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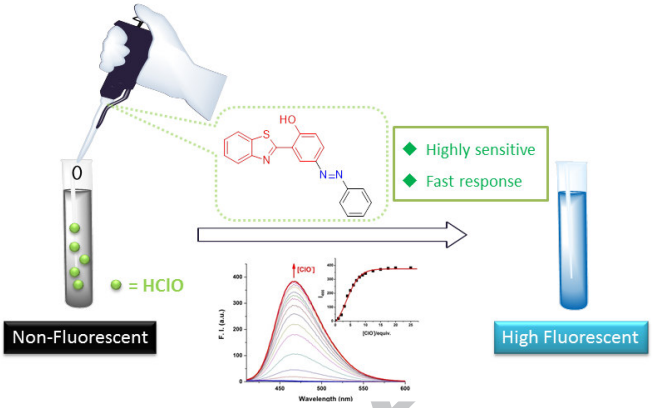

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Yimin Pan, Jing huang, and Yifeng Han*

Department of Chemistry, The Key Laboratory of Advanced Textile Materials and Manufacturing Technology, Zhejiang Sci-Tech University, Hangzhou, 310018, China.

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ABSTRACT

A new ESIPT-based fluorescent probe, **PHC2**, for the detection of hypochlorous acid has been rationally designed and developed. Endowed by the specific reaction between hypochlorous acid and phenyl azo group, **PHC2** features high degree of selectivity and sensitivity for HClO with a low detection limit (13.2 nM) under physiological conditions in neutral aqueous solution.

1. Introduction

As a well-known antiseptic germicidal agent, hypochlorous acid (HClO) is widely employed in our daily life such as household bleach, disinfectant of public water supplies, and as an antimicrobial.[1] Particularly, it is also an important member of the reactive oxygen species (ROS) family and plays important roles in the immune-defense system.[2] Endogenous HClO is produced mainly in leukocytes (including neutrophils, macrophages, and monocytes) from the peroxidation reaction of hydrogen peroxide (H_2O_2) and chloride ions (Cl^-) catalyzed by the enzyme myeloperoxidase (MPO).[3] However, the normal concentration range for HClO in biological systems is narrow, with both deficiency and excess causing many pathological states, such as arthritis, atherosclerosis, nephropathy, cardiovascular diseases, neuron degeneration and even cancer.[4] Consequently, it is strongly desirable to develop new analytical methods for the highly sensitive and accurate detection of HClO.

Conventional techniques used for quantification of HClO, such as potentiometry, and electroanalysis,[5] often need the complicated sample preparation and the expensive and sophisticated instrumentation, and are therefore not suitable for real-time and in situ detection. In comparison with those conventional methods for HClO, the non-invasive fluorescent probe methods display apparent advantages as exemplified by their ease of application both in solution and in living organism as well as their high selectivity and sensitivity for trace analytes with temporal and spatial resolution.[6]

To date, numbers of fluorescent probes have been developed and applied to the detection of HClO, most of which were designed by taking advantage of the strong oxidizing properties

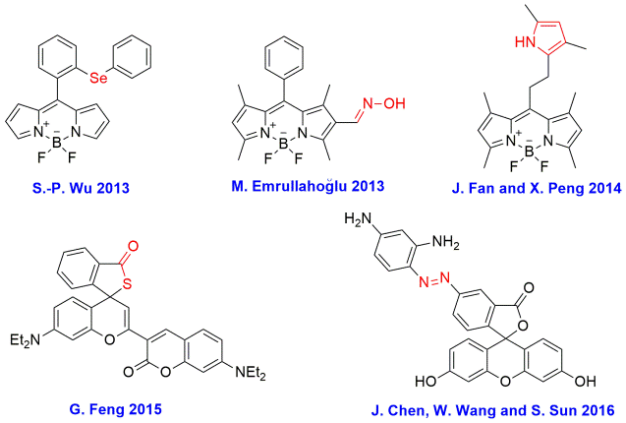
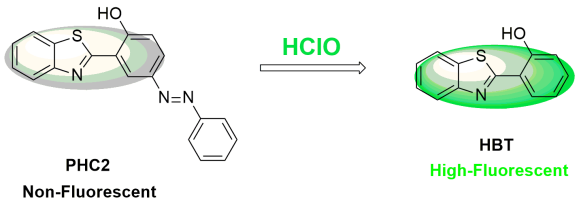


Figure 1. Structures of some reported HClO probes.

of HClO. Functional groups, such as *p*-alkoxyaniline, *p*-methoxyphenol, dibenzoylhydrazine, hydroxamic acid, oxime, thiol, and selenide, have been employed as reporters in fluorescent probe design due to their tendency of being easily oxidized by HClO.[7] However, most of these probes are still limited by low sensitivity, pH dependency, or sluggish detection kinetics, compromising their applicability for real-time detection of HClO. Therefore, the development of novel fluorescent probes with high selectivity, excellent sensitivity, and real-time capability to detect HClO still arouses much attention.

