Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supplementary Information for

An off-on fluorescent probe for detection of mitochondria-specific protein persulfidation

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Synthesis and verification of papain persulfide

A solution of papain (10 mg) in 1 mL of Tris-HCl (pH 7.4, degassed with N_2 for 10 min) was incubated with cysteine (1.7 mg) at room temperature for 10 min. Protein-containing fractions were incubated with DTNB (50 µL, 4 mM) at room temperature for 20 min and purified using a PD-10 column. A fraction of the pooled protein (200 µL, 120 µM) was incubated in a solution of Na_2S (4 µL, 30 mM) for 10 min and purified with a PD-10 column to obtain papain persulfide. Papain persulfide (20 µM) was incubated with IAM (2 mM) at room temperature for 1.5 h, and the products were analysed by MALDI-TOF-MS.

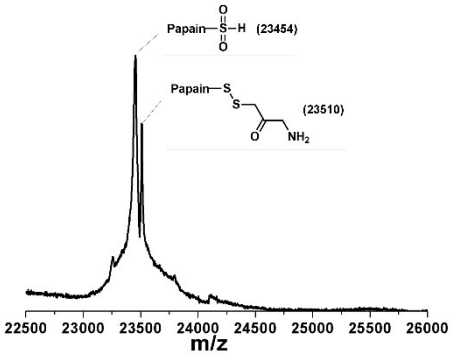


Figure. S1. Mass analyses of the reaction between papain-SSH and IAM.

Fluorescence spectra of HQO-SSH with GAPDH-SSH

A solution of GAPDH (5 mg) in 1 mL of Tris-HCl (pH 7.4, degassed with N_2 for 10 min) was incubated with DTT (10 mM) at room temperature for 1 h. Then DTT was removed using a PD-10 column. Then H_2O_2 and Na_2S were added to the GAPDH solution. The mixture was incubated at 37°C for 30 min. After that HQO-SSH (20 µM) was added in the solution. The fluorescence spectra of HQO-SSH and HQO-SSH+GAPDH-SSH was recorded.

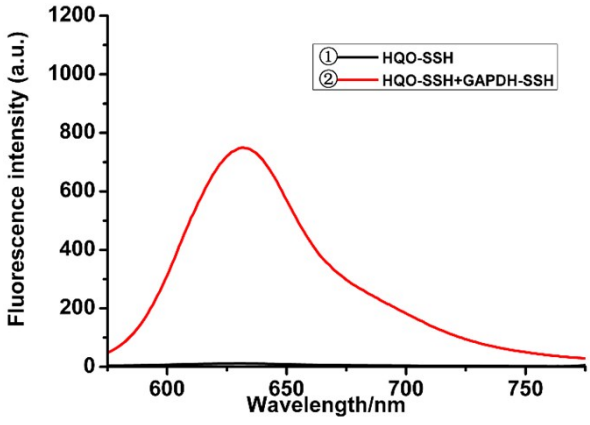


Figure S2. Fluorescence spectra of HQO-SSH (20 µM) with or without GAPDH-SSH.

