Supporting Information

Mitochondrion-Targeting Fluorescence Probe via Reduction Induced Charge Transfer for Fast Methionine Sulfoxide Reductases Imaging

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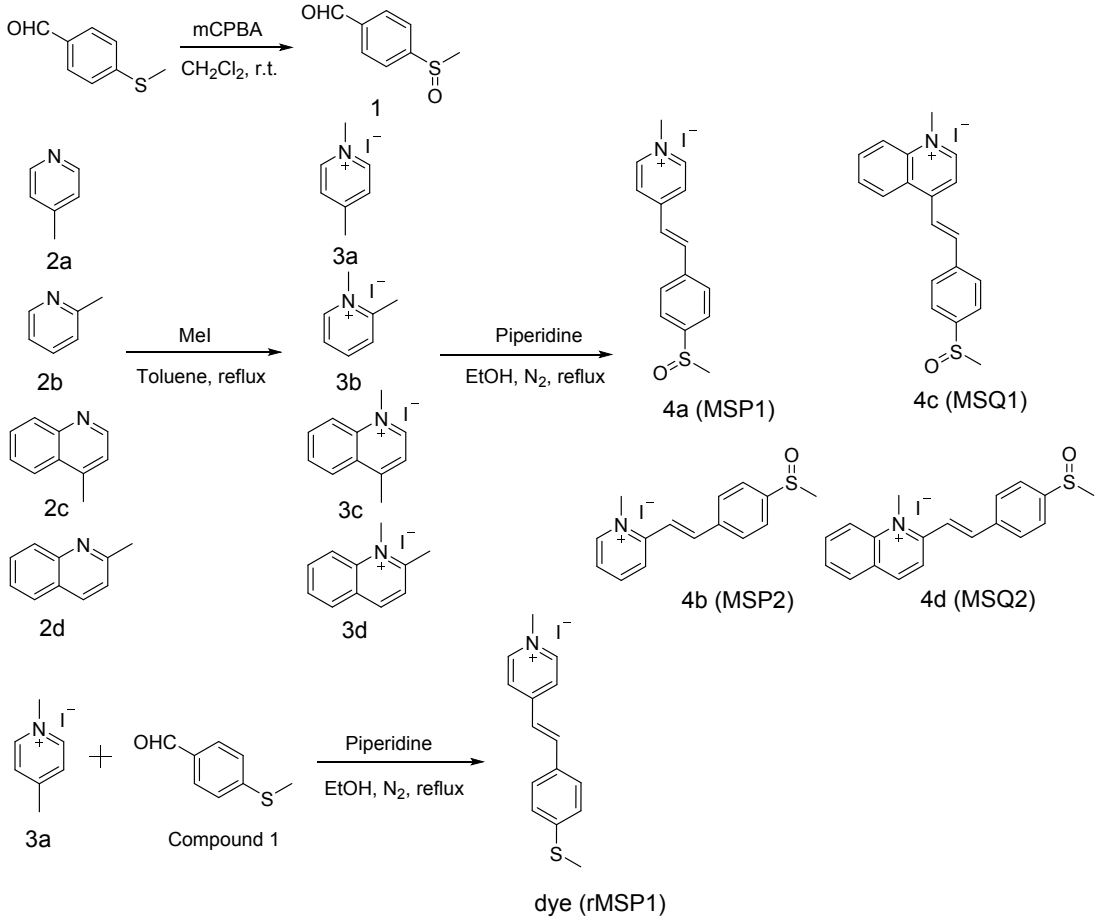
Scheme S1. Synthesis routes for MSP and MSQ.



EXPERIMENTAL SECTION

Materials. Methionine sulfoxide reductase A (MsrA) was purchased from Jena Bioscience (Thuringia, Germany). MsrA polyclonal antibody (anti-MsrA antibody, A12464) was purchased from ABclonal (Boston, USA). Anti-beta actin antibody (mAbcam 8226, loading control) was purchased from Abcam (Cambridge, England). Horseradish peroxidase-conjugated mouse anti-rabbit IgG was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Horseradish peroxidase-conjugated AffiniPure $F(ab')_2$ Fragment Goat Anti-Mouse IgG (H+L) was purchased from Jackson ImmunoResearch Laboratories, Inc. (Pennsylvania, USA). Nitrocellulose membrane were purchased from Pall Corporation (Westborough, USA). Dithiothreitol (DTT), Nonidet P 40 (NP-40), ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), glycerol, polyacrylamide, sodium dodecyl sulfate (SDS), phosphate buffered saline buffer (PBS, pH 7.4) were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). Tris(2-carboxyethyl)phosphine (TCEP), glutathione (GSH), cysteine (Cys), homocysteine (Hcy), vitamin c, bovine serum albumin (BSA), nitroreductase (NTR), NAD(P)H: quinone oxidoreductase isozyme 1 (NQO1), thioredoxin reductase (TrxR), 6-hydroxydopamine (6-OHDA) were all purchased from Sigma-Aldrich (MO, USA). HeLa cells (human cervical carcinoma cell line) and HEK-293T cells were obtained from the cell bank of Central Laboratory at Xiangya Hospital (Changsha, China). PC12 cells were purchased from Shanghai Cell Bank, Chinese Academy of Sciences. RPMI-1640 medium, Dulbecco's modified Eagles medium (DMEM, high glucose medium), penicillin, streptomycin, heat-inactivated fetal bovine serum (Invitrogen) and heat-inactivated horse serum were purchased from Thermo Fisher Scientific (MA, USA). Mito-Tracker Red (MitoTracker® Red) and Lyso-Tracker Red (LysoTracker® Red) were obtained from Thermo Fisher Scientific (MA, USA). All other reagents were of analytical grade and obtained from J&K Chemical (Beijing, China). Organic solvents were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) and used without further purification. Ultrapure water was obtained through a Millipore Milli-Q water purification system (Billerica, MA) and had an electric resistance >18.25 MΩ.

Instruments. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 spectrometer using $CDCl_3$ and DMSO-d_6 as solvent. The chemical shifts (δ) were reported in ppm (relative to TMS as internal standard) and coupling constants (J) are given in Hz. Signal multiplicities were represented by s (singlet), d (doublet), t (triplet),

dd (double doublet), and m (multiplet). Thin-layer chromatography (TLC) was performed on silica gel aluminum sheets with an F-254 indicator. The column chromatography was conducted using 200-300 mesh SiO_2 (Qingdao Ocean Chemical Products). UV-vis absorption spectrum was measured on Shimadzu UV2450 (Japan). Electrospray ionization mass spectrometry (ESI-MS) was determined using Finnigan LCQ Advantage MAX (Thermo Finnigan). High-resolution electrospray ionization mass spectra (HR-ESI-MS) was performed on an APEX IVFTMS instrument (Bruker Daltonics). Fluorescence spectra were collected on an F-7000 fluorescence spectrophotometer (Hitachi, Japan). All fluorescence images were acquired on Nikon TI-E+A1 SI confocal laser scanning microscope (Japan).

Synthesis of compounds

Synthesis of 4-(methylsulfinyl)benzaldehyde (1). Compound 1 was synthesized according to reported procedures.[S1] 3-Chloroperoxybenzoic acid (mCPBA) (85%, 4.06 g, 20 mmol) was added portionwise to a solution of 4 (methylthio)benzaldehyde (2.66 mL, 20 mmol) in CH_2Cl_2 (30 mL) at 0 °C. The mixture was stirred at 0 °C for 60 min and then stirred at room temperature for 12 h. Saturated sodium bicarbonate solution (80 mL) was added and the reaction mixture was extracted with CH_2Cl_2 (60 mL). The organic layer was dried with anhydrous Na_2SO_4 and concentrated in vacuum. The residue was purified by column chromatography using ethyl acetate/petroleum ether of 1:2 (v/v) as the eluent to yield Compound 1 as a white solid (2.12 g, 63.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 10.07 (1H, s, CHO), 8.02-8.05 (2H, m, Ar-H), 7.80-7.72 (2H, m, Ar-H), 2.77 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 191.16, 152.41, 138.11, 130.40, 124.17, 43.76.

General procedure for the synthesis of Compound 3a-3d. To a solution of iodomethane (2.50 mL, 40 mmol) in toluene (30 mL), Compound 2a-2d (20 mmol) was added. After stirring at 110 °C overnight, the solid product was collected by filtration, washed with EtOAc (100 mL) and diethyl ether (100 mL) and dried under vacuum to obtain pure product.

Synthesis of 1,4-dimethylpyridin-1-ium iodide (3a). Compound 2a (1.98 mL, 20 mmol) first reacted with iodomethane (2.50 mL, 40 mmol) in toluene (30 mL), which was then purified with EtOAc and diethyl ether to give a white solid product with a yield of 83.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.85 (2H, d, J = 6.4 Hz, Ar-H), 7.97 (2H, d, J = 6.4 Hz, Ar-H), 4.29 (3H, s, CH_3), 2.60 (3H, s, CH_3). ^{13}C NMR (100

MHz, DMSO-d_6) δ (ppm): 158.67, 144.96, 128.43, 47.69, 21.85. MS m/z (ESI): 108.11 $[M-I]^+$.

Synthesis of 1,2-dimethylpyridin-1-ium iodide (3b). Compound 2b (1.96 mL, 20 mmol) first reacted with iodomethane (2.50 mL, 40 mmol) in toluene (30 mL), which was then washed with EtOAc and diethy ether to give a white solid product with a yield of 79.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.05 (1H, d, J = 6.0 Hz, Ar-H), 8.49 (1H, d, J = 8.0 Hz, Ar-H), 8.07 (1H, d, J = 8.0 Hz, Ar-H), 7.96 (1H, t, J = 6.8 Hz, Ar-H), 4.26 (3H, s, CH_3), 2.81 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 156.34, 146.45, 145.43, 129.57, 125.65, 46.10, 20.60. MS m/z (ESI): 108.13 $[M-I]^+$.

Synthesis of 1,4-dimethylquinolin-1-ium iodide (3c). Compound 2c (1.98 mL, 20 mmol) was first reacted with iodomethane (2.50 mL, 40 mmol) in toluene (30 mL), which was then purified with EtOAc and diethyl ether to give a light yellow solid product with a yield of 86.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.38 (1H, d, J = 6.0 Hz, Ar-H), 8.48-8.55 (2H, m, Ar-H), 8.27 (1H, t, J = 7.2 Hz, Ar-H), 8.05-8.09 (2H, m, Ar-H), 4.59 (3H, s, CH_3), 3.01 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 158.62, 149.42, 138.13, 135.40, 130.12, 128.93, 127.27, 122.93, 120.01, 45.57, 20.14. MS m/z (ESI): 158.19 $[M-I]^+$.

Synthesis of 1,2-dimethylquinolin-1-ium iodide (3d). Compound 2d (2.70 mL, 20 mmol) was reacted with iodomethane (2.50 mL, 40 mmol) in toluene (30 mL) and then purified by washing with EtOAc and diethyl ether to give a grayish solid product with a yield of 71.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.11 (1H, d, J = 8.8 Hz, Ar-H), 8.60 (1H, d, J = 8.8 Hz, Ar-H), 8.41 (1H, d, J = 6.8 Hz, Ar-H), 8.23 (1H, t, J = 6.8 Hz, Ar-H), 8.13 (1H, d, J = 6.8 Hz, Ar-H), 7.99 (1H, t, J = 7.2 Hz, Ar-H), 4.46 (3H, s, CH_3), 3.10 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 161.63, 145.86, 139.66, 135.53, 130.78, 129.46, 128.25, 125.62, 119.45, 40.30, 23.67. MS m/z (ESI): 158.18 $[M-I]^+$.

General procedure for the synthesis of compound 4a-4d. To a solution of Compound 3a-3d (1 mmol) in dry EtOH (20 mL), Compound 1 (0.2 g, 1.2 mmol) and piperidine (0.1 mL) were added. Then the solution was refluxed under nitrogen atmosphere for 20 h. After removal of solvent, the residues were purified by alumina column chromatography using dichloromethane/methanol (10:1 to 20:1, v/v) as eluent to obtain Compound 4a-4d.

Synthesis of (E)-1-methyl-4-(4-(methylsulfinyl)styryl)pyridin-1-ium iodide (4a, MSP1). Compound 3a (0.23 g, 1 mmol) was first reacted with Compound 1 (0.20 g,

1.2 mmol) according to the general procedure, which was then purified by alumina column chromatography using dichloromethane/methanol (10:1, v/v) as eluent to afford Compound 4a with a yield of 31.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.90 (2H, d, J = 6.4 Hz, Ar-H), 8.27 (2H, d, J = 6.8 Hz, Ar-H), 8.08 (1H, d, J = 16.0 Hz, CH=), 7.94 (2H, d, J = 8.4 Hz, Ar-H), 7.75 (2H, d, J = 8.0 Hz, Ar-H), 7.66 (1H, d, J = 16.0 Hz, CH=), 4.29 (3H, s, CH_3), 2.78 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 152.44, 148.32, 145.63, 139.54, 137.84, 129.17, 125.40, 124.79, 124.30, 47.62, 43.53. HR-ESI-MS m/z: calcd 258.0947, found 258.1031 $[M-I]^+$.

Synthesis of (E)-1-methyl-2-(4-(methylsulfinyl)styryl)pyridin-1-ium iodide (4b, MSP2). According to the general method, the reaction of Compound 3b (0.23 g, 1 mmol) with Compound 1 (0.20 g, 1.2 mmol) produced the crude product, which was then purified by alumina column chromatography using dichloromethane/methanol (10:1, v/v) as eluent to afford Compound 4b with a yield of 17.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.06 (1H, d, J = 6.0 Hz, Ar-H), 8.53-8.61 (2H, m, Ar-H), 8.04-8.11 (3H, m, Ar-H and CH=), 7.97 (1H, t, J = 6.0 Hz, Ar-H), 7.77-7.82 (3H, m, Ar-H and CH=), 4.44 (3H, s, CH_3), 2.80 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 152.50, 148.93, 146.80, 144.98, 141.96, 137.60, 129.64, 126.05, 125.70, 124.70, 119.93, 46.64, 43.55. MS m/z (ESI): 258.07 $[M-I]^+$.

Synthesis of (E)-1-methyl-4-(4-(methylsulfinyl)styryl)quinolin-1-ium iodide (4c, MSQ1). Compound 3c (0.28 g, 1 mmol) first reacted with Compound 1 (0.20 g, 1.2 mmol) to produce the crude product which was further purified with alumina column chromatography using dichloromethane/methanol (20:1, v/v) as eluent to obtain Compound 4c with a yield of 22.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.14 (1H, d, J = 6.4 Hz, Ar-H), 8.89 (1H, d, J = 8.4 Hz, Ar-H), 8.35 (2H, t, J = 6.8 Hz, Ar-H), 8.12-8.23 (2H, m, Ar-H and CH=), 7.99-8.03 (2H, m, Ar-H and CH=), 7.83 (2H, d, J = 8.0 Hz, Ar-H), 4.48 (3H, s, CH_3), 2.96 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 152.97, 148.17, 142.76, 139.01, 135.53, 132.06, 129.80, 129.66, 126.61, 125.90, 119.52, 118.87, 116.36, 55.13, 45.16. MS m/z (ESI): 308.12 $[M-I]^+$.

Synthesis of (E)-1-methyl-2-(4-(methylsulfinyl)styryl)quinolin-1-ium iodide (4d, MSQ2). Compound 3c (0.28 g, 1 mmol) first reacted with Compound 1 (0.20 g, 1.2 mmol) to obtain the crude product which was then purified with alumina column chromatography using dichloromethane/methanol (20:1, v/v) as eluent to afford Compound 4d with a yield of 28.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.05 (1H, d, J = 8.8 Hz, Ar-H), 8.58 (2H, t, J = 8.8 Hz, Ar-H), 8.35 (1H, d, J = 8.0 Hz, Ar-

H), 8.17-8.24 (2H, m, Ar-H and CH=), 7.88-7.97 (4H, m, Ar-H and CH=), 7.39 (2H, d, J = 7.6 Hz, Ar-H), 4.56 (3H, s, CH_3), 3.08 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 156.62, 147.07, 144.32, 144.02, 139.65, 135.32, 131.63, 130.50, 130.14, 129.39, 128.17, 125.95, 121.64, 119.78, 118.47, 55.40, 46.70. MS m/z (ESI): 308.20 $[M-I]^+$.

Synthesis of (E)-1-methyl-4-(4-(methylthio)styryl)pyridin-1-ium iodide (dye, rMSP1). To a solution of compound 3a (0.23 g, 1 mmol) in dry EtOH (20 mL), 4-(methylthio)benzaldehyde (0.18 g, 1.2 mmol) and piperidine (0.1 mL) were added. Then the resulting solution was refluxed under nitrogen atmosphere for 20 h. After removal of solvent, the residues were purified by alumina column chromatography using dichloromethane/methanol (20:1, v/v) as eluent to afford the dye with a yield of 54.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.84 (2H, d, J = 6.8 Hz, Ar-H), 8.20 (2H, d, J = 6.4 Hz, Ar-H), 7.99 (1H, d, J = 16.4 Hz, CH=), 7.69 (2H, d, J = 8.4 Hz, Ar-H), 7.48 (1H, d, J = 16.4 Hz, CH=), 7.35 (2H, d, J = 8.4 Hz, Ar-H), 4.25 (3H, s, CH_3), 2.53 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 153.01, 145.42, 142.27, 140.64, 131.95, 129.05, 126.17, 123.76, 122.56, 47.36, 14.67. HR-ESI-MS m/z: calcd 242.0998, found 242.1076 $[M-I]^+$.

Determination of quantum yields. The quantum yields of MSP1 and rMSP1 in Tris-HCl buffers (25 mM, pH 7.4) were determined using quinine sulfate (Φ_R = 0.54 in 0.1 M H_2SO_4) as a reference. The quantum yields were calculated according to Equation 1.[S2]

$$\Phi_F = \frac{I}{I_R}\frac{A_R}{A}\left(\frac{n}{n_R}\right)\Phi_R \qquad (1)$$

where Φ_F is the quantum yield, I is the integrated area under the fluorescence spectra, A is the absorbance, n is the refractive index of the solvent, and R refers to the reference fluorophore.

***In vitro* assay of MsrA activity.** Stock solutions of MSP1 (500 μM) and DTT (100 mM) were prepared with Tris-HCl buffers (25 mM, pH 7.4). MSP1 and DTT with final concentrations of 10 μM and 5mM, respectively, were used throughout the *in vitro* experiments. A final concentration of 3.0 μg/mL was used for MsrA, unless otherwise indicated. Fluorescence spectra were all recorded on a F7000 spectrophotometer. Fluorescence spectra were recorded in the range from 460 to 705 nm, with an excitation wavelength of 385 nm. The excitation and emission slit widths were both 5 nm. To study the response of MSP1 toward MsrA, MSP1 (10 μM) and DTT (5 mM) were

mixed with MsrA (3.0 μg/mL) in Tris-HCl buffers (25 mM, pH 7.4) and incubated at 37 °C for 1 h. For inhibition assay, MSP1 (10 μM) and DTT (5 mM) were mixed with MsrA (3.0 μg/mL) and DMSO (0.05%) in Tris-HCl buffers (25 mM, pH 7.4) at 37°C for 1 h. Then, fluorescence spectrum was then recorded. To investigate the ability of MSP1 to quantify MsrA concentration, MSP1 (10 μM) was mixed with different concentrations of MsrA in Tris-HCl buffers (25 mM, pH 7.4) and fluorescence spectra were acquired after the samples were incubated at 37 °C for 1 h.

