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Two-Step Mechanism of Induction of the Gene Expression of a Prototypic Cancer-Protective Enzyme by Diphenols

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Cancer-preventive activity by exogenous molecules can be mediated by enhancing the expression of cytoprotective enzymes [e.g, glutathione-*S*-transferase (GST) or NAD(P)H−quinone oxidoreductase 1 (NQO1)] via antioxidant-response elements (AREs) present in the promoter regions of their genes. Previously, potency of induction of NQO1 has been linearly correlated with the ability to release an electron from different classes of inducers, including diphenols, phenylpropenoids, and flavonoids. In the present work, we focus on the induction of NQO1 by diphenols, which we consider as a model underlying the mechanisms of action of other phenolic inducers such as phenylpropenoids and flavonoids. A two-step mechanism of NQO1 activation is proposed involving (i) oxidation of diphenol inducers to their quinone derivatives and (ii) oxidation of two highly reactive thiol groups by these quinones of a protein involved in NQO1 induction. These two putative routes are supported by linear correlations between the inducer potencies and the redox properties of diphenols and of their corresponding quinones. The linear correlations demonstrate the possibility to predict the enhanced gene expression of enzymatic defenses by diphenols from quantum mechanical calculations (i) of the ability of diphenols to release electrons and (ii) of the electron affinity of their corresponding quinones.

Introduction

Oxidative stress, a constant hazard to aerobic life, is generated by reactive oxygen species (ROS) arising from respiration, immune responses to infection, interaction with xenobiotics such as drugs, tobacco, alcohol, asbestos, and metals, or irradiation by visible, UV, or ionizing radiations (*1, 2*). Accumulation of these oxidative stresses leads to oxidation of DNA, proteins, and lipids, tissue degeneration, mutagenicity, aging, and carcinogenesis (*2*).

Living systems have developed multiple lines of defense against these oxidative stresses. Prominent among these protective mechanisms is a family of phase 2 enzymes that are highly inducible and protect cells not only against the widespread damaging effects of ROS but also against toxicities of electrophiles and the damaging effects of inflammation. NADP(H)−quinone oxidoreductase 1 (NQO1) is a prototype phase 2 enzyme that is induced coordinately with other phase 2 proteins and has played a very useful role in the assessment of the potencies of phase 2 inducers, in the discovery and isolation of new inducers from natural sources, and in elucidating the chemistry of inducers (*3–5*).

Induction of NQO1 is regulated by the Keap1−Nrf2−ARE system. Certain inducers oxidize two highly reactive cysteine residues of the sensor protein Keap1, resulting in disulfide formation and conformation change, which allows Nrf2 to undergo nuclear translocation and binding to the antioxidant response element (ARE). The ARE, also known as the electrophile response element (EpRE), is represented by conserved upstream regulatory sequences that are present on many genes encoding proteins with various cytoprotective functions. After recruitment of the basic transcriptional machinery, the ultimate result is activation of the transcription of NQO1 and other ARE-regulated genes (*6–10*).

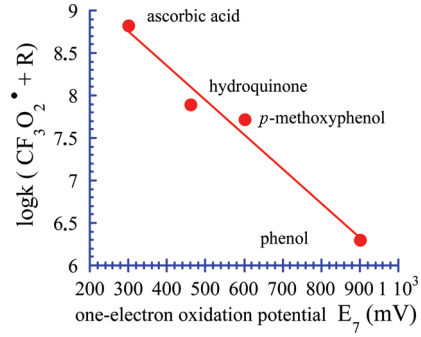

Figure 1. Log of the rate constant for the one-electron oxidation of reactant R by $CF_3O_2^{\bullet}$ peroxyl radical plotted vs the one-electron oxidation potential of R (data from Table 2).

One of the earliest clues to the structural requirements for phase 2 inducer activity was the finding that among diphenols (DP; see structures in Figures 2 and 3), only 1,2 diphenols (catechols) and 1,4-diphenols (hydroquinones) but not 1,3-diphenols (resorcinols) were inducers (*11*). This finding established that oxidative lability among these compounds was

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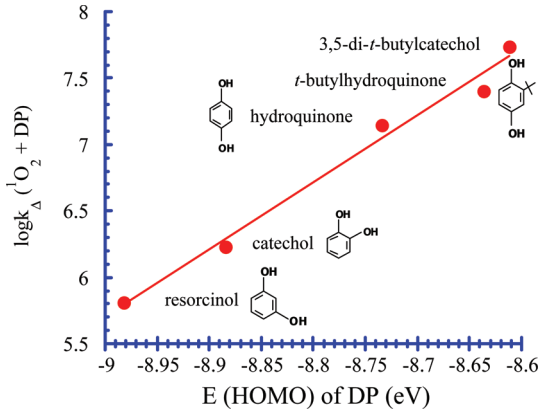


Figure 2. Log of the rate constant k_Δ for reaction of singlet oxygen $^1O_2$ with DP plotted vs the *E*(HOMO) of DP (data from Table 3).

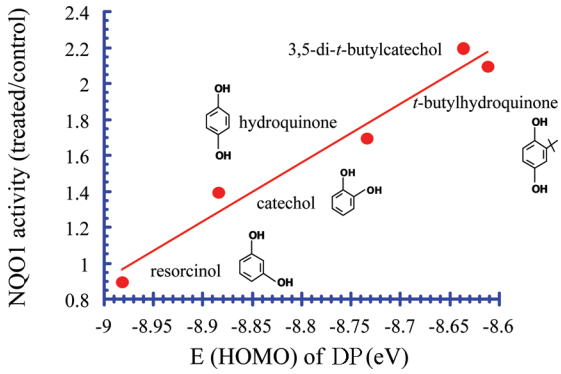


Figure 3. Relative induction of NQO1 activity (by 10 μM diphenols) measured in murine Hepa 1c1c7 cells plotted as a function of the *E*(HOMO) of the DP (data from Table 3).

essential for inducer activity but did not distinguish whether the redox process or the quinone products were the actual inducers. This issue was resolved by the subsequent realization that many inducers, such as quinones, were electrophilic Michael reaction acceptors (*12*). Collectively, these observations pointed to the universal reactivity of all ultimate inducers through their reduction by thiol groups and led to the recognition that cysteine residues of Keap1 are conclusive cellular sensors that react with inducers, thereby signaling phase 2 induction (*13*).

The potency for induction of NQO1 has been linearly correlated with the ability to release an electron among different classses of inducers including diphenols (DP) (*14*), phenylpropenoids (PP) (*15*), and flavonoids (F) (*16*). All NQO1 inducers, DP, PP and F, in their reduced form, are unable to oxidize thiols. Thus, the only possible route for this activation is a process involving an oxidized form of these inducers.

