Molecular orbital parameters and comparative QSAR in the analysis of phenol toxicity to leukemia cells

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Evidence is presented for a new type of phenol toxicity that is correlated with the parameter σ^+ or HOMO–LUMO gap calculations. The correlation equation: $\log 1/C = -1.58\sigma^+ + 0.21\log P + 3.10$ is based mostly on mono-substituted phenols, but more complex estrogenic phenols such as bisphenol A, diethylstilbestrol and estradiol are also well fit. Comparison with radical QSARs suggests that a radical mechanism may be involved.

Introduction

Since the advent of Quantitative Structure–Activity Relationships (QSARs) for biological systems[1] Hammett parameters have normally been used to account for the electronic effect of substituents on biological potency of sets of congeneric chemicals producing a standard response. They have the great advantage with our present system for regression analysis[2] in that they can be automatically loaded and analyzed. However, when more than simple substituent changes are involved the data are not suitable for QSAR analysis with Hammett parameters. One then has to resort to the use of molecular orbital calculations that have been valuable in formulating QSARs for a variety of studies of mutagenicity and carcinogenicity.[3] In the present report we compare their degree of importance with the Hammett parameter σ^+ formulated by H. C. Brown. Although Brown and his colleagues formulated σ^+ for aromatic electrophilic substitution reactions, it has been found by many others to be useful in correlating many kinds of radical reactions.[4]

Recent analysis indicates that the maldevelopment of rat embryos *in vitro* by X-phenols is correlated by σ^+ with slopes of -0.60 for four different types of end points.[5] Maldevelopment end points are quite difficult to quantify. A search for a simpler system of rapidly growing cells for comparison, led to the selection of L1210 leukemia cells. The molar concentrations (C) of monosubstituted *meta*- and *para*-phenols that induced 50% inhibition of growth in 48 h, were used as biological end points ($\log 1/C$). From these results the following two QSARs were derived.[6] The phenols were classified into two groups depending on their electron releasing (negative σ^+ values) or electron attracting (positive σ^+ value) attributes: (i) inhibition of growth by electron releasing substituents on phenols, eqn. (1), where

$$\log 1/C = -1.58(\pm 0.26)\sigma^+ + 0.21(\pm 0.06)\log P + 3.10(\pm 0.24) \quad (1)$$

$n = 23$, $r^2 = 0.898$, $s = 0.191$ and $q^2 = 0.868$; and (ii) inhibition of growth by electron attracting substituents on phenols, eqn. (2), where $n = 15$, $r^2 = 0.845$, $s = 0.232$ and $q^2 = 0.800$.

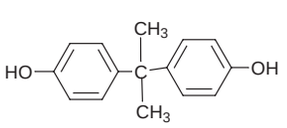
$$\log 1/C = 0.62(\pm 0.16)\log P + 2.35(\pm 0.31) \quad (2)$$

Log P represents the partition coefficient of each phenol, s is the standard deviation of the equation and q^2 is the cross-validated r^2.

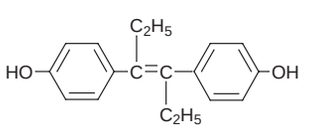
Our current database of 12 300 QSAR includes 7100 Hammett type equations from mechanistic studies in physical organic chemistry and 5200 equations from biological chemistry. An equation similar in scope to eqn. (1) for phenol toxicity is rare, except the aforementioned toxicity to rat embryos. However, there are a number of examples of enzymatic oxidation of phenols and other compounds that have $-\rho^+$ terms.[4] In the case of radical abstraction of H$^\bullet$ from phenols by a variety of simple chemical radicals we have found 25 examples of which 23 are best correlated by σ^+ with $-\rho^+$ values.[4] Hence, we presently believe that eqn. (1) is correlating a radical process in which the phenol is first converted to a phenoxyl radical in a rate limiting process. The radical then migrates and reacts with a sensitive site within the cell.

Eqn. (2) was an unusual finding. Adding electronic terms (σ, σ^+ or σ^-) to it did not improve the correlation. Eqn. (2) indicates that a weak radical is responsible for generating the phenoxyl radicals and that the process needs the assistance of electron releasing substituents and in fact is prohibited by electron attracting substituents. One such species from the cellular oxidation processes might be the rather weak oxidizing moiety HOO$^\bullet$. Eqn. (2) is a typical example of nonspecific hydrophobic toxicity. Hundreds of such simple equations are known.[3]

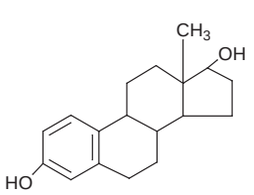
Our initial findings that the environmental estrogens 4-octylphenol and 4-nonylphenol are very well fit by eqn. (1), suggested that testing other estrogens might be of interest.



