Structure-Activity Correlations for a Series of Antiallergy Agents. 3. Development of a Quantitative Model

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A nonlinear regression model has been employed to investigate the activity of a series of 51 drugs in the rat passive cutaneous anaphylaxis assay. Although several classes of molecules are represented in the sample, there are certain common structural features that enable detailed geometric and electronic comparisons to be made. Ab initio Hartree–Fock SCF calculations were performed by using the molecular fragment approach to characterize the electronic structure and preferred conformations of the molecules. The statistical results establish the importance of nine structural factors in determining the potency as inhibitors of histamine release. Both the conformation of a drug and its capacity to act effectively as an electron acceptor in charge-transfer interactions are shown to be critical for high activity.

Oxanilic acid (molecule I in Figure 1) is the simplest representative of several classes of molecules that have been shown to exhibit activity in the rat passive cutaneous anaphylaxis (PCA) assay.[2-7] At certain dosage levels, agents incorporating key features of I (e.g., cromolyn sodium) prevent release of mediators of the allergic response from mast cells.[8] It has been postulated that cromolyn sodium acts by altering the phosphorylation of a protein associated with regulation of the secretory process.[9] However, the biochemical mechanism and site of inhibition have not yet been clearly established for cromolyn sodium and similar antiallergy drugs of the type under study.

In this report, the results are presented of an investigation into the electronic and geometric characteristics that enable molecules resembling I to effectively block release of histamine following antigen–antibody interaction on sensitized mast cells. The work builds upon the quantitative structure–activity relationship obtained in an earlier analysis[6] involving a series of oxanilic, quinaldic, and benzopyran-2-carboxylic acids. The present study has been expanded to include 51 molecules of the kind shown in Figure 1.

Methods

Biological Activities. As described elsewhere,[2,4-7] the rat PCA results were obtained from experiments in which the drug is administered iv, simultaneously with antigen challenge, as the salt of tris(hydroxymethyl)aminomethane. The findings are reported in terms of an activity index, A_{obsd}, given by eq 1, where

$$A_{obsd} = -\ln ED_{50} \quad (1)$$

the ED_{50} is the dose (micromoles per kilogram of rat body weight) required to produce 50% inhibition of histamine release in the PCA assay. For the series of compounds under study, the values of the ED_{50} cover a range of five orders of magnitude.

SCF-MO Calculations. The computational techniques and notation employed in this paper for characterizing electronic structure are the same as those described in the preceeding paper in this issue.[10] Each calculation has been performed with the acid form of the molecule to model the drug structure. The geometrical data employed in this study are tabulated as supplementary material (see paragraph at end of paper).

As measured by the correlation index, τ_{ij},[2] each of the molecules under study possesses a low-energy unoccupied orbital, designated hereafter as ϕ_a', which resembles the 8a″ MO of oxanilic acid. Since the energy, ϵ_a^*, of this orbital strongly correlates with A_{obsd} in the subset of drugs studied earlier, particular attention has been focused on this feature of the electronic structure in the current work. Owing to resonance effects of aromatic substituents, several molecules in the series under study contain a manifold of virtual orbitals with the characteristics of ϕ_a'. Contour density maps illustrating this resonance splitting of ϕ_a' are shown in Figures 2 and 3, where the molecules used as examples are, respectively, *N,N'*-(2-chloro-5-phenyl-*m*-phenylene)dioxamic acid and *m*-(2-pyrimidinyl)oxanilic acid. The complications arising from this effect will be discussed hereafter.

Development of Regression Model. Since the general model has been derived and employed successfully elsewhere,[6,11] it will only be summarized here in sufficient detail to define terms, explain major assumptions, and specify the nature of conformational effects that were not considered explicitly in studies on other systems. As demonstrated in ref 6, the theoretical activity index, A, may be given by eq 2 where k is Boltzmann's constant;

$$A = [kT \ln (fq_C/q_M q_R) - \Delta U - \Delta W]/kT \quad (2)$$

T is the absolute temperature; f is a drug-transport factor that expresses the fraction of the dose present in the receptor compartment during the critical period of the PCA reaction; q_C, q_M, and q_R are related, respectively, to the partition functions of the drug–receptor complex, C, the drug, M, and the receptor, R; ΔU is the change in solvation energy due to complex formation; and ΔW is the stabilization energy of the complex. The values of q_C, q_M, and q_R depend upon the manner in which the molecular energy is divided among the translational, rotational, vibrational, and electronic degrees of freedom. If the receptor is a macromolecule with mass much greater than that of the drug and the complex is loose so that the internal motions of M and R remain relatively unperturbed during association, some cancellation of terms in the statistical mechanical expression for $q_C/q_M q_R$ can be made so that eq 2 becomes eq 3, where q_{Mt} and q_{Mr} denote, respectively, the

$$A = [kT \ln (f/q_{Mt} q_{Mr}) - \Delta U - \Delta W]/kT \quad (3)$$

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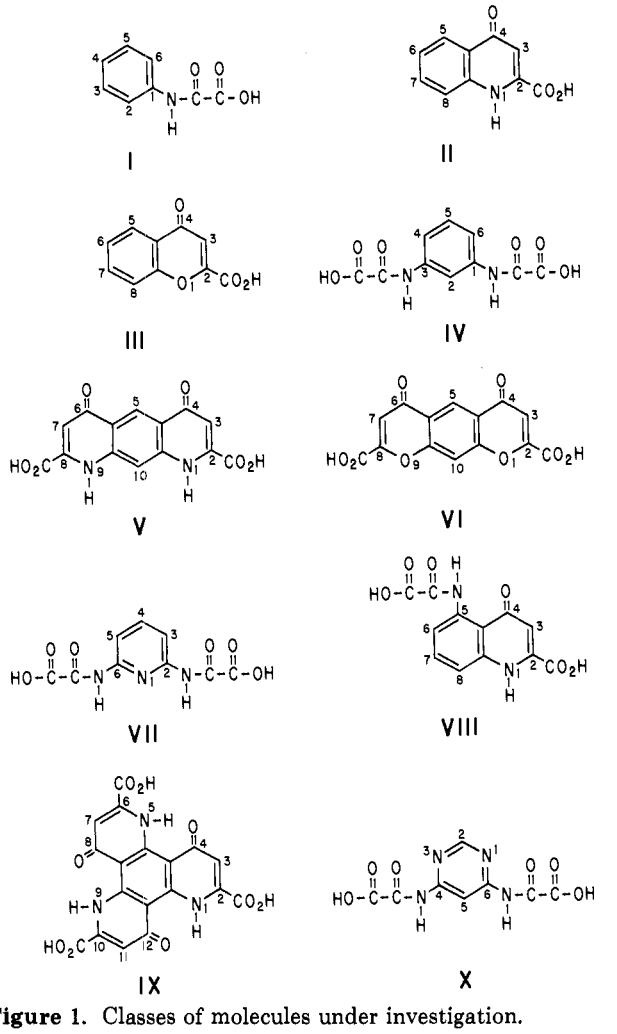

Figure 1. Classes of molecules under investigation.

translational and rotational partition-function terms for the drug molecule.

If each modification to the parent structure, or essential pharmacophore, in a series of drugs causes a small linear perturbation in the factors affecting activity, the value of A may be given by eq 4, where A_0 is the activity index of the parent drug,

$$A = A_0 + \sum_t (\partial A/\partial \omega_t)_{\omega_t=\omega_{t0}} \Delta\omega_t \tag{4}$$

ω_t is one of the variables upon which the terms in eq 3 depend, ω_{t0} is the value of ω_t in the parent drug, and $\Delta\omega_t = \omega_t - \omega_{t0}$. In order to recast eq 4 into a useful form for the case under study, it is necessary to consider the factors affecting A in somewhat more detail.

The contribution to eq 4 from changes in q_{Mt} and $q_{M'}$ may be explicitly determined from a knowledge of the drug structure. For example, if y is defined as eq 5 then the contribution, γ, to the

$$y = \ln (1/q_{Mt}q_{M'}) \tag{5}$$

sum in eq 4 due to variations in this factor may be written

$$\gamma = \left(\frac{\partial A}{\partial y}\right)_{y=y0} \Delta y \tag{6}$$

However, the partial derivative of A with respect to y is unity, so that $\gamma \equiv \Delta y$. Since oxanilic acid is the simplest molecule in the series, it may be taken as the parent structure for the purpose of evaluating γ (eq 7). In eq 7, m is the molecular weight of the

$$\gamma = -1.5 \ln (m/165.2) + \ln \sigma - 0.5 \ln (I_xI_yI_z/1.018 \times 10^{10}) \tag{7}$$

drug, σ is the rotational symmetry factor, $I_xI_yI_z$ is the product of the principal moments of inertia given in atomic units, 165.2 is the molecular weight of oxanilic acid, and 1.018×10^{10} is the value of $I_xI_yI_z$ for this reference molecule.

In order to discuss the contributions to eq 4 resulting from variations in ΔW, the factors affecting the stability of the drug–receptor complex must be treated in some detail. In ref 6, the complex was considered to be stabilized in part through a Mulliken charge-transfer interaction in which ϕ_a' acts as an electron acceptor and the donor orbital, ϕ_d, is associated with a receptor entity at the binding site. Due to the resonance splitting of ϕ_a' in certain molecules, it is necessary to employ a generalized form of the Mulliken theory to account for the fact that a set, $\{\phi_u^*\}$, of drug orbitals has the proper spatial distribution and symmetry properties to interact effectively with ϕ_d. Since the theory has been outlined in detail elsewhere,[11] it will only be summarized here.

