Rational design of a highly selective and sensitive fluorescent PET probe for discrimination of thiophenols and aliphatic thiols†

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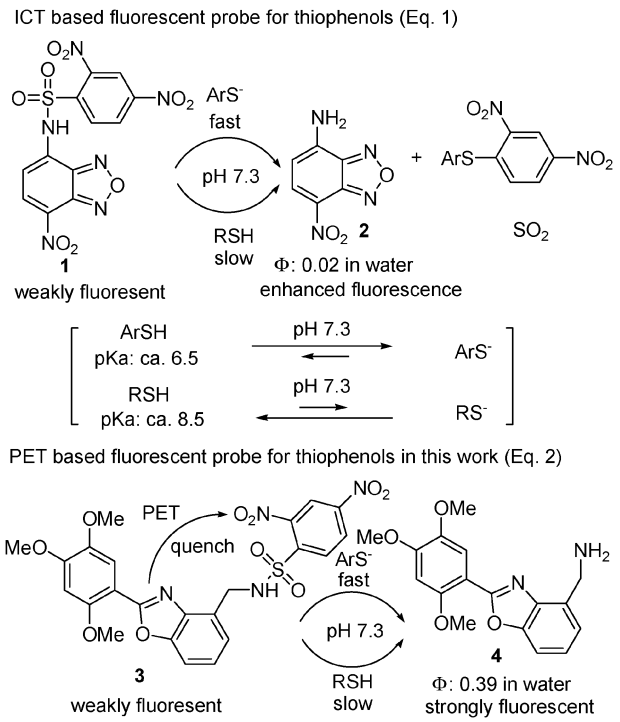
A novel highly sensitive and selective 'off-on' fluorescent probe for thiophenols has been developed by a PET mechanism through a rational design.

Sensitivity and selectivity are the most critical issues in the design of any artificial receptor. In addition, the binding event should be conveniently monitored in an 'off-on' signal output manner. It is extremely challenging to develop a sensor or probe capable of discriminating interesting analytes with close physical and chemical properties. Thiols are such a class of molecules. Aliphatic thiols such as cysteine,[1] homocysteine[2] and glutathione,[3] play important roles in biological systems. Nevertheless, thiophenols, in spite of their broad synthetic utility,[4] are highly toxic and pollutant compounds with LC_{50} (50% lethal concentration, *e.g.*, a dose required to kill half the members of a tested population) 0.01–0.4 mM for fish[5] and much more toxic than aliphatic ones.[6,7] Therefore, a detection technique that can selectively differentiate toxic thiophenols and biologically important aliphatic thiols is of considerable significance in the fields of chemical, biological and environmental sciences.

Recently, significant efforts have been directed toward the development of fluorescent sensors or probes for thiols.[8] It is noted that they are mainly designed for cysteine and homocysteine. However, in general they exhibit poor selectivity for aliphatic thiols and thiophenols. A sensor capable of distinguishing these substances has not been realized until recently when we developed the first highly selective fluorescent probe **1** for thiophenols based on an intramolecular charge transfer (ICT) mechanism (Scheme 1, eqn (1)).[9] Nevertheless, it has the drawbacks of the relatively weak fluorescence intensity of product **2** in aqueous solution as a result of its low quantum yield (Φ = 0.02) and relatively low sensitivity (detection limit at 2 μM). A "brighter" and more sensitive sensor for thiophenols is highly desirable from the practical application standpoint of view. Towards this end, in this communication, we wish to disclose a new mechanistically different, PET (photoinduced electron transfer)-based fluorescent probe **3** (eqn (2)) with several notable features in addition to high specificity to thiophenols: (1) a much higher quantum yield (Φ = 0.39) in an aqueous buffer; (2) >100-fold (*vs.* >50 folds for probe **1**) fluorescence intensity enhancement; (3) higher sensitivity (0.2 μM detection limit *vs.* 2.0 μM for probe **1**).

In the design of a novel PET based fluorescent probe[10] for thiophenols, we envision that the selection of 2,4-dinitrobenzenesulfonyl group as the critical component can kill two birds with one stone. On one hand, it serves as a recognition moiety due to its unique and high reactivity towards thiolate anions. The masked sulfonamide moiety can be facilely removed by a highly nucleophilic thiolate anion through a S_NAr process (Scheme 1).[11] Taking advantage of the difference in the acidity of thiophenols and aliphatic thiols, we envisioned that under the physiological pH, a thiophenol could quickly cleave the sulfonamide group since its corresponding more nuleophilic thiolate is the dominant species owning to its pK_a (*ca.* 6.5). Nevertheless, under the same reaction conditions, the aliphatic thiols with higher pK_a (*ca.* 8.5) remain largely in the less reactive neutral form and thus the reaction is very slow. The nitro moieties of the 2,4-dinitrobenzenesulfonamide are not only crucial for thiolates as essential nucleophiles in the S_NAr reaction, but also serve as an effective quencher in the newly designed PET fluorescent probe. A benzoxazole is selected as the fluorophore[12] since it exhibits a high quantum yield in water and can effectively participate in a PET process.



