Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary data for

A novel ratiometric fluorescent Probe for detection of hydrogen peroxide in human gastric carcinoma HGC-27 cells

Yong Xie[a#], Zhu Chen[c#], Linan Hu[c], Hailiang Zhang[b], Pengfei Xu[b]* and Ke Pan[a]*

[a]Department of General Surgery, The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, P.R. China;

[b]Institute of Clinical Pharmacy & Pharmacology, Jining First People's Hospital, Jining Medical University, Jining 272000, P.R. China;

[c]Departments of Radiology, The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, P.R. China;

1. Materials and Methods Materials.

All commercial chemicals were purchased from commercial suppliers and used without further purification. All solvents were purified before use. Liver cancer cell line HGC-27 cells was purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. Dulbecco's modified Eagle's medium (DMEM) were purchased from Sigma-Aldrich. Other medium components were obtained from Sigma-Aldrich too. Fetal bovine serum (FBS) was obtained from Hyclone Laboratories Inc. Hydrogen peroxide 30% was bought from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Fluorescence imaging of human gastric carcinoma HGC-27 cells were obtained using Olympus FV1000 confocal fluorescence microscope. ^{1}H NMR and ^{13}C NMR spectra were performed on Bruker Ascend 400 NMR spectrometer. Electrospray ionization mass spectra (ESI-MS) were collected on Agilent 6460 Triple Quadrupole LC/MS instrument. Absorbance spectra were recorded on Shimadzu UV-2450 UV-visible spectrophotometer. Fluorescence emission and excitation spectra were measured using Hitachi F-7000 spectrofluorometer. All UV/Vis and fluorescence titration experiments were performed using 10 μM of JNY-1 in a mixed buffer solution of DMF: PBS (30:70, v/v, pH=7.4, 10 mM) with varying concentrations of analytes at room temperature.

2. Cell Culture and Imaging

The HGC-27 cells were cultured in DMEM (Dulbecco'sModified Eagle's Medium) supplemented with 10% FBS (fetal bovine serum), 2 Mm of glutamine, penicillin (100 units/mL), and streptomycin (100 units/mL) under an atmosphere of 5% CO_2 and 95% air at 37 °C. These cells were then seeded in a 12-well plate at a density of 1.0×10^5 cells per well in culture media. Before the experiments, the culture medium in the well was removed and the HepG2 cells were washed by using PBS buffer (pH=7.4) solution three times. the cells were incubated in a PBS buffer solution (pH=7.4, containing 0.5 % DMSO, prepared from 2.0 mM stock solution in DMSO) with **JNH-1** (10.0 μM) at 37°C for 30 minutes, then removed the solution and washed the cells three times with PBS buffer solution to remove the extracellular **JNH-1**. Then the cells were treated with H_2O_2 (300 μM) or PBS for another 30 min at 37 °C and washed three times with

PBS buffer (pH 7.4), then collected fluorescence images.

3. Cell cytotoxicity

According to your suggestion, the toxicity of the probe **JNH-1** was studied in the HGC-27 cells by using MIT assay. HGC-27 cells were plated into a 96 well plate at a concentration of 5 x 10^3 cells/well in Dulbecco's modified Eagle medium (DMEM) with 10% FBS at 37 and 37 °C and 5% CO_2, and 24 h later, the cells were incubated with **JNH-1** with different concentrations (20 μM, 10 μm, 0 μM) for 2 h or 24 h, respectively. Old medium was taken out and new medium was added. Then,, 20 μL of the 5mg/mL MTT stock solution was added and incubated for 4 hours.Then the cell viability was calculated according to previous work.

