

FLUORESCENT DYADS AS SENSORS FOR PARTICULAR CATIONS AND ANIONS

A

Ph.D. thesis submitted in
fulfilment of the requirement of the degree of

Doctor of Philosophy

Submitted by

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Certificate

This is to certify that thesis entitled “**Fluorescent Dyads as Sensors for Particular Cations and Anions**” being submitted by Richa Rani in the fulfilment of the requirement for the award of Degree of Doctor of Philosophy to the School of Chemistry and Biochemistry, Thapar University, Patiala, is a record of candidate’s own independent and original research work carried out by her, under our 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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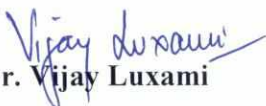
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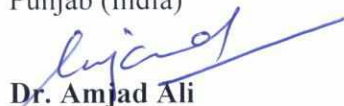
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Candidate's Declaration

I, hereby declare that the work presented in the thesis entitled “**Fluorescent Dyads as Sensors for Particular Cations and Anions**” in fulfilment 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. Kamaldeep Paul, Associate Professor and Dr. Vijay Luxami, Associate 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.


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Acknowledgements

I whole heartedly thank my might God for giving me the vision, power, spirit and endurance to complete this interesting research.

Though only my name appears on the cover of this thesis, a great many people have contributed to its production. I owe my gratitude to all those people who have made this dissertation possible and because of whom my graduate experience has been one that I will cherish forever. This thesis is the end of my journey in obtaining my Ph.D. I have not travelled in a vacuum in this journey. This thesis has been kept on track and been seen through to completion with the support and encouragement of numerous people including my well-wishers, my friends, colleagues who walked along and beside me to help support and motivate me all these years.

At the outset, I would like to express my gratefully appreciation to my supervisors Dr. Kamaldeep Paul, Associate Professor and Dr. Vijay Luxami, Associate Professor, School of Chemistry & Biochemistry, Thapar University, Patiala, for having faith in me and entrusting me with this opportunity to work under their guidance and supervision, providing all kinds of support and inspiration, wise counsel, constant encouragement, valuable suggestions and comments, expertise and fruitful advice throughout this exploration and preparation of the thesis. It's a great privilege to experience a sustained enthusiastic, involved interest, constructive criticism, tremendous enthusiasm and careful supervision from their side throughout the period of my work. As my supervisor, Dr. Vijay Luxami has constantly forced me to remain focused on achieving my goal. Her observations and comments helped me to establish the overall direction of the research and to move forward with investigation in depth. I thank her for providing me with the opportunity to work under her guidance and great effort she put into training me in the scientific field.

I express my gratitude to Director, Thapar University, Patiala and Dr. Amjad Ali, Head, School of Chemistry and Biochemistry, for their good wishes and motivation. I am profoundly obliged to Dr. O. P. Pandey, Dean, Thapar University, Patiala for all the facilities which have been immensely helpful for the completion of my work.

I would also like to thank my committee members, Dr. Susheel Mittal, Dr. Satyendra Kumar Pandey and Dr. Dinesh Goel for serving as my committee members even at hardship for their fruitful discussion, encouragement and suggestions. Special regards to all faculty members of the School of Chemistry and Biochemistry for providing suggestions and taking interest in the progress of work.

I am extremely thankful to Dr. Subodh Kumar, Professor, Guru Nanak Dev University Amritsar and Navneet Kaur, Assistant Professor, Punjab University, Chandigarh in helping us add new dimension to our research, coordination and support.

I am thankful to Mr. Chander Singh Thakur and other non-teaching staff, School of Chemistry and Biochemistry for the constant official help and cooperation.

I owe a debt of gratitude and heartfelt thanks to my lab mates and colleagues Mr. Iqbal Singh Brar, Dr. Alka Sharma, Dr. Meenakshi, Dr. Prinka Singla, Dr. Richa Goel, Mr. Akul Sen Gupta, Mr. Gulshan, Mrs. Ruhi, Ms. Richa Bansal, Ms. Sudesh Rani, Mr. Yuvraj Garg, Ms. Ramandeep Kaur, Ms. Suraksha, Ms. Rachna Pandey, Ms. Akansha, Mr. Rayees, Mr. Aadil Bathla, Ms. Tanushree basu, Dr. Rashmi Sharma, Dr. Bhupendra Pal, Dr. Rohit, Dr. Inder for their kind concern and critical observations in designing and planning experiments and above all their support, which always helped me endure hard times and to accomplish all my goals. I owe a word of thanks to all research scholars at the School of Chemistry and Biochemistry for providing constant support, encouragement and a good working atmosphere. I am thankful to all the students who worked with me and people, who taught me various lessons of life and helped me in polishing my skills of both personal and professional arena.

A special thanks to my family. Words cannot express how grateful I am to my loving father, my mother, my sister for their blessings and belief in me, who have always showered unconditional love on me, encouraged and supported me in every aspect. They formed a part of my vision and taught me the good things that really matter in life. Lastly, I thank my beloved brother (Vishal) for his continued and unfailing love, support and understanding in completion of my thesis and long journey.

I also thank University Grant Commission (UGC), New Delhi for fellowship and financial assistance. I am thankful to SAIF, Panjab University, Chandigarh for mass analysis, SAI Labs, Thapar University, Patiala for NMR studies.

Richa Rani

DEDICATED TO
MY
BELOVED COUNTRY
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LIST OF ABBREVIATIONS

ACN	Acetonitrile
ATP	Adenosine triphosphate
ALP	Alkaline phosphates
B3LYP	Becke's Lee Yang and Parr
CHEF	Chelation Enhanced Fluorescence
Cys	Cysteine
DFT	Density Functional Theory
DMSO	Dimethyl sulphoxide
DPA	Dipicolyl Amine
EDTA	Ethylenediaminetetraacetic acid
FRET	Forster Resonance Energy Transfer
GSH	Glutathione
Hcy	Homocysteine
HeLa	Henrietta Lacks
HEPES	(4-Hydroxyethyl)-1-piperazineethanesulfonic acid
H ₂ O	Water
HOMO	Highest Occupied Molecular Orbital
ICT	Internal Charge Transfer
LOD	Limit of Detection
LUMO	Lowest unoccupied Molecular Orbital
MeOH	Methanol
MLCT	Metal to Ligand Charge Transfer
M.Pt.	Melting Point
NBD	7-Nitrobenzo[c][1,2,5]oxadiazole
PET	Photoinduced Electron Transfer
ppb	Parts Per Billion
PPi	Pyrophosphate ions
ppm	Parts per million
TBAX	Tetrabutyl Ammonium halide
TBET	Through Bond Energy Transfer
TDSCF	Time-Dependent Self-Consistent Field
TLC	Thin Layer Chromatography

SYMBOLS USED

a.u	arbitrary unit
K	Stability Constant
ϕ	Quantum Yield
μM	Micro Molar
nM	Nano Molar
nm	Nano metre
λ_{max}	Absorption Maximum
λ_{em}	Emission Maximum
ns	Nano Seconds

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

The need for the development of sensors for recognition of different kinds of ions is substantively ubiquitous and confirmed by the efficient research efforts to improve the quality of lives. Furthermore, there are large numbers of metal ions and anions that play significant roles in human life, farming, industrial, biological processes as well as in the environment. The excess of these ions may be hazardous, therefore, detection of such ions in relevant systems is of particular interest.¹ Out of different sensors, chemosensors, especially fluorescent chemosensors, can afford real information and quantify the targets of particular interest.² A chemosensor is a miniaturized device having receptor with recognition site that is capable of transforming chemical information into electrical signal.³ In general, a chemosensor is an inexpensive and user friendly analytical tool that responds with excellent selectivity to a particular analyte (chemical substance) in organic, aqueous or semi-aqueous medium in order to produce a signal at particular concentration.⁴ Various types of chemosensors have been designed and constructed by several researchers. Chemosensors based on various moieties such as rhodamine,⁶ naphthalimide,⁷ coumarin,⁸ benzoxazoles,⁹ fluorescein,¹⁰ BODIPY,¹¹ squaraine,¹² phenanthrene¹³ etc. have been reported in the literature. Out of the above said moieties, naphthalimide, rhodamine, anthraquinone, thiazolidine, phenanthroimidazoles, 7-nitrobenzo[c][1,2,5]oxadiazole derivatives are chosen as excellent fluorophores because of their excellent stability, visible excitation and emission and large Stokes' shift that minimize the effects of the background fluorescence, easy use, high efficiency and low cost, high stability and quantum yield. Some of these moieties have been used as fluorescent chemosensors for metal cations and anions in recent years.¹⁴ These make these derivatives potential carriers for the preparation of new optical chemosensors.

In the literature, there is number of reports having colorimetric or fluorimetric chemosensors.¹⁵ Recently, investigations are made to construct such chemosensors which can act as sensors, incorporating response to all the properties like colorimetric, absorption, emission, ratiometric, selectivity, sensible and reversible etc.¹⁶ The Chemosensors in the presence of analyte showed different signaling mechanisms like photo-induced electron transfer (PET),¹⁷ intramolecular charge transfer (ICT),¹⁸ metal-to-ligand charge transfer (MLCT),¹⁹ excited state intramolecular proton transfer (ESIPT),²⁰ fluorescence resonance energy transfer (FRET),²¹

TBET (through-bond energy transfer)²² etc. FRET based sensors have drawn a special attention because these provide larger Stoke's shifts and spectral overlap. Dyes based on FRET processes are usually linked through a non-conjugated spacer, and the energy transfer takes place through space. FRET has been extensively used in biology and biophysics, where the participating moieties are labeled with highly emissive and large donors and acceptors. Latest examples include the study of protein folding, protein-protein interactions²³ and cellular signaling mechanisms in living cells.²⁴ FRET mechanism has also been responsible to elucidate folding and dynamics of RNA²⁵ as well as the stability and sequence-dependent structure of nucleosomes.²⁶ In comparison, TBET is not limited by such kind of spectral overlap and exhibits high energy transfer efficiency. These molecules have a donor connected to an acceptor through electronically conjugated linkers.

For the synthesis of FRET and TBET based molecules, numerous fluorescent dyes have been used. Fluorescent dyes, containing rhodamine, naphthalimide, anthraquinone moieties etc. have attracted considerable attention from chemists owing to their excellent photophysical properties such as high photostability, high fluorescence quantum yield, relatively longer emission wavelength and high absorption coefficient. Thus, two fluorochromophores of high quantum yields (homo or hetero) are combined together to form dyads, and the photophysical properties of these dyads towards various cations, anions and biological ions has been studied.

On the basis of different fluorescent moieties, mechanisms and the gaps in literature the whole thesis is categorized in different chapters and sub-chapters. Initially on the basis of different moieties and phenomenon, review of literature has been divided into two sections as mentioned below.

1.1 NBD based molecules

1.2 FRET/TBET based molecules

1.1 NBD based molecules

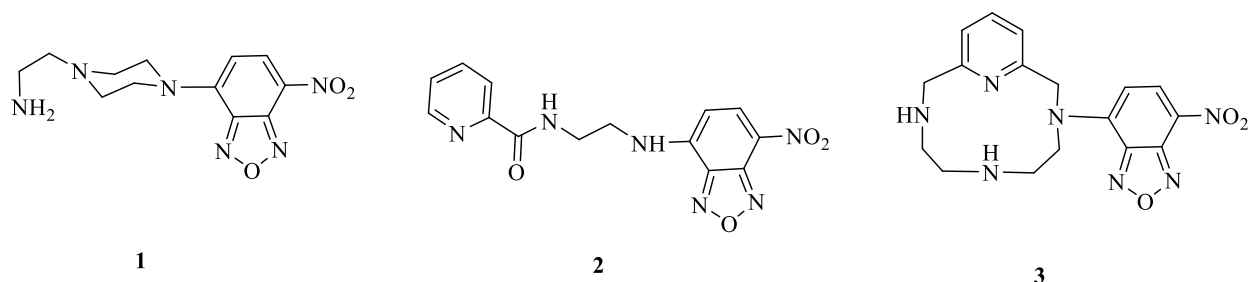
7-nitro-1,2,3-benzoxadiazole (NBD) exhibited strong chromogenic properties and allowed naked eye and inexpensive detection of various analytes. NBD molecules showed emission at a stronger wavelength, having high quantum yield, simple, high sensitive, low detection limit and good response time. Thus, NBD derivatives can be used as colorimetric and fluorogenic chemosensors. Moreover, on attaching with different moieties NBD gave rise to fluorescent

dyads with improved quantum yields and lower detection limits which could be further used for sensing of various ions. NBD moiety itself along with different receptor site can show the optical signal for sensing of various cations and anions. In another case, NBD moiety along with other fluorescent moiety and receptor site can show stronger emission/absorption changes for cations and anions. Thus, literature based on NBD moiety has been subdivided into two categories (i) NBD signalling unit as sensor for cations and anions; (ii) NBD based dyads as sensor for cations and anions.

1.1.1 NBD signalling unit as sensor for cations and anions

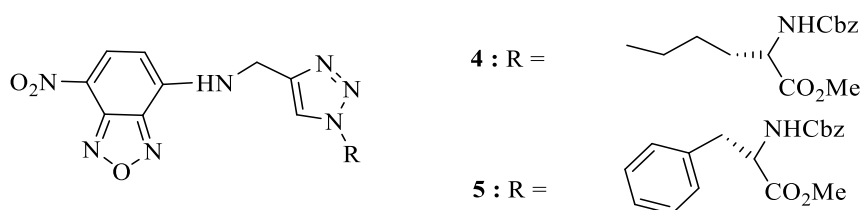
In such type of chemosensors, NBD moiety itself acts as receptor site and interacts with various analytes in various polar and nonpolar solvents. The interaction produced a detectable change as output in the form of the signal as an analyte with the binding site induces changes in the electronic properties of the NBD (signalling subunit), resulting in sensing of the target ions. This change is used to analyze various ions both qualitatively and quantitatively.

Xu *et al.* developed a simple 7-nitrobenzo[*c*][1,2,5]oxadiazole (NBD) based fluorescent probe **1** which showed absorption maxima at 470 nm and weak fluorescence emission at 542 nm and used for the selective estimation of Hg^{2+} ions in acidic conditions. Probe **1** was also used for selective estimation of S^{2-} ions with a color change from orange to pink in aqueous solutions and used as a visible kit for S^{2-} ions. Probe **1** behaved as first *pH* indicator showing H^+ -induced demetallation of mercury complex. Moreover, probe **1** was used for *in vitro* studies with HeLa cells.²⁷



Liu and Wu synthesized probe **2** showing absorption maxima at 472 nm and emission maxima at 544 nm. Probe **2** showed the selective change with the Cu^{2+} ions along with the minor interferences of other coexisting metal ions. Significant fluorescence quenching was observed with Cu^{2+} ions having binding constant $1.22 \times 10^3 \text{ M}^{-1}$. Probe **2** showed remarkable quenching effects at *pH* range of 6-10.²⁸ Wang *et al.* synthesized probe **3** that showed the fluorescence at

530 nm and underwent quenching on the addition of Cu^{2+} ions, therefore used for the selective detection of Cu^{2+} ions in HEPES buffer. Probe **3** was also used for the visualization of Cu^{2+} ions in the lysosomal cells.²⁹

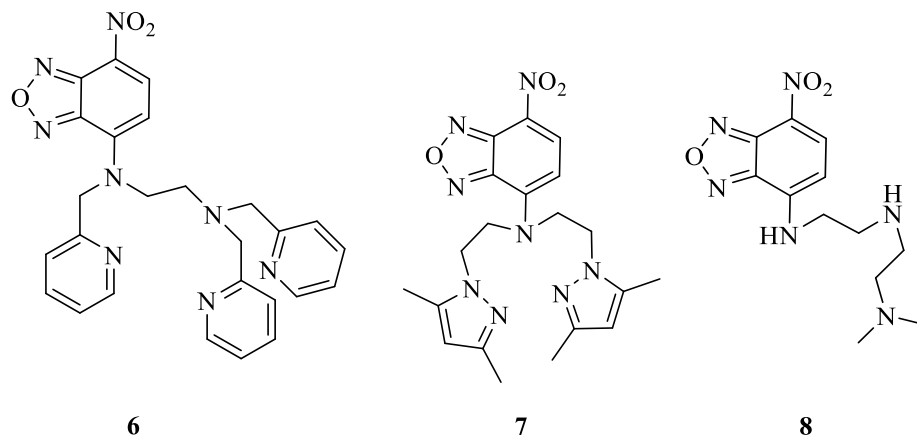


Ruan *et al.* reported NBD based compounds **4** and **5** bearing triazole moiety as the binding site for the colorimetric and fluorimetric determination of Hg^{2+} in an aqueous medium. Compound **4** exhibited characteristic absorption maxima at 456 nm and fluorescence band at 534 nm and on interaction with Hg^{2+} ions, underwent fluorescence quenching accompanied by red shifts in absorption and fluorescence spectra as well as color change. Compound **5** was used as the control compound to examine the complexation mode and significance of incorporation of amino acid and triazole ring to improve the binding ability of Hg^{2+} ions.³⁰

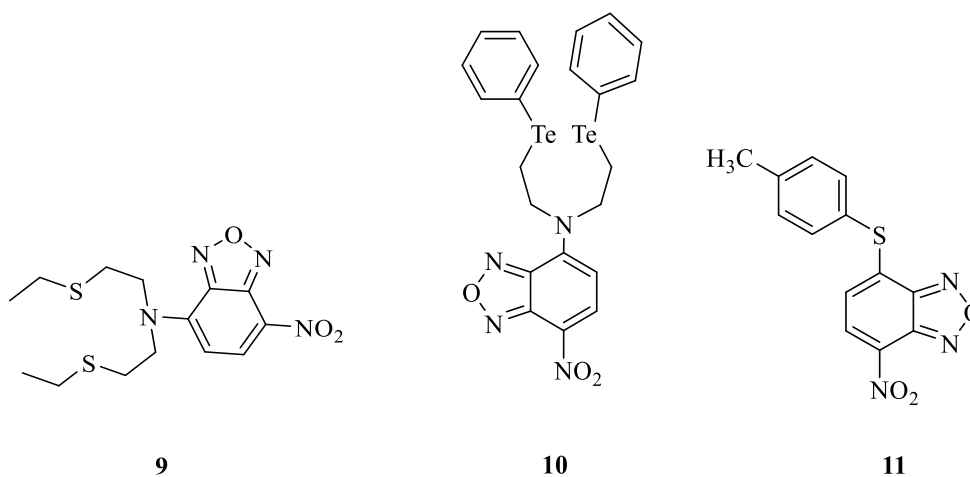
Xu *et al.* synthesized NBD based colorimetric and fluorimetric chemosensor **6** for the detection of Zn^{2+} ions. Chemosensor **6** showed two absorption bands at 350 nm and 487 nm and emission band at 537 nm. In the presence of Zn^{2+} ions, the intensities of absorption bands at 350 nm and 487 nm decreased with the concomitant increase in intensity at 400 nm with a color change from red to yellow. Enhancement in fluorescence intensity at 537 nm has been observed in the presence of Zn^{2+} ions in aqueous solution. Internal charge transfer (ICT) and photo induced electron transfer (PET) mechanisms were responsible for the spectral changes. The practical use of probe **6** was in the relevant detection of Zn^{2+} ions in the pancreatic β -cells.³¹ Liu *et al.* derived *turn-on* fluorescent probe **7** which exhibited reversible binding with Cu^{2+} ions and indicated a 20-folds increase in fluorescence intensity with a blue shift from 545 nm to 520 nm. The binding constant for Cu^{2+} ions was $2.62 \times 10^4 \text{ M}^{-1}$. Chemosensor **7** was also used for imaging of Cu^{2+} ions on HeLa cells by fluorescence microscopy.³²

Lee *et al.* synthesized NBD based fluorophores for the detection of Hg^{2+} ions and Ag^+ ions in $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (3:7, v/v). Only Hg^{2+} ions showed absorption changes selectively with a decrease in ligand peaks at 340 nm and 460 nm and increase in intensity at 400 nm. Upon addition of Hg^{2+}

and Ag^+ ions probe **8** showed turn on effect at 520 nm. Sensing abilities of probe **8** for Hg^{2+} and Ag^+ ions were successfully supported in real water samples and worked as fluorescent test strip with silica plate.³³



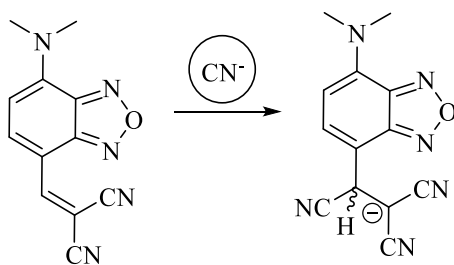
Jeong *et al.* reported a fluorescent chemosensor **9** for the estimation of Hg^{2+} ions in the semi-aqueous medium. The color change was observed from orange to yellow. After the interaction with Hg^{2+} ions, the absorption spectrum of probe **9** was shifted from 484 nm to 467 nm and weak emission spectrum shifted from 550 nm to 538 nm with increase in intensity. The binding constant was determined to be $4.00 \times 10^5 \text{ M}^{-1}$.³⁴



Kao *et al.* developed a fluorescent probe **10** for the selective recognition of Hg^{2+} ions in the semi-aqueous ($\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1,v/v)) medium. Probe **10** exhibited an absorption band at 490

nm and showed a color change from red to yellow in the presence of Hg^{2+} ions. Upon interaction with Hg^{2+} ions, an emission band at 525 nm was observed with green fluorescence. Probe **10** was used for the detection of Hg^{2+} ions in the wide pH range of 4.0-10.0. The detection limit for Hg^{2+} ions has been found to be 4.2 μM and binding constant was calculated as $3.81 \times 10^3 \text{ M}^{-1}$. Probe **10** was used for the detection of Hg^{2+} ions in living cells.³⁵

Wang *et al.* developed a NBD based chemosensor **11** for the detection of Cys over Hcy and amino acids. Upon treatment with Cys, the absorption band of chemosensor **11** at 435 nm quenched with the emergence of a new band at 476 nm and indicated a color change from yellow to orange. However, a drastic change in fluorescence spectra from very weak emission to the 100-folds enhancement at 540 nm was observed in the presence of Cys with a color change from yellow to green in fluorescence. Chemosensor **11** was effectively used for the fluorescence imaging of Cys in HeLa cells.³⁶



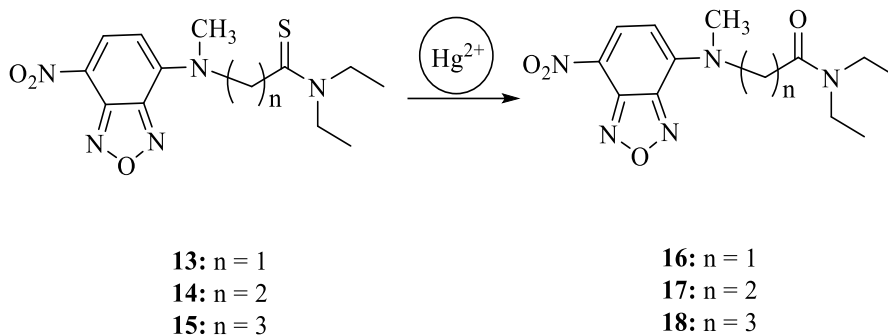
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Scheme 1.1.1: Sensing mechanism of binding with CN^- ions with Probe **12**.

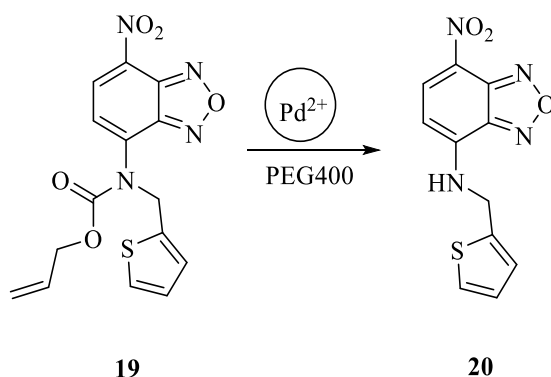
Liu *et al.* also prepared dicyanovinyl-substituted NBD probe **12** for the ratiometric, colorimetric determination of CN^- ions in aqueous acetonitrile solution. On the addition of CN^- ions, the intensities of absorption bands at 391 nm and 513 nm have been reduced accompanied with the formation of a new band at 441 nm with a color change from pink to colorless. The emission band at 581 nm underwent a blue shift to 526 nm with an isoemissive point at 549 nm after interaction with CN^- ions. Intramolecular charge transfer (ICT) was blocked by the addition of CN^- at α -position of dicyanovinyl group (Scheme 1.1.1), responsible for absorption and emission shifts.³⁷

Choi *et al.* developed a series of chemosensors **13-15** for the detection of Hg^{2+} ions. The UV-Visible spectrum of probe **13** showed an absorption band at 492 nm and further shifted to

481 nm after interaction with Hg^{2+} ions. Probe **13** exhibited an emission band at 542 nm which showed 34-folds enhancement in the presence of Hg^{2+} ions. These spectral changes were attributed to the Hg^{2+} -assisted desulfurization (Scheme 1.1.2) to form (**16-18**) thioamides which switching *on* the fluorescence emission. The signals produced by the probes strongly depended on the spacer between the NBD and thioamide moiety and decreased as the spacer length was reduced. The sarcosine based NBD-thioamide moiety (probe **16**) with methylene spacer has been found to be the best structure for the estimation of Hg^{2+} ions selectively (**16**). On the other hand, structures with ethylene and propylene spacers exhibited lower fluorescence enhancement and poor selectivity (**17**, **18**). The efficiency of photoelectron transfers between thioamide and NBD fluorophore has been decreased as the spacer length increased, therefore, the efficiency of probe **15** decreased. The detection limit of probe **13** was calculated to be $2.7 \times 10^{-6} \text{ M}$.³⁸



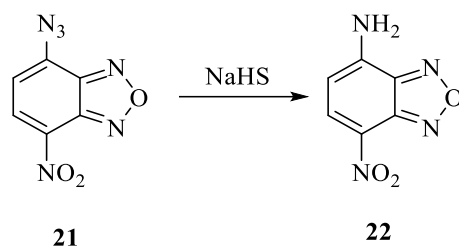
Scheme 1.1.2: Sensing mechanism of probes **13**, **14**, **15** binding with Hg^{2+} ions.



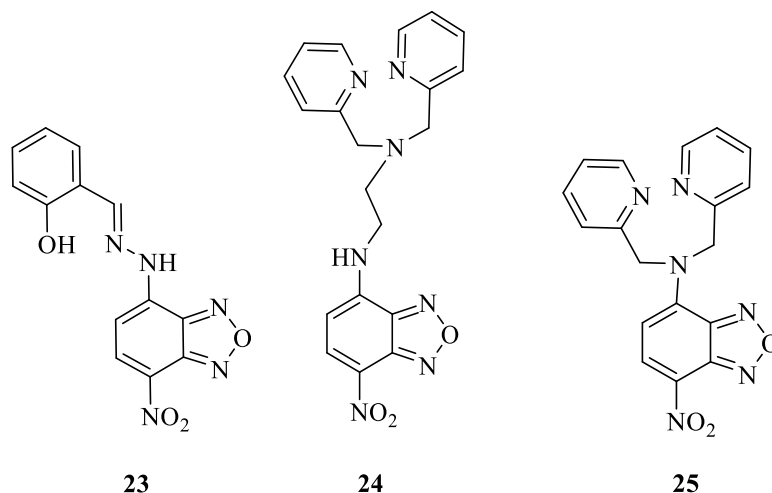
Scheme 1.1.3: Sensing mechanism of probe **19** for Pd^{2+} ions.

A NBD based probe **19** was synthesized by Xu *et al.* for the recognition of Pd²⁺ through the Tsuji-Trost reaction. The probe **19** upon titration with Pd²⁺ ions in PEG400 solution, showed a decrease in absorption intensity at 382 nm and 278 nm along with the emergence of new bands at 338 nm and 473 nm. Probe **19** showed 50-folds enhancement of emission intensity at 526 nm as probe **19** got transformed into **20** (Scheme 1.1.3) in the presence of Pd²⁺ ions. The detection limit of probe **19** was found to be 1.13×10^{-9} M.³⁹

Zhou *et al.* developed colorimetric and fluorescent chemosensor **21** having NBD unit linked with an H₂S-active azide group. Chemosensor **21** indicated sensitive response towards H₂S in aqueous medium through the conversion to compound **22** (Scheme 1.1.4). Upon the addition of NaHS, the absorption band at 396 nm led to a red shift to 468 nm and showed color change from pale yellow to deep yellow. The emission band at 550 nm was observed with a 16-folds enhancement and showed practical application for the detection of H₂S in living cells.⁴⁰



Scheme 1.1.4: Sensing mechanism of probe **21** for hydrogen sulfide.



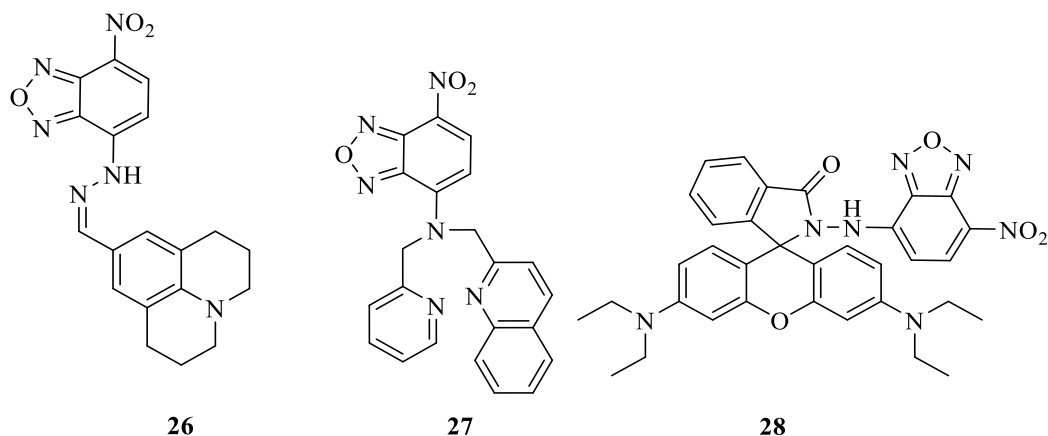
Lee *et al.* have synthesized chemosensor **23** containing NBD and phenol moieties which were linked through Schiff-base. In the presence of Ni^{2+} ions, the band at 515 nm shifted to 498 nm and simultaneously, the band at 400 nm was quenched with concomitantly increasing in intensities at 460 nm and 625 nm. A clear isosbestic point at 425 nm confirmed the formation of a stable complex between chemosensor **23** and Ni^{2+} ions. UV-Visible titration of **23** with Cu^{2+} ions showed a decrease in intensity at 500 nm with enhancement at 400 nm. A visible color change from pink to orange on interaction with Ni^{2+} ions and pink to brown on interaction with Cu^{2+} ions was observed. The sensing properties of chemosensor **23** were also explained by theoretical calculations and photophysical experiments.⁴¹

Jiang *et al.* developed NBD based probes **24** and **25** for the investigation of metal ions. Probe **24** showed weak fluorescence while **25** displayed an emission band at approx. 550 nm. Upon addition of Zn^{2+} ions, probe **24** showed 25-folds enhancement while probe **25** showed low affinity for Zn^{2+} ions due to strong electron withdrawing nature of NBD. PET was responsible for binding event in both cases.⁴²

1.1.2 NBD based dyads as sensor for cations and anions

In NBD based dyads, different fluorophores combined with NBD moiety to give to fluorescent chemosensors. As two fluorescent molecules, interplaying in sensing mechanisms, these tend to provide strong fluorescent signals, amplify the response, track multiple analytes with different mechanisms, improve selectivity, detection limit, sensitivity etc. Sensing mechanisms including ICT, FRET, TBET, PET, ESIPT etc. are responsible for the detection of various analytes in such type of dyads.

Lee *et al.* designed chemosensor **26** for the recognition of Cu^{2+} and Cr^{3+} ions in aqueous solution as a colorimetric chemosensor. Chemosensor **26** showed absorption maxima at 550 nm. In the presence of Cu^{2+} and Cr^{3+} ions, probe **26** showed a decrease in absorption intensity at 550 nm with the appearance of new band at 650 nm with four isosbestic points at 350 nm, 426 nm, 500 nm and 608 nm in case of Cu^{2+} ions and one isosbestic point at 602 nm in case of Cr^{3+} ions. Probe **26** showed color change from violet to yellow in the presence of Cu^{2+} ions and violet to blue in the presence of Cr^{3+} ions. The lowest detection limit for Cu^{2+} ions was determined to be 0.36 μM and for Cr^{3+} ions, 0.59 μM . The probe **26** showed practical applicability as visible test strip for the detection of Cu^{2+} and Cr^{3+} ions.⁴³

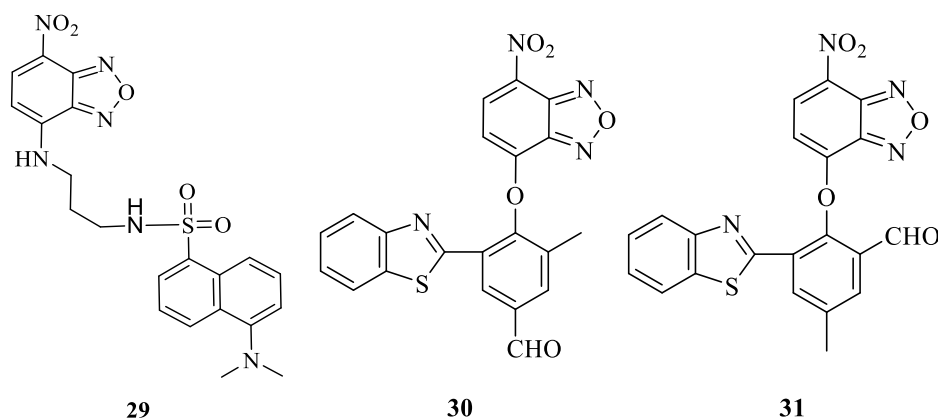


Kim *et al.* developed a new NBD based probe **27** as a chromogenic and fluorogenic sensor for the selective determination of Hg^{2+} ions with *on-off* fluorescence quenching effect. On addition of Hg^{2+} ions, absorption bands at 331 nm and 467 nm underwent quenching with the emergence of two new bands at 304 nm and 400 nm. Probe **27** selectively detected Hg^{2+} through a color change from yellow to colorless. Upon the subsequent addition of Hg^{2+} ions, emission band at 530 nm was quenched completely and showed high selectivity for Hg^{2+} ions over other potential competitor metal ions such as Cu^{2+} , Ag^+ , and Pb^{2+} . The binding mode of Hg^{2+} ions with probe **27** has been found to be a 1:2 complex formation which was confirmed through Job's plot.⁴⁴

Liu *et al.* developed probe **28** having NBD and rhodamine moieties for the recognition of Cu^{2+} and Fe^{3+} ions. In $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (1:1,v/v), showed remarkable selectivity towards Cu^{2+} ions. In the presence of Cu^{2+} ions, the absorption peak at 487 nm was gradually decreased and appearance of a new peak at 560 nm with a color change from pale orange to purple and emission spectrum, showed an enhancement at 573 nm. On the other hand in $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (99:1, v/v) the UV-Visible spectrum did not show any selectivity but fluorescence spectrum showed selectivity towards Fe^{3+} ions with the enhancement of weak band at 575 nm. The probe **28** used as a dual-channel chemosensor for the detection of Cu^{2+} and Fe^{3+} ions in a wide pH range of 2.6-10.9. The binding mechanism monitored through IR and mass spectrometric techniques, and has been found to be 1:1 stoichiometry between probe **28** and metal ions.⁴⁵

Bhoi *et al.* reported dyad **29** comprising two fluorescent units dansylamide and NBD. The dansyl group has been used as a receptor site for cations and NBD moiety functioned as a

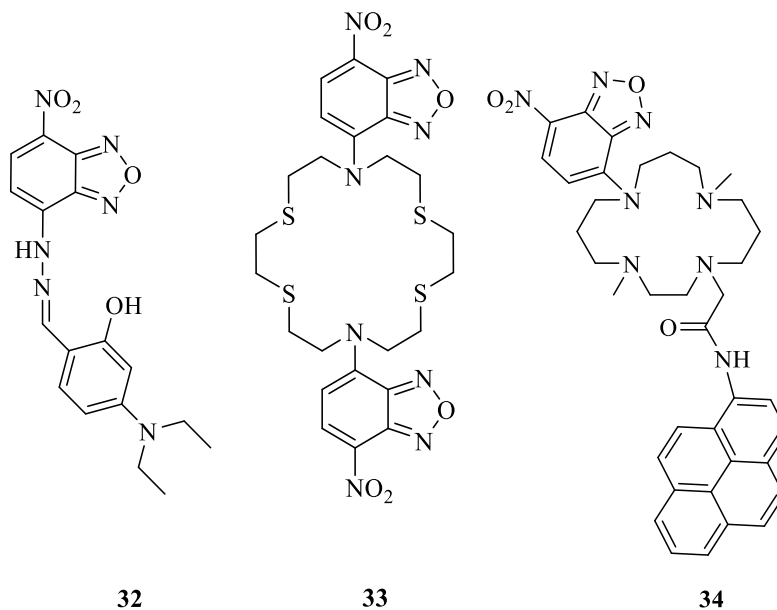
receptor site for anions within a single molecular system. Dyad **29** showed broad absorption band at 460 nm and broad emission band at 531 nm in acetonitrile. Zn^{2+} and Cu^{2+} ions induced fluorescence quenching while other metal ions led to fluorescence enhancement. Amongst various anions, only F^- ions showed remarkable change towards dyad **29**. Spectral changes were attributed to the disruption of PET phenomenon between dansyl and NBD moieties.⁴⁶



Ding *et al.* synthesized fluorescent probes **30** and **31** for the detection of H_2S . Both the probes exhibited selective, colorimetric and fluorescent *turn-on* responses. The absorption spectrum of probe **30** after addition of NaHS showed a color change from colorless to purple red. The emission spectrum of probe **30** showed significant enhancement at 550 nm with green fluorescence while probe **31** gave blue fluorescence at 455 nm after incremental addition of NaHS. These probes recognized H_2S even in the presence of other biothiols. Although probe **30** showed less stability, probe **31** was able to estimate H_2S over a wide pH range. Probe **31** was used to visualize H_2S in living cells and holds utility for imaging applications with low cytotoxicity.⁴⁷

Meng *et al.* developed NBD-based fluorescent probe **32** for the quantification of Cu^{2+} ions and S^{2-} ions in HEPES buffer. Probe **32** showed absorption band at 430 nm which gradually decreased on the addition of Cu^{2+} ions with two isosbestic points at 376 nm and 462 nm. Probe **32** displayed green emission centered at 519 nm which got quenched by the addition of Cu^{2+} ions. Binding of probe **32** with Cu^{2+} ions resulted in fluorescence quenching with 1:1 stoichiometry and detection limit of 1.6 nM. This quenching of probe **32**· Cu^{2+} complex can be recovered by the addition of S^{2-} ions and thus used for the estimation of S^{2-} ions through

displacement approach. Probe **32** was also capable of imaging and quantification of Cu^{2+} and S^{2-} in living cells.⁴⁸

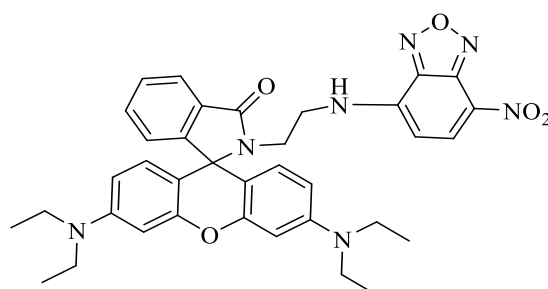


Kim *et al.* synthesized NBD derivative **33** with crown ether for the detection of Hg^{2+} ions in the mixed aqueous medium. Upon interaction with Hg^{2+} ions, the broad absorption band at 486 nm was shifted to 448 nm with a blue shift of 38 nm. Probe **33** also showed colorimetric change from light orange to yellow which allowed for the naked eye detection of Hg^{2+} ions. Probe **23** revealed a broad band centered at 541 nm, which underwent a blue shift to 529 nm and upon the addition of Hg^{2+} ions. Probe **33** could be used as a novel chemosensor for the recognition of sub-millimolar concentrations of Hg^{2+} ions in aqueous media.⁴⁹

Kim *et al.* also synthesized probe **34** for the selective detection of Hg^{2+} and Cu^{2+} ions in semi aqueous ($\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1,v/v)) medium. Probe **34** showed two absorption bands at 342 nm due to pyrene and 484 nm due to NBD. Upon addition of Hg^{2+} ions, a blue shift was observed in NBD region. Splitting in the pyrene absorption band with a color change from light orange to yellow was observed. Upon interaction with Cu^{2+} ions, an increase in absorbance in the pyrene region with a decrease in intensity accompanied by a blue shift of 11 nm in NBD absorption band was observed. Probe **34** exhibited two emission bands at 385 nm and 538 nm for the pyrene and NBD moieties, respectively. Upon addition of Hg^{2+} ions, the enhancement was observed at 538 nm while on the addition of Cu^{2+} ions, quenching was observed at 538 nm. The detection limits were found to be 7.9×10^{-6} M and 2.6×10^{-7} M for Hg^{2+} and Cu^{2+} ions,

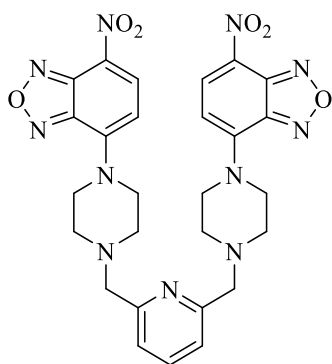
respectively. *Off-on-off* type signalling pattern has been used for the construction of supramolecular switching systems.⁵⁰

Liu *et al.* reported dyad **35** based on rhodamine-NBD conjugate for the selective recognition of Hg^{2+} ions. In the presence of Hg^{2+} ions, dyad **35** showed both ratiometric and colorimetric changes. In the presence of Hg^{2+} ions, the absorption bands of dyad **35** at 300 nm and 460 nm underwent a decrease in intensity with the concomitant increase in intensity at 525 nm and a color change from yellow to red. Free dyad **35** showed minimal emission but on the addition of Hg^{2+} ions, enhancement in intensity at 543 nm was observed.⁵¹

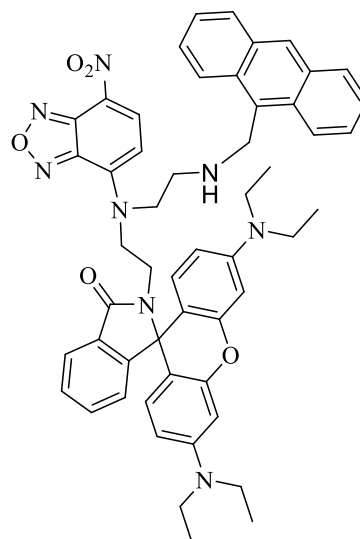


35

Kim *et al.* prepared a chemosensor **36** by combining NBD with 2,6-pyridyl moiety. In the presence of different metal ions, no significant change in the absorption band at 483 nm was observed but the emission spectrum showed a red shift from 543 to 550 nm in the presence of Hg^{2+} ions. The *turn-on* behavior of chemosensor **36** enabled the detection of Hg^{2+} ions in living cells.⁵²



36



37

Biswal *et al.* reported NBD, rhodamine, and anthracene based dyad **37** for the detection of Hg^{2+} ions selectively in $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1, V/V). Dyad **37** showed new absorption band at 557 nm and emission band at 580 nm corresponding to ring opened rhodamine. Dyad exhibited induced ratiometric fluorescence signalling through FRET.⁵³

1.2 FRET/TBET based molecules

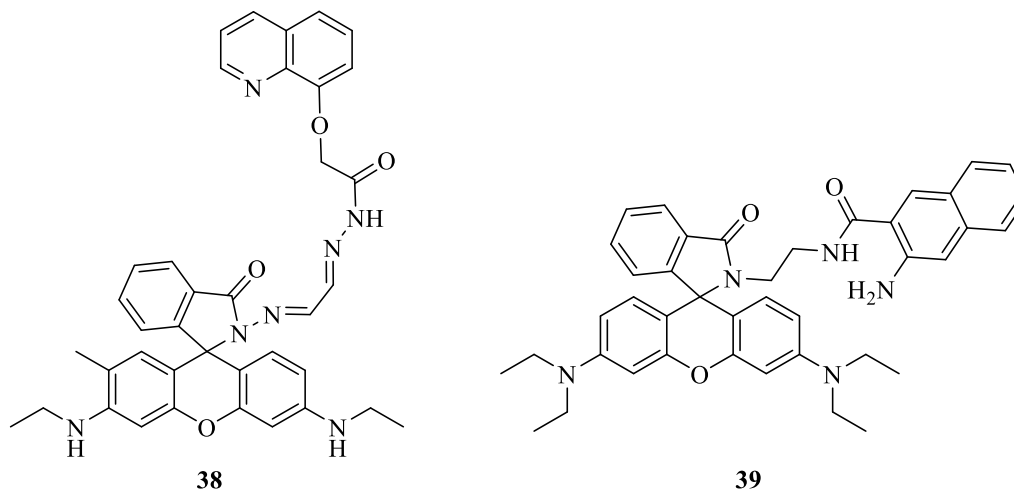
Moieties such as BODIPY, rhodamine, fluorescein, coumarin and naphthalimide derivatives have been widely used in bioimaging, biosensing, medical diagnosis, and environmental detection. However, the photophysical properties of various fluorophores has still limited the full potential of their applications. Besides this, exploration of dyads with energy donor–acceptor architectures can achieve large pseudo-Stokes' shifts. Such dyads could also afford simultaneous recording ratio of optical signals of two emission intensities at different wavelengths and used for DNA detection, protein labelling, nucleic acid regulation, and other biomarkers. Consequently, construction of such dyads could be very interesting and significant to studying the photophysical behavior towards different cations and anions. Depending on whether these donor-acceptor pair linked through non-conjugated or conjugated spacer they were divided in to two categories (i) fluorophores linked by a non-conjugated spacer are said FRET based dyads, and the energy transfer occurs through space; (ii) fluorophores linked by a conjugated spacer are said TBET based dyads, and the energy transfer occurs through bond.

1.2.1 FRET based molecules

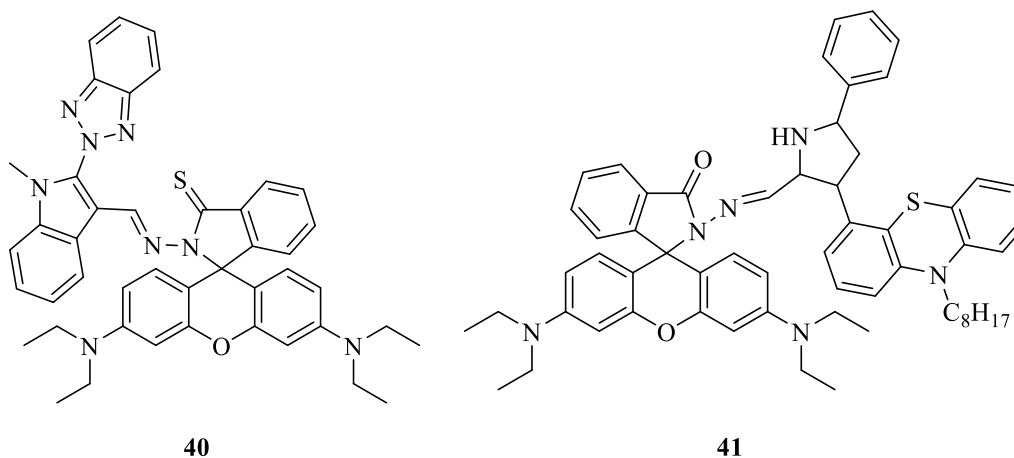
Qin *et al.* reported fluorescence resonance energy transfer (FRET) based rhodamine-quinoline dyad **38** and used for the detection of Fe^{3+} ions. Dyad **38** showed absorption spectrum at 300 nm due to quinoline group and on interaction with Fe^{3+} ions band at 300 nm increased with the emergence of two new bands at 350 nm and 530 nm with increased intensity. Dyad **38** displayed emission at 425 nm which on the addition of Fe^{3+} ions, dyad **38** showed large emission at 550 nm that was attributed to the FRET phenomenon from quinoline to rhodamine. The reversibility of the dyad **38** was confirmed by the addition of Fe^{3+} binding agent Na_2EDTA .⁵⁴

Sui *et al.* developed a ratiometric chemosensor **39** for the estimation of Pd^{2+} ions. Chemosensor **39** was synthesized by connecting rhodamine B ethylenediamine directly to 3-amino-2-naphthoic acid. Upon incremental addition of Pd^{2+} to chemosensor **39**, the emission band due to the naphthylamine moiety was decreased and a new emission band at 590 nm of the

rhodamine moiety was observed. The detection limit for the determination of Pd^{2+} was 45.9 nM. Chemosensor **39** was also practically applied for the detection of Pd^{2+} in mice.⁵⁵



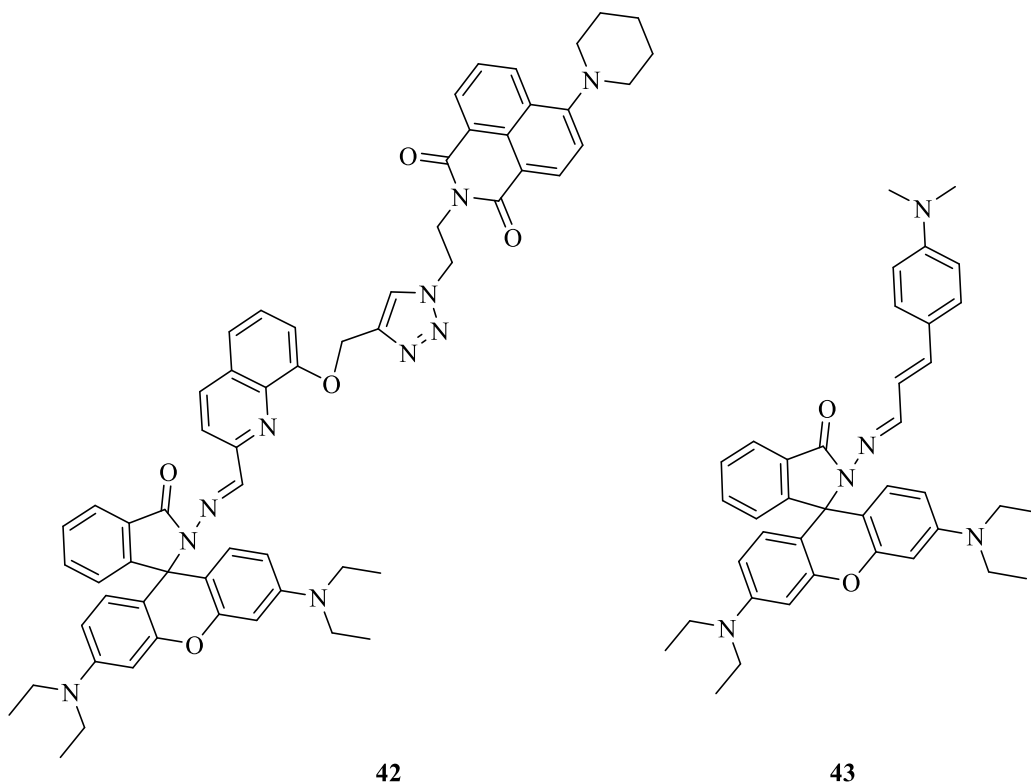
Liu *et al.* reported a fluorescent probe **40** comprising of a rhodamine acceptor and an indole-triazole donor, linked through a hydrazine moiety. Upon addition of Hg^{2+} ions, a marked increase in intensity at 565 nm was observed with a color change from colorless to pink. Probe **40** showed a large shift from 490 nm to 590 nm in emission spectrum upon the addition of Hg^{2+} ions. The detection limit of Hg^{2+} ions was found to be 11 nM. Probe **40** was used for the imaging of Hg^{2+} ions in MCF-7 cells.⁵⁶



Zhao *et al.* synthesized FRET based chemosensor **41** consisting of rhodamine acceptor and phenothiazine donor and functioned as a ratiometric fluorescent chemosensor for the detection of Hg^{2+} ions. The UV-Visible spectrum of **41** showed an absorption maximum at 415

nm due to the phenothiazine (donor) moiety. In the presence of Hg^{2+} ions, a new band at 550 nm due to the rhodamine open chain form was observed with a color change from yellow to pink. Upon excitation of chemosensor **41** at 415 nm, a red shift in emission band from 520 nm to 580 nm was observed in the presence of Hg^{2+} ions. The detection limit of Hg^{2+} was found to be 9.2×10^{-9} M. The energy transfer from donor (phenothiazine) to acceptor (rhodamine) was found to be 76.8%.⁵⁷

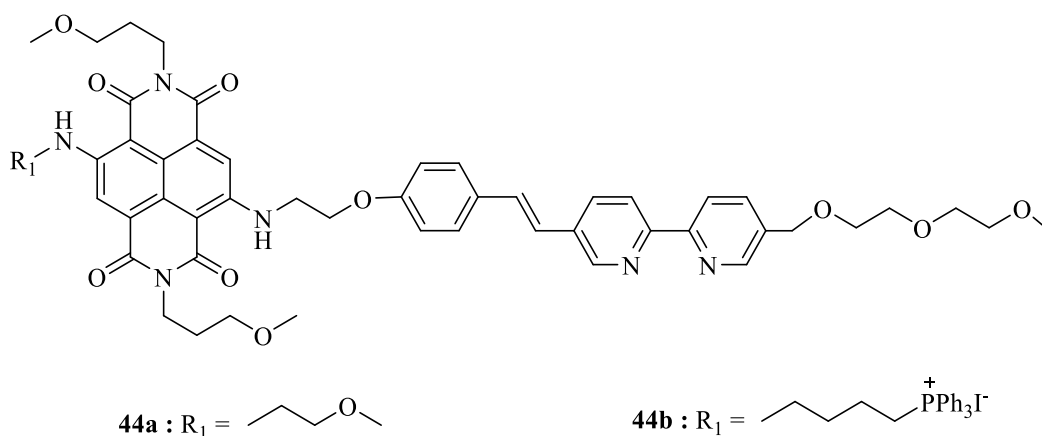
Cherreddy *et al.* synthesized dyad **42** through the combination of rhodamine acceptor and naphthalimide donor and showed FRET phenomenon in the presence of Fe^{3+} ions. Probe **42** showed an absorption band at 415 nm due to the naphthalimide moiety and upon the addition of Fe^{3+} ions, a linear increase at 560 nm was observed. The probe **42** showed emission spectra at 532 nm which ratiometrically shifted to 580 nm with an isoemissive point at 567 nm. Dyad **42** showed practical application for the intracellular imaging of Fe^{3+} ions.⁵⁸



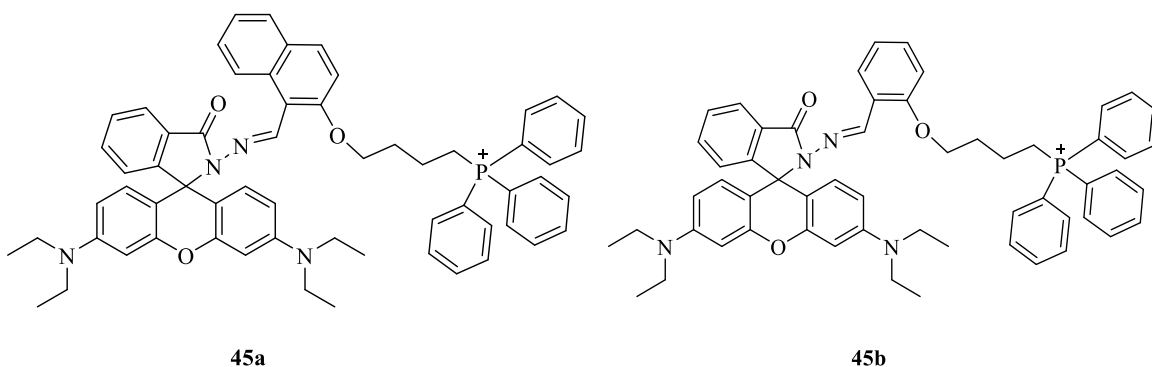
Kumar *et al.* reported dyad **43** consisting of rhodamine acceptor linked with dimethylaminocinnamaldehyde which showed FRET phenomenon in the presence of Fe^{3+} ions. The absorption spectrum showed two peaks at 308 nm and 380 nm and upon the addition of Fe^{3+}

ions, the peak at 380 nm diminished and a new peak at 554 nm appeared with a color change from colorless to pink. The emission spectrum of **43** was characterized by emission band at 470 nm which was shifted to 582 nm ratiometrically in the presence of Fe^{3+} ions. This ratiometric change facilitates sufficient overlap between the energy donor and energy acceptor and responsible for FRET phenomenon. The detection limit was found to be $60 \times 10^{-9} \text{ M}^{-1}$.⁵⁹

