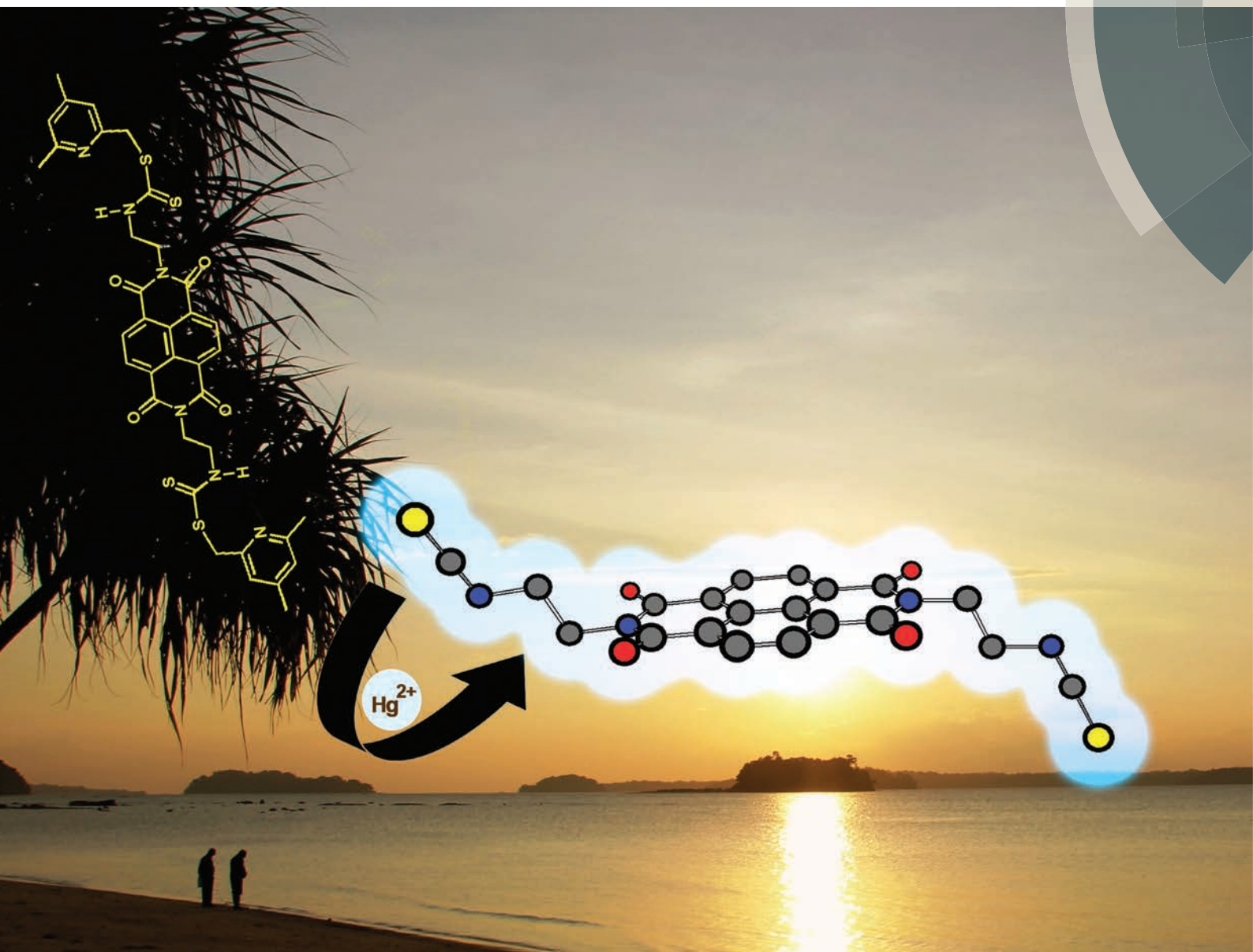


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A highly selective chemodosimeter for fast detection and intracellular imaging of Hg^{2+} ions based on a dithiocarbamate–isothiocyanate conversion in aqueous ethanol

A highly selective chemodosimeter for fast detection and intracellular imaging of Hg²⁺ ions based on a dithiocarbamate–isothiocyanate conversion in aqueous ethanol†

