ORIGINAL PAPER

A Fluorescent Turn-On Probe Based on Benzo [*E*] Indolium for Cyanide Ion in Water With High Selectivity

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Abstract A highly selective fluorescent turn-on probe for CN⁻ bearing a benzo [*e*] indolium group as a fluorophore and binding site was reported. The detection of CN⁻ was performed via the nucleophilic attack of CN⁻ toward the benzo [*e*] indolium group of the probe, resulting in a prominent fluorescence enhancement and an obvious color change. The probe shown a highly selectivity in water and could be performed in a range of pH value between 6 and 9. The detection limit was 4.6×10^{-8} M, which could be detected CN⁻ in drinking water. A simple paper test strip system for the rapid monitoring of CN⁻ was developed.

Keywords benzo [*e*] indolium · Cyanide-sensing · Chemosensor · Fluorescent turn-on

Introduction

Cyanide (CN⁻) is extremely toxic to mammals with even a small amount, and it can be absorbed through the lungs, gastrointestinal track and skin, leading to vomiting, convulsion, loss of consciousness, and eventual death. The highest allowable level of CN⁻ in drinking water is only 1.9 μM according to the World Health Organization (WHO) [1–3]. Nevertheless, cyanide has been produced in large quantities and used in various industrial processes, such as synthetic fibers, resins, herbicide and the gold-extraction [4], which has led to environmental contamination as well. Therefore, there is an emergency requirement to develop efficient and simple sensing systems to monitor CN⁻ from the contaminant sources. Multiple probes for CN⁻ have been developed over past 10 years through coordination ability and the nucleophilic reactivity of CN⁻ [5], such as Zn^{2+}-porphyrin, Ru^{2+}-pyridine,

Co^{2+}-salen [6–8], displacement [9–13], as well as bond-forming reaction between the CN⁻ and either an electrophilic carbon [14–32] or a boron center [33–35]. However, only a few probes can perform in water for the poor water-solubility of most probes [29, 36, 37].

Probes based on the chemical species induced changes in fluorescence appear to be particularly attractive due to the highly sensitive, quick, simple and real time monitoring of the fluorescence. Many fluorescent and colorimetric probes bearing an indoline moiety have been employed to detect CN⁻ for its highly reactive binding site and high selectivity [18, 24, 28, 30, 37–41]. However, the fluorescence of indoline is rather weak, and another fluorophore is essential to ensure the probe could be detected on a fluorescence spectrophotometer. Undoubtedly, the long synthetic pathways make the probes difficult to prepare, and also make the water-solubility of the probes worse. Here, we introduced a new fluorescence turn-on probe **1** bearing a positively-charged benzo [*e*] indolium fragments for detecting CN⁻ in water with high selectivity and sensitivity. CN⁻ is expected to be detectable by nucleophilic attack toward the carbon atom of the C=N group, which is activated by the strong electron-withdrawing feature of the positively-charged benzo [*e*] indolium fragments. We were attracted to benzo [*e*] indolium because (1) the benzo [*e*] indoline possesses an obvious fluorescence emission caused by a large π-conjugation after the addition of CN⁻; (2) any other fluorophore is unnecessary, the probe is easy-to-make, and (3) the positively-charged benzo [*e*] indolium fragments make the probe water-soluble.

Experimental Section

Apparatus and Materials

Absorption spectra were taken on a Shimadzu UV-1800 spectrophotometer. Fluorescence spectra were taken on a Hitachi F-

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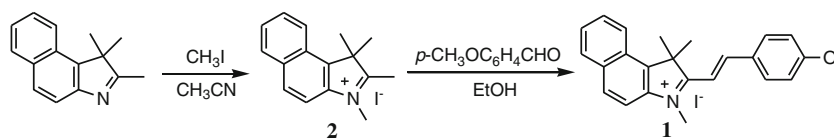
Scheme 1 Synthesis route of probe 1

2700 fluorescence spectrometer. ^{1}H NMR and ^{13}C NMR measurements were recorded at 600 and 150 MHz on a Brucker Avance 600-MHz spectrometer, respectively. Dimethyl sulfoxide (DMSO-d_6) was solvent, and tetramethylsilane (TMS) was used as internal standard. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; m = multiplet. ESIMS were taken on a Fourier transform ion cyclotron resonance mass spectrometry (Varian 7.0 T).

