Electronic Supplementary Material (ESI) for Analytical Methods

Electronic supplementary Information

A simple and sensitive intramolecular charge transfer fluorescent probe to detect CN^- in aqueous media and living cells

Mohammad Shahid and Arvind Misra*

Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi – 221 005. INDIA. Fax: +91-0542-2368127; Tel: +91-542-6702503; E-mail: arvindmisra2003@yahoo.com; amisra@bhu.ac.in.

Materials, Methods and Instruments:

Anthracene, melanonitrile and all anion salts were purchased from Sigma-Aldrich Chemicals Pvt Ltd and Merck India. Triple distilled water and spectroscopic grade solvents were used in spectroscopic studies. The absorption and emission spectra were recorded at room temperature on a Shimadzu 1700 spectrophotometer using a quartz cuvette (path length = 1cm) and CARY Eclipse (VARIAN) fluorescence spectrophotometer keeping band width constant (5 nm/5 nm) for emission spectra respectively. FT–IR spectra (KBr pellets) were recorded on a Varian-3100 spectrometer. ^{1}H NMR spectra (chemical shifts in δ ppm) were recorded on a JEOL AL 300 FT-NMR (300 MHz) spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a Micromass Quattro II spectrometer. Elemental analysis was carried out on CE-440 CHN Analyser (Exeter Analytical Inc.).

The association constant for a 1:1 stoichiometry was estimated by Benesi-Hildebrand methods using equation (1)

$$\mathbf{1/(\mathit{I} - \mathit{I}_o) = 1/(\mathit{I} - \mathit{I}_f) + 1/\mathit{K}\,(\mathit{I} - \mathit{I}_f)\,[M]} \qquad \mathbf{(1)}$$

Where K is the association constant, I is fluorescence intensity/absorbance of free receptor **1**, I_o is the observed fluorescence intensity/absorbance of a complex, **1-CN**$^-$ and I_f is the fluorescence intensity/absorbance at saturation point.

The quantum yields were estimated in MeCN/H_2O by secondary method using anthracene as known standard

$$\mathbf{Q = Q_R.\, \mathit{I}/\mathit{I}_R.\, OD_R/OD.\, n^2/n^2_R} \qquad \mathbf{(2)}$$

Where Q is the quantum yield, I is integrated area corresponding to the fluorescence intensity, OD is optical density, and n is the refractive index. The subscript R refers to the reference fluorophore of known quantum yield.

Fluorescence Imaging

HeLa cells were grown in a culture flask containing DMEM (Delbeco Modified Eagle's Medium) supplemented with 10% FBS (Fetal Bovine Serum) and 5% CO_2 at 37 ^{0}C. Cells were spun at 1200 rpm for 20 min and the sediment was suspended in 1X PBS (pH 7.4) buffer and

placed on a polylysine coated slide and incubated for one hour at room temperature for adherence. The HeLa cells were incubated with probe **1** (2 μM) for 30 min and washed thrice with 1X PBS. Then the cells were incubated with NaCN (2 μM) for 30 min and overnight. To remove the remaining CN^- ions cells were washed three times with PBS buffer. The fluorescence images after 30 min incubation time and after overnight incubation were taken by fluorescence microscope.

Experimental section:

Synthesis of fluorescent Probe 1

In a two-necked flask equipped with a reflux condenser and guard tube anthracene (1.78 g, 10 mmol) was suspended in *N,N'*–dimethylformamide (DMF) (2 ml, 26 mmol). Phosphorusoxychloride (15 mmol, 2.0 ml) was added dropwise for 30 min at room temperature. After complete addition reaction mixture was refluxed at 90 ^oC for 3 h then cooled in an ice-bath for 4 h and neutralized with sodium acetate (10 g in 20 ml water) solution. The reaction mixture was poured in cold water (250 ml) and further allowed to stand at 0^oC for 4 h. The precipitate so obtained was crystallized in methanol to get yellow colored crystals of 9-anthracenaldehyde **2.** Yield 65% (1.34 g, 6.5 mmol). M.p. 103-105 ^oC; R_f = 0.69 (EtOAc : hexane :: 1:9, v/v); IR (KBr) υ_{max} (cm^{-1}) 3047, 2770, 1667, 1552, 1443, 1338, 1248, 1162, 1047, 898, 730, 506; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 11.54 (s, 1H, CHO), 9.01 (d, 2H, *J* = 9.0 Hz), 8.71 (s, 1H), 8.09 (d, 2H, *J* = 8.4 Hz), 7.72 (t, 2H, *J* = 7.2, 7.8 Hz), 7.58 (t, 2H, *J* = 7.5, 7.2 Hz).

