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An efficient naphthalimide based receptor for selective detection of $Hg^{2+}$and Pb^{2+}ions

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Naphthalimide based receptor **1** with N-substituted benzothiazole and pyrrolidine subunit is designed, synthesized, and characterized using FT-IR,^{1}H and ^{13}C NMR spectroscopy and mass spectrometry techniques. The receptor **1** exhibits prominent optical response for $Hg^{2+}$and Pb^{2+}ions allowing the detection of these ions in acetonitrile (ACN). The formation of the receptor **1**:cation complexes have been investigated using UV-Vis and fluorescence emission titration. Further, the selectivity of the receptor **1**towards $Hg^{2+}$and Pb^{2+} ions on the presence of various interfering cations such as Mg^{2+}, Ba^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Ag^{2+}, Fe^{2+}, $Fe^{3+}$and Cr^{3+} has been confirmed by UV-Vis and fluorescence spectroscopy. The binding constant between receptor **1** and Hg^{2+} and Pb^{2+} was estimated by Benesi-Hildebrand plot and equations. The binding constants have been found to be K_a= 3.43286 × 10^{-6} and K_a= 2.84079 × 10^{-6} M for Hg^{2+} and Pb^{2+}, respectively. The limit of detection (LOD) for $Hg^{2+}$and $Pb^{2+}$by receptor **1**are down to 7.44 × 10^{-10} M and 1.26 × 10^{-9} M, respectively. In addition, Job's plot analysis reveals 1:2 binding stoichiometry between the receptor **1** and Pb^{2+} and Hg^{2+} cations.

Keywords: Benzothiazole, colorimetric, naphthaleneimide, pyrrolidine, sensor

The detection of heavy metal ion pollution in soil, water and environment such as Hg^{2+} and $Pb^{2+}$is of increasing importance due to their high toxicity[1,2]. Mercury in its organic and inorganic form once introduced into the human body can cause serious irreversible damage to the central nervous system *i.e.* autoimmune dysfunction[3]. Hg^{2+}, a toxic element, can easily enter into the body through the respiratory, digestive tract and readily absorbed through skin[4]. The presence of Hg^{2+} in environment is a serious threat to living organisms. Due to its toxicity, it has been listed as harmful pollutant by the United Nations Environment Programme. The maximal permissible level of Hg in drinking water is estimated at 1 µg/L (World Health Organization (WHO))[5,6]. Exposure to Hg^{2+} causes several diseases such as dermal toxicity, nephritis,uremia, reproductive failure, Minamata disease, pyrolysis, coma and heart disease[3,6-10]. Sources of Hg^{2+} and its derivatives to the environment stems from its use in medicinal, technological and agricultural applications[11]. Therefore, to monitor the presence of Hg^{2+} in water, environment and biological system, a simple, selective and effective detection method is needed[12].Furthermore, heavy metal element such as lead (Pb^{2+}) is also harmful to human and environment health[13]. According to WHO reports, the maximum Pb^{2+}contaminant limit in drinking water is currently set at 10 µg/L[5,6]. It is well documented that excess presence of Pb^{2+}physiologically affects human body systems including renal, neurological, hepatic, cancer, reproductive and hematopoietic systems[14]. Considering their toxicity, controlling the limits of heavy metal ion levels in environment and human body is of utmost importance. The detection and monitoring of trace amount of metals with highly selective and sensitive method can aid greatly.

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Literature survey revealed that there are several techniques, such as atomic absorption spectroscopy (AAS)[15], atomic fluorescence spectroscopy (AFS)[16] and inductively coupled plasma-mass spectrometry (ICP-MS)[17]have been successfully employed for the analysis and detection of low concentration of metal ions. Though these methods possess high sensitivity and precision, unfortunately these techniques are time-consuming, quite specialized, labor-intense and require sophisticated instruments. Moreover, these methods are not suitable for *in situ* metal ion monitoring in live cells and tissues. Fluorometric analysis of metal ions offers advantages to overcome these limitations while being highly sensitive, selective, fast and low-cost capable of probing solutions and biological samples[12].

In recent years, a number of fluorescent chemo-sensors such as triarylamine-triazine, coumarin, rhodamine, pyrene, porphyrin, dansyland bis-thiophene appeared to be more effective probes of Hg^{2+} due to their high sensitivity and selectivity[18-22]. Furthermore, the development of highly selective and sensitive fluorescent probes of Pb^{2+} are well documented in literature[19,23,24], and several fluorescence chemo-sensors were shown to be selective in detectingHg^{2+} and Pb^{2+} ions[18,19]. But it is notable that very few examples have been developed for the simultaneous detection of multi-metal ion coexisting in a sample[25-27]. Therefore, there is practical need to develop fluorescent receptors for the simultaneous selective and sensitive detection of combined presence of Hg^{2+} and Pb^{2+} ions.

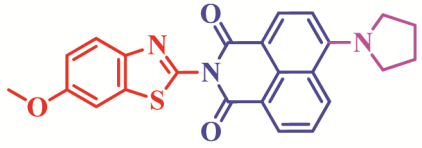
Naphthalimide derivatives, with their strong colorimetric and fluorescence detection ability, are widely employed as fluorescent chemo-sensor for heavy metal ion detection[19,28–30]. However, the naphthalimide based sensors for multi-ion detection are rarely investigated[31]. Therefore, the present investigation aimed to develop a cost-effective naphthalimide-based fluorescent chemo-sensor with an efficient selectivity and sensitivity towards Hg^{2+} and Pb^{2+} ion.

