**Electronic Supplementary Material (ESI) for RSC Advances**

# A ratiometric fluorescent probe with excited-state intramolecular proton transfer for benzoyl peroxide

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**Materials and instruments:**

Toluene-2-boronic acid (95%), *N*-bromosuccinimide (99%) and azobisisobutylonitrile (98%) were purchased from Sigma-Aldrich. 2-Aminothiophenol (98%) was obtained from Alladin (Shanghai, China). Acetonitrile (HPLC, 99.9%), 5-aminosalicylic acid (98.5%) were from J & K Chemicals. Thin layer chromatography (TLC) plates and silica gel (200-300 mesh) were purchased from Ocean Chemicals (Qingdao, China). Unless noted otherwise, all reagents were of analytical grade and used without further purification. 4-Acetamino-2-(benzo[d]thiazol-2-yl)phenol (**2**) and 2-(2-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**3**) were synthesized according to the reported literatures.[1] ROS were prepared according to reported literature.[2]

$^{1}$H NMR and $^{13}$C NMR spectra were recorded on a Bruker AMX-500 NMR spectrometer (tetramethylsilane as an internal standard). HRMS (high resolution mass spectrometry) were obtained on a micrOTOF-Q II mass spectrometer. The UV-Vis absorption spectra were measured using a Shimadzu UV-2450 spectrophotometer. The photoluminescence spectra were measured at room temperature using a Hitachi F-4600 spectrophotometer with the excitation and emission slit widths at 5.0 and 5.0 nm respectively at an excitation voltage of 700 V. Cell imaging was performed with an Olympus FV500 confocal microscope. The pH measurements were carried out with a pH meter (Leici PHSj-4a, Shanghai, China).

**Synthesis and characterization of A1:** To a stirred solution of **2** (0.525 g, 1.88 mmol) in DMF (18 mL) was added $K_2CO_3$ (0.55 g, 4 mmol) and the mixture was stirred for 5 min and subsequently 5 mL DMF solution of **3** (0.45 g, 1.51 mmol) was added dropwise. The reaction mixture was allowed for stirring at 80 $^{o}$C and the reaction was monitored by TLC. The crude product was poured into ice water and the precipitate was filtered and then purified by silica gel chromatography (70% ethyl acetate in hexane) to afford **A1** as white solid (0.61 g, 65%). $^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.28 (d, $J$ = 2.6 Hz, 1H), 8.02 (dd, $J$ = 14.0, 5.3 Hz, 2H), 7.93 (d, $J$ = 7.2 Hz, 1H), 7.84 (d, $J$ = 7.9 Hz, 1H), 7.59 (d, $J$ = 7.6 Hz, 1H), 7.47 (dd, $J$ = 17.7, 7.6 Hz, 2H), 7.42 – 7.36 (m, 2H), 7.34 (t, $J$ = 7.5 Hz, 1H), 7.15 (d, $J$ = 9.0 Hz, 1H), 5.61 (s, 2H), 2.17 (s, 3H), 1.21 (s, 12H). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 168.54, 162.86, 153.64, 151.83, 142.35, 136.30, 136.27, 131.46, 131.34, 128.23, 127.50, 125.95, 124.60, 124.28, 122.54,

122.29, 121.48, 120.45, 113.75, 83.81, 70.94, 24.82, 24.41. HRMS (TOF MS EI+) m/z: calcd. for $C_{28}H_{30}N_2O_4SB^+$ 501.2007, found 501.2029.