In the next step to a solution of **2** (2 mmol, 412 mg) in anhydrous dichloromethane (DCM; 10 ml), triethylamine (TEA; 0.1 ml) and malanonitrile (2 mmol, 132 mg) were added and stirred the reaction mixture for overnight. An orange colored precipitate was obtained which was filtered and washed with DCM followed by water and dried in air. Yield 83% (421 mg, 1.6 mmol). M.p. 211-213 ^oC; R_f = 0.41 (EtOAc : hexane :: 1:9, v/v); IR (KBr) υ_{max} (cm^{-1}) 3017, 2828, 2229, 1624, 1569, 1446, 1363, 1257, 1089, 737, 528; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.96 (s, 1H, Hv'), 8.65 (s, 1H, H5), 8.11 (d, 2H, *J* = 8.4 Hz, H1, H1'), 7.94 (d, 2H, *J* = 8.7 Hz, H4, H4'), 7.68 (t, 2H, *J* = 7.8 Hz, H2, H2'), 7.60 (t, 2H, *J* = 7.5 Hz, H3, H3'); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 188.0, 187.8, 160.5, 149.2, 132.5, 130.9, 129.5, 129.1, 128.3, 126.0, 123.8, 123.3, 112.9, 97.3; Anal. Calc. For $C_{18}H_{10}N_2$: C, 85.02; H, 3.96%; N, 11.02. Found: C, 84.95; H, 3.93; N, 11.12%.; ESI-MS $(M+H)^+$ m/z = 255.2.

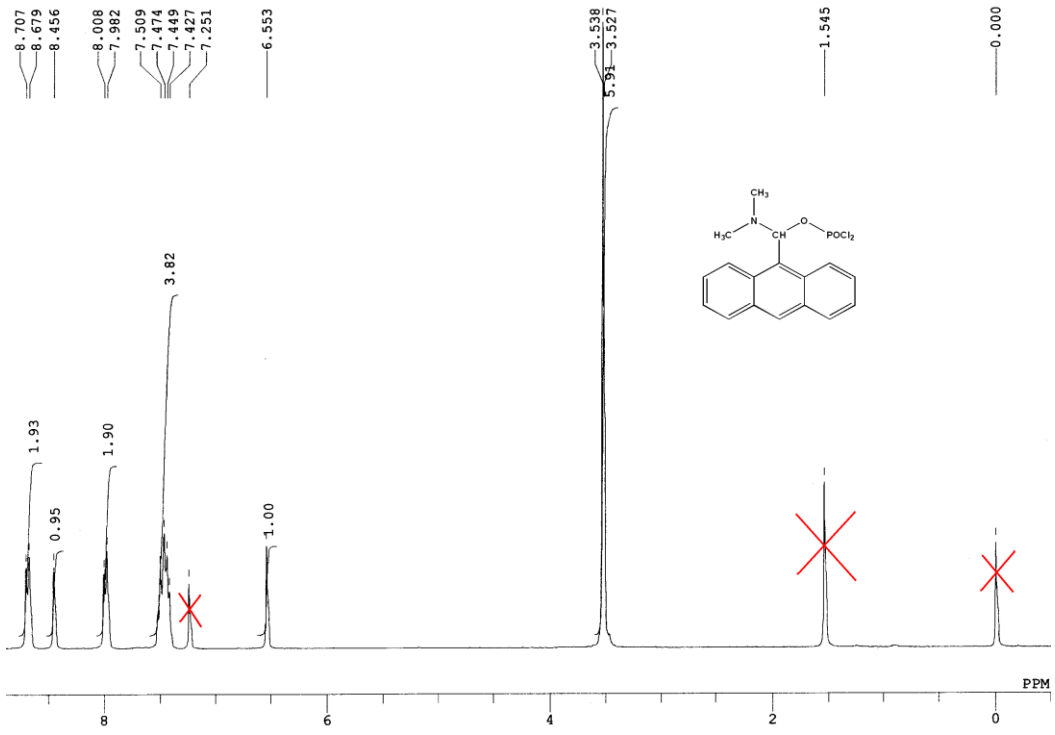

Figure S1: ^{1}H NMR spectrum of intermediate of **2** in $CDCl_3$.

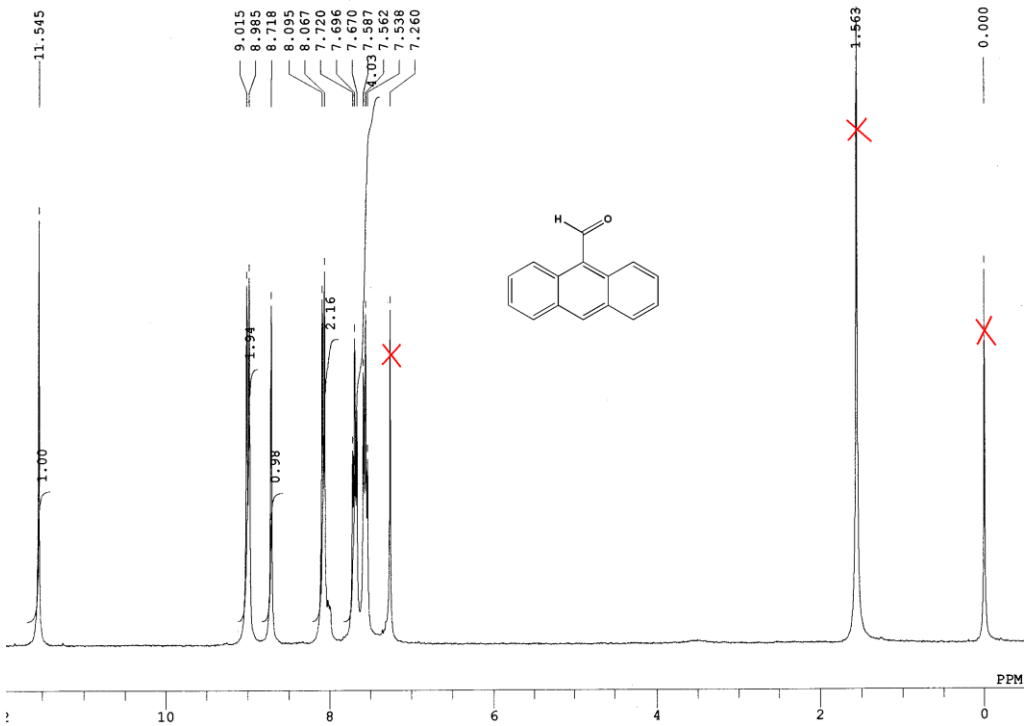

Figure S2: ^{1}H NMR spectrum of **2** in $CDCl_3$.

