Supplementary Material

A mitochondria-targeted rhodol fluorescence probe for imaging of hydrogen peroxide in living cells

Experimental Section

Instrumentation and Reagents

^{1}H-NMR spectra were recorded on an Avance NEO 400 NMR spectrometer (Bruker Inc., Switzerland). Mass spectra (MS) were recorded on a TSQ Quantum Access MAX triple-quadrupole mass spectrometer (Thermo Fisher Scientific, USA). An INESA Scientific PHS-3C pH meter was employed for the pH measurements of all solutions. Fluorescence spectra were collected on an F-7000 fluorescence spectrometer (Hitachi Co., Ltd. Japan) with a 1-cm quartz cell. Absorption spectra were recorded on a Cary 60 UV-spectrophotometer (Agilent Technologies, USA) with a 1-cm quartz cell. Cell imaging experiments were carried out on an LSM 710 laser scanning confocal microscope (Carl Zeiss, Oberkochen, Germany).

H_2O_2 and acetic acid were purchased from Aladdin Chemistry Co. Ltd (Shanghai, China). All reagents were of analytical reagent grade and used without further purification or treatment. All aqueous solutions were prepared with ultrapure water obtained by a Milli-Q water purification system (18.2 MΩ cm). A549 (Human lung cancer) cells and human serum were obtained from the Anshan Central Hospital.

Figures

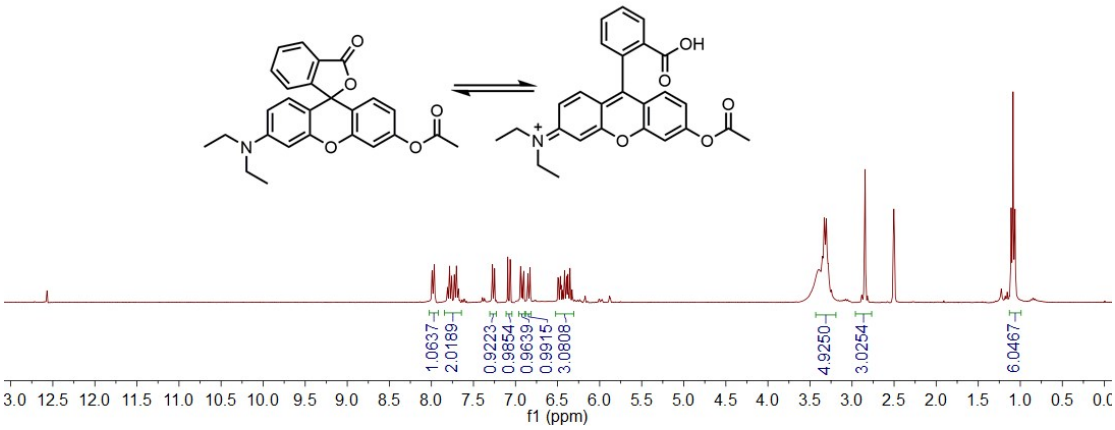

Fig. S1 ^{1}H-NMR spectrum of Rhodol-OAc in DMSO-d_6.

