Supporting information

An AIE probe for imaging mitochondria SO_2-induce stress and its level in heat stroke

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Contents

Experimental part

1. Instruments

^{1}H NMR and ^{13}C NMR spectra were taken on the Bruker DTX-400 spectrometer in $CDCl_3$ with TMS as internal standard. Mass spectral determination were performed with a Q-Exactive HR-MS spectroscopy (Thermo). Fluorescence spectra measurements were performed on a HITACHI F-4600 fluorescence spectrophotometer, the excitation and emission wavelength band passes were both set at 5 nm, excitation voltage was 700 V, scan range: 450 – 700 nm. Absorption spectra were recorded using a Lambda 35 UV/VIS spectrometer, PerkinElmer precisely. Fluorescence imaging and co-localization experiment were acquired on a LEICA TCS SP8 laser scanning confocal microscope.

2. Materials

All chemicals reagents were used as received from commercial sources without further purification. Solvents for chemical synthesis and analysis were purified according to standard procedures. The solutions of anions and amino acid were prepared from corresponding salts including: $NaHSO_3$, NaF, NaCl, NaBr, NaI, $NaHCO_3$, $NaCO_3$, NaAcO, Cys, Hcy, GSH, $NaNO_3$, $NaNO_2$, NaClO, H_2O_2, TBHP, ·OH, $ONOO^-$, O– 2, $^{1}O_2$, NO, Na_2S, NaHS, Na_2SO_4, $Na_2S_2O_3$, Na_3PO_4, NaN_3, KSCN were prepared according to reported literature.

3. Optical Studies

Stock solutions of **MITO-TPE** (1 mM) was prepared in DMSO. For optical study, 3.0 mL **MITO-TPE** (10 μM) in PBS buffer (10 mM, pH 7.4) solution was prepared in a bottle. The UV and fluorescent spectra were recorded excitation with 405 nm at 37 °C. All spectra were obtained in a quartz cuvette (path length 1 cm).

4. Cell Culture and Imaging

MCF-7 cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, at 37 °C, in 5% CO_2. For fluorescence imaging, probe MITO-TPE (10 μM) was added to MCF-7 cells and incubated at 37 °C for 30 min in 5% CO_2. Then, the living cells were washed with PBS buffer (pH 7.4) for three times. Next, the cells were treated with Na_2SO_3 (100 μM) solution for another 30 min in 5%CO_2 and then washed three times with PBS buffer. To observe the subcellular distributions of the probe, the MCF-7 cells were treated with a mitochondrial staining probe (500 nM) for 30 min. Then the cells continue to incubate with Na_2SO_3 (100 μM) for another 30 min. The media was removed and the cells were washed three times with PBS buffer (pH 7.4). Cells were imaged using Leica TCS SP8 laser scanning confocal microscope.

5. Zebrafish Culture and Imaging

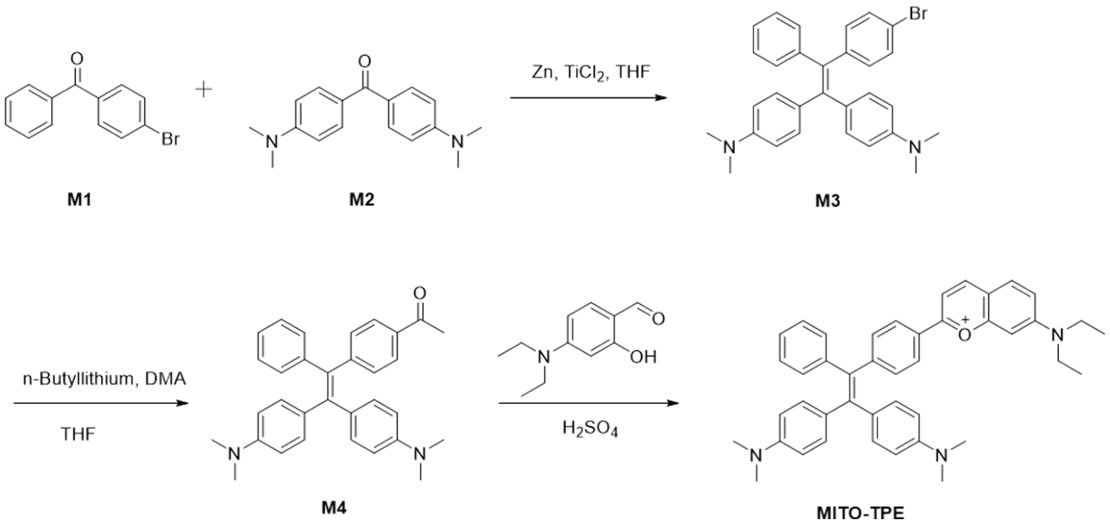
Wildtype zebrafish were obtained from the Nanjing Eze-Rinka Biotechnology Co., Ltd. Zebrafishes were fed in E3 embryo media (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 10-5% methylene blue; pH 7.5) at 28 ^{o}C. The 5-day-old zebrafish were incubated with

probe (10 μM) for 0.5 h, and then imaged after washing by PBS buffer, as the control group. As the negative control group. Zebrafish were pretreated with probe for 0.5 h, subsequently incubated with Na_2SO_3 (100 μM) for 0.5 h, and then imaged after washing by PBS buffer. As the experimental group, pretreated probe (10 μM) Zebrafish incubated with Na_2SO_3 (100 μM) and then treated with FA (200 μM).. Zebrafish were imaged using Leica TCS SP8 confocal microscope.

