

Design and Synthesis of a Pyridine Based Chemosensor: Highly Selective Fluorescent Probe For Pb^{2+}

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Received: 22 June 2013 / Accepted: 9 August 2013 / Published online: 20 August 2013
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Abstract A pyridine based imine-linked chemosensor has been synthesized and evaluated its binding affinity with library of transition metal ions. It has prominent selectivity towards Pb^{2+} among other metal ions in DMF/ H_2O (9:1, v/v) solvent system. The 1:1 stoichiometric was confirmed by job's plot and has a binding constant (K_a)= $5.142 \times 10^3 \text{ M}^{-1}$ on fluorescence. A B3LYP/6-31G and B3LYP/LanL2DZ basis sets were employed for optimization of **3** and **3**. Pb^{2+} .

Keywords Chemosensor · Fluorescent probe · Imine-linked · Association constant · DFT

Introduction

Due to indispensable roles of transition metal ions in biological and chemical processes, the design and synthesis of chemosensors capable of selective binding and sensing of

cations has been attracted much attention in recent years [1]. Various chemosensors already reported in literature for cation binding utilize non-covalent interaction like electrostatic interactions, charge induced dipole interaction etc. to bind the guest species [2, 3]. Some of transition metal (heavy metal) ions have too much adverse effect on ecosystem. In the category of heavy metal ions, lead, cadmium and mercury get considerable attention due to their non-biodegradable nature. Lead is the most toxic and abundant metal in environment; as it is extensively used in batteries, gasoline and pigments etc. [4]. If it finds a way to enter in food chain, then it may cause neurological, reproductive and cardiovascular disorders. Beside, Pb^{2+} may lead to be a serious threat to flora and fauna. There are various techniques to detect the metal ions like atomic absorption spectrometry and inductively coupled plasma mass spectrometry etc. [5]. However, these instrumental methods require tedious sample preparation, large detection time and off site detection. On the other hand, chromogenic receptors have advantage of onsite detection and low detection limit.

Recently, numerous efforts have been devoted by different research groups to develop fluorescent sensor for Pb^{2+} [6]. However, most of sensor showed quenching upon interaction with Pb^{2+} and some suffers from interfering background fluorescence, small enhancement [7]. A enhancement in spin-orbital coupling [8] or transfer of energy or electron [9, 10] was possible reasons behind quenching upon addition Pb^{2+} . Herein, we synthesized an imine-linked noncyclic chemosensor which showed enhancement in intensity upon interaction with Pb^{2+} . A noncyclic compounds gain to much attention because of their ability to complex with ionic and neutral molecules and have number of binding sites [11, 12]. Although many kinds of noncyclic compounds are already

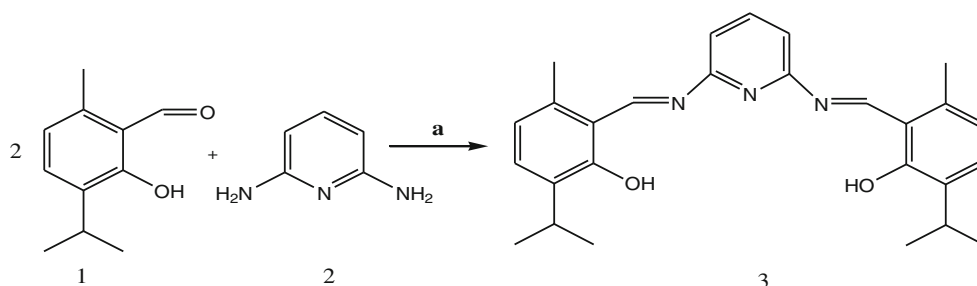
Electronic supplementary material The online version of this article (doi:10.1007/s10895-013-1287-6) contains supplementary material, which is available to authorized users.

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Scheme 1 Synthesis of receptor **3** (a) C₂H₅OH, refluxed 12 h



exist in reports but development of a cost effective synthetic route under mild conditions still challenging subject for synthetic chemists [13, 14]. Our main goal is to synthesize small non-cyclic imine-linked chemosensor for cation sensing, having multiple hydroxyl groups.