4. Synthesis details

Synthesis and Characterization of Compound **1**. To a 50 mL three-neck round-bottom flask were added fluorescein (1.7 g, 5.0 mmol) and MeOH (3 mL) at room temperature and the whole system was then cooled to 0 °C. A mixture of NaOH aqueous solution (15 g, 50%) and 15-crown-5 (20 μL) was added within 5 min. The resulting mixture was stirred for 10 min, and then allowed to warm gradually in an oil bath. $CHCl_3$ (10 mL) was added dropwise while the reaction temperature was maintained at 55 °C. The reaction mixture was further stirred for 10 h at this temperature, and then cooled to room temperature. The mixture is acidified with H_2SO_4 (2 mL, 10 M), and the purple-black precipitate appeared. This solid was filtered and dried and purified by flash column chromatographyon silica gel to afford the crude monoaldehyde-functionalized fluorescein, which could be recrystallized in acetone (10 mL) to give pure sample (252 mg, 14%) as a pale-yellow solid. ^{1}H NMR (DMSO-*d*6, 300 MHz) δ = 11.89 (s, 1H), 10.63 (s, 1H), 10.29 (s, 1H), 8.02 (d, J = 7.5 Hz, 1H), 7.81 (d, J = 7.2 Hz, 1H), 7.73 (d, J = 7.2 Hz, 1H), 7.32 (d, J = 7.5 Hz, 1H), 6.95 (d, J = 9.0 Hz, 1H), 6.85 (s, 1H), 6.71 (d, J = 9.0 Hz, 1H), 6.61 (brs, 2H) ppm. ^{13}C NMR (DMSO-*d*6, 75 MHz) δ = 192.9, 168.5, 162.9, 159.6, 152.4, 152.2, 150.9, 136.5, 135.8, 130.3, 129.0, 125.9, 124.8, 124.0, 113.5, 113.4, 109.7, 109.2, 109.1, 102.7, 81.8 ppm. ESI-MS: *m/z* 361.1 $[M + H]^+$.

Synthesis and Characterization of Compound **2**. To a solution of monoaldehyde-

functionalized fluorescein (720 mg, 2.0 mmol) and diethyl malonate (365 μL, 2.4 mmol) in dry EtOH (40 mL) were added piperidine (6 drops) and glacial acetic acid (2 drop) at room temperature. Then, the resulting mixture was heated gradually to 80 °C and reflux for 20 h. The above mixture was cooled to room temperature, followed by filtering and crystallization. The product was then washed with EtOH (40 mL) and dried in vacuum to afford the desired compound **2** as a solid (592 mg, 65%). ^{1}H NMR (DMSO-d_6, 400 MHz; ppm): δ10.39 (s, 1H), 9.01 (s, 1H), 8.04 (d, J = 7.6 Hz, 1H), 7.81 (td, J= 7.2, 0.8 Hz, 1H), 7.75 (td, J = 8.0, 0.8 Hz, 1H), 7.31 (d, J =7.6 Hz, 1H), 7.14 (dd, J = 13.6, 9.2 Hz, 2H), 6.96 (t, J = 1.2 Hz,1H), 6.65 (d, J = 1.2 Hz, 2H), 4.35 (q, J = 7.2 Hz, 2H), 1.36 (t,J = 7.2 Hz, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz; ppm): δ168.5, 162.5, 159.9, 155.6, 155.4, 152.3, 150.8, 147.7, 141.7,135.9, 133.8, 130.5, 129.1, 125.7, 124.9, 124.1, 117.6, 114.3,113.8, 112.2, 108.9, 107.4, 102.7, 81.5, 61.6, 14.1. ESI-MS: m/z 457.3 [M + H]+

Synthesis and Characterization of Probe **JNH-1**. 4-(Bromomethyl)Benzeneboroni-cacid-pinacolester (296 mg, 1.0 mmol) was added to a solution of compound **2** (246 mg, 0.5 mmol) and Cs_2CO_3 (325 mg, 1.0 mmol) in DMF (10 mL). The resulting mixture was heated to 80 ^{0}C and further stirred for 20 h. Water (50 mL) was added to the mixture, followed by extraction with CH_2Cl_2 (15 mL x 3). The combined organic phase was dried by anhydrous Na_2SO_4. The solvent was removed by evaporation, and the residue was purified by flash column chromatography on silica gel to afford probe **JNH-1** as a white solid (217 mg, 33%).^{1}H NMR (400 MHz, Chloroform-d) δ 9.13 (s, 1H), 8.06 (d, J = 6.8 Hz, 1H), 7.85 (d, J = 8.1 Hz, 2H), 7.74 – 7.63 (m, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.14 (d, J = 7.0 Hz, 1H), 7.02 (d, J = 1.7 Hz, 2H), 7.00 (d, J = 2.2 Hz, 1H), 6.78 (dd, J = 8.8, 2.3 Hz, 1H), 6.74 (d, J = 8.8 Hz, 1H), 5.17 (s, 2H), 4.49 (q, J = 7.1 Hz, 2H), 1.47 (t, J = 7.1 Hz, 3H), 1.35 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.88, 163.17, 160.72, 156.30, 156.02, 152.54, 151.29, 148.34, 142.77, 139.04, 135.47, 135.19, 133.83, 130.29, 129.28, 126.48, 126.07, 125.40, 123.83, 117.90, 114.75, 113.68, 112.33, 111.01, 107.85, 102.13, 83.93, 81.54, 70.31, 62.29, 24.88, 14.32. ESI-MS: m/z 673.03 $[M + H]^{+}$.