5. Synthesis.

Compound **M3** and **M4** were synthesized according to the reported literature.[1, 2]

Synthesis of **MITO-TPE**: in a 50 mL round bottom flask, compound **M4** (460.0 mg 1 mmol) and 4-(Diethylamino) salicylaldehyde (193.2 mg 1 mmol) were dissolved in 5 mL of 98% H_2SO_4 and reacted at 90 °C for 12 hours. After the reaction was completed, it was poured into 50 mL of ice water and 1 mL of perchloric acid was added to produce a solid. The crude product was obtained by suction filtration. Column chromatography (MeOH/DCM=1/20) gave 150.0 mg of a purple solid with a yield of 20.9%.^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.58 (d, J = 8.08 Hz, 1H), 7.88 (d, J = 8.76 Hz, 3H), 7.66 (d, J = 8.08 Hz, 1H),7.26 (m, 3H), 7.15 (m, 3H), 7.06 (m, 2H), 6.92 (m, 5H), 6.48 (d, J = 8.76 Hz, 2H), 6.44 (d, J = 8.80 Hz, 2H), 3.68 (m, 4H), 2.91 (s, 12H), 1.35 (t, J = 7.00 Hz 6H), ppm;^{13}C NMR ($CDCl_3$, 100 MHz): δ = 166.6, 159.4, 156.4, 153.5, 149.5, 149.3, 148.9, 145.5, 144.3, 135.1, 133.0, 133.0, 132.9, 132.8, 131.7, 131.2, 131.1, 128.1, 127.3, 126.3, 125.7, 118.8, 118.2, 111.5, 111.1, 109.1, 95.8, 45.5, 40.3, 40.3, 31.6. Mp: 191.0 °C-192.0 °C. HR-MS: m/z calcd for $[C_{43}H_{44}N_3O]^+$ = 618.3479, Found:618.3472.



Scheme S1. Synthetic route of probe **MITO-TPE**.

1. M. Kang, X. Gu, R. T. K. Kwok, C. W. T. Leung, J. W. Y. Lam, F. Li and B. Z. Tang, *Chem. Commun.*, 2016, **52**, 5957-5960.
2. E. Wang, E. Zhao, Y. Hong, J. W. Y. Lam and B. Z. Tang, *J. Mater.Chem. B*,

2014, **2**, 2013-2019.

Supporting figures

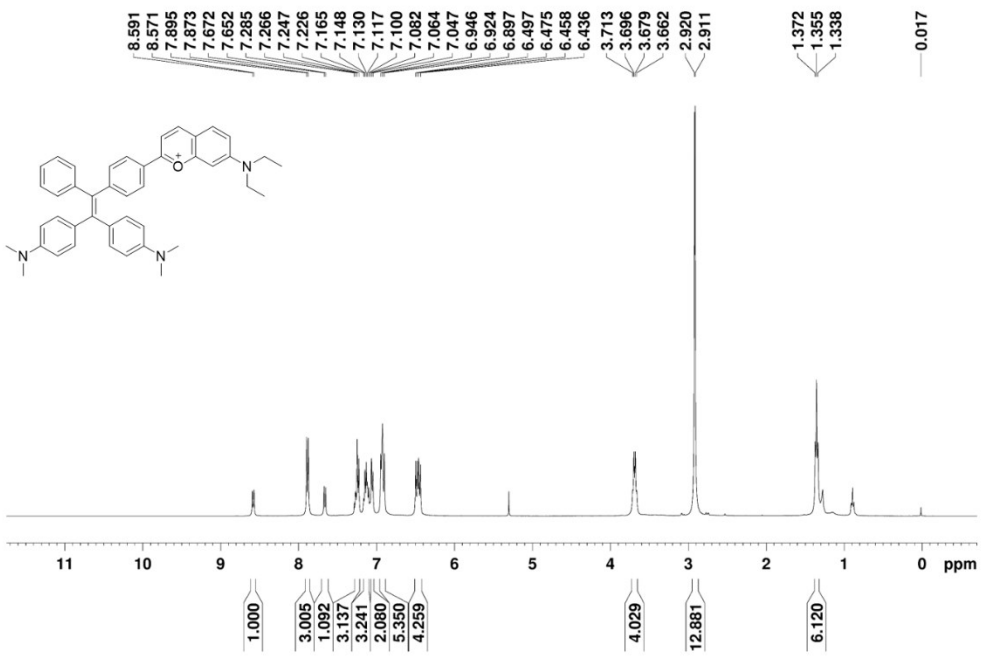


Figure S1. ^{1}H NMR spectra of **MITO-TPE** in $CDCl_3$.

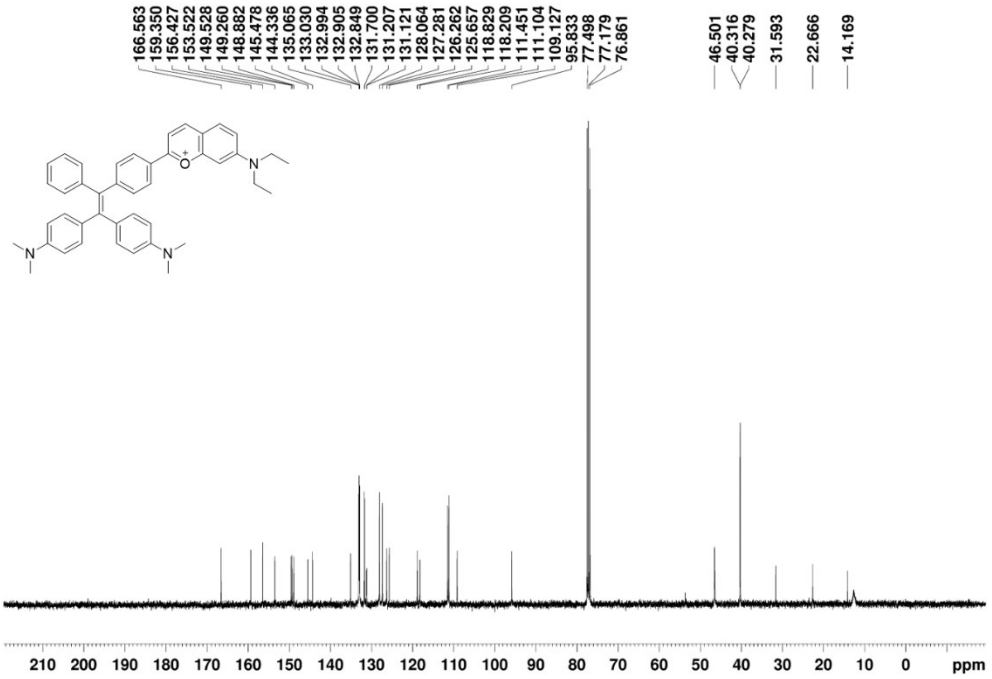


Figure S2. ^{13}C NMR spectra of **MITO-TPE** in $CDCl_3$.