Selectivity of HQO-SSH towards protein persulfidation

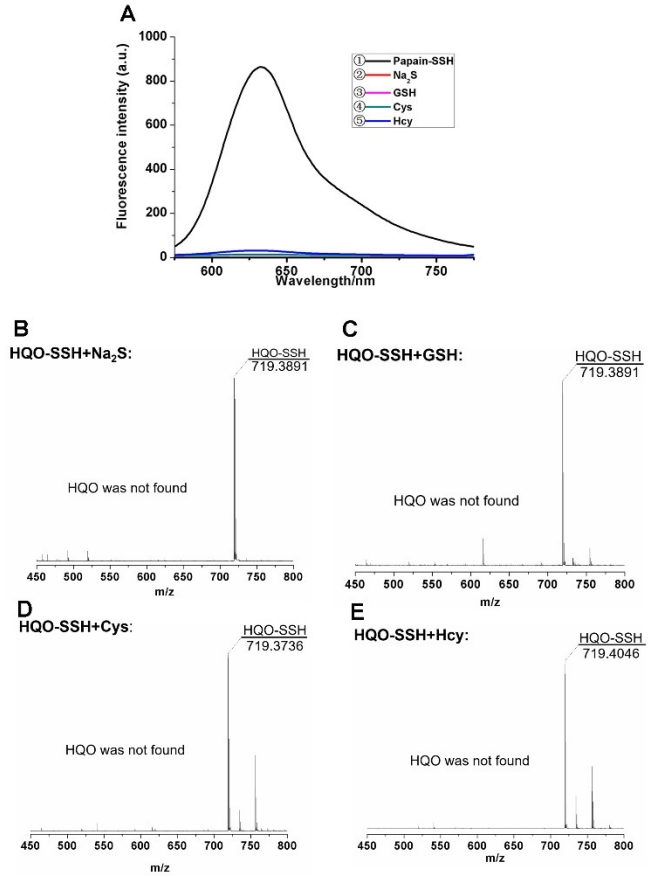


Figure S3. Selectivity of HQO-SSH towards protein persulfidation. A) Fluorescence spectra of HQO-SSH (20 μM) with papain-SSH (40 μM), Na2S (1 mM), GSH (10 mM), Cys (1 mM), and Hcy (100 μM) for 4 min. B) Mass analyses of the reaction of HQO-SSH with Na2S (1 mM), GSH (10 mM), Cys (1 mM), and Hcy (100 μM) for 4 min.

CCK-8 assay of HQO and HQO-SSH

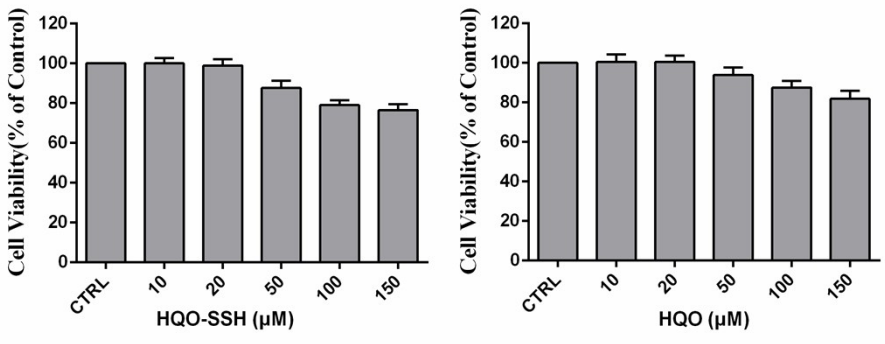


Figure. S4. Untreated or HQO-SSH/HQO-treated BEAS-2B cells were further cultured for 24 h. Cell

viability was assessed using the CCK8 assay. The experiment was repeated three times and the data are shown as mean (±S.D.).

Visualizing the change of mitochondrial protein persulfidation in SM-treated BEAS-2B cells

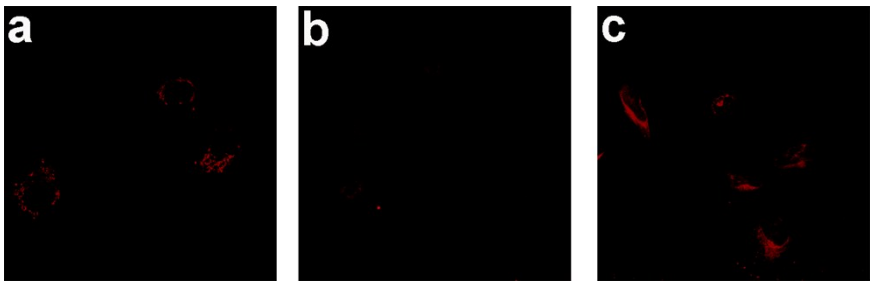


Figure. S5. Cells were incubated with HQO-SSH (10 μM) alone for 6 min (a). The cells were treated with sulfur mustard (50 μM) for 30 min, followed by incubation with HQO-SSH (10 μM) for 6 min (b). Sulfur mustard-treated cells were treated with Na_2S (70 μM) for 30 min, followed by incubation with HQO-SSH (10 μM) for 6 min (c).

