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Hg^{2+}-selective dual signaling probe based on a thio-functionalized rhodamine B hydroxamic acid

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ABSTRACT

A new Hg^{2+}-selective signaling system based on a thio-functionalized rhodamine B hydroxamic acid was investigated. A thio-analogue of rhodamine B hydroxamic acid had a distinct Hg^{2+}-selective signaling behavior in aqueous solution, whereas its parent hydroxamic acid was completely unreactive toward any metal ions. Selective chromogenic and fluorogenic signaling of Hg^{2+} by the thio-functionalized rhodamine B hydroxamic acid in aqueous solution was possible with a detection limit of 1.0×10^{-6} M. Hg^{2+} determination based on paper test strips was conducted as a practical application.

The development of smart sensors or probes for the selective sensing and visualization of important metal ions has attracted much research interest,[1] and many elaborate chemosensors based on fluorescein, rhodamine, and other functional dyes are constantly being designed for this purpose. Among the various molecular platforms used for the design of sensing materials, rhodamine fluorophores are particularly widely used because of their favorable optical properties.[2] In particular, hydroxamic acid derivatives of rhodamine have recently attracted much research interest. Rhodamine hydroxamic acids and their derivatives have exhibited interesting signaling ability toward Fe^{3+},[3] Cu^{2+},[4] hypochlorous acid,[5] and volatile acidic gases.[6] Rhodamine hydroxamic acid derivatives that are tethered to an alkyne or triazole moiety have been developed for the detection of Au^{3+} or Pt^{2+}.[7,8] Yang et al. reported that rhodamine hydroxamic acid with a 2-deoxyribose moiety could selectively detect cysteine and homocysteine in water.[9] Additionally, the Lossen rearrangement of rhodamine hydroxamic acid was applied to the detection of a nerve agent simulant, diethyl chlorophosphate.[10] However, despite the potential Hg^{2+} sensing ability of hydroxamic acid derivatives, no studies regarding Hg^{2+}-signaling have yet been conducted.

The replacement of an oxygen atom with a sulfur atom in a specific ligand system is often a powerful tool for the fine control of the metal affinity of the ligands.[11] Previously, the thionation of calix[4]arene-based amide derivatives was used to target thiophilic metal ions, such as Hg^{2+} and Cd^{2+}.[12] Simple rhodamine fluorophores have been transformed into thiolactones by thionation to change them into Hg^{2+}-selective sensors.[13] Switching the recognition preference of rhodamine B spirolactam by replacing one atom resulted in the design of rhodamine B thiohydrazide for the recognition of Hg^{2+} in aqueous solution.[14]

However, the thio-analogues of many dyes have been used as chemodosimeters for the signaling of many important species, such as Hg^{2+}, Cu^{2+}, and various oxidants. In particular, thio-functionalized chromophores and fluorophores are the basis of the design of desulfurization-based chemodosimeters, which exploit the thiophilicity of Hg^{2+} and its critical role in desulfurization reactions.[15] Since the development of Czarnik's anthracene-based thioamide,[11a] many probes have been developed using similar Hg^{2+}-induced desulfurizations of thiocarbonyl functional groups, such as thiocoumarins,[16] thioureas,[17] and 8-hydroxyquinoline-based thioamide derivatives.[18] In addition, many chemodosimeters based on the sequential reactions of Hg^{2+}-induced desulfurization followed by cyclization have also been reported.[19]

Hg^{2+} signaling is crucial from both an industrial and ecological viewpoint because of its high impact on the environment.[20] Many intricate sensors and reaction-based probes are being continuously developed for the signaling of this important species.[21] Among the many signaling motifs, rhodamine derived reaction-based probes have been particularly successful and attractive because of their marked chromogenic and fluorogenic responses

