The Chemical Biology of Naphthoquinones and Its Environmental Implications

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Annu. Rev. Pharmacol. Toxicol. 2012. 52:221–47

First published online as a Review in Advance on September 13, 2011

The *Annual Review of Pharmacology and Toxicology* is online at pharmtox.annualreviews.org

This article's doi: 10.1146/annurev-pharmtox-010611-134517

0362-1642/12/0210-0221$20.00

Keywords

quinone, electrophile, redox cycling, prooxidant, covalent bond, oxidative stress

Abstract

Quinones are a group of highly reactive organic chemical species that interact with biological systems to promote inflammatory, anti-inflammatory, and anticancer actions and to induce toxicities. This review describes the chemistry, biochemistry, and cellular effects of 1,2- and 1,4-naphthoquinones and their derivatives. The naphthoquinones are of particular interest because of their prevalence as natural products and as environmental chemicals, present in the atmosphere as products of fuel and tobacco combustion. 1,2- and 1,4-naphthoquinones are also toxic metabolites of naphthalene, the major polynuclear aromatic hydrocarbon present in ambient air. Quinones exert their actions through two reactions: as prooxidants, reducing oxygen to reactive oxygen species; and as electrophiles, forming covalent bonds with tissue nucleophiles. The targets for these reactions include regulatory proteins such as protein tyrosine phosphatases; Kelch-like ECH-associated protein 1, the regulatory protein for NF-E2-related factor 2; and the glycolysis enzyme glyceraldehyde-3-phosphate dehydrogenase. Through their actions on regulatory proteins, quinones affect various cell signaling pathways that promote and protect against inflammatory responses and cell damage. These actions vary with the specific quinone and its concentration. Effects of exposure to naphthoquinones as environmental chemicals can vary with the physical state, i.e., whether the quinone is particle bound or is in the vapor state. The exacerbation of pulmonary diseases by air pollutants can, in part, be attributed to quinone action.

INTRODUCTION

Polynuclear aromatic hydrocarbon (PAH): product of fossil fuel combustion that typically contains two or more fused aromatic rings

Quinones are a class of organic compounds whose chemical properties allow them to interact with biological targets by forming covalent bonds and by acting as electron transfer agents in oxidation-reduction reactions. The widespread nature of these interactions has been documented in multiple reviews (1–6). Of this class, the naphthoquinones are of particular interest because of their occurrence as natural products (6–8) and as environmental chemicals (9). In this review, we describe the role of quinone chemistry in the cellular and in vivo effects associated with the toxicology and pharmacology of naphthoquinone derivatives. Our particular interest has been in the actions of 1,2-naphthoquinone (1,2-NQ) (**1**, **Figure 1**; bold numbers in parentheses refer to structures) and 1,4-naphthoquinone (1,4-NQ) (**3**, **Figure 1**), two environmental quinones, in cellular changes associated with potential adverse health effects.

EXPOSURE

The most common exposure to the naphthoquinones is from the environment, as they are components of air pollutants from combustion of fossil (9) and diesel fuel (10–12) and from tobacco smoke (13). Atmospheric formation of quinones also occurs by direct and indirect ozone-based processes in which polynuclear aromatic hydrocarbons (PAHs) adsorbed on particle surfaces are

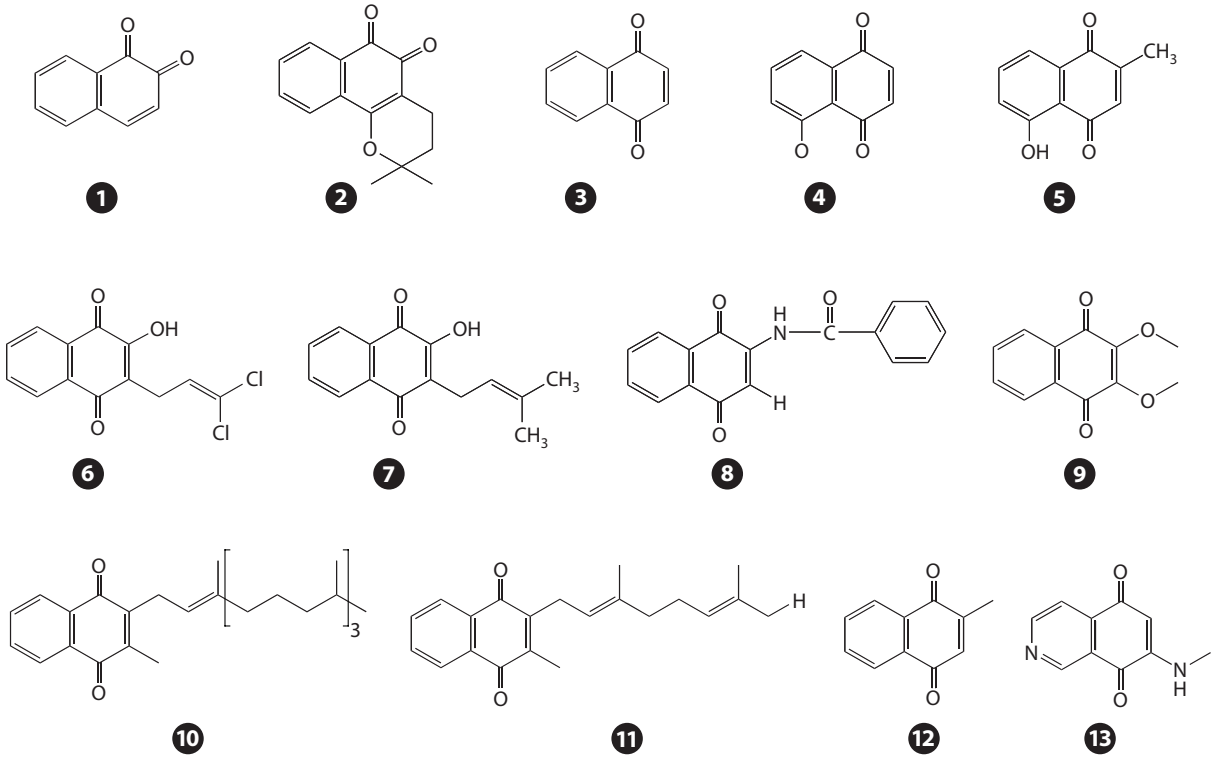
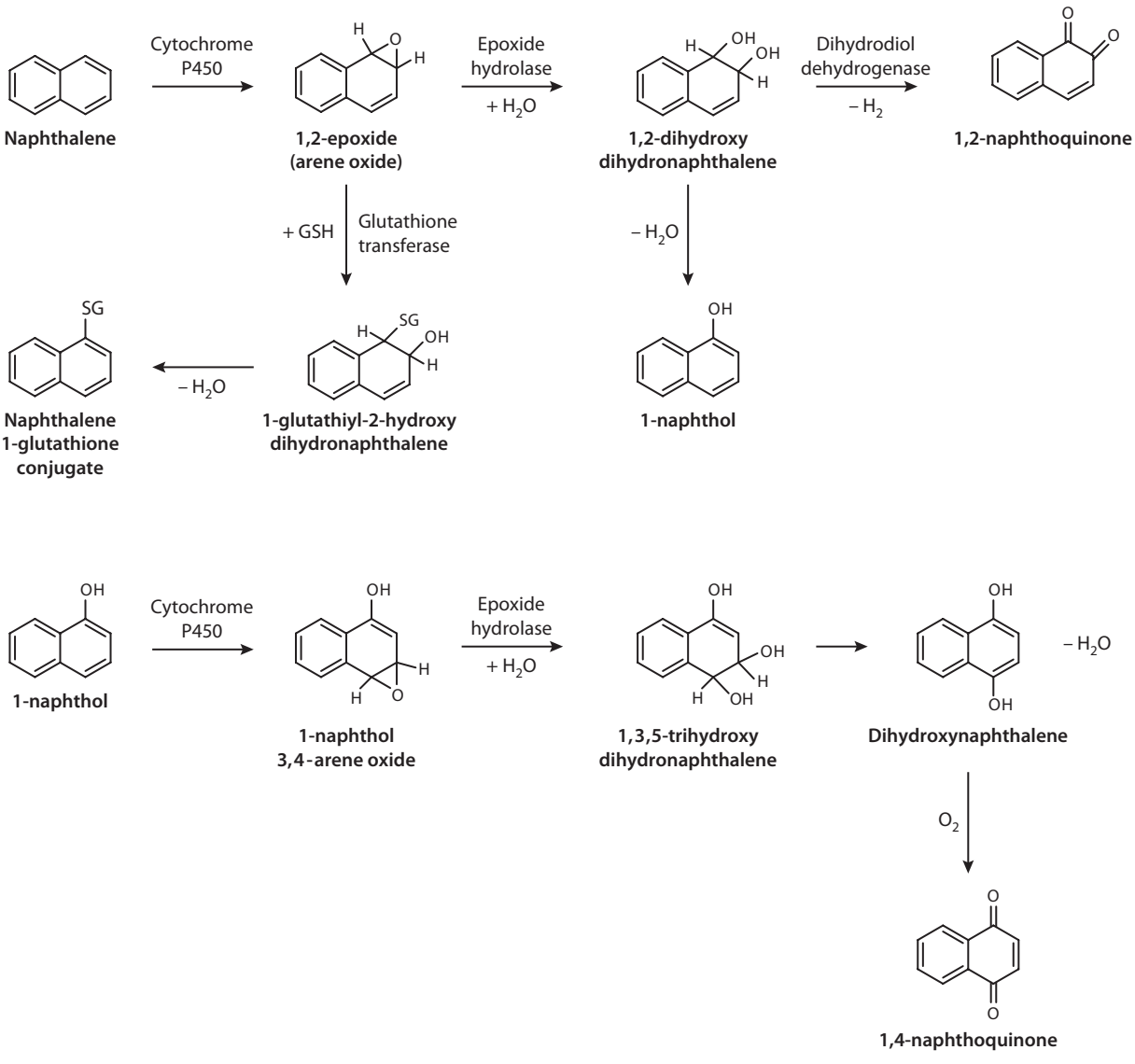


Figure 1

Structures of naphthoquinone derivatives. **1**, 1,2-naphthoquinone; **2**, β-lapachone; **3**, 1,4-naphthoquinone; **4**, juglone; **5**, plumbagin; **6**, dichloroallyl lawsone; **7**, lapachol; **8**, PPM-18; **9**, 2,3-dimethoxy-1,4-naphthoquinone; **10**, vitamin K_1 (phylloquinone); **11**, vitamin K_2 (menaquinone); **12**, vitamin K_3 (menadione); **13**, caulibugulone A.

oxidized by ozone itself (14) or an ozone-based oxidized alkene derivative (15). Although the levels of the naphthoquinones may be relatively low, exposure to their precursor, naphthalene, is significant (16–18). In the Los Angeles Basin, for example, more than 99% of the total PAH mass present in the atmosphere is naphthalene (16, 19). It also constitutes a high proportion of the PAH mass in cigarette smoke (20). Naphthalene is the simplest PAH, with two benzene rings. It is highly volatile and has a selective toxicity toward the lungs (21), an action proposed to reflect differences in metabolism between the lungs and other tissues (22). Conversion of naphthalene to 1,2-NQ and 1,4-NQ occurs biologically through well-established metabolic pathways associated with naphthalene toxicity (Scheme 1) (23–26).

Scheme 1



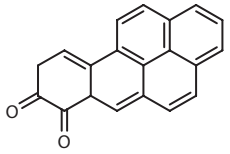
Critical intermediates in this metabolic pathway are the arene oxide and the dihydrodiol, 1,2-dihydroxydihydronaphthalene. The three-membered arene oxide ring is a reactive intermediate; it can be opened by electrophiles such as hydroxide and thiolate by enzymatic and chemical reactions. The hydrolysis product is the dihydrodiol (27), which can lose the elements of water to become a naphthol or be dehydrogenated by dihydrodiol dehydrogenase to form the quinone.

RELEVANT CHEMISTRY

Quinones are conjugated carbonyl groups in six-membered rings and are two-electron oxidation products of aromatic diols. The structure can be part of multiring systems such as the PAHs; an example is benzo[a]pyrene or benzene, as shown in the structures below.

