

## Supporting Information

### Radical-Triggered Ring-Opening of Aminocyclopropane for Detection of Hydroxyl Radicals in Living Cells

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## Experimental Section

**Materials and Instruments.** 7-(Diethylamino) coumarin-3-carboxylic acid, dichloromethane, thionyl chloride, aminocyclopropane, triethylamine, hydrochloric acid (HCl), thiazolyl blue (MTT) and acetonitrile were attained from Shanghai Macklin Biochemical Technology Co., Lt. Sodium hydroxide (NaOH), sodium chloride (NaCl), anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>), ferrous chloride (FeCl<sub>2</sub>), ferric chloride (FeCl<sub>3</sub>), cupric chloride (CuCl<sub>2</sub>), copper chloride (CuCl), sodium hypochlorite (NaClO), zinc chloride (ZnCl<sub>2</sub>), magnesium chloride (MgCl<sub>2</sub>), calcium chloride (CaCl<sub>2</sub>), sodium sulfite (Na<sub>2</sub>SO<sub>3</sub>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and sodium nitrite (NaNO<sub>2</sub>) were purchased from Sinopharm Chemical Reagent Co., Ltd. Doxorubicin (DOX) and Cisplatin were obtained from Shanghai Aladdin Bio-Chem Technology Co., LTD.

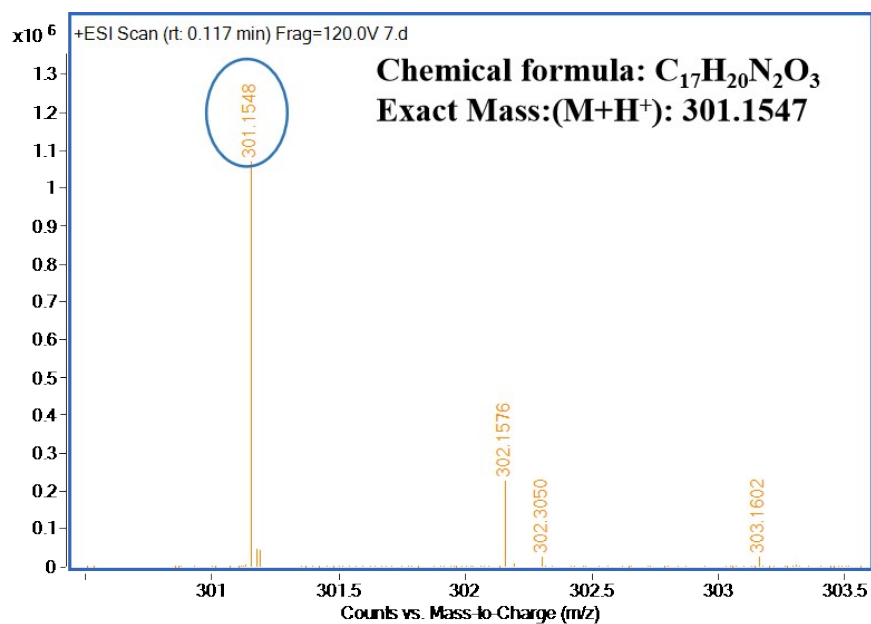
**Cell Culture.** 4T1 cells were cultured with RPMI-1640 medium containing 1% Penicillin-Streptomycin and 10% fetal bovine serum (FBS) and incubated in an incubator with 5% CO<sub>2</sub> at 37 °C. 293T cells were maintained in DMEM containing 10% FBS and 1% Penicillin-Streptomycin and incubated in a humidified incubator, which provided an atmosphere of 5% CO<sub>2</sub> and a constant temperature of 37 °C. Both cell lines were purchased from Wuhan Procell Life Science & Technology Co., LTD.

