CHAPTER 3

SYNTHESIS & RECOGNITION PROPERTIES

OF UREA/THIOUREA BASED TRIPODAL RECEPTORS

3.1. Introduction

Wide spread use of urea 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.¹ The H-bond donor tendencies can be enhanced by introducing electron withdrawing groups. To attain a particular selectivity, metal ions can be used. Complexation of ligand with metal ion can result in proper orientation of H-bond donor group and further provides a suitable binding site for anion.² Among various biologically important anions, phosphate ion and their derivatives, such as HPO$_4^{2-}$, pyrophosphate (PP$_i$, P$_2$O$_7^{4-}$), adenosine triphosphate (ATP), adenosine diphosphate (ADP), CTP$_3$, IP$_3$, and phosphoproteins have play essential role in signal transduction and energy storage in living systems and gene construction.³ A number of optical techniques have been reported in the literature for the recognition of phosphate containing anions. One of the main limitations of these compounds was that most of them have no or low solubility in water. Hence all the studies have to be performed in organic solvent or mixed aqueous organic solvent which restrict their applications in real samples. Conversion of the organic compound to fluorescent organic nanoparticles (FONPs) is one of the best ways for the real sample analysis (environmental samples (tap water, river water, sea salt etc.) and biological samples (urine, blood serum etc.)). Fluorescent organic nanoparticles (FONPs) are developed from organic compound by injecting the non-aqueous solution of organic compound in aqueous medium while sonicating. Lack of insolubility of organic compound in water force the organic molecules to get aggregated in roughly spherical particles. Size of the fluorescent organic nanoparticles (FONPs) can easily be controlled by the concentration of the compound, temperature (at which sonication performed), and speed of injecting. Fluorescent organic nanoparticles (FONPs) can be used for cation and anion sensing in aqueous medium. Moreover, fluorescent organic nanoparticles (FONPs) can also be used as chemosensors for the detection of biologically important ions and biomolecules e.g NAD, NADH, NADP, ATP, ADP, AMP, DNA, Cytosine, Uracil and Adenine, etc in aqueous medium.
3.2. Result and Discussion

3.2.1. Synthesis of urea/thiourea based tripodal receptors (33-40)

![Chemical Structure]

The receptors (33-40) were synthesized by the condensation of N,N-dimethyl-1,3-propanediamine and appropriate aldehyde (a-b), followed by reduction with NaBH₄ and further reaction of the reduced product with isothiocyanate/isothiocyanate (d-g) (Scheme 3.1). Progress of the reaction was checked by TLC. After the completion of the reaction, solvent was removed under reduced pressure and solid products were recrystallized in methanol to obtain pure products. The compounds (33-40) were fully characterized with CHN analysis, ¹H and ¹³C NMR and mass spectroscopy. Compound 31a formed from the condensation reaction between N,N-dimethyl-1,3-propanediamine and pyrene-1-carboxaldehyde using methanol as a solvent. Compound 31a was obtained in 90% yield.
Compound 32a was synthesized by reduction of compound 31a with NaBH₄ in methanol. Solvent was evaporated and the reduced product was extracted from CHCl₃:H₂O mixture. Compound 32a was obtained in 85% yield. Compound 33 was synthesized by refluxing solution of 32a and p-nitrophenyl isothiocyanate in dry CHCl₃ for 10 hours. A brown coloured product separated out in 72% yield, which was washed with CHCl₃ many times. The mass spectrum indicates MS m/z at 439. ¹H NMR of compound 33 showed two multiplets at 7.7 (3H, ArH), 1.4 (2H, CH₂), four triplets at 7.4 (1H, ArH), 7.3 (1H, ArH), 3.2 (2H, CH₂), 2.2 (2H, CH₂), three doublets at 8.2 (2H, ArH), 7.8 (2H, ArH), 7.0 (1H, ArH), and four singlets at 11.4 (1H, OH), 9.7 (1H, NH), 4.5 (2H, CH₂), 2.0 (6H, CH₃). ¹³C NMR of compound 33 showed signal at 56.2 due to CH₂N, at 51.9, 49.3 and 44.7 due to CH₂ and at 18.8 due to CH₃ and signals at 150.8, 142.6, 141.4, 131.0, 129.0, 128.2, 127.9, 126.3, 124.6, 124.2, 122.7, 120.6, 117.6 and 108.0 are due to aromatic carbons. Signal at 187.5 is due to C=S group. Compound 34 was synthesized by refluxing solution of 32a and p-nitrophenyl isocyanate in dry CHCl₃ for 10 hours. A yellow coloured product separated out in 69% yield, which was washed with CHCl₃ many times. The mass spectrum indicates MS m/z at 423. ¹H NMR of compound 34 showed three multiplets at 7.8 (2H, ArH), 7.4 (2H, ArH), 1.9 (2H, CH₂), four triplets at 7.3 (1H, ArH), 6.8 (1H, ArH), 3.5 (2H, CH₂), 2.5 (2H, CH₂), four doublets at 7.9 (1H, ArH), 7.7 (1H, ArH), 7.1 (1H, ArH), 6.9 (1H, ArH) and four singlets at 10.7 (1H, OH), 10.5 (1H, NH), 4.5 (2H, CH₂) 2.1 (6H, CH₃). ¹³C NMR of compound 35 showed signals at 48.3 due to CH₂N, at 40.4, 38.8 and 36.9 due to CH₂, at 18.3 due to CH₃ and signals at 151.8, 129.5, 129, 126.8, 124.8, 123.2, 120.6, 120.5, 119.5, 116.9, 116.8, 115.6, 113.4 and 112.5 are due to aromatic carbons. Signal at 154.7 is due to C=O group. Compound 35 was synthesized by refluxing solution of 32a and 1-naphthylisothiocyanate in dry CHCl₃ for 10 hours. A brown coloured product separated out in 68% yield, which was washed with CHCl₃ many times. The mass spectrum indicates MS m/z at 443. ¹H NMR of compound 35 showed five multiplets at 7.8 (2H, ArH), 7.4 (4H, ArH), 6.8 (1H, ArH), 3.7 (2H, CH₂), 1.8 (2H, CH₂), two triplets at 7.1 (1H, ArH), 2.3 (2H, CH₂), two triplets at 7.7 (1H, ArH), 6.7 (1H, ArH), 6.6 (1H, ArH), and four singlets at 10.7 (1H, OH), 9.2 (1H,NH), 2.0 (6H, CH₃), 5.1 (2H, CH₂). ¹³C NMR of compound 35 showed signal at 59.4 due to CH₂N, at 50.3, 40.1 and 18.9 is due to CH₂, at 13.9 due to CH₃ 162.1, 146.8, 145.4, 141.9, 140.4, 139.9, 131.7, 126.4, 122.0, 120.5, 116.7, 115.4, 114.7, 109.6 and 98.0 are due to aromatic carbons. Signal at 164.8 is due to C=S group. Compound 36 was synthesized by refluxing solution of 32a and 1-naphthylisocyanate in dry CHCl₃ for 10 hours. After the completion of the reaction, a brown coloured jelly is formed in 76% yield, which was washed with CHCl₃
many times. The mass spectrum indicates MS m/z at 428. \(^1\)H NMR of compound 36 showed four multiplets at 7.9 (2H, ArH), 7.6 (2H, ArH), 7.5 (4H, ArH), 1.7 (2H, CH\(_2\)), three triplets at 7.1 (1H, ArH), 3.4 (2H, CH\(_2\)), 2.2 (2H, CH\(_2\)), two doublets at 7.8 (1H, ArH), 6.6 (2H, ArH), and four singlets at 9.5 (1H, OH), 9.3 (1H,NH), 4.5 (2H, CH\(_2\)), 2.0 (6H, CH\(_3\)). \(^{13}\)C NMR of compound 36 showed signal at 54.5 due to CH\(_2\)N, at 48.6, 44.3, 43.7 due to CH\(_2\), at 24.7 due to CH\(_3\) and signals at 156.7, 140.2, 135.8, 134.3, 133.7, 129.2, 128.4, 128.2, 128.0, 125.9, 125.3, 123.7, 122.8, 122.6, 121.3, 120.4, 118.1, 117.4, 114.1 and 113.8 are due to aromatic carbons. Signal at 157.5 is due to C=O group. 9.5 (br, 1H, OH), 9.3 (br, 1H,NH), 8.1 (d, 1H, ArH), 7.9 (m, 2H, ArH), 7.8 (d, 1H, ArH), 7.6 (m, 1H, ArH), 7.5 (m, 2H, ArH), 7.1 (t, 1H, ArH), 6.7(d, 2H, ArH), 6.6 (d, 1H, ArH), 4.5 (s, 2H, CH\(_2\)), 3.4 (t, 2H, CH\(_2\)), 2.2 (t, 2H, CH\(_2\)), 2.0 (s, 6H, CH\(_3\)), 1.7 (m, 2H, CH\(_2\)); Compound 31b formed from the condensation reaction between N,N-dimethyl-1,3-propanediamine and 2-hydroxynaphthaldehyde using methanol as a solvent. Compound 32a was obtained in 90% yield. Compound 32b was synthesized by reduction of compound 31b with NaBH\(_4\) in methanol. Solvent was evaporated and the reduced product was extracted from CHCl\(_3\):H\(_2\)O mixture. Compound 32b was obtained in 90% yield. Compound 37 was synthesized by refluxing solution of 32b and p-nitrophenyl isothiocyanate in dry CHCl\(_3\) for 10 hours. A brown coloured product separated out in 74% yield, which was washed with CHCl\(_3\) many times. The mass spectrum indicates MS m/z at 497. \(^1\)H NMR of compound 37 showed four multiplets at 8.1 (6H, ArH), 7.9 (4H, ArH), 7.76 (2H, ArH), 1.3 (2H, CH\(_2\)), two triplets at 3.6 (2H, CH\(_2\)), 2.1 (3H, CH\(_2\)), one doublet at 8.3 (1H, ArH), 7.6 (2H, ArH), and three triplets at 11.8 (1H, NH), 5.9 (2H, CH\(_2\)), 2.2 (6H, CH\(_3\)). \(^{13}\)C NMR of compound 37 showed signal at 53.8 due to CH\(_2\)N, at 52.8, 46.8, 44.5 due to CH\(_2\) at 24.81 due to CH\(_3\) and signals at 148.3, 142.9, 131.2, 131.1, 130.7, 129.5, 129.4, 128.2, 127.5, 126.3, 126.2, 125.4, 124.9, 124.8, 124.6, 124.4, 122.8, 122.2 and 113.3 are due to aromatic carbons. Signal at 184.2 is due to C=S group. Compound 38 was synthesized by refluxing solution of 32b and p-nitrophenyl isocyanate in dry CHCl\(_3\) for 10 hours. A brown coloured product separated out in 70% yield, which was washed with CHCl\(_3\) many times. The mass spectrum indicates MS m/z at 481. \(^1\)H NMR of compound 38 showed three multiplets at 8.1 (5H, ArH), 7.9 (6H, ArH), 1.7 (2H, CH\(_2\)), two triplets at 2.8 (2H, CH\(_2\)), 2.3 (2H, CH\(_2\)), two doublets at 8.3 (d, 1H, ArH), 7.5 (d, 1H, ArH), and three singlets at 11.1 (brs, 1H, NH), 4.4 (2H, CH\(_2\)), 2.1 (6H, CH\(_3\)). \(^{13}\)C NMR of compound 38 showed signal at 58.0 due to CH\(_2\)N, at 51.8, 48.4 and 45.5 due to CH\(_2\), at 27.9 due to CH\(_3\) and signals at 147.8, 141.4, 133.8, 131.3, 131.2, 130.8, 130.7, 129.4, 128.1, 127.6, 127.4, 127.1, 126.8, 125.9, 125.2, 124.8, 124.7, 123.1, 122.9 and 117.5 are due to
aromatic carbons. Signal at 156.8 is due to C=O group. Compound 39 was synthesized by refluxing solution of 32b and 1-naphthylisothiocynate in dry CHCl₃ for 10 hours. A brown coloured product separated out in 71% yield, which was washed with CHCl₃ many times. The mass spectrum indicates MS m/z at 502. **¹H NMR** of compound 39 showed four multiplets at 8.1 (4H, ArH), 7.8 (5H, ArH), 7.5 (4H, ArH), 1.2 (2H, CH₂), two triplets at 3.7 (2H, CH₂), 2.0 (2H, CH₂), four doublets at 8.4 (d, 1H, ArH), 7.9 (1H, ArH), 7.7 (1H, ArH), 7.6 (1H, ArH) and three singlets at 11.2 (1H, NH), 5.9 (2H, CH₂), 1.8 (6H, CH₂). **¹³C NMR** of compound 39 showed signal at 54.4 due to CH₂N, at 54.3, 46.8 and 44.5 are due to CH₂, at 25.4 due to CH₃ and signals at 137.9, 134.5, 131.3, 131.0, 130.9, 129.7, 128.5, 128.0, 127.5, 127.4, 127.1, 127.0, 126.2, 126.1, 126.0, 125.7, 125.6, 125.3, 125.0, 124.9, 124.8, 123.7 and 123.6 are due to aromatic carbons. Signal at 185.8 is due to C=S group. Compound 40 was synthesized by refluxing solution of 32b and 1-naphthylisocyanate in dry CHCl₃ for 10 hours. A brown coloured product separated out in 72% yield, which was washed with CHCl₃ many times. The mass spectrum indicates MS m/z at 486. **¹H NMR** of compound 40 showed five multiplets at 8.2 (4H, ArH), 8.0 (3H, ArH), 7.8 (2H, ArH), 7.5 (4H, ArH), 1.3 (2H, CH₂); three triplets at 7.6 (2H, ArH), 3.5 (2H, CH₂), 2.2 (2H, CH₂), one doublet at 8.5 (1H, ArH) and three singlets at 10.3 (1H, NH), 5.4 (2H, CH₂), 1.9 (6H, CH₃). **¹³C NMR** of compound 40 showed signals at 61.52 due to CH₂N, at 53.9, 47.6 and 44.1 due to CH₂, at 25.0 due to CH₃ and signals at 135.8, 134.0, 132.6, 131.3, 131.0, 130.9, 129.5, 128.7, 128.5, 128.4, 127.9, 127.4, 127.3, 127.1, 126.2, 126.1, 126.0, 125.8, 125.5, 125.3, 125.0, 124.7, 124.1, 123.4, 122.3 and 120.2 are due to aromatic carbons. Signal at 158.0 is due to C=S group.

