Rhodamine-Based Fluorescent Probe for Highly Selective Determination of Hg$^{2+}$

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ABSTRACT: The determination of mercuric ions (Hg$^{2+}$) in environmental and biological samples has attracted the attention of researchers lately. In the present work, a novel turn-on Hg$^{2+}$ fluorescent probe utilizing a rhodamine derivative had been constructed and prepared. The probe could highly sensitively and selectively sense Hg$^{2+}$. In the presence of excessive Hg$^{2+}$, the probe displayed about 52-fold fluorescence enhancement in 50% H$_2$O/CH$_3$CH$_2$OH (pH, 7.24). In the meantime, the colorless solution of the probe turned pink upon adding Hg$^{2+}$. Upon adding mercuric ions, the probe interacted with Hg$^{2+}$ and formed a 1:1 coordination complex, which had been the basis for recognizing Hg$^{2+}$. The probe displayed reversible dual colorimetric and fluorescence sensing of Hg$^{2+}$ because rhodamine’s spirolactam ring opened upon adding Hg$^{2+}$. The analytical performances of the probe for sensing Hg$^{2+}$ were also studied. When the Hg$^{2+}$ concentration was altered in the range of 8.0 × 10$^{-8}$ to 1.0 × 10$^{-5}$ mol L$^{-1}$, the fluorescence intensity showed an excellent linear correlation with Hg$^{2+}$ concentration. A detection limit of 3.0 × 10$^{-8}$ mol L$^{-1}$ had been achieved. Moreover, Hg$^{2+}$ in the water environment and A549 cells could be successfully sensed by the proposed probe.

INTRODUCTION

Recently, developing highly selective and sensitive sensors for toxic metal ions have been in demand in environmental and biological studies.$^{1-3}$ Heavy metal mercury is toxic and bio-accumulating, can be generated by both natural resources and anthropogenic activities, and ubiquitously exists in the global environment. Mercury includes elemental mercury, inorganic mercury, and organic mercury. Mercuric ions (Hg$^{2+}$) are much more common than mercurous ions (Hg$^{+}$), and low-dose Hg$^{2+}$ in the body could cause long-standing irreversible harm to the human health.$^{4,5}$ Methylmercury is a dominant form of organic mercury and could be produced through microbiological transformation of Hg$^{2+}$ in the aquatic environment. Moreover, methylmercury could bio-accumulate in the human body by biological food chain and result in a variety of illnesses including cardiovascular diseases, Minamata disease, growth retardation, dyskinesia, and so forth.$^{6-9}$ The maximum amount of Hg$^{2+}$ allowed in drinking water is 2 ppb (10$^{-9}$ mol L$^{-1}$) according to the US Environmental Protection Agency.$^{10}$ Thus, sensing Hg$^{2+}$ has high significance in environmental and medical science.

To date, many analytic approaches have been employed for sensing Hg$^{2+}$ such as high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICP-MS),$^{11}$ HPLC coupled with atomic fluorescence spectrometry (HPLC–AFS),$^{12}$ ICP-atomic emission spectrometry (ICP-AES),$^{13}$ atomic absorption spectrometry (AAS),$^{14}$ ultraviolet–visible absorption spectrometry (UV–vis),$^{15}$ fluorometry,$^{16,17}$ electrochemical methods,$^{18,19}$ and so on. Among all the methods developed, fluorometry possess remarkable advantages because of its high sensitivity, inherent simplicity, instrument operability, in situ detection, and bioimaging analysis in vivo.$^{16,17}$ Hitherto, many fluorescent probes had been constructed to determine Hg$^{2+}$.$^{20-30}$ However, some analytical performances of these probes are unsatisfactory, including the slow response time,$^{20-23}$ cross

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interference, poor sensitivity, and so forth. Therefore, novel Hg$^{2+}$ fluorescent probes are still desirable, which possess fast response speed, excellent selectivity, and sensitivity.

Rhodamine and rhodamine derivatives have been applied widely as fluorescent probes owing to their remarkable optical characteristics including a high fluorescence quantum yield ($\Phi$), a large molar extinction coefficient ($\varepsilon$), and longer excitation and emission wavelengths. So far, many fluorescent probes utilizing rhodamine derivatives had been utilized to sense different cations including Cu$^{2+}$, Pb$^{2+}$, Cr$^{3+}$, Fe$^{3+}$, Al$^{3+}$, Zn$^{2+}$, and so forth. The sensing mechanism of these probes for cations is based on the transformation from a spirocyclic form into an open cyclic structure. In the absence of cations, these probes displayed no fluorescence and colorlessness because of their spirocyclic form. After adding cations, these probes emitted a powerful fluorescence and showed a pink color because the cyclic structure of the spirolactam or spirolactone was opened through a reversible complexation or nonreversible chemical interaction. The above sensing mechanism has also been applied to construct rhodamine-based Hg$^{2+}$ fluorescent probes. However, there are still more or less limitations for some of these Hg$^{2+}$ fluorescent probes including the delayed response, a narrow pH range, and cross interference. Thus, developing unusual rhodamine-based fluorescent probes for Hg$^{2+}$ is still very important.

