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A reaction-based ratiometric fluorescent probe for mercury ion detection in aqueous solution

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ABSTRACT

Mercury ions are crucially harmful to ecosystem and human being due to their characters of bioaccumulation and difficulty of biochemical degradation. Therefore, development of mercury ion detection methods has attracted increasing interests recently. In this study, we successfully synthesized a hydroxyphenylbenzothiazole (HBT)-based fluorescent probe **HBT-Hg** in an extremely simple manner for mercuric ions detection. The spectral studies revealed that the probe **HBT-Hg** could react with Hg^{2+} selectively and sensitively in PBS buffer (10 mM, pH = 7.40), showing ratiometric fluorescent changes from blue to light green. The response mechanism of the probe **HBT-Hg** and Hg^{2+} was finally confirmed by HPLC analysis, *viz.*, the probe **HBT-Hg** converted to its precursor compound **1**. Finally, the probe HBT-Hg was successfully applied in monitoring Hg^{2+} in living A549 cells.

1. Introduction

The detection methods of mercuric compounds have attracted increasing attentions of chemists and environmentalists due to their fatal toxicity to ecological environment and organisms [1–4]. Several traditional methods are popularly adopted for mercuric compounds detection, including colorimetric, atomic absorption/emission spectroscopy (AAS/AES), inductively coupled plasma mass spectrometry (ICP-MS) [1]. For colorimetric analysis, its main disadvantage is that the detection limit is too high, which is very unfavorable for mercuric analysis. Although the other two methods with high specificity and precision, the analysis methods essentially depend on the expensive instruments, particularly unsuitable for instant and fast detection. Fluorescent probe is a promising tool for analysis of various species in the fields of chemistry, medicine, biology and environment, which is developing rapidly to meet the current needs [5–22]. In recent years, a large number of Hg^{2+} probes have been developed based on different fluorophores [23–31], including coumarin, naphthalimides, rhodamine, resorufin, cyanine, BODIPY, and so on. Even so, there remains a lot of drawbacks to current fluorescent probes during their practical use, such as poor water solubility, slow response rate (especially for reaction-based probe) as well as low sensitivity [32]. In order to overcome these issues, novel fluorescent probes based on various fluorophores are still to be developed.

The development of novel fluorescent probes depends on two main factors, fluorescence signal transmission and recognition sites. As signal transducers in fluorescent probes, novel fluorophores with excellent optical performances and fine-tuning of its specific photo-physical properties remains a profound challenge. Recently, Hydroxyphenylbenzothiazole (HBT) derivatives have attracted great attentions because of their excellent optical performances [33–36]. Thus, HBT, as the initial skeleton, is expanded to form a mega library of HBT derivatives by conjugation with different functional groups and further used as fluorophores to design probe for different species analysis. In particular for 2-(2-hydroxyphenyl) benzothiazole, well-known for its ESIPT mechanism, were extended for design of target probes. However, a limited number of probes were developed based on 2-(4-hydroxyphenyl)benzothiazole. In our lab, we have developed a series of fluorescent probes based on this skeleton of HBT for different species analysis, such as metal ions and anions [37–40]. Different recognition sites adopted for mercuric detection were summarized in mainly two manners. One is ligands containing hetero-atoms can coordinate with Hg^{2+}, and the other is Hg^{2+}-induced reaction. However, coordination-based probes generally suffered from interferences of other metal ions with similar coordinate properties. Although Hg^{2+}-induced reaction for designing novel probe with high selectivity, most of current reaction-based Hg^{2+} fluorescent probe was performed in mixed

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solution containing a high proportion of organic solvent to ensure the reaction proceeds at a certain speed. There remains a great challenge to design a novel fluorescent probe for Hg^{2+} detection.

