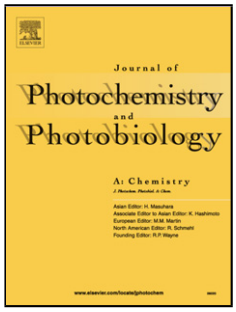
Journal Pre-proof



A colorimetric and fluorescent probe for sulfite/bisulfite based on conjugated benzothiazole derivative and imaging application in living cells

Li Zhang, Lei Wang, Xuan Zhang, Zhi-Jia Zhu

PII: S1010-6030(20)30297-5

DOI: https://doi.org/10.1016/j.jphotochem.2020.112498

Reference: JPC 112498

To appear in: *Journal of Photochemistry & Photobiology, A: Chemistry*

Received Date: 10 December 2019

Revised Date: 3 March 2020

Accepted Date: 8 March 2020

Please cite this article as: Zhang L, Wang L, Zhang X, Zhu Z-Jia, A colorimetric and fluorescent probe for sulfite/bisulfite based on conjugated benzothiazole derivative and imaging application in living cells, *Journal of Photochemistry and amp; Photobiology, A: Chemistry* (2020), doi: https://doi.org/10.1016/j.jphotochem.2020.112498

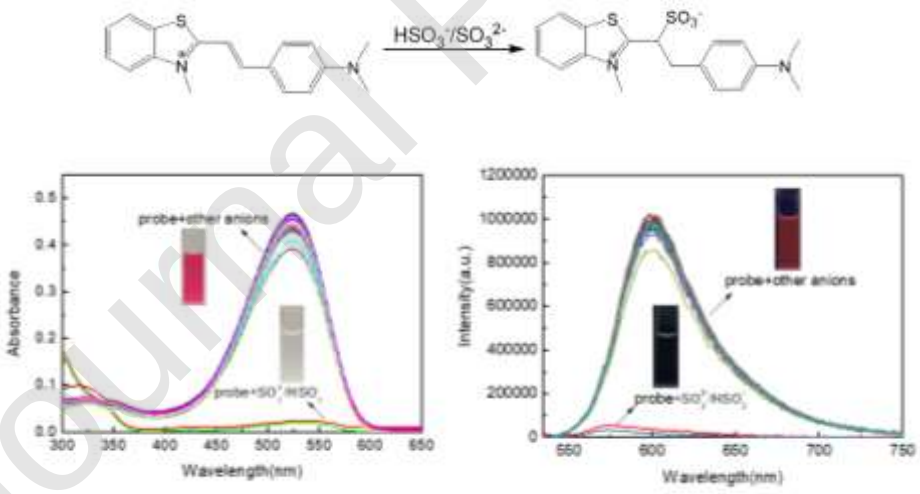
A colorimetric and fluorescent probe for sulfite/bisulfite based on conjugated benzothiazole derivative and imaging application in living cells

Li Zhang [a], Lei Wang [a], Xuan Zhang [a,b,*], Zhi-Jia Zhu [a]

[a] *Key Laboratory of Science and Technology of Eco-Textiles, Ministry of Education, College of Chemistry, Chemical Engineering & Biotechnology, Donghua University, Shanghai 201620, China;* [b] *State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116024, China.*

E-mail address: xzhang@dhu.edu.cn

Graphical Abstract



Highlight

- New fluorescent probe based on conjugated benzothiazole under an intramolecular sensing mechanism was developed.

- High selectivity toward SO_3^{2-}/HSO_3^- in aqueous solution.
- Imaging of SO_3^{2-}/HSO_3^- in living cells was achieved.

ABSTRACT

A new colorimetric and fluorescent probe **2** for SO_3^{2-}/HSO_3^- detection was developed based on a conjugated benzothiazole derivative. The probe **2** was red color in aqueous solution and became colorless in the presence of SO_3^{2-}/HSO_3^-, accompanied by a fluorescence quenching. It was rationalized by an intramolecular charge transfer (ICT) mechanism, where the addition of SO_3^{2-}/HSO_3^- to the vinyl double bond broken the conjugation and blocked the ICT process. This probe showed highly selective detection of SO_3^{2-}/HSO_3^- compared with other sulfur-containing species and featured a detection limit of 2.01 μM. Moreover, the present probe was also successfully used for the fluorescence imaging of intracellular sulfur dioxide derivatives in living HeLa cells.

Keywords: Colorimetric and fluorescent probe; sulfite/bisulfite; benzothiazole derivative; intramolecular charge transfer; fluorescence imaging

1. Introduction

Sulfur dioxide (SO_2) has been considered to be a serious environmental and air pollutant over the past few years [1]. Its derivatives (usually existed in SO_3^{2-}/HSO_3^-) have also been widely used in food, drinks and the pharmaceutical industry due to their excellent anti-oxidation and anti-microbial properties [2–5]. Furthermore, the sulfur dioxide derivatives were considered to

play a pivotal role in many physiological processes [5], and the exposure to high doses of SO_2 is closely associated with respiratory responses [6], lung cancer [7], cardiovascular disease [8], and some neurological disorders [9]. Thus, it is highly in demand to develop effective methods for the detection of SO_3^{2-}/HSO_3^-.

