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Rational design of a fluorescent hydrogen peroxide probe based on the umbelliferone fluorophore

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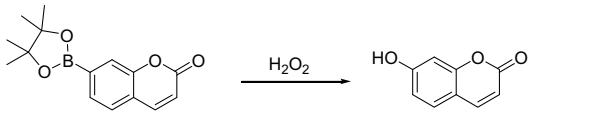
Abstract

In this study, we report a novel water-soluble umbelliferone-based fluorescent probe for hydrogen peroxide. This probe shows very large increases (up to 100-fold) in fluorescent intensity upon reaction with hydrogen peroxide, and good selectivity over other reactive oxygen species (ROS).

Keywords: Hydrogen peroxide; Reactive oxygen species (ROS); Hydroxycoumarin; Umbelliferone; Fluorescence

Produced during a number of physiological processes, such as Alzheimer's disease, apoptosis, and phagocytosis,[1–3] reactive oxygen species (ROS), including hydroperoxide (ROOH), superoxide (O_2^{-}), hydroxyl radical (·OH), and various peroxides (ROOR′) react with a large variety of easily oxidizable cellular components, such as NADH, NADPH, catecholamines, ascorbic acid, histidine, tryptophan, tyrosine, cysteine, glutathione, proteins, and nucleic acids.[4] ROS can also oxidize cholesterol and unsaturated fatty acids, causing membrane lipid peroxidation. Among all the ROS in biological systems, hydrogen peroxide (H_2O_2) is vasoactive and has been detected under various pathophysiological conditions such as inflammation, hypoxia-reoxygenation, and deficiency of a co-factor for nitric oxide (NO) synthesis. Hydrogen peroxide is generated in response to various stimuli, including cytokines and growth factors, and is also involved in regulating biological processes as diverse as immune cell activation and vascular remodeling in mammals,[5] and stomatal closure and root growth in plants.[6] Moreover, recent evidence demonstrated that hydrogen peroxide generated by the mitochondrial respiration is a potent inducer of oxidative damage and mediator of aging.[7] Although a number of reports have been published, the significance of hydrogen peroxide in biological system and the mechanism of its action are still poorly understood because of the limited availability of detection methods.

There have been several types of probes reported for the detection of hydrogen peroxide and other ROS, including fluorescein analogues,[8,9] rhodamine analogues, Amplex Red analogues,[10,11] phosphine-based fluorophores,[12,13] and lanthanide coordination complexes.[14,15] A recent review has summarized the current state of this field very well.[16] Each of the reported probes has its own advantages and disadvantages. An ideal probe should (1) be specific to the ROS of interest; (2) have the appropriate physicochemical properties to allow for permeation across the membrane barriers and to allow certain solubility in water; (3) give off a strong and easily detectable reporting signal upon encountering the appropriate ROS, (4) have the property



1 (weak fluorescence) **2** Umbelliferone (strong fluorescence)

Scheme 1. Reaction of compound **1** with hydrogen peroxide.

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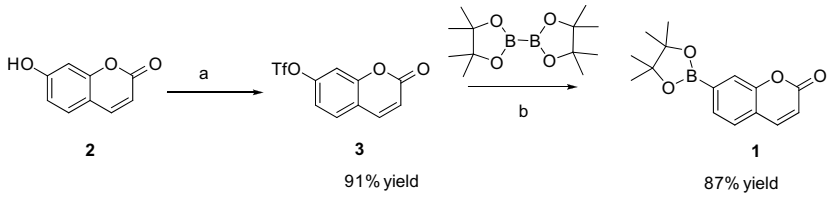

Scheme 2. Synthesis of coumarin boronate. Reagents and conditions: (a) $(TfO)_2O$, DMAP, DCM, rt; (b) $PdCl_2(dppf)/dppf$, KOAc, dioxane, 80 °C, microwave.

of turning on the signal upon encountering the appropriate ROS instead of turning off; and (5) easily synthesized from readily available starting materials. Among the detectable signals used, fluorescence is considered one of the more sensitive ones. Some important desirable features for a fluorescent probe include high quantum yield and emission in the visible region. Herein, we report the design and synthesis of a fluorescent hydrogen peroxide probe that has most of the desirable features prescribed for such a probe. This probe complements what is already available and should be very useful for the in vitro and in vivo detection of hydrogen peroxide formation.

