

# Cyanine-based Fluorescent Probe for Cyanide Ion Detection

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## Research Article

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# Abstract

Cyanine-based probe-possessing indolium and indole unit was synthesized in two-step with easy available raw material: a potential probe for the cyanide ion detection. The detecting ability of the probe was investigated and confirmed by a visual and instrumental approach. A noticeable color change from orange to colorless obtained only for cyanide ion and other added ions does not impart any changes visually and through UV and Fluorescence technique. To confirm the mechanism of sensing <sup>1</sup>H-NMR recorded. From the result, the peak belonging to N -methyl displayed an upfield shift from 4.01 δ ppm to 2.74 δ ppm due to the disappearance of indolium ion and the olefin protons peaks were shifted from 7.19 to 6.17 and 8.70 to 7.20 δ ppm confirms the nucleophilic addition of cyanide ion to the probe. Test kit from filter paper prepared for the real-time monitoring cyanide ion. The prepared strip is effective in detecting cyanide ion with a visual color change.

## Introduction

Cyanide is the primary pollutant found in our water surface due to its extensive industrial usage. It is involved in several chemical procedures such as silver extraction and gold extraction, metallurgy, tanning, plastic and medicine manufacturing, electroplating, etc. [1-5]. Besides, CN<sup>-</sup> gas uses eliminating vermin and insects and is used as a warfare chemical agent [6,7]. The world health organization has fixed a bearable amount of CN<sup>-</sup> in drinking water and is nearly 500 μM and 70 μM for short-term and long-term revelations, respectively [8]. Several methods are currently reported to recognize cyanide ions, such as titrimetric, potentiometric, chromatographic, voltammetric, and electrochemical approaches [9-12]. But, many of these approaches are time taking, costly, and difficult for on-site recognition. Hence, it is essential to produce extremely selective, sensitive, and suitable methods for recognizing cyanide in real samples. The simple, economical, and quick executions of optical sensors (colorimetric/fluorimetric) have been getting much attention recently [13-17]. In the optical chemosensors, various mechanisms were reported in the literature, such as hydrogen bonding, nucleophilic addition (chemodosimeter), and supramolecular self-assembly [18-24]. The maximum of developed probes suffered by challenging synthetic procedures, inadequate color response, lack of selectivity, and sensing limits in a complete aqueous medium. However, some of the receptors overcome these drawbacks to detect CN<sup>-</sup> ions reported in the literature [25-27]. Thus, the progress of developing a useful probe, which could be proficient at recognizing cyanide ion in maximum water-consuming medium, is getting extreme care. Considering these points, two easy to make cyanine based probe (**PI**) has been synthesized. The stable intramolecular transition (ICT) and extended conjugation between the electron-deficient indolium and electron-rich indole moiety make the examinations show brilliant recognizing properties. Likewise, this kind of probes holds a very responsive indolium moiety to identify cyanide ions [28-30]. Consequently, the cyanide ion reacts with probes' indolium site by the nucleophilic attack that interrupts the π-conjugation and thus blocked the ICT from indole to indolium group follow-on a substantial fluorescence and absorption variation can occur.

# Experimental

## 2.1. Materials and methods

2,3,3-Trimethyl-3*H*indole, 1*H*indole-3-carbaldehyde, iodomethane, tetrabutylammonium salts were purchased from Sigma Aldrich, Piperidine purchased from TCI chemicals. Toluene and ethanol HPLC grade solvents received from Rankem. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were reordered in order to confirm the compounds. Bruker AVANCE III spectrometer was used and operated at 600 MHz (<sup>1</sup>H NMR), and 150 MHz (<sup>13</sup>C NMR) at ambient temperature, DMSO-d<sub>6</sub> is used as a solvent. Bruker-micrOTOF QII mass spectrometer was used to record the HRMS. Agilent 8453 spectrophotometer was used to obtain UV-Vis absorption spectra and RF-5301PC spectrofluorophotometer was used for fluorescence emission spectra. For the fluorescence measurements, the excitation wavelength was fixed as 290 nm, and both the excitation and emission slit widths were 3 nm.

