Supporting Information

The fluorescence probe with targeted mitochondria for detecting hydrogen peroxide in vitro and diabetic mice

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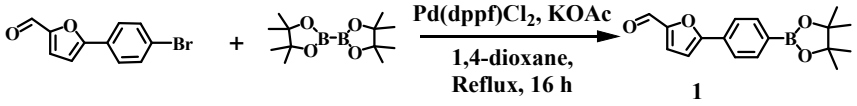
Table of Contents

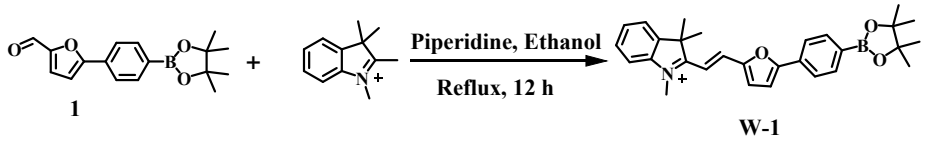
1. General information

All the solvents were purified and dried according to general methods. ^{1}H NMR spectra were recorded on a Bruker AVIII-500 MHz spectrometer. Chemical shifts (in ppm) were determined by reference to the residual solvent peak (DMSO-d_6: 2.5 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constants (Hz) and integration. ^{13}C NMR spectra were recorded on the same NMR spectrometer. Chemical shifts (in ppm) were determined by reference to the residual solvent peak (DMSO-d_6: 39.52 ppm). High- resolution mass spectra (HRMS) were measured with Thermo (orbitrap Elite). Absorption spectra were measured using a Thermo (BioMate 3S) UV/Vis spectrophotometer. Fluorescence measurements were carried out with a F97pro fluorospectrophotometer.

2. Synthesis of compounds



Synthesis of compound 1: 5-(4-Bromophenyl)furan-2-carbaldehyde (527 mg, 2.11 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (508 mg, 2.0 mmol), Pd(dppf)Cl_2 (43.9 mg, 0.0600 mmol) and KOAc (588 mg, 6.0 mmol) were dissolved in 1, 4-dioxane solution (10 mL). The mixture was stirred at 80 °C for 16 h. The mixture was allowed to cool to room temperature, diluted with CH_2Cl_2 and filtered. All volatiles are removed in vacuum to obtain compound **1** directly used in the next experiment.



Synthesis of compound W-1: Compound **1** (126 mg, 0.5 mmol) and 1,2,3,3-tetramethyl-3*H*-indol-1-ium (202 mg, 0.5 mmol) were dissolved in 10 mL ethanol and stirred until completely dissolved, the mixture was stirred and reflux for 12 h at 80 ℃.

TCL detected the reaction process. After the reaction was completed, it was cooled to room temperature, The solvent was removed by vacuum under reduced pressure, dichloromethane: methanol (100:1~20:1) was used as eluent, silica gel chromatography was performed. The product is obtained as red solid **W-1** (136 mg, yield 30%).**^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.34 (d, J = 16.0 Hz, 1H), 8.11 (d, J = 9.3 Hz, 2H), 7.88 (dd, J = 11.7, 7.0 Hz, 2H), 7.82 (d, J = 8.2 Hz, 2H), 7.70 (s, 1H), 7.67 – 7.58 (m, 2H), 7.55 (d, J = 3.8 Hz, 1H), 7.39 (d, J = 16.0 Hz, 1H), 4.15 (s, 3H), 1.78 (s, 6H), 1.33 (s, 12H).**^{13}C NMR** (125 MHz, DMSO-d_6) δ 180.96 , 151.90 , 143.81 , 142.40 , 137.35 , 135.52 , 129.44 , 127.10 , 125.05 , 123.32 , 115.31 , 112.70 , 109.31 , 84.45 , 52.18 , 34.68 , 25.94 , 25.19 , 22.16 .**HRMS (ESI)** m/z calcd for $C_{29}H_{33}O_3NB$ (M): 454.25480, Found: 454.25480, error: -0.00253 ppm.