For studying the real time response of MSP1 towards MsrA, MSP1 (10 μM), DTT (5 mM) were rapidly mixed with different concentrations of MsrA (0, 0.5, 1.0, 3.0, 5.0 μg/mL) in Tris-HCl buffers (25 mM, pH 7.4). Then, the fluorescence intensity at 547 nm was recorded in real time for 4800 s at 37 °C with an excitation wavelength of 385 nm on an F-7000 fluorescence spectrophotometer, using slit widths of 5 nm for both excitation and emission.

To investigate the effect of pH on the response of MSP1 towards MsrA, MSP1 (10 μM), DTT (5 mM) and MsrA (1.0 μg/mL) were first mixed in Tris-HCl buffers (25 mM) of different pH values. Then the solutions were incubated at 37 °C for 1 h and fluorescence spectra were then recorded. The fluorescence intensities at 547 nm were plotted against different pH values. The effect of pH on the stability of MSP1 was also studied under the same conditions.

To investigate the effect of temperature on the response of MSP1 towards MsrA, MSP1(10 μM), DTT (5 mM) and MsrA (0.6 μg/mL) were mixed in Tris-HCl buffers (25 mM, pH 7.4) at different temperature for 1 h. Fluorescence spectra were then recorded. The fluorescence intensities at 547 nm were plotted against different temperature. The effect of temperature on the stability of MSP1 was also studied under the same conditions.

To calculate the Michaelis constant (K_m) and the maximum rate (V_{max}), MsrA (3.0 μg/mL) and DTT (5mM) were mixed with different concentrations of MSP1 (2, 3, 4, 5, 6, 7, 8, 9, 10 μM) in Tris-HCl buffers (25 mM, pH 7.4). The mixture was then incubated at 37 °C for 10 min and fluorescence spectra were then recorded. Data was analyzed with the Lineweaver Burk plot.

For selectivity assay, MSP1 (10 μM), DTT (5 mM) were first mixed with different biologically relevant species, including DTT (5 mM), TCEP (1 mM), GSH (1 mM), Cys (1 mM), Hcy (1 mM), glucose (1 mM), ascorbic acid (1 mM), BSA (1 mg/mL, 15 μM), NADPH (0.2 mM), NTR (9.6 μg/mL, 400 nM), NQO1 (10.0 μg/mL, 323 nM),

TrxR (10.5 μg/mL, 300 nM), MsrA (3.0 μg/mL, 104.2 nM) respectively in Tris-HCl buffers (25 mM, pH 7.4). Then, the mixture was incubated at 37 °C for 1 h and fluorescence spectra were obtained. The fluorescence intensity at 547 nm was plotted against different species.

HPLC and HR-ESI-MS analysis. The HPLC chromatograms of MSP1 (50 μM), rMSP1 (50 μM), and the reaction products of MsrA (5.0 μg/mL) and MSP1 (50 μM) at 37 °C for 1 h were performed on a system with a C18 column (150 nm × 4.6 mm, 5 μm) and the conditions were as follows: methanol/H_2O = 50/50 (v/v); flow rate: 0.8 mL/min; detection wavelength: 360 nm. For further demonstration of the reaction mechanism, HR-ESI-MS was introduced to analyze the products of MSP1 (10 μM) after reaction with MsrA (3.0 μg/mL) at 37 °C for 1 h in positive mode.

Cell culture. HeLa cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere incubator containing 5 wt %/vol CO_2. HEK-293T cells were cultured in DMEM, supplemented with 10% fetal calf serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin under the same conditions. PC12 cells were cultured in DMEM medium supplemented with 10% heat-inactivated horse serum, 5% heat inactivated fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin at 37 °C under the same conditions. The cell density was determined using a TC20™ automated cell counter (BIO-RAD, USA).

Cytotoxicity study. The cytotoxicity of MSP1 was evaluated by a CellTiter 96® Aqueous One Solution Cell Proliferation Assay kit. HeLa cells were seeded at 5×10^3 cells per well, while HEK-293T cells and PC12 cells were seeded at 1×10^4 cells per wells. All the cells were first cultured at 37 °C for 24 h. Then MSP1 ((0-500 μM) was added and incubated for another 24 h. Afterwards, the culture medium was removed and cells were washed with 1×PBS (200 μL). Then, 20 μL CellTiter Reagent diluted with 100 μL of growth medium was added and incubated at 37 °C for 2 h. The absorbance measurements at 490 nm was obtained on a Thermo Scientific Multiskan Microplate Reader (Thermo Fisher, USA).

Fluorescence imaging of living cells. Fluorescence imaging of living cells was performed as follows: HeLa cells were plated on a 35 mm Petri dish with a 10 mm bottom well for 24 h. The cells were then washed with PBS and incubated with 1 mL culture medium including 100 μM of MSP1 for 4 h at 37 °C. For the inhibition

experiment, HeLa cells were incubated with 1 mL of the culture medium including DMSO (1%) and 100 μM of MSP1 for 4 h at 37 °C. For colocalization assay, HeLa cells were first incubated with 100 μM of MSP1 in 1 mL of the culture medium for 4 h at 37 °C. Then Mito-Tracker Red (100 nM) or Lyso-Tracker Red (100 nM) were added and incubated for another 15 min. Fluorescence emission of MSP1 was collected at 505-605 nm with an excitation wavelength of 405 nm. Fluorescence emission of Mito-tracker red and Lyso-Tracker Red were collected at 593-620 nm under excitation at 560 nm. Fluorescence imaging of HEK-293T cells was performed using the same protocol.

For fluorescence imaging of Msrs activity in a cellular model of Parkinson's disease model, the cellular model was established according to literature procedures.[S3] PC12 cells were plated on a 35 mm Petri dish with a 10 mm bottom well in the culture medium for 24 h. For establishing a cellular PD model, PC12 cells were treated with 6-OHDA (35 μM and 70 μM) for 16 h and then incubated with 100 μM of MSP1 in 1 mL of the culture medium for 4 h at 37°C. Fluorescence emission was collected at 505-605 nm under excitation at 405 nm. For colocalization assay, PC12 cells were treated with 6-OHDA (35 μM and 70 μM) for 16 h and then incubated with 100 μM of MSP1 in 1 mL of the culture medium for 4 h at 37°C, followed by Mito-Tracker Red (100 nM) for 15 min. Fluorescence emission of MSP1 was collected at 505-605 nm with an excitation wavelength of 405 nm while that for Mito-tracker red was collected at 593-620 nm under excitation at 560 nm.

Determining Msrs activity in cell lysates. For cell lysate extraction, cells were plated on a 25 cm^2 culture flask in the culture medium for 24 h. Approximately 3.0×10^6 cells were collected and centrifuged at 2000 r/min for 3 min at room temperature. The supernatant was carefully removed and the pellet was washed with cold PBS by centrifugation. Then, 200 μL of ice-cold lysis buffer (50 mM Tris-HCl, 100 mM NaCl, 2.5 mM EDTA, 2.5 mM EGTA, 1% NP-40, 5% glycerol) buffer was added. The pellet was gently resuspended in lysis buffer and incubated on ice for 30 min, which was kept at -20 °C for further use.

For determining the Msrs activity in HeLa cells, MSP1 (10 μM) was mixed with DTT (5 mM) and 40 μL cell lysates (1.2×10^6 cells) in Tris-HCl buffers (25 mM, pH 7.4) in a test tube and the solutions were incubated at 37 °C for 1 h. Fluorescence spectra were recorded in the range from 460 to 705 nm with an excitation wavelength of 385 nm, using slit widths of 5 nm for both excitation and emission.

For determining Msrs activity in lysates of cellular PD model, PC12 cells were plated on a 25 cm^2 culture flask for 24 h. PC12 cells treated with 6-OHDA (35 μM and 70 μM) for 16 h were collected and lysed with ice-cold lysis buffer. Then the cell lysates (40 μL, 1.2×10^6 cells) were incubated with MSP1 (10 μM) and DTT (5mM) in Tris-HCl buffers (25 mM, pH 7.4) at 37 °C for 1 h. Fluorescence spectra were recorded in the range from 475 to 650 nm with an excitation wavelength of 385 nm, using slit widths of 5 nm for both excitation and emission. The protein levels of MsrA in the cell lysates of cellular PD model were determined by Western blot.[S4]

References

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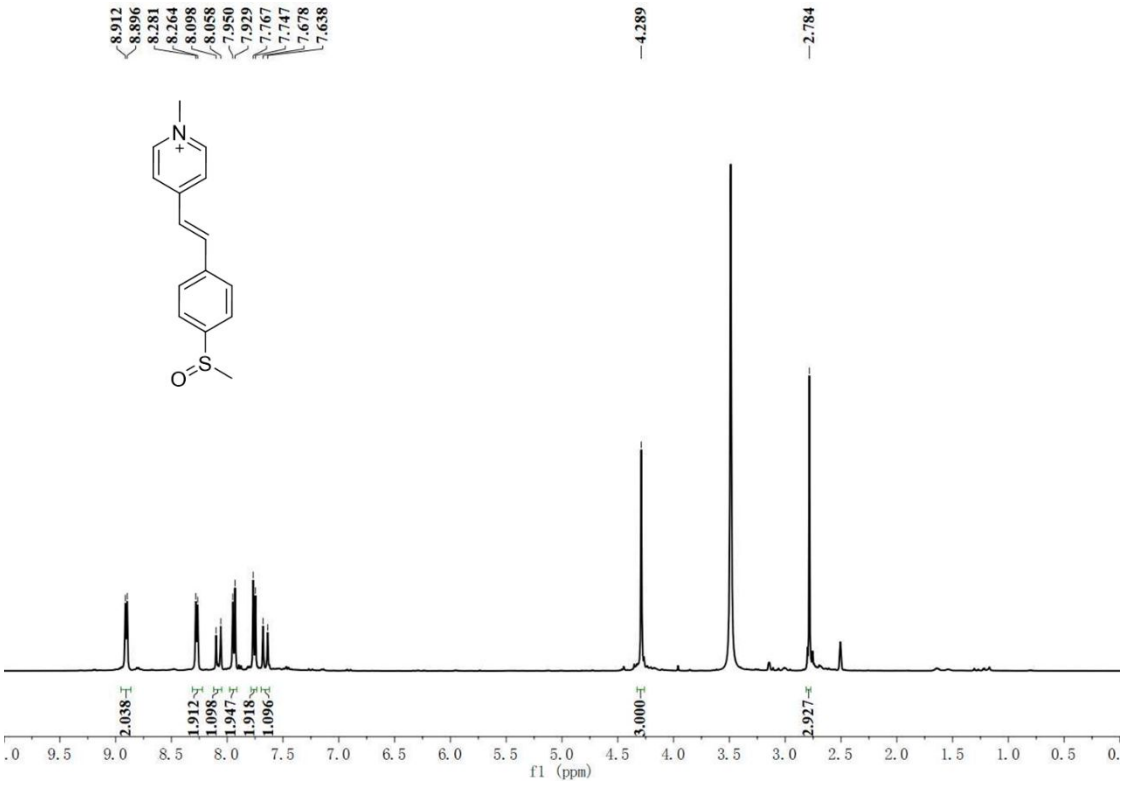

Figure S1. ^{1}H NMR spectrum of MSP1 in DMSO-d_6.

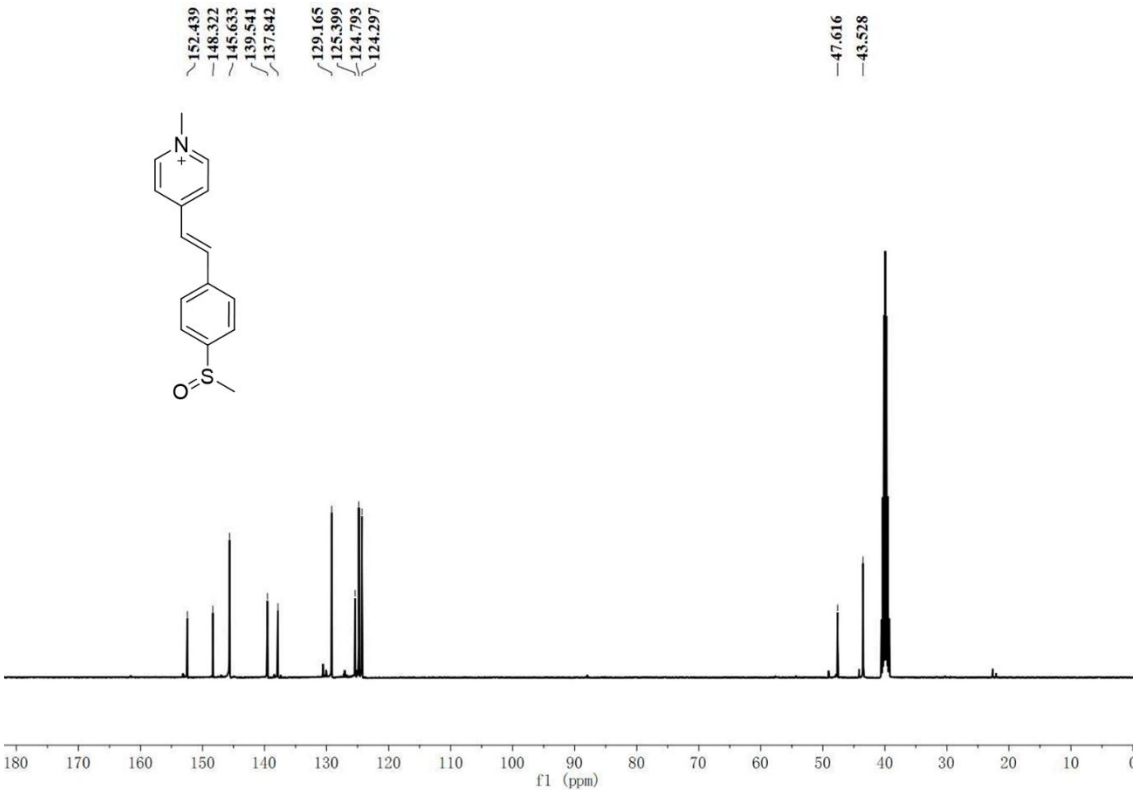

Figure S2. ^{13}C NMR spectrum of MSP1 in DMSO-d_6.

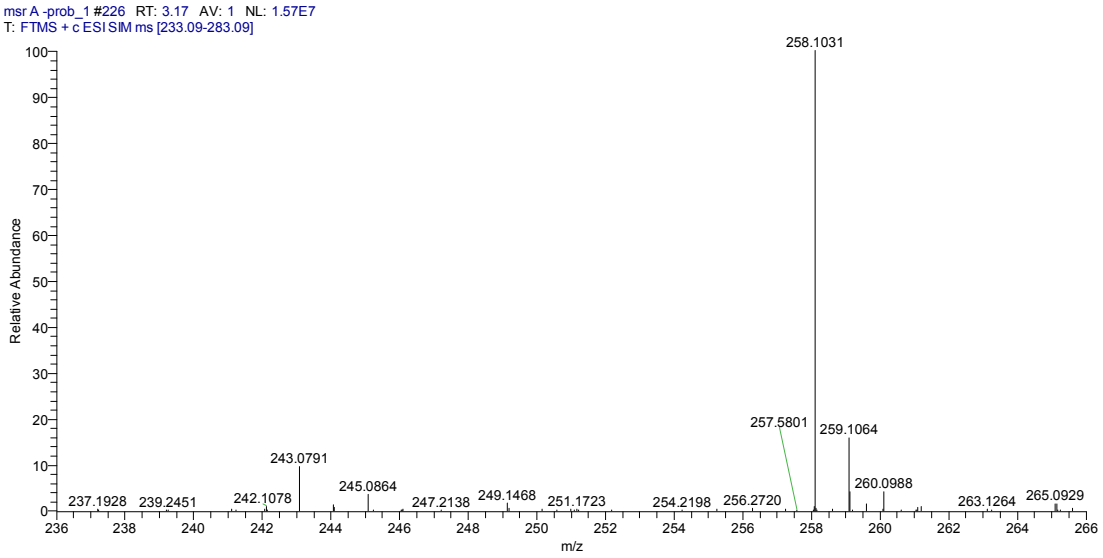

Figure S3. HR-ESI-MS spectrum of MSP1.

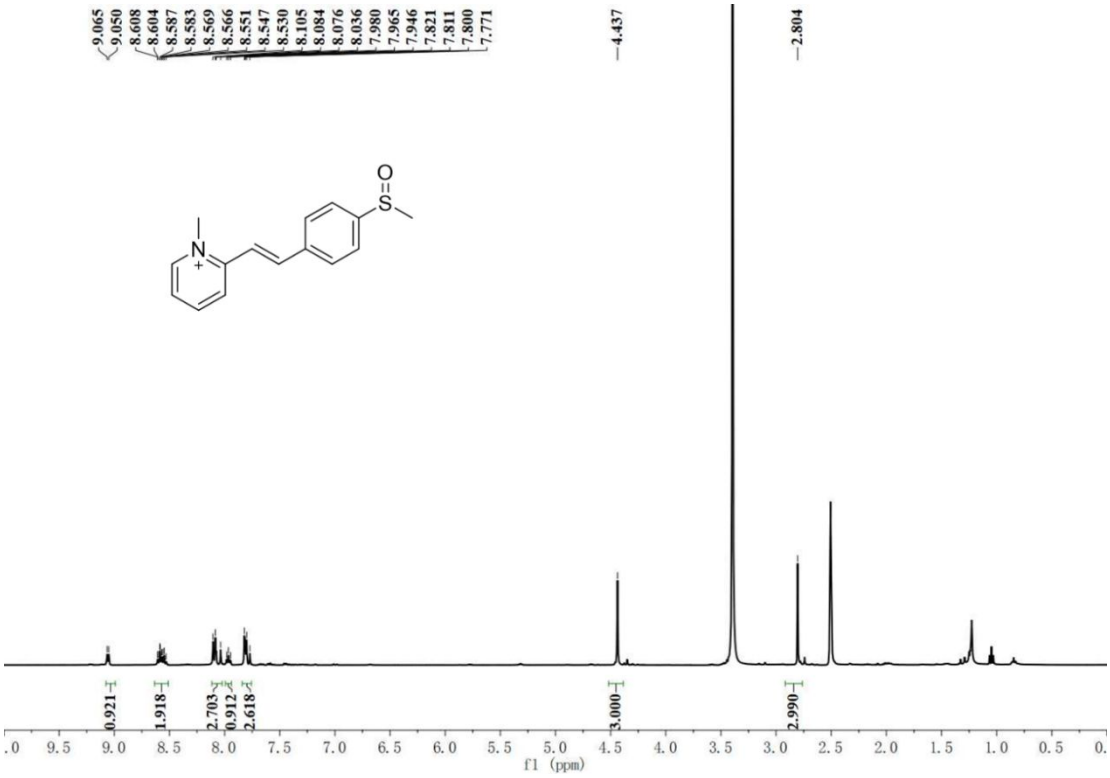

Figure S4. ^{1}H NMR spectrum of MSP2 in DMSO-*d*.