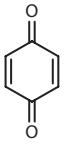
Structure 1

Benzo[a]pyrene-7,8-dione



Structure 2

1,4-benzoquinone

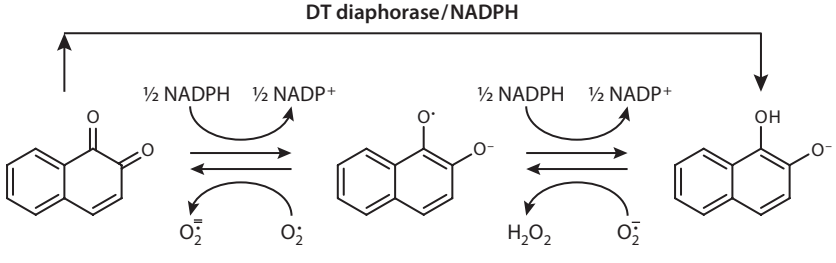


Quinones as Electron Transfer Agents

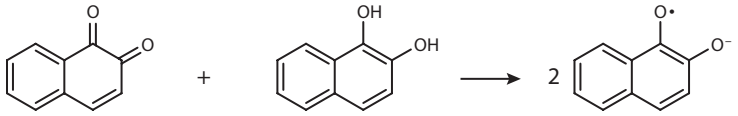
Electrophile: an electron-deficient compound that can accept an electron pair to form a covalent bond with its reaction partner (nucleophile)

Reactive oxygen species (ROS): chemically reactive molecules such as superoxide radical, hydroxyl radical, and hydrogen peroxide

Quinones can participate in the initiation of and the propagation of so-called free radical chain reactions. Free radicals are reactive chemical species with an unshared electron that can be transferred to other species, which, in turn, become free radicals. When oxygen is involved in these reactions, it is reduced to reactive oxygen species (ROS), a term commonly used to describe superoxide, hydroxyl radical, and hydrogen peroxide. In biochemical reactions, the quinone function can be reduced to the semiquinone, a free radical, then to the hydroquinone by a sequence of two one-electron reductions (Reaction 1, lower arrows) by cytochrome P450 reductase and other flavoprotein enzymes (27–30). The intermediate semiquinone can dissociate from the flavoproteins and be available for other reactions in the cell. However, DT diaphorase (NADPH quinone reductase) reduces quinones directly to hydroquinones (28) (Reaction 1, upper arrows), initiating a metabolic pathway, which, when followed by conjugation, leads to a less reactive ester, which is eliminated. Semiquinones are also generated nonenzymatically by the comproportionation of a hydroquinone and quinone (Reaction 2) (4, 32) and the superoxide-based oxidation of the hydroquinone anion (Reaction 3). The hydroquinone and semiquinone are potentially toxic to the cells for they can participate in a redox cycling chain propagation reaction involving Reaction 3, which generates the radical semiquinone, and Reaction 4 (31), which generates hydrogen peroxide. Because oxygen is the only other reactant, this reaction can occur as long as the hydroquinone is present in the cell.



Reaction 1



Reaction 2

The ability of quinones to participate in electron transfer reactions varies with the specific quinone, and in studies with the flavoprotein enzyme nitric oxide synthase (NOS), a limited number of quinones acted as electron acceptors, undergoing reduction with NADPH as the reducing agent or electron source. To be active, the quinones required one-electron reduction potentials between −240 and −100 mV; this criterion was met by 1,2-NQ and 1,4-NQ (33). Differences between the reduction potentials of 1,4-benzoquinone (1,4-BQ) and 9,10-phenanthroquinone (9,10-PQ) were attributed to differences in their mechanisms of toxicity. Thus, 1,4-BQ with a one-electron redox potential of 99 mV is not redox active in biological systems, whereas 9,10-PQ with a potential of −124 mV is capable of electron transfer in the biological milieu and can reduce oxygen to its reactive species in the presence of NADPH. When exposed to yeast, 9,10-PQ increases oxidized glutathione (GSH) and is more toxic under aerobic conditions compared with 1,4-BQ, whose toxicity appears to arise mostly from its electrophilicity (34).

Nucleophile: an electron-rich chemical that is attracted to electron-deficient compounds (electrophiles)

Michael reaction: the nucleophilic 1,4-addition of an α,β-unsaturated carbonyl compound to a nucleophile

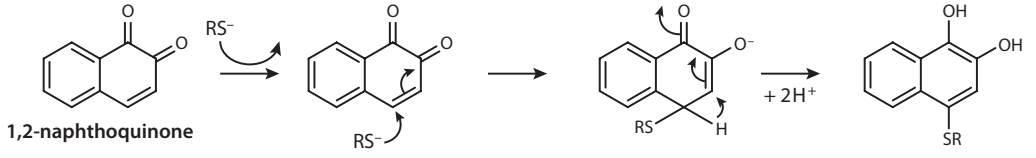
Reaction 3

Quinones as Electrophiles

As electrophiles, quinones form covalent bonds with nucleophilic functions in biological molecules in an arylation reaction. When the nucleophile is a thiol, the reaction generates a thioether, which is generally stable (35). Quinones contain the electrophilic α,β-unsaturated carbonyl system, also termed a Michael acceptor, for its ability to form covalent bonds with nucleophilic functions such as the thiolate in the Michael reaction shown in Reaction 5.

Reaction 4

The thiol adduct of quinones (see Reaction 5) is a hydroquinone that is also redox active. In a study of the reaction of 1,2-NQ with 2-mercaptoethanol, Smithgall et al. (36) found that the reaction product of 1,2-NQ with 2-mercaptoethanol was 1,2-dihydroxynapthalene 4-thioether, which readily undergoes oxidation to the corresponding quinone. These observations are relevant to the comments by Brunmark & Cadenas (28), who point out that although its redox potential is not substantially altered, the thioether hydroquinone is autoxidized at a faster rate than is the unsubstituted hydroquinone, suggesting a kinetic rather than thermodynamic role in the process.



Reaction 5

1,2-dihydroxynaphthalene 4-thioether

In summary, quinones exhibit two reactions that describe their interaction with biological systems. First, they react as electron transfer agents, transferring electrons from a reducing agent such as NADPH to oxygen; this generates initially superoxide, which, in turn, disproportionates into oxygen and hydrogen peroxide (Reaction 6). Second, they react as electrophiles, forming covalent bonds with nucleophilic functional groups in biological molecules.

Reaction 6

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH): a metabolic enzyme responsible for catalyzing one step in the glycolytic pathway, the reversible oxidative phosphorylation of glyceraldehyde 3-phosphate

Protein tyrosine phosphatase 1B (PTP1B): a negative regulator of tyrosine kinase growth factor signaling such as the EGFR pathway

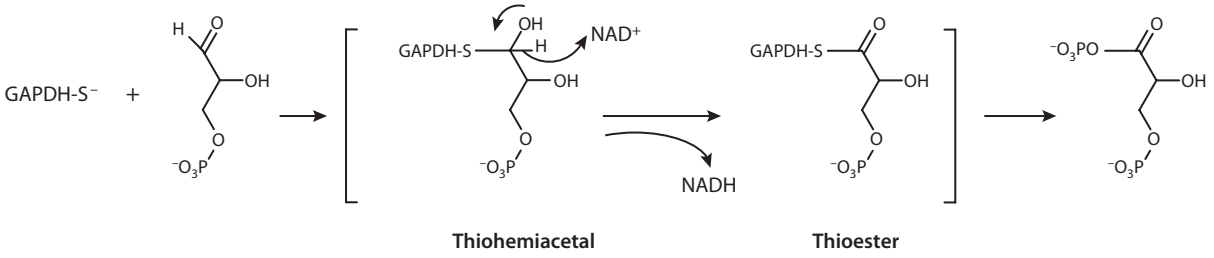
pKa value: a key parameter to predict the ionization state of a molecule with respect to pH

Nucleophilic Thiol Functions as Quinone Targets

The thiol of cysteine is the major redox-active and nucleophilic functional group in biological systems. This amino acid is a component of the redox-active peptide GSH and many proteins. GSH, a tripeptide of γ–glutamyl-cysteinyl-glycine, serves as a reductant and nucleophile in cells, protecting them from chemical insults that could lead to cellular distress. It is present in high concentrations (1–10 mM) in cells and is the cosubstrate for multiple reductive and conjugative pathways (30, 37–39). Cysteine thiols serve as key nucleophiles in the catalytic center of numerous enzymes that modify an electrophilic center. Two such enzymes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and protein tyrosine phosphatase 1B (PTP1B), are mentioned here because their interactions with 1,2-NQ are discussed further in this review.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). This glycolytic enzyme utilizes the thiolate of Cys152 (in the human enzyme) to form a covalent bond with the carbonyl carbon of glyceraldehyde-3-phosphate (Scheme 2).

Scheme 2

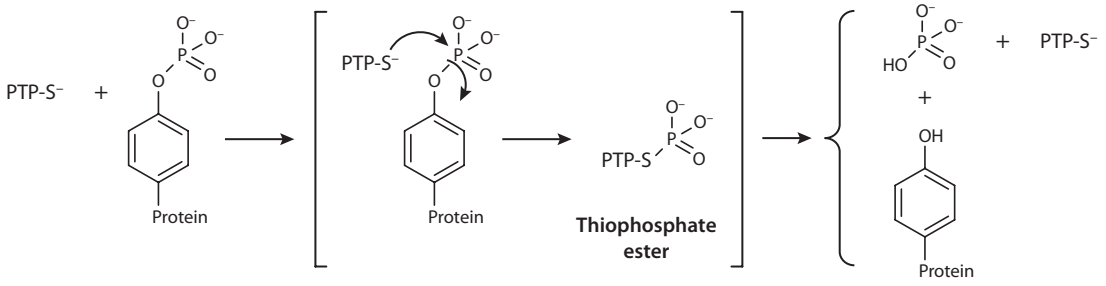


Thiohemiacetal **Thioester**

The resulting complex, a thiol acetal, transfers a hydride to NAD to form the thiol ester of glyceric acid-3-phosphate and NADH (40). The thiol ester is then cleaved by phosphorylation to generate the product, 1,3-diphosphoglyceric acid. The thiolate anion is critical to the reaction, and to promote its reactivity, neighboring groups serve to decrease its pKa from a typical thiol pKa of ~8 to 6. This decrease allows it to be extensively ionized and chemically available at physiological pH (41).

Protein tyrosine phosphatase 1B (PTP1B). Protein tyrosine phosphatases (PTPs) utilize serine hydroxyl or cysteine thiols to catalyze the hydrolysis of a tyrosine phosphate ester (42, 43) (Scheme 3).

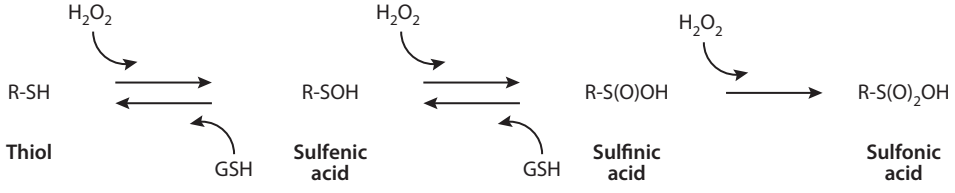
Scheme 3



PTP1B is a cysteine- or thiol-based phosphatase that utilizes the nucleophilic properties of the thiolate anion at the residue of Cys215 (in the human enzyme) to displace the tyrosine from the phosphate moiety, generating a phosphothioester. This ester is then hydrolyzed to phosphate, the thiolate enzyme, and the protein substrate.

As with GAPDH, the low pKa value of the thiol function raises the proportion of the reactive, thiolate state in the protein (44). With a lower thiol pKa value, proteins such as these contribute to the availability of reactive thiolate moieties in the cell because of their greater ionization, compared with GSH, whose pKa is approximately 9. In the thiolate state, oxidation is also easier, and oxidation of the thiol inactivates these enzymes because of the reduced nucleophilicity of the sulfur function. In the case of PTPs, this reaction appears to be a mechanism for their regulation.

Thiols can undergo two-electron oxidation reactions to the sulfenate, sulfinate, and sulfonate states sequentially, as shown in Reaction 7, with hydrogen peroxide as the electron acceptor. Hydrogen peroxide is a cell signaling molecule whose action depends on the oxidation of thiols (45–47).



Reaction 7

Except for the oxidation of sulfinic acid to sulfonic acids, the reactions are biochemically reversed by GSH-dependent reductases (48). Sulfenic acids can also be reduced to the thiol by dithiothreitol (DTT), a common thiol-reducing agent (49). Thiols and their oxidation products, sulfenic acids (46, 50) and sulfinic acids (51), are involved in cell signaling reactions that involve hydrogen peroxide as a second messenger.

Nucleophilic Nitrogen Functions as Quinone Targets

The terminal amino group in lysine and the imidazole group in histidine are also nucleophilic. However, the pKa of the terminal amino group in lysine has a pKa of approximately 10, so at

physiological pH, the group is almost completely charged unless a neighboring function can lower its pKa to allow electrophiles to react with its neutral, nucleophilic state (52, 53). The imidazole nitrogen is also nucleophilic; it has a pKa value of approximately 6, so a high percentage of the function is neutral at physiological pH. As such, it is an important nucleophile in the action of hydrolytic enzymes such as cholinesterases. The imidazole nitrogen is also a target for the electrophilic action of 1,2-NQ on PTP1B, with which it forms a covalent bond (54). 1,4-BQ is reported to be covalently bound to solvent-exposed lysine-rich regions of cytochrome *c* (55).

BIOCHEMISTRY AND CELLULAR BIOLOGY

This section summarizes the chemistry of quinone interaction with selected biological targets and its consequences. The structures of the quinones described are shown in **Figure 1**, and target proteins are summarized in **Table 1**.

Prooxidant Effects of Quinones

In their prooxidant interactions, quinones can transfer electrons from a biological substrate to oxygen, generating ROS that oxidize functional groups on proteins. The prooxidant capability is dependent on the reduction potential of the specific quinone. For example, 9,10-PQ acts primarily as a prooxidant, whereas 1,4-BQ acts primarily as an electrophile (56). 9,10-PQ does not have the electrophilic properties of benzo- and naphthoquinones and behaves more like an aromatic carbonyl compound. The most common functional group target of quinones is the ionized state of the thiol function, which is susceptible to oxidation (Reaction 7).

Numerous naphthoquinones undergo one- and/or two-electron reduction by flavin and non-flavin enzymes (1, 3, 4). For example, 1,2-NQ is a good electron acceptor and thus an efficient substrate for the aldo-keto reductase (AKR) isozymes (57), NAD(P)H quinone oxidoreductase 1 (NQO1) (58) and cytochrome P450 reductase (59). Results of detailed studies that investigate the prooxidant actions of quinones on proteins are summarized below.

