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PAPER

Rhodamine-based bis-sulfonamide as a sensing probe for Cu^{2+} and Hg^{2+} ions†

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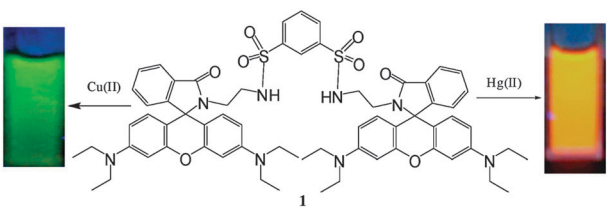
A new rhodamine-based bis-sulfonamide **1** has been designed and synthesized. The receptor selectively recognizes Cu^{2+} and Hg^{2+} ions in CH_3CN–water (4/1, v/v; 10 μM tris HCl buffer, pH 6.8) by showing different emission characteristics and color changes. While Cu^{2+} is sensed through increase in emission, Hg^{2+} is detected by a weak ratiometric change in emission of **1**. The receptor shows *in vitro* detection of both the ions in human cervical cancer (HeLa) cells.

Introduction

Design and synthesis of optical chemosensors for the selective recognition of metal ions has attracted a great deal of attention.[1] Of particular interest is the development of fluorescent sensors for heavy transition metal ions such as Hg^{2+} and Cu^{2+}, due to their biological and environmental importance.[2] Among the various transition metal ions, the copper ion causes significant environmental pollution and also serves as a catalytic cofactor for a variety of metalloenzymes.[3] However, exposure to a high level of copper, even for a short period of time, can cause gastrointestinal disturbance, while long-term exposure can cause liver or kidney damage.[4] Similarly, the mercury ion (Hg^{2+}) is considered to be dangerous as it can accumulate in the human body and cause a wide variety of diseases even at a low concentration, such as prenatal brain damage, serious cognitive disorders, and Minamata disease.[5] Therefore, it is of utmost interest to develop highly sensitive and selective assays for both Cu^{2+} and Hg^{2+} ions. In recent years, considerable efforts have been made so far to establish fluorescent chemosensors of different architectures for the selective sensing of Cu^{2+} and Hg^{2+} ions.[6,7] In this regard, most of the probes for Cu^{2+} show fluorescence quenching and the fluorescent probes which exhibit turn-on response[8] are less in number. On the other hand, the ratiometric probes for the detection of Hg^{2+} ions[9] are considerably important and they are relatively less in number than the 'turn-on' based receptors.[10] The ratiometric chemosensors give advantages over the conventional monitoring of fluorescence intensity at a single wavelength. A dual emission system can minimize the measurement errors because of the factors such as photo transformation, receptor concentrations, and environmental effects.[11]

During the course of our work on sensing of cations[6j,9e,12] and anions[13] of biological significance, we report in this full account a new rhodamine-based bis sulfonamide which recognizes both Cu^{2+} and Hg^{2+} ions by exhibiting different emission characteristics in a semi-aqueous system [CH_3CN–water (4/1, v/v; 10 μM tris HCl buffer; pH 6.8)]. In our recent publication,[9e] we have shown that Hg^{2+} ions over a series of ions (even Cu^{2+} ions) can be selectively detected by a rhodamine labeled tripodal receptor. Manipulation of functional groups and their placement under different spacers sometimes lead to a variety of new structures that show different recognition features. In relation to this, the present design in this account represents an example where Hg^{2+} and Cu^{2+} ions are simultaneously detected in a semi-aqueous system both colorimetrically and fluorometrically.



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Rhodamine B and its derivatives (RhB), with their good photo stability and high fluorescence quantum yield, act as chemosensors towards metal ions by exhibiting switching between the spirocyclic form (which is colorless and non-fluorescent) and the ring-opened amide form, which is pink and strongly fluorescent.[9,10]

A closer look into the literature[14a] reveals that although a number of rhodamine labeled receptors are known for sensing different metal ions, the simultaneous detection of both Cu^{2+} and Hg^{2+} ions by a rhodamine-labeled receptor module is less in number.[14b–f] In addition to the few examples on this

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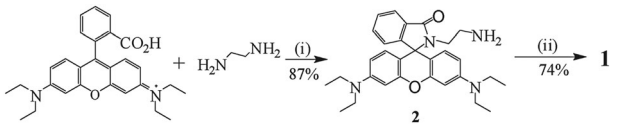
† Electronic supplementary information (ESI) available: Figures showing the change in fluorescence and UV-vis titrations of receptor **1** with various metal ions, Job plot, reversibility test, detection limit, spectral data for **1** and **3**. See DOI: 10.1039/c2nj40391a

aspect,[14b–f] the present simple structure is a new addendum as a dual probe for sensing both Cu^{2+} and Hg^{2+} ions.

Results and discussion

The receptor **1** was obtained according to Scheme 1. Coupling of benzene-1,3-disulfonyl dichloride with the rhodamine labelled amine **2**[14c] afforded the desired compound **1** in good yield.