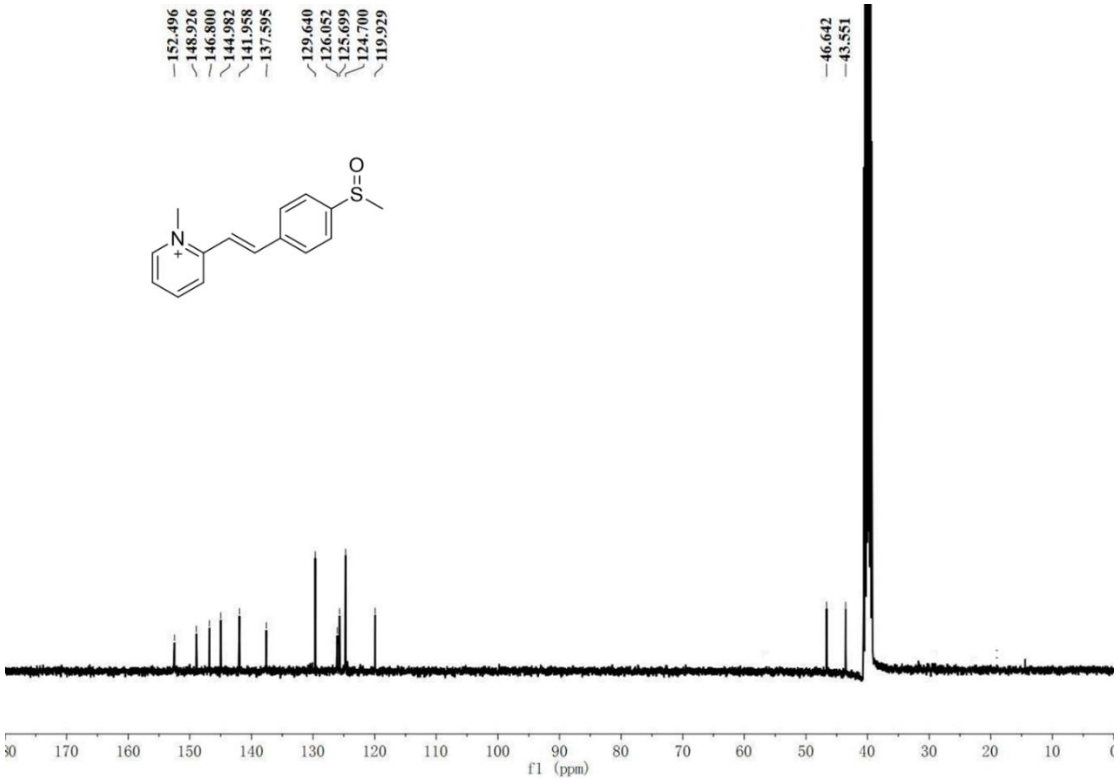

Figure S5. ^{13}C NMR spectrum of MSP2 in DMSO-d_6

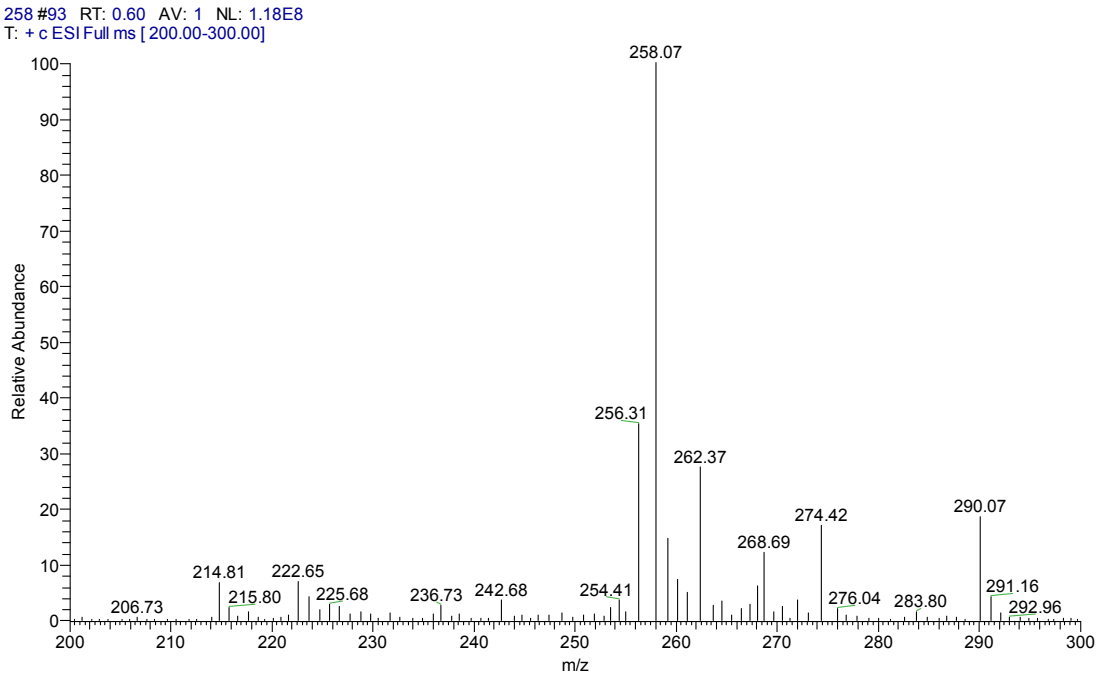

Figure S6. ESI-MS spectrum of MSP2.

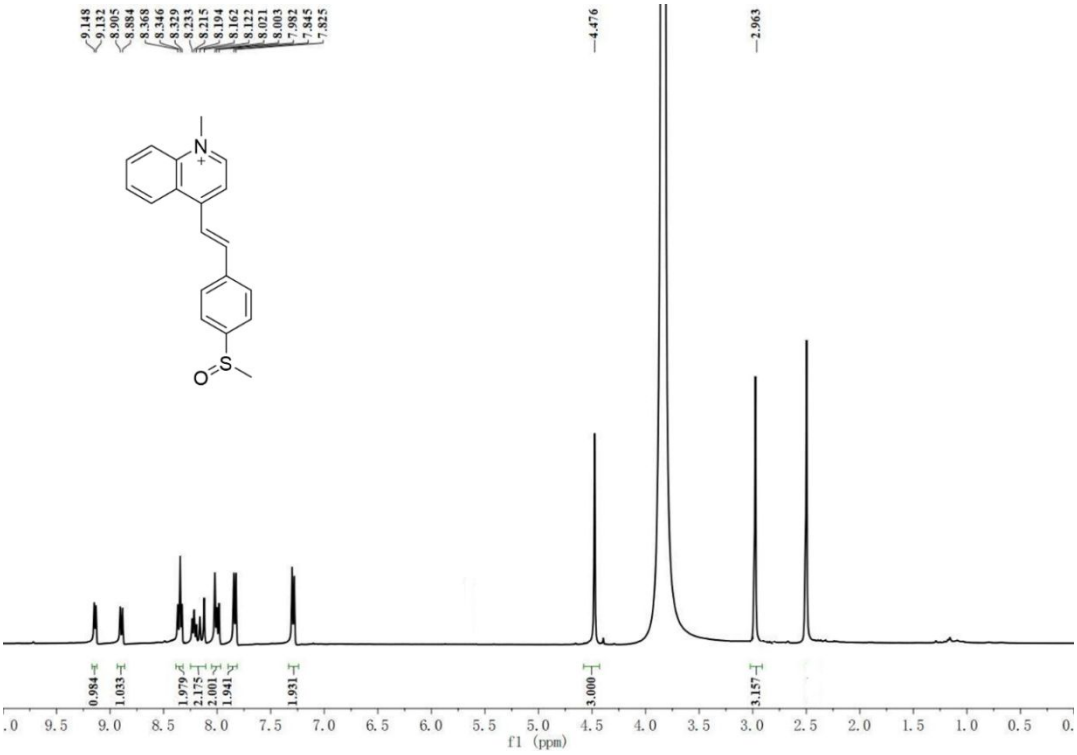

Figure S7. ^{1}H NMR spectrum of MSQ1 in DMSO-d_6.

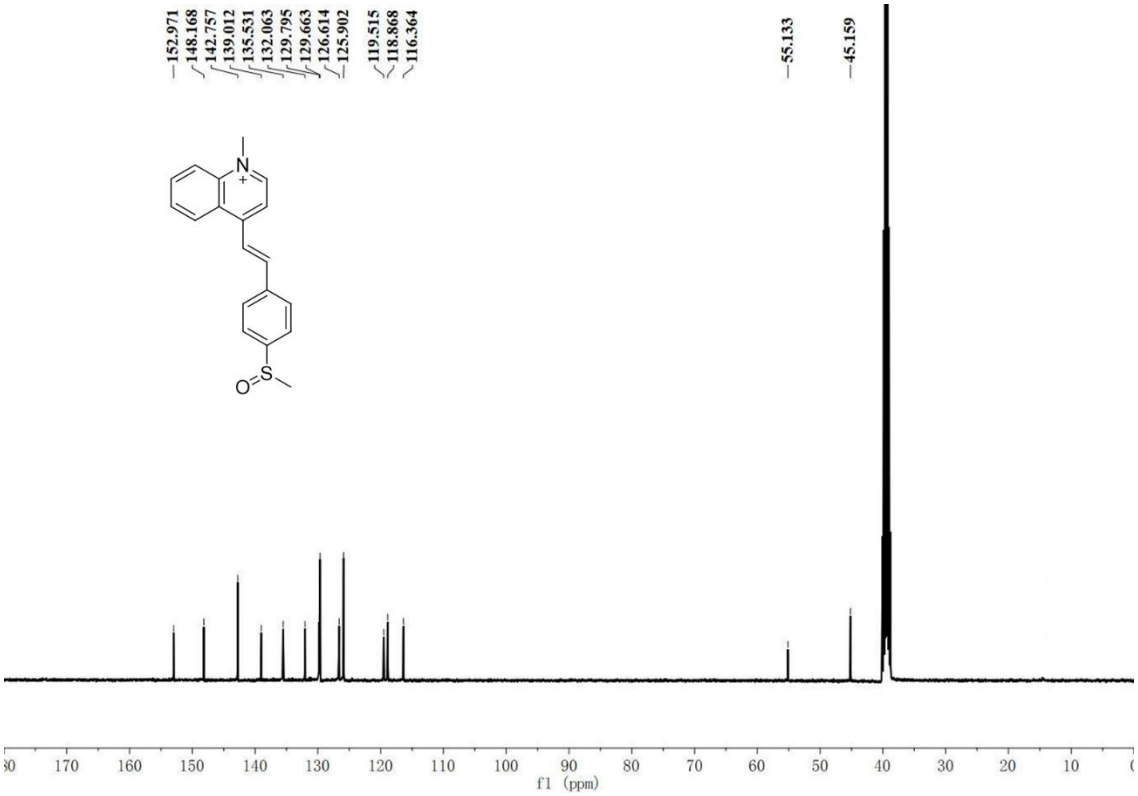

Figure S8. ^{13}C NMR spectrum of MSQ1 in DMSO-d_6.

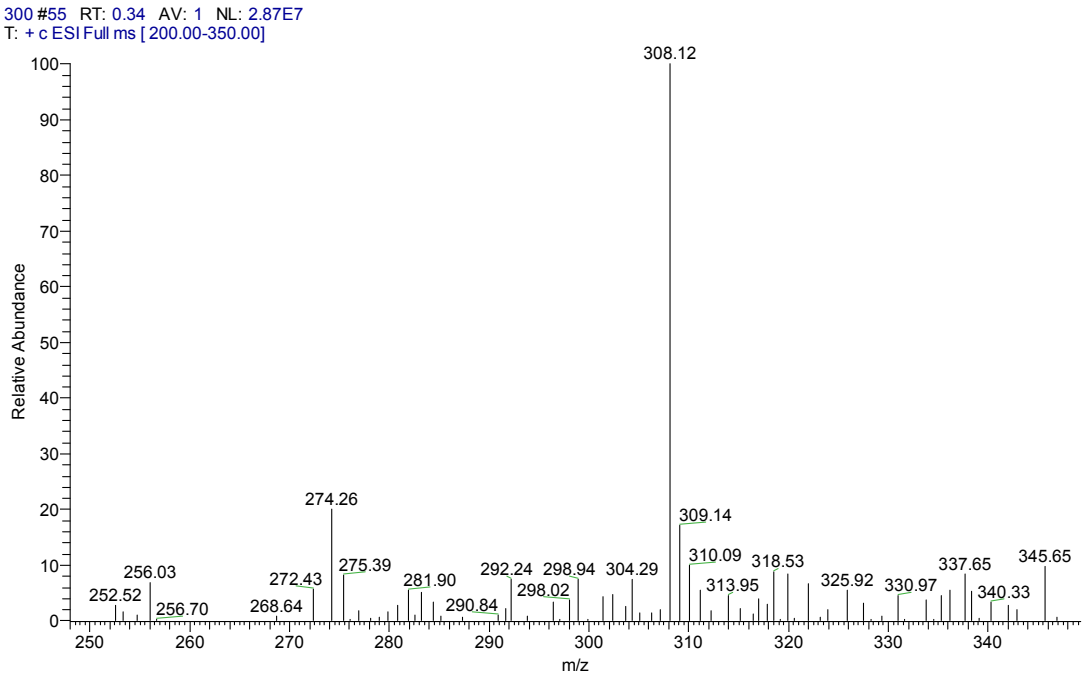

Figure S9. ESI-MS spectrum of MSQ1.

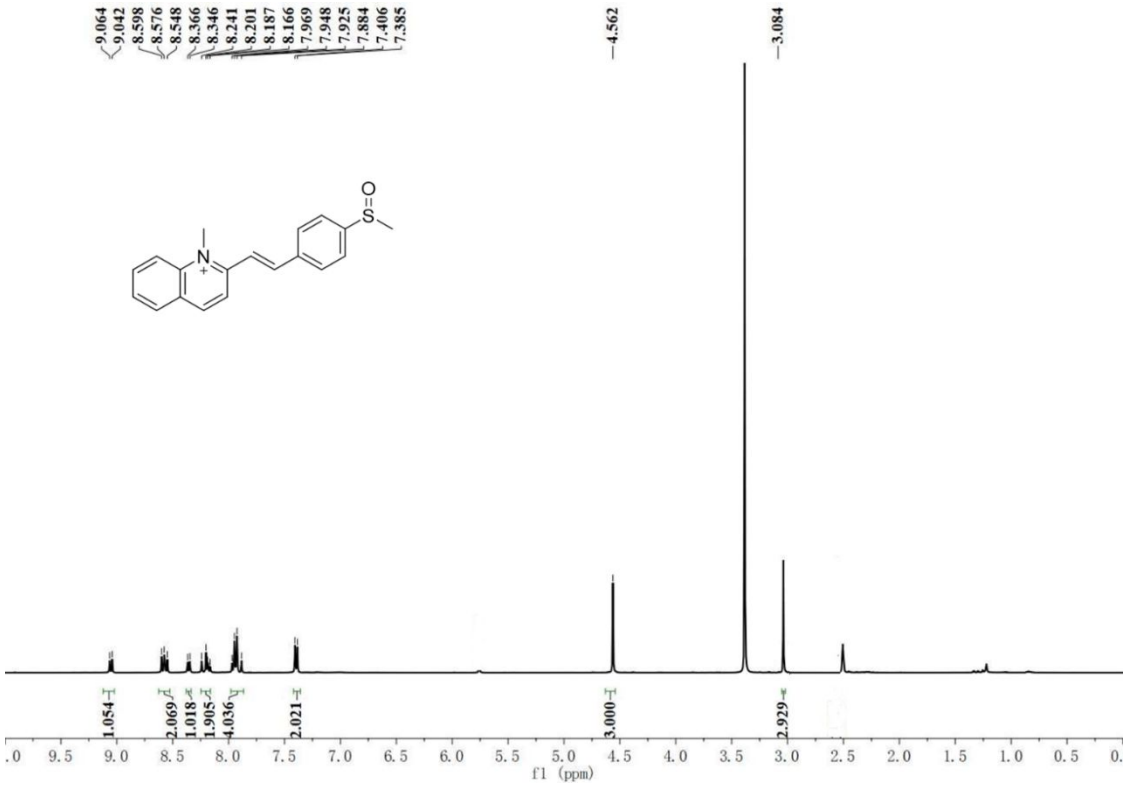

Figure S10. ^{1}H NMR spectrum of MSQ2 in DMSO-d_6.

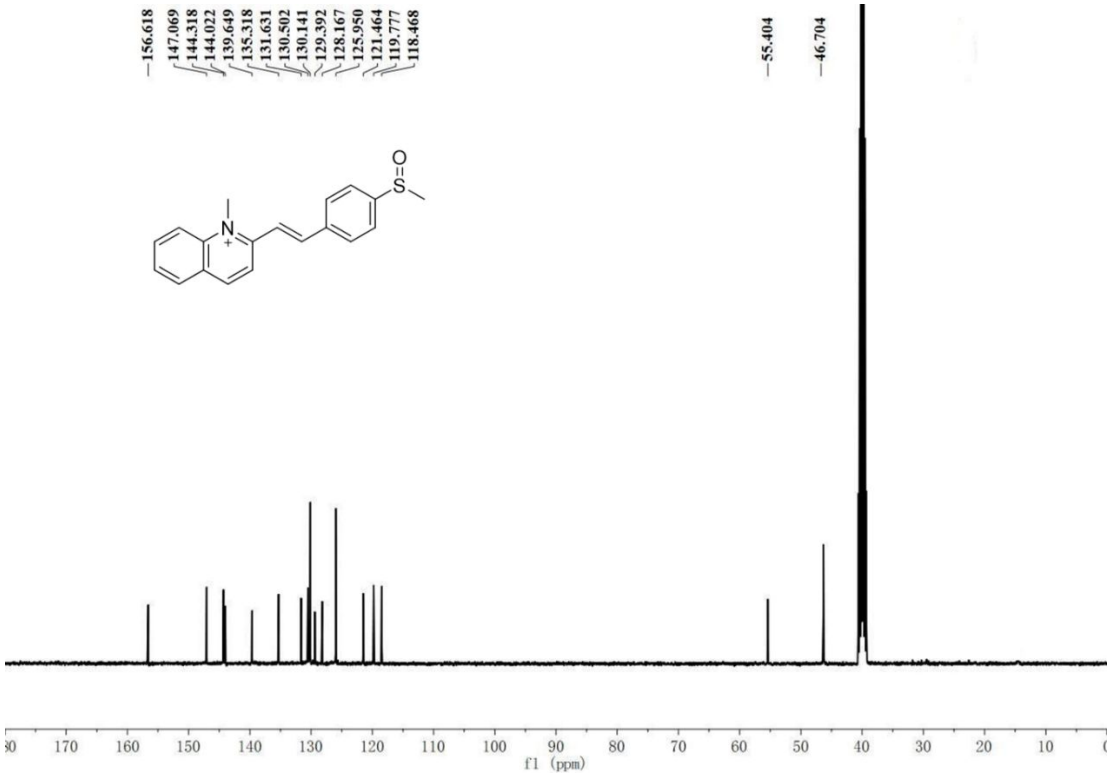

Figure S11. ^{13}C NMR spectrum of MSQ2 in DMSO-d_6.

