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A novel terephthalaldehyde based turn-on fluorescent chemosensor for Cu^{2+} and its application in imaging of living cells†

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A new terephthaldehyde-based chemosensor **1** bearing an aminophenol recognition unit has been synthesized and applied to the fluorescent sensing of metal ions. Molecular system **1** acts as a highly selective and sensitive fluorescence turn-on sensor for Cu^{2+}. The sensing mechanism has been explored. It is proposed that Cu^{2+} binds with the imine and hydroxyl moiety of **1** in 1 : 2 binding stoichiometry, thereby enhancing the fluorescence at 386 nm. The detection limit and association constant (K_a) of **1** with Cu^{2+} were found to be 0.62 μM and 6.67×10^4 M^{-1}, respectively. Chemosensor **1** has shown excellent specificity towards Cu^{2+} and has been successfully applied to the determination of Cu^{2+} in live L929 cells.

Introduction

Metal ions, though present in small amounts, play indispensable roles in biology, including structural support, storage, electron transfer, dioxygen (O_2) binding, and catalytic function.[1–4] There is increasing evidence that, irrespective of whether they are in surplus or deficient, they may induce various disorders. Cu^{2+} an essential micronutrient for all known life forms, playing the crucial role of cofactor in nearly 20 enzymes and taking the next place to iron and zinc among the transition metals in the body.[2] Equally, Cu^{2+} is a significant pollutant due to its excessive use all over the world.[3] Long-term exposure to high levels of Cu^{2+} has been reported to induce liver and kidney damage and can cause oxidative stress and disorders associated with neurodegenerative diseases such as Menkes syndrome, Wilson's disease, Alzheimer's disease and prion disease.[4] Owing to the continuous attention given to Cu^{2+} in the environment and its diagnostic role in biological systems, the development of a Cu^{2+} selective probe by a fluorescence technique has emerged as an axial point among the sensing community.[5–7] Detection based on simple fluorescent probes offers innate advantages over traditional techniques, such as appreciable detection sensitivity and selectivity, reliability, operational simplicity and easy signal detection readout compared to other traditional analytical methods.[8] However, most of the recently reported Cu^{2+} selective probes have disadvantages, such as multi-step synthesis, cross sensitivity towards other ions and lack of bio-compatibility.[9] All these limitations restricted their potential applications in the environment and in biology. On the other hand, selective fluorescence sensing of Cu^{2+} over other paramagnetic cations (Fe^{3+}) and heavy atoms (Hg^{2+} and Pb^{2+}) is still challenging because all of these metal ions can show quenching of fluorescence upon binding of the probe *via* energy/electron transfer or heavy atom enhanced intersystem crossing/spin–orbit coupling.[10] Therefore, the development of fluorescence sensors with high selectivity and sensitivity for sensing of Cu^{2+} from aqueous media is of current interest.

In this paper, we have successfully employed a novel aminophenol-substituted Schiff base ligand embedded for the determination of Cu^{2+} ions.[11] These components make up chemosensor **1**, a terephthaldehyde moiety which acts as a reporter and an aminophenol moiety which acts as a metal ion coordination site. Binding of Cu^{2+} ions to chemosensor **1** causes remarkable fluorescence enhancement at 386 nm. The selectivity of probe **1** was tested with all metal ions, but only **1**·Cu^{2+} shows fluorescence enhancement at 386 nm and furthermore

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the probe was successfully applied to the intracellular imaging of Cu^{2+} ions.

Experimental

All reagents and chemicals were purchased from Aldrich Chemicals Ltd and were used without further purification. The solvents were distilled before use. The reaction was monitored by thin-layer chromatography (TLC). The FT-IR and mass spectra were recorded by using a Shimadzu FTIR-8400 and Bruker Ultraflex II MALDI/TOF spectrometer, respectively. The ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AVANCE II 400 MHz spectrometer in DMSO-d_6 as solvent. Fluorescence measurements were made with a HORIBA JOBIN YVON Fluoromax-4 spectrofluorometer equipped with a xenon lamp. The excitation wavelength and emission slit width were 300 nm and 5/5 nm, respectively. UV-Vis absorption spectra were recorded on a Shimadzu UV-2450 spectrophotometer. All crystal structures were solved by direct methods. The program SAINT (version 6.22) was used for integration of the intensity of reflections and scaling. The program SADABS was used for absorption correction. The crystal structures were solved and refined using the SHELXTL (version 6.12) package.[12] All hydrogen atoms were included in idealized positions, and a riding model was used. Non-hydrogen atoms were refined with anisotropic displacement parameters. The data collection and structure solution parameters for compound **1** are given in ESI (Table S1†).

