Fluorescent Probes

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A Highly Selective Fluorescent Probe for Thiophenols**

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The development of highly sensitive and selective detection techniques for the discrimination of relevant biologically active and toxic molecules is of considerable importance in the fields of chemical, biological, and environmental sciences. Thiols are an important class of molecules in biological systems and chemical science. Aliphatic thiols are found in several biologically important molecules including cysteine,[1] homocysteine,[2] and glutathione,[3] which are associated with a wide range of biological functions, while thiophenols, in spite of their broad synthetic utility,[4] are a class of highly toxic and pollutant compounds.

The study of the toxicity of thiols in fish reveals that the median lethal dose (LC_{50}) values range from 0.01 to 0.4 mM.[5] Generally, thiophenols are more toxic than aliphatic alcohols.[6] Symptoms of exposure include a burning sensation, coughing, wheezing, laryngitis, shortness of breath, headache, nausea, and vomiting through targeting of the central nervous system, kidney, and liver.[7] In a worst-case scenario, they can result in death. The main sources of the production of toxic and pollutant thiophenols include oil and coal refineries,[8] the plastics and rubber industry,[9] and waste-deposit landfills.[10]

A variety of detection methods,[11] including sensitive fluorescent probes, have been reported.[12–14] However, the examination of these probes reveals that most of them suffer from poor selectivity toward aliphatic thiols and thiophenols. Accordingly, a fluorescent reagent that enables thiophenols and aliphatic thiols to be selectively differentiated is needed. It is fully realized that the design of such fluorescent reagents is a challenging task because of the similar chemical profiles of aliphatic thiols and thiophenols. To the best of our knowledge, such a probe has not been described before. Herein, we report a novel, sensitive fluorescent probe for the highly selective detection of thiophenols. The studies demonstrated that a >50-fold fluorescence intensity enhancement is observed for thiophenols, but there is no gain for aliphatic thiols under neutral aqueous conditions.

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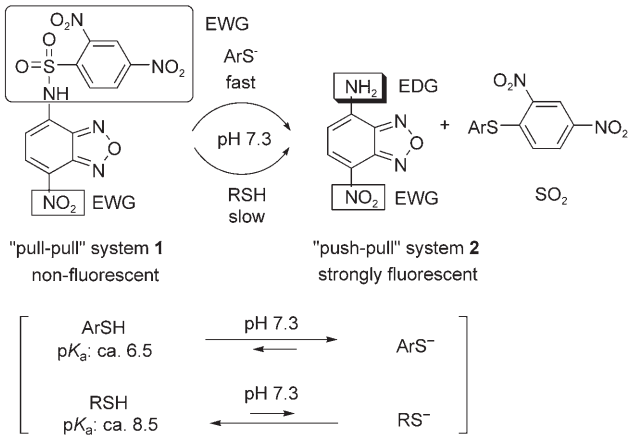
Manipulation of the electronic features of the substituents of a fluorophore may lead to a change in fluorescence emission profile, through either intramolecular charge transfer (ICT) or photoinduced electron transfer (PET) pathways.[15] The design of the PET-based fluorescent sensor is relatively easier because the efficiency of the PET process can be predicted. Nevertheless, the ICT type of fluorescent reagent can afford high sensitivity as it has a very low intrinsic fluorescence. A typical example is the nonfluorescent probe 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) as a result of two electron-withdrawing groups (EWGs; 4-chloro- and 7-nitro), which block the ICT process.[16] However, the substitution of the Cl atom by an amino moiety as electron-donating group (EDG) results in a dramatic increase in fluorescence. Based on this observation, we hypothesized that masking the amino group by an EWG would give rise to a nonfluorescent molecule. Removal of the protecting moiety of the amino group should lead to a highly fluorescent compound.

It is known that the strongly electron-withdrawing 2,4-dinitrobenzenesulfonyl group has been used for the protection of an amino group.[17] The resulting sulfonamide can be readily cleaved by a thiolate anion, derived from a thiol under basic conditions through an S_NAr process (Scheme 1). This



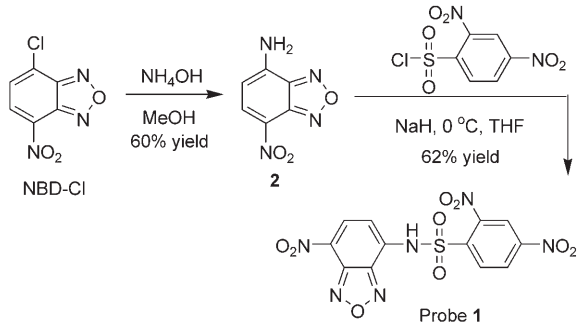
Scheme 1. Design of a fluorescent probe for thiophenols.

mechanism underlines the importance of the nucleophilic thiolate, which is an essentially reactive form for the reaction. The pK_a value of thiophenols is around 6.5, whereas that of aliphatic thiols is about 8.5. In a neutral reaction medium (for example, pH 7.3), the high degree of dissociation of thiophenols results in the predominant generation of the corresponding thiolate, which can effectively react with 2,4-dinitrobenzenesulfonamide (Scheme 1). However, under the same reaction conditions, the aliphatic thiols remain as a less

reactive neutral form and thus the cleavage of the sulfonamide is very slow.

