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ORIGINAL ARTICLE

A Turn on ESIPT Probe for Rapid and Ratiometric Fluorogenic Detection of Hg^{2+} and its Application in Live-Cell Imaging

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Abstract A probe based on 2-(2′-hydroxyphenyl) benzothiazole (HBT) and thiophosphate has been synthesized and used for the ratiometric detection of Hg^{2+}. The probe was designed in such a way that the excited state intramolecular proton transfer (ESIPT) of the HBT moiety get blocked. The probe exhibited a strong fluorescence enhancement upon addition of Hg^{2+} while showing almost no response to other cations in CH_3CN/HEPES buffer solution. The probe exhibited fast selectivity towards Hg^{2+} and could be completed in 1 min. Fluorescence imaging experiments of Hg^{2+} in living TE-1 cells demonstrated its value of practical applications in biological systems.

Keywords Hg^{2+} · ESIPT · Thiophosphate · Fluorescence · Deprotection

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Introduction

The design and development of fluorescent probes for the detection of heavy and transition metals are significant due to their vital role in biological and environmental application in recent years [1–4]. Mercury is one of the most prevalent toxic metals in both the environment and biological system [5, 6]. Even at a very low concentration, the mercuric ion (Hg^{2+}) which combines with both inorganic and organic ligands, can readily penetrate through biological membranes. Mercury can cause serious and irreversible DNA damage, mitosis impairment and nervous system defects [7–9]. Therefore, it is of great importance to develop advanced methods for detecting mercury ions in biological system and natural environment. Until recently, many excellent works of Hg^{2+} sensing by synthesized fluorescent probes have been reported and investigated [10–12].

Among these works been reported, many fluorescent probes were based on single emission intensity change [13–15]. However, changes in the emission intensity at a single wave length being the only detection signal, such turn-on probes tend to be affected by the variations in the sample and probe environment, illumination intensity or instrumental efficiency [16]. Ratiometric probes can eliminate most of these interferences through simultaneous recording ratio signals of two emission intensities at different wavelengths, which provided a built-in correction for the environmental effects [17, 18]. From this point of view, 2-(2′-hydroxyphenyl) benzothiazole (HBT) is very familiar because of its intramolecularly hydrogen-bonded property, which exhibits excited state intramolecular proton transfer (ESIPT) [19, 20]. There were a number of reactive probes reported based on the HBT moiety for the selective detection of different analytes via "protection-deprotection" sequence [21–23]. According to the strong thiophilic affinity of Hg^{2+}, many chemodosimeters

contained an "S″ group [7, 24, 25]. Thus, along with the leaving of HgS, the "protection-deprotection" reaction is accomplished, as well as the resulting ESIPT modulated fluorescence off–on response.

Herein, we present a simple and new fluorescent probe **BTP** for the detection of Hg^{2+} based on ESIPT mechanism. Probe **BTP** contained an "P = S″ group, and as expected, it exhibited a nonreversible, highly selective and sensitive recognition toward Hg^{2+} over other examined metal ions in CH_3CN/HEPES (10 mM, pH = 7.4, 1:4, *v*/v) solution. Additionally, according to the fluorescence imaging experiments of Hg^{2+} ions in living TE-1 cells, **BTP** could be used for detecting Hg^{2+} in biological samples.

Experimental Section

Apparatus

Fluorescence spectra were recorded on the F-7000 FL Spectrophotometer (Hitachi, Japan), and the excitation and emission wavelength band passes were both set at 5.0 nm. ^1H and ^{13}C NMR spectra were recorded using a Bruker DTX-400 spectrometer. Samples were dissolved in $CDCl_3$ and placed in 5 mm NMR tubes, TMS was used as internal reference. ESI mass spectra were carried out on an HPLC Q-Exactive HR-MS spectrometer (Thermo, USA) by using methanol as mobile phase. Fluorescence images experiments were carried out with a Zeiss-Axio Observer D1 inverted fluorescence microscope.

