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APPLICATION OF THE HARD AND SOFT, ACIDS AND BASES (HSAB) THEORY TO TOXICANT-TARGET INTERACTIONS

Richard M. LoPachin[8], **Terrence Gavin**[§], **Anthony DeCaprio**[¥], and **David S. Barber**[+]

[8]Department of Anesthesiology, Montefiore Medical Center, 111 E.210th St., Bronx, NY 10467

[§]Department of Chemistry, Iona College, New Rochelle, NY 10804

[¥]Department of Chemistry and Biochemistry, Florida International University, 11200 S.W. 8th St. Miami, FL 33199

[+]Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL 32611

Abstract

Many chemical toxicants and/or their active metabolites are electrophiles that cause cell injury by forming covalent bonds with nucleophilic targets on biological macromolecules. Covalent reactions between nucleophilic and electrophilic reagents are however discriminatory, since there is a significant degree of selectivity associated with these interactions. Over the course of the past few decades, the theory of Hard and Soft, Acid and Bases (HSAB) has proven to be a useful tool in predicting the outcome of such reactions. This concept utilizes the inherent electronic characteristic of polarizability to define, for example, reacting electrophiles and nucleophiles as either hard or soft. These HSAB definitions have been successfully applied to chemical-induced toxicity in biological systems. Thus, according to this principle, a toxic electrophile reacts preferentially with biological targets of similar hardness or softness. The soft/hard classification of a xenobiotic electrophile has obvious utility in discerning plausible biological targets and molecular mechanisms of toxicity. The purpose of this Perspective is to discuss the HSAB theory of electrophiles and nucleophiles within a toxicological framework. In principle, covalent bond formation can be described by using the properties of their outermost or frontier orbitals. Because these orbital energies for most chemicals can be calculated using quantum mechanical models, it is possible to quantify the relative softness (σ) or hardness (η) of electrophiles or nucleophiles and to subsequently convert this information into useful indices of reactivity. This atomic level information can provide insight into the design of corroborative laboratory research and thereby help investigators discern corresponding molecular sites and mechanisms of toxicant action. The use of HSAB parameters has also been instrumental in the development and identification of potential nucleophilic cytoprotectants that can scavenge toxic electrophiles. Clearly, the difficult task of delineating molecular sites and mechanisms of toxicant action can be facilitated by the application of this quantitative approach.

Keywords

α,β-unsaturated carbonyl derivatives; acrolein; 4-hydroxy-2-nonenal; oxidative stress; neuroprotection; HSAB theory

Corresponding Authors: Richard M. LoPachin, Ph.D., Department of Anesthesiology, Montefiore Medical Center, 111 E.210th St. Bronx, NY 10467, (718) 920-5054 (phone), (718) 515-4903 (fax), lopachin@einstein.yu.edu. Terrence Gavin, Ph.D. Department of Chemistry, Iona College, 402 North Ave. New Rochelle, NY 10804, (914) 633-2301 (phone), tgavin@iona.edu.

INTRODUCTION

Unlike the formation of drug-receptor complexes that primarily involve short distance forces such as hydrogen-bonding, hydrophobic or van der Waals interactions, many chemical xenobiotics and/or their active metabolites are electrophiles (electron deficient) that form covalent bonds (e.g., S_N2, Schiff base formation, Michael addition) with cognate nucleophilic (electron rich) sites on biological targets[1–10]. Cytotoxicity occurs because the formation of covalently bonded adducts can impair the functions of enzymes, DNA, cytoskeletal proteins and other macromolecules, thereby leading to defective cell processes. Thus, it is now recognized that electrophilic α,β-unsaturated carbonyl derivatives of the type-2 alkene chemical class (e.g., acrolein, acrylamide or methylvinyl ketone) cause broad organ system toxicity by forming covalent Michael-type adducts with nucleophilic sulfhydryl groups on functionally-critical proteins[11–20]. However, electrophiles do not react indiscriminately with nucleophiles and instead such interactions occur along a continuum of relative reactivity. The significant degree of selectivity that occurs in electrophile-nucleophile interactions is predicted by the Hard and Soft, Acid and Bases (HSAB) theory of Pearson[5,17,21]. Based on this concept, reactive molecules are assessed on the basis of their respective polarizability such that electrophiles and nucleophiles are classified as either soft (relatively polarizable) or hard (relatively non-polarizable). A useful theorem stemming from this principle is that toxic electrophiles react preferentially with biological targets of similar hardness or softness. Therefore, the α,β-unsaturated carbonyls can be viewed as soft electrophiles that cause cytotoxicity by selectively forming adducts with soft nucleophilic anionic thiolates on functionally critical proteins[9,14–16,18].

Whereas the HSAB theory initially described hardness and softness in terms of the experimental ionization potential and electron affinity of the reacting molecules, these parameters also can be related (e.g., by Koopmans theorem) to the respective energies of the outermost or frontier molecular orbitals (FMOs). Since small molecule FMO energies can be determined computationally using various quantum mechanical models, HSAB parameters such as the softness (σ) or hardness (η) of an electrophile and its nucleophilic target can be readily derived. Correspondingly, the values for σ and η can be used in a more recently developed algorithm to calculate the electrophilic index (ω) of a toxicant, the value of which reflects the propensity of the electrophile to form adducts with a given biological nucleophile. Although more complex, the respective σ and η values for the nucleophile can also be used to derive an index of nucleophilicity (ω^-, see ahead). On a pragmatic basis these parameters can be used to predict the toxic potential of electrophilic xenobiotic chemicals and to facilitate the identification of corresponding nucleophilic molecular sites of action[5,8,17]. Indeed, our research has shown that, excluding steric and other physiochemical issues (see ahead), the calculated values of these descriptors (σ, ω, ω^-) directly corresponded to the second order rate constants for α,β-unsaturated carbonyl adduction of cysteine sulfhydryl groups. For individual members of a α,β-unsaturated carbonyl series, the kinetic and HSAB parameters were closely correlated to the respective magnitude of in vitro toxicity; i.e., disruption of synaptosomal function[14–16,18]. This ability of HSAB parameters (σ, η, ω, ω^-) to predict the potency of a given chemical toxicant has significant implications for risk assessment[19,20]. In addition, these parameters are a useful tool in the search for macromolecular targets and subsequent elucidation of molecular mechanisms of toxicity. Therefore, in this Perspective, we will discuss the HSAB theory and define the physiochemical characteristics of hard and soft, electrophiles and nucleophiles. As we will point out, establishing structure-activity relationships (SAR) based on kinetic and HSAB properties can reveal the substructure (e.g., α,β-unsaturated aldehyde) or moiety (e.g., carbonyl group) responsible for the toxicity of electrophile. This forms a rational basis for categorizing a chemical series and for understanding the corresponding toxic mechanism[19,22,23]. Finally, in this Perspective we will present examples from the literature

as a framework for demonstrating how quantum chemical calculations can be effectively applied toward understanding molecular toxicodynamic issues.

