CHAPTER 2

SYNTHESES AND RECOGNITION PROPERTIES OF MONOMERIC
AND POLYMERIC RECEPTORS

2.1. Introduction

A remarkable progress has been made in the field of polymer chemistry since Staudinger first introduced various polymerization methods in the early 1920’s. Structural formulae for natural rubber, polystyrene, and polyoxymethylene was proposed by Staudinger. He is also credited for inventing the term “macromolecule” which is still commonly used today. The field of synthetic polymers advanced further in the 1930’s when Carothers developed two synthetic polymers, neoprene and nylon, which are extensively used in many industrial applications. A term invented by Lehn, “supramolecular chemistry”, which means chemistry beyond the molecule, leads to more advancement in polymer chemistry. Polymer based optical chemosensor have many advantages over small organic compounds based sensors. One of the most important advantage of polymer based sensors is signal amplification. Swager et al. first demonstrated that polyreceptor i.e. conjugated polymer sensors, with their receptors connected in conjugation with each other, as molecular wires, have several advantages over small molecules for sensing applications. They were determined to be more sensitive because of the efficient mobility of excitation energy between the receptors along the polymer backbone. The polymer structure also provided facile structural modification and good processability. Further, conjugated polymer chains have good binding efficiency due to the presence of multiple recognition sites. Variety of polymeric sensors available in literature e.g. polymer sensors bearing pendant ligands for recognition, dendrimer based sensors, conjugated polymer based sensors, and imprinted polymer based sensors etc. showing recognition properties towards various analytes. For analytes of environmental and biological importance, the sensing is desirable in aqueous medium, so the materials are planned to impart solubility of material in aqueous medium. The poor solubility of many organic receptors is due to the presence of hydrophobic moieties in the design of the chemosensor such as anthracene, naphthalimide, pyrene etc. Contrary to these types of receptors, the binding units constituted by the polyamine scaffolds may offer
good solubility in aqueous medium and the polyamine derivatives can form stable metal chelates.

2.2. Result and Discussion

2.2.1. Synthesis

2.2.1.1. Synthesis of various monomeric and dimeric units

2.2.1.1.1. Chromone based monomeric and dimeric receptors

The 3-formylchromone 1 is an important target for the heterocyclic synthesis; due to the occurrence of three electron deficient sites: (a) carbon C-2, (b) the aldehyde carbon and (c) the C-4 carbon of the carbonyl group. The compound 3 was synthesized through a condensation reaction of 3-formylchromone 1 and N,N-dimethylpropylamine 2 in dry methanol for 3 hours (Scheme 2.1). A solid was obtained following the crystallization from chloroform hexane mixture. A light yellow solid was obtained in 85% yield. Compound 3 was fully characterized by \(^1\)H and \(^{13}\)C NMR, and mass spectroscopy, and the purity of the sample was established with elemental analysis. Mass spectra of compound 3 confirmed that we get the desired product of molecular weight [M+Ca\(^{2+}\)] peak at 298. The \(^1\)H NMR (400 MHz, CDCl\(_3\)) spectrum showed three singlets at 8.77 (1H, CH=N), 7.60 (1H, C=CH) and 2.32 (6H, CH\(_3\)), a doublet at 7.75 (1H, ArH), two multiplets at 7.06 (2H, ArH) and 1.78 (2H, CH\(_2\)), three triplets at 7.40 (1H, ArH), 3.48 (2H, CH\(_2\)) and 2.52 (2H, CH\(_2\)). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) indicated a signal at 161.5 corresponding to Schiff base -CH=N and the signals at 195.8, 161.5, 153.7, 133.6, 131.7, 126.1, 118.1, 117.9, 117.6, 110.3, 56.2, 49.6, 45.5 and 28.7 were confirmed the number of carbon atoms in the compound 3.

![Scheme 2.1](image)

The compound 5 was prepared according to the literature method.\(^9\) The brief procedure include the refluxing of the 3-formylchromone 1 and an ethylenediamine 4 in dry methanol
yields the compound 5. The light yellow coloured solid 5 was obtained in 82% yield following the crystallization from chloroform hexane mixture (Scheme 2.2). The $^1$H NMR spectra was matched well with literature.$^9$

![Scheme 2.2](image)

By inspiring from the above work, it was planned to synthesize the other derivative of chromone by the reaction of chromone with triamine, tetramine and teramine. The products formed from these reactions were not soluble in any solvent. Hence characterization and photophysical studies were not possible.

2.2.1.1.2. Monomeric receptors having dihydropyrimidones moieties

It is well known fact that there is lot of difference in the recognition properties of monomeric and polymeric units.$^{10}$ The work planned under this series is devised for a comparative account of monomer with the polymers and here the monomeric units are potent to act as sensor for both cations and anions. The second advantage of this approach lies in the fact that these compounds are synthesized through one pot synthesis. There are many reactions in literature which make use of multi-components reaction in one pot, and the Biginelli reaction is the one of them.$^{11}$ Moreover, the reaction is explored with lots of catalyst and with variety of components. This is an inspiration to explore this reaction for the synthesis of monomeric unit and grafting of these units into polymeric framework.

In general, the three component Biginelli reaction used to produce 3,4-dihydropyrimidinones from an aldehyde, urea and a keto ester is proved to be a magnificent approach for fabrication of compounds of biological interest.$^{12}$ Apart from widely used acid catalysed condensation in ethanol for Biginelli reaction, several other modifications regarding solvents and catalyst are the subject of this synthesis.$^{13}$ Among other catalysts, zinc perchlorate hexahydrate is an effective catalyst for the synthesis of dihydropyrimidones and their respective thione derivatives as it is less time consuming and economical.$^{14}$
We synthesized 3,4-dihydropyrimidin-2-(1H)-ones (DHPM) 6-17 using one pot three component Biginelli condensation catalyzed with zinc perchlorate. The reaction involved the refluxing of various araldehyde (a-c), urea/thioura and ethyl/methyl acetoacetate in methanol using zinc perchlorate as a catalyst (Scheme 2.3). Metal perchlorates are strong contenders as catalysts for industrial use. The applicability of this catalyst resulted in decreased reaction time and increased yields of the biologically active dihydropyrimidine derivatives. The high efficiency of zinc perchlorate is due to its high oxophilic character i.e. higher charge with respect to size ($Z^2/r$) value ($5.33 \times 10^{-2}$). The lower hydrolysis constant (pK$_a$ value=9.6) of Zn$^{2+}$ ion facilitated to hold oxophilic property in the presence of water of hydration and trace of moisture. Also electron withdrawing nature of counter ion perchlorate makes it more electrophilic in nature.

$$
\begin{align*}
R-CHO + H_2N\text{-}NH_2 + CO\text{-}COR_1 & \xrightarrow{\text{ZnClO}_4\cdot6\text{H}_2\text{O}} \xrightarrow{\text{Methanol, Reflux}} R_1O^+\text{-}N=O\text{-}R \n\end{align*}
$$

6 R=a, X=O, R$_1$=Me; 7 R=a, X=O, R$_1$=Et; 8 R=a, X=S, R$_1$=Me; 9 R=a, X=S, R$_1$=Et
10 R=b, X=O, R$_1$=Me; 11 R=b, X=O, R$_1$=Et; 12 R=b, X=S, R$_1$=Me; 13 R=b, X=S, R$_1$=Et
14 R=c, X=O, R$_1$=Me; 15 R=c, X=O, R$_1$=Et; 16 R=c, X=S, R$_1$=Me; 17 R=c, X=S, R$_1$=Et

Scheme 2.3

Kappe proposed the mechanism (Scheme 2.4) with zinc perchlorate as a catalyst. According to Kappe the synthesis of DHPM involves the acid catalyzed formation of N-acyliminium ion 20 (intermediate formed from the aldehyde 19 and urea 18). Interception of the iminium ion 20 by keto ester 21 produces an open chain ureide 22, which subsequently cyclises to DHPM 23.\textsuperscript{15}
Scheme 2.4. Mechanism for one-pot synthesis of DHPM Biginelli condensation protocol.

The structures of 6-15 were fully confirmed by $^1$H NMR, $^{13}$C NMR and Mass Spectra. Light green coloured product 6 is formed in 72% yield. $^1$H NMR spectrum of 6 showed five singlets at 9.41 (1H, NH), 7.94 (1H, NH), 6.39 (1H, CH), 3.30 (3H, OCH$_3$), 1.16 (3H, CH$_3$), one multiplet at 8.28 (4H, ArH), three doublets at 8.59 (1H, ArH), 8.13 (1H, ArH) and 8.01 (1H, ArH), one triplet at 8.08 (1H, ArH). The $^{13}$C NMR showed a peak at 18.0 due to -CH$_3$, at 30.7 is due to -CH, at 50.1 is due to -OCH$_3$, 151.7 and 99.5 are due to the presence of =C=C, peak at 180.1 and 165.9 are due to the presence of =C=O, and all other peaks at 148.9, 138.9, 130.9, 130.4, 127.5, 127.4, 127.3, 127.1, 126.3, 125.6, 125.4, 125.0, 124.9, 124.1, 124.0 and 123.4 are due to aromatic carbons. Mass spectra of compound 6 confirmed that we get the desired product of molecular weight [M+1] peak at 371. Light green coloured product 7 is formed in 75% yield. $^1$H NMR spectrum of 7 showed four singlets 9.3 (1H, NH), 7.89 (1H, NH), 6.39 (1H, CH), 2.4 (3H, CH$_3$), three doublets at 8.5 (1H, ArH), 8.1 (2H, ArH), 8.02 (1H, ArH), two triplets at 8.08 (1H, ArH), 0.7 (3H, CH$_3$), two multiplets at 8.2 (4H, ArH) and 3.8 (2H,CH$_2$). The $^{13}$C NMR showed a peak at 17.8 and 13.7 are due to -CH$_3$, at 50.1 is due
to -CH, at 58.95 is due to -OCH₂, 148.5 and 99.4 are due to the presence of -C=C, peak at 165.2 and 151.4 are due to the presence of -C=O, and all other peaks at 139.0, 130.8, 130.2, 130.0, 127.3, 127.2, 126.9, 126.1, 125.5, 125.2, 124.9, 124.8, 123.9, 123.8, 123.43 and 99.6 are due to aromatic carbons. Mass spectra of compound 7 confirmed that we get the desired product of molecular weight [M+1] peak at 385.1. Yellow coloured product 8 formed in 85% yield. 

**1H NMR spectrum of 8** showed five singlets at 10.4 (1H, NH), 9.8 (1H, NH), 6.3 (1H, CH), 2.4 (3H, OCH₃) and five doublets at 0.7 (3H, CH₃), 8.6 (1H, ArH), 8.2 (1H, ArH), 8.1 (1H, ArH) and 7.9 (1H, ArH), one triplet at 8.0 (1H, ArH), one multiplet at 8.3 (4H, ArH). The ¹³C NMR showed a peak at 17.24 due to -CH₃, at 30.65 is due to -CH, at 50.11 is due to -OCH₃, 165.5 and 100.9 are due to the presence of -C=C, peak at 206.4 is due to the presence of -C=S, peak at 173.6 is due to the presence of -C=O, and all other peaks at 145.3, 137.3, 130.8, 130.3, 130.2, 127.5, 127.2, 127.1, 126.3, 125.5, 125.4, 125.1, 125.0, 123.8 and 123.3 are due to aromatic carbons. Mass spectra of compound 8 confirmed that we get the desired product of molecular weight [M+1] peak at 387.1. Yellow coloured product 9 formed in 81% yield. 

**1H NMR spectrum of 9** showed four singlets at 10.7 (1H, NH), 7.92(1H, NH), 6.28 (1H, CH), 2.44 (3H, OCH₃), 9.45 (1H, ArH), one multiplet at 8.12 (8H, ArH), one quateret at 3.80 (2H, CH₂), one triplet at 0.78 (3H, CH₃). The ¹³C NMR showed a peak at 13.9 and 18.4 are due to -CH₃, at 51.8 is due to -CH, at 60.4 is due to -OCH₂, at 121.8 due to the presence of -C=C, peak at 193.3 is due to the presence of -C=S, at 165.0 due to the presence of -C=O, and all other peaks at 131.4, 131.0, 130.9, 130.7, 130.6, 128.7, 128.0, 127.4, 127.2, 127.0, 126.7, 126.3, 125.8, 125.5, 125.3, 124.7, 123.2 are due to aromatic carbons. Mass spectra of compound 9 confirmed that we get the desired product of molecular weight [M+1] peak at 401. Yellow coloured product 10 is formed in 70% yield. 

**1H NMR spectrum of 10** showed six singlets at 8.0 (1H, OH), 7.6 (1H, NH), 6.4 (1H, NH), 5.5 (1H, CH), 3.8 (3H, OCH₃) and 2.3 (3H, CH₃), two triplets at 7.1 (2H, ArH), 7.0 (1H, ArH), two doublets at 7.2 (2H, ArH) and 6.8 (2H, ArH). The ¹³C NMR showed a peak at 16.3 due to -CH₃, at 44.03 is due to -CH, at 52.03 is due to -OCH₃, 152.9 and 95.2 are due to the presence of -C=C, peak at 169.1 and 155.7 are due to the presence of -C=O, and all other peaks at 136.7, 127.0, 121.6, 121.0, 119.6, 119.1, 113.3 and 11.9 are due to aromatic carbons. Mass spectra of compound 10 confirmed that we get the desired product of molecular weight [M+Na]⁺ peak at 338.4. Yellow coloured product 11 formed in 76% yield. 

**1H NMR spectrum of 11** showed five singlets at 7.7 (1H, OH), 6.8 (1H, NH), 6.3 (1H, NH), 5.5 (1H, CH) and 2.3 (3H, CH₃), two multiplets at 7.3 (2H, ArH) and 7.2 (4H, ArH), one quateret at
4.2 (2H, CH₂), one triplet at 1.3 (3H, CH₃). The $^{13}$C NMR showed a peak at 14.7 and 16.3 are due to -CH₃, at 44.03 is due to -CH, at 61.5 is due to –OCH₂, 155.7 and 95.7 are due to the presence of –C=C, peak at 169.4 and 156.2 are due to the presence of -C=O, and all other peaks at 136.7, 127.0, 121.6, 121.0, 119.6, 119.1, 113.3 and 111.9 are due to aromatic carbons. Mass spectra of compound 11 confirmed that we get the desired product of molecular weight $[M+1]$ peak at 338.4. Yellow coloured product 12 is formed in 85% yield. $^1$H NMR spectrum of 12 showed six singlets at 9.5 (1H, OH), 8.1 (1H, NH), 7.39 (1H, NH), 5.3 (1H, CH), 3.8 (3H, OCH₃) and 2.3 (3H, CH₃), one doublet at 7.10 (2H, ArH), one triplet at 7.16 (1H, ArH), two multiplets at 7.19 (2H, ArH) and 7.07 (1H, ArH). The $^{13}$C NMR showed a peak at 16.3 due to -CH₃, a triplet at 45.6 is due to -CH, at 61.5 is due to –OCH₂, 156.8 and 97.6 are due to the presence of –C=C, peak at 180.4 is due to the presence of -C=S, peak at 169.1 is due to the presence of -C=O, and all other peaks at 136.7, 127.0, 121.6, 121.0, 119.6, 119.1, 113.3 and 111.9 are due to aromatic carbons. Mass spectra of compound 12 confirmed that we get the desired product of molecular weight $[M+1]$ peak at 328.9. Yellow coloured product 13 formed in 68% yield. $^1$H NMR spectrum of 13 showed five singlets at 8.1 (1H, NH), 7.9 (1H, OH), 5.3 (1H, CH), 3.1 (1H, NH), and 1.9 (1H, OCH₃), three triplets at 7.2 (1H, ArH), 7.3 (1H, ArH), 1.3 (3H, CH₃), one quateret at 4.2 (2H, CH₂), one multiplet at 7.8 (2H, ArH). The $^{13}$C NMR showed a peak at 14.7 and 16.3 are due to -CH₃, at 45.6 is due to -CH, at 61.5 is due to –OCH₂, 159.7 and 98.0 are due to the presence of –C=C, peak at 180.4 is due to the presence of -C=S, 169.4 are due to the presence of -C=O, and all other peaks at 136.7, 127.0, 121.6, 121.0, 119.6, 119.1, 113.3, 111.9 are due to aromatic carbons. Mass spectra of compound 13 confirmed that we get the desired product of molecular weight $[M+1]$ peak at 343.4. Yellow coloured product 14 is formed in 73% yield. $^1$H NMR spectrum of 14 showed six singlets 12.4 (s, 1H, NH), 8.6 (s, 1H, NH), 8.1 (s, 1H, NH), 8.0 (s, 1H, CH), 3.8 (s, 3H, OCH₃), 2.0 (s, 3H, CH₃), two multiplets at 7.9 (m, 1H, ArH), 7.3 (m, 2H, ArH) and two doublets at 8.5 (d, 1H, ArH), 7.6 (d, 1H, ArH). The $^{13}$C NMR showed peaks at 13.8 and 17.6 due to -CH₃, at 59.0 is due to -CH, 96.7 and 115.9 are due to the presence of –C=C, peak at 165.1 is due to the presence of -C=O, and all other peaks at 161.5, 151.8, 149.2, 139.1, 131.0, 124.9, 123.9 are due to aromatic carbons. Mass spectra of compound 14 confirmed that we get the desired product of molecular weight $[M+1]$ peak at 323. Yellow coloured product 15 formed in 69% yield. $^1$H NMR spectrum of 15 showed three singlets at 11.8 (1H, NH), 8.5 (1H, NH), 2.0 (s, 3H, CH₃), three doublets at 8.3 (1H, ArH), 7.2 (1H, ArH), 7.0 (1H, ArH), one quateret at 4.2 (2H, CH₂), one triplet at 1.3 (3H, CH₃), one multiplet at 7.9 (3H, 2ArH & CH). The $^{13}$C NMR showed a peak at 14.0 is due to -CH₃, at
60.5 is due to –OCH₂, 113.5, 112.6, are due to the presence of –C≡C, peak at 163.9 is due to the presence of -C=O and all other peaks at 137.6, 136.4, 135.2, 133.9, 132.8, 124.7, 122.7, 121.1, 119.8 are due to aromatic carbons. Mass spectra of compound 15 confirmed that we get the desired product of molecular weight [M+1] peak at 300. Compound 16 and 17 were prepared, however these compounds were not soluble in any solvent, thus these compounds (16-17) could not be characterized. Some of the 3,4-dihydropyrimidin-2-(1H)-ones (DHPM) moieties were also planned by using aldehydes like 3-formylchromone and 2-hydroxy-4-nitrobenzaldehyde. Compounds were synthesized and it was found that these synthesized compounds were not soluble in any solvent. Hence no characterization studies have been performed on these compounds, and no further studies were performed.

2.2.1.2. Synthesis of various polymeric units

2.2.1.2.1. Polymeric receptors containing imine linkages

The work has been planned on functionalization of polymeric backbone bearing amine functional group 24 and this polymer is commercially available from Aldrich. The condensation reactions are planned with aldehyde, so as to obtain materials having desired photo-physical properties.
The synthesis of compounds 25(a-d) is detailed in Scheme 2.5 and these compounds were synthesized by the condensation reaction between the commercially available polyamine 24 and the corresponding aldehydes. The yellow coloured thick products were washed many times with methanol to give pure Schiff bases. The resultant imine linked products 25(a-d) were characterized by spectroscopic methods i.e. a singlet for –CH=N– in the $^1$H NMR spectra at 8.95 ppm, 8.17 ppm, 8.34 ppm and 8.30 ppm is corresponding to the structures of 25a (yield=68%), 25b (yield=77%), 25c (yield=81%) and 25d (yield=85%), respectively.

2.2.1.2.2. Polymeric units having dihydropyrimidones moieties

The Biginelli compounds synthesized in Scheme 2.3 (6-15) were grafted on the polyamine backbone 24 to synthesize polymers 26(a-e). The synthesis of compounds 26(a-e) is detailed in Scheme 2.6. These compounds were synthesized by the condensation reaction between the commercially available polyamine 24 and the 3,4-dihydropyrimidin-2-(1H)-ones (DHPM) through refluxing in methanol. The yellow coloured thick products were washed many times with methanol to get pure products (yield=82%, 70%, 76%, 74% and 80% for 26a, 26b, 26c, 26d and 26e, respectively).

![Scheme 2.6](image_url)
1H NMR of compound 26a showed six singlets at 9.3 (OH), 8.9 (NH), 7.9 (ArH), 5.3 (CH), 3.4 (CH2), 1.06 (CH3), two multiplets at 7.3 (ArH), 2.4 (CH2), one doublet at 6.6 (d, ArH); 13C NMR confirmed the number of carbon atoms in compound 26a; 170.1, 168.9, 168.6, 159.6, 157.6, 154.4, 149.6, 148.3, 129.0, 128.8, 128.4, 126.5, 123.3, 118.9, 118.4, 114.5, 96.2, 95.3, 83.1, 60.6, 60.3, 53.6, 49.6, 53.6, 49.6, 48.6, 26.5, 23.8. 1H NMR of compound 26b showed eight singlets at 9.0 (NH), 8.2 (NH), 7.9 (OH), 7.5 (ArH), 7.3 (ArH), 7.1 (ArH), 5.3 (CH), 1.0 (CH3), four multiplets at 6.6 (ArH), 3.6 (CH2), 3.1 (CH2), 2.4 (CH2), one doublet at 6.6 (ArH); 13C NMR confirmed the number of carbon atoms in compound 26b; 183.8, 176.5, 168.3, 168.0, 148.3, 130.6, 130.2, 128.7, 128.4, 127.1, 123.9, 121.9, 118.0, 81.5, 60.8, 60.5, 48.6, 42.5, 30.7, 23.1. 1H NMR of compound 26c showed five singlets at 8.5 (NH), 8.5 (NH), 6.3 (CH), 1.8 (CH3), 1.7 (CH3), six multiplets at 8.2 (ArH), 7.9 (ArH), 3.7 (CH2), 3.6 (CH2), 3.1 (CH2), 2.5 (CH2), one triplet at 8.0 (ArH), one doublet at 8.1 (ArH); 13C NMR confirmed the number of carbon atoms in compound 26c; 165.3, 161.2, 151.5, 148.7, 139.1, 127.4, 127.3, 126.3, 125.3, 125.0, 123.5, 99.6, 76.6, 59.0, 54.5, 53.0, 50.9, 49.1, 30.7, 28.8. Compound 26d was synthesized and it was found that it was not soluble in any solvent. Hence no characterization studies have been performed on this compound. 1H NMR of compound compound 26e showed five singlets at 9.3 (NH), 9.0 (NH), 5.9 (CH), 5.4 (CH), 1.9 (CH3), four multiplets at 8.2 (ArH), 7.9 (ArH), 3.0 (CH2), 2.4 (CH2); 13C NMR confirmed the number of carbon atoms in compound 26e; 176.5, 168.0, 148.3, 130.6, 130.2, 129.1, 128.8, 128.7, 128.4, 127.2, 124.0, 121.9, 118.1, 81.4, 60.9, 23.1.

2.2.1.2.3. Polymeric units having Urea/thiourea moieties

The wide spread use of urea/thiourea based derivatives in the designing of anion sensors is linked to their two H-bond donating tendencies. Introduction of hydrogen bond donor groups to an organic moiety results in host for anions. The H-bond donor tendencies can be enhanced by introducing electron withdrawing groups. The synthesis of compounds 27-30 is detailed in Scheme 2.7 and these compounds were synthesized by the condensation reaction between the commercially available polyamine 24 and the corresponding isocyanates/isothiocyanates in methanol. Reactions were performed by refluxing of polyamine 24 and the corresponding isocyanates/isothiocyanates. The thick products were washed many times with methanol to get pure products (yield=80%, 73%, 78% and 79% for 27, 28, 29 and 30, respectively).
Compound 27 and 28 were prepared, however these compounds were not soluble in any solvent, thus these compounds could not be characterized. $^1$H NMR of compound 29 showed two singlets at 8.4 (NH), 3.5 (CH$_2$), three multiplets at 7.9 (ArH), 7.4 (ArH), 2.5 (CH$_2$). $^{13}$C NMR confirmed the number of carbon atoms in compound 29: 150.5, 136.8, 134.5, 128.9, 128.6, 127.6, 127.5, 127.3, 125.7, 124.9, 122.3, 121.8, 118.4, 118.0, 108.5, 53.9, 52.7, 48.8, 48.5, 47.07. $^1$H NMR of compound 30 showed five singlets at 6.9 (NH), 5.6 (NH), 3.6 (CH$_2$), 1.9 (CH$_2$), 1.7 (CH$_2$), four multiplets at 7.9 (NH), 7.3 (ArH), 2.5 (CH$_2$), 1.5 (CH$_2$), one triplet at 7.1 (ArH); $^{13}$C NMR confirmed the number of carbon atoms in compound 30: 182.6, 161.7, 144.9, 134.7, 134.4, 130.3, 128.6, 127.2, 126.0, 124.1, 122.8, 115.8, 107.9, 56.2, 54.1, 52.6, 47.1, 42.6.

2.2.2. Recognition studies

The section deals with the evaluation of photophysical properties of monomeric and polymeric receptors. All of these receptors either contain imine linkage or –N/-O/-S donor sites. All the above synthesized compounds have been checked against a various environmentally and physiologically active metal ions/anions in aqueous media by developing organic nanoparticles. Cation/Anion recognition properties have been investigated by change in the fluorescence profile of the receptors. Organic nanoparticles
were synthesized using reprecipitation technique. In this technique, desired amount of compound is dissolved in a minimum amount of organic solvent. From the above solution, a very small volume is taken in a syringe and injected into distilled water while sonicating. After the injection, sonication was continued for 30 minute to ensure the preparation of stabilized organic nanoparticles. Recognition studies for nano-aggregates of organic receptors in aqueous medium are done on fluorescence spectroscopy by adding 5 eq. of different anion and cation solutions to host solution taken in different 5 ml volumetric flasks. All the recognition studies were performed at a temperature of 25±1°C. For cation binding studies of compound, cation solutions of 5 mM concentration were prepared in distilled water using nitrate salts of different metals. Concentration of anion solutions prepared in distilled water using tetrabutyl ammonium salts of anions is 1 mM. Before recording the fluorescence spectra, the solutions in volumetric flasks are shaken properly and then kept for some time to ensure the uniformity of solutions. Titrations are done by adding small increments of the selected ion to host solution in 10 mL volumetric flask followed by recording the spectra after each addition.