In this study, we made an attempt to incorporate benzothiazole subunit[21,32-34]as a strong chelating moiety to enhance the naphthalimide chromophore fluorescence performance. Pyrrolidine moiety was also conjugated to the naphthalimide core to enhance the colorimetric and emission propertiesof chemo-sensor (Scheme I). The introduction of benzothiazole subunit into the naphthalimide at its imide position resulted in improved selectivity and sensitivity towards Hg^{2+} and Pb^{2+}as shown by experimental results in acetonitrile (ACN).

Experimental Section

Materials and Methods

The6-methoxybenzo[d]thiazol-2-amine, 6-bromo-1*H*,3*H*-benzo[de]isochromene-1,3-dione, toluene, pyrrolidine, zinc acetate and all bivalent and trivalent metal chlorate or perchlorate were purchased from Sigma Aldrich (Bengaluru, Karnataka, India) and used without further purification, unless otherwise specified. The^{1}H NMR and^{13}C NMR spectra were recorded usingAVANCE 400spectrometer with 500 MHz and 125 MHz respectively(Instrument installed in CSIR-Indian Institute of Chemical technology, Hyderabad, India).TMS was used as internal reference and $CDCl_3$as solvent. The chemical shift (δ) of protons were represented in part per million (ppm) and coupling constant (*J*) was reported in Hertz (Hz). The splitting pattern was denoted as multiplet (m), triplet (t), doublet of doublet (dd), singlet (s) and doublet (d), and mass spectrometric data were obtained by positive electron spray ionization (ESI-MS) technique on an Agilent Technologies 1100 Series (Agilent Chemistation Software) mass spectrometer (Instrument installed in CSIR-Indian Institute of Chemical Technology, Hyderabad, India). High-resolution mass spectra (HRMS) were obtained using ESI-QTOF mass spectrometry (Instrument installed in CSIR-Indian Institute of Chemical Technology, Hyderabad, India). FT-IR spectra were recorded using a Perkin–Elmer FT-IR 400 spectrometer (Instrument installed in CSIR-Indian Institute of Chemical technology, Hyderabad, India). UV-Vis absorbance spectra were recorded by UV-Vis 1800 Shimadzu spectro-photometer (Instrument installed in CSIR-Indian Institute of Chemical Technology, Hyderabad, India). Fluorescence emission spectra were measured usingRF-6000 (Shimadzu, Japan) Spectrofluoro-



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Scheme I — Structure of receptor **1**

photometer (Instrument installed in CSIR-Indian Institute of Chemical Technology, Hyderabad, India).

Synthesis of compound C

A mixture of 6-bromobenzo[de]isochromene-1,3-dione (**A**), (0.5000 g 0.00180mol, 1 equivalent), zinc acetate (0.0331 g 0.000180mol, 0.1 equivalent) of 5 mL ethanol was taken in seal tube and stirred for 15 minutes at RT. Then 6-methoxy- benzo[d]thiazol-2-amine (**B**), (0.4228 g,0.0023mol, 1.3 equivalent) was slowly added to it in presence of N_2 gas. The reaction mixture was refluxed at 115°C with continues stirring for 10h. After the completion of reaction as monitored by TLC the reaction was stopped and cool at RT. The reaction mixture was washed with 25 mL of methanol and dried, cream colour solid powder 82% of C (0.650 g) was obtained without further purification. IR (KBr, ν cm^{-1}) 779.21, 833.62, 1227.56, 1347.99, 1460.49, 1577.23, 1686.02, 1719.98, 2925.51;^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (dd,*J* = 7.3, 1.1 Hz, 1H), 8.69 (dd, *J* = 8.5, 1.1 Hz, 1H), 8.50 (d,*J* = 7.9 Hz, 1H), 8.16 – 8.08 (m, 1H), 8.01 (d, *J* = 9.0 Hz, 1H), 7.92 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.39 (d,*J* = 2.5 Hz, 1H), 7.15 (dd, *J* = 9.0, 2.6 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.52, 155.03, 135.62, 134.22, 133.56, 132.74, 131.68, 131.01, 129.29, 129.02, 128.54, 127.61, 122.89, 120.62, 118.84, 104.12, 56.01; ESI-MS (m/z %): 479 (55) $[M+K]^+$.

