Fast detection of hypobromous acid in cells and water environment by a lysosome-targeted fluorescent probe

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Table of Contents

1. General information.

4-Bromo-1,8-naphthalic anhydride，methylene glycol monomethylether, N-(2-aminoethyl)-morpholine, N,N-diisopropylethylamine and other reagents were purchased from Innochem Technology Co., Ltd. All the chemicals we used in experiment were analytical reagent grade.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVB-400 spectrometer using TMS as the internal reference. ESI-MS spectra were acquired on Agilent Infinity Lab LC/MSD. HRMS (ESI) spectra were measured with a Waters e2695 spectrometer.The fluorescence spectra were obtained by Hitachi F-7000 spectrofluorometer with the excitation and emission slit widths at 5/2.5 nm. The absorption spectra were obtained by AOE A360 UV–Vis spectrophotometer. Fluorescence imaging of HBrO in HeLa cells was performed by Nikon AR1+ confocal microscope.

The solution of **SWJT-23** (1.0 mM) was prepared in DMSO for subsequent detection. HBrO was prepared by using 1.16g of NaOH dissolved in 10 mL ultrapure water and 386.66 μL of liquid bromine in an ice bath. The concentration of HBrO was calculated by Lambert-Beer's law (E_{329} = 332 $L \cdot mol^{-1} \cdot cm^{-1}$). Peroxynitrite ($ONOO^-$) was obtained by the reaction of H_2O_2 and $NaNO_2$, and the concentration was determined from the absorption at λ=302 nm (ε=1670 $L \cdot mol^{-1} \cdot cm^{-1}$) in a 0.1 M NaOH. Hydroxyl radical (·OH) was prepared by the classical Fenton reaction between H_2O_2 and Fe^{2+}. $^1O_2$ was generated by reaction between H_2O_2 and excessive NaClO, and concentrations of ·OH determined by H_2O_2 concentrations. Other analytes such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Br^-, ClO^-, SO_4^{2-}, Cys, Hcy, GSH, NO^{2-}, H_2O_2, Na_2S, NaSH were dissolved in ultrapure water to prepare 10.0 mM stock solutions. All experiments were performed at 37 ℃ in 10 mM HEPES buffer solution (1 % DMSO, pH = 7.4). The excitation wavelength in all fluorescence spectra is 435 nm.

Using quinine sulfate as a reference compound, the quantum yield was calculated by the following formula:

$$\Phi_u = \Phi_s (F_u/F_s) (A_s/A_u)$$

Φ_u and Φ_s represent the fluorescence quantum yields of **SWJT-23** and quinine sulfate, respectively. F_u and F_s were the fluorescence emission peak integral value of **SWJT-23** and

quinine sulfate, respectively. A_s and A_u represent the maximum absorbance value of **SWJT-23** and quinine sulfate, respectively.

The test paper was immersed in the DMSO solution containing 100.0 μM of **SWJT-23** for several minutes. Then, take out the test paper and different concentrations (0, 5.0, 10.0, 20.0, 30.0, 50.0 μM) of HBrO solution were sprayed on the test strip. The fluorescent color change of the test paper can be seen under the 365 nm ultraviolet lamp.

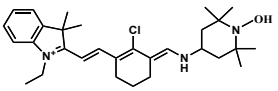
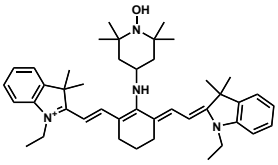
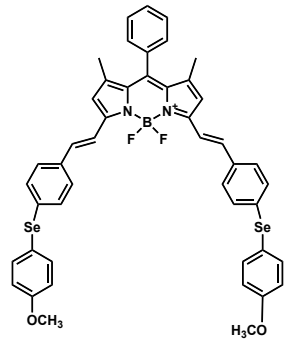
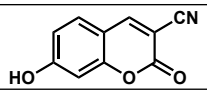
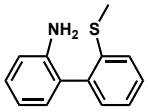
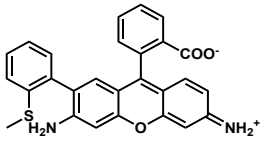
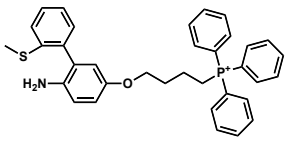
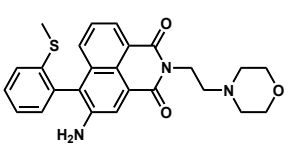
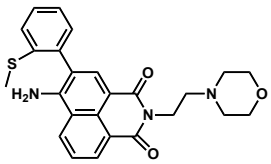
The cytotoxicity of **SWJT-23** on HeLa cells was detected by CCK-8 assay. HeLa cells were seeded at a 96-well culture plates and grown in 5 % CO_2 at 37 °C for 24 h, then incubated with **SWJT-23 (**0, 5.0, 10.0, 15.0, 20.0 μM) for 24 h. After the incubation, the CCK solution was added into each well, incubated for 2 h at 450 nm ELISA to measure the OD value and calculate the cell viability of each group.

