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# Hg<sup>2+</sup>-selective "turn-on" fluorescent chemodosimeter derived from glycine and living cell imaging

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### 1. Introduction

Mercury is one of the most dangerous and widespread global pollutant [1,2], as seen in such unfortunate incidents as Minamata disease [3] and mercury poisoning in Iraq [4]. As these cases showed, methylmercury is extremely toxic to aquatic organisms and birds than inorganic mercury, which eventually reaches the top of the food chain and accumulates in higher organisms, especially in large edible fish [5]. When consumed by humans, methylmercury triggers several serious disorders including sensory, motor, and neurological damage. The development of methods for the determination mercury is, therefore, of significant importance for environment and human health. Traditional analytical techniques for Hg<sup>2+</sup> include atomic absorption spectroscopy, cold vapor atomic fluorescence spectrometry and gas chromatography. These methods, however, require not only complicated instrumentation but also a long measuring time. Therefore, it is urgent to develop new methods for monitoring Hg<sup>2+</sup> with a low limit of detection, as well as rapid and facile detection. In particular, fluorescence has been regarded as the most powerful optical technique for detecting low concentrations of metal ions, and considerable efforts have been devoted to the development of fluorescent chemical sensors

#### ABSTRACT

A new nonfluorescent benzthiazole derivative of dithio-N-phthalovlglycine was prepared, and its fluorogenic chemodosimetric behaviors toward transition metal ions were investigated. The dithio-Nphthaloylglycine derivative showed highly Hg<sup>2+</sup>-selective fluorescence enhancing ("turn-on") properties in 20% aqueous acetonitrile solution ( $H_2O/CH_3CN = 80:20$ , v/v). The chemodosimetric behavior is based on the Hg<sup>2+</sup> triggered desulfurization of dithio-N-phthaloylglycine derivative into its oxygen analogue. To observe the cell permeability of 3 into Pollen grains, we also employed it for the fluorescence detection of the changes of intracellular Hg<sup>2+</sup> in cultured cells.

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that detect mercury ions [6-9]. Among them, a chemodosimetric approach frequently yields successful results in the selective and sensitive signaling of Hg<sup>2+</sup> ions [10–15]. Hg<sup>2+</sup>-triggered desulfurization and/or transformation of thioamide [16], selenolactone [17] and thione has been successfully utilized for the development of Hg<sup>2+</sup>-selective chemodosimeters, beginning with the classical anthracene-based dosimeter [18] developed by Czarnik and coworkers.

These methods may facilitate mercury analyses by the conversion of thiocarbonyl compounds into their carbonyl analogues via oxidative procedures that involve inorganic and organic reagents, as well as hydrolytic reactions [19]. Because mercury is a soft Lewis acid, a vast majority of the chemosensors and chemodosimeters for mercury contain sulfur atom(s) that can tightly coordinate the metal, thus weakening the C=S bond and as part of off-on fluorescence switches. So the probes for Hg<sup>2+</sup> generally consist of a chromophore and a reactive region that can be chelated [20-22] or reacted [16,23-30] with Hg<sup>2+</sup> ions. The absorption or emission properties of the probes change with the interactions between the reactive region and Hg<sup>2+</sup> ions. The development of novel chromophores with appropriate reactive regions for selective, sensitive detection remains an important goal for ion sensors. During our ongoing research on both cation [31,32] and anion [33,34] recognition, herein, we report the synthesis and application of a probe based on benzthiazole, 2-benzthiazol-2-ylmethylisoindole-1,3-dithione (3), for the selective, sensitive detection of  $Hg^{2+}$  ions in

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Scheme 1. Synthesis of chemodosimeters 3 and 4.

aqueous acetonitrile  $H_2O/CH_3CN = 80:20$ , v/v. The desulfurization reaction of carbonyl sulfide takes advantage of the high affinity of  $Hg^{2+}$  for sulfur. A simple substitution of the oxygen atom with sulfur results in a significant change in the optical properties of the compounds.

In the present case, our strategy for designing a chemical sensor for mercury was glycine amino acid to N-phthaloylglycine scaffold having an appended benzthiazole. As amino acids are water-soluble and biologically and environmentally compatible, new chemical sensors based on amino acids are a promising route of development. However, as none of the natural amino acids except Trp and Tyr have fluorescent properties [35,36], conjugation of a fluorophore into the amino acid is a critical step for the synthesis of fluorescent chemical sensors based on amino acids. Therefore, we speculated that the introduction of the benzthiazole ring to the dithio-N-phthaloylglycine-based probe based on the following considerations: (i) increase the affinity to Hg<sup>2+</sup> ions in competitive aqueous media; (ii) change the spatial effects within one molecule; (iii) realize the real-time detection (quickly induce the fluorescent response); (iv) improve the sensitivity of Hg<sup>2+</sup> ions.

