Relationship Between Phenol-Induced Cytotoxicity and Experimental Inhibition Rate Constant or a Theoretical Parameter

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Abstract: We synthesized various dimer forms of 2-methoxyphenols and 2-*tert*-butylphenols, as dimers such as curcumin exhibit potent antioxidant and anti-inflammatory activity. We investigated the QSARs between the cytotoxicity and independent variables; kinetic parameters (inhibition rate constant (k_{inh}/k_p), stoichiometric factor (*n)*) or DFT-based theoretical parameters (*i.e.* phenolic O-H bond dissociation enthalpy (BDE), ionization potential according to Koopman's theorem (IP), LUMO, absolute hardness (η), electronegativity (χ) and electrophilicity (ω)) for 2-methoxyphenols and 2-*tert*- or 2,6-di-*tert*-butylphenols. The cytotoxicity of these phenols against human tumor cells (HSG, HL60) and/or human gingival fibroblasts (HGF) showed a marked negative linear relationship to k_{inh}/k_p, suggesting that the cytotoxicity of phenols may be related to radical reactions. By contrast, a linear relationship between the cytotoxicity and η-term was demonstrated; 2-methoxyphenols showed a negative slope, whereas 2-*tert*- or 2,6-di-*tert*-butylphenols showed a positive slope. Also, the cytotoxicity of *tert*-butylphenols was linearly dependent on the LUMO-term, showing a positive slope. The cytotoxicity of methoxy-substituted monophenols toward both HSG and HGF cells was related to both log P and η-terms. Also, that of X-phenols toward murine L-1210 cells was related to both log P and η or IP-terms, determined from a dataset reported by Zhang *et al*., 1998. It was concluded that the phenol-induced cytotoxicity was attributable to radical reactions resulting from the terms (k_{inh}/k_p, IP, η, and LUMO) in QSAR. The LUMO-dependent cytotoxicity of 2-*tert*- or 2,6-di-tert-butylphenols may be related to their quinone oxidation products. Experimental and theoretical parameters provide a useful approach for analysis of the cytotoxicity for phenolic compounds.

Keywords: Cytotoxicity, 2-*tert*- or 2,6-Di-*tert*-butylphenols, Inhibition rate constants, 2-Methoxyphenols, Partition coefficients (log P), Phenol dimers, QSAR, Theoretical parameters.

1. INTRODUCTION

2-Methoxyphenols, such as eugenol (**1**), isoeugenol (**2**), and curcumin (**11**), are components of flavors used in the food industry. These compounds are of interest because of their anti-inflammatory and chemopreventive properties, which result from their antioxidant activity [1,2] Also, butylated hydroxyanisole (**12**) and butylated hydroxytoluene (**14**) are widely used as food antioxidants. The term "antioxidant" has been used in a wide sense to indicate compounds that prevent the formation of reactive oxygen species (ROS), or in a narrow sense to indicate those that eliminate previously formed ROS through a chain-breaking action [3]. These phenols are prototypic chain-breaking antioxidants that act during the autoxidation of polyunsaturated fatty acids in biological systems. Thus, the beneficial or adverse effects of these phenols are initiated by abstraction of the hydrogen atom from the hydroxy group of the phenol. In compounds **1**, **2**, **12** and **14**, the free radicals formed react *via* their mesomeric forms, primarily in the *ortho* position for the three former compounds and in the *para* position for **14**, and then oxidative coupling of the phenols leads to dimerization [4,5,6]. Oxidation of the phenolic compounds could give rise to similar radical species, semiquinone radicals, and the subsequent formation of a quinone methide that would be reactive with bioactive substances such as glutathione, amino acids, peptides, proteins, or with a second phenolic radical [7,8]. Thus, dimerization and oxidation-quinone (methide) formation play a crucial role in the biological activities of 2-methoxyphenols and 2-*tert*- or 2,6-di-*tert*butylphenols *in vivo*. Dimerization of these phenols can reduce their prooxidant activity, and the biological activities of these compounds may be enhanced by increasing their antioxidant activity. Therefore, we synthesized dimers from the corresponding monomers (Fig. **1**): EUG-dimer (3,3'-dimethoxy-5,5'-di-2-propenyl-1,1'-biphenyl-2,2'-diol) (**3**) from **1**, dehydrodiisoeugenol (2-(3-methoxy-4-hydroxyphenyl)-3-methyl-5-(1-propenyl)-7-methoxy-2,3-dihydrobenzofuran) (**4**) from **2**, and α-diisoeugenol (R-1-ethyl-5-hydroxy-*t*-3-(4-hydroxy-3-methoxyphenyl)-6-methoxy-*c*-2-methylindane) (**5**) from **2.** Also, we synthesized dimers from 2-*tert*-butylphenol monomers (Fig. **2**): **13** (3,3'-di-tert-5,5'-methoxy-1,1'-biphenyl-2,2'-diol) from **12**, **16** (3,3'-di-*tert*-butyl-5,5'-dimethyl-1,1'-biphenyl-2,2'-diol) from 2-*tert*-butyl-4-methylphenol **15**, and **19** (3,3',5,5'-tetra-*tert*-butyl-1,1'-biphenyl-2,2'-diol) from **18**. Investigations in our laboratory have focused on the mechanism of activity of a

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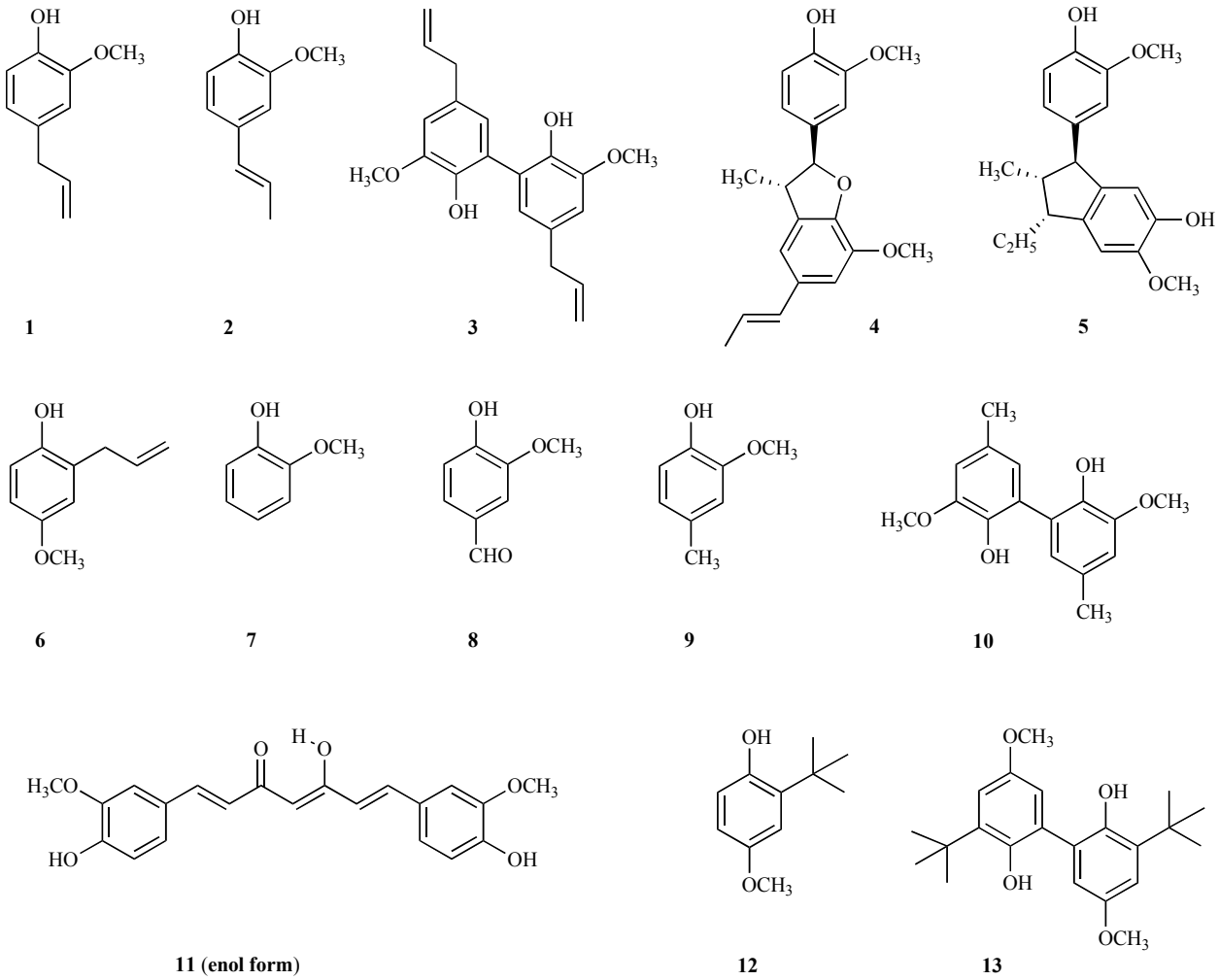

