DICYANO-BENZIMIDAZOLE MOLECULES AS RATIOMETRIC AND COLORIMETRIC SENSORS FOR ANIONS

Thesis submitted in partial fulfilment of the requirements for the award of the degree of

MASTER OF SCIENCE

IN

CHEMISTRY

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July 2015

CANDIDATE'S DECLARATION

This thesis titled "Dicyano-Benzimidazole Molecules As Ratiometric And Colorimetric Sensors For Anions" is a presentation of my original research work. Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature, and acknowledgement of collaborative research and discussions. The work was done under the guidance of Dr Vijay Luxami at Thapar University, Patiala.

Aparna Garg

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Date: 14.07.2015

In my capacity as supervisor of the candidate's thesis, I certify that the above statements are true to the best of my knowledge.

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CERTIFICATE

This is to certify that the thesis "**Dicyano-Benzimidazole Molecules As Ratiometric And Colorimetric Sensors For Anions**" being submitted by Aparna Garg in partial fulfillment of requirements for the award of degree of Master's in Science in Chemistry in School of Chemistry and Biochemistry, Thapar University, Patiala, is a bonafide work carried out under the supervision of Dr. Vijay Luxami and that no part of the thesis has been submitted for the award of any degree.

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INTRODUCTION

In the vast field of supramolecular chemistry, the design and utilization of chemosensors for ion and molecule recognition have developed at an extraordinary rate. Design of the chemosensors consists of three components; a chemical receptor capable of recognizing the guest usually with high selectivity; a transducer or signaling unit that converts the binding event into a measureable physical change and finally a method of gauging this change and translating it to useful information. There are three different approaches which have been employed by various groups in pursuing the synthetic receptors, each differing in the way the first two units are arranged with respect to each other.[1]

1) Binding site-signaling approach

2) Displacement approach

3) Chemodosimeter approach.

In the "binding site-signaling subunit" approach, the two units are linked through a covalent bond.[2] The interaction of the analyte with the binding site brings about changes in the electronic properties of the signaling subunit resulting in sensing of the target anion. The displacement approach is based on the formation of molecular assemblies of binding site signaling subunit, which on coordination of a particular anion with the binding site results in the release of signaling subunit into the solution with a simultaneous change in their optical properties.[3] In the chemodosimeter approach a specific anion-induced chemical reaction occurs which results in an optical signal.[4] Out of these three approaches the first one has been widely exploited. Cation vs. anion sensing, though booming in a big way, the research in anion-selective receptors design is still less developed than that reported for its cation counterpart. This lack of development can be related to the following differences that exist between anions and cations.[5]

- Ionic size – The anionic sizes are generally larger than cationic sizes and consequently call for larger binding sites. For e.g. 0.167 nm ionic radius of Cl^- is much larger than 0.133 nm of that of K^+.
- Shapes- Anions come in diverse geometrical shapes e.g. spherical (Cl^-), linear (CN^-), tetrahedral (SO_4^{2-}) and trigonal planar (NO_3^-).
- The pH range for existence of anions is narrower in contrast to cations as in the case of phosphate.
- Anions are more heavily hydrated than the cations of similar sizes.

Anions play an essential role in a broad range of chemical and biological processes, and numerous efforts have been dedicated to the development of abiotic receptors for anionic species. In an advanced supramolecular concept, recognition sites can be coupled to certain groups that are capable of "reporting" the anion coordination process.[6] In this case, the binding process is converted into a signaling event. Many chemosensors display changes in either colour or fluorescence in the presence of a certain guest although the changes in electrochemical properties such as the oxidation potential of redox active groups have also been extensively known. In this sensing process, information at the molecular level, such as the presence or absence of a certain guest in solution, is amplified to a macroscopic level; hence, sensing might lead to the qualitative or quantitative determination of certain guests. A general approximation to the development of anion chemosensors is the coupling of at least two units, each displaying a specific function: the binding site and the signaling subunit.[7] The former has the function of coordinating to a certain anion, whereas the latter alters some spectroscopic characteristics (color or fluorescence) upon anion coordination. Binding sites and signaling units could be covalently linked (binding site-signaling subunit approach) or not (displacement approach).[8] In addition to these systems, anion signaling using fluorescence or color changes can also be noticed using irreversible reactions.[9] The emission signal can incorporate various signaling mechanisms like PET, ICT, ESIPT, FRET etc. for sensing.[10]

Excited state intramolecular proton transfer (ESIPT) has gained much attention due to the applications in molecular probes, luminescent materials, and molecular logic gates etc. The most notable photophysical property of the ESIPT chromophores is the large Stokes shift, compared to the normal fluorophores such as fluorescein, rhodamine or boron-dipyrromethene (BODIPY).[11] The large Stokes shift is a looked-for feature for fluorophores because the self-absorption, or the

inner filter effect, can be avoided and the fluorescence analysis can be improved upon multifolds, though it is difficult to increase the Stokes shift of the conventional fluorophores by chemical modification. Another distinctive feature of the ESIPT chromophores is the transient nature of the ground state of the emissive species of the ESIPT chromophores, i.e. the keto tautomer.

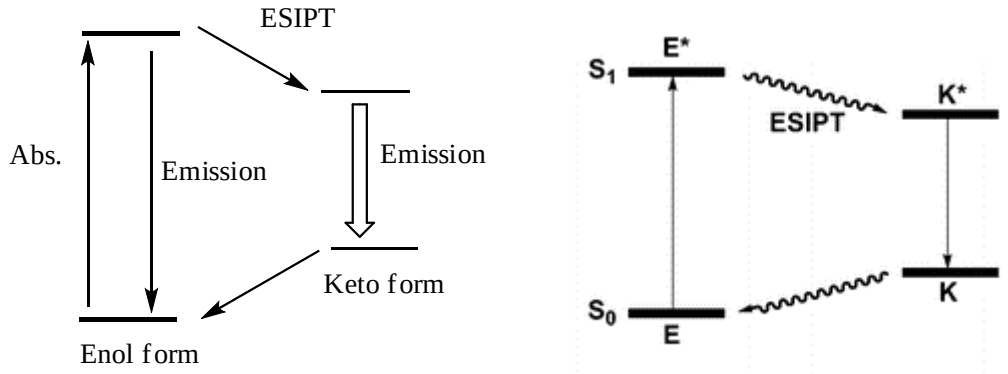


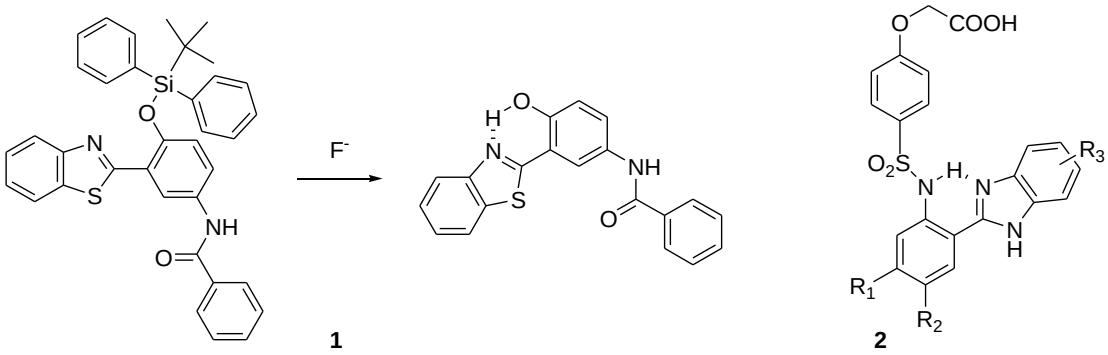
Figure a. Illustrates the mechanism of ESIPT.

In general, the ESIPT process necessitates a preformed intramolecular hydrogen bond (H-bond) between the proton donor (-OH, -NH_2) and proton acceptor (-C=O, - N=) groups which are in close proximity to each other in a molecule.[12] In the electronic ground state, typical ESIPT molecules exist exclusively in their enol (E) form, it being better stabilized by intramolecular H-bond. Upon photo excitation, however, the redistribution of electronic charge takes place, resulting in an increase in the acidity of the proton donor and the basicity of the proton acceptor. As an effect, fast proton transfer reaction from the proton donor to the proton acceptor occurs along the excited-state potential energy surface through the intramolecular H-bond, leading to a tautomeric conversion from the excited enol form (E^*) to the excited keto form (K^*) in a sub-picosecond time scale. After decaying radioactively to the ground state, reverse proton transfer occurs to its E form (Fig. a). Different absorbing (E) and emitting (K) molecular species in this intrinsic four-level photocycle often leads to total exclusion of self-absorption and the large Stokes' shifted emission. In addition, this process brings about the transient chemical change from E to K tautomer, resulting in the brief alternation of the electronic properties such as electron density distribution, energies of electronic state, and dipole moments. Due to the complexed photophysical process and transient changes involved in its four-level photocycle, ESIPT is readily affected by its environmental conditions, leading to fine spectral responses

LITERATURE REVIEW

The organic compounds containing functional groups or heterocyclic rings containing binding sites which can serve as selective and effective ion receptor systems has led to work towards developing synthetic receptors that can selectively recognize ions and act as sensors. Among such heterocyclic units, the imidazole ring behaves as an excellent hydrogen bond donor moiety in a synthetic anion receptor system, and the acidic properties of the NH proton of the imidazole can be tuned by changing the electronic properties of the imidazole substituents. On the other hand, the presence of a donor pyridine-like nitrogen atom within the ring which is capable of selectively binding cationic species also converts the imidazole derivatives into excellent metal ion sensors. In this sense, the binding properties of the imidazole core may be modulated by linear or angular annulation to aza-heterocycles leading to expanded imidazole derivatives bearing several binding sites. While the positively charged imidazolium derivatives have recently been widely used and comprehensively reviewed as selective anion receptors through the C2–H···anion typical ionic hydrogen bond, the dual capability of the imidazole ring to act as a selective molecular sensor of anions and/or cations even as ion-pair receptors has not been reviewed.