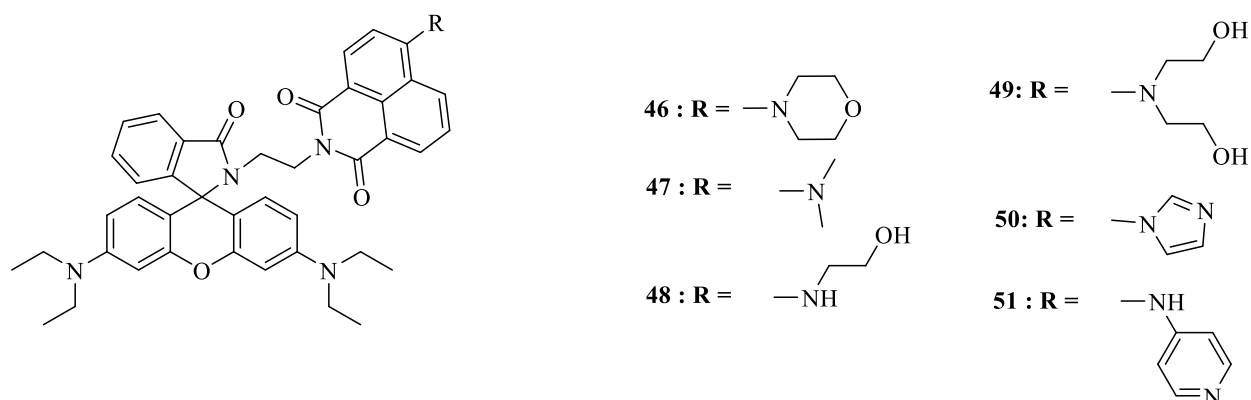
Sreenath *et al.* synthesized probes **44a** and **44b** having 5-(4-methoxystyryl)-5'-methyl-2,2'-bipyridine as FRET donor and diaminosubstituted naphthalenediimide as FRET acceptor for the recognition of Zn^{2+} ions. The fluorescent spectra of **44a** and **44b** exhibited weak fluorescence which underwent enhancement centered at 630 nm in the presence of Zn^{2+} ions. The intercellular fluorescence of probe **44a** was low because of the diaminosubstituted naphthalenediimide moiety and showed strong red emission due to the dispersion of probe **44b** into the inner mitochondrial membrane.⁶⁰



Chen *et al.* reported FRET based dyads **45a** and **45b** for the selective detection of Fe^{3+} ions. The structural characteristic of dyad **45a** comprised of a naphthalene chromophore and an alkyltriphenylphosphonium cation attached to rhodamine. In dyad **45b**, the naphthalene chromophore was replaced with a benzene ring. Dyads **45a** and **45b** indicated a new absorption band at 570 nm and a color change from colorless to orange in the presence of Fe^{3+} ions. In the presence of Fe^{3+} ions, enhancement of the emission was observed at the expense of emission at 431 due to FRET from naphthalene (donor) to rhodamine acceptor. Due to the absence of energy donor group, no FRET was observed in the case of dyad **45b**. The enhancement of fluorescence was observed in the presence of different metal ions due to ring opening of rhodamine moiety.⁶¹

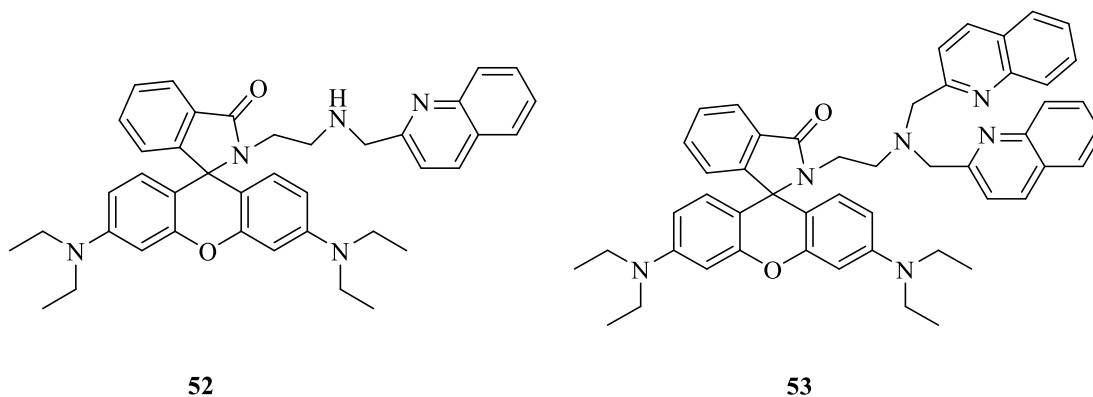


Wang *et al.* synthesized a series of ratiometric chemosensors **46-51** based on rhodamine acceptor and naphthalimide donors. During preliminary results, it was found that all these compounds exhibited selectivity towards Fe^{3+} ions with different degree of interference from Cr^{3+} ions. Chemosensor **47** showed the best selectivity towards Fe^{3+} as it contained a strong electron donating group (dimethylamino). Chemosensor **47** showed an absorption band at 412 nm and in the presence of Fe^{3+} ions, a new band at 554 nm was observed with a color change from yellow to pink. The emission band of dyad **47** shifted from 520 nm to 577 nm in the presence of Fe^{3+} ions and showed a 17-folds increase in intensity. The detection limit was calculated to be 0.418 ppm.⁶²

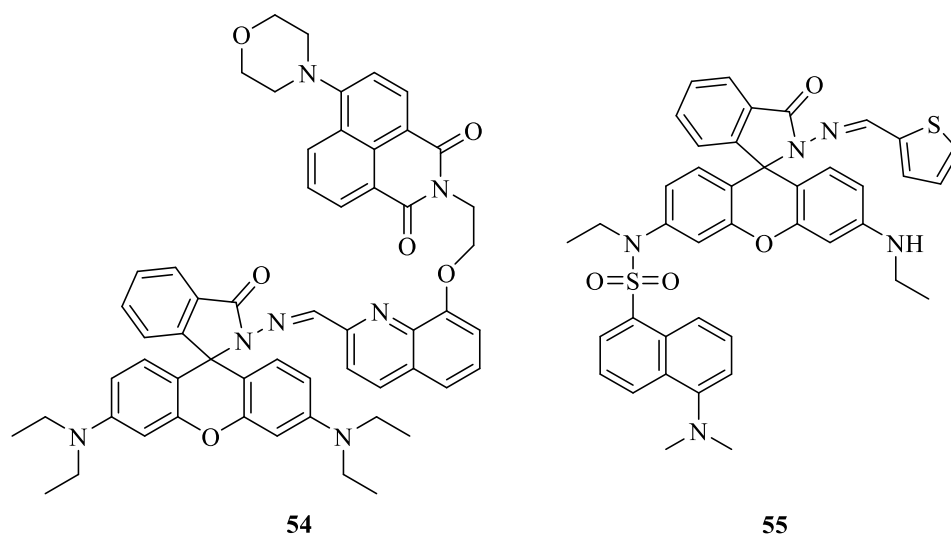


Adhikari *et al.* designed and synthesized probes **52** and **53** for the detection of Au^{3+} ions through FRET mechanism. Probes **52** and **53** were weakly emissive and enhancement at 440 nm was observed in the presence of Au^{3+} . Probes **52** and **53** revealed Au^{3+} ions in the presence of masking agent KI so that interferences with Hg^{2+} ions could be minimized. Probe **52** showed interference from Cr^{3+} ions while it was not in the case of probe **53**. Probe **52** also showed

reversibility with CN^- ions. Further, probe **52** was cell permeable and allowed imaging of Au^{3+} in living HeLa cells.⁶³

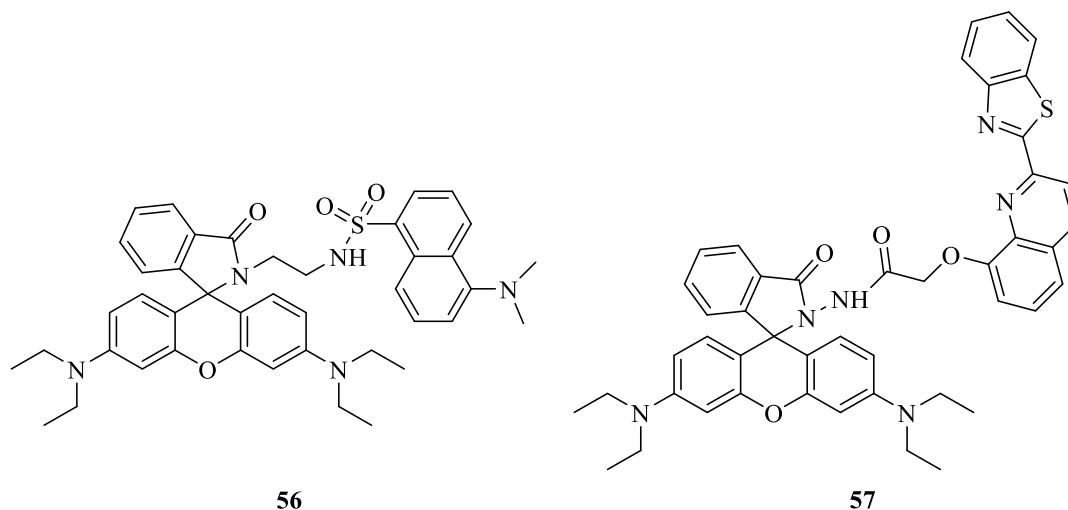


Li *et al.* synthesized a FRET based probe **54** having multi-chelating sites for Cr³⁺ ions. Probe **54** showed an absorption band at 380 nm and yellow emission at 544 nm in ethanol-water (2:1, v/v). The addition of Cr³⁺ ions produced a new band at 568 nm with a change in color from yellow to red. Upon excitation of probe **54** at 405 nm, emission at 544 nm was quenched with the emergence of a new band at 592 nm. The emission ratio at 592 nm and 544 nm has been changed from 0.77 to 5.63, having 8-folds enhancement.⁶⁴

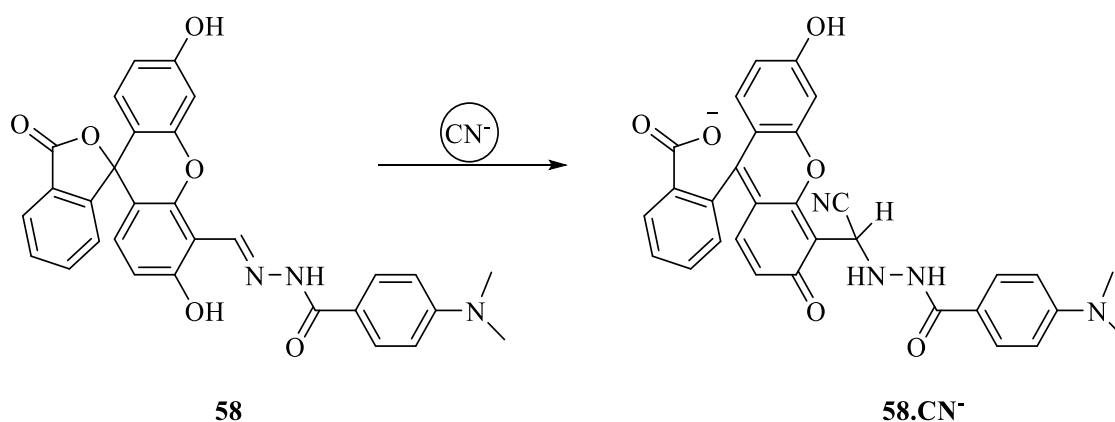


Suresh *et al.* synthesized rhodamine derivative **55** with *N*-dansyl and thiophene moieties for the recognition of Hg²⁺ ions in aqueous solution. Upon interaction with Hg²⁺ ions, a new band at 530 nm was observed due to the ring opening of rhodamine moiety. The ratio of acceptor

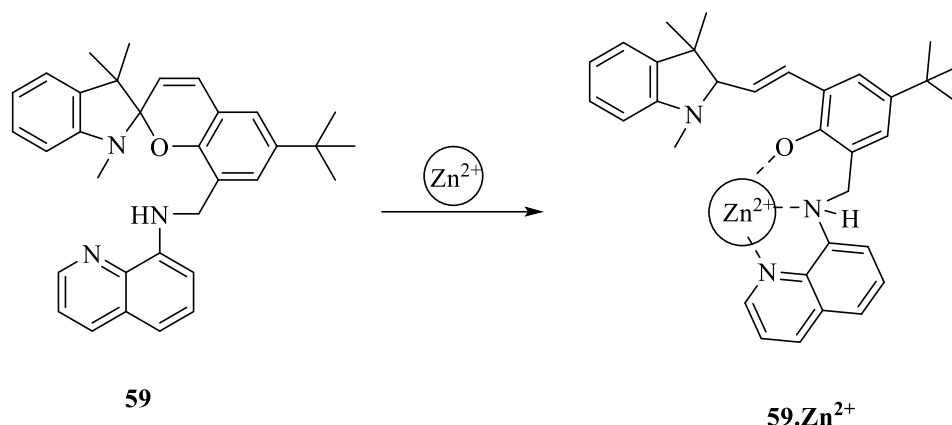
and donor emission intensity I_{555}/I_{483} , in the absence and presence of Hg^{2+} ions, varied from 0.83 to 22.46 and showed a 27-folds enhancement due to FRET phenomenon.⁶⁵



Piao *et al.* synthesized chemosensor **56** based on FRET phenomenon for the selective detection of Fe^{3+} ions. In the presence of Fe^{3+} ions, a color change from colorless to pink color was observed with the formation of the band at 555 nm. In fluorescence spectroscopy, a broad emission band from 450 nm to 650 nm was observed and on the addition of Fe^{3+} ions, strong emission band at 578 nm was also observed. Binding of Fe^{3+} ions induced the transfer of energy from a donor (dansyl) to acceptor (rhodamine) and showed an *off-on* FRET behavior. In addition, relatively high sensitivity for Fe^{3+} ions induced the possibility for potential use in complex samples containing different metal ions. Job's plot analysis showed 1:1 complex formation of the Fe^{3+} ions with chemosensor **56**.⁶⁶



Scheme 1.1.5: Sensing mechanism of probe **58** CN^- ions.



Scheme 1.1.6: Sensing mechanism of probe **59** Zn^{2+} ions.

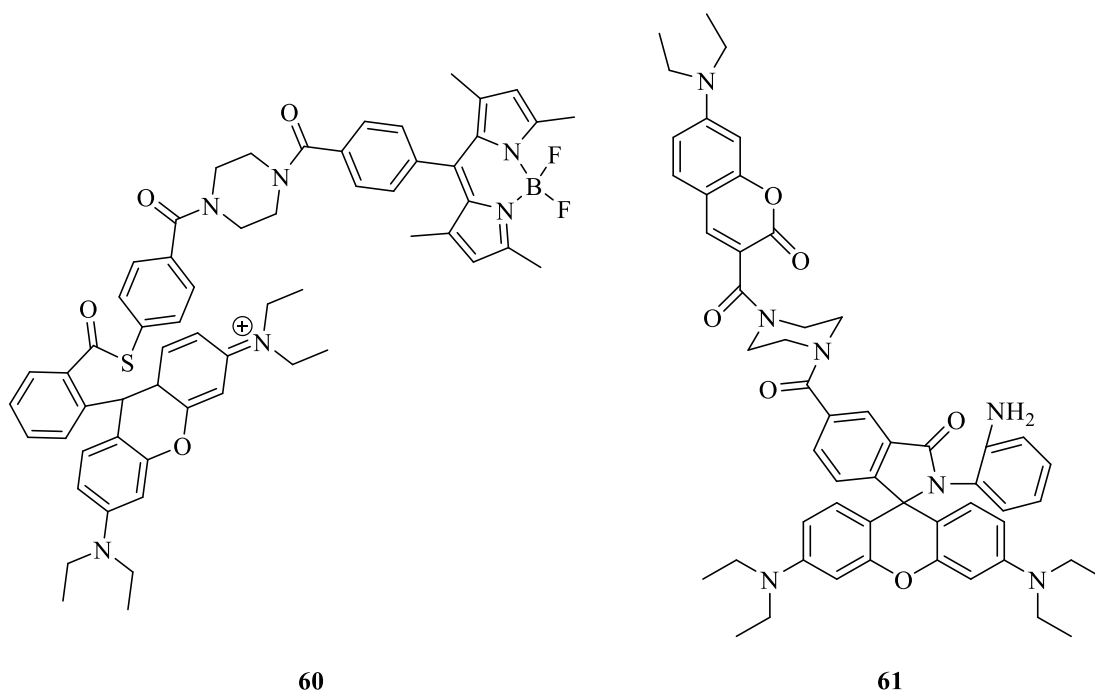
Aich *et al.* reported dyad **57** that showed colorimetric and ratiometric emission changes upon interaction with Cd^{2+} ions and *turned-on* the FRET process. Dyad **57** exhibited three absorption bands at 262 nm, 300 nm and 360 nm and displayed a new peak at 565 nm in the presence of Cd^{2+} ions, induced by the ring opening of rhodamine moiety. The color change from colorless to pink was observed due to the ring opening of rhodamine moiety. In the presence of Cd^{2+} ions, the band intensity of dyad **57** at 470 nm decreased and formation of a new band at 585 nm was observed. The color change from blue to red after illumination under UV light facilitated the naked eye detection. Dyad **57** was used as imaging agent for the detection of Cd^{2+} ions in living cells.⁶⁷

Guo *et al.* reported probe **58** as a FRET *off-on* system for the ratiometric detection of CN^- ions in DMF: H_2O (9:1, v/v). Probe **58** displayed an absorption band at 350 nm with a weak shoulder band at 515 nm, indicating trace amounts of the ring opened form of fluorescein (Scheme 1.1.5). Upon addition of CN^- ions, the intensity at 350 nm decreased while at 515 nm the intensity increased with a color change from colorless to brown. Upon addition of CN^- ions, the band intensity at 450 nm was quenched and at 530 nm, shifted to 550 nm. CN^- ion attacked as nucleophile to the hydrazone, followed by proton transfer of the acidic phenol proton to the developing nitrogen anion of probe **58**, inducing the ring-opening in the fluorescein moiety.⁶⁸

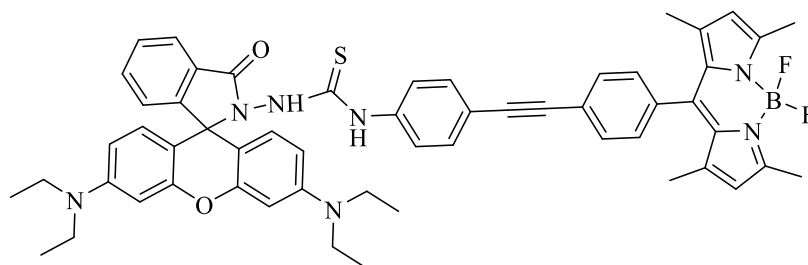
Chan *et al.* synthesized probe **59** by conjugating 8-aminoquinoline to a spiropyran backbone with a CH_2 spacer. Probe **59** constructed FRET cassettes through binding with Zn^{2+} ions in ethanol (Scheme 1.1.6). In the presence of Zn^{2+} ions, two broad peaks emerged at 380 nm and 540 nm with a color change from colorless to red. The probe **59** exhibited *turn-on* fluorescence and ratiometric response and showed selective change with Zn^{2+} ions. Upon excitation at 370

nm, the intensity of the emission band at 470 nm decreased and a new band at 645 nm appeared. The detection limit was calculated to be $7.2 \times 10^4 \text{ M}^{-1}$.⁶⁹

A FRET-based ratiometric thiol probe **60** was synthesized by Long *et al.* The probe **60** consisted of a rhodamine dye, a thioester group, a piperazyl moiety and a BODIPY dye. The BODIPY and rhodamine as the fluorophores and piperazyl moiety as the linker enabled the energy transfer between the BODIPY (donor) and the rhodamine (acceptor). The free probe in its ground state showed two bands at 500 nm and 562 nm. Upon excitation, the probe **60** showed emission at 590 nm, characteristics emission of rhodamine moiety, confirmed the FRET *on*. On the other hand, the addition of Cys to the free probe, the emission at 590 nm was quenched with a new peak of Bodipy at 510 nm indicating FRET *off*.⁷⁰

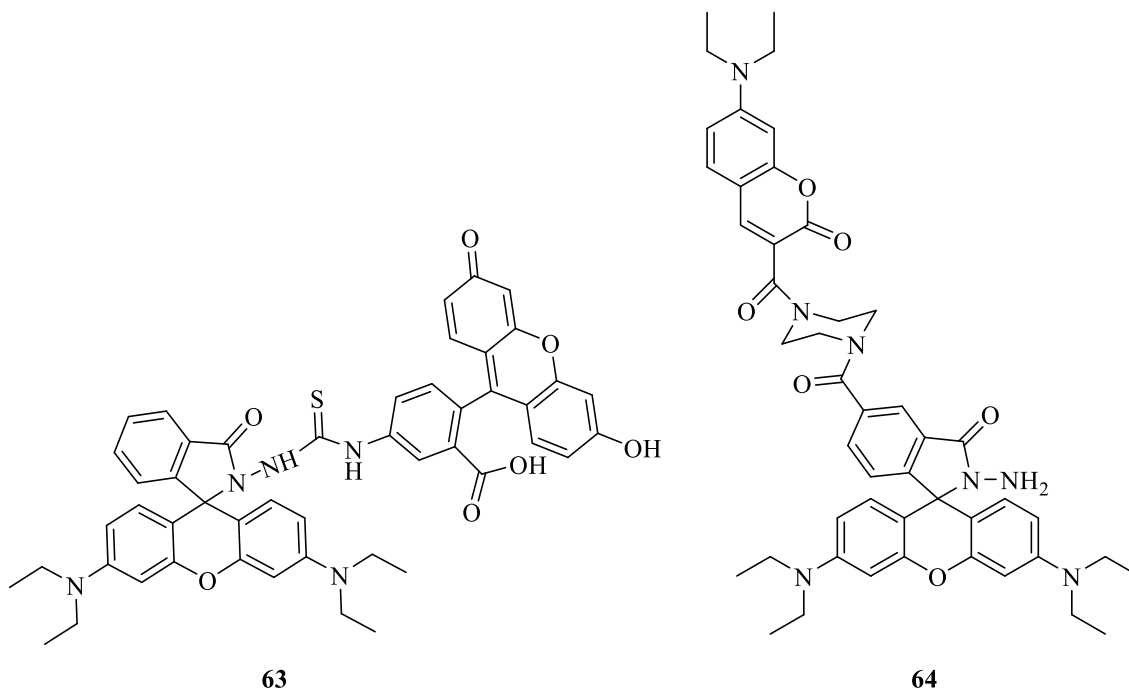


Yuan *et al.* developed coumarin-rhodamine based dyad **61** which was the first small-molecule for ratiometric fluorescent sensing of NO. Interestingly, dyad **61** showed a very large 420-folds variation in fluorescence intensity ratio from 473 nm to 583 nm which was attributed to FRET phenomenon. Dyad **61** showed some significant features as high sensitivity, specificity, low cytotoxicity, good membrane permeability and worked well at physiological pH. Dyad **61** showed practical application in imaging of NO in macrophage cells.⁷¹



62

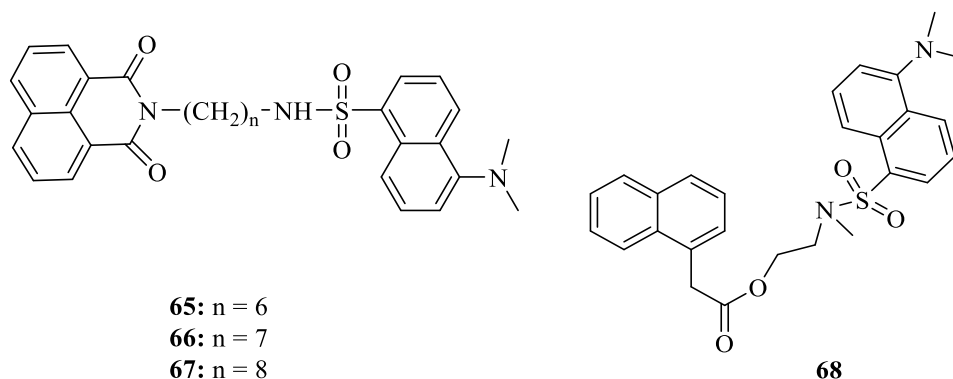
BODIPY (donor)-rhodamine (acceptor) based FRET *off-on* dyad **62** was reported by Zhang *et al.* as a ratiometric and intracellular Hg^{2+} sensor. The absorption profile showed a maximum at 501 nm and the addition of Hg^{2+} ions showed an increase in intensity at 560 nm due to the ring opened form of rhodamine with a color change from green to pink. Excitation of dyad **62** at 488 nm resulted in emission band at 514 nm which got quenched in the presence of Hg^{2+} ions with the emergence of a new band at 589 nm. Thus, dyad **62** exhibited a clear Hg^{2+} -induced change in the intensity ratio of the two well-separated and strong emission bands of BODIPY and rhodamine.⁷²



Shang *et al.* synthesized irreversible Hg^{2+} selective ratiometric fluorescence dyad **63** having a fluorescein fluorophore linked to a rhodamine B hydrazide through a thiourea spacer. The developed dyad exhibited an absorption band at 490 nm which was shifted to 565 nm on the

addition of Hg^{2+} ions and a remarkable yellow to magenta color change. Dyad **63** showed ratiometric fluorescence enhancement from 520 nm to 590 nm.⁷³

Coumarin-rhodamine based compound **64** was synthesized by Yaun *et al.* as a FRET ratiometric fluorescent chemodosimeters for ratiometric imaging of Cu^{2+} in living cells. These exhibited several favorable features, including a large variation in the emission ratio, well-resolved emission peaks, high selectivity, high sensitivity, low cytotoxicity, and good cell membrane permeability.⁷⁴

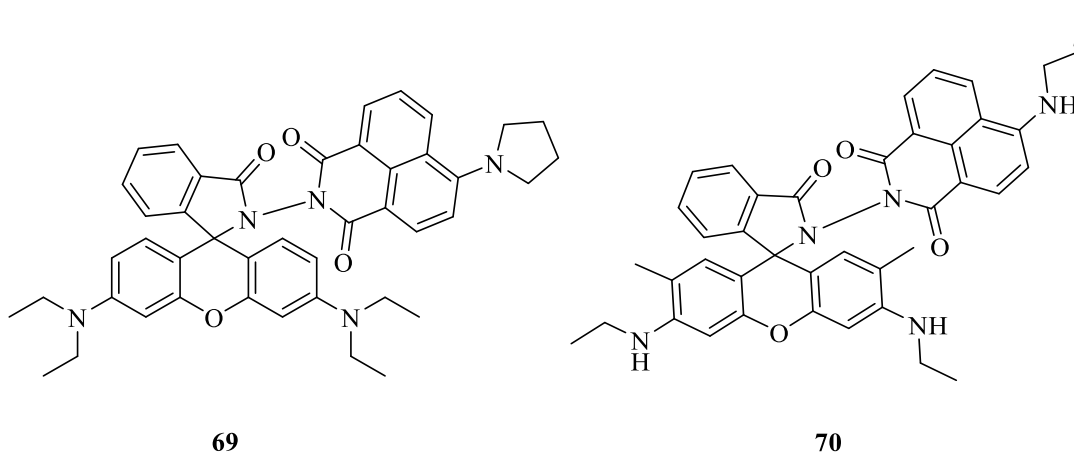


Chimeric dyads **65-67** based on dansyl and naphthalimide units linked through the polymethylene group were synthesized by Jisha *et al.* Their photophysical properties and interactions with various metal ions were investigated under different conditions. These dyads showed dual emission, due to the locally excited state of the naphthalimide chromophore and FRET from the dansyl moiety. The uniqueness of these dyads was that they formed stable 2:1 stoichiometric complex involving sulfonamide functionality and acted as visual fluorescence ratiometric probe for the selective determination of Cu^{2+} ions.⁷⁵

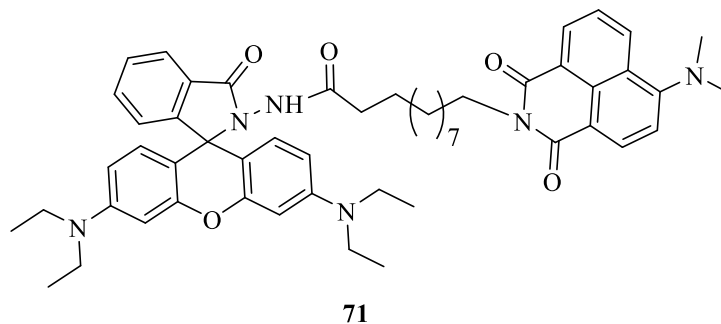
Xi *et al.* designed and synthesized probe **68** with 1-naphthylacetic moiety as the emission donor and dansyl group as the acceptor. FRET efficiency of free probe **68** has been found larger than 90%. Probe **68** displayed large emission shift from 500 nm to 338 nm after cleavage of ester bond between the two moieties.⁷⁶

Rhodamine-naphthalimide dyad **69** was synthesized by Mahato *et al.* and found to bind with Hg^{2+} or Cr^{3+} ions even in presence of excess of other competing ions, associated with changes in their optical and fluorescence spectral behavior. Dyad **69** showed absorption band at 455 nm and switched to 561 nm on the addition of Hg^{2+} or Cr^{3+} ions. These spectral changes

were significant in the visible region as the color change was observed from colorless to pink and allowed the naked-eye detection of these particular ions. The fluorescence spectrum showed maxima at 533 nm and shifted to 583 nm in the presence of Hg^{2+} or Cr^{3+} ions.⁷⁷



FRET based bichromophoric chemosensor **70** was designed by Bojinov *et al.* consisting of rhodamine 6G as acceptor and 1,8-naphthalimide as a donor. Compound **70** showed FRET phenomenon with sufficient overlap between the absorption spectrum of the acceptor (rhodamine) and the emission spectrum of the donor (naphthalimide). Chemosensor **70** showed an absorption band at about 400 nm-432 nm and at pH 5, and the appearance of the new band at 522-524 due to the opening of rhodamine ring. After excitation, donor emission decreased by 96-97% and acceptor emission increased by more than 33% which confirmed the FRET phenomenon at pH 5.⁷⁸

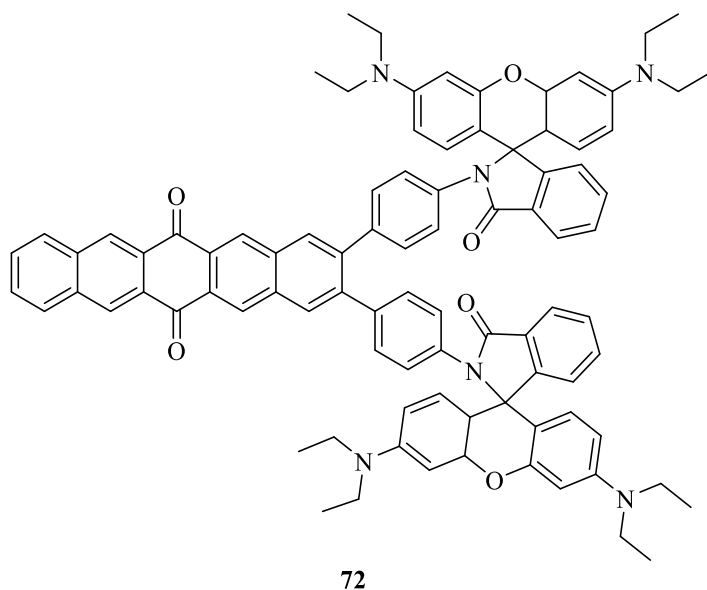


Wang *et al.* synthesized naphthalimide-rhodamine B derivative **71** as fluorescence *turn-on* chemodosimeter for the detection of Sn^{4+} ions. In the presence of Sn^{4+} ions, the absorption

band at 418 nm shifted to 555 nm with a color change from light yellow to red. Similar results were observed in the case of emission spectroscopy through emission color change from light yellow to orange and emission intensity changed from 523 nm to 580 nm. In addition, the compound was used as a fluorescent probe for the detection of Sn^{4+} ions in biological systems and might act as a tool to study the physiological functions of Sn^{4+} in the human body.⁷⁹

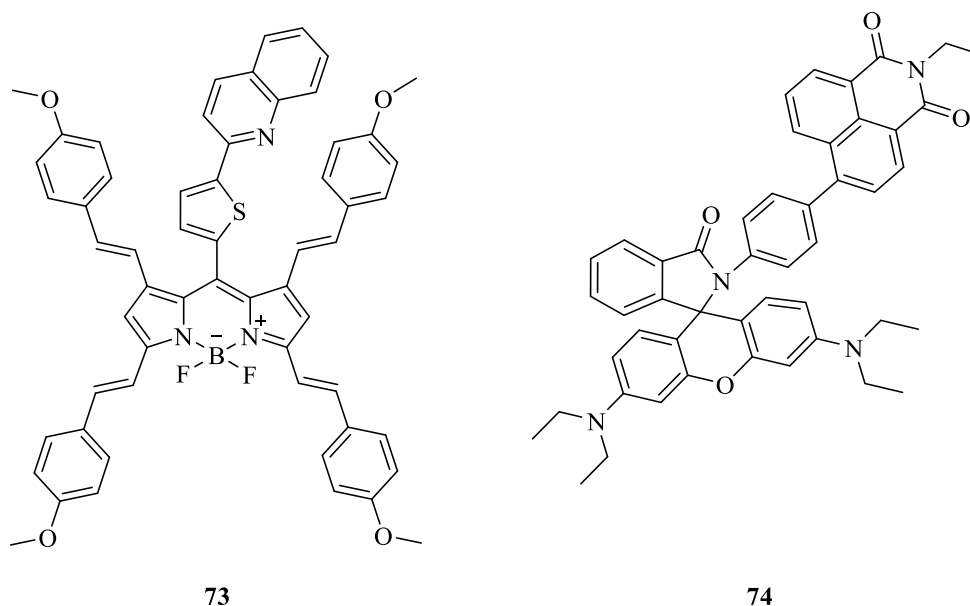
1.2.2 TBET based molecules

Bhalla *et al.* synthesized probe **72** by conjugate connection through an aryl group pentaquinone (donor) and rhodamine (acceptor) as through bond energy transfer (TBET) chemosensor. The mechanism was based on Hg^{2+} ions, induced spiro-ring opening. Probe **72** showed absorption bands at 275 nm and 322 nm. Upon addition of Hg^{2+} ions, the intensity of these bands increased and a new band appeared at 554 nm. The color change from colorless to pink facilitated the naked eye detection of Hg^{2+} ions. The solution of probe **72** when excited at 360 nm showed quenching in emission and on interaction with Hg^{2+} ions, a new band at 582 nm was observed. Biological application of probe **72** was explored for the detection of Hg^{2+} ions in prostate cancer (PC3) cell lines.⁸⁰



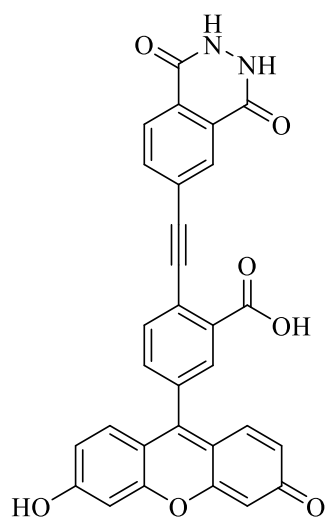
A new BODIPY based derivative **73** was synthesized by Qu *et al.* for the detection of Fe^{3+} ions selectively via TBET phenomenon. Probe **73** exhibited pseudo-Stokes' shift of 400 nm due to 99% energy transfer. Probe **73** showed absorption bands at 370 nm and 708 nm and an

emission band at 732 nm. Upon addition of Fe^{3+} ions, fluorescence quenching was observed due to binding with the donor entity in aqueous media. Probe **73** has been found practical application for the detection of Fe^{3+} ions in living cells.⁸¹

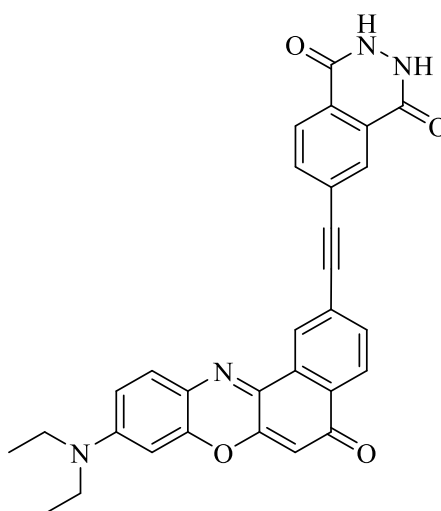


Kumar *et al.* synthesized a naphthalimide (donor) appended rhodamine (acceptor) based fluorescent probe **74** which showed through bond energy transfer (TBET) phenomenon in the presence of Hg^{2+} ions in semi aqueous media (THF: H_2O (99.5:0.5, v/v)). The probe **74** showed absorption bands at 320 nm and 363 nm. In the presence of Hg^{2+} ions, the intensity of the absorption band at 363 nm has increased with the emergence of a new band at 565 nm with a color change from colorless to pink. The fluorescence band was observed at 472 nm which underwent an increase with the appearance of a new band at 578 nm in the presence of Hg^{2+} ions.⁸²

Han *et al.* reported two cassettes **75** and **76** based on the fluorescein moiety that emitted at very long wavelengths. The energy transfer efficiency was determined to be 39% and 42% for cassettes **75** and **76**, respectively. Cassettes **75** and **76** showed absorption bands at 494 nm and 558 nm, respectively and emission bands at 518 nm and 628 nm, respectively. *In vitro* experiments showed that compounds **75** and **76** could be activated with peroxidase under physiological conditions.⁸³

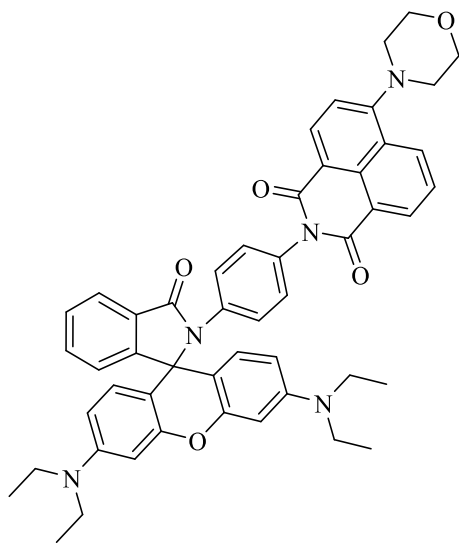


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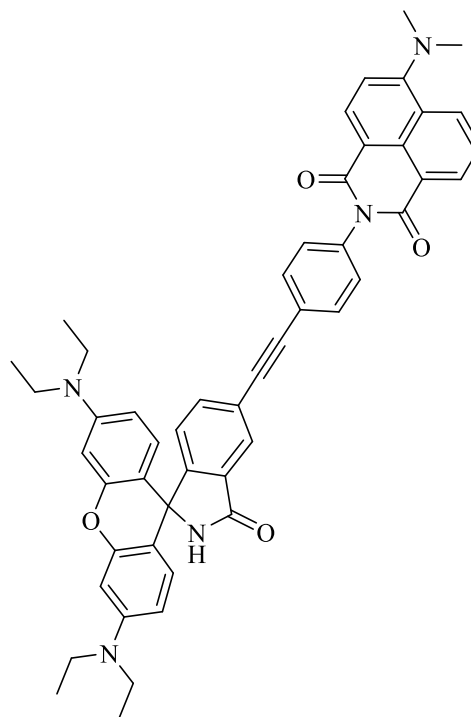


76

Wang *et al.* synthesized TBET based fluorescence, ratiometric chemosensor **77**. The absorption peak at 406 nm shifted to 562 nm and color change from yellow to pink was observed which facilitated naked eye detection of Fe^{3+} ions. The emission spectrum showed ratiometric behavior through shifting in wavelength from 535 nm to 586 nm. Probe **77** exhibited excellent selectivity towards Fe^{3+} ions through chelation of oxygen atom on the amide group in aqueous solution.⁸⁴

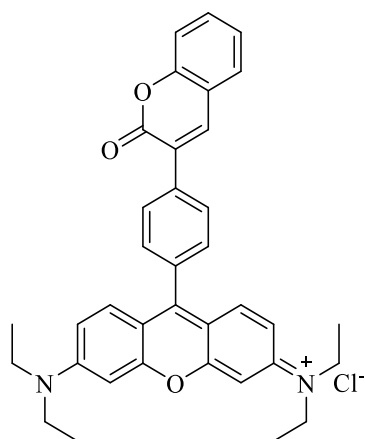


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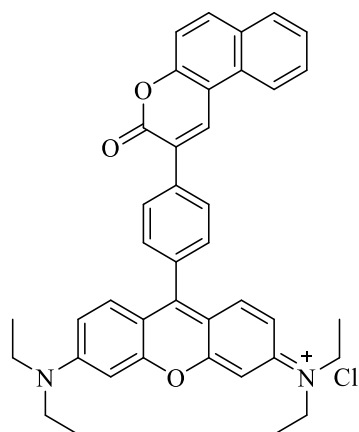


78

Fan *et al.* prepared rhodamine-naphthalimide based dyad **78**, exhibiting selective recognition of Cu^{2+} ions via hydrolysis and opening of the spirolactam ring of the rhodamine moiety in aqueous solution. Moreover, dyad **78** was used for the detection of Cu^{2+} ions in living cells. Dyad **78** showed a red shift in the absorption band of 115 nm from 431 nm to 546 nm with a color change from yellow-green to pink. The emission band showed characteristic change from 533 nm to 577 nm and attributed to TBET phenomenon in the presence of Cu^{2+} ions.⁸⁵



79

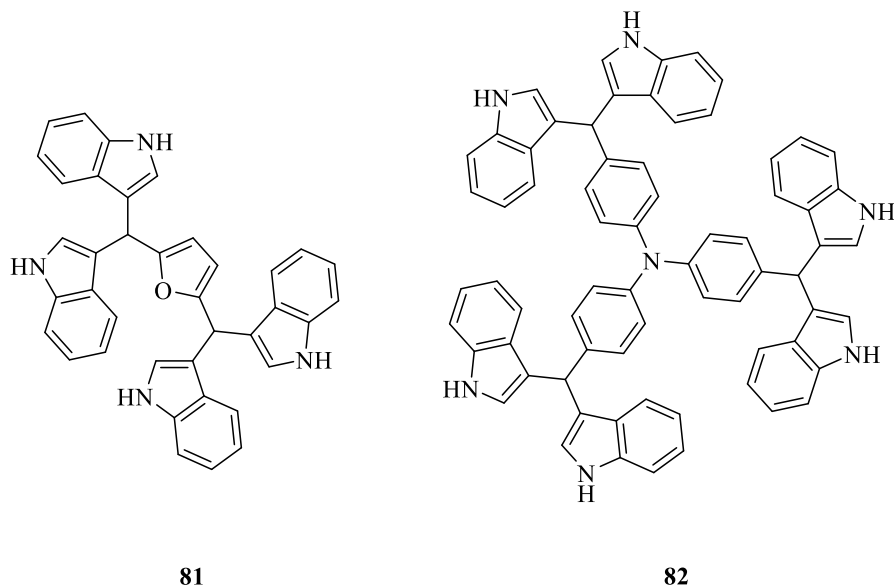


80

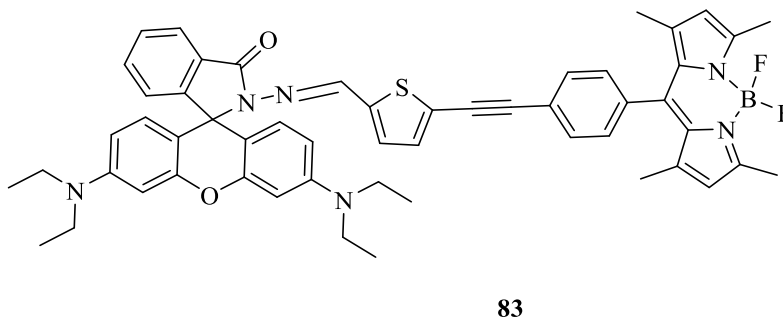
Lin *et al.* developed coumarin-rhodamine based TBET cassettes **79** and **80** having dual fluorescence ratiometric behavior with minimum spectral overlap between acceptor absorption and donor emission. Both of these cassettes exhibited large pseudo-Stokes' shifts of about 230 nm and emission shifts to about 170 nm. These cassettes were used for biological applications as having cell-membrane-permeability. These cassettes functioned in a wide pH range from 3.0 to 8.6. The emission ratio I_{587}/I_{465} changed from 46.5 at pH 3.0 to 0.2 at pH 8.6.⁸⁶

Sain *et al.* synthesized two receptors **81** and **82**, which exhibited remarkable colorimetric changes towards HSO_4^- ions in the mixed aqueous medium. In the presence of HSO_4^- ions, receptor **81** showed quenching at 290 nm and 282 nm and showed an increase in intensity at 513 nm (color change from yellow to red) while receptor **82** showed enhancements at 492 nm and 535 nm (color change from yellow to pink). Receptor **81** showed a quenched emission spectrum while **82** showed reversible enhancement with HSO_4^- ions. ^1H NMR experiments supported the TBET phenomenon for the complexation of HSO_4^- with both receptors. Both receptors exhibited 'on the spot' color change from yellow to pink towards the toxic HSO_4^- ions. HSO_4^- ions showed

toxic behavior as high doses of sulfate ions generated from HSO_4^- ions, lead to diarrhoea, irritation of the skin and eyes and respiratory paralysis.⁸⁷

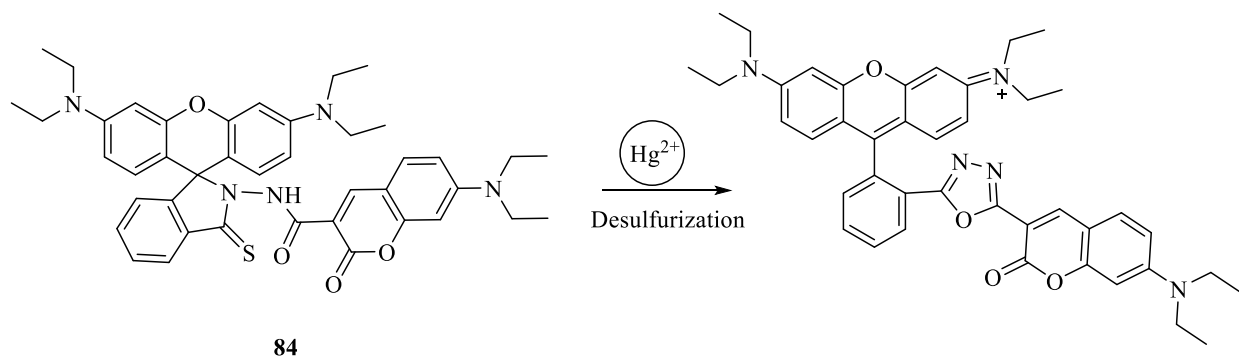


Cherreddy *et al.* synthesized novel BODIPY-rhodamine dyad **83** operating through TBET phenomenon and its utility for the selective detection of trivalent metal ions. Dyad **83** produced remarkable absorption and fluorescence changes only with Al^{3+} , Cr^{3+} , and Fe^{3+} metal ions and allowed ratiometric detection at ppb levels. Dyad **83** showed two absorption bands at 374 nm and 499 nm and interaction of trivalent cations induced a new band at 530 nm. In the presence of Al^{3+} , Cr^{3+} and Fe^{3+} ions, the ligand band at 517 nm was quenched and a new band formed at 585 nm. Reversibility was achieved with EDTA. Dyad **83** was also applicable for the imaging of Al^{3+} , Cr^{3+} and Fe^{3+} ions present in W138 cells.⁸⁸



Gong *et al.* reported TBET based probe **84** for the selective determination of Hg^{2+} ions as this probe promoted desulfurization reaction. Through the combination of TBET mechanism

with dual-switch sensing, probe **84** displayed two emission peaks with a difference of 110 nm and afforded selective detection of Hg^{2+} ions. Probe **84** showed a peak at 420 nm which shifted to 450 nm with the emergence of a new peak at 567 nm due to the ring opening of rhodamine (Scheme 1.1.7). A color change from yellow to red was observed which facilitated the naked eye detection of Hg^{2+} ions. Probe **84** showed an emission band at 470 nm but on the addition of Hg^{2+} ions, the emission intensity at 470 nm decreased and a new band was formed at 580 nm due to the opening of the rhodamine-moiety. Probe **84** could be used for the ratiometric imaging of Hg^{2+} in biological samples.⁸⁹



Scheme 1.1.7: Sensing mechanism of probe **84** with Hg^{2+} ions.

1.3 Summary of strengths and weaknesses of the review of literature

After extensive survey of the complete literature, it has been found that NBD based molecules having NBD moiety (receptor) or NBD based dyads (interplaying mechanisms as ICT, FRET, TBET, PET, ESIPT etc.) were used to detect various heavy metal ions such as Hg^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Pd^{2+} , Ni^{2+} etc. and anions such as S^{2-} , CN^- , H_2S , F^- etc. These were mainly used to study photophysical response, and their applications as bioimaging agents. In addition, these dyads with different sensing mechanisms have been improving the selectivity, the sensitivity, and the signal-to-noise ratio. Further other part of review of literature described different dyads based on FRET/TBET. Main focus on the dyads is the contribution of different linkers between the energy donor and acceptor according to FRET and TBET. If energy transfer through-space, then the donor chromophore, and acceptor fluorophore was connecting through a proper linker. If energy transfer through-bond, the donors are connected to acceptors via electronically conjugated linkers, which prevent the donor and acceptor fragments from being planar. There were numerous examples have been discussed in the review to channelize the FRET/TBET. It includes

the system of BODIPY–rhodamine, rhodamine–coumarin, rhodamine–fluorescein, rhodamine–naphthalimide, rhodamine–naphthalene, rhodamine–isoquinoline and rhodamine–dansyl, rhodamine–pentaquinone, fluorescein, rhodamine, naphthalimide with other molecules and so on. Their applications are for the detection of various metal ions and anions.

Based on above depiction of literature survey, we find some lacuna in the present study. It has been found that there are several examples having NBD as signalling unit but scarce examples are present based on NBD dyads. A few chemosensors have been explored for applicability of visible color change for the detection of various analytes. These kind of dyads could be used further as a tool kit for detection of various analytes in wide pH range. They can be applicable for detecting multiple analytes and for the fabrication of smart logic gates with multi-inputs and multi-outputs. Thus, such dyads would provide the ample opportunities for the generation of ratiometric fluorescent sensors for various cations anions and biological ions at the distinctly different wavelength.

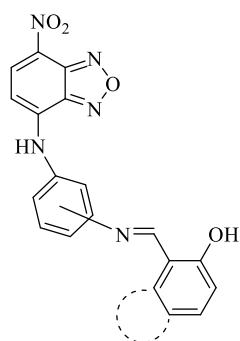
FRET/TBET based dyads are not much studied for the detection of multiple ions differentially, which can be boon for sensing various ions. Such mechanism based cassettes could be feasible for practical use such as ions detection in real samples, protein labelling, and organelles localization. Since there are advantages of fluorescent ratiometric measurement at two wavelengths, these dyads will become useful for quantification of ions. We consider that even more wonderful dyads based on NBD/FRET/TBET should be developed to satisfy different applications. Moreover, there are a large number of literature reports of chemosensors based on thiazole and its derivatives.⁹⁰ They are used as excellent chemosensors for sensing of various ions. Inspired from this, it was observed that thiazolidine scaffolds have attracted continuing attention in medicinal chemistry.⁹¹

Keeping in mind the above points, the following **objectives** have been designed.

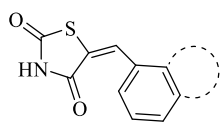
1. Synthesis of NBD based dyads to explore the chemosensing properties for detection of multiple ions.
2. Synthesis of hybrid molecules based on thiazolidine moiety to explore combined effect of sensing and quantification of optical signals for generation of molecular logic gates.
3. Synthesis of the dyads through combinations of various fluorescent dyes in conjugation or through some spacers and exhibiting FRET/TBET phenomenon for sensing of cations, anions, and biological ions.

1.4 Overview of designed work

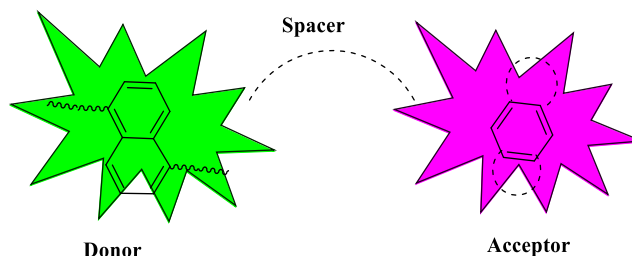
Optical sensors based on NBD, thiazolidine, FRET/TBET dyads operating *via* different mechanism have been synthesized as they have auspicious future. There were many nominal problems in the designing of these sensors which are trying to be overcome by this research work, which includes the considerations through different models. NBD based dyads through Schiff bases have been synthesized as colorimetric and fluorescent chemosensors (Model 1). These dyads are used to quantify Cu^{2+} , Ni^{2+} , Al^{3+} , Hg^{2+} , CN^- ions in organic and semi-aqueous solvents depending upon the dyad. Then, thiazolidine based molecules have been synthesized as novel sensors to sense Cu^{2+} , F^- , CN^- , H_2PO_4^- , HSO_4^- , Hg^{2+} , Al^{3+} etc. in different solvents (Model 2). These molecules further elaborated as logic devices. In the last, rhodamine-naphthalimide dyad operating *via* FRET and phenanthroimidazole-anthraquinone dyad operating *via* TBET has been synthesized and used for the investigations of photophysical properties in the presence of various ions (Model 3). These designed chemosensors in the present work used for the ion quantification, the ability to operate in water, having sufficient lifetime and sensitivity at wide pH range, recognize the various analytes in the aerobic and aqueous medium. Moreover, the design and synthesis of inexpensive and disposable sensor such as sensor strips have been highly desired. NBD and thiazolidine based sensors display significant color changes in the presence of particular ion solution, therefore, used as sensor strips. These optical sensors represented by three models; show high selectivity for a particular ion in the presence of other ions of similar nature. Interestingly, these sensors could detect small concentrations of the analyte of interest in the presence of other interfering ions, is of considerable interest.



Model 1



Model 2



Model 3

Models for the present study

Whole thesis is categorized in different chapters and sub-chapters as depicted in the following flowchart.