Fig. (1). The structure of 2-methoxyphenols and 2-*tert*-butyl-4-methoxyphenols.

variety of anticancer and antioxidant agents, namely dimers derived from 2-methoxyphenols and 2-*tert*-butylphenols, with the view to designing effective protocols for clinical trials [2]. We have investigated the radical production, prooxidation, radical-scavenging activity, and cytotoxicity of these compounds [2, 6, 9, 10]. Currently, QSARs (quantitative structure-activity relationships) are used as predictive tools for preliminary evaluation of the activity of chemical compounds through the use of computer-aided models [10-17]. Here, we present the results of our experiments and current knowledge, and discuss the mechanism of cytotoxicity for simple X-phenols and multisubstituted complex phenols, such as 2-methoxyphenols and 2-*tert*- or 2,6-di-*tert*-butylphenols, based on the QSARs using the experimental inhibition rate constant and a theoretical parameter.

2. CYTOTOXICITY *VERSUS* KINETIC PARAMETER (K_{inh}/K_p, *N*)

Many researchers have investigated the mechanism underlying the toxicity of phenolic compounds based on QSARs. For example, QSARs of phenolic compounds in L1210 leukemia cells have been shown previously to be related mostly to their BDE (phenolic O-H bond dissociation enthalpy) and overall hydrophobicity (octanol–water partition coefficient, log P), suggesting that phenol-induced toxicity is correlated with the radical reaction [12]. In addition to BDE and log P, an acceptable QSAR for the cytotoxicity of monophenols, which are simple phenols in terms of their ionization constant (pK_a) and the Brown variation of the Hammet electronic constant (σ^+), has been reported [18,19]. However, *ortho*-substituted or multisubstituted complex *tert*-butylphenols have been found to behave quite differently from simple phenols; QSARs of 2-alkyl- or 2,6-dialkyl-4-X-phenols for the cytotoxicity with BDE or σ^+ have yielded poor results, whereas those with the Taft steric parameter (E_{S-2}) and Otsu's radical parameter (E_R) have yielded good results [13]

These findings have provided valuable guidance for our QSAR studies of complex phenols. E_R and other radical parameters are necessary in order to derive meaningful QSARs for complex phenol antioxidants because the effects of free radicals in living systems cannot be ignored. Therefore, it is very important to accurately determine the radical-scavenging activity of phenolic compounds. The antioxidant activity of natural and synthetic phenols has been assessed using various tests such as inhibition of low-density

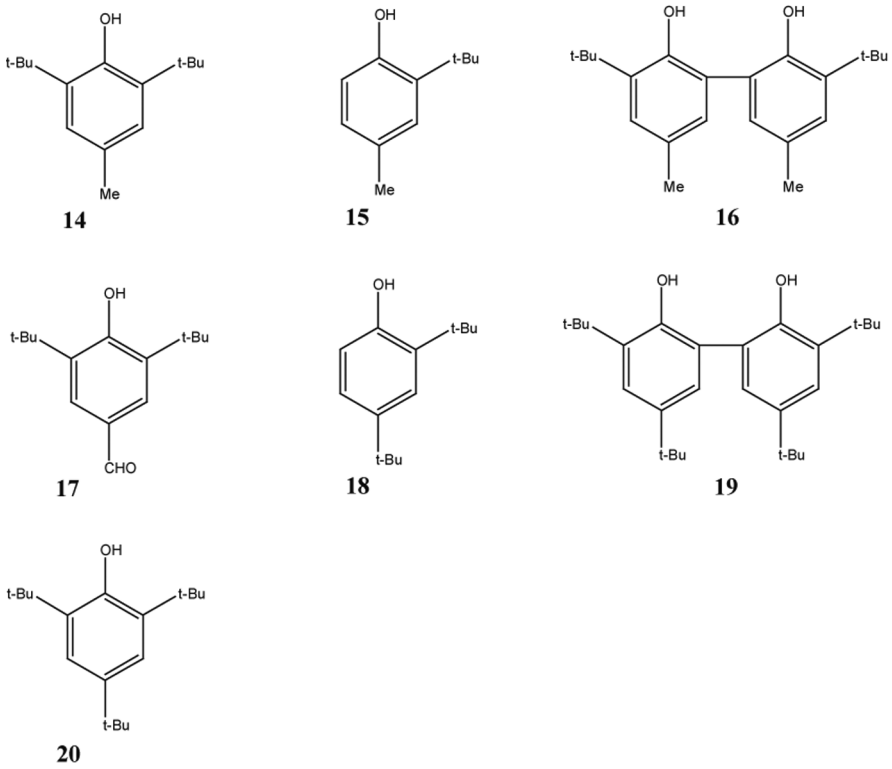

Fig. (2). The structure of 2-*tert*- or 2,6-di-*tert*-butylphenols.

lipoprotein oxidation [20], the lipid peroxidation inhibition capacity (LPIC) assay [21], 1,1-diphenyl-2-picrylhydrazyl (DPPH)-scavenging, and the inhibition of AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride)-induced peroxidation of linoleic acid in sodium dodecyl sulphate micelles [22] and the cupric ion reducing antioxidant capacity (CUPRAC) and trolox equivalent antioxidant capacity (TEAC) methods [23].

Also, the radical-scavenging activities of a wide variety of phenols have been determined previously using the induction period method for inhibiting the oxidation of styrene in the presence of a phenolic inhibitor by peroxyl radicals ($ROO^{\cdot}$) generated by 2,2'-azobisisobutyronitrile (AIBN) [24,25,26]. Note that AIBN, an alkyl radical ($R^{\cdot}$), is coverted to $ROO^{\cdot}$ in the presence of air.

We have previously used differential scanning calorimetry (DSC) and the induction period method for polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of AIBN or benzoyl peroxide (BPO) in order to investigate the radical scavenging activity of eugenol and related compounds under nearly anaerobic conditions [6,10,14,15]. This induction period method has proved to be reliable for evaluating the activity of phenolic compounds [6,10,14,15]. This is because the DSC technique is very sensitive and extraordinarily precise. Also, biological systems have a low oxygen tension [27] and cancer cells show anaerobic metabolism (*i.e.* they do not utilize oxygen) [28]. Therefore, our biomimetic system under nearly anaerobic conditions may be a good model for evaluating the antioxidant activity of anticancer drugs. Furthermore, the radical reaction takes place in more lipophilic media (*e.g.*, the peroxy radical reaction with vitamin E in the lipid bilayer of cells) [29], and therefore the mechanism of cytotoxicity of phenolic compounds in a biological system may be assumed from such DSC experiments in a lipophilic MMA medium. This mini-review presents the results obtained with the benzoate radical ($PhCOO^{\cdot}$), because it is an oxygen-centered radical, similar to the alkyl peroxyl radical ($ROO^{\cdot}$) generated in biological systems. The chemical structures of the investigated 2-methoxyphenols and 2-*tert*- or 2,6-di-*tert*-butylphenols are shown in Figs. (**1** and **2**), respectively. The ratio of the rate constant for inhibition to that for propagation (k_{inh}/k_p) determined by equation 8, and the stoichiometric factor *n* (the number of free radicals trapped by one mole of antioxidant moiety) determined by equation 5, for these phenols, are shown in Table **1**. Since k_p is constant, k_{inh}/k_p was denoted as the inhibition rate constant for practical convenience. The estimated value of the propagation rate constant (k_p) in this study was about 797 $M^{-1}s^{-1}$[10].