## 2.2. Synthesis

Fluorescent probe (**PI**) was easily synthesized in two steps, by using simple and inexpensive starting materials. In the first step, methylation of compound (**1**) with iodomethane gives compound (**2**) in excellent yields. In the second step, compound (**2**) undergoes a simple aldol condensation reaction with indole aldehyde to provide fluorescent probe (**PI**) in good yield. The synthesized compounds were confirmed by NMR (<sup>1</sup>H and <sup>13</sup>C) and HRMS.

**2.3. Experimental procedure for the synthesis of 1,2,3,3-tetramethyl-3*H*indolium iodide (**2**).** Compound **1** (0.5 g, 4.399 mmol) was dissolved in toluene (10 mL) and iodomethane (0.85 mL, 13.207 mmol) was added at room temperature and the reaction was refluxed for 6 h. during the reaction the solid precipitate was formed. The reaction mixture was cooled to room temperature and the solid was filtered and washed with diethyl ether (20 mL). The resulting white crystalline solid was used to next step without purification. Yield: (0.734 g, 96%), mp 258 – 260 °C, <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 600 MHz) δ 7.94 – 7.92 (m, 1H), 7.86 – 7.83 (m, 1H), 7.64 – 7.61 (m, 2H), 3.99 (s, 3H), 2.80 (s, 3H), 1.54 (s, 6H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 150 MHz) δ 196.4, 142.5, 142.0, 129.7, 129.2, 123.7, 115.6, 54.4, 35.3, 22.2. HRMS m/z calculated for C<sub>12</sub>H<sub>16</sub>N<sup>+</sup> (M)<sup>+</sup>: 174.1277, found: 174.1280.

**2.4. Experimental procedure for the synthesis of 2-(2-(1*H*indol-3-yl)vinyl)-1,3,3-trimethyl-3*H*indol-1-ium iodide (**PI**):** Compound **2** (0.5 g, 2.871 mmol) and indole-3-carbaldehyde (0.41 g, 2.871 mmol) was dissolved in ethanol (15 mL) and piperidine (3 μL, 0.12 mmol) was added and refluxed for 3 h under nitrogen atmosphere. Orange solid was obtained, filtered and washed with diethyl ether (20 mL) to produce bright solid. Yield: (0.95 g, 78%), mp 285 – 287 °C, <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 600 MHz) δ 12.76 (brs, 1H), 8.70 (d, *J* = 15.8 Hz, 1H), 8.69 (s, 1H), 8.28 (dd, *J* = 5.8, 3.0 Hz, 1H), 7.81 (d, *J* = 7.3 Hz, 1H), 7.76 (d, *J* = 7.9 Hz, 1H), 7.62 (dd, *J* = 5.8, 3.0 Hz, 1H), 7.57 (t, *J* = 7.5 Hz, 1H), 7.50 (t, *J* = 7.5 Hz, 1H), 7.39 (dd, *J* = 6.0, 3.0 Hz, 1H), 7.19 (d, *J* = 15.8 Hz, 1H), 4.01 (s, 3H), 1.81 (s, 6H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 150 MHz) δ 180.6, 149.3, 142.9, 142.5, 138.8, 129.7, 129.2, 129.1, 127.9, 125.0, 124.8, 123.6, 123.1, 121.9, 116.3, 115.5,

114.0, 113.9, 105.5, 54.3, 51.3, 22.1. HRMS  $m/z$  calculated for  $C_{21}H_{21}N_2^+$  (M)<sup>+</sup>: 301.1699, found: 301.1704.

## Results And Discussion

### 3.1 Visual reaction of probe PI

The binding reaction of probe **PI** ( $1 \times 10^{-5}$  M) with a numerous anions ( $1 \times 10^{-5}$  M) like  $AcO^-$ ,  $SCN^-$ ,  $CO_3^{2-}$ ,  $CN^-$ ,  $PF_6^-$ ,  $CNO^-$ ,  $HSO_4^-$ ,  $H_2PO_4^-$ ,  $Cl^-$ ,  $Br^-$ ,  $NO_3^-$ ,  $ClO_4^-$ ,  $F^-$ ,  $SO_4^{2-}$  and  $N_3^-$  was inspected in DMF:water (2:8, v/v) through colorimetric appreciation performance was exhibited in figure 1A. As per expectation, the probes **PI** display deep orange color which immediately changed to colorless with cyanide addition while the other added ions not generating such color variation. Similarly, the changes obtained were checked under UV lamp the probe and the added ion except cyanide ion showed little yellow fluorescence means no substantial change in the probe solution and with cyanide ion, it is showing blue fluorescence under UV light figure 1B. This primary examination exposed that the probes **PI** can selectively recognize  $CN^-$  ion with a discrete color variation.