3. Experiment methods

3.1 High glucose cell model

293T cells (8×10^4) were uniformly inoculated in confocal laser culture dishes and cultured in a microcell incubator for 24 h at 37℃, and then washed 3 times with PBS. The first group was treated with probe and incubated for 30 min; the second group was pretreated with glucose (30 mM) for 30 min, and then re-incubated with the medium containing probe for 30 min; the third group was treated with 30 mM glucose for 30 min and re-incubated with the medium containing 10 mM GSH for 30 min, and then re-incubated the medium containing probe for 30 min. 50 μL anti-fluorescence quenching sealing solution was added in a petri dish and observed by confocal laser microscope (293T medium RMI-1640).

3.2 Diabetic mice model

The streptozotocin (STZ, an analogue of n-acetylglucosamine and a specific toxin of islet beta cells) were injected intraperitoneally into C57 male mice to establish the

type 1 diabetes model. Before model, the mice were fed a 12 h diet. Then the mice were divided into the control group and the experimental group. The experimental group was injected intraperitoneally with STZ (180 mg/kg), while the control group was injected with the same dose of buffer solution, and the diet was given 3-4 h after administration. Before the experiment (6 h), the fasting blood glucose of mice in the experimental group was tested as 11.1 mmol/L, indicating that C57 mice suffer from diabetes. Animals were then imaged by the IVIS imaging system (Perkin Elmer, USA) after injecting **W-1** through the tail vein. After imaging, the mice were euthanized, and the fluorescence intensity of the isolated organs was evaluated by taking the heart, liver, spleen, lung, kidney and serum.

4. Figures

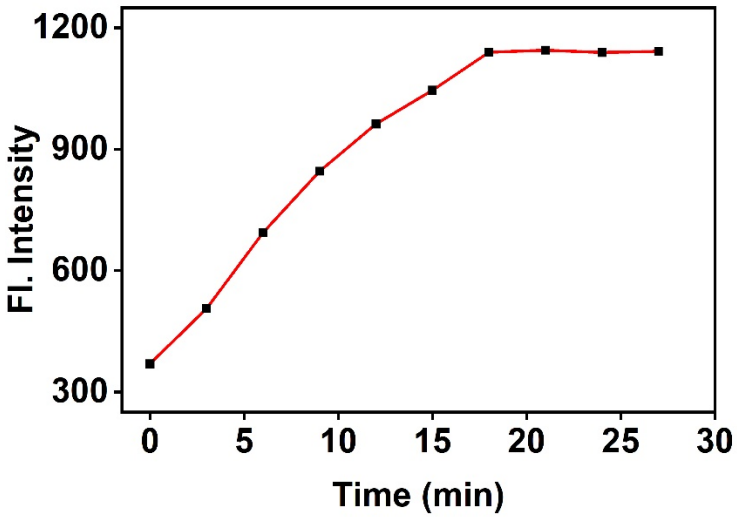


Figure S1 Time-dependent fluorescence change of **W-1** (10 μM) with addition of H_2O_2 in an aqueous PBS buffer (containing 5% DMSO, pH=7.4) under room temperature, λex = 521 nm.

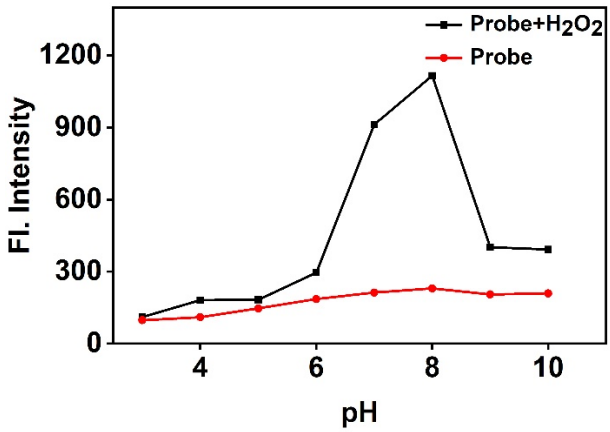


Figure S2 pH-Dependent fluorescence intensity changes of **W-1** and **W-1** toward H_2O_2 in an aqueous PBS buffer (containing 5% DMSO, pH=7.4) under room temperature, λex = 521 nm.

