dryness to give 16 (46 mg, 85%). The solid was recrystallized from EtOH–Et_2O: mp 193 °C (sintered from 100 °C); MS m/z 269 (M^+); UV λ_{max} (H_2O) 248 nm (ϵ 7300); UV λ_{max} (0.5 N NaOH) 249 nm (ϵ 9800); NMR (DMSO-d_6 + D_2O) 8.13 (s, 1 H, H-2), 7.20 (dd, 1 H, 5-C*H*=CH_2, J = 18.3, J = 12.2 Hz), 5.86 (dd, 1 H, 5-CH_c=C$H_a$$H_b$, $J_{a,b}$ = 1.5, $J_{a,c}$ = 18.3 Hz), 5.64 (d, 1 H, H-1′, $J_{1',2'}$ = 5.1 Hz), 5.50 (dd, 1 H, 5-CH_c=$CH_a$$H_b$, $J_{a,b}$ = 1.5, $J_{b,c}$ = 12.2 Hz), 4.31 (dd, 1 H, H-2′, $J_{2',1'}$ = 5.1, $J_{2',3'}$ = 4.9 Hz), 4.08 (dd, 1 H, H-3′, $J_{3',2'}$ = 4.9, $J_{3',4'}$ = 4.4 Hz), 3.92 (m, 1 H, H-4′), 3.60 (m, 2 H, H-5′a,b). Anal. ($C_{11}H_{15}N_3O_5$) C, H, N.

(*Z*)-5-[2-(Methylthio)vinyl]-1-β-D-ribofuranosylimidazole-4-carboxamide (17). A solution of sodium thiomethoxide (15% in H_2O, 1 mL) was added to a solution of **9b** (270 mg, 1.01 mmol) in MeOH (15 mL). The mixture was stirred for 7 h at room temperature. The mixture was neutralized with 1 N HCl and concentrated in vacuo. The residue was passed through a short silica gel column (1.5 × 2 cm) to remove the salt. The final purification was done by a HPLC (Inertsil-ODS, 20.0 × 250 mm, flow 9 mL/min) eluted with 20% MeOH in H_2O. The fractions having retention time at 8 min were collected, and the solvent was removed in vacuo to give 17 (248 mg, 78%) as a white foam: FABMS m/z 316 (M^+ + 1); UV λ_{max} (H_2O) 237 nm (ϵ 11 100), 281 nm (ϵ 4800); UV λ_{max} (0.5 N HCl) 286 nm (ϵ 3800); UV λ_{max} (0.5 N NaOH) 240 nm (ϵ 10 200), 283 nm (ϵ 4800); NMR (DMSO-d_6 + D_2O) 8.01 (s, 1 H, H-2), 6.63 (d, 1 H, 5-C*H*=CH, J = 10.4 Hz), 6.34 (d, 1 H, 5-CH=C*H*, J = 10.4 Hz), 5.48 (d, 1 H, H-1′, $J_{1',2'}$ = 4.4 Hz), 4.06 (dd, 1 H, H-2′, $J_{2',1'}$ = 4.4, $J_{2',3'}$ = 4.9 Hz), 4.03 (dd, 1 H, H-3′, $J_{3',2'}$ = 4.9, $J_{3',4'}$ = 3.8 Hz), 3.87 (dt, 1 H, H-4′, $J_{4',3'}$ = 3.8, $J_{4',5'}$ = 3.3 Hz), 3.59 (m, 2 H, H-5′a,b), 2.25 (s, 3 H, SC*H*$_3$). Anal. ($C_{12}H_{17}N_3O_5S \cdot {}^1/_5H_2O$) C, H, N, S.

Assay of in Vitro Antitumor Activity. In vitro antitumor activity was determined by using murine and/or human tumor cells. Roswell Park Memorial Institute Medium 1640 supplemented with 10% heat-inactivated fetal bovine serum and 50 μg/mL of kanamycin was used as the cell cultured medium. Tumor cells (1×10^4 cells/mL) were cultured in a CO_2 gas incubator at 37 °C for 72 h (or for 96 h for Table III and IV) in 1 mL of medium containing various concentrations of test compound. Their viability, estimated by use of a variation of a colorimetric [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay,[26] was compared to that of control cells incubated in the identical medium without the compound. The antitumor activity evaluated as IC_{50} (the concentration in μg/mL required for 50% inhibition of cell growth). The IC_{50} value was obtained by plotting the logarithm of concentration of the test compound vs the growth rate (percentage of control) of the treated cells. The results are representative of three separate experiments.

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Registry No. 1, 2627-69-2; **6a**, 131194-97-3; **6b**, 118908-07-9; **6c**, 118908-02-4; **6d**, 118908-04-6; **6e**, 118908-05-7; **6f**, 118908-03-5; **6g**, 131195-02-3; **6h**, 131195-04-5; **6i**, 131195-06-7; **6j**, 131195-08-9; **6k**, 131195-10-3; **8**, 118744-90-4; **9a**, 118908-01-3; **9b**, 126004-24-8; **9c**, 118934-03-5; **9d**, 118907-98-5; **9e**, 118907-99-6; **9f**, 118907-97-4; **9g**, 131195-01-2; **9h**, 131195-03-4; **9i**, 131195-05-6; **9j**, 131195-07-8; **9k**, 131195-09-0; **10a**, 118907-96-3; **10b**, 131195-00-1; **11**, 23192-63-4; **12**, 59354-00-6; **13a**, 126004-21-5; **13b**, 131195-12-5; **13c**, 126004-19-1; **13d**, 131195-11-4; **13e**, 131195-13-6; **13f**, 126004-18-0; **14a**, 131194-98-4; **14b**, 126004-13-5; **14c**, 126004-14-6; **14d**, 126004-15-7; **14e**, 131195-14-7; **14f**, 126004-22-6; **15**, 114485-26-6; **16**, 114485-11-9; **17**, 131194-99-5.

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Structure–Activity Relationship of Mutagenic Aromatic and Heteroaromatic Nitro Compounds. Correlation with Molecular Orbital Energies and Hydrophobicity

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A review of the literature yielded data on over 200 aromatic and heteroaromatic nitro compounds tested for mutagenicity in the Ames test using *S. typhimurium* TA98. From the data, a quantitative structure–activity relationship (QSAR) has been derived for 188 congeners. The main determinants of mutagenicity are the hydrophobicity (modeled by octanol/water partition coefficients) and the energies of the lowest unoccupied molecular orbitals calculated using the AM1 method. It is also shown that chemicals possessing three or more fused rings possess much greater mutagenic potency than compounds with one or two fused rings. Since the QSAR is based on a very wide range in structural variation, aromatic rings from benzene to coronene are included as well as many different types of heterocycles, it is a significant step toward a predictive toxicology of value in the design of less mutagenic bioactive compounds.

Introduction

The problem of toxicity in drug development becomes of ever greater importance as more sophisticated methods of epidemiology uncover more subtle forms of toxicity. Concern has shifted from acute toxicity to that resulting from long term exposure to drugs and/or their metabolic products. Such potential toxicity, when identified early in drug development, can avoid needless expense and loss of time. Kapeghian and Traina[1] point out in their review of experimental toxicology in the pharmaceutical industry that the time has come to move from "descriptive toxicology" to "predictive toxicology". This advice applies not only to medicinal chemistry and to the production of all industrial chemicals, pesticides, solvents, etc., but also to the recognition of dangers inherent in so called "natural environmental" compounds which may even be present in common foods. In this report we consider a rather general QSAR for correlating the mutagenicity of aromatic and heteroaromatic nitro compounds. Despite the fact that aromatic nitro compounds have been found to be both mutagenic and carcinogenic,[2-5] drugs containing this

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function are still in use and it is of interest to see what molecular features result in nitro compounds having little or no mutagenic activity.

Nitroaromatic compounds have also become of acute concern because of their carcinogenicity and their occurrence in automobile and diesel exhaust fumes.[2] Moreover, such substances are common intermediates in the synthesis of many industrial compounds and thousands are prepared each year as intermediates in numerous academic laboratories. Although carcinogenicity does not necessarily parallel mutagenicity the relationship is close enough so that tests for mutagenicity, such as the Ames test, can be used as an inexpensive means for uncovering hazardous chemicals. Indeed, a number of the highly mutagenic nitroaromatics have been found to be carcinogenic.[3,4]

The idea of using drugs, for example, which are mutagenic, but presumably not carcinogenic, is not attractive since mutagenicity so often involves damage to DNA. Long term exposure to chemicals interacting with DNA is an unsettling prospect and understanding of the relationship between structure and mutagenic activity enables one to avoid working in less profitable areas early on in a research program.

In certain areas such as antimicrobial agents it may not be possible to determine mutagenicity because of the high toxicity of the agent to test organisms. As discussed below we were unable to obtain satisfactory mutation data on chloramphenicol, but we can estimate its mutagenic activity from quantitative structure–activity relationships (QSAR) developed here.

For these reasons, and also to gain a more fundamental understanding of the mechanism of mutagenesis, we have undertaken a systematic quantitative structure–activity relationship (QSAR) study of aromatic and heteroaromatic compounds using the Ames test to define mutagenicity.[5-7]

Following up earlier leads[8-10] that mutagenicity was correlated with the energy of the nitroarene's lowest unoccupied molecular orbital (LUMO), we discovered that accounting for the hydrophobicity of the mutagens led to an improved and more extensive correlation.[5,7] Equation 1, based on the results of the Ames test using *S. typhimurium* TA98 bacteria, was derived.[7]

$$\log \text{TA98} = -2.29\ (\pm 0.41)\ \epsilon_{\text{LUMO}} + 1.62\ (\pm 0.28) \log P - 4.21\ (\pm 0.80) \log (\beta \cdot 10^{\log P} + 1) - 7.74\ (\pm 1.4) \quad (1)$$

$$n = 66,\ r = 0.885,\ s = 0.750,\ \log P_0 = 4.86,\ \log \beta = -5.06$$

In this expression TA98 represents mutagenic activity in revertants/nmol produced by the mutagen, ϵ_{LUMO} is the energy of the lowest unoccupied molecular orbital calculated with use of MNDO, and P is the mutagen's octanol/water partition coefficient. The statistical parameters describing the regression are n, the number of data points upon which the equation is based; r, the correlation coefficient; and s, the standard deviation from the regression.