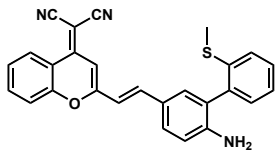
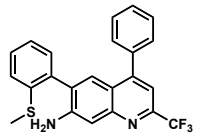
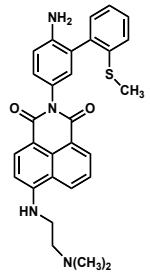
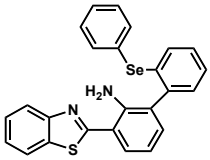
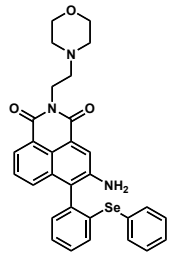
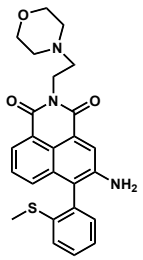
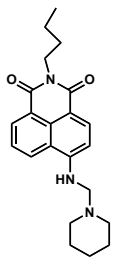
HeLa cells were placed at the bottom of a glass dish and cultured in an incubator at 37 ° C for 24 hours. The cells were washed three times with PBS, and the blank group was incubated with **SWJT-23** (10.0 μM) for 30 min and then imaged. Subsequently, imaging of endogenous HBrO in cells were implemented in two groups. In the first group, cells were incubated with NaBr (100.0 μM) for 30 min, then these cells were further incubated with **SWJT-23** (10.0 μM) for 30 min. In the second group, cells were pretreated with Br^- (100.0 μM) and N-acetylcysteine (100.0 μM) for 30 min, then these cells were loaded with **SWJT-23** (10.0 μM) for 30 min. In addition, imaging of exogenous HBrO in cells were carried out. Cells were incubated with HBrO (30.0 μM) for 30 min after preincubation with **SWJT-23** (10.0 μM) for 30 min. The above cells were washed three times with PBS and imaged under confocal laser scanning microscope.

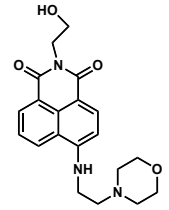
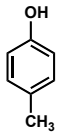
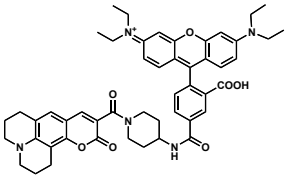
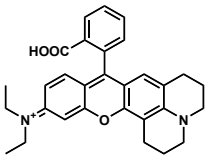
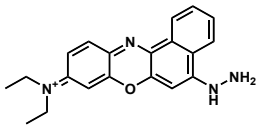
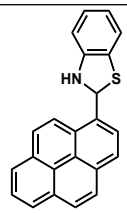
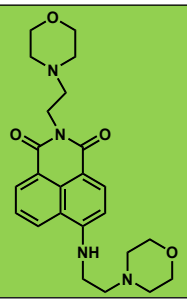
For colocalization experiments, the cells were divided into three groups, each group of cells were separately co-incubated with **SWJT-23** (10.0 μM) and Lyso-Tracker Red (50.0 nM) for 30 min. Then the above cells were washed three times with PBS and imaged under confocal laser scanning microscope. The Pearson's coefficients for the three groups of cells were 0.89, 0.92 and 0.95, and the overlap coefficients were 0.90, 0.93 and 0.93 (calculated by Image J).

2. Summary of the fluorescent probes for HBrO.

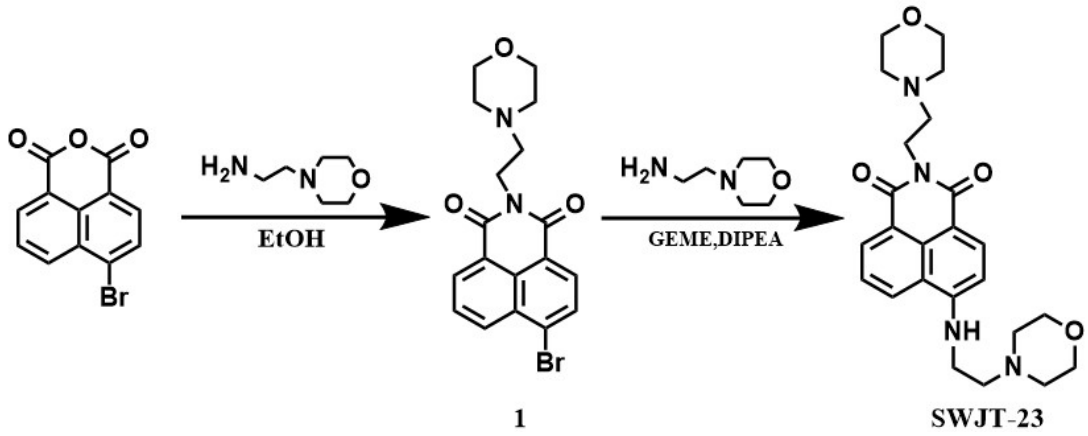
Table S1 Summary of the fluorescent probes for HBrO