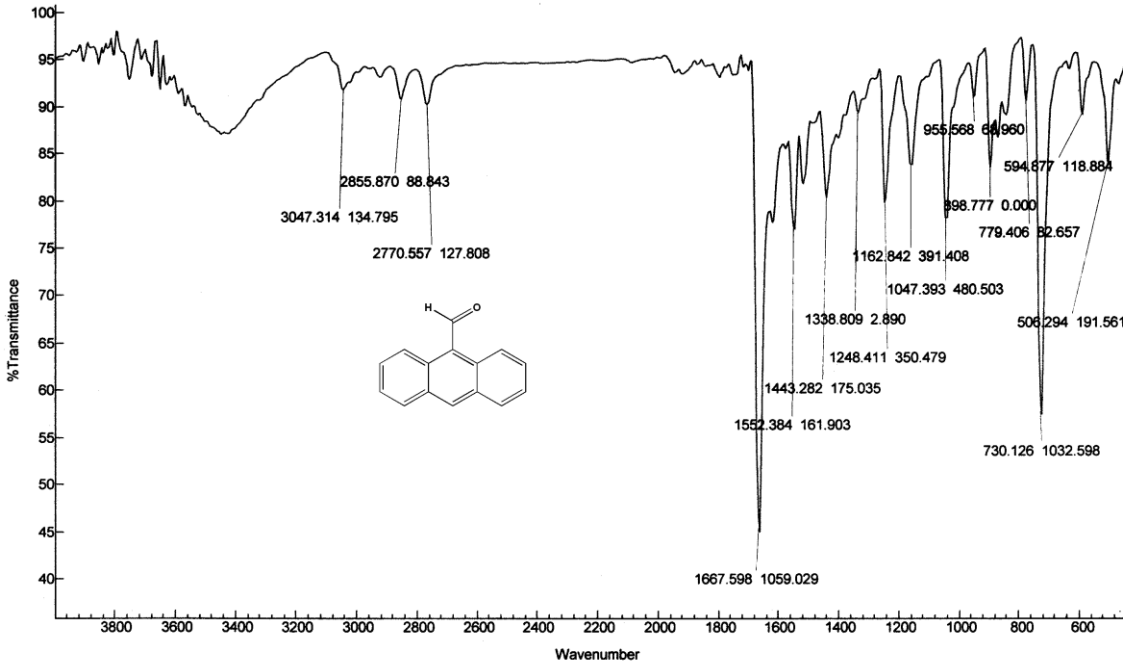

Figure S3: FT-IR spectrum of **2**.

