Comparative QSAR and the Radical Toxicity of Various Functional Groups

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Contents

I. Introduction

This review sets out to survey the important contributions of physicochemical descriptors that are utilized to characterize chemical reactivity as well as biological activity and to focus attention on certain functional groups that have a propensity to participate in radical-mediated reactions in vitro and in vivo.[1,2] The importance of radical species in homeostasis warrants a thorough study of their quantitative structure−activity relationships (QSAR), and thus, the process of "lateral validation" is used to establish and enhance lateral support for these QSAR models.

Since its advent almost 40 years ago, QSAR has evolved on many fronts.[3,4] Numerous parameters have been developed and various types of regression models have been devised.[5] Yet the original approach based on Hammett parameters, octanol/water partition coefficients, steric parameters, and polarizability factors presents the best systematized approach to comparative QSAR. It allows for comparisons among biological QSAR and between QSAR from physical organic chemistry and biological systems.[6] The extensive body of work that has been published using this approach and multiple linear regression (MLR) techniques allows for adequate comparison and meaningful interpretation based on the parameters and their statistical significance. Statistics alone, however, do not ensure a robust result.

The development of a science of QSAR is predicated on the ability to make lateral comparisons between physicochemical-based models and/or biological models in order to validate original hypotheses. This can only be accomplished with a uniform set of parameters with clearly defined meaning as building blocks. Since a biological target as simple as a single cell is remarkably complex, the perturbation of such a system with a set of 20 'congeneric' chemicals and the ability to obtain uniform data is a daunting task. Even the use of well-established parameters does not diffuse the uncertainty concerning the dependent variable and the uniformity of its values, i.e., do these values **all** represent a single ligand−receptor interaction with a minimum of side reactions?[7] One way to address this issue is to begin with a parent molecule that induces some specific activity and to thoughtfully make systematic changes that will yield evidence for or against the major effects: electronic, hydrophobic, and steric. Early toxicology studies were guilty of sequestering together heterogeneous classes of chemicals in order to develop an adequate model.[8] In some instances, this approach worked well, but in others a modest r^2 and a low q^2 indicated that the model was off kilter, i.e., all members of the test set were not affecting cellular biochemistry in a uniform manner. To recognize and understand such problems, considerable background in mechanistic organic chemistry, biochemistry, and biology, statistics, and computerized model building is needed. It is also beneficial to have the resources to facilitate the syntheses of a well-designed set of reasonable congeners and the biological testing of these molecules.

Various approaches have been used to describe structure−property correlations based on different descriptors. Topological and connectivity descriptors as first described by Randic and then extensively developed by Kier and Hall have seen substantial use in correlating chemical properties such as boiling point, molar refraction, and water solubility as well as biological activities.[9−11] The latter include applications in environmental toxicology and pharmacology.[12−14] Despite their use, models incorporating these descriptors are difficult to compare because of their paucity and lack of transparency (in terms of physicochemical interpretation). Many computational approaches consider numerous parameters with a variety of mathematical descriptors to obtain 'reasonable' QSAR, but none have the ability to compare the

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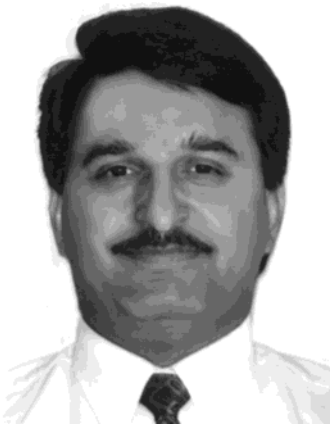
Rajni Garg received her M.Sc. degree in Chemistry (1984) from Meerut University and M.Phil. (1988) degree from Delhi University, India. Her M.Phil. dissertation work was on peptide synthesis. She was a faculty member in the Chemistry Department of Birla Institute of Technology and Science, Pilani, India, from 1991 to 1996, where she taught organic and physical chemistry. She received her Ph.D. degree in 1996 under the supervision of Professor S. P. Gupta. Her doctoral work was on QSAR studies on anti-HIV agents. In February 1997, she joined Professor Corwin Hansch as a postdoctoral researcher, and she is currently involved in building a C-QSAR databank. Her research interests include QSAR and computer-assisted drug design.

various models that are generated across functional groups.

The development of mechanistic organic chemistry provides some insight. Intense work by numerous physical organic chemists for most of the 20th century was required before a reasonable understanding of chemical reactivity in mathematical terms was obtained.[15−18] Chemical−chemical interactions are considerably different from mostly complex chemical−cellular interactions, although excellent progress in understanding has been made in cases where the dependent variable is uniform.[19,20] Lateral validation requires an extensive computerized database of QSAR based on data from all accessible literature sources. Such a database exists, and it contains 8 700 equations from mechanistic organic

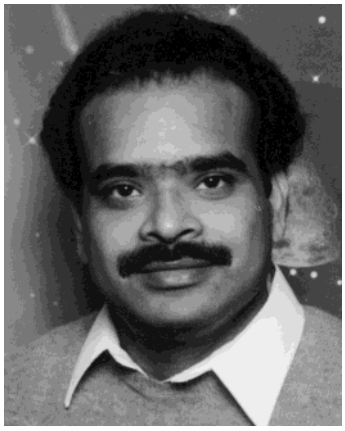


Sanjay Kapur graduated from the Panjab University Chandigarh, India, with his MSc(Hons) degree and received his Ph.D degree from the Postgraduate Institute of Medical Education and Research, Chandigarh, India, in 1993. His career interests have centered around research in toxicology and drug metabolism. The work for which he received the Young Scientist award was aimed at understanding the mechanisms of *N*-nitrosodiethylamine-induced toxicity in chemical carcinogenesis/drug metabolism. After receiving his doctorate degree, he spent two years teaching radiation safety and medical biophysics courses at PGIMER. Then he moved to CHUL Research Center in Quebec City, Canada, to hold a postdoctoral position pursuing research in the areas of signal transduction and free-radical toxicology. In 1997 he joined the renowned QSAR group at Pomona College, where he is currently involved in studying QSAR of free-radical-mediated cytotoxicity of substituted phenols and other active hydrogen compounds in various cell culture systems.

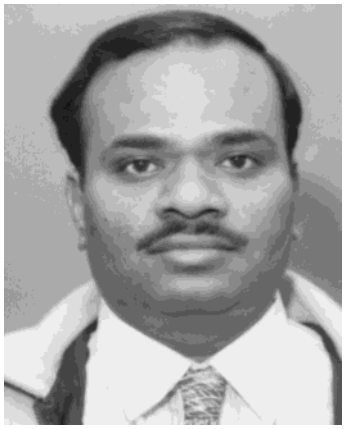
Alka Kurup received her undergraduate degree in Pharmacy in 1981 from Birla Institute of Technology and Science in Pilani, India. In 1988, she received her Masters degree from the College of Pharmacy in Manipal, India. For two years she assumed Inchargeship and Quality Control of the Pharmacy Manufacturing Wing at Kasturba Medical College in Manipal. In 1991, she joined Birla Institute of Technology and Science as a faculty member in the Department of Pharmacy. She completed her Ph.D. degree in 1997 under the supervision of Professor S. P. Gupta with her thesis regarding QSAR studies of anticancer drugs. She joined Professor Hansch's group in July 1998 to pursue postdoctoral research. Currently, she is involved in building the C-QSAR database. Her research interests include QSAR and computer-aided drug design.

chemistry and 8 700 from biological reactions.[6] The availability of suitable data allows for the generation of a statistically valued model and its subsequent inclusion in the database. It is of interest to note that a few unusual esoteric studies have been eye openers in this process.[21]

In 1961, a few parameters were explored to gain understanding of the elements of QSAR. However, it was not until the early 1980s when measurement of the activities of various carefully designed sub-



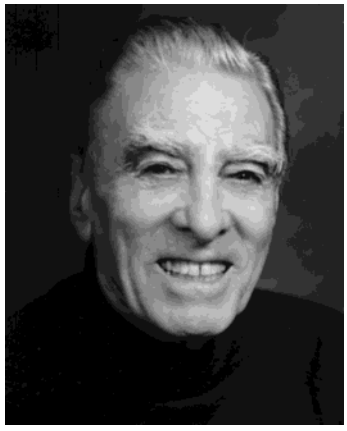
Rajeshwar P. Verma was born in 1966 in Barh (India). He received his B.Sc.Hons. (1984), M.Sc. (1988), and Ph.D. (1992) degrees in Chemistry from Magadh University, Bodh-Gaya. He spent a year at the same university as a postdoctoral fellow with Professor K. S. Sinha. He joined Roorkee University (Now IIT Roorkee) as a research associate and worked with Professor S. M. Sondhi (1994–1997). He also worked as a Lecturer of Chemistry at Gurukula Kangri University, Hardwar (1994–1995). He won a Research Associateship Award in December 1994 from the Council of Scientific & Industrial Research, New Delhi (India). In 1997, he moved to Pomona College to join renowned Professor Corwin Hansch and Cynthia Selassie's group as a research associate. His research interests include the following: Isolation, characterization, and synthesis of natural products derived from medicinal plants; synthesis of phenolic/active hydrogen compounds and biologically important heterocycles; application of principles of QSAR to the study of antifolates, multidrug resistance, and free-radical-mediated toxicity of phenolic/active hydrogen compounds.



Suresh B. Mekapati was born 1969 in India. He obtained B.S. degree in Pharmacy (1990) from Annamalai University, Chidambaram, and M.S. degree in Pharmacy from Birla Institute of Technology and Science (B.I.T.S.), Pilani, India. He was a faculty member in Pharmacy Department of B.I.T.S., Pilani, under the supervision of Professor S. P. Gupta. His doctoral work was on QSAR studies on anti-HIV agents. In February 2000 he joined Professor Hansch's laboratory at Pomona College to pursue postdoctoral research, and he is currently involved in building a C-QSAR database. His research interest include QSAR and computer-assisted drug design (CADD).

strates/inhibitors of highly purified enzymes (whose X-ray crystal structures were known) led to the formulation of rigorous models that confidence was instilled in this approach. The resulting QSAR were visualized via 3-D molecular graphics, which confirmed the earlier findings and validated this approach nearly 20 years after its birth.[22−25]

In this review, the chemical reactivity of various functional entities such as the nitro, amino, olefinic, hydroxy, and carboxaldehyde groups are examined. These reactivities are then compared to their biological activities versus isolated receptors in cellular systems or in vivo.



Corwin Hansch received his undergraduate education at the University of Illinois and his Ph.D. degree in Organic Chemistry from New York University in 1944. After working with the DuPont Company, first on the Manhattan Project and then in Wilmington, DE, he joined the Pomona College faculty in 1946. He has remained at Pomona except for two sabbaticals: one at the Federal Institute of Technology in Zurich with Professor Prelog and the other at the University of Munich with Professor Huisgen. The Pomona group published the first paper on the QSAR approach relating chemical structure with biological activity in 1962. Since then, QSAR has received widespread attention. Dr. Hansch is an honorary fellow of the Royal Society of Chemistry and recently received the ACS Award for Computers in Chemical and Pharmaceutical Research for 1999.

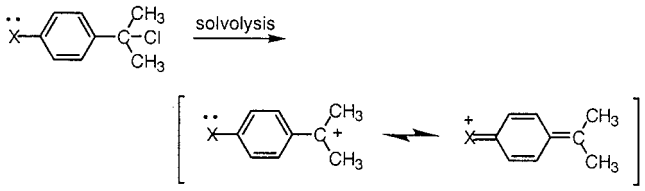
II. Methodology

All physicochemical descriptors are auto-loaded, and multiregression analyses (MRA) to derive the QSAR are executed with the C-QSAR program.[26] The utility of the QSAR program in comparative correlation analysis has been discussed.[19,27] While comparing different QSAR, however, it must be borne in mind that variations in quality of testing in different laboratories will have an effect that cannot be estimated. Calculation of bond dissociation energies (BDE) were done by utilizing Jaguar 3.0.[28]

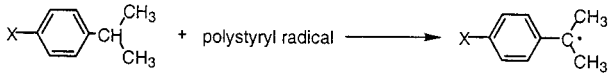
The parameters used in this report have been discussed in detail along with their applications.[19] However, in brief, Hammett σ, σ^-, and σ^+ constants are electronic parameters which apply to substituent effects on aromatic systems.[16,29−31] The normal σ for substituents on aromatic systems where strong resonance between substituent and reaction center does not occur is defined as $\sigma = \log K_X - \log K_H$, where K_H is the ionization constant for benzoic acid (normally in water or in 50% ethanol) and K_X is that for substituted benzoic acid.[29] σ^- and σ^+ are employed where there is a strong resonance interaction between substituent and reaction center. Of these, σ^- is defined using the ionization constants from phenols or anilines similar to σ: $\sigma^- = \log K_X - \log K_H$, where K refers to the ionization of anilines or phenols. While σ and σ^- are defined via equilibrium constants, σ^+ is the Brown variant of the Hammett σ constant and is defined by the rate of solvolysis of cumyl chlorides in 90% acetone/10% water as illustrated in Scheme 1.[31]

Taft's σ^* applies to electronic effects in aliphatic systems.[32] F is the field (inductive) effect of a substituent.[33] E_{LUMO} is the energy of the lowest unoc-

Scheme 1. Solvolysis of X-Cumyl Chlorides



Scheme 2. Abstraction of Benzylic Hydrogen



cupied molecular orbital and is a quantum chemical parameter that corresponds to the electron affinity of the molecule.[34] BDE is the homolytic bond dissociation energy.[35] E_R is Otsu's radical parameter and is based on radical abstraction of •H from $X-C_6H_4-CH(CH_3)_2$,[36] see Scheme 2.