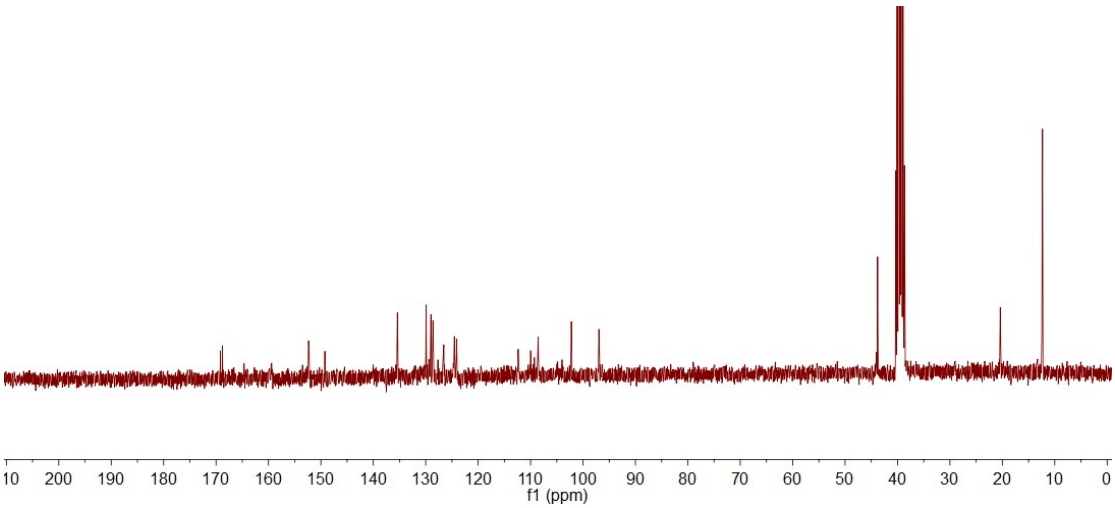

Fig. S2 ^{13}C-NMR spectrum of Rhodol-OAc in DMSO-d_6.

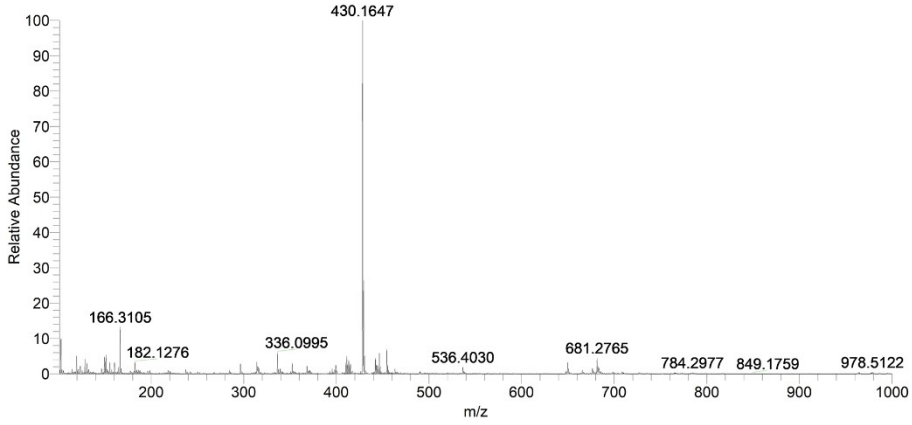

Fig. S3 ESI-MS spectrum of Rhodol-OAc.

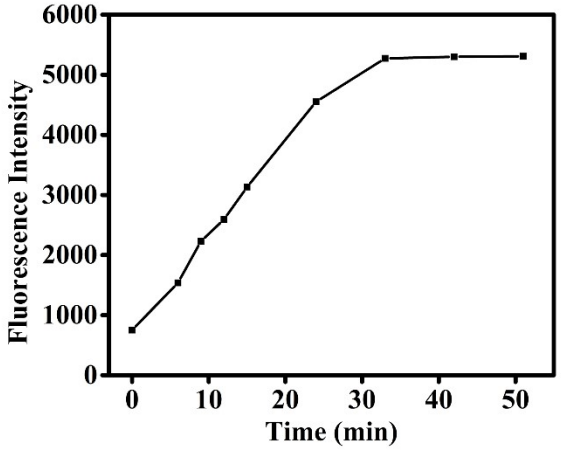

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Fig. S4 Time-dependent fluorescence intensity changes of Rhodol-OAc (10 μM) after the addition of H_2O_2 (200 μM) in PBS/EtOH (99:1, v/v, 10 mM PBS, pH 7.4). (λ_{ex}=500 nm)

