A highly sensitive fluorescent probe for detection of benzenethiols in environmental samples and living cells†

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A highly sensitive fluorescent probe with a detection limit of 1.8×10^{-9} M was constructed and employed to detect benzenethiols in environmental samples and living cells.

Benzenethiols are widely used in the production of pesticides, polymers, and pharmaceuticals.[1] However, exposure to benzenethiol liquid or vapor can induce grave central nervous system damage and other related systemic injuries including increased respiration, muscular weakness, paralysis of the hind limbs, coma, and even death.[2] Because of the high toxicity of the widely existent benzenethiols and their easy entrance into the human body by inhalation and skin absorption, it is very important to detect benzenethiols both in environmental and biological settings.

Although a number of fluorescent probes for sensing of thiols have been reported,[3–5] these probes cannot clearly discriminate benzenethiols over aliphatic thiols. So far, there is only one example of a selective benzenethiol fluorescent probe.[6] However, this probe has not been successfully applied to monitor benzenethiols in environmental samples and living systems probably due to its limited sensitivity (a detection limit of 2 μM). To the best of our knowledge, no fluorescent probes which are capable of detection of benzenethiols in environmental and biological samples have been previously constructed. In this contribution, we report a new type of highly sensitive and robust fluorescent probe (probe **1**, Fig. 1) for benzenethiols with a much lower detection limit of 1.8×10^{-9} M, over 1000-fold more sensitive than the current one. It is important to note that our new probe is the first selective benzenethiol fluorescent probe that is useful for fluorescence detection of benzenethiols in environmental and biological settings.

Fluorescent thiol probes have been constructed by exploiting the high nucleophilic reactivity[3,4] or transition metal-affinity[5] of the thiol group. In this work, we judiciously designed probe **1** (Fig. 1) as a new type of selective benzenethiol fluorescent probe based on the thiolysis of dinitrophenyl ethers.[7] Notably, although the thiolysis of dinitrobenzenesulfonylamides has been used for benzenethiol probe development,[6] the thiolysis of dinitrophenyl ethers has not previously been exploited in the design of selective benzenethiol fluorescent probes. Probe **1** contains a coumarin dye and a dinitrophenyl ether moiety. It is known that electron donating groups in the 7-position and electron withdrawing groups in the 3-position contribute to the high fluorescence quantum yields of coumarin dyes.[8] We thus anticipated that incorporation of a strong electron withdrawing group such as a dinitrophenyl group[9] in the 7-position of the coumarin fluorophore could significantly diminish the fluorescence. In other words, dinitrophenyl ether **1** should be much weaker than phenolic hydroxyl **2** in fluorescence. However, if dinitrophenyl ether **1** is able to be converted into phenolic hydroxyl **2** by benzenethiols under the appropriate conditions, we should observe a substantial fluorescence turn-on response.

Compound **1** was readily synthesized in only two steps (Scheme S1†). Indeed, as designed, dinitrophenyl ether **1** is essentially non-fluorescent (Φ_f = 0.006, see ESI†)[10] in the neutral aqueous conditions (25 mM sodium phosphate buffer, pH 7.0, containing 45% DMF as a co-solvent), whereas coumarin hydroxyl **2** is highly fluorescent (Φ_f = 0.50) around 494 nm in the same aqueous buffer. Thus, it is apparent that compound **1** is promising as a fluorescence turn-on probe for benzenethiols provided that compound **1** could be converted by benzenethiols to give compound **2**.

Kinetic studies indicate that probe **1** reacted rapidly with benzenethiol at room temperature in neutral aqueous conditions (Fig. S1†). We then proceeded to study the fluorescence response of probe **1** to various concentrations of benzenethiol. Addition of increasing concentrations of benzenethiol elicited a dramatic change in the emission spectra: an intense new peak around 494 nm was observed, and the fluorescence enhancement at 494 nm was up to 165-fold (Fig. 2). Furthermore, the introduction of benzenethiol turned the visual emission of the probe **1** solution from dark to bright green (Fig. 1), which further supports the fluorescence turn-on response. A linear calibration graph of the response to the benzenethiol concentrations from 4.0×10^{-9} M to 3×10^{-6} M was obtained (Fig. S2†), indicating that probe **1** can be potentially employed to quantitatively detect benzenethiol concentrations with a very large dynamic range from the low nanomolar to micromolar range. The detection limit of probe **1** towards

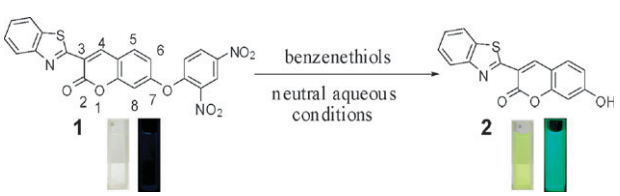

Fig. 1 The design of fluorescent benzenethiol probe **1**. Neutral aqueous conditions: 25 mM sodium phosphate buffer, pH 7.0, containing 45% DMF as a co-solvent.

