Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

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

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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_2O (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:

$$\Phi u = \Phi s\ (Fu/Fs)(As/Au)(\eta_u^{\,2}/\eta_s^{\,2})$$

"*Fu*" and "*Au*" represent the integral of the fluorescence emission and absorbance spectra of **SWJT-19**, respectively. "*Fs*" and "*As*" were the integral of the fluorescence emission and absorbance spectra of fluorescein, respectively. "Φu" and "Φs" represent fluorescence quantum yield of **SWJT-19** and fluorescein, respectively. "η_u" and "η_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 ℃ in a 5% CO_2 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 ℃ for 24 hours. The cells were washed with phosphate buffered saline (PBS) and added 10.0 μM of **SWJT-19** at 37 ℃ for 30 minutes, then washed with PBS and imaged. After incubating with 1.0 mM ClO^- for 30 min at 37 ℃, HeLa cells were washed with PBS and imaged again. HeLa cells were incubated with 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.

2. Synthesis of SWJT-19.

SWJT-OH (100.0 mg, 0.28 mmol) was dissolved in 20 mL of dichloromethane, then N,N-diisopropylethanamine (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_2Cl_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): δ = 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): δ = 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 $C_{24}H_{25}N_3O_3S$ $[M+H]^+$ 436.1617, found 436.1689.

3. ^{1}H, ^{13}C NMR and ESI-MS copies of SWJT-19.

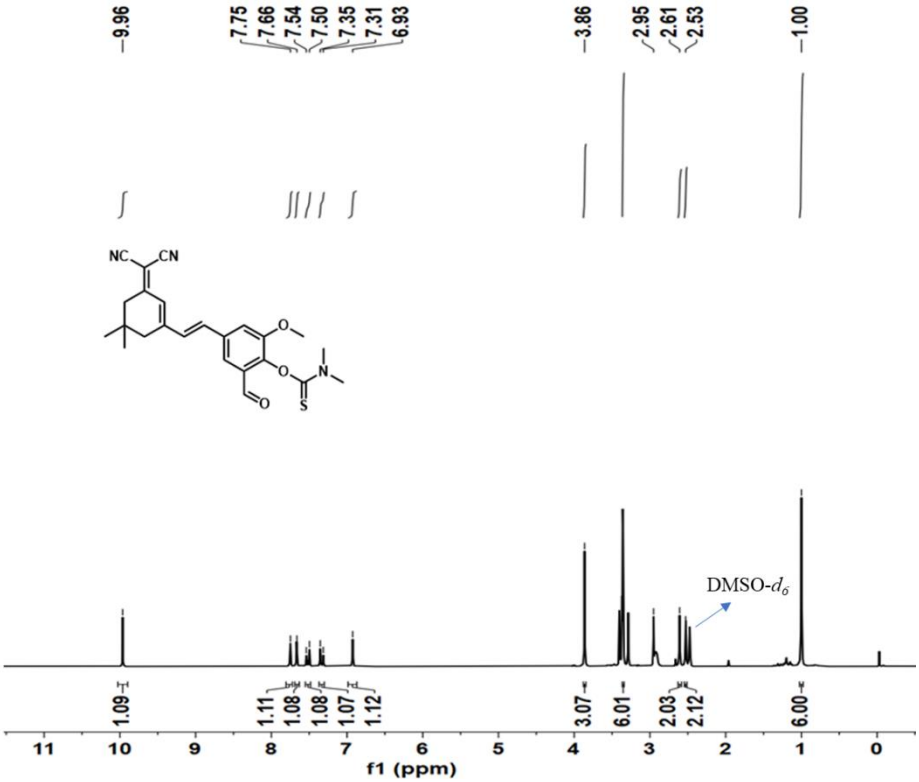

Figure S1. ^{1}H NMR spectrum of **SWJT-19** (400 MHz, DMSO-d_6).

