

Synthesis and Studies of Molecules Exhibiting Excited State Proton Transfer and Aggregation Induced Emission as Sensors and Logic Devices

A
Thesis submitted in
fulfillment of the requirement of the degree of

Doctor of Philosophy

Submitted by
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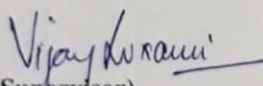
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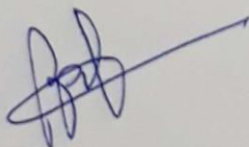
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Certificate

Certified that the thesis entitled “**Synthesis and Studies of Molecules Exhibiting Excited State Proton Transfer and Aggregation Induced Emission as Sensors and Logic Devices**” which is submitted by Mr. Akul Sen Gupta in fulfillment of the requirement for the award of the Degree of Doctor of Philosophy in the School of Chemistry and Biochemistry, Thapar University, Patiala, is a record of candidate’s own independent and original research work carried out by him under my supervision and guidance. The material embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree.


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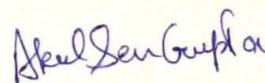
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Candidate's Declaration

I, hereby declare that the work presented in the thesis entitled "**Synthesis and Studies of Molecules Exhibiting Excited State Proton Transfer and Aggregation Induced Emission as Sensors and Logic Devices** " in fulfillment of the requirement for the award of the Degree of Doctor of Philosophy, School of Chemistry and Biochemistry, Thapar University, Patiala, is an authentic record of my own work carried out under the supervision of Dr. Vijay Luxami Assistant Professor, School of Chemistry & Biochemistry, Thapar University, Patiala, India. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any degree in India or Abroad.

Place: Patiala

Date: 5^h Oct, 2016



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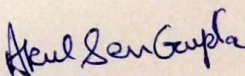
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Akul Sen Gupta

List of Abbreviations

ACQ	Aggregation Caused Quenching
AIE	Aggregation Induced Emission
ATP	Adenosine tri Phosphate
ALP	Alkaline phosphates
B3LYP	Becke's Lee Yang and Parr
CHEF	Chelation Enhanced Fluorescence
Cys	Cysteine
DFT	Density Functional Theory
DLS	Dynamic Light Scattering
DMSO	Dimethyl sulphoxide
DPA	Dipicolyl Amine
ESICT	Excited State Intramolecular Charge Transfer
ESIPT	Excited State Intramolecular Proton Transfer
FESEM	Field Emission Scanning Electron Microscopy
GSH	Glutathione
Hcy	Homocysteine
HEPES	(4-hydroxyethyl)-1-piperazineethanesulfonic acid
HOMO	Highest occupied molecular orbital
ICT	Internal Charge Transfer
LEDs	Light emitting diodes
LOD	Limit of Detection
LUMO	Lowest unoccupied molecular orbital
MeOH	Methanol
MLCT	Metal to ligand charge transfer
M.Pt.	Melting Point
OLEDs	Organic light emitting diodes
PET	Photoinduced Electron Transfer

PMMA	Poly(methyl methacrylate)
ppb	Parts per billion
PPi	Pyrophosphate
ppm	Parts per million
RAHB	Resonance Assisted Hydrogen Bonding
SEM	Scanning Electron Microscope
TBAX	Tetrabutyl ammonium halides
TBET	Through bond energy transfer
TDSCF	Time-dependent self-consistent field
TEM	Transmission Electron Microscopy
TLC	Thin layer chromatography
TPE	Tetraphenyl ethylene
XRPD	X-ray Powder Diffraction
XRD	X-ray Diffraction

Symbols Used

K	Stability Constant
α	Pre exponential factor
ϕ	Quantum Yield
μM	Micro Molar
nM	Nano Molar
λ_{max}	Absorption maximum
λ_{em}	Emission maximum
τ	Decay time
ns	Nano seconds

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CHAPTER 1

Introduction and Review of Literature

1.1 Introduction

Recognition of any kind of ion now a day is substantially ubiquitous with the aim of improving the quality of our lives in any technologically advanced application. According to the definition given by IUPAC, a sensor is a system that, stimulated by any form of energy, reacts with changing its own state and thus changes in one or more of its characteristics. Among different kinds of sensors, chemical sensors, i.e. those sensors that transform the chemical information into an analytically useful signal are of particular importance. Chemical sensors, however, are valuable not only since they are cheap and user friendly analytical tools; they also allow monitoring analyte concentrations in real time and real space.¹⁻³ In general, there is a receptor as recognition site which is attached to a fluorophore. The source of signal in a typical optical chemosensor converts the recognition event to the optical signal. Moreover, large numbers of small anions and cations play important role in human life which exist in organisms as well as in external environment. Thus, detection of such anions and cations is of particular interest to many scientists and researchers.

Anions have many fundamental and functional roles in many of the biological processes.⁴ Many cations are also functionally involved in all key biological processes, such as contraction of muscles, transmission in case of nerve impulses and cell activity regulation.⁵ Many heavy metal ions needs to be detected, as many of them are substances having priority hazardous and toxic, causing environmental and health problems. Moreover, cell functions such as death and proliferation depend on availability of particular cation.⁶

Among the different chemical sensors, fluorescence-based sensors have many advantages: fluorescence measurements are usually very sensitive (single molecule detection is possible), low cost, easily performed, and versatile, offering sub nanometer spatial resolution with submicron visualization. There are many fluorescent-sensing mechanism that have been extensively used, involving, internal charge transfer (ICT) or metal to ligand charge transfer (MLCT), photoinduced electron transfer (PET), formation of excimer/exciple etc. for sensing cations as well as anions.⁷ Amongst all these important phenomenon's, the excited state intramolecular proton transfer (ESIPT)⁸ has called upon a great importance due to its immeasurable applications

such as photostabilizer for ultraviolet, laser dyes, scintillators, probes for membrane and protein⁸ and also serves as potential component in case of photo based switches and OLEDs.⁹ ESIPT materializes in molecules containing hydrogen donor group like phenolic or amino moiety with basic site i.e. O, S, N elements in ground state via interactions of hydrogen bond. As depicted in Figure 1 the proton that covalently attached in the electronically excited state jumps to adjacent hydrogen-bonded atom having less than 2 Å distance which resulted in dissipation of surplus amount of excited state energy. This phenomenon formed phototautomer having lower energy emits at higher wavelength with unusual high stokes shift which can range from 100-500 nm. This equilibrates back to its normal i.e. ground state proton bounded to the original atom thermally. This stokes shift, which is very large in case of ESIPT, is the most important property for sensors as self-absorption and inner filter effect can be avoided leading to the improvement of the fluorescent analysis.

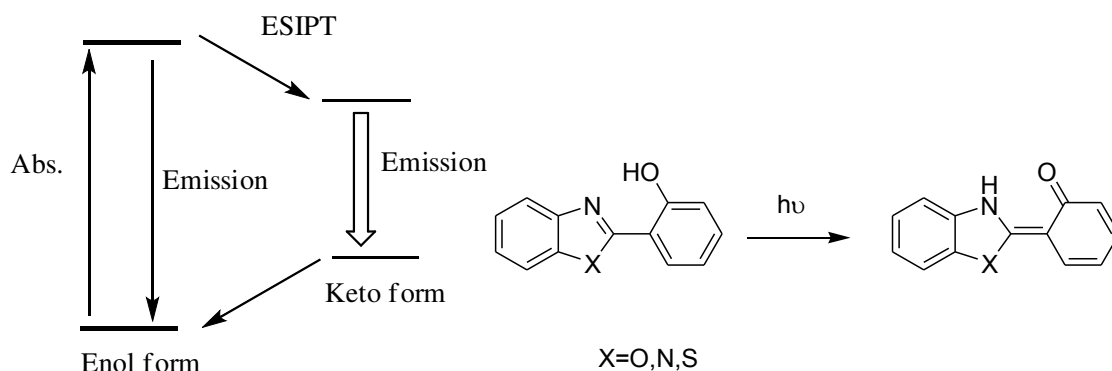


Figure 1: Schematic representation of ESIPT phenomenon

Therefore, molecules bearing ESIPT shows mainly two different type of emission channels – one because of normal emission i.e enol form and second is due to the excited state which is stabilized i.e keto form, through ESIPT having large Stokes shift. Due to this dual emission, the ESIPT bearing sensors have great advantage with large range emission spectra where various fluorescent colors can be changed by adding different analytes. In many cases the fluorescence of orthodox luminophores is often enfeebled or even quenched when dissipated in aqueous media or manufactured into solid film which greatly lemmatize its real-world applications. To overcome this problem a phenomenon named “aggregation-induced emission” (AIE)¹⁰⁻¹¹ was

immersed i.e. nonemissive molecules when dispersed in solution form, results in emission due to aggregate formation. Diminution of intramolecular torsional or rotational motions is the main reason for the AIE effect. Figure 2(A) shows an example of Aggregation Caused Quenching (ACQ) effect which is due to π - π stacking and leading to non-emissive solution state. But experimental and theoretical reports¹²⁻¹³ have concluded that the torsions as well as internal rotations within AIE active compounds deactivates the excited states, thus these compounds were almost non-fluorescent in solutions (Figure 2 (B)).¹⁴⁻¹⁷

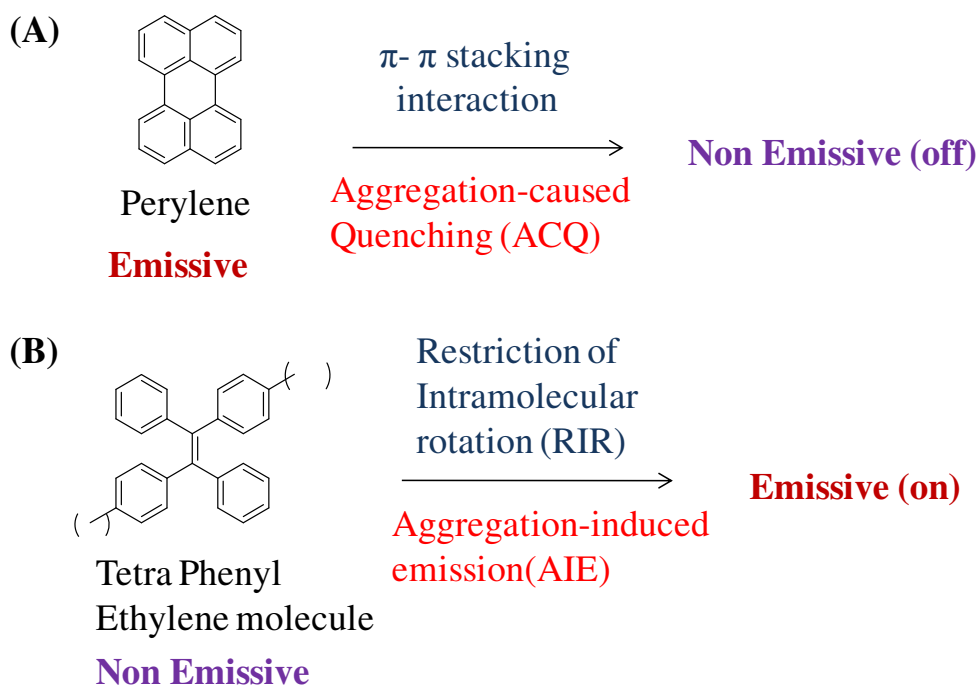


Figure 2: Diagrammatic representation of (A) ACQ and (B) AIE phenomenon.

Out of the different AIE luminogens, tetraphenylethene's (TPE's)¹⁸ are excellent emitters for fabricating light-emitting diodes but also are sensitive probes for the detection of biomolecules due to their abnormal fluorescence behaviours.¹⁹ The main fact is that the TPE molecules are almost non emissive/weakly fluorescent in solution but on aggregation they emit strongly. Incorporation of ESIPT and AIE moieties on to the single fluorophore has been scarcely studied in literature²⁰⁻²⁵ and the sensing properties towards cations and anions are further limited. On the basis of these gaps the present thesis has been categorized into following chapters and their subchapters.

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Chapter 2: Synthesis and photophysical properties of molecules exhibiting ESIPT phenomenon for cations and anions.	2.1 General Introduction 2.2 Ratiometric fluorescent chemosensor for fluoride ion based on inhibition of excited state intramolecular proton transfer 2.3 Benzimidazole based ratiometric chemosensor for detection of CN^- and Cu^{2+} ions in protic/aqueous system: Elaboration as XOR logic operation. 2.4 ESICT-ESIPT coupled fluorescent sensor for copper and hydrogen sulphate ions 2.5 Chromofluorescent probe for estimation of cyanide in human blood serum/plasma like solutions using chemodosimetric approach
Chapter 3: Synthesis of hybrid molecules showing ESIPT-AIE phenomenon and their sensing behavior	3.1 General Introduction 3.2 AIE-ESIPT coupled ratiometric fluorescent probe for detection of Cu^{2+} and F^- ions 3.3 ESIPT-AIE coupled probe for detection of trivalent metal ions (Al^{3+}, Cr^{3+} and Fe^{3+}) in aqueous media 3.4 Benzimidazole-TPE hybrid probe for ratiometric estimation of cations and anions
Chapter 4: Quantification of optical signals for generation of molecular logic gates	4.1 General Introduction 4.2 Dansyl–Antipyrine dyad as sequential XNOR logic device. 4.3 Naphthalene- Antipyrine dyad based ionic encoder and decoder

1.2 Research Gaps and Objectives

1.2.1 Research Gaps

- ❖ Aggregation induced emission phenomenon's based tetra phenyl ethylene molecules have not been used for sensing of anions and cations.
- ❖ Logic devices based upon ionic inputs have not been explored much like encoders and decoders.
- ❖ ESIPT incorporated on AIE phenomenon's is not much studied as it provides light having wide range from 400-800 nm which can be boon for sensing various ions.

1.2.2 Objectives:

- Synthesis of molecules exhibiting AIE and ESIPT phenomenon for sensing of cations, anions and biological ions.
- Synthesis of hybrid molecules showing ESIPT - AIE phenomenon to explore combined effect on sensing.
- Quantification of optical signals for generation of molecular logic gates.
- Theoretical studies of synthesized molecules towards certain cations and anions.

1.3 Review of Literature

Based upon the two different phenomenon's, review of literature has been divided into three sections as mentioned below.

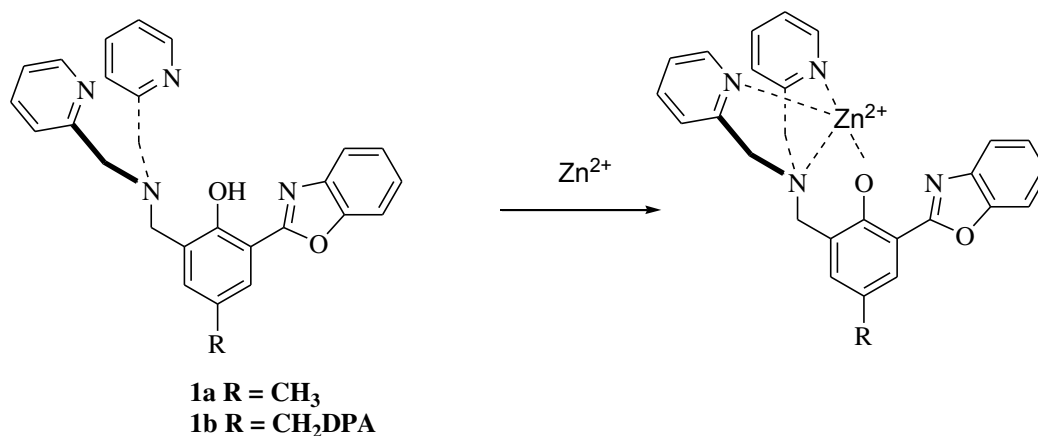
1.3.1 ESIPT phenomenon's based molecules for sensing

1.3.2 AIE phenomenon's based molecules for sensing

1.3.3 ESIPT coupled AIE molecules for sensing

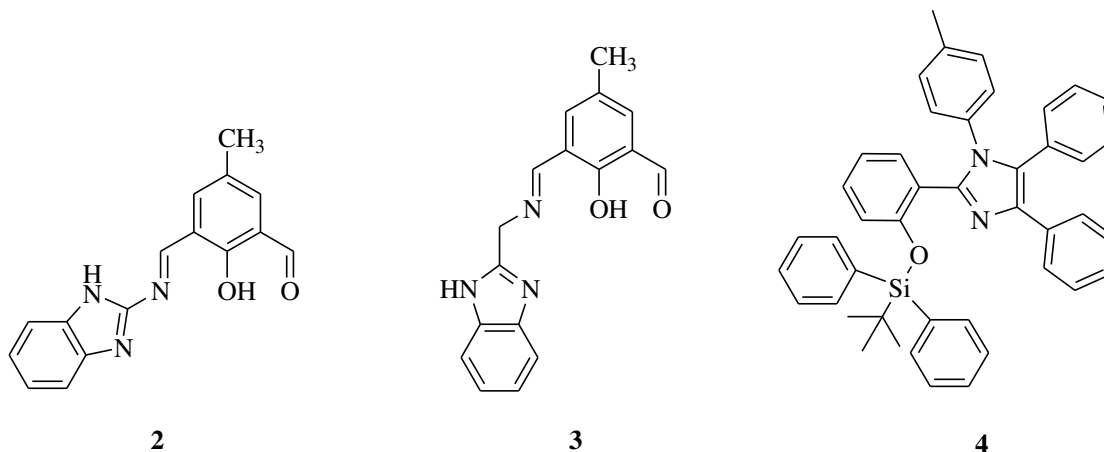
1.3.1 ESIPT phenomenon's based molecules for sensing

Pang *et al.* synthesized a benzothiazole derivative **1a** in which 2,2'-dipicolylamine (DPA) group has been used for Zn^{2+} binding. ^1H NMR and mass spectral techniques have been used to reveal the anion sensing mechanism. The absorption band at 343 nm has been shifted to 367 nm and blue shifted was observed in emission to 429 nm in the presence of Zn^{2+} ions. This Zn^{2+} complex was further used for solvent-switchable selectivity, responding to adenosine 5'-triphosphate (ATP) in water and pyrophosphate (PPi) in EtOH. Probe **1b** complex showed ESIPT "off" in the presence of Zn^{2+} ions and ESIPT "on" in the presence of pyrophosphate ions.²⁶



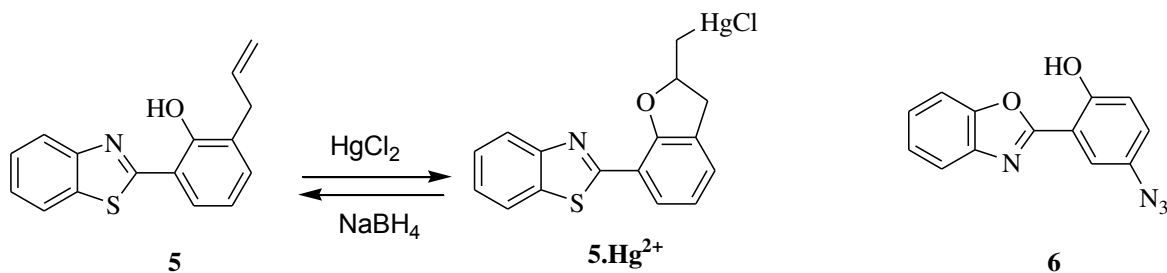
Roy *et al.* synthesized two schiff-bases **2** and **3** based on benzimidazole. Molecule **2** showed selective changes for Zn^{2+} ions. The compound **2** upon excitation at 445 nm showed the emission enhancement at 528 nm in the presence of Zn^{2+} ions. No significant changes in the presence of other metal ions were observed. The authors compared the photophysical properties of molecule **2** with **3** and observed that compound **3** had an effect both on absorption as well as fluorescence

peak positions due to methylene group. Molecules **2** and **3** were found selective towards Zn^{2+} ions with very low limit of detection (LOD) values. Molecule **2** was also used for cell imaging in human lung cancer.²⁷



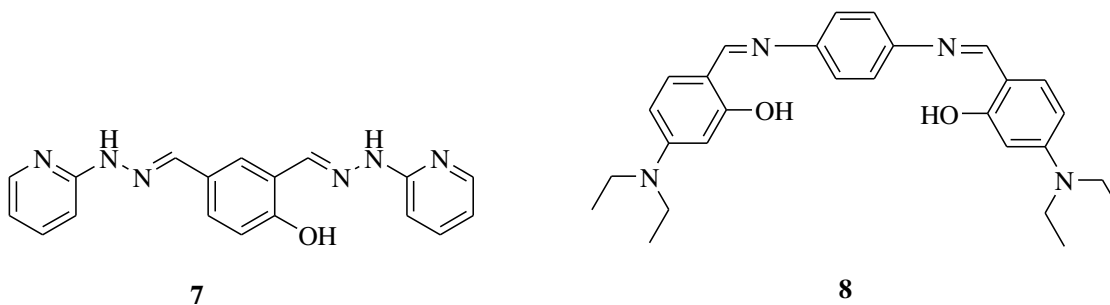
Venkatasubbaiah *et al.* synthesized probe **4** which was used for the detection of F^- ions. They used different analytical and spectroscopic techniques for characterization of probe **4**. They observed that the cleavage of Si-O bond was the main reason for exhibiting a multi-channel rapid response and selectivity as well as sensitivity towards F^- ions. In the absence of F^- ions the probe **4** showed no ESIPT but after the addition of F^- ions, ESIPT was turned “on” due to the cleavage of Si-O bond which resulted in the formation of OH group on probe **4** that can donate its proton to N atoms. These results were also correlated with time-dependant density functional theory (TD-DFT) calculations.²⁸

A ratiometric fluorescent chemodosimeter **5** for reusable detection of Hg^{2+} was developed by Liu *et al.* The chemodosimeter response was observed *via* interrupting the excited state intramolecular proton transfer process leading to selective estimation of Hg^{2+} with a color change from green to blue under fluorescence. The chemodosimetric approach was determined by the formation of **5.Hg**²⁺ complex. The main application reported was removal of Hg^{2+} from water that was further reused. Thus, the fluorescence response towards Hg^{2+} ions were reported with low detection limit (1.0 ppb), and was used to sense Hg^{2+} ions in drinking water. This was also used in imaging of Hg^{2+} ions in living cells.²⁹

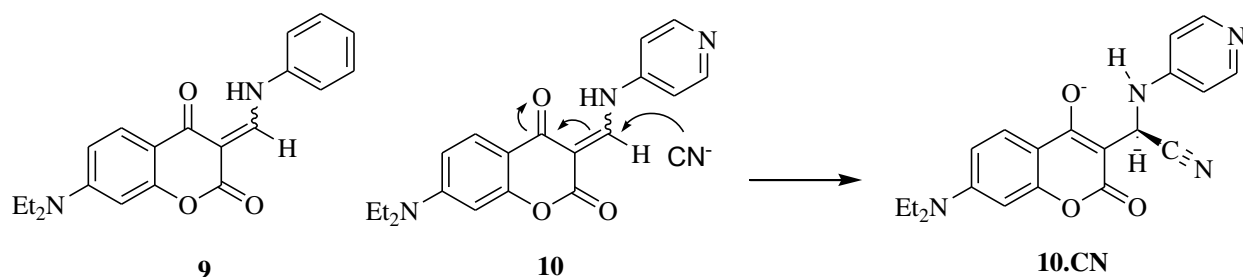


Xu *et al.* constructed a simple azido-substituted sensor **6** which showed turn-on response to glutathione (GSH) and cysteine (Cys) over homocysteine (Hcy), sulfide and other amino acids. The main reason for this selectivity was based on selective nucleophilic substitution–rearrangement type reactions which lead to conversion of azido group to amine group.³⁰

A pincer-type chemosensor **7** showed sensitivity and selectivity for Zn²⁺ ions *via* turn-on fluorescence behavior. Chemosensor **7** having pyridine group and a hydrazone bond moiety showed the inhibition of ESIPT phenomenon's along with the –HC=N– isomerization and Chelation Enhanced Fluorescence Enhancement (CHEF) for estimation of zinc ions.³¹ The detection limit of compound **7** for zinc ions was reported to be 1.39×10^{-8} M.

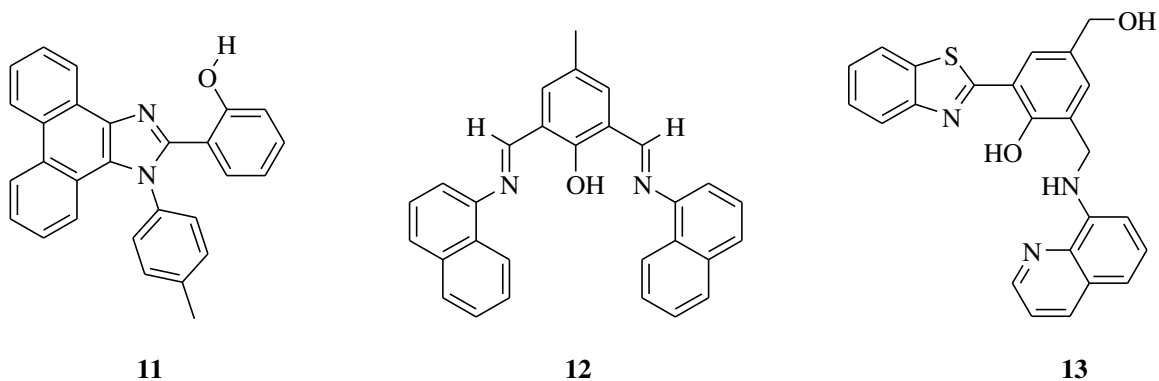


Singh *et al.* developed chemosensor **8** which showed selective as well as sensitive recognition of Fe³⁺ and Cu²⁺ ions. In case of Fe³⁺ ions, receptor **8** showed change in color from yellow to orange indicating red-shift and in case of Cu²⁺ ions, color changed from yellow to colorless indicating blue-shift. However, emission study showed that probe **8**, in the presence of Fe³⁺ ions observed emission enhancement along with a red-shift whereas fluorescence quenching was shown in case of Cu²⁺ ions. Probe **8** was used as an IMPLICATION type logic gate by using Cu²⁺/Fe³⁺ as the chemical inputs.³²



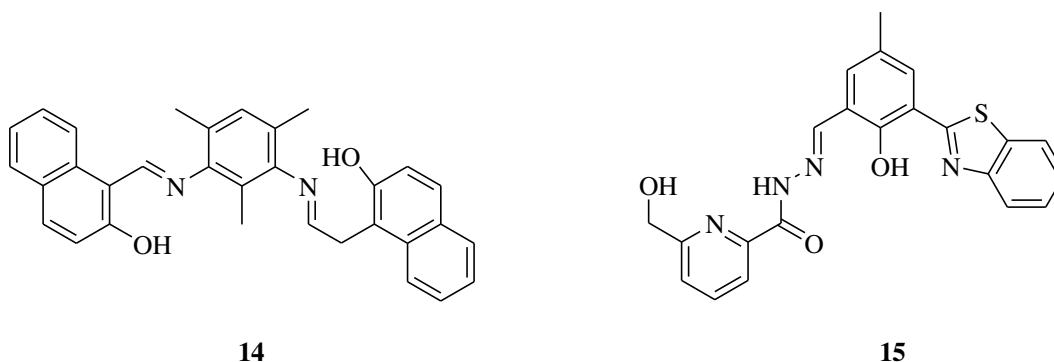
Two coumarin-enamine chemodosimeters **9** and **10** showed selective detection of cyanide ion with detection limit approximately 4.2 ppb. Probe **10** showed an absorption band at 405 nm that was shifted to 335 nm in presence of CN^- ions which was clearly distinguishable via naked eye. Chemodosimeter approach was observed *via* Michael addition to form **10.CN** adduct.³³

Zhang *et al.* developed a novel probe **11** which showed selectivity towards Hg^{2+} ions over other metal ions with large bathochromic shift (approx 100 nm). Dichloromethane solvent was introduced for extraction process of Hg^{2+} ions as well as for optical detection. The detection limit was 7.8×10^{-9} M because dichloromethane has enhancement effect on ESIPT. This probe was also used for the detection of Hg^{2+} in living cells through fluorescence images.³⁴



Das *et al.* developed X-ray structurally characterized naphthalene based probe **12** for selective detection of Sn^{2+} by colorimetric and fluorescence methods. Probe **12** in the presence of Sn^{2+} exhibited emission band at 420 nm i.e of monomer along with a strong red emission at 582 nm in acetonitrile of excimer mode. The dynamic excimer was observed upto low equivalents of Sn^{2+} but gradually converted to a static form above 2.5 eq. of Sn^{2+} . In aqueous methanol, probe **12** was used to detect Al^{3+} ions through the formation of green fluorescence. These photophysical results were correlated by different spectroscopy methods and also by DFT studies.³⁵

Bhardwaj *et al.* designed a benzothiazole based chemosensor **13** which showed fluorescence turn-on in the presence of Hg^{2+} ions. Detection limit of $0.11 \mu\text{M}$ Hg^{2+} was reported. The metal binding mechanism was correlated by NMR and Mass spectrometry as well as by DFT studies. Probe **13** showed cell membrane permeability and efficiency for the detection of Hg^{2+} ions in HeLa cells.³⁶

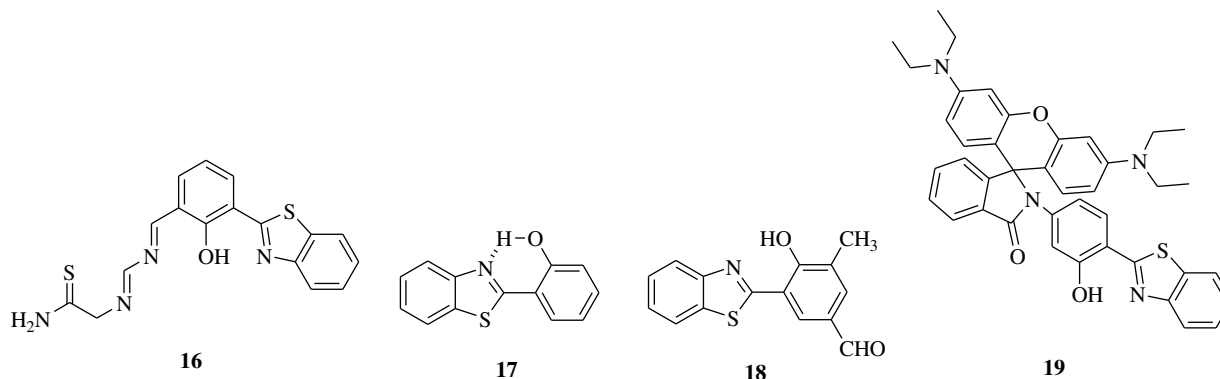


Imine conjugate Schiff base **14** was used for detection of Zn^{2+} ions selectively among the wide range of metal ions. Probe **14** showed an absorption band at 370 nm which decreased simultaneously with the formation of two new bands at 394 and 412 nm in the presence of Zn^{2+} ions. However, emission enhancement was observed at 454 nm in the presence of Zn^{2+} ions. Probe **14** showed fluorescence turn “on” with the Zn^{2+} ions having lowest detection value of $1.47 \mu\text{M}$. Single crystal X-ray studies of probe **14** were also performed to determine the binding of Zn^{2+} ions and revealed the formation of binuclear complex.³⁷

Goswami *et al.* designed and synthesized a new fluorescence ratiometric probe **15** based on Chelation Enhanced Fluorescence (CHEF) as well as excited state intramolecular proton transfer phenomenon's which was used for sensing of Al^{3+} ions. The photophysical properties of the probe were altered upon the introduction of Al^{3+} ions in a mixed aqueous solution. Except Cu^{2+} and Pb^{2+} ions, there was no significant change observed which indicated high affinity towards Al^{3+} ions. The complexation of the probe **15** with Al^{3+} ions inhibited ESIPT and induced the CHEF. The binding constant was calculated to be $6.72 \times 10^{-8} \text{ M}$.³⁸

Goswami *et al.* designed ESIPT-based sensor **16** for detecting perchlorate ions. They reported high selectivity as well as sensitivity with colour changes from orange to blue under UV light in the presence of perchlorate ions. Sensor **16** showed two emission bands at 476 (weak) and 562

nm, but in the presence of perchlorate ions the emission band at 476 nm emerged gradually and the band at 562 nm decreased simultaneously. The mechanistic approach observed was due to inhibition of 2-way excited-state intramolecular proton transfer mechanism in aqueous solution.³⁹

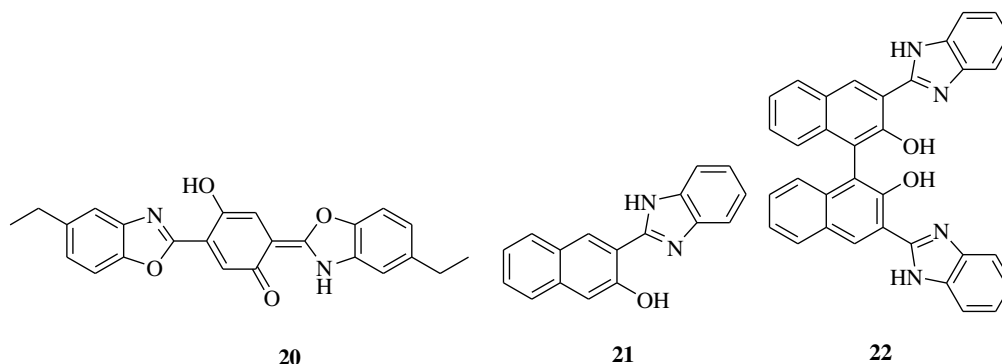


Zhao *et al.* introduced chromophores **17** based upon ESIPT phenomenon's and their applications as chemosensors and biological cell image as well as in white-light emitting materials.⁴¹ Mono-formylated benzoxazole derivative **18** was synthesized by Rodembusch *et al.* through duff method. Solvent effect was explored to great extent onto this molecule and was used for optical sensing of amines in solution form.⁴⁰

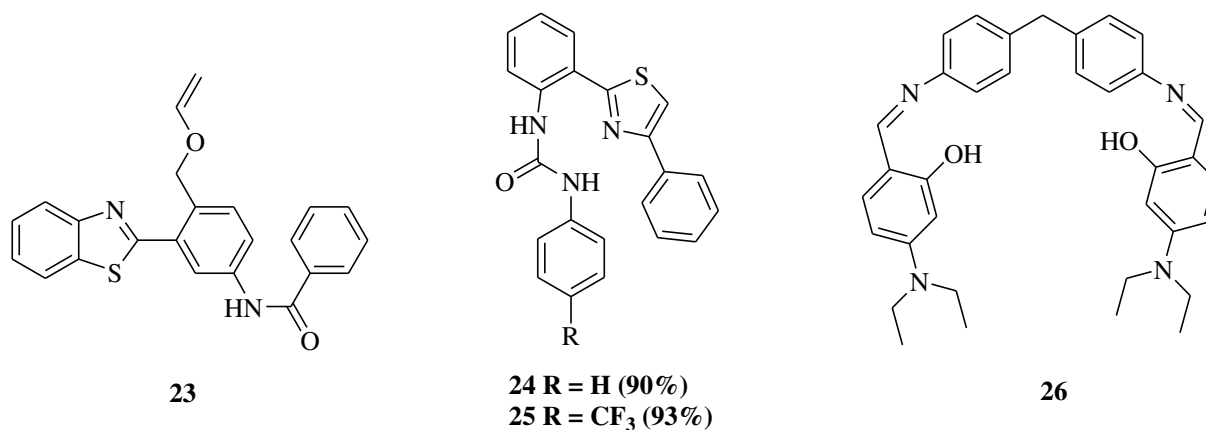
Zhang *et al.* designed a rhodamine based molecule **19** having high quantum yield. Compound **19** responded to acid/base and emitted Red Green Blue (RGB) light with a single molecule even in solution state and in Poly (methyl methacrylate) PMMA film. ESIPT and through bond energy transfer (TBET) led to the release of emission band at 365 nm having high quantum yield. Application was found in anti-counterfeiting and logic gates. The ESIPT and TBET mechanisms resulted in excellent optical properties which were confirmed by using X-ray single crystal diffraction as well as other spectroscopy techniques.⁴²

Wnuk *et al.* studied the compound **20** with different time resolved fluorescence techniques. The studies were performed on rate of proton transfer dependence upon solvent polarity. The potential of double-well for the S1 state of **20** was calculated which helped to confirm the nature of the phototautomer.⁴³ Probes **21** and **22** selectively bound with Zn^{2+} ions and gave new emission band at 490 nm. However, in case of probe **22**, Cu^{2+} ions caused only fluorescence

quenching. By using chemical inputs, different logic gates like NOR, INH and AND were formed.⁴⁴



Qian *et al.* synthesized a highly selective fluorescent probe **23** for the sensing of palladium species. The main group responsible for hydrolyzation was allyl group that was hydrolyzed by Pd^0 species via Tsuji–Trost reaction. Hydrolysis was catalysed by Pd^0 releasing the fluorophore which showed two different emission bands. The maximum emission properties were due to the enol and keto forms at 415 and 555 nm respectively. The palladium species were also detected by paper strip method.⁴⁵

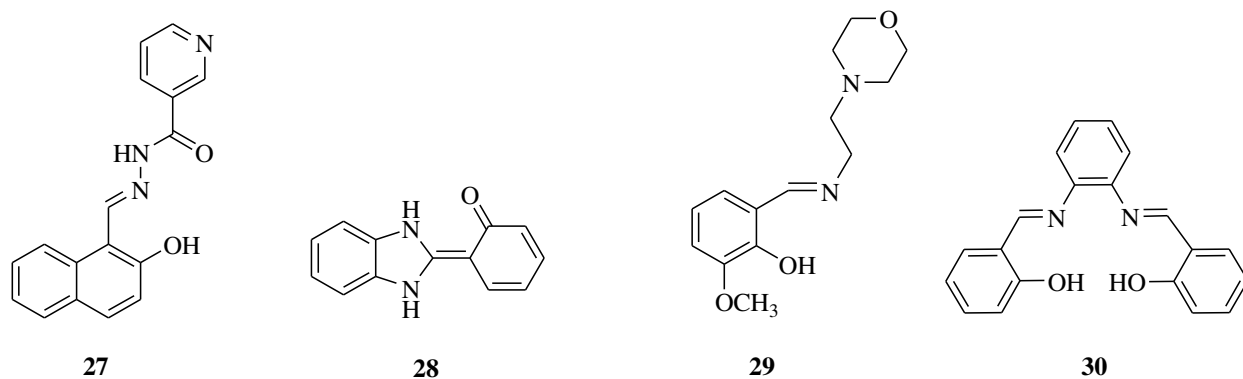


The fluorogenic sensors **24** and **25** have been prepared by Helal and Kim based upon urea derivative and were used for the recognition of anions. Chemosensor **24** was highly selective for acetate ions over other anion because of the structure matching of host and the guest. There was slight blue shift observed for absorption band in presence of acetate ions at 339 nm. Sensor **24** showed two emission bands at 389 nm (normal emission) and 556 nm (keto emission) which

disappeared and a new band was formed at 479 nm in presence of acetate ions. The acetate binding chances were tuned by fluctuating the acidity of the N-H proton found in urea moiety.⁴⁶

Imine based chemosensor **26** was synthesized and evaluated for cation sensing properties by Singh *et al.* Based upon ESIPT and charge transfer mechanism, chemosensor **26** showed high sensitivity as well as selectivity towards Zn^{2+} ions. The Zn^{2+} complex of the sensor **26** was used for phosphate quantification as well as for sensing of phosphorylated biomolecules via cation displacement approach.⁴⁷

An efficient fluorescent Al^{3+} sensor **27** has been designed and synthesized by Yang *et al.* This receptor showed “off-on” type fluorescent response towards Al^{3+} ions. Other metal ions caused almost no emission enhancement. Sensor **27** exhibited two absorption bands at 317 nm and 362 nm which decreased gradually and two new absorption bands appeared at 340 nm and 409 nm due to Al^{3+} ions binding. The emission of **27** was turned “on” at 474 nm upon binding with Al^{3+} ions due to the suppression of excited-state intramolecular proton transfer as well as photoinduced electron transfer phenomenon's.⁴⁸



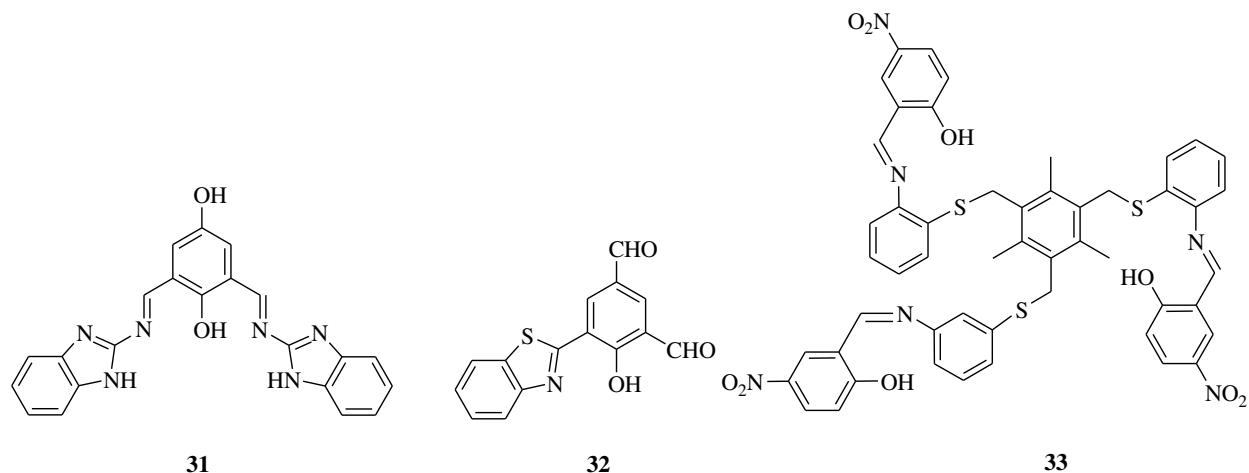
Srinivasan *et al.* prepared chemosensor **28** which behaved as a selective fluorescent sensor for the detection of Fe^{3+} ions. The chemosensor was characterized by single crystal XRD analysis which showed that imidazole ring was planar having monoclinic crystal form. Addition of Fe^{3+} ions to **28** resulted in dramatic fluorescence quenching. It showed the reversible fluorescence enhancement by adding H_3PO_4 which deactivated iron complex.⁴⁹

Schiff-base compound **29** was selective for Zn^{2+} ions which were explored by Roy *et al.* The emission intensity of **29** increased about 25 times in case of Zn^{2+} ions. The quantum yield as well

as the life-time of **29** was increased in the presence of Zn^{2+} ions. This enhancement in emission intensity of **29** was due to the restriction of ESIPT, PET and CHEF on complex formation. Job's plot analysis revealed 1:1 complex of Zn^{2+} ions with sensor **29**. The observed electronic transitions were in correlation with theoretical calculations. Probe **29** was used for imaging in case of DLD-1 as well as for human colorectal adenocarcinoma cell.⁵⁰

Velmathi *et al.* reported highly selective binding of Zn^{2+} ions by the salicylaldehyde based Schiff base receptor **30**. Receptor **30** detected Zn^{2+} ions in aqueous medium by both colorimetric and fluorescent response. Receptor **30** showed an absorption band at 340 nm which was shifted to 380 nm in the presence of Zn^{2+} ions. Due to inhibition of ESIPT receptor **30** on binding with Zn^{2+} ion, exhibited fluorescence enhancement.⁵¹ Dibenzimidazolodiimine sensor **31** was designed and synthesized by Goswami and coworkers for selective detection of acetate ion. Sensor **31** showed significant naked eye color change from light yellow to pink upon addition of acetate ion due to excited state intramolecular proton transfer (ESIPT).⁵²

An intense ESIPT molecule **32** lost its properties in mixed media because of resonance-assisted H-bond (RAHB) in absolute water which lead *o*-aldehyde more reactive than the *p*-aldehyde. By adding cysteine/homocysteine to the solution of **32**, it transformed into thiazolidine/thiazine ring, which in turn released the phenolic proton for transfer to nitrogen of the benzothiazole ring in excited form, leading to turning “on” of ESIPT mechanism.

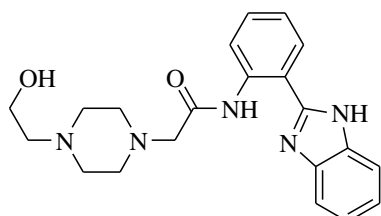


Hundal and coworkers synthesized a mesitylene based tripodal Schiff's base **33** having OH as end groups. Compound **33** was found highly sensitive and selective for Cu^{2+} ions in aqueous medium. The emission band at 528 nm due to ESIPT phenomenon's quenched up to 94%

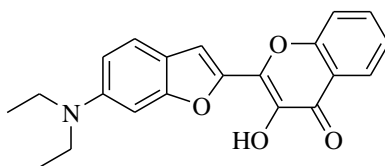
in the presence of Cu^{2+} ions *via* restricting ESIPT phenomenon's. The sensor **33** was used in live cell imaging and showed no cytotoxic effect.⁵⁴

Tang *et al.* developed a new benzimidazole based fluorescent sensor **34** that enabled relay recognition of Cu^{2+} and S^{2-} ions in water solution. Sensor **34** displayed excited state intramolecular proton transfer phenomenon's having two emission bands at 373 nm and 495 nm and were quenched in the presence of Cu^{2+} ions. The Cu^{2+} complex of **34** formed in-situ exhibited excellent selectivity to S^{2-} via fluorescence “off-on” response through Cu^{2+} displacement approach.⁵⁵

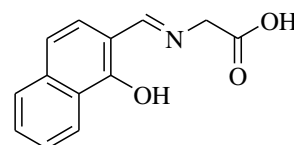
3-Hydroxyflavone based derivative **35** was synthesized by Wang *et al.* The complex of Zr-EDTA and **35** was developed as highly selective and sensitive sensor for the detection of F^- . The Zr-EDTA complex with **35** showed an absorption band at 510 nm and emission band at 610 nm. Absorption band of the complex at 510 nm shifted to 460 nm in the presence of F^- ions. On addition of F^- , emission intensity at 610 nm of Zr-EDTA-**35** complex increased and band at 470 nm decreased simultaneously with color change. The results were attributed to inhibition of ESIPT mechanism.⁵⁶



34

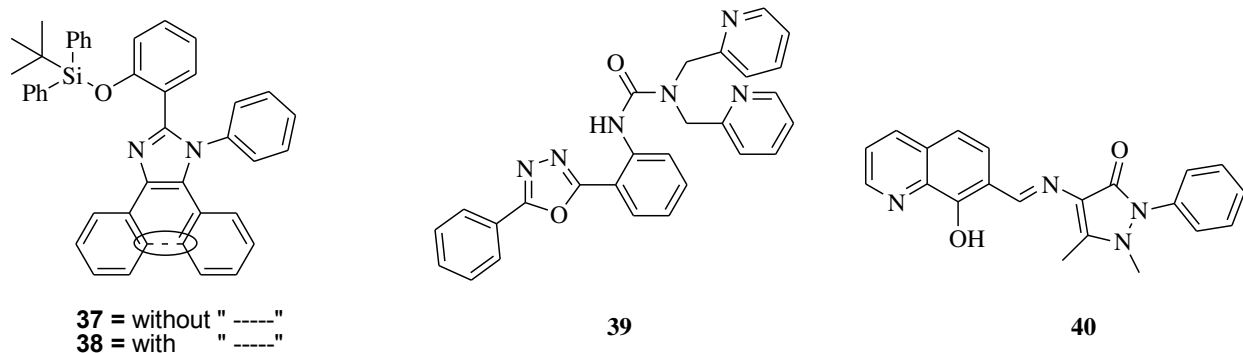


35



36

Kim *et al.* used sensor **36** as a turn on fluorescent sensor for both Zn^{2+} and F^- ions. Receptor **36** showed fluorescence enhancement in 100% aqueous solution in the presence of Zn^{2+} ions. Chemosensor **36** clearly distinguished Zn^{2+} from Cd^{2+} ions. Disruption of the internal charge transfer (ICT) caused inhibition of the C=N isomerization and the excited-state proton transfer phenomenon's was the main fact for selective response towards Zn^{2+} ions. The receptor **36** could also detect F^- ions selectively in DMSO. The fluorescence, UV-vis titration and ^1H NMR titration proved that F^- ion caused deprotonation of hydroxyl proton.⁵⁷



Two fluorescent chemosensors **37** and **38** having masked excited state intramolecular proton transfer phenomenon's were synthesized. In the presence of fluoride anion there was cleavage of Si–O bond which in turn activated the ESIPT phenomenon's. Both these sensors showed high sensitivity and selectivity towards F^- ions which was not even affected by the presence of hydroxide anion.⁵⁸

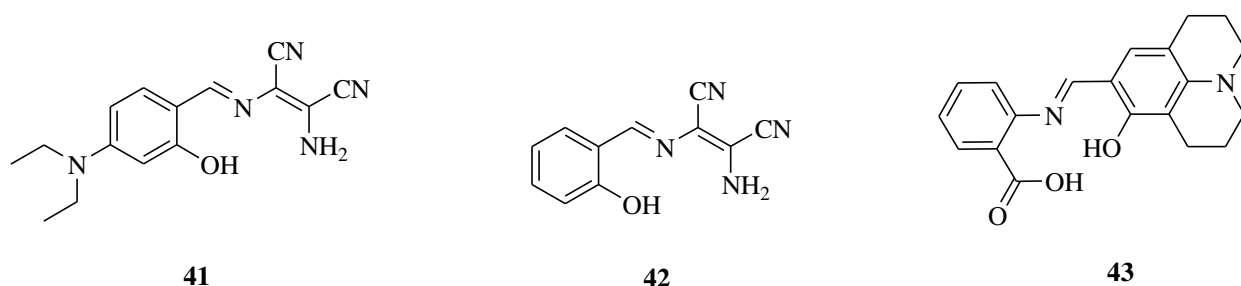
Tang *et al.* designed and synthesized a fluorescent sensor **39** for sensing of Zn^{2+} ions. Sensor **39** coordinated with Zn^{2+} through amide formation with 1:1 stoichiometry. The red-shift was observed along with emission enhancement upon complexation of **39** with Zn^{2+} ions which turn ESIPT process “on”. The sensor **39** was used for detection of Zn^{2+} ions in water samples as well as in living cells.⁵⁹

8-Hydroxyquinoline and amino-antipyrine based Schiff base **40** was designed for sensing of Al^{3+} ions by Yang *et al.* The two absorption bands at 285 nm and 384 nm decreased gradually with blue shift to respective 271 nm and 367 nm in the presence of Al^{3+} ions. The sensor **40** showed the weak emission at 495 nm which in the presence of Al^{3+} ions blue shifted to 466 nm with high emission intensity. The sensor showed high selectivity with Al^{3+} ions, ~26 times more enhancement in fluorescence intensity with detection limit as low as 10^{-7} M.⁶⁰

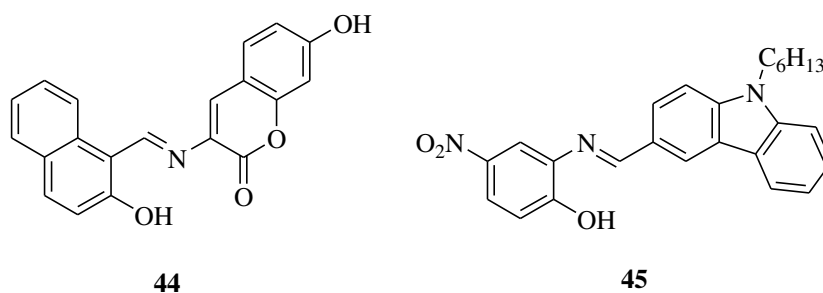
Upadhyay *et al.* evaluated two simple fluorescent chemosensors **41** and **42** having salicylidene imine groups for selective sensing of Al^{3+} ions. The dual emission channels were observed for **41** ($\lambda_{em} = 499$ nm and 535 nm) and **42** ($\lambda_{em} = 485$ nm and 515 nm) in the presence of Al^{3+} with associated green/blue color. This dual emission bands were due to ESIPT and ICT phenomenon. Moreover the fluorescence turn “on” was due to the inhibition of photoinduced electron transfer as well as C=N isomerization upon binding of Al^{3+} ions. The limit of detection

for Al^{3+} with **41** was found to be 10 times lower than that of **42** and the stoichiometry between **41/42** and Al^{3+} were found to be 1:1 and 2:1, respectively.⁶¹

A water-soluble carboxylic-functionalized chemosensor **43** was synthesized by Kim *et al.* which showed fluorescence enhancement in the presence of Al^{3+} ions having 1:1 complexation stoichiometry. The lowest detection value of **43** was found to be 24 nM for Al^{3+} ions. Chemosensor **43** was successfully applied for detecting Al^{3+} ions in living cells and real samples. The sensing mechanism was found to be due to inhibition of ESIPT and the CHEF effect which was further supported by theoretical calculations.⁶²



Qin and Yang designed and synthesized naphthalene schiff-base **44** as a fluorescent sensor for Zn^{2+} and Cu^{2+} ions. Due to the inhibition of ESIPT sensor **44** showed significant fluorescence enhancement in case of Zn^{2+} ions while in case of Cu^{2+} ions fluorescence was quenched which was due to its paramagnetic nature of Cu^{2+} ions. Sensor **44** was used for constructing INHIBIT logic gate using two metal ions as chemical inputs.⁶³

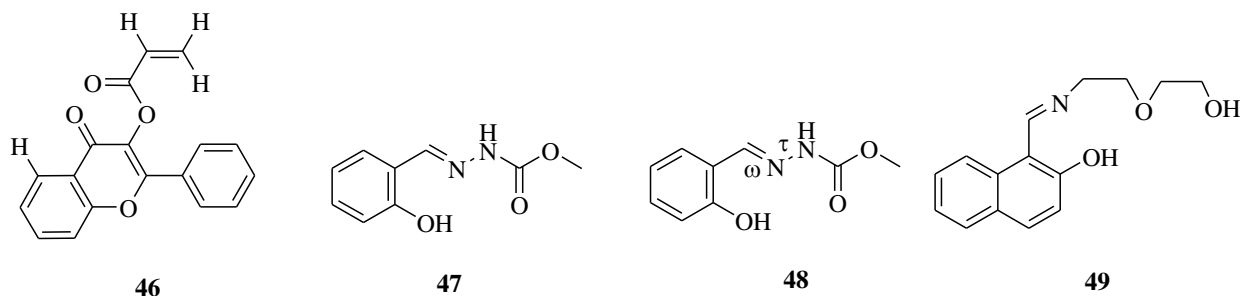


Carbazole-based Schiff-base **45** was used for selective estimation of Fe^{3+} and Cu^{2+} ions by Li *et al.* Job's plot method was used to determine stoichiometry ratio of **45**- Fe^{3+} and **45**- Cu^{2+} which was found to be 2:1 and 1:1 respectively. The detection limit was calculated to be 4.23×10^{-6} mol/L and 5.67×10^{-6} mol/L in the presence of Fe^{3+} and Cu^{2+} ions, respectively. The fluorescent

enhancement in case of $\text{Fe}^{3+}/\text{Cu}^{2+}$ ions was due to the inhibition of $\text{C}=\text{N}$ isomerization and the masked ESIPT of compound **45**.⁶⁴

A fluorescent chemodosimeter **46** having 4-oxo-2-phenyl-4*H*-chromen-3-yl acrylate moiety was synthesized by Pang *et al.* The probe was used for specific detection of cysteine amongst all the other biological thiols. The probe **46** showed very weak blue emission which upon addition of cysteine (Cys), changed to a strong green emission with 20-fold enhancement. The result achieved was attributed to reaction of Cys with **46** that induced acrylate hydrolysis which enabled the ESIPT process to shift the weak blue emission towards strong green emission. These results were correlated *via* ^1H NMR studies.⁶⁵

Pang *et al.* also reported non-fluorescent Schiff base of probe **47** which showed the turn-on blue fluorescence $\lambda_{\text{em}} \approx 475$ nm upon addition of H_2O in aprotic solvent CH_3CN . Various spectroscopic methods like ^1H NMR and dynamic light scattering were used to elucidate the results. Spectroscopic studies recorded at low temperatures revealed that dynamic response of **47** to temperature was due to “on” or “off” excited state intramolecular proton transfer (ESIPT) upon interaction with protic solvent. The probe **47** was used for estimation of Al^{3+} ions.⁶⁶

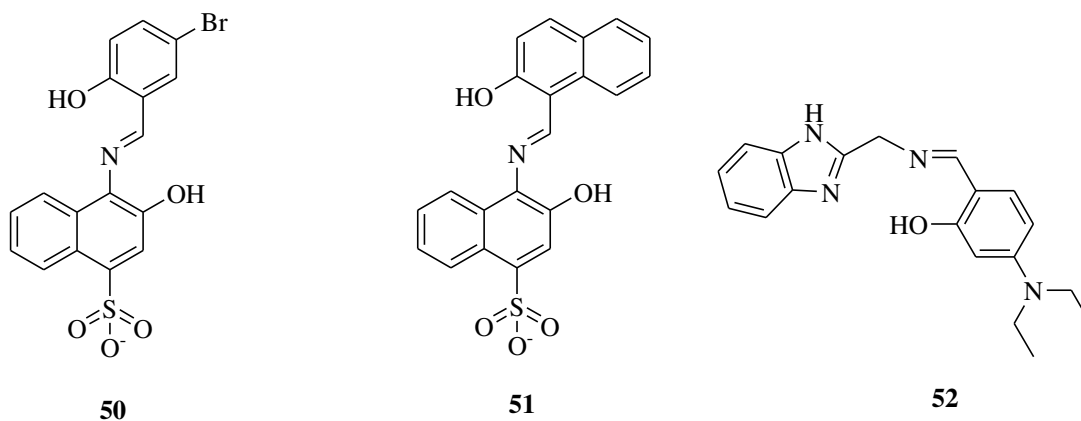


Budzak and Jacquemin synthesized Schiff base probe **48** which was used for detection of Al^{3+} ions. The non-emissive nature of **48** in aprotic solvents was due to the presence of an efficient conical intersection (CI) between the ground-states and the first singlet excited-states which was close to the Franck–Condon geometry; this excited-state pathway leading to the barrierless CI but implies large amplitude motions. They concluded that constraining of the molecule was by impeding the rotation around the imino bond which enabled excited-state intramolecular proton transfer. This same mechanism was used for sensing of Al^{3+} ions *via* turning of fluorescence emission after the formation of complex with Al^{3+} ions and dehydrogenated Schiff base.⁶⁷