Synthesis of receptor 1

After that synthesized 6-bromo-2-(6-methoxy-benzo[d]thiazol-2-yl)-1*H*-benzo[de] isoquinoline-1,3 (2*H*)-dione **C**(0.1500 g 0.00034mol, 1 equivalent), pyrrolidine **D**, (0.0315gm. 0.00044mol, 1.3 equivalent) were taken in round bottom flask then dry moisture free toluene (5 mL)were added it in presence of N_2 and stirred at 110°C for 6h. Progress of reaction was monitored by TLC. After completion of reaction, the reaction mixture was cooled to RT and dried over Rota-evaporator. The obtained crude product was purified by silica gel column chromatography (hexane: dichloromethane; 7:3) to yield yellow powder of **1**(0.104gm., 71.03%). IR (KBr, νcm^{-1}) 811.40, 1155.46, 1302.50, 1352.77, 1462.91, 1570.54, 1607.96, 1643.85, 1667.66, 2866.89, 2924.59; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.76 – 8.53 (m, 2H), 8.46 (d, *J* = 8.7 Hz, 1H), 8.00 (d, *J* = 8.9 Hz, 1H), 7.56 (dd,*J* = 8.5, 7.4 Hz, 1H), 7.37 (d, *J* = 2.5 Hz, 1H), 7.12 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.83 (d, *J* = 8.7 Hz, 1H), 3.91 (s, 3H), 3.83 (s, 4H), 2.13 (dd, *J* = 7.7, 5.0 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.15 (s), 154.08 (s), 153.32 (s), 145.07 (s), 138.27 (s), 134.21 (s), 132.95 (s), 131.96 (d, *J* = 20.8 Hz), 124.74 (s), 123.08 (s), 122.71 (s), 122.05 (s), 115.68 (s), 109.64 (s), 108.66 (s), 104.12 (s), 55.86 (s), 53.34 (s), 26.14 (s);ESI-MS (*m/z %*): 430 (100) $[M+H]^+$; HRMS: calculated for $C_{24}H_{20}O_3N_3S$ = 430.12199 Found $[M+H]^+$ = 430.12101.

Optical and Photophysical Detection

UV-Vis absorption spectroscopic study

The probe 1 was dissolved in acetonitrile (10 v) with c ≈ 1.0×10^{-5}M corresponding to the maximum of absorbance in range ≈ 0.1 to 0.25 for the UV experiments. The solution of the probe was placed in a quartz cuvette (*l*= 1 cm, V_0 =3 mL), and the various analyte solutions were added. At each addition, UV-Vis spectrum was recorded. The experiment was performed at RT.

Fluorescence spectroscopic study

The various metal ion solutions were added to a solution of (1.0×10^{-5}M) of probe **1** in acetonitrile. Upon each addition, the emission spectrum was recorded with wavelength of (λ_{ex} = 460 nm). The titrations were performed at RT.

Optical detection

The various metal ion solutions were added to a solution of (5.0×10^{-5}M) of probe **1** in acetonitrile. The addition of various metal ion solutions (10.0×10^{-5}M) image was recorded with well resolved camera at RT.

Time resolved fluorescence spectroscopy

Fluorescence lifetime measurements were carried on a pico second time-correlated single photon counting (TCSPC) setup (FluoroLog3-Triple Illuminator, IBH Horiba Jobin Yvon, Alabaster, Al, USA) (Instrument installed in CSIR-Indian Institute of Chemical technology, Hyderabad, India) employing a picosecond light emitting diode laser (Nano LED, λex= 365 nm). The samples were prepared in acetonitrile. The TCSPC measurements were performed evaluated using a 1 cm cuvette at 25 °C.

Results and Discussion

Design and Synthesis of Receptor 1

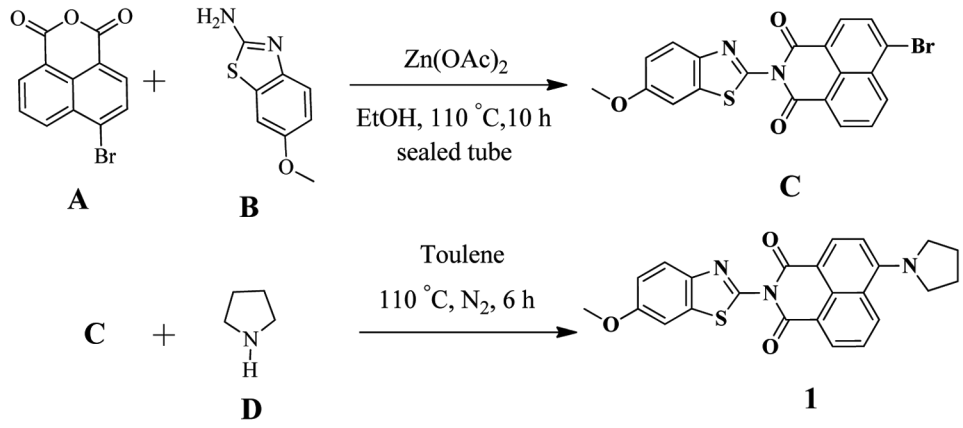
Fluorescence receptor have become powerful tools as chemo-sensor and imaging of trace ions in the food, environment and biological systems. Therefore,

designing and synthesizing such fluorescent probes with optical recognition and fluorescence response has attracted great attention in recent years. Our research group is interested in development of such probes. From literature search we have found that designing fluorescent receptor based on naphthalimide (NPI) is ideal. NPI exhibits good absorbance and fluorescence characteristics along with thermal stability. These properties of NPI extends its application range as chemo-sensor and biosensor. Benzothiazole was employed as one of the subunit in the probe as this moiety has been reported to have a strong ability to coordinate with metal ions[21,32–34]and has been previously utilized as a receptor in the design of novel fluorescent sensor for Hg^{2+} and Pb^{2+}.To achieve the desired requirements, herein, we designed a NPI-benzothiazole-pyrrolidine-based receptor **1** for high selectivity and sensitivity detection of Hg^{2+} and Pb^{2+}.