**Cytotoxicity Assay.** 4T1 cells and 293T cells were used to evaluate the cytotoxicity of CC-7 using MTT assay. 4T1 and 293T cells were seeded at a density of 3000 cells per well on 96-well plates and incubated overnight. MTT assay was performed on cells 48 hours after treatment with various concentrations of CC-7 (0

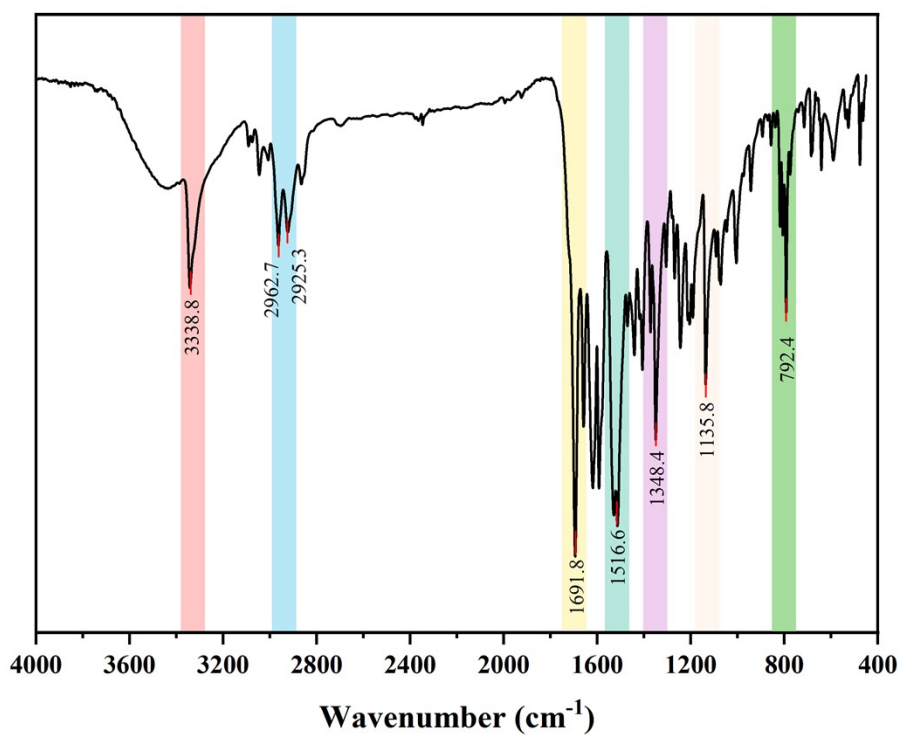
$\mu\text{M}$ , 1.0  $\mu\text{M}$ , 2.0  $\mu\text{M}$ , 4.0  $\mu\text{M}$ , 8.0  $\mu\text{M}$ , 16.0  $\mu\text{M}$ , 32.0  $\mu\text{M}$ , 64.0  $\mu\text{M}$  and 128.0  $\mu\text{M}$ ).

After 4 hours of incubation with MTT (5 mg/mL), the culture medium was removed and 150  $\mu\text{L}$  DMSO was added to each well to dissolve the formazan crystals. The wells were then shaken for 10 minutes at room temperature. The absorbance at 570 nm was recorded using a microplate reader (Varioskan LUX, Thermo-scientific).

