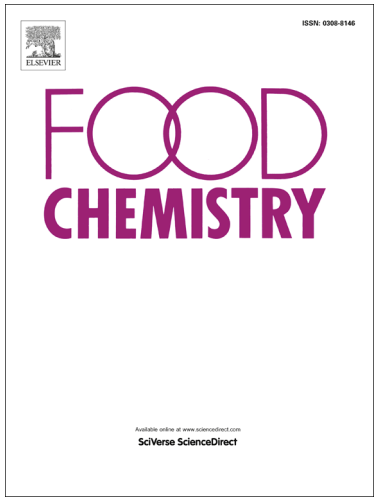

Analytical Methods

A rapid and visible colorimetric fluorescent probe for benzenethiol flavor detection

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A rapid and visible colorimetric fluorescent probe for benzenethiol flavor detection

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Abstract: Benzenethiols are a class of flavoring ingredients used in the food, pharmaceutical, cosmetics and chemical industries. A rapid and visible colorimetric fluorescent probe was developed for the detection of benzenethiol flavors. It provides rapid quantitative detection of benzenethiols at low levels, down to a limit of 10 nM. Test paper containing the probe changes color according to benzenethiol concentration (from colorless to pink, visible with the naked eye). The probe was also successfully used to test benzenethiol concentrations in real food samples. This study demonstrates that this novel probe can be employed as a benzenethiol testing tool.

Keywords: Benzenethiol flavors; visible colorimetric; fluorescent probe

Highlights:

Development of a new, rapid, visible colorimetric fluorescent probe for benzenethiols.
The detection limit of this fluorescent probe is 10 nM.
Benzenethiol was successfully detected in real samples using the new probe.

1. Introduction

Benzenethiols are a class of flavoring ingredients with similar chemical properties (Chinese Standards for Food Additives, 2015; Liu, & Sun, 2018). There are widely used in the food, pharmaceutical, cosmetics and chemical industries (Jung, Chen, Kim, & Yoon, 2013). Benzenethiols are recognized by the Flavor and Extract Manufacturers Association (FEMA) as being "generally recognized as safe", and include benzenethiol (FEMA 3616) (Oser, & Ford, 1979), o-toluenethiol (FEMA 3240) (Hall, & Oser, 1970), 2-ethylthiophenol (FEMA 3345) (Oser, & Ford, 1973), 2,6-dimethylthiophenol (FEMA 3666) (Oser, Ford, & Bernard, 1984) and 2-mercaptoanisole (FEMA 4159) (Smith, et al., 2003). The odors of benzenethiol, o-toluenethiol and 2-mercaptoanisole are considered meaty, but ethylthiophenol and 2,6-dimethylthiophenol are sulfurous. The odor description of benzenethiol is meaty, sulfuraceous, phenolic, rubbery and allicious (Oser, & Ford, 1979), while o-toluenethiol is meaty, smoked, sausage-like, smoky and phenolic (Hall, & Oser, 1970). 2-ethylthiophenol is sulfurous, roasted, smoky and meaty (Oser, & Ford, 1973), 2,6-dimethylthiophenol is sulfurous, roasted, meaty, metallic and phenolic (Oser, Ford, & Bernard, 1984), and 2-mercaptoanisole is meaty, smoked, sausage-like, smoky, and phenolic (Smith, et al., 2003). They are primarily useful for the preparation of onion, garlic, meat and nut fragrances. The maximized survey-derived daily intake of benzenethiol is 0.73 μg/person/day (Oser, & Ford, 1979), while that of o-toluenethiol is 17.00 μg/person/day (Hall, & Oser, 1970), 2-ethylthiophenol is 0.00012 μg/person/day (Oser, & Ford, 1973), 2,6-dimethylthiophenol is 1.30 μg/person/day (Oser, Ford, & Bernard, 1984), and 2-mercaptoanisole is 1.50 μg/person/day (Smith, et al., 2003). Excessive amounts of benzenethiols can induce central nervous system damage, vomiting, muscular weakness, coma, and even death (Amrolia, Sullivan, Stern, & Munday, 1989; Chen, Tang, & Lin, 2016; Pagidi, Kalluvettukuzhy, & Thilagar, 2018; Xiong, et al., 2017). The lethal dose (LD_{50}) of o-toluenethiol for mice is 100 mg/kg (Fairchild, & Stokinger, 1959; Meek, & Pettit, 1985). The LD_{50} of 2,6-dimethylthiophenol for rats is 3150 mg/kg (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), 2012). The LD_{50} of benzenethiol is 46 mg/kg for rats (Fairchild, & Stokinger, 1958), 24 mg/kg for birds and 25 mg/kg for mice (Schafer, 1972). The LD_{50} of 2-mercaptoanisole is 1560 mg/kg for mice and 1740 mg/kg for rats (Ioannides, Delaforge, & Parke, 1981). Hence, it is of great significance to develop a sensitive,

high selectivity, simple and rapid method of detecting benzenethiol flavors.