### 3.2. Absorption studies

The absorption modification of probe **PI** was examined with the existence of a number of anions as mentioned directly above. As seen from figure 2, the probe **PI** displayed a band pointed at 489 nm and this might be because of the ICT throughout the probes and this transition is accountable for the color of probes. Further, the added ions did not produce in the least alteration in the bands while the adding of cyanide ion showed a fresh band at 289 nm array with the disappearance of a band at 489 nm. This blue shift of probe with  $CN^-$  ion indicates that there was a strong binding between the probes and cyanide ion. To know the binding capability of probe **PI** ( $1 \times 10^{-5}$  M), a titration was conducted with increase in addition of  $CN^-$  ion to the probe solution (Fig. 3A). With the gradual quantities of  $CN^-$  ions ( $0-1 \times 10^{-5}$  M) added, the fresh band placed on 289 nm detected with growing intensity of band, which is attributed by clear color transformation, which could be seen above. The limit of detection of **PI** for  $CN^-$  was deliberated as  $1.73 \times 10^{-5}$  M, based on  $3\delta/k$  (Fig. 3B).

### 3.3. Fluorescence spectral studies

Similarly, the recognizing capability of **PI** with chosen anions has been inspected by emission spectral technique when exciting at 289 nm. As seen from figure 4, the probe **PI** excited at 289 nm. Then the emission peak appeared at 373 nm and was enhanced with the added  $CN^-$  ion, whereas no changes in the spectra were spotted with the other chosen anions. Thus, the above observation states that there is an interaction among the probes with cyanide ion.

The 'On-Off' response of fluorescence is effectively monitored with the steady addition of  $CN^-$  ion ( $0-1 \times 10^{-5}$  M) to the solution of probes. The figure 5A displayed that the probes shown its emission at 373

nm for **PI** were in progress to increase progressively with the CN<sup>-</sup> ion addition. The emission peak of the probe entirely enhanced with added cyanide ion (0-1x10<sup>-5</sup> M). The color variations of **PI** with cyanide ion in UV lamp was taken and kept in the figure 5A. With CN<sup>-</sup> ion addition, the probes turned its less fluorescent yellow color to deep blue fluorescent. From the obtained data from the fluorescence spectra, the binding constant can be calculated using the equation for cyanide ion  $(F_x - F_0)/(F_x - F_0) = 1/K[CN^-]$  it is 1.17x10<sup>5</sup> M<sup>-1</sup> (Fig. S7). The calculated detection limit by emission profile was found to be 1.8x10<sup>-8</sup> M for **PI** by means of 3σ/k (Fig. 5B).

### 3.4. pH effect of probe towards CN<sup>-</sup> ion

The recognizing aptitude of **PI** for cyanide ion was further checked by the effect of pH by absorption study. As seen in figure 6, in acidic and neutral pH (2-7), the free probes exhibited the absorbance at 489 nm while the absorbance is reduced with CN<sup>-</sup> ions. At basic pH (8-10), the probes absorbance gently weakened and touched the lower most λ<sub>max</sub> (pH 10), whereas no any absorbance was detected with the added CN<sup>-</sup> ions. The changes in the absorbance peak in basic pH are primarily because of the OH<sup>-</sup> ion interference, which binds intensely with the probes.