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† Electronic supplementary information (ESI) available: Experimental procedures and some spectra for probe **1**. See DOI: 10.1039/b922478e

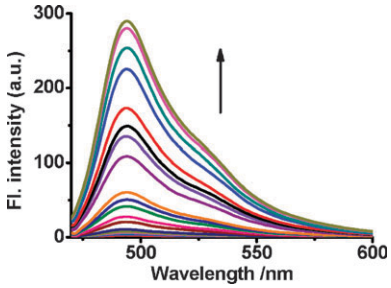

Fig. 2 Fluorescence spectral changes of probe **1** (1 μM) upon addition of increasing concentrations (0–5 equiv.) of benzenethiol (λ_{ex} = 461 nm) in the neutral aqueous conditions (25 mM sodium phosphate buffer, pH 7.0, containing 45% DMF as a co-solvent) for 30 min.

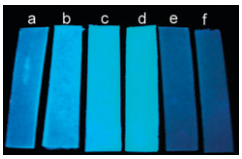

Fig. 3 Photographs of the probe **1** test papers: Test papers a–d were placed in a chemical storage cabinet for 30 min where an uncapped bottle of volatile benzenethiol was stored. As a reference, the test paper e was placed into a chemical storage cabinet where an uncapped bottle of volatile 2-mercaptoethanol was stored. The negative control test paper f was not exposed to either benzenethiol or 2-mercaptoethanol vapor. The test papers a–f were prepared from the probe **1** solution with concentrations of 10, 20, 60, 100, 100, and 100 μM, respectively.

benzenethiol was calculated to be 1.8×10^{-9} M (Fig. S3†). Notably, this detection limit is 3 orders of magnitude more sensitive than the previously reported fluorescent benzenethiol probe.[6]

To confirm that the fluorescence sensing response of the probe to benzenethiol is indeed due to the conversion of probe **1** to compound **2**, the reaction product of probe **1** with benzenethiol was isolated by column chromatography. The ^{1}H NMR spectrum of the isolated product is essentially identical with that of the standard compound **2** (Fig. S4†), in good agreement with the formation of compound **2**. This is further confirmed by the mass spectrometry analysis of the isolated product (Fig. S5a†). In addition, the absorption, emission, and excitation spectra of the isolated product were identical with those of the standard compound **2** (Fig. S6–S7†). Thus, the extensive studies of NMR, mass spectrometry, absorption, emission, and excitation spectroscopy corroborate that indeed, as designed, nonfluorescent probe **1** was transformed by benzenethiol to afford strongly fluorescent compound **2** for a fluorescence turn-on response.

Probe **1** was treated with a wide variety of species to evaluate the selectivity. As expected, the introduction of benzenethiol and its derivatives such as 4-chlorobenzenethiol, 4-bromobenzenethiol, or 4-aminobenzenethiol to the probe **1** solution elicited a significant enhancement in the fluorescent intensity at 494 nm (Fig. S8†). The addition of 4-mercaptobenzoic acid also induced a fluorescence turn-on response with an enhancement less than benzenethiol, probably due to the reduction of the thiol nucleophilicity by the electron withdrawing carboxylic acid group.[6] By contrast, no marked increase in the emission was noted upon addition of the representative amino acids, sugar, vitamins, aromatic alcohols and amines, or aliphatic thiols. Furthermore, the visual response of probe **1** to the various species (Fig. S9†) demonstrates that probe **1** has potential as a naked eye diagnostic tool for the "in-the-field" detection of benzenethiols. As the thiolysis of dinitrophenyl ethers may also occur by treating dinitrophenyl ethers with aliphatic thiols such as 2-mercaptoethanol under basic aqueous conditions,[11] we thus examined the pH effect on the selectivity of benzenethiol over 2-mercaptoethanol. As showed in Fig. S10†, the optimal selectivity was observed near neutral pH. This can be attributed to the distinct pK_a values of benzenethiols (pK_a = 6.5) and aliphatic thiols (pK_a = 8.5), and to the thiolysis of dinitrophenyl ethers proceeding *via* nucleophilic substitution by the nucleophilic thiolate.

As benzenethiol liquid is volatile and the resulting benzenethiol vapor can cause severe headache and irritation, it is of importance to detect benzenethiol vapor. We thus prepared a series of test papers from the probe **1** solutions with different concentrations (see ESI†). The test papers a–d were then placed into a chemical storage cabinet containing an uncapped bottle of benzenethiol. After 30 min, the test papers were removed and were photographed. As expected, test papers a–d exhibited bright emission (Fig. 3). By contrast, test paper e which was kept in a chemical storage cabinet containing an uncapped bottle of volatile 2-mercaptoethanol and the negative control test paper f which was not exposed to benzenethiol vapor showed very faint fluorescence. This indicates that the fluorescence system in the solid support could selectively sense benzenethiol over 2-mercaptoethanol vapor. Thus, probe **1** paper strips show the potential for practical on-site applications in selectively detecting benzenethiol vapor *via* a convenient handheld UV lamp or laser pen in diverse situations.

