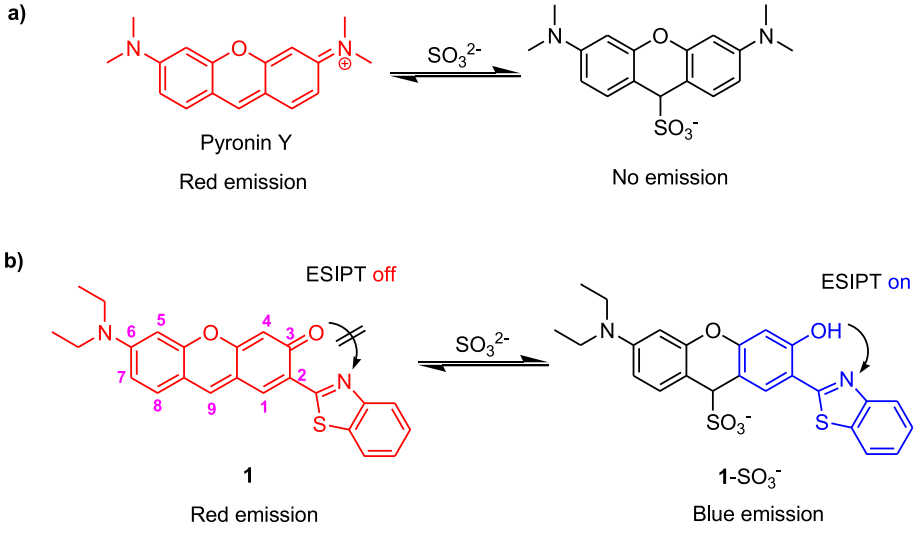
Sulfites are commonly used to prevent food from browning, discourage bacterial growth in wines, and maintain the stability and potency of some medications. However, high doses of sulfite may cause adverse reactions and acute symptoms [29]. Besides, many studies suggested that extended exposition to SO_2 and/or its derivatives could produce different toxicological effects such as cancer, cardiovascular diseases, neurological disorders, and the change of the characteristics of voltage-gated sodium and potassium channels [30–32]. Hence, the regulation and detection of sulfite attracted considerable attention. In recent years, a number of sulfite fluorescent probes have been developed, which are based on different signaling mechanisms, such as the reaction of sulfite with aldehydes [33–36], levulinate esters [37–40], Michael-type additions [41–46], and coordinative interactions [47,48]. However, most of these fluorescent probes respond to sulfite with changes only in fluorescence intensity. By contrast, ratiometric fluorescent probes allow the measurement of emission intensities at two different wavelengths, which should provide a built-in correction for environmental effects and can also increase the dynamic range of fluorescence measurement. Therefore, some ratiometric fluorescent probes for sulfite detection have been developed [38,42,43,45,46,48]. However, there is still plenty room for improvement in terms of selectivity, sensitivity and performance with a new sensing mechanism.

Recently, we observed that pyronin Y could react with sulfite in aqueous solution to afford both absorbance and fluorescence turn-off response due to interrupting the π-conjugation of xanthene ring at the C-9 atom (Scheme 1a, Fig. S1 in Supporting Information). More significantly, this nucleophilic reaction showed excellent selectivity toward sulfite over other common anions (Fig. S2, Supporting Information). Unfortunately, an unwanted fluorescence turn-off response was obtained in the above reaction. Generally, turn-on probes are more reliable than turn-off probes because their signal arises from a low background. False positives are rarely observed with turn-on probes because, unlike turn-off sensors, high background intensity and photobleaching minimally affect their overall response [49,50]. Although an undesired turn-off response was obtained upon treating pyronin Y with sulfite, this reaction inspired us to construct ratiometric fluorescent probes toward sulfite by using xanthene ring as the recognition moiety. In the above sensing process, the nucleophilic addition of sulfite to the pyronin C-9 atom was accompanied by an "imine-amine" transduction at its 3-position (Scheme 1a). We thus envisioned that an analogous "quinone–phenol" transduction would occur if pyronin ring was replaced by a rhodol scaffold. Furthermore, if a benzothiazole unit was introduced adjacent to the phenolic oxygen of the rhodol fluorophore, the "quinone–phenol" transduction could be utilized to modulate the ESIPT existing between the phenolic OH and the N atom of the benzothiazole ring within the dyes. Our approach is depicted in Scheme 1b. Free compound **1** is present in its strongly fluorescent quinoid form, and the ESIPT process cannot occur as no free protons are available in close proximity to benzothiazole. Thus, **1** will exhibit only rhodol emission. By contrast, if sulfite attacks at C-9 position of **1** to interrupt its π-conjugation system, "quinone–phenol" transduction occurs simultaneously and the desirable ESIPT process between the phenolic OH and the adjacent benzothiazole unit will occur upon photoexcitation, thus affording HBT-like emission. The above conversions offer a ratiometric response toward sulfite. Herein, as a proof-of-concept we report such a "quinone–phenol" transduction-activated ESIPT sensing scheme for sulfite. To the best of our knowledge, such a method was rarely used for switching the fluorescence of ESIPT chromophores [51,52].

2. Experimental section

2.1. Materials and instrumentation

All the chemical reagents and solvents were purchased from commercial suppliers and used without further purification unless for special needs. Doubly distilled water was used in the experiments. The fluorescence spectra and relative fluorescence intensity were measured with a Shimadzu RF-5301 spectrofluorimeter with a 10 mm quartz cuvette. UV–Vis spectra were made with a Shimadzu UV-2550 spectrophotometer. High-resolution mass spectra



Scheme 1. The proposed sensing mechanism for sulfite when using pyronin Y (a) or probe **1** (b).