### 3.2.2. Recognition studies of urea/thiourea based tripodal receptors

The recognition properties of the polymeric receptors (33-40) have been evaluated with the help of fluorescence spectroscopy.

#### 3.2.2.1. Recognition studies of receptor 33

A 5 μM concentration of receptor 33 in acetonitrile exhibited the emission band centred at λₘₚₓ = 344 nm. However, the same concentrations of receptor 33 in aqueous medium (by developing nano-aggregates of 33) exhibited red shift with emission band centred at 371 nm (Figure 3.1). The bathochromic shift of N33 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. DLS studies revealed that nano-aggregates of receptor N33 have size 20 nm at 5 µM concentration.

![Fluorescence emission spectra of receptor 33 in acetonitrile and of N33 in aqueous medium (5 µM) at λ_ex = 299 nm.](image)

**Figure 3.1.** Fluorescence emission spectra of receptor 33 in acetonitrile and of N33 in aqueous medium (5 µM) at λ_ex = 299 nm.

The metal binding affinity of nano-aggregates of N33 was evaluated in aqueous medium. A solution of nano-aggregates of N33 (5 µM) was mixed with aliquots of metal salt solution and the respective emission spectra were measured (λ_ex=299 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). The emission profile of nano-aggregates of N33 was not perturbed by any of the tested metal ions (Figure 3.2). Therefore, nano-aggregates of N33 have no binding affinity towards any of the tested metal ions. The anion binding abilities of nano-aggregates of N33 were assessed from the modulation of emission band of nano-aggregates of N33. 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 N33 was done by addition of 5eq. of tetrabutyl ammonium anions (5 mM) to the fixed concentration (5 µM) of nano-aggregates of N33 at excitation wavelength of 299 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 N33 with variety of anions have not shown any significant changes in emission spectra (Figure 3.3).
Figure 3.2. Changes in fluorescence intensity of nano-aggregates of N33 (5 µM) upon addition of a particular metal nitrates (5 eq.) in aqueous medium (λ<sub>ex</sub> = 299 nm).

Figure 3.3. Changes in emission profile of nano-aggregates of N33 (5 µM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salt (5 eq.) in aqueous media (λ<sub>ex</sub> = 299 nm).

Binding affinity of compound was checked against 10 biomolecules. For this experiment, initial screening was carried out with a library of 10 biomolecules (NAD, NADH, NADP, ATP, ADP, AMP, DNA, Cytosine, Uracil and Adenine). The anion binding tests of nano-aggregates of N33 was done by addition of 5 eq. of different biomolecules to the fixed concentration (5 µM) of nano-aggregates of N33 at excitation wavelength of 299 nm. Fluorescence spectras were recorded for each solution after proper shaking and keeping each solution for sufficient time. The nano-aggregates of N33 with variety of biomolecules have
not shown any significant changes in emission spectra (Figure 3.3).

**Figure 3.3.** Changes in emission profile of nano-aggregates of N33 (5 µM) in aqueous medium upon addition of a different biomolecules (5 eq.) in aqueous media (λ_{ex} = 299 nm).

To check utility of nano-aggregates of N33 as a sensor, the emission spectra response of nano-aggregates of N33 at different pH values was monitored. The experiments were carried out at a pH range from 2.0 to 12.0, with a concentration of N33 fixed at 10 µM (Figure 3.5). 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 N33, in both acidic (pH< 7) and basic conditions (pH>7), the pH has no effect on the emission spectra of nano-aggregates of N33.

**Figure 3.5.** Effect of pH on nano-aggregates of N33 (5 µM) in aqueous system (λ_{ex} = 299 nm).
3.2.2.2. Recognition studies of receptor 34

Graphical abstract 3.1. Cartoon representation showing enhancement in fluorescence intensity after interactions of N34 with NADP.

A 0.8 µM concentration of receptor 34 in acetonitrile exhibited the emission band centred at $\lambda_{\text{max}} = 356$ nm. However, the same concentrations of nano-aggregates of N34 in aqueous medium exhibited red shift with emission band centred at 351 nm (Figure 3.6). The bathochromic shift of nano-aggregates of N34 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. DLS studies revealed that nano-aggregates of receptor N34 have size 10 nm at 0.8 µM concentration.

