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ATROPISOMERIC CARBAMOYL TYPE INHIBITORS OF PANCREATIC CHOLESTEROL ESTERASE

Gialih Lin,* Hsiao-Chien Liu, and Yi-Chin Tsai

Department of Chemistry, National Chung-Hsing University, Taichung 402, Taiwan

Abstract: Atropisomers of 1,1'-bi-2-naphthyl-2,2'-di-*N*-butylcarbamate (**1**), 1,1'-bi-2-naphthyl-2-*N*-butylcarbamate-2'-ol (**2**), and 1,1'-bi-2-naphthyl-2-*N*-butylcarbamate-2'-butyrate (**3**) 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).

Recently there has been increased interest in pancreatic cholesterol esterase (CEase) due to 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 an investigation of enantiomeric inhibitors as serine protease inhibitors, atropisomeric carbamoyl type of inhibitors *R*-**1**,*S*-**1**, *R*-**2**,*S*-**2**, and *R*-**3**,*S*-**3** are prepared and evaluated for their effects on CEase.

Like 4-nitrophenyl-*N*-alkyl carbamates,[3,4] all the inhibitors listed in Table 1 are characterized as active site-directed irreversible inhibitors of CEase and meet some of the criteria proposed by Abeles and Maycock.[5] First, the inhibition is time-dependent and follows first-order kinetics; second, with increasing concentration of inhibitor the enzyme displays saturation kinetics; third, the enzyme can be protected by carbamates **1-3** in the presence of a competitive inhibitor such as phenylboronic acid. The mechanism for active site-directed irreversible inhibition in the presence of a substrate is shown in Scheme.

Scheme. Kinetic scheme for active site-directed irreversible inhibition of CEase in the presence of a substrate

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

$$E + I \overset{K_I}{\rightleftharpoons} EI \xrightarrow{k_2} EI' \xrightarrow{k_3} E + Q$$

Because the inhibition of CEase follows first-order kinetics over the observed time period, the rate of hydrolysis of EI' must be significantly slower than the rate of formation of EI'($k_2 >> k_3$).[6] Therefore, values of K_I and k_2 can be calculated from Equation 1:[3,7]

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

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.

Table. Kinetic Data for the CEase-Catalyzed Hydrolysis of 4-Nitrophenyl Butyrate in the Presence of Carbamates 1-3.[a]

Inhibitors[b]	$K_I/\mu M$[c]	$k_2/10^{-3}sec^{-1}$	$k_i/10^2M^{-1}sec^{-1}$	$k_i(S)/k_i(R)$
rac-**1**	29±7	1.2±0.1	0.4±0.1	-
R-**1**	30±1	0.95±0.02	0.32±0.01	-
S-**1**	2±1	1.6±0.9	8±6	30±20
rac-**2**	0.8±0.8	7±5	100±100	-
R-**2**	0.8±0.3	10±2	120±50	-
S-**2**	1.3±0.6	6±1	50±20	0.4±0.2
rac-**3**	10±5	0.6±0.3	0.6±0.4	-
R-**3**	20±4	0.8±0.4	0.4±0.1	-
S-**3**	9±3	0.6±0.2	0.8±0.4	2±1

a. General procedures: The CEase-catalyzed hydrolysis of 4-nitrophenyl butyrate 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 compounds were prepared from *rac*-, *R*-, or *S*-1,1'-bi-2-naphthyl-2,2'-diol (Aldrich) by general procedures. c. These values are in excellent agreement with those determined in the absence of substrate (zero time method)[7] and by initial velocities.[8]

The low K_I value observed with *S*-**1** compared with that of *R*-**1** may be due to 2:1 molecularity in binding. Thus, a 4-fold decrease in K_I is expected from *rac*-**1** to *S*-**1** because half of *rac*-**1** is *R*-**1** which "in-inhibits" the enzyme and increases K_I value 2-fold, and the other half of *rac*-**1** is *S*-**1** which also increases K_I value 2-fold due to the concentration. Similarly, a 4-fold decrease is expected from *R*-**1** to *rac*-**1**. This expectation is primarily come from the fact that the rate of phospholipase A2-catalyzed hydrolysis of a DL-phosphatidylcholine is about one quarter of that of hydrolysis of a L-phosphatidylcholine because the D-phosphatidylcholine is a competitive inhibitor of that enzyme.[9,10] Therefore, about a 16-fold increase in K_I value is observed from *S*-**1** to *R*-**1**. The k_2 value increases 1.7-fold from *R*-**1** to *S*-**1**. Therefore, a 25-fold increase in k_i is observed from *R*-**1** to *S*-**1** ($k_i(S)/k_i(R)$ = 30±20). The stereochemical preference (*S*>*R*) for carbamates **1** and **3** is the same as that of CEase-catalyzed hydrolysis of the substrate 1,1'-bi-2-naphthyl-2,2'-dibutyrate;[11-13] however, the stereochemical preference for carbamates **2** is different. There are two possible explanations why *S*-**1** is better inhibitor than *R*-**1**. First, one of *N*-butylcarbamate group of *R*-**1** is too close to the reaction center and prohibits the entry of the enzyme; second, the hydrophobic binding in *S*-**1** is better than that in *R*-**1** (Figure). The steric hindrance decreases from *R*-**1** to *R*-**3**, but the hydrophobic binding increases from *S*-**1** to *S*-**3**.

