Sensors and Actuators B 206 (2015) 268–275

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Development of a ratiometric fluorescent probe for sulfite based on a coumarin–benzopyrylium platform

Yanhong Chen [a], Xin Wang [a], Xiao-Feng Yang [a,*], Yaogang Zhong [b], Zheng Li [b], Hua Li [a]

[a] Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of Ministry of Education, College of Chemistry & Materials Science, Northwest University, Xi'an 710069, PR China
[b] College of Life Sciences, Northwest University, Xi'an 710069, PR China

ARTICLE INFO

Article history:
Received 31 July 2014
Received in revised form 2 September 2014
Accepted 13 September 2014
Available online 22 September 2014

Keywords:
Sulfite
Fluorescent probe
Ratiometric
Benzopyrylium
Coumarin

ABSTRACT

A ratiometric fluorescent probe, 2-(7-diethylamino-2-oxo-2H-1-benzopyran-3-yl)-7-hydroxyl-1-benzopyrylium (**1**), has been developed for sulfite sensing. The method employs the nucleophilic addition of sulfite to the electrically positive benzopyrylium moiety of **1** to block the π-conjugated system of the whole molecule, which in turn results in significant blue shifts in the absorption and emission spectra of the sensing system. The fluorescence intensity ratio at 485 and 640 nm (I_{485}/I_{640}) increases linearly with sulfite concentration in the range of 0.05–10 μM. The proposed probe shows excellent selectivity toward sulfite over other common anions and biothiols. The bioimaging experiment demonstrates the potential of probe **1** for the ratiometric fluorescent imaging of sulfite in living cells.

1. Introduction

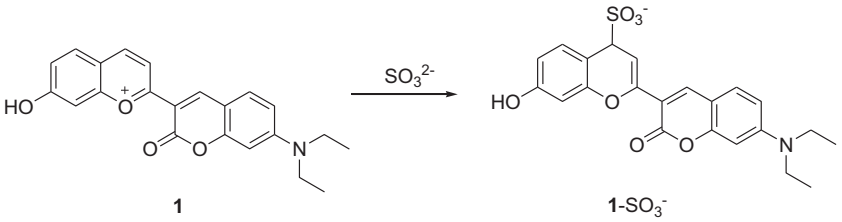
Sulfur dioxide (SO_2) is an environmental pollutant and is toxic at elevated concentrations. Inhaled SO_2 is easily hydrated to produce sulfite (SO_3^{2-}) and bisulfite (HSO_3^-) (3:1 M/M, in neutral fluid) [1], and the toxicity of SO_2 is mainly affected by the two derivatives. SO_2 at elevated concentrations is known to induce oxidative damage to biomacromolecules such as proteins, lipids, and DNA. Moreover, epidemiological studies indicate that extended exposure to SO_2 and/or its derivatives is associated with lung cancer, cardiovascular diseases, neurological disorders [2], and a change in the characteristics of voltage-gated sodium and potassium channels [3]. On the other hand, sulfite is widely used as a preservative for food and beverages to prevent oxidation and bacterial growth and inhibit the development of both enzymatic and nonenzymatic browning during production and storage [4]. Since high doses of sulfite may cause adverse reactions and acute symptoms, the threshold levels of sulfite in foodstuffs have been rigorously controlled [5]. Therefore, the development of a sensitive and selective method for sulfite and bisulfite assays is of great importance, especially the successful application in bioimaging of living cells.

Several methods such as spectrophotometry [6–8], chromatography [9,10], electrochemistry [11,12], and chemiluminescence [13,14] have been developed for sulfite detection. Fluorescent probes are valuable molecular tools for sensing and imaging trace amounts of samples due to their high sensitivity, exacting specificity, simplicity of implementation, and an ability to allow for real-time monitoring of target molecules in live cells or tissues. Accordingly, several fluorescent probes for sulfite (or bisulfite) have been exploited in recent years, based on the reaction of sulfite with aldehydes [15–19], levulinate esters [20–24], Michael-type additions [25–30], and coordinative interactions [31,32]. However, aldehyde-based probes can only be operated in acidic conditions [15], and may suffer from the interference of cysteine (Cys) and homocysteine (Hcy) [33]. Probes based on the levulinate group show certain drawbacks such as low sensitivity, or long response time [22]. Other reported probes perform poorly in pure water solution [29]. Therefore, there remains a need for developing simple and effective fluorescent probes for sulfite based on alternative detection principles.