Wright and Shadnia [29] previously focused on one important aspect of the toxicity: the rate constant for production of phenoxyl radicals. They reported a linear relationship between log k (inhibition rate constant) and ΔBDE (the difference from the BDE of phenol) for X-phenols with a negative slope and r^2=0.96. Note that log k had been determined using the induction period method for inhibiting the oxidation of styrene in the presence of antioxidants initiated by AIBN [25]. We also examined the correlation of k_{inh}/k_p with BDE_{1st}, IP_{1st} or IC_{50} for eleven 2-methoxyphenols at a concentration of 1 mM. The results are shown in Table **2**. For these phenols, there was a significant linear relationship between k_{inh}/k_p and IP_{1st} or IC_{50} with r^2 values of 0.80 and 0.99, respectively. By contrast, there was

Table 1. Radical-Scavenging Activities of Multisubstituted Phenols Using the Induction Period Method in the Benzoyl Peroxde (BPO) − Methyl Methacrylate (MMA) System and a DPPH (1,1'-diphenyl-2-picrylhydrazyl) assay and their DFT-Based Phenolic O-H Bond Dissociation Enthalpy (BDE) and Ionization Potential Provided by *Koopmans*' Theorem (IP)

	BPO-MMA		DPPH Assay	BDE	IP
Compound	**k_{inh}/k_p (n)[a]**	**k_{inh}/k_p (n)[b]**	**IC_{50}, mM**	**kJ/mol**	**eV**
1	11.19(1.4)	7.07(1.4)	0.06	346.8	5.45
2	8.78(1.7)	5.63(1.9)	0.05	339.2	5.18
3	7.76(2.3)	6.71(2.3)	0.015	336.5, 354*	5.19, 5.46*
4	18.29(0.8)	_	1.3	359.7	5.2
5	5.87(2.7)	_	0.05	343.2, 347.2*	5.28, 5.3*
6	10.08(2.0)	_	0.06	339.2	5.26
7	12.81(1.2)	_	0.51	364.6	5.53
8	104.44(0.1)	27.4(0.2)	27.4	361.9	6.08
9	10.57(1.4)	_	0.01	344.1	5.38
10	6.26(2.4)	_	0.024	338.1, 338.2*	5.11, 5.02*
11	4.53(3.8)	4.89(3.9)	0.04	344, 347*	5.27, 5.37*
12	15.93(1.4)	2.49 (1.8)	0.052	325	5.3
13	8.86(1.2)	4.50(3.3)	0.012	320.5, 319.6*	5.28, 5.37*
14	_	1.69(1.8)	0.1	319	5.52
15	_	3.75(2.0)	1.01	340	5.66
16	_	1.64(1.9)	0.05	327, 363*	5.70, 5.43*
17	_	11.05(0.3)	_	336	6.17
18	_	6.01(1.9)	_	341	5.68
19	_	1.83(1.8)	_	330, 362*	5.71, 5.44*
20	_	1.84(1.8)	_	320	5.54

For the number of compounds see Figs. (**1** and **2**), respectively.
[a]The ratio of the rate constant of inhibition to that of propagation. *n*, stoichiometric factor. Values were determined at the concentration of 1mM phenols in this work. Data represent the mean of three independent experiments. Errors < 7%.
[b]The values determined at 5 mM phenols were taken from Kadoma *et al*.[10].
BDE and IP were taken from Kadoma *et al*. [2, 10]. *The second BDE or IP.

Table 2. Linear Dependences of BDE_{1st} and IP_{1st} on Inhibition Rate Constants (k_{inh}/k_p)

			y=a+bx				
Phenols	**y**	**x**	**a**	**b**	**n**	**r^2**	**P**
A	k_{inh}/k_p	BDE	-849.2(±0.9)	2.5(±0.8)	11	0.53	P=0.01, P<0.05
	k_{inh}/k_p	IP	-494.7(±13.5)	95.8(±15.8)	11	0.80	P=0.0002, P<0.001
	k_{inh}/k_p	IC_{50}	3.5(±0.1)	8.8(±2.9)	11	0.99	P<0.001
B	k_{inh}/k_p	BDE	_	_	7		NS
	k_{inh}/k_p	IP	-78.1(±1.8)	14.4(±3.4)	7	0.78	P=0.008, P<0.01

A, 2-methoxyphenols; B, 2- or 2,6-di-*tert*-butylphenols with exception of compounds **12** and **13**.

also a linear relationship between k_{inh}/k_p and IP_{1st}, but not BDE_{1st}, for seven *tert*-butylphenols with the exception of compounds **12** and **13** (r^2=0.78). **12** and **13** possess a *methoxy*-substituent at the 4-position (Fig. **2**), and their radical-scavenging behavior appeared to differ greatly from that of other *tert*-butylphenols with an alkyl-substituent at the 4-position. This may be attributable to the methoxy substitients, strong electron-donor groups, in contrast to alkyl groups.

We investigated the relationships between cytotoxicity and k_{inh}/k_p for 2-methoxyphenols and 2-*ter*t- or 2,6-di-*ter*t-butylphenols using salivary gland carcinoma cells (HSG), human promyelocytic leukemia cells (HL60) and human gingival fibroblasts (HGF) [10]. The results obtained are shown in Table **3**. The relationship between cytotoxicity and k_{inh}/k_p for 2-methoxyphenols and 2-*tert*- or 2,4-di-*tert*-butylphenols is shown in Figs. (**3B** and **4B**), respectively. As can be seen in Fig. (**3B**), a significant linear relationship between log 1/C and k_{inh}/k_p was observed of eight 2-methoxyphenols against HSG, HL60 and HGF cells was observed, with the exception of compounds **4**, **5** and **8**. Their r^2 for HSG, HL60 and HGF cells was 0.81, 0.70 and 0.58 respectively. Note that the line for HL-60 cells was not shown in Fig. (**3B**) because of the overlapping of that for HSG cells. As shown in Table **2**, a relationship for k_{inh}/k_p vs IP_{1st} or BDE_{1st} was derived for 2-methoxyphenols. The relationship for k_{inh}/k_p vs BDE yielded a poor result, and therefore it was of interest to determine whether IP is involved in the cytotoxicity of phenolic compounds. There was a significant relationship between log 1/C and IP for 2-methoxyphenols when **4**, **5** and **11** were excluded (r^2=0.6-0.7 for indicated three cell lines). Compounds **4**, **5** and **11** exhibited strong cytotoxicity, which appeared not to be induced by IP alone. By contrast, for *tert*-butylphenols, there was no relationship between cytotoxicity and IP or BDE. These findings suggested that the cytotoxicity of *tert*-butylphenols differed considerably from that of 2-methoxyphenols.

The occurrence of dimerization can be estimated from the *n* value. In general, phenolic compounds possess a *n*

Table 3. The 50% Cytotoxic Concentration (C) of 2-Methoxyphenols and 2- or 2,6-di-tert-butylphenols against HSG, HL60 and HGF Cells

		HSG cells	HL60 cells	HGF cells	
Com.	**Phenols**	**[a]log 1/C, M**	**[b]log 1/C, M**	**[c]log 1/C, M**	**[d]log P**
1	Eugenol (EUG)	3.50	3.77	3.54	2.55
2	Isoeugenol	4.55	4.78	4.49	2.51
3	EUG-dimer	3.82	4.09	3.16	4.75
4	Dehydrodiisoeugenol	5.52	5.32[e]	5.51	4.06
5	alpha-Diisoeugenol	5.57	5.11[e]	5.55	4.40
6	2-Allyl-4-methoxyphenol	3.65	2.92[e]	3.39	2.55
7	Guaiacol	3.1	2.87[e]	2.25	1.51
8	Vanillin	2.89	2.6[e]	1.8	1.19
9	MMP	3.4	3.77[e]	2.68	1.98
10	MMP-dimer	4.33	4.05[e]	3.51	3.59
11	Curcumin	5.52	5.78	5.48	2.51
12	BHA	4.65	4.24[e]	4.56	3.14
13	BHA-dimer	3.47	3.05[e]	3.12	5.40
14	BHT	4.50	_	4.56	5.48
15	BMP	4.50	_	4.70	3.65
16	BMP-dimer	5.51	_	5.5	7.35
17	DTBHB	2.25	_	2.1	4.28
18	DTB	5.67	_	5.65	5.03
19	DTB-dimer	5.16	_	5.16	9.67
20	TBP	5.39	_	5.48	6.75

For the numbers of compounds see Fig. **1** and **2** respectively. C: 50% cytotoxic concentration.
[a-d]From Kadoma *et al*. [2,10]. [e]in this work.