Very recently, Su, Wang, and Chen et al. developed the first fluorescein-based fluorescent HClO-sensor by the oxidation reaction between the azo moiety and HClO, which shows highly



Scheme 1. The strategy for the design of **PHC2**.

selective and sensitive to detect HClO in absolute PBS with low detection limit (8.9 nM).[7z] 2-(2'-hydroxyphenyl) benzothiazole (HBT) is a well-known chromophore exhibiting excited state intramolecular proton transfer (ESIPT) through the keto-enol tautomerism which results in a large Stokes shift. Inspired by these works, we report here a new and simple HBT-based fluorescence turn-on probe, **PHC2**, a phenylazo derivative of HBT, for the detection of HClO. We envisioned that the presence of the electron-withdrawing phenylazo group would repress the fluorescence emission of **PHC2**.[8] However, HClO promoted oxidation of azo group would generate the free HBT moiety and in turn restore the strong fluorescence (Scheme 1).

2. Results and discussions

As shown in Scheme 2, **PHC2** can be readily prepared in two convenient steps under facile conditions with high yield starting with commercially available aniline and salicylaldehyde. The product (**PHC2**) was characterized by ^{1}H, and ^{13}C NMR (ESI†).

With **PHC2** in hand, we firstly assessed its UV-vis spectroscopic properties in PBS buffer solution (10 mM, pH 7.4, containing 1.0% EtOH) (Fig. 2 and S1, ESI†). **PHC2** (5.0 μM) displayed two moderate UV-vis absorption bands around 300 and 400 nm, respectively. Upon incremental addition of HClO (0-5.0 equiv.) to the 5.0 μM solution of **PHC2**, the peaks at 300 nm dramatically decreased, while a band at 400 nm increased simultaneously with a clear isosbestic point at 221, 236, 270 and 380 nm respectively, indicating that free HBT was formed in the presence of HClO (Fig. 2 and Scheme 1).[9] Furthermore, a good linear relationship (R^2 = 0.992) was observed between the absorbance at 400 ($A_{400\ nm}$) and concentrations of HClO in the range of 0-3.0 equiv. (Fig. 2 and S1, ESI†).

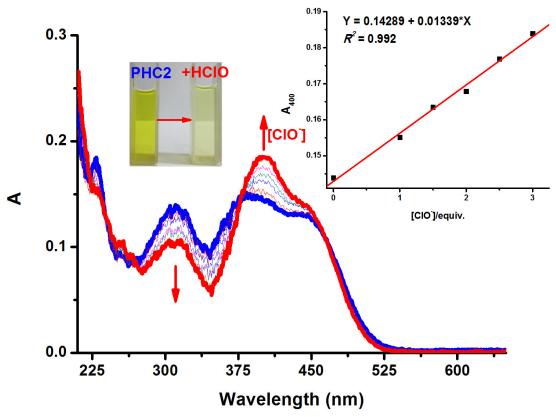


Figure 2. UV-vis absorption spectra of **PHC2** (10.0 μM) in the presence of different concentration of HClO (0-5.0 equiv.). Inset 1: the absorbance at 400 nm as a function of HClO concentration (0-3.0 equiv.). Inset 2: cuvette images of probe **PHC2** before and after addition of HClO. All spectra were acquired 5 min after HClO addition at room temperature in PBS buffer solutions (10 mM, pH 7.4, containing 1.0% EtOH).