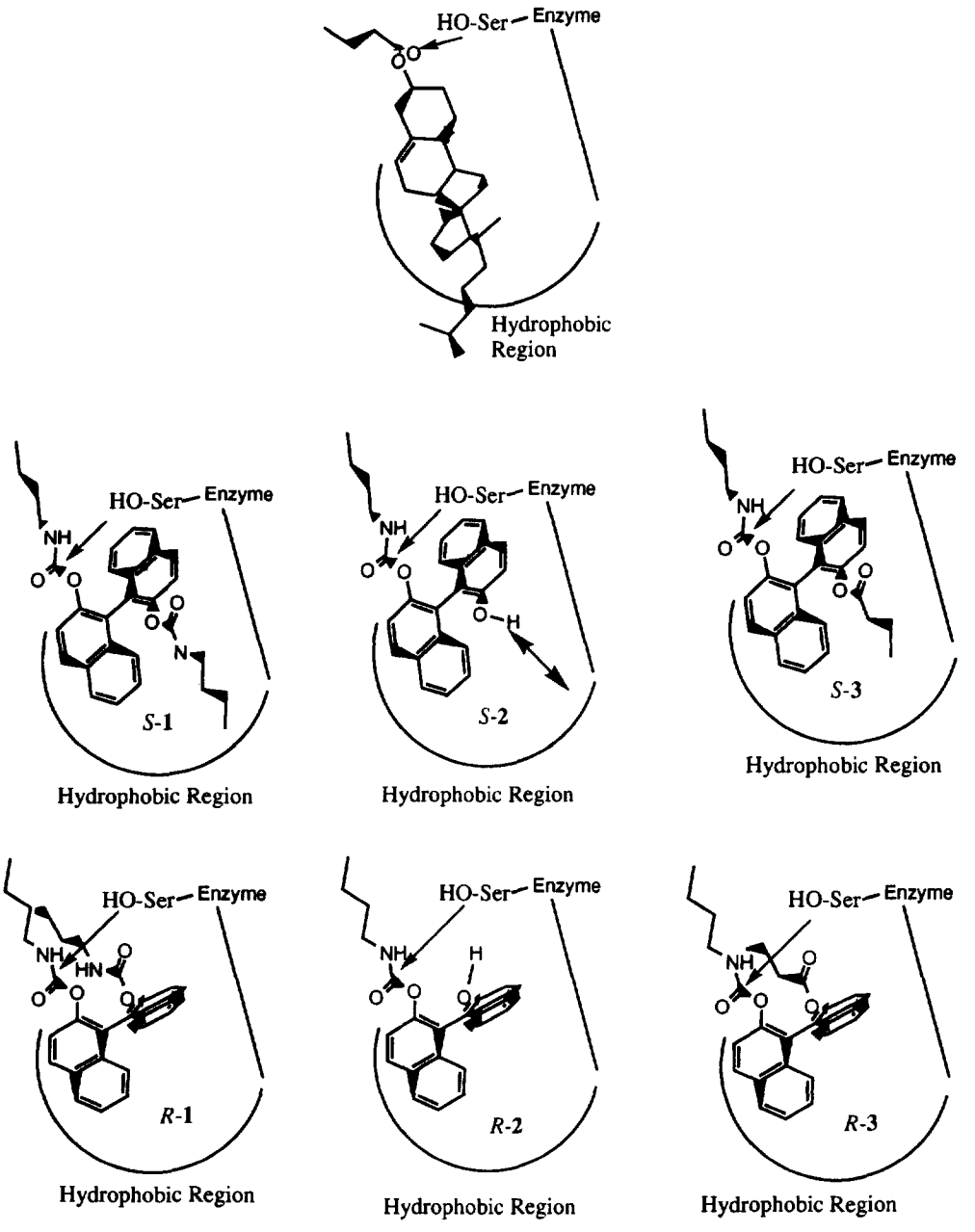

Figure. Interactions between CEase and cholesterol ester and CEase and inhibitors: Structures of cholesteryl butyrate and inhibitors **1-3** shown are obtained from MM-2 energy-minimization by CSC Chem 3D Plus™ (version 3.1.2) and are lopsided in the same direction as proposed by Kazlauskas.[11]

Therefore, the $k_i(S)/k_i(R)$ value of carbamate **1** is higher than that of carbamate **3**. On the other hand, the hydrophobic interaction in *S*-**2** is not favorable because of the hydroxyl group in that region. In the event, *R*-**2** is the most potent irreversible inhibitor of all inhibitors we examine. Besides the hydrophobic binding, another explanation for this is that the acidity of the hydroxyl group of *R*-**2**, on the same site of reaction center, is relatively high and facilitates the inhibitor to contact with the basic residue (likely His) of CEase by H-bonding. Differences in k_i values between *R*- and *S*-atropisomers of carbamates **2** and **3** are small but between those of carbamate **1** are large. From our previous result,[12,13] the CEase-catalyzed hydrolysis of 1,1'-bi-2-naphthyl-2-butyrate-2'-ol (early transition state) is much more reactive than that of 1,1'-bi-2-naphthyl-2,2'-dibutyrate (late transition state). Similarly, the $k_i(S)/k_i(R)$ value for carbamate **1** (late transition state) is greater than that of carbamate **2** (early transition state). This can be explained by Hammond postulate.[14]

Further investigations for other enantiomeric inhibitors on CEase, lipases, and acetylcholinesterase will be communicated in due course.

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