Experimental

All the required chemicals were purchased from Sigma Aldrich Ltd. and used without further purification. For UV–vis and fluorescence study the metal ions Cr³⁺, Mn²⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Hg²⁺ were added as their chlorides, Bi²⁺, Pb²⁺, Ag⁺, Cd²⁺, Th⁴⁺ were added as their nitrates, Nd³⁺ was added as its oxide, Ce⁴⁺ was added as its sulfate while U⁶⁺ was added as its acetate. For analytical studies, a stock solution of metal salts (1 mM) and receptor **3** (0.1 mM) was prepared in water and DMF/H₂O (90:10, v/v) solvent system respectively. The absorption spectra were recorded on Shimadzu made UV-2450 Spectrophotometer. Fluorescence studies were carried out on a HORIBA JOBIN YVON, Fluoromax-4 Spectrofluorometer. The solvent ratio DMF/H₂O (90:10, v/v) was kept constant during whole sets of experiments. The structural determinations were carried out by using FT-IR, ¹H-NMR, ¹³C-NMR and LC MS spectroscopic techniques. FT-IR spectra was recorded (Spectrophotometer Simadzu Asia Pacific Ptd. Ltd, Singapore) by uniformly mixing sample with KBr. ¹H NMR spectrum was recorded in CDCl₃ as a solvent (Varian Mercury YH-300 MHz Spectrometer) with tetramethylsilane (TMS) as an internal reference standard. Mass spectra were recorded under ESI mode, on Thermo Finnigan (model- LCQ Advantage MAX) mass spectrometer.

Synthesis of 6,6'-((1E)-(pyridine-2,6-diylbis(azanylylidene)-bis(methanylylidene))bis(2-isopropyl-5-methylphenol) (receptor **3**)

A solution of 2,6-diaminopyridine (0.11 g, 1 mmol) was added to a solution 2-hydroxy-3-isopropyl-6-methyl-benzaldehyde (0.36 g, 2 mmol) in ethanol (50 ml) and mixture was

refluxed for 12 h. After cooling, orange color solid product was separated out. The product was filtered and washed with petroleum ether (2 x 5 ml) and recrystallised from ethanol. Yield: 75 %; m.p. 142–144 °C; FT-IR (KBr): $\nu=3,300\text{--}3,050, 3,039, 1,597, 758, 653\text{ cm}^{-1}$; ¹H NMR (300 MHz, CDCl₃): $\delta=1.26\text{--}1.29$ (d, $J=7.2$ Hz, 12H, 4CH₃), 2.57 (s, 6H, 2CH₃), 3.37–3.46 (m, 2H, 2CH), 6.69–6.72 (d, $J=7.5$ Hz, 2H, 2Ar-H), 7.23–7.26 (d, 4H, 4Ar-H), 7.81–7.86 (t, $J=7.5$ Hz, 1H, Py-H), 9.97 (s, 2H, 2CH=N), 14.62 (bs, 2H, 2OH); ¹³C NMR (75 MHz, CDCl₃): $\delta=14.1, 25.3, 31.3, 100.5, 109.2, 114.5, 120.8, 122.6, 127.6, 130.3, 139.8, 147.6, 178.1$; MS (ESI): m/z requires 429.24, found 430.22.

Recognition Studies

The metal binding studies were performed in 5 ml volumetric flasks having fixed concentration of particular metal ions (1 mM) along with receptor **3** (0.1 mM) in DMF/H₂O (90:10, v/v) solvent system. The titration experiments were conducted manually stepwise addition of Pb²⁺ in receptor **3** solution (10 ml). To guarantee the uniformity of solution, enough time was given before recording any spectrum. The binding constant was calculated using Benesi-Hildebrand plot [15]. The stoichiometry of **3**.Pb²⁺ complex was confirmed through method of continuous variation (Job's plot) [16]. Similarly, association constant (K_a) was computed from the

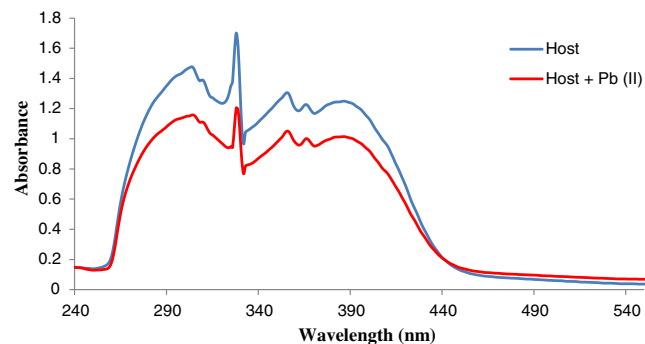


Fig. 1 Change in absorption spectrum of receptor **3** (0.00001 mM, 2,000 μ L) upon addition of Pb²⁺ ion (0.001 mM, 100 μ L)

