QSAR Studies on Enzyme Inhibitors

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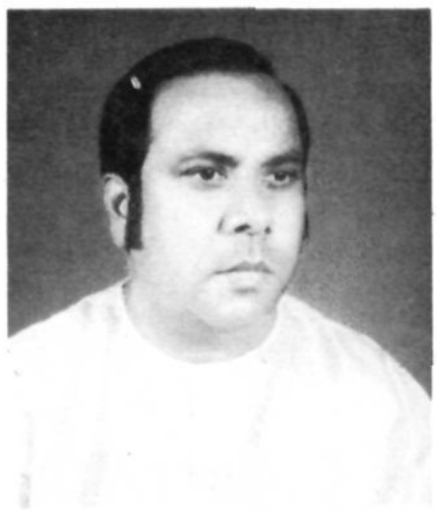

Satya P. Gupta is presently an Associate Professor of Chemistry at Birla Institute of Technology and Science (BITS), Pilani. Born in 1945, he received his M.Sc. (Physical Chemistry) in 1967 and Ph.D. in 1971 from the University of Allahabad, Allahabad. He then moved to Tata Institute of Fundamental Research (TIFR), Bombay, where he worked with Dr. G. Govil in the field of molecular biology. He joined BITS in 1973. He has worked in many areas such as classical potential functions, molecular orbital theory, molecular biology, and quantum pharmacology. His present interests, however, lie mainly in the theoretical aspects of drug design.

I. Introduction

Inhibitors of enzymic reactions have aquired a very vast dimension in biochemical, biophysical, medical, and pharamceutical research and in the treatment of a large number of diseases. They are used to study specific intracellular functions and related processes and provide tools by which the mechanisms of ligand binding, enzymic catalysis, and other aspects of enzyme chemistry can be elucidated. Blockade of the function of an essential enzyme for growth or cell division has been a favored target for anticancer and antiparasite chemotherapy. The partial or total blockade of a mammalian enzyme for control of the function of a specialized type of cell in mammals, such as nerve or brain cells, forms the targets for the medicinal chemists. The rational design of effective therapeutic agents, however, first requires the identification of a target enzyme that, if inhibited, will produce the desired therapeutic effect without causing toxicity to the host. After the target enzyme to be inhibited has been identified, one must attempt to find an effective inhibitor. Until recently, however, many widely used drugs that are enzyme inhibitors were actually found by trial and error methods. But now we have found ways that provide a rational basis to the design of enzyme inhibitors.

Long ago it was proposed that the biological activity of a compound is a function of its chemical structure. Today, biological activity is considered as a function of physicochemical properties. With this concept, structure–activity relationships (SAR) are developed when a set of physicochemical properties of a group of congeners are found to explain variations in biological responses of those compounds. This resulted in discovery, examination, and interpretation of SAR in a more systematic way, which led to the introduction of quantitative structure–activity relationships (QSAR) studies.

II. Introduction to QSAR Study

The QSAR study tries to explain the observed variations in biological activities of a group of congeners in terms of molecular variations caused by a change of the substituents. Two important applications of QSAR studies can be stated: the predictive aspect and the diagnostic aspect. The predictive aspect, as the name implies, deals with the extrapolation and interpolation of a correlation study. The diagnostic aspect, on the other hand, answers mechanistic aspects of the reaction. Some important approaches to QSAR studies are the nonparametric methods developed by Free and Wilson[1] or by Fujita and Ban,[2] the parametric method developed by Hansch,[3] discriminant analysis,[4] and the pattern recognition technique.[5] The choice of method depends on various factors such as quality of the biological data, number of compounds tested, degree of

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TABLE 1. Linear Free Energy Related Parameters

parameter	name	description
P	partition coefficient	log P taken as a measure of the hydrophobicity of the molecule; for measuring P, an octanol–water system preferred[6,7]
π	hydrophobic constant	π = log P_X − log P_H, where P_X is the partition coefficient of the substituted compound and P_H that of the unsubstituted reference compound[7]
R_M	hydrophobic constant from chromatography	log P linearly related[8] to R_M as log P = R_M + constant
k'	hydrophobic constant from high-pressure liquid chromatography (HPLC)	log P linearly related[9] to log k' as log P = log k' + constant
σ (σ_m, σ_p)	Hammett constant	defined[10] only for meta and para substituents to represent electronic character; positive value of σ denotes electron-withdrawing character and negative value of σ denotes electron-donating character; parameter may represent effects of ionization, hydrogen bonding, and charge–charge or charge–dipole interactions of compounds with the receptor
σ_0	apparent Hammett constant for ortho substituents	unrealiable and rarely used; hydrogen bonding to solvent decreases π so covariance may occur
σ^+, σ^-	Hammett constants	used, respectively, when substituents donate electrons to a positive site or withdrawn from a negative site by direct resonance interaction[11,12]
σ^*	Taft constant	measure of the electronic effect produced by aliphatic substituents[13]
σ_I	inductive constant	related[13] to σ^* as σ_I = 6.43σ^*
$\mathcal{R}$, $\mathcal{F}$	resonance and field constants	σ involves resonance effect ($\mathcal{R}$) and field (inductive) effect ($\mathcal{F}$) of a substituent,[14] i.e., σ = $a\mathcal{R}$ + $b\mathcal{F}$, where a and b are constants depending upon the type of σ (σ_m, σ_p); $\mathcal{R}$ and $\mathcal{F}$ more indicative of intrinsic resonance and field effects of a substituent; sign of $\mathcal{R}$ and $\mathcal{F}$ would indicate the sign of the charge that the substituents would place on the ring
pK_a	ionization constant	pK_a = −log K_a, K_a being the ionization constant of an acid
Q (Q^σ, Q^π)	charge of an atom	positive charge indicates interaction of atom with a negative center of the receptor; negative charge indicates interaction of the atom with a positive center of the receptor; Q^σ stands for the σ charge and Q^π for the π charge, calculated by quantum mechanical methods[15,16]
f_r^E, f_r^N	electrophilic and nucleophilic frontier orbital densities	quantum mechanical parameters used to discuss the electrophilic and nucleophilic reactions at a particular center in the molecule[17]
S_r^E, S_r^N	electrophilic and nucleophilic superdelocalizabilities	incorporate the concept of charge-transfer capabilities along with an orbital charge at a particular atom[18]
E_{HOMO}	energy of the highest occupied molecular orbital	quantum mechanical parameter that corresponds to the energy of the ionization potential of the molecule[15,16]
E_{LEMO}	energy of the lowest empty molecular orbital	quantum mechanical parameter that corresponds to the electron affinity of the molecule[15,16]
E_s	Taft steric constant	related to the acid-catalyzed hydrolysis[13] of α-substituted acetates (XCH_2COOR) and represents the steric effect affecting intramolecular and intermolecular hindrance to the reaction or binding
MR	molar refractivity	MR = $[(n^2 - 1)/(n^2 + 2)]MW/d$, where n is the refractive index for the sodium D line, MW is the molecular weight, and d is the density of the compound; MR can be used[19] as a steric parameter in the absence of E_s; measures the electronic effect also[19] and may reflect the dipole–dipole interaction at the active site[20]
MV	molar volume	MV,[21] V_w,[22] r,[23] R,[24] L and B,[25] MW,[19] etc., have also been used as steric parameters
V_w	van der Waals volume	
r	van der Waals radius	
R	interatomic distance	
L	length parameter	
B	width parameter	
χ	molecular connectivity index	topological parameter[26] defined to account for the effects of kinds of atoms, bonding type, adjacency environment, branching pattern, unsaturation, and heteroatom content in a molecule on its reactivity or activity

variance in the results, and ratio of the time required for synthesis and biological testing.

The most widely used approach continues to be the so-called Hansch approach,[3] where the variance in biological activity (BA) is explained by the variance of certain physicochemical and structural properties of molecules. The physicochemical properties include electronic characteristics, steric factors, and the solvent-partitioning or hydrophobic effects. Thus, the Hansch model proposes the dependence of the biological activity of drugs on their physicochemical and structural properties to be in the fashion as shown by eq 1, where π or log P is a hydrophobic parameter, σ

$$\text{BA} = a + b\pi \text{ (or log } P) + c\pi^2 \text{ (or log } P)^2 + d\sigma + eE_s + gS \quad (1)$$

an electronic parameter, E_s a steric factor, and S a structural parameter defining the shape, size, or topography of the molecule. All these parameters are briefly described in Table 1 in accordance with various sources.[6-26]

Equation 1 shows a nonlinear, i.e., a parabolic, dependence of activity on the hydrophobic character of molecules. Hansch, in fact, had assumed a "random walk" of the molecules, where hydrophilic molecules tend to remain in aqueous phases, hydrophobic molecules tend to go into lipid phases, and only molecules with an optimal hydrophilic/hydrophobic balance tend to reach their goal in reasonable time and concentration. For in vivo systems, the nonlinear dependence of the rate constants of drug transport through aqueous and bioorganic phases on lipophilicity seems to be the most reasonable explanation for the nonlinear dependence of activity on π or log P. For simple in vitro systems, e.g., enzyme inhibition, such nonlinear relationships result from equilibrium distribution of the drug toward different areas at the enzyme surface, from limited binding space at the active site, or from limited solubility of the more lipophilic congeners.

However, in many cases the relationships between activity and lipophilicity were found to be strictly linear,[3] and although the parabolic model proved to be extremely useful for practical purposes, there was an inconsistency between it and the linear model. While in most of the cases the ascending parts of nonlinear relationships were strictly linear, the ascending and descending parts of each parabola would be slightly curved. Although much less is known about the dependence of biological activities on lipophilic character beyond the point of optimal lipophilicity ($\log P_o$ or π_o), most often a linear relationship is observed with a negative slope beyond it.

To overcome such inconsistencies between the linear and nonlinear models, a number of different models[27-33] were proposed, out of which Kubinyi's bilinear model (eq 2 or 3) was found to be the most useful[34-40] after

$$\mathrm{BA} = a \log P - b \log (\beta P + 1) + c \qquad (2)$$

$$\mathrm{BA} = a\pi - b \log (10^{\pi}\beta + 1) + c \qquad (3)$$

Hansch's parabolic model to describe the nonlinear relationships. For this nonlinear model, however, one can not employ the usual least-squares method; hence, all the disposal parameters, a, b, c, and β, are evaluated by an iterative procedure. In the case of hydrophobic binding, one would find a linear increase in potency until a breaking point (π_o or $\log P_o$) is reached, and then the second term in eq 2 (or 3) takes over and a new linear relationship with slope $(a - b)$ is obtained. If $(a - b)$ is zero, one can assume that at point π_o one has reached the edge of a hydrophobic pocket, and the larger hydrophobic groups must at least in part extend beyond the enzyme in the aqueous space. Thus, from this bilinear model, one can easily find the size of the hydrophobic pocket or an enzyme by increasing the size of the substituents binding in this region. In simple regression analysis, this aspect of the study poses a problem. Nevertheless, most of the QSAR studies made so far on enzyme inhibitors have been based on the Hansch approach. No doubt many of them would yield better results if subjected to Kubinyi's bilinear model.

III. Limitations of QSAR

While QSAR studies can be successfully utilized to predict the activity of new analogues and discuss the mechanisms of drug–receptor interactions, they have many drawbacks and limitations as described below.[41]

The substituent effect on hydrophobicity is characterized by log P based on an octanol–water system; hence, even a very significant correlation cannot represent a true model for hydrophobic interaction between a drug molecule and the receptor. The value of log P also depends on the electronic characters and the hydrogen-bonding properties of the substituents.[42,43] Thus, if one gets a correlation with log P only, one cannot conclude that there is only hydrophobic interaction between drug and receptor and that no electronic interaction or hydrogen bonding takes place. Another factor that may influence log P values is the steric effect that can prevent the access of water to a hydrophilic group.[44] One may, however, choose to calculate log P and do QSAR analysis, but in spite of the work of many investigators, the calculation of log P is still characterized by a large set of empirical rules, interaction terms, and special adjustments.[45,46] Values determined for one system cannot be universally used for every system.

A more serious problem arises with the electronic parameters. In fact, details of the chemistry of drug–receptor interactions are usually not known; hence, the type of parameter needed to model this interaction is not properly defined. The Hammett constants do not reflect which portion of the drug molecule would be actually involved in the interaction with the receptor. Quantum mechanical calculations do provide some help in this direction, but they are time consuming and expensive.

Steric interactions are extremely difficult to extrapolate from system to system. With drugs, one usually is not even sure of the atom from which to base the steric effects. The use of parameters such as MR, MW, V_w, r, R, χ, etc., do not give any idea in what way steric effects would affect the drug–receptor interaction.

Even a successful QSAR study will provide only indirect information about the three-dimensional aspects of the drug–biomolecule interaction. Both drugs and biomolecules are three-dimensional objects whose chemical features are related to their three-dimensional shape. The interaction between them involves a complementarity or fit between the two objects. Although these three-dimensional considerations may be implicit in the use of E_s and factoring π and MR by position, no explicit terms have been designed to specifically describe the variation in conformation, conformational flexibility, or three-dimensional aspects of analogues. Regarding receptor mapping, most of the QSAR studies have been made with the assumption that drug receptors are relatively rigid molecules.[47,48] In the case of enzymes, however, one has little concept of how rigid and unbending a particular portion of the enzyme with which the inhibitor comes into contact may be, and in such situations it is extremely difficult to parametize the steric effects.[49] In many cases, it is difficult to know the exact dimensions of the active site of the enzymes where the inhibitors are binding.

Although molecules are usually represented on paper as rigid structures, they may in fact be quite different in solution and their dynamic nature should be recognized. There is considerable evidence that macromolecules—even in the crystalline state—exhibit a wide spectrum of motion.[50-54] These motions may be involved in some molecular conformational changes on substrate or drug binding. The binding of substrates to proteins, however, seems to have the general effect of lightening the molecular structure and reducing its flexibility.[55]

Many structural features that affect the activity but cannot be parametized by the usual variables such as π, σ, E_s, etc., are accounted for by the use of indicator variables. These indicator variables are arbitrarily assigned two values: one to indicate the presence of the specific structural feature and the other to indicate its absence. If the entire series of congeners is divided into two sets, one with and one without the specific structural feature, one would obtain two equations almost parallel, with a difference in their intercepts only. An indicator variable thus can be pictured simply as a constant that adjusts two parallel equations into one. If two sets are far apart in data space described by the

usual parameters, one builds in a large amount of variance with the indicator variable, leading to a much higher correlation coefficient (r).[56] Despite the better r, the new correlation may be a poorer one, and thus, one can be misled if other statistical parameters are not available.

Another serious problem in QSAR analysis is the problem of collinearity.[45] For example, π and MR most often turn out to be so collinear that it becomes impossible to tell whether one or both are involved in SAR. The MR-dependent QSAR in vitro may vanish in the in vivo QSAR, and in the case of enzyme inhibition, a QSAR of in vitro data may lead to a completely false interpretation for in vivo interaction.[49]

Over and above all, a QSAR study may be incorrectly interpreted if the biological property of interest is not correctly measured. A measured biological response may be a complex result of several processes, and an in vitro model of drug–receptor interaction does not always represent the true in vivo model.

IV. Inhibitors and Inhibitor Constants

Enzyme inhibitors are basically of two types: reversible inhibitors and irreversible inhibitors. Those that follow the inhibition mechanism as given by eq 4

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EI \tag{4}$$

$$E + I \xrightarrow{k} EI \tag{5}$$

$$E + I \underset{k_{-1}'}{\overset{k_1'}{\rightleftharpoons}} EI \xrightarrow{k_2} E\text{–}I \tag{6}$$

are called reversible inhibitors, and those that follow the inhibition mechanism as given by either eq 5 or 6 are called irreversible inhibitors. Of the latter, those following eq 5 are called nonspecific irreversible inhibitors and those following eq 6 are called specific irreversible inhibitors [active-site-directed[57] or k_{cat} (suicide) inhibitors[58–60]]. In eq 4–6, E stands for enzyme and I stands for inhibitor.

For the reversible inhibitors that follow the mechanism as shown by eq 4, the inhibitor constant K_i, which is actually the dissociation constant for complex EI, is given by eq 7. Equation 7 is derived with the assumption that the concentration of inhibitor as compared with that of enzyme is very large and that a very small amount of the inhibitor combines with the enzyme, so that the concentration of the inhibitor can be regarded as being unchanged after formation of the complex. This inhibitor constant K_i is usually characteristic of such a combination where the inhibition shows no time dependence, as the reaction does not involve formation of covalent bonds between the enzyme and the inhibitor. Since the interaction is reversible, inhibition can be reversed by dialysis, gel filtration, or dilution.

$$K_i = k_{-1}/k_{+1} = ([E] - [EI])\,[I]/[EI] \tag{7}$$

Reversible inhibitors are classified into a number of types, depending upon the effects they produce in the simple Michaelis–Menten equation[61] (eq 8), which is derived for the reaction of the substrate (S) with the enzyme (eq 9). Equation 8 is derived by steady-state approximation,[62] which gives K_m, the Michaelis–Menten constant, equal to $(k_2 + k_{-1})/k_1$.[63] V_i and V_{max} in eq 8 are the initial and the maximum velocities, respectively, of the substrate–enzyme reaction. From eq 8, K_m can be defined as the substrate concentration at which $V_i = {}^1/_2 V_{max}$. An inhibitor that increases the K_m value without affecting V_{max} is termed a competitive inhibitor, and one that decreases V_{max} without affecting K_m is termed a noncompetitive inhibitor. Inhibitors that cause an equal decrease in K_m and V_{max} are termed uncompetitive (for anticompetitive) inhibitors. Equations 10–13 describe all types of reversible inhibition.

$$V_i = V_{max}[S]/([S] + K_m) \tag{8}$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P \tag{9}$$

None

$$V_i = V_{max}/(1 + K_m/[S]) \tag{10a}$$

$$\text{or } \frac{1}{V_i} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}}\frac{1}{[S]} \tag{10b}$$

Competitive

$$V_i = \frac{V_{max}}{1 + (K_m/[S])(1 + [I]/K_i)} \tag{11a}$$

$$\text{or } \frac{1}{V_i} = \frac{1}{V_{max}} + \frac{K_m(1 + [I]/K_i)}{V_{max}}\frac{1}{[S]} \tag{11b}$$

Noncompetitive

$$V_i = \frac{V_{max}/(1 + [I]/K_i)}{1 + K_m/[S]} \tag{12a}$$

$$\text{or } \frac{1}{V_i} = \frac{(1 + [I]/K_i)}{V_{max}} + \frac{K_m(1 + [I]/K_i)}{V_{max}}\frac{1}{[S]} \tag{12b}$$

Uncompetitive

$$V_i = \frac{V_{max}}{(1 + [I]/K_i) + K_m/[S]} \tag{13a}$$

$$\text{or } \frac{1}{V_i} = \frac{(1 + [I]/K_i)}{V_{max}} + \frac{K_m}{V_{max}}\frac{1}{[S]} \tag{13b}$$

Inhibitor constant K_i for any type of inhibitor can be determined[63] by a Lineweaver–Burk plot,[64] also known as a double-reciprocal plot, where $1/V_i$ is plotted against $1/[S]$ for a given concentration of inhibitor, or by Dixon's plot,[65] where $1/V_i$ is plotted against varying inhibitor concentrations for a given concentration of substrate. The Linewaver–Burk plot for each type of inhibitor is shown in Figure 1. It is obvious from this figure that if the value of K_m for substrate–enzyme interaction (plot D) is known, one can easily evaluate the value of K_i for any kind of inhibitor for a given concentration from the vertical or horizontal intercept of the inhibitor's plot. In any type of inhibition, the magnitude of the inverse of the horizontal intercept of the plot is known as the apparent K_m (K_m(app)).

Of the various types of simple reversible inhibitors, those most commonly dealt with medically and pharmacologically are competitive inhibitors. They are normally based on analogues of the true substrate of the enzyme and are supposed to bind at the same site

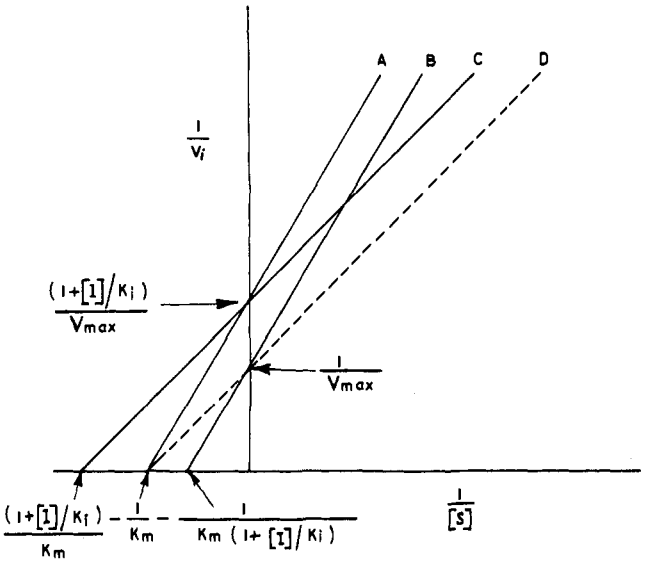


Figure 1. Lineweaver–Burk plot. Key: A, noncompetitive inhibition; B, competitive inhibition; C, uncompetitive inhibition; D, control with no inhibitor.

of the enzyme at which the substrate binds. Hence, the effect of a competitive inhibitor can be completely reversed by high concentrations of substrate. Because of this, the in vivo potency of a competitive inhibitor may be expected to be less than its in vitro potency measured with purified enzyme, as in the in vivo system the inhibition of an enzyme may lead to an increase in the steady-state concentration of the substrate.

Some inhibitors bind to enzymes so tightly that they can inhibit the enzyme at very low concentrations, comparable to that of the enzyme. For such tightly binding inhibitors, the concentration of inhibitors should not be kept constant in evaluating the value of K_i and, hence, eq 5 should not be valid. There would be an effective reduction in the concentrations of inhibitors, but this point is often ignored in estimating K_i.

For careful QSAR studies, it is therefore advisable that one finds 95% confidence limits on K_i.[66]

When the inhibitor does not bind very tightly, the Scatchard method[67] can be applied to analyze the binding data. Equation 7 may be rearranged into eq 14. In this equation [E] represents the enzyme concentration expressed in terms of its binding sites, i.e., if the enzyme has n binding sites per molecule, the value of [E] in this equation will be n times its molar concentration. If the molar concentration of enzyme is designated by [E′], then dividing eq 14 by [E′] gives eq 15. Thus, a graph of [EI]/[E′] vs. [EI]/[I][E′] will give a straight line with a slope of $-K_i$ and an intercept on the vertical axis equal to the value of n.

$$[EI] = [E] - K_i\frac{[EI]}{[I]} \qquad (14)$$

$$\frac{[EI]}{[E']} = n - K_i\frac{[EI]}{[I][E']} \qquad (15)$$

Another, but approximate, index of potency of an inhibitor is I_{50}, i.e., its concentration leading to 50% enzyme inhibition. Sometimes I_{50} is divided by S_{50}, the substrate concentration required for 50% enzyme inhibition, to express the inhibitor potency as (I_{50}/S_{50}). The expression of percentage inhibition given at a single fixed concentration of substrate is of little use, and in some cases it can be misleading, as in many cases different degrees of inhibition with different substrates have been observed, showing that more than one enzyme may be involved.

In the case of irreversible inhibitors, only the first-order rate constant (or second-order rate constant at very low inhibitor concentration) is necessary to define the potency of inhibitors obeying eq 5; and for those obeying eq 6, the enzyme–inhibitor dissociation constant is also required, since for eq 6 the kinetic equation is[68] eq 16. I_{50} values have also been used to express the potency of irreversible inhibitors, but they are not absolute values, as they vary with enzyme concentrations.

$$d[EI]/dt = k_2[E]/(1 + K_i/[I]) \qquad (16)$$

V. Binding Sites of Enzymes

Substrates or inhibitors act at a particular site on the enzyme known as the active site, and the lock-and-key or the template analogy of enzyme–substrate (or enzyme–inhibitor) interaction has been proposed[69] because of the high degree of specificity of the enzymes. Thus, the active site (also known as the substrate-binding site or catalytic site) of the enzyme is complementary in size, shape, and chemical nature to the substrate molecule. The chemical change that converts the substrate to the product can occur only when the substrate is anchored in the active site. If one examines the geometry of binding sites in proteins, one would generalize that binding sites, from the viewpoint of the protein, are invariably crevices or grooves in the protein surfaces. It may be therefore expected that small molecules fit into these crevices.

The enzymatic binding sites, however, have several common features: (1) they are usually composed of a combination of very polar and very nonpolar amino acids; (2) a significant fraction of enzymatic active sites contains metal ions; (3) enzymes are usually engaged in altering covalent bonds, and binding sites reflect this activity by having large electric fields in a largely water-free environment.[70]

Studies on protein flexibility and the finding that binding of small ligands to the protein can induce specific conformational changes have played a central role in understanding the mechanisms of enzyme action. Some X-ray studies have provided direct evidence for conformational changes in proteins induced by ligands,[71,72] and other studies have indicated that even extremely subtle changes in molecular geometry can significantly alter the binding behavior.[73,74] Thus, the flexible enzyme or induced-fit theory was proposed by Koshland[75] to explain certain anomalies in the lock-and-key hypothesis, such as compounds resembling the normal substrate chemically but possessing less bulky groups often failed to react, though they would have completely fit, and compounds with more bulky groups often failed to react (as expected), yet they were found to bind tightly to the enzyme. Many bireactant enzymes would not bind substrate B before substrate A, though the enzymes would have binding sites for both B and A. The flexible-enzyme hypothesis therefore assumes that the substrate induces a conformational change in the enzyme that results in a precise alignment

of the catalytic groups with the susceptible bonds on the substrate. Substrate analogues with larger or smaller groups may bind to the enzyme but may not induce the proper alignment of the catalytic groups. In ordered bireactant systems, substrate A is assumed to induce a conformational change that exposes the binding site for substrate B.

The active sites are not the only receptor sites on the molecule. Regulatory sites, also called allosteric sites, distinct from the active sites, have also been identified in may multisubunit enzymes. An allosteric site is complementary to the structure of the protein–substrate complex and binds to the complex specifically and reversibly.[76] This causes a slight reversible change, called allosteric transition, in the protein's structure that modifies the properties of the active site in the protein and changes its function.

Several other areas adjacent to the active site often help strengthen the binding between the enzyme and the substrate or inhibitor. The surface of an enzyme can be expected to have many polar groups such as the hydroxyl groups of serine and threonine, carboxylic groups of glutamic and aspartic acids, and sulfur of cysteine and methionine. These groups are good nucleophiles. Therefore, when an inhibitor with a properly positioned electrophilic (leaving) group is complexed to the active site of an enzyme, a fast neighboring group reaction with high specificity can occur between the nucleophilic group on the surface of the enzyme and the electrophilic group on the inhibitor, forming a covalent bond. This is what actually happens in active-site-directed or k_{cat} inhibition. The former differs from the latter only in the sense that while in active-site-directed inhibition the inhibitor reacts with the enzyme by virtue of its own reactivity; in k_{cat} inhibition the inhibitor is converted to a reactive form by the catalytic action of the enzyme.

There may be other types of interaction also, such as hydrophobic or van der Waals, between the substrate or inhibitor and the areas near the active site of the enzyme. A phenomenon called the bulk tolerance[57] exploits the differences in the topography of areas adjacent to similar areas of two different enzymes.

VI. Classification of Enzymes under Review

All the enzymes whose inhibitions have been subjected to QSAR studies are grouped according to the similarity of the chemical process involved in catalyzing a biochemical phenomenon. This classification may differ from that of the Nomenclature Commission of the I.U.B. (International Union of Biochemistry) in the sense that many groups of this classification may fall in only one group of the latter.[77] But the present classification has been done for the sake of convenience of review and further discussion. The classification is described below, and the members in each group whose inhibition has been discussed are listed in Table 2 along with their EC number and the biochemical process they catalyze.

A. Oxidoreductases. These enzymes are concerned with biological oxidation and reduction and hence play an important role in respiration and fermentation processes. In a majority of cases they are called dehydrogenases, as the substrate undergoes oxidation by donating hydrogen atoms. A variety of substances such as O_2, NAD^+, $NADP^+$, cytochrome, H_2O_2, quinone, etc., act as acceptors. They are sometimes called reductases. In processes where O_2 is the acceptor, the enzymes are better known as oxidases. Oxygenases incorporate molecular oxygen into the molecule being oxidized.

Among all oxidoreductases studied, we have put all those bearing the name oxidase or oxygenase into one group (A_1) and all those bearing the name dehydrogenase or reductase into a second group (A_2).

B. Methyltransferases. These enzymes mediate the process of biological methylation in which a methyl group is transferred to an acceptor. The transfer is normally from *S*-adenosylmethionine.

Certain enzymes transfer substituted or unsubstituted alkyl groups other than a methyl group and are called alkyltransferases. Any such enzyme belonging to the group of alkyltransferases has been discussed in the group of methyltransferases.

C. Acyltransferases. Enzymes that transfer acyl groups forming either esters or amides are called acyltransferases. In most cases the donor is an acyl-coenzyme A derivative.

D. Pentosyltransferases. These enzymes belong to the general category of glycosyltransferases, which catalyze the transfer of sugar residues to various acceptors, especially to an OH group of another sugar or a phosphate or to a nitrogen atom of a heterocyclic ring. The transfer of a pentosyl is brought about by pentosyltransferases.

E. Nucleotidyltransferases. Nucleotidyltransferases belong to the group of enzymes that transfer phosphate groups to acceptors. They transfer a substituted phosphate group from a pyrophosphate to another substituted phosphate group forming a new pyrophosphate. These enzymes are thus important in the synthesis of dinucleotides and analogous molecules.

F. Esterases. Esterases are the hydrolases that catalyze the hydrolysis of ester bonds in a variety of esters such as carboxylic esters, thioesters, phosphates, phosphoric diesters, sulfuric esters, etc.

G. Glycosidases. Glycosidases hydrolyze glycosyl compounds. They are classified under hydrolases, but some of them can also transfer glycosyl residues to oligosaccharides, polysaccharides, and other alcoholic acceptors.

H. Peptidyldipeptide Hydrolases. These enzymes belong to the subgroup of hydrolases that act on peptide bonds. They are exopeptidases, which split off dipeptide units from the C terminus of peptide chains.

I. Serine Proteinases. They are endopeptidases acting on the peptide bonds. They are called serine proteinases because they have serine and histidine in their active centers.

J. Thiol Proteinases. They differ from the serine proteinases only in the sense that they have cysteine in their active centers.

K. Amido- and Aminohydrolases. These hydrolases act on carbon–nitrogen bonds other than peptide bonds. They can hydrolyze amides, amidines, and nitriles.

L. Pyrophosphatases. These enzymes hydrolyze acid anhydrides. They act on diphosphate bonds in compounds such as nucleoside di- and triphosphates and on sulfonyl-containing anhydrides such as adenyl sulfate.

M. Hydrolyases. Enzymes that cleave C–C, C–O, C–N, and other bonds by means other than hydrolysis or oxidation are called lyases. Hydrolyases are those lyases that catalyze the breakage of C–O bonds leading to unsaturated products with elimination of water.

N. Synthetases. All the enzymes that are involved in the synthesis of any biochemical have been put in this group, even though they belong to different classifications as determined by the Nomenclature Commissions of the I.U.B.

VII. QSAR Results and Discussions

A_1. Oxidases

1. Glycolic Acid Oxidase

As mentioned in Table 1, glycolic acid oxidase (GAO) catalyzes the production of glyoxylate from glycolate, which is the most important immediate biosynthetic precursor of oxalate.[78] The majority of kidney stones in humans contain calcium oxalate. Calcium oxalate is the predominant component in kidney stones and serves as the nidus for the crystallization of stones of mixed composition.[79] In genetic primary hyperoxalurias, the inefficient utilization of glyoxylate in normal pathways results in overproduction of oxalate, leading to serious and frequently lethal consequences.[78] Inhibitors of GAO, therefore, may serve as potentially useful drugs for the treatment of those disease states in which the pathological consequences are due to crystallization of calcium oxalate, such as primary hyperoxalurias and calcium oxalate renal lithiasis. Inhibitors of GAO may reduce both the production of glyoxylate from glycolate as well as the subsequent oxidation of glyoxylate to oxalate.

According to Schumann and Massey,[80] GAO contains a hydrophobic bonding region and two positively charged groups in close proximity. To find deeper insight into the mechanism of GAO inhibition, some QSAR studies were recently made.

Randall et al.[81] attempted a QSAR study on three series of compounds, substituted glycolic acids (Table 3), substituted oxyacetic acids (Table 4), and substituted glyoxylic acids (Table 5). For glycolic acids, these authors found their inhibitory potency (pI_{50}) to be related to MR and π as shown in eq 17 and 18, where data

$$pI_{50} = 0.054\ (\pm 0.007)\mathrm{MR} + 1.36 \qquad n = 21,\ r = 0.878 \tag{17}$$

$$pI_{50} = 0.6\ (\pm 0.1)\pi + 1.66 \qquad n = 21,\ r = 0.786 \tag{18}$$

within the parentheses are the 95% confidence intervals (at places where they would be without the ± sign, they would mean the standard error of the coefficient of variables), n is the number of data points, and r is the correlation coefficient. For oxyacetic acids, the pI_{50} was found to be correlated with π (eq 19). MR was not

$$pI_{50} = 0.8\ (\pm 0.3)\pi - 0.10\ (\pm 0.05)\pi^2 + 1.80 \qquad n = 31,\ r = 0.672 \tag{19}$$

found to be significant in this case. Randall et al.[81] also treated the para-, ortho-, and meta-substituted derivatives separately. Including compound 22 in each case they found the correlations for the three different groups as shown by eq 20–22, respectively.

$$pI_{50} = 1.5\ (\pm 0.5)\pi - 0.21\ (\pm 0.08)\pi^2 + 1.04 \qquad n = 11,\ r = 0.830 \tag{20}$$

$$pI_{50} = 0.10\ (\pm 0.04)\pi^2 + 2.41 \qquad n = 10,\ r = 0.727 \tag{21}$$

$$pI_{50} = 0.65\ (\pm 0.1)\pi - 0.4\ (\pm 0.2)\sigma_m + 1.71 \qquad n = 9,\ r = 0.928 \tag{22}$$

For substituted glyoxylic acids (Table 5), the pI_{50} was found to be poorly correlated with π (eq 23); but when

$$pI_{50} = 0.03\ (\pm 0.02)\pi^2 + 3.68 \qquad n = 18,\ r = 0.261 \tag{23}$$

a dummy parameter D was created for a subgroup of six compounds containing heteroatoms in close proximity to the point of attachment of the substituent to the α-keto group of the glyoxylic acids, the correlation was found to be improved (eq 24). D was given a value of 1.0 for these six compounds (55, 56, 59, 68–70) and 0.0 for all others.

$$pI_{50} = 0.04\ (\pm 0.02)\pi^2 + 0.8\ (\pm 0.2)D + 3.26 \qquad n = 18,\ r = 0.768 \tag{24}$$

However, in no case other than with meta-substituted phenoxyacetic acids (eq 22) was any electronic parameter found to be relevant. On the basis of these correlations, Randall et al.[81] suggested that the hydrophobic character of inhibitors plays an important role in GAO inhibition. In all cases, the predominant hydrophobic term had a positive coefficient, but in eq 19 and 20 π^2 had negative coefficients, and these two equations showed a parabolic correlation. But for a series of inhibitors binding to a purified enzyme preparation, such a parabolic dependence should not be expected, as there are no transport barriers, such as intervening membranes or nonselective binding to extraneous biological material. Randall et al. therefore attributed this parabolic dependence to a limited steric bulk tolerance at the enzyme active site[82] or to the interaction of inhibitors with a hydrophobic region of limited area near the active site. However, if bulk steric effects contributed to eq 19 and 20, one would expect parallel correlations in MR, which were not found. A better picture would have been obtained had one tried Kubinyi's bilinear model.

In three cases, the ortho-substituted phenoxyacetic acids (eq 21) and the glyoxylic acids (eq 23, 24), the π^2 term was found to be more significant than the π term. Randall et al. felt that this was artifactual, however, for in all these cases the correlation between π and π^2 was greater than 0.97. This large correlation meant that the two parameters were nearly equivalent, and in a small set of data, the π^2 term might by chance be found to be more significant.

However, the electronic parameter was found to be important in only one case, the meta-substituted phenoxyacetic acids (eq 22). Since in no other case was any electronic parameter found to be significant, it is possible that the significance of σ_m in eq 22 is a coincidence resulting from the small number of compounds used in deriving this equation. Thus, the marginal importance of σ_m together with the lack of significance of σ_p, $\mathcal{F}$, and $\mathcal{R}$ indicates that these standard electronic parameters

TABLE 2. Descriptions of Enzymes Reviewed

enzyme (EC no.)	systematic name	reaction catalyzed
	Oxidases	
glycolic acid oxidase (1.1.3.1)	glycolate:oxygen oxidoreductase	glycolate + O_2 = glyoxylate + H_2O_2
xanthine oxidase (1.2.3.2)	xanthine:oxygen oxidoreductase	xanthine + H_2O + O_2 = urate + superoxide
D-amino acid oxidase (1.4.3.3)	D-amino acid:oxygen oxidoreductase (deaminating)	D-amino acid + H_2O + O_2 = a 2-oxo acid + NH_3 + H_2O_2
monoamine oxidase (1.4.3.4)	amine:oxygen oxidoreductase (deaminating, flavin containing)	$RCHNH_2$ + H_2O + O_2 = RCHO + NH_3 + H_2O_2
mixed-function oxidases	see text	see text
lipoxygenase (1.13.11.12)	linoleate:oxygen oxidoreductase	linoleate + O_2 = 13-hydroperoxyoctadeca-9,11-dienoate
dopamine β-hydroxylase (1.14.17.1)	3,4-dihydroxyphenylethylamine, ascorbate:oxygen oxidoreductase (β-hydroxylating)	3,4-dihydroxyphenylethylamine + ascorbate + O_2 = noradrenalin + dehydroascorbate + H_2O
succinate oxidase	see text	see text
	Dehydrogenases	
alcohol dehydrogenase (1.1.1.1)	alcohol:NAD^+ oxidoreductase	alcohol + NAD^+ = aldehyde or ketone + NADH
lactate dehydrogenase (1.1.1.27)	L-lactate:NAD^+ oxidoreductase	L-lactate + NAD^+ = pyruvate + NADH
malate dehydrogenase (1.1.1.37)	L-malate:NAD^+ oxidoreductase	L-malate + NAD^+ = oxaloacetate + NADH
glyceraldehyde 3-phosphate dehydrogenase (1.2.1.12)	D-glyceraldehyde 3-phosphate:NAD^+ oxidoreductase (phosphorylating)	D-glyceraldehyde 3-phosphate + orthophosphate + NAD^+ = 3-phospho-D-glyceroyl phosphate + NADH
inosinic acid dehydrogenase (1.2.1.14)	IMP:NAD^+ oxidoreductase	inosine 5′-phosphate + NAD^+ + H_2O = xanthosine 5′-phosphate + NADH
glutamate dehydrogenase (1.4.1.2)	L-glutamate:NAD^+ oxidoreductase (deaminating)	L-glutamate + H_2O + NAD^+ = 2-oxoglutarate + NH_3 + NADH
dihydrofolate reductase (1.5.1.3)	5,6,7,8-tetrahydrofolate:$NADP^+$ oxidoreductase	5,6,7,8-tetrahydrofolate + $NADP^+$ = 7,8-dihydrofolate + NADPH
ribonucleoside diphosphate reductase (1.17.4.1)	2′-deoxyribonucleoside diphosphate:oxidized thioredoxin 2′-oxidoreductase	2′-deoxyribonucleoside diphosphate + oxidized thioredoxin + H_2O = ribonucleoside diphosphate + reduced thioredoxin
	Methyltransferases	
hydroxyindole *O*-methyltransferase (2.1.1.4)	*S*-adenosyl-L-methionine: *N*-acetylserotonin *O*-methyltransferase	*S*-adenosyl-L-methionine + *N*-acetylserotonin = *S*-adenosyl-L-homocysteine + *N*-acetyl-5-methoxytryptamine
catechol *O*-methyltransferase (2.1.1.6)	*S*-adenosyl-L-methionine:catechol *O*-methyltransferase	*S*-adenosyl-L-methionine + catechol = *S*-adenosyl-L-homocysteine + guaiacol
phenylethanolamine *N*-methyltransferase (2.1.1.28)	*S*-adenosyl-L-methionine: phenylethanolamine *N*-methyltransferase	*S*-adenosyl-L-methionine + noradrenalin = *S*-adenosyl-L-homocysteine + adrenalin
methionine adenosyltransferase (2.5.1.6)	ATP:L-methionine *S*-adenosyltransferase	ATP + L-methionine + H_2O = orthophosphate + pyrophosphate + *S*-adenosyl-L-methionine
	Acyltransferases	
N-arylhydroxamic acid *N*,*O*-acetyltransferase (2.3.1.5)	acetyl-CoA:arylamine *N*-acetyltransferase	acetyl-CoA + arylamine = CoA + *N*-acetylarylamine
choline acetyltransferase (2.3.1.6)	acetyl-CoA:choline *O*-acetyltransferase	acetyl-CoA + choline = CoA + *O*-acetylcholine
lysophosphatidylcholine acyltransferase (2.3.1.23)	acyl-CoA:1-acylglycero-3-phosphocholine *O*-acyltransferase	acyl-CoA + 1-acylglycero-3-phosphocholine = CoA + 1,2-diacylglycero-3-phosphocholine
	Pentosyltransferases	
uridine phosphorylase (2.4.2.3)	uridine:orthophosphate ribosyltransferase	uridine + orthophosphate = uracil + α-D-ribose 1-phosphate
thymidine phosphorylase (2.4.2.4)	thymidine:orthophosphate deoxyribosyltransferase	thymidine + orthophosphate = thymine + 2-deoxy-D-ribose 1-phosphate
	Nucleotidyltransferases	
DNA polymerase (2.7.7.7)	deoxynucleoside triphosphate:DNA deoxynucleotidyltransferase	*n* deoxynucleoside triphosphate = *n* pyrophosphate + DNA_n
	Esterases	
acetylcholinestearase (3.1.1.7)	acetylcholine hydrolase	acetylcholine + H_2O = choline + acetate
cholinesterase (3.1.1.8)	acylcholine acylhydrolase	an acylcholine + H_2O = choline + a carboxylic acid anion
cAMP phosphodiesterase (3.1.4.17)	3′:5′-cyclic nucleotide 5′-nucleotidohydrolase	nucleoside 3′:5′-cyclic phosphate + H_2O = nucleoside 5′-phosphate
	Glycosidases	
neuraminidase (3.2.1.18)	acylneuraminyl hydrolase	hydrolysis of 2,3-, 2,6-, and 2,8-glycosidic linkages joining terminal nonreducing *N*- or *O*-acylneuraminyl residues to galactose, *N*-acetylhexosamine, or *N*- or *O*-acylated neuraminyl residues in oligosaccharides, glycoproteins, glycolipids, or colominic acid
	Peptidyldipeptide Hydrolases	
angiotensin converting enzyme (3.4.15.1)	peptidyldipeptide hydrolase	polypeptidyl-dipeptide + H_2O = polypeptide + dipeptide
	Serine Proteinases	
chymotrypsin (3.4.21.1)		preferential cleavage: Tyr, Trp, Phe, Leu
trypsin (3.4.21.4)		preferential cleavage:Arg, Lys

TABLE 2 (Continued)

enzyme (EC no.)	systematic name	reaction catalyzed
thrombin (3.4.21.5)		preferential cleavage: Arg; activates fibrinogen to fibrin
plasmin (3.4.21.7)		preferential cleavage: Lys > Arg; higher selectivity than trypsin
complement		see the text
	Thiol Proteinases	
papain (3.4.22.2)		preferential cleavage: Arg-, Lys-, Phe-X-(the peptide bond next-but-one to the carboxylic group of phenylalanine); limited hydrolysis of native immunoglobulins
ficin (3.4.22.3)		preferential cleavage: Lys, Ala, Tyr, Gly, Asn, Leu, Val
bromelain (3.4.22.4)		preferential cleavage: Lys, Ala, Tyr, Gly
actinidin (3.4.22.14)		specificity close to that of papain
	Amido- and Aminohydrolases	
urease (3.5.1.5)	urea amidohydrolase	urea + H_2O = CO_2 + $2NH_3$
cytosine nucleoside deaminase (3.5.4.1)	cytosine aminohydrolase	cytosine + H_2O = uracil + NH_3
guanine deaminase (3.5.4.3)	guanine aminohydrolase	guanine + H_2O = xanthine + NH_3
adenosine deaminase (3.5.4.4)	adenosine aminohydrolase	adenosine + H_2O = inosine + NH_3
	Phosphatases	
(Na^+-K^+)ATPase (3.6.1.3)	ATP phosphohydrolase	ATP + H_2O = ADP + orthophosphate
	Hydrolyases	
carbonic anhydrase (4.2.1.1)	carbonate hydrolyase	H_2CO_3 (or H^+ + HCO_3^-) = CO_2 + H_2O
	Synthetases	
prostaglandin synthetase (1.14.99.1)	8,11,14-eicosatrienoate, hydrogen-donor:oxygen oxidoreductase	8,11,14-eicosatrienoate + AH_2 + $2O_2$ = prostaglandin E_1 + A + H_2O
thymidylate synthetase (2.1.1.45)	5,10-methylenetetrahydrofolate:dUMP *C*-methyltransferase	5,10-methylenetetrahydrofolate + dUMP = dihydrofolate + dTMP
dihydropteroate synthetase (2.5.1.15)	2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine diphosphate: 4-aminobenzoate 2-amino-4-hydroxydihydropteridine-6-methenyltransferase	2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine diphosphate + 4-aminobenzoate = pyrophosphate + dihydropteroate

are, at best, of only limited importance in the inhibition of GAO.

Rooney et al.[83] recently prepared a new series of GAO inhibitors, 4-substituted 3-hydroxy-1*H*-pyrrole-2,5-dione derivatives (Tables 6 and 7), and made a QSAR study of them. For compounds 71–111, they correlated the inhibition activity with π and MR as shown in eq 25 and 26, where s and F are, respectively, the standard deviation and the F ratio between the variances of observed and calculated activities. However, as is obvious, correlations expressed by eq 25 and 26 were not very significant; hence, a dummy parameter D was included to discriminate those compounds in Table 7 that have a thiazole group from those in Table 6 that have no thiazole group, with a value of unity for the former and zero for the latter. Equations 27 and 28 were then obtained. For compound 96, D was put equal to zero,

$$pI_{50} = 0.45\ (\pm 0.08)\pi + 4.54$$
$$n = 41,\ r = 0.68,\ s = 0.736,\ F = 34.13 \quad (25)$$
$$pI_{50} = 0.056\ (\pm 0.010)\mathrm{MR} + 3.32$$
$$n = 41,\ r = 0.67,\ s = 0.749,\ F = 31.64 \quad (26)$$
$$pI_{50} = 0.50\ (\pm 0.05)\pi + 1.1\ (\pm 0.2)D + 3.91$$
$$n = 41,\ r = 0.87,\ s = 0.508,\ F = 57.76 \quad (27)$$
$$pI_{50} = 0.051\ (\pm 0.009)\mathrm{MR} + 0.65\ (\pm 0.22)D + 3.28$$
$$n = 41,\ r = 0.74,\ s = 0.683,\ F = 23.48 \quad (28)$$

although it belongs to Table 7. The reason was that the activity of this compound was comparable to that of compounds in Table 6. However, from these equations, Rooney et al.[83] concluded, as anybody would, that π, MR, and D are not sufficient to completely explain the GAO inhibition. Rooney et al. did not try any electronic parameter. Nonetheless, their study parallels to the one made by Randall et al.,[81] and one can therefore conclude that the hydrophobic character only partly accounts for the inhibition of GAO by these inhibitors and the property that complements the inhibition is yet to be found.

TABLE 3. Substituted Glycolic Acids and Their GAO Inhibitory Potencies

RCH(OH)COOH (*d,l*)

compd	R	pI_{50}
1	C_6H_5	2.40
2	4-IC_6H_4	3.79
3	4-ClC_6H_4	3.23
4	4-(C_6H_5O)C_6H_4	3.80
5	4-(C_6H_5)C_6H_5	4.40
6	4-FC_6H_4	2.40
7	4-(1-*c*-C_6H_4N)C_6H_4	4.16
8	4-BrC_6H_4	3.39
9	4-(*c*-C_6H_{11})C_6H_4	4.52
10	3-$NO_2C_6H_4$	2.75
11	2-ClC_6H_4	2.77
12	$CH_3CH_2SCH_2$	2.72
13	$C_6H_5CH_2$	3.79
14	$C_6H_5CH{=}CH$	3.33
15	*c*-C_6H_{11}	2.40
16	$(CH_3)_2CHCH_2$	2.60
17	5-($C_6H_5CH_2$)-2-(*c*-C_4H_3S)	3.98
18	5-(4-ClC_6H_4)-2-(*c*-C_4H_3S)	4.29
19	4-(C_6H_5)$C_6H_4SCH_2$	5.00
20	$CH_3SCH_2CH_2$	2.30
21	3,5,7-$(CH_3)_3$-(*c*-$C_{10}H_{15}$)	3.40

The GAO inhibition activity of a series of phenoxyacetic acids was shown to be related with hydropho-

TABLE 4. Substituted Oxyacetic Acids and Their GAO Inhibitory Potencies

$ROCH_2COOH$

compd	R	pI_{50}
22	C_6H_5	2.74
23	4-$NO_2C_6H_4$	2.96
24	4-$(CH_3)_3CC_6H_4$	3.64
25	4-HOC_6H_4	2.64
26	4-ClC_6H_4	3.80
27	4-$CH_3OC_6H_4$	2.64
28	4-$(C_6H_5)C_6H_4$	3.80
29	4-$(CH_3COCH{=}CH)C_6H_4$	3.85
30	4-$NH_2C_6H_4$	2.09
31	$NO_2CH{=}CHC_6H_4$	3.14
32	4-$[C(CH_3)_2CH_2C(CH_3)_3]C_6H_4$	3.27
33	2-$NH_2COC_6H_4$	2.57
34	2-$CH_3C_6H_4$	3.55
35	2-ClC_6H_4	3.27
36	2-$NO_2C_6H_4$	2.49
37	2-$CH_3OC_6H_4$	2.80
38	2-BrC_6H_4	2.31
39	2-HOC_6H_4	2.62
40	2-$(CH_2{=}CHCH_2)C_6H_4$	3.80
41	2-$(CH_3CH{=}CHCH_2)C_6H_4$	3.82
42	3-$CH_3OC_6H_4$	3.00
43	3-ClC_6H_4	3.43
44	3-$CH_3C_6H_4$	3.57
45	3-IC_6H_4	3.35
46	3-$CH_3CONHC_6H_4$	2.28
47	3-$NO_2C_6H_4$	3.09
48	3-$CF_3C_6H_4$	3.46
49	3-$C_2H_5OC_6H_4$	3.09
50	$C_6H_5CH{=}CHCH_2$	2.57
51	2-naphthyl	3.09
52	$C_6H_5CH_2$	3.10

TABLE 5. Substituted Glyoxylic Acids and Their GAO Inhibitory Potencies

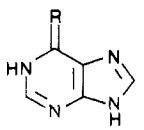
RC(O)COOH

compd	R	pI_{50}
53	4-$(C_6H_5)C_6H_4$	3.21
54	C_6H_5	2.64
55	2-NO_2-5-$(C_6H_5CH_2O)C_6H_3CH_2$	4.19
56	2-NO_2-6-$CH_3C_6H_3CH_2$	4.02
57	3,4-$(CH_3O)_2C_6H_3CH_2$	3.85
58	$C_6H_5CH_2$	4.12
59	2-$NO_2C_6H_4CH_2$	4.37
60	3-HO-4-$(CH_3O)C_6H_3CH_2$	3.64
61	4-$ClC_6H_4CH{=}CH$	3.57
62	3-$CH_3OC_6H_4CH{=}CH$	3.40
63	3,4-$Cl_2C_6H_3CH{=}CH$	4.10
64	2,4-$Cl_2C_6H_3CH{=}CH$	4.10
65	2-$ClC_6H_4CH{=}CH$	3.80
66	3,4-$(CH_3O)_2C_6H_3CH{=}CH$	2.92
67	$CH_3(CH_2)_3$	4.00
68	5-C_6H_5-2-(c-C_4H_3S)	4.52
69	5-$C_6H_5CH_2$-2-(c-C_4H_3S)	4.70
70	5-(4-ClC_6H_4)-2-(c-C_4H_3S)	5.05

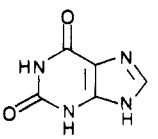
bicity by Lukens and Horsfall[84] also.

