Supporting Information

A fluorescein-based fluorescent probe for fast detection of malondialdehyde and its imaging study

Min Peng, Quan-Rong Du, Xue Yao, Chun-Ni Li, Yang Tian, Yu Peng,* and Ya-Wen Wang*

School of Chemistry & School of Life Science and Engineering

Southwest Jiaotong University, Chengdu 610031, People's Republic of China

ywwang@swjtu.edu.cn, pengyu@swjtu.edu.cn

Table of Contents

1. General Methods.

Compound **1** and ethanol (EtOH) were purchased from Innochem. Hydrate hydrazine (80%) was purchased from chronchem (Chengdu, China). 1,1,3,3-tetraethoxypropane (TEP) was obtained from Aladdin Industrial Inc Co. (Shanghai, China). The polyamide thin films were purchased from Sijia Biochemical Plastic Factory (Zhejiang, China). Unless otherwise specified, all the chemicals were analytical reagent grade and can be used directly without further purification. MDA solution was prepared from TEP as literature procedure and the concentration of MDA was measured by UV-vis absorption spectrum before use. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVB-400 spectrometer using TMS as the internal reference. HRMS (ESI) spectra were measured with a Waters e2695 spectrometer. Fluorescence spectra were recorded by an F7000 spectrofluorimeter from Hitachi PharmaSpec with the excitation and emission slit widths at 10/10 nm. Confocal imaging of HeLa cells was performed by Nikon AR1+ confocal microscope.

SWJT-17 was weighed and dissolved in DMSO to prepare 1.0 mM stock solution. Al^{3+}, Mg^{2+},Ca^{2+} Cd^{2+}, Zn^{2+}, formaldehyde, acetaldehyde, glyoxal, acetaldehyde, acrolein, glyoxylic acid, H_2O_2, OCl^-, Cys and GSH were dissolved in pure water prepare solution for standby. **SWJT-17** stock solution was diluted to 20.0 μM with DMSO-PBS (1:99, v/v pH = 7.24). For all fluorescence spectra, the excitation was set at 430 nm, and the excitation and emission gaps were 10/10 nm.

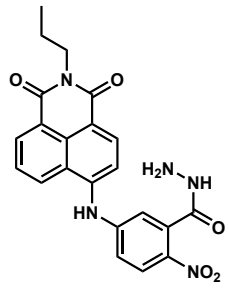
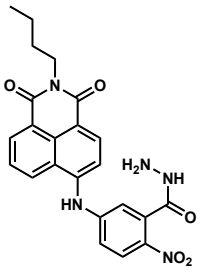
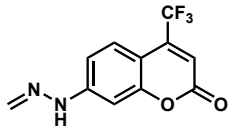
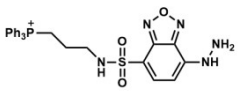
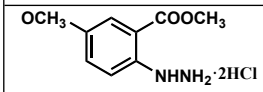
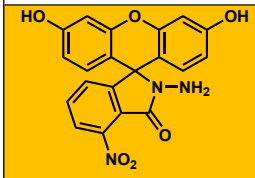
The HeLa cells were incubated in a glass-bottom petri dish (r = 15 mm) and adhered at 37 °C for 24 hours. The cells were washed with phosphate buffered saline (PBS) and added **SWJT-17** (20.0 μM) at 37 °C for 30 minutes, then washed with PBS and imaged. After incubating with **SWJT-17** (20.0 μM) at 37 °C for 30 min, washed with PBS and added 1.0, 2.0 and 5.0 mM MDA at 37 °C for 30 minutes. Fluorescence imaging of MDA in HeLa cells was recorded on a laser scanning confocal microscope. The excitation wavelength of the laser is 488 nm.

The Staphylococcus aureus were incubated in a glass-bottom petri dish (*r* = 15 mm) and adhered at 37 °C for 24 hours. The cells were washed with phosphate buffered

saline (PBS) and added **SWJT-17** (20.0 μM) at 37 °C for 1 h, then washed with PBS and imaged. After incubating with **SWJT-17** (20.0 μM) at 37 °C for 30 min, washed with PBS and added 1.0, 2.0 and 5.0 mM MDA at 37 °C for 30 minutes. Fluorescence imaging of acrolein in Staphylococcus aureus was recorded on a CLSM (LSM800). The excitation wavelength of the laser is 488 nm.

