On the toxicity of phenols to fast growing cells. A QSAR model for a radical-based toxicity

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The cytotoxicities of a series of simple phenols as well as estrogenic phenols such as octyl and nonyl phenols, Bisphenol A, diethylstilbestrol, estradiol, estriol, equilin and equilenin were studied in a fast growing murine leukemia cell line. The use of calculated homolytic bond dissociation energies (BDE) as the electronic parameter led to the development of a Quantitative Structure–Activity Relationship model with superior results; one which established the importance of relatively low BDE values in enhancing toxicity to rapidly multiplying cells. The correlation equation that emerged is as follows: $\log 1/C = -0.19\text{BDE} + 0.21 \log P + 3.11$. It suggests that toxicity is closely related to mostly homolytic cleavage of the phenolic O–H bond and overall hydrophobicity of the phenol.

Introduction

The phenolic hydroxy group has a wide range of cellular activities that have not been clearly examined. At present, there is intense interest in phytophenols and polyphenols from natural sources that appear to act as antioxidants or radical scavengers.[1] On the other hand, phenols have also been implicated in problems associated with estrogenic toxicity.[2] Estrogens have been known for some time to be carcinogenic and teratogenic: perinatal diethylstilbestrol (DES) exposure induces neoplastic and teratogenic effects in the reproductive tracts of male and female progeny of both humans and experimental animals.[3,4]

The Quantitative Structure–Activity Relationships (QSAR)[5–8] paradigm has been useful in elucidating the mechanisms of chemical–biological interactions in various biomolecules, particularly enzymes, membranes, organelles and cells. It is well established that many radical forming reactions of phenols are correlated by σ^+, the Brown variant of the Hammett electronic parameter.[8] Twenty five examples of radical formation by simple phenols have recently been examined and substituents that increase electron density on the aromatic ring have been shown to favor $^\bullet$H abstraction.[8]

The unusual results of Kavlock *et al.*[6] on the toxicity of simple phenols to rat embryos *in vitro* prompted this study. The maldevelopment of the embryos was found to correlate with σ^+ as was the inhibition of replicative DNA synthesis in Chinese hamster lung cancer cells. Thus, it seemed that fast growing cells with high oxygen demand would produce radicals which could oxidize phenols to toxic phenoxyl moieties. This was in line with the numerous examples of radical abstraction of $^\bullet$H from phenols that also correlated with σ^+ and had a negative rho ($-\rho^+$) value. Accordingly, a series of X-phenols were evaluated for their ability to inhibit fast growing L1210 cells. Using these cytotoxicity results, eqns. (1) and (2) were formulated for electron releasing and electron withdrawing phenols, respectively;[9] C represents the IC_{50}, the concentration of X-phenol that inhibits cell growth by 50%.

$$\log 1/C = -1.58(\pm 0.26)\sigma^+ + 0.21(\pm 0.06) \log P + 3.10(\pm 0.24) \quad (1)$$
$$n = 23, \quad r^2 = 0.898, \quad s = 0.191, \quad q^2 = 0.868$$

$$\log 1/C = 0.62(\pm 0.16) \log P + 2.35(\pm 0.31) \quad (2)$$
$$n = 15, \quad r^2 = 0.845, \quad s = 0.232, \quad q^2 = 0.800$$

A slightly larger, more heterogeneous data set revealed that σ^+ could be replaced with the HOMO–LUMO (L–H) gap parameter to yield eqn. (3).[10]

$$\log 1/C = -2.50\text{L–H gap} + 0.25 \log P + 26.58 \quad (3)$$
$$n = 26, \quad r^2 = 0.903, \quad s = 0.176 \quad q^2 = 0.874$$

For eqn. (3), only phenols that contain electron releasing substituents ($\sigma^+ < 0$) were included. Eqns. (1) and (3), based largely on simple, monosubstituted phenols, were surprisingly found to correlate to more complex 'estrogenic' phenols such as Bisphenol A, DES and estradiol. For these more complex phenols, it was necessary to estimate the values of σ^+ and it was found that eqn. (3) gives a slightly statistically better correlation with essentially the same dependence on log P.