Nowadays, several methods, including spectrophotometry [10], chromatography [11], chemiluminescence [12], and electrochemistry [13], have been developed to determine sulfur dioxide derivatives. Alternatively, fluorescence technique is highly sensitive, simple, rapid, and inexpensive, and attracted much attention. For example, fluorescent probes have been reported for SO_3^{2-}/HSO_3^- detection based on various strategies, such as selective deprotection of levulinate group [14–16], complexation with amines [17, 18], and selective reaction with aldehyde [19, 20]. Recently, the design of fluorescent probes based Michael addition reaction principle has received much interest due to nice selectivity towards the detection of $HSO_3^-$and SO_3^{2-} in aqueous solution [21–26]. However, of these reported fluorescent probes, some involved tedious synthesis procedures, while some suffered from long reaction time [15], acidic condition [19] or requirement of surfactant additive [22]. Furthermore, the positive-charged probes have become intriguing due to its promising application in bioimaging [24–26].

In this work, a new colorimetric and fluorescent probe (**2**, Scheme 1) for SO_3^{2-}/HSO_3^- detection was developed based on a positive-charged benzothiazole derivative. An intramolecular charge transfer (ICT) process in the probe **2** was blocked by Michael addition of SO_3^{2-}/HSO_3^- to vinyl double bond, and therefore both the color and fluorescence were quenched in aqueous solution. The probe exhibited a high selectivity and was successfully used for fluorescence imaging of sulfur dioxide derivatives in living cells.

2. Experimental

2.1 Materials and methods

All the chemicals are analytical grade and purchased from Sinopharm Chemical Reagents Corp. (Shanghai, China). Phosphate buffered solution (PBS, 10 mM, pH = 7.4) was prepared from K_2HPO_4 (0.1 M) and KH_2PO_4 (0.1 M). ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400 MHz spectrometer. Mass spectra were obtained on AB Sciex MALDI-TOF/TOF^{TM}MS. Fluorescence spectra were measured on an Edinburgh FS5 spectrofluorometer with Ex/Em slit widths of 5 nm. Absorption spectra were obtained on a SHIMADZU UV-1800 spectrophotometer. The pH was adjusted by using Mettler Toledo pH Meter. Fluorescence imaging experiments in living HeLa cells were carried out with a OLYMPUS DP72 microscope.

HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS (fetal bovine serum) at 37℃ in a 95% humidity atmosphere under 5% CO_2 environment, and then were seeded in a 12-well culture plate for one night before cell imaging experiment. The cells were then incubated with the probe **2** (20 μM) for 45 min at 37℃, then further incubated with the addition of SO_3^{2-} (300 μM) for another 10 min and observed under the fluorescence microscopy, respectively. All the cells were washed with PBS buffer (10 mM) three times to remove free probe.

2.2 General procedure for measurements

Probe **2** was dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution (1 mM). Deionized water was used to prepare stock solutions (10 mM) of SO_3^{2-}/HSO_3^- and other analytes for spectroscopic measurements. The probe solutions (50 μL) were separately transferred into a 5

mL sample tube from stock solution and followed by addition of an appropriate aliquot of the analytes to prepare series test solution. The test solutions were then diluted to 5 mL with PBS containing 50% DMSO. The resulting solution was shaken well at room temperature for 10 min before spectral measurements.

2.3 Synthesis

*Synthesis of **1***. Under N_2 atmosphere, 2-methyl phenylthiazole (508 uL, 4 mmol) and methyl iodide (1 mL, 16 mmol) were dissolved in acetonitrile (10 mL) and refluxed at 80℃overnight. After cooling to room temperature, a large amount of light green solid precipitate was obtained. The solid was washed three times with the mixed solvent of n-hexane and ethyl acetate (v/v = 3:1) and dried in vacuum. The product was then obtained to be 0.8403g (yield: 72%). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.45 (d, J = 8.0 Hz ,1H), 8.31 (d, J = 8.0 Hz ,1H)，7.90 (t, J = 8.0 Hz, 1H), 7.81 (t, J = 8.0 Hz, 1H), 4.20 (s, 3H), 3.17 (s, 3H).