Scheme 1 (i) EtOH, reflux, 9 h; (ii) benzene-1,3-disulfonyl dichloride, CH_2Cl_2, Et_3N, 10 h.

The metal ion binding properties of **1** towards metal ions such as Hg^{2+}, Cu^{2+}, Cd^{2+}, Fe^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Ag^{+} and Pb^{2+} (taken as their perchlorate salts) were investigated in CH_3CN–H_2O solution ($CH_3CN:H_2O$ = 4:1, v/v; 10 μM tris HCl buffer, pH 6.8). Without cations, **1** is almost non-fluorescent. However, on excitation at 510 nm, a non-structured emission at 524 nm increased to a significant extent upon interaction with only Cu^{2+} ions over the other metal ions studied. Fig. 1 shows the plot of change in emission of **1** at the monomer emission (524 nm) in the presence of 20 equiv. of the different metal ions. It is evident from Fig. 1 that the receptor is much selective to Cu^{2+} ions. Other metal ions except Hg^{2+} weakly perturbed the emission of **1** at this wavelength. On progression of titration of **1** with the metal ions, it is observed that only in the presence of Cu^{2+} and Hg^{2+} ions a new peak at 580 nm appears with significant intensity. Fig. 2, in this regard, shows the change in fluorescence ratio of **1** at 580 nm in the presence of 20 equiv. of the different metal ions. As can be seen from Fig. 2, the receptor is more sensitive to Cu^{2+} than to Hg^{2+} ions. Fig. 3 shows the emission titration spectra with Cu^{2+} ions and also the associated change in colour under illumination of UV light. Fig. 4, under identical conditions, describes the emission titration spectra obtained from the gradual addition of $Hg(ClO_4)_2$ solution to the solution of **1** (c = 2.14 × 10^{-5} M) in CH_3CN–H_2O (4/1, v/v; 10 μM tris HCl buffer; pH 6.8). In this case, while the emission centered at 524 nm decreases to a lesser extent,[14g] a new band at 580 nm begins to appear on progression of the titration of **1**

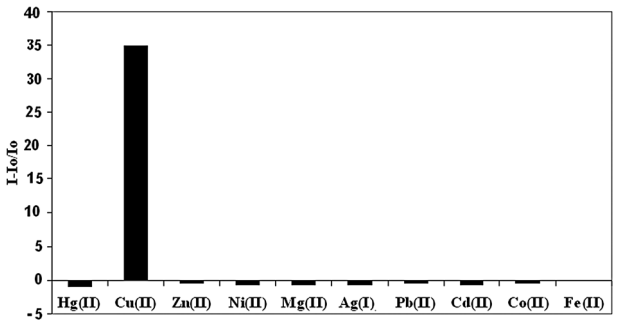


Fig. 1 Change in fluorescence ratio of **1** (c = 2.14 × 10^{-5} M) at 524 nm upon addition of 20 equiv. of cations.

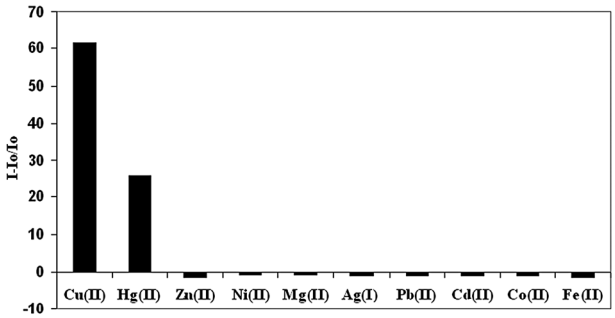


Fig. 2 Change in fluorescence ratio of **1** (c = 2.14 × 10^{-5} M) at 580 nm upon addition of 20 equiv. of cations.

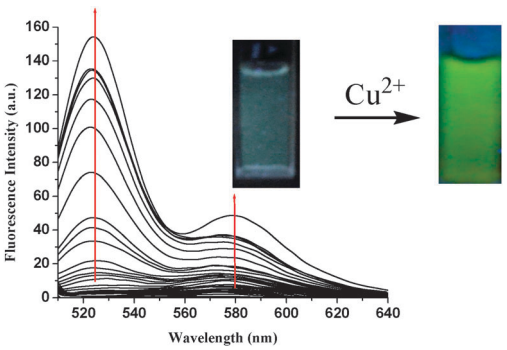


Fig. 3 Fluorescence titration spectra of **1** (c = 2.14 × 10^{-5} M) in CH_3CN–water (4/1, v/v; 10 μM tris HCl buffer, pH 6.8) upon addition of Cu^{2+} (c = 4.18 × 10^{-4} M) (λ_{ex} = 490 nm); inset: colour change of the receptor solution under illumination of UV light.