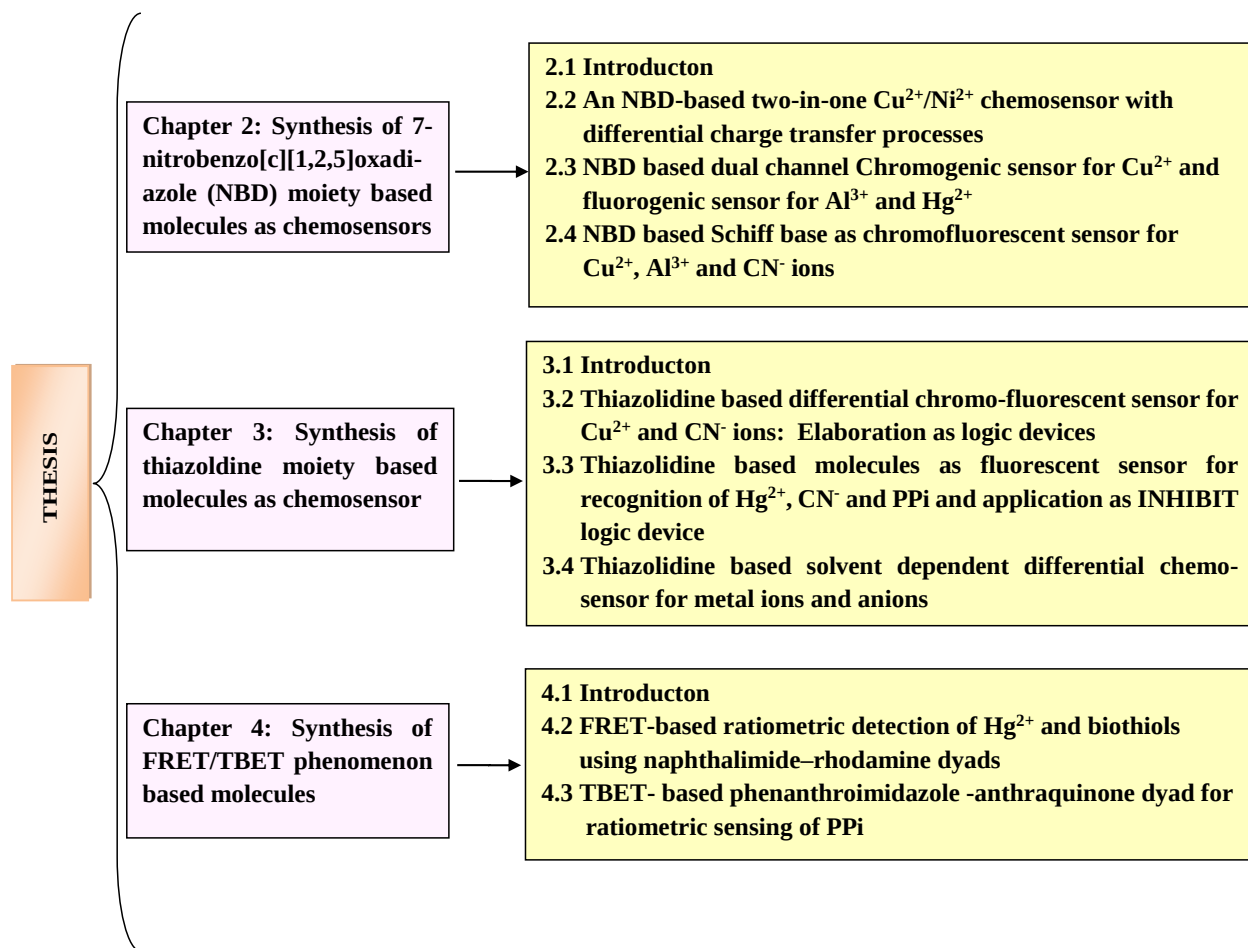


Figure 1.1: Flowchart of Thesis

Materials and Methods

The materials and methods were used to synthesize molecules and further studied their ion recognition through various spectroscopic techniques. Different materials and methods well used in chapters 2-4 and 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 was monitored by Thin Layer Chromatography. Thin Layer Chromatographic plates were made by coating Silica gel GF 254 on glass plates by using chloroform and methanol as solvents. All reactions were monitored through TLC experiment and seen under UV light. Different solvent systems (elutents) as mobile phase were used based on the polarity of the compounds. Melting points were carried out using the open capillary tube method and were uncorrected.
- ❖ **Characterization of compounds:** All the synthesized compounds were fully characterized by the NMR and mass spectrometry techniques. Deuterated solvents were used for NMR analysis with TMS as an internal standard. ^1H NMR and ^{13}C NMR spectra were carried out using JEOL ECS-400 MHz spectrometer in SAI Labs, Thapar University, Patiala, and Bruker- 400 MHz in SAIF, Punjab University, Chandigarh. All chemical shifts are reported in ppm relative to the TMS as an internal reference. Mass spectra of the synthesized compounds were recorded at Waters Micromass Q-Tof Micro 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–Visible 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 analytical/HPLC grade solvents. Tetrabutylammonium salts were used for the anions and perchlorate/nitrate salts in case of cations. Solutions were typically 20 μM or 10 μM for UV–Visible studies. The solution was placed in a quartz cell and its UV-Visible 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-Visible 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 spectrum was recorded. The addition of different concentrations of ion were 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/Equilibrium constant calculation:** Stability constants were determined using Benesi–Hildebrand Equation (Eq. (1))

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

where A_0 , A , $A_{\max}$ are the absorption in the absence of ion, at an intermediate, and at saturation. $[F^-]$ is a concentration of an ion, n is the stoichiometric ratio, K is binding constant, The association constant (K) could be calculated 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.

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

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 using analytical grade CH_3CN with 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).

$$\Phi_{fs} = \Phi_{fr} \times \frac{1 - 10^{-A_r L_s}}{1 - 10^{-A_s L_r}} \times \frac{N_s^2}{N_r^2} \times \frac{D_s}{D_r} \quad \dots \dots \dots (2)$$

- ❖ **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. 2 μL solution of a different concentration of analyte was added to this coated TLC plates and then observed under UV light.

- ❖ **Job's Plot:** To study the stoichiometry of probes with different analytes, Job's plots were plotted. The absorbance/emission in each case with different *host-guest* ratio but equal in volume was recorded. Job's plot was drawn by plotting $\Delta I \cdot X_{\text{host}}$ vs X_{host} (ΔI = change of intensity of the absorbance/emission 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.
- ❖ **Absorption and emission pH titrations:** Effect of pH on absorption and emission spectrum of probes and metal/anion complexes were studied by using an Equiptronics pH meter. The pH of the solutions was adjusted (pH = 2 to 13) using different concentrations of acids/base 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.
- ❖ **General procedure for the elaboration of logic operations:** UV-Visible studies were carried out on 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 a quartz cell and its UV-Visible 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-Visible or fluorescence spectrum.
- ❖ **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 the same hybrid functional and basis set. The low-lying first singlet excited state (S_1) of the different probes was relaxed 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.
- ❖ **Interference limits:** (i) Level of allowed interference for any interfering ion with particular analyte up to 30% was considered as a threshold; (ii) Secondly level of competitor concentration

was varied according to the capacity of interfering ion with any analyte up to which it can tolerate the interference from competitor ions. As in mostly cases, competitor concentration was 50, 100, 1000 times stronger than analyte but in some rare case as with dyad **15**, it was of equal strength.

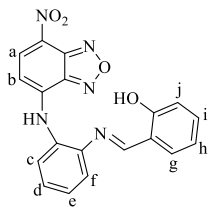
CHAPTER 2

SYNTHESIS OF 7-NITROBENZO[C][1,2,5]OXADIAZOLE (NBD)

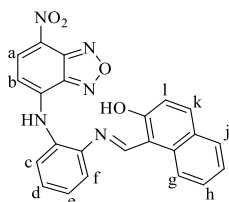
MOIETY BASED MOLECULES AS CHEMOSENSORS

2.1 Introduction

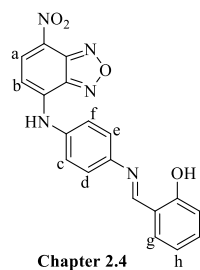
7-Nitro-1,2,5-benzoxadiazole (NBD) is an extensively used fluorescent probe in biochemical, biophysical and cell biological studies.⁹² It has many biological applications viz., labeling agent for proteins, fluorescent tagging reagent, molecular imaging agent etc.⁹³ NBD based molecular systems give fluorescence in the visible range, and show fluorescence lifetime sensitivity towards polar solvents.⁹⁴ This is due to the fact that NBD could covalently attach to polar lipids and thus NBD-labeled lipids have spectroscopic and microscopic applications. Furthermore, NBD exhibits emission at a longer wavelength and has some hydrophilic nature due to the presence of electron withdrawing groups and hetero atoms (N and O).⁴⁰ The presence of conjugated groups causes a change in emission intensity from weak to strong. These characteristics of NBD gives the opportunity to be used in biological systems as well as in analytical chemistry as a chemosensor.⁴¹ Therefore, such molecular systems are significantly used to investigate various ions such as metal ions, anions, inorganic/organic molecules, biological molecules etc.^{47,48} Additionally, Schiff bases with π electrons in the C=N group and nitrogen in the aromatic ring can offer a good possibility for chelation with metal ions.^{68,75} The chelation to metal ions would enhance ICT (intra-molecular charge transfer) transitions or make LMCT (ligand to metal charge transfer) transitions, which are utilized for the detection of metal ions. In this chapter, we describe the synthesis of NBD based derivatives with *o*-phenylenediamine and *p*-phenylenediamine in a combination with 2-hydroxybenzaldehyde and 2-hydroxy-1-naphthaldehyde, linked through Schiff bases. These dyads were used for study the photophysical behavior towards different metal ions and anions.



Chapter 2.2



Chapter 2.3



Chapter 2.4

These dyad combinations are discussed as per following subchapters:

2.2 An NBD-based two-in-one $\text{Cu}^{2+}/\text{Ni}^{2+}$ chemosensor with differential charge transfer processes.

2.3 NBD based dual channel chromogenic sensor for Cu^{2+} and fluorogenic sensor for Al^{3+} and Hg^{2+} .

2.4 NBD based Schiff base as a chromofluorescent sensor for Cu^{2+} , Al^{3+} , and CN^- ions.

2.2 An NBD-based two-in-one Cu²⁺/Ni²⁺ chemosensor with differential charge transfer processes

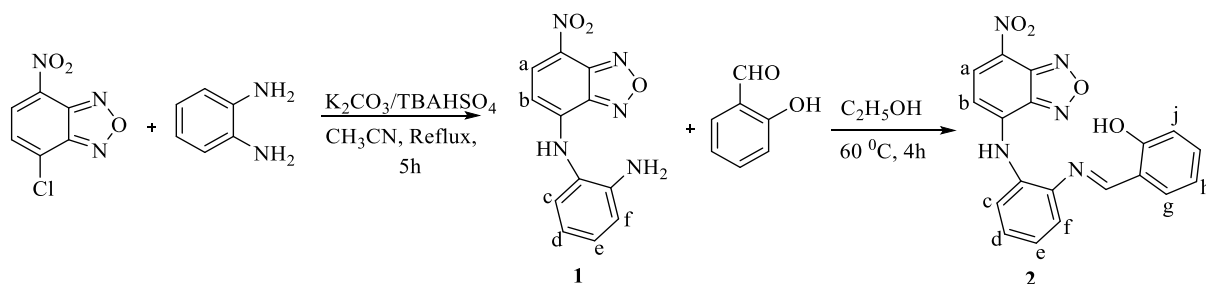
2.2.1 Abstract

A new multifunctional NBD-based dyad **2** has been developed and their photophysical behavior towards cations was investigated. Dyad **2** showed high sensitivity and selectivity towards Cu²⁺/Ni²⁺ over other metal ions over a wide pH range. The dyad **2** could be used for the simultaneous estimation of Cu²⁺ and Ni²⁺ ions through its visible color change from yellow to blue and light green, respectively, in semi-aqueous (CH₃OH:H₂O (1:1,v/v)1:1) system. The binding mode of dyad **2** to Cu²⁺ and Ni²⁺ was determined to be a 1:1 complexation stoichiometry using a Job's plot. Ethylenediamine tetraacetate (EDTA) showed high selectivity towards the *in situ* prepared Cu²⁺ complex over the Ni²⁺ complex, which was applied to distinguish Ni²⁺ and Cu²⁺ ions. The detection limits for Cu²⁺ (1.8×10⁻⁷ M) and Ni²⁺ ions (1.1×10⁻⁶ M), were lower than those recommended by the WHO guidelines for drinking water. Moreover, dyad **2** could be used as a practical, visible colorimetric test kit for Cu²⁺ ions. The sensing mechanisms of Cu²⁺ and Ni²⁺ ions were supported by DFT-calculations.

2.2.2 Synthesis of compounds **1** and **2**

4-Chloro-7-nitro-1,2,5-benzoxadiazole (NBD-Cl) and *o*-phenylenediamine in the presence of K₂CO₃ and TBAHSO₄ in acetonitrile were refluxed for 5 h and monitored through TLC. After the completion of the reaction, acetonitrile was distilled off and the residue was extracted using chloroform and water. The crude product was purified by column chromatography to get the pure product **1** in 70% yield as a reddish brown solid; M.pt. = 220 °C; MS (ESI) *m/z* 272.1 (M⁺ + H) (Scheme 2.2.1). ¹H NMR spectrum in CDCl₃ + DMSO-*d*₆, showed 1H singlet at δ 9.90 due to NH, 1H multiplet at δ 8.42 due to H_a, 2H multiplet at δ 7.20 due to H_a and, 1H multiplet at δ 6.92 due to H_{b,e}, 1H multiplet at δ 6.81 due to H_f, 1H multiplet at δ 6.10 due to H_d. The peak at δ 9.90 was due to NH, confirmed the formation of compound **1**. ¹³C NMR spectrum showed signals at δ 144.2, 143.7, 143.2, 142.6, 136.2, 128.6, 126.7, 123.0, 121.6, 117.8, 116.5, 100.8 for aromatic-C. On the basis of these spectral data, the compound N¹-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)benzene-1,2-diamine was assigned to the structure of compound **1**.

The dyad **2** was synthesized by stirring of compound **1** and 2-hydroxybenzaldehyde in ethanol at 60 °C for 4 h. Solid separated was filtered and washed with ethanol to get pure dyad **2** as a yellow solid in 80% yield; M.pt. = 220 °C; MS (ESI) m/z 376.3 ($M^+ + H$) (Scheme 2.2.1). ^1H NMR spectrum showed 1H singlet at δ 12.49 due to imine-CH, 1H singlet at δ 10.44 due to NH, 1H singlet at δ 8.71 due to H_a , 1H doublet at δ 8.42 due to H_b , 1H multiplet at δ 7.53-7.51 due to H_e , 4H multiplet at δ 7.49-7.35 due to H_{g-j} , 1H multiplet at δ 6.96-6.94 due to H_c , 1H doublet at δ 6.87 due to H_f , 1H doublet at δ 6.33 due to H_d . The peak at δ 12.49 was due to imine-CH, confirm the formation of Schiff base. ^{13}C NMR spectrum showed signals at δ 195.3 for imine-C, 164.1, 160.6, 144.6, 144.2, 143.4, 136.7, 133.6, 132.7, 131.3, 129.0, 127.9, 127.1, 123.4, 119.8, 119.1, 116.9, 101.5 for aromatic-C. The peak at δ 195.3 was due to the formation of Schiff base. On the basis of spectral data, the compound was assigned to the structure of 2-(((2-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)phenyl)imino)methyl)phenol (dyad **2**).



Scheme 2.2.1: Synthesis of compounds **1** and **2**

2.2.3 Results and discussion

2.2.3.1 Sensing behavior of dyad **2** towards various metal ions

To evaluate the binding affinity of dyad **2** towards different metal ions, UV-visible and fluorescence studies were performed in $CH_3OH:H_2O$ (1:1, v/v) at pH 7.2 (HEPES buffer, 10^{-2} M). The absorption spectrum of dyad **2** (20 μ M, $CH_3OH:H_2O$ (1:1, v/v)) is characterized by two bands at 340 nm and 475 nm. Upon addition of 1000 μ M of various metal ions such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Hg^{2+} , Pb^{2+} , Al^{3+} etc., as their perchlorate salts, to dyad **2** (20 μ M, $CH_3OH:H_2O$ (1:1, v/v) HEPES buffer), no significant visible or spectral change was observed except for Cu^{2+} and Ni^{2+} ions. The presence of Cu^{2+} and Ni^{2+} ions showed the visible color change from yellow to blue and light green, respectively (Figures 2.2.1 and 2.2.2a).

Upon incremental addition of Cu^{2+} ions to the solution of dyad **2** (20 μM), the absorption intensity of the bands at 477 nm and 340 nm decreased and concomitantly new absorption bands at 585 nm and 409 nm reached a maximum at 1.0 equiv. of Cu^{2+} ions. Four clear isosbestic points were observed at 315 nm, 365 nm, 465 nm and 512 nm, indicating that only one product

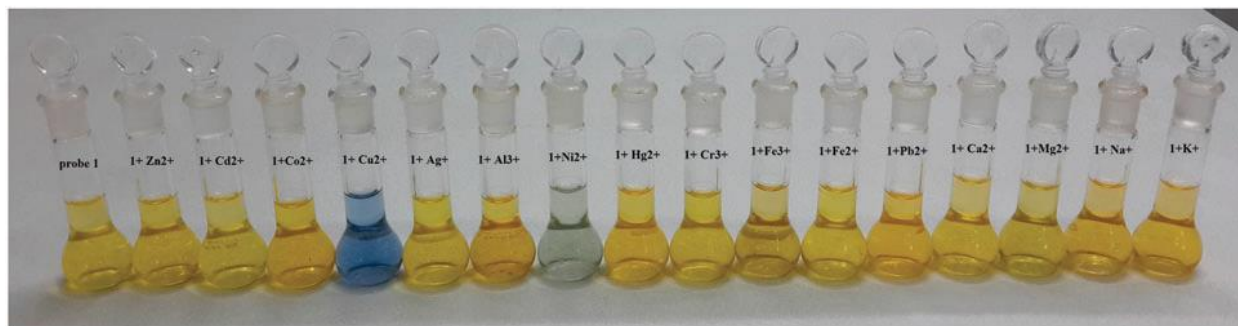


Figure 2.2.1: Visible color changes on addition of various metal ions to dyad **2**.

was generated from dyad **2** upon binding to Cu^{2+} ions (Figure 2.2.2b). The ratios of absorption intensities at 585 nm and 475 nm (A_{585}/A_{475}) vary from 0.0167 to 3.0, showing a 179-folds ratiometric change. Thus, dyad **2** can be used to quantify 0.1–8 μM of Cu^{2+} ions. Job's plot analysis showed a 1:1 stoichiometry of Cu^{2+} to dyad **2** (Appendix-A, Figure A1). Based on the UV-Visible titration of dyad **2** with Cu^{2+} , the association constant (K) of **2** with Cu^{2+} was calculated from the spectral curve fit of the data using Specfit 32. The K value was determined to be $3.62 \pm 0.01 \times 10^6 \text{ M}^{-1}$.

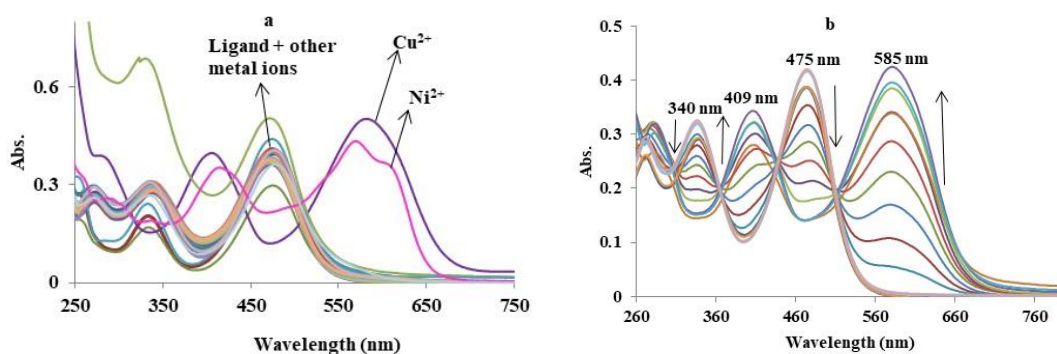


Figure 2.2.2: (a) UV-Visible spectra in the presence of other metal ions; (b) Absorption spectra of dyad **2** (20 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1, v/v), HEPES buffer) upon complexation with increasing concentrations of Cu^{2+} ions.

To further check the practical applicability of dyad **2** as a Cu^{2+} selective sensor, competition experiments were performed (Figure 2.2.3). Upon addition of 1.0 equiv. of Cu^{2+} ions in the presence of 100 equiv. of other metal ions, there was no obvious interference from the other metal ions. Thus, **2** could be used as a selective colorimetric sensor for Cu^{2+} ions in the presence of most competing metal ions. Fe^{3+} inducing 25% drop in absorbance but it was significant as 30% interference was considered threshold.

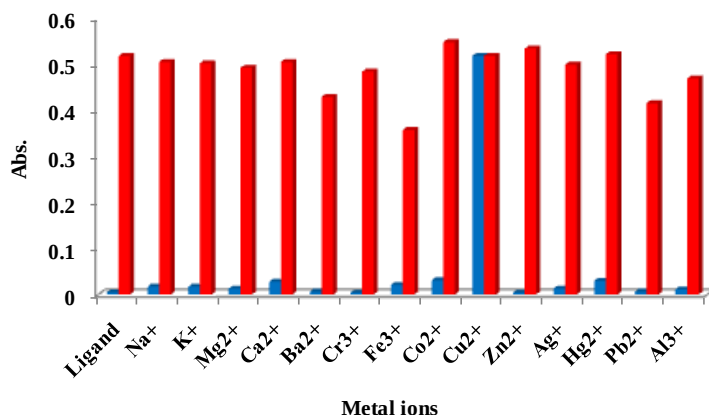


Figure 2.2.3: Blue bars show the effect of different metal ions on absorption response of dyad **2** (20 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1, v/v)) and red bars represent absorption response of dyad $2 \cdot \text{Cu}^{2+}$ (20 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1, v/v)) at 585 nm in the presence of various interfering metal ions (1 mM).

The effect of pH on the absorption response of **2** to Cu^{2+} ions in a series of solutions with pH values range from 2.5 to 13.0 was explored. The color of the $2 \cdot \text{Cu}^{2+}$ complex and the absorption changes remained unaffected between pH 4.0 and 12.0. These results indicated that Cu^{2+} could be clearly detected using dyad **2** over a wide pH range of 4.0–12.0 (Figure 2.2.4).

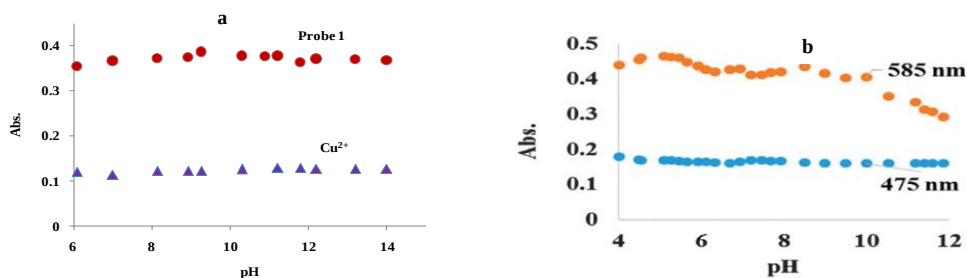


Figure 2.2.4: (a) The effect of pH on the absorption of dyad **2** (475 nm) and dyad $2 \cdot \text{Cu}^{2+}$ (20 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1, v/v), 585 nm); (b) absorption changes (585 nm and 475 nm) of the dyad $2 \cdot \text{Cu}^{2+}$ complex at different pH values (4–12).

Upon incremental addition of Ni^{2+} ions to dyad **2** (20 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1, v/v), HEPES buffer, pH = 7.0), the intensity of the absorption band at 475 nm decreased and a new absorption band was formed at 575 nm with a strong shouldered band at 607 nm associated with a visible color change from yellow to light green (Figure 2.2.5a). Four clear isosbestic points were observed at 300 nm, 370 nm, 445 nm and 505 nm, indicating that only one product was generated from dyad **2** upon binding to Ni^{2+} . The ratios of absorption intensities at 575 and 475 nm (A_{575}/A_{475}) vary from 0.0158 to 2.25 and showed a 142-folds ratiometric change on addition of Ni^{2+} ions (Figure 2.2.5b). Job's plot analysis showed a 1:1 stoichiometry for Ni^{2+} to dyad **2** (Figure A2). The association constant was calculated based on UV-Visible titration by using the Benesi–Hildebrand equation/spectral curve fit data. The K value was determined to be $3.03 \pm 0.01 \times 10^6 \text{ M}^{-1}$. The detection limit ($3\sigma/k$) of dyad **2** was found to be $1.1 \times 10^{-6} \text{ M}$, which was much lower than that recommended by the WHO for drinking water.

To further check the practical ability of dyad **2** as a Ni^{2+} sensor, competitive metal ion experiments were performed in the presence of 1.0 equiv. of Ni^{2+} and 50 equiv. of other interfering metal ions like Na^+ , K^+ , Ba^{2+} , Mg^{2+} , Ca^{2+} , Co^{2+} , Al^{3+} , Ag^+ , Hg^{2+} , Fe^{3+} , Cr^{3+} , Pb^{2+} , Zn^{2+} etc., as their perchlorate salts, and no significant interference was observed. However, there was some interference in the detection of Ni^{2+} , which was 10% from Co^{2+} and 12% from Fe^{3+} at higher equivalents (Figure 2.2.6). Nevertheless, the changes of color were still detectable in the presence of these interfering metal ions.

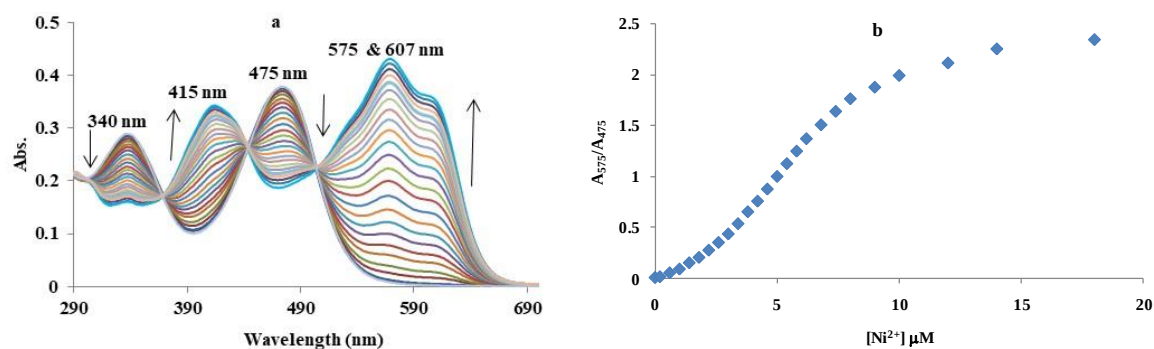


Figure 2.2.5: (a) Absorption spectra of dyad **2** (20 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1, v/v), HEPES buffer) upon complexation with increasing concentrations of Ni^{2+} ions; (b) ratiometric plot for the analysis of Ni^{2+} ions.

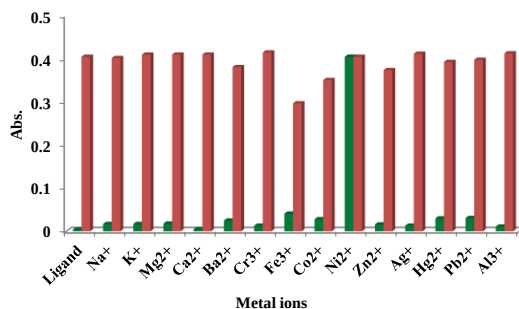


Figure 2.2.6: Green bars show the effect of different metal ions on absorption response of dyad **2** (20 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1, v/v)) and red bars absorption response of dyad **2**· Ni^{2+} (20 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1, v/v)) at 575 nm in the presence of various interfering metal ions (1 mM).

The excitation of dyad **2** (20 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1, v/v), HEPES buffer) at 340 nm showed a very weak emission. Upon addition of various metal ions like Na^+ , K^+ , Ba^{2+} , Mg^{2+} , Ca^{2+} , Co^{2+} , Al^{3+} , Ni^{2+} , Ag^+ , Hg^{2+} , Fe^{3+} , Cu^{2+} , Cr^{3+} , Pb^{2+} , Zn^{2+} etc., as their perchlorate salts, no significant change was observed except the presence of Al^{3+} ions (Figure 2.2.7a). Upon incremental addition of Al^{3+} to the solution of dyad **2**, the emission was *turned-on* at 485 nm and achieved a plateau after 50 μM of Al^{3+} ions due to the chelation enhanced fluorescence (CHEF) effect (Figure 2.2.7b). The Cu^{2+} and Ni^{2+} , due to the paramagnetic effect, showed emission quenching, which could not be determined due to the weak emission of dyad **2**. To check the practical ability of dyad **2** as a selective Al^{3+} sensor, we carried out competitive experiments in the presence of 10 μM of Al^{3+} and 1 mM of other interfering metal ions like Na^+ , K^+ , Ba^{2+} , Mg^{2+} , Ca^{2+} , Co^{2+} , Al^{3+} , Ni^{2+} , Ag^+ , Hg^{2+} , Fe^{3+} , Cu^{2+} , Cr^{3+} , Pb^{2+} , Zn^{2+} etc., as their perchlorate salts, and no significant interference was observed as shown in Figure 2.2.8. Fe^{3+} has been inducing 30% drop in absorbance but it was significant as 30% interference was considered threshold.

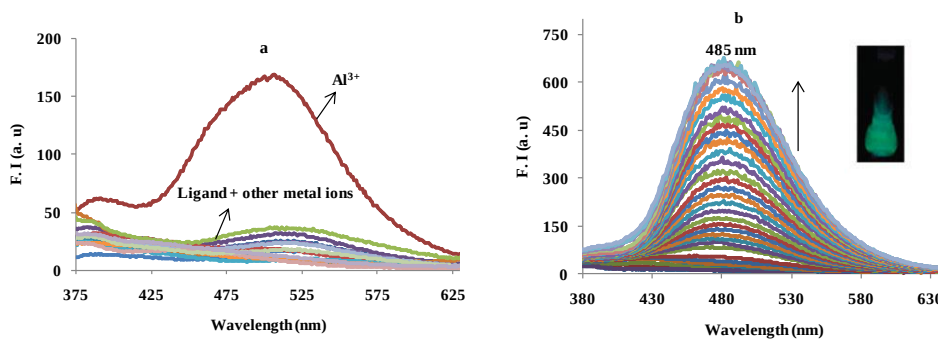


Figure 2.2.7: (a) Fluorescence response of dyad **2** (20 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1, v/v)) in the presence of various metal ions (50 equiv.); (b) fluorescence titration of Al^{3+} ions on excitation at 340 nm.

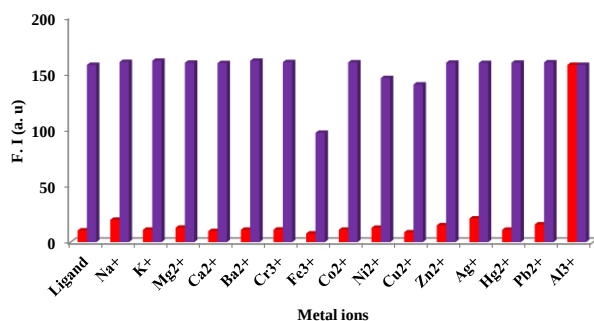


Figure 2.2.8: Red bars show the effect of different metal ions on emission response of dyad 2 (20 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1, v/v)) and purple bars represent emission response of dyad 2· Al^{3+} (20 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1, v/v)) in the presence of various interfering metal ions (1 mM).

2.2.3.2 Reversible binding of dyad 2

To examine the reversibility of the binding of dyad 2 to Cu^{2+} and Ni^{2+} ions, experiments were performed in the presence of EDTA. Upon addition of an aqueous solution of EDTA (2 equiv.) to the 2· Cu^{2+} complex, the absorption band at 585 nm disappeared and simultaneously reappearance of the absorption maximum at 475 nm was observed. Again, on the addition of Cu^{2+} to this solution mixture, having EDTA, the absorption band at 585 nm reappeared. The preferential binding of EDTA to Cu^{2+} led to the formation of Cu-EDTA and regeneration of dyad 2, confirming the reversible binding of dyad 2 to the Cu^{2+} ions (Figure 2.2.9a). This is attributed to the fact that binding constant for EDTA with Cu^{2+} is 6.3×10^{18} .

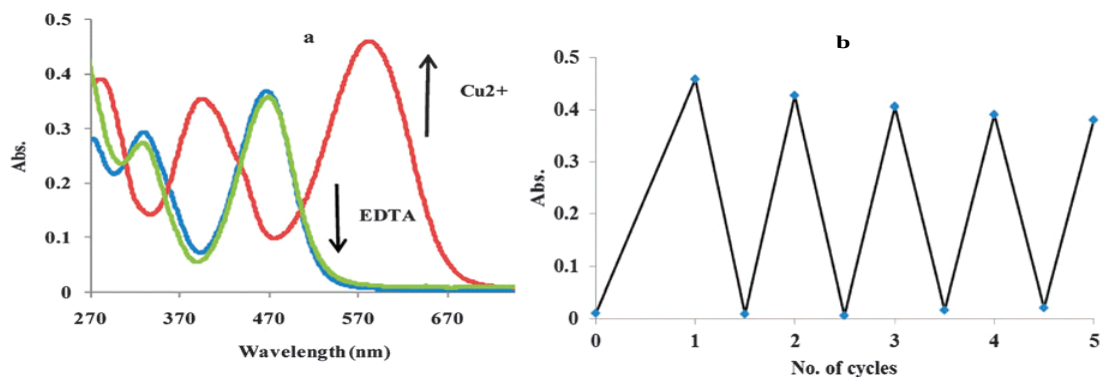


Figure 2.2.9: The absorption response showing the reversibility of (a) Cu^{2+} (10 μM) coordination to dyad 2 by EDTA (20 μM); (b) the switchable behavior in the absorbance at 585 nm by the alternate addition of Cu^{2+} and EDTA to dyad 2.

Interestingly, the alternate addition of Cu^{2+} and EDTA to dyad **2** gave rise to a switchable change in the absorbance at 585 nm and can be repeated more than 5 times (Figure 2.2.9b). However, no absorption reversal was observed when EDTA was added to the **2**· Ni^{2+} complex. This result indicated that EDTA is not able to remove Ni^{2+} from the **2**· Ni^{2+} complex and hence cannot restore the absorption of dyad **2**·EDTA even binding constant for EDTA with Ni^{2+} is 3.9×10^{18} . This fact was explained with the help of theoretical calculations. Therefore, dyad **2**·EDTA has high selectivity towards the *in situ* prepared Cu^{2+} complex over the Ni^{2+} complex, which can be applied to distinguish Ni^{2+} and Cu^{2+} .

2.2.3.3 Practical applicability

To further investigate the practical application of dyad **2**, test kits were prepared by immersing Whatman filter papers into the solution of dyad **2** and were dried under vacuum. The spots of different concentrations of the Cu^{2+} solutions on the paper strips showed the visible color change to blue. Thus, these paper strips were used for the sensing of copper ions (Figure 2.2.10). Moreover, another experiment was performed to show the application of this dyad in which the dyad **2** solution was adsorbed onto a silica gel 60–10 mesh. The presence of different concentrations of Cu^{2+} ions is the reason of the drastic color changes. Thus, this dyad can be used to sense and quantify the Cu^{2+} ions through these test kits as well.

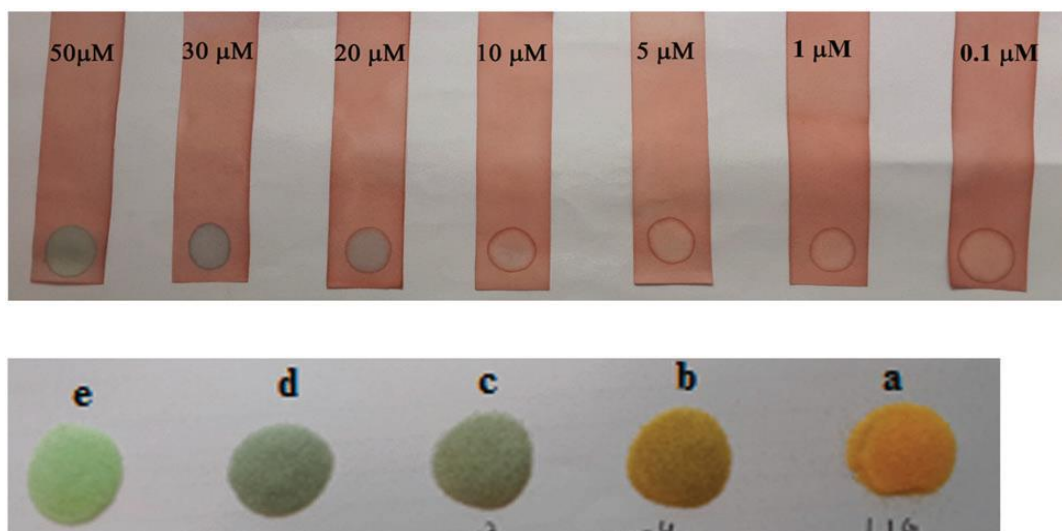


Figure 2.2.10: Photographs of a filter paper coated (upper) with dyad **2** (80 μM) and its color changes (in a circle) in the presence of different concentrations of Cu^{2+} ions. Bottom: color change of a silica gel coated with dyad **2** with increasing concentrations of Cu^{2+} ions: (a) dyad **2** only; (b) **2** + 2 μM Cu^{2+} ; (c) **2** + 4 μM Cu^{2+} ; (d) **2** + 6 μM Cu^{2+} ; and (e) **2** + 10 μM Cu^{2+} .

2.2.3.4 Theoretical studies

To understand the sensing mechanism of Cu^{2+} and Ni^{2+} binding to dyad **2**, theoretical calculations were performed in parallel to the experimental studies. As Job's plot analyses showed the 1:1 stoichiometric ratio in both the cases, so all theoretical calculations were performed with the 1:1 stoichiometry. The significant structural properties of the energy-minimized structure are shown in Figure 2.2.11.

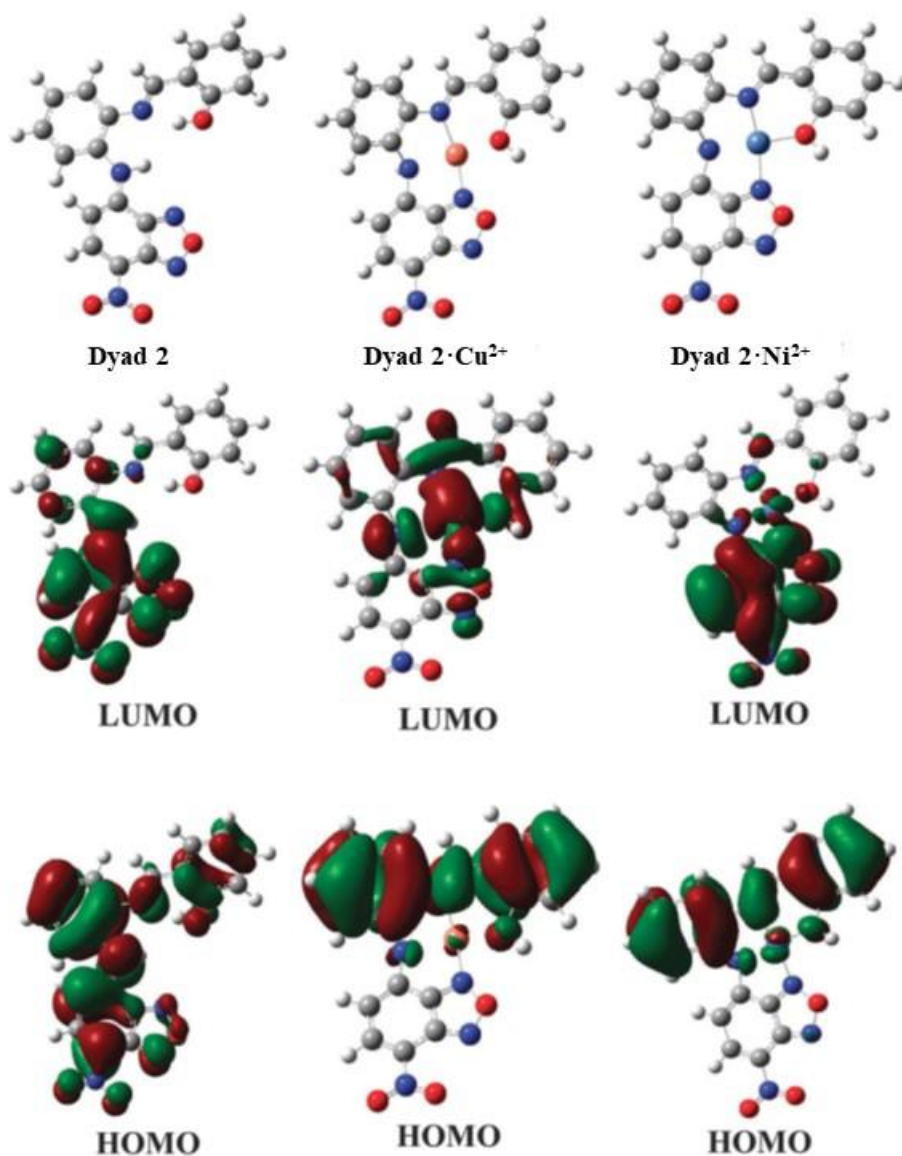


Figure 2.2.11: Energy minimized structure and frontier molecular orbital contribution of dyad **2** and its complexes with Cu^{2+} and Ni^{2+} ions.

The structure optimization of dyad **2** with Cu^{2+} and Ni^{2+} showed that the complexes attained the planarity and formed symmetrical square planar complexes. These optimized structures clearly predicted the LMCT process in the complexes. The dyad **2** in the presence of Ni^{2+} ions forms more planar stable square planar complex than that in the presence of Cu^{2+} ions, which can be clearly predicted from the bond distances of metal with the four heteroatoms involved in the formation of the square planar complex. Due to this reason, the addition of ethylenediaminetetraacetate (EDTA) caused the revival of dyad **2** absorption changes in the case of Cu^{2+} but not in the case of Ni^{2+} .

To interpret further the absorption properties of dyad **2** and its complexes with Cu^{2+} and Ni^{2+} , TD-DFT calculations were performed. In the case of dyad **2**, the main molecular orbital contribution of the first lowest excited state was determined for the HOMO – LUMO transition (450 nm), indicating the intramolecular charge transfer from the Schiff moiety to the NBD moiety. In the case of the **2**· Cu^{2+} complex, the 6th and 7th excited states (550 nm and 545 nm) were found to be relevant to the color change (yellow to blue), showing predominant LMCT and ICT. In the case of the **2**· Ni^{2+} complex, the 8th and 9th excited states (585 nm and 590 nm) were found to be related to the metal-to-ligand charge transfer (MLCT), and its contribution to the lowest-energy excitation was around 20%. Such an excitation corresponds to the charge transfer from the excited Ni^{2+} centre to NBD and Schiff base moieties. These transitions were found to be relevant to the color change (yellow to light green), showing predominant ICT and MLCT. These studies clearly predicted the differential charge transfer in these complexes.

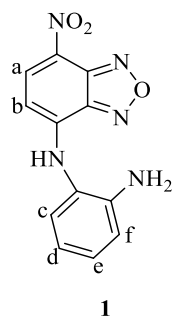
2.2.4 Conclusions

A NBD-based dyad **2** was developed for the detection of Cu^{2+} and Ni^{2+} ions in semi-aqueous systems. Dyad **2** could be used for the simultaneous estimation of Cu^{2+} and Ni^{2+} ions through its visible color change from yellow to blue and light green, respectively in semi-aqueous systems. The binding mode of dyad **2** to Cu^{2+} and Ni^{2+} was determined to be a 1:1 complexation stoichiometry through the Job's plot. The detection limits for Cu^{2+} , Ni^{2+} ions, 0.1-8 μM and 0.5-10 μM , respectively, were lower than those recommended by the WHO guidelines for drinking water. Moreover, dyad **2** could be used as a practical, visible colorimetric test kit for Cu^{2+} ions. The sensing mechanisms of Cu^{2+} and Ni^{2+} ions were also supported by DFT-calculations.

2.2.5 Experimental section

Synthesis of compound 1: Compound **1** was obtained through the reaction of 4-chloro-7-nitro-1,2,5-benzoxadiazole (1 g, 5.02 mmol) and *o*-phenylenediamine (0.54 g, 5.02 mmol) in the presence of K₂CO₃ (0.83 g, 6.02 mmol) and TBAHSO₄ in acetonitrile. The solution was refluxed for 5 h and monitored through TLC. After the completion of the reaction, the acetonitrile was distilled off under reduced pressure. The residue obtained was extracted using chloroform and water. The chloroform layer was collected and dried using sodium sulfate and distilled out on rota to get the compound. The crude product was purified by column chromatography to get the pure product **1**.

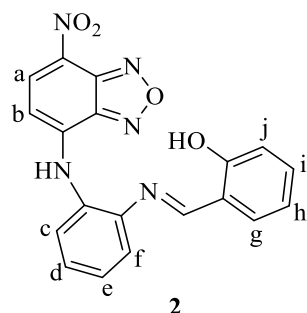
N¹-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)benzene-1,2-diamine (compound 1)



70% yield; reddish brown solid; M.pt. 220-222 °C; ¹H NMR (CDCl₃ + DMSO-*d*₆, 400MHz, ppm): δ 9.90 (s, 1H, NH), 8.42 (m, 1H, ArH_a), 7.20 (m, 2H, ArH_{b,e}), 6.92 (m, 1H, ArH_c), 6.81 (m, 1H, ArH_f) 6.10 (m, 1H, ArH_d); ¹³C NMR (CDCl₃ + DMSO-*d*₆, 100MHz): δ 144.2, 143.7, 143.2, 142.6, 136.2, 128.6, 126.7, 123.0, 121.6, 117.8, 116.5, 100.8 (ArC). ESI-MS: *m/z* 272.1 (M⁺ + H).

Synthesis of dyad 2: Compound **1** and 2-hydroxybenzaldehyde were stirred in ethanol at 60 °C for 4 h. The solid of dyad **2** was separated, filtered and washed with ethanol to get the pure dyad **2**.

2-(((2-((7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)phenyl)imino)methyl)phenol (dyad 2)



80% yield; yellow solid; M.pt. 220-222 °C; ¹H NMR (CDCl₃ + DMSO-*d*₆, 400 MHz, ppm): δ 12.49 (s, 1H, imine-CH), 10.44 (s, 1H, NH), 8.71 (s, 1H, ArH_a), 8.42 (d, 1H, *J* = 8.72 Hz, ArH_b), 7.53-7.51 (m, 1H, ArH_e), 7.49-7.35 (m, 4H, ArH_{g-j}), 6.96-6.94 (m, 1H, ArH_c), 6.87 (d, 1H, *J* = 8.24 Hz, ArH_f), 6.33 (d, 1H, *J* = 8.72 Hz, ArH_d); ¹³C NMR (CDCl₃ + DMSO-*d*₆, 100 MHz): δ 195.3, 164.1,

160.6, 144.6, 144.2, 143.4, 136.7, 133.6, 132.7, 131.3, 129.0, 127.9, 127.1, 123.4, 119.8, 119.1, 116.9, 101.5 (ArC). ESI-MS: m/z 376.3 ($M^+ + H$).

2.3 NBD based dual channel chromogenic sensor for Cu^{2+} and fluorogenic sensor for Al^{3+} and Hg^{2+}

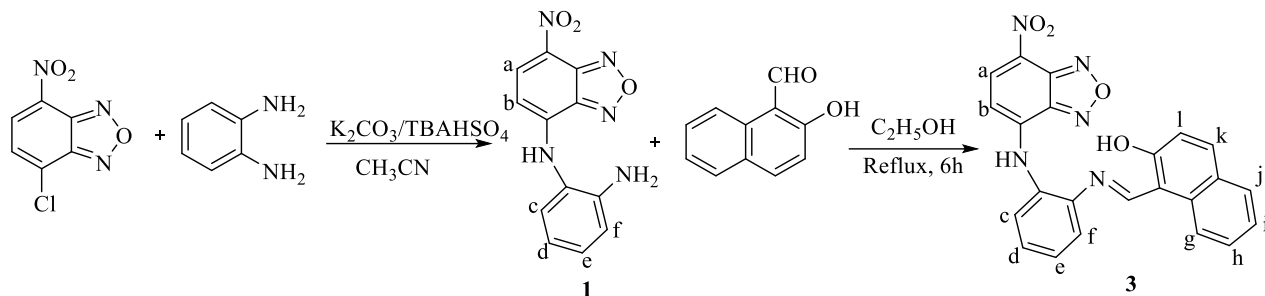
2.3.1 Abstract

In continuation of the previous chapter, we have synthesized a novel NBD based dual channel chromogenic sensor for Cu^{2+} ions and fluorescent sensor for Al^{3+} and Hg^{2+} ions. Dyad **3** showed absorption maxima at 445 nm and 320 nm. In the presence of Cu^{2+} ions, dyad **3** showed an instant color change from yellow to blue with a red shift of 150 nm and showed a new absorption band at 595 nm. Dyad **3** showed weak emission at 355 nm which *turned-on* in the presence of Hg^{2+} and Al^{3+} ions. Dyad **3** showed emission enhancement at 355 nm in the presence of Hg^{2+} ions whereas, quenching at 355 nm with the concomitant increase in intensity at 445 nm in the presence of Al^{3+} ions. The detection limit of Cu^{2+} ions was found to be 0.05 μM and $\text{Hg}^{2+}/\text{Al}^{3+}$ was 0.02 μM .

2.3.2 Synthesis of compounds **1** and **3**

Compound **1** was synthesized by the method discussed in chapter 2.2 and the synthetic route for the synthesis of dyad **3** has been outlined in Scheme 2.3.1. Dyad **3** was synthesized by coupling of compound **1** and 2-hydroxy-1-naphthaldehyde in ethanol as a red solid in 70 % yield; M.pt. = 230 °C; MS (ESI) m/z 426.4 ($\text{M}^+ + \text{H}$) (Scheme 2.3.1).

^1H NMR spectrum showed 1H singlet at δ 15.03 for OH, 1H singlet at δ 10.79 due to imine-CH, 1H singlet at δ 9.63 due to NH, 1H doublet at δ 8.44 due to H_a , 1H triplet at δ 8.36 due to H_e , 3H multiplet at δ 7.87-7.72 due to $\text{H}_{b,j,k}$, 3H multiplet at δ 7.59-7.53 due to $\text{H}_{c,d,f}$, 1H triplet at δ 7.45 due to H_h , 1H triplet at δ 7.36 due to H_i , 1H doublet at δ 6.91 due to H_g , 1H doublet at δ 6.26 due to H_l . The peak at δ 10.79 was due to imine-CH confirmed the formation of Schiff base. ^{13}C NMR spectrum showed signals at δ 195.1 for imine-C, 167.9, 163.7, 156.8, 144.3, 143.5, 141.9, 138.3, 136.7, 136.3, 132.7, 130.2, 128.8, 128.7, 127.8, 127.4, 127.1, 126.7, 123.4, 120.8, 119.5, 108.9 for aromatic-C. The peak at δ 195.1 was observed due to the formation of Schiff base. On the basis of these spectral data, the dyad **3** has been assigned the structure of 1-(((2-((7-Nitrobenzo [c][1,2,5]oxadiazol-4-yl)amino)phenyl)imino)methyl)naphthalen-2-ol.



Scheme 2.3.1: Synthesis of compounds **1** and **3**

2.3.3 Results and discussion

To evaluate the binding affinity of dyad **3** with different metal ions, UV-Visible and fluorescence studies were performed in methanol. The UV-Visible spectrum of dyad **3** (10 μ M, CH₃OH) was characterized by two absorption maxima at 320 nm and 445 nm. Upon addition of 50 equiv. of different metal cations such as Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Cr³⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Ag⁺, Hg²⁺, Pb²⁺, Al³⁺ etc., as their perchlorate salts, to dyad **3** (10 μ M, CH₃OH), only Cu²⁺ ions showed selective and visible change. Dyad **3** showed color change from yellow to greenish blue (Figure 2.3.1) in the presence of Cu²⁺ ions. Upon incremental addition of Cu²⁺ ions between 0.05 μ M -2.8 μ M, the intensity of absorption bands at 445 nm and 320 nm was decreased and a new absorption band was formed at 595 nm (Figure 2.3.2). The ratio of absorption intensities at 595 nm and 445 nm has been changed from 0.02 to 1.08 in the presence of Cu²⁺ ions. The 54-folds ratio change provided the opportunity for ratiometric estimation of Cu²⁺ ions. The lowest detection limit for Cu²⁺ ions was found to be 0.05 μ M through absorption method. The binding constant was determined to be $4.04 \pm 0.01 \times 10^5 \text{ M}^{-1}$ through Benesi Hildebrand equation.

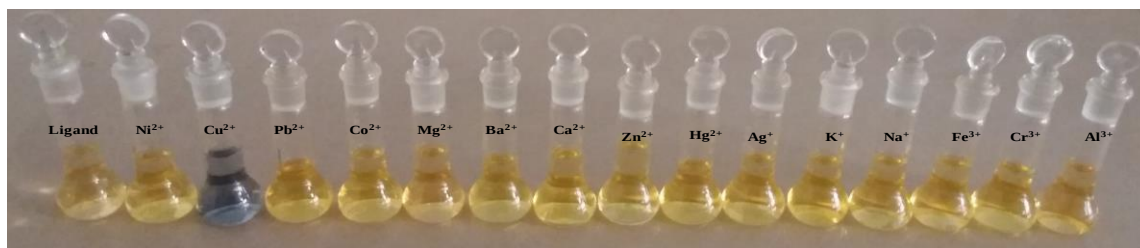


Figure 2.3.1: Photograph of visible color change on addition of various metal ions (1000 μ M) into dyad **3** (10 μ M, CH₃OH).

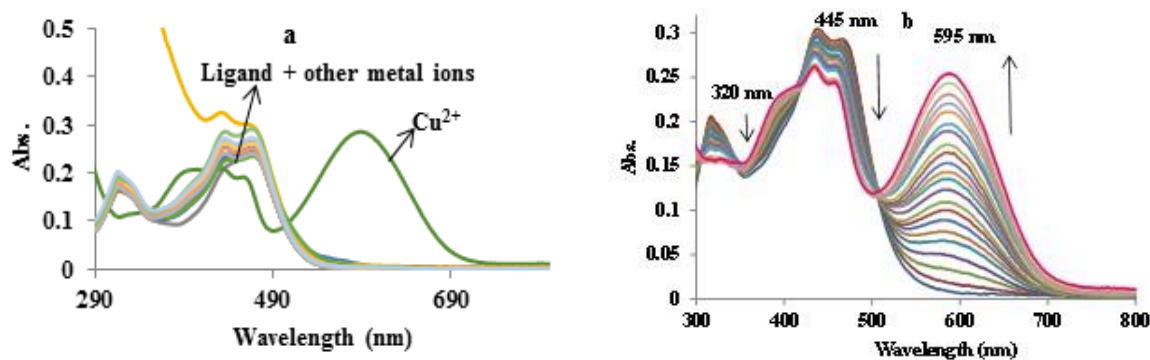


Figure 2.3.2: (a) UV-Visible spectra of dyad **3** (10 μM, CH₃OH) in the presence of various metal ions (50 equiv.); (b) effect of the addition of Cu²⁺ ions on dyad **3** (10 μM, CH₃OH).

To check the practical ability of dyad **3** as Cu²⁺ sensor, we carried out competitive experiments in the presence of 5 μM of Cu²⁺ and 1 mM of other interfering metal ions like Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Cr³⁺, Fe³⁺, Co²⁺, Ni²⁺, Zn²⁺, Ag⁺, Hg²⁺, Pb²⁺, Al³⁺ etc., as their perchlorate salts, no significant interference was observed with these metal ions as shown in Figure 2.3.3. Fe³⁺ is inducing 25% drop in absorbance but it was significant as 30% interference was considered threshold.