General procedures for spectra acquisition

The stock solution of probe **1** (1×10^{-5} M) was prepared in acetonitrile. The cationic solutions (1×10^{-4} M) such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cr^{3+}, Ba^{2+}, Cs^+, Cd^{2+}, Pb^{2+}, Ag^+ and Sr^{2+} were prepared in aqueous media. Each time, a 2 mL solution of **1** was put in a quartz cell of 1 cm optical path length, and then appropriate volumes of stock solutions of all cations (1×10^{-4} M) were added to it by using a micropipette. The binding stoichiometry of **1** with Cu^{2+} was determined by using a Job's plot. For the Job's plot analysis, a series of solutions with varying mole fractions of Cu^{2+} were prepared by keeping the total concentration of **1** and Cu^{2+} constant. The maximum fluorescence intensity at 386 nm for each solution was plotted against the mole fraction of Cu^{2+} ions.

Synthesis of receptor 1

2-Aminophenol (0.325 g, 2.0 mmol) was added to an ethanolic solution of terephthaldehyde (0.2 g, 1.0 mmol) and the mixture was stirred at room temperature for 4 h. The golden yellow solid obtained at room temperature was filtered, dried and further purified by recrystallization (84% yield). FT-IR (cm^{-1}): 3365.90, 3049.56, 1917.31, 1791.93, 1598.08, 1491.99, 1369.50, 1230.63, 1149.61, 842.92, 746.48, 589.27, 519.83. ^{1}H NMR (400 MHz, δ, ppm): 8.91 (s, 1H, OH), 8.76 (s, 1H, CH═N), 8.13 (s, 2H, Ar–H), 7.24 (dd, 1H, J = 1.2 and 6.6 Hz, Ar–H), 7.10 (dt, 1H, Ar–H), 6.92 (dd, 1H, J = 7.0 Hz and 0.96 Hz, Ar–H), 6.84 (dt, 1H, J = 7 Hz and 1.0 Hz, Ar–H); ^{13}C-NMR (100 MHz, δ, ppm): 115.93, 118.61, 119.39, 127.71, 128.94, 137.23, 138.46 (Ar), 151.48, 157.89 (CH═N); HR-MS calcd for $C_{20}H_{17}N_2O_2$ $(M + H)^+$ m/z = 317.1290, observed 317.1287.

Synthesis of 1·Cu^{2+} complex

Copper acetate in water (0.1 g, 1.0 mmol) was added dropwise to a solution of receptor **1** (0.316 g, 2.0 mmol) in acetonitrile and then the mixture was refluxed for 8 h. The dark maroon red colour solid obtained was filtered, dried and further purified by recrystallization (80% yield). FT-IR (cm^{-1}): 3336.00, 3043.77, 1904.77, 1681.02, 1580.72, 1471.74, 1386.86, 1283.67, 1166.97, 949.97, 838.10, 745.51, 595.06; LCMS calcd for $C_{40}H_{30}CuN_4O_4$ $(M + H)^+$ m/z = 694.24, observed 693.43.

In vitro cell imaging

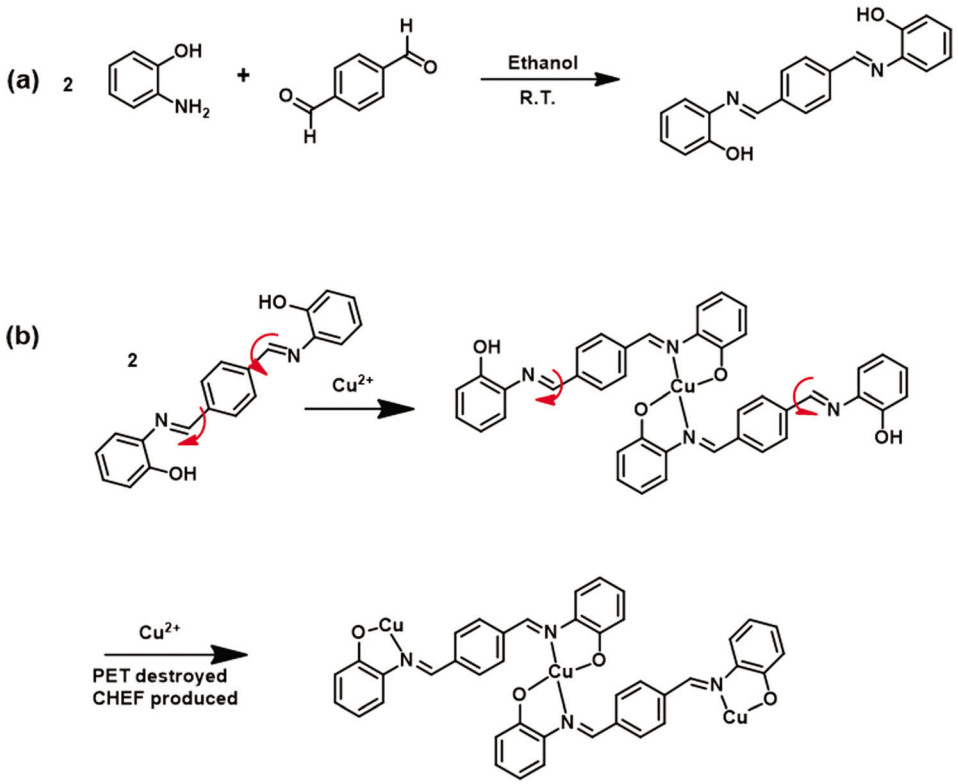
L929 live cells were seeded over sterilized glass cover slips in wells of 12 well plates with a concentration of 20 000 cells per well. Cells were incubated at 37 °C in 5% CO_2 under humidified conditions. After 24 hours of incubation, the cells were treated with 100 μL of compound **1** (DMSO + PBS, 1 : 9 ratio) at a concentration of 0.26 μM and kept for 30 minutes under incubation conditions. After 30 minutes, the cells were washed twice with PBS (pH 7.0) to remove extra compound. After that, 100 μL PBS and Cu^{2+} (0.26 μM) was added to the cell and it was again kept for 20 minutes of incubation. After incubation, the copper treated cells were washed with PBS to ensure the complete removal of extra salt. Then, the cells present in all wells were fixed by treatment with 1.0 mL of 3.5% formaldehyde solution for 15 minutes. After careful washing of fixed cells with PBS solution, they were imaged under CLSM.