Hence, consideration of above mentioned factors and combined our previous work, we have selected HBT fluorophores as signal transduction group to designed a fluorescent probe **HBT-Hg** in a step from compound **1** with a high yield of 83.9% (Scheme 1), and was fully characterized by ^1H NMR, ^{13}C NMR and HRMS spectra (Fig. S1-S3). In the presence of Hg^{2+}, a reaction was triggered. The probe **HBT-Hg** converted to compound **1**, accompanied with a ratiometric fluorescence change from blue to light green. The response mechanism was further confirmed by HPLC analysis. All the spectral studies demonstrate the probe **HBT-Hg** can be used for detection of Hg^{2+} with high selectivity and sensitivity.

2. Experimental

2.1. General methods

All reagents, including 2-aminobenzenethiol, *p*-hydroxybenzaldehyde, toluene, acetic acid, 1, 3-dimercaptopropane and hexamethylenetetramine, were analytical grade and purchased from commercial supplies without further purification. NMR spectra were performed on Bruker 500 MHz instruments (AVANCE IIITM 500). MS spectrum was obtained on LTQ-Orbitrap-ETD high resolution mass spectrometer (Thermo Scientific, Orbitrap Elite). Live cell imaging was carried out on Olympus fluorescent microscope (Fluo View FV1000). UV–vis absorption and fluorescence spectral studies were recorded on an Evolution 220 UV–Visible (Thermo) and FluroMax-4 Spectrophotometer (HORIBA Scientific), respectively.

2.2. Reaction mechanism studies

Reaction mechanism studies of the probe **HBT-Hg** with Hg^{2+} were performed on an Agilent Technologies 1260 Infinity HPLC system. All the prepared samples (**HBT—Hg**, Compound **1**, and their mixture) were passed through a 0.22 μm filter before each sample (20 μL) was loaded onto a reversed-phase column, Agilent ZORBAX SB-C18 (5 μm, 4.6 × 150 mm). The column was eluted using a mixture of methanol and water (70:30). The flow rate was set at 0.6 mL min^{-1}. A UV/vis detector was used to monitor the samples at the wavelength of 330 nm. The probe **HBT-Hg** (10 μM) was incubated with Hg^{2+} (100 μM) for 5 min in PBS buffer.

2.3. Fluorescence imaging

We used the live A549 cells for fluorescence imaging. A549 live cells (4×10^5) were cultured in 15 mm plates and allowed to grow overnight. The probe **HBT-Hg** (10 μM) was added to the plate and continued culture for 5 min, and further treatment with 100 μM Hg^{2+} for 2 min, 5 min, and 10 min. Then the cells were visualized and photographed on fluorescent microscope without washing.

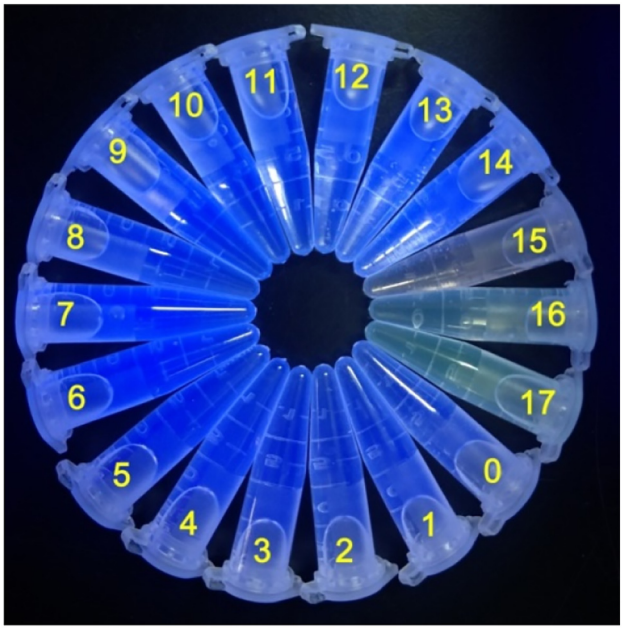
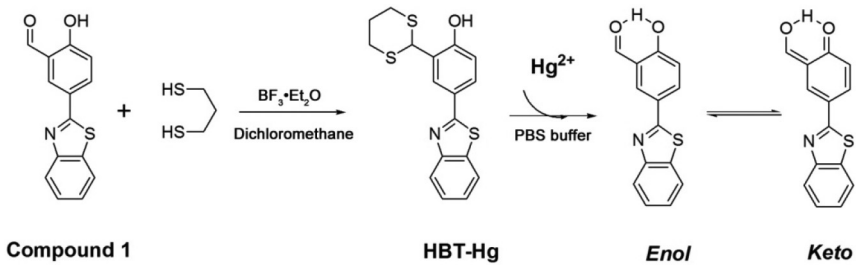