2-(7-Diethylamino-2-oxo-2H-1-benzopyran-3-yl)-7-hydroxyl-1-benzopyrylium (**1**) constructed by hybridizing coumarin and benzopyrylium moieties. Recently, these kinds of dyes have received great attention due to their good photochemical properties such as high molar extinction coefficients, large fluorescence quantum yields, and long excitation and emission wavelengths [34,35]. More significantly, the benzopyrylium unit can not only extend the absorption and fluorescence spectra of the fluorophore but also acts as a guest receptor, as its 4-position is an effective reaction site for nucleophiles. The nucleophilic

* Corresponding author. Tel.: +86 29 88302604; fax: +86 29 88303798.
E-mail addresses: xfyang@nwu.edu.cn, xfyang258@163.com (X.-F. Yang).

http://dx.doi.org/10.1016/j.snb.2014.09.052
0925-4005/



Scheme 1. Proposed mechanism for the ratiometric sensing of sulfite when using **1**.

addition to its benzopyrylium C-4 atom alters the large π-conjugated system of **1** and thereby affords remarkable blue shifts in the optical spectra of the sensing system. Since **1** contains a coumarin fluorophore, it can still afford coumarin emission even after nucleophilic addition to its benzopyrylium unit. The coumarin–benzopyrylium platform can therefore serve as a broadly applicable platform to construct ratiometric probes based on altering the π-conjugation system of the fluorophore, and ratiometric fluorescent probes for Cys/Hcy [36], H_2S [37] and Hg^{2+} [38] have been reported.

On the other hand, it was reported that sulfite can react with α,β-unsaturated compounds efficiently in aqueous solution [39]. Considering that this reaction would block the π-conjugation of the system, it is possible to design a chemosensing system for sulfite based on this process. Thus, according to this mechanism, we propose **1** as a ratiometric fluorescent probe for sulfite. The ratiometric sensing is realized via the nucleophilic attack of sulfite to the electrically positive benzopyrylium moiety of **1** to interrupt the large π-conjugated system of the original fluorophore. As a result, two well-resolved emission bands before and after adding sulfite are observed due to the distinct emission between **1** and the corresponding **1**-SO_3^- adduct (Scheme 1). The fluorescence intensity ratio at 485 and 640 nm (I_{485}/I_{640}) increases linearly with sulfite concentration in the range of 0.05–10 μM. The proposed probe shows excellent selectivity toward sulfite over other common anions and biothiols. Probe **1** has been successfully applied to the ratiometric imaging of sulfite in living HepG2 cells.

2. Experimental

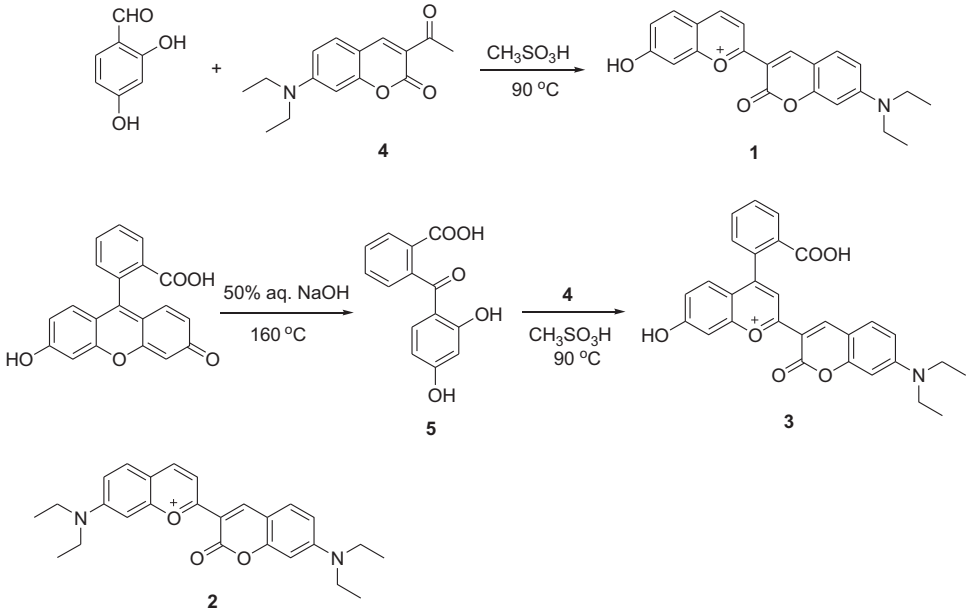
2.1. Materials and instrumentation

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. Flash chromatography was performed using Qingdao Haiyang silica gel (200–300 mesh). 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. IR spectra were recorded on a Bruker Vertex 70 FT-IR spectrometer using a diamond ATR attachment. High-resolution mass spectra (HRMS) were collected using a Bruker micrOTOF-Q II mass spectrometer (Bruker Daltonics Corp., USA) in electrospray ionization (ESI) mode. ^1H and ^{13}C NMR spectra were obtained on either a Varian INOVA-400 or Gemini 2000 (600 MHz) spectrometer with reference to solvent signals. The pH was measured with a Sartorius PB-10 pH meter. The fluorescence images were acquired with an Olympus BX 61 fluorescence scanning microscope (Tokyo, Japan).

2.2. Synthesis of compounds *1*, *2* and *3*

2.2.1. Compound *1*

Compound **4** was synthesized according to the reported procedure [40]. 2,4-Dihydroxybenzaldehyde (0.24 g, 1.738 mmol) and **4** (0.38 g, 1.448 mmol) were dissolved in methanesulfonic acid (5 mL)



Scheme 2. Synthesis of probe **1**, **3** and the structure of **2**.