

A rhodamine-piperazine conjugate as a fluorogenic sensor for mercury(II) ion in aqueous ethanol medium

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A *bis*-rhodamine piperazine conjugate has been synthesized in good yields as a pale yellow solid. The metal-free compound does not show any fluorescence upon excitation. However, specifically in presence of Hg^{2+} ion in aqueous ethanol, the color changes to pink and it exhibits high fluorescence upon excitation at 520 nm. Presence of other biologically relevant metal ions in the system does not affect the fluorescence output to any significant extent. Thus, this compound can be used as a chromogenic and fluorogenic sensor for Hg^{2+} ion in aqueous ethanol medium.

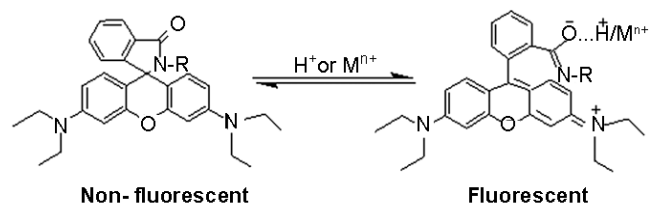
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Fluorogenic chemosensors are miniature signal transducers that can report the presence of an analyte via changes in the measurable photophysical property of the system¹. While both quenching as well as enhancement of fluorescence can be used to detect the presence of an analyte, it is the enhancement that is preferable in sensors for practical reasons. A chemosensor that can sense a specific analyte is potentially useful in chemical as well as biological systems. Specifically, fluorescence detection of heavy metal ions is of great importance as some of these metal ions are highly toxic and environmental pollutants.

Among the commonly occurring heavy metal pollutants, mercury is considered to be one of the most toxic and environmentally hazardous². It can be released in different forms through natural events as well as human activities and exhibit extreme disorders of the neurological, nephrological, immunological, cardiac and reproductive systems³⁻⁶. Some microorganisms, particularly sulfate-reducing bacteria⁷ produce methyl mercury, a potent neurotoxin, from other forms of mercury. Methyl mercury poses serious health problems by damaging the central nervous and endocrine systems, leading to several cognitive and motion disorders. Overall, the multiple pathways of spreading mercury through air, food, water, etc., is a serious concern because it persists in the environment and subsequently accumulates through the food chain. This, the use of sensor molecules for Hg^{2+} that present instantaneous

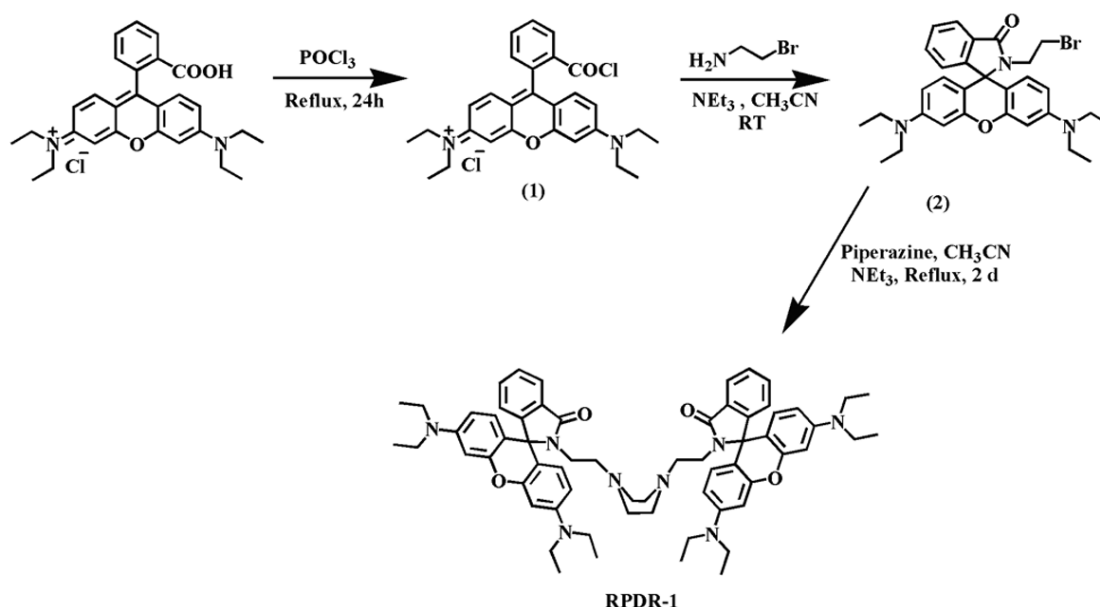
measurable optical response is highly desirable. A few examples of Hg^{2+} sensors are known that can selectively detect Hg^{2+} ion in aqueous medium⁸. Unfortunately, most of the reported chemosensors⁹⁻¹⁰ have some disadvantages, such as poor aqueous solubility, cross-sensitivities towards other metal ions and strict reaction conditions.

