Imidazole Fused Anthraquinone Derivatives as Chromogenic Sensors for Cations and Anions

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

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IN
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Submitted By:

AVNI REKHI

Registration No. 301402004

Supervisor:

Dr. Vijay Luxami

School of Chemistry and Biochemistry

Thapar University

Patiala

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Avni Rekhi

CANDIDATE'S DECLARATION

This thesis titled "**Imidazole fused Anthraquinone derivatives as chromogenic sensors for cations and anions**" is a presentation of my original research work. Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature, and acknowledgement of collaborative research and discussions. The work was done under the guidance of Dr Vijay Luxami at Thapar University, Patiala.

Patiala
Date: 16th June, 2016

Avni Rekhi
301402004

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

Dr. Vijay Luxami
Supervisor
SCBC, Thapar University
Patiala

Dr. Bohamali Pal
Professor and Head
SCBC, Thapar University
Patiala

CERTIFICATE

This is to certify that the thesis "**Imidazole fused Anthraquinone derivatives as chromogenic sensors for cations and anions**" is being submitted by Avni Rekhi in partial fulfillment of requirements for the award of degree of Master's in Science in Chemistry in School of Chemistry and Biochemistry, Thapar University, Patiala, is a bonafide work carried out under the supervision of Dr. Vijay Luxami and no part of the thesis has been submitted for the award of any degree.

Dr. Vijay Luxami

Assistant Professor and Supervisor,
School of Chemistry and Biochemistry,
Thapar University, Patiala

Dr. Bonamali Pal

Professor and Head
School of Chemistry and Biochemistry,
Thapar University,
Patiala, Punjab

Dr. S. S. Bhatia

Dean, Academic Affairs,
Thapar University,
Patiala, Punjab

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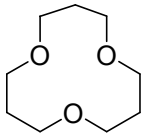
Introduction

Host guest chemistry is the chemistry in which there is a molecular entity that forms complexes with organic or inorganic guests or a chemical species that can accommodate guests within cavities of its crystal structure. Host is the organic molecule that contains convergent binding site while, guest are those molecules or ions that contain divergent binding sites. Guests are of two types cationic and anionic most of the receptors bind to cation via ion dipole interactions while in case of anions charge, size plays an important role. Complexes are formed when hosts and guests are held together in solution in definable structural relationships by electrostatic forces such as ion pairing, hydrogen bonding, metal ion-to-ligand attraction, π-π-stacking, dipole-dipole interactions, and van der Waals attraction and the entropic component of desolvation. Cryptands, Crown ethers, urea, thiourea are the certain examples. It generally works on Lock and Key method whose principle is size, shape and position of the binding sites within the active site of a host (enzyme) which are ideal for specific recognition of the guest (substrate). Host guest chemistry plays an important role in Catalysis because non-covalent interactions are extremely important in binding reactants into conformations suitable for reaction and lowering the transition state energy of reaction. Template-directed synthesis is a special case of supramolecular catalysis. Encapsulation systems such as micelles are also used in catalysis to create microenvironments suitable for reactions to progress that is not possible to use on a macroscopic scale. It also plays important role in developing new pharmaceutical therapies by understanding the interactions at a drug binding site. The area of drug delivery has also made critical advances as a result of supramolecular chemistry providing encapsulation and targeted release mechanisms. In addition, molecules have been designed to disrupt protein-protein interactions that are important to cellular function. Host guest chemistry has property to demonstrate computation functions on a molecular scale also. In many cases, photonic or chemical signals have been used in these components but electrical interfacing of these units has also been shown by host guest chemistry. Data storage has been accomplished by the use of molecular switches with photochromic and photoisomerizable units by electrochromic and redox-switchable units and even by molecular motion. Synthetic molecular logic gates have been demonstrated on a conceptual level.

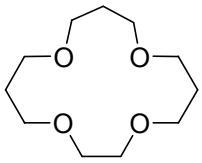
The field green chemistry is where reactions have been developed which proceed in the solid state directed by non-covalent bonding. Such procedures are highly desirable since they reduce the need for solvents during the production of chemicals. This type of chemistry is often pursued to develop new functions that cannot appear from a single molecule. These functions also include magnetic properties, light responsiveness, self-healing polymers, synthetic ion channels, molecular sensors, etc. Now days research is going on supramolecular interactions in biological systems. The binding of enzymes with their cofactors has been used as a route to produce modified enzymes, electrically contacted enzymes and even photoswitchable enzymes. The enzyme cytochrome C oxidase is an enzyme embedded into inner membrane of mitochondria. Its main function is to oxidise the protein called cytochrome C. Cytochrome C oxidase contains several metal active sites. The complex is composed of large membrane protein containing several prosthetic sites for metals. Two heme, two type of cytochrome and two copper centers are contained by the complex. In fact, the copper centers coordinate a OH^- in the fully oxidized state. DNA has been used both as a structural and as a functional unit in synthetic supramolecular systems. Interactions between DNA and DNA binding proteins and between DNA and small biologically active molecules are central to many of the vital processes in biological systems. DNA binds by DNA-ligand interactions i.e. intercalation between the base pairs of DNA. There is hydrogen bonding between different base pair of DNA which is kind of supramolecular interactions. If there are any changes in hydrogen bonding between base pair than it gets ruptured which can lead to various diseases in our body. Hemoglobin is found in red blood cells. A heme group consists of an iron Fe^{2+} held in a porphyrin ring in deoxy form. This porphyrin ring consists of four molecules of pyrrole linked together by bridges called methine bridges with the iron ion bound in the center. The iron ion is the site of oxygen binding. The iron is bound strongly to the globular protein via the N atoms of the ring called imidazole ring of histidine. A sixth position can reversibly bind oxygen by a coordinate covalent bond completing the octahedral group of six ligands. In oxy form Fe^{2+} changes its conformation to Fe^{2+}. O_2, NO, CO and H_2S can bind to the iron atom in heme proteins. Once bound to the prosthetic heme groups, these molecules can modulate the activity function of those hemeproteins, affording signal transduction.

Some toxic anions like cyanide binds to heme proteins and inhibit the terminal respiratory chain enzyme cytochrome C oxidase which leads to fatal consequences.

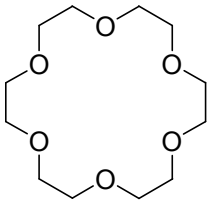
Valinomycin is a naturally occurring dodecadepsipeptide used in the transport of K^+ and as an antibiotic . It can interact with K^+ and can transport it where there is deficiency of it. It has specificity for K^+ rather than Na+ due to its corresponding size. Even 18-crown-6 has high affinity for K^+ cation, 15-crown-5 for Na^+ cation, and 12-crown-4 for Li^+ cation which all includes weak interactions which can be used for transportation or detection of certain metals in our body.



12 crown-4



15- crown -5



18- crown -6

Chemosensors are the devices that respond to particular analytes and convert the chemical stimuli into a signal which can be measured or recorded. Particular guest analytes bind molecules through noncovalent interactions, so called host–guest chemistry or supramolecular chemistry. A chemical sensor has three parts: a receptor, a signal transducer and a read out mechanism. The receptor is a moiety that interacts directly with the analyte and is capable to convert the changes in the chemical composition of the molecular environment like physical or chemical properties for e.g. The electron distribution energy of frontier orbital, redox potential etc.[1] The important feature of a receptor is the selectivity so that it interacts or binds with a particular analyte irrespective of other interfering molecules. The transducer transforms and amplifies the chemical changes into an observable analytical signal output. The read-out moiety is responsible for reporting the recognition event.