## Supporting Figures and Table



**Figure S1.** The high-resolution mass spectrometry (HRMS) of CC-7.



**Figure S2.** Fourier transform infrared spectroscopy (FTIR) of CC-7.

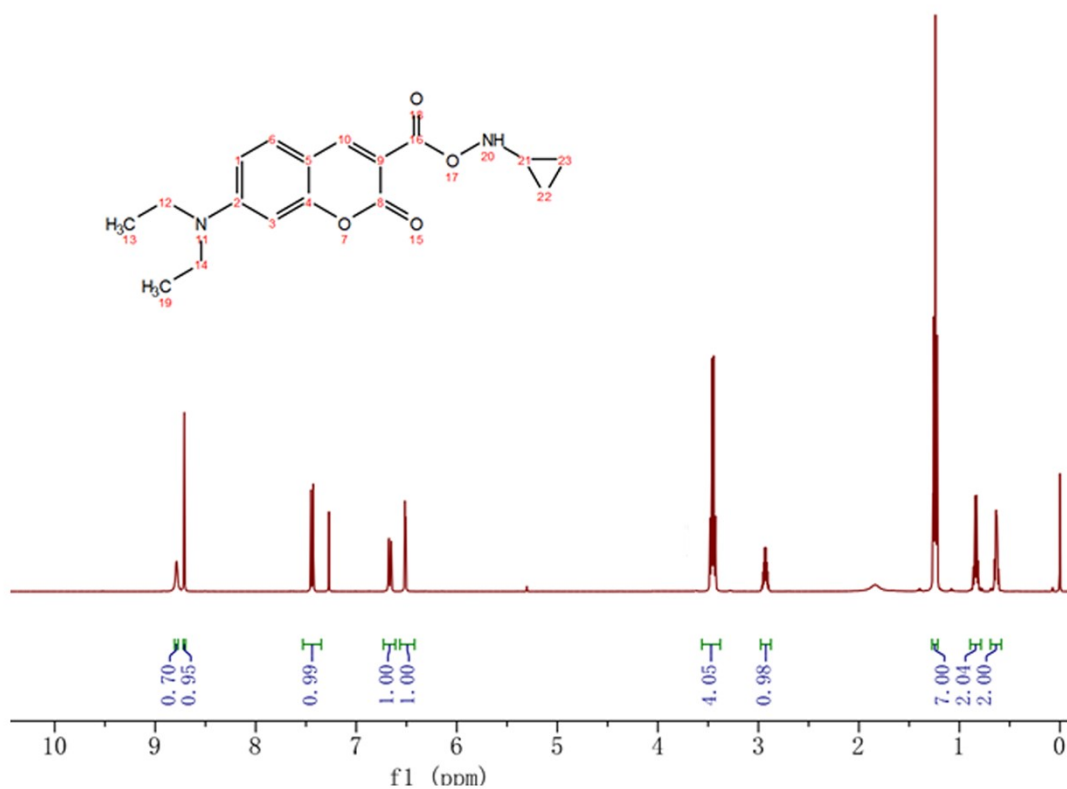


Figure S3. a) The  $^1\text{H}$  NMR spectrum of CC-7.