Our design strategy depends on the selective hydrogen peroxide-mediated conversion of arylboronates into phenols.[17,18] In this design, the coumarin moiety was selected as the fluorescent chromophore. The end product after hydrogen peroxide oxidation is 7-hydroxycoumarin (umbelliferone) (Scheme 1), which is a well-known fluorophore with high quantum yield.[19–21] Umbelliferone is also used in sunscreen lotion as an antioxidant and has a minimal toxicity.[22] In addition, the designed probe can be synthesized easily from readily available starting material

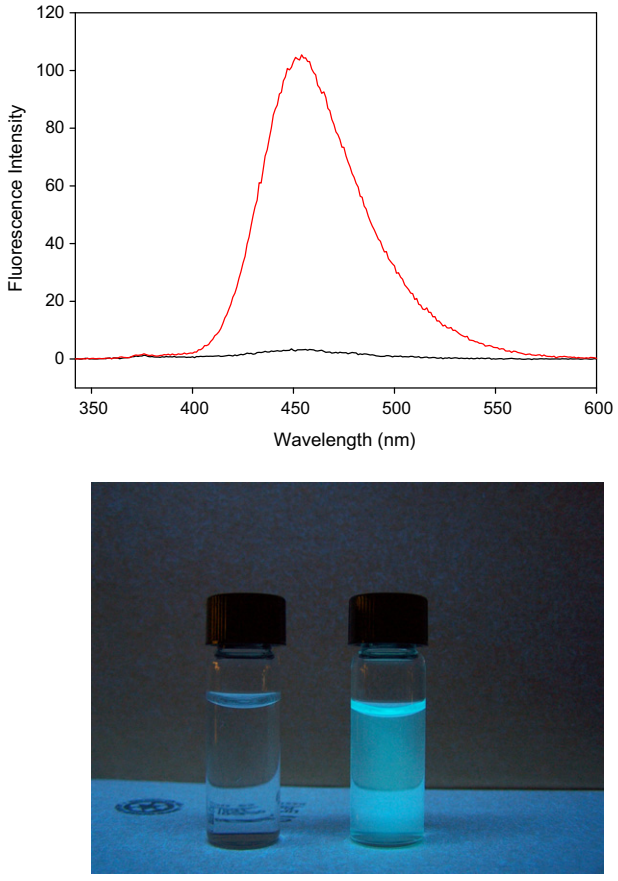

Fig. 1. Fluorescence response of 5 μM compound **1** to 100 μM H_2O_2 after 30 min. The black and red lines were recorded before and after H_2O_2 addition, respectively (top). The spectra were acquired in 0.1 M phosphate buffer, pH 7.4 ($\lambda_{ex} = 332$ nm). The bottom panel shows the color change before (left) and after hydrogen peroxide addition (right).