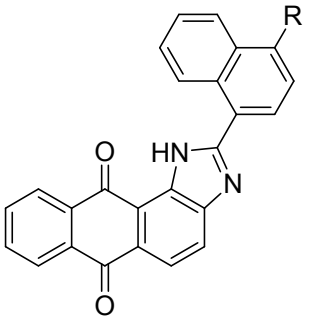
Chemo sensors which include cations, anions play a significant role in various biological and environmental processes. Ca^{2+} is one of the main constituent in building strong bones and teeth.[2,3] It suppresses muscle cramps and initiate reactions in the human body.

Similarly $Zn^{2+}$and Mg^{2+} prevents cytotoxicity. Fe^{3+} deficiencies are common in cancer patients.[4] Deficiency in K^{+} level leads to hypertension, stroke and seizures.[5] Deficiency of Cr^{3+} causes diabetes and cardiovascular diseases whereas an excess intake of Cr^{3+} leads to genotoxicity.[6] Anions also play a significant role in several biological processes. I^{-} anion actively participates in many neurological activities and thyroid functions. Its deficiency causes physiological disorders, which leads to growth and maturation of the organ system.[7] Cl^{-} anions regulate many cellular processes like membrane potential, neurotransmission, cell volume etc.[8-10] F^{-} gets accumulated in the body and effects bones, hair as it is toxic. Therefore, the recognition and sensing of these analytes are essential.

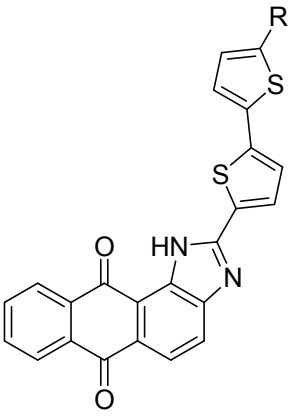
Several techniques are used for detecting anions and cations like High-performance liquid chromatography (HPLC), inductively coupled plasma atomic emission spectrometry, Mass spectrometry (MS) and Atomic absorption spectroscopy (AAS). However, these techniques are time consuming and pretreatment of the samples and sophisticated machinery is required. Hence best technique is Fluorogenic chemo sensors as they offer a low detection limit, high selectivity and sensitivity, small response time and on-site detection.[11,12] There are various fluorescent sensing mechanisms internal charge transfer (ICT), photoinduced electron transfer[13] (PET), Metal-Ligand charge transfer[14] (MLCT), Excited state intramolecular proton transfer (ESIPT). The most common is ESIPT as it has vast applications as laser dyes, UV-photostabilizer and Led.[15] ESIPT is a photochemical process that produces a tautomer with a different electronic structure from the original excited form.[16] It is a four-level photo-cycle (E - E* - K* - K) {(E) - enol to (K) – keto}. The remarkable properties of the ESIPT fluorophores are a large Stokes shift, dual emission, sensitivity to the surrounding medium. For ESIPT to occur the presence of an intramolecular hydrogen bonding between the proton donor (–OH and $–NH_2$) and the proton acceptor (=N– and -C=O) [17-19] groups in close proximity to each other. This covalently attached proton in the electronically excited state migrates to a neighboring hydrogen bonded atom.

LITERATURE REVIEW

Raposo *et al.*[20] synthesized **1** and **2** and characterized a colorimetric and fluorimetric chemosensors for F , CN and OH which contained anthraquinone and imidazole as signalling or binding sites. When F , CN and OH to acetonitrile solutions of compounds **1** and **2**, a marked colour change from yellow to pink was observed and the fluorescence emission of **1** was switched "on", whereas for **2** there was a fluorescence quenching. Considering recognition in organic aqueous mixture, it was found that selectivity for CN was achieved for both receptors, with an easily detectable colour change from yellow to orange. Moreover, sensors **1** and **2** showed good sensitivity with μM-level detection limit for CN in acetonitrile as well as in acetonitrile/water (9:1). Through spectrophotometric and spectrofluorimetric titrations of both anthraquinones with various anions, higher sensitivity for F , CN was observed, but with different fluorogenic behaviour: imidazo-anthraquinone **1** displayed a CHEF effect whereas anthraquinone **2** responded with a CHEQ effect. As for the colorimetric behaviour, straightforward naked-eye detection from yellow to pink was possible after addition of these anions.



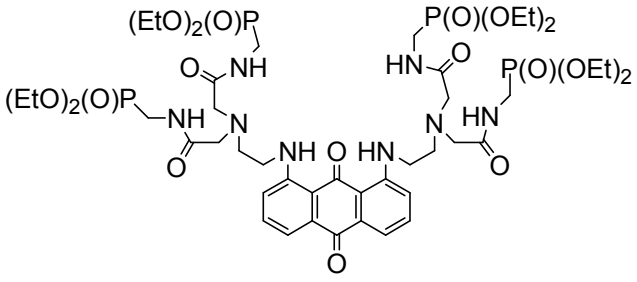
1 R=OMe
2 R=NMe_2



3a R = H
b R = OEt
c R = CN

Batista *et al.*[21] synthesized colorimetric or fluorimetric sensor **3a, 3b, 3c** for fluoride ion sensing containing anthraquinone as a chromogenic signaling unit and imidazo-2,2 c-bithiophene as binding sites. They reported new imidazoanthraquinone sensors which contained bithiophene moieties. The electronic nature of its substituents was used to tune the selectivity and the photophysical properties of the chemosensors. The systems after deprotonation of the imidazole NH upon addition of the fluoride ion proved to be efficient sensors for metal ions such as Zn^{2+}, Hg^{2+} and Cu^{2+} suggesting the formation of 1:2 complexes, except for the complex formed by **3a** with Zn^{2+} with a 1:1 molar ratio. Compound **3a** displayed a marked change from pink to yellow-gold colors upon complexation in Acetonitrile solution.

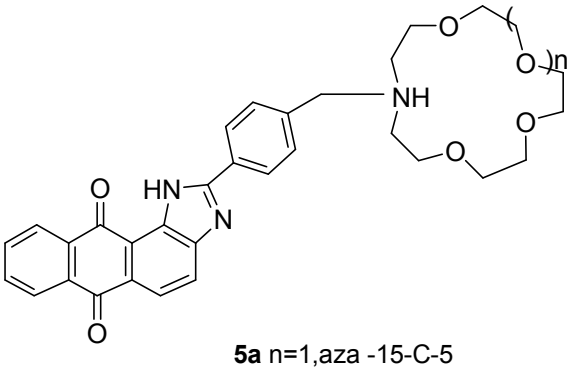
Ermakova *et al.*[22] synthesied a molecular sensor **4**, for detection of Hg^{2+} in water. This molecule combines a 1,8-diaminoanthraquinone signaling subunit and phosphonic acid esters that confer the water solubility to the dye quantitative recognition of Hg^{2+} at the ppm level was achieved with a polymer film doped with chemosensor **4** placed in the loculi of a Teflon plate. Visible colour change was observed at different detection level 2-1000 ppm of Hg^{2+} in aqueous solutions using small disks of an agarose polymer film doped with chemosensor **4**



4

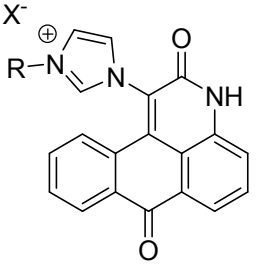
Yoshida *et al.*[23] synthesied fluorescent chemosensor in which an aza-crown is linked to a 2-phenylimidazoanthraquinone fluorophore (PIAQ–AC) **5a** and **5b** by a methylene spacer. They studied the effect on alkali and alkaline-earth metal ions. They figured out that enhancement was greatly dependent on both the charge and the size of metal ions with respect to the cavity size **of** the crown ring. They studied that Alkaline-earth metal cations induce much larger enhancement of fluorescence intensity than alkali metal cations. The cation whose size is closer to that of the cavity of the aza-crown seems to exhibit a larger fluorescence enhancement among cations of the same charge. These results confirmed that their sensors could recognise the size and the charge

of the metal ions and can transform their information precisely into the magnitude of the enhanced fluorescence.