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A new naphthalene diimide–dithiocarbamate based fluorescence probe was synthesized and its fluorogenic behavior towards various metal ions was studied. Upon addition of various metal ions, the probe afforded an irreversible change only with Hg²⁺ ions in aqueous-ethanol media (4 : 1 v/v) with a fourfold enhancement of the fluorescence ($\Phi = 0.03 \rightarrow 0.11$) along with a distinct 43 nm blue shift of the emission maxima. The mechanism of the chemodosimetric behavior of the probe has been attributed to a Hg²⁺ induced transformation of a weakly fluorescent dithiocarbamate to a highly fluorescent isothiocyanate which has been characterized by a number of spectroscopic techniques and a crystal structure. Intracellular detection of Hg²⁺ ions was achieved using the probe.

Introduction

The development of selective chemosensors for the detection of toxic heavy-metal ions draws particular interest because of the wide use of these metal ions and their important impact on the environment.¹ Among them, mercury is one of the most prevalent toxic pollutants in the environment because it is widely distributed in water and soil² and subsequently bioaccumulates in mammals through the food chain.³ Both the elemental form and the salt are converted to highly potent neurotoxin methyl mercury by certain anaerobic bacteria. Besides, even a very low concentration of mercury ions causes a variety of diseases and affects the central nervous system which is reflected in symptoms such as tremors, vision problems, headache, hearing and memory impairments.⁴ Despite its toxicity, mercury and its salts are still extensively used in industrial processes.⁵

Analytical techniques such as atomic absorption spectrometry (AAS), inductively coupled plasma-mass spectrometry (ICPMS), inductively coupled plasma-atomic emission spectrophotometry (ICP-AES) and highly sensitive electrochemical techniques such as ion-selective potentiometry offer unparalleled sensitivity.^{6,7} However, they bear several disadvantages

such as high instrument cost, a complicated procedure for sample preparation, and different modes for different charged states of an element. Therefore, detection of low concentrations of Hg²⁺ with simple spectrometric techniques is crucial. Lippard^{1b} and later, Kim⁸ have reviewed optical sensors for mercury ions. Among the various spectroscopic methods, use of fluorescent techniques for the detection of Hg²⁺ ions offers numerous advantages in terms of high sensitivity, selectivity, rapid response time, low cost and simple operation procedure.⁹ Most small-molecule based fluorescent detection relies on changes in the signal resulting from metal-ligand binding events.

In contrast, the chemodosimeter approach involves an irreversible chemical transformation between the dosimeter molecule and a specific analyte which is reflected in a cumulative response that is proportional to the concentration of the analyte.¹⁰ This approach has become an important strategy for fluorescence detection of metal ions owing to its high selectivity and sensitivity.¹¹ Recently Li and coworkers have written a comprehensive review on chemodosimeters for Hg²⁺ ions.¹² There are reports of development of chemodosimeters that are based on Hg²⁺-promoted desulfurization reactions, including cyclizations,¹³ hydrolysis,¹⁴ eliminations,¹⁵ decomposition,¹⁶ ring opening of spiro systems¹⁷ and conversion of thiocarbonyl compounds to their carbonyl analogues.¹⁸ However, often long reaction times and elevated temperatures are required for the desulfurization processes. Besides, in certain cases, less thiophilic metal ions such as Ag⁺ and Pb²⁺ can also mediate desulfurization.^{14f}

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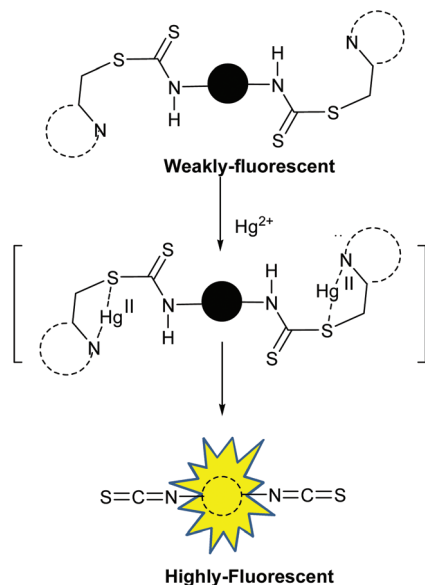


Fig. 1 Working principle of chemodosimeter **1**: Hg^{2+} induced transformation of a weakly emissive dithiocarbamate to a highly fluorescent isothiocyanate.