The aim of the present work was to find the role played by different species involved in the redox cascade of reactions leading to Nrf2 activation. Induction of NQO1 by diphenols (DP), measured in Hepa 1c1c7 cells, is discussed, with the possibility that the route leading to Nrf2 activation by DP is a model for the mechanism underlying activation by other phenolic inducers such as PP and F. A two-step mechanism of activation of Nrf2 is examined; it involves (i) formation of oxidized forms of the DP inducers and (ii) oxidation of two neighboring thiol groups of Keap1 by these oxidized species, which we propose are quinone derivatives Q (see structures in Figure 4).

These two putative routes are supported by quantitative structure–activity relationships correlating NQO1 induction by DP with redox properties (i) of DP and (ii) of DP oxidized

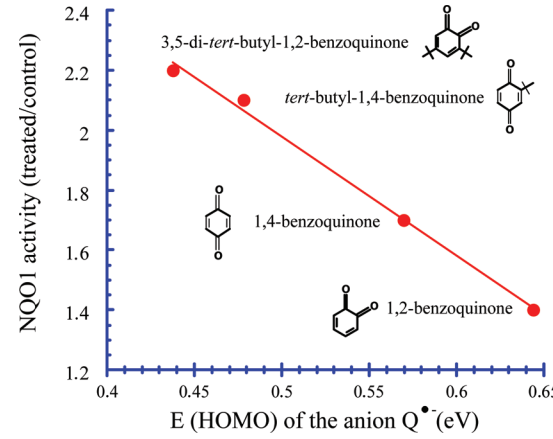


Figure 4. Relative induction of NQO1 activity (by 10 μM diphenols) measured in murine Hepa 1c1c7 cells plotted as a function of the electron affinity of the quinone Q expressed by the *E*(HOMO) of the corresponding anion $Q^{\bullet-}$ (data from Table 4).

species. The redox properties examined are (i) for the first step the one-electron oxidation potentials of the DP, $E(DP^{\bullet+}/DP)$ and (ii) for the second step the one-electron reduction potentials, $E(Q/Q^{\bullet-})$, of the quinone derivatives Q. Both redox properties were quantified by molecular orbital calculations.

Materials and Methods

Determination of the Redox Properties of the Different Species, S, under Study. Species S are neutral molecules or anions for which we need to know the one-electron reduction potentials of the couples $(S^{\bullet+}/S)$ (oxidized form/reduced form). The one-electron reduction potential, $E(S^{\bullet+}/S)$, characterizes the ease of oxidation of S to $S^{\bullet+}$, as well as the ease of reduction of $S^{\bullet+}$ to S and thus represents also the electron affinity of $S^{\bullet+}$.

Species S under study, that is, reduced forms of $(S^{\bullet+}/S)$ couples, are the following molecules: H_2O, ROH, ROOH, H_2O_2, vitamin C, hydroquinone, *p*-metoxyphenol, phenol, resorcinol, catechol, *t*-butyl-hydroquinone, and 3,5-*t*-butylcatechol. Species S are also anions, including the superoxide anion $O_2^{\bullet-}$, the reduced form of singlet oxygen $^1O_2$, and $Q^{\bullet-}$, the reduced form of quinones Q, such as 1,2-benzoquinone, 1,4-benzoquinone, *t*-butyl-1,4-benzoquinone, and 3,5-di-*t*-butyl-1,2-benzoquinone. For most of these molecules, reliable one-electron reduction potentials, $E_7(S^{\bullet+}/S)$, at pH 7 are already known from pulse radiolysis determinations (*18*).

However, in the absence of reliable one-electron reduction potentials for some DP such as resorcinol, catechol, *t*-butyl-hydroquinone, or 3,5-*t*-butylcatechol, we have used physicochemical parameters that are linearly correlated with $E_7(DP^{\bullet+}/DP)$. These redox properties are (i) a kinetic parameter, namely, the log of the rate constant (k_Δ) of the reaction of singlet oxygen ($^1O_2$) with DP, which we had previously determined (*14*), and (ii) the energy of the highest occupied molecular orbital *E*(HOMO), currently calculated by a simple, semiempirical quantum mechanical method, which represents the energy required to detach an electron from a molecule in the dilute gas phase. In a series of related compounds S, the log of the rate constant $k_\Delta(^1O_2+S)$ (*14*) and the *E*(HOMO) of S (*17*) can both be linearly correlated with the one-electron oxidation potential of S, $E(S^{\bullet+}/S)$, in solution.

The electron affinity of quinones Q (1,2-benzoquinone, 1,4-benzoquinone, *t*-butyl-1,4-benzoquinone, or 3,5-di-*t*-butyl-1,2-benzoquinone), which represents the ease of reducing Q or oxidizing $Q^{\bullet-}$, is the energy liberated when an electron adds to the molecule Q in the gas phase. It is linearly related to the

reduction potential, $E(Q/Q^{\bullet -})$, measured in solution and correlated also with the energy of the lowest unoccupied molecular orbital E(LUMO) of Q (*17*). As will be developed later, the calculation of the E(HOMO) of the $Q^{\bullet -}$ anions can be considered as a more precise way to calculate the electron affinity of the neutral Q than the calculation of the energy of the lowest unoccupied molecular orbital, E(LUMO), of Q.

It is important to emphasize that although the redox potentials can be strongly affected by the surrounding medium, linear correlations are observed between E(HOMO) of a series of related species S calculated in the gas phase and their ionization potential measured in the gas phase or their one-electron oxidation potential $E(S^{\bullet +}/S)$ measured in different solvents. These correlations were previously observed and discussed for series of substituted derivatives of aromatic hydrocarbons and of phenolic compounds (*17*). Similarly, in biological quantitative structure–activity relationships (QSAR) where physicochemical properties of a series of compounds are determined *ex vivo* and their biological activities measured *in vivo* or in cellular systems, it is common to find succcessful linear correlations when these studies are carried out on exogenous congeners of the same general structure (*18*).

In current IUPAC conventions, the oxidation potential of S, $E(S^{\bullet +}/S)$, should now be called the reduction potential of $S^{\bullet +}$. However, the old expression "oxidation potential" of S is still often used by literature data reported here.

For calculations of the E(HOMO) of S or the E(HOMO) of $Q^{\bullet -}$, we used the semiempirical AM1 quantum mechanical calculations, carried out with the Hyperchem 7.51 program. The restricted Hartree–Fock (RHF) formalism was used to complete these calculations. The conformation of the molecules was minimized by using the Polack−Ribiere minimization algorithm, until the root-mean-square of the energy gradient reached a value of 0.01 kcal/(mol Å) (*15*).

