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Aggregated-induced emission phenothiazine probe for selective ratiometric response of hypochlorite over other reactive oxygen species

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ABSTRACT

In order to perform a ratiometric fluorescent sensor to recognize hypochlorite over other reaction oxidation species, a simple phenothiazine probe (**QC1**) with double dioxaborolane moieties was designed and synthesized. **QC1** provided blue emission in aqueous and solid state. Double dioxaborolane and quaternary phosphonium salt was introduced into the molecule to prevent aggregation. The sulphur atom at the centre of phenothiazine was to respond to the hypochlorite at room temperature over other ROS because of its stronger oxidized ability. **QC1** gave good linear fitting in the ratiometric mode under both absorption and emission titration experiments. Moreover, **QC1** showed lower detection limited that reached 0.95 μM in absorption titration and 0.41 μM in fluorescent titration process.

1. Introduction

Reactive oxygen species (ROS) plays an indispensable role in a wide variety of biological processes [1–4]. For example, recent studies reported that cancer cells constantly generate high levels of intracellular ROS, due to oncogenic transformation [5]. Among the various ROS, hypochlorous acid (HClO) is a powerful microbicidal agent in the innate immune system for its strong oxidizing property. The endogenous HClO released by the neutrophil plays a vital role in killing a wide range of pathogens [6,7]. As a result, excessive generation of HClO always implicates in many human diseases, and can be found as inflammation-associated tissue injury [1,8–11]. Moreover, it is considered that HClO contributes to cardiovascular disease, and cell apoptosis through calcium dependent calpain activation. Therefore, there is urgent need to sense the various biological effects of HClO with lower detection limit, good selectivity and in real time [12,13].

With high sensitivity and adjustable structure, many small molecular fluorescent sensors were reported, such as cyanide dyes, fluorescein, rhodamine, BODIPY, naphthalimide, Dicyanomethylene-4H-pyran, and so on [14–26]. There are still some challenges to optimize the small molecular fluorescent sensors for HClO: 1) some commercial dyes show poor stability for oxidants. For example, the cyanide dyes are always bleached by ROS [27]; 2) the ratiometric response probe with different fluorescent channels is still not common [28–31].

To solve such problems, it is needed to develop stabile fluorophores and the useful redox reactions that can output the ratiometric signals for sensing HClO beyond other ROS. In 1985, Brubaker et al. reported a desulfurization technology with NaClO [32]. As we know, one component of the organic sulphur in coal is dibenzothiophene, an analogy of phenothiazine. Further research on the oxidation of phenothiazine indicated the Chlorite can oxidize the dye to sulfones under stronger oxidizing conditions such as the presence of fuming nitric acid and hydrogen peroxide [33]. Based on this knowledge, we hypothesize that the modified phenothiazine can only be oxidized by a strong oxidant, like ClO^-, and we designed the phenothiazine-based fluorescent sensor (**QC1**) to detect ClO^-. As shown in Scheme 1, we introduced two dioxaborolane moieties to enhance the steric of phenothiazine that

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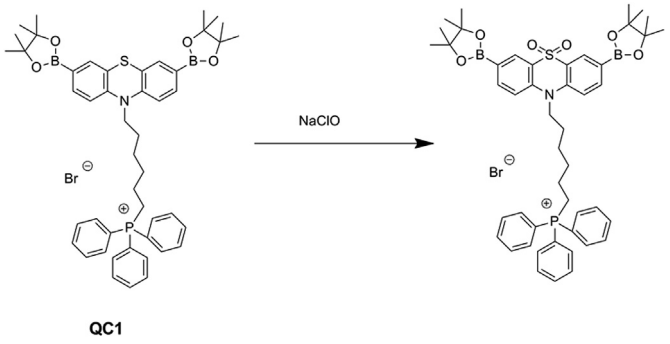


Scheme 1. The descript of probe **QC1** and its recognition mechanism towards NaClO.

can restrain π–π stacking of the rigidity dyes. The quaternary phosphonium salt attached on the N atom of phenothiazine also showed steric hindrance effect for the sensor.

2. Experimental

2.1. Materials and instruments

All reagents and solvents were used as received without further purification. Deionized water was used in the experiments throughout. Silica gel (100–200 mesh) was used for column chromatography. Mass determination was made on a GC-TOF MS spectrometry. NMR spectra were recorded on a Varian 400 MHz with chemical shifts reported as ppm (in DMSO-*d6* or $CDCl_3$, TMS as internal standard). Fluorescence measurements were performed on a FS-5 spectrophotometer (Edinburgh, Britain) and the slit width was set as 1 nm for excitation and emission, respectively. Absorption spectrum was measured on a SHIMADZU UV-3600 spectrophotometer. NO and ClO^- were produced from the dissolution of NOC13 [1-Hydroxy-2-oxo-3-(3-aminopropyl)-3-methyl-1-triazene] [34] and NaClO in the de-ion water, respectively. H_2O_2 was diluted from the stabilized 30% H_2O_2 solution. Hydroxyl radical (·OH) was generated by reaction of 1 mM Fe^{2+} with 200 μM H_2O_2. Tert-butoxy (t-BuOO•) was prepared from the reaction of 1 mM Fe^{2+} with 200 μM TBHP. $NaNO_3$ and $NaNO_2$ were used as the source of NO_3^- and NO_2^-, respectively.