HSAB DEFINITIONS: SOFT AND HARD, ELECTROPHILES AND NUCLEOPHILES

Physiochemical Principles Determining the Selective Interactions of Electrophile and Nucleophile

HSAB theory classifies reacting species as either relatively “hard” or “soft”, based on polarizability; i.e., the ease with which electron density can be displaced or delocalized to form new covalent bonds. Polarizability is an inherent characteristic of electron distribution in atoms or molecules. Thus, electrons that are far from the influence of the nucleus or that occupy a large volume (electron “cloud”) are more likely to be displaced; i.e., redistributed into a new bonding pattern. For example, the conjugated α,β-unsaturated carbonyl structure of many type-2 alkenes is considered to be a soft electrophile (Table 1) because the delocalized *pi* electrons are mobile. This mobility can be considered the extension of an electron cloud over four nuclear centers (C=C-C=O) based on interactions between the orbitals of the electron-withdrawing carbonyl group and those of the alkene moiety. The extended cloud is easily distorted hence, “soft” (polarizable). In contrast, hard electrophilic toxicants, e.g., chloroethylene oxide or vinyl chloride (Table 1), have highly localized, non-extended charge densitites at specific electrophilic centers; e.g., the respective C1 carbon atoms. Consequently, these chemicals are characterized by low electron polarizability. The softness or hardness of a nucleophile is also determined by the polarizability of corresponding electrons. Sulfur has a large atomic radius so that its valence electrons are relatively far from the nucleus and, as such, are highly polarizable. Upon ionization of the thiol (i.e., $SH \rightarrow S^{-}$), the consequent expansion of the anionic cloud yields the relatively soft (most easily polarizable) thiolate nucleophile; e.g., compare the respective thiol- and thiolate-states of the amino acid residue, cysteine (Table 2). In contrast, nitrogen and oxygen nucleophiles have relatively small atomic radii and, accordingly, their electron clouds are less readily distorted. Consequently, such compounds are much harder nucleophiles; e.g., the methoxide ion and the primary nitrogen of the amino acid lysine (Table 2). Expansion of the electron cloud via nearby conjugation with unsaturated sites yields a less hard nucleophile; e.g., the secondary amine of histidine, the phenolate ion or the enolate of 2-acetylcyclopentanone (Table 2).

According to the selectivity principle of the HSAB model, soft electrophiles preferentially form adducts with nucleophiles of comparable softness, whereas hard electrophiles form adducts with hard nucleophiles (see ahead). The data presented in Table 2 indicate that, among the biological nucleophiles, the sulfhydryl thiolate state of cysteine residues is the softest[5,8,17]. Therefore, the preferred nucleophilic cellular targets of the soft electrophilic α,β-unsaturated carbonyls are cysteine sulfhydryl thiolate groups. In contrast, the neurotoxic *n*-hexane metabolite, 2, 5-hexanedione is a hard electrophile (Table 1) that preferentially forms adducts with harder nucleophiles such as nitrogen atoms on ε-amino group of lysine residues. Additional hard nucleophilic targets include the secondary imidazole nitrogen of histidine and the carbon, nitrogen and oxygen groups of DNA/RNA.

HSAB Descriptors of Electrophile-Nucleophile Adduct Formation

As indicated above, covalent bond formation between reacting molecules can be described by using the properties of their outermost orbitals. The two most important of these are the highest energy orbital that contains electrons (known by the acronym HOMO = Highest Occupied Molecular Orbital) and the lowest energy orbital that is vacant (LUMO = Lowest Unoccupied Molecular Orbital). Covalent bonding such as adduct formation occurs when

electron density is transferred between the orbitals of the reacting species (nucleophile to electrophile) to form the new bonds of the product (the adduct). FMO energies (E_{HOMO}, E_{LUMO}) for a wide variety of chemical structures can be calculated from quantum mechanical models and the corresponding hardness (η) and softness (σ) can then be derived using equations (1) and (2).

$$\eta=[E_{LUMO} - E_{HOMO}]/2, \quad (1)$$

$$\sigma=1/\eta \quad (2)$$

In essence, softness measures the ease with which electron redistribution takes place during covalent bonding; i.e., nucleophile donation of electrons and acceptance by the electrophile. Therefore, with respect to electrophilic species, it is often the case that softness (higher σ value) is correlated with ease of adduct formation (expressed, for example, as a reaction rate). The electrophilic index (ω) is a parameter that combines softness and chemical potential (μ; equation 3)[24]. The latter parameter, μ, is the propensity of a species to undergo chemical change. Thus, ω is calculated according to equation (4) and represents a more comprehensive measure of electrophilic reactivity.

$$\mu=[E_{LUMO}+E_{HOMO}]/2) \quad (3)$$

$$\omega=\mu^2/2\eta \quad (4)$$

With respect to the nucleophile, the corresponding molecular orbital energies can also be used to calculate nucleophilic softness and chemical potential as shown above in equations (2) and (3). In addition, the likelihood that a given nucleophile (A) will form an adduct with a given electrophile (B) can be predicted by calculating a nucleophilicity index (ω^-) using equation (5)[25].

$$\omega^-=\eta_A(\mu_A - \mu_B)^2/2(\eta_A+\eta_B)^2] \quad (5)$$

This parameter considers the hardness (η) and chemical potential (μ) of both the electrophilic and nucleophilic reactants. The electrophilic (ω) and nucleophilic (ω^-) indices have been demonstrated to be reliable descriptors for a variety of electrophile-nucleophile interactions[8,18,27–28].

To perform the quantum mechanical computations numerous computer software packages such as Gaussian (http://www.gaussian.com), HyperChem (www.hyper.com), Q-Chem (http://www.q-chem.com), Spartan (www.wavefun.com), etc., are available commercially. In addition, there are many open source programs like GAMESS (www.msg.chem.iastate.edu/gamess), deMon (www.demon-software.com/public_html/index) and Abinit (www.abinit.org) among others that could presumably be used to make these types of calculations. While various models exist, density functional theory (DFT) provides the most consistently employed approach (the B3LYP model) for the purposes described by this review. However, in one case (see ahead) we used a Hartree-Fock (HF) model with good results. Although in most of the work described here molecular geometries (or energies) are calculated in water, we have also reported an application using gas phase (in vacuum) calculations. The use of either state can be rationalized but, in our applications, no differences in the results were observed as long as

the method chosen was consistently employed. Throughout this study only global (i.e., whole molecule) parameters are considered but the use of localized functions at sites of reactivity within a molecule has been successfully applied by others in the development of computational models[23,24].

TOXICOLOGICAL APPLICATION OF HSAB PRINCIPLES

Whereas exceptions exist, most toxic chemicals are electrophiles that produce toxicity by interacting with biological nucleophiles[5,6,8,10,29] and, therefore, the HSAB concepts should have broad applicability to the field of Molecular Toxicology. In the following sections we will demonstrate how the application of HSAB principles can help identify the sites and mechanisms of toxicant action. Thus, we will consider the application of HSAB principles to recent structure-toxicity studies involving acrolein, 4-hydroxy-2-nonenal and other α,β-unsaturated carbonyl and aldehyde derivatives. In addition, we will discuss the role of HSAB principles in the development of nucleophilic 1,3-dicarbonyl compounds that might be useful in treating disease processes involving endogenous α,β-unsaturated carbonyl compounds. Although the HSAB calculations are broadly applicable and straightforward, our discussions of these studies will also serve to identify certain physiochemical toxicant characteristics and intervening biological factors that can influence toxic expression and interpretation of the quantum mechanical data.

α,β-Unsaturated Carbonyl Derivatives: Toxicological Consequences of Exposure

Acrylamide (ACR), acrolein, 4-hydroxy-2-nonenal (HNE) and other structurally related derivatives (Table 1) are characterized by a conjugated α,β-unsaturated carbonyl substructure that is formed when an electron-withdrawing group (e.g., a carbonyl group) is linked to an alkene. These are therefore soft electrophiles of the type-2 alkene chemical class. Members of this class are used extensively in the manufacturing, agricultural and polymer industries and, consequently, occupational human exposure is significant. In addition, these chemicals are recognized as environmental pollutants and dietary contaminants; e.g., acrolein from combustion of petrochemical fuels, methyl vinyl ketone (MVK) in automobile exhaust, and ACR in French fries. α,β-Unsaturated carbonyl derivatives are also prevalent components of cigarette smoke; e.g., acrolein, ACR and acrylonitrile. Acute or chronic exposure to these type-2 alkenes has been linked to major organ system toxicity and to possible carcinogenicity in humans and laboratory animals[5,30–34]. In addition to environmental or exogenous intoxication, acrolein, HNE and certain other α,β-unsaturated aldehydes are highly toxic by-products of membrane lipid peroxidation associated with cellular oxidative stress. Growing evidence indicates that these endogenous type-2 alkenes play a pathogenic role in many diseases that have oxidative stress as a common molecular etiology; e.g., atherosclerosis, Alzheimer's disease, diabetes[5,13,17,35,36]. Clearly, the type-2 alkenes are an important class of chemicals with respect to environmentally acquired toxicity and endogenous disease pathogenesis. As described in the following subsection, deciphering the mechanism of type-2 alkene cytotoxicity can be aided significantly by application of HSAB concepts and quantum mechanical descriptors.

