

Fluorescent chemosensor: recognition of metal ions in aqueous medium by fluorescence quenching

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Abstract A thiourea based benzothiazole–naphthalene (BTNP) receptor is synthesized and their photophysical properties and binding affinity with various metal ions is investigated in aqueous medium. UV–Vis absorption as well as emission spectroscopy in HEPES buffer exhibited the sensitivity and selectivity of BTNP relatively, more for Hg^{2+} ion in which the fluorescence intensity was significantly quenched (65–70%) and intensity of a fluorescent blue colored solution diminished (*switch-off*). The receptor BTNP has shown 2 ppm level detection limit for the metal ion. The proposed mechanism for the metal ion recognition by BTNP is realized through the studies based on FT-IR, ^1H and ^{13}C NMR spectroscopy. The results support the probability of Hg^{2+} ion induced irreversible desulphurization chemical reaction leading to the formation of urea derivative and also about complexation reaction with Cu^{2+} ion. Jobs plot analysis suggests about the complexation in 1:1 and 2:1 stoichiometry between BTNP and Hg^{2+} and Cu^{2+} metal ions respectively. The association constants and quenching efficiency were estimated to understand the unique naked-eye sensitivity of BTNP for the recognition of both the metal ions.

Keywords Metal–ion recognition · Sensor · Thiourea · Benzothiazole–naphthalene (BTNP)

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Introduction

The design and development of selective chemosensors for the determination of heavy and transition metal ions are interesting because of their considerable effects on environment and in biological systems [1–5]. Mercury is considered as one of toxic environment pollutant, mainly generated from industrial sources and once the elemental mercury is converted into methyl-mercury with the help of microorganism it may reach to the organs of human and animal bodies through a food chain [6–8]. The accumulation of mercury in the human body may cause serious health problems, like prenatal brain damage, cognitive and motion disorders, and Minamata disease [9, 10]. Similarly, copper has also been considered as another biologically important transition metal–ion [11] because, their higher concentration may displace other metal ions, and/or cofactors that are useful for various biologically important enzymes catalyzed chemical reactions [12]. The fluctuation in Cu^{2+} ion concentration may also induce oxidative stress and their infection in neuronal cytoplasm may cause Alzheimer's or Parkinson's diseases [13]. In this prospective several chemosensors based on moieties, such as azo-derivatives [14], *N*-dansylcarboxamide [15], aminonaphthol [16], anthrylacetylamide [17], crown-ethers [18], thio-carbamate [19], naphthalimides [20], rhodamine [21–23], anthraquinone [24], benzothiazole [25], fluorescein [26], etc. have been developed.

The most of heavy and transition metal ions display nonspecific fluorescence quenching upon interaction with a probe/receptor molecules. For example, Hg^{2+} and paramagnetic Cu^{2+} ions generally induce fluorescence quenching due to enhanced spin–orbit coupling [27] and by energy or electron transfer mechanism [28, 29] respectively. Thus, a receptor having both chromo and fluorogenic properties, and

are sensitive to change in their optical properties [30] selectively, in the presence of metal-ions, like mercury will be of interest for a naked-eye visible recognition events [31]. Moreover, a molecular system capable in chelate formation, to monitor these metal ions, will be advantageous in respect of obtaining significant fluorescence responses by the mechanism of chelation-induced fluorescence quenching (CHEQ) or enhancement (CHEF) [32–34].

According to the hard and soft acid-base concept thiourea, being a soft base has affinity to bind with a soft acid like mercury and copper ions [35]. In fact, colorimetric sensors designed specifically for Hg^{2+} ion are crown ethers, containing nitrogen and sulfur atoms as potential coordinating sites [18, 36] therefore, we anticipated that a thiourea-based receptor having both sulfur and nitrogen atoms would also coordinate with these metal ions in the reaction medium. The thiourea-based systems have not been much explored as a receptor for the detection of heavy and transition metal ions [37] although, both urea and thiourea subunits owing to their ability to act as a hydrogen-bond donor, are frequently, utilized as chemosensors for the anions [38, 39]. Recently, some thiourea-based fluorescent organic nanoparticles have also been utilized to detect heavy and transition metal-ions [40, 41].

Thus, keeping above facts in mind we tried to utilize a simple thiourea-based, benzothiazole–naphthalene (BTNP) receptor, **1** for the detection of heavy and transition metal-ions. The binding affinity of the receptor for various metal-ions is investigated by spectroscopic methods such as, UV–Vis absorption, fluorescence, FT-IR and ^1H and ^{13}C NMR. The photophysical properties in the aqueous medium clearly demonstrated the affinity of receptor **1** for Hg^{2+} ion selectively, among the other metal ions. However, receptor **1** has also shown affinity for Cu^{2+} metal ion in the absence of other metal ions. Both Hg^{2+} and Cu^{2+} ions were able to quench the fluorescence intensity of **1** probably, by an irreversible mercury induced desulfurization chemical reaction [42, 43] and by the removal of a labile proton (deprotonation), respectively in complexation reaction involving N and S atoms of thiourea subunit and of benzothiazolyl ring [44, 45].

Result and discussion

Synthesis and photophysical properties

The present thiourea-based benzothiazole–naphthalene (BTNP) receptor, **1** has been synthesized in good yield utilizing dithiocarbamate approach in a single-step reaction [46]. It was kept in mind that thiourea, being a soft base will likely to react with most of heavy and transition metal ions having of soft acid character. Secondly, the BTNP due

to the presence of nitro group will able to reveal a significant optical property as a result of photoinduced electron transfer (PET) reaction and can be potentially utilized for the recognition of metal ions according to variation in the optical properties. The structure of the compound was confirmed from spectroscopic and analytical data. FT-IR spectra in KBr pellet for **1** shows (Fig. S1, Supporting Information) vibration band at 3453 and 3168 cm^{-1} and a medium band at 1570 and 1278 cm^{-1} for asymmetric and symmetric stretching vibrations for N–H and –CS respectively. The vibration bands at 1513, 1330 and 791 cm^{-1} may be attributed to stretching bands corresponding to the NO_2 and –CS of benzothiazole unit. The ^1H NMR spectrum (Fig. S2, Supporting Information) for **1** in $\text{DMSO}-d_6$ solution shows two broad signals at 10.47 and 9.79 δ ppm values, probably for NH1 and NH2 protons of thiourea subunit (as shown in model, Fig. S4, Supporting Information) respectively. The resonance signals at 8.71 δ (singlet), 8.1 δ (doublet) and at 8.08 δ (triplet) values are probably due to the H1, H2 ($J = 8.1$ Hz) and H' protons of benzothiazole (BT) unit respectively. A multiplet in between 7.4 and 7.66 δ values may be attributed to H_o , H_p , H_o' , H_m' (of NP ring) and H_3 (of BT ring) protons. ^{13}C NMR spectrum for **1** in CDCl_3 solution besides, a chemical shift at 178.38 δ ppm, attributable to the –C=S unit of thiourea subunit shows the characteristic resonance signal for respective carbons (Fig. S3, Supporting Information).

