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A near-infrared fluorescent probe for rapid detection of hydrogen peroxide in living cells

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ABSTRACT

A new near-infrared and colorimetric fluorescent molecular probe was developed for rapid detection of H_2O_2. The near-infrared fluorescence OFF–ON switch is triggered by transformation of phenylboronic acid unit to phenol in the presence of H_2O_2. No quinone methides are released in this process, which is preferable for in vivo studies. In addition, probe **1** at low concentration exhibits high quality optical imaging during a short period in in vitro cell study.

1. Introduction

Reactive oxygen species (ROS) are a class of oxygen-containing oxidants, which have important roles in regulation of many physiological processes.[1–4] ROS are formed as a natural by-product of the normal metabolism of oxygen. Moderate levels of ROS are crucial for diverse cellular responses required for normal cell functions.[5] ROS function predominantly depends on the redox modification of critical residues in proteins, which results in functional changes.[6] However, exogenous sources such as UV light, heat exposure and radiation can cause the increase of ROS levels.[7–9] The overproduction of ROS may damage biological molecules such as proteins, phospholipids, RNA and DNA, which leads to pathological states and contributes to aging[10–12] and diseases including cancer,[13–15] diabetes[16–18] and neurodegenerative disorders.[19,20]

There are multiple types of ROS and the most widely known ROS are hydroxyl radical (OH•), hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\bullet-}$).[1] In general, these species can convert through a three-step chain reaction. Superoxide radical is the most reactive among these oxidants. However, less reactive H_2O_2 is the major contributor to oxidative damage due to the longer half-life, which makes it possible to travel through cells and tissues.[21]

Recently, several specific and highly sensitive fluorescent probes have been developed for the detection of H_2O_2.[22–24] Some of these molecules have good performance in in vitro experiments. However, the limitation of conventional ROS fluorescent probes is their application in in vivo studies. Biological chromophores such as hemoglobin can strongly absorb visible light, which limit the depth penetration of probes with short wavelength emission. In addition, biological fluorophores including elastin[25] and collage[26] has emission in the visible range and can cause significant interference with these probes.

Over the past decade, near-infrared (NIR) fluorescent dyes have become promising tools for monitoring various biological processes in cells and organisms.[27–30] In general, NIR fluorophores are defined as substances with emit fluorescence in the range of 650–900 nm. Compared with most conventional fluorescent probes, NIR probes possess unique advantages in in vivo studies such as deeper tissue penetration ability, lower background autofluorescence and less damage to biological samples. So far, several NIR fluorescent probes have been reported to detect intracellular H_2O_2.[27] However, most of these works employ boronate-masked phenol group as the trigger moiety, which inevitably form *para*-quinone methide as the byproduct.[31–33] Quinone methides are unstable and can react with endogenous substances such as proteins, DNA as well as GSH, which may result in toxic effects and other undesired side effects in in vivo studies.[34]

Compared with other NIR fluorophores like squaraine and cyanine, dicyanomethylene-4*H*-pyran (DCM) fluorophore is more

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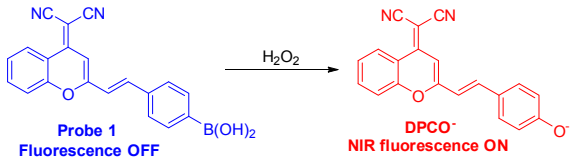
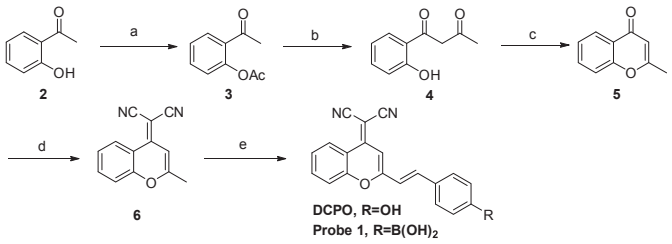
photostable and is widely used in fluorescence imaging.[35–40] Our strategy for detection of H_2O_2 bases on the introduction of styrene boronic acid moiety into benzopyran scaffold (Scheme 1). The near-infrared fluorescence OFF–ON switch is triggered by transformation of phenylboronic acid unit to phenol by the interaction with H_2O_2, which results in the formation of $DPCO^-$ without the release of quinone methides.



Scheme 1. Design of probe **1** and H_2O_2-promoted release of $DPCO^-$.

2. Results and discussion

Using 1-(2-hydroxyphenyl)ethan-1-one as starting material, probe **1** was synthesized smoothly in five steps (Scheme 2). We first tested the optical properties of probe **1** in PBS buffer (0.1 M, pH 7.4, 50% DMSO). As expected, the replacement of phenol moiety with phenylboronic acid group reduces the conjugated π-electron system of DPCO and thus turns OFF the fluorescence of the probe. UV–vis spectra of **1** (5 μM) exhibited two absorptions at around 420 nm and 450 nm. After treatment with 20 equiv of H_2O_2 for 60 min, the absorption at 420 nm and 450 nm apparently decreased, whereas a new absorption peak appeared at 560 nm (Fig. 1). Such a large red shift of 110 nm in the absorption behavior changed the color of the solution from yellow to purple, allowing colorimetric detection of H_2O_2 by naked eye (Fig. 1).



Scheme 2. Synthesis of probe **1** and DPCO. (a) AcCl, pyridine, DCM, 96%; (b) t-BuOK, THF, 22%; (c) 98% H_2SO_4, AcOH, 91%; (d) malononitrile, Ac_2O, 23%; (e) 4-hydroxybenzaldehyde or (4-formylphenyl)boronic acid, piperidine, CH_3CN, 35%.