2. Xanthine Oxidase

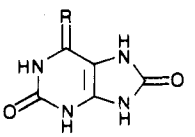
Xanthine oxidase oxidizes hypoxanthine (**1a**) to xanthine (**2**), which is further oxidized to uric acid (**3a**).[85] The overproduction of uric acid leads to an abnormally high concentration of sodium urate in extracellular fluids, and the relative insolubility of sodium urate leads to its deposition in synovial fluids, in and about joints, and in certain subcutaneous sites, ultimately causing gout.[85]



1a, R = O
b, R = S



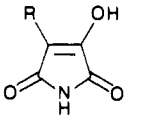
2



3a, R = O
b, R = S

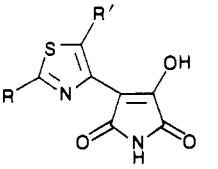
Similarly, 6-mercaptopurine (1b), which is a substrate analogue of hypoxanthine, is converted by this enzyme to thiouric acid (**3b**).[86] 6-Mercaptopurine is an antileukemic agent,[86] and its selective action on susceptible tumor cells has been correlated with the absence or a

TABLE 6. 4-Substituted 3-Hydroxy-1*H*-pyrrole-2,5-dione Derivatives and Their GAO Inhibitory Potencies



compd	R	pI_{50}
71	C_6H_5	4.87
72	4-BrC_6H_4	5.60
73	4-IC_6H_4	5.70
74	4-$NO_2C_6H_4$	5.10
75	4-$NH_2C_6H_4$	4.60
76	$C_6H_5CH_2$	5.03
77	4-(c-C_6H_{11})C_6H_4	5.98
78	4-(1-Ad)C_6H_4	5.74
79	4-$(C_6H_5)C_6H_4$	6.38
80	4-(4-BrC_6H_4)C_6H_4	7.06
81	4-(3,4-$Cl_2C_6H_3CH_2$)C_6H_4	6.66
82	4-(1,2,3,4-H_4-1-$C_{10}H_7$)C_6H_4	5.89
83	4-(2-C_6H_5-1-C_8H_6N)C_6H_4	6.30
84	4-$(C_6H_5)C_6H_4CO$	4.76
85	4-$(C_6H_5)C_6H_4O$	6.57
86	4-$(C_6H_5)C_6H_4S$	5.52
87	6-OCH_3-2-$C_{10}H_7$	5.66
88	*n*-$C_{10}H_{21}$	7.23
89	*n*-$C_{12}H_{25}$	7.09
90	NH_2CO	2.19
91	4-$(CH_3)_2CHC_6H_4$	5.34
92	4-(4-BrC_6H_4)-2-C_3H_2NS	6.63
93	5-(4-ClC_6H_4)-2-C_4H_3S	6.58

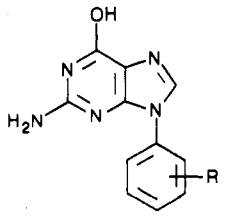
TABLE 7. 4-Heterocyclic-Substituted 3-Hydroxy-1*H*-pyrrole-2,5-dione Derivatives and Their GAO Inhibitory Potencies[a]



compd	R	pI_{50}
94	4-FC_6H_4	6.75
95	4-BrC_6H_4	7.00
96	4-BrC_6H_4	4.58
97	3-BrC_6H_4	6.82
98	4-ClC_6H_4	6.86
99	3-$CF_3C_6H_4$	6.57
100	4-$BrC_6H_4CH_2$	6.18
101	2,6-$Cl_2C_6H_3$	7.07
102	2,3-$Cl_2C_6H_3$	6.77
103	3,4-$Cl_2C_6H_3$	7.11
104	3-Cl-4-$CH_3C_6H_3$	6.92
105	2,6-$(CH_3)_2C_6H_3$	6.92
106	4-CH_3O-2,6-$Cl_2C_6H_2$	6.77
107	4-C_5H_4N	6.48
108	3-C_5H_4N	6.16
109	2,6-$(CH_3)_2$-4-C_5H_2N	6.26
110	2-pyrazinyl	5.33
111	4-thiazolyl	5.19

[a] R′ = CH_3 for **96**; for all others R′ = H.

TABLE 8. Xanthine Oxidase Inhibitors and Their Inhibition Activities



compd	R[a]	pI_{50}[b]	compd	R[a]	pI_{50}[b]
4	2-Cl	5.09	39	H	6.39
5	2-Br	5.11	40	4-O$(CH_2)_3$X-3′-SO_2F	6.40
6	3-X-3′-SO_2F, 4-OCH_3	5.25	41	4-O$(CH_2)_2$X-4′-SO_2F	6.48
7	3-Y-3′-SO_2F, 4-OCH_3	5.31	42	4-Y-3′-SO_2F	6.55
8	3-X-4′-SO_2F, 4-OCH_3	5.35	43	3-Cl	6.57
9	2,3-CH=CHCH=CH	5.38	44	4-$CH(CH_3)_2$	6.60
10	3-Y-4′-SO_2F, 4-OCH_3	5.39	45	4-C_6H_5	6.60
11[c]	4-NH_2	5.43	46	3-CH_3	6.62
12	4-Z-4′-SO_2F	5.60	47	3-NHCHO	6.64
13	4-$N(CH_3)_2$	5.68	48	3-OCH_3	6.66
14	4-$NHCOCH_2Br$	5.72	49	4-OH	6.68
15[c]	3-X-3′-SO_2F	5.74	50	4-O$(CH_2)_2$X-3′-SO_2F	6.74
16	4-Cl	5.74	51	3-CF_3	6.82
17	4-$C(CH_3)_2$	5.74	52	4-O$(CH_2)_2$X′-4′-CH_3, 3′-SO_2F	6.92
18	4-CH_3	5.80	53	3-Y-3′-SO_2F	6.96
19	4-CF_3	5.89	54	4-OC_2H_5	6.96
20[c]	3-Z-3′-SO_2F	5.89	55	3-$NHCOCH_2OC_6H_4$-4′-SO_2F	7.00
21	3,4-Cl_2	5.96	56	4-O$(CH_2)_2$X′-2′-Cl, 5′-SO_2F	7.04
22	4-O$(CH_2)_3$X-4′-SO_2F	6.00	57	3-Y′-4′-CH_3, 3′-SO_2F	7.04
23	4-Z-3′-SO_2F	6.02	58	4-O$(CH_2)_3C_6H_5$	7.08
24[c]	3-Z-4′-SO_2F	6.14	59	3-C_6H_5	7.09
25	3,4-$(OCH_3)_2$	6.14	60	3-$NHCOC_6H_5$	7.14
26	4-Y-4′-SO_2F	6.15	61	3-$NHCOCH_2Br$	7.15
27	4-O$(CH_2)_2$Y-4′-SO_2F	6.16	62	3-Y′-2′-Cl, 5′-SO_2F	7.15
28	4-O$(CH_2)_3$X-4′-SO_2F	6.16	63	4-O$(CH_2)_2$X′-2′-OCH_3, 5′-SO_2F	7.16
29	4-C_2H_5	6.17	64	3-X′-2′-Cl, 5′-SO_2F	7.28
30	4-O$(CH_2)_3$Y-3′-SO_2F	6.20	65	3-Y-4′-SO_2F	7.29
31	2-F	6.21	66	3-X′-3′-Cl, 4′-SO_2F	7.48
32	4-$(CH_2)_3CH_3$	6.21	67	4-$NHCO(CH_2)_2C_6H_4$-4′-SO_2F	7.58
33	3-NH_2	6.22	68	3-X-4′-SO_2F	7.62
34	4-O$(CH_2)_2$Y-3′-SO_2F	6.28	69	3-X′-4′-CH_3, 3′-SO_2F	7.74
35	4-OCH_3	6.30	70	3-X′-2′-OCH_3, 5′-SO_2F	7.80
36	4-O$(CH_2)_2$Y′-4′-CH_3, 3′-SO_2F	6.31	71	3-$NHCOCH_2C_6H_4$-4′-SO_2F	7.82
37	4-$CONH_2$	6.38	72	3-$NHCO(CH_2)_4C_6H_4$-4′-SO_2F	8.00
38	3,4-CH=CHCH=CH	6.39			

[a] X = $NHCONHC_6H_4$, Y = $NHCOC_6H_4$, Z = $NHSO_2C_6H_4$, X′ = $NHCONHC_6H_3$, Y′ = $NHCOC_6H_3$. [b] Reference 90. [c] Not used in regression.

low amount of xanthine oxidase in these tumor cell lines.[87] That is to say that tumor cell lines with high levels of xanthine oxidase would not be expected to be inhibited by 6-mercaptopurine; therefore, Baker proposed that a selective blockade of xanthine oxidase in a tumor cell line unresponsive to 6-mercaptopurine would be a useful adjunct to 6-mercaptopurine therapy.[86] As a result, Baker and co-workers synthesized series of xanthine oxidase inhibitors and studied their activities.[86,88] Silipo and Hansch[89,90] later compiled the series and the activity data to make a QSAR study on them.

Silipo and Hansch[89,90] made a QSAR study on the compounds as listed in Table 8. They are 9-(R-phenyl)guanines. For these compounds, the inhibition activity was found to be correlated with molar refractivity and the Taft steric parameter as shown in eq 29.[90]

$$pI_{50} = 0.267\ (\pm 0.06)(\text{MR-3}) - 0.647\ (\pm 0.12) \times (\text{MR-3}\cdot\text{MR-4}) + 1.291\ (\pm 0.39)(E_s\text{-2}) + 0.101\ (\pm 0.04)(\text{MR-4}) + 0.252\ (\pm 0.11)(E_s\text{-4}) + 4.552\ (\pm 0.45)$$

$$n = 65,\ r = 0.910,\ s = 0.308 \qquad (29)$$

In deriving this equation, compounds 11, 15, 20, and 24 were not included. They all were poorly fit, but no useful comments were made by Silipo and Hansch on their aberrant activity. Most of the congeners in Table 8 contain an SO_2F function. For those that had no SO_2F function, Silipo and Hansch[89] obtained the correlation as shown by eq 30. From eq 29 and 30, it

$$pI_{50} = 0.20(\text{MR-3,4}) + 1.26(E_s\text{-2}) + 0.43(E_s\text{-4}) + 4.33$$

$$n = 30,\ r = 0.924,\ s = 0.228 \qquad (30)$$

appears that a bulky group at the 3-position will enhance the activity. Since the coefficient of MR-3 in eq 29 is comparable to that of MR-3,4 in eq 30 and since the coefficient of MR-4 in eq 29 is very small, it can be said that a bulky group at the 4-position will not have much effect on the activity. However, the positive coefficients of E_s-4 in both equations show that a bulky substituent at the 4-position may produce some steric effects (the bulkier the group, the more negative would be the value of E_s). Similarly, from both the equations, the 2-substituent is also found to produce the steric hindrance. It may also be proposed that the interaction between the 3-substituent of the inhibitors and the

enzyme may be partly hydrophobic and partly a van der Waals type, as MR was shown[90] to have a moderate correlation with π and, as is obvious from Table 1, it is the function of molecular volume.

No indicator variable was found to be necessary for compounds containing the SO_2F function; hence, no specific role of the latter could be discussed. Also, Silipo and Hansch did not use any electronic parameter in their correlation, but since eq 29 and 30 both exhibited significant correlations without an electronic parameter, it may be assumed that no electronic interaction would be involved in the inhibition of this enzyme.

The role of the cross-product term (MR-3·MR-4) with a negative coefficient in eq 29 is difficult to explain.

3. D-Amino Acid Oxidase

D-Amino acid oxidase is a flavoenzyme (flavin adenine dinucleotide, FAD, linked). It oxidizes (deaminates) a D-amino acid to an α-keto acid and ammonia. The ammonia produced in this process may be utilized in protein synthesis. Any surplus amount of it must be detoxified; otherwise, it may lead to severe toxic signs and death. However, from a purely mechanistic point of view, the substrate specificity of the enzyme has been studied. Neims et al.[91] studied the oxidation of meta- and para-substituted *C*-phenylglycines and phenylalanines by hog kidney D-amino acid oxidase. The enzyme extracted from sheep kidney was also employed to study the oxidation of *C*-phenylglycines. Neim et al.[91] then studied the relationship of substrate specificity to $\sigma - P$. Plots of the log of the maximal rate (V_{max}) of oxidation were found to be asymmetrically biphasic in all the cases studied. Hansch and Kerley[92] then made a quantitative correlation study on *C*-phenylglycine derivatives. The oxidation of compounds as listed in Table 9 by hog kidney D-amino acid oxidase was correlated with electronic parameters as shown in eq 31–34. All these equations represent significant

$$\log V_{max} = 2.0\ (\pm 0.49)\sigma^+ + 0.138\ (\pm 0.38)$$
$$n = 11,\ r = 0.952,\ s = 0.499 \qquad (31)$$

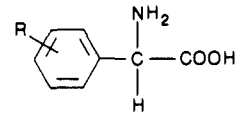
$$\log V_{max} = 3.646\ (\pm 1.69)\sigma^+ - 3.287\ (\pm 0.279)\sigma + 0.539\ (\pm 0.45)$$
$$n = 11,\ r = 0.975,\ s = 0.382 \qquad (32)$$

$$\log V_{max} = 2.988\ (\pm 0.48)\sigma - 6.383\ (\pm 1.99)E_R + 0.541\ (\pm 0.34)$$
$$n = 11,\ r = 0.986,\ s = 0.293 \qquad (33)$$

$$\log V_{max} = 2.592\ (\pm 0.77)\sigma - 2.705\ (\pm 1.43)\sigma^2 + 0.170\ (\pm 0.43)$$
$$n = 11,\ r = 0.966,\ s = 0.446 \qquad (34)$$

correlations, and eq 34 highly supports the emphasis of Neims et al. on parabolic dependence of oxidation on σ. Nonetheless, Hansch and Kerley[92] accepted eq 33 as most likely to describe the situation, as it also suggests the involvement of radicals in the oxidation through its E_R parameter and as there is considerable evidence for the involvement of radicals in the oxidation reactions of amino acid oxidases.[93-95] While a positive coefficient of σ indicates that electron withdrawal by the substituents promotes oxidation, the negative coefficient of E_R has been interpreted to mean that delocalization of an odd electron by substituents inhibits hydrogen abstraction of a benzylic hydrogen atom. In the derivation of eq 33 (and also others) however, compounds 6, 8, 9, 11, 13, and 15 were not included; 6 and 15 were not included as they were misfit in the equations, but the others were not included because of the lack of E_R values. Thus, the conclusion drawn by Hansch and Kerley is based on only 11 data points, which cannot be said to be sufficient enough to draw such specific conclusions, though these authors also found the oxidation of some of these compounds by sheep kidney to be equally significantly related to σ and E_R (eq 35). In eq 35, the number of data points

TABLE 9. Oxidation of *C*-Phenylglycines by Hog and Sheep Kidney D-Amino Acid Oxidases



compd	R	V_{max}[a]	V_{max}[b]	log $(1/K_m)$[c]
1	*p*-$N(CH_3)_2$	0.0003	0.002	2.07
2	*p*-NH_2	0.0016		2.19
3	*p*-OH	0.015	0.035	2.32
4	*p*-OCH_3	0.092	0.039	2.22
5	*p*-CH_3	0.25		2.52
6	*p*-F	6.7[d]		2.65
7	*p*-Cl	4.1	0.82	2.67
8	*p*-COO^-	0.002[d]	<0.001[e]	
9	*p*-$N^+(CH_3)_3$	<0.0001[d]	<0.001[e]	
10	H	7.8	1.5	2.83
11	*m*-NH_2	3.2[d]	0.45[e]	2.22
12	*m*-CH_3	3.5		2.64
13	*m*-OH	5.0[d]		2.18
14	*m*-OCH_3	1.9		
15	*m*-F	7.2[d]		2.85
16	*m*-Cl	5.9		2.85
17	*m*-NO_2	3.2	0.49	2.41

[a] With hog kidney D-amino acid oxidase. [b] With sheep kidney D-amino acid oxidase. [c] With hog kidney enzyme but recalculated in terms of the zwitterion form of the substrates.[96] [d] Not included in the derivation of eq 31–34. [e] Not included in the derivation of eq 35.

$$\log V_{max} = 1.784\ (\pm 1.02)\sigma - 5.172\ (\pm 4.5)E_R - 0.193\ (\pm 0.89)$$
$$n = 6,\ r = 0.962,\ s = 0.374 \qquad (35)$$

$$\log V_{max} = 4.238\ (\pm 1.05)\sigma^+ - 5.170\ (\pm 1.7)\sigma + 0.208\ (\pm 0.35)$$
$$n = 6,\ r = 0.996,\ s = 0.118 \qquad (36)$$

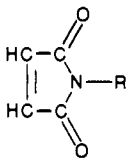
was however still smaller. In this case, in place of E_R, σ^+ had given a much better correlation (eq 36), but with so small a number of data points, not much confidence can be attached to any correlation.

In the study of Hansch and Kerley, π was not found to be useful, but Fujita[96] correlated the Michaelis constant (K_m) of these compounds (Table 9) with π, σ, and E_s as shown in eq 37. Equation 37 thus indicates the importance of not only electronic effects but also hydrophobic and steric effects.

$$\log 1/K_m = 0.300\ (\pm 0.236)\pi_3 + 0.593\ (\pm 0.321)\sigma_1 + 0.212\ (\pm 0.181)(E_s\text{-}3) + 2.339\ (\pm 0.164)$$
$$n = 14,\ r = 0.860,\ s = 0.161 \qquad (37)$$

The hydrophobic effect was also found to be important in the case of the inactivation of D-amino acid oxidase by a series of maleimides (I) studied by Fonda

and Anderson[97] as shown by eq 38, which was obtained



I: R = C_2H_5, n-C_4H_9-n-C_8H_{17}, C_6H_5

$$\log k = 0.339\pi + 4.705\sigma + 1.745$$
$$n = 8,\ r = 0.988,\ s = 0.085 \qquad (38)$$

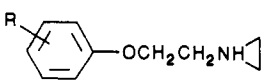
by Lien et al.[98] In eq 38, k is the inactivation constant of the compounds. For the alkyl derivatives, π alone was found to be very highly correlated, as expressed by eq 39. However, on the basis of eq 38 or 39, which uses a small number of data points, the importance of hydrophobic effects cannot be much stressed.

$$\log k = 0.395\pi + 1.051$$
$$n = 6,\ r = 0.999,\ s = 0.018 \qquad (39)$$

4. Monoamine Oxidase

Monoamine oxidase, MAO [amine:oxygen oxidoreductase (deaminating)], is a widely studied class of enzyme. It is an insoluble enzyme located on the outer membrane of the mitochondrion[99] and probably forms an intrinsic part of the structure of this membrane.[100] MAO plays an important role in the inactivation of both exogenously and endogenously formed amines.[101] The intestinal MAO inactivates the pressor amines of foodstuffs; blood vessel MAO protects the organs from the toxic effects of circulating amines; and MAO in tissues helps to regulate the intracellular concentration of certain monoamines, e.g., phenylethylamine (PEA), phenylethanolamine, tryamine, noradrenaline, dopamine, octopamine, 5-hydroxytryptamine (5-HT), tryptamine, N-methylhistamine, etc. The biological inactivation of neurotransmitters such as noradrenaline, dopamine, and 5-HT by MAO, however, then becomes the cause of mental depression. Since the discovery that the antidepressant activity of iproniazid is due to its high in vivo MAO inhibition,[102] series of MAO inhibitors have been studied. Some of the known MAO inhibitors[103,104] include (arylalkyl)hydrazines, aryl hydrazides, arylpropargylamines, arylcyclopropylamines, (aryloxy)cyclopropylamines, N-cyclopropyl(aryloxy)-ethylamines, β-carbolines, and α-methylated arylalkylamines. As discussed below, QSAR studies have been made on many of them in order to understand the mechanisms of MAO inhibition and substrate oxidation as well as the nature of the active site of the enzyme.

a. *N*-(Phenoxyethyl)cyclopropylamines. The QSAR study on N-(phenoxyethyl)cyclopropylamines II was first made by Fuller et al.[105] The rat liver mitochondrial MAO inhibition activities[106] of compounds as listed in Table 10 were found to be correlated with π, σ, and an arbitrary steric parameter γ (eq 40). γ was



II

$$pI_{50} = 0.865\gamma + 0.209\pi + 1.547\sigma + 5.928$$
$$n = 16,\ r = 0.905 \qquad (40)$$

TABLE 10. *N*-(Phenoxyethyl)cyclopropylamines II and Their Rat Liver Mitochondrial MAO Inhibitory Activities

compd	R	pI_{50}	compd	R	pI_{50}
1	4-Br	6.64	10	3-Cl	5.82
2	3,4-Cl_2	6.30	11	4-MeO	5.46
3	3-NO_2	5.76	12	3,4-Me_2	4.71
4	4-Me	5.69	13	3,5-Me_2	4.85
5	3,5-Cl_2	5.68	14	3-Me	4.78
6	3-CF_3	4.98	15	3,5-Me_2-4-Cl	4.70
7	3-Cl-4-Me	5.75	16	3,4,5-Me_3	3.54
8	3-Br	5.64	17	4-N=NC_6H_5	7.56
9	3-Me-4-Cl	6.06	18	4-NH_2	4.40

given a value of zero for a para substituent, −1.3 for a lone meta substituent, and −1.0 for a meta substituent in the presence of other substituents. Fuller et al.[105] did not include the last two compounds in the derivation of eq 40, as their activity data were not then available. However, the prediction of pI_{50} values for these compounds, 7.28 and 4.57, respectively, were very well substantiated by experiment later, with the values obtained as 7.56 and 4.40, respectively.[105] Later, Kutter and Hansch[107] included all 18 data points of Table 10 and obtained eq 41, which exhibited a more significant

$$pI_{50} = 0.923\ (\pm0.27)\gamma + 1.585\ (\pm0.52)\sigma + 0.285\ (\pm0.29)\pi + 5.924\ (\pm0.32)$$
$$n = 18,\ r = 0.940,\ s = 0.342 \qquad (41)$$

correlation than eq 40. Kutter and Hansch[107] also obtained the correlation using E_s in place of γ (eq 42), but not much difference was found in the significance of the correlation, and the coefficient of E_s was comparable to that of γ in eq 41.

$$pI_{50} = 0.702\ (\pm0.20)E_s + 1.640\ (\pm0.50)\sigma + 0.198\ (\pm0.27)\pi + 4.153\ (\pm0.42)$$
$$n = 18,\ r = 0.945,\ s = 0.342 \qquad (42)$$

Some of the compounds of Table 10, (2–6, 10, 11, 13, 17) were also studied for the inhibition of MAO extracted from human liver mitochondria.[106] Their pI_{50} values for MAO inhibition were 7.55, 5.83, 6.67, 6.20, 5.32, 6.35, 7.07, 5.10, and 8.83, respectively, and these were correlated with π, σ, and γ by Fuller et al.[105] (eq 43) and with π, σ, and E_s by Kutter and Hansch[107] (eq 44). From these equations, it is obvious that MAO

$$pI_{50} = 1.318\gamma + 0.813\pi + 0.727\sigma + 6.898$$
$$n = 9,\ r = 0.938 \qquad (43)$$

$$pI_{50} = 1.030\ (\pm0.39)E_s + 1.089\ (\pm1.2)\sigma + 0.398\ (\pm0.76)\pi + 4.541\ (\pm0.88)$$
$$n = 9,\ r = 0.955,\ s = 0.435 \qquad (44)$$

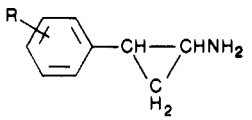
inhibition by this class of inhibitors will largely depend upon the hydrophobic, electronic, and steric properties of the molecules, but it is difficult to say in which way the first two properties will affect the activity, as the π and σ used were the sums of all π and σ values of all substituents in a compound. Thus, it is not clear which portion of the molecule will be interacting with the enzyme. However, Fuller et al.[105] as well as Kutter and Hansch[107] discussed the steric hindrance produced by meta substituents. There were two broad possibilities. The meta substituents might be involved in an intra- or an intermolecular steric repulsion. It was difficult to visualize how substituents in the meta position could interact strongly with the side chain; therefore, ac-

TABLE 11. In Vivo Activity of *trans*-Phenylcyclopropylamines IIIa against Rat MAO

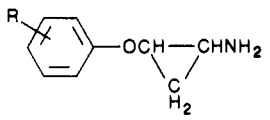
	compd									
	19	20	21	22	23	24	25	26	27	28
R	H	4-Cl	4-CF_3	4-CH_3	4-OCH3	3,4-Cl_2	3-Cl	3-CF_3	3,4-$(OCH_3)_2$	3,4-(CH_2O_2)
-log ED_{50}	5.96	5.77	5.96	5.52	5.92	5.36	5.19	4.80	4.96	5.54

cording to Kutter and Hansch,[107] it seemed most likely that meta substituents in some way hindered the binding of *N*-(phenoxyethyl)cyclopropylamines with the enzyme. However, nothing else can be said on the basis of these simple correlations.

b. Phenyl- and Phenoxycyclopropylamines. A QSAR study on phenylcyclopropylamines IIIa and phenoxycyclopropylamines IIIb was made by Fujita.[96]



IIIa



IIIb

The in vivo activities of 10 *trans*-phenylcyclopropylamines against rat MAO (Table 11)[108] were found to be related to π, σ, and E_s as shown in eq 45 and that of 6

$$-\log ED_{50} = 5.180\ (\pm 0.276) - 0.746\ (\pm 0.614)\pi + 1.858(\pm 1.370)\sigma_2 + 0.502\ (\pm 0.211)(E_s\text{-}3)$$
$$n = 10,\ r = 0.939,\ s = 0.179 \qquad (45)$$

phenoxycyclopropylamines (IIIb: R = H, 4-NMe_2, 4-OMe, 4-F, 4-Cl, 2-Me) having -log ED_{50} values as 4.82, 4.12, 4.25, 4.74, 3.66, and 4.51, respectively,[109] as shown[96] in eq 46 and 47. In eq 45, σ_2 refers to the electronic effect relative to the 2-position, and in eq 46, σ_1 refers to the same relative to the 1-position.

$$-\log ED_{50} = 3.743\ (\pm 1.176) - 0.489\ (\pm 1.306)\pi + 0.411(\pm 2.041)\sigma_1 + 0.986\ (\pm 1.253)(E_s\text{-}4)$$
$$n = 6,\ r = 0.936,\ s = 0.241 \qquad (46)$$

$$-\log ED_{50} = 3.569\ (\pm 0.761) + 0.966\ (\pm 0.866)(E_s\text{-}4)$$
$$n = 6,\ r = 0.840 \qquad (47)$$

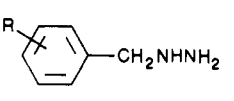
The in vitro anti-MAO (rat liver) activities of these six phenoxycyclopropylamines (pI_{50} = 6.73, 5.11, 6.00, 6.40, 6.10, and 6.73, respectively)[109] were related to σ and E_s as shown[96] in eq 48. In eq 45 and 46, the

$$pI_{50} = 5.349\ (\pm 0.811) + 1.803\ (\pm 1.461)\sigma_1 + 1.246\ (\pm 0.941)(E_s\text{-}4)$$
$$n = 6,\ r = 0.950,\ s = 0.244 \qquad (48)$$

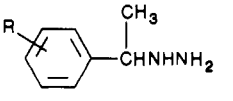
coefficient of π is negative, but eq 46 is not very significant, as there are too many variables for a small number of data points (six only). Therefore, for the in vivo activity of phenoxycyclopropylamines, eq 47 may be treated as decisive. The negative coefficient of π in eq 45 would thus mean that in the case of phenylcyclopropylamines the increase in hydrophobicity would lead to a decrease in in vivo activity by decreasing the effective concentration of compounds around the site of action. The more hydrophobic, the more inhibitor molecules would be trapped by lipid phases and possibly be metabolized to inactive compounds while on the way from the site of administration to the target enzyme.[96]

In no other case is π found to influence the activity, but the steric parameter E_s at varying positions is found to affect the activity in all cases, as was found with the *N*-(phenoxyethyl)cyclopropylamines. The electronic parameter relative to any position also appears to be equally important in most of the cases.

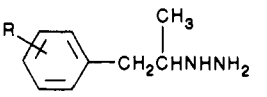
c. (Arylalkyl)hydrazines. Fujita[96] also made QSAR studies on some (arylalkyl)hydrazines such as benzylhydrazines IV, α-phenethylhydrazines V, and (phenylisopropyl)hydrazines VI. The in vitro activities



IV



V



VI

of eight benzylhydrazines (IV: R = H, 2-OCH_3, 4-Cl, 2-Cl, 3,4-Cl_2, 3,4-$(OCH_3)_2$, 2,3-$(CH)_4$, 4-*i*-C_3H_7; pI_{50} = 6.5, 6.7, 6.6, 6.7, 6.2, 5.9, 5.3, 5.6)[110] against guinea pig mitochondrial MAO, and the in vivo activities of seven phenethylhydrazines (V: R = H, 4-F, 4-Cl, 4-OC_2H_5, 4-C_6H_5, 4-OC_6H_5, 4-OCH_3; -log ED_{50} = 4.43, 4.31, 4.05, 3.95, 4.62, 4.95, 4.04)[111] and of seven (phenylisopropyl)hydrazines (VI: R = H, 4-OCH_3, 3,4-$(OCH_3)_2$, 3,4,5-$(OCH_3)_3$, 3,4-(CH_2O_2), 3-Cl, 2-CH_3; log A = 1.60, 0.98, 0.75, 0.51, 1.42, 1.39, 1.34)[112] against mouse MAO were correlated with different parameters[96] (eq 49–51).

$$pI_{50} = 5.832\ (\pm 0.209) - 0.545\ (\pm 0.125)\pi + 1.638\ (\pm 0.271)\sigma_2 + 0.516\ (\pm 0.161)(E_s\text{-}3)$$
$$n = 8,\ r = 0.996,\ s = 0.062 \qquad (49)$$

$$-\log ED_{50} = 3.343\ (\pm 0.898) + 0.606\ (\pm 0.464)\pi + 0.933(\pm 0.980)(E_s\text{-}4)$$
$$n = 7,\ r = 0.876,\ s = 0.214 \qquad (50)$$

$$\log A = 1.671\ (\pm 1.168)(E_s\text{-}4) - 0.675\ (\pm 0.761)\pi - 0.268\ (\pm 1.008)$$
$$n = 7,\ r = 0.894,\ s = 0.220 \qquad (51)$$

In eq 51, A is the activity relative to iproniazid. Again, in this case E_s appears to be more important than any other parameter. The variation in the coefficient of π from case to case provides no proper direction to discuss the dependence of inhibition on hydrophobicity.

d. Hydrazides. QSAR studies were made on hydrazides by Johnson,[113] Fulcrand et al.,[114] and Richard and Kier.[115] Johnson[113] correlated the in vivo potency of a series of aryl hydrazides (Table 12) determined against brain MAO by Zeller et al.[116] with π-electron density at the carbonyl oxygen (Q_0) or at the ring carbon (Q_1) to which the hydrazide group was attached. The best correlations that he obtained were eq 52 and 53,

$$MI = 13491 - 9511\ (\pm 1125)Q_0$$
$$n = 20,\ r = 0.894,\ s = 30,\ F = 71.5 \qquad (52)$$

$$MI = 863 - 748\ (\pm 171)Q_1$$
$$n = 20,\ r = 0.717,\ s = 43,\ F = 19.1 \qquad (53)$$

where MI, the Marsilid index, is defined as the ratio of

TABLE 12. *N*-Isopropylhydrazides and Their MAO Inhibitory Potencies

$ArCONHNHCH(CH_3)_2$

compd	Ar	MI
29	2-thienyl	198
30	3-thienyl	192
31	2-pyridyl	191
32	2-furyl	156
33	2-pyrazinyl	152
34	4-chlorophenyl	134
35	3-nitrophenyl	125
36	3-chlorophenyl	123
37	3-pyridyl	111
38	4-pyridyl	100
39	4-isopropylphenyl	97
40	3-pyrazolyl	84
41	3,4-dimethylphenyl	76
42	4-methoxyphenyl	63
43	2-aminophenyl	60
44	4-methylthiazol-5-yl	38
45	1,2,3-triazol-4-yl	38
46	4-aminophenyl	15
47	4-hydroxyphenyl	11
48	3-pyridazyl	0
49	2-hydroxyphenyl	0
50	2,4-dimethylpyrimid-5-yl	0
51	3,5-dimethylisoxazol-4-yl	0

the increase in serotonin in rat brain produced by a substance (in amount equimolar to 100 mg/kg) to the increase in serotonin produced by 100 mg of iproniazid (marsilid, 38)/kg. The MI of any compound is with reference to the MI of marsilid taken as equal to 100. In the derivation of eq 52, compounds 38, 48, and 51 were not included, however, as they were outliers. For the same reasons, compounds 29, 32, and 48 were not included in the derivation of eq 53.

For a subset of these compounds for which σ values were available, the correlation between MI and σ was as shown[113] in eq 54. Use of log P was found to be of

$$MI = 83.37 + 88.25\ (\pm 23.09)\sigma$$
$$n = 10,\ r = 0.804,\ s = 31.4,\ F = 14.6 \qquad (54)$$

no consequence in this case. These results of Johnson led him to suggest that substituents or heterocyclic rings that lead to decreased electron density in the region of the hydrazide group will increase the activity of the derivative as an MAO inhibitor.

Fulcrand et al.[114] correlated pI_{50} values of a series of (aryloxy)acetohydrazides (Table 13) with polarographic half-wave potential ($E_{1/2}$), ΔpK_a (a measure of the relative basicity of nitrogen), and E_s (eq 55). For the

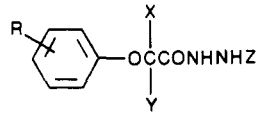
$$pI_{50} = 5.46 - 26.5E_{1/2} - 0.634(\Delta pK_a) + 0.307\ (E_s\text{-}6)$$
$$n = 24,\ r = 0.962,\ s = 0.163 \qquad (55)$$

same series of (aryloxy)acetohydrazides, Richard and Kier[115] introduced in the correlation their topological parameter χ, the molecular connectivity index, and obtained eq 56. Both eq 55 and 56, however, lead them

$$pI_{50} = -5.2 - 29E_{1/2} - 0.82^2\chi + 1.8^3\chi^v$$
$$n = 24,\ r = 0.941,\ s = 0.201,\ F = 52 \qquad (56)$$

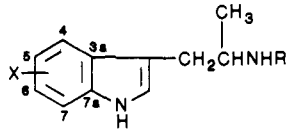
to simply suggest that electronic and steric parameters are important in the MAO inhibition by this class of inhibitors. χ does not add much to the knowledge about the mechanism of interaction; however, the relation of activity to the half-wave potential suggests that there might be some charge-transfer phenomenon involved in the inhibition mechanism.

TABLE 13. (Aryloxy)acetohydrazides and Their MAO Inhibitory Potencies



compd	R	X	Y	Z	pI_{50}
52	H	H	H	$CH(CH_3)_2$	5.42
53	2-Cl	H	H	$CH(CH_3)_2$	5.60
54	3-Cl	H	H	$CH(CH_3)_2$	5.40
55	4-Cl	H	H	$CH(CH_3)_2$	5.96
56	$2\text{-}CH_3$	H	H	$CH(CH_3)_2$	5.54
57	$3\text{-}CH_3$	H	H	$CH(CH_3)_2$	5.05
58	$4\text{-}CH_3$	H	H	$CH(CH_3)_2$	5.40
59	$2\text{-}OCH_3$	H	H	$CH(CH_3)_2$	5.62
60	$3\text{-}OCH_3$	H	H	$CH(CH_3)_2$	5.42
61	$4\text{-}OCH_3$	H	H	$CH(CH_3)_2$	5.52
62	H	H	CH_3	$CH(CH_3)_2$	5.00
63	2-Cl	H	CH_3	$CH(CH_3)_2$	5.16
64	3-Cl	H	CH_3	$CH(CH_3)_2$	4.96
65	4-Cl	H	CH_3	$CH(CH_3)_2$	5.00
66	H	CH_3	CH_3	$CH(CH_3)_2$	4.34
67	4-Cl	CH_3	CH_3	$CH(CH_3)_2$	4.80
68	$3\text{-}CH_3$	CH_3	CH_3	$CH(CH_3)_2$	4.90
69	H	H	H	C_2H_5	5.82
70	4-Cl	H	H	C_2H_5	6.00
71	H	H	H	$CH_2C_6H_5$	6.14
72	H	H	H	$CH(CH_3)C_6H_5$	5.70
73	$4\text{-}CH_3$	H	H	$CH(CH_3)C_6H_5$	6.05
74	$4\text{-}OCH_3$	H	H	$CH(CH_3)C_6H_5$	6.00
75	4-Cl	H	H	$CH_2C_6H_5$	6.96

TABLE 14. α-Methyltriptamines and Their in Vitro MAO Inhibitory Activities

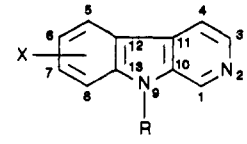


compd	X, R	pI_{50}	compd	X, R	pI_{50}
76	H	4.52	84	$N\text{-}CH_3$	4.63
77	$6\text{-}OCH_3$	4.12	85	$N\text{-}CH_3$, $6\text{-}OCH_3$	4.30
78	$6\text{-}CH_3$	4.00	86	$N\text{-}CH_3$, 6-Cl	4.00
79	$4\text{-}CH_3$	4.00	87	$N,6\text{-}(CH_3)_2$	3.67
80	$5\text{-}OCH_3$	3.42	88	$N,4\text{-}(CH_3)_2$	4.00
81	5-Cl	3.67	89	$N\text{-}CH_3$, $5\text{-}OCH_3$	3.37
82	$7\text{-}CH_3$	4.60	90	$N\text{-}CH_3$, 5-Cl	3.67
83	$5,7\text{-}Cl_2$	4.30			

e. α-Methyltryptamines. Fujita[96] in his studies on MAO inhibitors found the in vitro activity of a series of α-methyltryptamines (Table 14)[117] against guinea pig MAO to be correlated with various physicochemical parameters as given in eq 57, which shows that the effect of π, σ, and E_s on activity is the same as in the case of benzylhydrazines (eq 49).

$$pI_{50} = 3.152\ (\pm 0.4) - 1.085\ (\pm 0.620)\pi_{4,6} + 1.251\ (\pm 0.714)\sigma_{7a} + 1.071\ (\pm 0.439)(E_s\text{-}5)$$
$$n = 15,\ r = 0.862,\ s = 0.231 \qquad (57)$$

f. β-Carbolines. QSAR studies on β-carbolines VII were made by Fujita,[96] Tomas and Aullō,[118] and Lien et al.[98] The correlation obtained by Fujita for the in

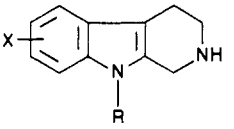


VII

TABLE 15. β-Carbolines VII and Their in Vitro MAO Inhibitory Activities

	compd											
	91	92	93	94	95	96	97	98	99	100	101	102
X	H	6-OMe	6-Me	6-Cl	6-NH_2	8-OMe	8-Me	8-NH_2	H	6-OMe	6-Me	8-Me
R	H	H	H	H	H	H	H	H	Me	Me	Me	Me
pI_{50}	4.54	4.37	3.92	4.62	3.20	3.92	4.14	3.24	5.00	4.55	4.15	4.70

TABLE 16. Tetrahydro-β-carbolines and Their in vitro MAO Inhibitory Activities



compd	X, R	pI_{50}	compd	X, R	pI_{50}
103	H	3.47	111	*N*-CH_3	5.00
104	6-OCH_3	2.88	112	*N*-CH_3, 6-OCH_3	4.00
105	6-CH_3	3.17	113	*N*,6-$(CH_3)_2$	4.00
106	6-F	3.28	114	*N*-CH_3, 6-F	3.92
107	6-Cl	3.38	115	*N*-CH_3, 6-Cl	3.74
108	6-Br	3.34	116	*N*,8-$(CH_3)_2$	4.80
109	8-CH_3	3.42	117	*N*-CH_3, 8-Cl	5.42
110	8-Cl	4.00			

vitro activity of compounds as listed in Table 15 against beef liver mitrochondrial MAO[119] is given in eq 58, where the dummy parameter $D = 1$ for R = NCH_3 and zero for R = H.

$$pI_{50} = 2.777\ (\pm0.503) + 0.590\ (\pm0.191)\pi_{6,8} + 0.720\ (\pm0.814)\sigma_{12} + 0.731\ (\pm0.290)(E_s\text{-}6,8) + 0.361\ (\pm0.230)D$$
$$n = 12,\ r = 0.979,\ s = 0.144 \qquad (58)$$

Tomás and Aullõ[118] tried to correlate the activity of these β-carbolines (excluding 94 and including two new congeners, 1-methyl-β-carboline and 1-amino-β-carboline[119]) with quantum mechanical parameters. They obtained eq 59, where Q_i and S_i^e represent the net

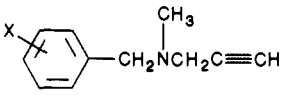
$$pI_{50} = 31.806 + 11.782Q_9 + 140.546S_9^e + 14.405Q_6 + 18.199S_6^e + 42.378Q_5 - 28.496S_5^e + 22.284Q_{13} - 101.543S_{13}^e + 63.959S_8^e$$
$$n = 13,\ r = 0.999,\ s = 0.011 \qquad (59)$$

charge density and the electrophilic superdelocalizability at atom i, respectively. Equation 59 is however totally meaningless, as there are 9 variables for only 13 data points. For a series of tetrahydro-β-carbolines (Table 16), Fujita[96] in fact had failed to show any effect of the electronic factor on the activity. The best equation that he could obtain for this series was eq 60.

$$pI_{50} = 2.586\ (\pm0.511) + 0.525\ (\pm0.515)\pi_{6,8} + 0.730\ (\pm0.417)(E_s\text{-}6) + 1.030\ (\pm0.392)D$$
$$n = 15,\ r = 0.909,\ s = 0.341 \qquad (60)$$

The activity of tetrahydro-β-carbolines was also determined against beef liver mitochondrial MAO.[120] Equations 58 and 60 both signify the importance of the hydrophobic character of the 6- and 8-substituents and the steric hindrance produced by the 6-substituent to the activity. Further, as observed from the coefficient of D, the methyl group at N_9 also appears to increase the activity. This is probably again due to the hydrophobic character of methyl group. The effect of the hydrophobic character of different kinds of substituents at N_9 was in fact shown by Lien et al.[98] by correlating

TABLE 17. Pargylines and Their Anti-MAO Activities



compd	X	pI_{50}	$-\log D_{min}$
118	H	6.05	4.20
119	2-Cl	7.30	4.58
120	2-Br	7.19	4.19
121	2,4-Cl_2	7.12	4.18
122	2-CH_3	6.77	3.84
123	4-*i*-C_3H_7	5.52	3.30
124	4-C_6H_5	6.70	3.37
125	3,4,5-$(OCH_3)_3$	6.77	3.39
126	4-$N(CH_3)_2$	5.46	3.60
127	2,3-$(CH)_4$	6.30	4.32
128	2-OCH_3	7.05	

the pI_{50} values of a small series of N-substituted β-carbolines (VII: X = H; R = H, CH_3, C_2H_5, *n*-C_3H_7, *n*-C_4H_9, *i*-C_5H_{11}, CH_2OCH_3, $COCH_3$; pI_{50} = 4.54, 5.00, 4.32, 3.82, 3.85, 2.74, 3.40, 3.82)[121] with log P as shown by eq 61. Though in in vitro inhibition the parabolic

$$pI_{50} = 3.719 \log P - 0.679 (\log P)^2 - 0.422$$
$$n = 8,\ r = 0.900,\ s = 0.360 \qquad (61)$$

dependence of activity on log P shows a limit of bulk tolerance, E_s was not found to have any correlation with the activity.[98] The large influence of hydrophobicity on the relative potency of β-carbolines against MAO was discussed by Martin and Biel also.[122]

g. Pargylines. Fujita[96] derived the correlation equations (eq 62 and 63) for pargylines (Table 17), where D_{min} represents the minimum dose to produce maximal response upon administration of DOPA in the mouse.[123] The pI_{50} values were against rat liver mitochondrial MAO.[123]

$$pI_{50} = 5.547\ (\pm0.588) + 0.389\ (\pm0.391)\pi + 1.192\ (\pm0.734)\sigma_2 + 0.764\ (\pm0.435)(E_s\text{-}4)$$
$$n = 11,\ r = 0.937,\ s = 0.271 \qquad (62)$$

$$-\log D_{min} = 3.432\ (\pm0.242) + 0.840\ (\pm0.464)\sigma_2 + 0.517\ (\pm0.255)(E_s\text{-}4)$$
$$n = 10,\ r = 0.928,\ s = 0.193 \qquad (63)$$

Martin et al.[124] analyzed a bigger series of pargyline analogues (Table 18). They related their pI_{50} values against rat liver mitochondrial MAO with pK_a and π as shown in eq 64, where D_2 is a dummy parameter to

$$pI_{50} = 4.38\ (\pm1.38)pK_a - 0.35\ (\pm0.10)pK_a^2 + 0.25\ (\pm0.19)\pi + 1.02\ (\pm0.45)D_2$$
$$n = 47,\ r = 0.87,\ s = 0.58 \qquad (64)$$

indicate the presence of a substituent at the 2-position of the aromatic ring with a value of unity. In the derivation of eq 64, compound 158 was not included, but its activity was correctly predicted by the equation. From their original list of compounds, Martin et al. excluded three more compounds that had bigger groups

TABLE 18. Pargyline Analogues and Their in Vitro MAO Inhibitory Activities

$RN(CH_3)CH_2C{\equiv}CH$ 129–161 $RNCH_3)CH_2C{\equiv}CCH_3$ 162

$RNHCH_2C{\equiv}CH$ 163–175 $RN(CH_3)CH_2C{\equiv}N$ 176

compd	R	pI_{50}
129	$CH_2C_6H_4$-2-OC_2H_5	7.5
130	$CH_2C_6H_4$-2-Cl	7.3
131	CH_2-2-$C_{10}H_7$	7.3
132	$CH_2C_6H_3$-2,4-Cl_2	7.1
133	$CH_2C_6H_3$-2,6-Cl_2	7.1
134	CH_2-1-$C_{10}H_7$	7.0
135	$CH_2C_6H_4$-2-OCH_3	6.8
136	$CH_2C_6H_4$-3-OCH_3	6.8
137	$CH_2C_6H_4$-2-CH_3	6.8
138	$CH_2C_6H_3$-3,4-OCH_2O	6.8
139	$CH_2C_6H_4$-4-C_6H_5	6.7
140	$CH_2C_6H_4$-2-F	6.7
141	$CH_2C_6H_3$-3,4-Cl_2	6.7
142	$CH_2C_6H_4$-4-Cl	6.6
143	$CH_2C_6H_4$-3-Cl	6.4
144	$CH_2C_6H_3$-2,4-$(OCH_3)_2$	6.4
145	$CH_2C_6H_4$-3-CH_3	6.4
146	$CH_2C_6H_4$-4-OCH_3	6.3
147	$CH_2C_6H_4$-4-$N(CH_3)_2$	6.3
148	$CH_2C_6H_4$-4-CH_3	6.3
149	$CH(CH_3)C_6H_5$	6.2
150	$CH_2C_6H_4$-3-OH	6.2
151	$CH_2C_6H_4$-4-OH	6.1
152	$CH_2C_6H_4$-4-O-C_6H_5	6.0
153	$CH_2C_6H_5$	6.0
154	$CH_2C_6H_3$-3,4-$(OCH_3)_2$	5.8
155	CH_2-2-C_5H_4N	5.5
156	$CH_2C_6H_4$-4-$CH(CH_3)_2$	5.5
157	CH_2-c-C_6H_{11}	5.4
158	$CH_2C_6H_2$-3,4,5-$(OCH_3)_3$	5.3[a]
159	$C(CH_3)_2C_6H_5$	5.2
160	CH_2-3-C_5H_4N	4.8
161	CH_2-4-C_5H_4N	4.7
162	$CH_2C_6H_5$	5.0
163	$CH_2C_6H_4$-4-Cl	6.0
164	1-$CH(CH_3)CH_2C_6H_5$	5.6
165	$(CH_2)_5C_6H_5$	5.3
166	$CH_2C_6H_5$	5.1
167	*d*-$CH(CH_3)CH_2C_6H_5$	5.0
168	$(CH_2)_3C_6H_5$	5.0
169	$CH(CH_3)C_6H_5$	4.6
170	CH_2-c-C_6H_{11}	4.3
171	$(CH_2)_4C_6H_5$	4.3
172	CH_3	4.0
173	$(CH_2)_5CH_3$	3.7
174	$C(CH_3)_2C_5H_{11}$	3.3
175	c-C_6H_{11}	3.0
176	$CH_2C_6H_5$	3.6

[a] Not included in the regression.

in place of CH_3 or H at the nitrogen. These compounds had much higher activity than predicted. Martin et al. correlated the concentrations of ionized and un-ionized drugs separately, but the equations obtained were not much different from eq 64. Equation 64 shows that π has little effect on the activity but that the electronic factor plays an important role. One can also conclude the same thing from eq 62 and 63, where the steric effect also appears to be important. In eq 64, D_2 speaks of almost the same effect.

From all these studies on different types of MAO inhibitors one finds that there is no consistency in the role played by the hydrophobic character of the molecules. In many equations, such as 45, 46, 49, 51, 57, etc., the coefficient of π has been negative, while in other equations it has been positive, and there have been cases where π has been found to produce no effect, for example hydrazides. It means that an increase in activity due to hydrophobicity of the molecules is possible only when the hydrophobic substituents of the molecules are properly oriented with respect to the hydrophobic site of the enzyme; otherwise, there is no effect of hydrophobicity. The decrease in activity due to hydrophobicity simply suggests that since hydrophobicity is related to the bulkiness of the group, substituents would be producing steric hindrance instead of leading to any hydrophobic interaction.

However, a majority of the cases exhibited the steric influence. The coefficient of E_s in each case was positive; hence, it can be said that in each case there was steric repulsion to the interaction of the side-chain amino group with the enzyme. This repulsion was effectively produced by the substituent either at the 3-position of the aromatic ring, as in *N*-(phenoxyethyl)-cyclopropylamines (eq 40–44, in vitro), phenylcyclopropylamines (eq 45, in vivo), and benzylhydrazines (eq 49, in vitro), or at the 4-position of the aromatic ring, as in phenoxycyclopropylamines (eq 46 and 47, in vivo; eq 48, in vitro), phenylhydrazines (eq 50, in vivo), and phenylisopropylhydrazines (eq 51, in vivo). But steric influence was produced from other positions also (see other cases).

The electronic effect is found to be a dominant factor in almost all cases. It is worth noting that in all cases where σ was introduced its coefficient was positive, indicating that electron-withdrawing groups increase the activity. Johnson's observation, based on eq 52 and 53 for aryl hydrazides, that substituents or heterocyclic rings that lead to decreased electron density in the region of the hydrazide group will increase the activity of the derivative is in total conformity with this.

In most of the cases, the side-chain amino group appears to be involved in the interaction with the enzyme. However, it is difficult to say what is the nature of the interaction. There are three possibilities: (1) a simple electrostatic interaction between the cationic form of the amino group and an anionic group in the enzyme; (2) specific nucleophilic attack of the free base form of the amino group on an electrophilic site on the enzyme; and (3) a charge-transfer type of interaction. However, in addition to this primary interaction, MAO inhibitors probably also interact through the aromatic ring.

The study of Martin et al.[124] on propynylamine derivatives (eq 64) suggested a parabolic dependence of MAO inhibition on the pK_a of the side-chain nitrogen. The derivatives had a range of pK_as from 3.5 to 9.0. Equation 64 gives the optimum value as pK_a 6.2. Thus, if the pK_a decreases from 9.0 to 6.2, the inhibitory activity will increase. This should be a consequence of electron withdrawal. Thus, one part of the parabola is consistent with eq 62 (or 63) obtained by Fujita for another series of pargyline derivatives. Below pK_a 6.2, however, electron withdrawal decreased the potency. This is, according to Martin et al.,[124] probably due to a change in the rate-limiting step of the inhibition mechanism.

From all these studies it is, however, observed that there is remarkable similarity in the electronic and steric effects in different types of MAO inhibitors. Regarding electronic effects, it can be generalized that the presence of electron-withdrawing groups on the

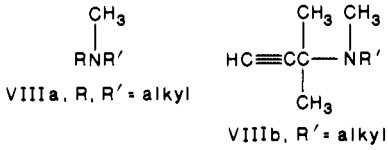
phenyl ring or replacement of the phenyl ring with certain types of heterocyclic rings will tend to increase the potency of the inhibitor in a predictable manner. However, the bulky substituents will tend to produce steric repulsion. Overall, the minimum requirement for a MAO inhibitor is that it should have an electron-rich functional group such as an amino nitrogen or acetylenic carbon which, according to Johnson,[113] should be in the plane of the aromatic ring and approximately at a distance of 5.25 Å from the center of the ring.

With reference to antidepressants, Fukunaga and Burger[125] made a passing reference to some of these QSAR studies.

5. Mixed-Function Oxidases

The hepatic microsomal enzymes that catalyze oxidation reactions that utilize molecular oxygen for the direct oxidation of the substrate have been termed mixed-function oxidases or oxygenases (MFO). The MFO system is active in the oxidative metabolism of xenobiotics to more polar substances.[126] The system is also implicated in the activation of polycyclic aromatic hydrocarbons to proximate carcinogens.[127] The system has been separated into three important components necessary for oxidation: (1) NADPH-dependent reductase; (2) cytochrome P-450 (a heme protein); (3) a heat-stable lipid fraction.[128] Cytochrome P-450 utilizes one molecule of oxygen and two reducing equivalents, usually from NADPH, to produce one molecule of water and the oxidized substrate. Besides being centrally implicated in the activation of carcinogens, this heme protein also produces certain drug-related toxicities. Attention therefore has been focused on inhibitors of the MFO system.