Clog *P* represents the calculated partition coefficient in octanol/water and is a measure of hydrophobicity of the whole molecule, while Mlog *P* is the measured partition coefficient. log *P* refers to the value of partition coefficient reported in the original publication. π is the hydrophobic parameter for substituents attached to benzene.[27]

CMR is the calculated molar refractivity for the whole molecule. MR is calculated from the Lorentz−Lorenz equation and is described as follows: $(n^2 - 1/n^2 + 2)(MW/d)$, where *n* is the refractive index, MW is the molecular weight, and *d* is the density of a substance. Since there is very little variation in *n*, MR is largely a measure of volume with a small correction for polarizability.[37] We have scaled our MR values by 0.1. MR can be used for a substituent or for the whole molecule. Clog *P* and CMR are the calculated values for the neutral form of partially ionized compounds.

B1, B5, and L are Verloop's sterimol parameters for substituents.[38] B1 is a measure of the width of the first atom of a substituent, B5 is an attempt to define the overall volume, and L is the substituent length. $B1_X$ pertains to *X*-position substituents on the phenyl ring (e.g., $B1_2$, $B5_2$, and L_2 are used for ortho-positioned substituents). *Es* is Taft's steric constant.[32] It is related to the acid-catalyzed hydrolysis of α-substituted acetates and represents the steric effect affecting intramolecular and intermolecular hindrance to the reaction or binding. The indicator variable *I* is assigned a value of 1 or 0 for special features with unique effects that cannot be parametrized and have been explained wherever used.

All the data have been collected from the literature (see individual QSAR for respective references). Various biological indices have been used in different QSAR. Although it is not possible to give the details (for a more detailed discussion readers are referred to the original publications), a brief explanation of some of the main parameters is provided. In most cases, log 1/*C* is the dependent variable and defines the biological parameter for these QSAR equations.[39] *C* is the molar concentration of a compound that induces a certain biological effect, e.g., LC_{50}, IC_{50}, or EC_{50}. LC_{50} is defined as the molar concentration of a compound that induces 50% lethality in organisms (bacteria, fish, etc.) under study. IC_{50} is the molar concentration of a compound that causes 50% inhibition in growth of cells, bacteria, fungi, plants, etc. EC_{50} represents the molar concentration of a compound that impacts a biological response by 50%.

Rate constants (*k*) have been utilized in different equations, and the subscripts 2 and 3 denote the order of the reaction rate. The relative rate of reaction of a substituted molecule versus an unsubstituted compound is represented by k_{rel}. Rates of reaction in enzyme−ligand interactions are governed by the Michaelis−Menten equation, and the following steady-state kinetic constants are utilized: K_M, k_{cat}, and k_{cat}/K_M.[40,41] K_M is the Michaelis constant, and k_{cat} is the catalytic constant or turnover number of the enzyme. Thus, k_{cat} is a first-order rate constant, while k_{cat}/K_M is an apparent second-order rate constant and is referred to as the "specificity constant". V_{max} is the limiting value of the initial rate γ of formation of products. Thus, V_{max}/K_M is a modified specificity constant, $k_{cat}[E_0]/K_M$, where E_0 is the concentration of the enzyme. In QSAR equations, *n* is the number of data points, *r* is the correlation coefficient, *s* is the standard deviation, *q* is a measure of quality of fit and calculated as described by Cramer et al.,[42] and the data within the parentheses are for the 95% confidence interval. All the QSAR reported here are derived by us and were not given with the original data sets taken from the literature as referenced.

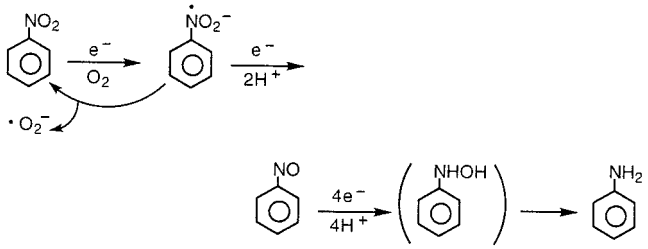
III. Results and Discussion

Free radicals play a critical and complex role in a diverse number of biological processes such as food oxidation, homeostasis, and the etiology of disease. They are produced in normal or aberrant cell metabolism from xenobiotics or ionizing radiation.[43,44] In aerobic organisms, ubiquitous electron acceptors such as molecular oxygen interact readily with other radical species to generate new radical species, which can mediate other cellular free-radical reactions. Thus, a chain reaction is set in motion.[45] Metabolic activation of organic compounds can also result in production of free radicals on nitrogen, oxygen, sulfur, and carbon. Radicals may be classed as cation radicals (positively charged), anion radicals (negatively charged), or neutral.[46] The following analysis of various reactions in physicochemical and biological systems will examine the role of various types of radicals in mediating chemical reactions and associated biological responses in various cells, bacteria, organisms, fish, and animals.

A. Aromatic Nitro Compounds

Nitroaromatic compounds by virtue of their prevalence in consumer products and industrial chemicals, coupled with their high potential as environmental pollutants, have engendered great interest in and extensive study of their reactivity and metabolism.[47]

Scheme 3. General Scheme for the Reduction of Nitroaromatics



It has been established that most of the reduction of the nitro group on nitrobenzene occurs in the intestinal microflora.[48] Microsomal and mitochondrial preparations are capable of one-electron reductions of nitro compounds to yield resonance-stabilized nitro anion radicals which are subsequently reduced to nitrosobenzenes and/or phenylhydroxylamines,[49] see Scheme 3. The formation of the critical nitro anion radical suggests that σ^- would be an effective descriptor of substituent effects in nitroarenes.

The following QSAR have been derived from data in the literature. QSAR 1−8 are derived from mechanistic organic chemistry for comparison with biological QSAR.[9−24]

Reduction of 4-X−$C_6H_4NO_2$ by $CH_3\dot{C}HOH$ in N_2O saturated solution[50]

$$\log k = 0.85(\pm 0.15)\sigma^- + 8.26(\pm 0.11) \quad (1)$$

$$n = 13,\ r^2 = 0.932,\ s = 0.125,\ q^2 = 0.911$$

outlier: X = H

Reduction of 4-X−$C_6H_4NO_2$ by aqueous solution of pyrimidine and nitrobenzene saturated with N_2O assayed by the radical reaction with 5-H, 6-Me-uracil[51]

$$\log k = 1.05(\pm 0.13)\sigma^- + 0.06(\pm 0.09) \quad (2)$$

$$n = 13,\ r^2 = 0.965,\ s = 0.120,\ q^2 = 0.944$$

omitted for lack of σ^- values: $-SO_3Me$, $-CH{=}N-OH$

Reduction of 4-X−$C_6H_4NO_2$ in aqueous solution containing pyrimidine and nitrobenzene saturated with N_2O and reaction with 5-OH, 6-Me-isocytosine[51]

$$\log k = 0.52(\pm 0.12)\sigma^- + 0.55(\pm 0.10) \quad (3)$$

$$n = 9,\ r^2 = 0.937,\ s = 0.075,\ q^2 = 0.916$$

Reduction of 4-X−$C_6H_4NO_2$ in N_2O saturated aqueous solution by $^{\bullet}CH_2OH$[50]

$$\log k = 0.86(\pm 0.41)\sigma^- + 7.16(\pm 0.41) \quad (4)$$

$$n = 6,\ r^2 = 0.893,\ s = 0.060,\ q^2 = 0.582$$

outlier: 4-$CONH_2$

Hydrogenation of 4-X−$C_6H_4NO_2$ in dioxan catalyzed by platinum-coated silica gel[52]

$$\log k = 0.83(\pm 0.33)\sigma^- + 0.88(\pm 0.16) \quad (5)$$

$$n = 5,\ r^2 = 0.955,\ s = 0.115,\ q^2 = 0.908$$

outlier: H

Hydrogenation of 4-X−$C_6H_4NO_2$ in toluene catalyzed by platinum-coated silica gel[52]

$$\log k = 0.47(\pm 0.16)\sigma^- + 1.66(\pm 0.08) \quad (6)$$

$$n = 5,\ r^2 = 0.966,\ s = 0.057,\ q^2 = 0.847$$

outlier: H

Hydrogenation of 4-X−$C_6H_4NO_2$ in ethyl acetate catalyzed by platinum-coated silica gel[52]

$$\log k = 0.68(\pm 0.08)\sigma^- + 1.15(\pm 0.04) \quad (7)$$

$$n = 5,\ r^2 = 0.966,\ s = 0.028,\ q^2 = 0.977$$

outlier: H

Hydrogenation of 4-X−$C_6H_4NO_2$ by dihydro-3-methyllumiflavin in dimethylformamide[53]

$$\log k_2 = 3.75(\pm 0.55)\sigma^- - 0.28(\pm 0.39) \quad (8)$$

$$n = 6,\ r^2 = 0.989,\ s = 0.235,\ q^2 = 0.980$$

outlier: 4-OMe

The above eight QSAR where reduction is carried out by a variety of agents are well correlated by σ^-. The Hammett parameter σ^- was designed for instances where there is direct resonance (through resonance) between the electrons of the substituent and the reaction center. It was developed from studies on the ionization of phenols, hence it is unusual that it works so well for the radical reduction of the nitro function. This suggests that a nitro anion radical may be a critical intermediate in the conversion to anilines. There is a high correlation between σ and σ^- in these nitrobenzenes but in most cases, the σ^- correlations are slightly better.

The uniformity of coefficients (ρ) with σ^- is also impressive considering the diversity of the reagents used in the reduction. It is noteworthy that for QSAR 5−7 where gaseous hydrogen is the reducing agent, σ^- holds and QSAR 5 and 7 have similar coefficients to QSAR 1−4. Absorption of hydrogen on platinum must cause homolytic dissociation of H_2. QSAR 8 with the unusual reducing agent is correlated by σ^- but with a much greater ρ value; weak reagents tend to yield larger ρ values, which implies that energetic assistance is provided by the substituents. Also, a strong role played by some solvents in catalysis should not be ruled out. Solvent contributions could enhance or minimize substituent effects in these reactions.[54]

An examination of the reduction of nitro compounds in biological systems reveals the following examples.

Reduction of X−$C_6H_4NO_2$ by milk xanthine oxidase under anaerobic conditions[55]

$$\log k = 0.92(\pm0.15)\sigma^- - 0.44(\pm0.20)B5_2 + 2.24(\pm0.24) \quad (9)$$

$$n = 27,\ r^2 = 0.871,\ s = 0.203,\ q^2 = 0.832$$

outliers: 4-SO_3^-, 4-SH

This is an excellent reference QSAR since it is directly related to Scheme 1. The $B5_2$ term, which is the sterimol parameter representing the bulk of ortho substituents, accentuates a small negative effect for sterically hindered ortho analogues. Seventy six percent of the variance in the data is explained by the electronic term and 11% by the steric term.

Reduction of 4-X−$C_6H_4NO_2$ to X−C_6H_4NHOH by NAD(P)H-linked methyl 4-nitrobenzoate reductase from E. coli[56]

$$\log k = 1.10(\pm0.26)\sigma^- + 1.50(\pm0.19) \quad (10)$$

$$n = 12,\ r^2 = 0.901,\ s = 0.214,\ q^2 = 0.861$$

outlier: 4-OH

The ρ value in QSAR 10 is similar to that of QSAR 9, despite differences in the reductases that are involved.

