A Ratiometric Fluorescence Chemodosimeter for Detecting Hg²⁺ in Aqueous Solutions and Living Cells

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Abstract

A new thiourea derivative (PT) bearing a water-soluble porphyrin fluorophore was synthesized and used as a highly sensitive and selective chemodosimeter for mercury ions (Hg^{2+}) in aqueous solution. PT exhibits large ratiometric absorption and fluorescence emission variations upon exposure to various Hg^{2+} concentrations based on the Hg^{2+} -promoted intramolecular cyclic guanylation of thiourea. This mechanism was verified *via* NMR, MS, and FT-IR spectral data. The chemodosimeter is highly selective for Hg^{2+} in the presence of alkali, alkaline earth, transition, and heavy metal ions in the physiological pH window with a detection limit of ppb level. The selectivity can also be visualized under UV light, whereby the red fluorescence of PT becomes orange only in the presence of Hg^{2+} . The potential practical application of PT to detect Hg^{2+} on paper strips is also described. Finally, in living cells, it exhibits excellent membrane permeability and low toxicity and thus can be used for confocal fluorescence imaging of intracellular Hg^{2+} .

Keywords: Mercury ion, Fluorescent Chemodosimeter, Ratiometric sensor, Porphyrin, Cell imaging

1. Introduction

Mercury ion (Hg^{2+}) is the most toxic as well as the most common heavy metal ion pollutant that poses severe health and environmental risks^[1-3]. Concerns over mercury exposure have motivated the development of new methods for monitoring aqueous Hg^{2+} in both environmental and biological samples. Because fluorescence detection is operationally simple and offers low cost, high sensitivity, and real-time monitoring, it has become the dominant strategy for Hg^{2+} sensing^[4-10]. Among them, chemodosimetric approach for efficient and selective Hg^{2+} signaling has been attracting much research interest^[11-16]. A chemodosimeter is a kind of chemosensor that recognizes specific ion through a highly selective chemical reaction (usually irreversible). The formed new species in the medium could change the photophysical behavior of dosimeter molecule that makes detection reliable and easier. Chemodosimetric reactions such as Hg^{2+} -promoted desulfation^[17-22], deselenization^[23], cyclization^[24-30], thiol elimination^[31-33], ring opening of rhodamine spiro systems^[34-38], and hydrolysis^[39-46], have been applied for specific Hg^{2+} sensing. However, many of the above chemodosimeters for Hg^{2+} have problems such as

poor water solubility^[18-20,22-24,28-33,36,37,42,46], short emission wavelength (<500 nm)^[17,19,21-23,41,42], fluorescence quenching^[26,30,39,41,45,46], and cross-sensitivities with other metal cations^[21,32,46], restricting their applications in biological systems to some extent. Furthermore, most Hg²⁺ chemodosimeters exhibit fluorescence changes mainly at a fixed wavelength. In most practical applications, fluorescence intensity changes can also be caused by many other poorly quantified or variable factors such as the excitation intensity, dye concentration, and environment around the dye (pH, polarity, temperature, and so forth)^[47]. The ratiometric method can normalize the variation of these effects and provide more robust and precise data for Hg²⁺ detection in the diversified microenvironments of living systems^[48]. Therefore, the development of a new ratiometric fluorescence chemodosimeter for sensitive and specific Hg²⁺ detection in natural water and living organisms is urgently needed.

Porphyrin and its derivatives are excellent building blocks in the design of fluorescent probes. They exhibit good photostability, high absorption coefficients over the 400–450 nm range, large Stokes shifts that minimize background fluorescence, and appreciable spectral shifts upon structure variation^[49-53]. It is well-known that thiourea can be easily transformed into guanidine derivatives because of high thiophilic nature of Hg²⁺, thus providing a molecular recognition/reactivity motif^[24,29,54-56]. The merits of porphyrins and thioureas are combined here in a new water-soluble porphyrin derivative (PT) for selective and sensitive fluorescence detection of Hg²⁺ over a broad pH range (Scheme 1). To the best of our knowledge, porphyrin-based, Hg²⁺-responsive fluorescent probes that depend on reactions have not yet been reported before. Here, the thiourea group undergoes Hg²⁺-triggered intramolecular cyclization coupled with ratiometric porphyrin fluorescence changes that eliminate most or all of the possible variability arising from differences in instrumental efficiency and environmental effects. Furthermore, this newly developed powerful fluorescent probe works effectively for intracellular Hg²⁺ imaging in HeLa cells which confirms its application in biological samples.