The equation is linear in ϵ_{LUMO} and bilinear in $\log P$, that is, activity increases linearly with $\log P$ with slope of 1.62 until $\log P$ equals 4.86 ($\log P_0$) and then decreases linearly with slope of $-2.59(1.62 - 4.21)$. Equation 1 includes a wide range of nitroaromatic structures ranging in size from 5-nitroindene to various nitrochrysenes. In addition, eq 1 correlates the mutagenicities of four heterocycles: nitroquinolines and nitrocarbazoles.

An equation similar to eq 1 correlates nitroarene mutagenicity in *S. typhimurium* TA100, another bacterial strain commonly employed in the Ames test.[7] Taken together, these two correlations demonstrate the significant role played by the hydrophobic character of the chemicals in determining mutagenic activity. Although the importance of hydrophobicity in mutagenicity was pointed out a decade ago,[11] it has generally been ignored by those working in the areas of mutagenicity and carcinogenicity. Since hydrophobicity is a property of molecular structure, any attempt to relate mutagenicity to structure must take this chemical property into account.

Another important feature of this study is the treatment of electronic effects. During the past two decades most of the QSARs derived for biological systems have relied on the use of Hammett–Taft σ constants to account for electronic variation associated with changes in molecular structure. As a result, these studies have often been limited to sets of congeners that could be treated by using Hammett–Taft substituent constants. A quantum chemical treatment of electronic effects, on the other hand, is potentially more powerful than the Hammett–Taft approach since it allows greater flexibility in the construction of the data set. An initial study by Klopman, et al.[8] followed by reports by Loew, et al.[9] and Maynard, et al.,[10] plus our own studies[6,12] have shown that the mutagenicity of aromatic nitro compounds is, in part, correlated to molecular orbital energies. This success has encouraged us to make a survey of the literature to find all examples where mutation rates have been reported for nitroaromatic and heteroaromatic compounds tested on *S. typhimurium* TA98.

Results

The data in Table I are best correlated by eq 2, the development of which is shown in eqs 3–5.

$$\log \text{TA98} = 0.65\ (\pm 0.16) \log P - 2.90\ (\pm 0.59) \log (\beta \cdot 10^{\log P} + 1) - 1.38\ (\pm 0.25)\ \epsilon_{\text{LUMO}} + 1.88\ (\pm 0.39)\ I_1 - 2.89\ (\pm 0.81)\ I_a - 4.15\ (\pm 0.58) \quad (2)$$

$$n = 188,\ r = 0.900,\ s = 0.886,\ \log P_0 = 4.93,\ \log \beta = -5.48,\ F_{1,181} = 48.6$$

$$\log \text{TA98} = 1.37\ (\pm 0.20) \log P - 3.85\ (\pm 0.85) \log (\beta \cdot 10^{\log P} + 1) - 3.06\ (\pm 0.67) \quad (3)$$

$$n = 188,\ r = 0.687,\ s = 1.47,\ \log P_0 = 5.00,\ \log \beta = -5.29,\ F_{1,182} = 73.5$$

$$\log \text{TA98} = 1.21\ (\pm 0.16) \log P - 3.05\ (\pm 0.68) \log (\beta \cdot 10^{\log P} + 1) - 1.65\ (\pm 0.32)\epsilon_{\text{LUMO}} + 5.23\ (\pm 0.68) \quad (4)$$

$$n = 188,\ r = 0.814,\ s = 1.18,\ \log P_0 = 5.06,\ \log \beta = -5.29,\ F_{1,183} = 104$$

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$$\log TA98 = 0.58\ (\pm 0.19)\log P - 2.35\ (\pm 0.58)\log(\beta \cdot 10^{\log P} + 1) - 1.32\ (\pm 0.28)\epsilon_{LUMO} + 1.91\ (\pm 0.44)I_1 - 3.91\ (\pm 0.65) \quad (5)$$

$n = 188$, $r = 0.872$, $s = 0.995$, $\log P_o = 4.78$, $\log \beta = -5.26$, $F_{3,184} = 54.8$

The parameters of eqs 2–5 have the same connotation as those of eq 1 except that two indicator variables I_1 and I_a have been added. The variable I_1 is set equal to 1 for all compounds containing three or more *fused* rings (e.g. anthracene, phenanthrene, chrysene, etc.). Congeners with two or less rings (e.g. benzene, quinoline, naphthalene, etc.) are assigned the value of 0. The positive coefficient with this term brings out the fact that the large ring compounds are more active than expected from log P and ϵ_{LUMO} alone. I_a takes the value of 1 for five examples of acenthrylenes and shows that these are much less active than expected for some unknown reason. All of these congeners contain a nitro group attached to a five-membered ring; however, this alone would not seem to be the single cause of low activity since 1-nitroacenaphthylene is well predicted. Still another instance of a nitro group on a five-membered ring is that of penta[*cd*]pyrene which is well fit if covered by the I_a variable. Nevertheless, we have omitted it because the pyrene structure is otherwise different from the acenthrylenes. Dropping the five acenthrylenes does not significantly change the parameters or the quality of the correlation of eq 2 ($n = 183$, $r = 0.901$, $s = 0.893$).

For the above correlations the figures in parentheses are for construction of the 95% confidence limits, n represents the number of data points supporting the QSAR, r is the correlation coefficient, s is the standard deviation from the regression, and log P_o is the optimum value of log P.

The ϵ_{LUMO} values used in eq 2 were obtained by using the AM1 method of calculation[13] and differ slightly from the MNDO values used to derive eq 1. Actually, the correlation between the two types of ϵ_{LUMO} is high (r = 0.921 for 146 examples) even though the geometries used in the calculations for the two methods were somewhat different. The AM1 method was chosen for this study because of its well-known ability to make superior predictions for nitro group and other substituent conformations in nitroaromatic compounds.

In the stepwise development of eq 2 the hydrophobic parameter is most important accounting for 47.2% of the variance (eq 3) in log TA98, while the ϵ_{LUMO} term accounts for 19.1%. The indicator variable I_1 accounts for 10.1% and I_a covers 5%.

The correlation matrix shows reasonable orthogonality between the variables except for log P and I_1.

correlation matrix for variables of eq 2

	ϵ_{LUMO}	log P	I_a	I_1
ϵ_{LUMO}	1	−0.17	−0.11	−0.33
log P		1	0.17	0.70
I_a			1	0.15

This collinearity results in a notable change in the coefficient in log P in going from eq 4 to eq 5. From past experience we would expect a coefficient with log P near 1 rather than 0.58 in eq 5 and 0.65 in eq 2. The value with eq 4 seems more reasonable although it is on the high side of 1.

Another point of interest is the highly unsymmetric bilinear relationship between activity and log P. Activity increases as 0.65 log P for compounds with $P < P_o$, and then decreases as −2.25 log P (0.65 − 2.90) for more hydrophobic compounds. Bilinear relationships between hydrophobicity and biological activity in microorganisms are often symmetrical, and so the rapid drop in activity seen for the largest mutagens in TA98 suggests that an additional factor, possibly steric, may reduce activity. In any case, one should not place too much emphasis on this part of the curve since it is determined by compounds having log $P > 5$. The limited solubility of such highly hydrophobic substances makes them difficult to test.

Equation 2 correlates the activity of 188 nitroaromatic and heteroaromatic compounds having an extremely wide range of structure, physicochemical, and mutagenic activity. The mutagenic rate varies by more than 8 powers of 10. Clearly the above results taken with our earlier studies[7,12] show that the hydrophobic character of organic chemicals is an important determinant of mutagenicity as well as carcinogenicity.[14] Moreover, these results demonstrate that any attempt to predict mutation rates quantitatively should take relative hydrophobicity into account as a potential contributing factor.

The following section contains a detailed discussion of the significance and limitations of eqs 2 and 6. However, it is evident from the amount of unexplained variance in the activity data and the rather large standard deviation of the regression, that our model needs further refinement. At the same time it should be recognized that the quality of fit is also due, in part, to experimental error and to systematic differences in assay techniques employed by many different laboratories. Where possible we have used average results from two or more laboratories and as demonstrated earlier this yields slightly better results than attempting to select a "best" set.[7] Furthermore, an additional source of error can be traced to the failure to employ highly purified nitroarenes for mutagenicity testing. Data on mononitropolycyclic aromatic hydrocarbons, in particular, is often suspect since these compounds are typically obtained from the nitration of the parent hydrocarbon—a method known to give mixtures of polynitrated products. A classic example regarding this was cited by Greibrokk, et al.[15] who demonstrated that two different commercial samples of 1-nitropyrene showed mutation rates of 11 000 and 5000 revertants/μg compared with 1900 for highly purified (>99.9%) material.

In order to refine our QSAR we shall not only need conceptual improvements in our modeling of structural properties, but also reliable mutagenicity data from a *single* laboratory on a few hundred *highly purified* nitroaromatic and heterocyclic compounds. For example, the heteroaromatic compounds as a group are less well fit than the carbocyclic nitro compounds. Also, substituent effects have not been carefully studied; efforts up to this point have been more concerned with ring variation. It is important to investigate the hydrophobic effect of aliphatic side chains to see if it parallels that of the flat aromatic ring systems.

Discussion

The strength of a correlation model, such as eq 2, is not determined entirely by statistical measures of quality of fit. In the following discussion we consider several issues, all of which bear on the reliability of eq 2 as a meaningful

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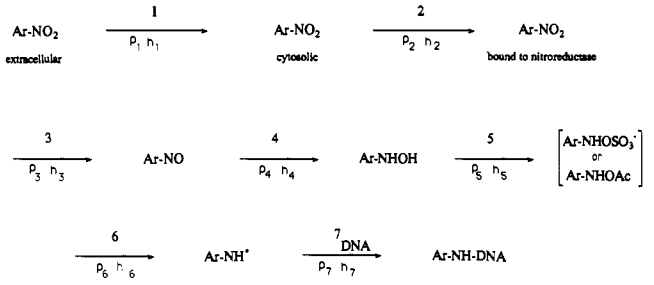
Scheme I. Mechanism of Mutagenesis by Aromatic Nitro Compounds, $ArNO_2$[a]



[a] ρ is the coefficient with the electronic terms (σ or ϵ_{LUMO}) and h is the coefficient with the positive hydrophobic term (log P).

QSAR for nitroaromatic mutagenicity. In particular, we shall examine the range of structural types, mutagenic activities, and structure-based parameters used to construct the equation. We shall also consider whether the equation is consistent with what is known about the mechanism of nitroaromatic mutagenesis. Finally, we shall focus on specific weaknesses of the model, such as the occurrence of outlier compounds, and what these failures say about the limitations of the model.