2.2. Synthesis of QC1

2.2.1. Synthesis of compound *2*

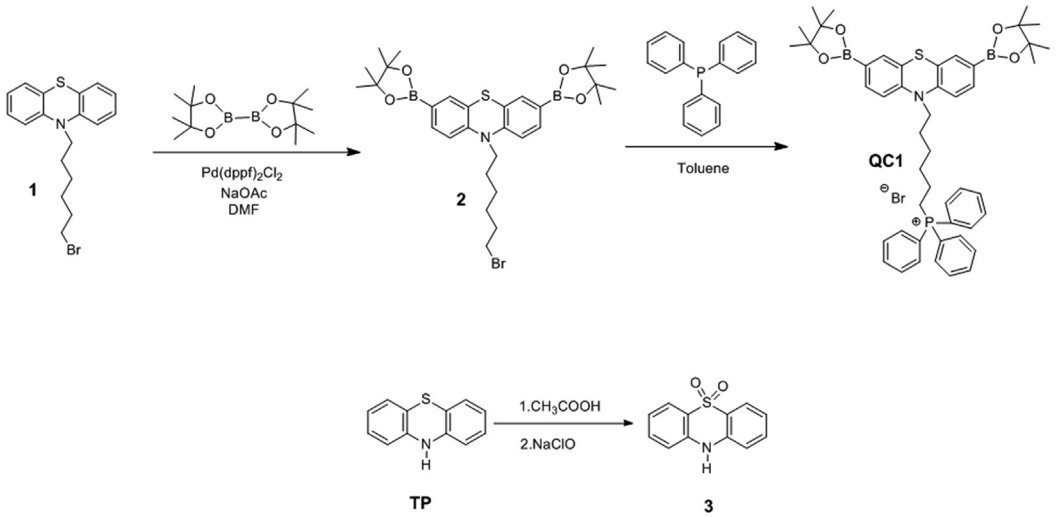
Compound **1** (180 mg, 0.5 mmol), bis(pinacolato)diboron (254 mg, 1.0 mmol), Pd(dppf)Cl_2 (36 mg, 0.05 mmol), and sodium acetate (287 mg, 3.5 mmol), were added to a dry Schlenk tube. Then 5 mL of dry DMF was added via syringe and the mixture was stirred under nitrogen at 80 °C for 2 h. After cooling to room temperature, the mixture was extracted with dichloromethane, washed with water, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (silica gel, petroleum ether/dichloromethane, 1/5, v/v) to give a light yellow solid (215 mg, 70.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.50–7.65 (m, 4H), 6.83 (d, $J = 8.03$ Hz, 2H), 3.90 (br. s., 2H), 3.38 (t, $J = 6.78$ Hz, 2H), 1.76–1.88 (m, 4H), 1.46 (br. s., 4H), 1.34 (s, 24H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 147.2, 134.0, 133.8, 124.3, 114.8, 83.7, 47.2, 33.7, 32.6, 29.7, 27.7, 25.9, 24.8.

2.2.2. Synthesis of *QC1*

A solution of compound **2** (306 mg, 0.5 mmol) and triphenylphosphine (145 mg, 0.55 mmol) in dry toluene (15 mL) was refluxed under nitrogen. The reaction was monitored by TLC. Upon completion, the solvent was removed under reduced pressure. The residue was purified by recrystallization to afford **QC1** (372 mg, 85% yield) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.68–7.82 (m, 15H), 7.54–7.63 (m, 2H), 7.47 (s, 2H), 6.75–6.90 (m, 2H), 3.87 (br. s., 2H), 3.53–3.66 (m, 2H), 1.69 (br. s., 2H), 1.59 (s, 4H), 1.39–1.46 (m, 2H), 1.32 (s, 24H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 147.28, 135.02, 134.21, 133.66, 133.56, 130.60, 130.48, 124.10, 118.64, 117.79, 115.20, 83.73, 46.84, 29.40, 25.90, 25.73, 24.84, 22.81, 22.30. HRMS(ESI): Found: [M–Br] 796.3917; requires [M–Br] 796.3927.

2.2.3. Synthesis of compound *3*

Sodium hypochlorite solution (7.0%, 2 mL) was added dropwise to a solution of phenothiazine (199 mg, 1 mmol) in acetic acid (10 mL) was stirred at 90 °C overnight. After cooled down, the water was added and extracted with ethyl acetate. The separated organic layer was and washed with water and brine, then dried over Na_2SO_4



Scheme 2. Synthesis Routine of probe **QC1**.