### 3.5. Anti-interference study of probes

Moreover, to inspect the anti-interference study of probe **PI** with CN<sup>-</sup> ion in the existence of added interfering anions, competitive investigation has been carried out (Fig. 7). The emission of probe **PI** (1x10<sup>-5</sup> M) with several chosen ions (1x10<sup>-5</sup> M) excepting CN<sup>-</sup> were initially monitored, afterwards the emission was measured for a second time with same samples with the addition of 1x10<sup>-5</sup> M of CN<sup>-</sup> ions. The figure pointed out that there is no or weak emission intensity for free probes and probes with the chosen ions, the peak intensity arise simply when the presence of cyanide ion. These observations suggested that the anti-interference of the probes for cyanide ion possibly would not be inclined by chosen competing ions. Hence, these probes confirm the exceptional selectivity for cyanide ion.

### 3.6. Probable mechanism by <sup>1</sup>H NMR study

In the synthetic scheme, *N*-methylation step (step-1) was confirmed by the appearance of sharp singlet at 3.99 (<sup>1</sup>H NMR) and 54.4 δ (<sup>13</sup>C NMR) ppm, corresponds to the methyl protons. These protons are more deshielded due to formation of indolium ion (strong electron-withdrawing ability (N<sup>+</sup>-CH<sub>3</sub>)). The HRMS spectra also further proved the *N*-methylation. In the second step, the fluorescent probe (**PI**) was confirmed by the disappearance of sharp singlet peak of methyl protons at 2.80 (<sup>1</sup>H NMR) and 35.3 δ (<sup>13</sup>C NMR) ppm, and appearance of indole protons as well as olefin bridge protons in the <sup>1</sup>H NMR spectrum. The stereo chemistry of the double bond (trans or “Entagagen”) was also confirmed by the <sup>1</sup>H NMR coupling constant of olefin protons (*J* = 15.8 Hz). This probe **PI** is used for the detection of CN<sup>-</sup> anion. The nucleophilic addition of CN<sup>-</sup> anion to the C=N bond in the indolium ring was also confirmed by the in situ analysis (NMR) of sample.

In the probe **PI** the chemical shift values of *N*-methyl protons appeared at 4.01 (HX)  $\delta$  ppm and the olefin protons was observed at 7.19 (HY) 8.70 (HZ)  $\delta$  ppm. In the next step, we dissolved this probe **PI** and TBACN in DMSO- $d_6$  solvent and recorded  $^1\text{H}$  NMR spectra. After  $\text{CN}^-$  anion attack, the chemical shift values were shifted to up field region on the  $^1\text{H}$  NMR scale (Figure 8). Moreover, a remarkable difference was observed in the chemical shift values of HX, HY and HZ. The peak belonging to *N*-methyl displayed an up field shift from 4.01  $\delta$  ppm to 2.74  $\delta$  ppm due to disappearance of indolium ion. Further, the olefin protons peaks were shifted from 7.19 to 6.17 (HY) and 8.70 to 7.20 (HZ)  $\delta$  ppm. From the above data, it is clearly confirmed that the **PI** molecule underwent  $\text{CN}^-$  nucleophilic addition reaction that cleanly converted the indolium ion to indoline-2-carbonitrile (**PI-CN**).

### 3.7. Practical application

The probes' practical use was monitored by making test strips for the immediate recognition of  $\text{CN}^-$  ions in aqueous solutions. Whatman paper was immersed into DMF solution ( $1 \times 10^{-3}$  M) of **PI** followed by drying. These strips were then tested with an aqueous solution containing different  $\text{CN}^-$  ion concentrations and thus were displayed in figure 9, where the strips of probes slowly decolorized. Therefore, the test kit prepared from filter paper appropriately senses cyanide ion in water medium without the requirement and the usage of any additional equipment. This experimentation creates that the probes are reasonably helpful for quick on-site recognition of  $\text{CN}^-$  ion.

## Conclusion

To conclude, we have developed a cyanine-based conjugated system of probes for the recognition of cyanide ion by nucleophilic addition mechanism. The recognition method of cyanide ion is visualized by a notable color variation to colorless from orange color. The lower most limit of detection for  $\text{CN}^-$  ion were proven by both fluorimetric approaches and thus below the tolerable limit set by WHO. The  $^1\text{H-NMR}$  investigation was established the proposed mechanism of probes with cyanide ion. Additionally, the prepared strips were useful for the recognition of  $\text{CN}^-$  ions short of resorting additional equipment.