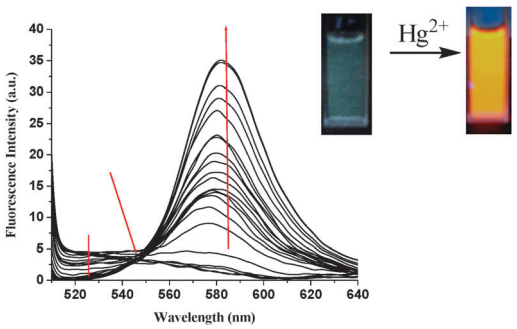


Fig. 4 Fluorescence titration spectra of **1** (c = 2.14 × 10^{-5} M) in CH_3CN–water (4/1, v/v; 10 μM tris HCl buffer, pH 6.8) upon addition of Hg^{2+} (c = 4.18 × 10^{-4} M); inset: colour change of the receptor solution under illumination of UV light.

with Hg^{2+} and results in a weak ratiometric change. Moreover, in the titration experiment the colour of the solution of **1** in the presence of Hg^{2+} was found to be pinkish yellow on illumination of UV light (inset of Fig. 4). This is in contrast to the case of Cu^{2+} ions (inset of Fig. 3). Thus, these two ions (Cu^{2+} and Hg^{2+}) are easily distinguishable from each other. It is further mentionable that under identical conditions they are distinguishable from the rest of the other ions examined as the peak at 580 nm was not observed during the course of titration (ESI†).

The pronounced OFF–ON type of Cu^{2+} selectivity was further established from fluorescence at 524 nm. Fig. 5, in this regard, describes the Cu^{2+} ion binding induced change in

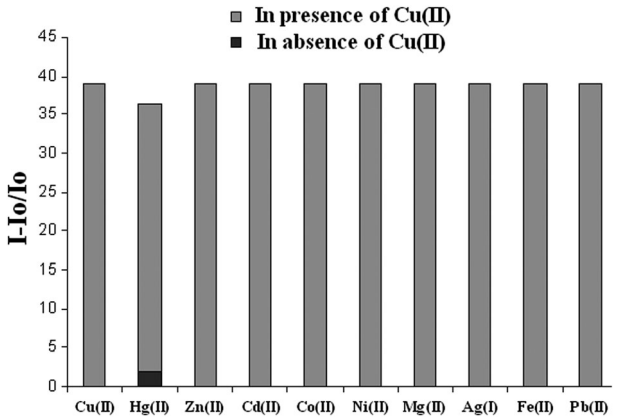


Fig. 5 Fluorescent response of sensor **1** ($c = 2.14 \times 10^{-5}$ M) to Cu^{2+} ($c = 4.18 \times 10^{-4}$ M) over the selected metal ions ($c = 4.18 \times 10^{-4}$ M).

emission of **1** in the presence and absence of 5 equiv. of other metal ions. As can be seen from Fig. 5, the interference of the metal ions considered in the present study is established to be negligible. The stoichiometries of the complexes[15] of **1** with both Cu^{2+} and Hg^{2+} ions were determined to be 1 : 1 and the binding constant[16] values (K_a) were found to be $(9.05 \pm 0.63) \times 10^4$ M^{-1} and $(7.87 \pm 0.55) \times 10^4$ M^{-1} for Cu^{2+} and Hg^{2+}, respectively. The values are close in magnitude and the small increase in K_a for Cu^{2+} is ascribed to its strong chelation at the binding core of **1**. As representative, Fig. 6a shows the Job plot for the Cu^{2+} complex of **1** and Fig. 6b shows the binding constant curve obtained from non-linear curve fitting of the emission titration data. Due to minor changes in emission we were unable to determine the binding constant values for other metal ions.

The selective fluorescence response of **1** towards Cu^{2+} ions in the study is explained as due to their best fit in the bis-sulfonamide cleft of **1**. The different mode of Cu^{2+}-induced change in emission of **1** from that of Hg^{2+} binding is presumably attributed to the involvement of the binding centre in different ways. To our belief, Cu^{2+} ions primarily interact with sulfonamide functionalities involving deprotonation of –NH protons (see Form **1A** in Fig. 7). As a consequence of this interaction the emission intensity at 524 nm gradually increases. After fixation of the metal ions by the sulfonamides, the closely spaced spirocyclic forms of the rhodamine parts are opened which provides

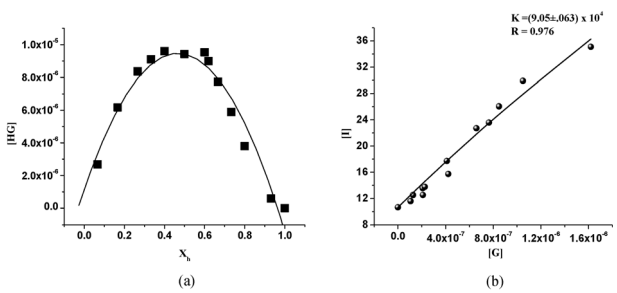


Fig. 6 (a) Fluorescence Job plot for **1** with Cu^{2+} in CH_3CN–water (4/1, v/v; 10 μM tris HCl buffer; pH 6.8) ([H] = [G] = 5×10^{-5} M); (b) nonlinear curve fitting of the fluorescence titration data for **1** ($c = 2.14 \times 10^{-5}$ M) with Cu^{2+} in CH_3CN–water (4/1, v/v; 10 μM tris HCl buffer; pH = 6.8).