Materials

All chemicals reagents were used as received from commercial sources without further purification. Solvents for chemical synthesis and analysis were purified according to standard procedures. Deionized water was used throughout the experiment. Chloride salts of metal ions (Li^+, K^+, Na^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Zn^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Cr^{3+}, Hg^{2+}, Al^{3+}) and the nitrate salts of Ag^+, Pb^{2+} and Fe^{3+} ions were prepared as 10.00 mM in water solution.

Synthesis of Probe BTP

The synthetic routine of probe **BTP** is outlined in Scheme 1. HBT was synthesized by a similar way described in a reported method [26]. Dimethylthiophosphinoyl chloride (105 μL, 1 mmol) in 20 mL dichloromethane was added to a mixture of HBT (136 mg, 0.60 mmol) and triethylamine (138 μL, 1 mmol) in 20 mL dichloromethane. The reaction mixture was stirred at room temperature for 4 h, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, hexane/ethyl acetate =2/1) to afford **BTP** (124 mg, 65 %) as a white powder. ^{31}P NMR (162 MHz, $CDCl_3$): δ = 96.16 ppm. ^1H NMR (400 MHz, $CDCl_3$, ppm) δ: 2.11 (d, 3 H, *J* = 4 Hz), 2.13 (d, 3 H, *J* = 4 Hz),7.35 (t, 1 H, *J* = 8 Hz), 7.50 (m, 3 H), 7.88 (d, 1 H, *J* = 8 Hz), 7.96 (d, 1 H, *J* = 8 Hz), 8.13 (d, 1 H, *J* = 8 Hz), 8.33 (d, 1 H, *J* = 4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ: 23.88, 24.61, 121.40, 121.69, 121.74, 123.31, 125.21, 125.38, 125.84, 125.89, 126.41, 130.81, 131.47, 135.54, 148.80, 148.89, 152.63, 162.45; HR-MS *m*/*z*: Calcd for $C_{15}H_{15}NOPS_2^+$ ($[M + H^+]^+$) 320.0333, found 320.0315 $[M + H^+]^+$, 342.0129 $[M + Na^+]^+$.

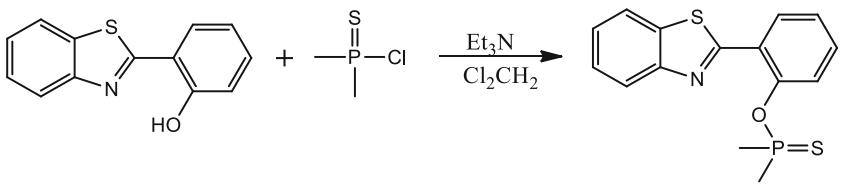
Results and Analysis

Probe **BTP** was dissolved in CH_3CN to make a 1 mM stock solution. Then the stock solution was further diluted to require concentration for measurement.

Fluorescence Spectral Responses of BTP

As is well known, the HBT and its derivatives produced the ESIPT tautomers (the keto forms), which showed fluorescence more powerfully at longer wavelengths compared to the phenol forms upon irradiation. The selectivity of **BTP** was observed in the fluorescence emission profile of **BTP** (10 μM) in a CH_3CN/HEPES (10 mM, pH = 7.4, 1:4, *v*/v) solution with appropriate amounts of metal ions (Fig. 1. inset). Probe **BTP** alone displayed an emission band centered at 377 nm, when excited at 310 nm. Upon addition of 10 eq. Hg^{2+}, the emission at 377 nm decreased, and a significant enhancement at 470 nm emerged quickly. This indicated that the chemical reaction between Hg^{2+} and the receptor (thiophosphinated phenolic) started at this minimum

