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RESEARCH ARTICLES

Thermodynamic Analysis of Structure–Activity Relationships of Drugs: Prediction of Optimal Structure

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Abstract □ A new quantitative and comprehensive approach relating structures of congeneric drugs to their relative biological activities is presented. The analysis is derived on the basis that structure–activity relationships represent a family, a different situation applying to each phenomenon such as drug absorption, drug transport, drug transformation, and drug excretion. The present treatment considers the relationship under equilibrium or quasiequilibrium conditions, thus permitting rigorous thermodynamic treatment. On the basis of the effect of structural changes on the distributive tendencies of the drug in various body tissues, including the receptor site, relationships have been derived which are surprisingly in good agreement with available experimental data. The approach suggests a rational way to predict the degree of lipophilicity which would result in maximal activity.

Keyphrases □ Structure–activity relationships, drugs—optimal structure prediction □ Thermodynamic analysis—structure–activity relationships □ Equilibrium conditions, model compartments—thermodynamic activity, drugs □ Energy change—aqueous–lipid partitioning

Persistent efforts have been made over many decades to bring some satisfactory order to the correlation of the relative activities of drugs with their molecular structure. The last few years have seen a great upsurge in interest in this direction. In this publication, the authors: (*a*) review many of the earlier hypotheses and theories dealing with structure–activity relationships, and (*b*) present a new formulation of the problem based on thermodynamics.

The proposed approach, which will be treated in depth later in this paper, assumes that any observed biological activity in the animal or any test system usually involves one or more time-independent situations and a large number of time-dependent processes such as drug absorption, drug transport, drug transformation, and drug excretion. Since structural alterations affect each of these differently, it would appear highly unlikely that any single relationship can account for the observed situation. The present treatment has been largely limited to analysis of the effects of structural changes on the time-invariant activity of drugs.

As a general approximation, overall interaction of a drug molecule with its receptor site appears to be resolvable into two parts. The first is highly specific in nature and is presumably responsible for the "lock and

key" relationship between the two interactants (1). It is suggested that this part of the interaction involves those portions of the drug and receptor species that are in intimate fixed contact with each other. The second, which is largely unspecific in nature, is generally considered to arise simply from hydrophobic interaction between the lipoidal parts of the drug molecule and various lipophilic portions of the receptor. The present treatment is limited essentially to the influence on drug activity arising from the effects of changes in its molecular structure on the latter contribution to drug–receptor binding.

It is recognized, however, that the effect on activity of any structural changes in the drug molecule cannot always be totally ascribed to one or the other of the two interaction categories. Introduction of a hydrophobic grouping in the near vicinity of the specific site, for example, may increase the nonspecific interactions while interfering with the "lock and key" relationship. However, if the specific-type binding is assumed to be limited to a fractionally small part of the molecular surface of the drug, it would appear that most structural changes would not affect the specific interaction but would manifest their effect essentially through their influence on hydrophobic binding. Most serious approaches relating group contribution to drug activity have been limited primarily to effects arising from the same unspecific part of the overall interaction.

EARLIER APPROACHES

Some of the more interesting earlier studies on structure–activity relationships were those made by Overton (2), Meyer (3), and Meyer and Hemmi (4), who related narcotic activity to partition coefficients and suggested that narcosis occurred when a definite molar concentration was reached in the receptive lipid biophase. In a normal homologous series, for example, the increase in activity upon the addition of a methylene group was shown to be in the narrow range of 2.5–3.3 times, depending on the nature of the drug series and the test organism. Many physicochemical properties of aliphatic compounds, which depend on an equilibrium between two phases, increase or decrease with change in chain length in a similar manner. The increase in biological response with chain length does not continue indefinitely as predicted by this relationship; instead, a cutoff point is reached where higher homologs have little or no activity. Hansch (5) rightly commented that such effects are of extreme importance and should be explained by any proposed model dealing with structure–activity relationships.

Ferguson (6) rationalized the picture of narcotic action using thermodynamics and suggested that an equilibrium exists between the extracellular phase and the phase at the site of action (receptor site) such that substances present at the same proportional saturation in a given medium have the same degree of biological action. Or, as stated by Brink and Posternak (7), "equal degrees of narcosis are produced at about equal thermodynamic activities."[1] This generalization, unlike the earlier theories of Overton (2) and Meyer (3), has the great advantage of not requiring any specific mechanism of action. Ferguson (6) also considered that physical toxicity involved no chemical reaction and that the narcotic substance left the body unchanged (8). For example, the substitution of a halogen in a hydrocarbon leaves the potency practically unchanged, and no specific narcotic character can be ascribed to the chlorine atoms in chloroform, their presence merely lowers the vapor pressure of methane to a level convenient for administration.

McGowan (9) related the bioactivity of organic compounds to their size (parachor), while Mullins (10) examined the problem with the help of the solubility parameter concept (11). The latter concluded that narcosis, by chemically inert molecules, took place when constant fraction of the total volume of some nonaqueous phase in the cell was occupied by narcotic molecules. If the narcotic behaved ideally in the biophase, the thermodynamic activity multiplied by the volume fraction of narcotic was a constant. Higher values of thermodynamic activity, which occurred when a homologous series was ascended, were attributed to an increase in the activity coefficient of the narcotic in the biophase.

Crisp and Marr (12) examined the action of a range of narcotic substances from a more strictly thermodynamic standpoint and concluded that the mechanism of narcotic action in small organisms is only consistent with an equilibrium condition between the narcotic in the biophase and in the external medium. They felt that no theory that relied solely on a rate process, such as diffusion through a lipoid layer, could account adequately for the facts. However, Hansch and Fujita (13) recently concluded that, in the great majority of cases, a true equilibrium condition is rarely achieved and that a probabilistic approach may be far more realistic. These aspects will be discussed at greater length with relation to the proposed model.