Fig. 1. Fluorescence changes of probe **HBT-Hg** (10 μM) towards various metal ions (100 μM) in PBS buffer (10 mM, pH = 7.40) under 365 nm lamp. 0 and 17 represent free probe **HBT-Hg** and Compound **1**, respectively. 1–16 represent the probe in presence of different metal ion including Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Al^{3+}, Cr^{3+}, Fe^{3+}, Cd^{2+}, Ni^{2+}, Fe^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, Ag^+, Hg^{2+}.

2.4. Synthesis of probe HBT-Hg

Compound **1** was easily obtained in two steps involving ring-forming condensation reaction and formylation reaction according to our previous procedures [37–39].

The probe **HBT-Hg** was synthesized according to reported literatures with some modification [27]. The compound **1** (1 mmol, 255 mg) and 1, 3-dimercaptopropane (1.1 mmol, 119 mg) was added to the solution of dichloromethane. Under stirring, the $BF_3 \cdot Et_2O$ (50 μL) was further added drop-wise to the mixture in an ice-water bath, and then the reaction was monitored by TLC until the reagents were basically consumed. And then the mixture was removed under reduced pressure. The crude product was purified by column chromatography with a yield of 83.9%, eluting with ethyl acetate/petroleum ether (1:5). ^1H NMR (500 MHz, DMSO-d_6) δ 10.31 (s, 1H), 8.30 (s, 1H), 8.08 (d, J = 16.3 Hz, 1H), 7.95 (dd, J = 8.8, 1.6 Hz, 1H), 7.90 (d, J = 8.9 Hz, 1H), 7.78 (d, J = 8.7 Hz, 1H), 7.24 (d, J = 16.3 Hz, 1H), 7.20–7.05 (m, 2H), 1.81 (s, 6H). ^{13}C NMR (126 MHz, Acetone-d_6) δ 167.99, 157.18, 155.34, 135.70, 129.59, 129.51, 128.01, 127.32, 126.83, 125.97, 123.67, 122.77, 117.07, 44.10, 32.70, 26.20. HRMS (m/z): 346.0389.$[M + 1]^+$, calcd. for $C_{17}H_{15}NOS_3$ = 345.032.



Scheme 1. Synthetic route of probe **HBT-Hg** and its response mechanism.