#### 2. Experimental

#### 2.1. Materials and apparatus

All the solvents were of analytic grade. All cations, in the form of perchlorate salts, were purchased from Sigma–Aldrich Chemical Co., stored in a desiccator under vacuum containing self-indicating silica, and used without any further purification. Solvents were dried according to standard procedures. Unless stated otherwise, commercial grade chemicals were used without further purification. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) using Spectrochem GF254 silica gel coated plates. The <sup>1</sup>H NMR spectra were recorded on Bruker-AM-400 spectrometer. The <sup>1</sup>H NMR chemical shift values are expressed in ppm ( $\delta$ ) relative to CHCl<sub>3</sub> ( $\delta$  = 7.26 ppm). UV–vis and fluorescence spectra measurements were performed on a JASCO V530 and a PerkinElmer LS-55 spectrofluorimeter respectively.

#### 2.2. General method

A  $2.0 \times 10^{-5}$  M solution of the probe **3** in CH<sub>3</sub>CN/H<sub>2</sub>O (20:80, v/v) was. Solutions of  $5.0 \times 10^{-4}$  M perchlorate salts of the respective cation were prepared in distilled CH<sub>3</sub>CN and were stored

under a dry atmosphere. All experiments were carried out in HEPES (20 mM, pH 7.05) buffer solution (containing 10% CH<sub>3</sub>CN as co-solvent).

### 2.3. Synthesis and characterization

The receptor 3 was synthesized according to Scheme 1. Nphthaloylglycine (1) was prepared in 82% yield from the reaction of glycine with phthalic anhydride in toluene at 100 °C in the presence of triethylamine. Benzothiazole derivative 2 was prepared by the reaction of N-phthaloylglycine with 2-aminothiophenol (PCl<sub>3</sub>, toluene, 75%). Dithio-derivative of 3 was prepared from the 2 by the reaction with Lawesson's reagent in good yield (62%) [37]. Compound 2 was reacted with Lawesson's reagent in refluxing dry toluene, and the resulting material was purified by column chromatography with silica gel using chloroform as the eluent. Compound 3 was obtained as chocolate brown solid with a yield of 62% (Scheme 1). The structures of 2 and 3 were fully confirmed by NMR spectroscopy and mass spectrometry (MS) (Figs. S1-S7). The structure of probe 2 was also confirmed by X-ray analysis [38] (Fig. 1) of its single crystal that was grown in acetonitrile. Thiocarbonyl derivative of 4 was similarly prepared from their Nphthaloylglycine (1) precursor.

### 2.3.1. Synthesis and characterization of receptor 2:

#### 2-benzothiazole-2-yl methyl-isoindole-1,3-dione

2-Aminothiophenol (138 mg, 1.1 mmol) and compound 1(189 mg, 1 mmol) were dispersed in dry toluene. The solution was warmed to  $40 \,^{\circ}$ C and phosphorus trichloride (0.1 mL, 1.1 mmol) was added dropwise to the reaction mixture. The mixture was heated 100  $^{\circ}$ C for 4 h and then cooled to room temperature. The reaction mixture was extracted with 20% sodium carbonate solution and washed with distilled water, and then evaporated to produce solid residue. The crude product was crystallized in dichloromethane/hexane to give compound 2 in 75% yield. Mp 189–191  $^{\circ}$ C. The structure of probe 2 was confirmed by X-ray analysis of its single crystal (CCDC 832376) that was grown in acetonitrile.

#### 2.3.2. Synthesis and characterization of receptor 3:

#### 2-benzothiazol-2-yl methyl-isoindole-1,3-dithione

Lawesson's reagent (203 mg, 0.5 mmol) was added to the solution of compound **2** (196 mg, 0.5 mmol) in dry toluene. The mixture was heated at 110 °C for 36 h and then allowed to cool slowly to



Fig. 1. X-ray crystal structure of 2. Hydrogen atoms are not shown for clarity.

room temperature. The coloured solid product obtained was filtered and purified by column chromatography using chloroform as the eluent to yield chocolate brown **3** (62%). Mp 173 °C.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.94 (d, *J* = 10.9 Hz, 1H), 7.84 (d, *J* = 13.4 Hz, 1H), 7.65 (m, 4H), 7.40 (t, *J* = 8.16 Hz, 1H), 7.35 (t, *J* = 9.76 Hz, 1H), 6.03 (s, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 171.57, 167.61, 167.59,134.63, 134.61, 134.53, 132.17,132.16, 126.57, 125.73,124.04, 123.93, 123.51, 121.87, 39.75, 38.76. ESI-MS: *m/z* 327.10 [M+1]<sup>+</sup>.