2. Summary of fluorescent probes for MDA.

Table S1

Probes	$\lambda_{ex}/\lambda_{em}$ nm	Detection limit	Reaction time	Solution	Reference
	370/553 480/520	0.6 μM	30 min	DMSO : PBS = 1 : 9	Ref. 18
	480/553	0.03 μM	30 min	DMSO : PBS = 1 : 9	Ref. 19
	365/440	0.13 μM	60 min	Unknown	Ref. 20
	373/554	0.454 μM	Unknown	Ethanol : PBS = 1:99	Ref. 21
	375/480	Unknown	Unknown	DMSO : PBS = 1 : 9	Ref. 22
	480/555	7.2 nM	6 min	0.01 M HCl	Ref. 23
	430/520	0.29 μM	≤ 60 s	DMSO : PBS = 1:99	*This work*

3. Synthesis of SWJT-17.

The hydrate hydrazine (48 μL 0.792 mmol) was dropped into the dissolved compound **1** (50.0 mg, 0.132 mmol) in 5 mL of ethanol. After refluxing and stirring at 80 °C for 4 h, the mixture was filtered and washed with ethanol for three times, then dried at 60 °C to obtain probe **SWJT-17** (41.5 mg, 80.4% yield) as a light beige solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.83 (s, 2H), 8.46 (s, 1H), 8.33 (s, 1H), 7.31 (s, 1H), 6.63 (s, 2H), 6.47 (s, 4H), 4.58 (s, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 163.9, 159.9 (2C), 157.3, 153.7 (2 C), 150.4, 132.9, 129.9 (2C), 129.1, 125.9, 119.0, 113.5 (2C), 109.7 (2C), 104.7 (2C), 66.3 ppm. HRMS(ESI): m/z calcd for $C_{20}H_{13}N_3O_6$ $[M+H]^+$: 392.0883, found: 392.0909 error 6.6 ppm.

4. ^{1}H, ^{13}C NMR and HRMS copies of SWJT-17.