Rhodamine spirolactam based chemosensors¹¹ have attracted a lot of attention since the pioneering work of Czarnik and his group¹² who showed that it was non-fluorescent to begin with while ring opening in presence of proton or Cu^{2+} ion gave a highly fluorescent product (Scheme 1). Since then, a number of rhodamine derivatives have been synthesized as chemosensors for different metal ions including mercury. These molecules are attractive due to excellent spectroscopic properties of large molar extinction coefficients and high emission quantum yields. Specificity of binding to the rhodamine derivative depends upon the type and spatial distribution of the donors attached. It is to be noted, however, that spirolactam ring opening is also influenced¹¹ by nature



Spirolactam ring-opening in presence of H^+ or a metal ion

Scheme 1



Synthetic route to the formation of RPDR-1

Scheme 2

of the solvents used. In the present paper, we describe a new chemosensor where two rhodamine moieties are attached through a piperazine spacer (RPDR-1 in Scheme 2). This compound undergoes irreversible ring opening in presence of Hg^{2+} ion to afford both chromogenic and fluorogenic response in aqueous ethanolic medium. Other workers in the field have shown that the spirolactum ring opening takes place specifically in presence of Hg^{2+} ion¹¹.

Materials and Methods

Reagent grade rhodamine B and all metal perchlorate salts were from Aldrich Chemical Company (USA), while reagent grade piperazine, 2-bromoethyl amine, anhydrous sodium sulfate and POCl_3 were from SD Fine Chemicals (India). These chemicals were used as received without further purification. All the solvents were acquired from SD Fine Chemicals (India) and were purified prior to use following standard methods. All the reactions were carried out under dinitrogen atmosphere. Chromatographic separation was done by column chromatography using 100-200 mesh silica gel obtained from Acme Synthetic Chemicals (India).

All the compounds were characterized by elemental analysis, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and ESI-MS spectra. Both $^1\text{H-NMR}$ (500 MHz) and $^{13}\text{C-NMR}$ spectra (125 MHz) of the compounds were recorded on a Jeol JNM-LA500 FT spectrometer in CDCl_3 with tetramethylsilane as the internal standard. Elemental

analyses were carried out using an Elementar Vario EL III Carlo Erba 1108 elemental analyzer. The EI-MS data were obtained in methanol from a WATERS-Q-T of Premier mass spectrometer. The ESI capillary was set at 2.8 kV and the cone voltage was 30 V. UV-vis spectra were recorded on a Shimadzu 2450 UV-vis spectrophotometer in aqueous ethanol at 298 K. Melting points were determined with an electrical melting point apparatus by PERFIT, India and are uncorrected. Steady-state fluorescence spectra were obtained using a Perkin-Elmer LS 50B luminescence spectrometer at 293 K with excitation and emission band-pass of 5 nm. Fluorescence quantum yields were determined by comparing the corrected spectrum with that of anthracene ($\phi = 0.297$) in ethanol¹³ taking the area under the total emission. The fluorescence measurements in solutions were carried out at $\sim 10^{-5}$ M concentration. The complex stability constant, K_a , was determined¹⁴ from the change in fluorescence intensity resulting from the titration of dilute solution ($\sim 10^{-5}$ M) of the dye against metal ion concentration. The reported values gave good correlation coefficients (>0.98).

Synthesis of the rhodamine derivative and precursor (2)

The synthetic route for the rhodamine-piperazine conjugate is illustrated in Scheme 2. All synthetic operations were carried out under dinitrogen unless otherwise mentioned.