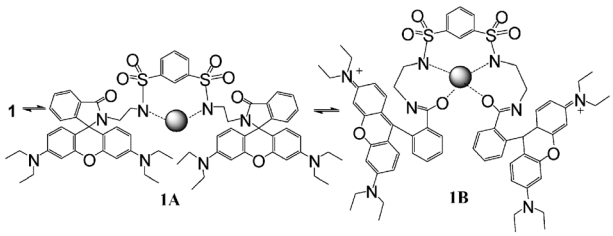


Fig. 7 Suggested modes of interaction of **1** with Cu^{2+}/Hg^{2+} ions in solution.

amide ions to chelate the metal ions strongly (Form **1B** in Fig. 7) for which a new peak at 580 nm begins to appear with significant growth on progression of the titration. We further presume that in contrast to Cu^{2+} binding, Hg^{2+} ions are initially involved in weak binding with the bis-sulfonamide moieties due to which change in emission of **1** at 524 nm is less. The rest of the part related to the spirolactam ring opening is similar to the case of Cu^{2+} and thus the fluorescence intensity at 580 nm increases although by a lesser extent. To resolve the issue on the binding of metal ions initially at the disulfonamide core of **1** followed by the spirolactam ring, we studied the interaction by considering a model compound **3** where the rhodamine part is absent (see ESI†). It is evident from Fig. S9a and S10 (ESI†) that upon addition of Cu^{2+} ions to the solution of **3** in CH_3CN–H_2O ($CH_3CN : H_2O$ = 4 : 1, v/v; 10 μM tris HCl buffer, pH 6.8), both the absorbance and emission underwent significant changes. This is in contrast to the case with Hg^{2+} ions (Fig. S9b and S11, ESI†) where complexation induced changes in absorbance and emission are less. Therefore, the appearance of the more intense emission peak at 524 nm for **1** while titration with Cu^{2+} ions is attributed to the greater interaction of Cu^{2+} ions at the disulfonamide core. The intensity of this peak is furthermore sensitive to the polarity of the solvent. While the solution of Cu-complex of **1** in CH_3CN was diluted with CH_3CN containing 25, 30, 40, 55 and 65% water, the peak at 524 nm gradually became intensified (see Fig. S16, ESI†). These findings demonstrate the case of charge transfer associated with the sulfonamide motif linked to the Cu^{2+} ions.[1f] To support the binding mechanism, we recorded the FTIR and ^{1}H NMR of **1** in the presence and absence of equiv. amounts of the metal salts. The formation of **1B** through the ring opening was established from both the ^{1}H NMR and FTIR studies. In FTIR, the amide carbonyl stretching of the spirolactam part that appeared at 1673 cm^{-1} was changed to a lower wavenumber 1671 cm^{-1} and 1647 cm^{-1} in the presence of Hg^{2+} and Cu^{2+} ions, respectively. The stretching frequency for the –SO_2– group that appeared at 1468 cm^{-1} in **1** was found to be unchanged during interaction with Hg^{2+} ions. In the case of interaction with Cu^{2+} ions a weak stretching band at 1466 cm^{-1} was noted and these results thus intimate that the –SO_2– motifs do not take part in the metal complexation (ESI†).

In ^{1}H NMR, the –NH protons that appeared at 7.32 ppm were invisible in the presence of 1 equiv. of Cu^{2+} and Hg^{2+} ions. The ring protons (e, f and g types; see the labeling of **1** in Fig. S12, ESI†) of the rhodamine part moved to the downfield directions in the presence of 1 equiv. of Cu^{2+} ions ($\Delta\delta_e = 0.04$, $\Delta\delta_f = 0.01$ and $\Delta\delta_g = 0.06$). Similar observation was found in

the presence of 1 equiv. of Hg^{2+} ions *i.e.* ring protons shifted downfield ($\Delta\delta_e$ = 0.02, $\Delta\delta_f$ = 0.07 and $\Delta\delta_g$ = 0.03) with significant broadening. Fig. S12 (ESI†), in this regard, shows the change in ^{1}H NMR of **1**. The downfield shifting of the 'e', 'f' and 'g' type protons indicated ring opening of the spirolactam part in **1**. During interaction while the 'h' type proton (8.23 ppm) showed a slight upfield shift of 0.05 ppm in the presence of Hg^{2+}, it remained positionally unaltered in the presence of Cu^{2+} ions. Such negligible or no change in chemical shift of the 'h' type proton indicates that the metal ion is more closely spaced to the spirolactam moiety of rhodamine rather than the disulfonamide core owing to charge–charge interaction.