I
Bisphenol A



II
Diethylstilbesterol



III
Estradiol

Accordingly, we tested Bisphenol A (**I**), diethylstilbestrol (DES) (**II**) and estradiol (**III**); all of which are well fit by eqn. (1) (see Table 1). For these three estrogens σ^+ values are not available

Table 1 Parameters used to derive eqns. (1), (3) and (4)

	Substituent	Obs. log 1/*C*	Calc. log 1/*C* [eqn. (4)]	Calc. log 1/*C* [eqn. (3)]	σ^+	log *P*	E_{HOMO}	E_{LUMO}	HOMO–LUMO gap
1	4-OMe	4.48	4.60	4.54	−0.78	1.34	−8.648	0.313	8.961
2	4-OC_2H_5	4.64	4.74	4.70	−0.81	1.81	−8.609	0.337	8.946
3	4-OC_3H_7	4.85	4.89	4.83	−0.83	2.33	−8.608	0.339	8.947
4	4-OC_4H_9	5.20	4.98	4.97	−0.81	2.90	−8.608	0.339	8.947
5	4-OC_6H_{13}	5.50	5.26	5.30	−0.81	4.22	−8.608	0.339	8.947
6	H	3.27	3.40	3.20	0	1.47	−9.114	0.398	9.512
7	4-F	3.83	3.57	4.17	−0.07	1.77	−9.093	0.059	9.152
8	4-NH_2	5.09	5.14	4.85	−1.30	0.04	−8.270	0.439	8.709
9	4-OH	4.59	4.66	4.40	−0.92	0.59	−8.725	0.220	8.945
10	4-Me	3.85	3.99	3.81	−0.31	1.94	−8.88	0.435	9.315
11	4-C_2H_5	3.86	4.08	3.95	−0.30	2.47	−8.856	0.456	9.312
12	4-OC_6H_5	4.97	4.58	5.17	−0.50	3.35	−8.797	0.116	8.913
13	Bisphenol A	4.07	4.25	4.19	−0.29[a]	3.32	−8.949	0.352	9.301
14	4-$C(Me)_3$	4.09	4.20	4.04	−0.26	3.31	−8.898	0.463	9.361
15	3-$C(Me)_3$	3.88	3.89	3.76	−0.10	3.05	−9.014	0.431	9.445
16	3-Me	3.54	3.61	3.58	−0.07	1.96	−9.013	0.397	9.41
17	3-NMe_2	4.11	3.67	4.59	−0.16	1.56	−8.492	0.472	8.964
18	3-C_2H_5	3.71	3.71	3.70	−0.07	2.40	−8.983	0.421	9.404
19	4-C_3H_7	4.04	4.18	4.03	−0.29	3.00	−8.902	0.433	9.335
20	4-C_4H_9	4.33	4.32	4.19	−0.29	3.64	−8.903	0.433	9.336
21	4-C_5H_{11}	4.47	4.41	4.29	−0.29	4.06	−8.903	0.433	9.336
22	4-C_8H_{17}	4.62	4.75	4.68	−0.29	5.68	−8.912	0.430	9.342
23	4-C_7H_{15}	4.49	4.64	4.56	−0.29	5.15	−8.903	0.433	9.336
24	4-C_9H_{19}	4.75	4.86	4.80	−0.29	6.21	−8.913	0.434	9.347
25	Estradiol	4.34	4.49	4.24	−0.35[a]	4.01	−8.978	0.372	9.350
26	DES	4.68	4.41	4.70	−0.16[a]	5.07	−9.017	0.256	9.273

[a] Estimated values.

so that they were not used to derive eqn. (1). However, using σ^+ for -$CH(CH_3)_2$ for bisphenol A, σ^+ for CH=CH_2 for DES and σ^+ of $CH(CH_3)_2$ and σ_m for CH_3 for estradiol provided a reasonable correlation ($r^2 = 0.883$). In order to gauge whether estimates of σ^+ for compounds **I**, **II** and **III** are ideal, molecular orbital indices for the series of phenols were calculated and a molecular orbital approach to the formulation of a mathematical model correlating inhibitory activity with physicochemical attributes was formulated.