5. Structure characterizations of probe JNH-1

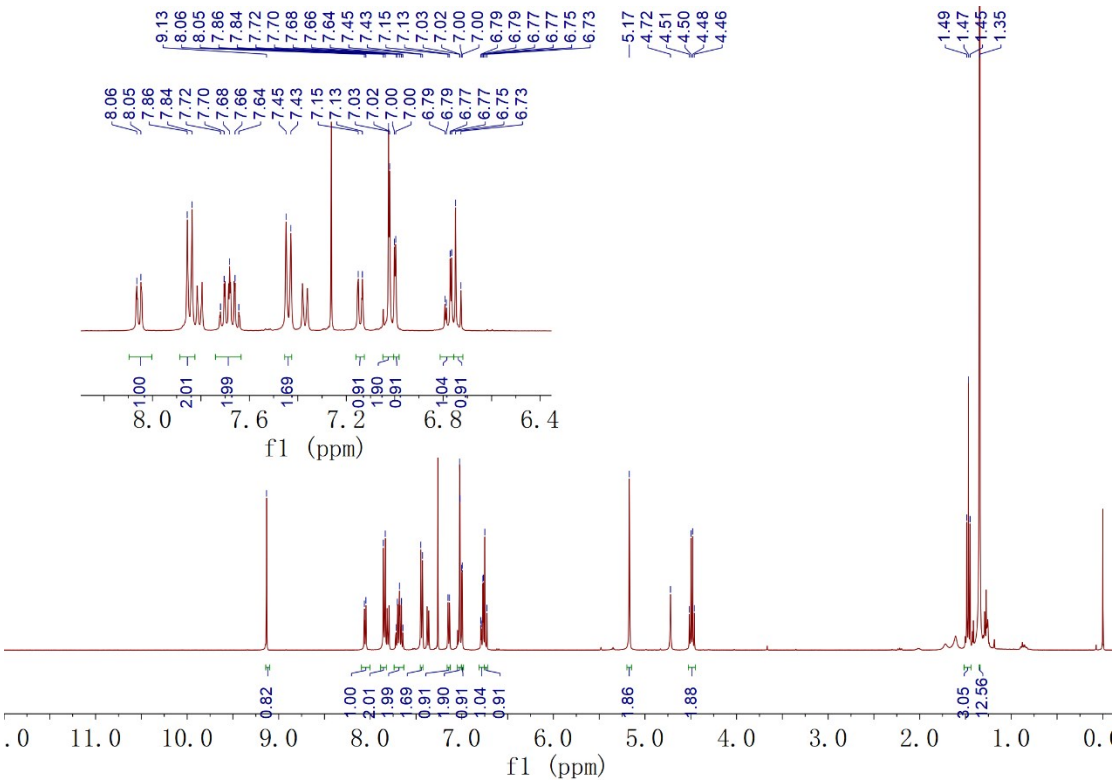

Figure S1. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) of **JNH-1**.