The precursor (2) was synthesized as follows: Rhodamine B hydrochloride (2 g; 4 mmol) was

allowed to reflux in 15 mL POCl_3 for 24 h and the remaining POCl_3 was distilled off to obtain a pink solid. This crude solid was used for the next step without any purification. It was dissolved in CH_3CN (25 mL) and to this solution was added 2-bromoethylamine hydrobromide (2.0 g, 10 mmol) followed by triethylamine (2.5 mL) and stirred for 24 h at room temperature. The mixture was then concentrated to a small volume (~3 mL) under reduced pressure, treated with 30 mL of water and then stirred for 15 minutes. The organic part was extracted with chloroform (3×50 mL). The chloroform layer after drying over Na_2SO_4 was evaporated off to obtain a pink solid. The crude product was purified by silica gel column chromatography using $\text{CHCl}_3/\text{CH}_3\text{OH}$ (99:1 v/v) as the eluent, affording (**2**) as a white solid. Yield ~30 %; ^1H -NMR (500 MHz, CDCl_3 , 25 °C, TMS) δ : 1.16 (t, 12H, $J = 5.7$ Hz), 2.97-3.00 (m, 1H), 3.12-3.15 (m, 1H), 3.29-3.37 (m, 8H), 3.43-3.46 (m, 1H), 3.49-3.53 (m, 1H), 6.25-6.27 (m, 2H), 6.37-6.43 (m, 4H), 7.05-7.07 (m, 1H), 7.41-7.45 (m, 2H), 7.89-7.90 (m, 1H); ^{13}C -NMR (125 MHz, CDCl_3 , 25 °C, TMS) δ : 12.5, 28.7, 40.6, 41.7, 44.3, 64.7, 97.6, 104.9, 108.1, 122.9, 123.8, 128.1, 128.6, 130.5, 132.6, 148.8, 153.1, 153.5, 168.1; ESI-MS: m/z (%): 550.179 (70) $[\text{M}+2]^+$; Anal. (%): calcd for $\text{C}_{30}\text{H}_{34}\text{N}_3\text{O}_2\text{Br}$: C 65.69, H 6.25, N 7.66; Found: C 64.87, H 7.32, N 7.25.

Synthesis of the bis-rhodamine piperazine conjugate, RPDR-1

To a solution of anhydrous piperazine (80 mg, 0.92 mmol) in acetonitrile (30 mL), triethylamine (1 mL, excess) was added and the resulting solution was allowed to stir for 30 min at room temperature. To this solution, compound (**2**) (1 g, 1.82 mmol) was added portion-wise over 10 min and finally allowed to reflux for 48 h. After cooling to RT, the bromide salt produced was removed by filtration. The pink colored filtrate was evaporated to dryness under reduced pressure to afford a pink solid. This solid was washed several times with water and then extracted with CHCl_3 . The organic layer, after drying over anhydrous Na_2SO_4 , evaporated to dryness to obtain a light pink solid. The crude product was then purified by silica gel column chromatography using $\text{CHCl}_3/\text{CH}_3\text{OH}$ (98:2 v/v) as the eluent, affording RPDR-1 as a light yellow solid. This solid was used for absorption and emission studies. Yield ~60 %; m. pt. 141 °C (uncorrected); ^1H -NMR (500 MHz, CDCl_3 , 25 °C, TMS) δ : 1.12 (t, 24H, $J = 7.15$ Hz), 2.01-2.04 (m, 4H), 2.16 (s, 8H), 3.16-3.19 (m, 4H), 3.28-3.35

(m, 16H), 6.20-6.22 (dd, 4H), 6.33-6.34 (d, 4H), 6.37-6.40 (m, 4H), 7.04-7.06 (m, 2H), 7.39-7.44 (m, 4H), 7.84-7.86 (m, 2H); ^{13}C -NMR (125 MHz, CDCl_3 , 25 °C, TMS) δ : 12.4, 12.7, 37.3, 44.5, 52.7, 55.6, 64.9, 97.8, 105.7, 108.1, 122.7, 123.8, 127.9, 129, 131.5, 132.3, 148.8, 153.4, 153.6, 167.9; ESI-MS: (m/z): 1021.58 (40%) $[\text{M} + \text{H}]^+$; Anal. (%): Calcd for $\text{C}_{64}\text{H}_{76}\text{N}_8\text{O}_4$: C, 75.26; H, 7.50; N, 10.97. Found: C, 74.86; H, 7.91; N, 10.58.

Results and Discussion

The dye RPDR-1 is stable in air and soluble in common organic solvents. Presence of the spirolactum bond is proven by the characteristic ^{13}C signal that appears¹⁵ at 64.9 ppm.

