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Detection of thiophenol in buffer, in serum, on filter paper strip, and in living cells using a red-emitting amino phenothiazine boranil based fluorescent probe with a large Stokes shift

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ABSTRACT

A novel red-emitting dye, **PB-NH$_2$**, was synthesized by incorporation of an electron rich phenothiazine moiety to classical boranil dye. **PB-NH$_2$** displayed excellent photophysical properties, such as long emission wavelength, large Stokes shift, strong emission both in solution and in solid state. Based on this attractive platform, **Probe 1**, was constructed for selective detection of thiophenol. Notably, **Probe 1** was ultrasensitive in response to thiophenol, and the corresponding detection limits for thiophenol in theoretical and in experimental were determined to be 1.4 and 10 nM, respectively. Significantly, **Probe 1** showed great potential for practical applications, and the applications in real water samples, in serum, on filter paper strips and in living cells were successfully demonstrated.

1. Introduction

Thiophenols have broad synthetic utility and they are widely used in chemical industry, such as the preparation of pesticides, medicine and various industrial products.[1–3] However, despite their great practical value, thiophenols have been listed as one of the prioritized pollutants by the United States Environmental Protection Agency (USEPA) due to the highly toxic property.[4] Long-term exposure of thiophenols liquid or vapor is extremely detrimental to human health, which may induce severe central nervous system damage and other related system injuries, even death.[5] Toxicological studies revealed that thiophenols process the median lethal concentration (LC50) values of 0.01–0.4 mM in fish and a median lethal dose (LD50) value of 46.2 mg/kg in mouse.[6,7] Therefore, the development of highly sensitive and selective methodologies for the detection of thiophenols is of considerable significance in the fields of chemical, biological and environmental science. Owing to the advantages of simplicity and high sensitivity as well as non-invasiveness and high spatiotemporal resolution, fluorescent methods have been recognized as the effective tools that can help monitor and visualize various cations, anions, and biomolecules both *in vitro* and *in vivo*.[8] In the past decade, great efforts have been devoted to the development of fluorescent probe for thiophenols over aliphatic thiols. Pioneered by Wang's group, the selective detection of thiophenols was realized by taking advantage of the thiophenols-mediated cleavage of sulphonamide.[9] From then on, a variety of fluorescent probes capable of detecting thiophenols selectively had been reported based on the extended version of the above-mentioned strategy.[10–34] Despite the significant advances have obtained so far, it is noteworthy that most of the reported probes suffer from short emission wavelength and relatively small Stokes shift. It is well known that fluorescent probes with emission in red or near-infrared range are optimal for biological imaging applications due to the deep tissue penetration, decreased light scattering, reduced autofluorescence and minimum photodamage.[35] Moreover, fluorophores with large Stokes shift are

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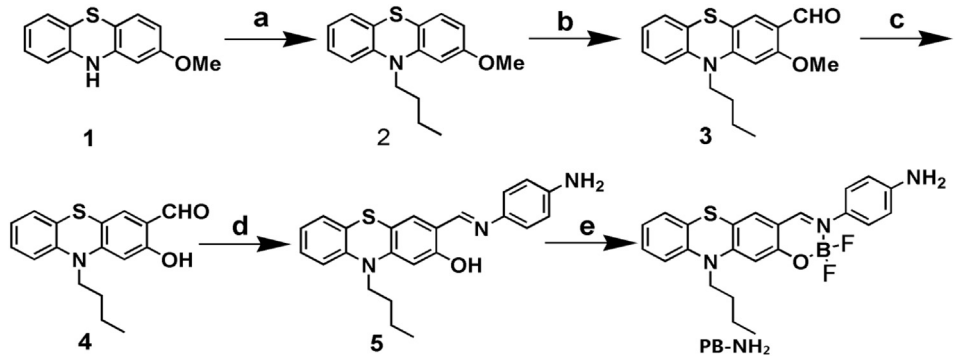

Scheme 1. Synthetic route of **PB-NH$_2$**. Reagents and conditions are as follows: (a) n-bromobutane, NaOH, KI, DMSO, 100 °C, Ar, 6 h; (b) $POCl_3$/DMF, 0 °C, Ar, 15 min, then compound 2, DMF, 60 °C, 4 h; (c) Aluminium powder, iodine, CH_3CN, room temperature, Ar, 30 min, then compound 3, CH_3CN, 80 °C, 6 h; (d) p-phenylenediamine, ethanol, room temperature. (e) $BF_3{\cdot}Et_2O$, DIEA, 1, 2-dichloroethane, reflux, 5 h.

quite suitable for fluorescence microscopy studies, because the large Stokes shift enables the clearly separated excitation and emission bands, which can effectively minimize the interferences caused by self-absorption or auto-fluorescence.[10] Unfortunately, red or near-infrared fluorescent probes for thiophenol with large Stokes shift are rare up until now. Among the reported thiophenol probes, three fluorescent probes emit in red or near-infrared region ($\lambda_{em} \geq 600$ nm) as well as display large Stokes shift ($\geq$120 nm); they could detect thiophenol in solutions, but not suitable for thiophenol detection in solid state due to the severe ACQ (aggregation-caused quenching) process (see Table S1). Fluorophores that exhibit large Stokes shift and strong red or near-infrared fluorescence both in solution and in solid state are highly desirable for thiophenol probes development, from the stand point of practical application.