UV absorption studies

The absorption spectrum of **1** was monitored in different solvents and was found optimum in acetonitrile–water mixture, in 1:1 ratio (v/v) and also in HEPES buffer of same solvent system (ACN/ H_2O ; 1:1, pH 7.4). Therefore, the binding affinity of **1** for various metal ions has been investigated in HEPES buffer solution. In the absence of metal ions the absorption spectrum of **1** (20 μM) illustrates two characteristic absorption bands at 378 ($\epsilon = 32450 \text{ M}^{-1} \text{ cm}^{-1}$) and a high energy band at 294 nm wavelengths, attributed to the $\pi \rightarrow \pi^*$ electronic transition of aromatic rings. The low energy band at 378 nm wavelength is attributed to the intramolecular charge transfer (ICT) reaction between the thiourea subunit to electron deficient benzothiazole (BT) unit. Upon addition of various metal ions (~ 10 equiv) such as, Na^+ , K^+ , Ca^{2+} , Cd^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Ag^+ , Pb^{2+} , Hg^{2+} and Cu^{2+} ions, as their nitrate salts, to the solution of **1** the absorption spectra is considerably modulated only in the presence of Hg^{2+} ion in which, the ICT band was hypsochromically shifted for 25 nm to appear at 353 nm wavelength and the low energy band at 294 nm wavelength shows significant enhancement in the absorbance due to relatively strong $\pi \rightarrow \pi^*$ electronic transitions (Fig. 1). The rest of metal ions have not

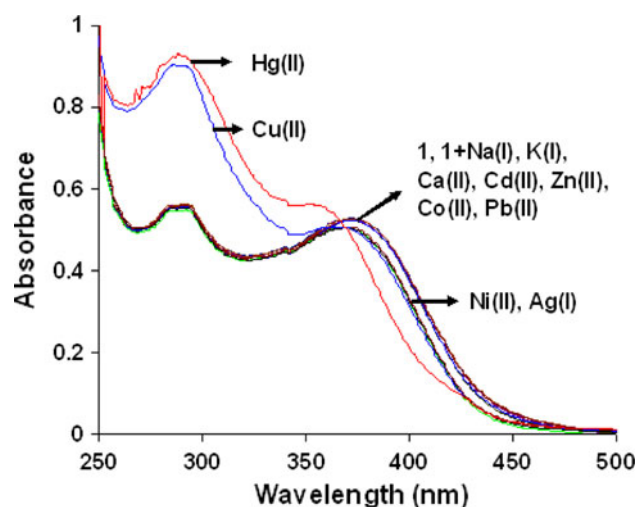


Fig. 1 UV-Vis spectra for **1** (20 μ M) in presence of varying metal ions in HEPES buffer solution

exhibited any significant change in the absorption spectrum of **1** except for Cu^{2+} ion. The absorption spectrum of **1** in the presence of Cu^{2+} ion reveals a relatively small decrease in the absorbance of ICT band along with a significant enhancement in the absorbance of high energy band at 294 nm. Consequently, upon interaction with both Hg^{2+} and Cu^{2+} metal ions a visible yellow–green color solution of **1** was almost disappeared, *switched-off* (inset of Fig. 2).

To ascertain the selective binding affinity of **1** for Hg^{2+} ion UV-Vis absorption titration experiment was carried out in ACN/ H_2O mixed (1:1; v/v) HEPES buffer (pH 7.4) system. To a 20 μ M concentration solution of **1** equivalents of Hg^{2+} ion (20 μ L = 0.4 equiv, $c = 10^{-3}$ M) was added sequentially. Upon addition of 2.4 equiv of Hg^{2+} ion to the

solution of **1**, ICT band was gradually enhanced and simultaneously, was blue-shifted to appear at 353 nm wavelength. Further, upon increasing the concentration of Hg^{2+} ion (2.8 equiv) the new transition band, centered at 353 nm wavelength starts decreasing and become saturated after the addition of 4.0 equiv of Hg^{2+} ion (Fig. 2). Similarly, absorption titration experiment was carried out with Cu^{2+} ion. Upon increasing the concentration of Cu^{2+} ion (0–20 equiv) to the solution of **1** no significant modulation in the absorption spectra was observed except a gradual enhancement in the absorbance of high energy band (inset of Fig. 8). Concomitantly, a yellow–green color of the receptor solution, become colorless or *switched-off* upon interaction with both Hg^{2+} and Cu^{2+} metal ions (inset of Fig. 2). The narrow changes in the absorption titration spectra with Cu^{2+} ion can be assumed to a rapid complexation reaction between **1** and Cu^{2+} ion involving both N and S atoms of thiourea subunit and thiazolyl ring of BT unit through the deprotonation of a labile proton in the medium [42, 43] (Fig. 3). However, inconsistent absorption spectra with Hg^{2+} ion have suggested about the possibility of Hg^{2+} ion-induced irreversible desulphurization chemical reaction leading to formation of urea/amide derivative, **4** by a complexation reaction with sulfur atom of thiourea subunit in the early stages.

Fluorescence properties

In order to understand the fluorescence property of receptor (BTNP), **1** first the emission spectra of naphthalene (NP) in HEPES buffer (ACN/ H_2O ; 1:1, v/v) was analyzed. Naphthalene (NP) upon excitation at 276 nm wavelength exhibited emission bands at 325 and 337 nm along with a

Fig. 2 Change in UV-Vis absorption spectra for **1** after the addition of **a** Hg^{2+} **b** Cu^{2+} ions in HEPES buffer solution ($\text{H}_2\text{O}/\text{ACN}$; 1:1, v/v, pH 7.4). Inset: Photographs illustrating the color change for **1** with Hg^{2+} and Cu^{2+} ions in HEPES buffer solution at pH 7.4