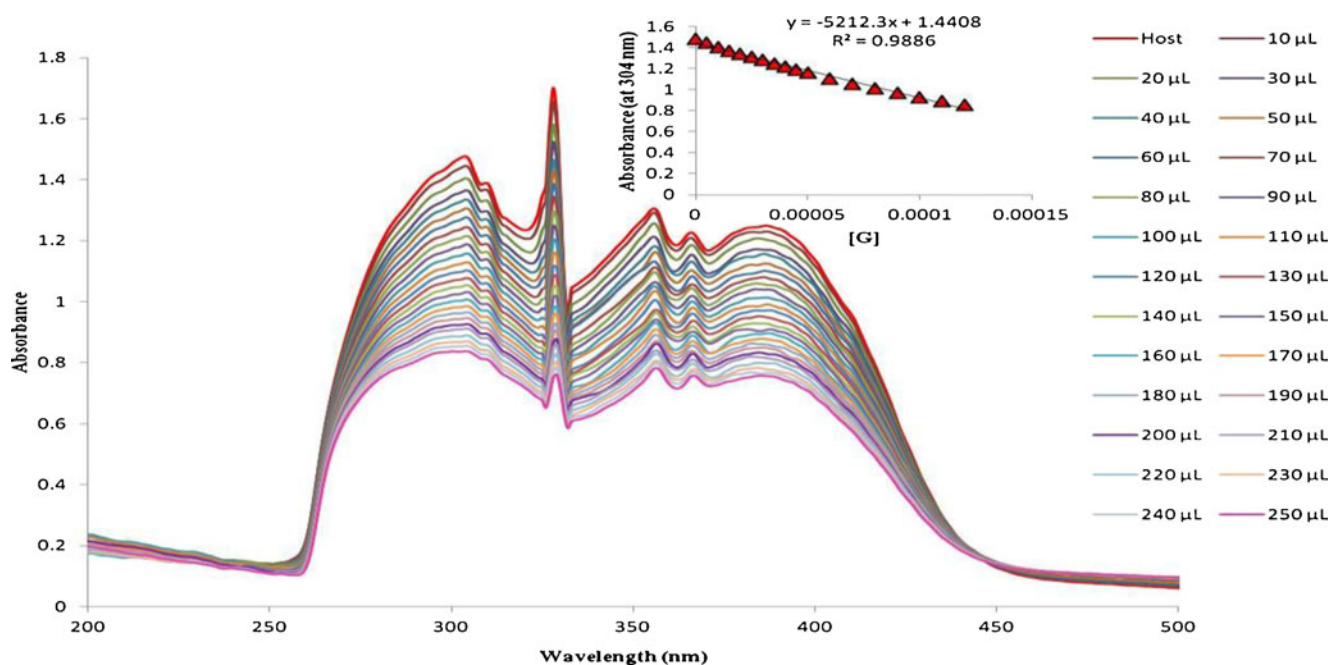


Fig. 2 A titration spectrum of receptor **3** (0.00001 mM), upon successive addition of Pb^{2+} ion (0.001 mM, 100 μL) in DMF/ H_2O (90:10, v/v) solvent system

UV-Visible titration data by using the same methodology. In case of UV-Visible titration, the experiment was carried through a stepwise addition of changeable amount of Pb^{2+} ion solution (0.001 mM) to a solution of receptor **3** (0.00001 mM) in DMF/ H_2O in 10 ml volumetric flask to maintain the absorbance nearby to one.

Results and Discussion

2-hydroxy-3-isopropyl-6-methyl-benzaldehyde (**1**) was synthesized by the reported method of Kuwar et al., [17]. Schiff's base **3** was prepared by condensation of two moles of 2-hydroxy-3-isopropyl-6-methylbenzaldehyde (**1**) with one mole of 2,6-diaminopyridine (**2**) in ethanol (Scheme 1). Synthesized receptor **3** was characterized by FT-IR, $^1\text{H-NMR}$, ^{13}C NMR spectroscopy and LC-MS. The spectral data obtained were found to be consistent with the structure of designed receptor **3**.