QSAR studies were first made by Hansch et al.[129] on the N-demethylation data of some amines (VIIIa,b).



The biological response, BR (the relative rate of in vitro demethylation by rat microsomes), was shown to be related to log P and pK_a as shown in eq 65, where ΔpK_a

$$\log BR = 0.470 \log P - 0.268\Delta pK_a - 1.305$$
$$n = 18,\ r = 0.890,\ s = 0.222 \qquad (65)$$

$= pK_a - 9.5$, 9.5 being the pK_a value for the reference compound $C_6H_5(CH_2)_3N(CH_3)_2$. Thus, eq 65 shows the importance of the lipophilic character of the molecules and states that the lower the electron density on nitrogen (as measured by $R_3N^+H \rightleftharpoons R_3N + H^+$), the greater the demethylation rate. However, the role of electron density on nitrogen in the demethylation mechanism is suggested to be complex. The introduction of the $(\Delta pK_a)^2$ term led to a better correlation (eq 66) than eq 65, suggesting a possible dual role of the lone-pair electrons.

$$\log BR = 0.484 \log P - 0.068(\Delta pK_a)^2 - 0.267\Delta pK_a - 1.225$$
$$n = 18,\ r = 0.924,\ s = 0.193 \qquad (66)$$

In this study, Hansch et al. used the calculated values of log P, so it did not take into account the degree of dissociation. In a later study, however, Hansch[130] used some experimental log P values and, by including a few more amines that contained aromatic rings, obtained eq 67. Thus, eq 66 and 67 both establish the impor-

$$\log BR = 0.33\ (\pm 0.10) \log P - 0.08\ (\pm 0.04) \times (\Delta pK_a)^2 - 0.15\ (\pm 0.04)\Delta pK_a - 0.44\ (\pm 0.32)$$
$$n = 22,\ r = 0.900,\ s = 0.163 \qquad (67)$$

tance of the lipophilic character of the molecules in the demethylation reaction. Prior to these studies, Gaudette and Brodie[131] showed that, for a wide variety of organic compounds, microsomes demethylated only those molecules that were quite lipophilic.

The importance of the hydrophobic character of molecules in the relative rate of oxidation of drugs by rat liver microsomes was also shown when Martin and Hansch[132] correlated the Michaelis constants for a wider variety of drugs (Table 19) with log P, which was measured for some compounds. Equation 68 was ob-

$$pK_m = 2.460\ (\pm 0.58) + 0.821\ (\pm 0.29) \log P$$
$$n = 14,\ r = 0.874,\ s = 0.508 \qquad (68)$$

tained without considering the ionization of any compound at pH 7.4, at which the rate of oxidation was studied. However, there were some compounds that were partially ionized at this pH. Hence, when the proper correction was made in the K_m values of these compounds (taking only the concentration of the unionized part at half-maximal velocity), eq 69 was ob-

$$pK_m(\text{corr}) = 2.90\ (\pm 0.38) + 0.698\ (\pm 0.19) \log P$$
$$n = 14,\ r = 0.920,\ s = 0.330 \qquad (69)$$

tained, showing even a better correlation than eq 68. The correlations expressed by eq 68 and 69 become more significant when one notices that a variety of reactions such as N-demethylation of substituted (dimethylammonio)anilines, O- and N-demethylation of complex heterocyclic compounds, and hydroxylation of aliphatic chains have been catalyzed. The high correlation of pK_m with log P reinforced the importance of hydrophobicity. The electronic effect was not found to play any role in this study.

A recent study on microsomal oxidation of some tertiary amines, however, did not show the positive dependence of pK_m on hydrophobicity.[133] For the N-demethylation of 12 para-substituted N,N-dimethylanilines catalyzed by phenobarbital-treated rat liver microsomes, Galliani et al.[133] found a negative dependence of pK_m on π (eq 70), and for the same re-

TABLE 19. Michaelis Constants of Various Substrates of Rat Microsomal NADPH Oxidase

no.	compd	pK_m
1	N,N-dimethyl-β-naphthylamine	5.63
2	m-chloro-N,N-dimethylaniline	4.94
3	m-methyl-N,N-dimethylaniline	4.73
4	p-methyl-N,N-dimethylaniline	4.70
5	p-amino-N,N-dimethylaniline	3.87
6	m-amino-N,N-dimethylaniline	3.85
7	N,N-dimethylaniline	4.19
8	pentobarbital	4.48
9	hexobarbital	4.22
10	codeine	3.36
11	ephedrine	1.97
12	barbital	2.77
13	physostigmine	2.94
14	caffeine	2.86

$$pK_m = -1.63 - 0.71\pi$$
$$n = 12, r = 0.828, s = 0.308 \quad (70)$$

action of those compounds catalyzed by untreated and β-naphthoflavone-treated rat liver microsomes, no correlation was found. Galliani et al. therefore suggested the possible intervention of other factors such as the basicity or the steric bulk of the substrates.

Ichikawa et al.[134] also pointed out that not all microsomal enzymatic reactions were heavily dependent upon log P. Their data on 4-hydroxylation of anilines were analyzed by Hansch,[130] and very poor correlations were obtained between the hydroxylation rate and the hydrophobicity (eq 71, 72). Equation 71 was obtained for 2-substituted anilines and eq 72 for 3-substituted ones.

$$\log BR = 1.73 (\pm 0.80) + 0.11 (\pm 0.49) \log P$$
$$n = 7, r = 0.253, s = 0.209 \quad (71)$$
$$\log BR = 1.00 (\pm 0.68) + 0.39 (\pm 0.34) \log P$$
$$n = 11, r = 0.653, s = 0.313 \quad (72)$$

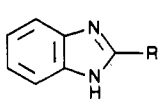
In the case of 2-substituted anilines, the electronic factor was found to be more important (eq 73), and in the case of 3-substituted anilines, the steric factor was found to play a major role (eq 74). Correlations expressed by eq 73 and 74 were, however, found to be improved by inclusion of log P (eq 75, 76). Thus, the overall hydroxylation reaction showed a small but significant dependence on the hydrophobic character of the substituents. However, since the set of substituents studied did not contain the ideal kind of variation necessary to delineate more sharply the effects of hydrophobic, electronic, and steric factors, the above conclusions on the role of these factors were only tentative.

$$\log BR = 1.76 (\pm 0.10) - 1.16 (\pm 0.50)\mathcal{R}$$
$$n = 7, r = 0.936, s = 0.076 \quad (73)$$
$$\log BR = 1.52 (\pm 0.16) + 0.41 (\pm 0.17)E_s$$
$$n = 11, r = 0.877, s = 0.199 \quad (74)$$
$$\log BR = 1.55 (\pm 0.19) - 1.17 (\pm 0.31)\mathcal{R} + 0.13 (\pm 0.11) \log P$$
$$n = 7, r = 0.984, s = 0.044 \quad (75)$$
$$\log BR = 1.03 (\pm 0.18) + 0.36 (\pm 0.07)E_s + 0.27 (\pm 0.09) \log P$$
$$n = 11, r = 0.982, s = 0.082 \quad (76)$$

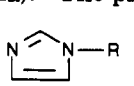
The pork liver microsomal NADPH-dependent N-oxidation of some tertiary amines was shown to have parabolic dependence on log P (eq 77).[135]

$$\log (\text{oxidn rate}) = 0.37 \log P - 0.03(\log P)^2 - 7.06$$
$$n = 10, r = 0.962, s = 0.106 \quad (77)$$

Recently, Murray et al.[136] studied the inhibition of a particular enzyme of the MFO system, namely aminopyrine N-demethylase (APDM), by a large series of benzimidazole derivatives (IXa). The pI_{50} values of 76

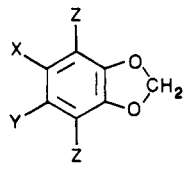


IXa



IXb, R = H, CH_3-$C_{12}H_{25}$

TABLE 20. Synergistic Activity of 1,3-Benzodioxoles with Carbaryl in Housefiles



compd	X	Y	Z	log SR5
15	H	H	H	1.49
16	H	CH_3	H	1.93
17	H	Cl	H	1.88
18	H	Br	H	2.19
19	H	NO_2	H	2.48
20	H	OCH_3	H	2.44
21	Cl	Cl	H	2.28
22	Br	Br	H	2.44
23	Cl	OCH_3	H	2.62
24	Br	OCH_3	H	2.66
25	Cl	Cl	Cl	2.34
26	Br	Br	Br	1.59
27	NO_2	OCH_3	H	2.73
28	NO_2	Cl	H	2.11
29	NO_2	Br	H	1.92
30	NO_2	NO_2	H	1.38

derivatives were correlated with log P, MR_2 (molar refraction of the 2-substituent), and $\sum\sigma$ as shown in eq 78, where all the parameters were significant at the 95%

$$pI_{50} = 0.440 (0.0652) \log P - 0.0409 (0.0088)(\log P)^2 + 0.207 (0.0222)MR_2 + 0.394 (0.0786)\sum\sigma + 2.664 (0.108)$$
$$n = 76, r = 0.931, s = 0.204, F_{4,71} = 115 \quad (78)$$

confidence level. This equation reestablished the importance of the hydrophobic character of molecules. The log P alone was shown to account for 65% of the variance in the activity (eq 79). The square term of log P in eq 78 did not have much weight, so no parabolic correlation was stressed.[136] However, in this study the dispersion interaction also appears to be important. The positive coefficient of MR_2 indicates that the 2-substituent is involved in the dispersion interaction. Similarly, the positive coefficient of $\sum\sigma$ indicates that an electron-withdrawing group at all positions will enhance the inhibitory effectiveness. Since eq 78 incorporates a large number of data points, the importance of dispersion and electronic effects in APDM inhibition cannot be ignored.

$$pI_{50} = 0.335 (0.0283) \log P + 2.982 (0.0962)$$
$$n = 76, r = 0.809, s = 0.323, F = 140 \quad (79)$$

In another study with a series of 1-alkylimidazoles IXb, an apparent parabolic relationship (eq 80) between π and inhibitory potency toward aldrin epoxidation was obtained,[137] indicating the existence of a hydrophobic pocket of limited size in the vicinity of cytochrome P-450. These 1-alkylimidazoles had shown a synergistic effect with carbaryl insecticide against houseflies.[137] In eq 80, SR5 is the weight:weight synergistic ratio of the LD_{50} of the carbaryl in the absence and presence of synergist, when the ratio of the latter to former was 5:1.

$$\log SR5 = 0.49\pi - 0.038\pi^2 + 0.44$$
$$n = 13, r = 0.922, s = 0.243 \quad (80)$$

For a different series of synergists, 1,3-benzodioxoles (Table 20), studied by Wilkinson,[138] Hansch[139] showed the electronic effect also. The two best correlations that were obtained were as shown by eq 81 and 82. In the

$$\log SR5 = 0.670\pi - 0.195\pi^2 + 1.316\sigma\cdot + 1.612$$
$$n = 13,\ r = 0.929,\ s = 0.171 \quad (81)$$

$$\log SR5 = 0.706\pi - 0.206\pi^2 + 1.460\sigma\cdot + 0.875E_s + 1.586$$
$$n = 16,\ r = 0.943,\ s = 0.164 \quad (82)$$

derivation of eq 81, compounds 28–30 were not included, as it was felt by Hansch that in these compounds the steric hindrance of large groups next to the nitro group would hinder its electronic interaction with the ring electrons. In eq 82, the value of E_s was, however, used for compounds 27–30. In these equations, $\sigma\cdot$ is a σ constant related to homolytic phenylation of substituted benzenes.[139] Involvement of this radical parameter led Hansch to suggest that these synergists are involved in radical reactions. The assumption was that synergists react with the microsomal enzyme to produce relatively stable free radicals that, if sufficiently lipophilic, tightly bind to the site of the enzyme that normally oxidizes and desorbs the insecticides. The ideal lipophilic character of a synergist for carbaryl on flies for optimum binding corresponded to a log P_0 of 3.8.

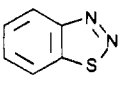
Equations 83 and 84 were obtained for 1:1 and 10:1 ratios of synergist and insecticide and were quite comparable to eq 82. Thus, all the equations from 81 to

$$\log SR1 = 0.689\pi - 0.192\pi^2 + 1.671\sigma\cdot + 0.909E_s + 1.191$$
$$n = 16,\ r = 0.941,\ s = 0.170 \quad (83)$$

$$\log SR10 = 0.704\pi - 0.201\pi^2 + 1.414\sigma\cdot + 0.851E_s + 1.709$$
$$n = 16,\ r = 0.940,\ s = 0.163 \quad (84)$$

84 establish the importance of lipophilic character and the ability of the molecules to form radicals in their synergistic activity, indicating simultaneously that large groups placed next to the strongly activating nitro group would produce steric hindrance. Correlations with several standard sets (σ_p, σ_r, σ_p^+) were found to be poor.

Gil and Wilkinson[140] drew the analogous conclusion regarding 1,2,3-benzodiazoles X, i.e., that their synergistic activity will depend on their lipophilic character and their ability to form radicals, when they derived eq 85. However, radical formation was not found to be



X

$$\log SR5 = 0.45\pi + 0.92\sigma\cdot + 1.78$$
$$n = 14,\ r = 0.882,\ s = 0.117 \quad (85)$$

an important phenomenon for these compounds in the inhibition of microsomal enzymes prepared from rat liver and armyworm gut.[141] The pI_{50} values for armyworm gut were found to be much better related to σ than to $\sigma\cdot$ (eq 86). Since the coefficient of σ is negative

$$pI_{50} = 0.76\ (\pm0.33)\pi - 0.25\ (\pm0.30)\pi^2 - 0.46\ (\pm0.34)\sigma - 3.83\ (\pm0.15)$$
$$n = 20,\ r = 0.942,\ s = 0.212 \quad (86)$$

in eq 86, the electron-donating group will enhance the activity. Here the inhibition will involve either the charge-transfer phenomenon or a nucleophilic attack at the enzyme. However, despite the difference in electronic response there was a reasonably good correlation between the pI_{50} values for armyworm and log SR values for carbaryl against houseflies.[141] This correlation was probably due to the fact that π was the dominant factor in both cases.

Testa[142] analyzed the effects of lipophilic, steric, and electronic factors in the inhibition of cytochrome P-450 mediated aniline hydroxylation by alcohols. On the basis of eq 87, which he first obtained for a series of

$$pI_{50} = 1.73\ (\pm0.19)\log P - 0.514\ (\pm0.088)(\log P)^2 - 1.05\ (\pm0.10)BULK_{lat}$$
$$n = 21,\ r^2 = 0.875,\ s = 0.334,\ \log P_0 = 1.68 \quad (87)$$

homologous and isomeric alcohols (Table 21),[143] he tried to establish the importance of the lipophilic and steric characters of the molecules. In eq 87, $BULK_{lat}$ is the total number of carbon atoms in the molecule divided by the number of carbon atoms in the main chain. This parameter was defined to measure the lateral bulk of the molecules. Log P was calculated according to Rekker and de Kort.[46] Testa, however, obtained another equation equally significant in terms of molar volume (V) and $BULK_{lat}$ (eq 88), which explained the

$$pI_{50} = 0.181\ (\pm0.024)V - 0.00127\ (\pm0.00021)V^2 - 1.29\ (\pm0.21)BULK_{lat} - 4.64\ (\pm0.66)$$
$$n = 21,\ r^2 = 0.878,\ s = 0.306,\ V_0 = 71.26 \quad (88)$$

parabolic dependence of activity on log P. Testa explained that hydrophobic interaction would be limited by the bulk tolerance of the active site of the enzyme.

The negative coefficient attached to the lateral bulk confirms the higher activity of unbranched alcohols.

TABLE 21. Inhibitory Potencies of Alcohols on Aniline Hydroxylation

no.	compd	pI_{50}
27	methanol	−3.09
28	ethanol	−1.10
29	1-propanol	−0.48
30	1-butanol	−0.05
31	1-pentanol	0.27
32	1-hexanol	0.54
33	1-heptanol	0.68
34	2-methyl-1-propanol	−0.39
35	2-methyl-1-butanol	−0.15
36	3-methyl-1-butanol	−0.19
37	2,2-dimethyl-1-propanol	−0.67
38	benzyl alcohol	0.32
39	2-propanol	−0.47
40	2-butanol	−0.35
41	2-pentanol	−0.07
42	2-hexanol	0.15
43	2-heptanol	0.25
44	3-pentanol	−0.37
45	3-hexanol	−0.47
46	2-methyl-3-pentanol	−0.89
47	2,4-dimethyl-3-pentanol	−1.38

Using some quantum mechanical parameters such as E_{HOMO}, E_{LUMO}, and charge densities at some atoms, Testa also tried to show the electronic effect. With inclusion of E_{HOMO} and E_{LUMO} he obtained eq 89 and 90, which do not show any improvement over the cor-

$$pI_{50} = 4.93\ (\pm1.90) + 1.66\ (\pm0.17)\log P - 0.47\ (\pm0.079)(\log P)^2 - 1.35\ (\pm0.20)\mathrm{BULK}_{lat} + 0.402\ (\pm0.163)E_{HOMO}$$

$$n = 21,\ r^2 = 0.895,\ s = 0.293,\ \log P_0 = 1.76 \quad (89)$$

$$pI_{50} = 1.15\ (\pm0.44) + 1.60\ (\pm0.18)\log P - 0.456\ (\pm0.081)(\log P)^2 - 1.29\ (\pm0.20)\mathrm{BULK}_{lat} - 0.240\ (\pm0.101)E_{LUMO}$$

$$n = 21,\ r^2 = 0.893,\ s = 0.295,\ \log P_0 = 1.75 \quad (90)$$

relation expressed by eq 87. Similarly, no charge density factor was found to have any effect in the correlation, and hence no electronic effect appears to be important in the inhibition of aniline hydroxylation by alcohols. Therefore, the conclusion drawn by Testa that charge-transfer processes would be involved in this phenomenon appears to be fictitious.

Recently, Bandiera et al.[144] did a study on the ability of some substituted halogenated biphenyls (4′-substituted 2,3,4,5-tetrachlorobiphenyls) to induce "P-448" dependent monooxygenases including aryl hydrocarbon hydroxylase (AHH) in vivo and in rat hepatoma cells. An initial and obligatory step in enzyme induction is believed to involve the reversible binding of inducers to a cytoplasmic receptor protein.[145] For certain polychlorinated dibenzo-*p*-dioxin analogues, the existence of an excellent correlation between their ability to induce AHH activity and their affinity for receptor binding was indicated.[145a-c] Bandiera et al.[144] analyzed the in vitro receptor-binding affinity of their substituted halogenated biphenyls in relation to physicochemical properties of substituents, and for 15 compounds of the series they obtained eq 91, where HB was a dummy

$$\log (1/EC_{50}) = 1.39\sigma + 1.31\pi + 1.12\mathrm{HB} + 4.20$$

$$n = 15,\ r = 0.916,\ s = 0.31 \quad (91)$$

parameter to indicate the hydrogen bonding. It was equal to 1 for substituents able to form hydrogen bonds and zero for those not able to form hydrogen bonds. From this equation, these authors showed the equal importance of both electronic and lipophilic characteristics of substituents for the binding of compounds with the receptor.

The three additional compounds that were studied but not included in obtaining eq 91 were the 4′-*n*-butyl, 4′-*tert*-butyl, and 4′-phenyl derivatives. Equation 91 predicted very high activity for these compounds as compared with their observed activity. The low activity observed for them was supposed to be due to steric hindrance produced by the bulky substituents.

6. Lipoxygenase

Lipoxygenase oxidizes linoleate to 13-hydroperoxyoctadeca-9,11-dienoate. Linoleic acid is one of the essential fatty acids, which are certainly required for good nutrition.

A very limited study was made on the inhibition of this enzyme. Certain aliphatic alcohols, methanol to heptanol, were found to inhibit it,[146] and their inhibition

TABLE 22. Fusaric Acid Analogues XI and Their Dopamine-β-hydroxylase Inhibitory Activities

compd	R	log RA	compd	R	log RA
1	H	−1.23	15	$(CH_2)_5Cl$	0.34
2	CH_3	−1.80	16	$(CH_2)_6Cl$	−0.13
3	C_2H_5	−1.40	17	$(CH_2)_2CHClCH_3$	0.16
4	C_3H_7	−0.57	18	$(CH_2)_3Br$	−0.49
5	C_4H_9	0.00	19	$(CH_2)_4Br$	0.24
6	C_5H_{11}	0.19	20	$(CH_2)_5C_6H_5$	−1.60
7	C_6H_{13}	−0.17	21	$CH_2CH(CH_3)_2$	−0.11
8	C_7H_{15}	−0.40	22	$(CH_2)_2CH(CH_3)_2$	0.21
9	C_8H_{17}	−0.96	23	$(CH_2)_2CHBrCH_2Br$	0.88
10	C_9H_{19}	−1.40	24	$(CH_2)_2CHBrCH_3$	0.88
11	$(CH_2)_4F$	−0.38	25	$(CH_2)_2CHClCH_2Cl$	0.88
12	$(CH_2)_5F$	−0.17	26	$NHCSN(CH_3)_2$	−1.12
13	$(CH_2)_3Cl$	−0.60	27	$CH_2OCON(CH_3)_2$	−1.89
14	$(CH_2)_4Cl$	0.38	28	SO_2NHCH_3	−3.00

activities were found to be well correlated with log *P* (eq 92)[146] and with a structural parameter *I* known as

$$pK_I = 0.944 \log P + 0.830$$

$$n = 12,\ r = 0.984 \quad (92)$$

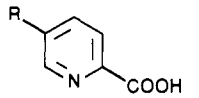
$$pK_I = 0.108\ (\pm0.0)I - 0.450\ (\pm0.12)$$

$$n = 12,\ r = 0.986,\ s = 0.140,\ F = 353 \quad (93)$$

negentropy (eq 93).[147] While eq 93 is only of predictive value, eq 92 shows the importance of the hydrophobic character of the molecules in the activity.

7. Dopamine β-Hydroxylase

The enzyme dopamine β-hydroxylase (DBH) catalyzes the conversion of dopamine to norepinephrine. Inhibitors of this enzyme may lower the norepinephrine levels and the blood pressure. Fusaric acid and many of its analogues (XI) have been found to act as inhib-



XI, R = C_4H_9 (fusaric acid)

itors of this enzyme.[148-151] Martin's group analyzed the inhibition activity of these compounds in relation to their physical properties in order to rationalize designing more potent drugs.[152] All inhibition data were found to be well related to the π of the substituents. Equation 94 relates the data of Suda et al.[148] on a series

$$pI_{50} = 1.53\pi - 0.31\pi^2 + 5.02$$

$$n = 10,\ r^2 = 0.81,\ s = 0.34,\ \pi_0 = 2.47 \quad (94)$$

$$pI_{50} = 3.03\pi - 0.63\pi^2 + 4.58$$

$$n = 14,\ r^2 = 0.88,\ s = 0.19,\ \pi_0 = 2.40 \quad (95)$$

that consisted of only straight-chain analogues (R = H–C_9H_{19}), and eq 95 relates the data of Umezawa et al.[150] on a series that contained halogen, a branched alkyl chain, or a benzene ring in the alkyl side chain. Equation 94 correctly predicted the activities of four new analogues studied by Umezawa.[149]

For a larger series (Table 22) studied by Hidaka et al.,[151] the inhibition activity (RA, relative to fusaric acid) was shown[152] to be related to π as in eq 96, which has

$$\log \mathrm{RA} = 1.25\pi - 0.27\pi^2 - 1.48$$

$$n = 28,\ r^2 = 0.67,\ s = 0.56,\ \pi_0 = 2.31 \quad (96)$$

a lower r^2 than eq 94 and 95 but is statistically signif-

icant. From eq 96, Martin et al.[152] assumed that some other factors, such as the fraction ionized, may be important as well.

From all three equations, however, it appears that the hydrophobic character of the molecules is an important factor in the inhibition of dopamine β-hydroxylase. Though Martin et al. did not comment on the parabolic dependence of activity on π, it seems that in vitro hydrophobic interaction with the enzyme would be limited by the bulk tolerance of the active site of the enzyme.

The electronic effect on DBH inhibition was recently shown by Dove et al.[153] by correlating the inhibition data from his own laboratory on some fusaric acid analogues with their acidity (pK_a) and basicity (pK_b) as shown by eq 97 and 98. Equation 97 relates the data

$$pI_{50}(Cu) = 39.739\ (\pm 6.563) - 0.572\ (\pm 0.166)pK_a - 2.366\ (\pm 0.447)pK_b$$
$$n = 12,\ r = 0.956,\ s = 0.303 \quad (97)$$

$$pI_{50} = 16.130\ (\pm 10.345) + 0.062\ (\pm 0.411)pK_a - 0.849\ (\pm 0.664)pK_b$$
$$n = 11,\ r = 0.851,\ s = 0.369 \quad (98)$$

measured for DBH incubated with copper sulfate, and eq 98 relates the same measured for the pure enzyme. In the derivation of eq 98, fusaric acid was not included. Since the mechanism of dopamine hydroxylation by DBH requires enzyme-bound Cu^{2+} and ascorbate as cofactors, $pI_{50}(Cu)$ was also found to be related to the copper complex formation ability (pK_{Cu}) measured for some of the analogues (eq 99). The high correlation

$$pI_{50}(Cu) = 1.273\ (\pm 0.392)pK_{Cu} - 3.900\ (\pm 2.960)$$
$$n = 7,\ r = 0.946,\ s = 0.422 \quad (99)$$

expressedby eq 99 simply emphasizes the participation of copper complex formation in the mechanism of action of fusaric acid analogues. Additionally, eq 97 suggests that the interaction of these compounds with copper-incubated DBH will be influenced by both the acidity and basicity of the molecules. In eq 98, pK_a is not significant, and inclusion of fusaric acid drastically reduced the correlation coefficient ($r = 0.655$); hence, eq 100 was obtained (including a few more compounds)

$$pI_{50} = 4.694\ (\pm 0.360) - 1.119\ (\pm 0.542)\sigma_p + 0.373\ (\pm 0.263)MR$$
$$n = 18,\ r = 0.794,\ s = 0.470 \quad (100)$$

to show that electron-donating and bulky groups at the 5-position will enhance the activity. Electron donation will probably increase the basicity of the carboxylic group which, as indicated by eq 98, will help in the inhibition, and the bulkiness of the group may lead to hydrophobic interaction, as there was a good correlation between MR and π.[154] However, π was not found by these authors to be exclusively related to inhibition activity; hence, polarizability may also be expected to play some role.

On the basis of these results, the conclusion that can be drawn is that both hydrophobic as well as electronic parameters are important in DBH inhibition.

8. Succinate and NADH Oxidases

The complete oxidation systems that seem to be located in mitochondria include β-oxidation of fatty acids, oxidations of succinate and NADH, and oxidative phosphorylation. The oxidation of succinate is brought about by succinate oxidase and that of NADH by NADH oxidase. Both enzymes are present in the inner membrane of the mitochondrion.

The partial inhibition of succinate oxidase from bovine liver and muscle by a group of miscellaneous compounds including alcohols and ketones was shown to depend upon hydrophobicity (eq 101–104).[154,3c] Excluding one compound, Kier et al.[155] correlated activity for 15–20% inhibition of the muscle enzyme with their molecular connectivity index (χ) (eq 105). Since for nonhomologous series χ may not be related to log P,[156] care should be taken in interpreting eq 105. This equation is only of predictive value and hardly throws any light on the mechanism of interaction.

$$pI_{15-20}(liver) = 0.80\ (\pm 0.14)\log P + 0.12\ (\pm 0.15)$$
$$n = 14,\ r = 0.963,\ s = 0.190 \quad (101)$$

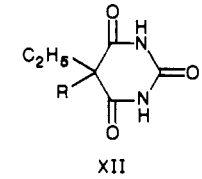
$$pI_{15-20}(muscle) = 0.76\ (\pm 0.12)\log P + 0.66\ (\pm 0.12)$$
$$n = 14,\ r = 0.972,\ s = 0.158 \quad (102)$$

$$pI_{100}(liver) = 0.76\ (\pm 0.14)\log P - 0.18\ (\pm 0.15)$$
$$n = 14,\ r = 0.958,\ s = 0.193 \quad (103)$$

$$pI_{100}(muscle) = 0.74\ (\pm 0.15)\log P - 0.21\ (\pm 0.16)$$
$$n = 14,\ r = 0.937,\ s = 0.203 \quad (104)$$

$$pI_{15-20}(muscle) = 0.916\ (\pm 0.073)\chi - 1.582\ (\pm 0.174)$$
$$n = 13,\ r = 0.966,\ s = 0.169 \quad (105)$$

The inhibition of NADH oxidation by a small group of barbiturates (XII: R = phenyl, isoamyl, 1-methylbutyl, cyclohexenyl, butyl, ethyl) was also shown to depend upon hydrophobicity as well (eq 106).[157] From these equations the importance of hydrophobicity in the inhibition of succinate and NADH oxidases is established.



XII

$$pI_{50} = 1.107\log P + 1.237$$
$$n = 6,\ r = 0.921,\ s = 0.261 \quad (106)$$

A_2. Dehydrogenases

1. Alcohol Dehydrogenase

Alcohol dehydrogenase (ADH) catalyzes the first step in alcohol metabolism and would be a rational target for inhibiting alcohol metabolism. Inhibitors of this enzyme would be useful for studying the metabolism of alcohols and for therapeutically preventing poisoning by methanol[158,159] and ethylene glycol.[160,161]

Hansch et al.[162] analyzed the activity of a variety of ADH inhibitors in relation to their physicochemical properties. The results obtained follow.

TABLE 23A. Amides and Their ADH Inhibition Constants

no.	compd	$-\log K_{ER,I}$	$-\log K_{E,I}$
1	FCH_2CONH_2	1.41	0.62
2	$F_2CHCONH_2$	1.12	0.55
3	F_3CCONH_2	1.33	1.09
4	$ClCH_2CONH_2$	2.12	1.24
5	$Cl_2CHCONH_2$	2.19	1.48
6	Cl_3CCONH_2	2.07	1.85
7	$BrCH_2CONH_2$	2.24	1.36
8	$Br_2CHCONH_2$	2.49	1.77
9	ICH_2CONH_2	2.72	1.62
10	$Et_2CHCONH_2$	3.41[a]	1.54[a]
11	Me_3CCONH_2	2.46[a]	1.37[a]
12	CH_2=$CHCONH_2$	2.11	1.77
13	CH_2=$C(Me)CONH_2$	3.02	2.06
14	MeCH=$CHCONH_2$	2.89	2.16
15	MeCH=$C(Me)CONH_2$	3.15	2.41
16	C_6H_5CONH	3.22	2.62
17	$Me_2CHCONH_2$	3.77	1.55

[a] Not used in correlations.

TABLE 23B. 4-Substituted Pyrazoles XIV and Their ADH Inhibition Constants

		log $(1/K_i)$		
compd	X	rat liver ADH	human liver ADH	rat hepatocytes
18	C_6H_{13}	9.15	7.78	6.90
19	C_5H_{11}	8.46	7.42	6.82
20	C_3H_7	7.56	6.32	6.47
21	CH_3	6.79	6.16	6.37
22	I	6.42	5.39	6.00
23	OC_3H_7	6.24	5.63	5.89
24	$OCH(CH_3)_2$	5.84	4.83	5.46
25	OC_2H_5	5.56	4.98	5.36
26	OCH_3	5.17	4.71	5.17
27	H	5.13	5.00	4.88
28	CN	4.71	4.03	4.48
29	NO_2	4.02	3.46	3.92
30	NH_2	3.74	3.79	3.61
31	$NHCOCH_3$	3.66		3.37

a. Amides with Liver Alcohol Dehydrogenase. Table 23A:[163]

$$-\log K_{ER,I} = 0.814\ (\pm 0.34) \log P - 0.768\ (\pm 0.20)\sigma^* + 3.528\ (\pm 0.33)$$

$$n = 15,\ r = 0.0.929,\ s = 0.302 \qquad (107)$$

$$-\log K_{E,I} = 0.805\ (\pm 0.39) \log P - 0.528\ (\pm 0.25)\sigma^* + 2.509\ (\pm 0.43)$$

$$n = 15,\ r = 0.859,\ s = 0.341 \qquad (108)$$

Small set of amides (formamide, acetamide, propionamide, butyramide, valeramide, hexanamide):[164]

$$-\log K_{E,I} = 1.050\ (\pm 0.36) \log P - 1.750\ (\pm 1.3)\sigma^* + 2.815\ (\pm 0.25)$$

$$n = 6,\ r = 0.996,\ s = 0.152 \qquad (109)$$

$$-\log K_{ER,I} = 1.144\ (\pm 0.52) \log P - 2.513\ (\pm 1.8)\sigma^* + 3.864\ (\pm 0.36)$$

$$n = 6,\ r = 0.994,\ s = 0.219 \qquad (110)$$

Another small set of amides (formamide, acetamide, butyramide, hexanamide, isobutyramide, trimethylacetamide, benzamide):[165]

$$-\log K_{E,S} = 0.548\ (\pm 0.34) \log P - 0.559\ (\pm 0.83)\sigma^* + 1.027\ (\pm 0.28)$$

$$n = 7,\ r = 0.934,\ s = 0.253 \qquad (111)$$

b. $RCOO^-$ (R = H–$C_{15}H_{31}$) with Liver Alcohol Dehydrogenase. Large set:[164]

$$-\log K_{E,I} = 0.693\ (\pm 0.09) \log P + 3.846$$

$$n = 14,\ r = 0.979,\ s = 0.341 \qquad (112)$$

$$-\log K_{EO,I} = 0.607\ (\pm 0.10) \log P + 4.857\ (\pm 0.25)$$

$$n = 14,\ r = 0.968,\ s = 0.368 \qquad (113)$$

Acetate, butyrate, hexanoate, octanoate, decanoate:[165]

$$-\log K_{E,I} = 0.917\ (\pm 0.29) \log P + 2.443\ (\pm 0.25)$$

$$n = 5,\ r = 0.970,\ s = 0.418 \qquad (114)$$

c. Alcohols with Liver Alcohol Dehydrogenase. Ethanol to pentanol:[166]

$$-\log K_{EO,S} = 0.666\ (\pm 0.29) \log P + 2.443\ (\pm 0.25)$$

$$n = 4,\ r = 0.990,\ s = 0.080 \qquad (115)$$

$$-\log K_{ER,S} = 0.814\ (\pm 0.36) \log P + 1.152\ (\pm 0.30)$$

$$n = 4,\ r = 0.990,\ s = 0.097 \qquad (116)$$

Ethanol to hexanol and 2,2,2-trifluoroethanol:[166]

$$-\log K_{E,S} = 0.650\ (\pm 0.29) \log P - 0.296\ (\pm 0.18)\sigma^* + 0.846\ (\pm 0.33)$$

$$n = 6,\ r = 0.986,\ s = 0.154 \qquad (117)$$

d. $RNH_3^+Cl^-$ (R = C_5H_{11}–C_8H_{17}, $C_{10}H_{21}$)[167] with Yeast Alcohol Dehydrogenase.

$$-\log K_I = 0.582\ (\pm 0.12) \log P + 2.100\ (\pm 0.12)$$

$$n = 5,\ r = 0.994,\ s = 0.074 \qquad (118)$$

e. 3-Carboxamido-*N*-alkylpyridinium Chloride (R = CH_3–$C_{10}H_{21}$)[168] with Yeast Alcohol Dehydrogenase.

$$-\log K_I = 0.505\ (\pm 0.03)\pi + 0.34\ (\pm 0.12)$$

$$n = 12,\ r = 0.997,\ s = 0.061 \qquad (119)$$

f. *N*-Alkylmaleimide (R = C_2H_5, C_4H_9–C_8H_{17})[169] with Yeast Alcohol Dehydrogenase.

$$\log K = 0.335\ (\pm 0.05) + 0.83\ (\pm 0.15)$$

$$n = 6,\ r = 0.994,\ s = 0.046 \qquad (120)$$

g. Reduction of 4-XC_6H_4CHO (X = 4-OCH_3, H, 4-CH_3, 4-Cl, 4-NO_2)[170] by Liver Alcohol Dehydrogenase.

$$\log K = 0.500\ (\pm 0.05)\pi + 1.024\ (\pm 0.05)\sigma + 1.994\ (\pm 0.02)$$

$$n = 5,\ r = 0.999,\ s = 0.010 \qquad (121)$$

In all these equations, terms on the left side have been defined as

$$K_{E,I} = \frac{[E][I]}{[EI]} \qquad K_{ER,I} = \frac{[ER][I]}{[ERI]}$$

$$K_{EO,S} = \frac{[EO][S]}{[EOS]} \qquad K_{E,S} = \frac{[E][S]}{[ES]}$$

where [E] refers to the concentration of free enzyme, [R] to the concentration of free NADH, [O] to the concentration of free NAD, [I] to the concentration of

free inhibitor, and [S] to the concentration of free substrate, and the terms in the denominators refer to the concentrations of the complexes after dissociation. K_I and K are the usual inhibitor and equilibrium constants, respectively.

From all these equations, Hansch et al.[162] tried to establish the importance of hydrophobicity in ADH inhibitions. Though in many cases the number of data points used in the correlation has been very small, the high r values support the effective role of hydrophobic character. The correlations are also important from another point of view in that the data used are from different laboratories and for different types of compounds.

The electronic effect, however, appears to be important only in the case of the amides. In eq 117 for alcohols and in eq 121 for benzaldehyde, the appearance of σ^* and σ, respectively, might be by chance only. Because of the small number of data points used in eq 117 and 121 and because of the absence of an electronic effect in many other cases, not much confidence can be attached to these equations. However, in the case of the amides, all eq 107–111 reflect is that electron-donating groups will increase the interaction of the molecules with the enzyme.

However, notwithstanding these conclusions, no set of congeners used in this study was ideally designed to assess properly the role of steric, electronic, and hydrophobic properties of molecules in ADH inhibition. Even the best set, the amides, had not included the more lipophilic analogues. In all cases, mostly calculated log P values were used. However, in another series of amides, para- and meta-substituted benzamides [with substituents like NO_2, Cl, Br, F, OH, OCH_3, CH_3, $(CH_3)_2N$, and $(CH_3)_2CH$], Hansch et al.[171] again found the activity being controlled by the hydrophobic and electronic characters of the molecules (eq 122). As with

$$-\log K_{ER,I} = 0.453\ (\pm 0.30)\pi_4 - 0.804\ (\pm 0.30)\sigma - 0.232\ (\pm 0.17)(E_s\text{-}4) - 2.369\ (\pm 0.20)$$
$$n = 14,\ r = 0.953,\ s = 0.168,\ F = 32.7 \quad (122)$$

aliphatic amides (eq 107), eq 122 also shows that electron-donating groups at ring positions will enhance the activity of benzamides. It therefore suggests that in each case the oxygen atom of the carbonyl group forms one of the centers of binding in complex formation. Complex formation may involve the charge-transfer phenomenon, where the oxygen atom may act as an electron donor. It was, however, shown by Sharma and Woronick[172] for the same series of benzamides that meta substituents produce better electronic effects (eq 123) than para substituents (eq 124). This may be due to strong resonance effects operating from the meta position.

$$-\log K_{ER,I} = -2.63 - 0.921\sigma$$
$$n = 7,\ r = 0.94,\ s = 0.130 \quad (123)$$
$$-\log K_{ER,I} = 2.30 - 0.325\sigma$$
$$n = 8,\ r = 0.36,\ s = 0.304 \quad (124)$$

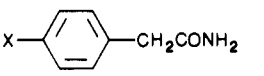
Regarding a hydrophobic effect, one would find from eq 122 as well as from eq 125 and 126 derived by Sharma and Woronick[172] that para substituents produce positive dominant effects. This shows that there is

$$-\log K_{ER,I} = 0.968\pi_4 - 2.45$$
$$n = 6,\ r = 0.88,\ s = 0.205 \quad (125)$$
$$-\log K_{ER,I} = -0.156\pi_3 - 3.00$$
$$n = 5,\ r = 0.36,\ s = 0.187 \quad (126)$$

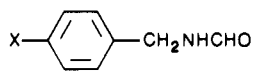
hydrophobic interaction directly involving the para substituent. Equation 122 further shows that this hydrophobic interaction is enhanced by the bulky nature of the substituent, as it also contains the steric parameter E_s whose coefficient is negative and significant at the 95% confidence level (the larger the group, the more negative is E_s). The bulky group would probably provide the better induced fit as suggested by Koshland.[173] Verloop et al.[25] accounted for this steric effect by their width parameter B_1 (eq 127). An effect of only para substituents in hydrophobic and steric effects shows the directional nature of the hydrophobic bonding.

$$-\log K_{ER,I} = 0.43\pi_4 - 0.81\sigma + 0.40B_{1,4} - 3.06$$
$$n = 14,\ r = 0.950,\ s = 0.172,\ F = 30.7 \quad (127)$$

In two other families of amide inhibitors (para-substituted phenylacetamides XIIIa and para-substituted formylbenzylamines XIIIb), the dominance of the hy-



XIIIa



XIIIb

drophobic character of the para substituent was recently shown by Hansch et al.[174] The data of Freudenreich et al.[175] on XIIIa [X = H, Cl, F, Br, I, OMe, OC_2H_5, $OCH(CH_3)_2$, OC_3H_7, OC_4H_9, $OCH_2CH_2CH(CH_3)_2$, OC_5H_{11}, $OCH_2C_6H_5$, $OCH_2C_6H_4$-4′-Br, $OCH_2C_6H_{11}$] for horse liver ADH inhibition was correlated with log P[174] (eq 128), and the data obtained by

$$\log (1/K_i) = 0.89\ (\pm 0.20)\ \log P + 3.56\ (\pm 0.29)$$
$$n = 11,\ r = 0.960,\ s = 0.197,\ F_{1,9} = 107 \quad (128)$$

these authors on a small series of XIIIb [X = H, OH, $O(CH_2)_3CH_3$, $O(CH_2)_4CH_3$, $OCH_2C_6H_5$, $(CH_2)_5CH_3$] for horse liver ADH inhibition was correlated with π[174] (eq 129). In these two cases, the electronic effect of sub-

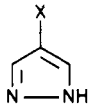
$$\log (1/K_i) = 0.84\ (\pm 0.58)\pi + 6.01\ (\pm 0.83)$$
$$n = 5,\ r = 0.935,\ s = 0.442,\ F_{1,3} = 20.9 \quad (129)$$

stituents was found to be absent, the reason being that substituents were insulated by the saturated CH_2 moiety from the carbonyl group, which forms the center of binding with the active site of the enzyme. Moreover, the substituents studied were of weak electronic character.

Four derivatives of XIIIa [X = $OCH(CH_3)_2$, $OCH_2CH_2CH(CH_3)_2$, $OCH_2C_6H_4$-4′-Br, $OCH_2C_6H_{11}$] and one of XIIIb [X = $(CH_2)_5CH_3$] were not included in the derivation of eq 128 and 129, respectively. These equations predicted very high activity for these analogues as compared with their observed values. This deviation of the observed values from their predicted values for these congeners was attributed, by molecular graphics, to the fact that their substituents had unfavorable contacts with the active surface of the enzyme.[174] The binding modes of ADH inhibitors in general involve interaction with a narrow hydrophobic

channel of the enzyme and coordination with the Zn atom in its active site.[176]

Along with the hydrophobic character, the electronic character of substituents was found to affect ADH inhibition significantly in the case of some 4-substituted pyrazoles XIV.[177] The inhibition constants for these



XIV

pyrazoles studied against ethanol oxidation by isolated rat hepatocytes[177] are listed in Table 23B. These inhibition constants were found to be correlated with the hydrophobic and electronic parameters of the substituents[177] (eq 130–132). Certain 4-substituted pyrazoles

Rat Liver ADH

$$\log (1/K_i) = 1.22 \log P - 1.80\sigma_m + 4.87$$
$$n = 14,\ r = 0.985,\ s = 0.316 \quad (130)$$

Human Liver ADH

$$\log (1/K_i) = 0.87 \log P - 2.06\sigma_m + 4.60$$
$$n = 13,\ r = 0.977,\ s = 0.303 \quad (131)$$

Isolated Rat Hepatocytes

$$\log (1/K_i) = 1.27 \log P - 0.20 (\log P)^2 - 1.80\sigma_m + 4.75$$
$$n = 14,\ r = 0.971,\ s = 0.320 \quad (132)$$

(X being mostly linear or branched alkyl groups or halogens) were studied by Dahlbom et al.[178] and Tolf et al.[179] against horse liver ADH also. Cornell et al.[177] correlated the horse liver ADH inhibition data with log P and σ (eq 133). Equations 130–133 all suggest that

$$\log (1/K_i) = 0.56 \log P - 1.11\sigma_m + 6.99$$
$$n = 16,\ r = 0.881,\ s = 0.404 \quad (133)$$

substituents that tend to donate electrons to the pyrazole ring make more potent inhibitors. This is consistent with the view[176] that the nitrogen at position 2 of pyrazole binds to the electron-deficient zinc atom at the enzyme active site and the nitrogen at position 1 reacts with the positively charged pyridine moiety of the coenzyme NAD^+.

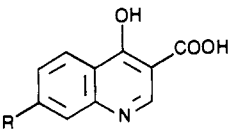
The parabolic correlation of activity with log P in the case of intact cells (eq 132) simply suggests, as usual, that the ability of compounds to cross the cell membrane to obtain access to the enzyme would be limited.

2. Lactate and Malate Dehydrogenases

Lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) are both oxidoreductases utilizing NAD^+ as acceptor. Lactate dehydrogenase converts lactate to pyruvate in the glycolytic process, and malate dehydrogenase plays a role in the Krebs cycle by the conversion of malate to oxalacetate.

Some cancer cells have exhibited abnormal levels or activities of lactate and malate dehydrogenases[180–183] and some other enzymes.[184,185] Selective inhibition of these enzymes in neoplastic tissue would therefore increase the prospects for chemotherapy of solid tumor systems. Inhibitors of these enzymes may be useful in the treatment of tumors that are slow in dividing and hence are resistant to inhibitors of DNA synthesis. Inhibition of either of them will ultimately lead to cessation of cellular respiration.

Recently, Coats et al.[186] synthesized a series of 7-substituted 4-hydroxyquinoline-3-carboxylic acids XV



XV

(Table 24) and studied their inhibition activities against mitochondrial MDH (m-MDH), cytoplasmic MDH (s-MDH), and skeletal muscle dehydrogenase (LDH-M_4). The inhibition activities were found to be well correlated with MR (eq 134–136),[186] V_w (van der Waals

$$pI_{50}(\text{m-MDH}) = 0.58\text{MR} + 2.46$$
$$n = 29,\ r = 0.96,\ s = 0.34,\ F_{1,27} = 329.86 \quad (134)$$

$$pI_{50}(\text{s-MDH}) = 0.29\text{MR} + 2.61$$
$$n = 17,\ r = 0.87,\ s = 0.38,\ F_{1,15} = 47.96 \quad (135)$$

$$pI_{50}(\text{LDH-M}_4) = 0.36\text{MR} + 2.79$$
$$n = 11,\ r = 0.96,\ s = 0.20,\ F_{1,9} = 95.60 \quad (136)$$

$$pI_{50}(\text{m-MDH}) = 1.96V_w + 2.28$$
$$n = 29,\ r = 0.96,\ s = 0.36,\ F_{1,27} = 290.61 \quad (137)$$

$$pI_{50}(\text{s-MDH}) = 0.96V_w + 2.57$$
$$n = 17,\ r = 0.89,\ s = 0.34,\ F_{1,15} = 57.51 \quad (138)$$

$$pI_{50}(\text{LDH-M}_4) = 1.24V_w + 2.67$$
$$n = 11,\ r = 0.95,\ s = 0.21,\ F_{1,9} = 85.50 \quad (139)$$

$$pI_{50}(\text{m-MDH}) = 0.65{}^1\chi^v + 2.45$$
$$n = 29,\ r = 0.96,\ s = 0.33,\ F_{1,27} = 347.53 \quad (140)$$

$$pI_{50}(\text{s-MDH}) = 0.32{}^1\chi^v + 2.65$$
$$n = 17,\ r = 0.89,\ s = 0.34,\ F_{1,15} = 54.65 \quad (141)$$

$$pI_{50}(\text{LDH-M}_4) = 0.41{}^1\chi^v + 2.78$$
$$n = 11,\ r = 0.96,\ s = 0.20,\ F_{1,9} = 105.61 \quad (142)$$

volume; eq 137–139),[187] and ${}^1\chi^v$ (first-order valence molecular connectivity index; eqs 140–142).[187] The hydrophobic parameter was not as well correlated with these enzyme inhibition activities; rather, the respiratory inhibition of the whole cell model measured by Shah and Coats[188] or Ehrlich ascites cells was found to be significantly correlated with π (eq 143).[188] Equation

$$pI_{50}(\text{ascites}) = 0.46\pi + 3.22$$
$$n = 14,\ r = 0.93,\ s = 0.28 \quad (143)$$

143 included only compounds 1–15, with the exclusion of **9**. Later, a few more compounds (16–19, 21) were evaluated for this activity by Coats et al.[186] and then, again with exclusion of 9, the activity was well correlated with the HPLC retention index (RI, eq 144).[186]

$$pI_{50}(\text{ascites}) = 0.21\text{RI} + 2.05$$
$$n = 19,\ r = 0.92,\ s = 0.31,\ F_{1,17} = 87.53 \quad (144)$$

TABLE 24. MDH, LDH, and Ascites Cell Respiration Inhibition Activities of 4-Hydroxyquinoline-3-carboxylic Acids XV

		pI_{50}			
compd	R	m-MDH	s-MDH	LDH-M_4	ascites
1	H				2.98
2	SO_2CH_3	3.18	3.36	3.39	2.75
3	OCH_3				3.28
4	OH	3.31	3.02	2.95	3.04
5	Cl	2.44	2.33	2.74	3.84
6	F	1.98	1.96		3.30
7	$CONH_2$	3.13	3.22		2.24
8	COOH	2.97	2.89	2.91	2.24
9	SO_3^-	2.67	2.51	3.06	2.88
10	SO_2NH_2	3.02	3.16	3.24	2.47
11	$COCH_3$	3.04	3.04	3.25	3.10
12	NO_2	2.72	2.92	3.02	3.24
13	$N(CH_3)_2$	3.32	3.43	3.50	3.33
14	$OCH_2C_6H_5$	4.49			4.41
15	$OCH_2C_6H_3$-3,4-Cl_2	5.32			4.82
16	$OCH_2C_6Cl_5$	5.32			4.37
17	OCH_2-α-$C_{10}H_7$	4.48			4.07
18	$OCH_2C_6H_4$-4-F	4.40			3.78
19	$OCH_2C_6H_4$-4-Br	5.17			4.27
20	OCH_2-β-$C_{10}H_7$	5.39			
21	$OCH_2C_6H_4$-4-OC_6H_5	5.83	4.51	4.56	4.00
22	$O(CH_2)_2C_6H_5$	4.42	3.81		
23	$O(CH_2)_2C_6H_4$-4-F	4.83			
24	$O(CH_2)_2C_6H_4$-4-Br	5.60	4.04	4.75	
25	$O(CH_2)_2$-β-$C_{10}H_7$	5.66	4.14		
26	$O(CH_2)_2C_6H_4$-4-OC_6H_5	5.74	4.21		
27	$O(CH_2)_2OC_6H_5$	4.22			
28	$O(CH_2)_2OC_6H_4$-4-F	4.74			
29	$O(CH_2)_2OC_6H_4$-4-Br	5.29			
30	$O(CH_2)_2O$-β-$C_{10}H_7$	5.80			
31	$O(CH_2)_2OC_6H_4$-4-OC_6H_5	5.61	4.09		

Equations 134–144 show the linear relationship. Squared terms of the variables were entered in some cases but were not significant. The pI_{50} for the whole-cell model was shown[188] to be poorly correlated with MR. Thus, the intracellular target enzyme model and the whole-cell model inhibition activities are not found to be related to a common variable. Notwithstanding this, we attempted[189] to show with the previous data of Shah and Coats[188] for compounds 1–15 that pI_{50}(ascites) and pI_{50}(m-MDH) could be correlated with a common parameter, V_w (eq 145, 146). Both eq 145 and 146 were significant at the 99% confidence level.

$$pI_{50}(\text{ascites}) = 2.644V_w^2 - 9.983V_w + 12.280$$

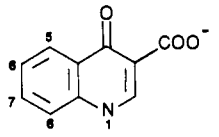
$$n = 15,\ r = 0.824,\ s = 0.442,\ F_{2,12} = 12.67 \qquad (145)$$

$$pI_{50}(\text{m-MDH}) = 2.32V_w - 1.171$$

$$n = 13,\ r = 0.925,\ s = 0.341,\ F_{1,11} = 65.55 \qquad (146)$$

Since MR, V_w, and $^1\chi^v$ were well correlated with each other,[187] it can be said that enzyme inhibition activities would be well controlled exclusively by the molecular size of the 7-substituents. This exclusive dependence of activity on molecular size and the lack of a relation of activity with the hydrophobic character of the molecules suggest[22,190] the involvement of dispersion interaction in any drug action; hence, LDH and MDH inhibitions appear to involve strong dispersion interaction. In this context, eq 145 seems to be important regarding the cell repiration inhibition. Notwithstanding the findings of Shah and Coats[188] (eq 143) or Coats et al.[186] (eq 144), eq 145 states that whole-cell respiration inhibition does not involve any different mechanism. However, being quadratic in nature, eq 145 suggests that up to a certain value of V_w (1.88×10^2 Å^3) the activity against cell respiration will decrease and then would start increasing as V_w increases. Up to this optimum value of V_w the substituents were more polar.[189] Therefore, it can be said that molecules with these substituents were trapped by the polar region of the cell membrane, and when the molecules acquired bigger substituents with less polar character, they were allowed to cross the membrane through the lipid phase and reach the target. At the target then the involvement of dispersion interaction is a possibility. Since there was no involvement of π^2 or significant RI^2 term in the correlations obtained by Shah and Coats or Coats et al., the role of lipid solubility in optimizing the activity could not be assessed. In deriving eq 145, no compound until then evaluated was excluded, while in eq 143, compound 9 with the SO_3^- group was totally misfit.