Results and discussion

Chemosensor **1** was synthesized by a condensation reaction of terephthaldehyde with 2.0 equivalents of aminophenol in ethanolic medium by following our previously reported procedure (Scheme 1a).[13] The structure of receptor **1** was well characterized by using ^{1}H NMR, ^{13}C NMR, FT-IR and HR-MS (Fig. S1–S4†).

X-ray crystallographic studies

Yellow needle crystals suitable for an X-ray diffraction study were obtained by slowly evaporating a hexane/dichloromethane solution of **1** at room temperature over a period of one week. Single-crystal X-ray diffraction structure analysis indicates that **1** crystallizes in a monoclinic system, $P2(1)/c$ space group (Table S1, ESI†). The CIF file for receptor **1** was deposited in the Cambridge Structure Database with CCDC no. 1524229.† Compound **1** exists as an S-trans type Schiff base chain with a terephthaldehyde group extending out of the aminophenol ring. From the dihedral angles of **1**, all atoms in **1** are located in almost the same plane (Fig. 1). The terminal phenyl rings are in plane with respect to each other and make an angle of



Scheme 1 (a) Synthetic scheme of receptor **1** and (b) the binding mode of **1** with Cu^{2+}.

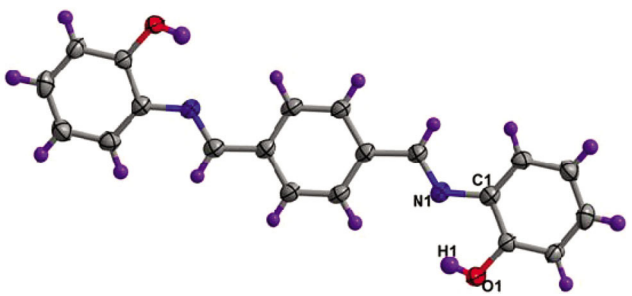


Fig. 1 X-ray crystal structure of receptor **1**.

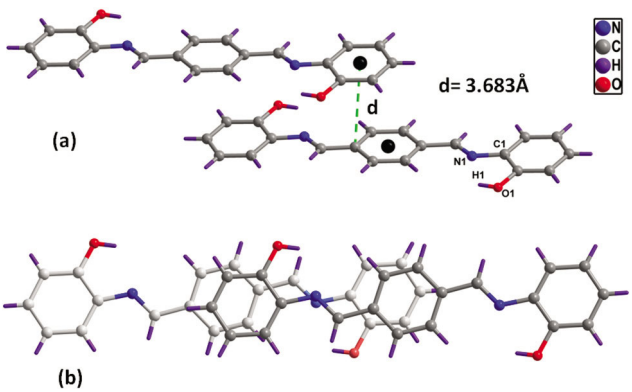


Fig. 2 Dimer of receptor **1**. Side (a) and top (b) views for the dimer formation *via* π–π interactions (broken line indicates weak interactions).