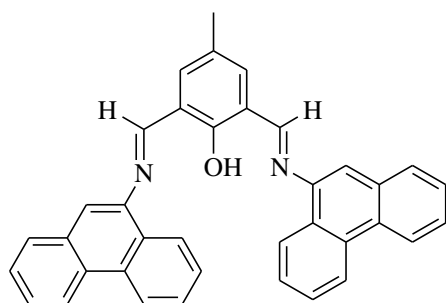
Naphthol-based probe **49** has been prepared by Wu *et al.* which was evaluated for the response to heavy metal ions in case of fluorescence. Probe **49** was used as “off-on” sensor for selective estimation of Zn^{2+} ions at 445 nm, due to the combined effects of chelation-enhanced fluorescence (CHEF) and C=N isomerization.⁶⁸

Upadhyaya *et al.* reported two optical probes **50** and **51** which were used for detection of Al^{3+} ions. The probes **50** and **51** showed emission enhancement upon interaction with Al^{3+} in ethanol and water mixture (4:1, v/v). This binding behavior was confirmed by ^1H NMR, Job's plot analysis and HRMS studies. The switching “on” phenomenon's of **50** and **51** towards Al^{3+} was attributed due to quenching of C=N isomerization, PET and ESIPT phenomenon's which was corroborated with theoretical studies.⁶⁹

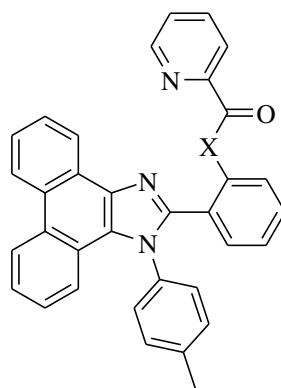
Kim *et al.* developed a colorimetric sensor **52** for $\text{Fe}^{2+/3+}$ as well as fluorometric detection of Zn^{2+} ions. In case of $\text{Fe}^{2+/3+}$ there was color change from colorless to dark green in almost near aqueous solution having low detection limits viz 1.21 μM for Fe^{3+} and 1.18 μM for Fe^{2+} . Probe **52** also sensed Zn^{2+} ions by ‘OFF–ON’ fluorescent response in aqueous solution having detection limit of 1.05 μM . The results were also proved by theoretical approaches.⁷⁰



Bhattacharyya *et al.* designed ESIPT based chemosensor **53** for dual sensing of Hg^{2+} and F^- ions in mixed aqueous medium and acetonitrile medium, respectively. Probe **53** showed emission bands at 466 and 617 nm, in presence of Hg^{2+} ions, emission at 466 nm remained unaffected whereas band at 617 nm slightly red shifted to 635 nm with about six fold fluorescence enhancements. The F^- ions induced the visible color change from pale yellow to pink. The probe **53** was also used for fluorescence imaging of Hg^{2+} ions in human cancer cells.⁷¹



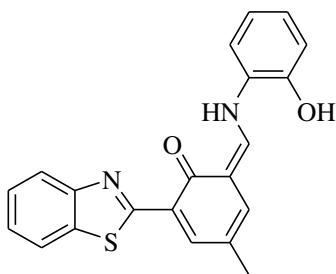
53



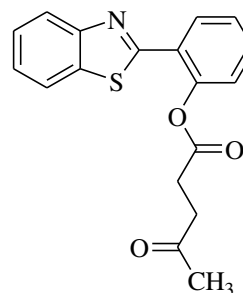
54 : X = NH
55 : X = O

Two probes **54** and **55** were synthesized by Li *et al.*, possessed ESIPT phenomenon's and were used for detection of Cu^{2+} ions. The probe **54** showed the fluorescence turn “off” whereas probe **55** showed turn “on” in the presence of Cu^{2+} ions. The detection limit was calculated as 1.6×10^{-9} M and 1.8×10^{-8} M for probes **54** and **55**, respectively. The recognition mechanisms for Cu^{2+} by probes were studied by Job's plot, NMR as well as mass spectrometry. The probes **54** and **55** were used for imaging Cu^{2+} within living cells with excellent cell permeability.⁷²

A benzothiazole based compound **56** was developed by Tang *et al.* for recognition of CN^- ions in DMSO: H_2O (95:5, v/v, HEPES at 10 mM, pH = 7.4). In the presence of CN^- , probe **56** displayed a blue shift accompanied with fluorescent enhancement along with color change from orange to yellow green. The mechanism of determination of CN^- was a typical nucleophilic addition to enaminone formed by compound **56**.⁷³



56



57

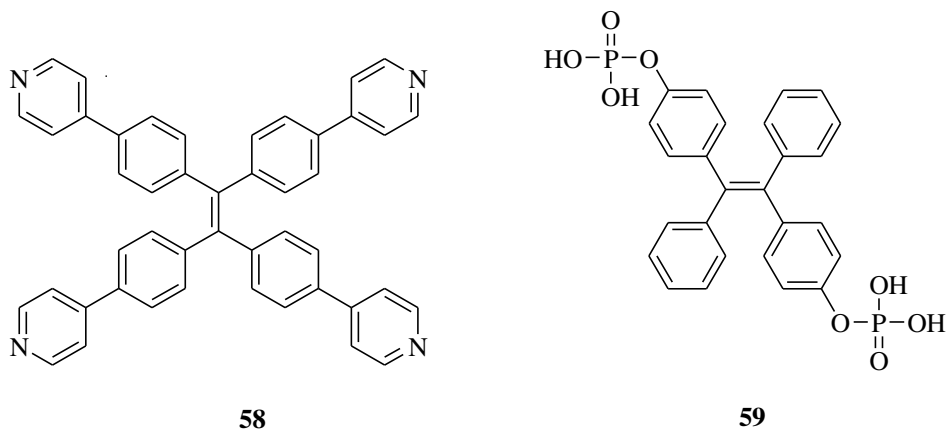
Song *et al.* identified and quantified sulfite anions through levulinate ester based probe **57**. The mechanism was based on the sulfite triggered intramolecular cleavage of levulinate moiety,

which decayed to its ground state through ESIPT mediated pathway. It was found selective for sulfite ions over other anions in nearly aqueous solution.⁷⁴

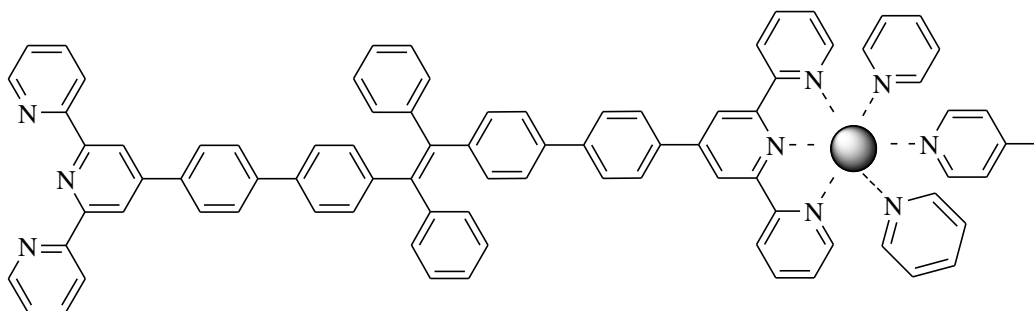
1.3.2 AIE phenomenon's based molecules for sensing

Huang *et al.* has described a probe **58** which showed AIE characteristics. Probe **58** showed switch on emission significantly by the interactions of Hg^{2+} and HSO_4^- ions based on aggregation mechanism with tetraphenylethylene molecules. Among the cations, addition of Hg^{2+} turned on the fluorescence of the molecule.⁷⁵

Liang *et al.* developed a fluorescent light-up probe **59** having AIE characteristics for sensing of alkaline phosphatase. In the presence of alkaline phosphates (ALP) the phosphate groups were cleaved *via* enzymatic hydrolysis which yielded a highly fluorescent product due to activated AIE process. The detection limit was 11.4 pM in tris buffer solution.⁷⁶



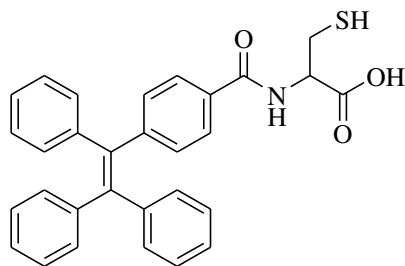
Hong *et al.* developed a fluorogenic Zn^{2+} and chromogenic Fe^{2+} sensor **60** based on aggregation induced emission characteristics. In the presence of Zn^{2+} ions, bathochromic shift of 50 nm in emission spectra was observed along with the associated visible color change from greenish blue to yellow for distinguishing Zn^{2+} ions from other metal ions. Due to the MLCT phenomenon's, TPE displayed magenta color upon binding with Fe^{2+} which allowed identification of Fe^{2+} in the aqueous media *via* naked eyes.⁷⁷



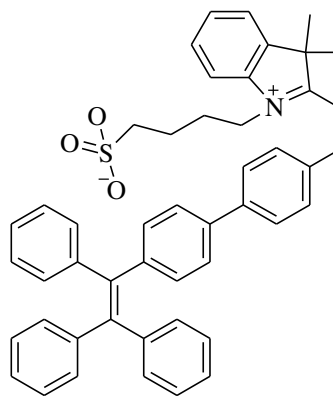
60

Baglan *et al.* reported selective and sensitive fluorescent sensor for arsenite which was based on cysteine fused TPE **61** having AIE characteristics. TPE based compound **61** was used for detection of As^{3+} in aqueous media with selective, label-free and at sub-ppb level of detection.⁷⁸

Chen *et al.* discriminated homocysteine, glutathione and cysteine *via* aggregation-induced emission-active dye **62**. This dye showed selectivity for homocysteine over cysteine and glutathione as well as other amino acids. The two absorption bands at 330 and 440 nm exhibited by compound **62** remained unaffected in the presence of Cys but addition of Hcy caused decrease in absorption intensity at 440 nm and the intensity of peak at 330 nm increased, which was in correlation to results obtained through addition of base.⁷⁹



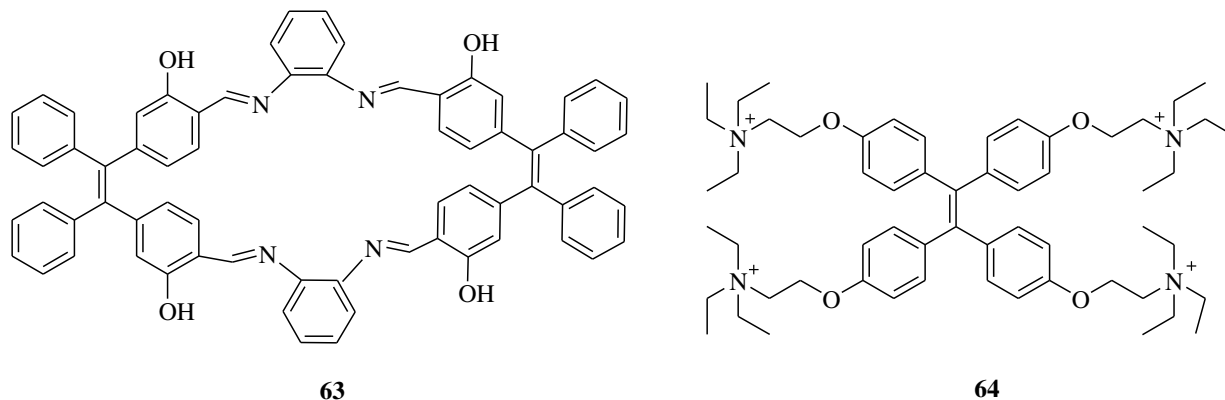
61



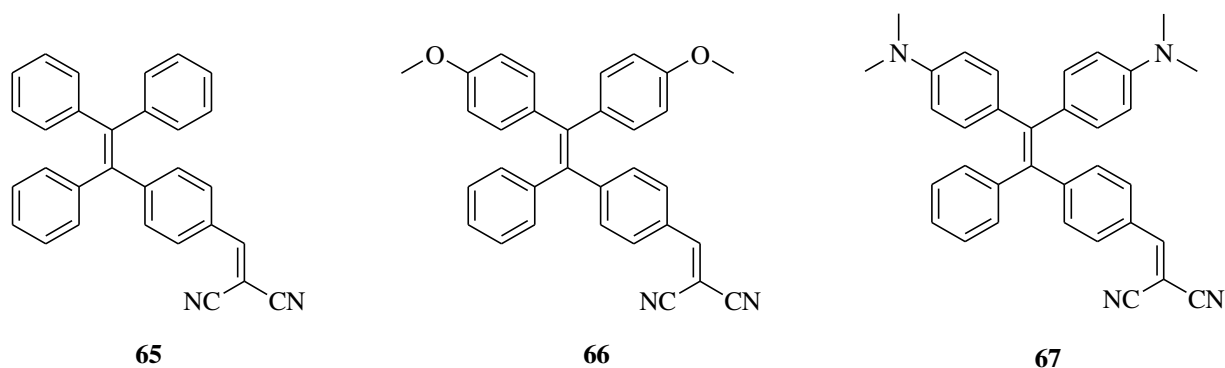
62

Feng *et al.* reported compound **63** for the visual detection of Cu^{2+} in water. A tetraphenyl ethylene Schiff base based aggregation-induced emission was synthesized by using condensation reaction. The macrocycle aggregated into nanofibers forms in aqueous solution, provided stable and fluorescent suspension. The absorption band at 335 nm for compound **63** was gradually decreased but two new absorptions at 450 and 305 nm gradually increased in the presence of Cu^{2+} ions having clear distinct isosbestic points at 381 and 316 nm. Copper ions led to quenching

of emission band at 595 nm for compound **63**, thus showed selective response towards Cu^{2+} ions.⁸⁰

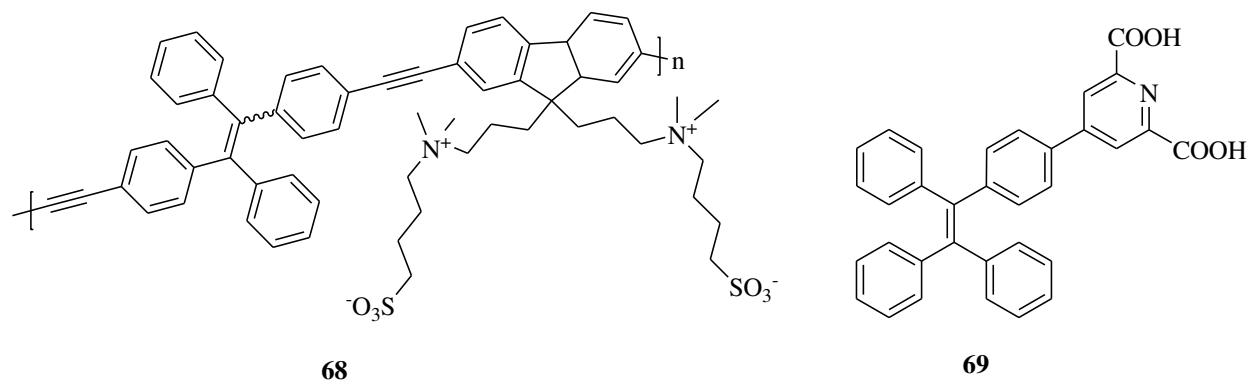


Zhu *et al.* reported tetraphenyl ethylene-based quaternary ammonium salt **64** as a DNA probe via coordination interaction. Due to coordination interactions which do not depend upon the size of the DNA, probe **64** exhibited more sensitivity for the detection of short single standard DNA.⁸¹ Liu *et al.* have synthesized three fluorogens **65-67** as paper-based assay having the application as portability, low cost and ease of operation. Amongst these three, **67** showed best response towards hydrazine having detection limit of 143 ppb and its stained paper strips offered fluorescence color change from dark red to light yellow in the presence of hydrazine. The high sensitivity of **67** was attributed due to strong electron donor sites.⁸²



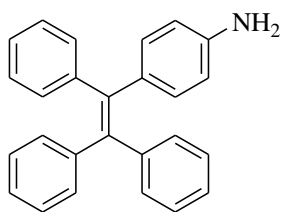
Wang *et al.* used palladium catalyzed Sonogashira reaction for the synthesis of TPE based probe **68**. Probe **68** showed AIE effect as it exhibited weak fluorescence in dilute DMSO solution but showed emission enhancement in case of DMSO/water mixtures. The average diameter of these aggregated particles was around 23 nm. Probe **68** showed an emission band at

505 nm that quenched by addition of Fe^{3+} ions. The probe was also used for detection of Fe^{3+} in A549 cells with low cytotoxicity and high photostability.⁸³

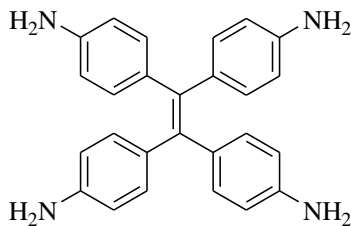


Wang *et al.* synthesized a novel fluorescent sensor **69** having TPE moiety based upon AIE phenomenon's. This probe was used for distinguishing visually Th^{4+} among lanthanides, transition metals as well as alkali metals in UV light even at ppb levels. Upon the addition of Th^{4+} , the emission value of the solution red-shifted to 512 nm and further stepwise addition of Th^{4+} led to significant enhancement in the fluorescence intensity. The fluorescence intensity at 512 nm was increased by a factor of 38 when the concentration of Th^{4+} reached 14 μM (1.4 equiv). The probe **69** was used as sensor in actinide metal recognition for the first time as well as having potential applications in environmental systems having thorium ion.⁸⁴

Banerjee *et al.* developed AIE based probe **70** for detection of trace amounts of nitrite and nitrate ions in water. The solution of probe **70** showed an intense blue fluorescence at λ_{max} 447 nm and upon the addition of nitrite ions, the fluorescence intensity decreased slowly, with almost no change in the absorption maxima. Nitrates were detected by reducing nitrates *in situ* and then making them to react with probe **70**. Probe **70** detected nitrites by “turn-off” fluorescence method by diazotization followed by the formation of non-fluorescent dye. They developed probe having cost effectiveness, high sensitivity, fast detection and low detection limit.⁸⁵



70



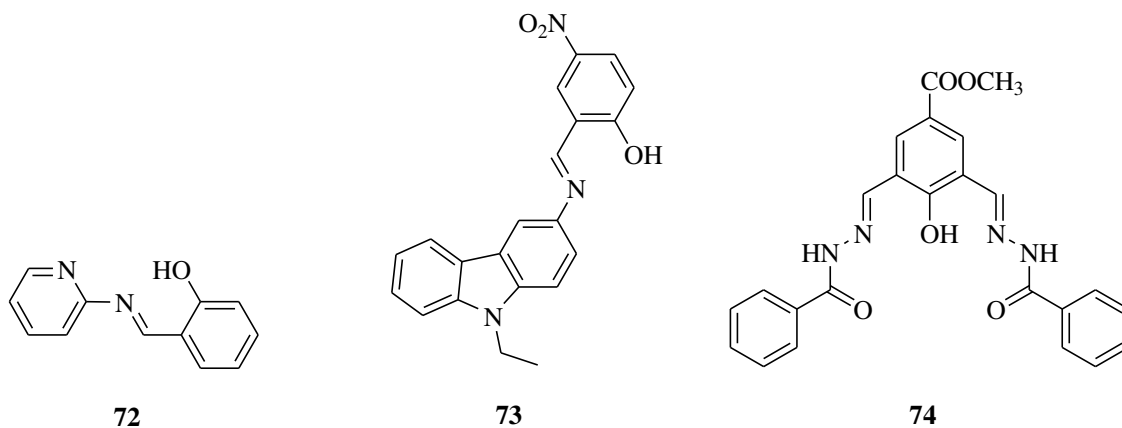
71

Bhosale *et al.* reported AIE active TPE probe **71** with high selectivity of nitrite ions. The probe **71** showed high selectivity towards nitrite ions over other anions with red shift of 30 nm. This probe was used for detection of nitrite due to visible color changes, shift observed in case of absorbance and enhancement of emission.⁸⁶

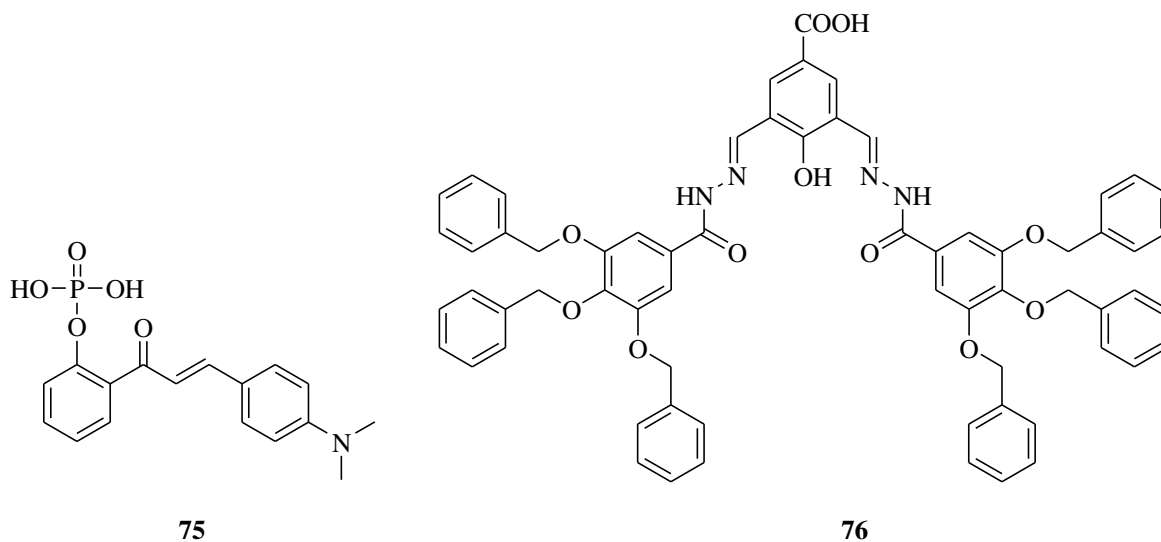
1.3.4 ESIPT coupled AIE molecules for sensing

Laskar *et al.* carried out solid state emission properties for Schiff base molecule **72**. The compound **72** showed bright emission in the solid state and was classified as an AIE active compound. This was classified as J-aggregation mediated AIE property coupled with excited state intramolecular proton transfer (ESIPT). Compound **72** showed mechano-fluorochromic (MFC) property (after grinding changed its emission color yellow to greenish-yellow). Compound **72** showed the selective and sensitive detection of F⁻ ions by turning the fluorescence on signal. It showed the detection limit of 14.0 pM and 1:1 binding stoichiometry with F⁻ ions. All the results were correlated by UV-Vis and ¹H NMR spectroscopy as well as by TD-DFT calculations.²⁰

A carbazole based compound **73** was synthesized by Pradeep *et al.* which exhibited both ESIPT as well as AIE phenomenon. The aggregate of **73** was used for estimation of 2-thiobarbituric acid. Compound **73** showed an emission band at 441 nm which quenched in the presence of 2-thiobarbituric acid. A comparative study was performed by taking barbituric acid, thiophene, urea, carbon disulphide as well as thiourea and found that among all these analytes tested **73** recognized 2-thiobarbituric acid with highest efficiency.²¹



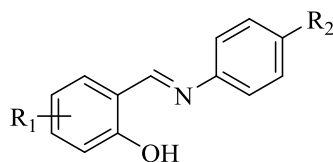
Schiff based chemosensor **74** was designed and synthesized by Yang *et al.* Sensor **74** was used for detecting Zn^{2+} ions on the basis of ESIPT coupled with AIE process. In the absence of Zn^{2+} , the sensor **74** displayed emission at 500 nm. Upon addition of Zn^{2+} to solution of **74**, significant fluorescence enhancements at 457 nm with the detection limit of 10^{-7} M for Zn^{2+} ions was observed.²²



Tang *et al.* developed a fluorescent probe **75** based on phosphorylated chalcone derivative for ALP activity assay and visualization in living cells. Probe **75** emitted greenish-yellow color in aqueous buffer due to the presence of ALP, the emission color changed from deep red gradually, having fluorescent response because of enzymatic product involved in ESIPT phenomenon's and AIE process. ALP measured in range 0-150 mU/mL with detection limit of

0.15 mU/mL. Due to excellent biocompatibility, probe **75** was used for ALP mapping in living cells.²³

Das *et al.* demonstrated compound **76** having ESIPT coupled AIE process. The J-aggregated state of **76** formed a gel in THF having luminescence property which was retained in case of solid state too. The J-type lamellar arrangement among copound **76** was revealed through photophysical studies and XRPD pattern. Finally compound **76** was exploited for affected transistors in case of organic field and for OLED's.²⁴



77	$R_1 = -N(CH_2CH_3)_2$	$R_2 = N(CH_3)_2$
78	$R_1 = -N(CH_2CH_3)_2$	$R_2 = -F$
79	$R_1 = -N(CH_3)_2$	$R_2 = -NO_2$

Qia *et al.* synthesized a series of schiff base derivatives having salicylaldehyde moieties **77-79**. These probes exhibited AIE effect with high fluorescent quantum yields in aggregated state which was based on ESIPT. Different substituent effects were studied through fluorescence as well as by theoretical approaches leading to conclusion that the salicylaldehyde derivatives could be used for Zn^{2+} ion sensing.²⁵

1.4 Materials and Methods

This sub-chapter reports the materials and methods used to synthesize molecules and further their ion recognition studies by using various spectroscopic techniques. Different materials and methods used in chapters 2-4 are described below:

- **General Experimental procedure for the synthesis:** All chemicals were purchased from Loba and Sigma Aldrich Chemical Co and used without further purification. The progress of the reactions were monitored through Thin Layer Chromatography technique. Thin Layer Chromatographic plates were made by using Silica gel GF 254 by using chloroform and methanol as solvents. All reactions were monitored by it and seen under UV light. Different solvent systems were adopted based upon the polarity of the compounds. Melting points were carried out by the open capillary tube method and uncorrected.
- **Characterization of compounds:** All the synthesized compounds were fully characterized by the NMR/Mass spectral techniques. The deuterated solvents were used for the NMR analysis with TMS as an internal standard. ^1H NMR and ^{13}C NMR spectra were carried out using a JEOL ECS-400 MHz spectrometer in SAI Labs, Thapar University, Patiala. . All chemical shifts are reported in ppm relative to the TMS as an internal reference. Mass spectra of the synthesized compounds were recorded at MAT 120 in SAIF, Punjab University, Chandigarh.
- **Techniques used for the photophysical studies:** All the solvents used for the photophysical studies were of analytical /HPLC grade. 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 recorded on Agilent Varian Cary Eclipse fluorescence spectrometer. All absorption and fluorescence scans were saved as ASC II files and further processed in Excel™ to produce all graphs shown. Stock solutions of probes were prepared at 10^{-3} M in distilled solvents. Tetrabutylammonium salts were used for the anions and perchlorate/nitrate salts in case of cations. Solutions were typically 20 μM for UV–Vis studies. The solution was taken in quartz cell and its UV-Vis or fluorescence spectrum was recorded. The addition of different concentrations of ion during titrations was carried out with a micropipette in aliquots of 1.5-3.0 μL in the same cell and each time the solution was allowed to stand for 3 min before recording the UV-Vis or fluorescence spectrum. Stock solutions of probes for NMR titrations were prepared at 5-2 mM in deuterated solvents The

solution was taken in NMR tube and its NMR spectra was recorded. The addition of different concentrations of ion was carried out with a micropipette in aliquots of 3.0-6.0 μL in the same tube and each time the solution was allowed to stand for 3 min before recording the NMR spectra

- **Stability constant calculation:** Stability constants were determined using Benesi–Hildebrand Equation (Eq. (1))

$$\frac{1}{(A - A_0)} = \frac{1}{(A_{\max} - A_0)} + \frac{1}{K[F^-]^n(A_{\max} - A_0)}$$

- **Eq.1:** Benesi-Hilderband Equation

where A_0 , A , $A_{\max}$ are the absorption considered in the absence of ion, at an intermediate, and at a concentration of saturation. K is binding constant; $[F^-]$ is concentration of ion. (n) is the stoichiometric ratio. The association constant (K) could be determined from the slope of the straight line of the plot of $1/(A - A_0)$ against $1/[M^{x+}]$.

- **Detection limit calculation:** The detection limit was calculated based on the titration. To determine the S/N ratio, the emission intensity of the probes was measured 5 times and the standard deviation of blank measurements was determined. The detection limit was then calculated using the equation.

$$\text{Detection limit} = 3\sigma_{bi}/m$$

where σ_{bi} is the standard deviation of blank measurements; m is the slope of intensity versus sample concentration. The detection limit was measured at $S/N = 3$.

- **Fluorescence quantum yield:** The fluorescence quantum yield Φ_f for the probe was determined at room temperature in analytical grade CH_3CN using optically matching solutions of resublimed standard. The quantum yield was calculated by using Eq (2), in which Φ_{fs} is the radiative quantum yield of the sample, Φ_{fr} is the radiative quantum yield of reference, A_s and A_r are the absorbances of the sample and the reference, respectively, D_s and D_r the areas of emission for the sample and reference, L_s and L_r are the lengths of the absorption cells, and N_s and N_r are the refractive indices of the sample and reference solutions (pure solvents were assumed).

$$\Phi_{is} = \Phi_{fr} \times \frac{1 - 10^{-A_{Lr}}}{1 - 10^{-A_{Ls}}} \times \frac{N_s^2}{N_r^2} \times \frac{D_s}{D_r}$$

Eq.2: Quantum yield Equation

- **Job's Plot:** To study the stoichiometry of probes with different analytes, Job's plots were plotted. The absorbance in each case with different *host–guest* ratio but equal in volume was recorded. Job's plot were drawn by plotting $\Delta I \cdot X_{\text{host}}$ 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). The mole fraction (X) of analyte was varied from 0.1 to 1.
- **Test Strip Method:** Aluminium TLC Sheets (precoated with fluorescent silica) were cut into 2×2 cm and coated with different probe solutions. Coated TLC plates were then dried under vacuum. 3 μL solution of different concentration of analyte was added to this coated TLC plates and then observed under UV light.
- **Absorption and emission pH Titrations:** Effect of pH on absorption and emission spectrum of probes and metal/anion complexes were studied by using pH meter. pH of the solutions was adjusted (pH = 2 to 13) using Equiptronics pH meter and the solutions were stirred before recording absorption and emission spectra. Solutions were of typically 10-20 μM for the pH titrations and acidity or basicity was increased by using 0.01 M HCl and 0.01 M NaOH respectively.
- **SEM/TEM/DLS sample preparations:** The SEM images were obtained with a JEOL JSM-7600. The TEM images were obtained with a HITACHI-7500 electron microscope. DLS experiments were performed on a Brookhaven 90 Plus particle size analyzer. Edinburgh FL920 Fluorescence Life Time Spectrometer was used for recording Time Resolved Fluorescence.
- **Theoretical Calculations:** The ground state (S_0) geometry of different probes was optimized using Density Functional Theory (DFT). The functional used was B3LYP and basis set used for all atoms were 6-31G(d,p) and LAN2DZ for cations. The vertical excitation energy and oscillator strengths at the ground state equilibrium geometries were calculated using with the same hybrid functional and basis set. The low-lying first singlet excited state (S_1) of the different probes was relaxed using to obtain its minimum energy geometry.

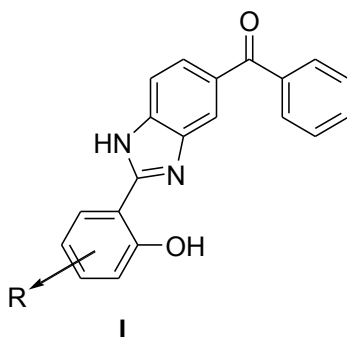
Frequency computations were also carried out on the optimized geometry of the low-lying vibronically relaxed first excited state of the probe with detected ion.

- **Experiments with human blood serum:** Incubated human blood serum was taken with thanks from the local Medical College and used as such. 1.0 ml of the serum was diluted in 10 ml biologically ultrapure water (phosphate buffer, pH = 7.2). The absorption of this solution was recorded. Then serum was treated with certain amount of CN^- solution and then probe solution was added to record absorption spectra.
- **General Procedure for elaboration of logic operations:** UV–Vis studies were carried out on a Specord PC machines using slit width of 1.0 nm and matched quartz cells. Fluorescence spectra were determined on a Varian Cary Eclipse fluorescence spectrometer. Solutions of fluorophores were prepared in doubly distilled acetonitrile. The solution was taken in quartz cell and its UV-Vis or fluorescence spectrum was recorded. The addition of different concentrations of ion was carried out with a micropipette in aliquots of 1.5-3.0 μL in the same cell and each time the solution was allowed to stand for 3 min before recording the UV-Vis or fluorescence spectrum.

CHAPTER 2

Synthesis and photophysical properties of molecules exhibiting ESIPT phenomenon for cations and anions

2.1 General Introduction: In this chapter ESIPT based probes **1-5** have been synthesized and their complexation behaviour towards different anions and cations has been investigated. Hydroxyaryl substituted benzoxazole have been extensively studied in literature, however most of the work has focussed on characterization and their binding behaviour on unsubstituted ESIPT platform. To explore the effect of electron donating and withdrawing on the ESIPT phenomenon's, a series of benzimidazole derivatives have been synthesized with general structure **I** containing bromo, diethyl amino, formyl groups etc. It has been clearly predicted that on changing the substituent from electron donating to electron withdrawing nature; analyte to be detected could be tuned.



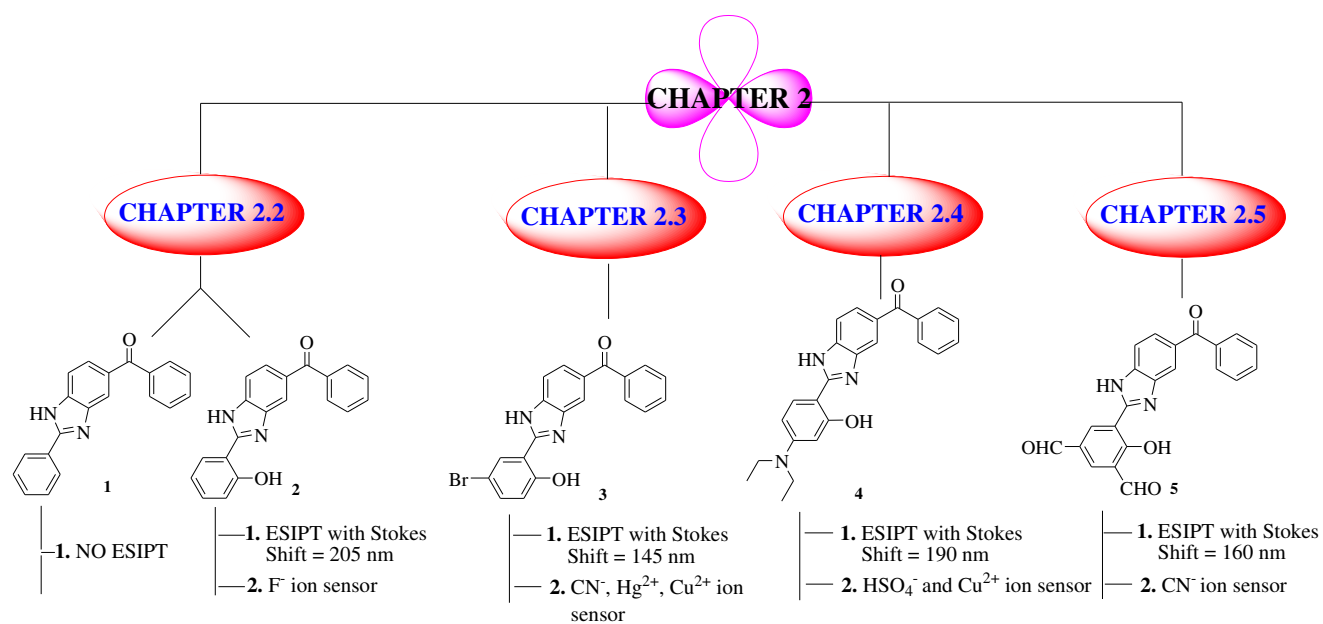
So, depending on the electron donating and withdrawing nature of substituent chapter 2 has been categorized into following also all these results are briefly compiled in flowchart 1.

2.2 Ratiometric fluorescent chemosensor for fluoride ion based on inhibition of excited state intramolecular proton transfer.

2.3 Benzimidazole based ratiometric chemosensor for detection of CN^- and Cu^{2+} ions in protic/aqueous system: Elaboration as XOR logic operation.

2.4 ESICT-ESIPT coupled fluorescent sensor for copper and hydrogen sulphate ions.

2.5 Chromofluorescent probe for estimation of cyanide in human blood serum/plasma like solutions using chemodosimetric approach.



Flowchart 1. Brief results of CHAPTER 2

2.2 Ratiometric fluorescent chemosensor for fluoride ion based on inhibition of excited state intramolecular proton transfer

2.2.1 Abstract

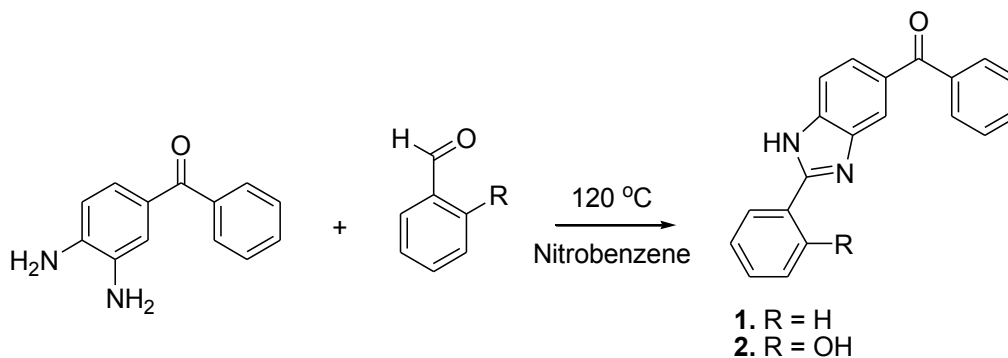
ESIPT phenomenon's based benzimidazole derivatives **1** and **2** have been synthesized and investigated their photophysical behavior towards various anions. Probe **1** was used as control probe for characterization of ESIPT properties of probe **2**. The probe **1** showed a very weak enol emission at 420 nm (as no proton donor sites were present) whereas probe **2** showed the keto emission band at 540 nm due to ESIPT (having proton donor sites). The probe **2** has been used for selective estimation of F⁻ ions as compared to other anions and signaled the binding event through formation of new absorption band at 360 nm and emission band at 420 nm. The probe **2** showed fluorescence behavior towards fluoride ions through hydrogen bonding interactions and restricted the ESIPT phenomenon's from OH to nitrogen of benzimidazole moiety to release its fluorescence at 420 nm. Furthermore, it has been theoretically confirmed that the added fluoride ion inhibited the ESIPT process in probe **2**. Probe **2** was successfully used for estimation of F⁻ ions *via* TLC test strip method.

2.2.2 Synthesis of probes 1 and 2

On refluxing solutions of 3,4-diaminobenzophenone and benzaldehyde in nitrobenzene, gave probe **1** as a colorless solid in 89 % yield; M.Pt. 220–221°C; MS (ESI) m/z 299 (M⁺+H) (scheme 1). ¹H NMR spectrum of probe **1** showed 2H multiplet at δ 8.25, 1H singlet at δ 8.17, 4H multiplet at δ 7.80, 7H multiplet at δ 7.58 for aromatic protons and 1H broad singlet at δ 4.97 due to NH (D₂O exchangeable). ¹³C NMR spectrum showed signals at δ 195.5 (C=O), 137.5, 130.9, 128.7, 127.9, 127.2, 123.1, 120.8, 117.3, 113.2, 110.9 for aromatic-C. This spectral data having peak at 195.5 which was due to C=O, corroborated the structure **1** for this compound.

Similarly the solution of 3,4-diaminobenzophenone and salicylaldehyde in nitrobenzene on refluxing gave probe **2** as a colorless solid in 92 % yield; m.p. 240–241 °C; MS (ESI) m/z 314.11 (M⁺+H) (scheme 1). Its ¹H NMR showed the 1H broad singlet at δ 12.57 due to NH (D₂O exchangeable), 1H broad singlet at δ 9.63 due to OH (D₂O exchangeable), two 1H singlets at δ 8.35 and δ 7.96, 1H doublet at δ 7.80, 4H multiplet at δ 7.59, three 1H triplets at δ 7.26, δ 7.12

and at δ 6.94, 1H multiplet at δ 6.83, 1H singlet at δ 5.77 for aromatic protons. The appearance of broad singlet at δ 12.57 and δ 9.63 due to NH and OH group confirmed respectively the formation of probe **2** over probe **1**. Its ^{13}C NMR spectrum showed signal at δ 195.5 (C=O), 158.3, 137.9, 131.7, 129.3, 127.9, 126.1, 124.8, 124.3, 120.7, 118.7, 117.1, 113.7, 112.1 and 110.9 for aromatic-C. This spectral data having peak at 195.5 which was due to C=O corroborate the structure of (2-(2-hydroxyphenyl)-1*H*-benzo[*d*]imidazol-5-yl)(phenyl)methanone (Probe **2**).



Scheme 1: Synthesis of probes **1-2**

2.2.3 Results and discussion

2.2.3.1 Photophysical properties of probes **1** and **2**

The UV-Vis spectroscopic properties of probes **1** and **2** were investigated in CH_3CN at 20 μM . Probe **1** and **2** displayed an absorption band centered at 320 nm and 335 nm respectively. Addition of different tetrabutylammonium anions viz. CN^- , F^- , Cl^- , Br^- , AcO^- , I^- , HSO_4^- , OH^- , H_2PO_4^- etc caused no significant change except presence of fluoride ions in case of probe **1**. Incremental addition of fluoride ions to probe **1** caused the decrease in absorption intensity at 320 nm and formation of new absorption band at 360 nm with two isobestic points at 275 and 335 nm on addition of 1000 μM solution of F^- ions. Addition of different tetrabutylammonium anions viz. CN^- , F^- , Cl^- , Br^- , AcO^- , I^- , HSO_4^- , OH^- , H_2PO_4^- etc caused no significant change except presence of fluoride and acetate ions in case of probe **2** (Figure 1(a)). Upon incremental addition of fluoride ions, absorption intensity at λ_{max} 335 nm decreased gradually with simultaneous increase in new absorption band at 360 nm with well defined isobestic points at 340 nm and 270 nm (Figure 2(a)). The presence of isobestic points showed that different species are in equilibrium and there is no stepwise formation of complexed species. The binding constant was calculated to be $6.6 \pm 0.02 \times 10^3 \text{ M}^{-1}$ with detection limit of 2 μM . The gradual addition of F^-

caused decrease in absorption intensity at 335 nm and increase at 360 nm provided the opportunity to determine F^- ions ratiometrically. The absorption ratio at these two wavelengths varied from 0.1356 to 1.383, indicating 10.19 fold absorption ratio change. Thus, probe **2** can be used to estimate a wide range of F^- ions between 2-2400 μM through ratiometric approach (Figure 2(b)). But, in case of acetate ions new bands at 272 nm and 350 nm appeared with addition of higher equivalents of acetate ions i.e. 1000 μM .

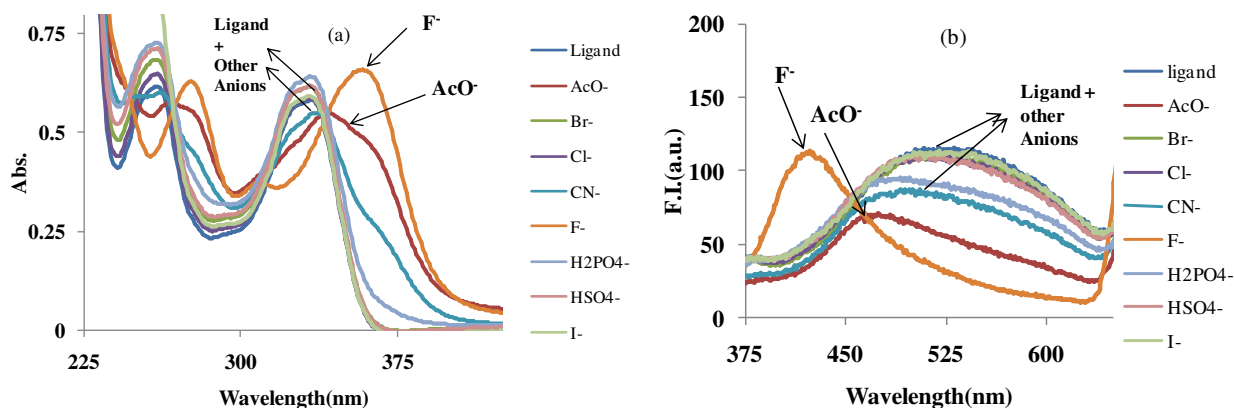


Figure 1: Effect of various anions on (a) absorption spectrum and (b) emission spectrum of probe **2** (20 μM , CH_3CN).

UV-Vis absorption changes of probe **1** in the presence of F^- ions were similar as that of probe **2**, which clearly indicated that both molecules in the ground state caused NH deprotonation which was also supported through theoretical calculations.

The probe **2** (20 μM , CH_3CN) on excitation at 340 nm displayed keto emission band at 540 nm. On addition of various anions like CN^- , F^- , Cl^- , Br^- , AcO^- , I^- , HSO_4^- , OH^- , $H_2PO_4^-$ etc, no significant change was observed except fluoride and acetate ions (Figure 1(b)). Upon incremental addition of fluoride ions, keto emission band at 540 nm was quenched and new emission band at 420 nm was formed. The new emission band at 420 nm was typically due to enol emission because presence of F^- ions caused deprotonation at $-OH$ group which restricted the ESIPT phenomenon's and thus released the enol emission at 420 nm (Figure 3(a)).

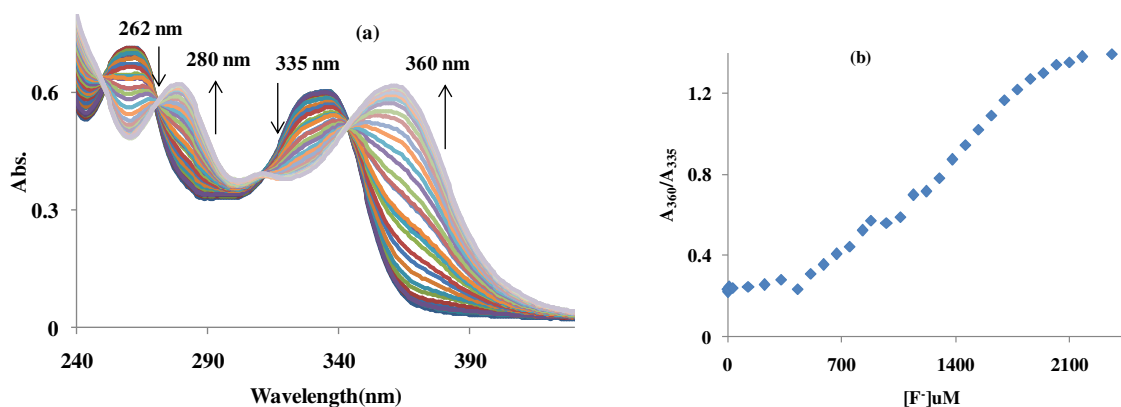


Figure 2: (a) Effect of addition of F^- ions on absorption spectrum of probe **2**; (b) Plot of absorption intensity ratio between 360 and 335 nm (A_{360} / A_{335}) vs $[F^-]$ ions of probe **2** at 20 μM in CH_3CN .

The binding constant was calculated to be $1.148 \pm 0.02 \times 10^4 M^{-1}$ with lowest detection limit of 2 μM . But in case of acetate ions new band at 420 nm appeared with addition of higher equivalents of acetate ions i.e. 1000 μM . The gradual addition of F^- ions caused decrease in emission intensity of keto tautomer at 540 nm and increase in emission intensity of enol tautomer at 420 nm and thus provided the opportunity to determine F^- ions ratiometrically. The emission ratio at these two wavelengths varied from 0.376 to 4.058, indicating 10.86 fold emission ratio changes. Thus, probe **2** can be used to estimate a wide range of F^- ions between 2-75 μM through ratiometric approach (Figure 3(b))

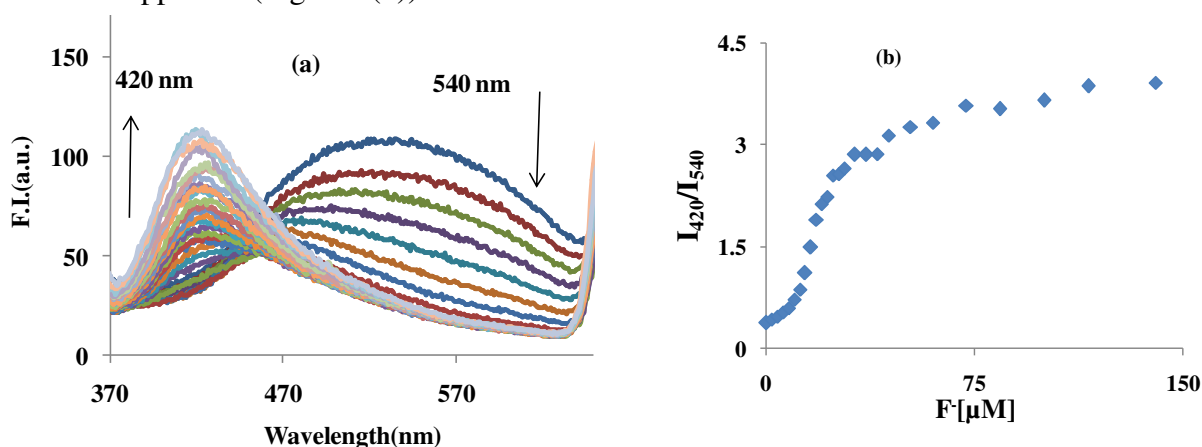


Figure 3: (a) Fluorescence spectrum of probe **2** (20 μM , $\lambda_{ex} = 340$ nm) in CH_3CN upon complexation with increasing concentration of F^- ions. (b) Plot of emission intensity ratio between 420 and 540 nm (I_{420} / I_{540}) vs $[F^-]$ ions of probe **2** at 20 μM in CH_3CN .

In case of probe **1**, absence of hydroxyl group led to exhibition of very weak enol emission at 420 nm with lesser Stokes shift of ~ 90 nm than probe **2** of ~ 200 nm at higher concentration. These studies clearly predicted that -OH participates in ESIPT phenomenon's.

2.2.3.2 Interference studies of probe 2

Addition of other anions (100 equiv.) such as Cl^- , Br^- , AcO^- , I^- , HSO_4^- , OH^- , H_2PO_4^- , no significant change in fluorescence intensity was observed by comparison with and without other anions. To determine the practical applicability of probe **2**, we carried out competitive experiments in the presence of 10 equiv. of F^- ions mixed with 50 equiv. of each of the various metal ions. The complex formed in the presence of acetate ions is very weak as compared to fluoride complex. Whereas, no significant variations in the fluorescence intensity were found when comparing the results with or without the other anions (Figure 4).

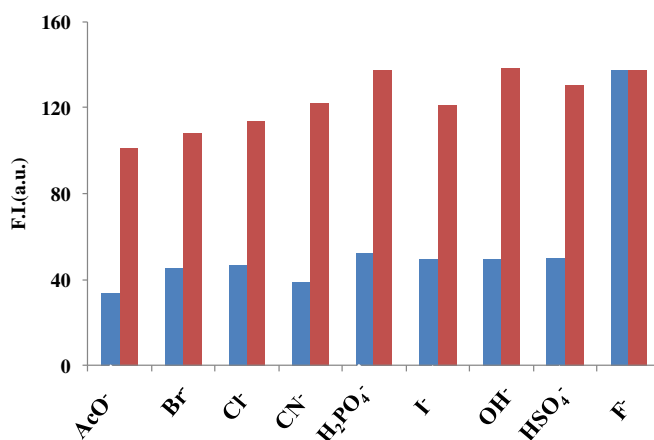


Figure 4: Red bars represent selectivity of probe **2** (20 μM , $\lambda_{\text{ex}} = 340 \text{ nm}$) upon addition of different metal ions in CH_3CN and blue bars show the competitive selectivity of probe **2** in the presence of interfering metal ions

2.2.3.3 Practical application of probe 2 by dip coating technique

In order to check the practical applicability of probe **2**, TLC strips were prepared by dip-coating a solution of probe **2**, followed by drying them in a vacuum in order to check for residual contamination in contact mode. We prepared several samples of solution-coated TLC strips and studied the response of their fluorescence towards F^- ions in contact mode and solution phase. Solution of F^- ions were placed over coated TLC strips for 5 sec. Upon illumination with a UV

lamp, blue spots were observed in the contact area (Figure 5). The minimum amount of F^- ions detectable by the naked eye was as low as, thereby registering a detection limit at 10^{-6} M.

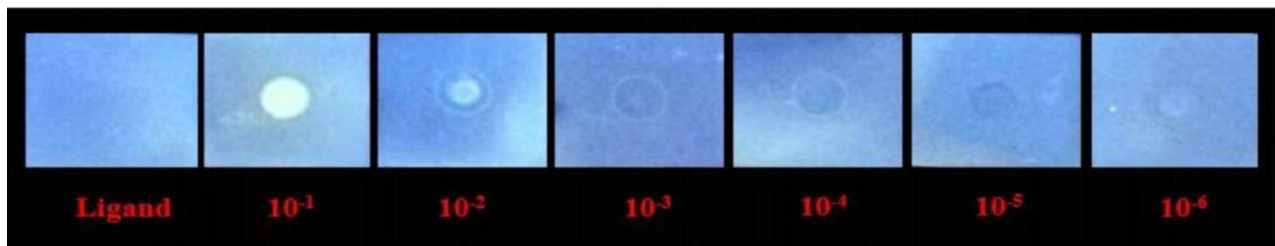


Figure 5: Photographs of probe **2** coated TLC strips under different experimental conditions.

2.2.4 Theoretical studies

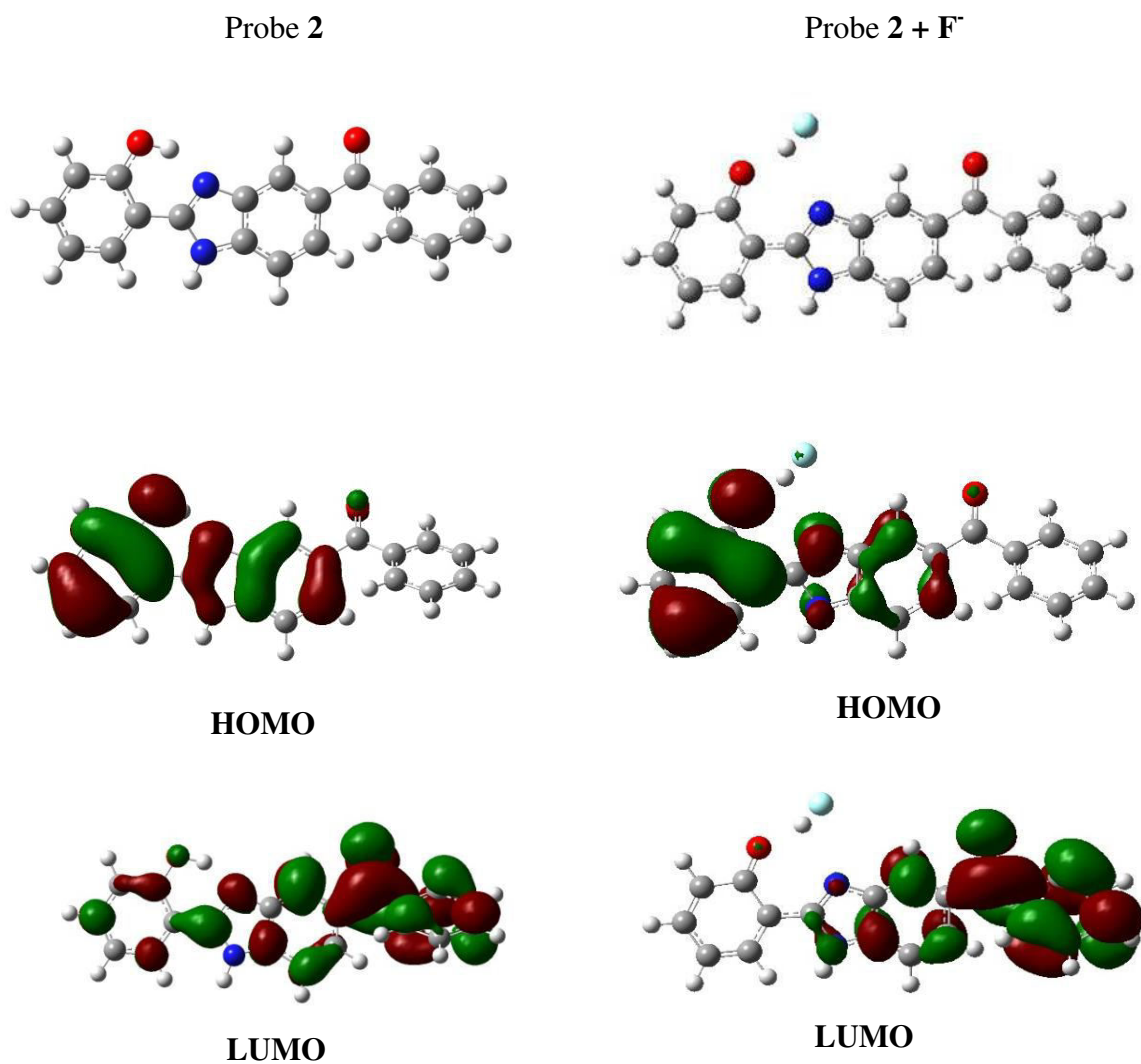
To explore the origin of each induced absorption band, theoretical calculation were employed to optimize the structure through DFT and TD calculation with B3LYP-631G methods. The probe **2** was optimized using DFT calculations. It was found that O-H bond acquires partial double bond character and hydrogen of O-H orient itself to the closer vicinity to imine nitrogen of benzimidazole moiety (Figure 6). The presence of F^- ions caused deprotonation at hydroxyl group and acquired keto form.