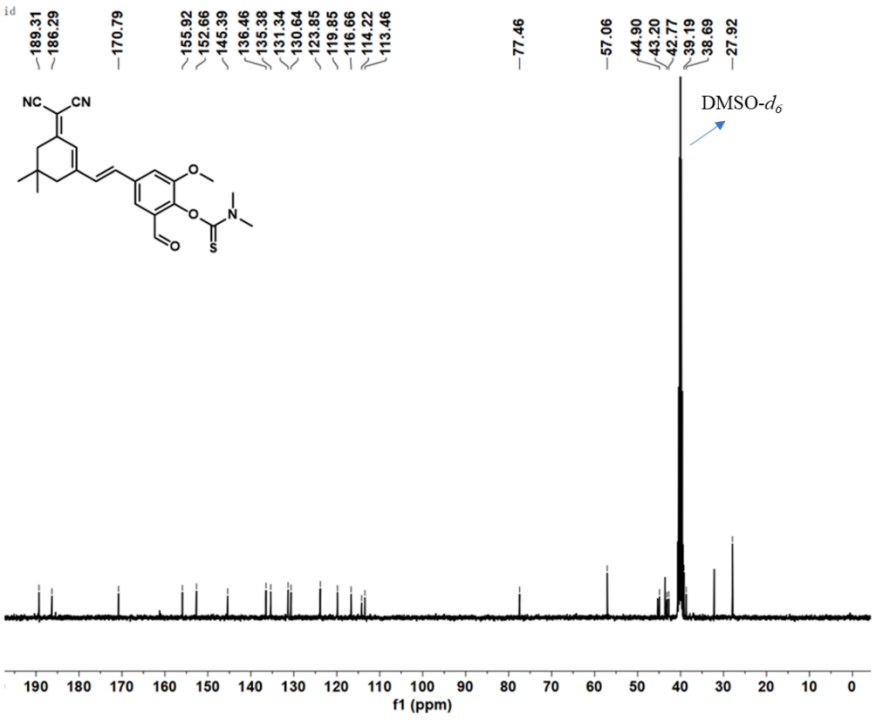

Figure S2. ^{13}C NMR spectrum of **SWJT-19** (100 MHz, DMSO-d_6).

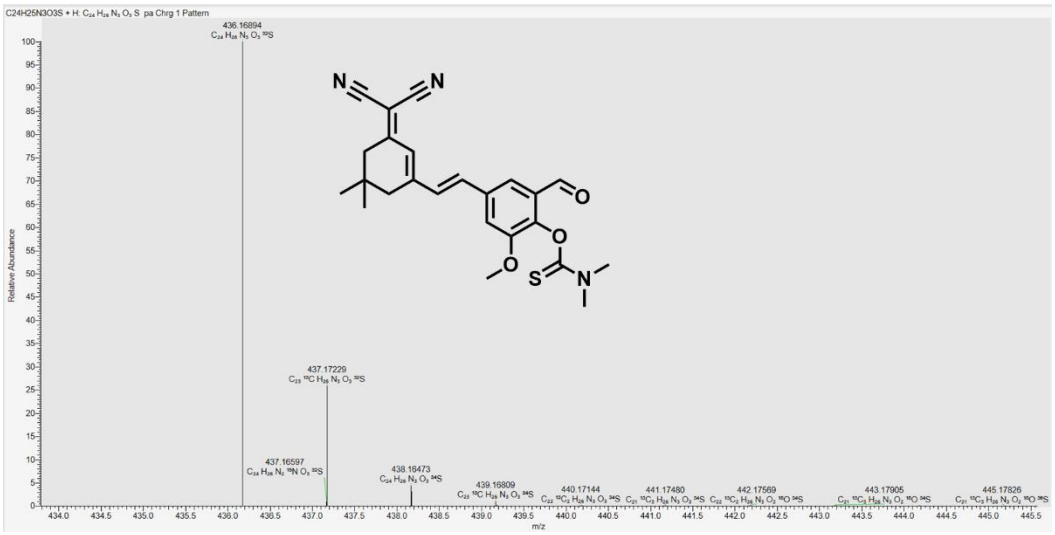

Figure S3. HRMS spectrum of **SWJT-19**.

