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Highly selective chemosensor for nano molar detection of Cu^{2+} ion by fluorescent turn-on response and its application in living cells

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ABSTRACT

A new, simple and symmetric thiophene based Schiff base **1** as highly selective and fluorescent sensor for Cu^{2+} ion detection in aqueous medium over other metal ions has been synthesized. Structure of the receptor **1** was confirmed by single crystal X-ray diffraction and other spectroscopic techniques. Absorption and emission spectra were used to study the cation sensing properties. Receptor **1** displays colorimetric response as yellow to colorless and fluorescence "turn-on" property in presence of Cu^{2+} ion. Receptor **1**- Cu^{2+} complex show 35 folds higher fluorescence response than that of receptor **1**. Binding constant for receptor **1**- Cu^{2+} complex was found as 4.23×10^5 with the detection limit of 0.418 nM. Further the recognition of Cu^{2+} ion in the living cell was achieved using fluoresce microscope. Theoretical calculations were done to support the above findings.

1. Introduction

To device fluorescent probe for the detection of biologically significant metal ions in the living cells or tissue is of current interest and important target in the field of cation sensors [1–3]. Generally, the receptor-cation interaction will be in the form of coordination bond, electrostatic and Vander walls interaction via electron or charge transfer mechanism. Recently many efforts have been taken for the recognition of transition metal ions [4–10] due to their own importance in the environmental and biological concern. Copper [11–14] was found as the third most essential element for plants, animals and human beings due to its involvement in the biological process like electron transfer, enzyme hydrolysis and being an efficient catalyst for redox process. The American Medical Association suggests the normal human body should contain 1.2–1.3 mg/day of copper. When the concentration of Cu^{2+} ion crosses its limit in living organism, it will produce reactive organic species (ROS) and consequently causes Alzheimer's, Parkinson's, and Wilson diseases.

To monitor the presence of excess Cu in cells and tissues of the human body, an efficient fluorescence sensor has to be devised and it can be visualized via fluorescence imaging [15–17]. In addition, performing an aqueous mediated [18–20] cation sensor only shows precise output in biological application. Due to the paramagnetic nature of Cu^{2+} ion, it is used as fluorescence quencher [21,22]. However fluorescence enhancement will give promising results than quenching in case of guest–host interaction and can be easily visualized in the bio imaging of living cells and drug delivery to the targeted cells. Recently rhodamine based compounds were reported as Cu^{2+} ion sensor with good fluorescence enhancement [23–25]. Hence to synthesize a selective and high fluorescent Cu^{2+} ion sensor in aqueous medium has gained additional importance for biological applications.

In this article, for the first time we are reporting the synthesis of thiophene based Schiff base (receptor **1**) in single step and studied its cation sensing properties. Receptor **1** showed good selectivity and sensitivity towards Cu^{2+} ion over other cations in aqueous medium. Weak fluorescence of the Receptor **1** is due to the effect of photo induced electro transfer mechanism through the lone pair of electrons present on the nitrogen of imine and amine group [26,27]. When Cu^{2+} is binding with receptor **1**, lone pair of electrons accompanies the vacant orbital of Cu^{2+} ion which inhibits PET mechanism and exhibits blue emission with large fluorescence intensity at 430 nm. Receptor **1** showed higher binding constant and detection limit towards Cu^{2+} ion in aqueous medium. Quantum yield calculation of receptor **1** implies that the

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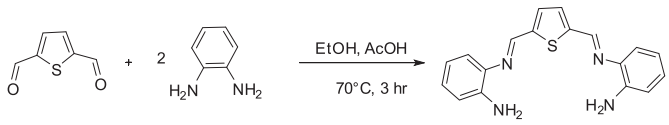

Scheme 1. Synthesis of receptor **1**.

emission after the detection of Cu^{2+} is 35 fold higher than before detection. Hence the utility of the receptor **1** was demonstrated for the recognition of Cu^{2+} ion in the living cells using confocal microscope. DFT calculations also carried out to prove the Cu^{2+} ion binding with receptor **1**.

2. Experimental section

2.1. Materials and instruments used

Solvents such as acetonitrile (ACN), ethanol (EtOH) and reagents used for spectroscopic experiments, synthesis were received commercially and used as such without any further purification. All metal salts as chloride, nitrate, acetate, sulfate were purchased and used for all colorimetric and spectroscopic titrations. Molecular weight of the receptor **1** was taken by Orbitrap Q exactive mass spectrometer. ^1H and ^{13}C NMR spectra were obtained on a BRUKER AV III-400 MHz Spectrometer using DMSO-d_6 as solvent. The structure of the receptor **1** was analyzed by X-ray diffraction method using Enraf Nonius CAD4-MV31 Bruker Kappa APEXII instrument. IR spectra were recorded on NICOLET IS5 instrument using KBr plates. UV–vis spectra were recorded on a Shimadzu UV-2600 Spectrophotometer with a quartz cuvette (path length = 1 cm) at room temperature (r.t). Fluorescence spectra were recorded on Shimadzu RF-5301 PC spectrophotometer. A 2.5×10^{-5} M solution of the receptor **1** in ACN and 1.5×10^{-3} M of all cations in H_2O were prepared and used for spectroscopic titrations. Bio imaging studies of *Escherichia coli* MTCC 2939 cell was carried out using confocal fluorescence microscope (Carl Zeiss, Germany).