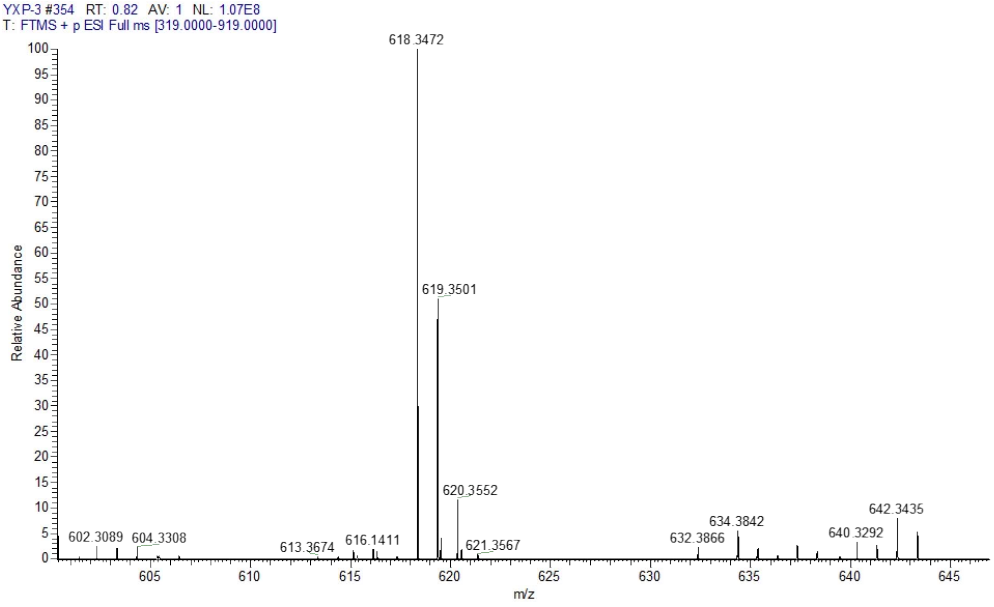


Figure S3. HR-MS spectra of **MITO-TPE**.

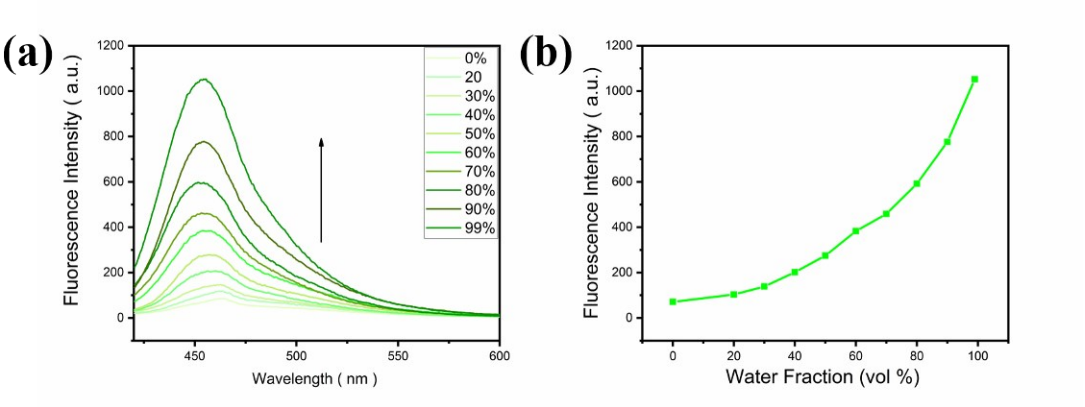


Figure S4. (a) Emission spectra of **MITO-TPE-SO$_2$** in DMSO–water mixtures with different water fractions (fw). (b) Plot of the relative intensity of **MITO-TPE-SO$_2$** versus the water fraction in DMSO–H_2O mixtures.

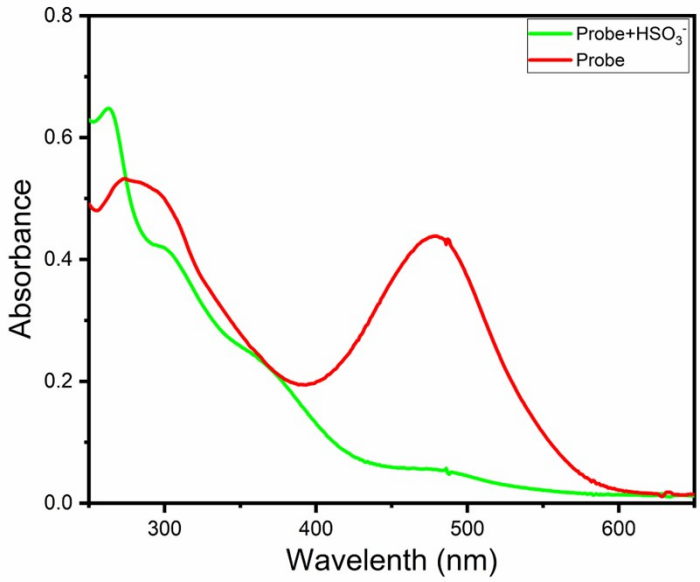


Figure S5. The UV-vis absorption spectra in the PBS buffer (10 mM, pH=7.4).

