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Rational designed benzochalcone-based fluorescent probe for molecular imaging of hydrogen peroxide in live cells and tissues

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ABSTRACT

Hydrogen peroxide (H_2O_2), a member of reactive oxygen species (ROS), usually acts as a significant oxidative stress marker in some diseases for its abnormal expression. Due to the merits of non-invasive and high selectivity, fluorescent probe has been proved to be an efficient method for H_2O_2 detection. Herein, we presented two novel boronate probes FD-1 and FD-2, in which the C-B bonds are well suitable for high selective detection of H_2O_2. Notably, these two probes were based on 4-hydroxybenzochalcone fluorophore, which exhibits excellent fluorescent properties through the intramolecular hydrogen bond. The results demonstrated that these two probes displayed satisfactory sensitivity and selectivity to H_2O_2, and probe FD-2 displayed faster response rate to H_2O_2 than FD-1. Importantly, FD-2 was successfully applied to image H_2O_2 in living cells and tissues, indicating probe FD-2 has great potential to monitor the H_2O_2 in biological system.

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1. Introduction

Hydrogen peroxide (H_2O_2) produced in the process of aerobic metabolism is one of the most important reactive oxygen species (ROS) [1,2] and plays significant roles in physiological process as signaling molecule [3–6]. H_2O_2 has been used as a significant oxidative stress marker in some diseases such as tumor, Parkinson's disease, Alzheimer's disease and inflammation, because this oxygenated chemical has been proved abnormal expression in these diseases [7–12]. It is believed that detection of H_2O_2 may serve as diagnosis of the aforementioned diseases [13]. Recently, several methods for H_2O_2 visualization in biological samples have been reported, including those using small-molecule [14–16], protein [17], and nanoparticle [18–20]. The fluorescent imaging possesses many advantages such as real time imaging, high selectivity, non-invasive and visual [21–24]. At present, numbers of fluorescent probes have been developed and applied for the H_2O_2 detection [25–31]. Although these fluorescent probes have all aforementioned merits but their detection sensitivity are generally not satisfactory for biological applications [32,33]. Therefore, development of high sensitivity fluorescence probes for monitoring H_2O_2 in living cells and tissues is of great meaning.

Due to the unique chemical reaction between boronate-to-phenol with H_2O_2, boronate ester has become an appropriate recognition moieties for H_2O_2 detection. [34,35] Several fluorescent probes based on boronate for tracking H_2O_2 have been developed [36,37]. These probes have advantages of high selectivity and response rate but does not perform well in the limit of detection [36]. To achieve the high sensitivity probe for monitoring H_2O_2, we designed two boronate probes based on 4-hydroxybenzochalcone fluorophore. The molecules of this type catch our eyes owing to its unique molecular structure [38]. Hydroxyl on the benzene ring is coupled with the carbonyl nearby forming hydrogen bond, more importantly, and this intramolecular hydrogen bond further augment the length of conjugation coplanar π-electron system to enhance the fluorescence capacity.

Herein, we designed and synthesized two boronate probes FD-1 and FD-2 based on 4-hydroxybenzochalcone fluorophore for detecting the H_2O_2 in living cells and tissues. In this paper, a series of tests were carried out to evaluate the practicability of the new fluorescent probes. The results indicated probes FD-1 and FD-2 displayed excellent selectivity for H_2O_2. The LOD of probes FD-1 and FD-2 were 78.89 nM and 58.13 nM, respectively, which demonstrated that these two probes were sensitive enough to track H_2O_2. Moreover, the time kinetics experiment established that two probes showed admirable response time to H_2O_2, especially probe FD-2, which exhibited faster reaction rate than FD-1. Finally, we

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further proved that probe FD-2 was capable of imaging H_2O_2 in living cells and tissues.