#### 3. Results and discussion

#### 3.1. UV-vis studies

The UV–vis spectroscopic behavior of **3** toward representative metal ions was investigated by treating it with physiologically important transition metal perchlorates in 20% aqueous acetonitrile solution ( $H_2O/CH_3CN = 80:20$ , v/v, 50 mM HEPES at pH 7.4). The absorption spectrum of **3** exhibited strong absorption bands at 243 and 360 nm. Fig. 2 shows the gradual increase in the UV–vis absorption spectra of **3** in 20% aqueous acetonitrile solution containing various concentrations of  $Hg^{2+}$  at room temperature. A clear isosbestic point was observed at 270 nm. The clear isosbestic point at 270 nm in the absorption spectra (Fig. 2) indicate that the reaction of **3** with  $Hg^{2+}$  produces a single component. A concomitant



**Fig. 2.** UV-vis absorption spectra of **3** (10  $\mu$ M) in H<sub>2</sub>O/CH<sub>3</sub>CN (H<sub>2</sub>O/CH<sub>3</sub>CN = 80:20, v/v, 50 mM HEPES at pH 7.4) containing different concentrations of Hg<sup>2+</sup> (0, 0.2, 0.4, 0.8, 1.0, 2.0, 4.0, 8.0, 10, 20, 40, 80, 100, 120, 150, 200  $\mu$ M). Inset: UV-vis Job plot of **3** with Hg<sup>2+</sup> by using continuous variation method.

chromogenic change from brown to colorless was observed. Upon interaction with various metal ions, significant changes in absorption spectra were observed particularly with Hg<sup>2+</sup>. Other metal ions caused relatively minor changes in the absorption spectra of **3** (Fig. S8). Judging from the titrations, the stoichiometries of the complexes were ascertained from the break of the UV–vis titration curves on the basis of the Job's plot method [39] (as shown in the inset of Fig. 2).

The stoichiometry of the Hg<sup>2+</sup> complex formed with **3** was determined by Job's plot (Fig. 2, inset) at 243 nm, and it was found to be 1:1. In the plot, a maximum at 0.5 mol fraction was observed that the large absorption change occurs via the 1:1 reaction. In addition, the absorption data shows clear isosbestic point.

#### 3.2. Fluorescent studies

To examine the sensitivity of **3**, fluorescence emission spectra were recorded in the presence of 50 equiv. of representative metal perchlorates in the same solvent system. It is expected that the designed probe 3 will display weak fluorescent. However, when it reacts with excess Hg<sup>2+</sup> to yield **2**, strong fluorescence should be observed gradually. As shown in Fig. 3a, 3 displays relatively weak fluorescence centered at about 360 nm in the absence of Hg<sup>2+</sup> with  $\lambda_{ex} = 243$  nm, and a pronounced 'off-on' type signaling toward Hg<sup>2+</sup> ions. Upon interaction with various metal ions, the fluorescence intensity at 360 nm was markedly enhanced with Hg<sup>2+</sup> ions (Fig. 3b), while other metal ions (Zn<sup>2+</sup>, Mn<sup>2+</sup>, Ni<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup>, and Pb<sup>2+</sup>) did not cause any noticeable responses (Fig. S9). The only exception was  $Cu^{2+}$ , which gave rise to a small fluorescent quenching response (Fig. S9b). The fluorescence titration profile of **3** versus Hg<sup>2+</sup>revealed that the maximum enhancement of the emission from **3** was obtained when 50 equiv. Hg<sup>2+</sup> was added to the solution (Fig. 3b).

The fluorescence ratio  $(I_0 - I)/I_0$  is displayed in Fig. 4a. For Hg<sup>2+</sup>, the value was almost 3 times. In contrast, a range of other ions showed ratios of less than 1. It can be concluded that **3** displays much higher selectivity for Hg<sup>2+</sup> ions than the other ions screened.

Furthermore, the emission color of probe **3** is displayed blue fluorescence and also metal-ion-dependent (Fig. 4b), demonstrating that the probe could be employed for a convenient visual sensing of  $Hg^{2+}$ .