Induction of NAD(P)H−Quinone Reductase (NQO1). Briefly, NQO1 induction was determined in Hepa 1c1c7 cell lines by measuring the rate of reduction of 2,6-dichloroindophenol by NADH reduced per minute per milligram of protein, according to the method of Prochaska et al. (*11*). Inductions of quinone reductase by DP were expressed as ratios of treated to controls. The choice of the particular cell line was determined by (i) its robust responsiveness to inducers, which allows quantitative comparisons of inducer potencies, and (ii) the fact that response to inducers in this cell line mimics closely the response to inducers that is observed *in vivo* in a number of murine tissues (*3*).

Statistical Calculations and Graph Plotting. Statistical calculations and graph plotting were performed using the Kaleidagraph software (version 3.6). This software calculates the linear curve fits, using the least-squared error method. The probability p-values were calculated using the standard statistical functions of Microsoft Excel 2003.

Results and Discussion

In a previous article (*14*), we had observed that the potency of NQO1 induction is higher when the electron-donating efficacy of DP inducers are larger, thus when the inducer DP is a better reducing species. The electron-donating efficacy was assessed *via* the log of the rate constant (k_Δ) of reaction of singlet oxygen ($^1O_2$) with DP (*14*). However, enzyme NQO1 induction is triggered by the inducer acting in its reaction with two thiol groups of the protein Keap1 not as a reducing species but as an oxidant (*13*). To become an oxidant, the DP inducer must become oxidized. For this reason, the focus of the present investigation is to establish the possibility of a two-step mechanism of NQO1 induction involving (i) the formation of oxidized forms of DP inducers, ultimately their corresponding quinones Q and (ii) the oxidation of two highly reactive thiol groups in a protein by Q, which activates the NQO1 induction.

Table 1. Reduction Potentials of ROS Involved in Oxidative Stress

couples involved in oxidative stress	E_7 (mV)[a]
$HO^{\bullet}$, H^+/H_2O	2730[b]
$RO^{\bullet}$, H^+/ROH (aliphatic alkoxyl radicals)	1600[c]
$HOO^{\bullet}$, H^+/H_2O_2	1060[d]
$ROO^{\bullet}$, $H^+/ROOH$ (alkyl peroxyl radicals)	770−1400[c]
$^1O_2(^1\Delta_g)/O_2^{\bullet -}$	650[b]

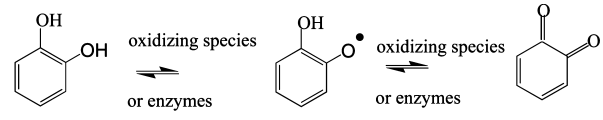
[a] The E_7 "reduction potentials" (at pH 7) reported for different couples involved in oxidative stress are equal to the "oxidation potentials" of the respective reduced species, H_2O, ROH, H_2O_2, ROOH, and $O_2^{\bullet -}$ of the different couples. [b] Data from ref *19*. [c] Data from ref *20*. [d] Data from ref *21*.

In part A, we examined the biochemical reactions that can drive the first step, DP oxidation and Q formation. Our aim is to find DP oxidation reactions that can depend on the redox potentials of the phenolic derivatives. Afterward, we will examine the reactions involved in a second step, where the Q oxidize thiol groups of the sensor protein Keap1.

In part B, we will try to establish correlations between the enhanced gene expression of NQO1 determined in cellular experiments and two parameters quantified by molecular orbital calculations: (i) the ability of DP to release electrons determined by their E(HOMO) with the enhanced NQO1 activity and (ii) the electron affinity of their corresponding Q determined by the E(HOMO) of their anions $Q^{\bullet -}$.

A. Reactions Involved in the Activation of NQO1 by DP. We examine the two-step mechanism leading to NQO1 activation: (i) the reactions leading to oxidations of the DP inducers in cellular systems and (ii) the reactions of the DP oxidized forms with the thiol groups of the sensor protein Keap1, ultimately leading to nuclear translocation of the transcription factor Nrf2.

1. First Step: Biochemical or Enzymatic Oxidations of the DP Leading to Phenoxyl Radicals XΦO$^{\bullet}$ and Quinones Q. As shown in the reactions of the catechol chosen as an example, two consecutive oxidations of DP (which are reduced quinones QH_2) can lead to the formation of phenoxyl radical XΦO$^{\bullet}$ and to quinone derivatives Q via equilibria 1. Let us consider some of the biochemical reactions that could lead to the oxidation of DP.



(1)

DP = QH_2 XΦO$^{\bullet}$ or QH$^{\bullet}$ Q

a. Oxidation of DP by Reactive Oxygen Species (ROS). One possible route of DP oxidation might occur via an attack by the radicals $HO^{\bullet}$, $RO^{\bullet}$, $ROO^{\bullet}$, and singlet molecular oxygen, $^1O_2(^1\Delta_g)$, species involved in oxidative stress, produced physiologically by aerobic metabolism, and often called reactive oxygen species (ROS). Their redox potentials at pH 7 are reported in Table 1.

Comparing the one-electron reduction potentials at pH 7, E_7, of the ROS involved in oxidative stress (Table 1) with those of the DP (Table 3), we note that the E_7 values of the active DP are below those of the ROS. Thus, using these E_7 values as a

Table 2. Rate Constants for Reactions of Halogenated Peroxyl Radicals

reactant R	E_7 (mV)[a]	log k ($CF_3O_2^\bullet$ + R)[b]	log k ($CCl_3O_2^\bullet$ + R)[b]	log k ($CBr_3O_2^\bullet$ + R)[b]
ascorbic acid	300	8.83	8.20	8.32
hydroquinone	460	7.90	7.0	7.25
p-methoxyphenol	600	7.72	6.53	6.98
phenol	900	6.30	<5	

[a] One-electron oxidation potentials, E_7, at pH 7 of the reactants R, which are equal to the reduction potential of the couple ($R^{\bullet+}$/R) (*19*). [b] Rate constants for one-electron oxidation of R by the $CF_3O_2^\bullet$, $CCl_3O_2^\bullet$, $CBr_2O_2^\bullet$ radicals in aqueous solutions (*26*).