2.2.2.1. Chromone based receptors

The recognition properties of the receptors 3 and 5 have been evaluated with the help of fluorescence spectroscopy.

2.2.2.1.1. Recognition studies of receptor 3

When excited at 330 nm, a 2 μM concentration of receptor 3 in DMSO exhibited the emission band centred at $\lambda_{max} = 438$ nm. However, the same concentrations of receptor 3 in aqueous medium (by developing nano-aggregates of receptor N3) exhibited slight blue shift with emission band centred at 415 nm, with simultaneous enhancement in intensity of 3 (Figure 2.1). The blue shift of receptor 3 in aqueous system can be explained by the changes in the conformation of the chromophore in aqueous system i.e formation of H-Type of aggregates where molecules are arranged in head to head/ tail to tail direction results in a blue shift of the emission band. DLS studies revealed that nanoaggregates of receptor N3 have size 4 nm at 2 μM concentration. The cation binding behaviour of nano-aggregates of N3 was studied in pure water. For this, changes in fluorescence emission spectra of nano-aggregates of N3 was observed upon the addition of 5 eq. of 19 different metal nitrate salts (such as Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, Cr³⁺, Mn²⁺, Fe²⁺, Co³⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, ...
Hg$^{2+}$ and Pb$^{2+}$) to nano-aggregates of N3 (2 μM) in aqueous medium. The solutions are shaken properly followed by recording the spectra for solution of each flask. The addition of different metal ions to the host solution did not affect the fluorescence profile of nano-aggregates of N3 (Figure 2.2).

**Figure 2.1.** Fluorescence emission spectra of receptor 3 in DMSO and of N3 in aqueous medium (2 μM) ($\lambda_{ex} = 330$ nm).

**Figure 2.2.** Changes in fluorescence intensity of nano-aggregates of N3 (2 μM) upon addition of a particular metal nitrates (5eq.) in aqueous medium ($\lambda_{ex} = 330$ nm).

Influence of the anion binding behaviour on the nano-aggregates of N3 in aqueous medium was also investigated. Fluorescence response of nano-aggregates of N3 was observed after the addition of the tetrabutyl ammonium salt of different anions (5 eq.) (such
as F-, Cl-, Br-, I-, CN-, CH₃COO-, HSO₄-, PO₄³⁻, NO₃⁻ and ClO₄⁻) to the nano-aggregates of N3 (2 μM). Fluorescence spectra were recorded for each solution after proper shaking and keeping each solution for sufficient time. The anion binding tests of nano-aggregates of N3 with variety of anions have not shown any significant changes in emission spectra of N3. (Figure 2.3)

![Figure 2.3](image1)

**Figure 2.3.** Changes in emission profile of nano-aggregates of N3 (2 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salt (5 eq.) in aqueous media (λ<sub>ex</sub> = 330 nm).

To check the utility of nano-aggregates of N3, the emission spectra of nano-aggregates was monitored at different pH. There was a negligible decrease in the fluorescence intensity of nano-aggregates of N3 on moving from pH 7 to 3. On moving towards basic pH, there was a slight increase in the fluorescence intensity of a N3 at 415 nm (Figure 2.4).

![Figure 2.4](image2)

**Figure 2.4.** Effect of pH on nano-aggregates of N3 (2 μM) in aqueous system (λ<sub>ex</sub> = 330 nm).
2.2.2.1.2. Recognition studies of receptor 5

A same concentration of receptor 5 (3.5 μM) in DMF and in aqueous medium showed different fluorescence profile when excited at 321 nm. The emission spectra of the 5 exhibited red shift with simultaneous enhancement in intensity (Figure 2.5). The bathochromic shift and enhancement in the fluorescence profile of receptor 5 in aqueous system can be explained by the changes in the conformation of the chromophore in aqueous system i.e formation of J-Type of aggregates where molecules are arranged in head to tail direction results in a bathochromic shift.18 DLS studies revealed that nanoaggregates of receptor N5 have size 5 nm at 2 μM concentration.

Evaluation in modulation of fluorescence signatures of nano-aggregates of receptor N5 in aqueous medium were investigated by addition of 2.5 μM concentration of different metal salts (such as Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, Cr³⁺, Mn²⁺, Fe²⁺, Co³⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺) to the fixed concentration (2.5 μM) of nano-aggregates of N5. Addition of Al³⁺ have pronounced enhancement in the fluorescence intensity of N5 with blue shift of Δλ_max = 27 nm (Figure 2.6). The unique metal recognition properties of N5 highlight the importance of preorganization of receptor pseudocavity in the architecture of

Graphical abstract 2.1. Cartoon representation showing interactions of N5 with Al³⁺ and CN⁻.
nano-aggregates of N5.

**Figure 2.5.** Fluorescence emission spectra of receptor 5 (3.5 μM) in different compositions of DMF/water.

To gain more insights into the binding behaviour of nano-aggregates N5 and Al$^{3+}$ ions, titration was performed in which molar ratio of Al$^{3+}$ ion was changed as compared to the fixed concentration of solution of nano-aggregates of N5 (2.5 μM). The successive additions of Al$^{3+}$ ion (0-2.5 μM) to the solution of N5 have followed the same trend as was observed in the metal binding tests and any particular amount of Al$^{3+}$ ion added to solution of nano-aggregates of N5 is directly proportional to the increase in the intensity of band at 421 nm (Figure 2.7).

This enhancement in the fluorescence intensity may be attributed to the rigidity in the design of receptor upon interaction of Al$^{3+}$ ions with ligand molecules. This rigidity make non-radiative decay of excited state less probable from the excited state of metal complex, which is expected to be an assembly of re-arranged ligand molecules in more static conformation through multivalent interactions. As the titration has shown a good linearity in a range of 0-2.5 μM concentration of Al$^{3+}$ (inset of Figure 2.7). Using this titration data, nano-aggregates of N5 can detect Al$^{3+}$ ion in the solution up to 100 nM. To realize the selectivity of nano-aggregates of N5 for Al$^{3+}$ ions, the competitive metal binding experiments were also performed to estimate Al$^{3+}$ (2.5 μM) in the presence of any of Li$^+$, Na$^+$, K$^+$, Cs$^+$, Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$, Ba$^{2+}$, Cr$^{3+}$, Mn$^{2+}$, Fe$^{2+}$, Co$^{3+}$, Cu$^{2+}$, Zn$^{2+}$, Ag$^+$, Cd$^{2+}$, Hg$^{2+}$ and Pb$^{2+}$ (2.5 μM). As shown in Figure 2.8, no significant variation in the fluorescence intensity was noticed by comparing
the profile of assembly of nano-aggregates of $\textbf{N5}$ and $\text{Al}^{3+}$ ($\textbf{N5.} \text{Al}^{3+}$) with and without other metal ions, means that nano-aggregates of $\textbf{N5}$ have a high selectivity for $\text{Al}^{3+}$ (Figure 2.8).

**Figure 2.6.** Changes in fluorescence intensity of nano-aggregates of $\textbf{N5}$ (2.5 µM) upon addition of a particular metal salts (1eq.) in aqueous medium (excitation at 321 nm).

**Figure 2.7.** Fluorescence spectra changes of nano-aggregates of $\textbf{N5}$ (2.5 µM) upon addition of $\text{Al}^{3+}$ (0-2.5 µM) in aqueous medium (excitation at 321 nm); (Inset: Linear regression graph between concentration of $\text{Al}^{3+}$ and increase in fluorescence intensity of $\textbf{N5}$ at 431 nm (LOD=100 nM)).
Figure 2.8. Competitive binding of nano-aggregates of N5 (2.5 μM) containing of Al$^{3+}$ over other selected metal ions at $\lambda_{ex} = 321$ nm. 1) Al$^{3+}$ only; 2) Al$^{3+}$ + Li$^+$; 3) Al$^{3+}$ + Na$^+$; 4) Al$^{3+}$ + K$^+$; 5) Al$^{3+}$ + Cs$^+$; 6) Al$^{3+}$ + Mg$^{2+}$; 7) Al$^{3+}$ + Ca$^{2+}$; 8) Al$^{3+}$ + Sr$^{2+}$; 9) Al$^{3+}$ + Ba$^{2+}$; 10) Al$^{3+}$ + Cr$^{3+}$; 11) Al$^{3+}$ + Mn$^{2+}$; 12) Al$^{3+}$ + Fe$^{3+}$; 13) Al$^{3+}$ + Co$^{2+}$; 14) Al$^{3+}$ + Cu$^{2+}$; 15) Al$^{3+}$ + Zn$^{2+}$; 16) Al$^{3+}$ + Ag$^+$; 17) Al$^{3+}$ + Cd$^{2+}$; 18) Al$^{3+}$ + Pb$^{2+}$; 19) Al$^{3+}$ + Hg$^{2+}$.

The anion binding tests of nano-aggregates of N5 with variety of anions (F, Cl, Br, I, CN, CH$_3$COO, HSO$_4$, PO$_4^{3-}$, NO$_3$ and ClO$_4$) have not shown any significant changes in emission spectra (Figure 2.9).

Figure 2.9. Changes in emission profile of nano-aggregates of N5 (2.5 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salt (5 eq.) in aqueous media ($\lambda_{ex} = 321$ nm).

Literature revealed the influence of pH on the photophysical properties of nano-aggregates,
thus the emission properties of nano-aggregates of N5 at different pH values (in the absence and presence of Al$^{3+}$) were monitored. For nano-aggregates of N5, in both acidic (pH< 7) and basic conditions (pH>7), the fluorescence intensity increased slightly, in other words we can say that pH has no or little effect on the emission spectra of nano-aggregates of N5. For aluminium complex of nano-aggregates of N5, fluorescence intensity decreased in both acidic and basic medium. The response of assembly of nano-aggregates of N5 and Al$^{3+}$ (N5.Al$^{3+}$) towards pH was also investigated. Figure 2.10 shows that assembly of nano-aggregates of N5.Al$^{3+}$ complex possesses good fluorescence response in the neutral pH range (6.0-8.0), which is favourable for its application in environmental samples, as most of such samples exist at neutral conditions.

![Figure 2.10](image.png)

**Figure 2.10.** pH studies of nano-aggregates of N5 and N5.Al$^{3+}$ complex.

Besides high selectivity, response time is one of the necessary criteria to check the real time applicability of a fluorescent chemosensor to determine Al$^{3+}$. To study the response time, fluorescence emission spectra of nano-aggregates of N5 was studied by varying the concentration of aluminium in the solution of nano-aggregates of N5. Experiment was performed by taking the solution of nano-aggregates of N5 in 5 different volumetric flasks and added different concentration of Al$^{3+}$ in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. As the concentration of Al$^{3+}$ was increased, time required to reach equilibrium was same i.e. sensor response time is independent of the concentration of guest (Figure 2.11).
Figure 2.11. Response time of nano-aggregates of N5 for Al\(^{3+}\) at \(\lambda_{ex} = 321\) nm.

To evaluate the effect of ionic strength, solution of nano-aggregates of N5 (2.5 µM) was made and then 150 equivalents of tetrabutyl ammonium perchlorate was added to a solution of nano-aggregates of N5 followed by keeping for half an hour to attain equilibrium. Fluorescence emission spectra of nano-aggregates of N5 remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of nano-aggregates of N5 with the increased number of ions in the solution (Figure 2.12).

Figure 2.12. Salt perturbation studies of N5 recorded with 2.5 µM concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of sensor and solvent system at \(\lambda_{ex} = 321\) nm.
Fluorescence intensity of nano-aggregates of N5 enhanced largely with a blue shift (from 430 nm to 403 nm) in the presence of Al\textsuperscript{3+}. The assembly of N5 and Al\textsuperscript{3+} (N5.Al\textsuperscript{3+}) (2.5 µM) is then titrated against different anions to investigate the utility of N5.Al\textsuperscript{3+} as an anion sensor. The fluorescence spectrum of N5.Al\textsuperscript{3+} (2.5 µM) upon addition of different selected tetrabutyl ammonium salts of anions (F\textsuperscript{−}, Cl\textsuperscript{−}, Br\textsuperscript{−}, I\textsuperscript{−}, CN\textsuperscript{−}, CH\textsubscript{3}COO\textsuperscript{−}, HSO\textsubscript{4}\textsuperscript{−}, PO\textsubscript{4}\textsuperscript{3−}, NO\textsubscript{3}\textsuperscript{−} and ClO\textsubscript{4}\textsuperscript{−}) (5 eq.) were studied. The data shows that the presence of anions has no influence on the magnitude of the fluorescence intensity of assembly of N5 and Al\textsuperscript{3+} (N5.Al\textsuperscript{3+}) except cyanide (Figure 2.13).

**Figure 2.13.** Changes in fluorescent intensity of assembly of nano-aggregates of N5 and Al\textsuperscript{3+} (N5.Al\textsuperscript{3+}) (2.5 µM) upon addition of a particular tetrabutyl ammonium anion salts (0.2 µM) in aqueous media (λ\textsubscript{ex} = 321 nm).

Titrations studies were performed by increasing the amount of cyanide in N5.Al\textsuperscript{3+} (2.5 µM). With the increase in the concentration of cyanide from 0 to 215 nM, quenching took place along with red shift (from 403 nm to 430 nm) in the fluorescence peak (Figure 2.14). Fluorescence intensity is showing a good linear response (96%) with increasing concentration of cyanide, with a detection limit of 28 nM (inset of Figure 2.14). Next, a series of competitive titrations were attempted to determine if cyanide could be measured in the presence of other competing anions. Figure 2.15 shows that cyanide could be measured accurately by assembly of N5 and Al\textsuperscript{3+} (N5.Al\textsuperscript{3+}) in the presence of equal amounts of any another tested anion. This may be due to the higher binding affinity of cyanide with assembly.
of N5 and Al$^{3+}$ (N5.Al$^{3+}$). The results of the spectroscopic studies indicate that the sensor N5 was recycled during the detection of cyanide ion.

**Figure 2.14.** Fluorescence spectra changes of assembly of N5 and Al$^{3+}$ (N5.Al$^{3+}$) (2.5 µM) upon addition of cyanide (0-215 nM) in aqueous medium ($\lambda_{ex} = 321$ nm); (Inset: linear regression graph between concentration of cyanide added and decrease in fluorescence intensity of N5.Al$^{3+}$ at 431 nm).

**Figure 2.15.** Competitive binding of assembly of N5 and Al$^{3+}$ (N5.Al$^{3+}$) containing CN$^-$ over other selected metal ions at $\lambda_{ex} = 321$ nm. 1) CN$^-$; 2) CN$^- + F^-$; 3) CN$^- + Cl^-$; 4) CN$^- + Br^-$; 5) CN$^- + I^-$; 6) CN$^- + CH_3COO^-$; 7) CN$^- + HSO_4^-$; 8) CN$^- + NO_3^-$ and 9) CN$^- + PO_4^{3-}$.
2.2.2.2. Monomeric receptors having dihydropyrimidones moieties

The recognition properties of the dihydropyrimidones receptors 6-17 have been evaluated with the help of fluorescence spectroscopy.

2.2.2.2.1. Recognition studies of receptor 6

Effect of water content on the photophysical properties for receptor 6 were explored by recording fluorescence emission spectra of receptor 6 in both DMF as well as aqueous system (by synthesizing nano-aggregates). The fluorescence spectra of receptor 6 in organic solvent system showed a manifest difference in the emission profile than nano-aggregates of N6 in aqueous system (Figure 2.16). The fluorescence spectra of receptor 6 in organic solvent showed monomer peaks ranging from (387-417 nm). Whereas the nano-aggregates of N6 showed broad, featureless emission centred at 441-510 nm. The formation of broad band at 441-510 nm is due the ground state excimer emission of pyrene in N6 after the formation of nano-aggregates in aqueous medium.

![Fluorescence emission spectrum of receptor 6 in DMF (40 μM) and of N6 in aqueous medium (13 μM) at λex = 335 nm.](image)

**Figure 2.16.** Fluorescence emission spectrum of receptor 6 in DMF (40 μM) and of N6 in aqueous medium (13 μM) at λex = 335 nm.

The effect of a wide range of environmentally and physiologically active metal ions was investigated for nano-aggregates of N6 in aqueous medium. For this 5 eq. of different metal nitrate salts (1 mM) (such as Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, Cr³⁺, Mn²⁺, Fe²⁺, Co³⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺) were added to the fixed concentration (13 μM). The addition of different metal ions to the host did not affect the spectra of host (Figure 2.17).
Figure 2.17. Changes in fluorescence intensity of nano-aggregates of N6 (13 μM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λ_{ex} = 335 nm).

Host solution of nano-aggregates of N6 was tested against a 9 tetrabutyl ammonium anions (F⁻, Cl⁻, Br⁻, I⁻, CN⁻, CH₃COO⁻, HSO₄⁻, PO₄³⁻, NO₃⁻ and ClO₄⁻). To check the anion binding studies 5 eq. of 9 tetrabutyl ammonium anions were added to the host solution of N6 (13 μM) (λ_{ex}=335 nm). Insignificant change in the emission profile of nano-aggregates of N6 was investigated in the presence of any of the tested anions (Figure 2.18).

Figure 2.18. Changes in emission profile of nano-aggregates of N6 (13 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media (λ_{ex} = 335 nm).
For the application of the sensor in environmental samples, the effect of pH on the fluorescence response of nano-aggregates of \textbf{N6} was also an important factor to consider. The experiments were carried out at a pH range from 3.0 to 12.0 with a concentration of \textbf{N6} fixed at 13 µM (Figure 2.19). In going from acidic to basic range, fluorescence intensity of nano-aggregates of \textbf{N6} first increase up to pH 5.1 and then slight decrease up to pH 12.

![Figure 2.19. Effect of pH on nano-aggregates of N6 (13 µM) in aqueous system (λ_{ex}=335 nm).](image)

2.2.2.2. Recognition studies of receptor 7

Effect of water content on the photophysical properties for receptor 7 were explored by recording fluorescence emission spectra of receptor 7 in both DMF as well as aqueous system (by synthesizing nano-aggregates). The fluorescence spectra of receptor 7 in organic solvent system showed a manifest difference in the emission profile than nano-aggregates of in aqueous system (Figure 2.20). The fluorescence spectra of nano-aggregates of \textbf{N7} in organic solvent showed monomer peaks ranging from (379-399 nm). Whereas the nano-aggregates of \textbf{N7} showed broad, featureless emission centred at 422-551 nm. The formation of broad band at 422-551 nm is due the ground state excimer emission of pyrene in \textbf{N7} after the formation of nano-aggregates in aqueous medium. Evaluation in modulation of fluorescence signatures of nano-aggregates of \textbf{N7} in aqueous medium were investigated by addition of 2.5 µM concentration of different metal nitrate salts (1 mM) (such as Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, Cr³⁺, Mn²⁺, Fe²⁺, Co³⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺) to the fixed concentration (17 µM) of nano-aggregates of \textbf{N7} at excitation wavelength of 335 nm. The solutions are shaken properly followed by recording the spectra for solution of each flask. The addition of different metal ions to the host did not affect the spectra of host (Figure 2.21).
Figure 2.20. Fluorescence emission spectra of receptor 7 in DMF (50 μM) and of N7 in aqueous medium (17 μM) at $\lambda_{ex} = 335$ nm.

Figure 2.21. Changes in fluorescence intensity of nano-aggregates of N7 (17 μM) upon addition of a particular metal nitrates (5eq.) in aqueous medium ($\lambda_{ex} = 335$ nm).

To evaluate the anion binding ability of nano-aggregates of N7, initial screening was carried out with a library of 9 tetrabutyl ammonium anions (F, Cl, Br, I, CN, CH$_3$COO, HSO$_4$, PO$_4^{3-}$, NO$_3^-$ and ClO$_4^-$). The anion binding tests of nano-aggregates of N7 was done by addition of 0.5 μM concentration of tetrabutyl ammonium anions (5 mM) to the fixed concentration (17 μM) of nano-aggregates of N7 at excitation wavelength of 277 nm. Fluorescence spectras were recorded for each solution after proper shaking and keeping each
solution for sufficient time. The anion binding tests of nano-aggregates of N7 with variety of anions have not shown any significant changes in emission spectra (Figure 2.22).

**Figure 2.22.** Changes in emission profile of nano-aggregates of N7 (17 µM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media (λ<sub>ex</sub> = 335 nm).

The effect of pH on the fluorescence emission profile of nano-aggregates of N7 was investigated. The experiments were carried out at a pH range from 3.0 to 12.0, with a concentration of N7 fixed at 17 µM (Figure 2.23). In going from pH 7 to 3 there was slight decrease in the pH. Similarly, from pH 7 to 12 there was slight decrease in the pH.

**Figure 2.23.** Effect of pH on nano-aggregates of N7 (17 µM) in aqueous system (λ<sub>ex</sub>=335 nm).
2.2.2.3. Recognition studies of receptor 8

A 0.5 μM concentration of receptor 8 in DMSO exhibited the characteristic monomer peaks ranging from 381 to 439 nm. Whereas the nano-aggregates of N8 (1 μM) showed broad, featureless emission centred at 435-544 nm (Figure 2.24). The formation of broad band at 450-496 nm is due the ground state excimer emission of pyrene in N8 after the formation of nano-aggregates in aqueous medium. DLS studies revealed that nano-aggregates of receptor N8 have size 40 nm at 5 μM concentration.

Figure 2.24. Fluorescence emission spectra of receptor 8 in DMSO (0.5 μM) and of N8 in aqueous medium (1 μM) (λex = 321 nm).
The metal recognition properties of nano-aggregates of N8 were inspected by changes in its fluorescence intensity in the presence of various metal ions viz., Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, Cr³⁺, Mn²⁺, Fe³⁺, Co²⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺ (as their nitrate salts). Kinetic effect on receptor behaviour was ruled out by allowing the solutions to stand for one hour and subsequently recording spectra. Results of investigations demonstrate that upon addition of different metal ions (5 eq.) in aqueous solution of nano-aggregates of N8 display no selectivity for any metal ion except Hg²⁺. However, addition of Hg²⁺ in aqueous solution of nano-aggregates of N8 fashioned a recognizable change in emission profile resulting in quenching of monomer and excimer emission of N8 (Figure 2.25).

![Figure 2.25](image-url)

**Figure 2.25.** Changes in fluorescence intensity of nano-aggregates of N8 (1 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λ<sub>ex</sub> = 321 nm).

Anion binding ability of nano-aggregates of N8 was investigated by changes in its fluorescence intensity in the presence of a library of 9 tetrabutyl ammonium anions (F⁻, Cl⁻, Br⁻, I⁻, CN⁻, CH₃COO⁻, HSO₄⁻, PO₄³⁻, NO₃⁻ and ClO₄⁻). The anion binding tests of nano-aggregates of N8 was done by addition of 5eq. of tetrabutyl ammonium anions to the fixed concentration (1 µM) of nano-aggregates of N8 at excitation wavelength of 321 nm. Fluorescence spectra were recorded for each solution after proper shaking and keeping each solution for sufficient time. The anion binding tests of nano-aggregates of N8 with variety of anions have not shown any significant changes in emission spectra (Figure 2.26).
Further, recognition studies of nano-aggregates of N8 were also performed. The addition of Hg\(^{2+}\) to the host solution (1 µM) results in a quenching of excimer emission with the blue shift of \(\Delta \lambda_{\text{max}} = 8\) nm (Figure 2.27). To gain more insights about the binding behaviour of nano-aggregates of N8 and Hg\(^{2+}\) ions, titration was performed in which molar ratio of Hg\(^{2+}\) ion were changed as compare to the fixed concentration of host solution (1 µM). Figure 2.27 shows continues decline of the fluorescence intensity of nano-aggregates of N8 with the successive addition of Hg\(^{2+}\) (0-4.4 µM) to a 1 µM solution of N8 in aqueous media. Titration has shown a good linearity in a range of 0-4.4 µM concentration of Hg\(^{2+}\) with a detection limit of 2.8 µM (Inset of Figure 2.27). To explore the possibility of using nano-aggregates of N8 as a practical ion selective fluorescent chemosensor for Hg\(^{2+}\) ion, competition experiments were carried out. Fluorescence emission spectroscopy was used to monitor the competitive events. For this purpose, the nano-aggregates of N8 was first mixed with 10 equivalents of a different metal ion Li\(^{+}\), Na\(^{+}\), K\(^{+}\), Cs\(^{+}\), Mg\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\), Ba\(^{2+}\), Al\(^{3+}\), Cr\(^{3+}\), Mn\(^{2+}\), Fe\(^{3+}\), Co\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Ag\(^{+}\), Cd\(^{2+}\) and Pb\(^{2+}\) (as their nitrate salts), followed by the addition of 5 equiv of Hg\(^{2+}\) ion. As can be seen from Figure 2.28, the presence of background metal ions has no effect on the excimer and monomer emission response of the nano-aggregates of N8 to the Hg\(^{2+}\) ion. Therefore, nano-aggregates of N8 shown to be a promising selective fluorescence sensor for Hg\(^{2+}\) in the presence of most competing metal ions.
Figure 2.27. Changes in emission profile of nano-aggregates of N8 (1 µM) upon successive addition of Hg²⁺ (0-4.4 µM) ($\lambda_{ex}$ = 321 nm); (Inset: Linear regression graph between concentration of mercury ion added and decrease in excimer emission of N8 ($\lambda_{ex}$ = 321 nm).

Figure 2.28. Competitive binding of nano-aggregates of N8 (1 µM) containing of Hg²⁺ over other selected metal ions at $\lambda_{ex}$ = 321 nm. 1) Hg²⁺ only; 2) Hg²⁺ + Li⁺; 3) Hg²⁺ + Na⁺; 4) Hg²⁺ + K⁺; 5) Hg²⁺ + Cs⁺; 6) Hg²⁺ + Mg²⁺; 7) Hg²⁺ + Ca²⁺; 8) Hg²⁺ + Sr²⁺; 9) Hg²⁺ + Ba²⁺; 10) Hg²⁺ + Al³⁺; 11) Hg²⁺ + Cr³⁺; 12) Hg²⁺ + Mn²⁺; 13) Hg²⁺ + Fe³⁺; 14) Hg²⁺ + Co²⁺; 15) Hg²⁺ + Cu²⁺; 16) Hg²⁺ + Zn²⁺; 17) Hg²⁺ + Ag⁺; 18) Hg²⁺ + Cd²⁺; 19) Hg²⁺ + Pb²⁺.