Figure 3.6. Fluorescence emission spectra of receptor 34 in acetonitrile (0.8 µM) and of N34 in aqueous medium (0.8 µM) at $\lambda_{\text{ex}} = 278$ nm.
To evaluate the metal binding ability of nano-aggregates of N34, initial screening was carried out with a library of 19 metal salts. A solution of nano-aggregates of N34 (0.8 µM) was mixed with aliquots of metal salt solution and the respective emission spectra were measured ($\lambda_{ex}=278$ 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). The emission profile of nano-aggregates of N34 was not perturbed by any of the tested metal ions (Figure 3.7). Therefore, nano-aggregates of N34 have no binding affinity towards any of the tested metal ions.

Figure 3.7. Changes in fluorescence intensity of nano-aggregates of N34 (0.8 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium ($\lambda_{ex} = 278$ nm).

Anion binding ability of nano-aggregates of N34 was checked by addition of 4 µM of 10 tetrabutyl ammonium anions (F$^-$, Cl$^-$, Br$^-$, I$^-$, CN$^-$, CH$_3$COO$^-$, HSO$_4^-$, PO$_4^{3-}$, NO$_3^-$ and ClO$_4^-$) in host solution of N34 (0.8 µM) in water ($\lambda_{ex} = 278$ 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. No changes were observed in the emission band of N34 at 350 nm (Figure 3.8).
**Figure 3.8.** Changes in emission profile of nano-aggregates of N34 (0.8 µM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salt (5 eq.) in aqueous media (λ<sub>ex</sub> = 278 nm).

**Figure 3.9** Changes in emission profile of nano-aggregates of N34 (0.8 µM) in aqueous medium upon addition of a different biomolecules (1 eq.) in aqueous media (λ<sub>ex</sub> = 278 nm).
The biomolecule binding studies of nano-aggregates of N34 were assessed from the modulation of emission spectra of nano-aggregates of N34. The biomolecule binding tests of nano-aggregates of N34 was done by addition of 1 eq. of different biomolecules to the fixed concentration (0.8 µM) of nano-aggregates of N34 at excitation wavelength of 278 nm. Fluorescence spectra were recorded for each solution after proper shaking and keeping each solution for sufficient time. The nano-aggregates of N34 with variety of biomolecules have not shown any significant changes in emission spectra. Whereas the additions of NADP into the solution of N34 resulted in enhancement in the fluorescence intensity of N34 with red shift (Figure 3.9).

To further check the binding action of nano-aggregates of N34 towards NADP ion, titration experiment was carried out by adding small aliquots of NADP (0-1 µM) to the solution of nano-aggregates of N34 (0.8 µM) in aqueous medium. With the increase in the concentration of host there was a continuous increase in the intensity of N34. Concentration of NADP was varied from 0 µM to 1 µM and titrations showed a good linearity in this concentration range of NADP ion (inset of Figure 3.10(A)). Limit of detection is estimated to be 0.2 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 9 volumetric flasks and then added the 1 µM of NADP ion solution to each flask. Then the addition of remaining 9 biomolecules (NAD, NADH, DNA, ATP, ADP, AMP, Cytosine, Uracil and Adenine) to the 9 volumetric flasks was done followed by recording fluorescence spectra for each solution after shaking the solutions properly. As shown in Figure 3.10(B). Nano-aggregates of N34 has a high selectivity for estimation of NADP, even in the presence of other metal ions.

In order to study the response time of nano-aggregates of N34 for NADP ion, the fluorescence spectra were recorded upon addition of different concentrations of NADP ion (6, 20, 30, 40 µM) to the solutions of nano-aggregates of N34 (4.5 µM) and each solution was analyzed as a function of time. The interpretation of results revealed that response time of sensor is indirectly proportional to the concentration of NADP ion. As the concentration of NADP increases the response time decrease i.e. at higher concentration of guest, sensor is taking less time to be stable (Figure 3.11).

To check utility of nano-aggregates of N34 as a sensor, the emission spectra response of nano-aggregates of N34 at different pH values was monitored. The experiments were
carried out at a pH range from 2.0 to 12.0, with a concentration of N34 fixed at 10 µM (Figure 3.12). 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 N34, 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 N34.

**Figure 3.10.** (A) Changes in emission profile of nano-aggregates of N34 (0.8 µM) upon successive addition of NADP (0-1 µM) (λex = 278 nm); (Inset: Linear regression graph between concentration of NADH added and increase in fluorescence intensity of N34 (λex = 278 nm); (B) Competitive binding studies of N34 (0.8 µM) containing NADP with selected biomolecules at λex = 278 nm. 1) NADP; 2) NADP + NAD; 3) NADP + NADH; 4) NADP + AMP; 5) NADP+ ADP; 6) NADP + ATP; 7) NADP + DNA; 8) NADP + Cytosine; 9) NADP + Uracil; 10) NADP + Adenine.
Figure 3.11. Response time of nano-aggregates of N34 for NADP at $\lambda_{ex} = 278$ nm.

![Graph showing response time of nano-aggregates of N34 for NADP at $\lambda_{ex} = 278$ nm.]

Figure 3.12. Effect of pH on nano-aggregates of N34 (0.8 $\mu$M) in aqueous system ($\lambda_{ex} = 278$ nm).

![Graph showing effect of pH on nano-aggregates of N34 (0.8 $\mu$M) in aqueous system ($\lambda_{ex} = 278$ nm).]

To evaluate the effect of ionic strength, solution of nano-aggregates of N34 (0.8 $\mu$M) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a solution of N34 and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of N34 remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of N34 with the increased number of ions in the solution (Figure 3.13).
Figure 3.13. Salt perturbation studies of N34 recorded with 0.8 μM concentration of nano-aggregates of N34 in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of N34 and solvent system at λ_{ex} = 278 nm.

3.2.2.3. Recognition studies of receptor 35

Effect of water content on the fluorescence profile of receptor 35 was evaluated by recording fluorescence spectra of receptor 35 in both acetonitrile as well as aqueous system (by developing nano-aggregates of 35). The fluorescence spectra of 35 in organic solvent system showed a significant difference in the emission profile than in aqueous system (Figure 3.14). Increased water content resulted in formation of aggregates, which induces slight blue shift in the fluorescence profile of 35. DLS studies revealed that nano-aggregates of receptor N35 have size 25 nm at 1 μM concentration. To check the metal binding ability of nano-aggregates of N35, initial screening was carried out with a library of 19 metal salts. A solution of nano-aggregates of N35 (1 μM) was mixed with aliquots of metal salt solution and the respective emission spectra were measured (λ_{ex}=278 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 N35 was observed with the addition of any other tested metal ions under the same conditions (Figure 3.15).
Graphical abstract 3.2. Cartoon representation showing quenching of fluorescence intensity after interactions of N35 with NADH.
Figure 3.14. Fluorescence emission spectra of receptor 35 in acetonitrile (2 µM) and of N35 in aqueous medium (1 µM) at $\lambda_{ex} = 278$ nm.

Figure 3.15. Changes in fluorescence intensity of nano-aggregates of N35 (1 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium ($\lambda_{ex} = 278$ nm).

To influence of the anions binding on the nano-aggregates of 35 in aqueous medium was investigated. Fluorescence response was observed after the addition of 5 eq. 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 N35 was done by addition of 5eq. of tetrabutyl ammonium anions to the fixed concentration (1 μM) of nano-aggregates of N35 at excitation wavelength of 278 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 N35 with variety of anions have not shown any significant changes in emission spectra (Figure 3.16).

![Figure 3.16. Changes in emission profile of nano-aggregates of N35 (1 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salt (5 eq.) in aqueous media (λex = 278 nm).](image)

Influence of the biomolecules addition on the nano-aggregates solution of 35 in aqueous medium was investigated. Fluorescence response was observed after the addition of the 10 biomolecules (NAD, NADH, NADP, ATP, ADP, AMP, DNA, Cytosine, Uracil and Adenine). The biomolecule binding tests of nano-aggregates of N35 was done by addition of 0.05 eq. of different biomolecules to the fixed concentration (1 μM) of nano-aggregates of N35 at excitation wavelength of 320 nm. Fluorescence spectra were recorded for each solution after proper shaking and keeping each solution for sufficient time. The nano-aggregates of N35 with variety of biomolecules have not shown any significant changes in emission spectra except NADH. Additions of NADH have pronounced quenching in the
fluorescence intensity of N35 with red shift (Figure 3.17).

![Fluorescence Spectra](image)

**Figure 3.17.** Changes in emission profile of nano-aggregates of N35 (1 µM) in aqueous medium upon addition of a different biomolecules (1 eq.) in aqueous media ($\lambda_{ex} = 278$ nm).

To learn more about the properties of N35 as a sensor for NADH, a fluorescence titration was carried out by adding incremental amount of NADH (0-20 nM) to the solution of N35 in aqueous medium. With the increase in the concentration of host there was a continuous decrease in the intensity of N35 (Figure 3.18). Concentration of NADH was varied from 0 nM to 20 nM and titrations showed a good linearity in this concentration range of NADH ion (inset of Figure 3.18). Limit of detection is estimated to be 3 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 10 volumetric flasks and then added the 1.8 nM of NADH ion solution to each flask. Then the addition of remaining 9 biomolecules (NAD, NADP, ATP, ADP, AMP, Cytosine, Uracil and Adenine) to the 9 volumetric flasks was done followed by recording fluorescence spectra for each solution after shaking the solutions properly.