10.39(7)° with the central phenyl ring. This suggests that the whole molecule is almost planar and conjugated. Moreover, rod-like molecules of the compound interact with neighboring molecules *via* hydrogen bonding interactions O1–H1···O1 2.702 Å to form a sheet like structure. Furthermore, this sheet like structure grows up and down *via* face-to-face stacking interactions π C(5)–H(10)···π(C1–C3) with a contact distance of [3.683(1)] Å to form a stair like 3D structural arrangement along the *b*-axis (Fig. 2).

Cation sensing behavior

The recognition ability of the receptor **1** (1×10^{-5} M, CH_3CN) towards various cations, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cr^{3+}, Ba^{2+}, Cs^+, Cd^{2+}, Pb^{2+}, Ag^+ and Sr^{2+} (1×10^{-4} M, H_2O) was investigated by fluorescence spectroscopy. In the absence of metal ions, receptor **1** showed a weak fluorescence band at 331 nm when excited at 300 nm. Among the various metal ions examined, receptor **1** showed significant fluorescence enhancement at 386 nm in the presence of Cu^{2+} (Fig. 3). The quantum yield (Φ) of receptor **1** (Φ = 0.001353) was increased in the presence of Cu^{2+} (Φ = 0.026638). However, the fluorescence of **1** remains unchanged with the addition of other ions, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cr^{3+}, Ba^{2+}, Cs^+, Cd^{2+}, Pb^{2+}, Ag^+ and Sr^{2+}, demonstrating the selectivity of receptor **1** towards Cu^{2+} (Fig. S5†).

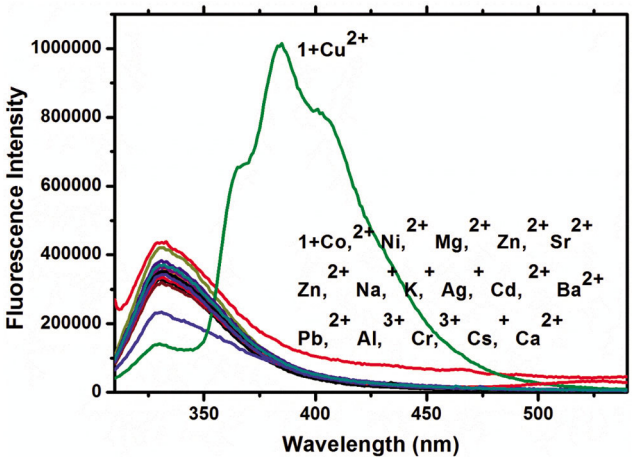


Fig. 3 Fluorescence changes of receptor **1** (1×10^{-5} M, CH_3CN) occurring upon addition of different metal ions, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cr^{3+}, Ba^{2+}, Cs^+, Cd^{2+}, Pb^{2+}, Ag^+ and Sr^{2+} (1×10^{-4} M, H_2O).

A competitive experiment was conducted to investigate the Cu^{2+} detection ability of **1** in the presence of other interfering metal ions. The competition experiments revealed that the Cu^{2+} (2 equivalents) induced fluorescence enhancement at 386 nm was unaffected even in the presence of 2 equivalents of environmentally relevant alkali or alkaline-earth metals and transition metal ions, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Ba^{2+}, Cs^+, Cd^{2+}, Pb^{2+}, Ag^+ and Sr^{2+}, except the trivalent metal ions $Al^{3+}$and Cr^{3+} (Fig. S6†).

To study the nature of the emission of **1** (1×10^{-5} M, CH_3CN), a fluorescence titration experiment was recorded with successive incremental additions of Cu^{2+} (10–1000 μL, H_2O) (Fig. 4). The fluorescence intensity at 331 nm was decreased up to the addition of one equivalent of Cu^{2+}. Upon further addition of Cu^{2+}, the fluorescence intensity was enhanced at 386 nm. The weak fluorescence from **1** may be due to the combined effects of photo-induced electron transfer (PET) and the C=N isomerization in the excited state. As described in Scheme 1b, the steady decrease in the fluorescence intensity of **1** at 331 nm observed up to the addition of one equivalent of paramagnetic Cu^{2+} is presumably due to the presence of an uncomplexed diminophenol unit of **1** that allowed the PET process as well as the conformational C=N isomerization in the excited state. However, the observed fluorescence enhancement at 386 nm upon further addition of Cu^{2+} can be explained by the inhibition of C=N isomerization due to the possible coordination of Cu^{2+} through the hydroxyl-O, imine-N atoms of receptor **1** that resulted in a chelation-enhanced fluorescence effect (CHEF) and also blocked the PET process.[14]