α,β-Unsaturated Carbonyl Derivatives: Softness and Electrophilicity

As discussed above, the relative softness and electrophilicity of a α,β-unsaturated carbonyl derivative can be calculated using LUMO and HOMO energies determined via quantum mechanical models. As the data in Table 3 illustrate, acrolein and HNE are the strongest electrophiles among the type-2 alkenes tested; i.e., the respective gas phase electrophilicity index (ω) is numerically larger than other constituents in the chemical series. In contrast, acrylamide (ACR) and methyl acrylate (MA) are substantially weaker electrophiles, whereas

MVK exhibits intermediate electrophilic reactivity. Overall, the type-2 alkene electrophilic indices suggest the following rank order of electrophilicity: acrolein ≈ HNE > MVK ≫ MA ≥ ACR. In this type-2 alkene series, the relative differences in electrophilicity are determined by substituent functional groups and their respective contributions to the electron density of the α,β-unsaturated carbonyl structure. Allyl alcohol and propanal do not contain extended pi electron systems and therefore, have substantially lower values of electrophilicity compared to acrolein and related conjugated analogs (Table 3). Since allyl alcohol and propanal lack a conjugated structure, neither of these substances can undergo Michael-type reactions; i.e., conjugate addition of a nucleophile. Subsequent experimental research showed that softness and electrophilicity were determinants of the chemical reactions that mediate type-2 alkene toxicity[14–16]. Excluding HNE (see below), the rank order of electrophilicity was closely correlated ($r^2 > 0.90$) to the corresponding second order rate constants for the adduct reaction with cysteine (log k_2) and to the respective potencies (log IC_{50}) for in vitro nerve terminal (synaptosomes) toxicity (Table 3). While this correlated data set consists of only four compounds, it should be recognized that each structure represents a different functional group (i.e., aldehyde, ketone, ester and amide) and thus, constitutes a significant range of potential variability. Within the confines of a particular functional group (e.g., esters), electrophilicity is consistently expressed (see Table 5). Molecular softness was also correlated to these experimental parameters but to a slightly lesser extent ($r^2 \approx 0.80$). These data suggest that electrophilicity governs the cysteine adduction rate and toxicity of a chemical and that softness also is an important factor.

α,β-Unsaturated Carbonyl Electrophilicity: Intervening Variables That Influence Interpretation

The preceding data indicate that, although values for HNE electrophilicity and softness were higher than those of acrolein and MVK[37], the rank order of the respective adduct rate constant (log k_2) and toxic potency (log IC_{50}) were substantially lower than predicted by the HSAB parameters. This discrepancy would seem to indicate a limited role for electrophilicity in determining toxic expression. However, the extended alkane tail of HNE (Tables 1 and 4) imposes significant steric hindrance that can impede access to the active site of many enzymes and thereby slow the corresponding kinetics of adduct formation and reduce toxic potency[37]. Such disagreement between direct chemical measurements and calculated HSAB parameters should be expected, since the respective algorithms for σ and ω do not consider steric factors. As an additional illustration of steric influences, Seiner et al.[38] demonstrated that acrolein and a series of structurally related aldehydes inhibited protein tyrosine phosphatase 1B (PTP1B) activity via covalent modification of a specific cysteine residue (Cys215). The order of potency was: acrolein ≫ crotonaldehyde > 3-methyl-2-butanal ≈ propanal. When the corresponding aqueous phase electrophilic indices (ω) were calculated retrospectively, the greater ability of acrolein to inactivate PTP1B was clearly related to correspondingly higher electrophilicity (ω = 3.82ev), whereas the reduced potency of crotonaldehyde was a function of lower electrophilicity (ω = 3.27ev). However, the ω value for 3-methyl-2-butanal (ω = 3.31) was significantly higher than that of propanal (ω = 2.32) and equivalent to that of crotonaldehyde (ω = 3.27). Similar to the previous HNE study (Table 4), this order of electrophilicity is not consistent with the rank order of potency. Nonetheless, as the authors indicate, it can be rationalized by steric hindrance imposed through the bulky tertiary alkene terminus of 3-methyl-2-butanal. It is noteworthy that, due to its significant ω value, this compound would likely inactivate PTP1B at higher concentrations and/or longer exposure times that overcome steric hindrance. However, this is not the case for propanal, since it is neither structurally nor electronically capable of irreversible sulfhydryl adduction.

As part of a structure-toxicity analysis of lipid electrophiles generated in vivo during phospholipid peroxidation, McGrath et al.[39] determined the corresponding half-lives ($t_{1/2}$) and second order rate constants (k_2) for the reactions of HNE, 4-oxonenal (ONE) and analogue metabolites (Table 4) with N-acetylcysteine (NAC). As indicated above these lipid derivatives are α,β-unsaturated carbonyl compounds that mediate cytotoxicity through irreversible modification of macromolecules and disruption of cellular response pathways[17,40,41]. When the corresponding electrophilicity values (ω) for the HNE analogues were calculated, the kinetic parameters ($t_{1/2}$, k_2) were only qualitatively correlated to ω unless the acidity of certain metabolites ($RCOOH \rightarrow RCOO^-$) was considered. Specifically, when structures of the dominant anionic species ($RCOO^-$) at pH 7.4 were used in a HF quantum mechanical model, correlation of both k_2 and $t_{1/2}$ with ω improved from $r^2 = 0.553$ to 0.906 and $r^2 = 0.752$ to 0.911, respectively. In addition, the lack of reactivity exhibited by HNEA in this study can be related to the extremely low value of ω calculated for its conjugate base (Table 4). It can also be seen from Table 4 that although the DF model yielded numerically different values for ω, the same result was obtained; i.e., rate data were well correlated to electrophilicity when the relevant anionic species were considered.