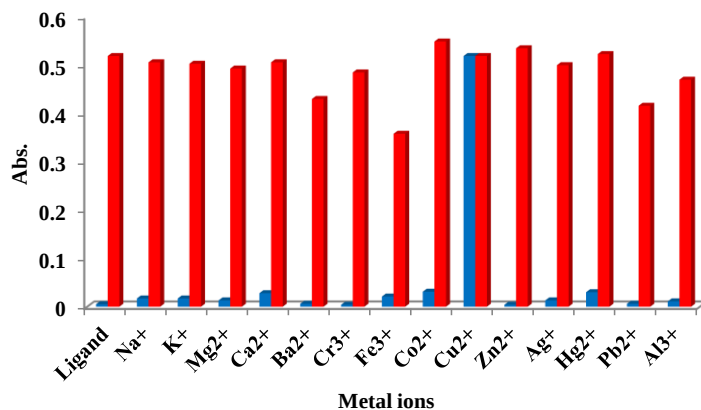


Figure 2.3.3: Blue bars show the effect of different metal ions on absorption response of dyad **3** (10 μM, CH₃OH) and red bars show the presence of interfering metal ions on dyad **3** + Cu²⁺ absorption intensity.

Upon excitation of dyad **3** (10 μM, CH₃OH) at 310 nm, no emission was observed. Upon addition of different metal ions such as Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Cr³⁺, Fe³⁺, Co²⁺, Ni²⁺, Zn²⁺, Ag⁺, Hg²⁺, Pb²⁺, Al³⁺ etc., as their perchlorate salts, the emission *turned-on* only in the presence of Al³⁺ and Hg²⁺ ions whereas other metal ions did not show any change (Figure 2.3.4). On titrating the dyad **3** (10 μM, CH₃OH) with different amounts of Al³⁺ (0.02-4 μM) the emission

was observed at 355 nm and on further addition of Al^{3+} (4-15 μM) the band at 355 nm was disappeared along with the appearance of a new emission band at 445 nm (Figure 2.3.5). The emission intensity at 355 nm increases from 37 to 374, showing a 10-folds change and the ratios of emission intensities at 445 and 355 nm (I_{445}/I_{355}) exhibit a drastic change from 0.08 in the absence of Al^{3+} to 5.74 in the presence of Al^{3+} , showing 71-folds variation in the emission ratio (Figure 2.3.6). The lowest detection limit was calculated to be 0.02 μM for Al^{3+} ions. The binding constant was determined to be $2.23 \pm 0.02 \times 10^5 \text{ M}^{-1}$ through Benesi Hildebrand equation.

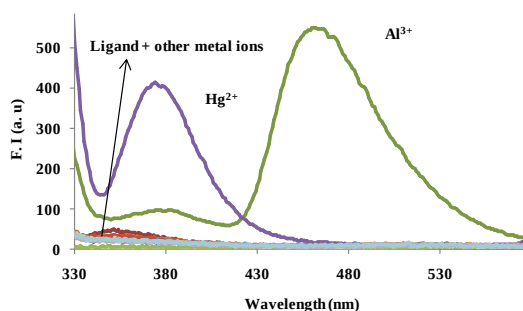


Figure 2.3.4: Fluorescence spectrum of dyad **3** (10 μM , CH_3OH) in the presence of various metal ions (50 equiv.).

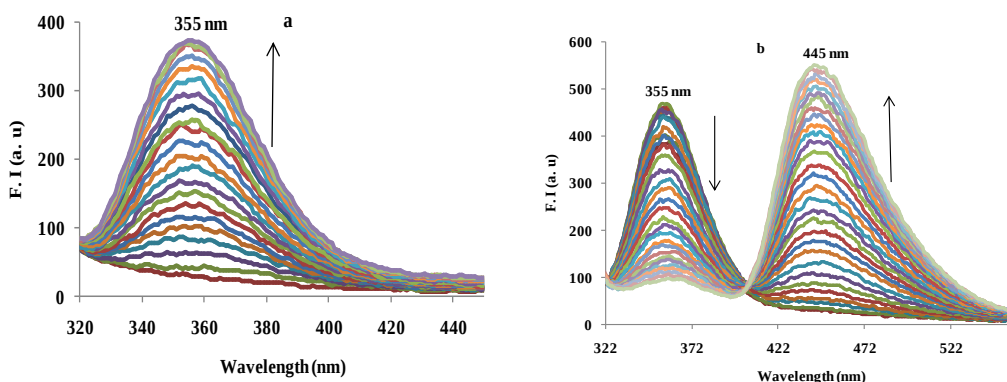


Figure 2.3.5: The fluorescence response of dyad **3** (10 μM , CH_3OH) (a) titration of Al^{3+} ions (0.02-4 μM); (b) titration of Al^{3+} ions (4-15 μM) on excitation at 310 nm.

However titrating the dyad **3** (10 μM , CH_3OH) with different amounts of Hg^{2+} (0.02-40 μM), a *turn-on* emission was observed at 355 nm (Figure 2.3.7a). Further increasing the concentration of Hg^{2+} , did not cause significant fluorescence emission changes. The emission intensity at 355 nm increases from 37 to 407 showed 11-folds change (Figure 2.3.7b) with $R^2 = 0.984$ (showed the excellent linear relationship between intensity and concentrations of Hg^{2+}). The lowest detection limit was calculated to be 0.02 μM for Hg^{2+} ions. The binding constant

calculated through Benesi-Hildebrand equation was $4.62 \pm 0.02 \times 10^4 \text{ M}^{-1}$. Al^{3+} showed large CHEF effect and Hg^{2+} relatively smaller CHEF effects as they were able to form a π -contact with the fluorophore.

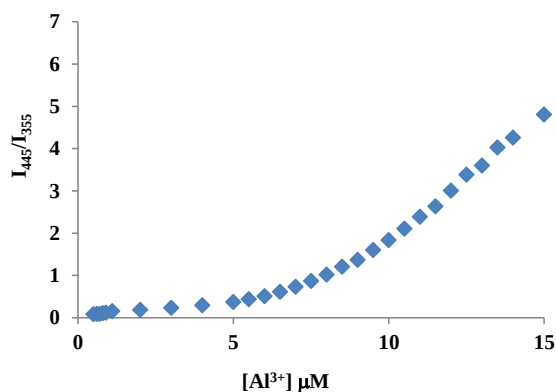


Figure 2.3.6: Emission ratiometric responses (I_{445}/I_{355}) of dyad **3** (10 μM) upon incremental addition of Al^{3+} ions.

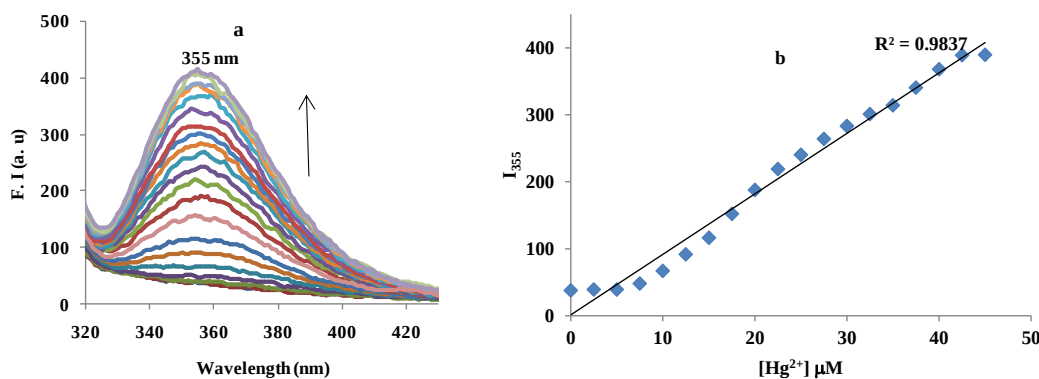


Figure 2.3.7: (a) The fluorescence response of dyad **3** (10 μM , CH_3OH) upon titration of Hg^{2+} ions (0.02-40 μM); (b) Linear fit emission change of dyad **3** (10 μM) at 355 nm upon incremental addition of Hg^{2+} ions.

To check the practical ability of dyad **3** as Al^{3+} and Hg^{2+} sensors, we carried out competitive experiments in the presence of 10 μM of $\text{Al}^{3+}/\text{Hg}^{2+}$ and 1 mM of other interfering metal ions like Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Pb^{2+} , etc., as their perchlorate salts, no significant interference was observed in both cases as shown in Figure 2.3.8. Fe^{3+} is inducing 20% drop in absorbance but it was significant as 30% interference was considered threshold.

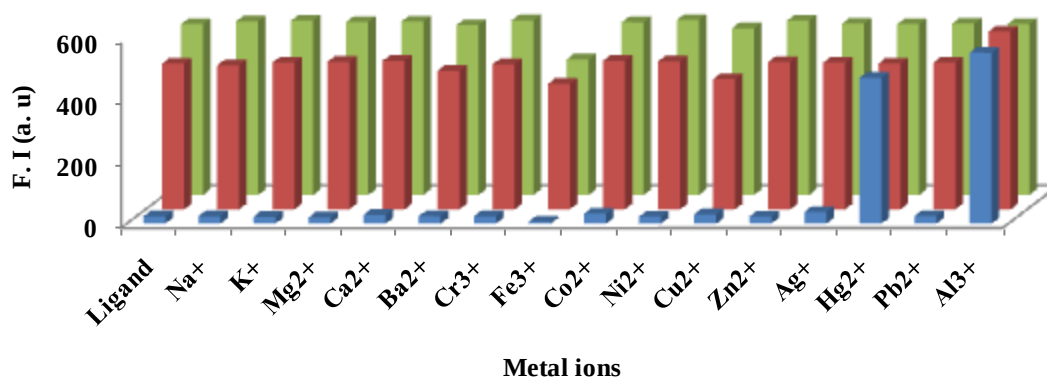


Figure 2.3.8: Blue bars show the effect of different metal ions on fluorescence response of dyad **3** (10 μ M, CH₃OH); red bars show the presence of interfering metal ions on dyad **3** + Hg²⁺; green bars show the presence of interfering metal ions on dyad **3** + Al³⁺.

2.3.4 Theoretical calculations

To know more about binding strategies of dyad **3** towards Cu²⁺ ions, geometry optimization were performed. The dyad **3** was optimized in ground state through DFT and TD-SCF calculation with B3LYP-631G methods.

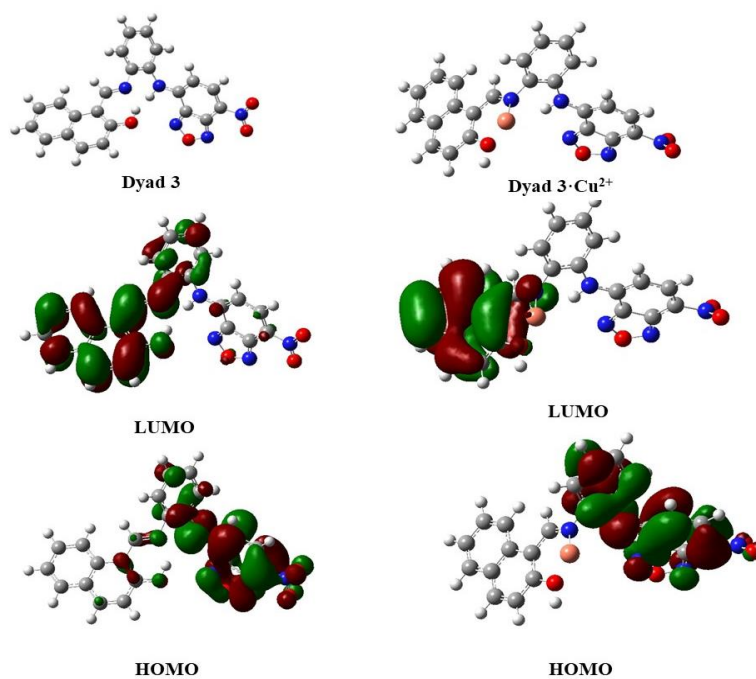


Figure 2.3.9: Energy minimized structure and frontier molecular orbital contribution of dyad **3** and its complexes with Cu²⁺ ions.

The complex of dyad **3** with Cu^{2+} ion, was optimized in gaseous phase using B3LYP-LAN2DZ basis set. The excite state of dyad **3** and its complex dyad **3**· Cu^{2+} showed the absorption maximum band at 440 nm and 636 nm in the gaseous state which is in good agreement with the experimental value at 445 nm and 595 nm respectively. The electronic transition can be considered as $n\text{-}\pi^*$ transition. It was investigated that Cu^{2+} ion showed its binding through Schiff base N atom with a bond distance of 1.87 Å, O of OH with a bond distance of 2.00 Å and form 1: 1 (dyad **3**: Cu^{2+}) complex. Frontier molecular orbital showed that HOMO of dyad **3** and its complex dyad **3**· Cu^{2+} with was completely located on NBD moiety while LUMO was distributed on naphthyl ring (Figure 2.3.9).

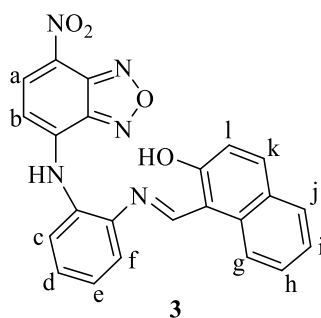
2.3.5 Conclusions

Easily synthesized dyad **3** showed selective colorimetric response towards Cu^{2+} ions and showed an instant color change from yellow to blue with a new absorption band at 595 nm. Dyad **3** opened two distinctly different emission channels at 355 nm and 445 nm in the presence of Hg^{2+} and Al^{3+} ions. Thus dyad **3** could be used for a dual sensor for Hg^{2+} and Al^{3+} ions through emission spectroscopy. The detection limit of Cu^{2+} ions was found to be 0.05 μM and $\text{Hg}^{2+}/\text{Al}^{3+}$ was 0.02 μM .

2.3.6 Experimental section

Synthesis of dyad 3: Compound **1** (0.1 g, 0.37 mmol) and 2-hydroxy-1-naphthaldehyde (0.06 g, 0.37 mmol) were refluxed in ethanol for 6 h. The red colored solid was separated out, filtered and washed with cold ethanol to get the pure dyad **3**.

1-(((2-((7-Nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)phenyl)imino)methyl)naphthalen-2-ol
(dyad **3**)



70% yield; red brown solid; M.pt. 230-232 °C; ^1H NMR ($\text{DMSO-}d_6$, 400 MHz, ppm): δ 15.03 (s, 1H, OH), 10.79 (s, 1H, imine-CH), 9.63 (s, 1H, NH), 8.44 (d, 1H, $J = 8.40$ Hz, ArH_a), 8.36 (t, 1H, $J = 7.64$ Hz, ArH_b), 7.87-7.72 (m, 3H, $\text{ArH}_{e,j,k}$), 7.59-7.53 (m, 3H, $\text{ArH}_{g,h,i}$), 7.45 (t, 1H, $J = 7.64$ Hz, ArH_l), 7.36 (t, 1H, $J = 6.88$ Hz, ArH_c), 6.91 (d, 1H, $J = 8.40$ Hz, ArH_f), 6.26 (d, 1H, $J = 8.40$ Hz, ArH_d); ^{13}C NMR ($\text{DMSO-}d_6$, 100 MHz): δ 195.1 (imine-C), 167.9, 163.7, 156.8,

144.3, 143.5, 141.9, 138.3, 136.7, 136.3, 132.7, 130.2, 128.8, 128.7, 127.8, 127.4, 127.1, 126.7, 123.4, 120.8, 119.5, 108.9 (ArC). ESI-MS: m/z 426.4 ($M^+ + H$).

2.4 NBD based Schiff base as chromofluorescent sensor for Cu^{2+} , Al^{3+} and CN^- ions

2.4.1 Abstract

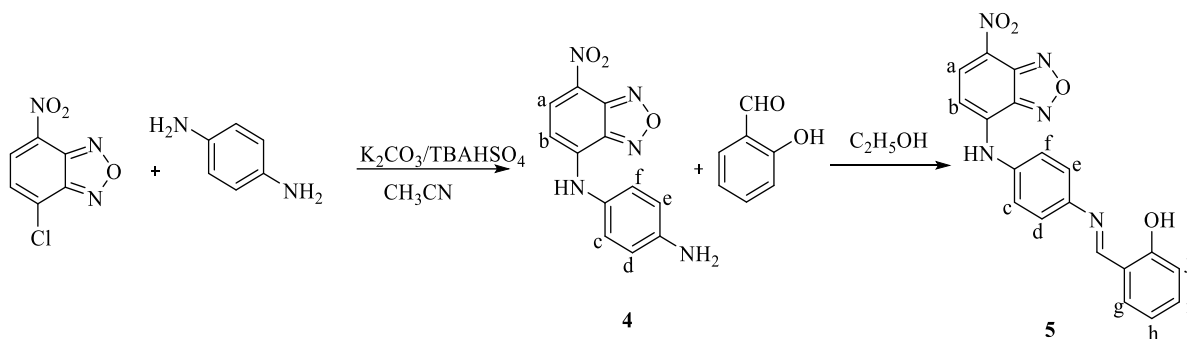
In continuation of our previous chapter, another NBD based Schiff base derivative **5** which includes NBD, salicylaldehyde and phenyl ring as linker has been designed and synthesized *via* simple condensation reaction between NBD amine derivative and salicylaldehyde. Its photophysical properties and selective recognition properties for various metal ions and anions in methanol have been investigated. In the presence of Cu^{2+} ions, dyad **5** showed a blue shift of 95 nm on binding with Cu^{2+} ions. Similarly, dyad **5** has been used to sense CN^- ions selectively with a color change from orange to yellow and showed a blue shift of 100 nm. The weak emission of dyad **5** was *turned-on* at 475 nm on addition of Al^{3+} and at 540 nm on addition of CN^- ions. Thus dyad **5** provides a convenient and selective detection of Cu^{2+} , Al^{3+} and CN^- ions.

2.4.2 Synthesis of compounds **4** and **5**

Compound **4** was synthesized by stirring the solution of 4-chloro-7-nitro-1,2,5-benzoxadiazole (NBD-Cl), *p*-phenylenediamine, potassium carbonate and TBSHSO_4 in acetonitrile at room temperature for 5 h, gave compound **4** as dark red solid in 75% yield; M.pt. = 235-240 °C; MS (ESI) m/z 272.2 ($\text{M}^+ + \text{H}$) (scheme 2.4.1). ^1H NMR spectrum showed 1H singlet at δ 10.08 for NH, 1H doublet at δ 8.41 due to H_a , two 2H doublets at δ 7.18 due to $\text{H}_{c,f}$ and 6.78 due to $\text{H}_{d,e}$, 1H doublet at δ 6.49 due to H_b , 2H singlet at δ 4.02 for NH_2 . The peaks at δ 10.08 due to NH and at δ 4.02 due to NH_2 , confirmed the formation of compound **4**. ^{13}C NMR spectrum showed signals at δ 146.1, 143.9, 143.6, 142.9, 136.2, 125.9, 124.8, 121.4, 114.4, 99.4 for aromatic-C. On the basis of these spectral data, this compound *N*¹-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)benzene-1,4-diamine has been assigned the structure of compound **4**.

Compound **4** was reacted with salicylaldehyde to form Schiff base (dyad **5**) as a red colored solid in 85 % yield; M.pt. = 240-242 °C; MS (ESI) m/z 376.1 ($\text{M}^+ + \text{H}$) (Scheme 2.4.1). ^1H NMR spectrum showed 1H singlet at δ 11.00 for NH, 1H singlet at δ 8.86 due to imine $\text{CH}=\text{N}$, 1H doublet at δ 8.52 due to H_a , 3H multiplet at δ 7.58-7.54 due to $\text{H}_{d,e,g}$, 2H doublet at δ 7.49 due to $\text{H}_{c,f}$, 1H triplet at δ 7.40 due to H_i , 2H multiplet at δ 6.99-6.96 due to $\text{H}_{h,j}$, 1H doublet at δ 6.83 due to H_b . The peak at δ 8.86 was due to imine $\text{CH}=\text{N}$ confirmed the formation of Schiff base. ^{13}C NMR spectrum showed signals at δ 171.6 for imine-C, 160.8, 147.9, 135.2,

133.9, 131.8, 131.6, 131.3, 129.9, 128.6, 127.9, 127.8, 127.6, 127.3, 126.7, 124.6, 122.7 for aromatic-C. This spectral data having an extra peak at 171.6 was due to the Schiff base that corroborated the structure 5 for (2-(((4-((7-Nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino) phenyl)imino)methyl) phenol).



Scheme 2.4.1: Synthesis of compounds 4 and 5

2.4.3 Results and discussion

2.4.3.1 Photophysical studies of dyad 5 towards different metal ions

The UV-Visible spectrum of dyad 5 (20 μ M, CH₃OH) was characterized by the absorption maxima at 485 nm with shouldered band at 340 nm. Addition of 50 equiv. of various metal ions such as Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Cr³⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Ag⁺, Hg²⁺, Pb²⁺, Al³⁺ etc., as their perchlorate salts, to dyad 5 (20 μ M, CH₃OH), only Cu²⁺ ions shows selective change (Figure 2.4.1a).

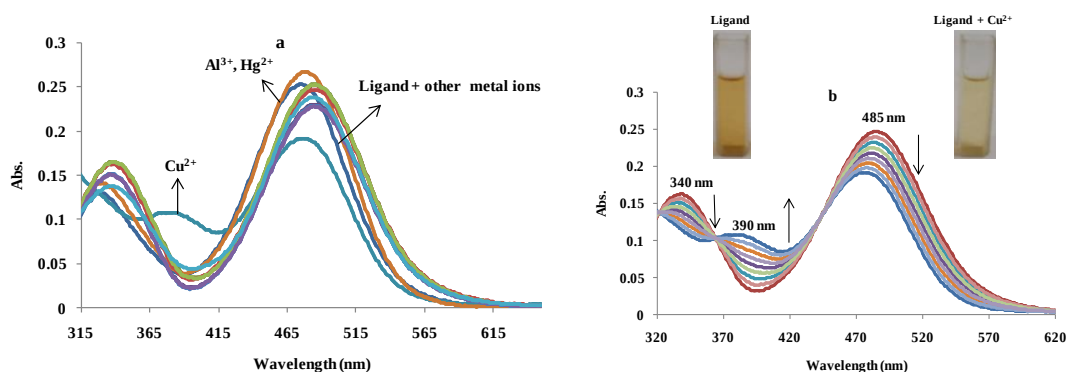


Figure 2.4.1: Absorption response of dyad 5 (20 μ M, CH₃OH) (a) with different metal ions; (b) on increasing the concentration of Cu²⁺ ions.

Incremental addition of 0-30 μM of Cu^{2+} to the dyad **5** (20 μM , CH_3OH), showed a new absorption band at 390 nm with the concomitantly decrease in the intensity at 485 nm and 340 nm (Figure 2.4.1b). The lowest detection limit was found to be 0.05 μM . The binding constant for the formation of a complex between dyad **5** and Cu^{2+} ions was found to be $5.0 \pm 0.01 \times 10^4 \text{ M}^{-1}$. To check the practical ability of dyad **5** as Cu^{2+} sensor, competitive metal ion experiment in the presence of 30 μM of Cu^{2+} and 1mM of other interfering metal ions such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Ag^+ , Hg^{2+} , Pb^{2+} , Al^{3+} etc., as their perchlorate salts, was carried out (Figure 2.4.2). The results showed that there was no interference of other metal ions in the estimation of Cu^{2+} ions.

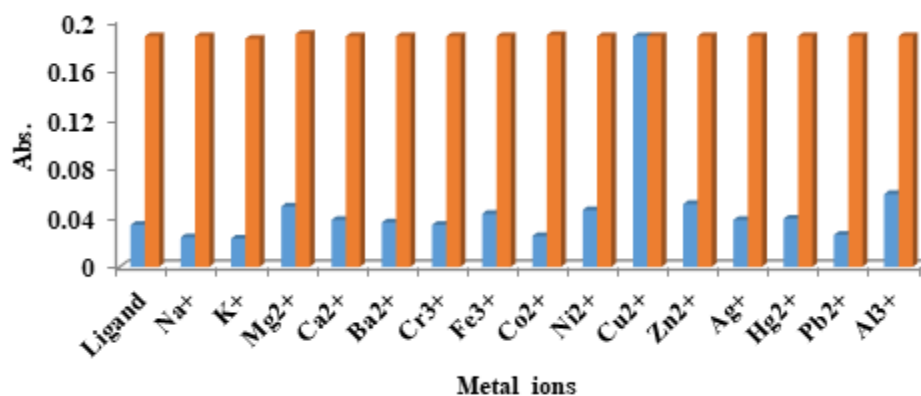


Figure 2.4.2: Blue bars show the effect of different metal ions on absorption response of dyad **5** (20 μM , CH_3OH) and green bars show the presence of interfering metal ions on dyad **5** + Cu^{2+} absorption intensity.

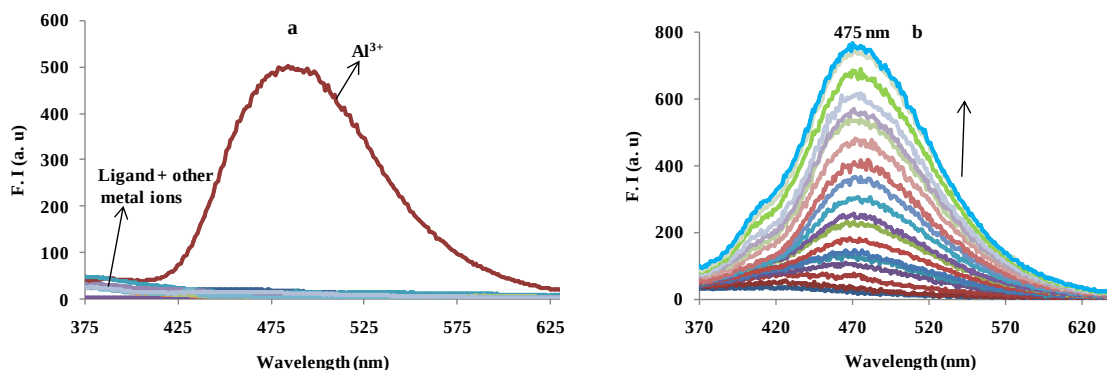


Figure 2.4.3: Emission response of dyad **5** (20 μM) (a) with different metal ions; (b) on increasing the concentration of Al^{3+} ions.

The dyad **5** (20 μM , CH_3OH) on excitation at 340 nm displayed weak emission. Dyad **5** underwent turned on the addition of Al^{3+} ions while other metal ions *viz.* Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Hg^{2+} , Pb^{2+} , etc., as their perchlorate salts, showed no effect (Figure 2.4.3a). Upon incremental addition of 5 μM solution of Al^{3+} ions, the emergence of a new band at 475 nm was observed due to the CHEF effect, and binding constant was determined to be $2.3 \pm 0.01 \times 10^4 \text{ M}^{-1}$ (Figure 2.4.3b). The emission intensity at 475 nm varied from 19 to 444, indicating 23-folds emission intensity change. To check the practical ability of dyad **5** as Al^{3+} fluorescent sensor, we carried out competitive experiments in the presence of 30 μM of Al^{3+} and 1 mM of other interfering metal ions such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Hg^{2+} , Pb^{2+} etc., as their perchlorate salts, no significant interference was observed (Figure 2.4.4).

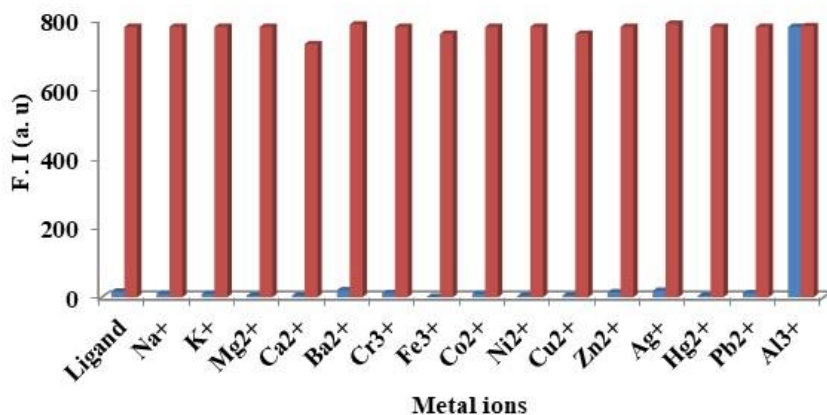


Figure 2.4.4: Blue bars show the effect of different metal ions on absorption response of dyad **5** (20 μM , CH_3OH) and green bars show the presence of interfering metal ions on dyad **5** + Al^{3+} absorption intensity.

2.4.3.2 Photophysical studies towards different anions

The absorption response of dyad **5** towards different anions such as F^- , CN^- , Cl^- , SCN^- , AcO^- , NO_3^- , Br^- , HSO_4^- , H_2PO_4^- , I^- etc., as their tetrabutylammonium salts, were investigated in CH_3OH . The absorption band due to dyad **5** at 485 nm remained unaffected in the presence of tested anions except CN^- ions (Figure 2.4.5a). Upon addition of 0-1 mM CN^- , the system exhibited a new band at 385 nm and decrease in intensity at 340 nm while no change was observed at 485 nm (Figure 2.4.5b). Moreover, this absorption change was also accompanied by a visual change in color from orange to yellow which offered naked eye detection of CN^- ions in CH_3OH .

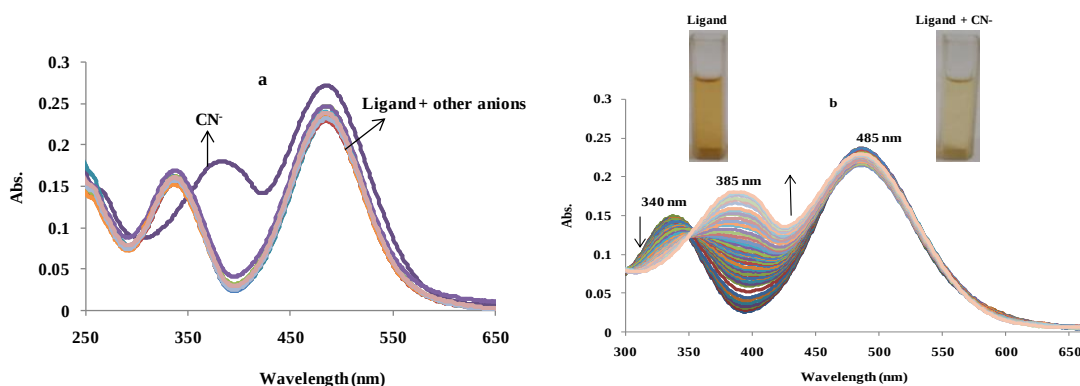


Figure 2.4.6: The absorption response of dyad **5** (20 μM , CH_3OH) (a) in the presence of various anions (50 equiv.); (b) in the presence of increasing concentration of CN^- ions.

The ratios of absorption intensities at 385 and 340 nm (A_{385}/A_{340}) exhibited a drastic change from 0.22 in the absence of CN^- to 1.40 in the presence of CN^- and with the detection limit of 0.05 μM , 6-folds variation in the absorption ratios. In order to check the mode of interaction of dyad **5** with CN^- , whether the absorption changes observed are due to the H-bonding interactions or CN^- binding to dyad, studies were performed in the presence of aqueous system. No change were observed. Thus, *in situ* 3 μl H_2O was added to dyad **5**• CN^- solution. The changes observed due to the presence of CN^- ions were reversed, which clearly predicted H-bonding interactions. The equilibrium constant was determined to be $9.85 \pm 0.03 \times 10^3 \text{ M}^{-1}$. To check the practical ability of dyad **5** as CN^- sensor, we carried out competitive experiments in the presence of 1 mM of CN^- and 1 mM of other interfering metal ions like F^- , Cl^- , SCN^- , AcO^- , NO_3^- , Br^- , HSO_4^- , H_2PO_4^- , I^- etc., as their tetrabutylammonium salts, no significant interference was observed (Figure 2.4.6).

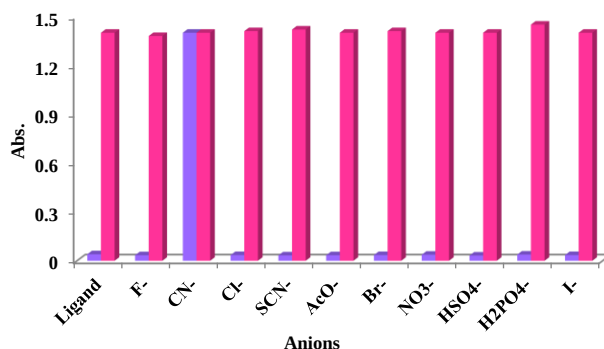


Figure 2.4.6: Blue bars show the effect of different anions on fluorescence response of dyad **5** (20 μM , CH_3OH) and pink bars show the presence of interfering anions on dyad **5** + CN^- absorption intensity.

The dyad **5** (20 μM , CH_3OH) on excitation at 340 nm showed a significant change only with CN^- ions out of various anions such as Br^- , Cl^- , CN^- , F^- , I^- , NO_3^- , SCN^- , HSO_4^- , H_2PO_4^- , AcO^- etc., as their tetrabutylammonium salts. Upon incremental addition of 7 μM solution of CN^- ions, the emergence of a new band at 540 nm was observed and equilibrium constant was found to be $5.00 \pm 0.02 \times 10^4 \text{ M}^{-1}$. The emission intensity at 540 nm varied from 6 to 69 indicating 11-folds emission intensity change (Figure 2.4.7).

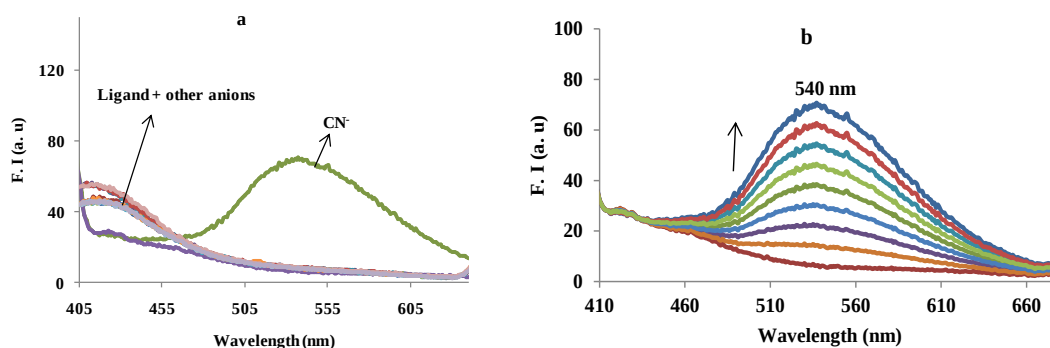


Figure 2.4.7: The emission response of dyad **5** (20 μM , CH_3OH) (a) in the presence of various anions (50 equiv.); (b) titration with increasing concentration of CN^- ions.

2.4.3.3 Theoretical calculations

To know more about binding strategies of dyad **5** towards Cu^{2+} ions and CN^- ions, geometry optimization were performed. The dyad **5** was optimized in ground state through DFT and TD-SCF calculation with B3LYP-631G methods. The complex of dyad **5** with Cu^{2+} ions and CN^- ions, was optimized in gaseous phase using B3LYP-LAN2DZ basis set. The excited state of dyad **5** and its complexes $\mathbf{5} \cdot \text{Cu}^{2+}$ and $\mathbf{5} \cdot \text{CN}^-$, showed the absorption maximum band at 544 nm, 434 nm and 427 nm in the gaseous state which is in good agreement with the experimental value at 485 nm, 390 and 385 nm respectively. The electronic transition can be considered as $\pi - \pi^*$ transition. It was investigated that Cu^{2+} ion showed its binding through Schiff base N atom with a bond distance of 1.87 Å, O of OH with a bond distance of 2.00 Å and form 1: 1 (dyad **5**: Cu^{2+}) complex. CN^- ions deprotonating the hydroxyl group and converge into keto form. Frontier molecular orbital showed that HOMO of dyad **5** and its complex $\mathbf{5} \cdot \text{Cu}^{2+}$ and $\mathbf{5} \cdot \text{CN}^-$ was completely located on phenyl ring while LUMO was distributed on NBD.

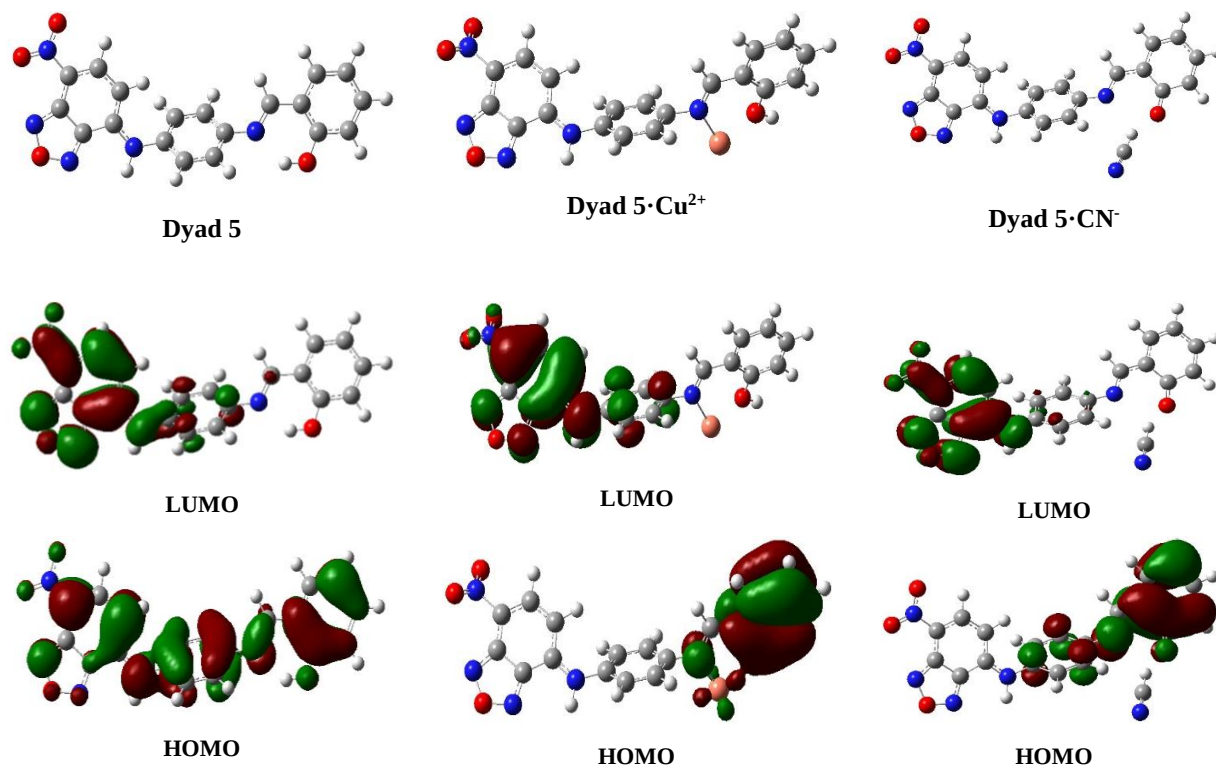


Figure 2.4.8: Energy minimized structure and frontier molecular orbital contribution of dyad 5 and its complexes with Cu²⁺ ions and CN⁻ ions.

2.4.4 Conclusions

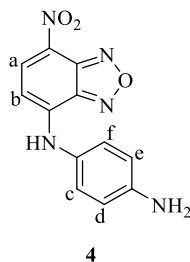
NBD based Schiff base dyad 5 has been used to sense Cu²⁺, Al³⁺, and CN⁻ ions selectively. In presence of Cu²⁺ ions, dyad 5 showed a blue shift of 95 nm on binding with Cu²⁺ ions. Similarly, dyad 5 was used to determined CN⁻ ions selectively with a color change from orange to yellow and showed a blue shift of 100 nm. Thus, dyad 5 worked as a chromogenic sensor for Cu²⁺ and CN⁻ ions. Weak emission of dyad 5 underwent *turn-on* upon addition of Al³⁺ and CN⁻ ions. The detection limit of Hg²⁺ and CN⁻ was 0.05 μ M. Thus, dyad 5 provides a convenient and selective detection of Cu²⁺, Al³⁺ and CN⁻ ions.

2.4.5 Experimental section

Synthesis of compound 4: To a solution of 4-Chloro-7-nitro-1,2,5-benzoxadiazole (1 g, 5.02 mmol), *p*-phenylenediamine (0.54 g, 5.02 mmol) in acetonitrile, potassium carbonate (0.83 g, 6.02 mmol) and TBAHSO₄ (catalytic amount) were added. The mixture was stirred at room

temperature for 5 h. The reaction was monitored through TLC. After the completion of the reaction, the acetonitrile was distilled off under reduced pressure. The residue obtained was extracted using chloroform and water. The chloroform layer was collected and dried using sodium sulfate and distilled out on rotary evaporator to get the compound. The crude product was purified by column chromatography to get the pure compound **4** as a dark red solid.

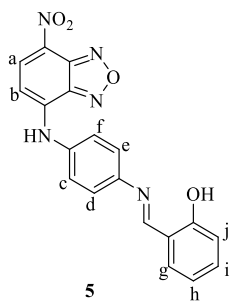
***N*¹-(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)benzene-1,4-diamine (compound 4)**



75% yield; dark red solid; M.pt. = 235-240 °C; ¹H NMR (CDCl₃ + DMSO-*d*₆, 400 MHz): δ (ppm) 10.08 (s, 1H, NH), 8.41 (d, 1H, *J* = 9.16 Hz, Ar*H*_a), 7.18 (d, 2H, *J* = 6.44 Hz, Ar*H*_{c,f}), 6.78 (d, 2H, *J* = 6.88 Hz, Ar*H*_{d,e}), 6.49 (d, 1H, *J* = 8.72 Hz, Ar*H*_b), 4.02 (s, 2H, NH₂); ¹³C NMR (CDCl₃ + DMSO-*d*₆, 100 MHz): δ (ppm) 146.1, 143.9, 143.6, 142.9, 136.2, 125.9, 124.8, 121.4, 114.4, 99.4 (ArC); MS(ESI) *m/z* 272.2 (M⁺ + H).

Synthesis of dyad 5: Schiff base reaction was carried out with compound **4** (0.1 g, 0.37 mmol) and salicylaldehyde (0.045 g, 0.37 mmol) in ethanol. After refluxing for 5 h, a solid separated out, was filtered and washed with ethanol to give pure red colored compound **5**.

2-(((4-((7-Nitrobenzo[*c*][1,2,5]oxadiazol-4-yl)amino)phenyl)imino)methyl)phenol (dyad 5)



85% yield; red solid; M. pt. = 240-242 °C; ¹H NMR (CDCl₃ + DMSO-*d*₆, 400 MHz): δ (ppm) 11.00 (s, 1H, NH), 8.86 (s, 1H, imine-CH), 8.52 (d, 1H, *J* = 8.72 Hz, Ar*H*_a), 7.58-7.54 (m, 3H, Ar*H*_{i,h,g}), 7.49 (d, 2H, *J* = 8.72 Hz, Ar*H*_{c,f}), 7.40 (t, 1H, *J* = 8.2 Hz, Ar*H*_j), 6.99-6.96 (m, 2H, Ar*H*_{d,e}), 6.83 (d, 1H, *J* = 8.72 Hz, Ar*H*_b); ¹³C NMR (CDCl₃ + DMSO-*d*₆, 100 MHz): δ (ppm) 171.6 (imine-C), 160.8, 147.9, 135.2, 133.9, 131.8, 131.6, 131.3, 129.9,

128.6, 127.9, 127.8, 127.6, 127.3, 126.7, 124.6, 122.7 (ArC); MS (ESI) *m/z* 376.1 (M⁺ + H).

Conclusion of chapter 2

Chapter 2 includes synthesis of NBD based Schiff bases and studied their photophysical behavior towards different metal ions and anions with following features:

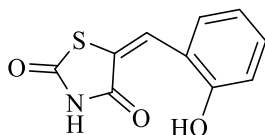
- An NBD-based dyad **2** was successfully synthesized the detection of multiple ions such as Cu^{2+} and Ni^{2+} ions in semi-aqueous systems as $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (1:1, v/v) through its visible color change.
- The detection limits for Cu^{2+} , Ni^{2+} ions, 0.1-8 μM and 0.5-10 μM , respectively, were lower than those recommended by the WHO guidelines for drinking water.
- Dyad **2** has a practical applicability through visible colorimetric test kit for Cu^{2+} ions.
- Dyad **3** acts as a multifunctional optical sensor with dual channel mode: Chromogenic sensor for Cu^{2+} and fluorogenic sensor for Al^{3+} and Hg^{2+} in CH_3OH .
- NBD based Schiff base dyad **5** has been developed to sense multi-ions as Cu^{2+} , Al^{3+} and CN^- ions selectively in CH_3OH .

CHAPTER 3

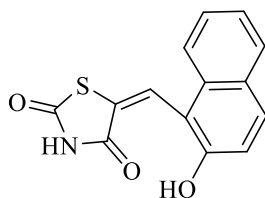
SYNTHESIS OF THIAZOLIDINE MOIETY BASED MOLECULES AS CHEMOSENSORS

3.1 Introduction

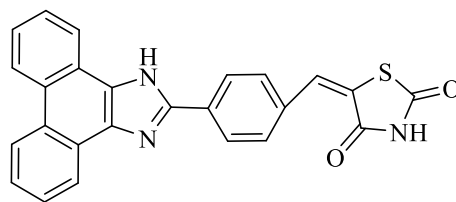
Thiazolidine derivatives have attracted continuing attention in the last few years,⁹⁵ because of having well-known scaffolds in medicinal chemistry.⁹⁶ Thiazolidine and its analogs have also found a prominent place in drug development for the treatment of various diseases.⁹⁷ In recent years, their analogs have attracted great attention as chemosensors.⁹⁸ A simple polynuclear hetero atom (N, S, and O) containing organic scaffolds explored as a potential chemosensor to detect various analytes. The thiazolidine heterocycle provides a wide range of molecular structure due to the presence of hetero atoms like N, S, and O, that gives the opportunity to behave as good chemosensor. To the best of our knowledge, there is no report in the literature where the thiazolidine moiety has been used as a sensor for cations and anions. In the present chapter, we describe the synthesis of thiazolidine based hybrid molecules to study their photophysical behavior towards various ions. The thiazolidine derivatives have been used for an elaboration of logic circuits. This categorization of these molecules has been discussed in detail as per following subchapters. The thiazolidine derivative has been synthesized by linking the thiazolidine with salicylaldehyde, hydroxy naphthaldehyde, and phenanthroimidazole.



Chapter 3.2



Chapter 3.3



Chapter 3.4

- 3.1** Thiazolidine based differential chromo-fluorescent sensor for Cu^{2+} and CN^- ions:
Elaboration as logic devices.
- 3.2** Thiazolidine based molecules as fluorescent sensor for recognition of Hg^{2+} , CN^- and PPi and application as INHIBIT logic device
- 3.4** Thiazolidine based solvent dependent differential chemo- sensor for metal ions and anions.

3.2 Thiazolidine based differential chromo-fluorescent sensor for Cu²⁺ and CN⁻ ions: Elaboration as logic devices

3.2.1 Abstract

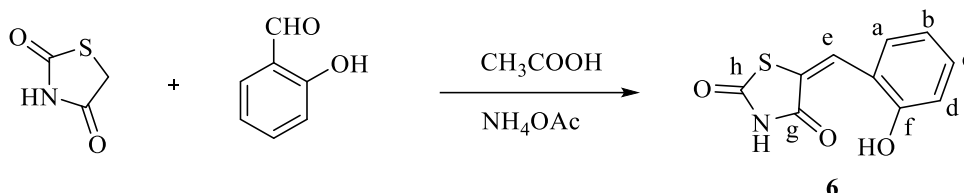
Thiazolidine based probe **6** has been synthesized and its photophysical behavior towards different metal ions and anions was investigated. Probe **6** was used for the selective detection of Cu²⁺ ions amongst other metal ions viz., Na⁺, K⁺, Ca²⁺, Ba²⁺, Cr³⁺, Mn²⁺, Fe³⁺, Fe²⁺, Co²⁺, Ni²⁺, Zn²⁺, Hg²⁺, Pb²⁺, Al³⁺ etc. The probe **6** displayed new absorption channels at 300 nm and 500 nm in the presence of Cu²⁺ ions. The ratiometric response has been observed in the presence of Cu²⁺ ions due to emission quenching at 425 nm and enhancement at 485 nm that resulted in *on-off-on* type sensing. Probe **6** has been used for detection of Cu²⁺ ions between 50 nM and 15 μ M, which is much lower than recommended by WHO in drinking water (30 μ M). Probe **6** has also shown selective ratiometric absorption behavior towards CN⁻ ions in CH₃CN: H₂O: 1:1. The ratiometric behavior of probe **6** in the presence of Cu²⁺ and CN⁻ ions has used as XOR logic function at λ_{em} 485 nm and YES logic gate at λ_{abs} 300 nm. The sensing mechanisms of probe **6** towards Cu²⁺ and CN⁻ ions have been theoretically supported by DFT and TD-DFT calculations. Probe **6** has been used for its practical applicability to sense Cu²⁺ and CN⁻ through a dip coating method.

3.2.2 Synthesis of probe 6

Probe **6** was prepared according to the synthetic route shown in Scheme 3.2.1. Thiazolidine-2,4-dione was synthesized *via* reaction between thiourea and chloroacetic acid in presence of concentrated HCl to give a white solid as a product in 85% yield, M.pt. = 123-125 °C; MS (ESI) *m/z* 117.5 (M⁺ + H). The ¹H NMR spectrum showed 1H singlet at δ 9.0 of NH and a 2H singlet at δ 4.70 for CH₂ protons. ¹³C NMR data showed signals at δ (ppm) 168.7 and 167.2 for C=O, at δ 116.4 for CH₂. This spectral data confirm the formation of thiazolidine-2,4-dione.

Probe **6** was synthesized through reaction of thiazolidine-2,4-dione with 2-hydroxybenzaldehyde in the presence of sodium acetate and glacial acetic acid to form a yellow colored solid in 90% yield; M.pt. = 235-237 °C; MS (ESI) *m/z* 221.1 (M⁺ + H) (scheme 3.2.1). ¹H NMR spectrum showed 1H singlet at δ 12.50 for OH, 1H singlet at δ 10.51 for NH, 1H singlet at δ 8.00 for H_e, two 2H multiplets at δ 7.32-7.26 due to H_{a,c} and 6.95-6.90 H_{b,d}. The peak at δ 8.00 confirmed the formation of probe **6**. ¹³C NMR spectrum showed signals at δ 168.4, 167.7 for

C=O, at δ 157.5 for C=C, at δ 132.2 for C-O, at δ 128.4, 127.6, 121.8, 120.2, 119.6, 116.2 for aromatic-C. This spectral data having extra peaks at δ 168.4 and 167.7 were due to carbonyl groups and at δ 157.5 for C=C corroborate structure **6**. On the basis of these spectral data, this compound has been assigned the structure of 5-(2-hydroxybenzylidene) thiazolidine-2,4-dione (probe **6**).



Scheme 3.2.1: Synthesis of probe **6**

3.2.3 Results and discussion

3.2.3.1 UV-Visible studies with different metal ions

Probe **6** (20 μ M, CH₃CN) showed absorption maxima at 347 nm. With the addition of various metal ions like Na⁺, K⁺, Ca²⁺, Ba²⁺, Cr³⁺, Mn²⁺, Fe³⁺, Fe²⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Hg²⁺, Pb²⁺, Al³⁺ etc., as their perchlorate salts, no significant change was detected, but in the presence of Cu²⁺ ions, a new absorption band at 300 nm associated with the color change from yellow to colorless. The incremental addition of 35 μ M of Cu²⁺ ions to probe **6** caused a decrease in absorption intensity at 347 nm with concomitant bathochromically shift to new absorption bands between 400 nm and 500 nm (Figure 3.2.1a).

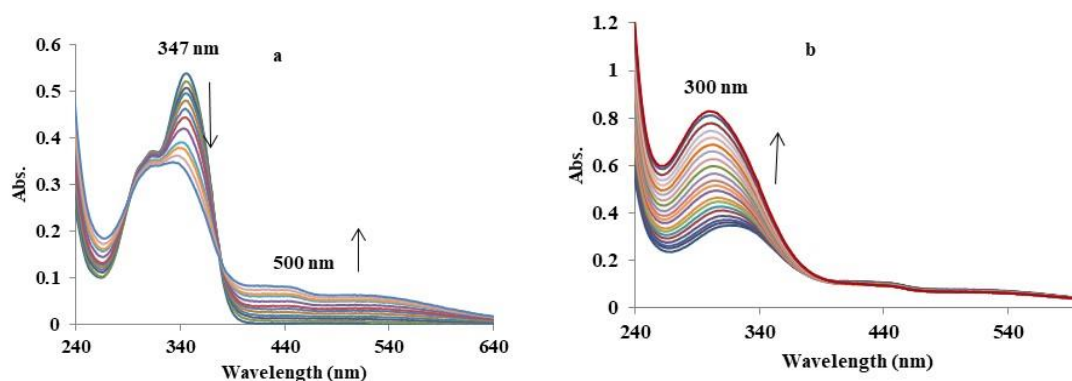


Figure 3.2.1: Absorbance spectrum of probe **6** (20 μ M, CH₃CN) on addition of Cu²⁺ ions from (a) 0-35 μ M; (b) 35-800 μ M.

Further addition of Cu²⁺ ions between 35 and 800 μ M caused the formation of the new absorption bands at 300 nm (Figure 3.2.1b). The complexation constant of Cu²⁺ ions with probe

6 was $5.1 \pm 0.01 \times 10^5 \text{ M}^{-1}$, calculated through Benesi–Hildebrand equation. To check the practical applicability of the probe **6** as a Cu^{2+} selective sensor, we carried out various metal ion competition experiment. Probe **6** was mixed with 1 equiv. Cu^{2+} ions in the presence of 100 equiv. of other metal ions. As shown in Figure 3.2.2, other metal ions had no interference with Cu^{2+} ions detection. Hence, these results suggested that the probe **6** could be an effective and selective sensor for Cu^{2+} ions. Fe^{2+} is inducing 15% and Fe^{3+} is showing 20% increase in absorbance but it was significant as 30% interference was considered threshold.

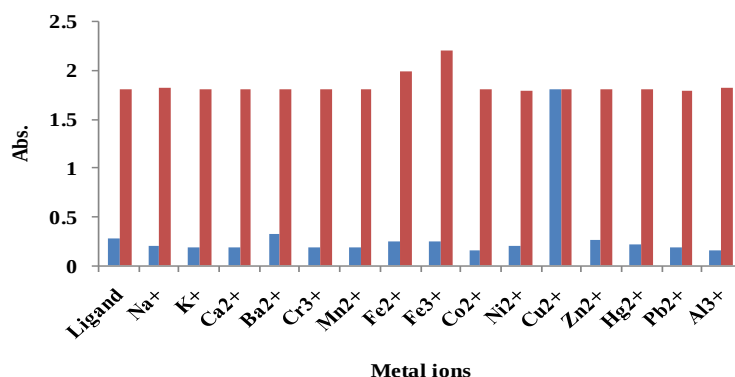


Figure 3.2.2: The blue bars represent the selectivity of the probe **6** towards Cu^{2+} ions (20 μM) upon addition of different metal ions in CH_3CN and the red bars show the competitive binding of the probe **6** in the presence of interfering metal ions.

3.2.3.2 Fluorescence studies with different metal ions

Probe **6** (20 μM , CH_3CN) on excitation at 350 nm showed an emission maximum at 425 nm. The addition of various metal ions like Na^+ , K^+ , Ca^{2+} , Ba^{2+} , Cr^{3+} , Mn^{2+} , Fe^{3+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Hg^{2+} , Pb^{2+} , Al^{3+} etc., as their perchlorate salts, no significant change was observed except Cu^{2+} ions (Figure 3.2.3a).

The presence of Cu^{2+} ions showed the formation of the new emission band at 485 nm. The incremental addition of Cu^{2+} ions to probe **6**, caused quenching of the emission band at 425 nm and formation of a new red shifted emission band at 485 nm. As emission at 425 nm was switched *off* and emission at 485 nm (Figure 3.2.3b) was switched *on* so, this switching *on-off* phenomenon provided the opportunity for ratiometric estimation of Cu^{2+} ions which is very rare due to the paramagnetic effect of Cu^{2+} ions. The emission bands at 425 nm and 485 nm linearly decreased and increased, respectively and were used to estimate 50 nM–15 μM of Cu^{2+} ions. The binding constant was $6.37 \pm 0.02 \times 10^5 \text{ M}^{-1}$, calculated through Benesi–Hildebrand equation.