Table 3. Redox Properties and Induction Efficacy of DP

diphenols	E(HOMO)[a]	log k_Δ ($^1O_2$ + DP)[b]	E_7(XΦO$^\bullet$,H$^+$/XΦOH) in mV vs NHE	NQO1 specific activity (treated/control)[c]
resorcinol	−8.9820	5.81	810[d]	0.9
catechol	−8.8841	6.23	530[d]	1.4
hydroquinone	−8.7344	7.15	460[d]	1.7
t-butyl-hydroquinone	−8.6120	7.74	260[e]	2.1
3,5-di-*t*-butylcatechol	−8.6365	7.40	290[e]	2.2

[a] Energy of the highest occupied molecular orbital calculated in the present work. [b] Rate constant k_Δ determined in CD_3OD taken from previous work (ref *14*). [c] Induction of quinone reductase in Hepa 1c1c7 cells by 10 μM of DP, expressed as relative specific activity (treated/control). Data extracted from Figure 3 of ref *11*. [d] Taken from ref *19*. [e] A linear correlation between E_7 of the couple phenoxyl radical/phenol (XΦO$^\bullet$, H$^+$/XΦOH) and E(HOMO) for resorcinol, catechol, and hydroquinone gives the relationship E_7(XΦO$^\bullet$, H$^+$/XΦOH) in mV vs NHE = −11237 − 1335E(HOMO), which allows us to estimate the unknown E_7 of the two other diphenols, *t*-butylhydroquinone and 3,5-di-*t*-butylcatechol, with an error of ±10%.

rough guide, we can predict that equilibrium 2, shown below, will shift to the right for any DP that is able to donate an electron (or a hydrogen atom) to any oxidizing species listed in Table 1. In equilibrium 2, the monoelectron oxidation of the DP leads to phenoxyl radical cations XΦOH$^+$, which rapidly lose a proton at pH 7 to form the neutral phenoxyl radicals XΦO$^\bullet$ (*22*):

$$\text{Oxidizing species } (HO^\bullet, RO^\bullet, RO_2^\bullet, H_2O^\bullet, {}^1O_2) + DP \rightleftharpoons H_2O, ROH, ROOH + \text{phenoxyl radical } X\Phi O^\bullet + H^+ \quad (2)$$

These phenoxyl radicals, XΦO$^\bullet$, are more stable, less energetic, and less reactive than the ROS radicals (*23*). Among these oxidations, those that are dependent on the redox potential of the DP inducers will be the important ones with rates that are the rate-determining step of the overall reaction leading to induction of NQO1, since it has been established that the NQO1 induction is correlated with the E(DP$^{\bullet+}$/DP) (*14*).

In the case of reactions with the HO$^\bullet$ radical, the most powerful oxidant, with a standard reduction potential E(HO$^\bullet$, H$^+$/H_2O) of 2.7 V (Table 1), the rate constants with hydroquinone, catechol, and resorcinol are all in the range of 10^{10} L mol^{-1} s^{-1} (*24*). Thus, these hydroxyl radical reactions with DP have rates in the range of diffusion-controlled and are independent of E(DP$^{\bullet+}$/DP). Pulse radiolysis studies have shown that the HO$^\bullet$ radical reacts by addition to yield dihydroxycyclohexadienyl radicals, which then may undergo a dehydration to yield the more resonance stabilized phenoxyl radical ΦO$^\bullet$ (*25*). Actually, the extremely oxidizing HO$^\bullet$ radical will react immediately at its site of formation with any substrate in its vicinity.

As examples of possible reactions of peroxyl radicals, $RO_2^\bullet$, with our five DP, we shall discuss the reactions of three halogenated peroxyl radicals, $CX_3O_2^\bullet$ ($CF_3O_2^\bullet$, $CCl_3O_2^\bullet$, and $CBr_3O_2^\bullet$) with substrates R, ascorbic acid, hydroquinone, *p*-methoxyphenol, and phenol, which have beeen studied by Huie et al. (*26*). These authors determined the rate constants k of the electron transfer reactions taking place from the different substrates R to the peroxyl radicals:

$$CX_3O_2^\bullet + R \rightarrow CX_3O_2^- + R^{\bullet+} \quad (3)$$

The logs of the rate constants of the reactions of these halogenated peroxyl radicals are reported in Table 2, as well as the one-electron potential at pH 7 of different substrates R, E_7($R^{\bullet+}$/R). They are linearly correlated with the reduction potential E_7($R^{\bullet+}$/R) with correlation coefficients r, according to the eqs a, b, and c:

$$\log k(CF_3O_2^\bullet + R) = 9.97 - 0.0040E_7(R^{\bullet+}/R) \quad r = 0.99 \quad (a)$$

$$\log k(CCl_3O_2^\bullet + R) = 9.79 - 0.0056E_7(R^{\bullet+}/R) \quad r = 0.98 \quad (b)$$

$$\log k(CBr_3O_2^\bullet + R) = 9.56 - 0.0045E_7(R^{\bullet+}/R) \quad r = 0.96 \quad (c)$$

The p values for correlations a, b and c are, respectively, 0.012, 0.13, and 0.19. This is in favor of the statistical significance of correlation a. The p values of correlations b and c are significantly higher. This is mainly because only three points enter the correlation, thus increasing the probability of correlation by chance. However, the existence of these two correlations is supported by the significance of correlation a and theoretically justified by the Marcus theory of electron transfer (*27–29*).

A graphical plot of equation a is shown in Figure 1 as an example of the linear correlations a, b and c.

These halogenated peroxyl radicals are not normally found in biological systems. However, electron transfer reactions from our DP to biological peroxyl radicals ROO$^\bullet$, similar to reaction 3, can be considered plausible (*30*). Indeed, the reduction potentials of the couple (DP$^{\bullet+}$/DP) are in the same range as those of the couples ($R^{\bullet+}$/R). Moreover the rate constants of peroxyl radicals such as peroxyl radical of thymidine-OH adduct or deoxycytidine-OH adduct with hydroquinone are, respectively, $k = 6.5 \times 10^6$ and 1.2×10^7 L mol^{-1} s^{-1} (*30*), in the range of those reported for the halogenated peroxyl radicals.

In the case of singlet oxygen ($^1O_2$) reactions with phenolic compounds, it can be noted that rate constants k_Δ reported in Table 3 are linearly correlated with the E(HOMO) of the five DP and thus with their reduction potential, E_7(DP$^{\bullet+}$/DP) (Figure 2). This linear correlation can be expressed by eq d:

$$\log k_\Delta({}^1O_2 + DP) = 51.22 + 5.07E(\text{HOMO of DP}) \quad r = 0.99 \quad (d)$$

Two types of singlet oxygen reactions have been observed, a physical quenching giving back ground-state oxygen and phenolic compound and a chemical quenching giving various products including corresponding quinones. In both cases, the reactions involved are electron transfer reactions from the DP to singlet oxygen as demonstrated by spectroscopic evidence of the formation of phenoxyl radicals ΦO$^\bullet$ (*31–33*).

In conclusion, we can observe that DP oxidations by ROS, including peroxyl radicals and singlet oxygen ($^1O_2$), can be dependent on the $E_7(DP^{\bullet+}/DP)$ as shown by the linear correlations a, b, c and d.