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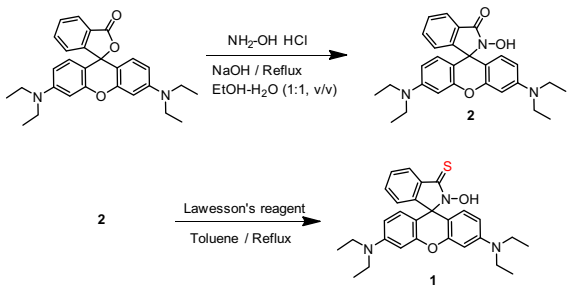
toward Hg^{2+} species.[22] A number of derivatives based on rhodamine hydrazide frameworks with an extra binding site have been reported for Hg^{2+} signaling.[23] However, rhodamine hydroxamic acid, which is another important rhodamine lactam platform, has not been tested because of its relative inertness toward most metal ions. We anticipated that the transformation of hydroxamic acid functionality into its thio-analogue by thionation would change the molecule into a probe with metal ion signaling responses.

Herein, we developed a new Hg^{2+}-selective probe by controlling the metal ion affinity of rhodamine hydroxamic acid by converting it into its thio-functionalized analogue. Through the introduction of a thio functional group into the hydroxamic acid, we obtained a new rhodamine-based probe with improved responses and marked sensitivity toward Hg^{2+} ions.

The thio derivative of rhodamine hydroxamic acid, **1**, was prepared by the thionation of hydroxamic acid, **2**, which was obtained using a literature procedure consisting of the reaction of rhodamine B base with hydroxylamine (NaOH),[10] using Lawesson's reagent (65%) (Scheme 1).[24] In fact, the thio-analogue of rhodamine hydroxamic acid was known, but its signaling behavior toward any analytes has not been studied.[14] Compound **1** has no absorption bands above 480 nm and very weak emissions because it is in its ring-closed thiolactam form (quantum yield $\Phi_{\mathbf{1}} = 0.0002$).[25]



Scheme 1. Synthesis of rhodamine B thiohydroxamic acid **1**.

The metal ion signaling behavior of **1** was preliminarily studied using UV–vis spectroscopy in a mixed solution of aqueous acetate buffer (pH 4.8) and DMSO (1:1, v/v). Hydroxamic acid **2** did not induce any changes in absorption behavior toward any of the surveyed representative alkali (Na^+, K^+), alkaline earth (Mg^{2+}, Ca^{2+}), or transition metal ions (Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, Pb^{2+}, Hg^{2+}). However, the thio-analogue, **1**, exhibited a prominent Hg^{2+}-selective signaling behavior under the same experimental conditions (Figs. 1 and S1, Supplementary data). The UV–vis spectrum of **1** revealed an intense absorption band at 574 nm exclusively in the presence of Hg^{2+} ions. This absorbance enhancement was very large (the absorbance ratio in the presence and absence of metal ions, A/A_0 at 574 nm = 550) because of the characteristic opening of the spirolactam moiety of rhodamine **1**. The other metal ions induced almost no response (A/A_0 ranged from 0.1 to 3.2). In particular, the interference of other thiophilic metal ions, including Ag^+ and Cu^{2+} was insignificant (the absorbance ratio in the presence and absence of metal ions at 574 nm, A/A_0, was 19 for Cu^{2+} and 5.5 for Ag^+).

The fluorescence signaling behaviors of **1** and **2** were measured in the same 50% aqueous DMSO solution. The parent hydroxamic acid **2** did not respond to any surveyed metal ions (Fig. S2, Supplementary data). In contrast, a prominent Hg^{2+}-selective fluorescence signaling was observed for the thio-analogue **1** (Figs. 2, and S3, Supplementary data). The fluorescence enhancement at 600 nm was larger than 950 fold ($\Phi_{\mathbf{1}+Hg(II)} = 0.11$).[25] The other metal ions only had minor responses and the fluorescence intensity ratios in the presence and absence of metal ions I/I_0 of **1** at 600 nm varied within a narrow range between 0.8 for Na^+ and 12 for Ag^+ ions.

