Tetrahedron Letters 53 (2012) 2332–2335

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

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

A benzothiazole-based fluorescent probe for thiol bioimaging

Wei Sun [a], Wenhua Li [a], Jing Li [a], Jian Zhang [b], Lupei Du [a], Minyong Li [a,*]

[a] Department of Medicinal Chemistry, Key Laboratory of Chemical Biology of Natural Products (MOE), School of Pharmacy, Shandong University, Jinan, Shandong 250012, China
[b] Institute of Immunopharmacology & Immunotherapy, School of Pharmacy, Shandong University, Jinan, Shandong 250012, China

ARTICLE INFO

Article history:
Received 20 December 2011
Revised 9 February 2012
Accepted 24 February 2012
Available online 3 March 2012

Keywords:
Benzothiazole
Fluorescent probe
Thiols
Cell imaging

ABSTRACT

This study reports a benzothiazole-based fluorescent probe with simple structure for thiols. This probe exhibited high on/off signal ratios and good selectivity toward thiols over other analytes, and was successfully applied to the imaging of thiols in living cells.

© 2012 Elsevier Ltd. All rights reserved.

Biological thiols/low molecular weight thiols, such as cysteine (Cys), homocysteine (Homo-Cys), and glutathione (GSH), which can regulate the intracellular redox state and higher-order structures of proteins, are active in the catalytic sites of numerous enzymes, and participate in intracellular signal transduction and gene regulation.[1] Generally, the levels of cellular thiols have been associated to toxic agents and diseases, including slowed growth, leucocyte loss, psoriasis, liver damage, cancer, and AIDS.[2] Consequently, selective detection of biological thiols should provide critical insight into pathological and biological sciences.

Among the various methods for detecting thiols, fluorescent molecular probes are of great interest because of their simplicity, low detection limit, wide range of dynamic response, and feasibility of intracellular detection.[3] Apart from reasonable physical properties (excitation/emission wavelength, Stokes shift, etc.), high response and good selectivity, an ideal fluorescent probe should be convenient to synthesize, act fast in mild condition, and have high permeability. In the present research, a simple benzothiazole fluorophore was masked with 2,4-dinitrobenzenesulfonyl (DNS), which was studied widely in the detection of thiols.[4] Thiols can release the fluorophore by the nucleophilic cleavage of the 2,4 -dinitrobenzenesulfonyl ester. This simple molecule has most of the desirable features, and thereafter should be particularly applicable for the in vitro and in vivo detection of thiols.

As depicted in Scheme 1, probe **1** started from commercially available 2-(benzo[d]thiazol-2-yl)phenol and was readily synthesized in only one step. 2-(Benzo[d]thiazol-2-yl)phenol was treated with 2,4-dinitrobenzene-sulfonyl chloride to obtain probe **1** in the presence of TEA in DCM with 80% yield.

The fluorescent properties of such a probe were evaluated under near physiological conditions (0.1 M phosphate buffer, pH 7.4). The absorption at 330 nm of 2-(benzo[d]thiazol-2-yl)phenol was dismissed after the connection of 2,4-dinitrobenzene-sulfonyl, and no fluorescence was found in probe **1**. The fluorescent intensity change of **1** was initially tested according to the reaction time with thiophenol (PhSH). As demonstrated in Figure 1, the fluorescent intensity reached the high plateau at 2 min after the addition of PhSH, and almost no change appeared when longer reaction time was examined.

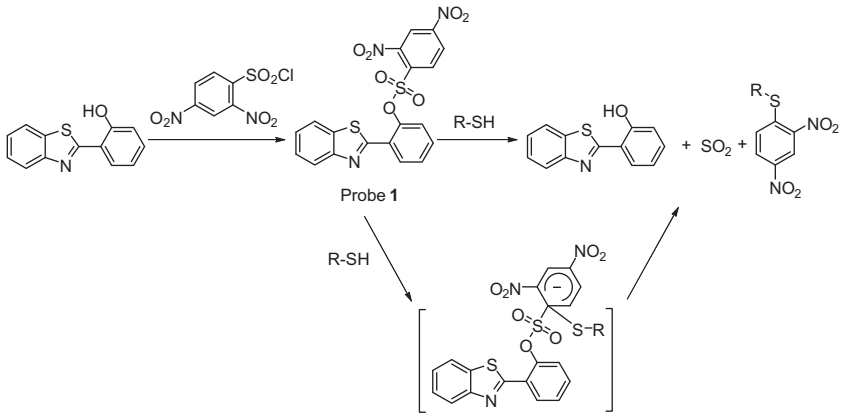
To determine the stability of the probe in PBS buffer solution, we treated it under various conditions by detecting the change of its fluorescent intensity (see Supplementary data, Fig. S1). It was found that the probe was essentially stable over a range of 5–9 under ambient light or protected from light, with a low level of its hydrolysis at pH 9.0

The sensitivity of probe **1** was then deliberated by fluorescence response toward different concentrations of PhSH. It was examined at 5 min after the addition of PhSH (Fig. 2). Upon the addition of PhSH (40 μM), the fluorescence intensity increases more than 25-fold with the equilibrium constant ($\log K$) being 12.1. And the fluorescence intensity of probe significantly increased as a corresponding function of PhSH concentration at more than 1.25 μM.