In this work, we have exploited a unique amino phenothiazine boranil dye **PB-NH$_2$**, which displays strong red emission not only in solutions but also in solid state. More importantly, the Stokes shift of **PB-NH$_2$** could reach to incredibly 172 nm in EtOH/PBS buffer (v/v = 1:1) solution. Thereby, **PB-NH$_2$** offers a robust platform upon which novel fluorescent probe for thiophenol was based. Herein, we synthesized a novel fluorescent **Probe 1** for thiophenol by incorporation of a dinitrobenzene sulfonate (DNBS) moiety to **PB-NH$_2$**, which could detect thiophenol both in aqueous solutions and in solid state.

2. Results and discussion

2.1. Design rational

In recent years, heteroatom-modified organic dyes, such as BODIPYs have received considerable attention due to their outstanding optical characteristics, such as relatively narrow absorption and emission band, high molar absorption coefficient, intense fluorescence quantum yield, satisfactory photostability and chemostability, and exceptional insensitivity to the polarity of solvents and to pH.[36] However, classical BODIPYs suffered from severe fluorescence quenching in solid state due to the tight intermolecular π-π stacking. Moreover, most BODIPYs exhibit very small Stokes shift (<20 nm, in most cases), which inevitably results in self-quenching and in measurement errors by excitation and scattering light.[41] Therefore, the development of novel red emissive boron difluoride complexes with large Stokes shift is of great importance. In fact, this also represents the current state-of-art in the field of novel BODIPY analogues exploitation.

Phenothiazine, which was first synthesized by Bernthsen in 1883,[38] has been widely used in a variety of industries including the manufacturing of dyes and pigments, drugs, dye-sensitized solar cells and photocopying materials.[39] Phenothiazine contains electron-rich nitrogen and sulfur heteroatoms in a heterocyclic structure with strong electron-donating ability. We anticipate that the introduction of a phenothiazine moiety to BODIPY analogues may serve as a robust strategy for developing red-emitting fluorescent dyes with large Stokes shift. Moreover, the nonplanar butterfly-like molecular conformation of phenothiazine can effectively preclude the intermolecular π-π stacking,[40] thereby leading to strong solid fluorescence.

As a proof-of-concept, we designed novel amino phenothiazine boranil dye, **PB-NH$_2$**, by incorporation of a phenothiazine moiety to classical amino boranil dye, and readily synthesized it from commercial available compound **1** in five steps (Scheme 1). Condensation of compound **1** with n-bromobutane to form compound **2**, the subsequent Vilsmeier acylation with $POCl_3$/DMF obtained phenothiazine-2-methoxy-3-aldehyde (compound **3**). Demethylation of compound **3** by AlI_3 (prepared in suit) afforded the key intermediate phenothiazine salicylaldehyde **4**, which was then treated with *p*-phenylenediamine formed Schiff base **5**. Finally, the BF_2 complexation of compound **5** and $BF_3{\cdot}Et_2O$ in 1,2-dichloroethane generated the target dye **PB-NH$_2$**. The chemical structures of all compounds were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS (see Supporting information).

With dye **PB-NH$_2$** in hand, we were eager to investigate its optical properties in different solvents. The absorption and emission spectra of **PB-NH$_2$** in different solvents (dichloromethane, EtOH, pH 7.4 PBS buffer/EtOH (v/v = 1:1) and CH_3CN) are shown in Fig. S1, and the corresponding photophysical data are summarized in Table 1. As designed, **PB-NH$_2$** displays emission peaks well into the red region, with maximum at 605–626 in different solvents. Moreover, **PB-NH$_2$** has the fluorescence quantum yields of 0.08–0.28 in different solvents, which are great values for red-emitting dyes with large Stokes shift. Remarkably, **PB-NH$_2$**

Table 1
Photophysical data of **PB-NH$_2$** in different solvents.

Solvent	λ_{abs}/nm[a]	λ_{em}/nm[b]	Stokes shift	Φ_f[c]	τ[d]/ns
dichloromethane	460	621	161	0.28	3.68
CH_3CN	453	613	160	0.20	2.81
EtOH	451	605	154	0.19	2.62
EtOH/PBS (1:1)	454	626	172	0.08	1.16

[a] The maximal absorption of the **PB-NH$_2$**.
[b] The maximal emission of the **PB-NH$_2$**.
[c] Φ_f is the relative fluorescence quantum yield estimated by using fluorescein (Φ_f = 0.79 in ethanol) as a standard.
[d] Fluorescence lifetime.