Chromosomal aberrations (EC_{50}) induced in human peripheral lymphocytes by X−$C_6H_4NO_2$[57]

$$\log 1/C = 1.30(\pm0.40)\sigma^- - 3.16(\pm0.44)I - 0.44(\pm0.29)B5_4 + 4.09(\pm0.46) \quad (11)$$

$$n = 18,\ r^2 = 0.955,\ s = 0.269,\ q^2 = 0.923$$

EC_{50} defines the molar concentration of the substituted nitrobenzenes that induce chromosomal aberrations in 50% of human peripheral lymphocytes. The indicator variable ($I = 1$) is utilized for seven examples where dinitro groups are present on the ring; these compounds tend to be more active than predicted. The indicator variable I and σ^- account for most (92%) of the variance in the data, while the sterimol parameter $B5_4$ accounts for only 4% of the variance. QSAR 12−18 pertain to the inhibition of growth of several organisms by substituted nitrobenzenes.

Inhibition of luminescence (EC_{50}) of photobacterium phosphorium by X−$C_6H_4NO_2$[58]

$$\log 1/C = 1.52(\pm0.41)\sigma^- + 1.97(\pm0.66) \quad (12)$$

$$n = 11,\ r^2 = 0.888,\ s = 0.294,\ q^2 = 0.846$$

outliers: 1,3-di-NO_2, 4-Br; 1,3-di-NO_2

LC_{50} of X−$C_6H_4NO_2$ to fathead minnows[58]

$$\log 1/C = 1.17(\pm0.25)\sigma^- + 2.37(\pm0.45) \quad (13)$$

$$n = 5,\ r^2 = 0.987,\ s = 0.123,\ q^2 = 0.957$$

outlier: 3-NO_2

In this particular dataset, drawn from the study of Zhao and Wang, although the correlation between σ and σ^- was very high, σ^- was used for consistency.

LC_{50} of X−$C_6H_4NO_2$ to fathead minnow (96 h)[59]

$$\log 1/C = 1.44(\pm0.31)\sigma^- + 3.85(\pm0.21) \quad (14)$$

$$n = 12,\ r^2 = 0.914,\ s = 0.242,\ q^2 = 0.866$$

outliers: 3,4-di-Cl; 4-Br

This set contains a number of disubstituted nitro congeners that are reasonably well fit without an indicator variable.

LC_{50} of X−$C_6H_4NO_2$ to fish (Cyprinus carpio)[60]

$$\log 1/C = 1.08(\pm0.24)\sigma^- - 0.40(\pm0.21)B5_3 + 4.33(\pm0.34) \quad (15)$$

$$n = 17,\ r^2 = 0.878,\ s = 0.237,\ q^2 = 0.832$$

outliers: H, 2-NH_2

σ^- accounts for 75% of the variance, while $B5_3$ accounts for 14%.

LC_{50} of X−$C_6H_4NO_2$ to fathead minnows[60]

$$\log 1/C = 1.10(\pm0.18)\sigma^- - 0.33(\pm0.18)L_3 + 4.61(\pm0.46) \quad (16)$$

$$n = 18,\ r^2 = 0.923,\ s = 0.211,\ q^2 = 0.881$$

outlier: H

Once again, σ^- accounts for most (84%) of the variance in the data.

LC_{50} of X−$C_6H_4NO_2$ to fathead minnows (96 h)[61]

$$\log 1/C = 0.83(\pm0.14)\sigma^- + 0.38(\pm0.18)L_{2,6} + 0.21(\pm0.20)B5_4 + 1.92(\pm0.98) \quad (17)$$

$$n = 30,\ r^2 = 0.894,\ s = 0.226,\ q^2 = 0.850$$

outliers: 2-Me, 5-NO_2; 2-NH_2, 4-Me, 5-NO_2; 2-OH, 3-Me, 5-NO_2

$L_{2,6}$ represents the sum of the lengths of substituents in the 2- and 6- positions. Thus, increased length in the ortho position and enhanced width in the para position of the substituents results in increased toxicity to the fish. Most of the toxicity can be ascribed to σ^- (81% of the variance in the data), while the steric terms combined contribute to only 8% of the variance in the data.

Inhibition of growth (IC_{50}) of pollen tubes in tobacco plants by X−$C_6H_4NO_2$[62]

$$\log 1/C = 0.85(\pm0.23)\sigma^- + 2.85(\pm0.43) \quad (18)$$

$$n = 8,\ r^2 = 0.932,\ s = 0.160,\ q^2 = 0.869$$

outliers: 2,3,6-tri-NO_2, 2,4,6-tri-NO_2

The IC_{50} serves as an acute toxicity endpoint and represents the inhibition of pollen tube growth by

50% after 24 h. Again, a positive dependence on σ^- is observed.

Inhibition of growth (IC_{50}) of Tetrahymena pyriformis by X−$C_6H_4NO_2$ after 48 h[63]

$$\log 1/C = 0.61(\pm 0.09)\text{Clog } P + 0.74(\pm 0.20)\sigma^- + 2.23\ (\pm 0.20) \quad (19)$$

$$n = 23,\ r^2 = 0.910,\ s = 0.179,\ q^2 = 0.863$$

outliers: 2-OH, 2-CHO, 2-COOH, 3-COOH

Using the data of Dearden et al.,[63] QSAR 19 was formulated. The carboxylic derivatives were omitted from the analysis since only un-ionized compounds were considered. The other two outliers (2-OH and 2-CHO) have their own inherent toxicity and hence could be clouding the overall picture. In QSAR 19, 65% of the variance can be explained by the hydrophobic term and 26% by the electronic term. An extended nitrobenzene toxicity study by Cronin and Schultz[64] led to the formulation of QSAR 20.

Inhibition of growth (IC_{50}) of Tetrahymena pyriformis by X−$C_6H_4NO_2$[64]

$$\log 1/C = 0.55(\pm 0.13)\text{Clog } P + 0.44(\pm 0.21)\sigma^- + 0.83(\pm 0.31)I - 0.92(\pm 0.35) \quad (20)$$

$$n = 41,\ r^2 = 0.918,\ s = 0.209,\ q^2 = 0.890$$

outliers: 6-Br, 3-NO_2

The indicator variable, I, acquires a value of 1 for 15 congeners bearing two nitro groups on the ring. These compounds tend to exhibit enhanced toxicity in comparison to mononitro benzenes. The electronic term, hydrophobic term, and indicator variable account for 74%, 11%, and 7%, respectively, of the variance in the data. QSAR 9−20 show a strong dependence on σ^- as opposed to σ, which indirectly implicates the nitro anion radical in their overall toxicity.

There are examples of biological QSAR of X−$C_6H_4NO_2$ that do not contain electronic terms. The following is an excellent example.

Minimum inhibitory zone concentration (MIC) of growth of S. cerevisiae (yeast) by X−$C_6H_4NO_2$[65]

$$\log 1/C = 0.94(\pm 0.12)\log P + 2.30(\pm 0.30) \quad (21)$$

$$n = 9,\ r^2 = 0.981,\ s = 0.055,\ q^2 = 0.970$$

outlier: 4-Br

From QSAR 21 it is clear that not all biological systems may contain a reductase capable of reducing a nitro group. There is thus a need for a wider range of studies on selected cells and organisms to establish a pattern for such activity. Hydrophobic compounds may disrupt a number of critical metabolic processes resulting in narcosis.[66] There is also a paucity of QSAR on lethality of various chemicals to whole animals. There is only one example of a QSAR based on the LD_{100} of humans by miscellaneous compounds that is based on a single parameter, Clog P.[67]

A MERLIN search on nitrobenzene allows one to sequester all datasets including those where only one congener contains a NO_2 group but all of the rest carry other substituents.[68]

Inhibition (EC_{50}) of acetylcholine hydrolase by X−$C_6H_4OP(=O)(C_2H_5)OCH_3$[69]

$$\log 1/C = 2.42(\pm 0.49)\sigma^- + 4.31(\pm 0.37) \quad (22)$$

$$n = 7,\ r^2 = 0.970,\ s = 0.309,\ q^2 = 0.941$$

In this study, a nucleophilic substitution is involved. In only one instance was X a nitro group.

Another example involves chloroamphenicol derivatives. Although it has long been known that most nitro compounds are toxic, caution must be exercised in assessing toxicity contributions, particularly when dealing with multifunctional compounds. Chloramphenicol, [4-NO_2−C_6H_4−CH(OH)CH($NHCOCHCl_2$)-CH_2OH], is a potent antibacterial agent, but its use has been curtailed because of the occurrence of serious blood dyscrasias, which are attributed to the presence of the nitro functionality. However, a study of chloramphenicol derivatives in which the nitro group was replaced by other substituents shed a different light on the problem.[70]

Inhibition of growth of E. coli by chloramphenicol analogues[70]

$$\log k = 3.04(\pm 0.77)E_R + 0.18(\pm 0.12)\log P + 0.61(\pm 0.21) \quad (23)$$

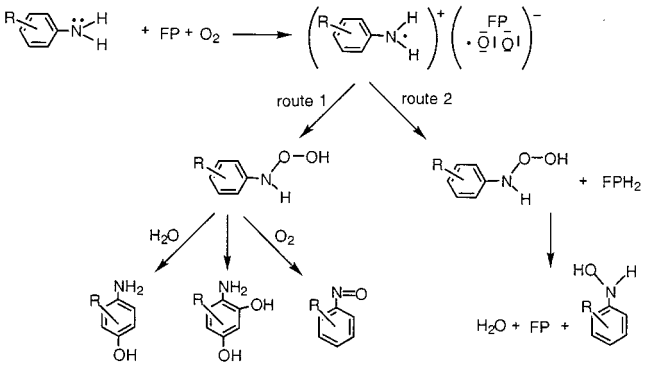
$$n = 9,\ r^2 = 0.947,\ s = 0.110,\ q^2 = 0.872$$

QSAR 23 covers the nitro substituent and eight other substituents that are all well correlated by Otsu's radical parameter, E_R, which was specifically delineated for free-radical reactions.[36] One line of evidence indicates that the conversion of the benzylic moiety to a toxic hydroxybenzyl radical may be responsible for the toxic manifestations. Thus, mechanistic insight gained from physical−organic chemistry is useful in understanding how certain chemical moieties will effect various biological systems.

B. Aromatic Amines

Beckett et al. proposed a general scheme for the metabolism of primary aromatic amines, which primarily involves microsomal N-oxidation.[71] This general scheme is outlined as follows. An electron from the nitrogen lone pair is transferred to triplet oxygen, which is subsequently oxidized to singlet oxygen in the presence of a flavoprotein. A complex of the nitrogen radical cation with the flavoprotein/oxygen is then formed. This complex can proceed along two distinct pathways. One route involves dissociation of the complex and ultimate formation of the *N*-hydroperoxide which under neutral aqueous conditions yields hydroxyaniline compounds (*o*-hydroxy and *p*-hydroxy aniline) and in the presence of air forms nitroso compounds. The nitroso compounds can be reduced metabolically to the phenyl hydroxylamine. Electron-attracting substituents tend to facilitate the formation of the hydroxy anilines, see Scheme 4.

Scheme 4. Metabolism of Aromatic Amines



The second route involves the reduction of the complex to a reduced flavoprotein−anion radical complex which eventually form the *N*-hydroperoxide that is metabolically cleaved to the phenylhydroxylamine. Thus, N-oxidation might provide a general pathway for the oxidation of anilines to the nitroso compound, para and ortho hydroxy anilines, as well as phenyl hydroxylamines. The formation of the critical intermediate, the nitrogen radical cation, in the metabolism of aromatic amines bodes well for the use of σ^+ in correlating biological data. The following nine examples are from mechanistic organic chemistry and deal with the oxidation of anilines.

Oxidation of X−$C_6H_4NH_2$ by chloramine-T in aqueous 10% ethanol[72]

$$\log k_2 = -0.82(\pm 0.26)\sigma^+ + 1.81(\pm 0.12) \quad (24)$$

$$n = 7,\ r^2 = 0.930,\ s = 0.101,\ q^2 = 0.884$$

outlier: 4-Br

Oxidation of X−$C_6H_4NH_2$ by peroxydisulfate in aqueous 40% tert-butyl alcohol at 45 °C[73]

$$\log k_2 = -1.07(\pm 0.15)\sigma^+ - 1.26(\pm 0.28)F_2 - 0.18(\pm 0.14)\mathrm{MR}_6 + 0.08(\pm 0.10) \quad (25)$$

$$n = 15,\ r^2 = 0.985,\ s = 0.093,\ q^2 = 0.971$$

σ^+, F_2, and MR_6 account for 85%, 12%, and 2%, respectively, of the variance in the data.

Oxidation of X−$C_6H_4NH_2$ by peroxydisulfate in aqueous 20% ethanol at 30 °C[74]

$$\log k_2 = -1.32(\pm 0.15)\sigma^+ - 1.35(\pm 0.30)\mathrm{F}_2 - 1.81(\pm 0.07) \quad (26)$$

$$n = 22,\ r^2 = 0.960,\ s = 0.130,\ q^2 = 0.948$$

outliers: 2-COOH; 4-NO_2

Once again, σ^+ accounts for 77% of the variance in the data and F_2 for an added 19%.