Table 1: Excitations for probe **2** that contribute to the transitions in the 250-700 nm and their expansion coefficients in gaseous phase

	λ_{max} (nm) Experimental	λ_{max} (nm) Theoretical	E_{exc} (eV)	Oscillator Strength	Excited State	Expansion Coefficient
Probe 2	335	330	4.0026	0.2424	H to L+1	0.63898
	262	280	4.3661	0.3049	H-3 to L	0.52184
Probe 2 + F^-	360	360	3.1007	0.2974	H to L+2	0.65909
	280	280	4.5082	0.1449	H-2 to L+2	0.60937

The calculation of probe **2**, showed that band at 330 nm was due to HOMO to LUMO + 1, which has oscillating strength 0.2424 and excitation energy 4.0026 eV. The HOMO of probe **2** locates mainly on benzimidazole ring and LUMO mainly on benzophenone moiety but for LUMO + 1 it exhibit an expanding π -nature orbital covering both benzimidazole and benzophenone moieties.

The other bands at 260 and 280 nm were due to HOMO - 1 to LUMO + 1 and HOMO - 3 to LUMO respectively (Table 1). In case of probe **2.F⁻** band at 360 nm was due to HOMO to LUMO + 2 transitions, which has oscillating strength 0.2974 and excitation energy 3.1007 eV. The HOMO of probe **2.F⁻** locates mainly on benzimidazole ring and LUMO mainly on benzophenone moiety but for LUMO + 2, it exhibit an expanding π -nature orbital covering both benzimidazole and benzophenone moiety. The other bands at 280 and 305 nm were due to HOMO - 2 to LUMO + 2 and HOMO - 3 to LUMO, respectively (Table 1).



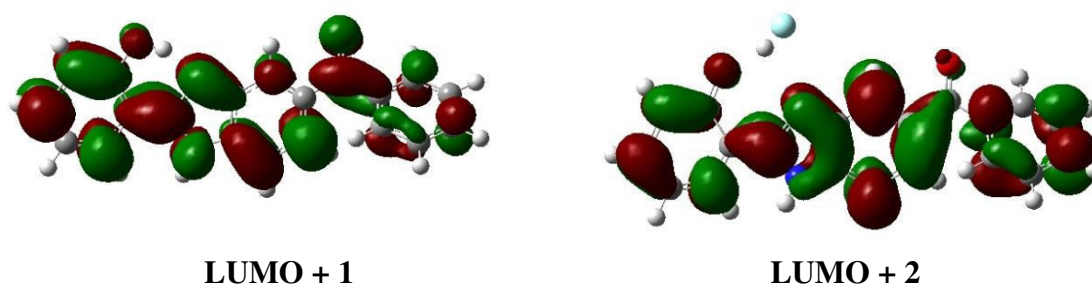


Figure 6: Optimized structures and HOMO and LUMO of probe **2**.

2.2.5 Conclusions

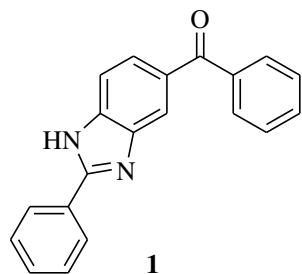
Hydroxyaryl benzimidazole derivative has been synthesized for selective sensing of fluoride ions. Probe **2** showed ratiometric absorption and emission behavior towards F^- ions to sense F^- ion between a broad range of 2–2400 μM . The presence of fluoride ions caused inhibition of keto tautomer and release of enol emission. The probe **2** showed practical applicability to sense fluoride ions at micromolar level through dip coating method.

2.2.6 Experimental section

2.2.6.1 General procedure for synthesis of probes **1** and **2**

Synthesis of probe 1: 3,4-diaminobenzophenone (500 mg, 2.3 mmol) and benzaldehyde (374 mg, 3.5 mmol) were dissolved in 10 mL nitrobenzene. The reaction mixture was refluxed for about 24 h, and then cooled to room temperature. The solid separated was filtered by using sintered crucible with the help of vacuum filtration method and washed with diethyl ether to obtain colorless solid of probe **1** (225 mg).

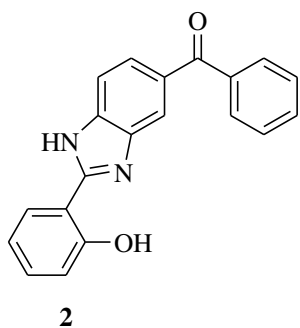
Phenyl(2-phenyl-1*H*-benzo[d]imidazol-5-yl)methanone (Probe **1**)



89% yield; colorless solid; M.pt. 220–221 °C; ^1H NMR ($CDCl_3$ + $DMSO-d_6$, 400 MHz): δ 8.26–8.24 (m, 2H, Ar), 8.17 (s, 1H, Ar), 7.85–7.76 (m, 4H, Ar), 7.66–7.48 (m, 7H, Ar), 4.97 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$ + $DMSO-d_6$, 100 MHz): δ 195.5 (C=O), 137.5, 130.9, 128.7, 127.9, 127.2, 123.1, 120.8, 117.3, 113.2, 110.9 (ArH); MS (ESI) m/z 299.11 ($M^+ + H$).

Synthesis of probe 2 – 3,4 Diaminobenzophenone (1 g, 4.7 mmol) and salicylaldehyde (630 mg, 5.1 mmol) were dissolved in 30 mL nitrobenzene. The reaction mixture was refluxed for about 24 h, and then cooled to room temperature. The solid separated was filtered and washed with diethyl ether to obtain yellow colored solid which was further purified by column chromatography to get pure probe **2** (528 mg).

(2-(2-hydroxyphenyl)-1*H*-benzo[*d*]imidazol-5-yl)(phenyl)methanone (Probe 2)



92% yield; yellow colored solid; Mpt. 240–241 °C; ¹H NMR (CDCl₃ + DMSO-*d*₆, 400 MHz): δ 12.57 (bs, 1H, NH), 9.63 (bs, 1H, OH), 8.35 (s, 1H, ArH), 7.96 (s, 1H, ArH), 7.88 (d, 1H, *J* = 8.28 Hz, ArH), 7.61–7.57 (m, 4H, ArH), 7.29–7.24 (t, 1H, *J* = 9.16 Hz, ArH), 7.14–7.10 (t, 1H, *J* = 11.0 Hz, ArH), 7.00–6.96 (t, 1H, *J* = 7.36 Hz, ArH), 6.86–6.79 (m, 1H, ArH), 5.77 (s, 1H, ArH); ¹³C NMR (CDCl₃ + DMSO-*d*₆, 100 MHz): δ 195.5 (C=O), 158.3, 137.9, 131.7, 129.3, 127.9, 126.1, 124.8, 124.3, 120.7, 118.7, 117.1, 113.7, 112.1, 110.9 (ArH); MS (ESI) *m/z* 314.11 (*M*⁺+H).

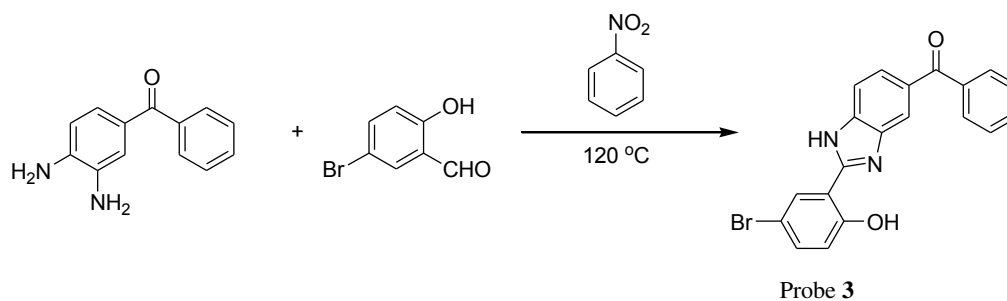
2.3 Benzimidazole based ratiometric chemosensor for detection of CN^- and Cu^{2+} ions in protic/aqueous system: Elaboration as XOR logic operation

2.3.1 Abstract

ESIPT based benzimidazole derivative probe **3** with electron withdrawing group has been synthesized and investigated their photophysical behaviour towards various anions. The main objective for the synthesis of probe **3** was to study the effect of electron withdrawing group on ESIPT phenomenon's. It was found that in the presence of electron withdrawing group at para position to the proton donor sites led to decrease in the value of Stokes shift when compared to that of probe **2** (having no group present at para position of proton donor). The probe **3** has been used for selective estimation of CN^- and signaled the binding event through formation of new absorption band at 380 nm and emission band at 430 nm with CN^- ions. The addition of various metal ions to probe **1** in aqueous system showed selectivity towards Cu^{2+} and Hg^{2+} ions as compared to other metal ions, and signal the binding event through formation of new absorption band at 375 nm and decrease in absorption intensity, respectively. Thus, probe **3** behaved as chemosensor for CN^- ions which restricts the ESIPT emission at 485 nm due to H-bonding interactions between O-H to nitrogen of benzimidazole moiety to release its enol emission. The chemical inputs of CN^- and Cu^{2+} ions to probe **3** mimicked a sequence dependent operation of an exclusive-OR (XOR) logic gate. The binding behavior of probe **3** towards CN^- and Cu^{2+} has also been confirmed through DFT calculations.

2.3.2 Synthesis of probe **3**

Solution of 3,4-Diaminobenzophenone and 5-Bromo-salicylaldehyde in nitrobenzene on refluxing gave probe **3** as a yellow colored solid in 92 % yield; m.p. 220–221 °C; MS (ESI) m/z 394.22 ($\text{M}^+ + \text{H}$) (Scheme 2). ^1H NMR spectrum showed the 1H doublet at δ 8.31, 1H singlet at δ 8.03, 4H multiplet at δ 7.78, 1H triplet at δ 7.67, 2H triplet at δ 7.54, 1H double doublet at δ 7.50 and 1H doublet at δ 7.02 for aromatic protons. ^{13}C NMR spectrum showed signals at δ 193.7 (C=O), 155.6, 136.2, 132.7, 130.4, 127.8, 127.2, 126.6, 117.8, 112.7, 108.6 for aromatic -C. This spectral data having peak at 193.7 which is due to C=O corroborate the structure **3** for this compound. On the basis of these spectral data, this compound has been assigned the structure of (2-(5-Bromo-2-hydroxyphenyl)-1*H*-benzo[*d*]imidazol-5-yl)(phenyl)methanone (Probe **3**).



Scheme 2: Synthesis of probe **3**

2.3.3 Results and discussion

2.3.3.1 Effect of anions on absorption spectrum of probe **3**

The sensing properties of probe **3** was examined in CH₃OH through the addition of tetrabutylammonium salt of various anions, including F⁻, Cl⁻, Br⁻, I⁻, SCN⁻, AcO⁻, NO₃⁻, H₂PO₄⁻, HSO₄⁻ and CN⁻ (Figure 7a). Upon the addition of 1000 μM of various anions, the absorption spectra of probe **3** did not show any significant change except CN⁻ ions. Nevertheless, in the presence of CN⁻ ion, showed the intensity decreased with emergence of a new absorption band at 380 nm. Upon incremental addition of CN⁻ ions to the solution of probe **3**, the absorption intensity at 340 nm decreased and a new absorption band appeared at 380 nm with addition of 1000 μM of CN⁻ ions (Figure 7b). A gradual spectral change has been shown in Figure 7b, where clear isobestic point was observed at 360 nm, indicating a clean transformation to a new species. The binding constant was calculated to be $0.45 \pm 0.02 \times 10^3 \text{ M}^{-1}$ with lowest detection value of 1 μM. The gradual decrease in absorption intensity at 340 nm and increase at 380 nm provided the opportunity to determine CN⁻ ions ratiometrically. The absorption ratio at 340 and 380 nm varied from 0.087 to 1.410, indicating 16.20 fold absorption ratio change. Thus, probe **3** can be used to estimate a wide range of CN⁻ ions between 1-1000 μM through ratiometric approach.

2.3.3.2 Effect of anions on emission spectrum of probe **3** On excitation of probe **3** (20 μM, CH₃OH) at 340 nm, the emission band at 485 nm was observed typically due to ESIPT phenomenon. Upon the addition of F⁻, Cl⁻, Br⁻, I⁻, SCN⁻, AcO⁻, NO₃⁻, H₂PO₄⁻ and HSO₄⁻, no substantial change was observed in the emission spectra of probe **3**. The only significant

response appeared in the presence of CN^- ions only (Figure 8a), which led to release of new emission band at 430 nm (ENOL emission) (Figure 8b).

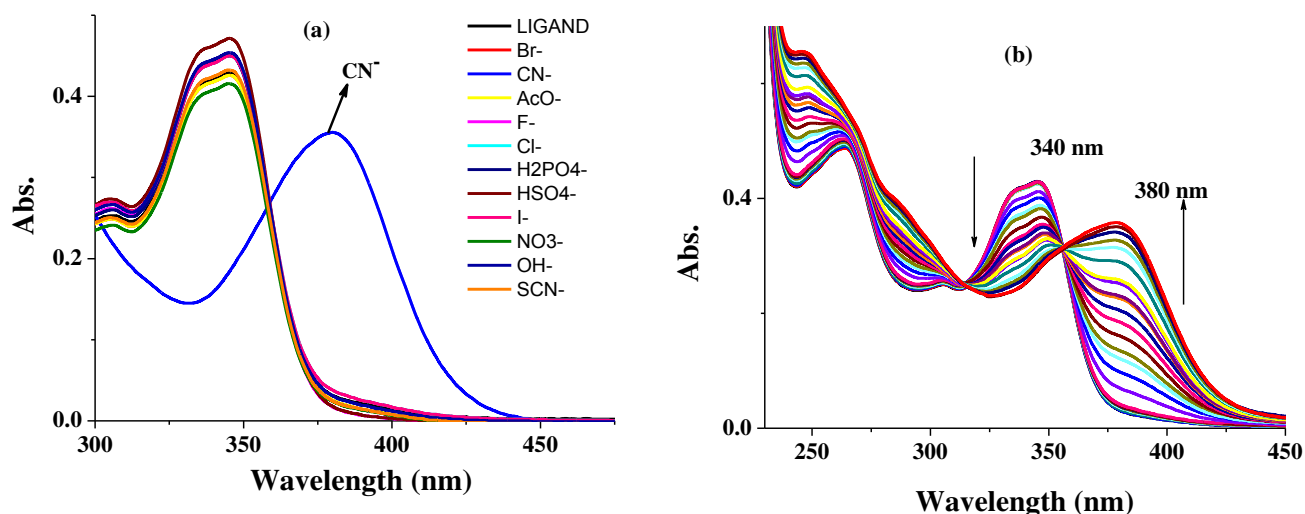


Figure 7: (a) Effect of addition various anions (1000 μM); (b) effect of incremental addition of CN^- ions on absorption spectrum of probe 3 (20 μM , CH_3OH).

The binding constant was calculated to be $7.44 \pm 0.01 \times 10^3 \text{ M}^{-1}$ with lowest detection limit of 2 μM , which is quite close to the maximum permissible level in drinking water (1.9 μM).

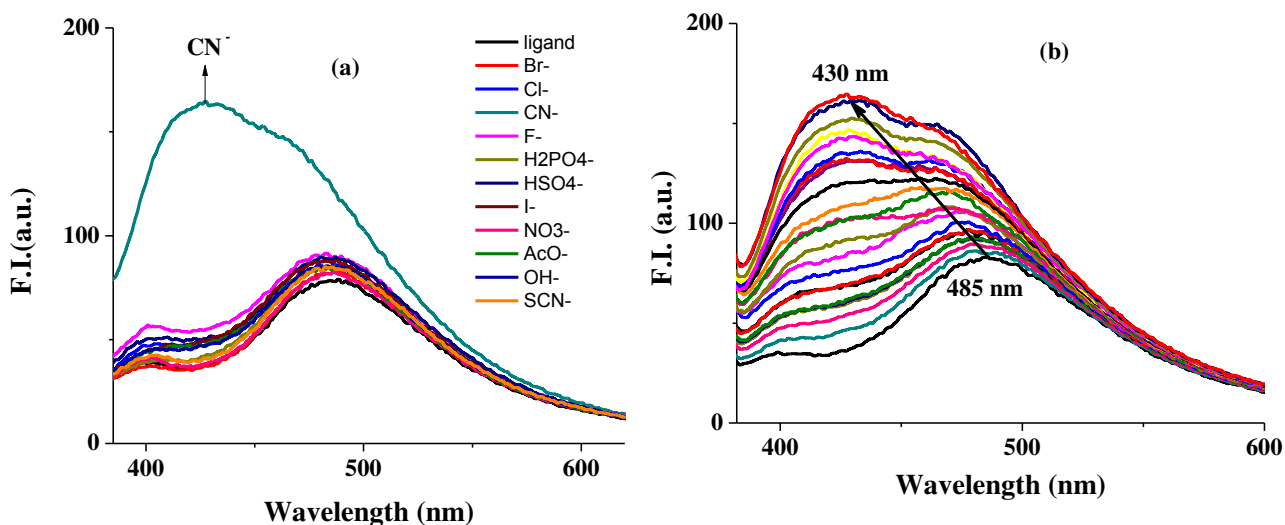


Figure 8: (a) Effect of various anions (1000 μM); (b) Effect of incremental addition of CN^- ions on emission spectra of probe 3 (20 μM , CH_3OH).

To further explore the utility of probe 3 as an ion-selective chemosensor for CN^- , competitive experiments were performed with 1000 μM of CN^- and 1000 μM of various other anions (F^- ,

Cl^- , Br^- , OH^- , I^- , SCN^- , AcO^- , NO_3^- , H_2PO_4^- and HSO_4^-) in a solution of probe **3** in CH_3OH (Figure 9). The absorption and emission spectra of probe **3** with CN^- were not influenced by the subsequent addition of competing anions. Thus, probe **3** can be used for the selective estimation of CN^- ions.

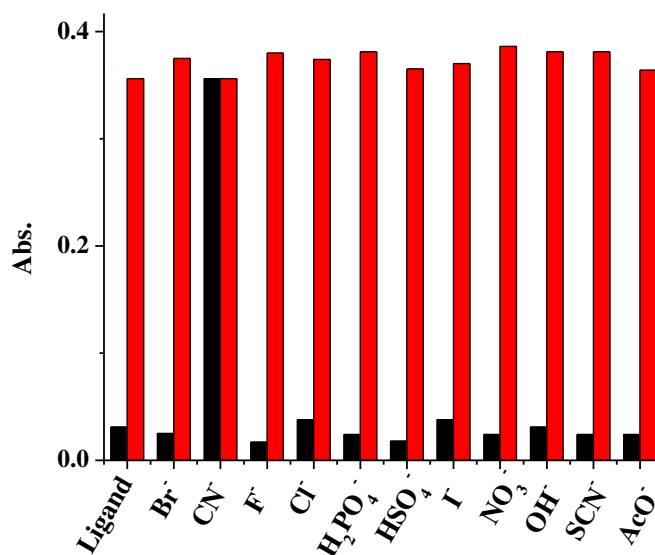


Figure 9: Black bars represent selectivity of probe **3** (20 μM , CH_3OH) upon addition of different anions and red bars shows the competitive selectivity of probe **3** in the presence of CN^- ions.

2.3.3.3 Effect of metal ions on absorption spectrum of probe **3**.

The sensing properties of probe **3** towards various metal ions were examined in $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ solutions (1:1 [v/v], containing 0.01 M HEPES, pH 7.2) through the addition of perchlorate salt of various cations, including Na^+ , K^+ , Ag^+ , Mg^{2+} , Ca^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Hg^{2+} , Pb^{2+} , Al^{3+} and Fe^{3+} (Figure 10a). The probe **3** ($\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1 [v/v], 0.01 M HEPES, pH 7.2) showed the λ_{max} at same position as observed in methanol solvent i.e. at 340 nm. Upon the addition of 200 μM of various metal ions, the absorption spectrum of probe **3** did not show any significant change except Cu^{2+} and Hg^{2+} ions. In the presence of Cu^{2+} ions, absorption intensity at 340 nm diminished with the concomitant increase in absorption intensity at 374 nm. However addition of Hg^{2+} ions absorption intensity at 265 nm and 340 nm decreased. Upon incremental addition of Cu^{2+} ions to the solution of probe **3**, the absorption intensity at 340 nm decreased and a new absorption band appeared at 375 nm. The incremental addition of Cu^{2+} induced a clear isobestic

point at 360 nm which clearly indicated for the formation of new species (Figure 10b). The binding constant was calculated to be $3.28 \pm 0.02 \times 10^6 \text{ M}^{-1}$ with lowest detection of $1 \mu\text{M}$. The decrease in absorption intensity at 340 nm and increase at 375 nm, in the presence of Cu^{2+} provided the opportunity to determine Cu^{2+} ions ratiometrically. The absorption ratio at 340 and 380 nm varied from 0.205 to 1.284, indicating 6.26 fold absorption ratio change. Thus, probe **3** can be used to estimate a wide range of Cu^{2+} ions between 1-20 μM through ratiometric approach.

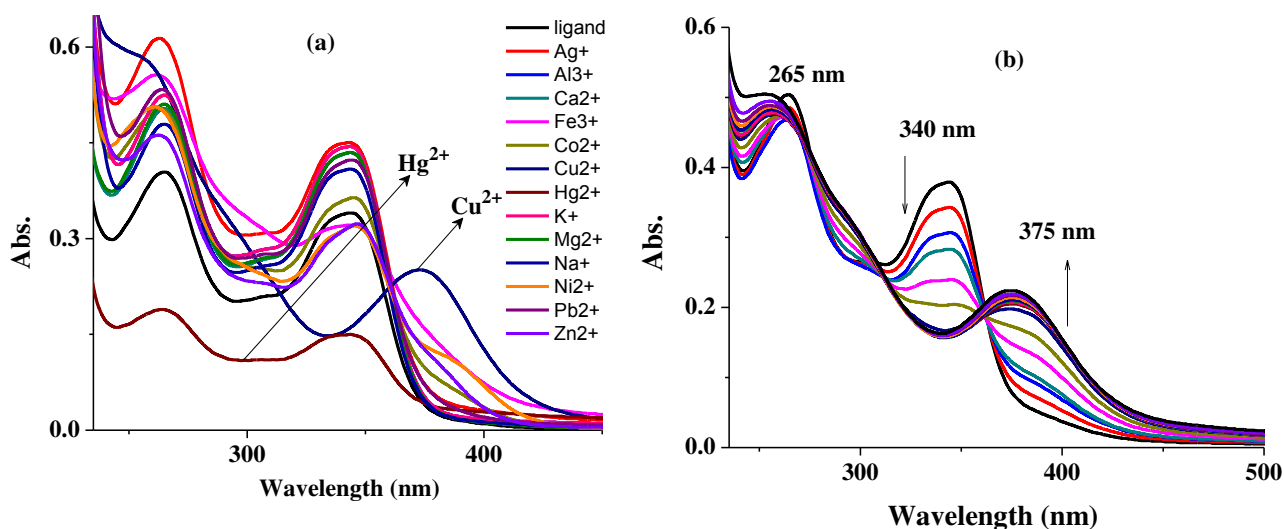


Figure 10: (a) Effect of various metal ions (200 μM); (b) addition of Cu^{2+} ions on absorption spectrum of probe **3** at 20 μM in $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ solutions (1:1 [v/v], containing 0.01 M HEPES, pH = 7.2).

Upon addition of Hg^{2+} ions to the solution of probe **3**, absorption intensity decreased and hence colour of solution bleached out (Figure 11a). The binding constant was calculated to be $1.06 \pm 0.02 \times 10^5 \text{ M}^{-1}$ with lowest detection of $1 \mu\text{M}$.

Interference studies for Cu^{2+} ions were performed in the presence of other metal ions and found there was no influence by the subsequent addition of competent metal ions was observed (Figure 12). Same concentration of Cu^{2+} and various other metal ions (Na^+ , K^+ , Ag^+ , Mg^{2+} , Ca^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Hg^{2+} , Pb^{2+} , Al^{3+} and Fe^{3+}) was taken with solution of probe **3** in $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ solutions (1:1 [v/v], containing 0.01 M HEPES, pH = 7.2).

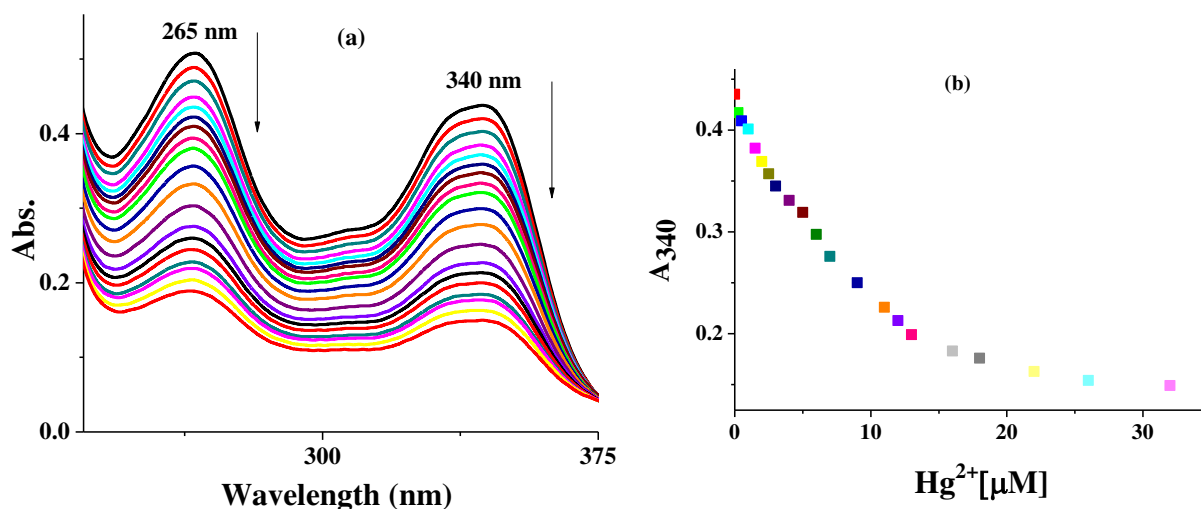


Figure 11: (a) Effect of addition of Hg^{2+} ions on absorption spectrum (b) absorption intensity at 340 nm (A_{340}) vs $[\text{Hg}^{2+}]$ ions of probe **3** at 20 μM in $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ solutions (1:1 [v/v], containing 0.01 M HEPES, pH = 7.2).

The absorption spectrum of probe **3** with Cu^{2+} at 375 nm was not influenced by the subsequent addition of competing metal ions (Figure 12). Thus, probe **3** can be used for the selective estimation of Cu^{2+} ions.

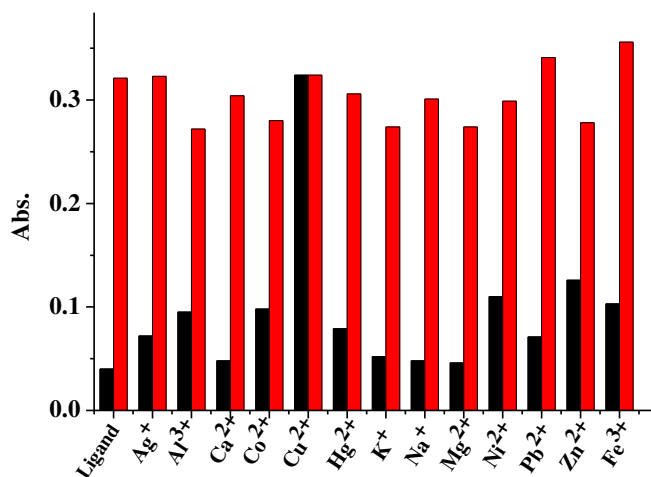


Figure 12. Black bars represent selectivity of probe **3** (20 μM) upon addition of different metal ions in $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ solutions (1:1 [v/v], containing 0.01 M HEPES, pH = 7.2) and red bars show the competitive selectivity of probe **3** in the presence of Cu^{2+} at 375 nm.

It is very significant from absorption studies that, probe **3** can be used for simultaneous analysis of Cu^{2+} and Hg^{2+} ions. The presence of Cu^{2+} ions induced the formation of new absorption band at 375 nm, whereas Hg^{2+} ions induced the decrease in absorption intensity at

340 nm. Thus, from the absorption signal one can get the information about presence of Hg^{2+} and Cu^{2+} ions in the solution (Figure 13).

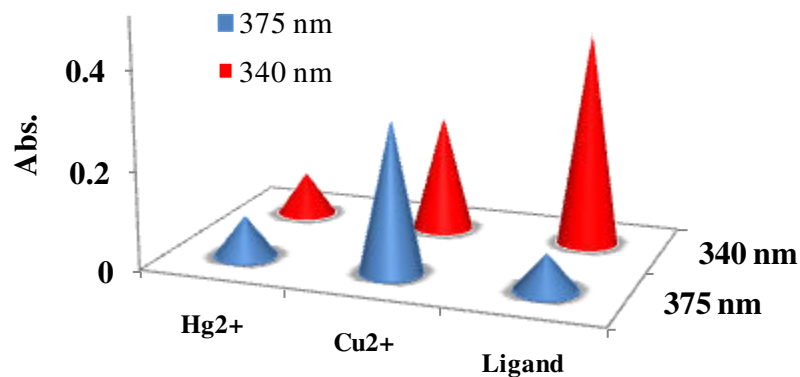


Figure 13: Effect of Cu^{2+} ions and Hg^{2+} ion on absorption spectra of probe **3** in $\text{CH}_3\text{OH}:\text{H}_2\text{O} :: 1:1$ (0.01 M HEPES, pH = 7.2) at 375 nm and 340 nm.

To know the binding properties of probe **3** with Cu^{2+} and Hg^{2+} job's plot analysis was performed. Stock solution of same concentration of probe **3** and respective ion was prepared in the order of 2.0×10^{-5} M in $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ solutions (1:1 [v/v], containing 0.01 M HEPES, pH = 7.2). The absorbance in each case with different *host-guest* ratio but equal in volume was recorded. Job's plot were drawn by plotting $\Delta I \cdot X_{\text{host}}$ 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). In case of Cu^{2+} and Hg^{2+} ions we found 2:1 stoichiometry i.e. two ligand molecules binds with one metal ion. To further examine the binding mode of probe **3** with Cu^{2+} and Hg^{2+} ions, a positive-ion ESI mass experiment was carried out. The major peaks at m/z 845.9 (Calc. 845.36) in case of probe **3**- Cu^{2+} complex and at m/z 945.9 (Calc. 946.06) in case of probe **3**- Hg^{2+} complex indicated 2:1 stoichiometry.

2.3.3.4 Effect of metal ions on emission spectrum of probe **3**

Excitation of probe **3** in $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ solutions (1:1 [v/v], containing 0.01 M HEPES, pH = 7.2), showed very weak fluorescence due to strong quenching effect of $\text{CH}_3\text{OH}:\text{H}_2\text{O}$. Thus the presence of metal ions could not be detected. Interestingly, as presence of CN^- ion showed the emission enhancement at 430 nm, thus probe **3**- CN^- complex was used for the determination of Cu^{2+} . The emission intensity due to probe **3**- CN^- complex at 430 nm shifted to 470 nm upon

addition of 100 μM Cu^{2+} (Figure 14a) and upon further addition Cu^{2+} ions between 100-600 μM , emission intensity at 470 nm got quenched (Figure 14b). By use of this displacement approach, probe **3**. CN^- complex was used to estimate 2-600 μM Cu^{2+} ions.

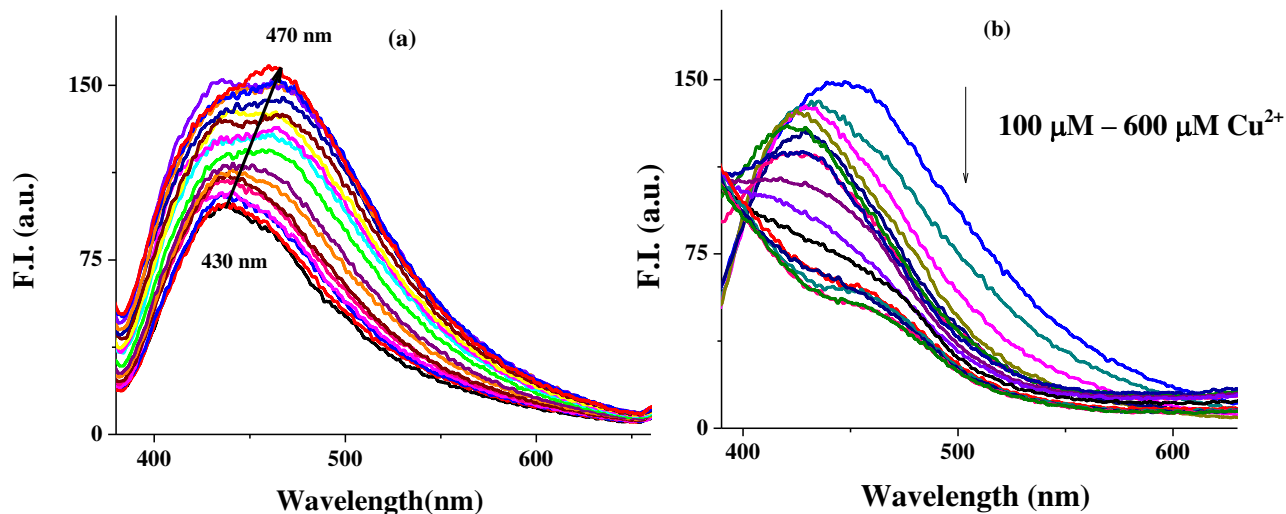


Figure 14: Effect of incremental addition of (a) 0-100 μM Cu^{2+} ; (b) 100-600 μM Cu^{2+} on emission spectra of probe **3** + CN^- (20 μM , CH_3OH).

2.3.3.5 Effect of pH on absorption changes

As pH variation affects the charge distribution on a molecule it can be responsible for changing the absorbance response of probe. So, effect of pH on absorption response of probe **3** and its complex with Cu^{2+} and Hg^{2+} has been investigated in the series of solutions with pH value ranging from 2 to 12 in $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ solutions (1:1[v/v]). The results indicated that probe is stable in pH range 2.5 - 9.1, whereas probe **3** + Cu^{2+} and probe **3** + Hg^{2+} complexes are stable in the range of 4.0 - 12.1 and 4.0 - 8.0 respectively.

2.3.3.6 Elaboration of probe 3 as XOR logic function

As absorbance output at 380 nm is in “on” state in the presence of both Cu^{2+} and CN^- . Thus, the molecular switching behavior of probe **3** with two chemical inputs (Cu^{2+} and CN^-) has been demonstrated with the help of binary logic. Absorption output at 380 nm was in “on” state only when both inputs were absent or present. Such absorption changes upon the actions of two

chemical inputs mimic the performance of an exclusive-OR (XOR) logic gate (Figure 15) with threshold value of 0.15. A XOR logic function is two input device and its output is “false” if the inputs are same and “true” if the inputs are different. Significantly, probe **3** restores the information if CN^- input comes first (“off” state) or Cu^{2+} first (“on” state). The development of such sequential circuits is important as combinational logic not enough to construct memory devices.

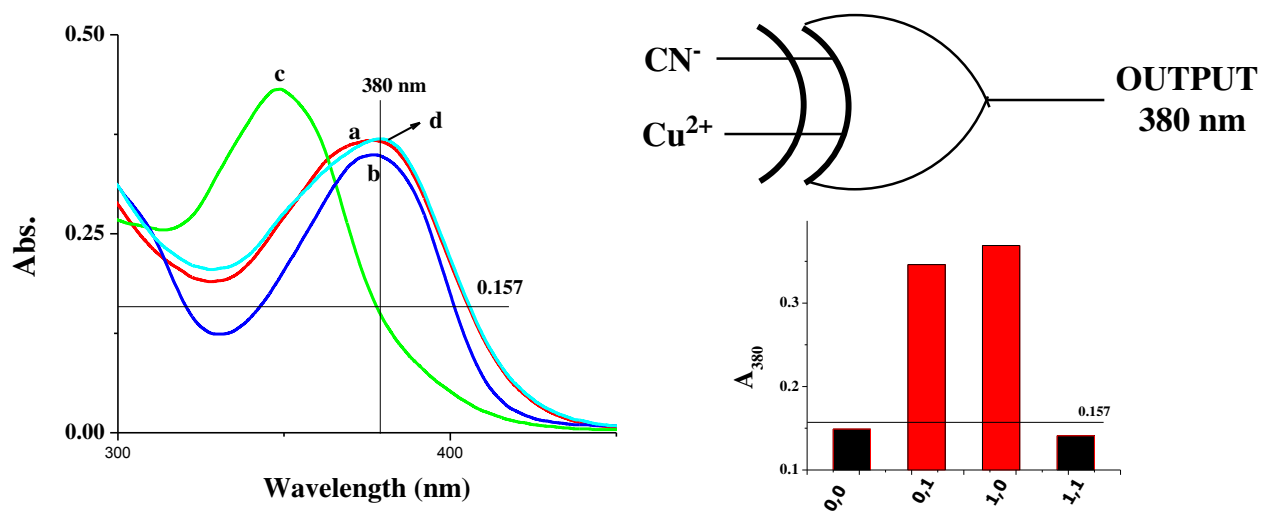


Figure 15: Absorption spectra of probe **3** (20 μM , MeOH) (a) in the presence of CN^- (2.5 eq.); (b) Cu^{2+} ions (1 eq.); (c) CN^- as first input (2.5 eq.) + Cu^{2+} (1 eq.); (d) CN^- as second input (2.5 eq.) + Cu^{2+} (1 eq.).

Table 2: Schematic representation of the XOR logic operation and Truth table for the sequential logic circuit, where 0 = *Off* and 1 = *On*.

INPUT 1 CN^-	INPUT 2 Cu^{2+}	OUTPUT $\lambda_{\text{abs}} = 380 \text{ nm}$
0	0	0
0	1	1
1	0	1
1(1 st)	1	0
1	1(1 st)	1

2.3.4 Theoretical studies

To know more insights into binding strategies of probe **3** towards CN^- , Hg^{2+} and Cu^{2+} ions, probe **3** was optimized in ground state through DFT and TD-SCF calculation with B3LYP-631G methods. It was observed that OH group acquired partial double bond characters moreover hydrogen attached to the oxygen group moved towards nitrogen group of benzimidazole ring due to H-bonding. The absorption band at 340 nm was due to HOMO-LUMO, having oscillating strength 0.6931 and excitation energy 3.6385 eV. We concluded that HOMO of probe **3** was located completely on benzimidazole ring but LUMO was expanding π -nature orbital covering both benzimidazole and benzophenone moieties. (Table 3)(Figure 16)

Probe **3**. CN^- ion was also optimized in gaseous phase using same basis sets as for probe **3** and found that CN^- ion deprotonates OH group present at benzimidazole ring thus band at 380 nm is due to HOMO to LUMO+2, having oscillating strength 0.2312 and excitation energy 3.1348 eV. Benzimidazole ring was responsible for HOMO of probe **3** + CN^- and both benzimidazole and benzophenone moiety for LUMO+2 as it exhibited an expanding π -nature orbital covering both moieties.

To know the binding of probe **3** with Cu^{2+} ion, optimization was done in gaseous phase using LAN2DZ method and other basis remaining same and found that Cu^{2+} ion in probe **3** showed its binding between two nitrogen atoms of benzimidazole and two oxygen atoms of hydroxyl phenyl group and form 2:1 (Probe **3**: Cu^{2+}) complex. The simulated absorption band at 375 nm was due to HOMO to LUMO+2 having oscillating strength 0.0208 and excitation energy 3.4250 eV.

However, binding with Hg^{2+} ion with probe **3** caused the deprotonation of hydroxyl phenyl proton as well as 2:1 (Probe **3**: Hg^{2+}) complex between two nitrogen atoms of benzimidazole and two oxygen atoms of hydroxyl phenyl group.

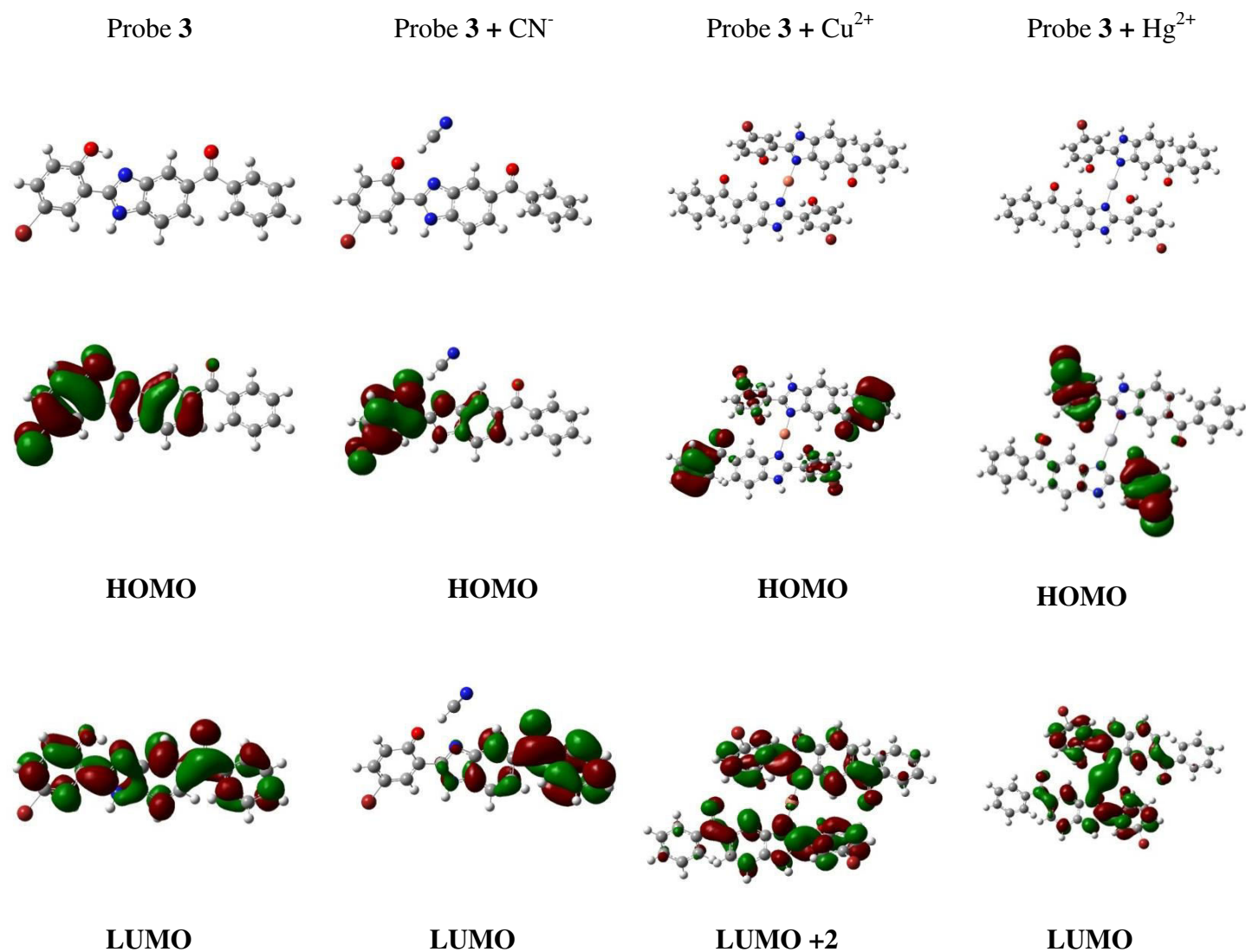


Figure 16: Optimized structures and HOMO and LUMO of probe 3, Probe 3 + CN⁻, Probe 3 + Cu²⁺ and Probe 3 + Hg²⁺.

Table 3: Excitations for probe **3** that contribute to the transitions in the 250-700 nm and their expansion coefficients in gaseous phase.

	λ_{abs} (nm) (Theoretical Value)	λ_{abs} (nm) (Exp. Value)	E_{exc} (eV)	Oscillator Strength	Excited State	% Contribution
Probe 3	340	340	3.6385	0.6931	$H \rightarrow L$	100
Probe 3 + CN^-	395	380	3.1348	0.2312	$H \rightarrow L+2$	96
Probe 3 + Cu^{2+}	362	375	3.4250	0.0208	$H \rightarrow L+2$	69
Probe 3 + Hg^{2+}	349	340	3.5512	0.1459	$H \rightarrow L$	70

2.3.5 Conclusions

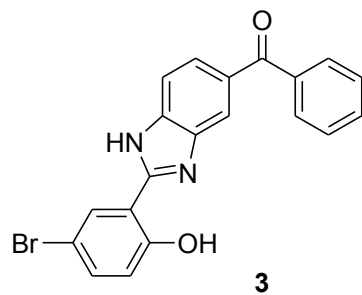
Benzimidazole derivative has been synthesized for selective sensing of CN^- and Cu^{2+} as well as Hg^{2+} ions in protic/aqueous solvents. Probe **3** showed ratiometric absorption behaviour towards CN^- ions with the formation of new absorption band at 380 nm and was used for estimation of broad range (1-1000 μM) of CN^- ions. Probe **3** also showed the differential behavior in the presence of Cu^{2+} and Hg^{2+} ions and signaled the binding event through formation of new absorption band at 375 nm and decrease in absorption intensity respectively. The probe **3**, in the presence of chemical inputs of CN^- and Cu^{2+} ions mimicked a sequence dependent operation of an exclusive-OR (XOR) logic gate.

2.3.6 Experimental Section

2.3.6.1 Synthesis of probe **3**.

In a 50 mL round bottom flask, 3,4 diaminobenzophenone (500 mg, 2.35 mmol) and 5-Bromosalicylaldehyde (473 mg, 2.35 mmol) was added and dissolved in 20 mL nitrobenzene. The reaction mixture was refluxed for about 24 h, solid separated out, cooled the reaction mixture at room temperature and then filtered. Solid was washed with diethyl ether to obtained yellow colored solid of probe **3** (590 mg)

(2-(5-Bromo-2-hydroxyphenyl)-1*H*-benzo[*d*]imidazol-5-yl)(phenyl)methanone (probe 3)



92% yield; Yellow colored solid; Mpt. 240–241 °C. ¹H NMR (CDCl₃ + DMSO-*d*₆, 400 MHz): δ 8.31 (d, 1H, *J* = 1.16 Hz, ArH), 8.03 (s, 1H, ArH), 7.79–7.77 (m, 4H, ArH), 7.69–7.65 (t, 1H, *J* = 7.8 Hz, ArH), 7.59–7.55 (t, 2H, *J* = 7.8 Hz, ArH), 7.52–7.49 (dd, *J*² = 2.28 Hz, *J*³ = 8.68 Hz, 1H, ArH), 7.02 (d, 1H, *J* = 9.16 Hz, ArH); ¹³C NMR (CDCl₃ + DMSO-*d*₆, 100 MHz): δ 193.7 (C=O), 155.6, 136.2, 132.7, 130.4, 127.8, 127.2, 126.6, 117.8, 112.7, 108.6 (ArH); MS (ESI) *m/z* 394.22 (*M*⁺+1).

2.4 ESICT-ESIPT coupled fluorescent sensor for copper and hydrogen sulphate ions

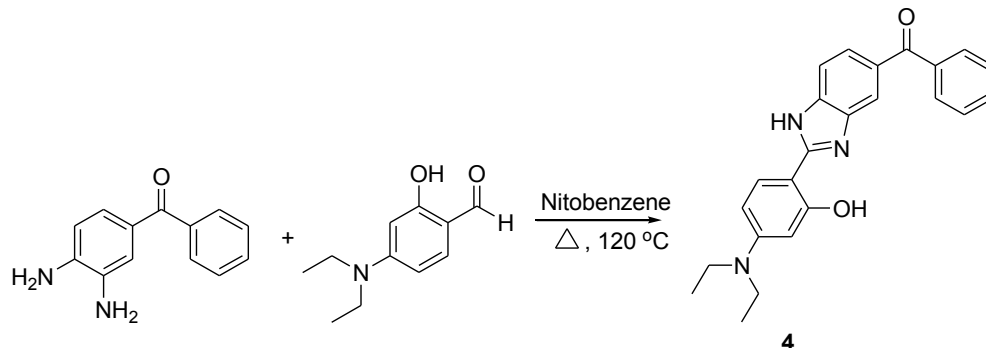
2.4.1 Abstract

In continuation to previous chapter i.e effect of substituent on ESIPT phenomenon, a new ESIPT-ESICT based benzimidazole has been synthesized and investigated their photophysical behaviour towards various cations and anions. The main objective was to study the effect of electron donating groups on ESIPT phenomenon. In the previous chapter effect of electron withdrawing group was studied which showed the decrease in Stokes shift upon introduction of electron-withdrawing group. However, incorporation of electron donating group at the meta-position of proton donor site (probe **4**) showed the enhanced Stokes shift as compared to probe **3**. The additional effect of charge transfer was also observed due to the presence of electron donating group which made one site of the molecule to be electron rich and other site having carbonyl group made it electron deficient. To the best of our knowledge only few reports^{7g} have been reported using ESIPT-ESICT couple phenomenon, used for anion sensing. It has been observed that probe **2** (without substituent) showed visible/optical changes with F^- ions however incorporation of electron donating strength of benzimidazole, selectivity shifted towards HSO_4^- and Cu^{2+} ions in case of probe **4**. The probe **4** showed the shifting of absorption band at 390 nm where as keto tautomer emission is quenched and enol tautomer band is released at 430 nm in the presence of HSO_4^- ions. The presence of Cu^{2+} ions showed the decreased absorption intensity at 370 nm and formation of new absorption band at 265 nm while emission band at 575 nm blue shifted to 540 nm at lower equivalents and then quenched to release normal emission band at 440 nm. The results obtained for the interactions of analytes were also correlated by theoretical and NMR studies.

2.4.2 Synthesis of Probe 4

On refluxing solution of 3,4-diaminobenzophenone and N,N'-diethyl salicylaldehyde in nitrobenzene gave probe **4** as a yellow colored solid in 92 % yield; m.p. 220–221 °C; MS (ESI) m/z 386.46 ($M^+ + H$) (scheme 3). ^1H NMR spectrum of probe **4** showed the 1H broad singlet at δ 12.80 due to NH proton (D_2O exchangeable), 1H singlet at δ 7.99, two 1H doublets at δ 7.90 and δ 7.75, 2H multiplet at δ 7.62, 2H triplet at δ 7.51, 1H double doublet at δ 6.30 for aromatic

protons and 4H multiplet at δ 3.32 due to N-CH₂, 6H triplet at δ 1.17 due to CH₃. ¹³C NMR spectrum showed signals at δ 195.9 (C=O), 160.1, 150.3, 131.2, 129.2, 127.6, 126.8, 103.0, 99.8, and 97.6 for aromatic –C and 43.8(N-CH₂), 12.1(CH₃). This spectral data having peak at 195.9 which is due to C=O and peaks at 43.8 and 12.1 due to N, N, diethyl group and their signal in ¹H NMR corroborate with the structure of [2-(4-Diethylamino-2-hydroxy-phenyl)-1*H*-benzimidazol-5-yl]-phenyl-methanone (Probe 4).



Scheme 3: Synthesis of probe 4

X-Ray Crystal Structure analysis

The crystals of probe 4 were grown in mixture of acetonitrile and ethanol by solvent evaporation method. Single crystal XRD studies showed that crystal has triclinic structure, having (*P*-1) space group with $R_1 = 7.88\%$ and GooF value to be 1.033 (Figure 17 and table 4). Unit cell dimensions were found to be as $a = 7.9374(3) \text{ \AA}$, $b = 11.7261(5) \text{ \AA}$, $c = 11.9156(5) \text{ \AA}$, $\alpha = 80.517(2)^\circ$, $\beta = 74.968(2)^\circ$, $\gamma = 74.505(2)^\circ$, Volume = $1026.72(7) \text{ \AA}^3$ and $Z = 2$.

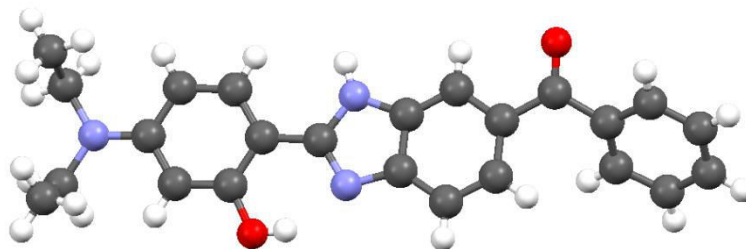


Figure 17: X-Ray crystal structure of probe 4.

Table 4: Summary of crystal data, data collection and structure refinement for probe **4**.**A. Crystal Data**

Identification code	SCE32	
Chemical formula	C ₂₄ H ₂₀ N ₃ O ₂	
Formula weight	382.43	
Temperature	296(2) K	
Wavelength	0.71073 Å	
Crystal system	triclinic	
Space group	P -1	
Unit cell dimensions	a = 7.9374(3) Å	α = 80.517(2)°
	b = 11.7261(5) Å	β = 74.968(2)°
	c = 11.9156(5) Å	γ = 74.505(2)°
Volume	1026.72(7) Å ³	
Z	2	
Density (calculated)	1.237 g/cm ³	
Absorption coefficient	0.080 mm ⁻¹	
F(000)	402	

B. Data collection and structure refinement

Theta range for data collection	1.78 to 25.04°
Index ranges	-9 ≤ h ≤ 9, -13 ≤ k ≤ 13, -14 ≤ l ≤ 14
Reflections collected	9578
Independent reflections	3617 [R(int) = 0.0368]
Coverage of independent reflections	99.4%
Absorption correction	multi-scan
Refinement method	Full-matrix least-squares on F ²
Refinement program	SHELXL-2013 (Sheldrick, 2013)
Function minimized	Σ w(F _o ² - F _c ²) ²
Data / restraints / parameters	3617 / 0 / 266
Goodness-of-fit on F ²	1.033

Final R indices	2138 data; $I > 2\sigma(I)$ $R_1 = 0.0788$, $wR_2 = 0.2322$
	all data $R_1 = 0.1276$, $wR_2 = 0.2782$
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.1798P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
Extinction coefficient	0.0280(90)
Largest diff. peak and hole	1.194 and $-0.216 \text{ e}\text{\AA}^{-3}$
R.M.S. deviation from mean	$0.092 \text{ e}\text{\AA}^{-3}$

2.4.3 Effect of solvent on emission spectrum of probe 4

The emission spectrum of probe **4** was observed in different solvents of varying polarity in order to study the solvent effect on ESIPT phenomenon. Probe **4** showed two absorption bands at 290 nm and 370 nm; the band at 290 nm was attributed to the $\pi-\pi^*$ transition and the band at 370 nm to an intramolecular charge transfer (ICT) transition. The emission spectrum of probe **4** exhibited large red-shift on increase in the solvent polarity, which indicated strong ICT characteristics for the excited states of the compound (Table 5). More importantly, the two emission band occurring in different solvents, is composed of a charge transfer emission (CTeq*) and a large Stokes shifted proton-transfer tautomer emission (PCTeq*) band maximized at 435 nm and 565 nm respectively. Therefore, a remarkable ESICT–ESIPT coupled system was evidently established (Figure 19). It should be noted that proton-transfer tautomer emission was dominant in polar solvents (dimethyl sulphoxide, and acetonitrile) due to the increase of solvent polarity, which resulted in a sharp decrease of the CTeq* energy.

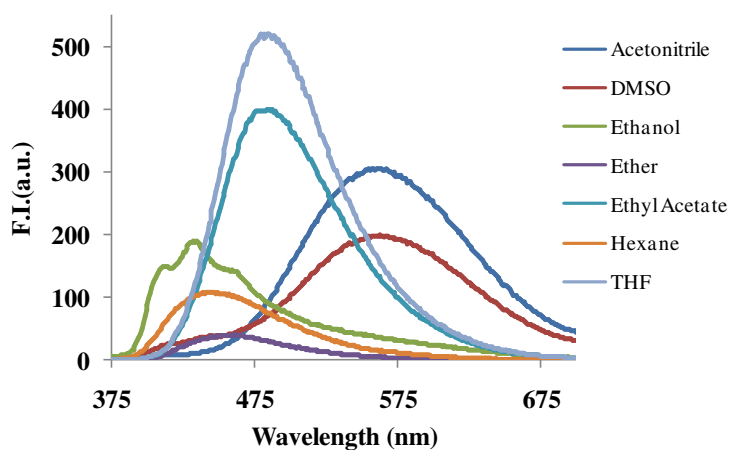


Figure 18: Effect of different solvents on emission spectra of probe **4** (10 μM , $\lambda_{\text{ex}} = 370 \text{ nm}$).

Table 5: Emission bands of probe **4** in different solvents.

Solvent	$\lambda_{em(N)}$	$\lambda_{em(K)}$	ϕ_N	ϕ_K
Acetonitrile	-	565	0.08	
DMSO	-	565	0.08	
Ether	430	-	-	
Ethanol	435	-	-	0.0069
Ethylacetate	485	-	-	
Hexane	435	-	-	
THF	485	-	-	

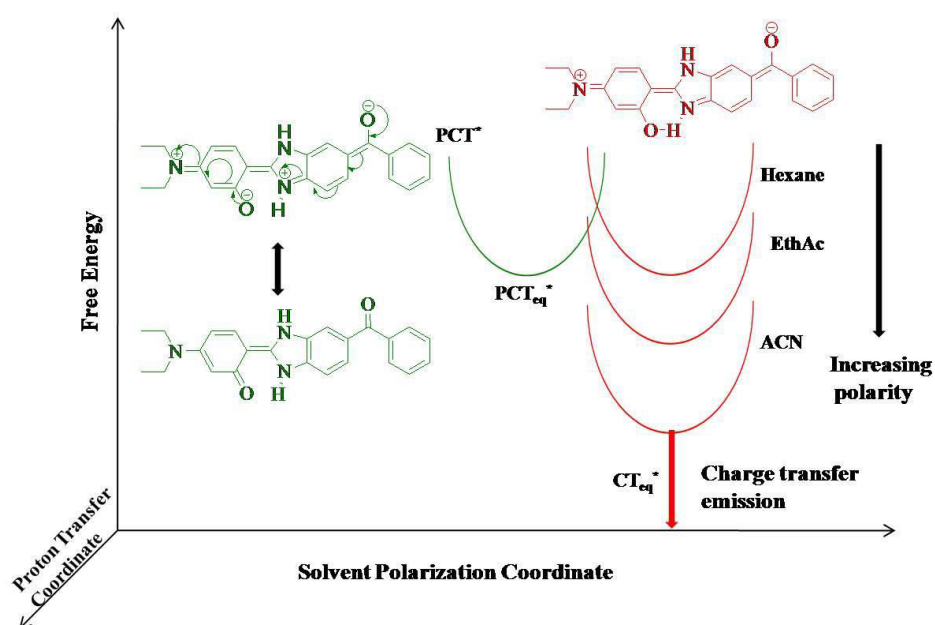


Figure 19: The induced barrier for ESIP as a function of solvent polarity focus on the event after solvent relaxation to the CTeq* state. The barrier is therefore increased as the solvent.