By far the most widely known and employed linear free energy approach to stucture–activity correlation is that due to Hansch (5, 14–16) and Hansch and Fujita (13). Originally, a four-parameter approach was suggested:

$$\log BR = k\pi^2 + k'\pi + \rho\sigma + k'' \qquad \text{(Eq. 1)}$$

where π is a constant derived from partition studies between water and 1-octanol, σ is the Hammett constant, and ρ is a reaction constant derived from regression analysis. Correlation between biological response (BR) and chemical structure was achieved with a great many diverse systems. When only the π-term was necessary for good correlation, the response was considered to be controlled by a physical process (*e.g.*, partitioning of the drug), whereas a chemical interaction was thought to be responsible for correlations dominated by the σ-term. However, this type of one-parameter approach was limited, no doubt, to the complex nature of the biological test systems which would include problems such as drug penetration, possible differential rates of metabolism and excretion, and steric, electronic, and hydrophobic interactions with critical sites in the biophase. Also of equal importance is the very limited accuracy of much of the biological data.

In free energy terms the response can be considered as being governed by one rate-limiting process for which K_{BR} is the equilibrium constant (14). Then:

$$\Delta F_{BR}^0 = \Delta F_{L/H}^0 + \Delta F_{\text{electronic}}^0 + \Delta F_{\text{steric}}^0 \propto \log K_{BR} \qquad \text{(Eq. 2)}$$

where L/H = hydrophobic and BR = biological response.

In partial terms for substituent effects and for a true equilibrium condition:

$$\delta_x F_{BR}^0 = \delta_x F_{L/H}^0 + \delta_x F_{\text{electronic}}^0 + \delta_x F_{\text{steric}}^0 \propto \delta_x \log K_{BR} \qquad \text{(Eq. 3)}$$

Attempts can then be made to associate the various free energy terms with definite physicochemical constants (Table I). For example,

$$\delta_x F_{L/H}^0 = f(\log P,\ \pi,\ R_M,\ \Delta R_M,\ \beta, \text{ and parachor}) \qquad \text{(Eq. 4)}$$

$$\delta_x F_{\text{electronic}}^0 = f[\sigma,\ \textit{etc.},\ \text{quantum mechanically calculated electron densities or chemical shifts (NMR)}] \qquad \text{(Eq. 5)}$$

The $\delta_x F_{\text{steric}}^0$ terms are somewhat difficult to ascribe to definite parameters, although E_S and E_S^C have been used. Often, a two-parameter approach seems to be successful.

Detailed examples of the use of the various parameters can be found in one of the many recent reviews of Hansch's work (13–15). In some cases, this has indicated that, while steric interactions are extremely important [for example, see Portoghese (17)], the concept of "lock and key" fit of drug and substrate has been overemphasized at the expense of hydrophobic bonding (14).

[1] The use of the term "thermodynamic activity" in this sense is questionable. As defined by G. N. Lewis (*Proc. Amer. Acad. Arts Sci.*, **37**, 49(1901), activity of Component A can only be compared to some other state of A. The authors will treat this in greater detail in a subsequent paper.

Hansch's approach is not without its shortcomings (18) and by and large it cannot cope with steric factors or with metabolic inactivation processes too successfully. Also, some surprising results appear in regression analysis. For example, in one case the derived equations would indicate that the mode of action of thiobarbiturates differs from that of the analogous barbiturates (19).

The linear free energy relations of Zahradnik (20, 21) are also of the basic Hammett type but are restricted in their application to aliphatic compounds of the type R—X. They can be written as:

$$\log(BR_i/BR_0) = \alpha\beta \qquad \text{(Eq. 6)}$$

where BR terms refer to the response of the species (i) and the reference species (0); β is a constant characterizing the alkyl substituent R and its value is independent of the nature of the functional group X; and α characterizes the susceptibility of the biological system to the influence of the substituents R. The two constants are mutually independent. The β is linearly related to the logarithm of the activity coefficient of the drug and Hansch's π (22). Success in correlation has been demonstrated for situations where specific electronic and steric effects are not crucial, and the approach has also been extended (21) using Hammett and Taft substituent constants.

In continuing investigations designed to elucidate structure–activity relationships, Purcell *et al.* (23) attempted correlation with a wide range of parameters, including dielectric and surface-active properties, dipole moments, and electronic structure. A number of attempts have also been made to apply molecular orbital methods, but progress has been slow due to the lack of a reasonable theoretical framework within which to work (24). Hansch (5) has not attempted to factor out hydrogen bonding, although Purcell *et al.* (23) have suggested ways of dealing with such a term.

Table I lists some of the parameters that have been used in linear free energy correlations of the Hansch type. In multiparameter correlations, the mathematical significance of the various terms, π, σ, *etc.*, as well as π^2, σ^2, and $\sigma\pi$, are determined using regression analysis. The physicochemical significance of the various squared and product terms is not clear.

Table I—Linear Free Energy Correlation Parameters (log BR = $a(1) + b(2) + \cdots + k$)