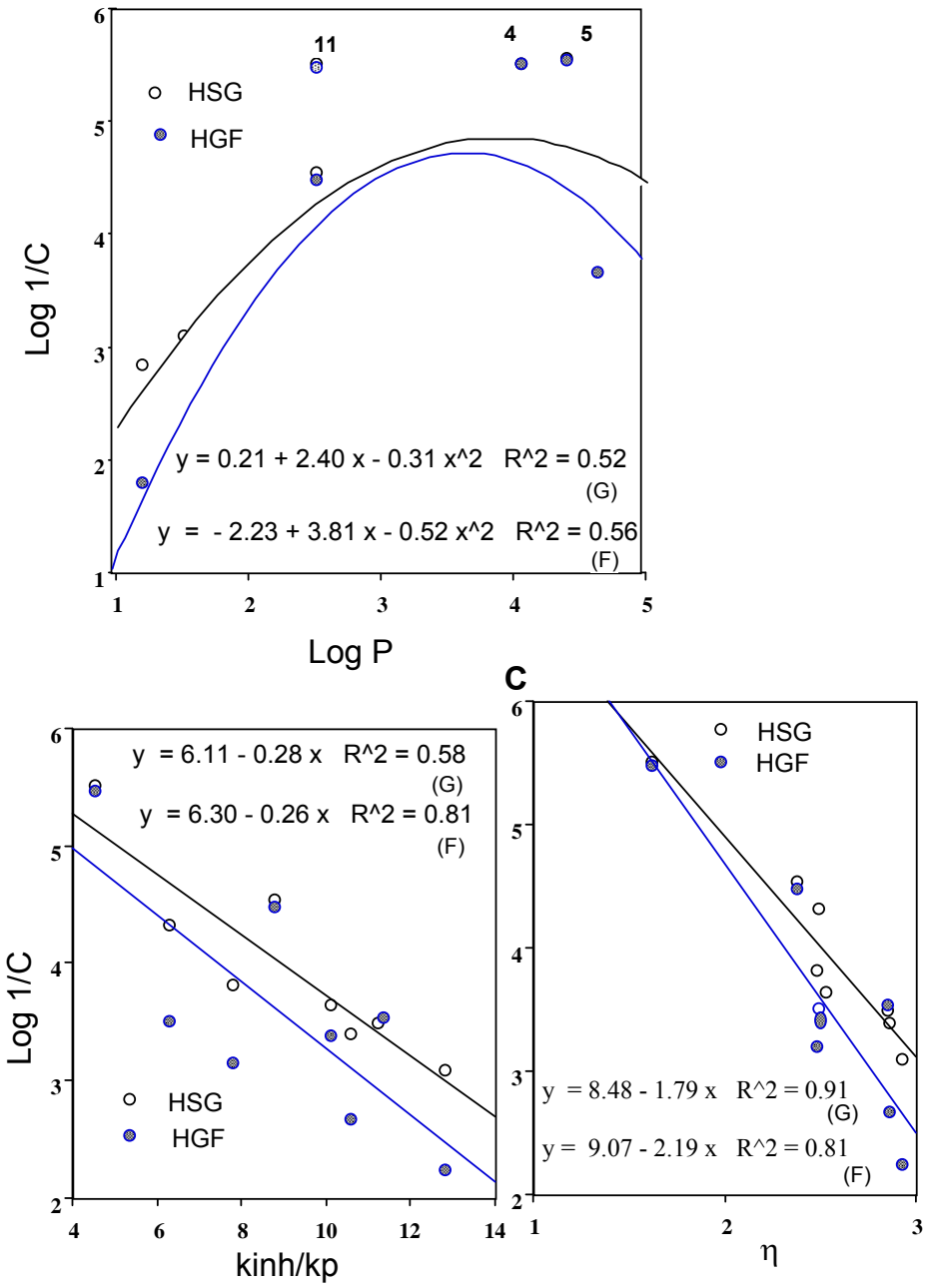

Fig. (3). Relationships between the cytotoxicity (log 1/C, M) and independent variables (log P (**A**), kinh/kp (**B**) and η (**C**)) for 2-methoxyphenols.

value of 2 [24]. When the *n* for monophenols is less than 2, and particularly near 1, dimerization preferably occurs [25]. Particularly, 2-methoxyphenols form intermolecular hydrogen bonds between 2-methoxy and phenolic O-H substituents, and consequently dimerization occurs due to the *ortho-ortho* coupling reaction derived from the original 2-methoxyphenol monomers [6]. As shown in Table **1**, the relative *n* value for 2-methoxymonophenols **1**, **4**, **7** and **9** was 1.4, 0.8, 1.2 and 1.4, respectively; thus their *n* value was considerably less than 2. On the other hand, the *n* value for *ter*t-butylmonophenols **12, 14**, **16**, **18** and **20** was 1.8, 1.8, 2.0, 1.9 and 1.8, respectively, thus approximating 2. This suggested that *ter*t-butylmonophenols may form a quinone and quinone methide due to two-electron oxidation. Indeed, the formation of **12**-quinone, **14**-quinone and **20**-quinone (or quinone methide) by oxidation has been reported previously [7,30,31]. Also, in general, the fully oxidized 2-methoxy dimer compounds of **3**, **5**, **10** and **11** bearing two phenolic OH groups would ideally have a *n* value of 4. The *n* value of *tert*-butyl-substituted dimer compounds **16** and **19** was about 2, whereas that of **13** was ca. 3, possibly due to the higher BDE and IP value at the first oxidation for the former than those for the latter. On the other hand, the IC_{50} value for some 2-*tert*- or 2,6-di-*tert*-butylphenols could not be determined using the DPPH method due to the sterically hindered molecular structure of these compounds (Table **1**).