Job's plot analysis of probe **6** showed the formation of 2:1 (probe **6**: Cu^{2+}) complex with Cu^{2+} ions (Figure A3).

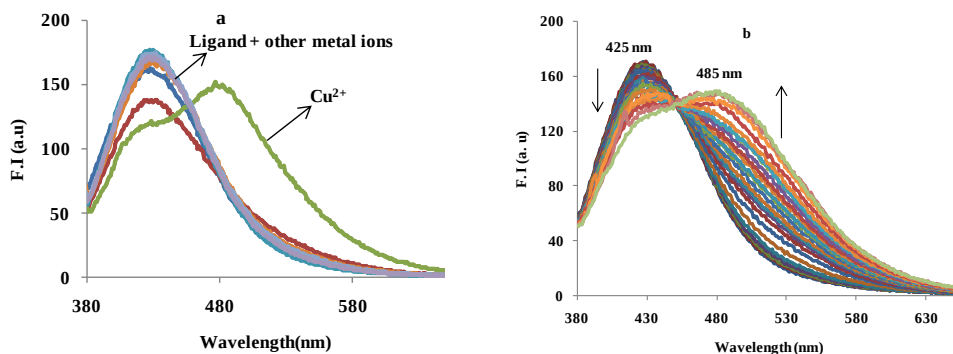


Figure 3.2.3: Emission spectrum of probe **6** (20 μM , CH_3CN) (a) in the presence of various metal ions; (b) on the incremental addition of Cu^{2+} ions.

3.2.3.3 UV-Visible studies with different anions in CH_3CN

On addition of various anions like F^- , CN^- , Cl^- , SCN^- , AcO^- , NO_3^- , Br^- , HSO_4^- , H_2PO_4^- , I^- etc., as their tetrabutylammonium salts, to probe **6** (20 μM , CH_3CN), no significant change was observed except in the presence of F^- ions (Figure 3.2.4a).

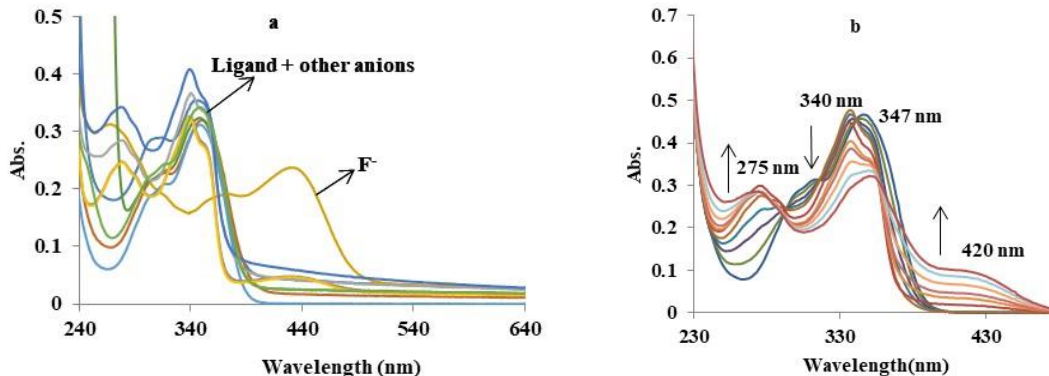


Figure 3.2.4: Absorption spectrum of probe **6** (20 μM , CH_3CN) (a) in the presence of various anions; (b) on the incremental addition of fluoride ions.

The presence of fluoride ions caused the formation of a new absorption band at 420 nm. The incremental addition of fluoride ions caused a decrease in absorption intensity of the band at 340 nm with concomitant formation of a new bathochromically shifted absorption band at 420 nm. A bathochromic shift was associated with a visible color change from colorless to bright yellow. Probe **6** showed an isosbestic point at 295 nm (Figure 3.2.4b) which indicated no

stepwise complexation and formation of single complexed species. Job's plot analysis of the probe **6** also showed the formation of 1:1 complex with fluoride ions (Figure A4).

3.2.3.4 Fluorescence studies with different anions in CH₃CN

Emission changes of the probe **6** were recorded in the presence of various anions viz., F⁻, CN⁻, Cl⁻, SCN⁻, AcO⁻, NO₃⁻, Br⁻, HSO₄⁻, H₂PO₄⁻, I⁻ etc., as their tetrabutylammonium salts. All of the F⁻, AcO⁻, H₂PO₄⁻, and CN⁻ ions showed the formation of a new emission band at a longer wavelength and no selectivity of ions was observed. In order to achieve selectivity, we studied binding in different solvents as a handy method to control anion nucleophilicity and sensing response. As stated above, in CH₃CN solutions, probe **6** induced changes with anions like F⁻, AcO⁻, H₂PO₄⁻, and CN⁻ but 50% vol. in water was enough to avoid completely the reaction of probe **6** with F⁻, AcO⁻, and H₂PO₄⁻. Thus, photophysical studies were performed in CH₃CN:H₂O (1:1, v/v), pH = 7.2) as explained above to achieve selectivity.

3.2.3.5 UV-Visible studies with different anions in CH₃CN: H₂O: 1:1, pH = 7.2)

In the presence of various anions like F⁻, CN⁻, Cl⁻, SCN⁻, AcO⁻, NO₃⁻, Br⁻, HSO₄⁻, H₂PO₄⁻, I⁻ etc., as their tetrabutylammonium salts, absorption spectrum of probe **6** (20 μM, CH₃CN: H₂O: 1:1, pH = 7.2) did not affect except cyanide ions (Figure 3.2.5a).

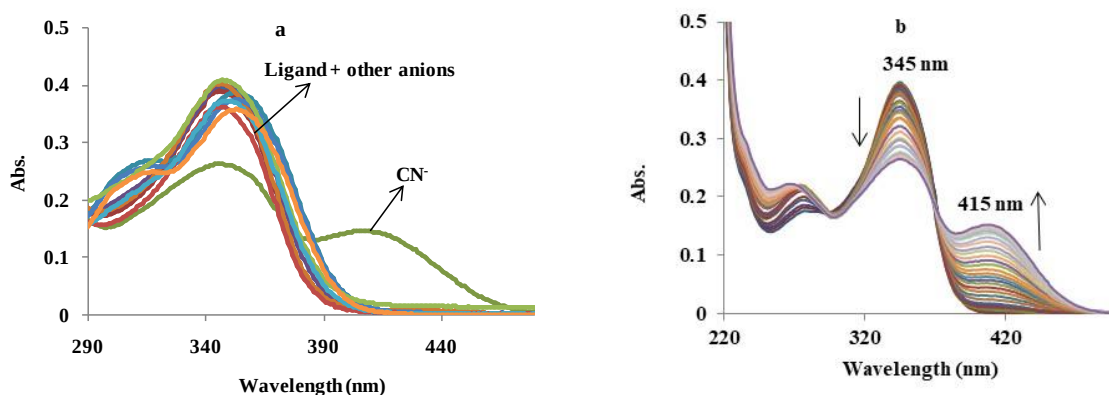


Figure 3.2.5: (a) Effect of various anions on absorption spectrum of probe **6** (20 μM, CH₃CN: H₂O: 1:1); (b) effect of incremental addition of cyanide ions on absorption spectrum of probe **6** (20 μM, CH₃CN: H₂O: 1:1, buffer, pH = 7.2).

The incremental addition of cyanide ions to probe **6** (20 μM, CH₃CN: H₂O: 1:1, pH = 7.2) caused a decrease in absorption intensity at 345 nm with concomitantly formation of new red shifted absorption band at 415 nm (Figure 3.2.5b). The probe **6** showed a clear isosbestic

point at 360 nm, indicating no stepwise complexation and formation of single complexed species. To check the practical applicability of probe **6** as CN^- selective sensor, we carried out anion competition experiments. Probe **6** was mixed with 10 equiv. of CN^- ions in the presence of 100 equiv. of other anions like F^- , Cl^- , SCN^- , AcO^- , NO_3^- , Br^- , HSO_4^- , H_2PO_4^- , I^- etc., as their tetrabutylammonium salts (Figure 3.2.6). No significant variation was observed in absorbance by the addition of interfering anions.

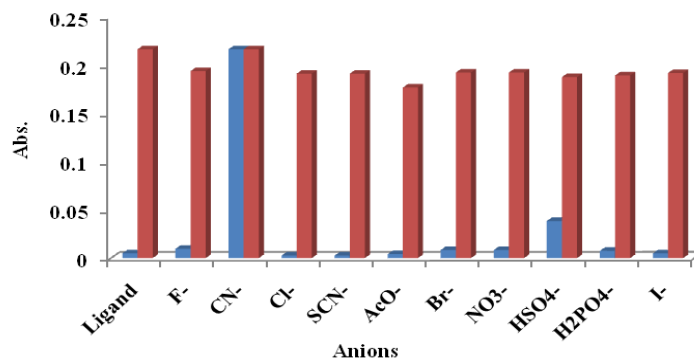


Figure 3.2.6: The blue bars represent the selectivity of probe **6** towards CN^- ions (20 μM , CH_3CN : H_2O : 1:1) upon addition of different anions and the red bars show the competitive selectivity of probe **6** in the presence of interfering anions.

3.2.3.6 Fluorescence studies with different anions in CH_3CN : H_2O : 1:1, pH = 7.2)

Upon excitation of probe **6** (20 μM , CH_3CN : H_2O : 1:1) at 350 nm, emission maxima at 420 nm were observed. The presence of various anions did not affect the emission spectrum of probe **6** except cyanide ions (Figure 3.2.7a).

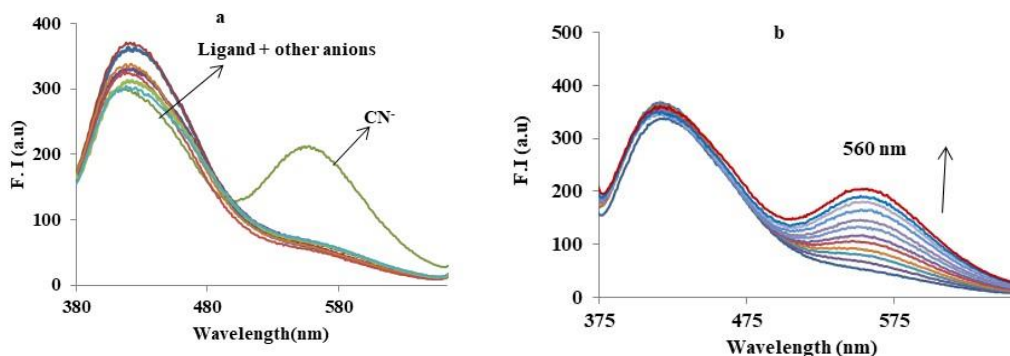


Figure 3.2.7: (a) Effect of various anions on emission spectrum of probe **6** (20 μM , CH_3CN : H_2O : 1:1); (b) effect of incremental addition of cyanide ions on emission spectrum of probe **6** (20 μM , CH_3CN : H_2O : 1:1, buffer, pH = 7.2).

The presence of cyanide ion caused the formation of a new emission band at 560 nm. Upon incremental addition of cyanide ions to probe **6** (20 μ M, CH₃CN: H₂O: 1:1), the emission band at 420 nm was not affected but led to the formation of red shifted band ($\Delta\lambda = 140$ nm) at 560 nm (Figure 3.2.7b). Job's plot analysis of probe **6** (Figure A5) also showed the formation of 1:1 complex with cyanide ions as explained theoretically. The complexation constant was determined to be $8.2 \pm 0.02 \times 10^4 \text{ M}^{-1}$. The lowest detection limit for CN⁻ was calculated using $3\sigma/\text{slope}$ method and was found to be 1 μ M.

3.2.3.7 Effects of pH on absorption and emission changes

Since the pH variation effects the charge distribution on a molecule, it can change the absorbance and fluorescence response of the probe. To check the versatility of this probe in different pH, the effects of pH on probe **6** and complex of probe **6** with cyanide ions in 1:1/CH₃CN: H₂O in UV–Visible and fluorescence were also studied. The results revealed that the probe **6** and its complex with cyanide ion are stable between pH range of 1.5–9.4.

3.2.3.8 Effect of CN⁻ on ¹H NMR spectrum

To understand the binding of cyanide with probe **6**, ¹H NMR titrations were performed in CD₃CN-*d*₃: D₂O:1:1. The hydroxyl proton signal of probe **6** was not observed due to D₂O exchange.

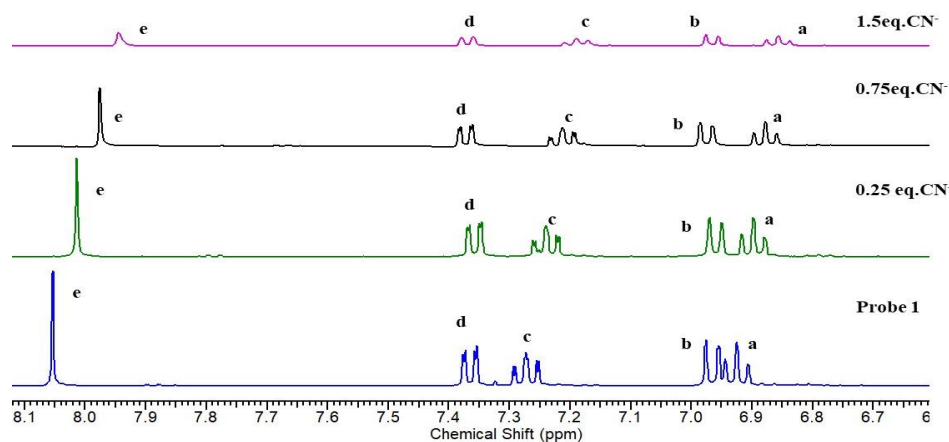


Figure 3.2.8: ¹H NMR titrations of probe **6** with TBACN in CD₃CN-*d*₃: D₂O: 1:1.

Upon addition of CN⁻ ions up to 1.5 equivalents, singlet of alkene H_e upfield shifted by 0.29 ppm from 8.07 ppm to 7.78 ppm and H_d proton of phenyl ring showed negligible shifting. Protons H_c

and H_b upfield shifted from 7.31 ppm to 7.15 ppm ($\Delta\delta = 0.16$ ppm) and 6.99 ppm to 6.96 ppm, respectively. NMR titrations clearly predicted the deprotonation of hydroxyl proton induced intramolecular charge transfer. The upfield shifts for phenyl and alkene protons in the presence of CN^- ions could be attributed due to electron delocalization induced shielding of protons (Figure 3.2.8). Also upon addition of H_2O to the probe **6**· CN^- complex the absorption band of probe **6** revived as availability of H^+ ion from water, inferring to the deprotonation of phenolic proton.

3.2.3.9 Practical application of probe **6** towards Cu^{2+} and CN^- ions

To check the practical applicability of probe **6** towards Cu^{2+} and CN^- ions, TLC strips were coated with 20 μM solution of probe **6** by dip-coating, followed by drying under vacuum to check the contamination through contact mode. We prepared different samples of solution coated strips and checked their fluorescence response towards Cu^{2+} and CN^- ions in contact mode and solution phase. Solutions of different concentrations of Cu^{2+} and CN^- ions were placed over coated strips for 10s for Cu^{2+} ions and 1-2 min for CN^- ions. Upon illumination with UV-lamp, blue spots were observed in the contact area in case of Cu^{2+} ions and bright yellow in the case of CN^- ions. The minimum amount of Cu^{2+} ions that can be detected with the naked eye was 10 μM (Figures 3.2.9 and 3.2.10).

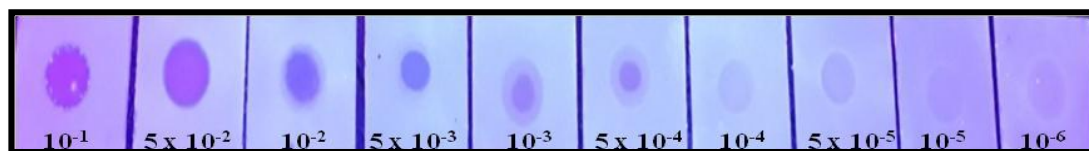


Figure 3.2.9: Photographs of test strips coated (upper) with probe **6** ($10^{-3} M^{-1}$) and its color changes (in a circle) in the presence of different concentrations of Cu^{2+} ions.

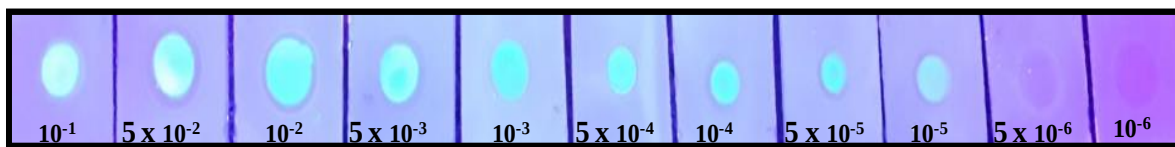


Figure 3.2.10: Photographs of test strips coated (upper) with probe **6** ($10^{-3} M^{-1}$) and its color changes (in a circle) in the presence of different concentrations of CN^- ions.

3.2.3.10 Logic gates

On the basis of chemical information about inputs and outputs of Cu^{2+} and CN^- ions in the absorption and emission channels, XOR and YES logic gates have been fabricated. It is always

difficult to achieve XOR logic gate chemically because, for XOR gate (0-0), two inputs (1-1) should have same spectroscopic properties for low signal output. In contrast, when there is a single input (0-1 and 1-0 states), there should have a high signal output.

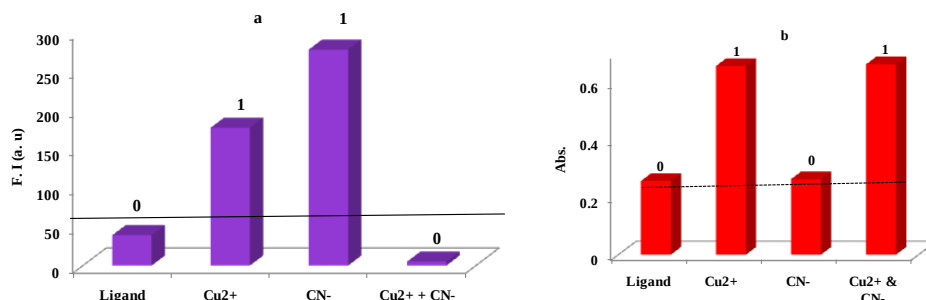


Figure 3.2.11: Bar diagrams of the logic circuits (a) XOR gate in emission spectroscopy at 485 nm; (b) YES gate in absorption spectroscopy at 300 nm, where 0 = *off* and 1 = *on*.

Table 1: Truth table for the sequential logic circuits XOR and YES gate, where 0 = *off* and 1 = *on*.

Input 1 Cu^{2+}	Input 2 CN^-	Output 2 $\lambda_{\text{em}} 485 \text{ nm}$	Output 2 $\lambda_{\text{Abs}} 300 \text{ nm}$
0	0	0	0
1	0	1	1
0	1	1	0
1	1	0	1

Chemical inputs for Cu^{2+} and CN^- have been represented as “1” for the presence and “0” for their absence. The output signal for probe **6** was measured at 485 nm in the case of emission spectroscopy and at 300 nm in the case of absorption spectroscopy. For fluorescence emission, the output is considered as “1” when intensity is above 40 a.u. (threshold value) and “0” when intensity is below this value. While in the case of absorption spectroscopy, the output is considered as “1” when absorption intensity is above 0.3 a.u. (threshold value) and “0” when intensity is below this value. The inputs and outputs of Cu^{2+} and CN^- ions corresponding to fluorescence and UV–Visible responses are given in Table 1. The emission at 485 nm is in *on* state when either of the two inputs (Cu^{2+} and CN^-) are present and in *off* state when neither is present or both are present. This behavior provided the elaboration of XOR logic function. The

outputs at $\lambda_{\text{abs}} = 300 \text{ nm}$ in the presence of chemical inputs Cu^{2+} and CN^- ions activated YES gate (Figure 3.2.11).

3.2.3.11 Theoretical studies

Theoretical calculations (DFT) have also been carried out on probe **6** to gain a better understanding of the binding interactions with Cu^{2+} and CN^- ions. Two stable conformations MODEL 1 and MODEL 2 were observed with optimization of probe **6** in a solvent system (Figure 3.2.12).

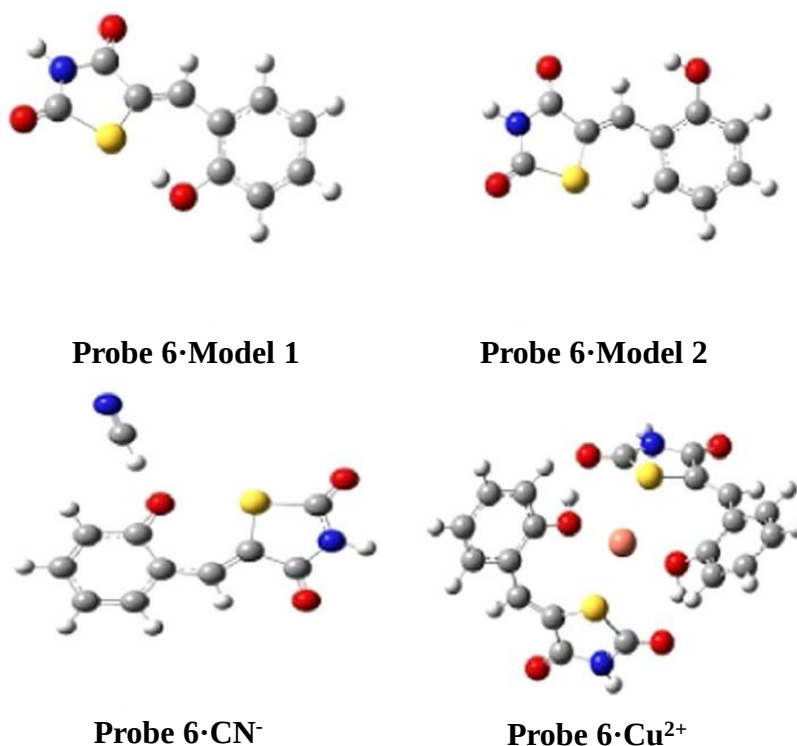


Figure 3.2.12: DFT optimized structure of probe **6** and their complexes with CN^- and Cu^{2+} ions.

Potential energy surface (PES) using a dihedral scan (atoms labeled C9, C8, C6, and C5) showed that MODEL 1 is 4.21 kJ mol^{-1} more stable than MODEL 2. TD-DFT calculations were performed on both MODEL 1 and 2 in a solvent phase to conclude conformation of probe **6**. MODEL 1 was found to be in close agreement with experimental data. So, further calculations were performed with MODEL 1. TD-DFT calculations to probe **6** showed the absorption maximum (λ_{max}) at 352 nm corresponding to transitions from $\text{HOMO} \rightarrow \text{LUMO}$ and $\text{HOMO-1} \rightarrow \text{LUMO}$ with orbital contributions, 90.8% , and 9.2% , respectively. On analysis of frontier molecular orbital, these transitions showed that HOMO is located at the π cloud of phenyl ring

containing hydroxyl, sulfur and ketone part. LUMO is distributed over π^* cloud of phenyl ring containing hydroxyl, sulfur and ketone part of the thiazolidine ring along with a small contribution of nitrogen atom that clearly predicted π - π^* transitions (Figure 3.2.12).

Probe 6



Probe 6•CN⁻



Probe 6•Cu²⁺

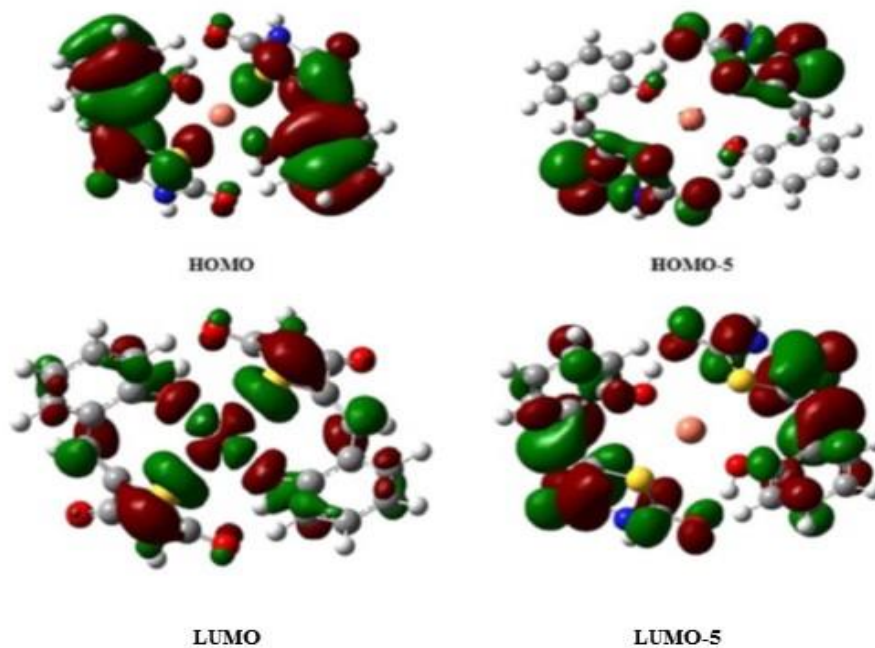


Figure 3.2.13: Frontier molecular orbital of probe **6** and its complexes (MO's with highest contributions are shown).

The structure of probe **6•CN⁻** was optimized using the same protocol as probe **6**. The optimized structure resulted in planar keto conformation at the phenyl ring by deprotonation of hydroxyl hydrogen and led to the formation of HCN molecule (Figure 3.2.13). TD-DFT studies revealed

that HOMO was distributed over the whole molecule except nitrogen atom, whereas LUMO was distributed over the whole molecule except sulfur atom with opposite charge densities. The electronic transition can be considered as a π - π^* transition with a smaller energy gap that caused a red shift in a spectrum which is in close agreement with the experimental results (from 352 nm to 438 nm). The electronic transition was contributed from HOMO $\rightarrow$ LUMO only. The structure optimization of probe **6**·Cu²⁺ complex showed the dimeric structure with slightly distorted square planar geometry. In the complex, Cu²⁺ symmetrically coordinates through a sulfur atom of thiazolidine moiety and the oxygen atom of hydroxyl phenyl (E = 1547.26 a.u.). The probe **6**·Cu²⁺ complex was stabilized through symmetrically strong hydrogen bonding interaction between phenolic hydrogen and thiazolidine oxygen. A TD-DFT calculation on the optimized geometry of complex showed that the energy difference between HOMO and LUMO was small as compared to probe **6**, resulted in a red shift in absorption spectra. Electronic transitions were mainly due to HOMO to LUMO and HOMO to LUMO+ 1 of π - π^* with a small contribution of ligand to metal charge transfer.

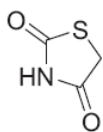
3.2.4 Conclusions

We have synthesized a fluorescent probe **6** for the selective recognition of Cu²⁺ and CN⁻ ions. Probe **6** has shown the ratiometric *on-off-on* type of emission response in the presence of Cu²⁺ ions and used for detection of Cu²⁺ between the detection limit of 50 nM–15 μ M. On the other hand, probe **6** was used for detection of cyanide ions between 4 μ M and 200 μ M in CH₃CN: H₂O:1:1. The binding behavior and optical response achieved in the presence of Cu²⁺ and CN⁻ ions, have been confirmed through DFT calculations. The probe **6** also mimicked the XOR and YES logic functions by using Cu²⁺ and CN⁻ ions chemical inputs. Probe **6** has also been used for its practical applicability to sense Cu²⁺ and CN⁻ ions through dip coating method.

3.2.5 Experimental section

Synthesis of thiazolidine-2,4-dione: To a stirring solution of chloroacetic acid (10 g, 0.11 mol) and thiourea (8.05 g, 0.11 mol) in 100 mL water, concentrated HCl was added and refluxed for 7 h. The white solid precipitate was filtered and washed with cold water to give the desired product.

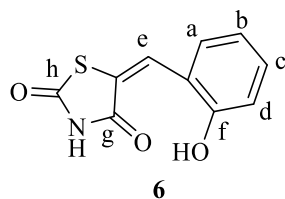
Thiazolidine-2,4-dione



85% yield; white solid; M.pt. = 123-125 °C; ^1H NMR (CDCl_3 , 400 MHz): δ (ppm) 9.00 (s, 1H, NH), 4.70 (s, 2H, CH_2); ^{13}C NMR ($\text{DMSO}-d_6$, 100 MHz): δ (ppm) 168.7, 167.2, 116.4; MS (ESI) m/z 117.50 ($\text{M}^+ + \text{H}$).

Synthesis of probe 6: A mixture of thiazolidine-2,4-dione (1.00 g, 8.5 mmol), 2-hydroxybenzaldehyde (1.04 g, 8.5 mmol), sodium acetate (0.70 g, 8.5 mmol) and glacial acetic acid (1 mL) were heated to the temperature of 140 °C for 6 h. After the completion of the reaction (TLC), the reaction mixture was poured into dilute HCl. The solid was separated out, filtered and dried to get the pure product.

5-(2-Hydroxybenzylidene)thiazolidine-2,4-dione (probe 6)



90% yield; yellow solid; M.pt. 235-237 °C; ^1H NMR ($\text{DMSO}-d_6$, 400 MHz, ppm): δ 12.50 (s, 1H, OH), 10.51 (s, 1H, NH), 8.00 (s, 1H, CH_e), 7.32–7.26 (m, 2H, $\text{ArH}_{a,c}$), 6.95–6.90 (m, 2H, $\text{ArH}_{b,d}$); ^{13}C NMR ($\text{CDCl}_3 + \text{DMSO}-d_6$, 100 MHz): δ 168.4 (C=O), 167.7 (C=O), 157.5 (C=C), 132.2 (C–O), 128.4, 127.6, 121.8, 120.2,

119.6, 116.2 (ArH); ESI-MS: m/z 222.1 ($\text{M}^+ + \text{H}$).

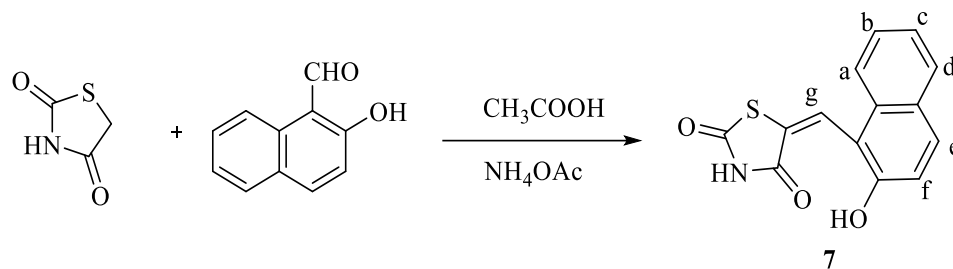
3.3 Thiazolidine based molecules as fluorescent sensor for recognition of Hg^{2+} , CN^- and PPI and application as INHIBIT logic device

3.3.1 Abstract

To explore the effect of increase of aromaticity to the thiazolidine derivatives, probe 7 with fused aromatic extension has been synthesized involving thiazolidine and hydroxy-1-naphthaldehyde for sensing of cations and anions. Also, 2-hydroxy-1-naphthaldehyde is often used as a precursor for fluorescent chemosensor. Consequently, the introduction of 2-hydroxy-1-naphthaldehyde resulted in a notable increase in the binding affinity, sensitivity, selectivity, leading to reversible fluorescent signalling. Probe 7 has been used for detection of Hg^{2+} ions amongst various metal ions. *In situ* formed $7 \cdot \text{Hg}^{2+}$ complex behaved as a selective fluorescent probe for PPI by a metal displacement approach, ensuing reversal to ligand from the $7 \cdot \text{Hg}^{2+}$ complex. It has also been used for the detection of PPI through UV-Visible and fluorescence spectrophotometer.

3.3.2 Synthesis of probe 7

Probe 7 was prepared according to the synthetic route shown in Scheme 3.3.1. Thiazolidine-2,4-dione was synthesized with thiourea and chloroacetic acid as discussed in chapter 3.2. Thiazolidine-2,4-dione on reaction with 2-hydroxy-1-naphthaldehyde in the presence of sodium acetate and glacial acetic acid formed probe 7 as yellow colored solid in 62% yield; M.pt. > 200 °C; MS (ESI) m/z 272.3 ($\text{M}^+ + \text{H}$) (scheme 3.3.1). The reaction intermediates and final products were fully characterized by different spectroscopic techniques from which satisfactory data was obtained. ^1H NMR spectrum showed 1H singlet at δ 8.63 due to H_g , three 1H doublets at δ 8.37 due to H_a , 8.14 due to H_d and 8.03 due to H_e , 4H multiplet at δ 7.82-7.68 due to $\text{H}_{c,b,f,\text{NH}}$. The peak at δ 8.63 was due to $\text{C}=\text{CH}$, confirmed the formation of probe 7. ^{13}C NMR spectrum showed signals at δ 176.2, 174.7 for $\text{C}=\text{O}$, at δ 150.2 for $\text{C}=\text{C}$, at δ 133.6 for $\text{C}-\text{O}$ and at δ 130.9, 129.3, 128.9, 127.9, 127.3, 126.0, 121.7, 116.9, 115.4 for aromatic-C. This spectral data having extra peaks at δ 176.2 and 174.7 were due to carbonyl groups, at δ 150.2 for $\text{C}=\text{C}$ and at δ 133.6 for $\text{C}-\text{O}$, corroborating structure 7. On the basis of these spectral data, this compound has been assigned the structure of 5-((2-hydroxynaphthalen-1-yl)methylene)thiazolidine-2,4-dione (probe 7).



Scheme 3.3.1: Synthesis of probe 7

3.3.3 Results and discussion

3.3.3.1 UV-Visible studies of probe 7 with metal ions

The recognition behavior of probe 7 towards metal ions has been investigated by absorption spectroscopic technique. In a typical experiment, a solution of 1000 μM metal ions, viz., Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Hg^{2+} , Pb^{2+} , etc., as their perchlorate salts were added to a solution of probe 7 (20 μM , CH_3CN). Amongst these, only Hg^{2+} ions showed significant changes in the absorption behavior of the probe (Figure 3.3.1a). Probe 7 displayed absorption band at 385 nm, the intensity of which upon addition of 0-250 μM of Hg^{2+} ions decreased along with the formation of a new band at 430 nm (Figure 3.3.1b).

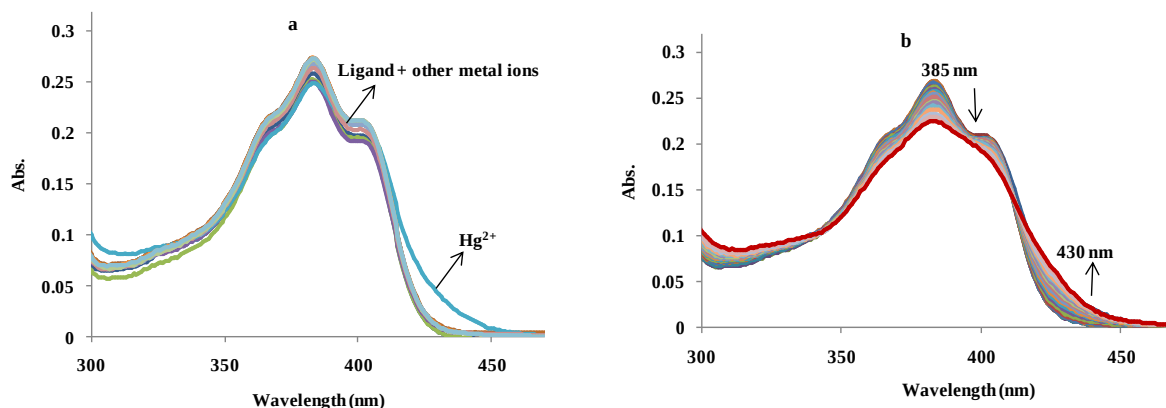


Figure 3.3.1: Absorption spectra of probe 7 (20 μM) (a) with different metal ions; (b) on increasing the concentration of Hg^{2+} ions in CH_3CN .

The absorption ratio of Hg^{2+} ions at 430 nm and 385 nm varied from 0.04 to 0.18, indicating 4-folds absorption ratio change. Thus, probe 7 was used to estimate a wide range of Hg^{2+} between 0.5- 250 μM through UV-Visible spectrophotometer having the lowest detection

limit of 500 nM. The stability constant for the Hg^{2+} in absorption spectroscopy was determined by Benesi-Hildebrand equation and found to be $2.00 \pm 0.02 \times 10^3 \text{ M}^{-1}$.

3.3.3.2 Fluorescence studies of probe 7 with metal ions

Probe 7 (20 μM , CH_3CN) on excitation at 380 nm displayed an emission band at 455 nm. The effect of various metal ions like Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Hg^{2+} , Pb^{2+} , Al^{3+} etc., as their perchlorate salts, were studied on emission spectrum of probe 7 (20 μM , CH_3CN). The emission band of probe 7 at 455 nm remained unaffected in the presence of tested metal ions except for Hg^{2+} (Figure 3.3.2a). The incremental addition of Hg^{2+} ions from 0 to 250 μM showed quenching of emission band at 455 nm with the formation of a new band at 485 nm. (Figure 3.3.2b). The emission ratio for Hg^{2+} ions at 485 nm and 455 nm varied from 0.67 to 1.44, indicating 2-folds emission ratio change. The binding constant was found to be $2.00 \pm 0.02 \times 10^4 \text{ M}^{-1}$, determined by Benesi-Hildebrand equation.

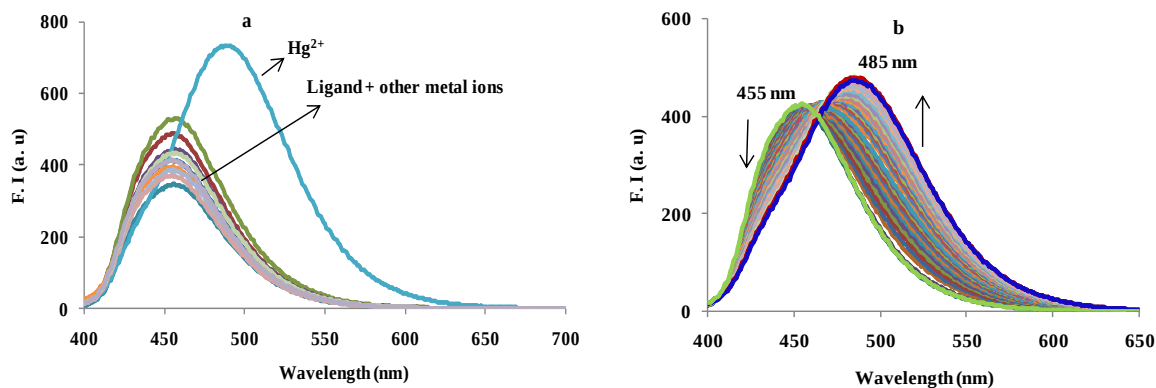


Figure 3.3.2: Emission spectra of probe 7 (20 μM) (a) with different metal ions; (b) on increasing the concentration of Hg^{2+} ions in CH_3CN .

3.3.3.3 Interference studies of probe 7 towards Hg^{2+} ions

The selectivity of probe 7 for Hg^{2+} was examined through measuring the absorption as well as fluorescence responses of probe 7 to different metal ions. As shown in Figures 3.3.1a and 3.3.2a, no noticeable absorption and fluorescence responses were observed to Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Pb^{2+} , Al^{3+} etc., as their perchlorate salts, except Hg^{2+} ions to which there were absorption responses at 430 nm and emission responses at 485 nm. For further evaluating the Hg^{2+} selectivity in complex backgrounds, competing experiments were carried out by measuring the absorption and fluorescence responses to Hg^{2+} in the presence of

interfering ions. Figure 3.3.3 showed the absorption response upon addition of various metal ions to the solutions of probe **7**. Hg^{2+} complex. The Hg^{2+} -induced absorption response was not affected by the presence of any other metal ions. The observations revealed that probe **7** is a highly selective towards Hg^{2+} ions.

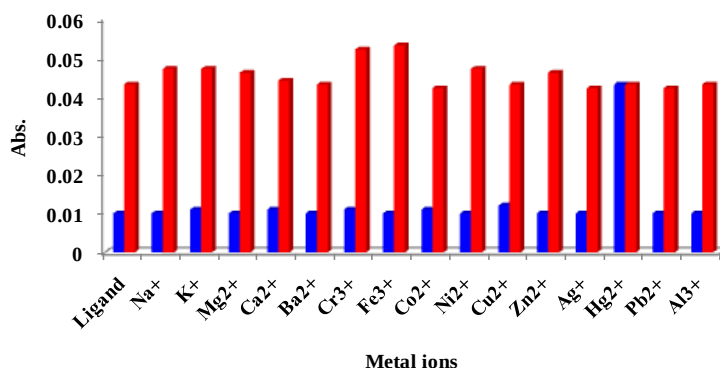


Figure 3.3.3: Blue bars represent the selectivity of probe **7** (20 μM , CH_3CN) upon addition of different metal ions and red bars shows the competitive selectivity of probe **7** in the presence of Hg^{2+} ions.

3.3.3.4 UV-Visible studies of probe **7** with anions

As probe **7** has a hydroxyl group, it prompted us to evaluate the binding behavior of probe **7** toward different anions. In a typical experiment, a solution of the anions, viz., F^- , CN^- , Cl^- , SCN^- , AcO^- , NO_3^- , Br^- , HSO_4^- , H_2PO_4^- , I^- , PPI etc., as their tetrabutylammonium salts were added to a solution of probe **7** (20 μM , CH_3CN).

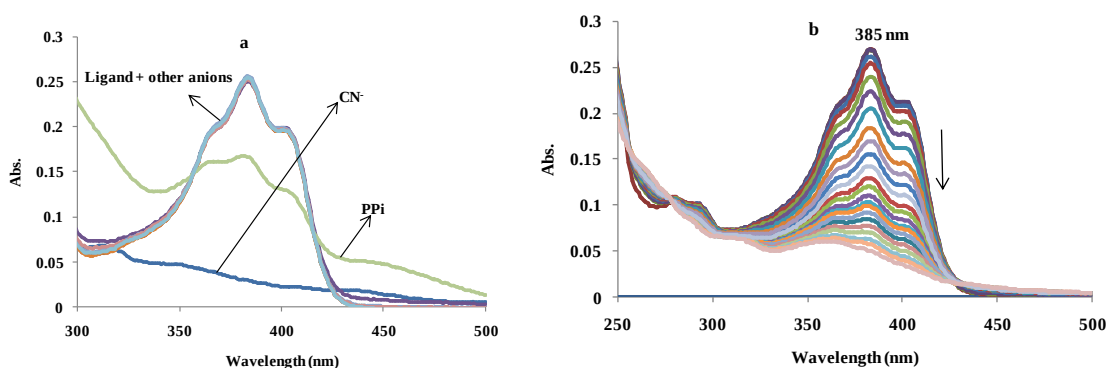


Figure 3.3.4: Absorption response of probe **7** (20 μM , CH_3CN) (a) with different anions; (b) on increasing the concentration of CN^- ions.

Amongst these anions, only CN^- and PPI resulted in significant changes in the absorption spectral behavior of the probe (Figure 3.3.4a). Incremental addition of CN^- ions to a solution of probe **7**

caused the decrease in absorption intensity at 385 nm (Figure 3.3.4b). Thus, probe 7 may be used for the detection of CN^- ions between 0.5-50 μM . To know the strength of binding of CN^- ions to probe 7, equilibrium constant was extracted to be $5.33 \pm 0.03 \times 10^4 \text{ M}^{-1}$. More interesting results were investigated upon addition of 1000 μM of PPI, the band at 385 nm underwent decrease with the simultaneous formation of new bands at 445 nm and 540 nm (Figure 3.3.5a). The absorption ratio for PPI ions at 445 nm and 385 nm varied from 0.003 to 0.31, indicating 102-folds absorption ratio change (Figure 3.3.5b) having a stability constant value of $4.41 \pm 0.03 \times 10^5 \text{ M}^{-1}$.

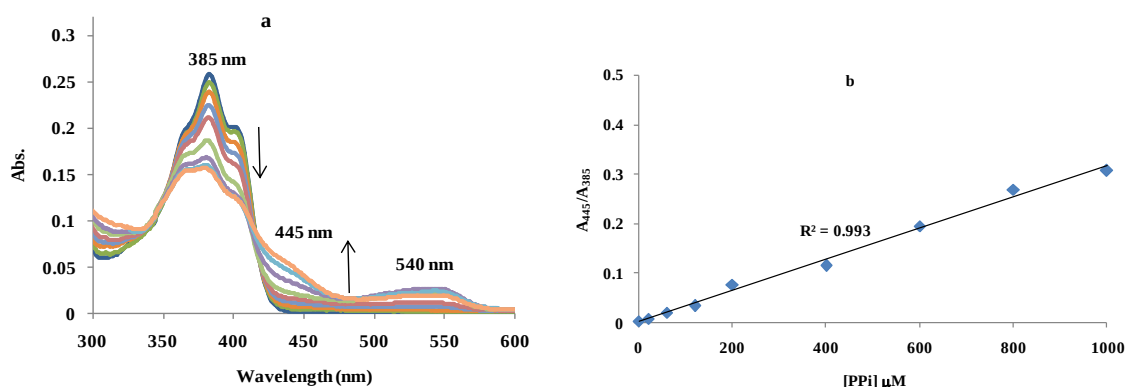


Figure 3.3.5: (a) Absorption on increasing the concentration of PPI in CH_3CN ; (b) absorption ratiometric responses A_{445}/A_{385} upon incremental addition of PPI of probe 7 (20 μM , CH_3CN).

3.3.3.5 Effect of various anions on emission spectrum of probe 7

On excitation at 380 nm, probe 7 showed the emission band at 455 nm which underwent change with CN^- with the interference from PPI at higher equivalents out of various anions, viz., F^- , CN^- , Cl^- , SCN^- , AcO^- , NO_3^- , Br^- , HSO_4^- , H_2PO_4^- , I^- , PPI etc., as their tetrabutylammonium salts (Figure 3.3.6a). Upon addition of 0-75 μM of CN^- ions (Figure 3.3.6b), band at 455 nm underwent quenching. The equilibrium constant was determined to be $2.17 \pm 0.02 \times 10^3 \text{ M}^{-1}$ by Benesi-Hildebrand equation. The lowest detection limit of CN^- was 500 nM. To determine the practical applicability of probe 7, we carried out competitive experiments in the presence of 1000 μM of CN^- ions mixed with 1000 μM of each of the various ions. No significant variation in the fluorescence intensity was observed when comparing with or without the anions (Figure 3.3.7). Due to hydrogen of hydroxyl group and one oxygen atom of thiazolidine molecule could have some hydrogen bonding interactions, which on addition of CN^- ions underwent disturbed and migration of proton resulted fluorescence quenching.

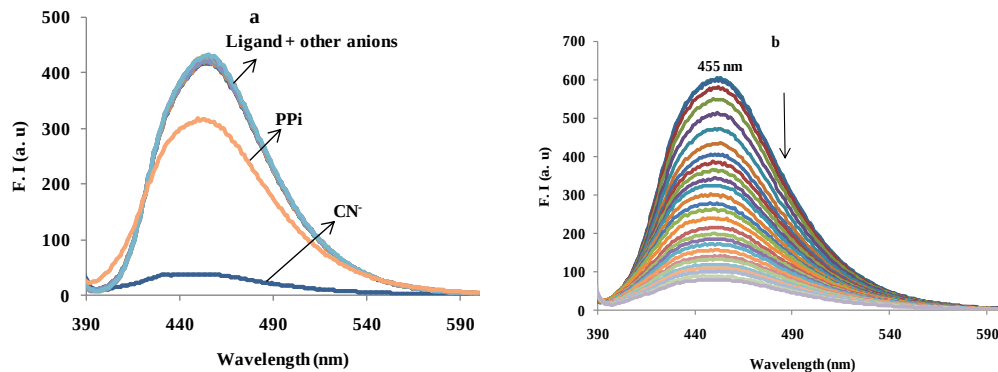


Figure 3.3.6: Emission response of probe 7 (20 μM , CH_3CN) (a) with different anions; (b) on increasing the concentration of CN^- ions.

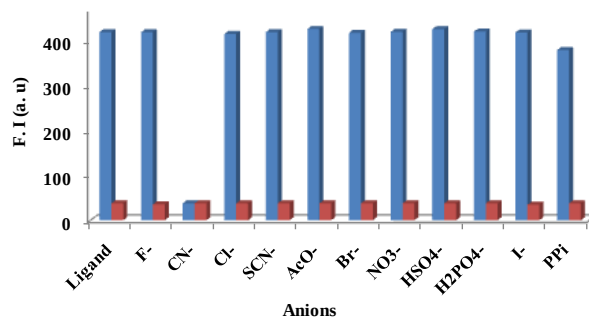


Figure 3.3.7: Blue bars represent the selectivity of probe 7 (20 μM , CH_3CN) upon addition of different anions and red bars show the competitive selectivity of probe 7 in the presence of CN^- ions.

3.3.3.6 Elaboration of probe 7 as INHIBIT logic gate

The emission response of probe 7 mimicked an INHIBIT logic gate with Hg^{2+} and PPI as chemical inputs and emission of both at 485 nm as output.

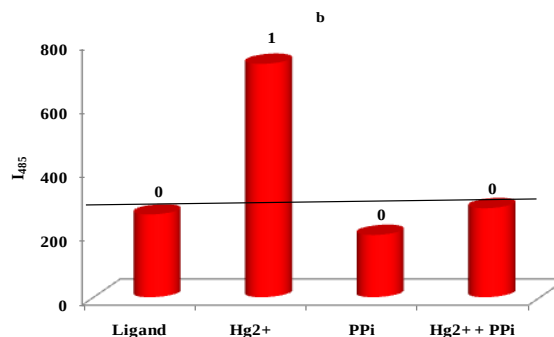
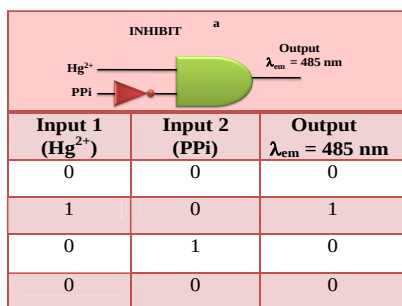


Figure 3.3.8: (a) Logic scheme and truth table for the INHIBIT logic gate of probe 7; (b) bar diagram of the logic circuit INHIBIT gate in emission spectroscopy at 485nm.

The INHIBIT logic gate is a combination of AND and NOT gates. The NOT gate converts input signals into the output response when the input is 1, the output is 0 and when the input is 0, the output is 1. The AND gate translates two input signals into one output. In the case of AND gate, the output is 1 only when both the inputs are 1 and 0 in other three cases. Probe 7 showed very weak emission at 485 nm (“0” state). In the presence of Hg^{2+} as chemical input, probe 7 exhibited fluorescence enhancement at 485 nm (“1” state), while in the presence of PPI and simultaneous presence of both Hg^{2+} and PPI as chemical inputs, produced weak emission (“0” state) (Figure 3.3.8). Therefore, these combinations mimicked INHIBIT logic gate with a threshold value of 278 nm.

3.3.3.7 Theoretical calculations

To know more about binding strategies of probe 7 towards Hg^{2+} ions, geometry optimization were performed. The probe 7 was optimized in ground state through DFT and TD-SCF calculations with B3LYP-631G methods. The complex of probe 7 with Hg^{2+} ion, was optimized in gaseous phase using B3LYP-LAN2DZ basis set.

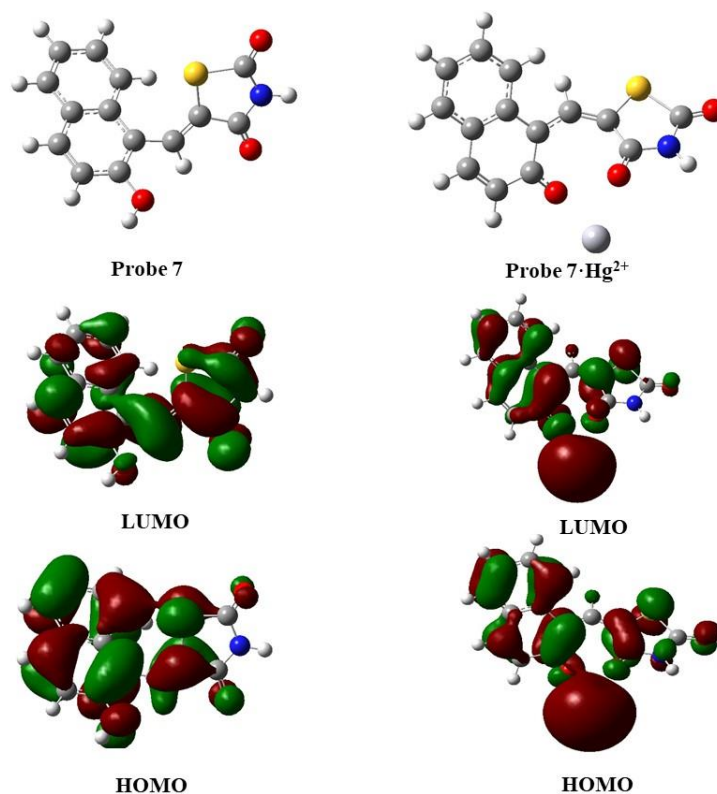


Figure 3.22: Energy minimized structure and frontier molecular orbital contribution of probe 7 and its complexes with Hg^{2+} ions.

The excited state of probe **7** and its complex probe **7·Hg²⁺** showed the absorption maximum bands at 383 nm and 433 nm in the gaseous state which is in good agreement with the experimental value at 385 nm and 430 nm, respectively. The electronic transition can be considered as π - π^* transition. It was investigated that Hg²⁺ ion showed its binding through O of naphthyl with a bond distance of 2.39 Å and O of thiazole ring with a bond distance of 2.5 Å. Frontier molecular orbital analysis showed that HOMO of probe **7** was located at naphthyl ring and LUMO was extended to thiazole ring. On the other HOMO of complex probe **7·Hg²⁺** was distributed over Hg²⁺ and naphthyl ring which was further extended on thiazole ring (Figure 3.3.9).

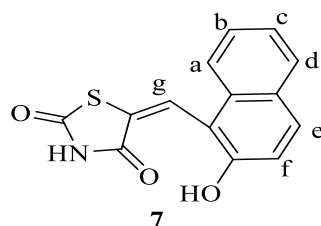
3.3.4 Conclusion

Thiazolidine based probe **7** has been synthesized and was used for selective determination of Hg²⁺ ions amongst various metal ions. It has also been used for the detection of CN⁻ and PPi ions through UV-Visible and fluorescence spectroscopies. The *in situ* formed **7·Hg²⁺** complex behaves as a selective fluorescent probe for PPi by a metal displacement approach, ensuing reversal to ligand from the **7·Hg²⁺** complex. The probe **7** was used for wide range determination of Hg²⁺ ions from 0.5-250 µM in CH₃CN. Depending on two inputs of Hg²⁺ and PPi and outputs at emission wavelength at 485 nm, INHIBIT logic gate has been mimicked.

3.3.5 Experimental section

Synthesis of probe 7: Synthesis of probe **7** was carried out with thiazolidine-2,4-dione (1 g, 8.5 mmol) and 2-hydroxynaphthaldehyde (2.32 g, 8.5 mmol) in the presence of sodium acetate (0.70 g, 8.5 mmol) in 5 mL glacial acetic acid. After 24 h heating to 120 °C, solid separated out and filtered to give the crude product which was further purified with the help of column chromatography and pure probe **7** obtained as yellow solid.