The signaling mechanism is based on  $Hg^{2+}$ -induced desulfurization of the thio derivative (Scheme 2). The thioketone group of **3** is readily transformed to its ketone function by reaction with  $Hg^{2+}$  ions [40] and the liberated sulfur atom forms a stable species of HgS with  $Hg^{2+}$  ions [41]. As a result of this transformation, the



**Fig. 3.** (a) Fluorescence titration of **3** with Hg<sup>2+</sup> ions. [c] = 1.0 × 10<sup>-5</sup> M, H<sub>2</sub>O/CH<sub>3</sub>CN (80:20) at pH 7.4 (50 mM HEPES).  $\lambda_{ex}$  = 243 nm. Inset: the photograph shows the fluorescence color of probe **3** before and after addition of 2 equiv. Hg<sup>2+</sup> ions. (b) Emission spectra of **3** (10  $\mu$ M) in the presence of 50 equiv. of Hg<sup>2+</sup> ions and 100 equiv. of each of Zn<sup>2+</sup>, Mn<sup>2+</sup>, Ni<sup>2+</sup>, Co<sup>2+</sup>, Cd<sup>2+</sup>, Cd<sup>2+</sup>, and Pb<sup>2+</sup> ions.  $\lambda_{ex}$  = 243 nm.



**Fig. 4.** (a) The fluorescence ratio of  $[I_t/I_0 = (I_0 - I)/I_0]$  of **3** at 360 nm upon addition of different metal ions (50 equiv. of each) in H<sub>2</sub>O/CH<sub>3</sub>CN = 80:20, v/v. (b) Visual fluorescence emission of probe **3** to metal ions (50 equiv. except 20 equiv. Hg<sup>2+</sup>) in H<sub>2</sub>O/CH<sub>3</sub>CN = 80:20, v/v (50 mM HEPES at pH 7.4).

weakly fluorescent dithio-N-phthoylglycine derivative  $\mathbf{3}$  ( $\phi = 0.002$  in 20% aqueous acetonitrile) is transformed into a strongly emitting phthoyl derivative of  $\mathbf{2}$  ( $\phi = 0.113$ ).

#### 3.3. <sup>1</sup>H NMR studies

To obtain further information about the proposed transformation of sensor **3**, <sup>1</sup>H NMR titration experiments were performed in CD<sub>3</sub>CN. Changes in the <sup>1</sup>H NMR spectra of sensor **3** before and after addition of Hg<sup>2+</sup> are shown in Fig. 5. In general, Hg<sup>2+</sup> induced resonance signals of the sensor molecule to become broad and slightly shifted to downfield region. For instance, the resonance signal corresponding to the aromatic protons were shifted downfield from 7.93 to 8.26 and 7.88 to 7.88 ppm upon addition of Hg<sup>2+</sup>. These changes indicated that indeed the sensor complexed with the metal ions. The chemodosimetric nature of the signaling was further verified by testing reversibility of the **3**·Hg<sup>2+</sup> system with EDTA treatment. The enhanced fluorescence of the **3**·Hg<sup>2+</sup> system was not significantly affected by treatment with chelating chemicals of EDTA (Fig. S10), but in the presence of EDTA, the fluorescence of **3** was not enhanced upon addition of Hg<sup>2+</sup> observations demonstrated that the **3**·Hg<sup>2+</sup> signaling system operates based on an irreversible chemodosimetric process.

#### 3.4. Mass spectral analysis

The another evidence for the 1:1 stoichiometry was suggested by ESI-MS analysis. In the absence of Hg<sup>2+</sup>, a peak was observed at m/z 327.1 corresponding to [**3**+H]<sup>+</sup> (Fig. S4). After the addition of 2 equiv. of Hg<sup>2+</sup> to the solution of **3**, the peak at m/z 327.1 disappeared. At the same time, a new peak at m/z 527.6 appeared, which is consistent with the compound [**3**+Hg<sup>2+</sup>+H]<sup>+</sup> (Fig. S5). In addition, changes in the fluorescence spectra of **3** in response to Hg<sup>2+</sup> ions also confirmed the proposed Hg<sup>2+</sup>-induced desulfurization process.