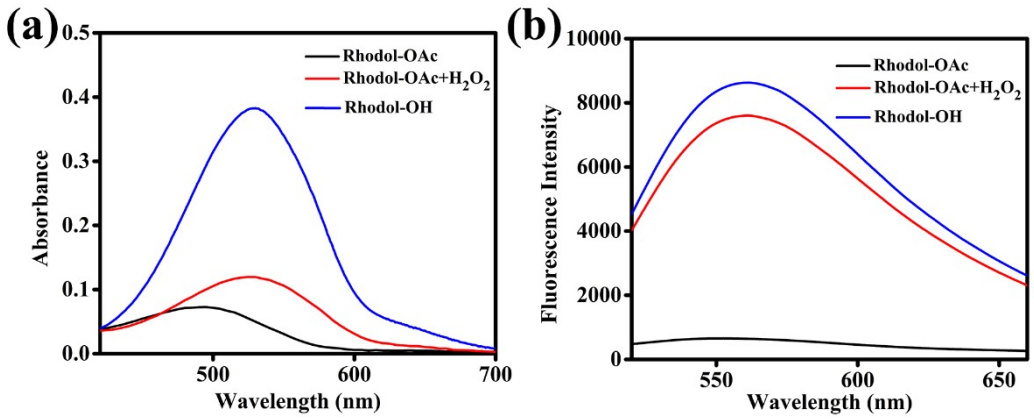


Fig. S5 (a) Absorption and (b) emission spectra of 10 μM Rhodol-OH and 10 μM Rhodol-OAc in the absence and presence of H_2O_2 (200 μM) in PBS/EtOH (99:1, v/v, 10 mM PBS, pH 7.4). (λ_{ex}=500 nm)