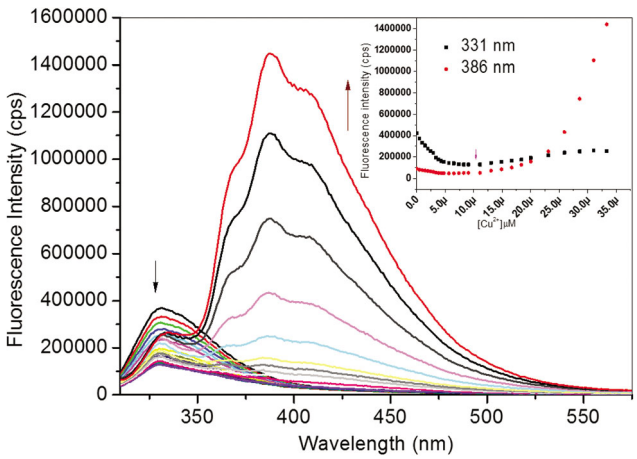


Fig. 4 Fluorescence emission spectra of **1** (1×10^{-5} M, CH_3CN) upon incremental addition of Cu^{2+} (1×10^{-4} M, H_2O).

The binding constant K_a of the complex species formed in solution was evaluated from the fluorescence titration profile by using the Benesi–Hildebrand equation.[15] From the fluorescence titration data, a binding constant of 6.67×10^{-4} M^{-1} was estimated (Fig. S7†). Also, the detection limit of probe **1** with Cu^{2+} was calculated to be 0.62 μM by applying the standard IUPAC method of 3σ. The obtained detection limit was comparable/superior to that of the recently reported Cu^{2+} selective chemosensors (Table S2†).[16–18] The binding stoichiometry of **1** with Cu^{2+} was also confirmed by the Job's plot method (Fig. S8†).[19] This clearly shows that probe **1** forms a complex with Cu^{2+} in a 2 : 1 binding stoichiometry. This was further supported by ESI-MS, wherein the molecular ion peaks at m/z = 693.43 correspond to $C_{40}H_{30}CuN_4O_4$ (Fig. S9†). The IR spectra of **1**·Cu^{2+} complexes show that a hydroxyl group peak still exists at 3336.00 and it revealed the involvement of only one hydroxyl group in Cu^{2+} ion binding (Fig. S10†). The hydroxyl group acts as a good binding site and plays an effective role in the binding of transition metal ions.

UV-Visible studies

Under similar conditions to those adopted for the fluorescence study, the selectivity of **1** with metal ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cr^{3+}, Ba^{2+}, Cs^+, Cd^{2+}, Pb^{2+}, Ag^+ and Sr^{3+} was investigated by using UV-Visible absorption spectroscopy. The absorption spectrum of probe **1** shows a band at 385 nm in acetonitrile solution that can be assigned to the n–π* transition. Addition of Cr^{3+}, Cu^{2+} and Al^{3+} resulted in a slight decrease in the absorbance of **1** at 385 nm, but no noticeable changes in the ground state electronic properties of **1** were observed with other tested metal ions (Fig. S11†). In the presence of Cu^{2+}, a broad absorption between 450 and 600 nm was also observed that cause a naked-eye detectable color change of **1** from light yellow to maroon red (Fig. 5). These results additionally support the formation of a complex between **1** and Cu^{2+} in the ground state.

The quantum mechanically computed structure of **1** and its complex with Cu^{2+} were theoretically obtained by applying the DFT method (B3LYP/6-31G**/LANL2DZ) in the gas phase. All calculations were performed by using the computational programmer Gaussian 09W.[20] The basis set LANL2DZ was considered only for the Cu atom, whereas 6-31G** was used for remaining C, H, N and O atoms. The calculated lowering in

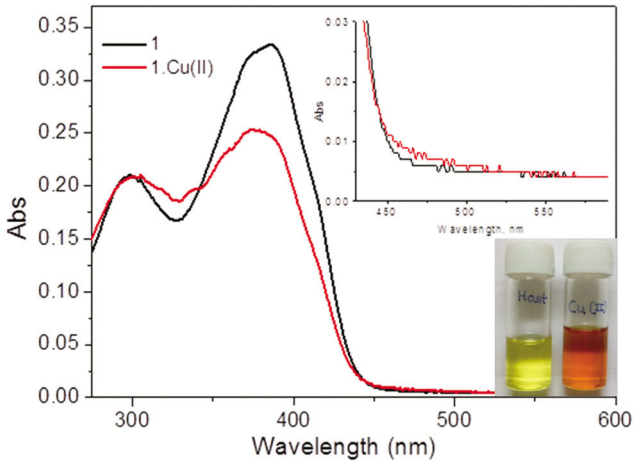


Fig. 5 UV-Visible spectra of probe **1** (1×10^{-5} M, CH_3CN) in the absence and presence of Cu^{2+}. Inset shows the colorimetric changes of **1** and **1** + Cu^{2+} ion.