Influence of other biomolecules on the sensing behaviour of N35 was investigated. Comparison of fluorescence spectra of host + NADH alone and of host + NADH in the presence of other metals showed that there is no interference from the other metals and sensor is highly selective for NADP (Figure 3.19).
Figure 3.18. Changes in emission profile of nano-aggregates of N35 (1 µM) upon successive addition of NADH (0-20 nM) ($\lambda_{ex} = 278$ nm); (Inset: linear regression graph between concentration of NADH added and decrease in fluorescence intensity of N35 ($\lambda_{ex} = 278$ nm).

Figure 3.19. Competitive binding studies of N35 (1 µM) containing NADH with selected biomolecules at $\lambda_{ex} = 320$ nm. 1) NADH; 2) NADH + NAD; 3) NADH + NADP; 4) NADH + AMP; 5) NADH + ADP; 6) NADH + ATP; 7) NADH + DNA; 8) NADH + Cytosine; 9) NADH + Uracil; 10) NADH + Adenine.

Further, response of nano-aggregates of N35 for NADH 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 N35 was studied by varying the concentration of NADH in the host solution. Experiment was performed by taking the host
solution of N35 in 3 different volumetric flasks and added different concentration of NADH in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. Response time of sensor is independent to the concentration of NADH ion (Figure 3.20).

![Figure 3.20. Response time of nano-aggregates of N35 for NADH at λ<sub>ex</sub> = 278 nm.](image)

To check utility of nano-aggregates of N35 as a sensor, the emission spectra response of nano-aggregates of N35 at different pH values was monitored. The experiments were carried out at a pH range from 2.0 to 12.0, with a concentration of N35 fixed at 1 µM (Figure 3.21). 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 N35, 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 N35.

![Figure 3.21. pH effect of nano-aggregates of N35 (1 µM) in aqueous system (λ<sub>ex</sub> = 278 nm).](image)
To evaluate the effect of ionic strength, solution of nano-aggregates of N35 (1 µM) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a solution of N35 and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of N35 remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of N35 with the increased number of ions in the solution (Figure 3.22).

![Fluorescence Emission Spectra](image)

**Figure 3.22.** Salt perturbation studies of N35 recorded with 1 µM concentration of N35 in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of N35 and solvent system at λ_{ex} = 278 nm.

3.2.2.4. Recognition studies of receptor 36

Effect of water content on the fluorescence profile of receptor 36 was evaluated by recording fluorescence spectra of receptor 36 in both acetonitrile as well as in aqueous system (by developing nano-aggregates). The fluorescence spectra of 36 in organic solvent system showed a significant difference in the emission profile than in aqueous system (Figure 3.23). Increased water content resulted in formation of aggregates, which induces decrease in the fluorescence intensity of 36 with slight red shift in the λ_{max}. This is due to the phenomena known as “aggregation caused quenching” (ACQ). DLS studies revealed that nano-aggregates of receptor N36 have size 20 nm at 0.3 µM concentration.
Graphical abstract 3.3. Cartoon representation showing interactions of N36 with Cs\(^+\) and DNA.

Figure 3.23. Fluorescence emission spectra of receptor 36 in acetonitrile (0.3 µM) and of N36 in aqueous medium (0.3 µM) at \(\lambda_{ex} = 282\) nm.

To evaluate the metal binding ability of nano-aggregates of N36, initial screening was carried out with a library of 19 metal salts. A solution of nano-aggregates of N36 (0.3 µM) was mixed with aliquots of metal salt solution and the respective emission spectra were
measured ($\lambda_{ex}=282$ 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 N36 was observed with the addition of any other tested metal ions under the same conditions except Cs$^+$ ion (Figure 3.24). With the addition of 5 eq. there was enhancement in the fluorescence intensity of the nano-aggregates of N36.

To evaluate the anion binding ability of N36, 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 N36 was done by addition of 5eq. of tetrabutyl ammonium anions (5 mM) to the fixed concentration (0.3 $\mu$M) of nano-aggregates of N36 at excitation wavelength of 300 nm. Fluorescence spectra was recorded for each solution after proper shaking and keeping each solution for sufficient time. The anion binding tests of nano-aggregates of N36 with variety of anions have not shown any significant changes in emission spectra (Figure 3.25).

**Figure 3.24.** Changes in fluorescence intensity of nano-aggregates of N36 (0.3 $\mu$M) upon addition of a particular metal nitrates (5eq.) in aqueous medium ($\lambda_{ex} = 282$ nm).
Figure 3.25. Changes in emission profile of nano-aggregates of N36 (0.3 μM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salt (5 eq.) in aqueous media (λ\text{ex} = 282 nm).

To check the binding ability of biomolecules for sensor N36, initial screening was carried out with a library of 10 biomolecules (NAD, NADH, NADP, ATP, ADP, AMP, DNA, Cytosine, Uracil and Adenine). The biomolecule binding abilities of nano-aggregates of N36 was done by addition of 5 eq. of different biomolecules to the fixed concentration (0.3 μM) of nano-aggregates of N36 at excitation wavelength of 282 nm. Fluorescence spectra were recorded for each solution after proper shaking and keeping each solution for sufficient time. The nano-aggregates of N36 with variety of biomolecules have not shown any significant changes in emission spectra except DNA. Additions of DNA have resulted in a quenching of fluorescence intensity of N36 (Figure 3.26). To gain more insights into the sensor activities of nano-aggregates of N36 and Cs\textsuperscript{+} ion, titration was performed by taking a fixed concentration of N36 and successive addition of Cs\textsuperscript{+} ion to the solution of N36. With the increase in the concentration of Cs\textsuperscript{+}, there was a continuous increase in the intensity of N36 (Figure 3.27). Concentration of Cs\textsuperscript{+} ion was varied from 0 μM to 1.5 μM and titrations showed a good linearity in this concentration range of Cs\textsuperscript{+} ion (inset of Figure 3.27). Limit of detection is estimated to be 0.08 nM (3σ method).
Figure 3.26. Changes in emission profile of nano-aggregates of N36 (0.3 µM) in aqueous medium upon addition of a different biomolecules (0.1 eq.) in aqueous media ($\lambda_{ex} = 282$ nm).

Figure 3.27. Changes in emission profile of nano-aggregates of N36 (0.3 µM) upon successive addition of Cs$^+$ (0-1.5 µM) ($\lambda_{ex} = 282$ nm); (inset: Linear regression graph between concentration of Cs$^+$ added and increase in fluorescence intensity of N36).
A chemosensor should have high selectivity. So competitive experiments were performed for the estimation of Cs\(^+\) (5 eq.) by nano-aggregates of 8c in the presence of any of Li\(^+\), Na\(^+\), K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\), Ba\(^{2+}\), Al\(^{3+}\), Cr\(^{3+}\), Mn\(^{2+}\), Fe\(^{2+}\), Co\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Ag\(^+\), Cd\(^{2+}\), Hg\(^{2+}\) and Pb\(^{2+}\) (5 eq.). As shown in Figure 3.28, no significant variation in the intensity was detected by comparing the intensity with and without other metal ions. Therefore, nano-aggregates of 8c have a high selectivity for estimation of Cs\(^+\), even in the presence of other metal ions.

**Figure 3.28.** Competitive binding studies of N36 containing Cs\(^+\) with selected Metal Ions at \(\lambda_{ex} = 282\) nm. 1) Cs\(^+\) only; 2) Cs\(^+\) + Li\(^+\); 3) Cs\(^+\) + Na\(^+\); 4) Cs\(^+\) + K\(^+\); 5) Cs\(^+\) + Mg\(^{2+}\); 6) Cs\(^+\) + Ca\(^{2+}\); 7) Cs\(^+\) + Sr\(^{2+}\); 8) Cs\(^+\) + Ba\(^{2+}\); 9) Cs\(^+\) + Al\(^{3+}\); 10) Cs\(^+\) + Cr\(^{3+}\); 11) Cs\(^+\) + Mn\(^{2+}\); 12) Cs\(^+\) + Fe\(^{3+}\); 13) Cs\(^+\) + Co\(^{2+}\); 14) Cs\(^+\) + Cu\(^{2+}\); 15) Cs\(^+\) + Zn\(^{2+}\); 16) Cs\(^+\) + Ag\(^+\); 17) Cs\(^+\) + Cd\(^{2+}\); 18) Cs\(^+\) + Hg\(^{2+}\); 19) Cs\(^+\) + Pb\(^{2+}\).

Further, response of nano-aggregates of N36 for Cs\(^+\) 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 N36 was studied by varying the concentration of Cs\(^+\) in the host solution. Experiment was performed by taking the host solution of N36 in 3 different volumetric flasks and added different concentration of Cs\(^+\) in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. The response time of the chemosensor to Cs\(^+\) ion is concentration-dependent, as the time required to reach equilibrium decrease as the concentration of Cs\(^+\) increased (as shown in Figure 3.29). However, in all cases, the stable reading could be obtained within 140 seconds. Therefore, this chemosensor could be used for real time monitoring of Cs\(^+\).
To gain more insights into the binding behaviour of nano-aggregates N36 and DNA ions, titration was performed by taking a fixed concentration of N36 and successive addition of DNA to the solution of N36. With the increase in the concentration of DNA there was a continuous decrease in the intensity of N36 (Figure 3.30). Concentration of DNA was varied from 0 nM to 80 nM and titrations showed a good linearity in this concentration range of DNA ion (inset of Figure 8). Limit of detection is estimated to be 8.3 nM (3σ method).