The ring opening in **1** to form the metal chelated species of type **1B** was further confirmed by ^{13}C NMR. Fig. 8 represents the change in ^{13}C NMR of **1** in the presence of 1 equiv. of each of Cu^{2+} and Hg^{2+} ions. The disappearance of the signal at 65.6 ppm for the tertiary carbon of the spirolactam ring of **1** (labeled 'i') is in favor of the opening of the spirolactam ring to introduce the form **1B**.

As shown in Fig. 9, without Cu^{2+} ions, **1** scarcely shows an absorption at 555 nm, indicating that **1** exists as spirolactam form. Addition of Cu^{2+} (Fig. 9) and Hg^{2+} (Fig. 10) separately to the solution of **1** ($c = 2.14 \times 10^{-5}$ M) in CH_3CN–H_2O (4/1, v/v; 10 μM tris HCl buffer; pH 6.8) brought about strong absorption at 555 nm along with clear colour change from colourless to pink, as is normally noticed for rhodamine-based probes. The appearance of pink color is attributed to the opening of the spirolactam rings and generation of the delocalized xanthene moieties.[14a] This was not observed when the titrations were conducted with other metal ions (ESI†). The stoichiometries of both Cu- and Hg-complexes in the ground state were established to be 1 : 1 (ESI†).

Careful scrutiny shows that the intensity of the pink colors arising from the addition of Cu^{2+} and Hg^{2+} ions is little different. It is mentionable that $Cu(NO_3)_2$ instead of $Cu(ClO_4)_2$ opened the spirolactam ring weakly as confirmed from the appearance of the faint pink colour of the solution as well as a weakly intense emission peak at 580 nm (ESI†). A similar situation was observed with the addition of $Hg(NO_3)_2$ to the solution of **1** (ESI†). This is assumed to be due to the interference of the nitrate ion at the bis-sulfonamide binding core of **1**.

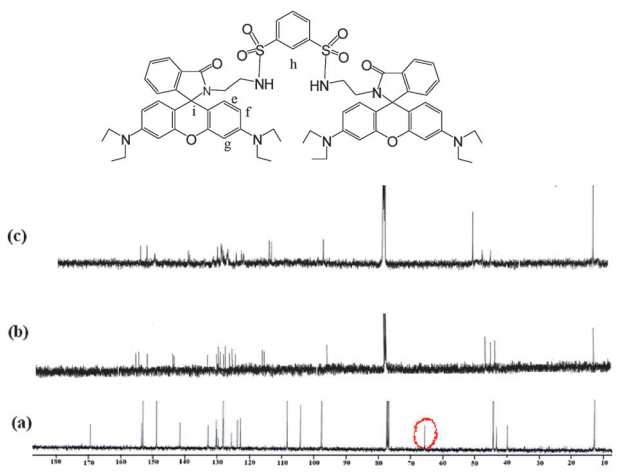


Fig. 8 ^{13}C NMR ($CDCl_3$, 100 MHz) of (a) **1**, (b) **1**·Cu^{2+} and (c) **1**·Hg^{2+}.

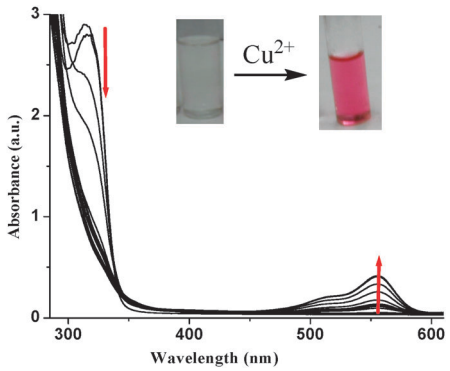


Fig. 9 Absorption titration spectra of **1** ($c = 2.14 \times 10^{-5}$ M) in CH_3CN–H_2O (4/1, v/v; 10 μM tris HCl buffer; pH 6.8) upon addition of Cu^{2+} ($c = 4.18 \times 10^{-4}$ M).

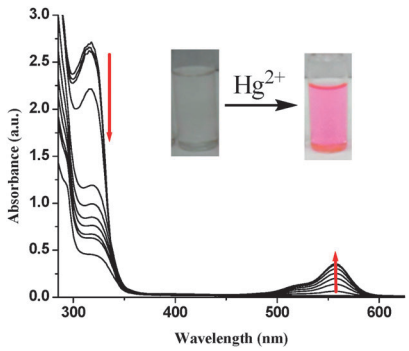


Fig. 10 Absorption titration spectra of **1** ($c = 2.14 \times 10^{-5}$ M) in CH_3CN–H_2O (4/1, v/v; 10 μM tris HCl buffer; pH 6.8) upon addition of Hg^{2+} ($c = 4.18 \times 10^{-4}$ M).