Until now, many methods for benzenethiol detection have been developed, such as gas chromatography (GC) (Beiner, Popp, & Wennrich, 2002), high performance liquid chromatography (HPLC) (Sun, et al., 2016) and nanomaterial-based sensors (Dreyer, et al., 2011; Zhao, et al., 2011). Recently, fluorescent detection has attracted interest due to its simplicity, high sensitivity, short response time and ease of observation (Chan, Dodani, & Chang, 2012; Collot, et al., 2018; Denis, et al., 2018; Wang, et al., 2018b). The first fluorescent probe for recognizing thiophenols was reported in 2007 based on a nucleophile substitution reaction mechanism (Jiang, et al., 2007). Up to now, many thiophenol fluorescent probes have been reported (Chen, Zhou, Peng, & Yoon, 2010; Guo, et al., 2018; Hong, Xia, Feng, & Feng, 2018; Lin, et al., 2017; Ma, et al., 2016; Wang, et al., 2018a; Yao, et al., 2018), but most have been designed and used for biological imaging. A fluorescent probe for benzenethiol flavors has not yet been reported. Most thiophenol probes still have shortcoming; for example, they may be unable to detect low concentrations of thiophenols due to having low sensitivity, slow fluorescent turn-on response and lack of obvious color changes, and have slow response speeds. Thus, there is a high demand for colorimetric fluorescent probes that can rapidly detect benzenethiol flavors with high sensitivity.

In order to find a more sensitive and visible colorimetric benzenethiol flavor fluorescent probe, a new probe based on (*E*)-6-(3-(4-nitrophenyl)-3-oxoprop-1-enyl)naphthalen-2-yl 2,4-dinitrobenzenesulfonate (Probe 1) is reported in this work. It uses (4-nitrophenyl)-3-oxoprop-1-enyl)naphthalene as the fluorophore, and 2,4-dinitrobenzenesulfonate as the reaction group. Probe 1 shows responsive and visible colorimetrics for benzenethiol with the naked eye, and has a low detection limit for benzenethiol flavors. In particular, the Probe 1 test paper reacts with different concentrations of benzenethiol to produce observable color changes. Hence, the Probe 1 test paper could be employed as a powerful tool for the detection of benzenethiol flavors.

2. Materials and methods

2.1 Chemicals

The chemicals 2-bromo-4’-nitroacetophenone (99%), trimethylamine (99%), triphenylphosphine (99%), 6-hydroxy-2-naphthaldehyde (98%), 2,4-dinitrobenzenesulfonyl chloride (98%), glutathione (GSH, 98%), hydrazine

hydrate (N_2H_4, 99%), cysteine (Cys, 99%), benzenethiol (PhSH, 99%), 2,6-dimethylthiophenol (DMTP, 99%), 2-methylbenzenethiol (2-MBT, 99%), allyl mercaptan (AMCT, 98%), mercaptopropionic acid (MCTP, 98%) and trimethylamine (99%) were purchased from Bailing Wei (Beijing, China). The analytically pure reagents sodium sulfide (Na_2S), $MgCl_2$ (magnesium chloride), KCl (potassium chloride), sodium bromide (NaBr), sodium dihydrogen phosphate (NaH_2PO_4), sodium chloride (NaCl), sodium sulfite (Na_2SO_3), sodium fluoride (NaF), sodium hydrogen sulfite ($NaHSO_3$), sodium sulfate (Na_2SO_4), potassium iodide (KI), tetrahydrofuran (THF), N,N-dimethylformamide (DMF), methanol (CH_3OH) and ethanol (C_2H_5OH) were obtained from Innochem Science and Technology Co., Ltd. (Beijing, China).

2.2 Instruments

Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AV 300 MHz NMR machine. High resolution mass spectrometer (HRMS) spectra were implemented on a Bulu Ke Apex IV Fourier-Transform Mass Spectrometer (FTMS). Fluorescence spectra were obtained on a Rili F-4600 fluorescence spectrometer.

2.3 Preparation of probe 1

As illustrated in scheme 1, 2-bromo-4’-nitroacetophenone (Compound 1; 4.88 g, 20 mmol) and triphenylphosphine (Compound 2; 5.24 g, 20 mmol) were dissolved in tetrahydrofuran (50 mL). The mixture was heated to reflux for 4 h, then cooled to 25 °C to obtain compound 3. NaOH (2 M, 20 mL), methanol (24 mL) and H_2O (28 mL) were added. The mixture was stirred at 25 °C overnight, then Compound 4 (a yellow solid) was obtained by filtration.

