**Supplemental Information**

# Redox Signaling by Reactive Electrophiles and Oxidants

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Table S1: A selection of HNE-sensitive proteins reported in the literature.[a]

| **Protein**[b] | **Method used**[c] | $k_{inact}/K_i$ | $k_{inact}$ | $IC_{50}$ **or** $K_i$ | **Residue(s) modified** | **Reference(s)** |
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| 20 S proteasome | Inhibition kinetics | Not reported | Not reported | Chymotrypsin, 50% inhibition after 1-2 min; 20 μM HNE. Trypsin, 50% inhibition after 100-200 min; 20 μM HNE. Postglutamyl low 20% inhibition after 250 min; 20 μM HNE. Crosslinking occurs over 30 min. | Not reported | (1) |
| Actin | MS | Not reported | Not reported | Not reported | C374; C257 | (2) |
| β-actin (ACTB) | MS; anti-HNE western blot | Not reported | Not reported | Not reported | H40 | (3) |
| α-actinin-1 (ACTN1) | MS | Not reported | Not reported | Not reported | C480 | (3) |
| α-actinin-4 (ACTN4) | MS | Not reported | Not reported | Not reported | C499 | (3) |
| Adenine nucleotide translocator (ANT) | Inhibition kinetics (mitochondria isolated from mice) | Not reported | Not reported | 700 μM (37 °C) | Not reported | (4) |
| Adenylyl cyclase associated protein 1 (CAP1) | MS; anti-HNE western blot | Not reported | Not reported | Not reported | C93 | (3) |
| Adipocyte fatty acid binding protein (FABP4) | MS; x-ray crystallography, mouse protein; one covalent | Not reported | Not reported | 50% labeling after 10 mins with 0.5 mM HNE (*R* or *S* enantiomer or racemic mixture) | C117 (for covalent adduct); GSTa4 shown to be reduced in obese | (5,6) |

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| | adduct and one low occupancy non-covalent structure disclosed (all on mouse protein) | | | | tissue | |
| Adipocyte fatty acid binding protein (FABP4) | | Not reported | Not reported | Not reported | C117 (for covalent adduct) | |
| ADP-ribosyltransferase (ART) | Inhibition kinetics | Not reported | Not reported | $K_i$ = 4 µM (Dickinson plot) | Not reported | (7) |
| Alcohol dehydrogenase (ADH) | Anti-HNE western blot; MS; inhibition assay; proteasomal stability assay (protein isolated from equine liver) | Not reported | Not reported | Not saturated at 200 µM HNE after 16 h; no effect on activity; bell-shaped effect on proteasomal stability | C46; C111 (involved in chelating zinc in active site) | (8) |
| Amyloid beta (Aβ) | Gel shift assay (Peptide containing residues 1-40 of Aβ) | Not reported | Not reported | Complex gel shift pattern around 50 µM protein and 50-500 µM HNE | Not explicitly reported, but the peptide investigated contains only one cysteine | (9) |
| Apolipoprotein B (APOB) | Gel shift assay; APOB degradation by macrophages | Not reported | Not reported | Gel shift observed upon treatment with 6 mM HNE; degradation by macrophages suppressed by ~50% upon treatment with 8 mM HNE | Not reported | (10) |

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| ATPase sarcoplasmic/endoplasmic reticulum $Ca^{2+}$ transporting 1 (ATP2A1 or SERCA1a) | Activity assay; MS; anti-HNE western blot; ABPP with FITC (S/ER vesicles isolated from rabbits) | Not reported | Not reported | Not reported | C471; C525; C561; C614; C636; C670; C674 or C675; K515 | (11) |
| Carbonic anhydrase (CA) | ABPP; HPLC shift; activity assay; anti-HNE western blot | Not reported | Not reported | 30% loss of activity upon treatment of enzyme with 1 mM HNE | Not reported | (3,12) |
| Cardiac Mitochondrial $NADP^+$-isocitrate Dehydrogenase ($mNADP^+$–ICDH) | Inhibition kinetics; anti-HNE western blot (Rat hearts and isolated mitochondria) | Not reported | Not reported | $IC_{50}$ ~ 20 μM (10 min treatment) | Not reported | (13) |
| Cathepsin B (CTSB) | Inhibition assay; anti-HNE western blot; MS (mouse macrophages) | Not reported | Not reported | $IC_{50}$ ~15-25 μM (3 h treatment) | C229 (active site); H150 | (14) |
| Cofilin 1 (COF1) | MS | Not reported | Not reported | Not reported | C139 | (3) |
| Creatine kinase B (CKB) | Inhibition assay; MS | Not reported | Not reported | $IC_{50}$ ~ 50 μM based on activity (2h treatment of 10 μM enzyme; possibly limited by enzyme) $IC_{50}$ ~ 10-30 μM based on C283 modification | C283 (10 μM HNE treatment) H7; H26; C141; C145; C254; C283 (30 μM HNE treatment) Many other modifications | (15) |