Recently, we have reported several dithiocarbamate-based fluorescent receptors for the detection and imaging of various heavy metal ions.¹⁹ Here, we report a compound **1** bearing collidine units (*i.e.*, 2,4,6-trimethylpyridine) attached *via* dithiocarbamate linkers with two ethylene diamine spacers at the two ends of a naphthalene diimide (NDI) fluorophore for the detection of Hg^{2+} . NDI based systems possess a large extinction coefficient in the visible range of absorption, high fluorescence quantum yield, and long absorption and emission wavelengths.²⁰ Compound **1** in response to Hg^{2+} ions shows a 43 nm blue shift in the emission maxima and a fourfold enhancement of fluorescence (quantum yield, Φ 0.03 \rightarrow 0.11). We have investigated the mechanism of the enhancement of fluorescence by carefully following the events after the addition of Hg^{2+} ions to compound **1** and attributed it to a unique Hg^{2+} promoted reaction of isothiocyanate formation from the dithiocarbamate unit (Fig. 1). To the best of our knowledge, this is the first example of a chemodosimeter that exploits the formation of an isothiocyanate from a thiocarbamate. The molecule was also used for bioimaging of Hg^{2+} ions in cells.

Experimental section

Synthesis

Compounds **2** and **3** were prepared following reported methods.^{20a}

Compound 4

NBS (1.76 g, 9.86 mmol) and AIBN (0.05 g) were added to 2,4,6-trimethylpyridine (1.0 g, 8.21 mmol) in carbon tetrachloride (100 mL) under a N_2 atmosphere. The reaction mixture was

stirred for 8 h at 85 °C and cooled to room temperature. The reaction mixture was concentrated under reduced pressure, which on chromatography (hexane–ethyl acetate, 98 : 2, v/v) yielded compound **4** (0.68 g, 40%) as a white oily liquid.²¹ ^1H NMR (400 MHz, CDCl_3) δ : 7.07 (s, 1H, ArH), 6.89 (s, 1H, ArH), 4.38 (s, 2H, CH_2), 2.50 (s, 3H, CH_3), 2.30 (s, 3H, CH_3). ^{13}C NMR (100 MHz, CDCl_3 , ppm) δ : 156.9, 154.6, 147.1, 122.4, 120.2, 32.9, 22.9, 19.5. ESI-MS m/z calc. $[\text{C}_8\text{H}_{10}\text{BrNH}]^+$, 200.0, found 200.1.

Compound 1

Compound **3** (0.20 g, 0.346 mmol), potassium carbonate (0.19 g, 1.38 mmol) and carbon disulfide (1.2 mL, 18.0 mmol) in dioxane–water (4 : 1, v/v, 10 mL) was stirred at room temperature for 1 h. Compound **4** (0.15 g, 0.75 mmol) in dioxane (4 mL) was added dropwise over a period of 10 minutes to the mixture and the stirring was continued for another 1 h at 25 °C. The yellowish solid thus obtained was dissolved in dichloromethane (20 mL) and washed with water. The organic layer was dried over anhydrous Na_2SO_4 and the volatiles were removed under reduced pressure. The residue on chromatography (hexane–ethylacetate, 1 : 1, v/v) yielded compound **1** (0.07 g, 40%) as a yellow solid, mp 173–175 °C. ^1H NMR (400 MHz, CDCl_3) δ : 11.92 (s, 2H, NH), 8.69 (s, 4H, ArH), 6.86 (2H, ArH), 6.85 (2H, ArH), 4.60 (t, 4H, $J = 4.3$ Hz, N- CH_2), 4.27 (q, 4H, $J = 4.5$ Hz, CH_2NH), 4.12 (s, 4H, Ar CH_2), 2.41 (s, 6H, CH_3), 2.28 (s, 6H, CH_3). ^{13}C NMR (125 MHz, CDCl_3) δ : 197.4, 163.1, 157.1, 156.8, 149.8, 130.9, 126.8, 126.6, 123.3, 121.1, 44.2, 41.22, 39.72, 23.95, 20.97. UV-vis (CHCl_3) λ_{nm} (ϵ , $\text{M}^{-1}\text{cm}^{-1}$): 358 (40 400), 378 (41 400); FT-IR (EtOH, ν/cm^{-1}): 3307, 2943, 2230, 2044, 1726, 1674, 1448, 1420, 1115. ESI-MS calc. for $\text{C}_{36}\text{H}_{34}\text{N}_6\text{O}_4\text{S}_4\text{H}^+$, 743.16, found 743.16.