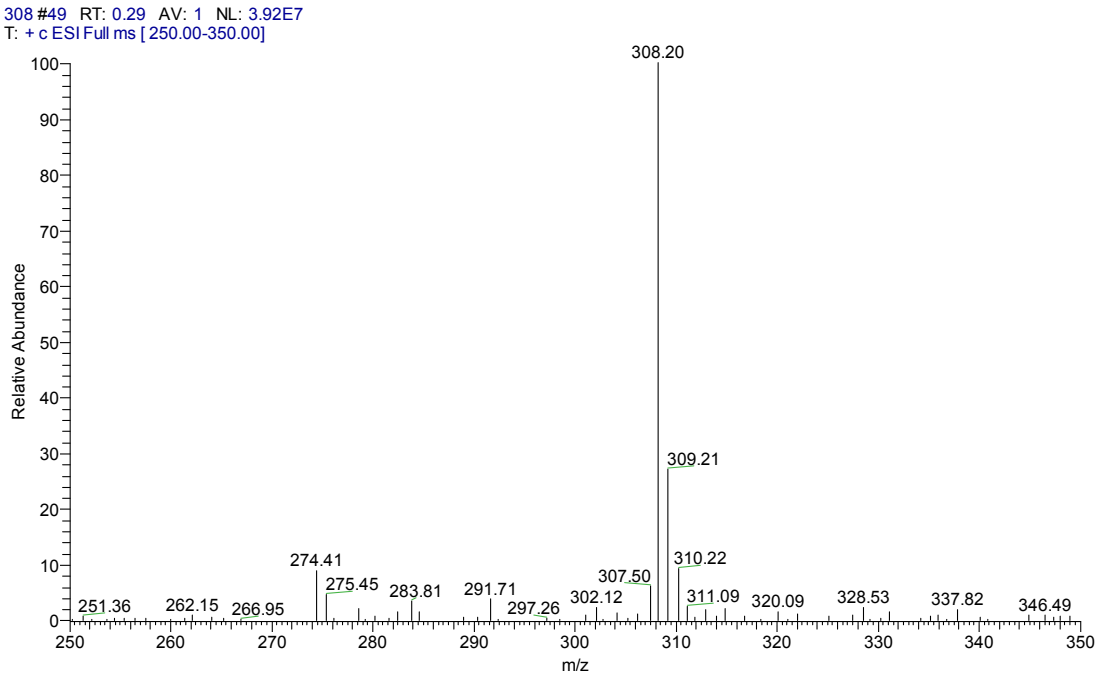

Figure S12. ESI-MS spectrum of MSQ2.

Table S1. *In vitro* responses of the probes.

Probes	λex/λem	DTT (F/F_0)	DTT+MsrA(F/F_0)
MSP1	385/547	1.11	38.45
MSP2	385/547	1.00	1.48
MSQ1	435/593	1.00	4.47
MSQ2	420/566	1.08	1.86

The assays were performed by incubating probes (10 μM) with DTT (5 mM) or DTT (5 mM) and MsrA (3.0 μg/mL) in Tris-HCl buffer (25 mM, pH 7.4) for 1 h at 37 ^{o}C. Fluorescence enhancement was expressed as F/F_0.

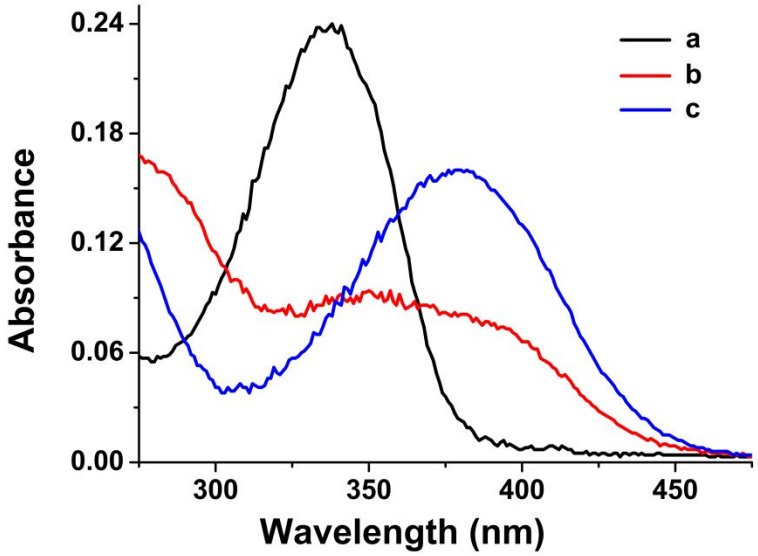

Figure S13. UV-*vis* absorption spectra of (a) MSP1 (10 μM), (b) MSP1 (10 μM) reacted with MsrA (5.0 μg/mL) and (c) rMSP1 (10 μM) in Tris-HCl buffer (25 mM, pH 7.4) for 1 h at 37 ^{o}C.

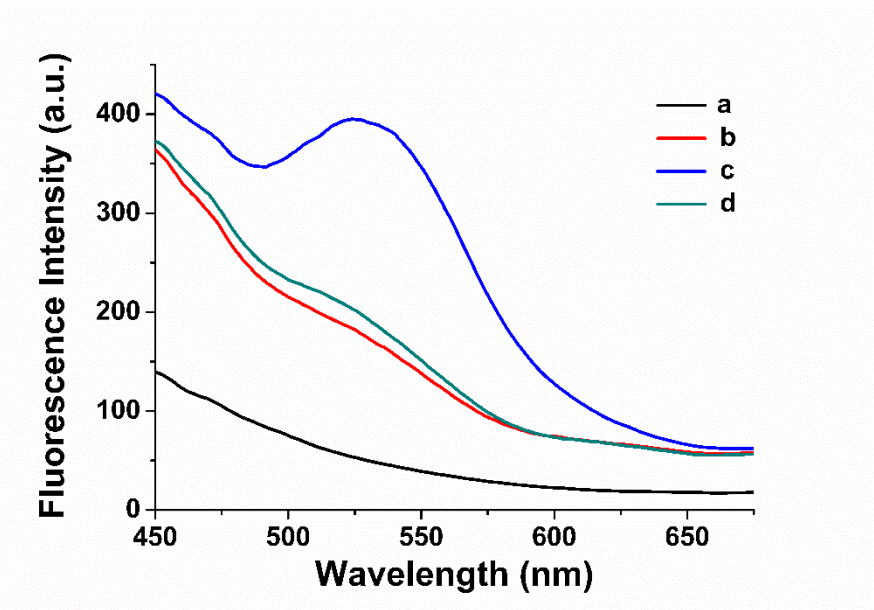

Figure S14. Fluorescence spectra of (a) MSP1 (10 μM) and DTT (5 mM), (b) HeLa cell lysates, (c) MSP1 (10 μM) incubated with HeLa cell lysates (40 μL), (d) MSP1 (10 μM) incubated with HeLa cell lysates (40 μL) and DMSO (1%) in Tris-HCl buffer (25 mM, pH 7.4) for 1 h at 37 °C.

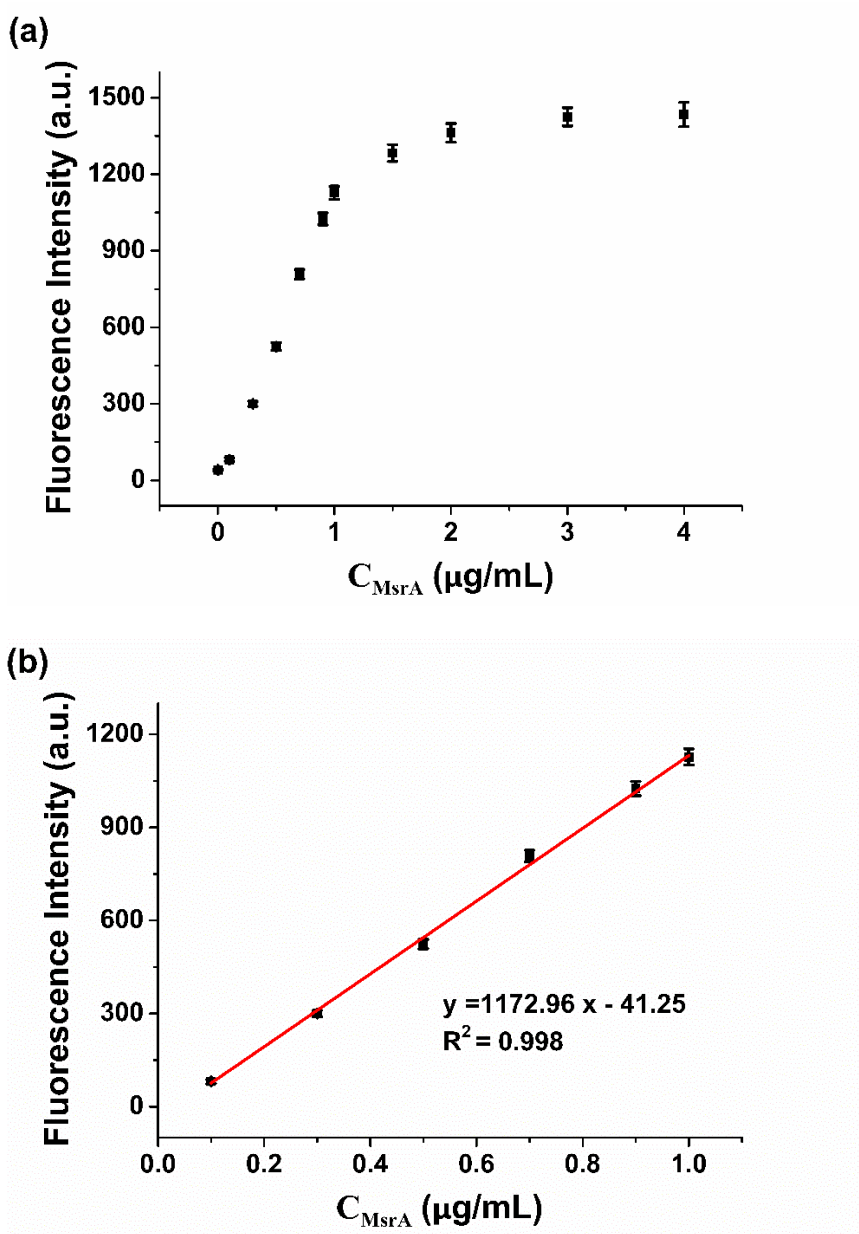

Figure S15. (a) Calibration curve of MSP1 (10 μM) in response to different concentrations of MsrA in Tris-HCl buffer (25 mM, pH 7.4) for 1 h at 37 °C. (b) Linear fitting of fluorescence intensity (F) toward the concentration (C) of MsrA from 0.1 to 1.0 μg/mL. λex/em = 385/547 nm.

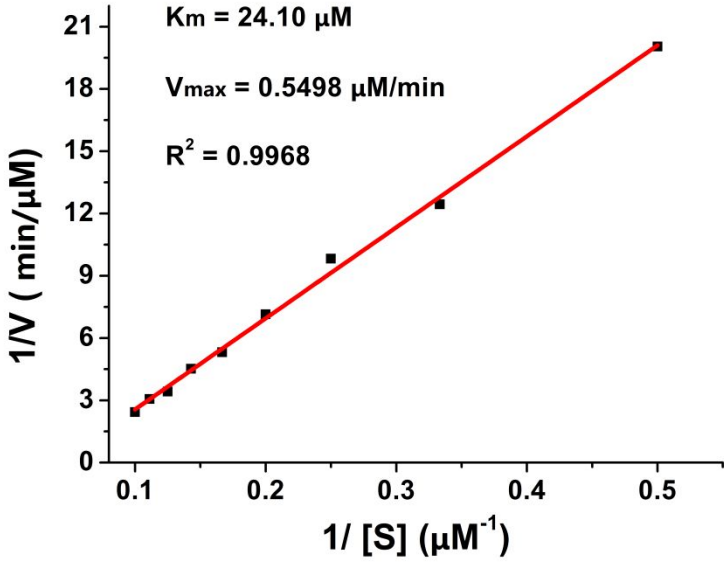

Figure S16. Lineweaver-Burk plot for the MsrA-catalyzed reaction. The Michaelis-Menten equation was described as: $V = V_{max}$ [probe]/ (K_m + [probe]), where V is the initial reaction rate, [probe] is the probe concentration (substrate), and the K_m is the Michaelis constant. Conditions: MsrA (3.0 μg/mL), 2-20 μM of MSP1 in Tris-HCl buffer (25 mM, pH 7.4) at 37 °C for 1 h. The measurements were performed at 37 °C with λex/em = 385/547nm.

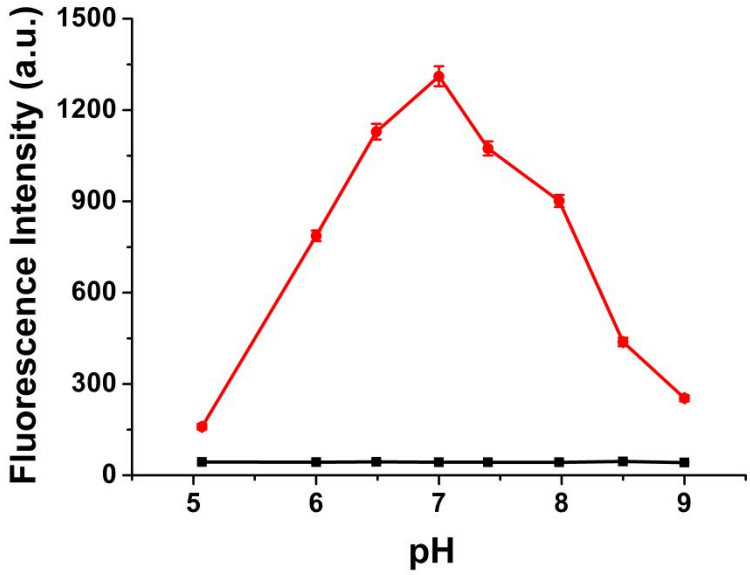

Figure S17. The effects of pH on the catalytic activity of MsrA. (■) MSP1 (10 μM). (●) MSP1 (10 μM) reacted with 1.0 μg/mL MsrA in Tris-HCl buffer (25 mM, pH 7.4) for 1 h at 37 °C. λex/em=385/547nm.

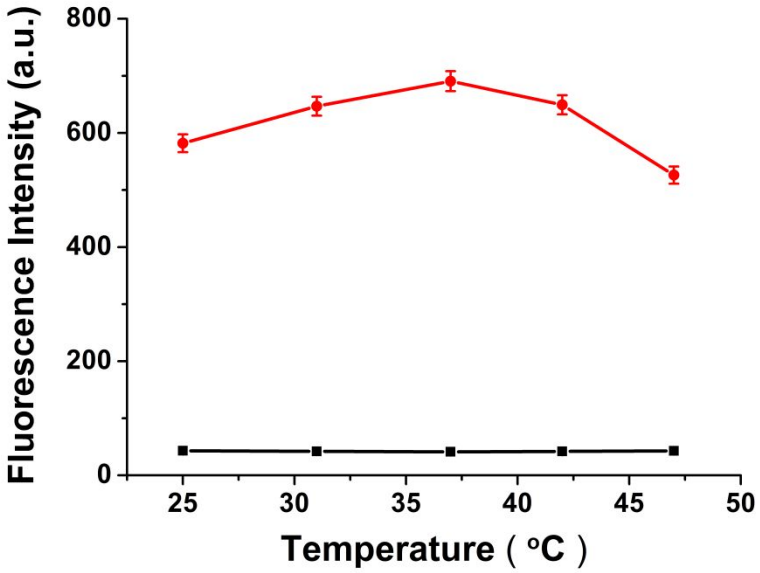

Figure S18. The effects of temperature on the catalytic activity of MsrA. (■) MSP1 (10 μM). (●) MSP1 (10 μM) reacted with and MsrA (0.6 μg/mL) in Tris-HCl buffer (25 mM, pH 7.4) for 1 h. λex/em = 385/547nm.

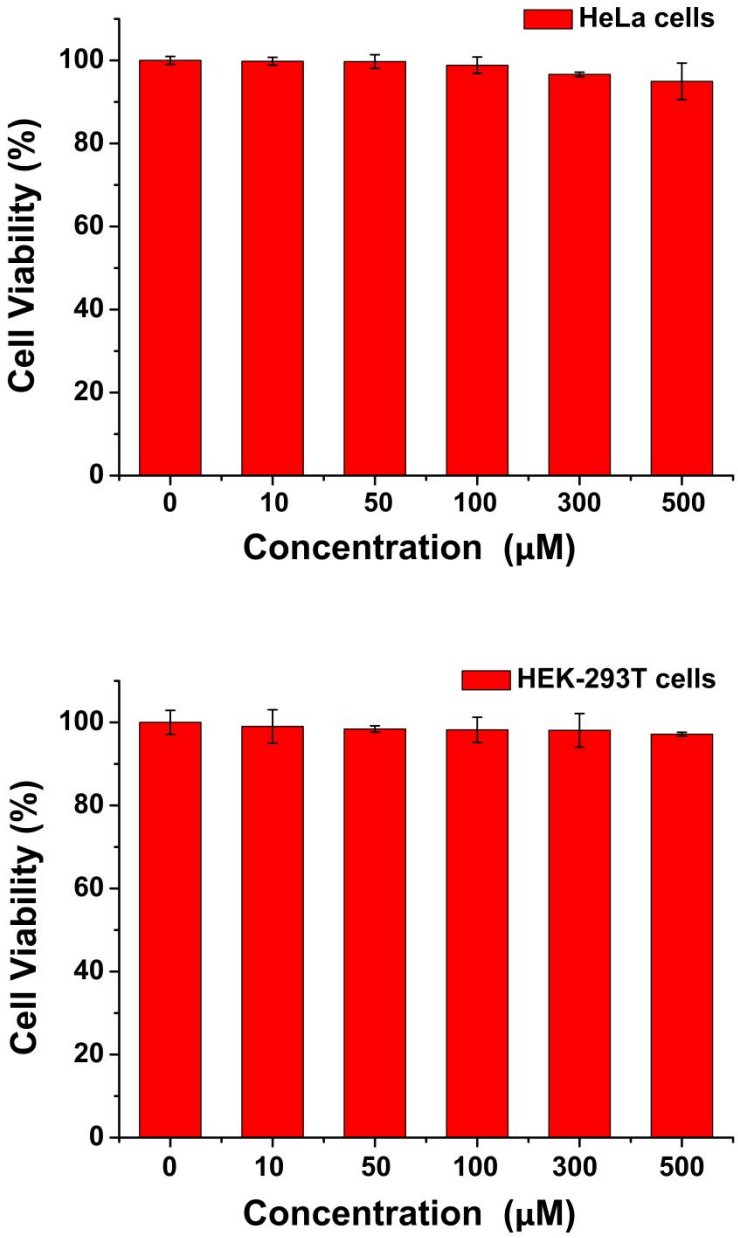

Figure S19. The effect of MSP1 (0-500 μM) on the viability of HeLa cells and HEK-293T cells. The viability of the cells without MSP1 is defined as 100%. The results are the means ± SD of three experiments.