Therefore, it is possible to design a fluorescent probe for the discrimination of aliphatic thiols and thiophenols under neutral, physiological conditions. By taking advantage of the unique reactivity profile and strong EWG capacity of the 2,4-dinitrobenzenesulfonamide, we envisioned that masking the amino group in the fluorescence-active 4-amino-7-nitro-2,1,3-benzoxadiazole (**2**) by the 2,4-dinitrobenzenesulfonyl moiety could generate a probe **1** for thiophenols (Scheme 1). It is expected that the designed probe **1** will not be fluorescent. However, when it reacts with a thiophenol to yield **2**, strong fluorescence should be observed.

To demonstrate the working hypothesis, we first synthesized the probe **1**. Its synthesis is straightforward in two steps (Scheme 2). Reaction of NBD-Cl with ammonium hydroxide



Scheme 2. Synthesis of probe **1**.

in methanol gave 4-amino-7-nitro-2,1,3-benzoxadiazole (**2**) in 60% yield. Introduction of the sulfonyl moiety into the amino group was achieved by reacting **2** with 2,4-dinitrobenzenesulfonyl chloride in the presence of NaH in THF to afford the target molecule **1** in 62% yield.

With probe **1** in hand, we first examined its fluorescence property in the absence and presence of a thiol and established the optimal measurement conditions. Notably, compound **1** had good solubility in water. Accordingly, the experiment was performed in an aqueous phosphate buffer (0.01 M, pH 7.3) containing **1** at a concentration of 2.0×10^{-5} M. As designed, probe **1** exhibited almost no fluorescence in the absence of a thiol at $\lambda_{ex}=465$ nm (Figure 1). When thiophenol (4.0×10^{-5} M, 2.0 equiv) was added, a significant increase in fluorescence intensity (> 50 times) was observed.

The reaction product **2** was monitored and confirmed by a comparison study with a standard pure compound **2** through ^{1}H NMR analysis. Remarkably, probe **1** showed a quick response toward thiophenol based on the study of reaction time. A pronounced intensity increase was obtained even after 5 min. The reaction reached completion after around 10 min. A limited change of fluorescence intensity was observed when longer reaction times were examined. Therefore, a reaction time of 10 min was selected to explore the selectivity of probe **1** toward thiols.

A variety of thiols were probed, including thiophenols, thioalcohols, cysteine, and glutathione, and other nucleophiles, such as NaCN and benzylamine ($BnNH_2$). As shown in Figure 2, probe **1** was highly selective to thiophenols. Significant fluorescence intensity enhancement was observed for 4-chloro, 4-methoxy-, and 4-methylthiophenols. An increase for 4-nitrothiophenol, which has a strong nitro EWG, was also obtained, but the magnitude was much smaller than those for the other thiophenols tested. A plausible reason is that the nitro group in 4-nitrothiophenol significantly decreases the nucleophilicity of the thiol, thus reducing its reactivity for the S_NAr reaction. Remarkably, as expected, no fluorescence intensity change was seen for molecules possessing aliphatic thiol moieties, such as *t*-butyl thioalcohol, cysteine, glutathione, NaCN, and $BnNH_2$. More significantly, in the presence

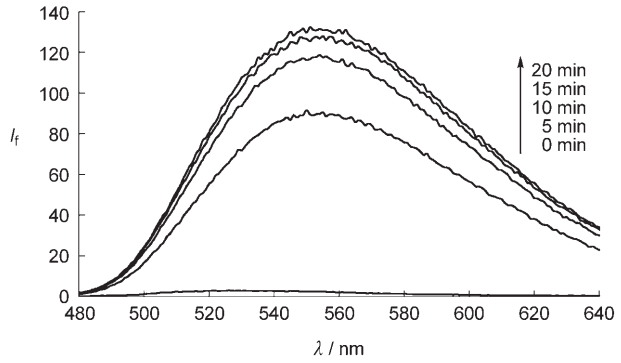


Figure 1. Reaction–time profile of probe **1** and thiophenol. Probe **1** (2×10^{-5} M), prepared from a stock solution (10 mM) in EtOH, was studied in a phosphate buffer (pH 7.3, 0.01 M) at room temperature in the absence and presence of thiophenol (2.0 equiv). The reaction solution was sampled for fluorescence measurement at $\lambda_{ex}=465$ nm after the specified time periods. I_f: Fluorescence intensity.