The hydrophobic character, particularly of 7-substituents, was not found to play any role in the inhibition of malate or lactate dehydrogenase even when a large series of 1,4-dihydro-4-quinoline-3-carboxylates XVI studied by the Baker group[191] were analyzed by



XVI

Yoshimoto and Hansch.[192] Equation 147, derived for

$$pI_{50}(\text{m-MDH}) = 0.699\ (\pm0.06)\pi_5 + 0.290\ (\pm0.08)MR_{6,7,8} - 1.121\ (\pm0.37)I_5 + 3.156\ (\pm0.18)$$

$$n = 75,\ r = 0.943,\ s = 0.385 \qquad (147)$$

$$pI_{50}(\text{s-LDH}) = 0.08\ (\pm0.02)MR_{1,5,6,8} + 0.487\ (\pm0.16)I_5 - 0.114\ (\pm0.09)I_1 + 3.853\ (\pm0.11)$$

$$n = 79,\ r = 0.836,\ s = 0.173 \qquad (148)$$

MDH, shows that there is some hydrophobic effect only from the 5-position; but simultaneously, the negative coefficient of the indicator parameter I_5, indicative of the presence of 5-$O(CH_2)_nOC_6H_5$ with $n = 3$ or 4, shows the steric effect produced by bulky 5-substituents. Equation 148, derived for cytoplasmic LDH, does not show any apparent hydrophobic effect; but since the coefficient of I_5, which is defined for the presence of 5-$(CH_2)_nC_6H_5$ and takes the value of unity for $n = 2$–6, is positive, it was assumed[192] that such groups at the 5-position might be involved in hydrophobic interaction. But since I_5 defines the bulkiness of the group, it is more proper to assume only the dispersion interaction from the 5-position. MR_5 is already well correlated with the activity. I_1 accounts for 1-H, which has a small negative effect on the activity.

Subjecting the first few compounds of Table 24 to principle-component analysis, Dove et al.[193] tried to establish that the activity against the whole-cell system could not be directly attributed to the inhibition of the enzyme systems. For the enzyme systems eq 149 was

$$pI_{50} = 0.20\ (\pm0.16)\mu + 0.81\ (\pm0.46)B_4 - 2.85\ (\pm1.30)$$

$$n = 8,\ r = 0.902,\ s = 0.305 \qquad (149)$$

obtained, and for the whole-cell system eq 150 was

$$pI_{50} = 0.39\ (\pm0.12)\pi - 0.12\ (\pm0.001)\mu + 3.71\ (\pm0.43)$$
$$n = 8,\ r = 0.949,\ s = 0.190 \qquad (150)$$

obtained, where μ is the dipole moment of the molecule and B_4 the steric width parameter of the substituent.[25] However, to firmly establish this conclusion, further study is needed.

3. Glyceraldehyde 3-Phosphate Dehydrogenase and Glutamate Dehydrogenase

The 4-quinoline-3-carboxylates studied by Baker's group[191] were also found to inhibit glyceraldehyde 3-phosphate dehydrogenase (GPDH) and glutamate dehydrogenase (GDH). Like MDH and LDH, GPDH and GDH are also important in the glycolytic pathway, and therefore, their inhibition will also lead to the inhibition of cell growth. Yoshimoto and Hansch[192] obtained correlations for their inhibition by 4-quinoline-3-carboxylates (eq 151 and 152). In eq 151, I_5 takes the

$$pI_{50}(\text{GPDH}) = 0.0906\ (\pm0.02)MR_{1,5,6,8} + 0.498\ (\pm0.18)I_5 - 0.149\ (\pm0.10)I_{1,5} + 3.127\ (\pm0.10)$$
$$n = 72,\ r = 0.849,\ s = 0.172 \qquad (151)$$

$$pI_{50}(\text{GDH}) = 0.491\ (\pm0.04)\pi_5 + 0.233\ (\pm0.05)MR_6 - 0.553\ (\pm0.17)I_5 + 3.355\ (\pm0.08)$$
$$n = 87,\ r = 0.948,\ s = 0.253 \qquad (152)$$

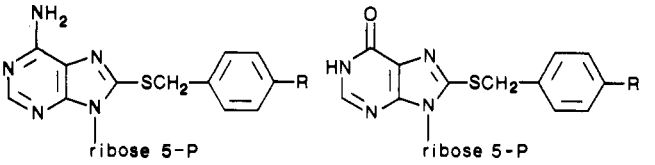
value of 1 for 5-$(CH_2)_2C_6H_4$-3′-X or -4′-X. This is however not an important parameter, as there were only four such groups. $I_{1,5}$ takes the value of 1 for congeners having H in both the 1- and 5-positions. This parameter suggests only a small negative steric effect. In totality, eq 151 is very much similar to eq 148 derived for LDH. Hence, the inhibition of GPDH can also be said to involve the dispersion interaction.

Likewise, eq 152, where I_5 takes the value of 1 for 5- or 6-$O(CH_2)_nC_6H_5$ (n = 2–5), is remarkably similar to eq 147 derived for MDH. The negative coefficient of I_5 shows a steric disturbance in the hydrophobic interaction, which otherwise will take place between the 5-substituent and the enzyme. The substituent at the 6-position appears to involve dispersion interaction. Substituents at other positions were not shown to be important in this case. For fruitful conclusions, further studies are demanded in the case of these two enzymes also.

4. Inosinic Acid Dehydrogenase

Inosine monophosphate (inosinic acid) dehydrogenase (IMPDH) catalyzes the conversion of IMP to xanthosine monophosphate (XMP). The conversion of IMP to XMP is the first step of the biochemical conversion of IMP to GMP (guanosine monophosphate). Hence, the enzyme IMPDH is of vital importance to rapidly growing cells, and therefore, the inhibition of this enzyme will lead to the inhibition of cell growth. This may be an important aspect to the design of anticancerous drugs.

Some AMP (adenosine monophosphate) and IMP analogues were found to act as IMPDH inhibitors,[194,195] and for a small series of 8-(para-substituted benzylthio)AMP and IMP analogues [XVIIa: R = H, F, Cl, OCH_3, CN, NO_2, $C(CH_3)_3$, COO^-; XVIIb: R = H, Cl, OCH_3, CN, NO_2, $C(CH_3)_3$], Skibo and Meyer[194] were able to correlate the inhibition constant with the field constant $\mathcal{F}$ (eq 153), where I takes the value of 1 for



XVIIa XVIIb

$$pK_i = 3.87\ (\pm0.11) + 0.75\ (\pm0.25)\mathcal{F} + 0.32\ (\pm0.14)I$$
$$n = 14,\ r = 0.933,\ s = 0.116,\ F_{2,11} = 36.8 \qquad (153)$$

IMP analogues and zero for AMP analogues. No other parameter was found to be correlated with K_i. Since only $\mathcal{F}$ gave a significant correlation, Skibo and Meyer assumed that some type of charge-transfer interaction with an electron-rich site in the active site was involved.

The pK_i values of these compounds were, however, found to be well correlated with V_w also (eq 154).[196]

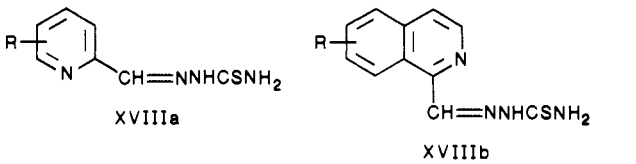
$$pK_i = 3.924 + 2.123\ (0.767)V_w - 3.720\ (0.993)V_w^2 + 0.316\ (0.074)I$$
$$n = 13,\ r = 0.907,\ s = 0.132,\ F_{3,9} = 13.96 \qquad (154)$$

Since $\mathcal{F}$ was not found to be correlated with V_w and since inclusion of $\mathcal{F}$ did not make any significant improvement in the correlation of eq 154, Gupta and Handa[196] assumed that there might be some dispersion interaction[22] between the substituent and the active site of the enzyme and that steric hindrance by comparatively bulker groups would limit this interaction. However, detailed study is needed to find the mechanism of IMPDH inhibition by AMP or IMP analogues.

5. Ribonucleoside Diphosphate Reductase

Like IMPDH, ribonucleoside diphosphate reductase (RDR) is also important to cell growth. It catalyzes the conversion of ribonucleotides to deoxyribonucleotides. Hence, the study of RDR inhibition is also important to the design of useful anticancerous drugs.

A number of α-N-formylheteroaromatic thiosemicarbazones such as XVIIIa and XVIIIb are known to inhibit ribonucleoside diphosphate reductase.[197] Dunn



XVIIIa XVIIIb

and Hodnett[198] did a QSAR study on these inhibitors with the enzyme extracted from rat Novikoff tumor and H.ep-2 tumor of human origin. Equation 155 was obtained for the inhibition of the RDR of H.ep-2 tumor by 2-formylpyridine thiosemicarbazones XVIIIa, and eq 156a and 156b were obtained, respectively, for the inhibition of H.ep-2 and rat Novicoff tumor enzymes by 1-formylisoquinoline thiosemicarbazones XVIIIb.

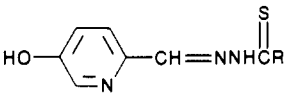
$$pI_{50} = 6.30 - 0.81\Sigma F_{3,5} + 0.29\Sigma\pi_{3,5} - 0.24MR_5$$
$$n = 28,\ r = 0.880,\ s = 0.330 \qquad (155)$$
$$pI_{50} = 6.70 - 1.81MR_5$$
$$n = 13,\ r = 0.80,\ s = 0.370 \qquad (156a)$$
$$pI_{50} = 7.67 - 0.44MR_5^2$$
$$n = 12,\ r = 0.930,\ s = 0.350 \qquad (156b)$$

TABLE 25. RDR Inhibitory Activities of 2-Formylpyridine Thiosemicarbazones XVIIIa

compd	5-R	pI_{50}[a]	compd	5-R	pI_{50}[a]
1	H	6.55	13	$OC_2H_4N(CH_3)_2$	4.62
2	CH_3	6.51	14	$O(C_2H_4O)_2C_2H_5$	5.69
3	C_2H_5	6.66	15	$OOCCH_3$	5.44
4	F	5.92	16	$OOCC_2H_5$	5.28
5	Cl	6.25	17	n-$OOCC_3H_7$	5.17
6	Br	6.30	18	n-$OOCC_{15}H_{31}$	3.96
7	I	6.39	19	$OOCCH_2OCH_3$	5.30
8	CF_3	5.62	20	$OOCCH_2OC_2H_5$	5.25
9	OH	5.17	21	$OOCCH_2N(CH_3)_2$	5.24
10	OCH_3	5.92	22	$OOCCH_2OC_6H_5$	4.89
11	OCF_3	5.60	23	$NHCOCH_3$	5.92
12	OC_2H_5	6.07	24	$N(CH_3)_2$	6.40

[a] Against H.ep-2 cells (human epidermoid carcinoma): French, F. A.; Blanz, E. J., Jr.; Shaddix, S. C.; Brockman, R. W. *J. Med. Chem.* **1974**, *17*, 172.

TABLE 26. RDR Inhibitory Activities of 4′-Substituted 5-Hydroxy-2-formylpyridine Thiosemicarbazones



compd	R	pI_{50}[a]	compd	R	pI_{50}[a]
25	NH_2	5.52	30	c-NC_4H_8	4.18
26	c-$N(CH_2CH_2)O$	4.16	31	SCH_3	4.94
27	c-NC_5H_{10}	4.38	32	NCH_2CH_2	4.10
28	c-$N(CH_2CH_2)_2NCH_3$	4.14	33	C_6H_5	4.31
29	c-$N(CH_2CH_2)S$	4.09			

[a] Against rat Novikoff ascites tumor cells: Agrawal, K. C.; Lee, M. H.; Booth, B. A.; Moore, E. C.; Sartorelli, E. C. *J. Med. Chem.* **1974**, *17*, 934.

In eq 155, F is the Swain–Lupton field constant. This equation shows the inductive effect as well as the hydrophobic effect from the 3- and 5-positions. But in the case of 1-formylisoquinoline thiosemicarbazones, eq 156a and 156b indicate a strong steric effect from the 5-position. The dominance of the steric effect of the 5-substituent was recently shown by Gupta et al.,[199] not only in the case if isoquinoline analogues but also in the case of pyridine analogues. These authors made an exhaustive QSAR analysis on these thiosemicarbazones in relation to V_w and the hydrophobic parameter log P. No correlations were found with log P, but V_w gave very significant correlations. Equations 157–161 were obtained, respectively, for 2-formylpyridine thiosemicarbazones (Table 25) against H.ep-2 cells, 4′-substituted 5-hydroxy-2-formylpyridine thiosemicarbazones (Table 26) against rat Novicoff ascites tumor cells, 1-formylisoquinoline thiosemicarbazones (Table 27) against H.ep-2 cells, and 1-formylisoquinoline thiosemicarbazones (Tables 28 and 29) against rat Novicoff ascites tumor cells. In eq 161, RA stands for relative activity, defined as the ratio of I_{50} for the parent molecule to that for the derivative. All the equations were significant at the 99% confidence level. Since all the correlations were linear and the coefficients of V_w invariably negative, it was inferred, in the absence of a hydrophobic effect, that the steric effect was a dominant factor in the inhibition of RDR by thiosemicarbazones. In the derivation of eq 157, compound 9 was not included, as it was reported not to exist purely in the phenolic form at physiological pH but as a mixture of the zwitterionic and phenolic forms. Its inclusion gave a little less satisfactory correlation. In the derivation of eq 158, compound 32 was not included as it was misfit in the correlation. Equation 158 shows that the steric factor is effective not only from the 5-position but from the linear chain as well.

$$pI_{50} = 6.360 - 1.070\ (0.158)V_w$$

$$n = 23,\ r = 0.827,\ s = 0.391,\ F_{1,21} = 45.58 \qquad (157)$$

$$pI_{50} = 6.272 - 1.799\ (0.245)V_w$$

$$n = 8,\ r = 0.949,\ s = 0.174,\ F_{1,6} = 54.02 \qquad (158)$$

$$pI_{50} = 7.068 - 4.978\ (0.588)V_w$$

$$n = 9,\ r = 0.954,\ s = 0.212,\ F_{1,7} = 71.69 \qquad (159)$$

$$pI_{50} = 8.184 - 3.452\ (0.539)V_w$$

$$n = 12,\ r = 0.897,\ s = 0.439,\ F_{1,10} = 41.08 \qquad (160)$$

$$\log RA = 0.087 - 1.409\ (0.162)V_w$$

$$n = 15,\ r = 0.923,\ s = 0.320,\ F_{1,13} = 75.18 \qquad (161)$$

TABLE 27. RDR Inhibitory Activities of 1-Formylisoquinoline Thiosemicarbazones XVIIIb

compd	5-R	pI_{50}[a]	compd	5-R	pI_{50}[a]
34	H	6.77	39	NO_2	5.75
35	OH	6.64	40	CN	5.66
36	F	6.46	41	COOH	5.00
37	Cl	5.77	42	SO_3H	5.00
38	CF_3	5.40			

[a] See footnote of Table 25.

TABLE 28. RDR Inhibitory Activities of 1-Formylisoquinoline Thiosemicarbazones XVIIIb

compd	5-R	pK_{50}[a]	compd	5-R	pK_{50}[a]
34	H	7.40	48	$N(C_2H_5)COCH_3$	5.70
43	$NHCOCH_3$	6.96	49	$NHCH_3$	7.52
44	NH_2	7.52	50	NHC_2H_5	6.89
45	$N(CH_3)(C_2H_5)$	6.03	51	$N(CH_3)_2$	6.64
46	$N(C_2H_5)_2$	5.07	52	N-succinimido	5.30
47	$N(CH_3)COCH_3$	5.75	53	N-pyrrolidinyl	5.07

[a] Against rate Novikoff ascites tumor cells: Mooney, P. D.; Booth, B. A.; Moore, E. C.; Agrawal, K. C.; Sartorelli, A. C. *J. Med. Chem.* **1974**, *17*, 1145.

TABLE 29. RDR Inhibitory Activities of 1-Formylisoquinoline Thiosemicarbazones XVIIIb

compd	5-R	pI_{50}[a]
34	H	0.00
54	$NHSO_2CH_3$	0.59
55	$NHCOC_6H_4(m\text{-}SO_2F)$	1.62
56	$NHCOC_6H_4(p\text{-}SO_2F)$	2.15
57	OSO_2CH_3	1.03
58	$OCO_2C_2H_5$	0.61
59	OH	0.63
60	$OCOC_6H_4(m\text{-}SO_2F)$	1.00
44	NH_2	0.00
61	$OCO_2C_6H_5$	1.54
62	$OCOC_6H_4(p\text{-}SO_2F)$	1.93
63	$OSO_2C_6H_4(o\text{-}SO_2F)$	2.11
64	$OSO_2C_6H_4(m\text{-}SO_2F)$	2.18
65	$OSO_2C_6H_4(p\text{-}SO_2F)$	2.20
66	$N(CH_2CH_2Cl)_2$	1.62

[a] Against rat Novikoff ascites tumor cells: Agrawal, K. C.; Booth, B. A.; Moore, E. C.; Sartorelli, A. C. *J. Med. Chem.* **1972**, *15*, 1154.

TABLE 30. RDR Inhibitor Activities of Aryl Hydroxamic Acids

RC(O)NHOH

compd	R	pI_{50}
67	pyridyl-2	3.30
68	pyridyl-3	3.10
69	unsubstituted phenyl	3.40
70	2-hydroxyphenyl	3.82
71	2-aminophenyl	3.92
72	3-hydroxyphenyl	3.46
73	3-aminophenyl	3.46
74	4-hydroxyphenyl	3.60
75	4-aminophenyl	3.82
76	4-methylaminophenyl	3.48
77	4-dimethylaminophenyl	3.30
78	4-methoxyphenyl	3.30
79	2,3-dihdyroxyphenyl	5.10
80	2,4-dihydroxyphenyl	3.60
81	2,5-dihydroxyphenyl	3.70
82	2,6-dihydroxyphenyl	4.00
83	3,4-dihydroxyphenyl	4.52
84	3.5-dihydroxyphenyl	3.40
85	2-hydroxy-3-methylphenyl	3.82
86	2-hydroxy-4-aminophenyl	3.70
87	3,4-dimethylphenyl	3.52
88	3,4-diaminophenyl	4.40
89	3,4-dimethoxyphenyl	3.60
90	2,4-dichlorophenyl	3.35
91	3,4-dichlorophenyl	3.60
92	2,3,4-trihydroxyphenyl	5.46
93	3,4,5-trihydroxyphenyl	5.00
94	3,4,5-trimethoxyphenyl	4.00

These studies, however, do not tell what the nature of the interaction is and what portion of the molecules is involved in the interaction. The ring nitrogen may be expected to be involved in some electronic interaction with the enzyme, which is hindered either by the substituent at the 5-position or by the bulky linear chain in either series of thiosemicarbazone.

A group of aryl hydroxamic acids (Table 30) were also studied for their RDR inhibition activity.[200,201] The pI_{50} values obtained for them against rat Novicoff hepatoma were then correlated by van't Riet et al.[202] as shown in eq 162. This equation is, however, of predictive value. No interpretation of this can be made in mechanistic terms.

$$pI_{50} = 2.36\ (\pm 0.04)^3\chi_p - 3.98\ (\pm 0.53)^0\chi^v + 0.97\ (\pm 0.05)(^1\chi^v)^2 + 9.20\ (\pm 5.50)$$
$$n = 28,\ r = 0.943,\ s = 0.21,\ F = 64 \qquad (162)$$

6. Dihydrofolate Reductase

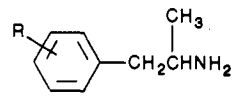
Dihydrofolate reductase (DHFR) or tetrahydrofolate dehydrogenase is an enzyme of central importance in biochemistry and medicinal chemistry. It catalyzes the reduction of dihydrofolate to tetrahydrofolate, a substance only one step short of the coenzyme for thymine synthesis. The inhibition of this enzyme provides a very important ground for designing anticancerous drugs. A tremendous amount of work has been done on the inhibitors of this enzyme. A recent review[203] on QSAR of DHFR inhibitors has extensively covered the literature; hence, repetition is avoided and readers are requested to refer to that paper. The essence of the work is that all steric, electronic, and hydrophobic parameters have been effective in DHFR inhibition, depending upon the source of the enzyme and type of inhibitors, but the hydrophobic parameter has been dominant.

B. Methyltransferases

1. Phenylethanolamine N-Methyltransferase

Phenylethanolamine *N*-methyltransferase (PNMT) is predominantly localized in the adrenal medulla, but recently it was also shown to be present in certain discrete areas of the brain stem.[204] This enzyme catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine (SAM) to norepinephrine with the formation of epinephrine.[205] Since PNMT acts at the final step in the biosynthesis of epinephrine, and since a high level of epinephrine may lead to increased blood pressure due to its vasoconstrictor effect on peripheral resistance vessels, selective inhibition of this enzyme may be of pharmacological and therapeutic importance. A selective inhibitor of PNMT can act as an antihypertensive agent by controlling epinephrine production without altering the norepinephrine level. Moreover, due to the importance of epinephrine in regulating cellular activity and in central regulatory roles,[204a,b] considerable attention has been paid to PNMT inhibitors.

A QSAR study was first made[206] on some amphetamines (XIX) that had shown PNMT inhibition activity. Equation 163 was obtained for a series of de-



XIX

$$pI_{50} = 0.455\pi + 2.18\sigma + 2.747 \qquad (163)$$
$$n = 17,\ r = 0.843$$

$$pI_{50} = 0.460\pi + 1.151\sigma + 2.732 \qquad (164)$$
$$n = 12,\ r = 0.894$$

rivatives that had substituent(s) at the 3- and/or 4-position(s), and eq 164 was obtained for a series of analogues that had substituents at the 2- and other positions. The substituents were mostly Cl, F, CH_3, OCH_3, *i*-Pr, or OC_6H_5. Compounds with an OH substituents were not included in the correlations. The activity of 4-OH compounds was poorly predicted by eq 163, however, that of the 3-OH derivatives was well predicted by it. These results led the authors[206] to suggest that 4-OH had a special affinity for the enzyme that could not be accounted for by hydrophobic or electronic properties but might be due to hydrogen bonding. Equations 163 and 164 were otherwise significant at the 99% confidence level and in each case showed hydrophobic and electronic effects on the activity. The π^2 and E_s terms were not found to affect the correlation. But when Hansch and Glave[207] considered all the compounds together, the steric effect from the 2-position had surfaced (eq 165). Equation

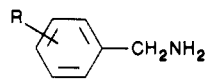
$$pI_{50} = 0.485\ (\pm 0.23)E_s\text{-}2 + 0.991\ (\pm 0.36)\pi_{2,3} + 1.408\ (\pm 0.37)\Sigma\sigma - 1.009\ (\pm 0.33)D + 2.550\ (\pm 0.28)$$
$$n = 32,\ r = 0.940,\ s = 0.288 \qquad (165)$$

165, where all OH derivatives except the one with 3,4-$(OH)_2$ were also included, shows a better correlation than eq 163 or 164. This equation, however, shows the hydrophobic effect only for the 2- and 3-positions, and the negative coefficient of the dummy parameter *D* defined for the presence of the 3-OCH_3 group indicates

that this group will reduce the activity. The reason for this anomalous behavior was not explained by the authors.

Substituents at the 4-position appeared to affect the activity by only their electronic character, i.e., electron-withdrawing nature.

For the inhibition of PNMT by monosubstituted benzylamines (XX), Fuller et al.[208] obtained eq 166–168.



XX

Ortho Derivatives (R = H, I, Br, Cl, F)

$$pI_{50} = 1.69\ (0.099)\pi + 2.986\ (0.432)\sigma + 3.115\ (0.073)$$
$$n = 5,\ r = 0.998,\ s = 0.07 \qquad (166)$$

Para Derivatives (R = H, CF_3, I, Br, Cl, F, CH_3)

$$pI_{50} = 0.670\ (0.262)\pi + 2.058\ (0.564)\sigma + 3.161\ (0.120)$$
$$n = 7,\ r = 0.984,\ s = 0.19 \qquad (167)$$

Meta Derivatives (R = H, CF_3, I, Br, Cl, F, CH_3O)

$$pI_{50} = 2.131\ (0.582)\pi - 0.597\ (0.478)\pi^2 + 1.971\ (0.413)\sigma + 3.054\ (0.144)$$
$$n = 7,\ r = 0.992,\ s = 0.16 \qquad (168)$$

All these equations represent highly significant correlations. Though the number of data points in each is very small, the high correlation coefficients establish the importance of the hydrophobic and electronic characters of the substituents. Unlike amphetamines (eq 165), in benzylamines the 4-substituent is also found to affect the activity hydrophobically in the same way as the 2- or 3-substituent. In eq 168, the π^2 term appears to be a chance occurrence and, moreover, with a comparatively large confidence interval it is not very significant.

However, when all ortho, meta, and para and some disubstituted derivatives were treated together, a comparatively poorer correlation was obtained.

The activity of a small group of *N*-alkylbenzylamines was shown to be affected by the acidity of the molecule and the steric hindrance of the substituent (eq 169). The correlation expressed by eq 169 was significant at the 95% level.

$$pI_{50} = 3.696\ (0.715) - 0.154\ (0.084)pK_a + 0.400\ (0.101)E_s$$
$$n = 8,\ r = 0.883,\ s = 0.23 \qquad (169)$$

To further study the electronic effect on PNMT inhibition activity of benzylamines, the entire series studied by Fuller et al.[208] was subjected to quantum chemical study.[209] Excluding iodo and bromo derivatives, Lokovits[209] related the activity of the rest with quantum mechanical parameters (eq 170) where X^1_1

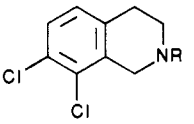
$$pI_{50} = 4.42 - 0.42\ (\pm 0.14)X^1_1 - 0.20\ (\pm 0.19)X^1_2$$
$$n = 22,\ r = 0.840,\ s = 0.57,\ F = 22.66 \qquad (170)$$

and X^1_2 are principal components obtained by principal quantum analysis. These components are related to various quantum chemical indices, hence, the mechanistic interpretation of eq 170 is complex. The conclusion of Lokovits that the inhibitory potency of benzylamines will involve charge-transfer phenomena in addition to electrostatic forces ought to be reaffirmed by further study.

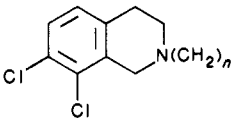
Nonetheless, from all these QSAR studies, it appears that all hydrophobic, electronic, and steric factors are important in PNMT inhibition. That the hydrophobic interactions are of primary importance for PNMT inhibition was also shown[210,211] qualitatively in the case of some nonaromatic analogues of phenylethanolamines. For a small group of phenylethanolamines, substrate activity was also found[212] to be related to hydrophobicity.

Additionally, the importance of molecular width and length and ethanolamine side-chain orientation with respect to the plane of the ring was also pointed out.[210,211]

For a new class of PNMT inhibitors,[213] 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline derivatives XXI, where



XXI



XXII

R was a group like XXII or an alkyl, phenyl, benzyl, or amino group, the activity was correlated[214] with V_w as shown by eq 171 where I is an indicator parameter

$$pI_{50} = 1.970V_w - 4.121I - 6.859$$
$$n = 14,\ r = 0.888,\ s = 0.585,\ F_{2,11} = 20.54 \qquad (171)$$

indicating the presence of a former type of group. Equation 171 is significant at the 99% confidence level and, in the context of the much discussed hydrophobic effect, shows only the hydrophobic effect, as the latter has been shown to be a function of V_w also.[22] However, the equation also shows the steric hindrance produced by a bigger group such as XXII.

2. Catechol and Hydroxyindole O-Methyltransferases

Catechol *O*-methyltransferase (COMT) plays an important role in extraneuronal inactivation of catecholamines and the detoxification of various xenobiotic catechols. It catalyzes the transfer of a methyl group from SAM (*S*-adenosyl-L-methionine) to catechol acceptors. The inhibition of this route of catecholamine metabolism has been the subject of considerable research interest. Studies on COMT inhibitors have been found to be useful in ascertaining the relative importance of COMT in the metabolism of norepinephrine[215] as well as in the elucidation of the mechanism of methyl transfer.[216] However, very limited QSAR studies are available on COMT inhibition.

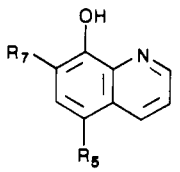
A QSAR study was attempted by Katz and Jacobson[217] on the data of Creveling et al.[218–220] for in vitro O-methylation of some substituted catechols by COMT. No significant correlation was obtained for K_m or V_{max}, but the meta to para ratio (m/p) of O-methylated products was found to have a satisfactory correlation with π and the steric parameter E_s as shown by eq 172

$$\log A = 2.00\ (\pm 0.12) + 0.18\ (\pm 0.12)\pi + 0.26\ (\pm 0.22)E_s + 0.49\ (\pm 0.31)E_s^2$$
$$n = 19,\ r = 0.82,\ s = 0.19 \qquad (172)$$

where A = (m/p) divided by the molecular weight of

the substrate. From this equation one finds that while an increase in the π value of the substituent will increase the meta product, the steric factor will optimize it. Meta methylation has been assumed to be the major pathway in vivo.[205c] However, certain recent studies have demonstrated the predominance of para methylation in vitro reactions.[221]

In a study on COMT inhibition by 5- and 7-substituted 8-hydroxyquinolines XXIII, Borchardt et al.[222] attempted to correlate the inhibition activity with electronic and steric parameters but failed to find any meaningful correlation. Equation 173, which they

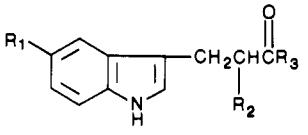


XXIII

$$pI_{50} = 4.61\ (\pm 0.12) + 0.44\ (\pm 0.14)\pi_7$$
$$n = 22,\ r = 0.82,\ s = 0.27,\ F_{1,20} = 42.38 \quad (173)$$

obtained relates activity with only the hydrophobic character of 7-substituents. But since there was a very small number of 7-substituted analogues in the entire series, this equation should be interpreted with the utmost care.

A very limited QSAR study was available on the inhibition of hydroxylindole *O*-methyltransferase (HOMT). HOMT forms 5-methoxytryptamine from 5-hydroxytryptamine and corresponding substances from other 5-hydroxyindoles with SAM as the methyl donor. The inhibition of this enzyme by a series of *N*-acyltryptamines XXIV[223] was found[98] to be correlated with the hydrophobicity of the molecules (eq 174).



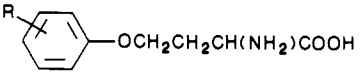
XXIV

$$pI_{50} = 0.561 \log P + 1.590 \quad (174)$$
$$n = 24,\ r = 0.870,\ s = 0.255$$

3. Methionine Adenosyltransferase

SAM, the active donor of methyl groups in various metabolic reactions such as those mentioned in the preceding sections, is actually synthesized by the enzyme methionine adenosyltransferase. Methionine adenosyltransferase (MAT) forms SAM by transferring the adenosyl group of ATP to methionine. MAT belongs to a heterogeneous group of enzymes called alkyltransferases. This group of enzymes transfers substituted or unsubstituted alkyl groups other than methyl groups.

Studies on MAT inhibition are also very scarce. A series of substituted *O*-phenyl-DL-homoserines (XXV;



XXV

X = H, *p*-F, *p*-Cl, *p*-Br, *p*-NO_2, *p*-OCH_3, *p*-CH_3, *m*-Cl, *m*-Br, *m*-NO_2, *m*-OCH_3) were found to inhibit MAT by Coulter et al.,[224] and the inhibition activity observed by these authors was shown by Hulbert[225] to be well correlated with the hydrophobic and electronic parameters of the substituents (eq 175). π and σ individually were

$$pI_{50} = 0.619\ (\pm 0.108)\pi + 0.614\ (\pm 0.128)\sigma + 1.62\ (\pm 0.06)$$
$$n = 11,\ r = 0.989,\ s = 0.056 \quad (175)$$

also found to be well correlated with the activity[224,225] (eq 176 and 177). Thus, all the equations (eq 175–177)

$$pI_{50} = 0.72\pi + 1.71 \qquad n = 11,\ r = 0.81 \quad (176)$$
$$pI_{50} = 0.76\sigma + 1.84 \qquad n = 11,\ r = 0.72 \quad (177)$$

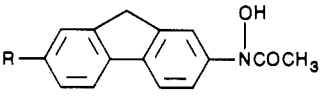
show that the hydrophobic character and the electron-withdrawing nature of the substituents will significantly affect the activity. However, on the basis of this single study, the actual mode of interaction of MAT inhibitors with the enzyme cannot be predicted.

C. Acyltransferases

1. N-Arylhydroxamic Acid N,O-Acyltransferase

N-Arylhydroxamic acid *N*,*O*-acyltransferase (AHAT) is a cytosolic enzyme present in the tissues of numerous mammalian species. It catalyzes the conversion of certain *N*-arylhydroxamic acids to *N*-acetoxyarylamines. In the absence of an arylamine acceptor, AHAT catalyzes the conversion of *N*-arylhydroxamic acids to reactive electrophilic intermediates that become irreversibly bound to cellular nucleophiles.[226,227]

A series of 7-substituted *N*-hydroxy-2-acetamidoflurenes XXVI were recently found to act as AHAT inhibitors,[228] and a QSAR study on them related their first-order inactivation rate constant (k_i) and the dissociation constant (K_D) to MR and σ[228] (eq 178 and 179). From eq 178 it appears that the electron-do-



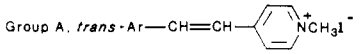
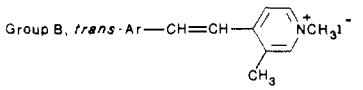
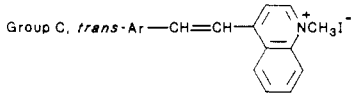
XXVI

$$\log k_i = 0.463\ (\pm 0.438) - 0.638\ (\pm 0.388)\mathrm{MR} - 1.537\ (\pm 0.739)\sigma$$
$$n = 10,\ r = 0.90,\ s = 0.29 \quad (178)$$
$$pK_D = 1.854\ (\pm 0.849)\mathrm{MR} - 2.464\ (\pm 0.746)$$
$$n = 9,\ r = 0.89,\ s = 0.47 \quad (179)$$

nating group, which after donation will become positively charged, will lead to a high inactivation rate but that the bigger substituents would reduce the rate. On the other hand, eq 179 shows that the binding of drugs to the enzyme would be strengthened by the larger substituents. Equation 179 had several anomalies, however; hence it is not very reliable. Substituents for the series studied were H, F, Cl, Br, I, CN, CH_3CO, CH_3O, CH_3CH_2O, and $CH_3(CH_2)_3O$. The last substituent was not included in eq 179. The equation predicts a very high pK_D value for this compound. A very low value is predicted for the acetyl derivative.

Thus, in the absence of detailed QSAR studies, it is difficult to draw any sound conclusion, but certain experiments with *N*-arylhydroxamic acids have pointed out that inactivation of AHAT involves a contribution by electrophilic species that diffuse away from the ac-

TABLE 31. Styrylpyridine Analogues and Their ChA Inhibitory Potencies

compd	Ar	pI_{50}	compd	Ar	pI_{50}
	Group A, *trans*-Ar—CH=CH— 				
1	1-naphthyl	6.33	7	C_6H_5	4.83
2	3-BrC_6H_4	5.83	8	4-$CH_3C_6H_4$	4.70
3	3-ClC_6H_4	5.64	9	4-FC_6H_4	4.68
4	4-BrC_6H_4	5.59	10	2-ClC_6H_4	4.56
5	3,4-$Cl_2C_6H_3$	5.48	11	3-$NO_2C_6H_4$	3.83
6	4-ClC_6H_4	5.16	12	4-$NO_2C_6H_4$	3.70
	Group B, *trans*-Ar—CH=CH— 				
13	1-naphthyl	6.40	16	C_6H_5	5.30
14	3-ClC_6H_4	5.80	17	2-ClC_6H_4	4.77
15	3,4-$Cl_2C_6H_3$	5.77	18	3-indolyl	4.41
	Group C, *trans*-Ar—CH=CH— 				
19	C_6H_5	5.46	21	3-pyridyl	3.22
20	2-thienyl	3.60			

tive site prior to becoming covalently bound to the enzyme.[229,230]

2. Choline Acetyltransferase

The neurotransmitter acetylcholine is synthesized from choline and acetyl coenzyme A with the help of the enzyme choline acetyltransferase (choline acetylase, ChA). The study of the inhibition of this enzyme would be of neuropharmacological importance. Inhibitors of ChA may act as muscle relaxants and thus produce anaesthetic effects.

A series of styrylpyridine analogues (Table 31) were studied for their ChA inhibitory potency,[231,232] and a QSAR study[233] on them correlated their potency with some HMO parameters (eq 180) and also with physi-

$$pI_{50} = 0.377\sum S^{E}_{Ar} - 2.309\sum|Q_{Ar}| + 3.864E_{LUMO} + 4.232$$
$$n = 19,\ r = 0.925,\ F = 29.85 \qquad (180)$$

$$pI_{50} = 1.725\pi - 0.807\sigma + 4.297$$
$$n = 11,\ r = 0.905,\ F = 18.02 \qquad (181)$$

cochemical parameters (eq 181). In eq 180, $\sum S^{E}_{Ar}$ is the sum of electrophilic superdelocalizabilities and $\sum|Q_{Ar}|$ the sum of the absolute charges of the aryl portion of the molecules. E_{LUMO} is the energy of the lowest unoccupied molecular orbital. While eq 180 is meant for all the compounds, eq 181 is derived only for group A, as π and σ were available for this group only. The 2-ClC_6H_4 derivative(s) was not included in either equation since inclusion led to a lower correlation in either case. Cavallito et al.[232] assumed that a 2-Cl substituent on the aryl portion of styrylpyridine-like compounds may detract from activity by decreasing coplanarity.

Since for a variety of aromatic systems the partition coefficient was shown[234] to be related to $\sum S^{E}$ and $\sum Q$, and since S^{E} represents the electron-donating capability, Allen et al.[233] suggested, on the basis of eq 180, that both charge-transfer and hydrophobic interactions may be occurring between the aryl moiety and ChA. The hydrophobic effect and the effect of electron donation by the substituents are shown to be important by eq 181 also.

Because of the involvement of E_{LUMO} in eq 180, Allen et al. also suggested that there would be an electron-acceptor interaction through a pyridinium-like moiety. However, the role of E_{LUMO} does not appear to be consistent, as with π it enters the equation with a negative sign (eq 182), opposite to that seen in eq 180.

$$pI_{50} = 1.216\pi - 1.657E_{LUMO}$$
$$n = 11,\ r = 0.948,\ F = 35.43 \qquad (182)$$

3. Lysophosphatidylcholine Acyltransferase

The plasma membrane bound enzyme lysophosphatidylcholine acyltransferase (lysolecithin acyltransferase) is thought to be responsible for regulating the proportion of saturated fatty acids present in phosphatidylcholines in the plasma membranes and to play an important role in the maintenance of membrane structure and integrity.[235] Some psychoactive cannabinoids have been found to inhibit this enzyme in mouse brain synaptosomes, and their K_i values (concentration at half-maximal inhibition) were related to the molar volume V_x as shown in eq 183. E_B is an

$$K_i = (2 \times 10^{-4}) + 10^{-(36000V_x - E_B)} \qquad (183)$$

interaction term giving the extent of interaction of the compound with some component of the aqueous or nonaqueous phase; however, the total term ($36000V_x - E_B$) defines the lipophilicity of the compound.[236] Thus, eq 183 shows that the enzyme inhibition activity of cannabinoids would be proportional to their lipophilicity.

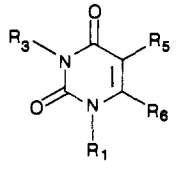
The inhibition of a membrane-bound enzyme responsible for maintenance of membrane integrity may have implications in neurotransmitter synthesis and release or in membrane excitation, but this scant study does not provide much ground for understanding the mechanism by which cannabinoids exhibit their psychoactivity.

D. Pentosyltransferases

Uridine and Thymidine Phosphorylases

Uridine phosphorylase (UP) and thymidine phosphorylase (TP) are important to RNA and DNA synthesis. While uridine phosphorylase catalyzes the phosphorolysis of uridine to uracil and α-D-ribose 1-phosphate, thymidine phosphorylase catalyzes the phosphorolysis of thymidine to thymine and 2-deoxy-D-ribose 1-phosphate. Thus, the inhibition of both UP and TP would be of importance to cancer chemotherapy.

Different series of uracil derivatives (XXVII) were found to inhibit UP and TP. Yoshimoto and Hansch[192] obtained eq 184 and 185, respectively, for the inhibition of uridine phosphorylase of Walker 256 tumor and of thymidine phosphorylase of *Escherichia coli*. In eq



XXVII

$$pI_{50} = 0.413\ (\pm0.08)(\pi\text{-L}) + 1.737\ (\pm0.29)(\text{I-1}) + 0.560\ (\pm0.25)(\text{I-2}) + 0.347\ (\pm0.25)(\text{I-3}) + 0.565\ (\pm0.26)(\text{I-4}) - 0.955\ (\pm0.27)$$

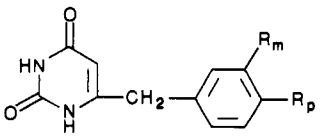
$$n = 89,\ r = 0.912,\ s = 0.392 \qquad (184)$$

$$\log (S_{50}/I_{50}) = 1.177\ (\pm0.23)(\text{I-3}) + 1.814\ (\pm0.32) \times (\text{I-1}) + 1.127\ (\pm0.35)\pi_o + 0.536\ (\pm0.22)(\text{I-2}) + 0.807\ (\pm0.29)\pi_m + 0.269\ (\pm0.12)(\pi\text{-1,3}) - 0.827\ (\pm0.37)(\text{I-4}) + 0.163\ (\pm0.08)(\pi\text{-6}) - 1.798\ (\pm0.29)$$

$$n = 136,\ r = 0.948,\ s = 0.431 \qquad (185)$$

184, π-L is the hydrophobic constant of the larger substituent in the 1- or 5-position, and I-1, I-2, I-3, and I-4 each takes the value of 1 for 5-$CH_2C_6H_5$/5-SC_6H_5, 1-$CH_2C_6H_5$, 1-H, and 6-$NHCH_2R$, respectively. Thus, eq 184 shows that the larger group at the 1- or 5-position will be involved in hydrophobic interaction. The fact, as suggested by indicator parameters, that groups such as $CH_2C_6H_5$ or SC_6H_5 at these positions make a positive contribution to the activity indicates that these groups might be involved in dispersion interaction. The positive contribution of 1-H may be due to hydrogen bonding and that of 6-$NHCH_2R$ may be again due to dispersion interaction, as the use of any electronic parameter was not found to be successful.

In eq 185, I-3 refers to 6-X-C_6H_5 groups, where X is NH, $NHCH_2$, O, S, or SO_2. For X = CH_2, I-2 has been given a value of unity. I-1 takes the value of 1 for 1- and 3-H. I-4 takes the value of 1 for the bridges 5-N–N– and 5-SO_2NH–. Groups with these bridges were few, and hence I-4 was not very important. Similarly, π-6 had little contribution. If these two parameters were excluded, there was a very insignificant decrease in the value of r (0.935), and only a little variation was shown when (π-1,3) was also removed (r = 0.925). The π_o and π_m terms are π for ortho and meta substituents (only on one side), respectively, in the phenyl group of the 6-XC_6H_5 substituent. Thus, the presence of a 6-XC_6H_5 group with hydrophobic substituents at the ortho and meta positions appears to be very important. These ortho and meta substituents may be involved in hydrophobic interactions. The effect of hydrogen at the 1- and 3-positions appears to be due to hydrogen bonding. Yoshimoto and Hansch, however, could not easily explain how 6-XC_6H_5 would be a better group with X = CH_2. It seems that the CH_2 allows the phenyl ring to acquire the desired conformation, while other bridges do not. This gets support from another QSAR study by the Hansch group[237] on a small series of XXVIII, where only the steric effects of R_m and R_p were found to control the activity (eq 186) by bringing the group into proper orientation.



XXVIII

$$pI_{50} = 1.72\ (\pm0.28) - 0.585\ (\pm0.19)(E_{s\text{-}m}) - 0.349\ (\pm0.27)(E_{s\text{-}p})$$

$$n = 7,\ r = 0.973,\ s = 0.193 \qquad (186)$$

But for a series of 6-substituted anilinouracils (CH_2 replaced by NH in XXVIII), Coats et al.[237] showed that

TABLE 32. Thymidine Phosphorylase Inhibition Activities of Substituted Uracils XXVII

compd	R	pI_{50}	compd	R	pI_{50}
		6-Substituted Uracils			
1	OC_6H_5	1.23	7	CF_3	0.63
2	CH_3SO_2	0.28	8	n-C_3H_7	−0.07
3	$(CH_2)_2C_6H_5$	0.22	9	C_6H_5	0.14
4	NH_2	0.17	10	H	0.00
5	$(CH_2)_3C_6H_5$	−0.04	11	CH_3	−0.05
6	n-C_5H_{11}	−0.07			
		5-Substituted Uracils			
12	NO_2	0.66	16	C_6H_5	−0.30
13	Br	0.35	17	$COCH_3$	−0.52
14	F	−0.11	18	H	−0.59
15	CH_3	−0.28	19	NH_2	−0.85
		1-Substituted Uracils			
20	$(CH_2)_5C_6H_5$	−0.32	26	i-C_6H_{13}	−1.17
21	$(CH_2)_4C_6H_5$	−0.60	27	c-C_5H_9	−1.28
22	$CH_2C_6H_5$	−0.76	28	i-C_5H_{11}	−1.30
23	$(CH_2)_2C_6H_5$	−0.80	29	n-C_4H_9	−1.35
24	$(CH_2)_3C_6H_5$	−1.11	30	CH_3	−2.30
25	n-C_5H_{11}	−1.15			

all ortho, meta, and para substituents would be involved in the hydrophobic interaction (eq 187) if the phenyl

$$pI_{50} = 1.234\ (\pm0.32)\pi_o + 0.734\ (\pm0.23)\pi_m + 0.435\ (\pm0.20)\pi_p + 1.144\ (\pm0.22)$$

$$n = 19,\ r = 0.929,\ s = 0.229 \qquad (187)$$

group is in proper orientation, which is probably controlled by the NH bridge. However, it was also shown that if the 6-position of uracil has substituents of the type as shown in Table 32, it would be the electronic character of these substituents that would be important (eq 188). The large positive coefficient of the field

$$pI_{50} = 0.018\ (\pm0.01)P_E - 0.930\ (\pm0.63)\mathcal{R} + 1.078\ (\pm0.51)\mathcal{F} - 0.72\ (\pm0.34)$$

$$n = 11,\ r = 0.928,\ s = 0.259 \qquad (188)$$

constant $\mathcal{F}$ suggests that electron withdrawal from N_1 increases the binding, and the large negative coefficient of the resonance constant $\mathcal{R}$ shows that electron donation by the group to the acceptor on the enzyme would be important. With all these, polarizability of the group also appears to affect the activity. Coats et al. have suggested that ionization of N_1 is essential for the activity.

The 5-substituents of uracil are also shown to affect the activity by the electron withdrawal from N_1 (eq 189). Equation 189 shows that the more ionized N_1 is,

$$pI_{50} = 2.178\ (\pm1.31) - 0.273\ (\pm0.15)pK_a$$

$$n = 8,\ r = 0.879,\ s = 0.257 \qquad (189)$$

$$pI_{50} = 0.535\ (\pm0.12)\pi - 2.495\ (\pm0.33)$$

$$n = 11,\ r = 0.959,\ s = 0.153 \qquad (190)$$

the better is the activity. Though the bigger groups at N_1 (Table 32) will mask the ionization, Coats et al. found that they compensate for the activity thus lost by their hydrophobic character (eq 190). However, the polarizability term in this case was also found to afford a good correlation (eq 191); hence, it is difficult to say

$$pI_{50} = 0.038\ (\pm0.01)P_E - 2.261\ (\pm0.40)$$

$$n = 11,\ r = 0.921,\ s = 0.212 \qquad (191)$$

what is the actual nature of the interaction of the 1-

substituent. A little better correlation with π was due to taking into consideration the possible interaction between the two π systems, the phenyl group of the substituent and the uracil ring, for the calculation of π.

In total, all these studies present a very complex mechanism of thymidine phosphorylase inhibition and also of uridine phosphorylase inhibition, for which only one QSAR study was attempted. Many of the compounds studied were misfit in the correlations, and hence excluded.

To show the importance of their molecular connectivity index, Kier et al. correlated the thymidine phosphorylase inhibition activity of 1-substituted uracils, as mentioned in Table 32, with χ[155] (eq 192). This also, in fact, shows that lengthening the substituent chain will increase the activity.

$$pI_{50} = 0.373\ (\pm 0.051)\chi - 3.415\ (\pm 0.325)$$
$$n = 11,\ r = 0.924,\ s = 0.207 \qquad (192)$$

E. Nucleotidyltransferases

DNA Polymerase

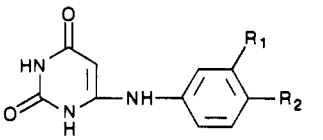
DNA polymerase is important in DNA replication. Replication can begin at any point in the double helix. The enzyme deoxyribonuclease splits open one strand by catalyzing the hydrolysis of a 3′-phosphate ester bond so that a deoxyribose with a free 3′-OH group is exposed. DNA polymerase then catalyzes the addition of nucleotides to the 3′-OH end, pairing appropriate nucleotides against the unbroken parent strand, which acts as a template. The selective inhibitors of this enzyme may be useful as anticancerous and antibacterial drugs.

A QSAR study was made[238] on a series of 6-anilinouracils (Table 33) for their inhibitory activity against the wild-type DNA polymerase III (pol III) and a mutant enzyme, pol III/azp-12, derived from *Bacillus subtilis*. For the two enzymes, Wright and Gambino[238] obtained eq 193 and 194, respectively, where K_i represents nothing but the concentration of the inhibitor required to achieve 50% inhibition of the enzyme activity. These equations simply show that the inhibition of either of the enzymes would involve hydrophobic interaction with both the meta and para substituents of the inhibitors, but that the interaction would be controlled by the relatively bigger size of the substituents. However, as is obvious from the coefficients of the variables, the extent of the interaction of substituents from the two different positions and the extent of the tolerance for the size of the substituents by the hydrophobic pocket of the enzymes are little different in the two enzymes.

$$pK_i = 1.00\ (0.13)\pi_m + 0.71\ (0.12)\pi_p + 3.87\ (0.48)MR_m - 2.30\ (0.27)(MR_m)^2 + 2.86\ (0.54)MR_p - 2.18\ (0.26)(MR_p)^2 + 2.50\ (0.30)$$
$$n = 37,\ r = 0.942,\ F_{6,30} = 40.01 \qquad (193)$$

$$pK_i = 1.26\ (0.13)\pi_m + 1.13\ (0.12)\pi_p + 2.51\ (0.46)MR_m - 1.67\ (0.26)(MR_m)^2 + 3.48\ (0.52)MR_p - 2.42\ (0.25)(MR_p)^2 + 2.39\ (0.29)$$
$$n = 37,\ r = 0.948,\ F_{6,30} = 45.35 \qquad (194)$$

TABLE 33. Inhibition Constants of 6-Anilinouracils against DNA Polymerase III and pol III/azp-12



compd	R_1	R_2	pK_i[a]	pK_i[b]
1	Et	Me	6.72	5.85
2	CH_2Cl	Me	6.59	6.82
3	I	Me	6.42	6.55
4	$-CH_2CH_2CH_2-$		6.38	5.71
5	Cl	Me	6.20	6.08
6	Me	Me	6.04	5.46
7	Cl	Cl	6.03	6.48
8	Me	Br	6.00	6.07
9	Et	H	5.54	4.73
10	I	H	5.33	4.69
11	CH_2Cl	H	5.12	5.26
12	Br	H	4.99	4.66
13	*n*-Pr	H	4.77	4.85
14	H	Me	4.76	4.16
15	Me	NH_2	4.72	3.80
16	Me	Et	4.72	5.38
17	H	Cl	4.60	4.85
18	Cl	H	4.60	4.38
19	H	Br	4.50	5.40
20	Me	H	4.37	3.75
21	H	Et	4.18	4.70
22	CH_2OH	Me	4.11	3.70
23	Me	*n*-Pr	4.01	4.86
24	H	CF_3	4.00	4.95
25	H	OMe	3.92	3.92
26	NH_2	Me	3.91	3.53
27	H	*i*-Pr	3.47	4.66
28	CH_2OH	H	3.38	2.29
29	H	*n*-Pr	3.35	4.28
30	Me	NHCOMe	3.33	2.81
31	NHCOMe	Me	3.33	2.73
32	H	NH_2	3.19	2.41
33	Me	*n*-Bu	3.16	3.88
34	*n*-Bu	H	3.16	3.33
35	H	OH	2.75	2.42
36	H	CH_2OH	2.50	2.71
37	H	*n*-Bu	2.31	2.66

[a] For pol III. [b] For pol III/azp-12.