---Scheme 1---

2. Experimental

2.1 Materials

5-(4-aminophenyl)-10,15,20-triphenylporphyrin (ATPP) was synthesized as reported previously^[57]. Analytical reagent grade *N*-methylethylenediamine, thiophosgene, and mercury

chloride were purchased from Sigma–Aldrich Chemical Co. All other reagents, cations (chloride salts), and solvents were obtained from Shanghai Chemical Agent Co. (China) and used without further purification, unless noted otherwise. Ultrapure water with electric resistance >18.2 MΩ·cm was supplied through a Millipore Milli-Q water purification system (Billerica, MA, USA). Dialysis was performed with a Spectra/ Por[®] Dialysis Membrane (USA).

2.2 Synthese

5-(4-aminophenyl)-10,15,20-triphenylporphyrin (ATPP) (0.5 g, 0.79 mmol) was added in a 250 ml round-bottom flask with 30 ml of concentrated sulfuric acid. The mixture was heated up to 75°C in an oil bath for 8 h. After cooling to room temperature, 50 ml of distilled water was slowly and carefully poured in to quench the reaction. The reaction mixture was transferred into vials for centrifugation for three times to remove most of the sulfuric acid. Then 1 M NaOH was added to neutralize the solution. Extensive purification was performed by ultracentrifugation filtration in a 1 000 MWCO membrane for several times with ultrapure water to remove the salt. The product was further purified by reverse-phase column chromatography (RP-18) and was lyophilized to obtain 5-(4-aminophenyl)-10,15,20-tris(4-sulfonatophenyl)-porphyrin (TPPSNH₂), Yield: 87.5%. Mp.: 319–320°C. ¹HNMR (500 MHz, DMSO-*d*₆): δ_H, ppm –2.86 (s, 2H), 5.69 (s, 2H), 7.02 (d, J=8 Hz, 2H), 7.91 (d, J=4 Hz, 2H), 8.05 (d, J=8.2 Hz, 6H), 8.18 (d, J=8 Hz, 6H), 8.83 (s, 6H), 8.98 (d, J=4 Hz, 2H). ¹³CNMR (125 MHz, DMSO-*d*₆): δ_C, ppm 148.70, 147.73, 141.43, 135.56, 133.66, 128.26, 124.15, 122.05, 119.39, 118.89, 112.56. MS (ESI): *m/z* 288.7233 (calcd. for M³⁻ 288.7016) (Figs. S1, S2, S3).

TPPSNH₂ (150 mg, 0.17 mmol) was dissolved in 20 ml ultrapure water in a reaction vial. Then thiophosgene (75 μL, 0.98 mmol) in 15 ml of dichloromethane was added and the two-phase mixture was vigorously stirred at room temperature for 4 h. The aqueous phase was extracted and washed three times with 10 ml of dichloromethane to remove the excess of thiophosgene. The product 5-(4-isothiocyanatophenyl)-10,15,20-tris(4-sulfonatophenyl)-porphyrin (TPPSNCS) was dried with a lyophilizer overnight to obtain a green powder (quantitative). Mp.: 331–332°C. ¹HNMR (500 MHz, DMSO-*d*₆): δ_H, ppm –2.92 (s, 2H), 7.89 (d, J=8.3 Hz, 2H), 8.05 (d, J=7.7 Hz, 6H), 8.18 (d, J=7.7 Hz, 6H), 8.31 (d, J=8.3 Hz, 2H), 8.84–8.89 (m, 8H). ¹³CNMR (125 MHz, DMSO-*d*₆): δ_C, ppm 147.64, 145.43, 142.75, 138.70, 135.84, 134.62, 127.96, 126.93, 124.23, 122.12, 120.55, 116.94. MS (ESI): *m/z* 302.3831 (calcd. for M³⁻ 302.6871) (Figs. S4, S5, S6).