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| | | | | | found at higher treatment concentrations | |
| Cytochrome C (CYCS) | MS (Protein isolated from equine heart) | Not reported | Not reported | Not reported | K5; K7; K8; K25; K27; H33; R38; K39; K55; K60; K72; K73; K79; K86; K87; K88; K99 | (16,17) |
| Cytochrome *c* oxidase (COX) | Inhibition kinetics [Isolated rat mitochondria, ref. (18); rat liver mitochondria, ref. (19)] | $k_{obs}$ = 0.001 $s^{-1}$ for inhibition [10 μM HNE treatment, ref. (18)] | Not reported | $IC_{50}$ ~ 8 mM [10 min treatment of mitochondrial fractions, ref. (18)]<br><br>$IC_{50}$ ~ 180 μM [1 or 2 h treatment of isolated protein or mitochondria, respectively, ref. (19)] | Not reported | (18,19) |
| D-3-phosphoglycerate dehydrogenase (SERA) | MS | Not reported | Not reported | Not reported | C369 | (3) |
| Dynein light chain Tetex-type 3 (DYLT3) | MS | Not reported | Not reported | Not reported | H7 | (3) |
| Elastin (ELN) | Anti-HNE western blot; activity assay | Not reported | Not reported | $IC_{50}$ ~ 60 μM based on activity (24 h treatment)<br><br>$IC_{50}$ ~ 10 μM based on western blot | Not reported | (20) |

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| | | | | (48 h treatment) | | |
| α-enolase (ENO1) | Anti-HNE western blot | Not reported | Not reported | Little quantitative information given | Not reported | (3,21) |
| Epithelial fatty acid binding protein (E-FABP) | Anti-HNE western blot; MS | Not reported | Not reported | Not reported | C120; C127; K115 | (22) |
| Eukaryotic elongation factor 2 (eEF-2) | ABPP; anti-HNE western blot [rats and rat liver homogenates, ref. (23)] | Not reported | Not reported | $IC_{50}$ ~ 75 μM | C41 | (23-25) |
| F-actin capping protein (CAPZB) | MS | Not reported | Not reported | Not reported | C93 | (3) |
| Fructosamine 3 kinase-related protein (FN3KRP) | ABPP | Not reported | Not reported | $IC_{50}$ ~60 μM | C24 | (24,25) |
| Fructose-bisphosphate aldolase A (ALDOA) | MS | Not reported | Not reported | Not reported | H246 | (3) |
| Glucose-6-phosphate dehydrogenase (G6PD) | Inhibition kinetics | Not reported | Not reported | $K_i$ = 1.5 mM (noncompetitive inhibition) | K205 | (26) |
| Glutamate transporter (GLT-1) | Anti-HNE western blot; activity assay (rat astrocytes) | Not reported | Not reported | $IC_{50}$ ~ 10–15 μM (3–5 h treatment) | Not reported | (27) |
| Glutathione peroxidase | Inhibition kinetics | Not reported | Not reported | $IC_{50}$ = 0.12 mM (30 min treatment) | Not reported | (28) |