The extremely broad range of molecular structures and mutagenicities correlated by eq 2, as well as the similarity of this equation with other mutagenicity QSARs is very encouraging. The data set for eq 2 includes such diverse molecules as 2,4-dinitrophenylhydrazine, nitrocoronene, mono-, di-, tri-, and tetranitroarenes, nitroindoles, nitroindazoles, nitrofurans, and nitrodiazines (see Table IV for structures of parent compounds). At the same time, the considerable variation in log TA98, log P, and ϵ_{LUMO} make it unlikely that eq 2 is the result of a chance correlation. Referring to Table I we find that log P ranges from −0.02 to 7.84, ϵ_{LUMO} from −3.41 to −0.57 eV, and the range in activity is roughly 1×10^8 revertants/nmol. Simply by varying ϵ_{LUMO} within the limits covered by the examples in Table I shows that log TA98 can be changed by 3.92 or TA98 by a factor of almost 10 000 by this factor alone. Variation in log P within the limits of −0.02 to log P_o indicates that log TA98 can be changed by 3.19. Designing a compound with optimum log P and ϵ_{LUMO} of −3.41 might yield a substance more mutagenic than the most potent nitro compound known, 1,8-dinitropyrene. Of course, it would be of interest to see how far the linear relationship between log TA98 and ϵ_{LUMO} holds. In attempting to make a more potent mutagen, one must not forget the confidence limits on the terms of eq 2.

Additional support for eq 2 can be obtained from a detailed consideration of the relationship between mutagenicity and the different structure parameters. Vance and his colleagues have rationalized many experimental observations using a mechanistic outline similar to the one shown in Scheme I.[16] After initial penetration of the cell (1) and diffusion to the activation site (2), the nitroarene is reduced to a hydroxylamine via a nitrosoarene intermediate (3,4). Amination of DNA by the hydroxylamine might occur via acetylation or sulfation of the hydroxylamine (5), subsequent formation of a nitrenium ion (6), and capture of the nitrenium ion by DNA (7). The sensitivity of each step in the mechanism to hydrophobic and electronic effects is symbolized by h_i and ρ_i (i = 1–7), respectively, where ρ_i is the coefficient with ϵ_{LUMO} and h_i is that with log P. Each step might also be sensitive to other structure-dependent properties, such as steric hindrance, which have not been incorporated into eq 2. The overall relationship between activity and hydrophobic and electronic effects, as given in eq 2, represents some combination of several h_i and ρ_i values.

Of course, Scheme I is an oversimplification and really constitutes only an outline of what must be considered in the mutagenic process. There are several reductases which may be involved in steps 3 to 4. In step 5 at least two different esterification reactions may occur and several enzymes may be involved, and finally, it is likely that the esters and/or nitrenium ion may attack several different sites on DNA. What eq 2 does is discuss, in numerical terms, an overview of a complex SAR and in fact we are surprised how many structures can be included in its purview.

Hydrophobicity, for example, can be expected to play two roles in the mechanism shown in Scheme I. On the one hand, relative hydrophobicity will affect penetration of the cell by the mutagen (h_1) and the random walk of the mutagen through the cell (h_2). Relative hydrophobicity may also affect the binding of the mutagen or its metabolites to enzymes involved in (de)activation (h_3–h_7). The contribution of each of these processes to the overall relationship between mutagenicity and hydrophobicity expressed in eq 2 cannot be determined, since many of the h_i values are unknown. However, it is still informative to compare the log P terms in eq 2 with those associated with other biological QSARs.

According to eq 2, activity is related to log P by a bilinear model, with a rising slope of about 0.7, an optimal log P_o of 5, and a falling slope of −2.25. This kind of behavior is well known in biological QSAR. For example, the optimal log P_o for nonspecific toxicants acting on microorganisms is generally in the range of 4–5.[17] A similar optimal log P_o was also found for the more limited data set in eq 1. The occurrence of an optimal log P value is probably due to the restricted movement of highly lipophilic compounds through the aqueous and lipid phases of cellular material which results in diminished activity.

A rising slope of 0.65 in the lower log P range (log P < 5) is reasonable but somewhat lower than we would expect. Experience has shown that it is unusual for the log P coefficient to be much greater than 1.1 in biological QSAR, and most values range from 0.3 to 1.1.[17]

It is also interesting to compare eq 2 with eq 6 which describes the mutagenic activity of triazenes in the Ames test. Equation 6 covers the action of aryltriazenes on *S. typhimurium* TA_{92},[12] including 17 examples of simple phenyltriazenes [X-C_6H_4N=$NN(CH_3)R$] and four heterocyclic triazenes.

$$\log 1/C = 0.97\ (\pm 0.24)\ \log P - 7.76\ (\pm 2.73)\ q_{HOMO} + 5.96 \quad (6)$$

$$n = 21,\ r = 0.931,\ s = 0.585$$

C is the molar concentration of triazene which produces 30 mutations above background in 10^8 TA92 bacteria, and q_{HOMO} represents the electron density in the HOMO that resides on the N containing the alkyl groups.[12] The aryltriazenes, unlike the nitroarenes require activation by a liver microsome fraction, S-9, before they can affect bacterial mutations. Although the coefficient with log P

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Table I. Compounds and Their Physicochemical Parameters Used to Derive Eqs 2–5

entry	compd		log revertants/nmol obsd	pred	dev	ϵ_{LUMO}	log P	I_a	I_l	ref
1	2-nitrophenanthrene		2.11	2.11	0.00	−1.246	4.23	0	1	57
2	8-nitroquinoline		−1.24	−1.25	0.01	−1.429	1.44[a]	0	0	69
3	5-nitroquinoline		−0.96	−0.94	−0.02	−1.456	1.86[a]	0	0	57, 3
4	1-nitrofluoranthene		2.74	2.76	−0.02	−1.591	4.69	0	1	57
5	2,5-difluoronitrobenzene		−0.79	−0.74	−0.05	−1.590	1.89	0	0	61
6	1,3,6,8-tetranitropyrene	(±0.54)[b]	4.99	4.93	0.06	−3.406	3.92	0	1	57
7	3-methyl-4-nitrobiphenyl		−0.10	−0.04	−0.06	−1.144	3.99	0	0	67
8	2-acetoxy-7-nitrofluorene		1.86	1.94	−0.08	−1.437	3.46	0	1	58
9	5-nitrobenzimidazole		−1.83	−1.74	−0.09	−0.982	1.64	0	0	*d*
10	2-methyl-7-nitrofluorene		2.36	2.46	−0.10	−1.387	4.62	0	1	58
11	9-nitrophenanthrene		2.25	2.12	0.13	−1.254	4.23	0	1	59
12	1-amino-8-nitropyrene		2.43	2.30	0.13	−1.627	3.63	0	1	57, 3
13	2,4-dinitrofluoranthene		3.78	3.65	0.13	−2.292	4.44	0	1	66
14	1-methyl-6-nitroindazole		−1.10	−1.23	0.13	−1.289	1.77	0	0	62
15	1-nitropyrene	(±0.32)[b]	2.78	2.91	−0.13	−1.698	4.69	0	1	57
16	3,4-dinitrofluoranthene		3.62	3.48	0.14	−2.172	4.44	0	1	70
17	2-nitro-*m*-phenylenediamine		−3.00	−2.86	−0.14	−0.529	0.87	0	0	76
18	3-nitro-9-fluorenone	(±0.64)[b]	2.13	2.27	−0.14	−1.861	3.06[a]	0	1	57
19	6-nitroquinoline	(±0.32)[b]	−1.08	−0.92	−0.16	−1.478	1.84[a]	0	0	57, 3
20	2-cyano-7-nitrofluorene		2.51	2.35	0.16	−1.764	3.40	0	1	58
21	4,3′-dinitrobiphenyl		0.23	0.41	−0.18	−1.665	3.52	0	0	68
22	2-iodo-7-nitrofluorene		2.97	2.79	0.18	−1.602	5.09	0	1	58
23	2-nitrobenz[*j*]acenthrylene		0.86	0.68	0.18	−2.164	5.07	1	1	63
24	1-nitrocoronene		0.45	0.63	−0.18	−1.728	6.79	0	1	57, 3
25	2-hydroxy-7-nitrofluorene		1.68	1.86	−0.18	−1.398	3.43	0	1	58
26	1-chloro-2,4-dinitrobenzene		0.30	0.11	0.19	−2.072	2.17[a]	0	0	61
27	1-nitrobenzo[*a*]pyrene		2.63	2.44	0.19	−1.801	5.87	0	1	3
28	2-fluoro-7-nitrofluorene		2.68	2.48	0.20	−1.558	4.11	0	1	58
29	2,4,7-trinitro-9-fluorenone	(±0.11)[b]	3.41	3.21	0.20	−2.837	2.42[a]	0	1	57
30	4-nitrobenz[*k*]acenthrylene		0.67	0.46	0.21	−2.005	5.07	1	1	63
31	1,2-dinitrofluoranthene		3.11	3.32	−0.21	−2.055	4.44	0	1	66
32	2,4,3′,4′-tetranitrobiphenyl		1.54	1.32	0.22	−2.562	3.00	0	0	68
33	2-[(trifluoroacetyl)amino]-7-nitrofluorene		2.81	2.59	0.22	−1.591	4.23	0	1	58
34	2-nitronaphthalene	(±0.29)[b]	−0.30	−0.06	−0.24	−1.451	3.24[a]	0	0	57
35	2-amino-7-nitrofluorene		1.56	1.32	0.24	−1.176	3.06[a]	0	1	58
36	3-methyl-2-nitronaphthalene		0.00	−0.24	0.24	−1.240	3.43	0	0	57, 3
37	4-nitrostilbene		0.69	0.45	0.24	−1.428	4.18	0	0	71
38	4-nitrobiphenyl		−0.30	−0.05	−0.25	−1.228	3.77	0	0	59
39	6-methoxy-8-nitroquinoline		−1.21	−0.95	−0.26	−1.443	1.87[a]	0	0	69
40	1-nitronaphthalene	(±0.49)[b]	−0.61	−0.35	−0.26	−1.266	3.19[a]	0	0	57
41	2-bromo-7-nitrofluorene		3.06	2.80	0.26	−1.600	4.83	0	1	58
42	2-methyl-5-nitroindazole		−1.10	−1.37	0.27	−1.190	1.77	0	0	62
43	2-nitrofluoranthene		3.01	2.73	0.28	−1.570	4.69	0	1	66
44	2-nitro-9,10-dihydrophenanthrene		1.99	2.27	−0.28	−1.265	4.53	0	1	59
45	1,4-dinitrobenzene		0.15	−0.13	0.28	−2.227	1.46	0	0	61
46	1-nitro-7,8,9,10-tetrahydrobenzo[*a*]pyrene		0.90	1.19	−0.29	−1.804	6.57	0	1	57, 3
47	2,7-dinitro-4,5,9,10-tetrahydropyrene		3.50	3.19	0.31	−1.880	5.02	0	1	59
48	2-nitroanthracene		2.95	2.62	0.33	−1.616	4.23	0	1	57
49	2,8-dinitrophenazine		2.75	3.09	−0.34	−2.808	2.29	0	1	65
50	1,5-dinitronaphthalene	(±0.01)[b]	0.52	0.18	0.34	−1.932	2.58[a]	0	0	57, 3
51	1,6-dinitro-9,10,11,12-tetrahydrobenzo[*e*]pyrene		2.41	2.76	−0.35	−2.184	6.01	0	1	20a
52	1,3-dinitro-9,10,11,12-tetrahydrobenzo[*e*]pyrene		2.41	2.77	−0.36	−2.189	6.01	0	1	20a
53	2,7-dinitrofluorene	(±0.41)[b]	3.22	2.86	0.36	−2.155	3.35[a]	0	1	57
54	7-nitro-2,3-dichlorodibenzo-1,4-dioxin		1.73	1.37	0.36	−1.464	6.24	0	1	74
55	1-methyl-2-nitronaphthalene		−0.70	−0.32	−0.38	−1.177	3.43	0	0	57, 3
56	2-nitrophenazine		2.06	2.44	−0.38	−2.234	2.52	0	1	65
57	2,4,4′-trinitrobiphenyl		0.66	1.04	−0.38	−2.242	3.26	0	0	68
58	*trans*-9,10-dihydro-3-nitrobenzo[*a*]pyrene-9,10-diol		2.80	2.42	0.38	−1.991	3.01	0	1	57, 3
59	2-nitrobenz[*l*]acenthrylene		0.26	0.65	−0.39	−2.140	5.07	1	1	63
60	1,3,8-trinitronaphthalene		0.35	0.74	−0.39	−2.468	2.30[a]	0	0	d
61	2-chloro-7-nitrofluorene		3.11	2.71	0.40	−1.556	4.68	0	1	58
62	2-amino-5-nitrophenol		−2.40	−2.00	−0.40	−0.923	1.36	0	0	77
63	*N*′-(5-nitro-2-furfuryliden)-5-nitro-2-furanacrylohydrazide		2.45	2.85	−0.40	−3.768	2.79[a]	0	0	72
64	2,7-dinitro-9-fluorenone	(±0.12)[b]	3.19	2.79	0.40	−2.338	2.84[a]	0	1	57
65	1,3-dinitronaphthalene		−0.05	0.37	−0.42	−1.952	2.83[a]	0	0	57, 3
66	4-amino-3′-nitrobiphenyl		−1.52	−1.10	−0.42	−0.959	2.68	0	0	80
67	2-hydroxy-1-nitrofluoranthene		2.26	2.68	−0.42	−1.536	4.66	0	1	66
68	3-nitrobenzo[*a*]pyrene		2.82	2.39	0.43	−1.766	5.87	0	1	3, 60
69	2,5-dinitrofluorene		3.20	2.77	0.43	−1.929	3.71	0	1	57, 3
70	5-nitroisatin		−0.94	−1.38	0.44	−1.786	0.47[a]	0	0	69
71	1,8-dinitro-9,10,11,12-tetrahydrobenzo[*e*]pyrene		2.19	2.64	−0.45	−2.095	6.01	0	1	20a
72	2-nitrofluorene	(±0.47)[b]	1.43	1.90	−0.47	−1.448	3.37[a]	0	1	57
73	3-amino-2′-nitrobiphenyl		−2.00	−1.53	−0.47	−0.648	2.68	0	0	80
74	3-nitrobenzo[*k*]fluoranthene		2.76	2.28	0.48	−1.689	5.87	0	1	57, 3
75	4,4′-dinitrobiphenyl		1.17	0.69	0.48	−1.870	3.52	0	0	59

