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Cite this: DOI: 10.1039/c0xx00000x

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PAPER

Pyrene Linked Thiourea as a Chemosensor for cation and simple fluorescent sensor for picric acid

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

Pyrene linked simple thiourea has been synthesized and receptor **1** acts as fluorescence and visual sensor for the detection of Cu²⁺ and Hg²⁺ ions. The sensor showed effective detection of Cu²⁺ and Hg²⁺ at a low detection limit. Receptor **1** has been successfully applied to fluorescence imaging of Cu²⁺ and Hg²⁺ ions in living cells. Also receptor **1** selectively detects picric acid via color change and fluorescence change.

Introduction

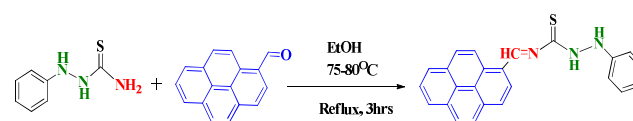
The detection of cations is an essential part of supramolecular chemistry and most important research topic in environmental, biology and medicinal chemistry.¹⁻² Among the available detection methods, chemosensors are predominantly attractive in terms of sensitivity, selectivity and response time.³ Copper is an essential trace heavy metal element in biological systems. Copper act as a cofactor for a variety of metalloenzymes and high level of Cu²⁺ has been reported to induce liver and kidney damage.⁴⁻⁸ Mercury, another heavy and toxic metal ion is widely distributed in the environment and one of the most notorious toxic metals. Mercury ions have high affinity for thiol groups in proteins and consequently causing many health problems in the brain, kidney, and central nervous system.⁹⁻¹³ Many fluorescent sensors for mercury and copper detection have been reported. But still the search of structurally simple receptor which can be easily synthesized and efficiently used has been of intense interest in the area of molecular recognition.¹⁴⁻¹⁸ Thus, developing methods for the detection of these two metal ions have been catching considerable attention in the medical, biological and environmental science. Among various nitroaromatics, picric acid (2, 4, 6- trinitrophenol) is a common reagent used in pharmaceutical, leather, fireworks, rocket fuels and dye industries. Picric acid is very harmful to human being like strong irritant, allergen and inhalation of picric acid can affect central nervous system, cardiovascular system, metabolism, kidney, urinary system and liver.^{19, 20} Recently the sensitive fluorescent based detection of nitroaromatics is gaining increasing attention. During the last few years, most of the reported fluorescent sensors exhibit sensitive response towards TNT compared to picric acid.^{21, 22} From this point of view, the development of highly selective and sensitive fluorescent molecule for trace detection of picric acid is still a challenge. Anthracene and pyrene have emerged as the most effective functional groups for fluorescence signaling. Pyrene is a polycyclic aromatic hydrocarbon (PAH). Pyrene derivative is evident as excellent

fluorophore and widely used in the developments of fluorescence sensors because of their excellent photoluminescence properties and chemical stabilities. Therefore, we synthesized the pyrene based thiourea Receptor **1** and evaluated its sensing properties. Receptor **1** has already been reported to act as fluorimetric chemosensor toward fluoride anions,²³ however their application as colorimetric sensor for different transition metal ions and picric acid has not been reported previously.

Herein, we report pyrene linked simple thiourea as a dual chemosensor for both Cu²⁺ and Hg²⁺ ions. Receptor display highly selective and sensitive recognition towards Cu²⁺ and Hg²⁺ ions over other cations via visual color change and shows significant changes in the absorption spectrum and great fluorescence enhancement upon the addition of Cu²⁺ and Hg²⁺ ions. Receptor **1** was further applied for the detection of Cu²⁺ and Hg²⁺ ions in living cells. Receptor **1** selectively detects picric acid over other nitroaromatic explosives. To the best of our knowledge this represents the first report on colorimetric/fluorescent chemosensor capable of detecting Cu²⁺, Hg²⁺ and picric acid by different response.