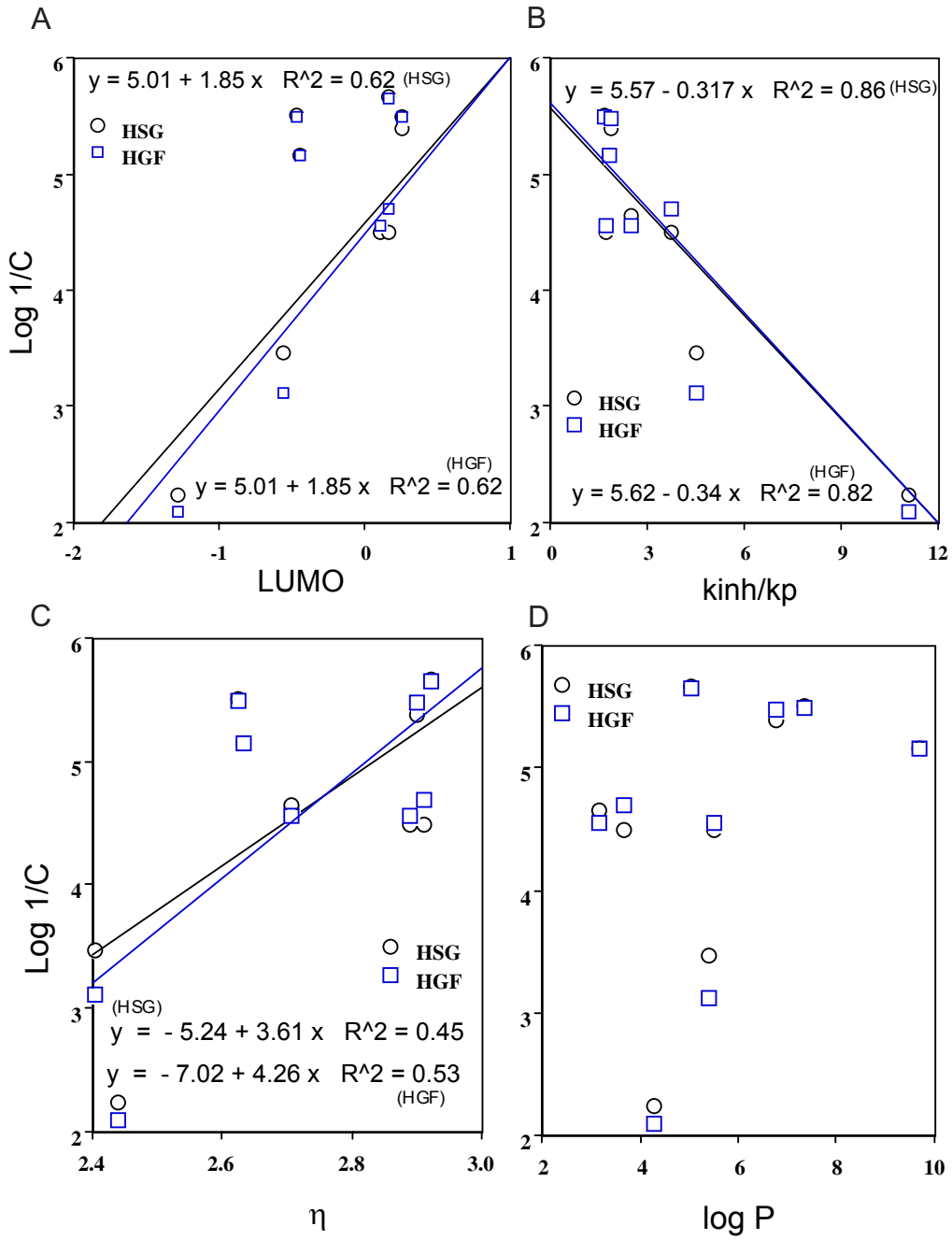

Fig. (4). Relationships between the cytotoxicity (log 1/C, M) and independent variables (LUMO (**A**), kinh/kp (**B**), η (**C**) and log P (**D**)) for 2-*tert*- or 2,6-di-*tert*-butylphenols.

The oxidized form of **18** preferably produces a precursor of 3,5-di-*tert*-butyl-*o*-benzoquinone, and consequently forms benzoquinone, as well as producing 4,6-di-*tert*-butyl-4-hydroproxy-2,5-cyclohexadiene-1-one [31]. Overall, since 2-*tert*- or 2,6-di-*tert*-butylmonophenols showed a *n* value of 1.8-2.0, their cytotoxic activity may be caused by quinones and quinone methides formed by autooxidation [32].

The number of scavenging radicals, *n*, was not directly involved in the cytotoxicity of 2-methoxyphenols and 2-*tert*- or 2,6-di-*tert*-butylphenols. By contrast, a causal link between cytotoxicity and k_{inh}/k_p was observed. The *n* value is correlating to a radical process in which the phenol is first converted to a phenoxyl radical during the induction period, as shown in Equation 5. The k_{inh}/k_p value can be calculated from both factors inhibited- propagation rate (Rp_{inh}) and *n*, as determined by Equation 8. Therefore, Rp_{inh} is important for determining the induction of cytotoxicity. When radicals oxidize phenolic antioxidants during the induction time, reactive intermediates such as quinone-methides, quinones, and dimer could be formed. The Rp_{inh} value could be considerably affected by one or more intermediate components formed by oxidation in rate-determining step, particularly when the components possess a newly acquired affinity for radicals. Therefore, the k_{inh}/k_p may provide decisive information about the reactive intermediates with cellular targets [10].

3. CYTOTOXICITY *VERSUS* THEORETICAL PARAMETER

QSARs are predictive tools for preliminary evaluation of the activity and properties of chemical compounds using computer-aided models. Currently, the effects of different substituents on phenol toxicity *in vitro* have been investigated by the Hansch group [11-13,18]. There has been increasing interest in the use of the LUMO-HOMO gap, an absolute hardness (η) in mechanistic analysis of not only organic reactions but also chemical-biological interactions in biosystems. Zhang *et al*. [11] have reported previously that a significant multiple linear regression equation for 26 X-phenols in L1210 leukemia cells can be denoted as log 1/C (which is a function [LUMO-HOMO gap (or σ^+), log P]) with r^2=0.90; as log P (hydrophobic term) increases, the cytotoxicity (C: the molar concentration inducing 50% inhibition of growth in 48 h) increases, and simultaneously as the LUMO-HOMO gap or σ^+-term decreases, the cytotoxicity is enhanced. In this model for electron-releasing phenols, a low coefficient with the log P-term has been reported, and therefore it has been concluded that phenol-induced cytotoxicity is related to the LUMO-HOMO gap or σ^+-term.

We calculated the IP according to Koopman's theorem [33] and also the η, χ and ω values using a dataset of HOMO and LUMO values [11] (Table **4**). Furthermore, we

Table 4. The Cytotoxicity (50% Growth Inhibition, C) of X-Phenols Against L1210 Leukemia Cells and their Independent Variables (IP, η, χ, ω, log P, BDE)

	Substituents	[a]Obs. Log 1/C	IP	η	χ	ω	[a]log P	BDE[#]
1	4-OMe	4.48	8.648	4.48	4.168	1.939	1.34	-0.61
2	4-OC_2H_5	4.64	8.609	4.473	4.136	1.912	1.84	-6.16
3	4-OC_3H_7	4.85	8.608	4.474	4.135	1.911	2.33	-6.23
4	4-OC_4H_9	5.2	8.608	4.474	4.135	1.911	2.90	-6.27
5	4-OC_6H_{13}	5.5	8.608	4.474	4.135	1.911	4.22	-6.30
6	H	3.27	9.114	4.756	4.358	1.997	1.47	0
7	4-F	3.83	9.093	4.756	4.517	2.229	1.77	-1.99
8	4-NH_2	5.09	8.27	4.355	3.916	1.760	0.04	-9.25
9	4-OH	4.59	8.725	4.473	4.253	2.022	0.59	-6.04
10	4-Me	3.85	8.88	4.658	4.223	1.914	1.94	-2.22
11	4-C_2H_5	3.86	8.856	4.656	4.2	1.894	2.47	-1.90
12	4-OC_6H_5	4.97	8.797	4.457	4.341	2.113	3.35	-4.55
13	Bisphenol A	4.07	8.948	4.651	4.3	1.987	3.32	-1.88
14	4-$C(Me)_3$	4.09	8.898	4.681	4.218	1.900	3.31	-1.54
15	3-$C(Me)_3$	3.88	9.014	4.723	4.292	1.950	3.05	0.12
16	3-Me	3.54	9.013	4.705	4.308	1.972	1.96	-0.25
17	3-NMe_2	4.11	8.492	4.482	4.01	1.794	1.56	-0.60
18	3-C_2H_5	3.71	8.983	4.702	4.281	1.949	2.40	0.13
19	3-C_3H_7	4.04	8.902	4.668	4.235	1.921	3.00	-2.01
20	4-C_4H_9	4.33	8.903	4.668	4.235	1.921	3.64	-2.08
21	4-C_5H_{11}	4.47	8.903	4.668	4.235	1.921	4.06	-2.13
22	4-C_8H_{17}	4.62	8.912	4.671	4.241	1.925	5.68	-2.17
23	4-C_7H_{15}	4.49	8.903	4.668	4.235	1.921	5.15	-2.17
24	4-C_9H_{19}	4.75	8.913	4.674	4.24	1.923	6.21	-2.17
25	Estradiol	4.34	8.978	4.675	4.303	1.980	4.01	-1.19
26	DES	4.68	9.017	4.637	4.381	2.069	5.07	-2.42

[a]From Zhang *et al*. [11]. The values for IP, η, χ and ω were calculated from a data set [11].
[#]Calculated from a data set presented by Pasha *et al*. [15]. [#]From Selassie *et al*. [13].

investigated the relationships between these parameters and the cytotoxicity. A significant linear relationship between log 1/C and independent variables (log P and IP or η) for 26 electron-releasing phenols was observed as follows:

$$\text{Log1/C} = 0.2(\pm 0.0)\ \log P - 2.1(\pm 0.3)IP + 20.0(\pm 0.3)\ (n=26,\ r^2=0.67,\ p<0.001) \quad (1)$$

$$\text{Log1/C} = 0.2(\pm 0.0)\ \log P - 4.0(\pm 0.4)\ \eta + 22.3(\pm 0.2)\ (n=26,\ r^2=0.81,\ p<0.001) \quad (2)$$

Good QSAR between log 1/C and both terms was found for X-phenols. However, there was no relationship between log 1/C and the variables (log P and χ or ω). On the other hand, a linear relationship between IP and σ^+ or BDE for these 26 phenols with exception of substituted groups such as 4-OMe, 4-F, 4-NH_2 and 3-NMe_2 yielded a good result (r^2=0.9), determined by data in Table **4**. Reasons for this anomalies are not obvious but these functional substituents may strongly affect the pi-cloud of the ring.