In addition to selectivity of nano-aggregates of N8 against other cations, for practical applications, the proper pH condition of the nano-aggregates of N8 was evaluated, as pH value is of great importance for the detection procedure. pH was varied by using dilute...
solutions of terabutyl ammonium hydroxide and hydrochloric acid. In the present study, effect of the pH value of the nano-aggregates of N8 on the emission intensity was studied in presence and absence of Hg$^{2+}$ ion and the results are shown in Figure 6. In both acidic (pH<7) and basic conditions (pH>7), there is slight decrease in the fluorescence intensity of nano-aggregates of N8 (Figure 2.29).

![Figure 2.29](image)

**Figure 2.29.** Effect of pH on nano-aggregates of N8 (1 µM) in aqueous system ($\lambda_{\text{ex}}=321$ nm).

Besides high selectivity, response time is one of the necessary criteria to check the real time applicability of a fluorescent chemosensor to determine Hg$^{2+}$. Sensor response time is independent of the concentration of guest (Figure 2.30).

![Figure 2.30](image)

**Figure 2.30.** Response time of receptor N8 for Hg$^{2+}$ ion at $\lambda_{\text{ex}}=321$ nm.
Fluorescence emission spectra of nano-aggregates of N8 remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of nano-aggregates of N8 with the increased number of ions in the solution (Figure 2.31).

**Figure 2.31.** Salt perturbation studies of nano-aggregates of N8 recorded with 1 µM concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of sensor and solvent system at λ<sub>ex</sub> = 321 nm.

2.2.2.2.4. Recognition studies of receptor 9

**Graphical abstract 2.3.** Cartoon representation showing interactions of N9 with Hg<sup>2+</sup> and I−.
Effect of water content on the photophysical properties for receptor 9 were explored by recording fluorescence emission spectra of receptor 9 in both DMF as well as aqueous system (by synthesizing nano-aggregates). The fluorescence spectra of receptor 9 in organic solvent system showed a evident difference in the emission profile than nano-aggregates of N9 in aqueous system (Figure 2.32). The fluorescence spectra of receptor 9 in organic solvent showed monomer peaks ranging from (381-399 nm). Whereas the nano-aggregates of N9 showed broad, featureless emission centred at 440-518 nm. The formation of broad band at 440-518 nm is due the ground state excimer emission of pyrene in N9 after the formation of nano-aggregates in aqueous medium. DLS studies revealed that nanoaggregates of receptor N9 have size 13 nm at 5 μM concentration. The metal recognition properties of nanoaggregates of N9 were inspected by changes in its fluorescence intensity in the presence of various metal ions viz., Li\(^+\), Na\(^+\), K\(^+\), Cs\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\), Ba\(^{2+}\), Al\(^{3+}\), Cr\(^{3+}\), Mn\(^{2+}\), Fe\(^{3+}\), Co\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Ag\(^+\), Cd\(^{2+}\), Hg\(^{2+}\), and Pb\(^{2+}\) (as their nitrate salts). Kinetic effect on receptors behaviour was ruled out by allowing the solutions to stand for one hour and subsequently recording spectra. Results of investigations demonstrate that upon addition of different metal ions (5 eq.) in aqueous solution of nano-aggregates N9 display no selectivity for any metal ion except Hg\(^{2+}\). However, addition of Hg\(^{2+}\) in aqueous solution of nano--aggregates N9 fashioned a recognizable change in emission profile resulting in enhancement of monomer emission of N9 with no effect on the excimer emission (Figure 2.33).

![Figure 2.32. Fluorescence emission spectra of receptor 9 in DMF (2 μM) and aqueous medium (6 μM) (λ\(_{\text{ex}}\) = 343 nm).](image-url)
Figure 2.33. Changes in emission profile of nano-aggregates of N9 (6 μM) in aqueous medium upon addition of 30 μM of a particular metal nitrate salts (λ<sub>ex</sub> = 343 nm).

To evaluate the anion binding ability of nano-aggregates of N9, initial screening was carried out with a library of 10 tetrabutyl ammonium anions (F<sup>-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, CN<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, HSO<sub>4</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, NO<sub>3</sub><sup>-</sup> and ClO<sub>4</sub><sup>-</sup>). The anion binding tests of nano-aggregates of N9 was done by addition of 5eq. of tetrabutyl ammonium anions to the fixed concentration (6 μM) of nano-aggregates of N9 at excitation wavelength of 343 nm. Fluorescence spectra were recorded for each solution after proper shaking and keeping each solution for sufficient time. The anion binding tests of nano-aggregates of N9 with variety of anions have not shown any significant changes in emission spectra (Figure 2.34).

Figure 2.34. Changes in emission profile of nano-aggregates of N9 (6 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media (λ<sub>ex</sub> = 343 nm).
Further, to gain more insight about the binding behaviour of nano-aggregates of \textbf{N9} with Hg$^{2+}$ ions, titration was performed in which molar ratio of Hg$^{2+}$ ion were changed as compare to the fixed concentration of host solution (6 \textmu M). Figure 2.35 showed continues enhancement of the fluorescence intensity of nano-aggregates of \textbf{N9} with the successive addition of Hg$^{2+}$ (0-300 nM) to a 6 \textmu M solution of nano-aggregates of \textbf{N9} in aqueous media. Titration has showed a good linearity in concentration range of 0-300 nM of Hg$^{2+}$ with a detection limit of 21 nM (inset of Figure 2.35). Further, in order to explore the possibilities of using nano-aggregates of \textbf{N9} as a practical ion selective fluorescent chemosensor for Hg$^{2+}$ ion, competition experiments were carried out. Fluorescence emission spectroscopy was used to monitor the competitive events. For this purpose, the nano-aggregates of \textbf{N9} was first mixed with 10 equivalents of a different metal ion Li$^+$, Na$^+$, K$^+$, Cs$^+$, Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$, Ba$^{2+}$, Al$^{3+}$, Cr$^{3+}$, Mn$^{2+}$, Fe$^{3+}$, Co$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Ag$^+$, Cd$^{2+}$ and Pb$^{2+}$ (as their nitrate salts), followed by the addition of 5 equiv of Hg$^{2+}$ ion. Presence of background metal ions has no effect on the excimer and monomer emission response of the nanoaggregate of \textbf{N9} to the Hg$^{2+}$ ion (Figure 2.36).

**Figure 2.35.** Changes in emission profile of nano-aggregates of \textbf{N9} (6 \textmu M) upon successive addition of Hg$^{2+}$ (0-300 nM) at $\lambda_{ex} = 343$ nm; (Inset: Linear regression graph between concentration of Hg$^{2+}$ added and increase in monomer emission of \textbf{N9} ($\lambda_{ex} = 343$ nm)).

In addition to selectivity of nano-aggregates of \textbf{N9} against other cations, for practical applications, the proper pH condition of the nano-aggregates of \textbf{N9} was evaluated, as pH value is of great importance for the detection procedure. For nano-aggregates of \textbf{N9}, in acidic
(pH < 7) conditions, at pH 6.1, there is a sudden drop in the fluorescence intensity of a nano-aggregates of N9 after 5.7 pH almost remain constant and in basic conditions (pH > 7), the fluorescence intensity decreased slightly (Figure 2.37).

**Figure 2.36.** Competitive binding of nano-aggregates of N9 (6 µM) containing of Hg²⁺ over other selected metal ions at λ<sub>ex</sub> = 343 nm. 1) Hg²⁺ only; 2) Hg²⁺ + Li⁺; 3) Hg²⁺ + Na⁺; 4) Hg²⁺ + K⁺; 5) Hg²⁺ + Cs⁺; 6) Hg²⁺ + Mg²⁺; 7) Hg²⁺ + Ca²⁺; 8) Hg²⁺ + Sr²⁺; 9) Hg²⁺ + Ba²⁺; 10) Hg²⁺ + Al³⁺; 11) Hg²⁺ + Cr³⁺; 12) Hg²⁺ + Mn²⁺; 13) Hg²⁺ + Fe³⁺; 14) Hg²⁺ + Co²⁺; 15) Hg²⁺ + Cu²⁺; 16) Hg²⁺ + Zn²⁺; 17) Hg²⁺ + Ag⁺; 18) Hg²⁺ + Cd²⁺; 19) Hg²⁺ + Pb²⁺.

Further, response of nano-aggregates of N9 for Hg²⁺ was also studied as function of time by monitoring the changes in the fluorescence spectra. To study the response time, fluorescence emission spectra of nano-aggregates of N9 was studied by varying the concentration of Hg²⁺ in the host solution. Experiment was performed by taking the host solution of N9 in 4 different volumetric flasks and added different concentration of Hg²⁺ in each flask. Thereafter, fluorescence spectra of each sample were taken after fixed interval of times. It was found that the change in the concentration of Hg²⁺ has no effect on the time required to reach equilibrium i.e. sensor response time is independent of the concentration of guest (Figure 2.38). Besides having high selectivity, response time is the necessary criteria to check the real time applicability of fluorescent chemosensor N9 to determine Hg²⁺. Response time of nano-aggregates of N9 was studied by varying the concentration of mercury ion in the host.
Figure 2.37. Effect of pH on nano-aggregates of N9 (6 µM) in aqueous system ($\lambda_{ex}$=343 nm).

Figure 2.38. Response Time of nano-aggregates of N9 for Hg$^{2+}$ ion at $\lambda_{ex}$ = 343 nm.

To evaluate the effect of ionic strength, solution of nano-aggregates of N9 (0.5 µM) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a solution of N13 and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of nano-aggregates of N9 remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of N9 with the increased number of ions in the solution (Figure 2.39).
Figure 2.39. Salt perturbation studies of nano-aggregates of N9 recorded with 6 µM concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of sensor and solvent system at $\lambda_{ex} = 343$ nm.

Further the assembly of nano-aggregates of N9 and Hg$^{2+}$ (N9.Hg$^{2+}$) was used to perform anion binding studies. The anion studies are performed by the similar methods as studies of anion recognition of nano-aggregates of N9. Fluorescence spectrum of assembly of nano-aggregates of N9 and Hg$^{2+}$ (N9.Hg$^{2+}$) (6 µM) upon addition of selected tetrabutyl ammonium salts of anions (F$^-$, Cl$^-$, Br$^-$, I$^-$, CN$^-$, CH$_3$COO$^-$, HSO$_4^-$, PO$_4^{3-}$, NO$_3^-$ and ClO$_4^-$) (5 eq.) were studied. The data showed presence of anions has no influence on the magnitude of the fluorescence intensity of assembly of nano-aggregates of N9 and Hg$^{2+}$ (N9.Hg$^{2+}$) except iodide (Figure 2.40).

Figure 2.40. Changes in emission profile of assembly of nano-aggregates of N9 and Hg$^{2+}$ (N9.Hg$^{2+}$) (6 µM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media ($\lambda_{ex} = 343$ nm).
Titrations were performed by increasing the amount of iodide in assembly of nano-aggregates of N9 and Hg^{2+} (N9.Hg^{2+}) (6 µM). With the increase in the concentration of iodide from 0 to 4.5 µM, result in a decrease in the emission of excimer and monomer. As the fluorescence spectrum was recorded within 20 seconds after iodide anion addition, and the intensity does not change with time, so the monitoring system is virtually real time stable. Fluorescence intensity is showing a good linear response with increasing concentration of iodide (Figure 2.41). Titration has shown good linearity in a range of 0-3.5 µM concentration of N9.Hg^{2+} with a detection limit of 0.2 nM (inset of Figure 2.41).

![Figure 2.41](image.png)

**Figure 2.41.** Changes in emission profile of assembly of nano-aggregates of N9 and Hg^{2+} (N9.Hg^{2+}) (6 µM) upon successive addition of iodide (0-44 µM) at λ_{ex} = 343 nm; (Inset: Linear regression graph between concentration of assembly of nano-aggregates of N9 and Hg^{2+} (N9.Hg^{2+}) and iodide added and decrease in fluorescence intensity of N9.Hg^{2+} (λ_{ex} = 343 nm)).

In order to check the selectivity of assembly of nano-aggregates of N9 and Hg^{2+} (N9.Hg^{2+}) towards I in the presence of other anions, competitive studies were carried out by fluorescence spectroscopy. The emission profile of the N9.Hg^{2+} complex was unperturbed in the presence of other anions, such as F, Cl, Br, CN, CH3COO, HSO4, and PO4^3-. These results show that assembly of nano-aggregates of N9 and Hg^{2+} (N9.Hg^{2+}) is highly selective fluorescent sensor for I (Figure 2.42).

The effect of pH on the fluorescence emission profile of nano-aggregates of N9 was
investigated. The experiments were carried out at a pH range from 3.0 to 12.0, with a concentration of N9 fixed at 20 µM (Figure 2.43). Fluorescence spectra were recorded at various pH values to study the effect of pH on the fluorescence spectra of host solution. No change in the emission spectra of assembly of nano-aggregates of N9 and Hg\(^{2+}\) (N9.Hg\(^{2+}\)) was observed in the pH range from 4.5-8.5. Response time of sensor is independent to the concentration of iodide (Figure 2.44).

**Figure 2.42.** Competitive binding of assembly of nano-aggregates of N9 and Hg\(^{2+}\) (N9.Hg\(^{2+}\)) containing iodide with selected metal ions at \(\lambda_{\text{ex}} = 343\) nm. 1) I; 2) I + F; 3) I + Cl; 4) I + Br; 5) I + CN; 6) I + CH\(_3\)COO; 7) I+ NO\(_3\); 8) I+ HSO\(_4\) and 9) I + PO\(_4^{3-}\).

**Figure 2.43.** Effect of pH on assembly of nano-aggregates of N9 and Hg\(^{2+}\) (N9.Hg\(^{2+}\)) (6 µM) in aqueous system (\(\lambda_{\text{ex}}=343\) nm).
2.2.2.2.5. Recognition studies of receptor 10

Figure 2.44. Response Time of assembly of nano-aggregates of \textbf{N9} and Hg$^{2+}$ (N9.Hg$^{2+}$) for I$^{-}$ ion at $\lambda_{ex} = 343$ nm.

**Graphical abstract 2.4.** Cartoon representation showing interactions of nano-aggregates of N10 with Cl$^{-}$.

A 0.1 $\mu$M concentration of receptor 10 in THF exhibited a very weak fluorescence emission band centred at $\lambda_{max} = 352$ nm. However, the same concentrations of receptor 10 in aqueous medium (by developing nano-aggregates of receptor 10) exhibited the two peaks one is 372 nm and another is at 324 nm. Also enhancement in fluorescence emission of 10 in aqueous media was observed as compared to the fluorescence spectra of the receptor in THF (Figure
2.45). Enhancement in fluorescence spectra of the receptor 10 in aqueous system can supported the aggregation induced emission due to the formation of J-type aggregation as a result molecules are arranged in head to tail direction and responsible for high fluorescence efficiency. DLS studies revealed that nanoaggregates of receptor N10 have size 30 nm at 0.1 μM concentration.

The nano-aggregates of N10 (0.1 μM) has been checked for its metal binding affinity from the changes in its fluorescence signature of upon addition of 5 eq. of different metal nitrates like Li+, Na+, K+, Cs+, Mg2+, Ca2+, Sr2+, Ba2+, Al3+, Cr3+, Mn2+, Fe2+, Co3+, Cu2+, Zn2+, Ag+, Cd2+, Hg2+ and Pb2+ in aqueous medium (Figure 2.46). No considerable change in the fluorescence signature of nano-aggregates of N10 was observed in the presence of any of these tested metal ions. Therefore, nano-aggregates of N10 have no binding affinity towards any of the tested metal ions.

![Fluorescence emission spectra of receptor 10 in THF and N10 in aqueous medium (0.1 μM) (λex = 287 nm).](image)

**Figure 2.45.** Fluorescence emission spectra of receptor 10 in THF and N10 in aqueous medium (0.1 μM) (λex = 287 nm).

To influence of the anions binding on the nano-aggregates of N10 in aqueous medium was investigated. Fluorescence response was observed after the addition of the tetrabutyl ammonium salt of different anions (5 eq.) (F−, Cl−, Br−, I−, CN−, CH3COO−, HSO4−, PO43−, NO3− and ClO4−) to the aqueous solution of nano-aggregates of N10. The anion binding tests of nano-aggregates of N10 with variety of anions have not shown any significant changes in emission spectra (Figure 2.47). However with the addition of 5 eq. of chloride ion there was enhancement in the fluorescence emission band of nano-aggregates of N10.
Figure 2.46. Changes in fluorescence intensity of nano-aggregates of N10 (0.1 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium ($\lambda_{ex} = 287$ nm).

Figure 2.47. Changes in emission profile of nano-aggregates of N10 (0.1 µM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media ($\lambda_{ex} = 287$ nm).

To gain more insights into the sensor activities of nano-aggregates of N10 for Cl⁻ ion, titration was performed by taking a fixed concentration of N10 and successive addition of Cl⁻ to the solution of N10. With the increase in the concentration of Cl⁻, there was a continuous increase in the intensity of N10 (Figure 2.48). Concentration of Cl⁻ ion was varied from 0 µM to 54 µM and titrations showed a good linearity in this concentration range of Cl⁻ ion (inset of Figure 2.48). Limit of detection is estimated to be 0.04 nM (3σ method). To check the
selectivity of the sensor, competitive binding test was performed. To perform this study host solution was taken in different 10 volumetric flasks and then added the 50 µM of Cl⁻ ion solution to each flask. Then the addition of remaining 9 tetrabutyl ammonium anions (F⁻, Br⁻, I⁻, CN⁻, CH₃COO⁻, HSO₄⁻, NO₃⁻, PO₄³⁻ and ClO₄⁻) to the 9 volumetric flasks was done followed by recording fluorescence spectra for each solution after shaking the solutions properly at 300 nm. Comparison of fluorescence spectra of host + Cl⁻ alone and of host + Cl⁻ in the presence of other anions showed that there is no interference from the other anions and sensor is highly selective for Cl⁻ (Figure 2.49).

![Graph showing fluorescence intensity against wavelength with concentration of chloride added from 0 µM to 0.4 µM](image)

**Figure 2.48.** Changes in emission profile of nano-aggregates of N10 (0.1 µM) upon successive addition of chloride (0-0.4 µM) (λ<sub>ex</sub> = 287 nm); (Inset: Linear regression graph between concentration of chloride added and increase in fluorescence intensity of N10 (λ<sub>ex</sub> = 287 nm)).

To check utility of nano-aggregates of N10 as a sensor, the emission spectra response of nano-aggregates of N10 at different pH values was monitored. The experiments were carried out at a pH range from 3.0 to 12.0, with a concentration of N10 fixed at 0.1 µM (Figure 2.50). Both acidic and basic titrations were conducted by changing pH of host solution using sodium hydroxide and hydrochloric acid and then fluorescence spectra was recorded at various pH values to study the effect of pH on the fluorescence spectra of host solution. For nano-aggregates of N10, in both acidic (pH< 7) and basic conditions (pH>7), the pH has no or little effect on the emission spectra of nano-aggregates of N10.
Figure 2.49. Competitive binding studies of nano-aggregates of N10 containing chloride over other selected tetrabutyl ammonium anions at $\lambda_{ex} = 287$ nm. 1) Chloride; 2) Chloride + Fluoride; 3) Chloride + Bromide; 4) Chloride + Iodide; 5) Chloride + Cyanide; 6) Chloride + Acetate; 7) Chloride + Sulphate; 8) Chloride + Phosphate; 9) Chloride + Nitrate and 10) Chloride + Perchlorate.

Figure 2.50. Effect of pH on nano-aggregates of N10 (0.1 µM) in aqueous system ($\lambda_{ex} = 287$ nm).

Further, response of nano-aggregates of N10 for Cl$^-$ was also studied as function of time by monitoring the changes in the fluorescence spectra. To study the response time, fluorescence emission spectra of nano-aggregates of N10 was studied by varying the concentration of Cl$^-$ in the host solution. Experiment was performed by taking the host solution of N10 in 3 different volumetric flasks and added different concentration of Cl$^-$ in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. The response time of the chemosensor to Cl$^-$ ion is concentration-independent, as the time
required reaching equilibrium unaltered with the concentration of Cl\textsuperscript{-}. In all cases, the stable reading could be obtained within 80 seconds. Therefore, this chemosensor could be used for real time monitoring of Cl\textsuperscript{-} (Figure 2.51).

![Figure 2.51](image_url)

**Figure 2.51.** Response time of nano-aggregates of N10 for Chloride ion at \( \lambda_{ex} = 287 \) nm

To evaluate the effect of ionic strength, solution of sensors N10 (0.1 µM) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a solution of N10 and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of N10 remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of N10 with the increased number of ions in the solution (Figure 2.52).

![Figure 2.52](image_url)

**Figure 2.52.** Salt perturbation studies of nano-aggregates of N10 recorded with 0.1 µM concentration of sensor in aqueous system with the respective fluorescence spectrum
recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of sensor and solvent system at $\lambda_{ex} = 287$ nm.

### 2.2.2.6. Recognition studies of receptor 11

**Graphical abstract 2.5.** Cartoon representation showing interactions of nano-aggregates of N11 with CN$^-$.

Effect of water content on the photophysical properties for receptor 11 were explored by recording fluorescence emission spectra of receptor 11 in both DMF (0.5 μM) as well as aqueous system (0.5 μM) (by synthesizing nano-aggregates of receptor 15). The fluorescence spectra of receptor 11 in organic solvent system showed a manifest difference in the emission profile than nano-aggregates of N11 in aqueous system (Figure 2.53). The fluorescence spectra of receptor 11 in organic solvent showed two peaks, one is at 319 nm and the second is broad emission band centred at 448 nm. Whereas in water the emission profile N11 have a new band centred at 365 nm with the blue shifting of peak at 465 nm. DLS studies revealed that nanoaggregates of receptor N11 have size 25 nm at 0.5 μM concentration. To evaluate the metal binding ability of nano-aggregates of N11, initial screening was carried out with a library of 19 metal salts. A solution of nano-aggregates of N11 (0.5 μM) was mixed with aliquots of metal salt solution and the respective emission spectra were measured ($\lambda_{ex}$=287 nm). To exclude, any kinetic effect, which may influence the fluorescence spectra, the solutions were kept for 60 minutes and fluorescence spectra were recorded again. Upon addition of an excess of 5 equivalents of various metal ions including Li$^+$, Na$^+$, K$^+$, Cs$^+$, Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$, Ba$^{2+}$, Al$^{3+}$, Cr$^{3+}$, Mn$^{2+}$, Fe$^{2+}$, Co$^{3+}$, Cu$^{2+}$, Zn$^{2+}$, Ag$^+$, Cd$^{2+}$, Hg$^{2+}$ and Pb$^{2+}$ (as their nitrate salts), No such significant change in the fluorescence intensity of N11 was
observed with the addition of any other tested metal ions under the same conditions (Figure 2.54).

![Fluorescence emission spectra of receptor 11 in THF and N11 in aqueous medium (0.5 µM) (λ\textsubscript{ex} = 287 nm).](image)

**Figure 2.53.** Fluorescence emission spectra of receptor 11 in THF and N11 in aqueous medium (0.5 µM) (λ\textsubscript{ex} = 287 nm).

![Changes in fluorescence intensity of nano-aggregates of N11 (0.5 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λ\textsubscript{ex} = 287 nm).](image)

**Figure 2.54.** Changes in fluorescence intensity of nano-aggregates of N11 (0.5 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λ\textsubscript{ex} = 287 nm).

To evaluate the anion binding ability of nano-aggregates of N11, initial screening was carried out with a library of 10 tetrabutyl ammonium anions (F\textsuperscript{−}, Cl\textsuperscript{−}, Br\textsuperscript{−}, I\textsuperscript{−}, CN\textsuperscript{−}, CH\textsubscript{3}COO\textsuperscript{−},...
HSO$_4^-$, PO$_4^{3-}$, NO$_3^-$ and ClO$_4^-$). The anion binding tests of nano-aggregates of **N11** was done by addition of 5eq. of tetrabutyl ammonium anions (5 mM) to the fixed concentration (5 μM) of nano-aggregates of **N11** at excitation wavelength of 287 nm. Fluorescence spectrums were recorded for each solution after proper shaking and keeping each solution for sufficient time. The anion binding tests of nano-aggregates of **N11** with variety of anions have not shown any significant changes in emission spectra except for cyanide anion (Figure 2.55). With the addition of 5 eq. of cyanide anion there was enhancement in the fluorescence emission band of **N11**. To gain more insights into the sensor activities of nano-aggregates of **N11** and CN$^-$ ion, titration was performed by taking a fixed concentration of **N11** and successive addition of CN$^-$ to the solution of **N11**. With the increase in the concentration of CN$^-$, there was a continuous increase in the intensity of **N11** (Figure 2.56). Concentration of CN$^-$ ion was varied from 0 μM to 2.5 μM and titrations showed a good linearity in the concentration range of 0 μM to 2.5 μM (inset of Figure 2.56). Limit of detection is estimated to be 38 nM (3σ method). Competitive experiments were performed for the estimation of CN$^-$ (2 eq.) by nano-aggregates of **N11** in the presence of any of 2eq. of F$^-$, Cl$^-$, Br$^-$, I$^-$, CH$_3$COO$^-$, HSO$_4^-$, NO$_3^-$, PO$_4^{3-}$ and ClO$_4^-$. As shown in Figure 2.57, no significant variation in the intensity was detected by comparing the intensity with and without other metal ions. Therefore, nano-aggregates of **N11** have a high selectivity for estimation of CN$^-$, even in the presence of other anions.

**Figure 2.55.** Changes in emission profile of nano-aggregates of **N11** (0.5 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media ($\lambda_{ex} = 287$ nm).
Figure 2.56. Changes in emission profile of nano-aggregates of N11 (0.5 μM) in aqueous medium upon successive addition of cyanide (0-2.5 μM) ($\lambda_{ex} = 287$ nm); (Inset: Linear regression graph between concentration of cyanide added and increase in fluorescence intensity of N11 ($\lambda_{ex} = 287$ nm)).