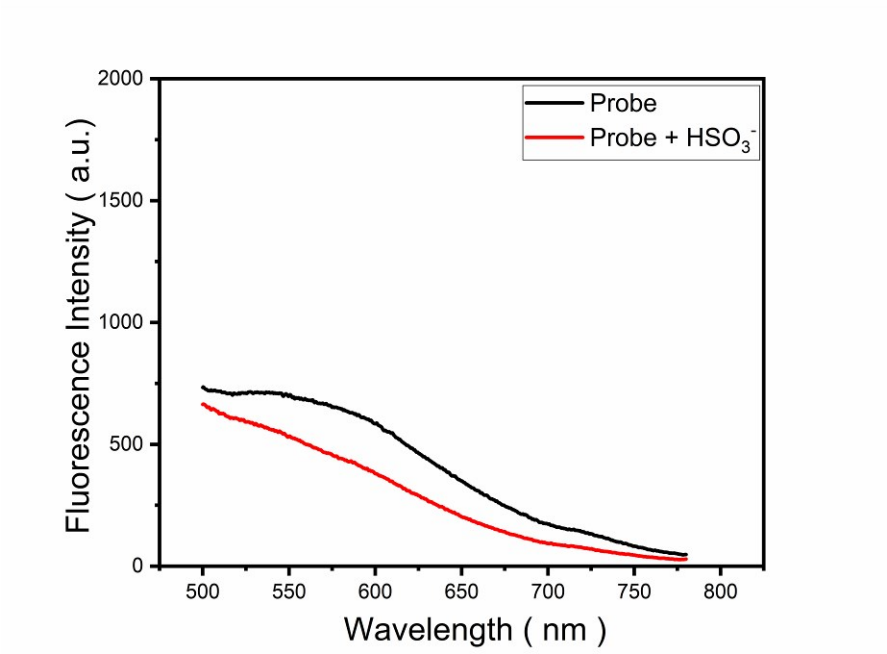


Figure S6. The fluorescence emission intensity of probe **MITO-TPE** (10 μM) upon treatment with $NaHSO_3$ (100 μM) under 490 nm excitation in the PBS buffer (10 mM, pH=7.4).

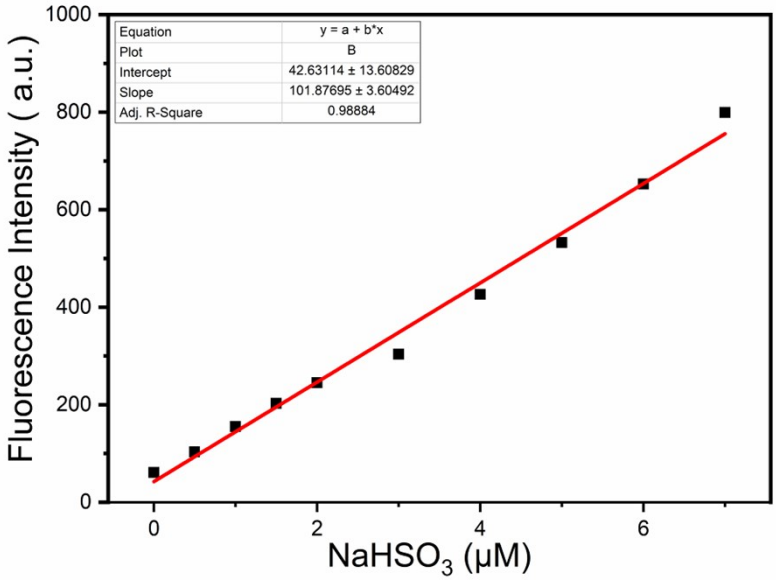


Figure S7. Linear plot of the fluorescence emission intensity against $NaHSO_3$ concentrations.

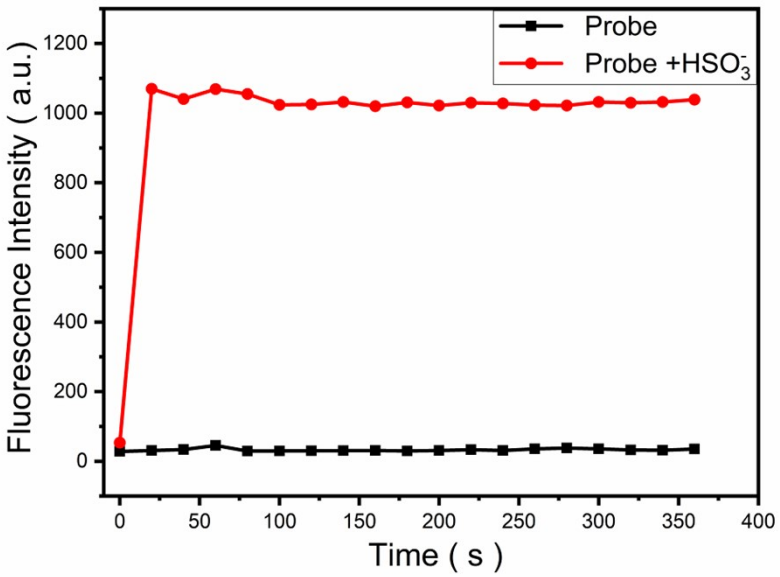


Figure S8. The fluorescence emission intensity of probe **MITO-TPE** (10 μM) upon treatment with $NaHSO_3$ (100 μM) with the progress of time in the PBS buffer (10 mM, pH=7.4).