2.2. Experimental procedure for the synthesis of the receptor 1

Receptor **1** was prepared by reacting 1 mmol of 2, 5- thiophene dicarboxaldehyde with 2 mmol of *o*-phenylene diamine in EtOH medium. A drop of acetic acid was added to the reaction mixture and allowed to reflux for 3 h at 70 °C. After cooling to r.t the precipitate formed was filtered, washed with EtOH and dried in a vacuum oven (Scheme 1). Orange color crystal of receptor **1** was obtained using dichloromethane as solvent by slow evaporation method. Yield: 70%. M.pt: 220 °C. IR (cm^{-1}, KBr): 3366, 3450, 1609, 1593, 1488, 1457, 1284, 1050. ^1H NMR (400 MHz DMSO-d_6): 8.82 (CH=N, 1H, s), 7.66 (Ar–H, 1H, s), 7.16–7.18 (Ar–H, 1H,d), 6.96–7.01 (Ar–H, 1H, t), 6.71–6.74 (Ar–H, 1H, d), 6.56–6.59 (Ar–H, 1H, t), 5.20 (NH_2, 2H, s).^{13}C NMR (100 MHz DMSO-d_6): 149.84, 146.59, 144.63, 134.70, 133.21, 128.58, 117.47, 116.73, 115.36. HRMS: 321.1170 (found), 321.1168 (calculated).

3. Results and discussion

Receptor **1** was synthesized in a single step (Scheme 1) and the structure was confirmed by spectroscopic (Figure S1), HRMS (Figure S2) and crystallographic method (Fig. 1). The single crystal XRD analysis tells the receptor **1** is monoclinic system with C2/c space group. The CCDC reference number is 942484–942485. All the other crystallographic data and packing diagram of receptor **1** is given in Figures S5 and S6.

3.1. Colorimetric and UV–vis analysis

Receptor **1** was treated with various cations like Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, and Sn^{2+} to study its sensitivity and selectivity towards particular metal ion over other cations. Among all cations, selectively Cu^{2+} ion showed colorimetric response as yellow to colorless and effective fluorescence "turn-on" under UV light with the addition of 0.8 equiv (Fig. 2). Other metal ions did not show significant color changes even with excess addition. Simultaneously, colorimetric result was further confirmed by UV–vis and fluorescence spectroscopy for the additional evidence of Cu^{2+} ion detection. In UV–vis titrations also, even with the addition of 0.8 equiv. of Cu^{2+} ion a new absorption band at 370 nm was appeared with higher intensity (Fig. 3a). Likewise, in fluorescence titrations maximum emission was observed at 430 nm in presence of 0.8 equiv. of Cu^{2+} ion (Fig. 3b). Additionally, 5 equiv. of Cr^{3+}, Sn^{2+} ions showed slight fluorescence emission at 465 nm with 35 nm red shift and all other cations did not show any significant color and optical changes even with excess addition.

In order to calculate the binding constant and detection limit values, spectroscopic titrations were carried out with gradual addition (0–0.8 equiv.). In UV–vis experiments, for each addition of Cu^{2+} ion to receptor **1**, the band in the visible region at 448 nm decreased and a new band was appeared at 370 nm with a blue shift of 78 nm (Fig. 4). This color and optical changes is due to the formation of a coordination bond between receptor **1** and Cu^{2+} ion via the transformation of lone pair of electrons of imine and amine into the vacant orbital of Cu^{2+} ion. Ultimately the conjugation of

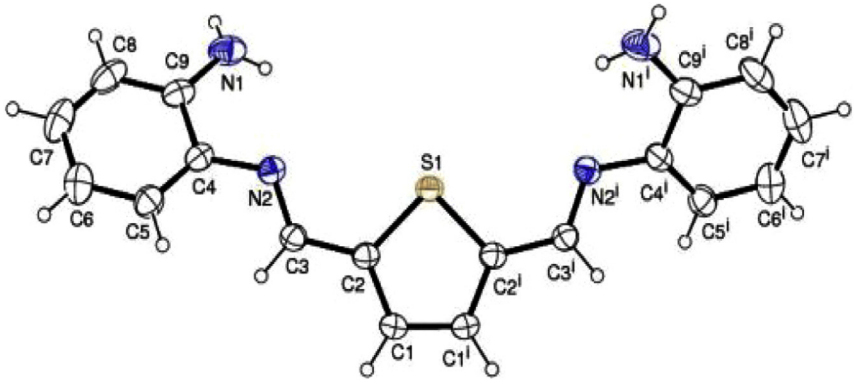

Fig. 1. ORTEP diagram of receptor **1**.