Figure 2.57. Competitive binding studies of nano-aggregates of N11 (0.5 μM) containing cyanide over other selected tetrabutyl ammonium anions at $\lambda_{ex} = 287$ nm. 1) Cyanide; 2) Cyanide + Fluoride; 3) Cyanide + Chloride; 4) Cyanide + Bromide; 5) Cyanide + Iodide; 6) Cyanide + Acetate; 7) Cyanide + sulphate; 8) Cyanide + Phosphate; 8) Cyanide + Nitrate and 9) Cyanide+ Perchlorate.
Both acidic and basic titrations were conducted by changing pH of host solution using sodium hydroxide and hydrochloric acid and then fluorescence spectra was recorded at various pH values to study the effect of pH on the fluorescence spectra of host solution. For nano-aggregates of N11, the pH has no or little effect on the emission spectra of nano-aggregates of N11 (Figure 2.58). Further, response of nano-aggregates of N11 for CN\(^{-}\) was also studied as function of time by monitoring the changes in the fluorescence spectra. To study the response time, fluorescence emission spectra of nano-aggregates of N11 was studied by varying the concentration of CN\(^{-}\) in the host solution. Experiment was performed by taking the host solution of N11 in 3 different volumetric flasks and added different concentration of CN\(^{-}\) in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. The response time of the chemosensor to CN\(^{-}\) ion is concentration-independent, as the time required to reach equilibrium did not alter with the concentration of CN\(^{-}\) (as shown in Figure 2.59). However, in all cases, the stable reading could be obtained within 30 seconds. Therefore, this chemosensor could be used for real time monitoring of CN\(^{-}\).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.58.png}
\caption{Effect of pH on nano-aggregates of N11 (0.5 \(\mu\)M) in aqueous system (\(\lambda_{\text{ex}} = 287\) nm).}
\end{figure}

To evaluate the effect of ionic strength, solution of nano-aggregates of N11 (5 \(\mu\)M) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a solution of N11 and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of N11 remain almost undisturbed in the presence of increased ionic strength showing
negligible interaction of nano-aggregates of N11 with the increased number of ions in the solution (Figure 2.60).

**Figure 2.59.** Response time of nano-aggregates of N11 for cyanide ion at $\lambda_{ex} = 287$ nm.

**Figure 2.60.** Salt perturbation studies of nano-aggregates of N11 recorded with 0.5 µM concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium nitrate under the same concentration of sensor and solvent system at $\lambda_{ex} = 287$ nm.
2.2.2.7. Recognition studies of receptor 12

Effect of water content on the photophysical properties for receptor 12 (20 μM) were evaluated by recording fluorescence spectra of receptor 12 in both DMF as well as aqueous system (by developing nano-aggregates of receptor 12). The fluorescence spectra of receptor 12 in organic solvent system showed a significant difference in the emission profile than nano-aggregates of N12 in aqueous system (Figure 2.61). Increased water content resulted in formation of aggregates, which induces decrease in the fluorescence intensity of 12 in aqueous medium. This is due to the phenomena known as “aggregation caused quenching” (ACQ). DLS studies revealed that nano-aggregates of receptor N12 have size 34 nm at 20 μM concentration.

![Fluorescence emission spectra of receptor 12 in DMF and N12 in aqueous medium (20 μM) (λ_exc = 287 nm).](image)

**Figure 2.61.** Fluorescence emission spectra of receptor 12 in DMF and N12 in aqueous medium (20 μM) (λ_exc = 287 nm).

Metal binding studies of nano-aggregates of N12 were performed in aqueous medium by addition of 5 eq. of different metal nitrate salts (1 mM) (such as Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, Cr³⁺, Mn²⁺, Fe²⁺, Co³⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺) to the fixed concentration (20 μM) of nano-aggregates of N12 at excitation wavelength of 287 nm. The solutions are shaken properly followed by recording the spectra for solution of each flask. The addition of different metal ions to the host did not affect the spectra of nano-aggregates of N12 (Figure 2.62). To evaluate the anion binding ability of nano-aggregates of N12, initial screening was carried out with a library of 10 tetrabutyl ammonium anions (F⁻, Cl⁻, Br⁻, I⁻, CN⁻, CH₃COO⁻, HSO₄⁻, PO₄³⁻, NO₃⁻ and ClO₄⁻). The anion binding tests of nano-
aggregates of N12 was done by addition of 5 eq. of tetrabutyl ammonium anions (5 mM) to the fixed concentration (20 µM) of nano-aggregates of N12 at excitation wavelength of 287 nm. Fluorescence spectra were recorded for each solution after proper shaking and keeping each solution for sufficient time. The anion binding tests of nano-aggregates of N12 with variety of anions have not shown any significant changes in emission spectra (Figure 2.63).

**Figure 2.62.** Changes in fluorescence intensity of nano-aggregates of N12 (20 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λ<sub>ex</sub> = 287 nm).

**Figure 2.63.** Changes in emission profile of nano-aggregates of N12 (20 µM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media (λ<sub>ex</sub> = 287 nm).

The effect of pH on the fluorescence emission profile of nano-aggregates of N12 was
investigated. The experiments were carried out at a pH range from 2.5 to 12.0, with a concentration of 3 fixed at 20 µM (Figure 2.64). Both acidic and basic titrations were conducted by changing pH of host solution using sodium hydroxide and hydrochloric acid and then fluorescence spectra was recorded at various pH values to study the effect of pH on the fluorescence spectra of host solution. No significant change in the emission spectra of nano-aggregates of N12 was observed in the pH range from 2.5 to 12.0.

**Figure 2.64.** Effect of pH on nano-aggregates of N12 (20 µM) in aqueous system (λ_{ex} = 287 nm).

### 2.2.2.2.8. Recognition studies of receptor 13

Effect of water content on the photophysical properties for receptor 13 (15 µM) were explored by recording fluorescence emission spectra of receptor 13 in both DMF as well as aqueous system (by synthesizing nano-aggregates of receptor 13). The fluorescence spectra of receptor 13 in organic solvent system showed a significant difference in the emission profile than nano-aggregates of N13 in aqueous system (Figure 2.65). DLS studies revealed that nanoaggregates of receptor N9 have size 35 nm at 15 µM concentration. Metal binding studies of nano-aggregates of N13 were performed in aqueous medium by addition of 5 eq. of different metal nitrate salts (1 mM) (such as Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, Cr³⁺, Mn²⁺, Fe²⁺, Co³⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺) to the fixed concentration (15 µM) of nano-aggregates of N13 at excitation wavelength of 287 nm. The solutions are shaken properly followed by recording the spectra for solution of each flask. The addition of different metal ions to the host did not affect the spectra of nano-aggregates of N13 (Figure 2.66).
Figure 2.65. Fluorescence emission spectra of receptor 13 in DMF and N13 in aqueous medium (15 μM) (λex = 287 nm).

To evaluate the anion binding ability of nano-aggregates of N13, initial screening was carried out with a library of 10 tetrabutyl ammonium anions (F\(^-\), Cl\(^-\), Br\(^-\), I\(^-\), CN\(^-\), CH\(_3\)COO\(^-\), HSO\(_4\)\(^-\), PO\(_4\)^3\(^-\), NO\(_3\)\(^-\) and ClO\(_4\)\(^-\)). The anion binding tests of nano-aggregates of N13 was done by addition of 5 eq. of tetrabutyl ammonium anions (5 mM) to the fixed concentration (15 μM) of nano-aggregates of N13 at excitation wavelength of 287 nm. Fluorescence spectrums were recorded for each solution after proper shaking and keeping each solution for sufficient time. The anion binding tests of nano-aggregates of N13 with variety of anions have not shown any significant changes in emission spectra (Figure 2.67).

Figure 2.66. Changes in fluorescence intensity of nano-aggregates of N13 (15 μM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λex = 287 nm).
Figure 2.67. Changes in emission profile of nano-aggregates of N13 (15 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media \((\lambda_{\text{ex}} = 287 \text{ nm})\). The effect of pH on the fluorescence emission profile of nano-aggregates of N13 was investigated at intensity of 350 nm and 473 nm. The experiments were carried out at a pH range from 3.0 to 12.0, with a concentration of nano-aggregates of N13 fixed at 15 μM (Figure 2.68). Both acidic and basic titrations were conducted by changing pH of host solution using sodium hydroxide and hydrochloric acid and then fluorescence spectra was recorded at various pH values to study the effect of pH on the fluorescence spectra of host solution and was found to be unstable with the change in pH.

Figure 2.68. Effect of pH on nano-aggregates of N13 (15 μM) in aqueous system \((\lambda_{\text{ex}} = 287 \text{ nm})\).
2.2.2.9. Recognition studies of receptor 14

Graphical abstract 2.6. Cartoon representation showing interactions of N14 with I−.

Effect of water content on the fluorescence profile of receptor 14 was evaluated by recording fluorescence spectra of receptor 14 in both DMF as well as aqueous system (by developing nano-aggregates). The fluorescence spectra of receptor 14 in aqueous medium was red shifted compared to the fluorescence spectra of receptor 14 in DMF (Figure 2.69). This is due to the formation of J-Type of aggregates where molecules are arranged in head to tail direction results in a bathochromic shift.16 DLS studies revealed that nano-aggregates of receptor N14 have size 10 nm at 1.2 μM concentration.

Figure 2.69. Fluorescence emission spectra of receptor 14 in DMF and N14 in aqueous medium (1.2 μM) (λ_{ex} = 403 nm).
The cation binding affinity of nano-aggregates of N14 was carried out by addition of 5 eq. of 19 metal nitrate salts (Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, Mn²⁺, Fe²⁺, Co³⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺) to the solution of N14 (1.2 μM) in aqueous medium (λₜₐₓ=403 nm). To exclude any kinetic effect, which may influence the fluorescence spectra, the solutions were kept for 60 minutes and fluorescence spectra were recorded again. The fluorescence profile of nano-aggregates of N14 was not perturbed by any of the tested metal ions (Figure 2.70). Therefore, nano-aggregates of N14 have no binding affinity towards any of the tested metal ions.

![Figure 2.70](image)

**Figure 2.70.** Changes in fluorescence intensity of nano-aggregates of N14 (1.2 μM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λₜₐₓ = 403 nm).

The anion binding abilities of nano-aggregates of N14 were assessed from the modulation of emission band of nano-aggregates of N14. Initial screening was carried out with a library of 10 tetrabutyl ammonium anions (F⁻, Cl⁻, Br⁻, I⁻, CN⁻, CH₃COO⁻, HSO₄⁻, PO₄³⁻, NO₃⁻ and ClO₄⁻). The anion binding tests of nano-aggregates of N14 was done by addition of 5eq. of tetrabutyl ammonium anions (5 mM) to the fixed concentration (1.2 μM) of nano-aggregates of N14 at excitation wavelength of 403 nm. Fluorescence spectras was recorded for each solution after proper shaking and keeping each solution for sufficient time. The anion binding tests of nano-aggregates of N14 with variety of anions have not shown any significant changes in emission spectra except for iodide anion (Figure 2.71). With the addition of 5 eq. of iodide ion there was enhancement in the fluorescence emission band of N14 centred at 470 nm.
Figure 2.71. Changes in emission profile of nano-aggregates of N14 (1.2 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media ($\lambda_{ex} = 403$ nm).

To gain more insights into the sensor activities of nano-aggregates of N14 and I\textsuperscript{-} ion, titration was performed by taking a fixed concentration of N14 and successive addition of I\textsuperscript{-} to the solution of N14. With the increase in the concentration of I\textsuperscript{-}, there was a continuous increase in the intensity of nano-aggregates of N14 (Figure 2.72). Concentration of I\textsuperscript{-} ion was varied from 0 μM to 5.8 μM and titrations showed a good linearity in this concentration range of I\textsuperscript{-} ion (inset of Figure 2.72). Limit of detection is estimated to be 0.06 μM (3σ method). To check the selectivity of the sensor, competitive binding test was performed. To perform this study host solution was taken in different 10 volumetric flasks and then added the 6 μM of I\textsuperscript{-} ion solution to each flask. Then the addition of remaining 9 tetrabutyl ammonium anions (F\textsuperscript{-}, Cl\textsuperscript{-}, Br\textsuperscript{-}, CN\textsuperscript{-}, CH\textsubscript{3}COO\textsuperscript{-}, HSO\textsubscript{4}\textsuperscript{-}, PO\textsubscript{4}\textsuperscript{3\textsuperscript{-}}, NO\textsubscript{3}\textsuperscript{-} and ClO\textsubscript{4}\textsuperscript{-}) (6 μM) to the 9 volumetric flasks was done followed by recording fluorescence spectra for each solution after shaking the solutions properly at 403 nm. Comparison of fluorescence spectra of host + I\textsuperscript{-} alone and of host + I\textsuperscript{-} in the presence of other anions showed that there is no interference from the other anions and sensor is highly selective for I\textsuperscript{-} (Figure 2.73).
Figure 2.72. Changes in emission profile of nano-aggregates of \textbf{N14} (1.2 \(\mu\text{M}\)) upon successive addition of iodide (0-5.8 \(\mu\text{M}\)) \((\lambda_{\text{ex}} = 403 \text{ nm})\); (inset: Linear regression graph between concentration of iodide added and increase in fluorescence intensity of \textbf{N14} \((\lambda_{\text{ex}} = 403 \text{ nm})\)).

![Graph showing changes in emission profile of nano-aggregates of N14 upon addition of iodide.](image)

\[ y = 98.186x + 459.09 \]
\[ R^2 = 0.9827 \]

Figure 2.73. Competitive binding studies of nano-aggregates of \textbf{N14} containing iodide over other selected tetrabutyl ammonium anions at \(\lambda_{\text{ex}} = 403 \text{ nm}\): 1) Iodide; 2) Iodide + Fluoride; 3) Iodide + Chloride; 4) Iodide + Bromide; 5) Iodide + Cyanide; 6) Iodide + Acetate; 7) Iodide + Hydrogen Sulphate; 8) Iodide + Phosphate and 9) Iodide + Nitrate and 10) Iodide + Perchlorate.

![Graph showing competitive binding studies of nano-aggregates of N14 containing iodide.](image)
In order to find a suitable pH span in which nano-aggregates of N14 can selectively detect I, acid/basic titrations was performed. In a pH range from 2.7 to 11.8, fluorescence intensity almost remains constant for nano-aggregates of N14 (1.2 µM) (Figure 2.74). In other words we can say that the nano-aggregates of N14 were insensitive to change in pH.

**Figure 2.74.** Effect of pH on nano-aggregates of N14 (1.2 µM) in aqueous system (λ<sub>ex</sub> = 403 nm).

Further, response of nano-aggregates of N14 for I was also studied as function of time by monitoring the changes in the fluorescence spectra. To study the response time, fluorescence emission spectra of nano-aggregates of N14 was studied by varying the concentration of I in the host solution. Experiment was performed by taking the host solution of nano-aggregates of N14 in 3 different volumetric flasks and added different concentration of I in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. The response time of the chemosensor to I ion is concentration-independent, as the time required to reach equilibrium is same irrespective of the concentration of I (as shown in Figure 2.75). However, in all cases, the stable reading could be obtained within 20 seconds. Therefore, this chemosensor could be used for real time monitoring of I. To evaluate the effect of ionic strength, solution of nano-aggregates of N14 (1.2 µM) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a solution of nano-aggregates of N14 and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of N14 remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of nano-aggregates of N14 with the increased number of ions in the solution (Figure 2.76).
Figure 2.75. Response time of nano-aggregates of N14 for Iodide ion at $\lambda_{ex} = 403$ nm.

Figure 2.76. Salt perturbation studies of nano-aggregates of N14 recorded with 1.2 µM concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of sensor and solvent system at $\lambda_{ex} = 403$ nm.
2.2.2.10. Recognition studies of receptor 15

When excited at 344 nm, a 10 μM concentration of receptor 15 in THF exhibited the emission band centred at $\lambda_{\text{max}} = 525$ nm. However, increased water content resulted in formation of aggregates, which induces decrease in the fluorescence intensity of 15 at 525 nm (Figure 2.77). This is due to the phenomena known as “aggregation caused quenching” (ACQ). DLS studies revealed that nanoaggregates of receptor N15 have size 26 nm at 10 μM concentration. The fluorescence behaviour of nano-aggregates of N15 in aqueous medium were investigated by addition of 5 equivalents of various metal ions including Li$^+$, Na$^+$, K$^+$, Cs$^+$, Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$, Ba$^{2+}$, Al$^{3+}$, Cr$^{3+}$, Mn$^{2+}$, Fe$^{2+}$, Co$^{3+}$, Cu$^{2+}$, Zn$^{2+}$, Ag$^+$, Cd$^{2+}$, Hg$^{2+}$ and Pb$^{2+}$ (as their nitrate salts) to the fixed concentration of N15 (5 μM). To exclude, any kinetic effect, which may influence the fluorescence spectra, the solutions were kept for 60 minutes and fluorescence spectra were recorded again. The emission profile of nano-aggregates of N15 was not perturbed by any of the tested metal ions (Figure 2.78). Therefore, nano-aggregates of N15 have no binding affinity towards any of the tested metal ions.

Graphical abstract 2.7. Cartoon representation showing interactions of N15 with CN$^-$. 
**Figure 2.77.** Fluorescence emission spectra of receptor 15 in THF and N15 in aqueous medium (10 μM) (λ<sub>ex</sub> = 344 nm).

**Figure 2.78.** Changes in fluorescence intensity of nano-aggregates of N15 (5 μM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λ<sub>ex</sub> = 344 nm).

To evaluate the anion binding ability of nano-aggregates of N15, initial screening was carried out with a library of 10 tetrabutyl ammonium anions (F<sup>-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, CN<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, HSO<sub>4</sub><sup>-</sup>, PO<sub>3</sub><sup>3-</sup>, NO<sub>3</sub><sup>-</sup> and ClO<sub>4</sub><sup>-</sup>). The anion binding tests of nano-aggregates of N15 were done by addition of 5 eq. of tetrabutyl ammonium anions (5 mM) to the fixed concentration (5 μM) of nano-aggregates of N15 at excitation wavelength of 344 nm. Fluorescence spectras was recorded for each solution after proper shaking and keeping each solution for sufficient time.
The anion binding tests of nano-aggregates of N15 with variety of anions have not shown any significant changes in emission spectra except for cyanide anion (Figure 2.79). With the addition of 5 eq. of cyanide anion there was enhancement in the fluorescence emission band of nano-aggregates of N15 centred at 441 nm.

![Figure 2.79](image-url)

**Figure 2.79.** Changes in emission profile of nano-aggregates of N15 (5 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media (λ<sub>ex</sub> = 344 nm).

To learn about the binding action of nano-aggregates of N15 towards CN<sup>-</sup> ion, titration experiment was carried out by adding small aliquots of CN<sup>-</sup> (0-4.5 μM) to the solution of N15 (5 μM) in aqueous medium. With the increase in the concentration of CN<sup>-</sup>, there was a continuous increase in the intensity of N15 (Figure 2.80). Concentration of CN<sup>-</sup> ion was varied from 0 μM to 4.5 μM and titrations showed a good linearity in the concentration range of 0 μM to 4.5 μM (inset of Figure 2.80). Limit of detection is estimated to be 59 nM (3σ method). In order to check whether the sensor could selectively monitor CN<sup>-</sup> ion in the presence of other competitive anions or not, the competitive experiments were carried out for the estimation of 5 equiv. of CN<sup>-</sup> in the presence of any one of the F<sup>-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, HSO<sub>4</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, NO<sub>3</sub><sup>-</sup> and ClO<sub>4</sub><sup>-</sup>). No significant change in the emission spectra was observed by comparing the spectra with and without other anions (Figure 2.81). Therefore, the receptor N15 could selectively monitor CN<sup>-</sup> even in the presence of other competitive metal ions.
Figure 2.80. Changes in emission profile of nano-aggregates of N15 (5 µM) upon successive addition of cyanide (0-4.5 µM) ($\lambda_{ex} = 344$ nm); (Inset: Linear regression graph between concentration of cyanide added and increase in fluorescence intensity of N15).

Figure 2.81. Competitive binding studies of nano-aggregates of N15 containing cyanide over other selected tetrabutyl ammonium anions at $\lambda_{ex} = 344$ nm. 1) Cyanide; 2) Cyanide + Fluoride; 3) Cyanide + Chloride; 4) Cyanide + Bromide; 5) Cyanide + Iodide; 6) Cyanide + Acetate; 7) Cyanide + sulphate; 8) Cyanide + Phosphate; 9) Cyanide+ Nitrate and 10) Cyanide+ Perchlorate.
To check utility of nano-aggregates of N15 as a sensor, the emission spectra response of nano-aggregates of N15 at different pH values was monitored. The experiments were carried out at a pH range from 2.0 to 10.5, with a concentration of N15 fixed at 5µM (Figure 2.82). Both acidic and basic titrations were conducted by changing pH of host solution using sodium hydroxide and hydrochloric acid and then fluorescence spectra was recorded at various pH values to study the effect of pH on the fluorescence spectra of host solution. For nano-aggregates of N15, in acidic (pH< 7) the pH has no effect on the emission spectra of nano-aggregates of N15. However In basic conditions from pH= 8 to 10.5, there was slight increase in the fluorescence intensity upto pH 8. After this fluorescence profile of N15 remains stable.

![Figure 2.82](image.png)

**Figure 2.82.** Effect of pH on nano-aggregates of N15 (5 µM) in aqueous system (λ<sub>ex</sub> = 344 nm).

Further, response of nano-aggregates of N15 for CN⁻ was also studied as function of time by monitoring the changes in the fluorescence spectra. To study the response time, fluorescence emission spectra of nano-aggregates of N15 was studied by varying the concentration of CN⁻ in the host solution. Experiment was performed by taking the host solution of N15 in 3 different volumetric flasks and added different concentration of CN⁻ in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. The response time of the chemosensor to CN⁻ ion is concentration-independent, as the time required to reach equilibrium did not alter with the concentration of CN⁻ (as shown in Figure 2.83). However, in all cases, the stable reading could be obtained within 40 seconds. Therefore, this chemosensor could be used for real time monitoring of CN⁻.
Figure 2.83. Response time of receptor N15 for cyanide ion at $\lambda_{ex} = 344$ nm.

To evaluate the effect of ionic strength, solution of nano-aggregates of N15 (5 µM) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a solution of N15 and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of N15 remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of nano-aggregates of N15 with the increased number of ions in the solution (Figure 2.84).

Figure 2.84. Salt perturbation studies of nano-aggregates of N15 recorded with 5 µM concentration of sensor in aqueous system with the respective fluorescence spectrum
recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of sensor and solvent system at $\lambda_{\text{ex}} = 344$ nm.

2.2.2.3. Polymeric receptors containing imine linkage

The recognition properties of the polymeric receptors 25(a-d) have been evaluated with the help of fluorescence spectroscopy.

2.2.2.3.1. Recognition studies of receptor 25a

Graphical abstract 2.8. Cartoon representation showing ratiometric response after interaction of N25a with Ag$^+$. A 0.5 $\mu$M concentration of receptor 25a in DMSO exhibited the emission band centred at $\lambda_{\text{max}} = 344$ nm. However, the same concentrations of receptor N25a in aqueous medium (by developing nano-aggregates) exhibited red shift with emission band centred at 351 nm. In addition to red shift an enhancement in intensity of N25a was also observed (Figure 2.85). DLS studies revealed that nanoaggregates of receptor N25a have size 23 nm at 0.5 $\mu$M concentration. The bathochromic shift of receptor N25a in aqueous system can be explained by the changes in the conformation of the chromophore in aqueous system i.e. formation of J-Type of aggregates where molecules are arranged in head to tail direction results in a bathochromic shift of the emission band. Enhancement in fluorescence spectra of the receptor N25a in aqueous system can also supported the aggregation induced emission due to the formation of J-type aggregation as a result molecules are arranged in head to tail direction and responsible for high fluorescence efficiency.
Figure 2.85. Fluorescence emission spectra of receptor 25a in DMSO and N25a in aqueous medium (0.5 μM) (λ<sub>ex</sub> = 277 nm).

Evaluation in modulation of fluorescence signatures of nano-aggregates of N25a in aqueous medium were investigated by addition of 2.5 μM concentration of different metal nitrate salts (1 mM) (such as Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Cs<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Sr<sup>2+</sup>, Ba<sup>2+</sup>, Al<sup>3+</sup>, Cr<sup>3+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Ag<sup>+</sup>, Cd<sup>2+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup>) to the fixed concentration (0.5 μM) of nano-aggregates of N25a at excitation wavelength of 277 nm. The solutions were shaken properly followed by recording the spectra for solution of each flask. The addition of different metal ions to the host did not affect the spectra of host except Ag<sup>+</sup>. Addition of Ag<sup>+</sup> has pronounced quenching in the fluorescence intensity of nano-aggregates of N25a at 351 nm with enhancement in the peak at 308 nm (Figure 2.86). Single wavelength based sensors has a drawback because results can be affected by the factors like environmental conditions, instrumental efficiency, etc. these drawbacks can be overcome by developing a ratiometric sensor which uses the ratio of two emissions at different wavelengths. This helps in providing more accurate analysis. The unique metal recognition properties of nano-aggregates of N25a for Ag<sup>+</sup> emphasize the importance of preorganization of receptor pseudocavity in the architecture of fluorescent organic nanoparticles.
Changes in fluorescence intensity of nano-aggregates of N25a (0.5 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium ($\lambda_{\text{ex}} = 277$ nm).

To evaluate the anion binding ability of nano-aggregates of N25a, initial screening was carried out with a library of 9 tetrabutyl ammonium anions (F, Cl, Br, I, CN, CH$_3$COO, HSO$_4$, PO$_4^{3-}$, NO$_3$ and ClO$_4$). The anion binding tests of nano-aggregates of N25a was done by addition of 0.5 µM concentration of tetrabutyl ammonium anions to the fixed concentration (0.5 µM) of nano-aggregates of N25a at excitation wavelength of 277 nm. Fluorescence spectras were recorded for each solution after proper shaking and keeping each solution for sufficient time. Insignificant change in the emission profile of nano-aggregates of N25a was investigated in the presence of any of the tested anions (Figure 2.87). To gain more insights into the binding behaviour of nano-aggregates N25a and Ag$^+$ ions, titration was performed in which molar ratio of Ag$^+$ ion was changed as compare to the fixed concentration of host solution (0.5 µM). The successive additions of Ag$^+$ ion (0-2.5 µM) to the host solution have followed the same trend as observed in the metal binding tests and any particular amount of Ag$^+$ ion added to host solution is directly proportional to the decrease in the intensity of band at 351 nm and increase in the intensity of band at 308 nm with slight blue shift in the frequency (5 nm) (Figure 2.88). The titration has shown a good linearity in a range of 0-2.25 µM concentration of Ag$^+$. Using this titration data, nano-aggregates of N25a can detect Ag$^+$ ion in the solution up to 0.2 nM level (3σ method, inset Figure 2.88).
Figure 2.87. Changes in emission profile of nano-aggregates of \textbf{N25a} (0.5 \, \mu\text{M}) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media ($\lambda_{\text{ex}} = 277$ \, nm).