Eqn. (2) differs radically from eqns. (1) and (3) in that no role could be found for electronic properties of these substituents and the coefficient with log P is consistent with that observed for numerous examples of nonspecific toxicity of phenols and other simple compounds. Thus, it appears that phenols containing electron-withdrawing substituents are not prone to radical formation. At pH 7.4 many of these phenols would be considerably ionized and could act as inhibitors of oxidative phosphorylation in mitochondria.[5] The importance of electron releasing substituents and the subsequent magnitude of the rho value in eqn. (1) suggest that an unreactive radical may be involved in the initiation step.[8]

Eqn. (3) poorly predicts the activity of 2-naphthol and equilenin, compounds that contain naphthalene rings. E_{LUMO} is relatively constant for the electron-donor phenols correlated by eqn. (3), making the L–H gap and E_{HOMO} strongly correlated, but this correlation fails for the naphthalene derivatives. We have found that calculated OH homolytic bond dissociation energies (BDE) can serve as a parameter that works well for both phenol and naphthol derivatives, and is superior to σ^+. We now consider the use of BDEs for *ortho* substituted phenols as well.

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Results

A. Cytotoxicity of 2-X-phenols

The *ortho* substituted phenols were first examined as an independent group with the expectation that a steric effect would be present. However, no role for steric effects could be ascertained with the usual steric parameters: Taft's steric parameter (E_s), molar refraction (MR) and Verloop's width parameters (B1 and B5). As in our first report[9] once again it was found that electron withdrawing substituents (NO_2, CN, CF_3, I, Br) did not fit in the same equation with electron releasing substituents. On excluding these electron withdrawing X-phenols, eqns. (4) and (5) were formulated, using σ^+ and BDE

$$\log 1/C = -1.50(\pm 0.45)\sigma^+ + 3.15(\pm 0.28) \quad (4)$$
$$n = 14, \quad r^2 = 0.816, \quad s = 0.323, \quad q^2 = 0.762$$

$$\log 1/C = -0.17(\pm 0.03)\text{BDE} + 3.18(\pm 0.16) \quad (5)$$
$$n = 14, \quad r^2 = 0.936, \quad s = 0.191, \quad q^2 = 0.915$$

values, respectively, while the correlation of cytotoxicities of these *ortho* substituted phenols with the L–H gap parameter was poor.

An assumption was made that $\sigma_o^+ = \sigma_p^+$ since many values are not available for σ_o^+. A correlation of 19 available σ_o^+ values with σ_p^+ reveals the following relationship:

$$\sigma_o^+ = 0.94(\pm 0.09)\sigma_p^+ + 0.04(\pm 0.03) \quad (6)$$
$$n = 14, \quad r^2 = 0.968, \quad s = 0.052, \quad q^2 = 0.962$$

Rho$^+$ is near 1 and the intercept is near 0. Adding an additional term to account for the added field/inductive effect of *ortho* substituents resulted in a very slight improvement that was lost when the larger set was correlated.

Rho$^+$ and the intercept of eqn. (4) are essentially the same as in eqn. (1). Also the intercepts of eqns. (4), (5) and (1) are the same, hence there is a consistency between the *ortho* substituted phenols and the other isomeric phenols. However, there is a very significant difference in that no hydrophobic term appears in either eqns. (4) or (5). An analysis of the correlation between the steric properties of *ortho* X-substituents as represented by Verloop's B1 parameter and the hydrophobicity of the *ortho* X-phenols, as represented by log P values, revealed that there was no negative dependence between these variables (r^2 = 0.336). Thus, in eqns. (4) and (5) a positive, hydrophobic effect is not being nullified by a deleterious steric effect in the *ortho* position. The fact that no steric effect could be established suggests that a simple radical is involved in the radical formation step and not an enzyme. The superoxide radical appears to be a potential candidate, although its exact role in cellular processes is difficult to delineate.

The lack of hydrophobic terms in eqns. (4) and (5), and their prominence in eqns. (1)–(3), suggests that the mediation of toxic action requires a specific receptor that can interact selectively with *meta* and *para* substituents. This must feature an extensive binding area since 4-octyl- and 4-nonylphenols, as well as DES (it is larger) are fitted well by the same model. Use of a parabolic or bilinear term for log P does not improve the correlation. The 2-ureido phenol is not well predicted by eqn. (5) and lack of a σ^+ value for it precludes its inclusion in eqn. (4). 2-Fluoro and 2-chloro are both fitted well by eqns. (4) and (5), while 2-bromo and 2-iodo are not, which would suggest a slight steric effect in the *ortho* position. The following phenols (all but one [2-ureido] having electron withdrawing substituents) were omitted in the formulation of eqns. (4) and (5): 2-CN, 2-NO_2, 2-I, 2-Br, 2-CF_3, 2-$NHCONH_2$.