It is important to monitor the level of benzenethiols in water samples as benzenethiols may be dumped as industrial waste into the environment and thus contaminate water sources. Probe **1** was employed to determine benzenethiol concentrations in water samples from YueLu spring and Xiang River (see ESI†). Added benzenethiol in the water samples could be accurately measured with good recovery (Table S1†), indicating that probe **1** is effective for quantitative detection of benzenethiol in water samples. Furthermore, the observation that benzenethiol with a concentration as low as 5×10^{-9} M could be accurately detected reinforces the high sensitivity of the fluorescence sensing system.

Another concern with possible benzenethiol contamination is soil. To determine whether fluorescent probe **1** could be applied to monitor benzenethiols in soil samples, we carried out a proof-of-concept experiment. As indicated in Fig. 4, the soil sample containing benzenethiol could be readily detected with a large fluorescence signal, while the soil sample containing aliphatic thiols represented by 2-mercaptoethanol caused no marked fluorescence response when compared with the soil blank. This not only indicates that the fluorescence detection system function wells in crude soil samples, but also suggests that the system is useful for selective sensing of benzenethiols over aliphatic thiols even in heterogeneous conditions.

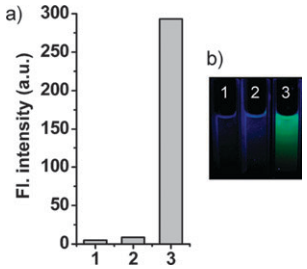

Fig. 4 (a) The fluorescence intensity and (b) fluorescence emission color changes of the probe **1** solution (1 μM) treated with the soil samples for 30 min. Sample 1: the soil blank; sample 2: the 2-mercaptoethanol-contaminated soil; sample 3: the benzenethiol-contaminated soil.

The usefulness of probe **1** for fluorescence imaging of benzenethiol in the living cells was also investigated. When Hela cells pre-treated with benzenethiol in a growth medium were incubated with probe **1** (see ESI†), bright fluorescence could be observed (Fig. 5a). By contrast, in a control experiment, incubation of Hela cells with only probe **1** provided no significant fluorescence (Fig. 5d). These data establish that probe **1** is cell membrane permeable and able to respond to benzenethiol in living cells. In another control experiment, the Hela cells were pre-treated with benzenethiol (1 μM), then incubated with *N*-ethylmaleimide[12] (1 mM, as a thiol-reactive reagent), and further treated with probe **1** (1 μM), no marked fluorescence was noted (Fig. 5e), indicating the selective reaction of probe **1** with benzenethiol.

In summary, we have rationally constructed a novel type of highly sensitive and robust fluorescent probe **1** for benzenethiols *via* the thiolysis of a dinitrophenyl ether. The key features of probe **1** include biocompatible assay conditions, excellent selectivity for benzenethiols over aliphatic thiols, and high sensitivity with a detection limit of 1.8×10^{-9} M. Importantly, we have demonstrated that the new probe is capable of detecting benzenethiol in water, soil, and living cells. In addition, benzenethiol vapor could be selectively detected over aliphatic thiol vapor by probe **1** on a solid support. Thus, probe **1** represents the first fluorescent probe that is useful for sensing benzenethiols in environmental samples and biological systems. One striking character of probe **1** is that the benzenethiol detection can be conveniently conducted by simple visual inspection without resorting to expensive instruments, indicating the practicality of the fluorescence sensing system. We anticipate that the probe will find wide on-site applications in industry, the laboratory, and the environment, and will be an effective chemical tool for studying the toxicity of benzenethiols in living systems at the molecular level.

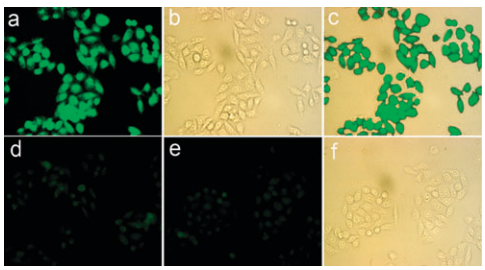

Fig. 5 Fluorescence and brightfield images of cells. (a) Fluorescence image of cells pre-treated with benzenethiol (1 μM) for 30 min and then incubated with probe **1** (1 μM) for 30 min; (b) Brightfield image of cells shown in pane a; (c) Overlay image of (a) and (b); (d) Fluorescence image of cells treated with only probe **1** (1 μM) for 30 min; (e) Fluorescence image of cells pre-treated with benzenethiol (1 μM) for 30 min, then incubated with *N*-ethylmaleimide (1 mM) for 30 min, and further treated with probe **1** (1 μM) for 30 min; (f) Brightfield image of cells shown in pane e.

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