2. Experimental section

2.1. Materials and instruments

All chemical materials were purchased through chemical reagent corporation and used without further purification. The absorption measurement we employed was One Drop Spectrophotometer (OD-1000+, Nanjing, China). Fluorescence spectrophotometer (PerkinElmer, LS55) was employed for the spectra detection. ^{13}C NMR and ^{1}H NMR spectra were tested on Bruker Advance 300-MHz Spectrometer; the values of δ are in ppm respect to TMS. Keep the record of Mass data (ESI) by quadruple mass spectrometry. Laser confocal cell imaging was taken by Laser confocal fluorescence microscopy (FluoView^{TM}, FV1000, Olympus, Japan). The images of tissue slices were obtained by Inverted fluorescence microscope (Leica EL6000, Germany). The ROS in the H_2O_2 selectivity experiments is as follows: H_2O_2, *tert*-butyl hydroperoxide (TBHP), OCl^- were obtained from the diluent of 30%, 65%, 5% aqueous solutions, respectively. $HO^•$ and t-$BuO^•$ were achieved by reaction of 100 mM Fe^{2+} with 20 mM H_2O_2 or 20 mM TBHP. $NO^•$ was added using diluent of sodium nitroprusside.

2.2. Synthesis of FD-1

1-(1-Hydroxynaphthalen-2-yl) ethan-1-one (680 mg, 3.5 mmol) and 4-methoxybenzaldehyde (364 mg, 3 mmol) were dissolved in CH_3OH (9 mL). Then $Ba(OH)_2$ (1.89 g, 6 mmol) was added and the solution was stirred at room temperature overnight. The reaction mixture was diluted with ethanol and filtered to give the intermediate product FD-OCH_3, which was used for the next step without further purification.

FD-OCH_3 (300 mg, 1 mmol) was dissolved in CH_2Cl_2 (10 mL). Then 10 mL BBr_3 was added at 0 °C under the nitrogen protection and stirred overnight. The reaction was extracted with ethyl acetate for three times. The intermediate chemical FD-OH was obtained after purified on the column of silica gel with analytical CH_2Cl_2. ^{1}H NMR (300 MHz, DMSO-*d*6) δ: 15.26 (s, 1H), 10.21 (s, 1H), 8.37 (d, *J* = 8.1 Hz, 1H), 8.29 (d, *J* = 9.1 Hz, 1H), 8.01 (s, 3H), 7.90 (m, 2H), 7.73 (t, *J* = 7.9 Hz, 1H), 7.61 (t, *J* = 7.5 Hz, 1H), 7.45 (d, *J* = 9.1 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-*d*6) δ: 193.8, 163.5, 161.2, 146.4, 137.4, 132.1, 130.7, 128.0, 126.5, 126.1, 125.4, 125.0, 124.0, 118.5, 117.6, 116.4, 113.8, 40.8, 40.5, 40.3, 40.0, 39.7, 39.4, 39.2, 30.8, 29.4. ESI–MS: 291.1 $[M+H]^+$.

FD-OH (120 mg, 0.4 mmol) was dissolved in 10 mL acetonitrile. Then 2-(4-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (147 mg, 0.5 mmol) and K_2CO_3 (114 mg, 0.8 mmol) were added to reflux react at 80 °C for 4.5 h. The solvent and the residue were separated by filtration under reduced pressure. Then the residue was purified on silica gel chromatography with petroleum ether/CH_2Cl_2 (V/V = 1:3) to obtain the desired product 143 mg FD-1. ^{1}H NMR (300 MHz, DMSO-*d*6) δ: 15.18 (s, 1H), 8.34 (s, 2H), 8.06 (d, *J* = 15.5 Hz, 1H), 7.95 (t, *J* = 5.3 Hz, 4H), 7.73 (t, *J* = 8.0 Hz, 3H), 7.61 (t, *J* = 7.3 Hz, 1H), 7.74 (t, *J* = 8.7 Hz, 3H), 7.13 (d, *J* = 8.7 Hz, 2H), 5.26 (s, 2H), 1.28 (m, 12H). ^{13}C NMR (75 MHz, DMSO-*d*6) δ: 193.4, 163.1, 160.7, 145.3, 140.0, 137.0, 134.5, 131.4, 130.3, 127.6, 127.4, 126.8, 126.1, 125.0, 124.5, 124.3, 124.2, 123.6, 118.5, 118.1, 115.3, 113.3, 83.6, 69.2, 40.3, 40.1, 39.8, 39.5, 39.2, 38.9, 38.7, 33.1, 31.2, 31.1, 29.8, 29.7, 28.9, 28.8, 28.6, 28.4, 28.2, 24.6, 22.0, 13.9.