Results and discussion

From the data in Table 1, eqns. (3) and (4) were formulated for the whole set, where for eqn. (3) $n = 26$, $r^2 = 0.903$, $s = 0.176$

$$\log 1/C = 0.25(\pm 0.05)\log P - 2.50(\pm 0.37)\text{L–H gap} + 26.58(\pm 3.3) \quad (3)$$

$$\log 1/C = 0.21(\pm 0.06)\log P - 1.57(\pm 0.26)\sigma^+ + 3.09(\pm 0.24) \quad (4)$$

and $q^2 = 0.874$, and for eqn. (4) $n = 26$, $r^2 = 0.883$, $s = 0.190$ and $q^2 = 0.853$. The quality of correlation of QSARs (3) and (4) is quite similar despite the different approaches to estimating the electronic effects of the substituents. The coefficients with the hydrophobic terms are in good agreement. Eqn. (3) indicates that a smaller LUMO–HOMO gap (L–H gap), enhances cytotoxicity of the X-phenols; *i.e.* the more easily an electron is promoted to an unoccupied orbital the more toxic the compounds. In recent years there has been an increasing interest in using the L–H gap in mechanistic analysis of organic reactions.[7–9] In the present example it is interesting that if the L–H gap term in eqn. (3) is replaced by E_{HOMO}, the correlation is considerably weaker: $r^2 = 0.752$. The unusually high intercept (26.58) in eqn. (3) is attributed to the large values of the L–H gap and their narrow range (8.709–9.512). Thus linear extrapolation of the L–H gap term to zero is meaningless since such values are not attainable.

The exact meaning of the utility of the molecular orbital terms is not clear; *i.e.* does the easy promotion of an electron to an unoccupied orbital indicate that H$^\bullet$ is more readily abstracted or does it imply that reaction may occur on the aromatic ring? The dearth of results in this area makes it difficult to compare eqn. (3) with similar type studies. In the case of eqn. (4) there is a large body of work with σ^+ for comparison.[4] The similarity of ρ^+ to hydrogen abstraction from phenols by radicals suggests that this is the rate limiting step that governs overall phenol toxicity.

Another revealing facet of this model is the good fit of the five estrogenic (compounds 13, 22, 24, 25 and 26 in Table 1) to eqns. (3) and (4). This calls for further study of the system in order to uncover the relationship between estrogenic activity, cell proliferation and DNA damage. It is of interest to note that the agent DES that was responsible for inducing carcinomas in the daughters of women taking it to prevent miscarriage, is significantly better fitted by eqn. (3) than by eqn. (4).

Bordwell and Cheng[10] have measured the redox potential of a limited set of phenols. Using these values, eqn. (5) was derived

$$\log 1/C = 0.22(\pm 0.27)\log P - 2.95(\pm 1.07)\text{redox } P + 4.73(\pm 0.30) \quad (5)$$

to delineate the effects of redox potentials on inhibitory potency, where $n = 8$, $r^2 = 0.940$, $s = 0.171$ and $q^2 = 0.687$ (see Table 2).

Thus as the redox potential (redox P) decreases, the inhibitory potency increases. Since the redox potential is on a different scale from σ^+ these parameters cannot be directly compared except to note that the redox value can replace σ^+ and yield a QSAR with an equivalent log *P* term, albeit with larger confidence limits due to the narrower range in log *P* values of the abbreviated dataset.

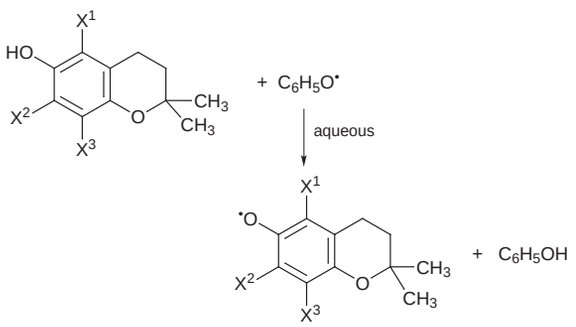
From data by Mukai *et al.*[11] on vitamin E analogs reacting with the phenoxyl radical (Scheme 1), eqn. (6) has been form-

$$\log k = 1.08(\pm 0.32)\sigma^+ + 0.37(\pm 0.28)\text{B1–3} + 2.35(\pm 0.39) \quad (6)$$

Table 2 Parameters used to derive eqn. (5)

	Substituent	Obs. log 1/*C*	Pred. log 1/*C*	Dev	Redox P^a	M log P^b
1	4-OMe	4.480	4.267	0.213	0.257	1.340
2	H	3.270	3.431	−0.161	0.550	1.470
3	4-NH_2	5.090	5.200	−0.110	−0.156	0.040
4	4-OH	4.590	4.609	−0.019	0.085	0.590
5	4-Me	3.850	3.838	0.012	0.447	1.940
6	4-CMe_3	4.090	4.159	−0.069	0.440	3.310
7	3-Me	3.540	3.630	−0.090	0.519	1.960
8	3-NMe_2	4.110	3.887	0.223	0.402	1.560

[a] Oxidation potential of corresponding phenoxide ion in DMSO. [b] M log *P* defines the measured partition coefficient.