B. Cytotoxicity of all X-phenols

Using the data in Table 1 containing 69 tested phenols, we have

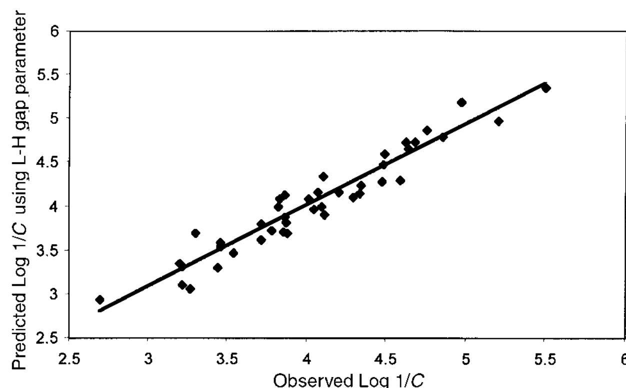

Fig. 1 Plot of observed *versus* predicted cytotoxicities (log 1/C) using L–H gap parameter of X-phenols.

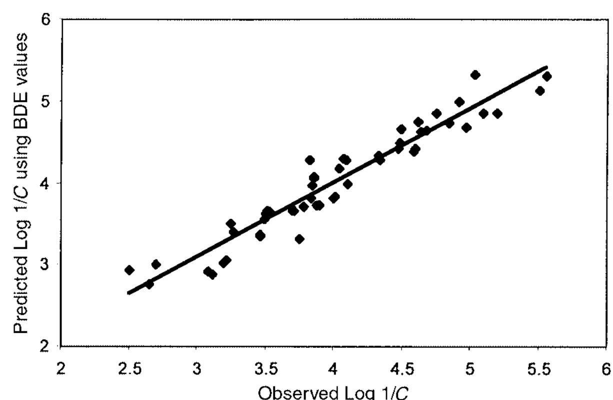

Fig. 2 Plot of observed *versus* predicted cytotoxicities (log 1/C) using BDE values of X-phenols.

derived eqns. (7)–(9) for the inhibition of growth in murine

$$\log 1/C = -1.35(\pm 0.15)\sigma^+ + 0.18(\pm 0.04)\log P + 3.31(\pm 0.11) \quad (7)$$
$$n = 51, \quad r^2 = 0.895, \quad s = 0.227, \quad q^2 = 0.882$$

$$\log 1/C = -0.19(\pm 0.02)\text{BDE} + 0.21(\pm 0.03)\log P + 3.11(\pm 0.10) \quad (8)$$
$$n = 52, \quad r^2 = 0.920, \quad s = 0.202, \quad q^2 = 0.909$$

$$\log 1/C = -2.64(\pm 0.34)\text{L–H gap} + 0.29(\pm 0.03)\log P + 27.76(\pm 3.11) \quad (9)$$
$$n = 42, \quad r^2 = 0.925, \quad s = 0.167, \quad q^2 = 0.912$$

leukemia cells by a comprehensive set of phenols. Although the concentrations that are utilized in this study to achieve a dramatic endpoint such as 50% inhibition of growth in 48 h may seem high, it is reasonable to assume that lower concentrations would induce perturbations in the critical initial stages of embryo development at especially sensitive sites, *e.g.*, germ cells. Note that three of the four components of the drug Premarin (estradiol, equilin and equilenin) are fitted well by eqns. (7) and (8) while the fourth, estrone, was too insoluble to test. Once again, phenols with electron withdrawing substituents were excluded from the analyses.

The following 18 substituted phenols have been omitted in this analysis: 2-OCH_3, 2-CN, 2-NO_2, 2-I, 2-SCH_3, 2-CH_2OH, 2-OC_2H_5, 2-CF_3, 3-NO_2, 3-$NHCOCH_3$, 3-Cl, 3-Br, 3-NH_2, 4-NO_2, 4-Cl, 4-$NHCOCH_3$, 4-CN and 4-Br. For all *ortho* substituted phenols including the 2-OH, 4-CH_3 analog, log P values of 0 have been assigned. The log P value for catechol is 0.88. The 3-NH_2 and 3-$N(CH_3)_2$ are both more active than predicted by eqn. (7). Reasons for these anomalies are not obvious but may involve ring oxidation. The parameters of eqn. (7)

Table 1 Cytotoxicities and parameters used to derive eqns. (7) through (9)

