DESIGN AND SYNTHESIS OF DIPODAL RECEPTOR

A

Thesis Submitted

In Partial Fulfilment of Requirements

For The Degree of

Master of Science in Chemistry

Submitted By

Prabhjot Kaur Gill
(Regd No. 300802013)

Supervised By

Dr. Navneet Kaur (Assistant Professor)
&
Dr. Susheel Mittal (Professor & Head)

School Of Chemistry and Biochemistry
Thapar University
Patiala 147004

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Acknowledgments

There are many people who have helped me to make the past such a meaningful time. Most of all I like to express my gratitude to my research mentors Dr. Navneet Kaur and Prof. Susheel Mittal for giving me chance to work on such an interesting topic. I would like to dedicate my work to my mentors Dr. Navneet Kaur and Prof. Susheel Mittal. Thank you to my friend and my lab mate Jasneet for supporting and helping me. I would like to express my sincere gratitude to School of Chemistry and Biochemistry for every care. Finally and most importantly, I must express my deepest appreciation to my family for their encouragement through the entire process.

Patiala:

Date: 15-July-2010

Prabhjot Kaur Gill

(Regd No. 300802013)

Candidate's Declaration

I, hereby declare that the work being presented in the dissertation entitled "DESIGN AND SYNTHESIS OF DIPODAL RECEPTOR", in partial fulfillment of the requirements for the award of degree of Masters in Chemistry, School of Chemistry and Biochemistry (SCBC), Thapar University, Patiala, is my own work during the period of Jan 2010 to May 2010, under the supervision of Dr. Navneet Kaur and Dr. Susheel Mittal. I have not submitted the matter embodied in this dissertation for the award of any other degree.

Patiala:

Date: 15-July-2010

Prabhjot Kaur Gill

(Regd No. 300802013)

This is to certify that the above statement made by the candidate is correct and true to the best of our knowledge.

Dr. Navneet Kaur
Supervisor,
Assistant Professor
School of Chemistry & Biochemistry
(Presently at UIEAST
Nanoscinece and Nanotechnology
Panjab University, Chandigarh)

Dr. Susheel Mittal
Supervisor,
Professor & Head,
School of Chemistry & Biochemistry

Certificate

This is to certify that the dissertation entitled "DESIGN AND SYNTHESIS OF DIPODAL RECEPTOR", being submitted by Ms. Prabhjot Kaur Gill in partial fulfillment of the requirements for the award of degree of Masters in Chemistry in the School of Chemistry and Biochemistry, Thapar University, Patiala, is a bonafide work carried out under the supervision of Dr. Navneet Kaur and Dr. Susheel Mittal and that no part of this work has been submitted for the award of any other degree.

Dr. Navneet Kaur
Supervisor,
Assistant Professor
School of Chemistry & Biochemistry
(Presently at UIEAST
Nanoscinece and Nanotechnology
Panjab University, Chandigarh)

Dr. Susheel Mittal 15/7/x
Supervisor,
Professor & Head,
School of Chemistry & Biochemistry

Dr. Susheel Mittal 15/7/x
Professor & Head
School of Chemistry & Biochemistry

Dr. R. K. Sharma
Dean, Academic Affairs

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INTRODUCTION

The functional chemosensors for the identification and quantification of important physiological and environmental analytes is of considerable importance.[1] The fluorescent probes have found widespread use in numerous bio-sensing applications including recognition of cations/anions, nucleic acid detection, immunoassays, cellular labeling and resonance energy transfer studies.[2] For the effective operational usage, high sensitivity and selectivity of these chemosensors are pre-requisites.[3] The fluorescence chemosensors are gaining much attention not only because of the sensitivity of the method, but also due to its effective applications in cellular environment.[4] With the appropriate choice of fluorophore, the measurements cause little or no damage to the host system.[5] Moreover, the time-resolved fluorescence has the special advantages for *in vivo* sensing of being relatively independent of light scattering in the tissues and of fluorophore concentration, thus correcting for photobleaching or fluorophore loss through diffusion or degradation.[6] These fluorescence techniques can provide information about the structure and micro-environment of molecules. For example, the conformation has changed and exposes the dye to solvent in a polarity-sensitive fluorophores linked to proteins i.e. acrylodan which is covalently attached to bacterial glucose-binding protein undergo quenching in fluorescence on addition of glucose. Thus, signaling the glucose-induced conformational change in this protein.[7]

Many synthetic fluorescent macrocyclic hosts such as podands, crown ethers, cryptands, cyclophanes and calixarenes have received considerable attention for sensor development.[8] In these macrocyclic receptors, many structural features control the fluorescence efficiency including double-bond torsion, low energy $n\pi^*$ levels, “heavy” atoms, weak bonds etc.[9] The rationale behind the working of chemosensor is based upon the idea that the binding of analyte with the chemosensor leads to the change in photo-physical properties (i.e. fluorescence) of chemosensors, and from these changes the concentration of analyte is determined in a system under observation.[10]

Mechanism in Fluorescent Chemosensor: The changes in photophysical properties of various chemosensors are governed by various types of mechanism, i.e. photoinduced electron transfer (PET), internal charge transfer (ICT), excimer & exciplex formation ,

electronic energy transfer (EET) or Förster resonance energy transfer (FRET). Therefore, using synthetic skills a wide range of sensors can be developed by modulating these structural features via chemical or physical means at the molecular level.

For the sake of simplicity, many PET sensors are reported with the design principal of "fluorophore-spacer-receptor". However, various electron donors can be engaged to provided that basic conditions of PET phenomena;[11] but the simplest and most popular design consists of an aliphatic amine as an electron donor and as a receptor for the cation.