For PT synthesis, the porphyrin isothiocyanate derivative TPPSNCS (40 mg, 0.041 mmol) and *N*-methylethylenediamine (3.05 mg, 0.041 mmol) were dissolved in 3 ml of degassed water/acetone (1:1). The mixture was stirred for 3 h at room temperature, and solvent was removed under reduced pressure. To remove water, toluene was added four times (4×2 ml) and evaporated. PT was isolated as a brown powder (yield > 99%). Mp.: 342–343°C. ^1H NMR (500 MHz, DMSO- d_6): δ_{H} , ppm –2.93 (s, 2H), 1.23 (d, 3H), 1.89–1.96 (m, 4H), 2.25–2.28 (m, 1H), 3.15–3.19 (m, 1H), 7.17–7.21 (m, 5H), 8.06 (d, $J=7.9$ Hz, 6H), 8.18 (d, $J=7.9$ Hz, 6H), 8.75–8.79 (m, 6H), 8.98 (d, $J=8.1$ Hz, 2H). ^{13}C NMR (125 MHz, DMSO- d_6): δ_{C} , ppm 184.69, 150.84, 150.77, 144.48, 143.78, 140.57, 137.03, 136.84, 129.29, 127.29, 126.20, 122.78, 32.14, 29.61, 21.66. MS (ESI): m/z 327.3072 (calcd. for M^{3-} 327.3819) (Figs. S7, S8, S9).

2.3 Apparatus

^1H NMR and ^{13}C NMR spectra were obtained on a Bruker (Advance DMX500) NMR spectrometer. Mass spectra (MS) were recorded with a Bruker Esquire 3000 Plus spectrophotometer (Bruker-Franzen Analytik GmbH, Germany). Ultraviolet visible (UV–vis) absorption spectra were acquired with a Shimadzu UV 2450 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were acquired with a Shimadzu RF-5301 PC fluorescence spectrophotometer (Shimadzu, Japan), with 3 nm excitation and emission slits. Fourier transform infrared (FT-IR) spectra of samples in KBr discs were recorded with a spectrometer (Nicolet, Nexus-470, USA). Cell imaging was performed on confocal laser scanning microscopy (CLSM) with a Leica TCS SP5 confocal setup mounted on a Leica DMI 6000 CS inverted microscope (Leica Microsystems, Germany) and was operated under the Leica Application Suite Advanced Fluorescence (LASAF) program, in which the wavelength of excitation is 488 nm, emission was collected at 575–620 nm. Melting points were obtained with an X-4 precise micro melting point cryoscope (Beijing FukaiInstrument Co., China). All pH measurements were made with a pH-10 C digital pH meter.

2.4 Preparation of stock solutions for fluorescent/UV–vis study

Stock solutions of 10 μM PT and 1.0×10^3 μM Hg^{2+} was prepared by dissolving PT and HgCl_2 in Tris-HCl (0.02 M) solution (containing 0.1 mM sodium phosphate, pH 7.4), respectively. The latter solution was further diluted stepwise to yield working solutions with concentrations ranging from 0.5×10^3 μM to 1 μM . In a typical experiment, test solution was prepared by adding 5 ml of the

stock solution of PT and 1 ml of the stock solution of Hg^{2+} to a 10 ml volumetric flask. The mixture was then diluted to 10 ml with Tris–HCl buffer solution (pH 7.4). The final concentrations of PT and Hg^{2+} in the complex solutions were 5 μM and 50–0.05 μM , respectively. A blank solution of PT was prepared under the same conditions without Hg^{2+} . Solutions of other metal ions were prepared by dissolving KCl, LiCl, NaCl, CaCl_2 , ZnCl_2 , PbCl_2 , BaCl_2 , FeCl_2 , CdCl_2 , $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ in Tris-HCl (0.02 M) solution (containing 0.1 mM sodium phosphate, pH 7.4) and then subsequently diluted to prepare working solutions. All solutions were protected from light and stored at 4 °C.

2.5 Cell cytotoxicity assays

HeLa cells ($2 \times 10^5 \text{ ml}^{-1}$) were plated in 96-well plates in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% bovine serum, 100 U ml^{-1} penicillin, and 100 $\mu\text{g ml}^{-1}$ streptomycin. The cells were incubated overnight (37 °C, 5% CO_2) and were treated with PT at different concentrations for 24 h. Non-treated cells were controls. The MTT (thiazolyl blue (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide)) assay was then performed according to the following procedure. 20 μL of a 5 mg ml^{-1} MTT solution in phosphate buffered saline (PBS) (0.01 M, pH 7.4) were added to each well. The cells were incubated for 3 h again and 100 μL of DMSO was added and shaken for 30 min at room temperature, and the absorbance of the solution was recorded using enzyme-linked immune detector at 570 nm. The cell viability (%) was calculated according to the following equation:

$$\text{Cell viability (\%)} = \frac{OD_{\text{Sample}}}{OD_{\text{Control}}} \times 100 \quad (1)$$

where OD_{sample} is the optical density of the wells treated with various concentration of PT, and OD_{control} is that of wells with untreated control cells.