Visualizing the change of mitochondrial protein persulfidation in SM-treated lung tissues

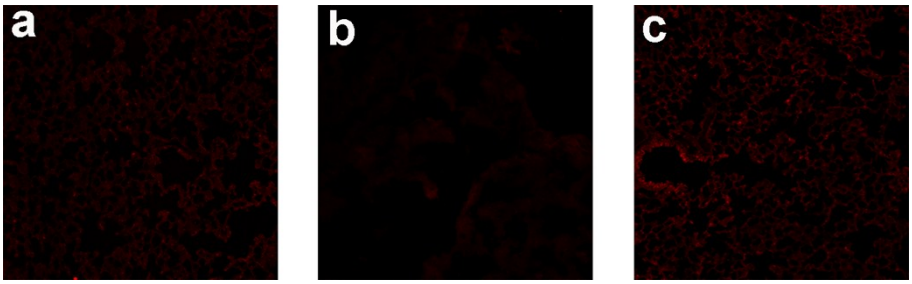
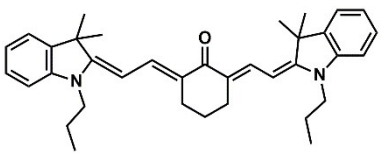
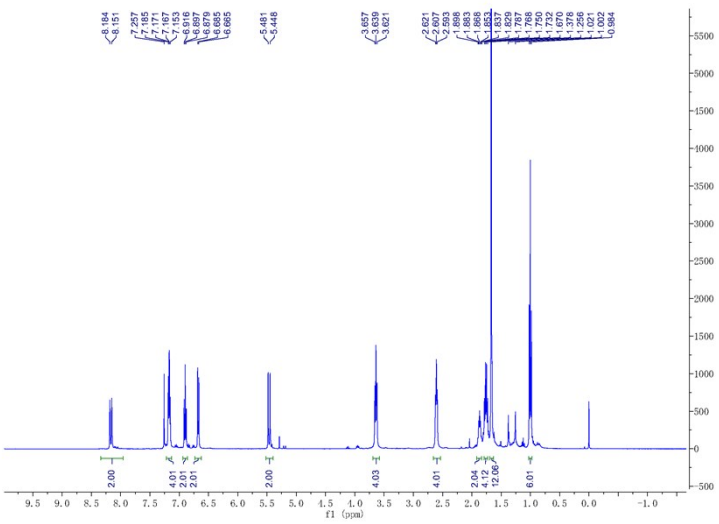


Figure. S6. Mice were injected subcutaneously with sulfur mustard (30 mg/kg), with or without intraperitoneal administration of 5 mg/kg NaHS. Additional NaHS was administered every day for three days. Control (a); sulfur mustard (30 mg/kg) treatment (b); sulfur mustard (30 mg/kg) with NaHS (5 mg/kg) treatment (c).

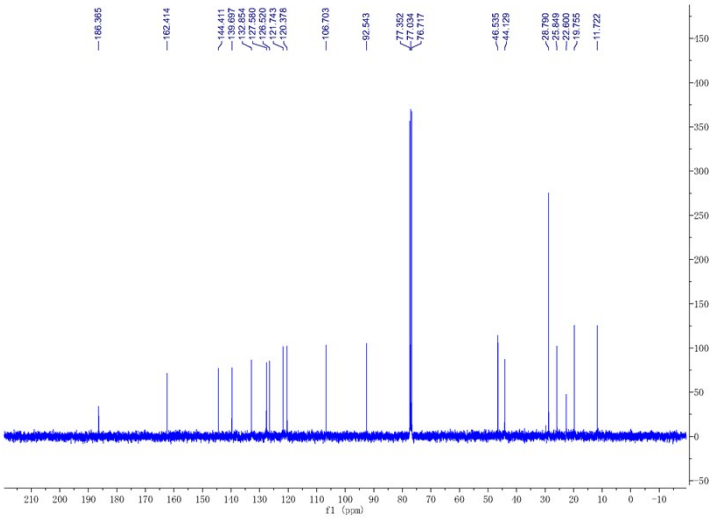
NMR and HRMS spectrum of compounds

HQO

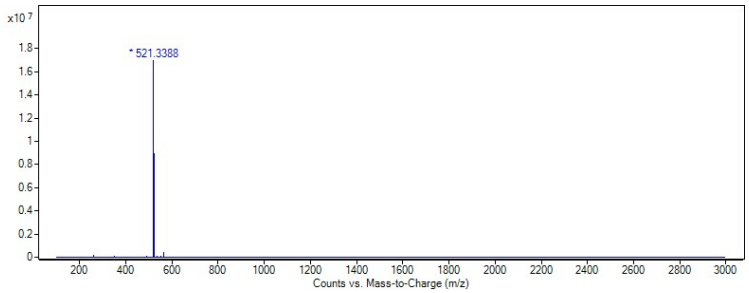




^{1}H NMR for HQO in $CDCl_3$ (ppm)