5-((2-Hydroxynaphthalen-1-yl)methylene)thiazolidine-2,4-dione (probe 7)



62% yield; yellow solid; M.pt. > 200 °C; ¹H NMR (CDCl₃, 400 MHz): δ (ppm) 8.63 (s, 1H, ArH_g), 8.37 (d, 1H, *J* = 8.40 Hz, ArH_a), 8.14 (d, 1H, *J* = 9.16 Hz, ArH_d), 8.03 (d, 1H, *J* = 8.40 Hz, ArH_e), 7.82-7.68 (m, 4H, ArH_{c,b,f,NH}); ¹³C NMR (CDCl₃, 100 MHz): δ (ppm) 176.2, 174.7

(C=O), 150.2 (C=C), 133.6 (C-O), 130.9, 129.3, 128.9, 127.9, 127.3, 126.0, 121.7, 116.9, 115.4 (ArC); MS (ESI) m/z 272.3 ($M^+ + H$).

3.4 Thiazolidine based solvent dependent differential chemosensor for metal ions and anions

3.4.1 Abstract

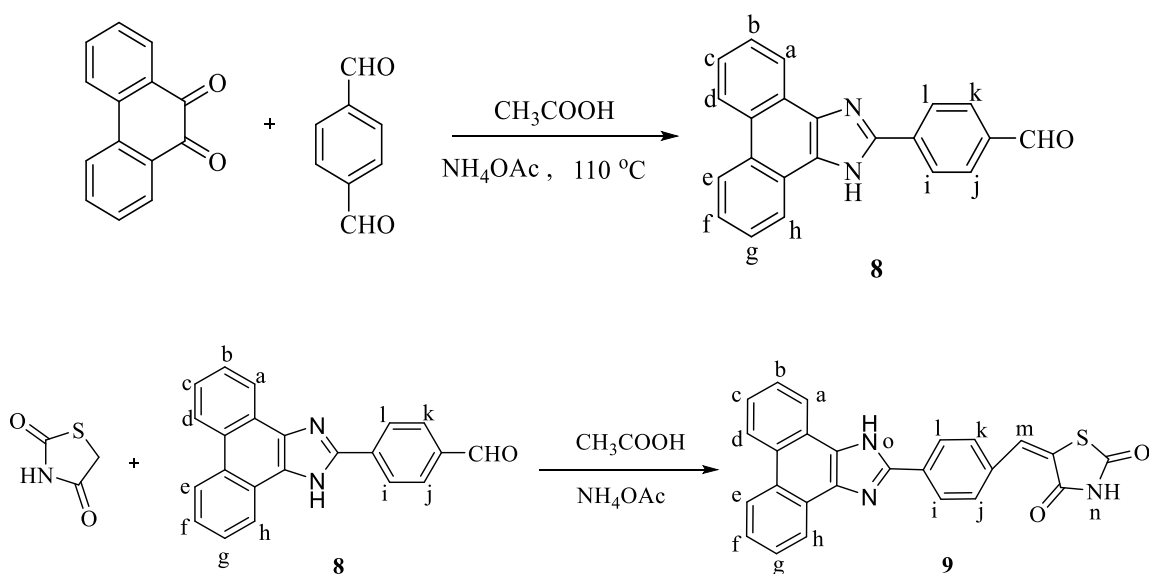
In continuation to synthesis of thiazolidine derivatives as chemosensors, a new derivative based on thiazolidine and phenanthroimidazol moieties has been synthesized. The main purpose to synthesize such molecules was to study of effect of hybridization with other fluorescent moiety on sensing of various ions. This hybrid molecule was investigated towards various cations and anions in polar as well as nonpolar solvents. Probe **9** exhibits absorption band at 395 nm and emission band at 560 nm in CH₃CN while in CH₃OH:H₂O (9:1, v/v) absorption band at 385 nm and emission band at 530 nm was observed. It has been used for detection of Hg²⁺ and Al³⁺ ions in both solvents (CH₃CN and CH₃OH:H₂O) amongst various metal ions. Interestingly, probe **9** showed drastic change with H₂PO₄⁻ in CH₃CN and HSO₄⁻ in CH₃OH:H₂O (9:1, v/v). Thus, a single probe worked differently in polar and nonpolar solvents.

3.4.2 Synthesis of compounds **8** and **9**

Probe **9** was synthesized according to the synthetic route as shown in Scheme 3.4.1. Thiazolidine-2,4-dione was synthesized with thiourea and chloroacetic acid as discussed in chapter 3.2. Compound **8** was synthesized by refluxing a solution of 9,10-phenanthrenequinone, terephthalaldehyde and ammonium acetate in the presence of glacial acetic acid for 1 h to obtain a yellow colored solid in 60% yield; M.pt. > 300 °C; MS (ESI) m/z 323.1 (M⁺ + H). The ¹H NMR spectrum showed 1H singlet at δ 13.71 for NH, 1H singlet at δ 10.07 due to CHO proton, six 2H doublets at δ 8.86 due to H_{d,e}, δ 8.59 due to H_{a,h}, δ 8.52 due to H_{j,k}, δ 8.13 due to H_{i,l}, δ 7.75 due to H_{c,f}, δ 7.67 due to H_{b,g}. The peak at δ 0.07 was due to CHO, confirmed the formation of compound **8**. The ¹³C NMR spectrum showed signals at δ 193.1 for aldehyde-C, 148.3, 130.6, 137.9, 136.5, 135.9, 128.8, 128.2, 127.3, 122.7, 127.8, 126.9, 126.3, 126.0, 124.7, 124.3, 122.7, 122.4 for aromatic-C. On the basis of these spectral data, this compound has been assigned the structure of 4-(1*H*-phenanthro[9,10-*d*]imidazol-2-yl) benzaldehyde (compound **8**).

Treatment of thiazolidine-2,4-dione with compound **8** in the presence of sodium acetate in glacial acetic acid afforded probe **9** in 65% yield as orange solid; M.pt. = 215-220 °C; MS (ESI) m/z 422.5 (M⁺ + H). The ¹H NMR spectrum showed 1H singlet at δ 13.62 for NH_o, two 2H singlet at δ 8.85 due to H_{d,e} and δ 8.56 due to H_{a,h}, 2H doublet at δ 8.43 due to H_{i,l}, 3H multiplet

at δ 7.82–7.79 due to $H_{m,c,f}$, 2H singlet at δ 7.74 due to $H_{j,k}$, 2H triplet at δ 7.64 due to $H_{b,g}$ and 1H singlet at δ 4.13 for NH_n . The peaks at δ 13.62 and δ 4.13 were due to NH, confirmed the formation of probe **9**. The ^{13}C NMR spectrum showed signals at δ 168.2, 168.1 for C=O, δ 148.1 for C=C, δ 133.6 for C-O and δ 131.5, 130.7, 127.8, 127.3, 126.7, 125.6, 124.5, 123.9, 122.1 for aromatic-C. This spectral data having extra peaks at δ 168.2 and 168.1 was due to carbonyl groups, δ 148.1 for C=C and δ 133.6 for C-O corroborating structure **9**. On the basis of these spectral data, this compound has been assigned the structure of 5-(4-(1*H*-phenanthro[9,10-*d*]imidazol-2-yl)benzylidene)thiazolidine-2,4-dione (probe **9**).



Scheme 3.4.1: Synthesis of compounds **8 and **9****

3.4.3 Results and discussion

The binding behavior of probe **9** was studied toward different cations and anions by UV-Visible and fluorescence spectroscopy in CH_3CN and $CH_3OH:H_2O$ (9:1, v/v).

3.4.3.1 UV-Visible studies of probe **9** towards different metal ions in CH_3CN

The absorption spectrum of probe **9** (10 μM , CH_3CN) was characterized by the presence of a typical absorption band at 395 nm. Out of various tested metal ions such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Hg^{2+} , Pb^{2+} , Al^{3+} etc., as their perchlorate salts, (1000 μM) only Hg^{2+} and Al^{3+} ions showed the spectral changes (Figure 3.4.1). The presence of other metal ions did not show any significant change. The incremental addition of Hg^{2+} ions (15

μM) and Al^{3+} ions ($35 \mu\text{M}$) to the solution of probe **9** showed a new absorption band at 370 nm with an isosbestic point at 383 nm (Figure 3.4.2a), indicating the formation of new species. The change in absorption ratio due to presence of Hg^{2+} ions at 370 nm and 395 nm varied from 0.772 to 1.27, indicating 2-folds absorption ratio changes and for Al^{3+} ions at 370 nm and 395 nm varied from 0.77 to 1.16, indicating 2-folds absorption ratio changes (Figure 3.4.2b). Thus, probe **9** has been used to quantify of Hg^{2+} and Al^{3+} between $0.5\text{--}15 \mu\text{M}$ and $0.5\text{--}35 \mu\text{M}$ through UV-Visible spectrophotometry having the lowest detection limit of 500 nM. The association constant for Hg^{2+} and Al^{3+} ions in absorption spectroscopy was determined by Benesi-Hildebrand equation which has been found to be $2.50 \pm 0.02 \times 10^5 \text{ M}^{-1}$ and $4.60 \pm 0.03 \times 10^4 \text{ M}^{-1}$, respectively.

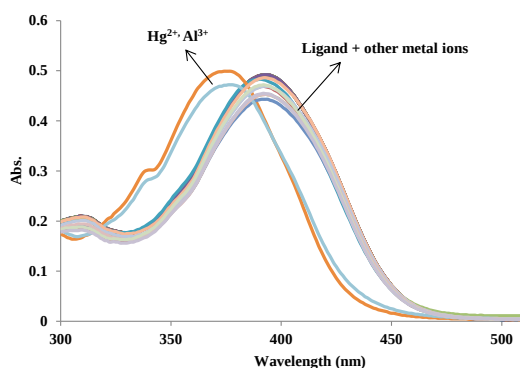


Figure 3.4.1: Absorption response of probe **9** ($10 \mu\text{M}$, CH_3CN) with different metal ions.

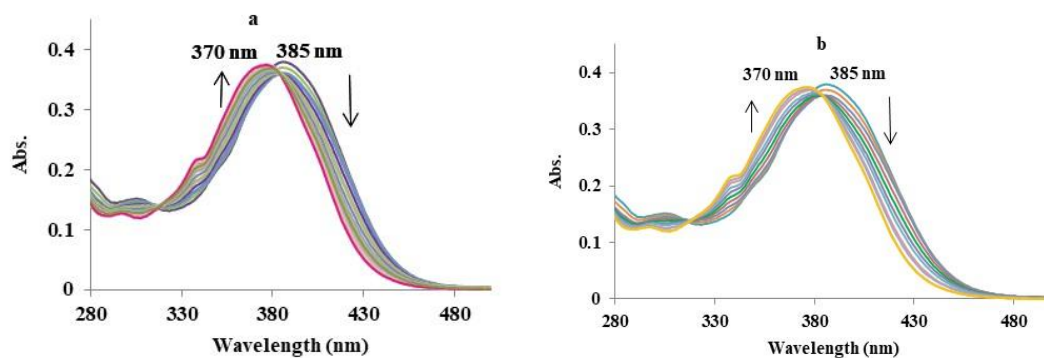


Figure 3.4.2: Effects of incremental addition of (a) Hg^{2+} ions; (b) Al^{3+} ions to the solution of probe **9** ($10 \mu\text{M}$, CH_3CN).

3.4.3.2 Fluorescence studies of probe **9** towards different metal ions in CH_3CN

The emission spectrum of probe **9** ($1 \mu\text{M}$, CH_3CN) showed a characteristic emission band at 560

nm upon exciting at 385 nm. Out of various metal ions tested (Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Hg^{2+} , Pb^{2+} , Al^{3+} etc., as their perchlorate salts, (1000 μM)) only Hg^{2+} and Al^{3+} ions showed the spectral changes. The presence of other metal ions did not induce any significant change (Figure 3.4.3). The probe **9** showed blue shift on the addition of Hg^{2+} and Al^{3+} ions. The incremental addition of 2.5 μM Hg^{2+} ions and 15 μM Al^{3+} ions to the solution of the probe **9**, showed the formation of a new band at 485 nm (Figure 3.4.4). The emission ratio at 485 nm and 560 nm varied from 0.42 to 3.23, indicating 8-folds emission ratio change for Hg^{2+} ions and 0.42 to 2.12, indicating 5-folds emission change for Al^{3+} ions. The binding constants for Hg^{2+} and Al^{3+} ions were $2.00 \pm 0.02 \times 10^6 \text{ M}^{-1}$ and $1.25 \pm 0.02 \times 10^3 \text{ M}^{-1}$, determined by Benesi-Hildebrand plot.

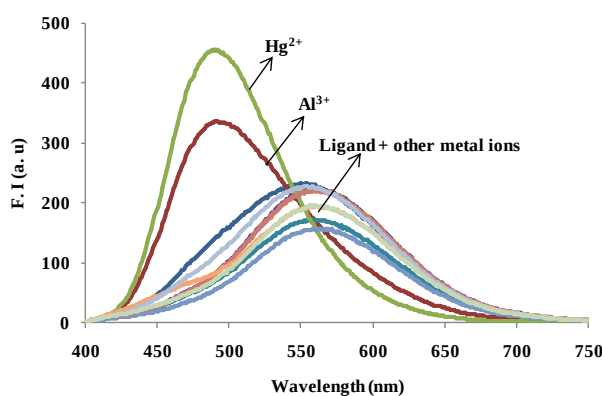


Figure 3.4.3: Emission response of probe **9** (1 μM , CH_3CN) with different metal ions.

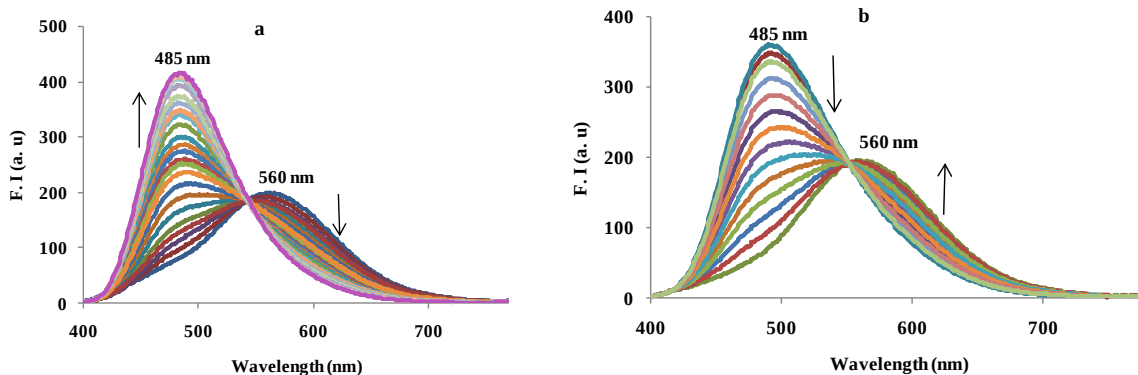


Figure 3.4.4: Effects of incremental addition of (a) Hg^{2+} ions; (b) Al^{3+} ions to the solution of probe **9** (10 μM , CH_3CN).

The emission response of probe **9** toward Hg^{2+} and Al^{3+} ions in the presence of 100 eq. of other metal ions such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Pb^{2+}

etc., as their perchlorate salts, were checked in CH₃CN, but no change in the emission intensity was observed which confirmed the selectivity of probe **9** towards Hg²⁺ and Al³⁺ ions (Figure 3.4.5).

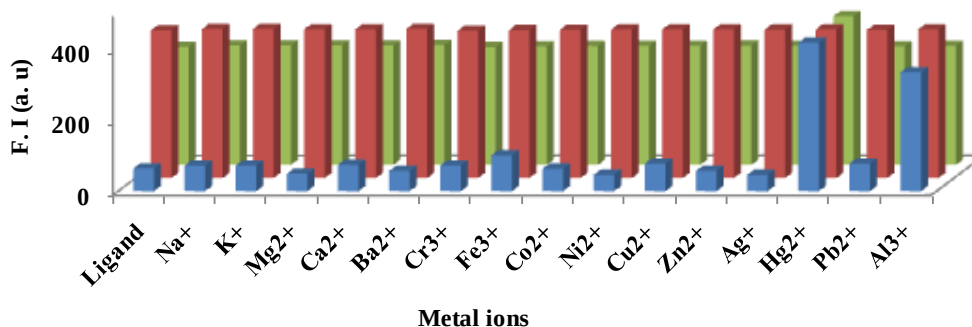


Figure 3.4.5: Blue bars show the effect of different metal ions on fluorescence response of probe **9** (1 μ M, CH₃CN); red bars show the presence of interfering metal ions on probe **9** + Hg²⁺; green bars show the presence of interfering metal ions on probe **9** + Al³⁺.

3.4.3.3 UV-Visible studies of probe **9** towards different anions in CH₃CN

As probe **9** comprises two NH groups, it persuaded us to check the effects of various anions. Only H₂PO₄⁻ ions caused changes in case of absorbance spectra in CH₃CN, out of all other anions like F⁻, CN⁻, Cl⁻, SCN⁻, AcO⁻, NO₃⁻, Br⁻, HSO₄⁻, I⁻ etc., as their tetrabutylammonium salts (Figure 3.4.6a). There was formation of a new band at 380 nm upon addition of 15 μ M solution of H₂PO₄⁻ ions with the formation of the clear isosbestic point at 390 nm which indicated for the formation of new species (Figure 3.4.6b)

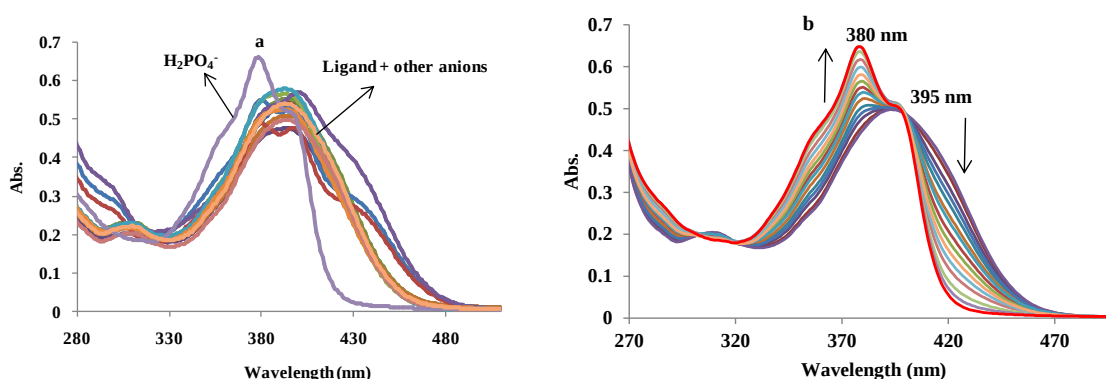


Figure 3.4.6: Absorption response of probe **9** (10 μ M, CH₃CN) (a) with different anions; (b) on increasing the concentration of H₂PO₄⁻ ions.

The absorption ratio at 380 nm and 395 nm varied from 0.91 to 1.27, indicating a 1.39-folds absorption change. Thus, probe **9** was used to estimate a wide range of H_2PO_4^- ions through UV-Visible spectrophotometry (0-15 μM). The equilibrium constant for H_2PO_4^- ions was determined to be, $8.64 \pm 0.01 \times 10^4 \text{ M}^{-1}$.

3.4.3.4 Fluorescence studies of probe **9** towards different anions in CH_3CN

Out of various anions tested F^- , CN^- , Cl^- , SCN^- , AcO^- , NO_3^- , Br^- , HSO_4^- , H_2PO_4^- , I^- etc., as their tetrabutylammonium salts (1000 μM), only H_2PO_4^- showed the spectral changes with the small interference of CN^- ions at higher equivalents whereas other anions did not show any significant change (Figure 3.4.7a). The incremental addition of H_2PO_4^- ions to probe **9** induced the formation of a new emission band at 470 nm, attributed to deprotonation of NH of probe **9** upon addition of 2 μM solution of H_2PO_4^- ions with 100 nM to be its lowest detection value (Figure 3.4.7b). The emission ratio at 470 nm and 560 nm varied from 0.22 to 6.98, indicating 31-folds emission change. Thus, probe **9** showed 90 nm blue shift and was used to quantify 0-2 μM of H_2PO_4^- ions through fluorescence spectrophotometry with equilibrium constant $5.12 \pm 0.01 \times 10^6 \text{ M}^{-1}$, determined by Benesi-Hildebrand equation. To assess the selectivity of probe **9** towards H_2PO_4^- ions among other ions, an experiment was performed by taking 1000 μM of various anions such as F^- , CN^- , Cl^- , SCN^- , AcO^- , NO_3^- , Br^- , HSO_4^- , I^- etc., and 1000 μM of H_2PO_4^- and emission was recorded. Results revealed that there was no significant variation in the emission band when comparing it with or without other anions (Figure 3.4.8).

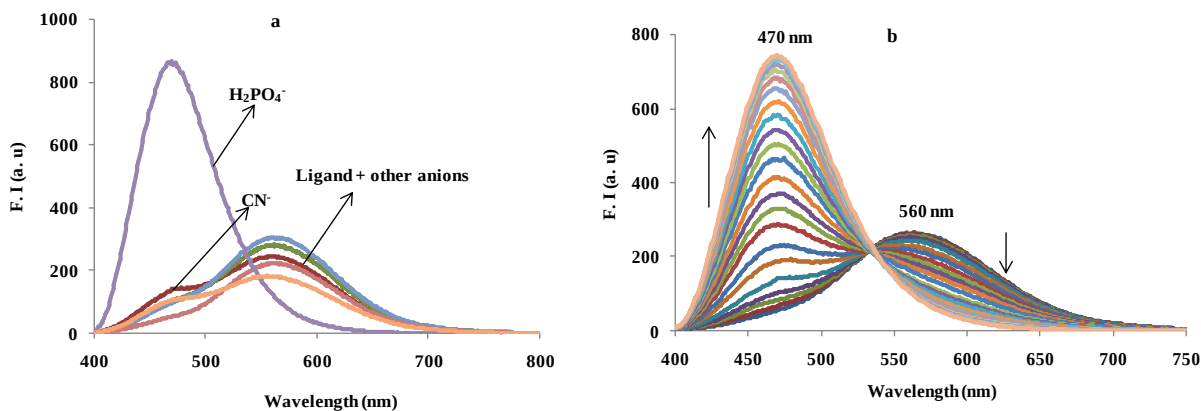


Figure 3.4.7: Emission response of probe **9** (1 μM , CH_3CN) (a) towards different anions; (b) on increasing the concentration of H_2PO_4^- ions.

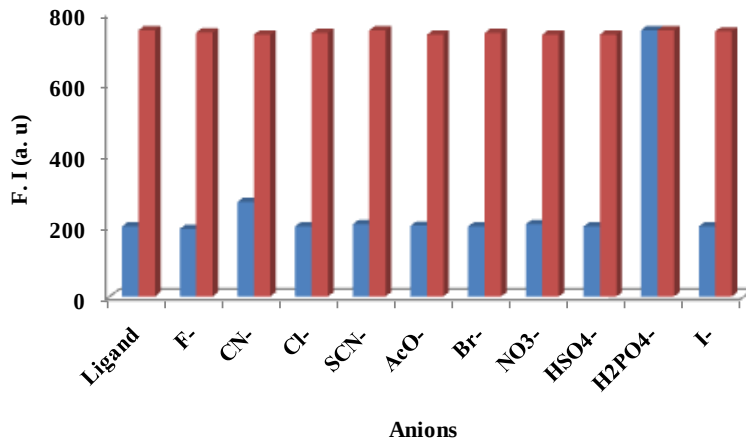


Figure 3.4.8: Blue bars represent the selectivity of probe **9** (1 μ M, CH₃CN) upon addition of different anions and red bars show the competitive selectivity of probe **9** (1 μ M, CH₃CN) in the presence of H₂PO₄⁻ ions.

3.4.3.5 UV-Visible response of probe **9** towards different metal ions in CH₃OH:H₂O (9:1, v/v).

The absorption spectrum of probe **9** (10 μ M, CH₃OH:H₂O (9:1, v/v)) was characterized by the presence of a typical absorption band at 385 nm (Figure 3.4.9). Out of various tested metal ions (Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Cr³⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Ag⁺, Hg²⁺, Pb²⁺, Al³⁺ etc., as their perchlorate salts, (1000 μ M)). Only Hg²⁺ and Al³⁺ ions showed the spectral changes whereas other metal ions did not show any significant change. The incremental addition of Hg²⁺ (100 μ M) and Al³⁺ ions (100 μ M), showed a new band at 370 nm with an isosbestic point at 381 nm to the solution of probe **9** (Figure 3.4.10). Thus, probe **9** was used to quantify a wide range of Hg²⁺ and Al³⁺ ions between 0.5-100 μ M through UV-Visible spectrophotometry, having the lowest detection limit of 500 nM. The association constants for Hg²⁺ and Al³⁺ ions in absorption spectroscopy were determined by Benesi-Hildebrand equation which have been found to be $9.14 \times 10^4 \text{ M}^{-1}$ and $5.77 \pm 0.01 \times 10^4 \text{ M}^{-1}$ respectively. We also checked the absorption response of probe **9** towards Hg²⁺ and Al³⁺ in the presence of other 100 eq. of metal ions as Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Cr³⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Ag⁺, Pb²⁺ etc., as their perchlorate salts, in the same conditions, no change in the absorption intensity was observed which confirmed the selectivity of probe **9** towards Hg²⁺ and Al³⁺ ions (Figure 3.4.11).

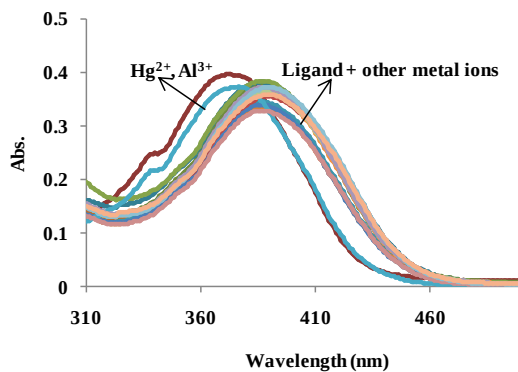


Figure 3.4.9: Absorption response of probe **9** (1 μ M, CH₃OH:H₂O (9:1, v/v)) with different metal ions.

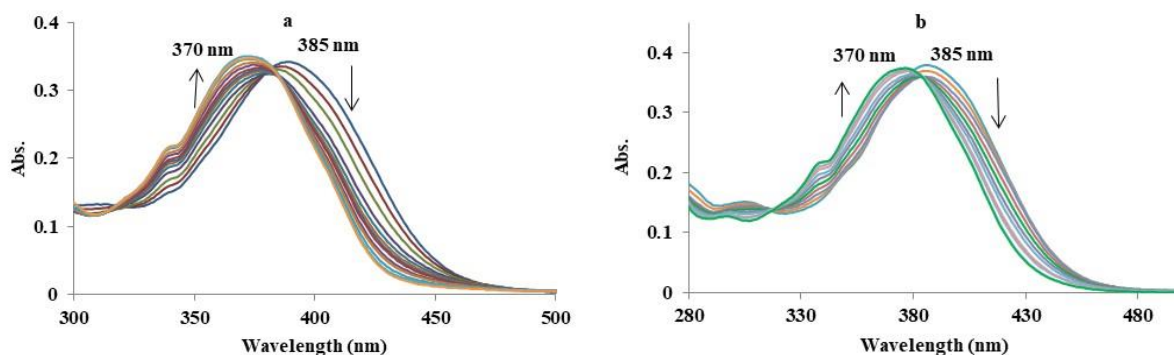


Figure 3.4.10: Effects of incremental addition of (a) Hg²⁺ ions; (b) Al³⁺ ions to the solution of probe **9** (10 μ M, CH₃OH:H₂O (9:1, v/v)).

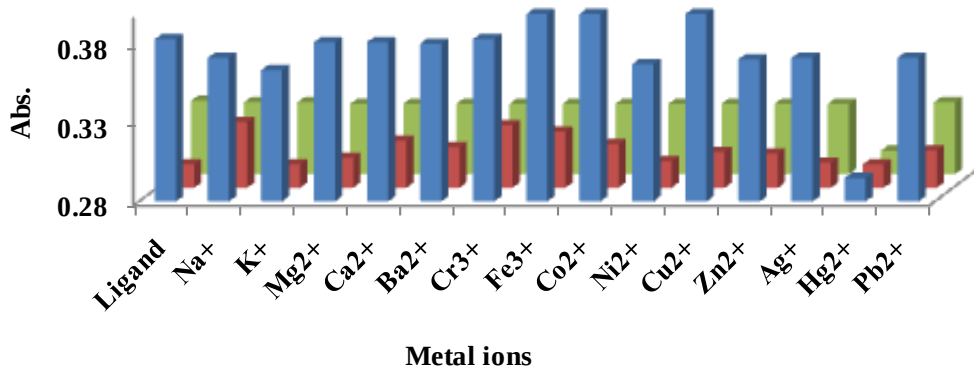


Figure 3.4.11: Blue bars show the effect of different metal ions on absorbance response of probe **9** (20 μ M, CH₃OH:H₂O (90:10, v/v)); red bars show the presence of interfering metal ions on probe **9** + Hg²⁺; green bars show the presence of interfering metal ions on probe **9** + Al³⁺.

3.4.3.6 Fluorescence studies of probe **9** towards different metal ions in CH₃OH:H₂O (9:1, v/v).

The emission spectrum of probe **9** (1 μ M, CH₃OH:H₂O (9:1, v/v)) showed a characteristic band at 530 nm upon excitation at 385 nm. Out of various metal ions tested (Na⁺, K⁺, Mg²⁺, Ca²⁺, Ba²⁺, Cr³⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Ag⁺, Hg²⁺, Pb²⁺, Al³⁺ etc., as their perchlorate salts, (1000 μ M)), only Hg²⁺ and Al³⁺ ions showed the spectral changes whereas other metal ions did not show any significant change (Figure 3.4.12).

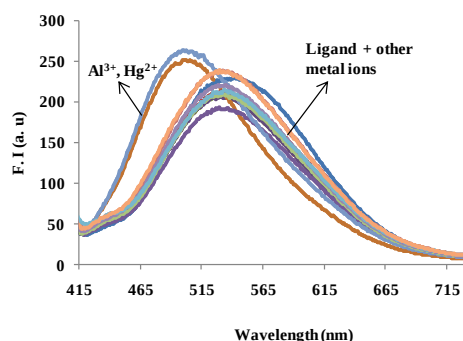


Figure 3.4.12: Emission response of probe **9** (1 μ M) with different metal ions in CH₃OH:H₂O (9:1, v/v).

The probe **9** showed blue shift on the addition of Hg²⁺ and Al³⁺ ions. The incremental addition of 1.2 μ M of Hg²⁺ ions and 2 μ M of Al³⁺ ions to a solution of probe **9**, quenching at 530 nm was observed and further addition of 35 μ M of both Hg²⁺ ions and Al³⁺ ions, a new emission band appeared at 500 nm.

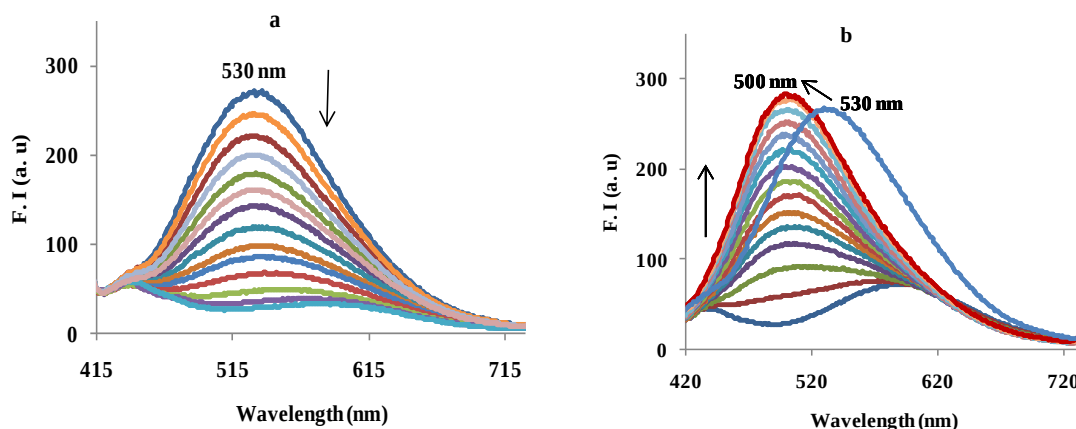


Figure 3.4.13: Emission responses of probe **9** (1 μ M) on increasing the concentration of Hg²⁺ ions from (a) 0.05-1 μ M; (b) 2-30 μ M in CH₃OH:H₂O (9:1, v/v).

The emission value at 500 nm varied from 29 to 277, indicated 10-folds emission change for Hg^{2+} ions and 32 to 219, indicating 7-folds emission change (Figures 3.4.13, 3.4.14). The binding constants for Hg^{2+} and Al^{3+} ions were $7.51 \pm 0.02 \times 10^4 \text{ M}^{-1}$ and $4.00 \pm 0.02 \times 10^4 \text{ M}^{-1}$, determined by Benesi-Hildebrand equation.

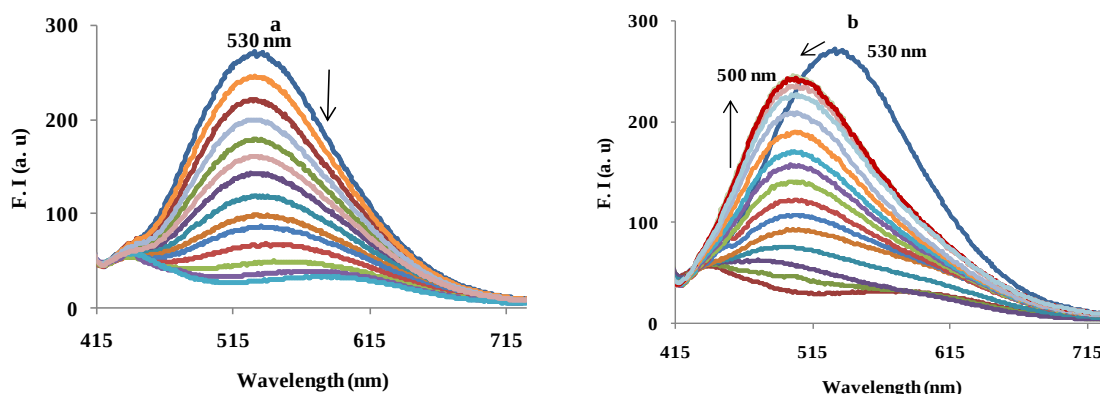


Figure 3.4.14: Emission response of probe **9** (1 μM) on increasing the concentration of Al^{3+} ions from (a) 0.05-2 μM ; (b) 5-40 μM in $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (9:1, v/v).

3.4.3.7 UV-Visible studies of probe **9** towards different anions in $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (9:1, v/v).

Effect of various anions like F^- , CN^- , Cl^- , SCN^- , AcO^- , NO_3^- , Br^- , HSO_4^- , H_2PO_4^- , I^- etc., as their tetrabutylammonium salts, on probe **9** (10 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (9:1, v/v)) was observed. No significant change in absorbance spectrum was observed except HSO_4^- ions.

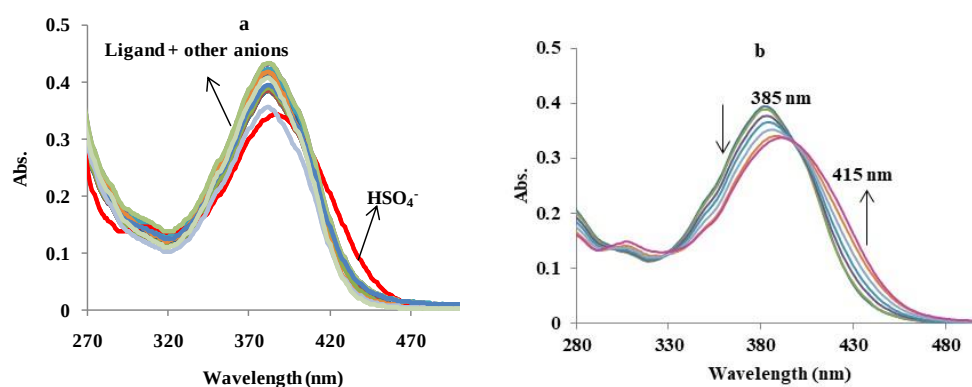


Figure 3.4.15: Absorption response of probe **9** (10 μM) (a) with different anions; (b) on increasing the concentration of HSO_4^- ions.

Upon incremental addition of HSO_4^- ions, absorbance intensity at 385 nm decreased with the formation of a new band at 415 nm with an isosbestic point 400 nm (Figure 3.4.15).

The absorption ratio at 415 nm and 385 nm varied from 0.48 to 0.78, indicating 2-folds absorption changes. The equilibrium constant for HSO_4^- ions was $5.95 \pm 0.01 \times 10^4 \text{ M}^{-1}$.

3.4.3.8 Fluorescence studies of probe 9 towards different anions in $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (9:1, v/v).

Out of various anions tested ions (F^- , CN^- , Cl^- , SCN^- , AcO^- , NO_3^- , Br^- , HSO_4^- , H_2PO_4^- , I^- etc., as their tetrabutylammonium salts (1000 μM)), only HSO_4^- showed the spectral changes whereas other anions did not show any significant change (Figure 3.4.16a). Upon incremental addition of 2 μM HSO_4^- ions, quenching of the band at 530 nm was observed. The emission value at 530 nm decreased which helps to quantify HSO_4^- ions with 100 nM to be its lowest detection value (Figure 3.4.16b). The equilibrium constant for HSO_4^- ions was $3.05 \pm 0.01 \times 10^6 \text{ M}^{-1}$, determined by Benesi-Hildebrand equation.

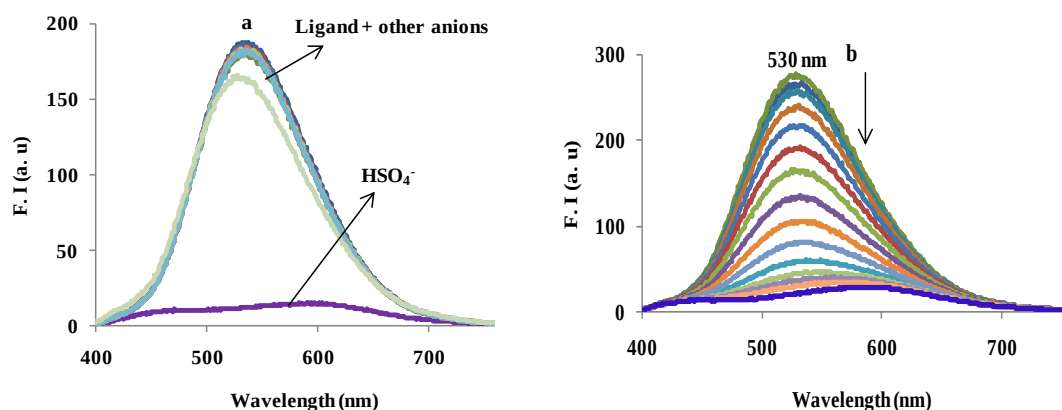


Figure 3.4.16: Emission response of probe 9 (1 μM) (a) with different anions; (b) on increasing the concentration of HSO_4^- ions.

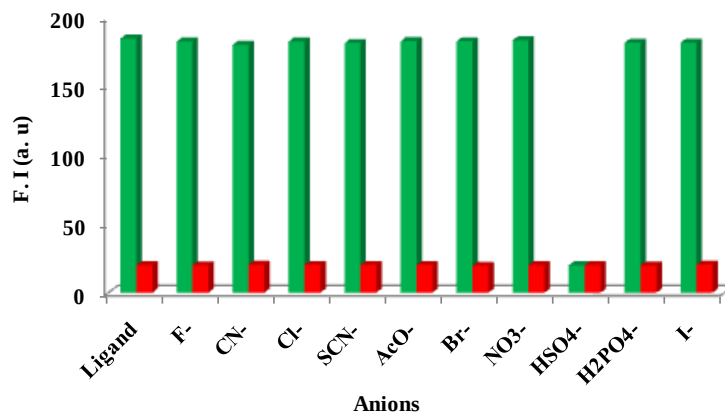


Figure 3.4.17: Green bars represent the selectivity of probe 9 (1 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (9:1, v/v)) upon addition of different anions and red bars show the competitive selectivity of probe 9 (1 μM , $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (9:1, v/v)) in the presence of HSO_4^- ions.

To determine whether probe **9** can be used for the selective detection of HSO_4^- ions among other ions, a simple experiment was performed by taking 1000 μM of various anions such as F^- , CN^- , Cl^- , SCN^- , AcO^- , NO_3^- , Br^- , H_2PO_4^- , I^- etc., and 1000 μM of HSO_4^- . Results revealed that there was no significant variation in the emission band when comparing it with or without the other anions (Figure 3.4.17).

3.4.3.9 Elaboration of probe **9** as XOR logic gate in CH_3CN

As emission output at 485 nm is in *on* state in the presence of both Hg^{2+} and H_2PO_4^- ions. Therefore, molecular switching behavior of probe **9** has been demonstrated by two chemical inputs (Hg^{2+} ions and H_2PO_4^- ions) using the binary logic system. Emission output at 485 nm was in *on* state only when either of the inputs were present. Such emission changes upon the actions of two chemical inputs mimic the exclusive-OR (XOR) logic gate (Figure 3.4.18) with a threshold value of 123.67. XOR logic function is two input device and its output is *true* if the inputs are different and *false* if the inputs are same. Same results were produced with the inputs of Hg^{2+} ions and H_2PO_4^- ions and output of both at 485 nm.

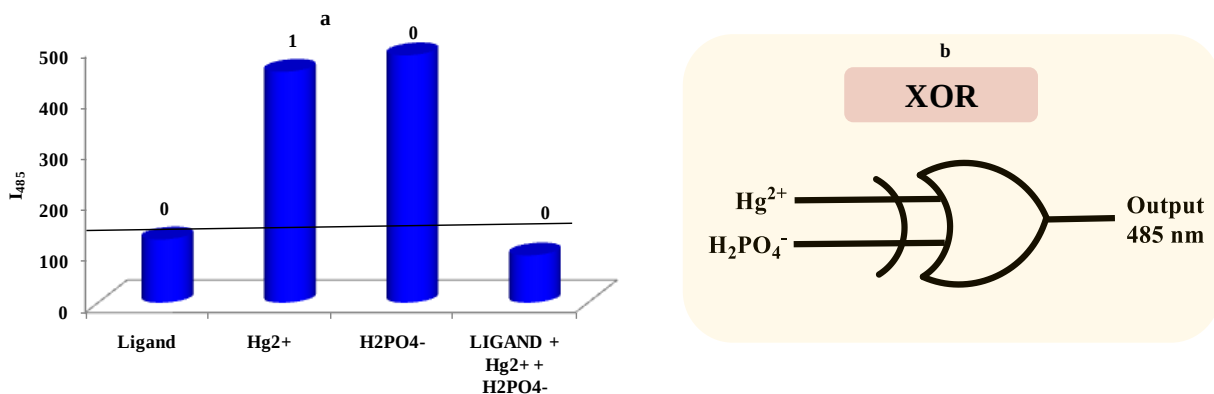


Figure 3.4.18: (a) Bar diagram of the XOR gate in emission spectroscopy at 485 nm IN CH_3CN ; (b) Circuit diagram for the XOR logic gate.

Conspicuously, probe **9** restores the information if Hg^{2+} ions first (*on* state) or H_2PO_4^- input comes first ions (*off* state) (Figure 3.4.18, Table 1). The production of such sequential circuits are important as logic gates are used to construct memory devices.

Table 1: Truth table for the sequential logic circuits XOR, where 0 = *off* and 1 = *on*.

Input 1 (Hg ²⁺)	Input 2 (H ₂ PO ₄ ⁻)	Output (I ₄₈₅ nm)
0	0	0
1	0	1
0	1	1
1	1	0

3.4.3.10 Theoretical calculations

To know more about binding strategies of probe **9** towards Hg²⁺, Al³⁺, H₂PO₄⁻, HSO₄⁻ ions, probe **9** was optimized in ground state through DFT and TD-SCF calculation with B3LYP-631G methods. The excited state of probe **9** and its complexes **9**·Hg²⁺, **9**·Al³⁺, **9**·H₂PO₄⁻, **9**·HSO₄⁻, showed the absorption maximum bands at 384 nm, 375 nm, 374 nm, 456 nm and 457 nm respectively in the gaseous state which is in good agreement with the experimental values at 385 nm, 370 nm, 370 nm, 395 nm and 415 nm, respectively. In case of **9**·Hg²⁺ and **9**·Al³⁺, blue shift was observed and in case of **9**·H₂PO₄⁻ and **9**·HSO₄⁻, red shift was observed, indicating that there were π - π^* transitions. To know the binding of probe **9** with Hg²⁺ and Al³⁺ ions, optimization was done in gaseous phase using LAN2DZ method and other basis remaining same and investigated that Hg²⁺ ion in complex showed its binding between S atom of thiazole moiety with a bond distance of 2.37 Å and form 1: 1 (probe **9**: Hg²⁺) complex. Al³⁺ ion binds to probe **9** through O atom of thiazole moiety with a bond distance of 1.92 Å. H₂PO₄⁻ and HSO₄⁻ bind through NH with bond distance of 1.64 Å and 1.68 Å respectively. Frontier molecular orbital showed that HOMO of probe **9** was completely located on phenanthrene and LUMO on thiazole moiety. HOMO and LUMO of probe **9**·Hg²⁺ complex located on thiazole moiety and phenanthrene. HOMO of probe **9**·Al³⁺ complex located on thiazole moiety and LUMO on phenanthrene ring. HOMO of **9**·H₂PO₄⁻, **9**·HSO₄⁻, was completely located on phenanthrene and LUMO was completely located on thiazole moiety (Figure 3.4.19).

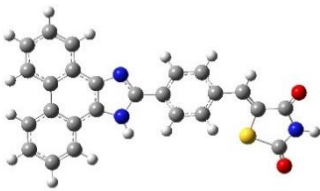
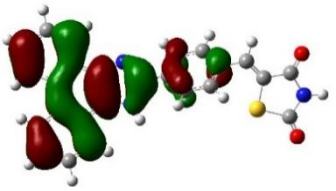
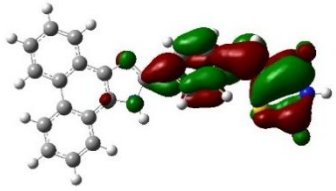
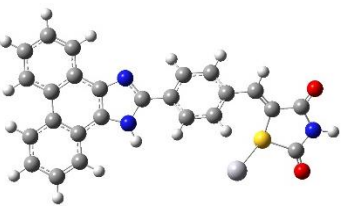
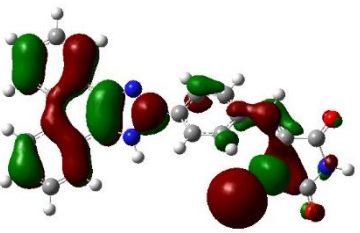
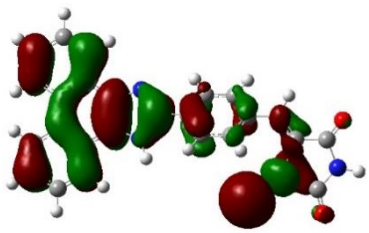
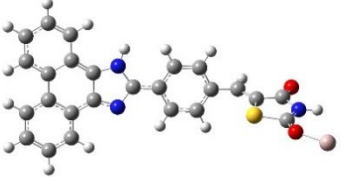
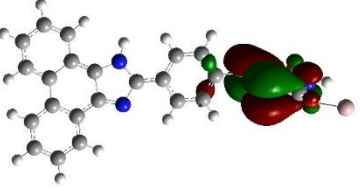
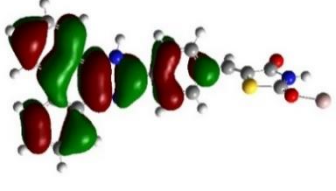
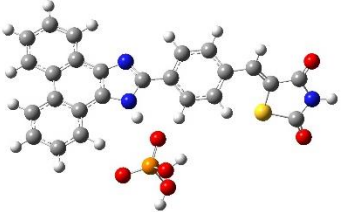
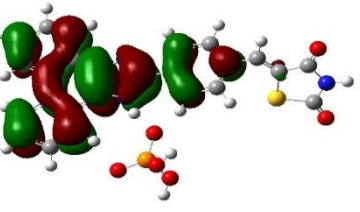
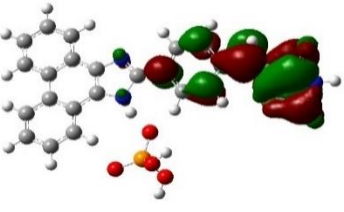
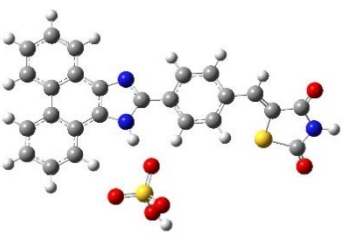
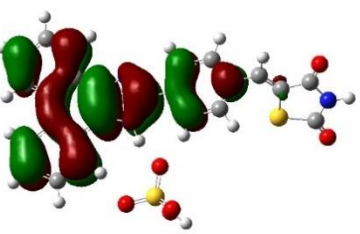
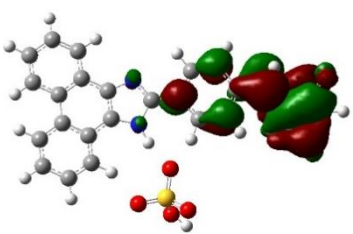
	Optimized Structure	HOMO	LUMO
Probe 9			
Probe 9·Hg ²⁺			
Probe 9·Al ³⁺			
Probe 9·H ₂ PO ₄ ⁻			
Probe 9·HSO ₄ ⁻			

Figure 3.4.19: Energy minimized structure and frontier molecular orbital contribution of probe 9 and its complexes with HSO₄⁻, H₂PO₄⁻, Hg²⁺, Al³⁺ ions.

3.4.3.11 Solvent effect

The development of sensors that work differentially in different solvents are of great significance because they are more efficient compared with others. Binding in different solvents controlled by solvation of different anions in different solvents. The degree of solvation depends on factors such as hydrogen bonding and Van der Waals forces. Thus, on the extent of hydrogen bonding of different anions behaves differently in protic polar and aprotic polar solvents. Thus probe **9** behaved differently towards different anions in polar and nonpolar solvents.

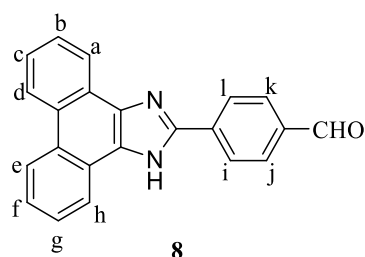
3.4.4 Conclusions

Probe **9** exhibited an absorption band at 395 nm, emission band at 560 nm in CH₃CN while in CH₃OH:H₂O (9:1, v/v) absorption band at 385 nm and emission band at 530 nm was observed. It has been used for detection of Hg²⁺ and Al³⁺ ions in both solvents (CH₃CN and CH₃OH:H₂O (9:1, v/v)) amongst various metal ions. Interestingly, probe **9** showed drastic change with H₂PO₄⁻ in CH₃CN and HSO₄⁻ in CH₃OH:H₂O (9:1, v/v). Thus a single probe works differently in polar and nonpolar solvents. Probe **9** has been elaborated as XOR logic gate.

3.4.5 Experimental section

Synthesis of compound 8: Compound **8** was readily synthesized in one step by coupling of 9,10-phenanthrenequinone (500 mg, 2.4 mmol) and terephthalaldehyde (966.34 mg, 7.2 mmol) in the presence of ammonium acetate (925.5 mg, 12.0 mmol) and glacial acetic acid as a solvent at refluxing temperature for 1 h. After cooled to room temperature, a copious amount of water was added to the mixture, the solid precipitate was filtered with buckner funnel under vacuum and washing was done with cold water which was further purified with the help of column chromatography to obtain pure yellow solid.

4-(1*H*-Phenanthro[9,10-*d*]imidazol-2-yl)benzaldehyde (compound 8)

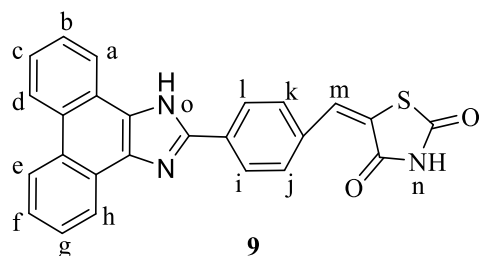


60% yield; yellow solid; M.pt. > 300 °C; ¹H NMR (DMSO-*d*₆, 400 MHz): δ (ppm) 13.71 (s, 1H, NH), 10.07 (s, 1H, CHO), 8.86 (d, 2H, *J* = 8.4 Hz, ArH_{*j,k*}), 8.59 (d, 2H, *J* = 7.64 Hz, ArH_{*i,l*}), 8.52 (d, 2H, *J* = 8.4 Hz, ArH_{*a,h*}), 8.13 (d, 2H, *J* = 8.4 Hz, ArH_{*b,g*}), 7.75 (d, 2H, *J* = 7.64 Hz, ArH_{*c,f*}), 7.67 (d, 2H, *J* = 6.88 Hz, ArH_{*d,e*});

^{13}C NMR ($\text{DMSO-}d_6$, 100 MHz): δ (ppm) 193.07 (CHO), 148.3, 130.6, 137.9, 136.5, 135.9, 128.8, 128.2, 127.3, 122.7, 127.8, 126.9, 126.3, 126.0, 124.7, 124.3, 122.7, 122.4 (ArC); MS (ESI) m/z 323.1 ($\text{M}^+ + \text{H}$).

Synthesis of probe 9: Probe **9** was synthesized with thiazolidine-2,4-dione (1 g, 8.5 mmol) and compound **8** (2.96 g, 8.5 mmol) in the presence of sodium acetate (0.70 g, 8.5 mmol) in glacial acetic acid. After 24 h, heating to 120 °C, a solid separated out and filtered to give the crude product which was further purified with the help of column chromatography to give an orange solid.

5-(4-(1*H*-Phenanthro[9,10-*d*]imidazol-2-yl)benzylidene)thiazolidine-2,4-dione (probe 9)



65% yield; orange solid; M.pt. = 215-220 °C; ^1H NMR ($\text{DMSO-}d_6$, 400 MHz): δ (ppm) 13.62 (s, 1H, NH_o), 8.85 (bs, 2H, $\text{ArH}_{j,k}$), 8.56 (bs, 2H, $\text{ArH}_{i,l}$), 8.43 (d, 2H, $J = 8.4$ Hz, $\text{ArH}_{a,h}$), 7.82-7.79 (m, 3H, $\text{ArH}_{m,b,g}$), 7.74 (s, 2H, $\text{ArH}_{c,f}$), 7.64 (t, 2H, $J = 6.84$ Hz, $\text{ArH}_{d,e}$), 4.13 (s, 1H,

NH_n); ^{13}C NMR ($\text{DMSO-}d_6$, 100 MHz): δ (ppm) 168.2, 168.1 (C=O), 148.1 (C=C), 133.6 (C-O), 131.5, 130.7, 127.8, 127.3, 126.7, 125.6, 124.5, 123.9, 122.1 (ArC); MS (ESI) m/z 422.5 ($\text{M}^+ + \text{H}$).

Conclusion of chapter 3

Chapter 3 includes synthesis of thiazolidine based derivatives and studied their photophysical behavior towards different metal ions and anions with following features:

- Probe **6** has been successfully synthesized to detect multi-ions and shown ratiometric *on–off–on* type of emission response in the presence of Cu^{2+} ions and used to estimate Cu^{2+} between the detection limit of 50 nM–15 μM and cyanide ions between 4 μM and 200 μM in $\text{CH}_3\text{CN}:\text{H}_2\text{O}:1:1$.
- The probe **6** also mimicked the XOR and YES logic function by using Cu^{2+} and CN^- ions as chemical inputs.
- Probe **6** has also been used for its practical applicability to sense Cu^{2+} and CN^- through dip coating method.
- The *in situ* formed $7\cdot\text{Hg}^{2+}$ complex behaved as a selective fluorescent probe for PPI by a metal displacement approach, ensuing reversal return of ligand from $7\cdot\text{Hg}^{2+}$ complex.
- Depending on two inputs of Hg^{2+} and PPI and outputs at emission wavelength 485 nm, INHIBIT logic gate has been mimicked by probe **7**.
- Probe **9** has been used for estimation of various ions selectively in both the solvents as CH_3CN and $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ and could be elaborated as XOR logic gate.

