A Highly Selective Fluorescent Probe for Hg$^{2+}$ Based on a 1,8-Naphthalimide Derivative

Meiju Tian, Chunyan Wang, Qiujuan Ma,* Yu Bai, Jingguo Sun, and Chunfeng Ding*

ABSTRACT: Hg$^{2+}$ has a significant hazardous impact on the environment and ecosystem. There is a great demand for new methods with high selectivity and sensitivity to determine mercury in life systems and environments. In this paper, a novel turn-on Hg$^{2+}$ fluorescent probe has been reported with a naphthalimide group. The Hg$^{2+}$ fluorescent probe was designed by the inspiration of the well-known specific Hg$^{2+}$-triggered thioacetal deprotection reaction. A 1,2-dithioalkyl group was chosen as the digestive system. Therefore, there is a great demand for new methods with high sensitivity and selectivity to monitor mercury in life systems and environments. In this paper, a novel turn-on Hg$^{2+}$ fluorescent probe based on a 1,8-naphthalimide derivative was designed by the inspiration of the well-known specificity and selectivity toward Hg$^{2+}$. Furthermore, the Hg$^{2+}$ fluorescent probe could work in a wide pH range. The linear relationship between the fluorescence intensity at 510 nm and the concentration of Hg$^{2+}$ was obtained in a range of Hg$^{2+}$ concentration from 2.5 × 10$^{-7}$ to 1.0 × 10$^{-5}$ M. The detection limit was found to be 4.0 × 10$^{-8}$ M for Hg$^{2+}$. Furthermore, with little cell toxicity, the probe was successfully applied to the confocal image of Hg$^{2+}$ in PC-12 cells.

INTRODUCTION

In recent years, heavy metals have been widely used in people’s production and daily life. However, the significant biological toxicity and nonbiodegradability of heavy metals cause serious pollution to the environment and threaten human health. Thus, detection of heavy metals has attracted extensive attention of researchers. Among them, mercury as a common toxic heavy metal is easily enriched by food chain bioamplification and eventually enters the human body. Mercury ions cause cell dysfunction due to their strong affinity for enzymes and thiols in proteins. Exposure to high levels of mercury in a long term can cause irreversible damage to the nervous system, endocrine system, immune system, and digestive system. Therefore, there is a great demand for new methods with high sensitivity and selectivity to monitor mercury in life systems and environments.

At present, many analytical methods have been developed to determine mercury ions, including high-performance liquid chromatography (HPLC), inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS), atomic emission spectrometry (AES), cold atomic fluorescence spectrometry, surface-enhanced Raman spectroscopy, ultraviolet–visible spectrophotometry (UV–vis), and electrochemical method. Although these methods are sensitive, selective, and precise for Hg$^{2+}$ detection, most of them are pretty sophisticated, time-consuming, and expensive as well as unsuited for real-time and on-site detection. Because of their superiority of simplicity, high sensitivity, and low price, most of the Hg$^{2+}$ fluorescent probes have been reported. It is reported that Hg$^{2+}$ can quench the fluorescence of the fluorescent probe molecule by coupling with the spin orbital of the fluorescent molecule, which is a common fluorescence quenching agent. Therefore, most fluorescent probes for the determination of mercury ions are designed by the inspiration of the fluorescence quenching mechanism and their sensitivity is lower than that of enhanced probes. However, only part of the measurement is based on the fluorescence enhancement principle for mercury ions. Therefore, the design of a fluorescence-enhanced Hg$^{2+}$ probe with high selectivity and sensitivity has great prospects and challenges.

Naphthalimide and its derivatives have been extensively applied in optical sensing owing to their favorable spectroscopic properties including superior light stability, high luminescence efficiency, and large Stokes shifts. Recently, naphthalimide-based probes have been reported to detect various substances including Hg$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, and so on.

