Structure–Activity Correlations for a Series of Antiallergy Agents. Oxanilic, Quinaldic, and Benzopyran-2-carboxylic Acids

B. Vernon Cheney,* John B. Wright, Charles M. Hall, Herbert G. Johnson,

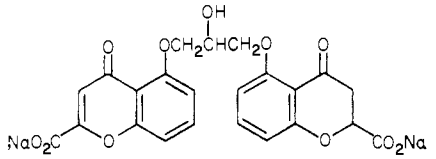
Research Laboratories of The Upjohn Company, Kalamazoo, Michigan 49001

and Ralph E. Christoffersen[1]

Department of Chemistry, University of Kansas, Lawrence, Kansas 66044. *Received March 6, 1978*

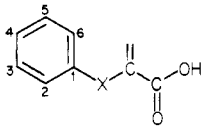
Ab initio Hartree–Fock SCF calculations with the molecular fragment technique have been performed on several drugs which exhibit activity in the rat passive cutaneous anaphylaxis (PCA) assay. Representative molecules of the following types were included in the series: oxanilic acids, 1,4-dihydro-4-oxoquinaldic acids, and 4-oxo-4*H*-1-benzopyran-2-carboxylic acids. A quantitative relationship has been established between the observed biological activity and an electronic index obtained from the calculations. The correlation is rationalized in terms of charge-transfer stabilization of the drug–receptor complex.

Recent research has produced a variety of agents that prevent mediators of the allergic response from being released after antigen–antibody interaction on sensitized mast cells. Cromolyn sodium (I), a clinically effective drug



I

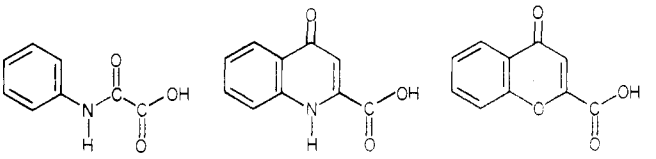
in the treatment of bronchial asthma, initially was found to exhibit this mode of action.[2] Subsequently, potential antiallergy agents which elicit effects similar to cromolyn sodium in animal test systems have been discovered among the following classes of molecules: xanthonecarboxylic acids,[3a] nitroindandiones,[3b] quinolonecarboxylic acids,[4] nitrocoumarins,[5] oxanilic acids,[6] dicarboxybenzodipyrans,[7] and fused-ring quinaldic acids.[8] Although the precise biochemical mechanism of action has not yet been clearly established, it is postulated that some of these drugs inhibit mediator release by acting at a common receptor. Support for this hypothesis is provided by certain similarities in molecular structure among several of the classes. For example, as a general rule, drugs of high activity incorporate the elements of moiety II within the framework of



II, X = O or NH

the molecule. The essential features of II are (a) a planar system with extended π bonding; (b) a benzene ring; (c) an attached oxygen or nitrogen heteroatom; and (d) a carboxyl group separated from the heteroatom by an sp^2-hybridized carbon atom which is designated by the incomplete double bond in the diagram.

Since some understanding as to the nature of drug–receptor interactions may often be gained by examining the electronic and steric properties of the drug molecules, a series of molecules which contain II as a fundamental moiety of the structure was selected for detailed investigation. Three classes of compounds are represented within the series—oxanilic acids (III), 1,4-dihydro-4-oxoquinaldic acids (IV), and 4-oxo-4*H*-1-benzopyran-2-carboxylic acids (V). It should be noted that the 13 drugs included in this study provide a 300-fold range of potencies in the rat passive cutaneous anaphylaxis (PCA) assay.



III IV V

Results

Table I contains a list of the molecules under consideration together with the biological activities. A quantitative measure of the biological effect is provided by the ED_{50}, which represents the dose required to produce 50% inhibition of the anaphylactic reaction in the PCA assay. Values of the ED_{50} for the compounds included in this study vary from 245 to 0.84 μmol/kg of rat body weight. In comparison, the observed ED_{50} for cromolyn sodium (I) is 6.1 μmol/kg. The procedure employed to determine the ED_{50} values is outlined in the discussion of biological activities.