2.4.4 Results and discussion

2.4.4.1 Photophysical properties of probe **4** towards metal ions

The recognition behaviour of probe **4** toward various metal ions has been investigated by absorption and emission spectroscopic techniques. In a typical experiment, a solution of cations, *viz.*, Pb^{2+} , Hg^{2+} , Fe^{3+} , Co^{2+} , Mg^{2+} , Ni^{2+} , Ag^{+} , Zn^{2+} , Cd^{2+} , Ba^{2+} , Ca^{2+} , Cu^{2+} , K^{+} and Na^{+} as their perchlorate salts was added to a solution of probe **4** (20 μ M, $CH_3CN:H_2O$, 95 : 5, v/v, HEPES

buffer, pH = 7.2). Among these, only Cu^{2+} ions resulted in significant changes in the absorption spectral behaviour of the probe (Figure 20a). Probe **4** displayed absorption band at 370 nm, intensity of which upon addition of 0-15 μM of Cu^{2+} ions decreased along with the formation of a new absorption band at 390 nm. Further addition of Cu^{2+} ions between 15 μM -50 μM , another new absorption band was observed at 275 nm (Figure 20b) with stability constant $8.8 \pm 0.01 \times 10^4 \text{ M}^{-1}$.

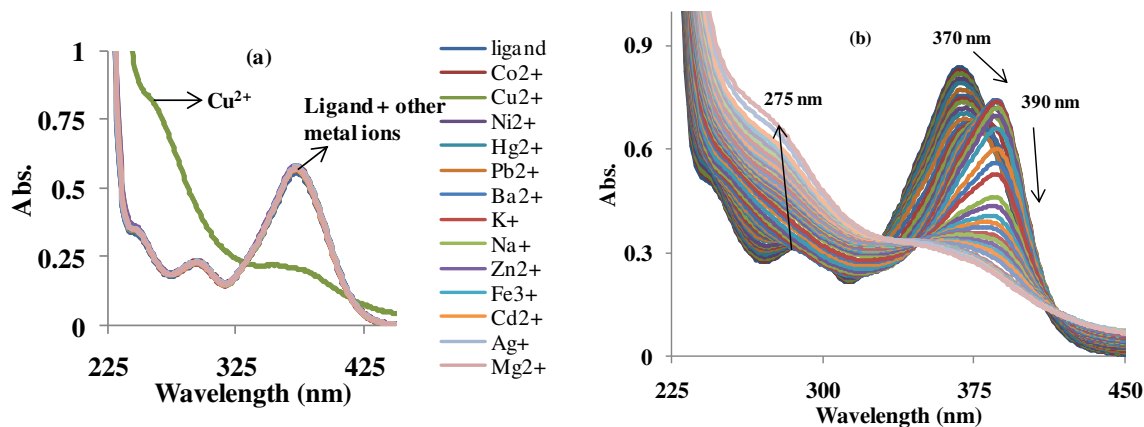


Figure 20: Effect of addition of (a) different metal ions; (b) incremental addition of Cu^{2+} ions on absorption spectrum of probe **4** (20 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}$, 95 : 5, v/v, HEPES buffer, pH = 7.2).

Effect of various metal ions like Cu^{2+} , Pb^{2+} , Hg^{2+} , Fe^{3+} , Co^{2+} , Mg^{2+} , Ni^{2+} , Fe^{2+} , Ag^+ , Zn^{2+} , Cd^{2+} , Ba^{2+} , Ca^{2+} , K^+ and Na^+ was studied on emission spectrum of probe **4** (20 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}$, 95 : 5, v/v, HEPES buffer, pH = 7.2).

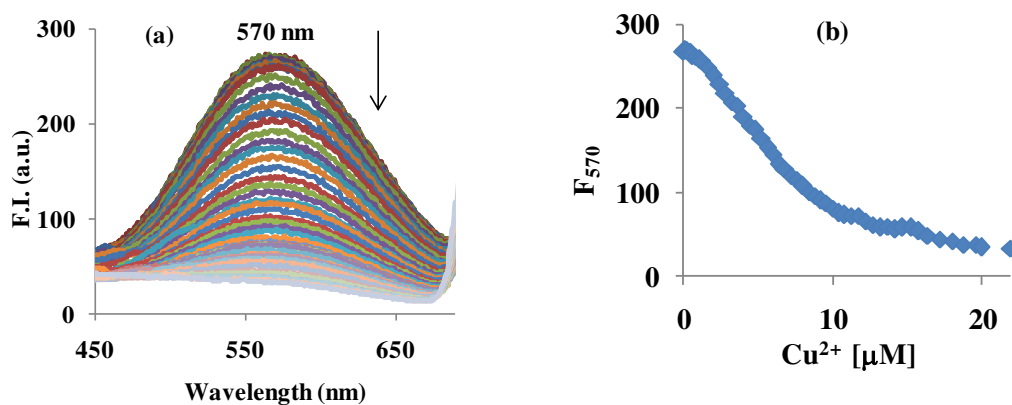


Figure 21: (a) Effect on emission spectrum of probe **4** (20 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}$, 95 : 5, v/v, HEPES buffer, pH = 7.2) on addition of Cu^{2+} ; (b) Emission response of probe **4** with Cu^{2+} ions.

The emission band of probe **4** at 570 nm remained unaffected in the presence of tested metal ions except Cu^{2+} with very small interference of Pb^{2+} and Fe^{3+} ions. The incremental addition of Cu^{2+} ions showed quenching at 570 nm band i.e. due to restriction of ESIPT phenomenon's (Figure 21) having stability constant to be $2.08 \pm 0.02 \times 10^3 \text{ M}^{-1}$.

2.4.4.2 Photophysical properties of probe **4** towards anions

As probe **4** is amphiphilic in nature, it has proton acceptor sites. So, it prompted us to evaluate binding behaviour of probe **4** toward different anions. In a typical experiment, a solution of the anions, *viz.*, CN^- , F^- , Cl^- , Br^- , CH_3COO^- , I^- , HSO_4^- and H_2PO_4^- as their tetrabutylammonium salts was added to a solution of probe **4** (20 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}$, 1 : 1, v/v, HEPES buffer, pH = 7.2). Among these, only HSO_4^- ions resulted in significant changes in the absorption spectral behaviour of the probe. Probe **4** in case of absorption band showed a red shift to 390 nm and band at 360 nm decreased simultaneously with formation of new band at 260 nm upon addition of 10 equivalents of HSO_4^- ions (Figure 22a). The absorption ratio at these two wavelengths varied from 0.8668 to 1.4969 indicating 1.726 fold absorption ratio changes. To know the effect of binding of HSO_4^- ions to probe **4**, stability constant was calculated to be $2.8 \pm 0.01 \times 10^4 \text{ M}^{-1}$. Thus, probe **4** was used to estimate a wide range of HSO_4^- ions between 2-150 μM through ratiometric approach (Figure 22b).

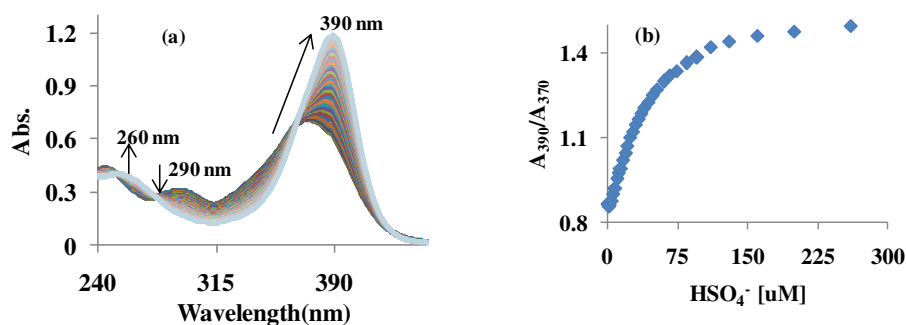


Figure 22: (a) Absorption spectrum of probe **4** (20 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}$, 1 : 1, v/v, HEPES buffer, pH = 7.2) upon complexation with increasing concentration of HSO_4^- ion; (b) Ratiometric response of probe **4** with HSO_4^- ions.

In case of fluorescence of probe **4** having same solvent system, band was observed at 560 nm which shifted to 430 nm upon addition of HSO_4^- ions with stability constant $6.1 \pm 0.1 \times 10^3 \text{ M}^{-1}$

(Figure 23a). The fluorescence ratio at these two wavelengths varied from 1.42 to 2.45 indicating 1.72 fold emission ratio change (Figure 23b).

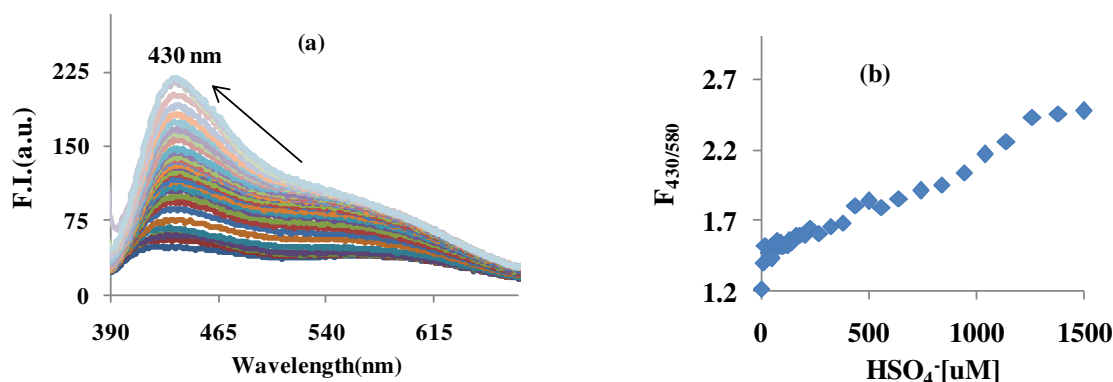


Figure 23: (a) Emission spectrum of probe **4** (20 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}$, 1 : 1, v/v, HEPES buffer, pH = 7.2) upon complexation with increasing concentration of HSO_4^- ion; (b) Ratiometric response of probe **4** with HSO_4^- ions.

The detection limit of HSO_4^- ions was determined to be 0.22 μM which is much lower than the literature reports. To determine the practical applicability of probe **4**, we carried out competitive experiments in the presence of 10 equiv. of HSO_4^- ions mixed with 50 equiv. of each of the various anions. No significant variation in the fluorescence intensity was observed when comparing the results with or without the other anions (Figure 24).

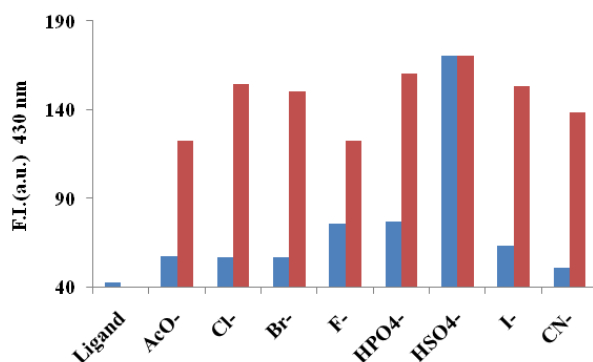


Figure 24: Red bars represent selectivity of probe **4** (20 μM , $\lambda_{\text{ex}} = 360 \text{ nm}$) upon addition of different anions in (20 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}$, 1 : 1, v/v, HEPES buffer, pH = 7.2) and blue bars shows the competitive selectivity of compound **1** in the presence of interfering anions

2.4.4.3 Practical applicability of probe 4

In order to check the practical applicability of probe 4, TLC strips were prepared by dip-coating a solution of probe 4, followed by drying them in a vacuum in order to check for residual contamination in contact mode. Several samples of probe solution-coated TLC strips were prepared and studied the response of their fluorescence towards Cu^{2+} and HSO_4^- ions in contact mode and solution phase. Solutions of Cu^{2+} and HSO_4^- ions were placed over coated TLC strips for 5 sec. Upon illumination with a UV lamp, blue spots were observed in the contact area. The minimum amount of Cu^{2+} and HSO_4^- ions detectable by the naked eye was as low as, thereby registering a detection limit at 10^{-6} M (Figures 25 and 26).

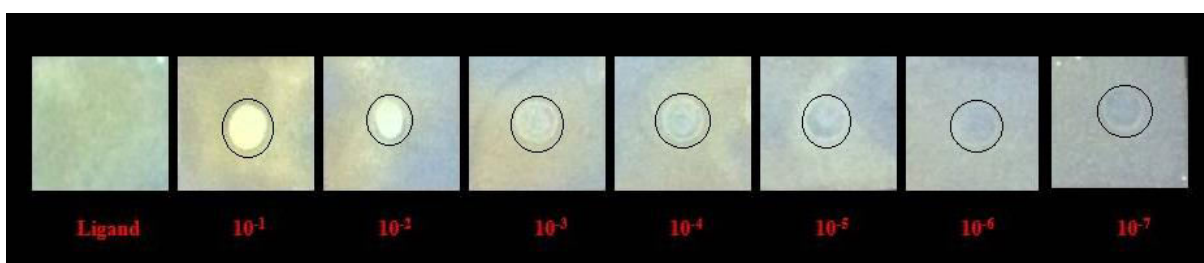


Figure 25: Photographs of probe 4 coated TLC strips under different Cu^{2+} ion concentrations.

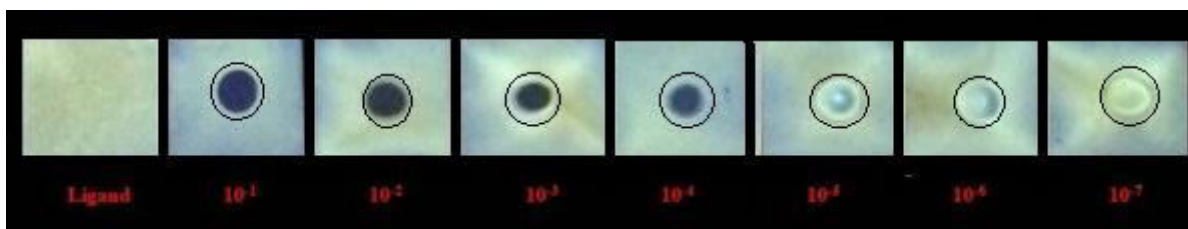


Figure 26: Photographs of probe 4 coated TLC strips under different HSO_4^- ion concentrations.

2.4.4.4 Effect of pH titrations on probe 4 and probe 4. HSO_4^-

As pH variation affects the charge distribution on a molecule it can be responsible for changing the absorbance response of probe. So, effect of pH on absorption response of probe 4 (20 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}$, 1 : 1, v/v) and its complex with HSO_4^- (1:1) ions has been investigated in the series of solutions with pH value ranging from 2 to 12. The results indicated that probe is stable within the pH range 5.6 - 11.9, whereas probe 4 + HSO_4^- complex showed the stability between pH range of 7.0 - 12.4 (Figure 27a and b).

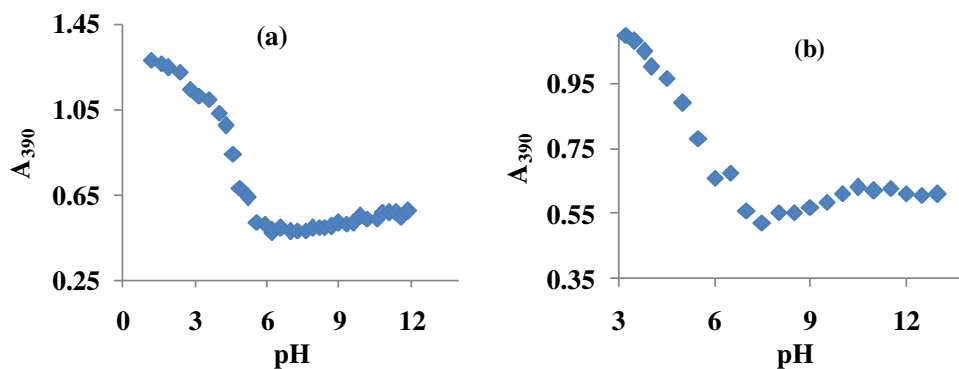


Figure 27: Effect of pH change on absorption at 390 nm for (a) probe **4** and (b) **4.HSO₄⁻** complex in CH₃OH:H₂O::1:1.

2.4.4.5 Effect of HSO₄⁻ on ¹H NMR spectrum

In order to evaluate the nature of the interaction of HSO₄⁻ ion with probe **4**, we commenced our investigations by monitoring the ¹H NMR spectral changes produced *via* the incremental addition of tetra-*n*-butylammonium hydrogen sulphate (TBAHSO₄) to a solution of probe **4** (5 mM) in CD₃CN-D₂O (1:1). Incremental addition of HSO₄⁻ ions lead to the downfield shift of H_a proton upon interaction with HSO₄⁻ molecules (Figure 28).

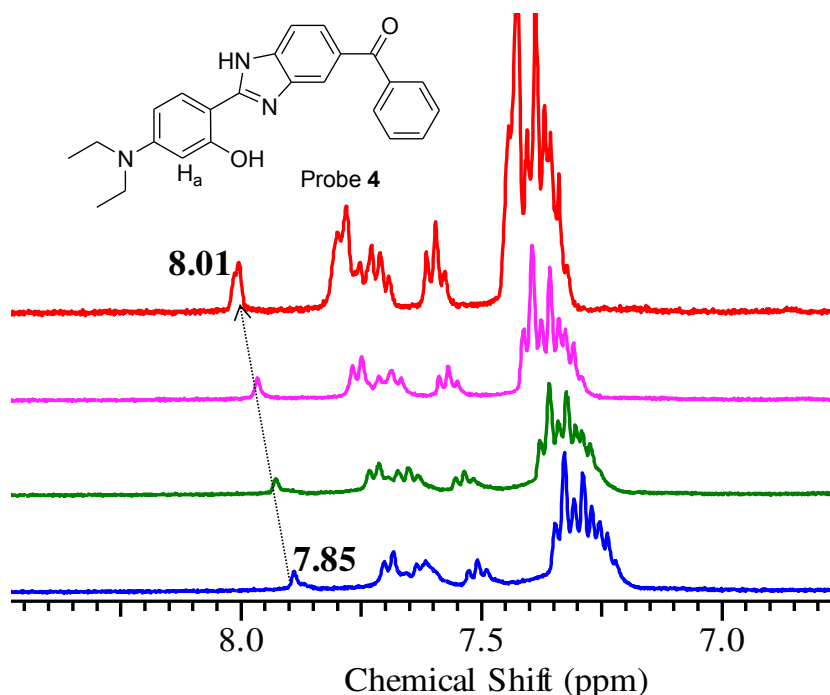


Figure 28: Partial ¹H NMR (400 MHz, CD₃CN:D₂O (1:1)) spectral changes upon addition of hydrogen sulphate anions (HSO₄⁻) to probe **4** (5 mM).

2.4.5 Theoretical Studies of probe 4

To explore the origin of each induced absorption band, theoretical calculations were employed to optimize the structure through DFT and TD-SCF calculation with B3LYP-631G methods for HSO_4^- and LAN2DZ method for Cu^{2+} ions. The structure optimization of probe 4 showed that OH group acquires partial double bond character and hydrogen appears in close vicinity of imine - N - to form NH in benzimidazole moiety.

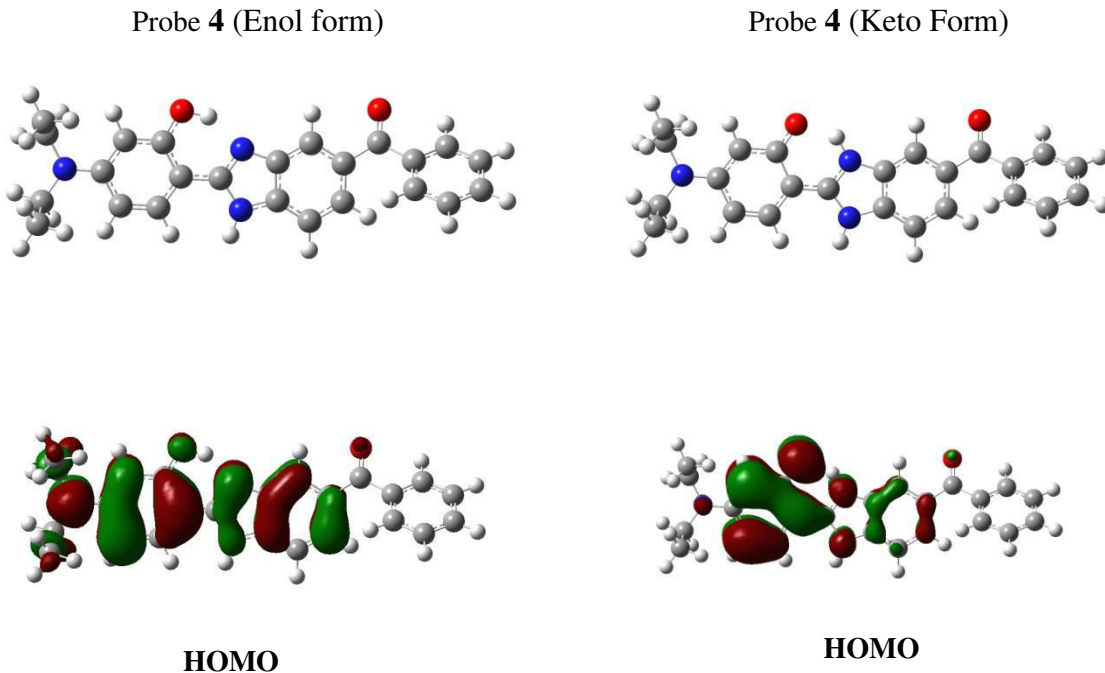
The optimization of probe 4 (enol form), in gaseous phase showed the absorption at 370 nm due to HOMO-LUMO, having oscillating strength 0.27 and excitation energy 3.2506 eV. The HOMO of probe 4 locates on benzimidazole ring and LUMO mainly on benzophenone moiety but for LUMO+1, it exhibited an expanding π -nature orbital covering both benzimidazole and benzophenone moieties. The other band at 290 nm contributes to HOMO-3 to LUMO transitions (Figure 29 and Table 6)

Table 6: Excitations for probe 4 that contribute to the transitions in the 250-700 nm range along with their relative contributions given by the expansion coefficients in gaseous phase.

Parameters	λ_{max} (Theoretically)	λ_{max} (Experimentally)	$E_{\text{exc}}(\text{eV})$	Oscillator Strength	Excited State	% Contribution
Probe 4 (Enol form)	381	370	3.2506	0.2700	H to L	100
Probe 4 (Enol form)	287	290	4.3162	0.1337	H-3 to L	97
Probe 4 (Keto form)	345	370	3.5914	0.8750	H - 1to L+1	67
Probe 4 (Keto form)	274	290	4.5209	0.133	H-1 to L+3	97

The probe 4 in keto form upon optimization in gaseous phase showed absorption band at 370 nm is due to HOMO - 1 to LUMO + 1, having oscillating strength 0.8750 and excitation energy 3.5914 eV. The HOMO of probe 4 locates mainly benzimidazole ring and LUMO mainly on benzophenone moiety but LUMO + 1, exhibited an expanding π -nature orbital covering both benzimidazole and benzophenone moieties. The other band at 290 nm was due to HOMO-1 to LUMO+3 (Figure 29 and Table 6).

Interaction of probe **4** with HSO_4^- ions showed that oxygen of HSO_4^- interacts with hydrogen of benzimidazole nitrogen and phenolic hydrogen thus showed absorption band at 390 nm due to HOMO to LUMO transitions having oscillating strength 0.2365 and excitation energy 2.9809 eV. The HOMO of probe **4** + HSO_4^- locates mainly on benzophenone ring and LUMO mainly on benzimidazole moiety whereas in case of LUMO + 1, it exhibited an expanding π -nature orbital covering both benzimidazole and benzophenone moiety. (Figure 30 and Table 7) The optimization of probe **4** binding properties towards Cu^{2+} ions were performed in acetonitrile using B3LYP-631G methods and LAN2DZ basis set and found that Cu^{2+} ions interacted with hydrogen of benzimidazole nitrogen. Two bands at 257 and 293 nm were observed. Band at 293 was due to HOMO - 1 to LUMO + 2 also due to HOMO - 2 to LUMO + 1 and band at 257 was mainly due to HOMO - 7 to LUMO + 2 and HOMO - 8 to LUMO + 2. HOMO located mainly on benzophenone ring except in case of HOMO - 7, which located mainly near copper, whereas in case of LUMO, it exhibited an expanding π -nature orbital covering both benzimidazole and benzophenone moieties. (Figure 31 and Table 7)



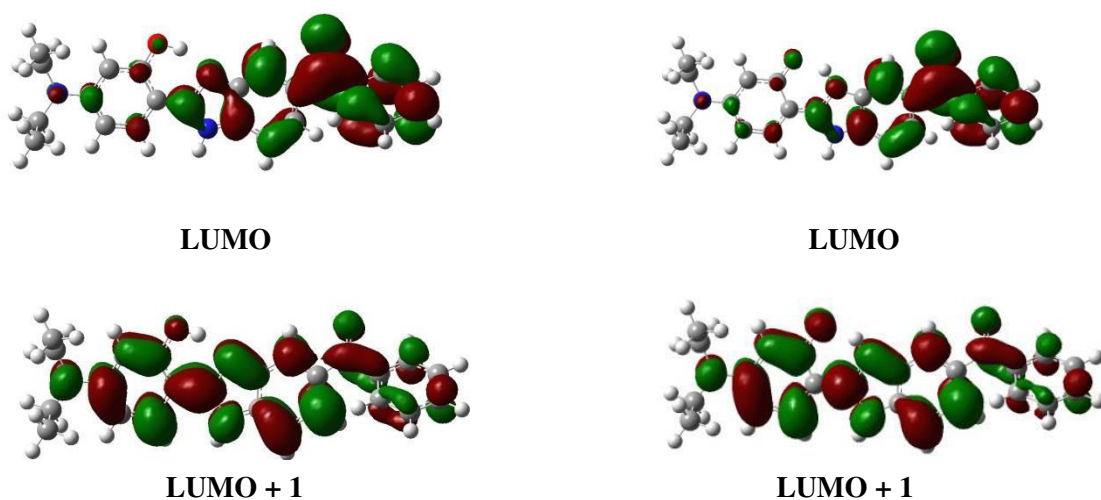


Figure 29: Optimized structures and HOMO and LUMO of probe 4.

Table 7: Excitations for probe 4 that contribute to the transitions in the 250-700 nm range along with their relative contributions given by the expansion coefficients in acetonitrile.

	λ_{max} (nm) (Theoretical values)	λ_{max} (nm) (Exp. values)	E_{exc} (eV)	Oscillator Strength	Excited State	% Contribut ion
Probe 4+ HSO_4^-	415	390	2.9809	0.2365	H to L	100
	323		3.8282	1.0379	H to L+1	100
Probe 4 + Cu^{2+}	367		3.3801	0.3462	H-1 to L+1 (ALPHA)	79
	257	260	4.8140	0.0325	H-7 to L+2 H-8 to L+2,	33 28
	293		4.2235	0.0805	H-1 to L+2 H-2 to L+1	30 15

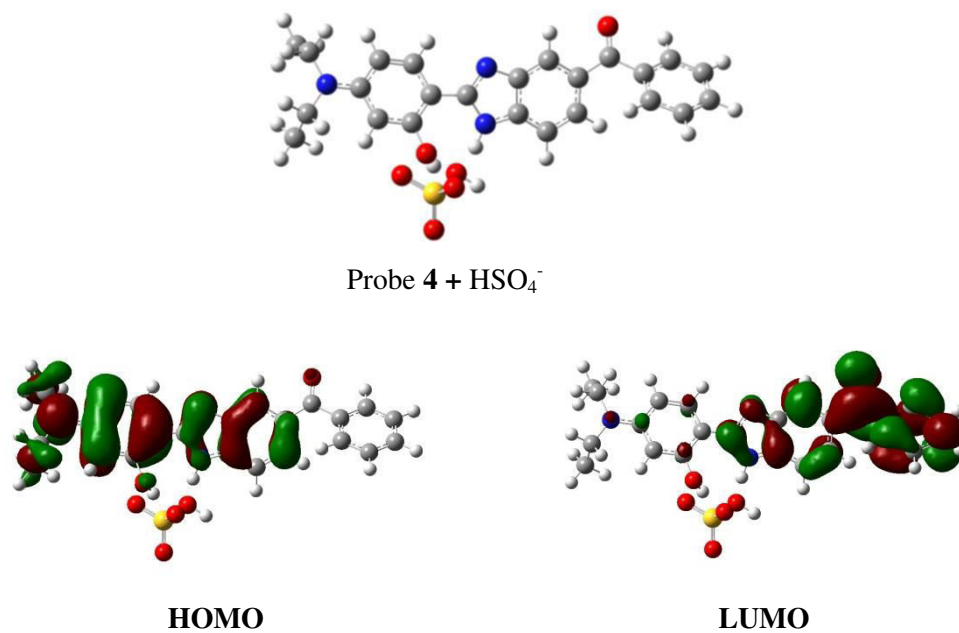


Figure 30: Optimized structures and HOMO and LUMO of probe 4 + HSO_4^- .

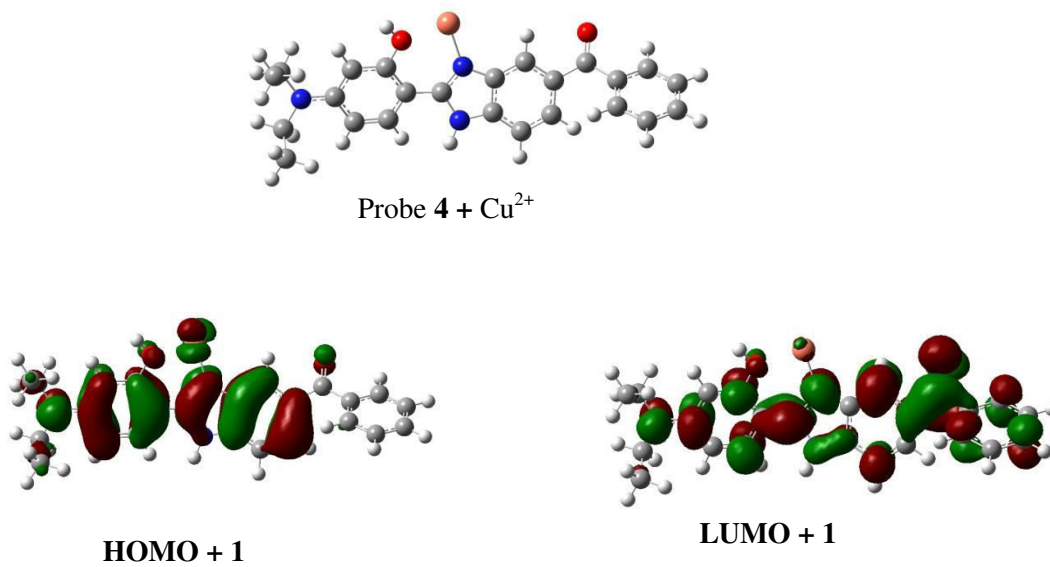


Figure 31: Optimized structures and HOMO and LUMO of probe 4 + Cu^{2+} .

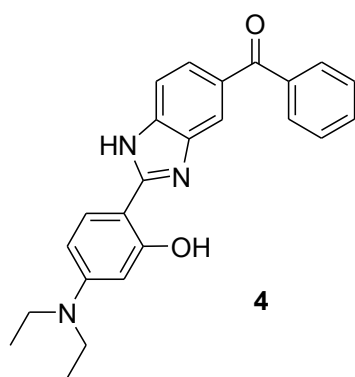
2.4.6 Conclusions

Benzimidazole substituted benzophenone has been synthesized which showed selectivity toward Cu^{2+} and HSO_4^- ions. Probe **4** showed fluorescence behaviour towards HSO_4^- ions through hydrogen bonding interactions and restricts ESIPT phenomenon's from OH to nitrogen of benzimidazole moiety. Probe **4** also showed the differential behavior in the presence of Cu^{2+} and signaled the binding event through formation of new absorption band at 275 nm and quenching of emission band at 570 nm. Probe **4** formed new bands with HSO_4^- at 390 nm and 430 nm in absorption and fluorescence spectroscopy respectively. Probe **4** showed practical applicability to sense Cu^{2+} and HSO_4^- ions through dip coating method. Results were also correlated by theoretical methods.

2.4.7 Experimental

2.4.7.1 General procedure for synthesis of probe 4: 3,4 diaminobenzophenone (500 mg, 2.3mmol) and N,N-diethyl salicylaldehyde (454 mg, 2.3 mmol) were dissolved in 30 mL nitrobenzene. The reaction mixture was refluxed for about 35 h, solid separated out, cooled the reaction mixture at room temperature and then filtered. Solid was washed with diethyl ether to obtained yellow coloured solid of probe **4** (414 mg)

[2-(4-Diethylamino-2-hydroxy-phenyl)-1H-benzimidazol-5-yl]-phenyl-methanone(Probe 4)



92% yield; Yellow colored; M.pt. 220–221 °C; ^1H NMR, (CDCl_3 + $\text{DMSO}-d_6$, δ ppm, 400 MHz): δ 12.80 (bs, 1H, NH), 7.99 (s, 1H, Ar), 7.93-7.85 (d, $J = 32$ Hz, 2H, Ar), 7.76-7.74 (d, $J = 8$ Hz, 2H, Ar), 7.65-7.58 (m, 2H, Ar), 7.53-7.49 (t, $J = 8$ Hz, 2H, Ar), 6.32-6.29 (dd, $J^1 = 1.84$ Hz, $J^2 = 2.32$ Hz, 1H, Ar), 6.18 (d, $J = 2.28$ Hz, 1H, Ar), 3.41-3.35 (m, 4H, $\text{N}(\text{CH}_2)_2$), 1.19-1.16 (t, $J = 6$ Hz, 6H, $(\text{CH}_3)_2$). ^{13}C NMR (CDCl_3 + $\text{DMSO}-d_6$, 100 MHz): δ 195.9 (C=O), 160.1, 150.3, 131.2, 129.2, 127.6, 126.8, 103.0, 99.8, and 97.6 ArC and 43.8($\text{N}-\text{CH}_2$), 12.1(CH_3).; MS (ESI) m/z 386.46 ($\text{M}^+ + 1$).

2.5 Chromofluorescent probe for estimation of cyanide in human blood serum/plasma like solutions using chemodosimetric approach

2.5.1 Abstract

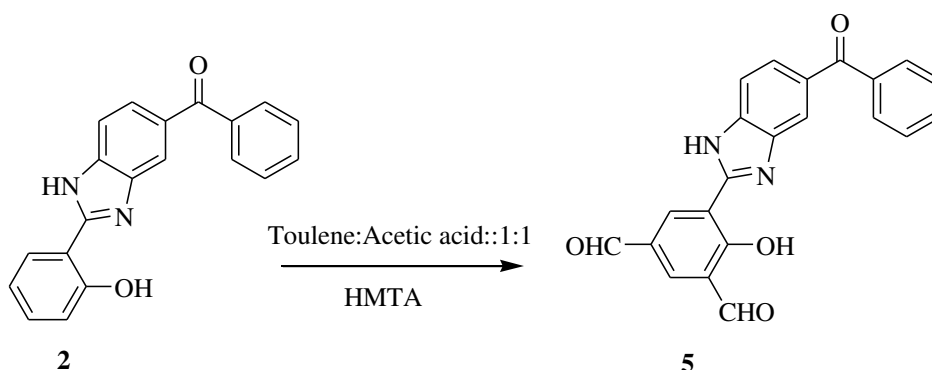
In continuation to study of effect of substituents (electron donating and electron withdrawing) on ESIPT phenomenon's, benzimidazole based probe **5** has been synthesized which exhibited Resonance-Assisted Hydrogen Bonds (RAHBs) and ESIPT phenomenon's on to single platform. Introduction of electron-withdrawing group (-CHO) at *ortho*-position of the proton donor site can induce Resonance-Assisted Hydrogen Bonds (RAHBs). RAHBs provide the high stability of intramolecular hydrogen bonds and rationalized by enhanced π -delocalization involving the hydrogen atom. RAHBs are formally a linear combination of two tautomeric forms. Thus, coupling of excited state intramolecular proton transfer (ESIPT) with RAHBs is one of the interesting phenomena which have not been explored much yet. ESIPT moiety with an aldehyde group at the *ortho*-position of the hydroxyl group take advantage of RAHBs and undergo more facile nucleophilic addition reactions.

With these realizations as well as those based on our experience in developing ESIPT based optical probes or methods for detection of biologically important species, probe **5** has been synthesized having hydroxyl phenyl-benzimidazole moiety typically for ESIPT phenomenon's, substituted with -CHO moiety at the *ortho*-position of the hydroxyl group to take advantage of RAHBs. This coupled ESIPT-RAHBs phenomenon's provide the advantage to selective and ratiometric estimation of cyanide ions. The probe **5** bound selectively with cyanide ions by forming new absorption bands at 380 nm and 415 nm and emission band at 410 nm. The simultaneous increase and decrease at two acquisition emission wavelengths have been used for ratiometric detection of cyanide ion. The interaction behaviour of CN^- with probe **5** has been confirmed through NMR titrations as well as DFT calculations. The applicability of probe **5** has been proved by analysing the CN^- ions in the human blood sample.

2.5.2 Synthesis of probe 5

On refluxing solution of probe **2** and hexamethylenetetramine in toluene: acetic acid in 1:1 ratio gave probe **5** as a yellow colored solid. MS (ESI) m/z 389.1 ($\text{M}^+ + 1 + \text{NH}_4$) (scheme 4). ^1H NMR spectrum of probe **5** showed two ^1H singlets at δ 10.55 and δ 9.92 due to two CHO protons, ^1H

singlet at δ 8.68, 1H doublet at δ 8.31, 2H multiplet at δ 7.85, 1H triplet at δ 7.63, 3H triplet at δ 7.53, 1H doublet at δ 7.18 and 1H triplet at δ 7.01 for aromatic protons and 1H broad singlet at δ 8.05 (D₂O exchangeable) for NH. The two additional peaks at 10.55 and 9.92 of CHO confirmed the structure for probe **5**. ¹³C NMR spectrum showed signals at δ 196.0, 190.0 for aldehydic -C, 163.6, 161.0, 137.7, 133.6, 132.3, 131.6, 130.4, 129.3, 127.9, 127.7, 124.9, 123.5, 118.5, 117.7, 114.0, 112.7 for aromatic -C. On the basis of these spectral data, this compound has been assigned the structure of probe **5**.



Scheme 4: Synthesis of probe **5**

2.5.3 Results and discussion

2.5.3.1 Sensing ability of probe **5** towards various anions

The recognition behaviour of probe **5** towards various anions has been investigated by absorption and emission spectroscopic techniques. In a typical experiment, a solution of anions, *viz.* CN⁻, F⁻, Cl⁻, Br⁻, CH₃COO⁻, I⁻, HSO₄⁻, H₂PO₄⁻, NO₃⁻ as their tetrabutylammonium salts was added to a solution of probe **5** (20 μ M, CH₃CN–H₂O, 4 : 1, v/v, HEPES buffer, pH = 7.2). Among these, only CN⁻ ion resulted in significant changes in the absorption spectral behaviour of the probe (Figure 32a). On gradual addition of CN⁻ ions (0.0–6.0 equiv.) to a solution of probe **5** led to a bathochromic shift for the absorption bands at 385 nm and 440 nm (Figure 32b). The spectral changes were associated with visible color change from colourless to yellow (Figure 33) and achieved plateau after addition of 100 μ M of CN⁻ ions. To know the effect of nucleophilic binding of CN⁻ ions to probe **5**, stability constant was calculated to be $1.1 \pm 0.1 \times 10^4$.

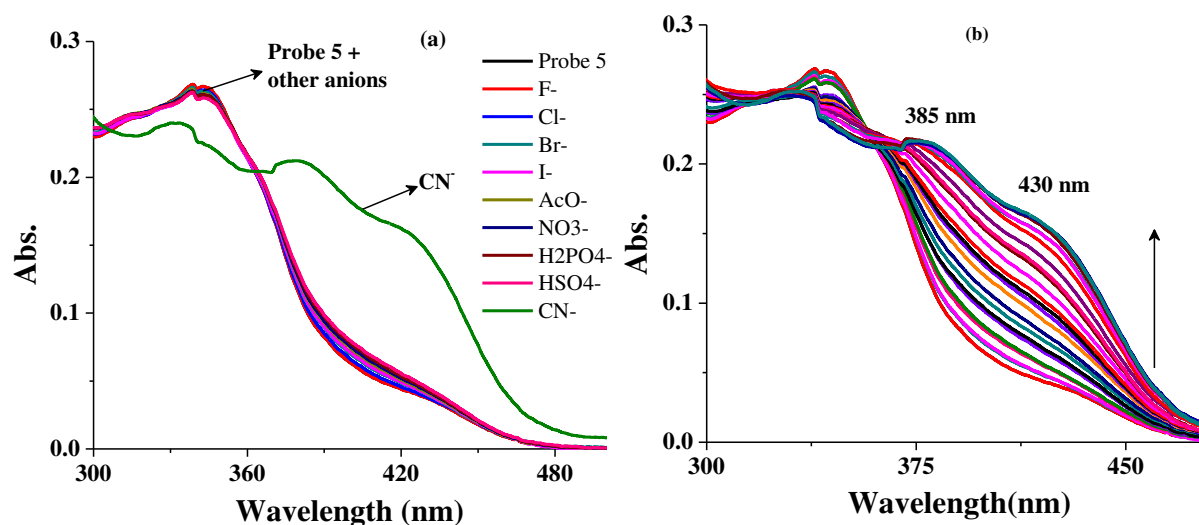


Figure 32: (a) Changes in absorption spectrum of probe **5** upon addition of 50 equivalents of various anions; (b) Effect of incremental additions of CN⁻ ions on absorption spectrum of probe **5** (20 μM, CH₃CN:H₂O::4:1).

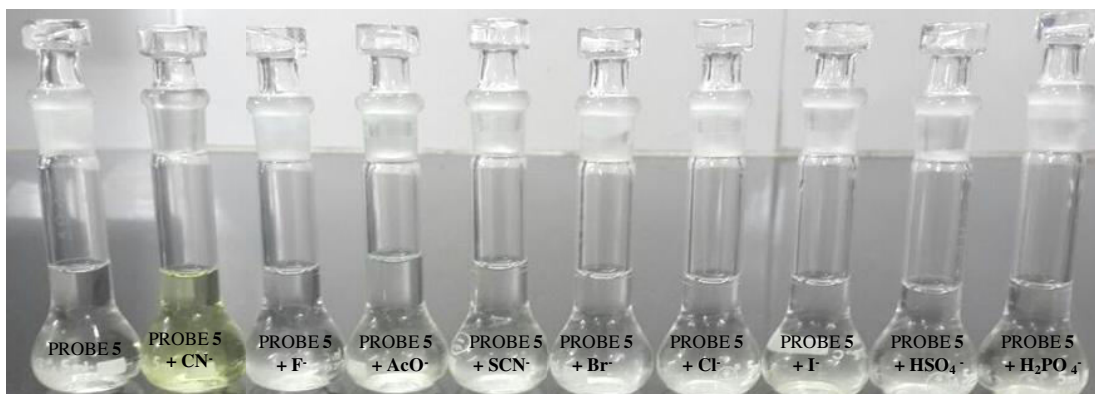


Figure 33: Visible color changes upon addition of various anions.

The probe **5** (10 μM, CH₃CN:H₂O, 4:1, v/v, HEPES buffer, pH = 7.2) upon excitation at ~340 nm exhibited a ESIPT emission band at 503 nm. The fluorogenic response of probe **5** has been observed by monitoring the changes in fluorescence behaviour after addition of the tested anions to a solution of probe **5**. Insignificant changes in the fluorescence behaviour of the probe were observed in the presence of the tested anions except for CN⁻ ions (Figure 34a). Gradual addition of CN⁻ ions to a solution of probe **5** (Figure 34b) resulted in a remarkable shift of the emission bands from 503 nm to 425 nm. The emission ratio at 425 nm and 503 nm varied from 0.1269 to 0.6218, indicating 4.89 fold emission ratio change. Binding constant was calculated to be $4.1 \pm 0.02 \times 10^4 \text{ M}^{-1}$. Notably, there was a linear dependence of the fluorescence intensity on the CN⁻ ion concentration in range from 25 nM to 500 nM ($R^2 = 0.9863$) with the detection limit of $2.0 \times 10^7 \text{ M}^{-1}$.

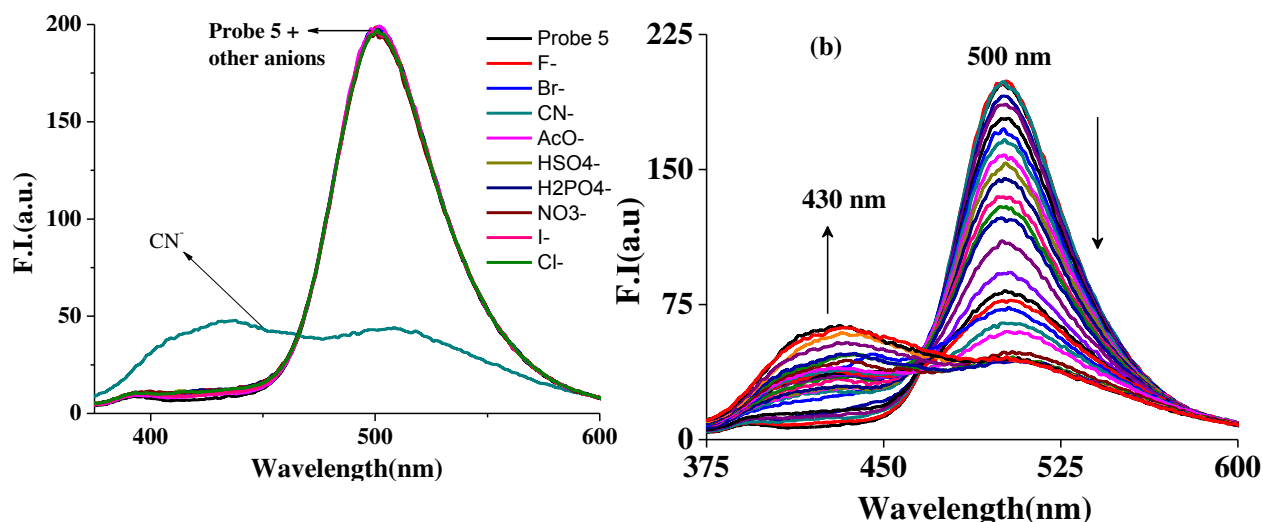


Figure 34: (a) Changes in emission spectrum of probe **5** upon addition of 50 equivalents of various anions (b) Effect of incremental additions of CN⁻ ions on emission spectrum of probe **2** (20 μ M, CH₃CN:H₂O::4:1).

To further evaluate the selectivity of probe **5** towards CN⁻, we also measured the absorption and emission intensities changes upon addition of varieties of anions. Specifically, 100 equiv. of each anion was added to the solution of probe **5** (20 μ M, CH₃CN:H₂O, 4:1, v/v, HEPES buffer, pH = 7.2) and incubated for 30 min at room temperature, before being subjected to spectral analysis. The absorption and emission intensities were enhanced remarkably only after addition of CN⁻ ions. The other studied anions like F⁻, Cl⁻, Br⁻, CH₃COO⁻, I⁻, HSO₄⁻, OH⁻, H₂PO₄⁻, NO₃⁻, SCN⁻, did not cause any remarkable change in the emission intensity. The competitive anion binding experiments were also conducted using 5.0 equiv. of CN⁻ ion in the presence of other anions (100 equiv.) and the same concentration of probe **5**. No noticeable changes were observed upon addition of other competing anions (Figure 35). This indicated that the probe **5** can be utilized conveniently for the detection of CN⁻ ion selectively.

In order to evaluate the nature of the interaction of CN⁻ ion with probe **5**, we commenced our investigations by monitoring the ¹H NMR spectral changes produced with the incremental addition of tetra-*n*-butylammonium cyanide (TBACN) to a solution of probe **5** (5 mM) in CD₃CN-D₂O. The proton signal of the hydroxyl group at δ 13.10 ppm exchanged due to fast exchange of H-bond on NMR time scale in the presence of D₂O. The signals at δ 9.93 ppm and δ 10.55 ppm were observed due -CHO protons *para* and *ortho* to O-H group, respectively (Figure 36).

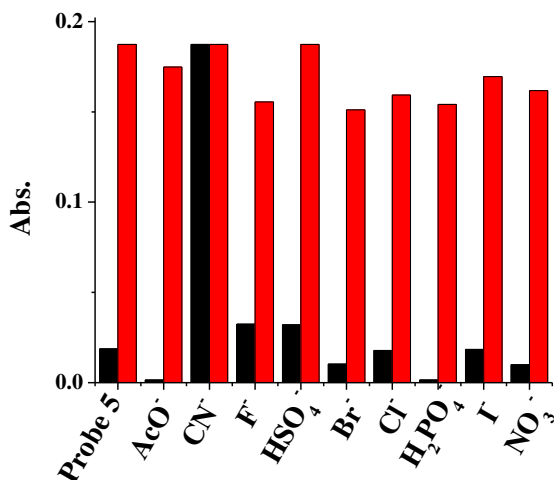


Figure 35: Red bars represent selectivity of probe **5** (20 μ M, CH₃CN:H₂O::4:1) upon addition of different anions and black bars shows the competitive selectivity of probe **5** in the presence of interfering anion.

The proton of -CHO group *ortho* to the -OH group appeared more downfield than the other due to strong intramolecular H-bonded 6-membered ring (due to RAHBs). On addition of CN⁻ ions, proton of *ortho* -CHO group at δ 10.55 disappeared and a new signal at δ 5.43 appeared. Whereas, -CHO proton *para* to the -OH group remained almost unchanged and aromatic protons shifted upfield (Figure 40).

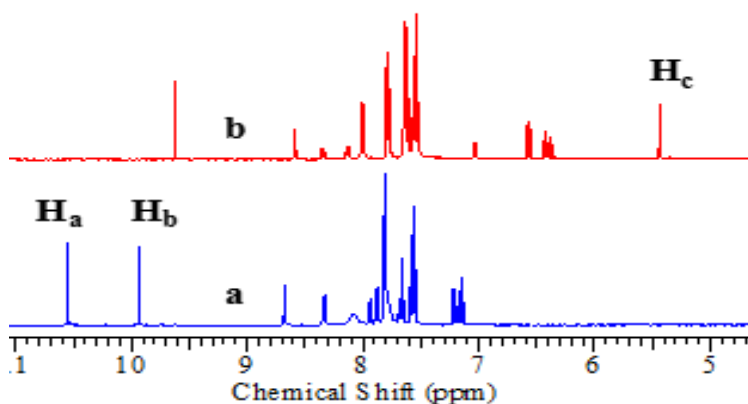
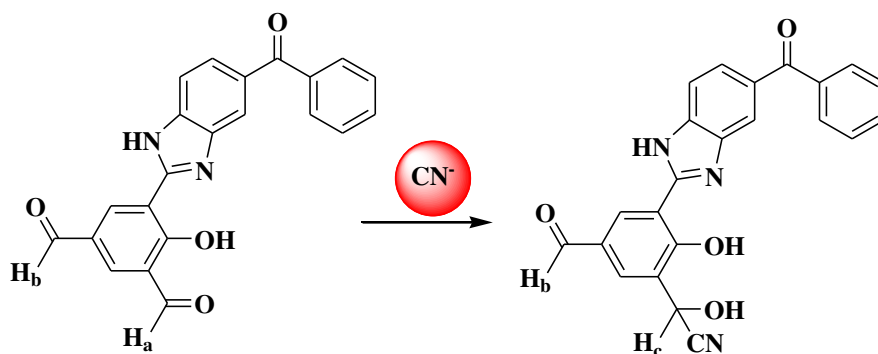


Figure 36: Partial ¹H NMR (400 MHz, CD₃CN:D₂O (4:1)) spectral changes upon addition of cyanide anions (CN⁻) to probe **5** (5 mM); (a) Probe **5** only; (b) Probe **5** + CN⁻ ions (1 equiv.).

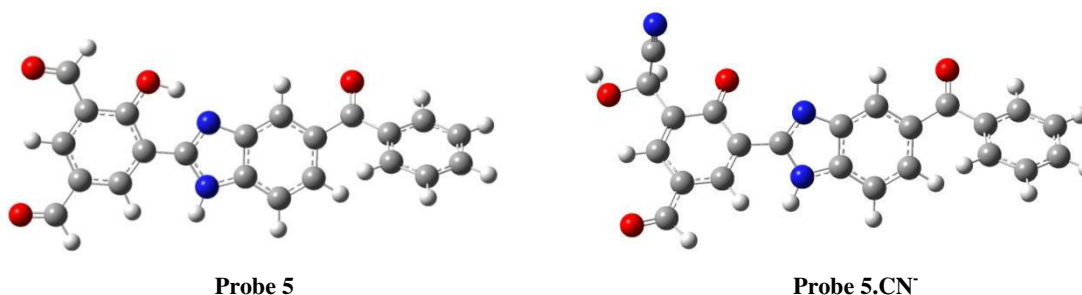
Thus, NMR studies clearly predicted the nucleophilic addition reaction of CN⁻ ion on the aldehyde group of probe **5** to form a cyanohydrin type compound (Scheme 5).



Scheme 5: Proposed mechanism of cyanide binding with probe **5**

2.5.4 Theoretical Studies

In order to seek more detailed information on the binding modes in these recognition processes, DFT and TD-DFT calculations in acetonitrile solvent at B3LYP/6-311 + G (d,p) (PCM) level were performed. These studies fully supported the ESIPT phenomenon's in probe **5** and the rapid nucleophilic attack of CN^- ion. The structure optimization of probe **5** in keto and enol tautomeric form showed the energy difference of 4.2 Kcal/mole which clearly predicted the extent of intramolecular hydrogen bonding in the ground state, required for ESIPT phenomenon's. Detailed information regarding the spectral changes upon the formation of probe **5**. CN^- complex was obtained from TDDFT calculations. The calculated excitation wavelength of probe **5**. CN^- complex was 355 nm, which red-shifted by 56 nm from that of probe **5** (411 nm). The magnitude of the calculated blue shift was in agreement with the experimentally observed one (62 nm). The HOMOs and LUMOs of probe **5** and probe **5**. CN^- complex are shown in Figure 37. The HOMO-LUMO transition contributed 100% and 97.4% to the excitation of probe **5** and **5**. CN^- complex, respectively (Figure 37).



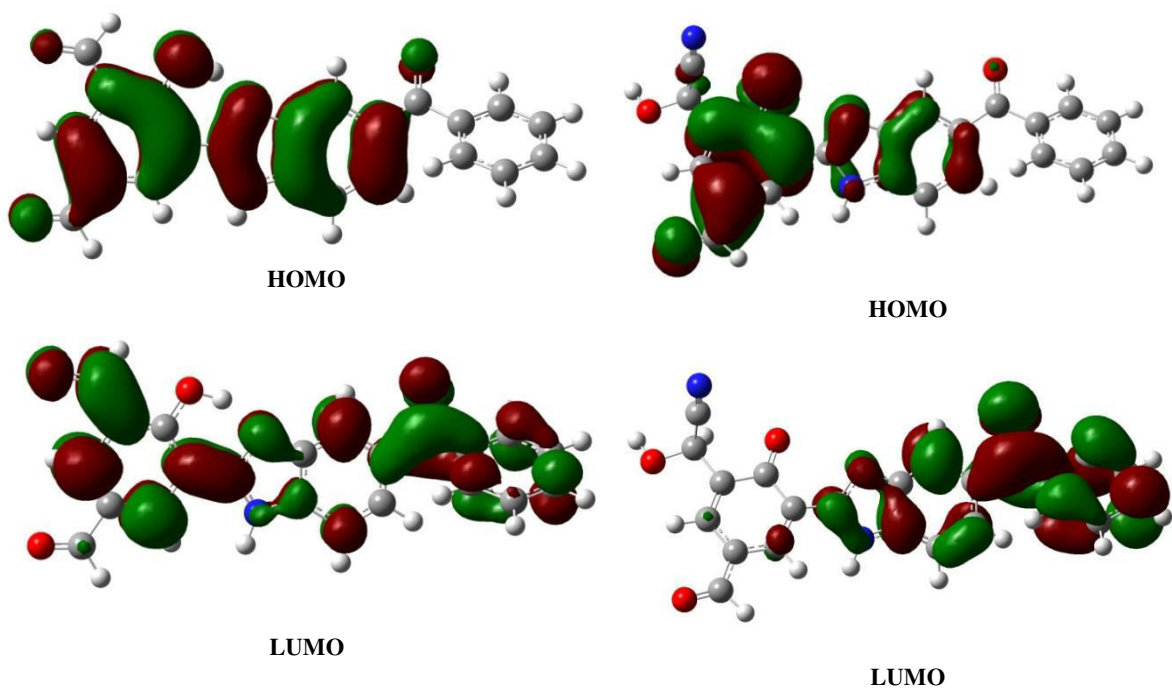


Figure 37: Structure optimization and frontier molecular orbitals of probe **5** and probe **5.CN⁻** complex.