Esters of acrylic acid and methacrylic acid are used extensively in the production of polymers for textiles, latex paints and medical/dental cements. The acrylates (CH_2=$CHCO_2R$) and methacrylates (CH_2=CCH_3CO_2R) are conjugated α,β-unsaturated ester derivatives. Several lines of evidence suggest that these compounds produce toxicity (e.g., carcinogenicity, skin sensitization) through adduction of protein sulfhydryl groups[20,42–44]. Early studies by Tanii and Hashimoto[42] determined the lethal oral doses (LD_{50}) in mice for a broad spectrum of acrylates/methacrylates. Results indicated that the LD_{50} was related to both solubility (as a partition coefficient, log P) and the reaction rate (log k) of these esters with glutathione in a buffered physiological solution. When the electrophilic index (ω) was calculated for the 14 compounds (Table 5), we found that the corresponding rate constants (log k) were highly correlated ($r^2 = 0.97$) to the ω parameter. In contrast, both log k ($r^2 = 0.65$) and ω ($r^2 = 0.60$) were only modestly correlated with the in vivo LD_{50} values. This discrepancy can, however, be explained by the variable solubility (log P; Table 5) of the acrylate and methacrylate compounds[18] and by the fact that toxicant solubility is a physiochemical determinant of tissue distribution and, therefore, target accessibility. This is evidenced by comparing the respective data for the water soluble 2-hydroxyethyl acrylate (log P = −0.21) to the less soluble ethyl acrylate (P = 1.33); i.e., both have virtually identical ω values (~3.200 ev), but significantly different reaction rates (log k = 1.92 vs 1.43, respectively) and toxic potencies (LD_{50} = 5.2 mmol/kg vs. 18 mmol/kg, respectively). Clearly the chemical factors that establish solubility do not necessarily produce commensurate changes in electronic characteristics such as electrophilicity. This is not surprising since, although the hydroxyl group improves solubility (i.e., CH_2=$CHCO_2CH_2CH_2OH$ vs CH_2=$CHCO_2CH_2CH_3$), this substituent is distant from the electrophilic site of the molecule (i.e., carbon-carbon double bond) and might have little effect on electrophilic reactivity. Thus, it should be evident from this example that, when individual differences in solubility are considered, the relative electrophilicity (ω) of the acrylates/methacrylates determines both the corresponding in vitro rate (log k) of cysteine adduct formation and the expression of in vivo toxicity[18,29,44].

The preceding discussion indicates that a variety of chemical toxicants can be classified according to the quantitative HSAB descriptors of hardness (η) and softness (σ), as determined by quantum mechanical calculations. Furthermore, the degree of electrophilic reactivity (ω) for these compounds can also be quantitatively determined. This latter parameter is critically important since it is directly related the second order rate constant (k_2) of the target adduct reaction and, therefore, reflects toxicological potential. Accordingly, ω has been suggested to be an *in silico* surrogate parameter for experimentally derived rate

constants (k_2) and, as such, might be a useful tool for predicting the toxicity of industrial chemicals[10,19,20,22,44]. The preceding discussion also pointed out that the HSAB algorithms do not account for possible intervening physiochemical variables; e.g., solubility, acid-base equilibrium and steric hindrance, that could independently modify toxicological outcome and lead to discrepancies in predicted vs. observed results. These issues have been addressed to some extent and, in at least one case, electrophilic and steric parameters were combined in a computational algorithm[24].

The α,β-unsaturated ester and carbonyl derivatives discussed here form adducts with cysteine and other amino acid targets on proteins via second order (covalent) reactions. The rates of these reactions are not only dependent upon the toxicant ω, but also the nucleophilic status of the target residues. Therefore, in the next section, we will discuss the application of HSAB parameters to the identification of amino acid sites of toxicant action.

α,β-Unsaturated Carbonyl Derivatives: Nucleophilic Targets and Molecular Mechanisms of Toxicity

According to the selectivity principle of the HSAB theory, soft electrophiles should preferentially react with soft nucleophiles. There are several nucleophilic amino acid side chains that might be relevant protein targets for the α,β-unsaturated carbonyl compounds: i.e., sulfhydryl groups on cysteine (Cys) residues, the primary nitrogen atoms of the lysine (Lys) ε-amino group and the secondary nitrogen atoms of the imidazole group of histidine (His). Although the type-2 alkenes can form adducts with lysine and histidine residues[40,45–52], the weight of kinetic and proteomic data indicate that cysteine sulfhydryl groups are the preferential targets for the type-2 alkenes[11,12,14,16,37,40,41,49,52–58]. On a quantitative basis, this cysteine preference is several orders of magnitude greater than that for the other biological nucleophiles. This differential is consistent with the fact that the side chain amino nitrogen groups of lysine and histidine are harder nucleophiles (Table 2) and have inherently lower reactivity for soft electrophiles such as the type-2 alkenes. The likelihood that a given nucleophile will form a covalent adduct with a type-2 alkene can be predicted by calculating an index of nucleophilicity (ω^-, see above). This comprehensive parameter considers the hardness (η) and chemical potential (μ) of both the electrophilic (type-2 alkene) and nucleophilic (cysteine, histidine or lysine) reactants[25]. As suggested by the respective ω^- values (Table 6), the type-2 alkenes preferentially form adducts with cysteine thiolate sites. However, based on a pK_a of 8.3, the cysteine sulfhydryl sidechain is mostly protonated at physiological pH (7.4) and, therefore, exists primarily as the neutral (0) thiol. Also at this pH, the imidazole secondary amine of histidine (pK_a of 6.0) is mostly deprotonated (0) and the primary ε-amino group amine of lysine (pK_a = 10.5) is completely protonated (+1). As the corresponding HSAB parameters indicate (Table 2), the relative nucleophilicity values for the thiol and nitrogen sidechain groups are low and hence, these sidechain states are not particularly reactive. Instead, a large body of evidence indicates that the α,β-unsaturated carbonyls selectively form adducts with the highly nucleophilic thiolate of cysteine sulfhydryl groups. This anionic state can be found within specialized pK_a-lowering microenvironments such as catalytic triads[5,17] and, as the respective HSAB descriptors (Tables 2 and 6) demonstrate, cysteine thiolates are a much softer and significantly more reactive nucleophile than the Lys, His or thiol sulfhydryl groups.

To provide experimental evidence for the thiolate-state preference of the α,β-unsaturated carbonyls, we determined the effects of pH on the corresponding rates of adduct formation. The thiolate concentration in solution will vary as a function of pH; i.e., given a cysteine pK_a of 8.3, at physiological pH (7.4) the majority (~90%) of sulfhydryl groups will be in the non-reactive thiol-state, whereas at higher pH, the thiolate/thiol ratio will become increasingly larger. Thus, we found that the reactions of L-cysteine (pK_a = 8.3) with selected type-2 alkenes were ten-fifteen times faster when the pH was increased from 7.4 to 8.8[14–16].

The observed changes in second-order rate constant (k_2) reflect the increased thiolate concentration at the higher pH value. The experimentally determined k_2 can be used to derive an anionic rate constant (k_{RS}^-) as a quantifiable measure of the inherent (concentration independent) nucleophilic strength[58]. Using the expression log ($k_{RS}^- - k_2$) = log k_2 + pKa − pH (where k_{RS}^- represents the anionic rate constant), a representative series of thiolate rate constants were calculated. For each α,β-unsaturated carbonyl derivative, the corresponding thiolate rate constants (k_{RS}^-) were highly correlated to ω^- ($r^2 = 0.91$; Table 6). Furthermore, the fact that ω^- and k_{RS}^- were closely correlated to the neurotoxic potencies (IC_{50}'s; Table 3) provided evidence that thiolate targeting by acrolein and HNE has toxicological relevance[15,16]. Similarly, previously reported rate data (k_2, k_{RS}^-) for the reactions of unhindered cysteine related thiolate nucleophiles[58] with acrylonitrile were well correlated ($r^2 > 0.97$) to our corresponding calculations of ω^- values (Table 7).