GAPDH (Scheme 2) is an important enzyme in yeast growth and reproduction, and toxicity studies showed that it was inactivated by both 9,10-PQ and 1,4-BQ but by mechanisms that reflect their differing chemistry (60). The prooxidant 9,10-PQ inactivated the enzyme by a DTT-reversible mechanism, whereas the electrophilic 1,4-BQ did not. These observations were interpreted to reflect the oxidation of the thiolate function in the enzyme by 9,10-PQ to the sulfenic acid or higher oxidation state (Reaction 7) or the formation of an intramolecular cys-cys disulfide, either of which would render the enzyme inactive. DTT could reduce both of these functions back to thiol states. Enzyme inactivated by 1,4-BQ was not reactivated under these conditions, reflecting the formation of an irreversible, covalent bond. In other studies with this enzyme, the redox-active 2,3-dimethoxy-1,4-NQ (**9**, **Figure 1**) (61) was incubated with GAPDH in the presence of ascorbate as an electron donor. The enzyme was inactivated under aerobic but not under anaerobic conditions, suggesting that the ROS generated by the quinone in the presence of the electron donor indirectly inactivated the enzyme. In contrast, incubation with the electrophile *N*-methyl maleimide resulted in a time-dependent inactivation that could be blocked by the continued presence of DTT, which acted as an alternate nucleophile (49). This inactivation of GAPDH by electrophiles under anaerobic conditions has been used as a general assay to demonstrate the presence of electrophiles in ambient air samples (62) and diesel exhaust particles (12).

All NOS isozymes are cytochrome P450–based enzymes that convert arginine to citrulline and the gaseous signaling molecule nitric oxide (NO). Naphthoquinones with one-electron reduction

Table 1 Target molecules of naphthoquinones and their cellular consequences

Number[a]	Naphthoquinone	Source examined[b]	Target(s)	Effect[c]	Consequence(s)[d]	Reference(s)
1	1,2-Naphthoquinone	a	EGFR phosphorylation	↑	Contraction of guinea pig trachea	80
		b	eNOS	↓	Suppression of vasorelaxation	65
		a, b	PTP activity, PTP1B activity	↓	Activation of EGFR signaling	54
		a, b	CREB	↓		116
		a	IKKβ/NF-κB/NO signaling	↓		106
		a	Inflammatory mediators	↑	Proinflammatory	103, 104
		a, b	Keap1	↓	Activation of Nrf2	89
2	β-lapachone	b	DNA topoisomerase I	↓		108
		b	DNA topoisomerase II	↓		107
		a	E2F1, S-phase checkpoint	↑	Apoptosis	112
		a	pRB, p21	↑	Proliferation inhibition	110
		a	NF-κB	↓	Apoptosis, growth inhibition	109
		a	Bcl-2	↓	Apoptosis, growth inhibition	111
		a	NF-κB, inflammatory cytokines	↓	Anti-inflammatory	102
3	1,4-Naphthoquinone	a	Nrf2	↑		89
4	Juglone	a	Tubulin, cyclin B	↓	Apoptosis, cell cycle arrest	121
		b	Pin1	↓		72
		b	RNA polymerase II	↓		146
		a	GM-CSF, IL-5	↓	Anti-inflammatory	101
		a	COX-2	↓	Anti-inflammatory	147
5	Plumbagin	a	PI_3K/Akt/mTOR pathway	↓	Autophagy, cell cycle arrest	123
		a	p53, JNK	↑	Apoptosis, cell cycle arrest	122
		a	NF-κB and its regulated genes	↓	Apoptosis, inhibition of cell invasion	148
		a	NF-κB, AP-1, MMP-2, u-PA	↓	Inhibition of cell invasion and migration	149
		a	STAT3, c-Src, JAK1, JAK2	↓	Apoptosis, proliferation inhibition	150
		a, b	p300 histone acetyltransferase	↓		73

(*Continued*)

Table 1 (*Continued*)

Number[a]	Naphthoquinone	Source examined[b]	Target(s)	Effect[c]	Consequence(s)[d]	Reference(s)
		a	NF-κB, inflammatory cytokines	↓	Anti-inflammatory	99
		a	Inflammatory mediators	↓	Anti-inflammatory	100
		a	Nrf2-ARE pathway	↑	Neuroprotection	90
		a	STAT3	↑	Proliferation	124
6	Dichloroallyl lawsone	a	Pyrimidine biosynthesis	↓		119
		a, b	Pyrimidine biosynthesis, dihydroorotate dehydrogenase	↓		118
		b	Dihydroorotate dehydrogenase	↓		120
7	Lapachol	b	DT diaphorase	↓		114
		a	DNA scission	↑		115
8	PPM-18	a	NF-κB, iNOS	↓	Anti-inflammatory	96
10–12	Vitamin K	a	EGFR, ERK	↑	Decrease of gap-junctional intercellular communication	79
		a	EGFR, ErbB2	↑		78
		a	NF-κB, inflammatory cytokines	↓	Anti-inflammatory	97
		a	NF-κB, TNF-α	↓	Anti-inflammatory	98
		a	Nrf2-target gene products	↑		91
13	Caulibugulone A	a	Cdc25	↓	Cell cycle arrest	66

[a]These numbers reflect the structures in **Figure 1**.

[b]a, cells; b, purified enzyme.

[c]Effect refers to increase (up) or decrease (down) in the response or [d]consequence of quinone action.

Abbreviations: AP-1, activator protein 1; ARE, antioxidant response element; COX-2, cyclooxygenase 2; CREB, cyclic adenosine monophosphate (cAMP) response element-binding; EGFR, epidermal growth factor receptor; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; GM-CSF, granulocyte-macrophage colony-stimulating factor; IKKβ, inhibitor of nuclear factor κB; IL-5, interleukin-5; iNOS, inducible nitric oxide synthase; JAK1/2, Janus kinase 1/2; JNK, c-Jun N-terminal kinase; Keap1, Kelch-like ECH-associated protein 1; MMP-2, matrix metalloproteinase 2; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor κB; NO, nitric oxide; Nrf2, NF-E2-related factor 2; PI_3K, phosphatidylinositol 3-kinase; pRB, retinoblastoma protein; PTP, protein tyrosine phosphatase; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor-α; u-PA, urokinase-type plasminogen activator.

potentials in the range of −203 mV to −36 mV shunt electrons away from the cytochrome P450 reductase component of neuronal NOS, thereby reducing the catalytic activity that generates NO (63). In this process, also known as uncoupling, the shunted electrons can be transferred to oxygen to generate superoxide as demonstrated by electron spin resonance analysis (63). Further studies revealed that 9,10-PQ and 1,2-NQ suppress endothelial NOS by this mechanism and block changes in vascular tone mediated by NO (64, 65). Additional examples of the redox-based inhibition of enzymes by quinones include the structurally related isoquinoline-1,4-quinones (e.g., caulibugulone A, **13**, **Figure 1**), which inhibit Cdc25 phosphatases primarily by their prooxidant action (66, 67).

Electrophilic Effects of Quinones

Proteomic analyses have shown that numerous cellular proteins undergo covalent modification by electrophilic reactions with naphthoquinones. For example, vitamin K_3 (**12**, **Figure 1**) modifies aldose reductase presumably through covalent bond formation with Cys298 (68). Following exposure of HepG2 cells to biotinylated vitamin K_2 (**11**, **Figure 1**), the quinone moiety of both the biotinylated and free vitamin K bound to 17β-hydroxysteroid dehydrogenase 4, apolipoprotein E, and 40S ribosomal proteins S7 and A14 (69). Both 1,4-BQ and 1,4-NQ form covalent bonds with multiple cellular proteins such as galectin-1, protein disulfide isomerase, and 60-kDa heat-shock protein upon incubation with human bronchial epithelial cells (70). The reactions of quinones with biological systems exhibit structural requirements in addition to the quinone functional group. In a study comparing the toxicities of juglone (**4**, **Figure 1**) and plumbagin (**5**, **Figure 1**), Inbaraj & Chignell (71) showed that the toxicity of plumbagin is primarily redox based, whereas that of juglone is electrophilic. Reflective of its electrophilic properties, juglone irreversibly inactivates the peptidyl-prolyl isomerase Pin1 by formation of covalent bonds with thiol groups at Cys41 and Cys69 of the *E. coli* enzyme (72). The ultimate inactivation of this enzyme appears to involve a rate-limiting conformational change following arylation to inactive protein. Plumbagin does have some non-redox-based actions, however, as it inhibits p300 histone acetyltransferase by hydrogen bond formation with the lysine 1358 of the enzyme (73).

Kelch-like ECH-associated protein 1 (Keap1): a negative regulator of Nrf2 and an electrophilic/oxidative stress sensor with reactive thiols

Modification of Signal Transduction by Quinones

Quinones have been shown to modify cell signaling pathways by reacting with key regulatory proteins, either directly as electrophiles or indirectly through the generation of ROS. Two examples of such regulatory proteins, protein tyrosine phosphotase (PTP) and Kelch-like ECH-associated protein 1 (Keap1), are described below.

Protein tyrosine phosphatase 1B (PTP1B). PTPs and dual-specificity phosphatases have important regulatory functions in cells (74–76). They are targets for anticancer therapeutic agents because of their role in cell proliferation, and several quinones, including vitamin K analogs, affect these enzymes by forming covalent bonds with specific nucleophiles. Several synthetic derivatives of menadione (**12**, **Figure 1**, also known as vitamin K_3) inhibit protein phosphatases such as Cdc25 and PTP1B through binding to key cysteine residues in the enzymes (74). Vitamin K_3 itself activates epidermal growth factor receptor (EGFR)/ErbB2 and EGFR/extracellular signal-regulated kinase (ERK) signaling through reduction of cellular PTP activity (77–79). In an environmentally relevant observation, 1,2-NQ caused a concentration-dependent contraction of guinea pig aortic rings through phosphorylation of EGFR (80). This action of 1,2-NQ was further investigated in human epithelial A431 cells. In this system, 1,2-NQ causes phosphorylation of EGFR that is coupled to a decline in PTP activity (54). Although the interaction of the EGFR agonist EGF produces ROS that reduce PTP1B activity by oxidizing the active site Cys215 (81), 1,2-NQ reduces PTP1B activity by covalent bond formation with its Cys121 (54) (**Figure 2*a***). These oxidative and electrophilic modifications of cysteine residues in PTP1B are associated with activation of EGFR in the cells because phosphatases negatively regulate EGFR (81). In transfection studies with a variety of PTP DNA, we have found that Src homology 2 domain-containing phosphatase 1 also has a dominant role in the blockage of EGFR activation caused by 1,2-NQ in A431 cells (N. Iwamoto & Y. Kumagai, unpublished observation).

Using a specific antibody (donated by Dr. Arne Ostman of the Ludwig Institute for Cancer Research in Sweden) that recognizes the SO_3H group of Cys215 in PTP1B, investigators showed

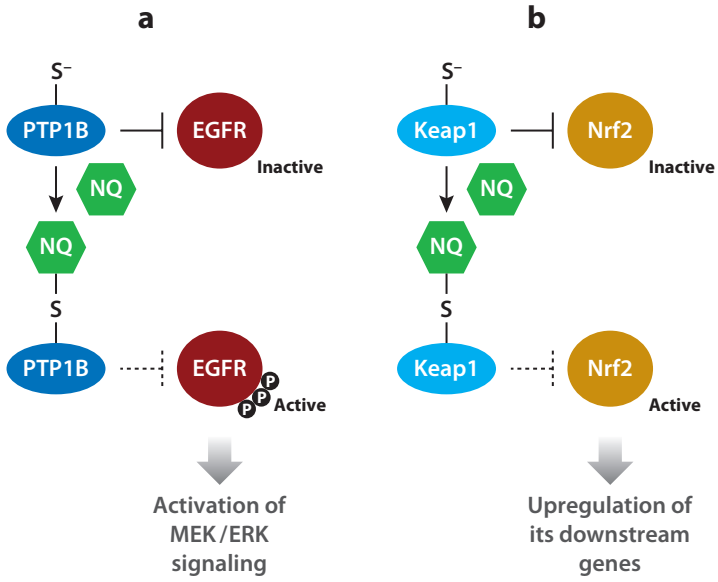


Figure 2

Naphthoquinone-mediated activation of cellular signaling pathways through covalent modification. (*a*) 1,2-NQ can modify the noncatalytic Cys121 (which is present as its thiolate ion at physiological pH), thereby forming an adduct with PTP1B. Crystallographic analysis revealed that this adduction affects the microenvironment of Cys215, resulting in inhibition of PTP1B activity accompanied by activation of EGFR by phosphorylation [Reference 54; S. Ito, N. Iwamoto & Y. Kumagai, unpublished observation]. The phosphorylation of EGFR then results in the activation of the MEK/ERK signaling pathway (N. Iwamoto, P. Chang & Y. Kumagai, unpublished observation). If the 1,2-NQ-PTP1B adduct does not undergo degradation, prolonged phosphorylation of EGFR occurs. (*b*) Covalent modification of Keap1 through cysteine residues causes activation of Nrf2, thereby increasing gene expression of glutamate-cysteine ligase, GSH *S*-transferases, NAD(P)H quinone oxidoreductase 1, aldo-keto reductases, and multidrug resistance–associated proteins, which contribute to the detoxification and excretion of 1,2-NQ (87, 88). Abbreviations: EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; Keap1, Kelch-like ECH-associated protein 1; MEK, mitogen-activated ERK kinase; Nrf2, nuclear factor (NF)-E2-related factor 2; PTP1B, protein tyrosine phosphatase 1B; NQ, naphthoquinone.

that the reaction of recombinant human PTP1B with 50 μM of 1,2-NQ for 10 min at 25°C causes an oxidative modification of the active cysteine residue in wild-type PTP1B, whereas there is no such further oxidation in the C215S mutant of PTP1B (N. Iwamoto & Y. Kumagai, unpublished observation). This finding suggests that oxidation of this thiol is due to a redox cycling of the thiol groups with an *ortho*-quinone such as 1,2-NQ (82). Because 1,2-NQ also covalently binds to Cys121 of PTP1B, both covalent attachment and ROS generation may occur to reduce PTP1B activity in A431 cells. Cys121 does not directly affect PTP1B activity because its mutation to serine does not affect PTP1B activity. The pKa value of this cysteine thiol is lowered by a lysine residue at position 120, making it a reactive nucleophilic target for 1,2-NQ. The noncatalytic property of Cys121 was confirmed by substitution of the lysine at this position by alanine; the modified protein was not alkylated by 1,2-NQ (N. Iwamoto & Y. Kumagai, unpublished observation).