## Declarations

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### Author's Declarations

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**Availability of data and material:** All data available.

**Code availability:** Not applicable.

**Author's contributions:** All authors (Mahesh Gosi, Nagaraju Marepu, and Yeturua Sunandamma) made substantial contribution in preparing the manuscript.

**Ethics approval:** Not applicable

**Consent to participate:** Informed consent obtained from all individual participants included in the study.

**Consent for publication:** Not applicable

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## Figures

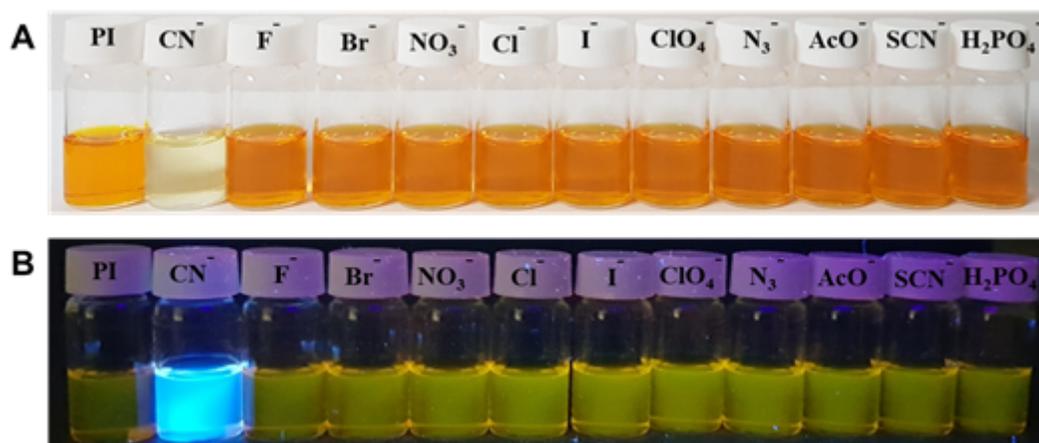


Figure 1

Color changes of PI with various anions (A) day light and (B) UV light

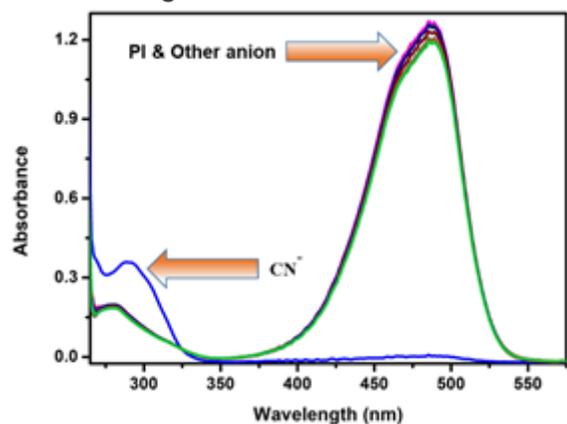
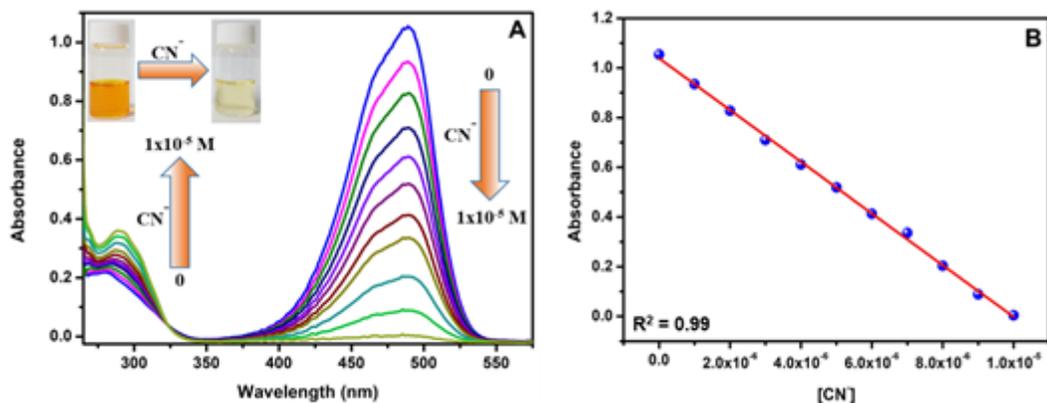