2.6 Cell imaging

HeLa cells were cultured in DMEM medium in an incubator (37 °C, 5% CO_2). The cells were plated on 6-well plate at 5×10^6 cells per well and allowed to adhere for 12 h. They were then washed with PBS three times and incubated in PT (10 μM) for 30 min at 37 °C. The solution was then removed, and the cells were washed twice with PBS to remove extracellular PT. Subsequently, the cells were divided into two groups. The first was a control group without exposure to Hg^{2+} , while the

other was treated with a 20 μM solution of Hg^{2+} for 30 min at 37 $^{\circ}\text{C}$. The PBS was then removed and the cells were washed twice with fresh PBS before imaging.

3. Results and Discussion

3.1. Preparation and characterization of PT

Tris-sulfonate porphyrins are water-soluble and often employed in biological studies^[58]. Compound PT was easily synthesized due to the high reaction efficiency between the amino and isothiocyanate groups to form the thiourea unit that can be easily transformed into guanidine derivatives with the promotion of targeted Hg^{2+} ion^[28]. The absorption spectrum of PT has characteristic porphyrin transitions, with an intense Soret band at 413 nm and four weak Q-bands at 513 nm, 547 nm, 587 nm, and 645 nm (Fig. 1). In agreement with this result, when excited at 413 nm, PT exhibited one typical fluorescence peak at 651 nm (Fig. 1). 5,10,15,20-tetra(4-sulfonatophenyl)-porphyrin (TPPS₄) was considered as standard ($\Phi_{\text{Ref}} = 0.16$), the fluorescence quantum yield (Φ_F) of PT in 50 mM HEPES buffer (pH 7.0), 100 mM KCl was determined to be 0.18, using the method described by Kalyanasundaram^[59]. This fact reveals that PT can perform as a suitable optical probe.

--- Fig. 1 ---

3.2. Hg^{2+} sensing properties of PT

As PT was soluble in aqueous solutions without an organic solvent, PT coordination with Hg^{2+} was investigated by spectrophotometric titration in an aqueous Tris-HCl buffer solution (pH 7.4), accordingly. As depicted in Fig. 2, the gradual addition of Hg^{2+} (0–10 equiv.) to a 5 μM PT solution caused the Soret band to bathochromically shift from 413 nm to 425 nm with an isosbestic point at ~ 420 nm. The Q bands also bathochromically shifted, indicating that a new compound was produced during the titration. That is, the addition of Hg^{2+} transforms the linear thiourea fragment into a circular imidazoline moiety^[24,29,54] which results in a slight distortion of the porphyrin ring due to steric hindrance^[60,61]. The observed 12 nm red shift in the Soret band is mainly due to the better conjugation of distorted porphyrin ring with imidazoline group in solution. It should be noticed that the peak numbers of PT in Q band were the same before and after reacted with Hg^{2+} , which means the porphyrin moiety does not coordinate with Hg^{2+} . Furthermore, when the amount of Hg^{2+} is beyond 1 equiv., the 425 nm absorbance tends to

saturation, indicating possible formation of a 1:1 complex between PT and Hg^{2+} . To further understand the binding behavior and determine the stoichiometry of the formed complex, a Job's plot for the absorbance is performed by keeping the sum of initial concentration of Hg^{2+} and PT at 10 μM and changing the molar ratio of Hg^{2+} ($X_M = ([\text{Hg}^{2+}] / ([\text{Hg}^{2+}] + [\text{PT}]))$) from 0 to 1. As shown in Fig. S10, the 425 nm absorbance vs. X_M has a maximum at a molar fraction of 0.5, indicating formation of a 1:1 stoichiometry for the PT and Hg^{2+} complex.