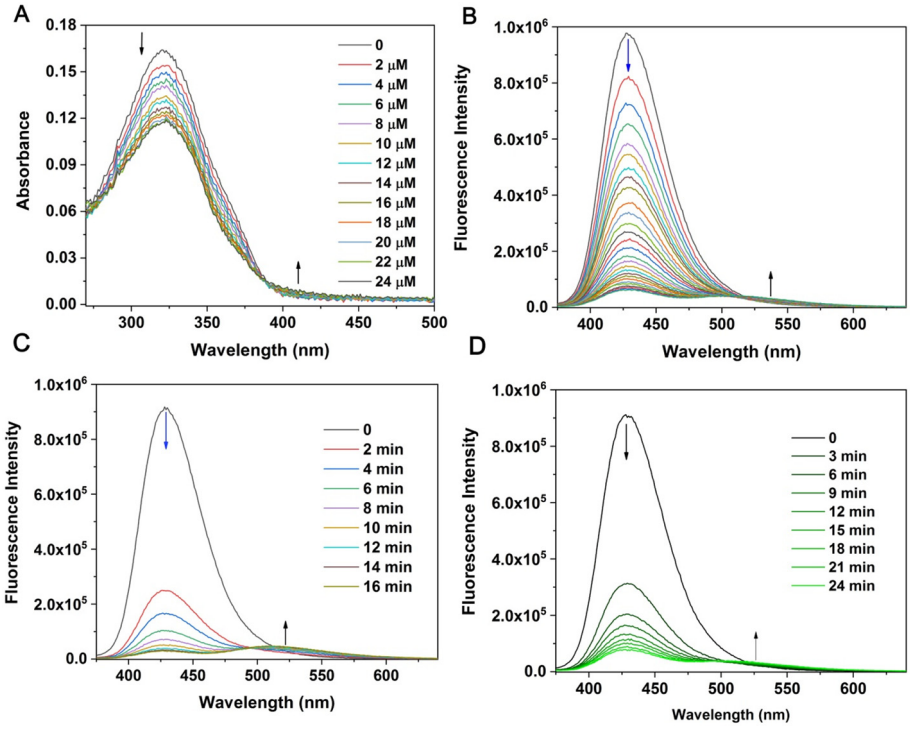


Fig. 2. Spectral studies of probe **HBT-Hg** towards Hg^{2+} in PBS buffer (10 mM, pH = 7.40). (A) Absorption spectra of probe **HBT-Hg** (10 μM) upon addition of various amount of Hg^{2+} (0–24 μM). (B) Fluorescent spectra of probe **HBT-Hg** (10 μM) upon addition of various amount of Hg^{2+} (0–54 μM). (C) The time-dependent fluorescent response of probe **HBT-Hg** (10 μM) towards Hg^{2+} (100 μM). (D) The time-dependent fluorescent response of probe **HBT-Hg** (10 μM) towards Hg^{2+} (10 μM). λ_{ex} = 350 nm.

3. Results and discussion

3.1. Fluorescence changes of probe HBT-Hg towards metal ions

With the probe **HBT-Hg** in hand, we firstly qualitatively investigated the fluorescence response of the probe by addition of 10 equiv. of different metal ions in PBS buffer (10 mM, pH = 7.40). As shown in Fig. 1, the probe **HBT-Hg** shows strong blue fluorescence (Eppendorf tube 0) under lamp 365 nm. There is no significant fluorescence signals change except two metal ions, Hg^{2+} and Ag^{+}. However, Ag^{+} only induced fluorescence quenching (Eppendorf tube 15) while the fluorescence change from blue to light green in presence of Hg^{2+} (Eppendorf tube 16) in accordance with the fluorescence of compound **1** (Eppendorf tube 17), suggesting only Hg^{2+} can induce the reaction that the probe **HBT-Hg** converted to compound **1**.

3.2. Spectral studies of probe HBT-Hg towards Hg^{2+}

Inspired by above initial results of fluorescent changes qualitatively observed by naked eye, UV–vis absorption and fluorescent spectral studies of the probe **HBT-Hg** response to Hg^{2+} were further carried out for quantitative Hg^{2+} analysis. As shown in Fig. 2A, there is only an absorption band center at 320 nm when the probe **HBT-Hg** in PBS buffer. The absorbance was gradually decreased after addition of Hg^{2+} from 0 to 24 μM, and a new absorbance band center at 420 nm formed with slight enhancement simultaneously, accompanied with an isosbestic point at 385 nm. Meanwhile, the fluorescence titration experiments were carried out and depicted in Fig. 2B. As the Hg^{2+} was added from 0 to 54 μM to the solution of probe **HBT—Hg**, the fluorescence of probe **HBT-Hg** at 430 nm was significantly quenched while a new peak formed at 520 nm.