6-Hydroxy-2-naphthaldehyde (Compound 5; 1.00 g, 5.8 mmol) and trimethylamine (0.61 g) were dissolved in DMF (10 mL), then placed in an ice bath. 2,4-dinitrobenzenesulfonyl chloride (Compound 6; 3.09g, 11.6 mmol) in DMF (10 mL) was slowly added. The reaction mixture was stirred for 3 h at 40 °C. The precipitate was collected by filtration, and then recrystallized from ethanol to give Compound 7. Then, Compound 7 (0.50 g, 1.2 mmol) and Compound 4 (0.63 g, 1.5 mmol) were dissolved in tetrahydrofuran (25 mL), refluxed for 8 h, distilled and recrystallized from $CHCl_3$ to obtain Probe 1 (0.32 g, Scheme 1).

2.4 Preparation of solutions of probe **1** and analytes

Ethanol, as a reagent, was used to dissolve Probe 1, PhSH, Cys, DMTP, GSH, AMCT, MCTP and 2-MBT. After mixing, a Probe 1 stock solution was obtained. The analytes Na_2S, $MgCl_2$, KCl, NaBr, NaH_2PO_4, NaCl, Na_2SO_3, NaF, $NaHSO_3$, Na_2SO_4,

KI and N_2H_4 were dissolved in distilled water to obtained 10 mM aqueous solutions. Various concentrations could be obtained by diluting these stock solutions with distilled water (Wang, et al., 2018b).

2.5 Preparation of real-world samples

Mineral water (bottle, 500 mL), coca cola (bottle, 500 mL), orange juice (bottle, 500 mL) and ham sausage (150 g) were purchased from wumei supermarket (Beijing, P.R. China). The solid ham sausage sample was accurately quantified (100.00 g), then chop up, ultrasonic extraction with ethanol (50 mL), filtered, concentration under vacuum to 1 mL. A 20 μL sample solution was used for the determination. Different concentrations of benzenethiol were added, and the 511 nm fluorescence signals of samples were recorded.

2.6 The procedures of benzenethiols determination

Preparation of the test system: 0.02 mL probe solution was added to a cuvette. Then, ethanol was poured into the cuvette to make up a volume of 2 mL. Finally, ion solution was added. After waiting a few minutes, it was mixed completely. Samples of mixture were analyzed in the fluorescence spectrometer using the conditions of λ_{ex} = 356 nm, λ_{em} = 511 nm, temperature = 25 °C, voltage = 700 V, and slit widths = 5 nm, 5 nm.

3. Results and Discussion

3.1 Probe synthesis

Probe 1 was synthesized by a four-step reaction (Scheme 1). First, the intermediate wittig reagent (Compound 4) was prepared by nucleophile substitution and elimination reactions. Second, intermediate Compound 7 was obtained from a nucleophile substitution reaction between Compounds 5 and 6. Lastly, Probe 1 was created from a wittig reaction of Compounds 4 and 7. This synthetic process and purification by recrystallization are classic organic chemistry methods. Probe 1 used ^{13}C NMR, HRMS and ^{1}H NMR for confirmation (Figs. A1-A3).

^{1}H NMR (300 MHz, DMSO), δ (ppm): 9.15 (d, *J* = 2.2 Hz, 1H), 8.59 (dd, *J* = 8.7, 2.3 Hz, 1H), 8.43 (d, *J* = 14.0 Hz, 5H), 8.33 – 7.79 (m, 8H), 7.38 (dd, *J* = 8.9, 2.4 Hz, 1H); ^{13}C NMR (75 MHz, DMSO), δ (ppm): 191.57, 154.09, 137.37, 133.83, 132.74, 130.63, 128.89, 128.65, 124.55, 122.19, 120.65, 119.75, 116.85; HRMS (ESI): calcd. for $[M-H]^+$ 548.039441, found 548.039739.

3.2 Sensing properties of Probe 1 towards benzenethiol

The benzenethiol flavors have similar chemical characteristics. Benzenethiol

(PhSH), 2,6-dimethylthiophenol (DMTP) and 2-methylbenzenethiol (2-MBT) were obtained for this work. Benzenethiol (PhSH) was selected as a typical benzenethiol flavor for testing the sensing properties of Probe 1 towards benzenethiol flavors.

The fluorescent response of Probe 1 to PhSH at different pHs from 3.0 to 10.0 was investigated (Fig. 1a). The fluorescent intensity of Probe 1 did not change from pH 3.0 to 7.4, but increased from pH 7.4 to 10.0. However, as PhSH was added, the fluorescent intensity of Probe 1 increased rapidly from pH 3.0 to 5.0, then stayed almost constant from pH 5.0 to 7.4, before increasing again from pH 7.4 to 10.0. The fluorescence intensity showed the greatest difference between Probe 1 and Probe 1-PhSH at pH 7.4; hence, pH-neutral conditions have the advantage of stabilizing benzenethiols. Therefore, pH 7.4 was selected as the most suitable condition for the subsequent experiments.