Oxidation of X−$C_6H_4NH_2$ to nitrobenzene by tert-butylhydroperoxide in benzene−20% chlorobenzene[75]

$$\log k_{\mathrm{rel}} = -1.42(\pm 0.41)\sigma^+ - 0.09(\pm 0.09) \quad (27)$$

$$n = 6,\ r^2 = 0.958,\ s = 0.078,\ q^2 = 0.916$$

outlier: 3-Br

Oxidation of X−$C_6H_4NH_2$ by Ti(III) in aqueous 20% acetic acid at 60 °C[76]

$$\log k_2 = -1.95(\pm 0.42)\sigma^+ - 0.05(\pm 0.10) \quad (28)$$

$$n = 7,\ r^2 = 0.966,\ s = 0.078,\ q^2 = 0.933$$

outliers: 4-Cl, 3-NO_2

Oxidation of X−$C_6H_4NH_2$ by periodate in aqueous 10% acetone[77]

$$\log k_2 = -1.40(\pm 0.45)\sigma^+ + 0.25(\pm 0.21) \quad (29)$$

$$n = 6,\ r^2 = 0.949,\ s = 0.160,\ q^2 = 0.898$$

outliers: 4-C_2H_5, 4-CH_3

It is of interest to note that the two alkyl substituents are poorly fit; the ethyl is more active than predicted, while the methyl is less active than predicted.

Oxidation of X−$C_6H_4NH_2$ by vanadium(V) in acetic acid 30% aqueous at 35 °C[78]

$$\log k_2 = -3.31(\pm 0.79)\sigma^+ + 0.58(\pm 0.41) \quad (30)$$

$$n = 7,\ r^2 = 0.958,\ s = 0.263,\ q^2 = 0.916$$

Oxidation of X−$C_6H_4NH_2$ by MnO_2 suspended in aqueous solution[79]

$$\log k = -3.80(\pm 1.28)\sigma^+ + 1.49(\pm 0.64) \quad (31)$$

$$n = 6,\ r^2 = 0.944,\ s = 0.567,\ q^2 = 0.876$$

In QSAR 24−31, the Hammett ρ^+ values are negative with varying magnitudes from −3.80 to −0.82. Electron-donating substituents clearly enhance oxidation. The higher ρ^+ values suggest that weaker oxidizing agents require greater energetic assistance from the substrates!

Hydrogen abstraction from X−$C_6H_4NH_2$ by α,α-diphenyl-β-picrylhydrazyl radical in CCl_4 at 41 °C[80]

$$\log k_3 = -2.83(\pm 0.64)\sigma^+ + 4.69(\pm 0.17) \quad (32)$$

$$n = 6,\ r^2 = 0.974,\ s = 0.138,\ q^2 = 0.932$$

Oxidation of X−$C_6H_4NH_2$ by borate radicals produced by flash photolysis of $NaBO_2$ and $K_2S_2O_8$ in aqueous solution at pH 11.5[81]

$$\log k_2 = -0.84(\pm 0.24)\sigma^+ + 8.51 \quad (33)$$

$$n = 5,\ r^2 = 0.977,\ s = 0.062,\ q^2 = 0.863$$

outlier: 4-Br

The strong dependence on σ^+ in QSAR 32 and 33 clearly point to radical reactions. This is in keeping

with the views of the original authors, who indicated that a radical species was involved in the reactions.

The following equations pertain to the interactions of $X-C_6H_4NH_2$ with biological systems.

Oxidation of $X-C_6H_4NH_2$ by horseradish peroxidase II[82]

$$\log k_{cat} = -5.45(\pm 3.40)\sigma^+ + 3.44(\pm 1.40) \quad (34)$$

$$n = 4,\ r^2 = 0.960,\ s = 0.483,\ q^2 = 0.633$$

This is a weak correlation as revealed by the high confidence intervals on σ^+ and the limited number of data points.

Oxidation of $X-C_6H_4NH_2$ by horseradish peroxidase I[83]

$$\log k_2 = -3.17(\pm 0.45)\sigma^+ + 0.34(\pm 0.88)\text{Clog } P + 5.14(\pm 0.21) \quad (35)$$

$$n = 9,\ r^2 = 0.990,\ s = 0.227,\ q^2 = 0.973$$

Oxidation of $X-C_6H_4NH_2$ by horseradish peroxidase II[84]

$$\log k_2 = -3.00(\pm 0.53)\sigma^+ + 0.25(\pm 0.10)\text{Clog } P + 4.52(\pm 0.24) \quad (36)$$

$$n = 8,\ r^2 = 0.988,\ s = 0.240,\ q^2 = 0.971$$

The ρ values in the above three QSAR are substantial, suggesting the presence of either a weak oxidizing agent such as the enzyme peroxidase or labile heteroatom−hydrogen bonds.

Oxidation of $X-C_6H_4NH_2$ by cytochrome C peroxidase[85]

$$\log k_2 = -2.86(\pm 0.43)\sigma^+ + 1.09(\pm 0.21) \quad (37)$$

$$n = 7,\ r^2 = 0.983,\ s = 0.193,\ q^2 = 0.962$$

outlier: 3-OMe

Oxidation of $X-C_6H_4NH_2$ by cytochrome C peroxidase (W51A)[85]

$$\log k_2 = -2.10(\pm 0.58)\sigma^+ + 3.07(\pm 0.29) \quad (38)$$

$$n = 7,\ r^2 = 0.945,\ s = 0.263,\ q^2 = 0.873$$

outlier: 4-Cl

Oxidation of $X-C_6H_4NH_2$ by Gram-negative bacterial oxidase[86]

$$\log k = 1.64(\pm 0.69)\text{Mlog } P - 1.01(\pm 0.53)\sigma^+ - 16.6(\pm 1.03) \quad (39)$$

$$n = 6,\ r^2 = 0.964,\ s = 0.215,\ q^2 = 0.846$$

outlier: H

Hydrophobicity as delineated by Mlog P (measured partition coefficients) accounts for 52% of the variance in the data, while σ^+ accounts for the other 42% of the variance.

Oxidation of $X-C_6H_4NH_2$ by cytochrome C peroxidase (W51F)[85]

$$\log k_2 = -1.60(\pm 0.58)\sigma^+ + 3.14(\pm 0.29) \quad (40)$$

$$n = 7,\ r^2 = 0.909,\ s = 0.263,\ q^2 = 0.829$$

outlier: 4-Cl

Oxidation of $X-C_6H_4NH_2$ by soil fungus peroxidase[87]

$$\log A = -1.20(\pm 0.17)\sigma^+ - 1.33(\pm 0.57)B1_6 + 0.54(\pm 0.15)\text{Clog } P - 0.81(\pm 0.31)F_2 + 3.05(\pm 0.55) \quad (41)$$

$$n = 32,\ r^2 = 0.907,\ s = 0.168,\ q^2 = 0.820$$

outlier: H, 3-OMe

A is defined as the biotransformation of anilines by *G. candidum* L3 peroxidase. In this case, the anilines were oxidized to mostly diazo compounds. $B1_6$ applies to the larger substituent when two ortho substituents are present. This is an unusual QSAR since it contains a significant hydrophobic term, a steric term, and a field/inductive parameter (F_2) for ortho substituents only. Nevertheless, there is still a strong dependence on σ^+, which accounts for 63% of the variance in the data, while the other parameters account for the rest.

Oxidation of $X-C_6H_4NH_2$ by chloroperoxidase from soil fungus[88]

$$\log V_{max}/K_M = -1.67(\pm 0.64)\sigma^+ - 1.02(\pm 0.32)\text{MR} + 3.46(\pm 0.31) \quad (42)$$

$$n = 9,\ r^2 = 0.950,\ s = 0.192,\ q^2 = 0.883$$

Sweet taste by humans of $X-C_6H_4NH_2$[89]

$$\log \text{RBR} = -0.66(\pm 0.28)\sigma^+ + 1.32(\pm 0.24)\text{Clog } P - 0.07(\pm 0.48) \quad (43)$$

$$n = 9,\ r^2 = 0.973,\ s = 0.132,\ q^2 = 0.936$$

Sweet taste by humans of $X-C_6H_4NH_2$[90]

$$\log \text{RBR} = -0.51(\pm 0.28)\sigma^+ + 1.19(\pm 0.24)\text{Clog } P + 0.25(\pm 0.46) \quad (44)$$

$$n = 18,\ r^2 = 0.894,\ s = 0.239,\ q^2 = 0.844$$

outliers: 3-NO_2, 6-OC_4H_9

QSAR 44 contains the data points from QSAR 43 plus others from various sources. The dependent variable RBR represents the relative biological response to sweet taste. In these two equations there is a reversal in importance of the two independent variables: Clog P and σ^+. Clog P accounts for most of the variance in the data (78−82%), while σ^+ only explains 10−15% of the variance. The two equations are in excellent agreement that $-\rho^+$ is significant and

suggest that oxidation of the amino group by taste receptors may be implicated in taste sensation.

Of the above 11 QSAR, 6 contain positive hydrophobic terms which suggests that amine oxidation is certainly more complex than nitro reduction. The dominating presence of the σ^+ variable is in harmony with the formation of a critical nitrogen radical cation−flavoprotein−oxygen complex. There are two instances where end products have been identified. In QSAR 41, the formation of $X-C_6H_4N{=}NC_6H_4X$ might be attributed to the condensation of $X-C_6H_4N{=}O$ and $X-C_6H_4NH_2$. QSAR 27 shows that the amino moiety can be converted to the nitro group, i.e., there is some interrelation between the two functional groups.

Overall, these results suggest that the aromatic amino group, which is prevalent in many endogenous and exogenous biomolecules, can be conjugated with a strong electron-releasing substituent to generate a reactive species of a radical nature that could have dire consequences, particularly after long-term usage.

C. Phenols

There are many examples drawn from the physical organic chemistry literature that conclusively demonstrate the propensity of the aromatic OH group to form radicals. Table 1 illustrates the point.[91]

Table 1. Hammett ρ Values for the Formation of X−Phenoxyl Radicals

solvents	reagent	ρ^+	T, °C
carbon tetrachloride	$Me_3CO^{\bullet}$	−1.81(±0.77)	130
styrene	styryl peroxy radical	−1.60(±0.08)	65
benzene	$Me_3CO^{\bullet}$	−1.46(±0.37)	130
styrene	styryl peroxide	−1.23(±0.10)	65
carbon tetrachloride	$Me_3CO^{\bullet}$	−1.13(±0.18)	120
acetonitrile	$Me_3CO^{\bullet}$	−1.02(±0.16)	130
benzene	$Me_3CO^{\bullet}$	−0.82(±0.08)	22
reactants	$Me_3CO^{\bullet}$	−0.82(±0.16)	25
chlorobenzene	$Me_3CO^{\bullet}$	−0.71(±0.10)	122
benzene	$(C_6H_5)_2CO^{\bullet}$	−0.60(±0.120)	22

Depending on the reaction conditions, there is a wide range in ρ^+ values and σ^+ is the parameter of choice.

In addition, there are many oxidation reactions of phenols that are correlated by σ^+. The following are illustrative.

Oxidation of $X-C_6H_4OH$ by peroxydisulfate in aqueous solution at 30 °C[92]

$$\log k_2 = -1.56(\pm 0.17)\sigma^+ + 0.20(\pm 0.07) \quad (45)$$

$$n = 34,\ r^2 = 0.919,\ s = 0.177,\ q^2 = 0.909$$

outliers: 4-CMe_3, 2-COOH

Oxidation of $X-C_6H_4OH$ by vanadium(V) in 30% acetic acid at 35 °C[78]

$$\log k_2 = -3.94(\pm 0.70)\sigma^+ + 0.10(\pm 0.24) \quad (46)$$

$$n = 7,\ r^2 = 0.977,\ s = 0.247,\ q^2 = 0.958$$

Oxidation of $X-C_6H_4OH$ by Mn(III) oxides in aqueous solution at 25 °C[93]

$$\log k_2 = -2.60(\pm 0.68)\sigma^+ - 6.48(\pm 0.19) \quad (47)$$

$$n = 7,\ r^2 = 0.950,\ s = 0.190,\ q^2 = 0.921$$

outlier: 4-COMe

Oxidation of $X-C_6H_4OH$ by chloramine-T in 0.1M NaOH at 60 °C[94]

$$\log k_2 = -1.41(\pm 0.49)\sigma^+ + 0.72(\pm 0.12) \quad (48)$$

$$n = 6,\ r^2 = 0.941,\ s = 0.107,\ q^2 = 0.870$$

outlier: 2-Cl

These results are of interest since Brown's σ^+ parameter was originally developed from studies on the solvolysis of cumyl chlorides. QSAR 45−48 suggest that the intermediate that is formed has distinct electrophilic character since σ^+ works surprisingly well for so many radical reactions.