Scheme 1 Synthetic route of probe **BTP**



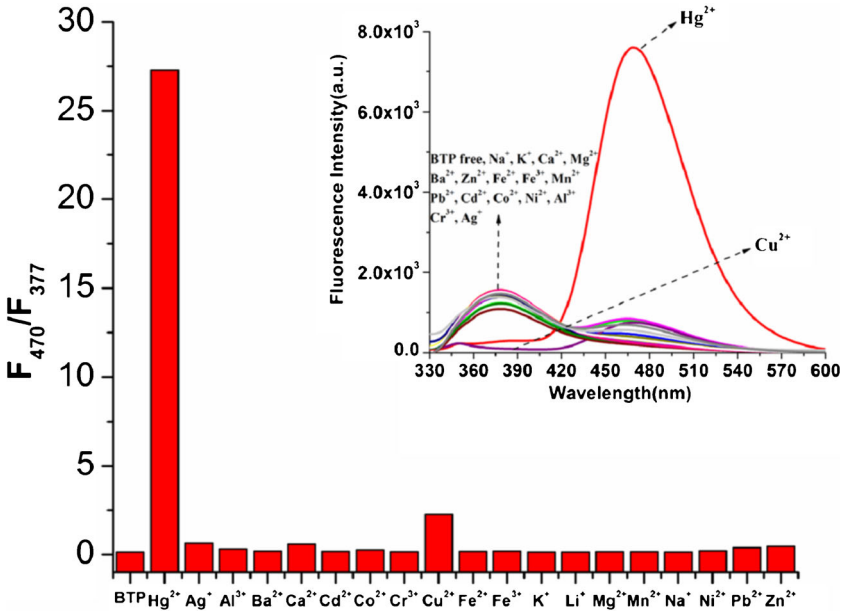


Fig. 1 Fluorescence intensity ratio (F_{470}/F_{377}) of **BTP** (10 μM) in the presence of 10 eq. different metal ions in CH_3CN/HEPES (10 mM, pH = 7.4, 1:4, *v*/v) solution. Inset: Fluorescence spectra of **BTP** (10 μM) in the presence of 10 eq. different metal ions in CH_3CN/HEPES (10 mM, pH = 7.4, 1:4, *v*/v) solution. Λ_{ex} = 310 nm, scan range 330–600 nm, slit width 5 nm

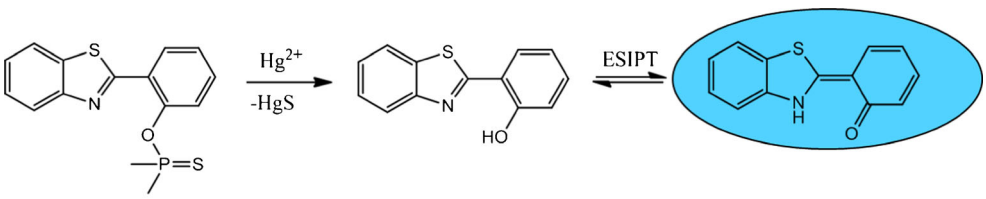
concentration and thus the ESIPT properties of HBT were demasked (Scheme 2). The fluorescence intensity ratio (F_{470}/F_{377}) of probe **BTP** toward different metal ions was recorded in Fig. 1, which exhibited a prominent enhancement of the fluorescence ratio (F_{470}/F_{377}) in the presence of 10 eq. Hg^{2+}. In the meantime, no response could be observed upon the addition of the same amount of other ions. This strongly suggested that **BTP** can serve as a high sensitivity for Hg^{2+}.

Furthermore, as shown in Fig. 2, the ratiometric fluorescence signal response of probe **BTP** toward Hg^{2+} in the presence of various coexistent anions such as NO_3^-, NO_2^-, Cl^-, Br^-, PO_4^{3-}, SO_4^{2-} and CO_3^{2-}, which revealed that all the tested anions have little interference on the detecting of Hg^{2+}. It was also investigated that the competitive experiment also confirmed that the background metal ions showed very low interference with the detection of Hg^{2+} (Fig. S5), only Cu^{2+} has posed a negligible effect on the fluorescence response of **BTP** for Hg^{2+}, it may due to the quenching effect of the paramagnetic Cu^{2+} [27]. Therefore, these results suggested that probe **BTP** has a high selectivity for Hg^{2+} in the presence of these tested foreign metal ions and anions.