CHAPTER 4

SYNTHESIS OF FRET/TBET PHENOMENON BASED MOLECULES

4.1 Introduction

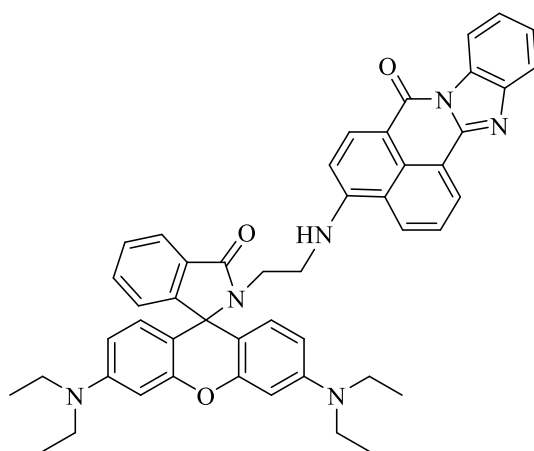
FRET based molecules are usually linked by a non-conjugated spacer, and the energy transfer occurs through space. These kind of molecules provides larger Stokes' shifts. The single excitation wavelength of a donor fluorophore results in the emission of the acceptor at a longer wavelength with a large shift in the spectral peak. FRET is a non-radiative process where an excited state donor 'D' (usual fluorophore) transfers the energy to a proximal ground state acceptor 'A' through long range dipole-dipole interactions. The rate of energy transfer is highly dependent on the extent of spectral overlap, the relative orientation of the transition dipoles, and most importantly the distance between donor and acceptor molecule.

Similarly, dyes which have a donor connected to an acceptor via electronically conjugated linkers, are TBET (through-bond energy transfer) fluorophores. TBET based sensors have also drawn special attention because TBET (through-bond energy transfer) is fast relative to non-radiative decay pathways. TBET is apparently not constrained by the requirement of spectrum overlap and would not necessarily involve loss of sensitivity at high resolutions. The general requirements for TBET to be operating are: (a) donor components with strong absorbance at the excitation wavelength; (b) acceptor components should fluoresce strongly; (c) functional groups that allow attachment linkers. The linker must prevent the donor and acceptor fragments from becoming planar. If they are planar then the system would behave as a single conjugated dye, conversely, the linker allows through-bond energy transfer (TBET) from the donor to the acceptor, that is rapid relative to non-radiative decay processes.

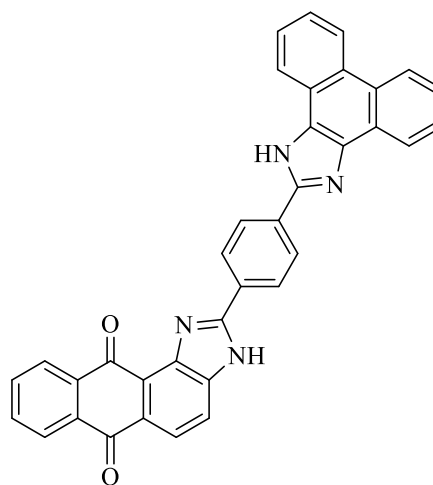
In order to synthesize FRET or TBET based molecules, numerous fluorescent dyes have been used. Out of these fluorescent dyes, the sets containing rhodamine,⁷⁴ naphthalimide,⁹⁰ anthraquinone,⁹⁹ phenanthrequinone¹⁰⁰ etc. dyes have attracted considerable interest from chemists due to their excellent photophysical properties such as high absorption coefficient, high fluorescence quantum yield, high photostability and relatively long emission wavelength. Rhodamine bearing a history of more than one hundred years, they nowadays still retain their strong vitality as it is strong acceptor. Also, anthraquinone is one of strong acceptor and has excellent photophysical properties.

Beside this, naphthalimide, phenanthroquinone, benzoxazoles²⁷ derivatives are also chosen as excellent fluorophores because of their excellent stability, visible excitation and emission, and large Stokes' shift that minimize the effects of the background fluorescence, easy use, high efficiency and low cost, high stability, and quantum yield. These make these derivatives as potential carriers for preparation of new optical chemosensors.

Naphthalimide derivative (in the first chapter) and phenanthroquinone (in the second chapter) have been chosen as the donor, which have a significant difference in spectral overlap integral between the acceptor and can be independently excited.



Chapter 4.2



Chapter 4.3

In order to synthesize FRET or TBET based molecules, numerous fluorescent dyes have been used. In the first section, we discussed the rhodamine based FRET dyad and in second section anthraquinone based TBET dyad. These molecules are discussed in detail as per following subchapters.

4.2 FRET-based ratiometric detection of Hg^{2+} and biothiols using naphthalimide–rhodamine dyads.

4.3 TBET- based phenanthroimidazole -anthraquinone dyad for ratiometric sensing of PPi

4.2 FRET-based ratiometric detection of Hg^{2+} and biothiols using naphthalimide–rhodamine dyads

4.2.1 Abstract

A new naphthalimide–rhodamine-based dyad **14** in CH_3CN -HEPES (1: 1) buffer solution exhibits fluorescence resonance energy transfer (FRET) from the naphthalimide to the rhodamine moiety on addition of only Hg^{2+} ions and allows ratiometric absorption and fluorimetric detection of Hg^{2+} ions between 50 nM (10 ppb) to 2 μM (0.4 ppm). FRET-induced fluorescence changes were recovered again by subsequent addition of thiol amino acids via reverse FRET. The interconversion of dyad **14** and **14**· Hg^{2+} via the complexation/decomplexation by the modulation of Hg^{2+} /Cys revealed a selective dyad for biothiols in real samples.

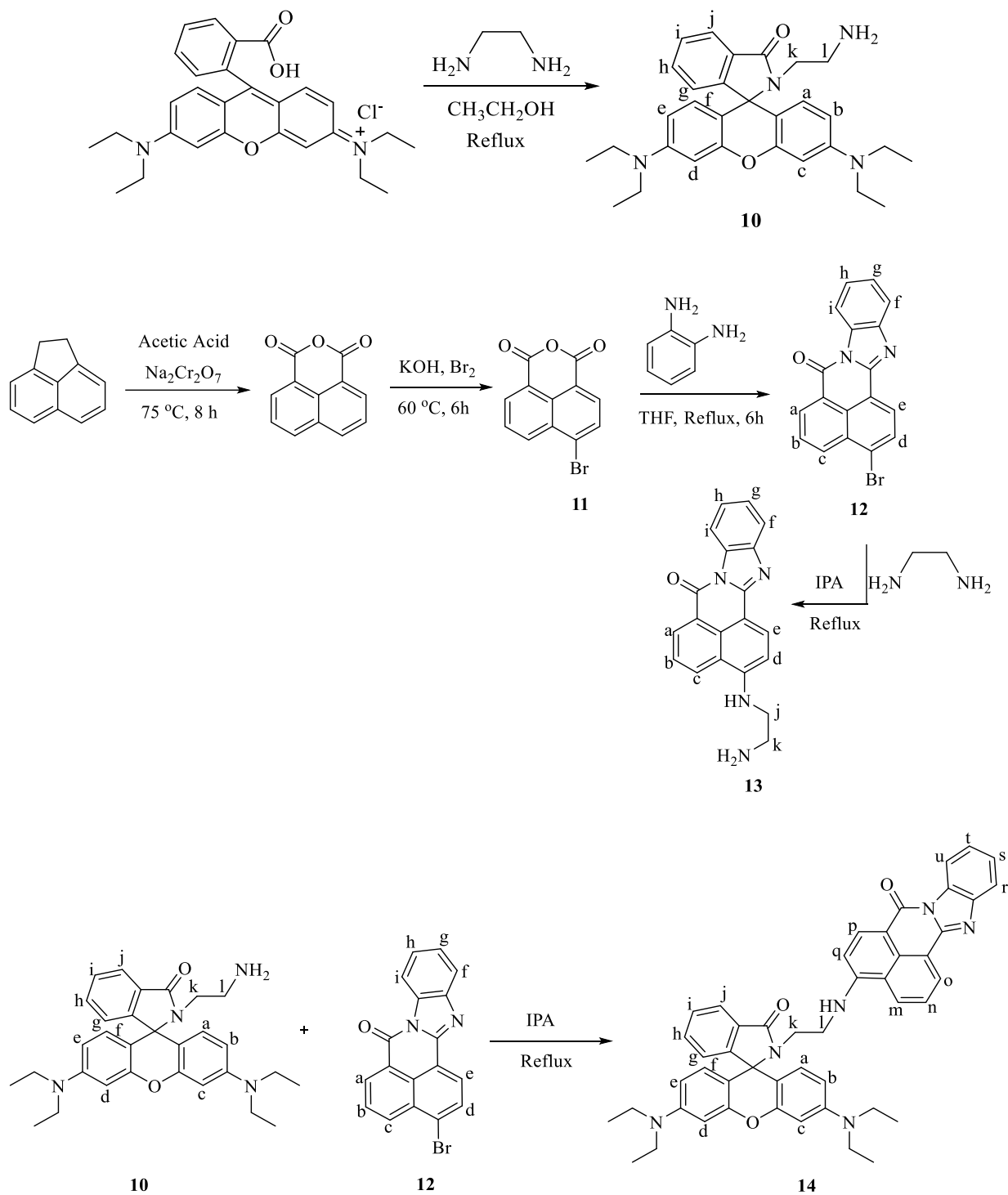
4.2.2 Synthesis of compounds 10-14

The dyad **14** was synthesized on the basis of the route shown in Scheme 4.2.1. Compound **10** was synthesized by refluxing the rhodamine B and ethylene-diamine in ethanol as a brown solid in 80% yield; M.pt = 215-217 °C; MS (ESI) m/z 485.3 ($\text{M}^+ + \text{H}$) (scheme 4.2.1). The ^1H NMR spectrum showed 1H multiplet at δ 7.86-7.81 due to H_j , 2H multiplet at δ 7.48-7.40 due to $\text{H}_{i,h}$, 1H multiplet at δ 7.09-7.03 due to H_g , 6H multiplet at δ 6.43-6.25 due to H_{a-f} , 8H quartet at δ 3.33 due to N-CH_2 , 2H triplet at δ 3.19 due to CH_{2k} , 2H triplet at δ 2.88 due to CH_{2l} , 12H triplet at δ 1.16 due to CH_3 . The ^{13}C NMR spectrum showed signals at δ 170.1, 153.6, 153.1, 148.9, 153.6, 153.1, 148.9, 132.9, 129.9, 128.2, 123.8, 122.9, 108.5, 103.8 of aromatic-C 97.8, 66.5, 44.2, 41.2, 40.2, 12.7 of aliphatic-C. On the basis of these spectral data, this compound has been assigned the structure of 2-(2-aminoethyl)-3',6'-bis(diethylamino)spiro[isoindoline-1,9'-xanthene]-3-one (compound **10**).

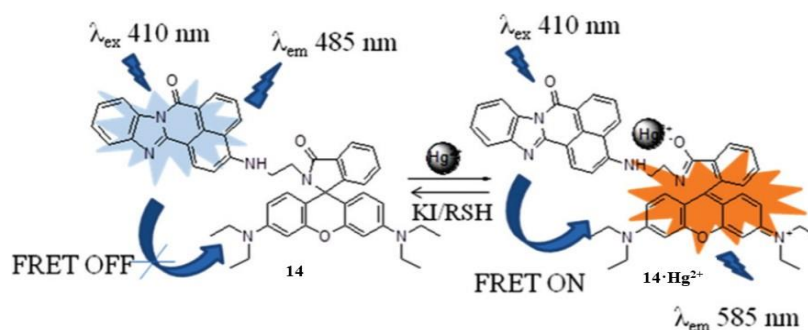
The target naphthalimide analogue was synthesized via multistep reactions from commercially available starting material acenaphthene. Oxidation of acenaphthene with acetic acid and sodium dichromate at 75 °C for 8 h gave 1,8-naphthalic anhydride with 80% yield followed by bromination with Br_2 and KOH at 60 °C for 6 h gave a yellow powder of 4-bromo-1,8-naphthalic anhydride (**11**) with 82% yield (M.pt. = 117-119 °C). Refluxing of compound **11** with *o*-phenylenediamine in THF for 6h obtained a brown powder of bromobenzo[*de*]benzo[4,5]imidazo[2,1-*a*]isoquinoline-7-one (**12**) in 63% yield; M.pt. = 190-192 °C; MS(ESI). The ^1H

NMR spectrum of compound **12** showed three ¹H double doublets at δ 8.90 due to H_d, 8.79 due to H_c and 8.04 due to H_b, two ¹H doublets at δ 8.53 due to H_e and 8.24 due to H_a, 3H multiplet at δ 7.74 due to H_{f,g,h} and 1H multiplet at δ 7.56 due to H_i. The ¹³C NMR spectrum of this compound showed signals at δ 165.7 due to C=O and 136.5, 134.5, 134.4, 133.4, 131.9, 131.7, 131.6, 131.1, 130.9, 129.3, 128.6, 128.2, 127.2, 124.9, 121.0, 119.9 of aromatic-C. Compound **12** and ethylenediamine were refluxed in the presence of isopropyl alcohol (IPA) for 12 h to give compound **13** (3-((2-aminoethyl)amino)-7H-benzo[de] benzo[4,5]imidazo[2,1-a]isoquinolin-7-one) in 80% yield; M.pt. = 170-172 °C; (ESI) m/z 329.2 (M⁺ + H). The ¹H NMR spectrum of compound **13** showed 2H doublet at δ 8.55 due to H_{c,d}, 1H doublet at δ 8.36 due to H_e, 1H singlet at δ 8.34 due to NH, 2H triplet at δ 7.83 due to H_{a,b}, 1H triplet at δ 7.15 due to H_g, 1H doublet at δ 6.98 due to H_f, 1H doublet at δ 6.87 due to H_f, 1H triplet at δ 6.73 due to H_i, 2H triplet at δ 4.73 due to N-CH_{2j}, 2H triplet at δ 4.21 due to CH_{2k}. The ¹³C NMR spectrum of this compound showed signals at δ 165.7, 136.5, 135.5, 134.4, 133.4, 131.9, 131.7, 131.6, 131.1, 129.3, 128.2, 127.2, 124.9, 121.0, 119.9 of aromatic-C and at δ 44.2, 30.2 of aliphatic carbons.

Compound **12** on reaction with rhodamine ethylene-diamine gave dyad **14** in 65% yield; M.pt. = 185-187 °C; (ESI) m/z 753.3 (M⁺⁺ + H). The ¹H NMR spectrum of dyad **14** showed 1H doublet at δ 8.84 due to H_m, 1H doublet at δ 8.58 due to H_o, 1H doublet at δ 8.47 due to H_q, 1H doublet at δ 8.34 due to H_p, 1H triplet at δ 7.95 due to H_n, 1H doublet at δ 7.83 due to H_j, 1H triplet at δ 7.76 due to H_i, 1H singlet at δ 7.55 due to H_a, 4H multiplet at δ 7.41-7.38 due to H_{r-u}, 1H multiplet at δ 7.10-7.00 due to H_h, 4H multiplet at δ 6.39-6.37 due to H_{d-g}, 1H doublet at δ 6.22 due to H_c, 1H doublet at δ 6.11 due to H_b, 2H triplet at δ 3.66 due to CH_{2k}, 8H quartet at δ 3.27 due to CH₂, 2H triplet at δ 2.95 due to CH_{2l}, 12H triplet at δ 1.12 due to CH₃. The additional protons in the aromatic and aliphatic regions confirmed the formation of dyad **14**. The ¹³C NMR spectrum of this compound showed signals at δ 165.7, 136.5, 135.5, 134.4, 133.4, 131.9, 131.7, 131.6, 131.1, 130.9, 129.3, 128.6, 128.2, 127.2, 124.9, 121.0, 119.9 for aromatic-C and at δ 44.2, 30.2 for aliphatic-C. On the basis of these spectral data, this compound 3',6'-bis(diethylamino)-2-(2-((7-oxo-7H-benzo[de]benzo[4,5]imidazo[2,1-a]isoquino-lin-4-yl)amino)thyl)spiro[isoin-dolin-1,9'-xanthen]-3-one has been assigned the structure of dyad **14**.



Scheme 4.2.1: Synthesis of compounds **10-14**



Scheme 4.2.2: Showing the FRET phenomenon

4.2.3 Results and discussion

4.2.3.1 UV-Visible studies of dyad **14** with metal ions

The absorption spectrum of dyad **14** (20 μ M, CH₃CN-HEPES buffer, 1: 1, pH = 7.12) showed an absorption maximum at 450 nm (ϵ = 21 000), predominantly due to an intra-ligand charge transfer (CT) transition in the naphthalimide moiety. On addition of metal cations viz. Na⁺, K⁺, Li⁺, Mg²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺, Pb²⁺ etc., as their perchlorate salts, a significant change in the electronic spectrum was observed only with Hg²⁺ ions. A new absorption band around 510 nm was appeared with a detectable change in solution color from yellow to pink. The Cu²⁺ ions also showed a small color change from yellow to light pink, but only upon addition of 200 μ M (10 equivalents) of Cu²⁺.

On gradual addition of Hg²⁺ ions to the solution of dyad **14** (20 μ M, CH₃CN-HEPES buffer (1:1)), the absorbance at 450 nm underwent a gradual decrease in its intensity with a concomitant increase in absorbance intensity at 510 nm, associated with two isosbestic points at 412 nm and 473 nm. This 60 nm red shift in the absorbance maxima pointed out to the rhodamine spirolactam ring opening through the interaction of **14** with Hg²⁺ ions (Figure 4.2.1a). The *off-on* switching behavior on the addition of Hg²⁺ ions to the solution of **14** at 450 nm and 510 nm provided dual absorption channels for elaborating a ratiometric approach, which permitted signal rationing and allowed the detection of analyte independent of the concentration of the receptor. The ratios of absorption intensities at 510 and 450 nm (I_{510}/I_{450}) exhibited a drastic change from 0.16 in the absence of Hg²⁺ to 3.82 in the presence of 20 μ M Hg²⁺, a 24-folds variation in the absorption ratios. The dyad **14** (CH₃CN-HEPES buffer, 1: 1) can be used for detection of Hg²⁺ ions between 50 nM (10 ppb)-20 μ M (0.4 ppm), (Figure 4.2.1b). The spectral fitting of these absorbance data showed the formation of a 1: 1 (**14**: Hg²⁺) stoichiometric complex [$\log \beta_{\text{LHg}^{2+}} = 5.19 \pm 0.12$] and was also confirmed by Job's plot.

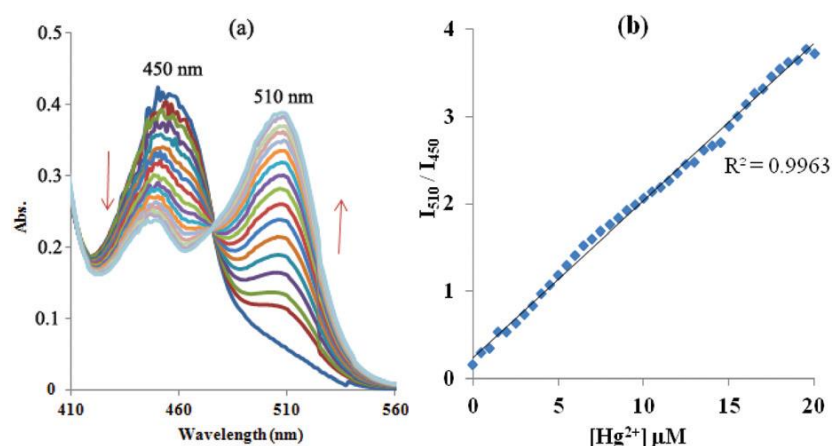


Figure 4.2.1: The effect of the addition of Hg^{2+} ions on (a) the UV-Visible spectrum of **14** (20 μM , CH_3CN –HEPES buffer (1: 1), $\text{pH} = 7.12 \pm 0.1$). (b) Absorbance ratiometric (20 μM) responses (A_{510}/A_{450}) of dyad **14** upon incremental addition of Hg^{2+} ions showing the linear fit.

4.2.3.2 Fluorescence studies of dyad **14** with metal ions

Upon excitation at 410 nm, the free dyad **14** displayed a single emission band centered at 485 nm ($\Phi = 0.21$), attributed to the emission of naphthalimide. There was no FRET in the free **14**, as the rhodamine acceptor was in the ring-closed form. On addition of Hg^{2+} , the fluorescence intensity at 485 nm significantly decreased with the simultaneous appearance of a new red-shifted emission band at around 585 nm (Figure 4.2.2a), ascribed to the emission of the rhodamine acceptor. This was also accompanied by a resonance color change from blue to red under 355 nm illumination of light. Also, the intensity of fluorescence emission at 485 nm and 585 nm, respectively decreased and increased linearly between 50 nM (10 ppb) and 1 μM (0.2 ppm) of Hg^{2+} ions (Figure 4.2.2b). Thus, the addition of Hg^{2+} elicited a large red shift (100 nm) in emission, which almost completely resolved the two emission peaks. This fluorescence enhancement at 585 nm is typically attributed to the opening of the spirolactam ring of rhodamine-B (Scheme 4.2.2). This ratio increases exponentially with an increase in the concentration of Hg^{2+} . However, the plot of the log of the ratio of intensities showed an excellent linear relationship with concentrations of Hg^{2+} between 100–800 nM with $R^2 = 0.9821$ (Figure 4.2.3a). The nonlinear regression analysis of the spectral data obtained on titration of the solution of **14** with Hg^{2+} ions showed the formation of 1: 1 stoichiometric complex [$\log \beta_{\text{LHg}^{2+}} = 6.22 \pm 0.24$]. The Job's plot analysis of the fluorescence titrations revealed a maximum at 0.5-mole

fraction (Figure 4.2.3b), also indicating 1: 1 binding stoichiometry. The ratios of fluorescence intensities at 585 nm and 485 nm (I_{585}/I_{485}) exhibited a drastic change from 0.11 in the absence of Hg^{2+} to 15.6 in the presence of $1.0 \mu\text{M}$ Hg^{2+} a 141-folds variation in the emission ratios. The response of dyad **14** to Hg^{2+} was quite fast and all absorption and fluorescence spectra were recorded within 2 min of adding Hg^{2+} .

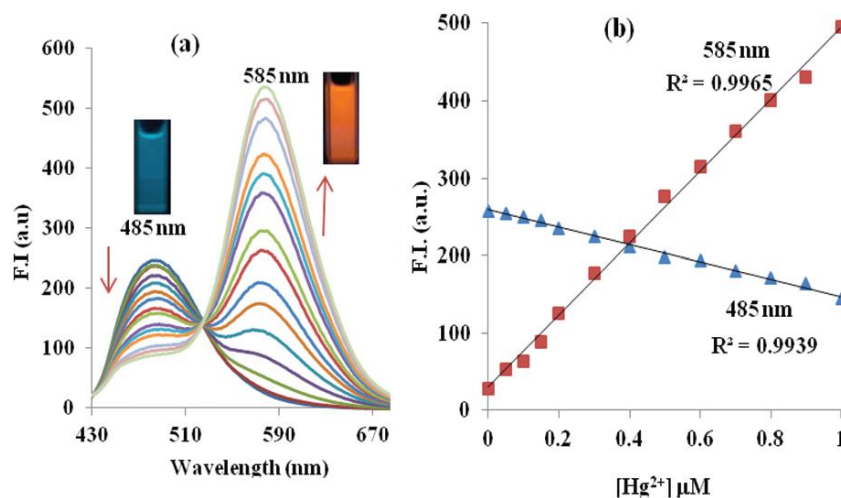


Figure 4.2.2: (a) The effect of gradual addition of Hg^{2+} ions on the fluorescence spectrum of dyad **14** ($1 \mu\text{M}$, CH_3CN –HEPES buffer (1: 1), $\text{pH} = 7.12 \pm 0.1$); (b) The linear increase in the fluorescence intensity vs. $[\text{Hg}^{2+}]$ at both 485 nm and 585 nm.

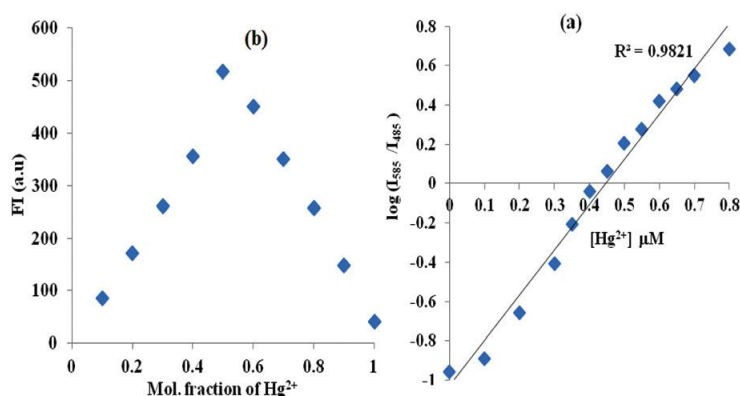


Figure 4.2.3: (a) Fluorescence ratiometric response (F_{585}/F_{485}) of dyad **14** versus Hg^{2+} concentration. (b) Job's plot showing the 1:1 stoichiometry between dyad **14** and Hg^{2+} ions.

4.2.3.3 Reversible binding of dyad **14**

In order to prove the reversibility of the dyad **14**, the experiments were performed in the presence of KI. On addition of an aqueous solution of KI to $\mathbf{14} \cdot \text{Hg}^{2+}$ complex, the emission band at 585 nm disappeared with the reappearance of the fluorescence maxima at 485 nm. Again, on

the addition of Hg^{2+} to this solution mixture, having KI, the emission band at 585 nm reappeared. Preferential binding of iodide ions to Hg^{2+} led to the formation of HgI_2 and the regeneration of the cyclic lactam form and confirmed the reversible binding of rhodamine dyad to Hg^{2+} (Figure 4.4).

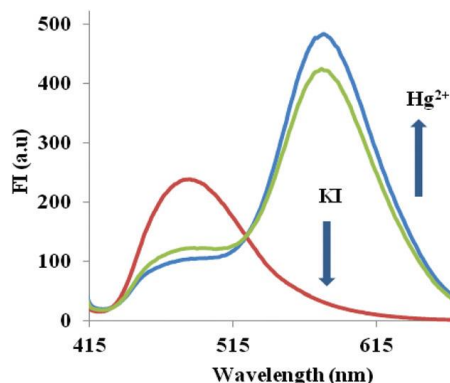


Figure 4.2.4: Fluorescence spectra showing the reversibility of Hg^{2+} coordination to dyad **14** by KI: blue line, 1 + 1 μM Hg^{2+} ; red line, 1 + 1 μM Hg^{2+} , 1 + 2 μM KI; green line, 1 + 1 μM Hg^{2+} , 1 + 2 μM KI + 5 μM Hg^{2+} ; in $\text{CH}_3\text{CN-H}_2\text{O}$ (1 : 1), HEPES buffer (pH = 7.12 ± 0.1) at $\lambda_{\text{ex}} = 410$ nm.

4.2.3.4 Selectivity to Hg^{2+} ions of dyad **14**

We also tested the fluorescence response of **14** to other metal ions, such as Na^+ , K^+ , Li^+ , Mg^{2+} , Cd^{2+} , Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Pb^{2+} etc., as their perchlorate salts, in CH_3CN –HEPES buffer (1:1) and no significant variation in the fluorescence spectrum of **14** was observed with any other metal ion except Cu^{2+} , which induced emission at 585 nm, but to a very small extent (Figure 4.2.5).

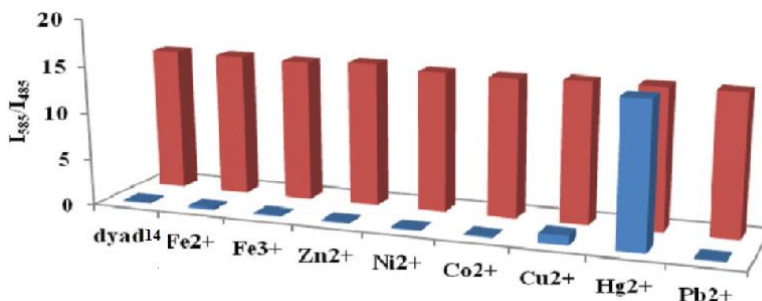


Figure 4.2.5: Fluorescence ratiometric response (I_{585}/I_{485}) of **14** (1 μM) upon addition of different metal ions in $\text{CH}_3\text{CN-H}_2\text{O}$ (1: 1), HEPES buffer (pH = 7.0 ± 0.1). Blue bars show the effect of different metal ions on dyad **14** fluorescence intensity and red bars show the presence of interfering metal ions on dyad **14** + Hg^{2+} fluorescence intensity.

To check the practical ability of dyad **14** as a Hg^{2+} -selective fluorescent sensor, we carried out competitive experiments in the presence of 1 μM of Hg^{2+} and 100 μM of the interfering metal ions like Fe^{2+} , Fe^{3+} , Pb^{2+} , Cu^{2+} , Zn^{2+} , Ni^{2+} , Co^{2+} . As shown in Figure 4.2.5, no significant variation in the fluorescence emission was observed by the addition of interfering metal ions.

4.2.3.5 Practical applicability of dyad **14**· Hg^{2+} complex to estimate biothiols in real samples

To investigate the ability of **14**· Hg^{2+} complex as an ensemble probe for thiols in biological media, absorption and emission changes of an aqueous solution of **14**· Hg^{2+} in CH_3CN –HEPES buffer (1: 1) were examined with the addition of various amino acids. The **14**· Hg^{2+} ensemble revealed remarkable emission changes with only the addition of thiol-containing amino acids, whereas no change was observed with other amino acids (Ala, Arg, Asp, Glu, Gly, His, Lys, Met, Pro, Ser, Thr, Trp, Tyr, Val) (Figure 4.2.6a). It was found that the emission spectrum of a mixture of **14**· Hg^{2+} and thiol is identical to that of **14**. The incremental addition of mercapto biomolecules, such as Cys, Hcy or GSH, to the aqueous solution of **14**· Hg^{2+} , resulted in revived UV-Vis absorption and fluorescence spectra of free dyad **14** and also returned the fluorescence quantum yield to 0.3. As seen in Figure 4.2.6b, when **14**· Hg^{2+} was titrated with Cys, the fluorescence intensity of the solution was proportional to the concentration of cysteine up to 2 equivalents. The detection limits estimated were 0.2 μM –2 μM for cysteine. Consistently, the visual emission color of **14**· Hg^{2+} solution turned from red to green as well. Among all of the amino acids tested, Cys gave the highest response toward the **14**· Hg^{2+} complex. As shown by Figures 4.2.6a and 4.2.7, fluorescence changes were highest at ~90% for Cys, 60% and 25% for Hcy and GSH, respectively, and below 5% for other amino acids.

The alternate addition of a constant amount of Cys and Hg^{2+} to dyad **14** gave rise to a switchable change in the fluorescence intensity at 585 nm. Such a reversible interconversion can be repeated more than 10 times by the modulation of Cys/ Hg^{2+} added, indicating that dyad **14** can be developed as a reversible fluorescence *off-on* dyad for Cys and Hg^{2+} (Figure 4.2.8). To further show the potential utility of **14**· Hg^{2+} in biological samples, **14**· Hg^{2+} was further studied as a dyad to detect biothiols in wheat samples, in which cysteine and other amino acids can be found free or linked to each other. It was confirmed that a trace amount of cysteine in a wheat sample can easily be detected using the **14**· Hg^{2+} dyad. For the determination of biothiols, a wheat sample (*Triticum aestivum*, cv. PBW343) was first introduced to extract the different proteins viz. albumin, globulin, prolamin etc., and then these proteins were hydrolysed to free

amino acids using 6N HCl. The fluorescence spectral changes observed with hydrolysed proteins were similar, as observed with dyad **14**· Hg^{2+} with cysteine (Figure 4.2.9). It shows that dyad **14** can be used to selectively estimate thiols in the wheat sample.

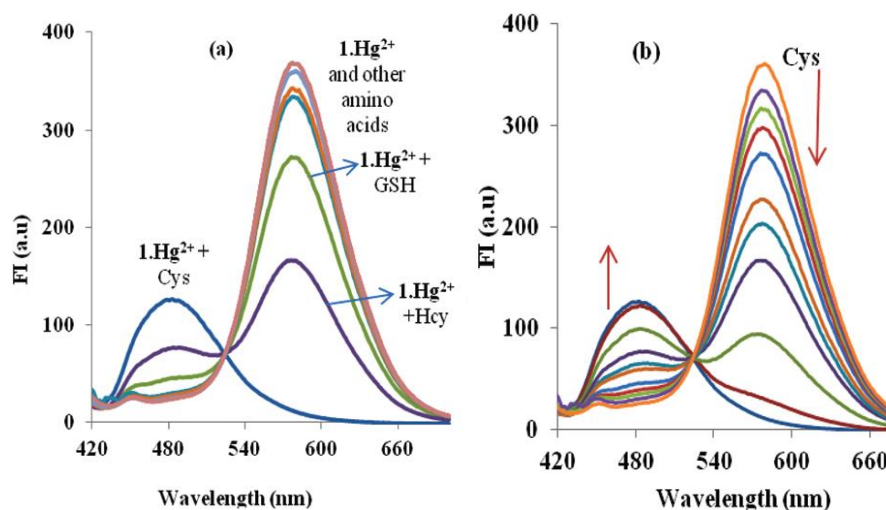


Figure 4.2.6: (a) The effect of different amino acids on the fluorescence spectrum of $14 \cdot \text{Hg}^{2+}$; (b) The effect of the incremental addition of cysteine on the fluorescence spectrum of $14 \cdot \text{Hg}^{2+}$ (1:1) complex at 1 μM concentration.

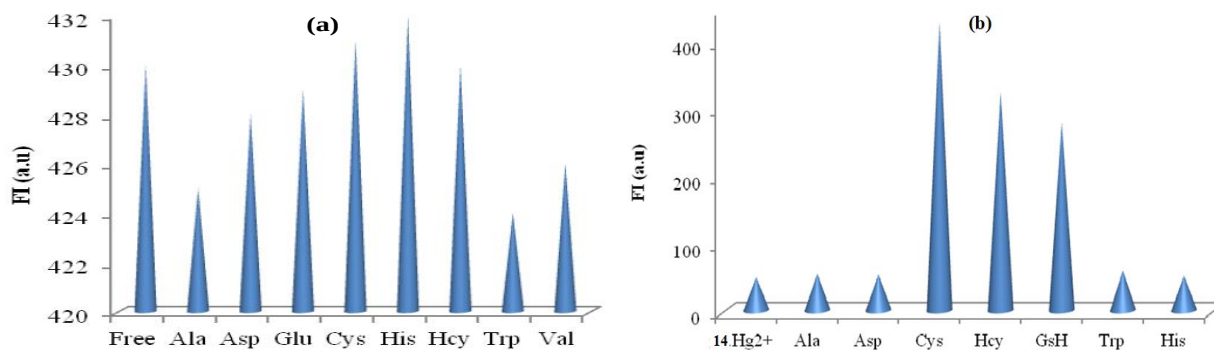


Figure 4.2.7: (a) Fluorescence intensity of dyad **14** (1 μM) at 485 nm in the presence of various amino acids; (b) Fluorescence intensity of dyad $14 \cdot \text{Hg}^{2+}$ complex (1 μM) at 485 nm in the presence of various amino acids.

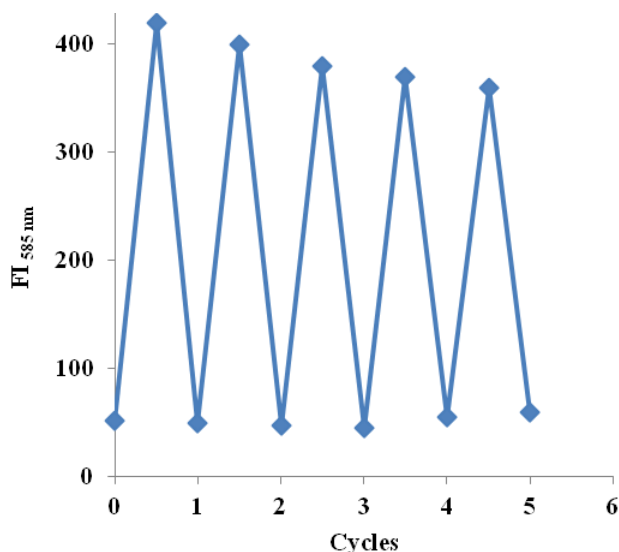


Figure 4.2.8: Fluorescent intensity of dyad **14** (1 μM) upon the alternate addition of Cys/ Hg^{2+} with several concentrations (0:0, 1:0, 1:1.5, 2:1.5, 2:3, 4:3, 4:6, 6:6, 6:9, and 10:9 μM).

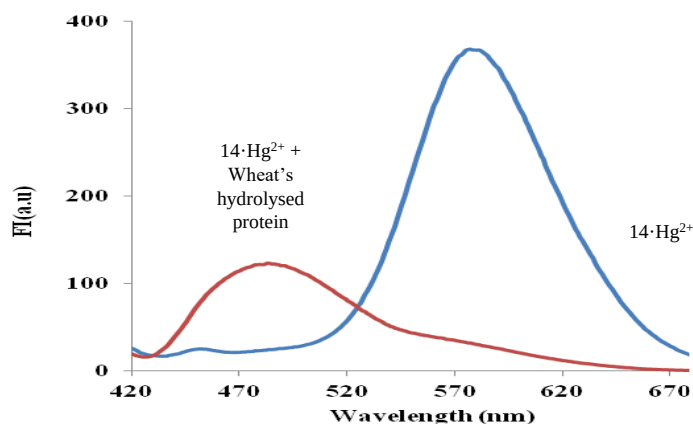


Figure 4.2.9: Effect of addition of wheat's hydrolysed proteins on fluorescence spectrum of $14\cdot\text{Hg}^{2+}$

4.2.3.6 Fluorescence resonance energy transfer (FRET) characteristics of dyad **14**

For an efficient FRET phenomenon, the overlap of the absorption spectrum of the acceptor with the emission spectrum of the donor, efficiency of energy transfer from donor to acceptor, donor–acceptor distance and relative orientation of the donor and acceptor transition dipoles, are essentially required. The absorption spectrum of the open spirocycle form of the rhodamine-B developed a significant overlap ($\text{JDA} = 3.8 \times 10^{13} \text{ M}^{-1} \text{ cm}^{-1}$) with the emission spectrum of **13** and clearly offers the possibility of a FRET process (Figure 4.2.10). The efficiency of the energy transfer (η_{EET}) was evaluated using the equation $\eta_{\text{EET}} = 1 - \Phi_{\text{F(donor in dyad 14)}} / \Phi_{\text{F(free donor)}}$. Here, $\Phi_{\text{F(donor in dyad)}}$ is the fluorescence quantum yield of the donor part in dyad **14** and $\Phi_{\text{F(free donor)}}$ is the fluorescence quantum yield of the donor when not connected to the acceptor. The

fluorescence quantum yield of donor **13** in CH₃CN–HEPES buffer was 0.215, whereas the quantum yield of the naphthalimide part in dyad **14** was 0.05. Thus, the efficiency of naphthalimide (η_{EET}) in the *on* state is very high i.e. up to 76.7%. Therefore, dyad **14** meets the criteria for the FRET mechanism. It is worth noting that the addition of Hg²⁺ ions to a solution of **13** caused fluorescence quenching only, no red shifted fluorescence band was observed, whereas **10** did not show any absorption and fluorescence changes on the addition of Hg²⁺ ions, which clearly depicted the FRET phenomenon from naphthalimide to rhodamine (closed form) in dyad **14** (Figure 4.2.11).

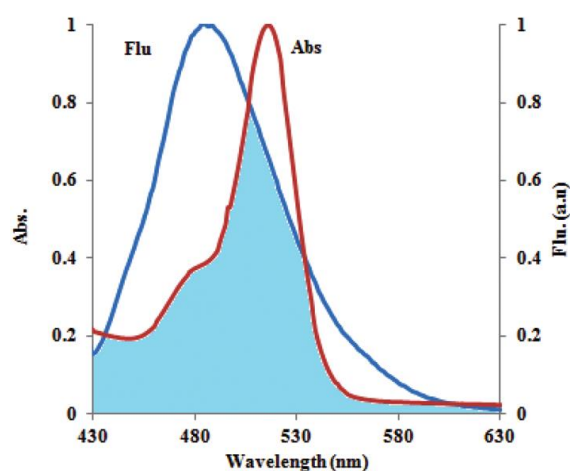


Figure 4.2.10: The overlap (shown with cyan shade) between the emission and absorption spectra of the donor (naphthalimide) and acceptor (rhodamine) moieties.

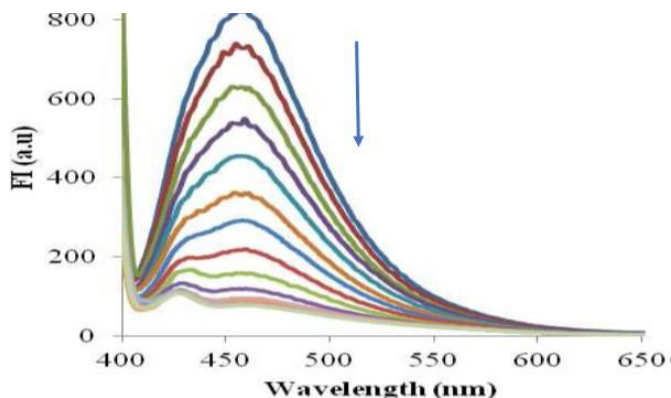


Figure 4.2.11: Effect of incremental addition of Hg²⁺ to solution of **13**

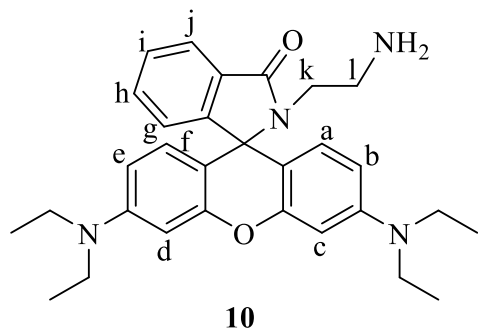
4.2.4 Conclusions

We have designed and synthesized of FRET-based dyad **14** as a new chromogenic and fluorescent chemosensor, suitable for ratiometric sensing of Hg^{2+} on the basis of the Hg^{2+} -induced *on-off-on* switching and ratiometric detection of biothiols based on FRET reversal. The well-resolved absorption and emission peaks, the high efficiency in energy transfer and large variation in the emission (I_{585}/I_{485}) ratios are the added advantages.

4.2.5 Experimental section

Synthesis of compound 10: Rhodamine B (4.80 g, 10 mmol) and ethylenediamine (5 mL) were dissolved in ethanol (60 mL) and refluxed for 24 h. The completion of the reaction was confirmed by TLC. Ethanol was evaporated and water was added to this reaction mixture, a solid was separated out, filtered, washed with water, a pale-pink solid of compound **10** was obtained.

2-(2-Aminoethyl)-3',6'-bis(diethylamino)spiro[isoindoline-1,9'-xanthen]-3-one (compound 10)



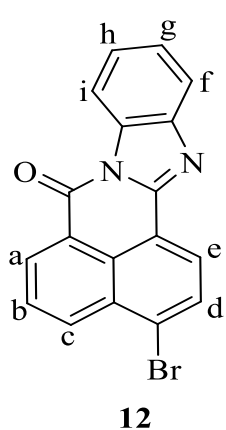
80% yield; pale-pink solid; M.pt. = 215-217 °C; ^1H NMR (CDCl_3 , 400 MHz): δ (ppm) 7.86-7.81 (m, 1H, ArH_j), 7.48-7.40 (m, 2H, $\text{ArH}_{i,h}$), 7.09-7.03 (m, 1H, ArH_g), 6.43-6.25 (m, 6H, ArH_{a-f}), 3.33 (q, J = 6.70 Hz, 8H, N- CH_2), 3.19 (t, J = 6.70 Hz, 2H, CH_k), 2.88 (t, J = 4.10 Hz, 2H, CH_l), 1.16 (t, J = 6.70 Hz, 12H, CH_3);

^{13}C NMR (CDCl_3 , 100 MHz): δ (ppm) 170.1, 153.6, 153.1, 148.9, 132.9, 129.9, 128.2, 123.8, 122.9, 108.5, 103.8, 97.8, 66.5, 44.2, 41.2, 40.2, 12.7; MS (ESI) m/z 485.3 ($\text{M}^+ + \text{H}$).

Synthesis of compounds 11, 12: To a solution of acenaphthene (2.31 g, 15.0 mmol) and acetic acid (60 mL), sodium dichromate (11.17 g, 40.0 mmol) was added with stirring at room temperature. The suspension was heated to 75 °C for 8 h. After the completion of the reaction, cold water was added to the reaction mixture to precipitate a solid, which was filtered, and washed with water. A yellow solid product of 1,8-naphthalic anhydride (2.6 g, 80%, M.pt. = 266-268 °C) was obtained. To a cooled solution of 1,8-naphthalic anhydride (1.98 g, 10.0 mmol)

in aqueous KOH (2.8 g in 12 mL water), bromine was added through a dropping funnel. The reaction mixture was heated to 60 °C for 6 h and then acidified with HCl solution. A solid was separated out and filtered to get crude product. The solid residue was heated with 5% NaOH solution, filtered and neutralized with HCl solution. After neutralization solid formed was filtered and washed with cold water. A yellow powder of 4-bromo-1,8-naphthalic anhydride (**11**, m.p. 117-119 °C) in 82% yield was obtained. 4-Bromo-1,8-naphthalic anhydride (2.00 g, 5.0 mmol) and *o*-phenylenediamine (0.618 g, 5.0 mmol) in THF were refluxed for 6 h. After the completion of reaction which was monitored by TLC, cooled the reaction mixture. A crude solid obtained was filtered and washed with THF to afford pure 3-bromobenzo[*de*]benzo[4,5]imidazo[2,1-*a*]isoquinolin-7-one (**12**).

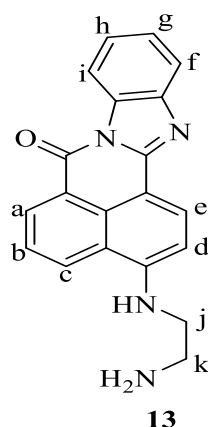
3-Bromo-benzo[*de*]benzo[4,5]imidazo[2,1-*a*]isoquinolin-7-one (**12**)



63% yield; brown solid; M.pt. = 190-192 °C; ¹H NMR (CDCl₃ + TFA, 400 MHz): δ (ppm) 8.90 (dd, ²*J* = 8.48 Hz, ³*J* = 0.68 Hz, 1H, ArH_d), 8.79 (dd, ²*J* = 7.36 Hz, ³*J* = 0.84 Hz, 1H, ArH_c), 8.53 (d, *J* = 7.92 Hz, 1H, ArH_e), 8.24 (d, *J* = 7.92 Hz, 1H, ArH_a), 8.04 (dd, ²*J* = 8.48 Hz, ³*J* = 7.48 Hz, 1H, ArH_b), 7.74 (m, 3H, ArH_{f,g,h}), 7.56 (m, 1H, ArH_i); ¹³C NMR (CDCl₃ + TFA, 100 MHz): δ 165.7, 136.5, 134.5, 134.4, 133.4, 131.9, 131.7, 131.6, 131.1, 130.9, 129.3, 128.6, 127.2, 124.9, 121.0, 119.9; MS (ESI) *m/z* 350.1 (M⁺ + H).

Synthesis of compound 13: Compound **12** (348 mg, 1 mmol) was dissolved in 2-propanol and ethylene diamine (500 mg, 10 mmol) was added. The resulting solution was refluxed for 12 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the 2-propanol was distilled off under reduced pressure. The residue obtained was extracted using chloroform and water. The chloroform layer was collected and dried using sodium sulfate and distilled out on rotary evaporator to get the compound. The crude product was purified through column chromatography to get pure compound **13**.

3-((2-aminoethyl)amino)-7H-benzo[de]benzo[4,5]imidazo[2,1-a]isoquinolin-7-one (13)

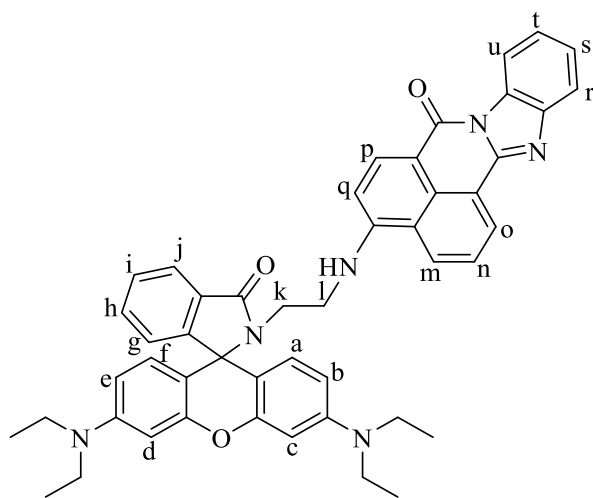


13

80% yield; creamish white solid; M. pt. 170-172 °C; ^1H NMR (CDCl_3 , 400 MHz): δ (ppm) 8.55 (d, $J = 6.44$ Hz, 2H, $\text{ArH}_{c,d}$), 8.36 (d, $J = 7.40$ Hz, 1H, ArH_e), 8.34 (s, 1H, NH), 7.83 (t, $J = 7.44$ Hz, 2H, $\text{ArH}_{a,b}$), 7.15 (t, $J = 7.12$ Hz, 1H, ArH_g), 6.98 (d, $J = 7.76$ Hz, 1H, ArH_i), 6.87 (d, $J = 8.08$ Hz, 1H, ArH_f), 6.73 (t, $J = 7.40$ Hz, 1H, ArH_h), 4.73 (t, $J = 7.40$ Hz, 2H, CH_{2j}), 4.21 (t, $J = 7.80$ Hz, 2H, CH_{2k}); ^{13}C NMR (CDCl_3 , 100 MHz): δ (ppm) 165.7, 136.5, 135.5, 134.4, 133.4, 131.9, 131.7, 131.6, 131.1, 130.9, 129.3, 128.6, 128.2, 127.2, 124.9, 121.0, 119.9 (ArC), 44.2 (CH_2), 30.2 (CH_3); MS (ESI) m/z 329.2 ($\text{M}^+ + \text{H}$).

Synthesis of dyad 14: Rhodamine-ethylenediamine **10** (242 mg, 0.5 mmol) was dissolved in 2-propanol and **12** (174 mg, 0.5 mmol) was added. The resulting solution was refluxed for 12 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature and evaporated to dryness. The residue was purified by column chromatography to get pure dyad **14**.

3',6'-Bis(diethylamino)-2-(2-((7-oxo-7H-benzo[de]benzo[4,5]imidazo[2,1-a]isoquinolin-4-yl)amino)ethyl)spiro[isoindoline-1,9'-xanthen]-3-one (dyad 14)



14

65% yield; yellow solid; M.pt. = 185-187 °C; ^1H NMR (CDCl_3 , 300 MHz): δ (ppm) 8.84 (d, $J = 7.2$ Hz, 1H, ArH_m), 8.58 (d, $J = 8.4$ Hz, 1H, ArH_o), 8.47 (d, $J = 8.4$ Hz, 1H, ArH_q), 8.34 (d, $J = 8.4$ Hz, 1H, ArH_p), 7.95 (t, $J = 8.1$ Hz, 1H, ArH_n), 7.83 (d, $J = 8.4$ Hz, 1H, ArH_j), 7.76 (t, $J = 8.1$ Hz, 1H, ArH_i), 7.55 (s, 1H, ArH_c), 7.41-7.38 (m, 4H, ArH_{r-u}), 7.10-7.00 (m, 1H, ArH_h), 6.39-6.37 (m, 4H, ArH_{d-g}), 6.22 (d, $J = 8.4$ Hz, 1H, ArH_a), 6.11 (d, $J = 8.4$ Hz, 1H, ArH_b), 3.66

(t, $J = 6.3$ Hz, 2H, CH_{2k}), 3.27 (q, $J = 7.2$ Hz, 8H, $4 \times \text{CH}_2$), 2.95 (t, $J = 6.3$ Hz, 2H, CH_{2l}), 1.12 (t, $J = 7.2$ Hz, 12H, $4 \times \text{CH}_3$); ^{13}C NMR ($\text{CDCl}_3 + \text{DMSO-}d_6$, 75 MHz): δ (ppm) 165.7, 136.5, 135.5, 134.4, 133.4, 131.9, 131.7, 131.6, 131.1, 130.9, 129.3, 128.6, 128.2, 127.2, 124.9, 121.0, 119.9 (ArC), 44.2 (CH_2), 30.2 (CH_3); MS (ESI) m/z 753.3 ($\text{M}^+ + \text{H}$).

4.3 TBET-based phenanthroimidazole-anthraquinone dyad for ratiometric sensing of PPI.

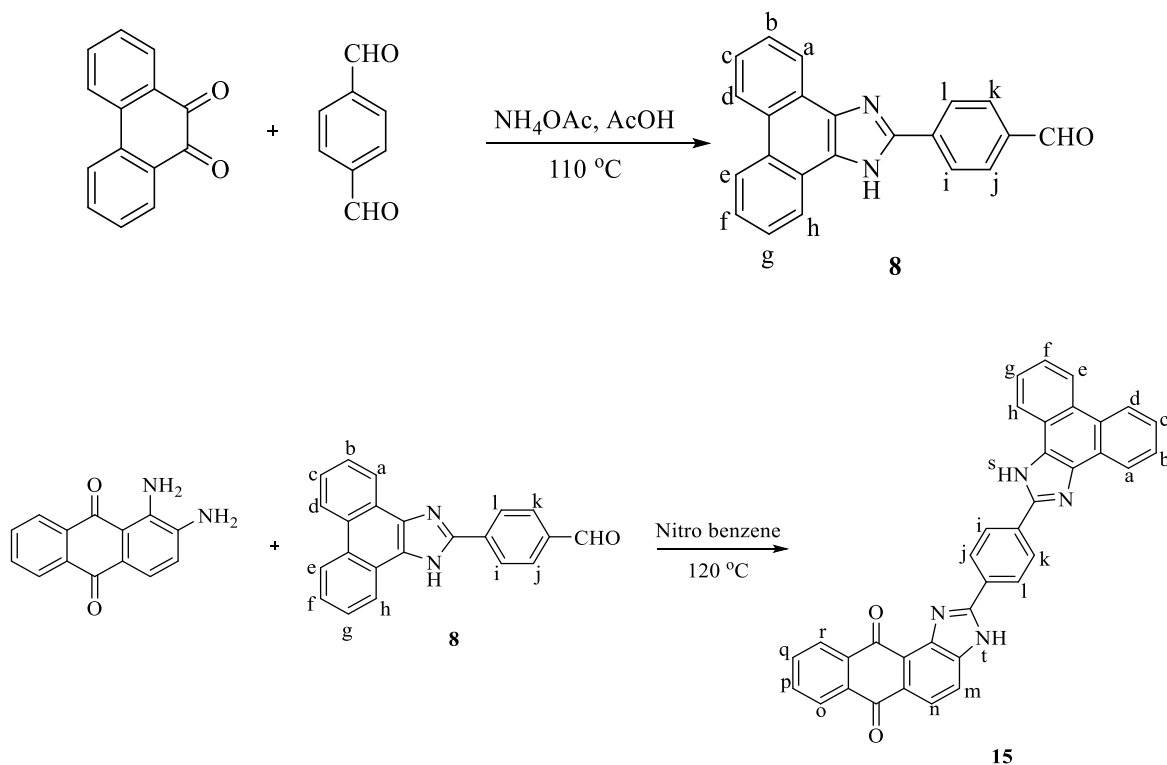
4.3.1 Abstract

Energy-donor-acceptor system based on FRET has been discussed in the previous chapter. FRET based dyad **14** has been synthesized which involves rhodamine as acceptor and naphthalimide as donor with non-conjugated spacer. In the continuity of the previous chapter, considerable attention has been paid to the exploration of TBET based dyad **15**. Now in this chapter, beside naphthalimide and rhodamine a different pair of donor-acceptor viz. anthraquinone as acceptor and phenanthraquinone as donor was selected. As anthraquinone is one of strong acceptor and has excellent photophysical properties. Phenanthraquinone²⁷ derivatives are also chosen as excellent fluorophores because of their excellent stability, visible excitation and emission and large Stokes' shift, easy use, high efficiency, high stability, and quantum yield. Dyad **15** has been used for the detection of Hg^{2+} and Al^{3+} , out of various metal ions through UV-Visible and fluorescence spectrophotometry. TBET based phenanthroimidazol-anthraquinone, dyad **15** showed selective detection of PPI. In dyad **15**, the donor (phenanthroimidazol) and acceptor (anthraquinone) joined by conjugated spacer, which prevents dyad **15** from becoming flat, with minimum spectral overlap, large pseudo-Stokes' shift (190 nm) and emission shifts on interacting with PPI. The quantum yield of the dyad **15** on complexation with PPI has been changed from 0.1 to 0.69.