Isothiocyanate 5

^1H NMR (400 MHz, CDCl_3 , ppm) δ : 8.83 (s, 4H), 4.56 (t, $J = 6.12$ Hz, 4H), 3.95 (t, $J = 6.08$ Hz, 4H). ^{13}C NMR (100 MHz, CDCl_3 , ppm) δ : 162.7, 131.5, 126.9, 126.4, 42.9, 39.5. Also see Table 1.

Crystallographic details (CCDC number 958704)

Compound **5**: Single crystals were obtained from the reaction of chemodosimeter **1** with Hg^{2+} followed by purification using preparative TLC and subsequent recrystallization from EtOH– CHCl_3 (1 : 1). The unit cell dimensions were determined by a least squares fit of 1141 machine centered reflections ($0^\circ < \theta < 20^\circ$) for chemodosimeter **1**. Thirty six standard reflections were used to check the crystal stability toward X-ray exposure, which showed no significant intensity reduction over the course of data collection. Six final cycles of refinement converged with discrepancy indices $R[F^2 > 2\sigma(F^2)] = 0.0439$ and $wR(F^2) = 0.0989$ for the chemodosimeter **1**.

Procedure for metal ion sensing

UV-vis spectroscopy. Absorption spectra of **1** (10 μM) were recorded with various metal ions Cr^{3+} , Mn^{2+} , Fe^{2+} , Fe^{3+} , Ba^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Pb^{2+} , Ag^+ , Ca^{2+} , Cd^{2+} , K^+ , Na^+ , Li^+ , and Hg^{2+}



Table 1 Crystallographic parameters for **1**

1	
Empirical formula	C ₂₀ H ₁₂ N ₄ O ₄ S ₂
F.w.	436.48
Space group	Monoclinic, <i>P2₁/c</i>
<i>a</i> (Å)	8.987(2)
<i>b</i> (Å)	13.788(3)
<i>c</i> (Å)	15.365(4)
β (°)	103.787(5)
<i>V</i> (Å ³)	1849.1(7)
<i>Z</i>	4
λ (Å)	0.71073
Crystal size (mm ³)	0.34 × 0.18 × 0.11
<i>T</i> (K)	100
μ (mm ⁻¹)	0.327
$R[F_o^2 > 2\sigma(F_o^2)]$	0.0439
$wR(F_o^2)$	0.0989
GOF	1.035
<i>W</i>	$1/[\sigma^2(F_o^2) + (0.0464P)^2 + 1.1540P]$ where $P = (F_o^2 + 2F_c^2)/3$

(20 μ M) and with increasing concentration of Hg²⁺ (0 to 50 μ M) in mixed solvent media (H₂O–EtOH, 4 : 1, v/v, with 1% DMSO as a cosolvent, 1 mM HEPES buffered at pH 7.0) at 25 °C.

Fluorescence spectroscopy. Fluorescence data of **1** (10 μ M) were recorded in mixed solvent media (H₂O–EtOH, 4 : 1, v/v, with 1% DMSO as a cosolvent, 1 mM HEPES buffered at pH 7.0) at 25 °C. The excitation wavelength was 350 nm and all excitation and emission slit widths were set at 5 nm unless otherwise indicated. The relative quantum yield of **1** was determined using quinine sulphate as a standard.

Results and discussion

The synthetic route for the chemodosimeter **1** is shown in Scheme 1. The ethylenediamine linked NDI intermediate **2** was synthesized based on a reported method by Licchelli.^{20a} Deprotection of the Boc group followed by treatment with carbon disulfide and subsequently with the bromomethyl compound **4** in dioxane–water afforded compound **1** in 40% yield (Scheme 1).