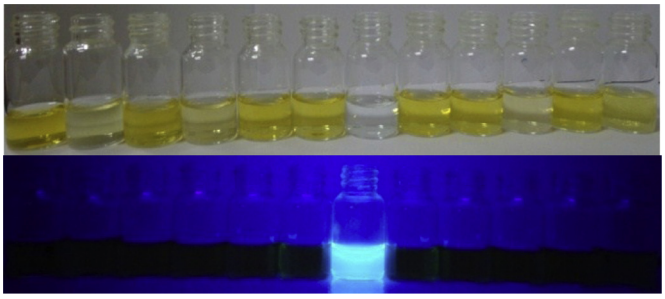

Fig. 2. Color change normal (top) and under UV light 365 nm (bottom) observed upon the addition of (0–5 equiv.) of various cations (1.5×10^{-3} M) to receptor **1** (2.5×10^{-5} M) (from left to the right: R, R + Cr^{3+}, R + Mn^{2+}, R + Fe^{3+}, R + Co^{2+}, R + Ni^{2+}, R + Cu^{2+} (0.8 equiv.), R + Zn^{2+}, R + Cd^{2+}, R + Sn^{2+}, R + Hg^{2+}, R + Pb^{2+}). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

free receptor **1** is disturbed when Cu^{2+} ion bind with receptor **1**, resulting a new band in the UV region.

3.2. Fluorescence titration

As like UV–vis titration, receptor **1** reached maximum fluorescence intensity in the blue region at 430 nm (Fig. 5) with the gradual titration of Cu^{2+} ion (0–0.8 equiv.). Also slight fluorescence enhancement with 35 nm red shift at 465 nm for Cr^{3+}, Sn^{2+} ions was

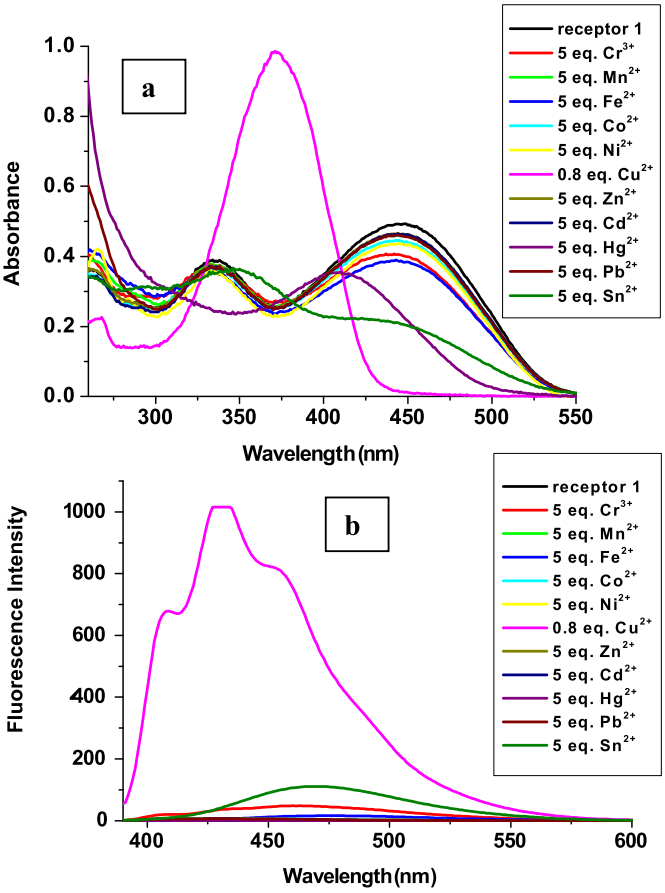

Fig. 3. (a) Absorption change (b) Emission change observed upon the addition of (0–5 equiv.) of various cations (1.5×10^{-3} M) to receptor **1** (2.5×10^{-5} M) (from left to the right: R, R + Cr^{3+}, R + Mn^{2+}, R + Fe^{3+}, R + Co^{2+}, R + Ni^{2+}, R + Cu^{2+} (0.8 equiv.), R + Zn^{2+}, R + Cd^{2+}, R + Sn^{2+}, R + Hg^{2+}, R + Pb^{2+}).