In order to determine the extent to which a given unfilled MO, ϕ_u^*, contributes to charge-transfer stabilization, it is useful to make the transformation given in eq 8, where $\{\phi_p'\}$ is an orthonormal

$$\phi_u^* = \sum_p c_{pu}'\phi_p' \tag{8}$$

set of localized fragment functions,[12] one of which is ϕ_a', the primary acceptor orbital. If ϕ_a' is the only fragment function that overlaps ϕ_d significantly, the stabilization energy of the complex may be given by eq 9, where Q is the energy of interaction between

$$\Delta W = Q - \kappa_{da} \sum_u \frac{(c_{au}')^2}{\epsilon_u^* - \epsilon_d - (c_{au}')^2\lambda_{da}} \tag{9}$$

the drug and receptor exclusive of charge-transfer effects, κ_{da} and λ_{da} are terms depending on the degree of overlap between ϕ_d and ϕ_a', c_{au}' is the coefficient of ϕ_a' in eq 8, and ϵ_d is the energy of ϕ_d. According to eq 9, the contribution of ϕ_u^* to the charge-transfer stabilization term depends upon its energy, ϵ_u^*, and the amount of ϕ_a' character that it possesses, as measured by the coefficient c_{au}'.

Substituents may affect the activity *indirectly* by perturbing the electronic structure of the essential pharmacophore. For example, inducing changes in ϵ_u^* and c_{au}' alters charge-transfer stabilization of the drug–receptor complex. On the other hand, a substituent may also exert a *direct* effect by virtue of its own steric and electronic properties. As shown in ref 11, when the substituents exert independent and additive effects on each variable ω_t, the following relationship may be obtained from eq 3–9

$$A = a + b\epsilon_{eff}^* + \sum_s c_s\delta_s + \gamma \tag{10}$$

where

$$\epsilon_{eff} = \sum_u (c_{au}')^2\epsilon_u^* + \lambda_{da}[1 - \sum_u (c_{au}')^4] \tag{11}$$

The terms in eq 10 are defined as follows: a, b, and c_s are coefficients to be determined by regression, δ_s is a factor that assumes a nonzero value only when the substituent denoted by subscript "s" is present, and γ is the contribution arising from the loss of the drug molecule's translational and rotational degrees of freedom upon binding to the receptor. The variable ϵ_{eff}^* may be considered as an "effective" acceptor energy produced by averaging the charge-transfer contributions from the manifold of acceptor orbitals. In computing ϵ_{eff}^*, it is possible to obtain values of ϵ_u^* and c_{au}' from the results of the SCF–MO calculations

(12) The appendix to ref 11 describes the procedure used for determining the coefficients (c_{pu}') once the set of localized fragment orbitals $\{\phi_p'\}$ has been found. In the present calculations, all molecular orbitals resembling ϕ_a' (the 8a″ MO of oxanilic acid) were found from comparisons using the correlation index τ_{ij}^2, as discussed in the preceding paper. The nature of the localized fragment orbitals that couple with ϕ_a' was then ascertained by inspecting each ϕ_u^* for contributions from particular combinations of p_π FSGOs in the aromatic substituent and oxamic acid side chain(s). Quantitative descriptions of the localized fragment orbitals other than ϕ_a' were obtained after decoupling the system through rotation of the moieties attached to the benzene ring to eliminate π-orbital overlap.

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on the drug molecule, but λ_{da} must be treated as a regression variable.

Conformational Effects. The use of eq 10 is straightforward in those cases where the drug molecule strongly favors a single geometry. However, in cases with some conformational freedom, the activity index must be found by means of an averaging process in which the contribution of each conformation is taken into account. Eq 10 then may be written as eq 12 where the symbols

$$\langle A \rangle = a + b\langle \epsilon_{eff}^* \rangle + \sum_s c_s \langle \delta_s \rangle + \langle \gamma \rangle \quad (12)$$

$\langle A \rangle$, $\langle \epsilon_{eff}^* \rangle$, $\langle \delta_s \rangle$, and $\langle \gamma \rangle$ denote average values of the activity index and the factors upon which it depends. If a factor of interest is denoted by Ω_t, the average determined from Boltzmann statistics may be given by eq 13, where Ω_{ti} is the value of the factor for the

$$\langle \Omega_t \rangle = G \sum_i g_i \Omega_{ti} \exp(A_i - E_i/RT) \quad (13)$$

ith conformational state, E_i is the energy of the unbound drug molecule in that state, A_i is the biological activity index of the ith conformer, g_i is the degeneracy of the state, and G is a coefficient defined below. In order to demonstrate more explicitly the dependence of $\langle \Omega_t \rangle$ on the calculated quantities, Ω_{ti} and E_i, eq 13 may be restated as eq 14 and 15, where the general form of eq 10 has been used to replace A_i.

$$\langle \Omega_t \rangle = G \sum_i g_i \Omega_{ti} \exp(\sum_r d_r \Omega_{ri} - E_i/RT) \quad (14)$$

$$G = 1/\sum_i g_i \exp(\sum_r d_r \Omega_{ri} - E_i/RT) \quad (15)$$

Since the factors in eq 12 that vary with conformation must be determined by means of eq 14, the resultant expression no longer retains linear form. Hence, iterative nonlinear regression techniques must be used to determine $\langle \epsilon_{eff}^* \rangle$, $\langle \delta_s \rangle$, and $\langle \gamma \rangle$, as well as a, b, and c_s.

In the cases under study, the conformational changes that exert the most significant effect on $\langle A \rangle$ generally involve a substituent undergoing rotation about the bond connecting it to the aromatic ring of the essential structural moiety. Such rotations may play a role in determining the magnitude of several factors—e.g., γ, ϵ_{eff}^*, and certain substituent terms, δ_s. If the torsional motion requires traversal of a high potential energy barrier similar to that exhibited by the oxamic acid moiety, the molecule may be limited to a few stable conformers. In such a case, the summations in eq 14 and 15 may be carried out directly. On the other hand, when the rotation is subject to a low potential energy barrier, the molecule can no longer be considered to exist in discrete conformational states. In this situation, since continuous variation of the substituent torsional angle, θ, must be taken into account, an enormous number of drug conformations has to be examined in order to evaluate the summations. However, the problem is made tractable by utilizing the results of a few calculations to determine approximate fitting functions for ϵ_{eff}^* and E. Then, eq 14 and 15 may be transformed into integrals with θ as the variable of integration. Once this has been accomplished, the calculation of G and $\langle \Omega_t \rangle$ proceeds in a straightforward manner by using the technique of numerical integration. The value of RT employed in these computations has been taken as 0.6 kcal/mol.

Since the actual functional relationship between ϵ_{eff}^* and θ cannot be expressed in simple analytical form, a piecewise linear fitting function is employed as an approximation. The end points of each linear segment are obtained from the results of SCF–MO calculations on the molecule of interest. In order to fit the calculated points of the conformational energy curve, a cosine expansion is used that has the form shown in eq 16 for an l-fold rotational barrier.

$$E(l,\theta) = \sum_k h_k \cos[l(k-1)\theta] \quad (16)$$

Results and Discussion

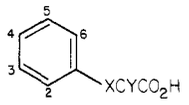
Features of the Molecular Structure. As established in the preceding paper, the various oxamic acids under study may often exist in more than one low energy conformation. An oxamic acid with an ortho or meta substituent may exhibit two different planar conformers, defined as syn (s) and anti (a). An ortho-substituted oxamic acid is locked into the a configuration due to severe steric interactions between the substituent and the amide carbonyl group when the molecule assumes the s configuration. A *m*-dioxamic acid may exist in three possible stereoisomeric forms, designated for convenience as the anti–anti (aa), syn–anti (sa), and syn–syn (ss) configurations. Certain conformational restrictions are imposed on a *m*-dioxamic acid molecule by the presence of a substituent bulkier than hydrogen ortho to one or both of the oxamic acid moieties. For example, if the substituent is located in the 2-position adjacent to both side chains, the molecule is fixed in the aa form. If it is situated in the 4-position, the molecule may exist in the sa or ss form, but cannot assume the aa conformation. Finally, a 4,6-disubstituted molecule exhibits only the ss form.

All of the conformational data employed to determine the regression parameters involved in the current study are presented in Tables I–III. (Concerning Tables II and III, see paragraph at end of paper about supplementary material.) In the discussion to follow, the various stable coplanar arrangements of the aromatic ring and attached oxamic acid chain(s) are designated as reference conformational states of the molecule. If the molecule also contains a substituent capable of rotation about its junction to the aromatic ring, the reference state for the substituent is arbitrarily taken as the orientation with zero torsional angle. A list of the conformational energies, degeneracies, and structural factors for the reference conformers of each molecule is presented in Table I. The effect of changes in θ on various factors involved in the calculation of biological activities is shown Table II. Three factors exhibit an important dependence on θ: the conformational energy; the energies of unoccupied molecular orbitals to which ϕ_a' contributes; and the coefficient, c_{au}', of the localized acceptor orbital in the expansion of ϕ_u^*. For the cases discussed in this report, rotation of a substituent has negligible effect on γ. Table III contains the coefficients, h_k, obtained from fitting the calculated conformational energies, $E(l,\theta)$, by means of eq 16.

As noted earlier,[6,10] the features characterizing the antibonding π orbital, ϕ_a', that interacts with the donor orbital of the receptor are (1) low energy, (2) high density at positions 2 and 5 of the essential six-membered aromatic ring, and (3) nodal surfaces separating atoms 2 and 5 from adjacent atoms. Most of the molecules in Table I exhibit only one orbital of this type. However, molecules **23** and **47–50** possess a manifold of molecular orbitals that incorporate the features of ϕ_a' as a result of resonance coupling with fragment orbitals (ϕ_b', ϕ_c', etc.) localized primarily in the aromatic substituents and oxamic acid moieties. According to the theory outlined in the previous section, a given molecular orbital, ϕ_u^*, will participate in receptor binding only if ϕ_a' contributes significantly to its description as measured by the coefficient c_{au}' in eq 8. These coefficients have been determined by the procedure outlined in the appendix of ref 11.

