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INHIBITION OF INTERLEUKIN-1β CONVERTING ENZYME BY N-ACYL-ASPARTYL ARYLOXYMETHYL KETONES

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Abstract: The synthesis of N-allyloxy-aspartyl aryloxymethyl ketones and their evaluation as inhibitors of interleukin-1β converting enzyme (ICE) are described. 3-Substituted 2-naphthyloxymethyl ketones were found to have K_i's of 90-500 nM. The SAR suggests that the substituents on the aryloxy group may have a specific binding interaction in the active site of ICE.

Interleukin-1β converting enzyme (ICE), a novel heterodimeric cysteine proteinase, has been demonstrated to be necessary and sufficient for the processing of interleukin-1β, a cytokine implicated as a major mediator of acute and chronic inflammation.[1-3] ICE-like enzymes have also been implicated in apoptotic processes by virtue of their homology to CED-3, a protein isolated from nematodes and shown to be involved in programmed cell death in that species.[4-6] Potent reversible[7-9] and irreversible[10-14] inhibitors of ICE have been described. Given the obvious limitations of peptidyl-based inhibitors for use as therapeutic agents, much effort has been directed toward the discovery of nonpeptidyl inhibitors of this proteinase. Single amino acid N-acyl-aspartyl methyl ketones substituted on the methyl group with phenylalkyl-X where X is O, S, NH, NCH_3, and O-C=O gave inhibitors of ICE with K_i's in the range of 4-90 μM.[15] Inclusion of a heteroatom on the methyl ketone is important for inhibition as the phenylpropyl ketone analog was inactive. Herein is described the synthesis of N-allyloxycarbonyl-aspartyl aryloxymethyl ketones and their evaluation as ICE inhibitors. Certain substituents on the aryl groups greatly improved enzyme inhibition and suggest an apparent specific binding interaction with the enzyme.

Chemistry

N-Allyloxycarbonyl-L-aspartic acid, β-t-buyl ester **1** was first treated with an alkyl chloroformate to form the mixed anhydride *in situ* which was subsequently reacted with excess diazomethane to form N-allyloxycarbonyl-3-amino-5-diazo-4-oxopentanoic acid, β-t-butyl ester **2**

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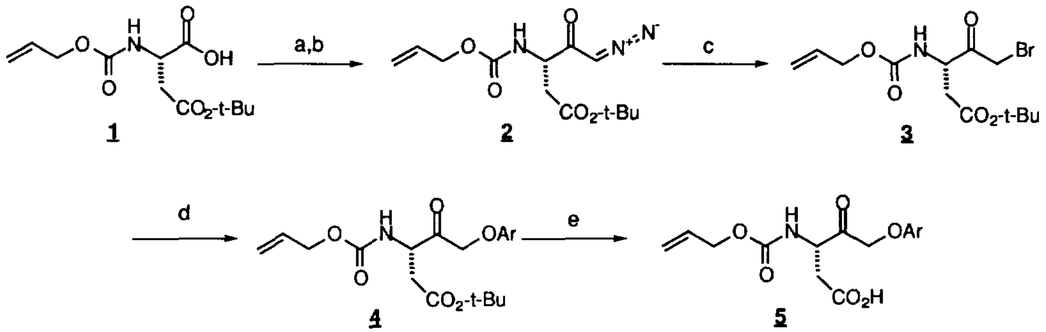
[Scheme 1]. Subsequent reaction of **2** with 48% hydrobromic acid / acetic acid in THF formed N-allyloxycarbonyl-3-amino-5-bromo-4-oxopentanoic acid, β-t-butyl ester **3**.[10] Reaction of **3** with the potassium salt of the desired aryloxy compound affected displacement of the bromide to form the aryloxymethyl ketone **4**. Removal of the β-t-butyl ester with 50% trifluoroacetic acid in CH_2Cl_2 gave the desired final product **5a-o**.

Scheme 1.



a) i-$C_4H_9OC(=O)Cl$, NMM, CH_2Cl_2; b) CH_2N_2, $(C_2H_5)_2O$; c) HBr, CH_3CO_2H; d) ArOH, K_2CO_3, DMF; e) CF_3CO_2H, CH_2Cl_2 or HCl, $(C_2H_5)_2O$