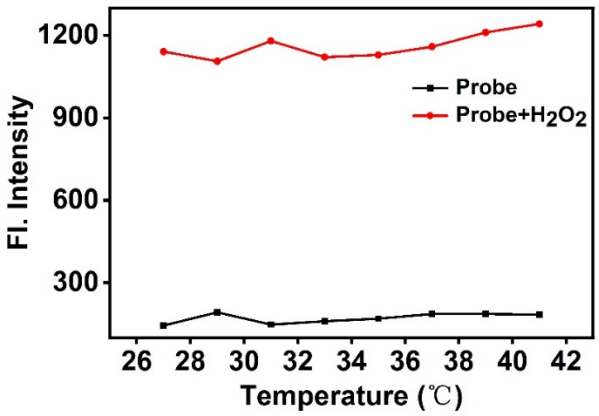


Figure S3 Temperature-dependent fluorescence intensity changes of **W-1** and **W-1** toward H_2O_2 in an aqueous PBS buffer (containing 5% DMSO, pH=7.4), λex = 521 nm.

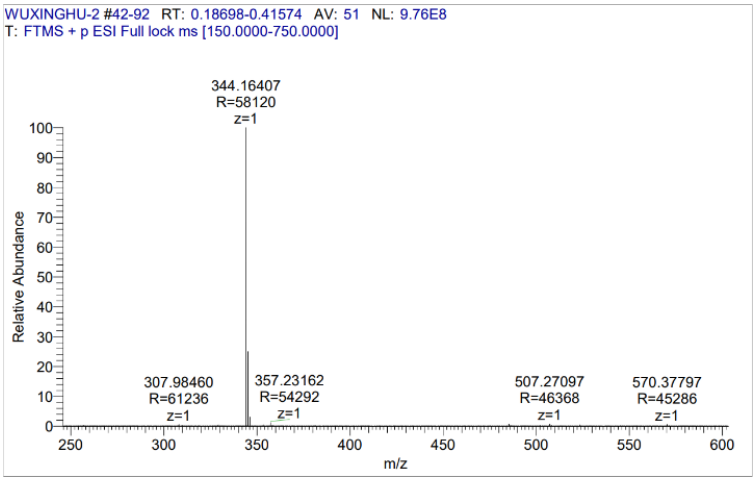


Figure S4 HRMS spectra of **W-1** toward H_2O_2

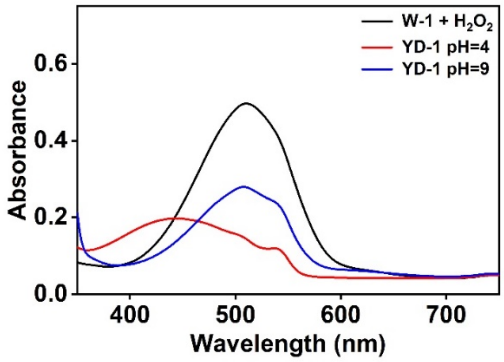


Figure S5 The absorption spectra of **W-1** in the presence of H_2O_2 in PBS buffer (containing 5% DMSO, pH=7.4) and **YD-1** in PBS buffer (containing 5% DMSO, pH 4/9) at room temperature.

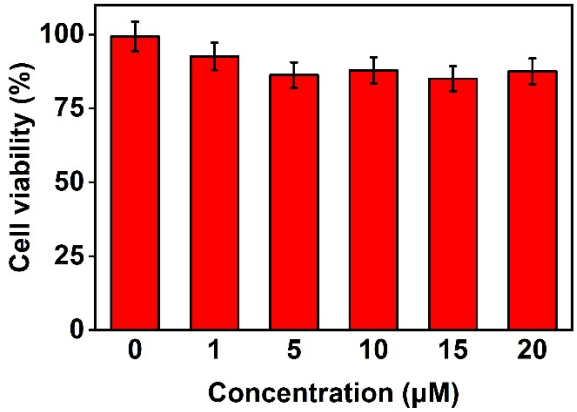


Figure S6 Cell viabilities with treatment of probe **W-1** in HeLa cells.