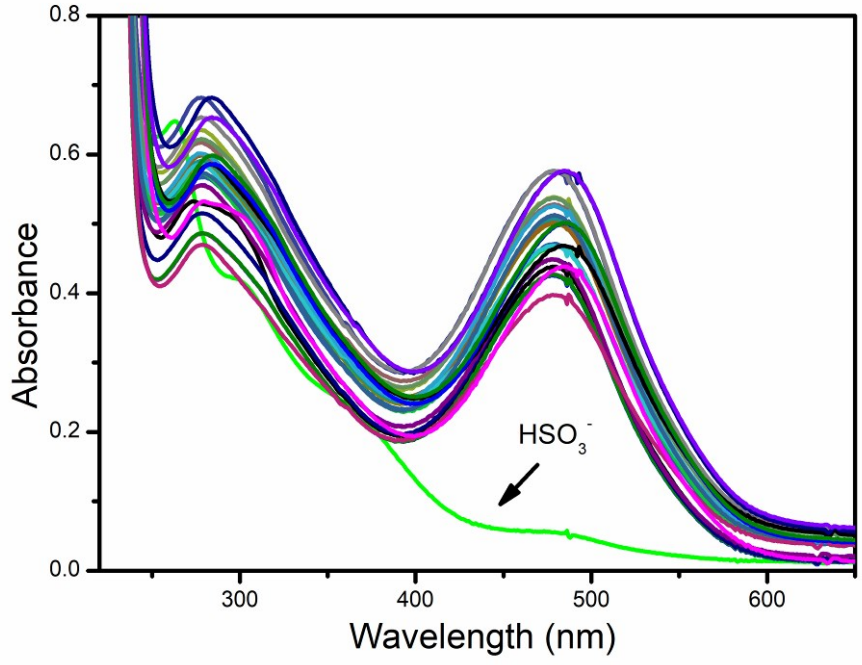


Figure S9. The UV-vis spectra of probe **MITO-TPE** (10 μM) with $NaHSO_3$ (100 μM) and other various anions (F^-, Cl^-, Br^-, I^-, AcO^-, HCO_3^-, CO_3^{2-}, GSH, Hcy, Cys , NO_3^-, NO_2^-, NO, HNO, $ONOO^-$, O_2^-, H_2O_2, ClO^-, TBHP, ·OH, $^1O_2$, S^{2-}, HS^-, SO_4^{2-}, $S_2O_3^{2-}$, PO_4^{3-}, N_3^-, SCN^-) (100 μM) in the PBS buffer (10 mM, pH=7.4).

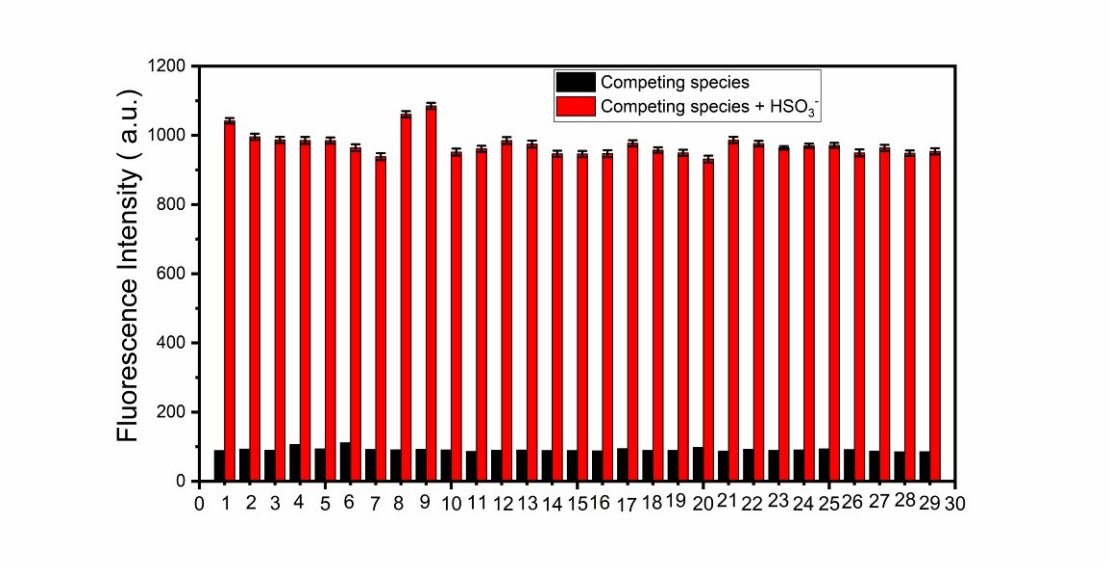


Figure S10. The fluorescence emission intensity of **MITO-TPE** (10 μM) upon the addition of various ions (F^-, Cl^-, Br^-, I^-, AcO^-, HCO_3^-, CO_3^{2-}, GSH, Hcy, Cys , NO_3^-, NO_2^-, NO, HNO, $ONOO^-$, O_2^-, H_2O_2, ClO^-, TBHP, ·OH, $^1O_2$, S^{2-}, HS^-, SO_4^{2-}, $S_2O_3^{2-}$, PO_4^{3-}, N_3^-, SCN^-) (100 μM) in the presence of $NaHSO_3$ (100 μM) in the PBS buffer (10 mM, pH=7.4).

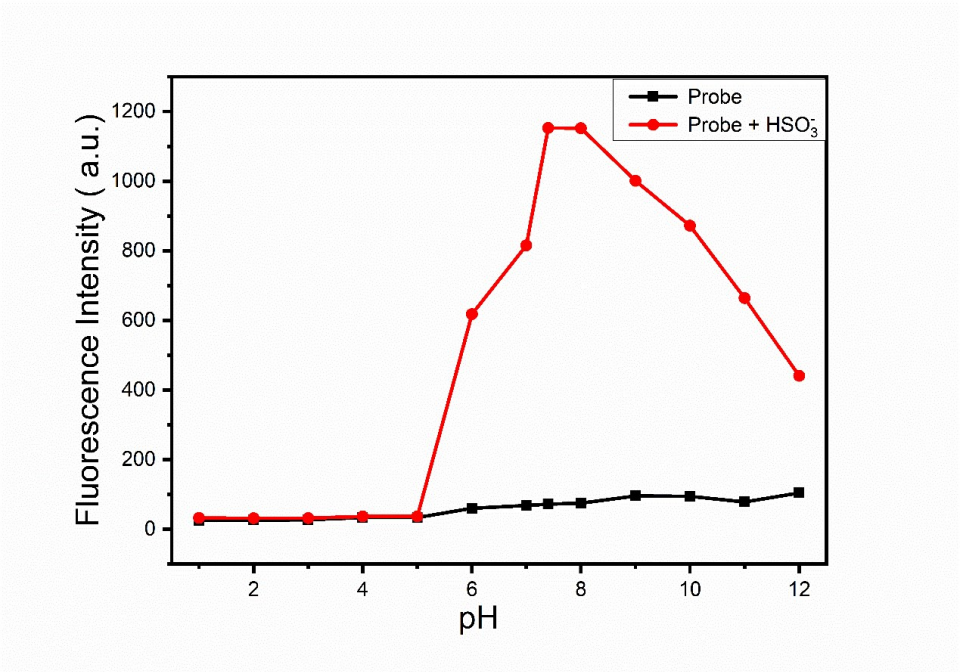


Figure S11. The fluorescence emission intensity of **MITO-TPE** (10 μM) in solution of different pH value (1-12).