the interaction energy ($E_{int} = E_{complex} - E_{receptor} - 2E_{Cu^{2+}}$) for the complexation between **1** and Cu^{2+} was found to be -240.49 kcal mol^{-1}, which indicates the formation of a stable complex (Fig. 6). Also, the planarity of **1** was destroyed upon complexation. The frontier molecular orbitals (FMOs) plots of **1** and its complex with Cu^{2+} were analyzed, which indicate the uniform distribution of charge density within the receptor **1** surface before complexation. After complexation, the charge density was mainly observed above the phenolate unit and the Cu^{2+}, which indicates that the intramolecular charge transfer (ICT) occurred between the metal ions and the receptor **1** (Fig. 6). Consequently, the band gap between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of **1** was lowered on complexation with Cu^{2+} due to the formation of a stable complex, which agrees well with the appearance of the maroon red coloration.

Intracellular imaging

The current research clearly revealed the selective complexation of **1** with Cu^{2+}, which resulted in a significant fluorescence enhancement at 386 nm. The efficient sensing ability of **1** with Cu^{2+} ions made us explore its application in live cell imaging of Cu^{2+} ions in live L929 cells (Fig. 7). Probe **1** was found to be non-toxic to the cells under the experimental conditions. L929 cells were incubated with probe **1** (0.26 μM) for 30 minutes and subjected to fluorescence measurement. The fluorescence and bright field images were taken through a confocal laser scanning microscope with 300 nm excitation. Insignificant fluorescence changes were observed in probe **1** treated cells. The cells were then washed thrice with HEPES

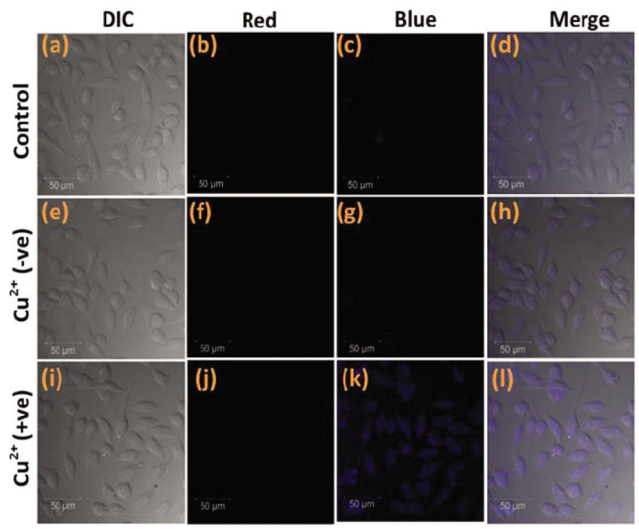


Fig. 7 Fluorescence intracellular imaging data: (a–d) control, (e–h) L929 living cells with receptor **1**, and (i–l) cells with probe **1** after incubation with Cu^{2+} for 30 minutes.

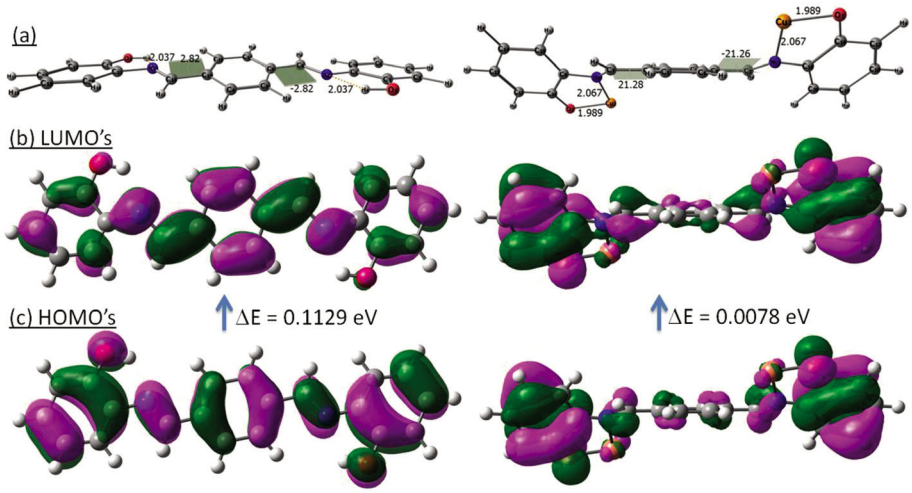


Fig. 6 DFT computed (a) optimized structure of receptor **1** and its complex with Cu^{2+}, and the (b) LUMO and (c) HOMO diagrams of **1** and its **1** – Cu^{2+} complex.

buffer in order to remove the excess probe present in the cells and then incubated with $CuCl_2$ (0.26 μM) for 30 minutes at 37 °C. The Cu^{2+} treated cells were imaged through a confocal fluorescence microscope. A bright blue fluorescence signal was observed, which is consistent with the fluorescence "turn-on" profile of the probe **1** with Cu^{2+} in the solution. Besides, bright field image measurement clearly shows that the cells are viable throughout the imaging experiments. In addition, an overlay of bright field and fluorescence images of probe and Cu^{2+} ions treated cells confirmed that the observed fluorescence signals are from the intracellular region.