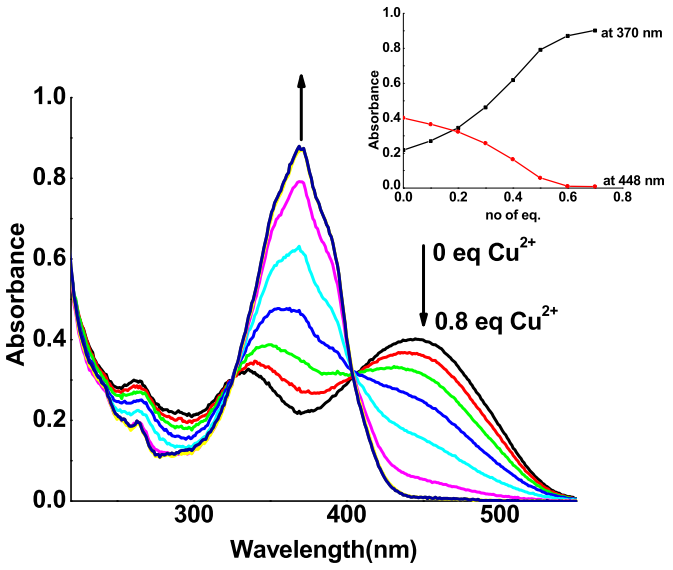

Fig. 4. Absorption spectra of the receptor **1** in ACN recorded (2.5×10^{-5} M in ACN) with gradual addition of (0–0.8 eq.) of Cu^{2+} ion (1.5×10^{-3} M in H_2O).

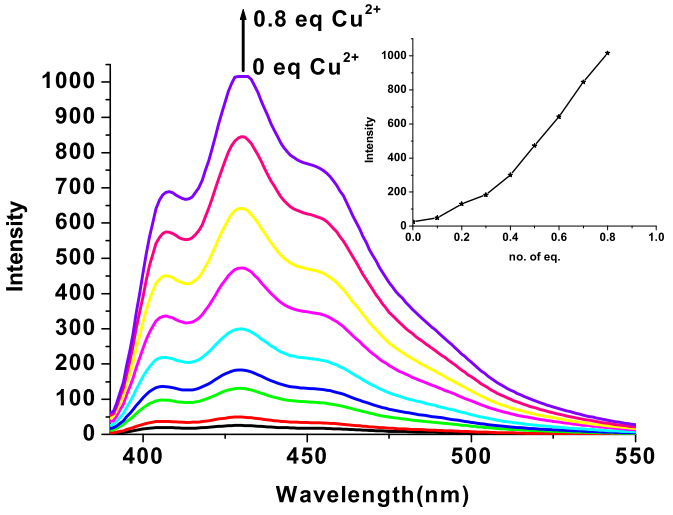

Fig. 5. Emission spectra of the receptor **1** in ACN recorded (2.5×10^{-5} M in ACN) with gradual addition of (0–0.8 eq.) of Cu^{2+} ion (1.5×10^{-3} M in H_2O). Inset: fluorescence image and linear increase in the formation of receptor **1**- Cu^{2+} complex.

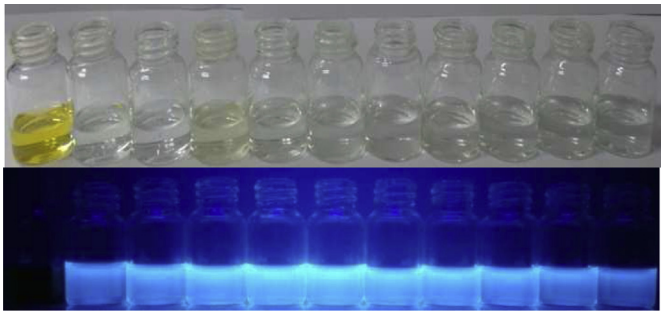

Fig. 6. Color Changes normal (top) and under UV light 365 nm (bottom) observed upon the addition of various cations to receptor **1**-Cu^{2+} complex. (From left to the right: R, R–Cu^{2+}+Cr^{3+}, R–Cu^{2+}+Mn^{2+}, R–Cu^{2+}+ Fe^{3+}, R–Cu^{2+}+Co^{2+}, R–Cu^{2+}+ Ni^{2+}, R–Cu^{2+}+Zn^{2+}, R–Cu^{2+}+Cd^{2+}, R–Cu^{2+}+Hg^{2+}, R–Cu^{2+}+Pb^{2+}, R–Cu^{2+}+ Sn^{2+}). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