In a preliminary attempt to establish structure–activity relationships for the oxanilic acids, various physicochemical parameters were employed in several regression equations.[9] The outcome revealed no significant correlation between the activity index, A_{obsd}, given by eq 1, and the lipophilicity

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

parameter, π. This finding seems to imply that access to the receptor site is not seriously restricted by diffusion across lipid barriers. Although the Hammett σ parameter was found to correlate with the activity index of those oxanilic acids containing only meta and para substituents, the lack of reliable parameter values for ortho substituents prevented extension of the analysis to the entire series. This partial correlation with σ gave an indication that electronic effects may play a dominant role in producing the observed biological responses.

In order to study the electronic structure of the drugs in some detail, ab initio molecular fragment SCF calculations[10] have been carried out on all of the molecules in the series. The results of the calculations reveal some notable parallels among the chemically interesting higher occupied and lower unoccupied molecular orbitals. For example, the highest occupied MO (HOMO) may be described in every case as a nonbonding π orbital with density concentrated to a large extent at the entity designated by X in moiety II. The next to highest MO (NHOMO) may be characterized as a nonbonding σ orbital with major contributions from the lone-pair orbitals of the carbonyl oxygens. Neither the HOMO nor the NHOMO exhibits significant density in the benzene-ring portion of

0022-2623/78/1821-0936$01.00/0

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

no.	class	substituents	ED_{50}[a]	A_{obsd} (η)[b]	A_{calcd}[c]	ΔA[d]	ϵ_a*[e]
1	III		245	8.3 (0.8)	8.6	−0.3	0.3011
2	III	4-CN	40.5	10.1 (1.0)	10.0	0.1	0.2793
3	III	2-CN	10.1	11.5 (0.5)	11.9	−0.4	0.2496
4	III	3-CN	3.38	12.6 (0.5)	11.9	0.7	0.2495[f]
5	III	3-CN,4-Cl	2.06	13.1 (0.7)	13.2	−0.1	0.2287[f]
6	III	2-Cl,5-CN	1.74	13.3 (1.2)	13.3	0.0	0.2278
7	III	3,5-$(CN)_2$	0.84	14.0 (1.2)	13.8	0.2	0.2204
8	IV	8-OH	114	9.1 (0.9)	8.7	0.4	0.2999
9	IV	6-OCH_3	82.2	9.4 (1.2)	10.0	−0.6	0.2791
10	IV	6-NH_2	48.3	9.9 (1.2)	9.5	0.4	0.2873
11	IV		42.4	10.1 (0.9)	10.4	−0.3	0.2733
12	V	6-NH_2	43.5	10.0 (0.8)	10.0	0.0	0.2785
13	V	6-NO_2	6.48	11.9 (1.0)	12.1	−0.2	0.2462

[a] The dose required for 50% inhibition of the anaphylactic response in rats is expressed in μmol/kg of body weight. All of the drugs were administered iv in tris(hydroxymethyl)aminomethane solution at the time of antigen challenge. [b] The experimental activity index, A_{obsd}, is obtained from eq 17 using the procedure outlined in the section on Biological Activities. Equation 18 or 19 is used to compute the estimated error, η, shown in parentheses following the value of A_{obsd}. [c] A_{calcd} is determined from eq 2. [e] The energy of the unoccupied antibonding π orbital of interest is given in atomic units. [f] Average of results from two planar conformations based on calculated populations using Boltzmann statistics. There were minor differences in the conformational energies and values of ϵ_a* between the two planar states of these meta-substituted molecules.

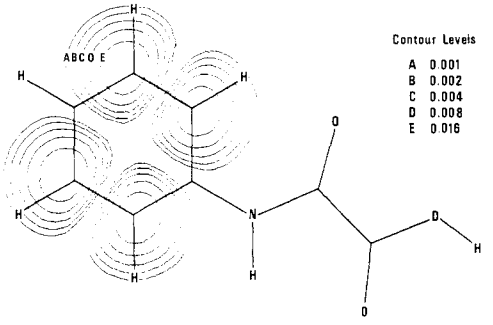


Figure 1. Contour map of density (holes per cubic atomic unit) in LUMO of oxanilic acid (1). The section of the map is located 0.8 above the nuclear plane.

the molecules. Both of the lowest unoccupied MO's (LUMO and NLUMO) in each molecule are antibonding π orbitals localized for the most part within the benzene ring. The LUMO of oxanilic acid (1), portrayed in Figure 1, possesses the following characteristics: (a) high density at C_2 and C_5 in the ring; (b) low density at atoms C_1 and C_4; and (c) nodal surfaces separating the p_π orbitals on C_2 and C_5 from adjacent p_π orbitals. The NLUMO of oxanilic acid has high density on C_1 and C_4 with nodes between these carbons and the adjoining atoms. In general, these descriptions may be applied to the LUMO and NLUMO of all the drugs under study if moiety II is employed as a template to specify corresponding benzene-ring positions in the different structures. However, it should be noted that the order of the two lowest unoccupied MO's is reversed in 4-cyanooxanilic acid (2) relative to the other molecules in the series.