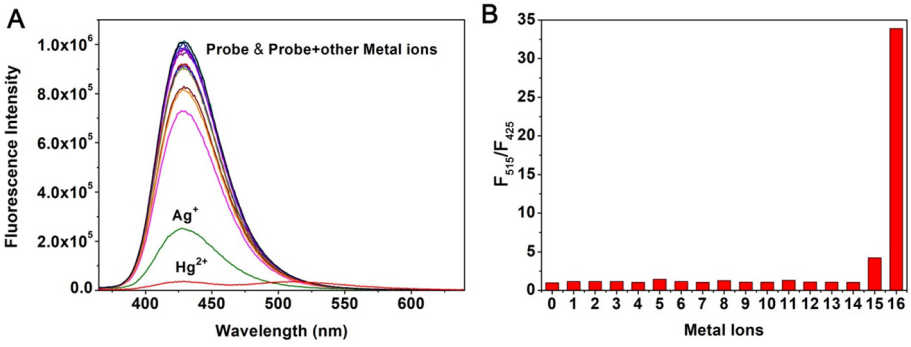


Fig. 3. Selectivity experiment of probe **HBT—Hg**. Fluorescence intensity changes of probe (10 μM) upon addition of various ions (100 μM): 0 represents free probe **HBT—Hg**. 1–16 represent the probe in presence of different metal ions including Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Al^{3+}, Cr^{3+}, Fe^{3+}, Cd^{2+}, Ni^{2+}, Fe^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, Ag^{+}, Hg^{2+}. λ_{ex} = 350 nm.

Subsequently, the time- and dose-depend fluorescent response of probe **HBT-Hg** (10 μM) towards Hg^{2+} (10, 100 μM) was performed and shown in Fig. 2C&2D. In order To clearly observe its ratiometric character, the amplified image of the long-wavelength peak was shown in Fig. S4. Significantly, brilliant fluorescent changes were observed when the probe was treated with Hg^{2+} as increasing time. By comparison, the probe **HBT-Hg** showed a fast response even if at 1 equiv. of Hg^{2+}. Overall, these preliminary dose- and time-depend investigations of absorption and fluorescence studies indicated the probe **HBT-Hg** could be used for rapid and sensitive monitoring Hg^{2+} in complete aqueous solution.