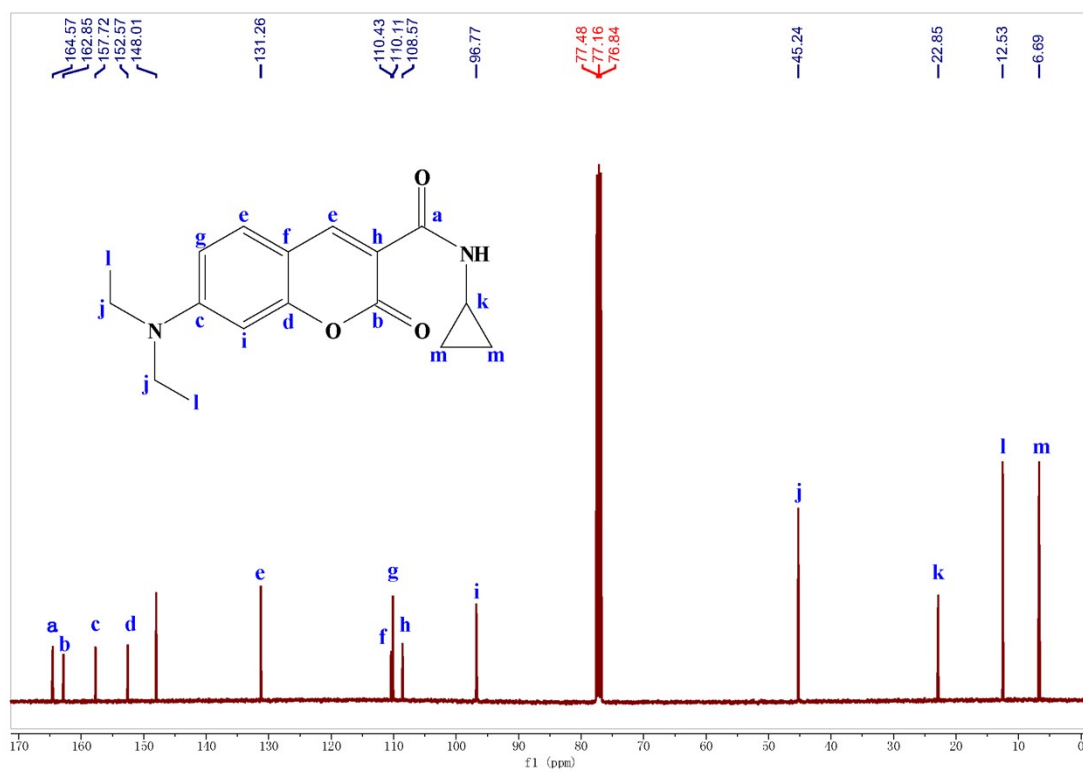
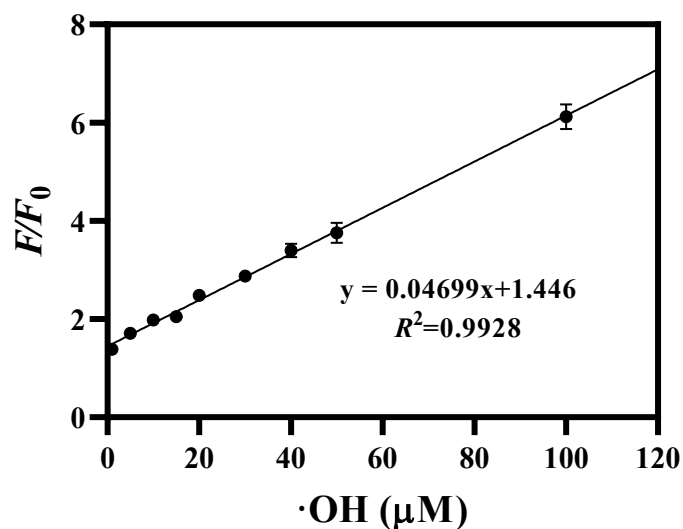
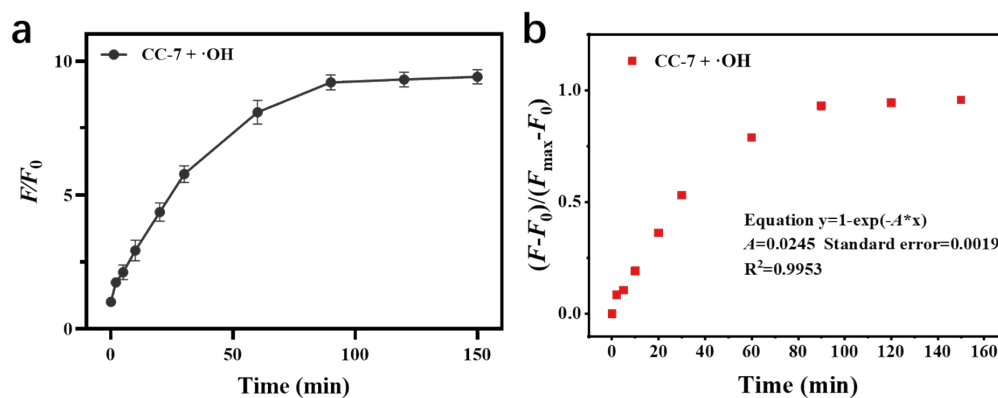


Figure S4. The  $^{13}\text{C}$  NMR spectrum of CC-7.