4. Effects of pH.

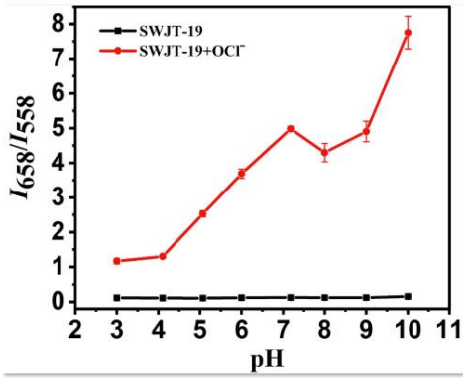

Figure S4. The effect of pH on the fluorescence intensity ratio (I_{658}/I_{558}) of probe **SWJT-19** (10.0 μM) in the absence or presence of ClO^- (1.0 mM) (λ_{ex} = 460 nm).

5. Linear concentration range of SWJT-19 with ClO^-.

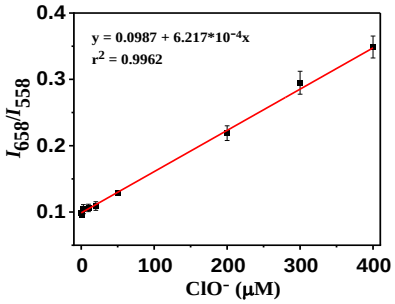

Figure S5. The fluorescence intensity ratio (I_{658}/I_{558}) of **SWJT-19** (10.0 μM) changes as concentrations of ClO^- (0–400.0 μM) (λ_{ex} = 460 nm) (LOD = 3σ/slope).

6. ESI-MS spectrum of probe SWJT-19 + ClO^-.

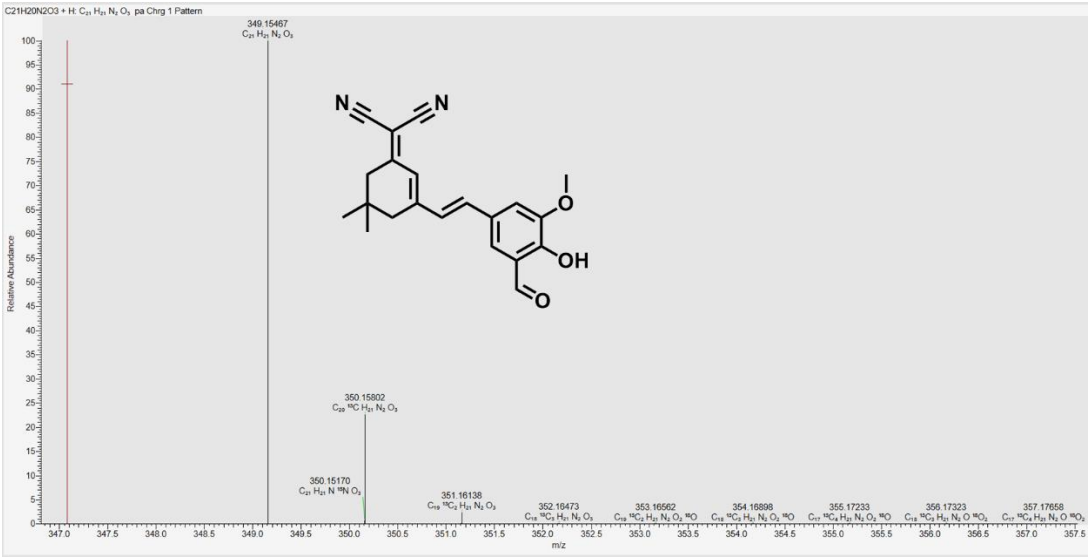

Figure S6. HRMS spectrum of probe **SWJT-19 +** ClO^-.

7. Stability of SWJT-19 solution.

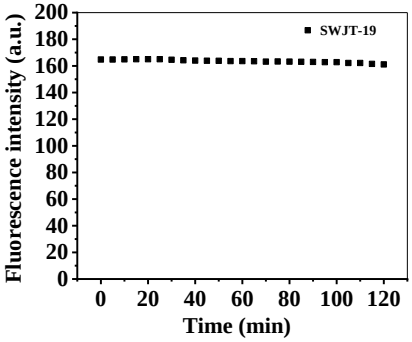

Figure S7. Spot chart of fluorescent intensity changes (**SWJT-19**, 10.0 μM) over time.