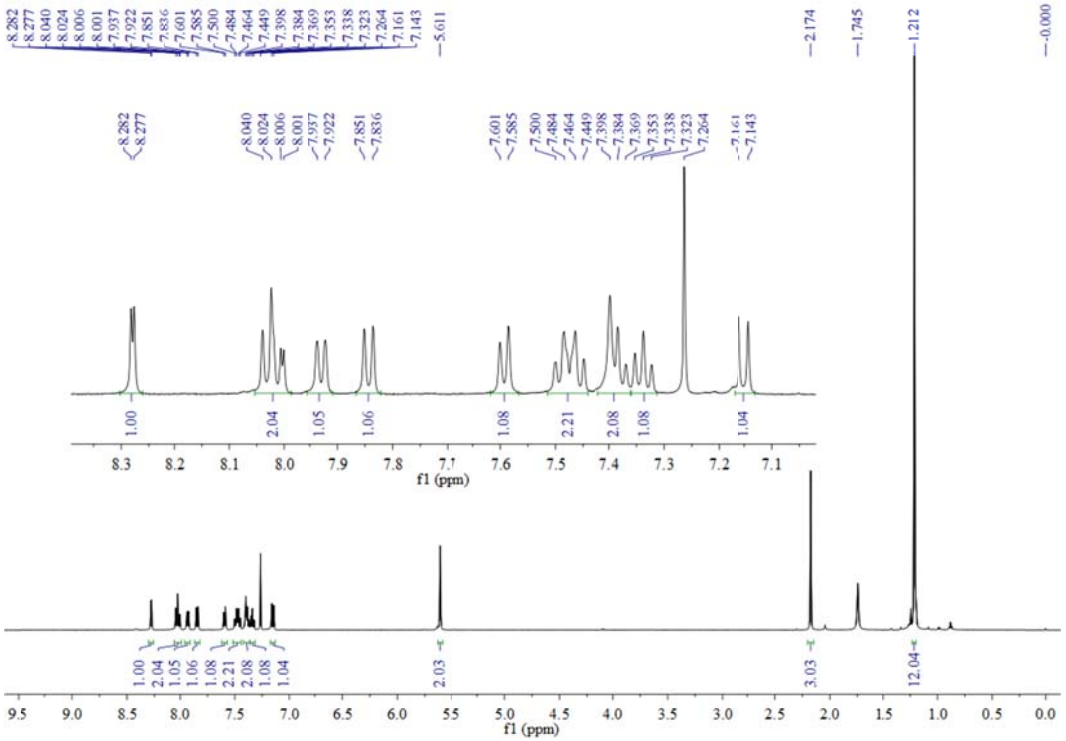

**Fig. S1** $^1$H NMR spectrum of **A1**.

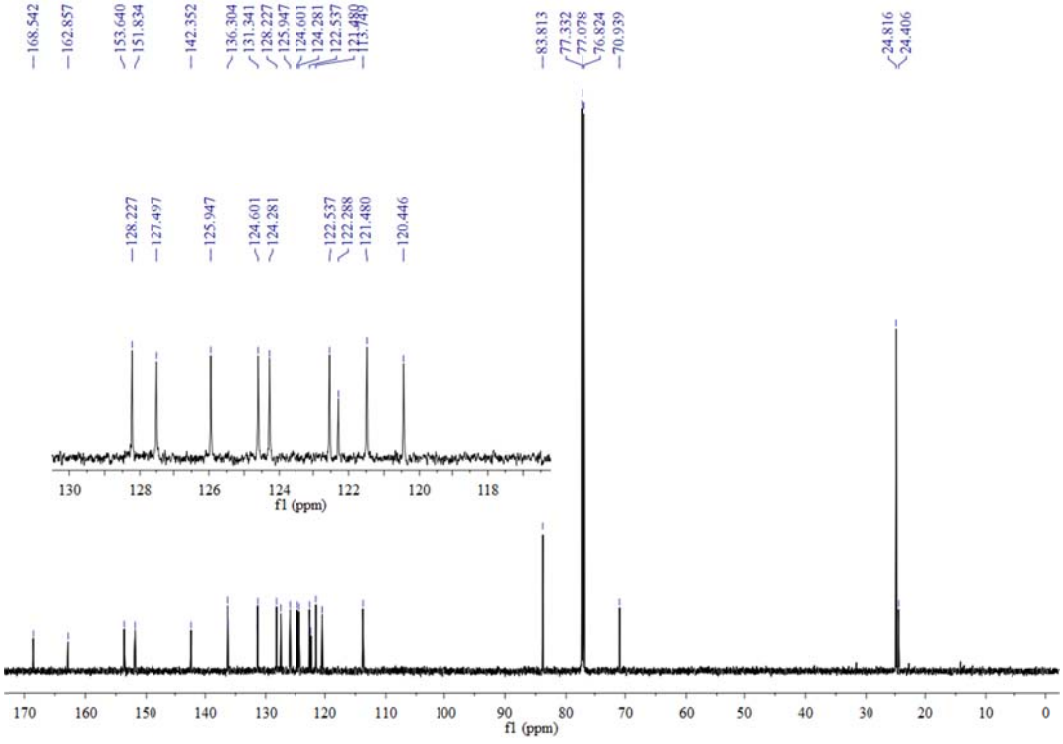

**Fig. S2** $^{13}$C NMR spectrum of **A1**.

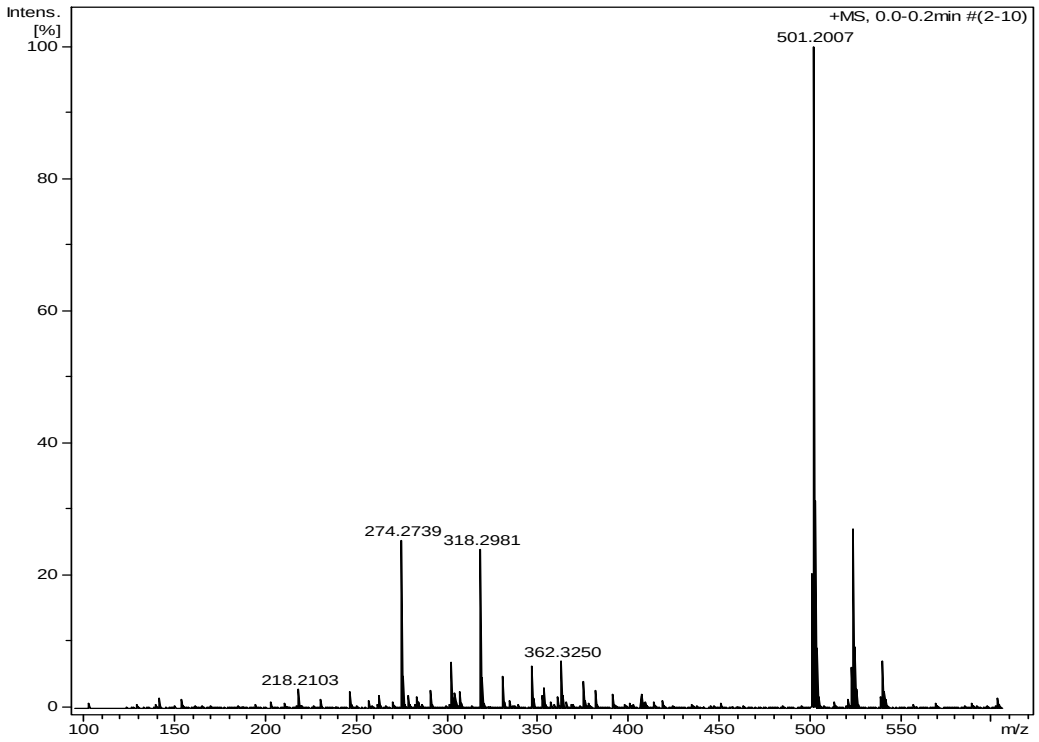


**Fig. S3** HRMS spectrum of **A1**.

## Experimental details

**Preparation of the testing solution:** A stock solution of **A1** (6 mM) was prepared in DMF. The testing solution was prepared by adding 3 μL **A1** stock solution into test tubes, followed by adding requisite volume of BPO sample solution. Then certain volumes of ethanol and PBS (10 mM) were added in turn to form 3 mL mixture solution containing 20% ethanol and 0.1% DMF.

**Detection of BPO in wheat flour:** The wheat flour containing a certain amount of BPO was prepared according to reported literature procedures[3] with some modifications. To begin with, 5 mL various concentrations (0, 0.5, 1.0, 5.0 mM) of BPO ethanol solution was mixed with 1.00 g wheat flour and then sonicated for 5 min. After standing for 2 min, the mixture was filtered through 0.22 μm membrane. Later, a certain volume of filtrate was added into the testing tube to make the final solution containing BPO in the range of 0.5–30 μM. Finally, the solutions were incubated with **A1** at 37 $^{o}$C for 60 min before fluorescence measurements.