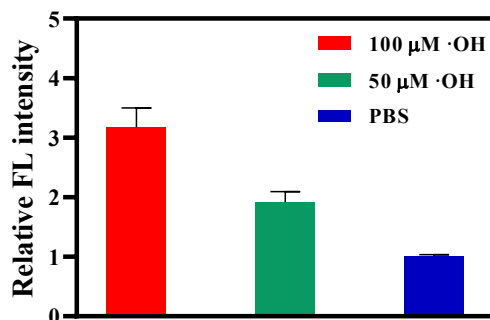


**Figure S5.** Linear calibration curve for the ratio fluorescence signal of CC-7 for  $\cdot\text{OH}$  concentration.

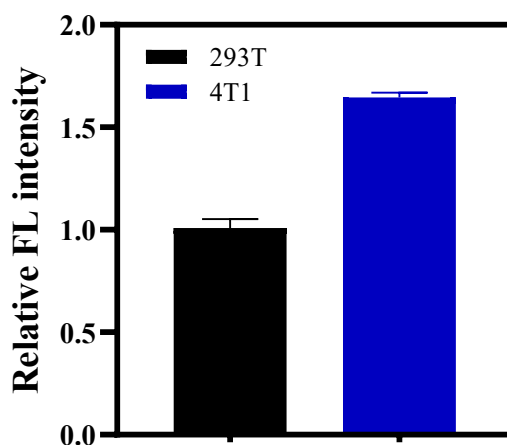


**Figure S6.** a) The ratio of the fluorescence signal ( $F/F_0$ ) of CC-7 in the presence of  $\cdot\text{OH}$  at various reaction times. b) Kinetic analysis of the probe's reaction with  $\cdot\text{OH}$ , fitting the normalized fluorescence intensity  $(F-F_0)/(F_{\text{max}}-F_0)$  to the equation  $y = 1 - \exp(-A \cdot x)$ , with  $A = 0.0245$ , standard error = 0.0019, and  $R^2 = 0.9953$ .

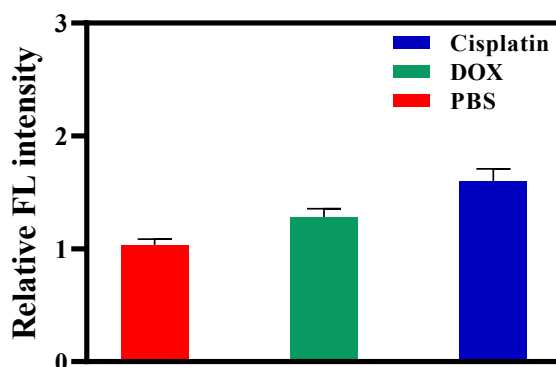




**Figure S7.** Quantitative statistical graph of intracellular fluorescence intensity after treatment with different concentrations of  $\cdot\text{OH}$ .



**Figure S8.** Quantitative statistical graph of fluorescence intensity of normal and cancer cells after treatment with the probe.



**Figure S9.** Quantitative statistical graph of intracellular relative fluorescence intensity after treatment with different chemotherapeutic agents.

**Table S1.** Detection of hydroxyl radical by CC-7 in real samples

Sample	$\cdot\text{OH}$ spiked ( $\mu\text{M}$ )	$\cdot\text{OH}$ recovered ( $\mu\text{M}$ )	Recovery (%)
Yangtze River	0	Not detection	----
	5	4.71 ( $\pm 0.07$ )	94.2
	10	10.51 ( $\pm 0.16$ )	105.1
	15	15.55 ( $\pm 0.27$ )	103.7
Sha Lake	0	Not detection	----
	5	5.18 ( $\pm 0.08$ )	103.3
	10	10.54 ( $\pm 0.27$ )	105.4
	15	14.76 ( $\pm 0.12$ )	98.4
East Lake	0	Not detection	----
	5	4.80 ( $\pm 0.10$ )	96.1
	10	9.76 ( $\pm 0.12$ )	97.6
	15	15.42 ( $\pm 0.21$ )	102.9
Tap water	0	Not detection	----
	5	4.91 ( $\pm 0.04$ )	98.2
	10	10.16 ( $\pm 0.08$ )	101.6
	15	14.61 ( $\pm 0.20$ )	97.4