Herein, a new Hg$^{2+}$ fluorescent probe 1 (Scheme 1) had been constructed, which chose rhodamine as the fluorophore. Because sulfur had strong affinity for Hg$^{2+}$, we introduced a sulfur-based functional unit to the probe in the present article. To prepare an optical chemical sensor (optode) for Hg$^{2+}$ in the next work, a terminal double bond was included in probe 1 to allow the probe to covalently immobilize on the activated surface of glass slides with the double bond by UV irradiation. When Hg$^{2+}$ was absent, the probe existed in a spirocyclic form and exhibited no fluorescence and colorlessness. When Hg$^{2+}$ was present, the probe emitted yellowish-red fluorescence, and the solution color changed to pink. The Hg$^{2+}$ probe exhibited excellent sensing performances including a fast response time, excellent sensitivity and selectivity, a broad pH working range, and so forth. In addition, nearly no cytotoxic reaction was found, and the probe could be successfully utilized to image Hg$^{2+}$ in A549 cells. Besides, the developed probe was also magnificently utilized to monitor Hg$^{2+}$ in water environments.

![Scheme 1. Preparation of Fluorescent Probe 1: (a) Hydrazine Monohydrate (85%), CH$_3$CH$_2$OH, Reflux, 12 h, 86%; (b) Lawesson’s Reagent, Toluene, Reflux, 4 h, 17%; (c) Allyl Bromide, Sodium Bicarbonate, Dormyl Dimethylamine, 70 °C, 48 h, 61%; and (d) CH$_3$CH$_2$OH, Reflux, 12 h, 75%](https://doi.org/10.1021/acsomega.2c03336)
RESULTS AND DISCUSSION

Spectral Characteristics of Probe 1. Figure 1 shows a graph depicting the variation in the fluorescence spectrum of probe 1 after adding different concentrations of mercury ions in Tris–HCl buffer (CH₃CH₂OH/H₂O, 1:1, v/v, pH 7.24). As illustrated in Figure 1, probe 1 emitted a very weak fluorescence when Hg²⁺ was not added. As the concentration of Hg²⁺ was increased, probe 1 exhibited enhanced fluorescence. When excess Hg²⁺ was added, the probe demonstrated a 52 times fluorescence increase at 586 nm. The experiment was based on these results to determine the concentration of mercury ions.

In order to explore the sensing mechanism of probe 1 toward Hg²⁺, the variation of the absorption spectrum of probe 1 with the gradual addition of mercury ions was recorded (Figure 2). As demonstrated in Figure 2, the UV−visible absorption spectrum of probe 1 showed a weak absorbance at 565 nm before the addition of mercury ions, which could be ascribed to the presence of the partial ring-opened structure of the spirolactam unit of probe 1. With the increment of Hg²⁺, probe 1 showed a gradual increase in absorption at 565 nm. When the Hg²⁺ concentration was 50 μM, the absorbance reached the maximum value. Meanwhile, the solution color change of probe 1 from colorless to pink after the addition of Hg²⁺ could be seen by the naked eye. The possible reason for absorption intensity enhancement at 565 nm was that the ring of the spirolactam structure of probe 1 opened upon the addition of mercury ions. The UV−visible absorption spectroscopy results also indicated that probe 1 interacted with Hg²⁺. Furthermore, a chemical shift of 64.23 ppm in the ¹³C NMR spectrum of probe 1 corresponded to the characteristic chemical shift value of the spirocyclic carbon, which also indicated that probe 1 existed in the spirolactam form before the addition of Hg²⁺.

Principle of Operation. To explore the linear correlation between the fluorescence intensity of probe 1 and mercury ions, the fluorescence intensity of probe 1 was studied after the addition of various concentrations of Hg²⁺. The fluorescence intensity of probe 1 at 580 nm linearly depended on the Hg²⁺ concentration when the Hg²⁺ concentration was varied from 8.0×10⁻⁸ to 1.0×10⁻⁵ mol L⁻¹ (Figure 3). The linear regression equation used was F = 25.1293 + 25.1931×10⁶×C (√(r²)= 0.9949); here, F is the fluorescence intensity, C represents the concentration of Hg²⁺, and r is the linear correlation coefficient. The detection limit was estimated to be 3.0×10⁻⁸ M based on 3S_B/m (where S_B is the standard deviation of 10 blank measurements and m is the slope of the linear regression equation),⁹⁵,⁵⁰ which was more sensitive than that of formerly developed Hg²⁺ fluorescent probes.⁹⁶−⁹⁹ Moreover, according to the UV−vis titration profile in Figure 2, the absorbance of probe 1 at 565 nm increased linearly with the change of the Hg²⁺ concentration of 8.0×10⁻⁸ to 2.0×10⁻⁵ mol L⁻¹ (Figure S1, Supporting Information). The linear regression equation used was A = 0.0569 + 0.1253×10⁶×C (r = 0.9975), where A is the absorbance, C represents the concentration of Hg²⁺, and r is the linear correlation coefficient.
Moreover, the detection limit was found to be $4.8 \times 10^{-8}$ mol L$^{-1}$ based on $3S_B/m$ (where $S_B$ is the standard deviation of 10 blank measurements and $m$ is the slope of the linear regression equation).