Nuclear factor (NF)-E2-related factor 2 (Nrf2): a transcription factor that regulates the expression of many cytoprotective genes

Kelch-like ECH-associated protein 1 (Keap1). The Keap1/nuclear factor (NF)-E2-related factor 2 (Nrf2) complex regulates cellular responses to chemical insults by increasing the expression of protective proteins such as GSH *S*-transferases (GSTs) and glutamate-cysteine ligase (GCL)

(83, 84). Keap1, a multithiol protein, is the negative regulator of the system, and when complexed with Nrf2, Keap1 mediates the rapid degradation of Nrf2 through interaction with cullin 3.

Several lines of evidence have indicated that the reactive thiols in Keap1, Cys151, Cys273, and Cys288 form covalent bonds with a variety of electrophiles, resulting in Nrf2 activation (85). Estrogen quinones, derived from 2- or 4-hydroxyestrogen, alkylate Keap1 through thiol groups that include Cys151, Cys273, and Cys288 (86). The chemically simpler 1,2-NQ, however, covalently binds to mouse Keap1 not only through Cys151, Cys273, and Cys288 but also through Cys257 and Cys488 (87, 88). These covalent modifications of Keap1 (see **Figure 2*b***) result in Nrf2 activation, which then upregulates antioxidant proteins such as GCL, phase II xenobiotic enzymes such as GSTs, uridine 5′-diphosphate (UDP)-glucuronyltransferases (UGTs), NQO1, AKRs, and phase III transporters such as multidrug resistance–associated proteins (MRPs) (**Figure 2*b***). Because these proteins are responsible for detoxification and excretion of quinoid compounds, Nrf2 clearly plays a critical role in cellular protection against quinones. Supporting this contention, experiments with primary hepatocytes from Nrf2 knockout mice revealed that deletion of Nrf2 enhances covalent modification of the cellular proteins and cellular toxicity (88). Related studies have shown that 1,4-NQ activates Nrf2 in human pulmonary artery endothelial cells (89) and that its derivative plumbagin activates the Nrf2-electrophile response element/antioxidant response element (Nrf2-EpRE/ARE) pathway in neuroblastoma SHSY-5Y cells as well as in primary cortical neurons (90). Menadione is thought to activate Nrf2 because it induces the expression of Nrf2-target gene products (91). As mentioned above, there is little evidence that oxidative modification of Keap1 thiols by ROS leads to activation of Nrf2. Pretreatment with the antioxidant trolox or with catalase conjugated with polyethylene glycol to scavenge hydrogen peroxide blocked the intracellular oxidant and/or ROS generation caused by estrogen quinones (86), 1,2-NQ (88), and *tert*-butyl-1,4-BQ (92), but these pretreatments did not affect Nrf2 activation during exposure to electrophiles. We have not detected an oxidative modification of Keap1 caused by 1,2-NQ, and most other studies have indicated that Nrf2 activation results from covalent modification of Keap1 as well. An exception is the report by Fourquet et al. (93), who showed that the intermolecular disulfide formation of Keap1 via Cys151 leads to Nrf2 activation following exposure of HeLa cells to NO or hydrogen peroxide. Endogenous electrophiles (e.g., 8-nitro-guanosine 3′,5′-cyclic monophosphate, 9- and 10-nitro-9-*cis*-octadecadienoic acid) are produced under nitrosative and oxidative stresses (94), and most endogenous electrophiles are capable of activating Nrf2 through covalent modification of Keap1 thiols (94, 95). We speculate, therefore, that endogenous electrophiles may play an indirect role in the nitrosative stress–mediated and oxidative stress–mediated Nrf2 activation.

Electrophile responsive element/antioxidant response element (EpRE/ARE): a consensus sequence (5′-RTGAG/CNNNGCR-3′) that binds Nrf2/small Maf heterodimer in genes

Quinones and Disease States

The chemical properties of the quinone function and their actions on cellular macromolecules have led to the use of quinones in the treatment of different disease states. In addition to their use in drug design, however, quinones also have adverse effects on the same systems being targeted for therapeutic agents. We summarize below actions of quinones on two disease states, inflammation and cancer.

Anti-inflammatory effects. Both 1,4- and 1,2-NQ derivatives exhibit anti-inflammatory actions. The synthetic 1,4-NQ derivative PPM-18 (**8**, **Figure 1**), a potent inhibitor of inducible NOS expression, acts by blocking the binding of nuclear factor κB (NF-κB) to the promoter (96), whereas vitamin K (**10–12**, **Figure 1**) (97, 98) and plumbagin (99, 100) exhibit anti-inflammatory effects by inhibiting NF-κB activation. Plumbagin also appears to have a selective effect on the

Nrf2-EpRE/ARE system, activating it through a PI_3 kinase/Akt pathway under conditions in which vitamins K_1, K_2, and K_3 are ineffective (90). Juglone, which lacks the 2-methyl group of plumbagin, selectively inhibits the parvulines or Pin1, a member of the three families of peptidyl-prolyl isomerases (72). This inhibitory property of juglone was used to investigate the role of Pin1 in inflammatory cytokine expression following eosinophil activation (101). In the study, rats were dosed with juglone, and, after allergen challenge, they exhibited a reduction in bronchoalveolar lavage fluid and pulmonary eosinophils, together with a reduction of granulocyte-macrophage colony-stimulating factor and interleukin-5 (101). The 1,2-NQ derivative β-lapachone (**2**, **Figure 1**) attenuates the expression of proinflammatory cytokines such as interleukin-1β, interleukin-6, and TNF-α through the inhibition of NF-κB activation in lipopolysaccharide (LPS)-stimulated BV2 microglia (102).

Proinflammatory effects. Proinflammatory actions of naphthoquinones have also been observed. When administered to mice, 1,2-NQ increased allergic airway inflammation as evidenced by increased lung expression of interleukin-4, interleukin-5, eotaxin, macrophage chemoattractant protein-1, and keratinocyte chemoattractant (103). 1,2-NQ was subsequently shown to induce/enhance airway hyperresponsiveness in the presence or absence of antigen in vivo and to potentiate pulmonary expression of interleukin-13 and MUC5AC in the presence of antigen (104). Experiments with inducible NOS knockout mice revealed that NO formation catalyzed by this enzyme has a protective role in acute lung inflammation that is caused by LPS (105). Although we reported that 1,2-NQ inhibits NOS-catalyzed NO production through an uncoupling reaction, 1,2-NQ was recently found to block LPS-mediated activation of inhibitor of nuclear factor κB (IKKβ)/NF-κB/NO signaling, resulting in the downregulation of iNOS expression in RAW264.7 cells and a substantial decrease in NO formation; similar results were also seen in the lungs of mice treated with LPS by intratracheal administration (106). These findings suggest that the disruption of the NF-κB pathway caused by 1,2-NQ exposure might facilitate LPS-dependent acute lung injury.

Anticancer effects. Numerous natural naphthoquinone derivatives exhibit actions that suggest a possible use as anticancer agents. The 1,2-NQ derivative β-lapachone is reported to be an inhibitor for DNA topoisomerase I and II (107, 108) and also affects signal transduction–related molecules, resulting in growth inhibition and apoptotic cell death (109–112). Lapachol (**7**, **Figure 1**) occurs in the wood of several species of the family *Bignoniaceae* and had been tested extensively as an antimalarial agent in the 1940s. This naphthoquinone was also known to exhibit antitumor activity (113). Although lapachol is a potent inhibitor of DT diaphorase (114), it undergoes one-electron reduction by cytochrome P450 reductase to generate ROS such as hydroxyl radicals that promote DNA scission associated with anticancer activity (115). The cyclic adenosine monophosphate (cAMP) response element-binding (CREB) protein is another transcription factor with conserved cysteine residues that regulate its DNA binding. Exposure of bovine aortic endothelial cells to 1,2-NQ disrupts CREB-dependent DNA binding activity, thereby downregulating Bcl-2 expression. This inhibitory action of 1,2-NQ on CREB activity was, at least in part, due to covalent modification of reactive thiols in CREB (116). A subsequent study showed that although CREB undergoes chemical modification by 1,2-NQ at Cys286, Lys290, and Lys319, the alkylation of Cys286 is essential for suppression of the DNA binding activity (117). CREB-regulated genes such as *Bcl-2* contribute to the inhibition of apoptosis, suggesting that 1,2-NQ might have an effect on apoptotic signaling. Dihydroorotate dehydrogenase, a key enzyme in pyrimidine nucleotide metabolism, is inhibited by the 1,4-NQ derivative dichloroallyl lawsone (**6**, **Figure 1**) (118–120). Juglone and plumbagin have also been examined in this context. Juglone induces apoptosis and

accumulation of cells in S phase by a mechanism distinct from its actions on Pin1 (see above). This compound affects microtubules and blocks mitotic spindle assembly, thereby preventing mitotic exit. Covalent bond formation with tubulin is suggested as a possible mechanism (121). Plumbagin inhibits cell proliferation and induces apoptosis through modulation of different signal transduction pathways such as NF-κB, STAT3 (signal transducer and activator of transcription 3), AP-1 (activator protein 1), p53, and Nox4 in a pulmonary A549 cell line (122). Other studies showed that plumbagin suppresses the PI_3K/Akt/mTOR pathway, thereby enhancing G_2-M arrest and autophagy in two breast cancer cell lines (MDA-MB23 and MCF-7) (123). In contrast to these antiproliferative effects, which used concentrations in excess of 1 μM, Luo et al. (124) recently reported a proliferative effect of plumbagin when embryonic progenitor cells were exposed to it at low (nanomolar) concentrations, so there is clearly a concentration dependency to the various actions of this compound.

***S*-transarylation:** a thioether exchange

Biochemical Fate of Modified Cellular Proteins

The biochemical fate of quinones follows their inherent chemical properties (1). They can undergo a two-electron reduction to the hydroquinone, which can then be conjugated with glucuronic acid, as shown for the redox-active 9,10-PQ (125). Their electrophilicity results in enzymatic and nonenzymatic conjugation with GSH to thioethers, which are rapidly excreted by MRPs (1). However, in terms of detoxication, GSH conjugation may not be as important as glucuronide formation of the hydroquinone because GSH depletion by buthionine sulfoximine did not affect 1,2-NQ toxicity, indicating that, like 9,10-PQ, the elimination of 1,2-NQ is dependent on glucuronic acid conjugation (88).

Although the protein adducts generated by the electrophilic actions of quinones may be considered as part of their metabolic fate, the arylated proteins retain the redox activity of the parent quinone, so they are termed active metabolites. In the context of their disposition, several reports indicate that the modified proteins may be reverted to their original active state by *S*-transarylation (126–128) or degradation of the protein, presumably resulting in a cysteine adduct. **Figure 3** summarizes the intracellular reactions of 1,2-NQ, its redox cycling, its detoxification, and the reactions that affect cell signaling (e.g., PTP1B/EGFR and Keap1/Nrf2, see **Figure 2**).

We examined the fate of 1,2-NQ arylated proteins in cells and found that proteins modified by this quinone may also be degraded by cellular processes. In a study using an antibody to 1,2-NQ to monitor arylated proteins (129) in A549 cells, a time-dependent loss in these proteins was observed; this loss was inhibited by the autophagic inhibitors such as chloroquine and 3-methyladenine (see **Figure 4**). A related report showed that the isoquinoline quinone caulibugulone A inactivated Cdc25A by promoting its degradation by a p38 kinase pathway (66). We have also observed a transarylation reaction involving GAPDH, which is discussed below.