2.3. Synthesis of FD-2

1-(1-Hydroxynaphthalen-2-yl)ethan-1-one (186 mg, 1 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (232 mg,1 mmol) were dissolved in ethanol/CH_2Cl_2 (10 mL/1 mL). Then two drops of piperidine were added to the reaction mixture and the whole mixture was stirred overnight. The residual was obtained out by rotary evaporation, which was purified on silica gel column with ethyl acetate/petroleum ether (V/V = 1:6) to obtain the target product FD-2 (335 mg, 0.8 mmol). ^{1}H NMR (300 MHz, DMSO-*d*6) δ: 14.94 (s, 1H), 8.34 (d, *J* = 8.3 Hz, 1H), 8.27 (d, *J* = 8.8 Hz, 1H), 8.16 (s, 1H), 7.19 (m, 4H), 7.73 (t, *J* = 12.4H, 3H), 7.57 (s, 1H), 7.42 (d, *J* = 8.9 Hz, 1H), 1.29 (s, 12H). ^{13}C NMR (75 MHz, DMSO-*d*6) δ: 193.4, 163.3, 144.6, 137.1, 137.0, 134.7, 130.4, 128.5, 127.5, 126.1, 125.0, 124.4, 123.6, 122.0, 118.2, 113.3, 83.8, 40.3, 40.1, 39.8, 39.5, 39.2, 39.0, 38.7, 24.6.

2.4. Sensing mechanism of FD-1 and FD-2 with H_2O_2

H_2O_2 (1 mL, 30%) and FD-1 (30 mg) were added into 10 mL DMSO/PBS (V/V = 1/1) solution. The reaction mixture oscillated in shaking table at 37 °C for 1 h. A needle shaped crystallite can be observed obviously in the mixture of FD-1 solution. Then the mixture was filtered to filter the crystallite. The filtrate was collected and was extracted with dichloromethane for three times. The collected organic layers were removed under reduced pressure. The residue was purified with silica gel column. The sensing mechanism of probe FD-2 was verified in the same way.

2.5. Cell culture

U87 cells, a human glioma cell line, were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA), cultured in DMEM containing 10% fetal bovine serum (FBS, Hyclone, containing 80 U/mL penicillin and 0.08 mg/mL streptomycin) at 37 °C in a 5% CO_2/95% air (v/v) incubator. The concentrations of cells for each confocal dish were adjusted to $5 \times 10^5 - 1 \times 10^6$ cells/mL for confocal imaging in high-glucose DMEM (4.5 g of glucose/L) containing 10% fetal bovine serum, cultures were cultured at 37 °C in a 5% CO_2/95% air (v/v) incubator.

2.6. MTT assay

U87 cells were cultured and removed to 96-well plates to a total volume of 100 μL (5×10^5–1×10^6 cells/mL) each well. Plates were maintained at 37 °C in a 5% CO_2/95% air (v/v) incubator for 24 h. Then the medium was replaced with 200 μL different probe concentrations of probe FD-2 (0, 5, 10, 20, 30, 40, 50, 60, 80, 100 μM) and cultured for 12 h. MTT solution (20 μL, 5 mg/mL) was then added to each well for another 4 h. The culture medium was removed and 150 μL of DMSO was added to each well to dissolve the crystallized product formazan. Finally the absorbance in each well of the 96-well plates was measured at 490 nm with a multi-well plate reader. The cell viability was calculated using the following formula: Cell viability = (the optical density of test wells − the optical density of medium control wells)/(the optical density of untreated wells − the optical density of medium control wells) × 100%.

2.7. Cell imaging

U87 cells were dispersed on 15 mm observation dish at 37 °C in a 5% CO_2/95% air (v/v) incubator for 24 h until adherence on the glass wall. Then cells were cultured with probe FD-2 (10 μM) for 30 min. Then the medium was removed and cells were washed with PBS (10 mM, pH 7.4) for three times. Fluorescent images were acquired on laser scanning confocal microscope (LSCM) with an objective lens (×10). The excitation wavelength was 488 nm. Following incu-

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