In addition, we also confirmed the stoichiometry of probe 1 with mercury ions by using Job’s plot (Figure 4). When the molar fraction of the amount of Hg$^{2+}$ was close to 0.5, the absorbance of probe 1 reached a maximum, indicating that probe 1 coordinated with Hg$^{2+}$ through a 1:1 stoichiometry. Based on the alteration in the absorption spectrum of probe 1 in the absence and presence of mercury ions and the coordination stoichiometry between probe 1 and Hg$^{2+}$, we proposed a possible structural model for the formation of complexes of probe 1 and mercury ions (Scheme 2). To further support the hypothesis, the NMR titration of probe 1 in the presence of Hg$^{2+}$ was carried out (Figure S2, Supporting Information). Hg$^{2+}$ is a heavy metal ion and could influence the proton signals near the Hg$^{2+}$ binding site.

**Figure 2.** UV–vis spectra of 5.0 μM probe 1 with the gradual addition of Hg$^{2+}$: 0, 0.08, 0.10, 0.20, 0.40, 0.50, 0.60, 0.80, 1.0, 2.0, 4.0, 5.0, 6.0, 8.0, 10, 20, 40, 50, 60, 80, and 100 μM from 1 to 21. Inset: the variation of absorbance of 5.0 μM probe 1 with Hg$^{2+}$ concentration.

**Figure 3.** Calibration curve between the fluorescence intensity of 5.0 μM probe 1 and the concentration of mercury ions. Inset: plot of fluorescence intensity of 5.0 μM probe 1 as a function of Hg$^{2+}$ concentration in the range of 0.08–1.0 μM.
S2, the $^1$H NMR signal of $H_a$ belonging to probe 1 was shifted to the upfield in the presence of $\text{Hg}^{2+}$, which demonstrated the binding between the oxygen atom of the hydroxyl group and $\text{Hg}^{2+}$. As displayed in Figure S2, the $^1$H NMR signal of $H_b$ in probe 1 also shifted upfield after the addition of $\text{Hg}^{2+}$, which illustrated the coordination between the nitrogen atom of the group ($–\text{CH}═\text{N}–$) and $\text{Hg}^{2+}$. The result of NMR titration experiment further confirmed the coordination mode between the probe and $\text{Hg}^{2+}$.

**Effect of pH.** For obtaining information about the pH effect, the fluorescence intensity changes of probe 1 before and after adding $\text{Hg}^{2+}$ are investigated in various pH conditions (Figure 5). As exhibited in Figure 5, the fluorescence intensity of probe 1 is substantially unchanged in the range of pH 4.50 to 12.
However, in the presence of Hg$^{2+}$, the probe exhibited a strong fluorescence in the range of 4.50−8.50. The above results demonstrated that the fluorescent probe was not affected in the pH range of 4.50−8.50 and thus could be exploited to sense Hg$^{2+}$ in the actual samples. When the pH was less than 4.50, probe 1 emitted enhanced fluorescence with reduced pH values, which could be attributed to the ring-opened structure of probe 1 under acidic conditions. Considering the response speed, sensitivity, and practical application, the pH 7.24 Tris−HCl buffer was used in this experiment.

**Selectivity.** In order to ensure that probe 1 could be used in a wide variety of environments, we examined the selectivity of probe 1 for Hg$^{2+}$ (Figure 6). It can be found from the black histogram in Figure 6 that the solution of Li$^+$, Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Fe$^{3+}$, Al$^{3+}$, Cd$^{2+}$, Zn$^{2+}$, Ag$^+$, Mg$^{2+}$, Pb$^{2+}$, Ba$^{2+}$, and Cr$^{3+}$ ions had almost no effect on the fluorescence intensity of probe 1 at a concentration of 10 times the Hg$^{2+}$ concentration. In addition, when the concentrations of Cu$^{2+}$, Co$^{2+}$, Ni$^{2+}$, and Mn$^{2+}$ were equal, probe 1 displayed a notable fluorescence increase only upon adding Hg$^{2+}$. The color change, fluorescence change, and UV−vis response of the probe in the absence and presence of those different ions mentioned above were also studied (Figure S3, Supporting Information). From Figures 6 and S3, the probe showed high selectivity for Hg$^{2+}$ by fluorometry. However, the detection of Hg$^{2+}$ was interfered by Cu$^{2+}$ by ultraviolet−visible absorption spectrometry. Moreover, competition experiments were executed in which the developed probe was used to sense Hg$^{2+}$ (1.0 × 10$^{-5}$ mol L$^{-1}$) in the coexistence solution of Hg$^{2+}$ and other metal ions (white histogram in Figure 6). The experimental results showed that the relative error of usual