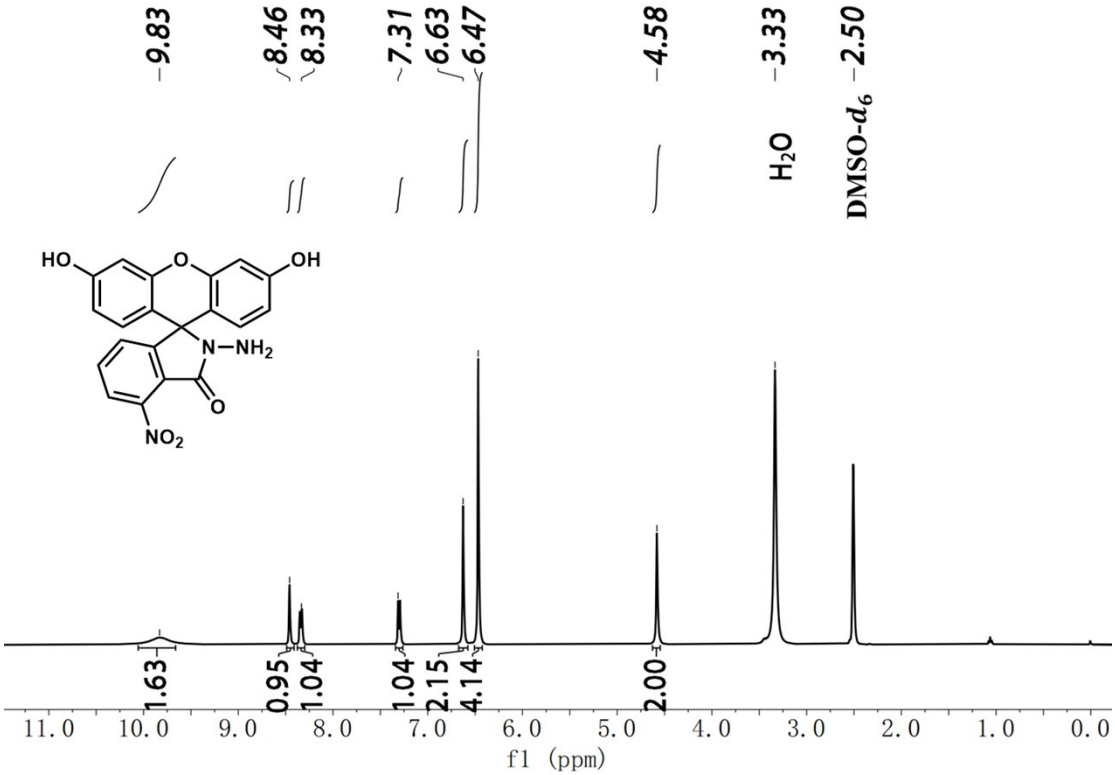


Fig. S1. ^{1}H NMR spectrum of probe **SWJT-17** (400 MHz, DMSO-d_6).

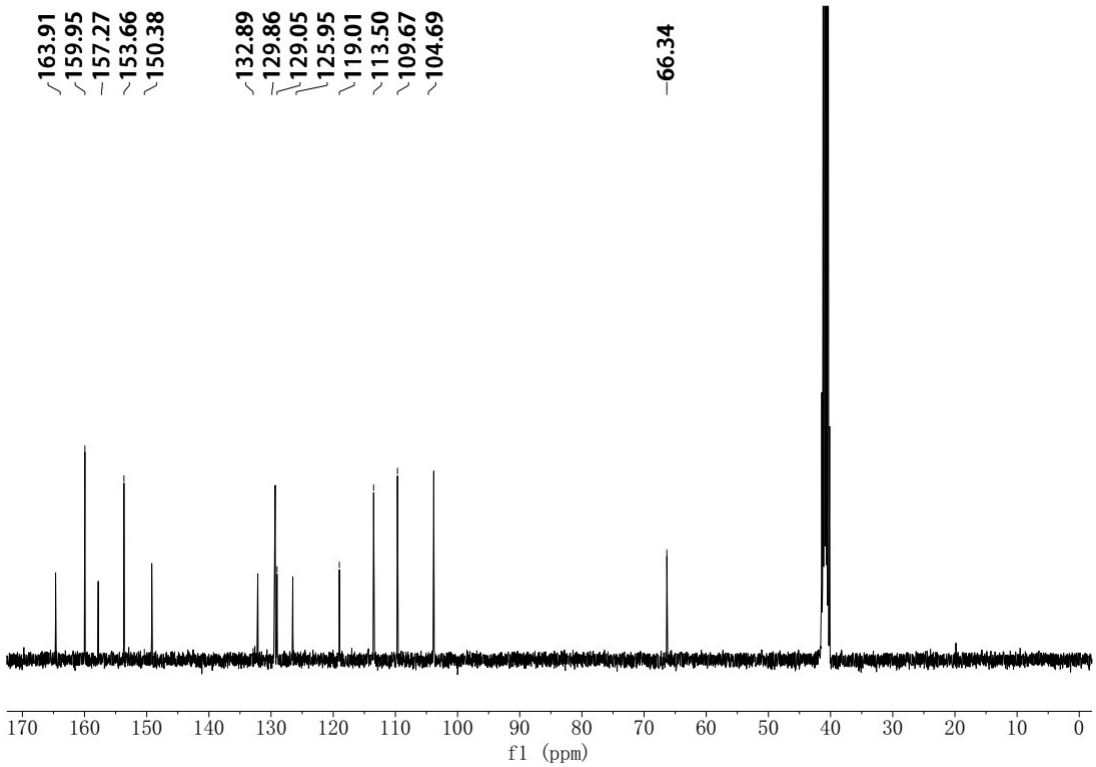


Fig. S2. ^{13}C NMR spectrum of probe **SWJT-17** (100 MHz, DMSO-d_6).