As a more relevant experimental approach to defining the interactions of type-2 alkenes with cysteine sulfhydryl groups, we determined the effects of acrolein, MVK and ACR on erythrocyte glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity[18]. Consistent with the concepts of the previous section, the softness (σ) and electrophilicity (ω) values of the selected type-2 alkenes were related to the corresponding second order rate constants (log k_2) and potencies (log K_I) for GAPDH inhibition (Table 8). Whereas earlier proteomic studies demonstrated the cysteine preference of type-2 alkene adduct formation[40,41,56,59,60], it is unlikely that these adducts all have toxicological relevance; i.e., the corresponding cysteine residues all play a vital role in protein function. Indeed, other studies indicated that the type-2 alkenes impaired protein function by reacting with specific cysteine residues on important cellular proteins; e.g., *N*-ethylmaleimide inhibits vesicle (H+)-ATPase activity by reacting with Cys254[61]; HNE adduct formation at Cys280 inhibits mitochondrial SIRT3 acitivity[62]. However, it was unclear why these residues were selectively targeted. Therefore, we used tandem mass spectrometry to quantify the adduct formation associated with graded concentrations of a weak electrophile, ACR[18]. Results indicated that lower in vitro concentrations of ACR inhibited GAPDH activity by selectively forming adducts with Cys 152 in the active site of this enzyme, whereas at higher concentrations, ACR also reacted with Cys 156 and Cys247. Calculations using the PROPKA program revealed a pK_a of 6.03 for Cys152, whereas the pK_a values for Cys156 and Cys247 were higher. Furthermore, we found that GAPDH inhibition by the selected type-2 alkenes was pH-dependent indicating thiolate mediation. These data suggest that Cys152 is contained within a low pK_a microenvironment[63,64] and that ACR reacted preferentially with the resulting sulfhydryl thiolate.

Considered together, our data indicate that α,β-unsaturated carbonyl chemicals produce cytotoxicity by forming Michael-type adducts with sulfhydryl groups on specific cysteine residues[5,17,59]. The elucidation of this mechanism was guided by HSAB-based calculations of softness and hardness, which indicated that the type-2 alkenes were electrophiles of varying softness and that, as such, these chemicals could form covalent bonds (adducts) with nucleophiles of comparable softness. Calculation of ω^- suggested nucleophilic anionic thiolates as potential molecular sites of electrophile action. Cysteine thiolates are present in the active sites of many proteins where they play critical roles in modulating protein activities and, therefore, post translational modification via adduct formation would cause cytotoxicity by inhibiting the function of important proteins; e.g., *N*-ethylmaleimide sensitive factor (NSF), GAPDH, ubiquitin carboxyl terminal hydrolase-L1 (UCH-L1), clathrin and vesicular-ATPase[11–14]. These predictions were corroborated by direct evidence from a variety of proteomic and kinetic experimental approaches in both *in vitro* and *in vivo* models of type-2 alkene toxicity[5,17,31,59]. Because these thiolate targets are acceptors for nitric oxide (NO) and other modulatory electrophiles, we have proposed that toxicity induced by α,β-unsaturated carbonyl exposure is mediated by disruption of cellular redox

signaling pathways[5,17,31,65]. As will be discussed, a similar approach was used to decipher the mechanism of protection against cellular oxidative stress provided by 1,3-dicarbonyl enols.

α,β-Unsaturated Carbonyl Derivatives: Relevance of HSAB Calculations to In Vivo Toxicokinetics and Toxicodynamics

The evidence reviewed above indicates that the type-2 alkenes cause cytotoxicity through a common molecular mechanism. This common mode of action suggests that members of this chemical family should, depending upon route of exposure, produce a similar pattern of organ system toxicity. However, ACR intoxication in laboratory animals or humans (e.g., occupational exposure or poisoning) produces selective neurotoxicity, whereas similar intoxication with acrolein, MVK or other type-2 alkenes causes peripheral organ toxicity[5,31]. This seemingly inconsistent diversity in toxicological responses is not due to variations in mechanism, but to relative differences in electrophilicity that correspondingly influences tissue distribution and toxicokinetics. As we have discussed, acrolein is a highly reactive soft electrophile (Table 1) that rapidly form adducts with soft nucleophilic thiolate sites on cysteine residues of proteins (Table 6). Following systemic intoxication with a reactive type-2 alkene, the rapid formation of protein adducts essentially limits tissue distribution. Consequently, the resulting toxic manifestations are determined by the site of absorption; e.g., acrolein inhalation (cigarette smoke, pollution) produces pulmonary toxicity, whereas systemic acrolein intoxication is associated with hepatic and/or vascular toxicity[66,67]. In contrast to more reactive type-2 alkenes, ACR is a weak water-soluble electrophile (Table 1) that slowly forms adducts with protein thiolate sites (Table 6). Correspondingly, ACR is less influenced by systemic “adduct buffering” and therefore has a relatively large volume of distribution that includes the central nervous system[68,69]. In accordance with these concepts, oral acrolein intoxication of rats (0.05–0.5 mg/kg) produced centrolobular liver necrosis and vascular hypertension[70], whereas oral ACR (10–50 mg/kg) caused selective neurotoxicity in this species[71]. However, the production of neurotoxicity is not simply a function of brain accessibility, since ACR has an equal propensity to form thiolate adducts in both nervous and non-nervous organ systems. Instead, our studies indicate that ACR neurotoxicity is mediated by selective nerve terminal damage and that several unique anatomical and functional characteristics predispose this neuronal region to electrophile-induced toxicity[5,17]. Specifically, as indicated above, we suspect that ACR (and other type-2 alkenes) causes cytotoxicity by reacting with thiolate sites that act as NO acceptors. Neurotransmission is a complex multistep processes that is highly regulated by NO signaling[59] and, therefore, disruption of this pathway is likely to have a significant impact on presynaptic activity. Furthermore, the nerve terminal is anatomically separated from the cell body and is, as a result, devoid of transcriptional and translational abilities. Therefore, unlike the cell body, the nerve terminal is vulnerable to electrophile attack, since it cannot mount transcription-based cytoprotective mechanisms such as the Nrf2-Keap1 response[72]. Finally, relative to proteins in other nerve regions or cell types, the turnover of nerve terminal proteins is exceptionally slow[73]. Thus, when these proteins are rendered dysfunctional through cysteine adduct formation, they are replaced slowly. This leads to the accumulation of nerve terminal adducts[11,12], progressive impairment of presynaptic processes[13,57] and cumulative neurotoxicity[71]. Thus, although the type-2 alkenes have a common mechanism of cytotoxicity, differences in electrophilic strength among these chemicals can influence respective tissue distributions and therefore their toxicological manifestations.

THE CYTOPROTECTIVE PROPERTIES OF 1,3-DICARBONYL ENOLS: MECHANISTIC INSIGHTS GAINED BY HSAB QUANTUM MECHANICAL CALCULATIONS

Cellular Oxidative Stress: Phytopolyphenol Cytoprotection

There is general agreement that cellular oxidative stress is a common molecular etiology in many chronic disease states (e.g., Alzheimer's disease, atherosclerosis) and acute injuries (e.g., stroke, traumatic spinal cord injury). A confluence of epidemiological data and clinical trials[74,75], corroborated by laboratory evidence[76–78], indicate that the course of these conditions can be significantly improved by the administration of plant-derived polyphenolic compounds such as curcumin (curry spice), resveratrol (red grapes) or phloretin (apple skins). It has been largely assumed that the protective effects of these phytopolyphenols were related to their abilities as antioxidants to trap reactive oxygen species. However, recent evidence indicates that polyphenolic cytoprotection involves scavenging of other electron-deficient species that also mediate oxidative stress; i.e., divalent metal ions (e.g., Fe^{2+}, Cu^{2+}) and toxic unsaturated aldehydes (e.g., acrolein, HNE)[79–81]. Structure-activity studies have indicated that the molecular determinant of these protective actions is resident enol moieties[82–84]. For example, the α-carbon hydrogen atoms on the central β-diketone bridge of curcumin are acidic and, upon ionization, in physiological buffers, a carbanionic enolate is formed as the corresponding conjugate base. Moreover, the characteristic phenols of flavonoids such as phloretin are enols, since they consist of a hydroxyl group attached to a carbon-carbon double bond. These enols will also form nucleophilic enolate ions. Despite therapeutic potential, however, the clinical utility of the phytopolyphenols could be limited by poor bioavailability, toxicity and chemical instability[36,85,86].

