Pergamon

Tetrahedron Letters, Vol. 37, No. 2, pp. 193-196, 1996

0040-4039/96 $15.00 + 0.00

0040-4039(95)02126-4

Linear Free Energy Relationships of the Inhibition of Pancreatic Cholesterol Esterase by 4-Nitrophenyl-*N*-Alkylcarbamate

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Abstract: 4-Nitrophenyl-*N*-alkylcarbamates (1) as active site-directed irreversible inhibitors of pancreatic cholesterol esterase are investigated for values of the dissociation constant (K_i), the carbamylation constant (k_2), and the bimolecular rate constant (k_i). Linear free energy relationships between $-\log K_i$, $\log k_2$, or $\log k_i$ and polar substituent constant (σ^*) are observed. Taft's E_S steric constants are not important in these correlationships. Multiple linear relationships of $-\log K_i$, $\log k_2$, or $\log k_i$ are observed on Charton equation ($\log k = \alpha\sigma_I + \beta\sigma_R + \psi\nu + h$).

Recently there has been increased interest in pancreatic cholesterol esterase (CEase) due to an observed correlation between enzymatic activity *in vivo* and absorption of dietary cholesterol.[1,2] An investigation into the mechanism of this enzyme may lead to the design of mechanism-based inhibitors which could be of future therapeutic use. As part of a program we directed to the design of such inhibitors, we therefore investigated the correlation of the structure of inhibitor with CEase inhibition.

4-Nitrophenyl-*N*-alkylcarbamates are potent active site-directed irreversible inhibitors of CEase.[3] The mechanism for inhibition in the presence of substrate is shown in Scheme 1.

$$E + S \underset{}{\overset{K_m}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

$$E + I \overset{K_i}{\rightleftharpoons} E\text{-}I \xrightarrow{k_2} E\text{-}I' \xrightarrow{k_3} E + Q$$

Scheme I. Kinetic scheme for inactivation of CEase in the presence of substrate

Therefore, values of K_I and k_2 can be calculated from Equation 1:[3,4]

$$k_{app} = \frac{k_2 [I]}{K_i(1+\frac{[S]}{K_m}) + [I]} \qquad (1)$$

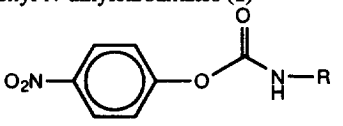
In Equation 1, k_{app} values are the first-order rate constants which can be obtained according to Hosie's method.[3] Bimolecular rate constant, $k_i = k_2/K_i$, is related to overall inhibitory potency.

For enzyme inhibition, less attention had been paid on the correlation with the structure of inhibitors.[5,6] We first reported there existed Hammett linear free energy relationships on the inhibition of CEase by substituted phenyl-*N*-butylcarbamate.[7] In this communication, we further drew attention to correlations of CEase inhibition with Taft-Ingold Equation[8-11] (2) or Charton Equation[12-14] (3):

$$\log (\frac{k}{k_0}) = \rho^*\sigma^* + \delta E_S \qquad (2)$$

$$\log k = \alpha\sigma_I + \beta\sigma_R + \psi\nu + h \qquad (3)$$

Table 1. Substituent Constants and Kinetic Data for the CEase-Catalyzed Hydrolysis of 4-Nitrophenylbutyrate in the Presence of 4-Nitrophenyl-*N*-alkylcarbamates (**1**)[a,b]



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R=	σ^*[c]	E_s[c]	σ_I[c]	σ_R[d]	ν[e]	$K_i/\mu M$[f]	k_2/min^{-1}	$k_i/M^{-1}min^{-1}$
Et	-0.1	-0.07	-0.054	-0.14	0.56	3.1	0.00385	1,240
n-Pr	-0.12	-0.36	-0.059	-0.13	0.68	2.9	0.00374	1,290
n-Bu	-0.13	-0.39	-0.01	-0.13	0.68	2.6	0.00378	1,450
$(CH_2)_5CH_3$	-0.15[g]	-0.40[h]	-0.07	-0.13	0.73	3.2	0.00300	938
$(CH_2)_7CH_3$	-0.13[g]	-0.33[i]	-0.07	-0.13	0.68	3.6	0.00375	1,040
$(CH_2)_2Cl$	0.39	-0.43[i]	0.05	-0.01	0.68	5.8	0.0121	208
CH_2Ph	0.22	-0.38	0.02	-0.08	0.70	4.4	0.00881	2,000
Allyl	0.10	-0.39	0.0007	-0.12	0.64	3.8	0.00600	1,580