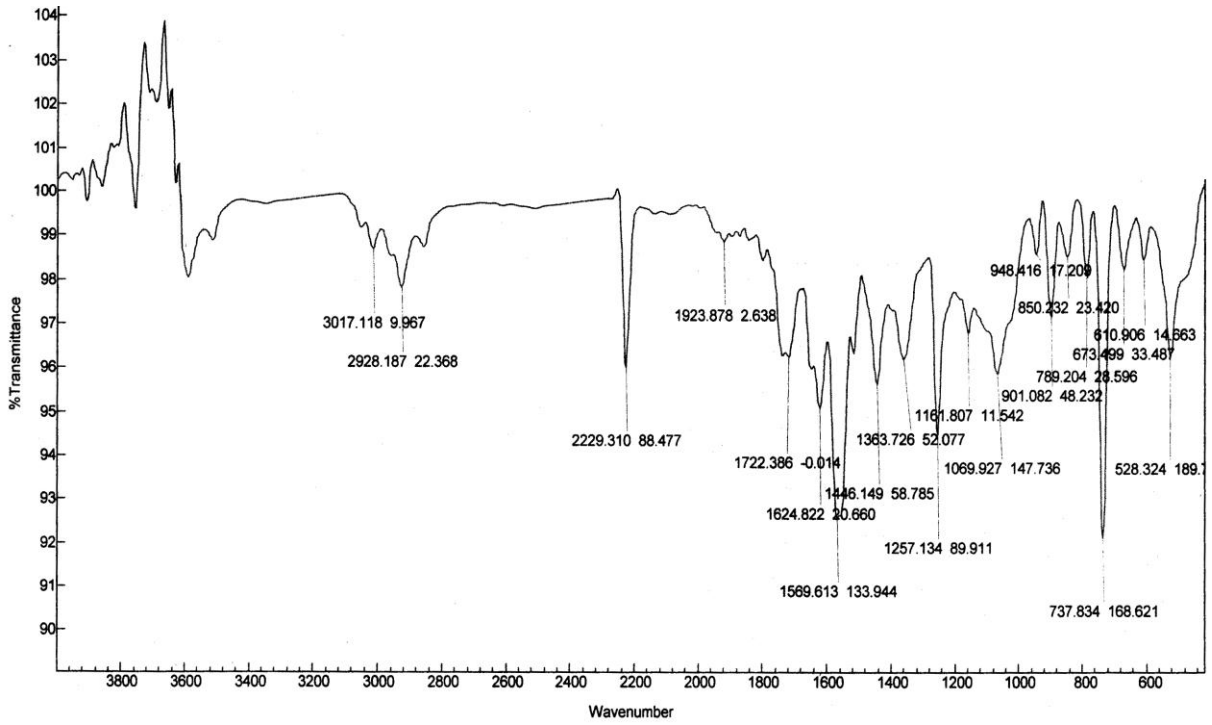

Figure S4: FT-IR spectrum of **1**.

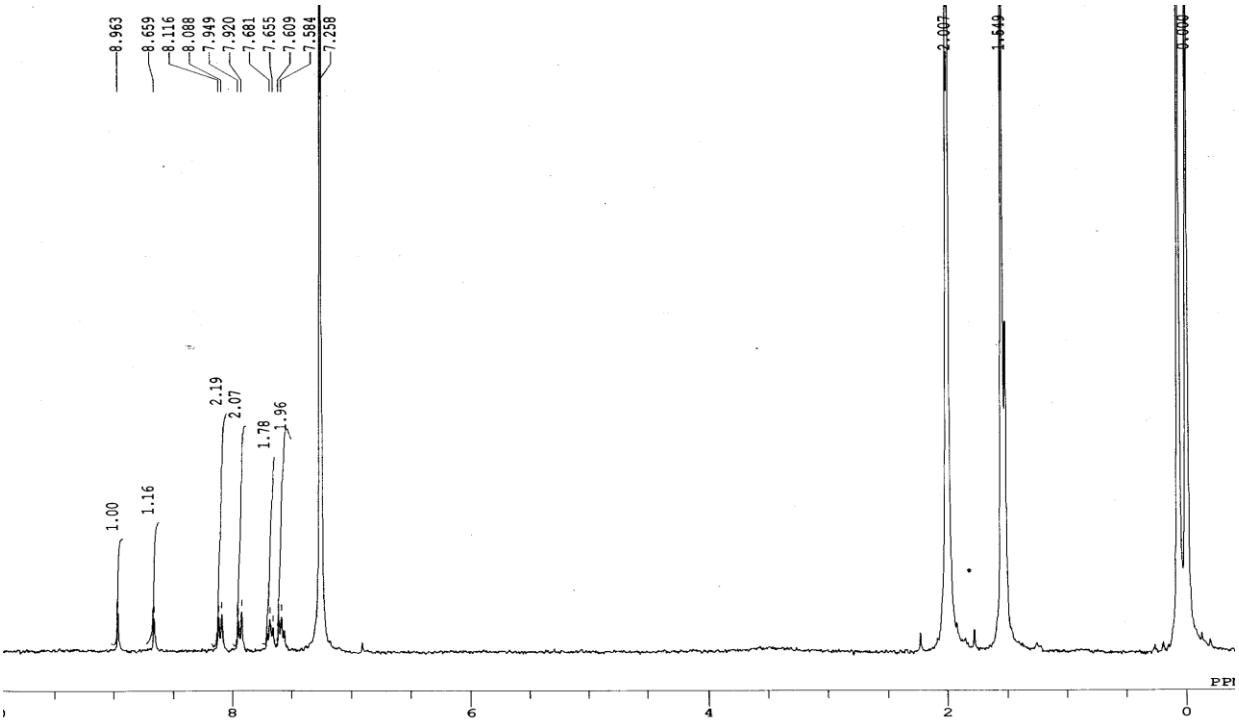

Figure 5: ^{1}H NMR spectrum of **1** in $CDCl_3$.