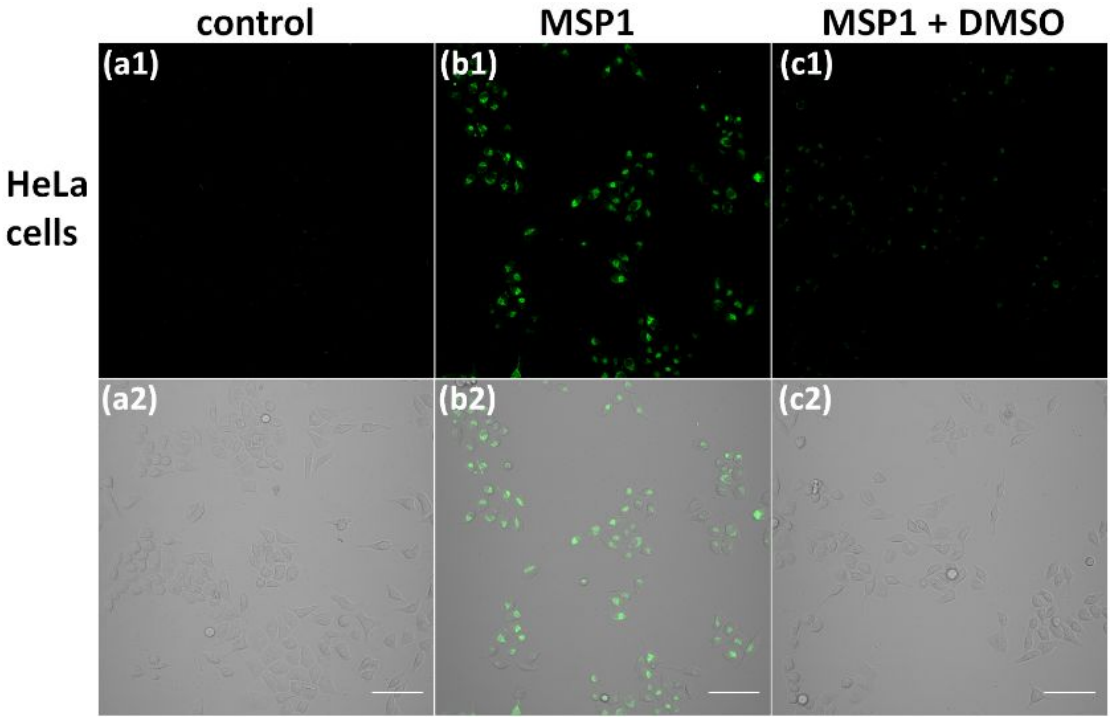

Figure S20. Fluorescence and differential interference contrast (DIC) merged images for HeLa Cells. (a1-a2) Only Cells. (b1-b2) Cells incubated with MSP1 (100 μM) for 4 h. (c1-c2) Cells sequentially treated with 1% DMSO for 1 h and then MSP1 (100 μM) for 4 h. Scale bar = 100 μm.

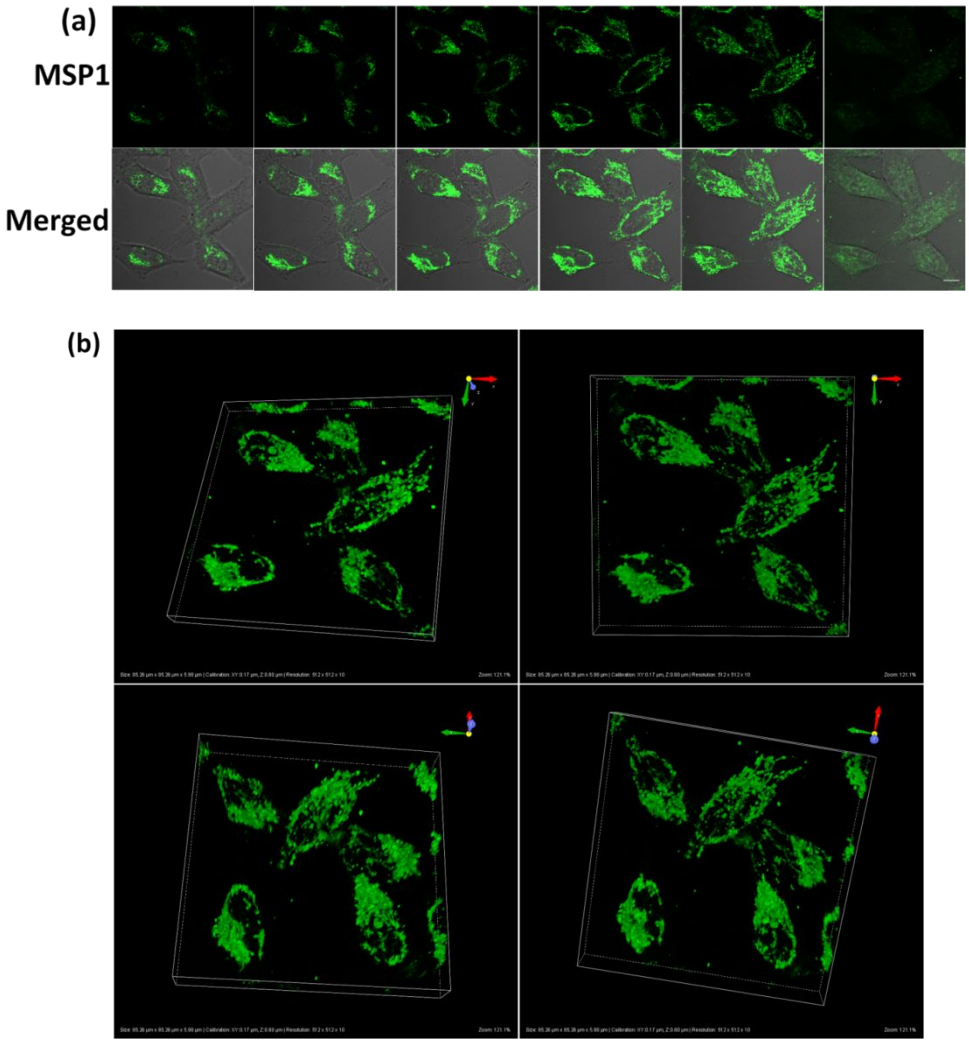

Figure S21. 3D confocal imaging of HeLa cells with 100 μM MSP1. (a) Cellular imaging at different sections through the z-axis were acquired with 0.9-μm intervals, Scale bar = 10 μm; (b) Projection mapping on the x-y plane of data acquired at 0.897-μm z-axis intervals. Width: 85.26 μm, height: 85.26 μm, depth: 5.38 μm.

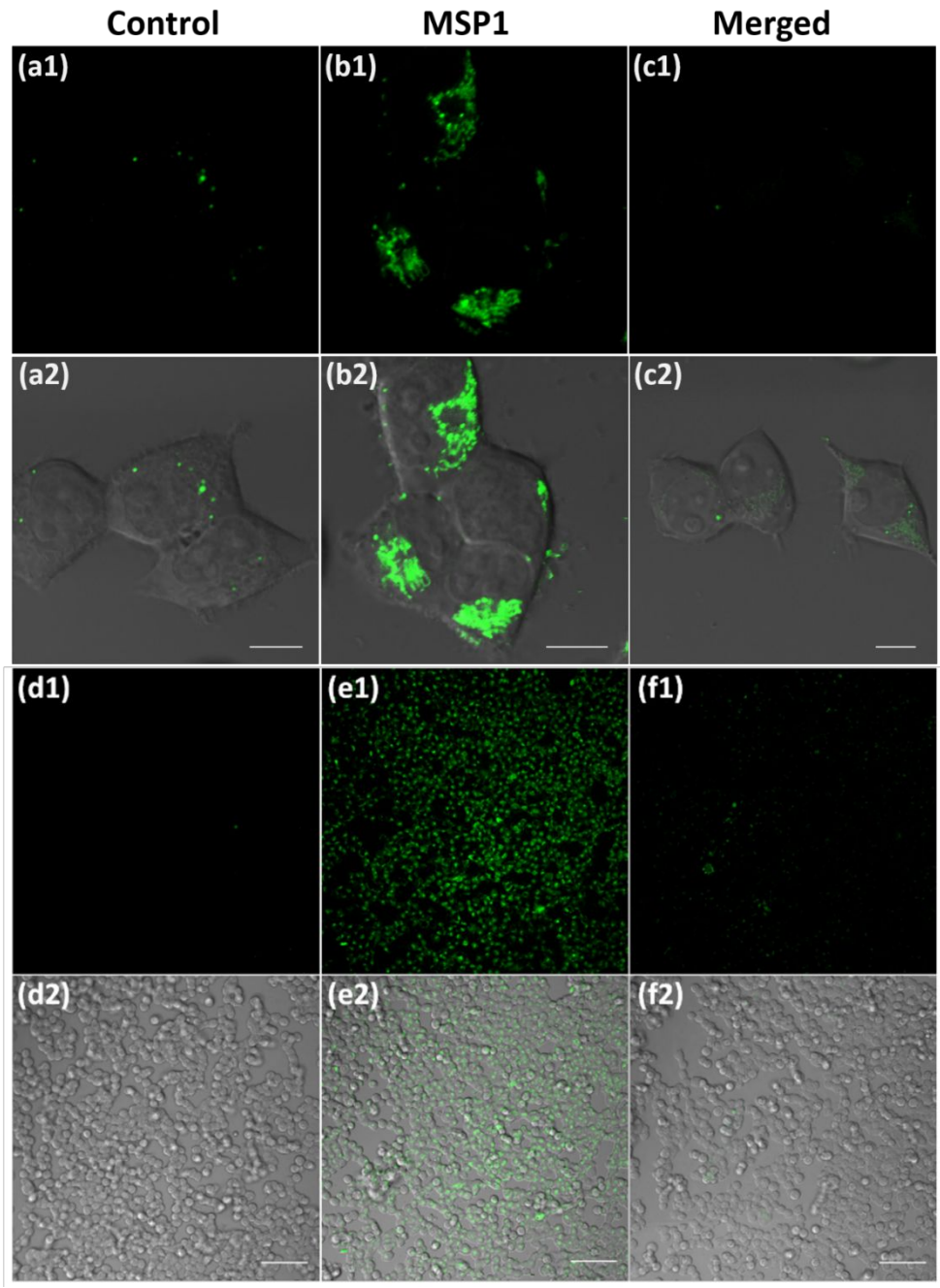

Figure S22. Fluorescence and DIC overlay images of HEK-293T cells. (a1, a2, d1, d2) Only cells. (b1, b2, e1, e2) Cells incubated with MSP1 (100 μM) for 4 h; (c1, c2, f1, f2) Cells treated with 1% DMSO for 1 h and then incubated with MSP1 (100 μM) for 4 h. a-c, Scale bar = 10 μm. d-f, Scale bar = 100 μm.

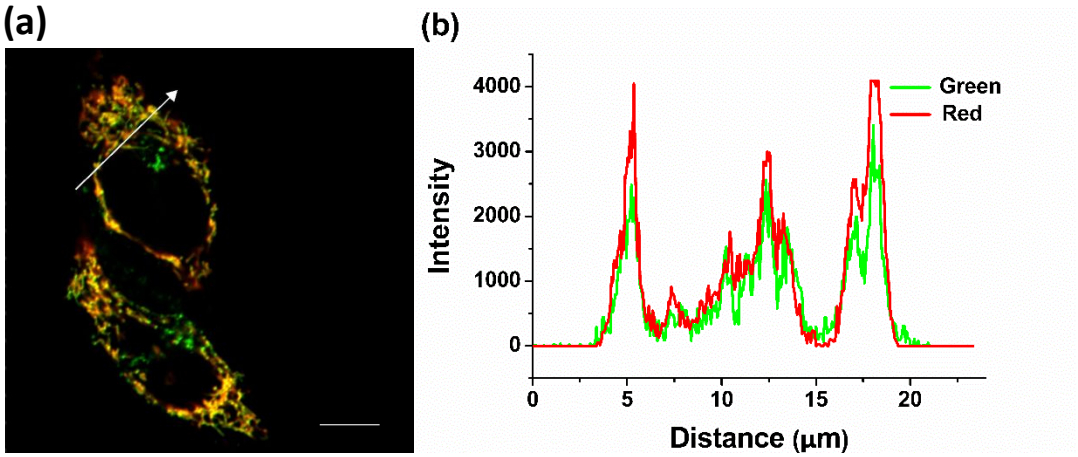

Figure S23. Fluorescence colocalization assay for HeLa cells. (a) Fluorescence overlay images of MSP1 (100 μM) and MitoTracker Red (100 nM) in Figure 3c in the manuscript. Scale bar=10 μm; (b) Fluorescence intensity variations of the white line across the cell, MSP1 (green), MitoTracker Red (Red).

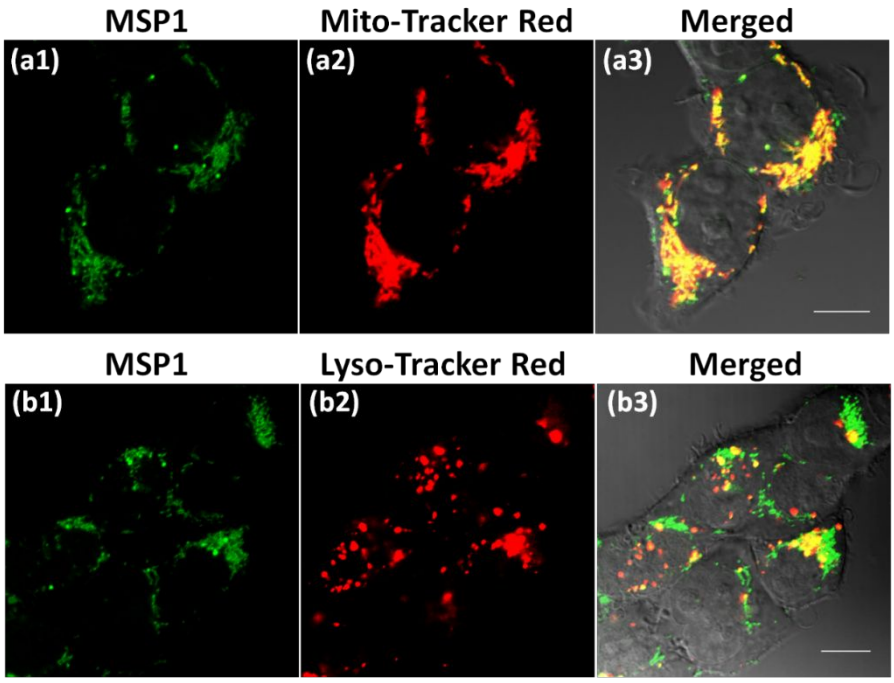

Figure S24. Fluorescence imaging of cellular localization of MSP1 in HEK-293T cells followed MitoTracker and LysoTracker staning. HEK293 cells were first treated with MSP1 (100 μM) for 4 h (a1, b1), and then incubated with 100 nM Mito-Tracker Red (a2) or 100 nM Lyso-Tracker Red (b2) for 15 min. Scale bar = 10 μm.

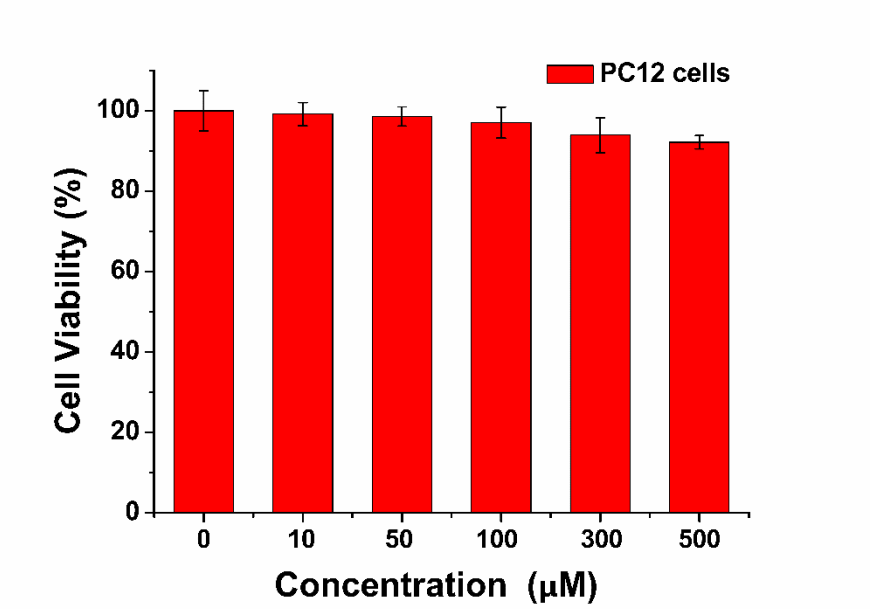

Figure S25. Effect of MSP1 (0-500 μM) on the viability of PC12 cells. The viability of the cells without MSP1 is defined as 100%. The results are the means ± SD of three experiments.