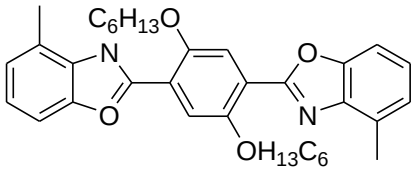
Yang and colleagues reported a ratiometric sensor **1** prepared by reaction of the proton donor group of ESIPT molecules with analyte-cleavable protecting groups, whose hydroxyl group was protected by tert-butyldiphhenylsilyl (TBDPS) group thus inhibiting the ESIPT process.[12,13] BTTPB emitted only blue-violet fluorescence in aqueous micellar solution of cetyltrimethylammonium bromide (CTAB) . Upon fluoride addition, the Si-O bond of BTTPB immediately cleaved and 3-BTHPB was generated, which exhibits a yellow K* emission. As a result, both the absorption and emission spectra of the solution showed ratiometric responses. The aqueous fluoride anion was detectable down to 100 ppb level without any significant interference from other anions such as $H_2PO_4^-$, AcO^-, or CN^-.



1 **2**

Christoph J. Fahrni et.al prepared and characterized a series of water-soluble 2-(2'-arylsulfonamidophenyl) benzimidazole derivatives **2** containing electron donating and accepting groups attached to various positions of the fluorophore system in aqueous solution at 0.1 M ionic strength.[14] The measured p*K*a's for deprotonation of the sulfonamide group of monosubstituted derivatives ranged between 6.75 and 9.33 and followed closely Hammett's free energy relationship. In neutral aqueous buffer, all compounds undergo efficient excited-state intramolecular proton transfer (ESIPT) to yield a strongly Stokes-shifted fluorescence emission from the phototautomer. Upon deprotonation of the sulfonamide nitrogen at high pH, ESIPT was interrupted to yield a new, blue-shifted emission band. The study provides valuable information regarding substituent effects on the photophysical properties of this class of ESIPT fluorophores in an aqueous environment and may offer guidelines for designing emission ratiometric pH or metal-cation sensors for biological applications.

Yi Pang et al. prepared a bis(benzoxazole) derivative with a metal-chelating ligand (DPA), **3** which exhibited a large fluorescence turn-on effect upon zinc-binding.[15] The metal chelation enabled ESIPT, giving rise to an additional emission band in the near-IR region with a large Stokes shift (230 nm). Upon binding with Zn^{2+} cation, the weakly fluorescent (the enol emission at 543 nm) can be enhanced by as much as 10-fold. In addition, the metal-binding enabled the ESIPT giving emission in NIR region which was in sharp contrast to mono-HBO derivatives of 1 whose metal binding disabled the ESIPT by removing the phenolic proton.



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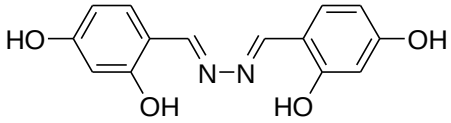
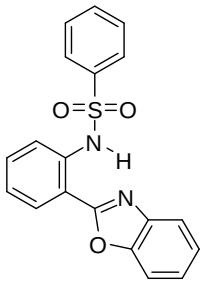


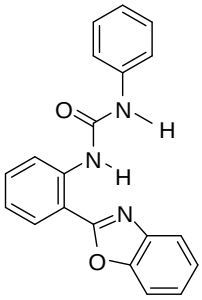
4

Jing Luo and co-workers synthesized a novel colorimetric and fluorescent double-sensor **4** based on Salicylaldehyde bis-Schiff.[16] The sensor exhibited high selectivity and sensitivity towards Cu^{2+} in aqueous solution with a visible colour change from colourless to yellow and with Al^{3+} a significant fluorescent enhancement in ethanol over a range of tested metal ions. Chemosensor **5** based on Salicylaldehyde bis-Schiff that had good selectivity at microlevel for Cu^{2+} and at a nanomolar level for Al^{3+} via turn-on fluorescence.

Xiaojun Peng et al. developed two anion sensors from condensation of 2-(2'-aminophenyl)benzoxazole with *p*-toluenesulfonyl chloride and phenyl isocyanate, (TABO and PUBO) **5a** and **5b,** which underwent excited-state intramolecular proton transfer (ESIPT) upon excitation.[17] For the acid receptor TABO, the ESIPT process was readily interrupted by basic anions such as F^-, CH_3COO^-, and $H_2PO_4^-$ by deprotonating the sulfonamide unit, whereas in the case of PUBO, being a good hydrogen-bonding donor, the ESIPT process was inhibited either by the fluoride induced deprotonation of the urea unit or by the formation of a strong CH_3COO^--urea intermolecular hydrogen bond complex, and these two types of inhibition mechanisms subsequently resulted in different ratiometric responses. But anions with less hydrogen-bonding acceptor abilities could not inhibit the ESIPT. By varying the acidity and the hydrogen bonding donor ability of the protons that are crucial to the ESIPT process, a combination of a good sensitivity and distinguish ability could be obtained.



5a



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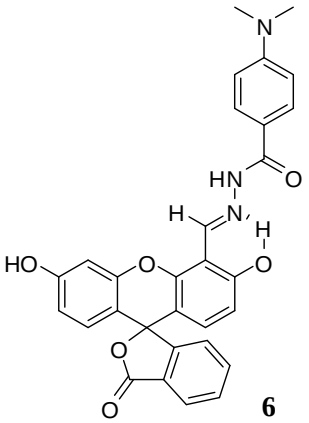
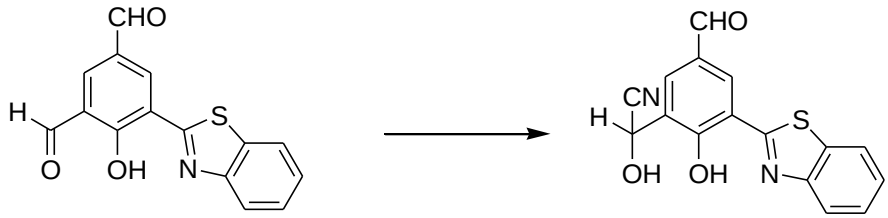


6

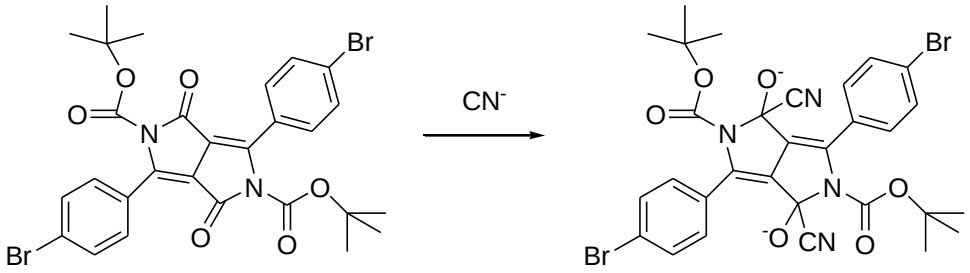
Wei Guo and co-workers, on the basis of FRET from 4-(*N*,*N*-dimethylamino)benzamide to fluorescein, developed a ratiometric fluorescence probe bearing a hydrazone binding unit **6** for highly selective and sensitive detection of CN^- in an aqueous solution.[18] The probe showed a clear CN^--induced change in the intensity ratio of the two well-separated emission bands of 4-(*N*,*N*dimethylamino)benzamide group and fluorescein. The significant changes in the color and fluorescence could be observed by the naked eye.

Intramolecular proton transfer (ESIPT) based sensor for ratiometric detection of CN^- in aqueous solution was developed by Goswami et al.[19,20] The sensor **7** contained a 2-(2-hydroxyphenyl)benzothiazole moiety and displayed green emission at 521 nm. CN^- addition to the moiety induced a sharp decrease in the intensity of emission at 521 nm and a simultaneous increase in the intensity of a band centered at 436 nm, with an iso-emissive point at 488 nm with a subsequent colour change from green to blue. The colorimetric and fluorescent changes were attributed to the formation of 7-I through nucleophilic addition of CN^- to the o-aldehyde group in **7**, which leads to inhibition of the ESIPT process.