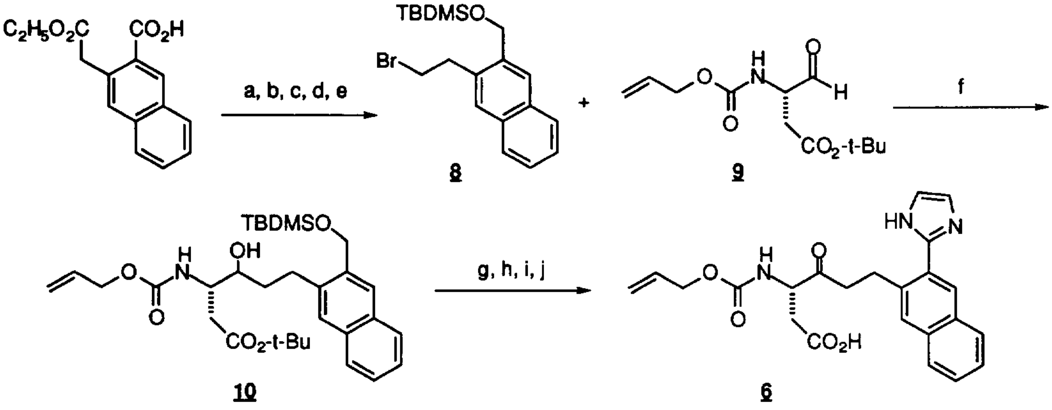
The synthesis of the 'carbon' analog **6** is outlined in Scheme 2. The crucial step in this sequence is the chromous chloride / vitamin B_{12} catalyzed condensation of bromide **8** with aldehyde **9** to give **10**.[16] Subsequent removal of the *tert*-butyldimethylsilyl group with tetrabutyl ammonium fluoride and oxidation with TPAP in the presence of N-methyl-morpholine-N-oxide afforded the keto-aldehyde. Reaction with glyoxal in aqueous ammonia introduced the imidazole group. The t-butyl ester was removed with 50% trifluoroacetic acid to form **6**.

In situ removal of the 'Alloc' protecting group in **5a** and **5o** with a palladium catalyst and tributyltin hydride[7] in the presence of Cbz-L-Val-L-Ala and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenztriazole (HOBt), and N-methylmorpholine afforded tripeptide analogs **7a** and **7b**.

Results and Discussion

The unsubstituted phenoxymethyl ketone **5a** was a modestly potent, competitive, reversible inhibitor of ICE. Whereas the phenoxy group in **5a** has the potential to be a leaving group in a mechanism of inhibition analogous to the acyloxymethyl ketones, no time dependent inhibition was observed during the course of the assay (~30 min). Addition of an *ortho*-carboxamide in **5b** did not improve potency nor was time dependent inhibition observed. The addition of a 4-phenyl substitution in **5c** provided an irreversible inhibitor ($k_{inact}/K_i = 3400\ M^{-1}s^{-1}$), comparable to some of

Scheme 2.



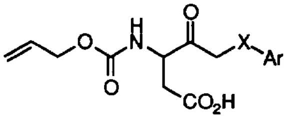
a) i-$C_4H_9OC(=O)Cl$, NMM, THF; b) $NaBH_4$, CH_3OH; c) TBDMSCl, imidazole, DMF; d) DIBAL, CH_2Cl_2; e) Ph_3P, NBS, DMF; f) $CrCl_2$, Vitamin B_{12};, DMF; g) $(n\text{-}C_4H_9)_4NF$, THF; h) TPAP, NMO, CH_2Cl_2, 4A sieves; i) glyoxal, aq. NH_3, CH_3OH; j) TFA, CH_2Cl_2

the better single amino acid acyloxymethyl ketones[10,11], whereas the 5-phenyl analog **5d** was again comparable to **5a**.

Naphthyloxymethyl ketones were also investigated. The unsubstituted naphthyl analogs **5e** and **5f** were comparable to **5a** and offered no particular insights into further improving potency versus ICE. However, the importance of the position of the carboxamide moiety on the naphthyl ring became obvious in the series **5g**-**5l**. Only the 3-carboxamido-2-naphthyloxy derivative **5i** was an inhibitor of ICE. At first, inhibition of ICE by the N-phenylamide **5j** and hydroxymethyl analog **5k** and the complete loss of inhibition by the dimethylamide **5l** suggested the possible influence of a hydrogen bond interaction. However, the corresponding methyl ester **5m** and nitrile **5n** were still inhibitors, albeit less active than **5l**. Replacement of the carboxamide in **5i** with a 2-imidazole further enhanced the potency versus ICE to give **5o** with a K_i of 92 nM. The most potent compounds in this series are characterized by relatively slow rates of formation of reversible enzyme-inhibitor complexes, with second-order rate constants in the range of 2-10 x 10^3 $M^{-1}s^{-1}$. Slow binding inhibition probably also applies to the less potent members of the series, although the high concentrations required to achieve inhibition in these cases precluded accurate measurements of this parameter.