![Graph showing emission profile changes](image)

To check the selectivity of the sensor, competitive binding test was performed. To perform
this study host solution was taken in different 19 volumetric flasks and then added the 0.5 µM of Ag⁺ ion solution to each flask. Then the addition of remaining 18 metal solutions (Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, Cr³⁺, Mn²⁺, Fe²⁺, Co³⁺, Cu²⁺, Zn²⁺, Cd²⁺, Hg²⁺ and Pb²⁺) to the 18 volumetric flasks was done followed by recording fluorescence spectra for each solution after shaking the solutions properly at 277 nm. Comparison of fluorescence spectra of host + Ag⁺ alone and of host + Ag⁺ in the presence of other metals showed that there is no interference from the other metals and sensor is highly selective for Ag⁺ (Figure 2.89). The performance of the chemosensor for target ion may be affected by the pH value of the environment around the fluorescent chemosensor either due to the hydrolysis reaction for the metal ions in the basic condition or protonation/deprotonation reaction for the fluorophore. The effect of pH on the fluorescence response of nano-aggregates of N25a was therefore investigated. The experiments were carried out at a pH range from 3.0 to 12.0, with a concentration of nano-aggregates of N25a fixed at 0.5 µM (Figure 2.90). Both acidic and basic titrations were conducted by changing pH of host solution using sodium hydroxide and hydrochloric acid and then fluorescence spectra were recorded at various pH values to study the effect of pH on the fluorescence spectra of host solution. For nano-aggregates of N25a, in both acidic (pH< 7) and basic conditions (pH>7), the pH has no or little effect on the emission spectra of nano-aggregates of N25a.

![Figure 2.89. Competitive binding studies of nano-aggregates of N25a containing Ag⁺ over other selected metal Ions at λex = 277 nm. 1) Ag⁺ only; 2) Ag⁺ + Li⁺; 3) Ag⁺ + Na⁺; 4) Ag⁺ + K⁺; 5) Ag⁺ + Cs⁺; 6) Ag⁺ + Mg²⁺; 7) Ag⁺ + Ca²⁺; 8) Ag⁺ + Sr²⁺; 9) Ag⁺ + Ba²⁺; 10) Ag⁺ + Al³⁺; 11) Ag⁺ + Cr³⁺; 12) Ag⁺ + Mn²⁺; 13) Ag⁺ + Fe³⁺; 14) Ag⁺ + Co²⁺; 15) Ag⁺ + Cu²⁺; 16) Ag⁺ + Zn²⁺; 17) Ag⁺ + Cd²⁺; 18) Ag⁺ + Hg²⁺; 19) Ag⁺ + Pb²⁺.

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Besides high sensitivity and selectivity, a short response time is another necessity for a fluorescent chemosensor to monitor Ag\(^+\) in real-time. To study the response time, fluorescence emission spectra of nano-aggregates of N25a was studied by varying the concentration of Ag\(^+\) in the host solution. Experiment was performed by taking the host solution of nano-aggregates of N25a in 3 different volumetric flasks and added different concentration of Ag\(^+\) in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. The response time of the chemosensor to Ag\(^+\) is concentration-independent, as the time required to reach equilibrium does not affect with Ag\(^+\) concentrations. However, in all cases, the stable reading could be obtained within 60 seconds. Therefore, this chemosensor could be used for real time monitoring of Ag\(^+\). From Figure 2.91 one can also discover that once a plateau is reached, the fluorescence intensity at 580 nm stays almost unchanged the rest of the time, indicating that the chemosensor is photo stable under irradiation with visible light. To evaluate the effect of ionic strength, solution of nano-aggregates of N25a (0.5 µM) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a solution of nano-aggregates of N25a and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of nano-aggregates of N25a remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of nano-aggregates of N25a with the increased number of ions in the solution (Figure 2.92).
Figure 2.91. Response time of nano-aggregates of N25a for Ag\(^+\) at \(\lambda_{\text{ex}} = 277\) nm.

Figure 2.92. Salt perturbation studies of nano-aggregates of N25a recorded with 0.5 µM concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of sensor and solvent system at \(\lambda_{\text{ex}} = 277\) nm.
2.2.3.2. Recognition studies of receptor 25b

**Graphical abstract 2.9.** Cartoon representation showing binding between N25b and Sr\(^{2+}\).

Effect of water content on the photophysical properties for nano-aggregates of N25b were explored by recording fluorescence spectra of receptor 25b in both DMF as well as aqueous system (by synthesizing nano-aggregates). The fluorescence spectra of receptor 25b in organic solvent system showed a marked difference in the emission profile than nano-aggregates of compounds in aqueous system (Figure 2.93). The fluorescence spectra of receptor 25b in organic solvent showed monomer peaks ranging from (407-424 nm). Whereas the nano-aggregates of N25b showed broad, featureless emission centred at 444-511 nm. The formation of broad band at 444-511 nm is due the ground state excimer emission of pyrene in N25b after the formation of nano-aggregates in aqueous medium. DLS studies revealed that nanoaggregates of receptor N25b have size 47 nm at 0.5 μM concentration.

To evaluate the metal binding ability of nano-aggregates of N25b, initial screening was carried out with a library of 19 metal salts. A solution of nano-aggregates of N25b (0.5 μM) was mixed with aliquots of metal salt solution and the respective emission spectra were measured (\(λ_{\text{ex}}=345\) nm). To exclude, any kinetic effect, which may influence the fluorescence spectra, the solutions were kept for 60 minutes and fluorescence spectra were recorded again. Figure 2.94 showed the influence of the addition of various metal nitrate salts on the fluorescence signature of nano-aggregates of N25b in aqueous system. Upon addition of an excess of 5 equivalents of various metal ions including Li\(^+\), Na\(^+\), K\(^+\), Cs\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\),
Ba\(^{2+}\), Al\(^{3+}\), Cr\(^{3+}\), Mn\(^{2+}\), Fe\(^{2+}\), Co\(^{3+}\), Cu\(^{2+}\), Zn\(^{2+}\), Ag\(^{+}\), Cd\(^{2+}\), Hg\(^{2+}\) and Pb\(^{2+}\) (as their nitrate salts); only maximum fluorescence quenching was observed for Sr\(^{2+}\) at 471 nm. No such significant change in the fluorescence intensity of nano-aggregates of N25b was observed with the addition of any other tested metal ions under the same conditions.

**Figure 2.93.** Fluorescence emission spectra of receptor 25b in DMF and N25b in aqueous medium (0.5 μM) (\(\lambda_{ex} = 345\) nm).

**Figure 2.94.** Changes in fluorescence intensity of nano-aggregates of N25b (0.5 μM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (\(\lambda_{ex} = 345\) nm).

Anion binding ability of nano-aggregates of N25b was done by addition of 5 eq. of tetrabutyl ammonium anions (F\(^{-}\), Cl\(^{-}\), Br\(^{-}\), I\(^{-}\), CN\(^{-}\), CH\(_3\)COO\(^{-}\), HSO\(_4\)\(^{-}\), PO\(_4\)\(^{3-}\), NO\(_3\)\(^{-}\) and ClO\(_4\)\(^{-}\)
to the fixed concentration (0.5 μM) of nano-aggregates of N25b at excitation wavelength of 345 nm. Fluorescence spectra were recorded for each solution after proper shaking and keeping each solution for sufficient time. Insignificant change in the emission profile of nano-aggregates of N26d was investigated in the presence of any of the tested anions (Figure 2.95).

**Figure 2.95.** Changes in emission profile of nano-aggregates of N25b (0.5 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salt (5 eq.) in aqueous media (λex = 345 nm).

To further evaluate the binding ability of nano-aggregates of N25b towards Sr²⁺ ion, titration of N25b (0.5 μM) was carried out upon the addition of successive amount of Sr²⁺ from 0 μM to 1.5 μM (Figure 2.96). With the increase in the concentration of Sr²⁺ ion there was decrease in the fluorescence intensity of the receptor. Titrations showed a good linearity in this concentration range of Sr²⁺ ion from 0 μM to 1.5 μM (inset of Figure 2.96). Using this titration data, nano-aggregates of N25b can detect Sr²⁺ ion in the solution up to 9 nM (3σ method). The selectivity of nano-aggregates of N25b (0.5 μM) for Sr²⁺ was explored by performing competitive Sr²⁺ binding in the presence of any Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Ba²⁺, Al³⁺, Cr³⁺, Mn²⁺, Fe²⁺, Co³⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺ (2 eq.). No significant change in the fluorescence spectrum was found by comparison with or without the other metal ions (Figure 2.97).
Figure 2.96. Changes in emission profile of nano-aggregates of N25b (0.5 μM) upon successive addition of Sr²⁺ (0-1.5 μM) (λ_ex = 345 nm); (Inset: Linear regression graph between concentration of Sr²⁺ added and decrease in fluorescence intensity of N25b (λ_ex = 345 nm)).

Figure 2.97. Competitive binding studies of nano-aggregates of N25b containing Sr²⁺ over other selected metal ions at λ_ex = 345 nm. 1) Sr²⁺ only; 2) Sr²⁺ + Li⁺; 3) Sr²⁺ + Na⁺; 4) Sr²⁺ + K⁺; 5) Sr²⁺ + Cs⁺; 6) Sr²⁺ + Mg²⁺; 7) Sr²⁺ + Ca²⁺; 8) Sr²⁺ + Ba²⁺; 9) Sr²⁺ + Al³⁺; 10) Sr²⁺ + Cr³⁺; 11) Sr²⁺ + Mn²⁺; 12) Sr²⁺ + Fe³⁺; 13) Sr²⁺ + Co²⁺; 14) Sr²⁺ + Cu²⁺; 15) Sr²⁺ + Zn²⁺; 16) Sr²⁺ + Ag⁺; 17) Sr²⁺ + Cd²⁺; 18) Sr²⁺ + Hg²⁺; 19) Sr²⁺ + Pb²⁺.

In order to find a suitable pH span in which N25b can selectively detect Sr²⁺, acid/basic titrations was performed. In a pH range from 2.7 to 11.2, fluorescence intensity
almost remains constant for nano-aggregates of \textbf{N25b} (0.5 \textmu M) (Figure 2.98). In other words we can say that the nano-aggregates of \textbf{N25b} were insensitive to change in pH.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.98.png}
\caption{Effect of pH on nano-aggregates of \textbf{N25b} (0.5 \textmu M) in aqueous system ($\lambda_{\text{ex}} = 345$ nm).}
\end{figure}

In order to study the response time of nano-aggregates of \textbf{N25b} for Sr$^{2+}$ ion, the fluorescence spectra were recorded upon addition of different concentrations of Sr$^{2+}$ ion (0.1, 0.6, 1.4 \textmu M) to the solutions of nano-aggregates of \textbf{N25b} (0.5 \textmu M) and each solution was analyzed as a function of time. The interpretation of results revealed that after 100 seconds, the fluorescence intensity of all three solutions is independent of time; in other words, the sensor response time of nano-aggregates of \textbf{N25b} for Sr$^{2+}$ ion is 100 seconds (Figure 2.99).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.99.png}
\caption{Response time of nano-aggregates of \textbf{N25b} for Sr$^{2+}$ at $\lambda_{\text{ex}} = 345$ nm.}
\end{figure}
To evaluate the effect of ionic strength on the nano-aggregates of \textbf{N25b}, the excitation spectrum of the nano-aggregates of \textbf{N25b} (0.5 µM) was compared with the excitation spectrum of the same material recorded upon addition of 100 equiv. of the TBA salt of perchlorate (Figure 2.100). There was no change in fluorescence spectra, thus concluding that salt has no effect on nano-aggregates of \textbf{N25b} (0.5 µM).

\textbf{Figure 2.100}. Salt perturbation studies of nano-aggregates of \textbf{N25b} recorded with 0.5 µM concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of sensor and solvent system at $\lambda_{ex} = 345$ nm.

2.2.2.3.3. Recognition studies of receptor 25c

\textbf{Graphical abstract 2.10}. Cartoon representation showing quenching of fluorescence after interaction of \textbf{N25c} with Hg$^{2+}$. 

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In order to investigate the recognition behaviour of nano-aggregates of N25c, following studies have been performed. A 3.2 μM concentration of receptors 25c in DMF exhibited the two small peaks at \(\lambda_{\text{max}} = 377\) nm and 395 nm. However, the same concentrations of nano-aggregates of N25c in aqueous medium (by developing nano-aggregates of receptor N25c) exhibited the same peaks but with enhancement in fluorescence emission as compared to the fluorescence spectra of the compound in DMF (Figure 2.101). Enhancement in fluorescence spectra of the receptor N25c in aqueous system can support the aggregation induced emission. DLS studies revealed that nanoaggregates of receptor N25c have size 20 nm at 3.2 \(\mu\)M concentration.

![Fluorescence emission spectra of nano-aggregates of 25c in DMF and N25c in aqueous medium (3.2 \(\mu\)M) (\(\lambda_{\text{ex}} = 337\) nm).](image)

**Figure 2.101.** Fluorescence emission spectra of nano-aggregates of 25c in DMF and N25c in aqueous medium (3.2 \(\mu\)M) (\(\lambda_{\text{ex}} = 337\) nm).

The cation recognition behaviour of nano-aggregates of N25c was evaluated from the changes in fluorescence intensity of nano-aggregates of N25c upon addition of a particular metal salts (Figure 2.102). This is done by the addition of 5 eq. of 19 metal ions (5 eq.) (such as Li\(^+\), Na\(^+\), K\(^+\), Cs\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\), Ba\(^{2+}\), Al\(^{3+}\), Cr\(^{3+}\), Mn\(^{2+}\), Fe\(^{3+}\), Co\(^{3+}\), Cu\(^{2+}\), Zn\(^{2+}\), Ag\(^+\), Cd\(^{2+}\), Hg\(^{2+}\) and Pb\(^{2+}\)) to the host solution of nano-aggregates of N25c (3.2 \(\mu\)M) in water. For all samples fluorescence spectra was recorded at an excitation wavelength of 337 nm. Upon Addition of Hg\(^{2+}\) to the host solution of nano-aggregates of N25c resulted in quenching of the fluorescence intensity of the host. The addition of other metal ions to the host did not affect the spectra of host except Hg\(^{2+}\).
Figure 2.102 Changes in fluorescence intensity of nano-aggregates of N25c (3.2 μM) upon addition of a particular metal nitrates (5eq.) in aqueous medium ($\lambda_{ex} = 337$ nm).

Host solution of nano-aggregates of N25c was tested against a 9 tetrabutyl ammonium anions (F$^-$, Cl$^-$, Br$^-$, I$^-$, CN$^-$, CH$_3$COO$^-$, HSO$_4^-$, PO$_4^{3-}$, NO$_3^-$ and ClO$_4^-$). To check the anion binding studies 5 eq. of 9 tetrabutyl ammonium anions were added to the host solution of nano-aggregates of N25c (3.2 μM) ($\lambda_{ex}=337$ nm). Insignificant change in the emission profile of nano-aggregates of N26d was investigated in the presence of any of the tested anions (Figure 2.103).

Figure 2.103. Changes in emission profile of nano-aggregates of N25c (3.2 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media ($\lambda_{ex} = 337$ nm).
To check the binding behaviour of nano-aggregates of **N25c** and Hg$^{2+}$ ions, titration was performed. As the Hg$^{2+}$ ion was added from 0 µM to 16 µM to the fixed concentration of host solution (3.2 µM), the fluorescence intensity of **N25c** decreases continuously (Figure 2.104). The linear fluorescence quenching of **N25c** (3.2 µM) toward amounts of Hg$^{2+}$ added was obtained in the range of 0.05–4.5 µM ($R^2 = 0.983$). The limit of detection (LOD) was attained of 1.36 µM, based on $3\delta_{\text{blank}}/k$ (where $\delta_{\text{blank}}$ is the standard deviation of the blank solution and $k$ is the slope of the calibration plot) (inset of Figure 2.104). The competitive binding experiments were also conducted for **N25c** in the presence of 5 equiv. of one cation out of Li$^+$, Na$^+$, K$^+$, Cs$^+$, Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$, Ba$^{2+}$, Al$^{3+}$, Cr$^{3+}$, Mn$^{2+}$, Fe$^{2+}$, Co$^{3+}$, Cu$^{2+}$, Zn$^{2+}$, Ag$^+$, Cd$^{2+}$ and Pb$^{2+}$ along with 5 equiv of Hg$^{2+}$. As shown in Figure 2.105 the emission profile of the **N25c** with Hg$^{2+}$ was unperturbed in the presence of different cations.

![Figure 2.104](image_url)

**Figure 2.104.** Changes in emission profile of nano-aggregates of **N25c** (3.2 µM) upon successive addition of Hg$^{2+}$ (0-16 µM) ($\lambda_{\text{ex}} = 337$ nm); (inset: Linear regression graph between concentration of Hg$^{2+}$ added and decrease in fluorescence intensity of **N25c** ($\lambda_{\text{ex}} = 337$ nm)).

For the application of the sensor in environmental samples, the effect of pH on the fluorescence response of nano-aggregates of **N25c** was also an important factor to consider. The experiments were carried out at a pH range from 3.0 to 12.0 with a concentration of **N25c** fixed at 3.2 µM (Figure 2.106). Fluorescence spectra of the nano-aggregates of **N25c** remain unaffected in the pH range from 5.5 to 8.0.
Figure 2.105. Competitive binding of nano-aggregates of N25c (3.2 µM) containing of Hg$^{2+}$ over other selected metal ions at $\lambda_{ex} = 337$ nm. 1) Hg$^{2+}$ only; 2) Hg$^{2+}$ + Li$^+$; 3) Hg$^{2+}$ + Na$^+$; 4) Hg$^{2+}$ + K$^+$; 5) Hg$^{2+}$ + Cs$^+$; 6) Hg$^{2+}$ + Mg$^{2+}$; 7) Hg$^{2+}$ + Ca$^{2+}$; 8) Hg$^{2+}$ + Sr$^{2+}$; 9) Hg$^{2+}$ + Ba$^{2+}$; 10) Hg$^{2+}$ + Al$^{3+}$; 11) Hg$^{2+}$ + Cr$^{3+}$; 12) Hg$^{2+}$ + Mn$^{2+}$; 13) Hg$^{2+}$ + Fe$^{3+}$; 14) Hg$^{2+}$ + Co$^{2+}$; 15) Hg$^{2+}$ + Cu$^{2+}$; 16) Hg$^{2+}$ + Zn$^{2+}$; 17) Hg$^{2+}$ + Ag$^+$; 18) Hg$^{2+}$ + Cd$^{2+}$; 19) Hg$^{2+}$ + Pb$^{2+}$.

Figure 2.106. Effect of pH on receptor N25c (3.2 µM) in aqueous system ($\lambda_{ex} = 337$ nm).

For the real time monitoring of Hg$^{2+}$ response time was also measured. To check the response time, different concentrations of Hg$^{2+}$ was added in 3 different volumetric flasks containing the fixed concentration of N25c. Then fluorescence spectra of each sample were taken after fixed interval of times. The response time of the chemosensor to Hg$^{2+}$ is concentration-independent, as the time required to reach equilibrium does not affect with Hg$^{2+}$ concentrations (Figure 2.107).
Figure 2.107. Response time of nano-aggregates of N25c for Hg^{2+} ion at $\lambda_{ex} = 337$ nm.

Effect of ionic strength on the fluorescence profile of nano-aggregates of N25c (3.2 µM) was investigated by adding 100 equivalents of tetrabutyl ammonium perchlorate added to a solution of nano-aggregates of N25c and then kept for half an hour to attain equilibrium. The excess amount of perchlorate had no effect on the emission spectra of nano-aggregates of N25c. This means that N25c was not interacted with the increased number of ions in the solution (Figure 2.108).

Figure 2.108. Salt perturbation studies of nano-aggregates of N25c recorded with 3.2 µM concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of sensor and solvent system at $\lambda_{ex} = 337$ nm.
2.2.3.4. Recognition studies of receptor 25d

Graphical abstract 2.11. Cartoon representation showing interaction of N25d with Co²⁺ and HSO₄⁻.

A same concentration of nano-aggregates of N25d (0.6 μM) in DMF and in aqueous medium showed different fluorescence profile when excited at 382 nm. In DMF, 25d showed the emission band centred at λₘₐₓ=448 nm. However, in water the emission spectra of nano-aggregates of N25d exhibited red shift with emission band centred at 514 nm, with simultaneous enhancement in intensity (Figure 2.109). The bathochromic shift and enhancement in the fluorescence profile of receptor N25d in aqueous system can be explained by the changes in the conformation of the chromophore in aqueous system i.e. formation of J-Type of aggregates where molecules are arranged in head to tail direction results in a bathochromic shift. DLS studies revealed that nanoaggregates of receptor N25d have size 19 nm at 0.6 μM concentration. The effect of a wide range of environmentally and physiologically active metal ions was investigated for nano-aggregates of N25d in aqueous medium. For this 5 eq. of different metal nitrate salts (such as Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, Cr³⁺, Mn²⁺, Fe²⁺, Co³⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺)
were added to the fixed concentration (0.6 μM) ($\lambda_{\text{max}}=382$ nm). The addition of different metal ions to the host did not affect the spectra of host except Co$^{2+}$. With the addition of 5 eq. Co$^{2+}$ there was decrease in the fluorescence intensity of nano-aggregates of N25d at 514 nm (Figure 2.110).

**Figure 2.109.** Fluorescence emission spectra of nano-aggregates of 25d in DMF and N25d in aqueous medium (0.6 μM) ($\lambda_{\text{ex}} = 382$ nm).

**Figure 2.110.** Changes in fluorescence intensity of nano-aggregates of N25d (0.6 μM) upon addition of a particular metal nitrates (5eq.) in aqueous medium ($\lambda_{\text{ex}} = 382$ nm).
To check the anion binding ability of nano-aggregates of N25d, initial screening was carried out with a library of 9 tetrabutyl ammonium anions (F\textsuperscript{−}, Cl\textsuperscript{−}, Br\textsuperscript{−}, I\textsuperscript{−}, CN\textsuperscript{−}, CH\textsubscript{3}COO\textsuperscript{−}, HSO\textsubscript{4}\textsuperscript{−}, PO\textsubscript{4}\textsuperscript{3−}, NO\textsubscript{3}\textsuperscript{−} and ClO\textsubscript{4}\textsuperscript{−}). The anion binding tests of nano-aggregates of N25d was done by addition of 3.6 μM concentration of tetrabutyl ammonium anions to the fixed concentration (0.6 μM) of nano-aggregates of N25d at excitation wavelength of 382 nm. The excitation spectra of the nano-aggregates of N25d remain unaffected after the addition of anions (Figure 2.111).

![Image](image.png)

**Figure 2.111.** Changes in emission profile of nano-aggregates of N25d (0.6 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media (λ\textsubscript{ex} = 382 nm).

A detailed investigation was carried out into the recognition of Co\textsuperscript{2+} by performing titration nano-aggregates of N25d. With the increase in the concentration of Co\textsuperscript{2+} ion (0-3 μM) there was decrease in the fluorescence intensity of nano-aggregates of N25d, which is exactly same as observed during metal binding test. Fluorescence intensity of the nano-aggregates of N25d was linearly proportional to the Co\textsuperscript{2+} ion concentration from of 0-3 μM concentration (Figure 2.112(A)). Using this titration data, nano-aggregates of N25d can detect Co\textsuperscript{2+} ion in the solution upto 6 nM level (3σ method, Figure 2.112(B)). A color change was also observed upon the addition of cobalt to the host. Colorless solution of host in water turned into yellow after the addition of cobalt (Figure 2.112(C)). In order to investigate the practical application for Co\textsuperscript{2+} detection, the effect of competitive metal ions was studied by adding Co\textsuperscript{2+} to the host solution of N25d in the presence 2 eq. of the various metal ions. No significant variations on the emission profile of N25d for detection Co\textsuperscript{2+} was observed, showing the excellent selectivity for Co\textsuperscript{2+} ions (Figure 2.113).
Figure 2.112. (A) Changes in emission profile of nano-aggregates of N25d (0.6 µM) upon successive addition of Co²⁺ (0-3 µM) (λₑₓ = 382 nm); (B) Linear regression graph between concentration of Co²⁺ added and decrease in fluorescence intensity of N25d (λₑₓ = 382 nm); (C) Color change after the addition of cobalt to host.

Figure 2.113. Competitive binding of nano-aggregates of N25d (0.6 µM) containing of Co²⁺ over other selected metal ions at λₑₓ = 337 nm. 1) Co²⁺ only; 2) Co²⁺ + Li⁺; 3) Co²⁺ + Na⁺; 4) Co²⁺ + K⁺; 5) Co²⁺ + Cs⁺; 6) Co²⁺ + Mg²⁺; 7) Co²⁺ + Ca²⁺; 8) Co²⁺ + Sr²⁺; 9) Co²⁺ + Ba²⁺; 10) Co²⁺ + Al³⁺; 11) Co²⁺ + Cr³⁺; 12) Co²⁺ + Mn²⁺; 13) Co²⁺ + Fe³⁺; 14) Co²⁺ + Cu²⁺; 15) Co²⁺ + Zn²⁺; 16) Co²⁺ + Ag⁺; 17) Co²⁺ + Cd²⁺; 18) Co²⁺ + Hg²⁺ and 19) Co²⁺ + Pb²⁺.

In order to find a suitable pH span in which nano-aggregates of N25d can selectively detect Co²⁺, acid/basic titrations was performed. The working range for nano-aggregates of N25d as evident from figure 2.114 is 5.3-12.0. Furthermore the response of excitation profile
of nano-aggregates of N25d was studied as a function of time, by tracking the change in the fluorescence spectra. In a typical experiment, three different concentrations of Co²⁺ (0.5, 1.6 and 2.5 µM) were added to the solutions of fixed concentration (0.6 µM) of nano-aggregates of N25d and fluorescence spectra were recorded after small intervals of time (Figure 2.115). Result showed that the nano-aggregates of N25d was interacted with the Co²⁺ ion in first 7-8 seconds, after that stable reading was obtained.

![Fluorescence Intensity vs pH range](image)

**Figure 2.114.** Effect of pH on nano-aggregates of N25d (0.6 µM) in aqueous system ($\lambda_{ex} = 382$ nm).

![Intensity Ratio vs Time](image)

**Figure 2.115.** Response time of nano-aggregates of N25d for Co²⁺ at $\lambda_{ex} = 382$ nm.
Perturbation of high ionic strength was ruled out by comparison of fluorescence spectra of nano-aggregates of N25d (0.6 μM) with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium nitrate under the same host concentration (Figure 2.116).