UV-vis absorption spectroscopy

In aqueous ethanol, the dye does not show any noticeable absorption in the region of interest. The perchlorate salts of alkali, alkaline earth and first row transition metals were used to evaluate the binding properties of the dye. Among the heavy metals, perchlorate salts of Cd^{2+} , Pb^{2+} and Hg^{2+} ions were used. It is observed that only in presence of Hg^{2+} ion, a strong absorption band (ϵ , $12470 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$) centering at 560 nm appears along with a prominent shoulder at ~520 nm. Among the other metal ions probed, only Fe^{2+} , Cu^{2+} and Pb^{2+} show very weak absorption at 560 nm (Fig. 1). The 560 nm band arises^{11,16} due to the spirolactum ring opening and formation of delocalized xanthine moiety of the rhodamine group. The disappearance of the characteristic peak at $\delta = 64.9$ ppm in the ^{13}C -NMR spectrum of the Hg^{2+} complex also supports cleavage of the

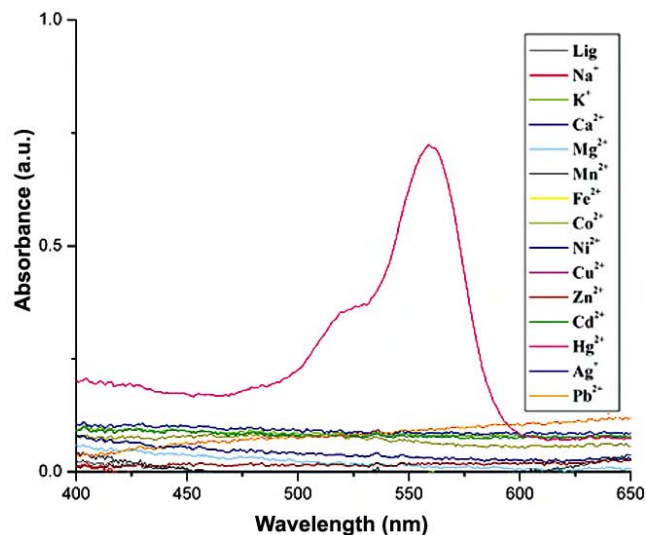


Fig. 1—Absorption spectra in presence of different metal ions.

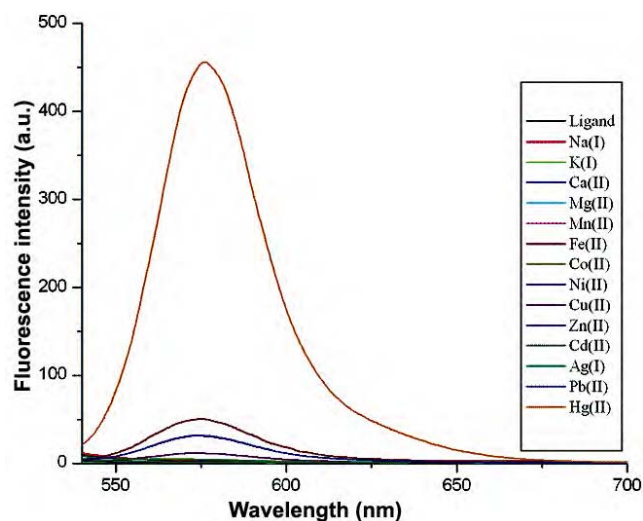


Fig. 2—Emission spectra in presence of different metal ions.

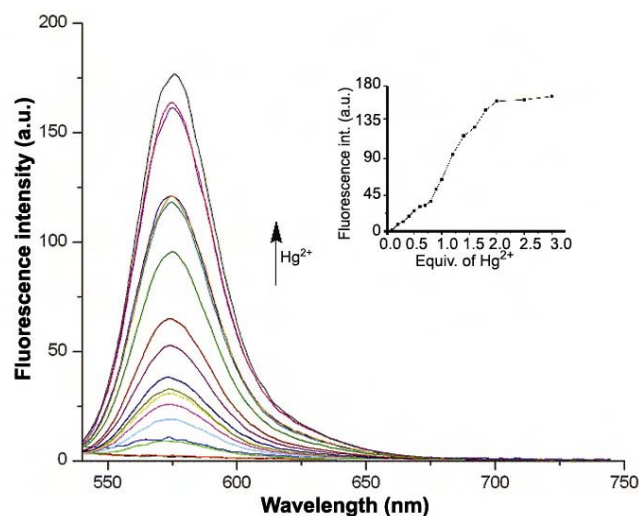


Fig. 4—Change of fluorescence spectra of the dye RPDR-1 ($\sim 10^{-5} M$) as a function of $[Hg^{2+}]$ ion in aqueous ethanol (1:1). The arrow indicates the trend of increasing $[Hg^{2+}]$. Excitation wavelength is 520 nm. Inset: Fluorescence enhancement at 575 nm as a function of $[Hg^{2+}]$.