--- Fig. 2---

To have an insight into the fluorescence signaling behavior of PT to Hg^{2+} , fluorescence titration with Hg^{2+} in varying concentrations was carried out. As plotted in Fig. 3, gradual addition of Hg^{2+} (0–10 equiv.) into PT solution (5 μM) reduced the 651 nm emission and induced a new peak at 601 nm with a quantum yield of 0.17. On further addition of Hg^{2+} , the ratiometric change of the fluorescence spectra became evident with a clear isoemission point at 630 nm. This also indicates that the formation of a Hg^{2+} -triggered new imidazoline derivative as well. The emission intensity at 601 nm (F_{601} , Fig. S11) and the intensity ratio (F_{601}/F_{651} , Fig. S12), increased upon the gradual addition of Hg^{2+} , which allowed the detection of Hg^{2+} by both normal and ratiometric fluorescence methods. It should be noticed that the excitation wavelength of the compound PT is in the visible range (≥ 400 nm), which is beneficial to minimize cell and tissue damage as compared to the fluorescent probe with UV excitation^[62]. In addition, the large Stokes shift (~ 50 nm) of PT is a great advantage for fluorescence imaging because it avoids self-quenching background levels from the excitation and scattered light. Moreover, because PT emits at relatively long wavelengths, interference from biomolecular absorption and autofluorescence is reduced, which facilitates tissue imaging at deeper penetration depths.

To characterize Hg^{2+} -induced cyclization leading to formation of the imidazoline derivative, ^1H and ^{13}C NMR spectra of PT were acquired in the absence and presence of Hg^{2+} . As shown in Fig. S13, the addition of Hg^{2+} resulted in the resonances of NH fragments, which directly attached to porphyrin unit (H1, δ 7.19, merged with protons of porphyrin ring) characteristically shifted upfield to appear at δ 6.91 ppm. Resonances at 2.25–2.28 and 3.15–3.19 ppm, attributed to $-\text{NH}$ protons (H2, H5), disappeared. In addition, the cyclization promoted a downfield shift for all protons of the ethylene linker arm and the methyl group (H3, H4 and H6). The signal appeared at 1.89–1.96 ppm, attributed to H3 and H4 protons, merged to appear at 2.34–2.37 ppm whereas H6

of the methyl unit shifted downfield to 1.66 ppm. Similarly, a typical resonance attributed to thiol carbon (C=S) at 184.69 ppm in the ^{13}C NMR spectrum of PT disappeared in the presence of Hg^{2+} . Simultaneously, a new resonance attributed to formation of an imine (C=N) in the imidazoline ring appeared at 163.59 ppm. The ethylene carbons of linker arm (C1 and C2), shifted downfield to appear at 36.08 and 37.09 ppm, respectively (Fig. S14).

FT-IR spectra of PT in the absence and presence of Hg^{2+} also indicate the formation of a new derivative. In Fig. S15, the absorption at $\sim 1235\text{ cm}^{-1}$, 3353 cm^{-1} , and 3234 cm^{-1} , which respectively corresponds to the stretching vibration of C=S, -N-H- (marked N-H2 and N-H3 in Fig. S15 (a)) groups in PT, disappeared in Fig. S15(b) after the addition of Hg^{2+} . This indicates that these groups participated in the Hg^{2+} -triggered transformation of PT. In the presence of Hg^{2+} , the -N-H- signal at 3427 cm^{-1} (marked N-H1 in Fig. S15(a)) preserves and moved to 3382 cm^{-1} in Fig. S15(b), indicates that this component was not involved in the interrelation with Hg^{2+} . Finally, the formation of a new molecule was confirmed by ESI-MS spectral data analysis, where the peak attributed to PT at $m/z\ 327.3072$ disappeared, and a new peak at $m/z\ 316.0747(\text{M}+\text{H}^+)$ for the imidazoline derivative appeared (Fig. S16). Thus, based on the above analysis, a plausible mechanism for Hg^{2+} induced cyclization reaction was proposed as depicted in Scheme 2.

--- Fig. 3---,---Scheme 2---

To calculate the limit of detection (LOD), we established a linear working curve by plotting the changes in fluorescence intensity ΔF (differences between the emission intensities of PT at 601 nm in the presence and absence of Hg^{2+}) vs. Hg^{2+} concentration over the range 0–5.5 μM , as shown in Fig. S17. The linear regression equation of calibration curve is $\Delta F = 30.82\text{ C} + 38.50$ (C: μM) with the correlation coefficient of 0.9967. While the limit of detection (LOD) for PT to detect Hg^{2+} was calculated on the basis of the following equation (2)^[29] and was found to be $1.8 \times 10^{-8}\text{ mol L}^{-1}$, suggesting that PT can be potentially used to detect Hg^{2+} contamination up to 3.6 ppb level (18 nM). This is relatively low according to allowed Hg^{2+} contamination in drinking water (1–2 ppb) and food gradients, as described by EPA, USA^[63].

$$LOD = \frac{3\sigma}{m} \quad (2)$$

In Eq. 2, σ is the standard deviation of the PT fluorescence intensity in solution without Hg^{2+} , and m is the slope of the calibration curve.