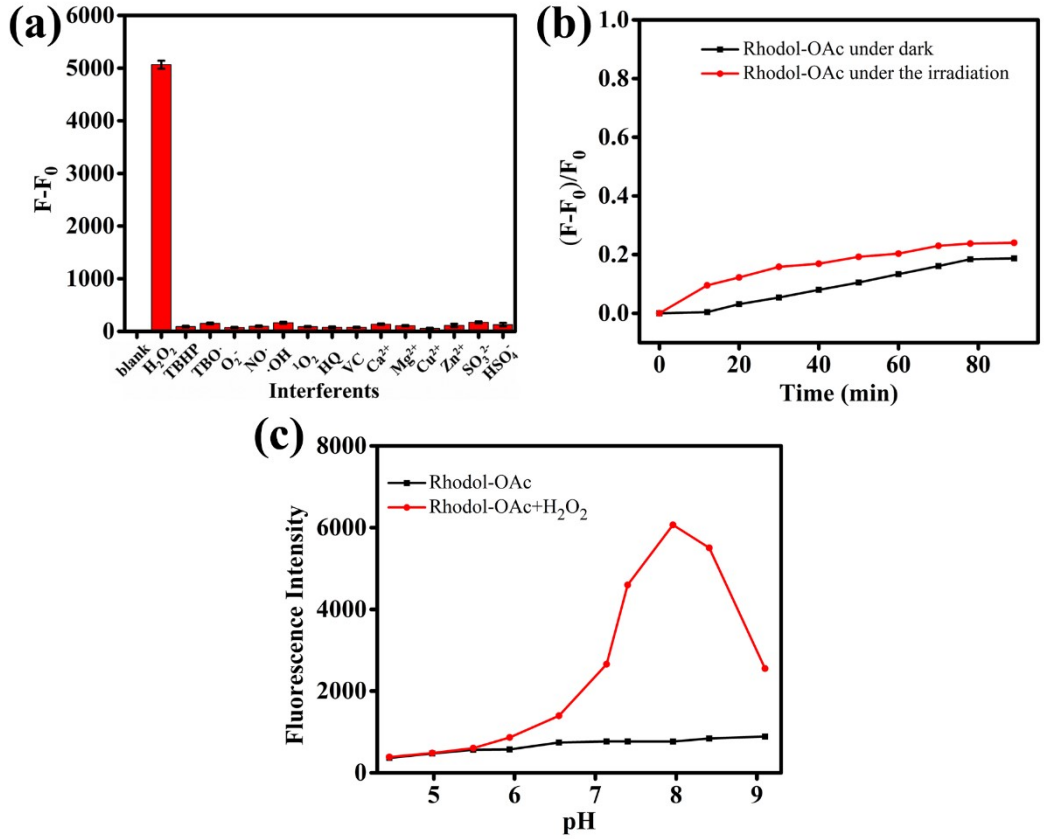


Fig. S6 (a) Fluorescence intensity increases of Rhodol-OAc (10 μM) after the addition of various interferents (200 μM). (b) Time-dependent ratio of fluorescence increase of Rhodol-OAc (10 μM) under irradiation of light (λ_{ex}=500 nm) or in darkness. Ratio of fluorescence increase was calculated according to the equation: $(F\text{-}F_0)/F_0$, where F_0 and F are fluorescence intensities of the testing system obtained at beginning and certain testing time, respectively. (c) Fluorescence intensity of Rhodol-OAc (10 μM) and Rhodol-OAc (10 μM) with H_2O_2 (200 μM) under various environmental pH at 37 ℃ for 30 min. λ_{ex}=500 nm, λ_{em}=560 nm.

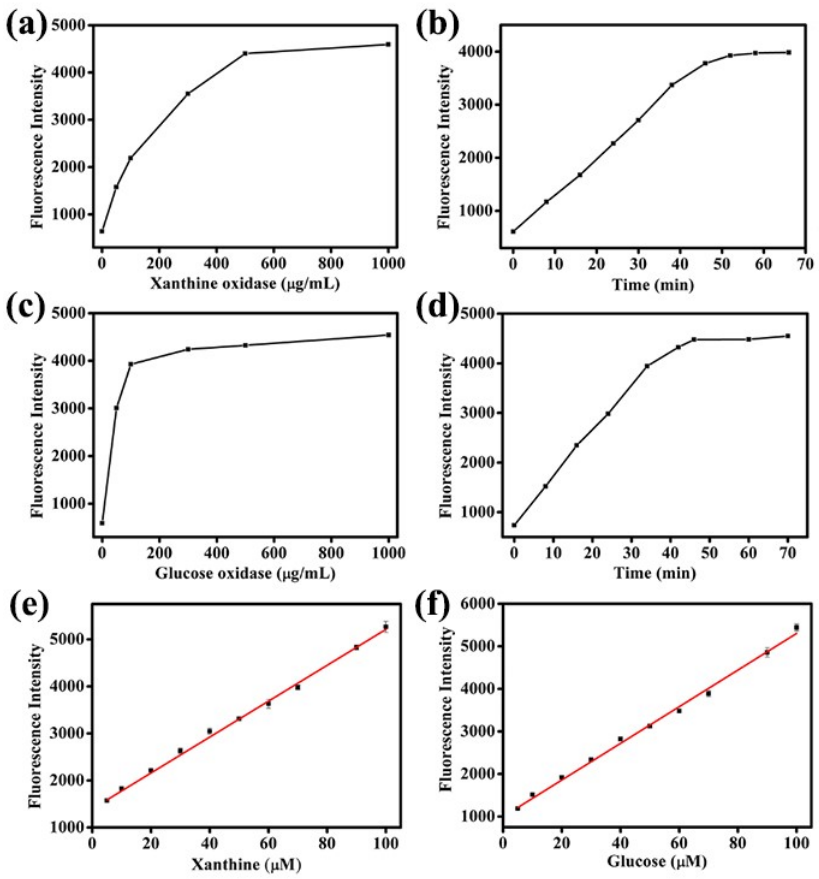


Fig. S7 Optimal concentrations of xanthine oxidase (a) and glucose oxidase (c). Reaction time between Rhodol-OAc and xanthine/xanthine oxidase (b) and that between Rhodol-OAc and glucose/glucose oxidase (d). Linear relationship between Rhodol-OAc and xanthine (e) and that between Rhodol-OAc and glucose (f).

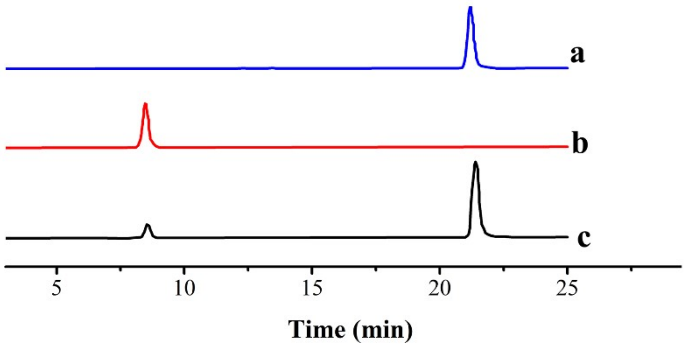


Fig. S8 HPLC analyses of different solutions. (a) Rhodol-OH (5 μM); (b) Rhodol-OAc (5 μM); (c) the reaction solution of 5 μM Rhodol-OAc with 50 μM H2O2 for 30 min. The detection wavelength was 500 nm.