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| (GPX) | | | | | | |
| Glutathione reductase (GSR) | Inhibition kinetics | $k_{inact}$ and $K_i$ likely uncoupled | $2.2\times10^{-4}$ $s^{-1}$ | $K_i$ = 0.5 μM | Not reported | (29) |
| Glutathione S-transferase α1 (GSTA1) | MS | Not reported | Not reported | Rate of disappearance of unadducted species ~ 0.17 $h^{-1}$ | Not reported | (30) |
| Glutathione S-transferase π1 (GSTP1) | MS; activity assay | Not reported | Not reported | Rate of disappearance of unadducted species ~ 0.31 $h^{-1}$ | K30; K55; K103; K128; C48; C102 | (30,31) |
| Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) | Inhibition kinetics | 3 $M^{-1}s^{-1}$ | Not reported | $IC_{50}$ ~ 20 μM (3h treatment) | Not reported | (32,33) |
| Heat shock protein 70 (HSP70) | Streptavidin-HRP detection of HNE-alkyne modified protein [yeast Ssa1, ref. (34)]; inhibition assay; anti-HNE western blot; MS | Not reported | Not reported | $IC_{50}$ ~ 400-500 μM [1 h treatment, ref. (34)]<br><br>No labeling saturation at 100 μM [16 h treatment of 1.6 μM protein, ref. (35)] | C303 [ref. (34)]<br><br>C267 [ref. (35)] | (34,35) |
| Heat shock protein 90 (HSP90) | Inhibition kinetics | Not reported | Not reported | $IC_{50}$ = 45 μM (40 min treatment)<br><br>$IC_{50}$ = 45 μM (30 min treatment)<br><br>$IC_{50}$ = 40 μM (20 min treatment) | C572 | (3,36) |
| Heme oxygenase 2 (HMOX2) | ABPP | Not reported | Not reported | $IC_{50}$ ~ 2 μM | C282 | (24) |

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| Human serum albumin (HSA) | MS | $k_{obs}$ for H242, 3–8 $h^{-1}$; $k_{obs}$ of other residues at least 1 order magnitude slower [5 μM HSA treated with 3.2 mM HNE, ref. (37)]<br><br>$k_{obs}$ for H105, $0.027 \pm 0.004$ $M^{-1}s^{-1}$; H367, $0.025 \pm 0.00$ $M^{-1}\,s^{-1}$; H67, $0.088 \pm 0.009$ $M^{-1}s^{-1}$; H510, $0.083 \pm 0.004\ M^{-1}\,s^{-1}$; | Not reported | Not reported | H67; H105; K199; K233; H242; H247; H288; H367; H510 | (37,38) |

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| | | H242/247 and H288, ~$0.2 \pm 0.1$ $M^{-1}$ $s^{-1}$; K199, ~$0.2 \pm 0.1$ $M^{-1}$ $s^{-1}$ [15 μM HSA treated with 1.5 mM HNE, ref. (38)] | | | | |
| IkB kinase (IKK) | Activity assay; gel shift assay | Not reported | Not reported | Labeling observed 30-60 μM HNE (10 min treatment) 30 μM HNE-treated and imunoprecipitated IKK was not active (30 min treatment) | Not reported | (39) |
| Leucine-rich repeat-containing protein 59 (LRC59) | MS | Not reported | Not reported | Not reported | H294 | (3) |
| Liver microsomal cytochrome P450 | Activity assay; tritium-labeled HNE incorporation into microsomes (mouse liver) | Not reported | Not reported | $IC_{50}$ ~ 250-500 μM for degradation (60 min treatment) $IC_{50}$ ~ 1 mM for tritiated HNE incorporation into microsomes | Not reported | (40) |
| Matrix metalloprotease | MS | Not reported | Not reported | ~20 min to reach 50% occupancy | H340/343; H251 | (41) |