We also investigated the time evolution of the response of PT to 2 equiv. of Hg^{2+} in aqueous solution (Fig. S18). We found that the interaction of PT with Hg^{2+} was complete in less than 10 min. Thus, this system could be used for real-time tracking of Hg^{2+} .

3.3. Effect of pH

To apply PT in more complicated systems, like in organism and environment, fluorescence intensity of PT at 601 nm in the presence of Hg^{2+} was evaluated in different pH values (Fig. 4). In the absence of Hg^{2+} , the fluorescence intensity of PT was unchanged between pH 4 and pH 10. Upon addition of Hg^{2+} , in the section of lower pH value, the fluorescence intensity of PT decreased with decreasing pH value, which might be attributed to protonation of compound PT. On the other hand, high pH leads to hydrolysis and precipitation of Hg^{2+} , which in turn, would reduce its reaction with PT. In a wide range of pH from 6.5 to 8.5, acidity does not affect the determination of Hg^{2+} with compound PT. In other words, the response of compound PT to Hg^{2+} is pH-independent in the biologically relevant pH range, which is convenient for practical applications in actual samples.

--- Fig. 4---

3.4. Selective and competitive experiment

High selectivity is necessary for an effective chemodosimeter. To investigate the selectivity of PT toward different metal ions, changes in fluorescence spectra of PT (5 μM) induced by Hg^{2+} (1 equiv.) and other metal ions (5 equiv. each) are recorded. As shown in Fig. 5(a), only Hg^{2+} showed distinct ratiometric responses, whereas very weak changes were observed for the other metal ions. These results confirm the high selectivity of PT for Hg^{2+} . Meanwhile, fluorescent emission color change of PT on Hg^{2+} binding is easily observed by a naked eye examination. Upon excitation at 365 nm under a UV lamp, the deep red fluorescence of PT becomes orange, only in the case of Hg^{2+} as compared to other metal ions (Fig. 5(b)). In addition, competition experiments on the signaling of Hg^{2+} by PT in the presence of common coexisting metal ions were also examined. In this study, 10 μM of Li^+ , Zn^{2+} , Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cd^{2+} , Ba^{2+} , Cu^{2+} , and Pb^{2+} , 1 mM of Na^+ , K^+ , Ca^{2+} , and Mg^{2+} were added to PT solutions (5 μM) as per relevance of the availabilities in physiological conditions. As shown in Fig. 6, significant enhancement of fluorescence intensity ratio (F_{601}/F_{651}) was detected in all cases after the addition of Hg^{2+} (5 μM) to the background metals-containing solutions. These results unambiguously demonstrate the

high selectivity of PT for Hg^{2+} over other competitive metal ions in aqueous medium. Moreover, it also confirmed the possibility of the formation of a new $\text{PT} + \text{Hg}^{2+}$ species in the medium and henceforth, PT could be termed as a potential chemodosimeter for Hg^{2+} .

--- Fig. 5---,--- Fig. 6---

3.5. Detection of Hg^{2+} on a cellulose paper strip

In order to make sure the analytical use of PT for Hg^{2+} detection, the test paper strips ($1.5 \times 0.5 \text{ cm}^2$) were prepared by immersing Whatman™ cellulose paper into an aqueous solution of PT (100 μM), followed by drying in air. Different concentrations (0.05 μM , 0.5 μM , and 5 μM) of HgCl_2 solution in water were dropped separately onto the test strips and dried in air. Same as in solution, the strips exhibited obvious fluorescent color variation from red to orange under 365 nm UV light excitation, and the intensity of orange color increased with the concentration of Hg^{2+} (Fig. S19). This result confirmed that the synthesized probe could also detect Hg^{2+} on cellulose paper strips.