1,3-Dicarbonyl Enols: A New Class of Cytoprotectants

The search for safe, water-soluble phytopolyphenol analogs led to the discovery that 1,3-dicarbonyl enols such as 2-acetylcyclopentanone (2-ACP) and acetylacetone (AcAc; Table 2) might be cytoprotective[87]. The structure of these simple soluble enols resembles the central diketone–bridge of curcumin. Like the phytopolyphenols, the α-carbon hydrogen atoms of these simple enols can also readily ionize to form highly nucleophilic soft enolates. Indeed, our studies[87] showed that the 1,3-dicarbonyl compounds provided levels of cytoprotection in several in vitro models of oxidative stress that exceeded those of the phytopolyphenols. The evidence suggested at least two mechanisms of 1,3-dicarbonyl cytoprotection in oxidative stress-induced cell injury. First, metal ion chelation mediated by bidentate coordination through the β-diketone structure. Presumably, the resulting inhibition or modification of the metal-catalyzed Fenton reaction reduces the cellular free radical burden. Second, the nucleophilic β-dicarbonyl enolates act as soft surrogate targets for free acrolein and other electrophilic α,β-unsaturated aldehydes. The reduction in toxic aldehyde content prevents secondary protein dysfunction in exposed cells. In studies that specifically examined the latter cytoprotective component, we identified the following rank order of 1,3-dicarbonyl analogs with respect to protection in acrolein-exposed neuronal cell culture systems: 2-ACP>AcAc, 1,1,1-tri-fluoro-2,4-pentanedione (TFPD)≫diethylmalonate (DEM). This sequence reflects the differences in second order rate constants (k_2) for the reaction of these 1,3-dicarbonyls with acrolein. The differential reactivity of the 1,3-dicarbonyls can be explained by the acidity (pK_a) of the parent compound and by the inherent nucleophilicity of the corresponding enolate. The pK_a values indicate the relative extent of ionization (enolate formation) and hence, reflect nucleophilic concentration as a factor contributing to reaction rate.

1,3-Dicarbonyl enols: HSAB Parameters

As discussed previously, the nucleophilic strength of an enolate carbanion can be quantified by ω^{-}[5,17]. The ω^{-} values computed for reactions between acrolein and various nucleophiles are presented in Table 9. A nucleophile of significant reactivity (high ω^{-} value) will increase the reaction rate (k_2) with a given electrophile by lowering the energy of the transition state. Therefore, ω^{-} values should correlate with the relative rate at which a given concentration of enolate reacts with a specific type-2 alkene (Table 9). Inherent differences in the respective ω^{-} and known pK_a values provide a molecular explanation of the corresponding adduct reaction rates and, hence, cytoprotective potency. Thus, 2-ACP is reasonably acidic (30–40% ionized at pH = 7.4; pK_a = 7.8) and forms an enolate that is a powerful nucleophile (relative ω^{-} = 401). Correspondingly, it exhibited superior protective capacity in all toxicity models. In contrast, AcAc is less reactive toward electrophiles, not only because it ionizes to a lesser extent (2% at pH 7.4; pK_a = 8.9), but also because it is a weaker nucleophile (ω^{-} = 160). Although TFPD is highly acidic (pK_a= 4.7), the resulting higher concentration of enolate at physiological pH is offset by its weakness as a nucleophile (ω^{-} = 108). Conversely, the enolate form of DEM is a significant nucleophile (ω^{-} = 228). However, the low acidity (pK_a = 12.9) of this chemical precludes any potential capability as a protectant, since less than 0.001% would be in the enolate state at physiological pH. NAC (pK_a = 9.5) ionizes poorly in biological solutions, but is protective against acrolein-induced thiol loss and toxicity because the nucleophilicity of the thiolate anion is relatively high (ω^{-} = 316). Phloretin has several enolizable sites and, although the nucleophilicity of these sites is modest (e.g., the relative ω^{-} of the C-3 enol on the A ring = 139), the corresponding pK_a value of 7.3 indicates significant (> 50%) ionization. Therefore, at physiological pH the high concentration of enolate and multiple site potential enable phloretin to provide significant thiol preservation through acrolein adduction. Together, these results indicate that the ability of 1,3-dicarbonyl compounds to act as acrolein targets is a predictable function of enolate formation and the inherent nucleophilicity of these carbanions. We are currently using the ability to quantify nucleophilicity (ω^{-}), in conjunction with a high level of chemical understanding (i.e., conformational flexibility, pK_a and nucleophilicity requirements) derived from extensive experimentation, to design and identify efficacious cytoprotective 1, 3-dicarbonyl analogs.

SUMMARY

In the field of Toxicology, the irreversible covalent interaction of a toxic electrophile and its cognate nucleophilic target is recognized as a basic reaction mediating chemical-induced cell injury. It is now understood that electrophile-nucleophile reactions exhibit a significant degree of selectivity, in that a given electrophile preferentially reacts with specific nucleophiles of comparable softness or hardness. This selectivity is based on electronic and structural characteristics that constitute the soft and hard classifications of the HSAB theory. In this Perspective, we have shown that the soft-soft interactions of electrophilic α,β-unsaturated carbonyl and aldehyde derivatives with their nucleophilic sulfhydryl thiolate targets can be understood and predicted through the calculation of HSAB parameters. Hard-hard interactions can also be quantitatively described and used to clarify toxicodynamics. Accordingly, we have employed HSAB calculations to describe the interactions of 2,5-hexanedione, the hard electrophilic metabolite of the parent compound, *n*-hexane, with possible hard nucleophilic targets in nervous tissue; e.g., ε-amino groups on lysine residues of axon cytoskeletal proteins[88]. Similar to the *n*-hexane/2,5-hexanedione relationship, many toxicants are metabolic derivatives of a less reactive parent or protoxicant; e.g., desulfuration of the relatively soft electrophilic chlorpyrifos parent to the highly reactive hard electrophilic oxon metabolite (chlorpyrifos oxon). Therefore, on a theoretical basis, HSAB calculations could also be used to determine the electrophilic reactivity and potential

toxicity of metabolites derived from less reactive protoxicants. However, despite these obvious beneficial applications of the HSAB calculations, in vivo and in vitro model systems and their presumed macromolecular targets are highly complex and, accordingly, accurate formulation of kinetic or mechanistic predictions is dependent upon knowledge of additional chemical features, such as steric hindrance, that might independently influence adduct reaction rates. Furthermore, it should be recognized that the relative electrophilicity of a toxicant will influence corresponding in vivo toxicokinetics and the organ systems affected, as evidenced in our study of type-2 alkene toxicity. Regardless, HSAB calculations provide molecular level information that can offer guidance for corroborative laboratory research and thereby help investigators discern corresponding electrophile molecular mechanisms and sites of toxicant action. The use of quantum chemical calculations has also been instrumental in the development and identification of potential nucleophilic "antagonists", which can scavenge electron-deficient species that mediate many diseases with a common etiology of cellular oxidative stress. Clearly, the difficult task of delineating molecular sites and mechanisms of toxicant action can be advanced by the application of quantum mechanical approaches.