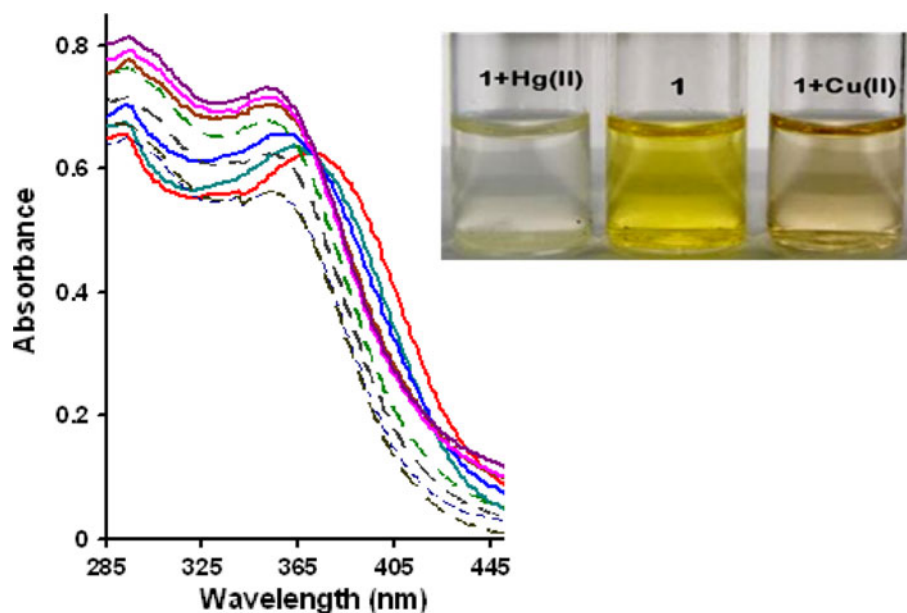


Fig. 3 The proposed complexation reaction for receptor **1** with Hg^{2+} and Cu^{2+} metal ions in HEPES buffer solution

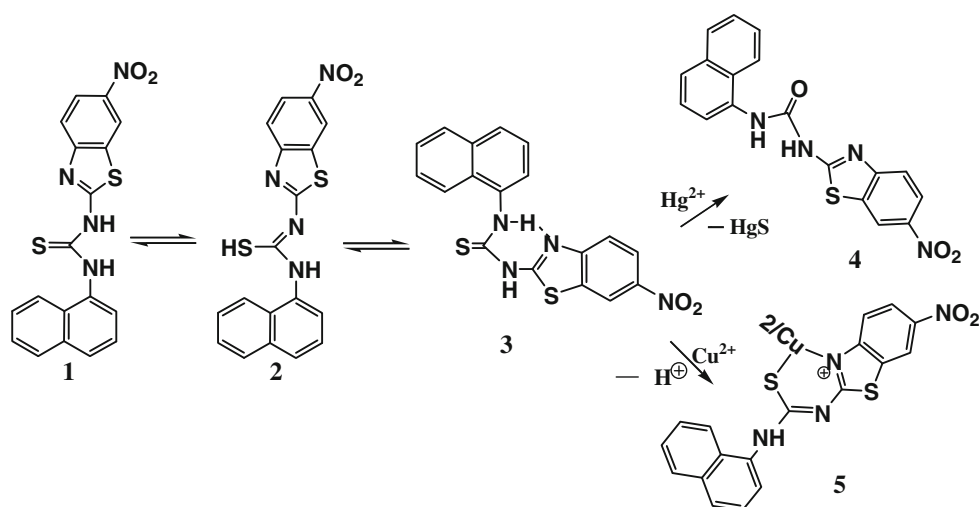
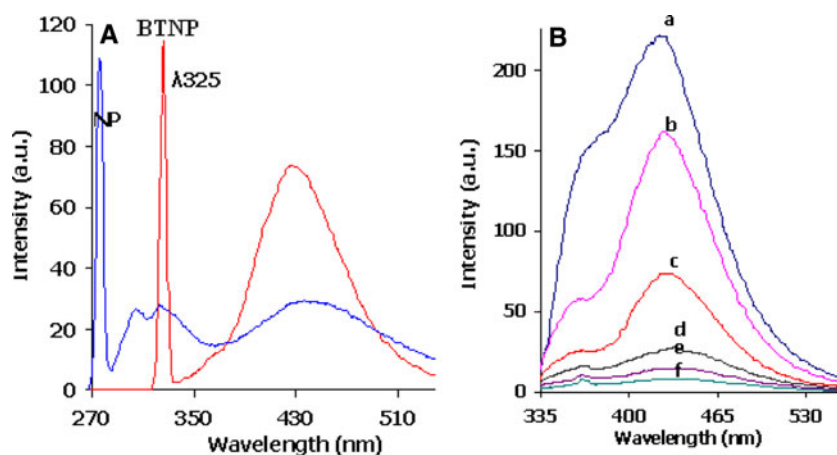


Fig. 4 a Comparative fluorescence spectra for naphthalene (NP) and **1**. **b** Change in relative fluorescence intensity for **1** upon dilution in the order (a) 10^{-5} M, (b) 5×10^{-6} M, (c) 10^{-6} M, (d) 7.5×10^{-7} M, (e) 5×10^{-7} M and (f) 10^{-7} M concentrations, at $\lambda_{\text{ext}} = 325$ nm excitation wavelengths in HEPES buffer (0.01 M, ACN/ H_2O in 1:1 ratio, v/v, pH 7.4) (bandwidth for excitation/emission 5/5 nm at 600 V PMT)



typical broad emission band at 431 nm wavelength, may be attributed for the structureless emission of a naphthyl excimer in the polar medium [47] (Fig. 4a). However, when benzothiazole–naphthalene (BTNP) receptor, **1** (1 μM) was excited at $\lambda_{\text{ext}} = 325$ nm wavelength enhanced fluorescence, similar to the naphthyl excimer was observed at 431 nm wavelength (Fig. 4). The enhanced excimer fluorescence intensity (~ 3 times) exhibited by **1** can be attributed to the extended conjugation through thiourea subunit and/or due to the intramolecular photo-induced electron transfer (PET) reaction involving thiourea subunit and electron deficient benzothiazole unit. Also, upon increasing the concentration of **1** from 10^{-7} to 10^{-5} M in HEPES buffer, a gradual increase in the relative fluorescence intensity with broadening in the emission spectra was observed (Fig. 4b). The observed fluorescence intensity signal indicates about the 100 nM range sensitivity of **1**. The quantum yield estimation for **1** was done by utilizing the secondary method [48] in acetonitrile solution and found $\phi_1 = 0.903$; 0.038 and 0.164 with respect to pyrene ($\phi_{\text{pyrene}} = 0.62$), anthracene ($\phi_{\text{anthracene}} = 0.3$) and naphthalene ($\phi_{\text{naphthalene}} = 0.2$) respectively.