The fluorescence response of Probe 1 (10 μM) to different concentrations of PhSH (0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM, 1.0 μM) was verified in 10 mM phosphate buffer saline (PBS; pH 7.4) with ethanol (v:v = 1:3) at 25 °C (Fig. 1b). As different concentrations of PhSH were added, the fluorescence intensity increased almost 2.5 times. The fluorescence signal at 511 nm increased constantly until 90 s, showing that Probe 1 only needs 90 s to respond to PhSH. The results suggest that Probe 1 is suitable for use for the rapid detection of PhSH.

The solution of Probe 1 in PBS (pH 7.4) with ethanol (v:v = 1:3) was added to different concentrations of PhSH (0.0 μM, 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM, 1.0 μM, 10 μM, 20 μM) and changes in fluorescence intensity were observed (Fig. 1c). The intensity of the highest fluorescence emission peak at 511 nm increased with the addition of PhSH (Fig. 1d). The fluorescence intensity was linearly correlated with concentrations of PhSH ranging from 0–1 μM (Fig. 1e). The detection limit (LOD) of Probe 1 for PhSH was 12 nM, based on LOD = 3 SD/B according to the definition of IUPAC (LOD =3SD/B, SD represents standard deviation of the blank solution and B is the slope of the linear regression) (Wang, et al., 2018b). These results suggest that Probe 1 is an appropriate tool for the quantitative detection of PhSH at low concentrations.

Benzenethiol could be detected by using paper strips containing Probe 1. Waterman filter paper was cut into 1 cm × 1 cm pieces and dipped in a 1 mM DMSO solution of Probe 1. The paper strips were then dried in a vacuum drying oven at 50 °C to rapidly evaporate the solvent (Xiong, et al., 2017). The prepared test paper was then placed

on a glass plate. 20 μL of PhSH of different concentrations (0.00 μM, 5 μM, 10 μM, 50 μM, 100 μM,) were added. After 30 s, the color of the test paper changed according to the concentration of PhSH, as observed by the naked eye under natural light or 254 nm UV light (Fig. 1f). All results indicate that Probe 1 can be applied as a sensor for the rapid detection of PhSH at concentrations of 0.00 mM, 0.05 mM, 0.10 mM, 0.50 mM and 1.00 mM.

To explore the detection sensitivity of Probe 1 for PhSH, various compounds were tested, including H_2S, HSO_3^-, N_2H_4, GSH, H_2O_2, Mg^{2+}, Na^+, K^+, SO_4^{2-}, Cl^-, F^-, Br^-, I^-, SO_3^{2-}, $H_2PO_4^-$, Cys, allyl mercaptan, mercaptopropionic acid, methylbenzenethiol and 2,6-dimethylthiophenol. As shown in Figure 1g, these competitors exhibited limited fluorescence responses, except for AMCT, 2-MBT and DMTP. The competitors MCTP, H_2S, GSH and Cys, which contain a sulfhydryl group, had no effect on PhSH. At the same time, competition experiments were conducted by adding PhSH to Probe 1 solutions containing the above competitors. The fluorescence response of Probe 1 was similar for the PhSH and PhSH + competitor samples. The fluorescence emission intensity of Probe 1 (10 μM) to 2-MBT was similar with PhSH, the fluorescence emission intensity of AMCT and DMTP was almost 10% less than PhSH. This indicates that Probe 1 is highly selective for benzenethiols.

2,6-dimethylthiophenol (DMTP) and 2-methylbenzenethiol (2-MBT) have similar chemical characteristics to PhSH (Liu, & Sun, 2018). Reaction times, and the relationship between fluorescence intensity and DMTP and 2-MBT concentrations using Probe 1, were examined at the same time. The fluorescence response of Probe 1 (10 μM) to different concentrations of DMTP (0.1μM, 0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM) was verified in 10 mM phosphate buffer saline (PBS; pH 7.4) in ethanol at 25 °C (Fig. 2a). The results show that Probe 1 needs 320 s to respond to DMTP. The fluorescence response of Probe 1 (10 μM) to different concentrations of 2-MBT (0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM, 1.0 μM) was verified under the same conditions (Fig. 2b). The results show that Probe 1 needs 75 s to respond to 2-MBT. The reaction rates of PhSH, DMTP and 2-MBT with Probe 1 were ranked 2-MBT > PhSH > DMTP. As this reaction is a nucleophile substitution reaction, the reaction rate is determined by the nucleophiles of the thiol group. Methyl groups are electron donating, such that the nucleophilicity of 2-MBT was increased. However, due to the steric effect, the nucleophilicity of DMTP was decreased.