2.5.5 Practical Applicability

Bearing in mind these favourable characteristics, to extend its performance to portable chemosensor kit, TLC strips of silica were coated with solution of probe **5** ($\text{CH}_3\text{CN}:\text{H}_2\text{O}::4:1$) and then dried under vacuum in order to check for residual contamination in contact mode. We prepared several samples of solution-coated TLC strips and studied the response of their fluorescence towards CN^- ions in contact mode and solution phase. Different concentrations of CN^- ions were placed over coated TLC strips for 5 sec. Upon illumination with UV lamp, blue spots were observed in the contact area (Figure 38). The minimum amount of CN^- ions detectable by the naked eye was as low as, thereby registering a detection limit at the 10^{-9} M.

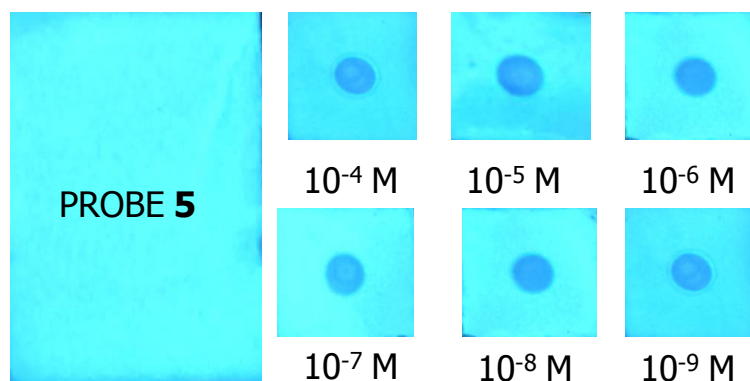


Figure 38: Photographs of probe **5** coated TLC strips under different experimental conditions.

The usability of probe **5** for determination of CN^- ions in biological samples has been shown by addition of cyanide ions into probe **5** in the presence of human blood serum. The general trends were quite similar to those observed in HEPES buffer. The addition of CN^- ions to the solution of probe **5** (20 μM , human blood serum–HEPES buffer–DMSO; 2 : 17 : 1; pH 7.4) is associated with ratiometric changes in emission intensity at 425 nm and 503 nm with an isosbestic point at 465 nm. As low as 100 nM (0.22 ppb) CN^- ions was measured in blood serum.

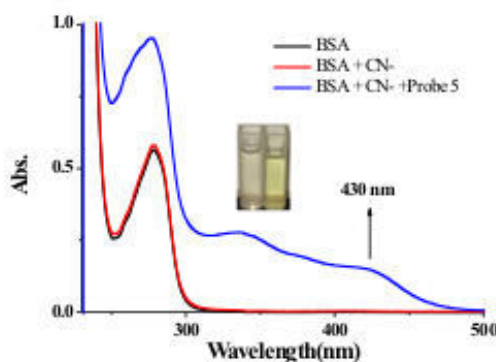


Figure 39: Effect of addition of probe **5** to plasma like solution containing BSA and CN^- ions on absorption spectrum in phosphate buffer

The similar kind of experiment was performed in plasma like solution containing BSA and CN^- (10 μM) in which probe **5** (20 μM) was added. Similar absorption changes were observed as in case of CN^- binding with probe **5** (Figure 39), which indicated that probe **5** can selectively

bound to CN^- in plasma like solution/blood serum in aqueous systems. Absorption spectra of this solution were recorded and displayed a sharp band at 278 nm with absorbance 1.01. Addition of 25 μL of cyanide ions decreased absorption intensity of 278 nm band to 0.822. Further addition of probe **5** (5 μM) to the above solution led to colour change from colourless to yellow and regeneration of absorption intensity back to 1.12 and a new hump near 380 nm region which is due to complexation of probe **5** with CN^- ions as shown previously. Titration was also done by adding different amounts of probe **5** to above solution. These results clearly indicated that cyanide bounded with blood serum can be set free by adding probe **5** which displaced cyanide from blood to itself.

2.5.6 Conclusion

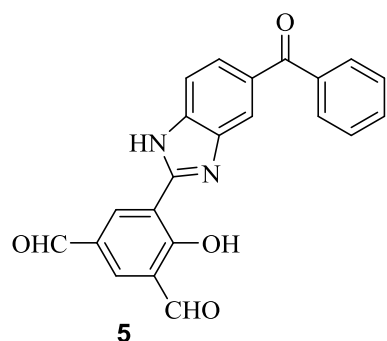
Benzimidazole substituted benzophenone probe **5** was synthesized, exhibiting RAHBs and ESIPT phenomenon's on single platform which was used for selective and ratiometric estimation of CN^- ions, using a chemodosimetric approach in aqueous system. Probe **5** led to the formation of two new absorption bands at 385 nm and 430 nm, while a new emission band at 430 nm in the presence of CN^- ions. This approach has been successfully used to detect CN^- ions in plasma like solutions as well as human blood serum. Probe **5** showed practical applicability to sense CN^- ions through dip coating method. Results were also correlated by NMR studies and theoretical methods. 385 nm and 430 nm

2.5.7 Experimental section

2.5.7.1 Synthesis of probe 5

Probe **2** (400 mg, 1.27 mmol) and hexamethylenetetraamine (356 mg, 2.54 mmol) were dissolved in 10 mL acetic acid and toluene (1:1) (scheme 4). The reaction mixture was refluxed for 8 h and reaction completion was monitored through TLC. After completion of reaction, the reaction mixture was cooled to room temperature and neutralized with ammonia solution. The solution was extracted with chloroform for 3 times. The organic layer was dried over sodium sulphate and concentrated to give yellow solid. The yellow solid was purified by column chromatography on silica gel using Hexane/EtOAc (6:4) as eluents to give pure probe **5** as a yellow solid.

Probe 5



78% yield; Yellow colored; M.pt. 240–241 °C; ^1H NMR (CDCl_3 + $\text{DMSO}-d_6$, 400 MHz): δ 10.55 (s, 1H, CHO), 9.92 (s, 1H, CHO), 8.68 (s, 1H, ArH), 8.31 (s, 1H, $J = 7.80$ Hz, ArH), 8.05 (bs, 1H, NH), 7.89–7.79 (m, 2H, ArH), 7.63 (t, 1H, $J = 7.78$ Hz, ArH), 7.53 (t, 3H, $J = 7.36$ Hz, ArH), 7.18 (d, 1H, $J = 8.28$ Hz, ArH), 7.01 (t, 1H, $J = 7.8$ Hz, ArH); ^{13}C NMR (CDCl_3 + $\text{DMSO}-d_6$, 100 MHz): δ 196.0, 190.0 (C=O), 163.6, 161.0, 137.7, 133.6, 132.3, 131.6, 130.4, 129.3, 127.9, 127.7, 124.9, 123.5, 118.5, 117.7, 114.0 and 112.7 (ArC); MS (ESI) m/z 389.1 ($\text{M}^+ + 1 + \text{NH}_4^+$).

2.5.5.2 Experiments with human blood serum: Human blood serum has been taken with thanks from the local Medical College and used as such. 1.0 ml of the serum was diluted in 10 ml biological ultrapure water (phosphate buffer, pH = 7.2). A certain amount of CN^- was added to this solution. Presence of 5 μM probe **5** in the serum solution turned its color dark yellow and release its actual absorbance of probe **5** with CN^- ions.

CHAPTER 3

Synthesis of hybrid molecules showing ESIPT-AIE phenomenon and their sensing behaviour

3.1 General Introduction: The sensors capable to analyse analytes in polar solvent like water have attracted the great attention due to their applicability in living system. We have discussed many sensors based upon ESIPT in previous chapters but to explore more effect of ESIPT based sensors in day to day life i.e. emission in polar solvents, AIE phenomenon was combined with it. Aggregation induced phenomenon i.e. AIE is a boon for science these days as even non-emissive molecules when dispersed in solution form, resulted in emission of higher intensity. The main reason for AIE effect is diminution of intramolecular torsional or rotational motions. Aggregation inhibits the internal rotations due to which the fluorescence of the compounds got switched “on” due to aggregation.

Advantages of AIE phenomenon's:

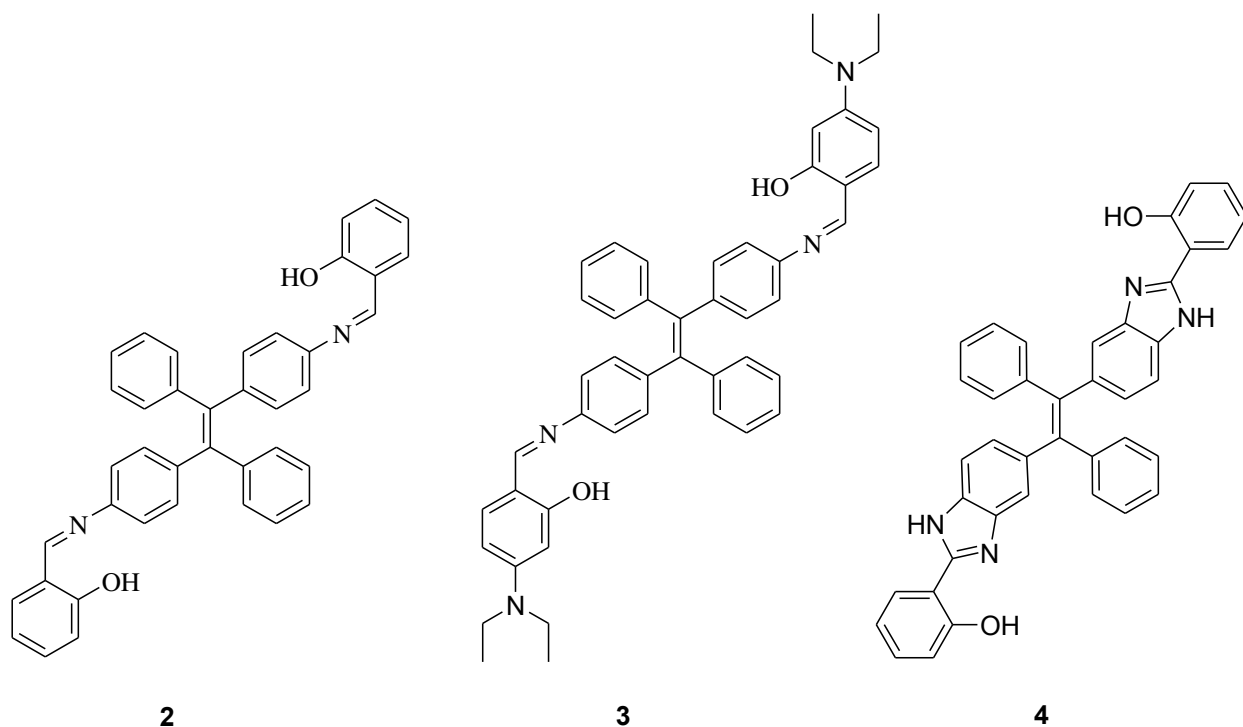
- Fluorogens i.e. Tetraphenylethylene (TPE) molecules with aggregation-induced emission (AIE) are novel class of materials used for biosensing and imaging applications.
- AIE fluorogens like tetraphenylethylene (TPE) and siloles derivatives experience increased emission when they are aggregated unlike conventional fluorophores that suffer from fluorescence quenching in high concentration or solid state due to π - π stacking interaction.
- These molecules have high quantum yields because of their unique propeller-like structure therefore intramolecular rotations are restricted in aggregated state, which prohibits energy dissipation *via* nonradiative channels.
- A number of AIE-based enzyme assays could be used for nuclease and phosphatase detection.

Advantages of ESIPT- AIE coupled phenomenon's:

- Molecules bearing both phenomenon's can emit white light having wide range from 400-800 nm that is boon for sensing biological ions.
- Self quenching effect at higher concentration of probe could be eradicated upon incorporation of ESIPT and AIE moieties onto single platform. .

- Large Stokes shift could be achieved with higher emission intensity in polar solvents.
- ESIPT incorporated with AIE phenomenon has not been studied yet.

Thus, we have synthesized different probes having ESIPT and AIE moieties onto single platform. Two different schiff bases of TPE were synthesized to study the effect of substituent on ESIPT as well as AIE. In final section benzimidazole as a ESIPT moiety and TPE as a AIE moiety has been coupled together and their photophysical properties has been studied. These molecules are discussed in detail as per following subchapters.



- 3.2 AIE-ESIPT coupled ratiometric fluorescent probe for detection of Cu^{2+} and F^- ions**
- 3.3 ESIPT-AIE coupled probe for detection of trivalent metal ions (Al^{3+} , Cr^{3+} and Fe^{3+}) in aqueous media**
- 3.4 Benzimidazole-TPE hybrid probe for ratiometric estimation of cations and anions**

3.2 AIE-ESIPT coupled ratiometric fluorescent probe for detection of Cu²⁺ and F⁻ ions

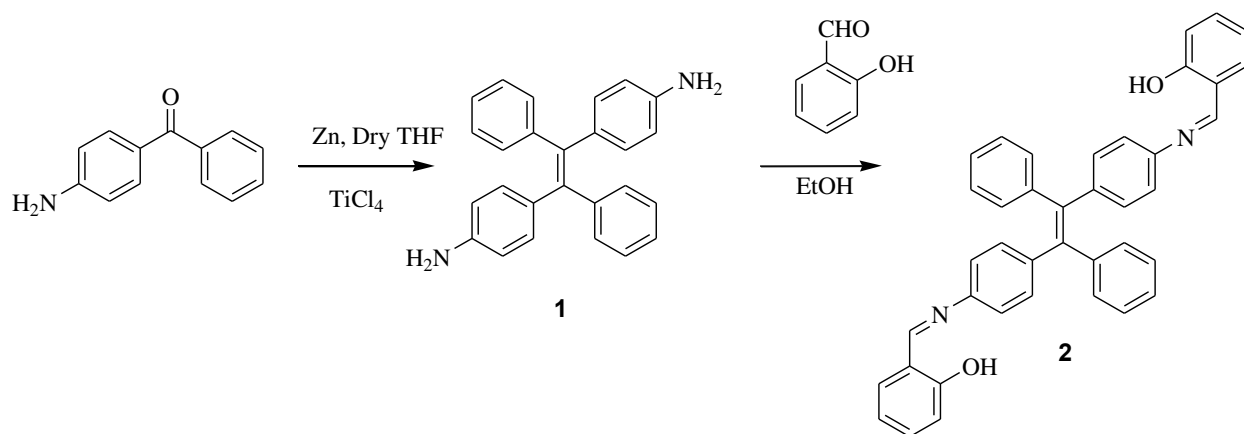
3.2.1 Abstract

We report a fluorescent probe **2** possessing hydroxyaryl schiff bases onto tetraphenylethylene (TPE) moiety having the characteristic of both aggregation induced emission (AIE) and excited-state intramolecular proton transfer (ESIPT). The coupled AIE and ESIPT shows the significant advantages, such as no self quenching at high concentration, large Stokes shift, high selectivity towards analyte etc. It has also been used for estimation of F⁻ and Cu²⁺ ions amongst anions and metal ions, respectively. Probe **2** showed the emission at 540 nm with a large Stokes shift of 170 nm and presence of Cu²⁺ ions induced the restriction of intramolecular motions led to high emission in the aggregated state (AIE) at longer wavelength (NIR region).

3.2.2 Synthesis of probes **1** and **2**

Synthesis- Probe **1** and **2** were prepared according to the synthetic routes shown in Scheme 1. Probe **1** was synthesized *via* McMurry coupling reaction between 4-amino benzophenone to give light yellow colored solid in 60 % yield. ¹H NMR spectrum showed two 4H multiplets at δ 7.23 and δ 7.12, 2H multiplet at δ 7.09 and two 4H multiplets at δ 6.50 and δ 6.85 for aromatic protons. ¹³C NMR spectrum showed signal at δ 142.8, 142.3, 142.2, 140.2, 132.8, 131.2, 131.1, 130.9, 128.0, 127.8, 126.9, 126.8, 120.7 and 120.6 of aromatic -C. This spectral data with absence of any peak beyond δ 150 confirmed that there is no C=O present corroborating the structure **1** for this compound.

Probe **1** on reaction with salicylaldehyde to form the schiff base (probe **2**) yellow colored solid in 94 % yield; m/e m/e 570.84 (M⁺)(Scheme 1). ¹H NMR spectrum showed the 2H singlet at δ 8.58 for OH (D₂O exchangeable), 4H multiplet at δ 7.35, 6H multiplet at δ 7.14, 14H multiplet at δ 7.05 and 2H triplet at δ 6.92 for aromatic protons. The extra peak at 8.58 confirmed the formation of Schiff base. ¹³C NMR spectrum showed signal at δ 162.0, 161.1 for imine -C 146.3, 143.4, 142.5, 140.5, 133.0, 132.4, 132.1, 131.4, 127.9, 126.8, 120.5, 119.2, 119.0 and 117.2 for aromatic -C. This spectral data having extra peaks at 162.0 and 161.1 than probe **1** was due to schiff bases that corroborates the structure **2** for this compound.



Scheme 1: Synthetic routes for probe **1** and **2**

We succeeded in growing the crystal of probe **2** in the mixture of THF and ethanol by solvent evaporation method, then single crystal XRD studies showed that crystal has triclinic structure, having (P-1) space group with $R_1 = 12.32\%$ and GooF value to be 1.033 (Figure 1 and Table 1). Unit cell dimensions were found to be as; $a = 9.26(2) \text{ \AA}$, $b = 11.45(3) \text{ \AA}$, $c = 14.67(4) \text{ \AA}$, $\alpha = 95.39(4)^\circ$, $\beta = 96.95(3)^\circ$, $\gamma = 107.68(2)^\circ$, Volume = $1457.7(7) \text{ \AA}^3$ and $Z = 2$

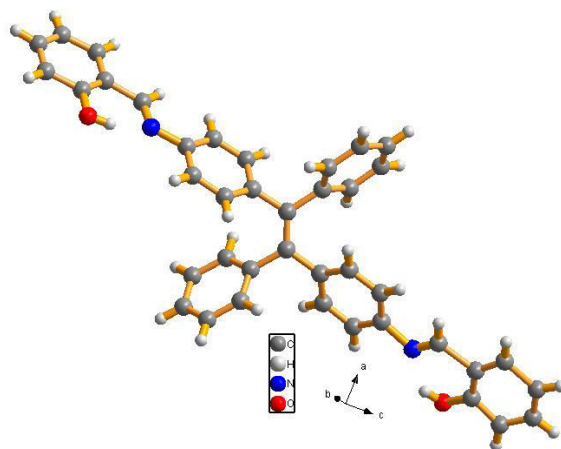


Figure 1: X-Ray crystal structure of probe **2**

Table 1: Summary of crystal data, data collection and structure refinement for probe **2**.

A. Crystal Data

Chemical formula	$C_{40}H_{30}N_2O_2$
Formula weight	570.66 g/mol
Temperature	296(2) K
Wavelength	0.71073 $\text{\AA}$

Crystal system	triclinic
Space group	P-1
Unit cell dimensions	$a = 9.26(2) \text{ \AA}$ $\alpha = 95.39(4)^\circ$ $b = 11.45(3) \text{ \AA}$ $\beta = 96.95(3)^\circ$ $c = 14.67(4) \text{ \AA}$ $\gamma = 107.68(3)^\circ$
Volume	$1457.(7) \text{ \AA}^3$
Z	2
Density (calculated)	1.300 g/cm^3
Absorption coefficient	0.080 mm^{-1}
F(000)	600

B. Data collection and structure refinement

Theta range for data collection	1.41 to 25.95°
Index ranges	$-11 \leq h \leq 11$, $-13 \leq k \leq 13$, $-17 \leq l \leq 17$
Reflections collected	14353
Independent reflections	5330 [R(int) = 0.0500]
Coverage of independent reflections	93.5%
Absorption correction	multi-scan
Refinement method	Full-matrix least-squares on F^2
Refinement program	SHELXL-2014/7 (Sheldrick, 2014)
Function minimized	$\sum w(F_o^2 - F_c^2)^2$
Data / restraints / parameters	5330 / 0 / 313
Goodness-of-fit on F^2	1.003
Final R indices	2181 data; $I > 2\sigma(I)$ $R_1 = 0.1232$, $wR_2 = 0.3252$ all data $R_1 = 0.2265$, $wR_2 = 0.4007$
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.1771P)^2 + 2.2973P]$ where $P = (F_o^2 + 2F_c^2)/3$
Absolute structure parameter	0.0(10)
Extinction coefficient	0.0090(40)
Largest diff. peak and hole	0.559 and $-0.512 \text{ e\AA}^{-3}$
R.M.S. deviation from mean	$0.073 \text{ e\AA}^{-3}$

3.2.3 Results and discussion

3.2.3.1 Aggregation-induced emission characteristics

To test the AIE characteristic of probe **2**, the effect of water volume fraction on emission was evaluated in H₂O/CH₃CN mixed media. A dilute solution of probe **2** (10 μ M, CH₃CN), emits faintly at 540 nm upon photoexcitation at 370 nm. However, with increasing the percentage of water fraction upto 90% (volume fraction) to the solution of probe **2** in CH₃CN, a gradual enhancement in emission was observed at 540 nm with slight hump at 570 nm (Figure 2). The enhanced emission induced by the formation of aggregates in water is typical phenomenon's for AIE-active compounds. The appearance of mixture was transparent and homogeneous, suggesting that the molecules aggregated and suspended in the mixture in nano-sized. In addition, Stokes shift as large as 170 nm was observed for probe **2** as a result of ESIPT. In low water volume fractions, ESIPT process of probe **2** was suppressed due to non-radiative way of excited states, which might be ascribed to free rotation around C=N. However because of high water volume fraction, rotation was restricted due to closely packed molecule in aggregated state, resulting in strong emission.

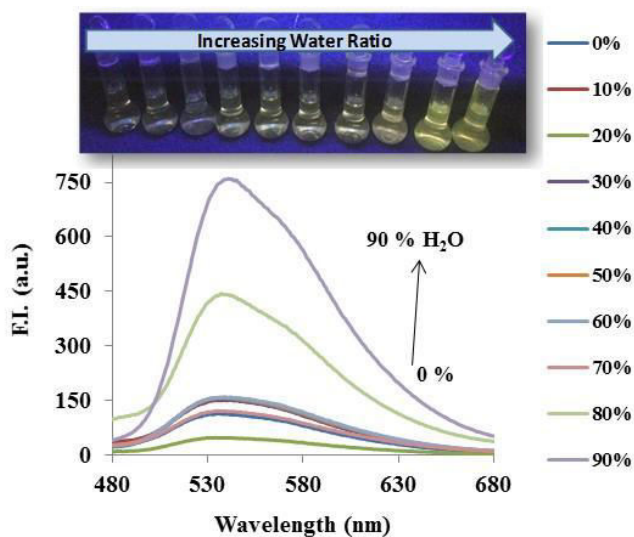


Figure 2: Emission spectrum of solution of probe **2** in CH₃CN and increasing CH₃CN /water ratio. Inset showed the emission color changes of probe **2** in CH₃CN and increasing water ratio under UV light.

To further investigate the aggregation behaviour of probe **2**, dynamic light scattering (DLS) studies FE-SEM and TEM analysis were performed by dissolving probe **2** in CH₃CN and its binary solution of 90% water/CH₃CN ratio. DLS measurements of probe **2** (10 μ M, H₂O–

CH₃CN; 9 : 1) solution showed the formation of aggregates with sizes ranging between 210 and 350 nm (average size 250 nm) with 0.364 PDI whereas in CH₃CN solvent aggregates could not be observed. FE-SEM and TEM images from binary solution of probe **2** (10 μM, H₂O– CH₃CN; 9 : 1) showed the formation of spherical aggregates with ~ 300 nm size (Figure 3a-d). Thin films obtained from the solution of probe **2** (CH₃CN) showed the presence of well-dispersed particles with sizes between 30 and 60 nm (Figure 2) which is in agreement with DLS studies.

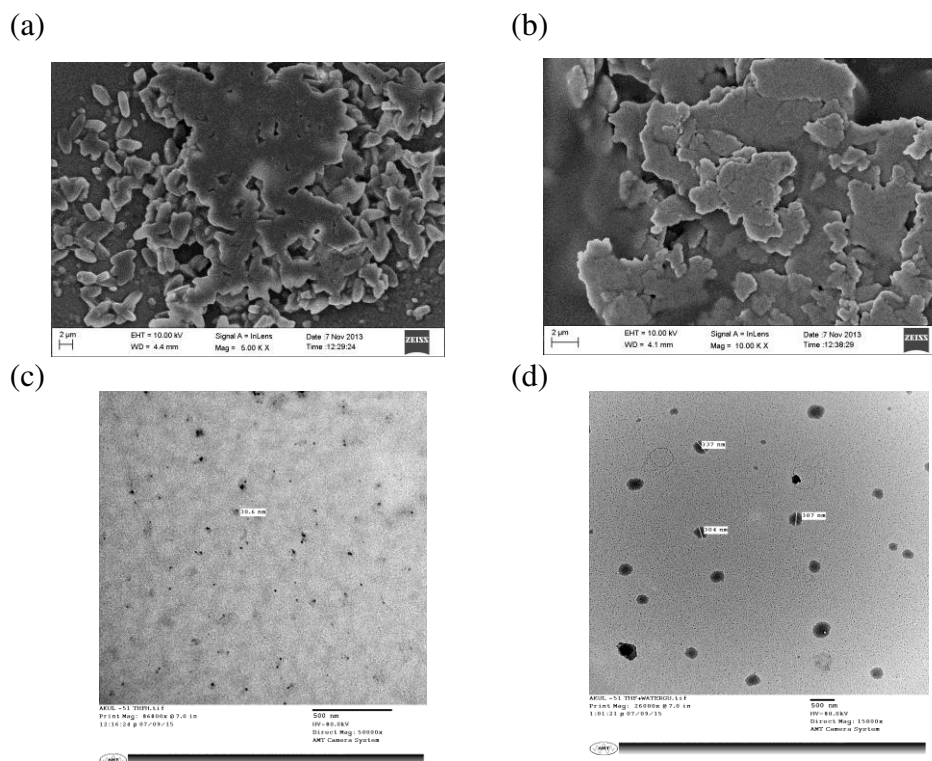


Figure 3: SEM images in (a) CH₃CN and (b) CH₃CN:H₂O::1:9; TEM images in (c) CH₃CN and (d) CH₃CN:H₂O::1:9 of probe **2**.

Probe **2** is therefore induced to emit light by aggregate formation; in other words, it is AIE active. In the dilute CH₃CN solution, the phenyl rings of the TPE core can rotate freely against the central olefinic double bond, which non-radiatively deactivates the excited species and renders the dye non-emissive. The intramolecular motions are restricted in the nano-aggregates in the CH₃CN/water mixture with high water fraction, which block the non radiative decay channels and make dye molecules highly luminescent.

Table 2. Time-resolved fluorescence decay parameters of probe **2** in different environments.

Solvent	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	α_1	α_2	α_3	τ_f (ns)
CH ₃ CN	0.08	-----	-----	8.22×10^8	---	---	0.08
CH ₃ CN :H ₂ O (1:9)	0.31	-----	-----	53330.84	---	--	0.31

The results were correlated with time resolved fluorescence experiments for probe **2** in CH₃CN and CH₃CN/water ratio. Results revealed a single exponential decay for probe **2** emission and the lifetime was found to be 0.08 ns in CH₃CN (Table 2). To lay emphasis on AIE effect, lifetime in case of CH₃CN:H₂O: 1:9 has been determined which was found to be 0.31 ns and single exponential decay. These results revealed that by adding water, the decay of the excited state was slower and remain for more time, which clearly depicted the presence of AIE phenomenon's (Figure 4).

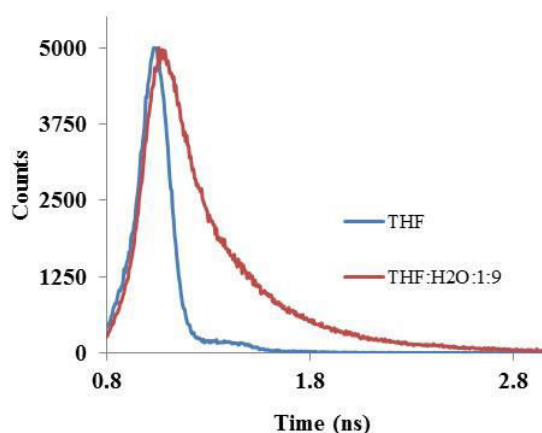


Figure 4: Typical time-resolved fluorescence decay profile of probe **2** in CH₃CN and CH₃CN:H₂O: 1:9 solvents as indicated in the figure legend.

3.2.3.2 Excited state intramolecular proton transfer (ESIPT) characteristics of probe **2**

Probe **1** (20 μ M, CH₃CN) showed absorption bands at 330 nm and 263 nm having emission band at 410 nm after exciting at 330 nm (Stokes shift = 80 nm) whereas probe **2** displayed absorption bands at 370 and 275 nm with emission band at 540 nm after exciting at 370 nm (Stokes shift = 170 nm) (Figure 5). Absorption band in case of probe **2** is on longer wavelength side due to

presence of additional conjugation of salicylaldehyde moiety whereas emission band is on higher side due to ESIPT phenomenon's in excited state as it has proton acceptor/donor sites. This confirms probe **2** displayed ESIPT phenomenon's having Stokes shift of 170 nm.

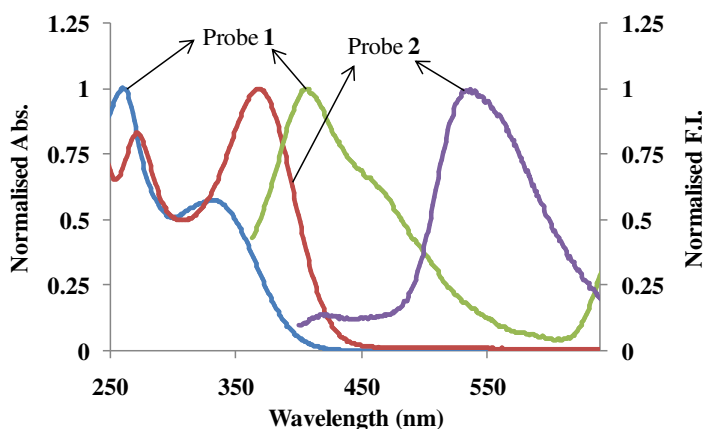


Figure 5: Normalized graph of absorption and emission spectra of probes **1** and **2**.

3.2.3.3 Sensing ability of probe **2** towards anions

The sensing ability of probe **2** to various anions was examined in CH_3CN . The probe **2** displayed an absorption maxima at 370 nm and no obvious interference after addition of 50 equiv. of anions viz., CN^- , Cl^- , Br^- , AcO^- , I^- , HSO_4^- , $\text{P}_2\text{O}_7^{4-}$, SCN^- , NO_3^- and H_2PO_4^- . However, upon addition of same amount of F^- ions, the absorption intensity at 370 nm decreased and new red shifted absorption band formed at 440 nm. In addition, color of the solution of probe **2** changed from colorless to extremely dark yellow (Figure 6a). Absorption titration results showed that intensity change ($A_{440 \text{ nm}}/A_{370 \text{ nm}}$) from 0.011 to 1.183, indicating 107 fold absorption intensity change for probe **2**. Probe **2** (CH_3CN) exhibited an emission band at 540 nm with a large Stokes shift of 170 nm after excitation at 370 nm. Upon addition of various anions, only F^- ion was able to trigger distinct fluorescence changes. The addition of F^- ions between 0.5 μM - 20 μM led to the formation of new emission band at 490 nm and further addition of F^- ions between 20 μM - 100 μM resulted in red shift to 565 nm (Figure 6b). The binding constant as calculated from Benesi Hildebrand plot was found to be $1.18 \pm 0.01 \times 10^5 \text{ M}^{-1}$. The lowest detection limit was determined to be 0.5 μM , which was much lower than that of the enforceable drinking water standard for F^- (210 μM) set by the United States Environmental Protection Agency (EPA). Thus, only F^- was able to trigger the distinct optical as well as colorimetric response of probe **2**,

whereas other anions like CN^- , Cl^- , Br^- , AcO^- , I^- , HSO_4^- , SCN^- , $\text{P}_2\text{O}_7^{4-}$, NO_3^- and H_2PO_4^- could cause negligible spectroscopic and visual signal changes. Therefore, probe **2** was demonstrated to be insusceptible to the interference from anions like CN^- , Cl^- , Br^- , AcO^- , I^- , HSO_4^- , $\text{P}_2\text{O}_7^{4-}$, SCN^- , NO_3^- and H_2PO_4^- .

To determine the practical applications of probe **2** towards F^- ions, competitive experiments were performed with 1000 μM of F^- and 1000 μM of other anions (CN^- , Cl^- , Br^- , I^- , SCN^- , AcO^- , NO_3^- , H_2PO_4^- , $\text{P}_2\text{O}_7^{4-}$ and HSO_4^-) in a solution of probe **2** in CH_3CN . The absorption/emission spectrum of probe **2** with F^- was not influenced by the subsequent addition of competing anions.

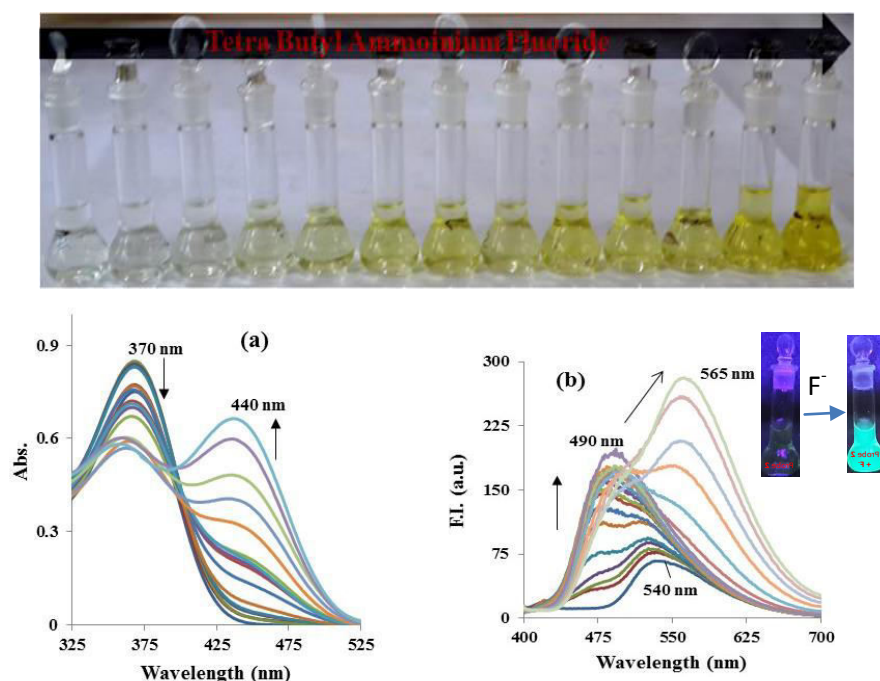


Figure 6: Effect of incremental addition of TBAF on (a) UV-vis absorption spectrum; (b) emission spectrum of probe **2** (20 μM in CH_3CN). Inset: photographs of optical changes upon addition of TBAF.

^1H NMR titrations in $\text{CD}_3\text{CN}-d_3$ was carried out to explore the sensing mechanism of probe **2** to F^- ions. It was easy to find out that the O-H proton of probe **2** at 13.28 ppm disappeared completely with increase in concentration of F^- ions, while the signals of other aromatic protons remain unaltered (Figure 7). The disappearance of signal clearly predicted the deprotonation of OH proton.

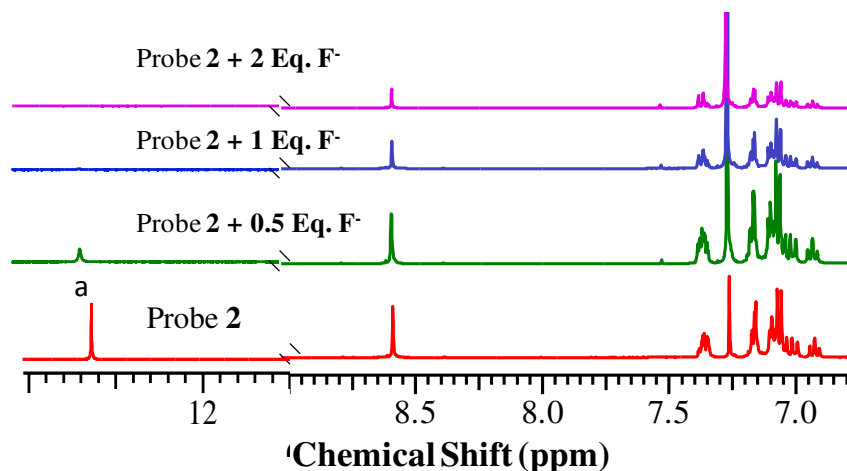


Figure 7: Partial ^1H NMR spectral (14 - 6.6 ppm region) of probe **2** (5 mM $\text{CD}_3\text{CN-d}_3$) in the presence of variable amount of TBAF.

3.2.3.4 Sensing ability of probe **2** towards cations

To get further insight into the sensing properties of probe **2** towards metal ions, absorption and emission variations caused by various metal ions, including Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Zn^{2+} , Cu^{2+} , Cr^{3+} , Co^{2+} , Ni^{2+} , Fe^{3+} , Ba^{2+} , Ag^+ , Hg^{2+} etc. were investigated. Only the presence of Cu^{2+} showed the absorption and emission changes towards probe **2**.

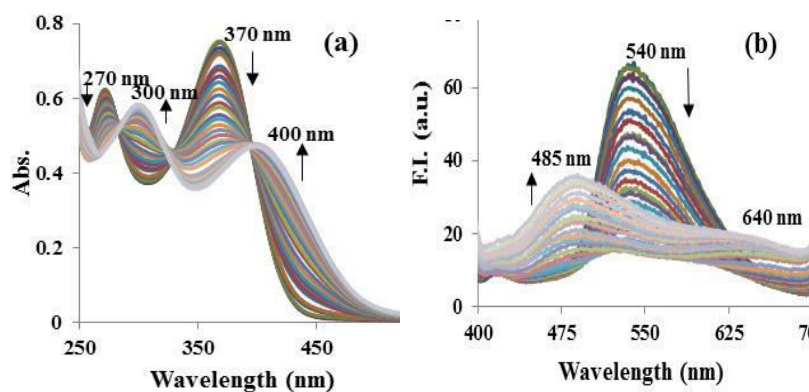


Figure 8: Effect of addition of Cu^{2+} ion on (a) absorption and (b) emission spectra of probe **2** (20 μM in CH_3CN).

Upon incremental addition of Cu^{2+} ions (200 μM) to the solution of probe **2**, the absorption intensity at 370 nm was gradually decreased and a new absorption band appeared at 400 nm (Figure 8a). This gradual addition of Cu^{2+} ions leading to decrease in absorption intensity at 370 nm and increase at 400 nm provided the opportunity to determine Cu^{2+} ions. The absorption ratio

at 370 and 400 nm varied from 0.513 to 1.118, indicating 2.18 fold absorption ratio change. Thus, probe **2** could be used to estimate a wide range of Cu^{2+} ions between 0.5-200 μM with lowest detection value of 500 nM.

Upon excitation of probe **2** at 370 nm, emission band was shown at 540 nm which upon addition of 0-20 μM Cu^{2+} ions quenched. However, further addition of Cu^{2+} ions between 20-100 μM showed the emergence of two new emission bands at 485 nm and 640 nm (vide supra NIR region) was observed (Figure 8b). The lowest detection limit was found to be 500 nM with binding constant of $5.3 \pm 0.01 \times 10^5 \text{ M}^{-1}$, further explore binding behavior of probe **2** with Cu^{2+} ion, Job's plot was plotted which revealed 1:1 stoichiometry in case of Cu^{2+} . While checking the interference of Cu^{2+} ions with other metal ions, a simple experiment was performed with 10 equiv of Cu^{2+} and 50 equiv. of various other cations (Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Zn^{2+} , Cr^{3+} , Co^{2+} , Ni^{2+} , Fe^{3+} , Ba^{2+} , Ag^+ , Hg^{2+}) in a solution of probe **2** in CH_3CN (Figure 9). The absorption/emission spectrum of probe **2** with Cu^{2+} was not influenced by the subsequent addition of competing metal ions.

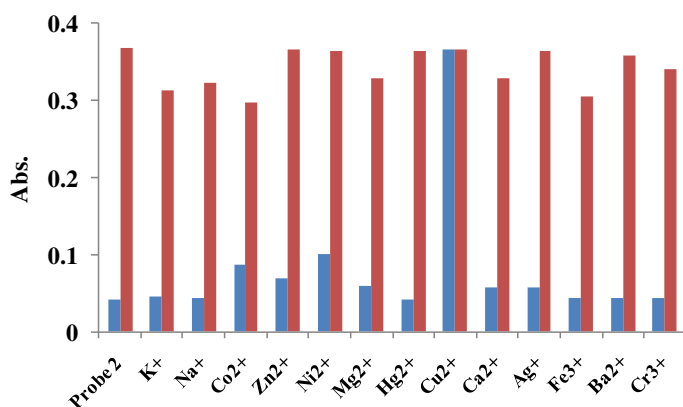


Figure 9: Blue bars represent selectivity of probe **2** (20 μM) upon addition of different metal ions in CH_3CN and red bars show the competitive selectivity of probe **2** in the presence of Cu^{2+} ions.

3.2.3.5 Time-resolved fluorescence decay behavior

Fluorescence lifetimes have been monitored under differential circumstances as in order to achieve a deeper insight into the photophysics of probe **2** and its modulation in the presence of metal ions. Figure 10 depicted the time resolved fluorescence decay profile of probe **2** under varied experimental conditions and the relevant data has been compiled in Table 3. The results

revealed a single exponential decay for probe **2** emissions and the lifetime was found to be 0.08 ns in CH₃CN. The presence of Cu²⁺ ions (100 μM) showed an even slower but bi-exponential decay at $\tau_1 = 0.08$ ns and $\tau_2 = 1.35$ ns). However, in case of 100 μM of F⁻ ions, the decay was more delayed ($\tau_1 = 5.40$ ns, $\tau_2 = 2.73$ ns and $\tau_3 = 0.07$ ns) and moreover, tri-exponential was observed.

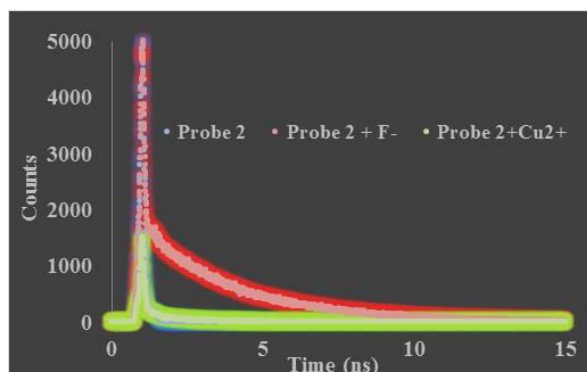


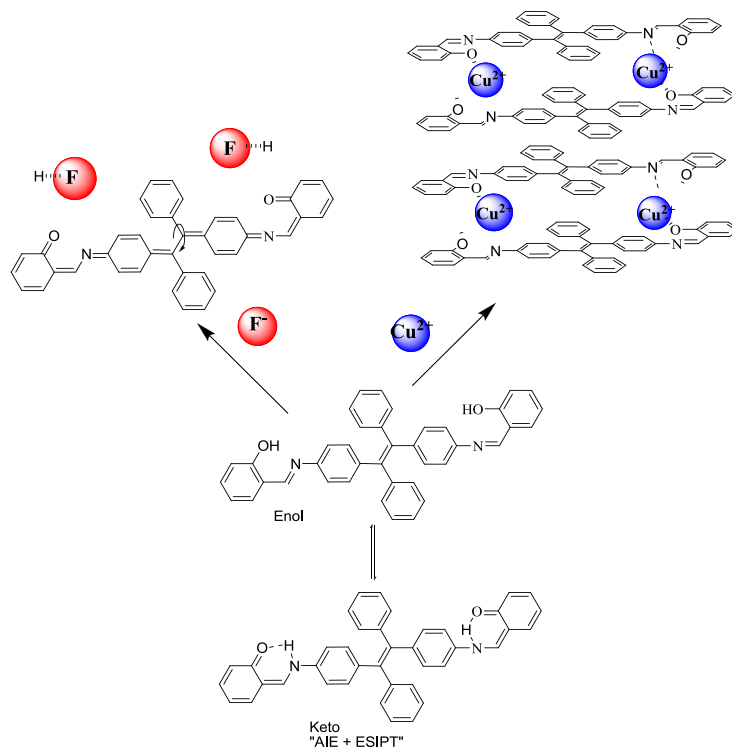
Figure 10: Typical time-resolved fluorescence decay profile of probe **2** in the presence of CH₃CN, 100 μM each of Cu²⁺ and F⁻ ions in ACN solvent.

Table 3: Time resolved fluorescence decay parameters of probe **2** in the presence of Cu²⁺ and F⁻ ions.

	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	α_1	α_2	α_3	τ_f (ns)
Probe 2	0.08	-----	-----	8046.94	-----	-----	0.08
Probe 2 + F ⁻	5.40	2.73	0.07	298.54	2127.36	1.96×10^{10}	0.07
Probe 2 + Cu ²⁺	0.08	1.35	-----	2.6×10^8	324.70	-----	0.08

Based on Job's plot and NMR titrations, a mechanism has been proposed for binding of probe **2** with F⁻ and Cu²⁺ ions. The schiff base moiety in the probe **2**, possessed ESIPT characteristics; it has both enol (E*) and keto (K*) emission, where K* emission formed through ESIPT. Because of AIE characteristics of TPE moiety in probe **2**, in non-aggregated state, the intramolecular hydrogen bonding of schiff base is deprotected by the presence of F⁻ ions, and E*/K* emission ratio increased *via* smooth ESIPT process. The presence of Cu²⁺ ions induced the aggregated state due to the strong coordination of N

and O atoms with Cu^{2+} ions. Therefore, in aggregated state, the intramolecular hydrogen bonding of schiff base is protected by the presence of Cu^{2+} ions, and K^*/E^* emission ratio increased. Thus, emission due to ESIPT+AIE phenomenon's was achieved at 640 nm (**Scheme 2**).



Scheme 2: Proposed mechanism of probe **2** with Cu^{2+} and F^- ions

3.2.4 Theoretical studies

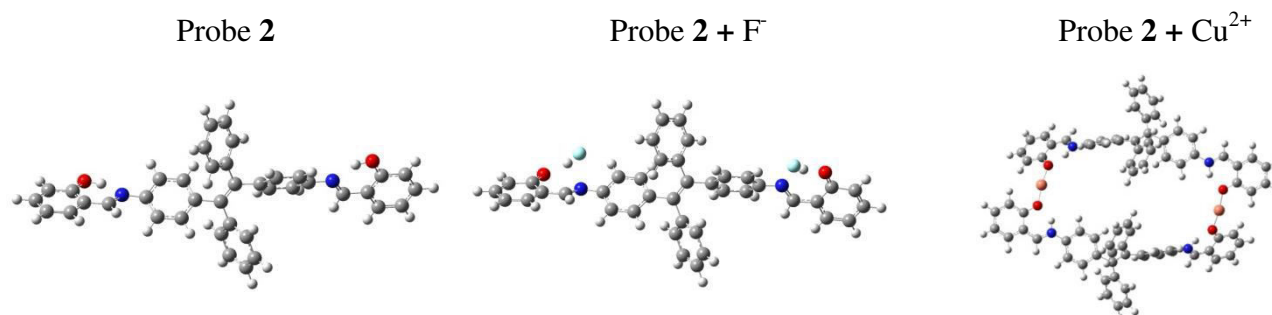
To know more regarding structure layout of probe **2**, optimization by using B3LYP functional in combination with 6-31G (d,p) has been performed. Probe **2** structural properties resembled with that of crystal data. It has been revealed that the molecule was non-planar, and the hydrogen of hydroxyl group bent towards imine bond on both ends (Figure 11).

In order to provide insight into the mode of complexation of probe **2** towards F^- and Cu^{2+} ions, the structures of probe **2**. F^- and probe **2**. Cu^{2+} complexes were optimized by using the B3LYP functional in combination with 6-31G (d,p) for probe **2** + F^- complex and LAN2DZ basis sets for complex of probe **2** + Cu^{2+} ions. The presence of fluoride ions caused deprotonation of O-H group on both ends as predicted by NMR whereas Cu^{2+} ion in proximity to probe **2**, showed

strong interactions with oxygen of OH group and link two molecules of probe **2** to form aggregated structure (Figure 11). Further, time dependent density functional theory (TD-DFT) calculations on probe **2** and its complexes with F^- and Cu^{2+} ions have also been performed. It has been observed that HOMO is distributed over the whole molecule and LUMO not covering the phenyl rings. These rings are almost perpendicular to the molecule and reverse in the probe **2** + F^- complex but associated with decrease energy gap between the HOMO and LUMO and thus the absorption band is shifted at longer wavelength. The new band formed was mainly because of HOMO to LUMO+1 having highest oscillator strength (Table 4). In case of probe **2** + Cu^{2+} complex, HOMO - 7 is distributed on whole of the molecule but LUMO + 1 is occupied mainly on both copper ions bound with two molecules of probe **2**.

Table 4: Excitations for probe **2** that contribute to the transitions in the 250-700 nm and their expansion coefficients in gaseous phase.

	λ_{exc} (nm) (Theoretical Value)	λ_{exc} (nm) (experimental Value)	E_{exc} (eV)	Oscillator Strength	Excited State	% Contri bution
Probe 2	425	370	2.9119	1.1143	H to L	100
Probe 2 + F^-	403	440	3.0799	0.7419	H to L+1	75
Probe 2 + Cu^{2+}	383	400	3.2368	.2656	H-7 to L+1 H-8 to L+2	55 35



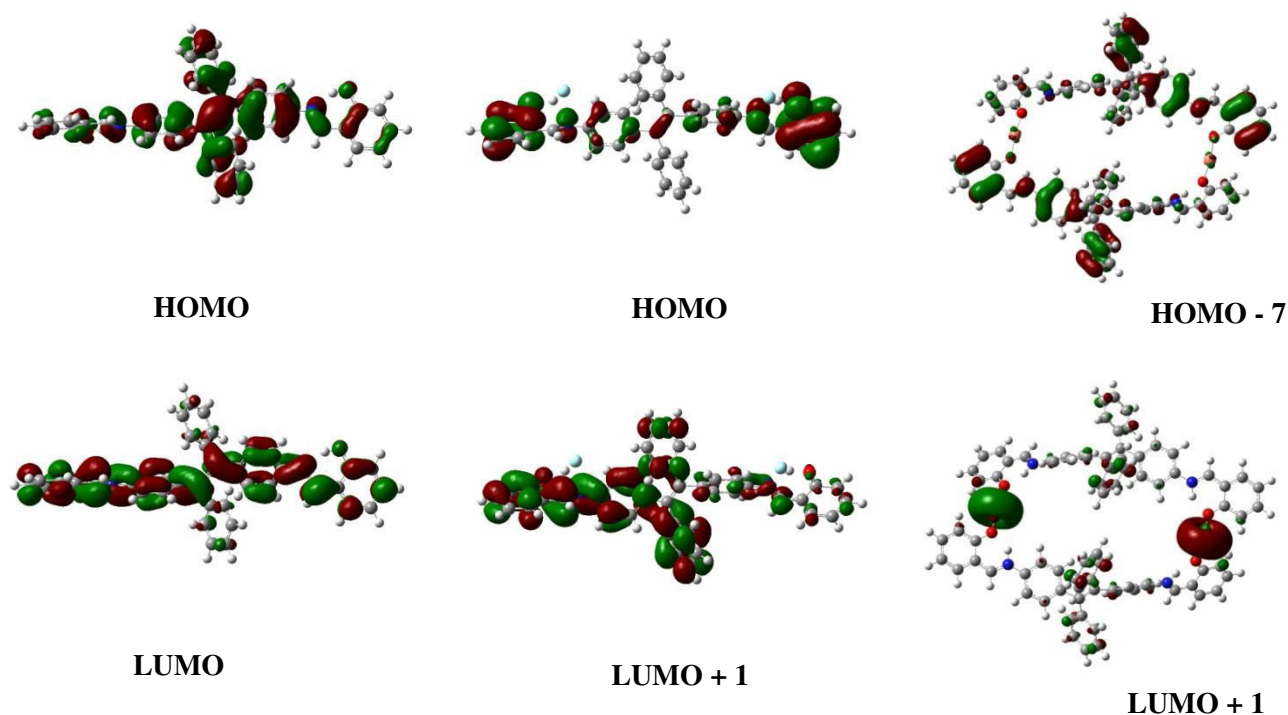


Figure 11: Optimized structures, HOMO and LUMO of respective probe **2**, probe **2** + F⁻ and probe **2** + Cu²⁺

3.2.5 Conclusion

We have designed a fluorescent probe **2**, possessing schiff base and TPE fluorogen onto a single platform with both AIE and ESIPT characteristics. The combination of these two emission mechanisms provide unique photophysical properties that are highly desirable for sensing of biologically important cations and anions. This combined phenomenon based platform provides the opportunity of large Stokes shift, self quenching free probe, and selective estimation of F⁻ and Cu²⁺ ions. The fluorescent response enables determination of F⁻ and Cu²⁺ with a detection limit of 5 μ M and 500 nM, respectively.

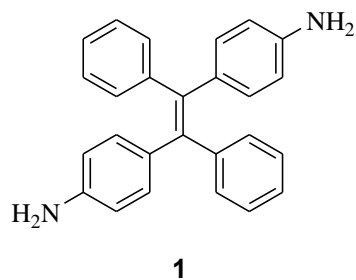
3.2.6 Experimental Section

3.2.6.1 Synthesis of probes **1** and **2**

Synthesis of probe 1 - Under N₂ atmosphere, a three-necked flask equipped with a magnetic stirrer was charged with zinc powder (1.97 g, 30 mmol) and 40 mL freshly distilled THF was added. The mixture was cooled to -5 °C, and TiCl₄ (1.66 mL, 15 mmol) was slowly added by a syringe with the temperature kept under 10 °C. The suspending mixture was warmed to room

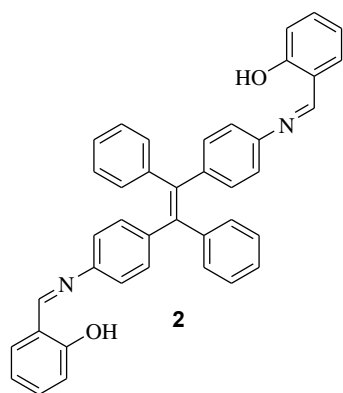
temperature and stirred for 0.5 h, then heated to reflux for 2.5 h. The mixture was again cooled to -5 °C, solution of 4-amino benzophenone (2g, 10 mmol) in 15 mL THF was added slowly. After addition, the reaction mixture was heated at reflux until the carbonyl compound was consumed (monitored by TLC). The reaction was quenched with 10% K₂CO₃ aqueous solution and extracted with EtOAc. The organic layer was collected and concentrated. The crude material was purified by column chromatography to give the desired products compound **1**.

4-((E)-2-(4-aminophenyl)-1,2-diphenylvinyl)benzenamine (Probe 1)



60% yield (1.1 g); Light yellow colored; M.Pt. > 300 °C; ¹H NMR (CDCl₃, 400 MHz), δ (ppm): 7.26–7.19 (m, 4H, ArH), 7.14–7.11 (m, 4H, ArH), 7.10–7.08 (m, 2H, ArH), 6.00–6.96 (m, 4H, ArH), 6.89–6.84 (m, 4H, ArH). ¹³C NMR (CDCl₃, 100 MHz), δ (TMS, ppm): 142.8, 142.3, 142.2, 140.2, 132.8, 131.2, 131.1, 130.9, 128.0, 127.8, 126.9, 126.8, 120.7, 120.6 (ArC).

Synthesis of Probe 2 - Compound **1** (100 mg, 0.27 mmol) and salicylaldehyde (67 mg, 0.55 mmol) in dry ethanol were stirred at refluxing temperature for 4-6 h. The solid was separated from the reaction mixture and was filtered and washed with ethanol to give pure yellow colored probe **2**.



94% yield (150 mg); Yellow colored; M.Pt. > 300 °C; ¹H NMR (CDCl₃, 400 MHz), δ (ppm): 8.58 (s, 2H, OH), 7.37–7.34 (m, 4H, ArH), 7.17–7.15 (m, 6H, ArH), 7.10–6.99 (m, 14H, ArH), 6.92 (t, *J* = 8.0 Hz, 2H, ArH). ¹³C NMR (CDCl₃, 100 MHz), δ (ppm): 162.0, 161.1, 146.3, 143.4, 142.5, 140.5, 133.0, 132.4, 132.1, 131.4, 127.9, 126.8, 120.5, 119.2, 119.0, 117.2 (ArC). MS (TOF): *m/e* 570.84 (M⁺).

