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# Supporting Information

**for**

# Ratiometric fluorescent and chromogenic chemodosimeter for cyanide detection in water and its application for bioimaging

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**General Information and Materials.** Unless otherwise mentioned, materials were obtained from commercial suppliers and were used without further purification. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Brucker 300 MHz instrument. For NMR spectra, DMSO-$d_6$ and $CDCl_3$ were used as solvent using TMS as an internal standard. Chemical shifts are expressed in δ ppm units and $^{1}H$–$^{1}H$ and $^{1}H$–C coupling constants in Hz. Mass spectra were carried out using a Waters QTOF Micro YA 263 mass spectrometer. Fluorescence spectra were recorded on a Perkin Elmer Model LS 55 spectrophotometer. UV spectra were recorded on a JASCO V-530 spectrophotometer. Elemental analysis of the compounds was carried out on Perkin-Elmer 2400 series CHNS/O Analyzer. Salts of different anions, viz., $Bu_4NF$, $Bu_4OAc$, $Bu_4NI$, $Bu_4NBr$, $Bu_4NCl$, KCN, $Bu_4H_2PO_4$, $Na_2SO_4$, NaSH, $NaNO_2$ were purchased from Spectrochem Pvt Ltd.(India).

Bulk solution of **ITP**, anion and cationic salts were made up in DMSO-water (5:95, v/v). For UV-vis and fluorescence titrations, stock solution of **ITP** was prepared (c = 1.0 x $10^{-6}$ M) in DMSO-$H_2O$ (5:95, v/v).The solution of the guest anions salts in the order of (4.0 x $10^{-6}$ M) was prepared in Millipore water.

**Cellular Imaging Methodology**.

**Cell Culture:** Frozen Human colorectal carcinoma cell line HCT 116 (ATCC : CCL-247) was obtained from the American Type Culture Collection (Rockville, MD, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma Chemical Co., St. Louis, MO, USA) supplem ented with 10% fetal bovine serum (Invitrogen), penicillin (100 μg/mL), and

streptomycin (100 μg/mL). The RAW 264.7 macrophages were obtained from NCCS, Pune, India and maintained in DMEM containing 10% (v/v) fetal calf serum and antibiotics in a CO2 incubator. Cells were initially propagated in 75 cm2 polystyrene filter-capped tissue culture flask in an atmosphere of 5% $CO_2$ and 95% air at 37°C humidified air till 70- 80% confluency. When the cells reached the logarithmic phase, the cell density was adjusted to 1.0 x $10^5$ per/well in culture media. The cells were then used to inoculate in a glass bottom dish, with 1.0 mL (1.0 x $10^4$ cells) of cell suspension in each dish. After cell adhesion, culture medium was removed. The cell layer was rinsed twice with phosphate buffered saline (PBS), and then treated according to the experimental requirement.

**Cell Imaging Study:** For fluorescence imaging studies RAW cells, 1.0 x $10^4$ cells in 150 μL of medium, were seeded on sterile 35 mm μ-Dish, glass bottom culture dish (ibidi GmbH, Germany), and incubated at 37 °C in a $CO_2$ incubator for 24-30 hours. The next day, cells were washed three times with phosphate buffered saline (pH 7.4) and fixed using 4% paraformaldehyde in PBS (pH 7.4) for 10 min at room temperature. Thereafter the cells were washed with PBS followed by permeabilization using 0.1% saponin for 10 min followed by incubation with $2.0 \times 10^{-5}$ M KCN dissolved in 100 μL DMEM at 37 °C for 1 h in a $CO_2$ incubator. Before microscopic imaging, all the solutions were aspirated and mounted on slides in a mounting medium containing DAPI (1 μg/mL) and stored in dark before microscopic images were acquired. The cells were observed under Andor spinning disk confocal microscope (SD-CM) with excitation at 488 nm monochromatic laser beam and the collected range of emission wavelength was between 510 and 560 nm (green channel).

Cells were imaged live by SD-CM 63× oil-immersion objective. Images were acquired in *z*-stacks of 28 planes at 0.3-μm intervals with 400-ms exposure times every 20 seconds over a period of 30 minutes. The cells were again washed thrice with PBS (pH 7.4) to remove any excess $CN^-$ and incubated in DMEM containing probe (**ITP**) to a final concentration of $1.1 \times 10^{-5}$ M followed by washing with PBS (pH 7.4) three times to remove excess probe outside the cells. In the same μ-Dish the cells were then treated with $1.0 \times 10^{-5}$ M of $AgNO_3$ solution for 1 h; the cells were washed with PBS three times to remove free compound and ions before microscopic observation as before with excitation at 488 nm. The emissions were obtained at 580 - 630 nm (red channel).