The affinity of various metal ions with receptor, BTNP has been further observed by monitoring the change in the fluorescence spectra after the addition of 20 equiv of tested metal ions to the solution of **1** (1 μM). Interestingly, a significant fluorescence quenching was observed only with Hg^{2+} ion, in which the fluorescence intensity was reduced by ~ 50 to 65%. Subsequently, a diminished (*switched-off*) fluorescent blue color solution of **1** was observed under the UV light (Fig. 5). The other metal ions such as, Na^+ , K^+ , Ca^{2+} , Cd^{2+} , Ag^+ , Co^{2+} , Pb^{2+} , Ni^{2+} and Zn^{2+} have not been able to induce any significant change in the fluorescence intensity of **1**. However, upon addition of Cu^{2+} ion to the solution of **1**, $\sim 33\%$ quenching in the fluorescence intensity was observed as a result of complexation reaction (Fig. 6). To observe the effect of different metal ions as interfering to each other excess amount of metal ions were added to the solution of **1** + Hg^{2+} complex in a particular order and emission was taken. The relative change in the fluorescence intensity was estimated as shown in the bar diagram (Fig. S5, Supporting Information). It can be seen that the extent of fluorescence quenching generated by the mercury ion remained unaffected after the addition of other

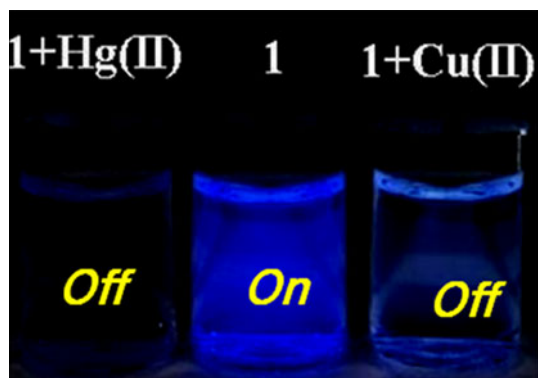


Fig. 5 Photographs obtained by illuminating respective solutions under UV-light at 256 nm wavelength

metal ions to the solution of **1** + Hg^{2+} complex and therefore, have clearly indicated about the high selectivity of chemosensor (BTNP) **1** for Hg^{2+} ion among the miscellaneous competitive metal ions.

To illustrate the sensitivity of **1** for Hg^{2+} ion fluorescence titration experiment was carried out by increasing the concentration of Hg^{2+} ion (10 μL = 1 equiv, $c = 2.5 \times 10^{-4}$ M) to the solution of **1** (1 μM) in HEPES buffer at room temperature. As the concentration of Hg^{2+} ion is increased in the solution of **1** (maximum 20 equiv) the intensity of emission band, at 431 nm wavelength, was gradually reduced, and $\sim 70\%$ fluorescence quenching was observed (Fig. 7) and concomitantly, the intensity of dark fluorescent blue color of chemosensor solution was diminished (*switched-off*) (Fig. 5). Similarly, in order to know the affinity of **1** with Cu^{2+} ion fluorescence titration experiments is carried out under similar condition and monitor the relative changes in the emission spectra. From Fig. 8 it can be seen that after the addition of 20 equiv of Cu^{2+} ion to the solution of **1** the fluorescence intensity was quenched by $\sim 65\%$ and, **1** could have able to detect both the metal ions up to 2 ppm concentration level. The 1:1 and

Fig. 6 a Fluorescence spectra for **1** (1 μM) upon interaction with various metal-ions (10 equiv) in HEPES buffer solution. **b** Barr diagram depicts the percentage of fluorescence quenching by various metal ions

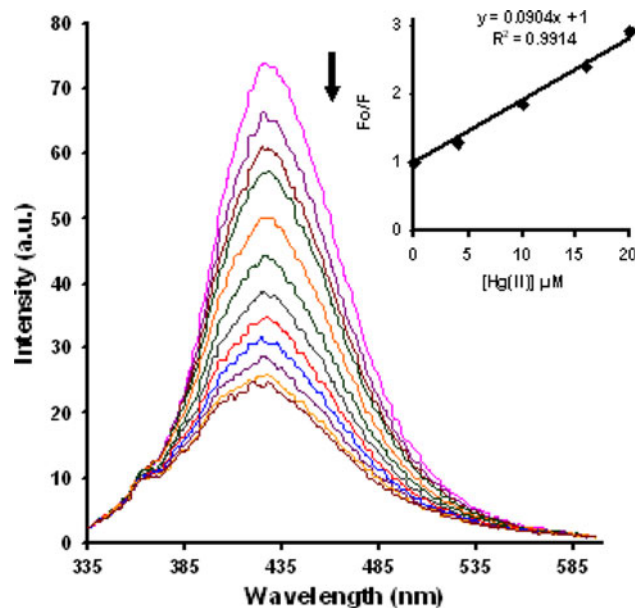
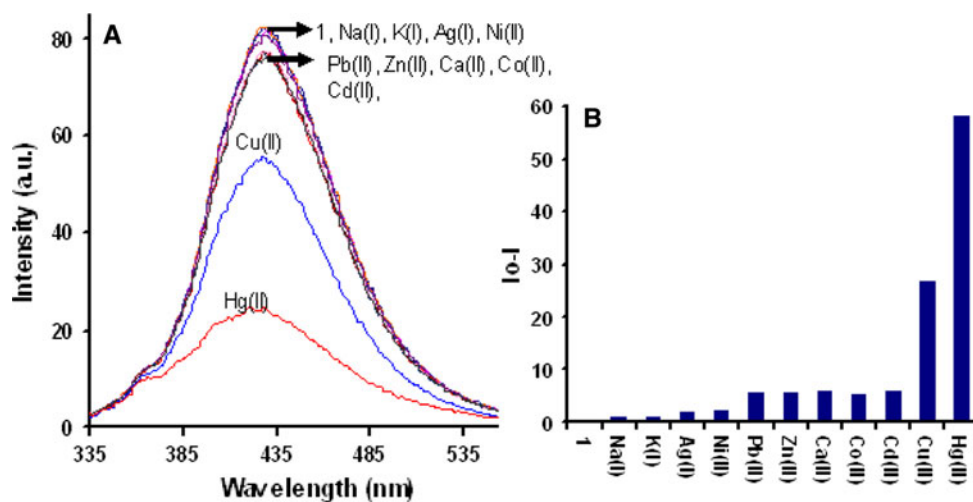


Fig. 7 Fluorescence titration spectra of receptor **1** (1 μM) with Hg^{2+} (0–20 equiv) at 325 nm excitation wavelength in HEPES buffer at pH 7.4

2:1 stoichiometry for complexation reaction of **1** with Hg^{2+} and Cu^{2+} metal ions in the solution have been realized by the analyses of Job's plot [49], obtained on the basis of fluorescence titration curves data (Fig. S6, Supporting Information). On the basis of nonlinear fitting of fluorescence titration curves [48–50] and by employing Benesi–Hildebrand method [51] the association constants were estimated using Eqs. 1 and 2 for 2:1 and 1:1 stoichiometry and was found $K_{\text{ass}} = 0.019 \times 10^4 \text{ M}^{-2}$ and $1.6 \times 10^4 \text{ M}^{-1}$ for Cu^{2+} and Hg^{2+} metal ions respectively (Fig. S7, Supporting Information). Thus, suggested about the mode of interaction between BTNP and metal ions during the recognition process. A quantitative measurement of fluorescence quenching has been carried out by obtaining