Scheme 1 Fluorescent probes for thiophenols.

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It is expected that the designed probe **3** will display weak fluorescence. However, when it reacts with a thiophenol to yield **4**, a strong fluorescent signal should be generated.

To prove the concept, we developed a synthetic route for the preparation of the newly designed PET probe **3** (see ESI†) and then carried out fluorescence studies. We first examined its fluorescence response toward thiophenol as a representative and meanwhile aimed at establishing the optimal measurement conditions. Its good water solubility enabled us to perform the investigation in an aqueous phosphate buffer (0.01 M, pH 7.3) at a concentration of 2.0×10^{-6} M. As designed, the probe **3** displayed almost no fluorescence in the absence of a thiol at $\lambda_{ex} = 335$ nm due to the efficient quenching by the nitro groups ($\varepsilon = 0.6 \times 10^4$ M^{-1} cm^{-1}, $\Phi < 0.01$) (Fig. 1).[13] Upon the addition of thiophenol (4.0×10^{-6} M, 2 equiv.), a dramatic fluorescence intensity increase was observed in a few seconds centered at 403 nm. After about 20 min, the fluorescence intensity reached the maximum and no significant change was seen in an extended reaction time. Notably, probe **3** exhibited a much stronger fluorescence response than previous probe **1** though its concentration is 10 times lower, and notably a >100 fold fluorescence intensity increase was obtained (Fig. 1). The reaction product was monitored and confirmed by a comparison study with a standard pure compound **4** through ^{1}H NMR analysis. The quantum yield of fluorescence for the product **4** was determined to be 0.39 ($\varepsilon = 1.2 \times 10^4$ M^{-1} cm^{-1}).[13]

In a control study, we synthesized compound **5**, bearing a *p*-methylbenzensulfonyl (Ts) group instead of a 2,4-dinitrobenzenesulfonyl moiety. Under the same conditions, it showed very strong fluorescence in the presence and absence of thiophenol and no response to thiophenol (see Fig. S1, ESI†). These results support our working hypothesis that the two nitro groups are essential for the high reactivity of the thiolate mediated S_NAr reaction and serve as an effective quencher of the PET sensor **3**.

Next we examined the sensitivity of the new probe **3** at 2×10^{-6} M for thiophenol under the same reaction conditions (Fig. 2). The fluorescence intensity increase displayed in a concentration dependent fashion and no obvious emission

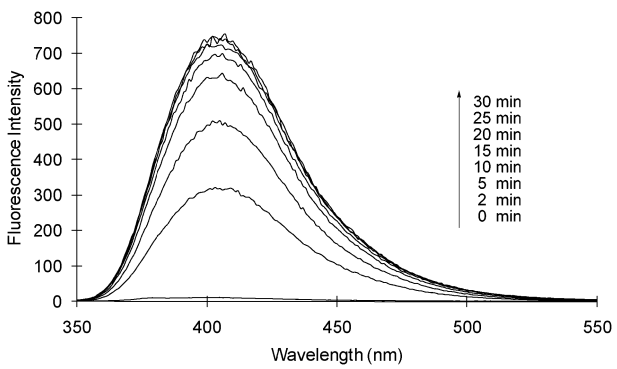

Fig. 1 Fluorescence emission time profile of probe **3** towards thiophenol. Probe **3** (2×10^{-6} M), prepared from a 1.0 mM stock solution in DMF, was studied in a phosphate buffer (pH 7.3, 0.01M) at room temperature in the absence and presence of thiophenol (2.0 equivalents). The reaction solution was sampled for fluorescence measurement at $\lambda_{ex} = 335$ nm at the specified time periods.