**Cytotoxic effect on Cells:** The cytotoxic effects of ITP, $CN^-$, ITP- CN complex and $Ag^+$ were determined by MTT assay following the manufacturer's instruction (MTT 2003, Sigma-Aldrich, MO). HCT cells were seeded onto 96-well plates (approximately $10^4$ cells per well) for 24 h. Next day media was removed and various concentrations of probe ITP, $CN^-$ and ITP- CN complex (0, 15, 25, 50, 75, and 100 μM) made in DMEM were added to the cells and incubated for 24 h. Solvent control samples (cells treated with DMSO in DMEM), no cells and cells in DMEM without any treatment were also included in the study. Following incubation, the growth media was removed, and fresh DMEM containing MTT solution was added. The plate was incubated for 3−4 h at 37°C. Subsequently, the supernatant was removed, the insoluble colored formazan product was solubilized in DMSO, and its absorbance was measured in a microtiter plate reader (Perkin-Elmer) at 570 nm.

The assay was performed in triplicate for each concentration of probe ITP, $CN^-$ and ITP- CN complex. The OD value of wells containing only DMEM medium was subtracted from all readings to get rid of the background influence. Data analysis and calculation of standard deviation was performed with Microsoft Excel 2007 (Microsoft Corporation).

**Preparation of ITP:**

2,3,3-Trimethyl-3H-indole was dissolved in dry $CHCl_3$. Drop wise methyl iodide was added into it and mixture was stirred at room temperature overnight when pale pink precipitate appeared. The cationic salt precipitate was filtered, washed with $CHCl_3$ for several times and collected. 6-(2-thienyl)-2-pyridinecarboxaldehyde (1.05 mmol, 100 mg) and N,2,3,3-tetramethylindolium cationic salt (1.05 mmol, 184 mg) were refluxed in 10 ml ethanol solution for 5 h. After reflux the mixture was stirred at room temperature for 1 hr. The solvent was evaporated in vacuum. The red residue was recrystallized by acetic ether/hexane to get the pure product as red crystalline solid (290 mg, 80%). Mp above 250°C. **$^1$H-NMR** ($CDCl_3$, 400 MHz): δ (ppm) 8.61(d, 1H, J=16.0 Hz), 8.28 (d, 1H, J=7.64 Hz), 8.17 (d, 1H, J=16.0 Hz), 7.76 (1H, t, J=8.40 Hz), 7.67 (d, 2H, J=8.30 Hz), 7.64 (d, 1H, J=3.08), 7.62 (m, 3H), 7.45 (d, 1H, J=4.6 Hz), 7.17 (t, 1H, J= 7.20 Hz), 4.51(s, 3H), 1.91 (s, 6H). **Anal. Calcd.** C 76.52, H 6.08, N 8.11, S 9.27; found: C 76.53, H 5.97, N 8.12, S 9.25; **MS (ESI MS)**: (m/z, %): **345.2457**[(**$ITP^+$**), 100 %]; Calculated for $C_{22}H_{21}N_2S$: **345.4884.** **$^{13}$C-NMR** (DMSO-d6, 75 MHz): δ(ppm) 181.68, 152.56, 150.87,149.38, 143.80,143.52, 141.82, 138.80, 130.03, 129.57, 129.13, 128.73, 126.88, 126.73, 121.80, 115.75, 115.69, 52.60, 34.52, 24.94.