**Figure 3.29.** Response time of nano-aggregates of N36 for Cs⁺ at λₑₓ = 282 nm.

**Figure 3.30.** Changes in emission profile of nano-aggregates of N36 (0.3 µM) upon successive addition of DNA (0-80 nM) (λₑₓ = 282 nm); (inset: Linear regression graph between concentration of DNA added and decrease in fluorescence intensity of N36).
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 2 µM of DNA ion solution to each flask. Then the addition of remaining 9 biomolecules (NAD, NADH, NADP, ATP, ADP, AMP, Cytosine, Uracil and Adenine) to the 9 volumetric flasks was done followed by recording fluorescence spectra for each solution after shaking the solutions properly at 398 nm. Comparison of fluorescence spectra of host + DNA alone and of host + DNA in the presence of other metals showed that there is no interference from the other metals and sensor is highly selective for DNA (Figure 3.31).

![Figure 3.31. Competitive binding studies of N36 (0.3 µM) containing DNA with selected biomolecules at λ<sub>ex</sub> = 320 nm. 1) DNA; 2) DNA + NAD; 3) DNA + NADH; 4) DNA + NADP; 5) DNA + AMP; 6) DNA + ADP; 7) DNA + ATP; 8) DNA + Cytosine; 9) DNA + Uracil; 10) DNA + Adenine.](image)

Further, response of nano-aggregates of N36 for DNA 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 N36 was studied by varying the concentration of DNA in the host solution. Experiment was performed by taking the host solution of N36 in 3 different volumetric flasks and added different concentration of DNA in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. Response time of sensor is independent to the concentration of DNA ion (Figure 3.32).
Figure 3.32. Response time of nano-aggregates of N36 for DNA at $\lambda_{ex} = 320$ nm.

To check utility of nano-aggregates of N36 as a sensor, the emission spectra response of nano-aggregates of N36 at different pH values was monitored. The experiments were carried out at a pH range from 2.0 to 12.0, with a concentration of N36 fixed at 0.3 µM (Figure 3.33). 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 N36, in both acidic (pH< 7) and basic conditions (pH>7), the emission spectra of nano-aggregates of N36 changed with the pH of the solution.

Figure 3.33. Effect of pH on nano-aggregates of N36 (0.3 µM) in aqueous system ($\lambda_{ex} = 282$ nm).

To evaluate the effect of ionic strength, solution of nano-aggregates of N36 (10 µM) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a
solution of N36 and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of N36 remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of N36 with the increased number of ions in the solution (Figure 3.34).

![Figure 3.34](image_url)

**Figure 3.34.** Salt perturbation studies of N36 recorded with 0.3 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> = 282 nm.

3.2.2.5. Recognition studies of receptor 37

Effect of water content on the fluorescence profile of receptor 37 was evaluated by recording fluorescence spectra of receptor 37 in both THF as well as aqueous system (by developing nano-aggregates). The fluorescence spectra of receptor 37 in organic solvent system showed a significant difference in the emission profile than in aqueous system (Figure 3.35). Increased water content resulted in formation of aggregates, which induces large red shift in the fluorescence intensity of 37. DLS studies revealed that nano-aggregates of receptor N37 have size 50 nm at 6 µM concentration. To evaluate the metal binding ability of nano-aggregates of N37, initial screening was carried out with a library of 19 metal salts. A solution of nano-aggregates of N37 (6 µM) was mixed with aliquots of metal salt solution and the respective emission spectra were measured (λ<sub>ex</sub>=320 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. Results of investigations demonstrate that upon addition of different metal ions (5 eq.) in aqueous solution of nano-aggregates of N37 display no selectivity for any metal ion (Figure 3.36).
Figure 3.35. Fluorescence emission spectra of receptor 37 in acetonitrile and of N37 in aqueous medium (6 µM) at $\lambda_{ex} = 320$ nm.

![Fluorescence emission spectra of receptor 37 and N37](image)

Figure 3.36. Changes in fluorescence intensity of nano-aggregates of N37 (6 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium ($\lambda_{ex} = 320$ nm).

To check the anion binding ability of sensor N37, 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 N37 was done by addition of 5eq. of tetrabutyl ammonium anions (5 mM) to the fixed concentration (6 µM) of nano-aggregates of N37 at excitation wavelength of 320 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 N37 with variety of anions have not shown any significant changes in emission spectra (Figure 3.37).
Figure 3.37. Changes in emission profile of nano-aggregates of N37 (6 µM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salt (5 eq.) in aqueous media (λ_{ex} = 320 nm).

Biomolecule binding studies of N37 were performed in aqueous medium by addition of 5 eq. of 10 biomolecules (NAD, NADH, NADP, ATP, ADP, AMP, DNA, Cytosine, Uracil and Adenine) to the fixed concentration (6 µM) of N37 at excitation wavelength of 320 nm. The solutions are shaken properly followed by recording the spectra for solution of each flask. The addition of different biomolecules to the host did not affect the spectra of N37. The nano-aggregates of N37 with variety of biomolecules have not shown any significant changes in emission spectra (Figure 3.38).

Figure 3.38. Changes in emission profile of nano-aggregates of N37 (6 µM) in aqueous medium upon addition of a different biomolecules (1 eq.) in aqueous media (λ_{ex} = 320 nm).
To check utility of nano-aggregates of N37 as a sensor, the emission spectra response of nano-aggregates of N37 at different pH values was monitored. The experiments were carried out at a pH range from 2.0 to 12.0, with a concentration of N37 fixed at 10 µM (Figure 3.39). 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 N37, 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 N37.

![Fluorescence spectra vs pH](image)

**Figure 3.39.** Effect of pH on nano-aggregates of N37 (6 µM) in aqueous system (λ_ex = 320 nm).

3.2.2.6. Recognition studies of receptor 38

![Graphical abstract 3.4](image)

**Graphical abstract 3.4.** Cartoon representation showing interactions of nano-aggregates of N38 with Cu²⁺.
Effect of water content on the fluorescence profile of 38 was evaluated by recording fluorescence spectra of receptor 38 in both THF as well as aqueous system (by developing nano-aggregates). The fluorescence spectra of receptor 38 in organic solvent system showed a significant difference in the emission profile than in aqueous system (Figure 3.40). Increased water content resulted in formation of aggregates results in a new fluorescence band at centered at 480 nm. DLS studies revealed that nano-aggregates of receptor N38 have size 28 nm at 3 µM concentration.

![Figure 3.40. Fluorescence emission spectra of receptor 38 in THF and of N38 in aqueous medium (3 µM) at λ_ex = 280 nm.](image)

The effect of a wide range of environmentally and physiologically active metal ions was investigated for nano-aggregates of N38 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 (3 µM) (λ_max=280 nm). Results of investigations demonstrate that upon addition of different metal ions (5 eq.) in aqueous solution of nano-aggregates of N38 display no selectivity for any metal ion except Cu2+. With the addition of 5 eq. Cu2+ there was decrease in the fluorescence intensity of N38 (Figure 3.41). To evaluate the anion binding ability of N38, initial screening was carried out with a library of 10 tetrabutyl ammonium anions (F−, Cl−, Br−, I−, CN−, CH3COO−, HSO4−, PO4 3−, NO3− and ClO4−). The anion binding tests of nano-aggregates of N38 was done by addition of 5eq. of tetrabutyl ammonium anions (5 mM) to the fixed concentration (3 µM) of nano-aggregates of N38 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 N38 with variety of anions have not shown any significant changes in emission spectra (Figure 3.42).

Figure 3.41. Changes in fluorescence intensity of nano-aggregates of N38 (3 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λ<sub>ex</sub> = 280 nm).

Figure 3.42. Changes in emission profile of nano-aggregates of N38 (3 µM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salt (5 eq.) in aqueous media (λ<sub>ex</sub> = 280 nm).
The biomolecule binding behaviour of nano-aggregates of \textbf{N38} was studied in aqueous medium. For this initial screening was carried out with a library of 10 biomolecules (NAD, NADH, NADP, ATP, ADP, AMP, DNA, Cytosine, Uracil and Adenine). The biomolecule binding tests of nano-aggregates of \textbf{N38} was done by addition of 5 eq. of different biomolecules to the fixed concentration (3 µM) of nano-aggregates of \textbf{N38} at excitation wavelength of 280 nm. Fluorescence spectra were recorded for each solution after proper shaking and keeping each solution for sufficient time. The nano-aggregates of \textbf{N38} with variety of biomolecules have not shown any significant changes in emission spectra (Figure 3.43).