	Parameter
1. π	Hydrophobic bonding constant from partition coefficients[a–e]
2. π^2	
3. σ	Hammett linear free energy constant[a–g]
4. σ^2	
5. σ^*	Taft aliphatic constant[h, i]
6. σ_I	Inductive parameter[j]
7. σ_+	Electrophilic radical constant[j]
8. σ_p	Electronic effect of substituent attached to side chain and positions *ortho* to it[k]
9. σ_m	
10. $\sigma^\cdot$	Radical constant[j]
11. $\pi\sigma$, *etc.*	?[l]
12. E_s	Taft steric parameter[m]
13. E_s^c	Hancock's corrected steric parameter[g]
14. N	Number of carbon atoms in substituent[n, o]
15. nH	Number of hydrogens attached to protonated nitrogen[g]
16. Log P	Partition coefficient[p]
17. Log VP	Vapor pressure (from GLC)[q]
18. P_E	Molar electronic polarizability[o]
19. γ	Arbitrary steric constant[r]
20. μ^2	Dipole moment[s]
21. $t_{1/2}$	Reaction parameter[q]
22. $S_o(Z)$	Superdelocalizability[t, u]
23. $f_{oxy.}(E)$	Frontier electron density (ether oxygen)[t, u]
24. ϵ	Electron density on nitrogen[v]
25. pKa	(*Reference 1*)
26. ΔpKa	Dissociation constant difference between parent and derivative[w]
27. E_R	Constant obtained from hydrogen abstraction reaction of nuclear-substituted cumenes[j]
28. Δk	Hydrogen bonding parameter[x]
29. P	Orientation polarization of amide groups[z]
30. A	Wheland's atomic localization energy[h]
31. [P]	Parachor[y]
32. E_A	Interaction parameter (hydrogen bonding)[z, aa]
33. F	Molar attraction constant[bb]
34. πF	?[bb]
35. R_M	Chromatography constant derived from R_f[cc]
36. *HOMO*	Energy of highest occupied molecular orbital[dd]
37. [P^*]	Adjusted parachor[ee, ff]
38. δ_c	Occupation number (extended Huckel theory)[gg]
39. e_c	Electron density by extended Huckel theory[gg]
40. ϵ_c	As 39, but by complete neglect of differential overlaps[gg]
41. θ	Total interaction energy hydride ion[gg]
42. Δ_E	Eigen value differences[gg]
43. δ_E	Incipient transition state energy differences[gg]
44. M	Molecular weight[ff]

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The choice of terms is often bewildering; and in many cases, little can be said about a particular parameter except that it "works" and gives improved correlation. The complexity of some of the more recent equations is also disturbing. A recent illustrative example cited by Hansch (25) but not recommended contains seven terms to correlate 16 data points:

$$\log BR = -0.123\,\pi^2 + 0.633\,\pi - 1.823\,\sigma^{\cdot 2} + 3.162\,\sigma^\cdot - 0.796(\pi\sigma^\cdot) + 0.639\,E_s + 1.450 \qquad \text{(Eq. 7)}$$

Leo *et al.* (26) recently examined some of the parameters currently used in structure–activity relationships and their improvement, if any, over simple correlations using molecular weight. In general, the octanol–water partition coefficient was more suitable for satisfactory correlation than polarizability, molar attraction constant, parachor, or adjusted parachor. A great number of linear free energy constants (27) have yet to be tested but this would appear to be only a matter of time.

Free and Wilson (28) developed a purely mathematical approach to structure–activity relationships from an original proposal by Bruice *et al.* (29). Here it is assumed that the contribution due to each substituent is additive and constant, regardless of substituent variation in the remainder of the molecule. Although being restricted to a series of chemically related species, it has the great apparent advantage over linear free energy methods in that no physicochemical data are required. Smithfield and Purcell (30) discussed the application and requirements of the method. These are: (*a*) closely re-

lated analogs that provide a gradual change in biological response; (*b*) accurate biological data; and (*c*) additive activity parameters. Although somewhat limited for these reasons, it has been used with success by Purcell and Clayton (31), Ban and Fujita (32), and many others. A similar additive method is that of Kopecký *et al.* (33) in which constants are fitted to a semiempirical equation using regression analysis. This too is limited, at present, to specialized classes of compounds.

Singer and Purcell (34) compared the linear free energy and Free and Wilson (28) types of correlation technique. Each has its own merits and disadvantages. In particular, the Free and Wilson additive constant group contribution concept is not suitable for cases where there is a parabolic relation between the partition coefficient and biological response.

Interrelationships between the various methods have also been discussed by Cammarata (24) who showed that many of the physicochemical approaches to the study of drug action can be related, in a quantitative manner, to the principle of hard and soft acids. Here the drug–receptor interaction was discussed in terms of each pair of interacting atoms making an independent contribution to the electronic, steric, and desolvation free energies of interaction.

A NEW PROPOSAL BASED ON THE EQUILIBRIUM MODEL

It is apparent from the preceding analysis of the various approaches to structure–activity relationships that no single proposed system has been widely accepted and, at the same time, been based on rational grounds. To a certain degree, this has been due apparently to the fact that a single, simple correlation was sought when none was possible for the multifaceted situation. The authors wish to suggest a more limited approach which seems to be rational, relatively simple, and perhaps more useful. It is designed for any test system that rapidly achieves distributive equilibrium or quasi-equilibrium with respect to the added drug. It assumes, in brief, that: (*a*) any test system, whether a culture of microorganisms, a mouse, or a man, consists of a number of widely differing physical regions having widely differing affinities for the added drug species, and (*b*) biological activity is determined by the relative amount distributed to the receptor from the total system.

For the purpose of analysis, the authors make the following conditions and fundamental assumptions.

1. A biological test system can be represented by t number of accessible compartments, w, 1, 2, 3 . . . $t + r$, where compartment w is the aqueous phase; 1, 2, 3, *etc.*, are tissue, lipoidal, protein, *etc.*, phases; and r is the receptor. The receptor can either be some definite site (*e.g.*, an enzyme surface) or some unspecialized region in some cells. The effective volume of each compartment is V_w, V_1, V_2, *etc.*

2. Thermodynamic equilibrium or quasiequilibrium is reached in all accessible phases, and the thermodynamic activity of the drug in the rth compartment is the same as that in the aqueous, first, second, *etc.*, all with reference to a common standard state. If a drug is added to the aqueous compartment, it will be distributed to all the other available compartments according to Nernst's distribution law.

3. For a series of drugs of closely related structures, biological activity is proportional to the fraction of the active sites occupied. If the fractions occupied are made the same, then equal biological response will be elicited.