Interactions of 1,2-Naphthoquinone with Glyceraldehyde-3-Phosphate Dehydrogenase

The glycolytic enzyme GAPDH is an important target in the inhibitory effects of quinones on yeast growth quinone toxicity (34), and direct studies with the enzyme and the electrophilic 1,4-BQ demonstrated its inhibition by an oxygen-independent irreversible process. Preliminary studies of the effects of 1,2-NQ on murine macrophage RAW264.7 cells (M. Shinyashiki, A. Eiguren-Fernandez & A.K. Cho, unpublished observation) and human pulmonary epithelial A549 cells (130) suggested, however, that this enzyme was unaffected when mammalian cells were exposed to the compound. These results indicate that the enzyme is resistant to the effects of the quinone

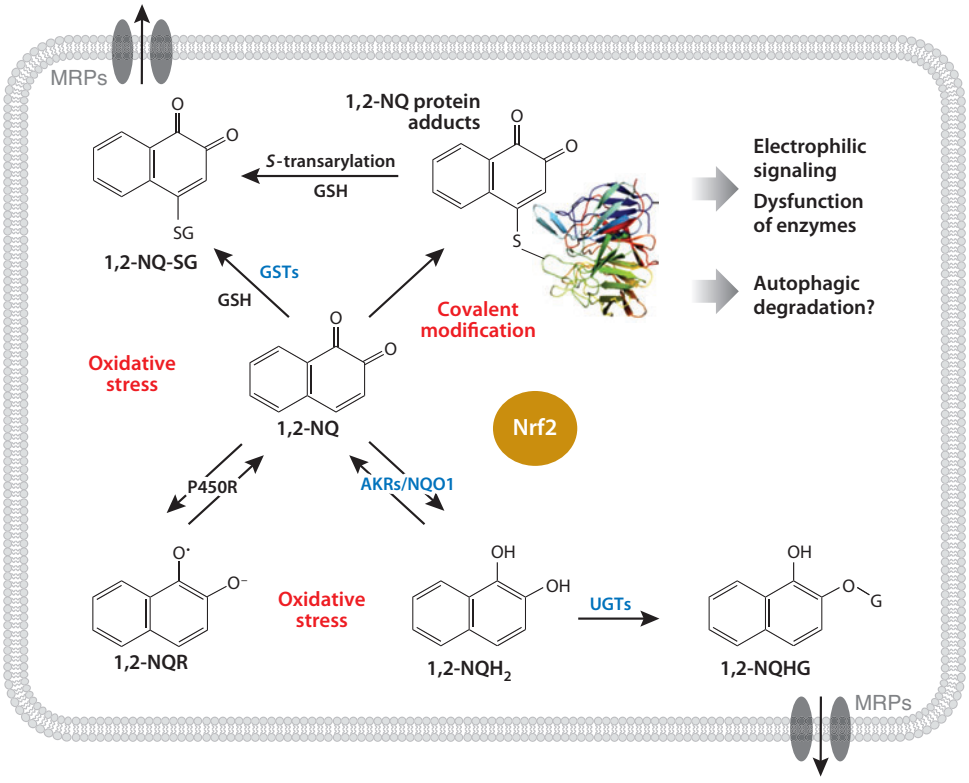


Figure 3

Biotransformation-based toxicological significance and biochemical fate of 1,2-naphthoquinone (1,2-NQ). Abbreviations: 1,2-NQH_2, 1,2-dihydroxynaphthalene; 1,2-NQHG, monoglucuronide of 1,2-NQH_2; 1,2-NQR, semiquinone radical of 1,2-NQ; 1,2-NQ-SG, glutathione (GSH) adduct of 1,2-NQ; AKR, aldo-keto reductase; GST, GSH *S*-transferase; MRP, multidrug resistance–associated protein; NQO1, NAD(P)H quinone oxidoreductase 1; Nrf2, nuclear factor (NF)-E2-related factor 2; P450R, cytochrome P450 reductase; UGT, uridine 5′-diphosphate (UDP)-glucuronyltransferase. Modified from Reference 88.

when exposure includes cellular processes. Subsequent studies showed that although several proteins undergo chemical modification by 1,2-NQ in A549 cells (130), there are proteins with the capacity to protect themselves or recover from chemical modification by 1,2-NQ. Examination of the 1,2-NQ-modified proteins with antibody and two-dimensional sodium dodecylsulfate polyacrylamide gel electrophoresis together with liquid chromatography/mass spectrometry analysis revealed that GAPDH was one such protein. As shown below, the interaction between 1,2-NQ and GAPDH is rather complex, with GAPDH acting both as a quinone reductase and as a catalyst for a transthiolation reaction.

GAPDH as a quinone reductase. GAPDH acts as a NADH-quinone reductase, transferring electrons from NADH to 1,2-NQ to form the corresponding hydroquinone that lacks electrophilicity (T. Miura, Y. Shinkai, R. Hirose, N. Iwamoto, A.K. Cho & Y. Kumagai, unpublished observation; **Figure 5**). However, 1,2-NQ is a selective substrate, approximately 30 times more reactive than 1,4-NQ and 20 times more reactive than 1,4-BQ. The two-electron reduction product,

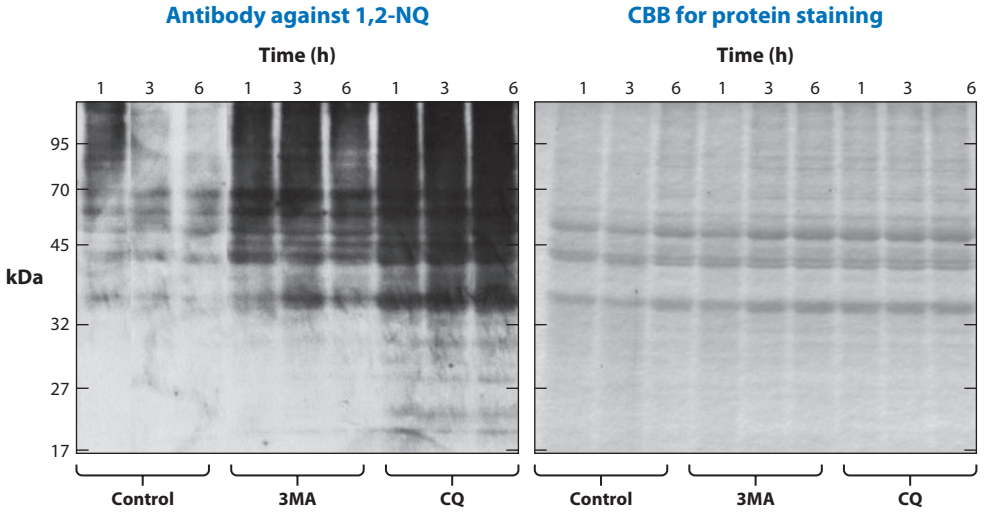


Figure 4

Effect of autophagy inhibitors on the chemical modification of cellular proteins during exposure of A549 cells to 1,2-naphthoquinone (1,2-NQ). (*Left*) Western blot analysis with specific antibody against 1,2-NQ. (*Right*) Protein staining. The cells were treated with 0.1 mM of chloroquine (CQ) for 12 h or 10 mM of 3-methyladenine (3MA) for 3 h prior to exposure to 20 μM of 1,2-NQ for 1, 3, or 6 h. The cellular proteins modified by 1,2-NQ were detected by Western blot analysis with a specific antibody against 1,2-NQ. Note the decline in the intensity of the modified proteins with time. The decline is reduced in the presence of the autophagy inhibitors. Abbreviation: CBB, Coomassie Brilliant Blue.

1,2-dihydroxynaphthalene, is rapidly glucuronidated by UGT, and this metabolite is transported into extracellular space through MRP transporters as reported previously for 9,10-PQ (125).

GAPDH as a catalyst for an *S*-transarylation reaction. The major thiol nucleophile in cells is GSH, which is important in quinone inactivation by conjugate formation. Its high intracellular concentration suggests that it may have a role in the apparent recovery from 1,2-NQ modification. With this in mind, we also examined its effect on the direct chemical interaction between 1,2-NQ and GAPDH (130). Addition of GSH to the inactive 1,2-NQ-modified GAPDH resulted in reactivation of the enzyme with the formation of the GSH conjugate of 1,2-NQ. The exchange involves a process in which the 1,2-NQ bound to the enzyme is displaced by GSH. The reaction has been postulated to include a reverse Michael reaction, in which a double conjugate of 1,2-NQ with GSH and GAPDH is formed; this double conjugate then dissociates with the liberation of the enzyme thiol (T. Miura, Y. Egara, R. Hirose, N. Iwamatot, Y. Shinkai, A.K. Cho & Y. Kumagai, unpublished information). The reaction likely reflects differences in the pKa values of the thiols involved. The pKa of the thiol in GAPDH is ~6, whereas that for GSH is ~9. Therefore, GAPDH, with the more acidic thiol, dissociates from the quinone more readily.

Song et al. (131) described an analogous reaction in which the chlorine atoms of polychlorinated biphenyl quinones were displaced by GSH. The authors argued that the loss of chlorine atoms reflects the better leaving-group properties of chloride compared with those of thiolate, which is consistent with observations made by us.

Subsequent siRNA screening studies by our laboratories have revealed that GAPDH does not affect other cellular proteins modified by 1,2-NQ action, i.e., they do not appear to be substrates for this transarylation reaction. However, cystathionine β-synthase and ubiquitin C-terminal

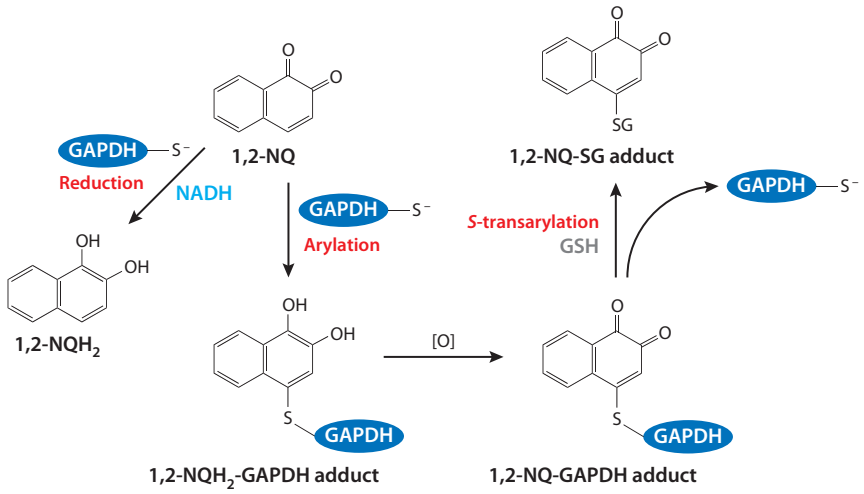


Figure 5

Actions of glyceraldehyde-3-phosphate dehydrogenase in the catalysis of the NADH-dependent two-electron reduction of 1,2-NQ and GSH-dependent *S*-transarylation of 1,2-NQ modified protein. GAPDH acts as a catalyst to form nonelectrophilic 1,2-NQH_2, thereby blocking potential chemical insult by 1,2-NQ. Cys152 is essential for the reduction of 1,2-NQ, but the reaction does not require its deprotonation to be regulated by His179 (130). The GSH-dependent *S*-transarylation reactions of GAPDH and 1,2-NQ consist of the following: 1,2-NQ is covalently bound to GAPDH at Cys152 by a Michael reaction; the generated 1,2-NQH_2-GAPDH adduct rapidly undergoes autoxidation to form the 1,2-NQ-GAPDH adduct; and a second Michael reaction leads to production of the dithiolated NQ moiety in the presence of GSH. This dithioether can then undergo a reverse Michael reaction, leading to the formation of 1,2-NQ-SG, which is coupled to the loss of 1,2-NQ-GAPDH adduct (130). Abbreviations: 1,2-NQ, 1,2-naphthoquinone; 1,2-NQ-GAPDH adduct, GAPDH modified by 1,2-NQ; 1,2-NQH_2, 1,2-dihydroxynaphthalene; 1,2-NQH_2-GAPDH adduct, GAPDH modified by 1,2-dihydroxynaphthalene; 1,2-NQ-SG adduct, GSH adduct of 1,2-NQ; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSH, glutathione. Modified from Reference 130.

hydrolase L1 are capable of suppressing covalent modification of cellular proteins by 1,2-NQ in A549 cells (T. Miura, T. Toyama, A. Yazawa, Y. Shinkai & Y. Kumagai, unpublished observations). In this case, 1,2-NQ reacts with SH^-, the anion of hydrogen sulfide (H_2S) produced by cystathionine β-synthase, to form the 1,2-NQ-SH adduct 4-thio-1,2-NQ and its dimeric ether, 4-thio-bis-1,2-NQ (1,2-NQ-S-1,2-NQ), both of which can then undergo glucuronic acid conjugation after two-electron reduction catalyzed by NQO1 and AKRs (130). We suggest that intracellular H_2S with a pKa value of 6.76 and GSH both can play a role in the inactivation of a variety of electrophiles including naphthoquinones.

GENERAL COMMENTS AND CONCLUSIONS

In their interactions with different cells, quinones can act as prooxidants, generating ROS by electron transfer reactions, and as electrophiles, reacting with nucleophilic centers on biological molecules to modify them through covalent bond formation. The focus of this review has been on 1,2- and 1,4-NQs and their derivatives. Experimental results have shown that 1,2-NQ is capable of reacting with multiple proteins as a prooxidant and electrophile. Experiments that made direct comparisons between 1,2- and 1,4-NQ showed that their reduction potentials are similar but that

there are selectivity differences in their electrophilic reactions. In this context, highly reactive 1,2-dihydroxy aryl or catechol moieties that can readily be oxidized to the corresponding quinones are found endogenously as neurotransmitters and as metabolites of steroids. The regulation of these reactive species is also critical, and, in addition to the more common metabolic pathways available, enzymes such as catechol-*O*-methyltransferase are important to the inactivation of these compounds.

ROLE OF QUINONES IN ENVIRONMENTAL TOXICOLOGY

The role of quinones in air pollution toxicity reflects their proinflammatory effects (10, 132–134). For example, air pollutant particles less than 2.5 μm in diameter activate the EGFR system (135) and promote airway remodeling by a prooxidant mechanism. This event, a secondary characteristic of asthma, results in an increase in extracellular matrix proteins, goblet cell and smooth muscle hyperplasia, and structural damage in the bronchial epithelium (136). Several investigators have presented histological evidence demonstrating increases in EGFR phosphorylation that accompany changes in bronchial epithelia (137–140). These events can be attributed to quinone action because vitamin K_3 has been shown to activate the EGFR-ErbB2 dimer by inhibition of PTP1B (78), as do the electrophilic 1,4-BQ and prooxidant 2,3-dimethoxy-1,4-NQ (77). As with 1,4-BQ, studies in our laboratories have documented the activation of EGFR by electrophilic actions of 1,2-NQ on PTP1B. This irreversible action on the enzyme may be more relevant to environmental exposure because, depending on the turnover rate of PTP1B and PTPs in general, it could result in a gradually increasing inactivation of this regulatory enzyme, which is the consequence of an irreversible effect on a slowly turning-over protein. Although their actual exposure concentrations are low, the cumulative effect of these electrophiles could lead to activation of EGFR and its downstream effects (54). However, although individual quinone actions support the role of quinones in the relevant inflammatory effects, evidence supporting atmospheric quinones and air pollution effects has been either correlative or indirect.