Receptor **3** exhibited broad absorption centred at 385 nm as shown in Fig. 1. This band may be assigned to transitions associated with phenol substituted ring. It was observed that decrease in absorbance of receptor **3** upon addition of Pb^{2+} ion in DMF/ H_2O (90:10, v/v) solvent system as depict in Fig. 1. This observation clearly indicated towards a charge transfer from nitrogen (donor) of imine to Pb^{2+} (acceptor) metal ion. To detail study titration was performed between receptor **3** with Pb^{2+} ion and found decrease in absorbance

with addition of Pb^{2+} , finally saturation in absorbance at 200 μL (Fig. 2). The inset of Fig. 2 illustrates linear relationship between absorbance versus $[\text{Pb}^{2+}]$ at 385 nm.

Fluorescence study was an important tool to evaluate the effect of metal binding on excited state of receptor **3**. The fluorescence response given by receptor **3** in DMF/water system (90:10 v/v, ≈ 0.68 mole fraction) towards the metal

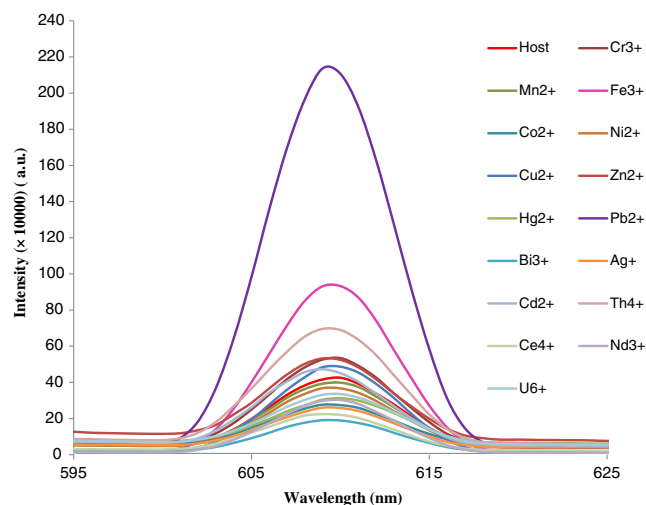
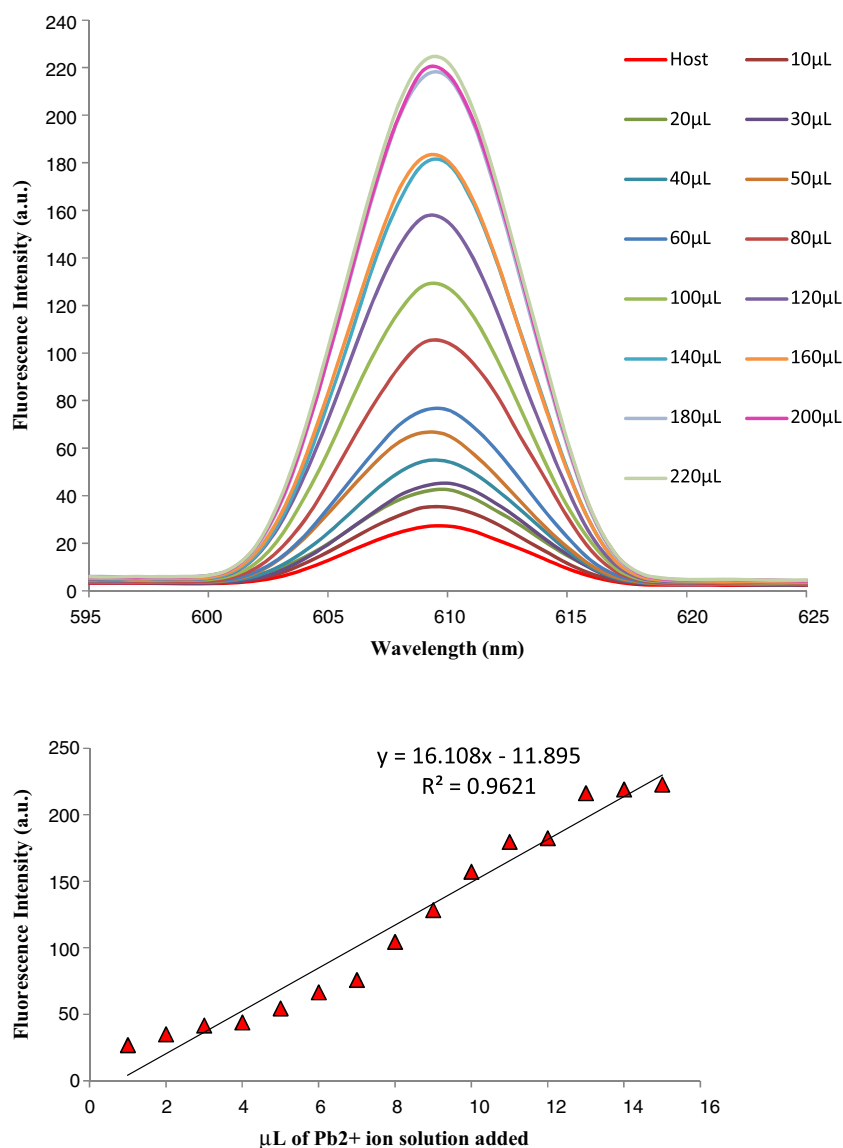