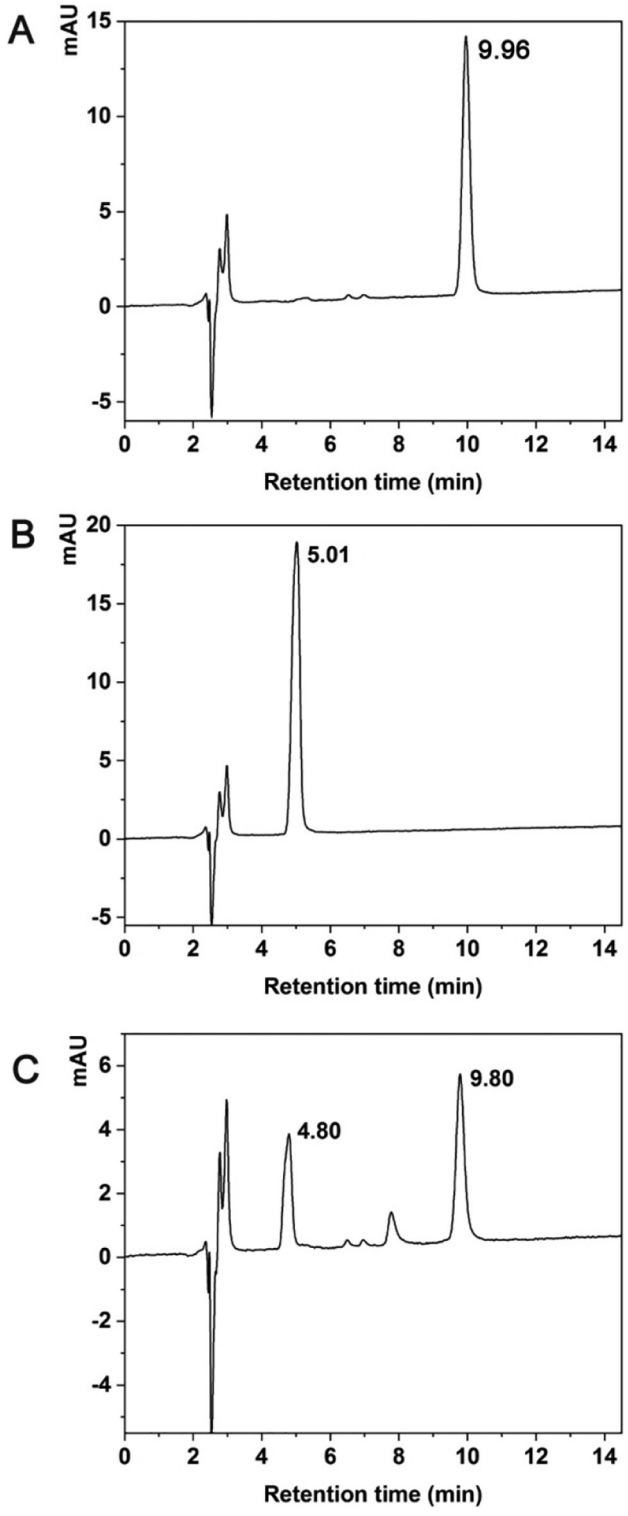


Fig. 4. Mechanism studies of probe **HBT-Hg** with Hg^{2+} by HPLC analysis. (a) The probe **HBT-Hg** (10 μM), (b) The compound **1** (10 μM), (c) The probe **HBT-Hg** (10 μM) was incubated with Hg^{2+} (100 μM) for 5 min in PBS buffer.

3.3. Selectivity experiments

In order to quantitatively evaluate the selectivity of the probe **HBT-Hg** towards Hg^{2+}, we further carried out fluorescent spectral studies of probe **HBT-Hg** with various metal ions, such as Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Al^{3+}, Cr^{3+}, Fe^{3+}, Cd^{2+}, Ni^{2+}, Fe^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, Ag^{+}, Hg^{2+}. As shown in Fig. 3, the results is highly similar to that described in Fig. 1, the emission peak at 430 nm was significantly quenched while a new peak was formed at 520 nm only when probe **HBT-Hg** was interacted with Hg^{2+}. Obviously, Ag^{+} just induced a certain extent fluorescence quenching at same peak center without a new peak formation. Furthermore, fluorescence titration experiments of the probe with Ag^{+} were carried out for comparison shown in Fig. S5. Meanwhile, there is no appreciable response by addition of other metal ions. By comparison of fluorescence ratio changes of F_{515}/F_{425}, only Hg^{2+} caused a noticeable fluorescent response reached as high as an approximate 34-fold enhancement (Fig. 3B). All these results clearly indicated the probe **HBT-Hg** with high selectivity for Hg^{2+} over other metal ion in PBS buffer.

3.4. Studying reaction mechanism by HPLC analysis

Although the solid spectral studies indicated that the probe **HBT-Hg** could react with Hg^{2+}, we performed HPLC experiments to support the reaction mechanism proceed as depicted in Scheme 1. All the samples of probe **HBT—Hg**, compound **1**, and their mixture (reaction for 5 min before load onto a reversed-phase column) were prepared in PBS buffer, and then were recorded on HPLC instrument. In Fig. 4c, the retention time of the mixture sample (The probe **HBT-Hg** interacted with Hg^{2+} for 5 min) was consistent with that of compound 1 (Fig. 4b), suggesting that the probe **HBT-Hg** converted to its precursor compound **1**.