We have attempted to address the ambiguity of the participation of multiple possible quinones in environmental toxicity using assays that provide a quantitative but chemically undefined measure of prooxidant (82, 141) and electrophilic (12, 49, 142) capabilities. In the case of prooxidant content, the activities correlate with the ability of the sample to stimulate expression of heme oxygenase-1 (143). This finding indicates that, for a limited sample number, prooxidant activity is predictive of a cellular response, in this case a protective or adaptive one. These assays, however, measure only the content of a particular class of chemical and do not consider the toxicokinetics of these active chemicals, so their predictive ability is limited.

One element of the toxicokinetics of exposure to air pollutants is the composition of the aerosols that constitute air pollution. Air pollution mixtures are typically described in terms of particulate and vapor phases, with particles described in terms of their diameters (133). Quinones distribute differently between the two phases, depending on their physical properties such as vapor pressure and size (9), and can be generated on the surfaces of particles in an oxidative reaction involving ozone and nitrogen dioxide (14, 144). There are differences between particle-bound and vapor-phase quinones that could also affect their toxicokinetics. Vapor-phase quinones would be expected to have ready access to the lung components, whereas particle exposure varies with size and content. For example, studies with radiolabeled benzo[a]pyrene have shown that when particle bound, in this case to diesel exhaust particles, this PAH persists for protracted periods relative to free, vapor-phase PAH (145). After exposure by inhalation, ultrafine particles (<0.2 μm) access tissues besides the lungs, including the central nervous system, so that particle-bound organic compounds can be distributed throughout the body. Organic species such as quinones can distribute to cellular components by dissociation from the particles in accordance with their partition characteristics.

SUMMARY POINTS

1. Naphthoquinones are found in the environment as products of fuel combustion, tobacco smoke, and plants.
2. Naphthoquinones can catalyze redox cycling to produce ROS and react with tissue nucleophiles to modify proteins covalently.
3. 1,2-NQ and related naphthoquinones affect signal transduction pathways such as PTP1B/EGFR signaling and the Keap1/Nrf2 system.
4. Exposure of cultured cells and experimental animals to naphthoquinones promotes not only inflammation but also anti-inflammation; most of the studied naphthoquinones exhibit anticancer effects, but a precursor, naphthalene, is recognized as a carcinogen.
5. GAPDH has a unique ability to resist electrophilic modification by 1,2-NQ through (*a*) NADH-dependent two-electron reduction and (*b*) GSH-dependent *S*-transalkylation.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank, for their contributions to the studies, Masaru Shinyashiki, Hiromi Nakajima, Yoshimi Tsurutani, Kazumi Midorikawa, Akiko Endo, Sachie Koide, Keiko Taguchi, Yang Sun, Rika Sugimoto, Shouta Kikuno, Sayako Fujii, Noriko Iwamoto, Megumi Shimada, Hai-Yan Jiang, Masako Akimori, Yumiko Numasawa, Akira Nishiyama, Yuko Egara, Yumi Abiko, Reiko Hirose, and Daigo Sumi, who were and are graduate students and staff in the Environmental Medicine Section, Doctoral Programs in Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba. We also thank Arantzazu Eiguren-Fernandez, Emma Di Stefano, and Debra A. Schmitz for their contributions to the research performed at UCLA. The work described in this review was supported in part by Grant-in-Aid for scientific research from the Ministry of Education, Science, Culture, and Sports of Japan (#23117703, #22406004, #21659154, #21117504, #20241015, #18406003, #18659167, #15390184, and #15659141 to Y.K.). The UCLA work was supported in part by the U.S. Environmental Protection Agency (EPA)–funded Southern California Center for Airborne Particulate Matter (Grant RD-83241301), the South Coast Air Quality Management District (award number 040624), and the National Institutes of Environmental Health Sciences–funded Southern California Environmental Health Science Center (Grant 5P30 ES07048). Although the research described in this article has been funded in part by the EPA, it has not been subjected to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency. No official endorsement should be inferred.

LITERATURE CITED

1, 3. Comprehensive reviews on quinone toxicity.

1. **Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ. 2000. Role of quinones in toxicology. *Chem. Res. Toxicol.* 13:135–60**
2. Cadenas E, Hochstein P, Ernster L. 1992. Pro- and antioxidant functions of quinones and quinone reductases in mammalian cells. *Adv. Enzymol. Relat. Areas Mol. Biol.* 65:97–146

3. **Monks TJ, Jones DC. 2002. The metabolism and toxicity of quinones, quinonimines, quinone methides, and quinone-thioethers. *Curr. Drug Metab.* 3:425–38**
4. O'Brien PJ. 1991. Molecular mechanisms of quinone cytotoxicity. *Chem. Biol. Interact.*80:1–41. Erratum. 1992. *Chem. Biol. Interact.* 81(1–2):219
5. Penning TM, Burczynski ME, Hung CF, McCoull KD, Palackal NT, Tsuruda LS. 1999. Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active *o*-quinones. *Chem. Res. Toxicol.* 12:1–18
6. Pinto AV, de Castro SL. 2009. The trypanocidal activity of naphthoquinones: a review. *Molecules* 14:4570–90
7. Abdelmohsen K, Patak P, von Montfort C, Melchheier I, Sies H, Klotz LO. 2004. Signaling effects of menadione: from tyrosine phosphatase inactivation to connexin phosphorylation. *Methods Enzymol.* 378:258–72
8. Shearer MJ, Newman P. 2008. Metabolism and cell biology of vitamin K. *Thromb. Haemost.* 100:530–47
9. Eiguren-Fernandez A, Miguel A, Di Stefano E, Schmitz D, Cho AK, et al. 2008. Atmospheric distribution of gas- and particle-phase quinones in Southern California. *Aerosol Sci. Technol.* 42:854–61
10. Chung MY, Lazaro RA, Lim D, Jackson J, Lyon J, et al. 2006. Aerosol-borne quinones and reactive oxygen species generation by particulate matter extracts. *Environ. Sci. Technol.* 40:4880–86
11. Jakober CA, Riddle SG, Robert MA, Destaillats H, Charles MJ, et al. 2007. Quinone emissions from gasoline and diesel motor vehicles. *Environ. Sci. Technol.* 41:4548–54
12. Shinyashiki M, Eiguren-Fernandez A, Schmitz DA, Di Stefano E, Li N, et al. 2009. Electrophilic and redox properties of diesel exhaust particles. *Environ. Res.* 109:239–44
13. Kautzman KE, Surratt JD, Chan MN, Chan AWH, Hersey SP, et al. 2010. Chemical composition of gas- and aerosol-phase products from the photooxidation of naphthalene. *J. Phys. Chem. A* 114:913–34
14. Chu SN, Sands S, Tomasik MR, Lee PS, McNeill VF. 2010. Ozone oxidation of surface-adsorbed polycyclic aromatic hydrocarbons: role of PAH-surface interaction. *J. Am. Chem. Soc.* 132:15968–75
15. Murray R, Singh M. 1997. Activation of PAH by ozone derived oxidants: results using fly ash as particulate [I]. *Polycycl. Aromat. Compd.* 12:51–60
16. Finlayson-Pitts B, Pitts J. 2000. *Chemistry of the Upper and Lower Atmosphere: Theory, Experiments, and Applications*. San Diego, CA: Academic
17. Nadal M, Mari M, Schuhmacher M, Domingo JL. 2009. Multi-compartmental environmental surveillance of a petrochemical area: levels of micropollutants. *Environ. Int.* 35:227–35
18. Preuss R, Koch HM, Wilhelm M, Pischetsrieder M, Angerer J. 2004. Pilot study on the naphthalene exposure of German adults and children by means of urinary 1- and 2-naphthol levels. *Int. J. Hyg. Environ. Health* 207:441–45
19. Eiguren-Fernandez A, Miguel AH, Froines JR, Thurairatnam S, Avol EL. 2004. Seasonal and spatial variation of polycyclic aromatic hydrocarbons in vapor-phase and $PM_{2.5}$ in Southern California urban and rural communities. *Aerosol Sci. Technol.* 38:447–55
20. Lu H, Zhu L. 2007. Pollution patterns of polycyclic aromatic hydrocarbons in tobacco smoke. *J. Hazard. Mater.* 139:193–98
21. Lin CY, Isbell MA, Morin D, Boland BC, Salemi MR, et al. 2005. Characterization of a structurally intact in situ lung model and comparison of naphthalene protein adducts generated in this model versus lung microsomes. *Chem. Res. Toxicol.* 18:802–13
22. Gram TE. 1997. Chemically reactive intermediates and pulmonary xenobiotic toxicity. *Pharmacol. Rev.* 49:297–341
23. DeStefano-Shields C, Morin D, Buckpitt A. 2010. Formation of covalently bound protein adducts from the cytotoxicant naphthalene in nasal epithelium: species comparisons. *Environ. Health Perspect.* 118:647–52
24. Lin PH, Chen DR, Wang TW, Lin CH, Chuang MC. 2009. Investigation of the cumulative tissue doses of naphthoquinones in human serum using protein adducts as biomarker of exposure. *Chem. Biol. Interact.* 181:107–14
25. Preuss R, Angerer J, Drexler H. 2003. Naphthalene—an environmental and occupational toxicant. *Int. Arch. Occup. Environ. Health* 76:556–76

26. Zheng J, Cho M, Jones AD, Hammock BD. 1997. Evidence of quinone metabolites of naphthalene covalently bound to sulfur nucleophiles of proteins of murine Clara cells after exposure to naphthalene. *Chem. Res. Toxicol.* 10:1008–14
27. Penning TM, Ohnishi ST, Ohnishi T, Harvey RG. 1996. Generation of reactive oxygen species during the enzymatic oxidation of polycyclic aromatic hydrocarbon *trans*-dihydrodiols catalyzed by dihydrodiol dehydrogenase. *Chem. Res. Toxicol.* 9:84–92
28. Brunmark A, Cadenas E. 1989. Redox and addition chemistry of quinoid compounds and its biological implications. *Free Radic. Biol. Med.* 7:435–77
29. Sugimoto R, Kumagai Y, Nakai Y, Ishii T. 2005. 9,10-Phenanthraquinone in diesel exhaust particles downregulates Cu,Zn–SOD and HO-1 in human pulmonary epithelial cells: intracellular iron scavenger 1,10-phenanthroline affords protection against apoptosis. *Free Radic. Biol. Med.* 38:388–95
30. Wardman P. 1990. Bioreductive activation of quinones: redox properties and thiol reactivity. *Free Radic. Res. Commun.* 8:219–29
31. Goin J, Gibson DD, McCay PB, Cadenas E. 1991. Glutathionyl- and hydroxyl radical formation coupled to the redox transitions of 1,4-naphthoquinone bioreductive alkylating agents during glutathione two-electron reductive addition. *Arch. Biochem. Biophys.* 288:386–96
32. Taguchi K, Fujii S, Yamano S, Cho AK, Kamisuki S, et al. 2007. An approach to evaluate two-electron reduction of 9,10-phenanthraquinone and redox activity of the hydroquinone associated with oxidative stress. *Free Radic. Biol. Med.* 43:789–99
33. Kumagai Y, Nakajima H, Midorikawa K, Homma-Takeda S, Shimojo N. 1998. Inhibition of nitric oxide formation by neuronal nitric oxide synthase by quinones: nitric oxide synthase as a quinone reductase. *Chem. Res. Toxicol.* 11:608–13
34. Rodriguez CE, Shinyashiki M, Froines J, Yu RC, Fukuto JM, Cho AK. 2004. An examination of quinone toxicity using the yeast *Saccharomyces cerevisiae* model system. *Toxicology* 201:185–96
35. Slaughter DE, Hanzlik RP. 1991. Identification of epoxide- and quinone-derived bromobenzene adducts to protein sulfur nucleophiles. *Chem. Res. Toxicol.* 4:349–59
36. Smithgall TE, Harvey RG, Penning TM. 1988. Spectroscopic identification of *ortho*-quinones as the products of polycyclic aromatic *trans*-dihydrodiol oxidation catalyzed by dihydrodiol dehydrogenase: a potential route of proximate carcinogen metabolism. *J. Biol. Chem.* 263:1814–20
37. Monks TJ. 1995. Modulation of quinol/quinone-thioether toxicity by intramolecular detoxication. *Drug Metab. Rev.* 27:93–106
38. Monks TJ, Hanzlik RP, Cohen GM, Ross D, Graham DG. 1992. Quinone chemistry and toxicity. *Toxicol. Appl. Pharmacol.* 112:2–16
39. Schafer FQ, Buettner GR. 2001. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic. Biol. Med.* 30:1191–212
40. Segal HL, Boyer PD. 1953. The role of sulfhydryl groups in the activity of D-glyceraldehyde 3-phosphate dehydrogenase. *J. Biol. Chem.* 204:265–81
41. Talfournier F, Colloc'h N, Mornon JP, Branlant G. 1998. Comparative study of the catalytic domain of phosphorylating glyceraldehyde-3-phosphate dehydrogenases from bacteria and archaea via essential cysteine probes and site-directed mutagenesis. *Eur. J. Biochem.* 252:447–57
42. Barford D, Flint AJ, Tonks NK. 1994. Crystal structure of human protein tyrosine phosphatase 1B. *Science* 263:1397–404
43. Guan KL, Dixon JE. 1991. Evidence for protein-tyrosine-phosphatase catalysis proceeding via a cysteine-phosphate intermediate. *J. Biol. Chem.* 266:17026–30
44. Jones DP. 2008. Radical-free biology of oxidative stress. *Am. J. Physiol. Cell Physiol.* 295:849–68
45. Forman HJ, Maiorino M, Ursini F. 2010. Signaling functions of reactive oxygen species. *Biochemistry* 49:835–42
46. Poole LB, Nelson KJ. 2008. Discovering mechanisms of signaling-mediated cysteine oxidation. *Curr. Opin. Chem. Biol.* 12:18–24
47. Saurin AT, Neubert H, Brennan JP, Eaton P. 2004. Widespread sulfenic acid formation in tissues in response to hydrogen peroxide. *Proc. Natl. Acad. Sci. USA* 101:17982–87
48. Claiborne A, Yeh JI, Mallett TC, Luba J, Crane EJ III, et al. 1999. Protein-sulfenic acids: diverse roles for an unlikely player in enzyme catalysis and redox regulation. *Biochemistry* 38:15407–16