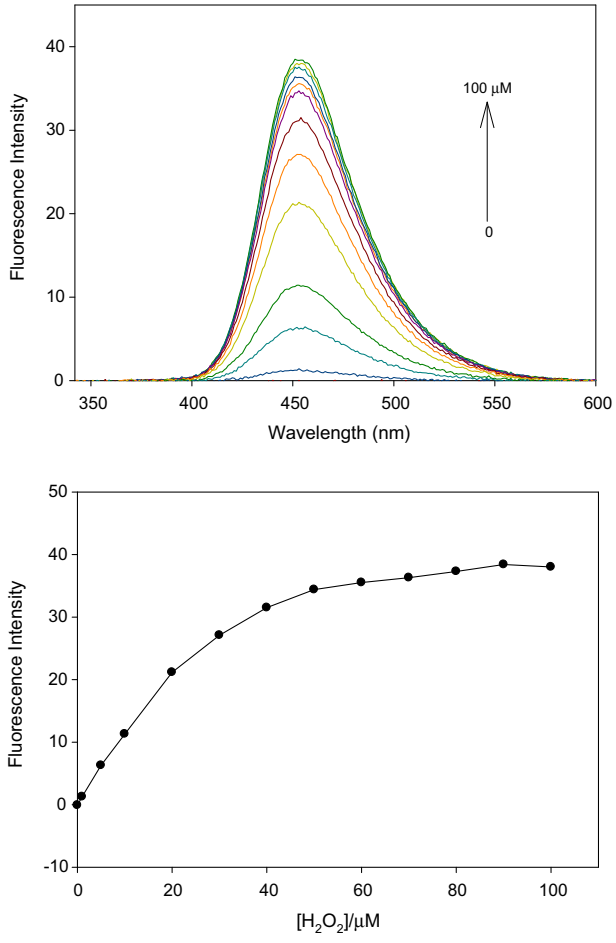

Fig. 2. Emission spectra (top) and concentration-dependent fluorescence intensity changes (bottom) of compound **1** at 454 nm at room temperature: experiments were conducted in 0.1 M phosphate buffer, pH 7.4 with excitation at 332 nm. The emission spectra were obtained 30 min after the addition of hydrogen peroxide to a 5 μM solution of compound **1**.

(umbelliferone) through a two-step conversion in high overall yield (about 80%).

The synthesis of the designed probe (**1**) started from commercially available 7-hydroxycoumarin (Scheme 2). After conversion to the corresponding triflate (**3**) in over 90% yield,[23] palladium-mediated borylation using pinacol-protected diborate gave the final probe (**1**) in about 87% yield.[24,25]

Compound **1** was evaluated for its ability to detect hydrogen peroxide under near physiological conditions (0.1 M phosphate buffer, pH 7.4). The probe itself (**1**) displays no fluorescence. The addition of hydrogen peroxide triggers a very significant fluorescence increase (about 100-fold) at 454 nm (Fig. 1). The NMR and MS experiments also confirmed that umbelliferone (**2**) was the product from the reaction of coumarin-7-boronate (**1**) with hydrogen peroxide.

Next, we investigated the concentration-dependent fluorescence response of compound **1** to the addition of hydrogen peroxide (Fig. 2). The fluorescence intensity of compound **1** increased as a function of hydrogen peroxide concentration at below 40 μM and then it leveled off.

We also investigated whether the fluorescence response of compound **1** was hydrogen peroxide-specific. Figure 3 compares the relative reactivity of compound **1** toward various ROS.[26] Selectivity data are displayed at several time points over 120 min. Compound **1** exhibits a 100-fold higher response to hydrogen peroxide over similar ROS such as hypochlorite (OCl^-), *tert*-butyl hydroperoxide (TBHP), and *tert*-butoxy radical (*t*-BuO·). This probe is also more than sixfold more responsive to hydrogen peroxide over hydroxyl radical (·OH) and over twofold more reactive over superoxide (O_2^-). It is particularly noteworthy that the fluorescence response of compound **1** was not influenced significantly by hypochlorite (OCl^-), hydroxyl radical (·OH), *tert*-butyl hydroperoxide (TBHP), and *tert*-butoxy radical (*t*-BuO·). Thus, we believe that compound **1** could be a very useful novel fluorescent probe for detecting hydrogen peroxide.