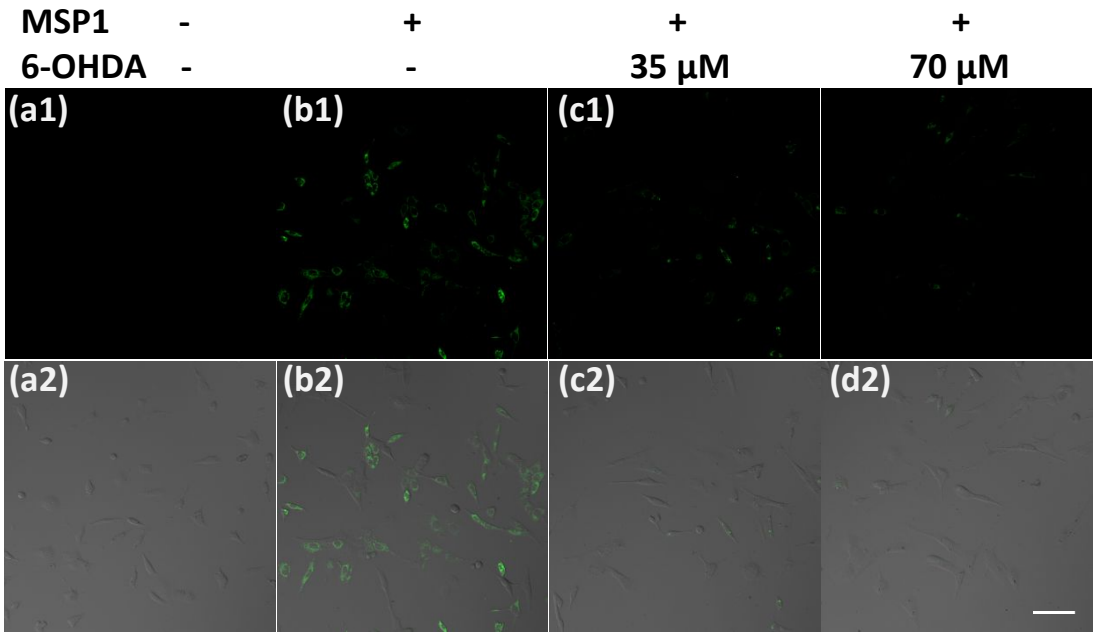

Figure S26. Fluorescence and DIC overlay images of PC12 cells. (a1, a2) Only cells. (b1, b2) Cells treated with MSP1 (100 μM) for 4h. (c1, c2) Cells treated with 6-OHDA (35 μM) for 16 h and then incubated with MSP1 (100 μM) for 4 h. (d1, d2) Cells treated with 6-OHDA (70 μM) for 16 h and then incubated with MSP1 (100 μM) for 4 h. Scale bar = 100 μm.

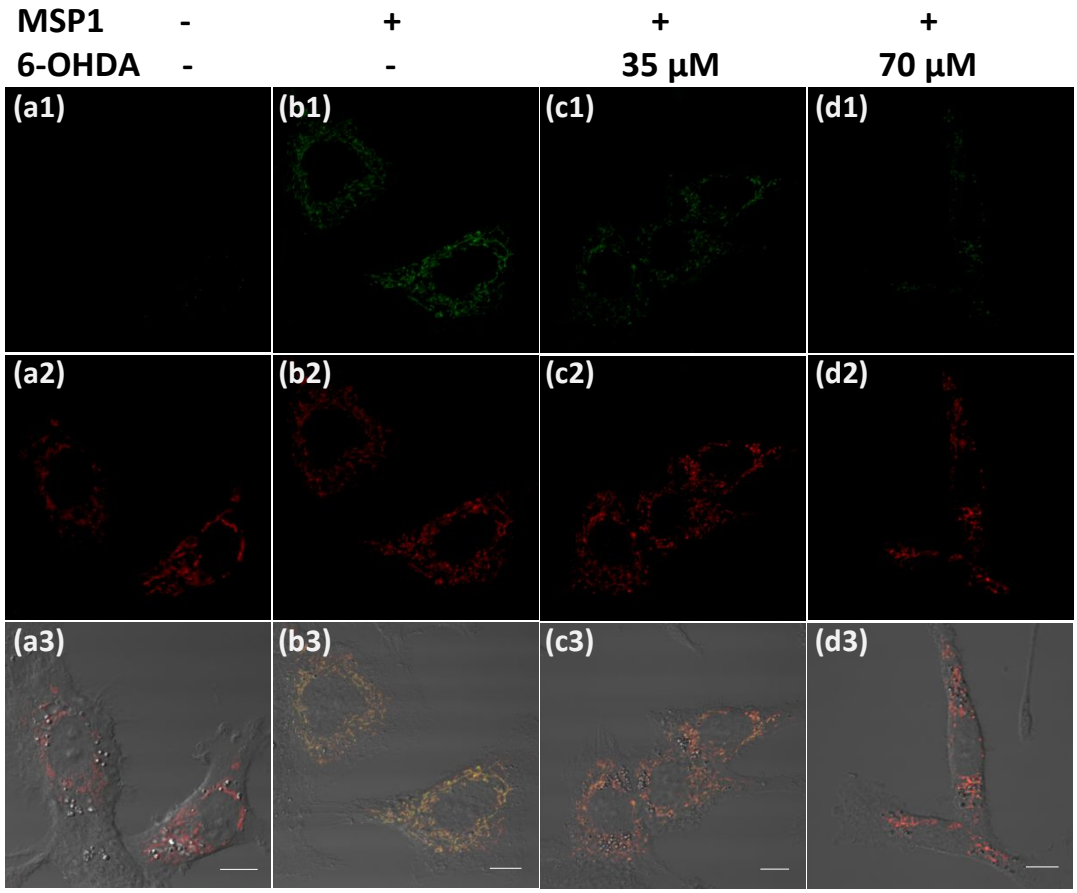

Figure S27. Fluorescence images of MSP1 in PC12 cells. (a1) Cell treated with Mito-Tracker Red for 15 min. (b1) Cells incubated with 100 μM MSP1 for 4 h and then Mito-Tracker Red for 15 min. (c1) Cells treated with 35 μM 6-OHDA for 16 h, then incubated with 100 μM MSP1 for 4 h and Mito-Tracker Red for 15 min. (d1) Cells treated with 70 μM 6-OHDA for 16 h, then incubated with 100 μM MSP1 for 4 h and Mito-Tracker Red for 15 min. (a1-d1) Fluorescence emission from MSP1. (a2-d2) Fluorescence emission from Mito-Tracker Red. (a3-d3) Merged images. Scale bar = 10 μm.

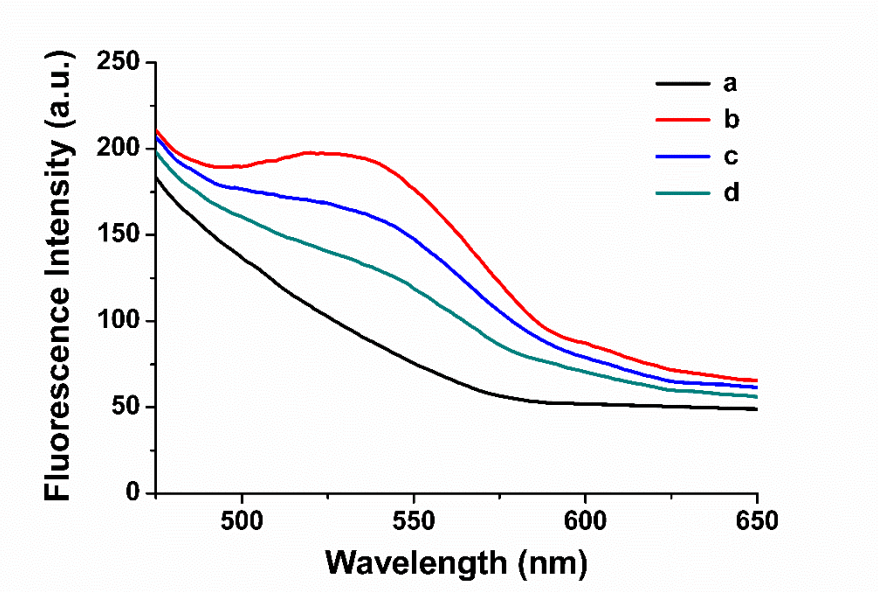

Figure S28. Fluorescence responses of PC12 cell lysates under different conditions. (a) PC12 cell lysates alone (40 μL). (b) PC12 cell lysates (40 μL) incubated with MSP1 (10 μM) in Tris-HCl buffer (25 mM, pH 7.4) for 1 h at 37 °C. (c) Cell lysates (40 μL) of PC12 cells treated with 35 μM 6-OHDA for 16 h then incubated with 10 μM MSP1 in Tris-HCl buffer (25 mM, pH 7.4) for 1 h at 37 °C. (d) Cell lysates (40 μL) of PC12 cells treated with 70 μM 6-OHDA for 16 h then incubated with 10 μM MSP1 in Tris-HCl buffer (25 mM, pH 7.4) for 1 h at 37 °C.

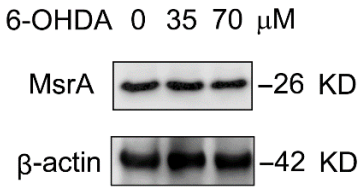

Figure S29. Determination of MsrA protein level in the PC12 cell lysates treated with or without 6-OHDA by Western blots.

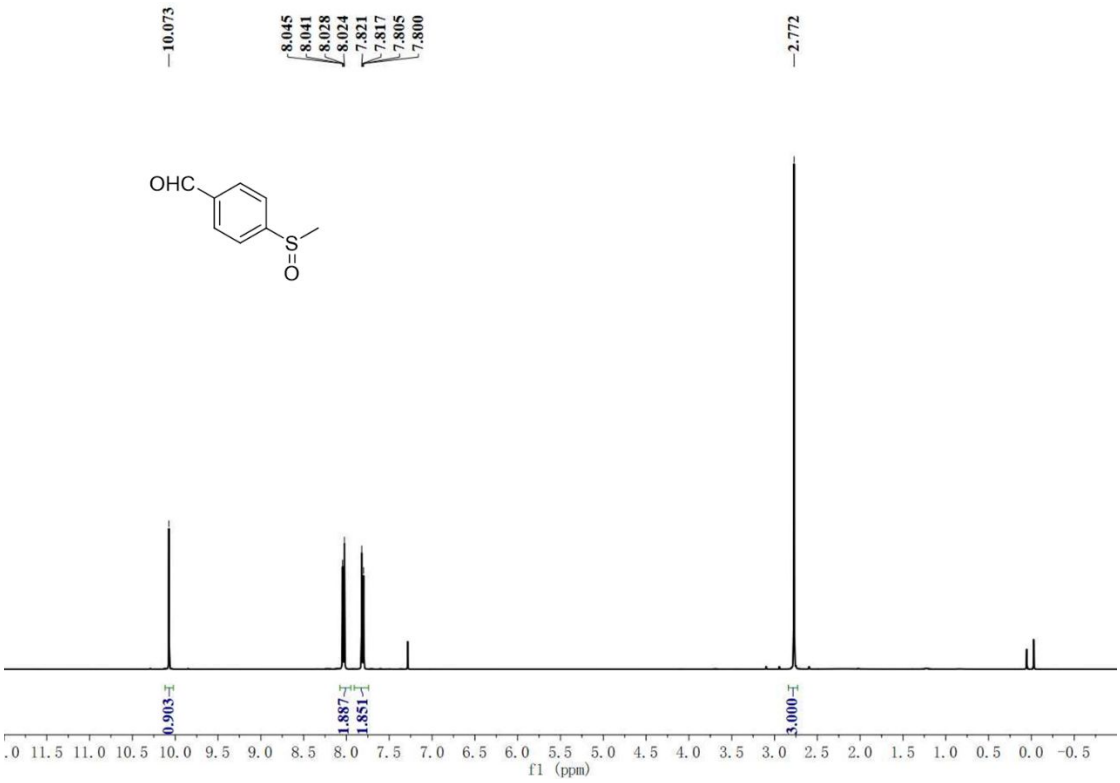

Figure S30. ^{1}H NMR spectrum of Compound 1 in $CDCl_3$.

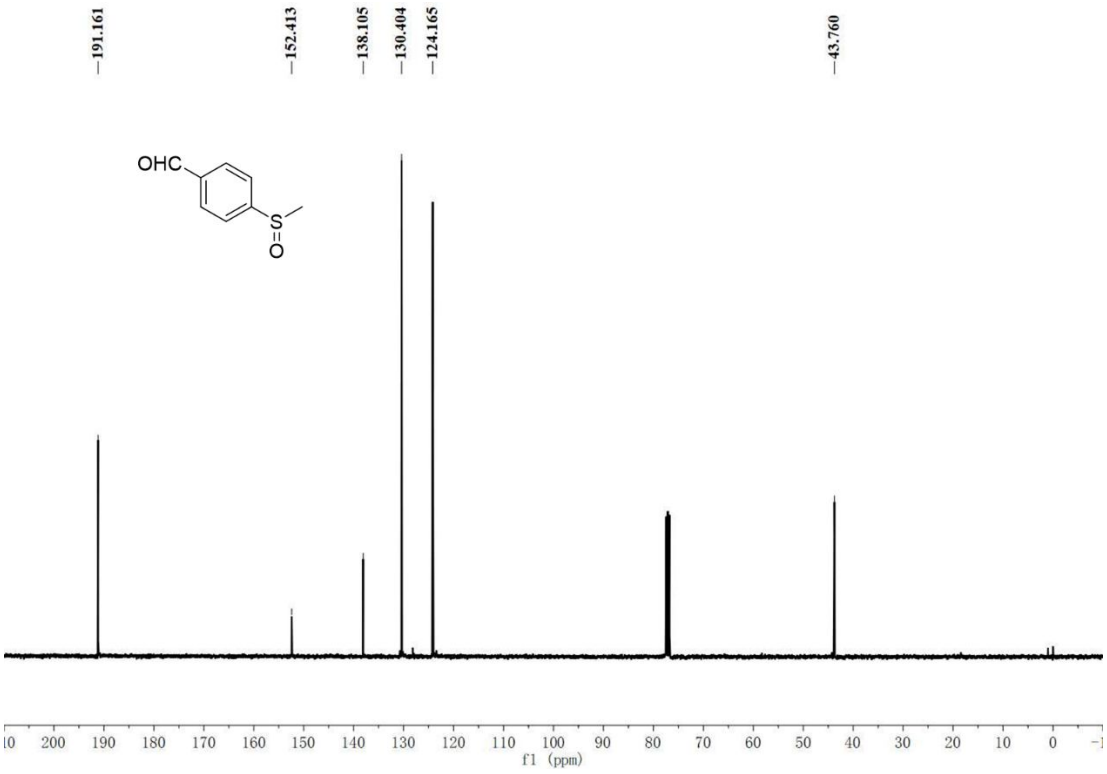

Figure S31. ^{13}C NMR spectrum of Compound 1 in $CDCl_3$.

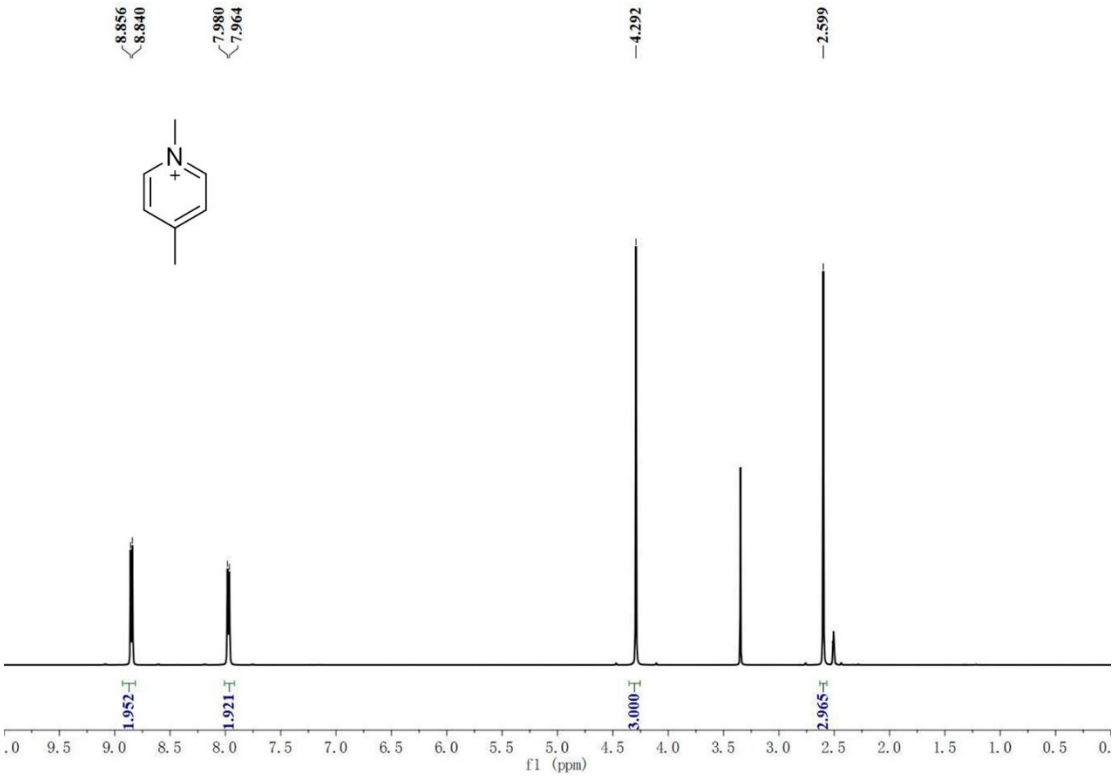

Figure S32. ^{1}H NMR spectrum of Compound 3a in DMSO-d_6.

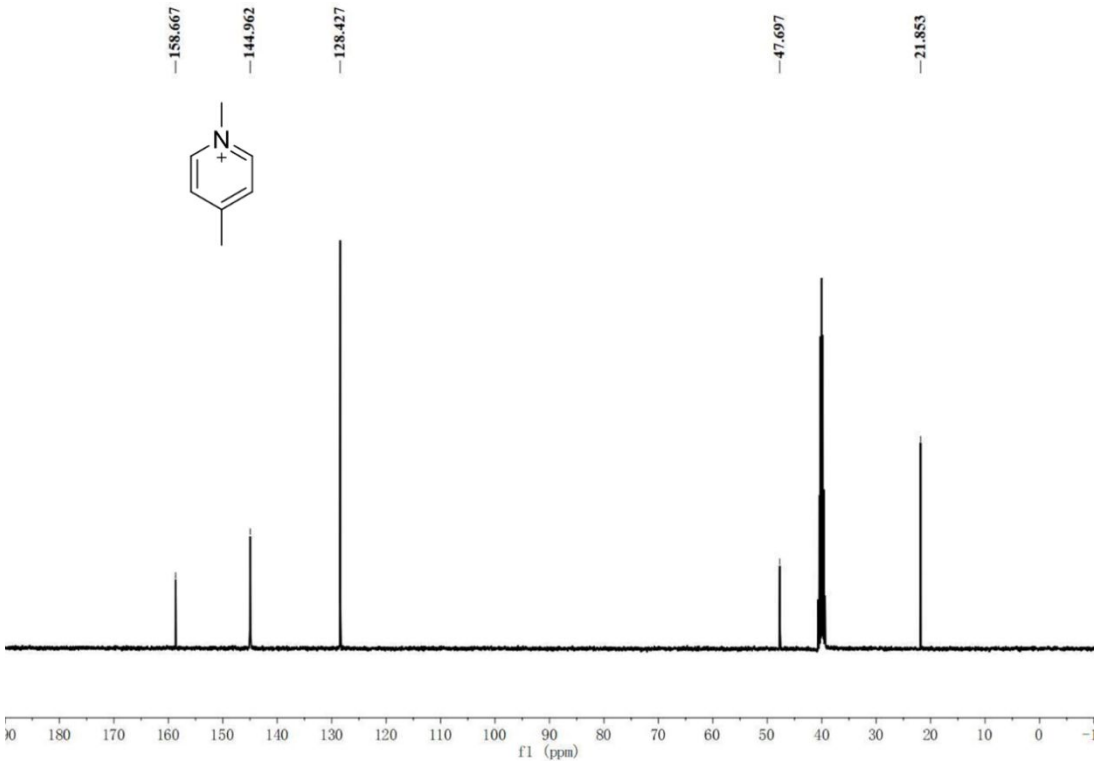

Figure S33. ^{13}C NMR spectrum of Compound 3a in DMSO-d_6.