The responses of the probe toward relevant aliphatic thiols, reactive sulfur species, common nucleophiles, and amino acid were considered as well. As shown in Figure 3, probe **1** encourages a significant response to aromatic thiols, including thiophenol (PhSH), 4-chlorothiophenol (4-Cl–PhSH), and 2-aminothiophenol

* Corresponding author. Tel./fax: +86 531 8838 2076.
E-mail address: mli@sdu.edu.cn (M. Li).

0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2012.02.098



Scheme 1. Synthesis of probe **1**.

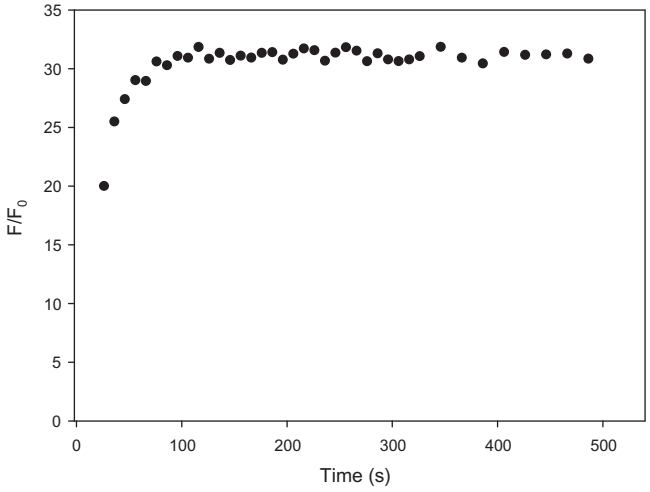


Figure 1. Time course of the interaction of probe **1** (20 μM) with thiophenol in PBS (pH 7.4, 0.2% DMSO as a cosolvent). The concentration of thiophenol is 20 μM. Fluorescence intensity was recorded at 460 nm with λ_{ex} = 340 nm.

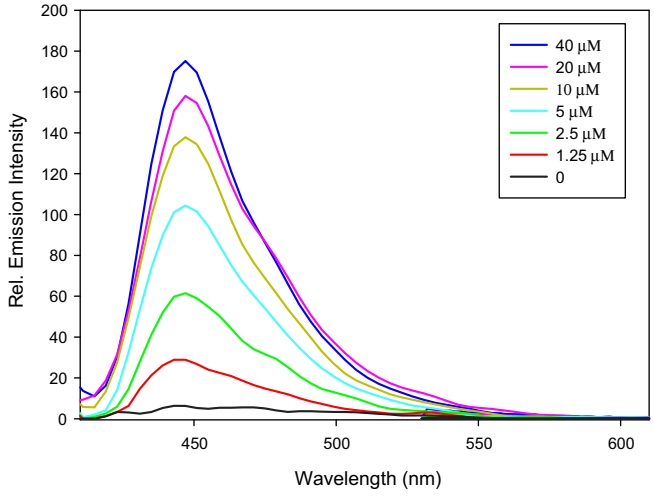


Figure 2. Concentration-dependent emission intensity changes of probe **1** at room temperature: Experiments were conducted in 0.1 M phosphate buffer, pH 7.4. The emission spectra were obtained at 5 min after the addition of PhSH to a 20 μM solution of probe **1** with λ_{ex} = 340 nm.

(2-NH_2–PhSH). In this case, aliphatic thiols, including L-glutathione (GSH), L-cysteine (Cys), and thioglycolic acid (TGA), can also induce weak responses, which are 1/2–1/3 those of aromatic thiols. The aniline and other active sulfur species, including sodium hydrosulfide (NaHS, a well-known donor of H_2S), the sulfide anion and bisulfite, which can also cleave benzenesulfonamide,[5] induced weaker responses (which are 1/3–1/4 those of aliphatic thiols), while the other analytes including common nucleophile (KI), amino acids (L-Gly and L-Glu) did not result in a significant fluorescent response. Considering the possible reaction of DNS-based fluorescent probes with ROS,[6] the fluorescence response to ROS of the probe was also detected. In brief, this probe has no response to KO_2 and H_2O_2.