3.3 ESIPT-AIE coupled probe for detection of trivalent metal ions (Al^{3+} , Cr^{3+} and Fe^{3+}) in aqueous media

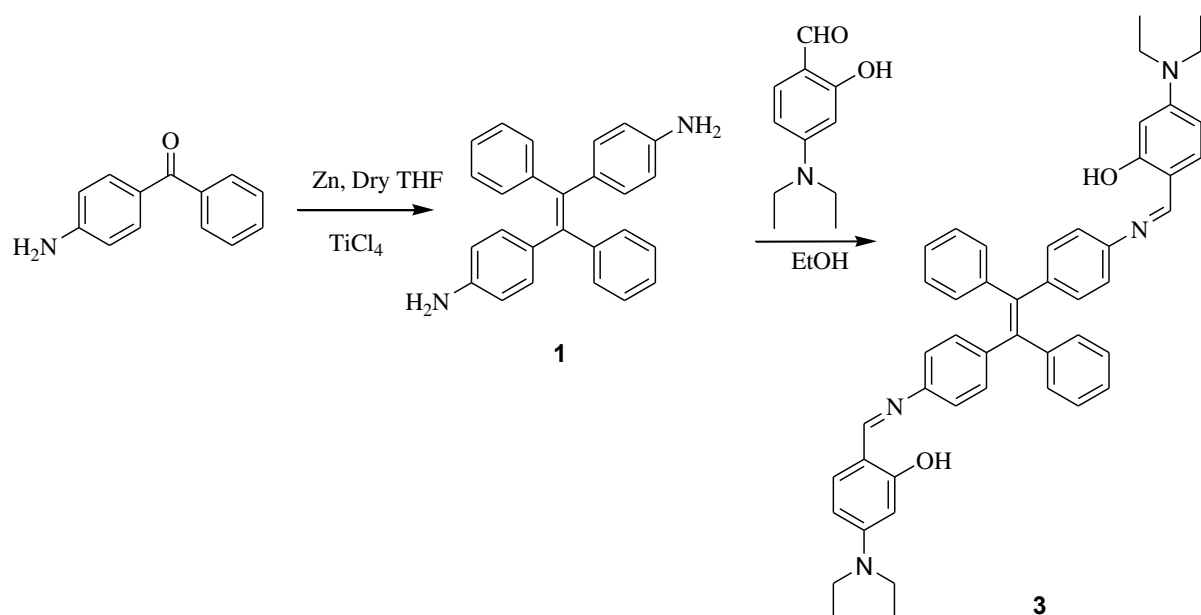
3.4.1 Abstract

In order to study the effect of substituent on AIE-ESIPT coupled phenomenon derivative probe **3** was synthesized. N, N-diethyl group has been incorporated at meta position to OH group, thus affecting the ESIPT properties. Probe **3** exhibited an absorption band at 400 nm which upon addition of trivalent cations like Al^{3+} , Cr^{3+} and Fe^{3+} shifts to two new bands at 440 nm and 460 nm. Probe **3** on aggregating displayed an AIE band at 525 nm which upon addition of trivalent cations, shifted to 580 nm due to disaggregation of probe **3** molecules. This was used as a backbone for estimation of trivalent cations even at very low concentrations. Moreover, repeatability of probe **3** from probe **3**. Fe^{3+} complex via EDTA was also achieved which formed the basis for construction of INHIBIT logic device and logic device having three chemical input.

3.3.2 Synthesis of probe 3

Probe **1** and **3** were prepared according to the synthetic routes shown in Scheme 3. Probe **1** was synthesized via McMurry coupling reaction between 4-amino benzophenone as discussed in chapter 3.2. Probe **3** was synthesized by schiff base formation of probe **1** with N,N-diethylsalicylaldehyde. The reaction intermediates and final products were fully characterized by spectroscopic techniques from which satisfactory data was obtained.

Probe **1** on reaction with N,N-diethyl salicylaldehyde to form the schiff base probe **3** yellow colored solid in 91 % yield; m/e 713.14 ($M^+ + 1$) (Scheme 3). ^1H NMR spectrum showed 2H singlet at δ 8.36 for OH protons (D_2O exchangeable), 12H multiplet at δ 7.10, 6H multiplet at δ 7.00, 2H double doublet at δ 6.21, 2H doublet at δ 6.16 for aromatic protons, 8H quartet at δ 3.33 and 12H triplet at δ 1.21 for aliphatic protons. ^{13}C NMR spectrum showed signals at δ 164.5, 159.6, 151.7, 146.3, 143.8, 141.2, 140.2, 133.6, 132.3, 131.4, 127.8, 126.4, 120.0, 109.1, 103.7, 97.7 for aromatic $-\text{C}$, 44.5 and 12.4 for aliphatic $-\text{C}$. This spectral data having extra peaks at 164.5 and 159.6 than probe **1** was due to schiff bases corroborating the structure **3** for this compound. On the basis of these spectral data, this compound has been assigned the structure of probe **3**.



Scheme 3: Synthesis of compound **1** and **3**

3.3.3 Results and discussion

3.3.3.1 Aggregation-Induced Emission studies of probe **3**:

To test the AIE characteristic of probe **3**, the effect of water volume fraction on emission was evaluated in H₂O/CH₃CN mixed media. A dilute solution of probe **3** (10 μM, CH₃CN) emits at 580 nm. However, with increasing the percentage of water fraction only upto 10% (volume fraction) to the solution of probe **3** in CH₃CN, a gradual shift in emission was observed towards 525 nm (Figure 12). This emission induced increased on increasing the water ratio and by the formation of aggregates in water i.e. typical phenomenon for AIE-active compounds. The appearance of mixture was transparent and homogeneous, suggesting that the molecules aggregates, suspended in the mixture in nano-sized. As water is a poor solvent of probe **3**, its molecules must aggregate in the aqueous mixtures with high water fractions. In ACN solution, the phenyl rings on the TPE molecules undergo active intramolecular torsional/rotational motions against the central double bond. The TPE molecules may adopt very twisted conformations and emit at shorter wavelength. In the aggregate state, the intramolecular torsional/rotational motions are inhibited. Thus TPE molecules acquire less twisted, most stable conformations, resulting in red-shifted emission.

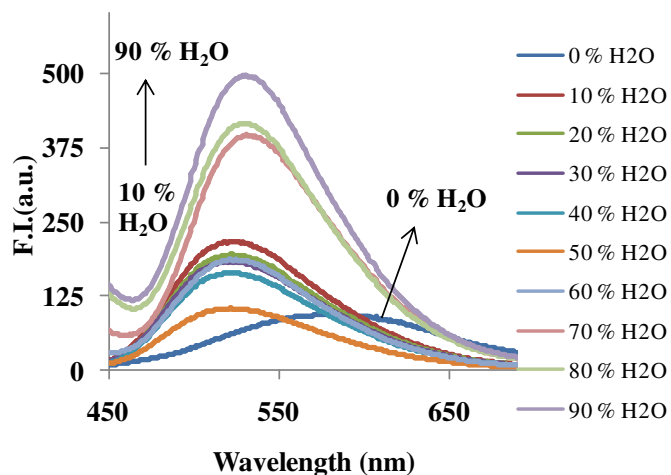
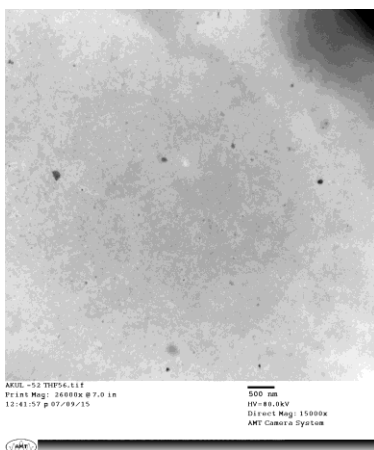


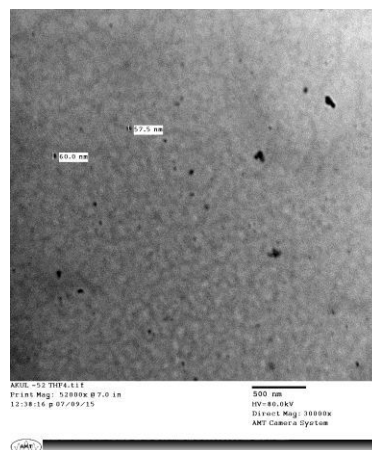
Figure 12: Emission spectrum of solution of probe **3** in ACN and increasing ACN/water ratio at 2 μ M.

This was clearly demonstrated by TEM images (Figure 13) that there is no aggregation in dilute solution of probe **3** in THF i.e. particles were nanosized but by adding water 70% Vol, the particle size increased ten times which clearly depicts that there is aggregation in case of water.

A



B



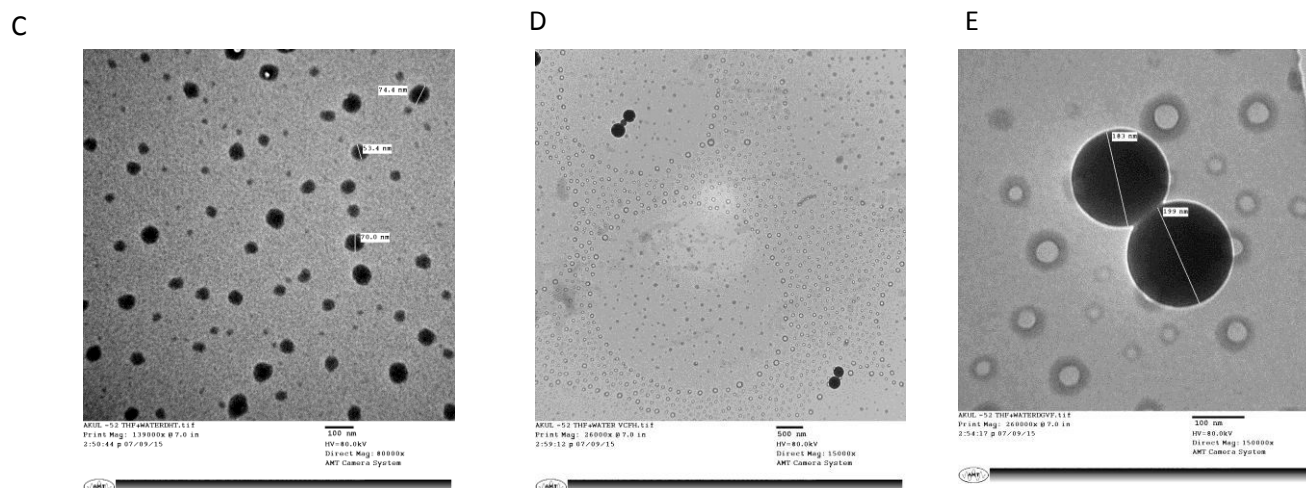


Figure 13: TEM images of probe **3** in (A-B) THF; (C-E) THF:H₂O::1:9

3.3.3.2 Sensing behavior of probe **2** towards various cations

The UV-Vis spectroscopic properties of probe **3** were investigated in CH₃CN: H₂O:: 1:1 at 10 μ M. Probe **3** displayed an absorption band centered at 400 nm. Addition of different metal ions viz. Ag⁺, Al³⁺, Cu²⁺, Fe³⁺, Hg²⁺, Mg²⁺, K⁺, Na⁺, Ni²⁺, Pb²⁺, Zn²⁺, Ba²⁺, Ca²⁺, Co²⁺, Cr³⁺ etc caused no significant change except presence of trivalent metal ions like Al³⁺, Cr³⁺ and Fe³⁺. Upon incremental addition of Al³⁺ ions, absorption intensity at λ_{max} 400 nm decreased gradually with simultaneous increase in intensity of new absorption bands at 435 nm and 460 nm (Figure 14a), with detection limit of 0.2 μ M. The absorption ratio at 460 nm and 400 nm wavelengths varied from 0.5838 to 1.5409 indicating 2.64 fold absorption ratio change (Figure 14b). Thus, probe **3** can be used to estimate a wide range of Al³⁺ ions between 0.2-150 μ M through absorption spectroscopy (Figure 14b).

Similarly in case of Cr³⁺ ions two new absorption bands at 435 nm and 455 nm were formed upon incremental addition upto 100 μ M solutions (Figure 15a), with detection limit of 0.2 μ M. The absorption ratio at 455 nm and 400 nm wavelengths varied from 0.6584 to 1.4082 indicating 2.14 fold absorption ratio change (Figure 15b).

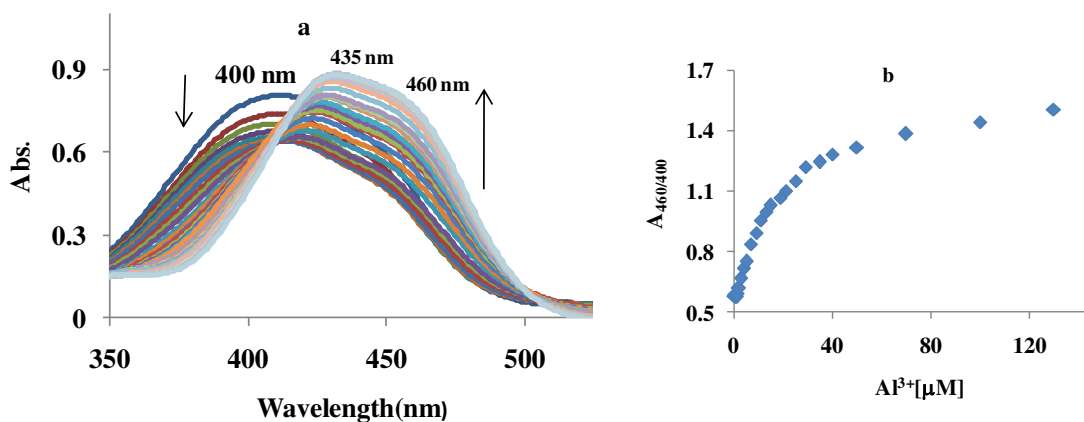


Figure 14: (a) Absorption spectrum of probe **3** (10 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$) upon complexation with increasing concentration of Al^{3+} ions; (b) Plot of absorption intensity ratio between 460 nm and 400 nm (A_{460} / A_{400}) vs $[\text{Al}^{3+}]$ ions of probe **3** at 10 μM in $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$.

Thus, probe **3** was used to estimate a wide range of Cr^{3+} ions between 0.2-120 μM through absorption spectroscopy (Figure 15b).

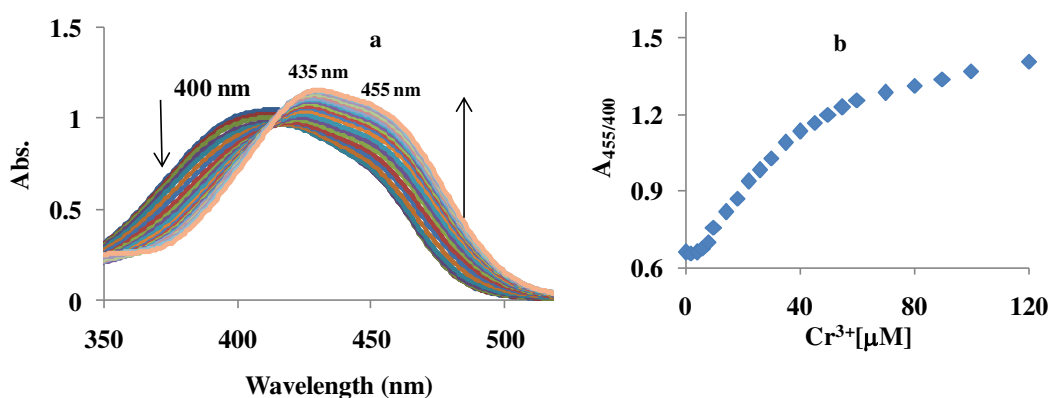


Figure 15: (a) Absorption spectrum of probe **3** (10 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$) upon complexation with increasing concentration of Cr^{3+} ions; (b) Plot of absorption intensity ratio between 455 nm and 400 nm (A_{455} / A_{400}) vs $[\text{Cr}^{3+}]$ ions of probe **3** at 10 μM in $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$.

Upon incremental addition of 180 μM solution of Fe^{3+} ions to probe **3** resulted in formation of two new bands at 435 nm and 464 nm (Figure 16a), with lowest detection limit of 0.5 μM . The absorption ratio at 464 nm and 400 nm wavelengths varied from 0.5974 to 1.500 indicating 2.14 fold absorption ratio change (Figure 16b). Thus, probe **3** was used to estimate a wide range of Fe^{3+} ions between 0.5-180 μM through absorption spectroscopy (Figure 16b).

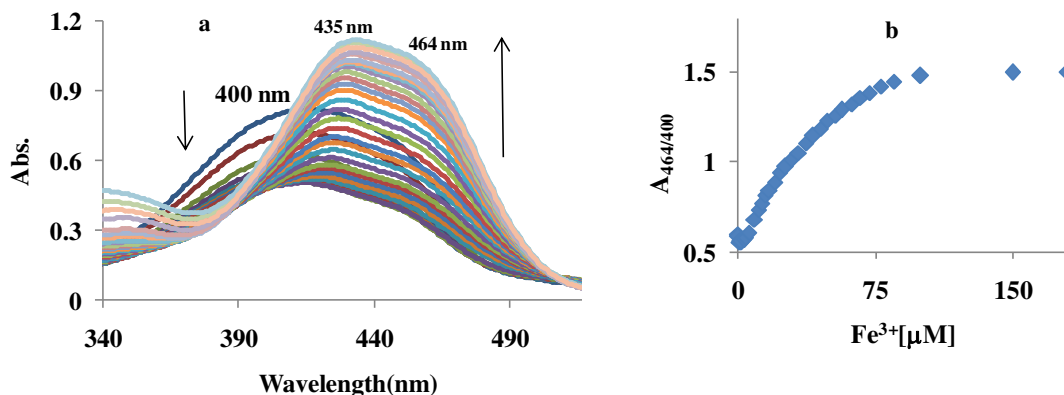


Figure 16: (a) Absorption spectrum of probe **3** (10 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$) upon complexation with increasing concentration of Fe^{3+} ions; (b) Plot of absorption intensity ratio between 464 nm and 400 nm (A_{464} / A_{400}) vs $[\text{Fe}^{3+}]$ ions of probe **3** at 10 μM in $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$.

The probe **3** (2.5 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$) on excitation at 410 nm displayed aggregation induced emission band at 525 nm. On addition of various cations viz., Ag^+ , Al^{3+} , Cu^{2+} , Fe^{3+} , Hg^{2+} , Mg^{2+} , K^+ , Na^+ , Ni^{2+} , Pb^{2+} , Zn^{2+} , Ba^{2+} , Ca^{2+} , Co^{2+} , Cr^{3+} etc. caused no significant change except presence of trivalent metal ions via Al^{3+} , Cr^{3+} and Fe^{3+} . Upon incremental addition of trivalent cations aggregation induced band at 525 nm was quenched and band at 580 nm was formed due to disaggregation of the aggregated probe **3**. Further to check the titrating properties the trivalent cations were titrated with the solution of probe **3** and for Al^{3+} ions only 200 μM solution was used (Figure 17a).

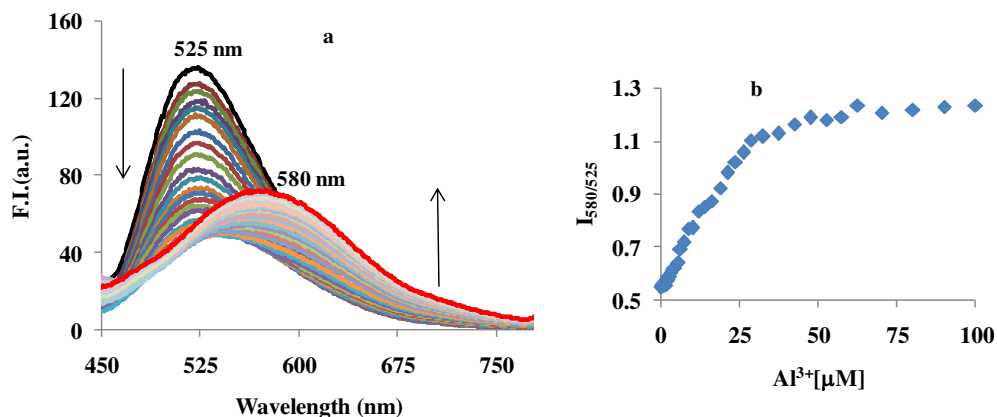


Figure 17: (a) Emission spectrum of probe **3** (2.5 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$) upon complexation with increasing concentration of Al^{3+} ions; (b) Plot of emission intensity ratio between 580 nm and 525 nm (I_{580} / I_{525}) vs $[\text{Al}^{3+}]$ ions of probe **3** at 2.5 μM in $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$.

The binding constant was calculated to be $1.47 \pm 0.01 \times 10^6 \text{ M}^{-1}$ with lowest detection limit of $0.3 \mu\text{M}$. The emission ratio at 580 nm and 525 nm varied from 0.5471 to 1.2328 indicating 2.25 fold emission ratio change (Figure 17b). Thus, probe **3** was used to estimate a wide range of Al^{3+} ions between $0.3\text{--}200 \mu\text{M}$ through emission spectroscopy (Figure 17b).

Moreover, $250 \mu\text{M}$ Cr^{3+} ions solution was responsible for forming new emission band at 580 nm (Figure 18a). The binding constant was calculated to be $1.33 \pm 0.01 \times 10^5 \text{ M}^{-1}$ with lowest detection limit of $0.25 \mu\text{M}$. The emission ratio at 580 nm and 525 nm varied from 0.5479 to 1.2942 indicating 2.36 fold emission ratio change (Figure 18b). Thus, probe **3** was used to estimate a wide range of Cr^{3+} ions between $0.25\text{--}250 \mu\text{M}$ through emission spectroscopy (Figure 18b).

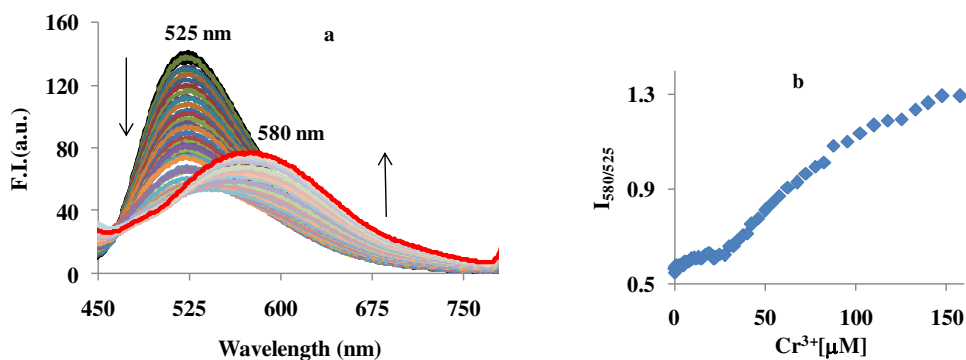


Figure 18: (a) Emission spectrum of probe **3** ($2.5 \mu\text{M}$, $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$) upon complexation with increasing concentration of Cr^{3+} ions; (b) Plot of emission intensity ratio between 580 nm and 525nm (I_{580} / I_{525}) vs $[\text{Cr}^{3+}]$ ions of probe **3** at $2.5 \mu\text{M}$ in $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$.

Upon incremental addition of $60 \mu\text{M}$ solution of Fe^{3+} ions to probe **3** resulted in formation of new band at 580 nm (Figure 19a). The binding constant was calculated to be $8.03 \pm 0.02 \times 10^5 \text{ M}^{-1}$ with detection limit of $0.6 \mu\text{M}$. The emission ratio at 580 nm and 525 nm wavelengths varied from 0.5448 to 1.4731 indicating 2.70 fold emission ratio change (Figure 19b). Thus, probe **3** was used to estimate a wide range of Fe^{3+} ions between $0.6\text{--}60 \mu\text{M}$ through emission spectroscopy (Figure 19b).

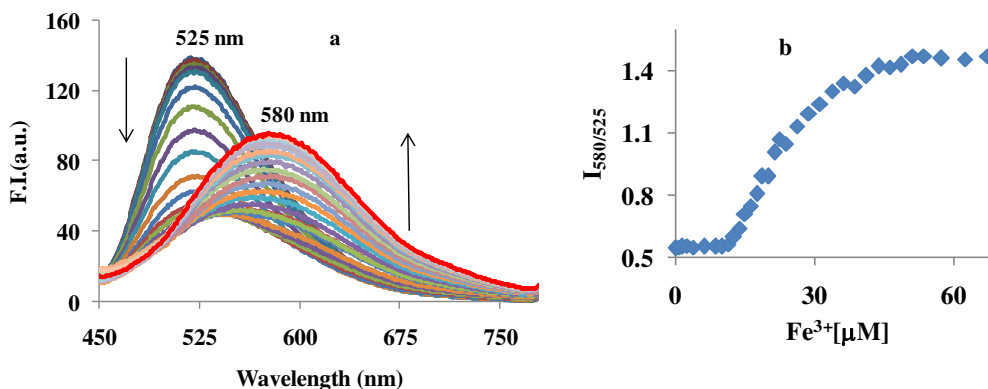


Figure 19: (a) Emission spectrum of probe **3** (2.5 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$) upon complexation with increasing concentration of Fe^{3+} ions; (b) Plot of emission intensity ratio between 580 nm and 525 nm (I_{580} / I_{525}) vs $[\text{Fe}^{3+}]$ ions of probe **3** at 2.5 μM in $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$.

3.3.3.3 Proposed mechanism – As it is very clear from the above results that band at 525 nm was due to aggregation caused by addition of 50 % water while checking photophysical properties of probe **3**. As all three trivalent cations are very acidic in nature and reactive i.e. disaggregates the aggregated molecules of probe **3** thus releasing its fluorescence back to its normal emission at 580 nm as depicted in scheme 4.



Scheme 4: Proposed mechanism for binding of probe **3** with trivalent cations

3.3.4 Practical Applicability: To examine the reversibility/practical applicability of the binding of probe **3** to detected trivalent metal ions, experiments were performed in the presence of EDTA. Upon addition of an aqueous solution of EDTA (2 equiv.) to the $\mathbf{3}.\text{Fe}^{3+}$ complex, the

emission band at 580 nm disappeared and simultaneously reappearance of the emission band at 525 nm was observed i.e. of probe **3** itself. Again, on addition of Fe^{3+} to this mixture, having EDTA, the emission band at 580 nm reappeared. The basic criteria found was that EDTA binds to Fe^{3+} ions leading to the formation of Fe–EDTA, thus regenerating emission of probe **3** which confirmed the reversal binding of probe **3** to the Fe^{3+} ions (Figure 20a). Moreover, the alternate addition of Fe^{3+} ions and EDTA to probe **3** gave rise to a switchable change in the emission at 580 nm and can be repeated more than 5-6 times (Figure 20b). However, no emission reversal was observed when EDTA was added to the 3.Al^{3+} and 3.Cr^{3+} complexes. These results indicated that EDTA is not able to remove Al^{3+} from 3.Al^{3+} complex and Cr^{3+} from 3.Cr^{3+} complex and hence cannot restore the emission of probe **3**. As per the literature, EDTA having six potential binding sites forms 1:1 complexes with all the metal ions. Metal-EDTA complexes are quite stable due to number of chelate rings formed. However, among Al^{3+} , Fe^{3+} and Cr^{3+} , values of formation constants of their complexes with EDTA are varied which forms the basis of only Fe-EDTA stable complexes. Formation constant in case of Fe-EDTA is maximum ($\text{Log } K_f = 25.1$) as compared to Al-EDTA and Cr-EDTA complexes. Hence, only Fe^{3+} forms stable complexes with EDTA while Al^{3+} and Cr^{3+} ions do not form stable complexes. This can lead to distinguish Fe^{3+} ions over Al^{3+} and Cr^{3+} .

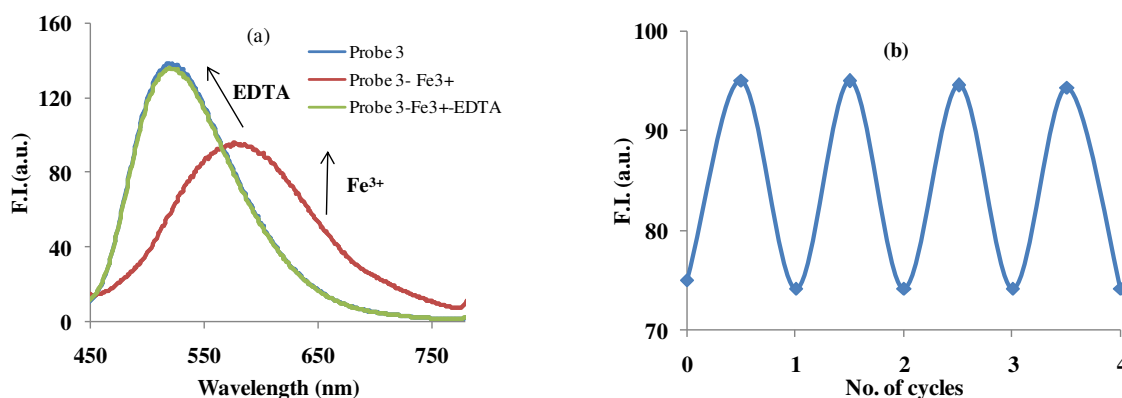


Figure 20: (a) The emission response showing the reversibility of (a) Fe^{3+} (40 μM) coordination to probe **3** by EDTA (40 μM) and (b) the switchable behaviour in the emission at 580 nm by the alternate addition of Fe^{3+} and EDTA to probe **3**.

3.3.4.1 Elaboration as LOGIC DEVICE:

As emission output at 580 nm is in “on” state in the presence of only Fe^{3+} ions. Thus, the molecular switching behavior of probe **3** with two chemical inputs (Fe^{3+} and EDTA) has been demonstrated with the help of binary logic. Emission output at 580 nm was in “on” state only when Fe^{3+} ions were present, such emission changes upon the actions of one chemical input mimic the performance of an INHIBIT (INH) logic gate (Figure 21 and Table 5) with threshold value of 0.15. An INH logic function is two input device and its output is “true” only if one input alone is present. Significantly, probe **3** restores the information if Fe^{3+} input comes first (“on” state) and rest all states as “off” states. Whenever EDTA was present, both inputs were absent thus output was in false state. Thus, developments of these sequential circuits have been important as combinational logic is not enough to construct memory devices.

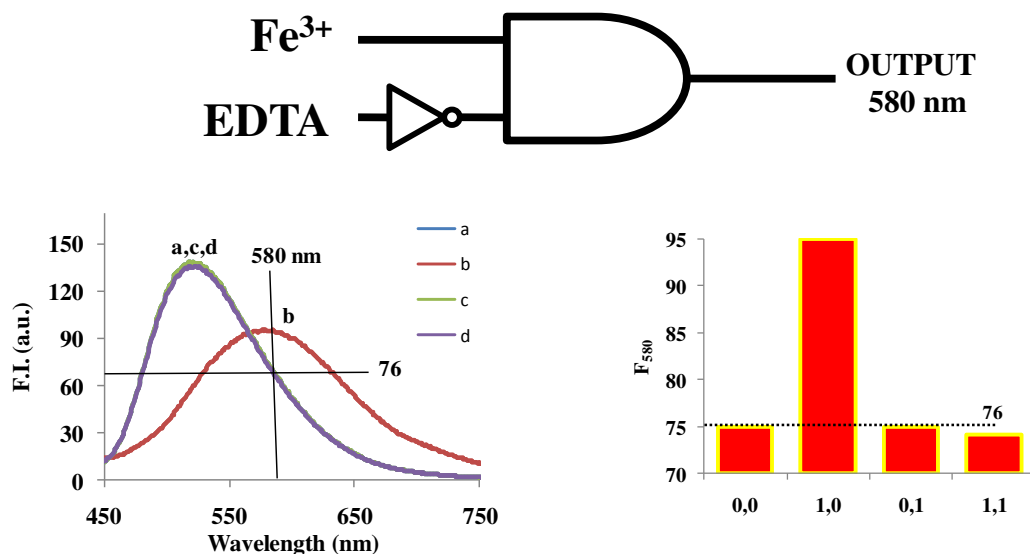


Figure 21: Emission spectrum of probe **3** (2.5 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}::1:1$) (a) in absence of both inputs; (b) in the presence of Fe^{3+} (40 μM); (c) EDTA (40 μM); (d) Fe^{3+} (40 μM) + EDTA (40 μM).

Table 5: Schematic representation of the INH logic operation and truth table for the sequential logic circuit, where 0 = *Off* and 1 = *On*.

INPUT 1 Fe^{3+}	INPUT 2 EDTA	OUTPUT $\lambda_{\text{em}} = 580 \text{ nm}$
0	0	0
1	0	1
0	1	0
1	1	0

Three Input Logic Devices: Probe **3** showed new band formation in presence of trivalent metal ions namely Al^{3+} , Fe^{3+} and Cr^{3+} . Same results were obtained in the presence of either two or all three trivalent ions. This concept was used to construct 3 input logic OR gate by taking output at 580 nm which is always in “*turn on*” state except one when no trivalent metal ions are present (Figure 22a and Table 6). At 525 nm which is always in “*off state*” except when all three trivalent metal ions are present, this led to construction of NOR logic operation at this wavelength (Figure 22b and Table 6).

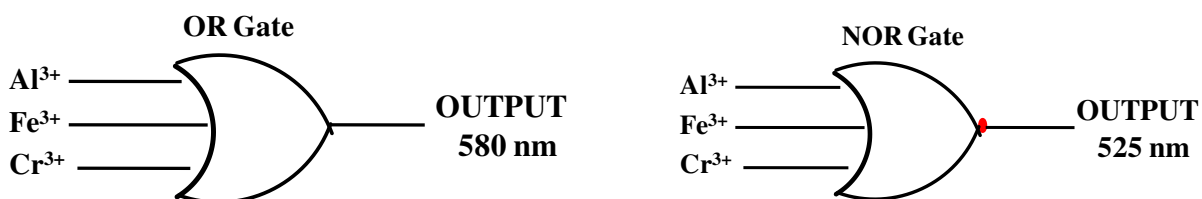


Figure 22: Schematic representation of the (a) OR; (b) NOR logic operations having three inputs.

Table 6: Truth table for OR and NOR logic operation, where 0 = *Off* and 1 = *On*.

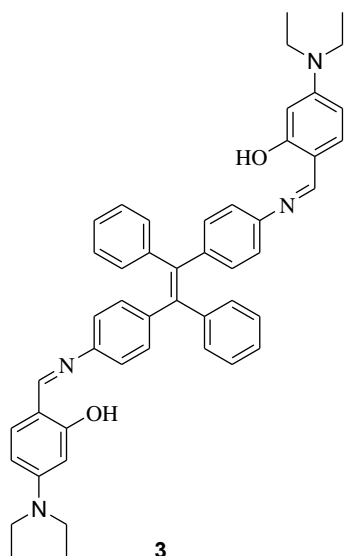
INPUT			OUTPUT ($\lambda_{em} = 580\text{ nm}$) OR GATE	OUTPUT ($\lambda_{em} = 525\text{ nm}$) NOR GATE
Al ³⁺	Fe ³⁺	Cr ³⁺		
0	0	0	0	1
0	0	1	1	0
0	1	0	1	0
1	0	0	1	0
1	1	0	1	0
0	1	1	1	0
1	0	1	1	0
1	1	1	1	0

3.3.5 Conclusions

An ESIPT coupled AIE phenomenon's bearing probe **3** has been synthesized and used for estimation of trivalent cations. Probe **3** exhibited an absorption band at 400 nm which was red shifted to 440 nm and 460 nm upon addition of trivalent cations. Probe **3** also displayed AIE effect which led to emission of molecule at 525 nm and thus by adding trivalent cations aggregation was disturbed leading to disaggregation which in turn released the fluorescence emission at 580 nm. This was used as a backbone for estimation of trivalent cations even at very low concentrations and for three chemical input based OR and NOR logic gates.

3.3.6 Experimental Section –

3.3.6.1 Synthesis of Probe 3 - Schiff base reaction was carried out with Probe **1** (100mg, 0.27 mmol) and N, N-diethyl salicylaldehyde (107 mg, 0.55 mmol) in dry ethanol were stirred at refluxing temperature for 4-6 h. The solid was separated from the reaction mixture and was filtered and washed with ethanol to give pure yellow colored probe **3**.



3

91% yield (180 mg); Yellow colored solid; M.Pt. > 300 °C ^1H NMR (CDCl_3 , 400 MHz), δ (ppm): 8.36 (s, 2H, OH), 7.15–7.07 (m, 12H, ArH), 7.02–6.96 (m, 6H, ArH), 6.23–6.20 (dd, $J^2 = 2.32$ Hz, $J^3 = 8.72$ Hz, 2H), 6.16 (d, $J = 2.28$ Hz, 2H), 3.41–3.36 (q, 8H, N- CH_2), 1.21 (t, $J = 6.8$ Hz, 12H, CH_3); ^{13}C NMR (CDCl_3 , 100 MHz), δ (ppm): 164.5, 159.6, 151.7, 146.3, 143.8, 141.2, 140.2, 133.6, 132.3, 131.4, 127.8, 126.4, 120.0, 109.1, 103.7, 97.7 (ArC), 44.5 (N- CH_2), 12.4 (CH_3); MS (TOF): m/e 713.14 ($\text{M}^+ + 1$).

3.4 Benzimidazole-TPE hybrid probe for ratiometric estimation of cations and anions

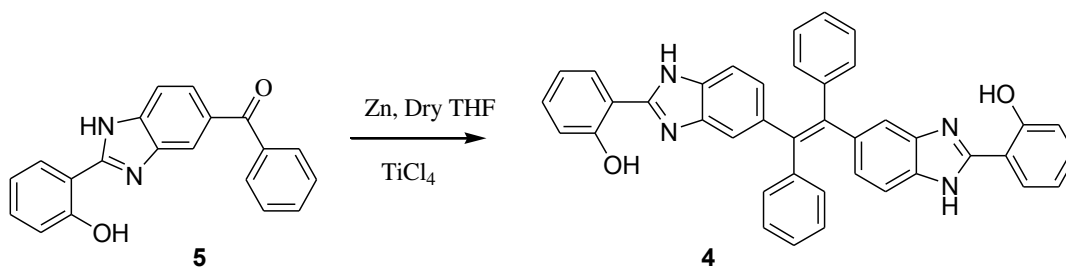
3.4.1 Abstract

ESIPT based benzimidazole moiety has been incorporated on TPE moiety *via* mcMurry coupling reaction. The probe **4** showed the ESIPT characteristic due to benzimidazole moiety and AIE characteristics due to TPE moiety. Probe **4** exhibited an absorption band at 335 nm and emission band at 460 nm. The addition of metal ions like Cu^{2+} , Pb^{2+} , Zn^{2+} and Fe^{3+} showed the shift of emission band at 460 nm to 375 nm. The probe **4** was used for sensing of CN^- and F^- ions *via* deprotonation by masking ESIPT band and releasing the normal emission band at 425 nm.

3.4.2 Synthesis of Probe 4

The compound **5** was synthesized by the method as discussed in chapter 2 and synthetic route for the synthesis of probe **4** has been outlined in Scheme 5.

Probe **4** was synthesized *via* McMurry coupling reaction of compound **5** as colorless solid in 60 % yield (Scheme 5). ^1H NMR spectrum of probe **4** showed the 2H double doublet at δ 7.90, two 8H multiplets at δ 7.30 and δ 7.21, two double doublets at δ 7.12 and 7.04 and one triplet at δ 6.93 for aromatic protons, 2H broad singlet at δ 4.11 due to NH protons (D_2O Exchangeable protons). The additional peaks of protons in aromatic regions confirmed the formation of probe **4**. ^{13}C NMR spectrum showed signals at δ 159.0, 141.9, 131.6, 129.2, 128.7, 126.3, 126.1, 119.0, 117.8 and 113.3 for aromatic -C. On the basis of these spectral data, this compound has been assigned the structure of probe **4**.



Scheme 5: Synthetic route for the synthesis of probe **4**

3.4.3 Results and discussion

3.4.3.1 Aggregation-Induced Emission- A dilute CH_3CN solution of probe **4** ($2\ \mu\text{M}$) emits faintly at 460 nm when photoexcited at 320 nm (Figure 23). In $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ mixture having 80 vol % of water, the band shifted at 370 nm with the increase in its intensity with H_2O ratio. It was clearly depicted that by increasing water ratio, there was incremental enhancement in emission properties i.e. AIE effect. As water is a poor solvent of probe **4**, its molecules undergo aggregation in the aqueous mixtures with high water fractions. The mixture appearance was transparent and homogeneous, suggesting that the dye aggregates suspended in the mixture are nanosized. In CH_3CN solution, the phenyl rings on the TPE molecules undergo active intramolecular torsional/rotational motions against the central double bond. The TPE molecules may adopt very twisted conformations and emit at shorter wavelength. In the aggregate state, the intramolecular torsional/rotational motions are inhibited. Thus, TPE molecules acquire less twisted, most stable conformations, resulting in red-shifted emission.

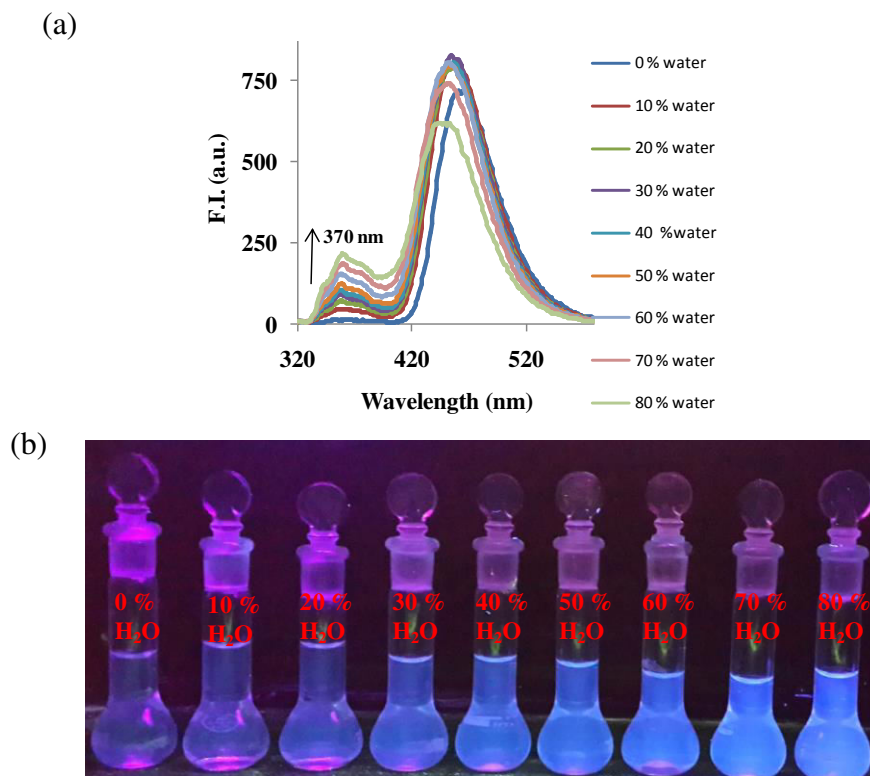


Figure 23: (a) Emission spectrum of solution of probe **4**; (b) Visible color changes under UV light in CH_3CN and increasing $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ ratio at $2\ \mu\text{M}$

3.4.3.2 Photophysical behavior of probe 4 towards metal ions

The UV-Vis spectroscopic properties of probe **4** were investigated in CH₃CN at 20 μ M. Probe **4** displayed absorption bands at 320 nm and 335 nm. Addition of different cations viz., Ag⁺, Al³⁺, Cu²⁺, Fe³⁺, Hg²⁺, Mg²⁺, K⁺, Na⁺, Ni²⁺, Pb²⁺, Zn²⁺, Ba²⁺, Ca²⁺, Co²⁺, Cr³⁺ etc caused no significant change except presence of metal ions via Cu²⁺, Zn²⁺, Pb²⁺ and Fe³⁺. Upon incremental addition of 100 μ M solution of Cu²⁺ ions, absorption intensity at λ_{max} 320 nm and 335 nm decreased gradually with simultaneous increase in absorption bands at 300, 325, 342 and 375 nm (Figure 24a). The detection limit was 1 μ M. In case of addition of 1000 μ M solution of Zn²⁺ ions, new band at 370 nm was formed (Figure 24b).

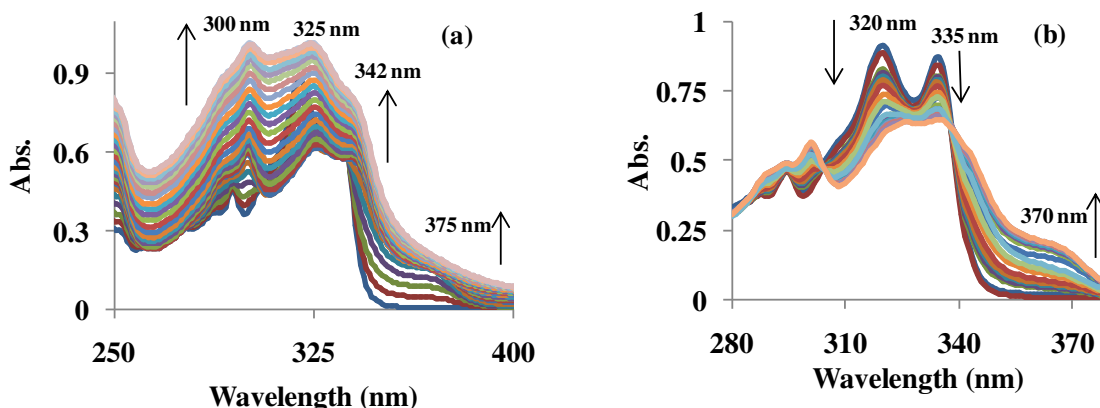


Figure 24: Absorption spectra of probe **4** (20 μ M, CH₃CN) upon complexation with increasing concentration of (a) Cu²⁺; (b) Zn²⁺ ions.

Similarly in the presence of Pb²⁺ ions, new absorption band was formed 340 nm (Figure 25a) and only 5 μ M solutions of Fe³⁺ ions was sufficient to decrease the probe **4** absorption intensity (Figure 25b).

The probe **4** (5 μ M, CH₃CN) on excitation at 320 nm displayed keto emission band at 460 nm having Stokes shift of 140 nm. On addition of various cations viz. Ag⁺, Al³⁺, Cu²⁺, Fe³⁺, Hg²⁺, Mg²⁺, K⁺, Na⁺, Ni²⁺, Pb²⁺, Zn²⁺, Ba²⁺, Ca²⁺, Co²⁺, Cr³⁺ etc caused no significant change except presence of metal ions like Cu²⁺, Zn²⁺, Pb²⁺ and Fe³⁺.

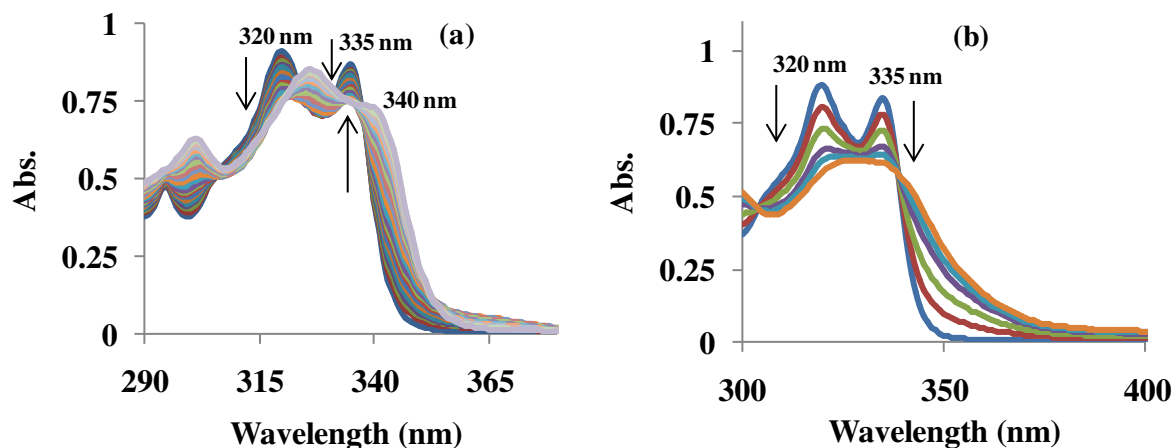


Figure 25: Absorption spectra of probe **2** (20 μM , CH_3CN) upon complexation with increasing concentration of (a) Pb^{2+} and (b) Fe^{3+} ions.

Upon incremental addition of 5 μM solution of Cu^{2+} ions band at 460 nm decreased along with formation of new band at 375 nm with a clear isobestic point at 425 nm having lowest detection value to be 0.125 μM and binding constant to be $1.04 \pm 0.02 \times 10^6 \text{ M}^{-1}$ (Figure 26a). The emission ratio at 375 nm and 460 nm varied from 0.0101 to 7.5 indicating 740 fold emission ratio changes and estimating a wide range of Cu^{2+} ions form 0.1-5 μM . Moreover, for Zn^{2+} ions, same changes were observed with 150 μM solution (Figure 26b). The binding constant was calculated to be $1.63 \pm 0.01 \times 10^5 \text{ M}^{-1}$ with lowest detection limit of 0.3 μM . The emission ratio at 375 nm and 460 nm varied from 0.0125 to 1.985 indicating 159 fold emission ratio change. Thus, probe **4** can be used to estimate a wide range of Zn^{2+} ions between 0.3-200 μM through emission spectroscopy.

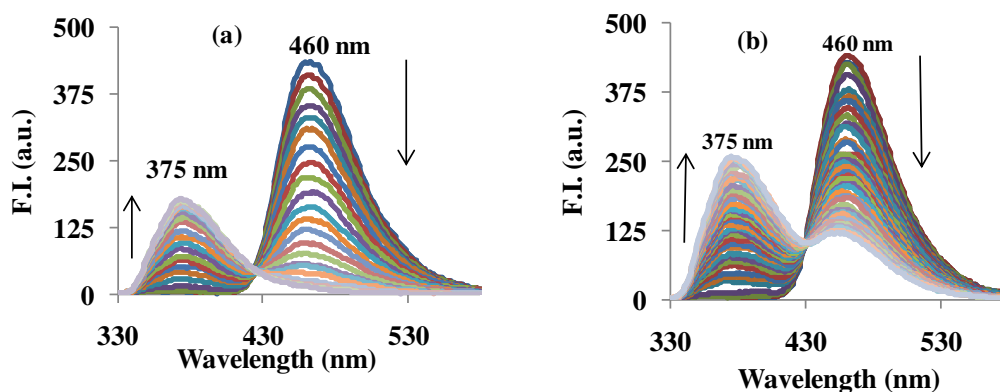


Figure 26: Emission spectra of probe **4** (5 μM , CH_3CN) upon complexation with increasing concentration of (a) Cu^{2+} and (b) Zn^{2+} ions.

In the presence of 50 μM Pb^{2+} ions, new emission band at 375 nm was formed with higher quantum yield than Cu^{2+} and Zn^{2+} ions (Figure 27a). The emission ratio at 375 nm and 460 nm varied from 0.0122 to 4.87 indicating 400 fold emission ratio change. The binding constant was calculated to be $7.41 \pm 0.01 \times 10^4 \text{ M}^{-1}$ with lowest detection limit of 5 μM . Thus, probe **4** can be used to estimate a wide range of Pb^{2+} ions between 5-50 μM through emission spectroscopy. Upon incremental addition of 45 μM solutions of Fe^{3+} ions, the new band at 375 nm was observed with simultaneous decrease at 460 nm band (Figure 27b). The emission ratio at 375 nm and 460 nm varied from 0.0106 to 14.045 indicating 1325 fold emission ratio change. The binding constant was calculated to be $26.3 \pm 0.1 \times 10^6$ with lowest detection limit of 0.25 μM . Thus, probe **4** can be used to estimate a wide range of Pb^{2+} ions between 0.25-45 μM through emission spectroscopy. The results were compiled in Table 7 for the detected metal ions with probe **4**.

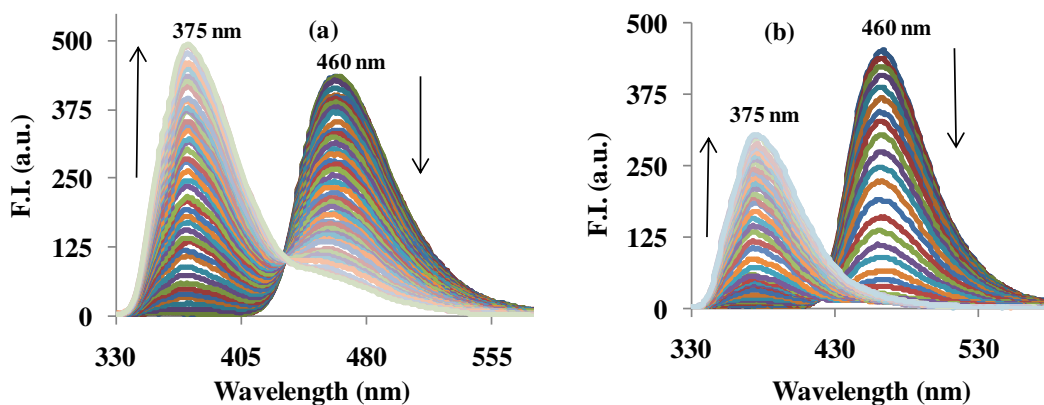


Figure 27: Emission spectra of probe **4** (5 μM , CH_3CN) upon complexation with increasing concentration of (a) Pb^{2+} and (b) Fe^{3+} ions.

Table 7: Important facts of probe **4** via fluorescence spectrophotometer

S.No.	Cation Detected	Stability Constant (M^{-1})	LOD (μM)
1	Cu^{2+}	$1.04 \pm 0.02 \times 10^6$	0.12
2	Zn^{2+}	$1.63 \pm 0.01 \times 10^5$	0.25
3	Pb^{2+}	$7.41 \pm 0.01 \times 10^4$	0.50
4	Fe^{3+}	$26.3 \pm 0.1 \times 10^6$	0.25

As probe **4** comprises of two OH groups, it persuaded us to check the complexation behavior towards different anions. Only CN^- and F^- ions caused changes in case of absorbance as well as fluorescence in CH_3CN out of all other anions via Cl^- , Br^- , I^- , SCN^- , AcO^- , NO_3^- , H_2PO_4^- and HSO_4^- as it led to deprotonation. In case of absorbance, there were formation of new band at 345 nm and 380 nm upon addition of 40 μM solution of CN^- ions or F^- ions (Figures 28a,b). The absorption ratio in case of CN^- ions at 380 nm and 335 nm varied from 0.0059 to 0.1884 indicating 32 fold absorption ratio changes and in case of F^- ions absorption ratio varied from 0.0170 to 0.1952 indicating 11.58 fold ratio change. Thus, probe **4** can be used to estimate a wide range of CN^- or F^- ions between 2 - 40 μM through UV-Vis spectrophotometer.

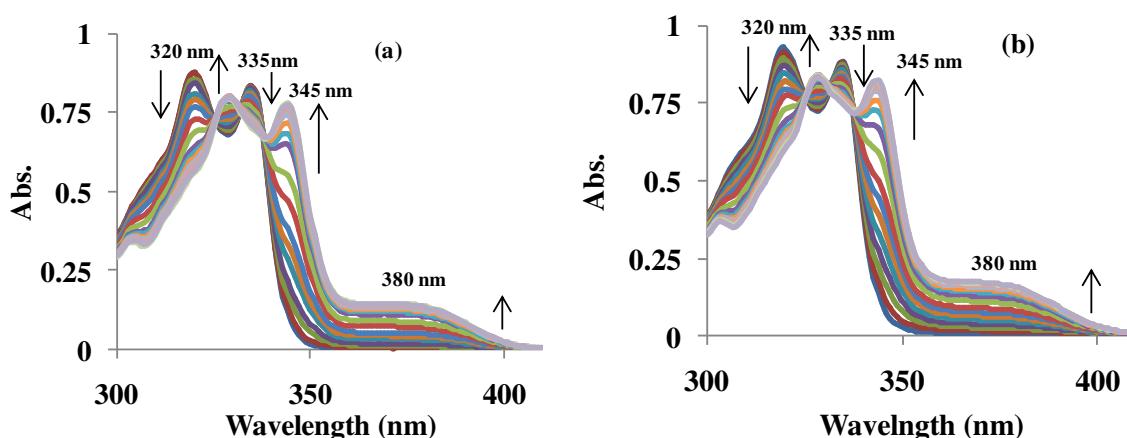


Figure 28: Absorption spectra of probe **2** (20 μM , CH_3CN) upon complexation with increasing concentration of (a) CN^- and (b) F^- ions.

In case of fluorescence there was formation of new emission band at 420 nm attributed to deprotonation of OH i.e. enol band of probe **4** upon addition of 25 μM solution of CN^- or F^- ions with 0.5 μM to be its lowest detection value (Figures 29a,b). The binding constant was calculated to be $1.6 \times 10^5 \text{ M}^{-1}$ for CN^- ions and $1.48 \times 10^5 \text{ M}^{-1}$ for F^- ions. The emission ratio for CN^- ions at 420 nm and 460 nm varied from 0.1392 to 1.692, indicating 12.15 fold emission ratio changes. Similarly, for F^- ions emission ratio changes at 420 nm and 460 nm varied from 0.1368 to 1.5691 indicating 11.47 fold emission ratio change. Thus, probe **4** can be used to estimate wide range of CN^- or F^- ions between 0.5 - 25 μM through fluorescence spectrophotometer.

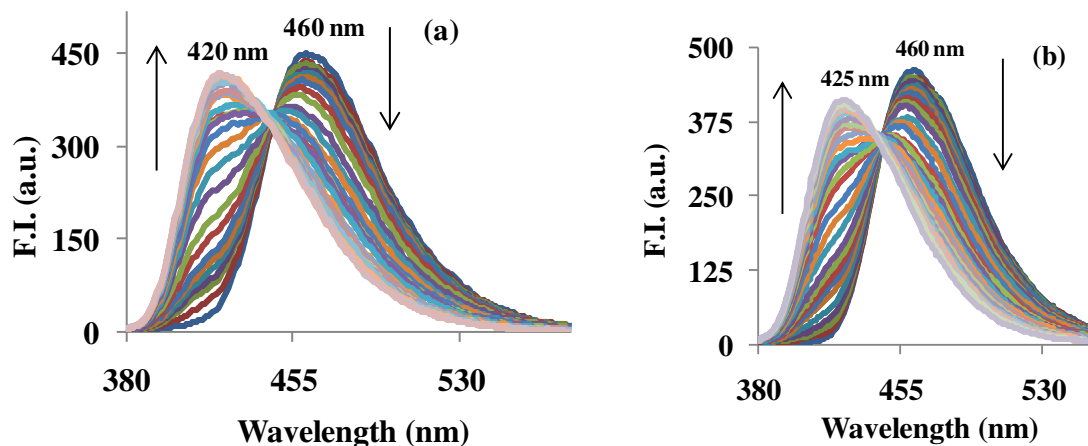


Figure 29: Emission spectra of probe **2** (5 μM , CH_3CN) upon complexation with increasing concentration of (a) CN^- and (b) F^- ions.

As it is very clear from the discussed results that band at 375 nm was due to aggregation while checking photophysical properties of probe **4**. All the detected cations via Cu^{2+} , Zn^{2+} , Pb^{2+} and Fe^{3+} led to aggregation of molecule, thus releasing its band at 375 nm. For both anions CN^- and F^- , there was deprotonation of OH group leading to release of enol form i.e. masking ESIPT band.