4. Essentially all of the administered drug will be distributed to the various accessible body compartments, and only an insignificant amount will actually be attached to the receptor site.

Apparent observed overall activities of a series of drugs in any animal test system will obviously be dependent on effects of structural changes on, for example, the process of absorption, the process of transport to the area of the receptor site, the process of excretion, the process of chemical transformation into metabolites, *etc.* As is apparent, the authors have in the present analysis restricted the definition of drug activity to equilibrium situations, the term drug activity being related to the intensity of biological response observed when the test drug is assumed to be completely distributed over all of the readily accessible tissue and fluid space. The equilibrium, or more correctly, the pseudoequilibrium definition of drug activity can be, to a certain extent, considered as the intrinsic (time-independent) activity, and other (time-dependent) effects can be considered modifications of it. The pseudoequilibrium situation commonly prevails, because a significant number of the body compartments of the test systems are not accessible to many drugs.

Table II—Affinity Constants for the CH_2 Group

Solvent	F
Carbon tetrachloride	4.6
Cyclohexane	4.5
Chloroform	4.4
Hexane	4.2
Benzene	4.2
Ethyl ether	3.6
Octanol	3.2
Oleic acid	3.1
Ethyl acetate	3.0
n-Butanol	2.6
2-Butanol	2.2
3-Pentanone	2.1

The influence of the distributive effects on drug activity can be formalized as follows. Consider the situation when an amount of drug, S, is administered to the test system. It is evident that

$$S = C_wV_w + C_1V_1 + C_2V_2 + \cdots C_iV_i$$
$$= C_wV_w + \sum_{i=1}^{i=t} C_iV_i \qquad \text{(Eq. 8)}$$

where C's refer to the effective concentrations in each accessible biophase compartment and V's to their volumes. As previously stated, the number of accessible compartments is taken as t, and the amount of drug incorporated into the receptor phase is normally negligible.

If the drug distribution between the aqueous phase and each biophase is assumed to follow a linear partition isotherm, a distribution constant can be defined:

$$K_i = \frac{C_i}{C_w} \qquad \text{(Eq. 9)}$$

and

$$S = C_w\left(V_w + \sum_{i=1}^{i=t} K_iV_i\right) \qquad \text{(Eq. 10)}$$

The effective concentration of the drug on the receptor can then be formulated by solving for C_w in Eq. 10:

$$C_r = K_rC_w = \frac{SK_r}{V_w + \sum_{i=1}^{i=t} K_iV_i} \qquad \text{(Eq. 11)}$$

or

$$E = \frac{C_r}{S} = \frac{K_r}{V_w + \sum_{i=1}^{i=t} K_iV_i} \qquad \text{(Eq. 12)}$$

E being the concentration of drug produced on the receptor per unit amount of drug administered. It relates directly to the expected relative activity of the drug.

What change in activity can be expected on this basis when, for example, a hydrogen in the reference (parent) drug is replaced by a methyl group? It is apparent from Eq. 12 that the activity of the new derivative will differ from that of the parent compound as the chemical change influences the various K, the partition coefficient, values in the equation. For a system where $V_w \gg \sum_{i=1}^{i=t} K_iV_i$ (that is, for a system where the bulk of the drug is in the aqueous phase), it is evident that the substitution of a methyl for a hydrogen will produce a marked increase in lipophilicity and activity corresponding to a similar increase in K_r (usually of the order of 2–3×), the partition coefficient of the receptor site. This relates as a first approximation to the amount of free energy necessary to bring a methylene group from aqueous to the receptor bond state. It is evident that if two methylene groups are introduced (*e.g.*, by substituting with C_2H_5 rather than CH_3), the increase in activity will correspond to the

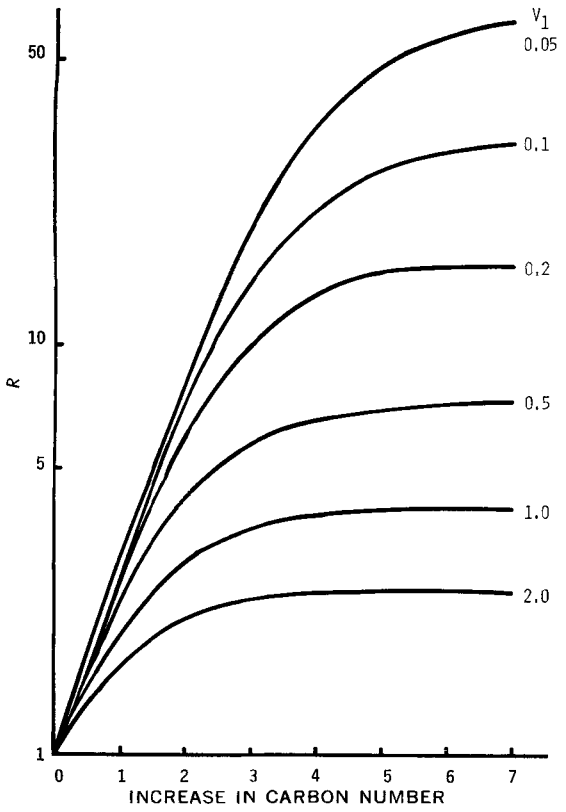

Figure 1—*Two-compartment model analysis of drug distribution:* $F_{(CH_2)r} = 3$ *and* $F_{(CH_2)l} = 3$.

square of the first; *i.e.*, if the first increase is by a factor of 2.5, the second will be approximately $(2.5)^2$ or 6.25. This follows essentially the group contribution approach developed by Hansch and others (5).