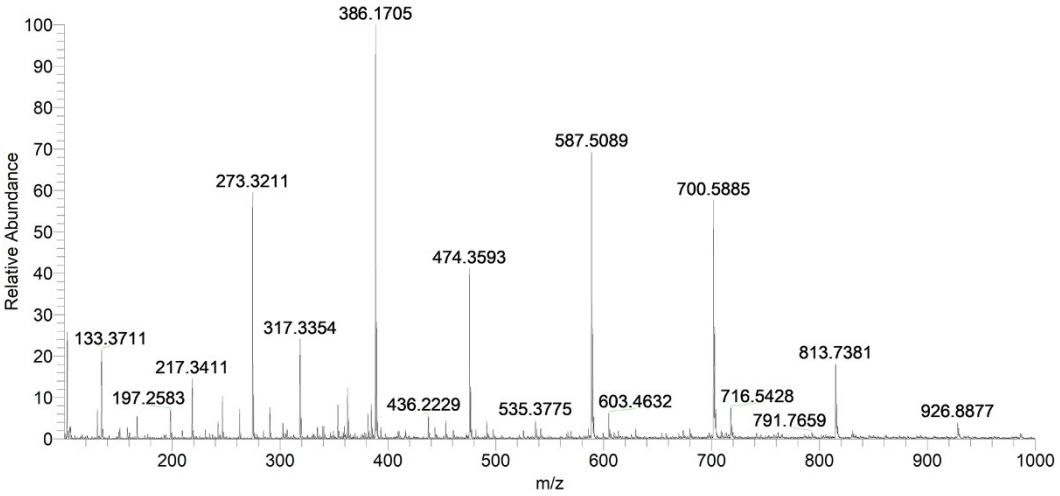


Fig. S9 ESI-MS spectrum of the product of reaction between Rhodol-OAc (10 μM) and H_2O_2 (200 μM).

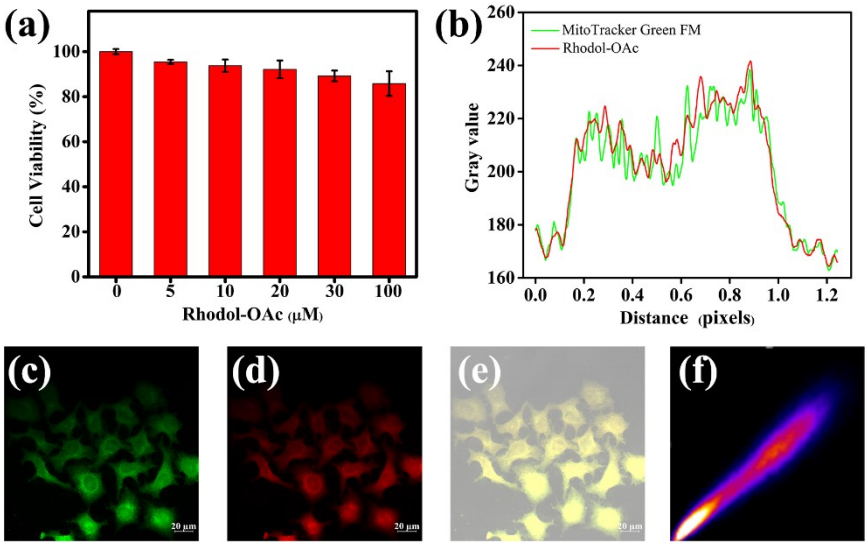
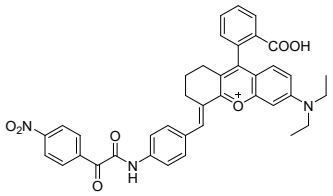
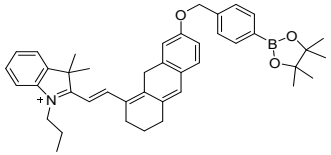
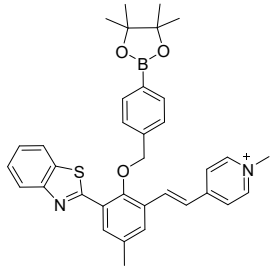
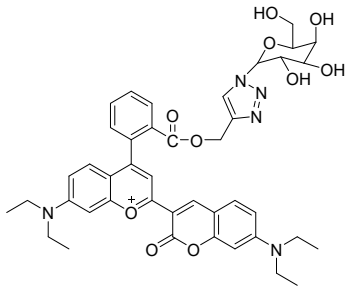
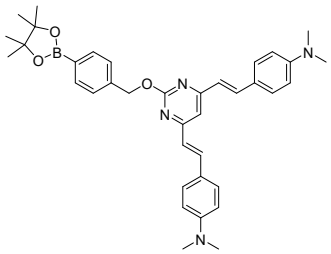