The designed receptor **1** was synthesized in two steps as outlined in Scheme II. The imide formation reaction of NPA (**A)** with 6-methoxy-2-amino benzothiazole (**B**) in presence of zinc acetate was performed to yield compound **C**. Amine ring system was introduced into the NPI structure *via* substitution reaction of the Br-substituted **C** with pyrrolidine in toluene as a solvent. Thus, the receptor **1**was successfully prepared with 71.03% yield as yellow powder.

Optical properties

At first optical recognition experimental method was carried out to receptor **1** in acetonitrile solution (5.0×10^{-5}M) with the addition of metal ions such as Mg^{2+}, Ba^{2+}, Ni^{2+}, Hg^{2+},Co^{2+},Cu^{2+}, Ag^{2+}, Pb^{2+},Fe^{2+}, $Fe^{3+}$and Cr^{3+} (2 equiv., *ca*. 10×10^{-5} M).The experimental solutions were monitored by visual (optical) method. The receptor **1** solution exhibits reddish colour in acetonitrile under UV light (λ_{ex} = 365 nm). The receptor **1** solution displayed only shows colour change from reddish to faint red in the presence of Hg^{2+} and Pb^{2+} ions (Figure 1). To the receptor **1**, addition of the other metal ions solution did not exhibit in appreciable color change. Moreover, the colour change of receptor **1** in presence of Hg^{2+} and Pb^{2+} ions increased with the increase in metal ion amount towards profound faint red. We presume that the receptor **1**•Hg^{2+} and **1**•Pb^{2+} complex formation takes place.



Scheme II — Synthesis of receptor **1**

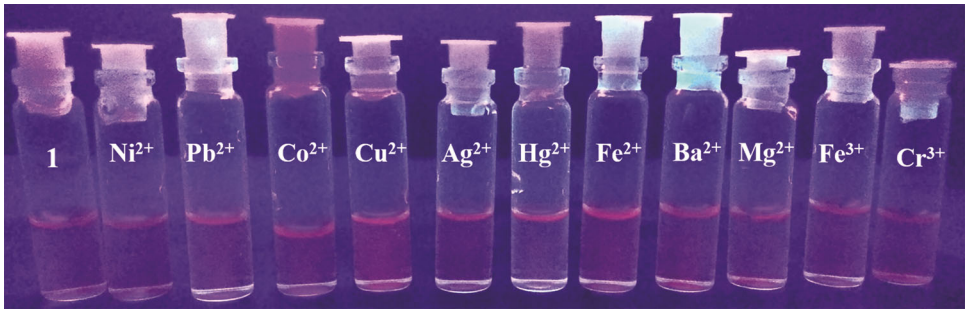


Figure 1 — Color change of receptor **1** solution (5.0×10^{-5} M) in acetonitrile upon addition 2 equiv. of various metal ion (10×10^{-5} M)

UV-Vis absorption spectral properties

The series of metal ion detection experiments were performed out to receptor **1** in acetonitrile. The UV-Vis electronic absorption spectra were employed to investigate recognition properties of **1** towards various bivalent and trivalent metal ions. The absorption spectral study was depicted in Figure 2a, 2b, and 2c. The UV-Vis absorption spectrum of receptor **1** displays only one prominent peak at 465 nm (Figure 2a). The absorption experiments of receptor **1** was first employed to monitor the detection of metal ions. The absorption changes of receptor **1** was monitored with the addition of metal ions such as Mg^{2+}, Ba^{2+}, Ni^{2+}, Hg^{2+},Co^{2+}, Cu^{2+}, Ag^{2+}, Pb^{2+},Fe^{2+}, $Fe^{3+}$and Cr^{3+} (0 to 2.3 equivalent). When 2.3 equivalent of Hg^{2+} ions were added to receptor **1**, the absorption peak intensity at 465 nm significantly decreases (Figure 2a). With the addition of Pb^{2+} ion to receptor **1** the same trend was observed (Figure 2a). After the addition of other metal ions *e.g.* Mg^{2+}, Ba^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Ag^{2+}, Fe^{2+}, $Fe^{3+}$and Cr^{3+} (0 to 2.3 equivalent), significant absorption spectral changes were also occurred (Figure 2a).Absorption titration experiment were performed at RT to investigate absorption spectral changes with the addition of Hg^{2+} (Figure 2b) and Pb^{2+} (Figure 2c) ions. As shown in Figure 2b and 2c, addition of Hg^{2+} (0.1 equivalent) and Pb^{2+}(0.1 equivalent) to the receptor **1** resulted into sudden decrease in absorption spectral peak intensity at 465 nm. When furtherHg^{2+}(0.2-2.3 equivalents) and Pb^{2+}(0.2-2.3 equivalents) ions were added to the receptor **1**, the absorption spectral peak intensity of **1** were gradually decreases and saturated at 2.3equivalent. We presume that the peak appeared at 465 nm downturn may be due to the complex formation between receptor **1** and Hg^{2+} and Pb^{2+} (Figure 2a). Thus, the changes in absorption peak indicates interaction between $Hg^{2+}$and Pb^{2+} with the receptor **1,**which in turn exhibits change in electronic properties.