The intensity of the highest fluorescence peak at 511 nm increased with addition of

DMTP (Fig. 2c) and 2-MBT (Fig. 2d). The fluorescence intensity was linearly related to the concentration of DMTP in the range 0–0.8 μM (Fig. 2e), and the LOD of Probe 1 for DMTP was 15 nM. A linear relationship between 2-MBT concentration and fluorescence intensity was found in the 0–1.0 μM range (Fig. 2f), and the LOD of Probe 1 for 2-MBT was 7 nM. These results suggest that Probe 1 is a wonderful tool for the quantitative detection of low levels of DMTP and 2-MBT.

3.3 Reaction mechanism

A possible response mechanism may contribute to the thiolysis of the 2, 4-dinitrobenzenesulfonyl group of Probe 1 via a nucleophile substitution reaction that generates Compound 8, (2,4-dinitrophenyl) (phenyl)sulfane (Compound 9) and SO_2 (Compound 10), as shown in Scheme 2 (Wang, et al., 2018b). To verify this response mechanism, Probe 1's reaction with PhSH was analyzed by MS. A peak at m/z = 318.30 was observed, which correlates with the formation of Compound 8 (Fig. A4). A peak at m/z = 277.36 correlates with the formation of Compound 9 (Fig. A5). The results suggest that the mechanism by which Probe 1 senses PhSH is most likely based on the thiolysis of the 2, 4-dinitrobenzenesulfonyl moiety.

3.4 Detection of PhSH in real samples

The results thus far indicate that Probe 1 has good recognition for PhSH in complex environments. The ability of Probe 1 to detect PhSH in real samples was demonstrated to prove its applicability. Four kinds of real samples (mineral water; Coca Cola; orange juice; ham sausage; 20 μL) were added to Probe 1 solutions (10 μM). Then, different amounts of PhSH (0.2 μM and 0.4 μM) were added due to the detection limit of Probe 1 was 10 nM and the linear ranges were from 0–1 μM. As shown in Table 1, amounts of 0, 0, 0 and 0.0618 μM PhSH were found in the four kinds of real samples (mineral water; Coca Cola; orange juice; ham sausage). Hence, Probe 1 could detect PhSH in these four real samples. The recovery values of 94.40 % to 101.0 % show that Probe 1 has good ability to detect PhSH in real samples (mineral water; Coca Cola; orange juice; ham sausage). The results show that Probe 1 is a feasible and practical testing method for detecting PhSH in real samples. As DMTP, 2-MBT, 2-ETP and 2-MP have similar chemical characteristics to PhSH, Probe 1 could be expected to detect concentrations of DMTP, 2-MBT, 2-ETP and 2-MP in real samples.

Most existing thiolphenol fluorescent probes have been designed and used for biological imaging (Chen, Zhou, Peng, & Yoon, 2010; Guo, et al., 2018; Hong, Xia,

Feng, & Feng, 2018; Lin, et al., 2017; Ma, et al., 2016; Wang, et al., 2018a; Yao, et al., 2018). A fluorescent probe for benzenethiol flavor detection has not been reported before. Probe 1 is a novel fluorescent probe that is very easy to synthesize. Paper strips containing Probe 1 changed color in 30 s when exposed to different concentrations of PhSH. These changes could be observed by the naked eye under natural light or 254 nm UV light. In addition, this visual change shows that Probe 1 can be used to develop a naked eye detection tool for the detection of PhSH. Furthermore, Probe 1 was successfully used to detect PhSH concentrations in real samples.

4. Conclusions

In summary, a rapid and visible colorimetric fluorescent probe for detecting PhSH was developed. The function of Probe 1 relies on the nucleophile substitution reaction of Probe 1 with PhSH, which generates (2,4-dinitrophenyl)(phenyl)sulfane, as verified by MS. Probe 1 is a rapid and useful quantitative tool for detecting benzenethiol flavors in low concentrations. Importantly, Probe 1 test paper reacted with different concentrations of PhSH to produce different colors, changing gradually from colorless to pink. This indicates that Probe 1 could be employed as a testing tool for PhSH. Furthermore, our work shows that Probe 1 is useful for testing PhSH levels in real-world samples.

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Appendix A. Supplementary data

HRMS, ^{13}C NMR and ^{1}H NMR spectra of probe **1**; MS spectra of compounds 8 and 9.

Declaration of Interest Statement

Declarations of interest: none. The authors declare no competing financial interest.

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Lists of Figures and Tables

Scheme 1. Synthesis of Probe 1.