Fig. S10 (a) Cell viability of A549 cells in the presence of Rhodol-OAc at various concentrations. Colocalization of Rhodol-OAc and Mito Tracker Green FM in the mitochondria of A549 cells. (b) Fluorescence intensity of the regions of interest across A549 cells. (c) A549 cells cultured with Mito Tracker Green FM (10 μM) for 30 min at 37°C. (d) A549 cells cultured with Rhodol-OAc (10 μM) for 30 min at 37°C. (c) Merged image of (a) and (e). (f) The correlation between the intensity of Rhodol-OAc and that of Mito Tracker Green FM.

69 Table S1 Comparison of HAA with other reported fluorescence probe for H_2O_2

Probe	Reaction medium	$\lambda_{ex}/\lambda_{em}$ (nm)	LOD (μM)	Ref.
Rhodol-OAc	PBS:EtOH (99:1, *v/v*; pH 7.4)	500/560	0.055	This work
	PBS:DMSO (999:1, *v/v*; pH 7.4)	590/730	0.061	[1]
	PBS (pH 7.4)	670/708	0.14	[2]
	PBS:DMSO (99:1, *v/v*; pH 7.4)	395/(650/500)	0.27	[3]

	PBS:MeOH (9:1, *v/v*; pH 7.4)	650/(482/706)	0.33	[4]
	PBS:DMSO (499:1, *v/v*; pH 7.4)	540/640	1.38	[5]

Reference

[1] T. Gu, S. Mo, Y. Mu, X. Huang, L. Hu, Detection of endogenous hydrogen peroxide in living cells with para-nitrophenyl oxoacetyl rhodamine as turn-on mitochondria-targeted fluorescent probe, Sensors and Actuators B: Chemical 309 (2020) 127731.

[2] J. Zhang, L. Shi, Z. Li, D. Li, X. Tian, C. Zhang, Near-infrared fluorescence probe for hydrogen peroxide detection: design, synthesis, and application in living systems, Analyst 144(11) (2019) 3643-3648.

[3] Y. Liu, L. Bai, Y. Li, Y. Ni, C. Xin, C. Zhang, J. Liu, Z. Liu, L. Li, W. Huang, Visualizing hydrogen peroxide in Parkinson's disease models via a ratiometric NIR fluorogenic probe, Sensors and Actuators B: Chemical 279 (2019) 38-43.

[4] W.-L. Jiang, W.-X. Wang, J. Liu, Y. Li, C.-Y. Li, A novel hepatocyte-targeting ratiometric fluorescent probe for imaging hydrogen peroxide

in zebrafish, Sensors and Actuators B: Chemical 313 (2020) 128054.

[5] X. Qiu, C. Xin, W. Qin, Z. Li, D. Zhang, G. Zhang, B. Peng, X. Han, C. Yu, L. Li, W. Huang, A novel pyrimidine based deep-red fluorogenic probe for detecting hydrogen peroxide in Parkinson's disease models, Talanta 199 (2019) 628-633.