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**RESULTS AND DISCUSSION**

**Spectroscopic Analytical Performance of Probe 1 toward Hg^{2+}.** To evaluate the fluorescence recognition ability of probe 1 for detection of Hg^{2+} in 0.01 M PBS buffer (ethanol/water = 2:8, v/v; pH = 7.40), the probe (5.0 μM) was dealt with various concentrations of Hg^{2+}, and the fluorescence spectra were obtained (as shown in Figure 1). With the increase of the concentration of Hg^{2+} (0–10 μM), the fluorescence emission intensity at 510 nm was enhanced, which constituted the base for the detection of Hg^{2+} using probe 1 in the work. In the presence of 10 μM Hg^{2+}, the probe showed the most significant fluorescence change and about 20-fold fluorescence enhancement at 510 nm was detected, which might be owing to the Hg^{2+}-triggered thioacetal deprotection reaction to generate the formyl group. Additionally, linear relationships in the range of Hg^{2+} concentration from 2.5 × 10^{-7} to 1.0 × 10^{-3} M were obtained by plotting between the fluorescence intensity of probe 1 at 510 nm and Hg^{2+} concentrations (Figure 2). The linear regression equation was $F = 96.5841 + 107.0940 \times C$ ($r = 0.9972$). Here, $F$ denotes the fluorescence intensity of probe 1 at 510 nm, $C$ stands for the concentration of Hg^{2+}, and $r$ indicates the linear correlation coefficient. The detection limit was determined to be 3S_B/m (where S_B represents the standard deviation of 10 blank measurements and $m$ is the slope of the calibrated relationship).
Hg²⁺ is added, the fluorescence intensity of the probe at 510 nm changes rapidly with the incubation time, reaching equilibrium after 10 min in the buffer of 0.01 M PBS (ethanol/water = 2:8, v/v; pH = 7.40). The response time of the probe to Hg²⁺ was much faster than that of the previously developed Hg²⁺ fluorescent probe. Without Hg²⁺, the fluorescence intensity at 510 nm did not change in the system, indicating that the probe was steady under the experimental conditions. The experimental results indicate that the probe can be applied for rapid determination of Hg²⁺. In this work, the assay time was optimized to 10 min.

Effect of pH. To detect Hg²⁺ sensitively, the pH effects were studied on probe 1’s fluorescence intensity under the conditions with and without Hg²⁺ (Figure 4). From Figure 4, in the pH range of 2.00–11.00, without Hg²⁺, the fluorescence intensity of probe 1 (5.0 μM) is stable. Meanwhile, in the pH range of 3.00–11.00, the fluorescence intensity of probe 1 showed significant emission enhancement with Hg²⁺ (10 μM). When the pH value is higher than 10.0, the fluorescence intensity of probe 1 was weak in the presence of Hg²⁺, which might be because Hg²⁺ was combined with OH⁻ to be Hg(OH)₂. The experimental results indicate that the Hg²⁺ probe can work in a wide pH range. The pH range of the physiological environment was covered in this range, resulting that the probe can be applied for biological detection.

Selectivity. High selectivity is an important factor in the preparation of excellent fluorescent probes. To study the specificity of the probe 1 to Hg²⁺, the fluorescence response of the probe 1 was investigated with various metal ions (Figure 5A). Hg(NO₃)₂ supplied mercury ions. Na⁺, K⁺, Ca²⁺, Ba²⁺, Mn²⁺, Ni²⁺, Fe³⁺, Al³⁺, and Cr³⁺ were supplied with their chlorides. Mg²⁺, Cd²⁺, Co²⁺, Cu²⁺, and Fe²⁺ were supplied with their sulfates. Zn²⁺, Pb²⁺, and Ag⁺ were supplied with their nitrates. As shown in Figure 5A, the fluorescence intensity of probe 1 significantly strengthened after adding Hg²⁺ and slightly increased in the presence of Ag⁺, while no significant fluorescence enhancement was observed after adding other metal ions. In biological and environmental analyses, the possible interference of Ag⁺ on the determination of Hg²⁺ could be successfully shielded by introducing an appropriate concentration of KBr solution. In addition, the selectivity of probe 1 for Hg²⁺ with the existence of other metal ions was also investigated (Figure 5B). From Figure 5B, when different metal ions and mercury ions coexisted, the fluorescence intensity of probe 1 exhibited little or almost no change. These results indicated that the probe had a good selectivity for Hg²⁺.