To investigate the affinity of **1** (10 μ M) for metal ions, UV-vis and fluorescence experiments were initially carried out using a variety of cations: Cr³⁺, Mn²⁺, Fe²⁺, Co²⁺, Ba²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Pb²⁺, Ag⁺, Ca²⁺, Cd²⁺, K⁺, Na⁺, Li⁺, and Al³⁺ in mixed solvent media (H₂O–EtOH, 4 : 1, v/v, with 1% DMSO as a cosolvent in

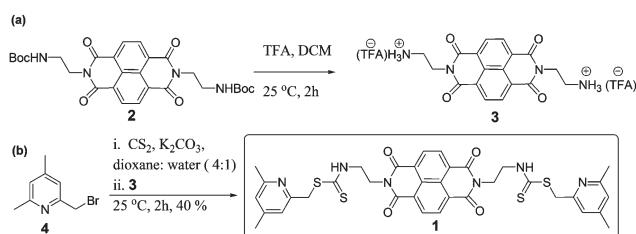
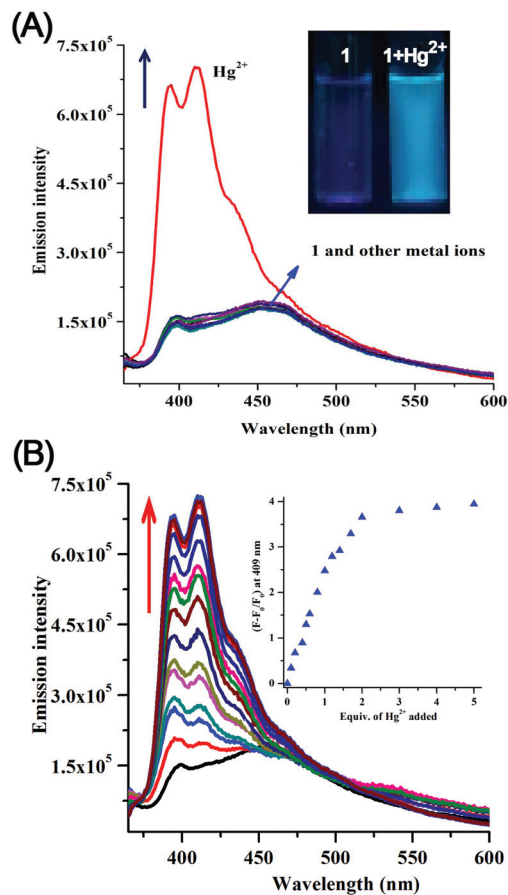
Scheme 1 Synthesis of chemodosimeter **1**.

Fig. 2 (A) Emission spectra of **1** (10 μ M) in the presence of different metal ions (50 μ M) in H₂O–EtOH (4 : 1, v/v) with 1% DMSO as a cosolvent, buffered at pH 7.0 with 1 mM HEPES at 25 °C; inset: fluorescence images under 366 nm UV light of **1** in the presence and absence of Hg²⁺; (B) emission spectra of **1** (10 μ M) in the presence of Hg²⁺ (0 to 50 μ M) in mixed solvent media; Inset: Hg²⁺ titration profile at 409 nm under the same conditions.

1 mM HEPES buffered at pH 7.0 at 25 °C). In the absence of any metal ions, compound **1** (10 μ M) displayed an absorption band centered at 358 and 378 nm. Of the sixteen metal ions mentioned above, significant spectral change was observed only with Hg²⁺ ions (Fig. S1, ESI[†]). The intensity of both the bands gradually increased upon addition up to 20 μ M of Hg²⁺ (Fig. S2, ESI[†]). The resultant solution was brightly luminescent under UV light (inset in Fig. 2A).

The fluorescence spectra of dosimeter **1** (10 μ M) upon excitation of the naphthalene diimide fluorophore ($\lambda_{\text{ex}} = 350$ nm, excitation and emission slit widths = 5 nm) displayed a weak emission band (quantum yield, $\Phi = 0.03$) at 452 nm in mixed aqueous–organic solvent media (H₂O–EtOH, 4 : 1, v/v, with 1% DMSO as a cosolvent, 1 mM HEPES buffered at pH 7.0) at 25 °C (Fig. 2A). On addition of Hg²⁺ (0–50 μ M) a fourfold enhancement ($\Phi = 0.11$) of the fluorescence intensity along with a 43 nm blue shift of emission maxima was observed (Fig. 2A).