All reagents and solvent were purchased from commercial source and used without further purification, if not otherwise stated. All reactions were carried out on the magnetic stirrers and their reaction process was monitored on thin layer chromatography (TLC).

Synthesis

Preparation of **2**

2, 3, 3-Trimethylbenz [e] indole (10.0 g, 47.78 mmol) was dissolved in acetonitrile (50 mL), then methyl iodide (3.57 mL, 57.34 mmol) was added and the reaction mixture was heated at reflux for 1 h with vigorous stir. The mixture was cooled to room temperature and the precipitate was collected by filtration, washed with diethyl ether, and dried *in vacuo*. Product **2** was obtained as a white solid in 86 % yield (14.41 g). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.36 (d, 1H, *J*=8.2), 8.29 (d, 1H, *J*=8.8), 8.22 (d, 1H. *J*=8.2), 8.10 (d, 1H, *J*=8.8), 7.78 (m, 1H), 7.72 (m, 1H), 4.09 (s, 3H), 2.87 (s, 3H), 1.75 (s, 6H).

Preparation of Probe **1**

2 (1.05 g, 3 mmol) was dissolved in ethanol (5 mL), then 4-methoxybenzaldehyde (0.36 g, 3 mmol) was added and the reaction mixture was heated at reflux for 12 h. When the mixture was cooled to room temperature and the precipitate was collected by filtration, washed with cold ethanol, and dried *in vacuo*. Probe **1** was obtained as an orange solid in 56 % yield (0.8 g). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.47 (m, 2H), 8.28 (m, 3H), 8.23 (d, 1H), 8.11 (d, 1H), 7.77 (m, 2H), 7.61 (d, 1H), 7.19 (m, 2H), 4.27 (s, 3H), 3.92 (s, 3H), 2.02 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 185.7, 167.1, 155.4, 142.8, 141.0, 136.4, 136.3, 134.2, 133.9, 133.4, 131.7, 130.8, 130.4, 130.0, 126.5, 118.3, 116.6, 113.5, 59.2, 56.9, 38.3, 28.7, 24.7. HRMS (ESI) calcd. for [**1**]$^+$ 342.1852, found 342.1855 Scheme 1.

Results and Discussion

Cyanide Binding Studies of **1**

^{1}H NMR analysis was firstly carried out to demonstrate the proposed addition mechanism. The solution of probe **1** (3×10^{-3} M) in DMSO-d_6 (450 μL) and D$_2$O (50 μL) was placed in the NMR tube, and potassium cyanide powder (1 equiv. and 2equiv) was added. All the potassium cyanide powder was soluble. As anticipate, after addition of potassium cyanide powder (1 equiv.) into the solution of **1**, the nucleophilic attack of CN$^-$ toward the positively-charged benzo [*e*] indolium group weakened its electron-withdrawing character and lead all the ^{1}H NMR signals upfield shifted (Fig. 1). It is clearly that the proton signal (H^a, at δ 4.23) of methyl group connected with N$^+$ was dramatically
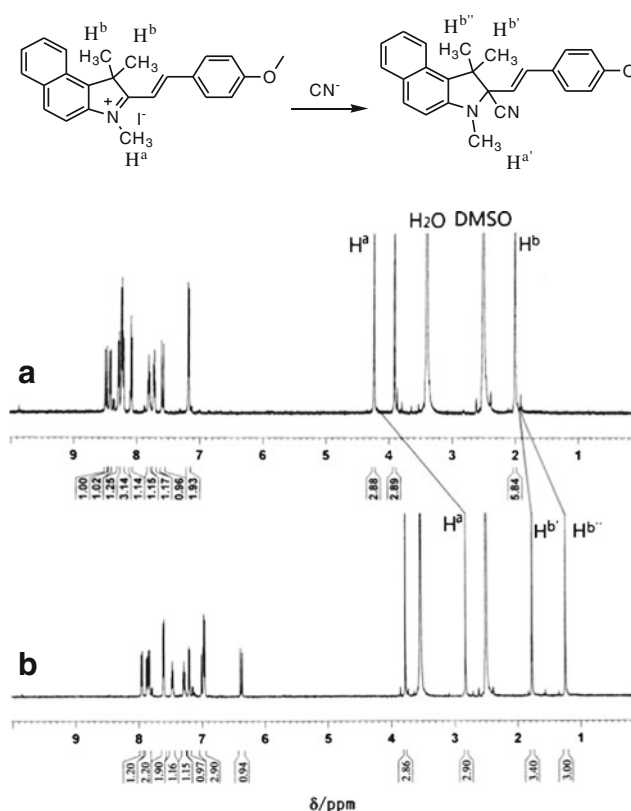