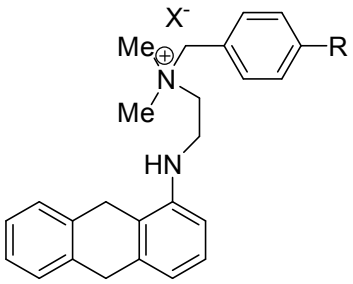


5a n=1,aza -15-C-5
5b n=2, aza-18-C-6

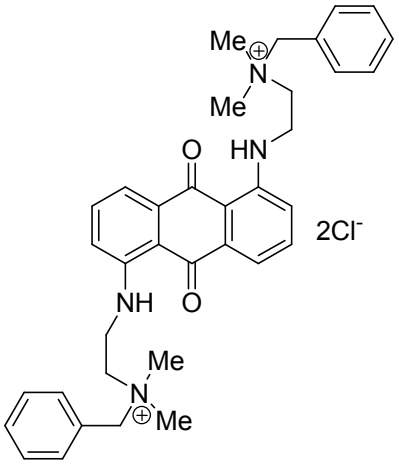
Kumar *et al.*[24] have developed *N*-aryl imidazolium based probes **6** and **7**, which enable naked eye and dual channel (absorption and fluorescence) detection of F^- and AcO^- ions respectively. When tetrabutylammonium fluoride (TBAF) was added to a solution of **6**, the color of the solution changed from light yellow to orange in CH_3CN-DMSO (20:1). The addition of acetate and dihydrogen phosphate to the chemosensor **6** showed significantly smaller changes than that observed for 2 equiv. of F^- anion. The fluorescence intensity for **6** at 480 nm (green emission) was turned off and simultaneously a new red-shifted fluorescence emission band at 580 nm appeared. For **7** in $CHCl_3$-MeOH, AcO^- ions showed a new absorption band at 475 nm was absorbed and similar fluorescence properties as F^- ions.



6 R= Benzyl,X= Cl^-
7 R=dodecyl,X=Br^-



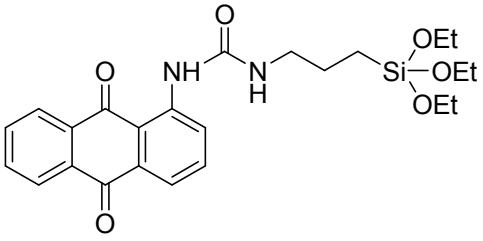
8 R = H,X=Cl
9 R = NO_2,X=Br



10

Luxami *et al.*[25] synthesized quaternary ammonium cation (for electrostatic interactions) and N–H (for H-bonding) based chromogenic and fluorescent chemosensors **8–10** which were selective for fluoride ion. The effect of a proximal positive charge on the spectroscopic behaviour of **8–10** was studied using absorption and steady-state fluorescence spectroscopy. Chemosensors **8–10** was the first examples, where intramolecular interactions of an anthracene-9, 10-dione derivative with a quaternary ammonium cation switch 'ON' the emission. The chemosensor **8** in CH_3CN–DMSO (9:1) exhibits two absorption bands at λ_{max} 310 nm and 480 nm. When tetrabutylammonium fluoride (TBAF) was added to a solution of **8** it resulted in a change in colour of the solution from yellow to pink. Chemosensors **8** and **9** on excitation at 450 nm exhibited emission band at λ_{max} 580 nm. But when fluoride was added the fluorescence intensity at 580 nm was completely turned off and a new blue-shifted fluorescence spectrum with two new emission bands at 505 and 540 nm was formed. In **10** with same solvent system on excitation at λ_{max} 450 nm exhibited emission at λ_{max} 585 nm but when fluoride was added quenching occurred.

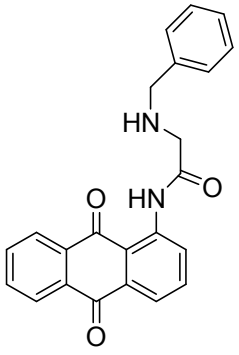
Anthraquinone-based fluorescent receptor **11** which was immobilized on mesoporous silica (AFMS). The sensing abilities of AFMS and AFSP were studied by addition of the anions F^-, Cl^-, Br^-, I^- and HSO_4^- to water suspensions of the assayed solid. The addition of fluoride ions only to a suspension of AFMS resulted in a large decrease in the fluorescence intensity of the anthraquinone of AFMS.



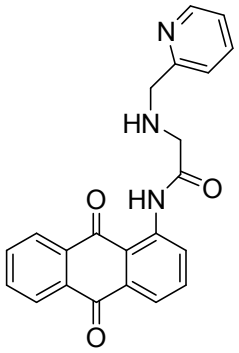
11

Wu *et al.*[26] synthesized 9,10-anthraquinone-based chemosensor **12** which indicates the presence of Cu(II) ions among other transition metal ions with high selectivity by a color change from yellow to dark red. Chemosensor **13** shows binding toward Cu^{2+}, Ni^{2+} and Co^{2+} with color changes from yellow to dark red, red and pale green respectively. Especially, Co(II) binding with

chemosensor **13** causes significantly green fluorescence. The only difference is the ring in the metal-chelating ligand: chemosensor **12** contains a benzene ring and chemosensor **13** contains a pyridine ring. This difference results in chemosensors **12** and **13** exhibiting different metal ion selectivity. Cu^{2+} was the only metal ion which resulted insignificant fluorescence quenching while other metal ions only caused minor changes in fluorescence intensity. Chemosensor **12** detected Cu^{2+} through a fluorescence quenching process. Co^{2+} binding with chemosensor **13** only resulted in a significant increase in fluorescence intensity.

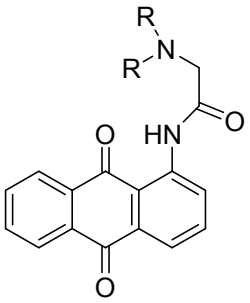


12

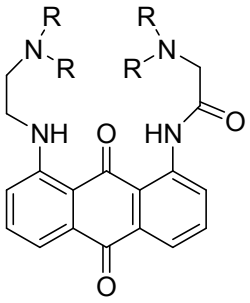


13

Kaur *et al.*[27] synthesized 1,4-Bis[2-aminoethylamino]anthracene-9,10-diones derivatives which selectively bind with Cu^{2+} to form complexes with unusual selectivity under basic conditions. The deprotonation of the aryl amine NH in the case of these chemosensors causes a bathochromic shift in the absorption band from 585 nm and 635 nm to 725 nm. They reported that aminoethyl functional groups bearing anthracene-9,10-diones **14** and **15** effectively which binds with Cu^{2+} amongst various alkali, alkaline earth and transition metal ions. The variation in substituent nature and their position on the anthracene-9,10-dione platform effectively controls the stability of their Cu^{2+} complexes across various pH ranges Thus, 1,4-bis[2-aminoethylamino]anthracene-9,10-diones in CH_3OH/H_2O (1:1) solution at pH > 7 selectively binds with Cu^{2+} even in the presence of alkali, alkaline earth and other heavy metal ions.