Proposed Mechanism. On the basis of the deprotection reaction of the dithiaoacetal triggered by Hg²⁺, it is supposed that the 1,2-disulfide group in probe 1 was deprotected to aldehyde by Hg²⁺, thus transforming probe 1 into compound 5 (Scheme 2). To validate the reaction mechanism of probe 1 for Hg²⁺, ultraviolet–visible absorption spectroscopy (Figure 6) and high-performance liquid chromatography (Figure 7) were employed to provide direct evidence. As depicted in Figure 6, without Hg²⁺, the probe exhibited an absorption peak at around 461 nm, and compound 5 has a major absorption peak at around 417 nm. In the presence of Hg²⁺ (50 μM), the probe showed an evidently blueshift from 461 to around 417 nm. Meanwhile, the color of the solution changed from pale yellow to light green and probe 1 could be applied as a visual tool to determine Hg²⁺. So, it may be attributed to the formation of the compound 5 produced via the reaction of probe 1 to Hg²⁺. Furthermore, from Figure 7, the probe 1 showed a peak at 16.14 min and the compound 5 exhibited a peak at 12.18 min. The retention time (12.15 min) of the probe with Hg²⁺ (1 mM) is equal to that of compound 5. The HPLC result also indicated that the newly emerging substance in probe 1 with Hg²⁺ was compound 5. A peak at m/z 296.0930 (Figure S12, Supporting Information) from the ESI-MS spectrum indicated a new product formed after mixing the probe 1 and Hg²⁺ for 10 min. As shown in Figure S1, the peak at m/z 296.0930 matched with the structure (compound...
The above results confirmed our hypothesis in Scheme 2.

Scheme 2. Proposed Possible Mechanism of the Response of Probe 1 to Hg^{2+}

5 – H]^−. The above results confirmed our hypothesis in Scheme 2.
EXPERIMENTAL SECTION

Materials and Instruments. 4-Bromo-1,8-naphthalic anhydride, trifluoroacetic acid, and 1,2-ethanedithiol were bought from Heowns Biochemical Technology Company. Anhydrous potassium carbonate was purchased from Tianjin Ruijinte Chemical Company. HI (55%) was provided by Energy Chemical (Shanghai, China). The reagents needed for high-performance liquid chromatography experiments were all chromatographically pure, which were bought from Tianjin Siyou Fine Chemical Company. Urotropine was bought from Tianjin Sailboat Chemical Reagent Technology Company.

Figure 7. (A–C) HPLC of (A) probe 1 (50 μM), (B) reaction mixture of probe 1 (50 μM) with Hg2+ (1 mM), and (C) compound 5 (50 μM). HPLC conditions: total flow rate, 1.0 mL/min; Agela Technologies Venusil XBP C18 5 μm, 4.6 × 250 mm; methanol/water = 70:30 (v/v); and detection wavelength at 440 nm.

Figure 8. (A, B) MTT assay of PC-12 cells in the presence of different concentrations of (A) probe 1 and (B) compound 5 (0, 2, 4, 8, and 16 μM) for 24 h at 37 °C.

Figure 9. Images of PC-12 cells treated with the presented probe 1. (a) Bright-field transmission image of PC-12 cells incubated with 5.0 μM probe 1 for 30 min at 37 °C. (b) Fluorescence image of the cells shown in panel (a). (c) Bright-field transmission image of PC-12 cells incubated with 5.0 μM probe 1 for 30 min and then treated with 10 μM Hg2+ for another 30 min. (d) Fluorescence image of cells shown in panel (c).
Company. All of chemicals used in synthesis were analytical reagent grade and used as obtained unless otherwise indicated. Ultrapure water has been utilized throughout, and water was purified using the distilling instrument of an SZ-93 automatic double pure water distiller from Shanghai Yarong Biochemical Instrument Factory. Silica gel 60 F254 was used in TLC analysis, and silica gel (200–300 mesh) was applied in column chromatography. Both of them were bought from Qingdao Ocean Chemicals (Qingdao, China).