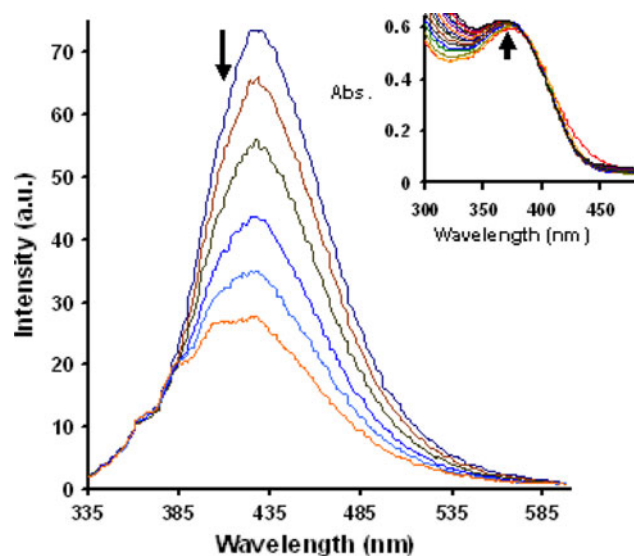


Fig. 8 Fluorescence titration spectra of receptor **1** (1 μM) with Cu^{2+} ion at 325 nm excitation wavelength and *inset shows* changes in absorption titration curves in HEPES buffer at pH 7.4

Stern–Volmer plots for both Hg^{2+} and Cu^{2+} metal ions. The almost linear S–V curves (Fig. 9) with values of $K_{\text{SV}} = 90400 \text{ M}^{-1}$ ($R^2 = 0.9914$) and 78000 M^{-1} ($R^2 = 0.9929$) for Hg^{2+} and Cu^{2+} metal ions respectively, have clearly suggested about the dynamic mode of quenching in the present recognition process, and, which was further evident by approximately two to threefold decrease in the quantum yields ($\phi_{1+\text{Hg}^{2+}} = 0.322; 0.0135; 0.055$ and $\phi_{1+\text{Cu}^{2+}} = 0.352, 0.0148; 0.056$) respectively.

To address the possibility of an irreversible Hg^{2+} ion mediated desulphurization reaction of **1** and a probable complexation reaction between **1** and Cu^{2+} ion, a strong chelating agent, EDTA was added systematically and both the electronic and fluorescence spectra were analyzed

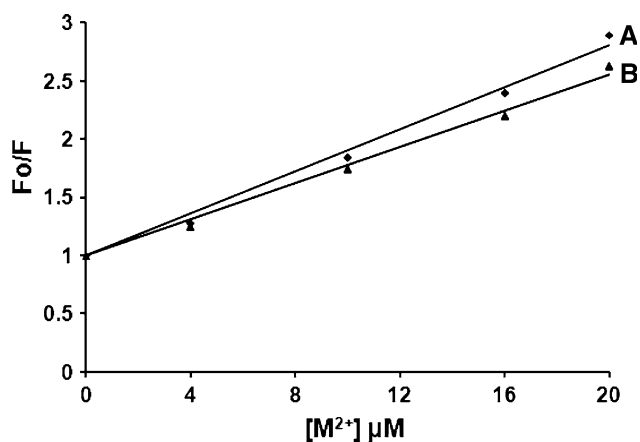


Fig. 9 The linear Stern–Volmer plots illustrates about dynamic mode of quenching in the fluorescence intensity of **1** in presence (a) Hg^{2+} and (b) Cu^{2+} ions at 325 nm excitation wavelength in HEPES buffer

(Fig. 10). BTNP does not show any significant affinity for EDTA as the fluorescence intensity of **1** remained unchanged upon the addition of excess amount of EDTA (250 equiv) to the solution of **1** (Fig. S8, Supporting Information). Also, upon addition of excess amount of Hg^{2+} and Cu^{2+} ions to the solution of **1**–EDTA insignificant change in fluorescence was observed. However, the absorption spectrum of **1**–EDTA solution upon addition of Hg^{2+} ion was blue-shifted with a significant enhancement in the absorbance of low energy band. Since, the addition of Hg^{2+} and Cu^{2+} metal ions to the solution of **1** quenches the fluorescence intensity and after the addition of EDTA to the probable **1**– Hg^{2+} and **1**– Cu^{2+} complex fluorescence does not regained and also, no significant change in the absorption spectra was observed, that clearly confirms the proposed irreversible mode of sensing mechanism in which the desulphurization and deprotonation (removal of proton from tautomeric forms, Fig. 3) reactions have occurred respectively. Thus, receptor **1** can be termed as a chemodosimeter, and that can be potentially used for the detection of both the metal ions with “On–Off” switching ability.

FT-IR, ^1H NMR and ^{13}C NMR analysis

The FT-IR spectra of **1** on treatment with Hg^{2+} ion shows the appearance of a strong and broad vibration bands at 1088 cm^{-1} $\nu(\text{C–O–M})$ and 1628 cm^{-1} $\nu(\text{C=O})$ respectively, along with a shift in the N–H stretching vibration from 3453 to 3448 cm^{-1} can be assigned to the formation of amide derivative as a consequence of Hg^{2+} ion induced irreversible desulphurization chemical reaction. Similarly, interaction of **1** with Cu^{2+} ion resulted a new strong band at 1384 cm^{-1} and can be attributed to **1** + Cu^{2+} complexation involving N and S atoms of thiourea and benzothiazolyl unit, “–CS– Cu^{2+} –N–” [42, 43] (Fig. S1, Supporting Information).

^1H NMR titration spectra analysis, upon addition of Hg^{2+} ion (1 equiv), show a significant high-field shift of 0.43 and 0.65 δ values for NH1 and NH2 protons of **1** in $\text{DMSO-}d_6$ solution respectively (Fig. 11). The H1 and H2 (8.23 δ) protons were shifted toward down field with 0.09 and 0.13 δ chemical shifts respectively. The resonance signals for the rest of protons of both NP and BT units were shifted toward the high-field region. However, resonance signals were almost remained constant on further increase in the concentration of Hg^{2+} ions (maximum 3.0 equiv). The significant changes occurred at the NH protons in the ^1H NMR signals is probably due to the more charge density on urea subunit obtained as a consequence of Hg^{2+} ion induced desulphurization reaction and thus, supporting the proposed mechanism for the detection of Hg^{2+} ion. Similarly, upon the addition of Cu^{2+} ion (1 equiv) the probable