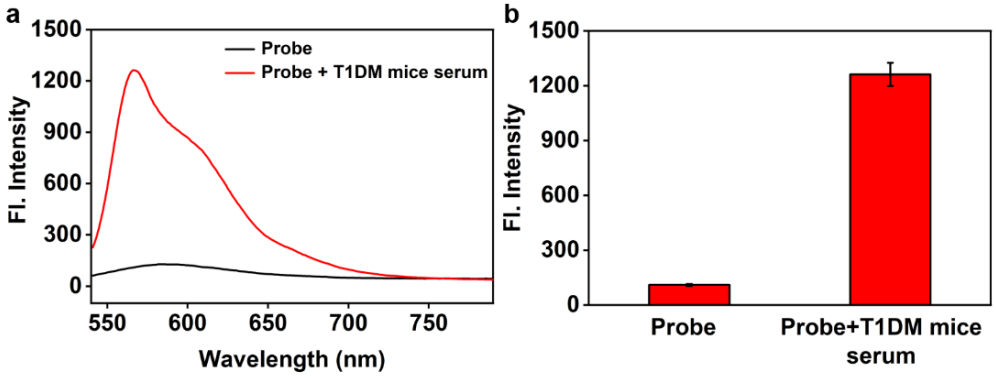
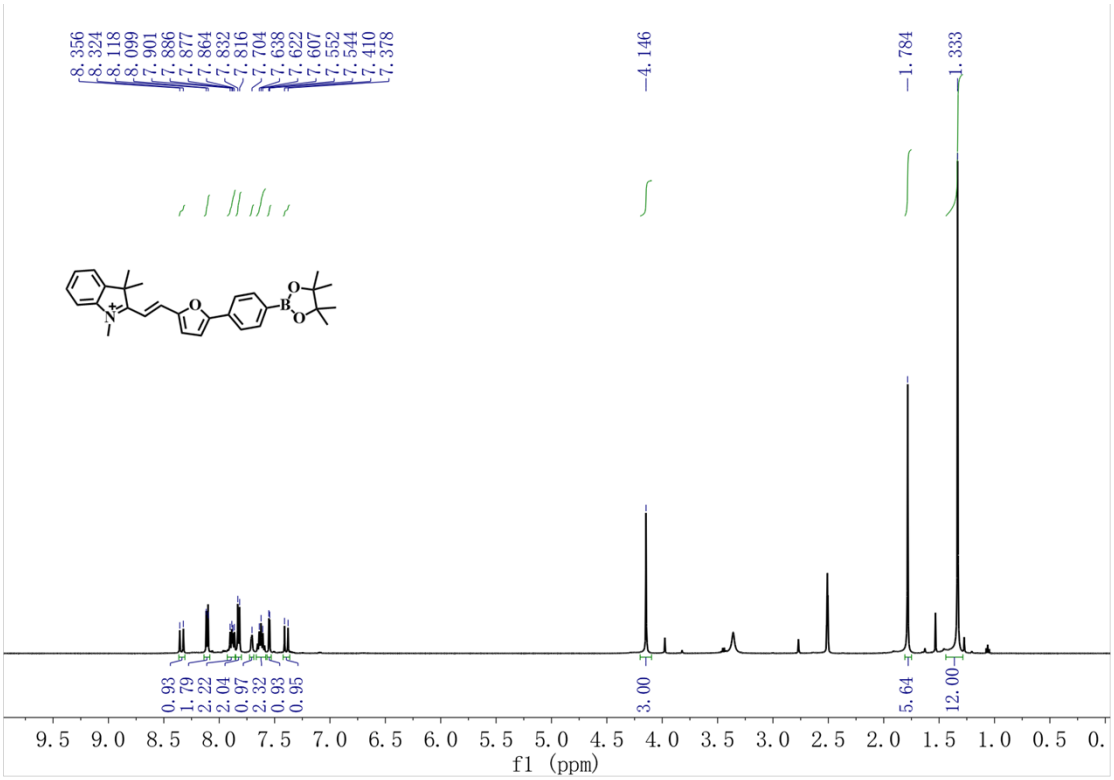
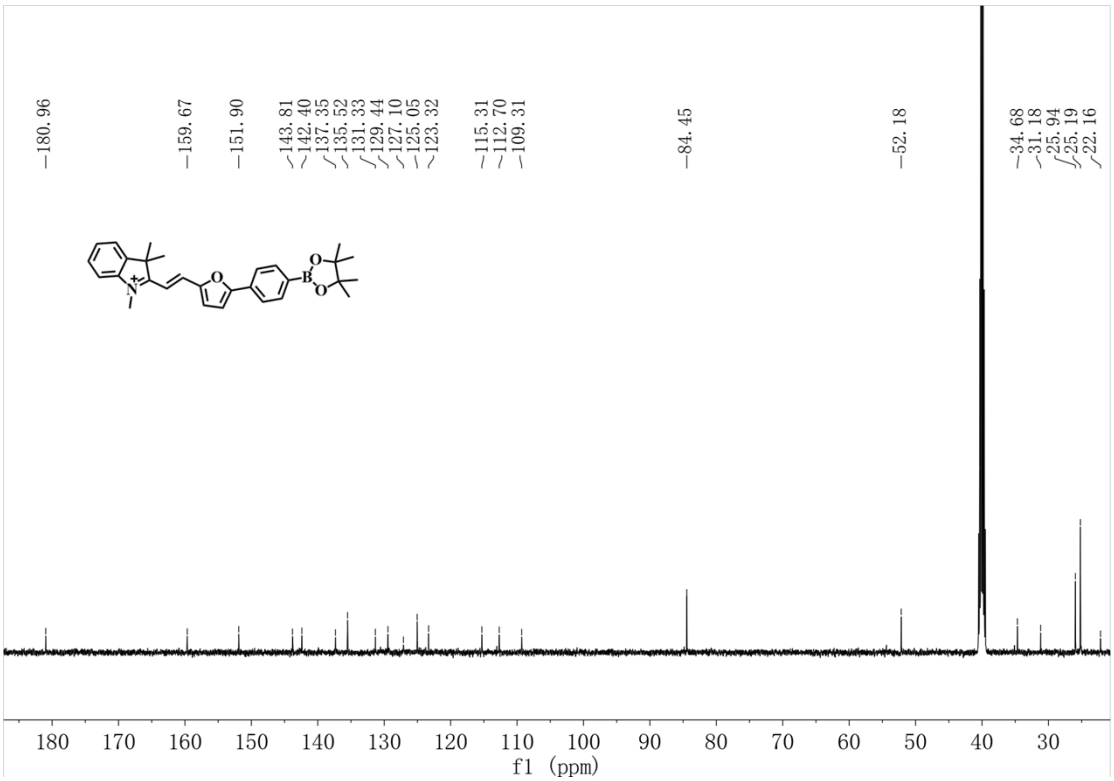