To evaluate the specificity of Hg²⁺, the emission behaviours of **1** (10 μ M) towards various metal ions (50 μ M) such as Cr³⁺,



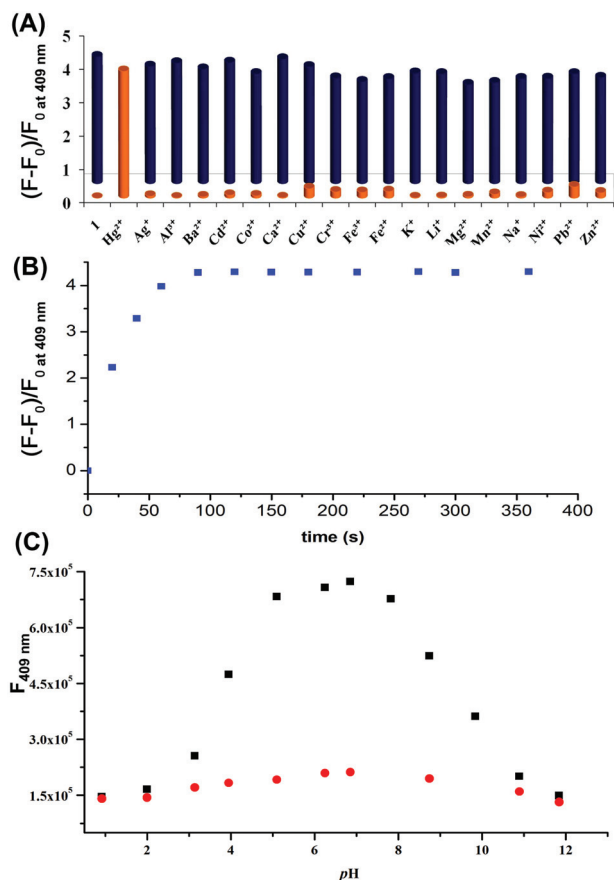


Fig. 3 (A) Orange bars: changes in fluorescence intensity of **1** (10 μM) upon addition of various metal salts (50 μM) in H_2O –EtOH (4 : 1, v/v); Blue bars: restoration of fluorescence intensity of sensor **1** (10 μM) towards Hg^{2+} (20 μM) in the presence of competitive cations (50 μM); (B) time-course analysis of the fluorescence response of Hg^{2+} with **1** at 409 nm; (C) change in the fluorescence intensity of **1** (10 μM) and **1**· Hg^{2+} at different pH at 409 nm.

Mn^{2+} , Fe^{2+} , Co^{2+} , Ba^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Pb^{2+} , Ag^+ , Ca^{2+} , Cd^{2+} , K^+ , Na^+ , Li^+ , and Al^{3+} were examined under the same conditions (H_2O –EtOH, 4 : 1, v/v). The results shown in Fig. 2A demonstrated the high specificity of compound **1** towards the detection of Hg^{2+} ions. Fluorescence titration of **1** (10 μM) was performed with various concentrations of Hg^{2+} (Fig. 2B). The plot of fluorescence intensity of **1** at 409 nm linearly increased up to the addition of 2 equiv. of Hg^{2+} . From the fluorescence titration plot, the detection limit²² of **1** for the analysis of Hg^{2+} in mixed aqueous–organic medium was estimated to be 2.1×10^{-7} (M) (Fig. S3, ESI[†]).

Up to this point it was thought that the changes in the absorption and fluorescence spectra were the consequences of a metal–ligand binding event as seen previously with many of our thiocarbamate based sensors.¹⁹ However, when we tried the reversibility experiments using metal ion chelators, it was realized that the change on addition of the Hg^{2+} ion was irreversible. Two metal ion chelators, EDTA and 8-hydroxyquinoline (100 μM each), were added separately to solutions containing compound **1** (10 μM) and $\text{Hg}(\text{NO}_3)_2$ (20 μM) in the