Moreover, for the R oxidations as well as for the DP oxidations, the rates of electron transfer are below diffusion-controlled rates, in the endergonic region, far from the exergonic Marcus inverted region (*27*). For this reason, the correlations a, b, c and d appear linear, instead of being parabolic as predicted by the Marcus theory of electron transfer (*27–29*).

b. Other Routes of DP Oxidations. Autoxidation and enzymatic oxidations may be other routes of DP oxidation. Autoxidation by ground-state triplet oxygen, $^3O_2$, is much slower than that by singlet oxygen. It becomes important only in nonphysiological alkaline solutions, where the phenol oxidation occurs via the phenolate ions by an electron transfer reaction, which is endothermic for hydroquinone, catechol, or trolox, taken as examples (*34*):

$$\Phi O^- + O_2 \rightarrow \Phi O^{\bullet} + O_2^{\bullet-}$$

However, this oxidation by O_2 occurs because it is pulled to the right by

$$2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$$

which is a very rapid reaction. Autoxidation is severely complicated by a number of secondary reactions recently reviewed (*34*).

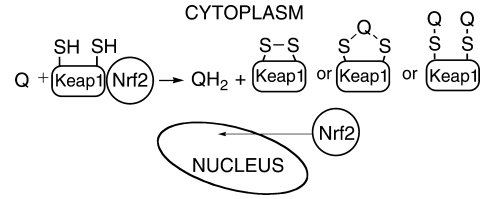
Enzymatic oxidations of phenols and dietary polyphenols by peroxidases, such as tyrosinase, are widespread in plants and animals. Peroxydases catalyze phenol oxidation at physiological pH and produce oxidant species including electrophilic quinones, melanin, and other pigments (*35*). They lead to the browning and blackening of fruits and vegetables when peeled or sliced. The quinones formed by phenol enzymatic oxidations *in vivo* can induce oxidative stress via depletion of reduced glutathione and redox cycling and are implicated in the potential oxidant toxicity of high concentrations of dietary polyphenols *in vivo* (*36*). This generation of cytotoxic quinone- or quinomethide-type cytotoxic oxidants by polyphenols acting as substrates for peroxidases and metalloenzymes has been used to explain the toxicity of 13 polyphenolic molecules against human promyelotic leukemia HL-60 cells, which is correlated with their ease of oxidation and their lipophilicity (*37*). A similar production of cytotoxic oxidants via peroxydase action on phenolic molecules can be proposed to explain the correlation observed between the ease of oxidation of seven phenolic flavones, PF, from *Lethedon tannaensis* and their toxicity toward human nasopharynx carcinoma (KB) cells (*38*).

In conclusion, ROS and enzymes can oxidize phenolic derivatives P with reactions depending on their oxidation potentials $E(P^{\bullet+}/P)$.

2. Second Step: Thiol Oxidation by Oxidized Diphenols, DP (QH$^{\bullet}$ or Q). The two oxidized forms of the DP (QH_2), which are (i) phenoxyl radicals $X\Phi O^{\bullet}$, also called semiquinone radicals $QH^{\bullet}$, and (ii) quinones Q, can react with thiols (RSH). The rate constants of thiol oxidation by phenoxyl radicals, including oxidation of cysteamine by trolox phenoxyl radicals and oxidation of glutathione by tocopheroxyl radicals, have been measured by pulse radiolysis. They are, respectively, at pH 7 1.0×10^5 and 2.5×10^1 M^{-1} s^{-1} (*39*).

Reactivity of *o*-quinones versus thiols has been studied by pulse radiolysis (*40, 41*). The *o*-quinones were generated via pulse radiolysis by rapid (microsecond) one-electron oxidation of the corresponding stable synthesized catechols, forming semiquinones, which disproportionated over milliseconds to *o*-quinones. The latter reacted with the thiols in a pH-dependent manner, indicative of increased nucleophilicity of the thiolate anions as compared with their protonated forms.

Scheme 1. Second Step of the Mechanism of Induction of NAD(P)H−Quinone Oxidoreductase, Which Occurs via Oxidation of Keap1 Thiols by Q and Ultimately Allows Transcription Factor Nrf2 To Migrate into the Nucleus



The reactivities of two thiols, glutathione and cysteine, with 16 4-substituted *o*-quinones, show statistically significant Hammett and Swain−Lupton correlations (*41*). The rate constants of the reactions with cysteine and glutathione (GSH), measured between pH 6.9 and 7.2, are

(i) for the nonsubstituted 1,2-benzoquinone, $k = 5.3 \times 10^6$ L mol^{-1} s^{-1} (cysteine) and 2.6×10^6 L $mol^{-1}s^{-1}$ (GSH)

(ii) for the most electron-attracting substituent, 4-$CH_2NH_3^+$, $k = 3.1 \times 10^7$ L mol^{-1} s^{-1} (cysteine) and 1.2×10^7 L mol^{-1} s^{-1} (GSH)

(iii) for the least electron-attracting substituent, 4-methoxy, $k = 4.0 \times 10^5$ L mol^{-1} s^{-1} (cysteine) and 3.0×10^5 L mol^{-1} s^{-1} (GSH). The correlations observed show that the rate constants increase with the electron-withdrawing capacities of the substituent groups of the *o*-quinones and thus with their electron affinity (*40, 41*).

The stoichiometry and kinetics of the reactions of 1,4-benzo- or naphtho-quinones with thiols have been measured by stopped flow spectrophotometry, and the main findings are summarized and discussed by Wardman (*42*). The stoichiometry of the reaction with representative compounds was 1:2 thiol/quinone, a finding consistent with the formation of hydroquinones. Moreover, these reactions can produce not only reduced quinones (QH_2) but also modified quinones (Q-SR) via:

$$2Q + RSH + H^+ \rightarrow Q\text{-}SR + QH_2 \qquad (4)$$

As discussed and established by Wardman (*42*), reaction 4 can be broken down into two steps, of which the first is slower and rate-limiting:

$$Q + RS^- + 2H^+ \rightarrow (Q\text{-}SR)H_2 \qquad (5)$$

$$(Q\text{-}SR)H_2 + Q \rightleftharpoons Q\text{-}SR + QH_2 \qquad (6)$$

Consistent with the reactions described by Wardman (*42*), are the reactions shown in Scheme 1, adapted from Dinkova-Kostova et al. (*6*), where two thiols of the protein sensor Keap1 that are in spatial proximity can be oxidized by Q. Such oxidation renders Keap1 unable to target Nrf2 for degradation, allowing the nuclear translocation of Nrf2 and activation of the ARE-mediated transcription of phase 2 genes.