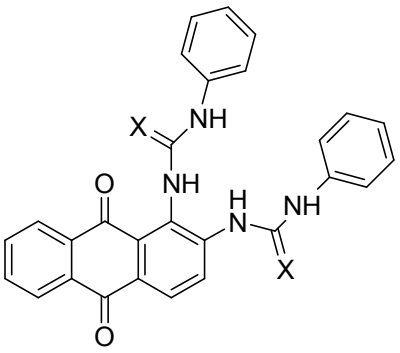


14a. R=H
14b. R=CH_3
14c. R=CH_2CONHC_8H17

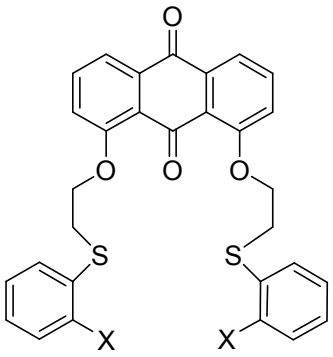


15a. R=H
15b. R=CH_3

Ganguly *et al.*[28] reported colorimetric receptors for selective fluoride ion sensing containing anthraquinone as chromogenic signaling subunit and urea, thiourea binding sites. These receptors have shown no affinity for other halide ions (Cl^-, Br^-, and I^- ions). Well-defined color change in the visible region of the spectrum was observed upon addition of fluoride ion in DMSO/CH_3CN solution of the receptors **16** and **17**. A bathochromic shift was observed on complexation with F^- for receptors **16** and **17**



16 X=O
17 X=S



18
X =NH_2

Pramila *et al.*[29] reported chemosensor **18** which estimated Cu(II) ions spectrophotometrically even in the presence of 10 mM Ni(II), Cd(II), Zn(II), Ag(I) and Pb(II) and 1 mM Hg(II) ions. The absorption spectrum of **18** does not show absorption after 520nm. On addition of Cu(II) nitrate a remarkable increase in absorption in the region 520–800 nm is observed . Visibly, a significant change in colour from light yellow (receptor **18**) to brown is also observed. The addition of Ni(II), Cd(II), Zn(II), Ag(I), Pb(II) and Hg(II) cations does not affect the absorption spectrum of **18**. Therefore, **18** shows an increase in absorption only on addition of Cu(II) and remains unaffected with other metal ions such as Ni(II), Cd(II),Zn(II), Ag(I), Pb(II) and Hg(II).

Gaps in Study

Based on detailed literature survey it has been found that there are only few reports where anthraquinone moiety id directly attached/fused to the aromatic ring. The fused aromatic system can increase the conjugation with the anthraquinone moiety and thus can show strong visible color changes in the presence of metal ions or anions. So, in the present research project we have synthesized the new chromogenic probes where anthraquinone moiety is fused with aromatic/heteroaromatic systems.

OBJECTIVES

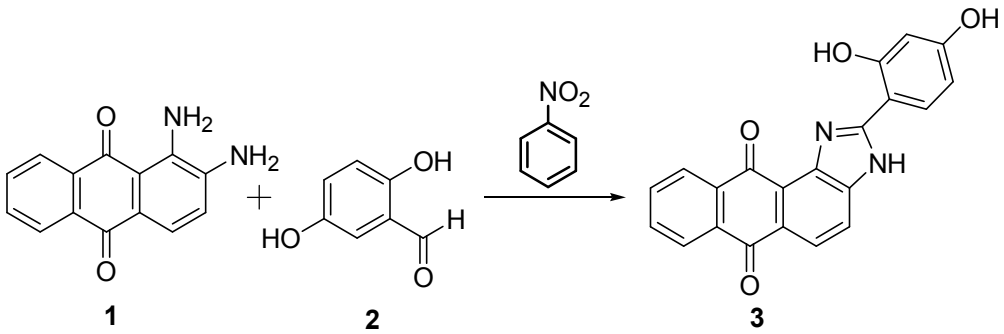
1) To synthesize imidazole fused anthraquinone derivatives.

2) To explore sensing ability of these molecules to sense various cations and anions.

RESULTS AND DISCUSSION

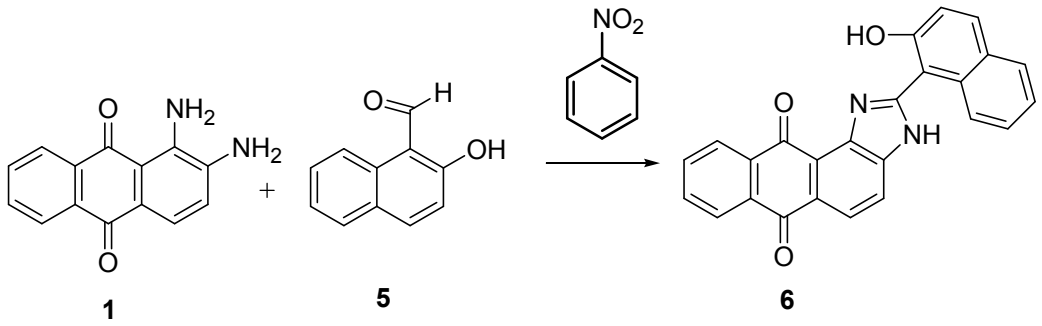
Pyrrole, thiourea, benzoimidazole, anthraquinone derivatives are hydrogen bonded molecules which have anion binding sites. Anthraquinone derivatives can be used for several optical applications in materials and medicinal chemistry. Although several imidazo-anthraquinone have been reported, up till now naphthyl-imidazo derivatives are still unknown. Due to its structure, it can impart interesting photophysical properties. Anthraquinone derivatives are electron acceptor groups that electronically connected with recognition sites and proved suitable receptors for the colorimetric sensing of certain anions. Thus we have designed anthraquinone based molecules (probe **3** and **6**).

Compound **3** was synthesized by the heating of a solution of 1,2-diaminoanthraquinone **1** (0.1 g, 0.42 mmol) and 2,4-dihydroxy benzaldehyde **2** (0.06 g, 0.49 mmol) in 10 mL nitrobenzene overnight. The reaction was monitored by thin layer chromatography (TLC). After completion of reaction, the reaction mixture was allowed to cool at room temperature. The cold diethyl ether was added to the reaction mixture. The precipitate formed in the solution was filtered and washed with diethyl ether to give brown pure solid of **3** with 50% yield (74.5 mg) (**Scheme 1**). ^{1}H NMR spectrum of compound **3** showed two 1H broad singlets at δ 12.54 and δ 11.99 for OH, 1H singlet at δ 10.21 for NH, 3H multiplet at δ 8.26-8.18, 1H singlet at δ 8.02, 3H multiplet at δ 7.90-7.89 and 2H multiplet at δ 6.48-6.45 for aromatic-H. ^{13}C NMR spectrum showed two C=O peaks at δ 183.8 and 182.1 and rest of the peaks were for aromatic-C. The NMR data confirming the structure for probe **3.**



Scheme 1: Synthesis of probe **3**

Compound **6** was synthesized by the heating of the solution of 1,2-diaminoanthraquinone **1** (0.1 g, 0.42 mmol) and 2-hydroxyl-1-napthaldehyde **5** (0.08 g, 0.49 mmol) in 10 mL nitrobenzene overnight at 110 °C. The completion of reaction was monitored by thin layer chromatography (TLC). After completion of reaction, reaction mixture was allowed to cool at room temperature. The cold diethyl ether was added to the reaction mixture. The precipitates formed in the solution were filtered and washed with diethyl ether to give black solid **6** with 60% yield (97.8 mg) (**Scheme 2**).



Scheme 2: Synthesis of probe **6**

^{1}H NMR spectrum of compound **6** showed 1H broad singlet at δ 13.16 for OH, 1H broad singlet at δ 11.29 for NH, 1H doublet at δ 8.84, 3H multiplet at δ 8.23-8.20, 1H doublet at δ 8.10, 1H doublet at δ 8.00, 3H multiplet at δ 7.93-7.89, 1H triplet at δ 7.53, 2H multiplet at δ 7.41-7.36 for aromatic-H; ^{13}C NMR spectrum showed two C=O peaks at δ 183.8 and 183.7 and rest of the peaks were for aromatic-C, confirming the structure for probe **6.**

Photophysical behavior of probe 3

Probe **3** showed an absorption band at 450 nm and very weak emission band was observed at 500 nm. Its sensing ability was studied in various solvent system like CH_3CN, CH_3OH and their different ration in H_2O. The sensing ability was specific in CH_3OH and H_2O (4:1). It showed distinct visible color changes in the presence of transition metal ions.

