

Susceptibility of the antioxidant selenoenzymes thioredoxin reductase and glutathione peroxidase to alkylation-mediated inhibition by anticancer acylfulvenes

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Supporting Information

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Figure S1. A reduction in the extracted ion intensity of the active site peptide after reactions with AFs provides further supporting evidence of active site modification. HPLC-ESI⁺-MS analysis of the tryptic digest of TrxR following AFs treatment. TrxR (5 μ M) was allowed to react with AFs (2 μ L, 125 mM) or 2 μ L of DMSO as control in a total volume of 0.2 mL. The reaction was diluted and trypsin digest was allowed to occur at 37 °C for 24 h. Spectra were obtained by full scan data acquisition performed within m/z 100-1500. A1. total ion chromatogram (TIC) of tryptic digest of control TrxR; A2. extracted ion chromatogram (EIC) of active site tryptic peptide (m/z 1142) from the control TrxR; A3. EIC of proposed AF-adducted active site tryptic peptide (m/z 1358) from the control TrxR; A4. EIC of proposed HMAF-adducted active site tryptic peptide (m/z 1388) from the control TrxR. B1. TIC of tryptic digest of AF-treated TrxR; B2. extracted ion chromatogram (EIC) of active site tryptic peptide (m/z 1142) from the AF-treated TrxR; B3. EIC of proposed AF-adducted active site tryptic peptide (m/z 1358) from the AF-treated TrxR. C1. TIC of tryptic digest of HMAF-treated TrxR; C2. extracted ion chromatogram (EIC) of active site tryptic peptide (m/z 1142) from the HMAF-treated TrxR; C3. EIC of proposed HMAF-adducted active site tryptic peptide (m/z 1388) from the HMAF-treated TrxR.

Figure S2. Inhibition curves for treatment of Gpx with iodoacetamide. Gpx (0.4 pmol) was allowed to react with iodoacetamide (1.87 mM) for 40 min in a total volume of 0.5 mL phosphate buffer (0.1 M, 1 mM EDTA, pH 7.0) at 37 °C. Average IC₅₀ for two trials is 2.4 ± 0.1 mM.

Figure S3. LC/MS spectra derived from AFs-treated Gpx. Gpx (1.6 nmol) was allowed to react with AFs (1.25 mM) in a total volume of 1 mL TE buffer. Gpx samples were then concentrated and unbound compound removed before LC/MS analysis.

Figure S4. Response of HeLa cells toward illudin S and AFs. Cells were treated with drugs for 12 h, which is the same treatment time for the cellular TrxR inhibition experiment. By assessing this toxicity first, we selected the drug concentration range that can ensure 80% of cells survive for cellular TrxR activity evaluation. Although high concentrations were tested, IC₅₀s could not be reached due to the short time treatment.

Figure S5. Induction of cellular TrxR by selenite (Na₂SeO₄). HeLa cells were cultured in medium with or without addition of sodium selenite (1 μ M) for three continuous days. The cellular TrxR protein level (A) and activity (B) were determined.

Table S1.

Illudin S (μM)	Average Activity (%)
0	100 \pm 31
0.5	119 \pm 41
2	125 \pm 34
20	128 \pm 36
200	110 \pm 24
1000	160 \pm 10

AF (μM)	Average Activity (%)
0	99 \pm 14
0.5	102 \pm 6
2	102 \pm 17
20	144 \pm 12
200	198 \pm 25
1000	196 \pm 34

HMAF (μM)	Average Activity (%)
0	100 \pm 59
0.25	111 \pm 46
0.5	129 \pm 58
2	162 \pm 12
200	155 \pm 22

Figure S1.