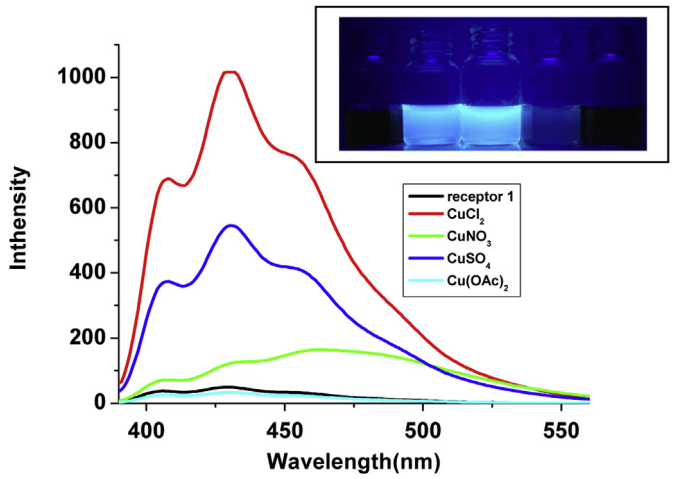

Fig. 7. Emission spectra of the receptor **1** recorded (2.5×10^{-5} M in ACN) after the addition of different copper salts (1.5×10^{-3} M in H_2O). Inset: colorimetric changes (From left to the right: R, R + $CuCl_2$, R + $Cu(NO_3)_2$, R + $CuSO_4$, R + $Cu(OAc)_2$).

observed in the gradual addition of (0–5 equiv.) of Cr^{3+}, Sn^{2+} ions (Figure S4). This large fluorescence enhancement at 430 nm of receptor **1**-Cu^{2+} complex is highly indicating the inhibition of photo induced electron transfer mechanism. The weak fluorescence of receptor **1** is due to the transfer of lone pair electron and delocalization of electron via PET to thiophene ring. The moment receptor **1** binds with Cu^{2+} ion, the transformation of delocalized electrons and photo induced electrons are blocked, hence the PET is inhibited and leads to increase in the fluorescence intensity at 430 nm. To examine the interference of other metal ions in the Cu^{2+} ion detection, the competitive experiment was performed. Five equiv. of all the metal ions were added to the Cu^{2+} ion complex of receptor **1** solution, but no changes were observed in the color of the receptor **1**-Cu^{2+} complex. This result is an added evidence for the high selectivity of the receptor **1** and can be used as selective Cu^{2+} ion sensor even in presence of other cations without any interference (Fig. 6).

In order to find out the effect of counter anions in the Cu^{2+} ion sensing mechanism of receptor **1**, colorimetric and fluorescence titrations were carried out with different copper sources like $CuCl_2$, $Cu(NO_3)_2$, $CuSO_4$, $Cu(OAc)_2$ in aqueous medium. Colorimetric results showed, the yellow color of receptor **1** was completely turned into colorless only with 0.8 equiv. of $CuCl_2$ and slight color change was observed with $CuSO_4$ whereas other copper salts induced no change. The same outcome was reflected in fluorescence titration also, maximum emission was observed with 0.8 equiv. of $CuCl_2$ and $CuSO_4$. Remaining copper sources did not show significant changes in colorimetric and emission spectrum (Fig. 7). Particular sensing of $CuCl_2$ indicates that the coordination nature of Cu^{2+} and counter anion Cl^- is favorable for the Cu^{2+} ion detection by receptor **1** over other sources. Such discriminate interaction of the receptor **1** toward copper chloride finds important usefulness in chemo-sensor application due to its highly selective binding to a single ionic copper compound. Such a peculiar response to single copper compound, as far as we are aware, has been reported elsewhere.

Both binding constant and binding mode were calculated using the measurement of absorbance. Binding constant (Ka) was