Effect of various metal ions on absorption spectrum of Probe 3

The chromogenic sensing ability of probe **3** was studied in CH_3OH : H_2O::4:1 in the presence of 1000 μM of different metal ions like Ag^{+}, Ba^{2+}, Al^{3+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+} etc. Out of these tested metal ions, only the presence of Cu^{2+}, Ni^{2+} and Zn^{2+} (Figure 1 and 2) showed significant visible color changes as well as spectral changes.

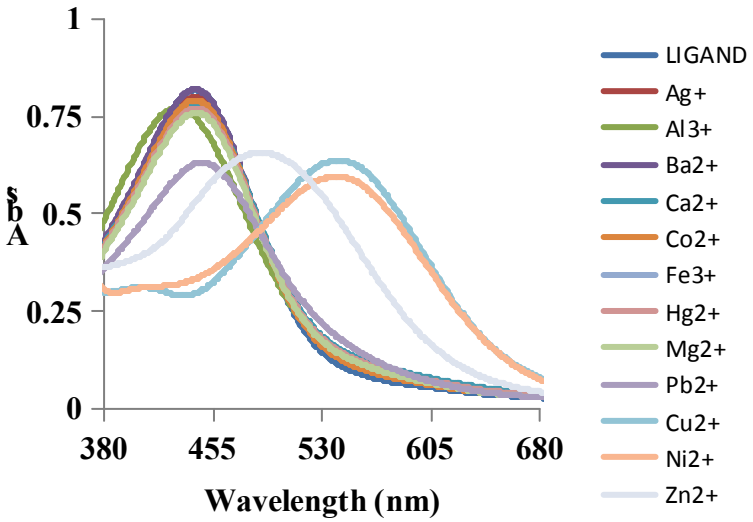


Figure 1: Effect of various cations on absorption spectra of probe **3** in CH_3OH: H_2O::4:1.

Figure 2: Photographs of probe **3** in the presence of different cation at 20 μM in CH_3OH / H_2O(4:1).

The probe **3** showed the absorption band at 450 nm and upon addition of Cu^{2+}, Ni^{2+} and Zn^{2+} ions a red shift was observed in the absorption spectra. Upon gradual addition of Cu^{2+} to the solution of Probe **3** (20 µM in CH_3OH/ H_2O(4:1), pH = 6.8), color changes from yellow to purple, absorption bands at 450 nm diminished steadily with simultaneous formation of new band at 550 nm till additions upto 8 µM of Cu^{2+} ions, with lowest detection limit of 0.25 µM (Figure 3a). Similarly upon gradual addition of Ni^{2+} to the solution of Probe **3** (20 µM in CH_3OH / H_2O(4:1) color changes from yellow to brown, absorption bands at 450 nm diminished steadily with simultaneous formation of new band at 560 nm till additions upto 66 µM of Ni^{2+} ions with detection limit of 1 µM (Figure 4a).

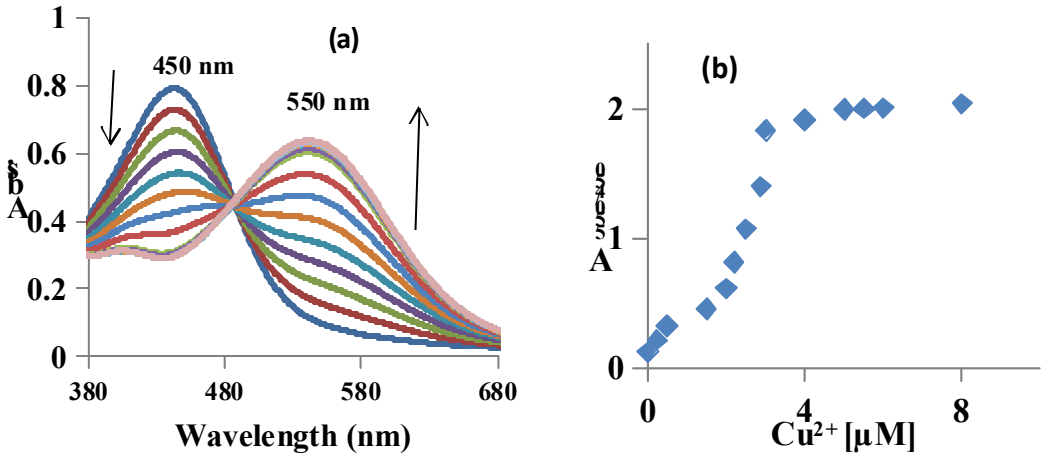


Figure 3: (a) Effect of incremental addition of Cu^{2+} on absorption spectrum of probe **3**(20 µM in CH_3OH / H_2O(4:1); (b) Ratiometric response between 450 and 550nm (A_{550} / A_{450}) vs Cu^{2+} [µM] ions on incremental addition of Cu^{2+} ions to probe **3** [CH_3OH / H_2O(4:1)].

Upon addition of Zn^{2+} ions to the solution of Probe **3** (20 µM in CH_3OH / H_2O(4:1), colour changes from yellow to pink, absorption bands at 450 nm diminished steadily with simultaneous formation of new band at 500 nm till additions upto 32 µM of Zn^{2+} ions, with detection limit of 0.5 µM (Figure 5a).

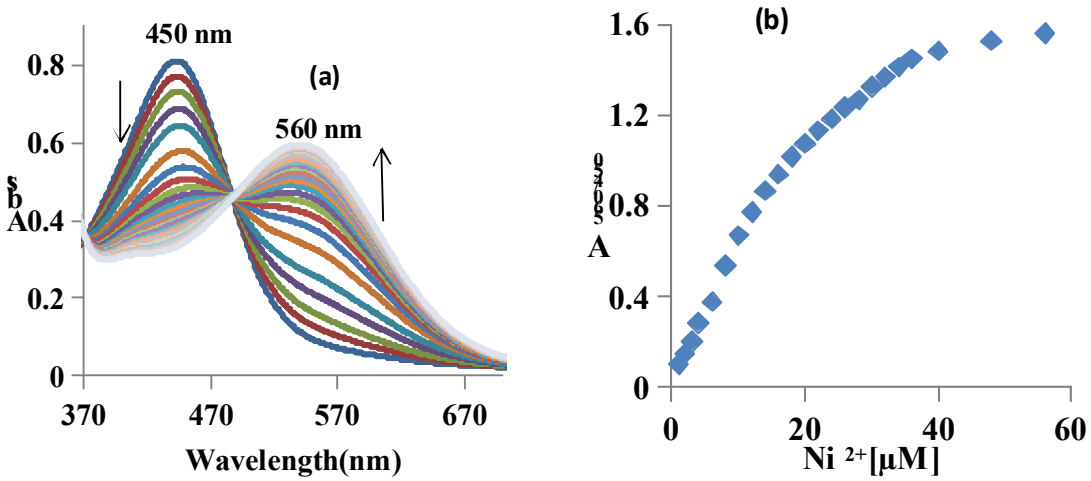


Figure 4: (a) Effect of incremental addition of Ni^{2+} on absorption spectrum of probe **3** in CH_3OH / H_2O(4:1); (b) Ratiometric response between 450 and 560 (A_{560} / A_{450}) vs Ni^{2+} [µM] ions on incremental addition of Ni^{2+} ions to probe **3** [CH_3OH /H_2O(4:1)].