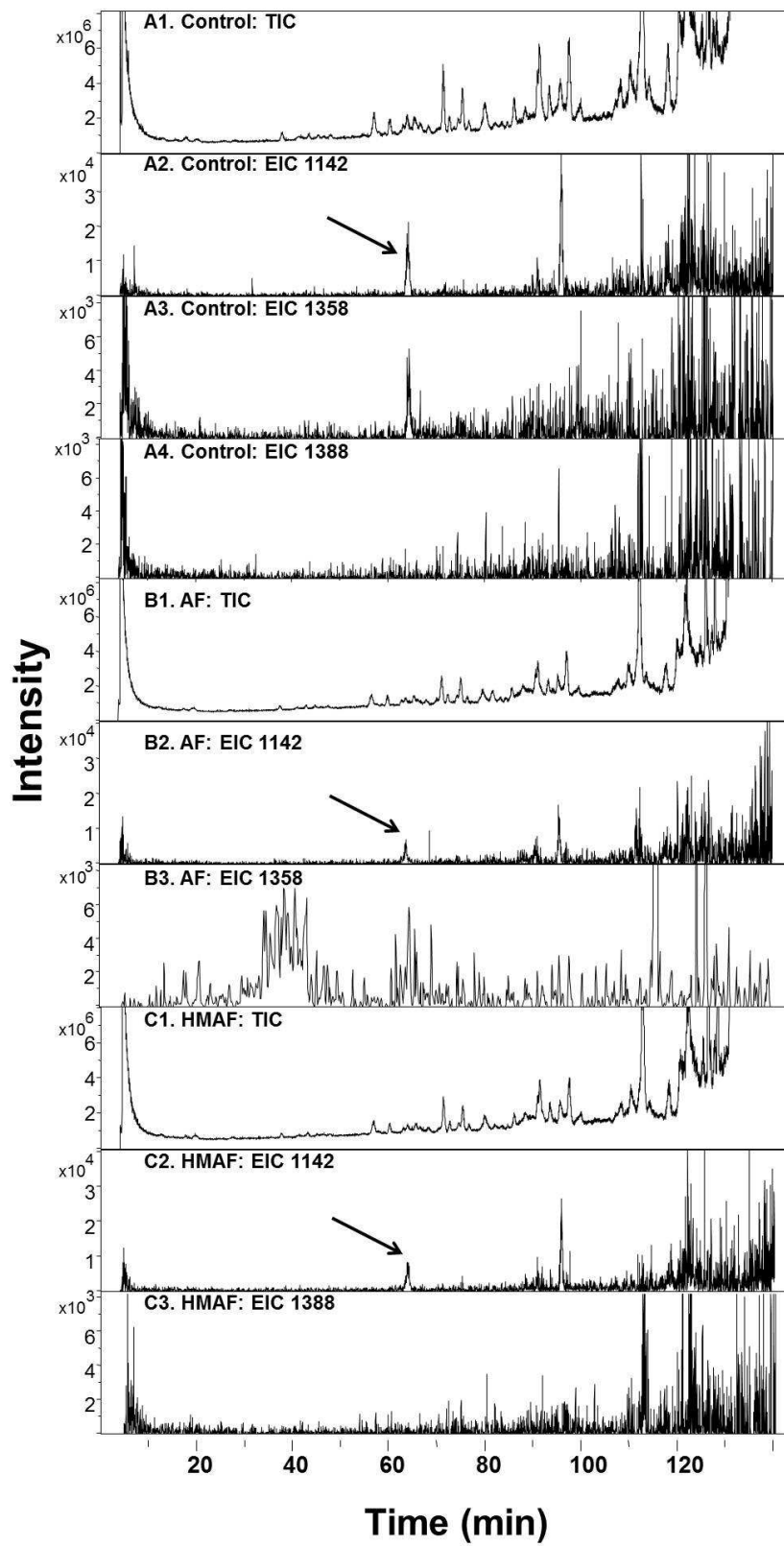


Figure S2.

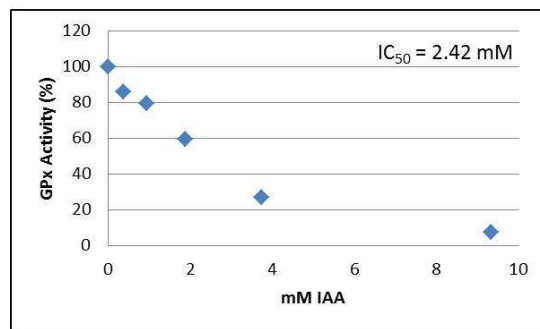
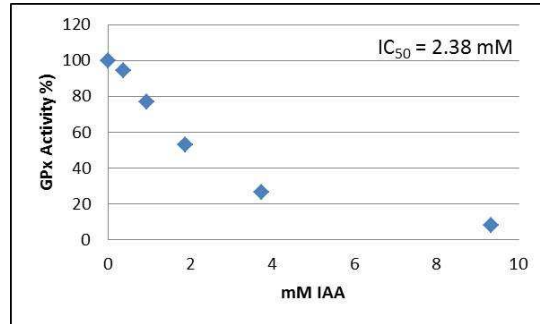


Figure S3.