In considering biological reactions, we find evidence for a variety of toxicities to occur via phenoxyl radicals. One of the most striking examples is that of the inhibition of growth of L1210 cancer cells. The impetus for this study was provided by evidence from an earlier EPA study on the toxicity of phenols to rat embryos in vitro where the QSAR obtained centered on a negative σ^+ term.[95] It was then assumed that fast growing embryos would produce more reactive oxygen species (ROS) that could convert the phenols to phenoxyl radicals and thus damage DNA. QSAR 49 and 50 were then developed.

IC_{50} of $X-C_6H_4OH$ and complex phenols to L1210 cells where X has negative σ^+ values[96]

$$\log 1/C = -1.35(\pm 0.15)\sigma^+ + 0.18(\pm 0.04)\log P + 3.31(\pm 0.11) \quad (49)$$

$$n = 51,\ r^2 = 0.895,\ s = 0.227,\ q^2 = 0.882$$

$$\log 1/C = -0.19(\pm 0.02)\mathrm{BDE} + 0.21(\pm 0.03)\log P + 3.11(\pm 0.10) \quad (50)$$

$$n = 52,\ r^2 = 0.920,\ s = 0.202,\ q^2 = 0.909$$

Besides the simple phenols, the following complex phenols in Figure 1 are covered by QSAR 49 and 50.

QSAR 49 suggests that the toxicity of X−phenols to rapidly growing cells is radical mediated and results from a critical interruption of cell machinery that inhibits proliferation. In the case of simple ortho-substituted phenols, log P is set to 0. Fourteen such examples yield QSAR 51 and 52.[96]

$$\log 1/C = -1.50(\pm 0.45)\sigma^+ + 3.15(\pm 0.28) \quad (51)$$

$$n = 14,\ r^2 = 0.816,\ s = 0.323,\ q^2 = 0.762$$

$$\log 1/C = -0.17(\pm 0.03)\mathrm{BDE} + 3.18(\pm 0.16) \quad (52)$$

$$n = 14,\ r^2 = 0.936,\ s = 0.191,\ q^2 = 0.915$$

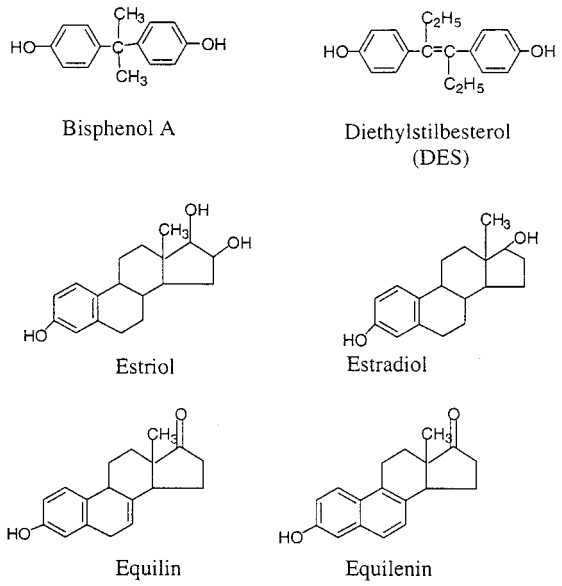


Figure 1. Structures of some estrogenic phenols.

QSAR 50 and 52 that utilize calculated bond dissociation energies of the O−H bond are superior to the ones that utilize σ^+. In part, this is due to the fact that some σ^+ values were not available and had to be estimated. Bond dissociation energies (BDE) were calculated from the following equation: $X-C_6H_4OH + C_6H_5O^\bullet \rightarrow X-C_6H_4O^\bullet + C_6H_5OH$. BDE values were based on B3LY/6-31G*/*/AMI energies and obtained using Jaguar 3.0.[28] They point directly to homolytic cleavage of the OH bond and confirm that σ^+ must also be invoking the same phenomenon. The above equations only hold with substituents or compounds that have negative ρ values or strongly negative BDE values. This is illustrated by QSAR 53.[97]

IC_{50} of $X-C_6H_4OH$ to L1210 cells where X has positive σ^+ values

$$\log 1/C = 0.62(\pm 0.16)\log P + 2.35(\pm 0.31) \quad (53)$$

$$n = 15,\ r^2 = 0.845,\ s = 0.232,\ q^2 = 0.800$$

outlier: 3-OH

These results clearly indicate that one must exercise caution in co-mingling together compounds that appear to be similar. Implications of the above-mentioned QSAR are important and suggest that consideration must be given to the physicochemical attributes of some functional groups before they are incorporated into pharmaceuticals for general use, e.g., phenol is not carcinogenic or mutagenic but *p*-methoxy phenol is carcinogenic to rodents.[98] This suggests that certain types of phenoxyl radicals interact with DNA which may result in carcinogenic and/or estrogenic endpoints. The estrogenic compounds diethylstilbestrol and nonyl phenol are well fit by QSAR 50, which also accounts for the cytotoxicity and perhaps the carcinogenicity of the female hormones[99] as seen in the case of the drug Premarin that contains several phenolic-based estrogenic components.

In this L1210 cell study, relatively high concentrations of phenols were required to inhibit cell growth. In the case of drugs inducing estrogenic toxicity in humans, small concentrations at the 'right' time in the early development of the fetus could trigger birth defects. Only a small percent of the women who use Premarin develop cancer, since there are protective mechanisms in humans to circumvent ill effects from radicals. The same is true of radicals formed in cigarette smoke that cause lung cancer. Long-term usage (>50 years) sometimes causes little or no problems, while some subjects contract cancer in 10 years. Thus, a strong basis in predictive toxicology will help weed out entities that only manifest untoward effects weakly, in the drug development stage. QSAR 50 is a valuable predictive model for the carcinogenicity or estrogenicity of phenols in animals or humans. The excellent prediction of the unusual and diverse compounds in Table 1 is beyond pure chance. Some phenol-based compounds that need more study are the flavonoids that are touted to have beneficial properties.

Thirty-five examples of phenol QSAR with $-\rho^+$ terms are available. Representative samples are described as follows.

LC_{50} of $X-C_6H_4OH$ to P. aeruginosa[100]

$$\log 1/C = -0.73(\pm 0.21)\sigma^+ + 0.86(\pm 0.12)\text{Clog } P - 0.42(\pm 0.13)B1_2 + 0.25(\pm 0.26) \quad (54)$$

$$n = 20,\ r^2 = 0.943,\ s = 0.103,\ q^2 = 0.918$$

outlier: 3-OMe

Hydrophobicity accounts for 54% of the variance in the data while σ^+ and the steric term ($B1_2$) each account for 20% of the variance in the data. The sterimol parameter B1 pinpoints the negative steric effect of ortho substituents.

EC_{50} of replicative DNA synthesis in Chinese hamster cells by $X-C_6H_4OH$[101]

$$\log 1/C = -0.74(\pm 0.34)\sigma^+ - 1.02(\pm 0.41)\text{CMR} + 6.98(\pm 1.6) \quad (55)$$

$$n = 9,\ r^2 = 0.915,\ s = 0.305,\ q^2 = 0.849$$

outliers: 4-OH, H

The EC_{50} represents the concentration of the phenol that inhibits the rate of [^{3}H]TdR incorporation in V79 Chinese hamster cells by 50%. Both descriptors σ^+ and the calculated molar refraction (CMR) contribute about equally to the variance in the data. The inhibition of DNA synthesis was directly attributed to the formation of a phenoxy tyrosyl radical species at the active site of ribonucleotide reductase. Quenching of the critical tyrosyl radical by activated phenols impeded normal DNA replicative synthesis.

EC_{50} of cyclooxygenase activity of prostaglandin synthase by $X-C_6H_4OH$[102]

$$\log 1/C = -1.08(\pm 0.40)\sigma^+ + 0.74(\pm 0.33)\text{Clog } P + 1.23(\pm 0.70) \quad (56)$$

$$n = 7,\ r^2 = 0.939,\ s = 0.132,\ q^2 = 0.794$$

Hydrophobicity and σ^+ equally account for the variance in the data.

Rate of oxidation of X−C$_6$H$_4$OH by horseradish peroxidase II[103]

$$\log k_2 = -2.41(\pm 0.52)\sigma^+ + 4.76(\pm 0.35) \quad (57)$$

$$n = 8,\ r^2 = 0.955,\ s = 0.324,\ q^2 = 0.900$$

outlier: 4-COOH

LC$_{20}$ of 8 day old flagfish larva by X−C$_6$H$_4$OH after 94 h[104]

$$\log 1/C = -0.94(\pm 0.32)\sigma^+ + 5.02(\pm 0.23) \quad (58)$$

$$n = 6,\ r^2 = 0.944,\ s = 0.201,\ q^2 = 0.898$$

outliers: 4-NO_2, 4-COOH, 4-CH_2OH

The nitro group has its own "intrinsic" type of toxicity as does the CH_2OH function. Thus, the toxicity expressed by nitrophenols could be a composite value attributed to the nitro anion radical and/or phenoxy radical cation. Another example comes from a study in our laboratory, which focused on the action of sterically hindered 2,6-disubstituted phenols acting on L1210 cells.[105]

IC$_{50}$ of L1210 cells by BHA/BHT analogues[105]

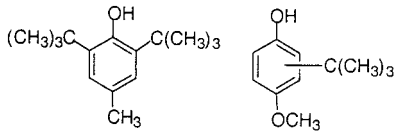
$$\log 1/C = 2.42(\pm 0.70)E_R - 0.47(\pm 0.10)E_S + 2.43(\pm 0.21) \quad (59)$$

$$n = 18,\ r^2 = 0.934,\ s = 0.136,\ q^2 = 0.905$$

outliers: 2,6-di-OMe; 2-CH_3, 4-NO_2; 2-CH_3, 4-$COCH_3$

E_S refers to the larger of the two ortho substituents. The negative coefficient with this term implies a positive steric effect since E_S values are negative. Steric hindrance around the reaction center appears to favor radical oxidation and thus the loss of the hydrogen. The impetus for this work comes from the commercial use of two radical scavenging compounds: butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) and the carcinogenicity of the latter. One would expect BHA to be more toxic because of the strong electron releasing OCH_3 function, Figure 2.

The aromatic OH group shows another type of toxicity in which σ is the parameter of choice as we see in QSAR 60 and 61.[106] Cronin and Schultz obtained the following QSAR in a comprehensive study of the toxicity of substituted phenols to *Tetrahymena pyriformis,* a unicellular ciliate.[107]



BHT BHA

Figure 2. Structures of BHT and BHA.

IC$_{50}$ of X−C$_6$H$_4$OH to Tetrahymena pyriformis[107]

$$\log 1/C = 0.67(\pm 0.02)\text{Clog } P - 0.67(\pm 0.55)E_{LUMO} - 1.12 \quad (60)$$

$$n = 120,\ r^2 = 0.893,\ s = 0.271$$

The following QSAR was reformulated using their data and utilizing σ in lieu of E_{LUMO}.

$$\log 1/C = 0.64(\pm 0.04)\text{Clog } P + 0.61(\pm 0.12)\sigma + 1.84(\pm 0.13) \quad (61)$$

$$n = 119,\ r^2 = 0.896,\ s = 0.265,\ q^2 = 0.887$$

Cronin and Schultz omitted a number of phenols with reactive moieties such as COOH, NH_2, NO_2, N═O, and *N*-acetyl substituents. QSAR 61 allows us to compare it to other QSAR based on σ. The ρ term is rather weak and σ cannot be replaced by σ^-. This suggests polarization of the OH moiety by the substituent may enhance its toxicity since through resonance is not involved. Ionization of simple phenols in aqueous solution is correlated by σ^- with $\rho^- \sim 2$. Eighty percent of the variance in the data is accounted for by Clog *P* while 10% of the variance is attributed to the Hammett constant.

Still another mode of phenol toxicity is correlated with σ^- suggesting that ionization is important.[108−110] The following two examples are representative of a number of examples in our system.