3.4.4 Conclusions

ESIPT-AIE based benzimidazole probe **4** has been synthesized which was used to determine different cations via Cu^{2+} , Zn^{2+} , Pb^{2+} and Fe^{3+} . Probe **2** exhibited an absorption band at 335 nm and emission band at 460 nm. Emission band got shifted to 375 nm in the presence of detected cations due to aggregation of probe **4**. In case of CN^- and F^- ions, the emission band has been shifted to 420 nm which was due to inhibition of ESIPT band and releasing the normal band.

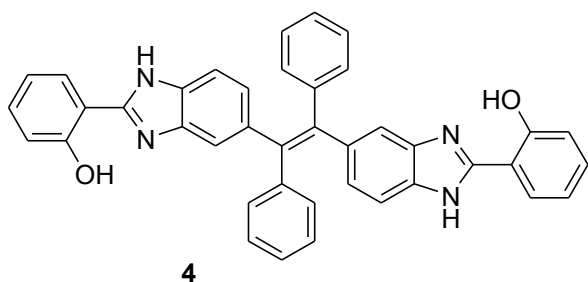
3.4.5 Experimental Section

3.4.5.1 Synthesis of probe **4**

Probe 4 – Compound **5** was synthesized according to our previous report. Under N_2 atmosphere, a three-necked flask equipped with a magnetic stirrer was charged with zinc powder (2.48 g, 60 mmol) and 40 mL freshly distilled THF was added. The mixture was cooled to $-5\text{ }^\circ\text{C}$, and TiCl_4 (2.00 mL, 30 mmol) was slowly added by a syringe with the temperature kept under $10\text{ }^\circ\text{C}$.

The suspending mixture was warmed to room temperature and stirred for 0.5 h, then heated at reflux for 2.5 h. The mixture was again cooled to -5 °C, solution of probe **5** (2g, 10 mmol) in 15 mL THF was added slowly. After addition, the reaction mixture was heated at reflux until the carbonyl compound was consumed (monitored by TLC). The reaction was quenched with 10% K₂CO₃ aqueous solution and extracted with EtOAc. The organic layer was collected and concentrated. The crude material was purified by column chromatography using 15 % ethylacetate and 85 % hexane to give the desired product probe **4**.

Probe 4



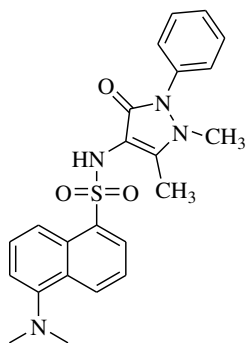
60% yield (1.1 g); Colorless solid; M.Pt. > 300 °C; ¹H NMR (CDCl₃, 400 MHz), δ (ppm): 7.92–7.89 (dd, $J^2 = 1.40$ Hz, $J^3 = 7.80$ Hz, 2H, ArH), 7.33–7.27 (m, 8H, ArH), 7.25–7.17 (m, 8H, ArH), 7.13–7.11 (dd, $J^2 = 1.36$ Hz, $J^3 = 8.68$ Hz, 2H, ArH), 7.06–7.03 (dd, $J = 8.0$ Hz, 2H, ArH), 6.93 (t, $J = 6.88$ Hz, 2H), 4.11 (bs, 2H, NH). ¹³C NMR (CDCl₃, 100 MHz), δ (ppm): 159.0, 141.9, 131.6, 129.2, 128.7, 126.3, 126.1, 119.0, 117.8, 113.3. MS (TOF): *m/e* 597.22 (M⁺ + 1 calcd. 596.22).

CHAPTER 4

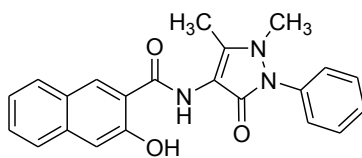
Quantification of optical signals for generation of molecular logic gates

4.1 General Introduction: The design and creation of new molecules⁸⁷ is the most creative art in chemistry as it arises from novel conceptual of well-known chemical reactions of well established molecules⁸⁸⁻⁹¹ but recently the molecules mimicking the operations of electronic devices have attained greater importance. The molecular approach to logic devices is usually associated with the mimicry of functions performed by silicon-based microprocessors and by moving beyond silicon-based technology simultaneously. It is becoming so much interesting these days that organic molecules can be used to perform digital data-processing functions as much as is done by the transistor switches in conventional electronics.⁹²⁻¹⁰⁰ The elementary logic operations, such as logic gates, encoders/decoders, molecular digital demultiplexers, and keypad locks, central to the functioning of electronic circuitry, can be performed by cleverly designing organic molecules, and can access even those areas which are inaccessible by the smallest electronics. Logic gates are a class of important and basic logic operations in which switches has output state (0 or 1) and depends on input conditions (0 or 1). Generally, there is one output mode which shows one logic function by combining two certain chemical inputs. More recently, construction of molecules, whose multi output logic functions can be modulated by multi-input combinations, has induced great interest. It would be possible to extend the information processing and computing to molecular level if only molecular logic gates are available, which could perform binary arithmetic and logical operations. Besides, receptor molecules obtained through simple synthetic protocols but capable of performing multiple logic operations are the first choice for any supramolecular chemist towards the construction of molecular devices and machines. Recently, devices from simple switches to produce more complex molecular systems that can perform a variety of classical logic functions,¹⁰¹ including extensions to switches on surfaces,¹⁰² and examples of half-adder,¹⁰³ full-adder,¹⁰⁴ keypad lock,¹⁰⁵ multiplexer,¹⁰⁶ multiplexer–demultiplexer,¹⁰⁷ multicomponent decoder¹⁰⁸ and encoder-decoder triad¹⁰⁹ has attained great importance. A digital encoder converts data into a code and a decoder converts the compressed data back into the original form. Gust *et al.*¹⁰⁹ reported a 4-to-2 encoder and 2-to-4

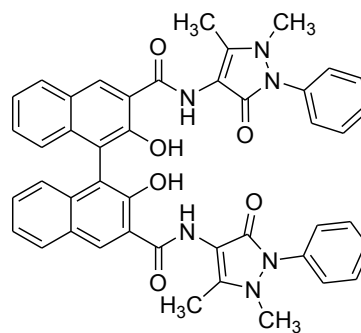
decoder based upon photonic inputs and outputs. Balzani *et al.*¹¹⁰ designed voltage and excitation based 4-to-2 encoder and 2-to-4 decoder. Bhalla *et al.*¹¹¹ reported for the first time 4-to-2 encoder based upon ionic inputs using pentaquinone derivative. Liu *et al.*¹¹² reported DNA based 2-to-1 encoder and 1-to-2 decoder. However, a molecular decoder with cations and anions as chemical inputs and fluorescence emission as an output is still unprecedented. Antipyrine is one of the biologically important moieties¹¹³ and has been widely used as sensors,¹¹⁴ for two-photon absorption, nonlinear optical and photoactive materials applications.¹¹⁵ In this chapter we have synthesized the dyads of antipyrine in combination with dansyl, hydroxyl naphthyl and BINOL moieties. These dyad combinations led to miniaturization of different logic devices like sequential XNOR logic gate, 2-1 encoder, 1-2 decoder and 4-2 bit encoder. Thus combination dyad based categorization of chapter is given below



1



2



3

4.2 Dansyl-antipyrine dyad as sequential XNOR logic device

4.3 Naphthalene-antipyrine dyad based ionic encoder and decoder

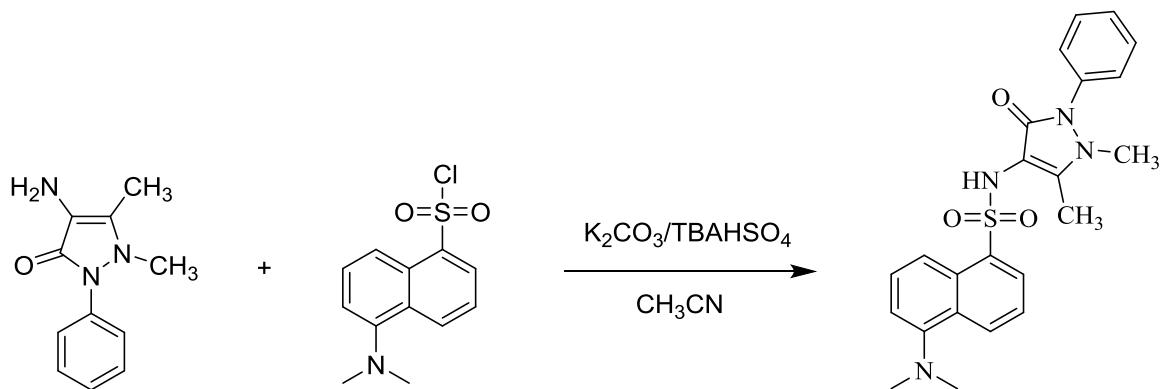
4.2 Dansyl-antipyrene dyad as sequential XNOR logic device

4.2.1 Abstract

Dyad **1** having dansyl and antipyrene hybrid molecular architectures was synthesized and their photophysical behavior towards various cations and anions was investigated. The dyad **1** was used for selective estimation of Cu^{2+} ions as compared to other metal ions and the binding event was indicated through formation of new absorption band at 465 nm and a decrease in fluorescence intensity at 528 nm. The dyad **1** showed ratiometric fluorescence behavior towards fluoride ions through hydrogen bonding interactions and restricted the photoinduced electron transfer from dansyl to antipyrene moiety to release its fluorescence at 428 nm. The chemical inputs of Cu^{2+} and F^- ions to dyad **1** mimicked a sequence dependent operation of an exclusive-NOR (XNOR) logic gate.

4.2.2 Synthesis of dyad **1**

The dyad **1** was synthesized by the reaction of 4-aminoantipyrene and dansyl chloride by using K_2CO_3 as base in dry CH_3CN . The reaction mixture was washed with water. The residue was column chromatographed on silica gel and recrystallized from ethanol to get pure dyad **1** as a brown colored solid in 84 % yield; MS (ESI) m/z 436.16 ($\text{M}^+\text{+H}$) (Scheme 1). ^1H NMR spectrum of dyad **1** showed 1H doublet at δ 8.49, 2H double doublet at δ 8.36, 2H multiplet at δ 7.46, 2H triplet at δ 7.36, 1H doublet at δ 7.23, 3H multiplet at δ 7.10 for aromatic protons, 1H broad singlet at δ 6.55 (D_2O exchangeable) for NH proton, 3H singlet at δ 3.04 due to N- CH_3 protons, 6H singlet at δ 2.83 due to N-(CH_3)₂ protons and 3H singlet at δ 2.32 due to CH_3 protons. The presence of three different peaks for 12 protons in aliphatic region confirmed the formation of dyad **1**. ^{13}C NMR spectrum showed signals at δ 160.9 (C=O), 153.5, 151.6, 130.6, 135.2, 134.1, 129.2, 128.9, 127.9, 127.1, 124.4, 123.2, 119.5, 114.9, 107.2 for aromatic -C, 45.4 (N- CH_3), 35.6 (N- CH_3) and 11.5 (CH_3). This spectral data having peak at 160.9 which was due to C=O corroborate the structure **1** for this compound. On the basis of these spectral data, this compound has been assigned the structure of 5-dimethylamino-naphthalene-1-sulphonic acid (1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4yl)-amide (Dyad **1**).



Dyad 1

Scheme 1: Synthesis of dyad **1**.

We succeeded in growing the crystal of dyad **1** in the mixture of THF and ethanol by solvent evaporation method, then single crystal XRD studies showed that crystal has monoclinic structure, having (*P* 2₁/c) space group with wR2 (reflections) = 0.1196 (Figure 1 and Table 1). Unit cell dimensions were found to be: *a* = 7.7031(7) Å, *b* = 17.8226(19) Å, *c* = 16.0433(16) Å, $\alpha = 90^\circ$, $\beta = 90.711(6)^\circ$, $\gamma = 90^\circ$, Volume = 2202.4(4) Å³ and *Z* = 4

Table 1: Summary of crystal data, data collection and structure refinement for dyad **1**

A. Crystal Data

Chemical formula	C ₂₃ H ₂₄ N ₄ O ₃ S	
Formula weight	436.53	
Temperature	200 K	
Wavelength	0.71073 Å	
Crystal system	monoclinic	
Space group	P 2 ₁ /n	
Unit cell dimensions	<i>a</i> = 7.7031(7) Å	$\alpha = 90^\circ$
	<i>b</i> = 17.8226(19) Å	$\beta = 90.711(6)^\circ$
	<i>c</i> = 16.0433(16) Å	$\gamma = 90^\circ$
Volume	2202.4(4) Å ³	
<i>Z</i>	4	
Density (calculated)	1.316 g/cm ³	
Absorption coefficient	0.179 mm ⁻¹	
<i>F</i> (000)	920	

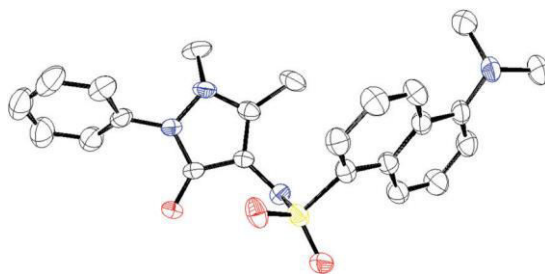


Figure 1: X-Ray crystal structure of dyad **1** (CCDC No. 963670)

4.2.3 Results and discussion

UV-Vis spectroscopic properties of dyad **1** were investigated in 20 μM CH_3CN . Dyad **1** which was colorless displayed an absorption band centred at 345 nm ($\varepsilon = 12,040 \text{ dm}^3\text{mol}^{-1}\text{cm}^{-1}$). On addition of different metal ions, viz., Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Hg^{2+} , Cd^{2+} , Cu^{2+} , Ag^+ and Pb^{2+} , to a solution of dyad **1**, there was no significant change in its UV-Vis spectrum except in case of addition of Cu^{2+} ions. On addition of Cu^{2+} to the solution of dyad **1**, the absorption intensity at 345 nm decreased and concomitantly a new absorption band at 465 nm was formed with an isobestic point at 380 nm (Figure 2a). The appearance of a broad range absorption band was associated with change in color of the solution from colorless to dark yellow and pointed to the deprotonation of sulfonamide NH and delocalization of the negative charge on both sulfonamide and antipyrine moieties. The spectral fitting of the absorption data obtained by titration of **1** with Cu^{2+} showed the formation of 1 : 1 stoichiometric **1**- Cu^{2+} complex ($\log \beta = 5.1 \pm 0.1$). Dyad **1** could be used to determine Cu^{2+} ions ratiometrically between 0.5-30 μM concentration (Figure 2b).

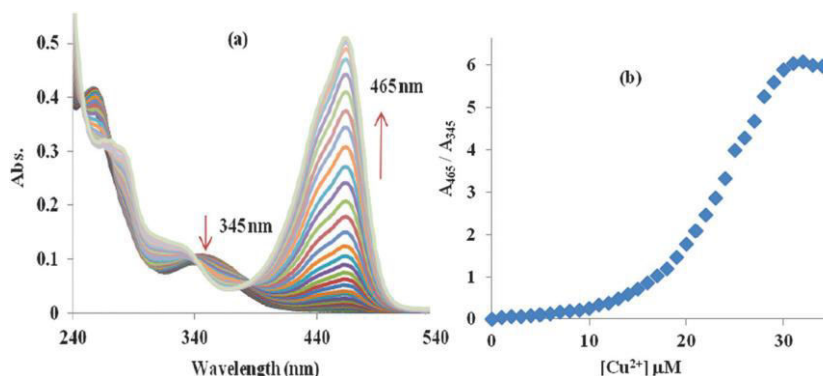


Figure 2: (a) Absorption spectrum of dyad **1** (20 μM , CH_3CN) upon complexation with increasing concentration of Cu^{2+} ions; (b) Ratiometric response of different concentrations of Cu^{2+} ions on dyad **1** (20 μM , CH_3CN).

To obtain an insight into the fluorescent properties of dyad **1** toward metal ions, the emission changes were investigated with different ions such as Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Hg^{2+} , Cd^{2+} , Cu^{2+} , Ag^+ and Pb^{2+} in CH_3CN . Dyad **1** ($5\ \mu\text{M}$, CH_3CN), on excitation at 370 nm, displayed emission band at 528 nm (typical of dansyl moiety) which underwent fluorescence quenching selectively on addition of Cu^{2+} (Figure 3), whereas other metal ions did not show any significant change. The fluorescence intensity of dyad **1** is linearly proportional to the Cu^{2+} concentration of 0-20 μM and the detection limit was calculated as 1.19×10^{-7} M. Job's plot analysis clearly showed 1 : 2 stoichiometry with **1** and Cu^{2+} . Fluorescence studies of dyad **1** were performed in the presence of other metal ions (Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Hg^{2+} , Cd^{2+} , Ag^+ and Pb^{2+}) under the same conditions as those used for the Cu^{2+} ions titration. However, no significant change in the emission spectrum was observed in the presence of other metal ions.

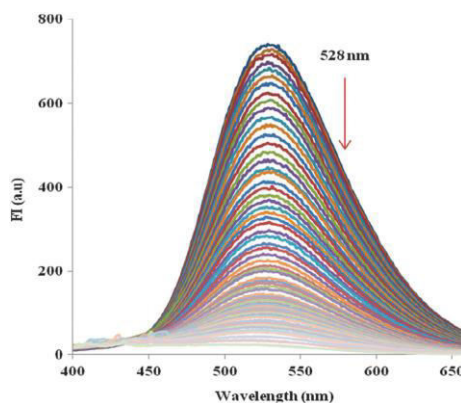


Figure 3: Fluorescence spectrum of dyad **1** ($5\ \mu\text{M}$, $\lambda_{\text{ex}} = 370\ \text{nm}$) in CH_3CN upon complexation with increasing concentration of Cu^{2+} ions.

To determine the practical applicability of dyad **1**, we carried out competitive experiments in the presence of 10 equiv. Cu^{2+} ions mixed with 50 equiv. of each of the various metal ions. No significant variation in the fluorescence intensity was found when comparing the results with or without other metal ions (Figure 4).

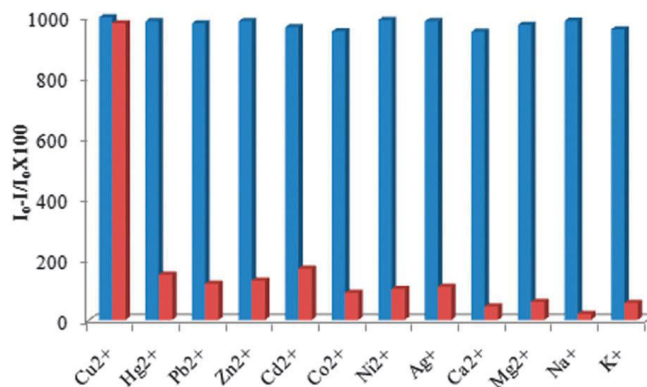


Figure 4: The red bars represent the selectivity of dyad **1** (1 μ M, λ_{ex} = 350 nm) upon addition of different metal ions in CH₃CN and the blue bars show the competitive selectivity of dyad **1** in the presence of interfering metal ions.

The presence of NH-group in dyad **1** prompted us to evaluate its binding behaviour towards different anions of biological interest. On addition of anions viz., F⁻, AcO⁻, Cl⁻, Br⁻, I⁻, SO₄²⁻, NO₃²⁻, PO₄²⁻, P₂O₅⁻, CN⁻ etc. a significant change in color (colorless to yellow) and absorption spectrum were observed only with F⁻ ions. On gradual addition of F⁻ ions to the solution of dyad **1**, the absorption intensity at 350 nm decreased with concomitant increase in absorption intensity at 310 (Figure 5a). Job's plot analysis and binding stoichiometry showed the 1: 1 (dyad **1**: F⁻) complexation with association constant $1.37 \pm 0.01 \times 10^5 \text{ M}^{-1}$. In case of fluorescence studies of dyad **1** (5 μ M, CH₃CN) with different anions, showed fluorescence changes only with F⁻ ions has been observed. On gradual addition of F⁻ to the solution of dyad **1**, the emission at 528 nm was quenched and a new emission band at 420 nm immersed (Figure 5b) that achieved its plateau after addition of 1 equiv. fluoride ions. The non-linear regression analysis showed the formation of 1 : 1 complexation with stability constant $1.5 \pm 0.02 \times 10^5 \text{ M}^{-1}$. With addition of other anions (100 equiv.) such as AcO⁻, Cl⁻, Br⁻, I⁻, SO₄²⁻, NO₃²⁻, PO₄²⁻, P₂O₅⁻ and CN⁻ no significant change in fluorescence intensity was observed by comparison with and without other anions except CN⁻ ions at more than 80 equivalents. With gradual addition of CN⁻ to the solution of dyad **1**, the emission band at 528 nm was quenched. The detection limit of F⁻ ions was determined to be 0.12 μ M. Therefore, in the ground state, dyad **1** complex with Cu²⁺ caused bathochromically shifted UV-Vis spectrum. However, in the excited state, the dansyl moiety of dyad **1** binds with two Cu²⁺ ions to form M₂L species. In excited state, the coordination of dimethylamino group of dansyl unit with Cu²⁺ caused quenching of fluorescence at 528 nm due to the dansyl moiety.

However, the presence of fluoride ion caused deprotonation of sulfonamide NH and restricted the photoinduced electron transfer from dimethylamino to antipyrine moiety to release its fluorescence at 420 nm. The simultaneous quenching and release of fluorescence were due to dansyl and antipyrine moieties, respectively that emulates into fluoride ion induced a ratiometric change (Scheme 2). The X-ray structure of dyad **1** and optimization at B3LYP/6-31G level showed an average distance of 6–7 Å between the two fluorophores and is sufficiently small for communication between the two fluorophores to operate the FRET phenomenon's.

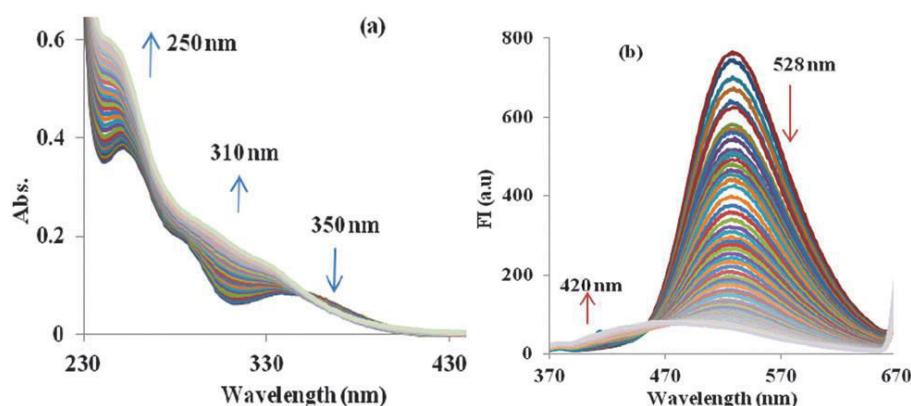
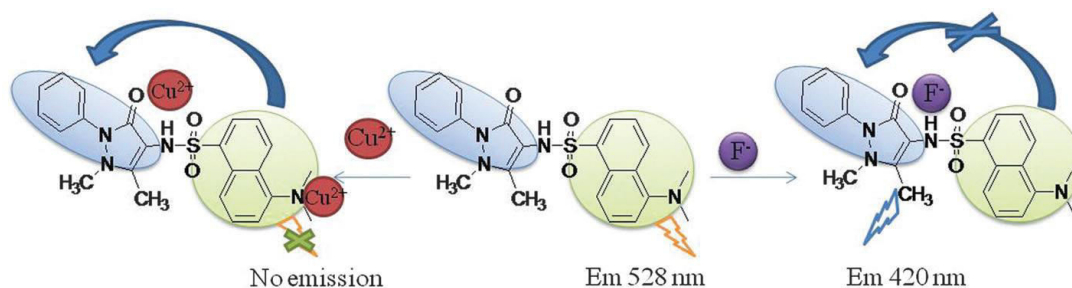


Figure 5: Effect of addition of fluoride ions on (a) absorption spectrum of dyad **1** (20 μM, CH₃CN); (b) emission spectrum of dyad **1** (5 μM, CH₃CN, λ_{ex} = 370 nm).



Scheme 2: Proposed mechanism for dyad **1** binding with Cu²⁺ and F⁻ ions.

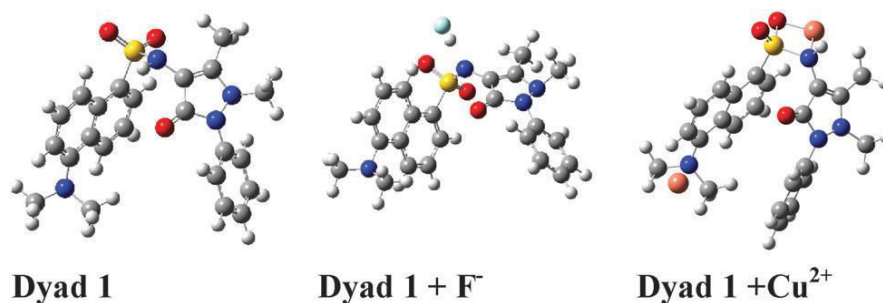


Figure 6: DFT optimized structure of dyad **1** and in presence of F^- and Cu^{2+} ions calculated at the B3LYP/6-31(d p) level.

4.2.4 Theoretical studies

In order to provide insight into the mode of complexation of dyad **1** towards Cu^{2+} and F^- ions, the structures of dyad **1** and its complexes with F^- and Cu^{2+} were optimized by using the B3LYP functional in combination with 6-31G (d,p) for dyad **1** and its F^- complex and LAN2DZ basis sets for complexes of dyad **1** with Cu^{2+} ions. The presence of fluoride ions caused deprotonation of sulphonamide NH whereas Cu^{2+} ion in proximity to dyad **1** and showed strong interactions with sulphonamide O and N, associated with rotation of H of NH towards the opposite direction (Figure 6). Further, time dependent density functional theory (TD-DFT) calculations on dyad **1** and its complexes with F^- and Cu^{2+} ions showed that HOMO is distributed over the antipyrine ring and LUMO over the dansyl ring and so, in the dyad **1**. F^- complex. This is associated with increased energy gap between the HOMO and LUMO and thus the absorption band is shifted at short wavelength. In case of dyad **1**. Cu^{2+} complex, HOMO is distributed on both the ring systems of dansyl and antipyrine and LUMO is mostly located on the antipyrine ring (Figure 7).

This indicated that dyad **1**. Cu^{2+} complex bears ICT character among the fluorophore units. Furthermore, the energy gap between HOMO and LUMO of Cu^{2+} complexed dyad **1** is smaller than that of dyad **1** which was in good agreement with the red shift in the absorption band of dyad **1** from 345 to 465 nm in the presence of Cu^{2+} ions.

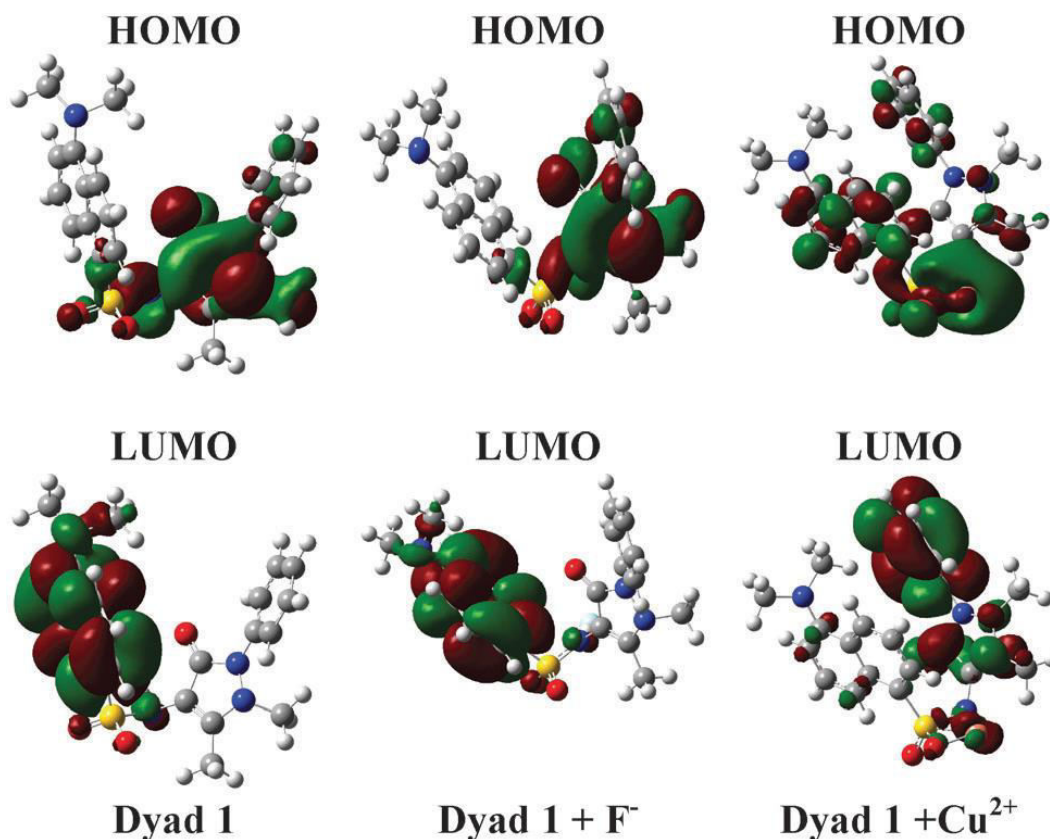
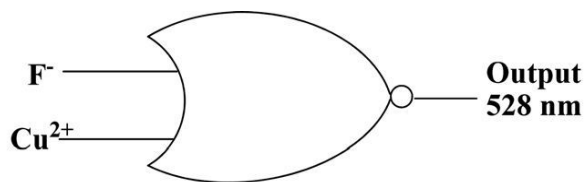


Figure 7: Contour diagrams of HOMO and LUMO of dyad **1**, dyad **1** + F⁻ and dyad **1** + Cu²⁺.

4.2.5 Application as logic device

Recently, the development of sequential logic devices involving the conversion of chemically encoded information into fluorescent signals has emerged as an active research area for unconventional computing. Sequential circuits are essential for the realization of memory devices capable of storing information and operating through feedback loops, where one of the output of the device functions as an input and is memorized as a “memory element”. Therefore, depending upon the different chemical inputs (F⁻ and Cu²⁺) and using the fluorescent signal as the output, an integrated and sequential logic circuit can be constructed (Table 2). Thus, the molecular switching behaviour of dyad **1** with two chemical inputs (Cu²⁺ and F⁻) was demonstrated with the help of binary logic. The fluorescence output at 528 nm was in “on” state only when both inputs were absent or present. Such fluorescence changes upon the actions of two chemical inputs mimic the performance of an exclusive-NOR (XNOR) logic gate (Figure 8).

Table 2: Schematic representation of the XNOR logic operation and truth table for the sequential logic circuit, where 0 = Off and 1 = On



Input 1 F^-	Input 2 Cu^{2+}	Output $\lambda_{em} 528 \text{ nm}$
0	0	1
1	0	0
0	1	0
1(1 st)	1	1
1	1(1 st)	0

A XNOR logic function is a two input device and its output is “true” if the inputs are the same and “false” if the inputs are different. Significantly, dyad **1** restores the information if F^- input comes first (“on” state) or Cu^{2+} first (“off” state). The development of such sequential circuits is important as combinational logic and not enough to construct memory devices.

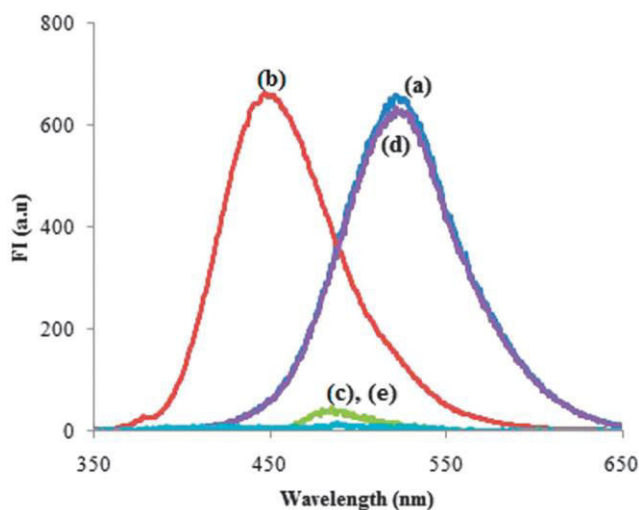


Figure 8: Fluorescence spectrum of (a) dyad **1** (5 μM , CH_3CN); (b) in the presence of fluoride ion (1 eq.); (c) Cu^{2+} ions (1 eq.); (d) F^- as first input (1 eq.) + Cu^{2+} (1 eq.); (e) F^- as second input (1 eq.) + Cu^{2+} (1 eq.).

4.2.6 Conclusions

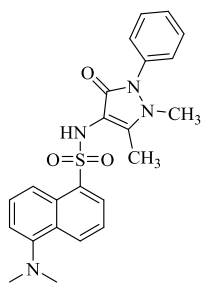
Dansyl–antipyrene dyad **1** was synthesized which showed selectivity toward Cu^{2+} and fluoride ions. Dyad **1** in the ground state interacts with only one Cu^{2+} ions to generate ICT induced color changes and in the excited state, the coordination of dimethylamino group of dansyl unit with Cu^{2+} causes quenching of the fluorescence due to the dansyl moiety. The dyad **1** showed ratiometric fluorescence behavior towards the fluoride ions through hydrogen bonding interactions and restricts the photoinduced electron transfer from dansyl to antipyrene moiety to release its fluorescence. The molecular switching has been observed between chemical inputs of Cu^{2+} and F^- ions which mimicked the sequence dependent operation of an exclusive-NOR (XNOR) logic gate.

4.2.7 Experimental section

4.2.7.1 Synthesis of dyad **1**

To the stirred solution of 4-aminoantipyrene (203 mg, 1 mmol) and K_2CO_3 (1.2 mmol) in dry CH_3CN (30 ml), the solution of dansyl chloride (269 mg, 1 mmol) in dry CH_3CN (10 ml) was added for 5 min and stirred the reaction mixture at refluxing conditions for 8 h. The reaction mixture was washed with water. The residue was column chromatographed on silica gel and recrystallized from ethanol to get pure dyad **1**.

5-Dimethylamino-naphthalene-1-sulphonic acid (1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-amide (Dyad **1**)



Dyad **1**

84% yield; Yellow colored solid; Mpt. 215–216 °C; ^1H NMR (CDCl_3 , 400 MHz): δ 8.49 (d, 1H, $J = 8.28$ Hz, ArH), 8.35–8.38 (dd, 2H, $^2J = 8.72$ Hz, $^3J = 5.96$ Hz, ArH), 7.44–7.49 (m, 2H, ArH), 7.34–7.37 (t, 2H, $J = 7.36$ Hz, ArH), 7.22–7.24 (d, 1H, ArH), 7.09–7.12 (m, 3H, ArH), 6.55 (bs, 1H, NH), 3.04 (s, 3H, N– CH_3), 2.83 (s, 6H, NCH_3), 2.32 (s, 3H, CH_3); ^{13}C NMR (CDCl_3 , 100 MHz): δ 160.9 (C=O), 153.5, 151.6, 130.6, 135.2, 134.1, 129.2, 128.9, 127.9, 127.1, 124.4, 123.2, 119.5, 114.9, 107.2 for aromatic –C, 45.4 (N– CH_3), 35.6 (N– CH_3), 11.4 (CH_3); MS (ESI) m/z 436.16 ($\text{M}^+ + 1$).

4.3 Naphthalene-antipyrine dyad based ionic encoder and decoder

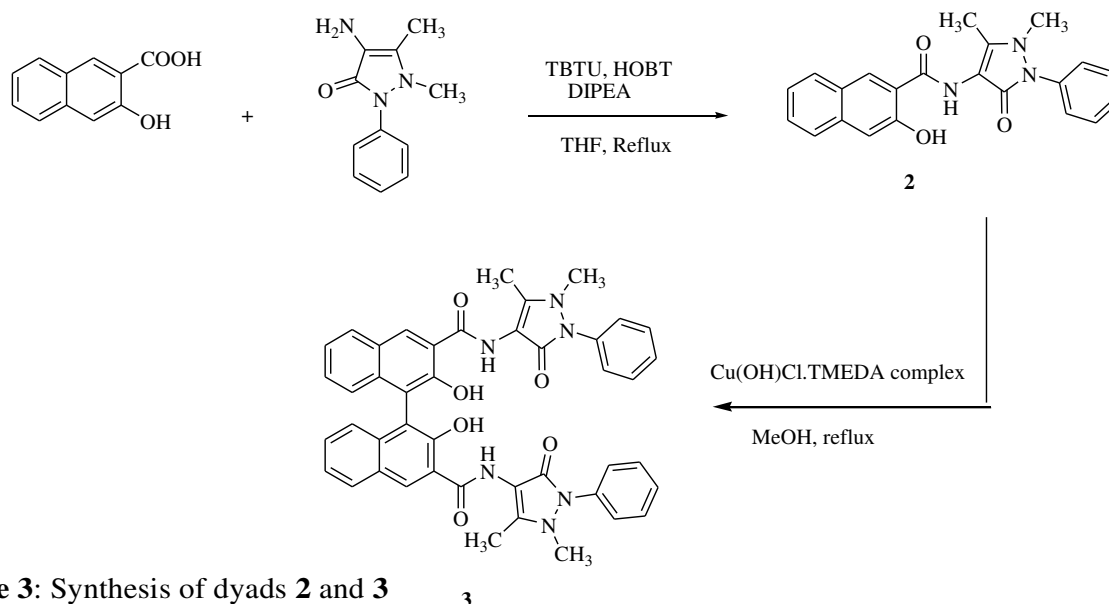
4.3.1 Abstract: Antipyrine and naphthoic acid based hybrid molecular architect containing amide group have been synthesized and investigated their photophysical behavior towards various cations and anions. The dyad **2** has been used for selective estimation of Al^{3+} ions as compared to other metal ions and signalled the binding event through formation of new absorption band at 405 nm and ‘turn-on’ fluorescence intensity at 505 nm via chelation enhanced fluorescence (CHEF) effect and restricting Photoinduced Electron Transfer (PET) phenomenon with lowest detection limit to be 2 μM . The dyad **2** showed selective estimation for CN^- ions among all other anions by forming new absorption band at 400 nm and fluorescence turn “on” at 520 nm with lowest detection limit to be 2 μM . Al^{3+} concentration was also detected by using dyad **2** in sewage water upto 7.4-8.6 ppm. To enhance the selectivity of dyad **2**, oxidative coupling was done to form dyad **3** which was used to miniaturized encoders and decoders. Dyad **3** with diamide group has been synthesized and investigated their photophysical behavior towards various cations and anions. The dyad **3** has been used for estimation of Al^{3+} and Cu^{2+} ions as compared to other metal ions and signaled the binding event through formation of new absorption bands at 415 nm and 430 nm. In case of fluorescence, there was new emission band formation for Al^{3+} at 515 nm and in case of Zn^{2+} at 485 nm while quenching was observed in case of Cu^{2+} ions. The dyad **3** showed selective estimation for CN^- ions among all other anions by forming new absorption band at 395 nm and new emission band at 495 nm with lowest detection limit to be 2 μM . Based on these results, anion/cation as well as cation/cation 2-to-1 encoder and 1-to-2 decoder were formed. Similarly 4-to-2 encoder was established by using cations as various inputs.

4.3.2 Synthesis of dyads 2 and 3

4.3.2.1 Synthesis of dyad 2 To the stirred solution of 3-hydroxy-2-naphthoic acid and 4-aminoantipyrine, *o*-(1*H*-Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate, *N,N* diisopropylethylamine and hydroxybenzotriazole in tetrahydrofuran were added. After completion of reaction and usual workup dyad **2** was obtained in 70% yield (Scheme 3); MS (ESI) *m/z* 389.17 (M^++1). ^1H NMR showed 1H

broad singlet at δ 11.85 (D_2O exchangeable) for OH proton, 1H singlet at δ 9.78 (D_2O exchangeable) for NH proton, 1H singlet at δ 7.90, 5H multiplet at δ 7.50, 2H multiplet at δ 7.39, 1H doublet at δ 7.33, 1H multiplet at δ 7.17, 1H singlet at δ 7.11 for aromatic protons, 3H singlet at δ 3.20 due to $N-CH_3$ protons and 3H singlet at δ 2.27 due to $-CH_3$ protons. The broad singlets at δ 11.55 and 9.78 of 1H due to OH and NH along with aliphatic proton peaks confirmed the formation of dyad **2**. ^{13}C NMR showed signals at δ 162.4, 167.2 due to $C=O$, 154.4, 150.8, 134.0, 136.6, 130.9, 129.5, 129.0, 127.9, 127.8, 127.0, 125.7, 125.3, 123.1, 118.8, 111.3, 107.1 for aromatic $-C$, 35.44 and 11.86 for aliphatic $-C$. This spectral data having peaks at 162.3, 167.2 which was due to $C=O$ and peaks at 35.4 and 11.8 for aliphatic $-C$ corroborated the structure **2** for this compound.

Synthesis of dyad 3: Refluxing of dyad **2** and $CuCl(OH).TMEDA$ in CH_3OH for 5-6 h gave diamide dyad **3** in 75% yield (Scheme 3); MS (ESI) m/z 744.27 (M^++1). ^1H NMR showed 2H broad singlet at δ 12.07 (D_2O exchangeable) for OH protons, 2H singlet at δ 10.37 (D_2O exchangeable) for NH protons, 2H singlet at δ 8.90, 2H multiplet at δ 7.95, 4H triplet at δ 7.51, 10H multiplet at δ 7.35, 2H doublet at δ 7.00 for aromatic protons, 3H singlet at δ 3.13 due to $-N-CH_3$ protons and 6H singlet at δ 2.26 due to $-CH_3$ protons. This additional number of protons confirmed the formation of dyad **3**. ^{13}C NMR showed peaks at δ 169.6, 161.6 due to $-C=O$, 154.3, 152.9, 135.8, 134.9, 129.7, 129.4, 129.2, 128.9, 126.5, 124.1, 123.8, 116.8, 116.5, 106.1 due to aromatic $-C$, 35.9 due to $-N-CH_3$ and 11.0 due to $-CH_3$. On the basis of these spectral data, this compound has been assigned the structure of (dyad **3**).



Scheme 3: Synthesis of dyads **2** and **3**

4.3.3 Results and discussion of dyad **2**

The UV-Vis spectroscopic properties of dyad **2** were investigated in CH₃OH (20 μM). Dyad **2** was colorless and displayed an absorption band centered at 350 nm and 280 nm. On addition of different metal ions, viz. Na⁺, K⁺, Ag⁺, Mg²⁺, Ca²⁺, Ba²⁺, Co²⁺, Cu²⁺, Ni²⁺, Zn²⁺, Hg²⁺, Pb²⁺, Fe³⁺ and Al³⁺ (1000 μM) to solution of dyad **2**, there was no significant change in its UV-Vis spectrum except in case of addition of Al³⁺ ions (Figure 9a).

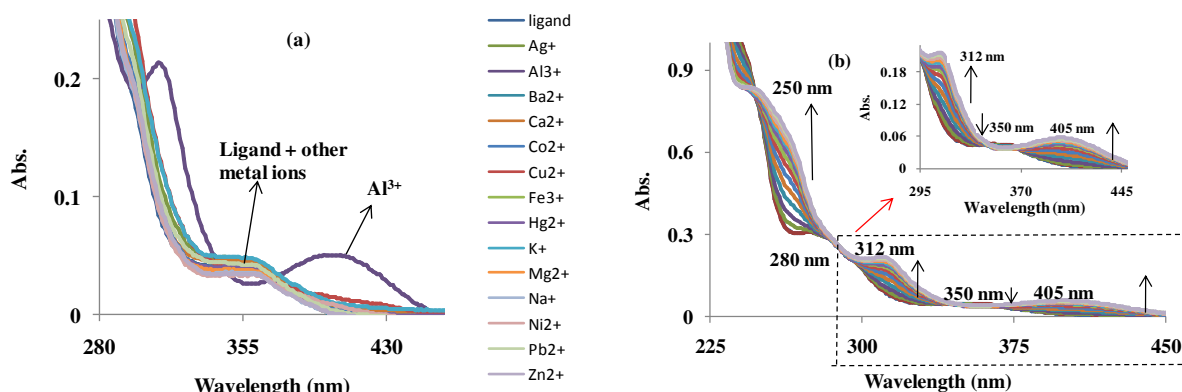


Figure 9: Effect of various (a) metal ions (1000 μM); (b) incremental addition of Al³⁺ (Inset: photograph of absorption spectrum from 295-445 nm upon addition of Al³⁺ on absorption spectrum of dyad **2** (20 μM, CH₃OH))

On addition of Al^{3+} (100 μM) ions to the solution of dyad **2**, absorption intensity at 350 nm decreased and concomitantly new absorption band at 405 nm was formed and the band at 280 nm shifted to 250 nm (Figure 9b). The absorption ratio at 350 nm and 405 nm varied from 0.136 to 1.225, indicating 9.00 fold absorption ratio change. Thus, dyad **2** was used to estimate a wide range of Al^{3+} ions between 5 - 100 μM through UV-Vis spectrophotometer. To obtain an insight into the fluorescent properties of dyad **2** toward metal ions, the emission changes were investigated with different metal ions such as Na^+ , K^+ , Ag^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Hg^{2+} , Pb^{2+} , Fe^{3+} and Al^{3+} (1000 μM) in CH_3OH . Dyad **2** on exciting at 360 nm was initially non fluorescent which underwent turn on emission in case of only Al^{3+} ions at 505 nm whereas no other metal ion was responsible for changes in emission properties (Figure 10a).

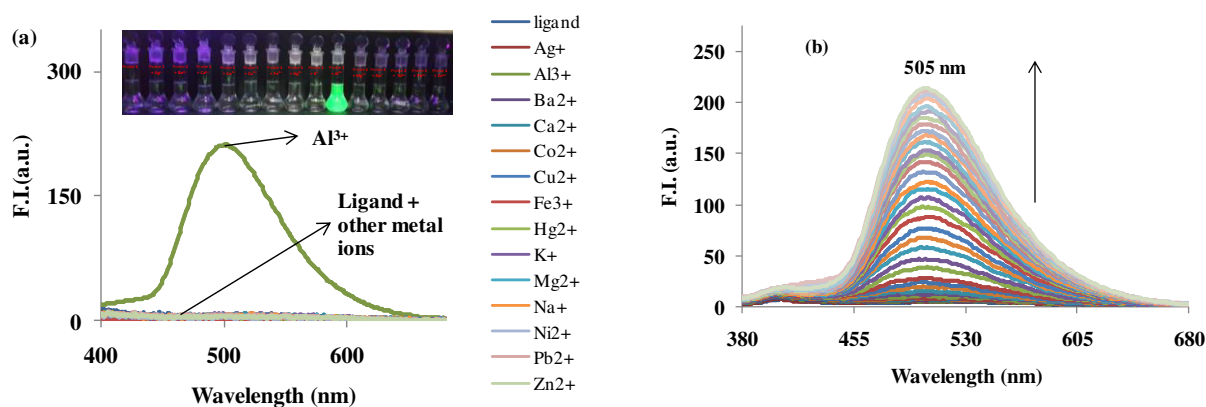


Figure 10: Effect of various (a) metal ions (1000 μM) and inset of photograph of dyad **2** with different metal ions under UV light; (b) Incremental addition of Al^{3+} on emission spectrum of dyad **2** (20 μM , CH_3OH).

Upon titrating dyad **2** with Al^{3+} ions, it has been found that with the addition of 200 μM Al^{3+} , there was formation of band at 505 nm, indicating fluorescence turn “on” (Figure 10b). The binding constant was calculated to be $5.2 \pm 0.02 \times 10^3 \text{ M}^{-1}$ with lowest detection of 2 μM . The emission intensity at 505 nm varied from 5.061 to 213.948 indicating 42.27 fold emission change. Thus, dyad **2** was used to estimate a wide range of Al^{3+} ions between 2 - 200 μM through fluorescence spectrophotometer.

Further, to explore the practical applicability of dyad **2** with Al^{3+} towards different metal ions, competitive experiments in the presence of 1000 μM of various metal ions via Na^+ , K^+ , Ag^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Hg^{2+} , Pb^{2+} and 1000 μM of Al^{3+} was performed. No significant variation in the absorption as well as emission band was found when comparing the results with or without the other metal ions (Figure 11).

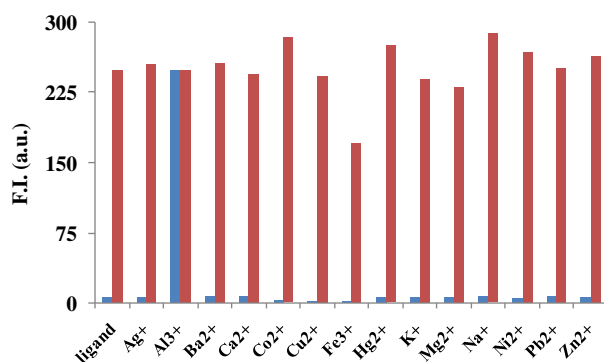


Figure 11: Blue bars represent selectivity of dyad **2** (20 μM) upon addition of different metal ions in MeOH and red bars show the competitive selectivity of dyad **2** in the presence of Al^{3+} .

In order to determine the binding properties of dyad **2** with Al^{3+} , NMR titrations were performed. It was observed that the aromatic proton peak at δ 8.6 splitted to give new peaks at δ 8.8 and 8.3, the peak at δ 7.8 shifted to δ 8.0, peak at δ 7.4 shifted to δ 7.7 due to binding of Al^{3+} to carbonyl and nitrogen of antipyrene moiety and oxygen of naphthoic acid ring (Figure 12).

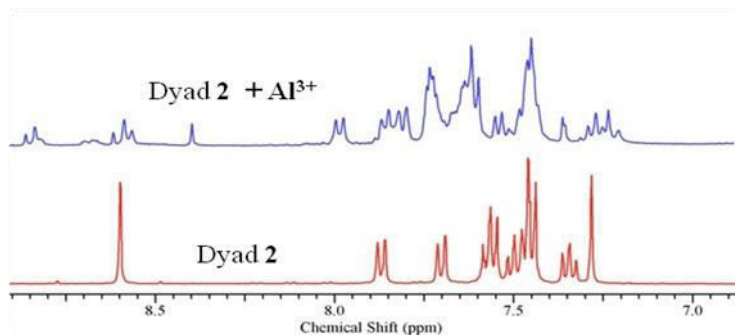


Figure 12: Partial ^1H NMR spectrum (9 - 6.6 ppm region) of dyad **2** (5 mM, CD_3OD) and in the presence of 1 equiv. of Al^{3+} ions.

The properties of dyad **2** for different anions were examined in CH₃OH solutions including F⁻, Cl⁻, Br⁻, I⁻, SCN⁻, AcO⁻, NO₃⁻, H₂PO₄⁻, HSO₄⁻ and CN⁻ ions. Upon the addition of 1000 μM of various anions, the absorption spectrum of dyad **2** did not show any significant change except for CN⁻ ion (Figure 13a). Upon the addition of CN⁻ ions, absorption band at 350 nm diminished with the concomitant appearance of a new band at 400 nm. Upon titrating solution of dyad **2** with CN⁻ ions, it was found that on addition of 300 μM CN⁻ ions, band at 350 nm diminished with formation of new band at 400 nm (Figure 13b).

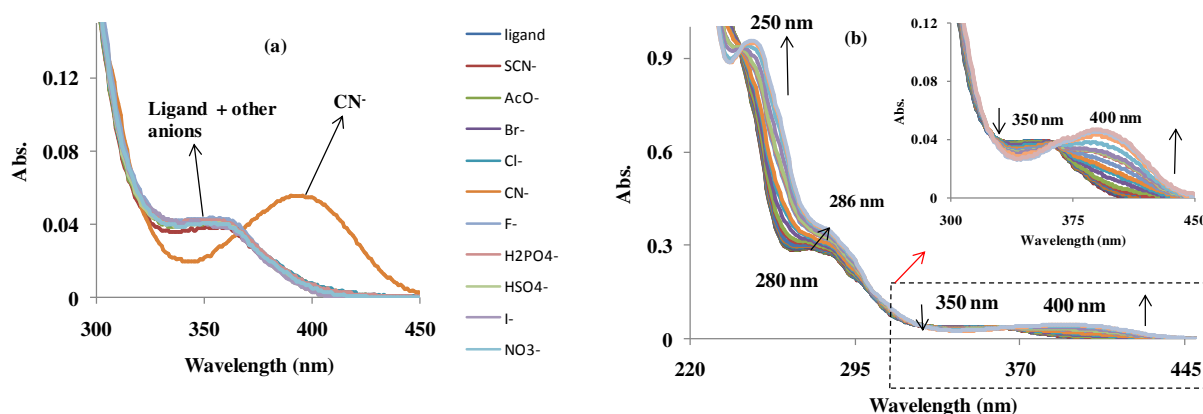


Figure 13: Effect of various (a) anions (1000 μM) (b) Incremental addition of CN⁻ (Inset: photograph of absorption spectrum from 220-445 nm upon addition of CN⁻) on absorption spectrum of dyad **2** (20 μM, CH₃OH)

The absorption ratio at 350 nm and 400 nm varied from 0.076 to 1.517, indicating 19.96 fold absorption ratio change. Thus, dyad **2** was used to estimate a wide range of cyanide ions between 2-300 μM through UV-Vis spectrophotometer. The emission properties of dyad **2** were investigated towards different anions such as F⁻, Cl⁻, Br⁻, I⁻, SCN⁻, AcO⁻, NO₃⁻, H₂PO₄⁻, HSO₄⁻ and CN⁻ (1000 μM) in CH₃OH on excitation at 360 nm. Dyad **2** showed “turn-on” fluorescence only in the presence of CN⁻ at 520 nm and no other anions participated (Figure 14a).

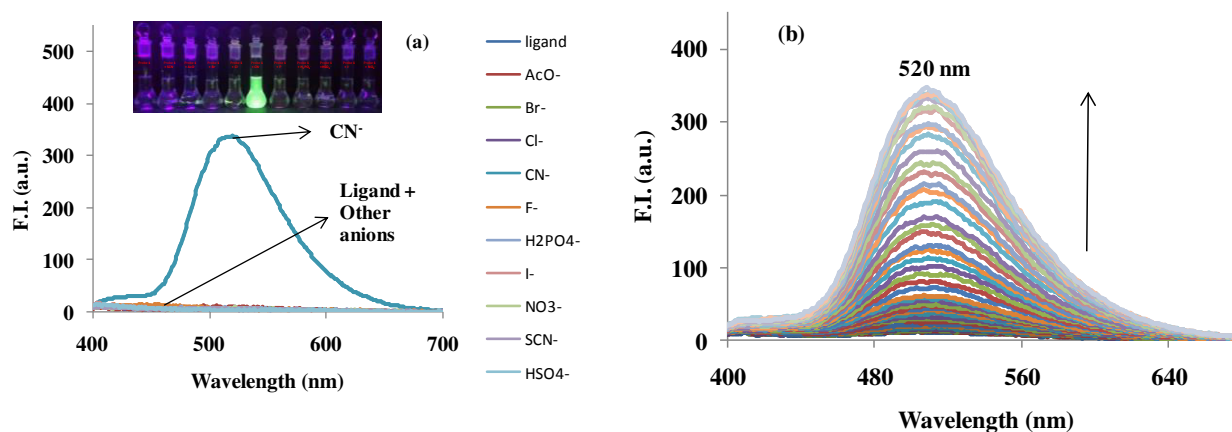


Figure 14: Effect of various (a) anions (1000 μM) (Inset: Photograph of dyad **2** with different anions under UV light); (b) Incremental addition of CN^- on emission spectrum of dyad **2** (20 μM , CH_3OH)

Upon incremental addition of CN^- ions to dyad **2** between 0.5 μM -500 μM , a linear increase in emission intensity was observed at 520 nm (Figure 14b). The binding constant was calculated to be $0.4 \pm 0.01 \times 10^5 \text{ M}^{-1}$ with lowest detection of 2 μM . The emission intensity at 520 nm varied from 10.854 to 320.766 indicating 30.30 fold emission change. Thus, dyad **2** was used to estimate a wide range of Al^{3+} ions between 2-780 μM through fluorescence spectrophotometer.

Further, to explore the practical applicability of dyad **2** with CN^- towards different anions, competitive experiments in the presence of 500 μM of various anions like Cl^- , Br^- , I^- , SCN^- , AcO^- , NO_3^- , F^- , H_2PO_4^- and HSO_4^- , and 1000 μM of CN^- was performed. No significant variation in the absorption as well as emission band was found when comparing the results with or without the other anions (Figure 15).

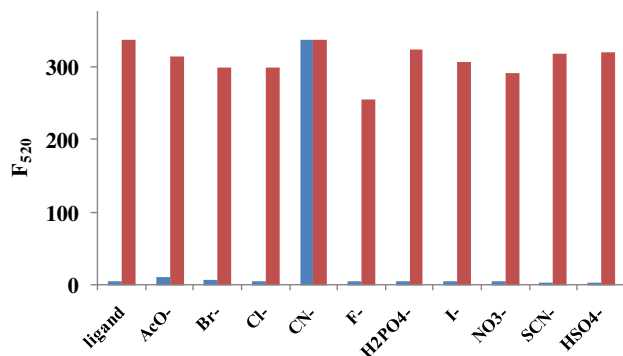


Figure 15: Blue bars represent selectivity of dyad **2** (20 μ M) upon addition of different anions in CH₃OH and red bars showed the competitive selectivity of dyad **2** in the presence of CN⁻.

Upon addition of 1 equiv. of CN⁻, ¹H NMR signal of dyad **2** revealed shifting of aromatic hydrogens of naphthalic ring due to deprotonation of hydroxyl group attached to it. Previously, the protons were deshielded due to presence of hydroxyl group but with addition of cyanide ions which led to deprotonation of this hydroxyl group. Thus all the aromatic hydrogen directly linked to this naphthalic ring i.e. H_a, H_b, H_c, H_f got shielded and shifted upfield (Figure 16 and Table 3).