1H NMR and 13C NMR spectra were collected by employing a Bruker DRX-500 NMR spectrometer using tetramethylsilane (TMS) as the internal standard. Mass spectra were obtained using an Agilent 6420 Triple Quadrupole LC–MS high-resolution mass spectrometer. The fluorescence spectra were measured using a Hitachi F-7000 fluorescence spectrophotometer with a 1 cm quartz cell (Tokyo, Japan). UV–vis absorption spectra were determined using an Evolution 260 Bio UV–vis spectrophotometer with a 1 cm quartz cell. High-performance liquid chromatograms were recorded using a Waters 1525 HPLC with an XBP C18 column (5.0 μm, 4.6 × 250 mm). The pH values were measured using a pH meter from the Mettler Toledo Company. Fluorescence imaging of living cells was performed using an FV1200 single-photon laser scanning confocal microscope from Olympus Company. A PBS (0.01 M, pH 7.40) buffer solution with EtOH as the cosolvent (EtOH/H2O = 2:8, v/v) was applied for all spectroscopic measurements. The fluorescence spectrum was acquired under the condition of the excitation wavelength at 414 nm and the range of the emission wavelength from 424 to 650 nm. The values of the excitation and emission slits were both 50 nm. Data processing was performed by using SigmaPlot software. Furthermore, except for the fluorescence data acquired by time scanning, all other data were obtained at 10 min after adding the mercury ion at 25 °C.

Syntheses. The synthesis pathway of fluorescence probe 1 is depicted in Scheme 1.

Synthesis of Compound 2. Compound 2 was prepared based on the previous paper. 4-Bromo-1,8-naphthalic anhydride (0.70 g, 2.5 mmol) and N-butylamine (0.37 g, 5 mmol) were added in 25 mL of pure ethanol and heated to reflux for 6 h. Then, the reaction solution was cooled down to 25 °C. The solvent was removed via vacuum, and the crude product underwent silica gel column chromatography for purification utilizing petroleum ether/ethyl acetate = 8:1 (v/v) to obtain compound 2 as a yellow solid (0.31 g, 77%).

Synthesis of Probe 1. The p-toluene sulfonic acid monohydrate (0.50 g, 1 mmol) and compound 2 (0.15 g, 0.5 mmol) were dissolved in 10 mL of pure ethanol, and 1,2-ethanediol (0.05 g, 0.55 mmol) was added dropwise. The mixed solution was heated to reflux for 24 h. The solvent was then removed under negative pressure. The crude product underwent silica gel column chromatography for purification utilizing dichloromethane/methanol = 50:1, v/v to obtain compound 3 as a brown-yellow solid (0.21 g, 70%). Further, except for the fluorescence data acquired by time scanning, all other data were obtained at 10 min after adding the mercury ion at 25 °C.

Synthesis of Compound 3. Compound 3 was synthesized in a previous paper. Compound 2 (0.66 g, 2 mmol) and anhydrous potassium carbonate (1.40 g, 10 mmol) were added to 20 mL of CH2OH, stirred, and heated to reflux for 14 h. Then, the solvent was removed and subjected to silica gel column chromatography with petroleum ether/ethyl acetate = 6:1 (v/v) as the eluent to obtain 3 as a gray solid (0.45 g, 79%).

Synthesis of Compound 4. Compound 4 was obtained referring to the report. Compound 3 (0.43 g, 1.5 mmol) was added to 30 mL of HI (57%), stirred, and heated to reflux for 8 h. The mixed solution was cooled to 25 °C and adjusted the pH value to neutral to obtain a precipitate. The precipitate was filtered, washed, and dried. Then, the solvent was removed under negative pressure and the resultant crude material underwent silica gel column chromatography for purification utilizing petroleum ether/ethyl acetate = 3:1 (v/v) to obtain compound 4 as a yellow solid (0.31 g, 77%).