**Detection of BPO in gel-like antimicrobial agent:** To 10 mL ethanol, 0.4844 g Benzoyl

Peroxide Gel (Laboratoires Galderma, France) containing 5% BPO was added. After sonicating for 5 min, the mixture was filtered. Then the filtrate was subjected to the analysis.

**Cell culture and fluorescence imaging:** Hela cells were grown in Dulbecco's modification of Eagle's medium Dulbecco (DMEM) supplemented with penicillin (100 U $mL^{-1}$), streptomycin (100 μg $mL^{-1}$) and fetal bovine serum (10%, v/v). After incubation for 24 h in a humidified incubator containing 5% $CO_2$ at 37 $^{o}C$, Hela cells were seeded in confocal dishes overnight. Hela cells were cultured with **A1** solution (100 μM) for 60 min, and then washed with PBS solution followed by incubated with 100 μM BPO DMF solution for 60 min. The fluorescence images were acquired with Olympus FV500 confocal microscope.

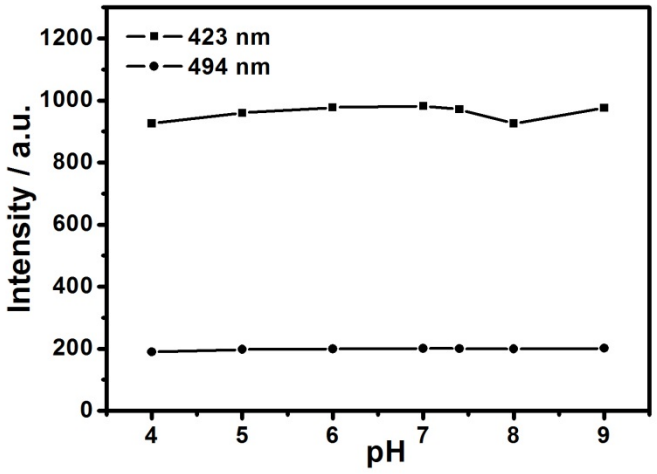


**Fig. S4** Fluorescence response of **A1** (6 μM) to various pH in 20 mM buffer solution with 20% ethanol and 0.1% DMF ($\lambda_{ex}$ 377 nm).

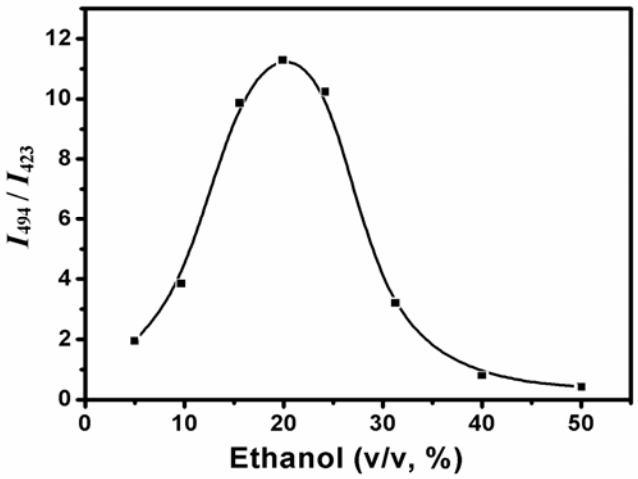


**Fig. S5** Effect of ethanol concentration on fluorescence intensity ratio ($I_{494}/I_{423}$) of **A1** (6 μM) towards

30 μM BPO. The assay was carried out in 20 mM PBS solution (pH 7.4) with 0.1% DMF at 37 °C for 60 min.

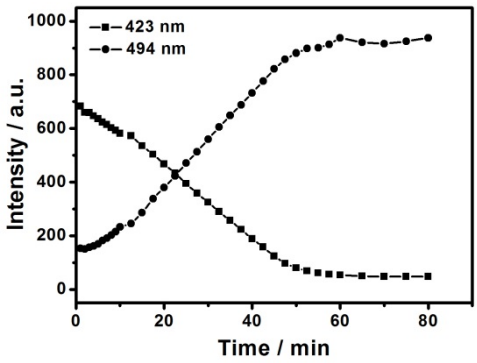


**Fig. S6** Time dependent changes of fluorescence intensity of **A1** (6 μM) with 30 μM BPO in 20 mM PBS solution (pH 7.4) with 20% ethanol and 0.1% DMF. The assay was carried out at 37 °C.