The implementation of the probe on fluorescent imaging was finally explored in living cells. After incubated with probe **1** (40 μM) for 30 min at 37 °C, human prostate cancer cell line (PC-3) was observed by fluorescence microscopy. As shown in Figure 4a, probe **1** can penetrate cell membrane to react with intracellular thiols, resulting in strong fluorescence emission. Furthermore, PC-3 cells were pretreated with 200 μM *N*-methylmaleimide (NEM) for 0.5 h to reduce the concentration level of biological thiols, and then they were incubated with the probe **1** (40 μM) for another 30 min. Distinct decrease of fluorescence response in PC-3 cells was observed (Fig. 4c and e). These interesting results demonstrated that probe **1** could be used for fluorescent imaging of thiols in living cells.

Additionally, we detected the fluorescent response of **1** toward different ratios of commercial rabbit plasma pretreated with triphenylphosphine which can reduce the disulfides to free thiols.[7] As depicted in Figure S2, the fluorescence intensity undertook linear relationship with the concentration of plasma. Such evidence indicates that probe **1** could detect free thiols in plasma quantitatively.

In conclusion, this report describes a selective benzothiazole-based fluorescent probe with small size and easy synthetic handling for the detection of thiols in cellulo. This probe has significant responses to thiophenols. Aliphatic thiols can also induce slight responses for this probe (1/2–1/3 those of aromatic thiols), while the other competitive analytes exhibit weak or no response. Furthermore, probe **1** is satisfactory for determining cellular thiols, which is substantiated by control experiments (with *N*-methylmaleimide) to be specific toward thiols in living cells. Consequently, the ramifications of this study suggest that this probe may offer a quick, simple, and selective way for the detection of thiols in vitro and vivo.

Acknowledgments

The present work was financed by grants from Shandong Natural Science Foundation (No. JQ201019) and Independent Innovation Foundation of Shandong University, IIFSDU (No. 2010JQ005).

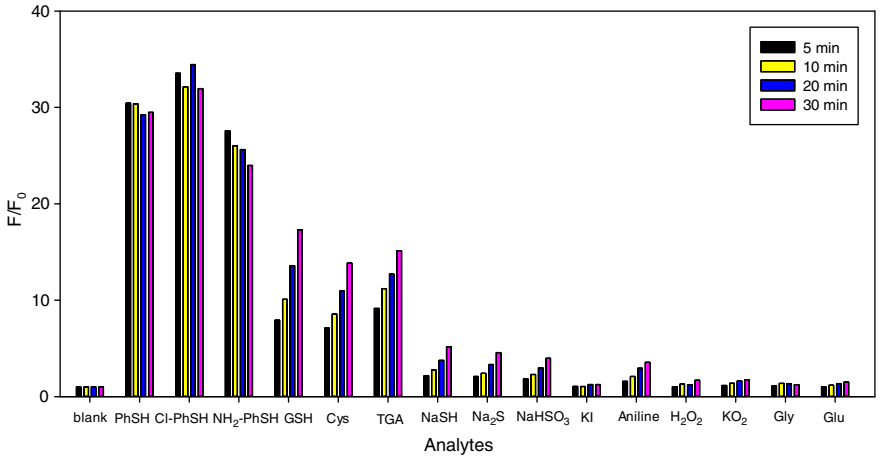


Figure 3. Fluorescence responses of probe **1** (20 μM) to different analytes. Relative florescence intensities of probe before and after incubation with analytes (25 μM) were acquired in 0.1 M phosphate buffer (0.2% DMSO), pH 7.4, and all data were obtained at 450 nm (λ_{ex} = 340 nm).