8. Fluorescence of SWJT-OH in different solvents.

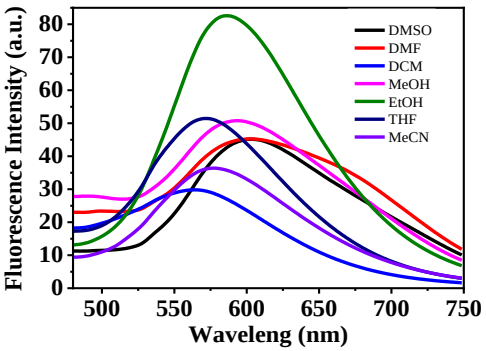

Figure S8. Emission spectra of **SWJT-OH** (10.0 μM) in all solvent (λ_{ex} = 427 nm).

9. Cytotoxicity assays.

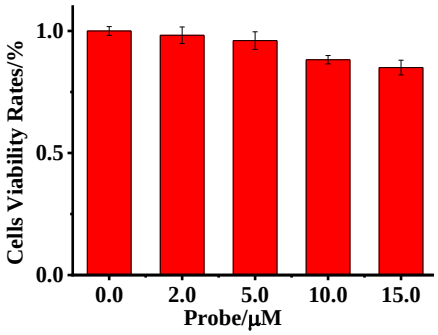

Figure S9. Cytotoxicity of **SWJT-19** at different concentrations (0.0, 2.0, 5.0, 10.0, 15.0 μM).

10. Intracellular imaging of and viscosity.

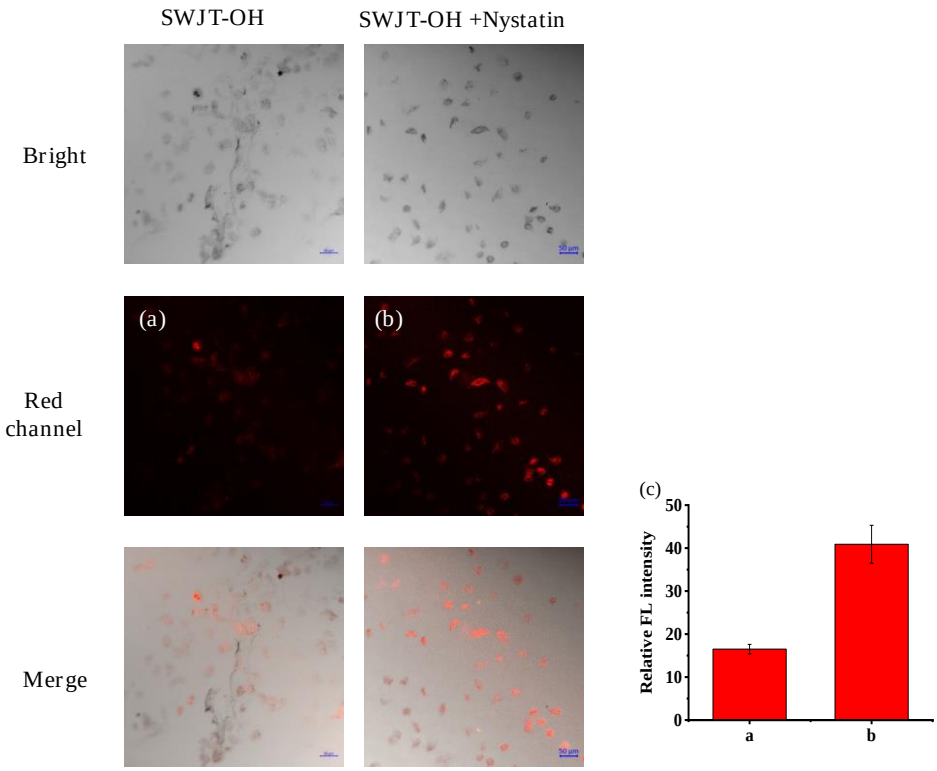

Figure S10. (a) HeLa cells were incubated with **SWJT-OH** (10.0 μM) for 30 min. (b) HeLa cells were incubated with Nystatin (10.0 μM) for 6 h and then with **SWJT-OH** (10.0 μM) for 30 min. (c) Relative fluorescence intensity output of groups (λ_{ex} = 500 nm).