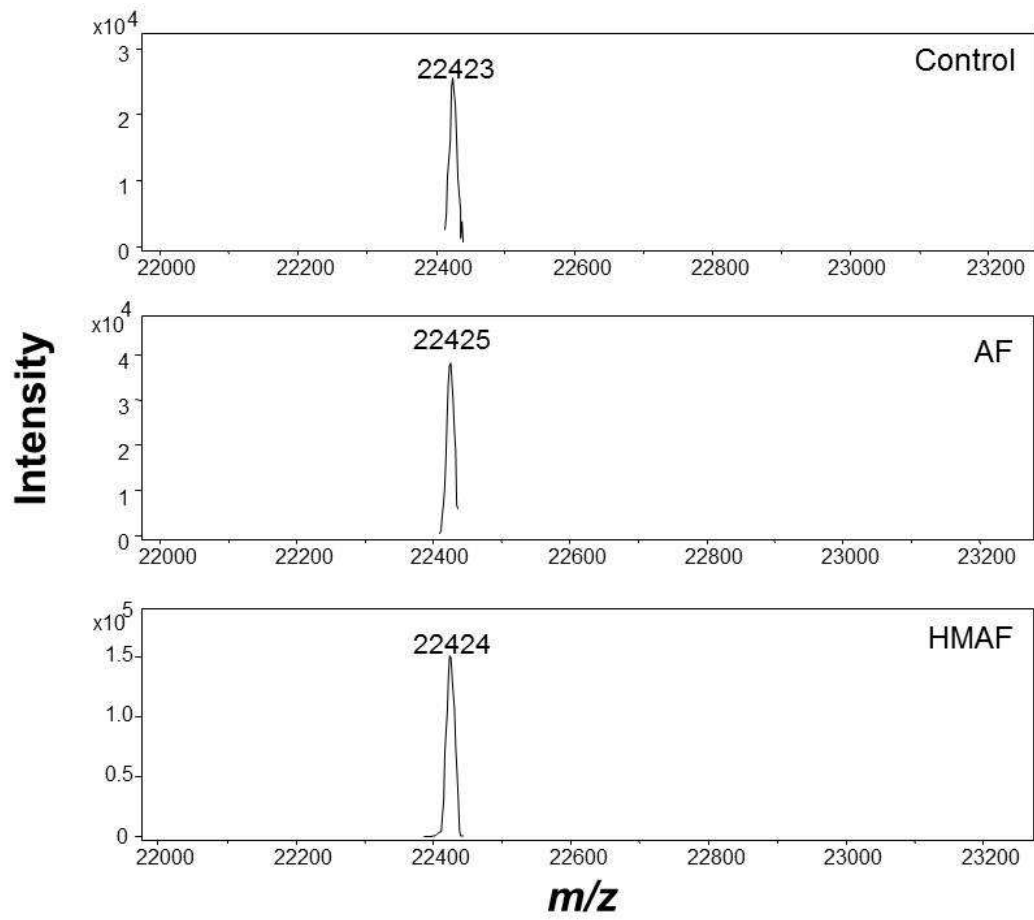


Figure S4.

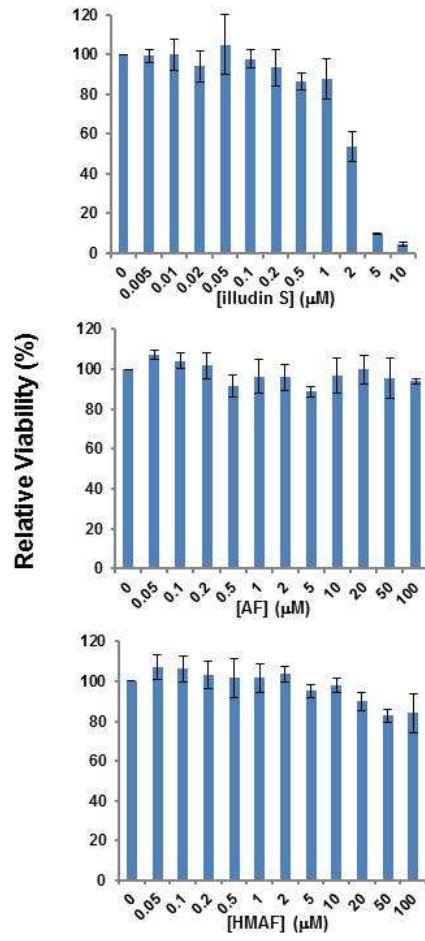


Figure S5.