Result and Discussion

A hot ethanolic solution of N-phenylthiosemicarbazide was slowly added to a solution of pyrene-1-carboxaldehyde in ethanol. The reaction mixture was reflux at 75-80 °C for 3 h, yielding the precipitate of receptor **1** (Scheme 1). After evaporating the solvent in vacuum, the residue was filtered and recrystallized with ethanol. Receptor **1** was characterized by ¹H NMR, FT-IR, mass and UV-vis spectroscopic techniques (Fig. S1-S3).

Scheme 1. Synthetic route of Receptor **1**

The recognition properties of receptor **1** towards different cations were studied by visual change method. Receptor **1** shows a color change from colorless to green in the presence of Cu^{2+} and Hg^{2+} (Fig 1). The other cations like Fe^{3+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} , Mn^{2+} , Sn^{2+} and Cr^{3+} were found to be insensitive with receptor **1**. The color changes are due to the formation of metal complexes with receptor **1**.

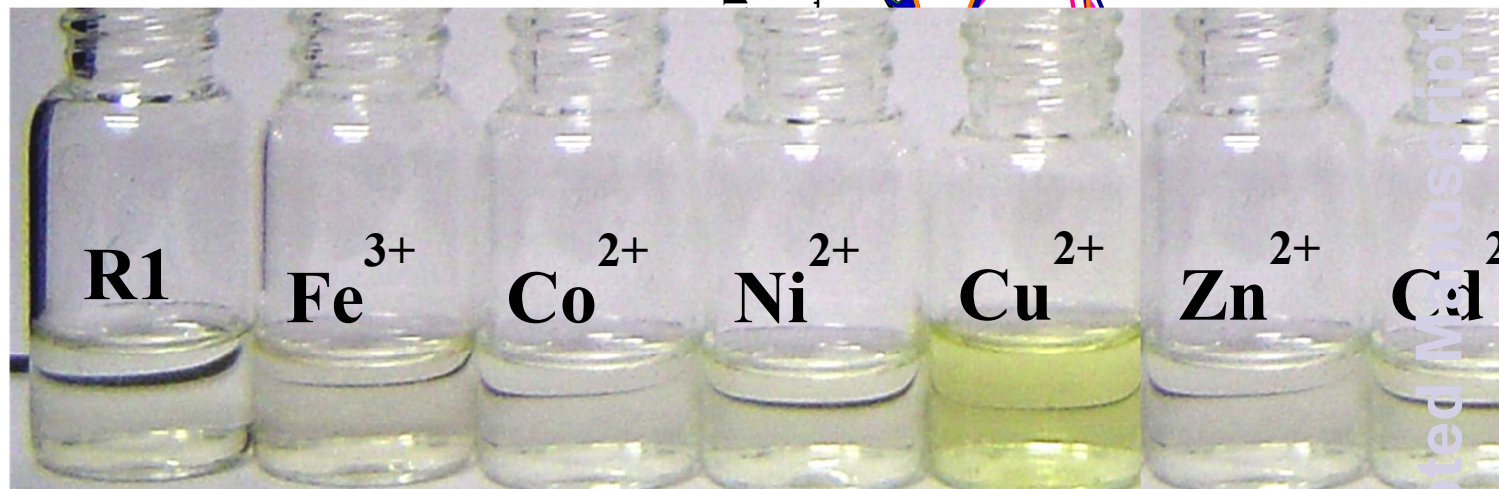


Fig 1. Color changes of receptor **1** (5×10^{-5} M soln in CH_3CN) before and after the addition of $200 \mu\text{L}$ of respective cations (1.5×10^{-3} M soln in H_2O).

The interaction of receptor **1** with different cations were evaluated through UV-vis titration method. The absorption spectrum of receptor **1** in CH_3CN exhibited an absorption maximum at 386 nm. To investigate the cation sensing property receptor **1** is treated with various metal ions: Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} , Hg^{2+} , Mn^{2+} , Sn^{2+} and Cr^{3+} in H_2O . The changes in the absorption spectra of receptor **1** upon the addition of all cations are shown in Fig. 2a. The absorption at 386 nm decreased with blue shift /red shift upon the addition of Hg^{2+} (Fig. S4) and Cu^{2+} (Fig. 2b) ions into receptor **1** respectively. In contrast, there was no significant optical change in the presence of other metal ions. The stoichiometry of the complexes formed was also determined by the changes in the UV-vis response of receptor **1** in the presence of varying concentrations of Cu^{2+} and Hg^{2+} ions, the results indicate the formation of 1:1 complexes with good binding constants ($K_{\text{app}} = 9.5 \times 10^5$ for R1-Cu^{2+} and 7.4×10^5 for R1-Hg^{2+}). The calculated detection limit is $3.2 \times 10^{-8} \text{ M}^{-1}$ and $0.56 \times 10^{-8} \text{ M}^{-1}$ for R1-Cu^{2+} and R1-Hg^{2+} respectively.