As a means of investigating relationships between molecular structure and biological activity, quantitative comparisons of corresponding MO's were made to establish whether or not significant trends exist in electronic indices such as orbital energy and density distribution. Although the density distributions in the orbitals of interest were found to vary slightly from one drug to another in the series, no pattern of differences could be discerned which correlates with the observed potencies in the PCA assay. On the other hand, a highly significant correlation was discovered between the biological activity index and the energy, ϵ_a*, of the unoccupied antibonding π orbital which has the form of the LUMO in oxanilic acid (1). Hereafter, the orbital of interest will be designated as ϕ_a*. Changes in the energies of other MO's could not be related to biological effects with confidence.

A simple linear equation of the form

$$A = -64.4\epsilon_a^* + 29.0 \quad (2)$$

was determined by regression analysis using the calculated values of ϵ_a* given in Table I. This relationship is considered to be highly significant since the correlation coefficient, r, and standard deviation, s, are respectively −0.98 and 0.37. It appears unlikely that additional parameters would yield a better fit as the value of s is well within the uncertainty limits in the measurements of A_{obsd}.

Discussion

In this section a rationale is proposed for the linear relationship between A_{obsd} and the calculated energy of ϕ_a*. The primary assumptions upon which the theory is based are (a) factors relating to drug transport and metabolism play a minor role so that activity differences essentially reflect events at the receptor; (b) occupancy of the receptor by the drug is required to produce the inhibitory effect;[11] (c) charge-transfer interactions are an important feature in stabilizing the drug–receptor complex; and (d) the unoccupied orbital which possesses the properties of ϕ_a* acts as an electron acceptor in the charge-transfer interactions. Since the receptor for these drugs has not been isolated and characterized, these assumptions cannot be verified through direct experimentation at the present time.

The validity of assumption (a) is strongly dependent upon the procedures employed in the PCA assay. Since mast cells tend to be located near vascular beds in connective tissue,[12] a drug which is administered iv may need only to pass through a relatively unrestrictive capillary membrane to obtain access to the receptor compartment. Also, simultaneous injection of drug and antigen results in a short time course for the experiment which minimizes problems associated with differences in metabolism or excretion rates. If the drugs are administered by a route other than iv, or if long delay times are interposed between injection of drug and antigen challenge, factors associated with adsorption, distribution, metabolism, and excretion do become important determinants of the activity.

For the purposes of this discussion, the in vivo system may be considered to consist of a large compartment in which the bulk of the drug is unevenly distributed and a number of extremely small compartments containing dilute

homogeneous concentrations of drug molecules, receptor entities, and drug–receptor complexes. The concentration, [M], of drug molecules in one of the small compartments may be expressed by eq 3, where D is the dose adminis-

$$[M] = fD \quad (3)$$

tered to the animal and f is a time-dependent function which involves factors relating to transport, metabolism, and excretion. If f changes relatively slowly with time, conditions within the compartment may be in a state of near equilibrium at any given instant. In this case, a valid description of the drug–receptor interaction may be given by eq 4, where m drug molecules, M, react with the re-

$$mM + R \rightleftarrows C \quad (4)$$

ceptor, R, to form the dissociable complex, C. Application of the law of mass action to this reaction gives eq 5, where

$$[C]/[M]^m[R] = K \quad (5)$$

K is the equilibrium constant. In order to form a weak complex, the concentration of drug must be in great excess. Hence, the amount of bound drug is assumed to be negligible when compared to the quantity of free drug in the compartment. Since the receptor-containing regions are expected to be relatively similar in nature, eq 3–5 should hold for the aggregate as well as the individual compartments.

