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A New Ring-opening Chromene Molecule: Colorimetric Detection of Cyanide Anion

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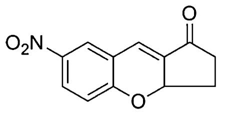
A new cyanide anion colorimetric probe was developed using a chromene derivative, 7-nitro-2,3-dihydro-1*H*-cyclopenta[*b*]chromen-1-one (**1**). The molecule exhibited much higher selectivity and sensitivity for detecting cyanide anion compared with other anions, F^-, Cl^-, Br^-, I^-, SCN^-, AcO^-, NO_3^-, ClO_4^-, SO_4^{2-}, PO_4^{3-}, SO_3^{2-}, CO_3^{2-}, and $C_2O_4^{2-}$, in aqueous media through a visual color change from colorless to yellow. The mechanism was proposed to be a novel chromene ring-opening, resulting from a nucleophilic attack on the probe **1** by CN^- anion.

The development of new anion chemosensors are of interest due to applicalility in chemical, biological, and environmental processes of particular relevance.[1] The cyanide anion (CN^-) is extremely toxic to living organisms[2] and is lethal to humans at 0.5–3.5 mg/kg of body weight.[3] Cyanide anion is also extremely detrimental and can lead to vomiting, convulsion, loss of consciousness, and eventual death.[4] The World Health Organization (WHO) and the Environmental Protection Agency (EPA) have set the maximum contaminant level (MCL) for cyanide to regulate the safe level for drinking water systems at $<1.9\,\mu M$[5] and at $0.2\,mg\,L^{-1}$,[6] respectively. Thus, the sensitive and selective detection of cyanide anion is of considerable importance and significant interest. Many assays have been carried out, including capillary electrophoresis,[7] electrochemical detection,[8] chemiluminescence sensors,[9] biosensors,[10] nanosensors,[11] UV–vis detection,[12] fluorescence detection,[13] colorimetric assays,[14] and others.[15] Among these assays, several color probes based on cyanide nucleophilic addition reaction of oxazine[14a,14b,14c] and spiropyran derivatives[14d] impress us and inspire us to explore new cyanide sensors. Most recently, our group has developed a new thiol probe[16] by employing a chromene derivative 7-nitro-2,3-dihydro-1*H*-cyclopenta[*b*]chromen-1-one (**1**)[17] (Scheme 1), which was simply and easily prepared by the reaction of 5-nitrosalicylaldehyde and 2-cyclohexen-1-one via Baylis–Hillman and intramolecular Michael addition in the presence of imidazole. Therein, the probe **1** response to thiols resulted from a nucleophilic addition. Further investigation showed that the probe **1** can detect selectively and sensitively cyanide anions in an aqueous environment with an evident color change from colorless to yellow.



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Scheme 1. Molecular structure of probe **1**.

Due to the lethality of cyanide anion under acidic condition, only the pH range (7.0–11.0) for determination of cyanide anion was also studied, and the results showed that the system had the UV–visible absorption maxium with no significant difference within the pH range 9.0–11.0. We, therefore, selected the conditions of a HEPES 50 mM, pH 10.0 buffer solution based on the pK_a of HCN being 9.3.

Figure 1a shows the change in the UV–visible spectra when the respective anions solution (100 equiv) is added to HEPES buffer (50 mM, pH 10.0) containing probe **1** (25 µM). As shown by absorption bands, the system exhibited higher selectivity toward cyanide anions (CN^-) than other common anions,

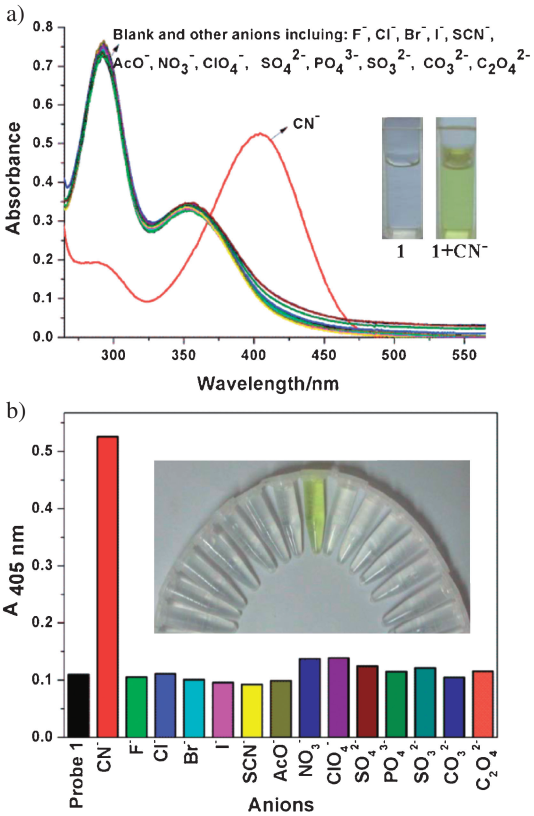


Figure 1. (a) UV–vis absorption spectra of the probe **1** (25 µM) in pH 10.0 aqueous buffer (HEPES 50 mM containing 0.1% EtOH) solution at 37 °C in the absence (blank) and presence of 2.5 mM other anions. Inset: A color change photograph for CN^- and other anions. (b) Optical density of the probe **1** (25 µM) at 405 nm upon addition of anions. Inset: A color change photograph of **1** upon addition of anions. Clockwise: only **1**, F^-, Cl^-, Br^-, I^-, SCN^-, AcO^-, CN^-, NO_3^-, ClO_4^-, SO_4^{2-}, PO_4^{3-}, SO_3^{2-}, CO_3^{2-}, and $C_2O_4^{2-}$.