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Abbreviations

HSAB	hard and soft, acids and bases
FMOs	frontier molecular orbitals
SAR	Stucture-Activity Relationship
LUMO	lowest unoccupied molecular orbital
HOMO	highest occupied molecular orbital
DFT	density-functional theory
HTT	Hartree-Fock theory
ACR	acrylamide
HNE	4-hydroxy-2-nonenal
MVK	methylvinyl ketone
MA	methyl acrylate
PTP1B	protein tyrosine phosphate 1B
NAC	*N*-acetylcysteine
LD_{50}	lethal oral dose for 50% of the population
GAPDH	glyceraldehydes 3-phosphate dehydrogenase
SIRT3	mitochondrial sirtuin3
NSF	N-ethylmaleimide sensitive factor
UCH-L1	ubiquitin carboxyl terminal hydrolase-L1
NO	nitric oxide

Nrf2/Keap1	nuclear factor erythroid 2-related factor 2/kelch-like erythroid cell-derived protein with CNS homology-associated protein 1
2-ACP	2-acetylcyclopentanone
AcAc	acetylacetone
TFPD	1,1,1-tri-fluoro-2,4-pentanedione
DEM	diethylmalonate

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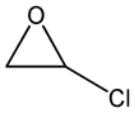
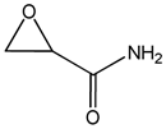
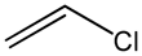
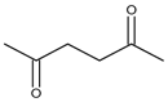
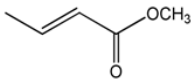
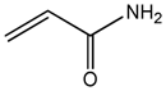
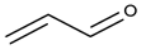
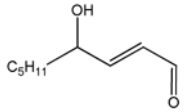
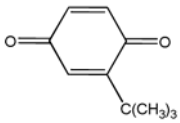
Table 1

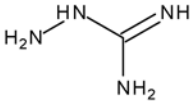
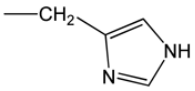
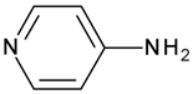
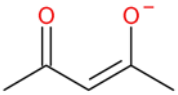
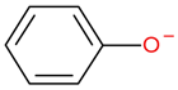
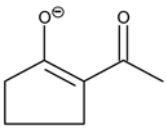
Hardness/Softness Values for Selected Electrophile[a]

Compound	Structure	Hardness (η, ev)	Softness (σ, ev^{-1})	Comments
chloroethylene oxide		4.26	0.235	Produced tumors via DNA adducts
glycidamide		3.72	0.269	Metabolite of acrylamide- possible carcinogen
vinyl chloride		3.55	0.282	Carcinogenic, neurotoxic and hepatotoxic industrial chemical
2,5-hexanedione		3.23	0.310	Metabolite of *n*- hexane- selective neurotoxicant
methyl crotonate		3.05	0.328	Industrial chemical
acrylamide		2.89	0.346	Selective neurotoxicant, suspected carcinogenic chemical
acrolein		2.64	0.379	Endogenous mediator of cellular oxidative stress
4-hydroxy-2- nonenal		2.55	0.393	Endogenous mediator of cellular oxidative stress
t-butylquinone		1.98	0.505	Induces Antioxidant Response Element (ARE)

[a]Ground state equilibrium geometries were calculated for each structure with DF B3LYP-6-31G* in water from 6-31G* initial geometries. LUMO and HOMO energies were generated exclusively from corresponding s-cis conformations and were used to calculate η and σ (see text).

Table 2

Hardness/Softness Values for Selected Nucleophiles[a]

Compound	Structure	Hardness (η, ev)	Softness (σ, ev^{-1})	Comments
aminoguanadine		3.73	0.268	Putative cytoprotectant
cysteine thiol (0)	$HSCH_2CH(NH_2)CO_2H$	3.54	0.282	Amino acid residue
lysine (0)	$H_2N(CH_2)_4CH(NH_2)CO_2H$	3.51	0.285	Amino acid residue
methoxide ion	CH_3O^-	3.47	0.288	Organic nucleophile
histidine (sidechain)		3.21	0.313	Amino acid residue
4-aminopyridine		2.93	0.341	Putative cytoprotectant
cysteine thiolate (−1)	$^-SCH_2CH(NH_2)CO_2H$	2.61	0.382	Amino acid residue
acetyl acetone anion		2.52	0.398	Putative cytorprotectant
phenolate ion		2.51	0.399	Organic nucleophile
acetylcyclopentanone anion (2-ACP)		0.537	1.87	Putative cytorprotectant

[a] Ground state equilibrium geometries were calculated for each structure with DF B3LYP-6-31G* in water from 6-31G* initial geometries. Values obtained were used to calculate σ and η (see text).

Table 3

Calculated and Experimental Parameters for Conjugated Type-2 Alkenes and Non-Conjugated Analogs

Type-2 alkene	[a] E_{LUMO} (ev)	E_{HOMO} (ev)	σ ($\times 10^{-3}$ ev^{-1})	ω (ev)	[b] Log k_2 (pH=7.4)	[c] Uptake Inhibition (log IC_{50})
Acrolein	−1.70	−6.98	379	3.57	2.596	−4.28
MVK	−1.46	−6.69	382	3.18	2.048	−3.48
HNE	−1.84	−6.93	393	3.78	0.938	−3.40
MA	−1.01	−7.36	315	2.76	−1.893	−0.34
ACR	−1.00	−6.78	346	2.62	−1.804	−0.36
Nonconjugated analogs						
[d] Propanal	−0.65	−6.84	323	2.26	-	-
[d] Allyl alcohol	+0.18	−7.06	276	1.63	-	-

[a] E_{LUMO} and E_{HOMO} were obtained after calculating ground state equilibrium geometries for each compound with DF B3LYP-6-31G* in vacuum from 6-31G* initial geometries. These values were generated exclusively from corresponding s-cis conformations and were used to calculate softness (σ) and the electrophilic index (ω) of each electrophile as described in LoPachin et al.[15].

[b] Second-order reaction rates (k_2) were determined for type-2 alkene reactions with L-cysteine at pH 7.4 (n=4–6 experiments).

[c] Inhibition of synaptosomal membrane ^{3}H-DA uptake was determined in striatal synaptosomes exposed to type-2 alkenes (n=4–6 experiments)[14].

[d] Does not undergo the Michael Reaction.

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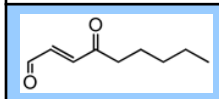
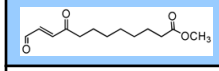
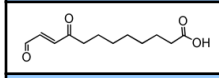
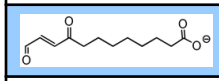
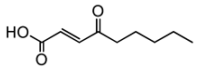
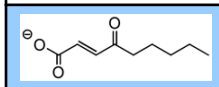
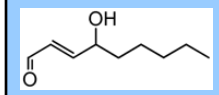
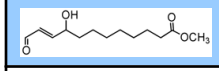
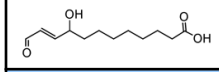
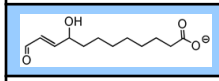
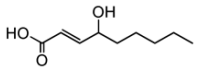
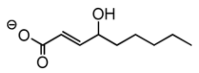
Table 4

Reaction of HNE/ONE analogues with N-acetylcysteine

Structure	Name[a]	k ($M^{-1}s^{-1}$)[a,b]	$t_{1/2}$ (s) [a,b]	ω (ev) [c]	ω (ev) [d]
	ONE	238	7.1	1.7354	5.4716
	ONE-CO_2Me	301	7.1	1.7354	5.4729
	ONE-CO_2H	169	13.9	1.7354	5.4495
	ONE-CO_2^-	169	13.9	1.6613	--[e]
	ONEA	1.72	580	1.7017	4.9213
	$ONEA^-$	1.72	580	1.3430	3.6997
	HNE	1.22	732	1.1819	3.8038
	HNE-CO_2Me	1.18	794	1.1798	3.8038
	HNE-CO_2H	0.79	1213	1.1798	3.7882
	HNE-CO_2^-	0.79	1213	1.1535	3.6105
	HNEA	NR	NR	1.2272	3.2497
	$HNEA^-$	NR	NR	0.7367	1.8417

[a] Data are from McGrath et al.[39] and shaded entries indicate dominant species (see corresponding text).

[b] Half-lives and rate constants from reaction with N-acetylcysteine at pH 7.4, 37C.