In the drugs with aromatic substituents, it has been found that the acceptor orbitals are composed of at least three localized fragment orbitals: (1) ϕ_a' in the essential aromatic ring, (2) ϕ_b' in the aromatic substituent, and (3) ϕ_c' in the oxamic acid group(s). Figure 2 illustrates these features of the electronic structure using molecule **23** as the example. There are two cases (molecules **47** and **48**) where an additional localized fragment orbital, ϕ_d', is found in the aromatic substituent. With regard to molecule **47**, ϕ_b' and ϕ_d' are respectively associated with the phenyl and vinylene moieties of the styryl group. In molecule **48**, ϕ_b'

Table I. Degeneracies, Conformational Energies, and Structural Factors for the Reference Conformational States of the Molecules[a]

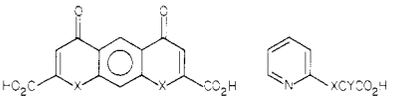


no.	type	substituents	conf	g	E^c	structural factors[b] γ	$\epsilon_1{}^{*d}$	δ_1	δ_2	δ_3	δ_4	δ_5	δ_6	δ_7	δ_8
1	I			1	0.0	0.0	0.3011	0	0	0	0	0	0	0	0
2	I	4-CN		1	0.0	−0.7	0.2793	0	0	0	0	0	0	0	0
3	I	2-CN	a	1	0.0	−0.8	0.2496	0	0	0	0	0	0	0	0
4	I	3-CN	a	1	0.25	−0.9	0.2491	0	0	0	0	0	0	0	0
			s	1	0.0	−0.9	0.2498	0	0	0	0	0	0	0	0
5	I	3-CN, 4-Cl	a	1	0.12	−1.5	0.2284	0	0	0	0	0	0	0	0
			s	1	0.0	−1.5	0.2290	0	0	0	0	0	0	0	0
6	I	2-Cl, 5-CN	a	1	0.0	−1.6	0.2278	0	0	0	0	0	0	0	0
7	I	3,5-CN	a	1	0.0	−1.6	0.2204	0	0	0	0	0	0	0	0
8	II	8-OH		1	0.0	−1.0	0.2999	0	0	0	0	0	0	0	0
9	II	6-OCH_3		1	0.0	−1.4	0.2791	0	0	0	0	0	0	0	0
10	II	6-NH_2		1	0.0	−1.0	0.2873	0	0	0	0	0	0	0	0
11	II			1	0.0	−0.6	0.2733	0	0	0	0	0	0	0	0
12	III	6-NH_2		1	0.0	−1.2	0.2785	0	0	0	0	0	0	0	0
13	III	6-NO_2		1	0.0	−0.5	0.2462	0	0	0	0	0	0	0	0
14	I	3-NH_2	a	1	0.9	−0.6	0.3276	1	0	0	0	0	0	0	0
			s	1	0.0	−0.6	0.3558	0	0	0	0	0	0	0	0
15	I	3-OCH_3	a	1	1.0	−0.9	0.3209	1	0	0	0	0	0	0	0
			s	1	0.0	−0.9	0.3409	0	0	0	0	0	0	0	1
16	I	2-Cl, 3-NH_2, 5-CN	a	1	0.0	−1.8	0.2493	1	0	0	0	0	0	0	0
17	I	2,4-Cl, 3-NH_2, 5-CN	a	1	0.0	−2.4	0.2276	1	0	0	0	0	0	0	0
18	II	7-NH_2, 8-CH_3		1	0.0	−1.3	0.2960	1	1	0	0	0	0	0	0
19	IV	2-Cl	aa	1	0.0	−1.9	0.2938	1	0	1	0	0	0	0	0
20	IV	2-Cl, 5-$CONH_2$	aa	1	0.0	−2.8	0.2875	1	0	1	0	0	0	0	0
21	IV	2-Cl, 5-CH_3	aa	1	0.0	−2.2	0.3060	1	1	1	0	0	0	0	0
22	IV	2-CN	aa	1	0.0	−1.9	0.2664	1	0	1	0	0	0	0	0
23	IV	2-Cl, 5-C_6H_5	aa	1	0.0	−3.2	0.2474	1	0	1	0	0	0	0	0
24	IV	2-Cl, 5-CN	aa	1	0.0	−2.4	0.2433	1	0	1	0	0	0	0	0
25	V	10-Cl		1	0.0	−2.6	0.2460	1	0	1	0	0	1	0	0
26	V	10-CH_3		1	0.0	−2.4	0.2679	1	1	1	0	0	1	0	0
27	V	5-CN, 10-Cl		1	0.0	−2.9	0.2161	1	0	1	0	0	1	0	0
28	VI	10-CH_3		1	0.0	−2.4	0.2573	1	1	1	0	0	1	0	0
29	IV	5-OCH_3	aa	1	0.0	−2.1	0.3574	1	0	1	0	0	0	0	1
			sa	2	−0.53	−2.8	0.3568	1	0	0	1	0	0	0	1
			ss	1	0.56	−2.1	0.3589	1	0	0	0	1	0	0	1
30	IV	5-$CONH_2$	aa	1	0.0	−2.5	0.3102	1	0	1	0	0	0	0	0
			sa	2	0.70	−3.2	0.3103	1	0	0	1	0	0	0	0
			ss	1	0.51	−2.5	0.3104	1	0	0	0	1	0	0	0
31	IV		aa	1	1.07	−1.5	0.3187	1	0	1	0	0	0	0	0
			sa	2	0.0	−2.2	0.3189	1	0	0	1	0	0	0	0
			ss	1	0.13	−1.5	0.3203	1	0	0	0	1	0	0	0
32	IV	5-CN	aa	1	0.94	−2.1	0.2637	1	0	1	0	0	0	0	0
			sa	2	0.0	−2.8	0.2631	1	0	0	1	0	0	0	0
			ss	1	0.2	−2.1	0.2631	1	0	0	0	1	0	0	0
33	IV	5-NO_2	aa	1	1.08	−2.4	0.2775	1	0	1	0	0	0	0	1
			sa	2	0.0	−3.1	0.2767	1	0	0	1	0	0	0	1
			ss	1	0.07	−2.4	0.2766	1	0	0	0	1	0	0	1
34	VII	4-OCH_3	aa	1	0.0	−2.1	0.3300	1	0	1	0	0	0	1	1
			sa	2	1.0	−2.8	0.3319	1	0	0	1	0	0	1	1
			ss	1	3.8	−2.1	0.3373	1	0	0	0	1	0	1	1
35	VII		aa	1	0.0	−1.5	0.3132	1	0	1	0	0	0	1	0
			sa	2	0.88	−2.2	0.3140	1	0	0	1	0	0	1	0
			ss	1	2.8	−1.5	0.3161	1	0	0	0	1	0	1	0
36	VII	4-Cl	aa	1	0.0	−2.2	0.2883	1	0	1	0	0	0	1	0
			sa	2	0.83	−2.9	0.2887	1	0	0	1	0	0	1	0
			ss	1	2.7	−2.2	0.2902	1	0	0	0	1	0	1	0
37	VIII	8-OCH_3		1	0.0	−3.0	0.3129	1	0	0	1	0	0	0	0
38	VIII	8-Cl		1	0.0	−3.0	0.2843	1	0	0	1	0	0	0	0
39	VIII	8-CH_3		1	0.0	−2.7	0.3122	1	1	0	1	0	0	0	0
40	IV	4-F	sa	2	0.0	−2.6	0.3155	1	0	0	1	0	0	0	0
			ss	1	0.13	−1.9	0.3171	1	0	0	0	1	0	0	0
41	IV	4-CN	sa	2	0.0	−2.7	0.2762	1	0	0	1	0	0	0	0
			ss	1	0.01	−2.0	0.2772	1	0	0	0	1	0	0	0
42	IV	4-Cl, 5-CN	sa	2	0.0	−3.2	0.2419	1	0	0	1	0	0	0	0
			ss	1	0.12	−2.5	0.2419	1	0	0	0	1	0	0	0
43	IV	4,6-F	ss	1	0.0	−2.0	0.3274	1	0	0	0	1	0	0	0
44	IV	4,6-Cl	ss	1	0.0	−2.5	0.2697	1	0	0	0	1	0	0	0
45	IV	5-$NHCOCO_2H$	C_{3h}	1	0.0	−2.7	0.3387	1	0	0	1	0	0	0	1
			C_s	3	1.2	−3.1	0.3409	1	0	1/3	1/3	1/3	0	0	1

Table I (*Continued*)

no.	type	substituents	conf	g	E^c	structural factors[b] γ	ϵ_1*[d]	δ_1	δ_2	δ_3	δ_4	δ_5	δ_6	δ_7	δ_8
46	IX			1	0.0	−3.1	0.2788	1	0	0	1	0	0	0	1
47	IV	5-*trans*-styryl[e]	aa	1	0.0	−3.5	0.2617	1	0	1	0	0	0	0	0
			sa	2	−0.3	−4.2	0.2592	1	0	0	1	0	0	0	0
			ss	1	0.1	−3.5	0.2582	1	0	0	0	1	0	0	0
48	IV	5-(2-benzimidazolyl)	aa	1	0.0	−3.6	0.2564	1	0	1	0	0	0	0	0
			sa	2	−1.9	−4.3	0.2546	1	0	0	1	0	0	0	0
			ss	1	0.18	−3.6	0.2538	1	0	0	0	1	0	0	0
49	IV	5-(2-pyrimidinyl)	aa	1	0.0	−3.0	0.2505	1	0	1	0	0	0	0	0
			sa	2	−1.5	−3.7	0.2483	1	0	0	1	0	0	0	0
			ss	1	−1.9	−3.0	0.2467	1	0	0	0	1	0	0	0
50	I	*m*-(2-pyrimidinyl)	a	1	−0.49	−2.0	0.2393	0	0	0	0	0	0	0	0
			s	1	0.0	−2.0	0.2414	0	0	0	0	0	0	0	0
51	X		aa	1	3.8	−1.5	0.3037	1	0	1	0	0	0	0	0
			sa	2	1.4	−2.2	0.3027	1	0	0	1	0	0	0	0
			ss	1	0.0	−1.5	0.3030	1	0	0	0	1	0	0	0