Table I (Continued)

entry	compd		log revertants/nmol obsd	pred	dev	ϵ_{LUMO}	log P	I_a	I_l	ref
76	2-chloronitrobenzene		−1.72	−1.23	−0.49	−1.069	2.24[a]	0	0	61
77	2-amino-3′-nitrobiphenyl		−1.52	−1.00	−0.52	−1.029	2.68	0	0	80
78	2′-methyl-4-nitrobiphenyl		−0.23	0.29	−0.52	−1.276	4.27	0	0	67
79	2-nitrodibenzo-1,4-dioxin		1.79	2.32	−0.53	−1.260	4.73	0	1	74
80	7-nitrofluoranthene		1.87	2.40	−0.53	−1.329	4.69	0	1	57
81	1,3-dinitrobenzene		0.03	−0.52	0.55	−1.937	1.49[a]	0	0	61
82	1,2,4-trinitrofluoranthene		3.56	4.11	−0.55	−2.710	4.18	0	1	66
83	2-nitrocarbazole		1.01	1.56	−0.55	−1.145	3.51	0	1	57, 3
84	4-nitrostyrene		−1.30	−0.74	−0.57	−1.256	2.61	0	0	71
85	6-nitrobenz[*e*]acenthrylene		0.04	0.61	−0.57	−2.113	5.07	1	1	63
86	5-nitrobenz[*k*]acenthrylene		0.92	0.34	0.58	−1.918	5.07	1	1	63
87	2-nitrodibenzofuran		1.64	2.23	−0.59	−1.488	3.83	0	1	*d*
88	4-nitroacetophenone		−1.54	−0.95	−0.59	−1.605	1.53[a]	0	0	71
89	1-methyl-7-nitroindazole		−1.00	−1.59	0.59	−1.028	1.77	0	0	62
90	1-nitrobenzo[*e*]pyrene		1.59	2.19	−0.60	−1.620	5.87	0	1	57, 3
91	*trans*-7,8-dihydro-3-nitrobenzo[*a*]pyrene-7,8-diol		3.08	2.47	0.61	−2.032	3.01	0	1	57, 3
92	2-nitro-4,5,9,10-tetrahydropyrene		1.58	2.19	−0.61	−1.208	5.28	0	1	59
93	3-nitrobenzo[*e*]pyrene		2.95	2.34	0.61	−1.729	5.87	0	1	57, 3
94	4-nitropyrene		3.39	2.76	0.63	−1.585	4.69	0	1	57, 3
95	2,3,5-trinitronaphthalene		1.51	0.85	0.66	−2.434	2.55	0	0	57, 3
96	1,3,6-trinitropyrene	(±0.54)[b]	4.99	4.33	0.66	−2.871	4.18	0	1	57
97	2-methoxy-7-nitrofluorene		2.79	2.12	0.67	−1.361	3.95	0	1	58
98	5-nitroisoquinoline		−1.55	−0.88	−0.67	−1.598	1.65	0	0	*d*
99	1-nitroacenaphthylene		1.77	2.47	−0.70	−2.159	2.72	0	1	57, 3
100	3-amino-3′-nitrobiphenyl		−1.70	−1.00	−0.70	−1.034	2.68	0	0	80
101	3-nitro-9,10,11,12-tetrahydrobenzo[*e*]pyrene		0.78	1.50	−0.72	−1.598	6.26	0	1	20a
102	2,4,5,7-tetranitro-9-fluorenone		2.93	3.66	−0.73	−3.172	2.40[a]	0	1	57, 3
103	2-nitropyrene		3.35	2.62	0.73	−1.487	4.69	0	1	57, 3
104	1-nitro-9,10,11,12-tetrahydrobenzo[*e*]pyrene		0.70	1.43	−0.73	−1.546	6.26	0	1	20a
105	5-nitro-1,10-phenanthroline		0.59	1.33	−0.74	−1.749	1.84	0	1	*d*
106	10-hydroxy-1-nitropyrene		1.89	2.66	−0.77	−1.607	4.34	0	1	57, 3
107	2,3,5-trinitrofluoranthene		3.44	4.22	−0.78	−2.791	4.18	0	1	66
108	3-nitrofluoranthene	(±0.49)[b]	3.67	2.88	0.79	−1.676	4.69	0	1	57
109	5,10-dinitrobenzo[*ghi*]perylene		3.60	2.80	0.80	−2.284	6.07	0	1	73
110	2,4,2′,6′-tetranitrobiphenyl		−0.07	0.74	−0.81	−2.140	3.00	0	0	68
111	4-nitrobenzaldehyde		−1.64	−0.82	−0.82	−1.687	1.56[a]	0	0	71
112	3-nitroacenaphthene		1.00	1.83	−0.83	−1.208	3.81	0	1	57, 3
113	2,4-dinitroaniline		−1.74	−0.91	−0.83	−1.491	1.84	0	0	79
114	2-amino-4′-nitrobiphenyl		−1.70	−0.84	−0.86	−1.148	2.68	0	0	80
115	5-nitroindene		0.08	−0.78	0.86	−1.161	2.74	0	0	57, 3
116	4-fluoronitrobenzene		−0.23	−1.10	0.87	−1.370	1.80[a]	0	0	61
117	2,4,3′-trinitrobiphenyl		0.03	0.90	−0.87	−2.142	3.26	0	0	68
118	3-nitro-7,8,9,10-tetrahydrobenzo[*a*]pyrene		0.30	1.19	−0.89	−1.800	6.57	0	1	57, 3
119	2,4,2′-trinitrobiphenyl		−0.19	0.70	−0.89	−1.995	3.26	0	0	68
120	1-methyl-5-nitroindazole		−0.82	−1.72	0.90	−0.937	1.77	0	0	62
121	1,2,5-trinitrofluoranthene		3.16	4.07	−0.91	−2.680	4.18	0	1	66
122	6-nitrochrysene	(±0.95)[b]	1.75	2.67	−0.92	−1.610	5.41	0	1	57, 3
123	3,2′-dimethyl-4-nitrobiphenyl		−0.84	0.08	−0.92	−1.056	4.49	0	0	67
124	1-nitro-2,4-difluorobenzene		−1.66	−0.73	−0.93	−1.596	1.89	0	0	61
125	2-nitro-4,5-dihydropyrene		3.27	2.33	0.94	−1.256	4.99	0	1	59
126	1,7-dinitrophenazine		2.02	2.96	−0.94	−2.718	2.29	0	1	65
127	3,4,3′-trinitrobiphenyl		1.92	0.98	0.94	−2.196	3.26	0	0	68
128	5-nitroacenaphthylene		1.91	2.86	−0.95	−2.149	3.36	0	1	57, 3
129	2,4-dinitrophenylhydrazine		−0.07	−1.01	0.95	−1.592	1.46[a]	0	0	69
130	2-nitroanisole		−2.70	−1.75	−0.95	−0.930	1.73[a]	0	0	69
131	4-nitrochalcone		−1.15	−0.21	−0.95	−1.538	2.83	0	0	71
132	2-nitrobenzaldehyde		−1.92	−0.96	−0.96	−1.499	1.74[a]	0	0	69
133	2-nitro-1,3,7,8-tetrachlorodibenzo-1,4-dioxin		−1.40	−0.42	−0.98	−1.492	7.13	0	1	74
134	2,7-dinitro-4,5-dihydropyrene		4.25	3.27	0.98	−1.951	4.73	0	1	59
135	3-chloro-4-fluoronitrobenzene		−1.21	−0.23	−0.98	−1.562	2.74	0	0	61
136	1-hydroxy-3-nitropyrene		3.87	2.89	0.98	−1.685	4.66	0	1	57
137	2,3-dinitrofluoranthene		2.62	3.61	−0.99	−2.263	4.44	0	1	66
138	2-methyl-6-nitroindazole		−0.41	−1.41	1.00	−1.157	1.77	0	0	62
139	4-chloro-2-nitroaniline		−2.00	−0.99	−1.01	−1.019	2.72	0	0	79
140	1,8-dinitronaphthalene		0.90	−0.11	1.01	−1.751	2.52[a]	0	0	57, 3
141	2,3-dichloronitrobenzene		−1.51	−0.49	−1.02	−1.228	3.05[a]	0	0	61
142	8-nitro-2,3,7-trichlorodibenzo-1,4-dioxin		−0.53	0.50	−1.03	−1.474	6.68	0	1	74
143	2-nitrophenetole		−2.22	−1.19	−1.03	−1.046	2.35	0	0	69
144	2,5-dichloronitrobenzene		−1.54	−0.51	−1.03	−1.288	2.90[a]	0	0	61
145	3-amino-4′-nitrobiphenyl		0.25	−0.80	1.05	−1.178	2.68	0	0	80
146	4-amino-4′-nitrobiphenyl		0.19	−0.90	1.09	−1.102	2.68	0	0	80
147	2-methyl-5-nitrobenzimidazole		−0.51	−1.61	1.10	−0.937	1.94[a]	0	0	69
148	3-nitroacenaphthylene		1.77	2.87	−1.10	−2.158	3.36	0	1	57, 3
149	2,7-dinitrophenazine		4.34	3.17	1.17	−2.870	2.29	0	1	65
150	metronidazole		−1.61	−2.79	1.18	−0.995	−0.02[a]	0	0	*d*