*Synthesis of **2***. The compound **2** was similarly synthesized according to the reported procedure with small modification [27]. Briefly, under N_2 atmosphere, compound **1** (0.2901g, 1 mmol) and 4-dimethylaminobenzaldehyde (0.1487g, 1 mmol) were dissolved in absolute ethanol (6 mL) and two drops of piperidine were added. The mixture solution was refluxed at 80℃for 4h, cooled to room temperature and quenched with diethyl ether (4mL). The solid was collected by filtration, washed with acetone, and dried under vacuum to afford a dark red solid product 0.2472g (yield: 59%). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.31 (d, J = 8.0 Hz ,1H), 8.08 (t, J = 16.0 Hz ,2H)，7.93 (d, J = 12.0 Hz, 2H), 7.79 (t, J = 8.0 Hz, 1H), 7.68 (m, 2H), 6.85 (d, J = 8.0 Hz, 2H), 4.28 (s, 3H), 3.11 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 171.28, 153.43, 150.06, 141.90, 132.79, 128.82, 127.39, 126.77, 123.77, 121.42, 115.90, 111.90, 106.22, 35.58.

MALDI-TOF-MS: m/z calcd for $C_{18}H_{19}N_2S$, 295.43; found 295.07 $[M]^+$.

3. Results and discussion

3.1 Selectivity of the probe 2 towards SO_3^{2-}/HSO_3^-

As shown in Fig. 1a, the probe **2** (10 μM) exhibits strong fluorescence at 600 nm (excitation wavelength is 525 nm) with a bright red emission color (Inset in Fig. 1a) in PBS buffer (10 mM, pH=7.4, 50% DMSO). Upon addition of 15 equiv of each analytes, including F^-, Br^-, Cl^-, CO_3^{2-}, HCO_3^-, NO_2^-, CH_3COO^-, SCN^-, $S_2O_3^{2-}$, ClO_4^-, $C_2O_4^-$, $H_2PO_4^-$, HPO_4^{2-}, SO_4^{2-}, S^{2-}, MnO_4^-, ClO^-, Cys, Hcy and GSH, the fluorescence emission spectra showed almost no significant change. However, the fluorescence intensity underwent a large quenching in the presence of SO_3^{2-}/HSO_3^- and the emission color of solution became dark (Inset in Fig. 1a). Fig. 1b showed the absorption spectra of the probe **2** in the absence and presence of various analytes. It can be seen that a remarkable color change of solution from pink to colorless (inset in Fig. 1b) in the presence of SO_3^{2-}/HSO_3^-, accompanied by disappearance of absorption at 525 nm. In contrast, others analytes did not give significant affection on the absorption spectra. This suggested that the probe **2** can serve as a colorimetric and fluorescent probe for highly selective detection of SO_3^{2-}/HSO_3^-. To further exemplify the selectivity towards SO_3^{2-}/HSO_3^- over other analytes, the competition experiment was conducted, where other analytes were firstly introduced into the probe solution and followed by addition of 15 equiv of SO_3^{2-}. As shown in Fig. 1c, the fluorescence was dramatically quenched without significant interference from these competing species. Furthermore, both the emission and solution color disappeared (inset in Fig. 1c).

3.2 pH effect and response time

To obtain the optimal experimental pH condition, the pH effect on fluorescence of the probe **2** in the absence and presence of SO_3^{2-} was investigated. As shown in Fig. 2a, without SO_3^{2-}, the fluorescence intensity at 600 nm exhibited a minor change over the pH range of 2−11, demonstrating the good stability of the probe. In contrast, in the presence of SO_3^{2-}, the fluorescence was significantly decreased at pH > 5. In this work, pH = 7.4 was selected to match with the application requirement in physiological environment. Considering the pKa_2 value (7.2) of H_2SO_3 [28], SO_3^{2-} was main species at pH = 7.4 and therefore used for the further titration. To examine the response time, the change of fluorescence intensity of the probe **2** was monitored with time upon addition of SO_3^{2-}. As shown in Fig. 2b, the fluorescence intensity at 600 nm was continually decreased and reaches constant after 10 min. Thus all of the measurements in this work were finished after standing 10 min.

3.3 Signaling mechanism

To understand the sensing mechanism of the probe **2** for SO_3^{2-}, MALDI-TOF-MS analysis of a mixed solution of the probe (10 μM) with 15 equiv of SO_3^{2-} was performed. As shown in Fig. 3, a prominent peak at m/z = 375.46 corresponding to [**2** + SO_3^{2-}]$^-$ was observed, confirming the formation of addition product between the probe **2** and SO_3^{2-}. As usually observed in conjugated benzothioazole derivatives, an intramolecular charge transfer (ICT) process is responsible for the long-wavelength fluorescence [29−33]. In the probe **2**, the dimethylaminophenyl group (electron donor) and benzothiazolium unit (electron acceptor) was connected by the vinyl double bond. The addition of SO_3^{2-} to the vinyl double bond broken the conjugation between the electron donor and electron acceptor, and therefore blocked the ICT process as depicted in Scheme 1. As a consequence, both the fluorescence and absorption were significantly quenched.