For the situation, $V_w < \sum_{i=1}^{i=t} K_i V_i$, *i.e.*, for systems in which the drug has been largely distributed into the tissue phases leaving only a small fraction in the aqueous, substitution of a methyl for a hydrogen may be expected to lead to a decrease in activity in instances where the receptor may be intermediate in polarity. This can be seen by using a highly simplified test system consisting only of an aqueous phase and a single lipoidal phase in addition to the receptor. For such a case:

$$E = \frac{K_r}{V_w + K_l V_l} \cong \frac{K_r}{K_l V_l} \quad \text{since } K_l V_l \gg V_w \quad \text{(Eq. 13)}$$

where subscript l refers to the lipoidal phase. It is evident that K_r may still increase by a factor of 2.5, but K_l may increase by a factor of 4.5, the expected decrease in activity in this example being 2.5/4.5 or by a factor of $^5/_9$. For the same system, introduction of C_2H_5 for CH_3 will be expected to produce a $(^5/_9)^2$ decrease in activity.

These concepts, as they apply to Eq. 12, can be generalized. Thus, when substituents α, β, *etc.*, are introduced, the effective drug concentration on the receptor is

$$E = \frac{K_r^*(F_\alpha)_r(F_\beta)_r \cdots}{V_w + \sum_{i=1}^{i=t} [K_i^*(F_\alpha)_i(F_\beta)_i \cdots]V_i} \quad \text{(Eq. 14)}$$

where the asterisk (*) refers to the partitioning properties of the parent reference drug into compartment i, and the F's are the factorial group contribution in modifying them with respect to each biophase. An F value, as used here, is the ratio of the partition coefficient of a substituted substance to the partition coefficient of the parent compound. For the example of the methyl substitution given in this illustration, $F_{(CH_2)r} = 2.5$ and $F_{(CH_2)l} = 4.5$. It is evident that for systems obeying equilibrium or pseudoequilibrium conditions, Eq. 14 will permit prediction of the effects of such substituents whose F values are known.

For comparison of relative activities of derived compounds with those of their parents, it is convenient to define another function:

$$R = E/E^* = \frac{K_r\left(V_w + \sum_{i=1}^{i=t} K_i^* V_i\right)}{K_r^*\left(V_w + \sum_{i=1}^{i=t} K_i V_i\right)} \quad \text{(Eq. 15)}$$

It is apparent that if $R > 1$, a derived compound is more active than its parent; conversely, if $R < 1$, it will be less active.

The relationships discussed can perhaps be seen more effectively graphically. For the two-compartment model discussed, select the situation such that $V_w = 1$ and $K_l^* = 0.33$. The resulting activity values expressed as R as functions of the number of added methylene groups are shown in Figs. 1–3. In all cases, R initially increases in magnitude with increase in chain length. When the volume of the lipoidal phase is somewhat less than that of the aqueous phase, this increase is more or less geometric in nature. However, the linear (geometric) region does not continue indefinitely, and a maximum or limiting value of R is obtained. If $F_{(CH_2)l} = F_{(CH_2)r}$, a plateau region is reached where further increase in chain length has little effect on R. If $F_{(CH_2)l}$ is greater than $F_{(CH_2)r}$, a maximum value for R is obtained. The exact shapes of the various hypothetical curves are dependent on the values of V_l and $F_{(CH_2)l}$, $F_{(CH_2)r}$. If $F_{(CH_2)l} = 2F_{(CH_2)r}$, a parabolic relationship results (Fig. 3). The position in the alkyl chain for maximum activity depends on the difference between the affinity factors (F values) of the receptor and lipoidal phase and the volume of the lipoidal phase. As the difference between $F_{(CH_2)r}$ and $F_{(CH_2)l}$ becomes greater, maximal R occurs at lower chain lengths. A similar result is obtained by increasing the volume of the lipoidal phase.

It is evident that for any real multicompartment system, the fractional distribution of drug into various compartments will be such that it will move from the aqueous phase gradually toward the most lipophilic compartment as the drug itself is made more lipoidal. In the extreme cases, the drug will be found largely in the aqueous or in the most lipoidal compartment (adipose tissues), which correspond roughly to the simplified example. The receptor sites, because of their locations, may be expected to offer intermediate environments

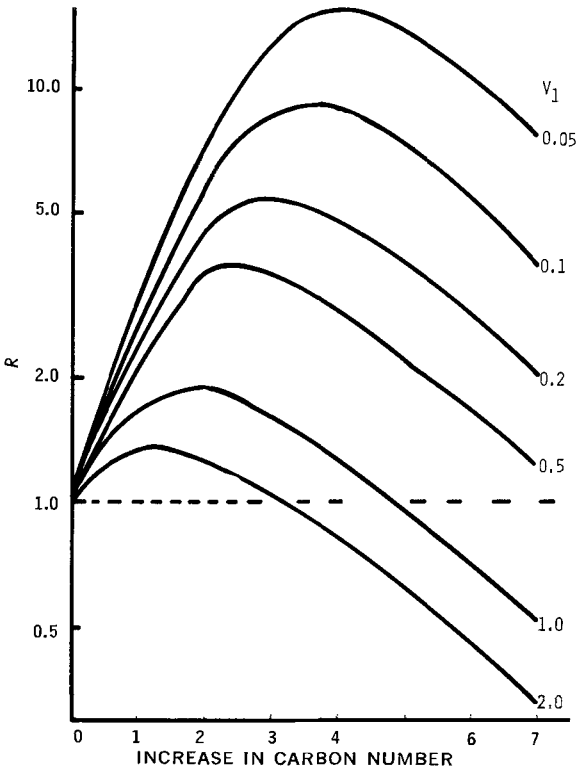

Figure 2—*Two-compartment model analysis of drug distribution:* $F_{(CH_2)r} = 3$ *and* $F_{(CH_2)l} = 4$.