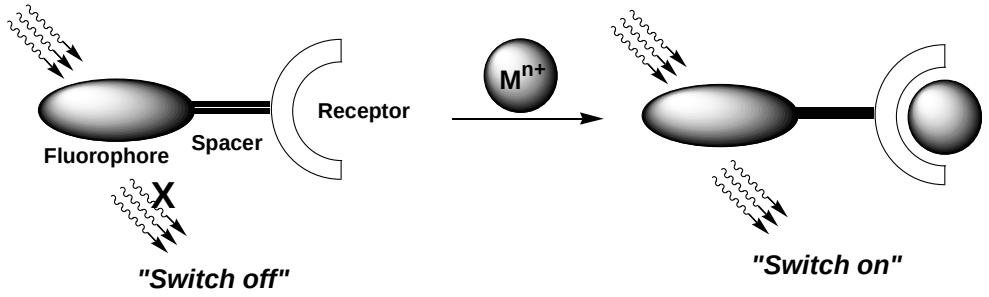


Fig 1: Working principle of chemosensor based upon photoinduced electron transfer (PET)

The PET path clearly originates at the nitrogen lone electron pair and terminates at the fluorophore.[12] Thus in the pure receptor; the fluorescence intensity is switched 'off' due to PET from the receptor. Upon binding of analyte, which is selected by the receptor causes the fluorescence intensity to be switched back 'on' again (Fig 1).[12] The 'off–on' nature of the analyte-induced switching and the modular nature of the system are used in the estimation of analyte.In contrast with PET systems with "fluorophore-spacer-receptor" model, there is another type of chemosensors, where the signaling sub-unit is directly integrated with a receptor. In this design, the orbitals overlap to an appreciable extend making the system with one terminal to be electron rich and the other electron poor, so that a substantial dipole is created.[13] Upon binding of an analyte (especially a charged one), into the receptor naturally causes an interaction with this excited state dipole (as shown in Fig 2). This interaction causes the shift in fluorescence spectrum and recently many researchers are interested in these types of receptors, which show in the shift in fluorescence spectrum upon cation binding than a simple 'off–on' nature of the chemosensor.[14]

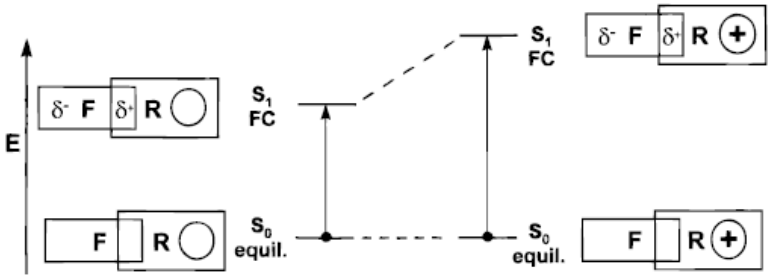


Fig 2. Cation effect on absorption or excitation spectral wavelength for a fluorophore with an ICT excited state

Like the receptors showing ICT mechanism, there are another set of chemosensors which upon cation binding can show the shift in fluorescence spectrum by the phenomenon of fluorescence (or Förster) resonance energy transfer (FRET).[15] In general, the term "Förster resonance energy transfer" is applied for phenomena where a donor chromophore, initially in its electronic excited state, may transfer energy to an acceptor chromophore through nonradiative dipole–dipole coupling.[16] When both chromophores are fluorescent, the term "fluorescence resonance energy transfer" is often used instead, although the energy is not actually transferred by fluorescence. For the design of chemosensor based upon FRET mechanism, the fluorescent emission peak of donor chromophore must overlaps the excitation peak of acceptor chromophore.[17]

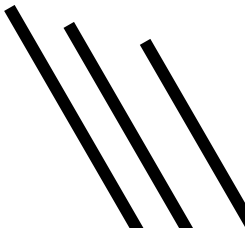
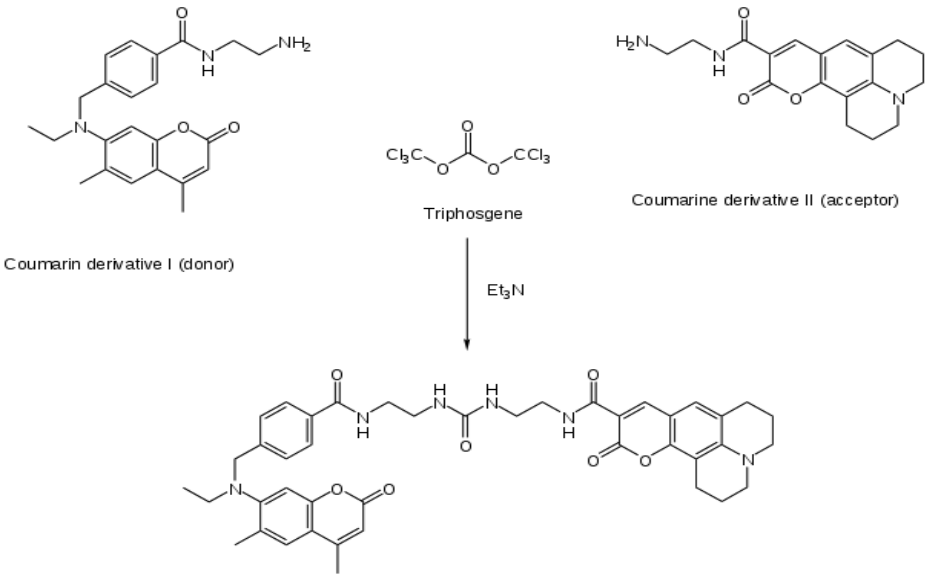


Fig 3: Working principle of chemosensor based upon photoinduced FRET



For example, a FRET pair has been applied in an experimental method for the detection of phosgene. In it, phosgene substitute serves as a linker between an acceptor and a donor coumarine (forming urea groups). The presence of phosgene was detected at $5x10^{-5}$ M with a typical FRET emission at 464 nm.[18]