3.4 Fluorescence and absorption titration

The fluorescence and absorption titration were performed at the optimal experimental conditions and the results were presented in Fig. 4. As shown in Fig. 4a, the fluorescence gradually decreased with increasing amounts of SO_3^{2-} up to 180 μM. A good linear relationship was found between the fluorescence intensity at 600 nm and SO_3^{2-} concentration (0−150 μM) (Fig. 4b). The detection limit was estimated to be 2.01 μM according to the ratio of signal to noise (S/N = 3). Notably, the probe **2** demonstrated the comparable and even better sensitivity than those of the previous fluorescent probes [14−16, 18, 23, 24], revealing the improved performance of the present probe (Table 1). Additionally, the absorption at 525 nm decreased with increasing SO_3^{2-} concentration (Fig. 4c) and a linear relationship was found in the range of 0−60 μM (Fig. 4d). Notably, a color decay change from the pink to the colorless (inset in Fig. 4d) with increasing the concentration of SO_3^{2-} was observed, suggesting that the present probe could serve as a "naked-eye" colorimetric probe for sulfur dioxide derivatives detection.

3.5 Fluorescence imaging in living cells

To evaluate the potential applications in the fluorescence imaging of SO_3^{2-} in living cells, the HeLa cells was incubated with probe **2** (20 μM) for 45 min at 37℃, and then observed by a fluorescence microscope. A very bright red fluorescence was observed inside cells (Fig. 5b), indicating that the probe is capable of applying in vivo imaging. Subsequently, 300 μM SO_3^{2-} was injected and further incubated for 10 min, the red fluorescence was completely quenched (Fig. 5d). These results demonstrated that the probe **2** could be successfully used to image intracellular sulfur dioxide level. The toxicity examination revealed that more than 80% cells remained alive even treated with 50 μM probe, demonstratling the present probe showed low cytotoxicity.

4. Conclusions

In summary, a new colorimetric and fluorescent probe for SO_3^{2-}/HSO_3^- detection was developed based on a conjugated benzothiazole derivative. The addition of SO_3^{2-}/HSO_3^- to the vinyl double bond broken the conjugation and blocked the intramolecular charge transfer (ICT) process, leading the quenching of fluorescence and absorption. The probe was highly selective for SO_3^{2-}/HSO_3^- and was successfully used for the fluorescence imaging in living cells. The intense changes of both color and fluorescence of the present probe make it possible to contribute to the design new colorimetric and fluorescent probes based on benzothiazole framework.

Credit author statement

Li Zhang: Investigation, Writing-Original draft preparation. **Lei Wang**: Investigation, Validation. **Xuan Zhang:** Conceptualization, Supervision, Writing-Reviewing and Editing. **Zhi-Jia Zhu**: Resources, Formal Analysis.

T **Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

his work was financially supported by the State Key Laboratory of Fine Chemicals, Dalian University of Technology (KF 1715).

References

[1] M. Kampa, E. Castanas, Human health effects of air pollution, Environ. Pollut. 151 (2008) 362–367.

[2] C. Winkler, B. Frick, K. Schroecksnadel, H. Schennach, D. Fuchs, Food preservatives sodium sulfite and sorbic acid suppress mitogen-stimulated peripheral blood mononuclear cells, Food Chem. Toxicol. 44 (2006) 2003–2007.

[3] S. C. Morgan, C. M. Scholl, N. L. Benson, M. L. Stone, D. M. Durall, Sulfur dioxide addition at crush alters Saccharomyces cerevisiae strain composition in spontaneous fermentations at two Canadian wineries, Int. J. Food Microbiol. 244 (2017) 96–102.

[4] R. Nassar, A. Trivella, S. Mokh, M. Al-Iskandarani, H. Budzinski, P. Mazellier, Photodegradation of sulfamethazine, sulfamethoxypiridazine, amitriptyline, and clomipramine drugs in aqueous media, J. Photochem. Photobiol. A 336 (2017) 176–182.

[5] J. Li, R. Li, Z. Meng, Sulfur dioxide up regulates the aortic nitric oxide pathway in rats, Eur. J. Pharmacol. 645 (2010) 143–150.

[6] S. Iwasawa, Y. Kikuchi, Y. Nishiwaki, M. Nakano, T. Michikawa, T. Tsuboi, S. Tanaka, T. Uemura, A. Ishigami, H. Nakashima, T. Takebayashi, M. Adachi,A. Morikawa, K. Maruyama, S. Kudo, I. Uchiyama, K. Omae, Effects of SO_2 on respiratory system of adult miyakejima resident 2 years after returning to the island, J. Occup. Health. 51 (2009) 38–47.

[7] M. Reist, P. Jenner, B. Halliwell, Sulphite enhances peroxynitrite-dependent α_1-antiproteinase inactivation. A mechanism of lung injury by sulphur dioxide? FEBS Lett. 423 (1998) 231–234.

[8] G. Li, N. Sang, Delayed rectifiier potassium channels are involved in SO_2 derivative-induced

hippocampal neuronal injury, Ecotoxicol. Environ. Saf., 72 (2009) 236–241.

[9] N. Sang, Y. Yun, H. Li, L. Hou, M. Han, G. Li, SO_2inhalation contributes to the development and progression of ischemic stroke in the brain, Toxicol. Sci. 114 (2010) 226–236.