4.3.2 Synthesis of dyad 15

Dyad **15** was synthesized according to the synthetic route shown in Scheme 4.3.1. Intermediate (4-(1*H*-phenanthro[9,10-*d*]imidazol-2-yl))benzaldehyde) was synthesized by refluxing a solution of 9,10-phenanthraquinone, terephthalaldehyde and ammonium acetate in the presence of glacial acetic acid. Dyad **15** was synthesized using the oxidative coupling of 1,2-aminoanthraquinone and 1*H*-phenanthro[9,10-*d*]imidazol-2-yl))benzaldehyde in nitrobenzene as a brown colored solid in 57% yield; M.pt. = 235-237 °C; MS (ESI) m/z 541.2 ($\text{M}^+ + \text{H}$). The ^1H NMR spectrum showed two 1H singlets at δ 13.62 and 13.29 for NH_t and NH_s , 2H multiplet at δ 8.89-8.8 due to $\text{H}_{o,n}$, 2H doublet at δ 8.68 due to $\text{H}_{a,h}$, 2H multiplet at δ 8.59-8.56 due to $\text{H}_{b,g}$, 2H doublet at δ 8.50 due to $\text{H}_{e,d}$, 4H multiplet at δ 8.27-8.09 due to H_{i-l} , 2H singlet at δ 7.94 due to $\text{H}_{m,r}$, two 2H multiplets at δ 7.79-7.71 due to $\text{H}_{c,f}$ and δ 7.68-7.62 due to $\text{H}_{p,q}$. The peaks at δ 13.62 and 13.29

were due to two NH protons, confirmed the formation of dyad **15**. The ^{13}C NMR spectrum showed signals at δ 185.2, 181.6 for C=O, 149.2, 140.0, 134.8, 133.9, 129.4, 128.7, 127.9, 126.8, 126.6, 126.3, 126.1, 125.2, 123.3, 122.8, 122.1, 120.2, 115.1, 112.1 for aromatic-C. The spectral data having peaks at δ 185.2, 181.6 due to C=O corroborated the structure **15** for this compound. On the basis of these spectral data, this compound has been assigned the structure of 2-(4-(1H-phenanthro[9,10-d]imidazol-2-yl)phenyl)-3H-anthra[1,2-d]imidazole-6,11-dione (dyad **15**).



Scheme 4.3.1: Synthesis of dyad **15**

4.3.3 Results and discussion

4.3.3.1 UV-Visible studies of dyad **15** with metal ions

UV-Visible spectroscopic properties of dyad **15** were investigated in $20\text{ }\mu\text{M}$ $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1, v/v). Dyad **15**, which was dark yellow color, displayed an absorption band centered at 430 nm ($\epsilon = 17500\text{ dm}^3/\text{mol}/\text{cm}$). Upon addition of different metal ions, viz., Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Hg^{2+} , Pb^{2+} , Al^{3+} etc., as their perchlorate salts, to the solution of dyad **15**, no significant change in its UV-Visible spectrum was observed except in case of addition of Hg^{2+} and Al^{3+} ions (Figure 4.3.1). On addition of $250\text{ }\mu\text{M}$ Hg^{2+} to the solution of dyad **15**, the absorption intensities at 430, 337 and 370 nm decreased and concomitantly a new

absorption band at 390 nm was formed (Figure 4.3.2a). The ratios of absorption intensities at 370 nm and 430 nm (A_{430}/A_{370}) exhibited a drastic change from 0.652 in the absence of Hg^{2+} to 3.08 in the presence of Hg^{2+} showing 5-folds variation in the absorption ratio having detection limit of 0.05 μM . Thus, dyad **15** was used to estimate a wide range of Hg^{2+} between 0.5-250 μM ratiometrically through UV-Visible spectrophotometer. The stability constant for the Hg^{2+} in absorption spectroscopy has been determined by Benesi-Hildebrand equation which was found to be $7.27 \pm 0.02 \times 10^3 \text{ M}^{-1}$. Upon the incremental addition of 0-20 μM Al^{3+} to the solution of dyad **15**, the absorption intensities at 430 nm and 337 nm underwent gradually decreased with the concomitant increase at 410 nm with the formation of isosbestic points at 423 nm and 375 nm (Figure 4.3.2b).

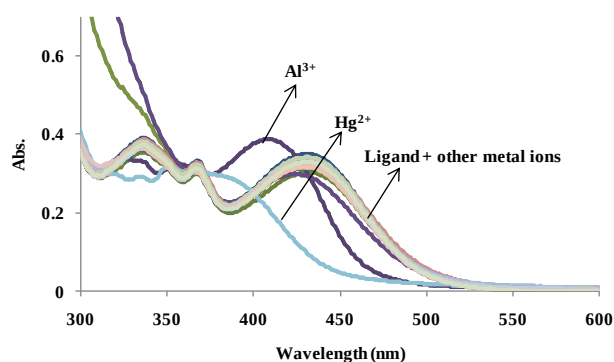


Figure 4.3.1: Absorption response of dyad **15** (20 μM) with different metal ions in $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1, v/v).

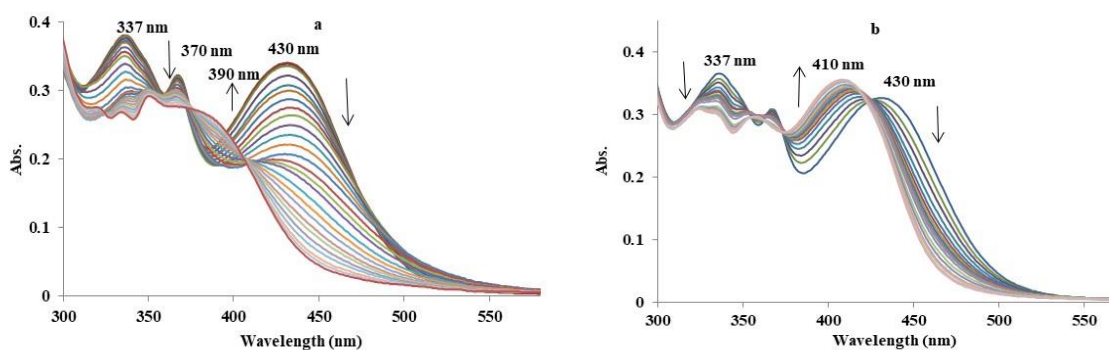


Figure 4.3.2: Absorption response of dyad **15** (20 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1, v/v)) (a) on increasing the concentration of Hg^{2+} ions; (b) on increasing the concentration of Al^{3+} ions.

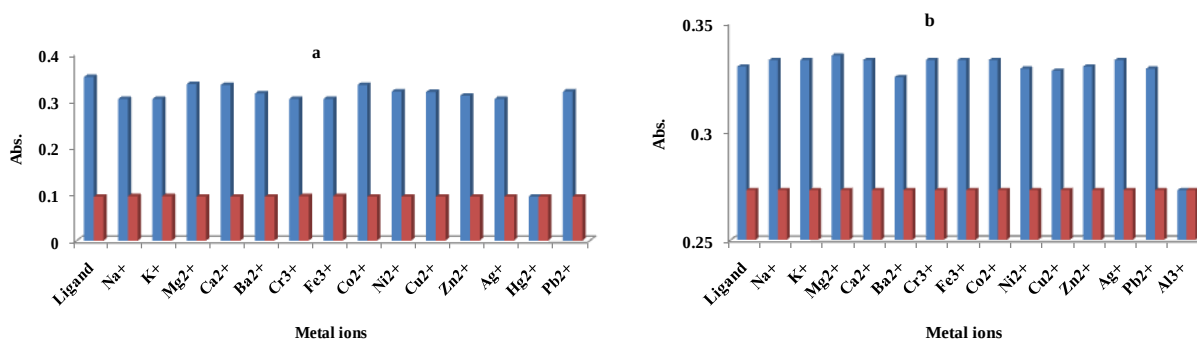


Figure 4.3.4: Blue bars represent the selectivity of dyad **15** (20 μM , CH_3CN) upon addition of different metal ions and red bars show the competitive selectivity of dyad **15** in the presence of (a) Hg^{2+} ions; (b) Al^{3+} ions.

The ratio of absorption intensities at 410 and 430 nm (A_{410}/A_{430}) exhibited a drastic change from 0.87 in the absence of Al^{3+} to 1.13 in the presence of Al^{3+} showing 1.3-folds variation in the absorption ratio having detection limit of 0.05 μM . The stability constant for the Al^{3+} ions in absorption spectroscopy was determined by Benesi-Hildebrand equation and found to be $2.87 \pm 0.01 \times 10^5 \text{ M}^{-1}$. To check the practical ability of dyad **15** as Hg^{2+} and Al^{3+} sensors, competitive experiments were carried out in the presence of 20 μM of Hg^{2+} and Al^{3+} and 1 mM of other interfering metal ions such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Pb^{2+} etc., as their perchlorate salts, no significant interferences were observed as shown in Figure 4.3.3.

4.3.3.2 Fluorescence studies of dyad 15 with metal ions

To obtain an insight into the fluorescent properties of dyad **15** towards metal ions, the emission change was investigated with different ions such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Ag^+ , Hg^{2+} , Pb^{2+} , Al^{3+} etc., as their perchlorate salts, in $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1, v/v). Dyad **15**, on excitation at 430 nm, displayed emission band at 530 nm (typical of anthraquinone moiety) which underwent fluorescence quenching selectively on the addition of Hg^{2+} with the small interference from Al^{3+} at high concentration whereas other metal ions did not show any significant change (Figure 4.3.4). The fluorescence intensity of dyad **15** was linearly proportional to the Hg^{2+} concentration of 0.05-5 μM and the binding constant was calculated to be $7.00 \times 10^4 \text{ M}^{-1}$. To determine the practical applicability of dyad **15**, competitive experiments were carried out in the presence of 10 equiv. Hg^{2+} ions mixed with 50 equiv. of each of metal ions. No significant variation in the fluorescence, as well as absorption intensity, was found when

comparing the results with or without other metal ions (Figure 4.3.5). Job's plot analysis clearly showed 1:1 stoichiometry with dyad **15** and Hg^{2+} (Figure A6).

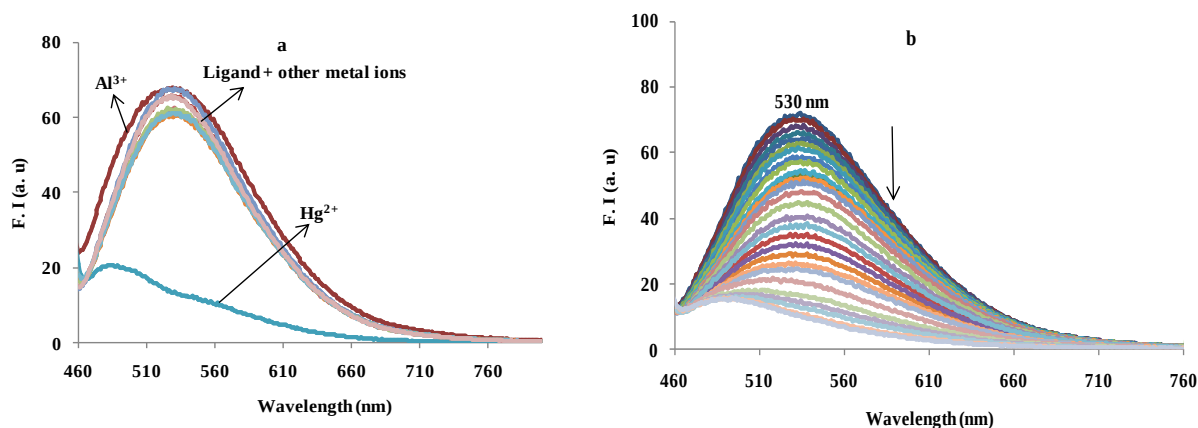


Figure 4.3.4: Fluorescence response of dyad **15** (20 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1, v/v)) (a) with different metal ions; (b) on increasing the concentration of Hg^{2+} ions.

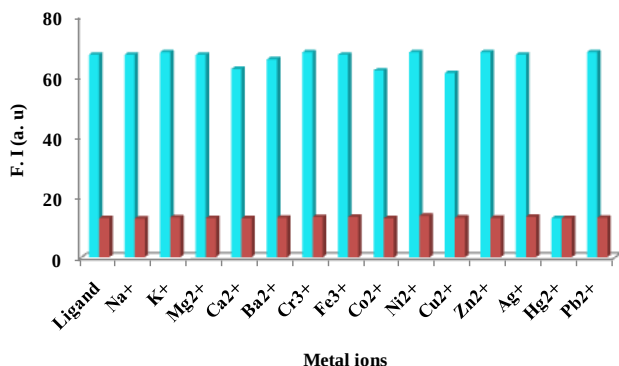


Figure 4.3.5: (a) Blue bars represent the selectivity of dyad **15** (20 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1, v/v)) upon addition of different metal ions and red bars show the competitive selectivity of dyad **15** in the presence of Hg^{2+} ions.

4.3.3.3 UV-Visible studies of dyad **15** with anions

The presence of NH-group in dyad **15** prompted us to evaluate its binding properties towards different anions of biological interest. On addition of anions viz., F^- , CN^- , Cl^- , SCN^- , AcO^- , NO_3^- , Br^- , HSO_4^- , H_2PO_4^- , I^- , PPi etc., as their tetrabutylammonium salts, a significant change in absorption was observed, only with PPi . On gradual addition of PPi ions to the solution of dyad **15**, the absorption intensity at 430 nm decreased with concomitant increase in absorption intensity at 495 nm. The equilibrium constant was determined to be $5.8 \pm 0.02 \times 10^4 \text{ M}^{-1}$ (Figure

4.3.6). The ratios of absorption intensities at 495 and 430 nm (A_{495}/A_{430}) exhibit a change from 0.18 to 1.38 showing 8-folds variation in the absorption ratio having a detection limit of 0.05 μM .

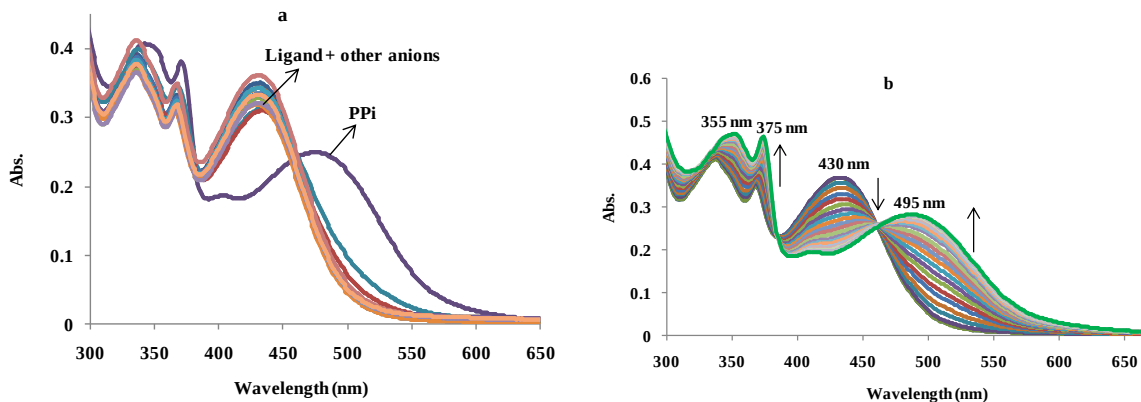


Figure 4.3.6: Absorption response of dyad **15** (20 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1, v/v)) (a) with different anions; (b) on increasing the concentration of PPI.

4.3.3.4 Fluorescence studies of dyad 15 with anions

In the case of fluorescence studies of dyad 15 with different anions *viz.*, F^- , CN^- , Cl^- , SCN^- , AcO^- , NO_3^- , Br^- , HSO_4^- , H_2PO_4^- , I^- , PPI etc., as their tetrabutylammonium salts, fluorescence changes only with PPI was observed (Figure 4.3.7a). On gradual addition of PPI to the solution of dyad 15, the emission at 530 nm was quenched and a new emission band at 620 nm emerged that reached its plateau after addition of 50 μM PPI (Figure 4.18b).

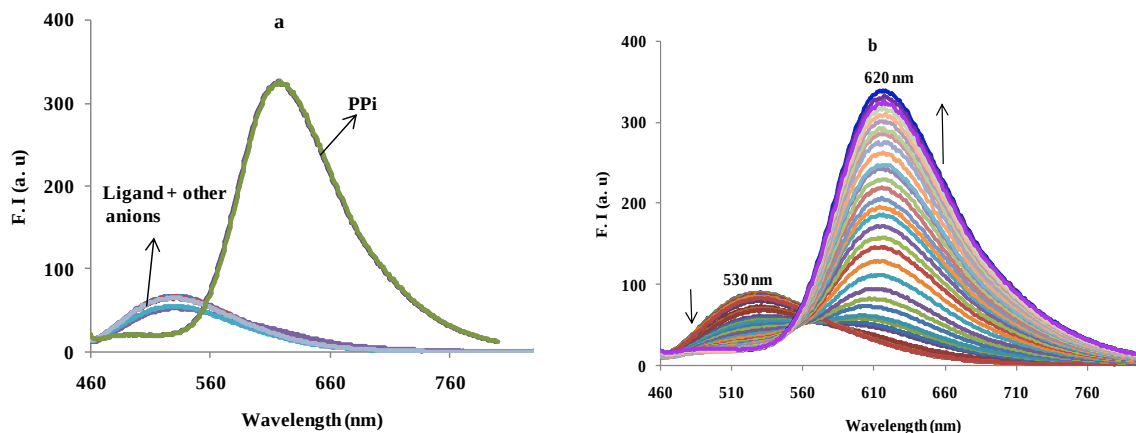


Figure 4.3.7: Fluorescence response of dyad **15** (20 μM , $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (9:1, v/v)) (a) with different anions; (b) on increasing the concentration of PPI.

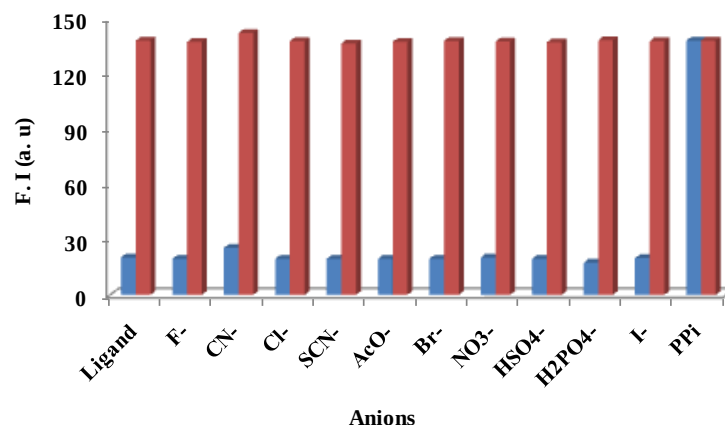


Figure 4.3.8: Blue bars represent the selectivity of dyad **15** (20 μ M, CH₃CN:H₂O (9:1, v/v)) upon addition of different anions and red bars show the competitive selectivity of dyad **15** in the presence of PPI.

The binding constant was determined to be $2.85 \pm 0.02 \times 10^5 \text{ M}^{-1}$. The ratios of absorption intensities at 620 and 530 nm (A_{620}/A_{530}) exhibited a change from 0.26 to 15.92 showing 60-folds variation in the absorption ratio having detection limit of 0.05 μ M. To determine the practical applicability of dyad **15**, competitive experiments were carried out in the presence of 1000 μ M of PPI mixed with 1000 μ M of each of the different ions. No significant variation in the fluorescence intensity was observed when comparing with or without the anions (Figure 4.3.8). The quantum yields of the dyad **15** and its complex with PPI were 0.1 and 0.69, respectively. Thus, dyad **15** showed higher sensitivity as well as faster response.

4.3.3.5 Energy transfer efficiency (ETE) calculations

To estimate the energy transfer efficiency (ETE) of dyad **15** upon addition of PPI, the absorption and emission spectra of phenanthroimidazole were investigated (Figure 4.3.9). The phenanthroimidazole showed a strong emission at around 400 nm and there was no change on addition of PPI to this compound. These results indicated that PPI has no effect on the emission spectra of phenanthroimidazole, so, TBET occurred between donor and acceptor. The ETE for dyad **15** on the addition of PPI was calculated to be 90.69 %. Many reports on TBET system indicated that there was some extent of FRET process resulting in the failure of 100% energy transfer. TBET cassettes have very large pseudo-Stokes' shifts of upto 190 nm and emission shift of 90 nm.

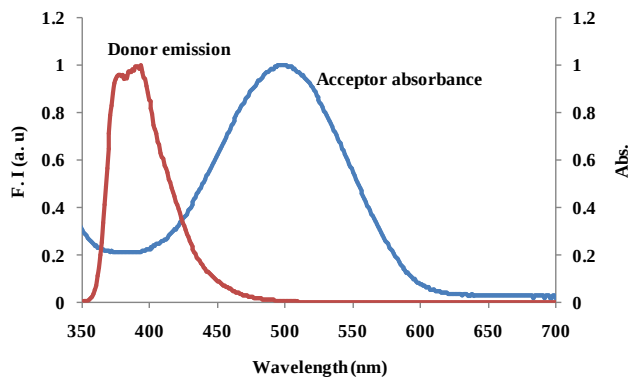


Figure 4.3.9: Normalized spectra of donor (phen) emission (red) and acceptor (anthraquinone) absorption (blue) and spectral overlap between them.

4.3.3.6 Theoretical calculations

Dyad **15** and its complex with PPI were optimized at B3LYP/6-31+G* theory. The excited state of dyad **15** and its complexes **15**·Cu²⁺ and **15**·CN⁻ showed the absorption maximum bands at 388 nm, 537 nm and 388 nm in the gaseous state which is in good agreement with the experimental values at 430 nm, 495 and 390 nm, respectively. The optimized structure of dyad **15** in ground state have trans orientation for NH groups of both benzimidazole rings. On the other hand, during the optimization of the complex (dyad **15**·PPI), the dyad **15** flipped through single bond leading to stabilization of complex through hydrogen bonding. The bond distance between anthraquinone benzimidazole and PPI oxygen atom was 2.7 Å and bond distance between phenanthroimidazole benzimidazole and PPI oxygen atom was 2.5 Å. Hg²⁺ ion binds with nitrogen and oxygen of anthraquinone ring with a bond distance of 1.9 Å and 2.3 Å. Further, the absorption properties of dyad **15** and its complexes with PPI, were predicted using TD-DFT calculations. Frontier molecular orbital analysis for dyad **15** showed electron density distribution at phenanthroimidazole and anthraquinone for HOMO and LUMO, respectively, indicating the intramolecular charge transfer. On the other hand, the HOMO and LUMO of complex dyad **15**·PPI have electron density distribution at PPI and anthraquinone, respectively. The complex also showed charge transfer from PPI to anthraquinone. HOMO of dyad **15**·Hg²⁺ distributed over phenanthroimidazole and LUMO was on phenyl, Hg²⁺ and anthraquinone moiety (Figure 4.3.10).

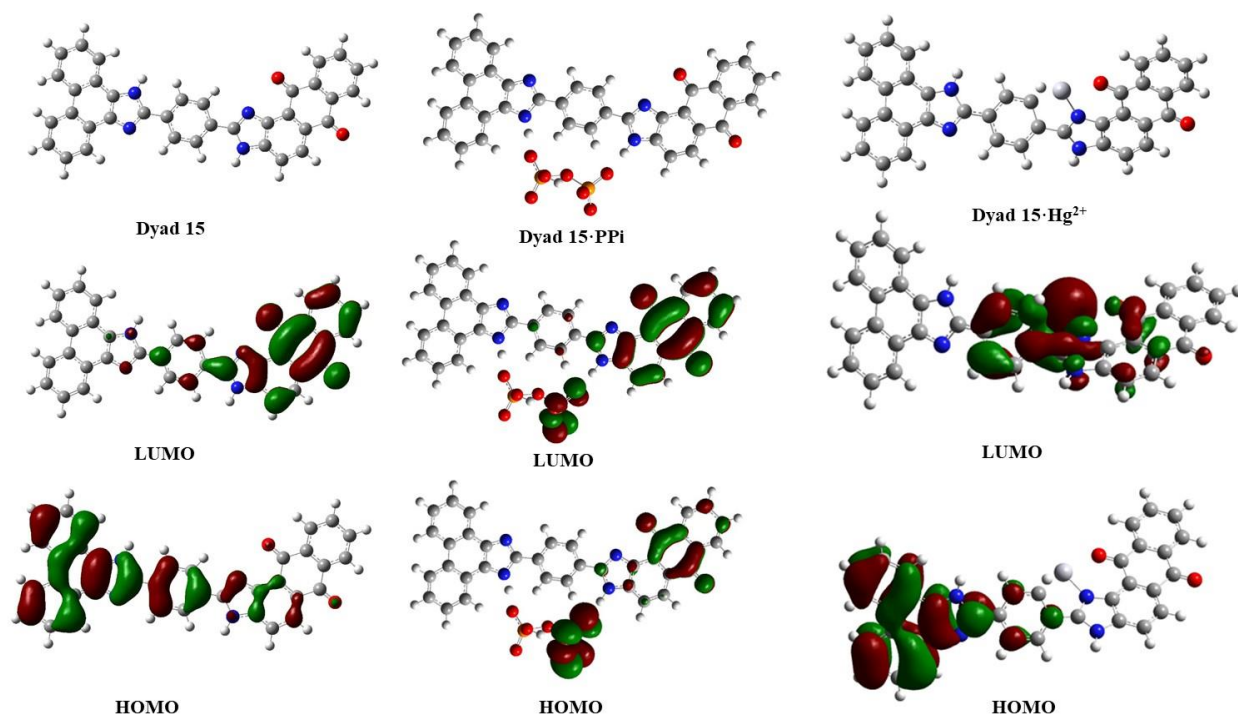


Figure 4.3.10: Energy minimized structure and frontier molecular orbital contribution of dyad **15** and its complexes with PPI ions.

4.3.4 Conclusions

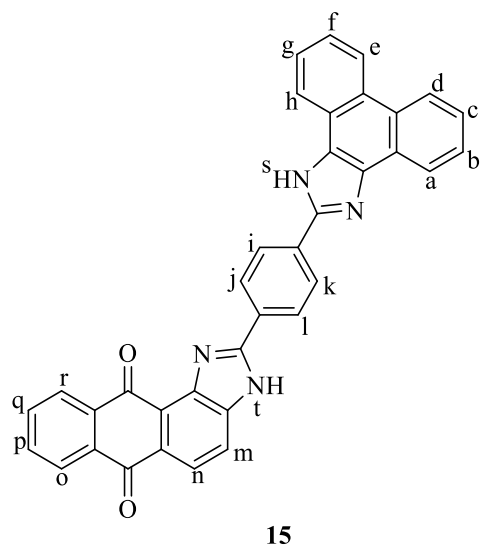
Through bond energy transfer based phenanthroimidazole-anthraquinone dyad **15** for ratiometric sensing of PPI has been synthesized. Dyad **15** has been used for the estimation of Hg^{2+} and Al^{3+} ions out of various metal ions through UV-Visible and fluorescence spectrophotometer. TBET based phenanthroimidazole-anthraquinone dyad **15** was also used for selective determination of PPI. Thus, dyad **15** showed minimal spectral overlap, large pseudo-Stokes' shift (190 nm) and emission shifts (90 nm) on interacting with PPI. The quantum yield of the dyad **15** on complexation with PPI was changes from 0.1 and 0.69.

4.3.5 Experimental section

Synthesis of dyad 15: A solution of 1*H*-(phenanthro[9,10-*d*]imidazol-2-yl)benzaldehyde (1 g, 3.1 mmol)) and 1,2-diaminoanthracene-9,10-dione (0.74 g, 3.1 mmol) in nitrobenzene (3 mL) was heated at 120 °C. The reaction was monitored through TLC and after completion of reaction (24 h), the reaction mixture was cooled to room temperature. A solid precipitate was separated

out, filtered and washed with diethyl ether which was further purified through column chromatography.

2-(4-(1*H*-phenanthro[9,10-*d*]imidazol-2-yl)phenyl)-3*H*-anthra[1,2-*d*]imidazole-6,11-dione
(dyad 15)



57% yield; red solid; M.pt. = 235-237 °C; ¹H NMR (CDCl₃ + DMSO-*d*₆, 400 MHz): δ (ppm) 13.62 (s, 1H, NH_t), 13.29 (s, 1H, NH_s), 8.89-8.83 (m, 2H, ArH_{o,r}), 8.68 (d, 2H, *J* = 7.64 Hz, ArH_{a,h}), 8.59-8.56 (m, 2H, ArH_{b,g}), 8.50 (d, 2H, *J* = 7.64 Hz, ArH_{e,d}), 8.27-8.09 (m, 4H, ArH_{i-l}), 7.94 (s, 2H, ArH_{p,q}), 7.79-7.71 (m, 2H, ArH_{c,f}), 7.68-7.62 (m, 2H, ArH_{m,n}); ¹³C NMR (CDCl₃ + DMSO-*d*₆, 100 MHz): δ (ppm) 185.2, 181.6, 149.2, 140.0, 134.8, 133.9, 129.4, 128.7, 127.9, 126.8, 126.6, 126.3, 126.1, 125.2, 123.3, 122.8, 122.1, 120.2, 115.1,

112.1 (ArC); MS (ESI) *m/z* 541.2 (M⁺ + H).

Conclusions of chapter 4

Chapter 4 includes FRET/TBET based dyads and studied their photophysical behavior towards different metal ions and anions with following features:

- FRET-based dyad **14** is used as a new chromogenic and fluorescent chemosensor suitable for ratiometric sensing of Hg^{2+} on the basis of the Hg^{2+} -induced *on-off-on* switching and ratiometric detection of biothiols based on FRET reversal.
- The well-resolved absorption and emission peaks, the high efficiency in energy transfer and large variation in the emission (I_{585}/I_{485}) ratios are the added advantages.
- TBET based dyad **15** donor (phenanthroimidazole) and acceptor (anthraquinone) are joined by conjugated spacer, which prevents dyad **15** from becoming flat.
- Thus dyad **15** is fluorescent dyad with minimum spectral overlap, large pseudo-Stokes' shift (190 nm) and emission shifts on interacting with PPI.
- The quantum yield of the dyad **15** on complexation with PPI was changes from 0.1 and 0.69.

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SUMMARY

Chemosensor is a miniaturized device having receptor with recognition site which on binding with a particular analyte converts the binding event to the optical signal. Chemosensors have some limiting features viz., selectivity, sensitivity, limit of detection, response time, concentration range etc. Thus, the development of molecular or polymeric systems capable of sensing biologically and/or environmentally important cations or anions has generated significant interest in recent years.¹ Among the various chemical sensors, fluorescence-based sensors have the benefit of being of non-destructive, rapidly performed, highly sensitive, versatile, low cost and can afford real information as well as quantify the targets.² Various sensors have been reported in literature such as rhodamine, naphthalimide, coumarin, benzoxazoles, fluorescein, BODIPY, squaraine, phenanthrene etc. due to their excellent stability, visible excitation and emission, and large Stokes' shift.³ Some of these sensors have been used as fluorescent chemosensors for pH, metal cations and anions in recent years.⁴

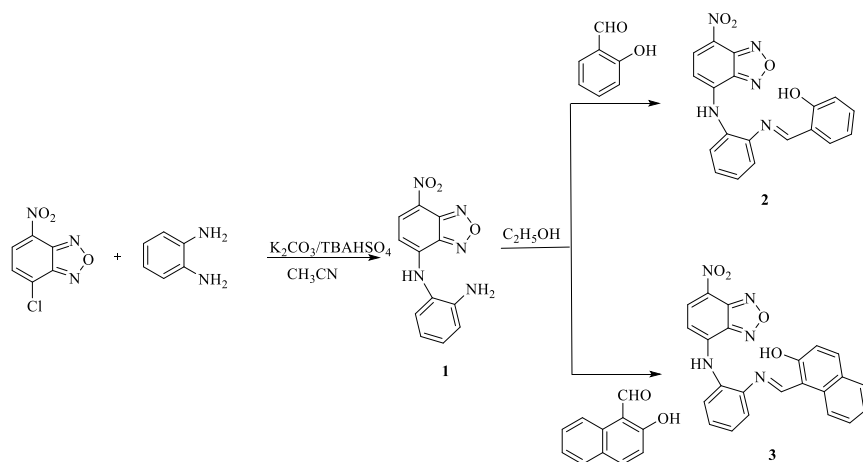
It has been found there are scarce examples based on NBD, thiazolidine, phenanthroimidazole, rhodamine and naphthalimide moieties. Also, there is still room to synthesize the dyads based on rhodamine, naphthalimide, coumarin, benzoxadiazole, BODIPY, etc. with varied spacer length to fine tune the various energy transfer processes like FRET, TBET, PET etc. The dyads through combinations of these fluorescent dyes in conjugation or through some spacers would provide ample opportunities for (i) generation of ratiometric sensors for various cations anions and biological ions at distinctly different wavelengths, (ii) absorption and emission changes can extend up to NIR regions, (iii) distinctly different absorption and emission channels can be used to generate molecular logic gates. The results of these investigations have been presented in chapters 2-4 which are preceded by a brief review of the literature on NBD and FRET/TBET based molecules in chapter 1.

Chapter 2: Synthesis of 7-nitrobenzo[c][1,2,5]oxadiazole (NBD) moiety based molecules as chemosensors

2.1 Introduction:-In this chapter, NBD based Schiff bases have been synthesized and their complexation behavior towards different anions and cations was investigated. These chemosensors depending on the different nature of binding sub-units are found to bind with different ions. The results of these findings have been discussed in three sections as given below:

2.2 NBD-based two-in-one $\text{Cu}^{2+}/\text{Ni}^{2+}$ chemosensor with differential charge transfer processes:- In this chapter, fluorescent dyad **2** possessing hydroxyphenyl Schiff base onto NBD moiety has been synthesized as depicted in Scheme 2.1. Dyad **2** showed high sensitivity and selectivity towards $\text{Cu}^{2+}/\text{Ni}^{2+}$ over other metal ions over a wide pH range. The dyad **2** could be used for the simultaneous detection of Cu^{2+} and Ni^{2+} ions through its visible color change from yellow to blue and light green, respectively, in semi-aqueous systems. The binding mode of dyad **2** to Cu^{2+} and Ni^{2+} was determined to be a 1:1 complexation stoichiometry using Job's plot. Ethylenediaminetetraacetate showed high selectivity towards *in situ* prepared Cu^{2+} complex over the Ni^{2+} complex, which has been applied to distinguish Ni^{2+} and Cu^{2+} ions.

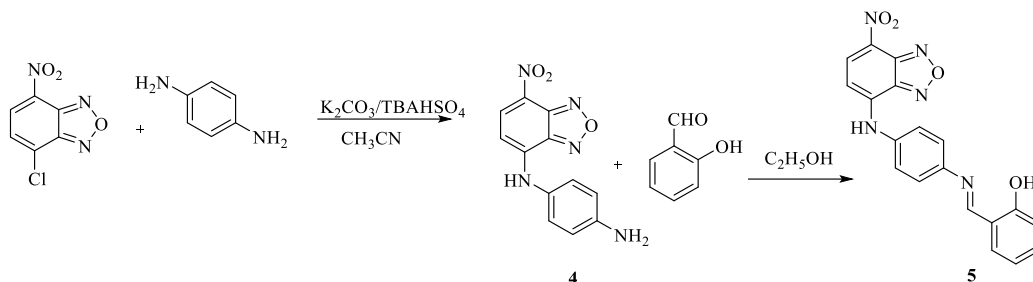
2.3 NBD based dual channel chromogenic sensor for Cu^{2+} and a fluorogenic sensor for Al^{3+} and Hg^{2+} :- NBD based Schiff base of 2-hydroxy-1-naphthaldehyde has been synthesized (Scheme 2.1). The dyad **3** acted as a dual channel mode chromogenic sensor for Cu^{2+} and a fluorogenic sensor for Al^{3+} and Hg^{2+} ions. In presence of Cu^{2+} ions, dyad **3** showed an instant color change from yellow to blue with a red shift of 150 nm. Dyad **3** worked as a fluorescent chemosensor for Al^{3+} and Hg^{2+} through *turn-on* the emission.



Scheme 2.1: Synthesis of dyads **2** and **3**

2.4 NBD based Schiff base as chromofluorescent sensor for Cu^{2+} , Al^{3+} , and CN^- ions:- A Schiff base derivative of NBD dyad **5** has been synthesized as depicted in Scheme 2.1. In presence of Cu^{2+} ions, dyad **5** showed a blue shift of 95 nm on binding with Cu^{2+} ions. Dyad **5** has been used to sense CN^- ions selectively with a color change from orange to yellow and showed a blue shift of 100 nm. Weak emission of dyad **5** underwent *turn-on* on at 475 nm on the

addition of Al^{3+} and at 540 nm on the addition of CN^- ions. Dyad **5** provided a convenient and selective detection of Cu^{2+} , Al^{3+} , and CN^- ions.



Scheme 2.2: Synthesis of dyad **5**

Chapter 3: Synthesis of thiazolidine moiety based molecules as chemosensors

3.1 Introduction:-In this chapter, we have synthesized the hybrid molecules by incorporating the thiazolidine moiety onto different fluorescent molecules. These molecules were used for the sensing of different cations and anions. This chapter has been further divided in following sub parts:

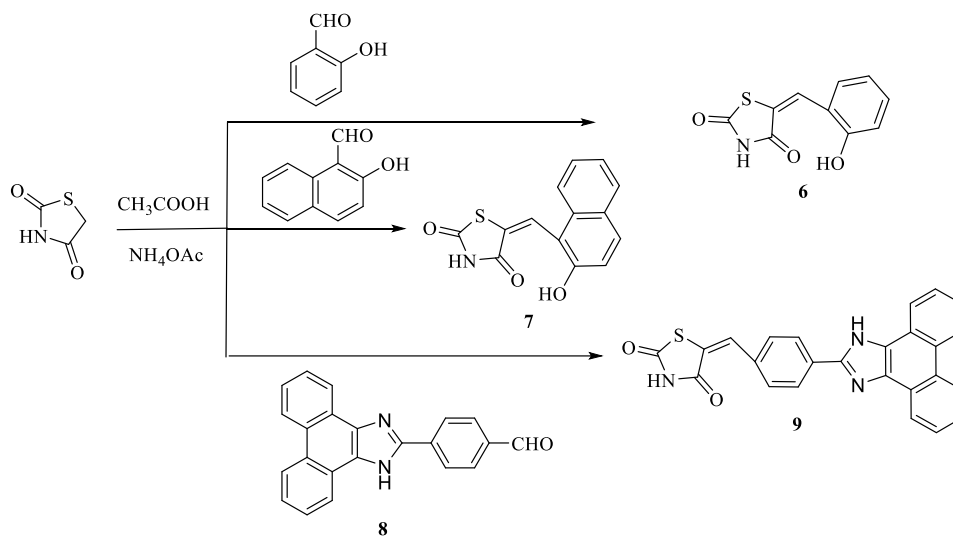
3.2 Thiazolidine based differential chromo-fluorescent sensors for Cu^{2+} and CN^- ions:

Elaboration as logic devices:-Thiazolidine based probe **6** has been synthesized (Scheme 3.1) and investigated their photophysical behavior towards different metal ions and anions. The probe **6** has opened new absorption and emission channels in the presence of Cu^{2+} ions and resulted in “ON–OFF–ON” type sensing. The probe **6** has also shown selective ratiometric absorption behavior towards CN^- ions in $\text{CH}_3\text{CN}:\text{H}_2\text{O}$: 1:1. The ratiometric behavior of the probe **6** in the presence of Cu^{2+} and CN^- ions have used as XOR logic function at λ_{em} 485 nm and YES logic gate at λ_{abs} 300 nm. Probe **6** has been used for its practical applicability to sense Cu^{2+} and CN^- through dip coating method.

3.3 Thiazolidine based molecules as a fluorescent sensor for recognition of Hg^{2+} , CN^- and PPI and application as INHIBIT logic device:-

Thiazolidine based probe **7** has been synthesized (Scheme 3.1) for selective determination of Hg^{2+} ions amongst various metal ions. The $\text{7}\cdot\text{Hg}^{2+}$ complex *in-situ* formed, behaved as a selective fluorescent probe for PPI by a metal displacement approach, ensuing reversal return of ligand from the $\text{7}\cdot\text{Hg}^{2+}$ complex. The probe **7** was used for wide range detection of Hg^{2+} ions from 0.5-250 μM in CH_3CN . It has also been used for the detection of CN^- ion, and PPI through UV-Visible and fluorescence

spectrophotometry. Depending on two inputs of Hg^{2+} and PPI and outputs at emission wavelength 485 nm, INHIBIT logic gate has been mimicked.



Scheme 3.1: Synthesis of compounds **6-9**

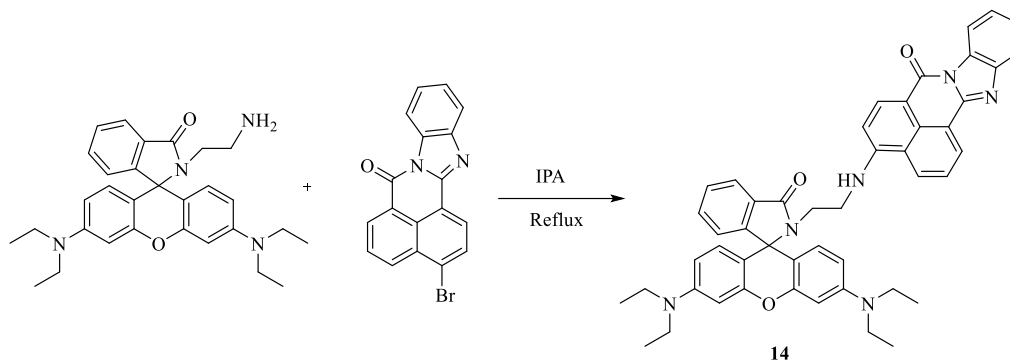
3.4 Thiazolidine based solvent dependent differential chemosensor for metal ions and anions:- Probe **9** based on thiazolidine (Scheme 3.1) has been used for detection of Hg^{2+} and Al^{3+} in both solvents such as CH_3CN and $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (9:1, v/v) amongst various metal ions. Interestingly, probe **9** showed drastic change with H_2PO_4^- in CH_3CN and HSO_4^- in $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ (9:1, v/v). Thus, a single probe worked differently in polar as well as nonpolar solvents. More interestingly, probe **9** could be elaborated as XOR logic gate.

Chapter 4: Synthesis of FRET/TBET phenomenon based molecules

4.1 Introduction:-The two fluorescent moieties on combining together communicate each other in different manners in the presence of metal ions depending on the binding subunits. The present chapter describes FRET and TBET phenomenon in the presence of metal ions.

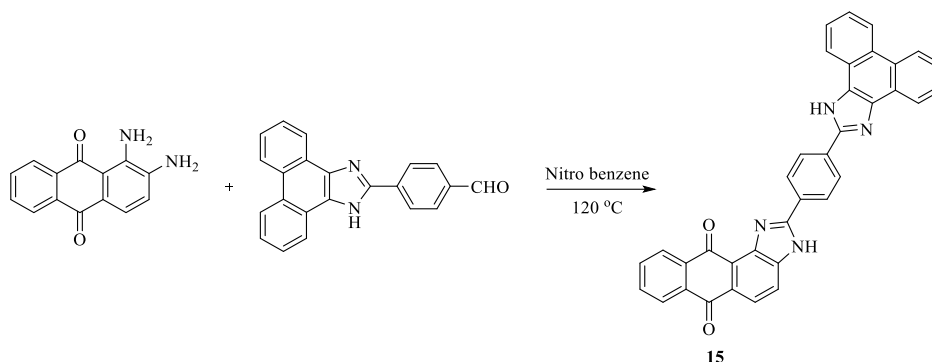
4.2 FRET-based ratiometric detection of Hg^{2+} and biothiols using naphthaimide–rhodamine dyads:- A naphthalimide–rhodamine-based dyad **14** (Scheme 4.1) in CH_3CN -HEPES (1:1) buffer solution exhibited fluorescence resonance energy transfer (FRET) from the naphthalimide to the rhodamine moiety on the addition of only Hg^{2+} ions. FRET-induced fluorescence changes were recovered again by the subsequent addition of thiol amino acids *via*

reverse FRET. The inter conversion of dyad **14** and **14**·Hg²⁺ via the complexation/decomplexation by the modulation of Hg²⁺/Cys, revealed a selective dyad for biothiols in real samples.



Scheme 4.1: Synthesis of dyad **14**

4.3 TBET- based phenanthroimidazole-anthraquinone dyad for ratiometric sensing of PPI: Dyad **15** has been synthesized by combining the phenanthroimidazole and anthraquinone moieties which exhibited through bond energy transfer (TBET) phenomenon for ratiometric sensing of PPI (Scheme 4.2). Dyad **15** has been used for the estimation of Hg²⁺ and Al³⁺ out of various metal ions through absorption and emission channels. The dyad **15** showed minimal spectral overlap, large pseudo-Stokes' shift (190 nm) and emission shifts upon interacting with PPI.



Scheme 4.2: Synthesis of dyad **15**

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APPENDIX-A

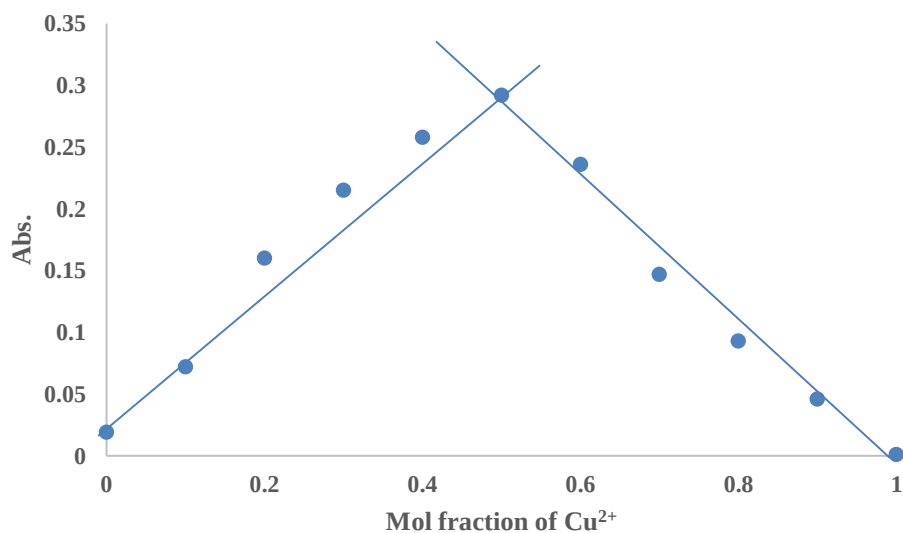


Figure A1: Job's plot showing the 1:1 stoichiometry between dyad 2 and Cu^{2+} .

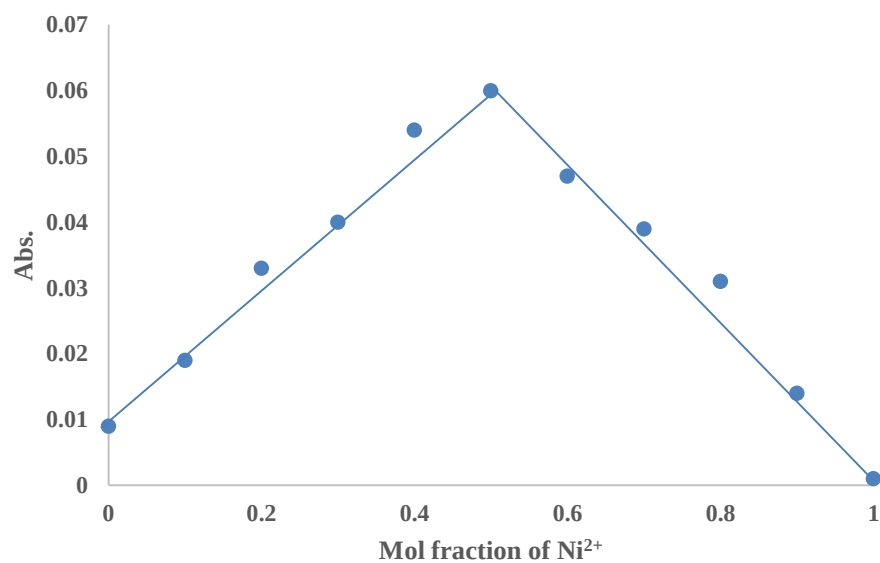


Figure A2: Job's plot showing the 1:1 stoichiometry between dyad 2 and Ni^{2+} .

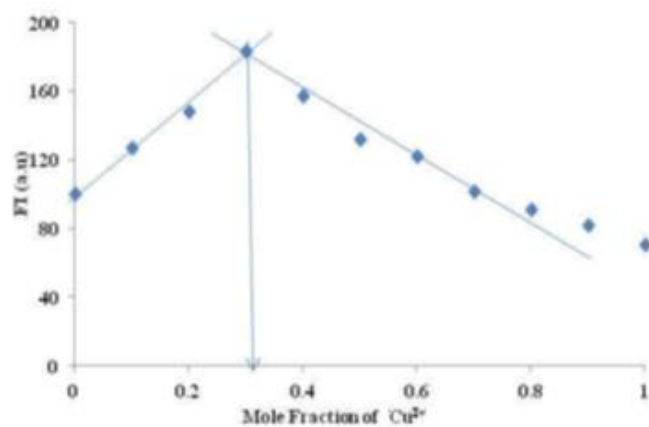


Figure A3: Job's plot showing the 1:2 ($2\text{L}:\text{Cu}^{2+}$) stoichiometry between probe 6 and Cu^{2+} .

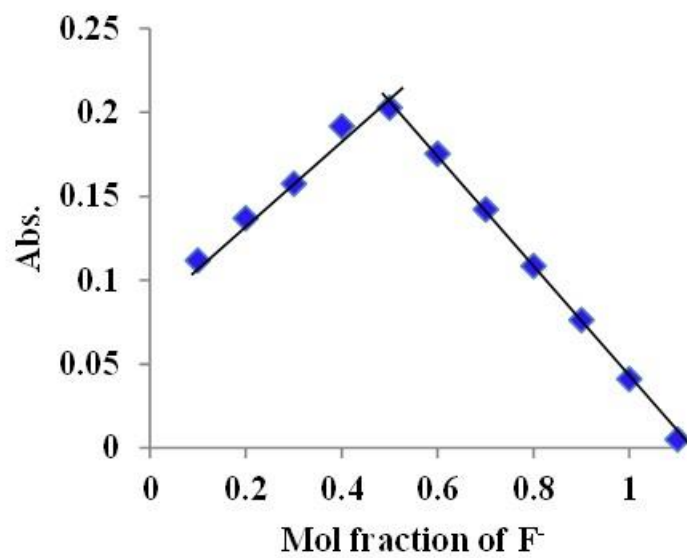


Figure A4: Job's plot showing the 1:1 stoichiometry between probe 6 and F^- .

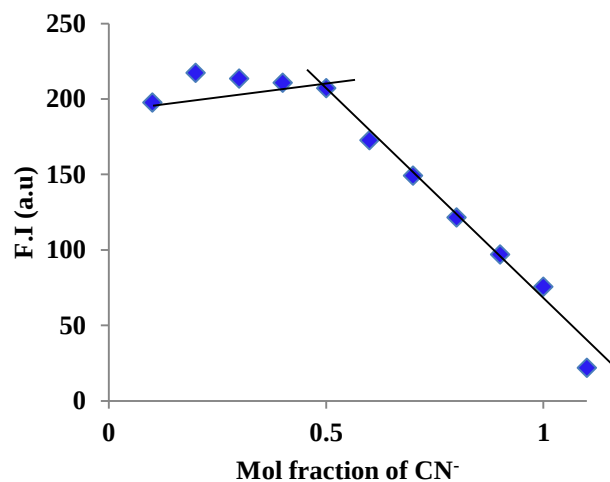


Figure A5: Job's plot showing the 1:1 stoichiometry between probe **6** and CN^- .

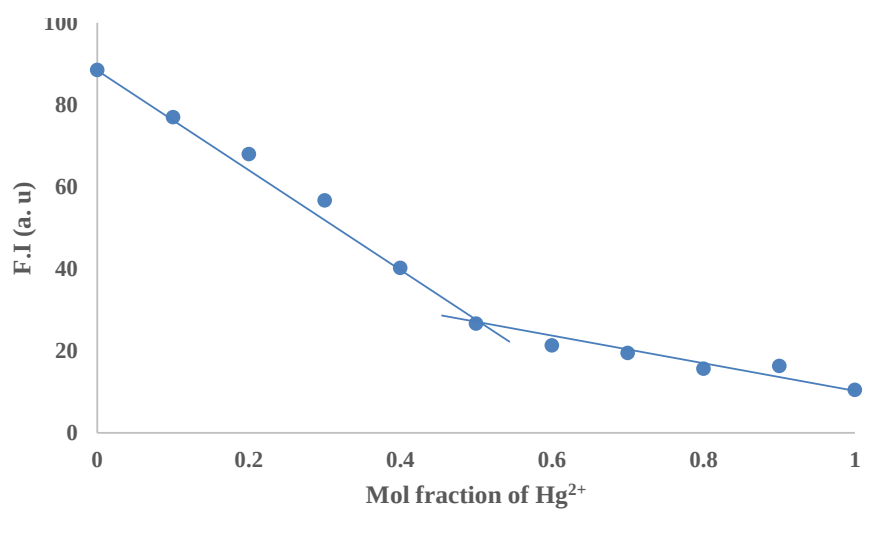


Figure A6: Job's plot showing the 1:1 stoichiometry between dyad **15** and Hg^{2+} .

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Papers in SCI/refereed journals

1. Vijay Luxami, Meenakshi Verma, Richa Rani, Kamaldeep Paul, Subodh Kumar, *Org. Biomol. Chem*, 2012, **10**, 8076-8081.
2. Navneet Kaur, Jasvinder Singh, Gargi Dhaka, Richa Rani, Vijay Luxami, *Supramol.Chem.*, 2015, **27**, 453-459.
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5. Richa Rani, Gulshan Sharma, Vijay Luxami, Kamaldeep Paul, *J. Lumin.*, 2016, **180**, 292-300.

Papers/ Posters in Conferences

1. Richa Rani, Vijay Luxami and Kamaldeep Paul, Presented (Poster) to the 15-CRSI-RSC National symposium in Chemistry (NSC-15), Banaras Hindu University, Varanasi, 1-3th February, 2013.
2. Richa Rani, Vijay Luxami and Kamaldeep Paul, Presented (Poster) to the National conference on innovative molecules or sustainable future (NSIMSF-15), Thapar University, Patiala, 24-26th October, 2013.
3. Richa Rani, Vijay Luxami and Kamaldeep Paul, Presented (Poster) to the SACOS at chemistry department in Punjabi University, Patiala, 19-20th Feb. 2015.
4. Richa Rani, Vijay Luxami and Kamaldeep Paul, Presented (Poster) to the 18-CRSI-RSC National symposium in Chemistry (NSC-18), Panjab University Chandigarh, 5-7th February, 2016.