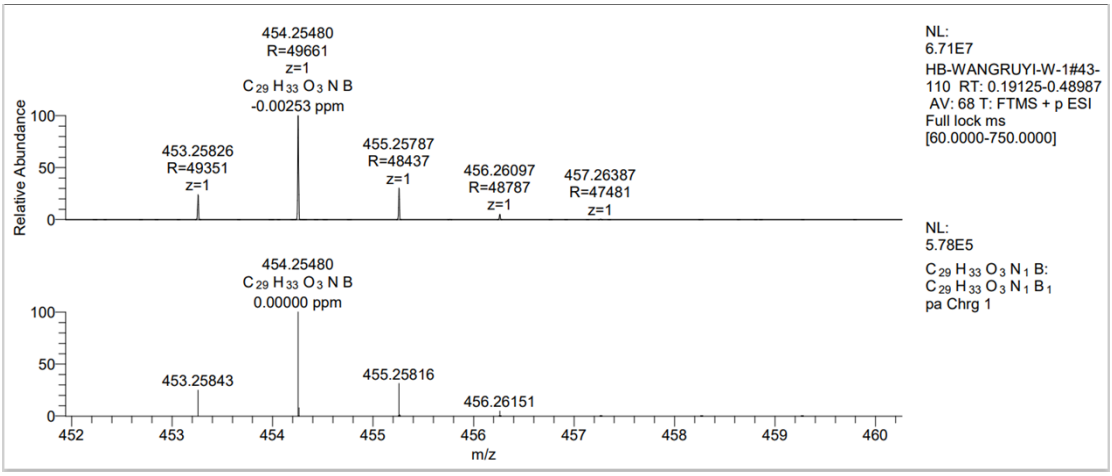
Figure S7 (a) Changes in the fluorescence of **W-1** after adding diabetic mouse serum (b) Quantitative analysis of the fluorescence intensity. The data are presented as the

mean ± SD (n = 3).

5. ^{1}H NMR, ^{13}C NMR and HRMS







HRMS spectra of **W-1**.