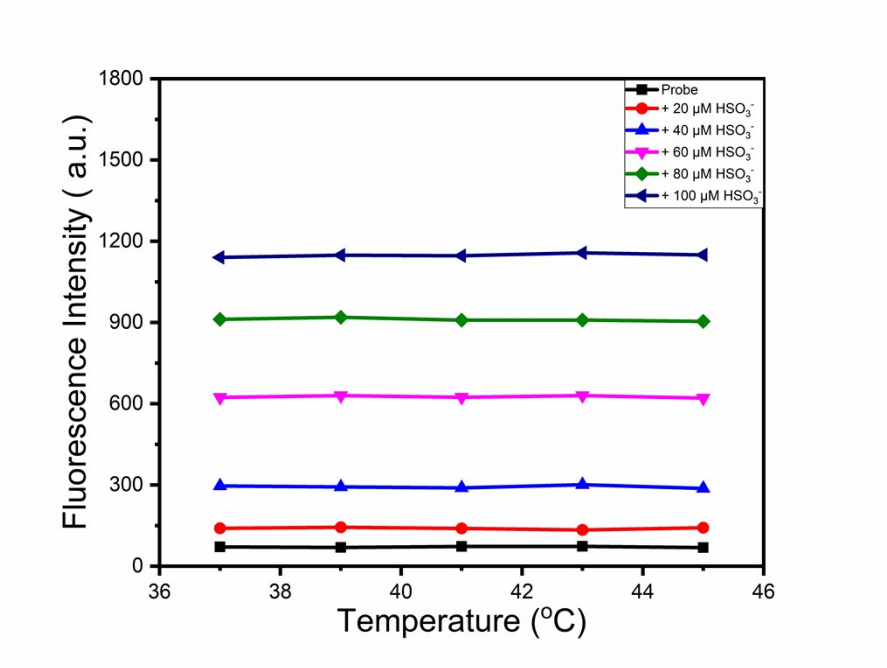


Figure S12. The fluorescence emission intensity of **MITO-TPE** (10 μM) in solution of different temperature.

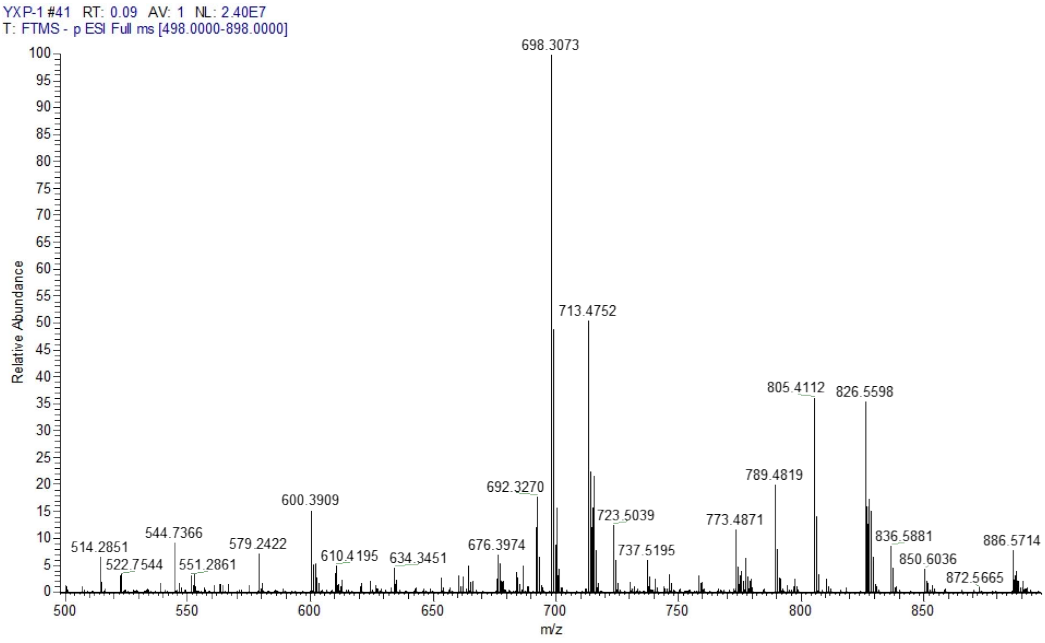


Figure S13. HR-MS spectra of **MITO-TPE** in the presence of $NaHSO_3$.

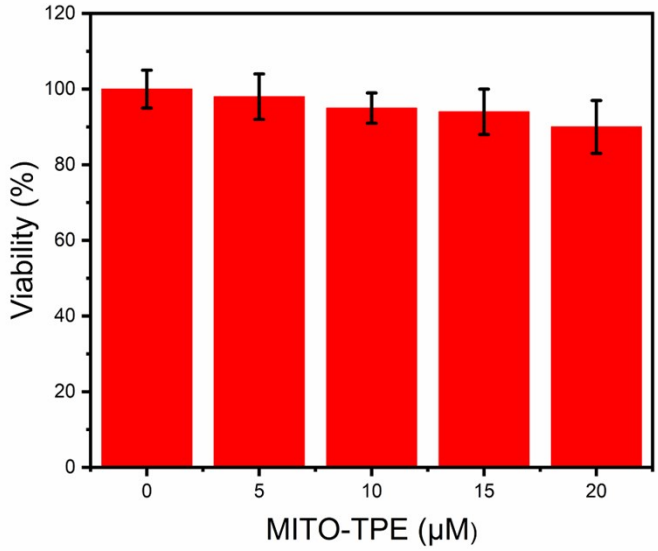


Figure S14. Cytotoxicity assay. MCF-7 cells were treated with different concentrations of probe **MITO-TPE**.