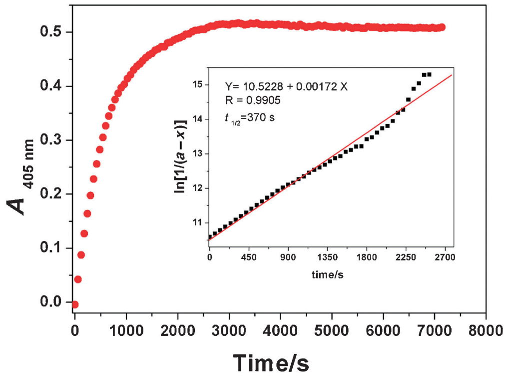
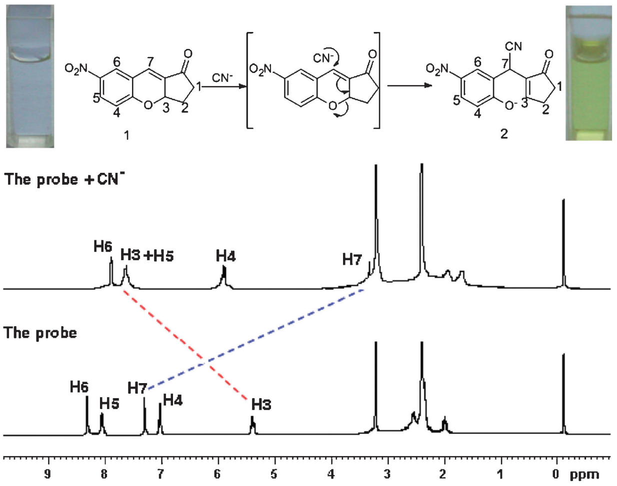


Figure 2. The kinetic study of the response of the probe **1** to cyanide anion at 37 °C under pseudo-first-order condition ([**1**] = 25 µM, [CN^-] = 2.5 mM).

fluoride (F^-), chloride (Cl^-), bromide (Br^-), iodide (I^-), acetate (AcO^-), thiocyanate (SCN^-), nitrate (NO_3^-), perchloride (ClO_4^-), sulfate (SO_4^{2-}), phosphate (PO_4^{3-}), sulfite (SO_3^{2-}), carbonate (CO_3^{2-}), and oxalate ($^-OOCCOO^-$). With cyanide anion presence, the absorption peak at 292 nm of probe **1** is shifted 405 nm (red shifted 113 nm) with an isosbestic point at 368 nm, indicating the formation of a new compound. When changes at 405 nm were monitored, good selectivity was observed for CN^- with a more than fivefold intensity increase in absorbance (Figure 1b). The effect caused by CN^- was very significant, and a distinct color change from colorless to yellow could be detected by the naked eye, while the color of the solutions containing the other anions remained relatively unchanged (Figure 1b inset). This indicates that the addition of other anions to the probe **1** system neither altered the color nor the UV–vis spectra in the system, and nor did their presence interfere with the detection of CN^-. The above results are very useful for biological analysis due to method's simplicity and the use of uncomplicated apparatus.

Figure 2 shows the kinetic study of the response of cyanide anion to probe **1** under pseudo-first-order conditions (25 µM probe **1** and 2.5 mM cyanide anion) at 37 °C. The reaction was finished within 40 min. Rate constants were obtained by fitting the initial concentration change (5–90%) according to a pseudo-first-order kinetics equation. The observed rate constant at pH 10.0 was $k_{obs} = 0.0017\,s^{-1}$, $t_{1/2} = 370\,s$ at 37 °C and $k_{obs} = 0.00078\,s^{-1}$, $t_{1/2} = 817\,s$ at 25 °C, respectively.

The response mechanism for cyanide anions was similar to probe **1** to thiols[16] and shown in Scheme 2 top. The 4-nitrophenolate group, which was released through chromene ring-opening resulting from nucleophilic attack of cyanide on probe **1**, should be responsible for the yellow color change. 1D ^1H NMR spectra (DMSO-d_6, 25 °C, TMS as an internal reference) in the absence and presence of cyanide anions were recorded (Scheme 2, bottom). With addition of CN^- (40 equiv) to probe **1** in DMSO-d_6, the signals of the aromatic protons shifted upfield (δ 6.02, 7.74, and 8.00) from their original resonances (δ 7.14, 8.16, and 8.41, respectively) due to the electron-rich phenolate oxygen atom. The resonance of the original chiral proton (H3) at 5.47 ppm disappeared and a peak at 7.74 ppm (=$CH_{cyclopent\text{-}2\text{-}enone}$) appeared. The signal of the chromene proton H7 changed to 3.53 ppm (assigned as a chiral proton) from the original 7.39 ppm. These implied the formation of the new proposed compound **2** as described in Scheme 2 by reacting cyanide anions with **1**.



Scheme 2. (Top) The proposed detection mechanism for probe **1**. (Bottom) ^1H NMR spectral change of the probe **1** (10 mM) upon addition of cyanide anions (400 mM) in DMSO-d_6. Note: the peak of proton H7 is overlaped partly with the broad peak of water at 3.43 ppm.

In summary, the current study has successfully developed a novel, highly sensitive and selective cyanide-reactive colorimetric probe, using a chromene molecule for the first time on the basis of a new chromene ring-open mechanism in an aqueous environment. The probe is easy to prepare via the Baylis–Hillman reaction and intramolecular Michael addition in the presence of imidazole. The whole recognition process brought evident color changes from colorless to yellow clearly visible to the naked eye. This is perhaps a model for developing new colorimeric or fluorescent cyanide anion probes, based on a number of chromene derivatives.[18] We believe this approach could be extended to many more receptors and may result in a new generation of optical sensors. Further cyanide anion probe designs are actively being researched in our laboratory.

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