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INHIBITION OF INTERLEUKIN-1β CONVERTING ENZYME BY N-ACYL-ASPARTIC ACID KETONES

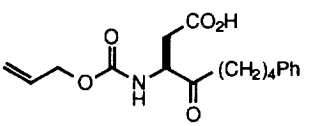
Adnan M. M. Mjalli†, Kevin T. Chapman, Justin J. Zhao,
Nancy A. Thornberry[1], Erin P. Peterson[1], Malcolm MacCoss*

Departments of Medicinal Chemistry and [1]Biochemistry
Merck Research Laboratories
Rahway, New Jersey 07065-0900

Abstract: N-Acyl aspartic acid ketones (**3a-3n**) were prepared from the corresponding bromomethyl ketone. The inhibition of interleukin-1β converting enzyme (ICE) by these single amino acid ketones is reported. The best compound had K_i of 3.5 μM versus ICE.

Peptidyl aldehydes and ketones have been demonstrated to be potent, reversible inhibitors of the cysteine proteinase interleukin-1β converting enzyme (ICE).[1-5] This novel proteinase has been found to be necessary for the processing of the mature form of the potent cytokine, IL-1β.[6-8] ICE-like activities have also been implicated in apoptotic processes in nematodes and neuronal cells.[9,10] It has been demonstrated from substrate specificity studies that four amino acids to the N-terminus of the cleavage site are necessary for efficient cleavage of peptides by ICE.[6] However, limitations to the development of peptidyl inhibitors is in part attributed to their physicochemical properties, lack of bioavailability and metabolic instability.[11,12] Therefore, discovery of non-peptidyl small molecule biomimetics remains a primary objective for any therapeutic target. Accordingly, we report herein a novel class of single amino-acid ketones and their evaluation as inhibitors of interleukin-1β converting enzyme.

Truncation of the peptidyl ICE inhibitor AcTyrValAlaAspCO$(CH_2)_4$Ph (K_i = 42 nM)[2] to the corresponding single amino acid **1** (Alloc-AspCO$(CH_2)_4$Ph) resulted in total loss of the activity against ICE. It was proposed that more potent inhibitors may result from enhancing the reactivity of the ketone carbonyl of **1** towards the active site cysteine of this enzyme.



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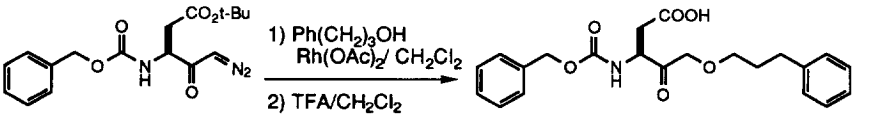
† Present address: Ontogen Corp., Carlsbad, CA 92009

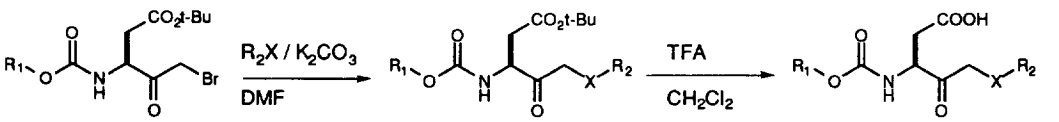
* Author to whom correspondence and reprint requests should be sent.

Compound **3a** was prepared from the reaction of diazomethyl ketone[13] **2** with 3-phenyl-1-propanol catalyzed with $Rh(OAc)_2$ in dichloromethane followed by cleavage of the t-butyl ester.



2 **3a**

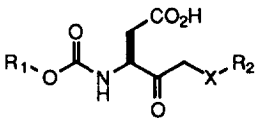
Compounds **3b-3n** were synthesized by reaction of the appropriate side chain (R_2X) with the bromomethyl ketone[13] **4** in dimethylformamide in the presence of powdered potassium carbonate in good yield (70-90%) to provide the corresponding ketone **5**. The t-butyl ester was cleaved in 50% trifluoroacetic acid in dichloromethane to afford the desired acids in near quantitative yield.