In accordance with assumption (b), the extent to which the anaphylactic response is blocked depends upon the fraction of receptors that has been deactivated (i.e., rendered unavailable for normal function in mediator release) by complexation with drug. Therefore, the observed percent inhibition, E, is given by eq 6, which may

$$E = 100[C]/([C] + [R]) \quad (6)$$

be rearranged to obtain eq 7. Substitution of eq 3 and

$$[C]/[R] = E/(100 - E) \quad (7)$$

7 into eq 5 yields the following relationship.

$$E/f^mD^m(100 - E) = K \quad (8)$$

The case of specific binding to form a loose one-to-one complex will be considered in further discussion so that m will be taken as unity henceforth. Quantitative comparisons of drug potencies are generally based on the dosage required to produce an effect amounting to one-half of the maximum possible response. Since $E = 50$ in such a case, eq 8 becomes

$$ED_{50} = 1/fK \quad (9)$$

where $D = ED_{50}$.

The methods of statistical mechanics may be employed to analyze, from a molecular standpoint, the factors contributing to the magnitude of the equilibrium constant.[13] If M, R, and C exist in very dilute concentrations, the value of K is given approximately by eq 10, where k

$$K = q_C e^{-(\Delta U + \Delta W)/kT}/q_M q_R \quad (10)$$

is Boltzmann's constant, T is the absolute temperature, ΔW is the stabilization energy of the drug–receptor complex, ΔU is the change in solvation energy that occurs upon complex formation, and q_i is related to the molecular partition function for the ith species. If the value of K from eq 10 is employed in eq 9, the expression for the ED_{50} becomes

$$ED_{50} = (q_M q_R/fq_C)e^{(\Delta U + \Delta W)/kT} \quad (11)$$

This expression for the ED_{50} may be substituted in eq 1 to yield eq 12, which has the general form of the linear

$$A = [kT \ln (fq_C/q_M q_R) - \Delta U - \Delta W]/kT \quad (12)$$

free-energy relationships that serve as the foundation for other structure–activity studies.[14-16]

If a major portion of the stabilization energy for the drug–receptor complex is provided by a Mulliken[17] charge-transfer interaction in which the drug acts as an electron acceptor, ΔW may be expressed approximately by eq 13, where Q is the energy of interaction between the

$$\Delta W = Q - \kappa/(\epsilon_a^* - \epsilon_d - \lambda) \quad (13)$$

drug and receptor exclusive of charge-transfer effects, κ and λ are terms which depend upon the degree of overlap between the donor and acceptor orbitals, and ϵ_d is the energy of the receptor donor orbital which will be denoted as ϕ_d in further discussion. Significant charge-transfer contributions to ΔW will exist only when ϕ_d and ϕ_a^* exhibit complementary features in the region of overlap with regard to local symmetry and density distribution. If such is not the case, κ (which is small at best) would become negligible. This is the basis for the "overlap and orientation" principle stated by Mulliken.[18] The energy gap, $\epsilon_a^* - \epsilon_d$, between the donor and acceptor orbitals also plays an important role in complex formation; the stability is enhanced if ϕ_a^* is a low-energy unoccupied MO and ϕ_d is a high-energy occupied MO.

Although it is not possible to explicitly determine Q, κ, ϵ_d, and λ in the absence of structural information concerning the receptor, there may be cases where significant variations in these quantities do not occur. For example, if the distribution of density in critical regions of the pharmacophore remains essentially constant for a given series of drug molecules, the values of Q, κ, and λ would not be expected to change greatly. As a result, the stability of the drug–receptor complex would appear to depend primarily on the value of ϵ_a^* if it were the only factor to differ within the set of molecules. Based on the evidence provided by the correlation in eq 2, these conditions prevail in the current study.

It is of interest to determine how the activity changes when various moieties are added to the essential pharmacophore which may be represented by the parent drug of the series. If the modifications cause small perturbations in the values of the factors affecting activity, a satisfactory description is provided by eq 14, where A_0 is the activity

$$A = A_0 + \sum_t (\partial A/\partial \omega_t)_{\omega_t = \omega_{t0}} \Delta\omega_t \quad (14)$$

index for the parent drug, $\omega_t \in [f, q_C, q_M, q_R, \Delta U, Q, \kappa, \lambda, \epsilon_d^*]$, ω_{t0} is the value of parameter ω_t in the parent drug, and $\Delta\omega_t = \omega_t - \omega_{t0}$. Equation 14 provides the basis for a linear regression model to fit the biological data.