[a] The various low-energy coplanar arrangements of the aromatic ring and attached oxamic acid chain(s) are designated as reference conformers of the molecule. The reference state for substituents that rotate about the bond of attachment to the aromatic ring is taken as $\theta = 0°$, where θ is the torsional angle that changes with rotation. [b] In defining the structural factors, use is made of the moiety above the column heads to describe elements of the essential pharmacophore, where X = NH or O and Y = O or CHC linked to benzene in a fused bicyclic system. Each of the structural factors δ_1–δ_5 and δ_8 represents a substituent of a given type and geometry attached to a particular position in the benzene ring; i.e.: δ_1 = 3-NHR or 3-OR; δ_2 = 2-, 4-, or 5-CH_3; δ_3 = 3-$XCYCO_2H$ in aa conformation; δ_4 = 3-$XCYCO_2H$ in sa conformation; δ_5 = 3-$XCYCO_2H$ in ss conformation; δ_8 = 5-NR_1R_2 or 5-OR. Factors δ_6 and δ_7 refer to variants of the essential pharmacophore in which the following entities appear:



δ_6 δ_7

[c] The conformational energy, E, is expressed in kilocalories per mole. [d] The value, ϵ_1*, given in the table corresponds to the energy of the lowest acceptor orbital in those cases where a manifold of acceptor orbitals exists. [e] The orientation of the vinylene moiety relative to the phenyl ring of the *trans*-styryl substituent is given by a torsional angle of 51.3°. See footnote *c* of Table II.

and ϕ_d' are both distributed over the entire benzimidazolyl substituent but exhibit distinctive density distributions and nodal patterns. An oxamic acid, molecule **50**, with a pyrimidinyl substituent also possesses a fourth localized orbital, ϕ_d', which exhibits density throughout the phenyl ring and attached oxamic acid chain. The acceptor manifold for molecule **50** is shown in Figure 3.

Preliminary Structural Factors. As a guide in developing the structure–activity relationship, it is useful to consider those elements of oxanilic acid that are common to all molecules under study as the critical pharmacophore. One may then conceptualize a region on the receptor overlaid by this pharmacophore. Any substituents that significantly affect the activity in a direct manner may indicate the location of critical points relative to the site thus defined. The various molecules under investigation may be considered as probes that aid in mapping the receptor surface. In order to facilitate comparisons among different classes of molecules as the "receptor map" is created, the positions of ring substituents will generally be given in this discussion with reference to the numbering scheme for the oxanilic acid structure (I).

It has been mentioned earlier that a substituent contributes indirectly to the activity by perturbing the energy of the orbital ϕ_a'. As an aid in discerning which substituents play a more direct role in the activity, a plot of A_{obsd} vs. ϵ_a* is shown in Figure 4 for a selected group of drugs. The 29 molecules included in this plot are those with well-established conformational preferences. Due to the importance of both the nature and position of substituents, the molecules with indeterminate conformations are not considered in this preliminary analysis. The large scatter in the points of Figure 4 clearly demonstrates that significant effects are exerted by structural factors other than ϵ_a*. Close inspection of the data reveals that the molecules may be collected into several sets that exhibit similar trends of activity, as illustrated by the series of lines labeled **R**–**W**. The specific structural features shared by the molecules within each set are discussed in succeeding paragraphs.

Molecules numbered from **1** to **13** in Table I fall into the group clustered about line **R**. Although three different classes (I–III) and a variety of substituents are represented in this series, the activity, A_R, of a molecule in the set may be determined quite accurately by eq 17, which is the linear

$$A_R = 28.0 - 64.4\epsilon_a^* \quad (17)$$

$$r_R = 0.98;\ s_R = 0.4$$

expression obtained in the first paper of this series.[6] This finding implies that the following phenyl-ring substituents do not exert a significant direct effect on the activity: CN in any position, 2-Cl, 4-Cl, 4-NH_2, 4-OCH_3, and 2-OH. Furthermore, the structural differences among oxanilic acid, 4(1)-quinolone-2-carboxylic acid, and chromone-2-carboxylic acid appear to affect the relative activity only by influencing the value of ϵ_a*. In this set of compounds, the various moieties attached to the basic structural unit apparently do not encounter either binding entities or steric hindrances at the receptor.

Three drugs in classes I and II are significantly more active than eq 17 would predict. These molecules, numbered **16**–**18** in Table I, exhibit a unique feature that the drugs in **R** lack; namely, an amino group in the 3-phenyl position. Another new substituent, the 2-methyl group, is found in molecule **18**. In Figure 4, an indication of the effect of 3-NH_2 on the activity is provided by line segment S drawn parallel to **R** through the midpoint between molecules **16** and **17**. Although experimental error and an

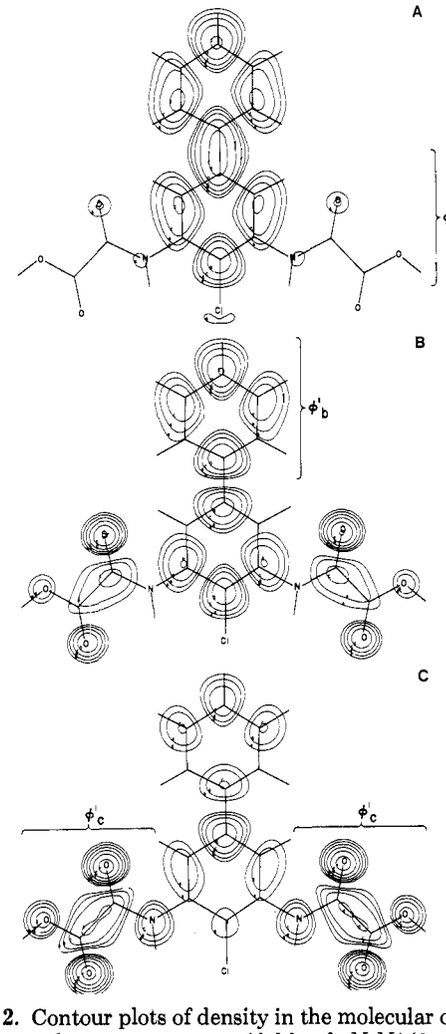


Figure 2. Contour plots of density in the molecular orbitals that constitute the acceptor manifold of *N,N'*-(2-chloro-5-*m*-phenylene)dioxamic acid (**23**) when $\theta = 0°$. A section 0.8 au (1 au = 0.529172 Å) above the plane of the nuclei has been selected for each map. The localized fragment orbitals discussed in the text make the following contributions to the acceptor orbitals: (A) $\phi_1^* = -0.711\phi_a' + 0.705\phi_b' - 0.098\phi_c'$; (B) $\phi_2^* = 0.606\phi_a' + 0.673\phi_b' - 0.423\phi_c'$; (C) $\phi_3^* = -0.350\phi_a' - 0.212\phi_b' - 0.906\phi_c'$.

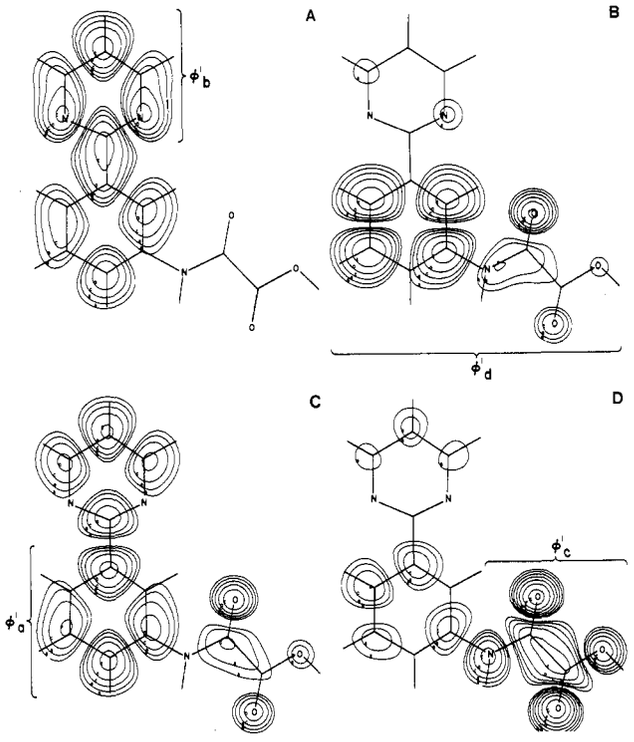


Figure 3. Contour plots of density in the molecular orbitals of the acceptor manifold in *m*-(2-pyrimidinyl)oxanilic acid (**50**). The molecule is shown in the s form with $\theta = 0°$. Each map is plotted at a height 0.8 au above the nuclear plane. The localized fragment orbitals discussed in the text make the following contributions to the acceptor orbitals: (A) $\phi_1^* = -0.573\phi_a' + 0.782\phi_b' - 0.088\phi_c' + 0.260\phi_d'$; (B) $\phi_2^* = 0.573\phi_a' + 0.127\phi_b' - 0.820\phi_d'$; (C) $\phi_3^* = -0.580\phi_a' - 0.582\phi_b' - 0.211\phi_c' - 0.502\phi_d'$; (D) $\phi_4^* = 0.072\phi_a' + 0.184\phi_b' + 0.976\phi_c' - 0.051\phi_d'$.

apparent combination of effects from the new substituents serve to obscure the trend of A_{obsd} with respect to ϵ_a^* in this set of molecules, the data can be reconciled with those of set **R** if the 3-amino and 2-methyl groups enhance the activity approximately 2 and 3 units, respectively. These factors are taken into account through assignment of structure factors δ_1 and δ_2 in Table I.