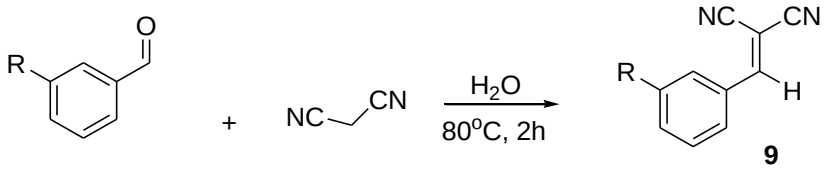
7

Another fluorescence probe for cyanide detection based on a carbonyl addition reaction was developed by Jang et al.[21] The diketopyrrolopyrrole **8** which displayed absorption at 442 nm and fluorescence emission at 512 nm, was observed to display a high sensitivity and selectivity for CN^-. Specifically, addition of cyanide to a CH_2Cl_2 solution of 10 promoted disappearance of the emission centered at 512 nm and a colour change from green to red. These optical changes were attributed to the formation of 10-I through nucleophilic addition of cyanide to both of the electron-deficient carbonyl carbons in the moeity. Among various anions (e.g., F^-, Cl^-, Br^-, I^-, ClO_4^- NO_3^-, $H_2PO_4^-$, HSO_4^-, AcO^-, SCN^- and HS^-), only cyanide induced strong fluorescence and colour changes of 8. Using this probe, cyanide could be detected at a micromolar level.



8

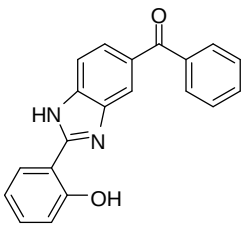
Tai-Bao Wei and colleagues designed and synthesized a chemosensor **9** by simple green chemistry procedure.[22] 9 displayed both colorimetric and fluorescence turn-off responses for cyanide (CN^-) ion in aqueous solution. The probe showed an instant visible color change from yellow to colorless and green fluorescence disappearance when CN^- was added. Investigation of the recognition mechanism indicated that the sensor recognized CN^- by a nucleophilic addition reaction. The coexistence of other anions did not interfere with the CN^- recognition process. The detection limit of the sensor **9** toward CN^- was 2.7×10^{-7} M, which was satisfactory for monitoring CN^- levels in physiological and environmental systems.



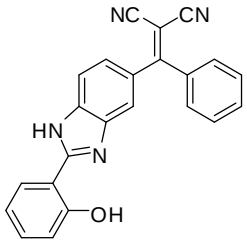
9

OBJECTIVE

ESIPT based benzimidazole molecule **1** which was selective for fluoride ions due to inhibition of ESIPT phenomenon has recently been reported[24] It was envisaged that replacement of carbonyl group with a more electron deficient di-cyano group can facilitates the attack of nucleophile[12] due to the introduction of the push-pull effect on the reported molecule. Thus cyanide ions which are known to be better nucleophiles than other anions can chemodosimeterically attack the probe **2**, whereas other anions will induce the ESIPT inhibition phenomenon. Further, introduction of $-N(C_2H_5)_2$ group as electron donating group to probe **4**, will further enhance the ESIPT phenomenon. This will lead towards highly selective, ratiometric and colorimetric change towards cyanide ions.



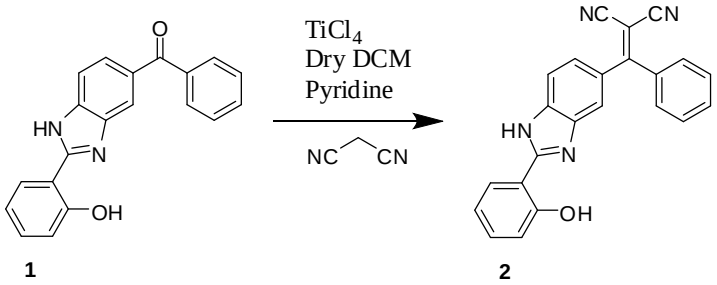
1



2

RESULTS AND DISCUSSIONS

Synthesis of Probe 2 – To a solution of **1**[23], (0.50 g, 1.29 mmol) and malononitrile (0.42 g, 6.35 mmol) in 30 ml dry CH_2Cl_2 were added 0.75 ml pyridine and 0.50 ml of $TiCl_4$ under N_2 atmosphere. The reaction mixture was refluxed overnight at 80°C. After completion of reaction, reaction mixture was allowed to cool to room temperature. The mixture was then diluted with dichloromethane and extracted with 2 N HCl solution. The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to get the crude product. The organic layer was evaporated, and the compound was purified by column chromatography using (65:35) (Hexane: Ethyl Acetate) as an eluent to give probe **2** (325 mg, 65% yield) (**Scheme 1**).

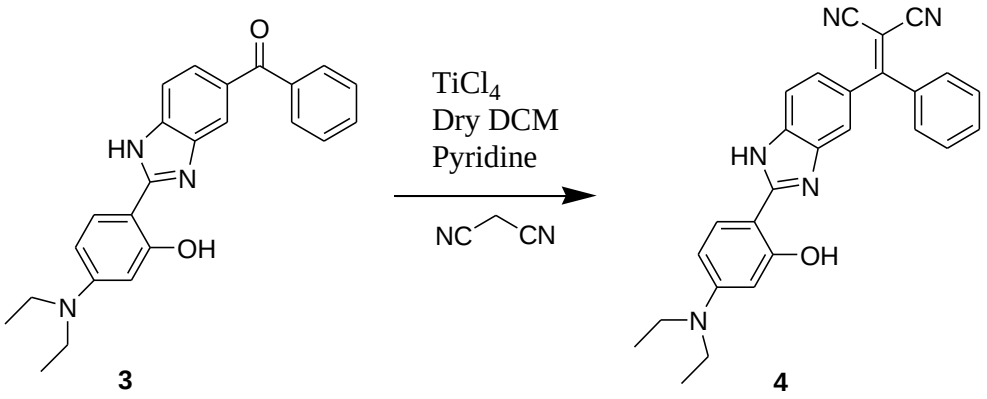


Scheme 1: Synthesis of probe 2

^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz) showed 1H doublet at δ 8.36 for aromatic-H, 1H singlet at δ 8.08 for aromatic-H, IH doublet at δ 7.99 for aromatic-H, 1H triplet at δ 7.67 for aromatic-H, 6H multiplet at δ 7.45-7.58 for aromatic-H, IH doublet at δ 7.32 for aromatic-H, 1H triplet at δ 7.09 for aromatic-H; ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 100 MHz): δ 173.87 (C=C), 158.03, 150.02, 141.49, 135.83, 134.90, 132.66, 132.29, 130.27, 128.93, 128.73, 127.28, 120.01, 117.44, 116.91, 114.65, 113.84, 113.72, 108 (ArH), 81.41(C-$(CN)_2$). ^{13}C NMR showed dicyano peak at 81.41 and a C=C peak at 173.87 and mass spectra showed m/z peak at 363.12, confirming the structure for probe **2.**

Synthesis of Probe 4 – To a solution of **3**[23], (1.29 mmol) and malononitrile (6.35 mmol) in 30 ml dry CH_2Cl_2 were added 0.75 ml pyridine and 0.50 ml of $TiCl_4$ under N_2 atmosphere. The

reaction mixture was refluxed overnight at 80°C. After completion, reaction mixture was allowed to cool to room temperature. The mixture was then diluted with dichloromethane and extracted with 2 N HCl solution. The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to get the crude product. The organic layer was evaporated, and the compound was purified by column chromatography using (65:35) (Hexane: Ethyl Acetate) as an eluent to give probe **4** (65% yield) (**Scheme 2**).



^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz) showed a broad singlet at δ 12.60 for OH group , 1H doublet at δ 7.74 for aromatic-H, 1H triplet at δ 7.62 for aromatic-H, 5H multiplet in the range of 7.52-7.47 for aromatic-H, 2H double doublet in the range of δ 6.34-6.31 for aromatic-H, 1H singlet at δ 6.26 for aromatic-H, 4H multiplet in the range of δ 3.44-3.38 for methylene protons attached to nitrogen, 6H triplet at δ 1.22 for methyl protons. ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 100 MHz): δ 175.11 (C=C), 160.26, 150.61, 136.40, 131.83, 130.13, 128.18, 127.04, 114.57, 114.22, 103.34, 99.63 (ArH), 97.656 (C-$(CN)_2$), 43.89 (N-$(CH_2)_2$), 12.21 (C-$(CH_3)_2$). ^{13}C NMR showed dicyano peak at 97.656, (N-$(CH_2)_2$) peak at 43.98 and a (C-$(CH_3)_2$) peak at 12.21 and mass spectra showed m/z peak at 434.19, confirming the structure for probe **4.**

Photophysical properties of Probe 2

Probe **1** showed an absorption band at 340 nm and emission band at 520 nm after exciting at 340 nm. In case of probe **2** we found a red shift in absorption at 370 nm due to the push-pull effect of dicyano group attached in place of carbonyl group. The emission band was at 505 nm after exciting at 320 nm i.e. stoke's shift of 185 nm was observed while it was 180 nm in case of

probe **1** (Figure 1)**.** Thus by introducing dicyano group, we have improved its absorption and emission properties.