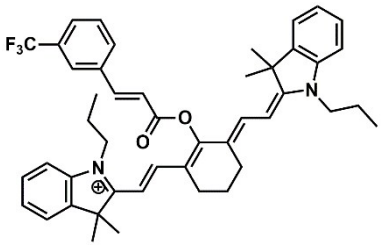


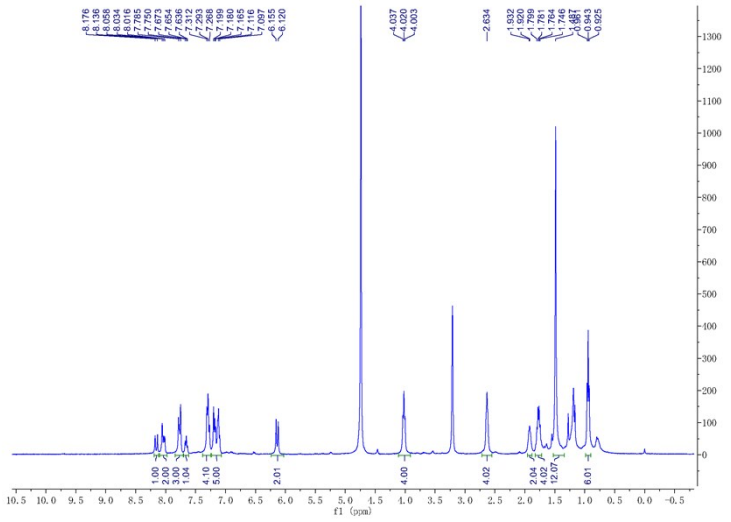
^{13}C NMR for HQO in $CDCl_3$ (ppm)



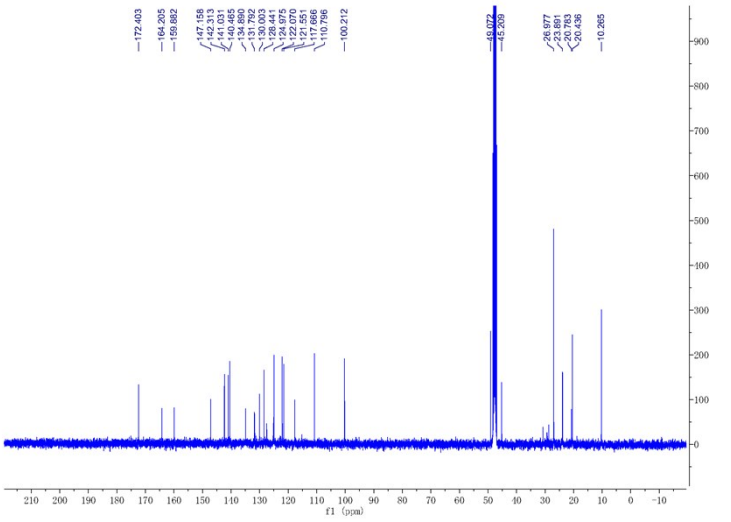
HRMS for HQO in $CHCl_3$

HQO-SSH

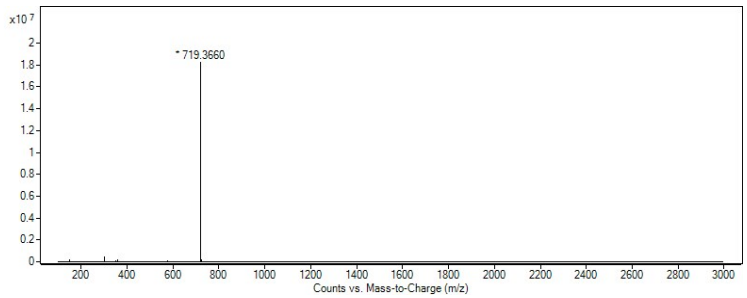




^{1}H NMR for HQO-SSH in CD_3OD (ppm)



^{13}C NMR for HQO-SSH in CD_3OD (ppm)



HRMS for HQO-SSH in $CHCl_3$