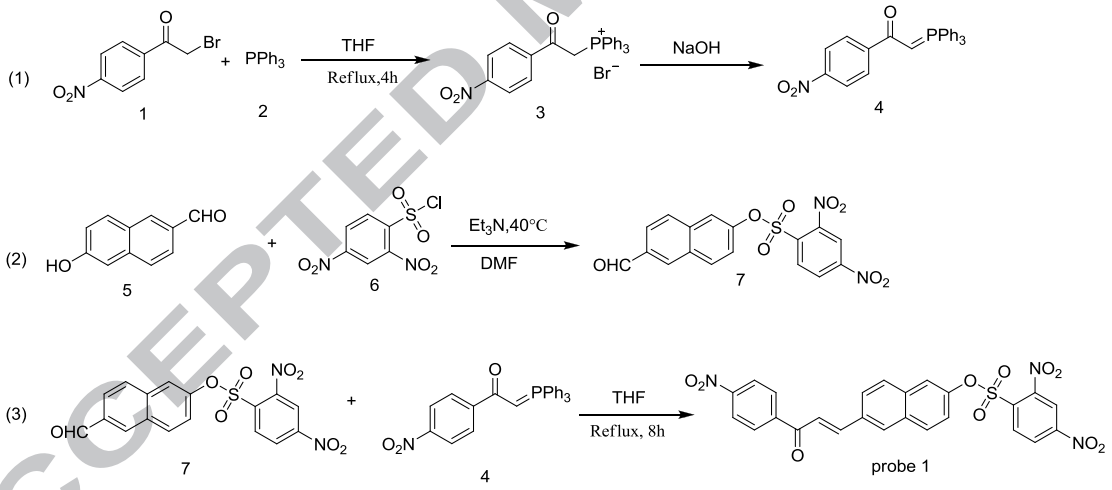
Scheme 2. The mechanism for reaction of Probe 1 with PhSH.

Figure 1. (a) Fluorescent responses of Probe 1 (10 μM) added to PhSH (300 μM) in buffer solution with pHs of 3–10). Tests were performed in triplicate. (b) Time-dependent fluorescence spectra of Probe 1 (10 μM) in the presence of PhSH (0.2, 0.4, 0.6, 0.8, 1.0 μM) in PBS (pH 7.4) with ethanol (v/v, 1:3) at 25 °C. Tests were performed in triplicate. (c) Fluorescence spectra of Probe 1 (10 μM) with PhSH (0, 0.2, 0.4, 0.6, 0.8, 1.0, 10, 20 μM). (d) Fluorescence intensity of Probe 1 (10 μM) with PhSH (0, 0.2, 0.4, 0.6, 0.8, 1.0, 10, 20 μM). (e) Plot of fluorescence intensity differences with PhSH amounts of 0 to 1.0 μM. (f) Photograph of Probe 1 paper strips subjected to PhSH (0, 5, 10, 50, 100 μM) under ambient light and 254 nm UV light. (g) Fluorescence intensity change of Probe 1 (10 μM) upon addition of various species (10 μM for each. 1, blank; 2, H_2S; 3, HSO_3^-; 4, N_2H_4; 5, GSH; 6, H_2O_2; 7, Mg^{2+}; 8, Na^+; 9, K^+; 10, SO_4^{2-}; 11, Cl^-; 12, F^-; 13, Br^-; 14, I^-; 15, SO_3^{2-}; 16, $H_2PO_4^-$; 17, Cys; 18, 2-MBT; 19, DMTP; 20, AMCT; 21, MCTP. 10 μM for PhSH). Tests were performed in triplicate.

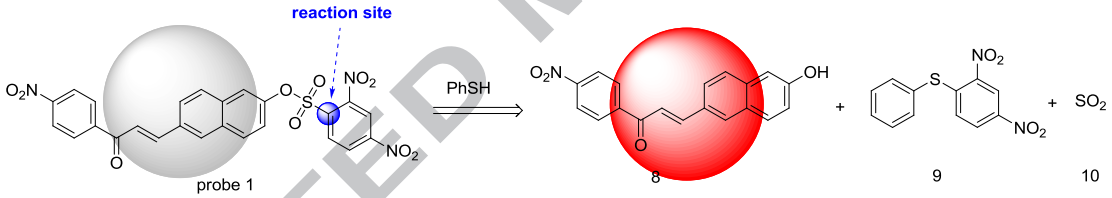
Figure 2. (a) Time-dependent fluorescence spectra of Probe 1 (10 μM) in the presence of DMTP (0.1, 0.2, 0.4, 0.6, 0.8 μM) in PBS (pH 7.4) with ethanol (v/v, 3:1) at 25 °C.