Table I (Continued)

entry	compd		log revertants/nmol obsd	pred	dev	ϵ_{LUMO}	log P	I_a	I_1	ref
151	7-nitroindazole		0.11	−1.07	1.18	−1.411	1.75	0	0	62
152	2,5-dinitrofluoranthene		2.32	3.51	−1.19	−2.191	4.44	0	1	66
153	5-nitroacenaphthene	(±0.18)[b]	0.58	1.78	−1.20	−1.151	3.85[a]	0	1	57
154	2-nitrobenzimidazole		0.00	−1.24	1.24	−1.362	1.59	0	0	62
155	2,4-dinitroanisole		−1.89	−0.64	−1.25	−1.737	1.72	0	0	69
156	6-nitroindoline		−0.48	−1.73	1.25	−0.854	1.92[a]	0	0	62
157	6-hydroxy-1-nitropyrene		1.34	2.61	−1.27	−1.623	4.19	0	1	57, 3
158	2-(acetylamino)-7-nitrofluorene		2.85	1.56	1.29	−1.340	3.08[a]	0	1	58
159	8-hydroxy-1-nitropyrene		1.49	2.78	−1.29	−1.742	4.19	0	1	57, 3
160	2-bromo-4,6-dinitroaniline		−1.32	−0.03	−1.29	−1.691	2.78	0	0	77
161	1,6-dinitrobenzo[*e*]pyrene		1.99	3.34	−1.35	−2.221	5.61	0	1	20a
162	3,4,3′,4′-tetranitrobiphenyl		2.85	1.49	1.36	−2.687	3.00	0	0	68
163	2,7-dinitropyrene		4.58	3.21	1.37	−1.974	4.44	0	1	59
164	1,3-dinitropyrene	(±0.26)[b]	5.04	3.67	1.37	−2.306	4.44	0	1	57
165	8-nitrofluoranthene		4.05	2.67	1.38	−1.522	4.69	0	1	57
166	1-nitro-3-acetoxypyrene		4.22	2.84	1.38	−1.709	4.42	0	1	57, 3
167	1,6-dinitropyrene	(±0.22)[b]	5.06	3.68	1.38	−2.310	4.44	0	1	57
168	4-nitroanisole		−2.70	−1.31	−1.39	−1.112	2.03[a]	0	0	69
169	2,4-dinitro-1-fluorobenzene		1.20	−0.19	1.39	−2.170	1.49	0	0	61
170	1-nitrophenazine		0.87	2.27	−1.40	−2.113	2.52	0	1	65
171	2,4,2′,4′-tetranitrobiphenyl		2.66	1.24	1.42	−2.508	3.00	0	0	68
172	6-nitroindazole		0.66	−0.77	1.43	−1.487	2.06[a]	0	0	62
173	3,4,4′-trinitrobiphenyl		2.60	1.16	1.44	−2.328	3.26	0	0	68
174	3,9-dinitrofluoranthene		5.02	3.54	1.48	−2.209	4.44	0	1	70
175	3-nitro-1,2,4,7,8-pentachlorodibenzo-1,4-dioxin		−0.33	−1.84	1.51	−1.616	7.84	0	1	74
176	6-nitroindene		0.96	−0.59	1.55	−1.304	2.74	0	0	57, 3
177	1,9-dinitrophenazine		1.26	2.82	−1.56	−2.614	2.29	0	1	65
178	2-methyl-7-nitroindazole		0.23	−1.33	1.56	−1.213	1.77	0	0	62
179	4,4′-dinitrochalcone		−1.42	0.22	−1.64	−1.889	2.73	0	0	71
180	5,8-dinitrobenzo[*ghi*]perylene		4.33	2.65	1.68	−2.182	6.07	0	1	73
181	2-nitro-*p*-phenylenediamine		−1.11	−2.81	1.70	−0.727	0.53	0	0	76
182	8-nitroquinaldine		−2.70	−0.98	−1.72	−1.366	1.99[a]	0	0	69
183	1,8-dinitropyrene	(±0.07)[b]	5.39	3.65	1.74	−2.294	4.44	0	1	57
184	3,7-dinitrofluoranthene		5.09	3.35	1.74	−2.074	4.44	0	1	70
185	3,4-dinitro-1-fluorobenzene		−1.84	−0.05	−1.79	−2.090	1.89	0	0	61
186	5-nitroindoline		−0.17	−2.02	1.85	−0.574	2.07[a]	0	0	62
187	2-nitrotriphenylene		4.09	2.21	1.88	−1.276	5.41	0	1	15
188	3-nitrocarbazole		−0.70	1.19	−1.89	−0.872	3.51	0	1	57, 3
189	1,3,6,8-tetranitronaphthalene[c]		−0.70	1.50	−2.20	−3.025	2.29	0	0	57, 3
190	5-nitroindole[c]		0.57	−1.68	2.25	−0.798	2.13	0	0	62
191	4-nitropenta[*cd*]pyrene[c]		0.77	3.40	−2.63	−2.138	4.35	0	1	63
192	5-nitrochrysene[c]		−0.22	2.41	−2.63	−1.429	5.41	0	1	57, 3
193	2-nitrochrysene[c]		−0.22	2.48	−2.70	−1.478	5.41	0	1	57, 3
194	4-nitro-*o*-phenylenediamine[c]		0.63	−2.40	3.03	−0.857	0.88	0	0	78
195	1,2,3-trichloro-4-nitrobenzene[c]		−2.94	0.18	−3.12	−1.465	3.61[a]	0	0	61
196	4-nitrocarbazole[c]		−2.00	1.49	−3.49	−1.092	3.51	0	1	57, 3
197	1-methyl-2-nitrobenzimidazole[c]		2.54	−1.35	3.89	−1.293	1.58	0	0	62

[a] Experimental log P. [b] Standard deviations of mean value. [c] These compounds were not included in deriving eqs 2–5. [d] Mutagenicity experiment was done by Microbiological Associates Inc., Rockville, MD.

in eq 2 is only 0.65, we believe that the coefficient 1.21 in eq 4, which has not been somewhat compromised by I_1, is closer to the true value and is in agreement with eq 6.

We believe that comparisons of this type are extremely important in developing an understanding of how chemicals react with biological systems and are too often neglected by researchers working in the QSAR area. In dealing with problems as complex as that posed by the data in Table I one cannot rely solely on the correlation coefficient and standard deviation to measure the QSAR's success. A more reassuring finding is that a given QSAR is in agreement with others dealing with the same or similar problems.

It is common for a bilinear log P–biological activity relationship to be symmetrical. Equation 2 deviates from this in that activity increases with a slope of 0.65 for compounds with $P < P_0$, and decreases with a slope of −2.25 for compounds with $P > P_0$. The drastic loss of activity observed in highly lipophilic compounds involves something more than the simple inhibition of movement of the mutagen in its random walk to its sites of action.[14,18a] Some kind of subacute toxicity which affects mutagenic expression may be involved; for example, there might be inhibition of the nitroreductase not caused by small molecules or there might be steric inhibition in the interaction with DNA. This point needs further study with a better selected set of congeners.