spirolactam bond. It follows, therefore, that only Hg^{2+} is able to cleave the spirolactam bond. As a result of this cleavage, the Hg^{2+} complex turns pink from the initial pale yellow color of the dye and can be easily detected by the naked eye.

Emission studies

The metal free RPDR-1 is non-fluorescent in nature in aqueous ethanol (1:1) medium. Upon addition of ~ 5 equivalent of $Hg(ClO_4)_2$, the color changes to pink which can be observed with naked eye for a $10^{-3} M$ solution. In presence of Hg^{2+} ion, a significant emission (quantum yield, $\phi = 0.51$) at

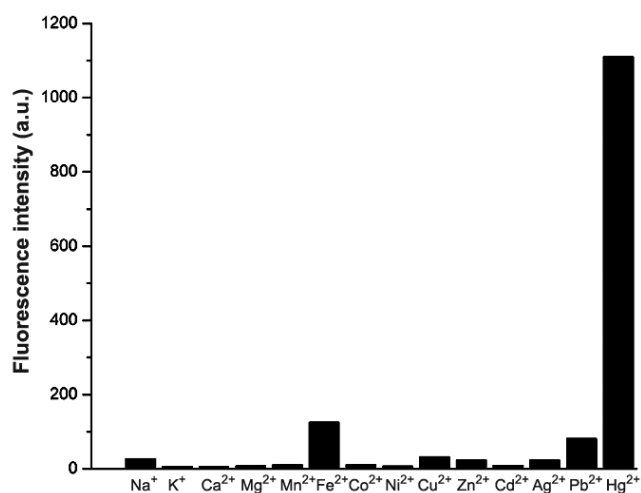


Fig. 3—Fluorescence enhancement of RPDR-1 (conc. $\sim 10^{-5} M$) in presence of different metal ions.

575 nm is observed. Other metal perchlorate salts show only negligible emission (Fig. 2). A competition experiment with biologically relevant alkali, alkaline earth, transition and heavy metal ions reveals that the dye is sensitive only to Hg^{2+} ion (Fig. 3). It is found that the fluorogenic response towards Hg^{2+} ion remains largely unaffected in presence of 10 equivalents of any of the biologically relevant metal ions in the background.

The fluorescence titration was carried out in aqueous ethanol (1:1) to evaluate the binding mode of Hg^{2+} ion with the dye. The experiment was carried out by gradual addition of $Hg(ClO_4)_2$ in equivalent amounts to the dye solution ($10^{-5} M$) and the fluorescence measured using the excitation wavelength of 520 nm. The association constant for Hg^{2+} is estimated to be 1.3×10^9 assuming 1:2 stoichiometry (Fig. 4). The 1:2 stoichiometry for the Hg^{2+} complex of the dye is further supported by the ESI-MS results¹⁷. The major peak is seen at 412.02 due to $[(RPDR-1) + 2(Hg^{2+}) + 4(CH_3CN) + 2(CH_3OH)]^{4+}$ ion.

Conclusions

In conclusion, we have synthesized a new bis-rhodamine piperazine conjugate that acts as a fluorogenic sensor selectively for Hg^{2+} ion in aqueous ethanol. The binding of the metal ion to the dye can be observed as a strong color change. This system is thus potentially useful to detect mercury in trace quantities and possible pollution management. Our preliminary studies indicate that this specificity is compromised when dry acetonitrile is used as the solvent instead of aqueous ethanol. It is known that

nature of solvent can influence spirolactum ring-opening process. It is imperative to probe different solvents for the ring opening process and we are presently working along these lines.

Supplementary Data

Supplementary data, viz., characterization data of the dye RPDR-1 and (2) including ^1H -NMR, ^{13}C -NMR and ESI-MS (Figs S1-S7) associated with this article may be obtained from the corresponding author on request.

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