**Figure 2.116.** Salt perturbation studies of nano-aggregates of N25d recorded with 0.6 μM concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of sensor and solvent system at λ<sub>ex</sub> = 382 nm.

The interaction of the assembly of nano-aggregates of N25d and Co<sup>2+</sup> (N25d-Co<sup>2+</sup>) with the anions was investigated by the addition of 5 eq. of tetrabutyl ammonium salts of anions (F<sup>-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, CN<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, HSO<sub>4</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, NO<sub>3</sub><sup>-</sup> and ClO<sub>4</sub><sup>-</sup>) into the host solution of N25d-Co<sup>2+</sup> (3.6 μM). When 18 μM of HSO<sub>4</sub><sup>-</sup> was added to the solution of N25d-Co<sup>2+</sup> (3.6 μM) an increase of the intensity of peak at 450 nm was observed. In the same conditions, no significant changes happened when 18 μM of other anions (F<sup>-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, CN<sup>-</sup>, CH<sub>3</sub>COO<sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, NO<sub>3</sub><sup>-</sup> and ClO<sub>4</sub><sup>-</sup>) were added (Figure 2.117). The assembly of nano-aggregates of N25d and Co<sup>2+</sup> (N25d-Co<sup>2+</sup>) is then titrated against HSO<sub>4</sub><sup>-</sup> to investigate the utility of N25d-Co<sup>2+</sup> as an anion sensor. Titrations were performed by increasing the amount of hydrogen sulphate in N25d-Co<sup>2+</sup> (3.6 μM). With the increase in the concentration of hydrogen sulphate from 0 to 18 μM, there was enhancement in the fluorescence intensity of the assembly of nano-aggregates of N25d and Co<sup>2+</sup> (N25d-Co<sup>2+</sup>). As the fluorescence spectrum was recorded within 20 seconds after hydrogen sulphate anion addition, and the intensity does not change with time, so the monitoring system is virtually real time stable. Fluorescence intensity is showing a good linear response with increasing concentration of hydrogen sulphate (Figure 2.118(A)). Titration has shown good linearity in a range of 0-18 μM concentration of HSO<sub>4</sub><sup>-</sup> with a detection limit of 0.4 nM (inset of Figure 2.118(B)). Colour of host was restored after
the addition of hydrogen sulphate ion to the complex of host.Co\textsuperscript{2+}. Yellow colour of host.Co\textsuperscript{2+} was changed back to colorless upon the addition of hydrogen sulphate to it (figure 2.118(C)).

**Figure 2.117.** Changes in emission profile of the assembly of nano-aggregates of N25d and Co\textsuperscript{2+} (N25d.Co\textsuperscript{2+}) (3.6 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media (λ\textsubscript{ex} = 382 nm).

![Fluorescence Intensity vs Wavelength](image)

\[ y = 35.891x + 154.98 \]
\[ R^2 = 0.9986 \]

**Figure 2.118.** Changes in emission profile of the assembly of nano-aggregates of N25d and Co\textsuperscript{2+} (N25d.Co\textsuperscript{2+}) (3.6 μM) upon successive addition of HSO\textsubscript{4}\textsuperscript{-} (0-18 μM) (λ\textsubscript{ex} = 382 nm); (B) Linear regression graph between concentration of hydrogen sulphate added and decrease in fluorescence intensity of N25d.Co\textsuperscript{2+} (λ\textsubscript{ex} = 382 nm); (C) Color restoration of host after the addition of hydrogen sulphate ion to the complex of host.Co\textsuperscript{2+}.
In order to check the selectivity of the assembly of nano-aggregates of N25d and Co$^{2+}$ (N25d-Co$^{2+}$) towards HSO$_4^-$ in the presence of other anions, competitive studies were carried out by fluorescence spectroscopy. The emission profile of the assembly of nano-aggregates of N25d and Co$^{2+}$ (N25d-Co$^{2+}$) was unperturbed in the presence of other anions, such as F$^-$, Cl$^-$, Br$^-$, I$^-$, CN$^-$, CH$_3$COO$^-$, PO$_4^{3-}$, NO$_3^-$ and ClO$_4^-$. These results show that assembly of nano-aggregates of N25d and Co$^{2+}$ (N25d-Co$^{2+}$) is highly selective fluorescent sensor for HSO$_4^-$ (Figure 2.119). Response time studies revealed that the response of the fluorescence profile of nano-aggregates of N25d and Co$^{2+}$ (N25d-Co$^{2+}$) is concentration-independent, as the time required to reach equilibrium does not affect with HSO$_4^-$ concentrations (Figure 2.120).

**Figure 2.119.** Competitive binding studies of assembly of nano-aggregates of N25d and Co$^{2+}$ (N25d-Co$^{2+}$) containing hydrogen sulphate over other selected tetrabutyl ammonium anions at $\lambda_{ex} = 382$ nm. 1) Hydrogen Sulphate; 2) Hydrogen Sulphate + Fluoride; 3) Hydrogen Sulphate + Chloride; 4) Hydrogen Sulphate + Bromide; 5) Hydrogen Sulphate + Iodide; 6) Hydrogen Sulphate + Cyanide; 7) Hydrogen Sulphate + Acetate; 8) Hydrogen Sulphate + Phosphate; 9) Hydrogen Sulphate + Nitrate and 10) Hydrogen Sulphate + Perchlorate.

**Figure 2.120.** Response time of nano-aggregates of N25d-Co$^{2+}$ complex for hydrogen sulphate ion at $\lambda_{ex} = 382$ nm.
pH studies were also performed in a similar manner as with N25d.Co²⁺ complex. Results showed that in the pH range from 6-12 there was no effect of pH on the fluorescence profile of N25d.Co²⁺. Even in the pH range from 3-6 there was not much change in the fluorescence profile of complex (Figure 2.121).

**Figure 2.121.** Effect of pH on nano-aggregates of N25d.Co²⁺ complex (3.6 µM) in aqueous system (λ<sub>ex</sub> = 382 nm).

Fluorescence emission spectra of nano-aggregates of N25d.Co²⁺ complex remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of nano-aggregates of N25d.Co²⁺ complex with the increased number of ions in the solution (Figure 2.122).

**Figure 2.122.** Salt perturbation studies of nano-aggregates of N25d-Co²⁺ complex recorded with 3.6 µM concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of sensor and solvent system at λ<sub>ex</sub> = 382 nm.
2.2.2.4. Polymeric receptors having dihydropyrimidones moieties

The recognition properties of the polymeric receptors 26(a-f) have been evaluated with the help of fluorescence spectroscopy.

2.2.2.4.1. Recognition studies of receptor 26a

Effect of water content on the photophysical properties for receptor 26a were evaluated by recording fluorescence spectra of 26a in both THF as well as aqueous system (by developing nano-aggregates). The fluorescence spectra of receptor 26a in organic solvent system showed a significant difference in the emission profile than 26a in aqueous system (Figure 2.123). Increased water content resulted in formation of aggregates, which induces decrease in the fluorescence intensity of N26a. This is due to the phenomena known as “aggregation caused quenching” (ACQ). DLS studies revealed that nanoaggregates of receptor N26a have size 60 nm at 3 µM concentration.

![Fluorescence emission spectra of receptor 26a in THF and N26a in aqueous medium (3 µM) (λ<sub>ex</sub> = 365 nm).](image)

Figure 2.123. Fluorescence emission spectra of receptor 26a in THF and N26a in aqueous medium (3 µM) (λ<sub>ex</sub> = 365 nm).

Metal binding studies of nano-aggregates of N26a were performed in aqueous medium by addition of 15 µM concentration of different metal nitrate salts (1 mM) (such as Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Cs<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Sr<sup>2+</sup>, Ba<sup>2+</sup>, Al<sup>3+</sup>, Cr<sup>3+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Ag<sup>+</sup>, Cd<sup>2+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup>) to the fixed concentration (3 µM) of nano-aggregates of N26a at excitation wavelength of 365 nm. The solutions are shaken properly followed by recording the spectra for solution
of each flask. The addition of different metal ions to the host did not affect the spectra of nano-aggregates of \textbf{N26a} except Zn$^{2+}$. With the 5 eq. addition of Zn$^{2+}$ showed a slight enhancement with Zn$^{2+}$ but further addition of Zn$^{2+}$ unable to bring any change in the spectra of \textbf{N26a} (Figure 2.124)

![Fluorescence spectra of nano-aggregates of N26a with various metal ions.](image)

**Figure 2.124.** Changes in fluorescence intensity of nano-aggregates of \textbf{N26a} (3 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium ($\lambda_{ex} = 365$ nm).

To evaluate the anion binding ability of nano-aggregates of \textbf{N26a}, initial screening was carried out with a library of 10 tetrabutyl ammonium anions (F, Cl, Br, I, CN, CH$_3$COO, HSO$_4$, PO$_4^{3-}$, NO$_3$ and ClO$_4$). The anion binding tests of nano-aggregates of \textbf{N26a} was done by addition of 15 µM concentration of tetrabutyl ammonium anions to the fixed concentration (3 µM) of nano-aggregates of \textbf{N26a} at excitation wavelength of 365 nm. Fluorescence spectra were recorded for each solution after proper shaking and keeping each solution for sufficient time. The anion binding tests of nano-aggregates of \textbf{N26a} with variety of anions have not shown any significant changes in emission spectra (Figure 2.125). The effect of pH on the fluorescence emission profile of nano-aggregates of \textbf{N26a} was investigated. The experiments were carried out at a pH range from 3.0 to 12.0, with a concentration of 3 fixed at 3 µM (Figure 2.126). Both acidic and basic titrations were conducted by changing pH of host solution using sodium hydroxide and hydrochloric acid and then fluorescence spectra was recorded at various pH values to study the effect of pH on the fluorescence spectra of host solution. In acidic conditions there was enhancement in the fluorescence intensity of nano-
aggregates of N26a, whereas in the basic conditions fluorescence emission remain stable till pH 10 after this there was slight increase in the fluorescence intensity of nano-aggregates of N26a.

**Figure 2.125.** Changes in emission profile of nano-aggregates of N26a (3 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media ($\lambda_{ex} = 365$ nm).

**Figure 2.126.** Effect of pH on nano-aggregates of N26a (3 μM) in aqueous system ($\lambda_{ex} = 365$ nm).
2.2.2.4.2. Recognition studies of receptor 26b

Graphical abstract 2.12. Cartoon representation showing quenching of fluorescence after interaction of N26b with Pb$^{2+}$.

Effect of water content on the photophysical properties for receptor 26b were explored by recording fluorescence emission spectra of receptor 26b in both THF as well as aqueous system (by synthesizing nano-aggregates). The fluorescence spectra of receptor N26b in water was blue shifted showed a significant difference in the emission profile than nano-aggregates of N26b in aqueous system (Figure 2.127). DLS studies revealed that nanoaggregates of receptor N26b have size 51 nm at 10 μM concentration. To evaluate the metal binding ability of nano-aggregates of N26b, initial screening was carried out with a library of 19 metal salts. A solution of nano-aggregates of N26b (10 μM) was mixed with aliquots of metal salt solution and the respective emission spectra were measured ($\lambda_{\text{ex}}$=344 nm). To exclude, any kinetic effect, which may influence the fluorescence spectra, the solutions were kept for 60 minutes and fluorescence spectra were recorded again. Figure 2.128 showed the influence of the addition of various metal nitrate salts on the fluorescence signature of nano-aggregates of N26b in aqueous system. Upon addition of an excess of 5 equivalents of various metal ions including Li$^+$, Na$^+$, K$^+$, Cs$^+$, Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$, Ba$^{2+}$, Al$^{3+}$,
Cr$^{3+}$, Mn$^{2+}$, Fe$^{2+}$, Co$^{3+}$, Cu$^{2+}$, Zn$^{2+}$, Ag$^+$, Cd$^{2+}$, Hg$^{2+}$ and Pb$^{2+}$ (as their nitrate salts); only maximum fluorescence quenching was observed for Pb$^{2+}$ at 422 nm. Not such significant change in the fluorescence intensity of nano-aggregates of N26b was observed with the addition of any other tested metal ions under the same conditions.

**Figure 2.127.** Fluorescence emission spectra of receptor 26b in THF and N26b in aqueous medium (10 μM) (λ$_{ex}$ = 344 nm).

**Figure 2.128.** Changes in fluorescence intensity of nano-aggregates of N26b (10 μM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λ$_{ex}$ = 344 nm).
To evaluate the anion binding ability of nano-aggregates of N26b, initial screening was carried out with a library of 10 tetrabutyl ammonium anions (F⁻, Cl⁻, Br⁻, I⁻, CN⁻, CH₃COO⁻, HSO₄⁻, PO₄³⁻, NO₃⁻ and ClO₄⁻). The anion binding tests of nano-aggregates of N26b was done by addition of 5eq. of tetrabutyl ammonium anions to the fixed concentration (10 µM) of nano-aggregates of N26b at excitation wavelength of 344 nm. Fluorescence spectras were recorded for each solution after proper shaking and keeping each solution for sufficient time. Insignificant change in the emission profile of nano-aggregates of N26d was investigated in the presence of any of the tested anions (Figure 2.129).

![Fluorescence intensity vs Wavelength](image)

**Figure 2.129.** Changes in emission profile of nano-aggregates of N26b (10 µM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media (λex = 344 nm).

To gain more insights into the binding behaviour of nano-aggregates N26b and Pb²⁺ ions, titration was performed by taking a fixed concentration of N26b and successive addition of Pb²⁺ to the solution of N26b. With the increase in the concentration of host there was a continuous decrease in the intensity of nano-aggregates of N26b (Figure 2.130). Concentration of Pb²⁺ was varied from 0 µM to 54 µM and titrations showed a good linearity in the concentration range of 1 µM to 54 µM (inset of Figure 2.130). Limit of detection is estimated to be 0.04 µM (3σ method). To check the selectivity of the sensor, competitive binding test was performed. Every excellent chemosensor must have high selectivity. So competitive experiments were performed for the estimation of Pb²⁺ (5 eq.) by nano-aggregates of N26b in the presence of any of Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, Cr³⁺, Mn²⁺, Fe²⁺, Co³⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺ and Hg²⁺ (5 eq.). As shown in Figure 2.131, no significant variation in the intensity was detected by comparing the intensity with and without
other metal ions. Therefore, nano-aggregates of N26b have a high selectivity for estimation of Pb\(^{2+}\), even in the presence of other metal ions.

**Figure 2.130.** Changes in emission profile of nano-aggregates of N26b (10 µM) upon successive addition of Pb\(^{2+}\) ion (0-54 µM) (λ\(_{ex}\) = 344 nm); (Inset: Linear regression graph between concentration of Pb\(^{2+}\) added and decrease in fluorescence intensity of N26b (λ\(_{ex}\) = 344 nm).

**Figure 2.131.** Competitive binding of N26b (10 µM) containing of Pb\(^{2+}\) over other selected metal ions at λ\(_{ex}\) = 344 nm. 1) Pb\(^{2+}\) only; 2) Pb\(^{2+}\) + Li\(^{+}\); 3) Pb\(^{2+}\) + Na\(^{+}\); 4) Pb\(^{2+}\) + K\(^{+}\); 5) Pb\(^{2+}\) + Cs\(^{+}\); 6) Pb\(^{2+}\) + Mg\(^{2+}\); 7) Pb\(^{2+}\) + Ca\(^{2+}\); 8) Pb\(^{2+}\) + Sr\(^{2+}\); 9) Pb\(^{2+}\) + Ba\(^{2+}\); 10) Pb\(^{2+}\) + Al\(^{3+}\); 11) Pb\(^{2+}\) + Cr\(^{3+}\); 12) Pb\(^{2+}\) + Mn\(^{2+}\); 13) Pb\(^{2+}\) + Fe\(^{3+}\); 14) Pb\(^{2+}\) + Co\(^{3+}\); 15) Pb\(^{2+}\) + Cu\(^{2+}\); 16) Pb\(^{2+}\) + Zn\(^{2+}\); 17) Pb\(^{2+}\) + Ag\(^{+}\); 18) Pb\(^{2+}\) + Cd\(^{2+}\); 19) Pb\(^{2+}\) + Hg\(^{2+}\).
The performance of the target ion somewhat affected by the pH value of the environment around the fluorescent chemosensor. For nano-aggregates of N26b, in acidic (pH < 7) conditions the pH has no effect on the fluorescence spectra, whereas from pH 7 to 10, the fluorescence emission decrease from 400 nm to 280 nm afterwards pH has no effect on the emission spectra of nano-aggregates of N26b (Figure 2.132).

![Fluorescence Intensity vs pH range graph]

**Figure 2.132.** Effect of pH on nano-aggregates of N26b (10 µM) in aqueous system (λ<sub>ex</sub> = 344 nm).

To study the response time, fluorescence emission spectra of nano-aggregates of N26b was studied by varying the concentration of Pb<sup>2+</sup> in the host solution. Experiment was performed by taking the host solution of N26b (10 µM) in 3 different volumetric flasks and added different concentration of Pb<sup>2+</sup> in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. The response time of the nano-aggregates of N26b to Pb<sup>2+</sup> ion is concentration-independent, as the time required to reach equilibrium does not affect with Pb<sup>2+</sup> concentrations. However, in all cases, the stable reading could be obtained within 30 seconds. Therefore, this chemosensor could be used for real time monitoring of Pb<sup>2+</sup>. From Figure 2.133 one can also discover that once a plateau is reached, the fluorescence intensity at 422 nm stays almost unchanged the rest of the time, indicating that the chemosensor is photostable under irradiation with visible light. To evaluate the effect of ionic strength, solution of nano-aggregates of N26b (10 µM) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a solution of nano-aggregates of N26b and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of nano-aggregates of N26b remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of N26b with the increased number of ions in the solution (Figure 2.134).
Figure 2.133. Response time of nano-aggregates of N26b for Pb^{2+} ion at $\lambda_{ex} = 344$ nm.

Figure 2.134. Salt perturbation studies of nano-aggregates of N26b recorded with 10 $\mu$M concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of sensor and solvent system at $\lambda_{ex} = 344$ nm.
2.2.2.4.3. Recognition studies of receptor 26c

Graphical abstract 2.13. Cartoon representation showing quenching of fluorescence after interaction of N26c with Ag⁺.

Effect of water content on the photophysical properties for receptor 26c (0.45 µM) were explored by recording fluorescence emission spectra of receptor N26c in both DMF as well as aqueous system (by synthesizing nano-aggregates). The fluorescence spectra of receptor N26c in organic solvent system showed a manifest difference in the emission profile than nano-aggregates of N26c in aqueous system (Figure 2.135). The fluorescence spectra of receptor N26c in organic solvent showed monomer peaks ranging from (382-394 nm). Whereas the nano-aggregates of N26c showed broad, featureless emission centred at 422-523 nm. The formation of broad band at 422-523 nm is due the ground state excimer emission of pyrene in N26c after the formation of nano-aggregates in aqueous medium. DLS studies revealed that nanoaggregates of receptor N26c have size 40 nm at 0.45 µM concentration.

Metal binding ability of nano-aggregates of N26c was carried out with a library of 19 metal salts. A solution of nano-aggregates of N26c (0.45 µM) was mixed with aliquots of metal salt solution and the respective emission spectra were measured (λₜₐₓ=330 nm). To exclude, any kinetic effect, which may influence the fluorescence spectra, the solutions were kept for 60 minutes and fluorescence spectra were recorded again. Figure 2.136 showed the influence of the addition of various metal nitrate salts on the fluorescence signature of nano-aggregates of N26c in aqueous system. Upon addition of an excess of 5 equivalents of various metal ions including Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, Cr³⁺, Mn²⁺, Fe²⁺, Co³⁺, Cu²⁺, Zn²⁺,
Ag\(^+\), Cd\(^{2+}\), Hg\(^{2+}\) and Pb\(^{2+}\) (as their nitrate salts); only maximum fluorescence quenching was observed for Ag\(^+\) around 470 nm. Not such significant change in the fluorescence intensity of nano-aggregates of N\textsubscript{26c} was observed with the addition of any other tested metal ions under the same conditions.

Figure 2.135. Fluorescence emission spectra of receptor \textbf{26c} in THF and N\textsubscript{26c} in aqueous medium (0.45 \(\mu\)M) (\(\lambda_{\text{ex}} = 330\) nm).

Figure 2.136. Changes in fluorescence intensity of receptor N\textsubscript{26c} (0.45 \(\mu\)M) upon addition of a particular metal nitrates (5eq.) in aqueous medium (\(\lambda_{\text{ex}} = 330\) nm).

To evaluate the anion binding ability of nano-aggregates of N\textsubscript{26c}, initial screening was carried out with a library of 10 tetrabutyl ammonium anions (F\(^-\), Cl\(^-\), Br\(^-\), I\(^-\), CN\(^-\), CH\(_3\)COO\(^-\), HSO\(_4\)\(^-\), PO\(_4\)\(^{3-}\), NO\(_3\)\(^-\) and ClO\(_4\)\(^-\)). The anion binding tests of nano-aggregates of
**N26c** was done by addition of 5eq. of tetrabutyl ammonium anions (5 mM) to the fixed concentration (0.45 μM) of nano-aggregates of **N26c** at excitation wavelength of 330 nm. Fluorescence spectra were recorded for each solution after proper shaking and keeping each solution for sufficient time. The emission profile of **N26c** complex was not affected by the presence of any of the anions as shown in Figure 2.137.

![Figure 2.137](image)

**Figure 2.137.** Changes in emission profile of nano-aggregates of **N26c** (0.45 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media (λ<sub>ex</sub> = 330 nm).

To learn more about the properties of nano-aggregates of **N26c** as a sensor for Ag<sup>+</sup>, a fluorescence titration was carried out by adding incremental amount of Ag<sup>+</sup> (0-2.2 μM) to the solution of nano-aggregates of **N26c** in aqueous medium (Figure 2.138). The addition of increasing amounts of Ag<sup>+</sup> (0-2.2 μM) to the solution of nano-aggregates of **N26c** resulted in quenching in the fluorescence intensity. Titrations showed a good linearity in this concentration range of Ag<sup>+</sup> ion (inset of Figure 2.138). Limit of detection is estimated to be 21 nM (3σ method). To check the selectivity of the sensor, competitive binding test was performed. To perform this study host solution was taken in different 19 volumetric flasks and then added the 2.5 μM of Ag<sup>+</sup> ion solution to each flask. Then the addition of remaining 19 metal solutions (Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Cs<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Sr<sup>2+</sup>, Ba<sup>2+</sup>, Al<sup>3+</sup>, Cr<sup>3+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, Co<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup>) to the 18 volumetric flasks was done followed by recording fluorescence spectra for each solution after shaking the solutions properly at 345 nm. Comparison of fluorescence spectra of **host** + Ag<sup>+</sup> alone and of **host** + Ag<sup>+</sup> in the presence of
other metals showed that there is no interference from the other metals and sensor is highly selective for Ag⁺ (Figure 2.139).

**Figure 2.138.** Changes in emission profile of nano-aggregates of N26c (0.45 μM) upon successive addition of Ag⁺ (0–2.2 μM) (λ<sub>ex</sub> = 330 nm); (Inset: Linear regression graph between concentration of Ag⁺ added and decrease in fluorescence intensity of N26c (λ<sub>ex</sub> = 330 nm).

**Figure 2.139.** Competitive binding studies of N26c containing Ag⁺ over other selected metal ions at λ<sub>ex</sub> = 330 nm. 1) Ag⁺ only; 2) Ag⁺ + Li⁺; 3) Ag⁺ + Na⁺; 4) Ag⁺ + K⁺; 5) Ag⁺ + Cs⁺; 6) Ag⁺ + Mg<sup>2+</sup>; 7) Ag⁺ + Ca<sup>2+</sup>; 8) Ag⁺ + Sr<sup>2+</sup>; 9) Ag⁺ + Ba<sup>2+</sup>; 10) Ag⁺ + Al<sup>3+</sup>; 11) Ag⁺ + Cr<sup>3+</sup>; 12) Ag⁺ + Mn<sup>2+</sup>; 13) Ag⁺ + Fe<sup>3+</sup>; 14) Ag⁺ + Co<sup>2+</sup>; 15) Ag⁺ + Cu<sup>2+</sup>; 16) Ag⁺ + Zn<sup>2+</sup>; 17) Ag⁺ + Cd<sup>2+</sup>; 18) Ag⁺ + Hg<sup>2+</sup>; 19) Ag⁺ + Pb<sup>2+</sup>.
The effect of pH on the fluorescence response of nano-aggregates of \textbf{N26c} was therefore investigated. The experiments were carried out at a pH range from 3.0 to 12.0, with a concentration of \textbf{N26c} fixed at 0.5 \( \mu \text{M} \) (Figure 2.140). Both acidic and basic titrations were conducted by changing pH of host solution using sodium hydroxide and hydrochloric acid and then fluorescence spectra was recorded at various pH values to study the effect of pH on the fluorescence spectra of host solution. For nano-aggregates of \textbf{N26c}, in both acidic (pH<7) and basic conditions (pH>7), the pH has no or little effect on the emission spectra of nano-aggregates of \textbf{N26c}.

\textbf{Figure 2.140}. Effect of pH on receptor \textbf{N26c} (0.45 \( \mu \text{M} \)) in aqueous system (\( \lambda_{\text{ex}} = 330 \text{ nm} \)).

Besides high sensitivity and selectivity, a short response time is another necessity for a fluorescent chemosensor to monitor \textbf{Ag}\(^{+}\) in real-time. To study the response time, fluorescence emission spectra of nano-aggregates of \textbf{N26c} was studied by varying the concentration of \textbf{Ag}\(^{+}\) in the host solution. Experiment was performed by taking the host solution of \textbf{N26c} in 3 different volumetric flasks and added different concentration of \textbf{Ag}\(^{+}\) in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. The response time of the chemosensor to \textbf{Ag}\(^{+}\) ion is concentration-independent, as the time required to reach equilibrium does not affect with \textbf{Ag}\(^{+}\) concentrations (Figure 2.141). However, in all cases, the stable reading could be obtained within 60 seconds. Therefore, this chemosensor could be used for real time monitoring of \textbf{Ag}\(^{+}\).
Figure 2.141. Response time of receptor N26c for Ag⁺ at $\lambda_{ex} = 330$ nm.

To evaluate the effect of ionic strength, solution of sensors N26c (0.45 µM) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a solution of N26c and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of N26c remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of N26c with the increased number of ions in the solution (Figure 2.142).