The negative coefficient with ϵ_{LUMO} has been justified on the basis of initial reduction of the nitro group as a rate-limiting step in nitroarene activation. That is, compounds with lower LUMO energies should be reduced more easily by cytosolic nitroreductases. Moreover, it was suggested that xanthine oxidase may be one of the enzymes responsible for nitroarene reduction.[19a] To investigate this possibility Tatsumi, et al. studied the reduction of a set of nitrobenzenes to the hydroxylamines by xanthine oxi-

(18) (a) Hansch, C. *Acc. Chem. Res.* **1969**, *2*, 232. (b) McCoy, E. C.; Rosenkranz, H. S. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 1362.

(19) (a) Harada, N.; Omura, T. J. *J. Biochem.* **1980**, *87*, 1539. (b) Tatsumi, K.; Kitamura, S.; Yoshimura, H.; Kawazoe, Y. *Chem. Pharm. Bull.* **1978**, *26*, 1713.

dase.[19b] Correlation of the reduction rates yielded the following QSAR:[7]

$$\log k = -1.53\ (\pm 0.36)\ \epsilon_{LUMO} - 0.06\ (\pm 0.50) \quad (7)$$

$$n = 21,\ r = 0.897,\ s = 0.242$$

$$\log k = 1.09\ (\pm 0.12)\ \sigma - + 1.73\ (\pm 0.11) \quad (8)$$

$$n = 21,\ r = 0.936,\ s = 0.192$$

In our earlier version[7] of eq 7 we employed ϵ_{LUMO} values calculated using MNDO and the correlation was poorer ($r = 0.804$) than the present equation based on AM1 ϵ_{LUMO} values. Especially significant is the observation that the addition of hydrophobic and/or steric terms does not improve either eq 7 or 8. If xanthine oxidase is the key nitroreductase in TA98, then, referring to Scheme I, we would suggest that $\rho_3 + \rho_4$ is rather large, and $h_3 + h_4 \approx 0$ (at least for small nitroaromatics). The behavior of the reduction step is strikingly different from that of the diffusion steps (1,2) where hydrophobicity should be important (h_1, h_2 large) and where electronic effects are expected to be absent ($\rho_1 \approx \rho_2 \simeq 0$). It is of interest that the coefficient with ϵ_{LUMO} in eq 7 is close to that in eq 2.

The positive ρ and its good agreement with ρ of eq 2 supports the conclusion that ρ in steps 3 and 4 should be positive.

An observation about the mutagenicity of nitro compounds, the mechanistic importance of which still eludes us, is the apparent requirement of esterification shown in Scheme I, step 5. It has been shown that *S. typhimurium* lacking the enzyme to acylate the hydroxylamine registers little mutagenicity with some nitroaromatics.[18b] What the electronic and hydrophobic requirements for this step would be are uncertain.

Reduction of the nitro- and nitrosoaromatics are both expected to be facilitated by electron-withdrawing ring systems, i.e., ρ_3 and ρ_4 should both be positive. Electron-withdrawing rings should also inhibit spontaneous conversion of the hydroxylamine to the nitrenium ion, and ρ_6 should be negative to promote potency. If nitrenium ion formation were rate limiting then the overall ρ would be substantially less than that of either ρ_3 or ρ_4. The very good agreement between the ϵ_{LUMO} terms in eqs 2 and 7 suggests that this is not the case. Vance, et al. has argued that inhibition of nitrenium ion formation in nitroarenes with electron-withdrawing rings should boost mutagenicity by increasing the lifetime of the hydroxylamine, thereby allowing it time to diffuse to the DNA.[16] If this were the case, there would be a negative relationship between mutagenicity and ϵ_{LUMO}, and the overall ρ would be negative as observed in eq 2.

Given the differing electronic requirements of steps 3–7 in Scheme I, we have also considered the possibility that ϵ_{LUMO} does not, by itself, adequately model the full range of electronic effects affecting mutagenicity. Attempts to find an optimum figure for ϵ_{LUMO} using either a parabolic or bilinear model were unsuccessful. Attempts to incorporate other molecular orbital parameters, such as the electron density on the N of NO_2, or the electron density on the carbon to which the NO_2 is attached, or the energy of the highest occupied molecular orbital (ϵ_{HOMO}), were also unsuccessful.

We have also considered the possibility that a statistical correction might be necessary for compounds containing more than one nitro group. Separate indicator variables 1 or 0 were assigned to compounds having 2, 3, or 4 nitro groups, however such variables did not improve eq 2. This is not entirely unexpected since even if 4 nitro groups were 4 times as active as one nitro group, this enhanced activity would still be less than the standard deviation of eq 2. One would need a much more carefully tested set of closely related polynitro compounds to establish the statistically expected effect of more than one nitro group. In such a study it would be important that the conformations of the nitro group be similar.

We were unable to find any typical steric factors which might be responsible for lowering the correlation of eq 2 except in the case of 9-nitroanthracene and its analogues (Table II). Of the 11 congeners of this type, two are moderately well predicted, although both are less active than expected: 6-nitrobenzo[*a*]pyrene and 6-nitro-4,5-dihydrobenzo[*a*]pyrene. The others are all very much less active than predicted. It has been noted that the nitro group in these compounds cannot be coplanar with the ring due to the presence of two peri hydrogens, and this steric effect has been used to explain the lower than expected activity that is observed.[16,20a]

It should be noted, however, that changes in nitro conformation can affect both the steric and electronic properties of the compound, either of which might lead to lowered activity. 1-Nitronaphthalene has a lower observed and calculated activity than 2-nitronaphthalene, but both compounds are well fit by eq 2 suggesting that the difference is due to electronic rather than steric factors. 2-Nitrobiphenyls are sometimes poorly fit by eq 2 compared to other biphenyls, but in other instances, the fit is good. While one logically expects steric effects to be significant for many compounds, the noise in the data in Table I seems to preclude establishing their identity.

Table II also contains a number of other compounds which have zero, or unspecified, activity. These compounds could not be used in deriving eq 2 and were not included in Table I. It is encouraging that most of the inactive substances are predicted to have low activity. However, two compounds, 3-nitroperylene and 1-nitrotriphenylene, are predicted to have high activity even though they are observed to be inactive (the activity of 3-nitroperylene was reported as <30 revertants/nmol[49]). We see nothing unusual about these two structures when comparing them to the others in Table I and therefore wonder about the biological test results.

Table I lists nine outlier compounds, marked by *c*, that were not used in the derivation of eq 2. Again, there is no discernable pattern among this group of deviant compounds.

In addition to the above problems, it seems unlikely that all of the variation in bioactivity for such a set of diverse chemicals can be correlated by log P and ϵ_{LUMO}. In fact, the necessity for two indicator variables reveals that additional factors do play a role in determining the mutagenicity of these two classes of congeners. The very low activity of the acenthrylenes is surprising in that most of the other large polycyclic aromatic compounds are reasonably well fit. This deviant group cries out for further investigation.

The halogenated nitroaromatics also form an interesting group. 2,4-Dinitrofluorobenzene is about 200 times as active as expected (eq 2), and the same is true for 4-nitrofluorobenzene. 2,4-Dinitrochlorobenzene is also more active than expected. It may be that these halogen compounds alkylate DNA directly. Other similar halogen compounds, such as 3,4-dinitrofluorobenzene, do not fit the pattern however.

(20) (a) Fu, P. P.; Ni, Y.-C.; Zhang, Y.-M.; Helflich, R. H.; Wang, Y.-K.; Lai, J.-S. *Mutat. Res.* **1989**, *225*, 121. (b) Private Communication from Dr. S. A. Kafafi, Division of Environmental Chemistry, Johns Hopkins University, Baltimore, MD.

Table II. Properties of Inactive Compounds, Those Without a Specific Value of Activity and Those Having 9-Nitroanthracene Type Structure

entry	compd	log revertants/nmol obsd	log revertants/nmol pred	ϵ_{LUMO}	log P	I_a	I_1	ref
1	6-nitrobenzo[*a*]pyrene[c]	1.64	2.39	−1.767	5.87	0	1	57, 3
2	6-nitro-4,5-dihydrobenzo[*a*]pyrene[c]	0.30	1.35	−1.350	6.16	0	1	57, 3
3	1-methyl-5-nitroindoline	*a*	−1.54	−0.560	2.86	0	0	62
4	1-methyl-5-nitroindole	*a*	−1.51	−0.746	2.50	0	0	62
5	nitrobenzene	*a*	−1.45	−1.089	1.85[b]	0	0	4, 61
6	1-methyl-7-nitroindole	*a*	−1.34	−0.868	2.50	0	0	62
7	4-nitroindole	*a*	−1.33	−1.050	2.13	0	0	62
8	5-nitroIndazole	*a*	−1.30	−1.117	2.03[b]	0	0	62
9	2-fluoronitrobenzene	*a*	−1.24	−1.321	1.69[b]	0	0	61
10	7-nitroindole	*a*	−1.22	−1.125	2.13	0	0	62
11	3-fluoronitrobenzene	*a*	−1.05	−1.358	1.90[b]	0	0	61
12	1,2-dinitrobenzene	*a*	−0.60	−1.834	1.58[b]	0	0	61
13	3-chloronitrobenzene	*a*	−0.79	−1.306	2.41[b]	0	0	61
14	4-chloronitrobenzene	*a*	−0.73	−1.360	2.39[b]	0	0	61
15	7-nitrobenz[*a*]anthracene[c]	−0.52	2.82	−1.723	5.41	0	1	57, 3
16	2,4-dichloronitrobenzene	*a*	−0.30	−1.351	3.09[b]	0	0	61
17	9-nitroanthracene[c]	−0.95	3.01	−1.755	4.78[b]	0	1	57, 3
18	3,4-dichloronitrobenzene	*a*	−0.02	−1.538	3.12[b]	0	0	61
19	*trans*-1,2-dihydro-9-nitroanthracene-1,2-diol[c]	*a*	0.54	−1.392	1.38	0	1	3
20	*trans*-3,4-dihydro-9-nitroanthracene-3,4-diol[c]	*a*	0.62	−1.447	1.38	0	1	3
21	6-nitro-7,8,9,10-tetrahydrobenzo[*a*]pyrene[c]	*a*	1.07	−1.717	6.57	0	1	3
22	*trans*-8,9-dihydro-7-nitrobenz[*a*]anthracene-8,9-diol[c]	*a*	1.63	−1.636	2.55	0	1	3
23	*trans*-3,4-dihydro-7-nitrobenz[*a*]anthracene-3,4-diol[c]	*a*	1.87	−1.808	2.55	0	1	3
24	1-nitrotriphenylene	*a*	1.98	−1.113	5.41	0	1	15
25	6-nitro-7-hydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene[c]	*a*	3.00	−1.748	4.78	0	1	3
26	11-hydroxy-7-nitrobenz[*a*]anthracene[c]	*a*	3.10	−1.820	5.06	0	1	64
27	3-nitroperylene	<1.48	2.50	−1.845	5.87	0	1	57

[a] Inactive. [b] Experimental log P. [c] 9-Nitroanthracene type structure.