a. General procedures: The CEase-catalyzed hydrolysis of 4-nitrophenylbutyrate was followed continuously at 410 nm in the presence and absence of inhibitor on a UV-visible spectrometer (HP 8452) that was interfaced to a microcomputer. Kaleida Graph™ (version 2.0) was used for all least-squares curve fittings. Bovine pancreatic CEase was purchased from Sigma. All the other procedures were the same as described by Hosie et al.[3] b. All inhibitors were prepared from the condensation of 4-nitrophenol with alkyl isocyanate in the presence of pyridine in dichloromethane at 25°C (80-95% yield). c. Obtained from references 8-11 d. $\sigma_R = \sigma_p - \sigma_I$ e. Obtained from references 9,12 f. These values are in excellent agreement with those determined in the absence of substrate (zero time).[4] g. Calculated from ν values assuming σ^* values are propotional to ν values[13] h. Taken as that of n-pentyl group i. Taken or calculated from reference 15

Table 1 summarized the polar substituent constant σ^*[8-11], Taft's E_s [9-11], Charton's σ_I, σ_R, and ν steric constants[12-14], and the values of K_i, k_2, and k_i for the CEase-catalyzed hydrolysis of 4-nitrophenylbutyrate in the presence of 4-nitrophenyl-*N*-alkylcarbamate inhibitors. All of $-\log K_I$, $\log k_2$, and $\log k_i$ values had linear relationships with σ^* values (Figure 1, Table 2) and multiple linear relationships with σ^*-E_s or σ_I-σ_R-ν values (Table 2). The formation constant of the covalent E-I tetrahedral intermediate (Scheme 1) was $1/K_I$ and the ρ^* value for this reaction was -0.50. This suggested that this step was insensitive to electronic perturbation. However, all the other serine hydrolases had positive ρ value for this step.[5-7,16,17] The mechanism of formation of the covalent E-S intermediate of a serine hydrolase was divided into two steps (the formation of an ES complex and the formation of a covalent E-S tetrahedral intermediate).[18] According to this, a two step mechanism of the formation of E-I tetrahedral intermediate was proposed (Fig. 2). The first step (K_{1C}) was the formation of the EI complex positioned the inhibitor with the correct conformation for reaction. In this step, the Asp-His-Ser charge relay network made the serine oxygen became partially negative. The serine oxygen then polarized the carbamate carbon and induced a partially positive charge on the carbamate carbon. Therefore, the K_{1C} step had a negative ρ value and was insensitive to electronic perturbation. The second step (K_{1I}) was the formation of a covalent E-I tetrahedral intermediate and had a positive ρ value. The E-I intermediate from substituted phenyl-*N*-butylcarbamate was very sensitive to electronic perturbation in this step

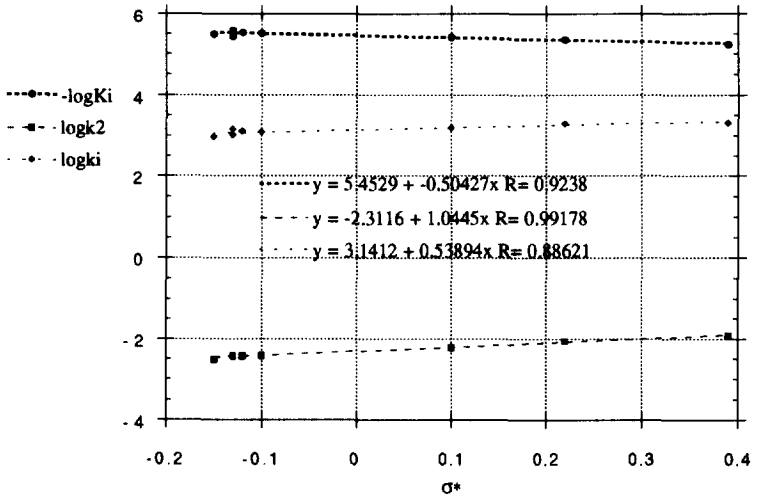

Figure 1. Taft-Ingold plots of $-\log K_i$, $\log k_2$, and $\log k_i$ against σ*