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| 13 (MMP13) | | | | (200 μM HNE treatment) | | |
| Mitogen-activated protein kinase 1 (MAPK1 aka ERK2) | Anti-HNE western blot | Not reported | Not reported | $IC_{50}$ ~ 5 μM (4 h treatment; some crosslinking observed at 100 μM HNE treatment) | H178; C63; H230 | (42) |
| $Na^+$-$K^+$-ATPase | Inhibition kinetics | Not reported | Not reported | $IC_{50}$ = 120 μM (30 min treatment) | Not reported | (43) |
| NADPH oxidase 2 (NOX2) | Labeling by HNE-alkyne biotin pulldown and western blot | Not reported | Not reported | Not reported | Not reported | (44) |
| Peroxiredoxin 6 (PRDX6) | MS; ABPP; Inhibition kinetics *in vitro* | Not reported | Not reported | 350 μM | C91 [refs. (3,45,46)] C47 [ref. (46)] | (3,45,46) |
| Phosphatase and tensin homolog (PTEN) | Activity assay; anti-HNE western blot; MS | Not reported | Not reported | $IC_{50}$ ~ 2 μM for activity inhibition (30 min treatment) | Not reported | (47) |
| Plasminogen activator inhibitor 1 RNA-binding protein (PAIR) | MS | Not reported | Not reported | Not reported | C11 | (3) |
| Protein arginine methyltransferase 1 (PRMT1) | ABPP, activity assay | Not reported | Not reported | $IC_{50}$ < 25 μM for both labeling and activity inhibition (30 min treatment) | C101 | (48) |
| Protein disulfide isomerase (PDI) | Inhibition kinetics | Not reported | Not reported | $IC_{50}$ ~ 30 μM (30 min treatment) | Not reported | (3,49,50) |
| Protein kinase C β | Activity assay (rat hepatocytes) | Not reported | Not reported | $IC_{50}$ ~ 4 μM (15 min treatment) | Not reported | (51) |

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| (PRKCB) | | | | | | |
| Protein kinase M2 (PKM2) | Activity assay; labeling by HNE-alkyne biotin pulldown and western blot | Not reported | Not reported | $IC_{50}$ ~ 40 μM for activity inhibition<br><br>$IC_{50}$ ~ 20 μM for labeling | C49; H272; C424; H439; K256 | (52) |
| RAC-β serine/threonine-protein kinase (Akt2) | Inhibition kinetics; Anti-HNE western blot; MS | Not reported | Not reported | $IC_{50}$ ~ 40 μM for labeling<br><br>$IC_{50}$ ~ 30 μM for activity inhibition | H196; H267; C311 | (53) |
| Reticulon-4 (RTN4) | ABPP | Not reported | Not reported | $IC_{50}$ ~ 75 μM | C1101 | (24,25) |
| Rhodopsin (RHO) | MS | Not reported | Not reported | Not reported | Not reported | (54) |
| Ro ribonucleoprotein | Anti-HNE western blot and antigen generation | Not reported | Not reported | Not reported | Not reported | (55) |
| SAM domain and HD domain binding protein (MOP-5) | MS | Not reported | Not reported | Not reported | C522 | (3) |
| Signal recognition particle 9 kDa protein (SRP09) | MS | Not reported | Not reported | Not reported | C48 | (3) |
| Sirtuin 3 (SIRT3) | Anti-HNE western blot; activity assay | Not reported | Not reported | 25% decrease in activity upon treatment with 100 μM HNE (30 min treatment) | C280; H354 | (56) |
| α- and β-spectrin | Anti-HNE | Not | Not | Labeling saturation reached | Not reported | (57) |

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| (SPTA1 and SPTB) | western blot | reported | reported | between 5-10 min upon 0.1 mM HNE treatment | | |
| Sterile α motif and leucine zipper containing kinase AZK (ZAK) | ABPP; inhibition kinetics | Not reported | Not reported | $IC_{50}$ ~15 μM [ref. (24)]<br><br>$IC_{50}$ < 10 μM [15 min on ice,15 min reaction–ref. (25)] | C22 | (24,25) |
| Superoxide dismutase (Cu, Zn, and Mn) | Gel shift assay and amino acid analysis | Not reported | Not reported | < 50% saturation obtained upon treatment with 2.5 mM HNE (6h treatment); no change in activity under these conditions | Lysines and histidines likely labeled, although not linked to function | (58) |
| Thioredoxin (TXN) | MS; Inhibition kinetics | Not reported | Not reported | Not reported | C32; C35 | (59) |
| Thioredoxin reductase 1 (TXNRD1) | MS; activity assay; inhibition kinetics | Not reported | Not reported | $IC_{50}$ = 3.8 μM (2h treatment) | C496; U497 | (59) |
| Transient receptor potential cation channel subfamily V member 1 (TRPV1) | Anti-HNE western blot | Not reported | Not reported | Not reported | C621 | (60) |
| Tubulin | Anti-HNE western blot; MS; activity assay [Bovine brain tubulin, ref. (61); 3T3 mouse fibroblasts, ref (62)] | Not reported | Not reported | $IC_{50}$ ~ 100-500 μM for polymerization inhibition (5 min treatment; possibly limited by protein concentration) | C295 [ref. (3)]<br><br>C347, C376, C308 [ref. (61)] | (3,61,62) |
| Vimentin | MS; anti-HNE | Not | Not | Not reported | C328 | (3) |