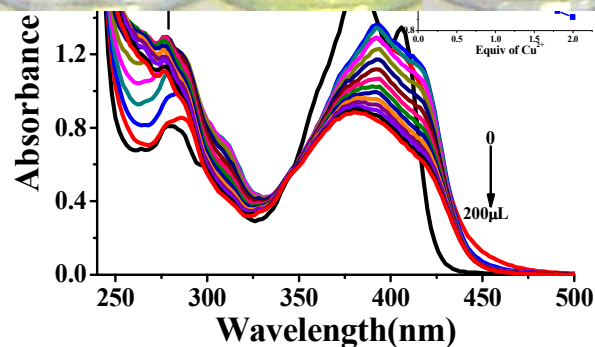
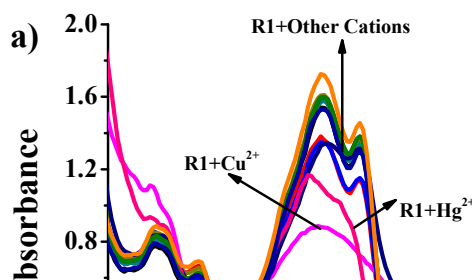


Fig 2b. UV-vis spectrum of receptor **1** (5×10^{-5} M, in CH_3CN) upon titration with Cu^{2+} (1.5×10^{-3} M, in H_2O) (Inset: Changes of absorbance upon addition of Cu^{2+} ion at 386 nm).

To further evaluate the binding affinity of receptor **1**, we investigated the emission spectrum of **R1** in the presence of various cations. The fluorescent titration of receptor **1** with different metal ions was investigated. On the addition of various metal ions into receptor **1**, Cu^{2+} and Hg^{2+} show a high fluorescence emission (Fig. 3a). Upon the gradual addition of Cu^{2+} into receptor **1**, an emission band at 440 nm shifted to 460 nm (red shift). After adding 2 equiv of Cu^{2+} , the emission intensity reached a maximum (Fig. 3b). The calculated quantum yield was 0.73, which is 16 fold higher than that of receptor **1** (0.045). During titration with Hg^{2+} , a band at 440 nm increased linearly up to 2 equiv of Hg^{2+} ions and the calculated quantum yield is 0.62 (Fig. 3c). The other cations like Fe^{3+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} , Mn^{2+} , Sn^{2+} and Cr^{3+} ions did not show any emission changes.

Fe³⁺, R+ Co²⁺, R+ Ni²⁺, R+ Cu²⁺, R+Zn²⁺, R+Cd²⁺, R+Pb²⁺,
 5 R+Hg²⁺, R+Mn²⁺, R+Sn²⁺ and R+Cr³⁺).

Sensors	LOD	Sensing Ions	Binding Constant	Linear Range (R ²)	Applications	References
Pyrene based sensor	3.91 μM 4.29 μM	Cu ²⁺ Hg ²⁺	1.0×10 ⁶ M ⁻¹ 8.4×10 ⁵ M ⁻¹	0.995 0.993	NA	24
Pyrene based sensor	2.17 μM	Cu ²⁺ Fe ³⁺	NA	0.991	HeLa cells (Fe ³⁺)	25
Pyrene based sensor	2.73 μM	Cu ²⁺	5.0 × 10 ⁸ M ⁻²	0.996	RAW 264.7 cells	26
Pyrene based sensor	4.69 μM	Hg ²⁺	NA	NA	NA	27
Pyrene based sensor	0.09 μM 0.09 μM	Cu ²⁺ Hg ²⁺	1.0 × 10 ⁴ M ⁻¹ NA	0.993 0.995	NA	28
Pyrene based sensor	0.79 nM	Hg ²⁺	2.4 × 10 ⁴ M ⁻¹	0.990	HeLa cells	29
Urea based sensor	NA	Cu ²⁺	9.3×10 ³ (R1) 3.3×10 ³ , 2.1×10 ⁴ (R2)	NA	NA	30
Pyrene linked Thiourea based sensor	3.20 Sub-nM 0.56 Sub-nM	Cu ²⁺ Hg ²⁺	9.5×10 ⁵ 7.4×10 ⁵	0.986 0.997	RAW 264.7 cells	This Work