Uncoupling (EC$_{50}$) of phosphorylation in Ascaris muscle mitochondria by X−C$_6$H$_4$OH[111]

$$\log 1/C = 0.93(\pm 0.20)\text{Clog } P + 2.04(\pm 0.21)\sigma^- + 0.47(\pm 0.48) \quad (62)$$

$$n = 21,\ r^2 = 0.967,\ s = 0.393,\ q^2 = 0.955$$

outliers: 4-$COCH_3$; 2,6-di-I-4-NO_2; 2-I, 4-CN-6-NO_2

IC$_{50}$ of Chlorella vulgaris by X−C$_6$H$_4$OH[112]

$$\log 1/C = 0.72(\pm 0.14)\text{Clog } P + 1.31(\pm 0.20)\sigma^- + 1.51(\pm 0.30) \quad (63)$$

$$n = 26,\ r^2 = 0.914,\ s = 0.195,\ q^2 = 0.883$$

outliers: 2-COMe, 2-OH, 4-CHO, 4-OH

In these examples, the need to use σ^- and the larger ρ values suggest that ionization may be important.

The simple aromatic OH has a complex character when it comes to toxicology. This may be symptomatic of functional groups in general. Thus, careful

delineation of beneficial and detrimental functional entities needs to be clearly established. There are beneficial phenols such as the flavonoids that need to be investigated and toxic phenols that need to be avoided. Thus, there is a strong push to mechanistically assess the "ambivalent" nature of the phenolic group in plants, bacteria, fungi, animals, and man.

D. Thiophenols

As one might expect, thiophenols behave in a manner similar to phenols as shown by QSAR 64.

IC_{50} of X−C_6H_4SH on L1210 leukemia cells[113]

$$\log 1/C = -0.98(\pm 0.20)\sigma^+ + 0.95(\pm 0.25)I + 3.92(\pm 0.15) \quad (64)$$

$$n = 22,\ r^2 = 0.854,\ s = 0.170,\ q^2 = 0.809$$

outliers: 4-$CHMe_2$, 4-NO_2, 4-OC_6H_5

The indicator variable, *I*, takes the value of 1 for the halogens Cl, Br, and I and the pseudo-halogen CF_3; its coefficient (0.95) indicates its positive influence on inhibitory potency. The unusual behavior of halogenated substituents is not well understood. BDE values give a slightly poorer correlation (r^2 = 0.818).

A search for comparative QSAR of thiophenols led to the following example.

Rate of hematin-catalyzed auto-oxidation of X−C_6H_4SH[114]

$$\log k = -0.80(\pm 0.16)\sigma^+ + 0.26(\pm 0.14) \quad (65)$$

$$n = 5,\ r^2 = 0.989,\ s = 0.087,\ q^2 = 0.964$$

The σ^+ term is very similar to that in QSAR 64, suggesting a radical mechanism for autoxidation of thiophenols and their radical mediated inhibition of cell growth. Unfortunately there are no examples from mechanistic organic chemistry for comparison with these two examples.

E. Thiomethyl Function

Few studies have been undertaken of the aromatic SH function, probably because of the difficulty of working with this easily oxidizable group. However, a considerable amount has been done with the thiomethyl moiety. Considering physicochemical studies, nine examples are identified and correlated by σ^+ with $-\rho^+$, of which the following are representative.

Oxidation of 4-X−$C_6H_4SCH_3$ by singlet oxygen in aqueous 50% methanol at 35 °C[115]

$$\log k_2 = -0.74(\pm 0.46)\sigma^+ + 7.36(\pm 0.45) \quad (66)$$

$$n = 4,\ r^2 = 0.960,\ s = 0.140,\ q^2 = 0.792$$

outlier: 4-OMe

Oxidation of 4-X−$C_6H_4SCH_3$ by singlet oxygen in benzene at 35 °C[115]

$$\log k_2 = -0.96(\pm 0.58)\sigma^+ + 5.94(\pm 0.57) \quad (67)$$

$$n = 4,\ r^2 = 0.962,\ s = 0.175,\ q^2 = 0.765$$

outlier: 4-OMe

Although the above correlations are not very sharp (because of the small number of data points), they certainly describe radical reactions. In both cases, the *p*-methoxy substituted is an outlier.

Oxidation of X−$C_6H_4SCH_3$ by peroxy diphosphate in aqueous 50% acetic acid at 35 °C[116]

$$\log k_2 = -0.78(\pm 0.10)\sigma^+ - 2.18(\pm 0.04) \quad (68)$$

$$n = 13,\ r^2 = 0.962,\ s = 0.66,\ q^2 = 0.939$$

Oxidation of 2-X−$C_6H_4SCH_3$ by Cr(VI) in aqueous 50% acetic acid at 40 °C[117]

$$\log k_2 = -0.85(\pm 0.41)\sigma^+ - 2.18(\pm 0.81)F_2 + 1.34(\pm 0.31) \quad (69)$$

$$n = 7,\ r^2 = 0.981,\ s = 0.142,\ q^2 = 0.927$$

F_2 is a statistically significant parameter that accounts for 83% of the variance in the whole set. It is noteworthy that after correction for the field/inductive effect of ortho substituents by F_2, the role of resonance is the same as the previous four examples.

Oxidation of X−$C_6H_4SCH_3$ by $CH_3CONHCl$ in 50% aqueous acetic acid at 25 °C[118]

$$\log k = -1.69(\pm 0.11)\sigma^+ - 1.01(\pm 0.18)B1_2 + 3.22(\pm 0.24) \quad (70)$$

$$n = 26,\ r^2 = 0.981,\ s = 0.157,\ q^2 = 0.971$$

outlier: 2-Me

In this example the sterimol parameter B1 is more significant than F_2 in QSAR 69. The weaker oxidizing agent results in a larger ρ^+, which suggests that greater energetic assistance is provided by the substituents. Eighty-four percent of the variance in the data is explained by σ^+, while $B1_2$ accounts for 14% of the variance.

Oxidation of X−$C_6H_4SCH_3$ by pyridinium chlorochromate (PCC) in chlorobenzene/50% nitrobenzene at 30 °C[119]

$$\log k = -0.98(\pm 0.21)\sigma^+ - 4.76(\pm 0.09) \quad (71)$$

$$n = 7,\ r^2 = 0.966,\ s = 0.097,\ q^2 = 0.929$$

outlier: H

The above examples provide reference points for the following examples drawn from biological systems.

Oxidation of 4-X−$C_6H_4SCH_3$ to the sulfoxide by horseradish peroxidase II[120]

$$\log k_{cat} = -0.73(\pm 0.22)\sigma^+ + 0.96(\pm 0.51)B1_4 + 4.56(\pm 0.74) \quad (72)$$

$$n = 8,\ r^2 = 0.937,\ s = 0.115,\ q^2 = 0.837$$

outlier: $NHCOCH_3$

This is not a robust correlation; the use of two parameters mandates the availability of at least 10 data points. Nevertheless, it still illustrates the importance of σ^+ in catalysis. This electronic descriptor accounts for 64% of the variance in the data, while the positive steric term handles 30%. Kobayashi et al. suggested that the large negative ρ^+ value they obtained was in accordance with the formation of a sulfide radical cation in the rate-determining step.[120]

Oxidation of 4-X−$C_6H_4SCH_3$ by horseradish peroxidase[121]

$$\log k_{cat} = -1.48(\pm 0.13)\sigma^+ + 0.13(\pm 0.08) \quad (73)$$

$$n = 4,\ r^2 = 1.00,\ s = 0.021,\ q^2 = 0.995$$

Oxidation of 4-X−$C_6H_4SCH_3$ by soybean sulfoxidase[122]

$$\log V_{max} = -0.65(\pm 0.29)\sigma^+ - 0.01(\pm 0.23) \quad (74)$$

$$n = 5,\ r^2 = 0.944,\ s = 0.148,\ q^2 = 0.890$$

As expected, release of electrons by X to S via resonance characterized by σ^+ occurs in every instance in the above examples.

F. Benzylamines

Examples of QSAR from physical organic chemistry include the following on the oxidation of benzylamines.

Oxidation of X−$C_6H_4CH_2NH_2$ by N-chlorosuccinimide in aqueous solution at 15 °C[123]

$$\log k_2 = -2.05(\pm 0.23)\sigma^+ + 1.08(\pm 0.32)B1_2 - 3.65(\pm 0.39) \quad (75)$$

$$n = 27,\ r^2 = 0.940,\ s = 0.253,\ q^2 = 0.916$$

outlier: 2-NO_2

The positive steric effect of ortho substituents characterized by B1 is unexpected but not unusual. A negative ρ value once again underscores the importance of electron-releasing groups in this oxidation processes.

Oxidation of X−$C_6H_4CH_2NH_2$ by $CH_3CONHBr$ in aqueous solution at 25 °C[124]

$$\log k_2 = -1.53(\pm 0.17)\sigma^+ + 0.53(\pm 0.20)B1_2 - 2.87(\pm 0.25) \quad (76)$$

$$n = 28,\ r^2 = 0.940,\ s = 0.180,\ q^2 = 0.912$$

outliers: 2-NO_2, 2-$COOCH_3$

Oxidation of X−$C_6H_4CH_2NH_2$ by 4-nitrobenzene sulfonyl peroxide in ethyl acetate at −78 °C[125]

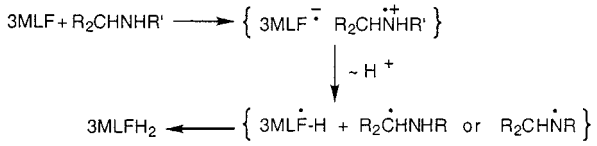
$$\log k_{rel} = -0.45(\pm 0.14)\sigma^+ + 0.02(\pm 0.04) \quad (77)$$

$$n = 5,\ r^2 = 0.973,\ s = 0.027,\ q^2 = 0.929$$

outlier: 4-Cl

In these three examples, σ^+ accounts for 82% (QSAR 75), 87% (QSAR 76), and 97% (QSAR 77) of the variance in the data. Using a photochemical model for catalysis of amine oxidative deamination, Mariano et al. examined the reaction of the triplet state of 3-methyl lumiflavin (3MLF) with an amine.[126] It was determined that the reaction was initiated by single electron transfer (SET) and proceeded via a neutral radical and an aminium cation radical intermediates, see Scheme 5.

Scheme 5. Mechanism for Photochemically Induced Oxidative Deamination of Benzylamine



In the case of benzylamines, the formation of an aminium cation radical intermediate suggests that electron-releasing substituents would enhance the stability of the transition state.

Considering biological QSAR on benzylamines, the following example on oxidation of X−$C_6H_4CH_2NH_2$ by monoamine oxidase (MAO) from bovine liver mitochondria is obtained. Extensive studies from Silverman's laboratory have established the importance of the SET mechanism in MAO B catalysis.[127] Note that QSAR 78 is for a binding constant, not oxidation.[128]

Oxidation of X−$C_6H_4CH_2NH_2$ by monoamine oxidase from bovine liver mitochondria[128]

$$\log k = -0.32(\pm 0.30)\sigma^+ - 0.62(\pm 0.19)B5_2 + 3.06(\pm 0.37) \quad (78)$$

$$n = 11,\ r^2 = 0.874,\ s = 0.193,\ q^2 = 0.661$$

outliers: H, 4-OH, 3-OMe

In the examples from physical organic chemistry, a consistent pattern of oxidation with negative σ^+ terms emerges. This is also true for QSAR 78, where oxidation by monoamine oxidase occurs. Since data on k_{cat} and K_M is not available, it is difficult to deconstruct the process.

G. Benzyl Alcohols

The following examples are drawn from the physical database and indicate that radical mechanisms once again predominate.

Oxidation of X−$C_6H_4CH_2OH$ by sodium N-bromoaminebenzenesulfonamide at 25 °C[129]

$$\log k = -2.83(\pm 0.15)\sigma^+ + 2.75(\pm 0.08) \quad (79)$$

$$n = 8,\ r^2 = 0.997,\ s = 0.090,\ q^2 = 0.995$$

Oxidation of X−$C_6H_4CH_2OH$ by N-bromosuccinimide in aqueous 20% acetic acid at 50 °C[130]

$$\log k_2 = -1.55(\pm 0.33)\sigma^+ - 1.00(\pm 0.16) \quad (80)$$

$$n = 8,\ r^2 = 0.957,\ s = 0.181,\ q^2 = 0.896$$

outlier: 4-F

Oxidation of X−$C_6H_4CH_2OH$ by bromine in aqueous 50% acetic acid at 25 °C[131]

$$\log k_2 = -2.10(\pm 0.22)\sigma^+ - 2.95(\pm 0.10) \quad (81)$$

$$n = 10,\ r^2 = 0.984,\ s = 0.075,\ q^2 = 0.975$$

outlier: 4-F

Oxidation of X−$C_6H_4CH_2OH$ by bromate in aqueous solution at 40 °C[132]

$$\log k_2 = -0.75(\pm 0.24)\sigma^+ - 1.43(\pm 0.14) \quad (82)$$

$$n = 6,\ r^2 = 0.948,\ s = 0.120,\ q^2 = 0.906$$

outlier: H

Oxidation of X−$C_6H_4CH_2OH$ by chloramines-T in aqueous 50% acetic acid at 25 °C[133]

$$\log k = -2.12(\pm 0.08)\sigma^+ - 5.37(\pm 0.04) \quad (83)$$

$$n = 9,\ r^2 = 0.998,\ s = 0.050,\ q^2 = 0.997$$

The above five QSAR strongly point to oxidation of the benzyl alcohols by reagents that are typically involved in radical oxidations.[91] The above results, all based on σ^+, can now be compared with those obtained in biological processes.

