Sequential detection of inflammation-related hypochlorite ions and viscosity with a relay fluorescent probe

Yu-Qing Zhang a, Zhi-Nan Kang a, Jing-Jie Ming a, Sheng-Nan Suo a, Jia-Ling Kang b, Hai Xu a, *, Ya-Wen Wang a, *, Yu Peng a

a School of Life Science and Engineering, School of Chemistry, Southwest Jiaotong University, Chengdu 610031, People’s Republic of China.
b Department of Clinical Pharmacy, West China Hospital, Sichuan University, Chengdu 610041, People’s Republic of China.

*Corresponding author.

E-mail addresses: xuhai@my.swjtu.edu.cn (H. Xu), ywwang@swjtu.edu.cn (Y.-W. Wang).

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1. General Methods.

Isophorone, malondicyanide, N,N-diisopropylethanamine, trifluoroacetic acid, and vanillin and other analytical reagents were all purchased from Innochem Technology Co., Ltd. Deionized water was used in analysis experiments.

$^{1}$H and $^{13}$C NMR spectra were recorded on a Bruker AVB-400 spectrometer using TMS as the internal reference. HRMS (ESI) spectra were measured with a Waters e2695 spectrometer. Fluorescence spectra were recorded by a F7000 spectrofluorimeter from Hitachi PharmaSpec. Fluorescence imaging of SWJT-19 in HeLa cells was recorded on a Nikon A1R+ (Japan) laser scanning confocal microscope. Fluorescence imaging of mice was collected on a Xenogen IVIS specturm (Xenogen Corporation, USA) small animal optical in vivo imaging system.

SWJT-19 was weighed and dissolved in ethanol to prepare 1.0 mM stock solution. Various analytes were dissolved in distilled water or ethanol prepare 0.1 mol/L solution for standby. SWJT-19 stock solution was diluted to 10.0 µM with EtOH-H$_2$O (3:7, v/v). For all fluorescence spectra, the excitation was set at 460 nm, the emission wavelength was in the range of 480-800 nm, and the excitation and emission gaps were 10/10 nm.

SWJT-OH was weighed and dissolved in ethanol to prepare 1.0 mM stock solution. For all fluorescence spectra, the excitation was set at 427 nm, the emission wavelength was in the range of 447-800 nm, and the excitation and emission gaps were 5/5 nm.

The quantum yield was calculated through the following formula:

$$\Phi_u = \Phi_s \left( \frac{F_u}{F_s} \right) \left( \frac{A_s}{A_u} \right) \left( \frac{\eta_u^2}{\eta_s^2} \right)$$

“$F_u$” and “$A_u$” represent the integral of the fluorescence emission and absorbance spectra of SWJT-19, respectively. “$F_s$” and “$A_s$” were the integral of the fluorescence emission and absorbance spectra of fluorescein, respectively. “$\Phi_u$” and “$\Phi_s$” represent fluorescence quantum yield of SWJT-19 and fluorescein, respectively. “$\eta_u$” and “$\eta_s$” are the refractive index of the solvent of SWJT-19 and fluorescein, respectively.

The cytotoxicity of SWJT-19 to HeLa cells was examined by CCK-8 assay method.
HeLa cells were seeded at a 96-well culture plate. After growth at 37 °C in a 5% CO₂ for 24 h, treated with 0.0, 2.0, 5.0, 10.0, 15.0 μM **SWJT-19**. After incubation for 12 h, the CCK solution was added into each well for further incubation for 4 h. The absorbance at 540 nm was measured.

The HeLa cells were incubated in a glass-bottom petri dish (Φ 15 mm) and adhered at 37 °C for 24 hours. The cells were washed with phosphate buffered saline (PBS) and added 10.0 μM of **SWJT-19** at 37 °C for 30 minutes, then washed with PBS and imaged. After incubating with 1.0 mM ClO⁻ for 30 min at 37 °C, HeLa cells were washed with PBS and imaged again. HeLa cells were incubated with Nystatin (10.0 μM) or LPS (1.0 μg/mL) for 6 h and then with **SWJT-19** (10.0 μM) for 30 min, then perform other experimental operations. Fluorescence imaging of intracellular **SWJT-19** in HeLa cells was recorded on a laser scanning confocal microscope. The excitation wavelength of the laser is 488 nm.

