Development of aminoethylpyridine based N,N,N,O-donor fluorescent probes for the detection of Fe$^{3+}$ and Hg$^{2+}$ in aqueous media

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Abstract. A couple of 2-aminoethylpyridine based fluorescent compounds (E)-1-((2-(5-nitropyridin-2-ylamino)ethylimino)methyl)naphthalen-2-ol (1) and (E)-4-nitro-2-((2-(5-nitropyridin-2-ylamino)ethylimino)methyl)phenol (2) have been designed and synthesized by setting up reactions of N-(2-aminoethyl)-5-nitropyridin-2-amine with 2-hydroxy-1-naphthaldehyde and 2-hydroxy-5-nitrobenzaldehyde, respectively. Both the probes have been characterized by elemental analyses, FTIR, $^1$H & $^{13}$C NMR, UV/vis and fluorescence spectral techniques. Furthermore the sensing behavior of 1 and 2 towards various metal ions were examined via UV/vis and fluorescence studies. The fluorescence of 1 and 2 enhanced upon interaction with Fe$^{3+}$ ion whereas Hg$^{2+}$ induced small fluorescence quenching of 1 and enhancement of fluorescence in 2. The selectivity and sensitivity assay ensured by 2 remains unaltered in presence of interfering metal ions. Hence, among these two chemo sensors only 2 may be useful for practical application of detection and estimation of trace Fe$^{3+}$ and Hg$^{2+}$ in real water samples and/or in biological systems.

Keywords. Heavy metal ions (HTMs), Aminoethylpyridine, N,N,N,O-donor, Fluorescent probes, Fluorescent quenching, Fluorescent Enhancement

1. Introduction

Heavy transition metal ions (HTMs) are essential elements both in the environmental and biological processes. These are considered indispensable owing to their involvement in various functions, such as electron transport, metabolism and catalysis. Therefore, it is of growing interest to design and develop novel, synthetically feasible, selective and sensitive fluorescent sensors for heavy metal ions (HTMs). Iron (Fe) is an essential trace element in living organisms (i.e. plants and animals) and plays a vital role in the life process, for instance, in enzyme catalysis, cellular metabolism, biological activities of organisms i.e. muscle contraction, nerve conduction, DNA/RNA synthesis and repair, electron transfer and enzymatic reactions, along with oxygen carrier in haemoglobin. The intake of excessive quantity of iron is detrimental or sometimes incurable to the body of the living organism whereas its deficiency causes diabetes, anemia, heart disease, cancer, and Parkinson’s disease along with organ dysfunction i.e. liver, heart and pancreas. In addition, accumulation of ferric ion (Fe$^{3+}$) causes oxidative stress, Alzheimer’s disease, and...
neurodegenerative diseases. Therefore, the development of the fluorescence sensor for selective detection of Fe$^{3+}$ in feasible medium is highly sought and quite important.

On the other hand, Mercury (Hg$^{2+}$) is a powerful neurotoxin in fishes, humans and wild species and it is widely distributed through the anthropogenic sources, barometers, thermometers, caustic soda, mercury lamps, food chain and natural environmental activities. The mercury intake in living organisms as well as in human beings through contaminated water is a growing issue, therefore its detection in water necessitates precise, ultra-sensitive, prompt and cost effective analytical methods. Furthermore, Hg$^{2+}$ exposure can lead to brain damage, heart failure, anaemia, pinks disease, and Minamata disease, along with and nervous system disorders.

Through this report, we develop the design and synthesis of aminoethylpyridine based N, N, N, O-donor fluorescent chemosensors 1 and 2 (Figure 1) for selective and sensitive detection of Hg$^{2+}$ and Fe$^{3+}$ in aqueous media (H$_2$O: EtOH, 6: 4). Notably, 1 could not function as efficient chemosensor for Hg$^{2+}$ and Fe$^{3+}$ whereas 2 serves as highly selective fluorescent ‘turn-on’ probe for these cations. Through this study, it can be claimed that probe 2 can be used as potential chemosensor for Hg$^{2+}$ and Fe$^{3+}$ ions in living biological systems / real water samples.

