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A ratiometric benzothiazole-based fluorescence probe for selectively recognizing HClO and its practical applications

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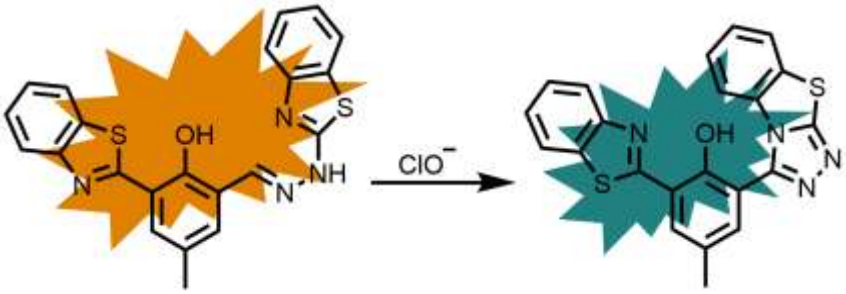
Graphical abstract



Abstract

A ratiometric probe (**HBT-HBZ**) bearing 2-hydrazino benzothiazole and 3(benzo[d]thiazol-2-yl)-2-hydroxy-5-methylbenzaldehyde for sensing hypochlorous acid (HClO) with high selectivity and sensitivity is reported in this article. The fluorescence intensity ratios ($I_{470\ nm}/I_{572\ nm}$) of the probe with different concentrations of analyte showed excellent selectivity and a linear response to minor changes in HClO. The detection limit of 24 nM suggests that the sensor is very sensitive to HClO. According to the series of performed experiments, **HBT-HBZ** has practical applications, such as the detection of hypochlorous acid residues in tap water, which has been rarely reported. In addition, confocal laser microscopy experiments confirmed that **HBT-HBZ** can selectively recognize HClO in HeLa cells.

Keywords: HClO; benzothiazole; fluorescence probe; tap water; bioimaging

On the basis of extensive reports, we realized that reactive oxygen species (ROS) actively participate in a variety of biological processes, and hypochlorous acid (HClO) is an important component of ROS; specifically, HClO is produced by immune cells to fight infections [1-3]. When bacteria invade the human body or when the epidermis is damaged, endogenous HClO is secreted from neutrophils in leukocytes in the body to resist these foreign bacteria and viruses by destroying their cell walls and making them unable to survive [4-8]. It is known that chlorine is commonly used to sterilize tap water. The sterilization effect is achieved because the formed HClO in an aqueous

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solution is a strong oxidant that kills harmful substances such as bacteria. Thus, residual amounts of hypochlorous acid in tap water are usually present. In addition, HClO discolors dyes and organic colors; thus, it is commonly used in industrial production or in daily life as a bleach, antioxidant, deodorant and disinfectant. However, excessive amounts of HClO are of great harm to the organism's health and result in tissue necrosis and diseases such as atherosclerosis and cancer [9-11].

For the qualitative and quantitative analysis of hypochlorous acid, many scientific methods have been invented, including mass spectrometry, HPLC analysis and bioanalytical methods etc. [12-14]. When implementing these methods of operation, the process is complicated, time-consuming and labor-intensive, and the equipment is difficult to master. To avoid the shortcomings, chemical sensors (e.g., fluorescence probes) that are designed to detect HClO are essential. It is known that fluorescence probes possess many advantages over the above methods, including high selectivity, sensitivity, and ease of operation. In addition, Fluorescent probes provide strategies for detecting hypochlorous acid in vitro and vivo [15-21].

Thus far, scientists have developed many fluorescence probes to detect HClO [22-31]. Strong oxidation of hypochlorous acid is a key consideration in the synthesis of these sensors [32-35]. However, some of the developed probes do not perform well in a physiological pH range and require excessive amounts of organic solvents. A fluorescent probe with a single emission peak response to hypochlorous acid does not perform well and is susceptible to environmental factors like he concentration of fluorescent probe, excitation intensity and so on [36-41]. Therefore, there is a pressing need to overcome these issues by creating new ratiometric fluorescence probes. By measuring the intensity changes of two emission peaks, ratiometric fluorescence probes tend to perform well in quantitative detection and offer more accurate data. In addition, successful application in living cells detection and some other useful practical applications can make fluorescence probes more attractive.