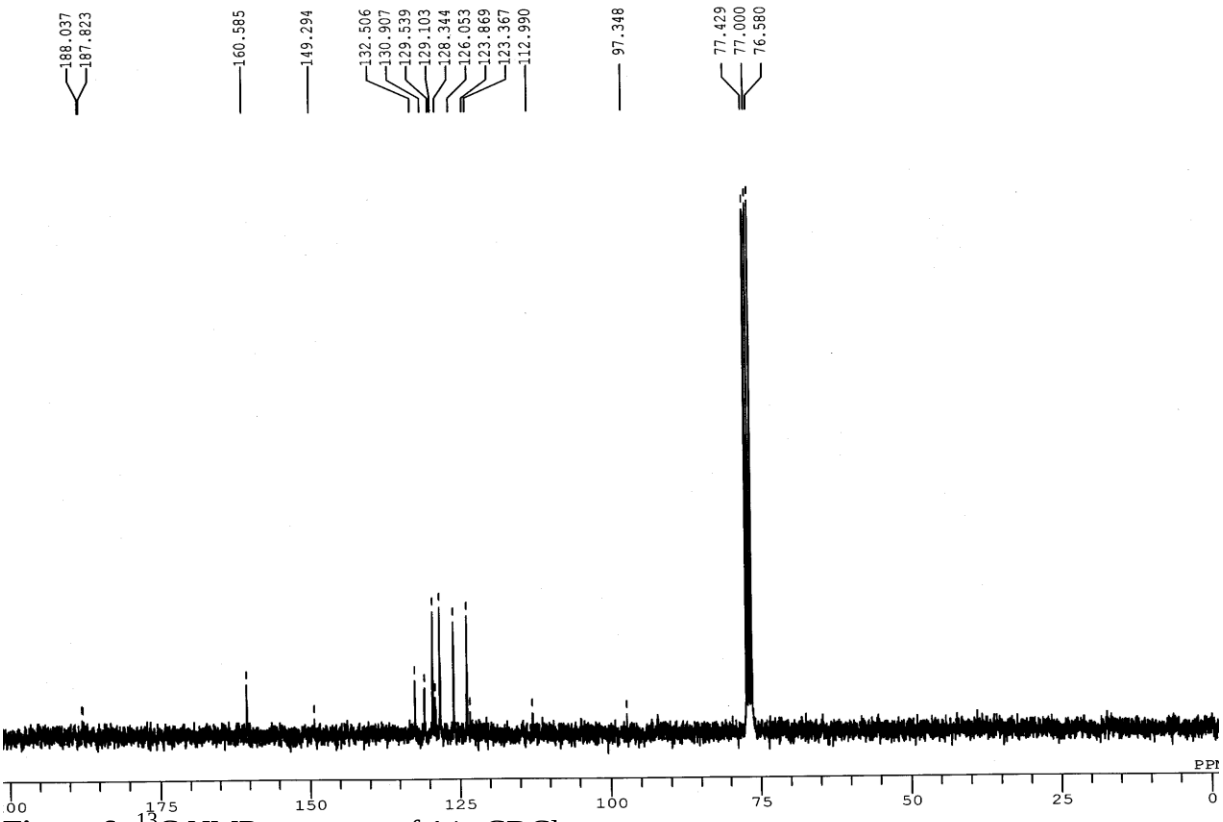

Figure 6: ^{13}C NMR spectrum of **1** in $CDCl_3$.

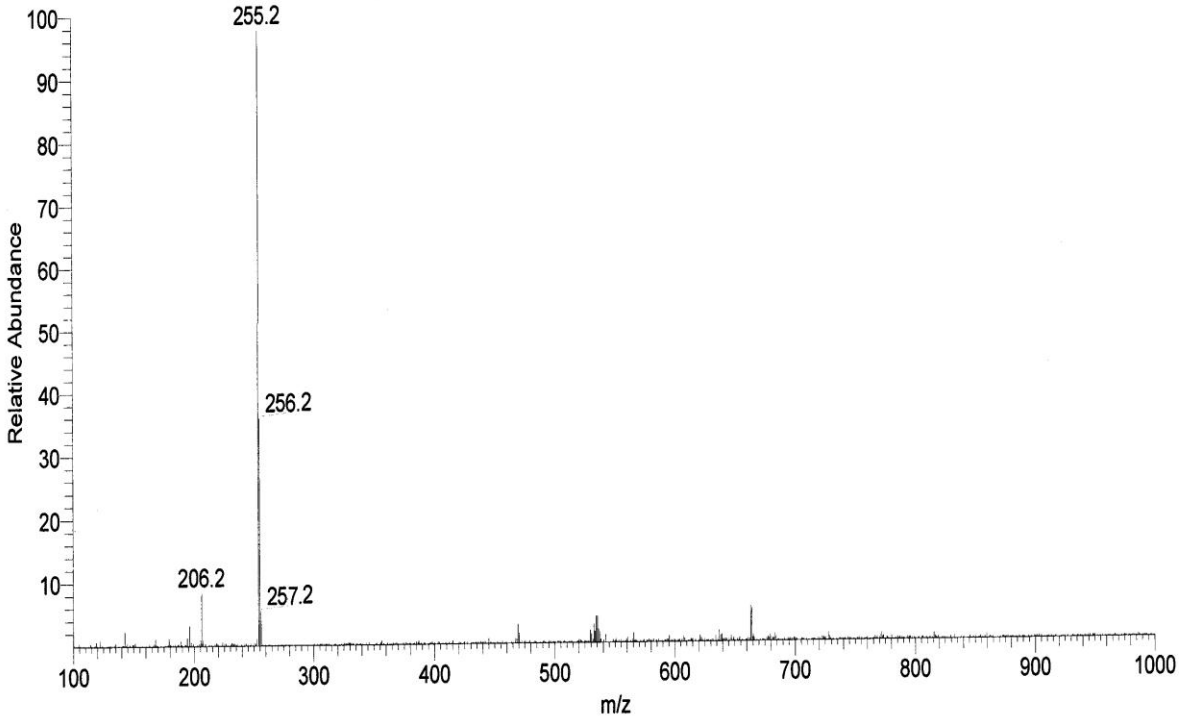

Figure S7: ESI-MS spectrum of **1** in $CHCl_3$.