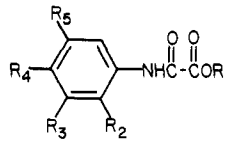
If ϵ_a^* is the only parameter which varies significantly for the set of molecules under consideration, eq 14 becomes

$$A = A_0 - [\kappa/(\epsilon_{a0}^* - \epsilon_d - \lambda)^2 kT](\epsilon_a^* - \epsilon_{a0}^*) \quad (15)$$

where ϵ_{a0}^* is the acceptor orbital energy in the parent molecule and $\partial A/\partial \epsilon_a^*$ has been determined from eq 12 and 13. Equation 15 demonstrates that A is a linear function of ϵ_a^* if the proper conditions exist in the system. Furthermore, since κ is a positive quantity,[19] the relationship yields a negative slope as found in the regression analysis. This result is consistent with the expectation that a reduction in the energy of the acceptor orbital will stabilize the drug–receptor complex when charge-transfer interactions play an important role.

Chemistry. Two of the oxanilic acid derivatives investigated, namely, oxanilic acid (**1**)[20] and 2-cyanooxanilic acid (**3**),[21] have been reported previously. Those deriv-

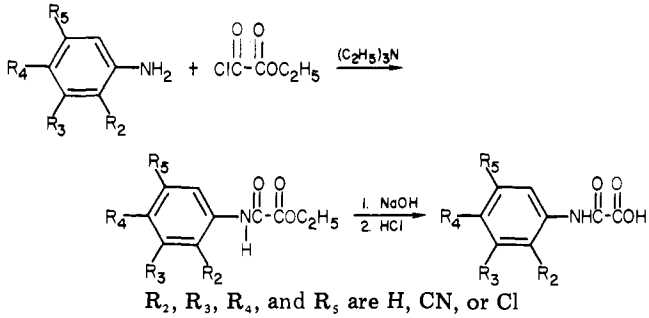
Table II. Derivatives of Oxanilic Acid



compd	R_2	R_3	R_4	R_5	R	procedure	yield, %	recrystn solvent[a]	mp, °C	mol formula	analyses
2	H	H	CN	H	H	B[b]	79	EtOH-H_2O	228 dec	$C_9H_6N_2O_3$	C, H, N
4	H	CN	H	H	H	B	71	EtOH	200 dec	$C_9H_6N_2O_3$	C, H, N
4a	H	CN	H	H	C_2H_5	A	32	EtOH	147-148	$C_{11}H_{10}N_2O_3$	C, H, N
5	H	CN	Cl	H	H	B	100		206 dec	$C_9H_5ClN_2O_3$	C, H, Cl, N
5a	H	CN	Cl	H	C_2H_5	A[c]	69	EtOH	184-185	$C_{11}H_9ClN_2O_3$	C, H, Cl; N[d]
6	Cl	H	H	CN	H	B	97	EtOH	193 dec	$C_9H_5ClN_2O_3$	C, H, Cl, N
6a	Cl	H	H	CN	C_2H_5	A	54	EtOH	82-83	$C_{11}H_9ClN_2O_3$	C, H, Cl, N
7	H	CN	H	CN	H	B	84	H_2O	233 dec	$C_{10}H_5N_3O_3$	C, H, N
7a	H	CN	H	CN	C_2H_5	A	80	EtOH	192-193	$C_{12}H_9N_3O_3$	C, H, N

[a] EtOH = ethanol. [b] Prepared from the methyl ester.[27] [c] DMF was omitted from the solvent mixture in the reaction. [d] N: calcd, 11.08; found, 11.69.

Scheme I



R_2, R_3, R_4, and R_5 are H, CN, or Cl

atives which have not been reported earlier are listed in Table II. Also presented in Table II are the previously unreported ethyl esters, which were intermediates in the preparation of the oxanilic acids. The general scheme of synthesis is outlined in Scheme I.

Biological Activities. Since a description of the rat PCA assay is given elsewhere,[4,8] it is sufficient to note that the ED_{50} computations are based on experiments in which the drug is administered iv at the time of antigen challenge as the salt of tris(hydroxymethyl)aminomethane (THAM). The procedure involves determination of the percent inhibition of the PCA reaction at three or more trial doses of drug. With these data, computation of the activity index, A_{obsd}, for a drug follows the method outlined in this section.