In this study, a new ratiometric benzothiazole-based fluorescent probe (**HBT-HBZ**) is reported. The developed probe exhibits excellent selectivity for HClO compared with other analytes. **HBT-HBZ** uses 3-(benzo[d]thiazol-2-yl)-2-hydroxy-5-methylbenzaldehyde as a donor. When **HBT-HBZ** is dissolved in a phosphate-buffered saline (PBS) solution, it exhibited weak orange fluorescence after the addition of some HClO into the solution; an intramolecular cyclization reaction immediately occurred between **HBT-HBZ** and HClO, and unbridged C=N transformed into bridged C=N [42]. The fluorescence of the mixture exhibited a strong cyan color. From this phenomenon, we concluded that **HBT-HBZ** can selectively identify HClO among other ions (e.g., ROS and RNS) in the in the PBS solution. Most importantly, **HBT-HBZ** provides an efficient approach for analyzing HClO residues in tap water. Finally, **HBT-HBZ** was used to image HClO in HeLa cells.

3-(benzo[d]thiazol-2-yl)-2-hydroxy-5-methylbenzaldehyde (Ⅱ) was synthesized according to our previous report [43]. The synthetic routes of **HBT-HBZ** are illustrated in **Scheme 1**. A Schiff base reaction occurred between Compound **II** and 2-hydrazino benzothiazole to form **HBT-HBZ**. Specific synthesis and characterization are located in the supporting information (Scheme S1, Figs. S1-S9).

A series of experiments of the **HBT-HBZ** response to ROS, RNS and other ions, (e.g., ClO^-, $ONOO^-$, $^tBuOO\bullet$, $ROO\bullet$, ClO_4^-, HPO_4^{2-}, H_2O_2, CO_3^{2-}, HSO_3^-, SO_4^{2-}, NO_2^-, NO_3^-, SCN^-, S^{2-}, Cl^-, Br^- and I^-), in the PBS solution (50 mM, PH = 7.4) were performed via fluorescence and UV spectroscopy to explore the selectivity of **HBTHBZ** for HClO. The **HBT-HBZ** solution showed an orange fluorescence band at 572 nm; after the addition of HClO (100 μM), the fluorescence intensity considerably increased at 470 nm and decreased at 572 nm. However, there was no major change in the fluorescence of the solution after the addition of other analytes (Fig. S10). The fluorescence intensity ratio between 470 and 572 nm after the addition of different

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analytes is shown in Fig. 1A. In addition, **HBT-HBZ** can clearly distinguish HClO and other analytes by UV spectroscopy (Fig. 1B); specifically, the absorbance of the solution clearly decreased in the range of 270 to 400 nm after adding HClO; other analytes did not show a similar decrease.

Then, fluorescence titration experiments were conducted to determine the sensitivity of **HBT-HBZ** for HClO. With the addition of HClO, a new peak appeared at 470 nm, and its intensity gradually increased, whereas the intensity of emission at 572 nm slightly decreased. Hypochlorous acid can be visually identified under a portable 365 nm UV lamp; after adding HClO, the fluorescence color changed from orange to cyan (Fig. 1C). The ratios of fluorescence intensity ($I_{470\ nm}/I_{572\ nm}$) were mapped as a function of the HClO amount (0 to 10 equiv.), and a linear relationship was observed (Fig. 1D). From the linear curve, 24 nM detection limit was obtained using a well-known formula (Formula. S1). Based on formula S2, the quantum yields of **HBT-HBZ** increased from 0.26 to 0.34 after the addition of HClO (10 equiv.).

We further explored the time effects of **HBT-HBZ** fluorescence with 10 equiv. HClO in the PBS solution (50 mM, pH = 7.4). When HClO was added into the solutions in which **HBT-HBZ** was dissolved, an immediate enhancement in fluorescence470 nm was observed; fluorescence intensity increased for approximately 1200 s and then remained stable (Fig. 2A). To investigate the appropriate pH range for HClO sensing, a series of pH-dependent experiments was performed. In the pH range of 4 to 10, the ratio of fluorescence intensity ($I_{470\ nm}/I_{572\ nm}$) of **HBT-HBZ** is very low and is approximately equal to zero. After adding 10 equiv. of HClO, the ratios showed considerable changes in the pH range of 5 to 10，which indicated that the probe was suitable for use under physiological conditions (Fig. 2B).