Fig. 3 Fluorescence spectra of receptor **3** (0.1 mM) upon addition of 100 μL of respective metal ions (1 mM) in aqueous phase for excitation wavelength at 304 nm

Fig. 4 Fluorescence spectra of **3** (0.1 mM) upon continuous addition of increasing amount of Pb^{2+} (0–200 μL) in DMF/ H_2O (9:1, v/v) solution (excitation wavelength at 304 nm)



ions was studied. In the organic-aqueous solution of DMF and water, water was used for homogenization of receptor **3** and the guest metal ion. A Fig. 3 illustrates changes in fluorescence of receptor **3** upon addition of selected metal ions. With an excitation wavelength (λ_{ex}) at 304 nm a distinct fluorescence enhancement in an emission peak with λ_{em} at 609 nm was observed. A change in fluorescence intensity of receptor **3** was observed upon addition of fixed amount (100 μL) of various metal ions. Upon addition Pb^{2+} , enhancement in emission of receptor **3** was observed. It means that receptor **3** has high selectivity for Pb^{2+} as compare to other transition metal ions like Cr^{3+} , Mn^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Hg^{2+} , Pb^{2+} , Bi^{3+} , Ag^+ , Cd^{2+} , Th^{4+} , Ce^{4+} , Nd^{3+} and U^{6+} .

Further, titration was performed to show gradual fluorescence enhancement in the emission peak of receptor **3** centered at 609 nm on adding changeable amounts of Pb^{2+} ion solution shown in Fig. 4. On titrating aqueous solution of Pb^{2+} ions with receptor **3** prepared in DMF/ H_2O (9:1; v/v) a progressive fluorescence enhancement was observed and reached the saturation point at 200 μL . These observations can be attributed to complete complexation. The detection limit (LOD) and limit of quantification (LOQ) is reasonably estimated to be 0.49 μM and 1.51 μM based on the ICH Q2B recommendations, below which the calibration graph is non linear [18].

$$\text{LOD} = 3.3 \sigma/S$$

$$\text{LOQ} = 10 \sigma/S$$

Fig. 5 **a** Fluorescence response (F_0-F/F_0) of receptor **3** (0.1 mM) in DMF/H₂O (9:1, v/v) upon addition of respective aqueous metal salt solutions (1 mM). **b** Interference of respective metals in Pb²⁺ ion detection with receptor **3**