	Substituent	Obs'd Log 1/*C*	Calc'd Log 1/*C* eqn. (7)	Calc'd Log 1/*C* eqn. (8)	Calc'd Log 1/*C* eqn. (9)	σ^+	Log *P*[g]	L–H gap	BDE[i]
1	H	3.27	3.57	3.41	3.06	0	1.47	−9.512	0
2	4-OCH_3	4.48	4.60	4.49	4.48	−0.78	1.34	−8.961	−6.01
3	4-OC_2H_5	4.64	4.72	4.64	4.65	−0.81[b]	1.81	−8.961	−6.16
4	4-OC_3H_7	4.85	4.84	4.74	4.80	−0.83	2.33	−8.947	−6.23
5	4-OC_4H_9	5.20	4.91	4.86	4.97	−0.81[b]	2.90	−8.947	−6.27
6	4-OC_6H_{13}	5.50	5.15	5.14	5.35	−0.81[b]	4.22	−8.947	−6.30
7	4-OC_6H_5	4.97	4.57	4.68	5.19	−0.50	3.35	−8.913	−4.55
8	4-CH_3	3.85	4.07	3.97	3.71	−0.31	1.94	−9.316	−2.22
9	4-C_2H_5	3.86	4.15	4.06	3.88	−0.30	2.47	−9.312	−1.90
10	4-C_3H_7	4.04	4.23	4.19	3.97	−0.29	3.00	−9.335	−2.01
11	4-C_4H_9	4.33	4.34	4.33	4.15	−0.29[c]	3.64	−9.336	−2.08
12	4-C_5H_{11}	4.47	4.42	4.42	4.28	−0.29[c]	4.06	−9.336	−2.13
13	4-C_7H_{15}	4.49	4.61	4.66	4.59	−0.29[c]	5.15	−9.336	−2.17
14	4-C_8H_{17}	4.62	4.70	4.76	4.73	−0.29[c]	5.68	−9.342	−2.17
15	4-C_9H_{19}	4.75	4.80	4.86	4.87	−0.29[c]	6.21	−9.347	−2.17
16	4-$C(CH_3)_3$	4.09	4.24	4.29	3.99	−0.26	3.31	−9.361	−1.54
17	4-$CONH_2$	2.48	2.88	2.94[a]	3.39[a]	0.36	0.33	−9.262	1.39
18	4-NO_2	3.45	2.58[a]	2.66[a]	2.85[a]	0.79	1.91	−9.637	4.66
19	4-I	3.86	3.64	4.08	4.13	0.14	2.91	−9.266	−0.65
20	4-SO_2NH_2	2.50	2.51	2.94	3.08[a]	0.60	0.06	−9.347	1.87
21	4-CHO	3.08	2.92	2.91	4.25[a]	0.47	1.35	−9.047	2.49
22	4-F	3.83	3.72	3.82	4.09	−0.07	1.77	−9.152	−1.99
23	4-NH_2	5.09	5.07	4.86	4.76[a]	−1.30	0.04	−8.709	−9.25
24	4-OH	4.59	4.65	4.39	4.30	−0.92	0.59	−8.945	−6.04
25	4-Cl	4.29	3.58[a]	3.90[a]	4.10	0.11	2.39	−9.219	−1.04
26	4-Br	4.20	3.57[a]	3.95[a]	4.16	0.15	2.59	−9.218	−0.42
27	4-CN	3.44	2.70[a]	3.05[a]	4.19[a]	0.66	1.60	−9.096	2.36
28	4-$NHCOCH_3$	3.73	4.21[a]	4.00[a]	4.54[a]	−0.60	0.51	−8.845	−4.39
29	3-NO_2	3.48	2.71[a]	2.94[a]	5.10[a]	0.71	2.00	−8.799	2.85
30	3-$NHCOCH_3$	2.65	3.16[a]	2.76	4.09[a]	0.21	0.73	−9.039	2.42
31	3-Cl	3.87	3.25[a]	3.37[a]	3.81	0.37	2.50	−9.339	1.32
32	3-$C(CH_3)_3$	3.88	3.98	3.74	3.69	−0.10	3.05	−9.445	0.12
33	3-CH_3	3.54	3.75	3.65	3.47	−0.07	1.96	−9.410	−0.25