#### 3.5. DFT calculation

To understand the interactions between probe **3** and the  $Hg^{2+}$  metal center, the quantum chemical calculations using at the DFT level were performed (Fig. S11). The molecular modeling showed (Fig. 6) that the cation probe **3** has concave structure. The ring S of benzthiazole and one of the S of dithio-N-phthaloylglycine









**Fig. 6.** Calculated optimized structure (B3LYP/6-31G<sup>\*</sup>) of **3**-Hg<sup>2+</sup> (E = -798.201 a.u.).



**Fig. 7.** Time trace of the Hg<sup>2+</sup>-signaling of **3**. [c] = 5.0 × 10<sup>-6</sup> M, [Hg<sup>2+</sup>] = 5.0 × 10<sup>-4</sup> M in H<sub>2</sub>O/CH<sub>3</sub>CN (H<sub>2</sub>O/CH<sub>3</sub>CN = 80:20, v/v, 50 mM HEPES at pH 7.4).  $\lambda_{ex}$  = 243 nm.

derivative of probe **3** are oriented into the cavity for the complexation of  $Hg^{2+}$ , owing to thiophilic character of  $Hg^{2+}$ . Due to the open-shell nature of the  $Hg^{2+}$ -containing complexes, the structure for the  $[\mathbf{3} \cdot Hg^{2+}]$  complex was calculated in a first step, following the experimentally observed 1:1 ligand/cation ratio. The 2:2 stoichiometry  $[\mathbf{3}_2 \cdot Hg_2]^{4+}$  was also checked but displayed a very endergonic complexation energy.

#### 4. Analytical application

To examine the sensitivity of **3** for  $Hg^{2+}$  ion sensing, its kinetic study was monitored for the reaction of  $Hg^{2+}$ -promoted desulfurization of compound **3**. Fluorescence titration was carried out to gain quantitative insight into the signaling behavior of **3** toward  $Hg^{2+}$  ions. From the  $Hg^{2+}$  concentration dependency of the signaling behavior, the detection limit of **3** for the analysis of  $Hg^{2+}$  ions was estimated to be  $1.0 \times 10^{-5}$  M. The  $Hg^{2+}$  signaling of **3** was relatively slow compared with that of other chemo dosimeters based on sulfur derivatives. A time trace revealed that signaling was complete within about 30 min at temperature of 25 °C (Fig. 7), these observation are some what undesirable for a practical applicability of sensor **3** as a  $Hg^{2+}$ -selective chemodosimeter.



**Fig. 8.** (a) Fluorescence microscope images of *Pollen* cells only, (b) images of cells + Hg<sup>2+</sup>(25 mM), (c) images of cells + Hg<sup>2+</sup> + **3** (5 μM), and fluorescence images (d-f) of *Pollen* cells incubated with 10 μM, 20 μM, 40 μM of **3** for 20 min, respectively.

On the other hand, imidazole **4**, which has a closely related structure to **3**, are very slowly photodegraded under the same experimental conditions about 0.5% after 30 min (Fig. S12). These observations suggest that the selective determination of  $Hg^{2+}$  ions by fluorescence enhancement could be practically feasible with sensor **3**.

#### 5. Cell imaging

Taking into account the ability of biosensing molecules to high sensitivity and selectivity of **3** for  $Hg^{2+}$ , **3** was used for imaging in vitro  $Hg^{2+}$  detection in living cells. To observe the cell permeability of  $Hg^{2+}$  and **3** into Pollen grains (Pollen grains were obtained from freshly collected mature buds of *Tecoma stans*, a common ornamental plant of *Bignoniaceae* family), all the cells were poisoned by incubated with  $Hg^{2+}$  (25 mM) for 30–40 min at 37 °C, and washed with 0.1 M HEPES buffer at pH 7.4 to remove the remaining  $Hg^{2+}$ .

Upon gradual addition of **3** into the cell, a series of images recorded (Fig. 8) in the absence and presence of  $HgCl_2$  and **3** by using the a Leica DM 1000 fluorescence microscope. Pollen cells incubated with  $Hg^{2+}$  initially display no fluorescent image, but the fluorescence image gradually becomes strong in the presence of different concentration of **3** (Fig. 8b–d). These results demonstrate that **3** might be used for detecting  $Hg^{2+}$  in biological samples.

#### 6. Conclusion

In summary, a new Hg<sup>2+</sup>-selective chemodosimeter system was developed based on simple glycine derivatives. The significant changes in the fluorescence color could be used for naked-eye detection. The signaling is due to the Hg<sup>2+</sup>-induced desulfurization of benzthiazole derivative of N-phthaloylglycine **3**. The significant enhancement in fluorescence after desulfurization in water-compatible solvents made **2** an excellent chemodosimeter for Hg<sup>2+</sup>. Mass spectra indicated that the high selectivity of **3** was due to an Hg<sup>2+</sup>-induced desulfurization reaction. It is anticipated that **3** could contribute to the development of mercury ion sensors. Furthermore, the sensor was applied for in vivo imaging of Hg<sup>2+</sup> using Pollen grains.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jphotochem.2012.05.001.

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