Then, we carefully studied the mechanism of hypochlorous acid by **HBT-HBZ**. First, we compared the changes in the ^{1}H NMR spectra with and without the addition of NaClO to the **HBT-HBZ** solution. With the addition of NaClO, the deprotonation of hydroxyl group is occurred, and two proton signals of d = 12.66 ppm (imine) and 12.34 ppm (amine) disappeared (Fig. S11), which indicated that the oxidation of **HBT-HBZ** by HClO occurs on the imine group that was oxidized into a triazolyl group; this was also confirmed by mass spectrometry measurements of the addition of HClO to **HBTHBZ** (Fig. S12). The mechanism of **HBT-HBZ** detection of HClO is shown in **Schemes 2** and **S2**. However, three infrared peaks at 2850, 2912, 2960 nm appeared after the addition of NaClO, which also confirmed that the imine group was oxidized into a triazolyl group (Fig. S14).

To evaluate its practical applications in industrial production and daily life, **HBTHBZ** was used to sense HClO in tap water. Although fluorescent probes for detecting hypochlorous acid have many practical applications, they are rarely used for detecting HClO in tap water. On the basis of the abovementioned, we developed a method for determining the concentration of HClO in tap water. After adding 2970 μL of deionized water and tap water to two quartz cuvettes, 30 μL of **HBT-HBZ** (1 mM) was added into quartz cuvettes and stirred. Then, we collected the fluorescence spectra and visually observed solution fluorescence with a portable UV lamp. Different from deionized water, the emission peak of the fluorescence spectrum at 572 nm considerably decreased after **HBT-HBZ** was added to tap water, whereas the fluorescence intensity at 470 nm increased. The insert in Fig. 3 shows that fluorescence of deionized water and tap water with **HBT-HBZ** are completely different under a portable 365 nm UV lamp (Fig. 3). From the abovementioned linear relationship, it was determined that **HBT-HBZ** was sensitive toward HClO in the tap water

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sample and HClO concentration was calculated as 2.0×10^{-5} M, which is higher than the required standard concentrations (8.4×10^{-6} M) [44,45]. According to GB 5749-2006, tap water in our laboratory is in compliance with the regulations [46]. Thus, we determined that **HBTHBZ** has a practical application in quantitatively determining the concentration of HClO in an environmental sample.

Inspired by the sensing properties of the extra-receptor **HBT-HBZ**, we explored the detection of HClO by **HBT-HBZ** imaging in cells. When HeLa cells were treated with **HBT-HBZ** (10 μM), they showed yellow fluorescence. After the treatment with NaClO (100 μM), different color of a bright cyan fluorescence was observed in HeLa cells (Fig. 4). The abovementioned experimental results showed that **HBT-HBZ** had another practical application in biological systems.

4. Conclusion

In conclusion, we developed a new type of ratiometric fluorescence probe (**HBTHBZ**), which is based on benzothiazole for the selective and sensitive discrimination of HClO in aqueous solutions. The probe exhibited high selectivity for HClO among other ROS, RNS and anions. The detecting mechanism was confirmed by MS, ^{1}H NMR and FT-IR analyses. In addition, **HBT-HBZ** has some practical applications such as the analysis of the HClO content in tap water successfully. Furthermore, confocal fluorescence microscopy imaging showed that **HBT-HBZ** can be applied for detecting HClO in living cells.

Declaration of interests

☒ 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.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