Probe	Detection Limit	Response time	Theoretical calculation support	Application in water environment	Application of portable test strips	References
	ND	900 s	✓	--	--	15
	ND	900 s	✓	--	--	15
	0.97 μM	3.0 min	✓	--	--	16
	30.6 nM	30 s	--	--	--	17
	17 pM	3.0 min	--	--	--	18
	20 pM	3.0 min	--	--	--	19
	1.82 nM	30 s	--	--	--	20
	99 nM	12 s	--	--	--	21
	33.5 nM	Immedia-ely	✓	--	--	22

	660 nM	8.0 min	--	--	--	23
	92 nM	10 s	--	--	--	24
	240 nM	4 s	--	--	--	25
	711 nM	4 min	--	--	--12	26
	296 nM	4 min	--	--	--12	26
	254 nM	5 s	✓	--	--	27
	200 nM	1 min	--	--	--	28

	15 nM	5 s	✓	--	--	29
	0.37 μM	--	--	--	--	30
	11.9 nM	10 s	--	--	--	31
	1.37 nM	4 s	--	✓	--	32
	3.8 nM	2 s	--	--	--	33
	3.0 pM	5 s	--	--	--	34
	119 nM	10 min	--	✓	--	35
	1.24 nM	3 s	✓	✓	✓	This work

3. Synthesis of SWJT-23.



Scheme S1. Synthesis of **SWJT-23.**

4. ^{1}H, ^{13}C NMR, and MS spectra of SWJT-23.

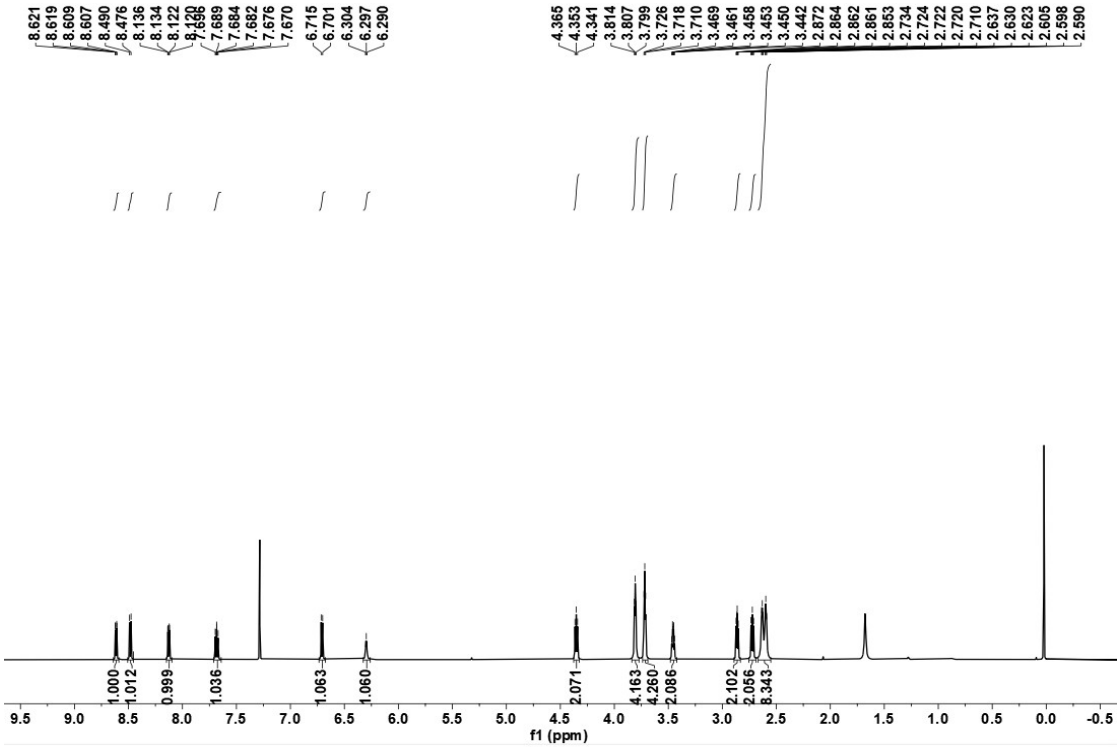


Fig. S1. ^{1}H NMR spectrum of **SWJT-23** (400 MHz, $CDCl_3$).