As shown in Fig. 3, the time dependence of the response of **BTP** to Hg^{2+} ions was investigated. It can be seen that the fluorescence intensity ratio signal of the **BTP** with Hg^{2+} ion increased for a few seconds, and leveled off as the time continues, while the fluorescence intensity of blank solution (only **BTP**, 10 μM) showed almost unchanged at the same conditions. The time-dependent change plot demonstrated the reaction could complete in about 1 min, which indicated the probe **BTP** had a fast response for Hg^{2+}. Therefore, a 1 min reaction time was selected in subsequent experiments in order to make the metal ions chelate with the sensors sufficiently.

To further investigate the interaction between Hg^{2+} and probe **BTP**, a fluorescence titration experiment was carried out. The fluorescence spectra of **BTP** (10 μM) exposed to CH_3CN/HEPES (10 mM, pH = 7.4, 1:4, *v*/v) solution containing different concentrations of Hg^{2+} were then recorded at an excitation wavelength of 310 nm (Fig. 4). Upon treatment with increasing concentrations of Hg^{2+}, the fluorescence intensity ratio (F_{470}/F_{377}) gradually increased, and reached saturation when the amount of Hg^{2+} was more than 12 μM (Fig. S6). Moreover, a linear relationship was found between the fluorescence intensity ratio (F_{470}/F_{377}) and the Hg^{2+} concentration from 4 to 12 μM (Fig. S7), the detection limit (3σ/slope) of probe **BTP** for the determination of Hg^{2+} was found to be 12 nM [28, 29].

Scheme 2 Hg^{2+}-promoted deprotection of **BTP** to compound **HBT**



Chemical Formula: $C_{15}H_{14}NOPS_2$
Exact Mass: 319.0254

Chemical Formula: $C_{13}H_9NOS$
Exact Mass: 227.0405

Highly Fluorescent

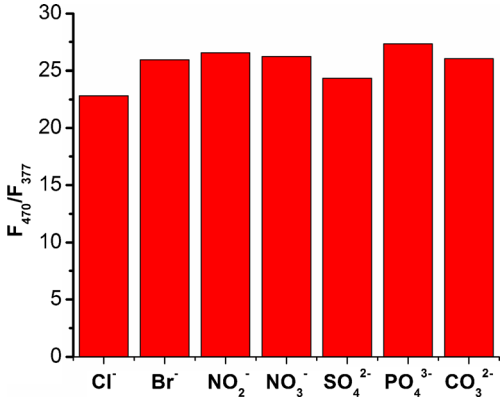


Fig. 2 Fluorescence intensity ratio (F_{470}/F_{377}) of **BTP** (10 μM) upon addition of 10 eq. Hg^{2+} in the presence of 10 eq. background various coexistent anions in CH_3CN/HEPES (10 mM, pH = 7.4, 1:4, *v*/v) solution. Λ_{ex} = 310 nm, scan range 330–600 nm, slit width: 5 nm

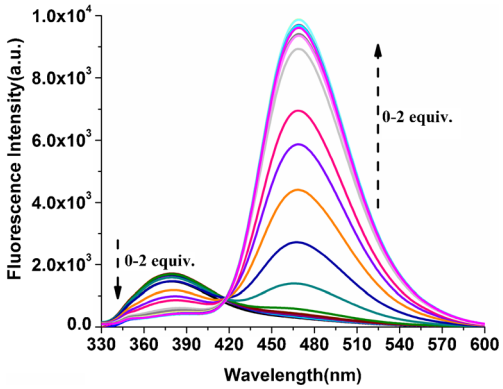


Fig. 4 Fluorescence emission spectra of **BTP** (10 μM) with gradual addition of various amounts of Hg^{2+} (from bottom 0–2 eq.) in CH_3CN/HEPES (10 mM, pH = 7.4, 1:4, *v*/v) solution. Λ_{ex} = 310 nm, scan range 330–600 nm, slit width: 5 nm

These results demonstrated that probe **BTP** could detect Hg^{2+} quantitatively.