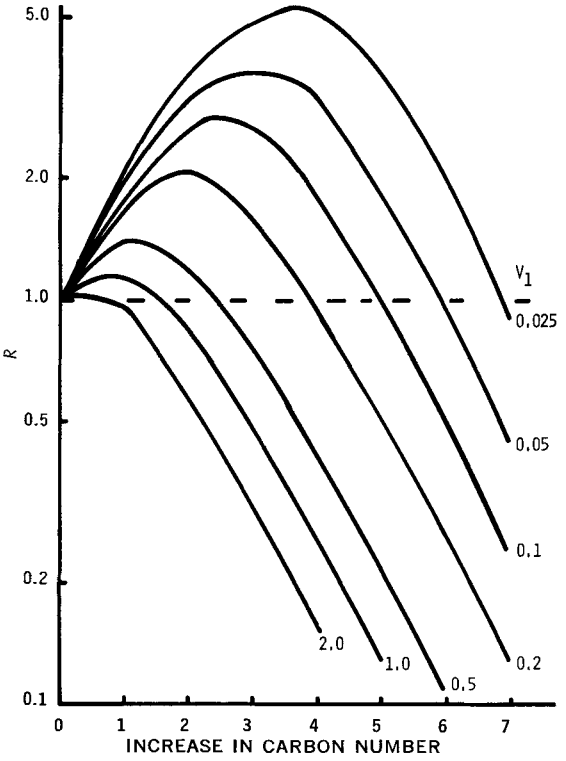

Figure 3—*Two-compartment model analysis of drug distribution:* $F_{(CH_2)_r} = 2$ *and* $F_{(CH_2)_l} = 4$.

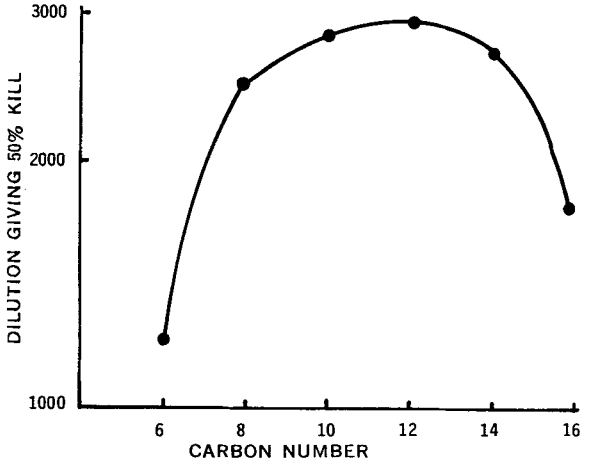

Figure 4—*Dilution of alkyl rhodanates required for 50% kill of green chrysanthemum aphid. [Plotted from the data of E. E. Bousquet, P. O. Salzberg, and H. F. Dietz,* Ind. Eng. Chem., *27, 1342(1935).]*

The authors found that for nearly all nonpolar lipoidal solvents such as benzene, cyclohexane, carbon tetrachloride, chloroform, and hexane, the free energy change in transferring a methylene function from water to the lipoidal environment is such that an addition of a CH_2 produces a partition coefficient increase by a factor close to 4.5 at 25° (Table II). Similarly, a CF_2 produces an increase by a factor of 5.5. It would appear, therefore, that if the effect on K_r is by a factor of 2–3, any significant increase in lipophilicity can be expected to lead to decreased activity at longer chain lengths. It is apparent that at some point of balanced lipophilicity, the optimal concentration of the drug on the receptor surface will be obtained. The so-called "parabolic" relationship between drug activity and lipophilicity, therefore, is a thermodynamically predictable situation, as shown in Figs. 1–3. Figure 4 shows an example of the "parabolic" relationship in biological response data between drug activity and lipophilicity.

Affinity constants, the F values, corresponding to effects of individual groupings on distributions of the drug between water and various lipoidal media (solvents) can most conveniently be estimated by partitioning experiments. For many systems, this would involve simply measuring the partition coefficient of a selected compound and that of the same compound containing in addition the grouping under study. The affinity constant for a methylene group, for example, can be evaluated by measuring the factorial increase in the partition coefficient of, for example, *p*-propylphenol as compared to that of *p*-butylphenol, the difference corresponding to the free energy of transfer of a methylene from water to the selected solvent. Some of these constants have been evaluated in the authors' laboratory in this manner, including the use of the ion-pair extraction procedure described earlier (35). It is evident that if the F values for all common substituent groupings were available, it would permit much greater insight into the potential distributive behavior of new drugs.

The $F_{(CH_2)}$ for inert hydrocarbon solvents, determined as described, is in the region of 4.2–4.5, while for polar liquids this value appears to be considerably lower and is dependent on the nature of the polar liquid in question. Some representative values calculated from partition data are shown in Table II. Values for other isolated groupings in various solvents can be also obtained from published data or from partition experiments. For example, $F_{(C_6H_6)}$ is 1000 in cyclohexane and 60 in octanol. The addition of a halogen atom brings about an increase in the partition coefficient in the majority of cases; for aromatic species, this increase can be correlated quite well with the size of the added groupings. Although different F values for the halogens are evident for different solvents, detailed accurate partition experiments will be necessary before a table of values similar to Table II can be derived for them.

Polar groupings, of course, present a very diverse picture, and a great variety of F values will be possible, depending on the nature of the lipid phase. To a first approximation, the hydroxyl group, for example, has an F value of 2×10^{-2} in octanol and 4×10^{-4} in cyclohexane. In general, polar groupings will have a solubilizing effect in water and will increase the hydrophilic nature of a drug species. Some insight into the possible variation in values of F can be gained by calculating activity coefficient values for various functional groups in different organic solvents (Table III). The magnitudes of the various contributions can be rationalized largely on the basis of hydrogen bonding interactions. When both hydrogen-donating and hydrogen-accepting groupings are present in a single drug molecule, there is always the possibility of intramolecular bonding resulting in marked departure from additivity situations for these groupings.