Tests were performed in triplicate. (b) Time-dependent fluorescence spectra of Probe 1 (10 μM) in the presence of 2-MBT (0.2, 0.4, 0.6, 0.8, 1.0 μM) in PBS (pH 7.4) with ethanol (v/v, 3:1) at 25 °C. Tests were performed in triplicate. (c) Fluorescence spectra of Probe 1 (10 μM) with DMTP (0, 0.1, 0.2, 0.4, 0.6, 0.8 μM). (d) Fluorescence spectra of Probe 1 (10 μM) with 2-MBT (0, 0.2, 0.4, 0.6, 0.8, 1.0 μM). (e) Plot of fluorescence intensity differences with DMTP ranging from 0 to 0.8 μM. (f) Plot of fluorescence intensity difference with 2-MBT ranging from 0 to 1.0 μM. Tests were performed in triplicate.

Table 1. Determination of benzenethiol concentrations in real samples



Scheme 1.



Scheme 2.

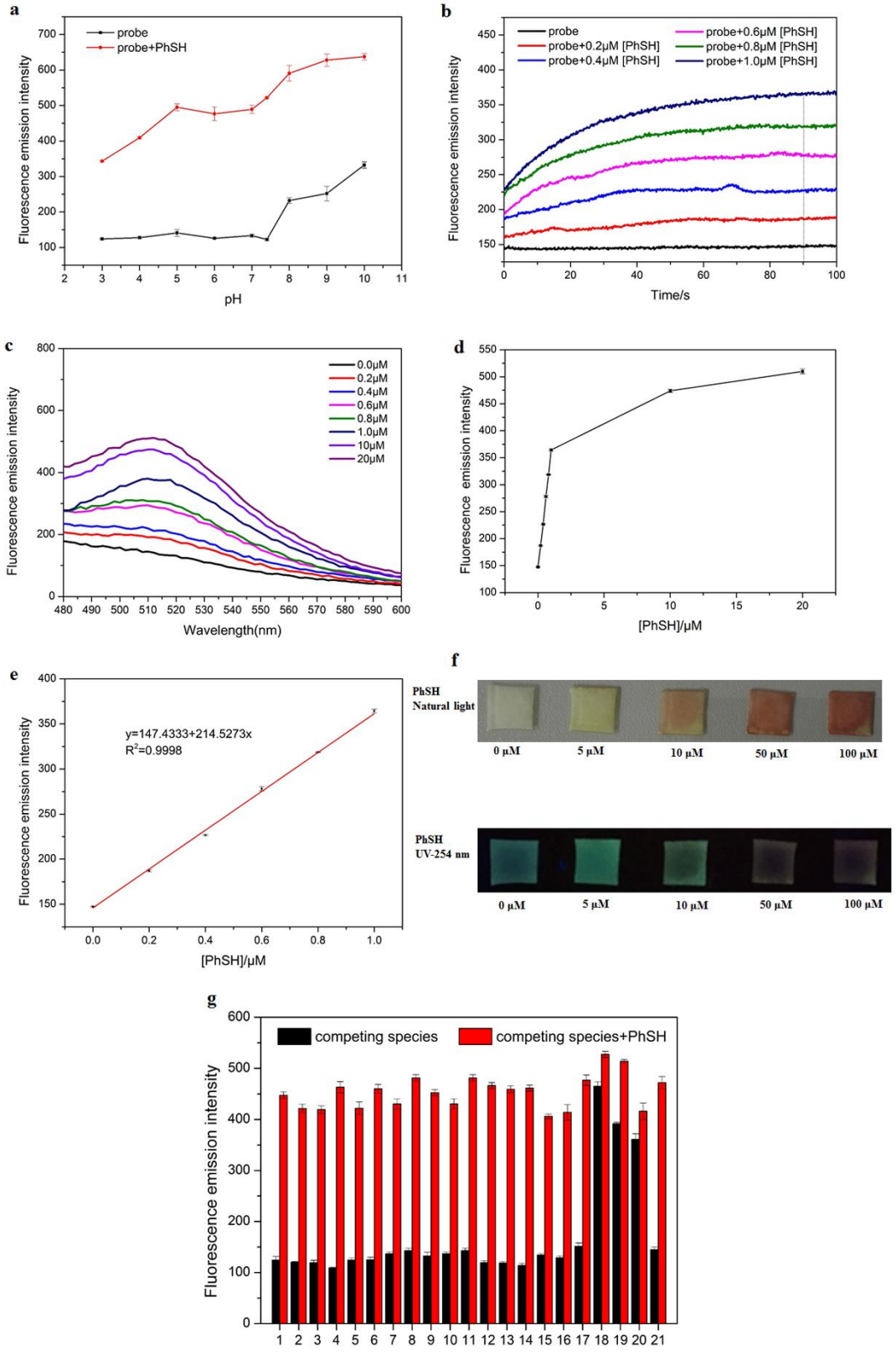


Figure 1.