![Figure 3.43. Changes in emission profile of nano-aggregates of N38 (3 µM) in aqueous medium upon addition of a different biomolecules (1 eq.) in aqueous media (λ\textsubscript{ex} = 280 nm).](image)

To learn the binding behaviour of nano-aggregates \textbf{N38} and Cu\textsuperscript{2+} ions, titration was performed by taking a fixed concentration of \textbf{N38} and successive addition of Cu\textsuperscript{2+} to the solution of \textbf{N38}. With the increase in the concentration of host there was a continuous decrease in the intensity of \textbf{N38} (Figure 3.44). Concentration of copper ion was varied from 0 µM to 16 µM and titrations showed a good linearity in this concentration range of Cu\textsuperscript{2+} ion (inset of Figure 3.44). Limit of detection is estimated to be 0.03 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 19 volumetric flasks and then added the 15 µM of Cu\textsuperscript{2+} ion solution to each flask. Then the addition of remaining 18 metal solutions (Li\textsuperscript{+}, Na\textsuperscript{+}, K\textsuperscript{+}, Cs\textsuperscript{+}, Mg\textsuperscript{2+}, Ca\textsuperscript{2+}, Sr\textsuperscript{2+}, Ba\textsuperscript{2+}, Al\textsuperscript{3+}, Cr\textsuperscript{3+}, Mn\textsuperscript{2+}, Fe\textsuperscript{2+}, Co\textsuperscript{3+}, Zn\textsuperscript{2+}, Ag\textsuperscript{+}, Cd\textsuperscript{2+}, Hg\textsuperscript{2+} and Pb\textsuperscript{2+}) to
the 18 volumetric flasks was done followed by recording fluorescence spectra for each solution after shaking the solutions properly. Comparison of fluorescence spectra of host + \( \text{Cu}^{2+} \) alone and of host + \( \text{Cu}^{2+} \) in the presence of other metals showed that there is no interference from the other metals and sensor is highly selective for \( \text{Cu}^{2+} \) (Figure 3.45).

**Figure 3.44.** Changes in emission profile of nano-aggregates of \( \text{N}38 \) (3 \( \mu \text{M} \)) upon successive addition of \( \text{Cu}^{2+} \) (0-18 \( \mu \text{M} \)) \((\lambda_{\text{ex}} = 280 \text{ nm})\); (Inset: Linear regression graph between concentration of \( \text{Cu}^{2+} \) added and decrease in emission of \( \text{N}38 \) \((\lambda_{\text{ex}} = 280 \text{ nm})\).

**Figure 3.45.** Competitive binding studies of \( \text{N}38 \) containing \( \text{Cu}^{2+} \) with selected Metal ions at \( \lambda_{\text{ex}} = 300 \text{nm} \). 1) \( \text{Cu}^{2+} \) only; 2) \( \text{Cu}^{2+} + \text{Li}^+ \); 3) \( \text{Cu}^{2+} + \text{Na}^+ \); 4) \( \text{Cu}^{2+} + \text{K}^+ \); 5) \( \text{Cu}^{2+} + \text{Cs}^+ \); 6) \( \text{Cu}^{2+} + \text{Mg}^{2+} \); 7) \( \text{Cu}^{2+} + \text{Ca}^{2+} \); 8) \( \text{Cu}^{2+} + \text{Sr}^{2+} \); 8) \( \text{Cu}^{2+} + \text{Ba}^{2+} \); 10) \( \text{Cu}^{2+} + \text{Al}^{3+} \); 11) \( \text{Cu}^{2+} + \text{Cr}^{3+} \); 12) \( \text{Cu}^{2+} + \text{Mn}^{2+} \); 13) \( \text{Cu}^{2+} + \text{Fe}^{3+} \); 14) \( \text{Cu}^{2+} + \text{Co}^{2+} \); 15) \( \text{Cu}^{2+} + \text{Zn}^{2+} \); 16) \( \text{Cu}^{2+} + \text{Ag}^+ \); 17) \( \text{Cu}^{2+} + \text{Cd}^{2+} \); 18) \( \text{Cu}^{2+} + \text{Hg}^{2+} \); 19) \( \text{Cu}^{2+} + \text{Pb}^{2+} \).
Besides high sensitivity and selectivity, a short response time is other one necessity for a fluorescent chemosensor to monitor Cu$^{2+}$ in real-time. To study the response time, fluorescence emission spectra of nano-aggregates of N38 was studied by varying the concentration of Cu$^{2+}$ in the host solution. Experiment was performed by taking the host solution of N38 in 3 different volumetric flasks and added different concentration of Cu$^{2+}$ in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. The response time of the chemosensor to Cu$^{2+}$ ion is concentration-independent, as the time required to reach equilibrium does not affect with Cu$^{2+}$ 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 Cu$^{2+}$ (Figure 3.46).

![Graph](image)

**Figure 3.46.** Response time of nano-aggregates of N38 for NADP at $\lambda_{ex} = 280$ nm.

To check utility of nano-aggregates of N38 as a sensor, the emission spectra response of nano-aggregates of N38 at different pH values was monitored. The experiments were carried out at a pH range from 2.0 to 12.0, with a concentration of N38 fixed at 10 µM (Figure 3.47). Both acidic and basic titrations showed not much variation in the fluorescence intensity of nano-aggregates of N38. To evaluate the effect of ionic strength, solution of nano-aggregates of N38 (3 µM) was made and then 100 equivalents of tetrabutyl ammonium perchlorate was added to a solution of N38 and then kept for half an hour to attain equilibrium. Fluorescence emission spectra of N38 remain almost undisturbed in the presence of increased ionic strength showing negligible interaction of N38 with the increased number of ions in the solution (Figure 3.48).
Figure 3.47. Effect of pH on nano-aggregates of N38 (3 µM) in aqueous system ($\lambda_{ex} = 280$ nm).

![Figure 3.47](image)

Figure 3.48. Salt perturbation studies of N38 recorded with 3 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 $\lambda_{ex} = 280$ nm.

3.2.2.7. Recognition studies of receptor 39

Effect of water content on the fluorescence profile of receptor 39 was evaluated by recording fluorescence spectra of receptor 39 in both THF as well as aqueous system (by developing nano-aggregates). The fluorescence spectra of 39 in organic solvent system showed a significant difference in the emission profile than in aqueous system (Figure 3.49). Increased water content resulted in formation of aggregates, which induces decrease in the fluorescence intensity of N39. This is due to the phenomena known as “aggregation caused quenching”
(ACQ). DLS studies revealed that nano-aggregates of receptor N39 have size 27 nm at 2 µM concentration.

**Graphical abstract 3.5.** Cartoon representation showing interaction of N39 with DNA.

**Figure 3.49.** Fluorescence emission spectra of receptor 39 in acetonitrile and of N39 in aqueous medium (2 µM) at λex = 320 nm.

To evaluate the metal binding ability of nano-aggregates of N39, initial screening was carried out with a library of 19 metal salts. A solution of nano-aggregates of N39 (2 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 N39 was observed with the addition of any other tested metal ions under the same conditions (Figure 3.50).

**Figure 3.50.** Changes in fluorescence intensity of nano-aggregates of N39 (2 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λ<sub>ex</sub> = 320 nm).

To evaluate the anion binding ability of sensor N39, 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 N39 was done by addition of 5eq. of tetrabutyl ammonium anions (5 mM) to the fixed concentration (2 µM) of nano-aggregates of N39 at excitation wavelength of 320 nm. Fluorescence spectra was recorded for each solution after proper shaking and keeping each solution for sufficient time. The anion binding tests of nano-aggregates of N39 with variety of anions have not shown any significant changes in emission spectra (Figure 3.51).

Binding affinity of biomolecules for N39 was carried out with a library of 10 biomolecules (NAD, NADH, NADP, ATP, ADP, AMP, DNA, Cytosine, Uracil and Adenine). The anion binding tests of nano-aggregates of N39 was done by addition of 5 eq. of different biomolecules to the fixed concentration (2 µM) of nano-aggregates of N39 at excitation wavelength of 320 nm. Fluorescence spectra were recorded for each solution after proper shaking and keeping each solution for sufficient time. The nano-aggregates of N39 with variety of biomolecules have not shown any significant changes in emission spectra except DNA. Additions of DNA have pronounced quenching in the fluorescence intensity of N39
Figure 3.51. Changes in emission profile of nano-aggregates of N39 (2 µM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salt (5 eq.) in aqueous media ($\lambda_{ex} = 320$ nm).

To gain more insights into the binding behaviour of nano-aggregates N39 and DNA ions, titration was performed by taking a fixed concentration of N39 and successive addition of DNA to the solution of N39. With the increase in the concentration of DNA there was a continuous decrease in the intensity of N39 (Figure 3.53). Concentration of DNA was varied from 0 µM to 1 µM and titrations showed a good linearity in this concentration range of DNA ion (inset of Figure 3.53). Limit of detection is estimated to be 5 nM ($3\sigma$ method).

Figure 3.52. Changes in emission profile of nano-aggregates of N39 (2 µM) in aqueous medium upon addition of a different biomolecules (1 eq.) in aqueous media ($\lambda_{ex} = 398$ nm).
Figure 3.53. Changes in emission profile of nano-aggregates of N39 (2 µM) upon successive addition of DNA (0-1 µM) ($\lambda_{ex} = 398$ nm); (inset: Linear regression graph between concentration of DNA added and increase in fluorescence intensity of N39).