4 **5** **3b-n**

As indicated in Table 1 replacement of the β-carbon atom in **1** with a heteroatom and/or electron withdrawing group resulted in inhibitory activity of these compounds versus ICE. Similar activity was obtained in **3a**, **3b** and **3j** (K_i = 24, 27 and 20 μM, respectively) regardless of the β-substituent. There was little difference in ICE inhibition of related compounds with Alloc or Cbz protecting groups (R_1 = allyl or benzyl), thus the relative potencies of **3a**, **3b** and **3c** reflect the effect of heteroatom replacement.[14] Significant improvement in activity was observed in the phenylethylamino methyl ketone **3d** over the phenylpropylaminomethyl analog **3c** (4.4 vs. 67 μM, respectively). N-Methylation of **3d** resulted in complete loss of activity in **3e** (>100 μM). These results suggest that these heteroatoms are not simply activating the carbonyl group, but they may have a specific interaction with the active site of the enzyme. Replacement of the ester group in compound **3j** (K_i = 20 μM) with a carbamate group in **3k** (K_i = 74 μM) resulted in a loss of activity. Replacement of the dihydrocinnamoyl group in compound **3j** with the conformationally restricted *trans*-cinnamoyl group in **3m** resulted in 3-4 fold decrease in potency. Compound **3n** with a cyclohexylethyl group showed a slight improvement in the activity over the phenylethyl analog **3j** (3.5 vs 20 μM, respectively). Despite the potential for the R_2X substituent to be a leaving group, none of the compounds in Table 1 exhibited time dependent inhibition under the assay conditions. Related tripeptide analogs also did not exhibit time dependent inhibition of ICE, however at higher

Table 1. Inhibition of Interleukin-1β Converting Enzyme by N-Acyl Aspartic Acid Ketones



Compd. No	R_1	X	R_2	K_i [a] (μM)
1	CH_2=$CHCH_2$	CH_2	$Ph(CH_2)_2$	>100
3a	$PhCH_2$	O	$Ph(CH_2)_3$	24
3b	CH_2=$CHCH_2$	S	$Ph(CH_2)_3$	27
3c	CH_2=$CHCH_2$	NH	$Ph(CH_2)_3$	67
3d	CH_2=$CHCH_2$	NH	$Ph(CH_2)_2$	4.4
3e	CH_2=$CHCH_2$	NMe	$Ph(CH_2)_2$	>100
3f	CH_2=$CHCH_2$	NBn	$Ph(CH_2)_2$	68
3g	CH_2=$CHCH_2$	NH	$PhCH_2$	>100
3h	CH_2=$CHCH_2$	NH	$(Ph)_2CH$	>100
3i	CH_2=$CHCH_2$	NH	$PhCH_2CH(Ph)$	70
3j	CH_2=$CHCH_2$	OC=O	$Ph(CH_2)_2$	20
3k	CH_2=$CHCH_2$	OC=ONH	$PhCH_2$	74
3l	CH_2=$CHCH_2$	OC=O	$PhO(CH_2)_2$	23
3m	CH_2=$CHCH_2$	OC=O	*trans*-PhCH=CH	90
3n	CH_2=$CHCH_2$	OC=O	Cyclohexyl$(CH_2)_2$	3.5

[a] Kinetic parameters were determined using a continuous fluorometric assay with the substrate, Ac-Tyr-Val-Ala-Asp-AMC.[6] The error in reproducing these values was typically 10-25%.

inhibitor concentrations or longer enzyme-inhibitor incubation times, the potential for irreversible inhibition does exist in a mechanism analogous to the acyloxymethylketones.[4,15]

This study introduces a novel class of reversible, single amino acid inhibitors of the IL-1β convertase. These small molecule inhibitors have the potential to serve as a core structure for the development of more potent non-peptidyl small molecule inhibitors of this important proteinase.[16]

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16. See following paper in this issue.

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