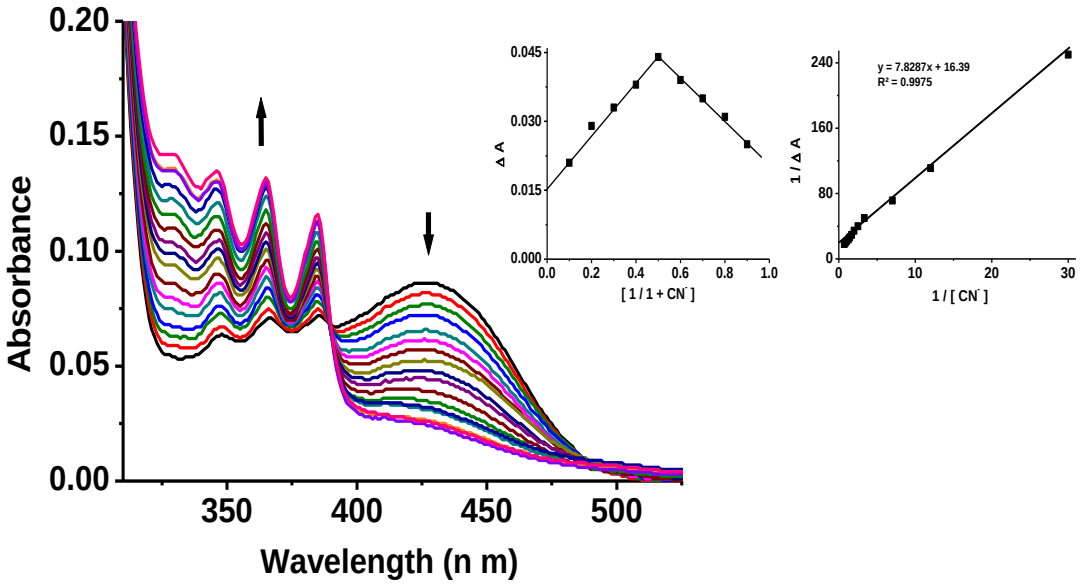

Figure S8: Absorption titration spectra of **1** (10 μM) upon addition of CN^- (0-2 equiv) in aqueous-MeCN (8:2, v/v). Inset shows Job's plot and Benesi-Hildebrand plot for **1** and CN^-.

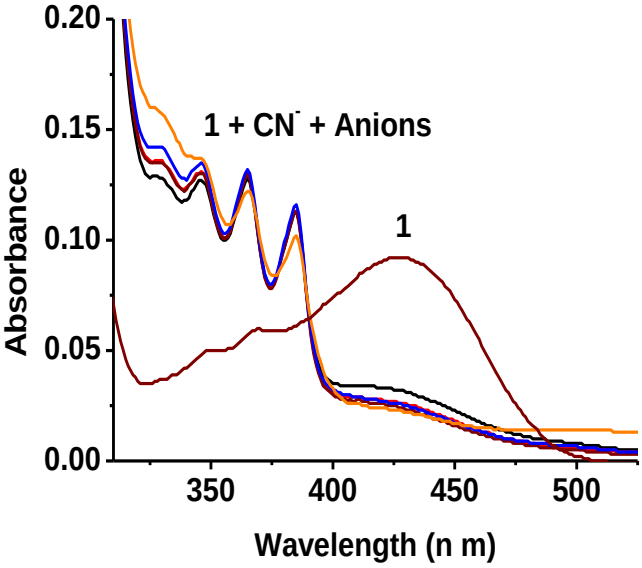

Figure S9: Interference studies of **1** (10 μM) upon addition of CN^- (3 equiv) and different anions (10 equiv) in H_2O-MeCN (8:2, v/v).

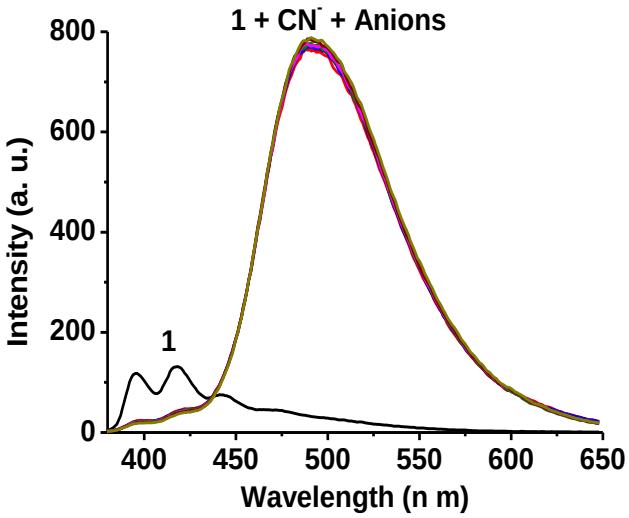

Figure S10: Competitive interaction study of **1** upon addition of tested anions (10 equiv) to a solution of **1**-CN^- .