Fig. 1 ^{1}H NMR spectral change of **1** (3×10^{-3} M) in the absent (**a**) and present of 1 equiv. of potassium cyanide (**b**) in DMSO-d_6:D$_2$O=9:1 (v/v).

shifted up-field to δ 2.83. Moreover, the proton signal (H^b, at δ 2.00) of two methyl groups was also shifted up-field and divided into two single signals (H$^{b'}$, at δ 1.78 and H$^{b''}$, at δ 1.24), which become non-equivalent after formation of **1**-CN. The unvaried integral intensity ratio between the methoxyphenyl and benzo [*e*] indolium moiety and the methyl signals indicates that the formation of **1**-CN keeps them intact. The ^{1}H NMR signals remained essentially unchanged when more than 1 equiv. cyanide was added into the solution, suggesting the 1: 1 binding stoichiometry between **1** and CN$^-$. In addition, the formation of the **1**-CN was further confirmed by mass spectrometry analysis, in which the peak at *m/z* 407.1520 (calcd. = 407.1512.) corresponding to [**1** + CN + K] $^+$ was clearly observed (Fig. 2).

Spectral Properties

Fluorescence and UV–vis absorption studies were performed using 10 μM and 20 μM solution of **1** in deionized water with appropriate amounts of anions (as their Na$^+$ salt, 10 mM and 100 mM). The excitation wavelength was 345 nm. The excitation and emission slit width were 5 nm and 5 nm, respectively. The detection was delayed 4 min to
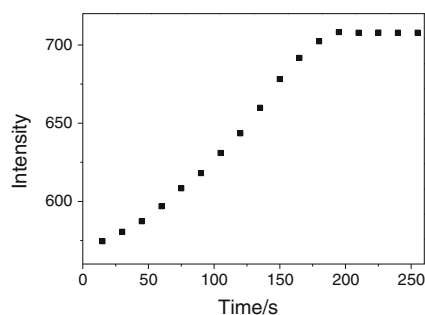

Fig. 3 Response time of reaction between **1** (10 μM) and CN$^-$ (3 equiv.). λ$_{ex}$=345 nm. Slits: 5 nm/ 5 nm

ensure complete reaction between **1** and CN$^-$ at room temperature (Fig. 3).