The possible interference of the Hg^{2+} signaling of **1** by the presence of coexisting metal ions was measured. The Hg^{2+}-selective signaling behavior of **1** was not strongly affected by the presence of other commonly encountered metal ions (Fig. 3). The variation of fluorescence intensity of the **1**–Hg^{2+} system at 600 nm in the presence and absence of coexisting metal ions $I_{(\mathbf{1}+\text{Metal ions}+Hg(II))}/I_{(\mathbf{1}+Hg(II))}$ under competitive conditions ([**1**]:[Hg^{2+}]:[M^{n+}] = 1:50:100) fluctuated only within a narrow range between 0.86 for Ag^+ and 0.97 for Li^+ ions.

The Hg^{2+}-selective signaling resulted from the Hg^{2+}-induced transformation of the hydroxythiolactam moiety of **1** into a nitrile (Scheme 2). Similar transformation of Hg^{2+}-induced conversion of rhodamine-thiolactam to rhodamine nitrile and hypochlorous acid-promoted oxidative transformation of 7-hydroxycoumarin-based oxime to its nitrile analogue has been reported.[26,27] Signaling is believed to proceed via initial formation of ring-opened thiohydroxamic acid that subsequently converted to fluorescent cyanide derivative with the concomitant elimination of HgS and water molecule. The suggested transformation was supported by NMR, IR, and mass spectrum measurements. The mass spectrum obtained for the signaling product of **1** in the presence of 10 equiv of Hg^{2+} ions revealed a diagnostic peak at $m/z = 424.5$ corresponding to nitrile **3** ($m/z = 424.2$ calculated for $C_{28}H_{30}N_3O$) (Fig. S4, Supplementary data). IR measurements revealed a characteristic nitrile band at 2233 cm^{-1} for **3** (Fig. S5, Supplementary

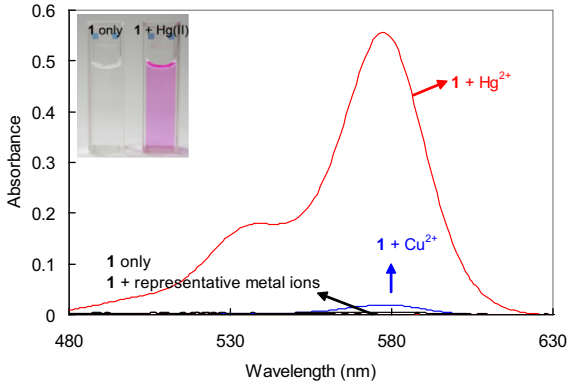


Figure 1. Changes in the UV–vis spectrum of **1** in the presence of various metal ions. [**1**] = 5.0×10^{-6} M and [M^{n+}] = 5.0×10^{-4} M. In acetate buffered (pH 4.8, final concentration = 10 mM) 50% aqueous DMSO solution.

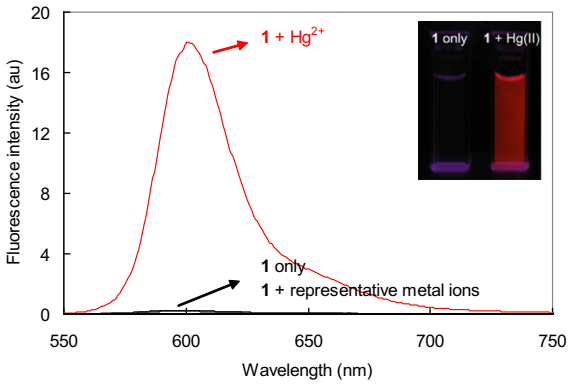


Figure 2. Changes in the fluorescence spectrum of **1** in the presence of various metal ions. [**1**] = 5.0×10^{-6} M and [M^{n+}] = 5.0×10^{-4} M. In acetate buffered (pH 4.8, final concentration = 10 mM) 50% aqueous DMSO solution. λ_{ex} = 540 nm.