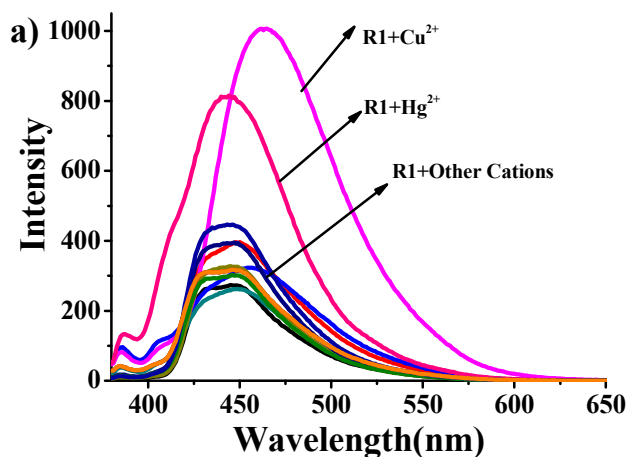


Fig 3a. Fluorescence spectrum of receptor **1** (5×10^{-5} M, in CH₃CN) upon titration with aqueous solution of cations (R, R+

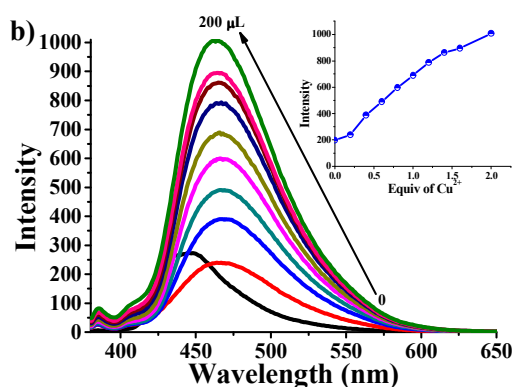


Fig 3b. Fluorescence spectrum of receptor (5×10^{-5} M, in CH₃CN) upon titration with Cu²⁺ (1.5×10^{-3} M, in H₂O) (Inset: Changes of emission upon addition of Cu²⁺ ion at 475 nm).

Table 1. Comparison of some reported chemosensors for Cu²⁺ and Hg²⁺

The receptor **1** was compared with some reported colorimetric/fluorescent chemosensors based on either pyrene or urea structural moiety for Cu²⁺ and Hg²⁺ ions (Table 1). While each chemosensor showed some advantages such as easy synthesis, high sensitivity, no interference, naked eye detection and application in living cell imaging. Our receptor **1** presents a number of attractive analytical features such as dual responsive, wide linear range, high selectivity, naked eye detection, and living cell imaging applications [22-30].

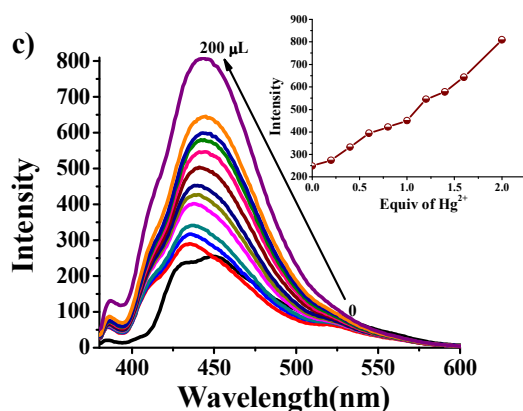


Fig 3c. Fluorescence spectrum of R1 (5×10^{-5} M, in CH₃CN) upon titration with Hg²⁺ (1.5×10^{-3} M, in H₂O) (Inset: Changes of emission upon addition of Hg²⁺ ion at 450 nm).