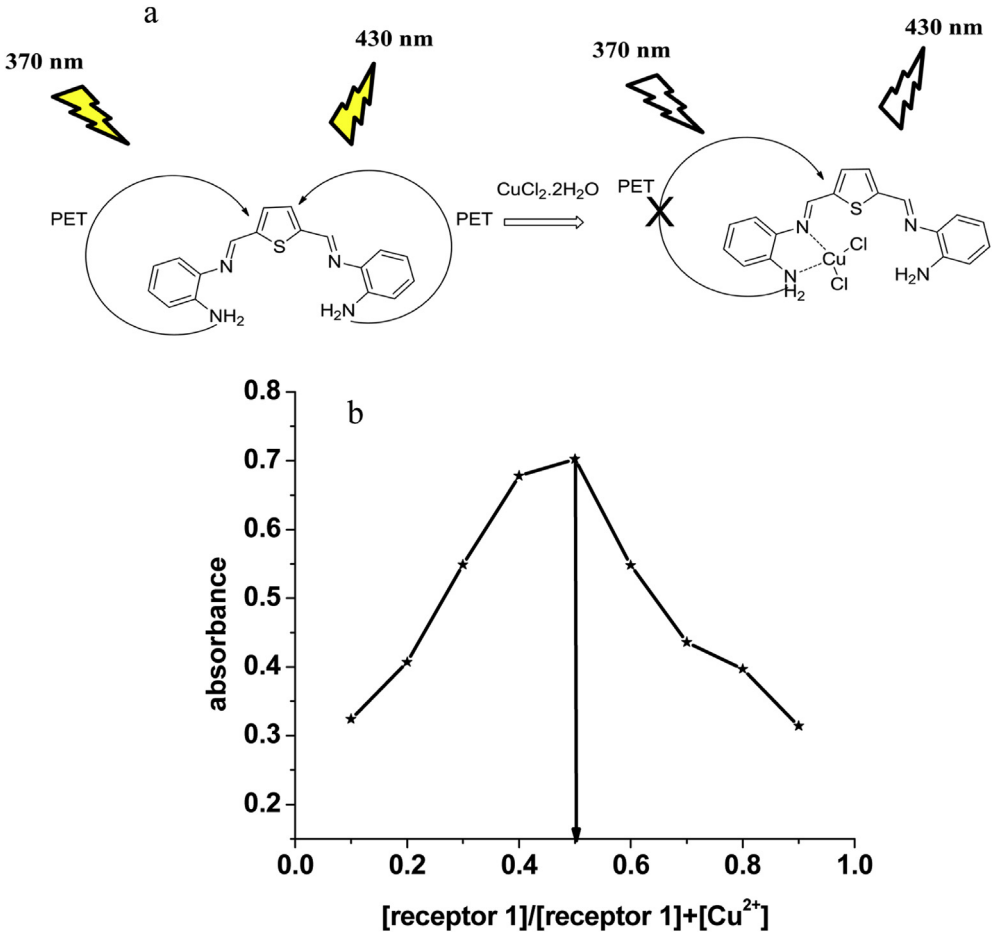

Fig. 8. (a) Binding mode and (b) Job's plot of receptor **1** with Cu^{2+} ion.

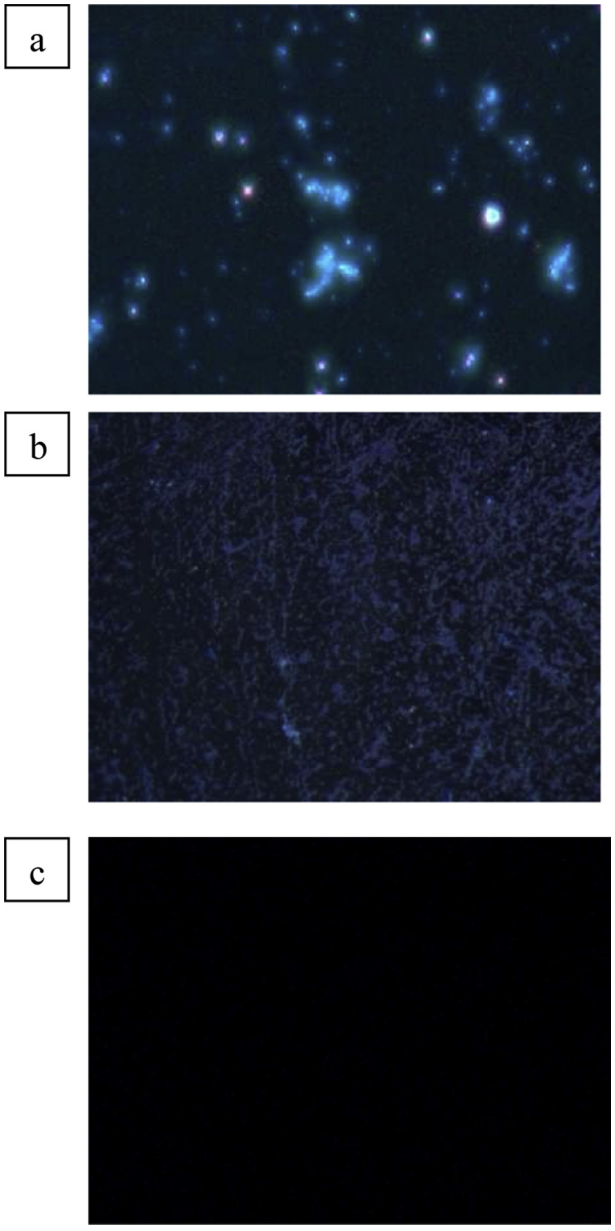

Fig. 9. Bio-imaging of copper ions in *E. coli* MTCC 2939 at 37 °C under fluorescence microscope. *E. coli* MTCC 2939 cells incubated with receptor **1** and (a) presence, (b) absence of copper ions and (c) *E. coli* MTCC 2939 cells alone under dark field microscope.