34	3-OCH_3	3.71	3.43	3.67	3.80	0.12	1.58	−9.243	−0.77
35	3-$N(CH_3)_2$	4.11	3.80	3.71[a]	4.53[a]	−0.16	1.56	−8.964	−0.60
36	3-C_2H_5	3.71	3.83	3.66	3.61	−0.07	2.40	−9.404	0.13
37	3-Br	3.82	3.25[a]	3.46[a]	3.99	0.39	2.63	−9.286	1.48
38	3-CN	3.11	2.86	2.88	4.30[a]	0.56	1.70	−9.068	2.75
39	3-F	3.46	3.19	3.35	3.59	0.34	1.93	−9.360	1.13
40	3-OH	3.46	3.29	3.36	3.54	0.12	0.80	−9.257	−0.18
41	3-NH_2	4.11	3.56[a]	3.42[a]	3.90	−0.16	0.21	−9.053	−0.51
42	2-CH_3	3.52	3.73	3.66	3.00[a]	−0.31	0	−9.368	−2.25
43	2-Cl	3.22	3.16	3.06	3.31	0.11	0	−9.254	1.00
44	2-F	3.20	3.40	3.03	3.35	−0.07	0	−9.240	0.47
45	2-OCH_3	3.78	4.36[a]	3.72	3.72	−0.78	0	−9.100	−2.70
46	2-C_2H_5	3.75	3.71	3.31	3.01[a]	−0.30	0	−9.369	−1.39
47	2-OH	4.92	4.55	4.99	3.50[a]	−0.92	0	−9.182	−10.11
48	2-NH_2	5.16	5.06	5.30	3.17[a]	−1.30	0	−9.309	−11.61
49	2-CN	3.30	2.42[a]	2.49[a]	3.69	0.66	0	−9.110	3.44
50	2-NO_2	3.34	2.25[a]	1.06[a]	4.14[a]	0.79	0	−8.940	13.19
51	2-Br	3.44	3.11	2.95[a]	3.30	0.15	0	−9.257	1.49
52	2-$C(CH_3)_3$	4.00	3.66	3.81	2.93[a]	−0.26	0	−9.397	3.19
53	2-I	3.95	3.12[a]	3.18[a]	2.44[a]	0.14	0	−9.583	0.10
54	2-SCH_3	3.70	4.12[a]	3.67	5.00[a]	−0.60	0	−8.612	−1.26
55	2-$CH(CH_3)_2$	3.50	3.69	3.63	2.91[a]	−0.28	0	−9.407	−2.56
56	2-$CH_2CH(CH_3)_2$	3.90	3.71	3.73	2.99[a]	−0.30	0	−9.376	−2.24
57	2-CH_2OH	2.70	3.36[a]	3.00	2.94	−0.04	0	−9.395	0.06
58	2-C_3H_7	3.49	3.69	3.56	2.96[a]	−0.28	0	−9.385	−1.34
59	2-CF_3	3.22	2.49[a]	2.67[a]	3.11	0.61	0	−8.694	3.79
60	2-OC_2H_5	3.25	4.40[a]	3.51	—	−0.81	0	—	−2.20
61	2-$NHCONH_2$	3.50	—	4.95[a]	6.53[a]	—	0	−8.034	−9.39
62	2-OH, 4-CH_3	5.03	4.97	5.33	3.83[a]	−1.23	0	−9.057	−11.57
63	Bisphenol A	4.07	4.28	4.30	4.16	−0.29	3.32	−9.301	−1.88
64	DES	4.68	4.42	4.65	4.74	−0.16[d]	5.07	−9.273	−2.42
65	β-Estradiol	4.34	4.49	4.28	4.23	−0.35[e]	4.01	−9.350	−1.47
66	Equilin	4.10	4.29	3.99	4.34	−0.35[e]	2.90	−9.186	−0.94
67	Estriol	4.01	4.21	3.84	4.08	−0.35[e]	2.45	−9.232	−1.16
68	Equilenin	4.60	4.43	4.43	7.47[a]	−0.42[f]	3.12	−8.025	−2.88
69	2-Naphthol	3.82	4.16	4.28	6.82[a]	−0.28	2.70	−8.225	−2.73
70	Estrone[a]	—[h]	4.33[a]	3.99[a]	[a]	−0.35	3.13	−9.230	−1.01