In order to be confirmed with the reversible nature of the complexation, fluorescence and absorption spectra of the copper and mercury complexes of **1** in CH_3CN–H_2O (4/1, v/v; 10 μM tris HCl buffer; pH 6.8) were observed upon addition of KI. Addition of KI did not bring the reverse change in both emission and absorption spectra. The pink color of the solution did not vanish. Similar experiments were also performed with addition of EDTA solution in large excess to the solutions of the complexes of **1** with Cu^{2+} and Hg^{2+} ions but we failed to prove the reversibility. Only in the presence of ethylene diamine the pink colour of the mercury complex of **1** was discharged and both the absorption and emission spectra of the free receptor were retrieved. In the case of the copper complex, the pink colour turned into faint blue color upon addition of ethylene diamine. Interestingly, while the emission spectrum for the free receptor was retrieved in this case, the absorption spectra appeared to be different. This occurred due to the simultaneous formation of the blue colored copper–ethylene diamine complex in solution (ESI†).

To determine the detection limit of the receptor **1** towards both Cu^{2+} and Hg^{2+} ions, emission titrations of **1** were conducted upon addition of the said metal ions in different concentrations. In regard to this, Fig. 11 represents the change in emission spectra of **1** as well as the associated colour change

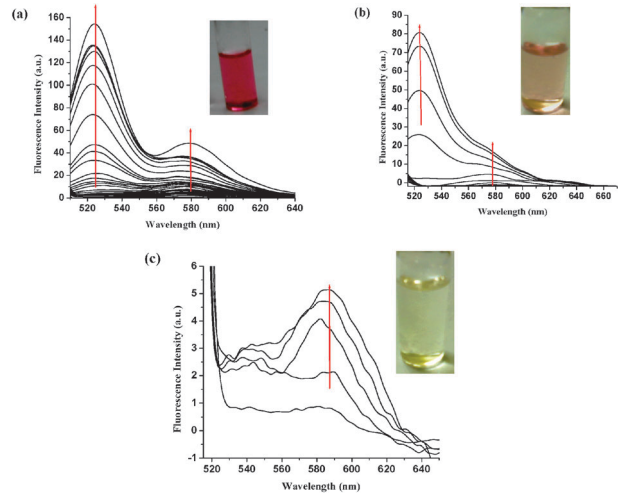


Fig. 11 Change in fluorescence spectra of **1** ($c = 2.14 \times 10^{-5}$ M) in CH_3CN–H_2O (4/1, v/v; 10 μM tris HCl buffer; pH 6.8) upon addition of (a) Cu^{2+} ($c = 4.18 \times 10^{-4}$ M), (b) Cu^{2+} ($c = 4.18 \times 10^{-5}$ M); (c) Cu^{2+} ($c = 4.18 \times 10^{-6}$ M). Associated colour changes are shown in the inset of the figures.

of the receptor solution with the variation of Cu^{2+} ion concentrations in CH_3CN–H_2O (4/1, v/v; 10 μM tris HCl buffer; pH 6.8). The spectral and colour changes of **1** upon the addition of Hg^{2+} ions of varying concentrations in CH_3CN–H_2O (4/1, v/v; 10 μM tris HCl buffer; pH 6.8) can be found in the ESI.† However, in both cases it is observed that the receptor **1** convincingly reports the detection of both Cu^{2+} and Hg^{2+} ions up to their concentration ranges of $\sim 10^{-5}$ M through colour change. Thus the detection limit of receptor **1** in the present case is observed to be ~21 μM which is comparable with the reported values 10–25 μM.[14b–f]

The potential biological application of the receptor was evaluated for *in vitro* detection of Cu^{2+} and Hg^{2+} ions in human cervical cancer (HeLa) cells. The HeLa cells were incubated with 5 μL of receptor **1** (10 μM in CH_3CN–H_2O (4/1, v/v)) in DMEM (Dulbecco's modified Eagle's medium) (without FBS) for 30 min at 37 °C and washed with phosphate buffered saline (PBS) buffer (pH = 7.4) to remove the excess of receptor **1**. DMEM (without FBS) was again added to the cells. The cells were next treated with 5 μL of $Cu(ClO_4)_2$ (30 μM) and incubated again for 30 min at 37 °C. A control set of cells which was devoid of Cu^{2+} ions was kept. A similar experiment was done for $Hg(ClO_4)_2$. Fig. 12a and b represent the bright field images of the cells before and after treatment of the cells with **1**, respectively. Cells incubated with receptor **1** without Hg^{2+} and Cu^{2+} (Fig. 12c) and cells incubated with Hg^{2+} without receptor **1** (Fig. 12d) did not show any fluorescence property. This was also true when cells were incubated with Cu^{2+} without receptor **1** (Fig. 12e). In contrast, cells incubated with the receptor **1** and then with Hg^{2+} ions showed the occurrence of red fluorescence (Fig. 12f). Again cells incubated with the receptor **1** and then with Cu^{2+} ions showed the occurrence of red fluorescence (Fig. 12g). These facts indicate the permeability of the receptor inside the cells and the binding of Hg^{2+}, Cu^{2+} ions with the receptor. Fig. 12h and i show the occurrence of green fluorescence upon incubation of receptor **1** and then Hg^{2+} (Fig. 12h) and Cu^{2+} (Fig. 12i) with the probe, respectively.

