Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 4, pp. 315-318, 1995
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0960-894X/95 $9.50+0.00

0960-894X(95)00027-5

STRUCTURAL AND STEREOCHEMICAL REQUIREMENTS OF TIME-DEPENDENT INACTIVATORS OF THE INTERLEUKIN-1β CONVERTING ENZYME

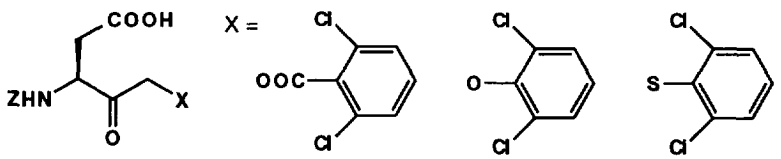
C.V.C. Prasad,*, † Catherine P. Prouty,† Denton Hoyer,† Tina M. Ross,† Joseph M. Salvino,† Mohamad Awad,† Todd L. Graybill,† Stanley J. Schmidt,† I. Kelly Osifo,† Roland E. Dolle,† Carla T. Helaszek,‡ Robert E. Miller,‡ and Mark A. Ator‡

Departments of † Medicinal Chemistry and ‡ Biochemistry
Sterling Winthrop Pharmaceuticals Research Division,
1250 South Collegeville Road, P. O. Box 5000, Collegeville, PA 19426

Abstract: Structural and stereochemical requirements of substrate based time-dependent inactivators of interleukin-1β converting enzyme were investigated. Hydrophobic amino acids with L-stereochemistry are preferred at the P_2 and P_3 positions. It appears that both D- and L-Asp are accepted by the enzyme at the P_1 position.

Interleukin-1β (IL-1β) is an important mediator of the biochemical cascade leading to inflammation.[1] Recent studies from Immunex and Merck have shown that the heterodimeric cysteine protease, interleukin-1β converting enzyme (ICE), is responsible for the endoproteolytic processing of a biologically inactive 31 kDa precursor IL-1β protein (pIL-1β) to the 17 kDa form (mIL-1β).[2-4] The severity of certain chronic inflammatory diseases correlate with IL-1β levels indicating ICE is a compelling therapeutic target for therapeutic intervention.[5] In connection with our interest in the discovery of novel antiinflammatory agents, we recently reported P_1 aspartate-based peptide α-((2,6-dichlorobenzoyl)oxy)methyl ketones as a potent time-dependent inhibitors of ICE.[6] Herein we report our investigations in identifying key structural and stereochemical recognition elements at the P_1-P_3 positions of substrate-based inhibitors of ICE.

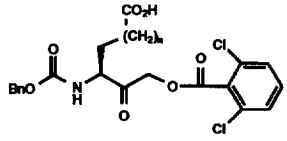
ICE is substrate selective cleaving only selected Asp-containing amide bonds. In light of the enzyme's substrate specificity,[4] our rational inhibitor design began with the synthesis of P_1 aspartate α-(aryl)oxy- and α-(arylacyl)oxymethyl ketones.[6] We observed that within the Asp and Xxx-Asp classes of compounds, the α-(arylacyl)oxymethyl ketones were consistently more active against the enzyme than the (aryl)oxy- counterparts. For example, benzoate **1** is >10-fold more potent than the structurally related phenolate **2** or thiophenolate **3.** As a result, we chose to focus our SAR efforts around structure **1.**[7]



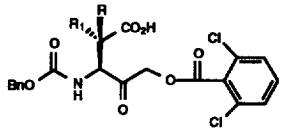
1: 7400 $M^{-1}s^{-1}$ **2**: <1000 $M^{-1}s^{-1}$ **3**: <1000 $M^{-1}s^{-1}$

Several structural analogs of **1** were synthesized featuring changes to the aspartic acid moiety in attempts to improve inhibitory potency and/or define key structural features required for recognition at the P_1 position. Inhibitors **4** and **5** were synthesized wherein the side chain carboxylate was homologated by one or two methylene groups. These inhibitors were synthesized from the corresponding protected glutamic acid or

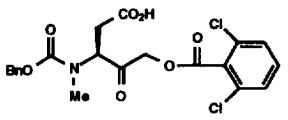
Figure 1: Structure-activity relationships at the P_1 position