![Figure 1 Chemical structure of 1 and 2.](image)

2. Experimental

2.1. Materials and Methods

All reagents (N-(2-aminoethyl)-5-nitropyridin-2-amine, 2-hydroxynaphthalene-1-carbaldehyde and 2-hydroxy-5-nitrobenzaldehyde) were purchased from Sigma Aldrich Chemical Co., USA. Unless otherwise stated. All the metal salts (Ag$^+$, Cd$^{2+}$, Cu$^{2+}$, Mn$^{2+}$, Fe$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Zn$^{2+}$, Hg$^{2+}$ and Pb$^{2+}$), other commonly usable reagents and solvents were purchased from HiMedia Laboratories Pvt. Ltd., India and solvents were dried and distilled prior to their use. The reagents were purchased from commercial sources and used after purification. Solvents were dried and distilled following standard literature procedures. UV-vis spectra and FT-IR spectra were recorded at room temperature using UV-1700 Pharma Spec, Shimadzu and PerkinElmer spectrometer (Spectrum two, UK), respectively. For FT-IR studies KBr pellets and for UV-vis studies aqueous colloid of the respective materials are used. Fluorescence measurements were done on a RF-5301 Fluorescence Spectrometer with excitation and emission slit widths of 10.0 nm, respectively, whereas $^1$H- and $^{13}$C-NMR spectroscopy was performed in d$_6$-DMSO using JEOL AL 500 FT-NMR machine at an operating frequency of 500 MHz ($^1$H) and 125 MHz ($^{13}$C). Chemical shifts (δ) are specified in parts per million (ppm) DMSO-d$_6$ as a solvent and tertiarmethylsilane (TMS) as an internal reference material and splitting patterns are labelled in terms of s (singlet), d (doublet) and t (triplet).

2.2. Synthetic procedure of probes

2.2.1. Common synthetic procedure for 1 and 2

Equimolar amount of N-(2-aminoethyl)-5-nitropyridin-2-amine and aldehyde in MeOH was refluxed for 4-6 h. The volume of resulting solution mixture was reduced to half under vacuum and left for slow evaporation. The precipitate thus obtained was filtered off and washed with methanol followed by diethyl ether, dried under vacuum and recrystallized using absolute alcohol.
2.2.1.1. Synthesis of (E)-1-((2-(5-nitropyridin-2-ylamino) ethylimino) methyl) naphthalen-2-ol; (I).

Yield (89 %); was confirmed by FTIR, $^1$H NMR, $^{13}$C NMR and elemental analyses; Analytical data: Anal. Calc. for C$_{18}$H$_{16}$N$_4$O$_3$ (336.34): C, 64.28; H, 4.79; N, 16.66; O, 14.27 Found: C, 64.61; H, 4.82; N, 16.68; O, 14.29 FT -IR (KBr; cm$^{-1}$): 3329 (w) (–OH stretch due to intermolecular hydrogen bonding,), 3059 (m), 2392 (m), 1653 (vs)(–C=N stretch),1636 (vs), 1613 (s), 1559 (s) (–C–H stretch), 1544 (s), 1506 (s), 1495 (s), 1490 (m), 1472 (s), 1363 (vs), 1208 (s) 1180(s), 1077(s), 832(s), 748(vs), 465(w).

$^1$H NMR (d$_6$-DMSO) δ (ppm): 9.18 (s, 1H, Ar), 8.46 (s, H, Ar), 8.12 (s, H), 7.93 (s, 1H, Ar), 7.77 (s, 1H, Ar), 7.73 (s, 1H, Ar), 7.46(s, 1H, Ar), 6.78(s, 1H, Ar), 5.18(s, 1H, –OH), 4.43 (s, 1H, –NH), 3.97(s, 2H), 3.56(s, 2H).

$^{13}$C NMR (d$_6$-DMSO) δ (ppm): 165.36, 161.16, 158.31, 145.71, 136.15, 133.54, 133.36, 132.82, 130.32, 129.01, 127.77, 126.48, 124.43, 119.73, 114.40, 111.41, 56.48, 53.91.