The inhibition of an RNA-instructed DNA polymerase by some rifamycin derivatives was also shown[239] qualitatively to be a function of the lipophilicity as measured by a reversed-phase thin-layer chromatographic technique.

F. Esterases

1. Cholinesterases

Cholinesterases catalyze the conversion of acylcholines to choline and, thus, are very important in the transmission of nerve impulses. When a nerve impulse arrives at a synapse, it releases an acylcholine (a neurotransmitter), which diffuses across the gap and, on reaching the far side, interacts with receptors on the postsynaptic membranes of a muscle or nerve cell. As soon as the neurotransmitter has acted, it should be removed from the synaptic cleft so that the synapse is restored to its resting state ready for another impulse. This is done by a cholinesterase by hydrolyzing the neurotransmitter. Inhibitors of cholinesterases thus lead to stoppage of the transmission of nerve impulses, resulting in death.

Cholinesterase is a general term that refers to two types of the enzyme, true (or specific) cholinesterase

and pseudo- (or nonspecific) cholinesterase. The main true cholinesterase is acetylcholinesterase (EC 3.1.1.7, AChE), which primarily hydrolyzes acetylcholine, and the main pseudocholinesterase is butyrylcholinesterase (EC 3.1.1.8, BuChE), which is primarily active against butyrylcholine. Both cholinesterases are present in mammals.

Extensive QSAR studies have been made on inhibitors of these enzymes in order to study the mechanism of inhibition and to design chemical defense agents (nerve gases) and agricultural insecticides. Organic phosphates, phosphonates, phosphoramidates, and carbamates constitute the important class of ChE inhibitors.[240,241] It is generally believed that these inhibitors exert their inhibitory effect upon the enzyme through enzymic hydrolysis[240,241] as shown for the carbamates, as an example, by eq 195, where the first step

$$R_1R_2NC(O)OR + EH \rightleftharpoons R_1R_2C(O)OR{\cdot}EH \rightarrow R_1R_2NC(O)E + ROH \quad (195)$$

involves the attack of the enzyme (EH) on a particular atom or bond of the inhibitor and the second step is the rate-determining step. Pullman and Pullman[242] proposed that practically all the fundamental types of biochemical substrates undergoing enzymic hydrolysis have the common feature of undergoing this reaction on a π-electron-deficient bond. Thus, while electronic character is indicated to be the dominant factor controlling the activity of ChE inhibitors, the hydrophobic and steric factors have also been found to affect the inhibition.

For a series of methylcarbamates (Table 34) studied by Metcalf and Fukuto[243] for the inhibition of flybrain cholinesterase, Hansch and Deutsch[244] obtained eq 196 for para-substituted and eq 197 for meta-substituted derivatives to show the effect of hydrophobicity. The

$$pI_{50} = 0.742\pi + 3.525$$
$$n = 23,\ r = 0.768,\ s = 0.458 \quad (196)$$
$$pI_{50} = 0.876\pi + 4.347$$
$$n = 30,\ r = 0.773,\ s = 0.592 \quad (197)$$

electronic parameter σ was found to be less significant than π, but inclusion of the former in the correlation resulted in a significant improvement over eq 196 and 197 (eq 198 and 199, respectively). In a later study,

$$pI_{50} = 0.714\pi - 0.868\sigma + 3.486$$
$$n = 23,\ r = 0.839,\ s = 0.399 \quad (198)$$
$$pI_{50} = 0.784\pi - 1.405\sigma + 4.618$$
$$n = 30,\ r = 0.845,\ s = 0.508 \quad (199)$$

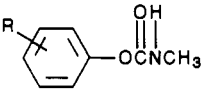
Hansch[245] combined meta and para isomers and obtained eq 200, where I was introduced as an indicator

$$pI_{50} = 0.69\pi - 0.95\sigma + 1.19I + 3.50$$
$$n = 53,\ r = 0.913,\ s = 0.415 \quad (200)$$

parameter and given a value of unity for meta isomers and of zero for para isomers. This equation shows that while hydrophobic and electron-donating groups will affect the activity, the effect would be more from the meta position than from the para position.

For seven 2-substituted analogues, the best correlation obtained[244] was as shown by eq 201 in which the steric parameter was also shown to affect the activity;

TABLE 34. Substituted Phenyl *N*-Methylcarbamates and Their ChE Inhibitory Activities



compd	R	pI_{50}	compd	R	pI_{50}
1	H	3.70[a]	31	3-C_2H_5	5.32
2	4-F	3.64	32	3-i-C_3H_7	6.47
3	4-Cl	3.62	33	3-s-C_4H_9	6.80
4	4-Br	4.00	34	3-c-C_5H_{11}	5.82
5	4-I	4.06	35	3-c-C_6H_{13}	5.70
6	4-CH_3	4.00	36	3-OCH_3	4.66
7	4-C_2H_5	4.42	37	3-OC_2H_5	5.22
8	4-i-C_3H_7	4.16	38	3-OC_3H_7	4.80
9	4-s-C_4H_9	5.74	39	3-i-OC_3H_7	5.04
10	4-c-C_5H_{11}	4.57	40	3-OC_4H_9	5.03
11	4-c-C_6H_{13}	5.05	41	3-s-OC_4H_9	5.15
12	4-OCH_3	4.10	42	3-$N(CH_3)_2$	5.10
13	4-OC_2H_5	4.16	43	3-SCH_3	5.15
14	4-OC_3H_7	3.96	44	3-SC_3H_7	5.96
15	4-i-OC_3H_7	4.06	45	3-i-SC_3H_7	5.74
16	4-OC_4H_9	4.70	46	3-SC_4H_9	6.11
17	4-s-OC_4H_9	4.49	47	3-SF_5	4.85
18	4-SCH_3	4.47	48	3,5-(i-C_3H_7)$_2$	7.48
19	4-SC_3H_7	4.92	49	3-CH_3-5-i-C_3H_7	7.25
20	4-i-SC_3H_7	5.04	50	3,5-(t-C_4H_9)$_2$	7.11
21	4-SC_4H_9	5.52	51	3,5-$(OCH_3)_2$	5.10
22	4-NO_2	2.52	52	3,5-Cl_2	4.92
23	4-$N(CH_3)_2$	3.62	53	3,5-$(CH_3)_2$	5.22
24	4-$Si(CH_3)_3$	4.81[b]	54	2-F	4.80
25	3-F	4.11	55	2-Cl	5.30
26	3-Cl	4.30	56	2-Br	5.66
27	3-Br	4.89	57	2-I	6.10
28	3-I	5.16	58	2-CH_3	3.89
29	3-t-C_4H_9	6.40	59	2-OCH_3	4.43
30	3-CH_3	4.85	60	2-NO_2	2.30

[a] Used for both para and meta. [b] Not used in eq 196–199. Used in eq 200.

but with such a small number of data points for three variables, not much confidence can be attached to the significance of this correlation.

$$pI_{50} = 3.845E_s + 2.799\pi + 4.246\sigma + 2.542$$
$$n = 7,\ r = 0.962,\ s = 0.494 \quad (201)$$

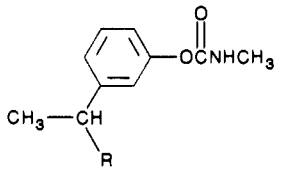
For a different series of meta- and para-substituted phenyl *N*-methylcarbamates, Jones et al.[246] obtained eq 202 (meta isomers) and 203 (para isomers). Verloop[247]

$$pI_{50} = 1.031\pi - 1.015\mathcal{T} + 4.394$$
$$n = 15,\ r = 0.855 \quad (202)$$
$$pI_{50} = 0.457\pi - 0.853\mathcal{T} - 1.180\mathcal{R} + 3.784$$
$$n = 13,\ r = 0.91 \quad (203)$$

made an improvement over eq 202 by adding $\mathcal{R}$ and E_s (eq 204). Again, while steric and electronic factors may

$$pI_{50} = 0.33\pi - 0.94\mathcal{T} - 1.85\mathcal{R} - 0.70E_s + 4.61$$
$$n = 15,\ r = 0.944 \quad (204)$$

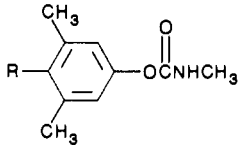
contribute to the binding, the hydrophobic factor appears to dominate the binding. That the larger contribution to hydrophobic interaction would be made by the meta substituent was shown by Magee[248] when he correlated Kohn's data[249] on meta-substituted derivatives of phenyl *N*-methylcarbamate of type XXIX (R = CH_3–C_3H_{17}) and obtained eq 205. Equation 205 however also shows that the binding would be limited by bigger substituents. The optimum interaction falls near R = C_3H_7.



XXIX

$$pI_{50} = 1.19\ (\pm 1.07)\pi - 0.38\ (\pm 0.23)\pi^2 + 0.83$$
$$n = 8,\ r = 0.954,\ s = 0.29 \qquad (205)$$

However, in many cases the electronic factor alone was shown to control the activity. For a series of para-substituted xylenyl carbamates (XXX), fly brain



XXX

$$pI_{50} = 6.41 - 0.83\sigma_p \qquad n = 12,\ r = 0.9 \qquad (206)$$

ChE inhibition activity was found to solely depend upon σ (eq 206),[250] and for the enzymatic (ChE) hydrolysis of substituted benzoylcholine esters, the apparent Michaelis constant, K_m(app), was found to be related to electronic parameters (eq 207 and 208).[251] Equation 207 was derived for ortho derivatives and 208 for meta and para derivatives. Substituents at any position were halides, methyl, methoxy, and nitro groups.

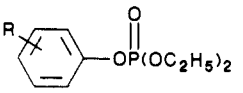
$$pK_m(app) = 4.814 - 0.608\mathcal{F} + 0.079P_E$$
$$n = 6,\ r = 0.942,\ s = 0.135 \qquad (207)$$
$$pK_m(app) = 4.816 - 1.313\sigma + 0.049P_E$$
$$n = 9,\ r = 0.980,\ s = 0.105 \qquad (208)$$

But for many other cases the role of π could not be avoided, and Fujita et al.[252,253] obtained eq 209 and 210

$$pK_d = 1.30\ (\pm 0.33)\pi + 2.24\ (\pm 0.61)\sigma^\circ + 1.68\ (\pm 0.46)\mathrm{HB} - 2.56\ (\pm 0.29)$$
$$n = 15,\ r = 0.944,\ s = 0.223 \qquad (209)$$
$$pK_d = 1.55\ (\pm 0.26)\pi - 1.93\ (\pm 0.52)\sigma^\circ + 1.53\ (\pm 0.36)\mathrm{HB} + 2.46\ (\pm 0.28)$$
$$n = 21,\ r = 0.967,\ s = 0.247 \qquad (210)$$

for the dissociation constant (K_d) of the enzyme–inhibitor reversible complex formed by some substituted phenyl *N*-methylcarbamates. Equation 209 was derived for ortho derivatives and eq 210 for meta derivatives. In these equations, HB is an indicator parameter to take into account the possibility of hydrogen bonding; hence, it was given a value of 1 for hydrogen-acceptor substituents such as NO_2, CN, CHO, Ac, COEt, and N(Me)$_2$. The term σ° was defined to account for the electronic effect in a biphase,[253] but as its coefficients in eq 209 and 210 are just opposite to each other in sign, it indicates a complex mechanism of electronic effect. However, the almost identical slopes of HB in these equations indicate that the basic substituents of the ortho and meta positions both associate, probably with a common hydrogen donor by a common mechanism. Similarly, almost equal coefficients of π in these equations suggest that the ortho and meta substituents both perhaps fit into a common hydrophobic region of the enzyme.

TABLE 35. ChE Inhibitory Activities of Diethyl Phenylphosphates



compd	R	pI_{50}	compd	R	pI_{50}
61	4-C(CH$_3$)$_3$	4.00	68	4-NO$_2$	7.59
62	4-Cl	4.52	69	3-SF$_5$	7.12
63	4-SCH$_3$	4.48	70	3-OCH$_3$	3.89
64	4-COOH	6.07	71	3-C(CH$_3$)$_3$	6.05
65	4-SO$_2$CH$_3$	6.60	72	3-NO$_2$	7.30
66	4-CHO	6.82	73	3-N$^+$(CH$_3$)$_3$	7.52
67	4-CN	6.89			

However, notwithstanding the above findings, the inhibition mechanism for a series of 97 phenyl methylcarbamates, where there was a variety of substituents in the phenyl ring, appeared to be quite complex. The in vitro anticholinesterase activity of these carbamates was found to be related to a number of variables as shown by eq 211,[254] where EC is the charge at pH

$$pI_{50} = 0.540\pi - 0.058\mathrm{MR} - 0.638\mathrm{EC} - 0.476\mathrm{HB} - 0.058\mathrm{CT} + 0.160\mathrm{MSD} - 5.16$$
$$n = 97,\ r = 0.80,\ s = 0.768 \qquad (211)$$

7, HB is the hydrogen-bonding parameter having a value of +1 for a donor and −1 for an acceptor, CT is the charge-transfer parameter having the value of +1 for nitro and nitrilo and −1 for amino or hydroxyl, and MSD is the minimal steric difference.

For a still bigger series of 269 derivatives of *N*-methylcarbamates that inhibit fly brain AChE, Goldblum et al.[255] obtained eq 212, where CHG and HB are

$$pI_{50} = 0.56\ (\pm 0.08)\mathrm{MR}_{3,4,5} + 1.56\ (\pm 0.20)\mathrm{MR}_2 - 0.61\ (\pm 0.09)E_s - 0.94\ (\pm 0.19)(\textstyle\sum\sigma_{o,p}^- + \sigma_m) + 1.43\ (\pm 0.31)\mathrm{CHG} - 0.23\ (\pm 0.04)(\mathrm{MR}_2)^2 - 5.24\ (\pm 1.27)F_{2,6}^2 + 3.47\ (\pm 0.90)F_{2,6} + 0.66\ (\pm 0.22)\mathrm{RGMR} - 0.62\ (\pm 0.22)\mathrm{HB} - 0.052\ (\pm 0.02)(\mathrm{MR}_3)^2 - 0.56\ (\pm 0.29)(E_{s-2})(E_{s-6}) + 3.46\ (\pm 0.21)$$
$$n = 269,\ r = 0.892,\ s = 0.485 \qquad (212)$$

indicator parameters to indicate the charged and hydrogen-bonding groups, respectively. In spite of such a large number of variables, there is still scope for improving the correlation. This correlation does not present any clear picture of the mechanism. But that charge transfer may often be involved in ChE inhibition was shown by Hetnarski and O'Brien[256] for some aryl methylcarbamates inhibiting bovine erythrocyte AChE. Equations 213 and 214 were obtained, where K_d is the

Para: $$K_d = 5.88 - 3.48\ (\pm 0.36)C_T - 2.65\ (\pm 0.22)\pi$$
$$n = 11,\ r = 0.989,\ s = 0.367 \qquad (213)$$

Meta: $$K_d = 2.44 - 1.23\ (\pm 0.36)C_T - 1.96\ (\pm 0.27)\pi$$
$$n = 9,\ r = 0.958,\ s = 0.322 \qquad (214)$$

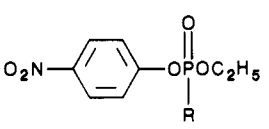
dissociation constant of the inhibitor–enzyme complex and C_T is the charge-transfer constant measured with reference to tetracyanoethylene (TCNE). In other studies as well, Hetnarski and O'Brien[257,258] observed

a parallelism between the inhibition constant and the association constant with TCNE, leading to the concept that a charge-transfer phenomenon was involved in AChE inhibition by carbamates.

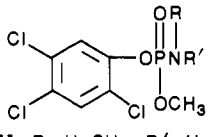
Studies on phosphates, phosphonates, phosphoramidates, etc., also revealed excellent correlations with hydrophobic, electronic, and steric parameters. Hansch[259] obtained eq 215 for a series of diethyl phe-

$$pI_{50} = 4.818\ (\pm0.41) - 0.556\ (\pm0.20)E_s + 2.452\ (\pm0.54)\sigma^- \quad n = 13,\ r = 0.962,\ s = 0.408 \qquad (215)$$

nylphosphates (Table 35) tested by Fukuto and Metcalf[260] against fly brain cholinesterase, and Hansch and Deutsch[244] derived eq 216 for some alkylphosphonic



XXXI: R = alkyl, phenyl



XXXII: R = H, CH_3; R′ = H, alkyl

$$\log K_i = 3.738E_s + 7.539 \quad n = 13,\ r = 0.901,\ s = 0.749 \qquad (216)$$

$$\log K_i = 2.359E_s - 3.913\sigma^* + 4.948 \quad n = 8,\ r = 0.939,\ s = 0.438 \qquad (217)$$

acid esters XXXI[261] and eq 217 for a small group of 2,4,5-trichloro-*N*-alkylphosphoramidates XXXII,[262] all studied against fly brain ChE. In both eq 216 and 217, K_i is the inhibition constant. Equation 216 was slightly improved when E_s was replaced by E_s^c, Hancok's corrected form of E_s (eq 218).[245,263] Inclusion of any electronic parameter had little effect over the correlations expressed by eq 216 or 218.

$$\log K_i = 2.58E_s^c + 7.94 \quad n = 13,\ r = 0.927,\ s = 0.648 \qquad (218)$$

For another series of XXXII where R = H and R′ = an alkyl group, Neely and Whitney[264] correlated their bimolecular rate constant (k_2) for fly brain AChE inhibition with π and k_h, the reactivity factor for the hydrolysis of inhibitors in 0.1 N NaOH at 50 °C, as shown in eq 219. For another series of XXXII, where

$$\log k_2 = 0.91\pi + 1.17 \log k_h + 3.21 \quad n = 9,\ r = 0.96,\ s = 0.315 \qquad (219)$$

both R and R′ were alkyl groups, Zerba and Fukuto[265] obtained eq 220. The log k_h in eq 219 incorporates the

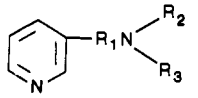
$$\log k_2 = 1.33\ (\pm0.65)E_s + 5.23 \quad n = 7,\ r = 0.923 \qquad (220)$$

steric effect. Thus, studies on phosphates, phosphoramidates, etc., show the dominance of steric influence in ChE inhibition. An electronic effect appears to be important only when the side chain aquires some preferred conformation. Thus, for a small set of diethyl phenyl phosphates, Aldridge and Davison[266] derived eq 221, which shows only the electronic effect in red cell

$$pI_{50} = 4.06\ (\pm0.75)\sigma^- + 2.95\ (\pm0.55) \quad n = 4,\ r = 0.998,\ s = 0.170 \qquad (221)$$

ChE inhibition. The early qualitative study of Fukuto and Metcalf[260a] and the later quantitative study of Hansch (eq 215) showed the greater electronic effect from the ring of the phenyl phosphates. It appears that by withdrawing the electrons the substituents of the ring increase the dipositive character of the labile O–P bond, facilitating the nucleophilic attack by the enzyme. The dependence of ChE inhibition on the lability of the O–P bond was observed with phenyl phosphates by Fukuto and Metcalf[260a] and with some phosphoramidates by Neely et al.[267]

TABLE 36. Antiacetylcholinesterase Activities of Nicotine Analogues



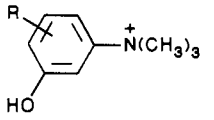
compd	$R_1NR_2R_3$	pI_{50}	compd	$R_1NR_2R_3$	pI_{50}
74	CH_2NH_2	0.96	84	$CH_2N(n\text{-}C_4H_9)_2$	2.85
75	$CH_2NH(CH_3)$	1.77	85	$CH_2N(CH_2)_4$	3.10
76	$CH_2NH(C_2H_5)$	2.24	86	$CH_2N(CH_2)_5$	3.00
77	$CH_2NH(C_3H_7)$	1.96	87	$CH_2N(CH_2)_4O$	0.20
78	$CH_2NH(i\text{-}C_3H_7)$	2.68	88	(±)-nornicotine	2.02
79	$CH_2NH(n\text{-}C_4H_9)$	1.96	89	(±)-nicotine	3.51
80	$CH_2N(CH_3)_2$	2.75	90	(±)-H_2-nicotyrine	3.64
81	$CH_2N(C_2H_5)_2$	3.35	91	(±)-anabasine	2.36
82	$CH_2N(C_3H_7)_2$	2.92	92	(±)-Me-anabasine	3.37
83	$CH_2N(i\text{-}C_3H_7)_2$	3.89			

Compounds not related to carbamates and phosphates were found essentially to involve electronic factors in their activity. For example, for certain β-pyridyl methylamino compounds (Table 36), eq 222 was

$$pI_{50} = 2.996 - 0.225\Sigma\pi - 2.619\Sigma\sigma^* \quad n = 19,\ r = 0.916,\ s = 0.397 \qquad (222)$$

$$pI_{50} = 5.322f - 1.247 \quad n = 4,\ r = 0.868 \qquad (223)$$

obtained,[268] and the AChE inhibition activities of four nicotinic acid derivatives (nicotinic acid, nicotinamide, 3-acetylpyridine, ethyl nicotinate) were correlated[269] with the frontier orbital density (f) of their carbonyl carbon (eq 223). Similarly, for a set of (3-hydroxyphenyl)trimethylammonium (3-HPTA) derivatives (XXXIII; R = 4-CH_3, 6-CH_3, 5-CH_3, 6-OCH_3, H, 4-OCH_3), the constant for AChE inhibition was related[270] to quantum mechanical parameters as shown by eq 224 and 225, where Q_1^π represents the π charge at C_1 and



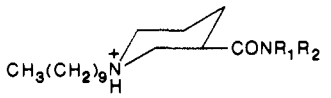
XXXIII

$$pK_i = 38.63Q_1^\pi + 11.88S_1^N - 0.29 \quad n = 6,\ r = 0.81,\ F = 2.85 \qquad (224)$$

$$pK_i = -2571Q_O^T - 2.83S_O^E - 645 \quad n = 6,\ r = 0.98,\ F = 44.44 \qquad (225)$$

S_1^N is the nucleophilic superdelocalizability index for it. Q_O^T and S_O^E are the total charge and the electrophilic superdelocalizability index, respectively, for the oxygen atom of hydroxyl group. Equations 223 and 224 are not statistically significant, but they indicate the possibility of interaction of the carbonyl carbon in nicotinic acid derivatives and of the ring C_1 in 3-HPTA derivatives with some anionic site of the enzyme. In

3-HPTA derivatives, eq 225 shows the interaction of the hydroxyl oxygen also with some cationic site of the enzyme. A similar concept can be derived from eq 226,



XXXIV

$$pI_{50} = 1124.571 - 51.877Q_C^T - 35.993Q_N^T - 3576.355Q_O^T$$
$$n = 7, r = 0.983, s = 0.101, F = 28.24 \quad (226)$$

which was derived for BuChE inhibition[271] by some N-substituted 1-decyl-3-carbamoylpiperidines [XXXIV; R_1R_2 = HCH_3, $(CH_3)_2$, $(CH_3)(C_2H_5)$, $(C_2H_5)_2$, pyrrolidyl, piperidyl, morpholinyl]. In eq 226, Q_C^T, Q_N^T, and Q_O^T represent the total σ and π charges at the carbonyl carbon, amide nitrogen, and carbonyl oxygen, respectively; hence, the equation shows the involvement of the entire –CON– groups. Gupta et al. had, therefore, argued that the polarizability of the group would be an important factor.[271] All these equations involving the quantum mechanical parameters and other electronic parameters also suggest the possibility of the involvement of charge-transfer phenomena in the inhibition, just as Hetnarski and O'Brien[256] had shown in the case of some carbamates (eq 213 and 214). The involvement of charge-transfer phenomena was shown even with some aromatic hydrocarbons such as chlorobenzene, benzene, toluene, anisole, *p*-xylene, *m*-xylene, and naphthalene, which were found to interact with AChE (eq 227).[256a]

$$K_d = 3.751 - 2.632\ (\pm 0.149)C_T - 1.385\ (\pm 0.130)\pi$$
$$n = 7, r = 0.997, s = 0.134 \quad (227)$$

However, it has been obvious from the studies mentioned hitherto that, if not in all cases then in most, the hydrophobic character of the molecules or substituents has been equally important, and there have been cases where ChE inhibition has been totally controlled by the hydrophobic factor. A very significant correlation was obtained between pI_{50} and π (eq 228)[269,272] for BuChE

$$pI_{50} = 4.17 + 0.561\pi$$
$$n = 6, r = 0.992, F = 279 \quad (228)$$

inhibition by a slightly different series of 1-decyl-3-carbamoylpiperidines [XXXIV; R_1R_2 = H_2, HCH_3, HC_2H_5, $(CH_3)_2$, $(C_2H_5)_2$, $(C_3H_7)_2$]. For the same series with one more derivative [R_1R_2 = $(CH_3)(C_2H_5)$], Kier et al.[155] correlated the pI_{50} with their $^1\chi$ (eq 229). Since

$$pI_{50} = 0.585\ (\pm 0.025)^1\chi + 0.617\ (\pm 0.241)$$
$$n = 7, r = 0.995\ s = 0.062 \quad (229)$$

$^1\chi$ is related to π, eq 229 has the same meaning as eq 228. The derivative with R_1R_2 = $(CH_3)(C_2H_5)$ was not included in eq 228, but the value predicted by this equation (pI_{50} = 5.05) was in good agreement with the observed value (5.01). However, eq 228 should not be taken as contradictory to eq 226 but as complementary. It may be assumed that the amide moiety (–CON–) would be involved in electronic interaction and the substituents at the nitrogen in hydrophobic interaction.

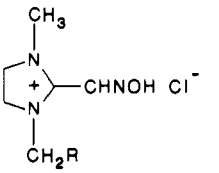
Over 30 years ago Burgmann[273] showed that the activities of alkyl derivatives of $RN^+(CH_3)_3$ against human plasma ChE and electric eel enzyme and that of $[(CH_3)_3{}^+N(CH_2)_n{}^+N(CH_3)_3$, n = 4–10] against the former were a significant function of the hydrophobicity (eq 230–232, respectively); and recently, for a set of

$$pI_{50} = 3.406\ (\pm 0.25) + 0.643\ (\pm 0.09)\log P$$
$$n = 7, r = 0.993, s = 0.089 \quad (230)$$

$$pI_{50} = 4.082\ (\pm 0.31) + 0.454\ (\pm 0.09)\log P$$
$$n = 7, r = 0.985, s = 0.094 \quad (231)$$

$$pI_{50} = 4.503\ (\pm 0.40) + 0.607\ (\pm 0.19)\log P$$
$$n = 7, r = 0.964, s = 0.199 \quad (232)$$

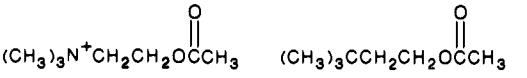
imidazolium compounds (XXXV), Bedford et al.[274] observed equally significant dependence of their eel AChE inhibition activity on their lipophilicity (eq 233).



XXXV: R = CH_3, $CH(CH_3)_2$, $CH_2C(CH_3)_3$, $CH(CH_3)C(CH_3)_3$, $(CH_2)_3CH_3$, $(CH_2)_7CH_3$, $CH_2C_6H_5$, $(CH_2)_3C_6H_5$, $CH_2C_{10}H_7$

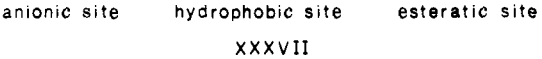
$$pI_{50} = 2.07\ (\pm 0.11) + 0.44\ (\pm 0.07)\log P$$
$$n = 9, r = 0.92 \quad (233)$$

From all these studies on ChE inhibition it becomes apparent that all three factors, hydrophobic, steric, and electronic, can contribute to the inhibition activity of molecules. Long ago it was proposed[275] that cholinesterase has two active sites, esteric and anionic. The esteric site is supposed to involve a serine hydroxyl group, which is acylated, phosphorylated, or carbamylated by an appropriate agent. For this phenomenon, the charge at the carbon or phosphorus of such an agent is important. The presence of an anionic site was postulated because the prominent-looking cationic charge on acetylcholine (XXXVIa) demanded it. Now,



XXXVIa XXXVIb

many QSAR studies have shown that the positive charge on some atoms in the inhibitors determines the activity. Thus, they simply verify the presence of an anionic site on the enzyme. But when it was found that 3,3-dimethylbutyl acetate (XXXVIb) and acetylcholine are equally easily hydrolyzed by AChE, it was realized that the van der Waals forces of the methyl groups could be just as important as a positive charge in the binding of substrate or inhibitor with the enzyme.[276] Wilson[277] emphasized that the binding is affected by ionic and dispersion forces, but today it is attributed to ionic and hydrophobic forces. QSAR studies support the idea that the enzyme must also possess a hydrophobic region, and it has been well established that hydrophobic regions near the active site influence the binding of organophosphorus[278,279] and other inhibitors.[280] Hence, a picture of the active zone of the enzyme can be proposed as XXXVII, and from QSAR



XXXVII

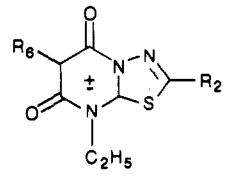
studies it can be said that for a cholinesterase inhibitor it is not essential to possess all hydrophobic, electronic, and steric properties to be effective; rather, any property leading to strong binding with the enzyme would suffice to produce the inhibition. The steric factors will influence the binding by disturbing the proper orientation of the interacting group with respect to the active sites of the enzyme.

The anionic site of the enzyme may occasionally be involved in charge-transfer phenomena also, since QSAR studies have also exhibited in many cases the formation of a charge-transfer complex.

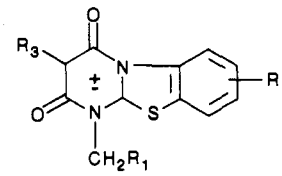
2. cAMP Phosphodiesterase

The enzyme adenosine cyclic 3′,5′-monophosphate (cAMP) phosphodiesterase (PDE) catalyzes the conversion of cAMP to adenosine 5′-monophosphate and, hence, is responsible, in part, for lowering the intracellular levels of this cyclic nucleotide. Many diseases are associated with lowered levels of cAMP. A correlation of a cAMP deficiency state has been observed in hypertension[281] and asthma,[282] and decreased intracellular concentrations of cAMP have been reported in the depressed immune response,[283] inflammation,[284] anaphylaxis,[285] and neurotransmitter release.[286] The selective inhibition of cAMP PDE would lead to selective increases in the level of cAMP in organs or cells.

A recent QSAR study[287] on some mesoionic 1,3,4-thiadiazolopyrimidines XXXVIIIa and benz-fused



XXXVIIIa: R_2 = H, alkyl; R_6 = alkyl, benzyl, phenyl



XXXVIIIb: R = OCH_3, OC_2H_5; R_1 = alkyl (linear or cyclic); R_3 = alkyl

mesoionic xanthine analogues (XXXVIIIb) showed that the inhibition activity measured for bovine heart phosphodiesterase[288,289] was a significant function of the size of the substituents. Equation 234 was obtained for

$$pI_{50} = 1.810\ (\pm0.583)V_w{\cdot}R_6 - 0.585\ (\pm0.377)I + 2.677\ (\pm0.398)$$

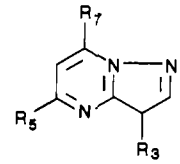
$$n = 15,\ r = 0.944,\ s = 0.26,\ F_{2,12} = 48.97 \quad (234)$$

$$pI_{50} = 3.901\ (\pm0.399) - 2.083(\pm1.539)V_w{\cdot}R_1 + 2.854\ (\pm1.379)(V_w{\cdot}R_1)^2 + 1.356\ (\pm0.524)V_w{\cdot}R$$

$$n = 15,\ r = 0.940,\ s = 0.10,\ F_{3,11} = 27.93 \quad (235)$$

the derivatives of XXXVIIIa and eq 235 was obtained for the derivatives of XXXVIIIb. In eq 234, I takes the value of 1 if R_2 is an alkyl group and zero if R_2 is simply H. Thus, this equation shows that while an increase in the size of the substituent at the 6-position will increase the activity, an alkyl group at the 2-position will decrease the activity by producing some steric hindrance in the drug–receptor interaction. The interaction is assumed to involve van der Waals forces. At a position equivalent to the 6-position of XXXVIIIa, the substituent R_3 in XXXVIIIb was not found to produce any effect on the activity. This may be, as shown by eq 235, due to the steric hindrance produced by the R_1 substituent; but since the dependence of activity on $V_w{\cdot}R_1$ is parabolic, there would be a positive effect on the activity by R_1 itself after the optimum value of $V_w{\cdot}R_1$ (0.365×10^2 Å^3) was reached. The reason for this positive effect by a bigger substituent may be that a bigger substituent may be able to reach the active site of the enzyme to have either hydrophobic or dispersion interaction. A significant positive coefficient of $V_w{\cdot}R$ suggests the positive effect on activity by the substituent at the fused phenyl ring also.

The attempt to correlate the PDE inhibition activity with π for a series of some 3,5,7-trisubstituted pyrazolo [1,5-*a*]pyrimidines XXXIX was not as successful (eq 236 and 237).[290] α_L in eq 236 and α_H in eq 237 represent



XXXIX: R_3 = H, NO_2, $CO_2C_2H_5$, Br; R_5 = alkyl, phenyl, CF_3; R_7 = mostly alkyl

$$\log \alpha_L = 0.45\ (\pm0.34)\pi_3 + 0.11\ (\pm0.29)\pi_5 + 2.12\ (\pm0.41)\pi_7 - 0.70\ (0.35)\pi_7^2 - 1.19\ (\pm0.72)$$

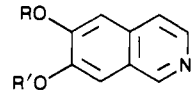
$$n = 25,\ r = 0.84,\ s = 0.34 \quad (236)$$

$$\log \alpha_H = 0.65\ (\pm0.42)\pi_3 + 0.28\ (\pm0.40)\pi_5 + 0.026\ (\pm0.31)\pi_7 - 0.49\ (\pm0.46)$$

$$n = 22,\ r = 0.654,\ s = 0.40 \quad (237)$$

the inhibition activity relative to theophylline against rabbit lung and beef heart PDE, respectively. The correlation is satisfactory only for the rabbid lung enzyme. π_5 is however not significant in this case also. Equation 236 therefore suggests that there might be some hydrophobic interaction in the inhibition of the rabbit lung enzyme, in which only substituents at the 3- and 7-positions of pyrazolopyrimidines would be involved. The equation also suggests that the interaction of the 7-substituent will be limited by its size.

The inhibition of bovine arterial cAMP PDE by a series of papaverine analogues (XL) could be correlated[291] with π as shown be eq 238, but a better correlation



XL

$$pI_{50} = 1.03\ \Sigma\pi - 0.16(\Sigma\pi)^2 + 2.62$$

$$n = 15,\ r = 0.82,\ s = 0.25,\ \Sigma\pi_o = 3.5 \quad (238)$$

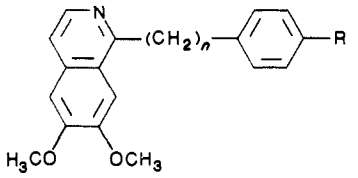
was obtained with V_w (eq 239)[292] for another study[293]

$$pI_{50} = 5.245 - 0.157V_w + 0.355I$$

$$n = 13,\ r = 0.920,\ s = 0.082,\ F_{2,10} = 27.38 \quad (239)$$

on bovine heart PDE inhibition by papaverine analogues as listed in Table 37. In eq 239, V_w is meant for the entire portion of the substituted phenyl or benzyl group. Thus, eq 239 shows that a phenyl or benzyl group at the 1-position of isoquinoline will reduce the activity by producing some steric hindrance, but the indicator parameter I, which was given a value of unity for all those substituents in the phenyl/benzyl groups with chlorine, indicates that the activity would be increased by such a substituent. This increase in the

TABLE 37. Papverine Analogues and Their PDE Inhibition Activities



compd	n	R	pI_{50}
1	0	NH_2	5.13
2	0	$NHCH_2CH_2Cl$	5.46
3	0	$NHCOCH_2Cl$	5.30
4	0	$N(CH_2CH_2Cl)_2$	5.28
5	0	$NHCOCH{=}CHCOOCH_3$ (cis)	5.28
6	0	$NHCOCH{=}CH_2$	4.92
7	0	$NHCOCH{=}CHCOOCH_3$ (trans)	4.85
8	1	NH_2	5.13
9	1	$NHCH_2CH_2Cl$	5.39
10	1	$NHCOCH_2Cl$	5.41
11	1	$N(CH_2CH_2Cl)_2$	5.28
12	1	$NHCOCH{=}CHCOOCH_3$ (cis)	5.40
13	1	$NHCOCH{=}CH_2$	5.00
14	1	$NHCOCH{=}CHCOOCH_3$ (trans)	5.10
15	1	3,4-$(OCH_3)_2$	5.06

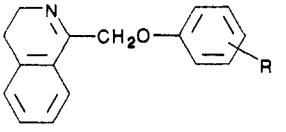
activity might be due to some electronic effect produced by the chlorine atom. The cis isomers of compounds 7 and 14 were not included in the regression, as to account for the conformational effect for just two compounds one more indicator parameter would have been required. The cis isomer was found to have more activity than the trans isomer.

These studies, however, do not provide much insight into the mechanism of phosphodiesterase inhibition.

G. Glycosidases

Viral Neuraminidase

The enzyme neuraminidase (*N*-acetylneuraminyl glycohydrolase, EC 3.2.1.18) is a component of surface protein in all strains of influenza and parainfluenza viruses. It assists in the release of the virus from infected cells.[294] Inhibition of this enzyme would be, therefore, expected to slow down the rate of viral release and thus delay the spread of the virus within the host tissue. A series of 1-(phenoxymethyl)-3,4-dihydroisoquinolines (XLI; R = H, 4-NO_2, 4-Br, 4-CN, 4-Cl, 4-F,



XLI

4-Me, 4-OMe, 4-OH, 4-OEt, 4-OPr, 4-OBu, 4-CMe_3, 3-Me, 3-F, 3-Cl) were studied for their neuraminidase inhibition activity[295] and subjected to QSAR analysis.[296] The QSAR analysis revealed that the activity was a significant function of the hydrophobic constant of the substituent (eq 240).[296] Additionally, the possibility

$$pC = 2.592 + 0.253\pi$$
$$n = 16,\ r = 0.834,\ s = 0.108 \qquad (240)$$

of dipole–charge interaction was also shown, as the component of the group dipole moment, μ_v, along the 1,4-axis of the substituted moiety was found to make a significant improvement over the correlation expressed by eq 240 (eq 241).[296] In eq 241, however, the

$$pC = 2.552 + 0.271\pi + 0.062\mu_v + 0.030\mu_v^2$$
$$n = 16,\ r = 0.937,\ s = 0.074 \qquad (241)$$

coefficient of μ_v was not significant at the 95% confidence level;[297] hence, μ_v was excluded and a new equation (eq 242) was obtained.[297]

$$pC = 2.548 + 0.265\ (\pm 0.032)\pi + 0.014\ (\pm 0.003)\mu_v^2$$
$$n = 16,\ r = 0.916,\ s = 0.081 \qquad (242)$$

But Cammarata et al.[297] showed that μ_v^2 was significantly related to σ^2 (r = 0.925), and since the latter was indicated to have a variety of possible origins,[298] Cammarata et al. argued that no definite interpretation could be given for the electronic term of eq 242. Thus, in order to find the actual mode of electronic interaction, further study is required.

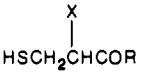
H. Peptidyldipeptide Hydrolases

Angiotensin Converting Enzyme

The formation of octapeptide angiotensin II, a potent vasoconstrictor agent and the main physiological stimulus for the release of aldosterone from the adrenal gland, from its precursor, decapeptide angiotensin I, is mediated by angiotensin converting enzyme (ACE), a peptidyldipeptide carboxyhydrolase. The ACE simply removes the C-terminal dipeptide histidylleucine from the decapeptide angiotensin I and yields the octapeptide angiotensin II.[299] The inhibitors of this enzyme, therefore, hold great promise in the treatment of hypertension.

Hydrophobicity is assumed to play an important role in ACE inhibition,[299] but a quantitative estimation of the role of hydrophobic character was, however, only recently made.[300] Data of various studies were analyzed in relation to calculated log P values.

Equation 243 was obtained for a series of mercaptoalkanoyl derivatives of amino acids (XLII) studied by Condon et al.[301] In the equation, I_1 with a value of 1

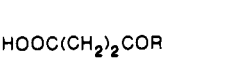


XLII: R = amino acid moiety; X = H, CH_3

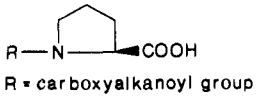
$$pI_{50} = 1.765 + 1.733\ (0.715)\log P - 0.244\ (0.129) \times (\log P)^2 + 0.831\ (0.488)I_1 + 1.555\ (0.373)I_2$$
$$n = 27,\ r = 0.915,\ s = 0.615,\ F_{4,22} = 28.31 \qquad (243)$$

was used to indicate whether the amino acid had any group that could form a hydrogen bond with the receptor and I_2 with a value of unity was used to indicate whether X was a methyl group with an S configuration (above the plane). Thus, eq 243 showed that, along with the hydrophobic character of the molecules, their ability to form the hydrogen bond and the specific configuration of a particular substituent were also important in ACE inhibition.

However, when series of carboxyalkanoyl amino acids (XLIII, XLIV)[302] were treated, no meaningful correlations were obtained.[300] Equation 244, which was ob-



XLIII, R = amino acid moiety



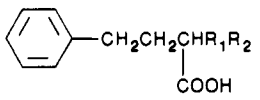
XLIV, R = carboxyalkanoyl group

$$pI_{50} = 2.715 + 0.014\ (0.035)\ \log P + 0.054\ (0.018)(\log P)^2$$
$$n = 10,\ r = 0.755,\ s = 0.207,\ F_{2,7} = 4.64 \quad (244)$$

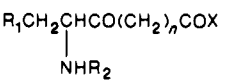
$$pI_{50} = 10.175 - 6.125\ (2.124)\ \log P + 1.236\ (0.474)(\log P)^2 + 1.976\ (0.215)I_2$$
$$n = 10,\ r = 0.972,\ s = 0.271,\ F_{3,6} = 33.68 \quad (245)$$

tained for the XLIII series where the amino acid moiety varied, was not statistically significant ($F_{0.05} = 4.74$). Equation 245, which was obtained for the XLIV series where the carboxylkanoyl moiety varied, was meaningless, as it implied huge activity for very lipophilic compounds, which is highly unlikely. In eq 245, I_2 had the same meaning as it had in eq 243.

For a small series of *N*-(1-carboxy-3-phenylpropyl) dipeptides (XLV),[303] the activity was related to hydrophobicity as shown by eq 246, and in the same



XLV: R_1, R_2 = amino acid moieties



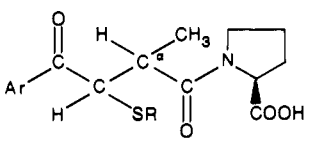
XLVI: R_1 = Ph, 4-HOPh, 4-Bzl-OPh, H, 3-pyridyl, 3,4-$(MeO)_2$Ph; R_2 = group like PhCO; X = amino acid, amino acid ester

$$pI_{50} = 4.607\ (2.396)\ \log P - 0.425\ (0.261)(\log P)^2 - 3.873$$
$$n = 8,\ r = 0.899,\ s = 0.301,\ F_{2,5} = 10.59 \quad (246)$$

$$pI_{50} = 2.276\ (0.532)\ \log P - 0.242\ (0.055)(\log P)^2 + 2.346$$
$$n = 22,\ r = 0.711,\ s = 0.832,\ F_{2,19} = 9.73 \quad (247)$$

fashion the activity of a series of (*S*)-1-[5-(benzoylamino)-1,4-dioxo-6-phenyl]-L-prolines (XLVI)[304] was found to be correlated with log *P* (eq 247). Equation 246 was significant at the 95% level, and eq 247, though its r value was not very high, was significant at the 99% level. From these two equations and eq 243, it was observed that in the series of compounds where the amino acid moiety varied, hydrophobicity affected the activity in a parabolic manner. Thus, the amino acid moiety probably interacts with the hydrophobic pocket of the enzyme but puts a limit also on the interaction.

However, in the case of 1-[3-(acylthio)-3-aroylpropionyl]-L-proline derivatives (XLVII)[305] where the



XLVII: R = CH_3CO, C_6H_5CO

structural variation was largely due to varying the aryl group, the ACE inhibition activity was not found to be related to hydrophobicity at all. Rather, the specific configuration of the α-methyl group was found to largely affect the activity. In the *S* configuration (above the plane) it increased the activity, and in the *R* configuration (below the plane) it decreased the activity.

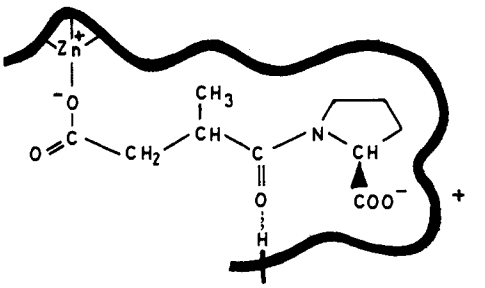


Figure 2. Hypothetical model for binding of ACE inhibitors with the enzyme. In the case of mercaptoalkanoyl derivatives, S will interact with Zn^+.

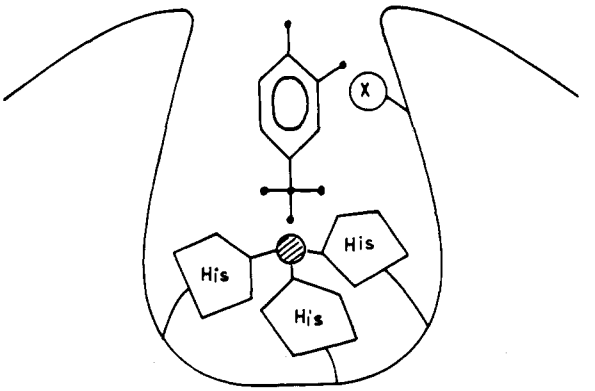
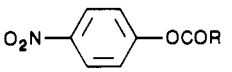


Figure 3. Representation of binding of sulfonamides with carbonic anhydrase.

TABLE 38. *p*-Nitrophenyl Esters and Their Chymotrypsin Hydrolysis Constants



compd	R	log k_3	compd	R	log k_3
1	$ClCH_2$	0.42	10	$Cl(CH_2)_3$	−1.29
2	H	0.18	11	$Cl(CH_2)_4$	−1.35
3	ICH_2	−0.24	12	$C_6H_5(CH_2)_2$	−0.75
4	CH_3OCH_2	−0.47	13	$C_6H_5(CH_2)_3$	−0.92
5	$Cl(CH_2)_2$	−1.68	14	$C_6H_5(CH_2)_4$	−1.73
6	$C_6H_5CH_2$	−1.73	15	H_2NCH_2	−0.46
7	CH_3	−2.00	16	L-CH_2CHNH_2	0.28
8	$(CH_3)_2CH$	−2.47	17	L-$(CH_3)_2(CH)_2NH_2$	0.83
9	$(CH_3)_3C$	−3.74	18	L-$C_6H_5CH_2CHNH_2$	1.57

The *S* configuration probably brings the α-methyl group very near to the hydrophobic region of the enzyme, and the *R* configuration keeps it away from the active site. The configuration of the methyl group would also affect the conformation of the entire side chain and thus would influence the other types of interaction also. The configuration of the SR group was also observed to affect the activity.[305]

All these findings regarding ACE inhibition are in total conformity to the interaction model proposed for these inhibitors (Figure 2).[299,306]

I. Serine Proteinases

1. Chymotrypsin

α-Chymotrypsin is a proteolytic enzyme (an endopeptidase) that hydrolyzes a wide variety of simple amide, peptide, and ester bonds. The systematic clinical usefulness of this enzyme is questionable. Since it is a foreign protein, severe anaphylactic reactions have followed its systematic use. However, of the hydrolytic

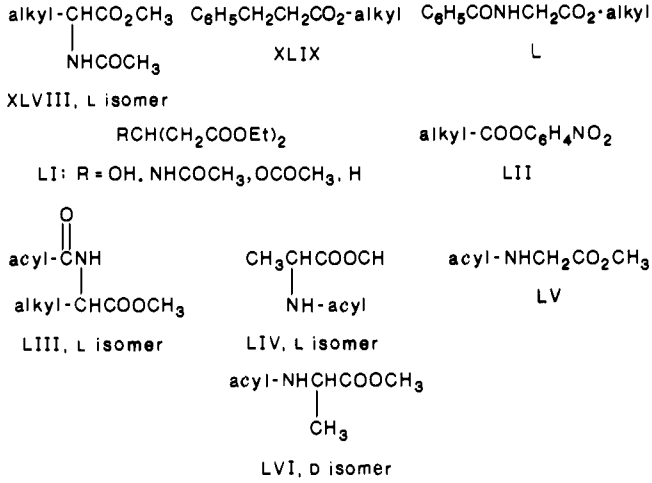
TABLE 39. $R_2CH(COOR_3)NHCOR_1$ Derivatives and Their pK_m Values for Chymotrypsin Interaction

compd	$NHCOR_1$	R_2	OR_3	pK_m
19	L-NHCOMe	Me	OMe	0.21
20	L-NHCOMe	Me	OEt	0.60
21	L-NHCOMe	*i*-C_3H_7	O-*i*-C_3H_7	0.75
22	L-NHCO-furyl-H_4	Me	OMe	0.88
23	L-NHCOMe	*i*-C_3H_7	OMe	0.95
24	L-NHCOMe	*i*-C_3H_7	OEt	0.96
25	L-NHCOMe	Et	OMe	1.28
26	L-NHCO-furyl	Me	OMe	1.31
27	L-$NHCOCH_2Cl$	*i*-C_3H_7	OMe	1.37
28	L-NHCO-3-pyridyl	Me	OMe	1.43
29	NHCOMe (symmetric)	COOEt	OEt	1.51
30	L-NHCOMe	C_3H_7	O-*i*-C_3H_7	1.51
34	L-NHCO-4-pyridyl	Me	OMe	1.54
32	L-NHCOMe	CH_2COOEt	OEt	1.64
33	L-NHCOMe	*i*-C_3H_7	OCH_2CH_2Cl	1.72
34	L-NHCO-2-pyridyl	Me	OMe	1.74
35	L-NHCO-2-thienyl	Me	OMe	1.82
36	L-NHCOMe	C_3H_7	OMe	1.99
37	L-NHCOMe	Me	OMe	2.01
38	L-NHCOMe	C_4H_9	OMe	2.17
39	L-NHCOPh	Me	OEt	2.22
40	L-$NHCOCH_2Cl$	C_3H_7	OMe	2.30
41	L-NHCOPh-2-NH_2	Me	OMe	2.33
42	L-NHCOPh	*i*-C_3H_7	OMe	2.34
43	L-NHCOMe	*i*-C_4H_9	OMe	2.42
44	L-NHCOMe	Ph	OEt	2.48
45	L-NHCOMe	C_6H_{13}	OMe	2.53
46	L-NHCOMe	CH_2Ph	R-O-*s*-C_4H_9	2.76
47	L-NHCOMe	C_5H_{11}	OMe	2.79
48	L-NHCOPh	Et	OMe	2.85
49	L-NHCOMe	CH_2Ph	OMe	2.90
50	L-NHCOMe	CH_2Ph	OEt	2.96
51	L-NHCOMe	CH_2Ph	(*S*)-O-*s*-C_4H_9	3.04
52	L-NHCOPh	C_3H_7	OMe	3.07
53	L-NHCOMe	C_3H_7	OCH_2CH_2Cl	3.10
54	L-NHCOMe	CH_2Ph-4-OH	OEt	3.15
55	L-NHCOMe	CH_2Ph	(*S*)-O-CH(Me)-*c*-C_6H_{11}	3.28
56	L-NHCOMe	CH_2Ph	(*R*)-O-CH(Me)-*c*-C_6H_{11}	3.31
57	L-NHCOMe	*i*-C_3H_7	OPh-4-NO_2	3.31
58	L-NHCOMe	Me	OPh-4-NO_2	3.32
59	L-NHCO-furyl	CH_2Ph-4-OH	OMe	3.38
60	L-NHCOMe	CH_2Ph-4-OH	OMe	3.49
61	L-NHCO-2-quinolyl	Me	OMe	3.66
62	L-NHCOMe	CH_2-*c*-C_6H_{11}	OMe	3.72
63	L-NHCOMe	CH_2-indolyl	OMe	4.02
64	L-NHCOMe	CH_2-indolyl	OEt	4.05
65	L-NHCOMe	CH_2Ph	(*S*)-O-CH(Me)Ph	4.22
66	L-NHCOMe	CH_2Ph	(*R*)-O-CH(Me)Ph	4.30
67	L-NHCOPh	CH_2Ph	OMe	4.46
68	L-$NHSO_2Me$	CH_2Ph	OPh	4.57
69	L-NHCOMe	CH_2Ph	OPh-4-NO_2	4.62
70	L-NHCOPh	CH_2Ph-4-OH	OEt	4.66
71	L-$NHCOOCH_2Ph$	Me	OPh-4-NO_2	4.70
72	L-$NHSO_2Me$	CH_2Ph	OPh-4-NO_2	4.70
73	L-NHCOPh	CH_2Ph-4-OH	OMe	4.74
74	L-$NHCOOCH_2Ph$	*i*-C_3H_7	OPh-4-NO_2	4.75
75	L-$NHSO_2Me$	CH_2Ph	OPh-4-OMe	4.82
76	L-$NHCOOCH_2Ph$	CH_2CONH_2	OPh-4-NO_2	4.87
77	L-$NHSO_2Me$	CH_2Ph	OPh-4-Cl	4.88
78	L-$NHSO_2Me$	CH_2Ph	OPh-4-COMe	4.89
79	L-$NHSO_2Me$	CH_2Ph	OPh-3-NO_2	5.06
80	L-$NHCOOCH_2Ph$	C_4H_9	OPh-4-NO_2	5.24
81	L-$NHCOOCH_2Ph$	*i*-C_4H_9	OPh-4-NO_2	5.32
82	L-$NHSO_2Me$	CH_2Ph	OPh-4-NO_2	5.37
83	L-$NHCOOCH_2Ph$	CH_2-indolyl	OPh-4-Cl	5.40
84	L-$NHCOOCH_2Ph$	C_3H_7	OPh-4-NO_2	5.57
85	L-$NHCOOCH_2Ph$	CH_2-indolyl	OPh-4-COMe	5.68
86	L-NHCOMe	CH_2-indolyl	OPh-4-NO_2	5.70
87	L-$NHCOOCH_2Ph$	Et	OPh-4-NO_2	5.74
88	L-$NHCOOCH_2Ph$	CH_2-indolyl	OPh-4-NO_2	5.92
89	L-$NHCOOCH_2Ph$	CH_2Ph	OPh-4-NO_2	6.32

enzymes, it has been most extensively studied for its mode of action.