Florescence emission spectral properties

Fluorescence emission spectroscopy technique was employed to explore the sensing properties of receptor **1** towards various heavy metal ion at RT and are depicted in Figure 3a, 3b and 3c. In the fluorescent experiments, upon excitation at λ_{ex} = 460 nm the receptor solution **1** in acetonitrile exhibits strong

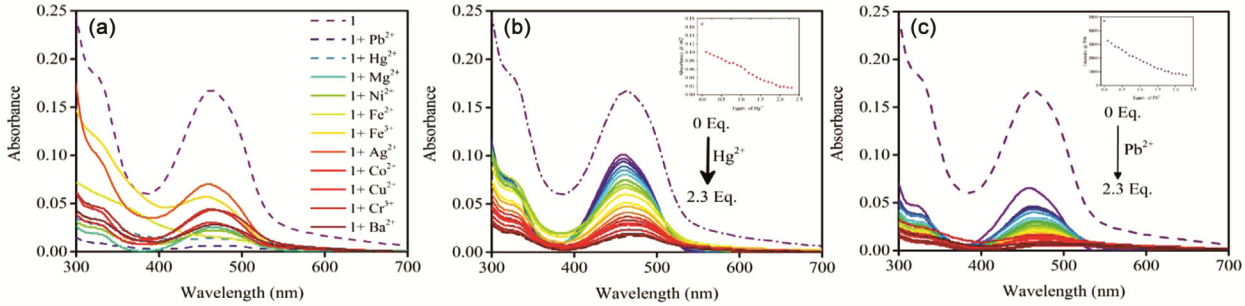


Figure 2 — (a) UV-Vis absorbance spectra of **1** (1.0×10^{-5} M) in ACN upon addition of 2.3 equivalent of various metal ion; (b) UV-Vis titration experiment of **1** in the presence of different concentration of Hg^{2+}, and (c) UV-Vis titration experiment of **1** in the presence of different concentration of Pb^{2+}

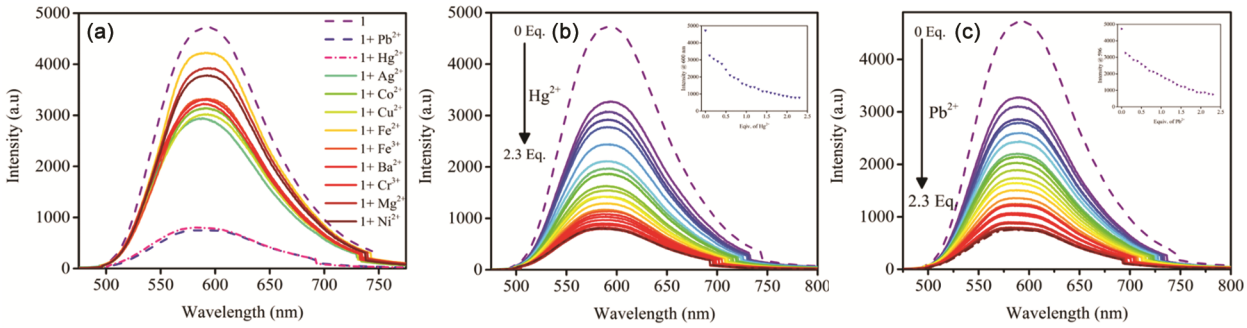


Figure 3 — (a) Fluorescence emission spectra (λ_{ex} = 460 nm) of **1** in the presence of 2.3 equivalent of various metal ion in acetonitrile and (b) Fluorescence titration experiment of **1** in the presence of different concentration of Hg^{2+}, and (c) Fluorescence titration experiment of **1** in the presence of different concentration of Pb^{2+} (λ_{ex} = 460 nm)

emission peaks at λ_{em} = 600 nm (Figure 3a). As revealed in Figure 3a, the fluorescence emission spectral measurement shows no significant change in the emission peak intensity of receptor **1** with the addition of Mg^{2+}, Ba^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Ag^{2+}, Fe^{2+}, Fe^{3+}andCr^{3+} (2.3 equivalent). Only when 2.3 equivalent of Hg^{2+} and Pb^{2+}were added, receptor **1**shows significant emission peak intensity quenching at 600 nm. To get comprehensive insight of emission quenching, fluorescence titration experiments were carried out at RT with the addition of Hg^{2+} and Pb^{2+} (0- 2.3 equivalent) to the receptor **1**. As shown in Figure 3b,Hg^{2+}ion (0-2.3 equivalent) were added gradually to receptor **1**. It was observed that the fluorescence emission peak at 600 nm was decreased gradually. When 2.3 equivalent of Hg^{2+} was added, the emission peak significantly decreased and reached its saturation point. Furthermore, in order to verify the sensitivity of receptor **1** towards Pb^{2+}, fluorescence titration measurements were carried out. As shown in Figure 3c, in the presence of Pb^{2+} ion (0-2.3 equivalent), the receptor **1** shows significant emission spectral peak changes at 600 nm. The fluorescence titration experiment results revealed significant spectral changes of receptor **1** with the gradual addition of Hg^{2+} and Pb^{2+}. This would urge that the receptor **1** could be an effective and highly sensitive fluorescent probe towards Hg^{2+} and Pb^{2+}(Figure 3b and 3c). It is presumed that the significant decrease in fluorescence emission peak intensity is due to complex formation between receptor **1** and Hg^{2+} and Pb^{2+} analytes, respectively.