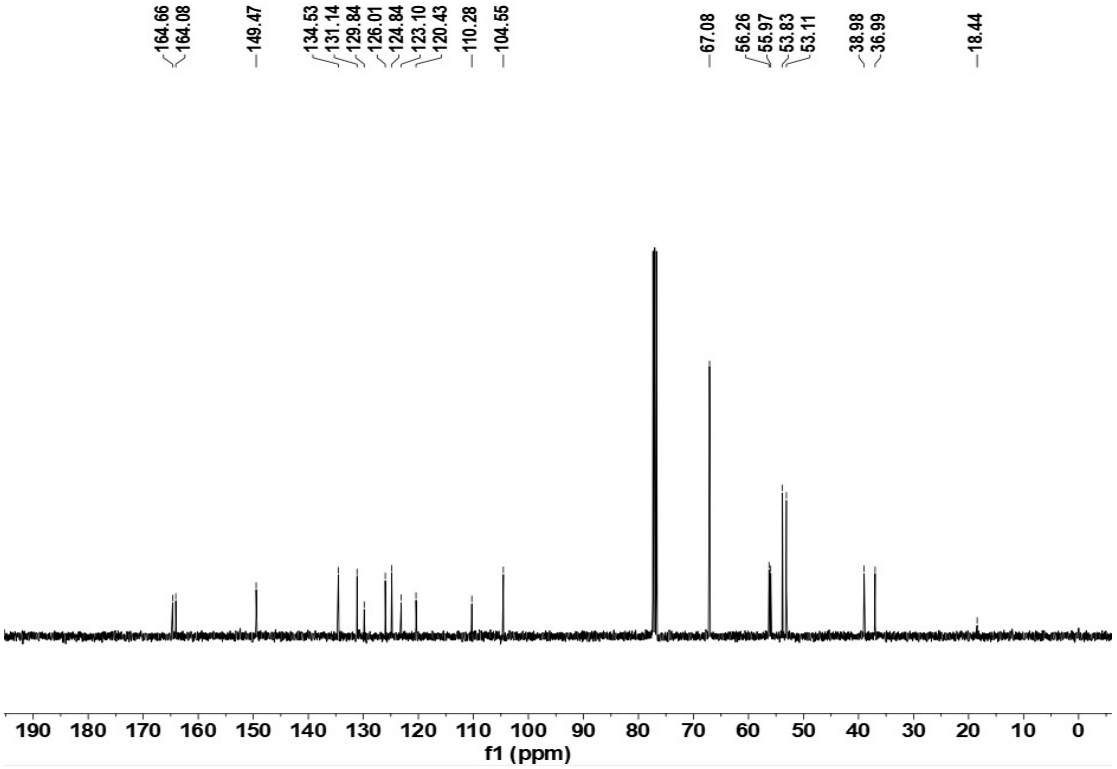


Fig. S2. ^{13}C NMR spectrum of **SWJT-23** (100 MHz, $CDCl_3$).