The Cu^{2+}, Ni^{2+} and Zn^{2+} addition resulted in decrease in absorption intensity at 450 nm and increase at 550nm, 560nm, 500nm simultaneously which provided an opportunity to determine all these anions ratiometrically (Figure 3b, 4b and 5b). The absorption ratio varied for Cu^{+2} from 0.218 to 2.038 at 450 nm and 550 nm indicating 9.6 fold absorption ratio changes. Thus, probe **3** can be used to estimate an extensive range of Cu^{2+} ions between 0.25-8 µM through ratiometric approach. The absorption ratio varied for Ni^{2+} from 0.151 to 1.562 at 450 nm and 560 nm indicating 10.4 fold absorption ratio changes. Thus, probe **3** can be used to estimate an extensive range of Ni^{2+} ions between 1-66 µM through ratiometric approach. The absorption ratio varied for Zn^{2+} from 0.418 to 1.122 at 450 nm and 500 nm indicating 0.89 fold absorption ratio changes. Thus, probe **3** can be used to estimate an extensive range of Zn^{2+} ions between 0.5-32 µM through ratiometric approach.

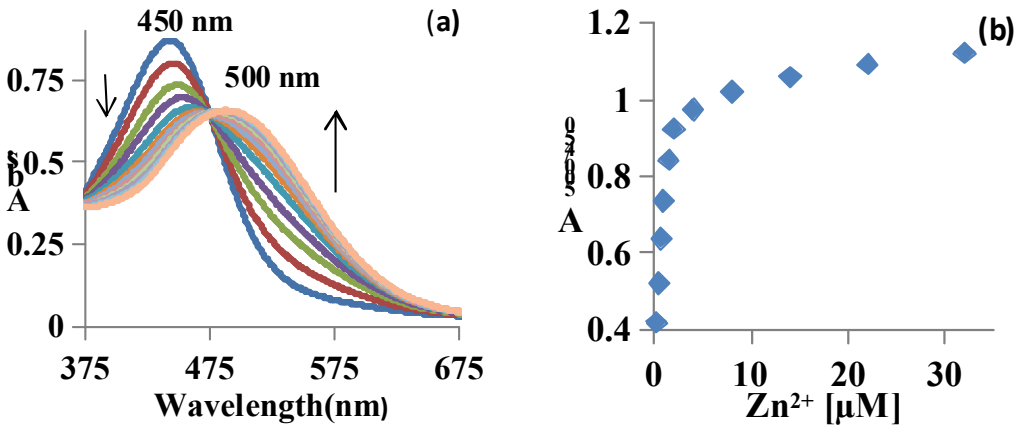


Figure 5: (a) Effect of incremental addition of Zn^{2+} on absorption spectrum of probe **3** in CH_3OH / H_2O(4:1); (b) Ratiometric response between 450 and 500 (A_{500} / A_{450}) vs Zn^{2+} [µM] ions on incremental addition of Zn^{2+} ions to probe **3** [CH_3OH / H_2O(4:1)].

Stability constants were calculated by using Benesi-Hilderband equation summarized in table 1.

Table 1. Stability constants of various cations through UV-Vis titration method

S. No.	Cation	Stability Constant (M^{-1})	Lowest detection limit (µM)	$\Delta\lambda_{max(nm)}$
1	Cu^{2+}	7.3×10^6	0.25	100
2	Ni^{2+}	0.24×10^6	1	110
3	Zn^{2+}	2.29×10^6	0.5	50

Photophysical properties of Probe 6

Probe **6** showed an absorption band at 430 nm and no emission was observed. Its sensing ability was studied in various solvent system like CH_3CN, CH_3OH, H_2O but sensing ability was specific in CH_3CN and H_2O(4:1) for cations and anions simultaneously. It showed visible color change with Ni^{2+} and Zn^{2+} cations and CN^- anion among the various studied cations and anions.

Effect of various anions on absorption spectrum of Probe 6

The chromogenic sensing ability of probe **6** was studied in CH_3CN / H_2O (4:1) in the presence of 1000 μM of different anions like CN^-, F^-, Cl^-, Br^-, AcO^-, I^-, HSO_4^-, OH^-, NO_3^-, SCN^-, $H_2PO_4^-$ etc. Out of these studied anions CN^- ion only showed significant visible color changes as well as spectral changes (Figure 6 and 7).

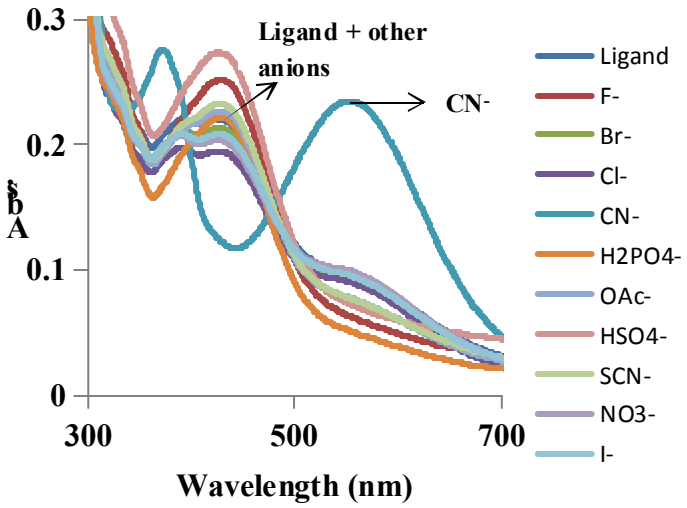


Figure 6: Effect of various anions on absorption spectra of probe **6** [CH_3CN / H_2O (4:1)]

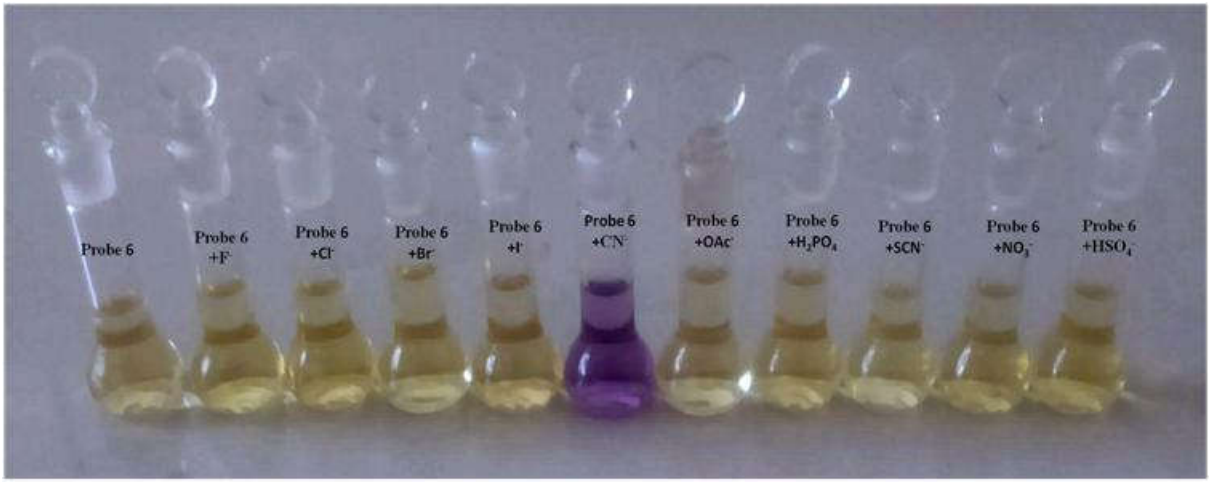


Figure 7: Photographs of probe **6** in the presence of different cation at 20 μM in [CH_3CN / H_2O(4:1)]

The probe **6** showed the absorption band at 430 nm and upon addition of CN^- to the solution of Probe **6** (20 μM, CH_3CN / H_2O(4:1)), color changes from yellow to purple, absorption bands at 430 nm diminished steadily with simultaneous formation of new bands at 375 nm and 560 nm with a clear isobestic point at 480 nm and 400 nm till additions upto 40 μM of CN^- ions, with detection limit of 1 μM (Figure 8a and 8b).