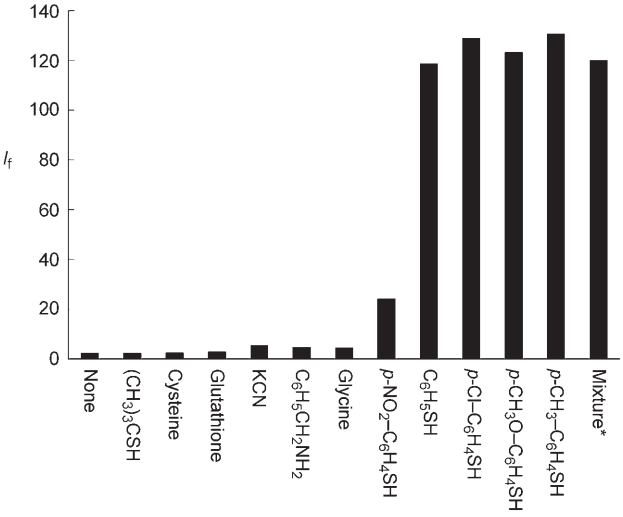


Figure 2. The selectivity of probe **1** toward thiols and other nucleophiles. Probe **1** (2×10^{-5} M) was studied in a phosphate buffer (pH 7.3, 0.01 M) at room temperature in the absence and presence of a thiol (2.0 equiv). After 10 min, the reaction solution was sampled for fluorescence measurement at $\lambda_{ex}=465$ nm. The fluorescence intensity at $\lambda_{em}=555$ nm is plotted versus analytes. *A mixture of PhSH, cysteine, KCN, and $BnNH_2$, each with a concentration of 4×10^{-5} M.

of other nucleophiles, such as cysteine, glutathione, NaCN, and $BnNH_2$, a similar fluorescence intensity increase was observed to that of a pure thiophenol, which indicates that probe **1** is particularly selective toward thiophenols without interference.

The sensitivity of the chemical reagent **1** at 2×10^{-5} M was examined next by using thiophenol as an analyte with a concentration ranging from 0.2 to 4.0×10^{-5} M under the same reaction conditions described above (Figure 3). The increase

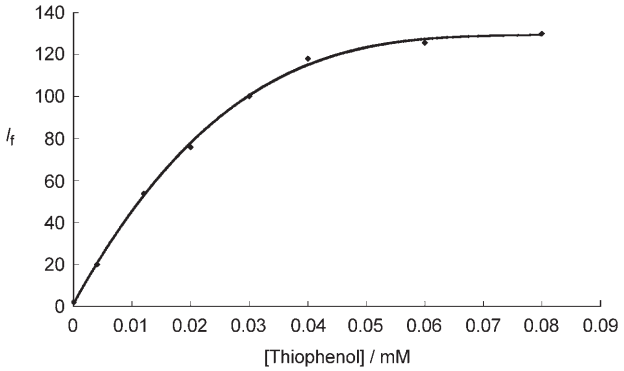


Figure 3. Effect of concentration of thiophenol on the fluorescence intensity of probe **1**. The fluorescence intensity at $\lambda_{em}=555$ nm is plotted versus concentration. See the legend of Figure 2 for experimental procedures.

in fluorescence intensity was displayed in a concentration-dependent manner. However, when more than three equivalents of thiophenol were used, the enhancement of fluorescence intensity reached a maximum without further alteration. Notably, a pronounced change in the fluorescence signal was observed even when the thiophenol concentration was as low as 0.2×10^{-5} M (0.2 equiv).

Finally, we evaluated the effect of reaction pH on probe **1**. As designed, no fluorescence intensity enhancement of **1** was observed for either thiophenols or aliphatic thiols at pH < 6 (see the Supporting Information), as these analytes exist as less nucleophilic neutral forms. It is expected that a large increase in fluorescence intensity would be observed at pH 7 to 9 as a result of the strong ionization of thiophenols. However, we found that probe **1** was not stable at high pH values (> 10). For example, at pH 12 it decomposed to give a nonfluorescent complex mixture.

In conclusion, we have successfully developed a novel, sensitive, and highly selective fluorescence probe **1** for thiophenols. The investigation demonstrates that the manipulation of the electronic nature of the substituents of a fluorophore can affect the fluorescence emission profile through the alteration of the ICT process. Moreover, it is possible to design a highly selective fluorescent reagent based on the analyte reactivity profile and reaction conditions. Dramatic fluorescence intensity enhancement is seen with thiophenols as a result of effective cleavage of the electron-withdrawing 2,4-dinitrobenzenesulfonyl moiety from nonfluorescent probe **1**, to generate highly fluorescent **2** in an aqueous neutral (pH 7.3) buffer with very short reaction times. However, no fluorescence is obtained with aliphatic thiols, including biologically interesting cysteine and glutathione, and other nucleophiles. Therefore, probe **1** can be used for the detection and quantification of highly toxic thiophenols in environmental science.

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