Sellasie *et al*. [12] previously investigated a QSAR in L1210 leukemia cells using a greater number of samples than those presented by Zhang *et al*. [11], and demonstrated a significant multiple linear regression for 42 X-phenols with a wide variety of substituents, which was denoted as log 1/C (for the function [LUMO-HOMO], log P) with r^2=0.91. By contrast, 14 2-X-phenols were related to the BDE-term (r^2=0.94), although the correlation of the cytotoxicity of 2-X-phenols with the LUMO-HOMO gap was poor. By contrast, phenols with substituents of an electron-attracting nature have been shown to exhibit log P-induced non-specific cytotoxicity [34].

Pasha *et al.* [15] have calculated the DFT-based reactivity indices for 49 2-X-, 3-X- and 4-X-phenols studied previously by Sellasie and co-workers [12], and investigated the relationships between these indices and the cytotoxicity against L1210 leukemia cells for these compounds. A significant multiple regression for a wide variety of phenols was denoted as log 1/C (which is the function [molecular weight, total energy (Hartree), η and χ] with r^2=0.82; the cytotoxicity was preferably related to the η− and χ-terms. However, to drive statistically significant multiple QSAR models for phenols using a theoretical technique, the descriptor selection from a number of congeners such as LUMO-HOMO gap, η, χ and ω may not be adequate to reflect the complexity of biological systems because these descriptors, as shown in Equations 9-11, are derived from both HOMO and LUMO. The addition of a number of physicochemical parameters such as log P and electronic parameters may be useful [11,12]. To clarify the causal relationship with each global descriptor based on the absolute hardness concept, we investigated the relationships between the cytotoxicity and each descriptor, including η, χ, ω, HOMO, LUMO and IP, using the dataset of Pasha *et al.* for the 49 X-phenols [15]. A linear relationship between log $1/C_{observed}$ and IP for 48 X-phenols, with the exception of 3-bromophenol, gave a statistically better correlation but its correlation was poor (r^2=0.47).

Although there was no relationship between log 1/C and χ, ω or the LUMO-term, there was a parabolic relationship between cytotoxicity and the η-term, despite its poor coefficient of determination. Together with our work, these findings suggest that the cytotoxicity for a wide variety of X-phenols may be related to the η− or IP-term. There are many ROS defense enzymes including superoxide dismutase, catalase and glutathione peroxidase, which are closely related to the cytotoxicity of phenolic compounds *in vivo* and *in vitro*. The cytotoxicity of phenols may be related to η- or IP-controlled enzymes.

The Hansch group has reported previously that phenol-induced cytotoxicity is related to the phenoxyl radicals, an oxygen-centered radical; this radical may interact with a receptor such as DNA, or it may represent the slightly enhanced transport of the phenoxyl radical in a cellular environment [34]. A possible relationship between ionization potential and horseradish peroxidase/hydrogen peroxide-catalyzed binding of aromatic hydrocarbons to DNA has been reported previously [35]. The good correlation between IP and BDE or σ^+ [11,12] suggested that the phenol-induced cytotoxicity may be attributable to the radical mechanism. The Hansch group has also indicated that a smaller LUMO-HOMO gap, and a smaller η, enhances the cytotoxicity of X-phenol; *i.e.* the more easily an electron is promoted to an unoccupied orbital, the more toxic the compound becomes [11]. The possible link between cytotoxicity and the η-term may be attributed to radical reactions, since there was no relationship between k_{inh}/k_p and the η-term (Table **2**).

The theoretical parameters used in the QSAR models can help to clarify the reasons behind various types of toxicity. Next, to clarify the mechanism of cytotoxicity of multisubstituted complex phenols, we investigated the relationships between log 1/C and log P, BDE, IP, HOMO, LUMO, η, χ or ω-term for 2-methoxyphenols and 2-*tert*-, or 2,6-di-*tert*-butylphenols. Descriptors were calculated according to DFT/B3LYP levels [2,10], and the results are shown in Table **5**. The relationships between cytotoxicity and various parameters for 2-methoxyphenols and 2-*tert*- or 2,6-di-*tert*-butylphenols were investigated, and the results are shown in Figs. (**3** and **4**), respectively.

For eight 2-methoxyphenols (monophenols **1**,**2**,**6**,**7**,**9**,**10**; biphenols **3**,**11**) with the exception of **4**, **5** and **8**, a significant linear relationship between log 1/C and the η-term was observed for HSG (r^2=0.91), HGF (r^2=0.81) and HL60 (r^2 =0.70) cells, respectively (Fig. **3C**). As the cytotoxicity became higher, the η−term became smaller, *i.e.* a negative slope was shown. A similar negative slope was obtained for X-phenols with electron-releasing substituents (Equation 2). By contrast, no relationship between log 1/C and LUMO, BDE, χ, or ω was observed, and this result was in accord with that for simple X-phenols, as described above. By contrast, a parabolic relationship between log 1/C and log P was observed for 2-methoxyphenols as a whole (Fig. **3A**), the r^2 value for HSG, HGF and HL60 cells being 0.52, 0.56 and 0.46, respectively. The curve for HL60 cells is not shown here because of the low coefficient. **4**, **5** and **11**, exerting potent cytotoxicity on tumor cells, were obvious outliers. The log P (*circa* 2.5) for **11** was smaller than that for **4** and **5**, but similar to that for **1** and **2**. However, the cytotoxicity of **11** was approximately 10-fold greater than that of **2**, and also approximately 100-fold greater than that of **1** (Table **3**). From these findings, the cytotoxicity of

Table 5. DFT-Based Descriptors for 2-methoxyphenols and 2-or 2,6-di-*tert*-bytylphenols

Com.	Phenols	E_{HOMO}, eV	E_{LUMO}, eV	η	χ	ω
1	Eugenol (EUG)	-5.448	0.247	2.848	2.6	1.186
2	Isoeugenol	-5.182	-0.429	2.377	2.806	1.656
3	EUG-dimer	-5.194	-0.236	2.479	2.715	1.487
4	Dehydrodiisoeugenol	-5.203	-0.422	2.391	2.813	1.655
5	alpha-Diisoeugenol	-5.283	0.141	2.712	2.571	1.219
6	2-Allyl-4-methoxyphenol	-5.257	-0.207	2.525	2.732	1.478
7	Guaiacol	-5.529	0.316	2.923	2.607	1.163
8	Vanillin	-6.075	-1.398	2.339	3.737	2.985
9	MMP	-5.376	0.332	2.854	2.522	1.114
10	MMP-dimer	-5.11	-0.134	2.488	2.622	1.382
11	Curcumin	-5.267	-2.038	1.6145	3.653	4.133
12	BHA	-5.300	0.109	2.705	2.596	1.246
13	BHA-dimer	-5.365	-0.562	2.402	2.964	1.829
14	BHT	-5.517	0.258	2.887	2.629	1.197
15	BMP	-5.664	0.1578	2.91	2.753	1.302
16	BMP-dimer	-5.699	-0.468	2.615	3.083	1.818
17	DTBHB	-6.168	-1.289	2.439	3.728	2.855
18	DTB	-5.682	0.163	2.92	2.759	1.304
19	DTB-dimer	-5.711	-0.444	2.633	3.078	1.799
20	TBP	-5.542	0.255	2.898	2.634	1.206