Figure 4 illustrates the absorption (A) and fluorescence (B) spectral changes for probe **1** upon addition of CN$^-$ in water. The absorption spectrum of **1** (20 μM) exhibits an obvious broad band at 445 nm, which is ascribed to the typical intramolecular charge transfer (ICT) band from the methoxy group to the positively-charged benzo [*e*] indolium fragments. Upon addition of CN$^-$, the band at 445 nm was gradually attenuated until saturation after 7 equiv., suggesting that the
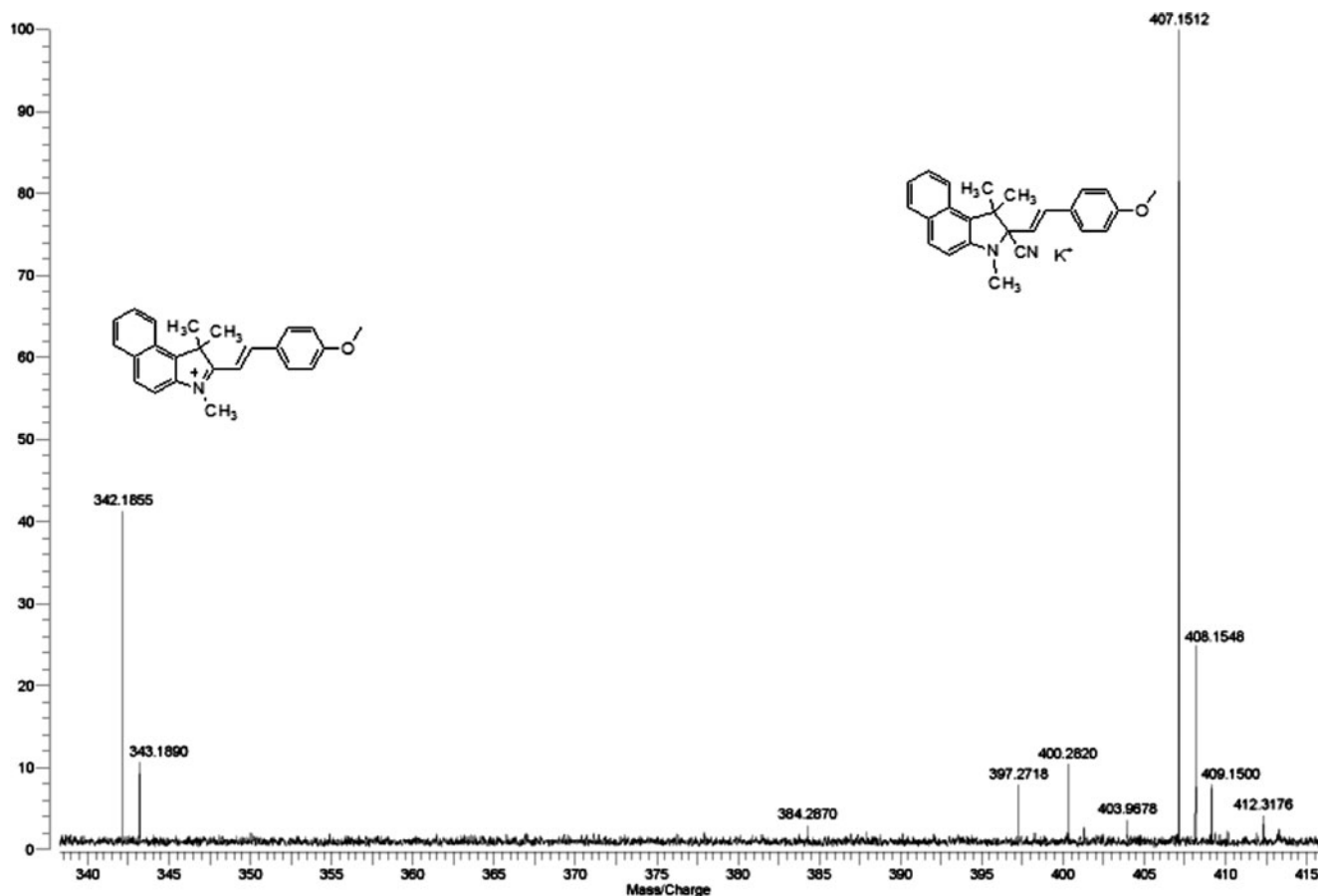