Interaction studies of 1 with anions in a Protein medium

The sensing behavior of **1** has been examined in the presence of human blood serum protein bovine serum albumin (BSA). Bovine serum albumin (BSA) is known to generate well defined hydrophobic-hydrophilic cavities in aqueous medium[12] and microenvironment sensitive fluorophore exhibits enhanced fluorescence in hydrophobic medium while the fluorescence quenching use to occur in hydrophilic medium possibly due to hindrance in charge transfer process. Thus, to understand probe-protein interaction emission property of **1** was examined in the presnece of human blood serum protein bovine serum albumin (BSA). Upon addition of BSA (0-3 μM) to a solution of **1** (10 μM) in aqueous-MeCN (8:2, v/v) fluorescence enhancement occurred corresponding to emission band at 380-430 nm when excited at 365 nm (Fig. S11, ESI). However, the emission observed was relatively weak when the emission spectra was acquired in the absence of BSA. Further the anion interaction study was performed in the presnece of BSA. Upon addition of CN^- anion (0-20 equiv; as their sodium salt in water) to a solution of **1** (10 μM) containing BSA (3 μM) fluorescence enhancement occurred in which a new emission band was observed at 487 nm along with enhancement in relative emission intensity centred at 380-430 nm (Fig. S12, ESI). The binding affinity of **1** with CN^- in BSA medium could be understood through the fluorescence titration experiment. Upon a gradual addition of CN^- ions to a solution of **1** containing BSA (3 μM) relative fluorescence intensity of emission band centered at 487 nm enhanced ~10 times and emission spectra become saturated after addition of 20 equiv of CN^- ions. The Job's plot analysis in the presence of BSA also suggested about a 1:1 stoichiometry between **1** and CN^- ions with an association constant, $K_{assoc} = 1.85 \times 10^4$ /M, estimated by the non linear fitting of emission titration curve data (Inset, Fig. S12, ESI). Thus, the preliminary experimental observations supported about the compatibility of **1** to detect CN^- ions in a protein medium without any significant hindrance and any additional undesirable contribution of protein on the optical behavior of **1.**

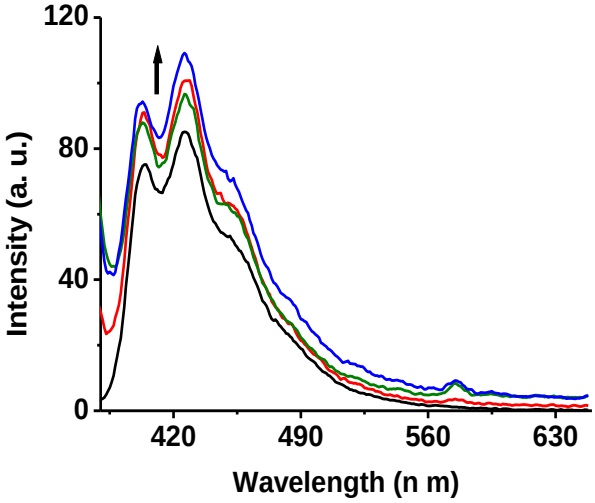

Figure S11: Change in emission spectra of **1** (10 μM) upon addition of Bovine serum albumin (BSA; 0-3 μM) in H_2O-MeCN (8:2, v/v).

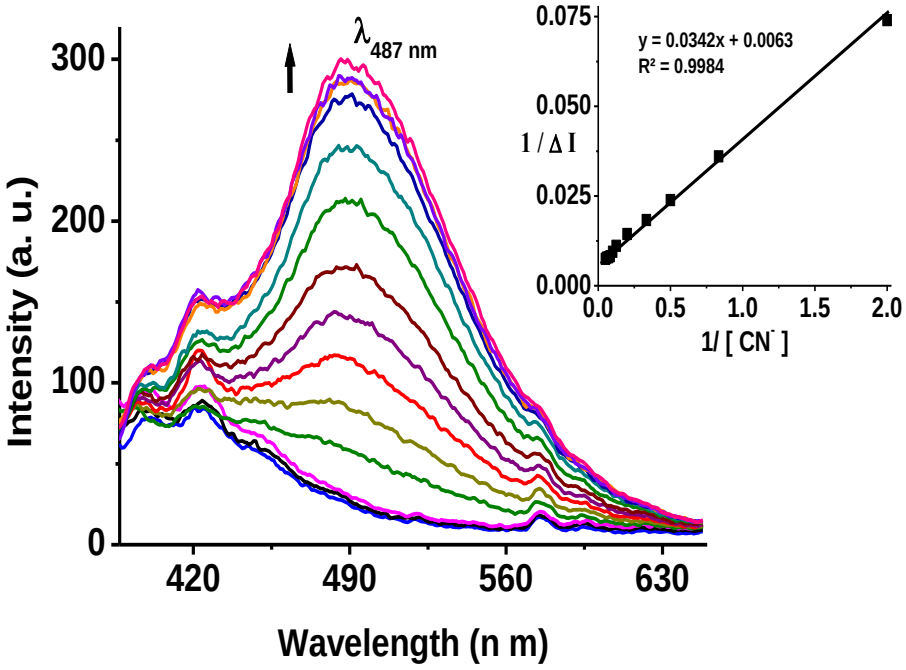

Figure S12: Emission titration spectra of **1** (10 μM) containing BSA (3 μM) upon addition of CN^- ions (0 – 20 equiv) in H_2O-MeCN (8:2; v/v). Inset: Benesi-Hildebrand Plot.