Earlier (QSAR 23), results on chloramphenicol derivatives acting on *E. coli* pointed toward a radical mechanism. A number of instances have been described where E_R (Otsu's parameter) correlates radical reactions better than σ^+. It is not clear what circumstances lie behind this observation. E_R has been delineated by the reaction shown in Scheme 2, and thus, one would presume it to be best for radical correlation studies, but only rarely does it appear to be more effective than σ^+.

In the following three examples, E_R is the parameter of choice.

Growth inhibition of molds (A. niger, penicillum, cladosporium, and mucor) by X−$C_6H_4CH_2OH$[134]

$$\log 1/C = 1.41(\pm 0.85)E_R + 0.67(\pm 0.08)\log P + 0.78(\pm 0.21) \quad (84)$$

$$n = 18,\ r^2 = 0.962,\ s = 0.160,\ q^2 = 0.940$$

outlier: 2-NO_2

C is the minimum inhibitory concentration of the benzyl alcohols in eqs 84−86. E_R makes a minor contribution to inhibitory activity (4%). Most of the variance in the data (93%) can be explained by hydrophobicity.

Growth inhibition of Gram-negative bacteria (Proteus vulgaris, E. coli, and Pseudomonas) by X−$C_6H_4CH_2OH$[134]

$$\log 1/C = 2.36(\pm 0.47)E_R + 0.67(\pm 0.06)\mathrm{Clog}\ P + 0.66(\pm 0.15) \quad (85)$$

$$n = 15,\ r^2 = 0.980,\ s = 0.092,\ q^2 = 0.965$$

outlier: 4-Cl

Growth Inhibition of Gram-positive bacteria (S. faecalis, S. albus, S. aureus) by X−$C_6H_4CH_2OH$[134]

$$\log 1/C = 1.72(\pm 1.30)E_R + 0.64(\pm 0.10)\mathrm{Clog}\ P + 0.87(\pm 0.24) \quad (86)$$

$$n = 10,\ r^2 = 0.978,\ s = 0.129,\ q^2 = 0.957$$

outliers: 4-Cl, 4-NO_2, 4-I, 3,5-di-CH_3

The weakest correlation (QSAR 86) is with the Gram-positive bacteria; nevertheless, E_R is superior to σ^+. In all these cases, this may be attributed to the binding of the substrates to the receptor for oxidation and the subsequent toxicity of reactive intermediates or products to bacterial cell growth.

Oxidation of X−$C_6H_4CH_2OH$ by trimethyl ester of coenzyme PQQ in acetonitrile[135]

$$\log k = -2.15(\pm 0.20)\sigma^+ + 6.07(\pm 0.18) \quad (87)$$

$$n = 4,\ r^2 = 0.999,\ s = 0.055,\ q^2 = 0.996$$

outlier: 4-Cl

Since chloramphenicol causes serious side reactions in humans, this raises questions about other types of benzyl alcohols or compounds having substituents readily converted to reactive moieties.

H. Benzaldehydes

QSAR 88 and 89 illustrate the radical-mediated reduction of benzaldehydes.[136]

Reduction of X−C_6H_4CHO by B-N-octyl-9-borabicyclo[3.3.1]nonane (9-BBN) in dodecane at 25 °C[136]

$$\log k_{rel} = 1.03(\pm 0.04)\sigma^+ + 0.01(\pm 0.03) \quad (88)$$

$$n = 7,\ r^2 = 0.999,\ s = 0.032,\ q^2 = 0.998$$

Reduction of X−C_6H_4CHO by morpholine−borane in 95% ethanol at 34.8 °C[137]

$$\log k = 1.23(\pm 0.17)\sigma^+ - 2.64(\pm 0.08) \quad (89)$$

$$n = 5,\ r^2 = 0.994,\ s = 0.054,\ q^2 = 0.967$$

QSAR 90 and 91 describe reductions of benzaldehydes in a biological setting.[137]

Reduction of X−C_6H_4CHO to X−$C_6H_4CH_2OH$ by horse liver alcohol dehydrogenase[138]

$$\log k_{cat} = 1.14(\pm 0.29)\sigma^+ + 1.92(\pm 0.25) \quad (90)$$

$$n = 5,\ r^2 = 0.981,\ s = 0.136,\ q^2 = 0.891$$

outlier: 4-NO_2

Reduction of X−C_6H_4CHO by bovine brain aldehyde reductase[139]

$$\log V_{max} = 0.47(\pm 0.17)\sigma^+ + 1.46(\pm 0.08) \quad (91)$$

$$n = 7,\ r^2 = 0.916,\ s = 0.080,\ q^2 = 0.876$$

outlier: 4-CN

The reduction of substituted benzaldehydes is quite complex and generally involves two steps—an equilibrium step and a hydride transfer.[136] Electron-withdrawing groups increase the rate of reduction by destabilizing the positively charged intermediate, thus enhancing its reactivity with a hydride donor. Positive ρ^+ values suggest that hydride transfer is dominant. In enzymatic systems, one could expect a similar mechanism of reduction to be deployed. Once again, σ^+ is the parameter of choice for correlation of radical reactions both in physical organic systems and in a biological milieu.

I. Substituted Toluenes

A very large amount of effort has been expended on studying the radical oxidation of toluenes. A recent review reported 57 examples correlated by σ^+ with ρ values ranging from about −2 to −0.32, depending on the type of radical and the reaction conditions.[91] Thus, there is a large amount of data on the propensity of the alkyl group to undergo radical reactions.

P450 hydroxylation of X−$C_6H_4CH_3$ by cytochrome P450[140]

$$\log k_{cat}/K_M = -0.71(\pm 0.62)\sigma^+ + 1.37(\pm 0.41)\mathrm{CMR} + 4.39(\pm 0.29) \quad (92)$$

$$n = 7,\ r^2 = 0.956,\ s = 0.157,\ q^2 = 0.847$$

outlier: 4-NO_2

The oxidation does show a modest dependence on σ^+, since 85% of the variance in the data is explained by CMR and about 10% by σ^+. Although we have only one direct biological based QSAR, there is an indirect relationship to aromatic methyl toxicity.

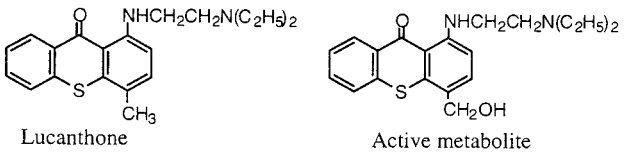


Figure 3. Structure of Lucanthone and its active metabolite.

The drug Lucanthone was developed for use against schistosomiasis. The active species is a metabolite with the CH_2OH moiety as the site of action, Figure 3.

Such a metabolite could have been expected from studies on the oxidation of X−$C_6H_4CH_3$. The strong electron-releasing NH unit is conjugated with the CH_3 group. The above examples reveal that whenever a $-CH_3$ or $-CH_2-$ is attached to appropriately substituted aromatic rings that can delocalize electrons via resonance, it is likely that one has an entity that can readily form radicals.

J. Olefins and Dienes

The olefin group is easily oxidized as is the diene moiety. In fact, it has long been known that butadiene is carcinogenic. First, careful analysis of the reactions of styrene from mechanistic organic chemistry (QSAR 93−96) reveal the following.

Reaction of X−$C_6H_4CH{=}CH_2$ with $CH_3-C_6H_4SO_2^\bullet$ in benzene at 43 °C[141]

$$\log k_{rel} = -0.49(\pm 0.11)\sigma^+ + 0.04(\pm 0.04) \quad (93)$$

$$n = 5,\ r^2 = 0.986,\ s = 0.024,\ q^2 = 0.968$$

Reaction of X−$C_6H_4CH{=}CH_2$ with malonyl radicals induced by cerium(IV) ammonium nitrate in methanol at 20 °C[142]

$$\log k_{rel} = -1.06(\pm 0.15)\sigma^+ - 0.02(\pm 0.06) \quad (94)$$

$$n = 5,\ r^2 = 0.994,\ s = 0.037,\ q^2 = 0.976$$

Oxidation of X−$C_6H_4CH{=}CH_2$ with thallium triacetate in acetic acid at 50 °C[143]

$$\log k = -2.26(\pm 0.47)\sigma^+ + 0.22(\pm 0.18) \quad (95)$$

$$n = 5,\ r^2 = 0.987,\ s = 0.128,\ q^2 = 0.970$$

outlier: 4-Me

Photoepoxidation of X−$C_6H_4CH{=}CH_2$ with $C_6H_5COOO^\bullet$ in benzene at 20 °C[144]

$$\log k_{rel} = -0.96(\pm 0.28)\sigma^+ - 0.09(\pm 0.12) \quad (96)$$

$$n = 5,\ r^2 = 0.975,\ s = 0.079,\ q^2 = 0.944$$

Unfortunately, there are only two biological examples for comparison.

ED_{50} of mice treated with X−$C_6H_4CH{=}CH_2$[145]

The ED_{50} is defined as the concentration of X−styrene that induces a hepatotoxic response in 50% of mice. The response is measured as an increase in the activity of alanine transaminase.

$$\log 1/C = -0.46(\pm 0.26)\sigma^+ + 3.22(\pm 0.18) \quad (97)$$

$$n = 6,\ r^2 = 0.862,\ s = 0.118,\ q^2 = 0.738$$

outlier: H

Although QSAR 97 is not very sharp, it does pass muster at the statistical level.

Inhibition of 5-lipoxygenase (EC_{50}) in rat leukemia cells by 2,6-X-4(2-(5-isoxazolyl)ethenyl) phenol[146]

$$\log 1/C = -0.82(\pm 0.17)\sigma^+ + 0.29(\pm 0.09)\text{Clog } P + 4.07(\pm 0.32) \quad (98)$$

$$n = 10,\ r^2 = 0.958,\ s = 0.111,\ q^2 = 0.907$$

outlier: 3,5-di-CMe_3

This QSAR could be regarded as phenol based or olefinic induced. It does suggest that leukemia cells would be useful for studying the toxicity of the vinylic moiety. σ^+ accounts for 64% of the variance in the data, while hydrophobicity accounts for the balance.

In considering the activity of butadienes, insight is drawn from one example from physical organic chemistry.

Rate of addition of dichlorocarbene to X−phenyl butadienes at 0 °C[147]

$$\log k_{rel} = -0.42(\pm 0.03)\sigma^+ - 0.01(\pm 0.02) \quad (99)$$

$$n = 9,\ r^2 = 0.994,\ s = 0.025,\ q^2 = 0.991$$

This free-radical reaction can be compared with the following biological example.[148]

IC_{50} of chloroquine resistant P. falciparum by methyl-(E)-3-methoxy-2−2(2-(E,E)-(4-X-phenylbuta-1,3-dienyl)phenyl)acrylates[148]

$$\log 1/C = -0.80(\pm 0.35)\sigma^+ + 1.35(\pm 0.26)B5_2 + 0.47(\pm 0.25)L_4 + 5.47(\pm 0.69) \quad (100)$$

$$n = 13,\ r^2 = 0.955,\ s = 0.225,\ q^2 = 0.893$$

IC_{50} is the concentration of the acrylate that inhibits the growth of chloroquine-resistant *P. falciparum* by 50%. Increased size of the substituents in the ortho and para positions as well as increased electron-donating capacity leads to enhanced toxicity to *P. falciparum*. An interesting feature of the butadiene unit is that it occurs in the important cholesterol lowering drugs Lovastatin and Pravastatin that have been shown to be carcinogenic in rats, Figure 4.[149]

From the limited studies pertaining to butadiene in terms of QSAR, these widely used drugs are expected to be carcinogenic in humans. The lack of observation of this phenomenon in humans may be attributed to the relative "newness" of these drugs and the slow manifestation of their long-term toxicity. It is of interest to examine drugs that have been pulled off the market or that have had cautionary labels attached to them. Lamisil is a drug that the

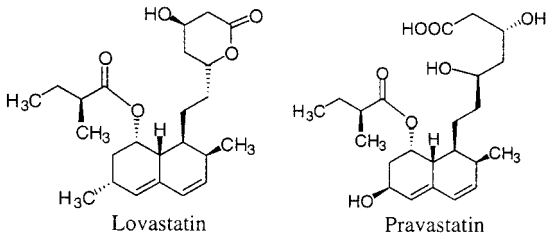


Figure 4. Structures of Lovastatin and Pravastatin.