![Figure 6. Metal-ion selectivity of 5.0 μM probe 1. The concentration of ions was 1.0 × 10$^{-5}$ M for Hg$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, and Mn$^{2+}$ and 1.0 × 10$^{-4}$ M for all other ions. Black bars: various metal ions were provided. White bars: various metal ions in the presence of Hg$^{2+}$ were provided.](https://doi.org/10.1021/acsomega.2c03336)

![Figure 7. Reversibility of probe 1 for Hg$^{2+}$ in Tris−HCl buffer (CH$_3$CH$_2$OH/H$_2$O, 1:1, v/v, pH 7.24). —:5.0 μM probe 1; —:5.0 μM probe 1 with 10 μM Hg$^{2+}$; —:5.0 μM probe 1 with 10 μM Hg$^{2+}$; and following the addition of 40 μM EDTA 2Na.](https://doi.org/10.1021/acsomega.2c03336)
interference including alkaline earth, alkali, and transition-metal ions was lower than ±5%, which was thought to be acceptable. These experiments demonstrated that the coexistence of different metal cations and mercury ions did not affect the determination of Hg\(^{2+}\), making the probe more likely to be used to determine actual samples.

For comparison, the complexation study with Hg\(^{2+}\) had been carried out using compound 2 (Figure S4, Supporting Information) or compound 3 (Figure S5, Supporting Information) in Tris–HCl buffer (CH\(_3\)CH\(_2\)OH/H\(_2\)O, 1:1, v/v, pH 7.24). As demonstrated in Figure S4, in Tris–HCl buffer (CH\(_3\)CH\(_2\)OH/H\(_2\)O, 1:1, v/v, pH 7.24), compound 2 showed fluorescence enhancement only in the presence of Cu\(^{2+}\). From Figure S5, compound 3 demonstrated a strong fluorescence increase and a pink color only in the presence of Cu\(^{2+}\) or Hg\(^{2+}\) (Figure S5 in the revised Supporting Information). Thus, probe 1 possessed higher selectivity for Hg\(^{2+}\) compared with compound 2 and compound 3.

**Reversibility and Response Time.** It was apparent to all that reversibility is a significant factor to prepare an excellent chemical sensor. We tested the reversibility of probe 1 by adding an EDTA solution (Figure 7). As demonstrated in Figure 7, the fluorescence intensity of probe 1 drastically increased after providing mercury ions, and the solution color transformed from colorlessness to pink. However, after the EDTA solution was added to the above pink solution, the fluorescence intensity decreased rapidly, and the solution color was altered from pink to colorlessness. These experimental consequences explained that the complexation process of probe 1 and Hg\(^{2+}\) was reversible.

At the same time, we also studied the time response of probes to mercury ions (Figure 8). Hg\(^{2+}\) (1.0 × 10\(^{-5}\) mol L\(^{-1}\)) were added to probe 1 solution, and the change in fluorescence intensity from 0.0 to 5.0 min was recorded to examine the time response of probe 1 for Hg\(^{2+}\). From Figure 8, it can be observed that the complexation speed of probe 1 and Hg\(^{2+}\) was very rapid, and the fluorescence intensity could reach the maximum within 60 s. The response time of the proposed probe for Hg\(^{2+}\) was shorter than that of formerly reported fluorescent probes for Hg\(^{2+}\).\(^{20–23}\) Therefore, the fluorescent probe would be utilized for real-time Hg\(^{2+}\) detection.

Compared with other probes for Hg\(^{2+}\) based on rhodamine derivatives (as displayed in Table S1, Supporting Information), this probe possessed many advantages including high sensitivity and specificity, fast response time, a wide pH working range, and so forth.

**Preliminary Analytical Application.** For verifying the application of probe 1 in actual sample analysis, probe 1 was utilized for the sensing of mercury ions in tap water and river water samples. The river water and tap water were directly used after being filtered by a 0.45 μm filter. The river water and tap water were measured by probe 1 for mercury ion content, which contained no Hg\(^{2+}\), and then the standard solutions of different concentrations of Hg\(^{2+}\) were separately added for the determination of the recovery rate (Table 1). As found from Table 1, the fluorescent probe has a satisfactory determination result of Hg\(^{2+}\) recovery rate in the tap water and river water. Thus, the probe could be effectively used to sense Hg\(^{2+}\) in the actual samples.