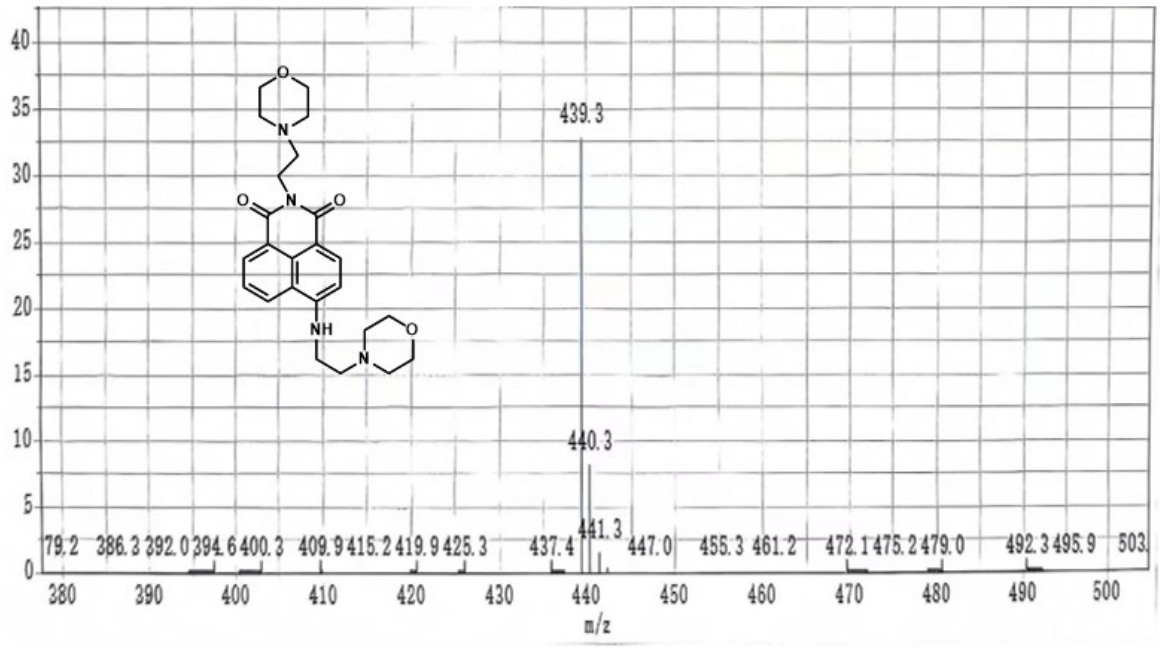


Fig. S3. ESI-MS spectrum of **SWJT-23.**

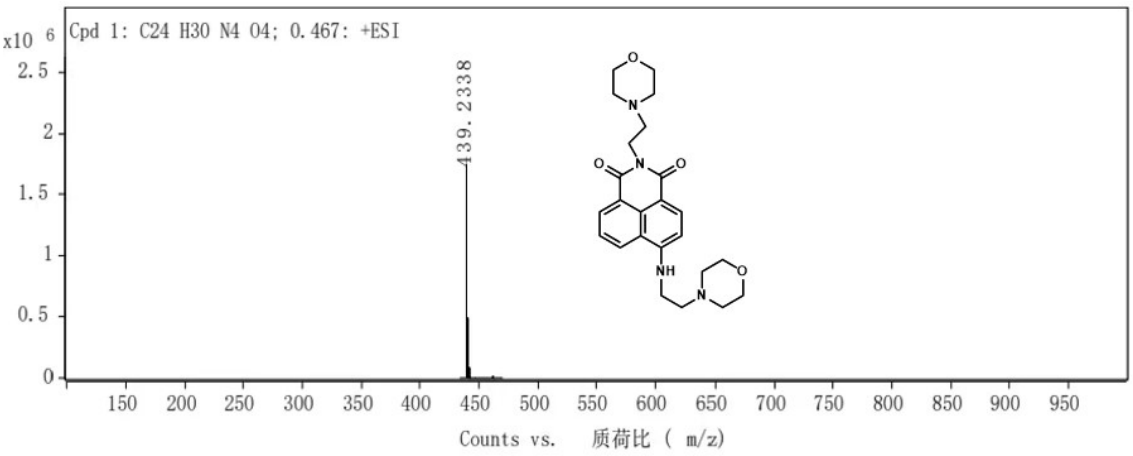


Fig. S4. HRMS spectrum of **SWJT-23.**

5. The pH effect of SWJT-23 and SWJT-23 +HBrO.

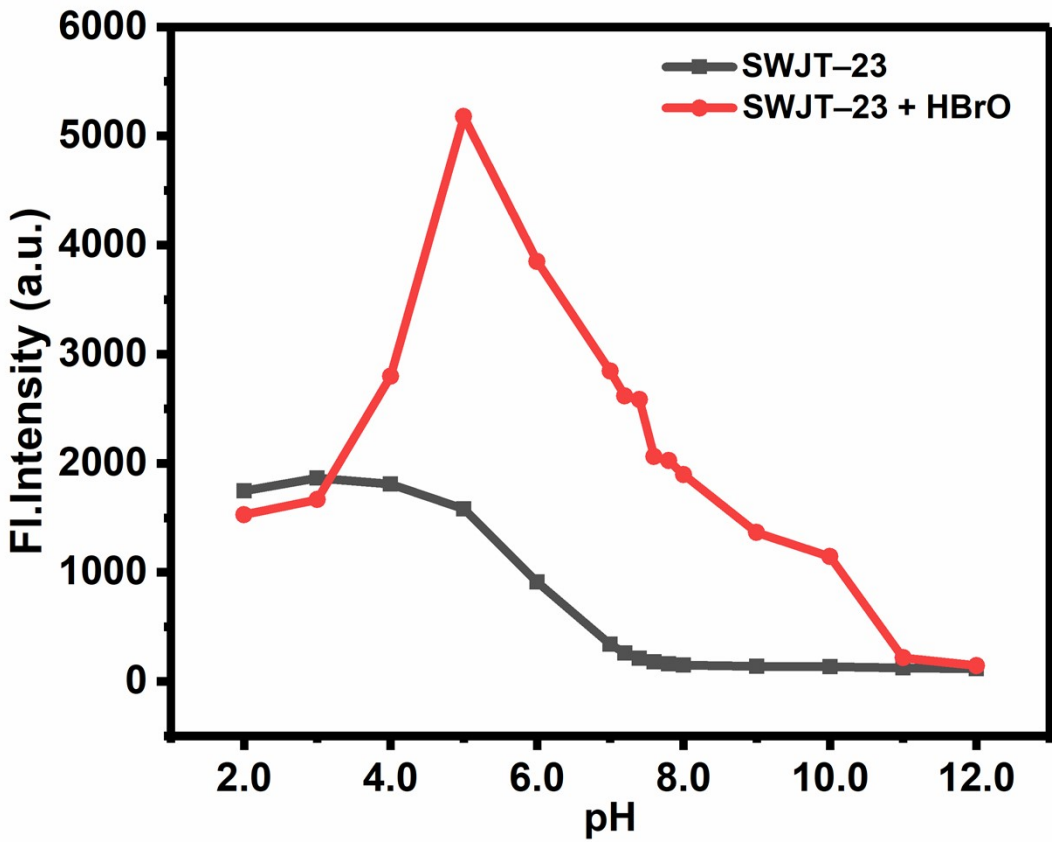