estimated using Benesi–Hildebrand plot and the plot was done by absorbance changes of consequent titration $(1/A - A_0)$ against $1/[Cu^{2+}]$. The magnitude of Ka was calculated from the intercept and slope of the straight line, estimated value is 4.23×10^5. Job's plot showed maximum mole fraction at 0.5 indicates the receptor binds with Cu^{2+} in 1:1 stoichiometry (Fig. 8a and b). The ESI-mass spectrum also support 1:1 binding mode and the molecular ion peak at 478.9022 suggests the complex formation as $[R1CuCl_2].H_2O$ and a base peak at 434.2151 suggest the removal of one chlorine ion and complex as $[R1CuCl]^{+}.H_2O$. FTIR spectra also sustain the binding of Cu with receptor **1**, which was confirmed with the significant shift. The peaks corresponding to NH_2 and CH=N at 3366 cm^{-1} and 1609 cm^{-1} shifted into 3157 cm^{-1} and 1628 cm^{-1} respectively. In addition to that, a strong and broad band at 3424 cm^{-1} was observed which suggests that H_2O molecule was coordinated in the complex. Fluorescence intensity was used to calculate quantum yield, showed 35 fold higher for receptor **1**- Cu^{2+} complex ($\varphi = 0.598$) than that of receptor **1** alone ($\varphi = 0.017$). Detection limit of Cu^{2+} ion by receptor **1** was calculated from the plot of fluorescence intensity vs concentration of Cu^{2+} ion [25]. From the plot, detection limit was calculated using the formula $3\sigma/m$. Where σ the standard deviation and 'm' is the slope of the straight line and it was found as 0.418×10^{-9} M. Thus the receptor **1** can be utilized as an efficient Cu^{2+} ion sensor at nano molar level.

3.3. Bio imaging of living cells

To assess the potential application of receptor **1** for an inevitable applications in bio-labeling and bio-imaging work in imaging research. It is revealed that *E. coli*, known to uptake copper ions in their microenvironment [28–30]. A bacterium, *E. coli* MTCC 2939 was chosen for the study and was exposed to copper ions in the concentration of 15 μM. The bacterial cells were then incubated with receptor **1**(25 μM) at 37 °C for 30 min and observed under fluorescence microscope. For the control, bacterial cells were incubated separately with copper ions and receptor **1** to understand the unique fluorescence property of receptor **1**. In this study we observed that the cells contain copper ions along with receptor **1** showed high fluorescence, whereas in cells with copper ions and receptor **1** alone not shown any fluorescence (Fig. 9a–c). Such fluorescence image indicates that the ability of the receptor **1** towards Cu^{2+} ion recognition in the living cells and has the good permeability, which is inevitable for bio-imaging experiments.

3.4. Theoretical calculations

For the complete understanding of the receptor **1** and its copper complex, DFT calculation and structure optimization were done using Gaussian09 program package [31] and the energies were compared with experimental observation. The Density Functional

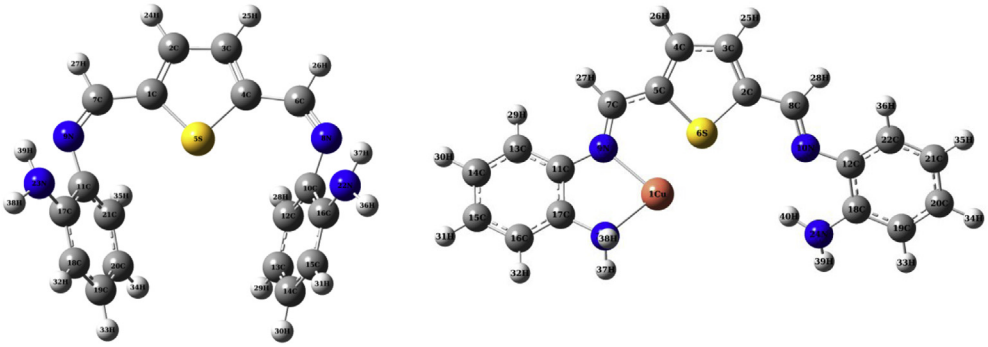

Fig. 10. Optimized structures of receptor **1** and Cu(II)-receptor **1** complex.