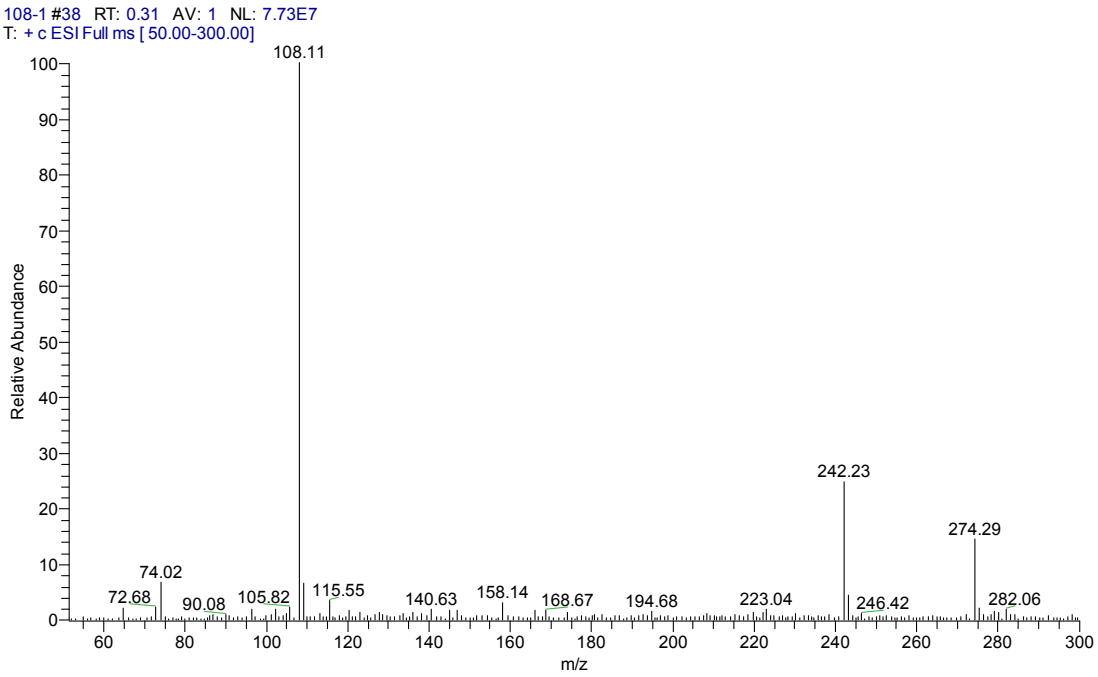

Figure S34. ESI-MS spectrum of Compound 3a.

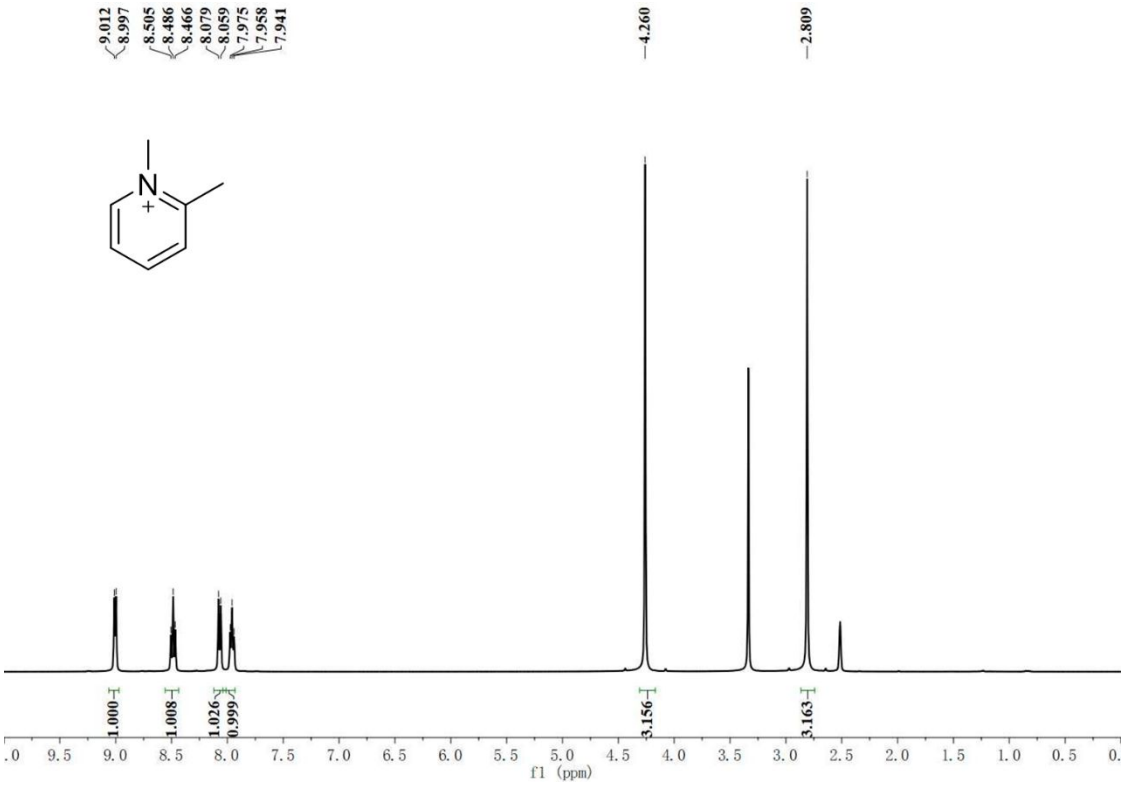

Figure S35. ^{1}H NMR spectrum of Compound 3b in DMSO-d_6.

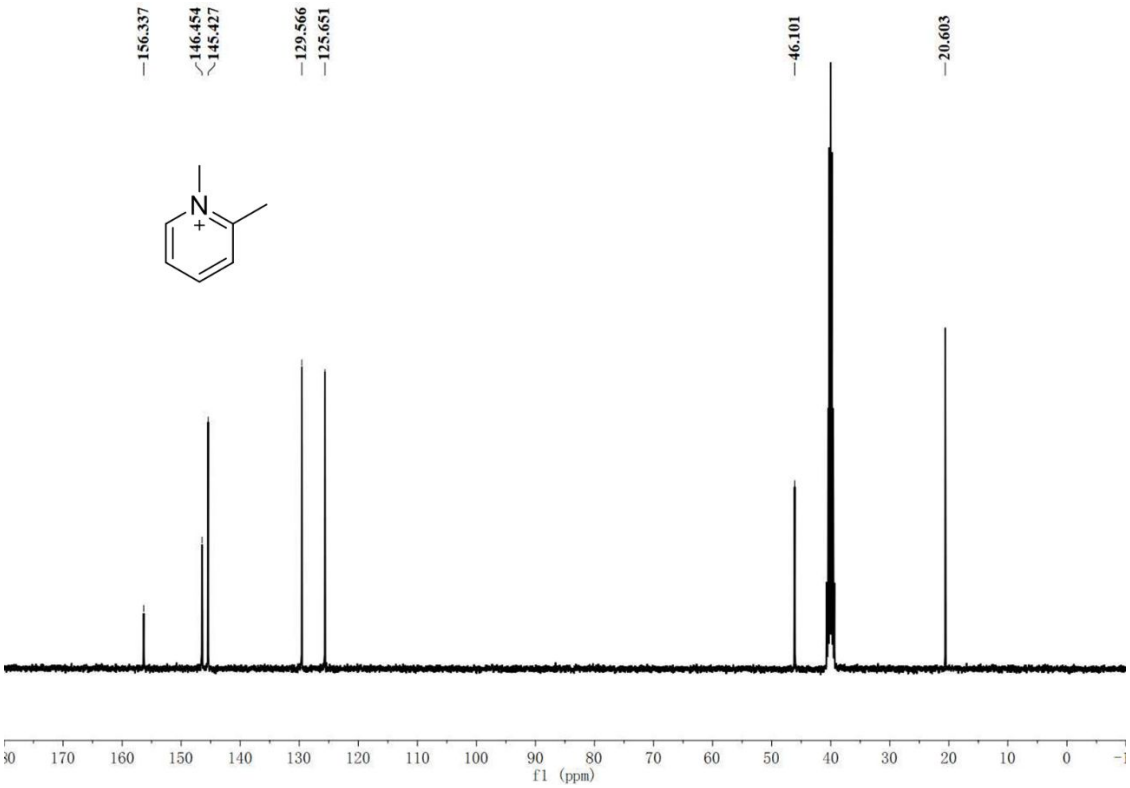

Figure S36. ^{13}C NMR spectrum of Compound 3b in DMSO-d_6.

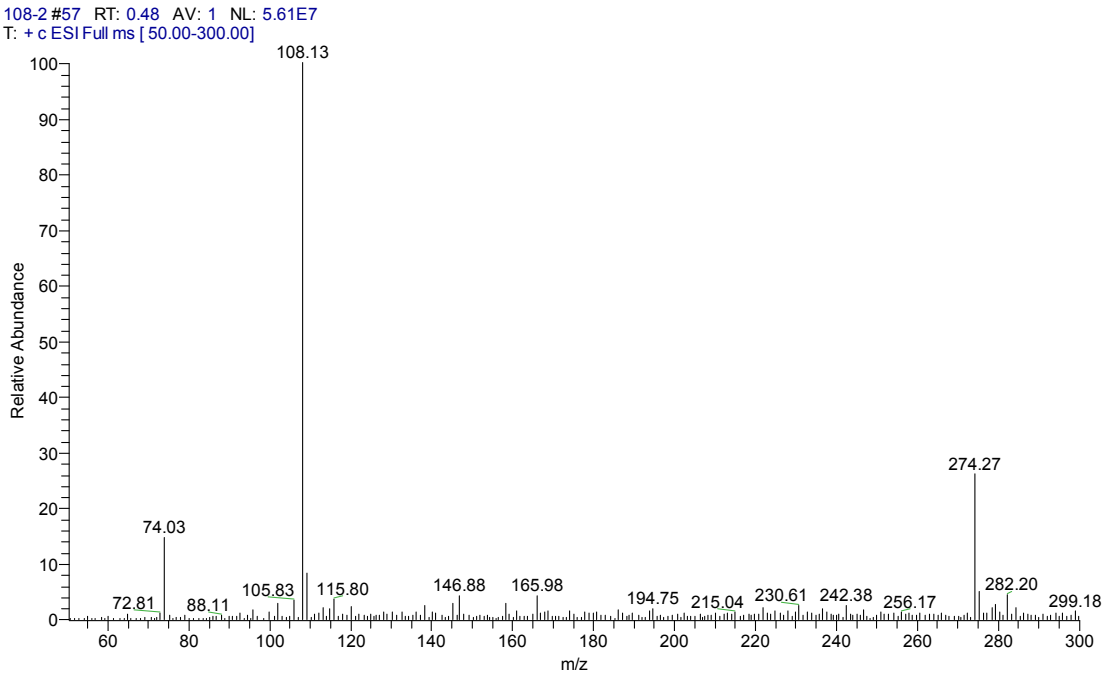

Figure S37. ESI-MS spectrum of Compound 3b.

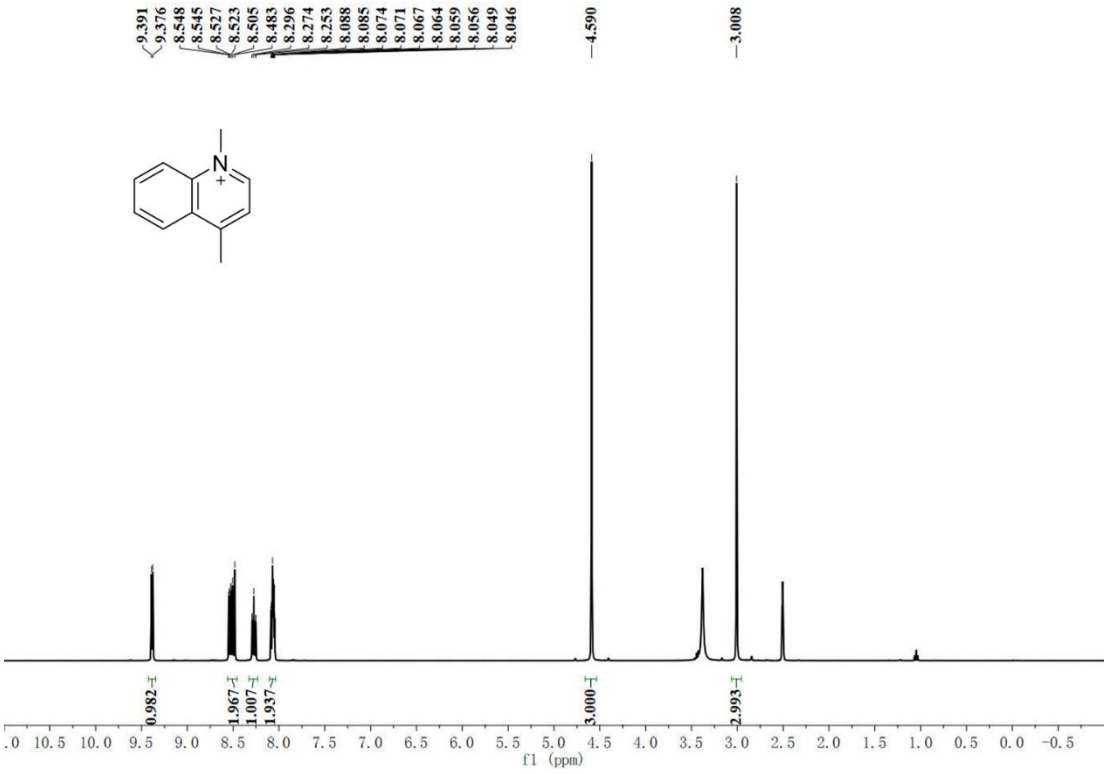

Figure S38. ^{1}H NMR spectrum of Compound 3c in DMSO-d_6.

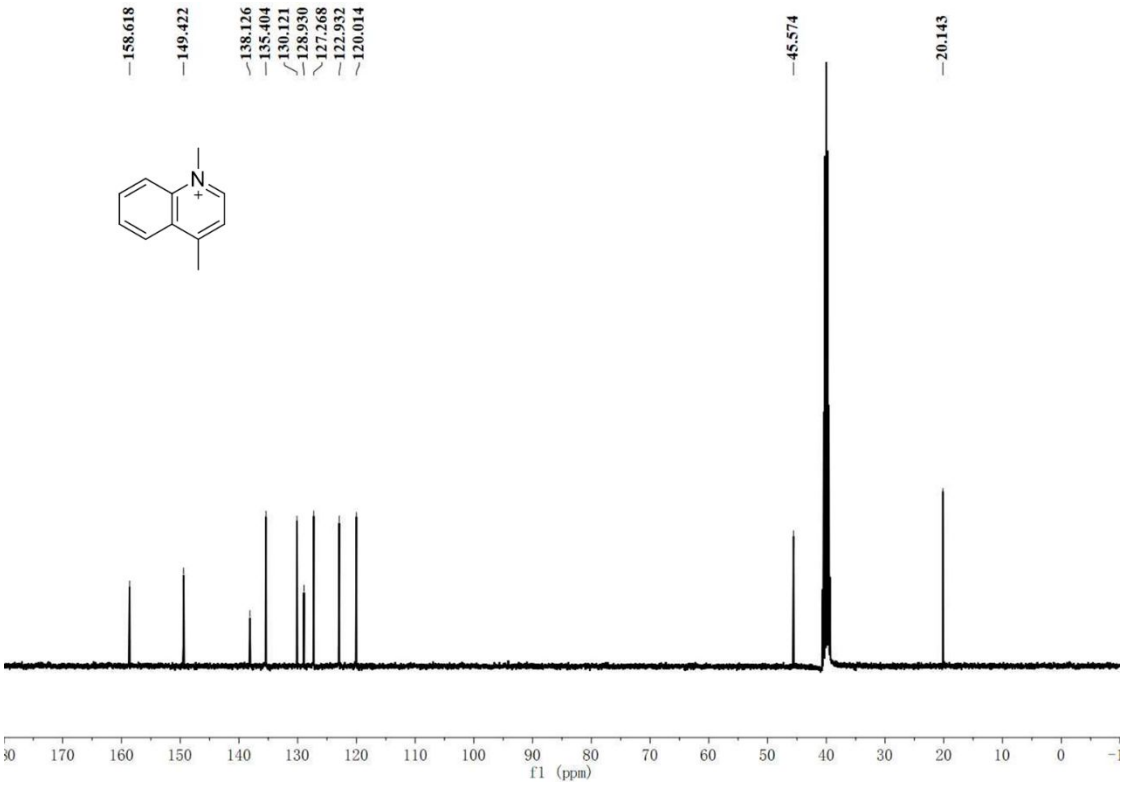

Figure S39. ^{13}C NMR spectrum of Compound 3c in DMSO-d_6.