Since the present system consists of two distinct binding sites for the metal ions and F⁻ ion. Receptor **1** has already been reported to act as fluoride ion chemosensor.²¹ We thought it is appropriate to examine the effect of competing F⁻ ion on the binding ability of receptor **1** for metal ions and vice versa. The binding ability of R1 with Cu²⁺ is higher than that of F⁻ ions. The sensing ability of

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R1 in the presence of competing ions is clearly illustrated in the schematic representation (Fig 4).

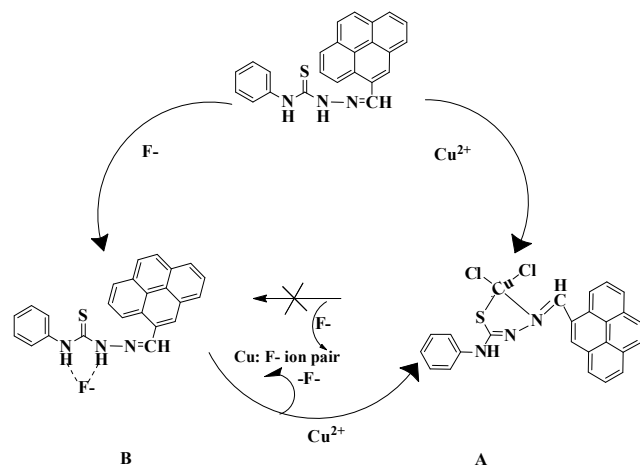


Fig 4. Possible structures of complexes formed between **R1** and F^-/Cu^{2+} ions.

Initial titration of **R1** with 2 equiv of Cu^{2+} , a blue shift of the band at 386 nm occurred followed by the reduction of intensity of the 382 nm band towards the addition of TBAF (Fig. S7). It can be concluded that the seizing of the metal ion to form an ion pair with F^- ion took place. In the next experiment, the addition of 200 μ L of F^- into **R1**, a new band at 475 nm due to the formation of **R1-F⁻** complex (Fig. S8). The blue shift of the band at 386 nm was resulted upon the incremental addition Cu^{2+} ions because F^- ions engage the metal ions in ion pair formation instead of complexation with **R1**. The competing ion study proves that Cu^{2+} ion has a strong affinity towards **R1** even in presence of F^- .

The binding of **R1** with tetrabutyl ammonium fluoride (TBAF) is also clear from the ¹H NMR spectroscopic titration method. ¹H NMR titrations were carried out in DMSO-*d*₆. The NH protons ($\delta=10.35$ ppm and $\delta=11.97$) of thiourea moiety of **R1** gets shifted and disappeared completely after the addition of 2equiv of F^- ions, indicating the interaction of the fluoride ions through hydrogen bond formation followed by deprotonation. The imine protons appeared at 9.40 ppm shifted to upfield with the addition of F^- ions (Fig. 5).

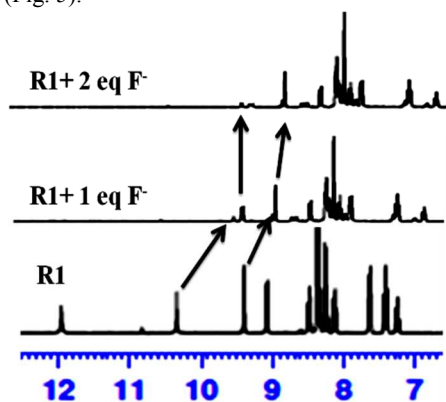


Fig 5. ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of **R1** on addition of 1.0 and 2.0 equivalents of F^- ion.