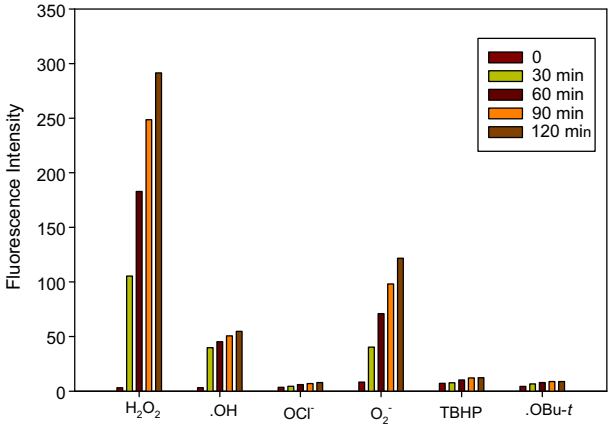

Fig. 3. Fluorescence response of compound **1** (5 μM) to various reactive oxygen species (ROS). Data shown are for 1.5 μM of OCl^-, 50 μM of O_2^-, 50 μM of *t*-BuO·, and 100 μM of all other ROS. Hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (TBHP), and hypochlorite (OCl^-) were diluted from 30%, 70%, and 5% aqueous solutions, respectively. Superoxide (O_2^-) was added as solid KO_2. Hydroxyl radical (·OH) and *tert*-butoxy radical (*t*-BuO·) were generated by the reaction of 1 mM Fe^{2+} with 100 μM H_2O_2 or 100 μM TBHP, respectively. The spectra were acquired in 0.1 M phosphate buffer, pH 7.4, and all the data were obtained after incubation with the appropriate ROS at room temperature. Emission intensity was collected at 454 nm ($\lambda_{ex} = 332$ nm).

In conclusion, we have designed and synthesized a novel water-soluble fluorescent probe (**1**) for hydrogen peroxide. This probe shows very large increases in fluorescent intensity upon reaction with hydrogen peroxide (up to 100-fold) at 5 μM. It also shows very good selectivity over other ROS. In addition, the probe also has the advantage of easy synthesis from readily available inexpensive starting materials. We hope that all these properties will make this coumarin-based fluorescent probe very useful for the in vitro and in vivo detection of hydrogen peroxide.

Acknowledgments

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Supplementary data

Supplementary data (spectroscopic data for **1** and **3**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.03.063.

References and notes

1. Mates, J. M.; Sanchez-Jimenez, F. M. *Int. J. Biochem. Cell Biol.* **2000**, *32*, 157–170.
2. Schoneich, C. *Biochim. Biophys. Acta* **2005**, *1703*, 111–119.
3. Simon, H. U.; Haj-Yehia, A.; Levi-Schaffer, F. *Apoptosis* **2000**, *5*, 415–418.
4. D'Autreaux, B.; Toledano, M. B. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 813–824.
5. Veal, E. A.; Day, A. M.; Morgan, B. A. *Mol. Cell.* **2007**, *26*, 1–14.
6. Slesak, I.; Libik, M.; Karpinska, B.; Karpinski, S.; Miszalski, Z. *Acta Biochim. Pol.* **2007**, *54*, 39–50.
7. Giorgio, M.; Trinei, M.; Migliaccio, E.; Pelicci, P. G. *Nat. Rev. Mol. Cell. Biol.* **2007**, *8*, 722–728.
8. Chang, M. C.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2004**, *126*, 15392–15393.
9. Miller, E. W.; Albers, A. E.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 16652–16659.
10. Votyakova, T. V.; Reynolds, I. J. *Arch. Biochem. Biophys.* **2004**, *431*, 138–144.
11. Miller, E. W.; Tulyathan, O.; Isacoff, E. Y.; Chang, C. J. *Nat. Chem. Biol.* **2007**, *3*, 263–267.
12. Onoda, M.; Tokuyama, H.; Uchiyama, S.; Mawatari, K.; Santa, T.; Kaneko, K.; Imai, K.; Nakagomi, K. *Chem. Commun.* **2005**, 1848–1850.
13. Soh, N.; Ariyoshi, T.; Fukaminato, T.; Nakano, K.; Irie, M.; Imato, T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2943–2946.
14. Wolfbeis, O. S.; Durkop, A.; Wu, M.; Lin, Z. *Angew. Chem., Int. Ed.* **2002**, *41*, 4495–4498.

