These studies reveal a potential problem of some magnitude in structure-activity correlations where many possible variables must be considered. This is particularly so in MO type correlations where many parameters can be calculated for a compound and frequently there is no valid reason to choose one over another. In these cases in order to adequately reduce the risk of chance correlations a large number of observations must be employed and these are not always available. Situations could easily arise in which the number of possible variables could not be adequately supported by the number of observations. Correlations obtained under these conditions would have greatly reduced significance. Some recently reported[7-9] correlations using MO parameters need to be reexamined in this context.

In Hansch type correlations the situation is less difficult since only a limited number of variables need be considered, representing possible hydrophobic, electronic, and steric effects. However, misleading correlations can still arise with an insufficient number of observations.

In Free and Wilson type correlations the phenomenon under discussion does not arise since each substituent is treated as a significant variable and therefore variables are not tested for possible inclusion using a multiple regression procedure.

The data presented allow an assessment to be made of the probable degree of chance correlation, when observations are examined for correlation with varying numbers of independent variables, as a function of the number of observations and the number of variables. Thus, for a given number of variables to be tested, the required number of observations to avoid undue risk of chance correlations can be estimated. For example, if $r^2 = 0.40$ is regarded as the maximum acceptable level of chance correlation then the minimum number of observations required to test five variables is about 30, for 10 variables 50 observations, for 20 variables 65 observations, and for 30 variables 85 observations.

Acknowledgment. The authors are indebted to Mr. M. Miller, Department of Computer and Scientific Information, Schering Corp., for the statistical studies.

References

(1) C. Hansch, *Annu. Rep. Med. Chem.*, **1966**, Chapter 34 (1967).
(2) C. Hansch, *ibid.*, **1967**, Chapter 33 (1968).
(3) W. P. Purcell and J. M. Clayton, *ibid.*, **1968**, Chapter 28 (1969).
(4) J. M. Clayton, O. E. Miller, Jr., and W. P. Purcell, *ibid.*, **1969**, Chapter 26 (1970).
(5) A. Cammarata, *ibid.*, **1970**, Chapter 24 (1971).
(6) P. N. Craig, C. Hansch, J. W. McFarland, Y. C. Martin, W. P. Purcell, and R. Zahradník, *J. Med. Chem.*, **14**, 447 (1971).
(7) A. Cammarata and R. L. Stein, *J. Med. Chem.*, **11**, 829 (1968).
(8) A. J. Wohl, *Mol. Pharmacol.*, **6**, 195 (1970).
(9) F. Peradejordi, A. N. Martin, and A. Cammarata, *J. Pharm. Sci.*, **60**, 576 (1971).

Structure–Activity Correlation for Substrates of Phenylethanolamine *N*-Methyltransferase (PNMT)

Ray W. Fuller* and Max M. Marsh

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206. *Received March 20, 1972*

Fujita and Ban[1] have reported a mathematical correlation of structure-activity relationships among PNMT substrates, using literature data.[2,3] The data they selected were measurements of substrate activity at a single substrate concentration; the concentration was very high, one at which inhibition by excess substrate occurs to different degrees among the various substrates.[4,5] Thus, the group contributions calculated by Fujita and Ban[1] probably relate mainly to *inhibitory* influences rather than to interactions favoring substrate activity. For instance, they showed a negative contribution of the 4-hydroxyl group. Such a negative contribution contrasts with the effect of the 4-hydroxyl group in the α-methylphenethylamine series that we recently reported as PNMT inhibitors.[6] There the 4-hydroxyl conferred an even greater affinity for PNMT than we were able to account for by σ and π values. We report here that correlation of structure with affinity of phenylethanolamines as PNMT substrates rather than with activity at a single excessively high concentration leads to conclusions different from those of Fujita and Ban.

We have calculated $-\log K_m$ values for phenylethanolamines as substrates for PNMT from rabbit adrenal.[5] The K_m values were calculated by the method of Wilkinson[7] from measurements of reaction velocity at 4–7 different substrate concentrations. All enzyme assays were done by a previously described method[8] in which the transfer of the labeled methyl group was measured after precipitation of *S*-[*methyl*-^{14}C]adenosylmethionine with Reinecke salt. The statistical evaluations were made by single and multiple linear regression analysis using Lilly Program S21, a modified and updated version of an original program submitted to the IBM 1620 user's group.