In the IR spectra the band at 1618 cm^{-1} is associated with C=N stretching vibration and is shifted to lower frequencies (1596 cm^{-1} & 1610 cm^{-1}) in the spectra of corresponding Cu^{2+} and Hg^{2+} complexes respectively. This indicated the coordination of azomethine nitrogen to the metal ion. A band which appeared in the region $3214\text{--}3285\text{ cm}^{-1}$ due to N-H in the ligand is disappeared on complexation. Further, a band due to C=S (742 cm^{-1}) which appeared in all the ligands has completely disappeared in the spectra of the complexes and a new band appeared around 700 cm^{-1} (Cu^{2+}) and 718 cm^{-1} (Hg^{2+}) (for C=S) due to enolization of -NH-C=S group of the ligand followed by deprotonation prior to coordination of the thiolate sulfur. Similarly in the ¹H NMR spectra of receptor **1** a singlet in the region 11.97 ppm for -NH proton, is absent in the spectra of $Cu(II)$ and $Hg(II)$ complexes confirms the above phenomenon. A doublet in the region 9.00–9.40 ppm in the complexes can be assigned to azomethine proton. Splitting of imine signal into a doublet is observed and attributed to coupling of imine proton with the metal ion (Fig. S9 & S10).

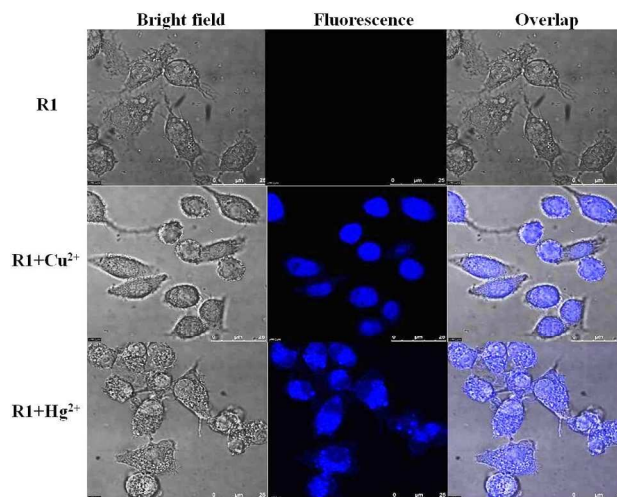


Fig 6. Fluorescence images of macrophage (RAW 264.7) cells treated with receptor **1** and Cu^{2+} and Hg^{2+} (Left) Bright field image; (Middle) fluorescence image; and (Right) merged image.

The chemosensor receptor **1** was used for imaging of living cells. To detect Cu^{2+} and Hg^{2+} in living cells, RAW 264.7 macrophage cells were cultured in DMEM supplemented with 10% FBS at 37°C under 5% CO_2 . Cells were plated on 18 mm glass coverslips and allowed to adhere for 24 h. RAW 264.7 cells macrophage were treated with $10\mu\text{M}$ Cu^{2+} and Hg^{2+} for 30 min and then washed with PBS three times. The cells were then incubated with the chemosensor receptor **1** ($10\mu\text{M}$) for 45 min and then washed with PBS three times to remove any remaining sensor. Images of the RAW 264.7 cells were obtained by using Fluorescence imaging with a Leica TCS-SP5-X AOBS Confocal microscope. Fig.6 shows images of RAW 264.7 cells with the chemosensor receptor **1** after treatment with Cu^{2+} and Hg^{2+} ions. An overlay of fluorescence images and bright-field images shows that the fluorescence signals are localized in the intracellular area, indicating subcellular distribution of Cu^{2+} and Hg^{2+} . It shows good cell-membrane permeability of the chemosensor receptor **1**.