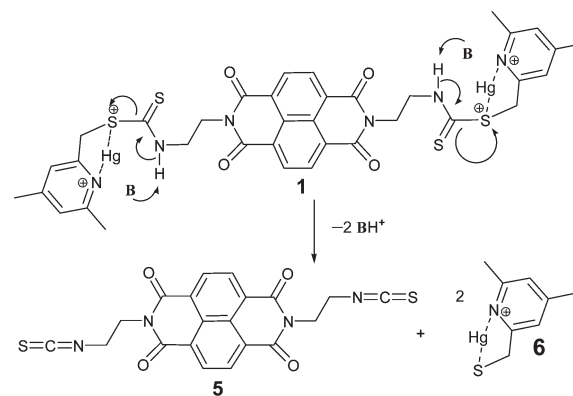


Fig. 4 Schematic representation of mechanistic insight of chemodosimeter **1**.

mixed solvent media buffered at pH 7.0 at 25 °C. The recorded fluorescence spectra revealed that none of the chelators changed the fluorescence intensity of the solutions (Fig. 6). Additionally, fluorescence enhancement was not observed when a mixture of Hg^{2+} and 8-hydroxyquinoline solution was added to a solution of **1**. These results indicated that the binding process of Hg^{2+} with **1** was irreversible.

Thus we started suspecting that the sensor molecule was reacting in the presence of Hg^{2+} . Thin layer chromatography (TLC) of the chemosensing mixture (**1** with Hg^{2+}) showed a new, highly fluorescent spot having less polarity than the original ligand. This was counterintuitive since the charged complex was expected to be more polar. The spot was separated and single crystals were obtained on slow evaporation from a mixture of EtOH– CH_2Cl_2 (1 : 1 v/v) at room temperature.

The crystal structure revealed the formation of an isothiocyanate from the thiocarbamate **1** (Fig. 5, compound 5). The lost fragment of the reaction was observed in mass spectrometric analysis. A peak corresponding to the formation of Hg -complex with the collidine unit was observed at $m/z = 372$ (Fig. S9, ESI[†]). All these lines of evidence helped us to gain the mechanistic insight into the formation of 5 from the chemodosimeter **1**. The proposed mechanism of the fluorescence enhancement is shown in Fig. 4. Thus compound **1** is a chemodosimeter for Hg^{2+} ions. Formation of isothiocyanate from a dithiocarbamic acid salt has been previously reported in the literature.²³

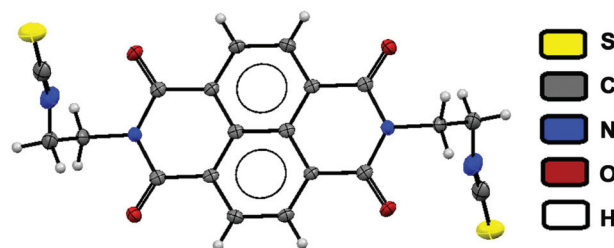


Fig. 5 Crystal structure of isothiocyanate analogies obtained on reaction between chemodosimeter **1** and Hg^{2+} in mixed media. (Thermal ellipsoids were drawn at the 50% probability level.)



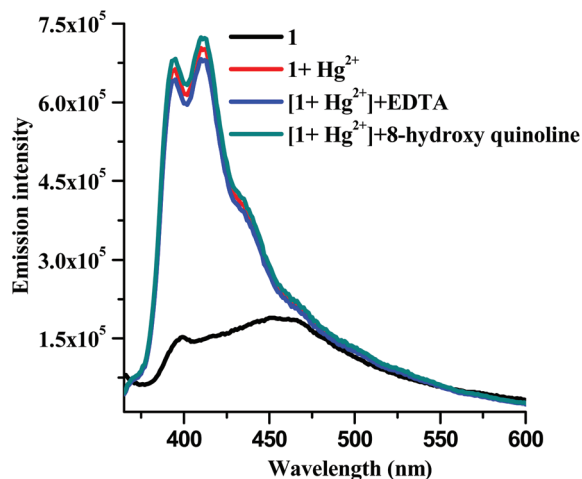


Fig. 6 Fluorescence spectral studies for establishing the irreversibility in binding of **1** to Hg^{2+} in mixed solvent media.