For practical applicability, the proper pH condition of this new probe for Hg^{2+} detection was also evaluated. We investigated the fluorescence properties of probe **BTP** and that probe **BTP** with Hg^{2+} (10 eq.) under different pH values, respectively (Fig. 5). Probe **BTP** was pH insensitive, and its ratiometric fluorescence response (F_{470}/F_{377}) was quite weak from pH 4 to 9. However, the fluorescent sensing toward Hg^{2+} was obviously affected by the change of pH values. Ratiometric fluorescence response (F_{470}/F_{377}) reached its maximum and kept constant around biologically relevant pH 4 to 9, indicating that its potential for application in biological systems.

Mechanism

In addition, the KI-adding experiments were conducted to examine the reversibility of this reaction as shown in Fig. S8. When excess KI (2 eq. of Hg^{2+}) was added to the **BTP** (10 μM) and Hg^{2+} (100 μM) in CH_3CN/HEPES (10 mM, 1:4, *v*/v) solution, the fluorescence intensity at 470 nm almost unchanged, indicating that the coordination of **BTP** with Hg^{2+} was chemically nonreversible.

According to the previous reported work [30–32], we proposed that the strong fluorescence enhancement was attributed to the deprotection of dimethyl- thiophosphinoyl group and concurrent generation of HBT. The observed change in presence of Hg^{2+} may arise from the HBT moiety which was released from **BTP** with the leaving of HgS. In order to verify this speculation, the reaction products of probe **BTP** and Hg^{2+} were subjected to ESI-HRMS spectrum. A major ion peak was founded at m/z = 228.0469 (Fig. S9), corresponding to the resulting HBT ($[M + H]^+$), clearly confirmed the proposed mechanism as shown in Scheme 2.

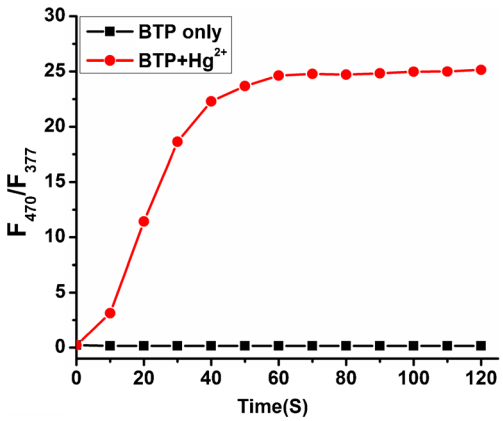


Fig. 3 Effect of reaction time on fluorescence intensity ratio (F_{470}/F_{377}) of **BTP** (10 μM) in the absence and presence of 10 eq. Hg^{2+} in CH_3CN/HEPES (10 mM, pH = 7.4, 1:4, *v*/v) solution. Λ_{ex} = 310 nm, scan range 330–600 nm, slit width 5 nm

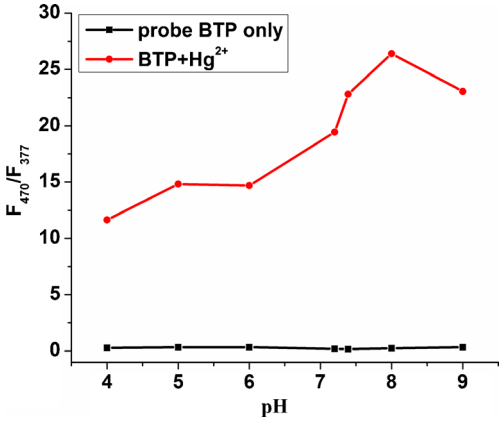


Fig. 5 Ratiometric fluorescence response (F_{470}/F_{377}) of free **BTP** (10 μM) and in the presence of 10 eq. Hg^{2+} in CH_3CN/HEPES (10 mM, 1:4, *v*/v) solution with different pH (10 mM HEPES) conditions. Λ_{ex} = 310 nm, scan range 330–600 nm, slit width: 5 nm