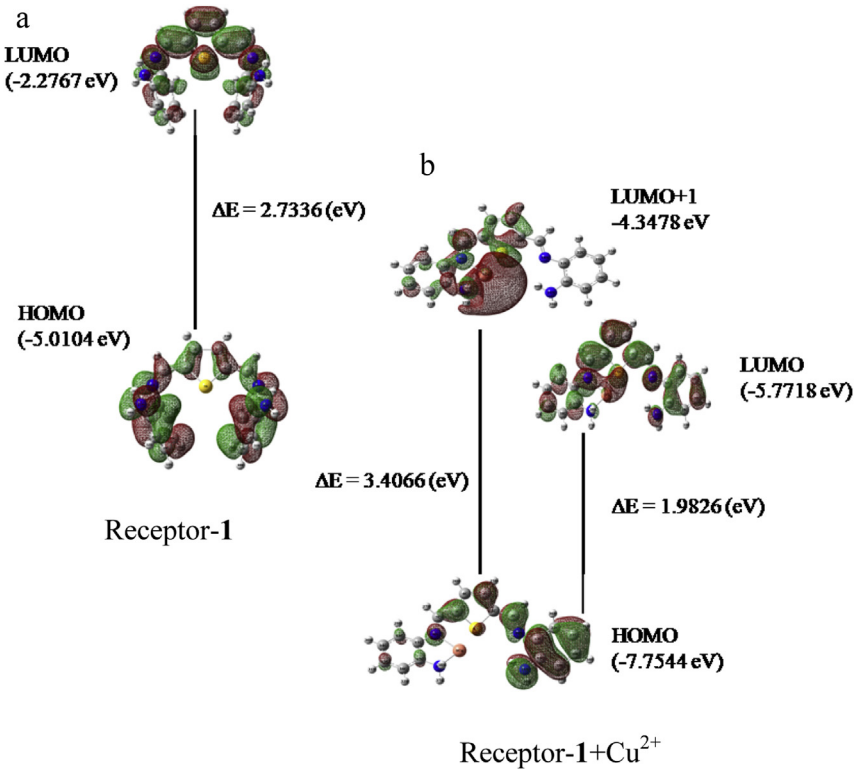

Fig. 11. (a) Energy levels of various HOMO and LUMO's of receptor **1** (b) Energy levels of various HOMO and LUMO+1 of Cu(II)-receptor **1** complex.

Theory (DFT) based Beck-3 Lee Young Parr (B3LYP)/6-31G(d,p) model chemistry was employed for L1 and effective core potential (ECP) based LANL2DZ basis set was incorporated to Cu complex. Fig. 10 represent the optimized energy structure of the receptor **1** and its Cu(II) complex. The Cu^{2+} ion is coordinated with the two nitrogen atom of the receptor **1** with a bond distance of 2.0804 A^0 and 2.0642 A^0. The calculated energy value from the HOMO-LUMO excitation reveals that the predominant absorption peak for receptor **1** observed at 453 nm is nearly close with the experimentally observed value at 448 nm. However, the energy of its receptor **1**-Cu^{2+} complex was calculated from HOMO-LUMO+1 excitation. In this case, main absorption peak at 448 nm was blue shifted to 370 nm which is also in well hold up with the theoretically predicted λ_{max} at 363 nm (Fig. 11). Table 1 clearly explains that the addition of copper ion blocks the conjugation of receptor **1** via the acceptance of lone pair of electrons present on nitrogen atom and leads to higher energy.

4. Conclusion

A new, selective aqueous mediated Cu^{2+} sensor **1** was successfully synthesized in single step and well characterized with single crystal XRD. The selective sensing of receptor **1** towards Cu^{2+} ion in aqueous medium was achieved by colorimetric, UV–vis and fluorescence spectroscopic methods with distinct color and optical changes without any interference even in presence of other metal ions. The application of receptor **1** for the Cu^{2+} ion recognition was successfully demonstrated in the living cells by means of receptor **1** as bio probe. This bio sensing of Cu^{2+} ion was confirmed by imaging the fluorescence nature of the *E. coli* cells in presence of receptor **1**-Cu^{2+} complex using fluorescence microscope. Receptor **1** was found to be able to act as a nano molar (0.423 nm) Cu^{2+} ion sensor with the binding constant of 4.23×10^5. DFT calculations also done to support the Cu^{2+} ion binding with receptor **1** and closely matched with the experimental observations.

Table 1
Calculated energies of receptor **1** and its copper complex.

Host-guest	Predominant excitation (eV)	ΔE (eV)	λ_{max} (nm)		Bond length (A^0)*	
			Calculated	Experimental	9N–1Cu	2.0804
R- **1**	L (−2.27679)	2.7336	453	448	23N–1Cu	2.0642
	H (−5.01047)				7C–9N	1.2996
R- **1** + Cu^{2+}	L + 1 (−4.3478)	3.4066	363	370	8C–10N	1.2954
	H (−7.7544)				17C–23N	1.4702
					18C–24N	1.3671

H-Highest occupied molecular orbital (HOMO), L- Lowest occupied molecular orbital (LUMO) *R- **1** + Cu^{2+} complex only.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dyepig.2014.01.001.

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