[c] $\omega = \mu^2/2\eta$ (see text). Orbital energies were generated exclusively from corresponding s-cis conformations and using ground state equilibrium geometries computed using HF 6-31G* in water starting from 3-21G geometry.

[d] Orbital energies were generated exclusively from corresponding s-cis conformations and were obtained from ground state equilibrium geometries computed using DF B3LYP 6-31G* in water starting from 6-31G* geometry.

[e] Failed to converge.

Table 5

Acrylate and Methacrylate Oral Lethality in Mice

Ester	LD_{50}[a] (mmol/kg)	Log P[a]	Log k[a]	ω (ev)[b]
methyl acrylate	9.6	0.8	1.602	3.2245
ethyl acrylate	17.97	1.33	1.428	3.1981
n-butyl acrylate	58.98	2.36	1.401	3.196067
isobutyl acrylate	47.63	2.22	1.431	3.196067
2-hydroxyethyl acrylate	5.177	−0.21	1.919	3.20026
2-hydroxypropyl acrylate	8.112	0.35	1.758	3.214
methyl methacrylate	51.97	1.38	−0.675	2.9967
ethyl methacrylate	68.64	1.94	−0.403	2.9729
isopropyl methacrylate	67.84	2.25	−0.876	2.9708
n-butyl methacrylate	142.7	2.88	−0.655	2.95895
isobutyl methacrylate	83.14	2.66	−0.769	2.9708
2-hydroxyethyl methacrylate	45.24	0.47	−0.273	3.0108
2-hydroxypropyl methacrylate	55.24	0.97	−0.273	2.9848
tert-butyl methacrylate	60.12	2.54	−0.632	2.9708

[a] Data from Tanii and Hashimoto[42].

[b] $\omega = \mu^2/2\eta$ (see text). Orbital energies were generated exclusively from corresponding s-cis conformations and were obtained from ground state equilibrium geometries computed using DF B3LYP 6-31G* in water starting from 6-31G* geometry.

Table 6

Calculated Nucleophilic Indices (ω^-) for Type-2 Alkene Reactions with Cysteine Sulfhydryl Groups

Electrophile	[a] ω^- Cys (−1)	ω^- Cys (0)	[b] log k_{RS^-}	[c] log IC_{50}
Acrolein	2.03	0.103	3.417	−4.28
HNE	1.93	0.083	1.759	−3.40
MVK	1.83	0.064	2.953	−3.48
MA	1.59	0.069	1.011	−0.34
ACR	1.50	0.036	0.767	−0.36

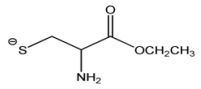
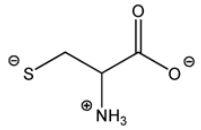
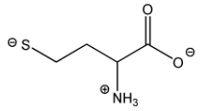
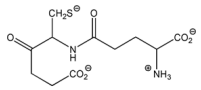
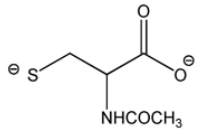
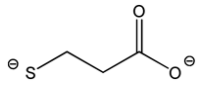
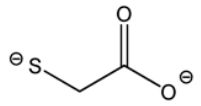
[a] The nucleophilicity index (ω^-) was calculated as $\omega^- = \eta A\ (\mu A - \mu B)^2/2(\eta A - \eta B)^2$, where $\eta = (E_{LUMO} - E_{HOMO})/2$, $\mu = (E_{LUMO} + E_{HOMO})/2$, A = reacting nucleophile and B = reacting electrophile[15]. FMO energies (E_{LUMO} and E_{HOMO}) were generated exclusively from corresponding s-cis conformations and were derived from equilibrium geometries of the electrophiles at the ground state calculated using DF B3LYP-6-31G* in water starting from 6-31G* geometries. For the sulfhydryl thiolate and thiol state, the respective ionization-states are presented in parentheses. The nucleophilicity index considers the respective hardness and chemical potential of the electrophilic (type-2 alkene) and nucleophilic (cysteine, histidine or lysine) reactants and is, therefore, a measure of the likelihood of subsequent adduct formation. As suggested by the respective ω^-values, the type-2 alkenes preferentially form adducts with cysteine thiolate sites as opposed to the thiol state.

[b] The k_2 values at pH 7.4 were corrected for the corresponding cysteine thiolate concentration (k_{RS^-}) according to the algorithm: log ($k_{RS^-} - k_2$) = log k_2 + pK_a − pH. Inhibition of membrane ^{3}H-dopamine transport was determined in rat striatal synaptosomes exposed in vitro to graded concentrations of each type-2 alkene.

[c] The concentration-response data for transport were fitted by nonlinear regression analysis and the respective IC_{50}'s were calculated by the Cheng-Prusoff equation[14,15]. Abbreviations: HNE = 4-hydroxy-2-nonenal, MVK = methyl vinyl ketone, MA = methyl acrylate and ACR = acrylamide.

Table 7

Reaction of thiolate nucleophiles with acrylonitrile at pH 8.1 and 30°C

Cysteine Analogue (pK_a)[a]	Nucleophile	Log k_2[a]	Log k[b]	ω^- (ev)[c]
cysteine ethyl ester (6.53)		−0.737	−0.726	1.94
cysteine (8.15)		−0.668	−0.341	2.16
homocysteine (8.70)		−0.921	−0.223	1.93
glutathione (8.56)		−0.762	−0.173	2.74
N-acetylcysteine (9.52)		−1.24	0.192	4.81
3-mercaptopropanoic acid (10.2)		−1.57	0.535	6.91
mercaptoacetic acid (10.05)		−1.41	0.546	7.54

[a] Data from Friedman et al.[58]

[b] Calculated as log (k − k_2) = log k_2 + pKa − pH.

[c] Orbital energies obtained from ground state equilibrium geometries computed using DF B3LYP 6-31G* in water starting from 6-31G* geometry.

Table 8

HSAB and Kinetic Parameters for Type-2 Alkene Interactions with GAPDH.

[a] Electrophile	σ (× 10^{-3} ev^{-1})	ω (ev)	log k_2	log K_I
[b] Acrolein	371	3.82	4.250	−4.419
MVK	363	3.38	3.885	−4.220
ACR	315	2.61	0.502	−0.607

[a] HSAB (σ, ω) and kinetic parameters (k_2, K_I) were calculated as described in Martyniuk et al.[18].

[b] Based on the HSAB parameters, acrolein and MVK are significantly softer and more reactive electrophiles than ACR; i.e., larger values of σ and ω, respectively. The rank orders of respective σ and ω values for each type-2 alkene were closely correlated to the corresponding rate constants (k_2; r^2 = 0.9996 and 0.9359, respectively) and relative potencies (K_I; r^2 = 0.9926 and 0.9004, respectively) for inhibition of GAPDH activity.

Table 9

HSAB and Kinetic Parameters for Reactions of 1,3-Dicarbonyl Compounds with Acrolein.

Compound	[a] ω^- ($\times 10^{-3}$ ev)	pK_a	[b] k_2 rate constant
2-ACP	401	7.8	0
AcAc	160	8.9	−24.9
TFPD	108	4.7	−52.1
DEM	228	12.9	−78.7
Phloretin	139	7.3	−1.9
NAC (−2)	316	9.5	nd

[a] The nucleophilic index (ω^-) was calculated based on the reaction of acrolein with the selected 1,3-dicarbonyl and was calculated according to the algorithm provided in the text. FMO energies were generated exclusively from corresponding s-cis conformations and were calculated as equilibrium geometries at the ground state using DF B3LYP-6-31G* in water starting from 6-31G* geometries.

[b] Second order rate constants (k_2) for the reactions of 1,3-dicarbonyls with acrolein are derived from LoPachin et al.[87] and are expressed as nM sulfhydryl loss s^{-1}.