All animal experiments were approved by the Southwest Military Region General Hospital and Southwest Jiaotong University Animal Experiment Ethics Committee, and regulations on the administration of experimental animals of People’s Republic of China. The Institute of Cancer Research (ICR) mice were kindly kept and were used in all the imaging experimental study. Representative fluorescence images of an ICR mouse given an intraperitoneal injection of LPS (20.0 μg/mL in saline) and a subsequent (8 h later) intraperitoneal injection of **SWJT-19** (50.0 μM) at the same region. Images were taken after incubation for 0, 2, 5, 10, 20, 25 and 30 min. The excitation wavelength of the laser is 500 nm.

SWJT-OH (100.0 mg, 0.28 mmol) was dissolved in 20 mL of dichloromethane, then N,N-diisopropylethylamine (150 µL) and dimethylthiocarbonyl chloride (142.0 mg, 0.91 mmol) were added to the above solution at room temperature. After stirring 4 hours, the mixture solution was extracted with CH$_2$Cl$_2$ (3× 150 mL). Finally, the organic layer was concentrated and purified on silica gel by column chromatography (petroleum ether : ethyl acetate = 10:1) to obtain SWJT-19 (43.9 mg, yield 35.2%).

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 9.96 (s, 1H), 7.75 (s, 1H), 7.66 (s, 1H), 7.52 (d, $J$ = 16.1 Hz, 1H), 7.33 (d, $J$ = 16.1 Hz, 1H), 6.93 (s, 1H), 3.86 (s, 3H), 3.36 (d, $J$ = 1.8 Hz, 6H), 2.61 (s, 2H), 2.53 (s, 2H), 1.00 (s, 6H) ppm. $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ = 189.3, 186.3, 170.8, 155.9, 152.7, 145.4, 136.5, 135.4, 131.3, 130.6, 123.9, 119.9, 116.7, 114.2, 113.5, 77.5, 57.1, 44.9, 43.2, 42.8, 39.2, 38.7, 27.9 (2C) ppm. HRMS (ESI): calcd for C$_{24}$H$_{25}$N$_3$O$_3$S [M+H]$^+$ 436.1617, found 436.1689.
3. $^1$H, $^{13}$C NMR and ESI-MS copies of SWJT-19.

Figure S1. $^1$H NMR spectrum of SWJT-19 (400 MHz, DMSO-$d_6$).

Figure S2. $^{13}$C NMR spectrum of SWJT-19 (100 MHz, DMSO-$d_6$).
Figure S3. HRMS spectrum of SWJT-19.
4. Effects of pH.

**Figure S4.** The effect of pH on the fluorescence intensity ratio ($I_{658}/I_{558}$) of probe SWJT-19 (10.0 μM) in the absence or presence of ClO$^-$ (1.0 mM) ($\lambda_{ex} = 460$ nm).
5. Linear concentration range of SWJT-19 with ClO⁻.

**Figure S5.** The fluorescence intensity ratio ($I_{658}/I_{558}$) of SWJT-19 (10.0 µM) changes as concentrations of ClO⁻ (0–400.0 µM) ($\lambda_{ex} = 460$ nm) (LOD = 3σ/slope).
6. ESI-MS spectrum of probe SWJT-19 + ClO⁻.

**Figure S6.** HRMS spectrum of probe SWJT-19 + ClO⁻.

**Figure S7.** Spot chart of fluorescent intensity changes \(\text{SWJT-19, 10.0 \, \mu M}\) over time.
8. Fluorescence of SWJT-OH in different solvents.

Figure S8. Emission spectra of SWJT-OH (10.0 μM) in all solvent (λ<sub>ex</sub> = 427 nm).
9. Cytotoxicity assays.

**Figure S9.** Cytotoxicity of SWJT-19 at different concentrations (0.0, 2.0, 5.0, 10.0, 15.0 μM).
10. Intracellular imaging of and viscosity.

Figure S10. (a) HeLa cells were incubated with SWJT-OH (10.0 μM) for 30 min. (b) HeLa cells were incubated with Nystatin (10.0 μM) for 6 h and then with SWJT-OH (10.0 μM) for 30 min. (c) Relative fluorescence intensity output of groups (λ_{ex} = 500 nm).