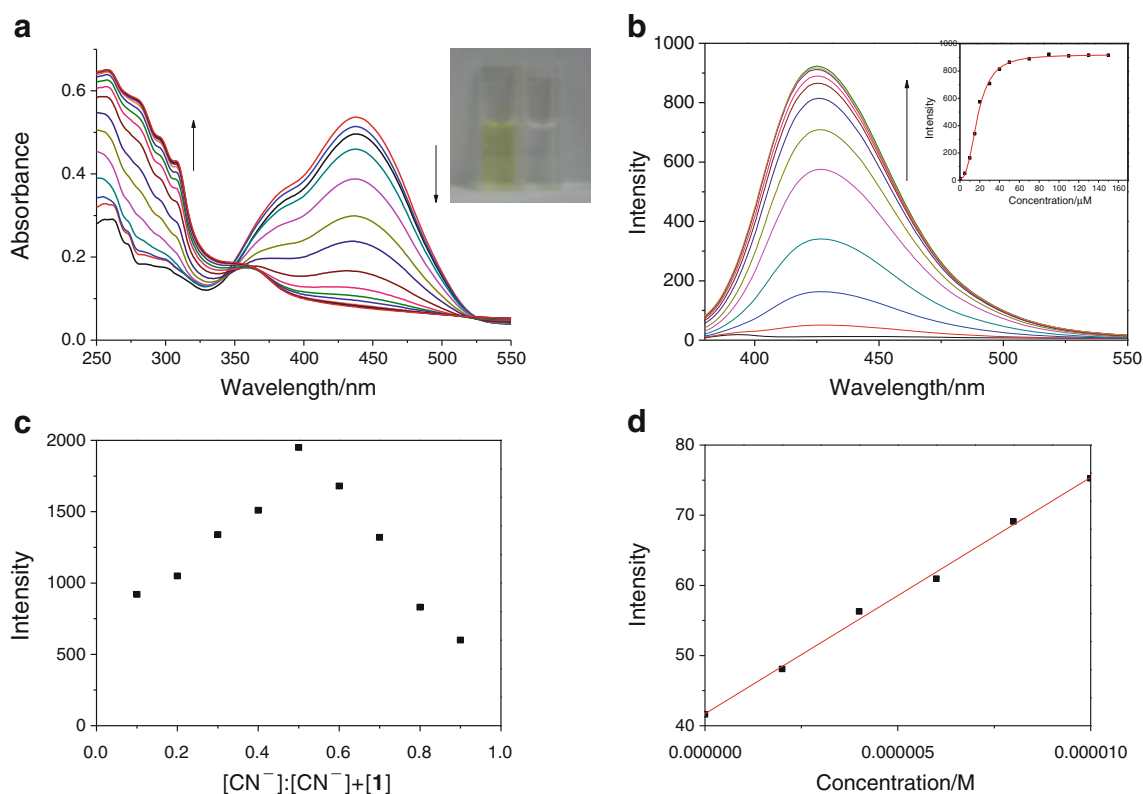**Fig. 2** The ESIMS spectral of **1** and **1**-CN

Fig. 4 **a** Absorption spectra of **1** (20 μM) upon the addition of CN⁻ in water. CN⁻ concentrations were varied in the following order: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100,120, 140, 160, 180, 200 μM. Inset: photos of **1** (20 μM) in the absence (left) or presence (right) of CN⁻ (100 μM) under the day light. **b** Fluorescence spectra of **1** (10 μM) upon the addition of CN⁻ in water. CN⁻ concentrations were varied in the following order: 0, 5, 10, 15, 20, 30, 40, 50, 70, 90, 110, 130, 150 μM. (C) The titration probe evaluated from fluorescent intensity at 425 nm. Job' plot for determining the stoichiometry of **1** and CN⁻ ([**1**]+[CN⁻]=100 μM). (D) The linear relation for concentration of CN⁻ in the range of 0–10 μM. λ_{ex}=345 nm. Slits: 5 nm/ 5 nm