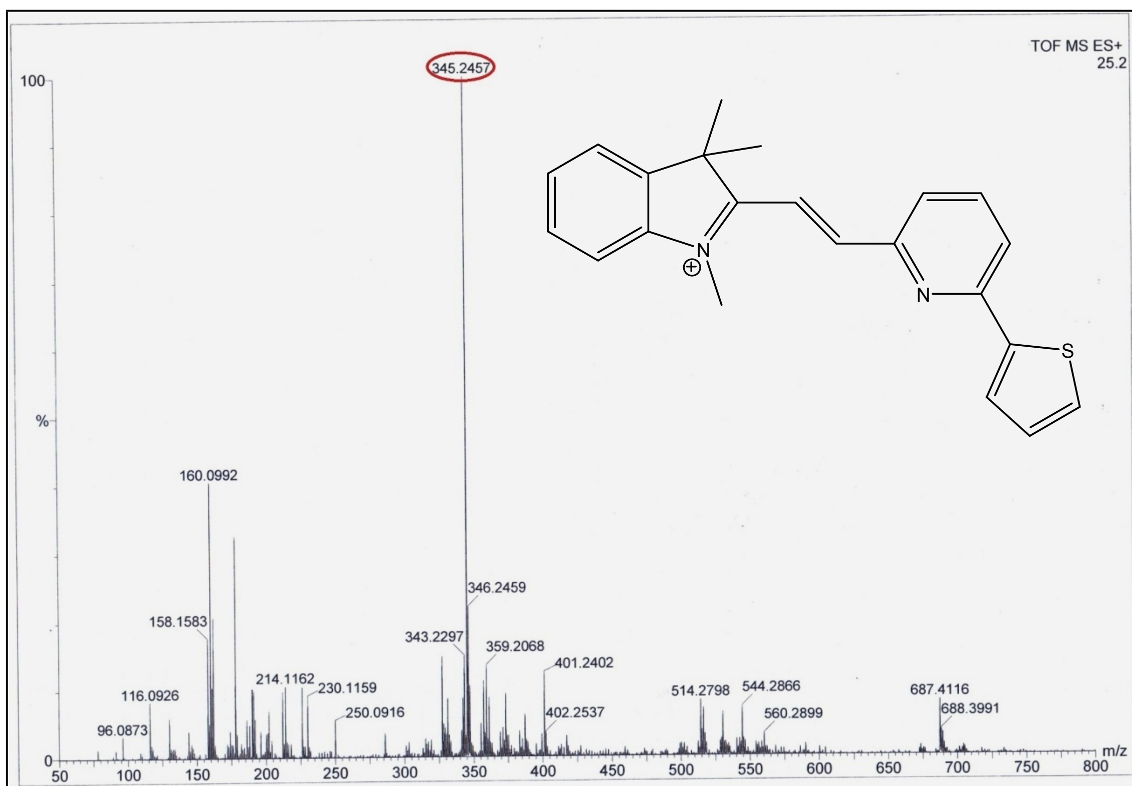


**Fig. S1**: ESI MS of **ITP.**

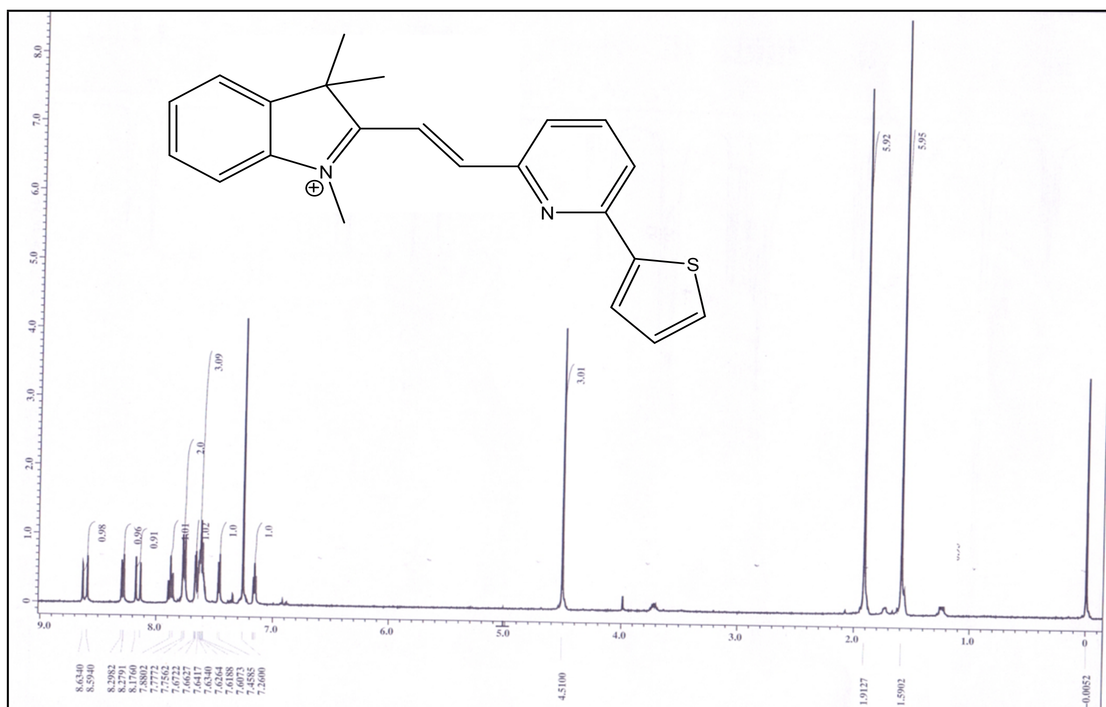

**Fig. S2**: $^1$H NMR of **ITP** in $CDCl_3$**.**

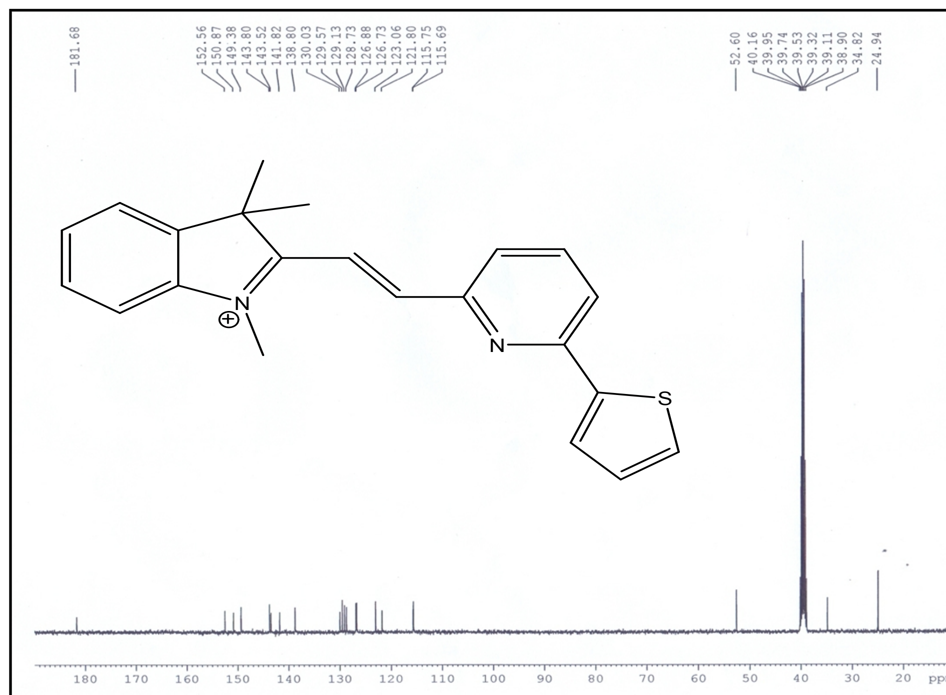


**Fig. S3**: $^{13}$C NMR of **ITP** in $d_6$-DMSO.

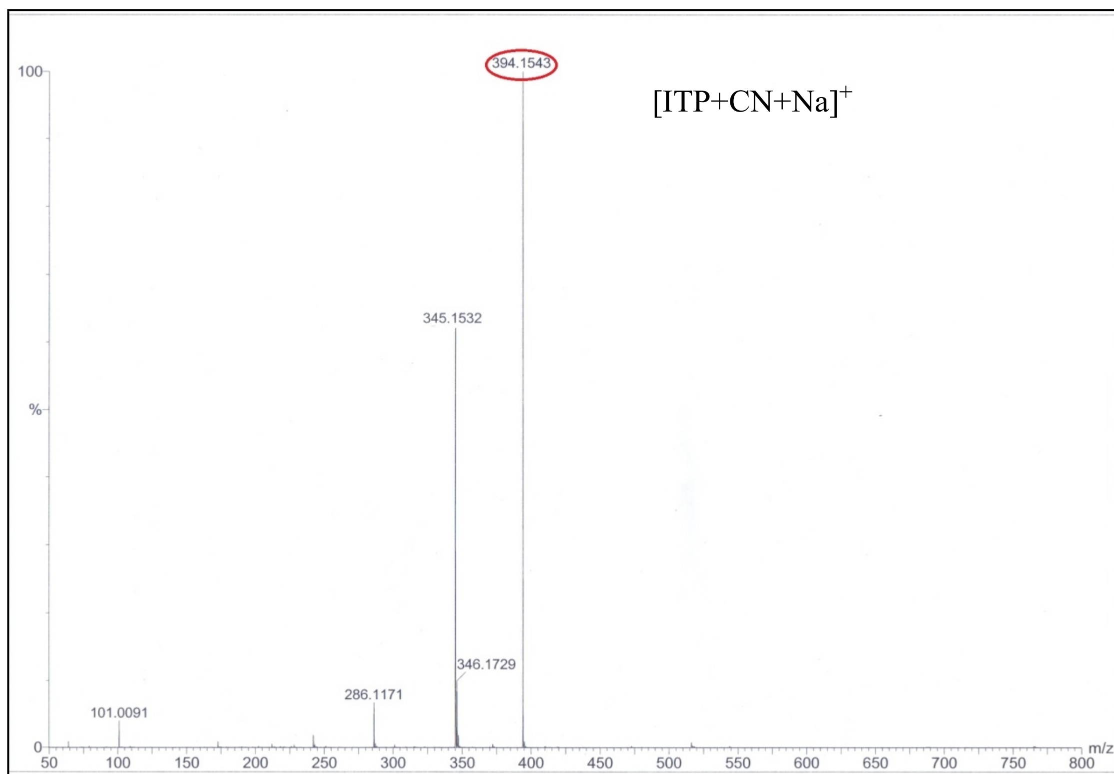


