Supporting Information

Carbonyl Sulfide (COS) Donor Induced Protein Persulfidation Protects against Oxidative Stress


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Figure S1: Structures of Mesalamine and 7a-7c.

Figure S2. Representative plots for the time course of H$_2$S release as monitored by methylene blue complex formation assay ($\lambda_{\text{max}}$ 676 nm) a) Kinetics of H$_2$S release from compound 1a. b) H$_2$S generation plot for compound 1b.

Figure S3. Representative plot for the time course of H$_2$S release from NQO1-Mes as monitored by methylene blue assay ($\lambda_{\text{max}}$ 676 nm). The rate constant for H$_2$S release was calculated to be 0.012 min$^{-1}$. 
Figure S4. Structure of dicoumarol, 8, a known inhibitor of NQO1 enzyme.

Figure S5. a) HPLC trace for compound 1a in buffer. b) Area under the curve for the formation of p-anisidine. c) Area under the curve corresponding to the formation of lactone. d) Area under the curve for decomposition of compound 1a.
Figure S6. Representative HPLC trace for compound NQO1-Mes in ACN (Retention time (RT) = 13.4 min). b) Representative HPLC traces for the decomposition of compound NQO1-Mes in the presence of NQO1 and NADH with concomitant release of lactone (RT = 5.8 min) and compound 9 (RT = 3.9 min). c) Area under the curve corresponding to the formation of mesalamine methyl ester, 9.

Figure S7. Detection of H$_2$S using sulfide selective electrode. Signal for H$_2$S was observed from compound NQO1-Mes in buffer containing NQO1 and NADH. No current corresponding to H$_2$S release was observed from NQO1-Mes alone in buffer or RPMI media containing 10% FBS.
Figure S8. a) Structures of CN-BOT and methyl sulfonylbenzothiazole (MSBT) used for the detecting cellular persulfidation levels. b) Protein persulfidation induced by NQO1 activated COS/H_2S donors.

Figure S9. Cell viability assay conducted with 1a for 72 h in human breast cancer, MCF-7 cells.
**Figure S10.** Cell viability assay conducted with compound 1a for 24 h in human breast cancer, MCF-7 cells was measured using LDH assay.

**Figure S11.** a) Structure of JCHD. b) Induction of cell death using JCHD in DLD-1 cells in 24 h measured using MTT assay.
Figure S12. a) Cell viability of compound 1a in human colon carcinoma, DLD-1 cells. b) Cytoprotective effects of varying concentrations of 1a against JCHD induced stress in DLD-1 cells in 24 h. c) Cell viability of compound 1b in DLD-1 cells after 24 h. d) Cytoprotective effects of varying concentrations of 1b against JCHD induced stress in DLD-1 cells in 24 h.
Figure S13. a) Cell viability for compounds 1a and 1b in wild type mouse embryonic fibroblast, WT-MEF cells. b) Protection against JCHD induced stress in WT-MEF cells in 24 h using varying concentrations of compounds 1a and 1b.

Figure S14. a) Cell viability for NQO1-Mes in wild type mouse embryonic fibroblast, WT-MEF cells. b) Cytoprotective effects of NQO1-Mes against JCHD (15 µM) induced oxidative stress in WT-MEF cells over a period of 24 h. Results are expressed as Mean ± SEM (n = 3). [ns – non significant]. Compound 5 (50 µM) represents the negative control and Mes represents mesalamine (50 µM).