Conclusion

In summary, we have synthesized a new terephthaldehyde–aminophenol conjugate **1** and applied it to the fluorescent sensing of metal ions. Probe **1** exhibited a high selectivity and sensitivity for the 'turn-on' fluorimetric detection of Cu^{2+} with a detection limit down to 0.67 μM. The fluorescence 'turn-on' sensing mechanism is proposed on the basis of complexation-induced inhibition of C=N isomerisation and PET process along with the CHEF. This probe shows no significant interference from other tested metal ions, and has been successfully applied in the bio imaging of Cu^{2+} in living L929 cells.

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References

1 (*a*) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515; (*b*) L. Prodi, F. Bolletta, M. Montalti and N. Zaccheroni, *Coord. Chem. Rev.*, 2000, **205**, 59; (*c*) C. Bargossi, M. C. Fiorini, M. Montalti, L. Prodi and N. Zaccheroni, *Coord. Chem. Rev.*, 2000, **208**, 17.

2 (*a*) R. L. Lieberman and A. C. Rosenzweig, *Nature*, 2005, **434**, 177; (*b*) K. Piontek, M. Antorini and T. Choinowski, *J. Biol. Chem.*, 2002, **277**, 37663; (*c*) K. C. Ko, J. S. Wu, H. J. Kim, P. S. Kwon, J. W. Kim, R. A. Bartsch, J. Y. Lee and J. S. Kim, *Chem. Commun.*, 2011, **47**, 3165.

3 (*a*) M. F. Yardim, T. Budinova, E. Ekinci, N. Petrov, M. Razvigorova and V. Minkova, *Chemosphere*, 2003, **52**, 835; (*b*) M. Royzen, Z. Dai and J. W. Canary, *J. Am. Chem. Soc.*, 2005, **127**, 1612.

4 (*a*) E. L. Que, D. W. Domaille and C. J. Chang, *Chem. Rev.*, 2008, **108**, 1517; (*b*) C. Vulpe, B. Levinson, S. Whitney, S. Packman and J. Gitschier, *Nat. Genet.*, 1993, **3**, 7; (*c*) D. J. Waggoner, T. B. Bartnikas and J. D. Gitlin, *Neurobiol. Dis.*, 1999, **6**, 221; (*d*) J. S. Valentine and P. J. Hart, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 3617; (*e*) D. R. Brown and H. Kozlowski, *Dalton Trans.*, 2004, 1907; (*f*) K. J. Barnham, C. L. Masters and A. I. Bush, *Nat. Rev. Drug Discovery*, 2004, **3**, 205; (*g*) B. E. Kim, T. Nevitt and D. J. Thiele, *Nat. Chem. Biol.*, 2008, **4**, 176; (*h*) G. J. Brewer, *Curr. Opin. Chem. Biol.*, 2003, **7**, 207; (*i*) G. L. Millhauser, *Acc. Chem. Res.*, 2004, **37**, 79; (*j*) S. P. Leach, M. D. Salman and D. Hamar, *Anim. Health Res. Rev.*, 2006, **7**, 97; (*k*) K. J. Barnham and A. I. Bush, *Curr. Opin. Chem. Biol.*, 2008, **12**, 222; (*l*) R. R. Crichton, D. T. Dexter and R. J. Ward, *Coord. Chem. Rev.*, 2008, **252**, 1189.

5 (*a*) *Metal Ions in Biological Systems: Properties of Copper*, ed. M. Dekker and H. Sigel, New York, 1981, p. 12; (*b*) E. L. Que, D. W. Domaille and C. J. Chang, *Chem. Rev.*, 2008, **108**, 1517; (*c*) E. Gaggelli, H. Kozlowski, D. Valensin and G. Valensin, *Chem. Rev.*, 2006, **106**, 1995; (*d*) H. N. Kim, M. H. Lee, H. J. Kim, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2008, **37**, 1465.