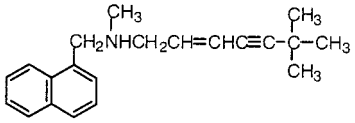


Figure 5. Structure of Lamisil.

FDA has recently earmarked for new label warnings. It is used for fungal infections of the skin and nails but has the propensity to cause severe liver damage, Figure 5.

There are two potential sites that could mediate radical toxicity in the liver. There is a benzylamine-type unit and a conjugated moiety comparable to butadiene. QSAR 93 indicates that olefinic linkages can participate in radical reactions, while QSAR 97 suggests that unsaturated linkages can be implicated in hepatotoxicity. Three QSAR can provide some insight on the Lamisil problem.

Addition of $^{\bullet}CCl_3$ to 4-X−$C_6H_4C{\equiv}CH$ in cyclohexane at 65 °C[150]

$$\log k_{rel} = -0.69(\pm 0.11)\sigma^+ - 0.04(\pm 0.05) \quad (101)$$

$$n = 13,\ r^2 = 0.942,\ s = 0.087,\ q^2 = 0.917$$

outlier: 4-COMe

Oxidation of 4-X−$C_6H_4C{\equiv}CH$ in benzene to X−phenyl−oxirane perbenzoic acid at 25 °C[151]

$$\log k_{rel} = -1.37(\pm 0.07)\sigma^+ - 0.03(\pm 0.03) \quad (102)$$

$$n = 6,\ r^2 = 0.999,\ s = 0.029,\ q^2 = 0.994$$

The above two reactions are radical in nature and suggest that the acetylenic (C≡CH) function behaves similarly to the ethylenic (CH═CH_2) moiety.

There is one biological QSAR for comparison.

Oxidation of X−$C_6H_4C{\equiv}CH$ to X−$C_6H_4CH_2COOH$ by cytochrome P450[152]

$$\log V_{max} = -0.98(\pm 0.56)\sigma^+ + 0.68(\pm 0.23) \quad (103)$$

$$n = 5,\ r^2 = 0.912,\ s = 0.158,\ q^2 = 0.681$$

Catalysis is directly proportional to V_{max}, and thus, catalysis is enhanced by electron-releasing substituents. The differences in structural features of Lamisil may not allow P450 oxidation, but radical formation via ROS may prevail. In reality, radical conversion to the epoxide (QSAR 102) may be more damaging. Styrene epoxides are quite mutagenic as shown by QSAR 104.[153]

$$\log k_2 = -1.93(\pm 0.57)\sigma^+ + 1.40(\pm 0.12) \quad (104)$$

$$n = 6,\ r^2 = 0.957,\ s = 0.093,\ q^2 = 0.911$$

outlier: 3-Cl

Epoxides can also readily undergo nucleophilic substitution with electron-rich functions, as QSAR 105 illustrates.

Reaction of X−C_6H_4−ethylene oxide with benzylamine to yield X−C_6H_4−$CH(NHCH_2C_6H_5)CH_2OH$ in ethanol[154]

$$\log k_2 = -1.06(\pm 0.31)\sigma^+ - 4.73(\pm 0.09) \quad (105)$$

$$n = 7,\ r^2 = 0.938,\ s = 0.093,\ q^2 = 0.891$$

outlier: 3-OMe

It is noteworthy that QSAR 105 contains a $-\sigma^+$ term. Two other drugs withdrawn from the market that contain unsaturation with the potential to yield epoxides are Xemilofibam and Rapacuronium bromide, Figure 6.

It is quite interesting that a common pattern exists among these examples that can be related to much simpler systems. Troglitazone (Rezulin) can be related to vitamin E, Figure 7.

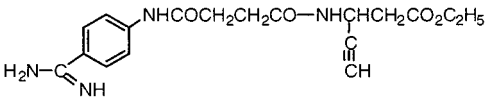
The encircled portion of troglitazone is the active portion of vitamin E. However, the vitamin contains a long carbon chain [$CH_2CH_2CH_2C(CH_3)CH_2CH_2$-$CH_2C(CH_3)CH_2CH_2CH_2C(CH_3)CH_3$] that makes it extremely hydrophobic, Clog $P = 12$. In fact, this cannot even be measured, while Rezulin has a Clog P value of 5.59. It is much more hydrophilic than vitamin E and therefore more easily accessible. Undoubtedly, the long chain of vitamin E evolved over the eons for a reason. It can anchor the vitamin into a large hydrophobic region with its less hydrophobic portion near the surface, of say a membrane, to scavenge radicals. The more hydrophilic troglitazone would be freer to move about, form a radical via reaction with ROS, and eventually cause cellular damage. To test this radical-scavenging ability, Mukai et al.[155] studied the following reaction from which we derived QSAR 106, Scheme 6.

$$\log k_2 = -1.08(\pm 0.32)\sigma^+ + 0.37(\pm 0.28)B1_3 + 2.35(\pm 0.39) \quad (106)$$

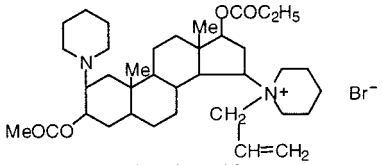
$$n = 10,\ r^2 = 0.908,\ s = 0.095,\ q^2 = 0.790$$

In this expression the sterimol parameter B1 for X_3 indicates a small positive steric effect that impacts aqueous solvation of the adjacent oxygen, making the lone pair electrons more available for delocalization of the radical electron. For the methyl groups, σ and σ^+ are highly collinear. σ^+ accounts for most of the variance (78%) in the data.

A more common kind of toxicity that has fascinated scientists through the ages pertains to ethanol. Considerable work has been done to elucidate the mechanism of oxidation of ethanol. The following QSAR focus on reagents that operate by a radical mechanism:[91]



Xemilofibam



Rapacuronium bromide

Figure 6. Structures of Xemilofibam and Rapacuronium bromide.

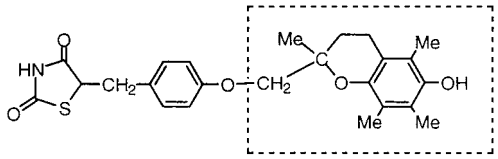
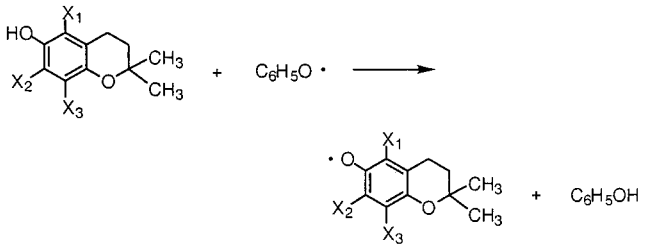


Figure 7. Structure of Troglitazone.

Scheme 6. Free-Radical-Mediated Hydrogen Abstraction of 2-Tocopherols



Oxidation of X−CH_2OH to X−CHO in 50% aqueous acetic acid by pyridinium hydrobromide perbromate[156]

$$\log k = -1.73(\pm 0.31)\sigma^* + 0.69(\pm 0.35)B1 + 0.33(\pm 0.62) \quad (107)$$

$$n = 8,\ r^2 = 0.987,\ s = 0.127,\ q^2 = 0.951$$

outlier: H

Oxidation of X−CH_2OH by N-bromoacetamide in 50% aqueous acetic acid at 35 °C[157]

$$\log k = -1.52(\pm 0.29)\sigma^* - 3.32(\pm 0.13) \quad (108)$$

$$n = 8,\ r^2 = 0.964,\ s = 0.137,\ q^2 = 0.905$$

outlier: $CHMe_2$

Oxidation of X−CH_2OH by 2,2′-bipyridinium chlorochromate in dimethyl sulfoxide at 40 °C[158]

$$\log k_2 = -1.44(\pm 0.07)\sigma^* - 0.62(\pm 0.05)E_S + 1.51(\pm 0.07) \quad (109)$$

$$n = 9,\ r^2 = 0.999,\ s = 0.032,\ \sigma^* = 0.998$$

These results are interesting in two respects. Negative ρ values indicate that electron-releasing substituents promote oxidation, and in two of the three examples there is positive steric effect. Recall

that E_S values are negative. The reason for the steric effects is not clear. Nevertheless, the results support a radical mechanism of oxidation.

The literature on the toxicology of ethanol continues to grow in leaps and bounds. The general consensus is that the first step in its toxic trek is its conversion to CH_3CHOH that is involved in liver damage. This species is then oxidized to the toxic metabolites acetaldehyde and acetic acid. The oxidation to acetic acid also involves σ^* terms with a negative ρ.

Oxidation of CH_3CHO to CH_3COOH by bovine brain dehydrogenase[159]

$$\log V_{max}/K_m = -1.06(\pm 0.34)\sigma^* + 8.63(\pm 0.23) \quad (110)$$

$$n = 11,\ r^2 = 0.849,\ s = 0.319,\ q^2 = 0.804$$

outlier: H

Unfortunately there is a dearth of comprehensive studies of the type covered by QSAR 107−109. These equations are in line with the many qualitative studies showing the dependence of toxicity on the formation of a radical, which is formed by action of the ROS on ethanol.[96] Numerous detoxifying mechanisms, such as superoxide dismutase, glutathione, etc., come into play, but the question remains as to how much alcohol is needed to overcome endogenous protective mechanisms.

IV. Conclusion

There are two reasons for studying QSAR. One is common to all areas of science, that of pure intellectual joy of understanding how systems work. The other affords us a practical understanding of how chemicals interact with each other and ultimately biological receptors. In recent years drugs have become much more effective at correcting fundamental deficiencies in human metabolism, and this necessitates usage for extended periods of time. Acute toxicity is easily recognized, but chronic toxicity and the development of malignant processes such as cancer remains a vexing problem. Epidemiology and statistics are key approaches for disentangling the many variables as was so well illustrated in the link between smoking and cancer.[160]

Radicals have been implicated in cancer and estrogenic toxicity.[44,91] These are not sudden onset diseases. Hence, it becomes of interest to know what types of chemicals have a propensity to form radicals in living systems. A long-term interest in characterizing the properties of various functional groups in terms of their biological activity has recently led to the creation of a database that has matured to the point where appropriate insight can be gained via comparative QSAR. However, when one thinks of all of the possible biological entities from DNA to cells to humans and the vast and increasingly complex numbers of organic compounds, it is apparent that a database of 17 400 QSAR is a very small start on an enormously complex problem. Nevertheless, progress is being made on this front.

It is increasingly evident that one cannot consider a substituent by itself. The electronic, hydrophobic, and steric attributes of other substituents in a molecule profoundly affect the reactivity of a leading functional group. As we have shown, substituents with electron-releasing σ^+ values produce estrogenic and carcinogenic phenols. Electron-attracting substituents are relatively benign. Phenols bearing substituents with positive σ^- values are toxic to mitochondria. The aromatic nitro function becomes worrisome if conjugated with substituents having σ^- with $+\rho^-$. However, toxicity may not be apparent if the system lacks enzymes that can reduce the nitro group to the nitro anion radical. Hence, it is not very toxic to L1210 cells. The aromatic amino entity is similar in reactivity to the phenolic function. It was a surprise to learn that it is not the nitro group that induces toxicity to bacteria in chloramphenicol, but actually it is the benzylic hydrogen activated by an OH group. It is not yet clear why in most instances radical activity can be accounted for by σ^+ but in other cases E_R is a better reflection of radical-forming ability.

In whole organisms the problems become more complex since ADME (adsorption, distribution, metabolism, and excretion) concerns have to be addressed. For example, in the discussion of radical toxicity, hydrophobicity of the chemicals is not an overriding issue, but in metabolism and elimination it can be crucial.[161] Early work has well established that one would want to develop the most hydrophilic drugs commensurate with efficacy to prevent drugs from crossing the blood−brain barrier.[67]

The importance of supporting one biological QSAR with another is obvious, but when the biological activities can be related to clearly delineated examples from physical organic chemistry, one can feel more secure in the conclusions, especially if a variety of examples can be mobilized to support a given view. Our early compilation of Hammett equations for chemical reactions was constructed almost 40 years ago, mostly out of curiosity. Today, collection is carried out with a purpose; a meager depository of oxidation reactions has now grown substantially. The current review shows the usefulness of this approach. In 1995, the discovery that deformation of rat embryos by limited small sets of phenols was correlated with σ^+ led to a vastly better understanding of the radical toxicity of phenols (QSAR 49). More recent work in the QSAR database has shown promise in uncovering allosteric reactions in vitro and in vivo.[162] Thus, a major side effect of database development has involved the genesis of unexpected insight.

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VI. References

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