A possible rationale for the contribution of the 3-amino group (δ_1) to the activity is based on certain features of the electronic structure displayed by the molecules in **S**. The lone-pair electrons associated with the nitrogen atoms attached to the phenyl ring are major contributors to the highest-occupied molecular orbital and another occupied orbital with slightly lower energy. Since sites with concentrated charge density in high-energy occupied orbitals may serve as electron-donating centers in a charge-transfer interaction, the nitrogen atom in the 3-phenyl position could very well exert its effect through binding with an electron-accepting entity at the receptor. If this is the case, other atoms with lone-pair electrons (e.g., O) may produce a similar effect while atoms without such electrons (e.g., H or C) would be ineffective.

In order to investigate the apparent enhancement of activity by CH_3 (δ_2), several other methyl-containing molecules in Table I must be considered. Only molecules **18**, **26**, and **28** exhibit a methyl group that unequivocally occupies the 2-phenyl position. Molecule **39** has a methyl substituent that could take either the 2-phenyl or 4-phenyl position depending upon the orientation of the drug with respect to the receptor. In addition, there is one molecule, **21**, with a methyl group in the 5-phenyl position. The point in Figure 4 corresponding to each of these molecules, with the possible exception of number **28**, is displaced upward from the activity expected as a result of the calculated value of ϵ_a^* and the trend established by the remaining members of the particular set of compounds. Therefore, methyl groups in the 2- and 5-phenyl positions appear to exert a positive influence on the activity. A 4-CH_3 substituent may also have such an effect based on the observed activity of molecule **39**. Due to limitations imposed by the quality of the data and the number of observations, it is impossible to determine whether the magnitude of the methyl effect is sensitive to the ring position of the group or not. However, an average contribution to the activity of roughly 2 units would seem to adequately account for the deviations arising from a methyl substituent in any ring position.

The structure of the methyl group is conducive only to very weak, nonspecific attractive interactions with the receptor, since the valence electrons all occupy stable bonding molecule orbitals. For this reason, the effect of the methyl substituent cannot be ascribed with any confidence to direct binding at the receptor. Since CH_3 increases the hydrophobic character of the molecule, the effect could reflect an advantageous change in factors relating to transport or solvation. In other words, the value of c_2 in eq 10 could be determined primarily by contributions from $\ln f$ and/or ΔU instead of the binding term, Q.

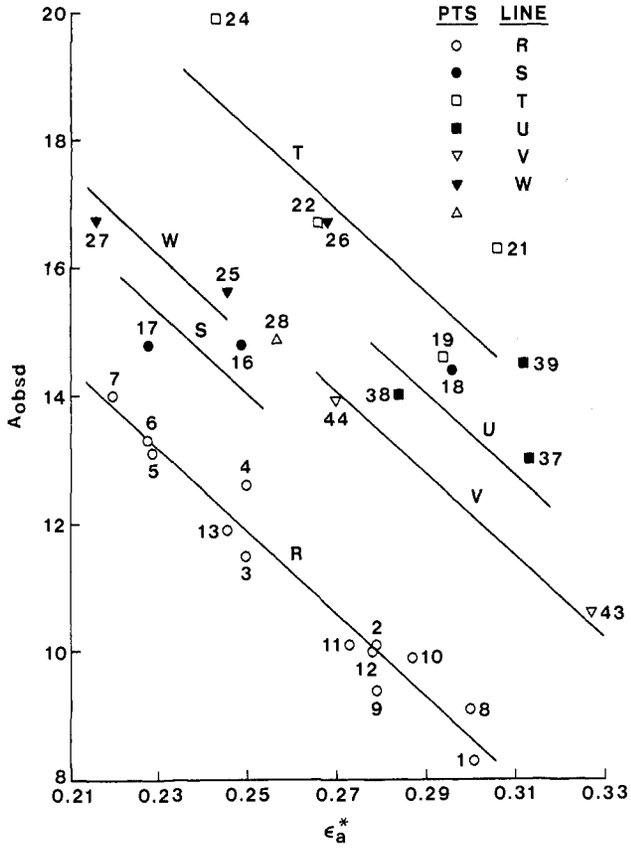


Figure 4. Activities of various molecules plotted against the energy, ϵ_a^*, of the unoccupied molecular orbital, designated as ϕ_a'. The number of each point corresponds to the number of the molecule in Table I. The parallel lines **R**–**W** reflect trends within sets of molecules differentiated by specific structural features as discussed in the text. Each line passes through the midpoint of the corresponding set, excluding the points (**18**, **21**, **26**, and **39**) of methyl-containing drugs. Although set **V** consists of only two conformationally restricted molecules in this preliminary graphical examination for significant structural factors, it is augmented in the regression analysis by inclusion of several drugs that exhibit population distributed among **V**, **U**, and **T**.

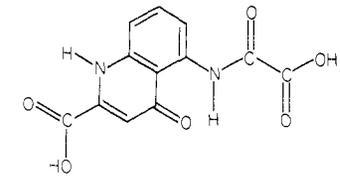
However, this hypothesis is not favored, since several other substituents should exert major direct effects on the activity if changes in these terms were important. For example, if Hansch π values are used as a measure of the substituent effect on the hydrophobic character of the molecule, Cl should be more effective than CH_3, and CN should exert a substantial negative effect. This is not consistent with the evidence provided by the **R** set of compounds where Cl and CN exhibit no significant direct effects on the activity. Since the foregoing considerations apparently rule out a direct methyl effect of the magnitude required, it would seem that the term serves to compensate for a deficiency in the simple FSGO basis sets used to describe the molecules under study. The deficiency is manifested by values of ϵ_a^*, which are much higher than expected for molecules containing a methyl group.

The molecules numbered **19**, **21**, **22**, and **24** in Table I constitute the set associated with line **T**. These drugs, which are all *N*,*N*′-(*m*-phenylene)dioxamic acids existing exclusively in the aa conformation, exhibit essentially the same trend of activity as a function of ϵ_a^* as the molecules in set **R**. Line **T** is displaced upward from line **R** by approximately 6.5 units. Of this total, roughly 2 units may be ascribed to the effect of the nitrogen atom in the 3-phenyl position. Thus, the $COCO_2H$ moiety attached to the nitrogen atom enhances the activity approximately 4.5 units—a value corresponding to a 90-fold increase in potency. This effect has been taken into account by means of factor δ_3.

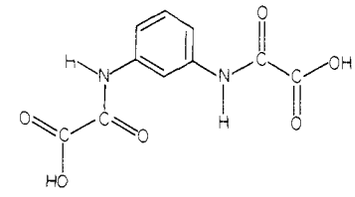
Analysis of the electronic structure of the molecules in group **T** reveals that the oxygen lone-pair electrons in the $COCO_2H$ moiety contribute strongly to several of the higher occupied molecular orbitals. Since electrophiles may be directed preferentially toward regions of high electron density in these orbitals, a positively charged center at the receptor could engage in binding with one or more of the oxygen atoms. If the acidic hydrogen were displaced by a properly located positive receptor entity, the drug–receptor interactions could have a very strong ionic component. Should binding interactions of this nature occur, the acid or salt would be considerably more effective than an ester in binding at the receptor.

Since the structure of the molecules in set **T** exhibits C_{2v} symmetry, there is an entropy contribution to the activity of 0.69 unit arising from the rotational symmetry factor, σ, which has a value of 2. However, this increase is overwhelmed by the decrease due to loss of translational and rotational degrees of freedom in binding. As a result, the net contribution of γ to the activity of the molecules is negative as shown by the values listed in Table I.

Set **U** is composed of three molecules from class VIII, numbered **37**–**39** in Table I, which are hybrids of the oxanilic and quinaldic acid structures. These compounds appear to be somewhat less active (~1.5 unit) than the drugs exhibiting similar values of ϵ_a^* in set **T**. The molecules of this set, like those in **T**, possess two $NHCCO_2H$ substructures attached to a six-carbon aromatic ring in a 1,3 relationship. However, with regard to the spatial arrangement of key functional groups, the molecules in set **U** resemble the sa conformer of *N*,*N*′-(*m*-phenylene)dioxamic acid, not the aa conformer, as the following structural diagrams show:



VIII



IV (sa)

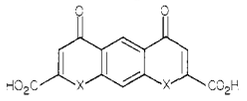
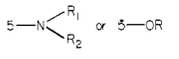
This comparison indicates that the 2-carboxy group in a **U**-type compound must occupy a different region of space than its counterpart in a **T**-type drug when the oxanilic acid moieties common to both sets of molecules are superimposed. As a consequence, the apparent downward displacement of line **U** relative to line **T** might be attributed in part to reduced binding between the carboxy in question and its center of attraction at the receptor. These conformational effects have taken into consideration by means of factor δ_4. Another factor contributing to the difference in activity between drugs in **T** and **U** is the enhancement associated with molecular symmetry; the molecules in set **T** possess a twofold rotational symmetry axis, while those in set **U** lack a rotational symmetry element.