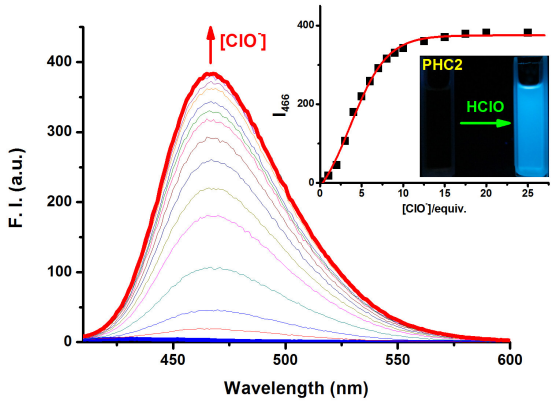


Figure 3. Fluorescence spectra of **PHC2** (5.0 μM) in the presence of different concentrations of HClO (0-25.0 equiv.). Inset 1: the fluorescent intensity at 466 nm as a function of HClO concentration. Inset 2: cuvette images of probe **PHC2** before and after addition of HClO taken under a hand-held UV-lamp (λ_{ex} = 365 nm). All spectra were acquired 5 min after HClO addition at room temperature in PBS buffer solutions (10 mM, pH 7.4, containing 1.0% EtOH) (λ_{ex} = 400 nm).

Furthermore, the fluorescence titration curve revealed that the fluorescence intensity at 466 nm increased linearly with increasing concentration of HClO (Fig. 3 and S2, ESI†) and further smoothly increased until a maximum was reached up to 50.0 μM of HClO (λ_{ex} = 400 nm, Φ = 5.18%, Table S1, ESI†). Owing to the specific reactivity of HClO-promoted oxidation reaction, **PHC2** displayed a high sensitivity toward HClO.

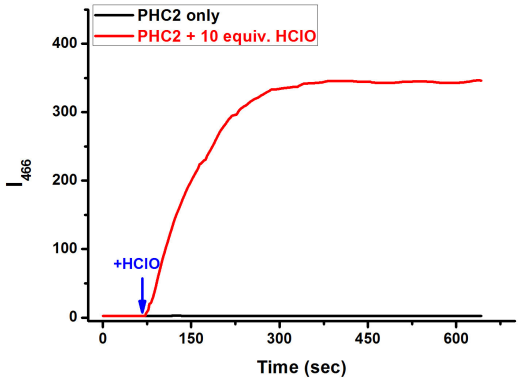


Figure 4. Time-dependent fluorescence intensity changes of **PHC2** (5.0 μM) upon addition of 10.0 equiv. of HClO at room temperature in PBS buffer solutions (10 mM, pH 7.4, containing 1.0% EtOH) (λ_{ex} = 400 nm).

Subsequently, the time-dependence of **PHC2** fluorescence was evaluated in the presence of HClO in PBS buffer solutions (10 mM, pH 7.4, containing 1.0% EtOH) (Fig. 4).The results showed that no change in fluorescence were detected before the addition of HClO to the 5.0 μM solution of **PHC2**. Furthermore, after the addition of 10.0 equiv. of HClO to the tested solution, the fluorescent intensity at 466 nm remarkably increased to their maximum value within 5 minutes, which indicated that **PHC2** possesses a real-time capability to measure HClO.

Continuing on, the fluorescence titration of **PHC2** towards various reactive oxygen species (ROS, including ·OH, $^1O_2$, H_2O_2, and ^{t}BuOOH), reactive nitrogen species (RNS, including $ONOO^-$, and NO), and cations commonly found in the biological systems was conducted to examine the selectivity (Fig. 5, 6 and S3, ESI†). Much to our delight, the tested analytes caused little change to the fluorescence profile of **PHC2**. It is noteworthy that **PHC2** still responds to HClO sensitively even in the presence of other relevant competing species. Therefore, these results suggest that **PHC2** displays high selectivity toward HClO.

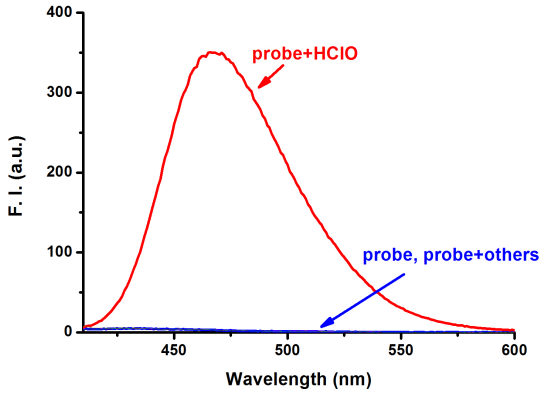


Figure 5. Fluorescence responses of **PHC2** (5.0 μM) to various ROS, RNS, and common coexisting ions (including ·OH, $^1O_2$, ^{t}BuOOH, Fe^{3+}, H_2O_2, NO, $ONOO^-$, and HClO) at 50.0 μM. All data were acquired 5 min after addition of each analyte at room temperature in PBS buffer solutions (10 mM, pH 7.4, containing 1.0% EtOH) (λ_{ex} = 400 nm).