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| (VIME) | western blot | reported | reported | | | |
| Voltage-dependent anion-selective channel protein 2 (VDAC2) | MS; ABPP | Not reported | Not reported | $IC_{50}$ ~5 μM | C47 [ref. (3)]<br><br>C210 [ref. (24)] | (3,24) |

[a]References are given in the last column. In cases where multiple sources reported different data for the same parameter(s), the particular reference is indicated next to the data point.
[b]Human gene name in brackets where specified in the original report.
[c]Where the human protein was not used, the species is indicated.

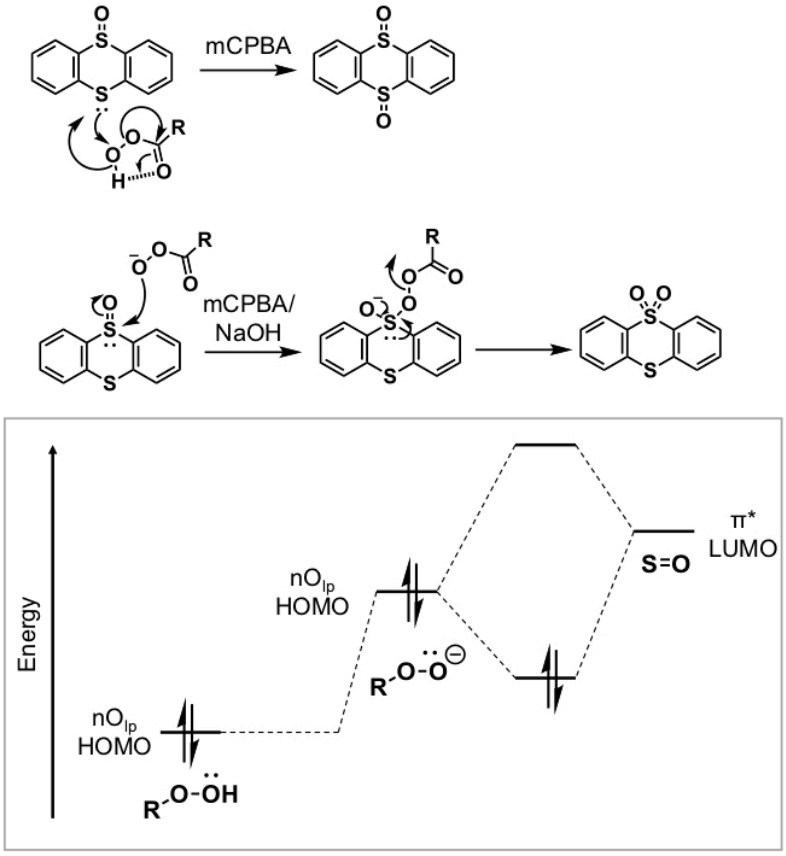


Figure S1: In thianthrene-5-oxide, a substrate bearing both a thioether and a sulfoxide, electrophilic oxidants such as mCPBA selectively oxidize the thioether whereas nucleophilic oxidants (e.g. mCPBA/NaOH) selectively oxidize the sulfoxide. This selectivity is explained by frontier-molecular orbital interactions (*inset*). The lone pairs on sulfur of the thioether (HOMO) are lower in energy and best matched to overlap with the LUMO of the protonated peracid. The lone pairs on the deprotonated peracid ($nO_{lp}$: non-bonding lone pairs on oxygen), however, are raised in energy (HOMO) and best matched to overlap with the π* of sulfoxide. For clarity, only the interaction with the sulfoxide π* is shown.

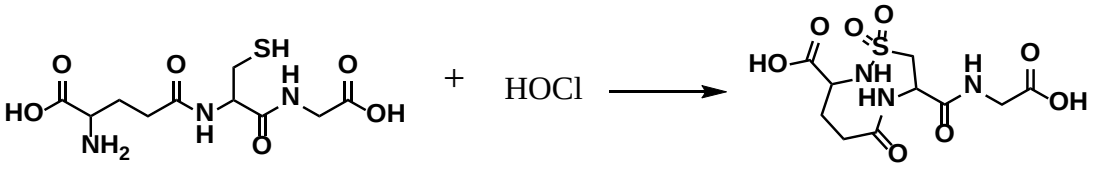


Figure S2: Oxidation of GSH by HOCl yields glutathione sulfonamide.