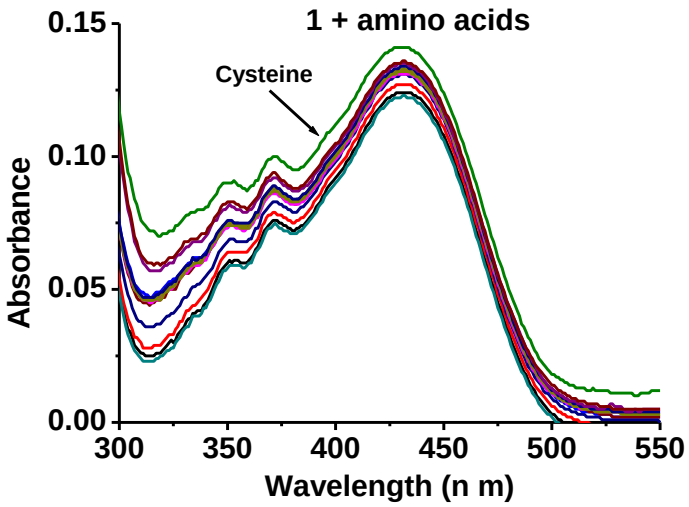

Figure S13: Interference studies of **1** (10 μM) upon addition of different amino acids (100 equiv) in H_2O-MeCN (8:2, v/v).

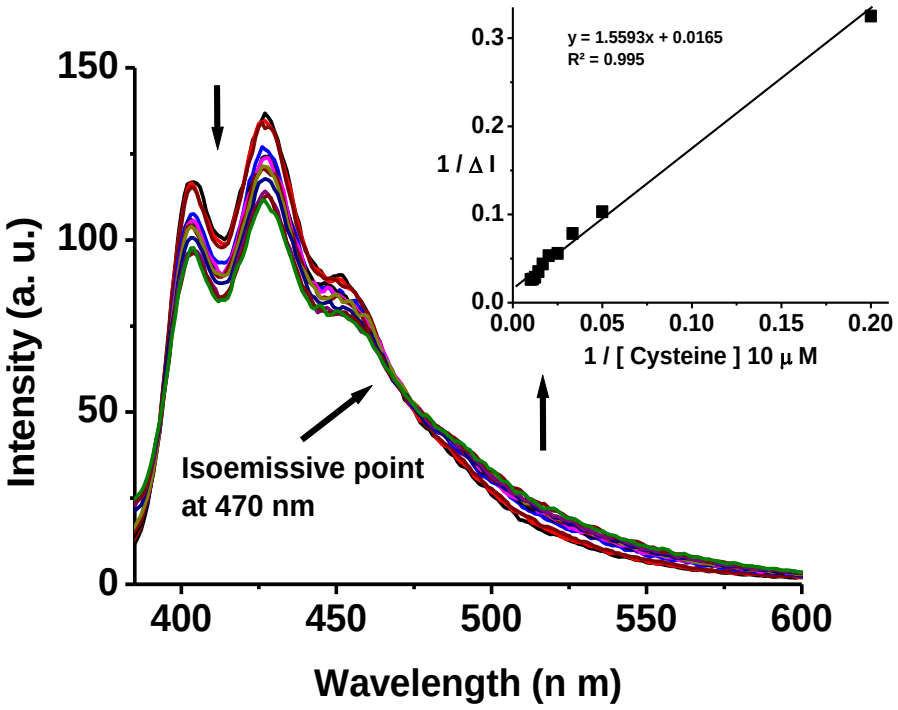

Figure S14: Emission titration spectra of **1** (10 μM) upon addition of Cysteine (0 – 100 equiv) in H_2O-MeCN (8:2; v/v). Inset shows the Benesi-Hildebrand graph for cysteine.

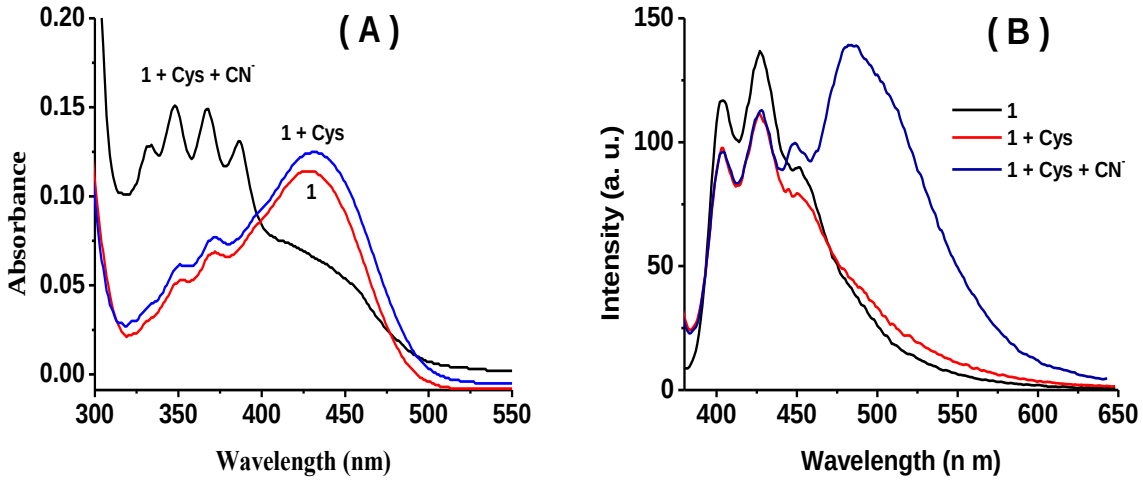

Figure S15: (**A**) Change in absorption and (**B**) emission spectra of **1** (10 μM) upon addition of Cysteine (100 equiv) and then CN^- (10 equiv) in H_2O-MeCN (8:2, v/v).