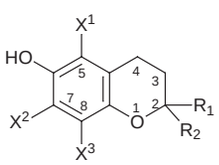
Scheme 1 Reaction of vitamin E analogs with phenoxyl radicals.

ulated,[4] where $n = 10$, $r^2 = 0.908$, $s = 0.095$ and $q^2 = 0.860$. In this expression, the positive B1–3 terms emphasize a small positive steric effect of X3. This is attributed to shielding of the adjacent oxygen atom that decreases interactions between the lone pair electrons and water thus facilitating delocalization of the radical electrons by the ring oxygen electrons. The value of ρ^+ in this free radical mediated reaction is similar to the one in eqn. (1).

Burton *et al.*[12] have analyzed the antioxidant activity of a wide variety of phenols, including various forms of vitamin E, by their ability to inhibit the oxidation of styrene by the peroxyl radicals generated from styrene by azobisisobutyronitrile. Although this "inhibited autoxidation of styrene" (IAS) method is rather complicated, it does produce constants that provide similar results with cells as shown by eqn. (7), where

$$\log k = -1.21(\pm 0.35)\sigma^+ - 2.39(\pm 0.22) \qquad (7)$$

$n = 13$, $r^2 = 0.836$, $s = 0.108$ and $q^2 = 0.769$, for vitamin E derivatives of the type **IV**. Two molecules whose deviations



IV

were considerably more than twice the standard deviation were omitted (2-Me, 2-COOH, 5,7,8-tri-CH_3 and 2-Me, 2-OMe, 5,7,8-tri-CH_3). All compounds except one contained an X^3 substituent so that comparison with the steric effect in eqn. (6) is not possible. However, ρ^+ for QSARs (6) and (7) are in good agreement. As with eqn. (6), steric effects of substituents X^1 and X^2 could not be detected.

From Burton *et al.*,[12] a study of hydrogen abstraction by peroxyl radicals of a set of multisubstituted-phenols, QSAR (8)

$$\log k = -1.97(\pm 0.32)\sigma^+ - 0.53(\pm 0.15)\text{MR-2} - 4.47(\pm 0.40) \qquad (8)$$

was formulated, where $n = 10$, $r^2 = 0.980$, $s = 0.129$ and $q^2 = 0.971$. One datapoint, 2,3,5,6-tetra-CH_3, 4-OCH_3 was omitted. The MR-2 term is basically an indicator variable since all compounds contained either 2,6-di-CH_3 or 2,6-di-$C(CH_3)_3$ substituents. Its negative coefficient pinpoints the deleterious effect of the *tert*-butyl groups. Eqns. (6) and (7) show that 2,6-di-CH_3 substitution does not produce a detectable steric effect, but eqn. (8) shows that large groups in the *ortho* positions of multisubstituted phenols may introduce steric effects. The value of ρ^+ obtained with their experimental conditions is significantly larger than that in eqn. (4) but it could be that electronic effects of substituents are somewhat mitigated in cellular systems.

Our results illustrate the advantage of having at hand a large database of easily accessible QSARs for comparative purposes.[2,4,13] When considered *in toto*, they suggest that the toxic effect of phenols on cancer cells may be mediated by their radical intermediates. When substituent parameters are available, the simple Hammett equation does as well as the more time consuming AMI method. The Hammett equation has another advantage in that the thousands of published examples enable one to easily relate newly derived equations to all sorts of other mechanistic studies made during the past 60 years. Hence, in initiating a structure–activity study it is advantageous to select substituents with known parameters. A wide selection of substituents with defined parameters are now available. At present we have 1999 σ_p and 1234 σ_m values for different substituents. There are 395 σ_p^+ values, 365 σ_p^-, 992, σ_I values and 1080 for π.[13] Once some mechanistic feeling has been developed for the reaction *via* comparative QSAR, one can then move to the computational intensive molecular orbital calculations to model the more complex structural changes.

This approach is especially valuable for biological reactions. In the case of phenols some reactions are best correlated by σ^+ or σ^-, but some show no dependence on electronic effects of substituents unless gross changes are introduced.

Experimental

Almost all of the octanol–water log *P* values have been experimentally determined. Calculated log *P* values were obtained using the Clog P program.[14] The σ^+ values are from our recent compilation.[15] Here we have used σ^+ from the smaller alkyl and alkoxy substituents for the larger substituents that have not yet been determined.[15] AMI methodology for the calculation of MO parameters was utilized. The structures of these molecules were constructed using SPARTAN SGI version 5.03X11 built under IRIX 6.2. The frontier molecular orbital energies (E_{HOMO}, E_{LUMO}) of these molecules were calculated based on the AM1 geometry optimization provided in Spartan.

Acknowledgements

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