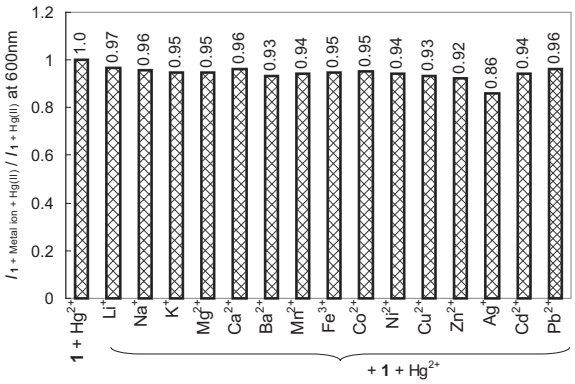
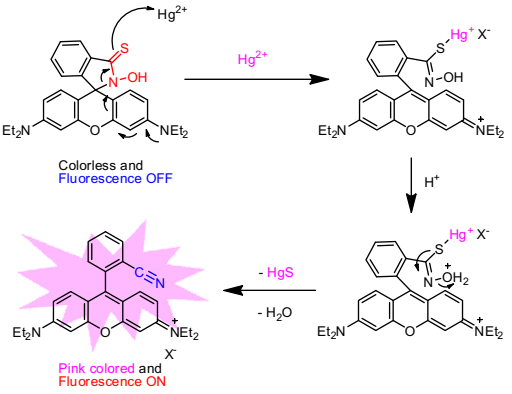


Figure 3. Competitive fluorescence signaling of Hg^{2+} by **1** in the presence of commonly coexisting metal ions. [**1**] = 5.0×10^{-6} M, [Hg^{2+}] = 2.5×10^{-4} M, and [M^{n+}] = 5.0×10^{-4} M. In acetate buffered (pH 4.8, 10 mM) 50% aqueous DMSO solution. λ_{ex} = 540 nm. The fluorescence intensity was measured at 600 nm.



Scheme 2. Plausible signaling mechanism of Hg^{2+} ions by probe **1**.

data). The ^1H and ^{13}C NMR spectra of the Hg^{2+} signaling product of **1** showed resonances that were expected for the postulated structure of nitrile **3** (Figs. 4 and S6, Supplementary data). The signaling was irreversible as demonstrated by the effect of the addition of EDTA to the **1**–Hg^{2+} signaling system (Fig. S7, Supplementary data). Upon treatment of **1** with 100 equiv of Hg^{2+} ions, the aforementioned chromogenic and fluorogenic signals were observed. However, upon treatment of the resulting solution with EDTA (2 equiv with respect to the added Hg^{2+}), the observed signals were not affected. This observation implied that the signaling of the **1**–Hg^{2+} system is an irreversible process.

The quantitative signaling behavior of **1** for Hg^{2+} ions was assessed using a fluorescence titration (Fig. 5). The fluorescence spectrum of **1** steadily increased as the concentration of Hg^{2+} increased up to 1.0×10^{-4} M. Using this concentration dependent absorption change, the detection limit for the determination of Hg^{2+} by **1** was estimated to be 1.0×10^{-6} M.[28]

The Hg^{2+} signaling of **1** was found to be pH dependent (Fig. S8, Supplementary data). As the pH of the solution increased from 4.8 to 8.0, the Hg^{2+} signaling slowly decreased, and after pH 8.0 no detectable signal was observed. However, the spectral change of **1** alone was not observed over the entire pH region from 4.8 to 9.0. This profile may be due to the involvement of protons in the postulated Hg^{2+} signaling mechanism (Scheme 2). At high pH region, the concentration of proton which is crucial for the activation of thiohydroxamic acid function is low that is unfavorable for the transformation to nitrile. From this observation, we confirmed that the Hg^{2+} signaling of **1** was relatively optimized using an acetate buffer at pH 4.8. On the other hand, the Hg^{2+}-selective signaling of **1** was complete within 5 min as monitored by a time trace plot