To find better insight into the mechanism of action of chymotrypsin, Hansch and Coats[307] subjected a variety of its substrates and inhibitors to QSAR study. For substrates XLVIII–LVI eq 248–257 were obtained, and for inhibitors LVII–LXII, some miscellaneous compounds, aromatic acids, and some hydrocarbons eq

258–266 were obtained. In all these equations, the



XLVIII: $pK_m = 1.419\ (\pm 0.40)\pi - 3.409\ (\pm 0.74)$

$n = 9, r = 0.955, s = 0.350$ (248)

XLIX: $pK_m = 0.210\ (\pm 0.22)\pi + 3.160\ (\pm 0.24)$

$n = 3, r = 0.997, s = 0.012$ (249)

L: $pK_m =$

$0.406\ (\pm 0.18)\pi + 0.400\ (\pm 0.30)E_s - 0.714\ (\pm 0.19)$

$n = 6, r = 0.972, s = 0.047$ (250)

Different Set of Data (L)

$pK_m = 0.251\ (\pm 0.31)\pi + 3.343\ (\pm 0.36)$

$n = 4, r = 0.925, s = 0.055$ (251)

LI: $pK_m = 0.518\ (\pm 0.61)\pi - 1.308\ (\pm 0.52)$

$n = 4, r = 0.932, s = 0.152$ (252)

LII: $\log (k_2/K_m) =$

$1.762\ (\pm 0.42)E_s + 0.789\ (\pm 0.40)\pi + 2.225\ (\pm 0.52)$

$n = 8, r = 0.981, s = 0.201$ (253)

LIII: $pK_m = 1.382\ (\pm 0.27)\pi(\text{alkyl}) +$

$0.082\ (\pm 0.02)P_E(\text{acyl}) - 3.876\ (\pm 0.58)$

$n = 21, r = 0.934, s = 0.331$ (254)

LIV: $pK_m = 0.103\ (\pm 0.023)P_E - 3.653\ (\pm 0.62)$

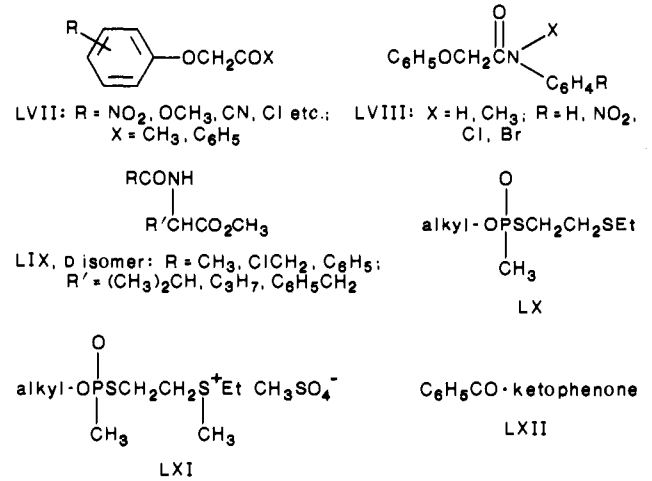
$n = 8, r = 0.975, s = 0.179$ (255)

LV: $pK_m = 0.042\ (\pm 0.015)P_E - 2.068\ (\pm 0.31)$

$n = 14, r = 0.873, s = 0.225$ (256)

LVI: $pK_m = 0.125\ (\pm 0.077)P_E - 3.887\ (\pm 1.822)$

$n = 7, r = 0.882, s = 0.270$ (257)



LVII: $\log (S/I)_{50} = 0.798\ (\pm 0.28)\pi +$

$0.459\ (\pm 0.45)\sigma + 0.868\ (\pm 0.40)D - 1.964\ (\pm 0.24)$

$n = 15, r = 0.913, s = 0.261$ (258)

$D = 1$ for X = C_6H_5; $D = 0$ for X = CH_3

LVIII: $\log (S/I)_{50} =$

$1.216\ (\pm 0.85)\pi_R - 1.289\ (\pm 0.51)$

$n = 6, r = 0.893, s = 0.297$ (259)

LIX: $pK_i =$

$2.874\ (\pm 0.90)\pi_{R'} + 0.089\ (\pm 0.02)P_{E\text{-}R'} - 6.793\ (\pm 1.49)$

$n = 7, r = 0.984, s = 0.193$ (260)

LX: $pK_i = 1.133\ (\pm 0.24)\pi - 1.151\ (\pm 0.53)$

$n = 7, r = 0.984, s = 0.242$ (261)

LXI: $pK_i = 1.284\ (\pm 0.32)\pi - 0.643\ (\pm 0.71)$

$n = 7, r = 0.978, s = 0.326$ (262)

LXII: $pK_i = 2.53\ (\pm 0.40) + 0.31\ (\pm 0.37)\pi$

$n = 3, r = 0.996, s = 0.020$ (263)

Miscellaneous

$pK_i = 0.977\ (\pm 0.06)\ \log P + 0.592\ (\pm 0.12)\text{HB} -$

$2.537\ (\pm 0.18)$

$n = 17, r = 0.994, s = 0.111$ (264)

Aromatic Acids

$pK_i = 0.942\ (\pm 0.58)\ \log P + 0.960\ (\pm 1.78)pK_a -$

$3.660\ (\pm 8.23)$

$n = 7, r = 0.917, s = 0.361$ (265)

Hydrocarbons

$pK_i = 1.473\ (\pm 0.43)\ \log P - 1.209\ (\pm 1.31)$

$n = 10, r = 0.942, s = 0.379$ (266)

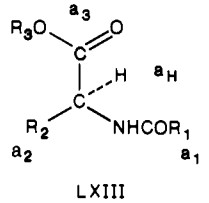
inhibition parameters $K_m = (k_{-1} + k_2)/k_1$ and $K_i = k_{-4}/k_4$, where the various rate constants were related to a double-displacement mechanism (eq 267–269) by which chymotrypsin is supposed to operate.[307] In this study, in most of the cases the number of data points was very small, but since a consistent correlation was observed for widely different series of substrates and inhibitors, Hansch and Coats described the binding of substrates and inhibitors with the enzyme as follows.

$$\text{RC(O)OR}' + \text{enzyme} \rightarrow \text{RC(O)–enzyme} \rightarrow \text{RCOOH} + \text{enzyme} + \text{R}'\text{OH} \quad (267)$$

$$\text{E} + \text{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{ES}' \ (+ \text{P}_1) \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \text{E} + \text{P}_2 \quad (268)$$

$$\text{ES}' + \text{H}^+ \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \text{ES}'\text{H} \quad (269)$$

The discussion was based on the Hein–Niemann model[308,309] of the interaction (LXIII). In this model,



LXIII

$NHCOR_1$ represents the *N*-acyl portion, R_2 is the side

chain in the α-position, C–OR_3 is the ester or amide bond that is hydrolyzed, and a_1, a_2, a_3, and a_H are the areas of the active site of the enzyme with which the four substituents on the α-carbon interact. Hence, from eq 248, 254, 258, 259, 261, 262, 264, and 266, Hansch and Coats suggested that hydrophobic binding occurred in the a_2 area and that it was common for substrates as well as for inhibitors. Hydrophobic binding at the a_3 area, though weaker than at a_2, was suggested from eq 249–251 and 263.

A steric inhibition by the alkyl group of the ester in binding of the sp^2 carbon of the carbonyl group, another possible interaction with a nucleophile at the a_3 site, was pointed out by eq 250 and 253. However, eq 254–257 and 260 led these authors to assume that the a_1 site is polar and that charge interactions occur between this and the polarizable substituents at amide nitrogen.

While eq 264 suggested that, among miscellaneous compounds, phenols (HB = 1) would be involved in hydrogen bonding with the a_3 site, which probably has a serine residue, eq 258 suggested that electron withdrawal by the substituent on the phenyl ring of LVII would probably make the carbonyl carbon more positive so that the latter interacts more strongly with the a_3 site. Similarly, in the case of the aromatic acids, the positive coefficient of pK_a suggested that a less ionized acid would be more effective. Probably, the positive charge of the carbonyl carbon remains more stabilized in the unionized fraction of the acid, or hydrogen is available from it for the hydrogen bonding.

Chymotrypsin hydrolysis of some *p*-nitrophenyl esters (Table 38) was qualitatively analyzed in relation to σ^* and E_s by Dupaix et al.,[310] but later Hansch[311] obtained, taking into consideration the hydrophobic effect also, a quantitative correlation for the first 13 compounds as shown by eq 270. For the last four

$$\log k_3 = 2.201\ (\pm 0.60)\sigma^* + 1.012\ (\pm 0.40)E_s + 0.374\ (\pm 0.22)\pi - 2.067\ (\pm 0.48)$$
$$n = 13,\ r = 0.969,\ s = 0.327 \qquad (270)$$

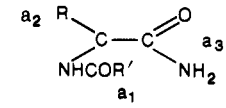
$$\log k_3 = 0.70\ (\pm 0.45)\pi - 0.31\ (\pm 0.73)$$
$$n = 4,\ r = 0.978,\ s = 0.219 \qquad (271)$$

compounds, which were distinctly different from the first set, the correlation obtained was as shown by eq 271. Similarly, for the chymotrypsin hydrolysis of seven derivatives of LII studied by Fife and Milstein,[312] Hansch[311] obtained eq 272. From these studies,

$$\log k_3 = 0.793\ (\pm 0.18)\pi + 1.539\ (\pm 0.14)E_s - 2.842\ (\pm 0.28)$$
$$n = 7,\ r = 0.998,\ s = 0.072 \qquad (272)$$

Hansch noted that step 3 in the hydrolysis reaction (eq 268) involved hydrophobic binding. However, he attributed this hydrophobic binding to the conformational change. Equations 270 and 272 exhibit the dominant steric effect also. The bulkiest group in the series (14, Table 38) produced a break in the structure–activity relationship; hence, it was not included in the regression. The electronic effect was noted only in eq 270. Since there was not sufficient variation in the σ^* value for the alkyl group in LII, no role for it could be seen in the hydrolysis of the derivatives of LII, and the addition of a second variable to eq 271 for such a small number of data points was meaningless.

The binding of some D- and L-N-acyl-α-aminoamides LXIV was, however, shown to depend upon the molar refractivity of the R and NHCOR′ groups.[313] For the



LXIV

L isomers that acted as substrates, K_m was related as shown by eq 273, and for the D isomers and some glycine derivatives that acted as inhibitors, K_i was related as shown by eq 274, where MR_l and MR_s refer to the molar refractivity of the larger and smaller of the two substituents (R and NHCOR′) at the α-carbon, respectively, and I_1 is an indicator variable with a value of 1 for hydrazides (NH_2 replaced by $NHNH_2$) and zero for all other cases. The positive coefficient of I_1 in both equations indicated that hydrazides bind more strongly than amides, and a higher positive coefficient of MR_l in eq 274 than in eq 273 suggested that inhibitors bind more tightly than the substrates. Since poorer correlations were obtained with π, Yoshimoto and Hansch[313] concluded that the binding pockets around the active site were not typically hydrophobic.

$$pK_m = 0.666\ (\pm 0.27)MR_l + 0.227\ (\pm 0.12)MR_s + 0.300\ (\pm 0.27)I_1 - 0.867\ (\pm 0.99)$$
$$n = 24,\ r = 0.826,\ s = 0.249 \qquad (273)$$

$$pK_i = 0.744\ (\pm 0.18)MR_l + 0.225\ (\pm 0.10)MR_s + 0.344\ (\pm 0.37)I_1 - 0.824$$
$$n = 21,\ r = 0.955,\ s = 0.234 \qquad (274)$$

In another study on ketones, amides, and esters (R_1COR_2; R_1 being mostly of the type $XC_6H_4OCH_2$ and R_2 being of the type CH_3, NHR, or OR), Yoshimoto and Hansch[192] showed that the inhibition activity related to only MR (eq 275). In eq 275, MR·R_1,R_2 represents the sum of the molar refractivities of the R_1 and R_2 groups. The square term of it indicates that very large groups will produce steric hindrance, but this term is not very significant. I_1 was given a value of unity for the cases where $R_2 = NHC_6H_4SO_2F$, and I_2 had a value of 1 for the cases where there were one or two halogens on the aromatic rings of R_1 and R_2. Thus, SO_2F in R_2 and Cl in the aromatic ring of any group were found to increase the activity. The effect of Cl was however attributed to a favorable conformational change.[192] I_3 and I_4 parametized the presence of COO^- on R_2 and R_1, respectively, and thus, their negative coefficients indicated that the highly hydrophilic COO^- had a deleterious effect, but less from the R_2 group and more from the R_1 group.

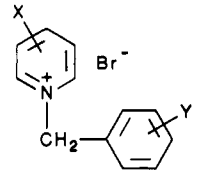
$$pI_{50} = 0.355\ (\pm 0.16)MR{\cdot}R_1,R_2 - 0.0099\ (\pm 0.0096) \times (MR{\cdot}R_1,R_2)^2 + 0.738\ (\pm 0.31)I_1 + 0.826\ (\pm 0.16)I_2 - 0.359\ (\pm 0.26)I_3 - 0.771\ (\pm 0.18)I_4 + 0.665\ (\pm 0.62)$$
$$n = 103,\ r = 0.944,\ s = 0.290 \qquad (275)$$

A new set of 33 data points for a similar series of inhibitors could be very well accommodated in eq 275, and a new equation (eq 276) was obtained[314] where the additional indicator parameter I_5 was used as a correction factor placing pK_i on the same basis as pI_{50}. The inhibition activity of the new set was measured in terms of K_i and not I_{50}.

$$pI_{50} = 0.33\ (\pm0.15)MR\cdot R_1,R_2 - 0.0083\ (\pm0.0087) \times (MR\cdot R_1,R_2)^2 + 0.75\ (\pm0.31)I_1 + 0.82\ (\pm0.13)I_2 - 0.35(\pm0.18)I_3 - 0.78\ (\pm0.18)I_4 + 0.23\ (\pm0.16)I_5 + 0.77\ (\pm0.55)$$

$$n = 136,\ r = 0.940,\ s = 0.292 \qquad (276)$$

Though for a set of benzylpyridinium bromides (LXV; X being a variety of substituents and Y being mostly NO_2, CH_3, Cl, and SO_2F), Yoshimoto and Hansch[192] correlated the inhibition activity with π (eq 277), the substitution of $MR_{X,Y}$ for $\pi_{X,Y}$ gave a correlation of comparable significance ($r = 0.869$). But since there was no high collinearity between $\pi_{X,Y}$ and $MR_{X,Y}$, these authors assumed that the active site of the enzyme was not very homogeneous but was well fit for either kind of interaction (hydrophobic or dispersion). Since in many other cases that would follow, Hansch and co-workers showed that the inhibition activity is better correlated with MR than with π, it could be that π is not so ideally measured as MR in these cases.



LXV

$$pI_{50} = 0.208\ (\pm0.06)\pi_{X,Y} + 1.135\ (\pm0.23)I_1 + 0.710\ (\pm0.23)I_2 + 0.276\ (\pm0.19)I_3 + 3.210\ (\pm0.26)$$

$$n = 56,\ r = 0.895,\ s = 0.291 \qquad (277)$$

In eq 277, I_1 and I_2 were each given a value of 1 to account for the effect of SO_2F at the 2- and 3-positions of the benzyl ring, respectively. It has been suggested that 2-SO_2F is favorably disposed in the ortho position to react with the hydroxyl group of the serine moiety. A smaller but beneficial effect of 3-SO_2F is also indicated. The indicator variable I_3 accounted for those cases where the nitrogen atom of an amide was attached directly to the pyridine ring. The slightly increased activity of such congeners was attributed to the electron donation by the amide nitrogen to the pyridinium ring.

As already mentioned, in a further study Hansch et al.[315] showed all interaction parameters like K_m, k_2, k_3, k_{cat}, K_i, etc., for compounds like $R_2CH(COOR_3)$-$NHCOR_1$ to be well related to MR. In varying series of $R_2CH(COOR_3)NHCOR_1$, the group $NHCOR_1$ was mostly the D or L form of NHCOMe, NHCOPh, $NHSO_2Me$, $NHCOOCH_2Ph$, NHCO-furyl, NHCO-2- or 3-pyridyl, etc., R_2 was mostly alkyl, phenyl, or benzyl, and OR_3 was mostly alkoxy, phenoxy, substituted phenoxy, etc. A long representative series is given in Table 39. For this series, K_m was related as shown by eq 278, and for a smaller series where $NHCOR_1$ was the D configuration, K_m was related as shown by eq 279. In these equations, MR-1, MR-2, and MR-3 referred to the molar refractivities of $NHCOR_1$ (or $NHSO_2R_1$), R_2, and OR_3, respectively. I_1 in eq 278 was given a value of 1 for $R_2 = CH(CH_3)_2$ and zero for other substituents. In eq 279, I_1 was used in place of MR-3, as OR_3 was either OMe or OPh-4-NO_2. It was equal to zero for the former, and 1 for the latter. σ_3* in eq 278 referred to the Taft constant of the R_3 group only.

$$pK_m = 1.09\ (\pm0.11)MR\text{-}2 + 0.80\ (\pm0.11)MR\text{-}1 + 0.52\ (\pm0.13)MR\text{-}3 - 0.63\ (\pm0.26)I_1 + 1.26\ (\pm0.28)\sigma_3^* - 0.057\ (\pm0.013)MR\text{-}1\cdot MR\text{-}2\cdot MR\text{-}3 - 1.61\ (\pm0.47)$$

$$n = 71,\ r = 0.979,\ s = 0.332 \qquad (278)$$

$$pK_m = 0.47\ (\pm0.31)MR\text{-}2 + 1.38\ (\pm0.59)MR\text{-}1 + 1.83\ (\pm0.72)I_1 + 2.76\ (\pm1.9)$$

$$n = 15,\ r = 0.993,\ s = 0.267 \qquad (279)$$

TABLE 40. Benzamidines LXVIII and Their Thrombin, Plasmin, Trypsin, and Complement Inhibition Activities

		pK_i			
compd	R	thrombin	plasmin	trypsin	complement
1	4-NO_2	2.5	2.4[a]	3.9	1.0[a]
2	3-CH_2OH	2.6	2.6	4.5	2.0
3	2-Me	1.0[a]	3.5	3.8	2.8
4	3-NO_2	2.6	3.0	4.1	2.8
5	3-COOH	2.7	3.4	2.3[a]	2.2
6	3-$CH_2C_6H_5$	3.4	3.9	5.2	3.2
7	H	2.9	3.3	5.1	3.2
8	3-NH_2	4.4[a]	4.0	5.2	3.2
9	3-C_6H_5	3.7	3.5	5.4	3.6
10	3-$N(CH_3)_2$	3.1	4.2	nt	3.7
11	3-OMe	3.1	3.5	4.9	3.8
12	3,4-Me_2	2.8	2.5[a]	5.1	3.9
13	3-Br	2.8	2.1[a]	nt	4.0
14	3,5-Me_2	2.0[a]	3.6	4.8	4.1
15	4-$CH_2COCOOH$	5.4[a]	4.9[a]	5.0[a]	4.9[a]
16	3-$O(CH_2)_3OC_6H_5$	3.8	4.7	4.0	5.0
17	4-OEt	2.8	nt[b]	4.0	<1[a]
18	4-OMe	2.7	nt	4.0	<1[a]
19	3-$CH_2COCOOH$	<1[a]	2.8	3.1[a]	<1[a]
20	3-naphthamidine	4.0[a]	4.8[a]	nt	<1[a]
21	4-CH_2OH	2.5	3.2	4.1	nt

[a] Not included in regressions. [b] Not tested.

For the K_m of some glycinates, $CH_2(COOR_3)$-$NHCOR_1$, eq 280 was obtained, and for the K_i of some D esters of an $R_2CH(COOR_3)NHCOR_1$ series, eq 281 was obtained. For a small group of aromatic acids

$$pK_m = 0.48\ (\pm0.10)MR\text{-}1 + 0.69\ (\pm0.10)MR\text{-}3 + 0.44\ (\pm0.23)\sigma_3^* - 0.20\ (\pm0.30)$$

$$n = 42,\ r = 0.981,\ s = 0.235 \qquad (280)$$

$$pK_i = 1.42\ (\pm0.25)MR\text{-}2 + 1.07\ (\pm0.27)MR\text{-}1 - 0.16\ (\pm0.08)MR\text{-}1\cdot MR\text{-}2\cdot MR\text{-}3 - 2.71\ (\pm0.81)$$

$$n = 12,\ r = 0.988,\ s = 0.207 \qquad (281)$$

(benzoic acid, *m*-toluic acid, *p*-toluic acid, hydrocinnamic acid, 4-phenyl-*n*-butyric acid, 2-naphthoic acid, 4-*tert*-butylbenzoic acid) and some miscellaneous compounds (cyclohexane, benzene, pentane, toluene, ethylbenzene, nitrobenzene, chlorobenzene, indene, naphthalene, azulene, anthracene), the inhibition activities were found to be related to MR as exhibited by eq 282 and 283, respectively.[315]

$$pK_i = 1.06\ (\pm0.20)MR - 1.49\ (\pm0.85)$$

$$n = 7,\ r = 0.987,\ s = 0.132 \qquad (282)$$

$$pK_i = 1.02\ (\pm0.25)MR - 0.38\ (\pm0.89)$$

$$n = 11,\ r = 0.952,\ s = 0.325 \qquad (283)$$

The acylation parameter k_2 for a series of L esters was also shown to depend upon MR (eq 284). MR-3 was not found to affect the correlation, possibly because there was not much variation in the R_3 group. I_1 was used for $R_2 = CH(CH_3)_2$.

$$\log k_2 = 1.10\ (\pm0.25)MR\text{-}2 - 0.52\ (\pm0.22)MR\text{-}1 - 1.56\ (\pm0.50)I_1 + 0.42\ (\pm0.85)$$

$$n = 18,\ r = 0.978\ s = 0.399 \qquad (284)$$

The k_2 data at pH 7 and 6 for X-$C_6H_4COOC_6H_4$-4-NO_2, where X was a small group such as Me, Cl, CF_3, NO_2, etc., were correlated by eq 285 and 286, and some k_2 values at pH 7 for X-$C_6H_4COOC_6H_3$-2,4-$(NO_2)_2$ were correlated by eq 287.

pH 7: $\log k_2 =$
$0.59\ (\pm0.23)\sum MR + 0.62\ (\pm0.24)\sum\sigma^+ + 3.09\ (\pm0.20)$

$$n = 14,\ r = 0.907,\ s = 0.163 \qquad (285)$$

pH 6: $\log k_2 =$
$0.83\ (\pm0.25)\sum MR + 0.49\ (\pm0.21)\sum\sigma^+ + 2.57\ (\pm0.20)$

$$n = 13,\ r = 0.933,\ s = 0.144 \qquad (286)$$

pH 7: $\log k_2 =$
$0.96\ (\pm0.27)$MR-p $+ 0.97\ (\pm0.25)\sigma^+ + 4.87\ (\pm0.18)$

$$n = 14,\ r = 0.949,\ s = 0.178 \qquad (287)$$

The deacylation step in chymotrypsin hydrolysis of some *p*-nitrophenyl esters (Table 38) was correlated by Hansch[311] as shown by eq 270. To this series were added additional data, and eq 288 was obtained.[315] The

$\log k_3 = 2.09\ (\pm0.34)\sigma^* + 1.21\ (\pm0.27)E_s +$
$0.34\ (\pm0.10)$MR $- 0.95\ (\pm0.71)I_1 - 1.91\ (\pm0.30)$

$$n = 36,\ r = 0.975,\ s = 0.320 \qquad (288)$$

replacement of MR by π here gave an inferior correlation. The indicator parameter I_1 was used for the cases where the substituent involved a substituted or unsubstituted phenyl ring. However, the data on deacylation of compounds belonging to the $R_2CH(COOR_3)$-$NHCOR_1$ series were found to depend only on MR-2 (eq 289). R_3 does not participate in deacylation, and

$\log k_3 = 0.75\ (\pm0.14)$MR-2 $- 1.79\ (\pm0.28)I_1 -$
$1.48\ (\pm0.26)I_2 - 0.31\ (\pm0.30)$

$$n = 33,\ r = 0.977,\ s = 0.289 \qquad (289)$$

Hansch et al. assumed in this case that R_1 was out of contact with the enzyme. I_1 in eq 289 takes the value of 1 for D isomers and zero for L isomers, and I_2 is assigned a value of 1 for $R_2 = CH(CH_3)_2$.

For a few cases, the activity was measured in terms of k_{cat}, which is defined as $k_{cat} = k_2k_3/(k_2 + k_3)$. This too was found to be related to MR. Equation 290 was obtained for a series of $R_2CH(COOR_3)NHCOR_1$, and eq 291 was obtained for a series of $CH_2(COOR_3)$-$NHCOR_1$.[315] In the former, I_1 was used for $R_2 = CH(CH_3)_2$, and in the latter, I_1 was used for R_1 being an aromatic moiety.

$\log k_{cat} = 1.79\ (\pm0.33)$MR-2 $-$
$0.24\ (\pm0.08)$(MR-2)$^2 - 1.45\ (\pm0.26)I_1 -$
$0.01\ (\pm0.009)$MR-1·MR-2·MR-3 $- 1.51\ (\pm0.31)$

$$n = 57,\ r = 0.959,\ s = 0.291 \qquad (290)$$

$\log k_{cat} = 0.42\ (\pm0.10)$MR-3 $- 0.27\ (\pm0.19)$MR-1 $+$
$0.64\ (\pm0.34)I_1 - 1.29\ (\pm0.49)$

$$n = 36,\ r = 0.905,\ s = 0.291 \qquad (291)$$

All these studies on substrates and inhibitors of chymotrypsin showed that their activities were dominantly the function of molar refractivity. However, the meaning of so high a dependence of activities on MR was not clear, but the working hypothesis of these authors was that MR space was different from π space and consisted of predominantly polar amino acid residues.

To further verify their observations and hypothesis, the Hansch group synthesized[316] a new series of $R_2CH(COOR_3)NHCOR_1$ with R_2 = H and COR_1 = 3-COC_5H_4N. Changing only the R_3 group, they measured the k_{cat} and K_m values for the compounds and subjected them to a QSAR analysis.[316] For the K_m values of 14 compounds studied (R_3 being mostly alkyl or substituted alkyl groups), the best equation obtained was eq 292. The use of π-3 in place of MR-3 gave a much poorer correlation (r = 0.893). This supported the view that the a_3 space was not typically hydrophobic.

$pK_m =$
$0.46\ (\pm0.20)$MR-3 $+ 1.36\ (\pm0.28)\sigma_3^* + 1.05\ (\pm0.40)$

$$n = 14,\ r = 0.957,\ s = 0.198 \qquad (292)$$

This new set of data could be well combined with those used to derive eq 278 to give eq 293. The coef-

$pK_m = 0.77\ (\pm0.11)$MR-1 $+ 1.13\ (\pm0.11)$MR-2+
$0.47\ (\pm0.11)$MR-3 $- 0.56\ (\pm0.25)I_1 + 1.35$
$(\pm0.22)\sigma_3^* - 0.055\ (\pm0.01)$MR-1·MR-2·MR-3 $-$
$1.64\ (\pm0.46)$

$$n = 85,\ r = 0.977,\ s = 0.333 \qquad (293)$$

ficients of eq 293 closely agree with those of eq 278, and the quality of fit is essentially the same. It is also worth noting that the coefficients of MR-3 and σ_3^* of eq 292 are almost the same as in eq 293 (or eq 278).

Combining their k_{cat} values with those used to obtain eq 290 and some measured by Béchet et al.[317] and Dorovska et al.,[318] Grieco et al.[316] derived eq 294 for k_{cat}/K_m. In eq 294, I_1 was equal to 1 not for only the

$\log (k_{cat}/K_m) = 0.76\ (\pm0.14)$MR-1 $+$
$3.19\ (\pm0.35)$MR-2 $+ 0.56\ (\pm0.13)$MR-3 $+$
$1.30\ (\pm0.26)\sigma_3^* - 2.27\ (\pm0.28)I_1 - 0.32$
(±0.08)(MR-2)$^2 - 0.067\ (\pm0.02)$MR-1·MR-2·MR-3 $-$
$3.21\ (\pm0.61)$

$$n = 77,\ r = 0.988,\ s = 0.369 \qquad (294)$$

isopropyl group but for the *sec*-butyl group also at R_2. This equation showed that the initial increase in the value of MR-1, MR-2, and MR-3 favored the hydrolysis. As with the isopropyl and *sec*-butyl groups in a_2 space, the *tert*-butyl group was found to produce steric hindrance in hydrolysis in the a_3 space, so the *tert*-butyl congener was misfit in eq 294.

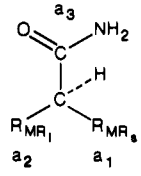
Hansch et al.[315] compared the binding of esters with that of (acylamino)amides LXIV. The two equations (273 and 274) obtained for the D and L isomers of (acylamino)amides could be combined to a single equation (eq 295)[313,315] where I_1 was used for hydrazides

$pK = 0.72\ (\pm0.13)MR_l + 0.230\ (\pm0.07)MR_s +$
$0.32\ (\pm0.20)I_1 + 0.31\ (\pm0.15)I_2 - 1.06\ (\pm0.45)$

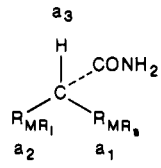
$$n = 45,\ r = 0.928,\ s = 0.235 \qquad (295)$$

and I_2 was given a value of 1 for D isomers and glycine amides and zero for L isomers. Equation 295 was important in the sense that substrates (L isomers) as well as inhibitors (D isomers and glycine derivatives) both could be well accounted for by a single equation. This equation also indicated that D isomers and glycine derivatives bind about twice as strongly as do L isomers. The assumed picture of binding of the two types of

congeners was given[315] as shown by LXVIa and LXVIb, where the $CONH_2$ group of the D isomer was shown to be in a_H space and not in a_3 space where hydrolysis is supposed to occur; hence, D isomers did not act as substrates.



LXVIa, L isomer



LXVIb, D isomer

Notwithstanding this, some D esters for which eq 279 was derived acted as substrates. Since for them MR-1 (for $NHCOR_1$) > MR-2 (for R_2), $NHCOR_1$ was assumed to lie in a_2 space, R_2 in a_1 space, and hence the R_3 group in a_3 space. For those D esters acting as inhibitors and for which eq 281 was obtained, MR-2 > MR-1, the positions of $NHCOR_1$ and R_2 had interchanged, and hence the R_3 group could not lie in a_3 space. Hansch et al., however, were not able to explain why all of the L esters that gave eq 278 acted only as substrates, while many of them had MR-2 > MR-1. However, the cross-product term MR-1·MR-2·MR-3 in eq 278, 293, or 294 indicated that placing too much bulk in any space, a_1, a_2, or a_3, produced hindrance in the interaction. The role of σ_3^* was doubtful as there was a considerable collinearity between σ_3^* and MR-3.

In the case of hydrolysis of glycinates (eq 280), where R_2 = H for each derivative and hence MR-1 was always greater than MR-2, one would have safely assumed that R_3 would be in a_3 space to bring about the hydrolysis. But Hansch et al. assumed a different picture and put R_3 in the a_2 space and H, i.e., R_2 in a_3 space, and thus complicated the explanation.

The acidic and neutral inhibitors for which eq 282 and 283 were obtained were suggested to bind in a_2 space in the same way as L esters, as the coefficients of their MR were almost equal to the coefficient of MR-2 in eq 278.

The coefficient of MR-2 in eq 284, which correlates the acylation step of L esters, is also equal to that of MR-2 in eq 278. This shows that the binding of R_2 in a_2 space is important in the acylation itself. However, the negative coefficient of MR-1 in eq 284 shows that a large R_1 group will hinder the acylation.

Equations 285–287, meant for nitrophenyl esters, do not provide any clear picture of acylation. The X substituent can be speculated to bind in a_2 space.

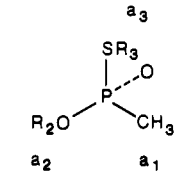
That the deacylation step also involved the binding of R_2 in a_2 space was obvious from eq 289, but the negative coefficient of I_1, the indicator parameter to isolate the D isomers, showed that D isomers were difficult to deacylate. The binding space for deacylation of substrates of the type $RCOOC_6H_4$-4-NO_2 was, however, not clear from eq 288. Since the coefficient of MR in eq 288 was very low as compared with that of MR-2 in eq 289, Hansch et al. assumed the binding to occur in a_3 space in this case. The parameters E_s and I_1 also indicated the steric hindrance in the binding of $RCOOC_6H_4$-4-NO_2 derivatives.

The importance of the binding in a_2 space was also shown by eq 290 and 294, which were obtained for k_{cat} and k_{cat}/K_m. But for $CH_2(COOR_3)NHCOR_1$ derivatives for which k_{cat} was related by eq 291, Hansch et al. assumed,[315] in the absence of an R_2 group (bigger than H), the R_3 group to bind in a_3 space. While eq 290 showed that a bigger group in the esters in any space, but particularly in a_2, will hinder the interaction, eq 291, with a positive value of I_1, showed that in glycinates the R_1 group, if an aromatic moiety, will bind more strongly.

Equations 278 and 292–294 all establish the importance of binding in a_3 space. Binding in a_1 space also appears to be equally important. However, all these studies do not support the classical view that chymotrypsin has a usual type of hydrophobic pocket. This is especially true for a_1 and a_2 spaces. Both of these regions have been studied with congeners for which π and MR were reasonably orthogonal, and in almost every instance MR was found to be far superior to π in the correlation equations.

In most of the cases, an indicator parameter was used to indicate whether R_2 was an isopropyl group. In almost all the cases, its coefficient was negative. It therefore indicated that this group will hinder interaction in a_2 space. The types of ligands binding in a_2 space have not been as extensively varied as those binding in a_1 and a_3 spaces; nonetheless, MR-2 consistently gave better results than π-2.

A great similarity was found in the interactions of acylamino acid esters and a series of phosphonates with chymotrypsin. Phosphonates LXVII were found to act



LXVII

as inhibitors of chymotrypsin,[319] but were called[319] "quasi-substrates" because they acylated the enzyme. Deacylation did not occur with them. The inhibition activity of 53 alkyl phosphonates [LXVII; R_2 = alkyl, R_3 = alkyl, $(CH_2)_nSC_2H_5$, $(CH_2)_nS^+(CH_3)C_2H_5$] studied by Aaviksaar[319] was related to MR and σ^* as shown in eq 296,[320] where the indicator parameter I was used with

$$\log k_i = 1.47\ (\pm 0.10)MR_{OR_2} + 0.34\ (\pm 0.09)MR_{SR_3} + 1.25\ (\pm 0.19)\sigma_3^* - 1.06\ (\pm 0.31)I - 3.43\ (\pm 0.74)\log(\beta \cdot 10^{MR_{OR2}} + 1) - 5.26\ (\pm 0.38)$$

$$n = 53,\ r = 0.985,\ s = 0.243 \qquad (296)$$

a value of unity for congeners having $R_3 = (CH_2)_nS^+(CH_3)C_2H_5$. The close correspondence of eq 296 with eq 278 or 293 shows how similar is the interaction of chymotrypsin with acylamino acid esters that act as substrates and with phosphonates that are quasi-substrates and act as inhibitors. Equation 296 could also be well compared with eq 297, which was obtained[320]

$$\log (k_2/K_s) = 1.78\ (\pm 0.21)MR\text{-}2 + 0.44\ (\pm 0.18)MR\text{-}1 - 2.13\ (\pm 0.46)I - 1.06\ (\pm 0.70)$$

$$n = 20,\ r = 0.990,\ s = 0.372 \qquad (297)$$

for k_2/K_s of a small set of ester substrates, congeners of $R_2CH(COOR_3)NHCOR_1$, studied by Berezin et al.[321] In the derivation of eq 297, the MR-3 term was not included, as there was little variation in the R_3 group. The terms MR-1, MR-2, MR-3, and I have the same meanings as in eq 278 or 293.

For a subgroup of the phosphonate series, where all congeners had a constant R_3 group (C_4H_9), the correlation obtained was as shown by eq 298 (or 299),[320]

$$\log k_i = 1.60\ (\pm 0.22)MR_{OR_2} - 3.85\ (\pm 1.17)\log(\beta \cdot 10^{MR_{OR2}} + 1) - 4.76\ (\pm 0.51)$$
$$n = 19,\ r = 0.978,\ s = 0.258 \qquad (298)$$

$$\log k_i = 2.87\ (\pm 0.84)MR_{OR_2} - 0.35\ (\pm 0.15)(MR_{OR_2})^2 - 5.80\ (\pm 1.1)$$
$$n = 19,\ r = 0.961,\ s = 0.331 \qquad (299)$$

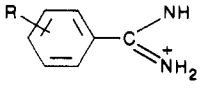
which particularly pointed out the same mode of binding of phosphonates in a_2 space of chymotrypsin as that of esters. A large OR_2 group will produce steric hindrance in the binding of phosphonates also.

The dependence of chymotrypsin binding on MR was studied qualitatively by Rapp et al. also.[322] However, in spite of numerous discussions in favor of MR, the importance of hydrophobic interaction in chymotrypsin–ligand binding cannot be totally ignored.[323]

2. Trypsin, Thrombin, Plasmin, and Complement

The essential feature of blood coagulation, complement activation, fibrinolysis, and digestion is the activation of serine proteinases, namely trypsin, thrombin, plasmin, and complement, which specifically hydrolyze protein substrates.[324] All these enzymes bind to protein substrates at lysine and/or arginine residues. Human trypsin is a pancreatic, digestive enzyme consisting of a single polypeptide chain. It has a specificity toward lysyl and arginyl residues and cleaves the C-terminal end to these residues in any protein. Human plasmin, consisting of two polypeptide chains, is a serum proteinase and cleaves the C-terminal end to both lysine and arginine in a wide variety of proteins, including casein, I_gG, insulin, fibrin, and others. Human thrombin is a proteolytic enzyme that is generated from the procoagulant factor prothrombin. It contains two polypeptide chains and cleaves the C-terminal end to arginine in fibrinogen, its primary protein substrates. Complement (C_{1s}) is a mixture of 11 serum proteins and is known to possess hydrolytic activity similar to thrombin and plasmin.[325]

All four enzymes have been found to be competitively inhibited by benzamidine, a small organic molecule that is an excellent model for the cationic side chains of arginine and lysine.[326] In order to study the structural basis of the substrate-binding specificity, certain QSAR studies on the derivatives of benzamidine LXVIII were made.



LXVIII

In a study on derivatives as listed in Table 40, Andrews et al.[327] obtained eq 300 for human trypsin inhibition, where MW represents the molecular weight of the compound. In this expression, the molar refractivity was used for only meta substituents, while

$$pK_i = 4.77\ (\pm 0.16) + 0.88\ (\pm 0.33)MR_m - 0.24\ (\pm 0.11)MW$$
$$n = 15,\ r = 0.84,\ s = 0.32 \qquad (300)$$

there were some para- and orthosubstituted derivatives also. The effect of 4-substituents is therefore shown to be only steric, as MW was used for all types of analogues. Since MW was used for the 3-substituted analogues also, its negative coefficient and the positive coefficient of MR_m show an ambivalent effect of 3-substituents in trypsin inhibition. No role for an electronic effect was found by Andrews et al. Since there was only one 2-substituted analogue (2-methyl derivative) to be included in eq 300, an effect of the 2-substituent could not be analyzed.

Excluding the 2-substituted congener, Recanatini et al.[328] made a more detailed QSAR study on the data of Andrews et al. and obtained eq 301–303. In these

$$pK_i = 5.37\ (\pm 0.49) - 0.44\ (\pm 0.27)B_4$$
$$n = 14,\ r = 0.713,\ s = 0.395 \qquad (301)$$

$$pK_i = 5.48\ (\pm 0.39) - 0.49\ (\pm 0.19)B_4 - 0.80\ (\pm 0.58)\sigma$$
$$n = 14,\ r = 0.855,\ s = 0.305 \qquad (302)$$

$$pK_i = 5.30\ (\pm 0.39) - 0.42\ (\pm 0.20)B_4 - 0.71\ (\pm 0.52)\sigma + 0.18\ (\pm 0.18)\pi_3$$
$$n = 14,\ r = 0.904,\ s = 0.263 \qquad (303)$$

equations, B_4 is the width parameter of Verloop et al.,[25] which has been used here for para substituents. The negative coefficient of it shows the importance of steric effects produced by the 4-substituents. From these equations, the electronic character of both the 3- and 4-substituents are found to be important. Electron release by the substituents from any position will lead to an increase in activity. Equation 303 shows the possibility of hydrophobic interaction from the 3-position (π_3 was derived from the nitrobenzene system).

For a small series of only 4-substituted benzamidines studied by Mares-Guia et al.[329] with bovine trypsin, Recanatini et al.[328] derived eq 304 and 305. These

$$pK_i = 4.46\ (\pm 0.20) - 0.90\ (\pm 0.51)\sigma$$
$$n = 10,\ r = 0.820,\ s = 0.279 \qquad (304)$$

$$pK_i = 4.89\ (\pm 0.47) - 0.75\ (\pm 0.45)\sigma - 0.20\ (\pm 0.21)B_4$$
$$n = 10,\ r = 0.902,\ s = 0.225 \qquad (305)$$

equations exhibited more of the electronic than the steric effects from the 4-substituents in bovine trypsin inhibition as opposed to that seen in human trypsin inhibition (eq 301–303). For their data on bovine trypsin, Mares-Guia et al.[329] had themselves shown the dominance of the electronic effect (eq 306). In addition to deriving eq 306, these authors noted a positive correlation between pK_i and the net π-electron density at the central atom of each substituent as calculated by an ω-technique.

$$pK_i = 4.40 - 0.88\sigma \qquad n = 10,\ r = 0.818 \qquad (306)$$

In the analysis of the data of Mares-Guia et al. or those of Andrews et al., congeners containing the COO^- or COOH group were not included. They were found to be poorly fit in the correlating equations. The reason for this poor fit may be attributed to the fact that σ constants for charged groups (COOH may be ionized as COO^- and H^+) are not constant.[330]

The use of MR_4 in place of B_4 in all these analyses made by Recanatini et al. was found to give a poorer correlation.

TABLE 41. Trypsin Inhibition Constants of Benzamidines LXVIII[a]

compd	R	pI_{50}
22	4-CON(CH$_3$)C$_6$H$_5$	3.10
23	4-X$_4$C$_6$H$_4$-4′-YC$_6$H$_4$-4′-YC$_6$H$_4$-4″-SO$_2$F	3.22
24	4-COCH$_3$	3.44
25	H	4.49
26	4-X$_3$C$_6$H$_5$	4.82
27	4-O(CH$_2$)$_3$C$_6$H$_4$-4′-YCH$_2$Br	5.04
28	4-O(CH$_2$)$_3$C$_6$H$_5$	5.10
29	3-X$_3$C$_6$H$_5$	5.12
30	3-X$_4$C$_6$H$_4$-4′-ZC$_6$H$_4$-4″-SO$_2$F	5.14
31	4-X$_3$C$_6$H$_4$-4′-YC$_6$H$_4$-4″-SO$_2$F	5.21
32	4-X$_4$C$_6$H$_4$-4′-YCH$_2$Br	5.27
33	4-X$_2$C$_6$H$_4$-3′-ZC$_6$H$_4$-4″-SO$_2$F	5.32
34	4-X$_4$C$_6$H$_4$-4′-YC$_6$H$_4$-3″-SO$_2$F	5.35
35	4-X$_4$C$_6$H$_4$-3′-YC$_6$H$_4$-4″-SO$_2$F	5.36
36	4-X$_2$C$_6$H$_4$-3′-ZC$_6$H$_3$-4″-CH$_3$-3″-SO$_2$F	5.39
37	4-X$_4$C$_6$H$_4$-4′-YC$_6$H$_4$-4″-SO$_2$F	5.39
38	4-X$_3$C$_6$H$_4$-4′-ZC$_6$H$_4$-4″-SO$_2$F	5.40
39	4-X$_2$C$_6$H$_4$-3′-ZC$_6$H$_4$-3′-SO$_2$F	5.44
40	4-X$_3$C$_6$H$_4$-4′-YC$_6$H$_4$-3″-SO$_2$F	5.47
41	4-X$_4$C$_6$H$_4$-4′-ZC$_6$H$_4$-3″-SO$_2$F	5.49
42	4-X$_2$C$_6$H$_4$-3′-YC$_6$H$_4$-4″-SO$_2$F	5.52
43	4-X$_4$C$_6$H$_4$-4′-ZC$_6$H$_3$-2″-OCH$_3$-5″-SO$_2$F	5.55
44	4-X$_4$C$_6$H$_3$-2′-Cl-4′-ZC$_6$H$_4$-3″-SO$_2$F	5.60
45	4-X$_4$C$_6$H$_4$-4′-ZC$_6$H$_3$-4″-OCH$_3$-3″-SO$_2$F	5.60
46	4-X$_4$C$_6$H$_3$-2′-Cl-4′-ZC$_6$H$_4$-4″-SO$_2$F	5.62
47	4-X$_4$C$_6$H$_4$-4′-ZC$_6$H$_3$-4″-CH$_3$-3″-SO$_2$F	5.64
48	4-X$_4$C$_6$H$_4$-4′-ZC$_6$H$_3$-4″-OC$_2$H$_5$-3″-SO$_2$F	5.66
49	4-X$_2$C$_6$H$_4$-3′-ZC$_6$H$_3$-2″-Cl-5″-SO$_2$F	5.74
50	4-X$_4$C$_6$H$_4$-4′-ZC$_6$H$_3$-2″-Cl-4″-SO$_2$F	5.80
51	4-X$_4$C$_6$H$_4$-4′-ZC$_6$H$_3$-3″-CH$_3$-4″-SO$_2$F	5.80
52	4-X$_4$C$_6$H$_4$-4′-ZC$_6$H$_3$-2″-Cl-5″-SO$_2$F	5.82
53	4-X$_4$C$_6$H$_4$-4′-ZC$_6$H$_4$-4″-SO$_2$F	5.85
54	4-X$_4$C$_6$H$_3$-3′-CH$_3$-4′-ZC$_6$H$_4$-4″-SO$_2$F	5.85

[a] X$_n$ = O(CH$_2$)$_n$O, Y = NHCO, Z = NHCONH.

For another set of data on bovine trypsin inhibition by 4-substituted benzamidines studied by Markwardt et al.,[331] Coats[332] could find the correlation as shown by eq 307, which exhibited the electronic effect but not the steric effect of the 4-substituents. Rather, a role for the hydrophobic character of these substituents was shown. A dominant role for the hydrophobic character of the 3-substituents was found by Coats (eq 308)[332] when he analyzed the bovine trypsin inhibition data for a small set of 3-substituted benzamidine analogues studied by Markwardt et al.[331] The inclusion of σ was not found to make any improvement over the correlation shown by eq 308.

$$pK_i = 0.20\ (\pm 0.07)\pi - 0.58\ (\pm 0.43)\sigma + 1.16$$
$$n = 26,\ r = 0.85,\ s = 0.27 \qquad (307)$$

$$pK_i = 0.22\ (\pm 0.06)\pi + 1.46$$
$$n = 8,\ r = 0.97,\ s = 0.08 \qquad (308)$$

The importance of the hydrophobic and electronic characters of the 4-substituents of benzamidines in bovine trypsin inhibition was also shown by Yoshimoto and Hansch[192] when they analyzed the data of Baker and Erickson[326b,333] (Table 41). Excluding compounds 22 and 23 of Table 41, Yoshimoto and Hansch obtained eq 309, which has close correspondence with eq 307, except that in place of σ σ^-, which described the electron-withdrawal effect by direct resonance interaction, was used. This close correspondence of eq 309 with eq 307 occurred in spite of the fact that congeners used to derive the former had comparatively very large substituents. However, since the use of MR in place of π in eq 309 had given an equally high correlation, Yoshimoto and Hansch assumed that, as with chymotrypsin, true hydrophobic interactions were also not involved in trypsin inhibition.

$$pI_{50} = 0.184\ (\pm 0.05)\pi - 0.978\ (\pm 0.32)\sigma^- + 4.463$$
$$n = 31,\ r = 0.949,\ s = 0.155 \qquad (309)$$

The importance of the molar refractivity of the 4-substituents in benzamidines was shown by Recanatini et al.[328] with the data of Labes and Hagen.[334] For compounds 55–112 of Table 42, they obtained eq 310–312. It is surprising that the coefficient of MR_4 is positive in eq 310 and 311, but negative in eq 312. However, since eq 312, representing a bilinear model, was statistically more significant, Recanatini et al. based their interpretation on this equation only and argued that until the optimum value of MR_4 = 1.11 the smaller substituents would produce the steric effect comparable to that found in eq 303 and 305. This steric effect was assumed to be due to the bad contact of the smaller substituents with the enzyme. However, beyond the optimum value of MR_4, the large lengthy substituents were expected to produce the positive effect of MR.

$$pK_i = 0.27\ (\pm 0.06)MR_4 + 0.85\ (\pm 0.18)$$
$$n = 55,\ r = 0.778,\ s = 0.329 \qquad (310)$$

$$pK_i = 0.24\ (\pm 0.05)MR_4 - 0.76\ (\pm 0.26)\sigma + 0.95\ (\pm 0.15)$$
$$n = 55,\ r = 0.875,\ s = 0.256 \qquad (311)$$

$$pK_i = -0.52\ (\pm 0.45)MR_4 + 0.84\ (\pm 0.50)\log(\beta \cdot 10^{MR_4} + 1) - 0.73\ (\pm 0.24)\sigma + 1.41\ (\pm 0.31)$$
$$n = 55,\ r = 0.900,\ s = 0.235,\ MR_4(\text{opt}) = 1.11 \qquad (312)$$

Using an indicator variable I_1 for 12 carbonyl-containing side chains (compounds 113–124), Recanatini et al. obtained eq 313.

$$pK_i = -0.59\ (\pm 0.52)MR_4 + 0.88\ (\pm 0.55)\log(\beta \cdot 10^{MR_4} + 1) - 0.74\ (\pm 0.23)\sigma + 0.51\ (\pm 0.16)I_1 + 1.38\ (\pm 0.30)$$
$$n = 67,\ r = 0.928,\ s = 0.233,\ MR_4(\text{opt}) = 1.03 \qquad (313)$$

For meta-substituted benzamidines studied by Labes and Hagen (Table 42), Recanatini et al. first derived eq 314, which included only compounds 126–152, and then derived eq 315, which included the remaining 12 compounds also characterized by two indicator variables I_2 and I_3. I_2 with a value of unity characterized substituents of the types 3-CH$_2$CO$_2$R and 3-CH$_2$COC$_6$H$_4$X (compounds 153–159), and I_3 with a value of unity characterized substituents of the types 3-CH=CHCO$_2$R and 3-CH=CHCOC$_6$H$_4$X (compounds 160–164). The coefficient of π_3 of eq 314 or that of eq 315 agrees well with that of eq 303, showing the importance of the hydrophobic character of the 3-substituents. π_3 was derived in this case also from the nitrobenzene system.

$$pK_i = 0.21\ (\pm 0.09)\pi_3 - 0.79\ (\pm 0.62)\sigma + 1.63\ (\pm 0.22)$$
$$n = 25,\ r = 0.823,\ s = 0.231 \qquad (314)$$

$$pK_i = 0.23\ (\pm 0.07)\pi_3 - 0.75\ (\pm 0.53)\sigma + 0.43\ (\pm 0.19)I_2 + 0.65\ (\pm 0.21)I_3 + 1.61\ (\pm 0.18)$$
$$n = 37,\ r = 0.909,\ s = 0.204 \qquad (315)$$

There is a great similarity among the coefficients of I_1, I_2, and I_3 of eq 313 and 315. They all showed the increase in activity by carbonyl-containing substituents.