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 2 µM of DNA ion solution to each flask. Then the addition of remaining 9 biomolecules (NAD, NADH, NADP, ATP, ADP, AMP, Cytosine, Uracil and Adenine) to the 9 volumetric flasks was done followed by recording fluorescence spectra ($\lambda_{ex} = 320$ nm) for each solution after shaking the solutions properly. Comparison of fluorescence spectra of host + DNA alone and of host + DNA in the presence of other metals showed that there is no interference from the other metals and sensor is highly selective for DNA (Figure 3.54).

Further, response of nano-aggregates of N39 for DNA 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 N39 was studied by varying the concentration of DNA in the host solution. Experiment was performed by taking the host solution of N39 in 3 different volumetric flasks and added different concentration of DNA in each flask. Then fluorescence spectra of each sample were taken after fixed interval of times. Response time of sensor is directly proportional to the concentration of DNA ion (Figure 3.55).
Figure 3.54. Competitive binding studies of N39 (2 µM) containing DNA with selected biomolecules at λ<sub>ex</sub> = 320 nm. 1) DNA; 2) DNA + NAD; 3) DNA + NADH; 4) DNA + NADP; 5) DNA + AMP; 6) DNA+ ADP; 7) DNA + ATP; 8) DNA + Cytosine; 9) DNA + Uracil; 10) DNA + Adenine.

Figure 3.55. Response time of nano-aggregates of N39 for NADP at λ<sub>ex</sub> = 320 nm.

In order to find a suitable pH span in which N39 can selectively detect DNA, acid/basic titrations was performed. In a pH range from 3 to 12.0, there was negligible change in the fluorescence intensity of nano-aggregates of N39 (2 µM) (Figure 3.56). In other words we can say that the nano-aggregates of N39 were insensitive to change in pH.
Figure 3.56. Effect of pH on nano-aggregates of N39 (2 μM) in aqueous system (λ_Ex = 320 nm).

Perturbation of high ionic strength was ruled out by comparison of fluorescence spectra of nano-aggregates of N39 (2 μM) with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium nitrate under the same host concentration (Figure 3.57).

Figure 3.57. Salt perturbation studies of N39 recorded with 2 μM concentration of nano-aggregates of N39 in aqueous system with the respective fluorescence spectrum recorded upon addition of 100 equiv. of tetrabutyl ammonium perchlorate under the same concentration of nano-aggregates of N39 and solvent system at λ_Ex = 320 nm.
3.2.2.8. Recognition studies of receptor 40

Effect of water content on the fluorescence profile of receptor 40 was evaluated by recording fluorescence spectra of receptor 40 in both THF as well as aqueous system (by developing nano-aggregates). The fluorescence spectra of receptor 40 in organic solvent system showed a significant difference in the emission profile than in aqueous system (Figure 3.58). Increased water content resulted in formation of new band centered at 472 nm. DLS studies revealed that nano-aggregates of receptor N40 have size 20 nm at 3 µM concentration.

![Figure 3.58. Fluorescence emission spectra of receptor 40 in acetonitrile and of N40 in aqueous medium (3 µM) at λex = 283 nm.](image)

To evaluate the metal binding ability of nano-aggregates of N40, initial screening was carried out with a library of 19 metal salts. A solution of nano-aggregates of N40 (3 µM) was mixed with aliquots of metal salt solution and the respective emission spectra were measured (λex=283 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). The metal binding studies suggested that none of the metal ions i.e. Li+, Na+, K+, Cs+, Mg2+, Ca2+, Sr2+, Ba2+, Al3+, Cr3+, Mn2+, Fe2+, Co3+, Cu2+, Zn2+, Ag+, Cd2+, Hg2+ and Pb2+ was unable to change the fluorescence profile of nano-aggregates of N40 (Figure 3.59).
Figure 3.59. Changes in fluorescence intensity of nano-aggregates of N40 (3 µM) upon addition of a particular metal nitrates (5eq.) in aqueous medium (λ<sub>ex</sub> = 283 nm).

To evaluate the anion binding ability of N40, 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 N40 was done by addition of 5eq. of tetrabutyl ammonium anions (5 mM) to the fixed concentration (3 µM) of nano-aggregates of N40 at excitation wavelength of 283 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 N40 with variety of anions have not shown any significant changes in emission spectra (Figure 3.60).

Figure 3.60. Changes in emission profile of nano-aggregates of N40 (3 µM) in aqueous medium upon addition of a particular tetrabutyl ammonium anion salt (5 eq.) in aqueous media (λ<sub>ex</sub> = 283 nm).
To check the binding ability of biomolecules for N40, initial screening was carried out with a library of 10 biomolecules (NAD, NADH, NADP, ATP, ADP, AMP, DNA, Cytosine, Uracil and Adenine). The anion binding tests of nano-aggregates of N40 was done by addition of 5 eq. of different biomolecules to the fixed concentration (3 µM) of nano-aggregates of N40 at excitation wavelength of 283 nm. Fluorescence spectra were recorded for each solution after proper shaking and keeping each solution for sufficient time. The nano-aggregates of N40 with variety of biomolecules have not shown any significant changes in emission spectra (Figure 3.61).

![Fluorescence spectra of nano-aggregates of N40 with various biomolecules](image)

**Figure 3.61.** Changes in emission profile of nano-aggregates of N40 (3 µM) in aqueous medium upon addition of a different biomolecules (5 eq.) in aqueous media (λ_{ex} = 283 nm).

To check utility of nano-aggregates of N40 as a sensor, the emission spectra response of nano-aggregates of N40 at different pH values was monitored. The experiments were carried out at a pH range from 2.0 to 12.0, with a concentration of N40 fixed at 2 µM (Figure 3.62). 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 going from pH 7 to 3 there was sudden increase in the fluorescence intensity and then it almost remains constant. Whereas, going from pH 7 to 11.2 there was slight decrease in the fluorescence intensity.

237
3.3. Experimental

3.3.1. 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-$d_6$ 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).

3.3.2. Synthesis

**Compound 33**: Compound 33 was synthesized by refluxing the solution of 32a (727 mg, 2.8 mmol) and 1-isothiocyanato-4-nitrobenzene (504 mg, 2.8 mmol) in dry CHCl$_3$. The reaction mixture was refluxed for 10 hours. A brown coloured product separated out, which was washed with CHCl$_3$ many times. Yield = 72%; $^1$H NMR (400 MHz, DMSO) $\delta$: 11.4 (brs, 1H, OH), 9.7 (brs, 1H, NH), 8.2 (d, 2H, ArH), 7.8 (d, 2H, ArH), 7.7 (m, 3H, ArH), 7.4
(t, 1H, ArH), 7.3 (t, 1H, ArH), 7.0 (d, 1H, ArH), 4.5 (s, 2H, CH₂), 3.2 (t, 2H, CH₂), 2.2 (t, 2H, CH₂), 2.0 (s, 6H, CH₃), 1.4 (m, 2H, CH₂); 
\(^{13}\text{C NMR}\) (100 MHz, DMSO) δ: 187.5, 150.8, 142.6, 141.4, 131.0, 129.0, 128.2, 127.9, 126.3, 124.6, 124.2, 122.7, 120.6, 117.6, 108.0, 56.2, 51.9, 49.3, 44.7, 18.8; ESI-MS m/z = 439 [M+1]^+; CHN analysis calculated for (C\(_{23}\)H\(_{26}\)N\(_4\)O\(_3\)S\(_1\)): C, 62.99; H, 5.98; N, 12.78%; Found: C, 62.95, H, 5.99; N, 12.70%.

**Compound 34:**- Compound 34 was synthesized by refluxing the solution of 32a (727 mg, 2.8 mmol) and 1-isocyanato-4-nitrobenzene (459 mg, 2.8 mmol) in dry CHCl\(_3\). The reaction mixture was refluxed for 10 hours. A yellow coloured product separated out, which was washed with CHCl\(_3\) many times. Yield = 69%; 
\(^1\text{H NMR}\) (400 MHz, CDCl\(_3\)) δ: 10.7 (brs, 1H, OH), 10.5 (brs, 1H, NH), 7.9 (d, 1H, ArH), 7.8 (m, 2H, ArH), 7.7 (t, 1H, ArH), 7.4 (m, 2H, ArH), 7.2 (t, 1H, ArH), 7.1 (d, 1H, ArH), 6.9 (d, 1H, ArH), 6.8 (t, 1H, ArH), 4.5 (s, 2H, CH₂), 3.5 (t, 2H, CH₂), 2.5 (t, 2H, CH₂), 2.1 (s, 6H, CH₃), 1.9 (m, 2H, CH₂);
\(^{13}\text{C NMR}\) (100 MHz, CDCl\(_3\)) δ: 154.7, 151.8, 129.5, 129, 126.8, 124.8, 123.2, 120.6, 120.5, 119.5, 116.9, 116.8, 115.6, 113.4, 112.5, 48.3, 40.4, 38.8, 36.9, 18.3; ESI-MS m/z = 423 [M+1]^+; CHN analysis calculated for (C\(_{23}\)H\(_{26}\)N\(_4\)O\(_4\)): C, 65.39; H, 6.20%; N, 13.26% Found: C, 65.30; H, 6.22%; N, 13.20%.