2.2.1.2. Synthesis of (E)-4-nitro-2-((2-(5-nitropyridin-2-ylamino) ethylimino) methyl) phenol; (2)

Yield (86%); was confirmed by FTIR, $^1$H NMR, $^{13}$C NMR and elemental analyses; Anal. Calc. for C$_{14}$H$_{13}$N$_5$O$_5$ (331.09): C, 50.76; H, 3.96; N, 21.14; O, 24.15 Found: C, 50.78; H, 4.00; N, 21.16; O, 24.19 FT-IR (KBr; cm$^{-1}$): 3343 (w) (–O-H stretching), 2962 (m) (–C–H Stretch aliphatic) 1654 (vs)(–C=N stretch), 1607 (s) (–C–H stretch), 1540 (s), 1534 (s), 1499 (s), 1471 (s), 1452 (m), 1433 (s), 1343 (vs), 1306 (s) 1298 (w)1275(s), 1244(vs), 1214(s), 1176(s), 1110(vs), 1003(w), 903(s), 846(s), 832(s), 692(s),; $^1$H NMR (d$_6$-DMSO) δ (ppm): 9.12 (s, 1H, Ar), 8.42 (d, 1H, Ar), 8.23(s, 1H), 8.12(s, 1H,Ar), 7.26(s, 1H), 6.99(s, 1H, Ar), 5.12 (s, 1H, OH), 4.12 (s, 1H, Amine), 3.84 (s, 2H), 3.53(s, 2H), $^{13}$C-NMR (d$_6$-DMSO) δ (ppm): 168.25, 163.16, 161.31, 143.55, 133.34, 132.82, 130.32, 129.01, 127.77, 126.48, 124.43, 119.73, 114.40, 111.41, 56.48, 53.91.

3. FTIR studies of probes 1 and 2 and their complexes with Fe(III) and Hg(II) metal ions.

The effect of cation binding on 1 and 2 was further investigated through FTIR analysis. The characteristic of azomethine (–C=N-) stretching vibrations frequencies of 1 and 2 appeared at 1653 and 1654 cm$^{-1}$ respectively. However, in 1, the binding of Fe$^{3+}$ and Hg$^{2+}$ could not cause significant shift in the vibrations due to 1 whereas in 2, the binding of Fe$^{3+}$ and Hg$^{2+}$ cations lead to the lowering of -C=N- stretching vibrations with appearance of strong bands at 1616 cm$^{-1}$ and at 1619 cm$^{-1}$ respectively. The shifting in the -C=N- frequencies after metalation strongly suggested that 2 formed the complexes by detection of these cations.

4. Result and Discussion

4.1. Preparation of stock solution for cation detection studies

The stock solutions of probe 1 and 2 (10 $\mu$m) were prepared in aqueous medium with distilled water (pH = 7.2) at r.t. The stock solutions (10 mM) of nitrate salts of Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Mn$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{+}$, Zn$^{2+}$, Ag$^+$, Cd$^{2+}$, Hg$^{2+}$ and Pb$^{2+}$; 10 mM in water were also prepared separately.

4.2. UV/vis spectral analysis of 1 and 2

The UV/vis spectrum of 1 demonstrates absorption bands at 369 and 240 nm while 2 shows bands at 355 nm and 239 nm (Figure 3a and 3b). The low energy (LE) bands are attributed to the n–$\pi^*$ and high energy (HE) bands possibly will be ascribed to the $\pi$–$\pi^*$ transitions. Further, to examine the binding effect of the cation on absorption behaviour of 1 and 2, the solution of above mentioned various cations (1 equiv.) were added to the solutions of probes. Among tested cations, only Fe$^{3+}$ and Hg$^{2+}$ induced significant changes in the spectral feature of probes 1 and 2. Addition of Fe$^{3+}$ (5 $\mu$L) to the solution of 1 causes large hyperchromic shifts both in the HE and LE bands along with significant blue shift which led to the absorption maximum at 323 nm. Likewise, addition of Fe$^{3+}$ to the solution
of 2 exhibits large hyperchromic shifts both in the LE and HE as well as noteworthy blue shift in LE band leading to the emergence of a new band at 302 nm.