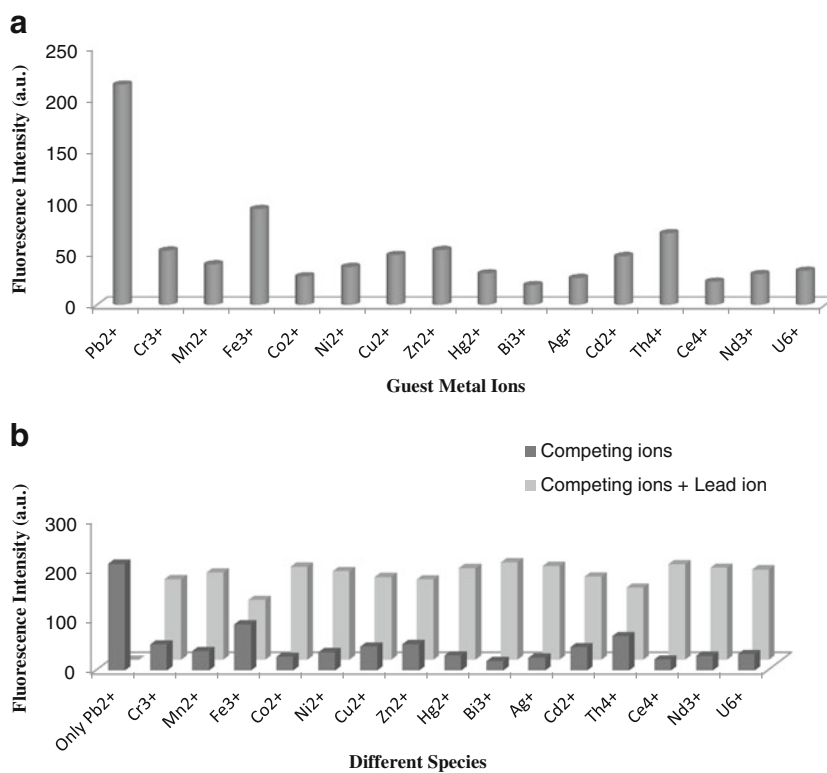


Fig. 6 Job's plot between **3** and Pb²⁺. The concentration of [HG] was calculated as $[HG] = \Delta F/F_0 \times [H]$

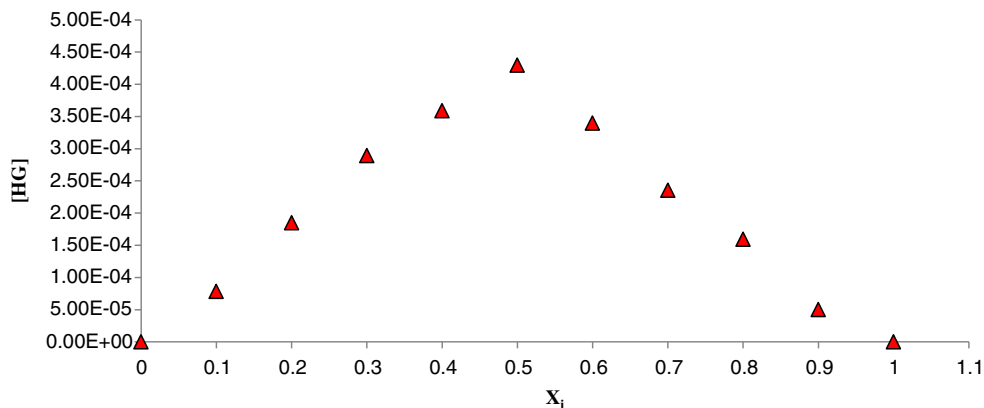


Fig. 7 Benesi-Hildebrand Plot from fluorescence of $1/\Delta F$ versus $1/[G]$, $K_a = \text{Intercept/slope} = 5.142 \times 10^3 \text{ M}^{-1}$ and from UV of $1/\Delta A$ versus $1/[G]$, $K_a = \text{Intercept/slope} = 5.213 \times 10^3 \text{ M}^{-1}$

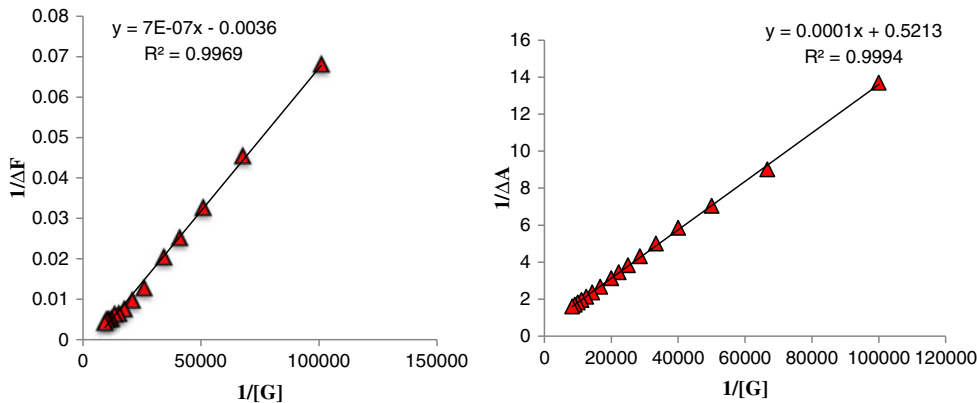
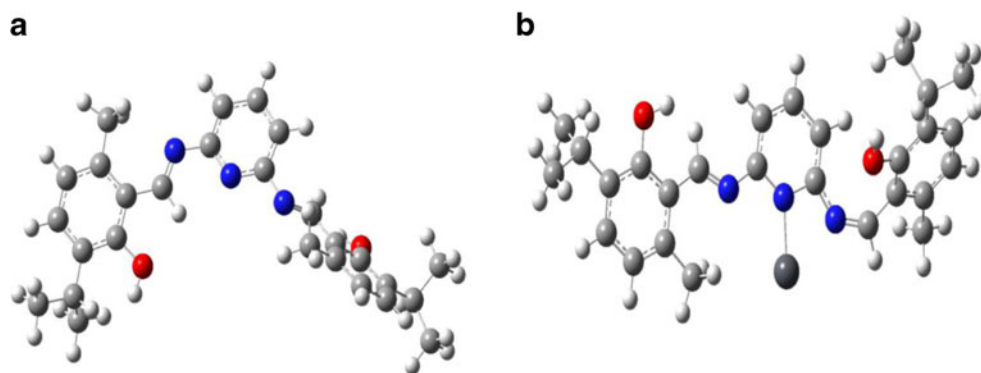