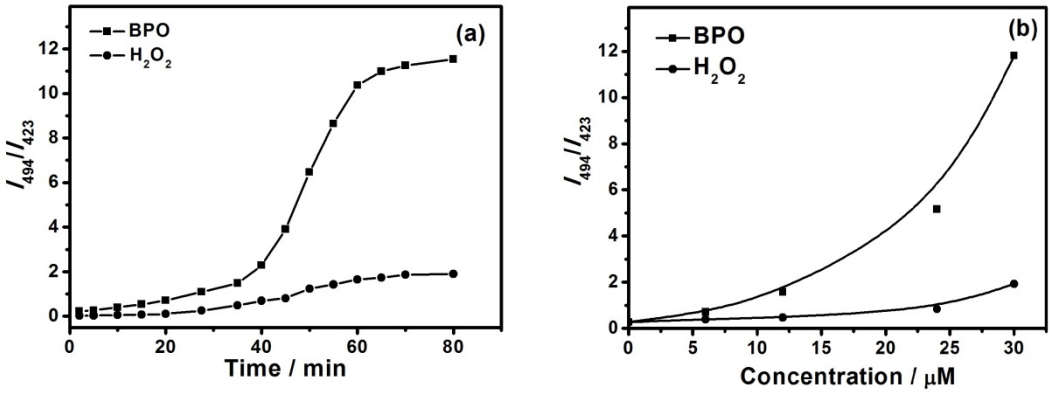


**Fig. S7** (a) Effect of reaction time on the fluorescence intensity ratio ($I_{494}/I_{423}$) of **A1** (6 μM) reacting with 30 μM of $H_2O_2$ or BPO, respectively; (b) Relative reactivity of **A1** (6 μM) towards various concentrations of $H_2O_2$ or BPO, respectively. The assay was carried out in PBS solution (pH 7.4) with 20% ethanol and 0.1% DMF at 37 °C for 60 min.