From the observed results with the six phenylethanolamines listed in Table I, with meta or para substituents on the ring, we derived an equation (eq 1) that fit the data at a level of significance $P = 0.01$. The square of the correlation coefficient was 0.909. The correlation was not improved

$$-\log K_m = \underset{(\pm 0.243)}{} 1.240\pi + \underset{(\pm 0.196)}{} 4.339 \quad (\pm 0.152) \qquad (1)$$

by adding a π^2 term. Standard errors of the terms are included in parentheses. As shown in the table, $-\log K_m$ values calculated from this equation agreed well with those derived from experimental observations. Hammett σ values for the substituents were not useful in the correlation. That these should not greatly influence the methylation reaction could be surmised from the fact that the pK_a's are not greatly influenced by aromatic substitution.

Two ortho-substituted phenylethanolamines, not included in the derivation of the equation, were estimated reasonably well by it. The calculated and observed $-\log K_m$ values were 5.06 and 4.80, respectively, for *o*-chlorophenylethanolamine and 4.39 and 4.10, respectively, for *o*-fluorophenylethanolamine.

Three hydroxy derivatives were, on the other hand, not

Table I. Observed and Calculated Activity of PNMT Substrates

Substrate	π^b	$-\log K_m$ value[a] Obsd	Calcd	Difference
Phenylethanolamine	0.0	4.10	4.34	+0.24
3-Fluorophenylethanolamine	0.19	4.60	4.57	−0.03
4-Fluorophenylethanolamine	0.14	4.80	4.51	−0.29
3-Bromophenylethanolamine	0.91	5.20	5.47	+0.27
4-Bromophenylethanolamine	0.90	5.60	5.46	−0.14
3,4-Dichlorophenylethanolamine	1.38	6.10	6.05	−0.05

[a] Units of K_m values were in molar substrate concentration. [b] π values from Fujita, *et al.*[10]

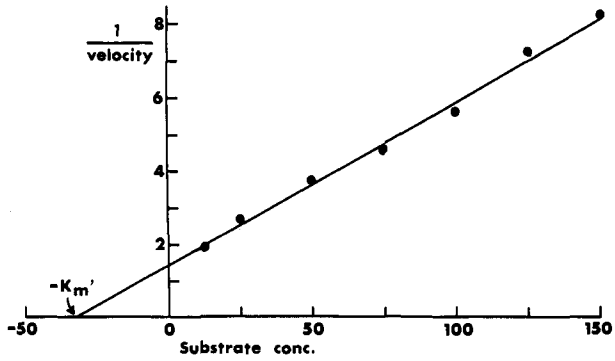

Figure 1. Substrate inhibition of PNMT by 3,4-dichlorophenylethanolamine. The reciprocal of velocity in pmoles/min is plotted *vs.* micromolar concentration of 3,4-dichlorophenylethanolamine. All concentrations were chosen to be in the range of inhibition by excess substrate.

Table II. Self-inhibition by PNMT Substrates at High Concentrations

Substrate	π^b	Concentration range studied, m*M*	$-\text{Log } K_m'$ value[a]
2-Chlorophenylethanolamine	0.59	0.5–8	1.72
2-Fluorophenylethanolamine	0.04	0.5–8	1.81
Phenylethanolamine		4–20	2.26
4-Fluorophenylethanolamine	0.14	0.2–3.2	2.38
3,4-Dihydroxyphenylethanolamine	−1.13	0.2–1.6	2.57
4-Hydroxyphenylethanolamine	−0.61	0.2–3.2	2.74
3-Bromophenylethanolamine	0.91	0.025–0.15	4.12
3,4-Dichlorophenylethanolamine	1.38	0.01–0.15	4.49
4-Bromophenylethanolamine	0.90	0.01–0.15	4.62

[a] K_m' value was determined graphically as shown in Figure 1. Units of K_m' values were in molar substrate concentration. [b] *Cf.* Table I.

consistent in their behavior. Whereas 3-methoxy-4-hydroxyphenylethanolamine (normetanephrine) had an observed $-\log K_m$ value (3.50) in good agreement with the calculated value (3.63), both 4-hydroxyphenylethanolamine (octopamine) and 3,4-dihydroxyphenylethanolamine (norepinephrine) were better substrates than the equation predicted. The $-\log K_m$ values observed for octopamine and norepinephrine were 5.00 and 4.90, respectively, whereas the calculated values were only 3.58 and 2.95, respectively. These compounds resembled the amphetamines[6] in that the 4-hydroxyl contributed to binding with PNMT in a manner over and above its effect on lipophilicity.