Indeed, when mammalian cells overexpressing Keap1 were exposed to the potent NQO1 inducer bis(2-hydroxybenzylidene)acetone, the presence of intermolecular disulfide-linked dimers of Keap1 was detected by Western blot analysis of the cellular proteins, which were separated by two-dimensional gel electrophoresis (*43*).

This second step in the NQO1 induction involving quinones had been predicted by Prochaska et al. (*11*) and Dinkova-Kostova et al. (*44*). We have emphasized a necessary property for three classes of NQO1 inducers (diphenols, aromatic diamines, and azodyes), which is their capacity to undergo complete oxidation to the corresponding quinones. This property was based on the experimental evidence that 1,3-diphenols and their substituted analogues are unable to give rise to the corresponding quinones and are inactive as inducers.

B. Quantitative Structure–Activity Relationships between Redox Properties of Diphenols DP and Quinones Q with Their NQO1 Induction *in Vitro*

The ability of DP to release electrons determined by their E(HOMO) and the electron affinity of their corresponding Q, determined by the E(HOMO) of their anions $Q^{\bullet -}$, will allow us to observe interesting correlations with the NQO1 acivation by DP measured in Hepa 1c1c7 cell lines.

1. Correlations of *E*(HOMO) of DP with Their NQO1 Induction Efficacy. In Table 3, we report the redox properties and induction efficacy of DP expressed as a relative specific activity for the five DP previously studied. In Table 3, as stated above, $X\Phi O^{\bullet}$ represents the phenoxyl radicals resulting from the one-electron oxidation of the DP. As indicated in a previous section, the one-electron reduction potential of the couple ($X\Phi O^{\bullet},H^{+}/X\Phi OH$), E_7 at pH 7, is equal to the one-electron oxidation potential of the DP.

We note that the three parameters, (i) one-electron reduction potential of the couple ($X\Phi O^{\bullet},H^{+}/X\Phi OH$), E_7 at pH 7, (ii) log $k_{\Delta}(^1O_2+$ DP), and (iii) energy of the highest occupied molecular orbital E(HOMO) of the DP, are linearly correlated with the potency of induction of NQO1 (*11*), with the following respective equations:

$$\text{NQO1 activity} = 2.77 - 0.0027E_7(DP^{\bullet +}/DP) \quad \text{with a correlation coefficient of} \quad r = 0.98 \text{ (e)}$$

$$\text{NQO1 activity} = -2.62 + 0.62\log k_{\Delta}(^1O_2 + DP) \quad \text{with} \quad r = 0.95 \text{ (f)}$$

$$\text{NQO1 activity} = 30.36 + 3.27E(\text{HOMO}) \quad \text{of DP with} \quad r = 0.98 \text{ (g)}$$

The last correlation g is displayed in Figure 3.

We point out that the E(HOMO) and the E_7 are inverse functions. In other words, the signs of the E(HOMO) are negative, while those of the E_7 are positive, and both absolute values decrease when the molecule is more reducing (i.e., the molecule releases an electron more easily). The three correlations e, f, and g show that the stronger the electron-donating property of the inducer, the greater its induction of quinone reductase. These three correlations also indicate that the DP oxidation, occurring via electron transfer, is the rate-determining step of the overall process leading to activation of NQO1.

The correlations observed occur in a narrow range of parameter values. However, in the case of equation f, the correlation occurs with a change of log k_{Δ} involving almost 2 orders of magnitude. Moreover, similar linear correlations have been observed for larger range of parameter values between the E(HOMO) of 30 phenylpropenoids (PP) (*15*) or 21 flavonoids F and their NQO1 induction (*16*).

We have observed earlier that different types of ROS and different enzymes can oxidize phenolic derivatives P with rate constants that depend on the rate constant of electron transfer and thus on their reduction potentials $E(P^{\bullet +}/P)$, which can be expressed by their E(HOMO). The fact that similar correlations involving E(HOMO) carry over to cellular events, such as NQO1 induction by phenolic derivatives, demonstrates that phenolic oxidation occurs as a first step toward NQO1 activation.

Table 4. *E*(HOMO) of the Quinone Anions and Their Induction Efficacy

Q	E(HOMO) of $Q^{\bullet -}$ (eV)	NQO1 relative specific activity[a] (treated/control)
1,2-benzoquinone	0.64363	1.4
1,4-benzoquinone	0.56943	1.7
t-butyl-1,4-benzoquinone	0.47822	2.1
3,5-di-*t*-butyl-1,2-benzoquinone	0.43736	2.2

[a] Induction of quinone reductase in Hepa 1c1c7 cells by 10 μM DP, expressed as relative specific activity (treated/control). Data extracted from Figure 3 of ref *11*.

2. Correlations of Electron Affinity of Q with the NQO1 Induction Efficacy. The reduction potentials of the quinone/semiquinone couples are very important for understanding of their reactions with thiols. In the absence of reliable one-electron reduction potentials $E_7(Q/Q^{\bullet -})$, we have determined the corresponding E(HOMO) of the oxidation of the anion of each quinone, $Q^{\bullet -}$, These E(HOMO) corresponding to 1,2-benzoquinone, 1,4-benzoquinone, *t*-butyl-1,4-benzoquinone, 3,5-di*t*-butyl-1,2-benzoquinone anions are reported in Table 4. The calculation of the E(HOMO) of the $Q^{\bullet -}$ anions can be considered as a more precise way to determine the electron affinity of the neutral Q than the calculation of the energy of the lowest unoccupied molecular orbital E(LUMO) of Q.[1]

As underlined in discussing the data presented in Table 3, the E(HOMO) have a sign that is the inverse of those of their corresponding E_7. Here we are dealing with the reduction potential E_7 of the couple ($Q/Q^{\bullet -}$).Thus, the redox ranking goes from the least oxidizing (1,2 benzoquinone) to the most oxidizing (3,5-di-*t*-butyl-1,2-benzoquinone) Q.

Plotting the induction of NQO1, expressed as relative specific activity of the enzyme (Table 3), as a function of E(HOMO) of the anion $Q^{\bullet -}$, which corresponds to the electronic affinity of the neutral molecule Q and to the reduction potential of the couple ($Q/Q^{\bullet -}$) (Figure 4) with a changed sign, we observe a linear correlation h:

$$\text{NQO1 activity} = 3.97 - 3.98E(\text{HOMO}) \text{ of anion } Q^{\bullet -} \text{ with } r = 0.99 \text{ (h)}$$

This correlation h indicates that the stronger the electron-attracting property of the quinone, the greater is its induction potency of quinone reductase (NQO1) synthesis. This is consistent with the fact that the rate constant of thiol oxidation by 16 4-substituted *o*-quinones is higher when their electron affinity is greater (*40, 41*).