Table III. Heterocyclic Compounds Poorly Predicted by Eq 2 and Their Physicochemical Parameters

entry	compd	log revertants/nmol obsd	log revertants/nmol pred	dev	ϵ_{LUMO}	log p[a]	I_a	I_1	ref
1	5-nitro-2-furaldehyde	0.64	−1.04	1.68	−1.785	1.01	0	0	72
2.	5-nitro-2-furanacrolein	1.97	−0.97	2.94	−1.851	0.96	0	0	72
3	nitrofurazone	1.02	−2.05	3.08	−1.412	0.23	0	0	72
4	nitrofurantoin	1.03	−2.18	3.21	−1.645	−0.47	0	0	72
5	niridazole	2.59	−1.43	4.02	−1.526	0.95	0	0	72
6	furazolidone	2.24	−2.11	4.35	−1.503	−0.04	0	0	72

[a] Experimental log P.

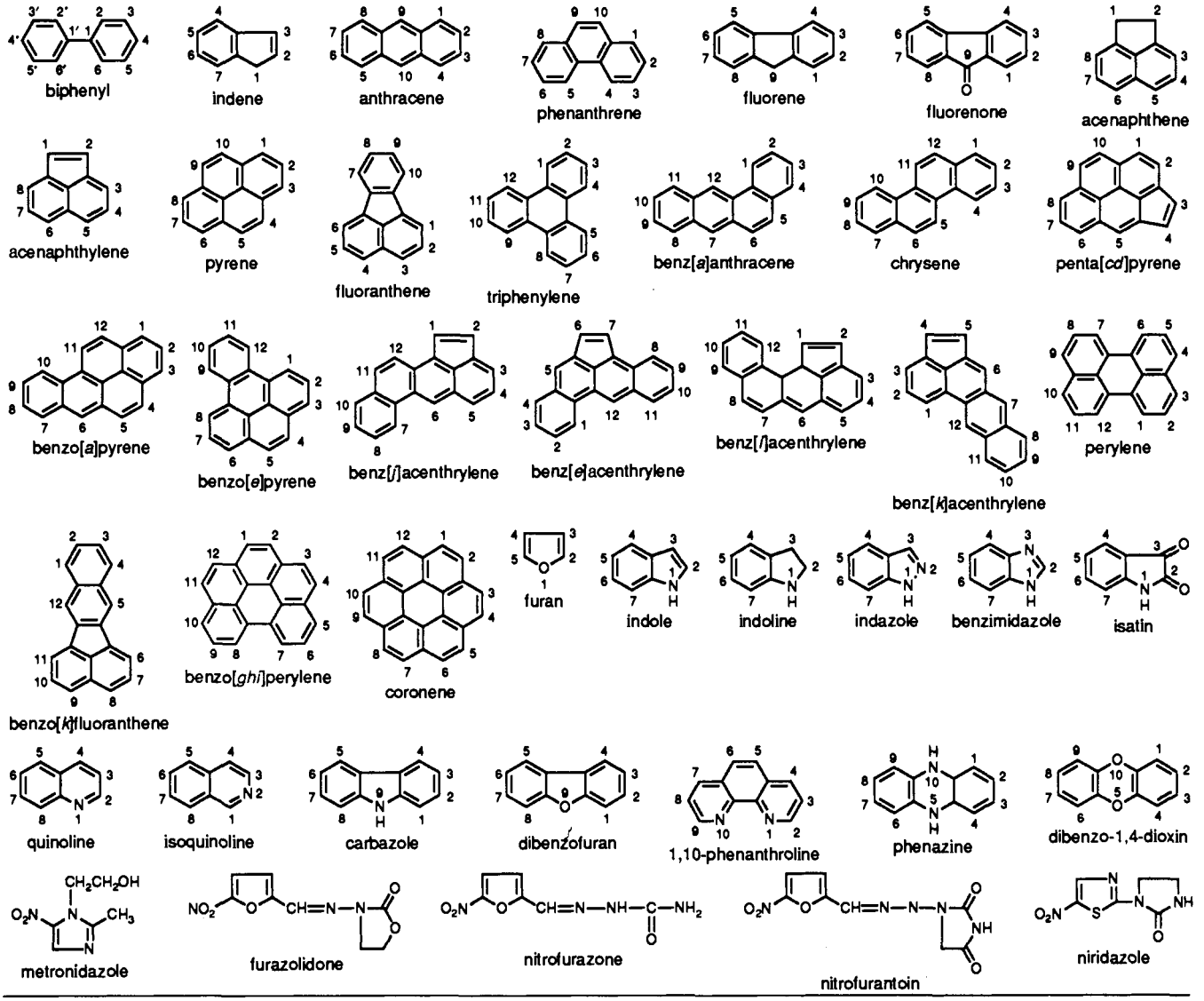
In Table III there are 5 examples of complex heterocycles containing a nitrofuran moiety which are all much more active than predicted by eq 2. This maybe due to their unusual structure, a different mechanism of reaction or to a shortcoming in the AM1 calculations. Kafafi has observed that the properties of simple furans (in contrast to those that are part of a large aromatic system, e.g. dibenzofuran) are not well estimated by the AM1 methodology.[20b] One thiazol, niridazole is also much more active than predicted, and we have found a similar difficulty with this ring system in another QSAR of mutagenicity.[12]

One other family of interest is the nitrofluoranthenes. Vance, et al.[21a] have carefully analyzed these compounds in terms of peri effects and their influence on the orientation of the nitro group. The low activity of 7-nitrofluoranthene was attributed to the nonplanarity of the nitro group according to this analysis. Table I contains data for 16 nitrofluoranthenes, including six mononitrofluoranthenes, seven dinitrofluoranthenes, and three trinitrofluoranthenes. Among the mononitrofluoranthenes, only 8-nitrofluoranthene is poorly fit, being much more active than expected. Among the dinitrofluoranthenes, only 3,7-dinitrofluoranthene is poorly fit. All three trinitrofluoranthenes are reasonably well fit. Vance, et al. suggests that beyond the conformation of the NO_2 groups one must also consider the geometry and the stability of the hydroxylamine groups. For example, electron-withdrawing elements should promote reduction and stabilize the hydroxylamine so that the ϵ_{LUMO} term could account for both problems. Vance, et al. also noted the depressing effect of mutagenicity by electron-releasing substituents pointing out that *p*-dinitrobenzene is mutagenic while *p*-nitrophenol is not. Such comparisons are important, but one must also consider the hydrophobic character of structural changes.

The indicator variable I_1 in eq 2 brings to light an important structural feature of these mutagens, namely, a 75-fold increase in mutagenicity for compounds containing three or more rings. Since we have also found this same I_1 term (unpublished results) in a QSAR describing the mutagenicity of aromatic amines in TA98 we believe that I_1 constitutes a structural variable that is independent of hydrophobic and electronic properties. It is generally believed that activation of nitro compounds and amines proceed through a common hydroxylamine intermediate, generated by either reduction of the nitro compound or oxidation of the amine respectively. Rosenkranz and Mermelstein[57] have noted in qualitative terms that large polycyclic aromatic nitro compounds appear to be more

(21) (a) Vance, W. A.; Levin, D. E. *Environ. Mutagen.* 1984, *6*, 797. (b) Rosenkranz, H. S. *Mutat. Res.* 1988, *196*, 1.

Table IV. Parent Structures of Aromatic and Heterocyclic Compounds



potent with strains TA98 and TA1538 and suggested that this effect is due to frame shift mutation, possibly arising from intercalation of the mutagen into DNA. The unusually high mutagenic (and possibly carcinogenic) activity associated with the larger mutagens has definite implications for drug design. Compounds with three or more rings, and which contain moieties that can be metabolically transformed into electrophiles should be regarded as poor starting points for drug development.

It has been pointed out that nitroaromatics per se tend to give erratic results in the Ames test. Our attempts at modeling nitroaromatic mutagenicity have also been complicated by the varying quality of the test results produced by the many different laboratories from which the data comes. In order to get some notion of the magnitude of this error, six highly purified compounds were tested in TA98 (see Experimental Section) yielding the following deviations from the mutation rates predicted by eq 2: 5-nitrobenzimidazole, −0.09; 2-nitrodibenzofuran, −0.59; 1,3,8-trinitronaphthalene, −0.39; metronidazole, 1.18; 5-nitroisoquinoline, −0.67; and 5-nitro-1,10-phenanthroline, −0.74. The average *absolute* deviation for the six compounds is 0.61 and is significantly less than the standard deviation (0.886) of eq 2. On the one hand, this result indicates the reliability of eq 2 and its ability to handle wide variations in structure. For example, all but one of the six compounds is a heterocycle, and yet relatively few heterocycles were used to construct eq 2. Likewise, the dibenzofuran is well fit in contrast to the small furans listed in Table III. On the other hand, the smaller average absolute deviation suggests that the careful purification and testing of nitroaromatics could yield a considerably sharper QSAR.

Many applications can be found for eq 2, in addition to correlating a broad range of mutagenicity data. For example, eq 2 provides a means for verifying the quality of experimental mutation rate measurements. A poorly predicted compound is a natural candidate for further purification and retesting, and other similar chemicals should also be tested to validate each particular deviation. There are also instances (cancer chemotherapy for example) where one might want to use a nitro group in a drug, and eq 2 shows how one can construct a structure containing minimal mutagenic activity. Since log P and ϵ_{LUMO} are easily calculated, even for hypothetical structures, those preparing nitro compounds as research intermediates can readily estimate the hazards associated with various nitro compounds. For example, it was clear from our QSAR on aromatic triazenes that by varying log P and quantum chemical parameters one could modulate the pharmacophore to produce nonmutagenic or extremely mutagenic triazenes (approaching aflatoxin B).[12]

Equation 2 can also be used to estimate the mutagenic properties of molecules for which an experimental mutation rate cannot be measured. For example, accurate mutagenic rates for antimicrobial agents in *S. typhimurium* are difficult to determine due to the compounds' toxicity. We have attempted to measure the mutation rate of chloramphenicol in TA98 and were unable to establish a significant mutation rate at any dose less than the toxic dose. Rosenkranz[21b] has also reviewed chloramphenicol toxicity and mutagenicity and has described the many difficulties associated with studying this highly effective antibacterial agent. However, by combining chloramphenicol's ϵ_{LUMO} and log P values, −1.311 eV and 1.14, respectively, with eq 2 we can estimate its mutagenic rate as −1.61 revertant/nmole.