[10] N. Altunay, R. Gurkan, A new simple UV-Vis spectrophotometric method for determination of sulfite species in vegetables and dried fruits using a preconcentration process. Anal. Methods 8 (2016) 342−352.

[11] S. Theisen, R. Hansch, L. Kothe, U. Leist, R. Galensa, A fast and sensitive HPLC method for sulfite analysis in food based on a plant sulfite oxidase biosensor, Biosens. Bioelectron. 26 (2010) 175−181.

[12] H. Meng, F. W. Wu, Z. K. He, Y. E. Zeng, Chemiluminescence determination of sulfite in sugar and sulfur dioxide in air using Tris(2,2′-bipyridyl) ruthenium(II)-permanganate system, Talanta 48 (1999) 571−577.

[13] H. Beitollahi, S. Tajik, P. Biparva, Electrochemical determination of sulfite and phenol using a carbon paste electrode modified with ionic liquids and graphene nanosheets: application to determination of sulfite and phenol in real samples, Measurement 56 (2014) 170−177.

[14] M.G. Choi, J. Hwang, S. Eor, C. SK, Chromogenic and fluorogenic signaling of sulfite by selective deprotection of resorufin levulinate, Org. Lett. 12 (2010) 5624−5627.

[15] S. Chen, P. Hou, J. X. Wang, X. Z. Song, A highly sulfite-selective ratiometric fluorescent probe based on ESIPT, RSC Adv. 2 (2012) 10869−10873.

[16] X. F. Gu, C. H. Liu, Y.C. Zhu, A boron-dipyrromethene-based fluorescent probe for colorimetric and ratiometric detection of sulfite, J. Agric. Food Chem. 59 (2011) 11935−11939.

[17] D. M. Rudkevich, A.V. Leontiev, Revisiting noncovalent SO_2-amine chemistry: an

indicator-displacement assay for colorimetric detection of SO_2, J. Am. Chem. Soc. 127 (2005) 14126−14127.

[18] Y. M. Sun, C. Zhong, R. Gong, H. L. Mu, E. Q. Fu, A ratiometric fluorescent chemodosimeter with selective recognition for sulfite in aqueous solution, J. Org. Chem. 74 (2009) 7943−7946.

[19] S. Y. Qiang, P. Wang, J. Liu, J. Y. Zhang, W. Guo, A fluorescent turn-on probe for bisulfite based on hydrogen bond-inhibited C=N isomerization mechanism, Analyst 137 (2012) 3430−3433.

[20] C. M. Yu, M. Luo, F. Zeng, S. Z. Wu, A fast-responding fluorescent turn-on sensor for sensitive and selective detection of sulfite anions, Anal. Methods., 4 (2012) 2638−2640.

[21] T. T. Niu, T. Yu, G. X. Yin, H. M. Chen, P. Yin, H. T. Li, A novel colorimetric and ratiometric fluorescent probe for sensing SO_2 derivatives and their bioimaging in living cells. Analyst 144 (2019) 1546−1554.

[22] Y. C. Sun, Z. Chen, F. Z. Chen, H. Liu, H. P. He, X. H. Zhang, S. F. Wang, An HBT-based near-infrared fluorescent probe for colorimetric and ratiometric detection of bisulfite and its applicationin living cells, J. Fluoresc. 27 (2017) 1405−1411.

[23] Y. P. Zhao, Y. Y. Ma, W. Y. Lin, A near-infrared and two-photon ratiometric fluorescent probe with a large Stokes shift for sulfur dioxide derivatives detection and its applications in vitro and in vivo, Sens. Actuators B. 288 (2019) 519−526.

[24] X. L. Yang, Y. T. Yang, T. T. Zhou, M. Jin, X. Q. Jing, C. H. Miao, W. Li, A mitochondria-targeted ratiometric fluorescent probe for detection of SO_2 derivatives in living cells and in vivo, J. Photoch. Photobio. A. 372 (2019) 212−217.

[25]Y. H. Yao, Q. Sun, Z. Y. Chen, R. Huang, W. B. Zhang, J. H. Qian, A mitochondria-targeted

near infrared ratiometric fluorescent probe for the detection of sulfite in aqueous and in living cells, Talanta 189 (2018) 429−436.

[26] H. Huang, W. Liu, X.-J. Liu, Y.-Q. Kuang, J.-H. Jiang, A novel mitochondria-targeted near-infrared fluorescence probe for ultrafast and ratiometric detection of SO_2 derivatives in live cells, Talanta 168 (2017) 203−209.

[27] P. J. Coelho, M. R. Castro, M. M. Raposo, Fast (hetero)aryl-benzothiazolium ethenes photoswitches activated by visible-light at room temperature, Dyes Pigm. 117 (2015) 163−169.