In order to further investigate the complex formation and stoichiometry between receptor **1** and Hg^{2+} and Pb^{2+} analytes in acetonitrile Job's plot were employed (Figure S10 and S11). It was observed that, the fluorescence emission intensity reached an extreme value when the molar fraction value of Hg^{2+} was0.3 (Figure S10). This indicates that the stoichiometric binding ratio of receptor **1** and Hg^{2+} ion was 1:2. Furthermore, as shown in Figure S11 when the molar faction of Pb^{2+} was 0.3, the emission peak intensity reached at its extreme value, suggesting that the stoichiometric ratio of receptor **1** and Pb^{2+} was 1:2. Thus, the fluorescence emission results with Job's plot revealed that one molecule of receptor **1** binds to two Hg^{2+} as well as Pb^{2+} analytes.

Time correlated single photon counting (TCSPC) studies

Time correlated single photon counting (TCSPC) experiments were performed to investigate the dynamic kinetics of emission for receptor **1**. The fluorescence emission response of chromophore **1** in acetonitrile solutions, in the presence and absence of Hg^{2+} and Pb^{2+} were examined. The system was excited at 460 nm and the emission response at 591 nm, 592 nm, 593 nm and 594 nm were recorded and the results are illustrated in Figure 4, and summarized in Table I. The decay profile of receptor **1**,**1**: Hg^{2+} (2.3 equivalent) and **1**:Pb^{2+} (2.3 equivalent) were fitted with bi-exponential function. As shown in Table I, the experimental fluorescence life-time measurement values obtained for receptor **1**,**1**: Hg^{2+} (2.3 equivalent) were 0.47 ns (93.6%) and 0.58 ns (100%), respectively. It is observed that τ_1 values of **1**: Hg^{2+} (2.3 equivalent) is higher than that of the chromophore **1**. The increase in τ_1 values follow the order **1**: Hg^{2+} (2.3 equivalent) >**1**,possibly due to binding of Hg^{2+} to the receptor **1**. In case of receptor **1**: Pb^{2+} (2.3 equivalent) the obtained fluorescence emission life time measurement value was 0.63 ns. The order of τ_1 values are **1**< **1**: Pb^{2+} (2.3 equivalent). The prolonged life time for **1**: Pb^{2+} (2.3 equivalent) as compared to dye **1** was observed.

Binding constant

The binding constant $K_a$of the receptor **1**•Hg^{2+} and **1**•Pb^{2+} complex formation were estimated by Benesi-

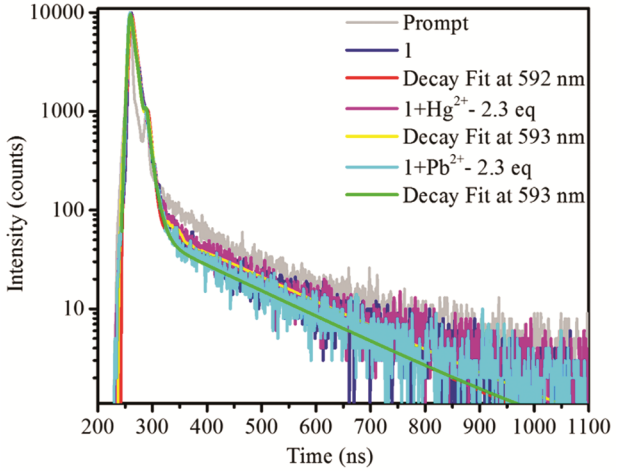


Figure 4 — Fluorescence lifetime measurement of receptor **1** in various ratios of Hg^{2+} and Pb^{2+}, respectively

Table I — Fluorescence life time of measurement receptor **1** in the various concentrations of Hg^{2+} and Pb^{2+}, respectively

Compd	τ_1 (ns)	Contribution(%)	τ_2 (ns)	Contribution(%)	τ_2 (ns)	Contribution(%)
1	0.47	43.34	8.30	0.99	0.44	55.65
1+Hg^{2+}(2.23 eq.)	0.58	49.75	9.70	0.03	0.58	50.20
1+Pb^{2+}(2.23 eq.)	0.63	33.54	0.30	60.62	9.36	5.84

Hildebrand equation. The fluorescence emission titration experiments were performed to determine the association constant K_a. The linearly fitted data were obtained from receptor **1**/I-I_0 *versus* 1/[Hg^{2+}] and 1/[Pb^{2+}], respectively. The Benesi-Hilderbrand equation [35,36] was utilized to evaluate the association constant. The equation is as shown below.

$$1/I\text{-}I_0 = 1/I_{max}\text{-}I_0 + 1/[I_{max} - I_0]K_a[C]$$

where, I_0 = the fluorescence emission intensity of free receptor **1**, I = the emission intensity of receptor **1** measured with added analytes such as Hg^{2+} and Pb^{2+}. I_{max} = the emission of receptor **1** measured with excess amount of the analytes at 600 nm. K_a = association constant and [C] is the concentration of added analytes Hg^{2+} and Pb^{2+}, respectively. The Benesi-Hildebrand equation used for determination of binding constant is valid for 1:2 complex formation between the receptor **1** and Hg^{2+} and Pb^{2+}.