curcumin-related compounds appeared to be log-P-independent, but η−dependent (Fig. **3C**). On the other hand, ROS generation for these compounds has been investigated previously, and shown to decline in the order **11**>>**2**>**1** [9]. This suggested that the pronounced cytototoxic activity of **11** may be related to its intracellular ROS generation. It is well known that in HL 60 cells, intracellular ROS generation is closely related to the induction of apoptosis and intracellular glutathione controlled-ROS generation [2]. Therefore, we investigated the cytotoxicity and apoptosis induction of **2**, **4**, **5** and **11** in HL60 cells with or without glutathione ethyl ether (GSHE) treatment and results were as follows (unpublished data): It was found that the 50% cytotoxic concentration (mM) declined in the order **2** (0.70) >**5** (0.08) >**4** (0.05)>**11** (0.04), as determined by the MTT method, whereas induction of DNA fragmentation (apoptosis) by **11**, **4**, **5**, and **2** occurred at ca. 0.01 mM, 0.03 mM, 0.03 mM and 0.50 mM, respectively, as determined by agarose gel electrophoresis. Also, with GSHE treatments, the cytotoxicity of both **4** and **11** was considerably reduced, whereas the cytotoxicity of both **2** and **5** was hardly reduced. These experiments were carried out using medium containing ca. 5% serum, rather than 1% serum, because growth of HL60 cells hardly occurs in serum-free conditions. We had also shown previously that **11** greatly induced apoptosis in HL-60 cells, whereas **5** did so only slightly, as judged by externalization of annexin-V-targeted phosphatidylserine residues. Although compounds **5** and **11** exhibited very similar cytotoxicity, **11** preferentially induced apoptotic cell death, whereas **5** induced predominantly necrotic cell death [36]. These findings supported that the cytotoxicity of phenols toward murine leukemia cells involves minimum apoptosis [37]. The cytotoxicy of phenols should be evaluated based on the type of cell death, *i.e.* apoptosis, necrosis, or autophagy.

Next, we investigated here QSARs for eight methoxy-substituted monophenols (compounds **1**,**2**,**4**, **6-9** and **12**) using the data in Tables **3** and **5** and equations 3 (for HSG cells, cancer cells) and 4 (HGF cells, normal cells) were formulated as follows:

$$\text{Log1/C} = 0.9(\pm0.1)\ \log P - 0.7(\pm0.5)\ \eta + 3.7(\pm0.3) \quad (n=8,\ r^2=0.90,\ p<0.01) \qquad (3)$$

$$\text{Log1/C} = 1.3(\pm0.2)\ \log P - 0.5(\pm0.6)\ \eta + 1.8(\pm0.4) \quad (n=8,\ r^2=0.93,\ p<0.01) \qquad (4)$$

Two equations were quite similar, despite the different cell lines and showed that the contribution of log P in modeling log 1/C in the η−term is favorable. A good QSAR

in both log P and η–terms for methoxy-substituted monophenols was in agreement with that of the 26 X-phenols in Equation 2, but the intercept (2-4) in Equations 2 was about 10-fold greater than that in equations 3 and 4. Reasons are not obvious but the sensitivity of phenols to cell lines may have been involved. Selassie *et al*. previously reported a good relationship between log 1/C toward murine L1210 cells and independent variables (log P and BDE or σ^+) for electron-releasing phenols, resulting in the intercept of about 3 [12]. This intercept value was similar to that obtained by our experiments despite the difference of cell lines. By contrast, for HL-60 cells, there was no statistically significant QSAR in terms of log P and η for these eight methoxyphenols. As shown in Table **3**, the cytotoxicity of **1**-**3** and **9** toward HL-60 cells was greater than the corresponding cytotoxicity toward HSG and HGF cells. Conversely, the cytotoxicity of **6**, an isomer of **1**, toward HL-60 cells was smaller than that toward HSG and HGF cells. Compounds **1**-**3** and **9** were highly sensitive to HL 60 cells. The specificity of these phenols for HL-60 cells was resulted in a poor QSAR. Also, for three cell lines, there was no correlation between log 1/C and kinetic parameters (k_{inh}/k_p and *n*) for the eight methoxy-substituted monophenols. This was likely identified as the cause of the large k_{inh}/k_p value of **8** and **4**. The function groups of vanillin, **8** have aldehyde, ether and phenol. Furthermore, biphenols **3**, **4**, **10** and **11** showed no QSAR for three cell lines in independent variables (log P, IP, η, k_{inh}/k_p and *n*), possibly due to the structural complexity of these compounds. Further studies using an appropriate number of congeners will be necessary to clarify the cytotoxic mecanism of biphenols.

Selassie *et al*. [37] have previously reported a QSAR model for the activation of caspase by phenols and subsequent apoptosis in a murine leukemia cell line to determine if caspase-mediated apoptosis plays a major role in the overall cytotoxicity of phenols. A significant multiple linear regression equation for phenols in L1210 leukemia cells using a specific apoptosis assay has been denoted as log $1/I_{50}$ (which was a function [B52, B53, $\pi_{2,4}$] (n=51, r^2=0.89), where I_{50} is the concentration of substituted phenols inducing caspase-mediated apoptosis by 50%. B52 is Verloop's sterimol descriptor, which is a measure of the width of the larger substituents in the *ortho* position. B53 represents the larger substituent in the *meta* position. The hydrophobic parameter $\pi_{2,4}$ represents the sum of the hydrophobicity of substituents in the *para* position and the bulky *ortho* position. The steric parameter B52 accounts for 81% of the variance in the data. This result indicated the strong dependence of caspase-mediated apoptosis on mostly steric parameters, suggesting that the process involves receptor-mediated interaction with caspases or mitochondrial protein and the cytotoxicity for electron-releasing phenols involves minimal apoptosis. We previously investigated caspase-mediated apoptosis induction by 2-*tert*- or 2,6-di-*tert*-butylphenols such as **12**, **14** and **15,** potent artificial antioxidants against HL60 cells, and found it declined in the order **12**>**15**>**14** [38]. **14**, a hindered phenol, showed less potent apoptosis induction than compounds **12** and **15**, which are less hindered phenols. This suggested that apoptosis induction may be related to a steric parameter [37], although it involves many factors such as anti-apoptotic proteins (Bcl-2, BclXL) and apoptotic proteins (caspases, BAX)[1]. In contrast, Loader *et al*. [39] have investigated a dataset of cytotoxicity for *ortho* alkyl-substituted phenols studied previously by the Hansch group [13] using the quantum topological molecular similarity (QTMS) method with electronic descriptors derived from *ab initio* wave functions of geometry-optimized molecules. It was revealed that the steric factor was not important for the phenolic compounds investigated; the cytotoxicity of these phenols was primarily attributable to electronic and radical effects. Further studies will be needed to elucidate the cytotoxicity and apoptotic mechanism of *ortho* alkyl-substituted phenols.

Fig. (**4**) also shows the relationship between log 1/C and LUMO (A), or η (C) for nine 2-*tert*- or 2,6-di-*tert*-butylphenols. For both HSG and HGF cells, a linear relationship for log 1/C and η was observed, with r^2= 0.60. Fig. (**4C**) shows a positive relationship between log 1/C and η. This slope was an inverse version of that for 2-methoxyphenols (Fig. **3C**). For both HSG and HGF cells, a linear relationship between log 1/C and the LUMO-term for these phenols was derived with a positive slope (A), the r^2 value being 0.62 and 0.53 for HSG and HGF cells, respectively. **4** and **8** may have been outliers due to their large k_{inh}/k_p , whereas **5** may have formed its quinone, the *n* value being about 3. By contrast, a significant relationship between log 1/C and k_{inh}/k_p for eight 2-*tert*- or 2,6-*di*-*tert*-butylphenols, with the exception of **18**, was observed for HSG and HFG cells with r^2 values of 0.86 and 0.82, respectively (Fig. **4B**); the r^2 for the compounds as a whole was about 0.6. The appearance of **18** as an outlier from the regression curve (Fig. **4B**) may be attributed to its formation of quinones, and consequently to the prominence of toxicity. These findings suggest that the phenol-induced cytotoxicity may be attributable to the inhibition rate constants of phenolic compounds.