In several cases, when a dimer is formed from the receptor (mono-unit) upon complexation with analyte, then a shift in emission is observed. The term excimer (excited state complex) is, strictly speaking, limited to cases in which a true dimer is formed; i.e. both components of the dimer are the same molecule.[19] The term exciplex refers to the heterodimeric case.[20]

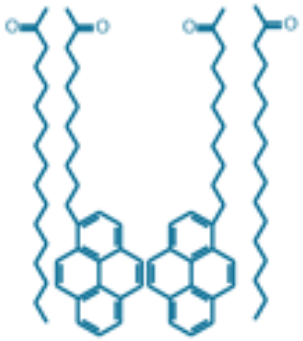


Fig 4. Pyrene eximer fluorescence ~470 nm

In continuation to the mechanism showing shifts in emission spectra, there is another type of mechanism where the receptor upon binding with metal ions show shift in emission spectra via involving keto-enol tautomerism.[21]

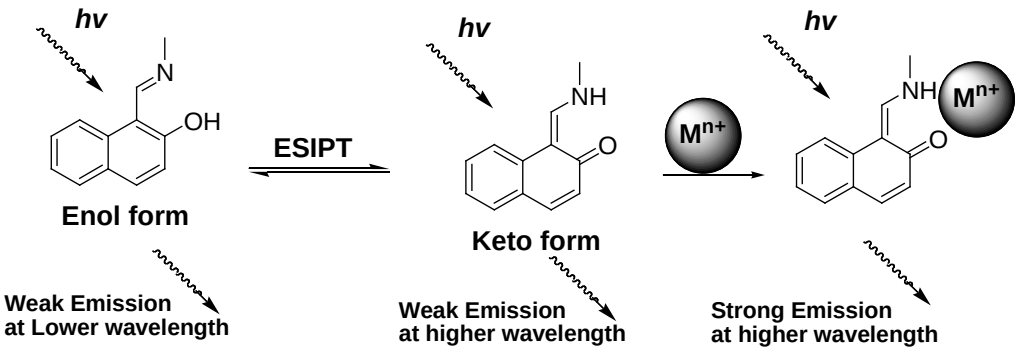


Fig 5: Mechanism showing the woking of chemosensor based on ESPT

This mechanism is operated in the way that in the absence of any metal ion the enol form is in equilibrium with its keto tautomer in the excited state. As metal ion binds to the keto form, then this form is removed from the equilibrium; consequently, the equilibrium has to adjust by producing more keto tautomer. Thus, the intensity due to the enol tautomer decreases while that from the metal-bound keto tautomer increases. Therefore, such receptor can be used for selective ratiometric estimation of metal ions and for the development of molecular logic gates.[21] Fluorescent based chemo sensor have received attention in recent years because fluorescence measurement can be obtain from chemo sensor in solution or at interference at low concentration of analyte. The fluorescent chemo sensor for alkali metal, alkaline earth metal and some heavy metal ion involve the covalently linked crown ether substituents and their derivatives. These chemosensors form complex with a particular cation and/or anion selectively and show change in absorption or/and fluorescence spectra. Recent advances in molecular recognition and supramolecular chemistry have made it possible to developed highly selective novel receptors for specific guest. Molecular recognition occurs through design and synthesis of completely artificial receptors which bind to small biological analytes such as cations, anions, metabolites, aminoacid and nucleotides. Most of the receptors mainly based upon the judicial approach of binding site and signaling subunit. The binding site

usually consists of various combinations of donor sites and the signaling subunit based upon chromogenic or fluorogenic moieties. By inserting the binding moieties of the ligand the number of donor atom are very important, which tends to fulfill the coordination environent of the metal ion.

Aims and Objectives

1,8-Naphthalimide derivatives are highly fluorescent and photostable, and are used as fluorescent dyes for synthetic polymers, as liquid-crystal additives, as electro-optically sensitive materials, in laser technology and as fluorescent markers in medicine and biology [22] Their high fluorescence quantum yield and photostability makes them excellent candidates for dye lasers, where a high quantum yield and low level of re-absorption are extremely important. 1,8-naphthalimide derivatives have been studied as DNA intercalators.[23] and there are very few reports proposing the quantification of metal cations.[24] and anions[25] This project aims at designing and synthesis of 1,8- naphthalimide based dipodal receptor to examine its binding behavior towards selected cations. The receptor planned for these studies is shown in figure 5.

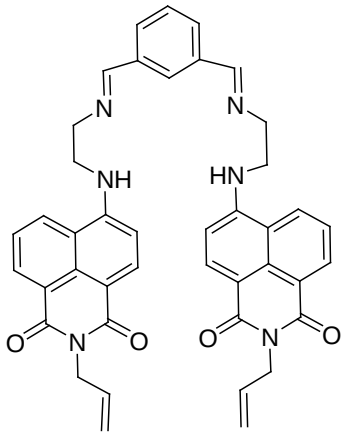


Fig 5: Design of receptor as fluorescent probe

The receptor is expected as a good probe for the fluorescent recognition of metal ions, the receptor is expected to adopt one or more of the following mechanism(s) for acting as fluorescent probe:

1. For the sake of simplicity, many PET sensors are reported with the design principal of “fluorophore-spacer-receptor”. However, various electron donors can be engaged to provided that basic conditions of PET phenomena; but the simplest and most popular design consists of an aliphatic amine as an electron donor and as a receptor for the cation. Thus in present design PET path clearly originates at the nitrogen lone electron pair and terminates at the fluorophore. Hence in the pure receptor, the fluorescence

intensity is switched ‘off’ due to PET from the receptor. Upon binding of metal ion, which is selected by the receptor causes the fluorescence intensity to be switched back ‘on’ again.