Molecules **43** and **44** in Table I, which constitute the set designated by **V**, are *N*,*N*′-(*m*-phenylene)dioxamic acids

Table IV. Correlation Matrix of Structural Factors

	$\langle\epsilon_{eff}^*\rangle$	$\langle\delta_1\rangle$	δ_2	$\langle\delta_3\rangle$	$\langle\delta_4\rangle$	$\langle\delta_5\rangle$	δ_6	δ_7	$\langle\delta_8\rangle$
$\langle\epsilon_{eff}^*\rangle$	1.0	0.31	0.08	0.05	0.27	0.15	−0.29	0.23	0.41
$\langle\delta_1\rangle$		1.0	0.22	0.52	0.40	0.23	0.19	0.17	0.20
δ_2			1.0	0.20	0.00	−0.12	0.39	−0.08	−0.12
$\langle\delta_3\rangle$				1.0	−0.27	−0.23	0.44	0.37	0.06
$\langle\delta_4\rangle$					1.0	0.06	−0.18	−0.14	0.26
$\langle\delta_5\rangle$						1.0	−0.10	−0.09	−0.09
δ_6							1.0	−0.08	−0.11
δ_7								1.0	0.18
$\langle\delta_8\rangle$									1.0

Table V. Nonlinear Regression Data Obtained from Correlation of Biological Activities with Structural Features by Eq 12

factor[a]	structural feature[b]	no. of molecules exhibiting feature	parameter	parameter value	standard error of parameter	t value
$\langle\epsilon_{eff}^*\rangle$	effective energy of acceptor-orbital manifold	50	b	−64.6	4.1	−15.8[c]
$\langle\delta_1\rangle$	3-NHR or 3-OR	39	c_1	3.1	0.39	8.2[c]
δ_2	2-, 4-, or 5-CH_3	5	c_2	1.8	0.41	4.4[c]
$\langle\delta_3\rangle$	3(aa)-$XCCO_2H$	24	c_3	4.8	0.40	12.0[c]
$\langle\delta_4\rangle$	3(sa)-$XCCO_2H$	20	c_4	2.8	0.44	6.4[c]
$\langle\delta_5\rangle$	3(ss)-$XCCO_2H$	15	c_5	1.1	0.62	1.8[d]
δ_6		4	c_6	−3.6	0.51	−7.1[c]
δ_7		3	c_7	1.3	0.49	2.7[e]
$\langle\delta_8\rangle$		8	c_8	1.9	0.39	4.9[c]
			a	29.2	1.1	26.5[c]
			λ_{da}	−0.093	0.011	−8.5[c]
multiple correlation coefficient (r)				0.96		
goodness of fit factor[f] (R^2)				0.92		
standard error of estimate (s)				0.85		
degrees of freedom				39		

[a] Factors denoted by a symbol of the type $\langle\Omega_t\rangle$ are conformationally averaged in the fitting process using eq 14. [b] X denotes either NH or O in the structural features corresponding to factors δ_3–δ_6. [c] $p < 0.001$. [d] $p < 0.1$. [e] $p < 0.02$. [f] The goodness of fit factor is defined as $R^2 = 1-[\Sigma(A_{obsd} - A_{calcd})^2/\Sigma(A_{obsd} - \bar{A}_{obsd})^2]$, where the summations are carried out over the 50 drugs of the study and $\bar{A}_{obsd}$ is the average of the observed activity indexes for the drugs.

existing in the ss conformation. Since line **V** falls roughly midway between lines **T** and **R**, dioxamic acids in the ss conformation apparently do not bind to the receptor as well as those in the aa conformation but are significantly more effective than the simple oxamic acids. Thus, a new factor, δ_5, must be employed for molecules in this category.

In order to maintain charge-transfer interactions involving ϕ_a', the **V**-type drugs must assume an orientation at the receptor with the phenyl ring and nitrogen atoms located in the positions taken by the corresponding entities of the aa and sa structures. However, both of the carboxycarbonyl moieties in the ss conformer then suffer displacement from the receptor sites occupied by the corresponding moieties in the aa structure; the shift leaves just two carboxy atoms, O(2) and O(2′), near the positions occupied in the aa form. In this case, the enhancement due to the nitrogen atom in the 3-phenyl position would be retained, but the effects of the carboxy groups would be reduced.

The three drugs in set **W** are tricyclic structures with C_{2v} symmetry from class V. These molecules exhibit several structural similarities to the molecules in set **T**. For example, in molecules **25**–**27**, the $NHCCO_2H$ substructures extending through the outer rings are attached to the central ring in a manner resembling the arrangement in the aa conformer of *N*,*N*′-(*m*-phenylene)dioxamic acid. Although the molecules in **W** and **T** possess similar acti-

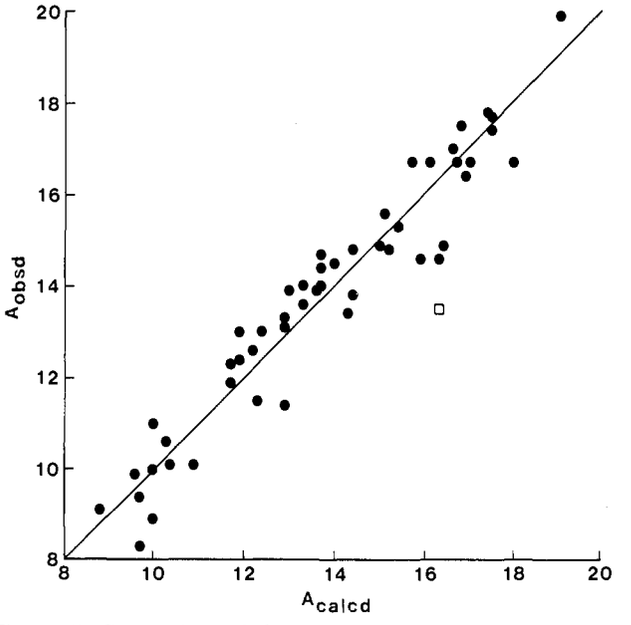


Figure 5. Comparison of observed activities of drugs in the rat PCA test with activities calculated from eq 12. The points are shown in relation to the theoretical line of unit slope. Although molecule **48** was not included in the regression analysis, its point is shown as an open square on the graph.

Table VI. Observed and Calculated Activities of 51 Molecules in the Rat PCA Assay

								conformationally averaged structural factors[f]								
no.	class[a]	substituents	ED_{50}[b]	A_{obsd} (η)[c]	A_{calcd}[d]	ΔA[e]	$\langle\gamma\rangle$	$\langle\epsilon_{eff}^*\rangle$	$\langle\delta_1\rangle$	δ_2	$\langle\delta_3\rangle$	$\langle\delta_4\rangle$	$\langle\delta_5\rangle$	δ_6	δ_7	$\langle\delta_8\rangle$
1	I		245	8.3 (0.8)	9.7	−1.4	0.0	0.3011	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	I	4-CN	40.5	10.1 (1.0)	10.4	−0.3	−0.70	0.2793	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	I	2-CN	10.1	11.5 (0.5)	12.3	−0.8	−0.80	0.2496	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	I	3-CN	3.38	12.6 (0.5)	12.2	0.4	−0.90	0.2495	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	I	3-CN, 4-Cl	2.06	13.1 (0.7)	12.9	0.2	−1.50	0.2287	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	I	2-Cl, 5-CN	1.74	13.3 (1.2)	12.9	0.4	−1.60	0.2278	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	I	3,5-CN	0.84	14.0 (1.2)	13.3	0.7	−1.60	0.2204	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	II	8-OH	114	9.1 (0.9)	8.8	0.3	−1.00	0.2999	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	II	6-OCH_3	82.2	9.4 (1.2)	9.7	−0.3	−1.40	0.2791	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	II	6-NH_2	48.3	9.9 (1.2)	9.6	0.3	−1.00	0.2873	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	II		42.4	10.1 (0.9)	10.9	−0.8	−0.60	0.2733	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	III	6-NH_2	43.5	10.0 (0.8)	10.0	0.0	−1.20	0.2785	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	III	6-NO_2	6.5	11.9 (1.0)	11.7	0.2	−1.6	0.2462	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	I	3-NH_2	131	8.9 (0.6)	10.0	−1.1	−0.60	0.3325	0.83	0.0	0.0	0.0	0.0	0.0	0.0	0.17
15	I	3-OCH_3	16.9	11.0 (1.2)	10.0	1.0	−0.90	0.3209	0.47	0.0	0.0	0.0	0.0	0.0	0.0	0.53
16	I	2-Cl, 3-NH_2, 5-CN	0.35	14.8 (1.2)	14.4	0.4	−1.80	0.2493	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17	I	2,4-Cl, 3-NH_2, 5-CN	0.38	14.8 (1.2)	15.2	−0.4	−2.40	0.2276	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18	II	7-NH_2, 8-CH_3	0.54	14.4 (0.4)	13.7	0.7	−1.30	0.2960	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
19	IV	2-Cl	0.47	14.6 (0.5)	16.3	−1.7	−1.90	0.2938	1.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
20	IV	2-Cl, 5-$CONH_2$	0.58	14.9 (1.0)	15.0	−0.1	−2.80	0.2992	1.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
21	IV	2-Cl, 5-CH_3	0.083	16.3 (0.8)	17.0	−0.7	−2.20	0.3060	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0
22	IV	2-CN	0.056	16.7 (1.3)	18.0	−1.3	−1.90	0.2664	1.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
23	IV	2-Cl, 5-C_6H_5	0.029	17.4 (1.2)	17.5	−0.1	−3.20	0.2540	1.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
24	IV	2-Cl, 5-CN	0.0023	19.9 (1.1)	19.1	0.8	−2.40	0.2429	1.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
25	V	10-Cl	0.17	15.6 (1.0)	15.1	0.5	−2.60	0.2460	1.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0
26	V	10-CH_3	0.056	16.7	15.7	1.0	−2.40	0.2679	1.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0
27	V	5-CN, 10-Cl	0.057	16.7 (1.2)	16.7	0.0	−2.90	0.2161	1.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0
28	VI	10-CH_3	0.33	14.9 (1.2)	16.4	−1.5	−2.40	0.2573	1.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0
29	IV	5-OCH_3	1.18	13.6 (0.6)	13.3	0.3	−2.23	0.3573	1.0	0.0	0.81	0.19	0.01	0.0	0.0	1.0
30	IV	5-$CONH_2$	0.90	13.9 (1.1)	13.0	0.9	−2.70	0.3222	1.0	0.0	0.70	0.28	0.02	0.0	0.0	0.0
31	IV		0.40	14.7 (0.5)	13.7	1.0	−1.78	0.3189	1.0	0.0	0.54	0.40	0.06	0.0	0.0	0.0
32	IV	5-CN	0.024	17.5 (0.6)	16.8	0.7	−2.36	0.2634	1.0	0.0	0.58	0.37	0.05	0.0	0.0	0.0
33	IV	5-NO_2	0.018	17.8 (0.5)	17.4	0.4	−2.69	0.2771	1.0	0.0	0.51	0.42	0.07	0.0	0.0	1.0
34	VII	4-OCH_3	0.075	16.4 (0.7)	16.9	−0.5	−2.11	0.3300	1.0	0.0	0.99	0.01	0.0	0.0	1.0	1.0
35	VII		0.037	17.0 (0.6)	16.6	0.4	−1.52	0.3132	1.0	0.0	0.97	0.03	0.0	0.0	1.0	0.0
36	VII	4-Cl	0.020	17.7 (0.8)	17.5	0.2	−2.22	0.2883	1.0	0.0	0.97	0.03	0.0	0.0	1.0	0.0
37	VIII	8-OCH_3	2.21	13.0 (1.2)	11.9	1.1	−3.00	0.3129	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0
38	VIII	8-Cl	0.81	14.0 (1.0)	13.7	0.3	−3.00	0.2843	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0
39	VIII	8-CH_3	0.51	14.5 (1.2)	14.0	0.5	−2.70	0.3122	1.0	1.0	0.0	1.0	0.0	0.0	0.0	0.0
40	IV	4-F	3.93	12.4 (1.2)	11.9	0.5	−2.44	0.3159	1.0	0.0	0.0	0.78	0.22	0.0	0.0	0.0
41	IV	4-CN	1.58	13.4 (0.7)	14.3	−0.9	−2.51	0.2765	1.0	0.0	0.0	0.73	0.27	0.0	0.0	0.0
42	IV	4-Cl, 5-CN	0.055	16.7 (1.2)	16.1	0.6	−3.03	0.2419	1.0	0.0	0.0	0.76	0.24	0.0	0.0	0.0
43	IV	4,6-F	24.9	10.6 (1.2)	10.3	0.3	−2.00	0.3274	1.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0