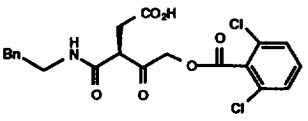
4: n=1, $k_{obs}/[I]$= <1000 $M^{-1}s^{-1}$
5: n=2, $k_{obs}/[I]$= <1000 $M^{-1}s^{-1}$



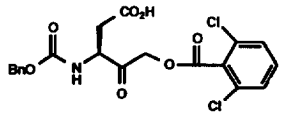
6: R= H, R_1= Me, $k_{obs}/[I]$= <1000 $M^{-1}s^{-1}$
7: R= Me, R_1= H, $k_{obs}/[I]$= <1000 $M^{-1}s^{-1}$



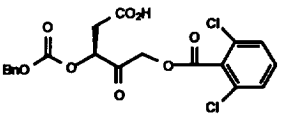
8: $k_{obs}/[I]$= <1000 $M^{-1}s^{-1}$



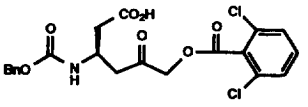
9: $k_{obs}/[I]$= <1000 $M^{-1}s^{-1}$



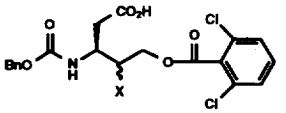
1: $k_{obs}/[I]$= 7400 $M^{-1}s^{-1}$



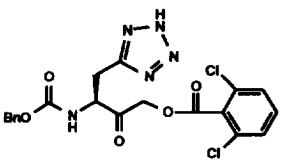
10: $k_{obs}/[I]$= <1000 $M^{-1}s^{-1}$



11: $k_{obs}/[I]$= <1000 $M^{-1}s^{-1}$



12: X= OH, $k_{obs}/[I]$= <1000 $M^{-1}s^{-1}$
13: X= H, $k_{obs}/[I]$= <1000 $M^{-1}s^{-1}$



14: $k_{obs}/[I]$= <1000 $M^{-1}s^{-1}$

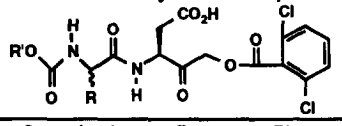
aminoadipic acid using the synthetic protocol described by us.[6] Compounds **4** and **5** were considerably less potent ($k_{obs}/[I]$ = <1000 $M^{-1}s^{-1}$) than **1** suggesting that the length of the amino acid side chain (Asp) is optimal at the P_1 position. Methyl substitution of the β-carbon in **1**,[9] introduced to reduce conformational freedom of the side chain around α-carbon, also led to less potent analogues **6** and **7**. Similarly, isosteric replacement of the side chain carboxylate with the tetrazole moiety as in **14** results in loss of enzyme affinity.

The relative contributions of amido NH and ketone carbonyl in **1** to enzyme recognition was investigated. N-methylated analogue **8**, reverse amide **9** and carbonate **10** were synthesized to assess the importance of amido NH functionality. Each of the compounds **8-10** are less potent than **1** ($k_{obs}/[I]$ = <1000 $M^{-1}s^{-1}$). The role of the carbonyl group was evaluated by synthesizing homo-ketone **11**, alcohol **12** and benzoate **13**. These compounds are also inactive with respect to **1** indicating that the carbonyl group plays a key role in P_1 residue recognition. This later observation is in keeping with SAR studies of peptide chloromethyl and (arylacyl)oxymethyl ketone inhibitors of papain and cathepsin B.[9]

A selection of amino acid residues were introduced at the P_2 and P_3 positions (Tables 1 and 2). The natural substrate for ICE, pIL-1β, contains a histidine residue at P_2. This residue was one of the first to be incorporated into **1** yielding dipeptide **15.** This dipeptide is 3-fold less active (**15** = 2200 $M^{-1}s^{-1}$) than **1** (7000 $M^{-1}s^{-1}$). The requirement for a P_2 side chain, however, is indicated by the lack of affinity of the glycine analogue **16**. Further introduction of hydrophobic amino acids at P_2 gave rise to enhanced potency, **17-20.** The hydrophobic nature of the active site cleft is evident upon considering the 10-fold increase in the rate of inactivation of benzyloxycarbamate **20** versus methoxycarbamate **19**. The strong preference for L-stereochemistry

at the P_2 residue is substantiated upon substitution of D-Ala for L-Ala (**17** versus **21**) at this position. A similar SAR emerged from the analysis of the P_3 position (Table 2). Hydrophobic amino acids having L-stereochemistry afforded inhibitors having the greatest inactivation rates. The relative insensitivity of the size of the hydrophobic residues in **15-20** and **22-24** to potency is consistent with these side chains residing in clefts (as opposed to discrete pockets) on the enzyme.[10, 11]