2. It is known that Schiff base containing fluorescent sensors are weakly emissive due to cis-trans isomerisation about the C=N bond that quenches fluorescence by non-radiative decay, and that a metal ion binding event that prevents this rotation leads to an enhancement in fluorescence.
3. The sp^3 nitrogen of binding sites is inserted in the signaling sub-unit, thus is directly integrated with a receptor. In this design, the orbitals overlap to an appreciable extend making the system with one terminal to be electron rich and the other electron poor, so that a substantial dipole is created. Upon binding of an analyte (especially metal ion), into the receptor naturally causes an interaction with this excited state dipole. This interaction causes the shift in fluorescence spectrum and recently many researchers are interested in these types of receptors, which show in the shift in fluorescence spectrum upon cation binding than a simple ‘off–on’ nature of the chemosensor.
4. When a dimer is formed from the receptor (mono-unit) upon complexation with analyte, then a shift in emission is observed. In the design of receptor 4, the free receptor has two signaling sub units and if metal ion bind in its receptor subunit, then it may leads to π-π stacking. The term excimer (excited state complex) is, strictly speaking, limited to cases in which a true dimer is formed; i.e. both components of the dimer are the same molecule.

Experimental

All the commercial chemicals were of reagent grade and were used without further purification. NMR spectra were recorded on a Bruker AVANCE 400 MHz spectrometer. Chemical shifts are reported in parts per million, downfield of TMS. Multiplicity is indicated by the following abbreviations: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (b). IR spectroscopy data was obtained from a Perkin Elmer Spectrum 100 FT-IR spectrometer. Elemental analyses were recorded on an Elementar Vario EL spectrometer. Absorbance measurements were recorded on Agilent UV–Vis spectrometer using 10 mm quartz cuvettes. Fluorescence measurements were recorded on a Perkin-Elmer LS55 luminescence spectrometer using 10 mm quartz cuvettes. Excitation slit size was 10 nm and emission slit size was 10 nm. Scan speed was set at 500. All the measurements were carried out at room temperature.

A. Synthesis of target compounds

(1) Synthesis of 2-allyl-6-bromo-benzo[*de*]isoquinoline-1,3-dione (2)

4-Bromo-1,8-naphthalic anhydride (**1**, 5 g, 18 mmol) and allylamine (1.7 g, 30 mmol) were refluxed at 50^0 in DMF with stirring for 6 h in glacial acetic acid (40 mL). The suspension was poured into ice–water (150 mL) and filtered to give a brown solid. Recrystallization from chlorobenzene gave pale gray needles (4.2 g, 74%). Product was characterized by melting point only.

(2) Synthesis of 2-allyl-6-(2-amino-ethylamino)-benzo[*de*]isoquinoline-1,3-dione (3)

1 (1 g, 3.18 mmol) was dissolved in an excess of ethylene diamine (3 mL) and heated at 80 ^oC for 18 h. The reaction mixture was poured slowly into water and the resulting precipitate was collected by filtration. The product was dried in vacuum to yield the product as a yellow solid (47%). Further the compound was crystallized from chlorobenzene. The solution of crude **2** in chlorobenzene (15ml) was refluxed for 20 min and then cooled in an ice bath to get needle shaped yellow crystals. The crystals were filtered to afford the product in 36% yield. Mp 155-157 ^oC (Literature value 155-158 ^oC) ^1H (400 MHz, $CDCl_3$): 8.53 (1H, d, J = 7.2 Hz, Naph-H), 8.41 (1H, d, J = 8.4 Hz,Naph-H), 8.12 (1H, d, J = 8.0 Hz, Naph-H),

7.56 (1H, t, J = 7.8 Hz, Naph-H), 6.65 (1H, d, J = 8.0 Hz, Naph-H), 6.13 (1H, br s, NH), 5.94 (1H, m, CH), 5.23 (1H, d, J_{ac} = 17.0, J_{bc} = 1.6 Hz, =CH), 5.10 (1H, d, J_{ab} = 10.0, J_{bc} = 1.6 Hz, =CH), 4.72 (2H, d, J = 5.6 Hz, =C-CH_2), 3.35 (2H, t, J = 5.6 Hz, -NCH_2), 3.12 (2H, t, J = 5.6 Hz, -NCH_2).

IR (cm^{-1}**):** 3357 (NH_2),1709 (sC=O) 1679 (asC=O) 1585 (C=C) 1375 CNC (imide)

CHN analysis: Theoretical C (69.1), H(5.8), N(14.2); Found C (69.4), H(5.7), N(14.4).

(3) Synthesis of Dipodal receptor 4

The compound was prepared by the condensation reaction of isophthaldehyde (125mg, 1.0 mmol) with amine **3** (172mg, 1.0 mmol) in dry methanol. The reaction mixture was allowed to stir at room temperature for 30 min. The yellow coloured precipitate was filtered, washed with methanol and dried (mg, 92%).

^{1}H (400 MHz, $CDCl_3$): 8.61 (1H, t, ArH), 8.57 (2x 1H, d, ArH), 8.53 (2 x 1H, d, J = 7.2 Hz, Naph-H), 8.46 (2x1H, s, -CH=N), 8.41 (2 x 1H, d, J = 8.4 Hz,Naph-H), 8.25 (1H, s, ArH), 8.14 (2 x 1H, d, J = 8.0 Hz, Naph-H), 7.82 (2 x 1H, t, J = 7.8 Hz, Naph-H), 6.66 (2 x 1H, d, J = 8.0 Hz, Naph-H), 6.13 (2 x 1H, br s, NH),6.03 5.31 (2 x 1H, m, CH), 5.23 (2 x 1H, d, J_{ac} = 17.0, J_{bc} = 1.6 Hz, =CH), 5.19 (2 x 1H, d, J_{ab} = 10.0, J_{bc} = 1.6 Hz, =CH), 4.80 (2 x 2H, d, J = 5.6 Hz, =C-CH_2), 4.06 (2 x 2H, t, J = 5.6 Hz, -NCH_2), 3.78 (2 x 2H, t, J = 5.6 Hz, -NCH_2).