6 (*a*) S. Seo, H. Y. Lee, M. Park, J. M. Lim, D. Kang, J. Yoon and J. H. Jung, *Eur. J. Inorg. Chem.*, 2010, 843; (*b*) H. H. Wang, L. Xue, Z. J. Fang, G. P. Li and H. Jiang, *New J. Chem.*, 2010, **34**, 1239; (*c*) W. Wang, A. Fu, J. You, G. Gao, J. Lan and L. Chen, *Tetrahedron*, 2010, **66**, 3695; (*d*) J. P. Sumner, N. M. Westerberg, A. K. Stoddard, T. K. Hurst, M. Cramer, R. B. Thompson, C. A. Fierke and R. Kopelman, *Biosens. Bioelectron.*, 2006, **21**, 1302.

7 (*a*) M. H. Kim, H. H. Jang, S. Yi, S. Chang and M. S. Han, *Chem. Commun.*, 2009, 4838; (*b*) Z. Xu, J. Yoon and D. R. Spring, *Chem. Commun.*, 2010, **46**, 2563; (*c*) J. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2007, **129**, 9838; (*d*) Y. Xiang, A. Tong, P. Jin and Y. Ju, *Org. Lett.*, 2006, **8**, 2863.

8 (*a*) A. P. S. Gonzales, M. A. Firmino, C. S. Nomura, F. R. P. Rocha, P. V. Oliveira and I. Gaubeur, *Anal. Chim. Acta*, 2009, **636**, 198; (*b*) J. S. Becker, A. Matusch, C. Depboylu, J. Dobrowolska and M. V. Zoriy, *Anal. Chem.*, 2007, **79**, 3208; (*c*) Y. Liu, P. Liang and L. Guo, *Talanta*, 2005, **68**, 25; (*d*) P. Pathirathna, Y. Yang, K. Forzley, S. P. McElmurry and P. Hashemi, *Anal. Chem.*, 2012, **84**, 6298.

9 (*a*) Y.-Q. Weng, F. Yue, Y.-R. Zhong and B.-H. Ye, *Inorg. Chem.*, 2007, **46**, 7749; (*b*) K. L. Ciesienski, L. M. Hyman, S. Derisavifard and K. J. Franz, *Inorg. Chem.*, 2010, **49**, 6808; (*c*) S.-P. Wu, K.-J. Du and Y.-M. Sung, *Dalton Trans.*, 2010, **39**, 4363.

10 (*a*) S. H. Choi, K. Pang, K. Kim and D. G. Churchill, *Inorg. Chem.*, 2007, **46**, 10564; (*b*) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515; (*c*) B. Valeur and I. Leray, *Coord. Chem. Rev.*, 2000, **205**, 3.

11 (*a*) H. Chen, Y. Wu, Y. Cheng, H. Yang, F. Li and P. Yang, *Inorg. Chem. Commun.*, 2007, **10**, 1413; (*b*) C. Sousa, P. Gameiro, C. Freire and B. Castro, *Polyhedron*, 2004, **23**, 1401; (*c*) O. Oter, K. Ertekin, R. Kilincarslan, M. Ulusoy and

B. Çetinkaya, *Dyes Pigm.*, 2007, **74**, 730; (*d*) N. Aksuner, E. Henden, A. Çukurovali and I. Yilmaz, *Sens. Actuators, B*, 2008, **134**, 510.

12 (*a*) *SMART: Bruker Molecular Analysis Research Tools, version 5.618*, Bruker Analytical X-ray Systems, Madison, WI, 2000; (*b*) G. M. Sheldrick, *SAINT-NT, version 6.04*, Bruker Analytical X-ray Systems, Madison, WI, 2001; (*c*) G. M. Sheldrick, *SHELXTL-NT, version 6.10*, Bruker Analytical X-ray Systems, Madison, WI, 2000; (*d*) B. Klaus, *DIAMOND, version 2.1 c*, University of Bonn, Bonn, Germany, 1999.

13 A. Kuwar, R. Patil, A. Singh, S. K. Sahoo and N. Singh, *J. Mater. Chem. C*, 2015, **3**, 453–460.

14 M. Yang, W. Meng, X. Liu, N. Su, J. Zhou and B. Yang, *RSC Adv.*, 2014, **4**, 22288.

15 H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703.

16 L. Gao, J. Q. Wang, L. Huang, X. X. Fan, J. H. Zhu, Y. Wang and Z. G. Zou, *Inorg. Chem.*, 2007, **46**, 10287.

17 W. Lin, L. Yuan, X. Cao, B. Chen and Y. Feng, *Sens. Actuators, B*, 2009, **138**, 637.

18 M. Ganjali, M. Emami and M. Salavati-Niasari, *Bull. Korean Chem. Soc.*, 2002, **23**, 1394.

19 P. Job, *Ann. Chim.*, 1928, **9**, 113–203.

20 M. J. Frisch, *et al.*, *Gaussian 09, G09W®*, Gaussian Inc., Wallingford, USA, 2009.