[a] Data points omitted in correlation analysis. [b] For these substituents σ^+ assumed to be the same as for 4-OC_2H_5. [c] For these substituents σ^+ assumed to be the same as for 4-C_3H_7. [d] For DES σ^+ for –CH=CH_2 was used. [e] For estradiol, estriol and equilin the sum σ^+ of –$CH(CH_3)_2$ (−0.28) and σ^+ for CH_3 (−0.17) were used. [f] For the naphthalene ring σ^+ of –CH=CHCH=CH– (−0.28) and −0.14 for the two carbon attachments to the naphthalene ring (equilenin). [g] Experimental values except for large R and OR that were calculated *C* Log *P* values. [h] Not tested due to solubility problems. [i] BDE values are defined as the reaction energy (in kcal mol^{-1}) for X–PhOH + PhO$^•$→X–PhO$^•$ + PhOH. BDE values are not corrected for changes in ZPE.

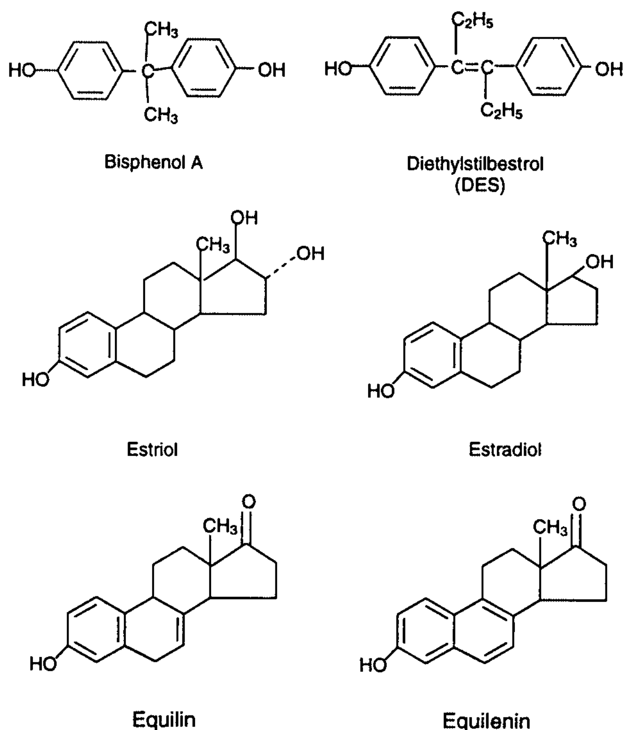

Fig. 3 Structures of synthetic and naturally occurring complex phenols.

are in reasonable agreement with eqn. (1). A plot of the predicted *versus* experimentally determined values of the biological activity in Fig. 1, shows good correspondence between the two variables.

The use of molecular orbital indices (charges, energies or multiple moments) have been well documented.[11] They are particularly useful when accurate and precise values for σ^+ are unavailable as in the case of complex or heterocyclic systems. Eqn. (8) illustrates the use of calculated homolytic bond dissociation energies for this data set.

Seventeen compounds that were omitted included: 2-CN, 2-NO_2, 2-I, 2-Br, 2-$NHCONH_2$, 2-CF_3, 3-NO_2, 3-Cl, 3-Br, 3-NH_2, 3-$N(CH_3)_2$, 4-NO_2, 4-Cl, 4-$NHCOCH_3$, 4-CN, 4-Br and 4-$CONH_2$. The results are similar to eqn. (7), but the correlation is significantly better using BDE. Also the agreement between the intercepts and the log *P* terms in eqns. (7), (8) and (1) is excellent. BDE appears to be the superior parameter. A plot of the predicted values *versus* experimentally determined values for log 1/*C* reveals no deviations from linearity. See Fig. 2. This assessment is pertinent because BDE directly measures the thermodynamics of phenoxyl radical formation as embodied in the following step: $X–C_6H_4OH + C_6H_4O^{\bullet} \rightarrow X–C_6H_4O^{\bullet} + C_6H_5OH$.

Using the L–H gap parameter, eqn. (9) was obtained, for the comprehensive set of phenols.

The 27 outliers are listed as follows: 2-$NHCONH_2$, 2-NH_2, 2-SCH_3, 2-I, 2-OH, 2-OH-4-CH_3, 2-$CH(CH_3)_2$, 2-CH_3, 2-C_2H_5, 2-NO_2, 2-$CH_2CH(CH_3)_2$, 2-$C(CH_3)_3$, 2-C_3H_7, 2-$NHCONH_2$, 3-NO_2, 3-$NHCOCH_3$, 3-CN, 3-$N(CH_3)_2$, 4-CHO, 4-$CONH_2$, 4-SO_2NH_2, 4-$NHCOCH_3$, 4-CN, 4-NO_2, 4-NH_2, 2-naphthol and equilenin. The major difference between eqn. (9) and eqns. (7) and (8) is the poor fit of the *ortho* X-phenols, 2-naphthol and equilenin using the L–H gap parameter. The coefficient with log *P* is similar to that in the other QSAR.