[28] M. Sun, H. Yu, K. Zhang, Y. Zhang, Y. Yan, D. Huang, S. Wang, Determination of gaseous sulfur dioxide and its derivatives via fluorescence enhancement based on cyanine dye functionalized carbon nanodots, Anal. Chem. 86 (2014) 9381−9385.

[29] N. Karton-Lifshin , L. Albertazzi , M. Bendikov , P. S. Baran and D. Shabat , "Donor-two-acceptor" dye design: A distinct gateway to NIR fluorescence, J. Am. Chem. Soc. 134 (2012) 20412−20420.

[30] N. Narayanaswamy, S. Das, P. K. Samanta, K. Banu, G. P. Sharma, N. Mondal, S. K. Dhar, S. K. Pati, T. Govindaraju, Sequence-specific recognition of DNA minor grooveby an NIR-fluorescence switch-on probe and itspotential applications, Nucleic Acids Res., 43 (2015) 8651−8663.

[31] X. Zhang, J.-Y. Liu, W.-W. Ma, M.-L. Yang, Near-infrared fluorescence of π-conjugation extended benzothiazole and its application for biothiol imaging in living cells, J. Mater. Chem. B 4 (2016) 6662−6669.

[32] X. Zhang, L. Zhang, W.-W. Ma, Y. Zhou, Z.-N. Lu, S.-Y. Xu, A near-infrared ratiometric fluorescent probe for highly selective recognition and bioimaging of cysteine, Front. Chem. 7

(2019) 32.

[33] Y. Zhou, L. Zhang, X. Zhang, Z.-J. Zhu, Development of a near-infrared ratiometric fluorescent probe for glutathione using an intramolecular charge transfer signaling mechanism and its bioimaging application in living cells, J. Mater. Chem. B 7 (2019) 809−814.

Figure Captions:

Fig. 1 Fluorescence (a) and absorption (b) spectra of the probe **2** (10 μM) in the absence and presence of 15 equiv various analytes in PBS buffer (pH = 7.4, 50% DMSO). Change of the fluorescence intensity at 600 nm upon addition of 15 equiv SO_3^{2-} in the presence of various analytes (c). Insets are photographs obtained under 365 nm lamp (a) and natural light (b) irradiation in the absence and presence of SO_3^{2-}/HSO_3^-.

Fig. 2 The change of fluorescence intensity of the probe **2** (10 μM) with pH (a) and response time (b) in PBS buffer (pH = 7.4, 50% DMSO). The concentration of SO_3^{2-} is 150 μM and the fluorescence emission and excitation wavelengths are 600 nm and 525 nm.

Fig. 3 Mass spectrum of the mixture of probe **2** (10 μM) upon addition of 15 equiv. of SO_3^{2-} in PBS buffer (pH = 7.4, 50% DMSO).

Fig. 4 Fluorescence (a) and absorption (c) titration spectra of the probe **2** (10 μM) with the addition of various amounts of SO_3^{2-} in PBS buffer (pH = 7.4, 50% DMSO). The corresponding linear relationships between the fluorescence intensity at 600 nm (b) and the absorbance at 525 nm (d) and concentration of SO_3^{2-}.

Fig. 5 Fluorescence imaging of SO_3^{2-} in living HeLa cells in PBS buffer (pH = 7.4, 50% DMSO). Bright-field (a) and corresponding fluorescence (b) images of HeLa cells incubated with the probe **2** (20 μM). Bright-field (c) and corresponding fluorescence (d) images of HeLa cells incubated

with the probe **2** (20 μM) and further incubated with SO_3^{2-} (300 μM).

Scheme1. Synthesis of probe **2** and the proposed sensing mechanism for SO_3^{2-}/HSO_3^-.