The linear relationship fitted data obtained from plotting 1/I-I_0 against 1/[Hg^{2+}] and 1/[Pb^{2+}] are depicted in Figure S12 and Figure S13, respectively. The calculated association constant (K_a) for binding between receptor **1** and $Hg^{2+}$was determined to be 3.43286×10^{-6} M, whereas between receptor **1** and Pb^{2+} was 2.84079×10^{-6} M. The obtained results indicate that the receptor **1** is competitively binds with Hg^{2+} and Pb^{2+}.

Limit of detection

In order to confirm the practical applicability and sensitivity of receptor **1** towards Hg^{2+} and Pb^{2+}limit of detection (LOD) were estimated from fluorescence emission measurements. The fluorescence emission response of **1** at different concentration of Hg^{2+}at 600 nm were recorded (Figure S14). We utilized an equation 3SD/ρ, where SD- is the standard deviation of the three blank solutions and ρ is the slope of the calibration curve between emission intensity against concentration of Hg^{2+} and Pb^{2+} analytes. As shown in Figure S14 the receptor **1** displayed a linear relationship between the emission peak intensity *versus* concentration of Hg^{2+}. The calculated LOD for Hg^{2+} was found out to be 7.44×10^{-10} M, suggesting that in practical applications receptor **1** can detect low concentration of Hg^{2+}. Furthermore, as shown in Figure S15, the fluorescence emission titration of **1** with Pb^{2+} was performed. From the linear region of calibration curve, the LOD for Pb^{2+} estimated to be 1.26×10^{-9} M. Thus, nanomolar concentration of Pb^{2+} ions were sufficient to monitor the change in fluorescence emission intensity of receptor **1**. Thus, these results revealed that the receptor **1** can be potentially useful and competitive to detect Hg^{2+} and Pb^{2+} ions. The comparative detection of limit for Hg^{2+} and Pb^{2+} ions by employing different dyes are comprised in Table S1. These results indicating that the method displayed herein is very much competitive.

Competitive binding studies of metal ions

In order to further confirm the selectivity of receptor **1** with Hg^{2+} and Pb^{2+}, competitive binding studies with other metal ions such as Mg^{2+}, Ba^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Ag^{2+}, Fe^{2+}, Fe^{3+}andCr^{3+} were performed. To investigate the effect of interfering metal ions the fluorescence emission spectral properties were monitored. As shown in Figure 5a, the emission peak intensities of the receptor **1**did not change significantly with the addition of various metal ions (2.3 equivalent). However, the addition of Hg^{2+} to **1** in the presence of other metal ions resulted in a significant emission spectral intensity changes at 600 nm (Figure 5a). The fluorescence emission spectral results confirm that receptor **1** selectively binds Hg^{2+} over the other interfering metal ions.

Furthermore, by using fluorescence emission response, the selectivity and sensitivity of **1** towards Pb^{2+} was also examined in the presence of other metal ions. As shown in Figure 5b, the receptor **1** solution containing various metal ions (2.3 equivalent) exhibited significant decrease in fluorescence emission spectral peak intensities after the addition of Pb^{2+} ions (2.3 equivalent), suggesting the tested metal ions did not interfere with the Pb^{2+} ions detection. Such selective sensing behaviour displayed by receptor **1** towards $Pb^{2+}$is clearly advantageous. Since

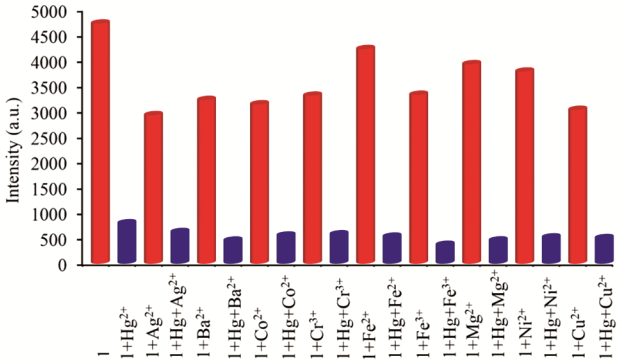


Figure 5a — Competitive binding study of 1 (1.0×10^{-5} M) with the selected metal ions Hg^{2+} in acetonitrile solution by fluorescence emission spectra @ 600 nm (λ_{ex} = 460 nm), in presence of various interfering metal ions

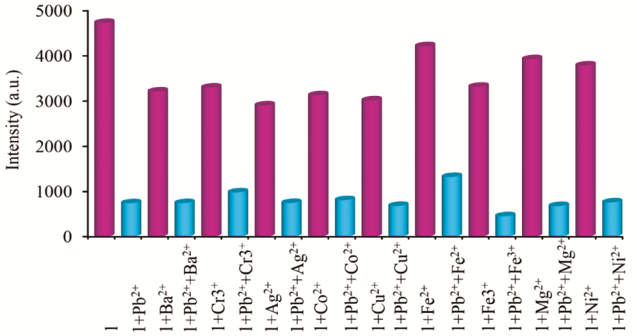


Figure 5b — Competitive binding study of 1 (1.0×10^{-5} M) with the selected metal ions Pb^{2+} ions (2.3 equivalent) in acetonitrile solution by fluorescence emission spectra @ 600 nm (λ_{ex} = 460 nm), in presence of various interfering metal ions

selective detection of Pb^{2+} ions in the presence of interfering metal ions is a major challenge[18,19].

Thus, the results obtained from UV-Vis and fluorescence emission spectroscopic measurements of receptor **1** revealed that it has a capability to detect both Hg^{2+} and Pb^{2+} ions in the presence of other metal ions.