Fig. S5. The pH effect of **SWJT-23** and **SWJT-23** + HBrO (λ_{ex} = 435 nm).

6. The stability of SWJT-23 and SWJT-23 +HBrO.

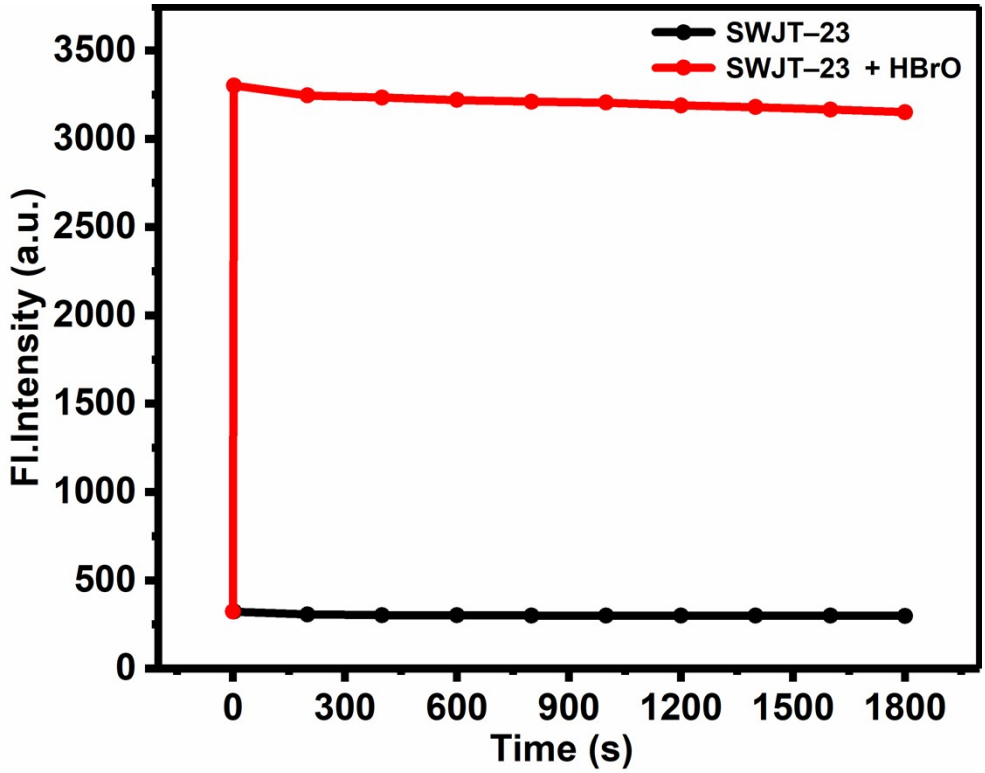


Fig. S6. The stability of **SWJT-23** and **SWJT-23 +HBrO** (λ_{ex} = 435 nm).