Figure 2.142. Salt perturbation studies of N26c recorded with 0.45 µM concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium nitrate under the same concentration of sensor and solvent system at $\lambda_{ex} = 330$ nm.
2.2.2.4.4. Recognition studies of receptor 26e

Graphical abstract 2.14. Cartoon representation showing (A) ratiometric response of fluorescence intensity after interaction of N26e with CN⁻; (B) quenching of fluorescence after interaction of N26e with Cu²⁺.

To evaluate the effect of water on the photophysical properties of receptor 26e, fluorescence spectroscopic studies were carried out. The excitation spectrum of 26e (4 µM) was recorded in THF, which showed a peak at 360 nm. However, in aqueous medium, fluorescence intensity of N26e at 360 nm decrease while a new peak originate around 410 nm (Figure 2.143). The metal binding studies of nano-aggregates of N26e were assessed from the modulation of emission spectra of nano-aggregates of N26e in the presence of metal ions. For this a solution of nano-aggregates of N26e (4 µM) was mixed with aliquots of metal salt solution and the respective emission spectra were measured (λex=300 nm). To exclude, any kinetic effect, which may influence the fluorescence spectra, the solutions were kept for 60 minutes and fluorescence spectra were recorded again. Upon addition of an excess of 5 equivalents of various metal ions including Li⁺, Na⁺, K⁺, Cs⁺, Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺, Al³⁺, Cr³⁺, Mn²⁺, Fe²⁺, Co³⁺, Cu²⁺, Zn²⁺, Ag⁺, Cd²⁺, Hg²⁺ and Pb²⁺ (as their nitrate salts), no such significant change in the fluorescence intensity of N26e was observed with the addition of any other tested metal ions under the same conditions except Cu²⁺ (Figure 2.144). With the addition of 5 eq. of Cu²⁺ there was decrease in fluorescence intensity of nano-aggregates of
**N26e.** DLS studies revealed that nanoaggregates of receptor **N26e** have size 57 nm at 4 μM concentration.

![Fluorescence emission spectra of receptor 26e in THF and N26e in aqueous medium (4 μM) (λ<sub>ex</sub> = 300 nm).](image1)

**Figure 2.143.** Fluorescence emission spectra of receptor 26e in THF and N26e in aqueous medium (4 μM) (λ<sub>ex</sub> = 300 nm).

![Changes in fluorescence intensity of nano-aggregates of N26e (4 μM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λ<sub>ex</sub> = 300 nm).](image2)

**Figure 2.144.** Changes in fluorescence intensity of nano-aggregates of N26e (4 μM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λ<sub>ex</sub> = 300 nm).

To check anion binding ability of sensor **N26e**, initial screening was carried out with a
library of 10 tetrabutyl ammonium anions (F\(^-\), Cl\(^-\), Br\(^-\), I\(^-\), CN\(^-\), CH\(_3\)COO\(^-\), HSO\(_4\)\(^-\), PO\(_4\)\(^{3-}\), NO\(_3\)\(^-\) and ClO\(_4\)\(^-\)). The anion binding tests of nano-aggregates of \textbf{N}26e was done by addition of 0.1 eq. of tetrabutyl ammonium anions (5 mM) to the fixed concentration (4μM) of nano-aggregates of \textbf{N}26e at excitation wavelength of 300 nm. Fluorescence spectras were recorded for each solution after proper shaking and keeping each solution for sufficient time. The anion binding tests of nano-aggregates of \textbf{N}26e with variety of anions have not shown any significant changes in emission spectra except for cyanide ion (Figure 2.145). With the addition of 5 eq. of cyanide ion there was decrease in the fluorescence emission band of \textbf{N}26e centred at 353 nm and simultaneous increase in the fluorescence intensity at 417 nm.

![Figure 2.145](image-url)

**Figure 2.145.** Changes in emission profile of nano-aggregates of \textbf{N}26f (4 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media (λ\(_{ex}\) = 300 nm).

To gain more insights into the binding behaviour of nano-aggregates \textbf{N}26e and Cu\(^{2+}\) ions, titration was performed by taking a fixed concentration of \textbf{N}26e and successive addition of Cu\(^{2+}\) to the solution of \textbf{N}26e. With the increase in the concentration of Cu\(^{2+}\) from 0 μM to 20 μM, there was a continuous decrease in the intensity of \textbf{N}26e (Figure 2.146). Titrations showed a good linearity in this concentration range of Cu\(^{2+}\) ion (inset of Figure 2.146). Limit of detection is estimated to be 2 nM (3σ method). To check the selectivity of the sensor, competitive binding test was performed. To perform this study host solution was taken in different 19 volumetric flasks and then added the 2.5 μM of Cu\(^{2+}\) ion solution to each flask.
Then the addition of remaining 18 metal solutions (Li\(^+\), Na\(^+\), K\(^+\), Cs\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\), Ba\(^{2+}\), Al\(^{3+}\), Cr\(^{3+}\), Mn\(^{2+}\), Fe\(^{2+}\), Co\(^{3+}\), Zn\(^{2+}\), Ag\(^+\), Cd\(^{2+}\), Hg\(^{2+}\) and Pb\(^{2+}\)) to the 18 volumetric flasks was done followed by recording fluorescence spectra for each solution after shaking the solutions properly at 345 nm. Comparison of fluorescence spectra of host + Cu\(^{2+}\) alone and of host + Cu\(^{2+}\) in the presence of other metals showed that there is no interference from the other metals and sensor is highly selective for Cu\(^{2+}\) (Figure 2.147).

**Figure 2.146.** Changes in emission profile of nano-aggregates of N26e (4 µM) upon successive addition of Cu\(^{2+}\) (0-20 µM) (\(\lambda_{ex}\) = 300 nm); (Inset: Linear regression graph between concentration of Cu\(^{2+}\) added and decrease in emission of N26e (\(\lambda_{ex}\) = 300 nm).

**Figure 2.147.** Competitive binding studies of N26e containing Cu\(^{2+}\) over other selected metal ions at \(\lambda_{ex}\) = 300nm. 1) Cu\(^{2+}\) only; 2) Cu\(^{2+}\) + Li\(^+\); 3) Cu\(^{2+}\) + Na\(^+\); 4) Cu\(^{2+}\) + K\(^+\); 5) Cu\(^{2+}\) + Cs\(^+\); 6) Cu\(^{2+}\) + Mg\(^{2+}\); 7) Cu\(^{2+}\) + Ca\(^{2+}\); 8) Cu\(^{2+}\) + Sr\(^{2+}\); 8) Cu\(^{2+}\) + Ba\(^{2+}\); 10) Cu\(^{2+}\) + Al\(^{3+}\); 11) Cu\(^{2+}\)
+ Cr³⁺; 12) Cu²⁺ + Mn²⁺; 13) Cu²⁺ + Fe³⁺; 14) Cu²⁺ + Co²⁺; 15) Cu²⁺ + Zn²⁺; 16) Cu²⁺ + Ag⁺; 17) Cu²⁺ + Cd²⁺; 18) Cu²⁺ + Hg²⁺; 19) Cu²⁺ + Pb²⁺.

Besides high sensitivity and selectivity, a short response time is another necessity for a fluorescent chemosensor to monitor Cu²⁺ in real-time. To study the response time, fluorescence emission spectra of nano-aggregates of N26e was studied by varying the concentration of Cu²⁺ in the host solution. Experiment was performed by taking the host solution of N26e in 3 different volumetric flasks and added different concentration of Cu²⁺ in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. The response time of the chemosensor to Cu²⁺ ion is concentration dependent, as the time required to reach equilibrium affect with Cu²⁺ concentrations. Response time of sensor is indirectly proportional to the concentration of Cu²⁺ ion (Figure 2.148). As the concentration of Cu²⁺ increases the response time decrease i.e. at higher concentration of guest, sensor is taking less time to be stable.

![Figure 2.148](image)

**Figure 2.148.** Response time of nano-aggregates of N26e for Cu²⁺ at λex = 300 nm.

To gain more insights into the sensor activities of nano-aggregates of N26e and CN⁻ ion, titration was performed by taking a fixed concentration of N26e and successive addition of CN⁻ to the solution of N26e. With the increase in the concentration of CN⁻, there was a continuous decrease in the fluorescence emission band of nano-aggregates of N26e centred at 354 nm and simultaneous increase in the fluorescence intensity at 414 nm (Figure 2.149). Concentration of CN⁻ ion was varied from 0 µM to 5 µM and titrations showed a good linearity in this concentration range of CN⁻ ion (inset of Figure 2.149). Limit of detection is estimated to be 0.3 nM (3σ method). To check the selectivity of the sensor, competitive
binding test was performed. In order to verify the interference caused by the anions on the binding of cyanide to N26e complex, the competitive experiments were accomplished in the presence of 5 equiv. of one anion out of F\(^-\), Cl\(^-\), Br\(^-\), I\(^-\), CH\(_3\)COO\(^-\), HSO\(_4^\)\(^-\), NO\(_3^\)\(^-\), PO\(_4^3^-\) and ClO\(_4^-\) along with 5 equiv of CN\(^-\). The fluorescence profile of the N26e complex with CN\(^-\) was not affected by the presence of different anions (Figure 2.150).

**Figure 2.149.** Changes in emission profile of nano-aggregates of N26e (0.45 \(\mu\)M) upon successive addition of CN\(^-\) (0-5 \(\mu\)M) (\(\lambda_{ex} = 300\) nm); (Inset: Linear regression graph between concentration of CN\(^-\) added and ratiometric change in fluorescence emission of N26e (\(\lambda_{ex} = 300\) nm).

**Figure 2.150.** Competitive binding studies of nano-aggregates of N26e containing cyanide over other selected tetrabutyl ammonium anions at \(\lambda_{ex} = 300\) nm. 1) Cyanide; 2) Cyanide +

Further, response of nano-aggregates of **N26e** for CN\(^-\) was also studied as function of time by monitoring the changes in the fluorescence spectra. To study the response time, fluorescence emission spectra of nano-aggregates of **N26e** was studied by varying the concentration of CN\(^-\) in the host solution. Experiment was performed by taking the host solution of **N26e** in 3 different volumetric flasks and added different concentration of CN\(^-\) in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. The response time of the chemosensor to CN\(^-\) ion is concentration-dependent, as the time required to reach equilibrium decrease as the concentration of CN\(^-\) increased (Figure 2.151). However, in all cases, the stable reading could be obtained within 140 seconds. Therefore, this chemosensor could be used for real time monitoring of CN\(^-\).

![Graph showing the response time of nano-aggregates of N26e for CN\(^-\) at λ\(_{ex}\) = 300 nm](image)

**Figure 2.151.** Response time of nano-aggregates of **N26e** for CN\(^-\) at λ\(_{ex}\) = 300 nm.

The effect of pH on the fluorescence emission profile of nano-aggregates of **N26e** was investigated. The experiments were carried out at a pH range from 3.0 to 13.0, with a concentration of **N26e** fixed at 20 µM (Figure 2.152). Both acidic and basic titrations were conducted by changing pH of host solution using sodium hydroxide and hydrochloric acid and then fluorescence spectra was recorded at various pH values to study the effect of pH on the fluorescence spectra of host solution. No change in the emission spectra of nano-aggregates of **N26e** was observed in the pH range from 6-9.
To evaluate the effect of ionic strength, solution of nano-aggregates of N26e (4 µM) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a solution of N26e and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of N26e remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of N26e with the increased number of ions in the solution (Figure 2.153).

**Figure 2.152.** Effect of pH on nano-aggregates of N26e (4 µM) in aqueous system ($\lambda_{ex} = 330$ nm).

**Figure 2.153.** Salt perturbation studies of nano-aggregates of N26e recorded with 4 µM concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium nitrate under the same concentration of sensor and solvent system at $\lambda_{ex} = 300$ nm.
2.2.2.5. Polymeric units having urea/thiourea moieties

2.2.2.5.1. Recognition studies of receptor 29

A 8.2 μM concentration of receptor 29 in THF exhibited the emission band centred at \( \lambda_{\text{max}} = 372 \) nm. However, the same concentrations of receptor N29 in aqueous medium (by developing nano-aggregates of receptor N29) exhibited red shift with emission band centred at 379 nm, with simultaneous enhancement in intensity of N29 (Figure 2.154). The fluorescence spectrum of nano-aggregates of N29 in water is blue shifted with enhancement in the emission profile. This phenomenon can be explained on the basis of formation of J-type aggregation. DLS studies revealed that nanoaggregates of receptor N29 have size 25 nm at 8.2 μM concentration.

![Fluorescence Emission Spectra](image)

**Figure 2.154.** Fluorescence emission spectra of receptor 29 in THF and N29 in aqueous medium (8.2 μM) (\( \lambda_{\text{ex}} = 300 \) nm).

Metal binding studies of nano-aggregates of N29 were performed in aqueous medium by addition of 5 eq. of different metal nitrate salts (1 mM) (such as Li\(^+\), Na\(^+\), K\(^+\), Cs\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\), Ba\(^{2+}\), Al\(^{3+}\), Cr\(^{3+}\), Mn\(^{2+}\), Fe\(^{2+}\), Co\(^{3+}\), Cu\(^{2+}\), Zn\(^{2+}\), Ag\(^+\), Cd\(^{2+}\), Hg\(^{2+}\) and Pb\(^{2+}\)) to the fixed concentration (8.2μM) of nano-aggregates of N29 at excitation wavelength of 300 nm. The solutions are shaken properly followed by recording the spectra for solution of each flask. The addition of different metal ions to the host did not affect the spectra of host (Figure 2.155) To evaluate the extent to which any anion may lead to change in the fluorescence.
profile of nano-aggregates of N29 (8.2 µM), 5 eq. of salts such as (F, Cl, Br, I, CN, CH₃COO, HSO₄, NO₃ and PO₄³⁻) were added in a solution containing receptor N29 (Figure 2.156). Fluorescence profile of N29 did not affect by the addition of any anion.

**Figure 2.155.** Changes in fluorescence intensity of nano-aggregates of N29 (8.2 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λ<sub>ex</sub> = 300 nm).

**Figure 2.156.** Changes in emission profile of nano-aggregates of N29 (8.2 µM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salts (5 eq.) in aqueous media (λ<sub>ex</sub> = 300 nm).

The effect of pH on the fluorescence emission profile of nano-aggregates of N29 was
investigated. The experiments were carried out at a pH range from 3.0 to 13.0, with a concentration of \textbf{N29} fixed at 8.2 µM (Figure 2.157). Both acidic and basic titrations were conducted by changing pH of host solution using sodium hydroxide and hydrochloric acid and then fluorescence spectra was recorded at various pH values to study the effect of pH on the fluorescence spectra of host solution. Basic condition did not affect the emission profile, whereas with the decrease in the pH there was enhancement in the fluorescence emission of N29.

![Figure 2.157. Effect of pH on receptor N29 (8.2 µM) in aqueous system (λ<sub>ex</sub> = 300 nm).](image)

2.2.2.5. Recognition studies of receptor 30

![Graphical abstract 2.15. Cartoon representation of binding between N30 and PO₄³⁻.](image)
A same concentration of receptor 30 (6 μM) in acetone and aqueous medium showed different fluorescence profile when excited at 280 nm. In acetone, 30 showed the emission band centred at $\lambda_{\text{max}}=345$ nm. However with the same concentration of the receptor 30 there was quenching in the fluorescence intensity of 30 (Figure 2.158). The quenching in the fluorescence profile of nano-aggregates of N30 in aqueous system can be explained by the changes in the conformation of the chromophore in aqueous system i.e. formation of H-Type of aggregates where molecules are arranged in head to head/ tail to tail direction. DLS studies revealed that nanoaggregates of receptor N30 have size 40 nm at 6 μM concentration.

![Fluorescence emission spectra of receptor 30 in acetone and N30 in aqueous medium (6 μM) at $\lambda_{\text{ex}} = 280$ nm.](image)

To evaluate the metal binding ability of nano-aggregates of N30, initial screening was carried out with a library of 19 metal salts. A solution of nano-aggregates of N30 (6 μM) was mixed with aliquots of metal salt solution and the respective emission spectra were measured ($\lambda_{\text{ex}}=280$ nm). To exclude, any kinetic effect, which may influence the fluorescence spectra, the solutions were kept for 60 minutes and fluorescence spectra were recorded again. Upon addition of an excess of 5 equivalents of various metal ions including Li$^+$, Na$^+$, K$^+$, Cs$^+$, Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$, Ba$^{2+}$, Al$^{3+}$, Cr$^{3+}$, Mn$^{2+}$, Fe$^{2+}$, Co$^{3+}$, Cu$^{2+}$, Zn$^{2+}$, Ag$^+$, Cd$^{2+}$, Hg$^{2+}$ and Pb$^{2+}$ (as their nitrate salts). No such significant change in the fluorescence intensity of N30 was observed with the addition of any of the tested metal ions (Figure 2.159).
Changes in fluorescence intensity of nano-aggregates of N30 (6 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium ($\lambda_{ex} = 280$ nm).

To evaluate the anion binding ability of sensor N30, initial screening was carried out with a library of 10 tetrabutyl ammonium anions (F$^-$, Cl$^-$, Br$^-$, I$^-$, CN$^-$, CH$_3$COO$^-$, HSO$_4^-$, PO$_4^{3-}$, NO$_3^-$, and ClO$_4^-$). The anion binding tests of nano-aggregates of N30 was done by addition of 3 eq. of tetrabutyl ammonium anions to the fixed concentration (6 µM) of nano-aggregates of N30 at excitation wavelength of 280 nm. Fluorescence spectras was recorded for each solution after proper shaking and keeping each solution for sufficient time. The anion binding tests of nano-aggregates of N30 with variety of anions have not shown any significant changes in emission spectra except for phosphate anion (Figure 2.160). With the addition of 5 eq. of phosphate anion there was enhancement in the fluorescence emission band of N30 centred at 340 nm.

Changes in emission profile of nano-aggregates of N30 (6 µM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salt (3 eq.) in aqueous media ($\lambda_{ex} = 280$ nm).
To gain more insights into the sensor activities of nano-aggregates of \textbf{N30} and \textit{PO}_4^{3-} ion, titration was performed by taking a fixed concentration of \textbf{N30} and successive addition of \textit{PO}_4^{3-} to the solution of \textbf{N30}. With the increase in the concentration of \textit{PO}_4^{3-}, there was a continuous increase in the intensity of \textbf{N30} (Figure 2.161). Concentration of \textit{PO}_4^{3-} ion was varied from 0 \(\mu\text{M}\) to 18 \(\mu\text{M}\) and titrations showed a good linearity in this concentration range of \textit{PO}_4^{3-} ion (inset of Figure 2.161). Limit of detection is estimated to be 0.8 nM (3\(\sigma\) method).

To check the selectivity of the sensor, competitive binding test was performed. To perform this study host solution was taken in different 9 volumetric flasks and then added the 20 \(\mu\text{M}\) of \textit{PO}_4^{3-} ion solution to each flask. Then the addition of remaining 9 tetrabutyl ammonium anions (\textit{F}, \textit{Cl}, \textit{Br}^-, \textit{I}^-, \textit{CN}^-, \textit{CH}_3\text{COO}^-, \textit{HSO}_4^-, \textit{NO}_3^- \text{and} \textit{ClO}_4^-) to the 9 volumetric flasks was done followed by recording fluorescence spectra for each solution after shaking the solutions properly at 300 nm. Comparison of fluorescence spectra of \textbf{host} + \textit{PO}_4^{3-} alone and of \textbf{host} + \textit{PO}_4^{3-} in the presence of other metals showed that there is no interference from the other metals and sensor is highly selective for \textit{PO}_4^{3-} (Figure 2.162).

\textbf{Figure 2.161.} Changes in emission profile of nano-aggregates of \textbf{N30} (6 \(\mu\text{M}\)) upon successive addition of phosphate (0-18 \(\mu\text{M}\)) (\(\lambda_{\text{ex}} = 280 \text{ nm}\)); (Inset: Linear regression graph between concentration of phosphate added and increase in fluorescence intensity of \textbf{N30} (\(\lambda_{\text{ex}} = 280 \text{ nm}\))).
Competitive binding studies of N30 containing phosphate over other selected tetrabutyl ammonium anions at $\lambda_{ex} = 280$ nm. 1) Phosphate; 2) Phosphate + Fluoride; 3) Phosphate + Chloride; 4) Phosphate + Bromide; 5) Phosphate + Iodide; 6) Phosphate+Cyanide; 7) Phosphate + Acetate; 8) Phosphate + Sulphate; 9) Phosphate + Nitrate; 10) Phosphate + Perchlorate.

Further, response of nano-aggregates of N30 for PO$_4^{3-}$ was also studied as function of time by monitoring the changes in the fluorescence spectra. To study the response time, fluorescence emission spectra of nano-aggregates of N30 was studied by varying the concentration of PO$_4^{3-}$ in the host solution. Experiment was performed by taking the host solution of N30 in 3 different volumetric flasks and added different concentration of PO$_4^{3-}$ in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. The response time of the chemosensor to PO$_4^{3-}$ ion is concentration-dependent, as the time required reaching equilibrium decrease as the concentration of PO$_4^{3-}$ increased (as shown in Figure 2.163). However, in all cases, the stable reading could be obtained within 200 seconds. Therefore, this chemosensor could be used for real time monitoring of PO$_4^{3-}$.

To check utility of nano-aggregates of N30 as a sensor, the emission spectra response of nano-aggregates of N30 at different pH values was monitored. The experiments were carried out at a pH range from 3.0 to 12.0, with a concentration of N30 fixed at 6 µM (Figure 2.164). Both acidic and basic titrations were conducted by changing pH of host solution using sodium hydroxide and hydrochloric acid and then fluorescence spectra was recorded at various pH values to study the effect of pH on the fluorescence spectra of host solution. For nano-aggregates of N30, in both acidic (pH< 7) and basic conditions (pH>7), the pH has no effect on the emission spectra of nano-aggregates of N30.
Figure 2.163. Response time of nano-aggregates of N30 for Phosphate ion at $\lambda_{ex} = 280$ nm.

To evaluate the effect of ionic strength, solution of nano-aggregates of N30 (6 µM) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a solution of N30 and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of N30 remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of N30 with the increased number of ions in the solution (Figure 2.165).

Figure 2.164. Effect of pH on nano-aggregates of N30 (6 µM) in aqueous system ($\lambda_{ex} = 280$ nm).
Figure 2.165. Salt perturbation studies of N30 recorded with 6 μM concentration of sensor in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of sensor and solvent system at λ_{ex} = 280 nm.

2.3. Experimental

2.3.1. General Information

All reagents were commercially available and used as received. Analytical grade solvents were used without further purification. \(^1\)H and \(^{13}\)C NMR spectra were recorded in DMSO-d6 on a Bruker Avance II 400 spectrometer (400 MHz with TMS as internal standard; chemical shifts are expressed in ppm). Mass spectra was recorded by Regional sophisticated instrumentation centre on JEOL 5×102/DA-6000 mass spectrometer of Panjab University, Chandigarh. The CHN analysis was performed using a Perkin Elmer 2400 CHN Elemental Analyser. The Photoluminescence measurements were carried on a Shimadzu Spectrofluorometer (RF-5301 PC) with fixed scanning speed, excitation and emission slit width was 10 nm. pH measurements were carried out on an ME/962P instrument. The size distribution of the complex was recorded using a Metrohm Microtrac Ultra Nanotrac Particle Size Analyser (Dynamic Light Scattering).

2.3.2. Synthesis

**Compound 3:** A solution of 3-formylchromone 1 (174 mg, 1 mmol) and N,N-dimethylpropylamine 2 (102 mg, 1 mmol) in 10 mL of dry methanol was refluxed at 80 °C for 3h. After the
completion of reaction (monitored with TLC); the solvent was evaporated under reduced pressure. The light yellow coloured solid 3 was obtained following the crystallization from chloroform hexane mixture. Yield = 85%; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 8.77 (s, 1H, CH=N), 7.75 (d, 1H, ArH), 7.60 (s, 1H, C=CH), 7.40 (t, 1H, ArH), 7.06 (m, 2H, ArH), 3.48 (t, 2H, CH$_2$), 2.52 (t, 2H, CH$_2$), 2.32 (s, 6H, CH$_3$), 1.78 (m, 2H, CH$_2$) ; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 195.8, 161.5, 153.7, 133.6, 131.7, 126.1, 118.1, 117.9, 117.6, 110.3, 56.2, 49.6, 45.5, 28.7; MS (ESI): m/z 298 (M + Ca$_2^+$); Anal. Calcd. For C$_{15}$H$_{18}$N$_2$O$_2$: C, 69.74; H, 7.02; N, 10.84; Found: C, 69.78; H, 7.02; N, 10.80%.

**Compound 5:** The compound 5 was prepared according to the literature method. Briefly, a solution of 3-formylchromone 1 (348 mg, 2 mmol) and ethylenediamine 4 (60 mg, 1 mmol) in 10 mL of dry methanol was stirred at room temperature for 2 h. After the completion of reaction (monitored with TLC), the solvent was evaporated under reduced pressure. The light yellow coloured solid 5 was obtained following the crystallization from chloroform hexane mixture. A yellow solid was obtained in 82% yield (276 mg). The $^1$H NMR spectra was matched well with literature.