**Fig. S4**: HRMS of **ITP-CN**

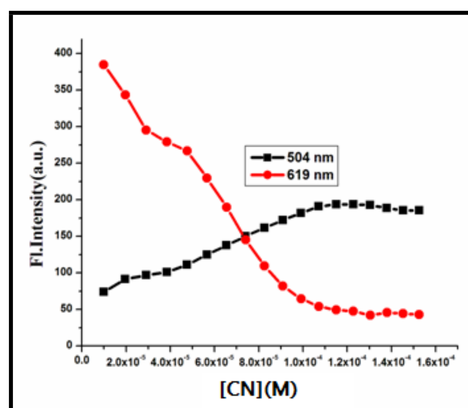


**Fig. S5**: Change in fluorescence intensity at 504 nm and 619 nm as the function of **CN**$^{-}$ concentration.

**Calculation of Detection limit:**

The detection limit (DL) of **ITP** for CN$^{-}$ were determined from the following equation:

DL = K* Sb1/S

Where K = 2 or 3 (we take 2 in this case); Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

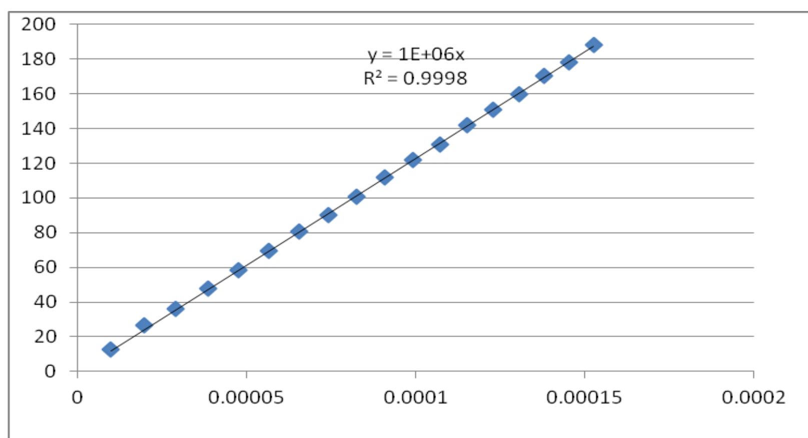


**Fig. S6**: Calibration curve for fluorescence titration of **ITP** with CN$^{-}$.

From the graph we get slope (S) = $1\times 10^{6}$. Standard deviation (Sb1=0.77)