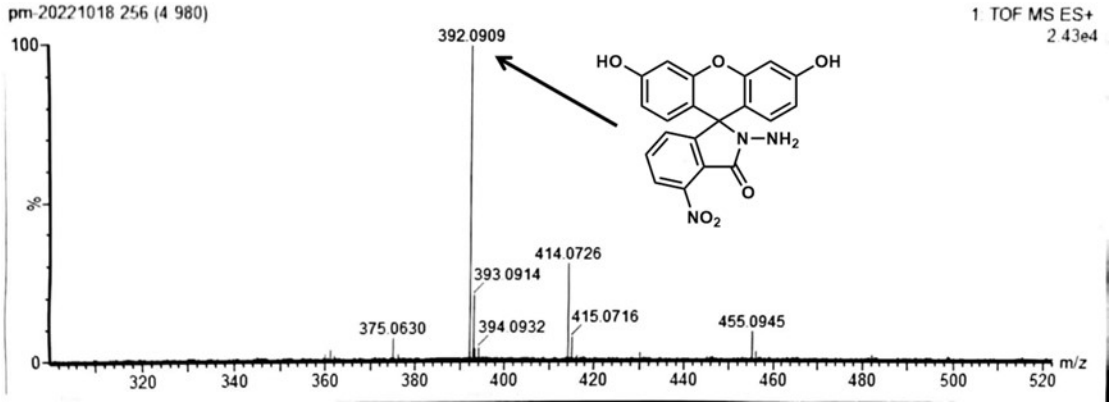


Fig. S3. HRMS spectrum of probe **SWJT-17**.

5. The pH effect of SWJT-17 to MDA.

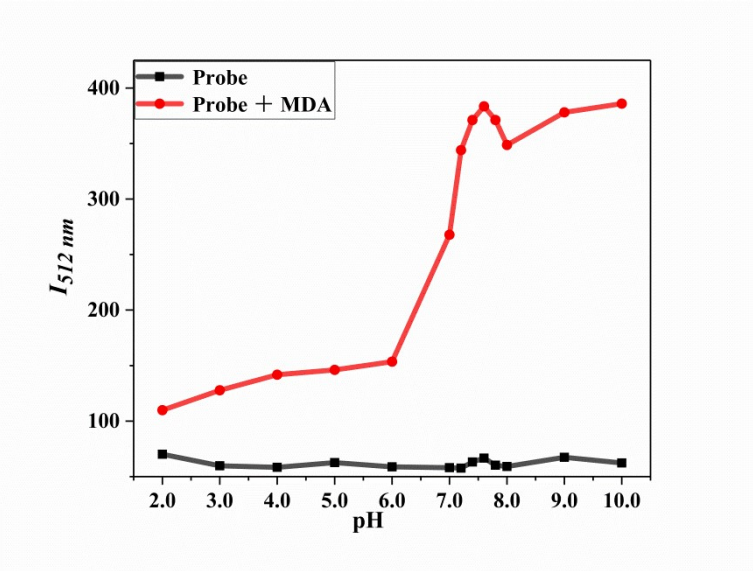


Fig. S4. The fluorescence intensity of **SWJT-17** (20.0 μM) at 520 nm in the presence and absence of MDA (1.0 mM) in DMSO/PBS (1:99 v/v) buffer solution under different pH values (λ_{ex} = 430 nm).