Accordingly, the emission at 670 nm dramatically appeared upon excitation at 560 nm (Fig. 2). The Stokes shift is as large as 110 nm, which is desirable for high quality optical imaging. Upon the treatment of 20 equiv H_2O_2 for 60 min, the conversion was almost complete and a 16-fold turn-on response of the fluorescent signal was observed (Fig. 2), suggesting that the phenylboronic acid group of **1** can be converted efficiently into fluorescent DPCO. HPLC analysis also supported the formation of DPCO upon the addition of H_2O_2 (Fig. S5). Different concentrations of H_2O_2 were added into the test solution, and the fluorescence intensity at 670 nm shows a linear relationship with H_2O_2 concentrations from 1 up to 10 μM (Fig. 2 and Fig. S2). Thus, the detection limit (3δ/slope) was as low as 1.65 μM. On the other hand, the fluorescence intensity of probe **1** at 670 nm gradually increases to a plateau within 100 min in the presence of 20 equiv H_2O_2 (Fig. S3 and S4).

Subsequently, we tested the turn-on response of probe **1** for different oxidants including hydrogen peroxide (H_2O_2), hydroxyl radical (OH•), TBHP, *tert*-butoxy radical (TBHP•) and Fe^{3+}. In the test system, 20 equiv of different oxidants were added, respectively, to a 5 μM solution of probe **1** and the fluorescence response was recorded over 100 min. As shown in Fig. 3, only H_2O_2 could cause

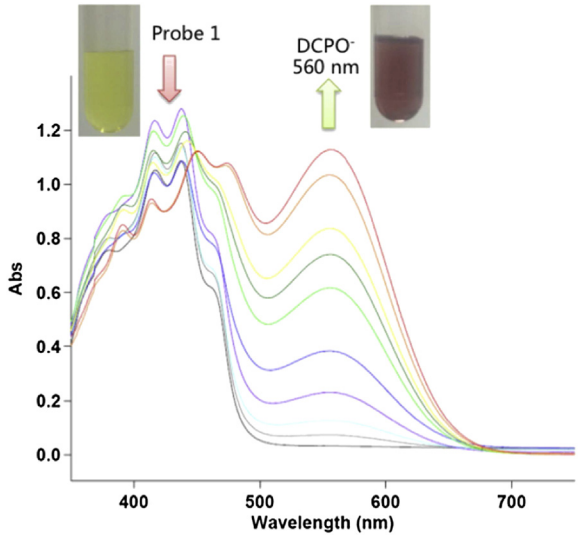


Fig. 1. Absorption spectra of probe **1** (5 μM) in the presence of different concentrations of H_2O_2 (0, 1.0, 2.5, 5, 10.0, 15.0, 20.0, 25.0, 100.0 μM; 60 min); PBS buffer (0.1 M, pH 7.4, 50% DMSO) at room temperature.

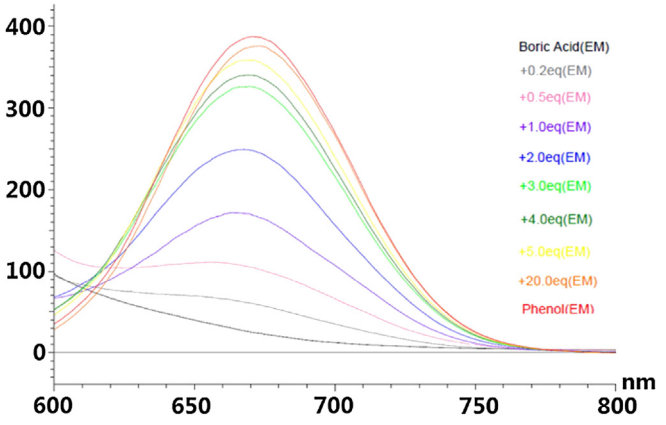


Fig. 2. Emission spectra of probe **1** (5 μM) in the presence of different equivalents of H_2O_2 (0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 20.0 equiv; 60 min) excited at 560 nm.

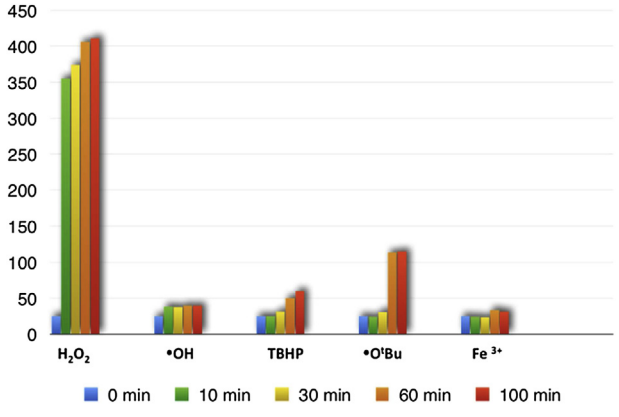


Fig. 3. Fluorescence intensity of probe **1** (5 μM) at 670 nm excited at 560 nm in the presence of various oxidants (100 μM). Data were acquired in PBS buffer (0.1 M, pH 7.4, 50% DMSO).

robust fluorescence intensity enhancement at 30 min while others exhibited almost no appreciable changes in fluorescence behavior. Samples treated with *tert*-butoxyl radical over 60 min showed a weak response. The selectivity for H_2O_2 over other relative