**Table 1. Sensing Hg\(^{2+}\) in Tap and River Water Samples with Probe 1**

| sample       | Hg\(^{2+}\) spiked (mol L\(^{-1}\)) | Hg\(^{2+}\) recovered (mol L\(^{-1}\)) | recovery (%) |
|--------------|-----------------------------------|--------------------------------------|--------------|
| river water  | 1 0                                | not detected                         |              |
|              | 2 5.00 × 10\(^{-6}\)               | (5.15\(^{±}\)  ± 0.10\(^{a}\))       | 103.0        |
|              | 3 1.00 × 10\(^{-5}\)               | ×10\(^{-6}\)                          | 102.0        |
|              |                                    | (1.02\(^{±}\) ± 0.02\(^{b}\)) ×10\(^{-5}\) |              |
| tap water    | 1 0                                | not detected                         |              |
|              | 2 5.00 × 10\(^{-6}\)               | (5.18\(^{±}\) ± 0.12\(^{a}\))       | 103.6        |
|              | 3 1.00 × 10\(^{-5}\)               | ×10\(^{-6}\)                          | 98.0         |
|              |                                    | (0.98\(^{±}\) ± 0.03\(^{b}\)) ×10\(^{-5}\) |              |

\(^{a}\)Mean values of three determinations. \(^{b}\)Standard deviation.
We also conducted a bio-imaging application research of probe 1. To assess the biocompatibility of probe 1, we performed a cytotoxicity assay using the MTT colorimetric assay (Figure S6, Supporting Information). From Figure S1, the cell viability of A549 cells was found to be higher than 90% when probe 1 was present, indicating that probe 1 was almost not cytotoxic. Next, we verified whether probe 1 could be employed for sensing Hg²⁺ in living cells by laser confocal fluorescence imaging experiments (Figure 9). As can be seen from Figure 9a, after culturing A549 cells in medium containing probe 1 (5.0 μM) for 30 min, the cells showed substantially no fluorescence. However, when A549 cells were cultured for 30 min with 1.0 μM Hg²⁺ under the same conditions, the cells exhibited a strong fluorescence (Figure 9d). According to the research results, probe 1 could be applied to fluorescence imaging of Hg²⁺ in living cells.

**CONCLUSIONS**

On the whole, a new fluorescent probe has been planned and prepared to quantify Hg²⁺, which used rhodamine as the fluorophore. After providing Hg²⁺, the probe displayed a strong fluorescence emission and a pink color due to its open-cyclic structure of the corresponding spirolactam via a reversible coordination. The fluorescent probe exhibited excellent sensing abilities, including excellent sensitivity and specificity, rapid response, a wide pH working range, and so forth. The fluorescent probe had been applied to sense Hg²⁺ in both tap and river water samples and demonstrated acceptable consequences. Furthermore, the probe also demonstrated superior biocompatibility, which permitted us to obtain fluorescence imaging of Hg²⁺ in A549 cells.

**EXPERIMENTAL SECTION**

**Materials and Instruments.** Lawesson’s reagent (97%) and allyl bromide were purchased from Aldrich. Rhodamine B and hydrazine monohydrate (85%) were bought from Shanghai Sinopharm Group Company. Before toluene was utilized, it was freshly distilled through adding sodium. Unless otherwise stated, other chemical reagents used in this work were of analytical grade and could be applied directly. Water used in all assays was ultrapure water.

UV–vis and fluorescence spectra were measured on a UV-2600 spectrometer and a Hitachi F-7000 spectrophotometer, respectively. A Bruker DRX-500 NMR spectrometer was utilized to measure the NMR spectra. Fluorescence images of living cells were acquired through an Olympus FV1200-MPE multiphoton laser scanning confocal microscope with a 40× objective lens. The pH of the solution was measured with a Mettler Toledo Delta 320 pH meter. SigmaPlot software was used to perform the data processing.

**Syntheses.** The method of preparation of fluorescent probe 1 is demonstrated in Scheme 1. During the process, product 2 was obtained through the interaction of hydrazine monohydrate (85%) and rhodamine B, as reported in the previous literature. Compound 3 was obtained by the chemical reaction between Lawesson’s reagent and compound 2 following an earlier developed method. 4-Formyl-3-hydroxyphenyl allyl ether (compound 4) was synthesized from 2,4-dihydroxybenzaldehyde and allyl bromide following a formerly developed procedure.