**Compound 35:**- Compound 35 was synthesized by refluxing the solution of 32a (727 mg, 2.8 mmol) and 1-isothiocyanatonaphthalene (518 mg, 2.8 mmol) in dry CHCl\(_3\). The reaction mixture was refluxed for 10 hours. A brown coloured product separated out, which was washed with CHCl\(_3\) many times. Yield = 68%; 
\(^1\text{H NMR}\) (400 MHz, DMSO) δ: 10.7 (brs, 1H, OH), 9.2 (brs, 1H, NH), 7.8 (m, 2H, ArH), 7.7 (d, 1H, ArH), 7.4 (m, 4H, ArH), 7.1 (t, 1H, ArH), 6.8 (m, 1H, ArH), 6.7(d, 1H, ArH), 6.6 (d, 1H, ArH), 6.5 (s, 2H, CH₂), 3.7 (m, 2H, CH₂), 2.3 (t, 2H, CH₂), 2.0 (s, 6H, CH₃), 1.8 (m, 2H, CH₂); 
\(^{13}\text{C NMR}\) (100 MHz, DMSO) δ: 164.8, 162.1, 146.8, 145.4, 141.9, 140.4, 139.9, 131.7, 126.4, 122.0, 120.5, 116.7, 115.4, 114.7, 109.6, 98.0 59.4, 50.3, 40.1, 18.9, 13.9; ESI-MS m/z = 443 [M]^+; CHN analysis
Calculated for \( \text{C}_{27}\text{H}_{29}\text{N}_{3}\text{O}_{1}\text{S}_1 \): C, 70.10; H, 6.59; N, 9.47 Found: C, 70.19; H, 6.92; N, 9.50%.

**Compound 36:** Compound 36 was synthesized by refluxing the solution of 32a (727 mg, 2.8 mmol) and 1-isocyanatonaphthalene (473 mg, 2.8 mmol) in dry CHCl₃. The reaction mixture was refluxed for 10 hours. A brown coloured product separated out, which was washed with CHCl₃ many times. Yield = 76%; \(^1\)H NMR (400 MHz, DMSO) \( \delta \): 9.5 (br s, 1H, OH), 9.3 (br s, 1H, NH), 7.9 (m, 2H, ArH), 7.8 (d, 1H, ArH), 7.6 (m, 2H, ArH), 7.5 (m, 4H, ArH), 7.1 (t, 1H, ArH), 6.7 (m, 2H, ArH), 6.6 (d, 1H, ArH), 4.5 (s, 2H, CH₂), 3.4 (t, 2H, CH₂), 2.2 (t, 2H, CH₂), 2.0 (s, 6H, CH₃), 1.7 (m, 2H, CH₂); \(^{13}\)C NMR (100 MHz, DMSO) \( \delta \): 157.5, 156.7, 140.2, 135.8, 134.3, 133.7, 129.2, 128.4, 128.2, 128.0, 125.9, 125.3, 123.7, 122.8, 122.6, 121.3, 120.4, 118.1, 117.4, 114.1, 113.8, 54.5, 48.6, 44.3, 43.7, 24.7; ESI-MS m/z = 428 [M+1]+; CHN analysis calculated for \( \text{C}_{23}\text{H}_{29}\text{N}_{3}\text{O}_2\text{S}_1 \): C, 75.85; H, 6.84; N, 9.83 Found: C, 75.75; H, 6.80; N, 9.80%.

**Compound 37:** Compound 37 was synthesized by refluxing the solution of 32b (886 mg, 2.8 mmol) and 1-isothiocyanato-4-nitrobenzene (504 mg, 2.8 mmol) in dry CHCl₃. The reaction mixture was refluxed for 10 hours. A brown coloured product separated out, which was washed with CHCl₃ many times. Yield = 74%; \(^1\)H NMR (400 MHz, CDCl₃) \( \delta \): 11.8 (br s, 1H, NH), 8.3 (d, 1H, ArH), 8.1 (m, 6H, ArH), 7.9 (m, 4H, ArH), 7.6 (d, 2H, ArH), 5.9 (s, 2H, CH₂), 3.6 (t, 2H, CH₂), 2.2 (s, 6H, CH₃), 2.1 (t, 3H, CH₂), 1.3 (m, 2H, CH₂); \(^{13}\)C NMR (100 MHz, CDCl₃) \( \delta \): 184.2, 148.3, 142.9, 131.2, 131.1, 130.7, 129.5, 129.4, 128.2, 127.5, 126.3, 126.2, 125.4, 124.9, 124.8, 124.6, 124.4, 122.8, 122.2, 113.3, 53.8, 52.8, 46.8, 44.5, 24.81; ESI-MS m/z = 497 [M+1]+; CHN analysis calculated for \( \text{C}_{29}\text{H}_{28}\text{N}_{4}\text{O}_{2}\text{S}_1 \): C, 70.14; H, 5.68; N, 11.28 Found: C, 70.12; H, 5.65; N, 11.30%.

**Compound 38:** Compound 38 was synthesized by refluxing the solution of 32b (886 mg, 2.8 mmol) and 1-isocyanato-4-nitrobenzene (459 mg, 2.8 mmol) in dry CHCl₃. The reaction mixture was refluxed for 10 hours. A brown coloured product separated out, which was
washed with CHCl₃ many times. Yield = 70%; ¹H NMR (400 MHz, CDCl₃) δ: 11.1 (brs, 1H, NH), 8.3 (d, 1H, ArH), 8.1 (m, 5H, ArH), 7.9 (m, 6H, ArH), 7.5 (d, 1H, ArH), 4.4 (s, 2H, CH₂), 2.8 (t, 2H, CH₂), 2.3 (t, 2H, CH₂), 2.1 (s, 6H, CH₃) 1.7 (t, 2H, CH₂); ¹³C NMR (100 MHz, CDCl₃) δ: 156.8, 147.8, 141.4, 133.8, 131.3, 131.2, 130.8, 130.7, 129.4, 128.1, 127.6, 127.4, 127.1, 126.8, 125.9, 125.2, 124.8, 124.7, 123.1, 122.9, 117.5, 58.0, 51.8, 48.4, 45.5, 27.9; ESI-MS m/z = 481 [M+1]⁺; CHN analysis calculated for (C₂₉H₂₈N₄O₃): C, 72.48; H, 5.87; N, 11.66 Found: C, 71.23; H, 6.585; N, 11.55%.

**Compound 39:** Compound 39 was synthesized by refluxing the solution of 32b (886 mg, 2.8 mmol) and 1-isothiocyanatonaphthalene (518 mg, 2.8 mmol) in dry CHCl₃. The reaction mixture was refluxed for 10 hours. A brown coloured product separated out, which was washed with CHCl₃ many times. Yield = 71%; ¹H NMR (400 MHz, CDCl₃) δ: 11.2 (brs, 1H, NH), 8.4 (d, 1H, ArH), 8.1 (m, 4H, ArH), 7.9 (d, 1H, ArH), 7.8 (m, 4H, ArH), 7.7 (d, 1H, ArH), 7.6 (d, 1H, ArH), 7.5 (m, 4H, ArH), 5.9 (s, 2H, CH₂), 3.7 (t, 2H, CH₂), 2.0 (t, 2H, CH₂), 1.8 (s, 6H, CH₃), 1.2 (m, 2H, CH₂); ¹³C NMR (100 MHz, CDCl₃) δ: 185.8, 137.9, 134.5, 131.3, 131.0, 130.9, 129.7, 128.5, 128.0, 127.5, 127.4, 127.1, 127.0, 126.2, 126.1, 126.0, 125.7, 125.6, 125.3, 125.0, 124.9, 124.8, 123.7, 123.6, 54.4, 54.3, 46.9, 44.5, 25.4; ESI-MS m/z = 502 [M+1]⁺; CHN analysis calculated for (C₃₃H₃₁N₃S₁): C, 79.0; H, 6.23; N, 8.38 Found: C, 78.98; H, 6.26; N, 9.005.

**Compound 40:** Compound 40 was synthesized by refluxing the solution of 32b (886 mg, 2.8 mmol) and 1-isocyanatonaphthalene (473 mg, 2.8 mmol) in dry CHCl₃. The reaction mixture was refluxed for 10 hours. A brown coloured product separated out, which was washed with CHCl₃ many times. Yield = 72%; ¹H NMR (400 MHz, CDCl₃) δ: 10.3 (brs, 1H, NH), 8.5 (d, 1H, ArH), 8.2 (m, 4H, ArH), 8.0 (m, 3H, ArH), 7.8 (m, 2H, ArH), 7.6 (t, 2H, ArH), 7.5 (m, 4H, ArH), 5.4 (s, 2H, CH₂), 3.5 (t, 2H, CH₂), 2.2 (t, 2H, CH₂), 1.9 (s, 6H, CH₃), 1.3 (m, 2H, CH₂); ¹³C NMR (100 MHz, CDCl₃) δ:
158.0, 135.8, 134.0, 132.6, 131.3, 131.0, 130.9, 129.5, 128.7, 128.5, 128.4, 127.9, 127.4, 127.3, 127.1, 126.2, 126.1, 126.0, 125.8, 125.5, 125.3, 125.0, 124.7, 124.1, 123.4, 122.3, 120.2, 61.52, 53.9, 47.6, 44.1, 25.0; ESI-MS m/z = 486 [M+1]; CHN analysis calculated for (C$_{33}$H$_{31}$N$_{3}$O$_{1}$): C, 81.62; H, 6.43; N, 8.65 Found: C, 80.00; H, 6.40; N, 9.13%.

3.4. References