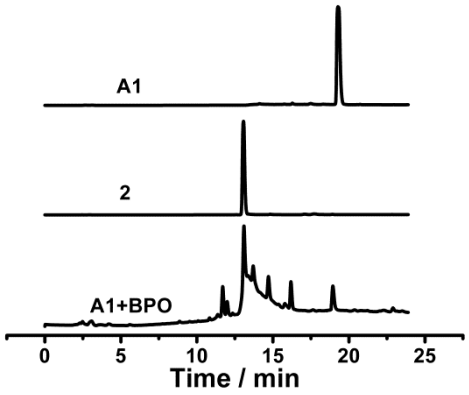


**Fig. S8** HPLC of **A1**, **2** and the product of **A1** reacting with BPO in PBS solution.

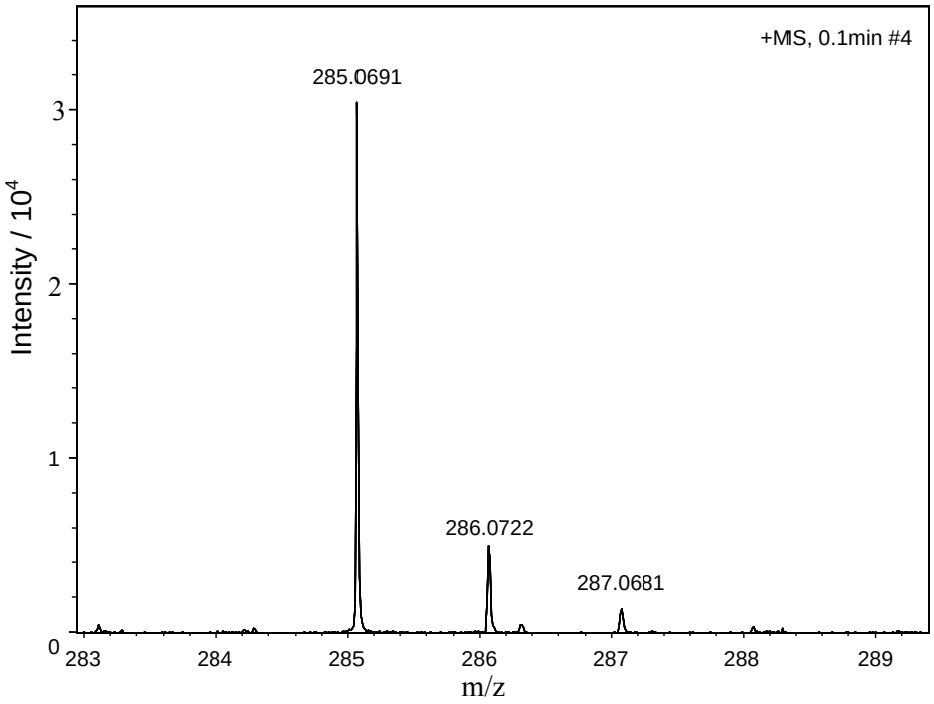


**Fig. S9** HRMS spectrum of the isolated product of (**A1** + BPO).

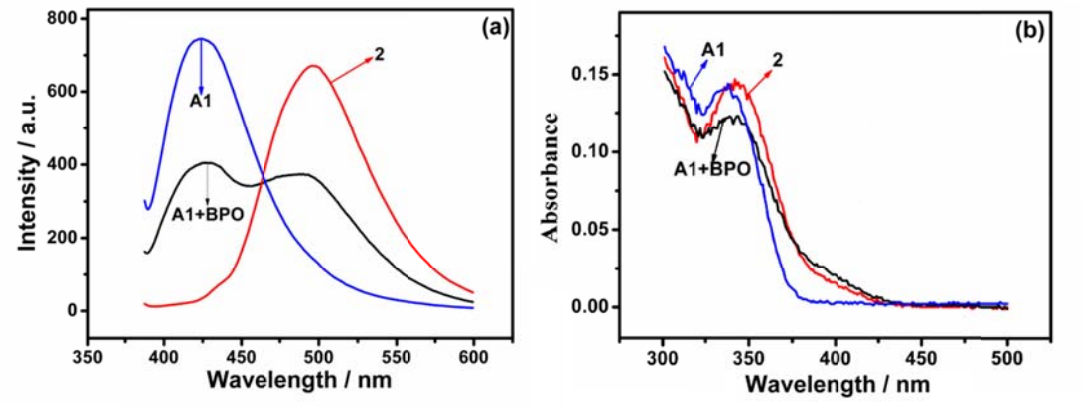


**Fig. S10** (a) Fluorescence spectra of probe **A1** (6 μM) in the absence or presence of BPO (10 μM), and **2** (~6 μM), excited at 377 nm. (b) Absorbance spectra of the probe **A1** (12 μM) in the absence or presence of BPO (28 μM), and **2** (12 μM).

**Table S1** Determination of BPO concentration in wheat flour.

| Sample | BPO added (mg) | BPO recovered (mg)[a] | Recovery (%) |
|---|---|---|---|
| Wheat flour A | 0 | Not detected | — |
| Wheat flour B | 6.1 | 5.8±0.06 | 96.4 |
| Wheat flour C | 12.1 | 11.7±0.05 | 96.2 |
| Wheat flour D | 60.6 | 58.0±0.15 | 95.7 |

[a] Relative standard deviations were calculated from the data of three measurements

**References:**

1. (a) E. Barni, P. Savarino, M. Marzona and M. Piva, *J. Heterocycl. Chem.*, 1983, **20**, 1517; (b) M. Santra, B. Roy and K. H. Ahn, *Org. Lett.*, 2011, **13**, 3422; (c) D. K. Scrafton, J. E. Taylor, M. F. Mahon, J. S. Fossey and T. D. James, *J. Org. Chem.*, 2008, **73**, 2871.
2. Z.-N. Sun, H.-L. Wang, F.-Q. Liu, Y. Chen, P. K. H. Tam and D. Yang, *Org. Lett.*, 2009, **11**, 1887.
3. W. Chen, Z. Li, W. Shi and H. Ma, *Chem. Commun.*, 2012, **48**, 2809.