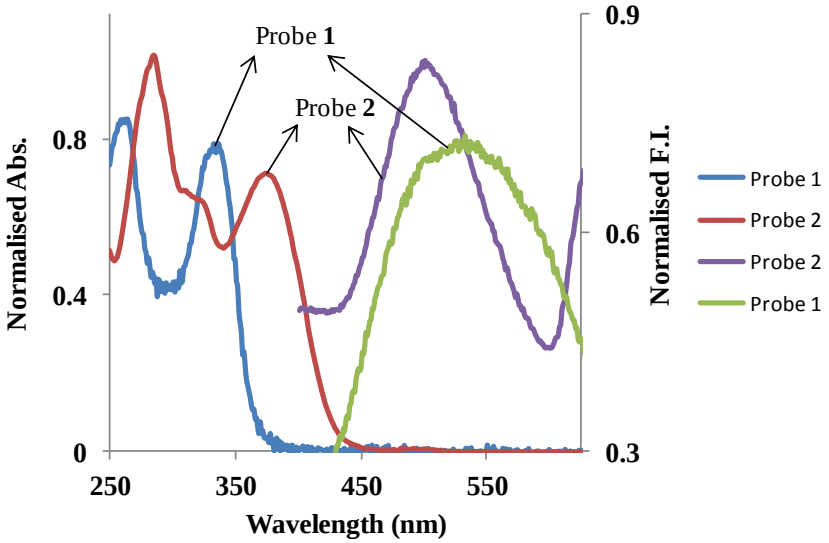


Figure 1. Comparison of absorption and emission spectra of probe **1** and **2**

Effect of various anions on absorption spectrum of Probe 2

The chromogenic sensing ability of **2** studied in THF in the presence of 1000 μM of different anions (CN^-, F^-, Cl^-, Br^-, AcO^-, I^-, HSO_4^-,$P_2O_7^{4-}$, OH^-, NO_3^-, SCN^-, $H_2PO_4^-$) only showed significant color changes in the presence of CN^-, F^-, AcO^-,$H_2PO_4^-$ and $P_2O_7^{4-}$ions (Figure 2 and 3).

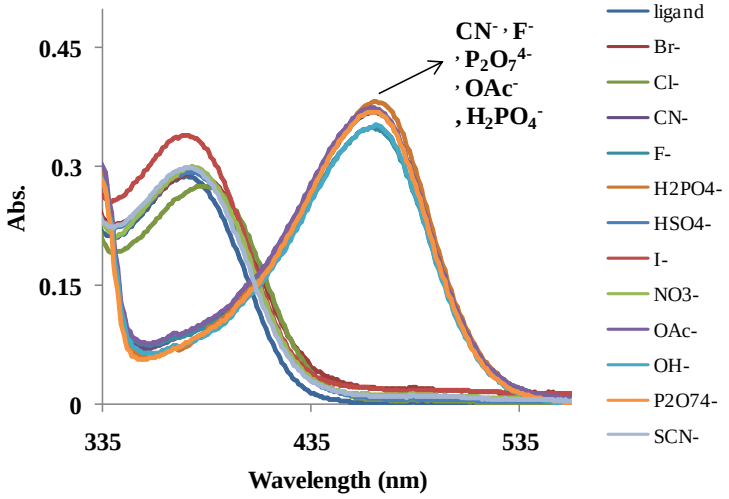


Figure 2: Effect of various anions on absorption spectra of probe 2 (20 μM, THF).

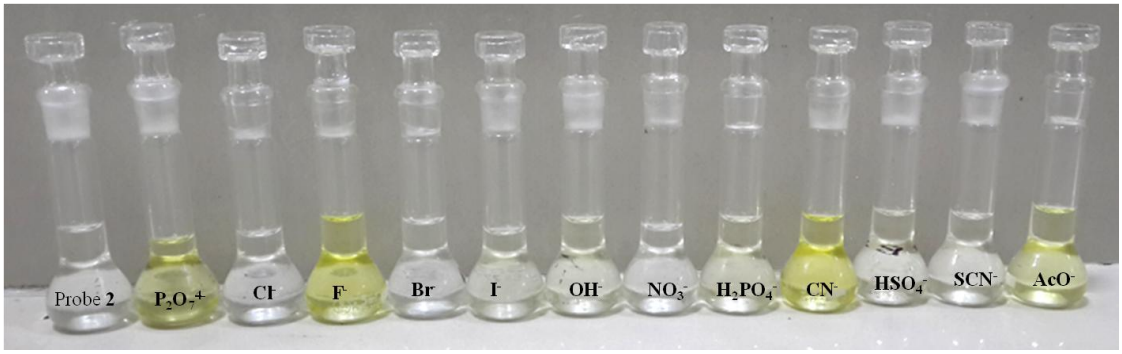


Figure 3: Photographs of probe **2** in the presence of different anions at 20 μM in THF

The probe **2** showed the absorption band at 370 nm and upon addition of CN^-, F^-, AcO^-, $P_2O_7^{4-}$, $H_2PO_4^-$ ions a blue shift was observed in the absorption spectra by 95 nm. Upon gradual addition of CN^- to the solution of Probe **2** (20 μM in THF), absorption bands at 370 nm and 285 nm diminished steadily with simultaneous formation of new bands at 465 nm, 315 nm and 260 nm with a clear isobestic point at 405 nm till additions upto 400 μM of CN^- ions, with detection limit of 4 μM (Figure 4a). The cyanide addition resulted in decrease in absorption intensity at 370 nm and increase at 465 nm provided an opportunity to determine cyanide ions ratiometrically (Figure 4b). The absorption ratio varied from 0.200 to 6.21 at 370 and 465 nm indicating 31.05 fold absorption ratio changes. Thus, probe **2** can be used to estimate an extensive range of cyanide ions between 4-400 μM through ratiometric approach.

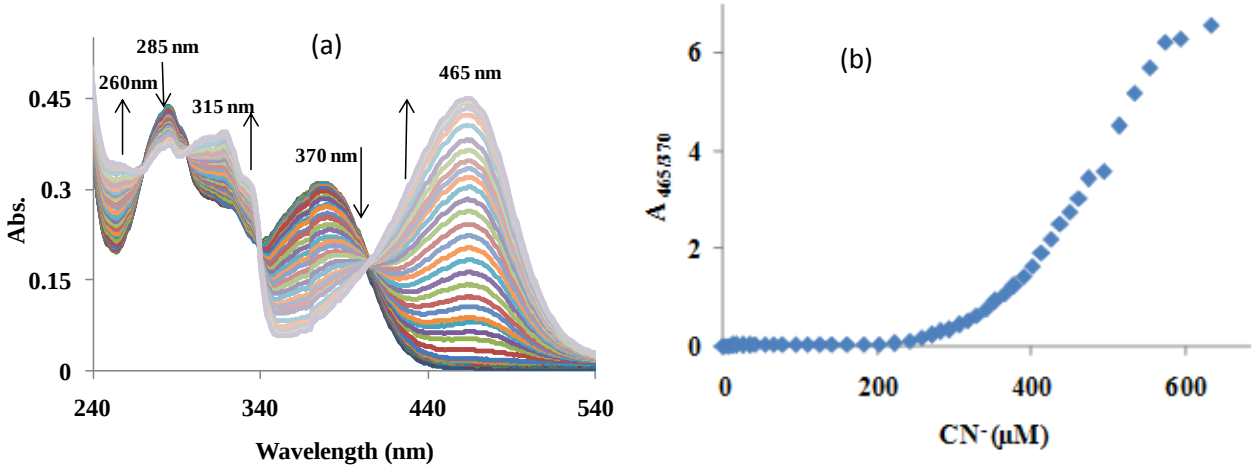


Figure 4: (a) Effect of incremental addition of cyanide on absorption spectrum of probe **2** (20 mM, THF); (b) Ratiometric response between 465 and 370 nm (A_{465} / A_{370}) vs [CN^-] ions on incremental addition of cyanide ions to probe **2** (20 mM, THF).

Similar results were observed in case of F^-, $H_2PO_4^-$, AcO^- and $P_2O_7^{4-}$ (Figure 5a-b, 6a-b) with varied stability constants which were calculated by using Benesi-Hilderband equation summarized in Table 1.