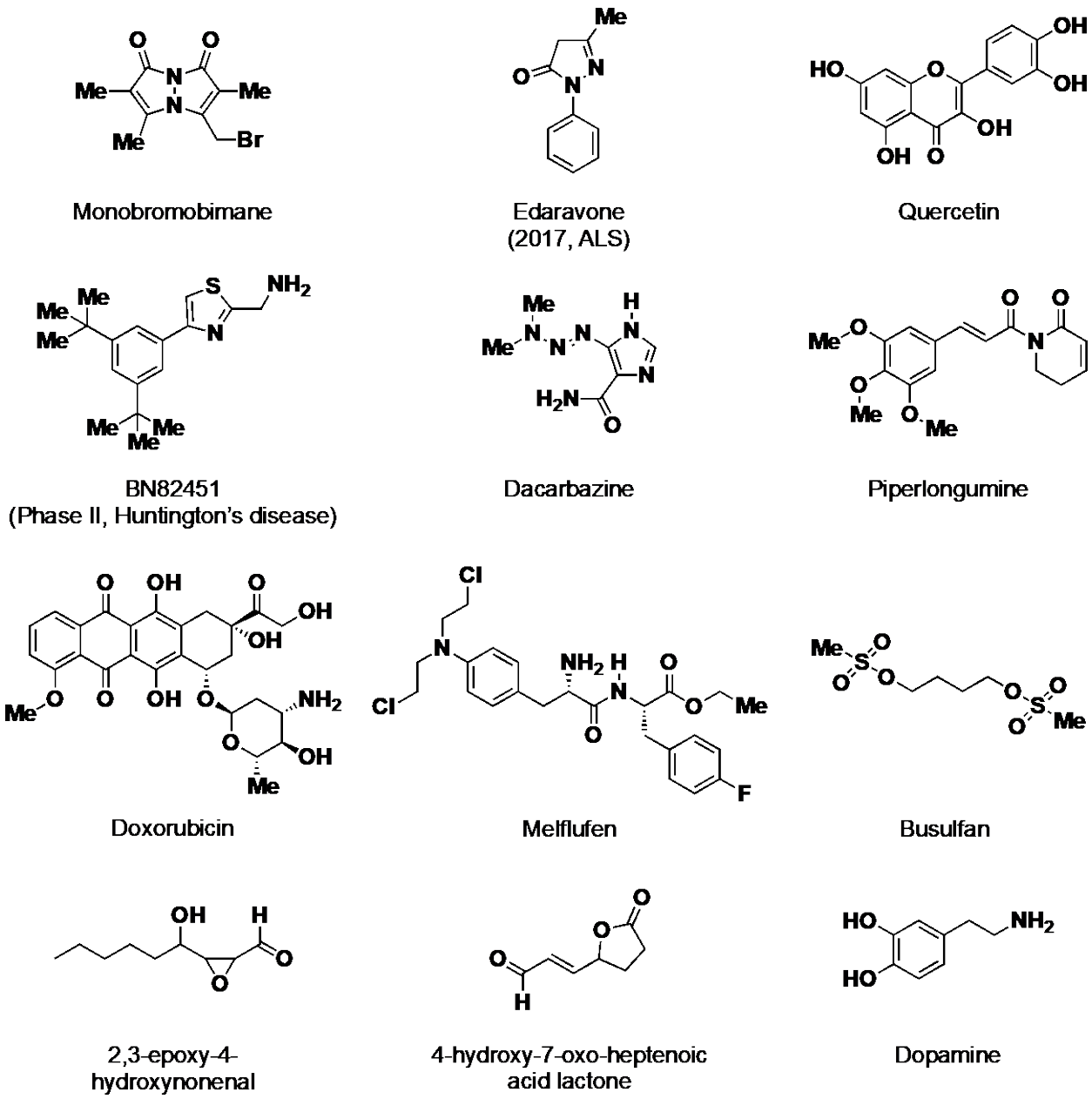


Figure S3: Structures of other various compounds discussed in the review (in cases where the compounds are used therapeutically, either the year of FDA approval or the clinical trial stage reached is given in brackets). ALS, amyotrophic lateral sclerosis.