To evaluate the efficacy of dosimeter **1** for practical use as a Hg^{2+} selective probe in the presence of other coexisting metal ions, competitive experiments were carried out with **1** ($10\ \mu\text{M}$) and Cr^{3+} , Mn^{2+} , Fe^{2+} , Ba^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Pb^{2+} , Ag^+ , Ca^{2+} , Cd^{2+} , K^+ , Na^+ , Li^+ and Al^{3+} ($50\ \mu\text{M}$) in H_2O - EtOH (4 : 1, v/v), with 1% DMSO as a cosolvent, buffered at pH 7.0 with HEPES at $25\ ^\circ\text{C}$, and Hg^{2+} ($20\ \mu\text{M}$) was subsequently added to it. The fluorescence intensity of the solutions at 409 nm shown in Fig. 3A (blue bars) clearly demonstrated that probe **1** can be used for the detection of Hg^{2+} without interference by other metal ions.

The fluorescence spectra of **1** in the presence of Hg^{2+} were examined over a wide range of pH from 1 to 12 (Fig. 3C). The recorded emission spectra revealed that probe **1** can effectively detect the Hg^{2+} ions at a biologically relevant pH 4.5 to 9.

Longer reaction times with chemodosimeters can be problematic for real-time analysis of Hg^{2+} .^{15b,20c,24} The time for the fluorescence response to Hg^{2+} with dosimeter **1** was also investigated. The fluorescence spectra of a solution containing **1** with 2 equivalents of Hg^{2+} were recorded at various time intervals. The plot of fluorescence intensity of **1** at 409 nm against time revealed that the reaction was fast and was completed within a minute (Fig. 3B). These results encouraged us to study chemodosimeter **1** for the real time intracellular monitoring of Hg^{2+} .

Chemodosimeter **1** was used for intracellular fluorescence imaging of Hg^{2+} ions using an epifluorescence microscope (Fig. 7). Human epithelial carcinoma cells, HeLa S3, were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 100 units mL^{-1} penicillin, and $100\ \mu\text{g}\ \text{mL}^{-1}$ streptomycin, at $37\ ^\circ\text{C}$ and 5% CO_2 . The cells were plated on 12 mm cover slips and were allowed to adhere for 24 h. The culture cell lines were exposed to $\text{Hg}(\text{NO}_3)_2$ ($10\ \mu\text{M}$) in DMEM for 1 h at $37\ ^\circ\text{C}$ and were subsequently washed with PBS to remove the excess Hg^{2+} ions. It was subsequently incubated with **1** ($10\ \mu\text{M}$ in DMSO) in PBS

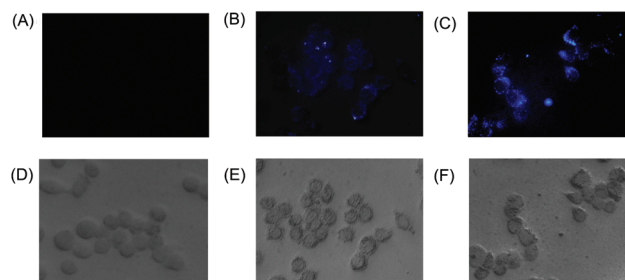


Fig. 7 Fluorescence microscope image of the HeLa S3; (A) only cells; (B) cells incubated with **1** ($10\ \mu\text{M}$); (C) cells exposed to both Hg^{2+} ($10\ \mu\text{M}$) and **1** ($10\ \mu\text{M}$); (D–F) the corresponding bright field images.

for 10 min at $25\ ^\circ\text{C}$, followed by washing the cells using PBS to remove the residual dye from the cells. The cells incubated with only **1** displayed a weak intracellular fluorescence (Fig. 7B), whereas the cells treated with both Hg^{2+} and **1** showed a bright intracellular fluorescence (Fig. 7C).

Conclusion

In conclusion, we have reported a novel chemodosimeter that detects Hg^{2+} with *ca.* fourfold enhancement of the fluorescence intensity and a 43 nm blue shift of the emission maxima. The sensor displayed a high selectivity for mercury ions in aqueous-ethanol media (4 : 1 v/v). The analytical detection limit of the sensor is $2.1 \times 10^{-7}\ \text{M}$. Probing of Hg^{2+} ions in cells is also possible using the dosimeter exploiting its high cell permeability and the subsequent formation of the isothiocyanate in the presence of intracellular Hg^{2+} ions.

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