In continuation of cation sensing, the sensing ability of nitroaromatics was studied using naked-eye and fluorescence spectroscopic methods. The addition of various nitroaromatics into R1, a color change from pale yellow to yawning green was observed for 2, 4, 6-trinitrophenol (Fig. 7a insert). Pyrene is a polycyclic aromatic hydrocarbon (PAH), which can emit strong monomer fluorescence at 420 nm and the formation of excimer fluorescence at 440 nm. Upon the addition of TNP into R1 ($\Phi=0.045$), a fluorescence quenching ($\Phi=0.015$) was observed and forms the non-fluorescent complex. Increasing the concentration of TNP upto 2 equiv, the emission band was totally decreased (Fig. 7b). This result indicated the formation of R1-TNP complex. The other nitroaromatics like 4-Nitroaniline (NA), 2-Nitrophenol (NP), Nitrobenzene (NB), 2-Nitrobenzaldehyde (NBZ), 1-Bromo-2-nitrobenzene (BNB) and 4-Nitrobenzoylchloride (NBCl) were found to be insensitive with receptor 1 (Fig. 5a). The fluorescence titrations were best fitted to 1:1 stoichiometry and the association constants of picric acid with R1 was found to be 6.0×10^4 . The detection limit was found to be $2.0 \times 10^{-7} \text{M}^{-1}$. Based on the complete quenching of fluorescence intensity, the Stern–Volmer quenching constant (K_{SV}) of $4 \times 10^6 \text{M}^{-1}$ was calculated for picric acid.

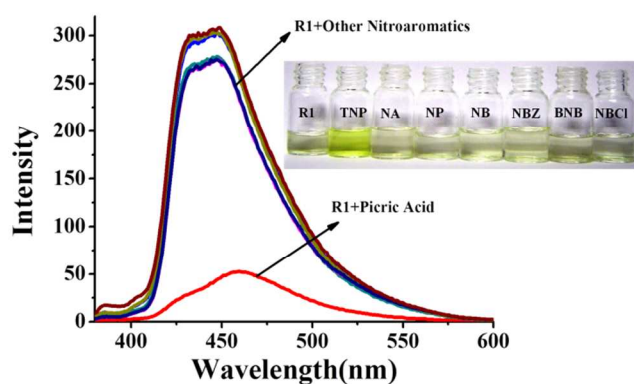


Fig 7a. Fluorescence spectrum of R1 ($5 \times 10^{-5} \text{M}$, in CH_3CN) with various nitrocompounds (2.0 equiv.) in CH_3CN . Inset: Color change of receptor 1 with 2, 4, 6-Tri nitro phenol (TNP), 4-Nitroaniline (NA), 2-Nitrophenol (NP), Nitrobenzene (NB), 2-Nitrobenzaldehyde (NBZ), 1-Bromo-2-nitrobenzene (BNB), 4-Nitrobenzoylchloride (NBCl).

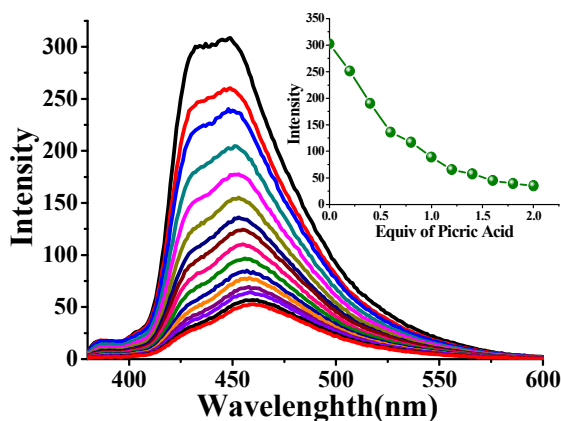


Fig 7b. Fluorescence quenching of receptor 1 upon the addition of TNP.

Conclusion

In summary, highly sensitive pyrene linked thiourea based receptor 1 act as colorimetric and fluorescence detector of Cu^{2+} , Hg^{2+} and picric acid (TNP). Receptor 1 can achieve double-channel detection of Cu^{2+} and Hg^{2+} and shows a significant color changes and fluorescent enhancement upon the binding with Cu^{2+} and Hg^{2+} ions in aqueous medium. Fluorescence microscopy experiments demonstrate that receptor 1 may have application as a fluorescent probe for detecting Cu^{2+} and Hg^{2+} in living cell imaging. Thus, the colorimetric and fluorescent receptor 1 can be used to determine Hg^{2+} and Cu^{2+} ions. Interestingly receptor 1 act as a sensitive and selective sensor for picric acid (TNP) and the detection limit is in the range of micromolar level.

Acknowledgement

Authors express their thanks to DRDO (ERIP/ER/1006004/M/01/1333 dated 23-05-2011) for financial assistance in the form of a major sponsored project.

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