Table 2. Simple and Multiple Linear Regression Analyses of the inhibition of Pancreatic Cholesterol Esterase by 4-Nitrophenyl-*N*-alkylcarbamates[a]

	SLR[b] ρ*	SLR R[e]	MLR-2[c] ρ*	MLR-2 δ	MLR-2 R	MLR-3[d] α	MLR-3 β	MLR-3 ψ	MLR-3 R
$-\log K_I$	-0.50	0.92	-0.51	-0.01	0.92	0.36	-2.66	-0.21	0.91
$\log k_2$	1.0	0.99	1.05	0.04	0.99	2.28	2.62	-0.45	0.97
$\log k_i$	0.54	0.89	0.55	0.03	0.89	2.65	-0.04	-0.24	0.96

a. StatWork™ (version 1.2) was used for multiple regression analysis. b. Single linear regression on log $(k/k_0) = \sigma^*\rho^*$ c. Multiple linear regression on Equation 2 d. Multiple linear regression on Equation 3 e. Correlation coefficient

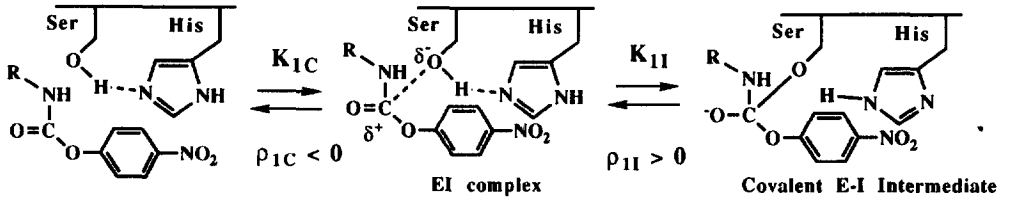

Figure 2. The Proposed Mechanism for the Formation of E-I Tetrahedral Intermediate

but that from 4-nitrophenyl-*N*-alkylcarbamate was not because alkyl group was one nitrogen away from the reaction center (the carbamate carbon). Because the Taft-Ingold model had not produced a complete separation of inductive and steric effects,[9-11] the ρ* values we obtained might have some steric contribution and made

them slightly negative. The Charton model successfully separated these two effect and the α value for the inductive effect was +0.36. The carbamylation step (k_2) was also proposed to be divided into two steps:[7] the pre-equilibrium step (ρ_{2pe} positive) and the dissociation step (ρ_{2d} negative). In this step, all ρ and α values obtained were positive. This meant that the pre-equilibrium step is more rate limiting than the other because the latter step did not involve a charge separation. The steric contribution for all correlationships we obtained was not important.

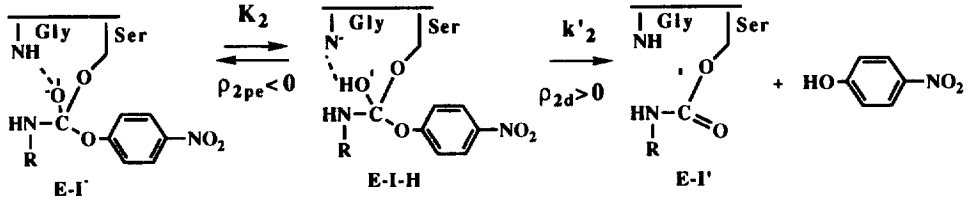

Figure 3. The Proposed Mechanism for the Carbamylation (k_2) step

Further investigations of linear free energy relationships on the inhibition of acetylcholinesterase will communicated in due course.

Acknowledgement: We thank the National Science Council of Republic of China for financial support.

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(*Received in Japan* 4 *August* 1995; *revised* 6 *November* 1995; *accepted* 10 *November* 1995)