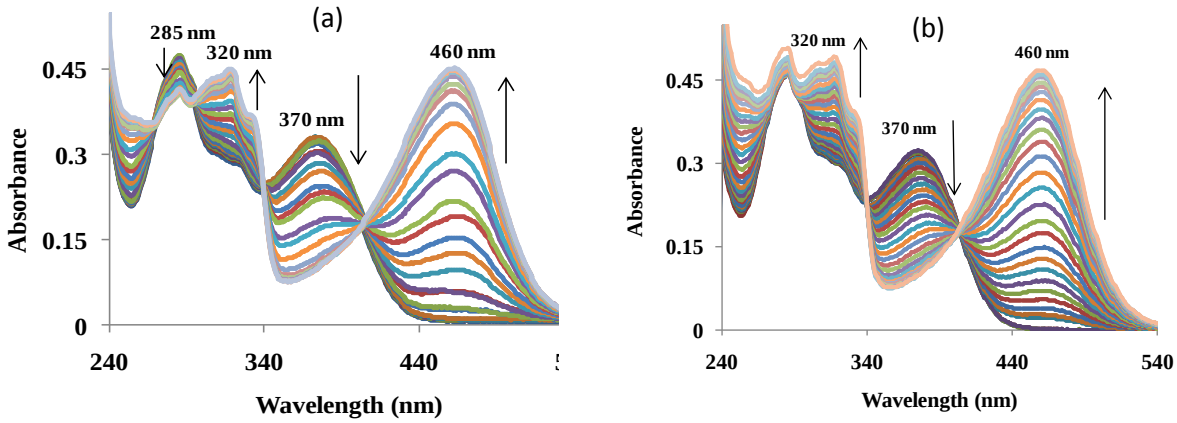


Figure 5: Effect of addition of (a) fluoride ions (b) pyrrophospahte ions on absorption spectrum of probe **2** (20 µM, THF)

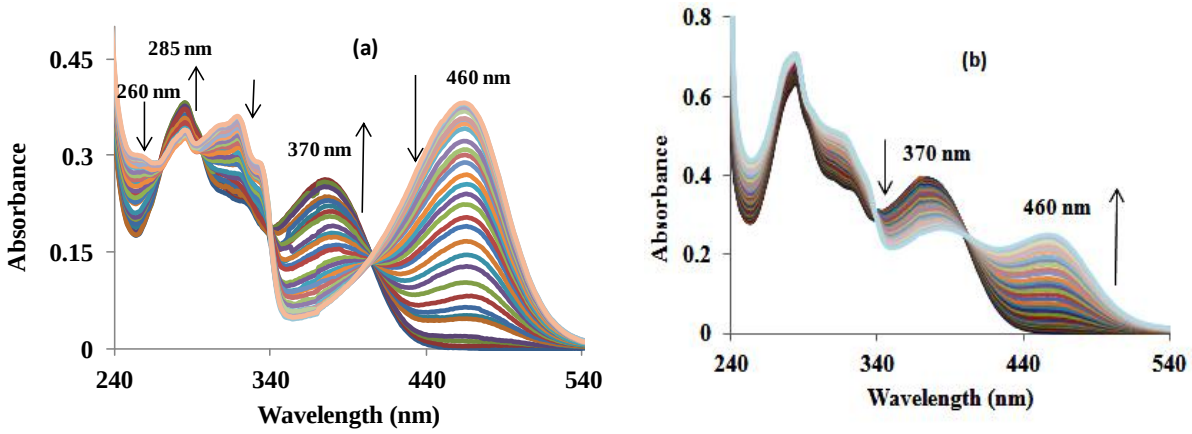


Figure 6: Effect of addition of (a) acetate ions (b) dihydrogen phosphate ions on absorption spectrum of probe **2** (20 µM, THF).

Effect of various anions on emission spectrum of Probe 2

The probe **2** (20 µM, THF) on excitation at 320 nm exhibited keto emission band at 505 nm. On addition of various anions, no significant change was observed except for CN^-, F^-, AcO^-, $H_2PO_4^-$

and $P_2O_7^{4-}$ ions. The changes observed in emission in case of CN^- and F^- were different from AcO^-, $H_2PO_4^-$ and $P_2O_7^{4-}$ ions (Figure 7).

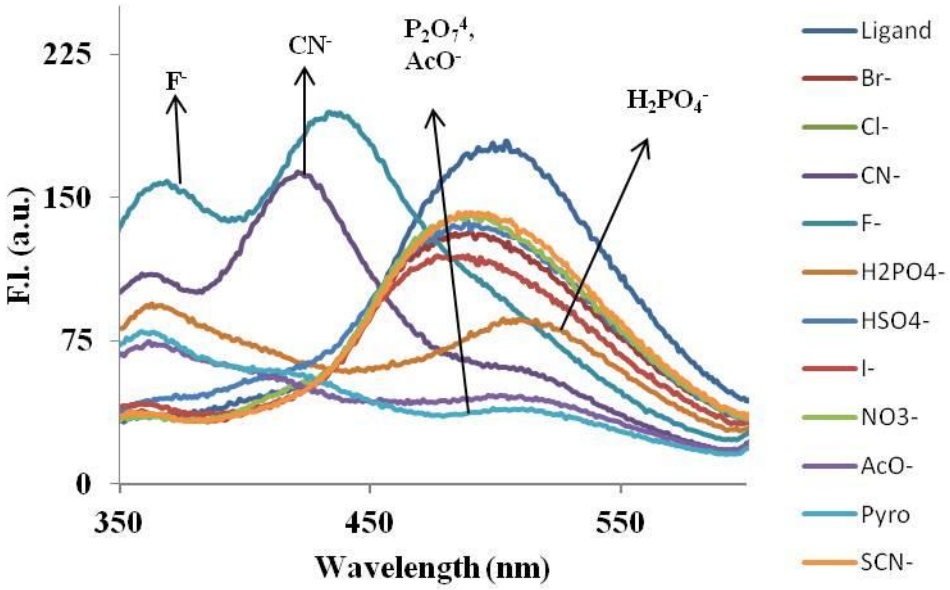


Figure 7: Effect of addition of various anions on emission spectrum of probe **2** (20 µM, THF).

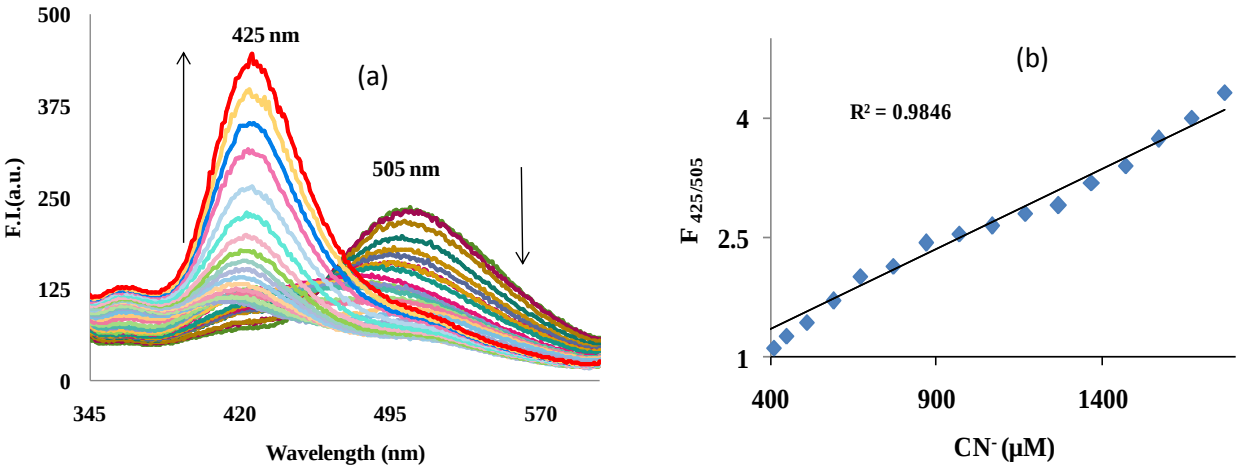


Figure 8: (a) Effect of addition of CN^- ions on emission spectrum (b) emission intensity ratio between 425 and 505 nm (F_{425} / F_{505}) vs [CN^-] ions of probe **2** at 20 µM in THF

The fluorescence quenching was observed in case of CN^- and F^- a clear formation of a new band at 425 and two new bands at 435 and 365 nm respectively. To check the binding behavior of probe **2** towards CN^- ions, solution of probe **2** was titrated against CN^- ions. We established that upon addition of 600 µM of CN^- ions, fluorescence intensity was completely quenched and on further addition, there was formation of a new band at 425 nm (Figure 8a). The binding constant was calculated to be 3.41 X 10^4 M^{-1} with detection limit of 4 µM. The emission ratio varied from 1.11 to 4.32 at 505 and 425 nm indicating 3.89 fold emission ratio changes (Figure 8b). Consequently, probe **2** can be used to estimate a wide range of cyanide ions between 4-400 µM.