15. Kozhevnikov, V. N.; Mandl, C.; Miltschitzky, S.; Duerkop, A.; Wolfbeis, O. S.; Koenig, B. *Inorg. Chim. Acta* **2005**, *358*, 2445–2448.
16. Soh, N. *Anal. Bioanal. Chem.* **2006**, *386*, 532–543.
17. Kuivila, H. G.; Wiles, R. A. *J. Am. Chem. Soc.* **1955**, *77*, 4830–4834.
18. Kuivila, H. G.; Armour, A. G. *J. Am. Chem. Soc.* **1957**, *79*, 5659–5662.
19. Aaron, J.-J.; Buna, M.; Parkanyi, C.; Antonious, M. S.; Tine, A.; Cisse, L. *J. Fluoresc.* **1995**, *5*, 337–347.
20. Moriya, T. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 1873–1886.
21. Azuma, K.; Suzuki, S.; Uchiyama, S.; Kajiro, T.; Santa, T.; Imai, K. *Photochem. Photobiol. Sci.* **2003**, *2*, 443–449.
22. Ramesh, B.; Pugalendi, K. V. *Life Sci.* **2006**, *79*, 306–310.
23. *Synthesis of 2-oxo-2H-chromen-7-yl trifluoromethanesulfonate* (**3**): To a solution of 7-hydroxycoumarin (0.81 g, 5 mmol) and 4-dimethylaminopyridine (DMAP, 0.61 g, 5 mmol) in DCM (15 mL) was added dropwise trifluoromethanesulfonic anhydride (1 mL, 6 mmol) at 0 °C. The resulting reaction mixture was stirred at room temperature overnight and quenched with saturated aqueous sodium carbonate solution. The organic layer was separated and dried over anhydrous Na_2SO_4. After the solvent removal, the crude product was purified by flash chromatography on silica gel with ethyl acetate–hexane (1:4) as the eluent to afford a yellow solid in 91% yield, mp = 75–77 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 7.74 (d, $J = 9.6$ Hz, 1H), 7.61 (d, $J = 8.4$ Hz, 1H), 7.28 (d, $J = 2.4$ Hz, 1H), 7.24 (dd, $J = 8.4$ and 2.4 Hz, 1H), 6.50 (d, $J = 9.6$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 159.2 (s), 154.5 (s), 150.8 (s), 142.1 (d), 129.4 (d), 118.8 (s), 118.6 (q, $J = 319$ Hz; s), 117.74 (d), 117.70 (d), 110.5 (d). GC–MS (EI): 294 (M^+).
24. Ishiyama, T.; Itoh, Y.; Kitano, T.; Miyaura, N. *Tetrahedron Lett.* **1997**, *38*, 3447–3450.
25. *Synthesis of 7-(4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-chromen-2-one* (**1**): A mixture of bis(pinacolato)diboron (0.14 g, 0.55 mmol), bis(diphenylphosphino) ferrocene (3 mol %), 1,1′-bis(diphenylphosphino)-ferrocene]dichloropalladium(II) (3 mol %), and potassium acetate (0.15 g, 1.5 mmol) was flushed with nitrogen; then the triflate (**3**, 0.15 g, 0.5 mmol) and dioxane (3 mL) were added. The resulting reaction mixture was irradiated at 80 °C for 1.5 h using a microwave reactor, diluted with benzene, washed with brine, and then dried over anhydrous Na_2SO_4. After the removal of solvent, the crude was purified by flash chromatography on silica gel with ethyl acetate–hexane (1:9) as the eluent to afford a yellow solid in 87% yield, mp = 173–175 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 7.74–7.67 (m, 3H), 7.47 (d, $J = 7.6$ Hz, 1H), 6.45 (d, $J = 9.6$ Hz, 1H), 1.37 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): 160.8 (s), 153.4 (s), 143.1 (d), 130.2 (d), 127.0 (d), 122.8 (d), 120.8 (s), 117.7 (d), 84.4 (s), 24.8 (q). GC–MS (EI): 272 (M^+).
26. *Fluorometric analysis*: The buffer reagents were bought from Aldrich and Acros and were used without purification. Water used for the fluorescence studies was doubly distilled and further purified with a Mill-Q filtration system. Quartz cuvettes were used in all studies. A Shimadzu UV-1700 UV–visible spectrometer was used for all the absorbance studies. A Shimadzu RF-5301PC fluorometer was used for all the fluorescent studies. The solutions of compound **1** (5×10^{-6} M) were prepared in 0.1 M phosphate buffer at pH 7.4. Then the solutions were transferred into a 1 cm quartz cell and UV absorbance data were recorded immediately. The UV λ_{max} was used as the fluorescence excitation wavelength.