Discussion

The new varied group of *ortho* substituents once again confirms earlier findings that strong electron withdrawing substituents block the σ^+ type of toxicity. However, it is not clear why 2-F and 2-Cl are fitted well, while 2-Br and 2-I are under predicted. There may be a subtle steric effect that is not easily discernible. Although the *tert*-butyl derivative is not omitted from the analysis, it is also mispredicted. The parent phenol (2-H) is fitted well by eqns. (1), (7), (8), and (9), as well as eqn. (2). It appears to mark the boundary line between nonspecific toxicity and radical toxicity. The lack of a hydrophobic effect for *ortho* substituents and the existence of one for *meta* and *para* substituents, suggests that receptor binding is involved most likely not in the initial radical-forming step, but in the next cytotoxic binding step. It could also indicate that *ortho* substituents bind in an unrestricted region or in an alternate binding mode, such that they do not contact the receptor but extend into aqueous space.

In the initial stage of this study, it was not at all anticipated that complex phenols such as Bisphenol A, DES, estradiol, estriol, equilin and equilenin (Fig. 3) would be fitted by simple models, as delineated in Models 7 and 8. Two prominent environmental estrogens, 4-octylphenol and 4-nonylphenol, are also well predicted by these models. The excellent fit of these bulky, hormonally-active compounds suggests that the receptor must be extensive, hydrophobic and bulk tolerant. Since the components of Premarin and even simple phenols such as 4-methoxyphenol and 4-methylcatechol (both having $\sigma^+ < 0$) are known to be carcinogenic,[12,13] while DES and nonylphenol are teratogenic,[3] DNA damage may be involved in these phenomena although teratogenicity may well be a developmental abnormality of hormonal origin. Radical mediated oxidation of DNA has been extensively discussed.[14] If DNA damage is the underlying reason for the cytotoxicity in cancer cells, then the binding site must have an unusual configuration to accommodate the hydrophobic region revealed by the QSAR models. Attempts to explain estrogen toxicity have focused on the estrogen receptor (ER) as a possible source of interaction. An X-ray crystallography study of the ligand-binding domain of ER has established that it has an extensive hydrophobic binding site.[15,16] Recent analysis of the binding of various ligands to the ER in terms of QSAR reveals little evidence for the involvement of electronic effects in binding.[7] In this case, the estrogen receptor-binding step is ruled out since murine leukemia cells are not ER positive. This observation eliminates radical generation at the ligand-binding domain of the ER, but does not preclude radical binding after generation of the stable phenoxyl radical in ER positive cells. Thus, there may be more than one mechanism for various types of toxicity in the whole animal (*e.g.*, estrogenicity, carcinogenicity, mutagenicity and aging) and this cellular model focuses on only two: cancer and estrogenic effects. It has implications beyond environmental toxicology, particularly in the development of new drugs where one would want to be cautious in developing molecules having a phenolic OH with a low BDE.

Experimental

Boiling points were measured under reduced pressure (10 mm) and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 400 NMR spectrometer with TMS as the internal standard; chemical shifts are given in δ (ppm) scale. IR spectra were recorded on a Perkin Elmer 1600 series FTIR and only principal, sharply defined IR peaks are reported. Mass spectra were taken on a Hewlett Packard GC/MS system HP 6890 series with mass selective detector.

All the phenols are commercially available compounds except the following: 2-ureidophenol, 2-isobutylphenol and 2-methylmercaptophenol. 2-Ureidophenol (2-hydroxyphenylurea) was prepared according to literature reports.[17] The other two phenols, *i.e.*, 2-isobutylphenol and 2-methylmercaptophenol, were prepared as follows.