[1] Gaston Berthier, personal communication. Indeed, according to Mulliken [see note 19 in Mulliken, R. S. (1949) Quelques aspects de la théorie des orbitales moléculaires. *J. Chim. Phys. 46*, 497–542], the calculation of the oxidation potential and of the electron affinity of a molecule A, using (i) the energy of the highest occupied molecular orbital, E(HOMO) and (ii) the energy of the lowest unoccupied molecular orbitals, E(LUMO), via the Koopmans' theorem [Koopmans, T. (1933) Ordering of wave functions and eigenenergies to the individual electrons of an atom. *Physica 1*, 104–113], introduces two sources of error. These errors can compensate for each other in the calculation by E(HOMO) of the first oxidation energy of a molecule A [i.e., the reduction potential of the couple ($A^{\bullet +}/A$)]. However, these errors add to one another in the calculation by E(LUMO) of the electron affinity [i.e., the reduction potential of the couple ($A/A^{\bullet -}$)]. For this reason, it is possible to assume that the E(HOMO) value of the anion of A can be a better approximation for the estimation of the electron affinity of A than the E(LUMO) of the neutral A.

These calculations therefore provide strong confirmation of a large body of experimental evidence that the potency of inducers is related to their electrophilicity (*45–47*). This investigation also provides a better understanding of the NQO1 induction by inducers that are not electrophilic molecules, such as DP, PP, or F, and indicates how their induction requires a first step involving their oxidation into electrophilic species.

Conclusion

It can be considered that the NAD(P)H−quinone reductase (NQO1) induction by our diphenols occurs in two steps, which are regulated by redox mechanisms: (i) oxidation of diphenols DP by ROS or peroxidase enzymes yielding ultimately corresponding quinones Q, with rates that are the rate-determining step of the overall reaction leading to induction of NQO1 and with a correlation indicating that the stronger the electron-donating property of the DP, the greater is its inducer potency, and (ii) oxidation of highly reactive thiols of the protein sensor Keap1 by quinones Q, giving back the corresponding diphenols and activating transcription factor Nrf2, with a correlation indicating that the stronger the electron-attracting property of Q, the greater its inducing potency.

For phenylpropenoids (PP) (*15*) and flavonoids (F) (*16*), it has been observed that their ability to activate an Nrf2-mediated gene expression is correlated with the *E*(HOMO) of these inducers or, in other words, with their ability to release one electron (i.e., to become oxidized). The present results on diphenols (DP) suggest the existence of a similar two-step mechanism for Nrf2 activation by PP and F inducers.

Moreover, the observed correlations demonstrate that determination of the electron-releasing efficacy of inducers (DP, PP, or F), expressed by their HOMO energy, can be a fruitful strategy with predictive value for the design of new and more effective congeners, enhancing NQO1 induction.

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References

(1) Gilbert, D. L., Ed. (1981) *Oxygen and living processes*, Springer Verlag, New York.
(2) Halliwell, B., and Gutteridge, J. M. C. (1999) *Free radicals in biology and medicine*, 3rd ed., Oxford University Press, Oxford, U.K.
(3) Fahey, J. W., Dinkova-Kostova, A. T., Stephenson, K. K., and Talalay, P. (2004) The "Prochaska" microtiter plate bioassay for inducers of NQO1, in *Quinones and quinone enzymes, part B*, Methods in Enzymology, volume 382 (Sies, H., and Packer, L., Eds.) pp 243–258, Academic Press, San Diego, CA.
(4) Kensler, T. W. (1997) Chemoprevention by inducers of carcinogen detoxication enzymes. *Environ. Health Perspect. 105* (Suppl. 4), 965–970.
(5) Talalay, P. (2000) Chemoprotection against cancer by induction of phase 2 enzymes. *Biofactors 12*, 5–11.
(6) Dinkova-Kostova, A. T., Holtzclaw, W. D., Cole, R. N., Itoh, K., Wakabayashi, N., Katoh, Y., Yamamoto, M., and Talalay, P. (2002) Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc. Natl. Acad. Sci. U.S.A. 99*, 11908–11913.
(7) Dinkova-Kostova, A. T., Holtzclaw, W. D., and Kensler, T. W. (2005) The role of Keap1 in cellular protective responses. *Chem. Res. Toxicol. 18*, 1779–1791.
(8) Motohashi, H., and Yamamoto, M. (2004) Nrf2-Keap1 defines a physiologically important stress response mechanism. *Trends Mol. Med. 10*, 549–557.
(9) Kobayashi, M., and Yamamoto, M. (2006) Nrf2-Keap1 regulation of cellular defense mechanisms against electrophiles and reactive oxygen species. *Adv. Enzyme Regul. 46*, 113–140.
(10) Kobayashi, M., and Yamamoto, M. (2005) Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. *Antioxid. Redox Signaling 7*, 385–394.
(11) Prochaska, H. J., De Long, M. J., and Talalay, P. (1985) On the mechanisms of induction of cancer-protective enzymes: a unifying proposal. *Proc. Natl. Acad. Sci. U.S.A. 82*, 8232–8236.
(12) Talalay, P., De Long, M. J., and Prochaska, H. J. (1988) Identification of a common chemical signal regulating the induction of enzymes that protect against chemical carcinogenesis. *Proc. Natl. Acad. Sci. U.S.A. 85*, 8261–8265.
(13) Holtzclaw, W. D., Dinkova-Kostova, A. T., and Talalay, P. (2004) Protection against electrophile and oxidative stress by induction of phase 2 genes: the quest for the elusive sensor that responds to inducers. *Adv. Enzyme Regul. 44*, 335–367.
(14) Scurlock, R., Rougée, M., and Bensasson, R. V. (1996) Redox properties of diphenols and their correlation to induction of enzyme synthesis. *Z. Phys. Chem. 196*, 85–92.
(15) Zoete, V., Rougée, M., Dinkova-Kostova, A. T., Talalay, P., and Bensasson, R. V. (2004) Redox ranking of inducers of a cancer protective enzyme via the energy of their highest occupied molecular orbital. *Free Radical Biol. Med. 36*, 1418–1423.
(16) Lee-Hilz, Y. Y., Boerboom, A. M., Westphal, A. H., Berkel, W. J., Aarts, J. M., and Rietjens, I. M. (2006) Pro-oxidant activity of flavonoids induces EpRE-mediated gene expression. *Chem. Res. Toxicol. 19*, 1499–1505.
(17) Pullman, B., and Pullman, A. (1963) *Quantum Biochemistry*, pp 128–135 and pp 495–497, Interscience Publishers, New York.
(18) Hansch, C. and Leo, A. (1995) *Exploring QSAR, Fundamentals and Applications in Chemistry and Biology*, ACS Professional Reference Book, American Chemical Society, Washington, DC.
(19) Wardman, P. (1989) Reduction potentials of the one-electron couples involving free radicals in aqueous solution. *J. Phys. Chem. Ref. Data 18*, 1637–1755.
(20) Koppenol, W. H. (1990) Oxyradical reactions: from bond-dissociation energies to reduction potentials. *FEBS Lett. 264*, 165–167.
(21) Koppenol, W. H., and Butler, J. (1985) Energetics of interconversion reactions of oxyradicals. *J. Adv. Free Radical Biol. 1*, 91–131.
(22) Dixon, W. T., and Murphy, D. (1976) Determination of the acidity constants of some phenol radical cations by means of electron spin resonance. *J. Chem. Soc., Faraday Trans. 2 72*, 1221–1230.
(23) Buettner, G. R. (1993) The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate. *Arch. Biochem. Biophys. 300*, 535–543.
(24) Buxton, G. V., Greenstock, C. L., Helman, W. P., and Ross, A. B. (1988) Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals in aqueous solutions. *J. Phys. Chem. Ref. Data 17*, 513–886.
(25) Land, E. J., and Ebert, M. (1967) Pulse radiolysis studies of aqueous phenol. Water elimination from dihydroxycyclohexadienyl radicals to form phenoxyl. *Trans. Faraday Soc. 63*, 1181–1190.
(26) Huie, R. E., Brault, D., and Neta, P. (1987) Rate constants for one-electron oxidation by the $CF_3O_2^{\bullet}$, $CCl_3O_2^{\bullet}$, and $CBr_3O_2^{\bullet}$ radicals in aqueous solutions. *Chem. Biol. Interact. 62*, 227–235.
(27) Marcus, R. A. (1964) Chemical and electrochemical electron transfer theory. *Annu. Rev. Phys. Chem. 15*, 155–196.
(28) Scandola, F., Balzani, V., and Schuster, G. B. (1981) Free-energy relationships for reversible and irreversible electron transfer-processes. *J. Am. Chem. Soc. 103*, 2519–2533.
(29) Amatore, C. (1991) Basic concepts, in *Organic Electrochemistry: An Introduction and a Guide*, 3rd ed., (Lund, H., and Baizer, M. M., Eds.), pp 11–119, Dekker, New York.
(30) Neta, P., and Huie, E. (1990) Rate constants for reactions of peroxyl radicals in fluid solutions. *J. Phys. Chem. Ref. Data 19*, 413–513.
(31) Wasserman, H. H., Murray, R. W., Eds. (1979) *Singlet Oxygen*, Academic Press, New York.
(32) Frimer, A. A., Ed. (1985) *Singlet* O_2, CRC Press, Boca Raton, FL.
(33) Gorman, A. A. (1992) *The Bimolecular Reactivity of Singlet Molecular Oxygen*, in Advances in Photochemistry, volume 17 (Volman, D., Hammond, G., and Neckers, D., Eds.) pp 217–274, John Wiley & Sons, New York.
(34) Steenken S. and Neta P. (2003) Transient Phenoxyl Radicals: Formation and Properties in aqueous solutions, in *The Chemistry of Phenols*, Patai series: The chemistry of functional groups (Rappaport Z., Ed.), pp 1107–1152, John Wiley, Interscience Publication, New York.
(35) Everse, J., Everse, K. E., and Grisham, M. B., Eds. (1991) *Peroxidases in Chemistry and Biology*, CRC Press, Boca Raton, FL.
(36) Lambert, J. D., Sang, S., and Yang, C. S. (2007) Possible controversy over dietary polyphenols: Benefits vs risks. *Chem. Res. Toxicol. 20*, 583–585.