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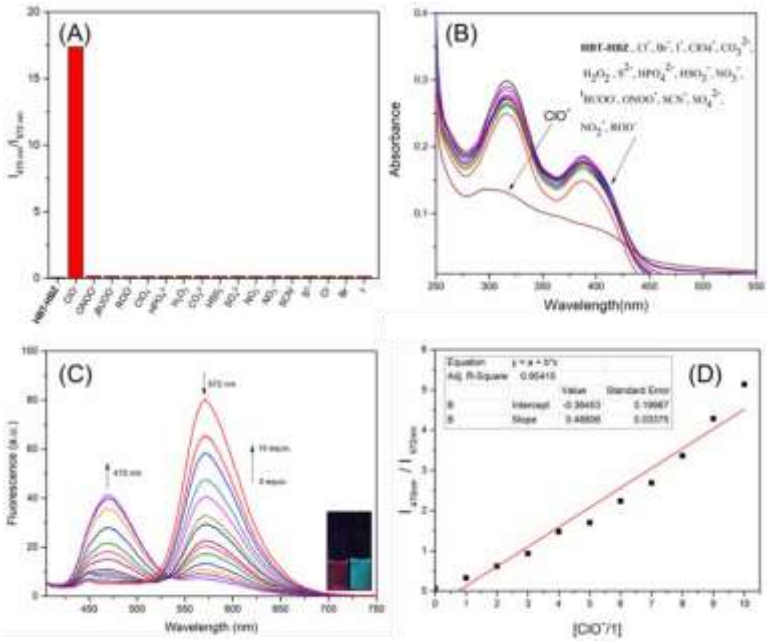

Fig. 1. (A) Fluorescence intensity ratio ($I_{470\ nm}/I_{572\ nm}$) changes of **HBT-HBZ** (10 μM) with different analytes (100 μM) in the PBS solution (50 mM, pH = 7.4) (λ_{ex} = 400 nm. Slit: 5 nm/10 nm). (B) Absorption spectra of **HBT-HBZ** (10 μM) with ClO^- (100 μM) and other anions (100 μM) in the PBS (50 mM, pH = 7.4) solution. (C) Fluorescence titration spectra of **HBT-HBZ** (10 μM) with increasing HClO concentration (λ_{ex} = 400 nm. Slit: 5 nm/10 nm). Inset: Fluorescence color of **HBTHBZ** (10 μM) (left), fluorescence color of **HBT-HBZ** with 10 equiv. of HClO (right) under a portable 365 nm UV lamp. (D) Linear relationship between fluorescent intensity ratios ($I_{470\ nm}/I_{572\ nm}$) and HClO concentrations (λ_{ex} = 400 nm. Slit: 5 nm/10 nm)

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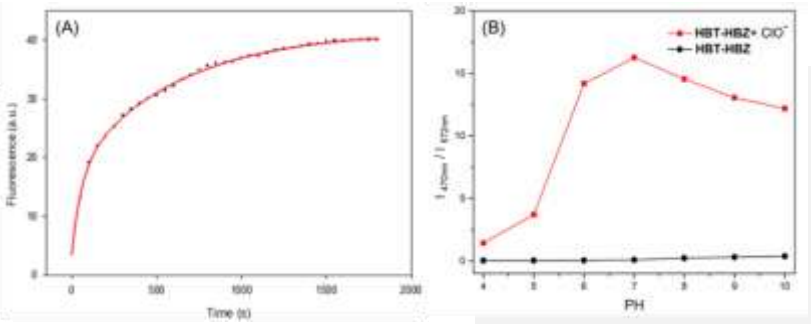

Fig.2. (A) Time-spectrum of fluorescence at 470 nm of **HBT-HBZ** (10 μM) in the PBS solution (50 mM, pH = 7.4) after the addition of HClO (λ_{ex} = 400 nm. Slit: 5 nm/10 nm). (B) Changes in the fluorescence intensity ratios ($I_{470\ nm}/I_{572\ nm}$) before and after the addition of HClO (100 μM) to **HBTHBZ** (10 μM) at different pH in the PBS solution (50 mM, pH = 7.4) (λ_{ex} = 400 nm. Slit: 5 nm/10 nm).

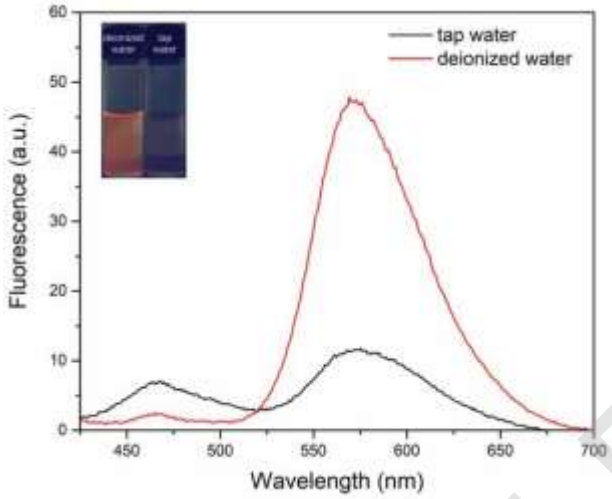

Fig.3. Fluorescence spectra of **HBT-HBZ** (10 μM) in deionized water and tap water (2970 μL) (λ_{ex} = 400 nm. Slit: 5 nm/10 nm). Inset: Fluorescence color of **HBT-HBZ** (10 μM) in deionized water and tap water under a portable 365 nm UV lamp.