Fig. 8 The DFT optimized structure of receptor **3** (a) and (b) **3**.Pb²⁺ calculated at the B3LYP/6-31G/LanL2DZ level respectively. The red, blue, gray, and dark gray spheres refer to O, N, C, and Pb²⁺ atoms respectively



Where, S and σ represents the slope and the standard deviation of the intercept of regression line of the calibration curve.

From the titration, it is clear that receptor **3** is almost non-fluorescent and addition of Pb²⁺ caused blocking of quenching pathway of receptor **3**. The possible mechanism of sensing is cancellation of PET and inhibition of C=N isomerization through complexation with Pb²⁺. The hydroxyl group works as an effective coordination sites for noncovalent interaction with the guest metal ions, especially transition series metal ions [19]. The importance of the phenolic -OH groups for inclusion of Pb²⁺ could be easily revealed from the binding pattern of receptor **3**. Therefore, it appears that the coordination site to accommodate the Pb²⁺ in the cavity of receptor **3** was nitrogen of imine-linkages and the phenolic -OH groups.

The competition experiments were conducted in presence of 1 equiv. of Pb²⁺ mixed with excess different metal ions (2 equiv.) and fluorescence profile of receptors **3** was almost not affected (Fig. 5 and b). These results indicate that receptor **3** shows a good sensitivity and selectivity towards Pb²⁺ ion in the presence of other competitive metal ions.

The stoichiometry of complex **3**.Pb²⁺ was studied using continuous variation method [16, 20]. The plot between $[HG]$ and $[H]/([H]+[G])$ has maxima at 0.5 which represent the 1:1 stoichiometry of complex **3**.Pb²⁺ (Fig. 6). This data was further confirmed by mass spectroscopy analysis. The LC-MS data showed the formation of 1:1 complex between ligand (receptor **3**) and a metal ion. It corresponds to $[\text{receptor } \mathbf{3} \cdot \text{Pb}^{2+} + 2\text{Na}^+]$ MW=682.27; calcd for $[\text{C}_{27}\text{H}_{29}\text{N}_3\text{O}_2\text{Pb}^{2+} + 2\text{Na}]$, 681.20. LC-MS of **3**.Pb²⁺ spectra are shown in supporting data.

Using the Benesi-Hildebrand [15, 21] (Eq. 1) and (Eq. 2) methodologies, we made the calculation of binding constant (K_a).

$$\frac{1}{F-F_0} = \frac{1}{(F-F_0)K[G]} + \frac{1}{(F_\infty-F_0)} \quad (1)$$

$$\frac{1}{A-A_0} = \frac{1}{(A_\infty-A_0)K[G]} + \frac{1}{(A_\infty-A_0)} \quad (2)$$

Where,

$\Delta F = F - F_0$	differential fluorescence intensities
F	Fluorescence intensity during titration of metal ion with receptor.
F_0	Fluorescence intensity of free receptor
F_∞	Fluorescence intensity after titration.
A_0	Absorbance of free receptor
A	Absorbance of receptor 3 during titration with metal ion