49. Shinyashiki M, Rodriguez CE, Di Stefano EW, Sioutas C, Delfino RJ, et al. 2008. On the interaction between glyceraldehyde-3-phosphate dehydrogenase and airborne particles: evidence for electrophilic species. *Atmos. Environ.* 42:517–29
50. Poole LB, Karplus PA, Claiborne A. 2004. Protein sulfenic acids in redox signaling. *Annu. Rev. Pharmacol. Toxicol.* 44:325–47
51. Jacob C, Holme AL, Fry FH. 2004. The sulfinic acid switch in proteins. *Org. Biomol. Chem.* 2:1953–56
52. Jacobs AT, Marnett LJ. 2010. Systems analysis of protein modification and cellular responses induced by electrophile stress. *Acc. Chem. Res.* 43:673–83
53. Labenski MT, Fisher AA, Lo HH, Monks TJ, Lau SS. 2009. Protein electrophile-binding motifs: Lysine-rich proteins are preferential targets of quinones. *Drug Metab. Dispos.* 37:1211–18
54. Iwamoto N, Sumi D, Ishii T, Uchida K, Cho AK, et al. 2007. Chemical knockdown of protein tyrosine phosphatase 1B by 1,2-naphthoquinone through covalent modification causes persistent transactivation of epidermal growth factor receptor. *J. Biol. Chem.* 282:33396–404
55. Fisher AA, Labenski MT, Malladi S, Gokhale V, Bowen ME, et al. 2007. Quinone electrophiles selectively adduct "electrophile binding motifs" within cytochrome *c*. *Biochemistry* 46:11090–100
56. Wardman P. 1989. Reduction potentials of one-electron couples involving free radicals in aqueous solution. *J. Phys. Chem. Ref. Data* 18:1637–756
57. O'Connor T, Ireland LS, Harrison DJ, Hayes JD. 1999. Major differences exist in the function and tissue-specific expression of human aflatoxin B1 aldehyde reductase and the principal human aldo-keto reductase AKR1 family members. *Biochem. J.* 343(Pt. 2):487–504
58. Lind C, Cadenas E, Hochstein P, Ernster L. 1990. DT-diaphorase: purification, properties, and function. *Methods Enzymol.* 186:287–301
59. Kumagai Y, Arimoto T, Shinyashiki M, Shimojo N, Nakai Y, et al. 1997. Generation of reactive oxygen species during interaction of diesel exhaust particle components with NADPH-cytochrome P450 reductase and involvement of the bioactivation in the DNA damage. *Free Radic. Biol. Med.* 22:479–87
60. Rodriguez CE, Fukuto JM, Taguchi K, Froines J, Cho AK. 2005. The interactions of 9,10-phenanthrenequinone with glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a potential site for toxic actions. *Chem. Biol. Interact.* 155:97–110
61. Gant TW, Rao DN, Mason RP, Cohen GM. 1988. Redox cycling and sulphydryl arylation; their relative importance in the mechanism of quinone cytotoxicity to isolated hepatocytes. *Chem. Biol. Interact.* 65:157–73
62. Eiguren-Fernandez A, Shinyashiki M, Schmitz DA, Di Stefano E, Hinds W, et al. 2010. Redox and electrophilic properties of vapor- and particle-phase components of ambient aerosols. *Environ. Res.* 110:207–12
63. Kumagai Y, Midorikawa K, Nakai Y, Yoshikawa T, Kushida K, et al. 1998. Inhibition of nitric oxide formation and superoxide generation during reduction of LY83583 by neuronal nitric oxide synthase. *Eur. J. Pharmacol.* 360:213–18
64. Kumagai Y, Hayashi T, Miyauchi T, Endo A, Iguchi A, et al. 2001. Phenanthraquinone inhibits eNOS activity and suppresses vasorelaxation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 281:25–30
65. Sun Y, Taguchi K, Sumi D, Yamano S, Kumagai Y. 2006. Inhibition of endothelial nitric oxide synthase activity and suppression of endothelium-dependent vasorelaxation by 1,2-naphthoquinone, a component of diesel exhaust particles. *Arch. Toxicol.* 80:280–85
66. Brisson M, Foster C, Wipf P, Joo B, Tomko RJ Jr, et al. 2007. Independent mechanistic inhibition of Cdc25 phosphatases by a natural product caulibugulone. *Mol. Pharmacol.* 71:184–92
67. Brisson M, Nguyen T, Wipf P, Joo B, Day BW, et al. 2005. Redox regulation of Cdc25B by cell-active quinolinediones. *Mol. Pharmacol.* 68:1810–20
68. Bhatnagar A, Liu SQ, Petrash JM, Srivastava SK. 1992. Mechanism of inhibition of aldose reductase by menadione (vitamin K3). *Mol. Pharmacol.* 42:917–21
69. Otsuka M, Kato N, Ichimura T, Abe S, Tanaka Y, et al. 2005. Vitamin K2 binds 17β-hydroxysteroid dehydrogenase 4 and modulates estrogen metabolism. *Life Sci.* 76:2473–82
70. Lame MW, Jones AD, Wilson DW, Segall HJ. 2003. Protein targets of 1,4-benzoquinone and 1,4-naphthoquinone in human bronchial epithelial cells. *Proteomics* 3:479–95

71. Inbaraj JJ, Chignell CF. 2004. Cytotoxic action of juglone and plumbagin: a mechanistic study using HaCaT keratinocytes. *Chem. Res. Toxicol.* 17:55–62
72. Hennig L, Christner C, Kipping M, Schelbert B, Rucknagel KP, et al. 1998. Selective inactivation of parvulin-like peptidyl-prolyl *cis/trans* isomerases by juglone. *Biochemistry* 37:5953–60
73. Ravindra KC, Selvi BR, Arif M, Reddy BA, Thanuja GR, et al. 2009. Inhibition of lysine acetyltransferase KAT3B/p300 activity by a naturally occurring hydroxynaphthoquinone, plumbagin. *J. Biol. Chem.* 284:24453–64
74. Kar S, Lefterov IM, Wang M, Lazo JS, Scott CN, et al. 2003. Binding and inhibition of Cdc25 phosphatases by vitamin K analogues. *Biochemistry* 42:10490–97
75. Samet JM, Tal TL. 2010. Toxicological disruption of signaling homeostasis: tyrosine phosphatases as targets. *Annu. Rev. Pharmacol. Toxicol.* 50:215–35
76. Tabernero L, Aricescu AR, Jones EY, Szedlacsek SE. 2008. Protein tyrosine phosphatases: structure-function relationships. *FEBS J.* 275:867–82
77. Abdelmohsen K, Gerber PA, von Montfort C, Sies H, Klotz LO. 2003. Epidermal growth factor receptor is a common mediator of quinone-induced signaling leading to phosphorylation of connexin-43: role of glutathione and tyrosine phosphatases. *J. Biol. Chem.* 278:38360–67
78. Beier JI, von Montfort C, Sies H, Klotz LO. 2006. Activation of ErbB2 by 2-methyl-1,4-naphthoquinone (menadione) in human keratinocytes: role of EGFR and protein tyrosine phosphatases. *FEBS Lett.* 580:1859–64
79. Klotz LO, Patak P, Ale-Agha N, Buchczyk DP, Abdelmohsen K, et al. 2002. 2-Methyl-1,4-naphthoquinone, vitamin K_3, decreases gap-junctional intercellular communication via activation of the epidermal growth factor receptor/extracellular signal-regulated kinase cascade. *Cancer Res.* 62:4922–28

80. Identification of 1,2-naphthoquinone as a chemical causing contraction of guinea pig trachea.

80. Kikuno S, Taguchi K, Iwamoto N, Yamano S, Cho AK, et al. 2006. 1,2-Naphthoquinone activates vanilloid receptor 1 through increased protein tyrosine phosphorylation, leading to contraction of guinea pig trachea. *Toxicol. Appl. Pharmacol.* 210:47–54

81. Tonks NK. 2003. PTP1B: from the sidelines to the front lines! *FEBS Lett.* 546:140–48
82. Kumagai Y, Koide S, Taguchi K, Endo A, Nakai Y, et al. 2002. Oxidation of proximal protein sulfhydryls by phenanthraquinone, a component of diesel exhaust particles. *Chem. Res. Toxicol.* 15:483–89
83. Kobayashi A, Kang MI, Watai Y, Tong KI, Shibata T, et al. 2006. Oxidative and electrophilic stresses activate Nrf2 through inhibition of ubiquitination activity of Keap1. *Mol. Cell. Biol.* 26:221–29
84. Wakabayashi N, Dinkova-Kostova AT, Holtzclaw WD, Kang MI, Kobayashi A, et al. 2004. Protection against electrophile and oxidant stress by induction of the phase 2 response: fate of cysteines of the Keap1 sensor modified by inducers. *Proc. Natl. Acad. Sci. USA* 101:2040–45

85. A comprehensive review of the molecular mechanisms of the Keap1/Nrf2 system.

85. Taguchi K, Motohashi H, Yamamoto M. 2011. Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution. *Genes Cells* 16:123–40

86. Sumi D, Numasawa Y, Endo A, Iwamoto N, Kumagai Y. 2009. Catechol estrogens mediated activation of Nrf2 through covalent modification of its quinone metabolite to Keap1. *J. Toxicol. Sci.* 34:627–35
87. Kobayashi M, Li L, Iwamoto N, Nakajima-Takagi Y, Kaneko H, et al. 2009. The antioxidant defense system Keap1-Nrf2 comprises a multiple sensing mechanism for responding to a wide range of chemical compounds. *Mol. Cell. Biol.* 29:493–502
88. Miura T, Shinkai Y, Jiang HY, Iwamoto N, Sumi D, et al. 2011. Initial response and cellular protection through the Keap1/Nrf2 system during the exposure of primary mouse hepatocytes to 1,2-naphthoquinone. *Chem. Res. Toxicol.* 24(4):559–67
89. Nakayama Wong LS, Lame MW, Jones AD, Wilson DW. 2010. Differential cellular responses to protein adducts of naphthoquinone and monocrotaline pyrrole. *Chem. Res. Toxicol.* 23:1504–13
90. Son TG, Camandola S, Arumugam TV, Cutler RG, Telljohann RS, et al. 2010. Plumbagin, a novel Nrf2/ARE activator, protects against cerebral ischemia. *J. Neurochem.* 112:1316–26
91. Ishii T, Itoh K, Takahashi S, Sato H, Yanagawa T, et al. 2000. Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. *J. Biol. Chem.* 275:16023–29
92. Abiko Y, Miura T, Phuc BH, Shinkai Y, Kumagai Y. 2011. Participation of covalent modification of Keap1 in the activation of Nrf2 by tert-butylbenzoquinone, an electrophilic metabolite of butylated hydroxyanisole. *Toxicol. Appl. Pharmacol.* 255:32–39