7. The detection limit of SWJT-23 to HBrO.

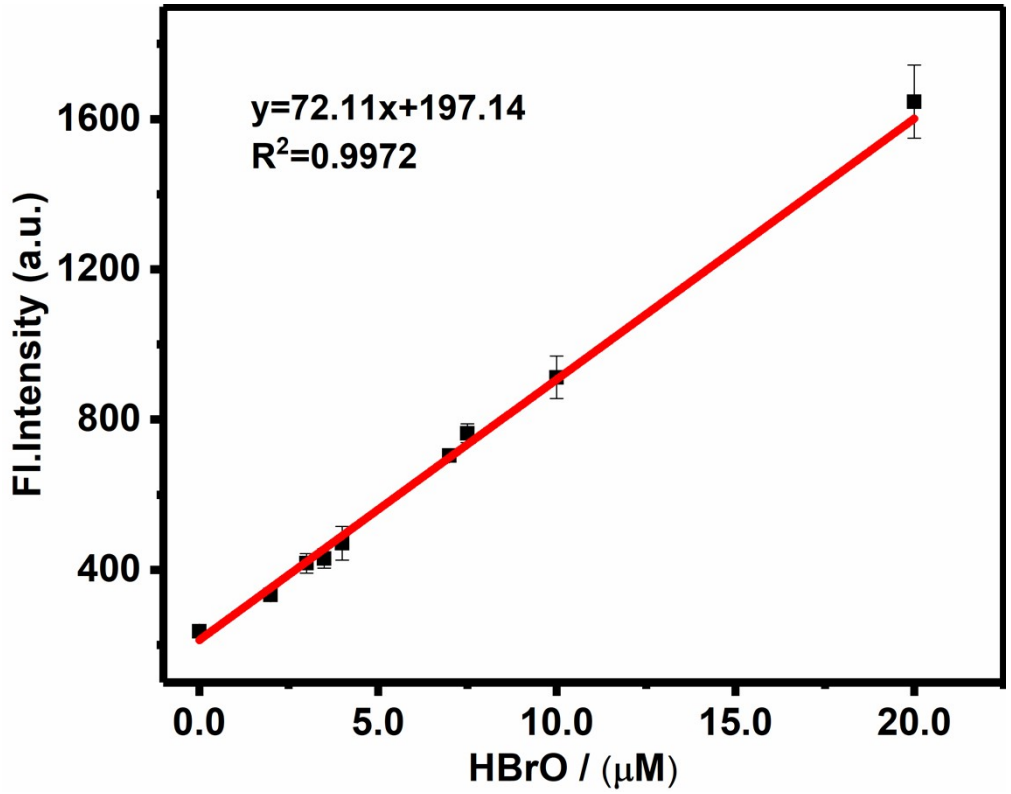


Fig. S7. Linear regression equation of **SWJT-23** (10.0 μM) upon the addition of HBrO (0 – 20.0 μM).

The limits of detections (LOD) and limits of quantification (LOQ) of the **SWJT-23** for **HBrO** were determined according to the following equation:

$$DL = K*Sb1/S.$$

Where K = 3 (LOD) and 10 (LOQ), respectively; Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

Linear Equation: $Y = 72.11X + 197.14$

$S = 72.11$

$Sb1 = 0.03$

$LOD = K*Sb1/S = 1.24$ nM (K=3)

$LOQ = K*Sb1/S = 4.16$ nM (K=10)