IR (cm^{-1}**):** 1683 (-C=O) 1641 (-C=N) 1579 (C=C) 1369 CNC (imide)

CHN analysis: Theoretical C (73.2), H(5.3), N(12.2); Found C (73.4), H(5.1), N(12.1);

B. Recognition studies

(1) Cation recognition studies

The cation binding ability of **4** was determined by preparing solutions containing 25 nM solution of receptor along with standard solution of a particular metal salt in THF:H_2O (9:1, v/v). The fluorescence spectrum of each solution was recorded with excitation at λ_{max} = 437

nm. The cation recognition behaviour of any receptor for the binding of a particular cation was evaluated from the changes in fluorescence spectrum of receptor upon addition of that metal salt.

(2) Receptor vs metal ion titration

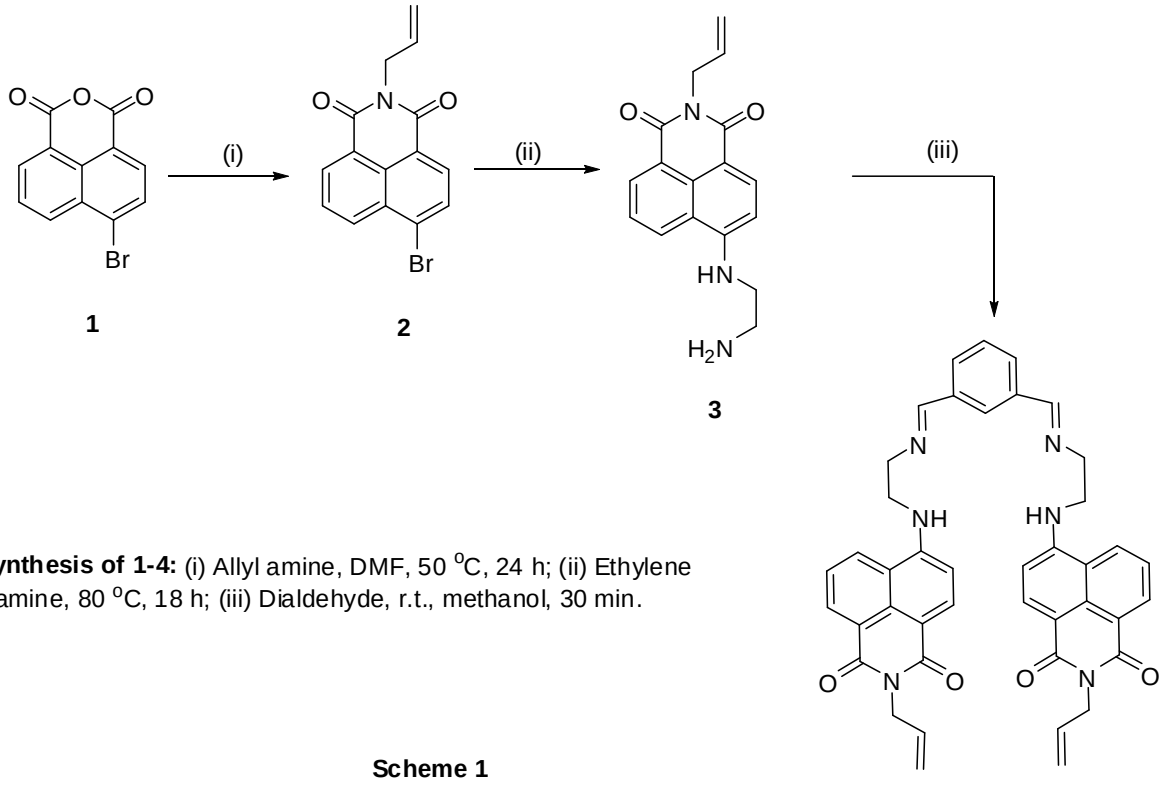
Volumetric flasks were taken each containing 25 nM of **4** along with varied amounts of a particular metal salt in THF:H_2O (9:1, v/v). The solutions were shaken thoroughly and their fluorescence spectra were recorded with excitation at λ_{max} = 437 nm.

(3) Stoichiometry determination

In order to determine stoichiometry of the complex formed from receptor **4** and and particular matter, solutions of **4** and metal salt were prepared as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 in THF:H2O (9:1, v/v). These solutions were kept at 25±1 ºC for 3 h, and were shaken occasionally. Their fluorescence spectra were recorded with excitation at λ_{max} = 437 nm and fluorescence intensity at λ_{max} = 515 nm was used for calculations. The concentration of [HG] was calculated by the equation $[HG] = \Delta I/I_o \times [H]$.It was done with the help of job plot .

Results & Discussion

The dipodal receptor **4** was synthesised by a series of steps as shown in scheme 1. The synthesis of **2-3** was performed following the slightly modified literature procedure.[8] 4-Bromo-1,8-naphthalic anhydride (0.1 mol) was taken in 50 ml. of chloroform along with allylamine (0.1 mol) was added. The solution was refluxed and the course of the reaction was monitored using TLC. After 8 h, when the reaction was completed, the solvent was evaporated. The crude product was dissolved in methanol and then water was poured to obtain product **2.** The product was washed and dried. The product **3** was prepared by taking compound **2** (0.05 mol) dissolved in an excess of ethylene diamine (10 mL) and heated at 80^{o}C for 18 h. The reaction mixture was poured slowly into water and the resulting precipitate collected by filtration. The product was dried in vacuum to yield the product as a yellow solid.