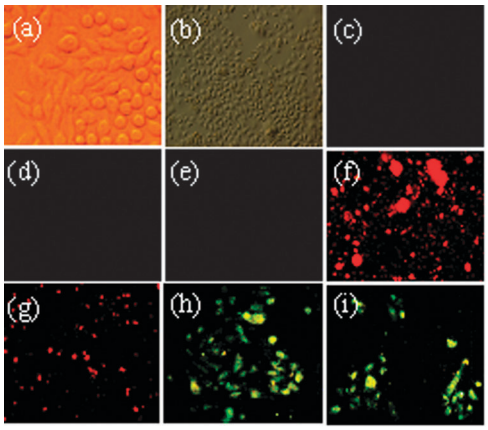


Fig. 12 Fluorescence and bright field images of HELA cell lines: (a) bright field image of normal cells, (b) bright field image of cells treated with probe **1** (10.0 μM) for 1 h at 37 °C, (c) fluorescence image of cells treated with probe **1** (10 μM) for 1 h at 37 °C, (d) fluorescence image of cells treated with $Hg(ClO_4)_2$ (30 μM) for 1 h, (e) fluorescence image of cells treated with $Cu(ClO_4)_2$ (30 μM) for 1 h, (f) red fluorescence images of cells upon treatment with probe **1** (10.0 μM) and then with $Hg(ClO_4)_2$ (30.0 μM) for 1 h, (g) red fluorescence images of cells upon treatment with probe **1** (10.0 μM) and then with $Cu(ClO_4)_2$ (30.0 μM) for 1 h (for red fluorescence image: λ_{ex} = 510 nm), (h) green fluorescence images of cells upon treatment with probe **1** (10.0 μM) and then with $Hg(ClO_4)_2$ (30.0 μM) for 1 h, (i) green fluorescence images of cells upon treatment with probe **1** (10.0 μM) and then with $Cu(ClO_4)_2$ (30.0 μM) for 1 h (for green fluorescence image λ_{ex} range: 511 nm to 534 nm and for red fluorescence image λ_{ex} range: 567 nm to 617 nm).

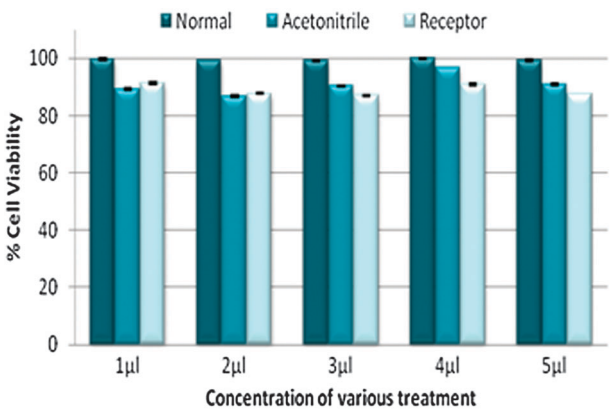


Fig. 13 MTT assay for the receptor **1**.

The addition of receptor **1** to the cells did not show any cytotoxicity as evident from the morphology of the cells (Fig. 12) as well as from the MTT assay (Fig. 13). The viability was more than 90%, 90% and 91% for normal, acetonitrile- and receptor-treated cells respectively. Since the percentage of viable cells of all the series was above 90% this would suggest that the receptor was not cytotoxic when exposed to cultured cells *in vitro*.

Conclusions

In conclusion, we have shown that the rhodamine labeled receptor **1** is capable of detecting Cu^{2+} and Hg^{2+} ions

simultaneously in aq. CH_3CN by exhibiting different emission characteristics as well as color. Among few examples[14b–f] on rhodamine labeled receptors for simultaneous detection of Cu^{2+} and Hg^{2+} ions, the present example in this full account is a new addendum. The dimension of the isophthaloyl cleft and the cooperative action of the sulfonamides along with the amide parts of the rhodamines favor the strong chelation of Cu^{2+} and Hg^{2+} ions over the other cations examined. Furthermore, the chemosensor **1** is found to be efficient in reporting the presence of both Cu^{2+} and Hg^{2+} ions inside the cell without showing any cytotoxicity.