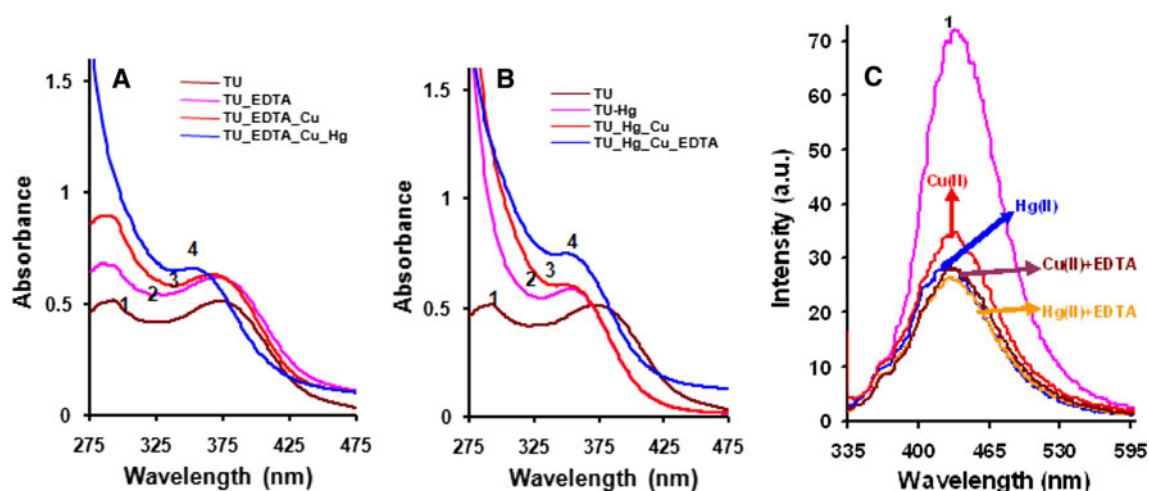


Fig. 10 Change in UV–Vis absorption (a, b) and fluorescence (c) spectra for **1** upon interaction with Hg^{2+} and Cu^{2+} ions in HEPES buffer solution (0.01 M, ACN/ H_2O , pH = 7.4) and upon interaction

with EDTA (250 equiv) to the same solution. $\lambda_{\text{ext.}} = 325$ nm; slit-width 5 nm/5 nm for both excitation and emission wavelengths, 600 V PMT

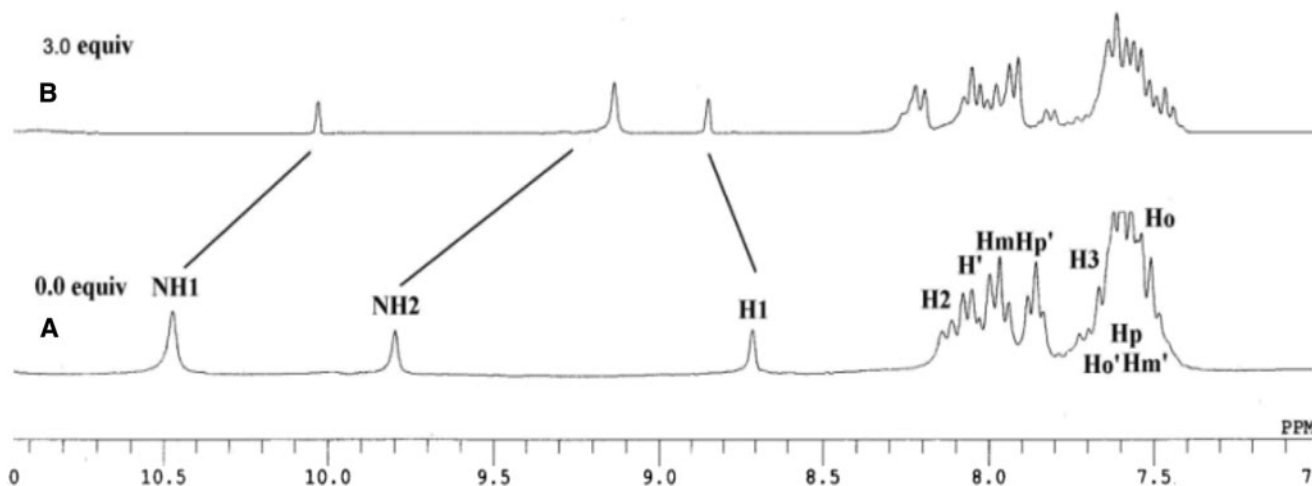


Fig. 11 ^1H NMR spectra for **1** a ($c = 2.6 \times 10^{-2}$ M) in $\text{DMSO}-d_6$ solution and b after addition of Hg^{2+} ions

resonance signals (Fig. 12) for NH1 and H' protons were shifted in the high-field region to appear at 9.94 and 7.94 δ ppm while the NH2 and H1 protons were shifted toward low-field to appear at 10.51 and 8.74 δ respectively, along with a new signal generated at 10.06 δ ppm. Upon increasing the concentration of Cu^{2+} ion (2.0 equiv) NH2 proton was significantly down field shifted to appear at 12.40 δ . This can be attributed to the more de-shielding on –NH2 subunit as a consequence of complexation reaction between **1** and Cu^{2+} ion and, the two close signals, appeared after the addition of 1 equiv of Cu^{2+} ion were resolved and shifted toward the high-field region to appear at 9.29 and 9.98 δ ppm values. Upon further increase in Cu^{2+} ions concentration (3.0 equiv) both the resolved signals become broadened and/or almost disappeared while the resonance signal for NH2 proton was remained. The H2

proton (doublet) of BT unit was broadened to appear as singlet without any significant shift, while the H' proton of NP unit was shifted in the high field region and merged together with other aromatic ring protons. The high field shift for NH1 proton can be attributed to greater charge density at thiourea subunit as a result of proton transfer reaction leading to generation of thione (–SH) in equilibrium. We hypothesized that the possibility of complexation reaction between **1** and Cu^{2+} ion can only be understood after considering removal of a proton from thiourea and/or isothiuronium tautomeric form in equilibrium of the complexation system. The resonance signals corresponding to the NH1 and –SH protons and their respective chemical shifts ($\Delta\delta$ 0.77 ppm) upon increasing the concentration of Cu^{2+} ion actually, validate the proposed mechanism of complexation reaction. Moreover, when the solution of