If eq 5 is transformed to give eq 16, a more tractable

$$\ln [E/(100 - E)] = m \ln D + \ln f^m K \qquad (16)$$

expression for analyzing the dose–response relationship is obtained. In theory, a plot of $\ln [E/(100 - E)]$ vs. $\ln D$ should produce a straight line with slope m and intercept $\ln f^m K$ providing E is greater than 0 and less than 100%. Both m and $\ln f^m K$ can be determined if there are two or more data points within the required range of E. Once the slope and intercept of the line have been found, the activity index may be computed using eq 17.

$$A_{obsd} = -\ln ED_{50} = (\ln f^m K)/m \qquad (17)$$

The expected error in the measurement of E is approximately 8% judging from the results of repeated experiments with a 19 μmol/kg dose of cromolyn sodium (I).[22] As a result, the slope of the least-squares line which best fits the observed data often deviates significantly from the ideal theoretical line. However, the calculated values of m for a series of drugs which act by the same mechanism should tend to cluster around the integer corresponding to the number of drug molecules associated with the receptor. The average slope ($\bar{m}$ = 1.1 ± 0.3) for nine compounds in the series under study is suggestive of a one-to-one complex between the drug and receptor. Therefore, in the remaining four cases (molecules **6**, **7**, **9**, and **10**), where only a single data point fell within the range $0 < E < 100\%$, the activity index was estimated by assuming $m = 1$.

The estimated error, η, in the activity index for a drug molecule is given by eq 18, where the various terms are

$$\eta = 100\Delta E[\sum_i [(p - 1)E_i^2(100 - E_i)^2]^{-1}]^{1/2} \qquad (18)$$

defined as follows: ΔE is the expected error in E for a single determination; p is the total number of usable dose–response data points for the compound of interest; and E_i is the observed inhibition for the ith trial dose of drug. A minimum error is expected when all of the doses tested yield inhibitions between 20 and 80%. According to eq 18, η becomes very large if E_i approaches 0 or 100%. However, a practical upper limit to the estimated error is established when the ED_{50} is bracketed by two doses, D_{100} and D_0, which respectively yield complete inhibition and no inhibition. Thus, η does not go to infinity as predicted by eq 18 but assumes a value given approximately by eq 19. The error estimates for molecules **6**, **7**, **9**, and **10** were

$$\eta = \tfrac{1}{2} \ln (D_{100}/D_0) \qquad (19)$$

obtained by means of eq 19.

SCF–MO Computations. The basis functions employed in the ab initio molecular fragment procedure are floating spherical Gaussian orbitals (FSGO) which have been described previously.[10,23] Molecular orbitals for the system are written as linear combinations of the FSGO functions using the relationship

$$\psi_i = \sum_{A=1}^{T} \sum_{r=1}^{NA} c_{ri}^A G_r^A \qquad (20)$$

where G_r^A is the rth FSGO in fragment A and c_{ri}^A is an expansion coefficient to be determined. The summation indices A and r, respectively, span all fragments of the molecule and all Gaussians within a fragment. Optimization of the molecular wave function is performed by a procedure similar to the standard LCAO–MO–SCF method.[24] Convergence of the SCF computations was judged by a maximum absolute difference of less than 10^{-4} in the elements of the charge-density and bond-order matrix, P, between successive iterations, where

$$P_{rs}^{AB} = 2\sum_{i=1}^{occ} c_{ri}^{A} c_{si}^{B} \quad (21)$$

The molecular geometry for the oxanilic acids was based on a moiety from the X-ray crystallographic structure[25] of *N,N'*-(*m*-phenylene)dioxamic acid. The 1,4-dihydro-4-oxoquinaldic acid and chromone-2-carboxylic acid geometries were modifications of the chromone moiety in the crystal structure of cromolyn sodium.[26] A table of bond lengths and angles employed in the calculations is available (see paragraph at end of paper regarding supplementary material).