Thus using the formula we get the detection limit=1.5 µM

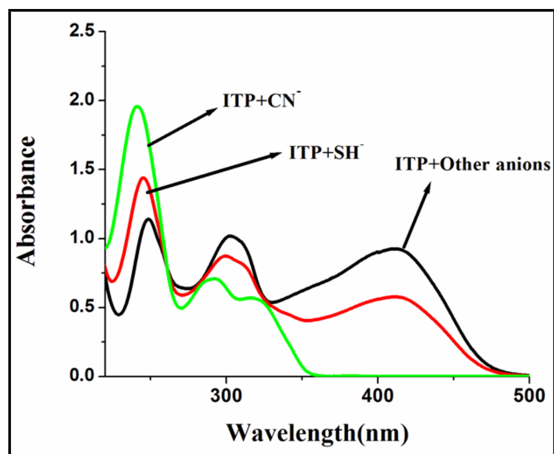


**Fig. S7:** Change in absorbance spectra of **ITP** when treated with $CN^-$ and other anions.

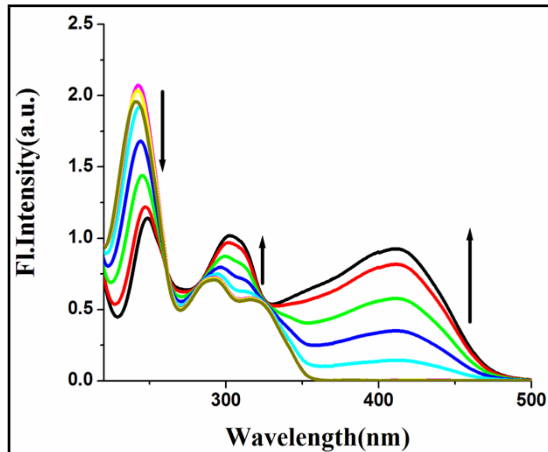


**Fig. S8**: UV-vis spectral changes of **ITP-CN** ($1.0 \times 10^{-6}$ M) in DMSO-$H_2O$ (5:95 V/V; pH 9.3) upon addition of $Ag^+$ ($2.0 \times 10^{-5}$ M ).

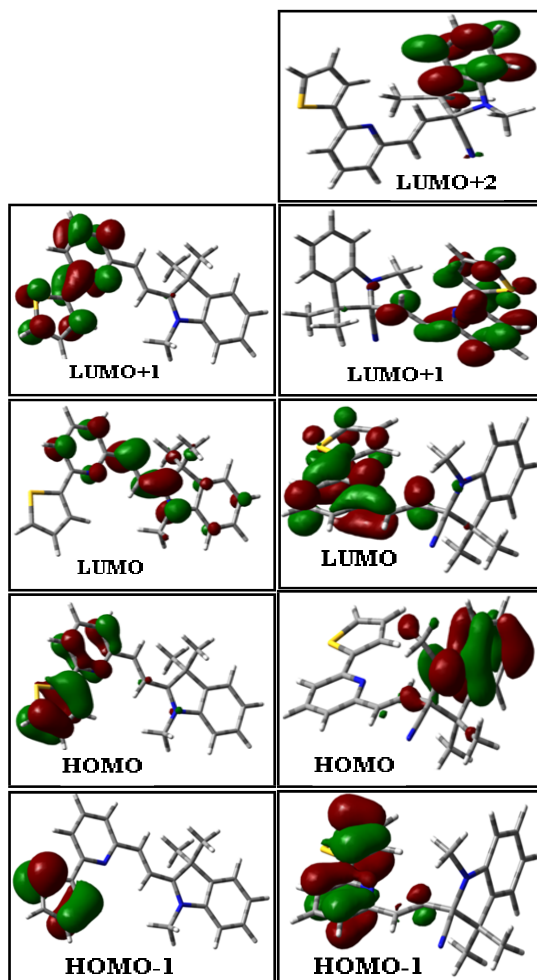


**Fig. S9**: Molecular orbitals ar **ITP** tronic cor **ITP-CN** f the relevant excitations of **ITP** and **ITP-CN**.

## Table S1:

| | Nature of transition | Wave lenth(nm) | Oscillator strenth(f) | % of transition |
|---|---|---|---|---|
| ITP | HOMO-1 → LUMO | 405.02 | 0.7130 | 64.66 |
| | HOMO→ LUMO+1 | 315.28 | 0.3748 | 57.53 |
| ITP-CN | HOMO-1 → LUMO | 330.68 | 0.2516 | 66.33 |
| | HOMO-1 → LUMO+1 | 301.48 | 0.3196 | 64.51 |
| | HOMO→ LUMO+2 | 281.12 | 0.5537 | 70.89 |