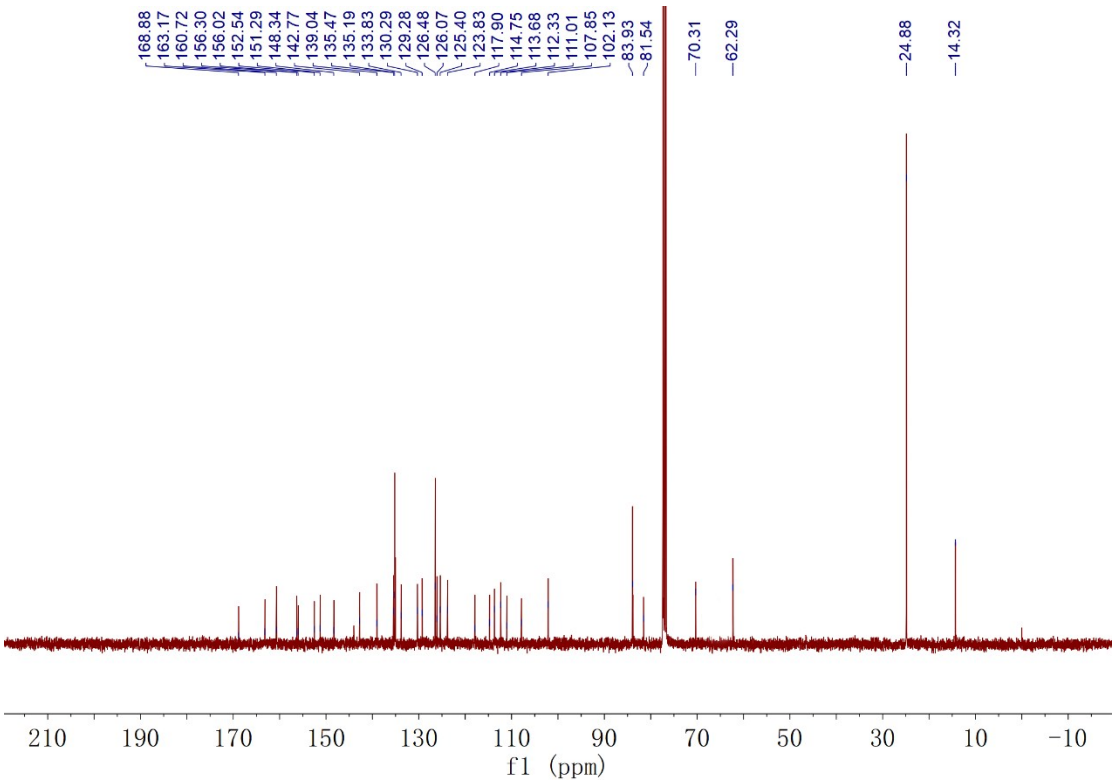

Figure S2. ^{13}C NMR (100 MHz, $CDCl_3$-*d*) of **JNH-1**.