![Figure 2 FT-IR spectrum of 1 and 2 in KBr pallets.](image)

**Scheme 1** Synthetic route of probes 1 and 2.

4.3. UV/vis titration study of 1 and 2

Considering substantial changes in the presence of Fe$^{3+}$ and Hg$^{2+}$ as well as to have deep insight about probe-cation binding, UV/vis titration experiment were performed (Figure 4). Gradual addition of Fe$^{3+}$ (0.5 µl, 0.1 equiv.) to a solution of 1 leads to ratiometric hyperchromic shift along with blue shift in the LE band to have band maxima at 343 nm (Figure 4a). Further additions of Fe$^{3+}$ (0.5-5.0 µl) lead to the incessant hyperchromic shift at 343 nm and at 240 nm. The large change in the LE band relative to that of HE band clearly suggests that Fe$^{3+}$ binding with 1 influence n–π* transition which is indicative of probe interaction through heteroatoms. Similarly, aliquot addition of Hg$^{2+}$ (0.5-5.0 µl) causes changes in LE band relatively small to that Fe$^{3+}$ with blue shift having absorption maxima at 352 nm thereby pointing towards Hg$^{2+}$ binding with 1 (Figure 4b). Besides, addition of Fe$^{3+}$ (0.5 µl,
0.1 equiv.) to the solution of 2 leads to the remarkable ratiometric change through hyperchromic shift and blue shift in the LE absorption band to appear band maxima at 332 nm whereas HE band exhibits only hyperchromic shift and without any wavelength shift (Figure 4c). Further additions of Fe$^{3+}$ (1.0-5.0 μl) caused further blue shift to appear band maxima at 308 nm along with continuous hyperchromic shift. Likewise, addition of Hg$^{2+}$ (0.5-5.0 μl) to the solution of 2 exhibited noticeable ratiometric change with blue shift to emerge band maxima 337 nm (Figure 4d) in the LE absorption band as well as hyperchromic shift in the HE band.

4.4. Fluorescence spectral analysis of 1 and 2

Fluorescence bands for 1 and 2 are 440 nm and 441 nm upon excitation at its LE band ($\lambda_{ex}$, 369 nm) and ($\lambda_{ex}$, 355 nm), respectively. The metal ion detection ability of 1 was examined in presence of aforesaid metal ions wherein only Fe$^{3+}$ and Hg$^{2+}$ induced minuscule fluorescence quenching (Figure 5a). The high spin paramagnetic Fe$^{3+}$ ($d^5$) stimulate charge/electron transfer procedure which leads to the quenching in the fluorescence. Likewise, 1 displays minute fluorescence quenching upon addition of Hg$^{2+}$ ion which pointed towards that interaction of 1 with Hg$^{2+}$ is too weak to persuade complex formation significantly. On the other hand, 2 exhibits noteworthy fluorescence enhancement after addition of Fe$^{3+}$ and Hg$^{2+}$ ions (Figure 5b).

Figure 3 UV/vis spectra of 1 (a) and 2 (b) (10 μM) in presence of various metal ions in aqueous solution (H$_2$O: EtOH, 6 : 4; pH = 7.2).