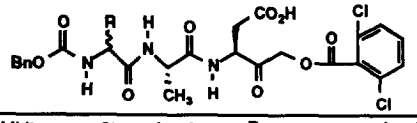
Table 1: Structure-activity relationships of dipeptides



Inhibitor	Stereochemistry at P_2	R	R'	$k_{obs}/[I]$ $(M^{-1}s^{-1})^a$
15	L		OMe	2200
16	L	H	OMe	<1000
17	L	CH_3	OMe	7000
18	L		OMe	2500
19	L	$CH(CH_3)_2$	OMe	4900
20	L	$CH(CH_3)_2$	OBn	41,000
21	D	CH_3	OBn	<1000

[a]For a description of ICE assay (pH 7.4, 37°C), see reference 6.

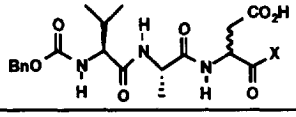
Table 2: Structure-activity relationships of tripeptides



Inhibitor	Stereochemistry at P_3	R	$k_{obs}/[I]$ $(M^{-1}s^{-1})^a$
22	L	CH_3	115,770
23	L	$CH(CH_3)_2$	406,700
24	L	CH_2Ph	232,950
25	D	CH_3	10,000

[a]For a description of ICE assay (pH 7.4, 37°C), see reference 6.

Table 3: Stereochemistry at P_1[a]



Inhibitor	Stereochemistry at P_1	X	$k_{obs}/[I]$ $(M^{-1}s^{-1})$	K_i (nM)
23	L	DCB[b]	406,700	
26	D	DCB	565,000	
27	L	PTP[b,12]	280,000	
28	D	PTP	298,000	
29	L	H		15
30	D	H		15

(a) For a description of ICE assay (pH 7.4, 37oC), see reference 6.
(b) DCB = (2,6-dichlorobenzoyl)oxy; PTP = ((1-phenyl-3-(trifluoromethyl)-pyrazol-5-yl)oxy.

The stereochemical requirement at the P_1 position was investigated. Surprisingly, diastereomeric inhibitor pairs **23** and **26** and **27** and **28** are equipotent against the enzyme (Table 3).[13] A similar result is seen in the reversible aldehyde-based inhibitor pair **29** and **30**.[14] Although this tolerance for D-stereochemistry at a P_1 position is unprecedented for the cysteine protease superfamily, the result is somewhat complicated as epimerization of the α-carbon occurs at the P_1-Asp residue. For inhibitor **23**, the $t_{1/2}$ for epimerization is 12-14 hours in assay buffer and *ca.* 3 hours in dog plasma. We found that the rate of epimerization is independent of leaving group, inhibitor concentration, and buffer concentration. The rate is pH dependent with the pH profile breaking at the carboxylate pK_a (*ca.* 4.5). These data are consistent with intramolecular abstraction of the α-proton by the β-carboxylate oxygen.[15a] We observed that the second order rate constant of inactivation by the D-Asp

inhibitors is invariant with increasing concentration of the L-Asp diastereomer. Addition of 1% up to 30% of the L-diastereomer **27** to the pure D-diastereomer **28** (keeping the total (I) constant) does not effect in any way the rate of inactivation of **28**. At present, we are unable to unequivocally distinguish whether the D- and L-diastereomers bind with equal affinity or whether their equal activity is a consequence of the epimerization in the assay.[15b]

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13. The dipeptides Z-Val-(L)-Asp-PTP and Z-Val-(D)-Asp-PTP also showed a similar trend and have comparable inactivation rates.
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15. (a) Details of these studies will be published elsewhere. (b) In contrast to the reversible and irreversible inhibitor classes, only the L-Asp is tolerated in a substrate. For example, Ac-Tyr-Val-Ala-(L)-Asp-NHMe is turned over by enzyme whereas the (D)-Asp analog is not.

(*Received in USA* 5 *December* 1994; *accepted* 11 *January* 1995)