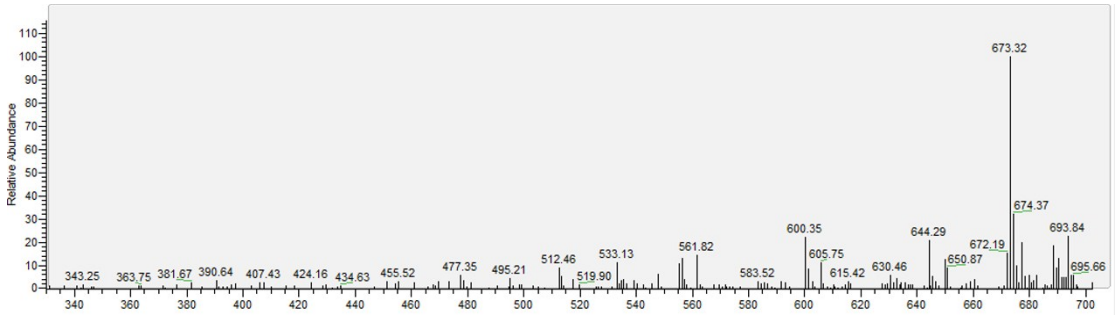

Figure S3. ESI MS spectra of **JNH-1**

6. UV-vis spectra of JNY-1 upon addition of H_2O_2.

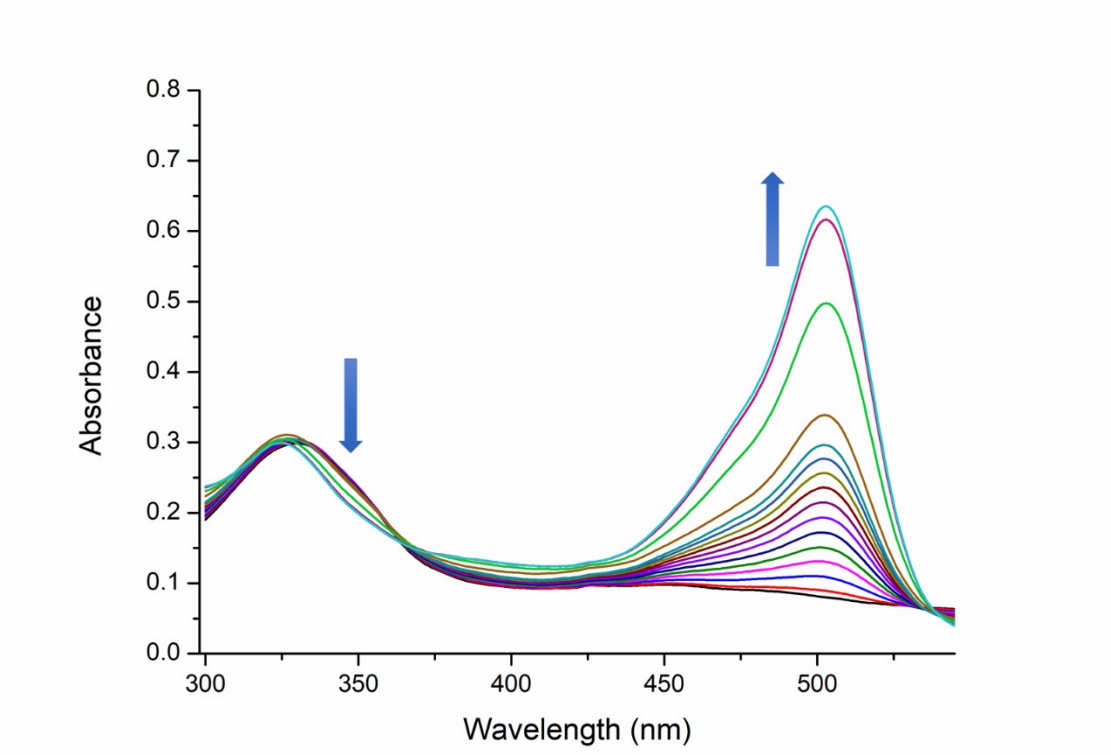

Figure S4. UV-vis spectra of **JNH-1** upon addition of H_2O_2.

