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Addendum

Multiple Regression Analyses of the Relationship between Structure and Inhibitory Effects of a Series of Substituted O-Phenyl-DL-homoserines on Adenosine Triphosphate:L-Methionine S-Adenosyltransferase

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SUMMARY

HULBERT, PETER B.: Multiple regression analyses of the relationship between structure and inhibitory effects of a series of substituted *O*-phenyl-DL-homoserines on adenosine triphosphate:L-methionine *S*-adenosyltransferase. *Mol. Pharmacol.* **10,** 315-318 (1974).

The correlation among the inhibitory potencies for rat liver ATP:L-methionine *S*-adenosyltransferase of a series of 11 substituted *O*-phenyl-DL-homoserines and various Hammett σ and Hansch π coefficients has been examined. Regression analyses by the method of least squares gave the best correlation by the use of a linear combination of ordinary σ values and π values for phenoxyacetic acid derivatives. The analysis gave the linear free energy relationship $pI_{50} = 0.619\pi + 0.614\sigma + 1.62$ for $n = 11$. The correlation coefficient of $r = 0.989$ indicated that an almost complete correspondence of structural properties with potency was achieved

In the accompanying paper, Coulter, Lombardini, and Talalay (1) have shown that *O*-phenyl-DL-homoserine and analogues bearing substituents on the aromatic ring are inhibitors of the ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6) of rat liver. The inhibitory potencies of these compounds were correlated with the positive magnitudes of the Hammett σ values, suggesting that electron-withdrawing substituents on the phenyl ring exert favorable influences on the inhibitory properties.

My colleagues (1) carried out a least-squares linear regression analysis of the correlation between Hammett σ values and pI_{50} values [$\log_{10}(1/I_{50})$, where I_{50} is the molar concentration of inhibitor required to achieve 50% inhibition under standard conditions]. Among the 11 compounds analyzed, the *m*- and *p*-nitrophenyl derivatives appeared to behave anomalously, but the remaining nine compounds gave a linear regression line expressed by the equation $pI_{50} = 1.45\sigma + 1.82$, with a correlation coefficient $r = 0.883$ ($r^2 = 0.780$). I report here various efforts to obtain linear regression analyses which reconcile the values for the nitro derivatives, give better correlations for all the measurements, and yield an almost complete correspondence of struc-

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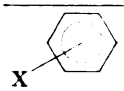
TABLE 1

Inhibitory potencies for ATP:L-methionine S-adenosyltransferase of rat liver: Hammett σ and Hansch π values for a series of substituted O-phenyl-DL-homoserines

 X =	$OCH_2CH_2CH(NH_2)CO_2H$	σ	π	Concentration required for 50% inhibition[a]	pI_{50}	ΔpI_{50}[b]
				M		
H		0	0	0.023	1.64	0.02
p-F		0.062	0.15	0.022	1.66	−0.09
p-Cl		0.227	0.70	0.0061	2.21	0.02
p-Br		0.232	1.02	0.0044	2.36	−0.03
p-NO_2		0.778	0.24	0.0051	2.29	−0.05
p-OCH_3		−0.268	−0.04	0.036	1.44	0.02
p-CH_3		−0.170	0.52	0.015	1.82	−0.01
m-Cl		0.373	0.76	0.0052	2.28	−0.03
m-Br		0.391	0.94	0.0032	2.49	0.06
m-NO_2		0.710	0.11	0.0087	2.06	−0.06
m-OCH_3		0.115	0.12	0.0146	1.84	0.07

[a] The I_{50} values are those of Coulter *et al.* (1).

[b] ΔpI_{50} = pI_{50} (experimental) − pI_{50} (calculated).

tural properties with the inhibitory potency of these compounds.

The molar concentrations required for 50% inhibition of enzyme activity for the 11 racemic compounds in this series are shown in Table 1. The corresponding pI_{50} values are also given. The σ values for substituents were obtained from Hammett (2), and the Hansch lipophilicity values (π), from Fujita, Iwasa, and Hansch (3). The π values in Table 1 refer to the analogues of phenoxyacetic acid, which represent a system comparable to the *O*-phenyl-DL-homoserines. Other values for Hammett functions (σ^+, σ^-, σ^n) were also used (2), but are not recorded.

If the electronic characteristics of all 11 compounds are subjected to linear regression analysis by the method of least squares for the correlation between pI_{50} and Hammett σ, the best fit corresponds to the equation

$$pI_{50} = 0.756\sigma + 1.84$$

where $n = 11$, $r = 0.717$, and $r^2 = 0.514$. The correlation coefficient, r, is significant at the 95% confidence level but not at the 99% level. The value of r^2 is a measure of the degree to which the variance of pI_{50} can be ascribed to the regression constants. In this case it is 51%. It will be seen that inclusion of the two nitro derivatives causes the value of the correlation coefficient to drop from $r = 0.883$ to $r = 0.717$.

When similar analyses were carried out with other Hammett functions, the following correlation coefficients were obtained:

pI_{50} and σ^+: $r = 0.730$
pI_{50} and σ^-: $r = 0.644$
pI_{50} and σ^n: $r = 0.702$

Consequently no substantial improvement over the ordinary σ values was obtained by utilizing the specialized Hammett function values.