1 **2** **3** **4**

Synthesis of 1-4: (i) Allyl amine, DMF, 50 ^{o}C, 24 h; (ii) Ethylene diamine, 80 ^{o}C, 18 h; (iii) Dialdehyde, r.t., methanol, 30 min.

Scheme 1

The final product **4** was prepared by simple condensation reaction of aldehyde and amine. The compound **3** (0.03 mol) was taken in methanol along with dialdehyde (0.01) and the reaction mixture was stirred at room temperature. Yellow colour ppts were separated out. These ppts were filtered and washed with methanol. All the compounds **2-4** were characterized with ^{1}H NMR. The melting points of compounds **2-3** were recorded and found to be well in agreement with the literature reported values. The final product **4** also shows the band for imine linkages (-CH=N-) in its IR spectrum and the purity of product was checked with elemental analysis.

b) Metal binding affinity of receptor 4

To evaluate the metal binding affinity of receptor 4, the changes in fluorescence intensity of **4** upon addition of different metal salts were recorded (Fig. 1).

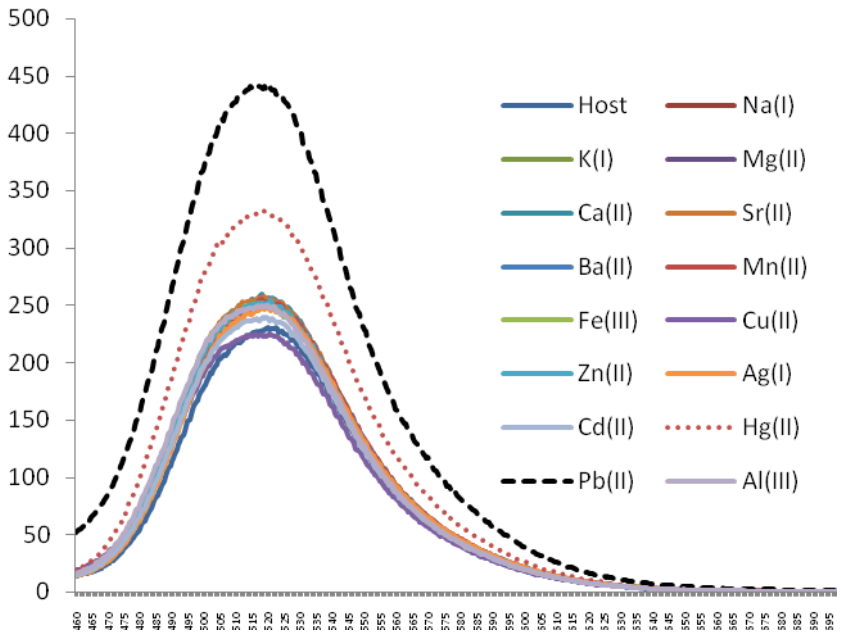


Fig 1. Changes in fluorescence intensity of **4** (25nM) upon addition of standard solution of a particular metal ion salt in THF/H_2O (9:1, v/v) solvent system (excitation at λ_{max} = 437 nm)

Figures 1 and 2 clearly show that there is a marked enhancement in fluorescence intensity only upon addition of Hg(II) or Pb (II). On the other hand, no such significant changes in fluorescence spectra were observed when receptor **4** was exposed to other metal salts under

the same experimental conditions. Upon complexation of either of Hg(II) or Pb (II) ion, the sp^2 N of the –C=N group is involved in coordination with the metal ion hindering the PET phenomenon and fluorescence is restored resulting in an off-to-on signal. On the other hand, the cis-trans isomerisation about the C=N bond that quenches fluorescence by non-radiative decay, and that a metal ion binding event that prevents this rotation leads to an enhancement in fluorescence.[26]

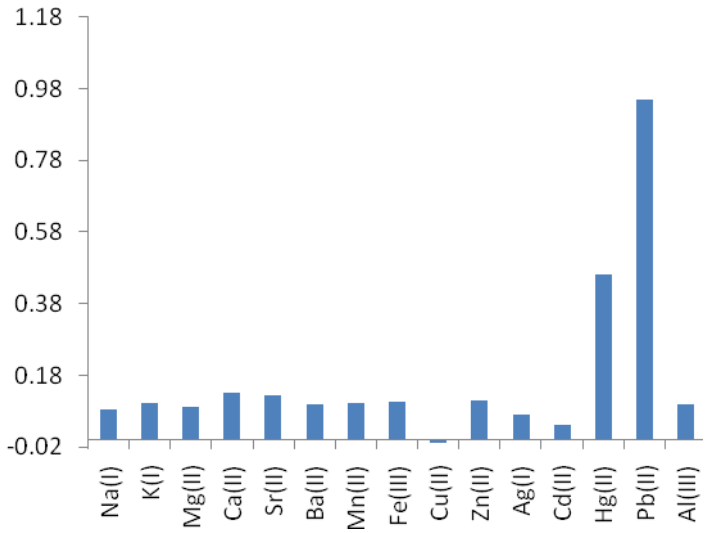


Fig 2. Fluorescence ratio $[(I_0\text{-}I)/I_0]$ of **4** (25nM) at 515 nm upon addition of standard solution of a particular metal ion salt in THF/H_2O (9:1, v/v) solvent system (excitation at λ_{max} = 437 nm). Where, I_0 and I are intensities in the absence and presence of the metal ion, respectively

c) Titration of receptor 4 w.r.t. metal ions

To learn more about the properties of **4** as a receptor for Pb(II), fluorescence titration was carried outon . Figure 3 illustrates the emission response of chemosensor **4** with increase in concentration of Pb(II) ion. The fluorescence intensity of a 25nM solution of **4** at λ_{max} = 515 nm enhanced, while shape of the emission band is not changed. The receptor **4** exhibited a high sensitivity toward Pb(II), enhancing its fluorescence intensity about two fold with 2.0 equiv of lead. The inset of figure 3 is showing the linear relationship of between the fluorescence intensity and addition of Pb(II). Thus receptor 4 can be used for the estimation of Pb(II) in a given sample upto a concentration range of 0-100 μM.