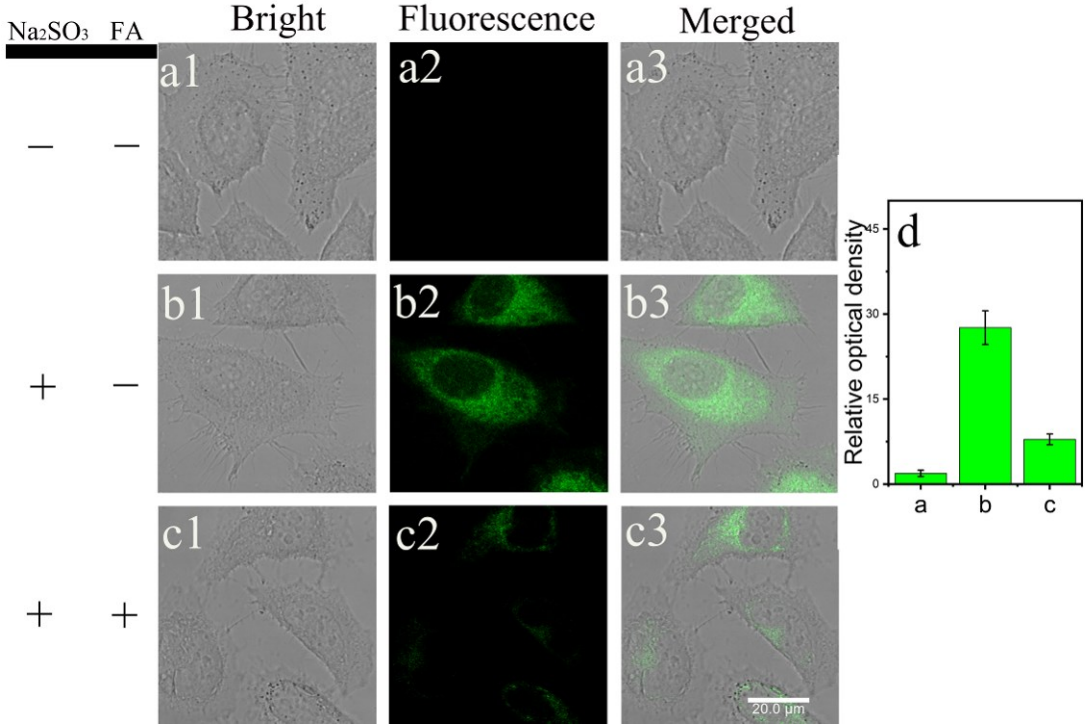


Figure S15. Fluorescence images of probe **MITO-TPE** respond to Na_2SO_3 with in MCF-7 cells. (a) Cells treated with probe **MITO-TPE** (10 μM) only. (b) Cells incubated with probe **MITO-TPE** (10 μM) and then treated with Na_2SO_3 (100 μM). (c) Probe-pretreated **MITO-TPE** (10 μM) cells incubated with Na_2SO_3 (100 μM) and then treated with FA (200 μM). (d) Relative optical density of the respect wells. (λ_{ex}= 405 nm, emission: 425-485 nm, Scale bar: 20 μm).

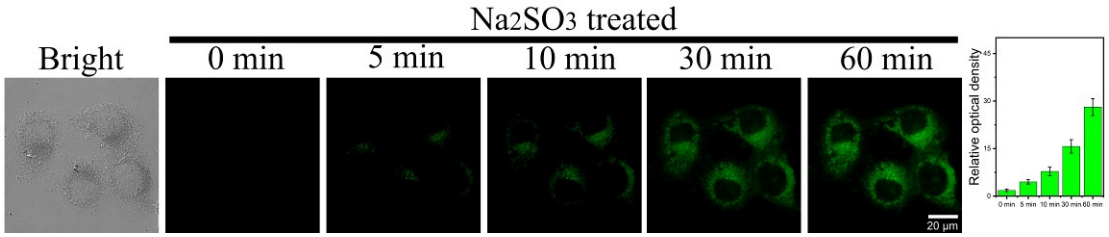


Figure S16. Time-dependent confocal images of exogenous HSO– 3 in MCF-7 cells. Relative optical density of the respect wells. (λ_{ex} = 405 nm, emission: 425-485 nm, Scale bar: 20 μm).

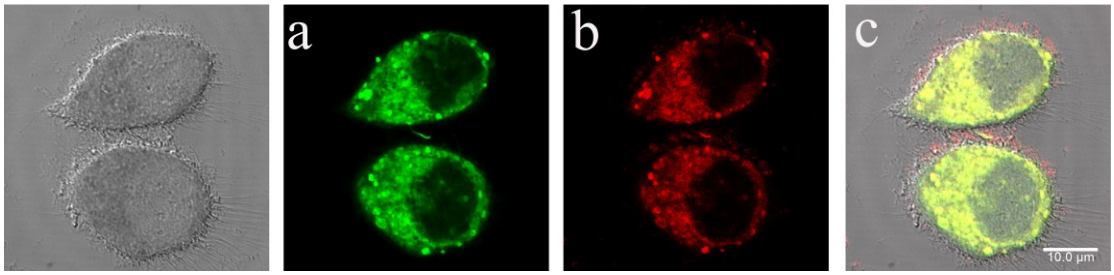
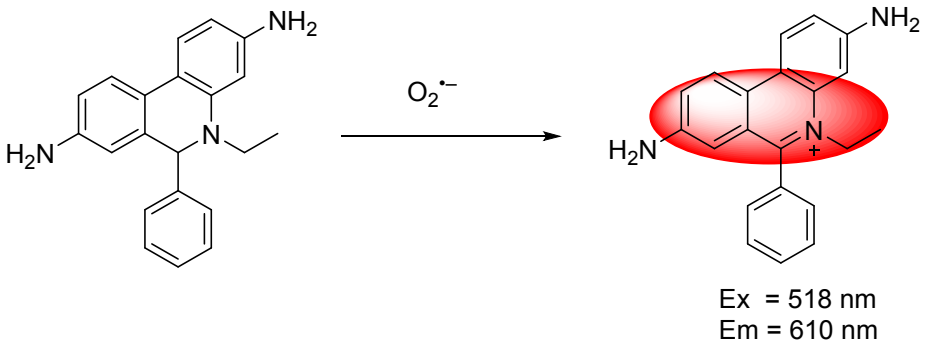


Figure S17. Fluorescence images of **MITO-TPE** colocalized to the mitochondria in MCF-7 cell. (a) **MITO-TPE** (10 μM), Channel 1 (green) (b) Mito-Tracker Red (100 nM), Channel 2 (red). (c) The merged pattern of a and b. (Channel 1: λ_{ex} = 405 nm, emission 425-485 nm, channel 2: λ_{ex} = 552 nm, emission 560-620 nm, Scale bar: 10 μm).