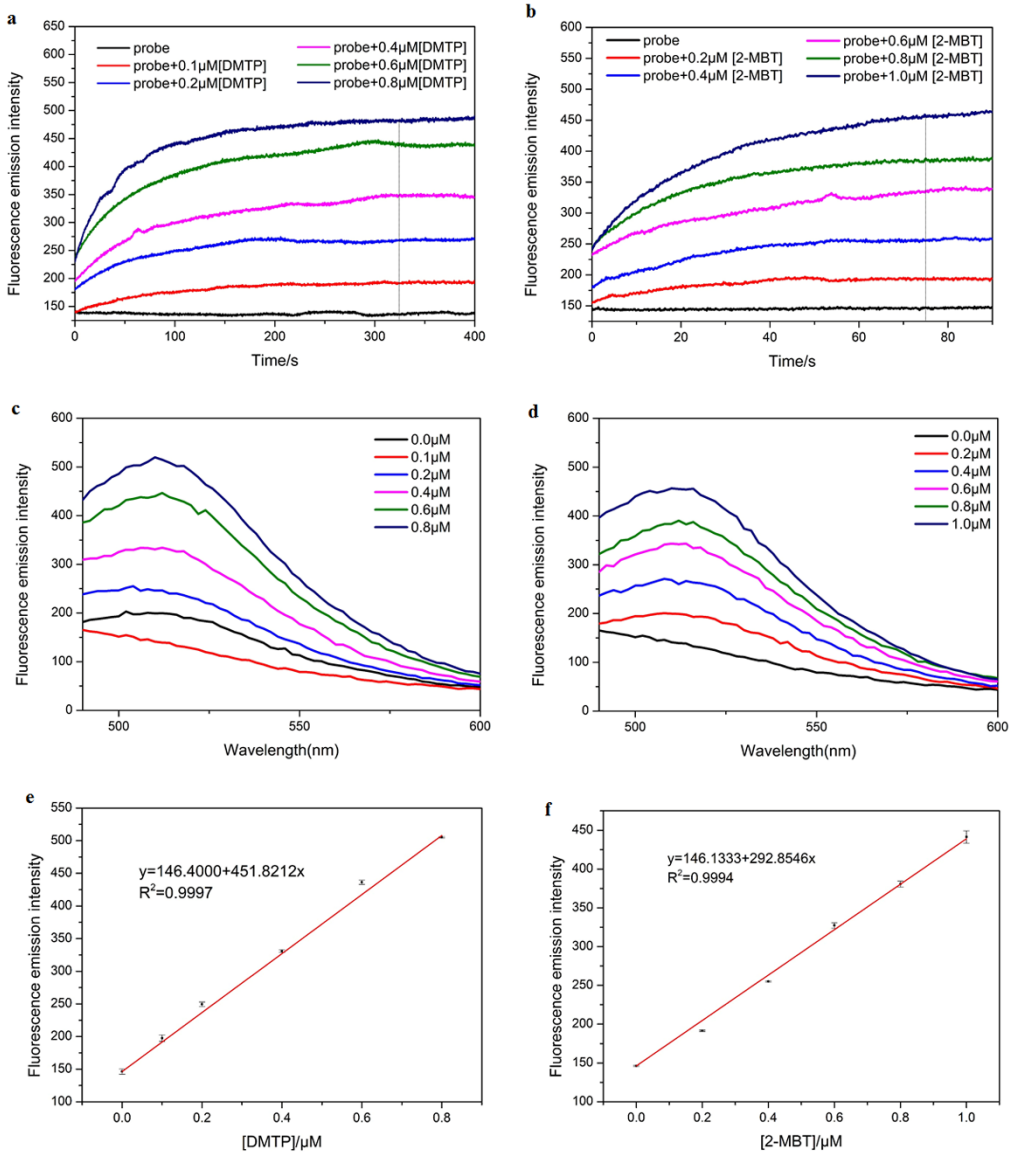


Figure 2.

Sample	PhSH amount originally present (μM)	PhSH amount added (μM)	PhSH amount detected (μM)	Recovery	s
Mineral water	0.0000	0.2000	0.2020	101.0%	0.0061
		0.4000	0.4012	100.3%	0.0037
Coca Cola	0.0000	0.2000	0.2064	103.2%	0.0046
		0.4000	0.3996	99.90%	0.0038
Orange juice	0.0000	0.2000	0.1924	96.20%	0.0057
		0.4000	0.3817	95.43%	0.0028
Ham sausage	0.0618	0.2000	0.2506	94.40%	0.0038
		0.4000	0.4595	99.43%	0.0045

Tests were performed in triplicate. s, standard deviation.

Table 1.

Highlights:

Development of a new, rapid, visible colorimetric fluorescent probe for benzenethiols.

The detection limit of this fluorescent probe is 10 nM.

Benzenethiol was successfully detected in real samples using the new probe.