By contrast, there was no relationship between log 1/C and log P for the nine *tert*-butylphenols as a whole (Fig. **4D**). Also, no relationship between the cytotoxicity and HOMO, IP, BDE, χ or ω-term was obtained for these phenols. These findings were in accord with those of a previous report [13]; the cytotoxicity of 2-alkyl or 2,6-dialkyl-4-X-phenols against tumor cells was not related to the BDE or σ^+. These findings demonstrate that the mechanism of cytotoxicity of multisubstituted *tert*-butylphenols differs greatly from that of 2-methoxyphenols. The energy of LUMO is directly related to electron affinity, and there are some reports of LUMO-induced toxicity of aromatic compounds in biological systems. Cronin *et al.* [40] reported toxicity data for 203 substituted aromatic compounds containing a nitro or cyano group, evaluated using the *Tetrahymena pyriformis* population growth impairment assay, and demonstrated a causal relationship between the toxicity and LUMO. Also, LUMO energy has recently been reported to correlate well with the tumor cell cytotoxicity of heterocyclic quinone anti-tumor agents [41]. Together with these findings, the data suggest that quinones and quinone methide formed by phenol enzymatic oxidation may be closely related to the cytotoxicity of *tert*-butylphenols. This generation of cytotoxic quinone- or quinon methide-type oxidants derived

from *ter*t-butylphenols may be interpreted from their *n* value of about 2 (Table **1**). There may be a difference in the mechanism of cytotoxicity between 2-methoxyphenols and 2-*tert*- or 2,6-di-*tert*-butylphenols.

4. CONCLUSION

We have reviewed the results of our previous studies on the basis of published materials and current knowledge. A QSAR model for the cytotoxicity of multisubstituted complex phenols with methoxy and *tert*-butyl substituents against HSG, HL60 and HGF cells demonstrated the mechanistic basis of the phenol-induced cytotoxicity as being dependent on k_{inh}/k_p-related radical reactions. Also, the cytotoxicity for simple X-phenols and 2-methoxyphenols was attributed to the absolute hardness (η)- and ionization potential (IP)-term, whereas that for 2-*tert*- or 2,6-di-*tert*-butylphenols was attributed to the LUMO-term. These parameters provide a deeper insight into the phenol-induced cytotoxicity of multisubstituted phenols. One or a combination of mechanisms; *i.e*. mitochondrial uncoupling, phenoxy radicals, or phenol metabolism to quinone methides and quinones, contribute to the cytotoxicity of phenols towards both tumor cells and primary cells, depending on the chemical structure of the phenol [19]. To elucidate the mechanism responsible for the cytotoxicity and induction of apoptosis induced by phenolic compounds, it will be necessary to evaluate the activity of a wide variety of phenols by using radical parameter and computer-aided models.

5. EXPERIMENTAL

Inhibition Rate Constant

The k_{inh}/k_p and *n* determined by the induction period method have been taken with their activities from previous our papers [10]. Briefly, the experimental resin consisted of MMA and BPO with or without additives. BPO was added at 0.1M and the additives were used with 0.1mM. Approximately 10 μl of the experimental resin (MMA: 9.12-9.25 mg) was loaded into an aluminum sample container and sealed by applying pressure. The container was placed in a DSC (model DSC 3100; Mac Science Co., Tokyo, Japan) kept at 70 °C, and the thermal changes induced by polymerization were recorded for the appropriate periods. The heat due to the polymerization of MMA was 13.0 kcal/mol in these experiments. The conversion of all samples, as calculated from DSC thermograms using the integrated heat evoked by polymerization of MMA. Time-convesion curves break when a phenolic inhibitor is consumed. These breaks are sharp and provide a reliable measure of the induction period of the inhibitors. The induction time (IT) was calculated from the difference between the IT of specimens and that of controls. The initial rates of polymerization in the absence (Rp_{con}) and presence (Rp_{inh}) of an phenolic inhibitor were calculated from the slope of the plots during the initial linear phase of the conversion rate of MMA polymerization (tangent drawn at the early polymerization stage).

Measurements of stoichiometric factor (*n*) and inhibition rate constant (k_{inh}) are as follows:

$$n=\{R_i[IT]\}/[IH] \quad (5)$$

The relative *n* value in Equation 5 can be calculated from the induction time in the presence of inhibitors:

where [IT] is the induction period in the presence of an inhibitor. The number of moles of peroxy radicals trapped by the antioxidant was calculated with respect to 1 mole of inhibitor moiety unit. The R_i value for BPO at 70°C was 2.28×10^{-6} mol $l^{-1}s^{-1}$.

When R_i is constant, i.e. when new chains are started at a constant rate, a steady-state treatment can be applied and the initial rate of polymerization of MMA is given by Equation 6:

$$Rp_{con}=\{k_p[MMA]R_i^{1/2}\}/(2k_t)^{1/2} \quad (6)$$

where MMA represents methyl methacrylate and k_p and k_t are the rate constants for chain propagation and termination, respectively. The Rp_{inh} rates are determined by Equation 7:

$$Rp_{inh}=\{k_p[MMA]\ R_i\}/\{nk_{inh}[IH]\} \quad (7)$$

in which Rp_{inh} is the initial rate of inhibited polymerization, [MMA], *n*, [IH] and k_p are defined above, and k_{inh} is the rate constant for scavenging (inhibition) of MMA radicals by an antioxidant. From Equations 2 and 3, k_{inh}/k_p can be calculated by Equation 8:

$$k_{inh}/k_p=[MMA]/\{[IT]\times[Rp_{inh}]\} \quad (8)$$

The k_{inh}/k_p and *n* were used for this QSAR study as independent variables.

Cytotoxicity

Briefly, HSG, HL-60 and HGF cells were inoculated into 96-well plates at density of 4×10^3 cells/well in 0.1 ml of MEM or a-MEM supplemented with 10%FBS and then cultured at 37°C for 2 days [10]. The medium was replaced by serum-free medium 1h before the assay, except in the case of HL60 cells. A stock solution of 100 mM of test compounds was prepared in dimethyl sulfoxide (DMSO). Each was diluted with DMSO and added to each wells at 1/500volume (the final concentration of each phenolic compound was 10^7 to10^3 M). After incubation of the cultures for 2days, viable cell numbers were assessed by MTT method. Also, HL-60 cells/ml of medium containing 1% FBS were incubated with each compound for 24 h, and the number of viable cells was determined by trypan blue dye exclusion [31]. The relative number of viable cells was expressed as percentage of the number in the experimental well to that in the control well (without test compound) from nine independent determinations. The 50% cytotoxic concentration (C) was determined from dose–response curves. Standard errors were <15%.

Dependent variables (log 1/C) for HSG, HL-60 and HGF cells were used for this QSAR study, respectively.

Theoretic Descriptors

BDE and IP

Using (DFT)/B3LYP, calculated BDE and IP values of phenolic compounds became reasonable. BDE was calculated as follows: First, the lowest and second-lowest-

energy conformers of both the phenol derivatives and their phenoxyl radical species were identified as candidates for geometry optimization using the conformer search procedure of the MMFF (Merck molecular mechanics force fields) calculation. Then, the tentative conformers were optimized in geometry by *ab initio* molecular orbital calculations on a Hartree-Fock model with *ab initio* 6-31G* (HF//6-31G*) for the phenols and UHF//6-31G* level for the phenoxyl radicals *in vacuo* to afford the respective energetic minimized structures. The electronic energy calculation was further proceeded by single point calculation of density functional theory (DFT) using the B3LYP functional on the 6-31G* basis set. Then, BDE = Hr + Hh – Hp, where Hr is the enthalpy of the phenoxyl radical generated by H-abstraction, Hh is the enthalpy of the hydrogen radical, and Hp is the enthalpy of the parent phenol.

The energy values of both the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) energy of the fully optimized phenol derivatives were calculated from the HF//6-31G*level basis set molecular orbital calculation. The absolute value of HOMO energy was adopted as an approximate ionization potential value (IP) according to Koopman's theory [33]. All of the molecular modeling and calculation were with Spartan 04 (Wavefunction Inc., Irvine, CA, USA).

Absolute Hardness (η), Electronegativity (χ) and Electrophilicity (ω)

These values were calculated using Equations 9-11, respectively, as follows:

$$\eta=\{E_{LUMO}-E_{HOMO}\}/2 \quad (9)$$

$$\chi=-\{E_{LUMO}+E_{HOMO}\}/2 \quad (10)$$

$$\omega=\chi^2/2\eta \quad (11)$$

Where E_{LUMO} and E_{HOMO} are the energy levels for the frontier orbitals.

Independent variables (BDE, IP, η, χ, ω) were used for this QSAR study.

CONFLICT OF INTEREST

None declared.

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