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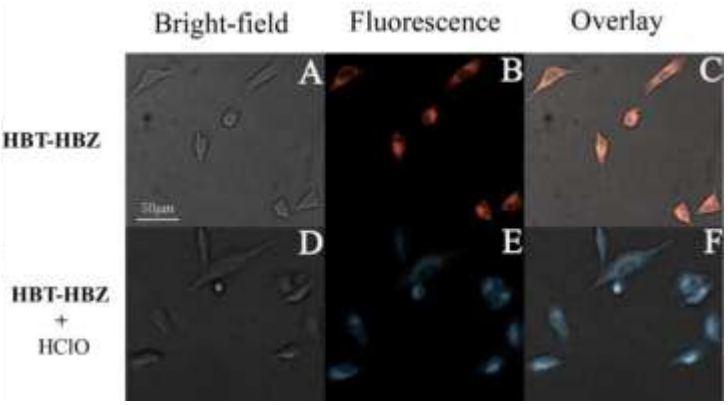

Fig.4. Fluorescence images. (A, B, C) HeLa cells incubated with **HBT-HBZ** (10 μM) for 15 min at 37 ^{0}C. (D, E, F) HeLa cells treated with **HBT-HBZ** (10 μM) before and after incubation with HClO (100 μM) for another 15 min at 37 °C.

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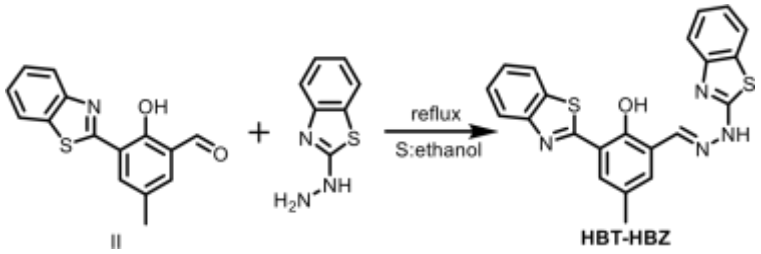
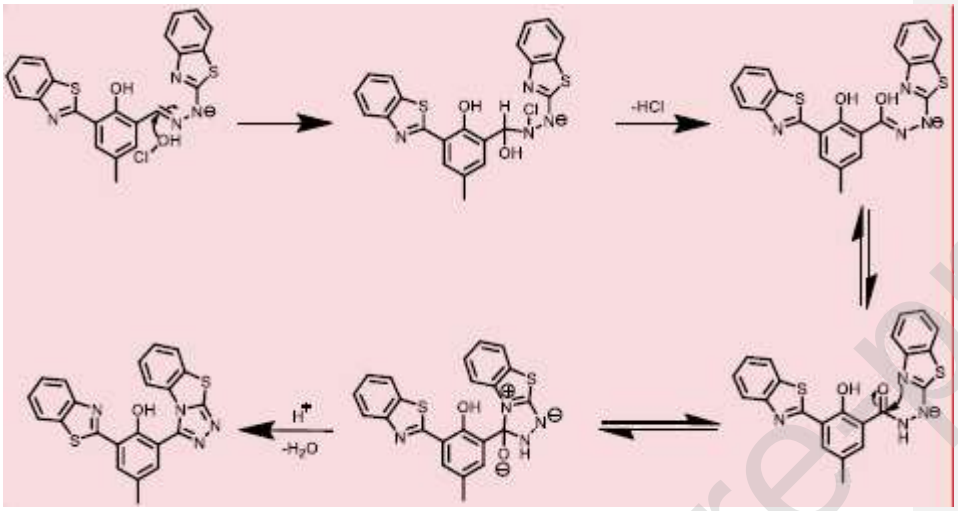


Scheme 1. Synthetic routes for **HBT-HBZ**



Scheme 2. Mechanism of the recognition process

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