Table 3: NMR alterations of dyad **2**.CN⁻

	Nature of peak	Actual position before addition of CN ⁻ (ppm)	Position after addition of CN ⁻ (ppm)	Chemical shift (ppm)
H _a	Singlet	8.60	8.47	0.13
H _b	Doublet	7.87	7.64	0.23
H _c	Doublet	7.69	7.52	0.17
H _f	Singlet	7.28	6.98	0.30

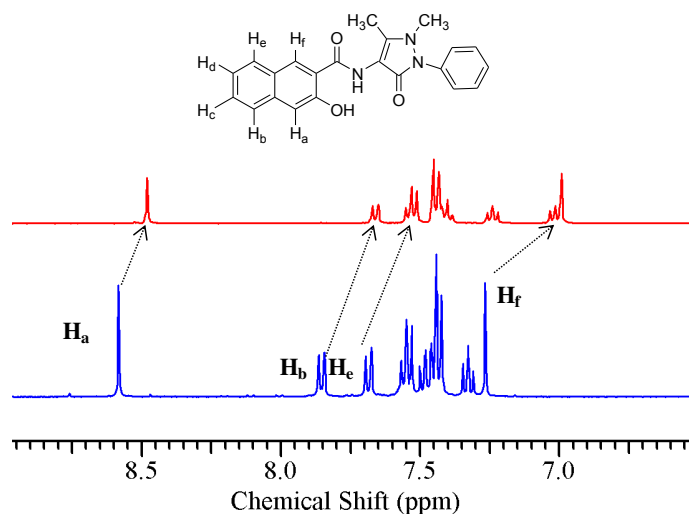
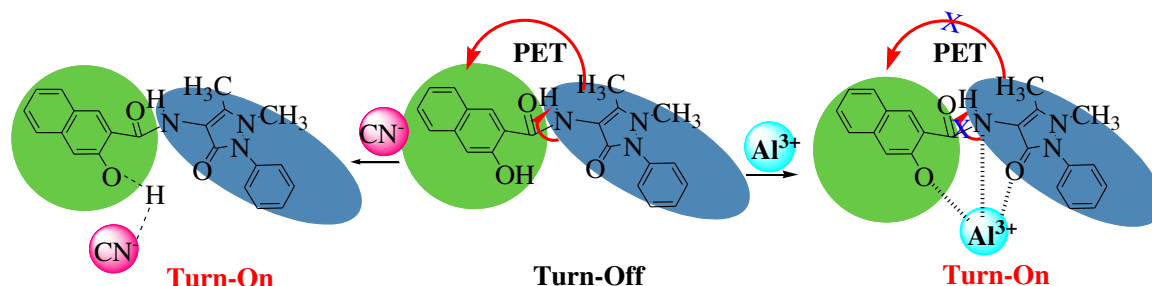


Figure 16 Partial ^1H NMR spectral (9 - 6.6 ppm region) changes of dyad **2** (5mM) in CD_3OD in the presence of TBACN (1 eq.)

Proposed binding approach of the complex

Based on the above results, a plausible binding mechanism of $\mathbf{2}.\text{Al}^{3+}$ complex is shown in Scheme 4. Dyad **2** has weak fluorescence due to intramolecular photo-induced electron-transfer (PET) process and the unrestricted torsional rotation between the C–C single bond which covalently links acid and antipyrine units. Thus, leading to no structural rigidity for the sensor due to the intramolecular rotation wherein the charge transfer-excited singlet state is rapidly deactivated. PET mechanism is responsible for deactivation of the excited state of a fluorophore by addition of an electron to one of its excited-state frontier orbitals, leading the fluorophore in a nonemissive state. It can be better understand if we considered the free electron pair of a functional group attached to the fluorophore which quench its fluorescence intramolecularly due to a PET process. Moreover, metal coordination to this group rendered it a less efficient electron donor, thereby interrupting the PET process and enhanced the fluorescence output via chelation-enhanced fluorescence (CHEF) effect. Here, the emission intensity of dyad **2** has been very low which was due to quenching by the amide nitrogen lone pair electrons through PET mechanism. However, addition of Al^{3+} ions to **2** led to remarkable fluorescence enhancement due to the PET inhibition process and restricted bond rotation due to the structural rigidity of the complex between the sensor and Al^{3+} ions. Based on the Job's

plot, it has been observed that there was 1:1 binding ratio between the dyad **2** and Al^{3+} complex. However, presence of cyanide ion caused the deprotonation of OH (Scheme 4).



Scheme 4: Proposed mechanism of binding of dyad **2** with CN^- and Al^{3+} ions.

4.3.4 Determination of Al^{3+} in sewage water samples

To examine the sensitivity and analytical performance conceptual experiment for dyad **2** was conducted. Two sewage water samples (Thapar University, Patiala, India) were collected and used for sensing of Al^{3+} ion. Both the samples were filtered through a 0.5 μL membrane and showed 7–10 ppm of Al^{3+} present in it by the Atomic Absorption Spectroscopy (AAS analysis) after spiking the samples with aluminum. Then the water samples were compared with standard Al^{3+} solution and analyzed with the dyad **2** (20 μM) (Table 4). The results for the sewage water samples showed satisfactory results, revealing that the receptor can be used for the determination of Al^{3+} in environmental sample analysis.

Table 4: Determination of Al^{3+} in water samples

Sample	Amount of Al^{3+} detected by AAS in ppm after spiking	Amount of Al^{3+} detected by fluorescence in ppm
Sewage Water 1	8.74	8.65 ± 0.02
Sewage Water 2	7.54	7.45 ± 0.01

4.3.5 Results and discussion of dyad 3

As dyad **2** showed sensitivity towards Al^{3+} ions only, in order to increase the more binding sites, dyad **3** was synthesized. Dyad **3** has more binding sites due to the presence of heteronuclear atoms in closed vicinity for interaction with cations. The binding behavior of dyad **3** was studied toward different cations and anions by UV-vis and fluorescence spectroscopy in CH_3OH . The absorption spectrum of **3** (20 μM , CH_3OH) was characterized by the presence of a typical absorption band at 370 nm (Figure 17a). Out of the various tested metal ions (K^+ , Na^+ , Ag^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Hg^{2+} , Pb^{2+} , Fe^{3+} and Al^{3+}), only Cu^{2+} and Al^{3+} ions showed the spectral changes whereas other metal ions did not show any significant changes. The incremental addition of Al^{3+} ions (140 μM) showed a new band at 415 nm upon the addition of 1000 μM of Al^{3+} with an isosbestic point at 385 nm to the solution of **3** indicating formation of new species (Figure 17b).

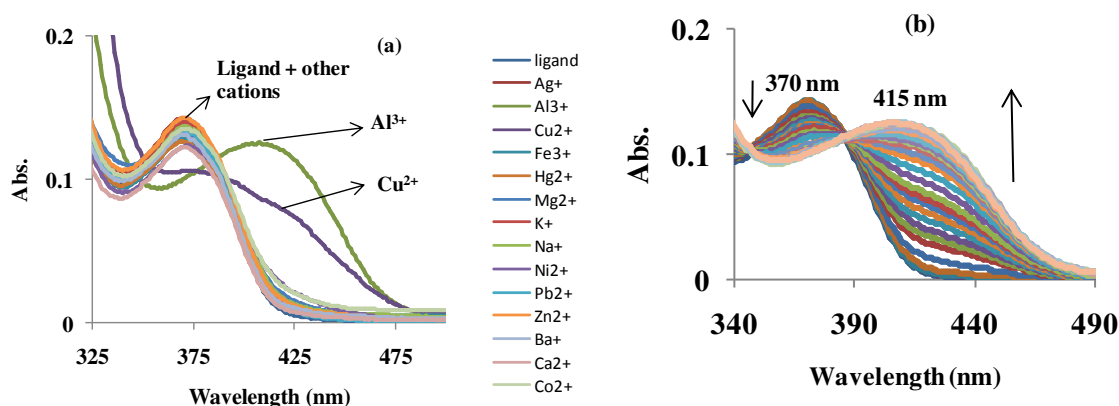


Figure 17: Effect of addition of (a) different metals ions; (b) Al^{3+} on absorption spectra of dyad **3** (20 μM , CH_3OH)

The absorption ratio at 415 nm and 370 nm varied from 0.085 to 1.23, indicating 14.47 fold absorption ratio change. Thus, dyad **3** was used to estimate a wide range of Al^{3+} ions between 0.5 - 140 μM through UV-Vis spectrophotometer. The incremental addition of Cu^{2+} (200 μM) led to formation of new band at 430 nm having lowest detection value to be 1 μM . The absorption value at 430 nm varied from 0.006 to 0.087, indicating 14.5 fold

absorption change. Thus, dyad **3** was used to estimate a wide range of Cu^{2+} ions between 1 - 200 μM through UV-Vis spectrophotometer.

The emission spectrum of dyad **3** (20 μM , CH_3OH) showed a characteristic band at 590 nm upon excitation at 350 nm. Out of the various metal ions tested (K^+ , Na^+ , Ag^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Hg^{2+} , Pb^{2+} , Fe^{3+} and Al^{3+}) only Cu^{2+} , Al^{3+} and Zn^{2+} ions showed the spectral changes whereas other metal ions did not show any significant changes (Figure 18). The Cu^{2+} ions showed the quenching effect due to its paramagnetic nature whereas Zn^{2+} and Al^{3+} showed the enhancement effect. The presence of Zn^{2+} ions showed blue emission whereas Al^{3+} ions showed bright yellow and Cu^{2+} ions showed light yellow emission.

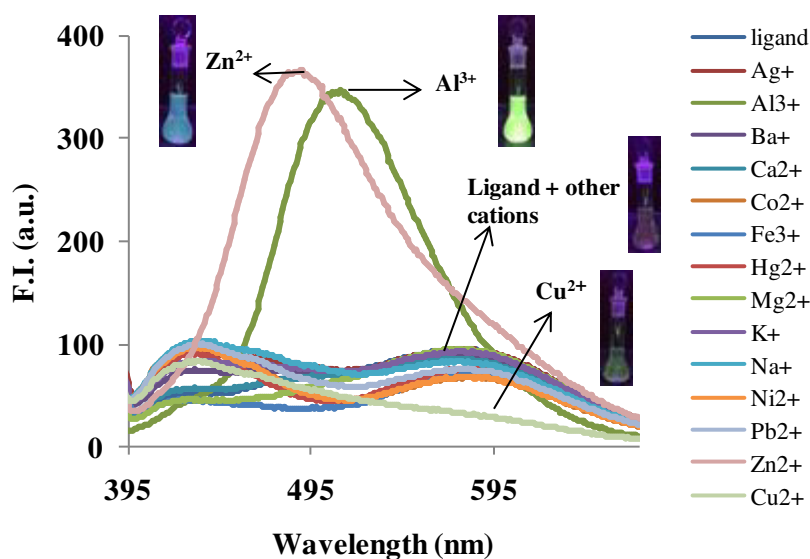


Figure 18: Effect of addition of metals ions on emission spectrum of dyad **3** (20 μM , CH_3OH). Insets of photographs of dyad **3** with detected metal ion under UV-light.

The incremental addition of 3 μM Al^{3+} ions to a solution of **3**, led to formation of new band at 460 nm (Figure 19a). Further addition of Al^{3+} ions (200 μM) the band at 460 nm got shifted towards 515 nm (Figure 19b). The emission value at 515 nm varied from 66.92 to 342.65, indicating 5.12 fold emission change.

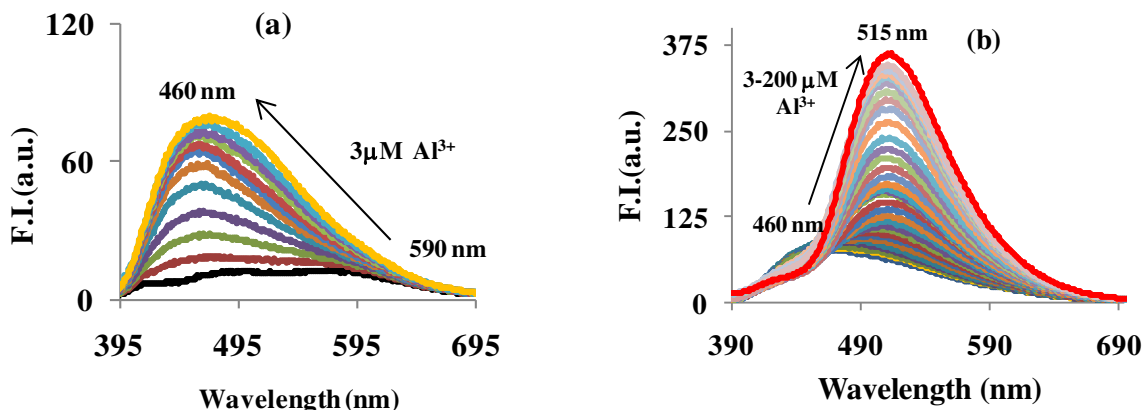


Figure 19: Effect of addition of (a) 3 μM Al^{3+} ions solution; (b) 3 -200 μM Al^{3+} ions solution on emission spectrum of dyad **3** (20 μM , CH_3OH)

Upon incremental addition of 400 μM , Cu^{2+} ions quenching at 590 nm band was observed with formation of slight hump at 425 nm (Figure 20a). An interesting result was obtained by adding only 15 μM solution of Zn^{2+} ions to dyad **3** leading to formation of new band at 485 nm with lowest detection value 200 nM (Figure 20b). Moreover emission value at 485 nm varied from 66.94 to 429.81, indicating 6.42 fold emission change. Binding constants along with detection limit for the detected metal ions were compiled in Table 4.

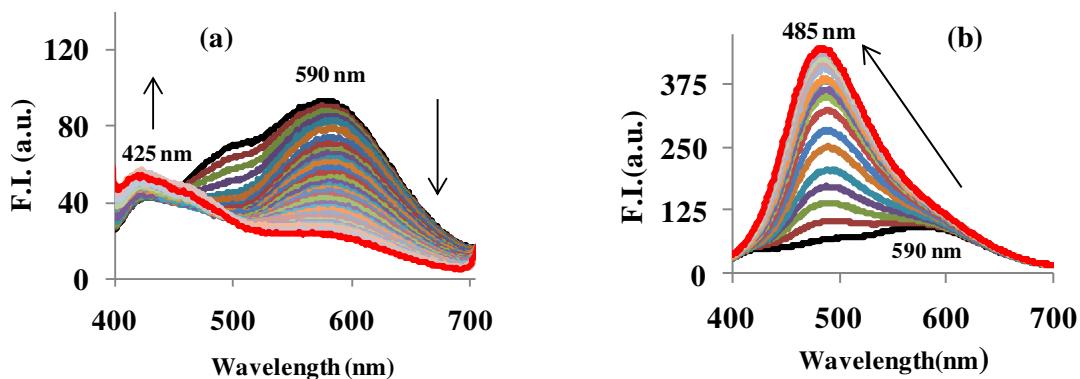
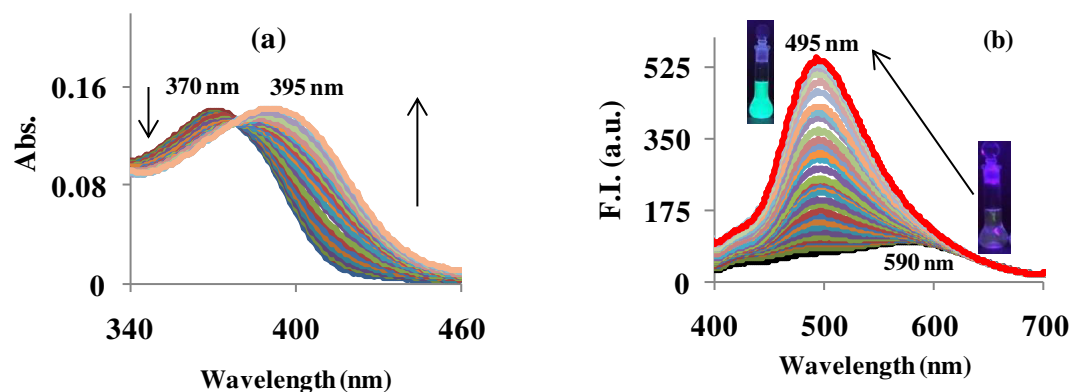


Figure 20: Effect of addition of (a) Cu^{2+} ; (b) Zn^{2+} on emission spectra of dyad **3** (20 μM , CH_3OH)

Table 5 Compiled results for detected cations in case of dyad **3**.

S.No.	Metal ion detected	Binding Constant	Detection Range
1	Al ³⁺	$2.4 \pm 0.01 \times 10^4 \text{ M}^{-1}$	0.1-200 μM
2	Cu ²⁺	$1.3 \pm 0.02 \times 10^5 \text{ M}^{-1}$	0.2-400 μM
3	Zn ²⁺	$3.2 \pm 0.01 \times 10^5 \text{ M}^{-1}$	0.2-15 μM

As dyad **3** comprises of two OH groups, it persuaded us to check the effect of various anions. Only CN⁻ ions caused changes in case of absorbance as well as fluorescence in CH₃OH out of all other anions like F⁻, Cl⁻, Br⁻, I⁻, SCN⁻, AcO⁻, NO₃⁻, H₂PO₄⁻ and HSO₄⁻. Color changes were observed under UV light in case of CN⁻ ions i.e. bright yellow. In case of absorbance, there was formation of new band at 395 nm upon addition of 700 μM solution of CN⁻ ions with formation of clear isosbestic point at 380 nm which indicated for the formation of new species i.e. due to deprotonation of CN⁻ ions (Figure 21a). The absorption ratio at 395 nm and 370 nm varied from 0.579 to 1.175, indicating 2.02 fold absorption ratio change. Thus, dyad **3** was used to estimate a wide range of CN⁻ ions between 10 - 700 μM through UV-Vis spectrophotometer.

**Figure 21:** Effect of addition of CN⁻ ions on (a) absorption; (b) emission spectra of dyad **3** (20 μM , CH₃OH); Insets of photographs of dyad **3** with and without CN⁻ ions under UV-light.

The incremental addition of CN⁻ ions to dyad **3** showed the formation of new emission band at 495 nm attributed to deprotonation of OH of dyad **3** upon addition of 200 μM solution of CN⁻ ions with 2 μM to be its lowest detection value (Figure 21b). Thus, dyad **3** was used to estimate a wide range of CN⁻ ions between 2 - 200 μM through

fluorescence spectrophotometer. To determine whether dyad **3** can be used for selective determination of CN^- ions among other ions, a simple experiment was performed by taking 1000 μM of various anions such as F^- , Cl^- , Br^- , I^- , SCN^- , AcO^- , NO_3^- , H_2PO_4^- and HSO_4^- and 1000 μM of CN^- . Results revealed that there was no significant variation in the absorption as well as emission band when comparing it with or without the other anions.

4.3.6 Elaboration of chemical inputs as Logic operations

An encoder is an electronic device, circuit, transducer, software program or algorithm that converts information from one format or code into another for the purpose of standardization, speed, secrecy, security or saving space by size shrinking and decoder compressed data back into the original form. Therefore, an encoder can perform both the function of protector and compressor of information, which is of great advantage. Different signaling with different ions prompted us to use these as chemical inputs for development of logic operations such as encoders and decoders. In order to pursue research mimic electronic devices, the dyad system **3** was examined as a molecular encoder and decoder. To acquire a 2-to-1 encoder, two different combinations having anion/cation as input and second having cation/cation as input was required. So, CN^- ions and Zn^{2+} ions were used as input A, input B and absorption at 405 nm as output Z (Figure 22a-c). When input A (CN^-) was in “on” state and input B (Zn^{2+}) was in “off” state to system 1 then the output Z (A_{405}) was in “on”. However when input A was “off” and input B was “on” as Zn^{2+} ions have no change on dyad **3** in UV-Vis spectroscopy output Z was in “on”. Similarly 2-to-1 encoder could also be formed by using another cation/cation based inputs i.e. Al^{3+} as input C, Zn^{2+} as input D and absorbance at 415 nm as output Y (Figure 22d-f).

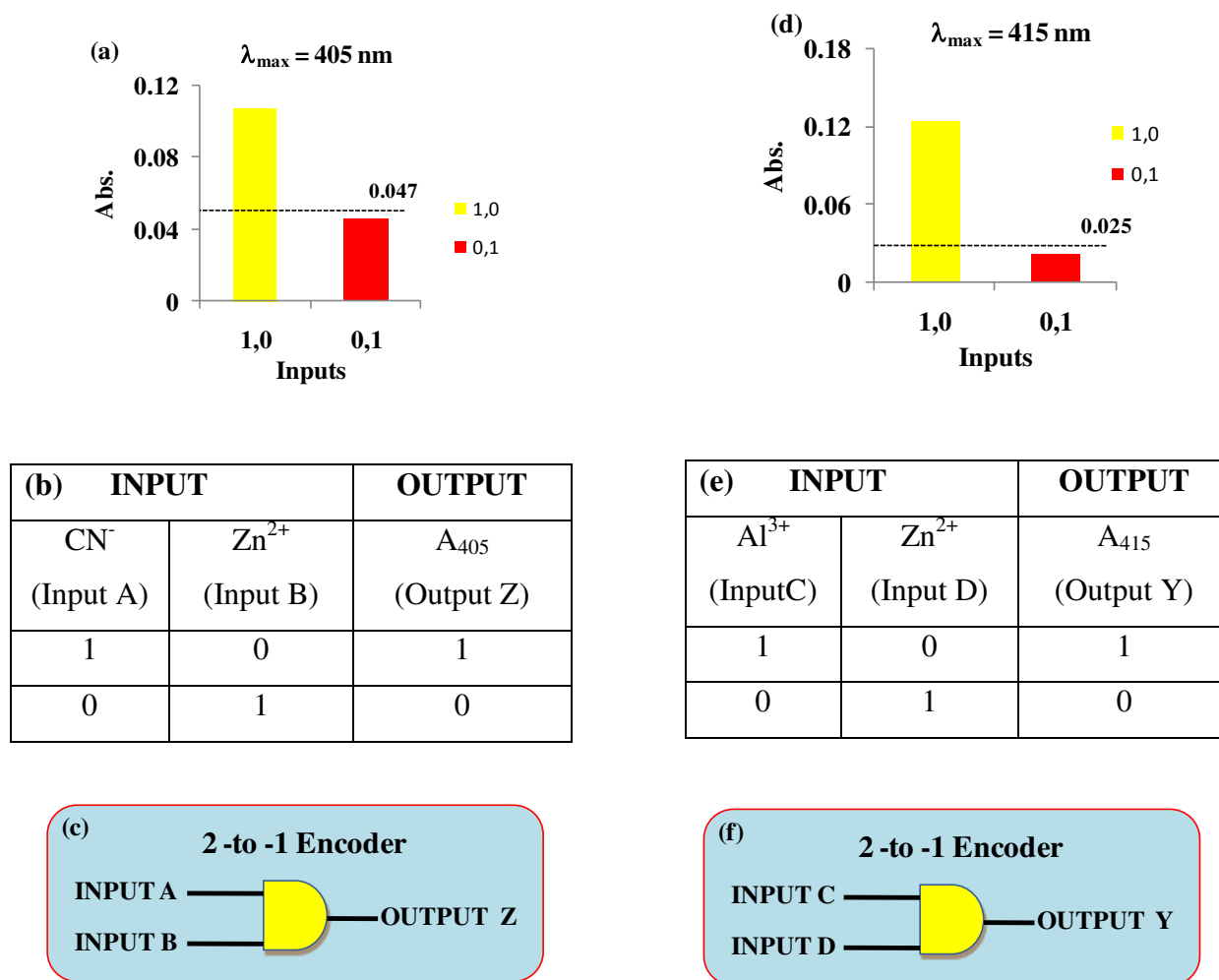


Figure 22: Absorption peak responses after application of (a) Inputs A and B; (d) Inputs C and D Truth table for (b) anion/cation; (e) cation/cation based 2-to-1 encoder and (c and f) schematic representation for the 2-to-1 encoder. The dashed lines show the thresholds separating output signals.

1-to-2 decoder was formed by using CN⁻ ions as input E and absorbance value at 370 nm as 1st output W and 2nd output X is absorbance at 405 nm. In the presence of input E (CN⁻), output W (A₃₇₀) was in “off” state and output X (A₄₀₅) was “on” state but in the absence of input E, output W was in “on” state and output X was in “on” state fulfilling the condition for 1-to-2 decoder (Figure 23a-c). On similar basis another 1-to-2 decoder is

formed using Al^{3+} ions as input F and absorbance at 370 nm as output U and absorbance at 415 nm as output V (Figure 23d-f).

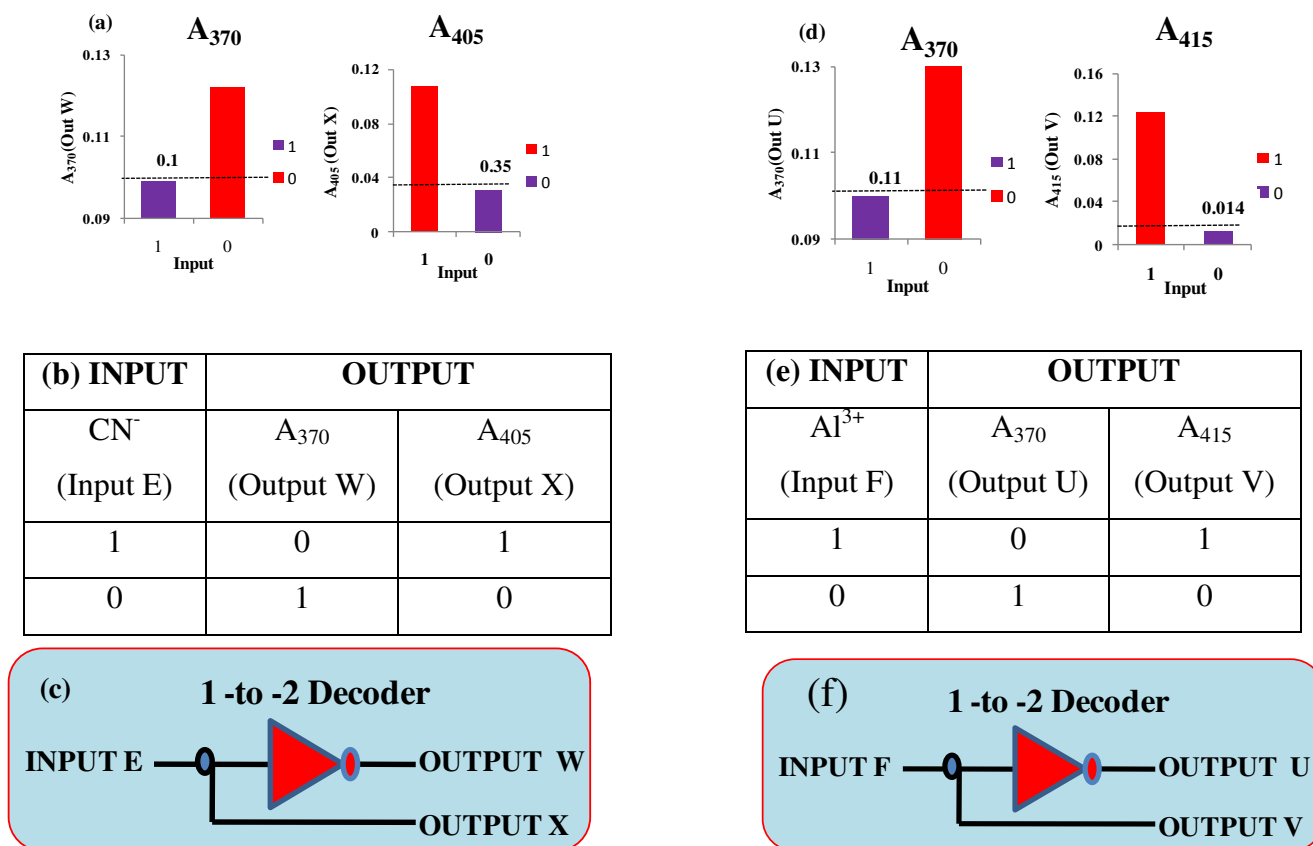


Figure 23: Absorbance peak responses after application of (a) Inputs E; (d) Inputs F. Truth table for (b) anion; (e) cation based 1-to-2 decoder and (c and f) schematic representation for the 1-to-2 decoder. The dashed lines show the thresholds separating output signals.

To acquire a 4-2 bit photonic encoder, we envisaged **In**₀ = Metals *via* K⁺, Na⁺, Ag⁺, Mg²⁺, Ca²⁺, Ba²⁺, Co²⁺, Ni²⁺, Hg²⁺, Pb²⁺ and Fe³⁺ (500 μM), **In**₁ = Cu²⁺ (500 μM), **In**₂ = Zn²⁺ (500 μM), and **In**₃ = Al³⁺ (500 μM) as four chemical inputs and emission wavelength of 505 nm absorbance wavelength at 415 nm as two photonic outputs, ‘**Out**₁’ and ‘**Out**₂’, respectively. By the operation of input **In**₀ to system **3**, neither emission at 505 nor absorbance at 415 nm was observed (Figure 24) *i.e.* both outputs ‘**Out**₁’ and ‘**Out**₂’ were in the “*off*” state ‘0’ (Table 6).

Reset of the system was achievable upon the addition of CN[−] ions (*vide supra*). The operation of input **In**₁ to the system quenched the emission at 505 nm and formed an absorption band at 415 nm, *i.e.* **Out**₁ was in the “*off*” state ‘0’ and **Out**₂ was in the “*on*” state ‘1’ (Table 6) (Figure 24). The operation of input **In**₂ to system **1** led to the formation of emission band at 505 nm without any absorbance at 415 nm (Figure 24), *i.e.* **Out**₁ was in the “*on*” state ‘1’ and **Out**₂ was in the “*off*” state ‘0’. When system **3** was operated by input **In**₃, both absorption and emission at 415 nm and 505 nm turned “*on*” respectively (Figure 24). Therefore, both outputs ‘**Out**₁’ and ‘**Out**₂’ were in the “*on*” state, *i.e.* ‘1’ (Table 6). From table 6, the outputs are expressed as:

$$\mathbf{Out}_1 = \mathbf{In}_2 + \mathbf{In}_3; \mathbf{Out}_2 = \mathbf{In}_1 + \mathbf{In}_3$$

Table 6: Truth table for 4-to-2 encoder where 0 = *off* and 1 = *on*

INPUT				OUTPUT	
In ₀	In ₁	In ₂	In ₃	Out ₀	Out ₁
1	0	0	0	0	0
0	1	0	0	0	1
0	0	1	0	1	0
0	0	0	1	1	1

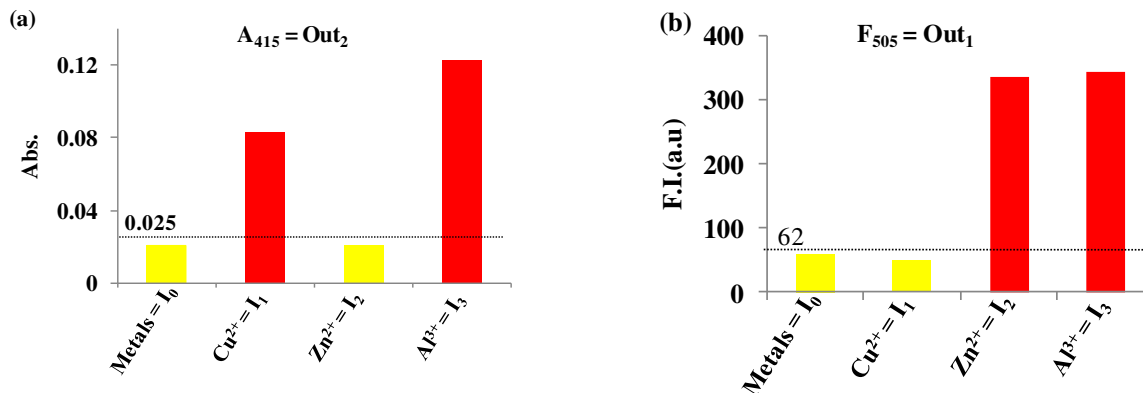


Figure 24: (a) Absorbance peak (b) fluorescence peak responses after application of four different inputs. The dashed lines show the thresholds separating output signals.

Therefore, a logic circuit diagram for the 4-2 encoder was obtained, as shown in Figure 25.

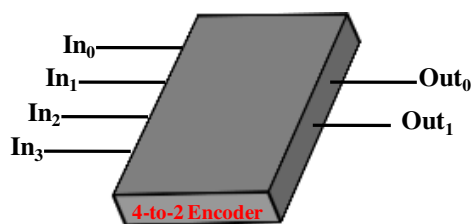


Figure 25: Schematic representation and truth table for a 4-to-2 encoder based on dyad **3**

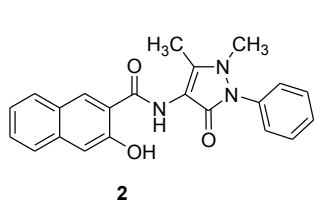
4.3.7 Conclusions

Antipyrine- Naphthoic acid based diamide **3** has been synthesized and investigated their photophysical behavior towards various cations and anions. Encoder and decoders were established using dyad **3**. Anion/cation as well as cation/cation 2 to 1 encoders were formed. Also anion and cation based 1 to 2 decoders were established. 4 to 2 encoder was constructed by using chemical inputs.

4.3.8 Experimental section

4.3.8.1 Synthesis of dyad 2: To the stirred solution of 3-hydroxy 2- naphthoic acid (200 mg, 1mmol) and 4-aminoantipyrine (259 mg, 1.2 mmol) was added O-(1*H*-Benzotriazol-1-yl)-*N,N,N',N'* tetramethyluronium tetrafluoroborate (409 mg, 1.2 mmol) , *N,N* diisopropylethylamine (206 mL, 1.2 mmol) and hydroxybenzotriazole (172 mg, 1.2 mmol) in tetrahydrofuran (30 ml). The reaction mixture was refluxed for 24 h till the reactants were consumed and progress of the reaction was monitored by TLC. The reaction mixture was washed with water and extracted with CHCl₃ (3 times), the solvent was removed under vacuum and dried over sodium sulphate. The residue was column chromatographed on silica gel by using CHCl₃: MeOH (99:1) to get pure amide as 280 mg (dyad 2).

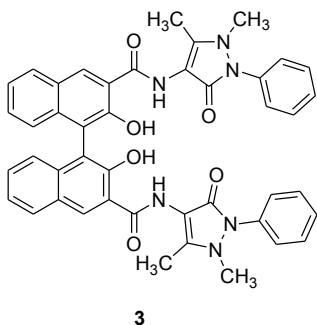
Dyad 2:



70% yield; Yellow colored solid; ¹H NMR (CDCl₃, 400 MHz): δ 11.85 (bs, 1H, OH), 9.78 (s, 1H, NH), 7.90 (s, 1H, ArH), 7.46-7.55 (m, 5H, ArH), 7.37-7.40 (m, 2H, ArH), 7.33 (d, *J* = 7.64 Hz, 1H, ArH), 7.15-7.19 (m, 1H, ArH), 7.11 (s, 1H, ArH), 3.20 (s, 3H, N-CH₃), 2.27 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 162.4 and 167.2 (C=O), 154.4, 150.8, 134.0, 136.6, 130.9, 129.5, 129.0, 127.9, 127.8, 127.0, 125.7, 125.3, 123.1, 118.8, 111.3, 107.1 (ArC), 35.4 (N-CH₃) and 11.8 (CH₃); MS (ESI) *m/z* 389.17 (*M*⁺+1).

4.3.8.2 Synthesis of dyad 3: Cu(OH)Cl.TMEDA mixture was prepared as per known procedure. A mixture of dyad 2 (500 mg) and CuCl(OH).TMEDA(10 mol %) in CH₃OH (30 ml) was refluxed for 5-6 h till the reactants were consumed and progress of the reaction was monitored by TLC. The solvent was removed under vacuum and purified through column chromatography on silica gel by using CHCl₃: MeOH (95:5) to get pure diamide as 750 mg (dyad 3).

Dyad 3:



75% yield; Yellow colored solid; ^1H NMR (CDCl_3 , 400 MHz):

δ 12.07 (bs, 2H, OH), 10.37 (s, 2H, NH), 8.90 (s, 2H, ArH), 7.94-7.97 (m, 2H, ArH), 7.51 (t, $J = 8.0$ Hz, 4H, ArH), 7.32-7.39 (m, 10H, ArH), 7.00 (d, $J = 4.0$ Hz, 2H, ArH), 3.13 (s, 6H, N- CH_3), 2.26 (s, 6H, CH_3); ^{13}C NMR (CDCl_3 , 100 MHz): δ 169.6 and 161.6 (C=O), 154.3, 152.9, 135.8, 134.9, 129.7, 129.4, 129.2, 128.9, 126.5, 124.1, 123.8, 116.8, 116.5, 106.1

(ArC), 35.9 (N- CH_3) and 11.0 (CH_3). MS (ESI) m/z 744.27 ($\text{M}^+ + 1$).

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Conclusion and Future perspectives of thesis

The research investigations in the present thesis revealed that the presence of electron donating and withdrawing groups like containing bromo, diethyl amino, formyl on the hydroxyary-benzimidazoles/substituted benzimidazoles highly affect sensing ability of the molecules. It has been clearly predicted that on changing the substituent from electron donating to electron withdrawing group, analyte to be detected could be tuned.

Furthermore, incorporations of ESIPT and AIE moieties onto single platform showed the wide range emission from 400-800 nm. Self-quenching effect at higher concentration of molecules could be eradicated upon incorporation of ESIPT and AIE moieties onto single platform. Importantly, large Stokes shift could be achieved with higher emission intensity in polar solvents. ESIPT-AIE molecules have high quantum yields because of their unique propeller-like structure therefore intramolecular rotations are restricted in aggregated state, which prohibits energy dissipation *via* nonradiative channels in the presence of different analyte. The synthesis of dyad combinations has led to miniaturization of different logic devices like sequential XNOR logic gate, 2-1 encoder, 1-2 decoder and 4-2 bit encoder which has the futuristic applications in digital data-processing functions in conventional electronic industry.

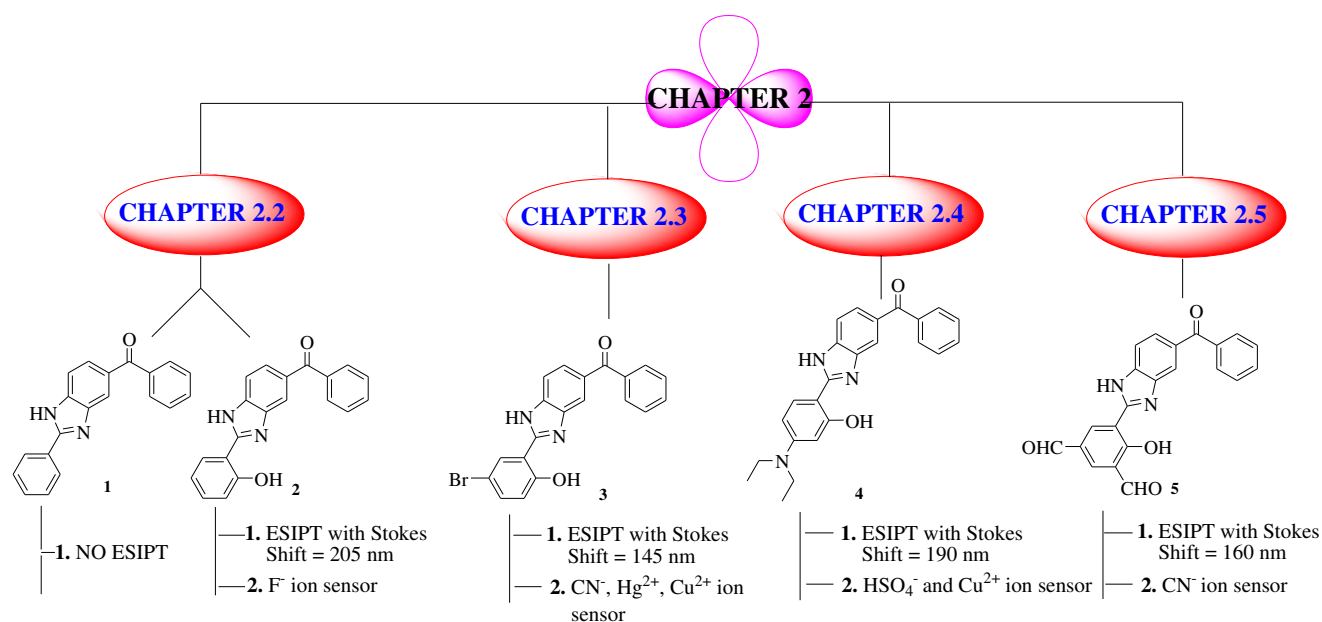
Thus, fluorescent chemosensors with dual interplaying sensing mechanisms tend to provide diverse fluorescence signals or amplify the response signals, which are propitious to simultaneously track multiple analytes or to improve the selectivity and sensitivity of the chemosensors. ESIPT–AIE coupled phenomenon can be exploited for designing sensors with large signal to noise ratios (SNRs) or big pseudo-Stokes shifts; could be used to engineer sensors for monitoring multiple analytes. With their unique merits, it is believe that dual and multiple mechanism-based fluorescent sensors will be employed for unprecedented and exciting applications in diverse fields, especially in high SNR fluorescence bioimaging, investigating interactions of multiple biological species, OLEDs, and developing optical logical devices with multiple inputs and outputs.

SUMMARY

The need to develop chemosensors for different target analytes is well-recognized. Chemosensors are emerging as a wide tool in supramolecular chemistry by using emission intensity. Moreover, these have been developed as the most competent tools for sensing of various different analytes.¹⁻¹² There are many phenomenons which can be used for sensing purposes out of which, the excited state intramolecular proton transfer (ESIPT) has called upon a great importance due to its immeasurable applications such as photostabilizer for ultraviolet, laser dyes, scintillators, probes for membrane and protein and also serves as potential component in case of photo based switches and OLEDs.¹³⁻¹⁵ In most of the cases fluorescence of luminophores is often enfeebled or even quenched when dissipated in aqueous media, to overcome this problem a phenomenon named “aggregation-induced emission” (AIE) was immerged i.e. nonemissive molecules when dispersed in solution form, results in emission due to aggregate formation (AIE).¹⁶⁻¹⁸ In the present research work, the tailor made design and synthesis of photo responsive (absorption or emission or both) molecules having ESIPT or AIE or both has been carried out. The photophysical behavior of the synthesized molecules towards varied inputs viz. anions, cations, pH etc. has been investigated. The optical outputs achieved during these investigations have been elaborated in terms of applications of these molecules in selective qualitative/quantitative analysis of the target analytes. The results of these investigations have been presented in chapters 2-4 which is preceded by brief review of literature on ESIPT and AIE in chapter 1.

Chapter 2: Synthesis and photophysical properties of molecules exhibiting ESIPT phenomenon for cations and anions

2.1 General Introduction: In this chapter ESIPT based benzimidazole probes have been synthesized and their complexation behaviour towards different anions and cations has been investigated. These chemosensors depending on the different nature of ESIPT phenomenon on benzophenone moiety are found to bind with different ions. The results of these findings have been discussed in four sections as given below also all these results are briefly compiled in flowchart 1.

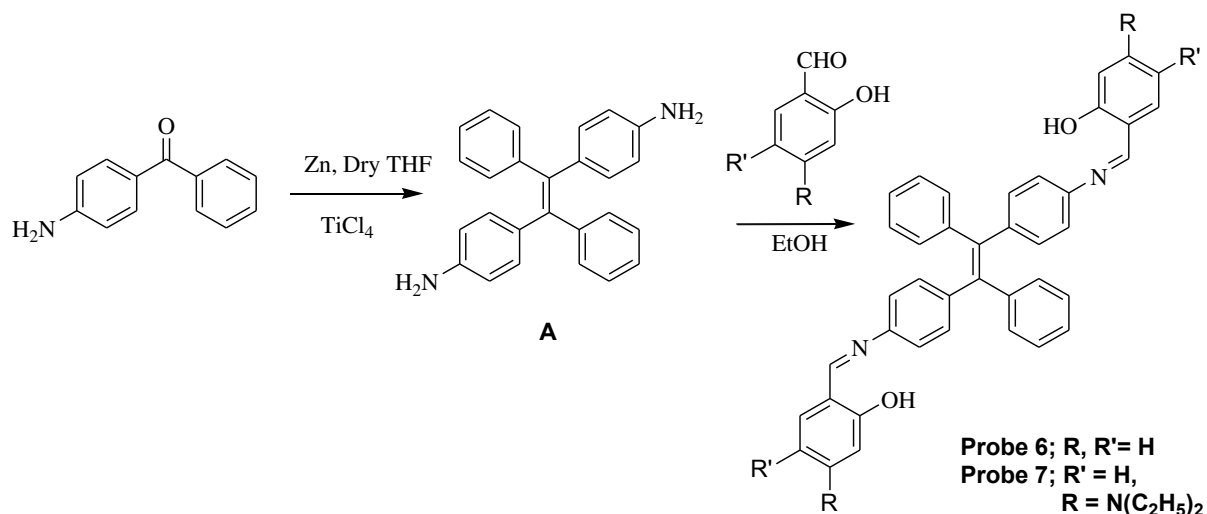


Flowchart 1: Brief results of Chapter 2

Chapter 3: Synthesis of hybrid molecules showing ESIPT-AIE phenomenon and their sensing behaviour

3.1 General Introduction: In this chapter we have synthesized the hybrid molecules by incorporating the ESIPT and AIE molecules onto the single platform to take the advantages of both phenomenon. Keeping in mind the advantage effects of ESIPT and AIE this chapter is divided in following sub parts

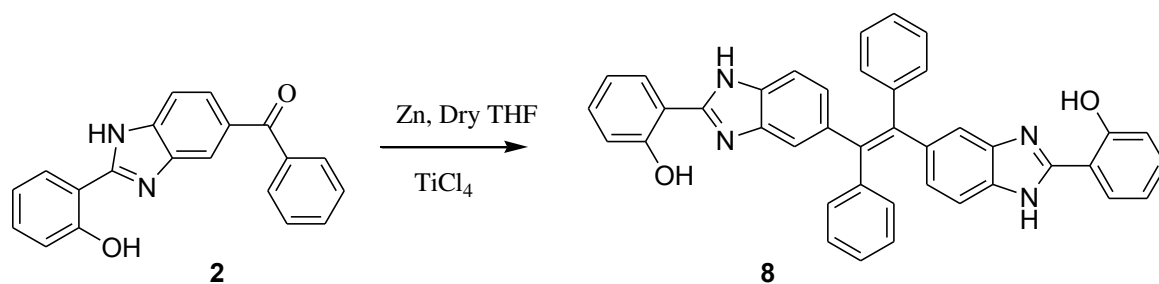
3.2 AIE-ESIPT coupled ratiometric fluorescent probe for detection of Cu²⁺ and F⁻ ions: In this subchapter fluorescent probe **6** possessing hydroxyaryl Schiff bases onto tetraphenylethylene (TPE) moiety having the characteristic of both aggregation induced emission (AIE) and excited-state intramolecular proton transfer (ESIPT) has been synthesized as depicted in scheme 1. The coupled AIE and ESIPT showed the significant advantages, such as no self quenching at high concentration, large Stokes shift, high selectivity towards analyte etc. It has also been used for ratiometric estimation of F⁻ and Cu²⁺ ions amongst anions and metal ions respectively. Probe **6** showed the emission at 540 nm with a large Stokes shift of 170 nm and presence of Cu²⁺ ions induced the restriction of intramolecular motions led to high emission in the aggregated state (AIE) at longer wavelength (NIR region).



Scheme 1: Synthesis of probes **6-7**

3.3 ESIPT-AIE coupled probe for detection of trivalent metal ions (Al³⁺, Cr³⁺ and Fe³⁺) in aqueous media: An ESIPT coupled AIE phenomenon bearing probe **7** has been synthesized (Scheme 1) and used for estimation of trivalent metal ions. Probe **7** exhibited an absorption band at 400 nm which upon addition of trivalent metal ions (Al³⁺, Cr³⁺ and Fe³⁺) bathochromically shifted to 440 and 460 nm. Probe **7** displayed the emission band at 525 nm typically due to AIE phenomenon. Upon addition of trivalent cations aggregated state was disturbed and opened a new emission channel at 580 nm due to the disaggregated emission state. The coupled phenomenon has been successfully used for estimation of trivalent cations even at nanomolar level.

3.4 Benzimidazole-TPE hybrid probe for ratiometric estimation of cations and anions: Benzimidazole-TPE hybrid has been synthesized as depicted in scheme 2 via mcmurry coupling with probe **8**. Probe **8** exhibited an absorption band at 335 nm and emission band at 460 nm which upon addition of cations namely Cu²⁺, Pb²⁺, Zn²⁺ and Fe³⁺ shifted to a new band at 375 nm. Probe **8** on aggregation displayed an AIE band at 375 nm. Probe **8** was used for sensing of CN⁻ and F⁻ ions by releasing the emission band at 425 nm.

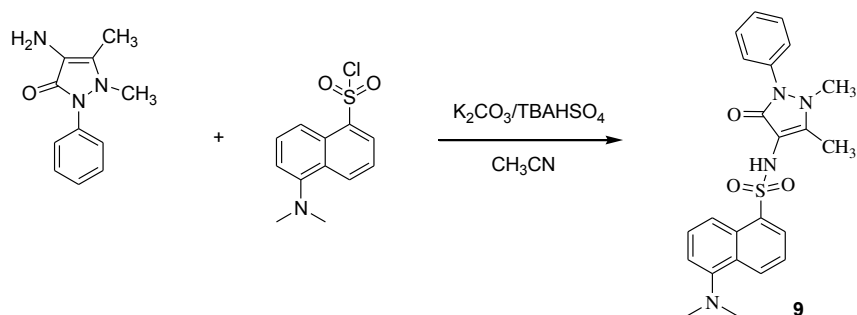


Scheme 2: Synthesis of probe **8**

Chapter 4: Quantification of optical signals for generation of molecular logic gates

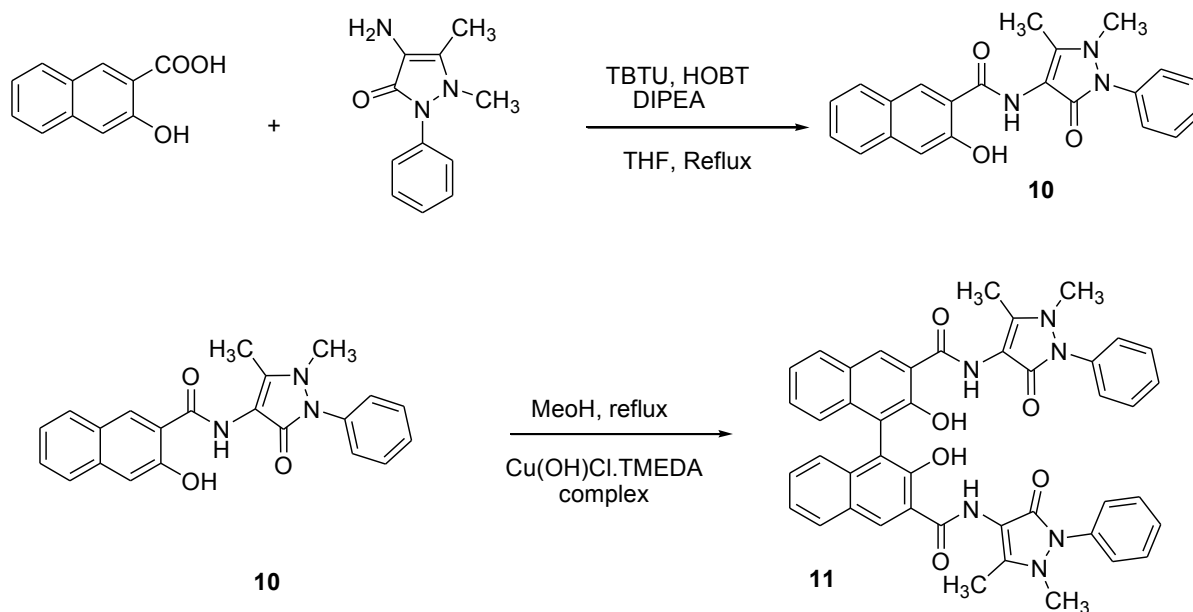
4.1 General Introduction: Molecules mimicking the operations of electronic devices has attained greater importance. Organic molecules can be used to perform digital data-processing functions as much as performed by conventional electronic devices. Initial reports of such systems were of simple switches or single-function logic gates but the field has recently developed from simple switches to produce more complex molecular systems that can perform a variety of classical logic functions, including extensions to switches on surfaces, and examples of half-adder, full-adder, keypad lock, multiplexer, multiplexer–demultiplexer and, moreover a multicomponent decoder and an encoder-decoder triad. We have discussed dyad with logic device application part in this chapter which is further divided in following sub parts.

4.2 Dansyl-antipyrene dyad as sequential XNOR logic device: Dansyl and antipyrene based hybrid dyad **9** was synthesized (Scheme 3) and their photophysical behaviour towards various cations and anions were investigated. The dyad **9** was used for selective estimation of Cu^{2+} ions as compared to other metal ions and the binding event was indicated through formation of a new absorption band at 465 nm and a decrease in fluorescence intensity at 528 nm. The dyad **9** showed ratiometric fluorescence behavior towards fluoride ions through hydrogen bonding interactions and restricted the photoinduced electron transfer from dansyl to antipyrene moiety to release its fluorescence at 428 nm. The chemical inputs of Cu^{2+} and F^- ions to dyad **9** mimicked as sequence dependent operation of an exclusive-NOR (XNOR) logic gate.



Scheme 3: Synthesis of dyad **9**

4.3 Naphthalene-antipyrine dyad based ionic encoder and decoder: Dyad **10** was synthesized by using coupling method as shown in scheme 4.



Scheme 4: Synthesis of dyad **10** and **11**.

Dyad **10** was used for estimation of Al^{3+} and CN^- ions. Practical application of dyad **10** was done by estimating Al^{3+} in sewage water samples. Dyad **11** having diamide group has been synthesized (Scheme 4) and investigated their photophysical behavior towards various cations and anions. Dyad **11** has been used for estimation of Al^{3+} and Cu^{2+} ions as compared to other metal ions and signaled the binding event through formation of new absorption band at 415 and 430 nm. New emission band was observed with Al^{3+} at 515 nm and with Zn^{2+} at 485 nm while

emission quenching was observed in case of Cu^{2+} ions. Dyad **11** showed selective estimation for CN^- ions among all other anions by forming new absorption band at 395 nm and new emission band at 495 nm. The differential behavior in the presence of metals and anions has been used for miniaturization of anion/cation, cation/cation 2-to1 encoder and 1-to2 decoder respectively. 4-to-2 encoder was established by using (cations/cation)² as inputs.

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LIST OF PUBLICATIONS

Papers in SCI/refereed journals

1. Vijay Luxami*, **Akul Sen Gupta** and Kamaldeep Paul, Dansyl-antipyrene dyad as fluorescent sensor for Cu^{2+} and F^- ; Sequential XNOR logic operation, *New J. Chem.*, 2014, **38**, 2841-2846.
2. **Akul Sen Gupta**, Kamaldeep Paul and Vijay Luxami*, Ratiometric fluorescent chemosensor for fluoride ion based on inhibition of excited state intramolecular proton transfer, *Spectrochim. Acta, Part A*, 2015, **38**, 67–72.
3. **Akul Sen Gupta**, Kamaldeep Paul and Vijay Luxami*, Benzimidazole based ratiometric chemosensor for detection of CN^- and Cu^{2+} ions in protic/aqueous system: Elaboration as XOR logic operation, *Inorg. Chim. Acta.*, 2016, **443**, 57–63.
4. **Akul Sen Gupta**, Aparna Garg, Kamaldeep Paul and Vijay Luxami*, Differential sensing of fluoride and cyanide ions by using dicyano substituted benzimidazole probe, *J. Lumin.*, 2016, **173**, 165-170.
5. **Akul Sen Gupta**, Kamaldeep Paul and Vijay Luxami* A fluorescent probe with “AIE + ESIPT” characteristics for Cu^{2+} and F^- ions estimation, *Sens. Actuators B: Chem.*, 2017, **246**, 653-661.
6. **Akul Sen Gupta**, Kamaldeep Paul and Vijay Luxami* ESIPT Coupled RAHB Probe for Estimation of Cyanide in Human Blood Serum/Plasma like Solutions using Chemodosimetric Approach, *Chemistry Select*, 2017, **2**, 800-804.
7. **Akul Sen Gupta**, Kamaldeep Paul and Vijay Luxami* ; Amide based fluorescence ‘Turn-On’ sensor for Al^{3+} and CN^- ions (*To be submitted*)
8. **Akul Sen Gupta**, Kamaldeep Paul and Vijay Luxami* Naphthalene-Antipyrene dyad based ionic encoder-decoder (*To be submitted*)
9. **Akul Sen Gupta**, Kamaldeep Paul and Vijay Luxami* ESIPT-AIE coupled probe for detection of trivalent ions in aqueous media: Elaboration as logic device (*To be submitted*)
10. **Akul Sen Gupta**, Kamaldeep Paul and Vijay Luxami* ESICT-ESIPT coupled fluorescent sensor for copper and hydrogen sulphate ions (*Under Preparation*)
11. **Akul Sen Gupta**, Kamaldeep Paul and Vijay Luxami* Benzimidazole based probe comprising of ESIPT and AIE for ratiometric detection of various anions and cations (*Under Preparation*)

CONFERENCES AND WORKSHOPS ATTENDED

1. Poster presented at NCIMS in School of Chemistry & Biochemistry, Thapar University, Patiala (24-26th October, 2013).
2. Participated in the workshop on “NMR/MRI: From molecules to Human Behaviour” held at Department of Chemistry, Guru Nanak Dev University, Amritsar (06-11th October, 2014).
3. Poster presented at 17th CRSI national conference at National Chemical Laboratory, Pune (6-8th Feb, 2015).
4. Poster presented at SACOS in Department of Chemistry, Punjabi University, Patiala (19-20th February, 2015).
5. Participated in the workshop on “Short term course on Advance material and Characterization Techniques” held at Department of Chemistry, National Institute of Technology, Jalandhar (01-07th June, 2015).
6. Poster presented at 18th CRSI national conference at Department of Chemistry, Panjab University, Chandigarh (5-7th Feb, 2016).