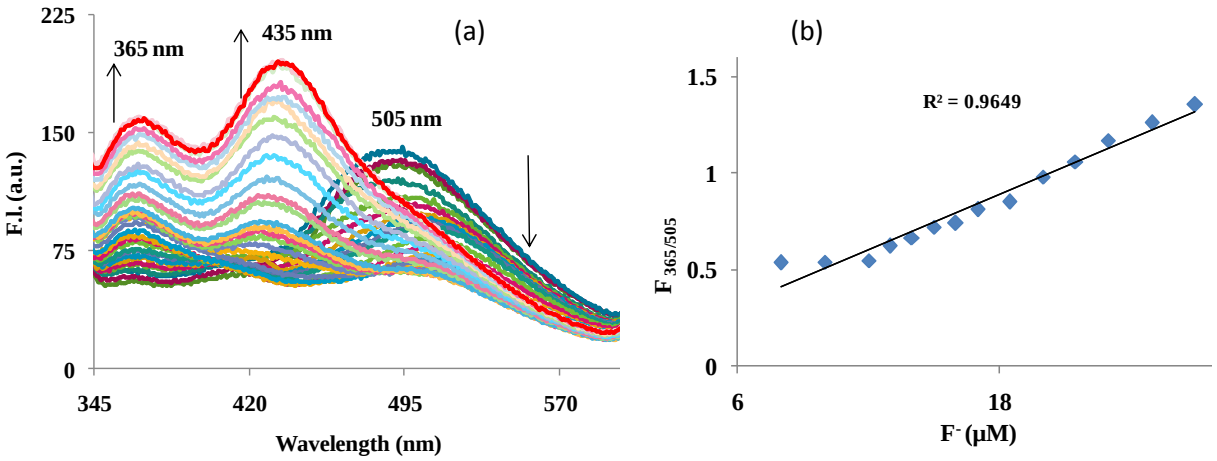


Figure 9: (a) Effect of addition of F^- ions on emission spectrum (b) emission intensity ratio between (b) 365 and 505 nm (A_{435} / A_{505}) vs [F^-] ions.

In case of addition of F^- ions, quenching of the ligand band and subsequent formation of two new emission bands i.e. at 435 and 365 nm simultaneously were observed (Figure 9a). The binding constant was calculated to be 1.78 X 10^4 M^{-1} with detection limit of 0.25 µM. The emission ratio varied from 1.36 to 2.06 at 505 and 435 nm and 0.53 to 1.35 at 505 and 365 nm indicating 1.514 and 2.55 fold emission ratio changes simultaneously (Figure 9b). Hence, probe **2** can be utilized to determine a wide range of flouride ions between the range of 0.25-200 µM.

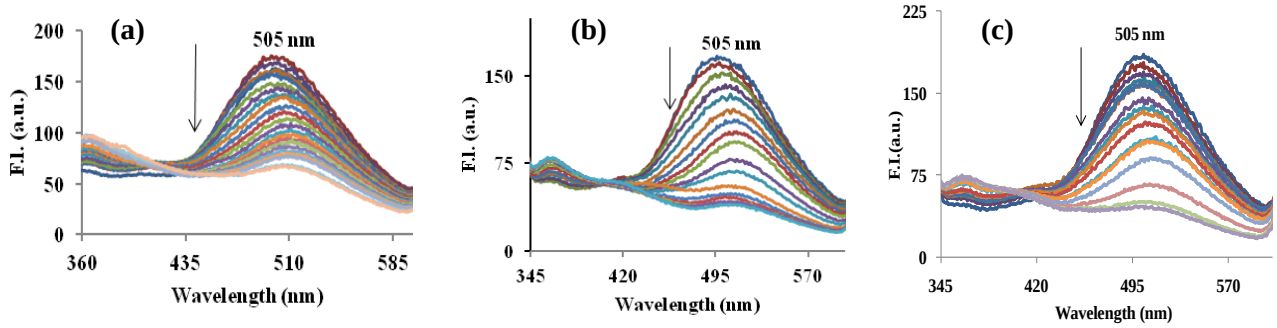


Figure 10: Effect of incremental addition of (a) $P_2O_7^{4-}$ ions (b) $H_2PO_4^-$ (c) AcO^- ions on emission spectrum of probe **2** (20 µM, THF).

In case of AcO^-, $H_2PO_4^-$ and $P_2O_7^{4-}$ ions, there was considerable amount of quenching (Figure 10a-c). The varied stability constants are summarized in Table 1.

Table1. Stability constants of various anions through UV-Vis and Fluorescence

S.No.	Anion	Stability Constant (Per Mole) UV-Visible	Stability Constant (Per Mole) Fluorescence
1.	CN^-	0.58×10^3	3.42×10^4
2.	F^-	4.04×10^4	1.78×10^4
3.	AcO^-	5.51×10^4	1.33×10^4
4.	$H_2PO_4^-$	1.18×10^3	6.46×10^3
5.	$P_20_7^{4-}$	1.40×10^4	1.9×10^4

Effect of anions on NMR spectrum of Probe 2

In order to observe the effect of anion binding towards probe **2**, NMR titrations were performed. In case of CN- ions addition, a new singlet peak at 5.00 ppm was appeared. As cyanide ions act as chemodosimeter and therefore attack at the electron deficient carbon adjacent to the di-cyano group, thus peak at 5.00 ppm is due to hydrogen of C(a) (Figure 11) Thus in scheme 3 we have proposed the mechanism of binding cyanide ions with probe **2**.

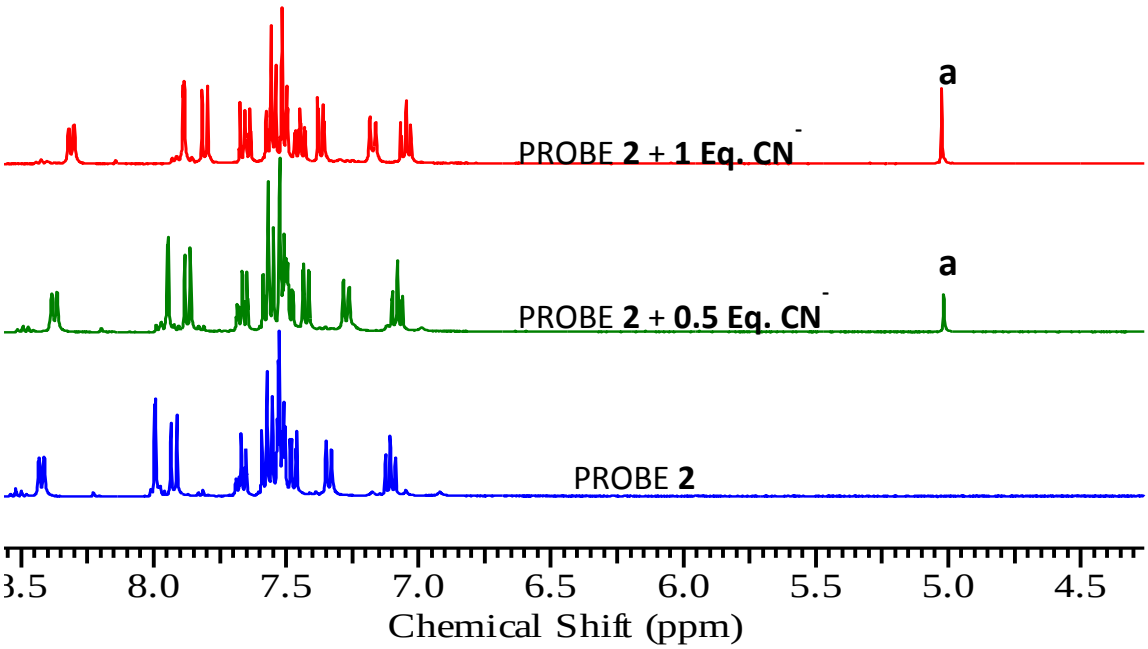


Figure 11: NMR titrations of Probe **2** with TBACN in CD_3CN at 5mM.