A semiquantitative concept of the effect of adding various functional groups to a hypothetical drug molecule can be obtained by using the F values given, employing arbitrary values for K_l, V_l, and V_w, and considering that in the simple two-compartment model the lipoidal phase is similar to an inert hydrocarbon (cyclohexane) and the receptor biophase is similar to a long-chain alcohol (octanol). The calculated R values (Table IV) show that the quantity of substituted drug reaching the receptor site, as compared to the parent drug, depends on the partition coefficient of the parent drug and the volume

Table III—Activity Coefficients of Aliphatic Functional Groups in Different Organic Solvents

Group	Solvent					
	Benzene	Chloroform	Carbon Tetrachloride	Octanol	Ether	Cyclohexane–Hexane
CH_2	1.00	0.96	0.92	1.32	1.15	1.00
COOH	64.5	45.7	74.2	0.58	1.41	50.0
C=O	2.6	0.56	6.1	2.6	2.0	(7)
OH	(1)	5.5	35	0.5	7	65
NH_2	1.5	0.25	2		0.75	5

Table IV—Substitution of a Hypothetical Drug; Effect of Partition Coefficient and Lipid Phase Volume on the Ratio R

Partition Coefficient of Parent Drug	Volume of Lipid Phase	R —CH_2	R —C_6H_5	R —OH
0.01	0.1	3.2	30.0	0.020
	1.0	3.1	5.5	0.0202
	10.0	2.5	0.65	0.022
0.1	0.1	3.1	5.5	0.0202
	1.0	2.5	0.65	0.022
	10.0	1.13	0.12	0.04
1.0	0.1	2.5	0.65	0.022
	1.0	1.13	0.12	0.04
	10.0	0.76	0.066	0.22
10.0	0.1	1.13	0.12	0.04
	1.0	0.76	0.066	0.22
	10.0	0.72	0.060	1.96
100.0	0.1	0.76	0.066	0.22
	1.0	0.72	0.060	1.96
	10.0	0.71	0.060	14.4

of the lipoidal compartment, all other factors being equal. The usual generalization that the addition of a hydrophobic grouping results in a greater quantity of drug at the receptor site and the converse for a hydrophilic grouping are of limited value. The CH_2 and C_6H_5 groups will give increased drug concentration at the receptor site and, hence, biological activity when K_l and V_l are small. However, when K_l and V_l become larger, the drug will be contained almost exclusively in the lipoidal phase. This is especially true for the C_6H_5 grouping in the present example, where R becomes very much less than unity, indicating a greatly reduced activity for the substituted form. At the other extreme, a hydroxyl group brings about reduced activity at low K_l and V_l but has the opposite effect at higher values.

One major point of interest resulting from this approach is that it suggests that within the stated limitations it is possible to predict the degree of lipophilicity required to elicit the optimal activity for a given drug series. As pointed out earlier, whether a given substituent will effect an improvement in the drug activity will depend on whether R as defined in Eq. 15 is greater than 1 or not, the optimal lipophilicity for the drug usually corresponding to $R = 1$ when a single CH_2 grouping is added. It is evident that if, for example, a methylene group is added to a parent drug molecule and the resulting derivative drug possesses essentially the same biological activity, R would be close to unity and these structures would represent nearly the peak in the parabolic relationship between activity and the number of, for example, methylene carbons. For the simple two-compartment model systems consisting only of the aqueous and lipoidal phases, R can be readily estimated by using Eq. 15 in terms of the relative amounts of the drug found in the aqueous phase (V_w) and in the lipid phase (V_l). In Fig. 5, R corresponding to addition of a single methylene is shown for $F_{(CH_2)l} = 4.5$ as a function of the logarithm of $K_l^* V_l/V_w$, the ratio of amount of drug concentrated in the lipid phase to that in the aqueous for several values of $F_{(CH_2)r}$, the affinity constant for the receptor site. Although the selection of the affinity constant for the receptor affects the ratio at which point $R = 1$, the maximum point in the parabolic relationship, the effect is relatively small, the ratio of the amounts of the drug in the two phases for $R = 1$ being 0.40 for $F_{(CH_2)r} = 2.00$, 0.56 for $F_{(CH_2)r} = 2.25$, and 1.25 for $F_{(CH_2)r} = 3.0$. Since the $F_{(CH_2)}$ values for most receptors would usually fall in the range of 2.25–2.50, assuming that the receptor site has an intermediate polar nature, the ratio would normally be expected to be within the range of 0.40–1.00 for maximum drug activity. Since this variance is well within the range produced by a single methylene group, it would appear that normally a rather sharp maximum in activity could be expected when approximately one-half to an equivalent amount of drug is concentrated in the adipose and other lipoidal tissues, as compared to that found free in the aqueous phase of the test system. This postulate is readily amenable to experimental test.

Although this approach was based on the simplified two-phase model, it is more widely applicable to real animal systems than it may first appear. The adipose tissue in man is normally the largest and the most important lipophilic depot, along with fat deposits in the circulatory and other systems. It is evident that it is largely to these accessible, similar, essentially nonpolar lipid deposits that lipophilic drugs tend to accumulate as they are made increasingly hydrophobic.