ICT process is blocked due to the nucleophilic attack of CN⁻; meanwhile, an obvious color change from yellow to colorless was also observed (inset of Fig. 4a). Moreover, a clear isosbestic point was also noted at 343 nm, indicating the formation of the **1**-CN adduct. Only 7 equiv. of CN⁻ used in water is impressive compared to many reported CN⁻ probes require high equivalents of CN⁻ to reach a maximal spectral signal. Figure 1b shows the fluorescence spectra of **1** (10 μM) after addition of different amounts of CN⁻ in water. The aqueous solution of **1** was almost non-emissive in the absence of CN⁻. However, the fluorescence increases gradually after addition of cyanide ions. The fluorescence at 425 nm belonged to benzo [*e*] indoline increases by more than 90 - fold when the concentration of CN⁻ reaches 7 equiv. in the solution of **1**. Job' plot analysis of the fluorescence titrations revealed a maximum at about 0.5 mol fraction suggesting 1: 1 binding between **1** and CN⁻ (Fig. 4c). The titration profile at 425 nm *versus* concentration of CN⁻ is shown in the inset of Fig. 4b, and the association constant for CN⁻ was estimated to be 5.2×10^3 (R^2=0.9914).

The fluorescent spectral response of **1** in the absence and presence of CN⁻ at different pH values was also evaluated as shown in Fig. 5. At pH values lower than 5, no obvious characteristic fluorescent of **1** could be observed regardless of the presence and absence of CN⁻, indicating that no reaction occurs between **1** and CN⁻. This could be attributed to the protonation of CN⁻ decreases the actual concentration of CN⁻ in the sample solution. Between pH 6 and 9, upon addition of CN⁻, **1** responded stably to CN⁻ without any interference by protons. At pH higher than 9, the fluorescent
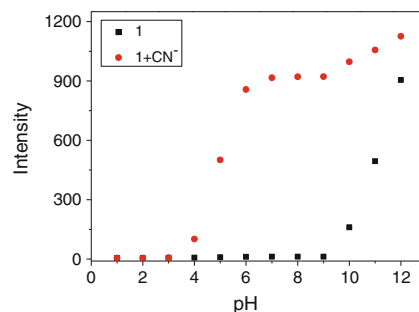

Fig. 5 Changes in fluorescence spectra of **1** (10 μM) in the absence and present of CN⁻(10 equiv.) in water as a function of pH. pH value were adjusted by the dilute solutions of NaOH and HCl, respectively. λ_{ex}=345 nm. Slits: 5 nm/ 5 nm

intensity at 425 nm of **1** increased dramatically. This could be ascribed to the nucleophilic attack by OH^- to **1**. The results indicate that **1** can successfully react with CN^- and allows CN^- detection at a range of pH values from 6 to 9.

Detection Limit Studies

The detection limit for **1** was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of **1** without CN^- was measured by 10 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and the CN^- concentration could be obtained in the range of $0–1\times10^{-5}$ M ($R^2=0.9876$), as shown in the inset of Fig. 4d. The detection limit was then calculated with the equation: detection limit$=3\sigma_{bi}/m$, where σ_{bi} is the standard deviation of blank measurements, m is the slope between intensity versus sample concentration. The detection limit was measured to be 4.6×10^{-8} M. This means that our proposed methods based on probe **1** is sensitive enough to monitor CN^- concentrations in water.

Selectivity Experiment

The fluorescent intensity of **1** (10 μM) caused by CN^- (5 equiv.) and other miscellaneous competing species (100 equiv.) including F^-, Cl^-, Br^-, I^-, AcO^-, SCN^-, N_3^-, ClO_4^-, NO_3^-, SO_4^{2-}, HSO_4^-, PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$ and HS^- in water were demonstrated in Fig. 6. It can be seen that these competitive species, just like F^-, AcO^-, HSO_4^{2-}, PO_4^{3-}, HPO_4^{2-} and $H_2PO_4^-$ which often show strong interference to CN^- detection, did not disturb the fluorescence emission of **1**. HS^- could attack to the same binding site as its nucleophilicity is comparable to CN^- [42], and an intensity
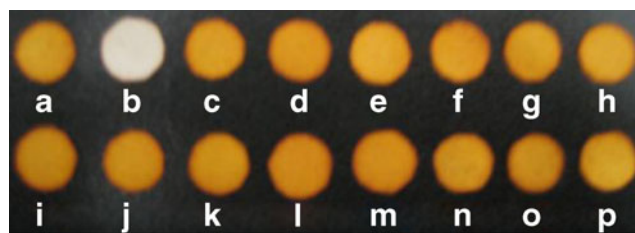