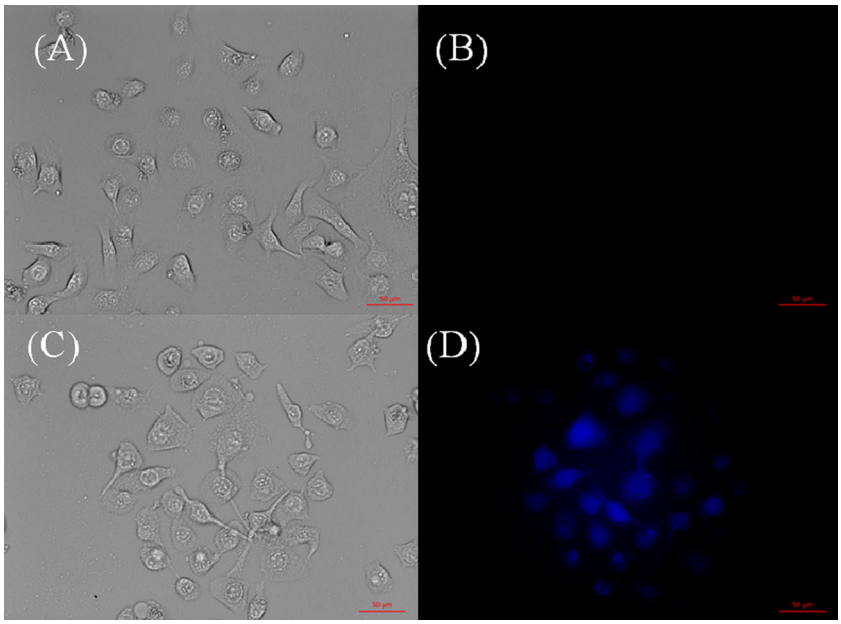


Fig. 6 Fluorescence images of Hg^{2+} in TE-1 cells with 10 μM solution of **BTP** in PBS buffer for 30 min at 37 °C, bright-field transmission images (**a**, **c**) and fluorescence images (**b**, **d**) of TE-1 cells incubated with 0 μM, 30 μM of Hg^{2+} for 30 min, respectively (λ_{ex} = 340 nm, blue channel)

Bioimaging Applications of Probe BTP in TE-1 Cells

We further investigated the practical application of **BTP** in biological systems [33, 34]. Fluorescent imaging inside TE-1 cells was monitored by fluorescence microscopy. As shown in Fig. 6, very weak fluorescence of **BTP** inside the living TE-1 cells was observed (Fig. 6b). After washing with water twice, 30 μM of Hg^{2+} were then supplemented to the cells. After incubated at 37 °C for another 30 min, a significant increase in the fluorescence from the intracellular area was observed (Fig. 6d). A bright field transmission image of cells with **BTP** and **BTP** with Hg^{2+} confirmed that the cells were viable throughout the imaging experiments (Fig. 6a and c). Therefore, these results demonstrated that probe **BTP** was cell membrane permeable and capable of fluorescence imaging of Hg^{2+} in biological samples.

Conclusion

In summary, a new ESIPT-based ratiometric fluorescence probe **BTP** for Hg^{2+} was prepared and reported. Probe **BTP** exhibited highly selective binding with Hg^{2+} over other metal ions and various coexistent anions in CH_3CN/HEPES (10 mM, 1:4, *v*/v) solution. Moreover, the preliminary experimental results demonstrated that probe **BTP** could be used for detecting Hg^{2+} in biological samples.

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