Replacement of the oxygen linkage to the methyl ketone with carbon in **6** completely eliminated any inhibition. A modest improvement in potency is seen in the tripeptide analogs going from the phenoxymethyl ketone **7a** to the 3-carboxamido-2-naphthyloxy derivative **7b**.

Table 1. Inhibition of Interleukin-1β Converting Enzyme by N-Allyloxycarbonyl Aspartyl Aryloxymethyl Ketones.



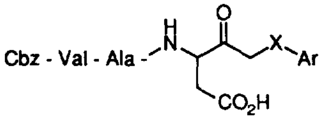
Compd. No.	X	Ar	K_i [a] (μM)	k_{on} [a] ($M^{-1}s^{-1}$)
5a	O	Ph	2.7	
5b	O	Ph-2-CONH$_2$	3.8	
5c	O	Ph-2-CONH$_2$-4-Ph		3400 [b]
5d	O	Ph-2-CONH$_2$-5-Ph	2.0	
5e	O	1-napthyl	9.5	
5f	O	2-naphthyl	4.0	
5g	O	1-naphthyl-2-CONH$_2$	>22	
5h	O	2-naphthyl-1-CONH$_2$	>18	
5i	O	2-naphthyl-3-CONH$_2$	0.32	5600
5j	O	2-naphthyl-3-CONHPh	0.5	4100
5k	O	2-naphthyl-3-CH$_2$OH	1.2	
5l	O	2-naphthyl-3-CON(CH$_3$)$_2$	>28	
5m	O	2-naphthyl-3-CO$_2$CH$_3$	2.6	
5n	O	2-naphthyl-3-CN	0.62	4500
5o	O	2-naphthyl-3-(2-imidazolyl)	0.09	12000
6	CH$_2$	2-naphthyl-3-(2-imidazolyl)	>100	

[a] Kinetic parameters were determined using a continuous fluorometric assay with the substrate, Ac-Tyr-Val-Ala-AMC.[2] The error in reproducing these values was typically 10-25%. [b] Exhibited time dependent inhibition, thus this value is a measure of k_{inact}/K_i.

Differences in the binding of tri- and tetrapeptide and single amino acid inhibitors of ICE have been reported.[11]

The substitution pattern required for a 3-substituted-2-naphthyloxy group (**5i** vs **5b**), the nature of that substituent (**5i** and **5o** vs **5m**, **5l**, and **5k**), and the requirement for a linking heteroatom (**5o** vs **6**) collectively suggest that these single amino acid inhibitors of ICE are binding across the active site, possibly into the 'prime-side' that would be occupied by Ala^{117}-Pro^{118} of proIL-1β. It is intriguing but highly speculative to suggest that the linking ether oxygen may hydrogen bond to His^{237} [17,18], the naphthyl group into the 'Pro^{118} space', and the carboxamide

Table 2. Inhibition of Interleukin-1β Converting Enzyme by Tripeptidyl Aryloxymethyl Ketones.



Compd. No.	X-Ar	K_I (nM)	k_{on} ($M^{-1}s^{-1}$)
7a	OPh	4.9	1.1×10^6
7b	O-2-naphthyl-3-$CONH_2$	1.7	4.3×10^5

continuing the substrate backbone. Given the potential for irreversible inhibition by these inhibitors at longer enzyme incubation times, conclusions of specific binding interactions of these inhibitors with ICE need a firmer, structure-based analysis. At longer enzyme-inhibitor incubation times, all of the inhibitors in this series will eventually irreversibly inhibit ICE through expulsion of the aryloxy leaving group to form the thiomethylketone with the active site cysteine, in direct analogy to the mechanism of irreversible inhibition by acyloxymethylketones and chloromethylketones[13]. Although the rate of this process is relatively slow for compounds such as **5a** ($k_{inact} / K_i < 10\ M^{-1}s^{-1}$), several of members in this series have second order rate constants for inactivation approaching those observed for single amino acid acyloxymethyl ketones (eg., **5o**, $k_{inact} / K_i \sim 1400\ M^{-1}s^{-1}$). The results presented herein do suggest that potent, small molecular weight, non-peptidyl inhibitors that span the active site of ICE can be realized.