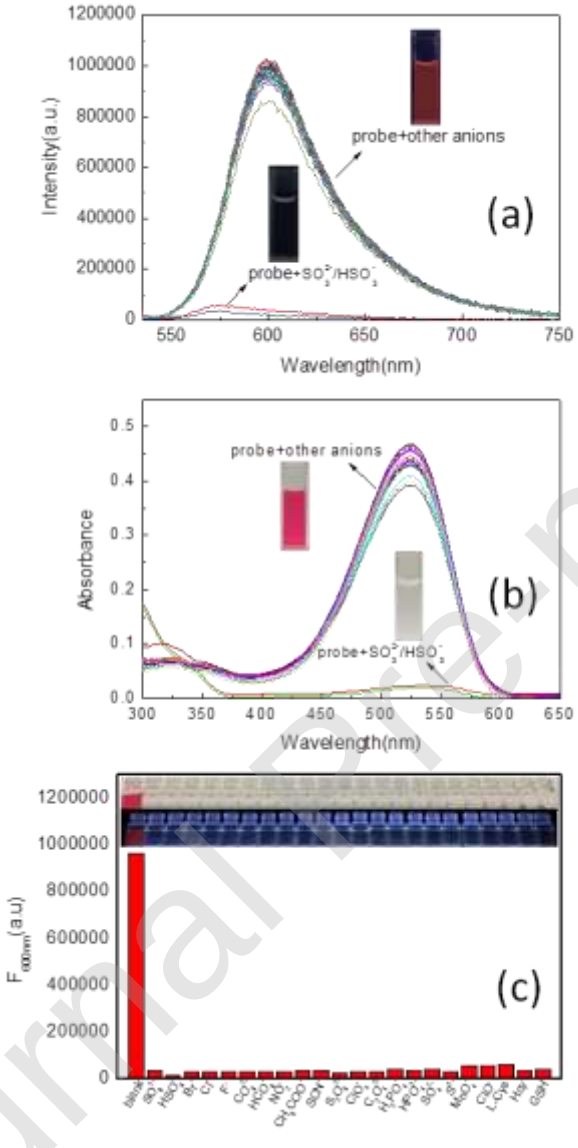

Intensity(a.u.)
Wavelength(nm)
probe+other anions
probe+SO_3^{2-}/HSO_3^-
(a)
Absorbance
Wavelength(nm)
probe+other anions
probe+SO_3^{2-}/HSO_3^-
(b)
F_{600nm}(a.u)
(c)