Synthesis of 2-isobutylphenol

2-Isobutylphenol was synthesized by the rearrangement of methylallylphenyl ether to the corresponding (methylallyl)-phenol, followed by catalytic reduction as shown in the literature.[18] The starting material, methylallyl phenyl ether, was prepared by the reaction of phenol with methylallyl chloride. Methylallyl phenyl ether: (65%) bp 65–66 °C (10 mm) [lit.,[18] 70 °C (8 mm)]; δ_H (400 MHz; $CDCl_3$): 1.92 (s, 3H, CH_3), 4.5 (s, 2H, $-CH_2-$), 5.03 (s, 1H, $=CH_2$), 5.18 (s, 1H, $=CH_2$), 7.02 (m, 3H, Ph), 7.38 (m, 2H, Ph); *m/z* 148(M^+, 26%) 133(42), 94(30), 55(100). 2-(Methylallyl)phenol: (70%) bp 92 °C (10 mm) [lit.,[18] 95 °C (9 mm)]; δ_H (400 MHz, $CDCl_3$): 1.72 (s, 3H, CH_3), 3.55 (s, 2H, $-CH_2-$), 4.80 (s, 1H, $=CH_2$), 4.88 (s, 1H, $=CH_2$), 5.5 (s, 1H, –OH), 6.82 (m, 2H, Ph), 7.1 (m, 2H, Ph); ν_{max}/cm^{-1} 3638 (OH), *m/z* 149(M^+ + 1, 9%), 148 (M^+, 85), 133(100), 105(70), 91(22), 77(52). 2-Isobutylphenol: (30%) bp 57 °C (10 mm) [lit.,[18] 86 °C (6 mm)]; δ_H (400 MHz; $CDCl_3$): 0.93 (d, *J* 7.5, 6H, $2 \times CH_3$), 1.94 (m,1H, –CH<), 2.48 (d, *J* 6.5, 2H, $-CH_2-$), 5.12 (s, 1H, –OH), 6.74 (d, *J* 8, 1H, Ph), 6.85 (t, *J* 7, 1H, Ph), 7.06 (t, *J* 7, 2H, Ph); δ_C (400 MHz, $CDCl_3$): 23.067 (s, $2 \times CH_3$), 29.272 (s, –CH<), 39.702 (s, $-CH_2$), 115.715 (s, C6), 120.981 (s, C4), 127.514 (s, C5), 128.042 (s, C3), 131.712 (s, C2), 154.046 (s, C1); ν_{max}/cm^{-1} 3637.6 (OH), *m/z* 150(M^+, 22%), 107(100), 77(14).

Synthesis of 2-methylmercaptophenol

2-Methylmercapto derivative of the phenol was synthesized from 2-methylmercaptoaniline by a diazo reaction.[19] (61.5%) bp 96–97 °C (10 mm) [lit.,[20] 105 °C (22 mm)]; δ_H (400 MHz; $CDCl_3$): 2.35 (s, 3H, $-SCH_3$), 6.73 (s, 1H, –OH), 6.91 (t, *J* 7.5, 1H, Ph), 7.02 (d, *J* 8.25, 1H, Ph), 7.27 (t, *J* 7.5, 1H, Ph), 7.53 (d, *J* 8.25, 1H, Ph); δ_C (400 MHz, $CDCl_3$): 20.197 (s, $-SCH_3$), 115.192 (s, C6), 121.415 (s, C4), 131.072 (s, C2), 134.792 (s, C5), 135.480 (s, C3), 156.643 (s, C1); ν_{max}/cm^{-1} 3638.3 (OH), 1300(SCH_3), *m/z* 142(M^+ + 2, 5%), 141(M^+ + 1, 9), 140(M^+, 100), 125(47), 97(55).

Cytotoxicity studies

The IC_{50} values in the L1210 cell line were determined according to previously published protocols.[9] The IC_{50} is defined as the concentration of X-phenol that inhibits growth by 50%.

QSAR Analysis

The C-QSAR suite of programs was used in the derivation of the various models.[21] *P* represents the octanol–water partition coefficient of the phenol. Most of the log *P* values were experimentally determined while in a few cases, they were calculated using the CLOGP program. The agreement between measured (*M* log *P*) and calculated partition coefficients (*C* log *P*) is excellent: $M \log P = 0.96\ C \log P + 0.02$ ($n = 40$, $r^2 = 0.973$, $s = 0.183$). Sigma-plus (σ^+) is the Brown variation of the Hammett electronic substituent constant. σ^+ represents σ^+ for *ortho* substituents while σ_p^+ represents σ^+ in the *para* position. The excellent correspondence between σ_o^+ and σ_p^+, led to the usage of σ_p^+ for all the phenols in this study. In all equations, *n* represents the number of data points, *r* is the correlation coefficient, and *s* is the standard deviation of the regression equation while q^2 comprises the cross validated r^2. BDE values were calculated as follows: BDE values are based on B3LYP/6-31G**//AM1 energies[22–24] and were obtained using Jaguar 3.0[25] (default numerical grids) and Spartan 5.0.[26] Effective core potentials were used for bromine- and iodine-substituted molecules (LACVP**).[27] BDE values for diphenols refer to the lower-energy phenoxyl radical. The L–H gap was calculated according to previously delineated procedures.[10]

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