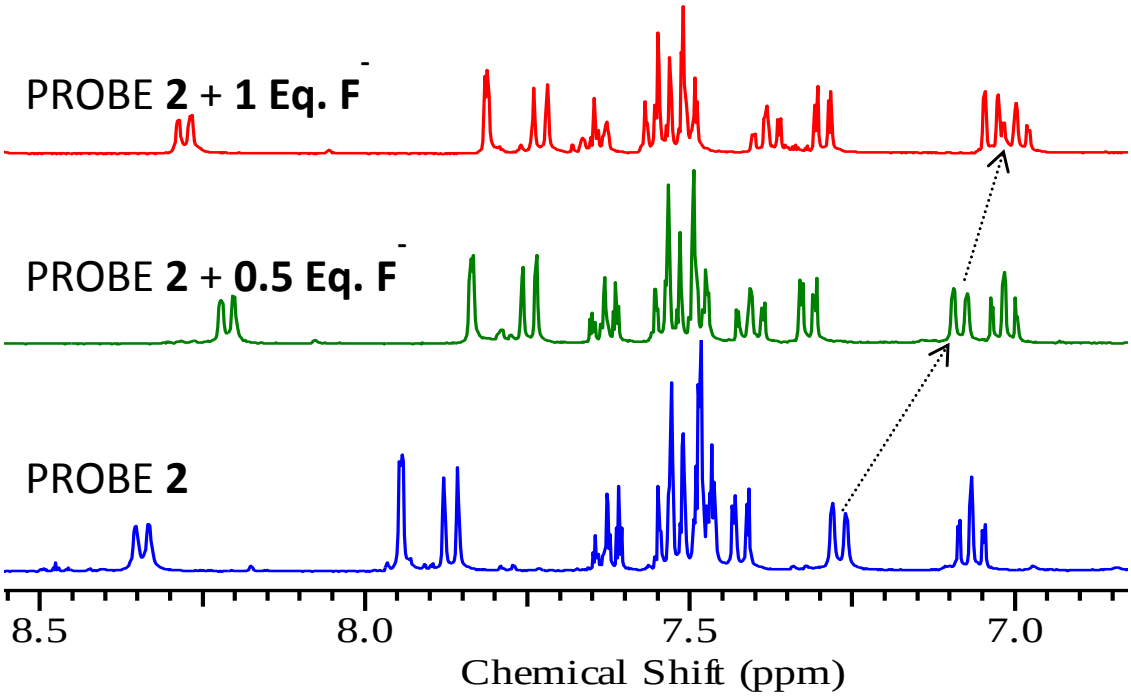


Figure 12: NMR titrations of Probe **2** with TBAF in CD_3CN at 5mM

Binding of fluoride was found to be different wherein the doublet near 7.5 ppm gets merged with the triplet near 7.00 ppm, due to the shielding of protons near to OH group causing it to deprotonate in the presence of fluoride (Scheme 2)(Figure 12). Similar changes were observed in case of AcO- , $P_20_7^{4-(}$Figure 13-14). We concluded that cyanide acts as chemodosimeter and all other anions are deprotanating the hydroxyl proton.

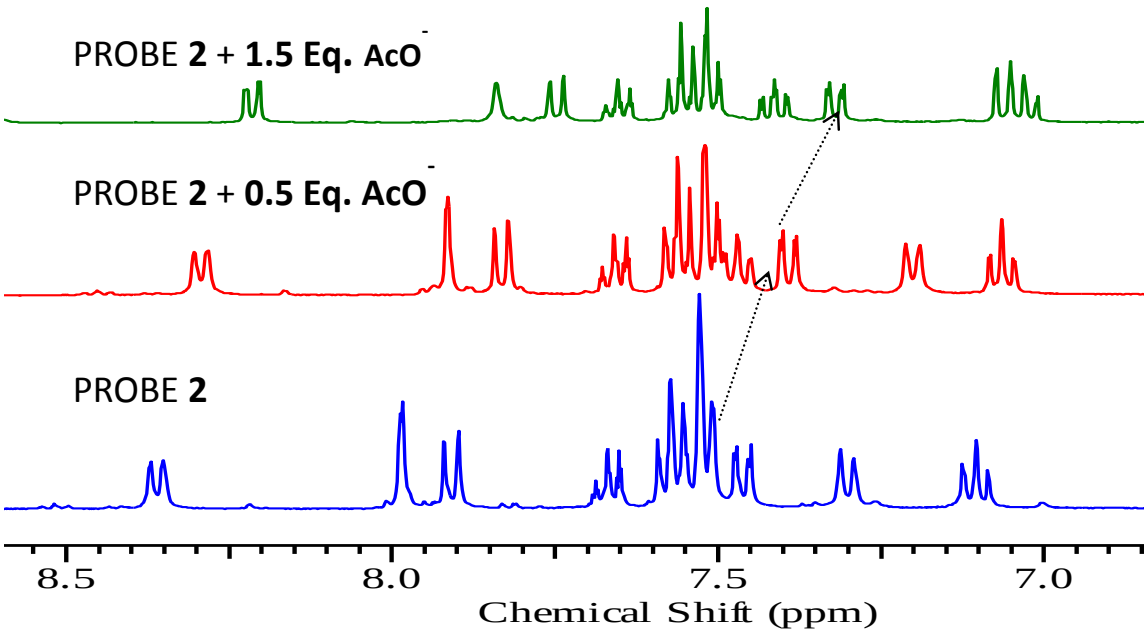


Figure 13: NMR titrations of Probe **2** with AcO^- in CD_3CN at 5mM.

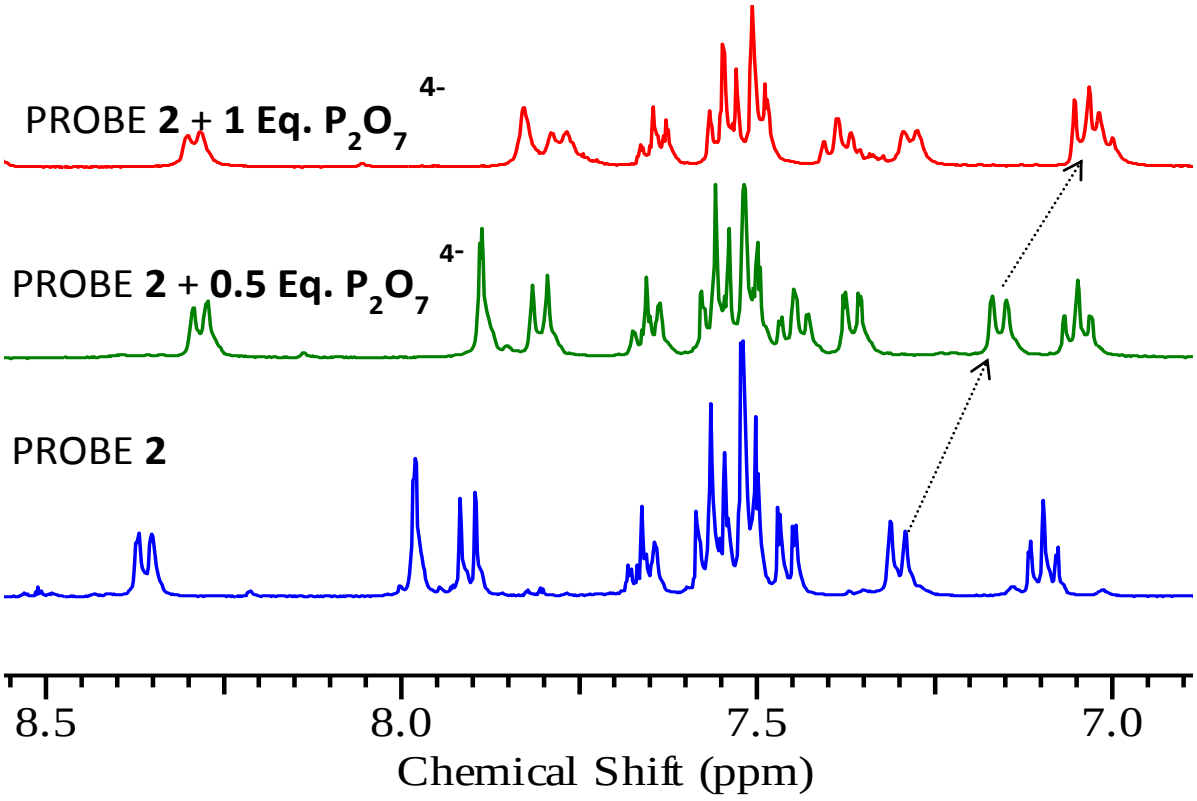
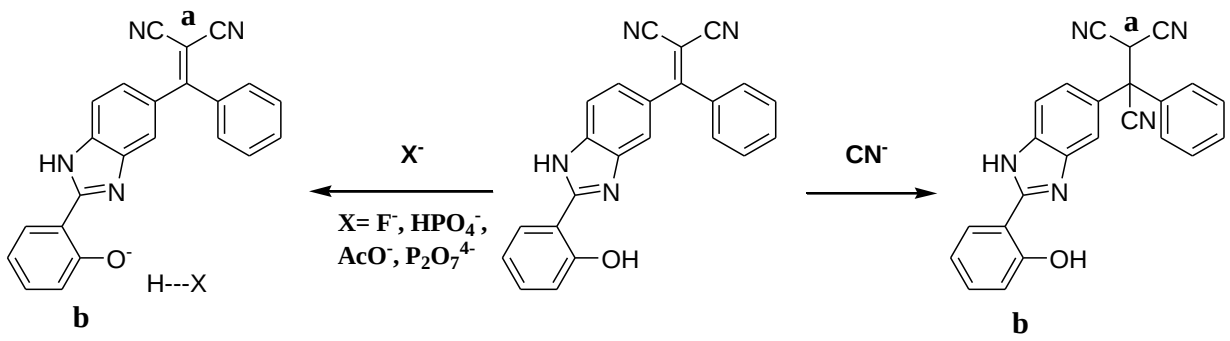


Figure 14: NMR titrations of Probe **2** with $P_2O_7^{4-}$ in CD_3CN at 5mM.



Scheme 3: Proposed mechanism of attack of anions$^-$ to probe **2**

Practical Applicability of Probe 2

In order to check the interference of other anions with CN^-, other anions were added (50 equiv. each) such as Cl^-, Br^-, I^-, OH^-, NO_3^-, SCN^-, HSO_4^- and no significant change in the absorption intensity was observed. To determine the practical applicability of probe **2**, competitive anion experiments was carried out in the presence of 10 equivalents of CN^- ions mixed with 50 equiv.

of each of the other anions. No noteworthy variation in the absorbance intensity was found on comparing the results with the ones in the presence and absence of the other anions (Figure 15).