Molecular Modelling

Theoretical density functional theory (DFT) and time dependent density functional theory (TDDFT) calculations were performed using the ORCA software package (Version 4.0.1.2) with CPCM water solvent model[37].Calculation was conducted using Becke, 3-parameter, Lee–Yang–Parr hybrid functionals (B3LYP) to consider the non-local density dependent dispersion correction. Because the NL correction increases the computational cost, the calculation was conducted in combination with RIJCOSX hybrid functional technique, where the computational overhead is marginal if it is done non-selfconsistently. The results show that both HOMO (−5.537 eV) and LUMO (−2.322 eV) of **1** are concentrated on the naphthalimide moiety of the molecules (Figure 6).

The density of state (DOS) spectrum give 3.215 eV HOMO-LUMO gap, while the simulated spectrum gives the HOMO-LUMO singlet transition at 446.3 nm with 0.3512 oscillation strength (Figure 7) as calculated using Gauss-Sum 3.0 program respectively[38]. These values are in close agreement with the experimental values here. Since the HOMO-LUMO singlet transition occurs on electrons on the naphthalimide moiety of the molecules (Table S2), when **1** act as a ligand to metal cations this causes a deficiency in electron density in these orbitals resulting in diminishing oscillator strength of the transition as observed in the experimental results.

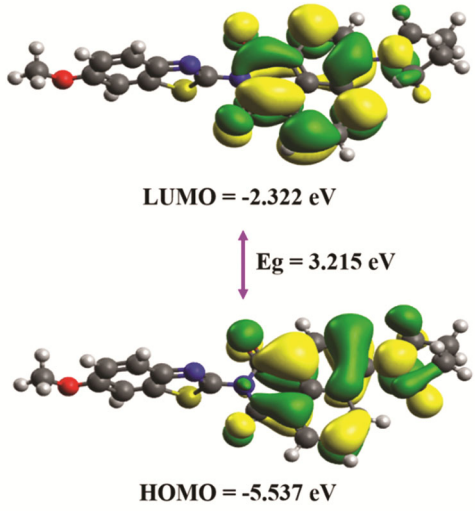


Figure 6 — The frontier molecular orbitals HOMO and LUMO wave function and energy levels of **1** in water as calculated using TDDFT-NL at B3LYP def2-TZVP def2/J RIJCOSX level of theory

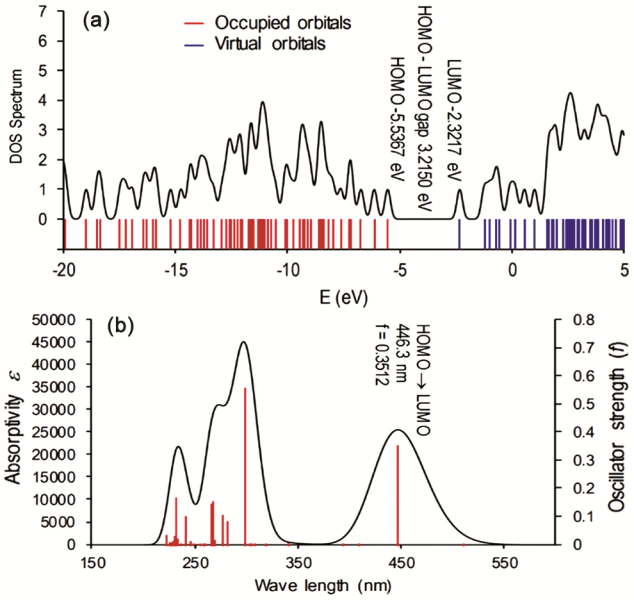


Figure 7 — The DOS and UV-Vis spectra of **1** in water as calculated using B3LYP def2-TZVP def2/J RIJCOSX level of theory

Conclusions

In summary, a naphthalimide-benzothiazole conjugate **1** functionalized with pyrrolidine derivative has been designed and synthesized. The receptor **1** was employed to investigate optical detection of metal ions such as Hg^{2+} and Pb^{2+} in acetonitrile. The probe **1** shows red fluorescence under UV light illumination at 365 nm. The receptor **1** selectively detect Hg^{2+} and Pb^{2+}ions in the presence of other interfering metal

ions such as Mg^{2+}, Ba^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Ag^{2+}, Fe^{2+}, Fe^{3+}andCr^{3+} (2.3equivalent).Only when 2.3 equivalent of Hg^{2+} and Pb^{2+} were added, receptor **1**displays significant fluorescence "on-off" strategy. Moreover, Job's plot analysis revealed 1:2 binding stoichiometry of **1**with Hg^{2+}as well as Pb^{2+}ions. Furthermore, the estimated binding constant (K_a) of receptor **1** towards Hg^{2+} and Pb^{2+}ions were 3.43286×10^{-6} M and 2.84079×10^{-6} M, respectively. The fluorescence titration experiments were employed to estimate the LOD and found down to be 7.44×10^{-10} Mfor Hg^{2+} and 1.26×10^{-9} M for Pb^{2+}. These results revealed the ultra-sensitivity of receptor **1** towards Hg^{2+} and Pb^{2+}ions. Thus, the present detection method has huge potential for real time applications.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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Competing financial interests

The authors declare no competing financial interests.

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