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References

1. Cerretti, D. P.; Kozlosky, C. J.; Mosley, B.; Nelson, N.; Van Ness, K.; Greenstreet, T. A.; March, C. J.; Kronheim, S. R.; Druck, T.; Cannizaro, L. ; Huebner, K.; Black, R. *Science* **1992**, *256*, 97.
2. Thornberry, N. A.; Bull, H. G.; Calacay, J. R.; Chapman, K. T.; Howard, A. D.; Kostura, M. J.; Miller, D. K.; Molineaux, S. M.; Weidner, J. R.; Aunins, J.; Elliston, K. O.; Ayala, J. M.; Casano, F. J.; Chin, J.; Ding, G. J.-F.; Egger, L. A.; Gaffney, E. P.; Limjuco, G.; Palyha, O. C.; Raju, S. M.; Ronaldo, A. M.; Sally, J. P.; Yamin, T.-T.; Lee, T. D.; Shively, J. E.; MacCoss, M.; Mumford, R. A.; Schmidt, J. A.; Tocci, M. J. *Nature* **1992**, *356*, 768.
3. Li, P.; Allen, H.; Banerjee, S.; Franklin, S.; Herzog, L.; Johnston, C.; McDowell, J.; Paskind, M.; Rodman, L.; Salfeld, J.; Towne, E.; Tracey, D.; Wardwell, S.; Wei, F.-Y.; Wong, W.; Kamen, R.; Seshadri, T. *Cell* **1995**, *80*, 401.

4. Yuan, J.; Shaham, S.; Ledoux, S.; Ellis, H. M; Horvitz, H. R. *Cell* **1993**, *75*, 641.
5. Miura, M.; Zhu, H.; Rotello, R.; Hartweig, E. A.; Yuan, J. *Cell* **1993**, *75*, 653.
6. Gagliardi, V.; Fernandez, P.-A.; Lee, R. K. K.; Drexler, H. C. A.; Rotello, R. J.; Fishman, M. C.; Yuan, J. *Science* **1994**, *263*, 826.
7. Chapman, K.T. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 613.
8. Mjalli, A. M. M.; Chapman, K., T.; MacCoss, M.; Thornberry, N. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2689.
9. Mullican, M. D.; Lauffer, D. J.; Gillespie, R. J.; Matharu, S. S.; Kay, D.; Porritt, G. M.; Evans, P. L.; Golec, J. M. C.; Murcko, M. A.; Luong, Y.-P.; Raybuck, S. A.; Livingston, D. J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2359.
10. Dolle, R. E.; Hoyer, D.; Prasad, C. V. C.; Schmidt, S. J.; Helaszek, C. T.; Miller, R. E.; Ator, M. A. *J. Med. Chem.* **1994**, *37*, 563.
11. Thornberry, N. A.; Peterson, E. P.; Zhao, J. J.; Howard, A. D.; Griffin, P. R.; Chapman, K. T. *Biochemistry* **1994**, *33*, 3934.
12. Dolle, R. E.; Singh, J.; Rinker, J.; Hoyer, D.; Prasad, C. V. C.; Graybill, T. L.; Salvino, J. M.; Helaszek, C. T.; Miller, R. E.; Ator, M. A. *J. Med. Chem.* **1994**, *37*, 3863.
13. Dolle, R. E.; Singh, J.; Whipple, D.; Osifo, I. K.; Speier, G.; Graybill, T. L.; Gregory, J. S.; Harris, A. L.; Helaszek, C. T.; Miller, R. E.; Ator, M. A. *J. Med. Chem.* **1995**, *38*, 220.
14. Prasad, C.V.C.; Prouty, C. P.; Hoyer, D.; Ross, T. M.; Salvino, J. M.; Awad, M.; Graybill, T. L.; Schmidt, S. J.; Osifo, I. K.; Dolle, R. E.; Helaszek, C. T.; Miller, R. E.; Ator, M. A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 315.
15. Mjalli, A. A. M.; Chapman, K. T.; Zhao, J. J.; Thornberry, N. A.; Peterson, E. P.; MacCoss, M. *Bioorg. Med. Chem. Lett.* preceding paper in this issue.
16. Takai, K.; Nitta, K.; Fujimura, O.; Utimoto, K. *J. Org. Chem.* **1989**, *54*, 4732.
17. Wison, K. P; Black, J. F.; Thomson, J. A.; Kim, E. E.; Griffith, J. P; Navia, M. A.; Murcko, M. A.; Chambers, S. P.; Aldape, R. A.; Raybuck, S. A.; Livingston, D. J. *Nature* **1994**, *370*, 270.
18. Walker, N. P. C.; Talanian, R. V.; Brady, K. D.; Dang, L. C.; Bump, N. J.; Ferenz, C. R.; Franklin, S.; Ghayur, T.; Hackett, M. C.; Hammill, L. D.; Herzog, L.; Hugunin, M.; Houy, W.; Mankovich, J. A.; McGuiness, L.; Orlewicz, E.; Paskind, M.; Pratt, C. A.; Reis, P.; Summani, A.; Terranova, M.; Welch, J. P; Xiong, L.; Moller, A.; Tracey, D. E.; Kamen, R.; Wong, W. W. *Cell* **1994**, *78*, 343.

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