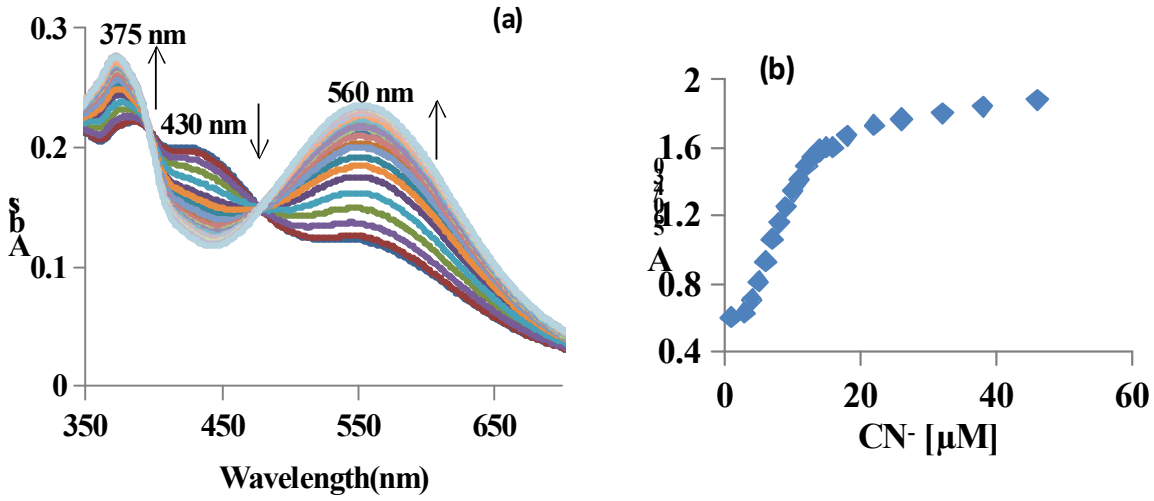


Figure 8: (a) Effect of incremental addition of CN^- on absorption spectrum of probe **6** in CH_3CN / H_2O(4:1); (b) Ratiometric response between 430 and 560 (A_{560} / A_{430}) vs CN^-[μM] ions on incremental addition of CN^- ions to probe **6** (CH_3CN / H_2O(4:1).

The cyanide addition resulted in decrease in absorption intensity at 430 nm and increase at 560 nm provided an opportunity to determine cyanide ions ratiometrically (Figure 8b). The absorption ratio varied from 0.027 to 1.87. at 430 and 560 nm indicating 0.33 fold absorption ratio changes. Thus, probe **6** can be used to estimate an extensive range of cyanide ions between 1-40 μM through ratiometric approach. Stability constant of CN^- ion was calculated by using Benesi-Hilderband equation summarized in table 2.

Effect of various cations on absorption spectrum of Probe 6

The chromogenic sensing ability of **6** studied in CH_3CN / H_2O(4:1) in the presence of 1000 μM of different metal ions like Ag^+, Ba^{2+}, Al^{3+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} ions as their perchlorate salts. Out of the tested metal ions, the presence of Ni^{2+} and Zn^{2+} ions only showed significant color changes as well as spectral changes (Figure 9 and 10).

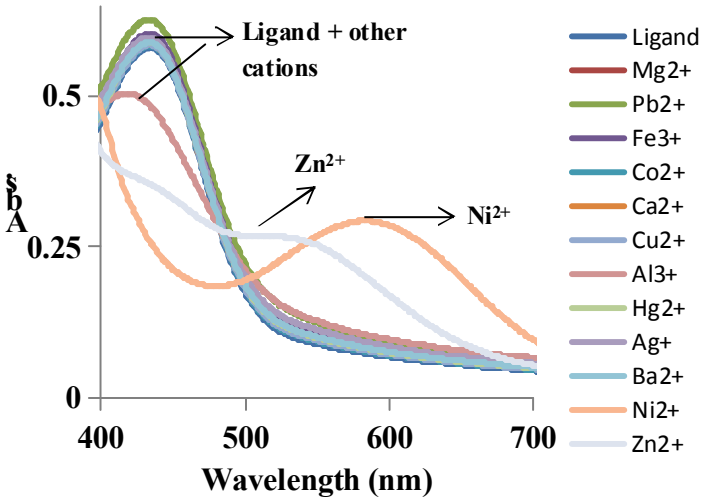


Figure 9: Effect of various anions on absorption spectra of probe **6** [CH_3CN / H_2O(4:1)]

Figure 10: Photographs of probe **6** in the presence of different cation at 20 μM in CH_3CN / H_2O(4:1).

The probe **6** showed the absorption band at 430 nm and upon addition of Ni^{2+} and Zn^{2+} ions,a red shift was observed in the absorption spectra .Upon gradual addition of Ni^{2+} to the solution of Probe **6** (20 μM in CH_3CN / H_2O(4:1),colour changes from yellow to blue absorption bands at 430 nm diminished steadily with simultaneous formation of new band at 590 nm till additions upto 168 μM of Ni^{2+} ions with detection limit of 0.5 μM (Figure 11a).

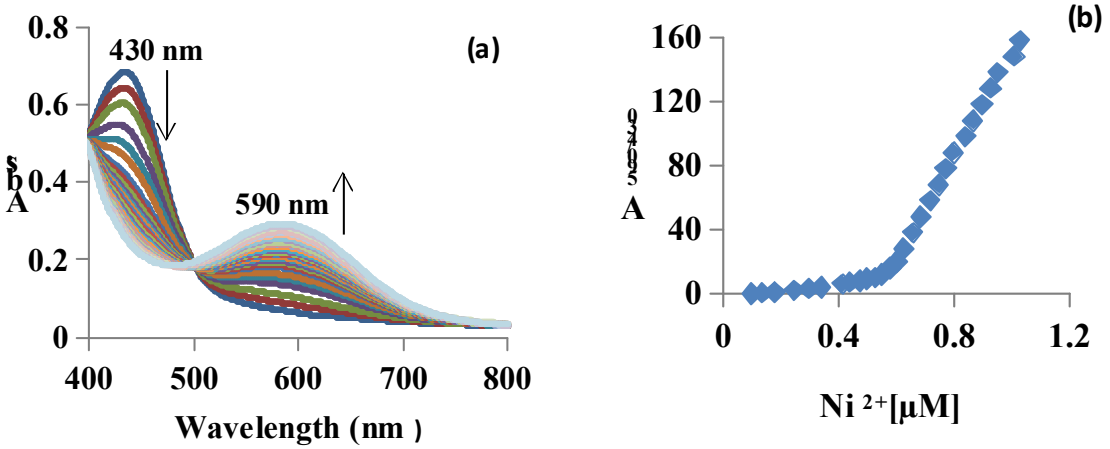


Figure 11: (a) Effect of incremental addition of Ni^{2+} on absorption spectrum of probe **6** in (CH_3CN / H_2O(4:1)) (b) Ratiometric response between 430 and 590 (A_{590} / A_{430}) vs Ni^{2+} [μM] ions on incremental addition of Ni^{2+} ions to probe **6** (CH_3CN / H_2O(4:1)).

Similarly, upon gradual addition of Zn^{2+} to the solution of Probe **6** (20 µM in CH_3CN / H_2O(4:1)), color changes from yellow to pink, absorption bands at 450 nm diminished steadily with simultaneous formation of new band at 550 nm till additions upto 47 µM of Zn^{2+} ions with detection limit of 0.5 µM (Figure 12a). The Ni^{2+} and Zn^{2+} addition resulted in decrease in absorption intensity at 430 nm and increase at 590 nm and 550 nm simultaneously provided an opportunity to determine both cations ratiometrically (Figure 11b and 12b).