**Compound 6:** Compound 6 was synthesized with condensation reaction of 1-pyrenecarboxaldehyde (230 mg, 1 mmol), urea (90 mg, 1.5 mmol) and methyl acetoacetate (139 mg, 1.2 mmol), catalyzed with Zn(ClO$_4$)$_2$.6H$_2$O (2 mol%) using methanol as a solvent. The reaction mixture was heated at reflux for 6-8 hours. The completion of the reaction was monitored by TLC (Thin layer Chromatography). After completion of reaction, crushed ice was added to the reaction mixture so that product precipitates out. These precipitate were filtered and washed with water and recrystallized from acetone/water solvent system to afford light green coloured product. Yield = 72%; $^1$H NMR (400 MHz, DMSO): $\delta$ = 9.41 (s, 1H, NH), 8.59 (d, 1H, ArH), 8.28 (m, 4H, ArH), 8.13 (d, 2H, ArH), 8.08 (t, 1H, ArH), 8.01 (d, 1H, ArH), 7.94 (s, 1H, NH), 6.39 (s, 1H, CH), 3.30 (s, 3H, OCH$_3$), 1.16 (s, 3H, CH$_3$) ; $^{13}$C NMR (100 MHz, DMSO): $\delta$ = 180.1, 165.9, 151.7, 148.9, 138.9, 130.9, 130.4, 127.5, 127.4, 127.3, 127.1, 126.3, 125.6, 125.4, 125.0, 124.9, 124.1, 124.0, 123.4, 99.5, 50.1, 30.7, 18.0; Mass (ESI): m/z 371 [M + 1]; Anal. Calcd. For C$_{23}$H$_{18}$N$_2$O$_3$: C, 77.95; H, 5.12; N, 7.90; found: C, 77.82; H, 5.01; N, 7.78%.
**Compound 7:** Compound 7 were synthesized with condensation reaction of 1-pyrenecarboxaldehyde (230 mg, 1 mmol), urea (90 mg, 1.5 mmol) and ethyl acetoacetate (156 mg, 1.2 mmol), catalyzed with Zn(ClO$_4$)$_2$.6H$_2$O (2 mol%) using methanol as a solvent. The reaction mixture was heated at reflux for 6-8 hours. The completion of the reaction was monitored by TLC (Thin layer Chromatography). After completion of reaction, crushed ice was added to the reaction mixture so that product precipitates out. These precipitate were filtered and washe with water and recrystallized from acetone/water solvent system to afford light green coloured product 7. Yield = 75%; $^1$H NMR (400 MHz, DMSO): $\delta$ = 9.3 (s, 1H, NH) , 8.5 (d, 1H, ArH), 8.2 (m, 4H, ArH), 8.1 (dd, 2H, ArH), 8.08 (t, 1H, ArH), 8.02 (d, 1H, ArH), 7.89 (s, 1H, NH), 6.39 (s,1H, CH), 3.8 (m, 2H,CH$_2$), 2.4 (s, 3H,CH$_3$), 0.7 (t, 3H, CH$_3$); $^{13}$C NMR (100 MHz, DMSO): $\delta$ = 165.2, 151.4, 148.5, 139.0, 130.8, 130.2, 130.0, 127.3, 127.2, 126.9, 126.1, 125.5, 125.2, 124.9, 124.8, 123.9, 123.8, 123.43, 99.6, 99.4, 58.95, 50.1,17.8,13.7; Mass (ESI): m/z 385.1 [M+1]; Anal. Calcd. For C$_{24}$H$_{20}$N$_2$O$_3$: C, 71.11; H, 5.19; N, 15 7.21 Found C, 71.47; H, 5.54; N, 7.28.

**Compound 8:** Compound 8 was synthesized with condensation reaction of 1-pyrenecarboxaldehyde (230 mg, 1 mmol), thiourea (114 mg, 1.5 mmol) and methyl acetoacetate (139mg, 1.2 mmol), catalyzed with Zn(ClO$_4$)$_2$.6H$_2$O (2 mol%) using methanol as a solvent. The reaction mixture was heated at reflux for 6-8 hours. The completion of the reaction was monitored by TLC (Thin layer Chromatography). After completion of reaction, crushed ice was added to the reaction mixture so that product precipitates out. These precipitate were filtered and washed with water and recrystallized from acetone/water solvent system to afford yellow coloured product 8. Yield = 85 %. $^1$H NMR (400 MHz, DMSO): $\delta$ = 10.4 (s, 1H, NH), 9.8 (s, 1H, NH), 8.6 (d, 1H, ArH), 8.3 (m, 4H, ArH), 8.2 (d, 1H, ArH), 8.1 (d, 1H, ArH), 8.0 (t, 1H, ArH), 7.9 (d, 1H, ArH), 6.3 (s, 1H, CH), 2.4 (s, 3H, OCH$_3$), 0.7 (s, 3H, CH$_3$); $^{13}$C NMR (100 MHz, DMSO): $\delta$ = 173.6, 165.5, 145.3, 137.3, 130.8, 130.3, 130.2, 127.5, 127.2, 127.1, 126.3, 125.5, 125.4, 125.1, 125.0, 123.8, 123.3, 100.9, 50.89, 50.11, 30.65, 17.24; Mass (ESI): m/z 387.1 [M+1]; Anal. Calcd. For C$_{23}$H$_{18}$N$_2$O$_5$S: C, 74.59; H, 5.74; N, 7.25; Found C, 74.64; H, 5.97; N, 7.33%.
**Compound 9**: Compounds 9 were synthesized with condensation reaction of 1-pyrenecarboxaldehyde (230 mg, 1 mmol), thiourea (114 mg, 1.5 mmol) and ethyl acetoacetate (156 mg, 1.2 mmol), catalyzed with Zn(ClO$_4$)$_2$.6H$_2$O (2 mol%) using methanol as a solvent. The reaction mixture was heated at reflux for 6-8 hours. The completion of the reaction was monitored by TLC (Thin layer Chromatography). After completion of reaction, crushed ice was added to the reaction mixture so that product precipitates out. These precipitate were filtered and washed with water and recrystallized from acetone/water solvent system to afford yellow coloured product 9. Yield = 81%; $^1$H NMR (400 MHz, DMSO): $\delta = 10.7$ (s, 1H, NH), 9.45 (d, 1H, ArH), 8.12 (m, 8H, ArH), 7.92 (s, 1H, NH), 6.28 (s, 1H, CH), 3.80 (m, 2H, CH$_2$), 2.44 (s, 3H, OCH$_3$), 0.78 (t, 3H, CH$_3$); $^{13}$C NMR (100 MHz, DMSO): $\delta = 193.3$, 165.0, 131.4, 131.0, 130.9, 130.7, 130.6, 128.7, 128.0, 127.4, 127.2, 127.0, 126.7, 126.3, 125.8, 125.5, 125.3, 124.7, 123.2, 121.8, 60.4, 51.8, 18.4, 13.9; Mass (ESI): m/z 401 [M+1]; CHN analysis calcd (for C$_{24}$H$_{20}$N$_2$O$_2$S): C, 63.14; H, 5.30; N, 8.18; Found: C, 71.89; H, 4.92; N, 6.86%.

**Compound 10**: Compound 10 was synthesized with condensation reaction of 2-hydroxynapthaldehyde (172 mg, 1 mmol), urea (90 mg, 1.5 mmol) and methyl acetoacetate (139 mg, 1.2 mmol), catalyzed with Zn(ClO$_4$)$_2$.6H$_2$O (2 mol%) using methanol as a solvent. The reaction mixture was heated at reflux for 6-8 hours. The completion of the reaction was monitored by TLC (Thin layer Chromatography). After completion of reaction, crushed ice was added to the reaction mixture so that product precipitates out. These precipitate were filtered and washed with water and recrystallized from acetone/water solvent system to afford yellow coloured product 10. Yield = 70%; $^1$H NMR (400 MHz, DMSO): $\delta = 8.0$ (s, 1H, OH), 7.6 (s, 1H, NH), 7.2 (d, 2H, ArH), 7.1 (t, 1H, ArH), 7.0 (t, 1H, ArH), 6.8 (d, 2H, ArH), 6.4 (s, 1H, NH), 5.5 (s, 1H, CH), 3.8 (s, 3H, OCH$_3$), 2.3 (s, 3H, CH$_3$); $^{13}$C NMR (100 MHz, DMSO): $\delta = 169.1$, 155.7, 152.9, 136.7, 127.0, 121.6, 121.0, 119.6, 119.1, 113.3, 11.9, 95.2, 52.03, 44.03, 16.3; Mass (ESI): m/z 338.4 [M + Na]$^+$; Anal. Calcd. For C$_{17}$H$_{16}$N$_2$O$_4$: C, 71.11; H, 5.19; N, 7.21; Found C, 71.47; H, 5.54; N, 7.28%.

**Compound 11**: Compound 11 were synthesized with condensation reaction of 2-hydroxynapthaldehyde (172 mg, 1 mmol), urea (90 mg, 1.5 mmol) and ethyl acetoacetate
(156 mg, 1.2 mmol), catalyzed with Zn(ClO$_4$)$_2$.6H$_2$O (2 mol%) using methanol as a solvent. The reaction mixture was heated at reflux for 6-8 hours. The completion of the reaction was monitored by TLC (Thin layer Chromatography). After completion of reaction, crushed ice was added to the reaction mixture so that product precipitates out. These precipitate were filtered and washed with water and recrystallized from acetone/water solvent system to afford yellow coloured product 11. Yield = 76%; $^1$H NMR (400 MHz, DMSO): $\delta$ = 7.7 (s, 1H, OH) , 7.3 (m, 2H, ArH), 7.2 (m, 4H, ArH), 6.8 (s, 1H, NH), 6.3 (s, 1H, NH), 5.5 (s, 1H, CH), 4.2 (q, 2H, CH$_2$), 2.3 (s, 3H, CH$_3$), 1.3 (t, 3H, CH$_3$); $^{13}$C NMR (100 MHz, DMSO): $\delta$ = 169.4, 156.2, 155.7, 136.7, 127.0, 121.6, 121.0, 119.6, 119.1, 113.3, 111.9, 95.7, 61.5, 44.03, 16.3, 14.7; Mass (ESI): m/z 327.2 [M+1]; Anal. Calcd. For C$_{18}$H$_{18}$N$_2$O$_4$: C, 65.38; H, 5.16; N, 8.97 Found: C, 65.38; H, 5.16; N, 8.96%.

**Compound 12:** Compound 12 was synthesized with condensation reaction of 2-hydroxynapthaldehyde (172 mg, 1 mmol), thiourea (114 mg, 1.5 mmol) and methyl acetoacetate (139 mg, 1.2 mmol), catalyzed with Zn(ClO$_4$)$_2$.6H$_2$O (2 mol%) using methanol as a solvent. The reaction mixture was heated at reflux for 6-8 hours. The completion of the reaction was monitored by TLC (Thin layer Chromatography). After completion of reaction, crushed ice was added to the reaction mixture so that product precipitates out. These precipitate were filtered and washed with water and recrystallized from acetone/water solvent system to afford yellow coloured product 12. Yield = 85%; $^1$H NMR (400 MHz, DMSO): $\delta$ = 9.5 (s, 1H, OH), 8.1 (s, 1H, NH), 7.39 (s, 1H, NH), 7.19 (m, 2H, ArH), 7.16 (t, 1H, ArH), 7.10 (dd, 2H, ArH), 7.07 (m, 1H, ArH), 5.39 (s, 1H, CH), 3.87 (s, 3H, OCH$_3$), 2.3 (s, 3H, CH$_3$); $^{13}$C NMR (100 MHz, DMSO): $\delta$ = 180.4, 169.1, 156.8, 136.7, 127.0, 121.6, 121.0, 119.6, 119.1, 113.3, 111.9, 97.6, 52.0, 45.6, 16.3; Mass (ESI): m/z 328.9 [M]; Anal. Calcd. For C$_{17}$H$_{16}$N$_2$O$_3$S: C, 62.18; H, 4.91; N, 8.53 Found: C, 62.20; H, 4.86; N, 8.50%.

**Compound 13:** Compound 13 were synthesized with condensation reaction of 2-hydroxynapthaldehyde (172 mg, 1 mmol), thiourea (114 mg, 1.5 mmol) and ethyl acetoacetate (156 mg, 1.2 mmol), catalyzed with Zn(ClO$_4$)$_2$.6H$_2$O (2 mol %) using methanol as a solvent. The reaction mixture was heated at reflux for 6-8 hours. The completion of the
reaction was monitored by TLC (Thin layer Chromatography). After completion of reaction, crushed ice was added to the reaction mixture so that product precipitates out. These precipitate were filtered and washed with water and recrystallized from acetone/water solvent system to afford yellow coloured product.

Yield = 68%; \(^1\)H NMR (400 MHz, DMSO): \(\delta = 8.1\ (s, 1H, OH), 7.9\ (s, 1H, NH), 7.8\ (m, 2H, ArH), 7.3\ (t, 1H, ArH), 7.2\ (t, 1H, ArH), 7.0\ (m, 2H, ArH), 5.3\ (s, 1H, CH), 4.2\ (q, 2H, CH\(_2\)), 3.1\ (s, 1H, NH), 1.9\ (s, 3H, OCH\(_3\)), 1.3\ (t, 3H, CH\(_3\)); \(^1^3\)C NMR (100 MHz, DMSO): \(\delta = 180.4, 169.4, 159.7, 136.7, 127.0, 121.6, 121.02, 119.6, 119.1, 113.3, 111.9, 98.0, 61.5, 45.6, 16.3, 14.7;\) Mass (ESI): \(m/z\ 343.41\ (M+1);\) Anal. Calcd. For C\(_{18}\)H\(_{18}\)N\(_2\)O\(_3\): C, 63.14; H, 5.30; N, 8.18 Found: C, 63.12; H, 5.32; N, 8.15%.

**Compound 14:** Compounds 14 was synthesized with condensation reaction of indole-3-carboxaldehyde (145 mg, 1 mmol), urea (90 mg, 1.5 mmol) and methyl acetoacetate (139 mg, 1.2 mmol), catalyzed with Zn(ClO\(_4\))\(_2\).6H\(_2\)O (2 mol %) using methanol as a solvent. The reaction mixture was heated at reflux for 6-8 hours. The completion of the reaction was monitored by TLC (Thin layer Chromatography). After completion of reaction, crushed ice was added to the reaction mixture so that product precipitates out. These precipitate were filtered and washed with water and recrystallized from acetone/water solvent system to afford pure products. Yellow coloured product in 73 % yield. \(^1\)H NMR \(\delta(ppm)\) 12.4 (s, 1H, NH), 8.6 (s, 1H, NH), 8.5 (d, 1H, ArH), 8.1 (s, 1H, NH), 8.0 (s, 1H, CH), 7.9 (m, 1H, ArH), 7.6 (d, 1H, ArH), 7.3 (m, 2H, ArH), 3.8 (s, 3H, OCH\(_3\)), 2.0 (s, 3H, CH\(_3\)); \(^1^3\)C NMR \(\delta(ppm)\) 165.1, 161.5, 151.8, 149.2, 139.1, 131.0, 124.9, 123.9, 115.9, 96.7, 59.0, 49.9, 17.6, 13.8; Mass (ESI): \(m/z\ 323\ [M+K]^+;\) CHN analysis calcd (for C\(_{15}\)H\(_{16}\)N\(_3\)O\(_3\)): C, 62.93; H, 5.63; N, 14.68; O, 16.76; found: C, 62.91; H, 5.65; N, 14.68; O, 16.76.

**Compound 15:** Compound 15 were synthesized with condensation reaction of indole-3-carboxaldehyde (145.16 mg, 1 mmol), urea (90 mg, 1.5 mmol) and ethyl acetoacetate (156 mg, 1.2 mmol), catalyzed with Zn(ClO\(_4\))\(_2\).6H\(_2\)O (2 mol%) using methanol as a solvent. The reaction mixture was heated at reflux for 6-8 hours.
The completion of the reaction was monitored by TLC (Thin layer Chromatography). After completion of reaction, crushed ice was added to the reaction mixture so that product precipitates out. These precipitate were filtered and washed with water and recrystallized from acetone/water solvent system to afford yellow coloured product 15. Yield = 70%, $^1$H NMR (400 MHz, DMSO): $\delta$ = 11.8 (s, 1H, NH), 8.5 (brs, 1H, NH), 8.3 (d, 1H, ArH), 7.9 (m, 3H, 2ArH + CH), 7.2 (d, 1H, ArH), 7.0 (d, 1H, ArH), 4.2 (q, 2H, CH$_2$), 2.0 (s, 3H, CH$_3$), 1.3 (t, 3H, CH$_3$); $^{13}$C NMR (100 MHz, DMSO): $\delta$ = 163.9,137.6, 136.4, 135.2, 133.9, 132.8, 124.7, 122.7, 121.1, 119.8, 113.5, 112.6, 60.5, 14.0; Mass (ESI): m/z 300 [M+1]; Anal. Calcd. For C$_{16}$H$_{17}$N$_3$O$_3$: C, 64.20; H, 5.72; N, 14.04 found: C, 63.97; H, 5.90; N, 14.02%.

**Compound 25a:** Compound 25a was prepared by dissolving polymer 24 (650 mg) and 2-hydroxynapthaldehyde (688 mg) in dry methanol separately. The two solutions were mixed and the reaction mixture was refluxed with stirring for 6h. Upon completion of reaction, the semi solid material was separated out. The material was washed with CH$_3$OH (50 mL) and dried under vacuum. Yield = 68%, $^1$H NMR (400 MHz, DMSO) $\delta$: 8.95 (brs, -CH=N), 7.99 (d, ArH), 7.6 (m, ArH), 7.26 (d, ArH), 7.23 (d, ArH), 7.09 (t, ArH), 6.70 (s, ArH), 3.21-3.62 (m, CH$_2$), 2.82 (s, -CH$_2$), 2.52 (s, CH$_2$); $^{13}$C NMR (100 MHz, DMSO): $\delta$ 160.7, 139.8, 134.5, 132.8, 132.7, 131.3, 131.1, 127.8, 123.9, 113.8, 62.4, 62.0, 57.8, 56.4, 54.1, 53.8, 52.3.

**Compound 25b:** Compound 25b was prepared by dissolving polymer 24 (650 mg) and pyrene-1-carboxaldehyde (919 mg) in dry methanol separately. The two solutions were mixed and the reaction mixture was refluxed with stirring for 6h. Upon completion of reaction, the semi solid material was separated out. The material was washed with CH$_3$OH (50 mL) and dried under vacuum. Yield = 77%; $^1$H NMR (400 MHz, DMSO): $\delta$ 8.17 (brs, CH=N), 7.6 (d, ArH), 7.4 (t, ArH), 7.3 (t, ArH), 7.1 (t, ArH), 6.9 (d, ArH), 3.13 (m, CH$_2$), 2.49 (m, -CH$_2$), 1.78 (m, CH$_2$), 0.91 (brs, CH$_2$); $^{13}$C NMR (100 MHz, DMSO) $\delta$: 131.0, 130.8, 130.1,
129.3, 129.0, 127.5, 125.1, 124.9, 124.3, 124.1, 118.7, 56.6, 54.3, 53.0, 49.4, 44.9, 37.7, 22.32.

**Compound 25c:** Compound 25c was prepared by dissolving polymer 24 (650 mg) and indole-3-carboxaldehyde (580 mg) in dry methanol separately. The two solutions were mixed and the reaction mixture was refluxed with stirring for 6h. Upon completion of reaction, the semi solid material was separated out. The material was washed with CH$_3$OH (50 mL) and dried under vacuum. Yield = 81%; $^1$H NMR (400 MHz, DMSO) δ: 11.03 (brs, NH), 9.87 (s, NH), 8.34 (brs, CH=N), 8.14 (m, ArH), 7.9 (brs, ArH), 7.61 (d, ArH), 7.48 (d, ArH), 6.9 (m, ArH), 6.3 (s, ArH), 3.27 (s, CH$_2$), 2.48 (m, CH$_2$); $^{13}$C NMR (100 MHz, DMSO) δ: 156.4, 137.1, 135.9, 130.8, 125.3, 120.9, 120.1, 118.8, 114.5, 111.8, 111.4, 101.0, 61.5, 54.2, 53.07, 50.73, 49.1, 35.9, 35.0, 34.0, 31.4, 28.6.

**Compound 25d:** Compound 25d was prepared by dissolving polymer 24 (650 mg) and 3-formylchromone (695 mg) in dry methanol separately. The two solutions were mixed and the reaction mixture was refluxed with stirring for 6h. Upon completion of reaction, the semi solid material was separated out. The material was washed with CH$_3$OH (50 mL) and dried under vacuum. Yield = 85%; $^1$H NMR (400 MHz, DMSO) δ: 8.3 (s, CH=N), 7.9 (m, ArH), 7.5 (brs, ArH), 7.2 (d, ArH), 6.8 (d, ArH), 6.3 (brs, NH), 6.0 (brs, NH), 5.7 (brs, NH), 3.2 (m, CH$_2$), 2.9 (s, CH$_2$), 2.0 (d, CH$_2$), 1.4 (brs, CH$_2$), 1.19 (m, CH$_2$); $^{13}$C NMR (100 MHz, DMSO) δ: 136.8, 134.5, 128.9, 128.6, 127.6, 127.5, 127.3, 125.7, 124.9, 122.34, 121.8, 118.4, 118.0, 108.5, 53.9, 52.3, 48.5, 48.5, 47.0, 41.0.
**Compound 26a:** Compound 26a was prepared by dissolving polymer 24 (650 mg) and 11 (1.30 g) in dry methanol separately. The two solutions were mixed and the reaction mixture was refluxed with stirring for 6h. Upon completion of reaction, the semi solid material was separated out. The material was washed with CH$_3$OH (50 mL) and dried under vacuum. Yield = 82 %; $^1$H NMR (400 MHz, DMSO): δ 9.3 (brs, OH), 8.9 (brs, NH), 7.9 (s, ArH), 7.3 (m, ArH), 6.6 (d, ArH), 5.3 (s, CH), 3.4 (brs, CH$_2$), 2.4 (m, CH$_2$), 1.06 (brs, CH$_3$); $^{13}$C NMR (100 MHz, DMSO) δ: 170.1, 168.9, 168.6, 159.6, 157.6, 154.4, 149.6, 148.3, 129.0, 128.8, 128.4, 126.5, 123.3, 118.9, 118.4, 114.5, 96.2, 95.3, 83.1, 60.6, 60.3, 53.6, 49.6, 53.6, 49.6, 48.6, 26.5, 23.8.

**Compound 26b:** Compound 26b was prepared by dissolving polymer 24 (650 mg) and 13 (1.37 g) in dry methanol separately. The two solutions were mixed and the reaction mixture was refluxed with stirring for 6h. Upon completion of reaction, the semi solid material was separated out. The material was washed with CH$_3$OH (50 mL) and dried under vacuum. Yield = 70 %. $^1$H NMR (400 MHz, DMSO): δ 9.0 (brs, NH), 8.2 (s, NH), 7.9 (brs, OH), 7.5 (brs, ArH), 7.3 (s, ArH), 7.1 (s, ArH), 6.6 (m, ArH), 6.6 (d, ArH), 5.3 (s, CH), 3.6 (m, -CH$_2$), 3.1 (m, -CH$_2$), 2.4 (m, -CH$_2$), 1.0 (s, -CH$_3$); $^{13}$C NMR (100 MHz, DMSO) δ: 183.8, 176.5, 168.3, 168.0, 148.3, 130.6, 130.2, 128.7, 128.4, 127.1, 123.9, 121.9, 118.0, 81.5, 60.8, 60.5, 48.6, 42.5, 30.7, 23.1.
**Compound 26c:** Compound 26c was prepared by dissolving polymer 24 (650 mg) and 7 (1.54 g) in dry methanol separately. The two solutions were mixed and the reaction mixture was refluxed with stirring for 6h. Upon completion of reaction, the semi solid material was separated out. The material was washed with CH$_3$OH (50 mL) and dried under vacuum. Yield = 76 %. $^1$H NMR (400 MHz, DMSO): δ 8.5 (s, NH), 8.5 (s, NH), 8.2 (m, ArH), 8.1 (dd, ArH), 8.0 (t, ArH), 7.9 (m, ArH), 6.3 (s, CH), 3.7 (m, CH$_2$), 3.6 (m, CH$_2$), 3.1 (m, CH$_2$), 2.5 (m, CH$_2$), 1.8 (s, CH$_3$), 1.7 (s, CH$_3$); $^{13}$C NMR (100 MHz, DMSO) δ: 165.3, 161.2, 151.5, 148.7, 139.1, 127.4, 127.3, 126.3, 125.3, 125.0, 123.5, 99.6, 76.6, 59.0, 54.5, 53.0, 50.9, 49.1, 30.7, 28.8.

**Compound 26e:** Compound 26e was prepared by dissolving polymer 24 (650 mg) and 15 (1.20 g) in dry methanol separately. The two solutions were mixed and the reaction mixture was refluxed with stirring for 6h. Upon completion of reaction, the semi solid material was separated out. The material was washed with CH$_3$OH (50 mL) and dried under vacuum. Yield = 80 %. $^1$H NMR (400 MHz, DMSO): δ 9.3 (brs, NH), 9.0 (brs, NH), 8.2 (m, ArH), 7.9 (m, ArH), 5.9 (brs, CH), 5.4 (s, CH), 3.0 (m, CH$_2$), 2.4 (m, CH$_2$), 1.9 (s, CH$_3$); $^{13}$C NMR (100 MHz, DMSO) δ: 176.5, 168.0, 148.3, 130.6, 130.2, 129.1, 128.8, 128.7, 128.4, 127.2, 124.0, 121.9, 118.1, 81.4, 60.9, 23.1.
**Compound 29:** Compound 29 was prepared by dissolving polymer 24 (650 mg) and 1-naphthylisocyanate (656 mg) in dry methanol separately. The two solutions were mixed and the reaction mixture was refluxed with stirring for 6h. Upon completion of reaction, the semi solid material was separated out. The material was washed with CH$_3$OH (50 mL) and dried under vacuum. Yield = 78%; $^1$H NMR (400 MHz, DMSO): $\delta$ 8.4 (s, NH), 7.9 (m, ArH), 7.4 (m, ArH), 3.5 (brs, CH$_2$), 2.5 (m, CH$_2$). $^{13}$C NMR (100 MHz, DMSO) $\delta$: 150.5, 136.8, 134.5, 128.9, 128.6, 127.6, 127.5, 127.3, 125.7, 124.9, 122.3, 121.8, 118.4, 118.0, 108.5, 53.9, 52.7, 48.8, 48.5, 47.07.

**Compound 30:** Compound 30 was prepared by dissolving polymer 24 (650 mg) and 1-naphthylisothiocyanate (740 mg) in dry methanol separately. The two solutions were mixed and the reaction mixture was refluxed with stirring for 6h. Upon completion of reaction, the semi solid material was separated out. The material was washed with CH$_3$OH (50 mL) and dried under vacuum. Yield = 79%; $^1$H NMR (400 MHz, DMSO): $\delta$ 7.9 (m, NH), 7.3 (m, ArH), 7.1 (t, ArH), 7.0 (d, ArH), 6.9 (brs, NH), 6.6 (d, ArH), 5.6 (brs, NH), 3.6 (brs, CH$_2$), 2.5 (m, CH$_2$), 2.3 (d, CH$_2$), 1.9 (brs, -CH$_2$), 1.7 (s, CH$_2$) 1.5 (m, CH$_2$); $^{13}$C NMR (100 MHz, DMSO) $\delta$: 182.6, 161.7, 144.9, 134.7, 134.4, 130.3, 128.6, 127.2, 126.0, 124.1, 122.8, 115.8, 107.9, 56.2, 54.1, 52.6, 47.1, 42.6.
2.4. References


