Supplementary Material (ESI) for Chemical Communications

Supporting information

Strong red fluorescent probes suitable for detecting hydrogen peroxide generated by mice peritoneal macrophages

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Chemicals and Instruments

Xanthine oxidase (grade I from buttermilk, Catalog No. X18750), hypoxanthine (HPX), L-glutathione reduced (Catalog No. G4251), tert-butyl hydroperoxide solution (Catalog No. B2633), 3-morpholinosydnonimine hydrochloride (SIN-1, Catalog No. M184), dimethylsulfoxide (DMSO, Catalog No. D2650), 3-(aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene (NOC-5, Catalog No. A5456), and phorbol 12-myristate 13-acetate (PMA, Catalog No. P1585) were purchased from Sigma Chemical Co. and used without further purification. 1,4-Hydroquinone (Catalog No. 53965) and 1,6-dihydroxynaphthalene (Catalog No. 37765) were purchased from Fluka Chemical Co.

Fluorescence images were acquired using a LSM510 confocal laser scanning microscope (Carl Zeiss Co., Ltd.). The fluorescence spectra and fluorescence intensity were measured on Cary Eclipse spectrofluorimeter with a xenon lamp (Varian, Australia).

Preparation

Naphthofluorescein: Under an argon atmosphere, 1,6-dihydroxynaphthalene (8.36 g, 60.0 mmol) and phthalic anhydride (4.44g, 30 mmol) were combined in methanesulfonic acid (150 ml). The mixture was heated at 100°C for 12 hrs. Upon cooling to room temperature, the solution was poured into 8 volumes of ice-cold water. The resulting red precipitate was collected and dried *in vacuo* to give crude product. Purification by column chromatography (silica gel, 9:1 dichloromethane/methanol) furnished naphthofluorescein as a brick red powder (10.23g, 79% yield). ^{1}HNMR (DMSO-d_6, the naphthofluorescein adopts

a lactone form in the DMSO-d_6 solution, and two hydroxy hydrogen protons have no signal, 300 MHz):δ =8.68(d, $^3J_{H,H}$ = 9.1Hz, 2H), 8.08 (dd, $J_{H,H}$ =6.1, 2.1 Hz,1H), 7.76 (m, 2H), 7.48 (d, $^3J_{H,H}$ = 8.7 Hz, 2H), 7.36 (dd, $J_{H,H}$ = 9.1, 2.3 Hz, 2H), 7.28 (dd, $J_{H,H}$ = 6.1, 2.8 Hz, 1H), 7.18(d, $J_{H,H}$ = 2.3 Hz, 1H), 6.72 (d, $^3J_{H,H}$ = 8.7 Hz, 2H). FTIR (KBr, the solid naphthofluorescein adopts a quinone form): v = 3412, 3132(OH, br), 1712, 1650(CO, s), 1630, 1607, 1532 (C=C) cm^{-1}. Elemental analysis (%) calcd for $C_{28}H_{16}O_5$: C 77.78, H 3.75; found: C 77.90, H 3.70.

NFDS-1: A mixture of naphthofluorescein (1.3g, 3.0mmol) and *p*-methylbenzenesulfonyl chloride (1.9g, 10mmol) in pyridine (50ml) was stirred at room temperature overnight. Upon concentration to dryness, the residue was taken into ethyl acetate and washed with 1M HCl (2x50ml), water (2x50ml) and brine (2x50ml). The organic layer was dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give crude product. NFDS-**1** was purified with column chromatography (silica gel, 2:1 hexane / ethyl acetate) to obtain a gray-white powder (1.47g, 66% yield). M.p.195-198°C. ^{1}HNMR (300 MHz, DMSO-d_6, TMS): δ = 8.86 (d, $^3J_{H,H}$ = 9.0 Hz, 2H), 8.11 (dd, $J_{H,H}$ = 6.2, 2.3 Hz, 1H), 7.78 (d, $^3J_{H,H}$ = 8.0 Hz, 4H), 7.75 (m, 2H), 7.48 (d, $^3J_{H,H}$ = 8.0 Hz, 4H), 7.40 (dd, $J_{H,H}$ = 9.0, 2.1 Hz, 2H), 7.38 (d, $^3J_{H,H}$ = 8.5 Hz, 2H), 7.30 (dd, $J_{H,H}$ = 6.2, 2.9 Hz, 1H), 7.20 (d, $J_{H,H}$ = 2.1 Hz, 2H), 6.90 (d, $^3J_{H,H}$ = 8.5 Hz, 2H), 2.40 (s, 6H). FTIR (KBr): v = 1765 (CO, s), 1648, 1600, 1572, 1468 (C=C, m), 1402, 1370 (S=O) cm^{-1}. Elemental analysis (%) calcd for $C_{42}H_{28}O_9S_2$: C 68.11, H3.78, S 8.65; found: C 68.32, H3.75, S 8.40

NFDS-2: This compound was prepared in a manner similar to NFDS-1 as described above, except that perfluorooctanesulfonyl chloride was used instead of *p*-methylbenzenesulfonyl chloride. NFDS-**2** (0.42g, 10% yield) was yellow oil. ^{1}HNMR (DMSO-d_6, 300 MHz): δ = 8.98 (d, $^3J_{H,H}$ = 8.9 Hz, 2H), 8.12 (dd, $J_{H,H}$ = 6.1, 2.2 Hz, 1H), 7.72 (m, 2H), 7.50 (dd, $J_{H,H}$ = 8.9, 2.0 Hz, 2H), 7.41 (d, $^3J_{H,H}$ = 8.4 Hz, 2H), 7.35 (d, $J_{H,H}$ = 2.0 Hz, 2H), 7.31 (dd, $J_{H,H}$ = 6.1, 2.5 Hz,

1H), 6.96 (d, $^{3}J_{H,H}$ = 8.4 Hz, 2H). FTIR (KBr): v = 1767 (CO, s), 1646, 1610, 1550 (C=C, m), 1400, 1375 (S=O) cm-1. Elemental analysis (%) calcd for $C_{44}H_{14}F_{34}O_9S_2$: C 37.82, H 1.00, S 4.58; found: C 37.90, H 1.10, S 4.48.

Confocal fluorescence imaging

Evaluation of the H_2O_2-selectivity of NFDS-**1** with peritoneal macrophages: The probe was dissolved in DMSO to obtain 10 mM stock solution. The cells ($2.5x10^4$) obtained from the peritoneum cavity of a mouse (Balb/c) were seeded onto glass slides, and were incubated at 37°C for 2h. Then, the cells were loaded with NFDS-**1** (10μM, HEPES buffer, pH 7.4) by incubation at 37°C for 30 min. Probe-loaded macrophages were washed twice with phosphate-buffered saline (PBS), then were stimulated with PMA ($2ngml^{-1}$) or treated with H_2O_2 (10 or 100nM) at 37°C for 10 min, respectively. Following cellular washing with PBS, slides were sealed. All images were taken at the same magnification using a LSM510 confocal laser scanning microscope (Carl Zeiss Co., Ltd.) with an objective lens (x40). The excitation wavelength was 633nm, and the emission was filtered using a 650nm barrier filter.