Fig. 7 Color changes of the filter paper containing **1** treated with various anions: **a** (**1**), **b** (CN^-), **c** (F^-), **d** (Cl^-), **e** (Br^-), **f** (I^-), **g** (AcO^-), **h** (SCN^-), **i** (N_3^-), **j** (ClO_4^-), **k** (NO_3^-), **l** (SO_4^{2-}), **m** (HSO_4^{2-}), **n** (PO_4^{3-}), **o** (HPO_4^{2-}) and **p** ($H_2PO_4^-$)

enhancement was observed at 465 nm which was attributed to the ICT process from HS to the naphthalene moiety. Thus, probe **1** shows a high selectivity toward to CN^-. Moreover, in the presence of the competing anions, the CN^- still resulted in similar intensity changes (Fig. 6b).

Filter Paper Test

A preliminary paper test strip system was constructed as shown in Fig. 7. The neutral filter papers were dipped into the stock solution of **1** (10 mM) and dried. Then, the prepared test papers were dipped into the various solutions of anions (1 mM). Only CN^- made the color fade from dark yellow to colorless. This phenomenon demonstrated that probe **1** could be used as a simple paper test for the rapid detecting CN^-.

Conclusion

In summary, probe **1** bearing a benzo [e] indolium group as the binding site and fluorophore was synthesized, and its absorption and fluorescence spectral properties in the presence
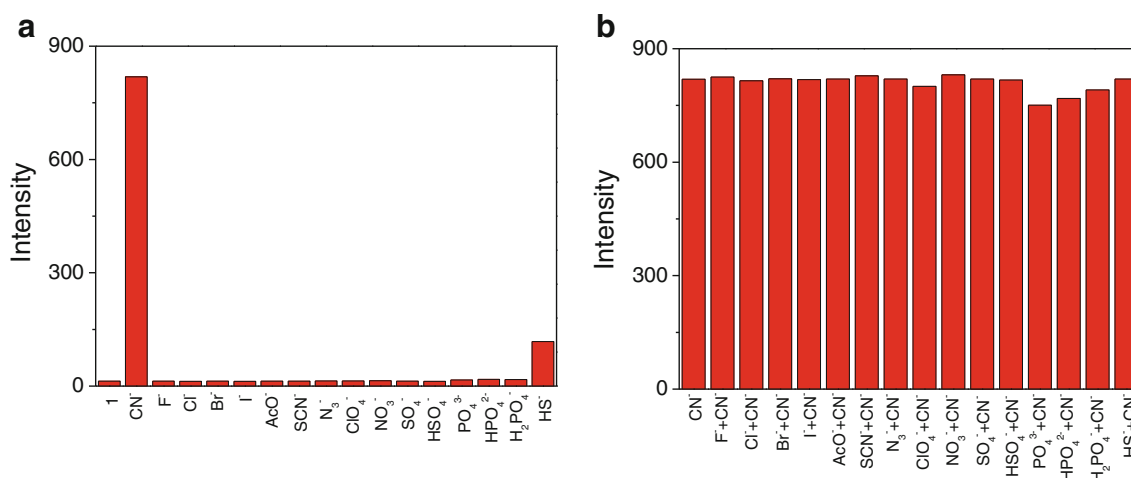

Fig. 6 **a** Fluorescence intensity at 425 nm of **1** (10 μM) upon addition of various anions (100 equiv.). **b** Intensity changes in the presence of the competing anions (100 equiv. respectively) followed by CN^- (5 equiv.). $\lambda_{ex}=345$ nm. Slits: 5 nm/ 5 nm

of anions were evaluated. Turn-on fluorescent and color change upon the addition of CN⁻ was observed in water system. Probe **1** allowed the detection of cyanide with high selectivity in water at a very low concentration (4.6×10^{-8} M), which were below the WHO detection level. Moreover, **1** could be developed as a simple paper test strip system for the rapid monitoring of CN⁻.

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