Conclusion

In summarizing the results of this study, the following points may be made: (1) a statistically valid, empirical relationship has been established between biological activity in the PCA assay and an electronic index, the energy of a low-lying unoccupied molecular orbital; (2) a derivation is given to rationalize and interpret the form of the empirical relationship; and (3) charge-transfer stabilization of the drug–receptor complex is indicated as a major factor in determining the activities of the molecules. Of particular interest is the establishment of a quantitative structure–activity correlation involving three seemingly distinct classes of molecules which act as inhibitors of mediator release in the PCA assay. This finding illustrates the value of molecular orbital techniques for revealing electronic similarities among ostensibly different molecules.

Experimental Section

Melting points were taken in an oil bath and are uncorrected. The IR spectra were measured on a Perkin-Elmer 421 or Digilab FTS 140 spectrometer. The NMR spectra were measured on a Varian A-60 or a Varian T-60 spectrometer. The IR and NMR spectra were consistent with the assigned structure in all cases. The results of elemental analysis were within ±0.4% of the theoretical values except where noted.

Ethyl 3'-Cyanooxanilate (4a) (Procedure A). To a solution of 17.72 g (0.15 mol) of 3-aminobenzonitrile in 20 mL of Me_2SO and 100 mL of ethyl acetate was added 18.18 g (0.18 mol) of triethylamine. To the stirred mixture at 0 °C was added, dropwise, 24.6 g (0.18 mol) of ethyloxalyl chloride. The mixture was stirred at 0 °C for 1 h and then allowed to warm to room temperature. The precipitate was removed by filtration and washed with ethyl acetate. The combined filtrate and washes were evaporated to dryness, and the residue was poured into water. The solid was removed by filtration and recrystallized from ethanol.

3'-Cyanooxanilic Acid (4) (Procedure B). To a solution of 5.46 g (0.025 mol) of ethyl 3'-cyanooxanilate in 50 mL of methylene chloride in a separatory funnel was added 25 mL of 1 N NaOH solution, and the mixture was shaken for 10 min. To the mixture was then added 400 mL of water and the aqueous phase was separated and acidified with 3 N hydrochloric acid. The precipitate was removed by filtration and washed with water.

Sodium 6-Amino-4-oxo-4*H*-1-benzopyran-2-carboxylate Hemihydrate (12a). To 6.49 g (0.0276 mol) of 6-nitro-4*H*-1-benzopyran-2-carboxylic acid (13) was added 2.32 g (0.0276 mol) of sodium bicarbonate, 150 mL of water, and 500 mg of palladium-on-charcoal catalyst. The mixture was hydrogenated at 3 atm of hydrogen.

The catalyst was removed by filtration. The yellow filtrate was frozen and the solvent removed under high vacuum. The resulting yellow powder was titrated with ethanol. Anal. ($C_{10}H_6NNaO \cdot 0.5H_2O$) H, N, Na; C: calcd, 50.85; found, 50.37.

6-Nitro-4*H*-1-benzopyran-2-carboxylic Acid (13). To a stirred solution of 28.55 g (0.15 mol) of 4*H*-1-benzopyran-2-carboxylic acid in 50 mL of concentrated sulfuric acid was added over the course of 50 min a mixture of 12.5 mL of concentrated nitric acid and 12.5 mL of concentrated sulfuric acid.

The mixture turned to a dark brown and an exothermic reaction took place. The mixture was heated on the steam bath for 30 min, cooled, and poured into ice–water. The precipitate was removed by filtration and recrystallized from ethanol. There was obtained 17.3 g (49%) of material melting at 265 °C dec. An additional recrystallization raised the melting point to 267 °C dec (lit.[28] mp 268 °C dec). Anal. ($C_{10}H_5NO_6$) C, H, N.

Acknowledgment. We are grateful to Mr. Norman H. Knight and his co-workers of these laboratories for determining the elemental analyses and to Mr. Paul A. Meulman for measuring and interpreting the IR spectra. We express our gratitude also to Mr. Nelson Major for providing expert technical assistance.

Supplementary Material Available: Bond lengths and bond angles employed in the MO calculations (4 pages). Ordering information is given on any current masthead page.