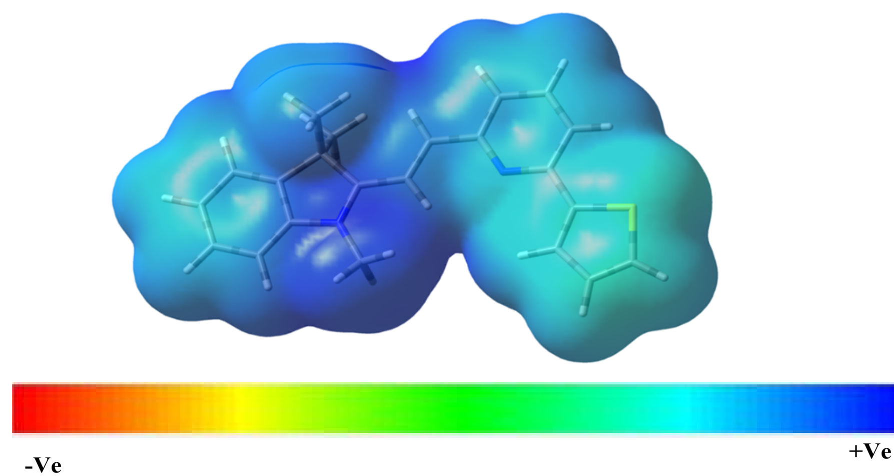


**Fig. S10**: The placement of charge density of **ITP**.

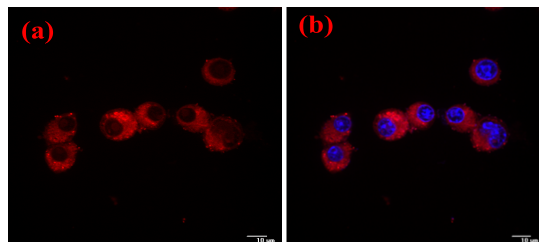


**Fig. S11:** Confocal fluorescence images of probe in Raw 264.7 cells (40× objective lens). (a) Stained with probe **ITP** only at concentration 1.0 x $10^{-6}$ M (b) overly image in dark field.

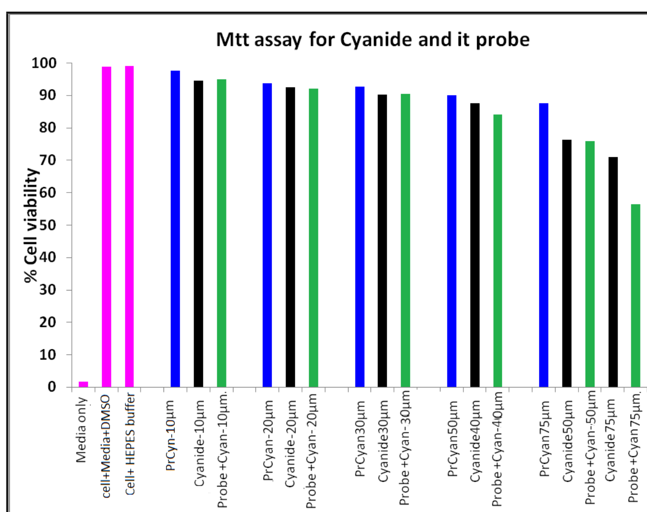


**Fig. S12:** MTT assay to determine the cytotoxic effect of **ITP** and **ITP-CN** complex on HCT cells (* PrCyan denotes Probe for cyanide i.e. **ITP**).

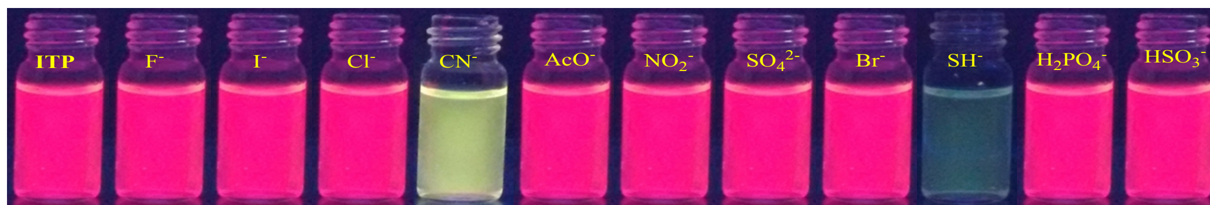


**Fig. S13:** Naked eye fluorescence change of **ITP** when different anions were added to it.

**Kinetic Studies:**

The reaction of sensor **ITP** ($2.0 \times 10^{-6}$M) with $CN^-$ in DMSO was monitored using the fluorescence intensity at 504 nm ($\lambda_{exc}$ = 411nm). The reaction was carried out at room temperature with an excess amount of $CN^-$ (10-12eq) present in the reaction vessel. The apparent rate constant for the reaction was determined by fitting the fluorescence intensities of the samples to the pseudo first-order equation (1):

$$\mathrm{Ln}((F_{max} - F_t) / F_{max}) = -k' t \quad (1)$$

Where $F_t$ and $F_{max}$ are the fluorescence intensities at 504 nm at times $t$ and the maximum value obtained after the reaction was complete. $k'$ is the apparent rate constant.