(37) Sergediene, E., Jönsson, K., Szymusiak, H., Tyrakowska, B., Ivonne, M., Rietjens, and Cenas, N. (1999) Prooxidant toxicity of polyphenolic antioxidants to HL-60 cells: description of quantitative structure-activity relationships. *FEBS Lett. 462*, 392–396.
(38) Bensasson, R. V., Jossang, A., Zahir, A., Bodo, B., and Land, E. J. (1999) Redox regulation of tumor cell toxicity by flavones from *Lethedon tannaensis*. *Free Radical Biol. Med. 27*, 95–99.
(39) Neta, P., and Grodowski, J. (2005) Rate constants for reactions of phenoxyl radicals in solution. *J. Phys. Chem. Ref. Data 34*, 109–199.
(40) Cooksey, C. J., Jimbow, K., Land, E. J., and Riley, P. A. (1992) Reactivity oforthoquinones involved in tyrosinase-dependent cytotoxicity: differencesbetween alkylthio- and alkoxy-substituents. *Melanoma Res. 2*, 283–93.
(41) Cooksey, C. J., Land, E. J., Rushton, F. A. P., Ramsden, C. A., and Riley, P. A. (1996) Tyrosinase-mediated cytotoxicity of 4-substituted phenols: Use of QSAR to forecast reactivities of thiols towards the derived ortho-quinones. *Quant. Struct.−Act. Relat. 15*, 498–503.
(42) Wardman, P. (1990) Bioreductive activation of quinones: Redox properties and thiol reactivity. *Free Radical Res. Commun. 8*, 219–229.
(43) Wakabayashi, N., Dinkova-Kostova, A. T., Holtzclaw, W. D., Kang, M. I., Kobayashi, A., Yamamoto, M., Kensler, T. W., and Talalay, P. (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. U.S.A. 101*, 2040–2045.
(44) Dinkova-Kostova, A. T., Fahey, J. W., and Talalay, P. (2004) Chemical Structures of Inducers of Nicotinamide Quinone Oxidoreductase 1 (NQO1). In *Quinones and Quinone Enzymes, part B*, Methods in Enzymology, Volume 382 (Sies, H., and Packer, L., Eds.) pp 423–448, Academic Press, San Diego, CA.
(45) Spencer, S. R., Xue, L., Klenz, E. M., and Talalay, P. (1991) The potency of inducers of Nad(P)H:(quinone-acceptor) oxidoreductase parallels their efficiency as substrates for glutathione transferases. Structural and electronic correlations. *Biochem. J. 273*, 711–717.
(46) Prestera, T., Zhang, Y., Spencer, S. R., Wilczak, C. A., and Talalay, P. (1993) The electrophile counterattack response: Protection against neoplasia and toxicity. *Adv. Enzyme Regul. 33*, 281–296.
(47) Prestera, T., and Talalay, P. (1995) Electrophile and antioxidant regulation of enzymes that detoxify carcinogens. *Proc. Natl. Acad. Sci. U.S.A. 92*, 8965–8969.

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