3.6. In vitro toxicity assay

Favorable fluorescence properties of PT for Hg^{2+} sensing prompted us to establish its utility for the determination of Hg^{2+} in living cells. Before the cell imaging test, we investigated the cytotoxicity of PT *in vitro*. After incubating HeLa cells (human cervical cancer) with PT at concentrations of 0/control, 5, 10, 20, 50, and 100 μM for 24 hours, the MTT assay was performed to determine the surviving fraction. The relative percentage of cell viability was determined as the ratio of formazan intensity in viable cells treated with PT to the intensity in control cells. As shown in Fig. S20, there was >87% cell viability even though the concentration of PT was added up to 100 μM . Thus, PT should be safe for bioimaging in living cells.

3.7. In vitro cell Imaging

To further demonstrate the practical application of the probe, we carried out experiments in living cells. HeLa cells were incubated with PT (10 μM) for 30 min at 37 °C, followed by addition of Hg^{2+} (20 μM) and incubation for another 30 min. With an emission filter of 575–620 nm, the enhancement of fluorescence could be observed after treatment with Hg^{2+} (Fig. 7). The results suggested that PT could penetrate the cell membrane and primarily nontoxic to the cell culture thus might be used for imaging of Hg^{2+} in living cells and *in vivo* potentially.

--- Fig. 7---

4. Conclusions

In conclusion, we have designed and synthesized a new ratiometric fluorescent chemodosimeter PT, which showed high sensitivity and selectivity toward Hg^{2+} . PT reacts irreversibly with Hg^{2+} *via* an intramolecular guanylation reaction to form a new imidazoline derivative in pure aqueous media, achieving a detection limit at nano-molar level. Both its water solubility and its ratiometric fluorescence response to the analyte enhance the practical utility of the probe. Furthermore, PT with low cytotoxicity has been successfully used for bioimaging, which might be favorable for biological applications to monitor and research the bioaccumulation and bioavailability of Hg^{2+} in living cells. More significantly, the general strategy described here affords a new avenue for the design of novel fluorescence sensors, which constitutes our future endeavors for sensor development.

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Figure Captions:

Scheme 1. Schematic of PT synthesis.

Scheme 2. Proposed Hg^{2+} -promoted intramolecular guanylation of PT.

Fig. 1. Absorption (left) and fluorescence emission (right) spectra of PT (5 μM) in Tris- HCl (0.02 M) solution (containing 0.1 mM sodium phosphate, pH 7.4).

Fig. 2. UV-vis spectra of PT (5 μM) in the presence of different concentration of Hg^{2+} in Tris-HCl (0.02 M) solution (containing 0.1 mM sodium phosphate, pH 7.4).

Fig. 3. Fluorescence emission spectra of PT (5 μM) in the presence of different concentration of Hg^{2+} in Tris-HCl (0.02 M) solution (containing 0.1 mM sodium phosphate, pH 7.4), $\lambda_{\text{ex}} = 413$ nm.

Fig. 4. Fluorescence intensities of PT (5 μM) at 601 nm (F_{601}) in the absence and presence of Hg^{2+} (1 equiv.) at various pH values, $\lambda_{\text{ex}} = 413$ nm.

Fig. 5. (a) Fluorescence response of PT (5 μM) to Hg^{2+} (1 equiv.) and other metal ions (5 equiv. each) in Tris-HCl (0.02 M) solution (containing 0.1 mM sodium phosphate, pH 7.4), $\lambda_{\text{ex}} = 413$ nm; (b) Photographs of PT (5 μM) upon adding 1 equiv. of various metal ions in Tris-HCl (0.02 M) solution (containing 0.1 mM sodium phosphate, pH 7.4), which was observed under a UV lamp ($\lambda_{\text{ex}} = 365$ nm).

Fig. 6. Metal ion selectivity of PT. The blue bars represent the fluorescence intensity ratio (F_{601}/F_{651}) of PT (5 μM) in the absence and presence of the chosen metal ions. The red bars represent the fluorescence intensity ratio (F_{601}/F_{651}) of PT (5 μM) treated with the chosen metal ions and then followed by adding Hg^{2+} (1 equiv.)

Fig. 7. CLSM images of Hg^{2+} in HeLa cells. (a-c) HeLa cells incubated with PT (10 μM) for 30 min; (d-f) HeLa cells incubated with PT(10 μM) for 30 min, washed three times, and then further incubated with 20 μM Hg^{2+} for 30 min, $\lambda_{\text{ex}} = 488$ nm. (a),(d) Bright-field transmission images; (b),(e) Fluorescence images; (c),(f) Overlay of the fluorescence and bright-field transmission images.

Bright-field**Fluorescence****Overlay****(a)****(b)****(c)****(d)****(e)****(f)**