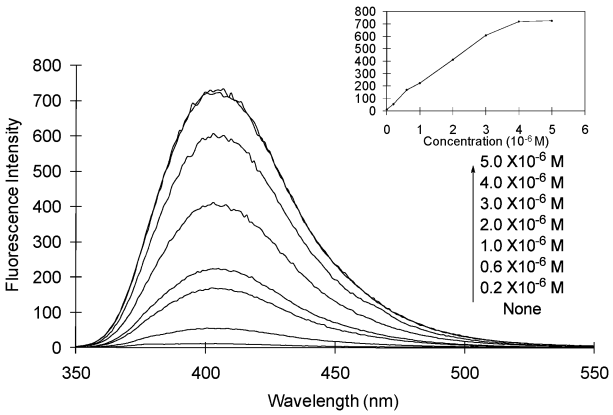

Fig. 2 Effect of thiophenol concentration on the fluorescence emission of probe **3**. Probe **3** (2×10^{-6} M) was studied in a phosphate buffer (pH 7.3, 0.01M) at room temperature in the absence and presence of a thiophenol at different concentrations. After 10 min, the reaction solution was sampled for emission measurement ($\lambda_{ex} = 335$ nm, fluorescence intensity at $\lambda_{ex} = 403$ nm is plotted *vs.* concentration).

shift in fluorescence spectra and almost no change absorption spectra (see Fig. S2, ESI†) were observed, which was in agreement with a typical PET process. Higher concentrations of thiophenol afforded a quicker and more dramatic response. For example, when 4 equiv. of thiophenol (8×10^{-6} M) was used, the enhancement of fluorescence intensity reached the maximum in 10 min. Further increase of thiophenol concentration did not result in additional enhancement of fluorescence intensity (Fig. 2, inset). Notably, even at 2×10^{-7} M concentration, a pronounced fluorescence signal change was observed, indicative of its higher sensitivity than that of probe **1** (detection limit: 2×10^{-6} M).

A survey of relevant aliphatic thiols and other common nucleophiles revealed that probe **3** displayed excellent selectivity towards thiophenol (Fig. 3). Aliphatic thiols tested including 2-methyl-2-propanethiol, cysteine and glutathione did not lead to a fluorescent response to the probe **3** 20 min after addition of the thiol agents. A similar trend was observed for other nucleophiles such as CN^-, I^-, PhOH, and $PhNH_2$. Moreover, in the presence of other nucleophiles such as a mixture of cysteine, glutathione, CN^-, I^-, and $BnNH_2$, a similar fluorescence intensity increase was observed to that of a pure thiophenol, indicating that the probe **3** is particularly selective to thiophenols without interference.

It is expected that the pH of the tested buffer solution would affect the fluorescence intensity and the reactivity of probe **3**. The probe itself was inert to pH change in a wide range (from 4 to 12) and no pH-dependent fluorescence change was obtained. However, as expected, in the presence of thiophenol, the fluorescence intensity alternation of probe **3** was pH dependent. At low pH (<5), almost no fluorescence was observed due to the very slow cleavage of the sulfonamide group by the weakly nucleophilic neutral thiophenol. However, under neutral to weakly basic conditions (pH 7 to 9), probe **3** exhibited the most sensitive response with significant enhancements of fluorescence with thiophenols as a result of the strong ionization of thiophenol.

In conclusion, taking advantage of the unique property and reactivity of 2,4-dinitrobenzenesulfonyl moiety, we have

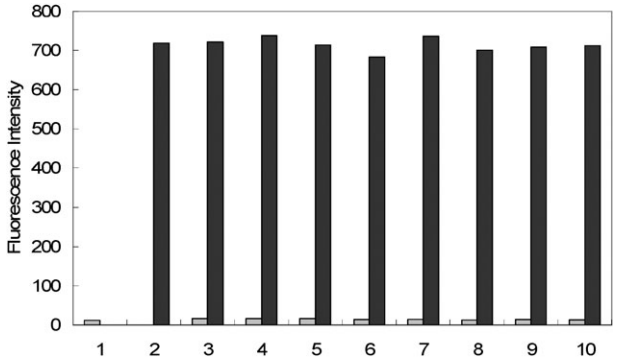

Fig. 3 The selectivity of probe **3** toward thiols and other nucleophiles. Gray bar: the fluorescence intensity of only a single analyte at 10 μM with the probe (2 μM), black bar: the fluorescence intensity of a mixture of a nucleophilic reagent at 10 μM and PhSH at 4 μM with the probe (2 μM). (1) probe **3** only, (2) PhSH, (3) cysteine, (4) $(CH_3)_3CSH$, (5) gluotathione, (6) glycine, (7) KCN, (8) KI, (9) PhOH, (10) $PhNH_2$. All data (λ_{em} = 403 nm) were acquired at 20 min after addition of analyte(s) in a phosphate buffer (pH 7.3, 0.01 M) at room temperature with λ_{ex} = 335 nm.

developed a novel fluorescence probe **3** for the detection of thiophenols in aqueous solution with excellent specificity. The probe was rationally designed based on the PET mechanism, which is different from probe **1** relying on the ICT pathway. Importantly, the sensitivity has improved significantly with a much higher quantum yield (Φ = 0.39) and >100-fold fluorescence intensity enhancement. These features of probe **3** mean it has great application potential for the detection and quantification of highly toxic thiophenols in environmental science.

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