**Synthesis of Compound 1.** Compound 3 (0.19 g, 0.40 mmol) and compound 4 (0.096 g, 0.54 mmol) were put in 50 mL CH₂CH₂OH. The mixture was warmed to refluxing for 12 h. After the solvents were cleaned in decomposition, purification of the crude product was done through column chromatography (CH₂COOCH₂CH₂/petroleum ether = 1:10, v/v) to produce probe 1 (0.19 g, 75%) as a pale yellow solid.

³H NMR (500 MHz, CDCl₃): δ (ppm) 11.50 (1H, s), 8.62 (1H, s), 8.09 (1H, dd, J = 5.8 Hz, 3.2 Hz), 7.41 (2H, dd, J = 5.8 Hz, 3.2 Hz), 7.19 (1H, d, J = 8.5 Hz), 7.13–7.11 (1H, m), 6.74 (2H, d, J = 8.8 Hz), 6.49–6.42 (2H, m), 6.32–6.27 (4H, m), 6.05–5.97 (1H, m), 5.39 (1H, dd, J = 17.2 Hz, 1.4 Hz), 5.27 (1H, dd, J = 10.5 Hz, 1.2 Hz), 4.51 (2H, dd, J = 5.3 Hz), 3.51 (8H, q, J = 7.0 Hz), 1.14 (12H, t, J = 7.0 Hz) (Figure S7, Supporting Information). ¹³C NMR (125 MHz, CDCl₃): δ (ppm) 170.13, 162.38, 161.97, 161.07, 155.13, 151.93, 148.33, 135.19, 133.02, 132.67, 132.16, 130.11, 127.91, 127.12, 122.21, 117.99, 111.75, 110.17, 108.28, 107.63, 101.92, 97.53, 68.83, 64.23, 44.37, 12.60 (Figure S8, Supporting Information). MS (ESI) m/z: 633.2902 (M + H)⁺ (Figure S9, Supporting Information).

**Measurement Procedures of Fluorescence Intensity.**

The preparation of 1.0 × 10⁻⁵ mol L⁻¹ probe 1 was done by adding the needed quantity of probe 1 to CH₂CH₂OH. 8 × 10⁻⁷–1.0 × 10⁻⁵ mol L⁻¹ Hg²⁺ stock solution was obtained by gradually diluting 1.0 × 10⁻² mol L⁻¹ mercury nitrate solution with 0.05 mol L⁻¹ Tris–HCl buffer (pH, 7.24). The 0.05 mol L⁻¹ Tris–HCl solution was adjusted by adding HCl or NaOH solution to obtain a range of pH buffer solutions. 12.50 mL of 1.0 × 10⁻⁵ mol L⁻¹ probe 1 and 2.50 mL of different concentrations of Hg²⁺ solution were put into a 25 mL volumetric flask and then it was made up to 25 mL using 0.05 mol L⁻¹ Tris–HCl buffer. A solution containing 5 × 10⁻⁹ mol L⁻¹ probe 1 and 8 × 10⁻⁸–1 × 10⁻⁴ mol L⁻¹ Hg²⁺ was obtained. The same procedure without adding Hg²⁺ was used to obtain the blank solution of probe 1.

All solutions were kept at 4 °C in the dark. The excited wavelength was 520 nm, and both the entrance and exit slits were of 2.5 nm, and the fluorescence intensity was recorded in the range of 540–650 nm.
ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c03336.

1H NMR spectra of probe 1 before and after adding Hg(II); absorbance of probe 1 at 565 nm with different metal ions; fluorescence and color changes of probe 1 with different metal ions; absorbance and fluorescence of compound 2 at 586 nm with different metal ions; fluorescence and color changes of compound 2 with different metal ions; absorbance and fluorescence of compound 3 at 586 nm with different metal ions; fluorescence and color changes of compound 3 with different metal ions; comparison of fluorescent probes for determination of Hg(II) based on rhodamine derivatives; assay of A549 cells in the presence of different concentrations of probe 1 (0, 2, 4, 8, and 16 μM) for 24 h at 37 °C; 1H and 13C NMR spectra of compound 1; and ESI-MS spectra of compounds 1 (PDF)

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Notes
The authors declare no competing financial interest.