Scheme S2. Proposed reaction mechanism of **HE** towards $O_2^{\cdot-}$.

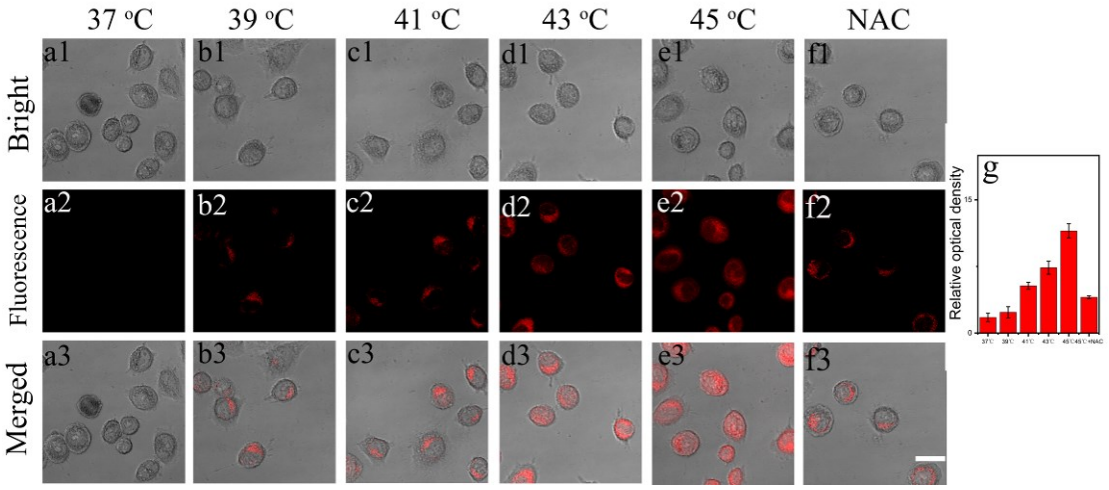


Figure S18. (a) Fluorescence images of MCF-7 cells incubated with **HE** (10 μM) at different temperatures (37, 39, 41, 43 and 45 °C). (g) Relative optical density of the respect wells. (λ_{ex} = 488 nm, emission: 575-635 nm, Scale bar: 20 μm).

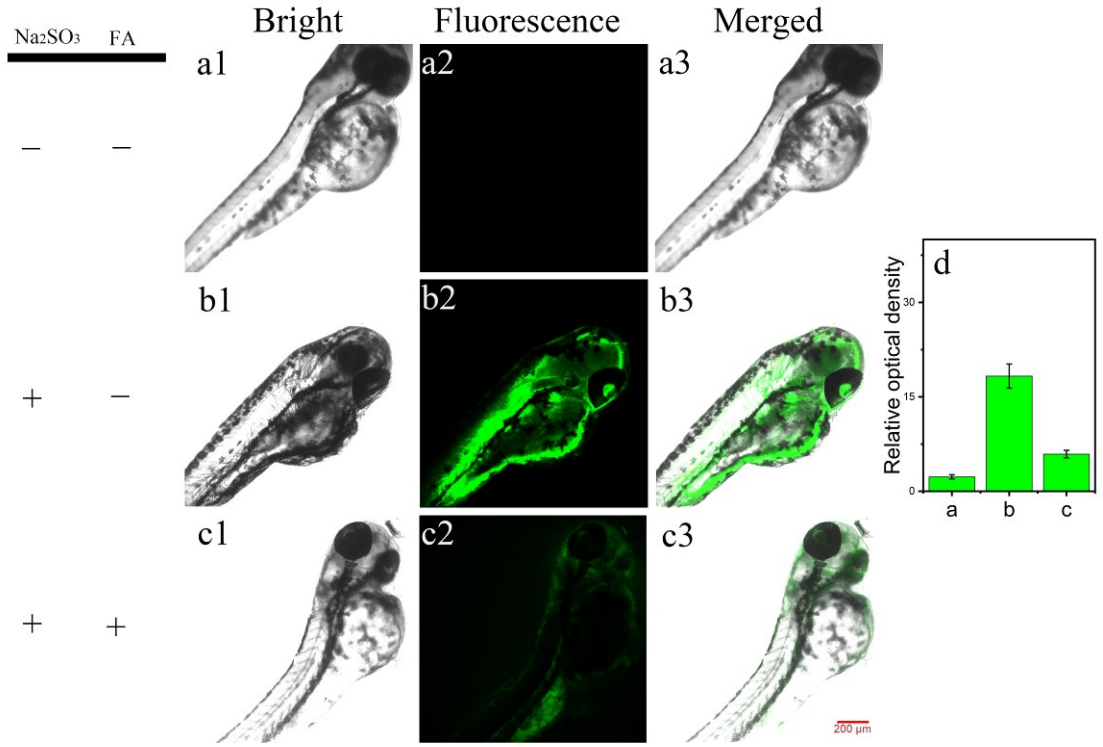


Figure S19. Fluorescence images of probe **MITO-TPE** respond to Na_2SO_3 in zebrafish. (a) Treated with probe MITO-TPE (10 μM) only. (b) Incubated with probe **MITO-TPE** (10 μM) and then treated with Na_2SO_3 (100 μM). (c) Zebrafish pretreated with **MITO-TPE** (10 μM), then incubated with Na_2SO_3 (100 μM), and last treated with FA (200 μM). (d) Relative optical density of the respect wells. (λ_{ex} = 405 nm, emission: 425-485 nm, Scale bar: 200 μm).