Fig. 1

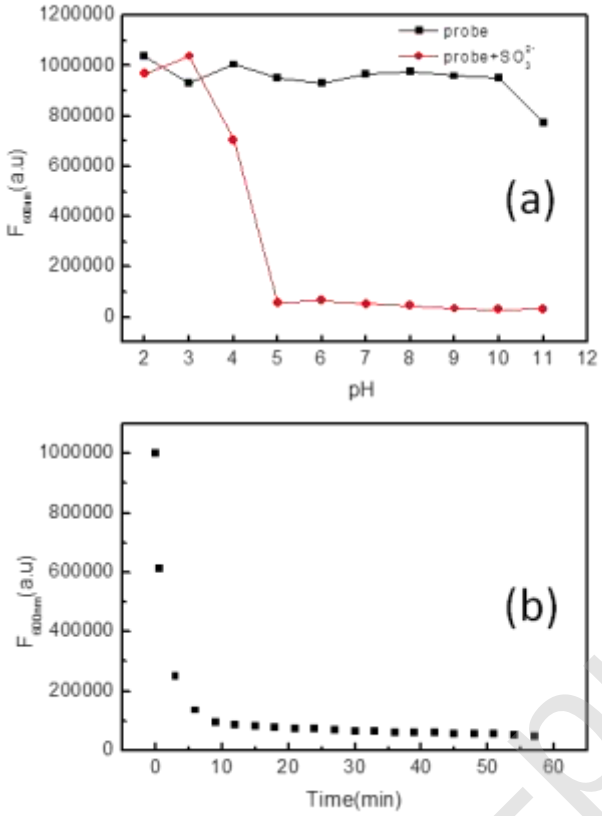


Fig. 2

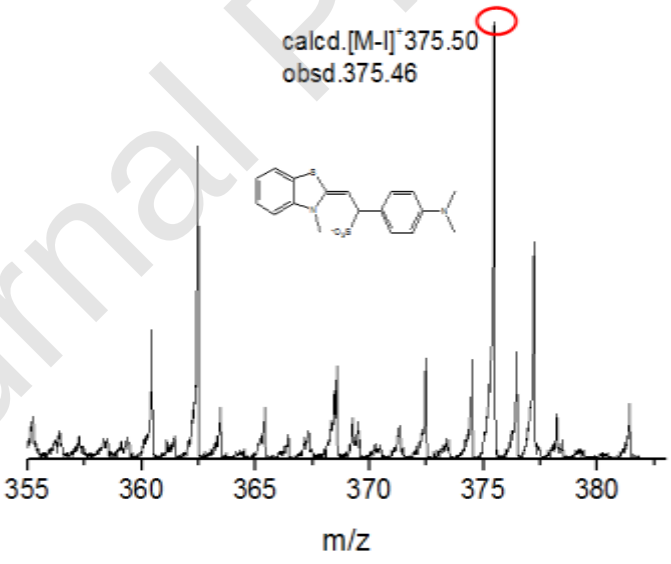


Fig. 3

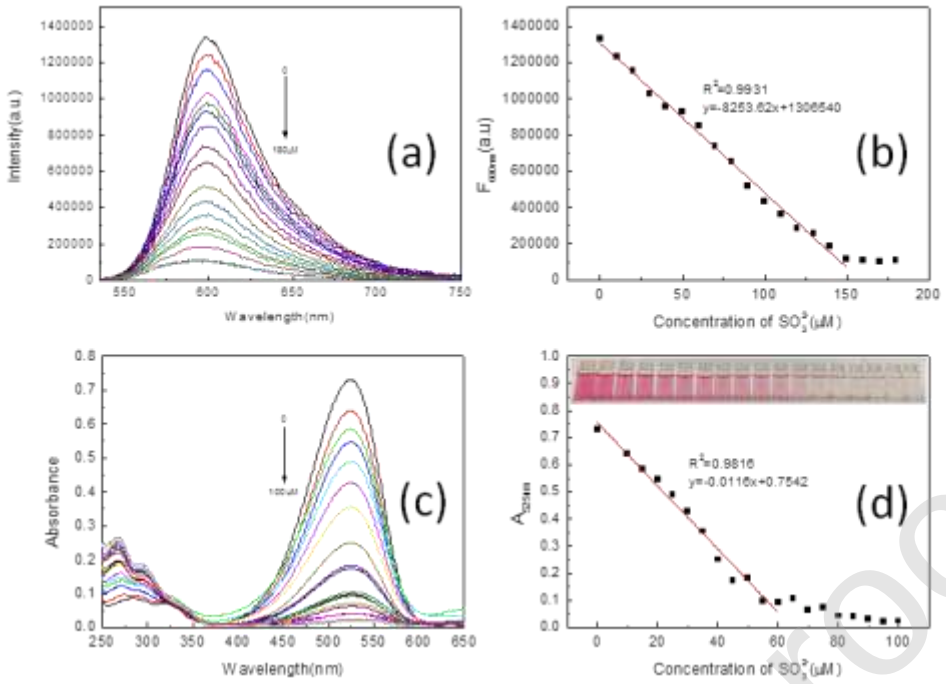


Fig. 4

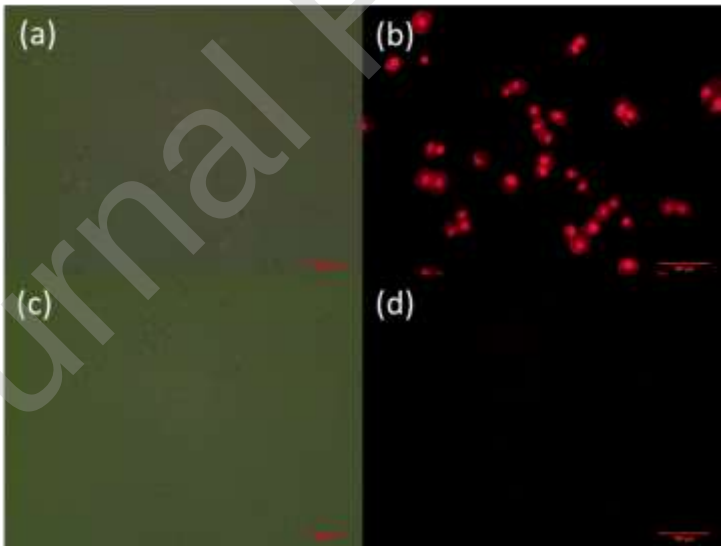
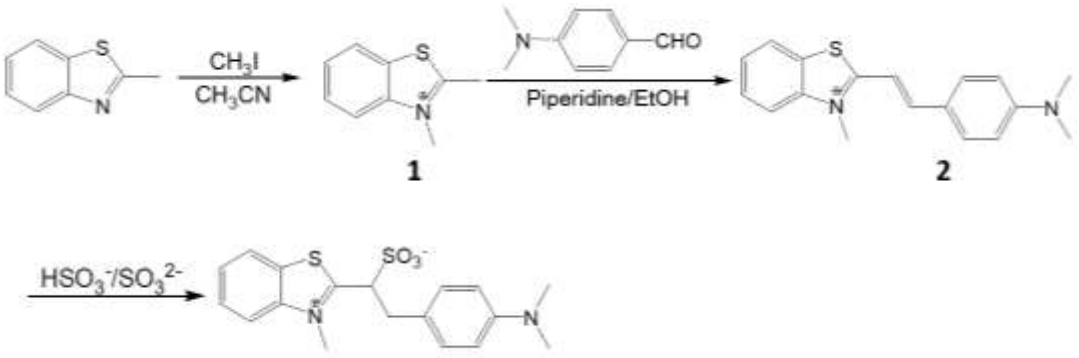


Fig. 5



Scheme1

Table 1 Performance comparison of various fluorescent probes for SO_3^{2-}/HSO_3^-.

References	Detection limit (μM)	Test medium and reaction time	Imaging application
14	49	HEPES-CH_3CN (98:2, pH 7.0), 20 min	None
15	5	HEPES-CH_3CN (1:1, pH 7.4), 60 min	None
16	58	H_2O-DMSO (1:1), 20 min	None
18	780	Tris-HCl-DMSO (9:1, pH 7.2), 10 min	None
19	0.1	Acetate-DMSO (1:1, pH 5.0), 5 min	None
22	0.101	PBS-CTAB (PBS 7.4), 3 min	MCF-7 cells
23	1.22	HEPES-DMF (9:1, pH 7.4), 5 min	BT-474 cells
24	5.12/1.06	PBS-CH_3CN (8:2, pH 7.4), 30 min	Hela cells
This work	2.01	PBS-DMSO (1:1, pH 7.4), 10 min	Hela cells