Synthesis of Probe 5. Compound 5 was prepared from the methods of a reported paper. A mixture of compound 4 (0.27 g, 1 mmol) and uracil-5 (0.28 g, 1 mmol) in trifluoroacetic (10 mL) was heated to reflux for 21 h. Then, the mixed solution was cooled to 25 °C, neutralized with sodium bicarbonate, and then extracted by dichloromethane. The organic layer was purified with water, dried over anhydrous Na2SO4, filtered, and concentrated. The crude product underwent silica gel column chromatography for purification utilizing dichloromethane/methanol = 50:1, v/v to obtain compound 5 as a brown-yellow solid (0.21 g, 70%). Further, except for the fluorescence data acquired by time scanning, all other data were obtained at 10 min after adding the mercury ion at 25 °C.

Cytotoxicity Assay. The tetrazolium-based colorimetric assay (MTT assay) was applied to evaluate the cytotoxicity of the probe. First, PC-12 cells were grown in an incubator containing 5% CO2 at 37 °C with the Dulbecco’s modified Eagle’s medium (DMEM). Approximately 1 × 104 cells were placed in each well of 96-well plates for 24 h. After the loading media was removed, different concentrations of probe 1 (0, 2, 4, 8, and 16 μM) and compound 5 (0, 2, 4, 8, and 16 μM)
were added, respectively. The cells were cultured at 37 °C in an atmosphere of 5% CO2 for 3 h. Then, the drug-containing medium was removed and the cells were fed with the new culture medium for 24 h. Whereafter, 10 μL of MTT (5 mg·mL⁻¹) was introduced and cultured for another 4 h. Finally, 150 μL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals and the 96-well plate was placed on a shaker for 10 min. The absorbance was recorded at 490 nm using a microplate reader (Synergy 2, BioTek Instruments Inc.), and A/A₀ × 100% was used to calculate the survival rate (A and A₀ stand respectively for the absorbance of the experimental group and control group).

**Confocal Imaging in Living Cells.** One day before imaging, PC-12 cells were seeded in 20 mm laser confocal dishes for about 24 h to assure that they were well cultured and fully adherent. PC-12 cells were treated with a medium including fluorescent probe 1 (5.0 μM) alone for 30 min; after which, the cells were cleaned with Dulbecco’s phosphate-buffered saline (DPBS) three times and imaged. In a control experiment, PC-12 cells were treated with a medium including fluorescent probe 1 (5.0 μM) for 30 min, cleaned three times with DPBS buffer to eliminate spare extracellular probe 1, and then cultured with Hg²⁺ (10 μM) for 30 min. Finally, PC-12 cells were cleaned with DPBS three times and imaged again. Confocal fluorescence images were recorded on an Olympus FV1200MPF single-photon confocal microscope with a 60X objective lens.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c01790.

1H NMR and 13C NMR spectra of compounds 1–5 and the ESI-MS spectrum of the reaction mixture of probe 1 with Hg²⁺ for 10 min (PDF)

**AUTHOR INFORMATION**

**Corresponding Authors**

Qiujuan Ma — School of Pharmacy, Henan University of Traditional Chinese Medicine, Zhengzhou 450046, PR China; Zhengzhou Key Laboratory of Chinese Medicine Quality Control and Evaluation, Zhengzhou 450046, PR China; orcid.org/0000-0001-9785-2733; Phone: +86-371-65676656; Email: maqiujuan104@126.com; Fax: +86-371-65660028

Chunfeng Ding — Henan Key Laboratory of Laser and Optoelectric Information Technology, School of Information Engineering, Zhengzhou University, Zhengzhou 450001, PR China; Email: ichilding@zzu.edu.cn

**Authors**

Meiju Tian — School of Pharmacy, Henan University of Traditional Chinese Medicine, Zhengzhou 450046, PR China

Chunyan Wang — School of Pharmacy, Henan University of Traditional Chinese Medicine, Zhengzhou 450046, PR China

Yu Bai — School of Pharmacy, Henan University of Traditional Chinese Medicine, Zhengzhou 450046, PR China

Jingguo Sun — School of Pharmacy, Henan University of Traditional Chinese Medicine, Zhengzhou 450046, PR China

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c01790

**Notes**

The authors declare no competing financial interest.

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