93. Fourquet S, Guerois R, Biard D, Toledano MB. 2010. Activation of NRF2 by nitrosative agents and H_2O_2 involves KEAP1 disulfide formation. *J. Biol. Chem.* 285:8463–71
94. Sawa T, Zaki MH, Okamoto T, Akuta T, Tokutomi Y, et al. 2007. Protein S-guanylation by the biological signal 8-nitroguanosine 3′,5′-cyclic monophosphate. *Nat. Chem. Biol.* 3:727–35
95. Tsujita T, Li L, Nakajima H, Iwamoto N, Nakajima-Takagi Y, et al. 2011. Nitro-fatty acids and cyclopentenone prostaglandins share strategies to activate the Keap1-Nrf2 system: a study using green fluorescent protein transgenic zebrafish. *Genes Cells* 16:46–57
96. Yu SM, Wu JF, Lin TL, Kuo SC. 1997. Inhibition of nitric oxide synthase expression by PPM-18, a novel anti-inflammatory agent, in vitro and in vivo. *Biochem. J.* 328(Pt. 2):363–69
97. Ohsaki Y, Shirakawa H, Miura A, Giriwono PE, Sato S, et al. 2010. Vitamin K suppresses the lipopolysaccharide-induced expression of inflammatory cytokines in cultured macrophage-like cells via the inhibition of the activation of nuclear factor κB through the repression of IKKα/β phosphorylation. *J. Nutr. Biochem.* 21:1120–26
98. Tanaka S, Nishiumi S, Nishida M, Mizushina Y, Kobayashi K, et al. 2010. Vitamin K_3 attenuates lipopolysaccharide-induced acute lung injury through inhibition of nuclear factor-κB activation. *Clin. Exp. Immunol.* 160:283–92
99. Checker R, Sharma D, Sandur SK, Khanam S, Poduval TB. 2009. Anti-inflammatory effects of plumbagin are mediated by inhibition of NF-κB activation in lymphocytes. *Int. Immunopharmacol.* 9:949–58
100. Luo P, Wong YF, Ge L, Zhang ZF, Liu Y, et al. 2010. Anti-inflammatory and analgesic effect of plumbagin through inhibition of nuclear factor-κB activation. *J. Pharmacol. Exp. Ther.* 335:735–42
101. Esnault S, Rosenthal LA, Shen ZJ, Sedgwick JB, Szakaly RJ, et al. 2007. A critical role for Pin1 in allergic pulmonary eosinophilia in rats. *J. Allergy Clin. Immunol.* 120:1082–88
102. Moon DO, Choi YH, Kim ND, Park YM, Kim GY. 2007. Anti-inflammatory effects of β-lapachone in lipopolysaccharide-stimulated BV2 microglia. *Int. Immunopharmacol.* 7:506–14
103. Inoue K, Takano H, Hiyoshi K, Ichinose T, Sadakane K, et al. 2007. Naphthoquinone enhances antigen-related airway inflammation in mice. *Eur. Respir. J.* 29:259–67
104. Inoue K, Takano H, Ichinose T, Tomura S, Yanagisawa R, et al. 2007. Effects of naphthoquinone on airway responsiveness in the presence or absence of antigen in mice. *Arch. Toxicol.* 81:575–81
105. Speyer CL, Neff TA, Warner RL, Guo RF, Sarma JV, et al. 2003. Regulatory effects of iNOS on acute lung inflammatory responses in mice. *Am. J. Pathol.* 163:2319–28
106. Sumi D, Akimori M, Inoue K, Takano H, Kumagai Y. 2010. 1,2-Naphthoquinone suppresses lipopolysaccharide-dependent activation of IKKβ/NF-κB/NO signaling: an alternative mechanism for the disturbance of inducible NO synthase-catalyzed NO formation. *J. Toxicol. Sci.* 35:891–98
107. Krishnan P, Bastow KF. 2000. Novel mechanisms of DNA topoisomerase II inhibition by pyranonaphthoquinone derivatives—eleutherin, α lapachone, and β lapachone. *Biochem. Pharmacol.* 60:1367–79
108. Li CJ, Averboukh L, Pardee AB. 1993. β-Lapachone, a novel DNA topoisomerase I inhibitor with a mode of action different from camptothecin. *J. Biol. Chem.* 268:22463–68
109. Choi BT, Cheong J, Choi YH. 2003. β-Lapachone-induced apoptosis is associated with activation of caspase-3 and inactivation of NF-κB in human colon cancer HCT-116 cells. *Anticancer Drugs* 14:845–50
110. Choi YH, Kang HS, Yoo MA. 2003. Suppression of human prostate cancer cell growth by β-lapachone via down-regulation of pRB phosphorylation and induction of Cdk inhibitor $p21^{WAF1/CIP1}$. *J. Biochem. Mol. Biol.* 36:223–29
111. Lee JI, Choi DY, Chung HS, Seo HG, Woo HJ, et al. 2006. β-Lapachone induces growth inhibition and apoptosis in bladder cancer cells by modulation of Bcl-2 family and activation of caspases. *Exp. Oncol.* 28:30–35
112. Li Y, Sun X, LaMont JT, Pardee AB, Li CJ. 2003. Selective killing of cancer cells by β-lapachone: direct checkpoint activation as a strategy against cancer. *Proc. Natl. Acad. Sci. USA* 100:2674–78
113. Rao KV, McBride TJ, Oleson JJ. 1968. Recognition and evaluation of lapachol as an antitumor agent. *Cancer Res.* 28:1952–54
114. Preusch PC. 1986. Lapachol inhibition of DT-diaphorase (NAD(P)H:quinone dehydrogenase). *Biochem. Biophys. Res. Commun.* 137:781–87

94. Discovery of an endogenous electrophile 8-nitro-cGMP and its signaling functions.

115. Kumagai Y, Tsurutani Y, Shinyashiki M, Homma-Takeda S, Yoshikawa T, et al. 1997. Bioactivation of lapachol responsible for DNA scission by NADPH-cytochrome P450 reductase. *Environ. Toxicol. Pharmacol.* 3:245–50
116. Endo A, Sumi D, Kumagai Y. 2007. 1,2-Naphthoquinone disrupts the function of cAMP response element-binding protein through covalent modification. *Biochem. Biophys. Res. Commun.* 361:243–48
117. Endo A, Sumi D, Iwamoto N, Kumagai Y. 2011. Inhibition of DNA binding activity of cAMP response element–binding protein by 1,2-naphthoquinone through chemical modification of Cys-286. *Chem. Biol. Interact.* 192(3):272–77
118. Bennett LL Jr, Smithers D, Rose LM, Adamson DJ, Thomas HJ. 1979. Inhibition of synthesis of pyrimidine nucleotides by 2-hydroxy-3-(3,3-dichloroallyl)-1,4-naphthoquinone. *Cancer Res.* 39:4868–74
119. Kemp AJ, Lyons SD, Christopherson RI. 1986. Effects of acivicin and dichloroallyl lawsone upon pyrimidine biosynthesis in mouse L1210 leukemia cells. *J. Biol. Chem.* 261:14891–95
120. Knecht W, Loffler M. 2000. Redoxal as a new lead structure for dihydroorotate dehydrogenase inhibitors: a kinetic study of the inhibition mechanism. *FEBS Lett.* 467:27–30
121. Fila C, Metz C, van der Sluijs P. 2008. Juglone inactivates cysteine-rich proteins required for progression through mitosis. *J. Biol. Chem.* 283:21714–24
122. Hsu YL, Cho CY, Kuo PL, Huang YT, Lin CC. 2006. Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) induces apoptosis and cell cycle arrest in A549 cells through p53 accumulation via c-Jun NH_2-terminal kinase-mediated phosphorylation at serine 15 in vitro and in vivo. *J. Pharmacol. Exp. Ther.* 318:484–94
123. Kuo PL, Hsu YL, Cho CY. 2006. Plumbagin induces G_2-M arrest and autophagy by inhibiting the AKT/mammalian target of rapamycin pathway in breast cancer cells. *Mol. Cancer Ther.* 5:3209–21
124. Luo Y, Mughal MR, Ouyang TG, Jiang H, Luo W, et al. 2010. Plumbagin promotes the generation of astrocytes from rat spinal cord neural progenitors via activation of the transcription factor STAT3. *J. Neurochem.* 115:1337–49
125. Taguchi K, Shimada M, Fujii S, Sumi D, Pan X, et al. 2008. Redox cycling of 9,10-phenanthraquinone to cause oxidative stress is terminated through its monoglucuronide conjugation in human pulmonary epithelial A549 cells. *Free Radic. Biol. Med.* 44:1645–55
126. Batthyany C, Schopfer FJ, Baker PR, Duran R, Baker LM, et al. 2006. Reversible post-translational modification of proteins by nitrated fatty acids in vivo. *J. Biol. Chem.* 281:20450–63
127. Lin D, Saleh S, Liebler DC. 2008. Reversibility of covalent electrophile-protein adducts and chemical toxicity. *Chem. Res. Toxicol.* 21:2361–69
128. Rudolph TK, Freeman BA. 2009. Transduction of redox signaling by electrophile-protein reactions. *Sci. Signal.* 2:re7
129. Miura T, Kumagai Y. 2010. Immunochemical method to detect proteins that undergo selective modification by 1,2-naphthoquinone derived from naphthalene through metabolic activation. *J. Toxicol. Sci.* 35:843–52
130. Miura T, Kakehashi H, Shinkai Y, Egara Y, Hirose R, et al. 2011. GSH-mediated *S*-transarylation of a quinone glyceraldehyde-3-phosphate dehydrogenase conjugate. *Chem. Res. Toxicol.* In press
131. Song Y, Wagner BA, Witmer JR, Lehmler HJ, Buettner GR. 2009. Nonenzymatic displacement of chlorine and formation of free radicals upon the reaction of glutathione with PCB quinones. *Proc. Natl. Acad. Sci. USA* 106:9725–30
132. Baeza-Squiban A, Bonvallot V, Boland S, Marano F. 1999. Airborne particles evoke an inflammatory response in human airway epithelium. Activation of transcription factors. *Cell Biol. Toxicol.* 15:375–80
133. Donaldson K, Borm P, eds. 2007. *Particle Toxicology*. Boca Raton, FL: CRC. 424 pp.
134. Squadrito GL, Cueto R, Dellinger B, Pryor WA. 2001. Quinoid redox cycling as a mechanism for sustained free radical generation by inhaled airborne particulate matter. *Free Radic. Biol. Med.* 31:1132–38
135. Blanchet S, Ramgolam K, Baulig A, Marano F, Baeza-Squiban A. 2004. Fine particulate matter induces amphiregulin secretion by bronchial epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 30:421–27
136. Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST. 2003. Airway remodeling in asthma: new insights. *J. Allergy Clin. Immunol.* 111:215–25; quiz 226

126, 127. Key reports providing evidence for reversibility of covalent modification of cellular proteins by electrophiles.

128. Comprehensive overview of the concepts and molecular mechanisms related to electrophile signaling.

137. Amishima M, Munakata M, Nasuhara Y, Sato A, Takahashi T, et al. 1998. Expression of epidermal growth factor and epidermal growth factor receptor immunoreactivity in the asthmatic human airway. *Am. J. Respir. Crit. Care Med.* 157:1907–12
138. Casalino-Matsuda SM, Monzon ME, Forteza RM. 2006. Epidermal growth factor receptor activation by epidermal growth factor mediates oxidant-induced goblet cell metaplasia in human airway epithelium. *Am. J. Respir. Cell Mol. Biol.* 34:581–91
139. Hamilton LM, Puddicombe SM, Dearman RJ, Kimber I, Sandstrom T, et al. 2005. Altered protein tyrosine phosphorylation in asthmatic bronchial epithelium. *Eur. Respir. J.* 25:978–85
140. Puddicombe SM, Polosa R, Richter A, Krishna MT, Howarth PH, et al. 2000. Involvement of the epidermal growth factor receptor in epithelial repair in asthma. *FASEB J.* 14:1362–74
141. Cho AK, Sioutas C, Miguel AH, Kumagai Y, Schmitz DA, et al. 2005. Redox activity of airborne particulate matter at different sites in the Los Angeles Basin. *Environ. Res.* 99:40–47
142. Iwamoto N, Nishiyama A, Eiguren-Fernandez A, Hinds W, Kumagai Y, et al. 2010. Biochemical and cellular effects of electrophiles present in ambient air samples. *Atmos. Environ.* 44:1483–89
143. Li N, Sioutas C, Cho A, Schmitz D, Misra C, et al. 2003. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environ. Health Perspect.* 111:455–60
144. Shiraiwa M, Garland R, Poeschl U. 2009. Kinetic double-layer model of aerosol surface chemistry and gas-particle interactions (K2-SURF): Degradation of polycyclic aromatic hydrocarbons exposed to O_3, NO_2, H_2O, OH and NO_3. *Atmos. Chem. Phys.* 9:9571–86
145. Sun JD, Wolff RK, Kanapilly GM, McClellan RO. 1984. Lung retention and metabolic fate of inhaled benzo(a)pyrene associated with diesel exhaust particles. *Toxicol. Appl. Pharmacol.* 73:48–59
146. Chao SH, Greenleaf AL, Price DH. 2001. Juglone, an inhibitor of the peptidyl-prolyl isomerase Pin1, also directly blocks transcription. *Nucleic Acids Res.* 29:767–73
147. Jeong HG, Pokharel YR, Lim SC, Hwang YP, Han EH, et al. 2009. Novel role of Pin1 induction in type II collagen-mediated rheumatoid arthritis. *J. Immunol.* 183:6689–97
148. Sandur SK, Ichikawa H, Sethi G, Ahn KS, Aggarwal BB. 2006. Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) suppresses NF-κB activation and NF-κB-regulated gene products through modulation of p65 and IκBα kinase activation, leading to potentiation of apoptosis induced by cytokine and chemotherapeutic agents. *J. Biol. Chem.* 281:17023–33
149. Shih YW, Lee YC, Wu PF, Lee YB, Chiang TA. 2009. Plumbagin inhibits invasion and migration of liver cancer HepG2 cells by decreasing productions of matrix metalloproteinase-2 and urokinase-plasminogen activator. *Hepatol. Res.* 39:998–1009
150. Sandur SK, Pandey MK, Sung B, Aggarwal BB. 2010. 5-hydroxy-2-methyl-1,4-naphthoquinone, a vitamin K3 analogue, suppresses STAT3 activation pathway through induction of protein tyrosine phosphatase, SHP-1: potential role in chemosensitization. *Mol. Cancer Res.* 8:107–18

RELATED REVIEWS

Marnett LJ, Riggins JN, West JD. 2003. Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein. *J. Clin. Investig.* 111:583–93

Kensler TW, Wakabayashi N, Biswal S. 2007. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu. Rev. Pharmacol. Toxicol.* 47:89–116

Akaike T, Fujii S, Sawa T, Ihara H. 2010. Cell signaling mediated by nitrated cyclic guanine nucleotide. *Nitric Oxide* 23:166–74

Annual Review of Pharmacology and Toxicology

Volume 52, 2012

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