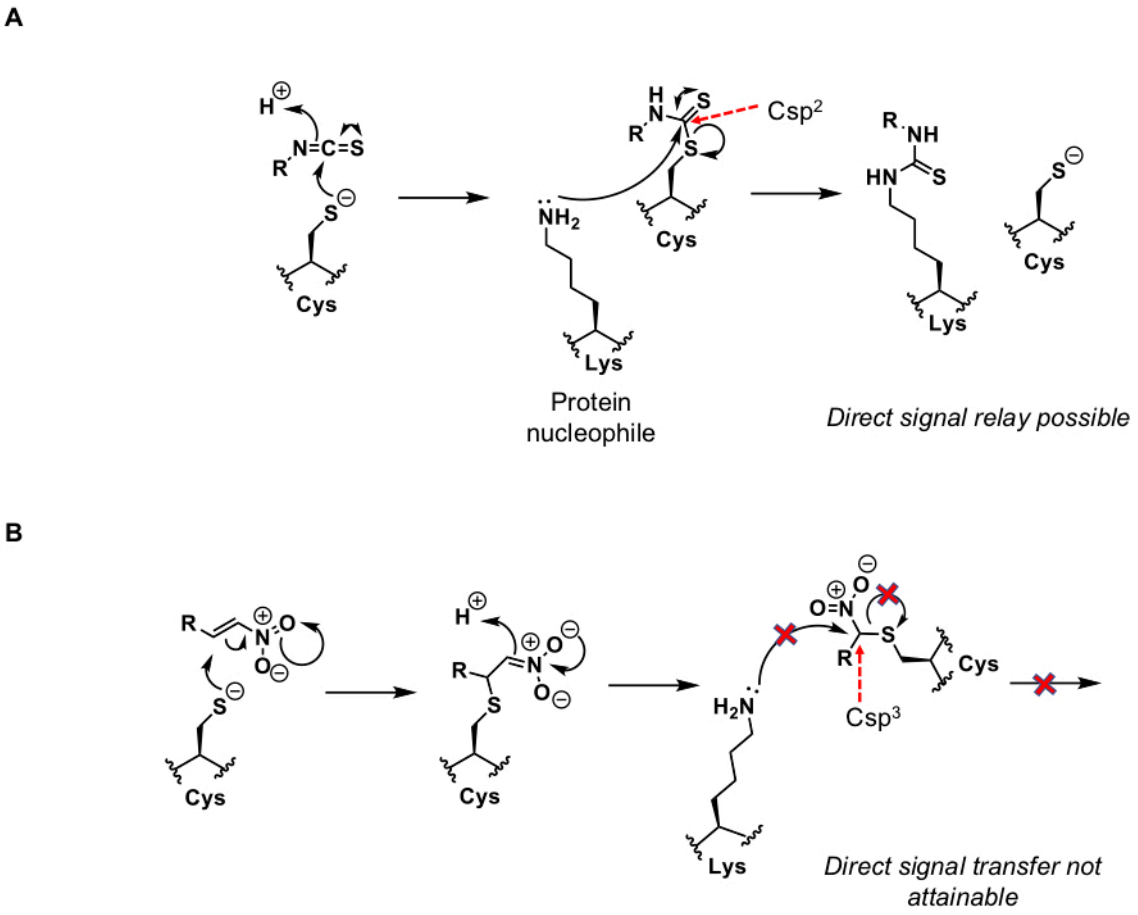


Figure S4: (A) Cysteine adducts to isothiocyanates (ITCs) are reversible. The $sp^2$-hybridized carbon of the thiourea can undergo nucleophilic attack by a protein nucleophile (e.g. lysine), resulting in a tetrahedral intermediate. Subsequent cysteine thiolate departure results in signal transfer. (B) Cysteine adducts to nitroolefins are not directly transferable. Nucleophilic attack on the $sp^3$-hybridized carbon is not a viable route to cleave the carbon-thiolate bond as a means to directly transfer the signal to the proximal lysine residue.

**Supplemental References**


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