TABLE 42. Labes and Hagen Data on Trypsin Inhibition by Benzamidines LXVIII

compd	R	pK_i	compd	R	pK_i
55	H	1.46	110	4-CH=CHCOC$_6$H$_5$	1.82
56	4-CH$_3$	1.52	111	4-CH=CHCOC$_6$H$_4$-4′-OCH$_3$	1.96
57	4-C$_2$H$_5$	1.16	112	4-CH=CHCOC$_6$H$_4$-3′-NO$_2$	1.72
58	4-C$_3$H$_7$	1.42	113	4-OCH$_2$COOCH$_3$	2.10
59	4-C$_4$H$_9$	1.55	114	4-OCH$_2$COOC$_2$H$_5$	2.19
60	4-C$_5$H$_{11}$[a]	2.10	115	4-OCH$_2$COC$_6$H$_5$	2.30
61	4-Cl	1.40	116	4-(CH$_2$)$_2$COOCH$_3$	1.92
62	4-Br	1.40	117	4-(CH$_2$)$_2$COOC$_2$H$_5$	1.85
63	4-NO$_2$	0.48	118	4-(CH$_2$)$_2$COCH$_3$	2.38
64	4-OH	1.30	119	4-(CH$_2$)$_2$COC$_6$H$_5$	2.57
65	4-CHO	1.01	120	4-(CH$_2$)$_2$COC$_6$H$_4$-4′-CH$_3$	2.52
66	4-CH$_2$Br	1.38	121	4-(CH$_2$)$_2$COC$_6$H$_4$-4′-OCH$_3$	2.72
67	4-COOCH$_3$	0.52	122	4-(CH$_2$)$_2$COC$_6$H$_4$-4′-Cl	2.46
68	4-COOC$_2$H$_5$	0.70	123	4-(CH$_2$)$_2$COC$_6$H$_4$-4′-OC$_6$H$_5$	2.82
69	4-COOC$_3$H$_7$	1.05	124	4-(CH$_2$)$_2$COC$_6$H$_4$-4′-OC$_6$H$_5$	3.42
70	4-COO-*i*-C$_3$H$_7$	0.92	125	4-SO$_2$NHC$_6$H$_5$[a]	0.70
71	4-COOC$_8$H$_{17}$	1.80	126	3-CH$_3$	1.50
72	4-COCH$_3$	0.50	127	3-Cl	1.38
73	4-CONHCH$_3$	0.85	128	3-NO$_2$	0.89
74	4-COC$_6$H$_5$	0.92	129	3-OH	1.48
75	4-COCH$_2$OCH$_3$	1.19	130	3-COOCH$_3$	1.06
76	4-OCH$_3$	1.50	131	3-COOC$_2$H$_5$	1.19
77	4-OC$_2$H$_5$	1.00	132	3-COO-*i*-C$_3$H$_7$[a]	1.00
78	4-OC$_3$H$_7$	1.05	133	3-COOCH$_2$C$_6$H$_5$	1.52
79	4-OC$_4$H$_9$	1.42	134	3-COCH$_3$	1.62
80	4-OC$_5$H$_{11}$	1.70	135	3-COC$_6$H$_5$	1.50
81	4-OC$_6$H$_{13}$	1.77	136	3-CONH$_2$	1.38
82	4-OC$_7$H$_{15}$	2.00	137	3-CONHCH$_3$	0.82
83	4-OC$_8$H$_{17}$	2.22	138	3-CONHCH$_2$C$_6$H$_5$	1.82
84	4-OC$_9$H$_{19}$	2.40	139	3-CONHC$_6$H$_5$	1.63
85	4-OC$_{10}$H$_{21}$	2.30	140	3-COC$_6$H$_4$-4′-CH$_3$	2.00
86	4-OC$_{11}$H$_{23}$[a]	2.40	141	3-COC$_6$H$_4$-4′-OCH$_3$	2.00
87	4-OC$_{12}$H$_{25}$[a]	2.16	142	3-COC$_6$H$_4$-4′-Cl	1.89
88	4-OCH$_2$C$_6$H$_5$	2.04	143	3-OCH$_3$	1.60
89	4-O(CH$_2$)$_3$C$_6$H$_5$	2.17	144	3-OC$_2$H$_5$	1.57
90	4-O(CH$_2$)$_2$C$_6$H$_5$	1.85	145	3-OC$_4$H$_9$	1.92
91	4-O(CH$_2$)$_2$OH	1.32	146	3-OC$_5$H$_{11}$	1.96
92	4-NHCOC$_6$H$_5$	1.72	147	3-OC$_6$H$_{13}$	2.10
93	4-NHCOC$_6$H$_4$-4′-NO$_2$	2.22	148	3-OC$_7$H$_{15}$	2.19
94	4-NHCOC$_6$H$_4$-4′-NH$_2$	1.96	149	3-O(CH$_2$)$_2$OH	1.80
95	4-CH$_2$OC$_6$H$_5$	2.05	150	3-CH$_2$SC$_6$H$_5$	2.31
96	4-CH$_2$SC$_6$H$_5$	1.82	151	3-CH$_2$OC$_6$H$_5$	2.05
97	4-CH$_2$COCH$_3$	1.40	152	3-NHCOC$_6$H$_5$[a]	1.32
98	4-CH$_2$COCH$_2$Cl	1.40	153	3-CH$_2$COOCH$_3$	1.89
99	4-CH$_2$COCH$_2$OH	1.40	154	3-CH$_2$COOC$_2$H$_5$	1.85
100	4-CH$_2$COC$_6$H$_5$	1.92	155	3-CH$_2$COC$_6$H$_5$	2.33
101	4-CH$_2$COC$_6$H$_4$-4′-CH$_3$	1.75	156	3-CH$_2$COC$_6$H$_4$-4′-CH$_3$	2.35
102	4-CH$_2$COC$_6$H$_4$-4′-Cl	2.41	157	3-CH$_2$COC$_6$H$_4$-4′-OCH$_3$	2.37
103	4-CH$_2$COC$_6$H$_4$-4′-OH	1.48	158	3-CH$_2$COC$_6$H$_4$-4′-Cl	2.36
104	4-CH$_2$COC$_6$H$_4$-4′-OCH$_3$	2.05	159	3-CH$_2$COOCH$_2$C$_6$H$_5$	2.35
105	4-CH$_2$COC$_6$H$_4$-4′-OC$_6$H$_5$	2.92	160	3-CH=CHCOOCH$_3$	2.22
106	4-CH$_2$COC$_6$H$_4$C$_6$H$_5$	2.23	161	3-CH=CHCOOC$_2$H$_5$	2.26
107	4-CH$_2$COCH$_2$OC$_2$H$_5$	1.60	162	3-CH=CHCOC$_6$H$_5$	2.22
108	4-CH=CHCOOCH$_3$	1.70	163	3-CH=CHCOC$_6$H$_4$-3′-NO$_2$	2.19
109	4-CH=CHCOOC$_2$H$_5$	1.72	164	3-CH=CHCOOCH$_2$C$_6$H$_5$	2.89

[a] Not used in correlation analyses.

Labes and Hagen[334] postulated that this increase in activity was due to the interaction of the positively charged carbon of the carbonyl group with the OH group of the Ser-195 of the enzyme. Equations 313 and 315 were well combined[328] to give eq 316, where the new

$$pK_i = 1.38\ (\pm0.28) - 0.59\ (\pm0.49)MR_4 + 0.88\ (\pm0.52)\log(\beta\cdot10^{MR_4} + 1) + 0.23\ (\pm0.07)\pi_3 - 0.74\ (\pm0.20)\sigma + 0.20\ (\pm0.30)I_m + 0.51\ (\pm0.15)I_1 + 0.43\ (\pm0.19)I_2 + 0.65\ (\pm0.22)I_3$$

$$n = 104,\ r = 0.924,\ s = 0.222,\ MR_4(\text{opt}) = 1.03 \quad (316)$$

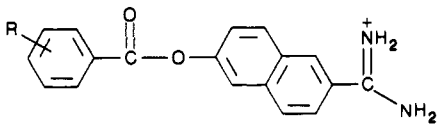
indicator parameter I_m was included to characterize the meta- and para-substituents, with a value of 1 for the former and zero for the latter. Certain data points were not included in the derivation of eq 310–316, as they were misfit in these equations. All such data points are indicated in the table (Table 42). Because of some other complexities, Recanatini et al.[328] excluded a few more compounds (not mentioned in the table) in their analysis, but Labes and Hagen considered[334] all the compounds and derived eq 317, where HP was used as an indicator parameter with a value of unity to indicate all carbonyl-containing substituents. It was zero for all other substituents.

$$pK_i = 0.21\pi - 0.43\sigma + 0.58HP + 1.39$$

$$n = 125,\ r = 0.82,\ s = 0.32 \quad (317)$$

An entirely different series of amidines (LXIX) were studied for their trypsin inhibition activity by Aoyama

et al.[335] Recanatini et al.[328] analyzed the data of Aoyama et al. (Table 43) and found the activity of the 4-substituted derivatives to be related to MR_4 and σ as shown in eq 318. Equation 318, with a significant positive



LXIX

$$pK_i = 2.64\ (\pm 0.28) + 0.41\ (\pm 0.21)MR_4 - 1.48\ (\pm 0.43)\sigma \quad n = 18,\ r = 0.922,\ s = 0.326 \qquad (318)$$

coefficient of MR_4 and the fact that replacement of MR_4 by π_4 yielded a poorer correlation,[328] stressed again the point that interaction of the 4-substituents with the enzyme is not typically hydrophobic. A few 3- and 3,4-disubstituted congeners (Table 43) could also be included in the correlation with MR = 0 for all 3-substituents without any loss to the quality of fit (eq 319). Certain outliers (Table 43) were not included in the derivation of eq 318 and 319. There were no obvious reasons for these outliers.

$$pK_i = 2.59\ (\pm 0.24) + 0.47\ (\pm 0.19)MR_4 - 1.40\ (\pm 0.40)\sigma \quad n = 21,\ r = 0.915,\ s = 0.322 \qquad (319)$$

In all the studies on trypsin inhibition, σ was found to play an almost uniform role.

For plasmin inhibition, Andrews et al.[327] correlated the data of Table 40 as shown by eq 320, and Coats[332]

$$pK_i = 3.23\ (\pm 0.16) + 0.25\ (\pm 0.12)\pi - 1.11\ (\pm 0.43)\mathcal{R} \quad n = 14,\ r = 0.89,\ s = 0.26 \qquad (320)$$

$$pK_i = 0.60\ (\pm 0.18) + 0.10\ (\pm 0.10)\pi + 1.57\ (\pm 1.90)\sigma \quad n = 8,\ r = 0.90,\ s = 0.11 \qquad (321)$$

$$pK_i = 0.02\ (\pm 0.01)P_E + 0.27\ (\pm 0.22) \quad n = 26,\ r = 0.70,\ s = 0.31 \qquad (322)$$

obtained eq 321 for 3-substituted benzamidines and eq 322 for the 4-substituted analogues. From only these equations, which are also not statistically significant, plasmin inhibition appears to involve only a small role for the hydrophobic and electronic characters of the substituents.

Thrombin inhibition by 3-substituted analogues was related[332] by eq 323 and that by 4-substituted analogues by eq 324, and the data of Table 40 on thrombin inhibition were related[327] by eq 325. All these equations

$$pK_i = 0.51\ (\pm 0.27) + 0.29\ (\pm 0.13)\pi - 0.96\ (\pm 0.75)\sigma \quad n = 11,\ r = 0.94,\ s = 0.20 \qquad (323)$$

$$pK_i = 0.21\ (\pm 0.18) + 0.30\ (\pm 0.08)\pi - 0.99\ (\pm 0.41)\sigma \quad n = 27,\ r = 0.93,\ s = 0.30 \qquad (324)$$

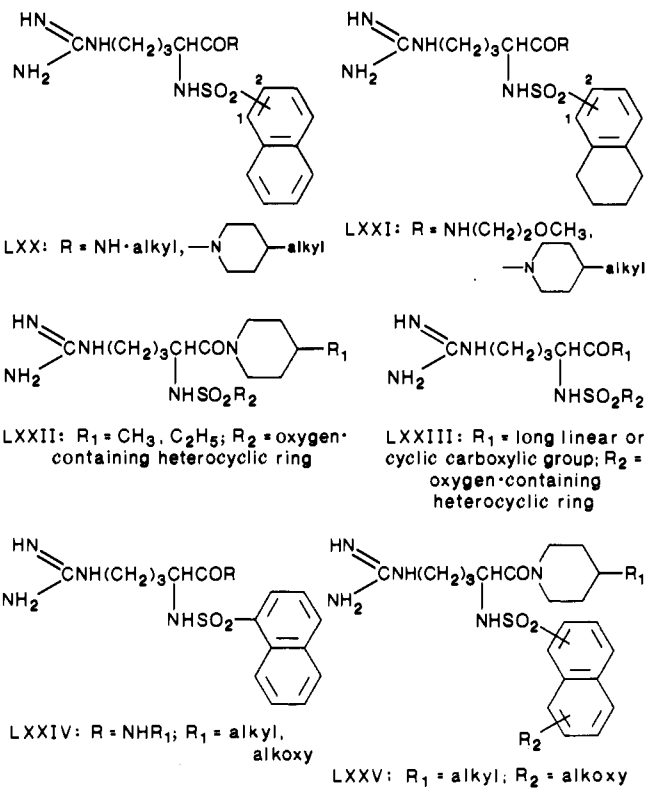
$$pK_i = 2.80\ (\pm 0.16) + 0.39\ (\pm 0.14)\pi \quad n = 15,\ r = 0.84,\ s = 0.29 \qquad (325)$$

exhibit the consistent role of π in thrombin inhibition. That hydrophocity could be an important factor in thrombin inhibition was recently shown in an exhaustive study by Gupta et al.[336] on derivatives of N^{α}-substituted L-arginine derivatives. It is well-known that synthetic arginine esters, such as N^{α}-tosyl-L-arginine methyl ester (TAME), are hydrolyzed by thrombin and inhibit the clotting activity of thrombin. Since the binding specificities of arginine derivatives would be determined by the structure of both sides of arginine, i.e., amino as well as carboxylic sides, a series of studies were recently undertaken by Okamoto et al.[337-339] to obtain potent and specific inhibitors of thrombin by modifications of the N^{α}-substituent and methyl ester portions of TAME. The linear relationships of activity with log P were obtained for series of LXX–LXXIIIas

TABLE 43. Trypsin Inhibition by Amidines LXIX

compd	R	pK_i
165	H	2.40
166	4-CH_3	3.00
167	4-t-C_4H_9	3.52
168	4-OCH_3	3.30
169	4-OC_4H_9	4.40
170	4-$OCH_2C_6H_5$	4.40
171	4-OH	3.52
172	4-NH_2	4.22
173	4-$N(CH_3)_2$[a]	3.52
174	4-$OCOCH_3$	3.40
175	4-SCH_3	2.70
176	4-F	2.52
177	4-Cl	2.52
178	4-Br	2.40
179	4-$NHCOCH_3$[a]	2.52
180	4-$COOCH_3$[a]	3.52
181	4-$COCH_3$	2.70
182	4-$CH{=}NOCH_3$	3.05
183	4-SO_2NH_2	2.00
184	4-CN	2.00
185	4-NO_2	2.00
186	4-$CH_2NHCOOCH_2C_6H_5$[a]	3.22
187	3-CH_3	2.70
188	3-CF_3	2.10
189	3,4-di-CH_3	2.70
190	3,4-OCH_2O[a]	3.70

[a] Not used in correlation analyses.



LXX: R = NH·alkyl, —N—alkyl

LXXI: R = $NH(CH_2)_2OCH_3$, —N—alkyl

LXXII: R_1 = CH_3, C_2H_5; R_2 = oxygen-containing heterocyclic ring

LXXIII: R_1 = long linear or cyclic carboxylic group; R_2 = oxygen-containing heterocyclic ring

LXXIV: R = NHR_1; R_1 = alkyl, alkoxy

LXXV: R_1 = alkyl; R_2 = alkoxy

$$pI_{50} = 5.960 + 1.503\ (0.423)\ \log P$$
$$n = 9,\ r = 0.803,\ s = 0.486 \quad (326)$$

$$pI_{50} = 5.789 + 0.797\ (0.105)\ \log P$$
$$n = 7,\ r = 0.959,\ s = 0.221 \quad (327)$$

$$pI_{50} = 5.723 + 0.531\ (0.082)\ \log P$$
$$n = 12,\ r = 0.899,\ s = 0.384 \quad (328)$$

$$pI_{50} = 6.116 + 0.532\ (0.111)\ \log P$$
$$n = 11,\ r = 0.847,\ s = 0.528 \quad (329)$$

$$pI_{50} = 6.171 + 0.073\ (0.334)\ \log P - 0.290\ (0.130)(\log P)^2$$
$$n = 18,\ r = 0.838,\ s = 0.472 \quad (330)$$

$$pI_{50} = 6.608 + 0.592\ (0.133)\ \log P - 0.456\ (0.154)(\log P)^2$$
$$n = 18,\ r = 0.816,\ s = 0.408 \quad (331)$$

shown by eq 326–329, respectively, and parabolic correlations were obtained for series of LXXIV and LXXV as shown by eq 330 and 331, respectively. In all these correlations, log P values calculated according to Hansch and Leo[45] were used. While all linear and parabolic correlations indicate the hydrophobic binding, the parabolic correlations also show that some bigger groups might produce steric hindrance too. All the correlations are significant at the 99% confidence level, but their r values still demand the inclusion of more parameters. An electronic character may be expected to complement the binding. This is indicated by eq 323 and 324.

For complement inhibition, Andrews et al.[327] correlated the data of Table 40 as shown by eq 332, and Coats[332] obtained eq 333 from the data of Table 44 listing some 3-substituted benzamidines. While eq 332

$$pK_i = 2.99\ (\pm0.29) + 0.41\ (\pm0.22)\pi - 1.11\ (\pm0.75)\mathcal{R}$$
$$n = 14,\ r = 0.82,\ s = 0.45 \quad (332)$$

$$pI_{50} = 0.02\ (\pm0.01)P_E - 1.13\ (\pm0.56)\sigma - 0.53\ (\pm0.23)$$
$$n = 25,\ r = 0.89,\ s = 0.24 \quad (333)$$

describes the effect of hydrophobicity, the use of π in place of P_E in eq 333 led to a poorer correlation.[332] However, it was difficult to say which effect, hydrophobicity or polarizability, was really involved in the interaction, as Hansch and Yoshimoto[340] obtained equally significant correlations with π (eq 334) and with

$$pI_{50} = 0.211\ (\pm0.05)(\pi\text{-}1,2) + 1.345\ (\pm0.13)D_1 + 0.620\ (\pm0.29)D_2 + 0.565\ (\pm0.14)D_3 + 2.440\ (\pm0.12)$$
$$n = 108,\ r = 0.931,\ s = 0.267 \quad (334)$$

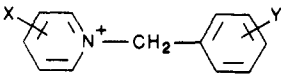
$$pI_{50} = 0.146\ (\pm0.03)(\text{MR-}1,2) + 1.068\ (\pm0.13)D_1 + 0.520\ (\pm0.28)D_2 + 0.429\ (\pm0.14)D_3 + 2.425\ (\pm0.12)$$
$$n = 108,\ r = 0.935,\ s = 0.258 \quad (335)$$

MR (eq 335) for a very complex and large set of benzamidine congeners, even though there was not a high collinearity between the two parameters ($r^2 = 0.36$). In the series, many substituents were of the type O-$(CH_2)_nOC_6H_4X$, $(CH_2)_4C_6H_4X$, or $O(CH_2)_4C_6H_4X$. For such substituents, a dummy parameter $D_1 = 1$ was used. For all other cases D_1 was equal to zero. If X in the substituents of these types was $NHCOC_6H_5$, $NHCONHC_6H_5$, $NHCONH(CH_2)_2C_6H_5$, $NHCOCH_2C_6H_5$, or $NHCOCH_2OC_6H_5$ with or without further substitution in the C_6H_5 moiety, a parameter D_3 with a value of 1 was used to account for this. The parameter D_2 was given a value of 1 if the substituent R had a pyridine moiety at the end. MR-1/π-1 accounted for the molar refractivity/hydrophobicity of the groups for which $D_1 = 0$, and MR-2/π-2 did so for X only in the substituents for which $D_1 = 1$.

TABLE 44. Complement Inhibition by Benzamidines LXVIII

compd	R	pI_{50}
191	H	−0.61
192	CF_3	−0.78
193	NO_2	−1.62
194	OC_3H_7	−0.16
195	OC_4H_9	−0.15
196	OC_5H_{11}	0.13
197	O-*i*-C_5H_{11}	0.14
198	$OCH_2C_6H_5$	0.02
199	$O(CH_2)_3OC_6H_5$	0.34
200	C_4H_9	−0.05
201	*i*-C_5H_{11}	−0.19
202	C_6H_5	0.10
203	$CH_2C_6H_5$	−0.34
204	$(CH_2)_2C_6H_5$	0.03
205	$CH{=}CHC_6H_5$	−0.06
206	$(CH_2)_2$-4-pyridyl	0.34
207	OCH_3	−0.48
208	Br	−0.48
209	$O(CH_2)_4C_6H_5$	0.62
210	$(CH_2)_4$(4-$CH_3CONHC_6H_4$)	0.40
211	$(CH_2)_2$-2-pyridyl	0.46
212	$(CH_2)_2$-3-pyridyl	0.39
213	$(CH_2)_4$-3-pyridyl	0.62
214	3,4-$(CH_3)_2$	−0.54
215	3,5-$(CH_3)_2$	−0.22

Though Hansch and Yoshimoto favored eq 335 in this study and assumed the dispersion interaction to be involved, in their two successive studies[341,342] on benzylpyridinium ions LXXVI they could correlate the



LXXVI

activity with π only. For a smaller set of Baker's data, they obtained[341] eq 336 and for a larger set including

$$pI_{50} = 0.18\ (\pm0.04)\pi_X + 0.46\ (\pm0.14)\pi_Y + 1.01\ (\pm0.28)\sigma_X^+ + 0.72\ (\pm0.12)D_1 + 2.50\ (\pm0.13)$$
$$n = 69,\ r = 0.939,\ s = 0.198 \quad (336)$$

$$pI_{50} = 0.16\ (\pm0.03)\pi_X + 0.38\ (\pm0.11)\pi_Y + 0.91\ (\pm0.25)\sigma_X^+ + 0.71\ (\pm0.010)D_1 + 2.58\ (\pm0.10)$$
$$n = 132,\ r = 0.945,\ s = 0.213 \quad (337)$$

all 69 data points of eq 336 they obtained[342] eq 337. Thus, new data points were well accommodated in the correlation. In both equations, the parameter D_1 was used for Y = 2-SO_2F. Thus, this group was to produce a positive effect on the activity. These equations also indicated the effect of the electronic character of the X group. The effect of the SO_2 group might be due to its electronic character only. However, from all these studies on complement inhibition, the actual nature of the interaction could not be derived.

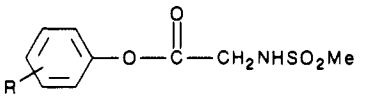
Thus in fact QSAR studies have not provided any clear picture of the mechanism of inhibition of any serine proteinase. Data analyzed by Andrews et al.[327] and those by Coats[332] could be well correlated with V_w

also,[343] but no new information was obtained.

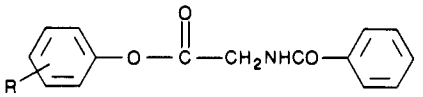
J. Thiol Proteinases

Papain, Ficin, Bromelain, and Actinidin

The thiol proteinases, papain from papaya, ficin from figs, bromelain from pineapple, and actinidin from kiwi fruit, are closely related to the serine proteinases. In place of serine, they all have cysteine in their active center. Of these thiol proteinases, papain has been most extensively studied by certain QSAR studies. A QSAR study made by Hansch and Calef[56] correlated the data of Williams et al.[344] on papain hydrolysis of esters of types LXXVIIa and LXXVIIb (R = OH, OMe, Me, CHO, F, Cl, COMe, NO_2, H, etc., at the 3- or 4-position) by eq 338 and 339, respectively. A combination of both



LXXVIIa



LXXVIIb

$$pK_m = 0.53\ (\pm 0.23)MR + 0.37\ (\pm 0.20)\sigma + 1.88\ (\pm 0.13)$$
$$n = 13,\ r = 0.935,\ s = 0.105 \quad (338)$$

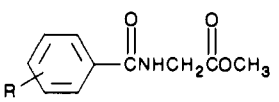
$$pK_m = 0.77\ (\pm 0.67)MR + 0.73\ (\pm 0.37)\sigma + 3.62\ (\pm 0.34)$$
$$n = 7,\ r = 0.971,\ s = 0.148 \quad (339)$$

equations with an indicator parameter I (equal to zero for LXXVIIa and 1 for LXXVIIb) resulted in eq 340,

$$pK_m = 0.57\ (\pm 0.26)MR + 0.56\ (\pm 0.19)\sigma - 1.92\ (\pm 0.15)I + 3.74\ (\pm 0.17)$$
$$n = 20,\ r = 0.990,\ s = 0.148 \quad (340)$$

indicating that congeners of LXXVIIb would be less effective than those of LXXVIIIa. Thus, the indicator parameter accounts for the steric effect, as LXXVIIb has a bulkier side chain.

While π had a very poor correlation in the above study, Hansch et al.[345] found the K_m of LXXVIII congeners that had substituents of a similar nature as LXXVIIa and LXXVIIb congeners to be related to π only (eq 341). But for a series of 4-substituted ana-



LXXVIII

$$pK_m = 1.005\ (\pm 0.11)\pi + 1.459\ (\pm 0.10)$$
$$n = 16,\ r = 0.981,\ s = 0.165 \quad (341)$$

logues of LXXVIIb (Table 45), Hansch and co-workers again found[346] the correlation to be significant with MR only (eq 342). Whereas, for 3- and 3,5-substituted

$$pK_m = 0.75\ (\pm 0.46)MR + 0.69\ (\pm 0.34)\sigma + 3.66\ (\pm 0.30)$$
$$n = 10,\ r = 0.944,\ s = 0.81 \quad (342)$$

congeners of LXXVIIb, they found the hydrophobic

TABLE 45. Papain, Actinidin, and Ficin Hydrolyses of Substituted Phenyl Hippurates LXXVIIb

		pK_m		
compd	R	papain[a]	actinidin[b]	ficin[c]
1	H	3.86	2.77	3.90
2	4-NH_2	3.59	2.55	*d*
3	4-F	3.78	2.72	3.53
4	4-CH_3	3.99	2.95	3.82
5	4-OCH_3	4.05	2.87	3.82
6	4-Cl	4.20	3.04	3.91
7	4-CN	4.23	3.62	4.27
8	4-SO_2NH_2	4.28[e]	3.22[e]	*d*
9	4-$CONH_2$	4.60	3.47	4.17
10	4-$COCH_3$	4.95	3.47	4.30
11	4-NO_2	5.01	3.80	4.50
12	3-NH_2	3.62	*d*	3.72
13	3-$NHCOCH_3$	3.83	3.18	3.74[e]
14	3-F	4.03	3.01	3.81
15	3-$CONH_2$	4.12	3.15	3.99
16	3-CN	4.32	3.08	4.15
17	3-NO_2	4.37	3.53	4.36
18	3,5-$(NO_2)_2$	4.48	3.90	4.69
19	3-CH_3	4.58	3.26	4.17
20	3-SO_2NH_2	4.55	3.42	*d*
21	3,5-$(CH_3)_2$	4.69	3.37	4.54
22	3,5-$(OCH_3)_2$	4.49[e]	3.60[e]	4.52[e]
23	3-Cl	4.73	3.63	4.43
24	3-I	4.91	3.93	4.64
25	3,5-$(Cl)_2$	4.91	3.84	4.98
26	3-CH_3-5-C_2H_5	4.97	3.52	4.79
27	3-CF_3	5.06	3.47	4.85
28	3,4,5-$(Cl)_3$	*d*	4.01	*d*
29	3-*t*-C_4H_9	*d*	3.66	5.14
30	3-Br	*d*	3.64	4.75
31	3-OCH_3	*d*	*d*	4.09
32	3-$COCH_3$	*d*	*d*	4.11
33	4-*t*-C_4H_9	*d*	*d*	4.33
34	4-Br	*d*	*d*	4.13

[a] Reference 346. [b] Reference 349. [c] Reference 350. [d] Not studied. [e] Not included in correlations.

parameter to be more important than MR and obtained eq 343. In eq 343, however, the π' means the π of only

$$pK_m = 0.93\ (\pm 0.27)\pi' + 0.53\ (\pm 0.29)\sigma + 3.95\ (\pm 0.19)$$
$$n = 16,\ r = 0.906,\ s = 0.202 \quad (343)$$

those meta substituents that have a positive value of π and, in the 3,5-disubstituted congeners, the π of that substituent that has the larger positive value of π. Thus, eq 343 was derived assuming that only one meta substituent can interact with the hydrophobic region of the enzyme. When π for all substituents was used instead of π', a very poor correlation was obtained (r = 0.768).[346] From eq 342, it was however shown that the 4-substituent will interact in some polar region of the enzyme. Compound 8 was not included in the derivation of this equation, as it was badly misfit, and for the same reason, compound 22 was not included in the derivation of eq 343. The reason for the misfit of these compounds in the respective equations was not clear.

When the two equations (342 and 343) were combined avoiding the inclusion of the parent analogue twice, eq 344 was obtained.[346] A similar equation (eq

$$pK_m = 1.03\ (\pm 0.25)\pi_3' + 0.61\ (\pm 0.29)MR_4 + 0.57\ (\pm 0.20)\sigma + 3.80\ (\pm 0.17)$$
$$n = 25,\ r = 0.0907,\ s = 0.208 \quad (344)$$

$$pK_m = 0.61\ (\pm 0.09)\pi_3' + 0.46\ (\pm 0.11)MR_4 + 0.55\ (\pm 0.20)\sigma + 2.00\ (\pm 0.12)$$
$$n = 32,\ r = 0.945,\ s = 0.178 \quad (345)$$

345) was obtained for a similar series of LXXVIIa by Carotti et al.,[347] where the parameters had the same meaning as in eq 344; but in this equation the coefficient of π_3' was much smaller than that in eq 344.

Due to the difference in the coefficients of π_3' in eq 344 and 345, Carotti et al.[347] concluded that hydrophobic binding of meta substituents to the active site of the enzyme is different in the two cases. They suggested that since the $NHCOC_6H_5$ moiety of LXXVIIb is very hydrophobic (π = +1.05) as compared with the $NHSO_2CH_3$ moiety (π = −1.18) of LXXVIIa, the former would anchor the substrate hydrophobically to the enzyme much more firmly than the latter, but this suggestion contradicts eq 340, which showed that congeners of LXXVIIb would be less effective than those of LXXVIIa. There the $NHCOC_6H_5$ group appeared to produce steric hindrance.

The role of σ, however, has been consistent in almost all papain hydrolyses studied. This means that a substituent from any position of the ring produces almost similar electron-withdrawal effects at the susceptible position of the side chain. The susceptible position may be the C–O bond, and the dipositivity of this bond may attract some nucleophilic region of the enzyme for hydrolysis. The current mechanism[347] of papain hydrolysis is as shown by eq 346. σ was found to be very well

$$RC(O)OC_6H_4X + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2}$$

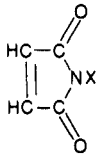
$$RC(O)S\text{-}E\ (+\ XC_6H_4O^-) \xrightarrow[HOH]{k_3} RCOOH + E \quad (346)$$

correlated (eq 347)[346] with nonenzymatic acid hydrolysis of LXXVIIb congeners, and σ^- was found to be equally well correlated (eq 348)[347] with their alkali hydrolysis.

$$\log k = 1.91\sigma - 3.94$$
$$n = 26,\ r = 0.987,\ s = 0.134 \quad (347)$$

$$\log k = 1.66\sigma^- - 2.39$$
$$n = 23,\ r = 0.975,\ s = 0.166 \quad (348)$$

Binding of different kinds of ligands was also studied. For example, Anderson and Vasini[348] studied the irreversible inactivation of papain by a set of *N*-alkylmaleimides (LXXIX) and determined the apparent second-order rate constant values for the inactivation. The log k values were then related by Hansch et al.[345] as shown by eq 349 and 350. Both equations represent



LXXIX: X = alkyl, phenyl

$$\log k = 0.615\ (\pm 0.13)MR + 1.290\ (0.41)$$
$$n = 9,\ r = 0.974,\ s = 0.180 \quad (349)$$

$$\log k = 0.551\ (\pm 0.12)\pi + 1.456\ (\pm 0.38)$$
$$n = 9,\ r = 0.974,\ s = 0.181 \quad (350)$$

exactly equally significant correlations. The reason for this was the high correlation between MR and π (r = 0.985). Hence, it cannot be said whether these compounds bind in the polar or hydrophobic region.

As compared with papain, actinidin, ficin, and bromelain hydrolyses are less studied. However, in a recent study on actinidin[349] and ficin[350] hydrolyses of LXXVIIb derivatives of Table 45, Carotti et al. obtained similar correlations (eq 351 and 352) as those for papain hydrolysis (eq 344).

Actinidin: $pK_m = 0.50\ (\pm 0.13)\pi_3' + 0.24\ (\pm 0.21)MR_4 + 0.74\ (\pm 0.15)\sigma + 2.90\ (\pm 0.12)$

$$n = 27,\ r = 0.927,\ s = 0.158 \quad (351)$$

Ficin: $pK_m = 0.84\ (\pm 0.14)\pi_3' + 0.41\ (\pm 0.18)MR_{4,5} + 0.57\ (\pm 0.16)\sigma + 3.60\ (\pm 0.17)$

$$n = 28,\ r = 0.941,\ s = 0.147 \quad (352)$$

The use of MR_4 in place of $MR_{4,5}$ in the case of ficin had given a little more inferior correlation.[350] The 5 in $MR_{4,5}$ refers to the more hydrophilic of the two meta substituents, which is assumed not to be contacting the hydrophobic region. Thus, while the mechanism of actinidin hydrolysis appears to be the same as that of papain hydrolysis (it is to be noted that as compounds 8 and 22 were misfit in eq 344, they were misfit as well in eq 351), the mechanism of ficin hydrolysis is indicated to be slightly different. With papain and actinidin, the 5-substituents did not appear to make any contact with the enzyme in any fashion, but in ficin they appeared to have some contact with the polar region. In the case of ficin the two poorly fit points were 13 and 22. The reason for their being misfit was also not clear.

The hydrolysis of a small set of LXXVIIb by bromelains B and D was related[345] as shown by eq 353 and 354, respectively. Similarly, bromelain B hydrolysis

$$\log (k_0/K_m) = 0.505\ (\pm 0.34)MR + 0.653\ (\pm 0.23)\sigma + 2.605\ (\pm 0.21)$$
$$n = 10,\ r = 0.961,\ s = 0.125 \quad (353)$$

$$\log (k_0/K_m) = 0.460\ (\pm 0.12)MR + 0.635\ (\pm 0.09)\sigma + 2.219$$
$$n = 9,\ r = 0.995,\ s = 0.041 \quad (354)$$

$$\log (k_0/K_m) = 0.464\ (\pm 0.20)MR + 0.526\ (\pm 0.13)\sigma^- + 1.131\ (\pm 0.10)$$
$$n = 9,\ r = 0.991,\ s = 0.063 \quad (355)$$

of a small set of LXXVIIa was related by eq 355. These equations show the binding of substituents only in the polar region. π was found to give poorer correlations in all these equations. The majority of substituents in both sets were, however, at the 4-position.

The Hammett constant σ or σ^- has played the same role in actinidin, ficin, and bromelain hydrolysis as in papain.

K. Amido- and Aminohydrolases

1. Urease

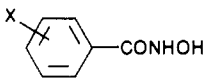
Urease (urea amidohydrolase) hydrolyzes urea to CO_2 and NH_3. Its importance in animal tissues is not well studied, but its inhibition has been studied. Hydroxamic acids (RCONHOH) have been found[351] to be potent inhibitors of this enzyme. A QSAR study on these inhibitors was made by Kumaki et al.[352]

For aliphatic hydroxamic acids (R = *n*-alkyl), Kumaki et al.[352] found the inhibition activity to be significantly correlated with π as shown by eq 356. This equation

$$pI_{50} = 5.975 + 0.407\pi - 0.080\pi^2$$
$$n = 11, r = 0.985, s = 0.119 \quad (356)$$

gave an optimum π value equal to 2.54, which means that an alkyl group larger than *n*-C_5H_{11} (π = 2.50) will produce steric hindrance. The electronic parameter was not found to be important here.

The electronic factor was not shown to affect activity in the case of benzohydroxamic acids LXXX also. The



LXXX: X = H, Cl, NO_2, CH_3, OH, alkoxy

best correlations that Kumaki et al.[352] obtained for meta- and para-substituted analogues of LXXX were as shown by eq 357 and 358, respectively. The π^2 term

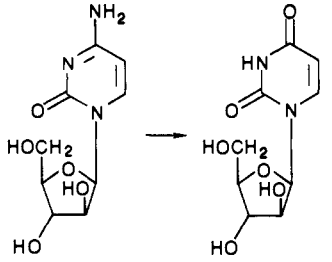
$$pI_{50} = 6.305 + 0.260\pi - 0.134\pi^2$$
$$n = 8, r = 0.942, s = 0.132 \quad (357)$$

$$pI_{50} = 6.440 - 0.432\pi$$
$$n = 9, r = 0.915, s = 0.269 \quad (358)$$

was not found to be statistically significant in the case of the para-substituted analogues. Since these equations relate in vitro activity, the negative effect of π can be attributed to only some steric role of the substituents. However, from this single study only, not much can be said about the mechanism of urease inhibition.

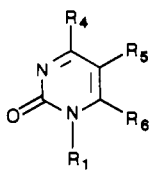
2. Cytosine Nucleoside Deaminase

Cytosine nucleoside deaminase catalyzes the deamination of nucleosides such as ara-C to ara-U (LXXXI).



LXXXI

Since ara-C, but not ara-U, has been effective against certain types of cancers, it was worthwhile to find inhibitors of cytosine nucleoside deaminase that could be effective selectively against the tumor enzyme but not against the human enzyme. The initial study was done by Baker and Kelley,[353] and their inhibition data on uracil analogues (LXXXII) were correlated by Yoshimoto and Hansch[192] as shown by eq 359. There were



LXXXII

$$pI_{50} = 0.283\ (\pm0.06)\pi_5 + 0.188\ (\pm0.07)\pi_{1,6} + 0.265\ (\pm0.16)I_1 + 2.257\ (\pm0.24)$$
$$n = 71, r = 0.927, s = 0.227 \quad (359)$$

a variety of substituents in LXXXII, and the parameter I_1 was used with a value of unity for 4-NH_2, 4-SH, and 4-NHOH functions. These groups are thus shown to produce positive effects on the activity. However, Yoshimoto and Hansch did not make any clear decision about the nature of the 1,5,6-space, as there was sufficiently high collinearity between π and MR, though replacement of π by MR in this case gave a relatively inferior correlation. Studies on further data may probably give a better insight.

3. Guanine Deaminase

Guanine deaminase hydrolytically deaminates guanine to xanthine. Hence, the selective inhibitors of this enzyme can also be exploited as anticancerous drugs. Inhibitors of the type as mentioned in Table 7 for xanthine oxidase were also studied for the inhibition of guanine deaminase,[88a-c,354] and their guanine deaminase inhibition activity was correlated by Silipo and Hansch[90] as shown in eq 360, where I_1 has been used

$$pI_{50} = 1.176\ (\pm0.25)MR_3 + 0.403\ (\pm0.11)\pi_4 - 0.127\ (\pm0.05)MR_3^2 - 3.417\ (\pm0.44)I_1 - 0.618\ (\pm0.25)I_2 + 1.608\ (\pm0.29)I_3 + 0.994\ (\pm0.43)(E_s\text{-}2) + 3.659\ (\pm0.50)$$
$$n = 92, r = 0.941, s = 0.366 \quad (360)$$

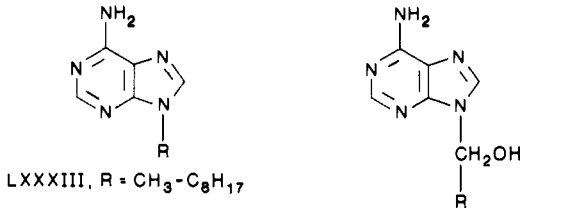
with a value of 1 to account for the presence of 4-OCH_3 as a second substituent and I_2, with a value of 1, to account for the presence of an SO_2F group in the substituents. The third parameter I_3 was given a value of 1 for a 4-OR group, where R was a variety of functions. For a subset of this series, Silipo and Hansch[355] obtained the correlation as shown by eq 361. An obvious

$$pI_{50} = 0.69MR_3 + 0.38\pi_4 + 1.10(E_s\text{-}2) + 1.34I_3 + 3.75$$
$$n = 32, r = 0.928, s = 0.363 \quad (361)$$

interpretation of eq 360 and 361 was that while the 4-substituents may be involved in hydrophobic interaction, the 3-substituents may be involved in dispersion interaction to the extent of bulk tolerance. Further, while a 4-OR group appears to produce a positive effect on the activity, a 4-OCH_3 group present as a second substituent appears to decrease the activity, and the presence of an SO_2F function in the substituents always leads to a negative effect.

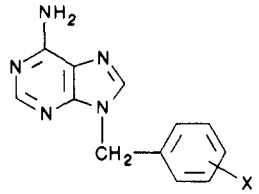
4. Adenosine Deaminase

Like guanine deaminase, adenosine deaminase hydrolytically deaminates adenosine to inosine. The selective inhibitors of this enzyme would, therefore, also be of importance to the treatment of tumors. Schaeffer et al. studied 9-alkyladenines LXXXIII,[356] 9-(1-hydroxy-2-alkyl)adenines LXXXIV,[357] and 9-benzyladenines LXXXV[358] for their adenosine deaminase inhibition activity and made a QSAR study[358] on them. In their QSAR study, they obtained eq 362 for 9-alkyladenines and eq 363 and 364 for 9-(1-hydroxy-2-alkyl)adenines. In the case of the latter, eq 363 included



LXXXIII, R = CH_3-C_8H_{17}

LXXXIV, R = CH_3-C_9H_{19}



LXXXV

$$\log (S/I)_{50} = 0.452\ (\pm 0.06)\pi - 1.194\ (\pm 0.15)$$
$$n = 8,\ r = 0.992,\ s = 0.078 \qquad (362)$$

$$\log (S/I)_{50} = 0.932\ (\pm 0.21)\pi - 0.483\ (0.41)$$
$$n = 6,\ r = 0.987,\ s = 0.157 \qquad (363)$$

$$\log (S/I)_{50} = 0.19\pi + 1.66$$
$$n = 3,\ r = 0.996,\ s = 0.012 \qquad (364)$$

only methyl to hexyl derivatives, and the remaining compounds were used to derive eq 364. The coefficient of π in eq 363 is much larger than that in eq 362. This led Schaeffer et al.[358] to suggest that the hydrophobic interactions of two different types of compounds are different. This difference in interactions was attributed to the possibility of conformational change in the enzyme brought out by congeners of LXXXIV, so that the hydrophobic region of the enzyme was more accessible to these congeners. But eq 364 derived for congeners of LXXXIV with bigger substituents indicates, with a much smaller coefficient of π, the limitation of the bulk tolerance.

The eight data points of the 9-alkyladenines that were correlated with π by eq 362 were as well correlated with χ by Kier et al.[155] as shown by eq 365. Since χ had a high correlation with π, eq 365 suggests only the hydrophobic interaction.

$$\log (S/I)_{50} = 0.449\ (\pm 0.025)\chi - 2.801\ (\pm 0.151)$$
$$n = 8,\ r = 0.991,\ s = 0.082 \qquad (365)$$

In the case of 9-benzyladenines (Table 46), Schaeffer et al.[358] related the activity of meta isomers as shown by eq 366, but failed to find any correlation for para isomers. In the case of meta isomers also, π as a single

$$\log (S/I)_{50} = 0.296\ (\pm 0.17)\pi + 1.096\ (\pm 0.43)\sigma - 0.039\ (\pm 0.17)$$
$$n = 9,\ r = 0.963,\ s = 0.132 \qquad (366)$$

parameter was poorly correlated ($r = 0.687$), suggesting that substituents at the meta position were not much favored for the hydrophobic interaction; but eq 366 shows that the electron-withdrawing nature of the substituents will help the interaction from this position. It may be that meta substituents, after withdrawing the electron, interact electrostatically with some cationic site of the enzyme.

Compound 9 was not included in eq 366, as it was misfit in the correlation. The reason for its high activity was, however, not known. Since there was no correlation obtained for para isomers, it was difficult to explain the mode of interaction of these isomers.

TABLE 46. Adenosine Deaminase Inhibition Activities of 9-Benzyladenines LXXXV

compd	X	log $(S/I)_{50}$	compd	X	log $(S/I)_{50}$
1	3-COOEt	0.69	10	H	−0.20
2	3-NO_2	0.52	11	4-NHAc	0.32
3	3-CN	0.48	12	4-COOMe	0.08
4	3-COOMe	0.44	13	4-CH_2Br	−0.15
5	3-CH_2Br	0.32	14	4-NH_2	−0.33
6	3-NHAc	−0.16	15	4-CN	−0.55
7	3-CH_2OH	−0.27	16	4-NO_2	−0.56
8	3-NH_2	−0.48	17	4-CH_2OH	−0.59
9	3-Ac	0.61			

L. Phosphatases

(Na^+-K^+)ATPase

A number of different adenosinetriphosphatase (ATPase) enzymes are known in which the hydrolysis of ATP is coupled to the transport of ions across a membrane. The Na^+-K^+-activated ATPase associated with the plasma membrane is one of them. Studies on the inhibition of this enzyme can be exploited to design local anaesthetics, cardiovascular agents, insecticides, and diuretics.

Grisham and Barnett[359] studied the effect of some aliphatic alcohols on the activity of (Na^+-K^+)ATPase of lamb kidney. Unger[360] related the ATPase inhibition activity of these alcohols (ethanol, butanol, 2-methyl-2-propanol, pentanol, heptanol, octanol) to hydrophobicity as shown by eq 367. In another study, Unger and

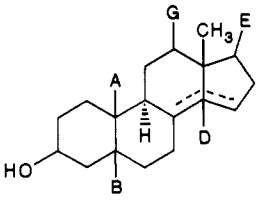
$$pI_{50} = 0.81 \log P - 1.20$$
$$n = 6,\ r = 0.998, s = 0.080 \qquad (367)$$

Chiang[361] related the data of Davis and Brody[362] on the inhibition of (Na^+-K^+)ATPase in rat brain by certain amines (promazine, chloropromazine, promethazine, trifluoperazine, thioradazine, prochlorperazine, perphenazine, imipramine, trifluopromazine) to lipophilicity measured by k' (eq 368). k' was obtained by use

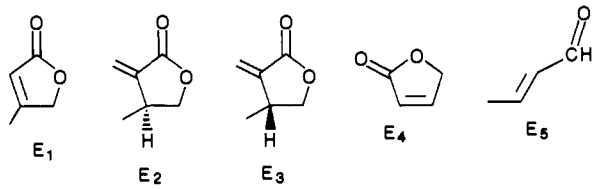
$$\text{logit } \% = 1.40 \log k' - 2.92$$
$$n = 9,\ r = 0.962,\ s = 0.269 \qquad (368)$$

of an optimized HPLC (high-performance liquid chromatography) system with an isotonic pH 7.4 1-octanol-saturated phosphate buffer with added 4 mM dimethyloctylamine on a persilated reversed-phase-18 column packing material. This parameter was found to be in excellent agreement with the 1-octanol bulk-phase shake-flask distribution coefficient.[361] The activity parameter logit % is log [%/(100 − %)], where % is the percentage of inhibition at 1×10^{-4} M. From the difference in slopes of eq 367 and 368, Unger[360] assumed that alcohols and amines may bind in somewhat different regions of the lipid. The hypothesis is that binding of the drug occurs to the membrane lipids, inducing a conformational change indirectly in the enzyme, which is then deactivated.[361]

The pharmacological effects of cardiac glycosides, digitalis genins, is supposed to be due to the inhibition of (Na^+-K^+)ATPase.[363] For a series of nine analogues of LXXXVI. Fullerton et al.[364,365] showed that ATPase



LXXXVI: A = CH_3, CHO; B = H, OH; C = H, OH; D = H, OH, nil; E = E_1, E_2, E_3, E_4, E_5



inhibition activity was a function of the position of the carbonyl oxygen of the E moiety relative to that in digitoxigenin (A = CH_3, B = G = H, D = OH, E = E_1) when the structurally similar parts of a digitalis genin with energetically favored conformation of E was superimposed upon the latter. In the superimposed situation, the distance (D) between the carbonyl oxygen of each analogue and that of digitoxigenin was found, and a correlation as shown by eq 369 was obtained.[364] However, the limited number of compounds and the clustering of D values around 5 and 1 did not permit a generalized conclusion.

$$pI_{50} = 6.47 - 0.457D \qquad r = 0.997 \qquad (369)$$

Repke et al.[366] were able to correlate the ATPase inhibition activity of some cardenolides with their permanent dipole moment (r = 0.95). However, Takiura et al.[367] discussed the importance of lipophilicity in ATPase inhibition.

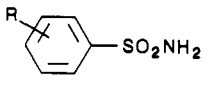
The importance of lipophilicity in (Na^+–K^+)ATPase inhibition was also shown by Uchida et al.[368] when they related the inhibition activity of certain unrelated alcohols and of lindane and its three isomers to log P as shown by eq 370. These compounds were active against cockroach nerve conduction and yeast growth.

$$pK_{50} = 0.77\ (\pm 0.08)\ \log P + 0.53\ (\pm 0.23)$$
$$n = 14,\ r = 0.988,\ s = 0.237 \qquad (370)$$

M. Hydrolyases

Carbonic Anhydrase

Carbonic anhydrase (CA) is an extremely efficient catalyst of the reversible hydration of carbon dioxide and plays an important role in respiration as well as in other physiological and pharmacological processes.[369-371] The inhibitors of this enzyme are, therefore, of immense importance in studies related to its physiological and pharmacological functions. Aromatic sulfonamides (LXXXVII), which may also act as diuretics, have been



LXXXVII

found to be specific inhibitors of this enzyme, and their unsubstituted SO_2NH_2 group has been found to play an important role in the inhibition.[369] A large number of sulfonamides (Table 47) were studied, and their inhibition activities at two different temperatures, 0.2 and 15 °C, were reported.[372] In a QSAR study then, Kakeya et al.[373] obtained eq 371–376, for the meta and para analogues at both the temperatures. These equations

TABLE 47. Carbonic Anhydrase Inhibition Activities of Sulfonamides LXXXVII

compd	R	pK_i[a] (0.2 °C)	pK_i[a] (15 °C)
1	H	0.215	0.124
2	*p*-NH_2	−0.363	−0.398
3	*p*-Cl	0.721	0.959
4	*p*-CH_3	0.420	0.496
5	*p*-CN	0.959	1.187
6	*p*-Br	0.921	0.959
7	*p*-NO_2	1.046	1.260
8	*p*-CH_3O	0.347	0.301
9	*p*-CH_3NH	−0.176	−0.046
10	*p*-CH_3CO	0.959	0.886
11	*m*,*p*-$(Cl)_2$	1.400	1.522
12	*m*-NO_2-*p*-Cl	1.769	1.602
13	*m*-CF_3-*p*-NO_2	1.854	1.658
14	*m*-Cl	0.638	0.921
15	*m*-CH_3	0.301	0.223
16	*m*-NO_2	0.886	1.125
17	*o*-CH_3	−0.204	−0.080
18	*o*-Cl	0.496	0.620
19	*o*-NO_2	0.331	0.455

[a] K_i is in units of 10^{-5} M.

0.2 °C:
$$pK_I = 0.702\pi + 0.439$$
$$n = 16,\ r = 0.789,\ s = 0.435 \qquad (371)$$
$$pK_I = 1.021\sigma + 0.474$$
$$n = 16,\ r = 0.938,\ s = 0.208 \qquad (372)$$
$$pK_I = 0.276\pi + 0.800\sigma + 0.413$$
$$n = 16,\ r = 0.965,\ s = 0.160 \qquad (373)$$

15 °C:
$$pK_I = 0.670\pi + 0.508$$
$$n = 16,\ r = 0.758,\ s = 0.462 \qquad (374)$$
$$pK_I = 1.018\sigma + 0.551$$
$$n = 16,\ r = 0.939,\ s = 0.212 \qquad (375)$$
$$pK_I = 0.223\pi + 0.839\sigma + 0.481$$
$$n = 16,\ r = 0.958,\ s = 0.176 \qquad (376)$$

show that the electronic parameter plays a major role in CA inhibition. However, while the electron-withdrawal nature of the substituents will affect the electronic character of the SO_2NH_2 group, their hydrophobic character will make them interact with some hydrophobic site of the enzyme.