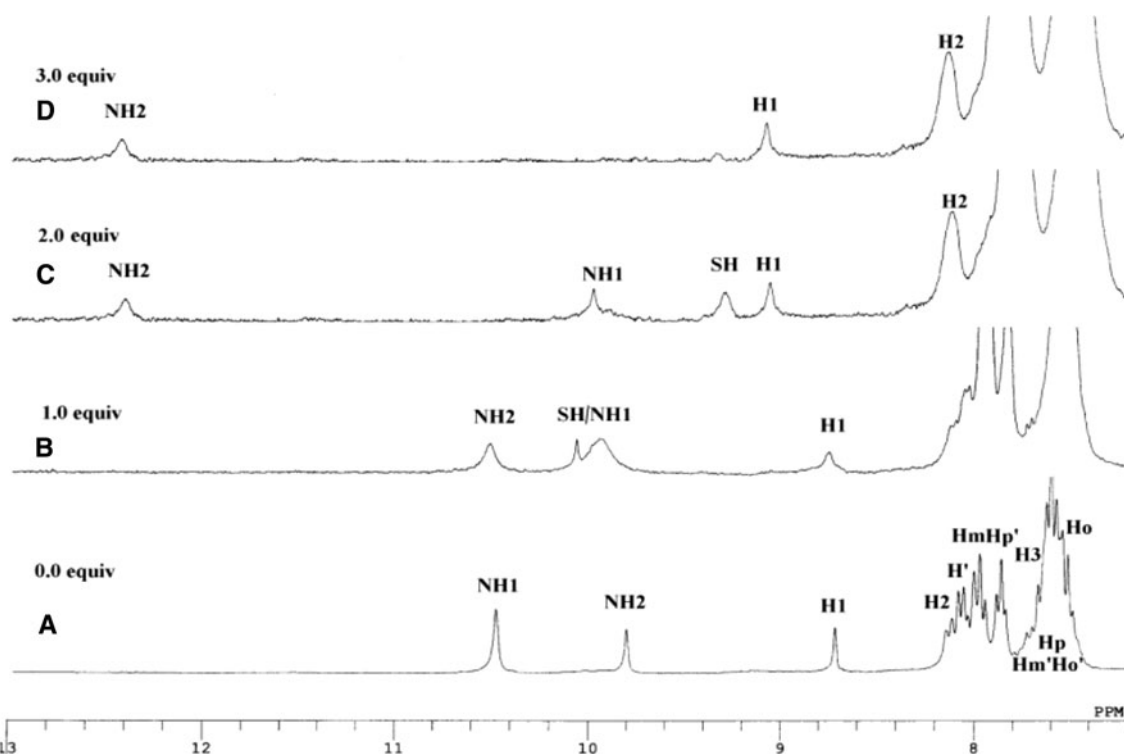


Fig. 12 ^1H NMR spectra for **1 a** ($c = 2.6 \times 10^{-2}$ M) in $\text{DMSO-}d_6$ solution and **b–d** after addition of Cu^{2+} ion

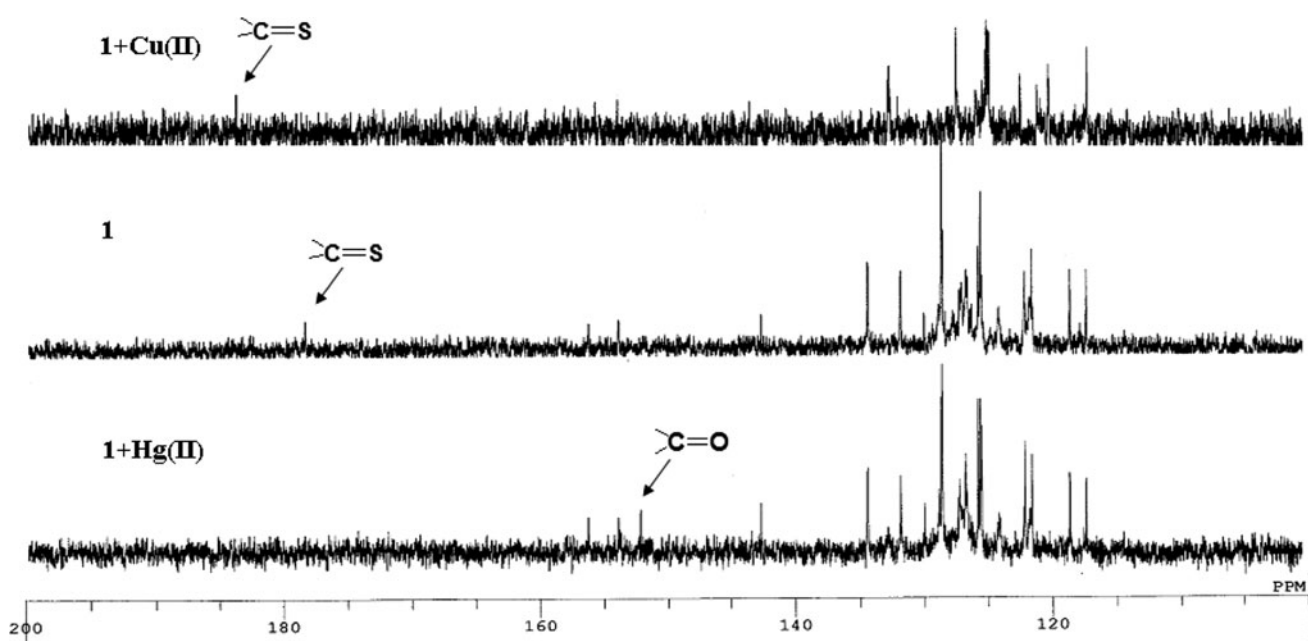


Fig. 13 ^{13}C NMR spectra for **1** ($c = 6.3 \times 10^{-2}$ M) in CDCl_3 solution and after addition of Cu^{2+} and Hg^{2+} ions

complex treated with D_2O the probable proton signals corresponding to the $-\text{NH}/-\text{SH}$ subunits were disappeared (Fig. S9, Supporting Information). Thus, clearly confirms the irreversible mode of sensing mechanism with Cu^{2+} ion through deprotonation reaction.

Moreover, a resonance signal attributable to $-\text{C}=\text{S}$ of thiourea subunit (Fig. 13) at 178.38δ ppm in ^{13}C NMR (CDCl_3) was disappeared upon the addition of Hg^{2+} ion and a new signal was appeared at 152δ ppm. The significant high field chemical shift ($\Delta\delta$ 26.4 ppm) is presumably

due to the Hg^{2+} ion induced desulphurization chemical reaction leading to the formation of urea derivative which have relatively more bond order and/or charge density on the urea/amide subunit than on a thioamide subunit [52–55]. However, the ^{13}C NMR ($\text{DMSO-}d_6$) spectrum for **1** + Cu^{2+} complex was crucial to understand. The probable resonance signals at 142.6, 118.6 and 131.8 δ ppm of benzothiazolyl and naphthyl rings were shifted to down field region and signals corresponding to the naphthyl unit at 128.6 and 127–126 δ ppm in the high-field region, upon interaction with Cu^{2+} ion. The significant shift in the resonance signal from 178.38 δ ($-\text{C}=\text{S}$ of thiourea subunit) to 184 δ ppm clearly supports the complexation reaction between **1** and Cu^{2+} ion involving thiourea subunit and nitrogen atom of thiazolyl ring of BT unit.