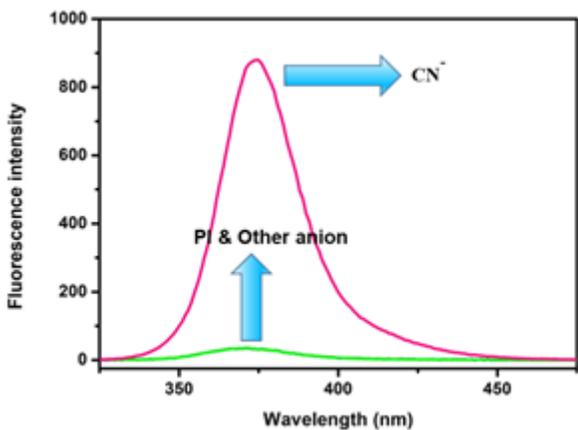
Figure 2

Absorption spectral changes of PI with several anions



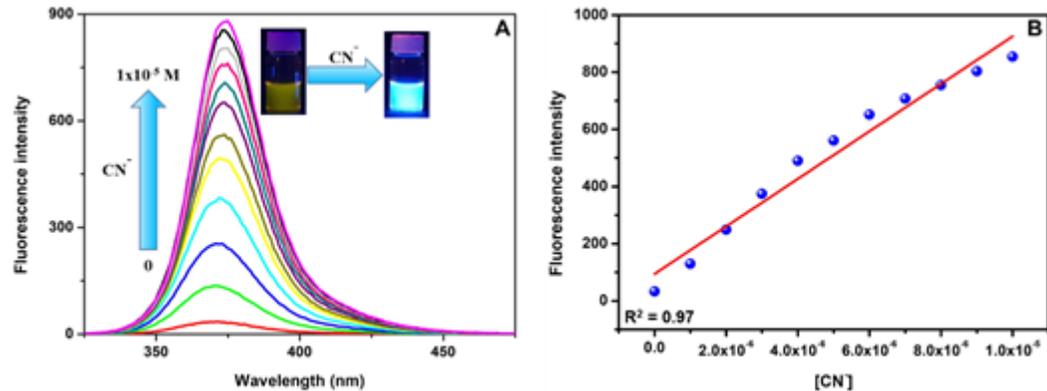
**Figure 3**

(A) Absorption changes of PI ( $1 \times 10^{-5}$  M) with  $\text{CN}^-$  ion ( $0-1 \times 10^{-5}$  M) addition in 2:8 v/v DMF: Water, (B) Plot for limit of detection calculation



**Figure 4**

Fluorescence spectral changes of PI with various anions



**Figure 5**

(A) Fluorescence spectral changes of PI ( $1 \times 10^{-5}$  M) with  $\text{CN}^-$  ion ( $0-1 \times 10^{-5}$  M) addition in 2:8 v/v DMF: Water, (B) Plot for limit of detection calculation