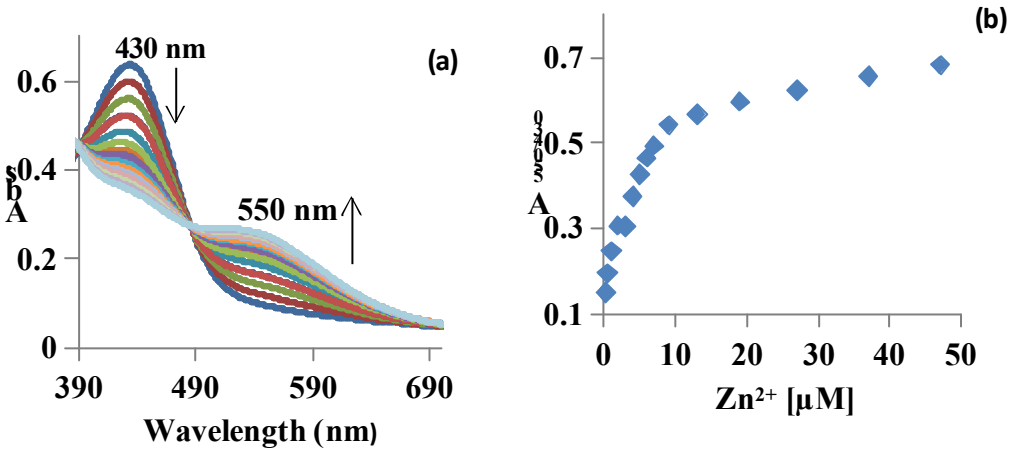


Figure 12: (a) Effect of incremental addition of Zn^{2+}on absorption spectrum of probe **6** in CH_3CN / H_2O(4:1) (b) Ratiometric response between 430 and 550 (A_{550} / A_{430}) vs Zn^{2+} [µM] ions on incremental addition of Zn^{2+} ions to probe **6** (CH_3CN / H_2O(4:1)) .

The absorption ratio varied for Ni^{2+} from 0.195 to 0.683 at 430 and 590 nm indicating 3.57 fold absorption ratio changes. Thus, probe **2** can be used to estimate an extensive range of Ni^{2+}ions between 0.5-168 µM through ratiometric approach. The absorption ratio varied for Zn^{2+} from 0.098 to 0.195 at 430 and 550 nm indicating 0.08 fold absorption ratio changes. Thus, probe **2** can be used to estimate an extensive range of Ni^{2+} ions between 0.5-47µM through ratiometric approach. Stability constants of cations was calculated by using Benesi-Hilderband equation summarized in table 2 .

Table 2. Stability constants of Anion and Cations through UV-Vis titration method

S. No.	Anion and Cations	Lowest detection limit (μM)	Stability constant (M^{-1})	$\Delta\lambda_{max(nm)}$
1	CN^-	1	0.20×10^6	130
2	Ni^{2+}	0.5	0.36×10^6	160
3	Zn^{2+}	0.5	0.06×10^6	120

EXPERIMENTAL

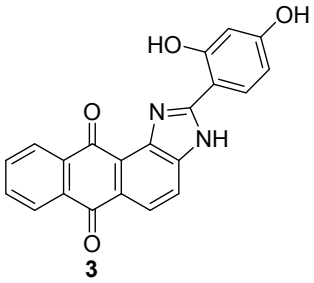
Materials and Equipments

Chemicals used were purchased from Sigma Aldrich and Loba chemie and used without further purification. All reactions were monitored by thin layer chromatography. Melting points were recorded by the open capillary tube method and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were carried out using JEOL ECS-400 MHz spectrometer in SAI Labs, Thapar University, Patiala. All chemical shifts are reported in ppm relative to the TMS as an internal reference. UV-Vis studies were carried out on Shimadzu UV-2600 machine using slit width of 1.0 nm and matched quartz cells. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrometer. Stock solutions of probes **3** and **6** were prepared at 10^{-3} M in distilled CH_3CN. All absorption and fluorescence scans were saved as ACS II files and further processed in Excel™ to produce all graphs shown. Solutions of **3** and **6** were typically 20 μM for UV-Vis studies. Tetrabutylammonium salt was used for anionic studies and perchlorate salts for cationic studies. Binding constants were calculated according to the Benesi-Hildebrand equation.(**1**) K_a was calculated following the equation stated below:

$$1/(A\text{-}A_o) = 1/\{K(A_{max}–A_o)\ [M_x+]n\} + 1/[A_{max}\text{-}A_o] \quad \text{----- 1}$$

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

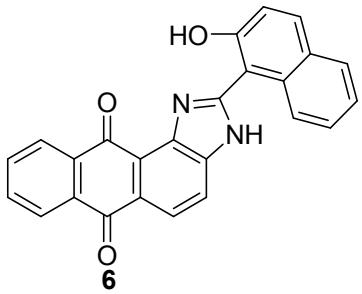
Synthesis of probe 3: To a solution of 1,2-diaminoanthraquinone **1** (0.1 g, 0.42 mmol) in 10 mL nitrobenzene, 2,4-dihydroxy benzaldehyde **2** (0.06 g, 0.49 mmol) was added and heated overnight at 110 °C. The completion of reaction was monitored through thin layer chromatography (TLC). After the completion of reaction; reaction mixture was allowed to cool to room temperature. Cold diethyl ether was added to the reaction mixture. The precipitates formed in the solution were filtered and washed with diethyl ether to get pure compound **3**.



3

Brown solid, yield: 50% (74.5 mg), m.pt.: 210 °C, ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 12.54 (bs, 1H, OH), 11.99 (bs, 1H, OH), 10.21 (s, 1H, NH), 8.26-8.18 (m, 3H, ArH), 8.02 (s, 1H, ArH), 7.90-7.89 (m, 3H, ArH), 6.48-6.45 (m, 2H, ArH); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ 183.8, 182.1 (C=O), 161.7, 158.9, 156.7, 147.9, 134.6, 134.3, 133.2, 132.7, 130.9, 126.9 126.2, 123.5, 120.9, 117.4, 108.5, 105.1, 102.8(ArC).

Synthesis of probe 6: The solution of 1,2-diaminoanthraquinone **1** (0.1 g, 0.42 mmol) and 2-hydroxyl-1-napthaldehyde **5** (0.08 g, 0.49 mmol) in 10 mL nitrobenzene was heated overnight at 110 °C. The completion of reaction was monitored through thin layer chromatography (TLC). After the completion of reaction, reaction mixture was allowed to cool to room temperature. Cold diethyl ether was added to the reaction mixture. The precipitates formed in the solution were filtered and washed with diethyl ether to yield pure compound **6**.



6

Black solid, yield: 60% (97.8 mg), m.pt.: 180°C, ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ 13.16 (bs, 1H, OH), 11.29 (bs, 1H, NH), 8.84 (d, 1H, *J* = 7.60 Hz, ArH), 8.23-8.20 (m, 3H, ArH), 8.10 (d, 1H, *J* = 8.40 Hz, ArH), 8.00 (d, 1H, *J* = 4.0 Hz, ArH), 7.93-7.89 (m, 3H, ArH), 7.53 (t, 1H, *J* = 7.64 Hz, ArH), 7.41-7.36 (m, 2H, ArH); ^{13}C NMR (DMSO-*d*$_6$,100 MHz):183.8, 183.7(C=O), 155.7, 155.6, 134.5, 134.2, 133.2, 133.1, 132.5, 132.3, 128.4, 128.0, 127.6, 127.1, 126.5, 125.2, 124.7, 123.4, 120.6, 118.2, 108 (ArC)

Conclusion

- ✓ New anthraquinone based chromogenic sensors have been synthesized for the detection of metal ions.
- ✓ Differential behavior of metal ions has been studied using different substituent.
- ✓ Easy and visible detection of different metal ions in the pool of metals.
- ✓ Detection of biologically important transition metal ions in μM/nM concentrations.

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