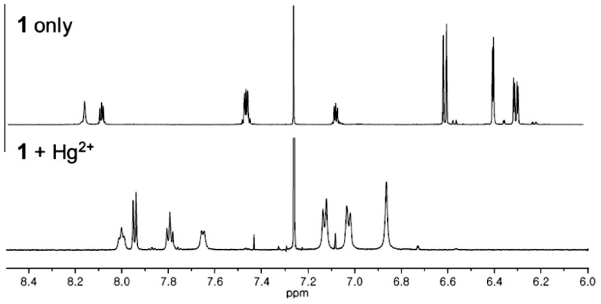


Figure 4. Partial ^1H NMR spectra of **1** and signaling product of **1** with Hg^{2+} in $CDCl_3$. [**1**] = 10 mM and [Hg^{2+}] = 50 mM. In acetate buffered (pH 4.8, 10 mM) 50% aqueous acetonitrile solution. The signaling mixture was extracted using dichloromethane and was purified by column chromatography.

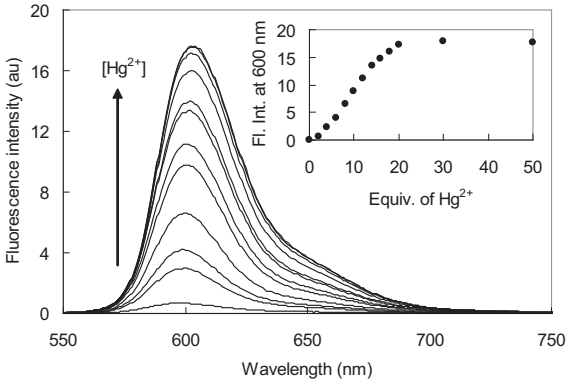


Figure 5. Fluorescence titration of **1** with Hg^{2+}. [**1**] = 5.0×10^{-6} M. In acetate buffered (pH 4.8, final concentration = 10 mM) 50% aqueous DMSO solution. The inset shows the fluorescence intensity enhancement at 600 nm as a function of [Hg^{2+}]. λ_{ex} = 540 nm.

obtained using fluorescence measurements (Fig. S9, Supplementary data). Compound **1** itself was stable and no noticeable changes in fluorescence were observed even 5 h after sample preparation.

Finally, the application of Hg^{2+}-selective signaling behavior of **1** was tested using test strip experiments. Test strips were prepared by impregnating filter paper (Whatman No. 2) with an acetonitrile solution of **1**. Upon treatment of the test strips with increasing amount of Hg^{2+}, distinct pink-colored spots were observed (Fig. 6). The color of the developed spots was analyzed using Adobe Photoshop (Figs. S10 and S11, Supplementary data). As shown in Figures S10 and S11, the developed color was well correlated to the [Hg^{2+}] of the analytes. This observation suggested that the test strips could be used for the rapid and semi-quantitative visualization of Hg^{2+} in aqueous environments.

In summary, a new Hg^{2+}-selective optical probe was developed through the thionation of rhodamine hydroxamic acid. The thiohydroxamic acid derivative had a pronounced dual signaling behavior

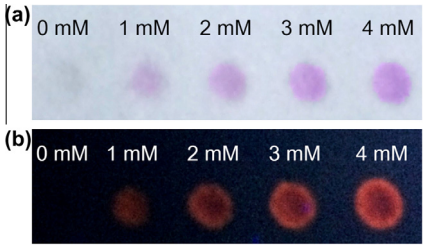


Figure 6. Pictures of the developed spots of **1** on test strips under (a) daylight and (b) UV light illumination in the presence of varying [Hg^{2+}]. [Hg^{2+}] = 0–4.0×10^{-3} M.

toward Hg^{2+} in the presence of other metal ions, which included thiophilic Cu^{2+} and Ag^{+} ions. Tuning the structure of rhodamine hydroxamic acid by thionation of the hydroxamic acid lactam moiety resulted in a remarkably selective and effective signaling behavior. The reported results provide useful information for the development of Hg^{2+} probes by fine-tuning probe structures using simple thionation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.06.085. These data include MOL files and InChiKeys of the most important compounds described in this article.

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