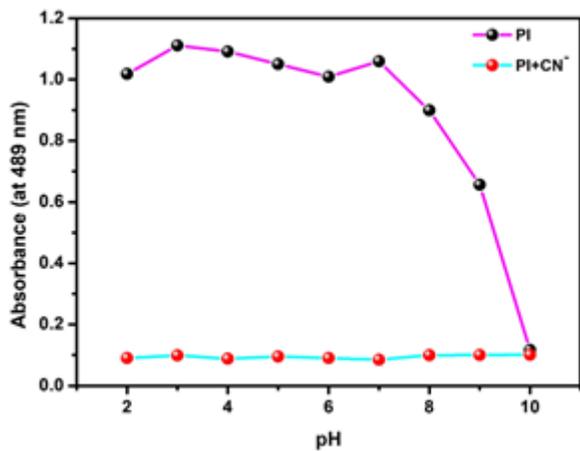


Figure 6

The pH effect of probe PI and its cyanide complex

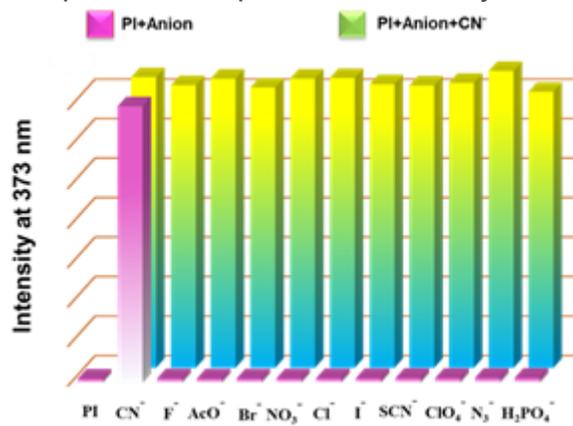
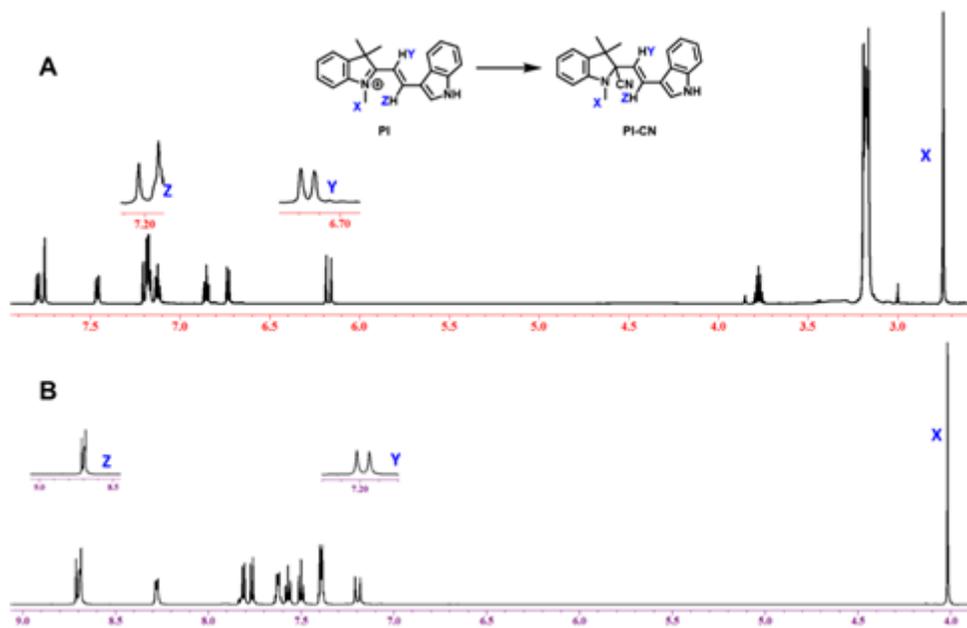


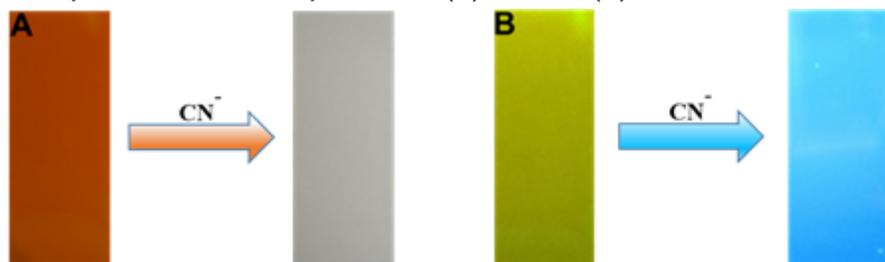
Figure 7

Competitive study of probe PI with cyanide ion in presence of different anions



**Figure 8**

The partial  $^1\text{H-NMR}$  spectra of (A) PI and (B) PI-CN in DMSO- $d_6$



**Figure 9**

Color change of test strips of PI with  $\text{CN}^-$  ion (A) day light, (B) UV light

## Supplementary Files

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