44	IV	4,6-Cl	0.9	13.9 (1.2)	13.6	0.3	−2.50	0.2697	1.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
45	IV	5-NHCOCO$_2$H	2.33	13.0 (1.2)	12.4	0.6	−2.78	0.3392	1.0	0.0	0.07	0.86	0.07	0.0	0.0	1.0
46	IX		0.45	14.6 (1.1)	15.9	−1.3	−3.10	0.2788	1.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0
47	IV	5-*trans*-styryl	0.23	15.3 (1.1)	15.4	−0.1	−3.71	0.2676	1.0	0.0	0.65	0.30	0.04	0.0	0.0	0.0
48	IV	5-(2-benzimidazolyl)	0.94	13.5 (0.6)	(16.3)[g]	(−2.8)[g]	−3.99	0.2431	1.0	0.0	0.33	0.56	0.11	0.0	0.0	0.0
49	IV	5-(2-pyrimidinyl)	1.0	13.8 (1.2)	14.4	−0.6	−3.39	0.2755	1.0	0.0	0.33	0.56	0.11	0.0	0.0	0.0
50	I	*m*-(2-pyrimidinyl)	4.4	12.3 (1.1)	11.7	0.6	−2.00	0.2401	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
51	X		11.5	11.4 (0.9)	12.9	−1.5	−1.72	0.3029	1.0	0.0	0.04	0.32	0.64	0.0	0.0	0.0

[a] Figure 1 defines the structural classes and numbering conventions used to designate substituent positions in the molecules. [b] The dose required for 50% inhibition of the anaphylactic response in rats is expressed in micromoles per kilogram of body weight. All of the drugs were administered iv as salts of tris(hydroxymethyl)amino methane at the time of antigen challenge. [c] The experimental activity index, A_{obsd}, and estimated error, η, have been determined by the methods described in ref 6. [d] A_{calcd} is determined from eq 12 with the structural factors shown in this table and the regression coefficients from Table V. [e] $\Delta A = A_{obsd} - A_{calcd}$. [f] The structural factors are defined in footnote *b* of Table I. Equation 7 defines the term γ, which arises from loss of translational and rotational degrees of freedom upon binding. The values of $\langle\delta_3\rangle$, $\langle\delta_4\rangle$ and $\langle\delta_5\rangle$ respectively predict the fraction of bound drug in the aa, sa, and ss conformations of the *m*-dioxamic acids. [g] Since molecule **48** was not included in the final regression calculation, the value of A_{calcd} is an estimate obtained from the established structure–activity relationship.

vating structural features, there is roughly a 3.5-unit difference in the level of activity. The reduced activity of the drugs in set **W** could possibly result from steric factors that greatly weaken binding interactions at the receptor. However, data from in vitro experiments[16] indicate that the drugs in set **W** may be no less effective in blocking 48/80-induced histamine release from isolated rat mast cells than the compounds in set **T**. Since the hypothesis of hindered receptor binding does not seem to be consistent with this evidence, the unusually low activity of the **U**-type drugs observed in vivo probably results from impeded transport into the receptor compartment. Structural factor δ_6 has been defined for molecules of this type.

There is one example of an additional structural type shown in Figure 4, namely, molecule **28**, which bears strong resemblance to the compounds in set **W**, but with oxygen atoms replacing the NH groups in positions 1 and 9 of the three-ring system. As a result of this structural resemblance, the transport and binding characteristics of molecule **28** and the compounds in set **W** could be much alike. Furthermore, since the oxygen atom is similar to the nitrogen in that both possess lone-pair electrons in conjugation with the π system, the oxygen-containing species could interact with the receptor in a manner very similar to the **W**-type drugs. For this reason, factor δ_6 has been applied to molecule **28** as well as those in **W**.

Nonlinear Regression Analysis. The foregoing analysis suggests that six structural factors in addition to ϵ_d* may be required in eq 10 to rationalize the observed activities of the 29 drugs included in Figure 4. A summary of these variables, designated δ_1–δ_6, is given in Table I. Whether or not this set of factors is sufficient to account for the activities of all molecules under study must be assessed by the results of the regression analysis.

Prior to discussion of the analysis, it should be noted that several molecules are capable of binding in more than one conformation. In this case, the magnitude of a structural factor depends upon two things: (1) the presence or absence of the particular structural feature in each conformation, and (2) the distribution of the bound drug population among the different conformers. With regard to the structural factors considered thus far, only δ_2 and δ_6 are conformationally independent terms that can be evaluated simply by inspection. The average magnitude, $\langle\delta_s\rangle$, for δ_1 and δ_3–δ_5 must be determined by nonlinear regression methods using eq 14 in conjunction with eq 12.

The statistical computations in this work have been carried out with the NONLIN program package of Metzlar et al.[17] After the initial trial using $\langle\epsilon_{eff}$*$\rangle$, $\langle\delta_1\rangle$, δ_2, $\langle\delta_3\rangle$, $\langle\delta_4\rangle$, $\langle\delta_5\rangle$, and δ_6 as the set of structural factors in eq 12, the following molecules remained as prominent outliers: (1) the dioxamic acids in class VII, (2) drugs with 5-substituents in which N or O adjoins the benzene ring, and (3) molecule **48**. These findings revealed the necessity for including additional structural factors in the analysis.

Structural factor δ_7 takes into account the effects of substituting a 2-pyridyl moiety for the phenyl ring in the essential pharmacophore. Although each pyridine-containing molecule possesses a low-lying antibonding π orbital with the characteristics of ϕ_a', several other features of the electronic structure (e.g., the charge distribution in the ring and the dipole moment) are significantly different from its benzene-containing congener. Thus, the enhanced activity represented by the δ_7 effect cannot be easily as-

(16) H. G. Johnson, private communication. Lodoxamide tromethamine (**24**) produces 44% inhibition of 48/80-induced histamine release at a concentration of 18 nM, while the fused-ring quinaldic acid (**26**) yields 53% inhibition at 30 nM.

cribed to one particular property of the pyridine moiety. Furthermore, the possibility exists that the factor serves to compensate for an error in the relative values of ϵ_a* caused by a slight imbalance in the FSGO basis sets used to describe the aromatic rings.

The structural factor δ_8 applies to substituents of the type 5-NR_1R_2 and 5-OR. Since the lone-pair electrons on the atoms N and O contribute to high-energy occupied molecular orbitals (including the frontier MO), these substituents markedly enhanced the capacity of the molecule to interact with an electrophilic receptor entity located near the 5-position of the essential aromatic ring.

In order to reconcile A_{calcd} with A_{obsd} for molecule 48, a large negative substituent effect (ca. −3 units) must be postulated for the 5-(2-benzimidazolyl) group. Although there is insufficient evidence to determine the source of the effect, it could possibly result from hindered transport to the receptor or steric inhibition of binding. The latter possibility merits consideration, since when the essential pharmacophores of all the molecules under study are overlaid, portions of the bulky benzimidazole moiety may be clearly seen to project into a region of space unoccupied by any other substituent. This region could be forbidden owing to the topography of the receptor. Unfortunately, the lack of additional examples of this substituent effect made it necessary to omit molecule 48 from the set used in further analysis.

Since no significant colinearities among the nine structural factors were revealed in the correlation matrix shown in Table IV, the analysis was completed with this set. A summary of the statistical data obtained from the final regression calculation is given in Table V. All ten parameters prove to be significant, and the analysis yields satisfactory measures (r = 0.96, R^2 = 0.92, and s = 0.85) for the quality of the fit. Neither the inclusion of molecule 48 in the data set nor the deletion of any other single molecule causes parameter values to change notably in the regression analysis from those given in Table V. Thus, the equation appears to be quite robust. Although the graph of A_{obsd} vs. A_{calcd} in Figure 5 reveals scatter about the line of perfect correlation, the computed point for molecule 48 is the only one lying at a distance greater than ±2s, where s is the standard error of the estimate. Table VI contains the values of A_{obsd}, A_{calcd}, and the conformationally averaged structural factors for all of the molecules. Since the uncertainty in A_{obsd} (expressed by the index η in Table VI) is generally of the same order as s, additional structural factors are unlikely to provide any meaningful improvement in the correlation.