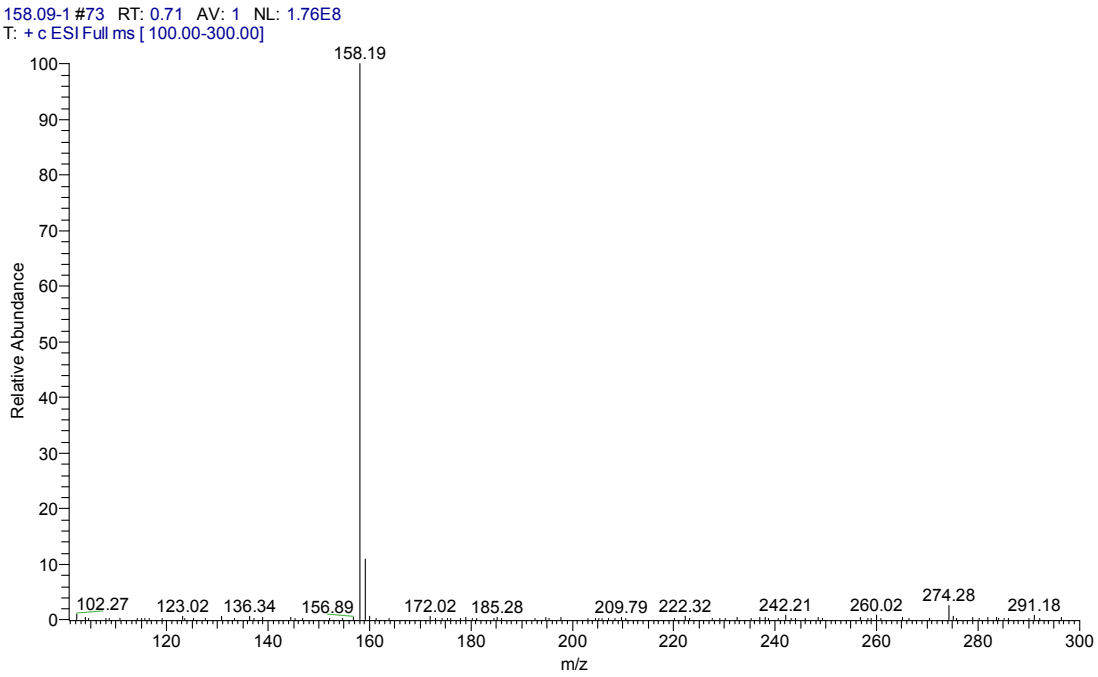

Figure S40. ESI-MS spectrum of Compound 3c.

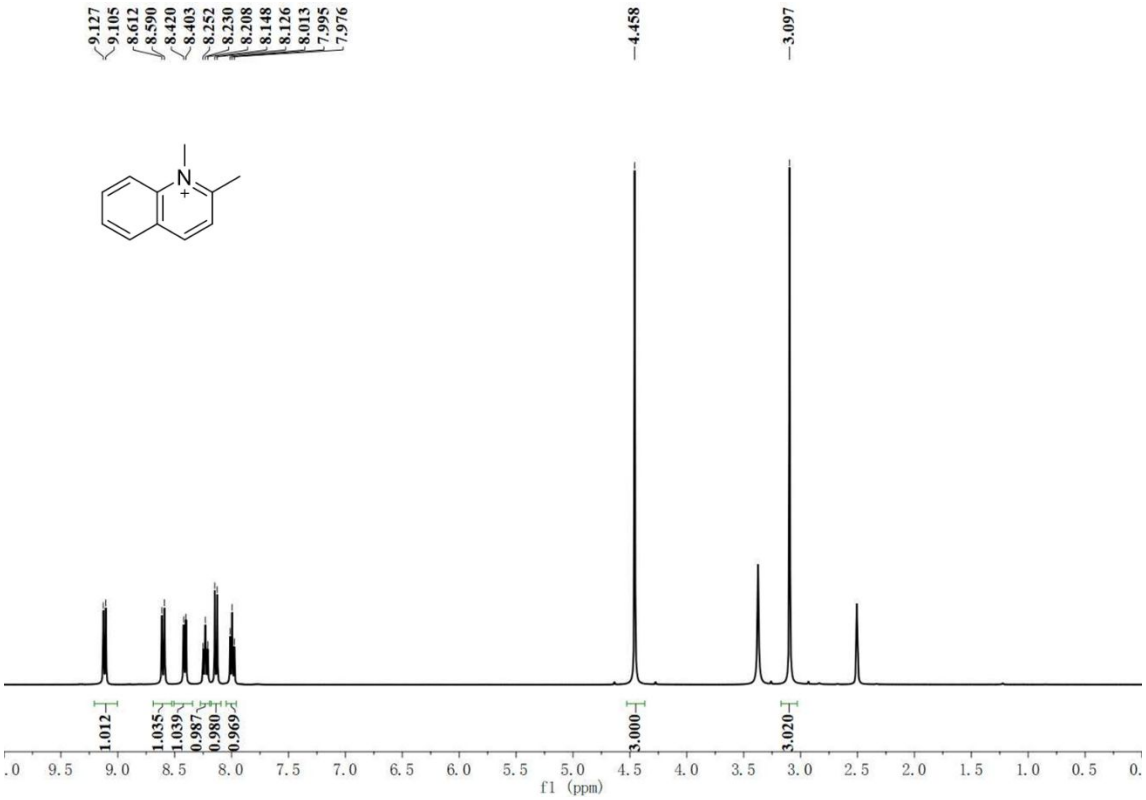

Figure S41. ^{1}H NMR spectrum of Compound 3d in DMSO-d_6.

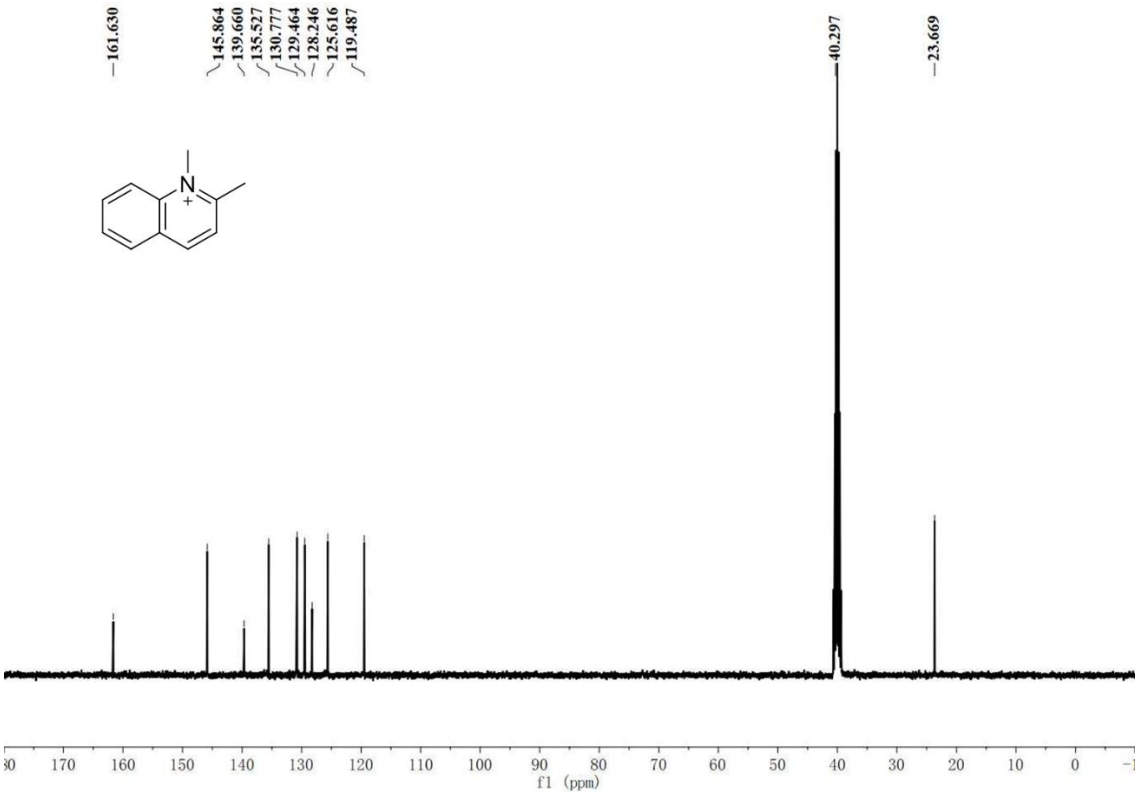

Figure S42. ^{13}C NMR spectrum of Compound 3d in DMSO-d_6.

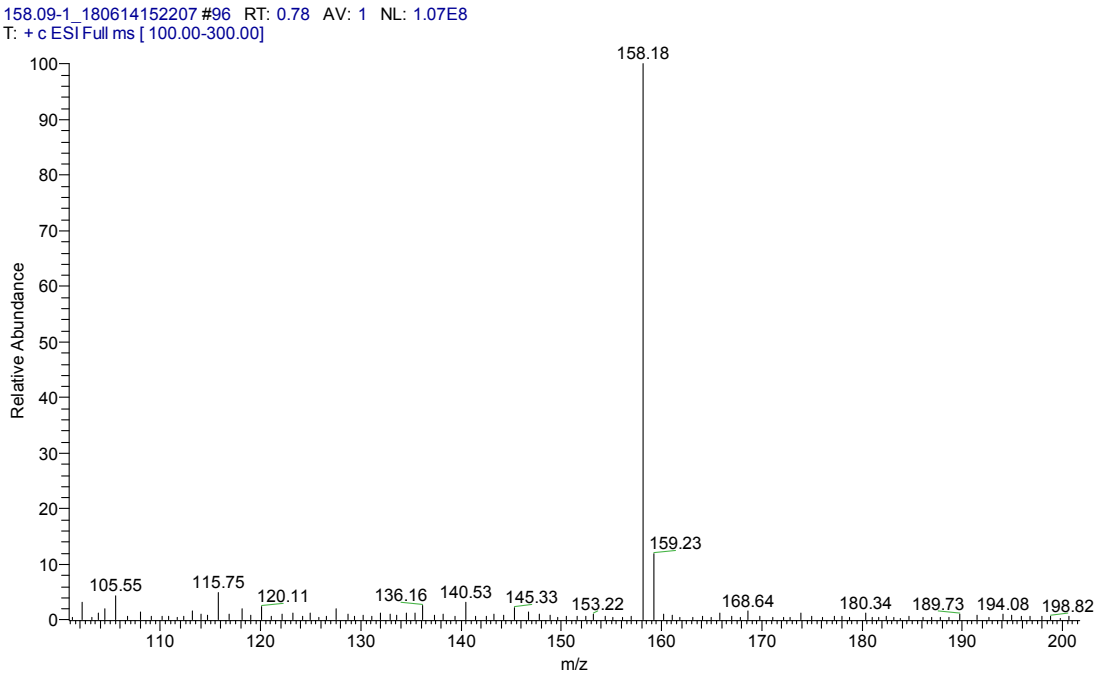

Figure S43. ESI-MS spectrum of Compound 3d.

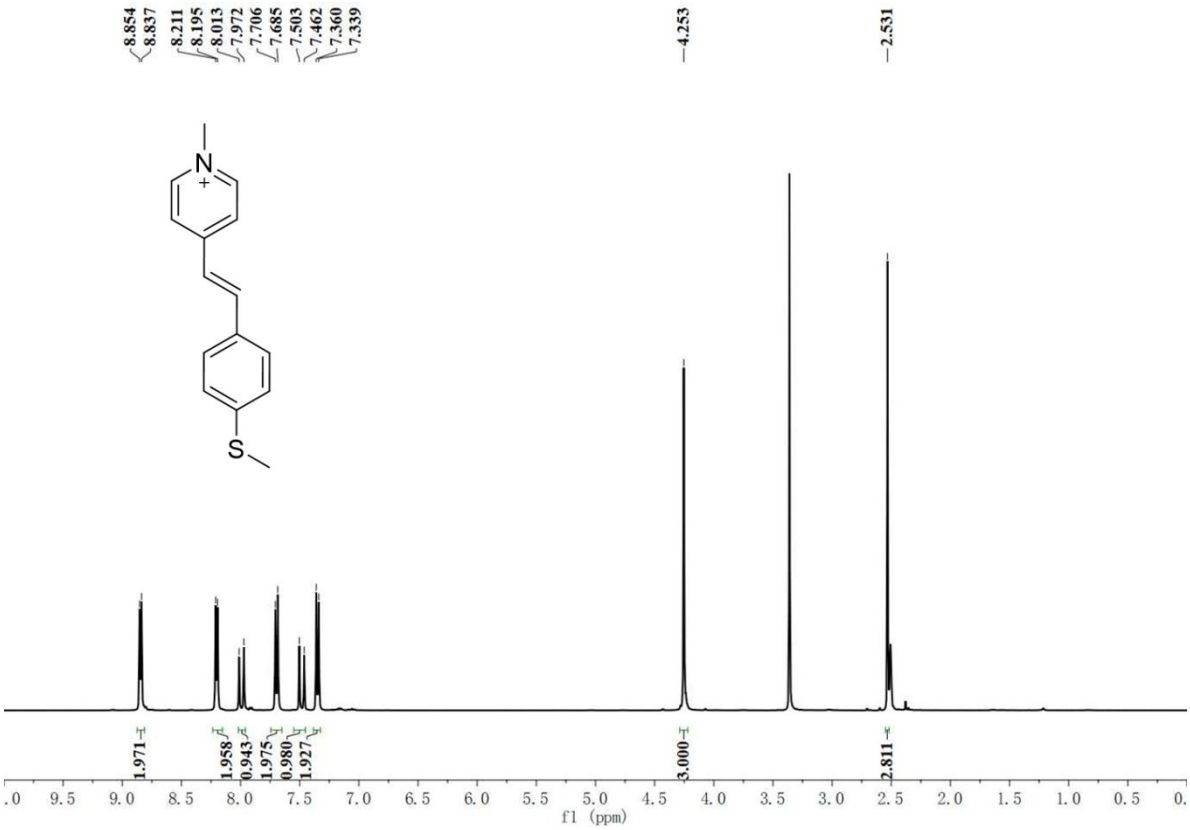

Figure S44. ^{1}H NMR spectrum of dye (rMSP1) in DMSO-d_6.

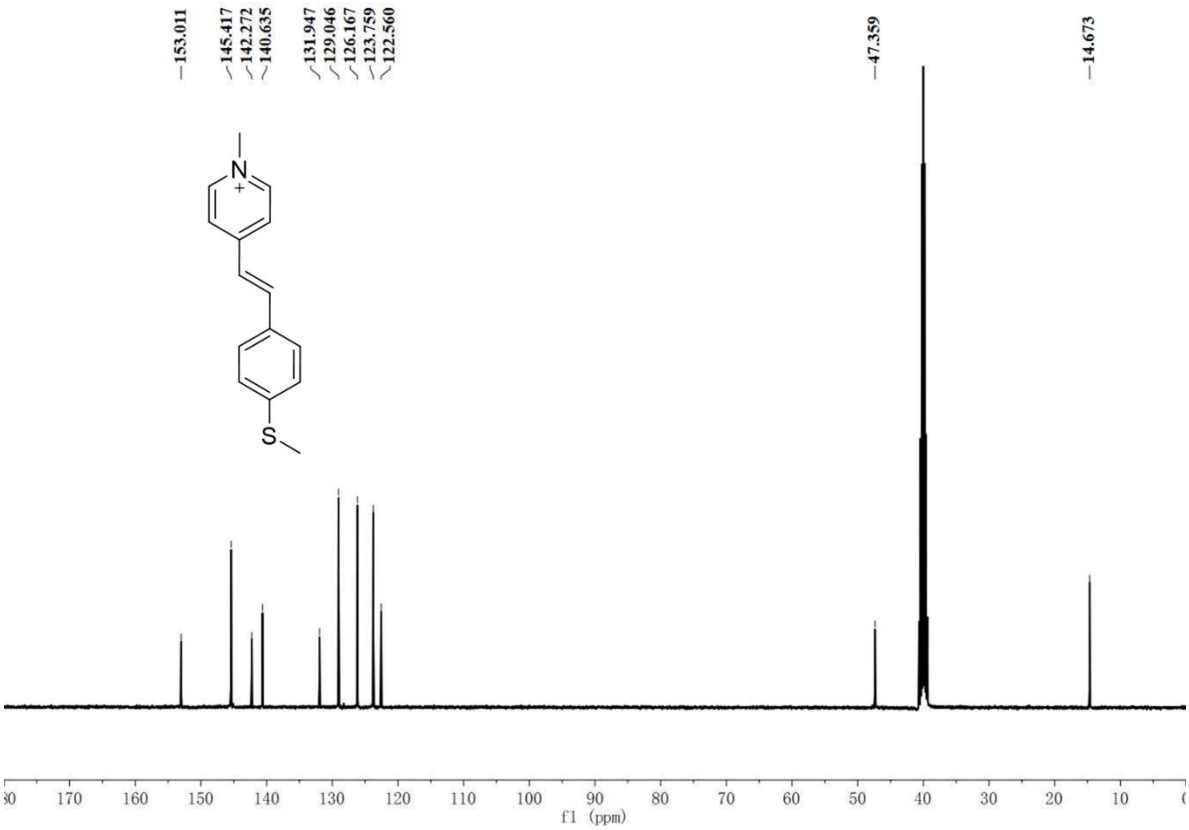

Figure S45. ^{13}C NMR spectrum of dye (rMSP1) in DMSO-d_6.

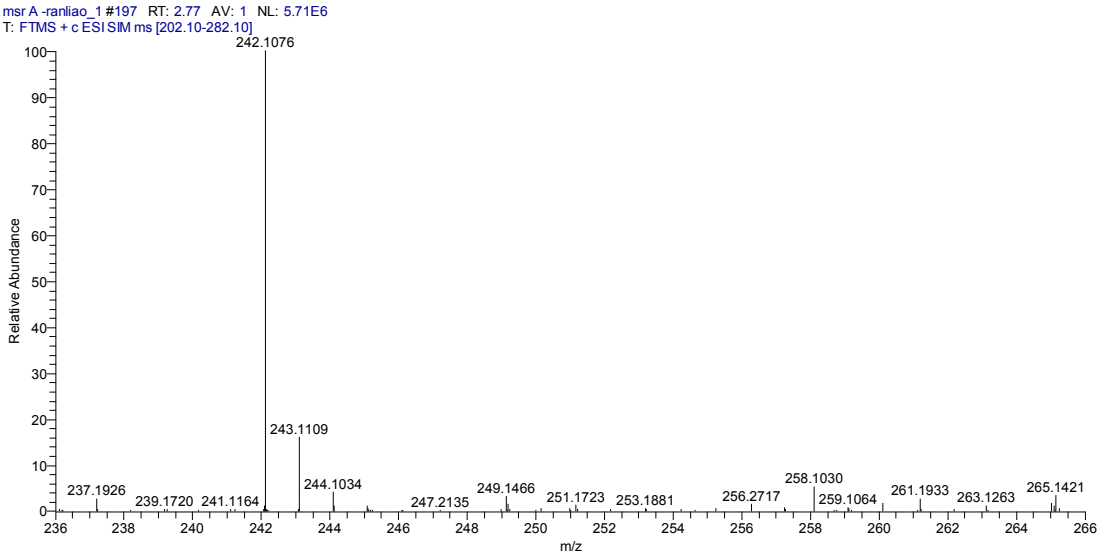

Figure S46. HR-ESI-MS spectrum of dye (rMSP1).