4.5. Fluorescence titration study of 1 and 2

Considering small fluorescence quenching both in presence of Fe$^{3+}$ and Hg$^{2+}$ ions in 1, the fluorescence titration experiments could not be performed (Figure 5a). However, in case of 2, Fe$^{3+}$ and Hg$^{3+}$ persuades ‘turn-on’ fluorescence and therefore the fluorescence titration studies were performed to have better insights into probe-cation binding by addition of Fe$^{3+}$ and Hg$^{2+}$ independently to the solution of 2 (Figure 6). Aliquot superfluities of Fe$^{3+}$ (0.5–5.0 μl) exhibited approximately ‘turn-on’ fluorescence (~37%) deprived of any significant blue shift in emission maxima (Figure 6a). Likewise, gradual introduction of Hg$^{2+}$ (0.5–5.0 μl) also displayed ‘turn-on’ fluorescence (~44%) with inconsequential hypsochromic shift in the fluorescence maxima (Figure 6b). The analogous fluorescence ‘turn-on’ response and spectral feature in the presence of both Fe$^{3+}$ and Hg$^{2+}$ suggests 2 may be intermingling through these cations through similar binding site. Moreover, the ‘turn-on’ fluorescence of 2 upon binding with Fe$^{3+}$ and Hg$^{2+}$ may be attributed to the suppression of PET upon binding through N,N,N,O-donor sites of 2 which is known as chelation enhanced fluorescence.
The Job's plot analysis revealed 1:1 stoichiometry both for Fe$^{3+}$ and Hg$^{2+}$.

The binding/association constants ($K_a$) for Fe$^{3+}$ and Hg$^{2+}$ in 1:1 molar ratio have been determined to be $1.02 \times 10^5$ M$^{-1}$ and $1.23 \times 10^6$ M$^{-1}$, respectively by using Benesi–Hildebrand equation ($\Delta F_{\text{max}} / \Delta F = 1 + (1 / K_{BH}[M])$, $K_{BH}$ is the complexation binding constant and [M] is the variant’s concentration. A plot of $\Delta F_{\text{max}} / \Delta F$ vs. $1 / [M]$ will give straight line with slope $1 / K_{BH}$. Binding constant was determined by inverse of slope. Here the intercept should be 1.) Furthermore, the selectivity ensured by fluorescent probe can only be claimed provided none of the competing analytes can interfere the detection of targeted cations. With this objective, numerous cations were added separately to the solution encompassing Fe$^{3+}$ and Hg$^{2+}$ wherein none of the added cation could influence the fluorescence intensity ensured by Hg$^{2+}$. On the other hand, the fluorescence intensity of Fe$^{3+}$ was slightly enhanced upon addition of Hg$^{2+}$ ion which suggests better selectivity of Fe$^{3+}$ toward Hg$^{2+}$ over Fe$^{3+}$ ion.

**Figure 4** UV/vis titration spectra of 1(a), (b) and 2(c), (d) in presence of (a, c) Fe$^{3+}$ and (b, d) Hg$^{2+}$ ions aqueous media (H$_2$O: EtOH; 6 : 4; pH = 7.2).
Figure 5 Fluorescence spectra of 1(a) and 2(b) (10 μM) in presence of various metal ions in aqueous solution (H₂O: EtOH; 6 : 4; pH = 7.2) upon excitation at 369 and 355 nm, respectively.

Figure 6 Fluorescence titration spectra of 1(a) and 2(b) in presence of Fe³⁺ and Hg²⁺ ions aqueous media (H₂O: EtOH; 6 : 4; pH = 7.2) upon excitation at 369 and 355 nm, respectively.
5. Conclusion

In summary, a couple of aminoethyl-pyridine based novel fluorescent probes 1 and 2 have been developed wherein only 2 demonstrated high selectivity for the reaction of N-(2-aminoethyl)-5-nitropyridin-2-amine and 2-hydroxynaphthalene-1-carbaldehyde leads to the formation of 1 while reaction of N-(2-aminoethyl)-5-nitropyridin-2-amine and 2-hydroxy-5-nitrobenzaldehyde afforded 2. Both the compounds have been characterized by elemental analyses, FTIR, $^1$H NMR, $^{13}$C NMR, UV/vis and Fluorescence spectral techniques. Furthermore the metal ion sensing behaviour of probes 1 and 2 has been examined via UV/vis and fluorescence spectral analyses. Probe 1 and 2 exhibit change in their UV/vis spectral pattern selectively for Hg$^{2+}$ and Fe$^{3+}$ whereas only 2 displays highly selective fluorescence ‘turn-on’ response for Hg$^{2+}$ and Fe$^{3+}$.

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7. References

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