References and Notes

(1) Supported in part by a grant from The Upjohn Co., Kalamazoo, Mich.
(2) J. S. G. Cox, J. E. Beach, A. M. J. N. Blair, A. J. Clarke, J. King, T. B. Lee, D. E. E. Loveday, G. F. Moss, T. S. C. Orr, J. T. Ritchie, and P. Sheard, *Adv. Drug Res.*, **5**, 115 (1970).
(3) (a) J. J. Pfister, R. W. Ferraresi, I. T. Harrison, W. H. Rooks, A. P. Roszkowski, A. VanHorn, and J. H. Fried, *J. Med. Chem.*, **15**, 1032 (1972); (b) D. R. Buckle, N. J. Morgan, J. W. Ross, H. Smith, and B. A. Spicer, *ibid.*, **16**, 1334 (1973).
(4) (a) C. M. Hall, H. G. Johnson, and J. B. Wright, *J. Med. Chem.*, **17**, 685 (1974); (b) J. B. Wright and H. G. Johnson, *ibid.*, **20**, 166 (1977).
(5) D. R. Buckle, B. C. C. Cantello, H. Smith, and B. A. Spicer, *J. Med. Chem.*, **18**, 391 (1975).
(6) J. Sellstedt, C. J. Guinosso, A. J. Begany, S. C. Bell, and M. Rosenthale, *J. Med. Chem.*, **18**, 926 (1975).
(7) J. R. Bantick, H. Cairns, A. Chambers, R. Hazard, J. King, T. B. Lee, and R. Minshull, *J. Med. Chem.*, **19**, 817 (1976).
(8) C. M. Hall, J. B. Wright, H. G. Johnson, and A. J. Taylor, *J. Med. Chem.*, **20**, 1337 (1977).
(9) P. J. Goodford, *Adv. Pharmacol.*, 11, 51 (1973).
(10) R. E. Christoffersen, D. Spangler, G. M. Maggiora, and G. G. Hall, *J. Am. Chem. Soc.*, **95**, 8526 (1973).
(11) A. J. Clark, *J. Physiol.*, **61**, 530 (1926).
(12) E. P. Benditt and D. Lagunoff, *Prog. Allergy*, **8**, 195 (1964).
(13) F. H. Johnson, H. Eyring, and M. J. Polissar, "The Kinetic Basis of Molecular Biology", Wiley, New York, N.Y., 1954, pp 88–97.
(14) C. Hansch, "Annual Reports of Medicinal Chemistry", C. K. Cain, Ed., Academic Press, New York, N.Y., 1966, p 347.
(15) A. Cammarata, "Molecular Orbital Studies in Chemical Pharmacology", L. E. Kier, Ed., Springer-Verlag, New York, N.Y., 1971, p 156.
(16) J. K. Seydel, "Drug Design", Vol. I, E. J. Ariens, Ed., Academic Press, New York, N.Y., 1971, p 343.
(17) R. S. Mulliken and W. B. Person, "Molecular Complexes", Wiley, New York, N.Y., 1969.
(18) R. S. Mulliken, *Recl. Trav. Chim. Pays-Bas*, **75**, 845 (1956).
(19) $\kappa = (H_{01} - S_{01}W_{el})^2$ where H_{01} and S_{01} are resonance and overlap integrals involving the wave functions ψ_0 and ψ_1 which respectively represent the neutral, MR, and dative, M^-R^+, configurations. W_{el} is the total electronic energy of the complex.
(20) O. Aschan, *Ber.*, **23**, 1820 (1890).
(21) A. Reissert and F. Grube, *Ber.*, **42**, 3710 (1909).
(22) In a total of 32 test runs, each using a dose of 19 μmol/kg of cromolyn sodium, the average inhibition was found to be 89% with a standard deviation of 8.3%.
(23) L. L. Shipman, R. E. Christoffersen, and B. V. Cheney, *J. Med. Chem.*, 17, 583 (1974).
(24) (a) C. C. J. Roothaan, *Rev. Mod. Phys.*, **23**, 69 (1951); (b) G. G. Hall, *Proc. R. Soc. London, Ser. A*, **205**, 541 (1951).
(25) D. J. Duchamp, The Upjohn Co., private communication.
(26) S. Hamodrakas, A. J. Geddes, and B. Sheldrick, *J. Pharm. Pharmacol.*, **26**, 54 (1974).
(27) M. T. Bogert and L. E. Wise, *J. Am. Chem. Soc.*, **32**, 1494 (1910).
(28) V. A. Zaqorevskiĭ, D. A. Zykov, and E. A. Orlova, *Zh. Obshch. Khim.*, **30**, 3894 (1960); *Chem. Abstr.*, **55**, 22301*h* (1961).