In summary, our QSAR analysis permits the following conclusions.

1. Hydrophobicity is clearly a major factor in the mutagenic potency of aromatic nitro compounds. Surprisingly this fact has been almost completely overlooked. Associated with this is the fact that bioconcentration of xenobiotics is highly correlated with log P.[22-25] These two factors support the concept of striving for minimal hydrophobicity in drug design[26,27] and in the development of industrial chemicals.

2. Electron-attracting elements conjugated with nitro groups enhance mutagenicity.

3. Compounds with three or more fused rings are much more mutagenic, other factors being equal, than those with one or two.

4. Finally, a most important message from this study for those involved in the design of bioactive molecules is that quantum chemistry has clearly reached the point where it can free us from some of the constraints of the Hammett equation. Although it is clear that Hammett constants can yield sharper correlations than molecular orbital indices,[12] the latter can provide mechanistic insight where structural variation is far beyond the reach of the Hammett approach as in the present case. The question arises as to why more examples of structure–activity relationships based on quantum chemical calculations have not appeared. We believe that the primary reason is that theoretical chemists have been very slow to appreciate the overriding importance of the hydrophobic properties of chemicals in modifying their biological properties. Unless hydrophobicity is properly accounted for it can completely mask the role of electronic effects on structural changes.

A special set of compounds, nitrocompounds containing an amino group, has also been included in Table I. This group was withheld in the early stages of the study because of its ambivalent character of carrying two known mutagenic moieties. However, since the amino group requires microsomal activation to achieve full mutagenic potency and our data set is limited to studies without such activation we have included this group assuming that the amino group affects mutagenicity only by its effect on log P and ϵ_{LUMO}.

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(24) Van Gestel, C. A. M.; Otermann, K.; Canton, J. H. *Regulat. Toxic. Pharmacol.* 1985, *5*, 422.
(25) Saarikoski, J.; Viluksela, M. *Ecotox. Environ. Safety* 1982, *6*, 501.
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(27) Hansch, C.; Björkroth, J. P.; Leo, A. J. *Pharm. Sci.* 1987, *76*, 663.

Table V. Comparison of Experimental and Calculated log P Values

entry	compd	log P expt	log P calcd	dev
1	2,4,7-trinitro-9-fluorenone	2.42	2.59	−0.17
2	2,7-dinitro-9-fluorenone	2.84	2.81	0.03
3	2,7-dinitrofluorene	3.35	3.41	−0.06
4	3-nitro-9-fluorenone	3.06	2.99	0.07
5	2-nitronaphthalene	3.24	3.06	0.18
6	2-nitrofluorene	3.37	3.67	−0.30
7	1-nitronaphthalene	3.19	3.06	0.13
8	5-nitroacenaphthene	3.85	3.81	0.04
9	2-(acetylamino)-7-nitrofluorene	3.08	2.82	0.26
10	1,3-dinitronaphthalene	2.83	2.80	0.03
11	1,8-dinitronaphthalene	2.52	2.80	−0.28
12	5-nitroquinoline	1.86	1.86	0.00
13	6-nitroquinoline	1.84	1.86	−0.02
14	9-nitroanthracene	4.78	4.23	0.55
15	2-amino-7-nitrofluorene	3.06	2.57	0.49
16	2,4,5,7-tetranitro-9-fluorenone	2.40	2.35	0.05
17	1,5-dinitronaphthalene	2.58	2.80	−0.22
18	1,3,8-trinitronaphthalene	2.30	2.55	−0.25
19	1,3-dinitrobenzene	1.49	1.63	−0.14
20	1,3,5-trinitrobenzene[a]	1.18	1.37	−0.19
21	4-nitrotoluene[a]	2.34	2.38	−0.04
22	2,4-dinitrotoluene[a]	2.04	1.89	0.15
23	2,6-dinitrotoluene[a]	2.02	1.87	0.15
24	nitrobenzene	1.85	1.89	−0.04
25	1-methyl-5-nitroimidazole[a]	0.16	−0.01	−0.17
26	2-methyl-5-nitroimidazole[a]	0.06	0.27	−0.21
27	1,2-dimethyl-5-nitroimidazole[a]	0.30	0.26	0.04
28	2-nitroanisole	1.73	1.82	−0.09
29	4-nitroanisole	2.03	2.10	−0.07
30	2-nitrobenzaldehyde	1.74	1.50	0.24
31	8-nitroquinoline	1.44	1.86	−0.42
32	6-methoxy-8-nitroquinoline	1.87	2.16	−0.29
33	4-nitroacetophenone	1.53	1.49	0.04
34	4-nitrobenzaldehyde	1.56	1.50	0.06
35	nitrofurantoin	−0.47	[b]	
36	nitrofurazone	0.23	0.20	0.03
37	2,4-dinitrophenylhydrazine	1.46	1.72	−0.26
38	2-fluoronitrobenzene	1.69	1.75	−0.06
39	3-fluoronitrobenzene	1.90	2.03	−0.13
40	4-fluoronitrobenzene	1.80	2.03	−0.23
41	2-chloronitrobenzene	2.24	2.32	−0.08
42	3-chloronitrobenzene	2.41	2.60	−0.19
43	4-chloronitrobenzene	2.39	2.60	−0.21
44	2,5-dichloronitrobenzene	2.90	3.03	−0.13
45	2,3-dichloronitrobenzene	3.05	3.03	0.02
46	2,4-dichloronitrobenzene	3.09	3.03	0.06
47	3,4-dichloronitrobenzene	3.12	3.31	−0.19
48	1,2,3-trichloronitrobenzene	3.61	3.74	−0.13
49	1,2-dinitrobenzene	1.58	1.63	−0.05
50	1-chloro-2,4-dinitrobenzene	2.17	2.06	0.11
51	8-nitroquinaldine	1.99	[b]	
52	furazolidone	−0.05	[b]	
53	5-nitro-2-furanacrolein	0.97	[b]	
54	5-nitro-2-furaldehyde	1.01	0.68	0.33
55	5-nitroindoline	2.07	2.02	0.05
56	6-nitroindoline	1.92	2.02	−0.10
57	5-nitroindazole	2.03	1.75	0.28
58	6-nitroindazole	2.06	1.75	0.31
59	2-methyl-5-nitrobenzimidazole	1.94	1.71	0.23
60	5-nitrolsatin	0.47	0.59	−0.12
61	*N'*-(5-nitro-2-furfurylidene)-5-nitro-2-furanacrylohydrazide	2.79	[b]	
62	niridazole	0.95	0.03	0.92
63	metronidazole	−0.02	−0.59	0.57

[a] Not included in deriving eq 2. [b] No value due to missing fragments.

Experimental Section

Chemicals. A number of chemicals were commercial products or synthesized by us. These substances were purified by HPLC. The purities were shown by HPLC to be 99.9% or better. 5-Nitrobenzimidazole, metronidazole, 5-nitro-1,10-phenanthroline, and 5-nitroisoquinoline were purchased from Aldrich Chemical Company, Milwaukee, WI. 2-Nitrodibenzofuran was synthesized

by following the method of Ames and Opalko,[28] mp 153–54 °C (lit.[28] mp 153–55 °C). 1,3,8-Trinitronaphthalene was synthesized by direct nitration of 1,8-dinitronaphthalene with concentrated sulfuric acid and nitric acid (*d* 1.5), mp 217–218 °C (lit.[29] mp 223 °C). The structure was verified by X-ray crystallography. The details of X-ray crystallographic analysis of this molecule will be published separately.

Electronic Descriptors. The electronic descriptor, ϵ_{LUMO}, was calculated by using the AM1 method (VAX version 4.10, Quantum Chemistry Program Exchange no. 455) developed by Dewar and co-workers.[13]

The ring structure of molecules containing the following ring systems were all taken from X-ray crystallographic studies: anthracene,[30,31] phenanthrene,[32] fluorene,[33] 9-fluorenone,[34,35] acenapthene,[36] pyrene,[37] fluoranthene,[38] benzo[*a*]pyrene,[39] perylene,[40] benzo[*ghi*]perylene,[41] furan,[42] indazole,[43] benzimidazole,[44] isatin,[45] quinoline,[46] isoquinoline,[47] carbazole,[48] phenazine,[49] metronidazole,[50] biphenyl,[51] anisole,[52] and chalcone.[53]

The starting ring geometries of simple benzene derivatives (except biphenyls, anisoles, and chalcones) were constructed from standard bond lengths and bond angles and then completely optimized by using the AM1 method. Ring geometries for the remaining molecules were obtained by using the CHEMLAB-II program.[54] All rings were regarded as planar unless there was X-ray evidence of deviations from planarity, or when the molecule included partially saturated rings.

Substituent geometries were optimized by using the AM1 method assuming the substituent to be coplanar with the adjacent ring. Substituent conformations were optimized in those cases where there was obvious steric hindrance due to adjacent substituents or peri hydrogens. Nitro group geometries were completely optimized in all cases by using the AM1 method.

Octanol/Water Partition Coefficient. The octanol/water partition coefficient of 63 compounds reported in Table V were measured in our laboratory by following a reported method[55] or obtained from the literature and showed good agreement with the calculated log *P* values except for metronidazole and niridazole. Seven of the 63 compounds in Table V were not needed in our present study but were included to show that experimental and calculated log *P* values for both aromatic and heterocyclic compounds agree well. The log *P* values for the rest of the nitro compounds were calculated by using CLOGP program release 3.54.[56]

Mutagenicity Assay. The mutagenicity of several nitro compounds was determined for us by Microbiological Associates of Rockville, MD. 5-Nitrobenzimidazole, metronidazole, 5-nitro-1,10-phenanthroline, 5-nitroisoquinoline, 2-nitrodibenzofuran, and 1,3,8-trinitronaphthalene were tested by following the methods described by Ames, et al.,[76] deSerres and Shelby,[77] Maron and Ames,[78] and Yahagi, et al.[79] on *S. typhimurium* TA98 without using metabolic activation (S9).

The mutagenicity data of the rest of the nitro compounds were obtained from the literature (see Table I).

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