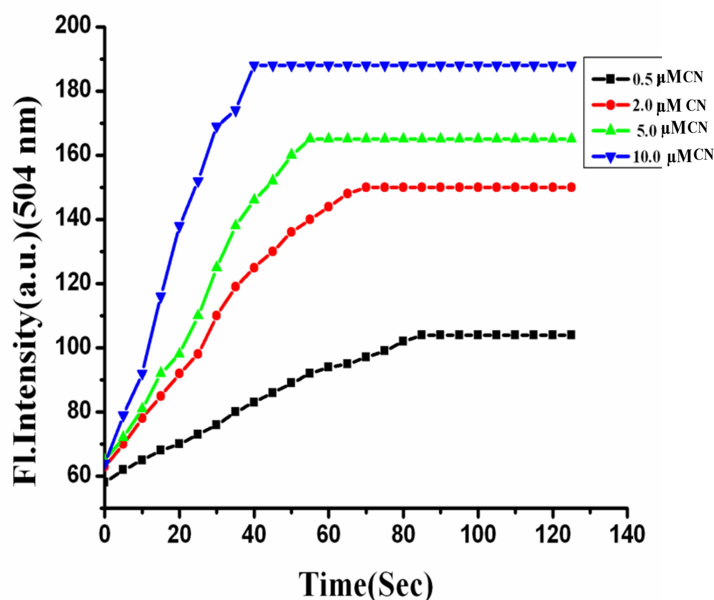


**Fig.S14:** Change in fluorescence intensities of **ITP** ($2.0 \times 10^{-6}$M) (at 504 nm) ($\lambda_{exc}$ = 411nm) with time for different concentration of $CN^-$.

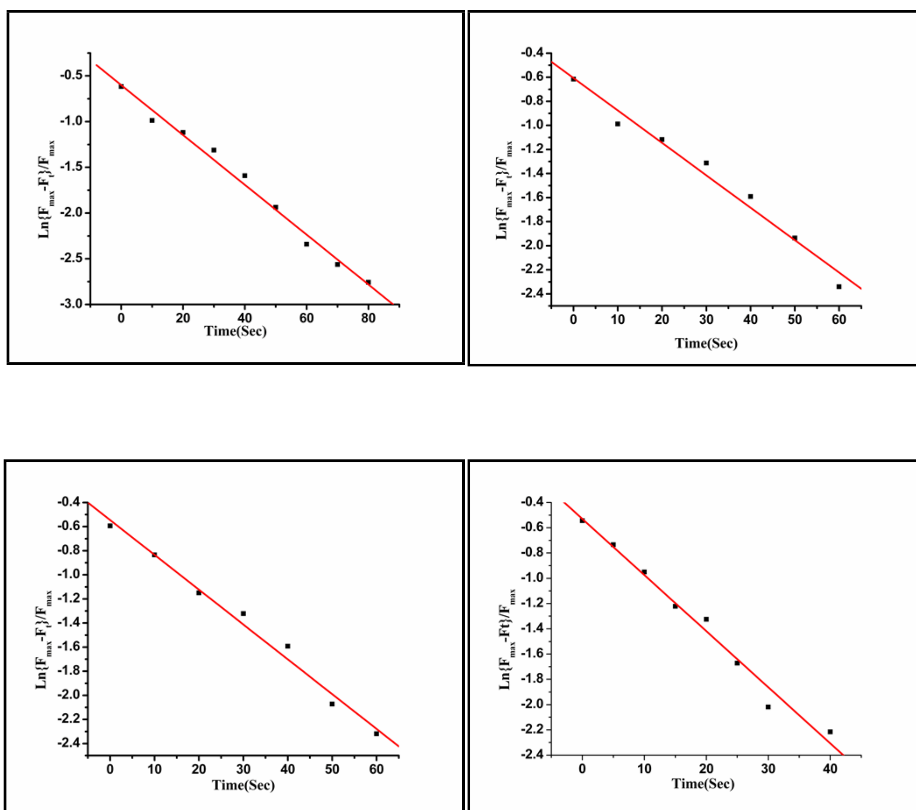


**Fig.S15:** Pseudo-first-order kinetic plot of **ITP** with $CN^-$ in DMSO: (a) **ITP**(1.0 x $10^{-6}$M) with $CN^-$(0.5μM) (b) **ITP**(1.0 x $10^{-6}$M) with $CN^-$(2.0 μM) (c) **ITP**(1.0 x $10^{-6}$M) with $CN^-$(5.0 μM) (d) **ITP**(1.0 x $10^{-6}$M) with $CN^-$(10.0 μM).

Figure S15 shows that the reaction with $CN^-$ is a typical pseudo-first-order process. Consistently the rate constant remains almost unchanged (k = 0.026 $sec^{-1}$) for different initial concentrations of **ITP**.