8. ESI-MS spectra of SWJT-HBrO.

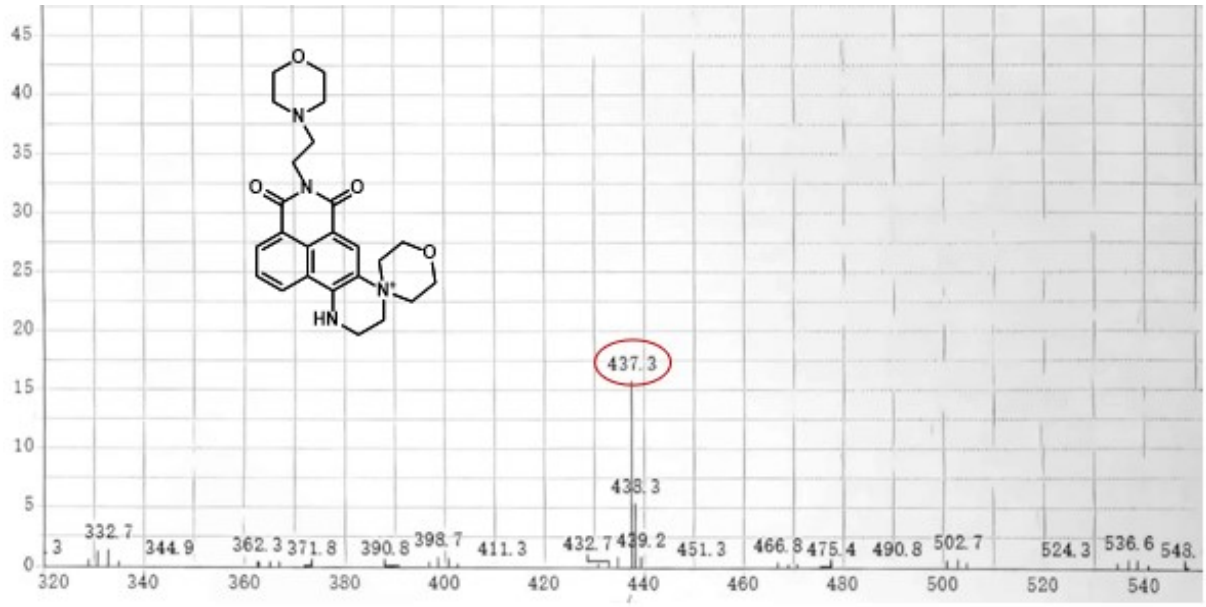


Fig. S8. ESI-MS spectrum of **SWJT-HBrO.**

9. Application of SWJT-23 in water samples.

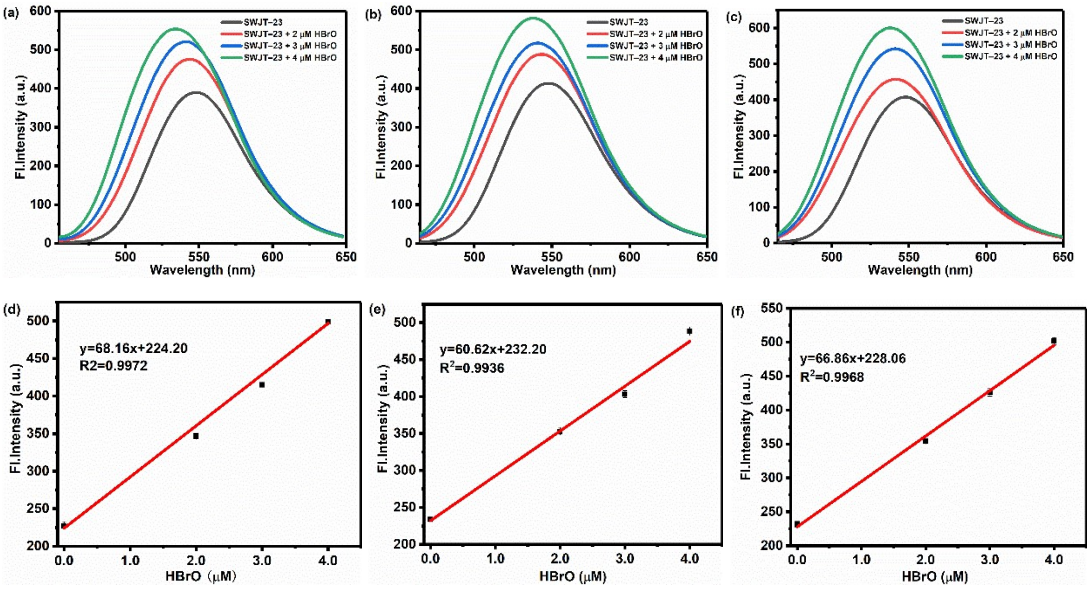


Fig. S9. Fluorescence spectra of **SWJT–23** (10.0 μM) spiked with different concentrations of **HBrO** ((2.0 to 4.0 μM) in (a) tap water (b) pool water and (c) lake water; Linear relationships of fluorescence intensities of **SWJT–23** spiked with different concentrations of **HBrO** (2.0 to 4.0 μM) in (d) tap water (e) pool water and (f) lake water.

10. Cell viability of SWJT-23.

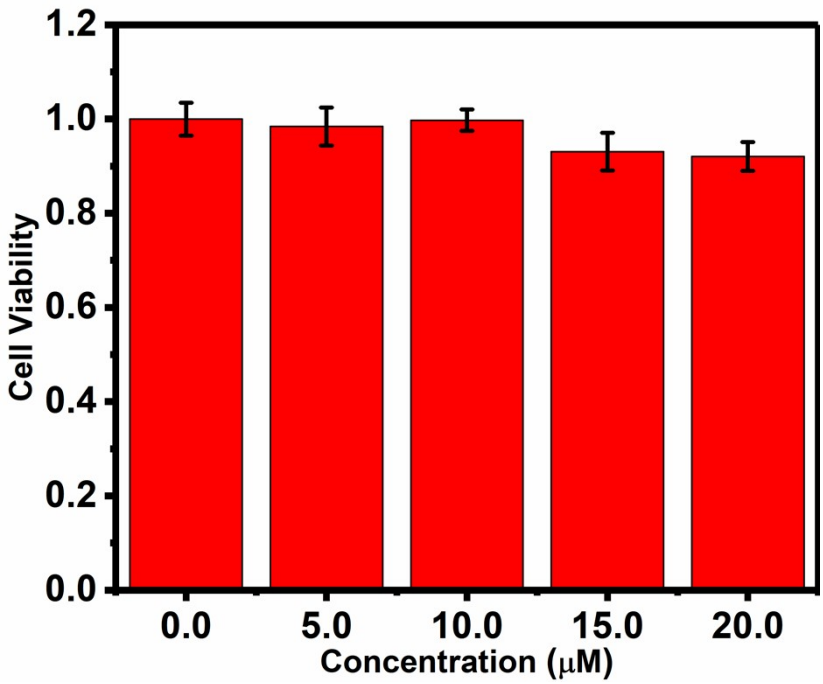


Fig. S10. Cytotoxicity of **SWJT-23** at different concentrations (0.0, 5.0, 10.0, 15.0, 20.0 μM).