7. Effect of pH.

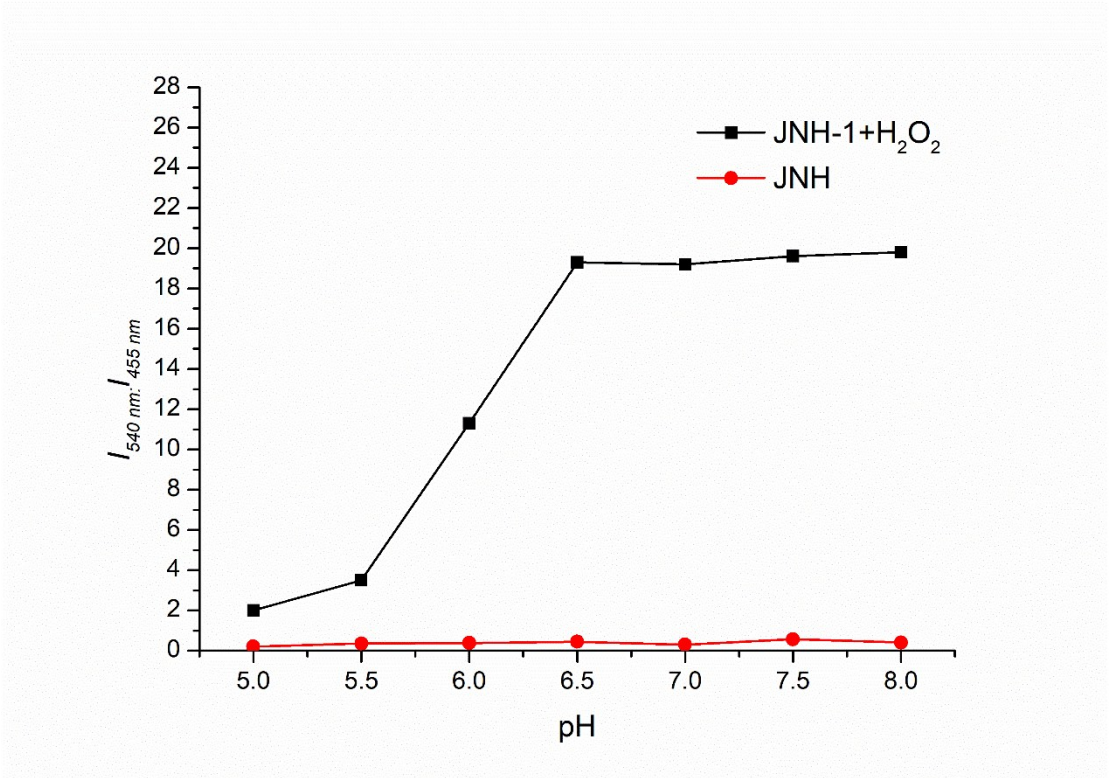

Figure S5. pH effect on the I_{540}/I_{455} of JNH-1 (10 μM) in the absence and presence of H_2O_2 (300 μM).

8. Color changes of probe upon addition of H_2O_2.

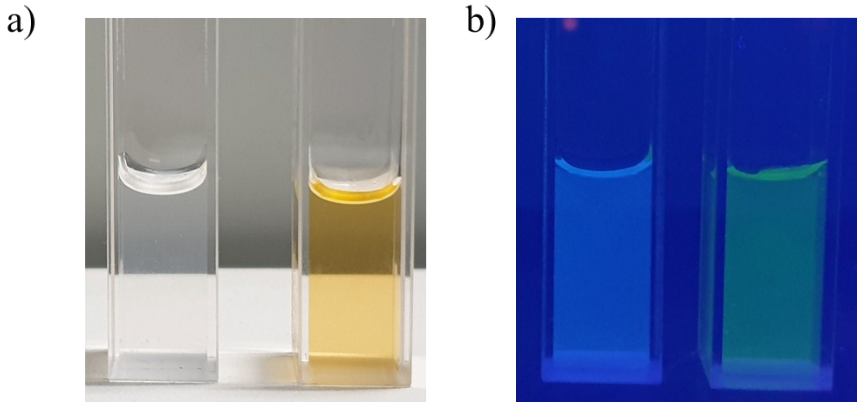

Figure S6. a): Color changes of probe **JNH-1** (10 μM) upon addition of H_2O_2 (300 μM) b): Emission color changes of probe **JNH-1** (10 μM) upon addition of H_2O_2 (300 μM).

9. Time-dependent changes in the fluorescence intensity at 540 nm in the presence of 300 μM H_2O_2.

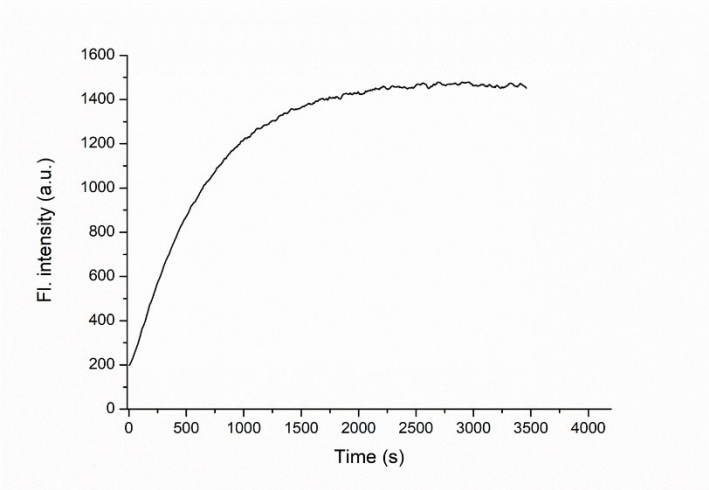

Figure S7. The time-dependent changes in the fluorescence intensity at 540 nm in the presence of 300 μM H_2O_2.