3.5. Fluorescence imaging

To verify practicability of the probe **HBT—Hg**, we carried out fluorescence imaging for visualization of Hg^{2+} in living A549 cells. The blue fluorescence signals were collected (the channel from 410 nm to 510 nm) using 405 nm as excitation. As shown in Fig. 5b, after incubation with **HBT-Hg** for 5 min, the A549 cells exhibit a bright blue fluorescence. The cells were further pretreated with Hg^{2+} for different time (2 min, 5 min and 10 min). The fluorescence signal was obviously quenched in Fig. 5c-e. The spectral studies firmly demonstrated that the probe **HBT-Hg** could sense Hg^{2+} with ratiometric character. Therefore, we also try to collect the green fluorescence signals (the channel from 500 nm to 600 nm). Regrettably, we did not obtain the positive results which may relative with slight fluorescence enhancement at 515 nm. Even so, the current results of imaging suggested the probe **HBT-Hg** had ability to monitor Hg^{2+} in living cells.

4. Conclusion

In summary, we have successfully developed a HBT-based fluorescent probe **HBT-Hg** by a simple synthesized step with a high yield for ratiometric detection of Hg^{2+}. The solid evidences confirmed that the probe **HBT-Hg** could sensitively and selectively react with Hg^{2+} in complete aqueous solution, and accompany with a change of red-shift in wavelength. Furthermore, the reaction mechanism was determined by HPLC analysis that the probe **HBT-Hg** converted to its precursor compound **1**. Finally, the probe **HBT-Hg** was successfully applied in monitoring Hg^{2+} in living A549 cells. All results demonstrated the probe **HBT-**

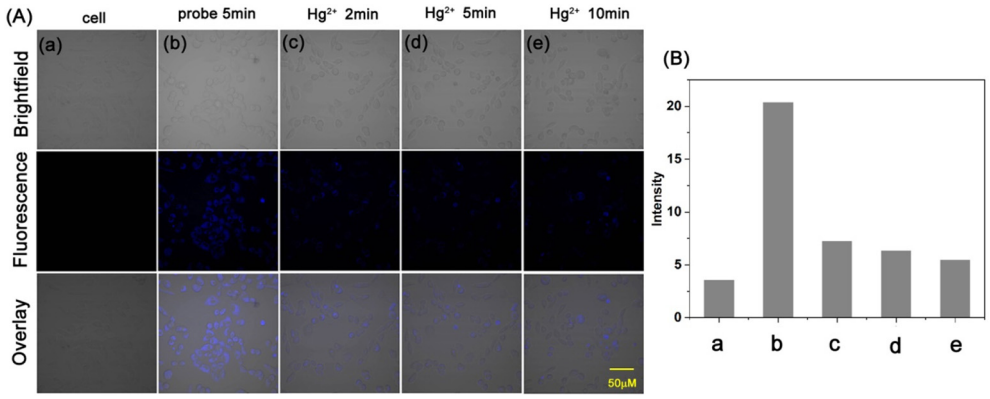


Fig. 5. (A) Fluorescence imaging of Hg^{2+} in living cells using **HBT—Hg**. The A549 cells (a). The cell treated with probe **HBT-Hg** (10 μM) for 5 min (b). The cells were further incubated with Hg^{2+} (100 μM) for 2 min(c), 5 min (d) and 10 min (e). (B) Relative fluorescence intensity was quantified by imageJ.

Hg is a useful tool for Hg^{2+} monitoring with potential application prospects.

CRediT authorship contribution statement

Shudi Liu: Fluorescent probe synthesis, spectral analysis, Writing - Original draft preparation and revising; **Di Feng**: Fluorescent probe synthesis, spectral studies; **Liangwei Zhang**: Spectral analysis, Writing - Original draft preparation and revising; **He Song**: Fluorescent probe synthesis, spectral studies; **Yue Wang**: Fluorescence imaging; **Xia Zhang**: Reaction mechanism studies, **Qingjun Zhao**: Fluorescent probe synthesis, spectral studies; **Lingxin Chen**: Conceptualization, Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2020.118817.

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