6. The detection limit of SWJT-17 to MDA.

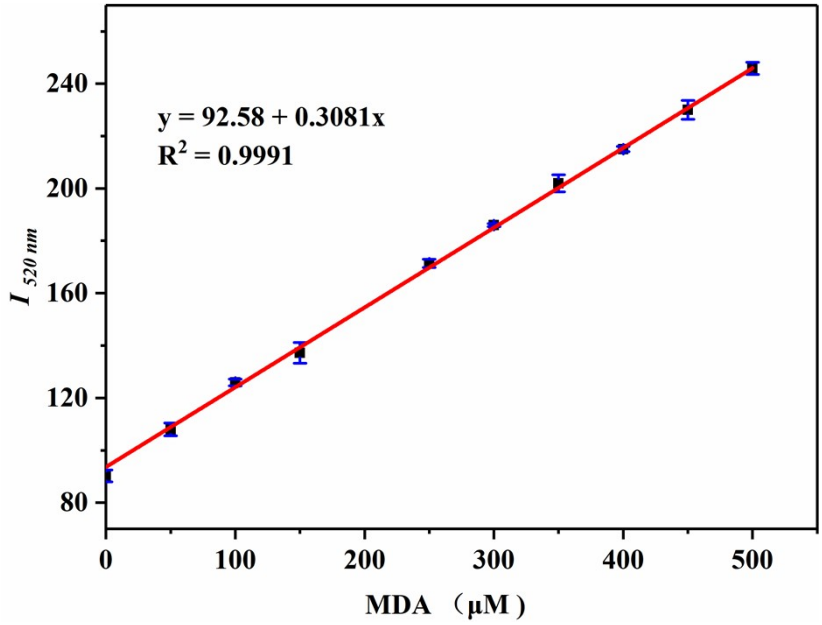


Fig. S5. Linear equation of **SWJT-17** (20.0 μM) upon the addition of MDA (0-500.0 μM) (λ_{ex} = 430 nm).

The detection limit is calculated according to the formula:

LOD = K × δ/S

Linear Equation: Y = 0.3081 X + 92.58

S = 0.3081

δ = 0.03

K = 3

LOD = K × δ/S = 0.29 μM

7. HRMS spectrum of compound SWJT-MDA.

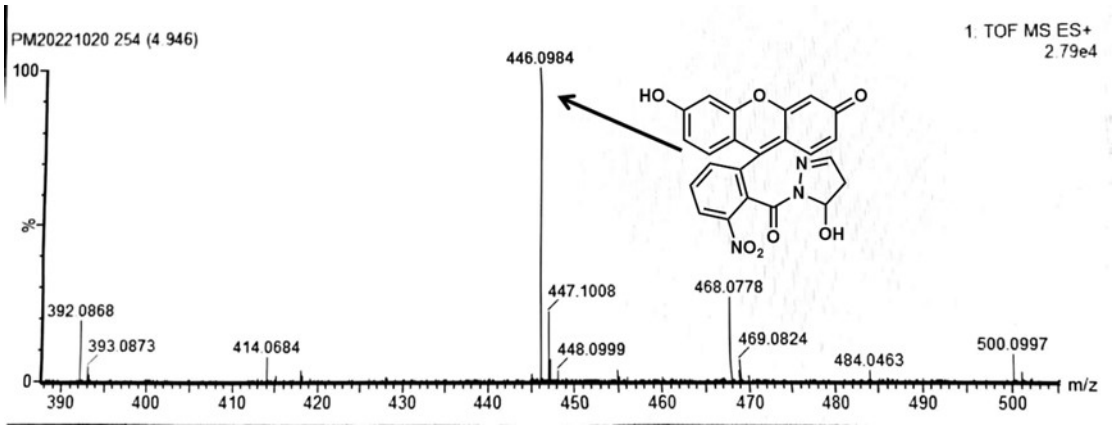


Fig. S6. HRMS spectrum of **SWJT-MDA**.

8. The cytotoxicity evaluation of SWJT-17 in living HeLa cells.

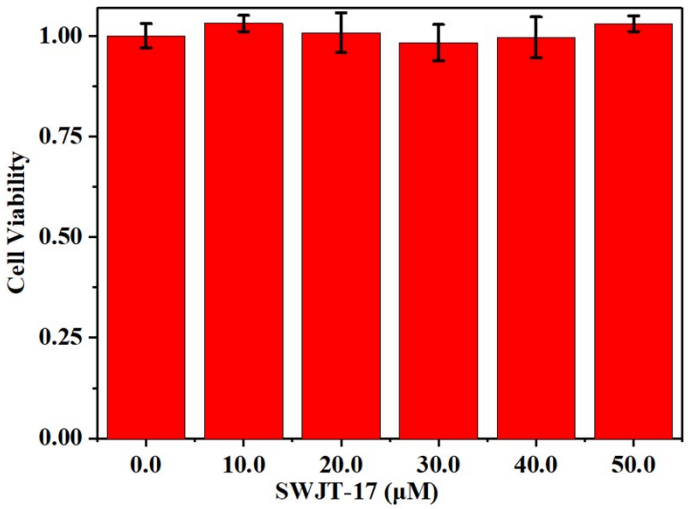


Fig. S7. The cytotoxicity evaluation of **SWJT-17** in living HeLa cells after incubation for 24 h. Each error bar shows the standard deviation of six independent measurements.