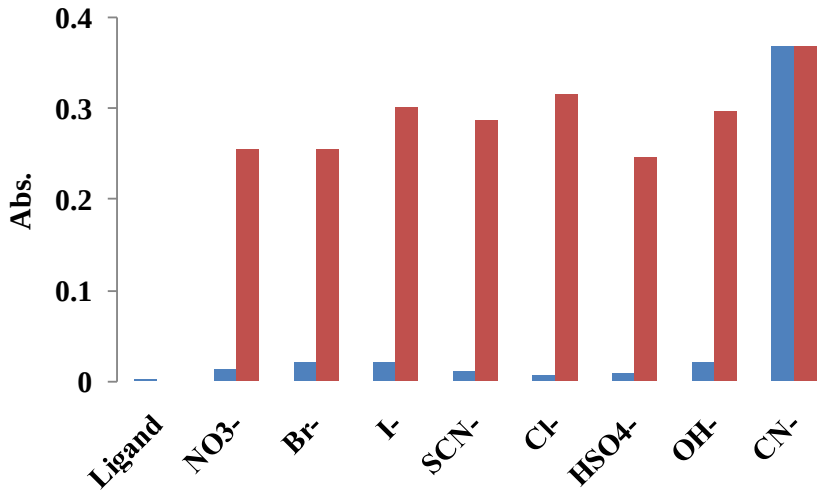


Figure **15**: Blue bars represent selectivity of probe **2 (**20 μM) upon addition of different anions in THF and red bars shows the competitive selectivity of probe **2** in the presence of interfering anion.

Binding behaviour of probe 2 towards CN^-

To determine complexation behavior of CN^- towards probe **2,** Job's Plot was drawn (Figure 16). Stock solution of the same concentration of Probe **2** and CN^- were prepared of the order of 2.0×10^{-5} (M) in THF. The absorbance in each case with different *host–guest* ratio but equal volume was recorded. Job plots were drawn by plotting $\Delta I.X_{host}$ *vs* X_{host} (ΔI = change of intensity of the absorbance spectrum during titration and X_{host} is the mole fraction of the host in each case, respectively) and found that there exists a 1:1 stoichiometry.

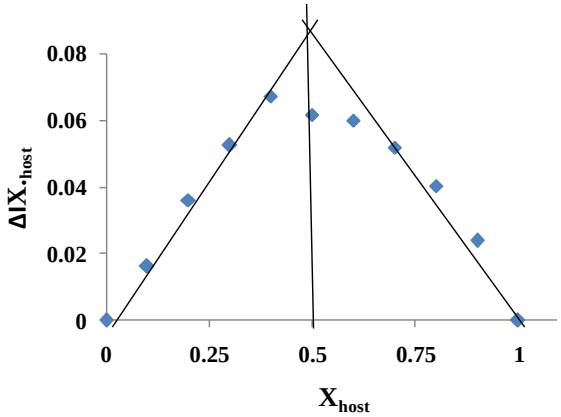


Figure 16: Job's plot diagram of Probe **2** with cyanide ions in THF.

EXPERIMENTAL SECTION

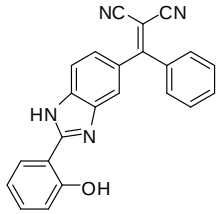
- **Materials and equipments**

All chemicals were from Loba and Sigma Aldrich Chemical Co and used without further purification. Column chromatography was performed using silica gel (60–120 mesh). All reactions were monitored by thin layer chromatography. Hexane:Ethyl Acetate was the adopted solvent system. Melting points were recorded by the open capillary tube method and uncorrected. ^{1}H NMR and ^{13}C NMR spectra were carried out using a JEOL ECS-400 MHz spectrometer in SAI Labs, Thapar University, Patiala with TMS as an internal reference. Mass Spectra of the synthesized compounds were recorded at MAT 120 in SAIF, Punjab University. All chemical shifts are reported in ppm relative to the TMS as an internal reference. UV-vis studies were carried out on a Shimadzu UV-2600 machine using slit width of 1.0 nm and matched quartz cells. Fluorescence spectra were determined on a Varian Cary Eclipse fluorescence spectrometer. Stock solution of probes **2** was prepared at 10^{-3} M in distilled CH_3CN. All absorption and fluorescence scans were saved as ACS II files and further processed in Excel™ to produce all graphs shown. Solutions of **2** were typically 20 µM for UV-vis studies. Tetrabutylammonium salt was used for anionic studies. Binding constant was calculated according to the Benesi-Hildebrand equation. K_a was calculated following the equation stated below.

$$1/(A-A_o) = 1/\{K(A_{max}-A_o)\,[M^{x+}]^n\} + 1/[A_{max}-A_o]$$

Here A_o is the absorbance of receptor in the absence of guest, A is the absorbance recorded in the presence of added guest, A_{max} is absorbance in presence of added $[M^{x+}]_{max}$ and K is the association constant.

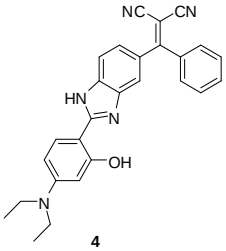
General Procedure for the synthesis of Probe 2 – To a solution of **1**[23], (0.50 g, 1.29 mmol) and malononitrile (0.42 g, 6.35 mmol) in 30 ml dry CH_2Cl_2 were added 0.75 ml pyridine and 0.50 ml of $TiCl_4$ under N_2 atmosphere. The reaction mixture was refluxed overnight at 80°C. After completion of reaction, reaction mixture was allowed to cool to room temperature. The mixture was then diluted with dichloromethane and extracted with 2 N HCl solution. The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to get the crude product. The organic layer was evaporated, and the compound was purified by column chromatography using (65:35) (Hexane: Ethyl Acetate) as an eluent to give of probe **2** (325 mg, 65% yield) (**Scheme 1**).



2

^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): δ 8.36 (d, 1H, *J* = 7.8 Hz, ArH), 8.08 (s, 1H, ArH), 7.99 (d, 1H, *J* = 8.72 Hz, ArH), 7.67 (t, 1H, *J* = 8.32 Hz, ArH), 7.45-7.58 (m, 6H, ArH), 7.32 (d, 1H, *J* = 8.72 Hz, ArH), 7.09 (t, 1H, *J* = 7.34 Hz, ArH); ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 100 MHz): δ 173.87 (C=C), 158.03, 150.02, 141.49, 135.83, 134.90, 132.66, 132.29, 130.27, 128.93, 128.73, 127.28, 120.01, 117.44, 116.91, 114.65, 113.84, 113.72, 108 (ArH), 81.41(C-$(CN)_2$). MS (ESI) m/z 363 (M^++1).

General Procedure for the synthesis of Probe 4: To a solution of **3**[23], (1.29 mmol) and malononitrile (6.35 mmol) in 30 ml dry CH_2Cl_2 were added 0.75 ml pyridine and 0.50 ml of $TiCl_4$ under N_2 atmosphere. The reaction mixture was refluxed overnight at 80°C. After completion, reaction mixture was allowed to cool to room temperature. The mixture was then diluted with dichloromethane and extracted with 2 N HCl solution. The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to get the crude product. The organic layer was evaporated, and the compound was purified by column chromatography using (65:35) (Hexane: Ethyl Acetate) as an eluent to give of probe **4** (65% yield)



4

^{1}H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): δ 12.60 (bs, OH), 7.74 (d, 1H, *J* = 8.7 Hz, ArH), 7.62 (t, 1H, *J* = 7.2 Hz, ArH), 7.52-7.47 (m, 5H, ArH), 6.34-6.31 (dd, 2H, ^{2}J = 2.32 Hz, ^{3}J = 9.16, ArH), 6.26 (s, 1H, *J* = 2.3 Hz, ArH), 3.44-3.38 (m, 4H, $N(CH_2)_2$), 1.22 (t, 6H, *J* = 8 Hz, $(CH_3)_2$);

^{13}C NMR ($CDCl_3$ + DMSO-d_6, 100 MHz): δ 175.11 (C=C), 160.26, 150.61, 136.40, 131.83, 130.13, 128.18, 127.04, 114.57, 114.22, 103.34, 99.63 (ArH), 97.656 (C-$(CN)_2$), 43.89 (N-$(CH_2)_2$), 12.21 (C-$(CH_3)_2$). MS (ESI) m/z 434.19 (M^{+}+1).

CONCLUSION

- We have synthesized dicyano probes to study the push-pull effect.
- The dicyano probed has been successfully used for the estimation of cyanide ions chemodosimetrically.
- A selective ratiometric detection of cyanide was reached due to its change in binding behavior from other anions.
- Differential behavior was observed for detection of various anions as sensors and chemodosimeters.
- An easy and selective low cyanide concentrations detection method has been developed.

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