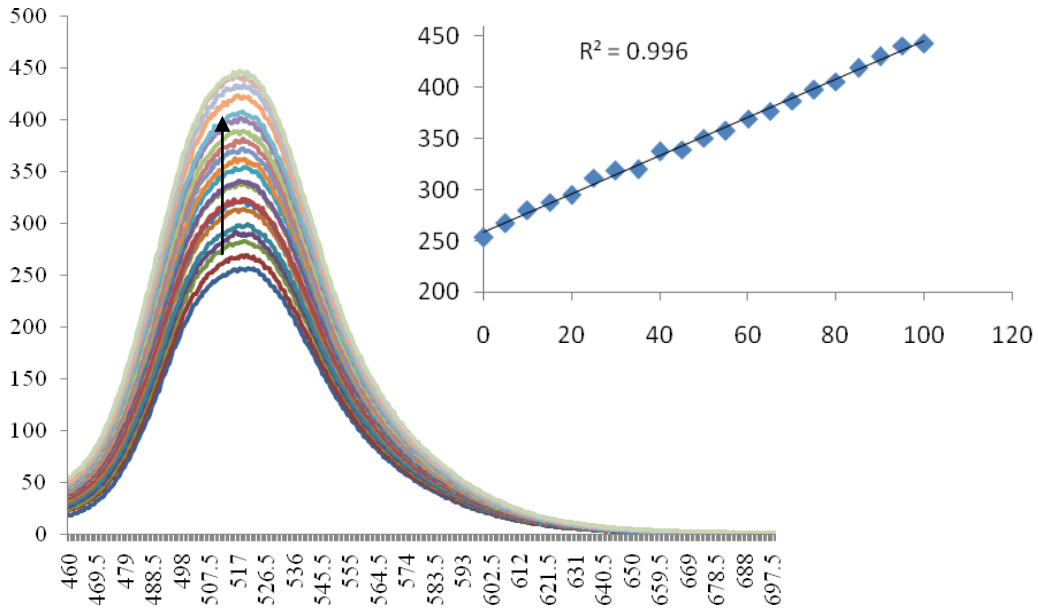


Figure 3. Fluorescence spectra changes of receptor **4** 25nM) upon addition of Pb(II) salt (0-100 μM) in THF/H_2O (9:1, v/v) solvent system (excitation at λ_{max} = 437 nm)

Secondly, the figure 1-2 are showing some enhancement with Hg(II); although this enhancement is not so prominent as it was found in case with Pb(II), but is significantly better than the other metal ions. Thus a titration is also planned with receptor **4** and Hg(II). Figure 4 illustrates the emission response of chemosensor **4** with increase in concentration of Hg(II) ion. The fluorescence intensity of a 25nM solution of **4** at λ_{max} = 515 nm enhanced, while shape of the emission band is not changed.

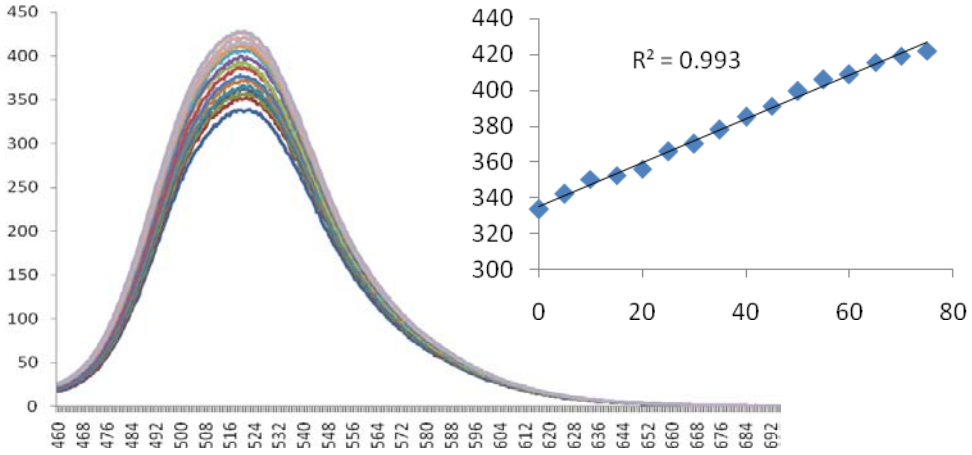


Fig 4. Fluorescence spectra changes of receptor **4** (25nM) upon addition of Hg(II) salt (0-80 μM) in THF/H_2O (9:1, v/v) solvent system (excitation at λ_{max} = 437 nm)

The inset of figure 4 is showing the linear relationship of between the fluorescence intensity and addition of Hg(II). Thus receptor **4** can be used for the estimation of Hg(II) in a given sample upto a concentration range of 0-80 μM.