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REFERENCES

1. Chen, X.; Pradhan, T.; Wang, F.; Kim, J. S.; Yoon, J. Fluorescent chemosensors based on spiroring-opening of xanthenes and related derivatives. Chem. Rev. 2012, 112, 1910–1956.
2. Li, X.; Gao, X.; Shi, W.; Ma, H. Design strategies for water-soluble small molecular chromogenic and fluorogenic probes. Chem. Rev. 2014, 114, 590–659.
3. She, M.; Wang, Z.; Chen, J.; Li, Q.; Liu, P.; Chen, F.; Zhang, S.; Li, J. Design strategy and recent progress of fluorescent probe for noble metal ions (Ag, Au, Pd, and Pt). Coord. Chem. Rev. 2021, 432, 213712.
4. Tchounwou, P. B.; Ayensu, W. K.; Ninashvili, N.; Sutton, D. Review: Environmental exposure to mercury and its toxicopathologic implications for public health. Environ. Toxicol. 2003, 18, 149–175.
5. Counter, S. A.; Buchanan, L. H. Mercury exposure in children: a review. Toxicol. Appl. Pharmacol. 2004, 198, 209–230.
6. Harris, H. H.; Pickering, I. J.; George, G. N. The chemical form of mercury in fish. Science 2003, 301, 1203.
7. Weiss, B. Why methylmercury remains a conundrum 50 years after Minamata. Toxicol. Sci. 2007, 97, 223–225.
8. Zahir, F.; Rizwi, S. J.; Haq, S. K.; Khan, R. H. Low dose mercury toxicity and human health. Environ. Toxicol. Pharmacol. 2005, 20, 351–360.
9. Diez, S. Human health effects of methymercury exposure. Rev. Environ. Contam. Toxicol. 2009, 198, 111–132.
10. Mercury Update: Impact on Fish Advisories. EPA Fact Sheet EPA-823-F-01-011; EPA, Office of Water: Washington, DC, 2001.
11. Zhou, D. B.; Xiao, Y. B.; Han, F.; Lv, Y. N.; Ding, L.; Song, W.; Liu, Y. X.; Zheng, P.; Chen, D. Magnetic solid-phase extraction based on sulfur-functionalized magnetic metal-organic frameworks for the determination of methylmercury and inorganic mercury in water and fish samples. J. Chromatogr. A 2021, 1654, 462465.
12. Chen, D. Y.; Lu, L.; Zhang, H.; Lu, B.; Feng, J. L.; Zeng, D. Sensitive mercury speciation analysis in water by high-performance liquid chromatography–atomic fluorescence spectrometry coupling with solid-phase extraction. Anal. Sci. 2021, 37, 1235–1240.
13. Anthemidis, A. N.; Zachariadis, G. A.; Michos, C. E.; Stratis, J. A. Time-based on-line preconcentration cold vapour generation procedure for ultra-trace mercury determination with inductively coupled plasma atomic emission spectrometry. Anal. Bioanal. Chem. 2004, 379, 764–769.
14. Miranda-Andrades, J. R.; Letichevsky, S.; González Larrudé, D. R. G.; Aucelo, R. Q. Photo-generation of mercury cold vapor mediated by graphene quantum dots/TiO2 nanocomposite: on line time-resolved speciation at ultra-trace levels. Anal. Chim. Acta 2020, 1127, 256–268.
15. Monisha, K.; Shrivas, T.; Kant, S.; Patel, R.; Devi, N. S.; Dahariya, S.; Pervez, M. K.; Deb, M. K.; Rai, J.; Rai, J. Inkjet-printed paper-based colorimetric sensor coupled with smartphone for determination of mercury (Hg²⁺). J. Hazard. Mater. 2021, 414, 125440.
16. Chen, S. Y.; Li, Z.; Li, K.; Yu, X. Q. Small molecular fluorescent probes for the detection of lead, cadmium and mercury ions. Coord. Chem. Rev. 2021, 429, 213691.
17. Wang, Y.; Zhang, L.; Han, X.; Zhang, L.; Wang, X.; Chen, L. Fluorescent probe for mercury ion imaging analysis: strategies and applications. Chem. Eng. J. 2021, 406, 127166.
18. Yantasee, W.; Lin, Y. H.; Zemanian, T. S.; Fryxell, G. E. Voltammetric detection of lead(II) and mercury(II) using a carbon paste electrode modified with thiol self-assembled monolayer on mesoporous silica (SAMMS). Analyst 2003, 128, 467–472.
19. Zhang, J. L.; Zhu, Z. W.; Di, J. W.; Long, Y. M.; Li, W. F.; Tu, Y. F. A sensitive sensor for trace Hg²⁺ determination based on ultrathin-C-N4 modified glassy carbon electrode. Electrochim. Acta 2015, 186, 192–200.
20. Tian, M.; Wang, C.; Ma, Q.; Bai, Y.; Sun, J.; Ding, C. A highly selective fluorescent probe for Hg²⁺ based on a 1,8-naphthalimide derivative. ACS Omega 2020, 5, 18176–18184.
21. Huang, L.; Yang, Z.; Zhou, Z.; Li, Y.; Tang, S.; Xiao, W.; Hu, M.; Peng, C.; Chen, Y.; Gu, B.; Li, H. A dual colorimetric and near-infrared fluorescent turn-on probe for Hg²⁺ detection and its applications. Dyes Pigm. 2019, 163, 118–125.
A novel fluorescent probe for Hg\(^{2+}\) detection in a wide pH range and its application in living cell imaging. *Anal. Methods* **2018**, *10*, 5554–5558.