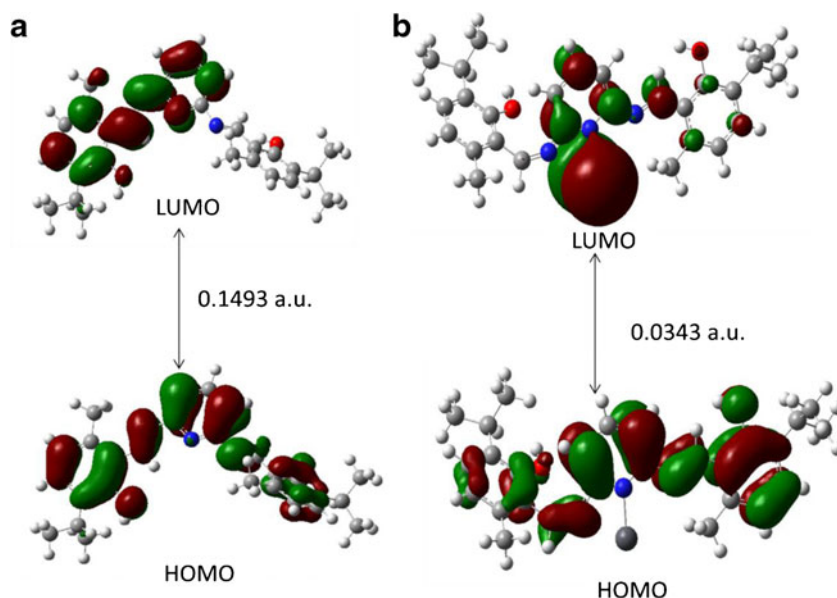
From Benesi-Hildebrand equation, computed association constant (K_a) from fluorescence and UV data was $K_a = 5.142 \times 10^3 \text{ M}^{-1}$ and $5.213 \times 10^3 \text{ M}^{-1}$ respectively. A Fig. 7 illustrated plot of adjusted Benesi-Hildebrand plot of $1/\Delta F$ versus $1/[G]$ and $1/\Delta A$ versus $1/[G]$, where ($\Delta A = A_0 - A$).

The computational study was carried out by using Density Functional Theory (DFT) in an attempt to better understand the nature of receptor and its interaction with metal ion. The B3LYP function was employed for calculations with 6-31G basis set on Gaussian 09 programmer except for Pb²⁺, for this

Table 1 An optimized energy value calculated at B3LYP/6-31G/LanL2DZ level

	Receptor 3	3 .Pb ²⁺
Energy (a.u.)	0.1493	0.0343

Fig. 9 The HOMO-LUMO gap of: (a) receptor **3** and (b) $3.Pb^{2+}$ calculated at the B3LYP/6-31G/LanL2DZ level respectively



LanL2DZ was used [22–25]. The receptor **3** has three dimensional structure and B3LYP/6-31G basis set was employed for its optimization. In order to reduce the stress from steric crowding, two arms are in different direction as shown in Fig. 8a. From Fig. 8a, it was observed that nitrogen atoms constitute the pseudo cavity for metal ion. However, $3.Pb^{2+}$ was optimized by using B3LYP/6-31G and LanL2DZ basis set for Pb^{2+} (Fig. 8b). On coordination of Pb^{2+} , there is increase in the stability of the whole system which confirmed from value of energy optimization (Table 1). The gap between HOMO-LUMO is less for $3.Pb^{2+}$ as compare to receptor **3** alone as depicted in (Fig. 9). Further, the HOMO-LUMO of the $3.Pb^{2+}$ showed that LUMO is main contributor in electronic transition [22, 23].

Conclusions

In summary, we designed and synthesised a noncyclic fluorescent receptor **3**, which is selective and sensitive towards Pb^{2+} ion, in organic-aqueous medium. The presence of Pb^{2+} ion gave rise to distinct fluorescent enhancement and resulted in colour change which can be easily detected by the naked eye under UV irradiation. Furthermore, interference of other ions in Pb^{2+} ion detection with the receptor **3** was found negligible. The Stoichiometry for the host guest complex formation realized from the Job's continuous variation method was 1:1 and the association constant (K_a) value obtained from fluorescence and UV titration data using Benesi-Hildebrand plot was $K_a=5.142 \times 10^3 M^{-1}$ and $5.213 \times 10^3 M^{-1}$ respectively which were found to be in harmony with each other.

Acknowledgements ASK gratefully acknowledge financial support from the Department of Science and Technology (DST under Fast Track Scheme-SR/FT/CS-160/2011), Government of India, New Delhi.

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