Stoichiometry of complex formed between receptor 4 and metal ions [Pb(II)/ Hg(II)]

Method of Continuous Variation, also known as Job plot, is used to determine the stoichiometry of a binding event. This method is widely used in analytical chemistry & instumental analysis In solutions where two species are present, one species (A) may bind to the other species (B). In some cases, more than one A will bind with a single B. One way to determine the amount of A binding to B is by using a Job plot .In this method, the total molar concentration of the two binding partners i:e metal and host are held constant, but their mole fraction are varied. Absorption signal is plotted against the mole fractions of these two components. The maximum or minimum on the plot corresponds to the stoichiometry of the two species, if sufficiently high concentrations are used. Continuous variation methods were used to determine the stoichiometric ratios of the receptor and metal ions [Pb(II) / Hg(II)].[27] Figure 5 shows Job's plots of the fluorescence intensity of free receptor **4** and the intensity of the system with the molar fraction of the host {[H]/([H]+[G])} for a series of solutions in which the total concentration of host and metal ion was constant, with the molar fraction of host continuously varying. The results illustrate that in both cases [Pb(II) / Hg(II)], receptor-guest complex concentration approaches a maximum when the molar fraction of host is about 0.5, meaning that both the metals form 1:1 complex with the receptor.

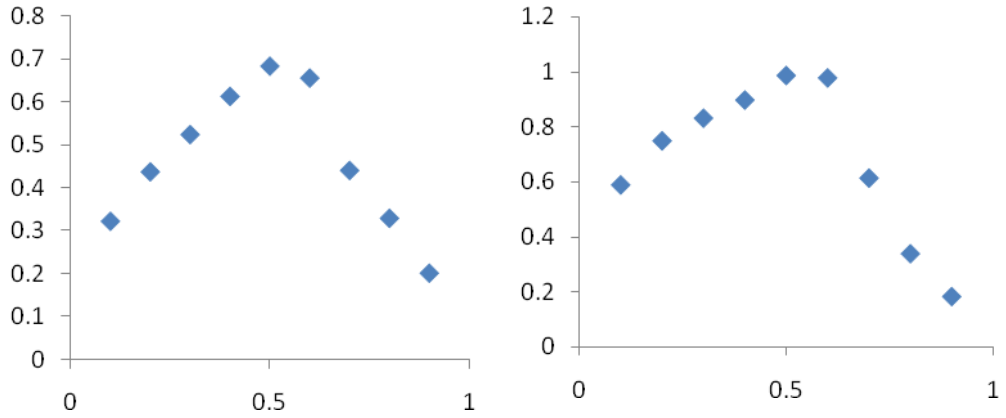


Figure 5. Job's plot between receptor **4** and metal ions Pb(II) [A] / Hg(II) [B]. The concentration of [HG] was calculated by the equation $[HG] = \Delta I/I_0 \times [H]$.

e) Binding constant of complex formed between receptor 4 and metal ions [Pb(II) / Hg(II)]

The binding constant is a special case of the equilibrium constant K. The equilibrium state of molecular binding, i.e. the balance between the binding and dissociation processes after infinite reaction time, may be formalized as the unbound compounds (reactants, A and B) transforming into a complex (product, C):

$$A + B \rightleftharpoons C$$

This directionality of reaction defines the association equilibrium constant Ka. The reverse directionality is used K_{diss}. Since the binding constant is related to the molar free enthalpy, it is used to quantify the affinity of binding. The Benesi-Hildebrand method is a mathematical approach used in the determination of the equilibrium constant K and stoichiometry of nonbonding interactions. This method has been typically used to study reaction equilibriums that form 1:1 guest-host complexes.

$$H + G \rightleftharpoons HG$$

When one of the reactants either host or guest is present in excess amounts over the other reactant, the characteristic absorbance spectra of the other reactant will be transparent in the collective absorbance or emission range of the reaction system. Therefore, by collecting the absorbance spectra of the reaction before and after the formation of the product and its equilibrium, the association constant of the reaction can be determined.

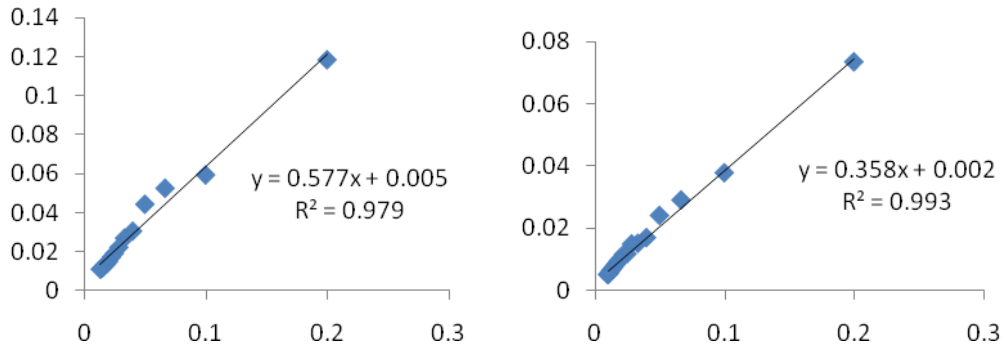


Fig 6. Benesi–Hilderbrand plot for the determination of stability constant between receptor **4** and metal ions Pb(II) [A] / Hg(II) [B]

Association constants K_a of 4 and metal ions [Pb(II) / Hg(II)] were calculated on the basis of the Benesi–Hilderbrand plot (Fig. 6), and it was found to be in the range of 10^5 M^{-1} for [Pb(II) / Hg(II)].[28]

Conclusions

A new fluorescent receptor was synthesized. The final receptor was prepared by Schiff's base condensation reaction. This reaction has been performed by reacting a dipodal aldehyde with fluorescent amine. The newly synthesized fluorescent receptor (4) was found to be a sensor for Hg(II) and Pb(II). It is found that upon complexation of either of Hg(II) or Pb (II) ion, the sp^2 nitrogen of the –C=N group is involved in coordination with the metal ion, hindering the PET phenomenon. On the other hand, the cis-trans isomerisation about the C=N bond that quenches fluorescence by non-radiatve decay, leads to an enhancement in fluorescence upon metal ion binding event that prevents this rotation.

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