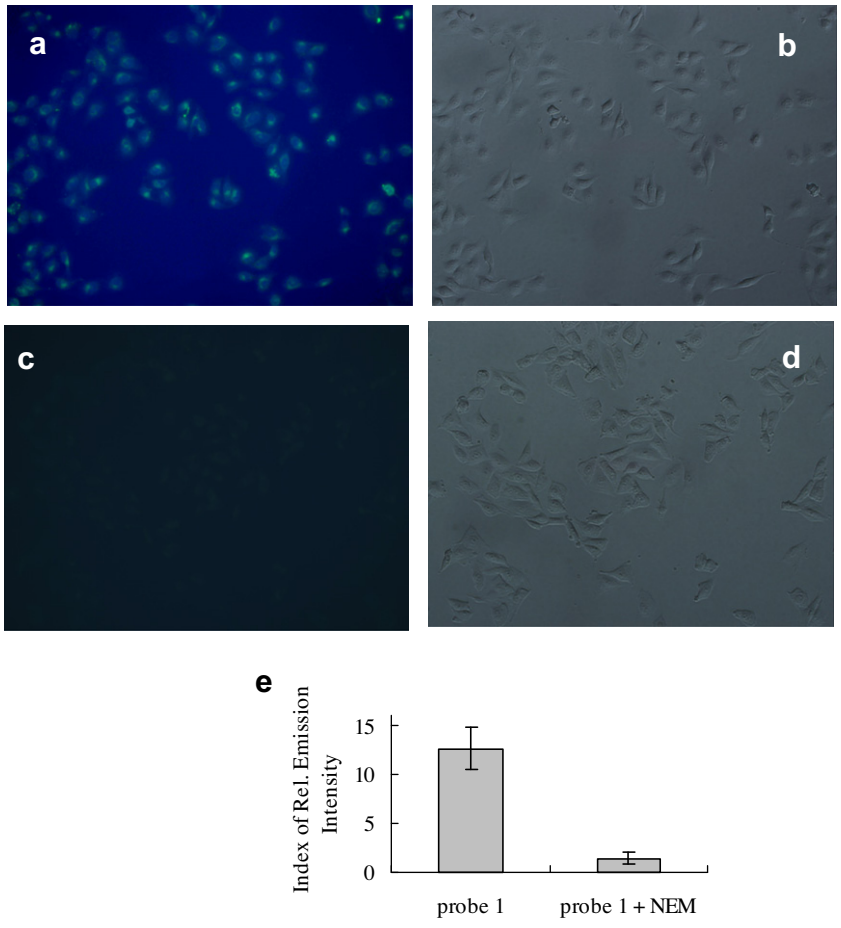


Figure 4. Fluorescence microscopic images of live PC-3 cells. Cells were incubated with probe 1 (40 μM) for 30 min at 37 °C before imaging. (a) Is the fluorescence image of cells incubated with probe **1**; (b) is the corresponding bright field image of (a); (c) represents the fluorescence image of cells incubated with probe **1**, after preincubation with NEM (200 μM) for 30 min; (d) represents the corresponding bright field image of (c); (e) shows the mean fluorescent photons and relative ratio of images (a) and (c) processed by ImageJ software.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2012.02.098. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. (a) Kooistra, T.; Millard, P. C.; Lloyd, J. B. *Biochem. J.* **1982**, *204*, 471–477; (b) Hwang, C.; Sinskey, A. J.; Lodish, H. F. *Science* **1992**, *257*, 1496–1502; (c) Lipton, S. A.; Choi, Y. B.; Takahashi, H.; Zhang, D. X.; Li, W. Z.; Godzik, A.; Bankston, L. A. *Trends Neurosci.* **2002**, *25*, 474–480.

2. (a) Shahrokhian, S. *Anal. Chem.* **2001**, *73*, 5972–5978; (b) Townsend, D. M.; Tew, K. D.; Tapiero, H. *Biomed. Pharmacother.* **2003**, *57*, 145–155; (c) Njålsson, R.; Norgren, S. *Acta Paediatr.* **2005**, *94*, 132–137.
3. (a) Xu, Z.; Chen, X.; Kim, H. N.; Yoon, J. *Chem. Soc. Rev.* **2010**, *39*, 127–137; (b) Cho, D. G.; Sessler, J. L. *Chem. Soc. Rev.* **2009**, *38*, 1647–1662; (c) Blom, H.; Kastrup, L.; Eggeling, C. *Curr. Pharm. Biotechnol.* **2006**, *7*, 51–66; (d) Kim, S. A.; Schwille, P. *Curr. Opin. Neurobiol.* **2003**, *13*, 583–590; (e) de Silva, A. P.; Gunaratne, H. Q.; Gunnlaugsson, T.; Huxley, A. J.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566.
4. (a) Maeda, H.; Matsuno, H.; Ushida, M.; Katayama, K.; Saeki, K.; Itoh, N. *Angew. Chem., Int. Ed.* **2005**, *44*, 2922–2925; (b) Wang, W.; Jiang, W.; Fu, Q. Q.; Fan, H. Y.; Ho, J. *Angew. Chem., Int. Ed.* **2007**, *46*, 8445–8448; (c) Ji, S.; Yang, J.; Yang, Q.; Liu, S.; Chen, M.; Zhao, J. *J. Org. Chem.* **2009**, *74*, 4855–4865; (d) Shao, J.; Guo, H.; Ji, S.; Zhao, J. *Biosens. Bioelectron.* **2011**, *26*, 3012–3017.
5. Yang, X. F.; Wang, L. P.; Zhao, M. L.; Qi, H. P.; Wu, Y. *Chin. J. Chem.* **2010**, *28*, 1469–1474.
6. Maeda, H.; Yamamoto, K.; Nomura, Y.; Kohno, I.; Hafsi, L.; Ueda, N.; Yoshida, S.; Fukuda, M.; Fukuyasu, Y.; Yamauchi, Y.; Itoh, N. *J. Am. Chem. Soc.* **2005**, *127*, 68–69.
7. Shiu, H. Y.; Chong, H. C.; Leung, Y. C.; Wong, M. K.; Che, C. M. *Chem. Eur. J.* **2010**, *16*, 3308–3313.