The importance of charge-transfer interactions involving the acceptor orbital, ϕ_a', in the aromatic ring of the essential pharmocophore has been established by the dependence of the PCA activity on the structural factor $\langle\epsilon_{eff}^*\rangle$, including the donor–acceptor interaction term λ_{da}. It should be noted that the negative coefficient of $\langle\epsilon_{eff}^*\rangle$ is consistent with the prediction of eq 9 that a reduction in the energy of the acceptor orbital(s) will stabilize the drug–receptor complex. According to eq 11, λ_{da} becomes important when resonance effects create a manifold of acceptor orbitals. Although the magnitude of λ_{da} is not large, it is highly significant ($p < 0.001$) from a statistical viewpoint. The small value for this parameter indicates a condition of low overlap between the donor MO, ϕ_d, and the primary acceptor orbital, ϕ_a', as expected for a weak charge-transfer complex.

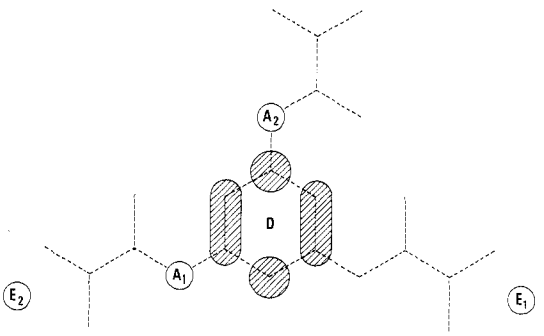
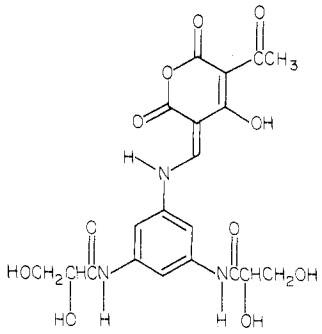


Figure 6. Schematic representation of possible receptor-binding sites superimposed upon outline (dashed structure) of C_s conformer of *N,N',N''*-1,3,5-benzenetriyltrioxamic acid. The donor site, D, specifies where the key aromatic ring of the drug is located, showing the regions of high density (shaded areas) in ϕ_a' where maximal overlap with the receptor orbital ϕ_d would occur. E_1 and E_2 designate electrophilic receptor entities with strong electrostatic attractions for the oxygens of the carboxy groups. A_1 and A_2 denote electrophilic receptor entities that interact with certain substituents in positions 3 and 5, respectively, of the key aromatic ring; enhanced activity results for groups containing oxygen or nitrogen adjoining the ring.

The calculations performed in this study reveal details of electronic structure that provide insight into factors underlying direct substituent effects. For example, all of the groups that enhance PCA activity, except for CH_3, exhibit electron-rich sites well suited for attractive interactions with cationic or polar species. In view of this fact, it is unlikely that the receptor presents a nonpolar, lipid environment for binding. This conclusion is supported by the results of Cramer et al.,[18] who found that increased substituent hydrophilicity is associated with enhanced PCA potency in a series of pyraneamines. Although electronic and geometric aspects of substituent structure were not considered explicitly in the pyranenamine study, it is interesting to note that the most potent compound (XI) of the series possesses several features that have been elucidated as activating factors in this investigation.



XI

The critical structural features discovered by the regression analysis suggest a receptor model similar to the one portrayed in Figure 6. In this construct, an electron-donating entity, D, lies at the center of the binding site. In order to effectively serve as a donor, D must possess a high-energy filled MO, ϕ_d, with a density distribution that complements the primary acceptor orbital, ϕ_a'. This fixes the location and orientation of the essential aromatic ring during association with the receptor. Two positively charged electrophilic entities, E_1 and E_2, are situated on either side of D near the sites occupied by the

(17) C. M. Metzler, G. L. Elfring, and A. J. McEwen, *Biometrics*, **30**, 562 (1974).

(18) R. D. Cramer III, K. M. Snader, C. R. Willis, L. W. Chakrin, J. Thomas, and B. M. Sutton, *J. Med. Chem.*, **22**, 714 (1979).

carboxy groups of a *m*-dioxamic acid. By displacing the acidic protons, E_1 and E_2 provide a strong ionic component to the receptor-binding energy. These receptor features are considered to underlie the tremendous enhancement in PCA activity represented by factors δ_3–δ_5. Differences in activity among the aa, sa, and ss structures may be explained by the positions of the carboxy oxygen atoms relative to E_1 and E_2 in the complexes of the three conformers. Receptor moieties, A_1 and A_2, with electron-accepting capacity are located near the positions occupied by C(3) and C(5) of the aromatic ring. A rationale for the role of δ_1 and δ_8 is provided by the existence of A_1 and A_2, respectively. If the drug molecule contains atoms with lone-pair electrons in juxtaposition to these entities, the stability of the complex will be increased through the resultant donor–acceptor interactions.

Conclusion

In an effort to discover which structural features affect the activity of drugs in the rat PCA assay, an investigation has been carried out involving a series of 51 molecules from several classes of compounds. The theoretical model employed in the study takes into account the following factors: (1) charge-transfer interactions between the receptor and the essential pharmacophore of the drug, where the latter may possess a manifold of acceptor orbitals; (2) effects of specific modifications in drug structure; (3) entropy contributions due to molecular size, shape, and symmetry; and (4) the distribution of bound drug among different conformational states of the molecule. These considerations led to the development of a nonlinear regression equation relating the biological activity index, $A = -\ln ED_{50}$, to a variety of calculable structural indexes. The ab initio SCF–MO molecular fragment method was employed to determine the electronic and geometric properties required by the theoretical model. The statistical findings lend support to the model and establish the importance of nine structural features for activity in the PCA assay. The magnitude of A_{calcd} for 50 drugs proved to be the same as A_{obsd} within the 95% confidence limits established by the experimental error. The existence of the single low-activity outlier, molecule **48**, suggests that bulky substituents could be used as probes to explore the extent of the binding site. Finally, inferences have been made regarding the cause of certain substituent effects based upon calculated indicators of reactivity, such as the density distribution in the higher filled molecular orbitals. From these inferences, a schematic receptor map has been created that may prove useful in designing further studies of this system.

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Registry No. **1**, 500-72-1; **2**, 58446-15-4; **3**, 61068-77-7; **4**, 58446-13-2; **5**, 58446-17-6; **6**, 58446-11-0; **7**, 58446-19-8; **8**, 67283-71-0; **9**, 52930-06-0; **10**, 52980-10-6; **11**, 13593-94-7; **12**, 67283-72-1; **13**, 30095-78-4; **14**, 101-09-7; **15**, 72269-26-2; **16**, 84944-23-0; **17**, 58446-09-6; **18**, 52979-88-1; **19**, 53882-08-9; **20**, 67451-36-9; **21**, 53882-32-9; **22**, 84944-24-1; **23**, 58763-12-5; **24**, 53882-12-5; **25**, 49635-52-1; **26**, 49635-47-4; **27**, 63920-88-7; **28**, 25201-04-1; **29**, 53882-30-7; **30**, 53882-14-7; **31**, 53882-05-6; **32**, 53882-10-3; **33**, 79808-23-4; **34**, 60494-53-3; **35**, 56216-25-2; **36**, 60494-55-5; **37**, 60722-37-4; **38**, 60722-36-3; **39**, 60722-35-2; **40**, 67451-34-7; **41**, 67451-33-6; **42**, 53882-17-0; **43**, 84944-25-2; **44**, 53882-22-7; **45**, 84944-26-3; **46**, 54046-97-8; **47**, 84944-27-4; **48**, 84944-28-5; **49**, 84944-29-6; **50**, 84944-30-9; **51**, 84944-31-0.

Supplementary Material Available: Geometrical data; variation of E, ϵ_u^*, and c_{au}' with rotation of certain substituents (Table II); and coefficients in the cosine expansion (eq 16) used to fit calculated values of $E(l, \theta)$ for molecules with rotating substitutents (Table III) (9 pages). Ordering information is given on any current masthead page.

Synthesis and Biological Distribution of Radiolabeled Ammineruthenium(III)–Amino Acid Complexes as Potential Pancreatic Imaging Agents

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Complexes of ammine[^{103}Ru]ruthenium(III) with L-histidine, β-(4-pyridyl)-α-alanine, and *S*-[β-(4-pyridyl)ethyl]-L-cysteine were synthesized in low specific activity and evaluated in mice as potential radiodiagnostic agents for pancreatic imaging. The biological distribution of each complex was determined in normal mice at 15 min, 1 h, and 2 h following intravenous administration. All four complexes were rapidly cleared through the kidneys, with 50% of the injected dose concentrated in the urine within 15 min. None of the complexes showed a tendency to accumulate in any major organ. Major differences in distribution were found in lungs, heart, spleen, stomach, intestine, bone, and soft tissues. A significant relative difference in pancreatic uptake was observed. Only the β-(4-pyridyl)-α-alanine complex exhibited pancreas to liver ratios significantly greater than 1. The pancreas to liver ratio of 17 was reached 1 h following injection of this ruthenium complex, which is considerably higher than commonly reported values of 2.5 for [^{75}Se]selenomethionine. The β-(4-pyridyl)-α-alanine complex is therefore a promising candidate for evaluation as a pancreatic imaging agent when labeled with cyclotron-produced ruthenium-97.

The rapid protein turnover in the pancreas relative to other organs formed the basis for the development of L-[^{75}Se]selenomethionine as a pancreatic imaging agent.[1-5] However, this radiopharmaceutical is far from ideal due

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