Since the binding of many drugs to proteins is related to their lipophilicity (4), the possible dependence of inhibitory potency on lipophilicity was examined by the use of Hansch π values. A linear regression analysis of the 11 compounds gave the relationship

$$pI_{50} = 0.720\,\pi + 1.71$$

where $n = 11$, $r = 0.808$, and $r^2 = 0.652$. The correlation coefficient is significant above the 99% confidence level, and 65% of the variance in pI_{50} can be attributed to the regression. Inhibitory potency rises with increasing π values. The use of π values obtained for different series of compounds leads to either better or worse correlations. Thus calculations based on π values for

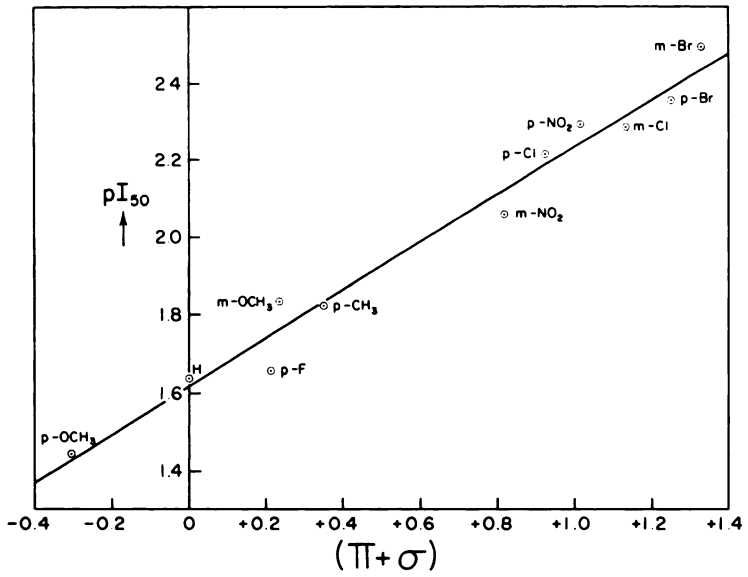


FIG. 1. *Analysis of inhibitory potencies of substituted O-phenyl-DL-homoserines in terms of electronic and lipophilic contributions of substituent groups*

The inhibition index (pI_{50}) is plotted as a function of the algebraic sum of Hammett σ and Hansch π values for a series of 11 *O*-phenyl-DL-homoserines substituted in the *meta* and *para* positions of the phenyl ring by the groups indicated. The linear regression analysis by the method of least squares gave the equation $pI_{50} = 0.617(\pi + \sigma) + 1.62$. The correlation coefficient, r, is 0.989.

substituents on the parent phenols give $r = 0.914$ ($r^2 = 0.836$), and π values for substituents on the parent benzenes give $r = 0.529$. It will be shown below that the π values for the phenoxyacetic acids are adequate to provide excellent correlations.

These findings show that inhibitory activity among the compounds under consideration depends on both the electronic character of the substituents and their lipophilic contribution. In particular, it becomes apparent that the nitrophenyl derivatives are poorer inhibitors than might be expected on the basis of their electronic character, but stronger inhibitors than expected from their lipophilicity.

Using both Hammett σ and Hansch π constants together, multiple linear regression analysis by the method of least squares was performed. It gave the linear free energy relationship

$$\begin{array}{lccc} pI_{50} = & 0.619\pi + & 0.614\sigma + & 1.62 \\ & (\pm 0.108) & (\pm 0.128) & (\pm 0.06) \end{array}$$

where $n = 11$, $r = 0.989$, $r^2 = 0.979$, and $s = 0.0565$. In this case r is the multiple correlation coefficient for the dependence pI_{50} on both π and σ. It is highly significant, since for 99% confidence limits with 8 degrees of freedom (11 points, three parameters) r need only be 0.765. Again, 98% of the variance is attributable to the constants chosen for the regression. s is the standard error of the equation, and 95% confidence limits are shown.

No improvement in the multiple correlation coefficient was obtained by the use of other values for π or σ. A number of such combinations were computed: pI_{50} with respect to π and σ^+ gave $r = 0.984$; π and σ^- gave $r = 0.986$; π and σ^n gave $r = 0.972$; π and σ, using π values for benzenes, gave $r = 0.979$; π and σ, using π values for phenols, gave $r = 0.970$. Thus the best correlation exists between the pI_{50} values and a linear combination of the ordinary Hammett values and the Hansch π values for substituted phenoxyacetic acids. The finding that the coefficients for π and σ in the multiple regression analysis equation are closely similar indicates that the inhibition index (pI_{50}) in-increases by a similar amount for an in-

cremental unit of π as for σ. No further physical significance can be attributed to this similarity at present. The value of the coefficient for π (0.62) lies in the range (0.60 $\pm$ 0.13) given for binding of other small molecules to proteins (4). The difference (ΔpI_{50}) between the experimentally determined pI_{50} values and those calculated from the above equation is shown in Table 1. All compounds obey the multiple regression equation well.

For the purposes of two-dimensional illustration only, the following equation was obtained:

$$pI_{50} = 0.617(\pi + \sigma) + 1.62$$

This is shown in Fig. 1, together with the experimental values. The nitrophenyl compounds fall close to the calculated regression line.

The ability to obtain highly satisfactory correlations between inhibition indices and a linear combination of π and σ values stresses the probable importance of both electronic and hydrophobic factors in the binding process. Furthermore, as such a highly satisfactory correlation was obtained without the introduction of steric parameters, it is unlikely that there is much steric hindrance to the binding of these compounds to the enzyme. Finally, the correlation indicates that an almost complete correspondence of structural properties with inhibitory potency has been achieved in this series of substituted *O*-phenyl-DL-homoserines.

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