Prabhu, J.; Velmurugan, K.; Nandhakumar, R. A highly selective and sensitive naphthalene-based chemodosimeter for Hg\(^{2+}\) ions. *J. Lumin.* **2014**, *145*, 733–736.

Tian, M.; Liu, L.; Li, Y.; Hu, R.; Liu, T.; Liu, H.; Wang, S.; Li, Y. An unusual OFF-ON fluorescence sensor for detecting mercury ions in aqueous media and living cells. *Chem. Commun.* **2014**, *50*, 2055–2057.

Rani, B. K.; John, S. A. Fluorogenic mercury ion sensor based on pyrene-amino mercapto thiadiazole unit. *J. Hazard. Mater.* **2018**, *343*, 98–106.

Bozkurt, E.; Gul, H. I. Selective fluorometric “Turn-off” sensing for Hg\(^{2+}\) with pyrazoline compound and its application in real water sample analysis. *Inorg. Chem. Acta* **2020**, *502*, 119288.

Jiang, X. D.; Zhao, J.; Li, Q.; Sun, C. L.; Guan, J.; Sun, G. T.; Xiao, L. J. Synthesis of NIR fluorescent thienyl-containing azabODIPY and its application for detection of Hg\(^{2+}\): electron transfer by bonding with Hg\(^{2+}\). *Dyes Pigm.* **2016**, *125*, 136–141.

Erdemir, S.; Oguz, M.; Malkondu, S. A NIR fluorescent sensor based on thiazoline-isophorone with low cytotoxicity in living cells for Hg\(^{2+}\), 99. *ACS Omega* **2022**, *7*, 119288.

Xu, J.; Li, Z.; Ma, W. A highly sensitivity fluorescent probe based on rhodamine for naked-eye detection of Hg\(^{2+}\) in aqueous solution. *Int. J. Environ. Anal. Chem.* **2021**, *0*, 1–11.

Wang, Y.; Ding, H.; Wang, S.; Fan, C.; Tu, Y.; Liu, G.; Pu, S. A ratiometric and colorimetric probe for detecting Hg\(^{2+}\) based on napthalimide-rhodamine and its staining function in cell imaging. *RSC Adv.* **2019**, *9*, 11664–11669.

Gauthama, B. U.; Narayana, B.; Sarojini, B. K.; Suresh, N. K.; Sangappa, Y.; Kudva, A. K.; Satyanarayana, G.; Raghu, S. V. Colorimetric “off-on” fluorescent probe for selective detection of toxic Hg\(^{2+}\) based on rhodamine and its application for in-vivo bioimaging. *Microchem. J.* **2021**, *166*, 106233.

Cui, C.; Gao, X.; Jia, X.; Jiao, Y.; Duan, C. A rhodamine B-based turn on fluorescent probe for selective recognition of Hg(II) ions. *Inorg. Chem. Acta* **2021**, *520*, 120285.

Lv, H.; Sun, H. A lysosome-targetable two-photon excited near-infrared fluorescent probe for visualizing hypochlorous acid-involved arthritis and its treatment. *Spectrochim. Acta, Part A* **2021**, *249*, 119326.

Tian, T. J.; Xu, J. H.; Ma, Q. J.; Li, L. K.; Yuan, H. M.; Sun, J. G.; Zhu, N. N.; Liu, S. Z. A novel lysosome-located fluorescent probe for highly selective determination of hydrogen polysulfides based on a napthalimide derivative. *Spectrochim. Acta, Part A* **2022**, *268*, 120708.

Shen, Y. M.; Zhang, X. Y.; Zhang, Y. Y.; Li, H. T. L.; Dai, C.; Peng, X. J.; Peng, Z.; Xie, Y. T. A fluorescent sensor for fast detection of peroxynitrite by removing of C=N in a benzothiazole derivative. *Anal. Chim. Acta* **2018**, *1014*, 71–76.

McClure, D. S. Spin-orbit interaction in aromatic molecules. *J. Chem. Phys.* **1952**, *20*, 682–686.

Ma, Q. J.; Zhang, X. B.; Zhao, X. H.; Jin, Z.; Mao, G. J.; Shen, G. L.; Yu, R. Q. A highly selective fluorescent probe for Hg\(^{2+}\) based on a rhodamine-coumarin conjugate. *Anal. Chem. Acta* **2010**, *663*, 85–90.

Ma, Q. J.; Zhang, X. B.; Zhao, X. H.; Gong, Y. J.; Tang, J.; Shen, G. L.; Yu, R. Q. A ratiometric fluorescent sensor for zinc ions based on covalently immobilized derivative of benzoxazole. *Spectrochim. Acta, Part A* **2009**, *73*, 687–693.