The negative contribution calculated by Fujita and Ban for the 4-hydroxyl group arose largely because norepinephrine had low activity according to the data they used, *e.g.*, much lower than phenylethanolamine itself. Paradoxically, the reason for the low relative activity of norepinephrine in those conditions is because its affinity for the enzyme is so great; the high substrate concentration was further above the optimum concentration and hence inhibition by excess substrate was greater with norepinephrine than with any of the other substrates in their list.

Figure 1 illustrates the inhibition that occurs at excess substrate levels in the case of 3,4-dichlorophenylethanolamine. The plot of the velocity reciprocal *vs.* substrate concentration permits calculation of a K_m' value.[9] The K_m' is a measure of the inhibition by excess substrate just as K_m is a measure of the substrate activity. Other phenylethanolamines were studied, and the data were plotted as in Figure 1. The values were fitted to a straight line by linear regression analysis, and the K_m' value was determined as the x intercept. Table II lists the calculated $-\log K_m'$ values for nine PNMT substrates. The $-\log K_m'$ values roughly paralleled the K_m values. Excluding the compounds in Table II with ortho or with hydroxyl substituents, we correlated the $-\log K_m'$ values for the remaining five compounds according to eq 2. The square of the correlation coefficient was

$$-\log K_m' = -1.484\pi^2 + 3.813\pi + 2.094 \quad (2)$$
$$(\pm 0.323)\quad (\pm 0.765)\quad (\pm 1.031)\quad (\pm 0.262)$$

0.96. Note that in this case the fit was improved by adding the π^2 term. On the basis of these limited data, it appears that the self-inhibition requirements are about the same as the substrate requirements. An optimum π value for self-inhibition, based on the derivatives of eq 2, is about 0.84. 4-Hydroxyl compounds again have higher $-\log K_m'$ values than would be predicted. That observation is consistent with our finding with amphetamines as inhibitors.[6]

References

(1) T. Fujita and T. Ban, *J. Med. Chem.*, **14**, 148 (1971).
(2) J. Axelrod, *J. Biol. Chem.*, **237**, 1657 (1962).
(3) J. Axelrod, *Pharmacol. Rev.*, **18**, 95 (1966).
(4) R. W. Fuller and J. M. Hunt, *Biochem. Pharmacol.*, **14**, 1896 (1965).
(5) R. W. Fuller, B. J. Warren, and B. B. Molloy, *Biochim. Biophys. Acta*, **222**, 210 (1970).
(6) R. W. Fuller, J. Mills, and M. M. Marsh, *J. Med. Chem.*, 14, 322 (1971).
(7) G. N. Wilkinson, *Biochem. J.*, **80**, 324 (1961).
(8) R. W. Fuller and J. M. Hunt, *Anal. Biochem.*, **16**, 349 (1966).
(9) M. Dixon and E. C. Webb, "Enzymes," Academic Press, New York, N. Y., 1958.
(10) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964).

Are Calculated Electron Populations Suitable Parameters for Multiple Regression Analyses of Biological Activity?

P. R. Andrews

Department of Physical Biochemistry, John Curtin School of Medical Research, The Australian National University, Canberra, Australian Capital Territory 2601, Australia.

Received March 27, 1972

A major objective of medicinal chemistry is identification of the salient structural properties of biologically active molecules. Hansch in particular[1] has sought quantitative correlations between activity and molecular properties by applying linear multiple regression analysis to combinations of substituent constants in free energy relationships similar to the Hammett equation. He has commonly employed hydrophobic, steric, and electronic constants, but has suggested[2] replacing the latter with the electron populations on atoms chosen during regression analysis of data from molecular orbital calculations.

The calculation of atomic electron populations by suitably partitioning molecular wave functions is familiar for π-electron systems, and many other structures have become accessible to quantum chemistry through the introduction of semiempirical all-valence-electron techniques such as extended Hückel theory (EHT)[3] and the complete neglect of