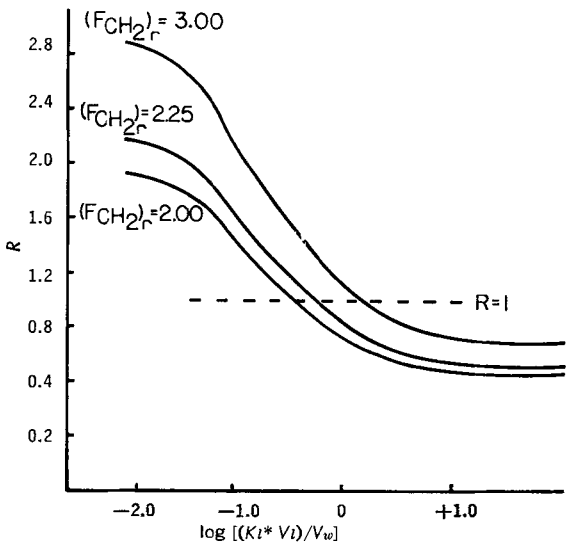

Figure 5—*Influence of distribution ratio and affinity constant for receptor site on the relative activity of drug containing CH_2 grouping over that of the parent compound.*

The present general approach has been described in terms of equilibrium interaction with a definite, although usually unidentified, receptor site. It is evident that essentially the same development and conclusions would apply to systems in which the drug activities are governed by rates of transport across membrane barriers. Since in such situations the rate is directly influenced by the equilibrium concentration on the surface of the barrier, all of the relationships derived apply with equal validity.

The authors recognize the fact that the present treatment attempts to treat an extremely complex problem in a simplistic way. Situations have been ignored that involve irreversible interaction with the receptor, induced conformational changes in the receptor, nonlinear distribution function (for the receptor and any of the remaining biophases), and any other complicating factor. The authors have not considered the specific interactions between the receptor and the drug nor between the competing sites in other biophases. The analysis treats the test system as being in equilibrium, a state that is not altogether realistic for any large living organism. Yet the present approach points to the start that must be taken in recognizing the influence of substituents on distributive tendencies to compartments other than the receptor in affecting the observed apparent activity.

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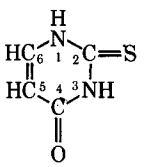
Metal Complexes of Thiouracils I: Stability Constants by Potentiometric Titration Studies and Structures of Complexes

EDWARD R. GARRETT and DENNIS J. WEBER

Abstract □ The divalent metals, Cu^{++}, Pb^{++}, Cd^{++}, Ni^{++}, and Zn^{++}, complex with the 5- and/or 6-alkyl-substituted thiouracils, HU. Significant concentrations of MU^+ and MU_2 complexes in homogeneous solution for all but Cu^{++} permitted estimation of the respective K_1 and K_2 stability constants by potentiometric titrations, where the log K_1 values were directly proportional to the pKa values of the parent thiouracils. Thus, the complex with 5,6-dimethyl-2-thiouracil (pKa′ 8.08) was the most stable, and the complex with 5-carboethoxy-2-thiouracil (pKa′ 6.43) was the least stable of those studied. The initial MU^+ complex is formed by the covalent bonding of the divalent cation at the anionic sulfur. When sulfhydryl formation in thiouracil is blocked by prohibiting tautomerization, as with 6-methyl-*N,N′*-diethyl-2-thiouracil, or by alkylation of the sulfur, as with 2-ethylmercapto-4-hydroxypyrimidine, no complexation with metal ions was observed. Pb^{++} and Cd^{++} ions have stability constants, K_1, for MU^+ formation with thiouracils that are 100 times greater than with Ni^{++} or Zn^{++}. No complexation of thiouracils with Fe^{+++}, Fe^{++}, Co^{++}, Ca^{++}, or Mn^{++} was observed. The MU_2 complex is formed by the covalent bonding of the divalent metal to two sulfur anions; this bis(6-*n*-propyl-2-thiouracil)-cadmium (II) is the first complex to precipitate from solution on the titration of cadmium ion and 6-*n*-propyl-2-thiouracil at 25 and 35°. The structure was confirmed by elemental analysis and IR spectra of synthesized compounds. In all other cases of studied complexation of Cd^{++} and Pb^{++} with 2-thiouracil, 6-methyl-2-thiouracil, 5-methyl-2-thiouracil, 5,6-dimethyl-2-thiouracil, 5-carboethoxy-2-thiouracil, and 6-*n*-propyl-2-thiouracil, the first complex that precipitated on potentiometric titration had a 1:1 stoichiometry and was most probably the cyclic dimer, M_2U_2, bis(thiouracil-metal) (II), although the polynuclear polymer, M_nU_n, was possible. The heightened acidity of the 4-OH of the initial MU^+ complex promoted dissociation at low pH values and subsequent covalent bonding of the divalent cation to the sulfur of one thiouracil and the oxygen of another. The resultant M_2U_2 or M_nU_n structure was confirmed by elemental analysis and IR spectra of synthesized complexes. The formed and precipitated complexes of Pb^{++} and Cd^{++} as MU^+, MU_2, and M_2U_2 were stable, at least in mildly alkaline solutions, whereas those of Ni^{++} and Zn^{++} were destroyed in mild alkali with the final precipitation of the metal hydroxides.

Keyphrases □ Thiouracils–metals—complexation □ Complexes, thiouracil–metal—stability constants □ Metal–thiouracil complexes —structure □ Solubility, aqueous—thiouracils □ Potentiometric titration—analysis □ IR spectrophotometry—structure □ UV spectrophotometry—structure

The antithyroid activities of 5-methyl-, 6-methyl-, and 5,6-dimethyl-substituted thiouracils have been claimed to be 0.7, 1.0, and 1.2 relative to 2-thiouracil (Structure I*a*) (1–3).



HU I*a*

The present antithyroid derivative of choice, because of its claimed maximal activity and low toxicity in the intact animal, is 6-*n*-propyl-2-thiouracil (1–5). Alkylation of thiouracil at the N-1, N-3, or sulfur positions greatly reduced (2), and substitution by electronegative groups at the 5- or 6-position practically eliminated, any antithyroid activity (1–3).

Cupric ion has been implicated in thyroid function (6). The copper content of the normal and pathologic thyroid has been determined (7) and verified by Kasanen and Viitanen (8) who found elevated copper levels in toxic and nontoxic goiters. The formation of diiodoty-