Kakeya et al. excluded ortho derivatives because of their poor correlation with π and σ, but Lien et al.[98] used log P in place of π and obtained eq 377 for all

$$pK_I = 0.259\ \log P + 0.886\sigma + 5.314$$
$$n = 19,\ r = 0.923,\ s = 0.247 \qquad (377)$$

ortho, meta, and para derivatives. Surprisingly, the coefficient of log P in eq 377 is not much different from that of π in eq 373 or 376. Similarly, the coefficients of σ in all three equations are almost same. Ortho substituents, however, were shown to produce steric effects. For the three ortho derivatives, Kakeya et al.[373] obtained eq 378 and 379 (value of r was not mentioned). But since these equations include only three data points, much confidence cannot be attached to their significance.

$$pK_I(0.2\ °C) = 1.388\sigma^* + 1.190E_s - 0.204 \quad (378)$$

$$pK_I(15\ °C) = 1.231\sigma^* + 0.975E_s - 0.080 \quad (379)$$

Kumar et al.[374] made a different analysis using only V_w. They found that activities of meta analogues (compounds 1 and 11–16) at both temperatures were well correlated with V_w (eq 380 and 381) but those of

$$pK_I(0.2\ °C) = 3.069V_w - 0.096$$
$$n = 7,\ r = 0.958,\ s = 0.211 \quad (380)$$

$$pK_I(15\ °C) = 2.741V_w + 0.038$$
$$n = 7,\ r = 0.902,\ s = 0.302 \quad (381)$$

$$pK_I(0.2\ °C) = 3.278V_w - 0.313$$
$$n = 13,\ r = 0.737,\ s = 0.447 \quad (382)$$

$$pK_I(15\ °C) = 1.203V_w + 0.254$$
$$n = 13,\ r = 0.350,\ s = 0.541 \quad (383)$$

para analogues (1–13) were poorly correlated with V_w (eq 382 and 383). In separated groups, π was however found to be very poorly correlated in either case, and Kumar et al. also noted that π was very poorly correlated with V_w in either case. Hence, these authors argued that meta-substituted sulfonamides had a highly complementary binding site, and that their interaction with the latter would depend upon the size of the meta substituent. As shown in Figure 3, the primary active site of the enzyme consists of a Zn^{2+} ion with three ligands from the protein, all of which are histidyl residues.[369,375-377] The fourth ligand is either a water molecule or hydroxyl ion.[378] The zinc coordination is distorted tetrahedral. The active site is situated in a deep pocket leading into the center of the enzyme molecule. The depth of this active site should be good enough to accommodate all the sulfonamides studied, and the inhibitors may lie well within the enzyme molecule. A good correlation between V_w and the inhibition activity in the case of the meta analogues indicates that the meta group comes in the vicinity of some amino acid residue near the primary binding site and interacts with it, and since this interaction depends upon the molecular size, it may be of the van der Waals type.[22,374] Since at the higher temperature, the correlation is a little inferior and the coefficient of V_w is also smaller, it may be assumed that at the higher temperature the distance between the meta substituent and the secondary binding site of the enzyme is disturbed. This difference can also be seen in the coefficients of π in eq 373 and 376.

However, in a recent study, the bigger substituents at the meta position were shown to produce steric hindrance also.[379] Using the data of King and Burgen (Table 48),[380] Hansch et al.[379] derived eq 384 for 4- and 3-substituted congeners of LXXXVII. In this equation, the indicator parameter I_1 was given a value of unity for 3-substituents and zero for 4-substituents. Thus, eq 384 shows that by some kind of steric effect the 3-substituents lower the activity by a factor of 100. The activity parameter K here is not an inhibition constant but a binding constant for absorption of the sulfonamides to human CA isoenzyme C prepared from erythrocytes.

$$\log K = 1.55\ (\pm0.40)\sigma + 0.62\ (\pm0.09)\log P - 2.07\ (\pm0.23)I_1 + 6.98\ (\pm0.20)$$
$$n = 24,\ r = 0.982,\ s = 0.210,\ F_{3,20} = 177 \quad (384)$$

TABLE 48. King and Burgen Data on Human Carbonic Anhydrase Inhibition by Sulfonamides LXXXVII

compd	R	log K	compd	R	log K
20	H	6.69	35	4-$CONHC_4H_9$	8.49
21	4-CH_3	7.09	36	4-$CONHC_5H_{11}$	8.75
22	4-C_2H_5	7.53	37	4-$CONHC_6H_{13}$	8.88
23	4-C_3H_7	7.77	38	4-$CONHC_7H_{15}$	8.93
24	4-C_4H_9	8.30	39	3-CO_2CH_3	5.87
25	4-C_5H_{11}	8.86	40	3-$CO_2C_2H_5$	6.21
26	4-CO_2CH_3	7.98	41	3-$CO_2C_3H_7$	6.44
27	4-$CO_2C_2H_5$	8.50	42	3-$CO_2C_4H_9$	6.95
28	4-$CO_2C_3H_7$	8.77	43	3-$CO_2C_5H_{11}$	6.86
29	4-$CO_2C_4H_9$	9.11	44	2-CO_2CH_3	4.41
30	4-$CO_2C_5H_{11}$	9.39	45	2-$CO_2C_2H_5$	4.80
31	4-$CO_2C_6H_{13}$	9.39	46	2-$CO_2C_3H_7$	5.28
32	4-$CONHCH_3$	7.08	47	2-$CO_2C_4H_9$	5.76
33	4-$CONHC_2H_5$	7.53	48	2-$CO_2C_5H_{11}$	6.18
34	4-$CONHC_3H_7$	8.08			

Still more dominant steric effects were found to be produced by 2-substituents. Including 2-substituted derivatives also, Hansch et al.[379] derived eq 385, where

$$\log K = 1.55\ (\pm0.38)\sigma + 0.64\ (\pm0.08)\log P - 2.07\ (\pm0.22)I_1 - 3.28\ (\pm0.23)I_2 + 6.94\ (\pm0.18)$$
$$n = 29,\ r = 0.991,\ s = 0.204,\ F_{4,24} = 324 \quad (385)$$

I_2 was given a value of unity for the 2-substituents and zero for all others. For only the 4-substituted analogues, hydrophobicity was, however, found to be a dominant factor (eq 386),[379] and the electronic parameter was found to play the next better role (eq 387).[379] But the comparison of coefficients of σ and log P of eq 384 and 385 and also the intercepts of these equations with those of eq 387 shows that all 2-, 3-, and 4-substituents seem to be acting in the same fashion once correction is made for some sort of steric effect produced by the 2- and 3-substituents.

$$\log K = 0.65\ (\pm0.22)\log P + 7.30\ (\pm0.40)$$
$$n = 19,\ r = 0.834,\ s = 0.457,\ F_{1,17} = 39.1 \quad (386)$$

$$\log K = 1.55\ (\pm0.39)\sigma + 0.65\ (\pm0.10)\log P + 6.93\ (\pm0.20)$$
$$n = 19,\ r = 0.971,\ s = 0.204,\ F_{2,16} = 69.4 \quad (387)$$

It is, however, to be noted that in the study by Kakeya et al. (eq 371–376) the electronic factor was found to play a better role than the hydrophobic one, while in the study of Hansch et al. (eq 386, 387) the hydrophobic factor was shown to play a better role than the electronic one. This discrepancy may be due to the difference in the structures of enzymes obtained from different sources. The amino acid composition of CA is known to vary with its source.[375] The data used by Kakeya et al. were for bovine CA, and those used by Hansch et al. were for human CA. Moreover, the different experimental conditions and the use of different activity parameters may also be responsible for this discrepancy.

Testa and Purcell[381] also analyzed the King and Burgen data of Table 48. Including a few thiophene analogues, they obtained eq 388, in which AC corre-

$$\log AC = 0.26\ (\pm0.14)\log P + 0.0226\ (\pm0.0061)V_s + 1.08\ (\pm0.04)D$$
$$n = 34,\ r^2 = 0.995,\ s = 0.536 \quad (388)$$

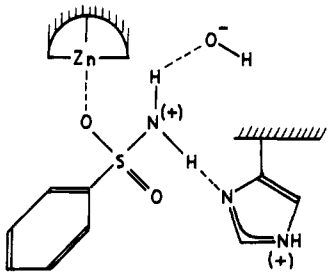


Figure 4. Molecular model as proposed by the Shinagawas for the interaction of sulfonamides with the active center of carbonic anhydrase.

sponds to K, V_s is the molar volume of substituents calculated according to Bondi, and D is the distance in angstroms between the S atom in the sulfonamide and the first C atom falling into one of four possible sectors of the enzyme. From this equation, Tests and Purcell concluded that "the affinity constant of the sulfonamides for carbonic anhydrase is massively structure-dependent, and depends only to a very limited extent on the partition properties of the ligand". The electronic characters of the substituents were not found by these authors to play any role.

However, the primary binding does involve the electronic character of the SO_2NH_2 group, which is influenced by the electronic nature of the substituents. Subbarao and Bray[382] showed that CA inhibition by these sulfonamides can be related to the ^{14}N nuclear quadrupole resonance difference frequency ν_d which is a measure of $(q_{NH} - q_{NS})$, the difference in electron densities of N–H and N–S bonds. For compounds 1–4, 8, and 17 of Table 47, they related pK_I with ν_d as shown by eq 389 and 390. These equations are, however, not very informative, as they include only six data points, and a few outliers were found to these equations.[382]

0.2 °C: $$\nu_d \text{ (kHz)} = 63.7\ pK_I + 979$$
$$n = 6,\ r = 0.86,\ s = 17 \text{ kHz} \quad (389)$$

15 °C: $$\nu_d \text{ (kHz)} = 56\ pK_I + 978$$
$$n = 6,\ r = 0.88,\ s = 16 \text{ kHz} \quad (390)$$

Shinagawa and Shinagawa[383] made a quantum mechanical (HMO) calculation on a different series of sulfonamides studied by Krebs[384] and related the CA inhibition activity with the frontier electron density of the lowest vacant orbital at the amide group position ($f_{NH_2}^N$, eq 391). Similarly, for a small group of thia-

$$pI_{50} = 5.51 f_{NH_2}^N + 3.13 \quad n = 22,\ r = 0.774 \quad (391)$$

zides,[385] they obtained eq 392. Slightly better corre-

$$pI_{50} = 8.62 f_{NH_2}^N + 1.69 \quad n = 6,\ r = 0.875 \quad (392)$$

lations were however found with the formal charge Q_{NH_2} at the amide group position, as shown by eq 393 for sulfonamides and by eq 394 for thiazides. From these

$$pI_{50} = 11.2 Q_{NH_2} - 1.32 \quad n = 22,\ r = 0.780 \quad (393)$$

$$pI_{50} = 19.8 Q_{NH_2} - 8.13 \quad n = 6,\ r = 0.885 \quad (394)$$

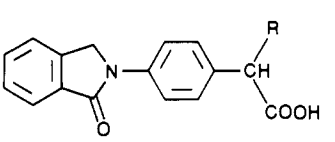
equations, the Shinagawas therefore assumed that the NH_2 group is probably involved in hydrogen bonding with the OH^- ion on one side and the imidazole ring on the other side in the enzyme. An oxygen atom of the SO_2 in sulfonamide is supposed to bind with the Zn^{2+} of the enzyme. The interaction model presented by the Shinagawas is as shown in Figure 4.

N. Synthetases

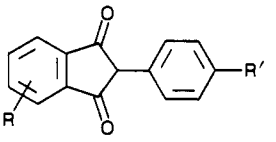
1. Prostaglandin Synthetase

Prostaglandin synthetase (PGS) is an oxidoreductase and is involved in the biosynthesis of human prostaglandin E_2 (PGE_2) starting from arachidonic acid.[386] Another prostaglandin obtained from human tissues is prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The letters E and F were originally derived from the observation that two types of prostaglandins could be partially separated by differential partitioning between ether (E) and phosphate buffer (F; from fostat, a Swedish word). Prostaglandins are one class of mediators liberated locally in tissues during inflammatory reactions. Since the discovery that aspirin inhibits prostaglandin biosynthesis,[387-389] it is believed that aspirin-like nonsteroidal antiinflammatory drugs block prostaglandin synthetase,[386] and in a recent review article[390] it was pointed out that in many cases the in vivo assays of antiinflammatory activity of nonsteroidal drugs were well correlated with their PGS inhibition activity. Hence, the in vitro study of PGS inhibition is considered to be one of the most promising rational approaches for prediction of antiinflammatory activity.

However, not many QSAR studies are available on PGS inhibition. For a very limited series of indoprofen analogues LXXXVIII, Ceserani et al.[391] related the PGS inhibition activity to the partition coefficient K_p (eq 395) and for a relatively bigger series of 2-aryl-1,3-



LXXXVIII



LXXXIX

$$pI_{50} = 0.49 \log K_p - 2.19 \quad n = 5,\ r = 0.97 \quad (395)$$

$$pI_{50} = 0.40\pi + 1.64\sigma + 3.47$$
$$n = 24,\ r = 0.912,\ s = 0.239 \quad (396)$$

indandione derivatives LXXXIX, Van de Berg et al.[392] related the activity to π and σ (eq 396). Similarly, for some phenols Dewhirst[393] obtained eq 397–399. Equation 397 correlates the activity of alkylphenols, eq 398 of 2-alkoxyphenols, and eq 399 of other 2-substituted phenols.

$$pI_{50} = 0.28\pi - 4.25\sigma + 3.00$$
$$n = 20,\ r = 0.92 \quad (397)$$

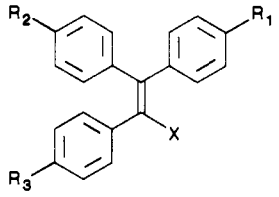
$$pI_{50} = 0.77\pi - 0.25\sigma + 3.95$$
$$n = 6,\ r = 0.99 \quad (398)$$

$$pI_{50} = 0.30\pi - 0.60\sigma + 3.58$$
$$n = 8,\ r = 0.99 \quad (399)$$

All these studies show the importance of the hydrophobic character of the molecules in PGS inhibition. Regarding electronic character, while eq 396 shows the increase in activity by electron withdrawal by the substituents in the phenyl ring, eq 397–399 show the same effect to be produced by electron donation.

Recently, some triphenylacrylonitriles (XC; X = CN) and triphenylethylenes (XC; X = H, CH_2NH_2, CH_2N-

$HCOCH_3$, $CONH_2$) were studied for their PGS inhibition activity.[394] Gupta et al.[395] correlated the activity data as shown by eq 400. This equation verified the



XC: R_1, R_2, R_3 = H, OH, CH_3, OCH_3, Cl, F, NH_2, etc.

$$pI_{50} = 5.916 + 1.281\ (\pm 0.538)\pi\text{-}R_1 - 0.005\ (\pm 0.437)\pi\text{-}R_2 + 0.592\ (\pm 0.426)\pi\text{-}R_3 - 1.41\ (\pm 0.600)I - 2.766\ (\pm 1.856)\sigma\text{-}R_1$$

$$n = 33,\ r = 0.864,\ s = 0.470,\ F_{5,27} = 15.92 \qquad (400)$$

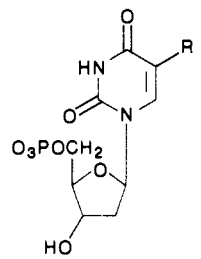
hydrophobic effect of the substituents and supported the positive role of electron donation. However, the electron-donating character of the substituents was found to be effective from only one ring. The equation was significant at the 99% confidence level, but the $\pi\text{-}R_2$ parameter was not very significant. The indicator parameter I was given a value of 1 for four triphenylethylene derivatives and zero for all triphenylacrylonitriles. The negative coefficient of this indicator parameter showed that an X other than CN will reduce the activity.

All the above studies are, however, not sufficient to provide any clear mechanism of PGS inhibition, except that hydrophobic interaction would be an important factor for all kinds of PGS inhibitors.

2. Thymidylate Synthetase

Thymidylate synthetase (ThS), in fact, belongs to the group of enzymes that are important in DNA synthesis. It catalyzes the formation of thymidylate from 2′-deoxyuridylate with transfer and reduction of one carbon unit of 5,10-methylenetetrahydrofolate. Hence, like others, this enzyme is also a popular target for antitumor agents.

Certain 5-substituted 2′-deoxyuridylates (XCI) were studied[396] for their binding with thymidylate synthetase of *Lactobacillus casei*, and the binding constant (K_i) was correlated[396] as shown by eq 401. This equation

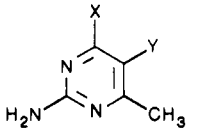


XCI: R = H, F, Cl, Br, I, CH_3, CF_3, CHO, CH_2OH

$$pK_i = 1.58\ (\pm 1.17)\sigma^- + 3.49\ (\pm 2.33)\mathcal{F} - 1.43\ (\pm 1.11)\mathrm{MR} + 5.88\ (\pm 0.84)$$

$$n = 9,\ r = 0.953,\ s = 0.461 \qquad (401)$$

indicates that electron withdrawal from the uracil ring will increase the binding power but, simultaneously, a steric hindrance will also be produced by the substituent. However, when Baker's data on 2-amino-6-methylpyrimidines XCII were correlated by Yoshimoto and Hansch[192] (eq. 402), a positive effect of MR of the



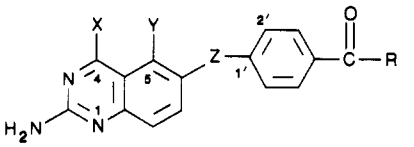
XCII

$$\log (S/I)_{50} = 0.255\ (\pm 0.05)\mathrm{MR}_5 + 0.905\ (\pm 0.29)I_1 - 0.664\ (\pm 0.23)I_2 - 2.910\ (\pm 0.32)$$

$$n = 41,\ r = 0.914,\ s = 0.299 \qquad (402)$$

5-substituents was exhibited. The series had X = OH, SH, H, NH_2, or $N(Me)_2$. With each of the last three substituents, there was only one derivative. A majority of the compounds had X = OH or SH. The indicator parameter I_1 was given a value of 1 for the cases where X = SH and zero for all others. Barring only four derivatives where Y = $(CH_2)_3C_6H_5$, $(CH_2)_4C_6H_5$, $NHCH_2CH{=}CHC_6H_5$, and $NH(CH_2)_3C_6H_5$, in all other congeners Y was of the type $(CH_2)_3NR_1R_2$, where R_1 and R_2 were a variety of functions. For Y where R_2 = $COCH_3$, COC_6H_5, and $COOC_6H_5$, the parameter I_2 was used with a value of unity. Thus, eq 402 shows that an SH group at the 4-position will increase the activity, and a function of the type described by I_2 in the Y substituent will decrease the activity. It is, however, not clear how these functions will decrease the activity and an SH at the 4-position will increase the activity, and not much can be said about the positive role of MR of Y, as its coefficient is very small.

In the case of a series of quinazolines XCIII, the ThS inhibition activities were related to only some indicator parameters. The parameters were defined as follows:



XCIII: X = OH, SH, NH_2; Y = H, CH_3; Z = $NHCH_2$, CH_2NH, CH_2S, CH_2O, $CH_2N(CHO)$, CH_2NCH_3, $CH_2N(CHO)$; R = Glu, $Glu(Et)_2$, OH, OC_2H_5

I_1 = 1 for X = OH or SH and zero for X = NH_2; I_2 = 1 for Y = CH_3 and zero for Y = H; I_3 = 1 for Z = $NHCH_2$ and zero for all others; I_4 = 0 for Z = CH_2NH or $NHCH_2$ and 1 for all others; I_5 = 0 for R = Glu or $Glu(Et)_2$ and 1 for R = OH or OC_2H_5; I_6 = 0 for R = Glu and 1 for all others. With these parameters, Chen et al.[397] correlated the L1210 mouse leukemic cell ThS inhibition activity as shown by eq 403 and the *L. casei*

$$pI_{50} = 5.98\ (0.14) + 0.75\ (0.23)I_4 - 2.01\ (0.20)I_5$$

$$n = 29,\ r = 0.905,\ s = 0.525,\ F_{2,26} = 58.96 \qquad (403)$$

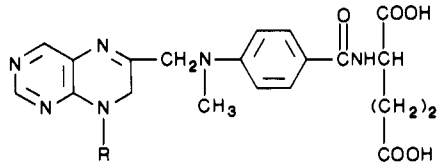
$$pI_{50} = 4.638\ (0.173) - 0.395\ (0.110)I_1 + 0.391\ (0.105)I_3 - 0.809\ (0.113)I_6 + 0.426\ (0.093)\pi_6$$

$$n = 28,\ r = 0.911,\ s = 0.246,\ F_{4,23} = 28 \qquad (404)$$

ThS inhibition activity as shown by eq 404. Since eq 403 and 404 are based on only indicator parameters and many compounds were outlier to them, no mechanistic conclusion can be drawn from them. However, these equations show that enzymes from two different sources are quite dissimilar in nature. Chen et al. also performed cluster analysis, factor analysis, and discriminant analysis for thymidylate synthetase inhibition

activity of these quinazolines, but no mechanistic aspect was discussed.

For a small series of 8-substituted 7,8-dihydromethotrexate analogues XCIV, Prabhakar et al.[398] correlated the data of Chaykovasky et al.[399] on *L casei* ThS inhibition with V_w (eq 405). Since no other QSAR studies were available on this class of inhibitors and since the present equation was based on only nine data points, the authors were not able to discuss any mechanistic aspect of ThS inhibition by this class of inhibitors.

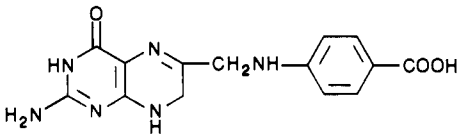


XCIV: R = H, alkyl, benzyl, chlorobenzyl, 1-naphthylmethyl

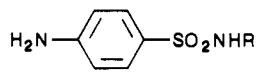
$$pI_{50} = 6.007 - 2.959\ (0.924)V_w\cdot R + 1.597\ (0.630)(V_w\cdot R)^2$$
$$n = 9,\ r = 0.853,\ s = 0.288,\ F_{2,6} = 8.05 \quad (405)$$

3. Dihydropteroate Synthetase

In the sequential pathway of folate synthesis in bacteria, the incorporation of *p*-aminobenzoic acid (PAB), the bacterial growth factor, into dihydropteroic acid (XCV) is mediated by the enzyme dihydropteroate synthetase. Studies[400-404] on sulfonamides (XCVI) by



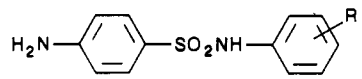
XCV



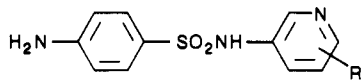
XCVI

now have established that these drugs exert their antibacterial action by inhibiting this enzyme by competition with PAB, and not by forming dihydropteroic acid analogues.

Miller et al.[405] obtained a cell-free system from *E. coli* and determined the effects of N^1-phenylsulfonamides XCVII and N^1-3-pyridylsulfonamides XCVIII on the synthesis of dihydropteroic acid. For both series (Table



XCVII



XCVIII

49), the cell-free (enzyme) inhibitory activity was shown to be very well correlated with the N^1 pK_a (eq 406 and 407). These authors also showed that there was a

$$pI_{50} = 2.26 - 0.43pK_a$$
$$n = 14,\ r = 0.975,\ s = 0.028 \quad (406)$$
$$pI_{50} = 2.99 - 0.55pK_a$$
$$n = 8,\ r = 0.978,\ s = 0.048 \quad (407)$$

perfect correlation (eq 408) between the cell-free inhibitory activity and the antibacterial activity (MIC, the minimum inhibitory concentration necessary to prevent the visible growth of *E. coli*) for both series in combination. A slight deviation to this correlation was shown by only a few compounds (11–15), and they were, therefore, not included in the derivation of eq 408.

$$\log I_{50} = 0.78 + 0.63 \log \text{MIC}$$
$$n = 18,\ r = 0.951,\ s = 0.051 \quad (408)$$

The cell-free inhibitory activity was also shown[405] to be well correlated with the NMR chemical shift (ppm) of the NH_2 proton of the corresponding substituted anilines. Equation 409 is for N^1-phenylsulfonamides

$$pI_{50} = 0.59\ \text{ppm} - 4.43$$
$$n = 14,\ r = 0.927,\ s = 0.069 \quad (409)$$
$$pI_{50} = 1.09\ \text{ppm} - 6.79$$
$$n = 8,\ r = 0.98,\ s = 0.094 \quad (410)$$

and eq 410 for N^1-pyridylsulfonamides. For a larger series of monosubstituted N^1-phenylsulfonamides, the MIC values against *E. coli* and *Mycobacterium smegmatis* were also[406] equally well correlated with the chemical shift (eq 411 and 412). For a subgroup of

$$\log (1/\text{MIC}) = 0.89\ \text{ppm} - 5.65 \quad (411)$$
$$n = 25,\ r = 0.976$$
$$\log (1/\text{MIC}) = 0.84\ \text{ppm} - 5.37 \quad (412)$$
$$n = 25,\ r = 0.971$$

these monosubstituted N^1-phenylsulfonamides, Seydel and Schaper[407] related the MIC values significantly to pK_a also (eq 413). Seydel and Schaper have in fact reviewed[407] all such work of their group and discussed the mode of interaction of sulfonamides.

$$\log (1/\text{MIC}) = 4.76 - 0.66pK_a$$
$$n = 18,\ r = 0.92,\ s = 0.17 \quad (413)$$

Long ago, Bell and Roblin[408] showed graphically the existence of a parabolic correlation between antibacterial activity and pK_a values (ranging from 3 to 11) for a long series of 46 sulfonamides. This parabolic correlation showed that there was an increase in activity as pK_a decreased from 11 to 7 but that there was a decrease in activity as pK_a decreased further. This led Bell and Roblin to suggest that a moderate increase in ionization (to $pK_a = 7$) favored the activity, as the ion is more active than the neutral species. But the high degree of ionization ($pK_a < 7$) suffered a loss of electronic charge at the SO_2N group due to electron withdrawal by the substituent. The crucial property of sulfonamide was thus suggested to be the electronic charge existing at the oxygen atom of the SO_2 group and not the ionization. Cowles' hypothesis was, however, that only the anion had significant activity but only the neutral form was able to penetrate the cell wall.[409] Brueckner[410] agreed to Cowles' hypothesis but assumed different intra- and extracellular pHs. On the basis of the linearity of the correlation as shown by eq 406 and 407, Miller et al.[405] differed with Bell and Roblin; but the observation that the five compounds (11–15) of

TABLE 49. Cell-Free Inhibitory and Antibacterial Activities of N^1-Phenylsulfonamides XCVII and N^1-3-Pyridylsulfonamides XCVIII

compd	R (N^1-phenyl)	I_{50}, μM	MIC, μM	N^1-pK_a	compd	R (N^1-3-pyridyl)	I_{50}, μM	MIC, μM	N^1-pK_a
1	4-OCH_3	75.0	34.50	9.34	13	2-NO_2-4-CF_3	5.0	3.00	6.10
2	H	45.0	16.00	9.10	14	2-Br-4-NO_2	4.0	2.30	5.70
3	4-Cl	35.0	13.00	8.56	15	2-Cl-4-SO_2NH_2		0.85	6.51
4	4-I	25.0	11.25	8.17	16	6-$(C_2H_5)_2N$	95.0	90.00	9.00
5	2-Cl-4-OCH_3	19.0	16.60	8.81	17	6-$(CH_3)_2N$	80.0	32.00	8.85
6	3-CF_3	15.0	5.60	7.98	18	6-CH_3O	55.0	28.80	8.37
7	2-Cl	13.5	2.80	8.18	19	2-Cl-6-CH_3O	18.5	9.50	7.95
8	4-$COCH_3$	10.5	2.00	7.52	20	H	14.0	4.00	7.57
9	4-CN	7.0	1.00	7.36	21	6-CH_3S	12.0	5.60	7.66
10	4-NO_2	7.0	1.00	6.97	22	6-Cl	6.5	1.40	7.07
11	2-OCH_3-4-NO_2	6.0	4.80	7.27	23	2-Cl	6.5	0.70	6.76
12	2-Cl-4-NO_2	6.0	10.80	6.17					

TABLE 50. N^1-Substituted Sulfonamides and Their Cell-Free Inhibition Indexes and pK_a Values

no.	compd	I_{50}/S	pK_a
24	N^1-ethylsulfanilamide	28	10.88
25	N^1-methylsulfanilamide	21	10.77
26	sulfanilamide	10	10.43
27	N^1-phenylsulfanilamide	1.90	8.97
28	sulfapyridine	0.67	8.43
29	sulfasomidine	0.45	7.40
30	sulfamethazine	0.51	7.34
31	sulfathiazole	0.34	7.12
32	sulfamoxole	0.27	7.00
33	sulfadiazine	0.78	6.48
34	sulfadimethozine	0.27	5.90
35	sulfamethoxazole	0.25	5.70
36	sulfaethylthiadiazole	0.43	5.45
37	sulfacetamide	3.50	5.38
38	sulfanilyl-3,4-xylamide	0.55	4.95
39	sulfisoxazole	0.46	4.90
40	sulfabenzamide	0.95	4.57
41	sulfanilylcyanamide	5.20	2.92

Table 49, which were highly ionized under the test conditions, were misfit in eq 408 led them to support Cowles' hypothesis that highly ionized sulfonamides do not easily penetrate the cell. The hydrophobicity in general was, however, not found to play any role in the activity.

Equations 411 and 412 show that the mechanism of inhibition of the whole cell of any kind of bacteria must be exactly the same. This is possible only when the drugs attack a common receptor, i.e., the enzyme.

The cell-free inhibition by a heterogeneous series of N^1-substituted sulfonamides (Table 50) with sufficient variation in pK_a values (2.92–10.88) was examined by Thijssen[411] also. In his study, he found that the plot of cell-free inhibition vs. pK_a was of exactly the same nature (parabolic) as that of antibacterial activity vs. pK_a studied by Bell and Roblin. He thus substantiated the Bell and Roblin theory. However, he also showed, by studying the activity of a few nonacidic molecules, that un-ionized molecules also contribute to the activity.

A quantum mechanical study[412] showed a rough parallelism between the biological activity of sulfonamides and the formal positive charge at their N^1 atom. Though there were a lot of approximations involved in the calculation, this finding was consistent with the assumption that the negative charge at the oxygen atom of the SO_2 group was an important property for the activity. The highly electronegative oxygen will make N^1 positive. The electron-withdrawing capability of the oxygen will depend upon the substituent in the phenyl ring.

Spectroscopic studies[404,413] were also in favor of the Bell and Roblin theory of the anionic feature of sulfonamide activity. Rastelli et al. measured the infrared stretching frequency (ν_s) of the SO_2 group and found it to be related to antibacterial activity[413] as well as to the dihydropteroate synthetase inhibition activity[404] of the anions. No quantitative correlation was shown for the antibacterial activity, but eq 414 was obtained for the enzyme inhibition.[404] For acidic sulfonamides, the

$$pI_{50} = 60.54 - 0.05\nu_s$$

$$n = 17,\ r = 0.932,\ s = 0.26,\ F = 99.08 \quad (414)$$

pI_{50} values were corrected according to the approximation that the whole activity of these compounds can be ascribed to the anionic forms only. Contribution of the corresponding molecular form was ignored. Thus, the inhibitions of the whole cell as well as the dihydropteroate synthetase by sulfonamides were to be related to the high polarization of the S–O bond, which could be the function of high negative charges on the oxygen atoms. The more polarized was the bond, the less was the stretching frequency and, thus, the greater the activity.

All these studies establish that the anionic form of the sulfonamides is important in cell-free enzyme inhibition as well as whole cell inhibition, and that the electronic character of the SO_2 group is important in the binding of the compounds with the receptor.

VIII. An Overview

It seems appropriate to judge all QSAR studies on the following points: (1) Is there any physicochemical, electronic, or steric property common to all inhibitors inhibiting the enzymes of the same group, and on this basis, can one find the common structural features of the enzymes having common biochemical functions? (2) Do the different types of inhibitors inhibiting the same enzyme involve the same mechanism of interactions? (3) How far have QSAR studies been consistent with experimental observations, and how far have they supplemented knowledge on the mechanisms of enzyme inhibitions and the biochemical functions of the enzymes?

As to the first point, one would find that the fundamental property of the molecules that is overwhelmingly involved in enzyme inhibition is hydrophobicity. The greatest contribution of QSAR study is that it has provided a systematic and fairly complete understanding in quantitative terms of the role of hydrophobicity in drug action. Hydrophobicity is not only related to absorption and distribution phenomena but also to the interactions with the receptor sites. The

critical role of hydrophobicity in in vitro activity has provided valuable information about receptor sites.

Normally, one otherwise finds no similarity in the mechanisms of inhibition of enzymes belonging to the same group and involving essentially the same chemical phenomenon (oxidation, reduction, hydrolysis, bond cleavage, etc.) in bringing out the related biochemical processes. Among the oxidases, one would notice that while in glycolic acid oxidase inhibition hydrophobic interaction appears to be important, in D-amino acid oxidase inhibition the electronic interaction appears to play a dominant role. In monoamine oxidase inhibition, electronic and steric factors dominate over the hydrophobic character, and in xanthine oxidase inhibition it is the polarizability that seems to be important in addition to the steric factor. The inhibitions of mixed-function oxidases, however, dominantly involve hydrophobic interaction. Similarly, there has been no consistency in the mechanism of inhibition of enzymes within any group. All the enzymes thus have different specificities, even if they are involved in quite similar biochemical processes.

It is, however, surprising that in the inhibition of the same enzyme there is often inconsistency in the modes of interaction if there are different types of inhibitors. Take, for example, monoamine oxidase. a variety of inhibitors were studied for the inhibition of this enzyme, but QSAR study showed that while there was a positive role of the hydrophobicity of the ring substituent of *N*-(phenoxyethyl)cyclopropylamines (eq 40–44), there was no effect of π of the ring substituent in phenoxycyclopropylamines (eq 48), and there was a negative effect in alkylhydrazines (eq 49) when one compared in vitro activity only. This is the inconsistency when all these types of inhibitors differ only in their side chains. Similarly, while in the case of α-methyltryptamines the coefficient of $\pi_{4,6}$ is negative (eq 57), in the case of a similar type of inhibitors, β-carbolines, the coefficient of $\pi_{6,8}$ is positive (eq 58). $\pi_{4,6}$ and $\pi_{6,8}$ both represent the hydrophobicity of the substituent in the benzene ring of the two series. Thus, there are some other inconsistencies as regards the role of π, but we have not given much weight to it in our discussion. The steric and electronic factors have been common to almost all MAO inhibitors. One can, however, note that in the case of tetrahydro-β-carbolines (eq 60) and some N-substituted β-carbolines (eq 61), no electronic factor was found to be important, and that the in vivo activity of hydrazides was totally a function of the electronic factor (eq 52–54).

Another good example can be cited for cholinesterase inhibitors. For methylcarbamates, π and electronic factors have been very important (eq 198–200, 202, 203, 209, 210, etc.), and the steric parameter has been only occasionally effective (eq 204); but for phosphates, phosphonates, phosphoramidates, etc., E_s, the steric parameter appears to be most important (eq 215–220). Compounds not related to carbamates and phosphates were shown to involve predominantly the electronic factors (eq 222–227). For compounds of the type $RN^+(CH_3)_3$ and imidazolium derivatives, hydrophobicity alone was found to be important (eq 230–233).

Readers would find a few more examples of this type of inconsistency. This inconsistency can be attributed to the fact that enzymes may have more than one active site, differing from each other with respect to their physicochemical nature, so that an inhibitor of any physicochemical characteristic finds the opportunity to interact with the enzyme.

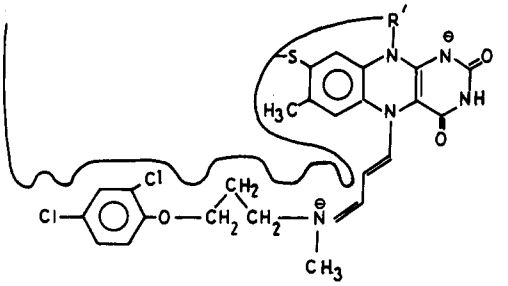
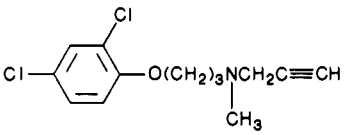


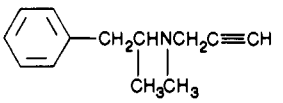
Figure 5. Diagrammatic representation of the binding of clorgyline to MAO-A.

Let us now see how far QSAR studies have been consistent with observations. We can start from MAO inhibition itself, as it has been extensively studied, theoretically as well as experimentally. We have seen from all QSAR studies in this case that there is remarkable similarity in electronic and steric effects in different types of inhibitors. Regarding an electronic effect, it could be generalized that electron-withdrawing groups on the phenyl rings or on heterocyclic rings replacing the latter will tend to increase the potency of the inhibitor in a predictable manner.

Knoll[101] described that there are two main forms of mitochondrial MAO: one that deaminates 5-HT and is highly sensitive to the inhibitor clorgyline (XCIX),



XCIX



C

developed by Johnston;[414] the other that deaminates benzylamine and meta-idobenzylamine[415] as well as phenylethylamine[416] and is insensitive to clorgyline but sensitive to deprenyl (C).[417–419] Johnston[414] named the first MAO-A and the second MAO-B. MAO-A is specialized for binding and metabolizing the ethylamine side chain of a substrate if it is attached to a 5-hydroxyindole ring, and MAO-B is specialized for recognizing and metabolizing phenylethylamine.[420–422] Therefore, the essential structural requirements of an MAO inhibitor are an aromatic ring, an amine group, a short carbon chain between them, and a proper 'enzyme-killing' group. The 'killing group' can be formed by (1) changing the amino group to a hydrazide, (2) changing the short carbon chain between the aromatic ring and the nitrogen to a cyclopropyl moiety, and (3) attaching a propargyl or cyclopropyl moiety to the nitrogen.

According to the model (Figure 5) proposed by Knoll,[421] the binding site for the nitrogen is in the vicinity of the covalently bound flavin group of the enzyme. This model, although shown for the inhibition of MAO-A by clorgyline, is applicable for the inhibition of MAO-B by deprenyl as well. This model is fully consistent with the findings of QSAR. It explains very well how the substituents on the phenyl ring will hinder the interaction of the inhibitor with the enzyme. The observation of Fuller et al.[105] and of Kutter and Hansch[107] in the case of (phenoxyethyl)cyclopropyl-

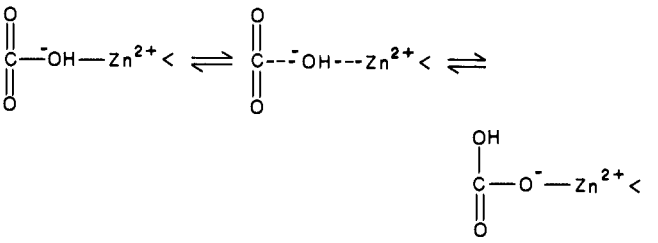
amines that there would be a dominant steric effect by a meta substituent (eq 40–44) is in good agreement with the model. In some cases, as in phenoxycyclopropylamines (eq 48), the steric effect was shown from the para position. These para substituents may hinder the proper orientation of the phenyl ring with respect to the active site at the enzyme, while the meta substituents will directly influence the fit of the phenyl ring with the active site.

The essential positive charge on the nitrogen will be increased by the electron withdrawal by the substituents, and thus, the interaction of nitrogen with the anionic site of the enzyme will be increased. Thus, the occurrence of σ with a positive sign in almost all the correlation equations is well substantiated. The small carbon chain between the phenyl ring and the nitrogen appears to hydrophobically bind with the enzyme.

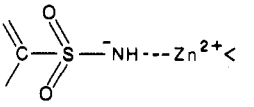
Regarding cholinesterases, which are another set of enzymes equally well studied, we have already pointed out in section VII F that QSAR studies have led to the same picture of inhibition as provided by other studies. That cholinesterase inhibitors exert their inhibitory effect upon the enzyme through enzymic hydrolysis according to the exemplary reaction as shown by eq 195 is firmly established,[240,241,423,424] and the model (XXXVII) proposed for the active sites of the enzyme is well-founded.[240,423] The anionic site of AChE is of critical importance and is believed to be due to a glutamic acid residue. The esteric site consists of a serine residue activated by an imidazole group (histidine). Adjacent to the anionic site there exists a large hydrophobic area that is, according to Steinberg et al.,[425] conformationally flexible and tends to assume a near planar form. In another study, Abou-Donia et al.[426] supported this concept and indicated a planar or slightly curved surface area with a radius greater than 10 Å. Because of this hydrophobic area, QSAR has shown excellent correlation with π.

The involvement of electronic factors suggests the occurrence of either charge transfer interactions or dipolar interactions. Substituents in phosphates are normally strongly polar and may force the ring to bind in a more constrained position to take advantage of dipolar interactions. Substituents in carbamates were either electronically neutral or only slightly polar. However, anomalies in the binding of aromatic phosphates and carbamates is due to the multiplicity of available binding sites on the enzyme.

Thus, for many enzymes, we have discussed in the Results and Discussions section itself how far QSAR study is consistent with the experimental findings. We can further discuss the case of carbonic anhydrase inhibition. The model proposed by the Shinagawas[380] (Figure 4) on the basis of QSAR equations (eq 391–394) is well supported by observations. Many investigators favor the basic proposal that a water molecule at the active site of the enzyme ionizes near neutrality, producing a metal-bound hydroxide ion that attacks the substrate in the hydration reaction.[427-430] Further, X-ray has revealed[431] that in addition to Zn^{2+} ion, the enzyme also has an imidazole residue in its active center. The model thus is in agreement with the two-center model of Inouye et al.[432] and with the chemical study made by Pocker and Storm.[433] However, the avidity of sulfonamides for the active site of carbonic anhydrase has remained an enigma. One possible explanation is that the bound sulfonamide group closely mimics the transition state of the reactants in the reversible hydration of CO_2. According to Coleman,[434] the favored mechanism of hydration of CO_2 involves the attack of OH^- ion on a CO_2 molecule and the possible transition state is



Therefore, Kumar et al.,[435] who found from resonance Raman spectroscopic studies that the SO_2NH^- group was involved in the complex, proposed that the bound sulfonamide must be in the form



Most fluorescence and absorption spectroscopic studies[434] have indicated that sulfonamides bind in a hydrophobic environment in the protein, but the model proposed by Kumar et al.[374] (Figure 3) for secondary binding of the phenyl ring through the meta substituent involving dispersion interaction provides additional information yet to be verified by experiment.

For angiotensin converting enzyme inhibition, it has already been discussed that findings from QSAR studies are in total conformity with the interaction model (Figure 2) proposed from experiments.[299,306]

Now we can finally discuss the inhibitions of chymotrypsin and papain. Among the serine proteinases, the chymotrypsin–ligand interaction is most extensively studied. The essence of QSAR studies in this case was the high dependence of the binding of substrates or inhibitors on molar refractivity. Generally there was high collinearity between MR and π, but in some cases, for example benzylpyridinium bromides for which eq 277 was obtained, MR and π were not collinear, yet they were related to activity with equal significance. This had led Yoshimoto and Hansch[192] to assume that the binding pocket around the active site in chymotrypsin was not typically hydrophobic. It was well fit for dispersion interaction also. This was well supported by the analysis of Dickerson and Geis.[436] Further, Franks[437] recently presented evidence for a second type of "hydrophobic bonding" in which groups with their surrounding flickering clusters of water are held together in solution without desolvation playing the major role. Yoshimoto and Hansch[313] therefore assumed that a high correlation with MR reflects this type of interaction. Thus, QSAR study on chymotrypsin has provided a new dimension of thought. Brot and Bender[438] concluced from a study that bindings in the a_1 and a_2 spaces in the Hein–Niemann model (LXIII) were independent processes. QSAR studies show that this is true only up to a point.

In the case of trypsin inhibition, QSAR studies showed a remarkable consistency for the role of an electronic factor in benzamidines for all different sets

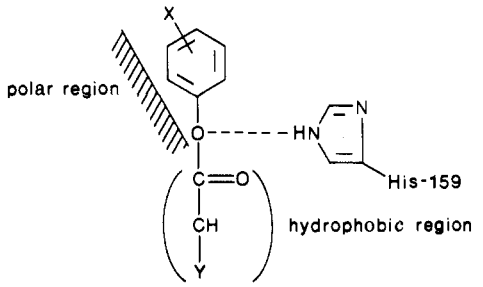


Figure 6. Hansch and Calef model for papain–ligand interaction.

of data obtained in different laboratories. The essence of the study was that electron release by substituents would increase the inhibition potency of benzamidines. However, the role of electron release by substituents is not completely obvious. Mares-Guia et al.[329] and later Recanatini et al.[328] rejected the idea of a charge-transfer interaction. One idea, which was not explored, is the effect of substituents on the hydrophobicity of the benzene ring. It has been known for some time that strong electron withdrawal by electron-attracting groups can, in some instances, decrease the hydrophobicity of neighboring parts of a molecule.[45] Hence, it is conceivable that reduction in hydrophobicity by the strong electron-attracting amidine group could be restored in part by strong electron-releasing groups. This point is worthy of further study.

As in chymotrypsin inhibition, the involvement of hydrophobic interaction was not very obvious in trypsin inhibition also. In most of the equations derived for 4-substituted benzamidines, the activity was found to be related to MR or some steric parameter. This simply suggests that the interaction of 4-substituents occurs with some polar region of the enzyme and is hindered by a sufficiently bulky group. True hydrophobic interaction was hard to be assumed, when Yoshimoto and Hansch found that the replacement of π in eq 309 by MR gave an equally high correlation.[192]

For other serine proteases such as thrombin, plasmin, and complement, QSAR studies could not discern properly the roles of hydrophobic and dispersion interactions.

In the case of papain–ligand interaction also, the molar refractivity was found to play a dominant role. However, the electronic factor was also important in this case. With this and the effective role of π in certain cases as shown by eq 341, 344, and 345, Hansch and Calef[56] proposed a model (Figure 6) for the interaction of ligand with papain. The structure of papain[439] and the ligand–enzyme interaction[440–442] are well studied. The model proposed is based on these studies and on the representation of the enzyme by Dickerson.[443] This model now provides directions to further studies.

A very consistent role of MR has been shown by QSAR studies in the interactions of ligands with ficin, bromelain, and actinidin, which belong to the group of serine proteases.

Similarly, QSAR studies on all other enzymes provide clues to the mechanism of inhibition and to the mode of interaction of the enzymes with their substrates. The QSARs on dihydropteroate synthetase established that the anionic form of sulfonamides is important in cell-free enzyme inhibition as well as whole cell inhibition and that the electronic character of the SO_2 group is important in the binding of the compounds with the receptor. Many QSAR studies are not significant, but they provide direction for thought and further studies.

QSAR analyses have been able to show that enzyme inhibition is not the function of any particular property of molecules but can depend upon a variety of factors. The QSARs have pointed out in particular that the potency of inhibitors depends not necessarily on overall log P or MR but possibly on π or MR at a specific site on the molecule. The use of such site-specific factors fits very well with the analysis of well characterized receptor binding assays.

QSAR analyses have not only established the relations between the biological potency and the physicochemical properties, but they have also been a guide to discover, quantitate, and evaluate possible relationships. The QSAR equations have described in quantitative terms the forces involved in the interactions.

Recently, the use of computer graphics has enormously facilitated the study of macromolecules. One can now easily visualize from graphics what kind of (polar or hydrophobic) surfaces are involved in the interactions of ligands with enzymes and what parts of macromolecules are causing steric effects. Hence, a combination of QSAR analyses with computer graphics may be of very high value. A few reports have been recently published that illustrate the value of color stereographics in enlarging our understanding of the mode of interaction of ligands with macromolecules.[174,203,328,346,347,349,379,444–452] With molecular graphics studies, the QSAR equations of certain enzymes, e.g., alcohol dehydrogenase,[174] trypsin[328] papain,[346,347] actinidin,[349] carbonic anhydrase,[379] etc., have been able to provide much a better picture of the inhibition mechanism. By molecular graphics modeling, the active site of ADH is characterized with a channel ca. 20 Å long from solution to the catalytic zinc atom.[453] Initially, this channel is very narrow but widens near the zinc atom. The channel widens again near the outer surface of the enzyme and becomes more polar. The narrowest part of the channel is essentially hydrophobic except near the catalytic zinc atom. The linear dependence of ADH inhibition activity of amides and pyrazoles on hydrophobicity (eq 128–131 and 133) led to the suggestion that these inhibitors bind in the narrow hydrophobic channel and coordinate to the zinc atom.[174] But the channel is so narrow that the water of solvation must be removed from substituents in the binding process.

In the case of trypsin inhibition, the graphics model in combination with QSAR analyses has described that benzamidine inhibitors are bound in a hydrophobic pocket in the active site of the enzyme and form an electrostatic bond between the positively charged amidine and the negative carboxylate of Asp-189 at the back of the hydrophobic pocket.[328] However, the small para substituents of benzamidines do not contact the hydrophobic space but, instead, collide with the polar OH of Ser-195. When π or a combination of π and MR do not correlate substituent effects while MR or steric parameters do, one can assume this to be diagnostic of interaction occurring with the polar enzymic space. Thus, small para substituents are in line with this assumption, as graphics allows one to visualize.[328]

The QSAR analyses for papain hydrolysis of esters were found to be in excellent agreement with molecular

graphics.[346,347] Equations 342 and 343 derived for phenyl hippurates LXXVIIb were quite in line with molecular graphics observations[346] that 4-R groups contact a polar region of the active site and remain exposed to the solvent and that 3-R groups bind to a moderately sized hydrophobic pocket near the active site, as one would expect from eq 343. The R groups of LXXVIII esters, as expected from eq 341, were visualized[346] to be bound in a simple, nondirectional hydrophobically driven association to a very large pocket of the active site channel formed by the side chains of the enzyme.

A little different mode of binding of the 3-R groups of glycinates LXXVIIa with papain was suggested by QSAR analysis (eq 345). As the coefficient of π_3' in eq 345 being smaller than that in eq 344 of phenyl hippurates would suggest, 3-R groups of glycinates do not contact the enzyme surface as effectively as those of phenyl hippurates. From molecular graphics, these groups of glycinates are visualized to lie along the surface of the enzyme in the long active-site groove, rather than being completely desolvated in the hydrophobic pocket.[347] The correlation analysis and the molecular modeling have, however, pointed out that the $NHSO_2CH_3$ moiety of glycinates and the $NHCOC_6H_5$ moiety of phenyl hippurates both bind in the hydrophobic space.

The QSAR model for actinidin hydrolysis of phenyl hippurates (eq 351) suggests that actinidin binds with hippurates in almost the same way as papain does with glycinates. This similarity in binding was well substantiated by molecular models with little difference.[349]

Regarding the binding of aromatic sulfonamides with carbonic anhydrase, the model as shown in Figure 3 presented a very simplistic picture. An elaborate picture was presented by Hansch et al.[379] with the help of a color stereomolecular graphics model of the enzyme–inhibitor complex and the QSAR of the King and Burgen data on sulfonamides.

The active site cavity of carbonic anhydrase is divided into two halves:[375-377] a hydrophobic half consisting of the residues Tyr-7, Asn-61, His-64, Asn-67 Glu-69, Gln-92, His-94, His-96, Glu-106, His-119, Thr-199, and Thr-200, and a hydrophobic half consisting of the residues Ala-65, Ile-91, Val-121, Phe-131, Leu-141, Val-143 Gly-145, Pro-201, *cis*-Pro-202, Val-207, Trp-209, and Val-211. The activesite surface is extended to include the residues Asp-62, Val-135, and Leu-198.[378] In the molecular model, it was possible to orient the para substituents of sulfonamides so that the alkyl chains lie along the hydrophobic wall near Phe-131 and Val-135 or in a small channel between Asp-62 and Asn-67. Clearly, the area around Phe-131 and Val-135 is more hydrophobic than the channel and provides favorable interactions with the alkyl chains. In addition, there is the possibility of hydrogen bonding between the ester or amide groups and Gln-92.

The meta substituents were found to be able to occupy the same two sites in the active-site cavity.[379] A binding mode near Phe-131 was again chosen as the most likely location for substituents due to the increased hydrophobicity.

All ortho substituents were found to display an unfavorable interaction with Pro-201, and their alkyl chains were constrained to occupy the area along the hydrophobic wall near Leu-198. This area was well contacted by meta substituents. The ester or amide substituents of the ortho position were expected to form a hydrogen bond with Thr-200.

The surprising aspect of such QSAR graphics analyses has been that, although enzyme structures are quite flexible, the features of the active site obtained via QSAR analyses made on data of studies in solution agree well with those obtained from the crystallized form of the enzyme by X-ray crystallography.

In conclusion, the combined use of QSAR and molecular graphics models provide a better picture of the interaction of organic compounds with biological receptors. The insight gained from such attempts will enable us to design more effective substrates and inhibitors for biological processes leading eventually to the design of more effective drugs.

IX. Acknowledgment

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