10. Plausible mechanism between probe JNH-1 and H_2O_2.

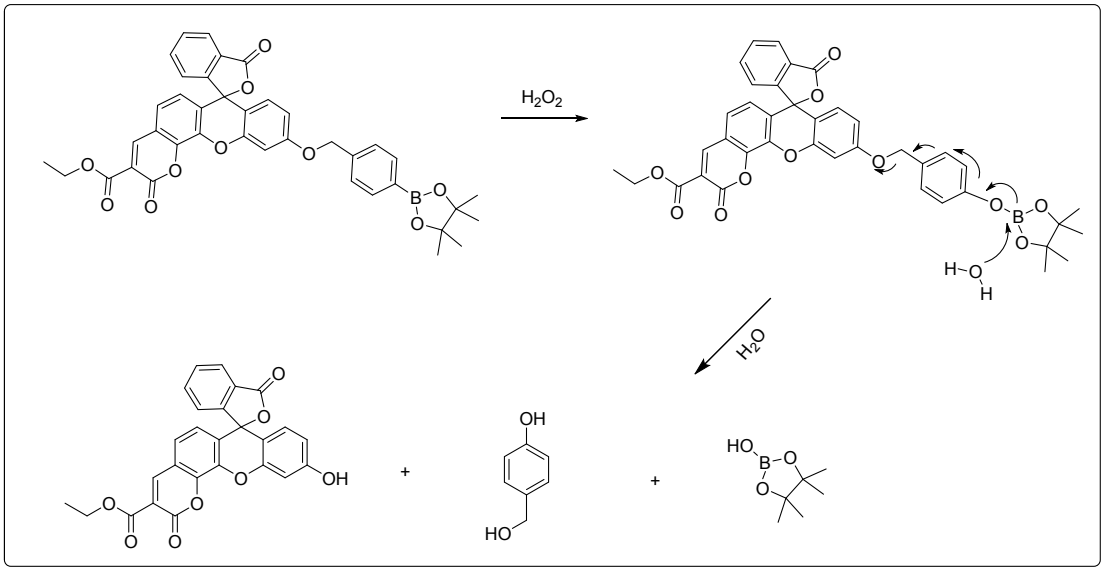

Figure S8. Plausible mechanism between probe **JNH-1** and H_2O_2.

11. Cell cytotoxicity

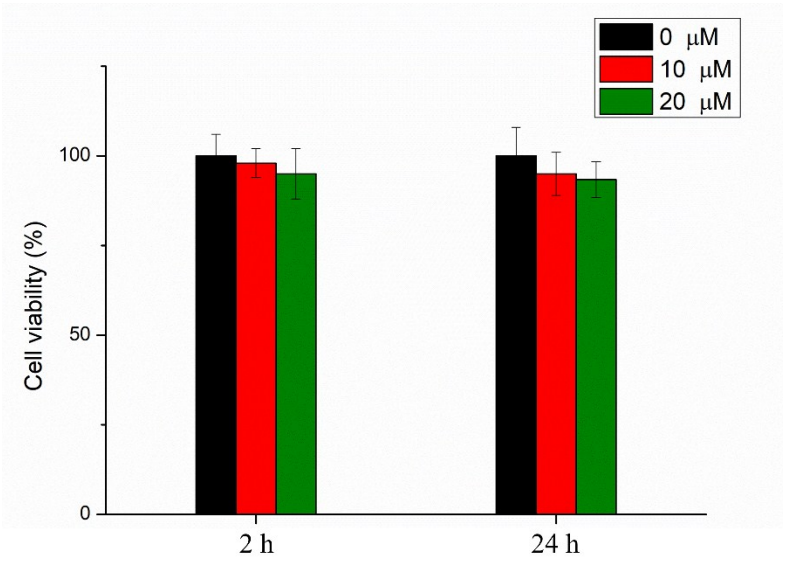

Figure S9. The cytotoxicity of **JNH-1** in living HepG2 cells for 2 h or 24 h: 0 μM, 10.0 μM and 20.0 μM