Experimental

2-(Benzyl(2-hydroxyethyl)amino)-*N*-(2-(3′,6′ bis(diethylamino)-3-oxospiro[isoindoline-1,9′-xanthene]-2-yl)ethyl)acetamide (1)

To a stirred solution of benzene-1,3-disulfonyl dichloride (0.055 g, 0.197 mmol) in dry CH_2Cl_2 (20 mL), the amine **2** (0.2 g, 0.412 mmol), which was obtained according to the reported procedure,[14c] was added dropwise followed by the addition of Et_3N. Stirring was continued for 12 h. After completion of reaction, monitored by TLC, solvent was evaporated and water was added to the residue. The aqueous layer was extracted with $CHCl_3$ (25 mL × 3) and dried over anhydrous Na_2SO_4. Purification of the crude mass by silica gel column chromatography using 3% CH_3OH in $CHCl_3$ as eluent yielded the desired compound **1** (0.170 g, 74%), mp 142 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (s, 1H), 7.91–7.87 (m, 4H), 7.48–7.42 (m, 5H), 7.31 (t, 2H, J = 4 Hz), 7.01 (m, 2H), 6.33–6.30 (br s, 6H), 6.19–6.13 (s, 6H, unresolved), 3.33–3.28 (m, 16H), 3.13–3.05 (m, 4H), 2.75–2.74 (m, 4H), 1.14 (s, 24H); ^{13}C NMR (100 MHz, d_6-$CDCl_3$): δ 169.6, 153.5, 153.1, 148.9, 141.6, 132.9, 130.3, 130.2, 129.7, 128.3, 128.2, 125.6, 123.8, 122.9, 108.3, 104.3, 97.7, 65.6, 44.3, 44.3, 39.9,12.5; FT IR (ν in cm^{-1}, KBr): 3171, 2969, 2928, 1677, 1633, 1546, 1468; MS (FAB) m/z 1171.5 $(M + H)^+$, 1170.4 (M^+).

General procedure for fluorescence and UV-vis titrations

Stock solutions of the receptor were prepared in 4 : 1 (v/v) CH_3CN : H_2O containing 10 mM Tris–HCl buffer (pH = 6.8) in the concentration range $\sim 10^{-5}$ M. 2.5 mL of the receptor solution was taken in a cuvette. Stock solutions of guests in the concentration range $\sim 10^{-4}$ M were prepared in the same solvents and were individually added in different amounts to the receptor solution. Upon addition of metal ions, the change in emission of the receptor was noted. The same stock solutions for receptor and guests were used to perform the UV-vis titration experiment. Solution of the metal salts was successively added in different amounts to the receptor solution (2.5 mL) taken in the cuvette and the absorption spectra were recorded. Both fluorescence and UV-vis titration experiments were carried out at 25 °C.

Job plots

The stoichiometry was determined by the continuous variation method (Job Plot).[15] In this method, solutions of host and guests of equal concentrations were prepared in the solvents used in the experiment. Then host and guest solutions were mixed in different proportions maintaining a total volume of 3 mL of the mixture. All the prepared solutions were kept for 1 h with occasional shaking at room temperature. Then emission and absorbance of the solutions of different compositions were recorded. The concentration of the complex *i.e.*, [HG] was calculated using the equation $[HG] = \Delta I/I_0 \times [H]$ or $[HG] = \Delta A/A_0 \times [H]$ where $\Delta I/I_0$ and $\Delta A/A_0$ indicate the relative emission and absorbance intensities respectively. [H] corresponds the concentration of the pure host. Mole fraction of the host (X_H) was plotted against concentration of the complex [HG]. In the plot, the mole fraction of the host at which the concentration of the host–guest complex [HG] is maximum gives the stoichiometry of the complex.

Method for MTT assay

Reagents. MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,*S*-diphenyltetrazolium bromide] and all other reagents were purchased from Sigma-Aldrich Inc. (St-Louis, MO, USA); Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, penicillin, streptomycin, neomycin (PSN) antibiotics were purchased from Gibco BRL (Grand Island, NY, USA).

Cell culture. Human cervical cancer cell line HeLa was procured from NCCS Pune, India. The cells were cultured at 5×10^5 cells per mL in DMEM supplemented with 10% fetal bovine serum and 1% PSN antibiotic at 37 °C, 5% CO_2 for experimental purpose.

Assessment of percentage of viable cells. The percentage viability of HeLa cells, after being exposed to the receptors, was evaluated by MTT assay (Mossman, 1983). The cells were incubated in 96-well microplates for 24 hours along with the receptors at different concentration. A series of normal cells (without any exposure) and a series of positive control cells (exposed to similar concentrations of acetonitrile, the "vehicle solvent" of the receptors, as that of the exposure of the receptors) were taken. The intracellular formazan crystals formed were solubilized with dimethyl sulfoxide (DMSO) and the absorbance of the solution was measured at 595 nm by using a microplate reader (Thermo scientific, Multiskan ELISA, USA). The calculated cell survival rate = percentage of MTT inhibition as follows: percentage of survival = (mean experimental absorbance/mean control absorbance) × 100%.

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