Experimental

General methods

For the UV–Vis absorption spectroscopy a 20 μM concentration solution of **1** was prepared in HEPES buffer (0.01 M, $\text{ACN}/\text{H}_2\text{O}$; 1:1, v/v, $\text{pH} = 7.4$) solution. For the absorption titration spectra aqueous solution of different metal ions ($c = 10^{-3}$ M, 20 $\mu\text{L} = 0.4$ equiv) as their nitrate salts were prepared and equivalents were added increasingly to a fix concentration of receptor solution. Similarly, the fluorescence studies in same buffer solution keeping a constant concentration for **1** (1 μM) and varying the concentration of metal ions (3 $\mu\text{L} = 0.1$ equiv, $c = 6.66 \times 10^{-4}$ M) was carried out on a Cary Eclipse fluorescence spectrophotometer. The fluorescence spectra were recorded at 10 min time interval after each addition of metal ions. The ^1H NMR spectra was recorded on a JEOL FT-NMR (300 MHz) spectrometer using TMS as an internal standard. The ^1H NMR titration studies was done by treating the receptor molecule ($c = 2.6 \times 10^{-2}$ M in $\text{DMSO-}d_6$) with Hg^{2+} and Cu^{2+} ions as their nitrate salt. The FT-IR spectra using KBr pellets were recorded on a Varian 3100 FTIR spectrometer. On the basis of fluorescence data the association constant were analyzed by Benesi–Hildebrand method [51] employing Eqs. 1 and 2 for 2:1 and 1:1 stoichiometry respectively.

$$1/(I - I_o) = 1/(I - I_f) + 1/K(I - I_f)[M]^{1/2} \quad (1)$$

$$1/(I - I_o) = 1/(I - I_f) + 1/K(I - I_f)[M] \quad (2)$$

where K is the association constant, I is the fluorescence intensity of the free receptor **1**, I_o is the observed fluorescence intensity of the **1** – Cu^{2+} complex, and I_f is the fluorescence intensity at saturation. The quantum yields were estimated with respect to the pyrene, anthracene and

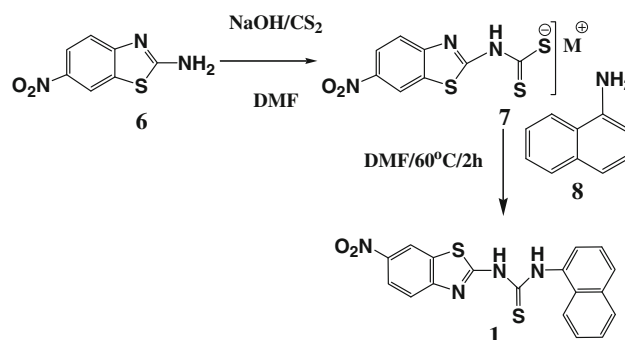
naphthalene as standard in acetonitrile solution by secondary methods [48] using Eq. 3

$$Q = Q_R \cdot I/I_R \cdot \text{OD}_R/\text{OD} \cdot n^2/n_R^2 \quad (3)$$

where Q is the quantum yield, I is the integrated intensity, OD is the optical density, and n is the refractive index. The subscript R refers to the reference fluorophore of known quantum yield. MM2 optimized minimum energy model picture (Fig. 4) was generated by the Chem 3D Ultra, 7.0 version software, Combridgesoft (UK).

Synthesis of **1**, [1-(naphthalene-1-yl)-3-(6-nitrobenzothiazolyl-2-yl)]thiourea

The synthesis of **1** is carried out (Scheme 1) with little modification as reported previously [46]. In brief, 2-amino-6-nitrobenzothiazole in N,N' -dimethylformamide (DMF) in the presence was reacted with carbon disulfide (CS_2) in the presence of sodium hydroxide (NaOH) under anhydrous condition for 1–2 h. When the reaction was completed (monitored on TLC) chloroform was added to the reaction mixture to obtain an orange color solid of dithiocarbamic salt **7** in 97% yield. The salt, **7** and 1-naphthylamine, **8** were taken in DMF and stirred the reaction mixture for 2 h at 60 $^\circ\text{C}$. Excess amount of water was added to precipitate out **1** in 65% yield. M.p. 137–140 $^\circ\text{C}$. R_f 0.65 ($\text{EtOAc} : \text{Hexane}:: 4 : 6$, v/v); IR (ν_{max} , KBr , cm^{-1}): 3325, 3166, 3056, 1568, 1511, 1445, 1329, 1228, 778; ^1H NMR ($\text{DMSO-}d_6$) δ (ppm): 10.40 (br, 1H, NH), 9.72 (br, 1H, NH), 8.64 (s, 1H, benzothiazole), 8.01–7.98 (d, 1H, $J = 8.1$ Hz, benzothiazole), 7.96–7.93 (d, 1H, $J = 7.8$ Hz, naphthyl), 7.93 (t, 1H, $J = 7.5$ and 8.1 Hz, naphthyl), 7.79–7.76 (d, 1H, $J = 7.5$ Hz, naphthyl), 7.66–7.63 (d, 1H, $J = 8.1$ Hz, benzothiazole), 7.59–7.44 (m, 3H, naphthyl), 7.41–7.39 (d, 1H, $J = 8.1$ Hz); ^{13}C NMR (CDCl_3 , 75.45 MHz) δ (ppm): 178.3, 156.2, 153.9, 142.7, 134.4, 131.8, 128.6, 127.3, 127.1, 126.7, 125.8, 125.6, 125.5, 122.1, 121.8, 121.6, 118.6, 117.3; Anal. Calc. for $\text{C}_{18}\text{H}_{12}\text{N}_4\text{O}_2\text{S}_2$; C, 56.83; H, 3.18; N, 14.73%. Found: C, 56.35; H, 3.41; N, 14.84%.



Scheme 1 Synthesis for receptor **1**

Conclusion

A thiourea-based benzothiazole–naphthalene (BTNP) receptor has been utilized for the recognition of Hg^{2+} and Cu^{2+} transition-metal ions with relatively higher sensitivity among the various metal ions. BTNP receptor has demonstrated “On–Off” sensing ability in the aqueous medium. The ^1H and ^{13}C NMR data and EDTA experiments have suggested the irreversible mode of sensing mechanism in which Hg^{2+} ion induced desulphurization reaction and Cu^{2+} ion-induced deprotonation of thiourea–isothioureia/thione proton occurred. Thus, we have demonstrated the optical properties and affinity of BTNP to work as a chemodosimeter to detect metal ions in aqueous medium.

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