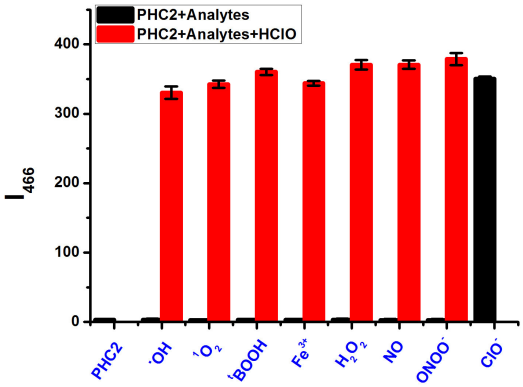


Figure 6. Fluorescence responses of **PHC2** (5.0 μM) to various ROS, RNS, and common coexisting ions (including ·OH, $^1O_2$, ^{t}BuOOH, Fe^{3+}, H_2O_2, NO, $ONOO^-$, and HClO) at 50.0 μM. Bars represent the emission intensity of **PHC2** at 466 nm. Data were collected under the same conditions as Fig. 5.

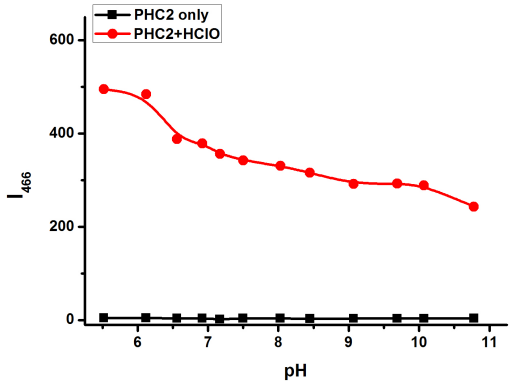


Figure 7. Effect of the pH on the fluorescence emission of **PHC2** (5.0 μM) alone and **PHC2** (5.0 μM) reacted with HClO (10.0 equiv.). All data were acquired 5 min after HClO addition at room temperature in PBS buffer solutions (10 mM, pH 7.4, containing 1.0% EtOH) (λ_{ex} = 400 nm).

Moreover, the HClO-sensing ability of **PHC2** at a wide range of pH values was investigated. As depicted in Fig. 7, **PHC2** alone was inert to pH in the range of 5.5-10.7. On the other hand, it still responded to HClO sensitively within this pH range (5.5-10.7). These results indicate that **PHC1** could be used in neutral natural systems, or a mildly acidic or basic environment.

For practical purposes, the detection limit of **PHC2** for the analysis of HClO was also an important parameter. The fluorescence titration curve revealed that the fluorescence intensity of **PHC2** at 466 nm increased linearly with the amount of HClO in the range of 0-12.0 μM (R^2 = 0.994) (Fig. S4, ESI†). Thus, the detection limit of **PHC2** for HClO was calculated to be 1.32×10^{-8} M, which indicated that **PHC2** could be a sensitive fluorescent probe for quantitative detection of HClO.

3. Conclusions

In conclusion, we have rationally developed a novel and simple ESIPT-based sensitive fluorescence probe, **PHC2**, for the detection of HClO under physiological conditions in neutral aqueous solution *via* HClO-induced oxidation of phenyl azo group. Based on this specific reaction, the probe has the unique advantages of easy-preparation, good water solubility, and excellent selectivity and sensitivity response towards HClO, with a low detection limit (13.2 nM). We anticipate that the experimental results of this study will inspire the future designing of ROS sensors for a variety of chemical and biological applications.

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Supplementary Material

Supplementary data (additional spectroscopic of SPd2) associated with this article - copies of ^{1}H and ^{13}C NMR Spectra can be found, in the online version, at http://dx.doi.org.

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Highlights

1. A new ESIPT-based fluorescent probe for the detection of HClO has been developed.
2. The probe shows high selectivity and sensitivity for HClO in aqueous solution.
3. The detection limit of probe for HClO is 13.2 nM.