

CONFORMATIONALLY CONSTRAINED INHIBITORS OF CASPASE-1 (INTERLEUKIN-1 β CONVERTING ENZYME) AND OF THE HUMAN CED-3 HOMOLOGUE CASPASE-3 (CPP32, APOPAIN)

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Abstract: A systematic study of interleukin-1 β converting enzyme (ICE, caspase-1) and caspase-3 (CPP32, apopain) inhibitors incorporating a P_2 - P_3 conformationally constrained dipeptide mimetic is reported. Depending on the nature of the P_4 substituent, highly selective inhibitors of both Csp-1 or Csp-3 were obtained.

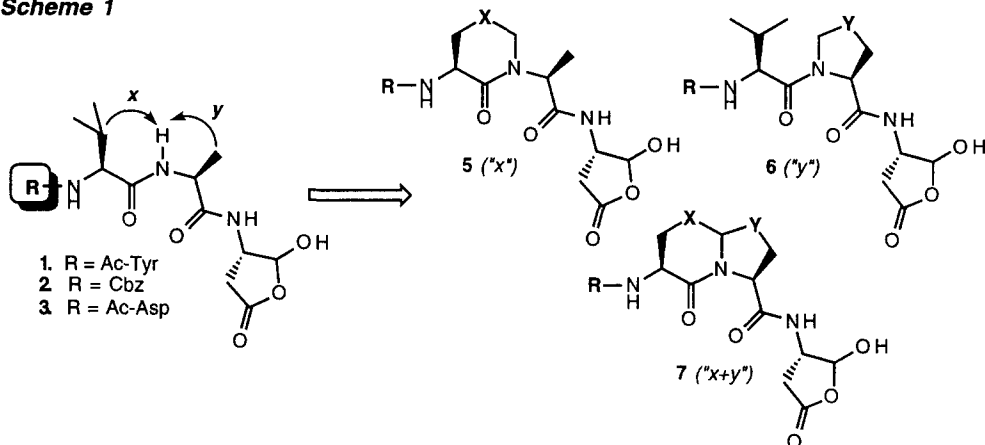
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Introduction

Interleukin-1 β converting enzyme (ICE, caspase-1)¹ catalyzes the cleavage of the biologically inactive 31KDa IL-1 β precursor at Asp¹¹⁶-Ala¹¹⁷ to generate the 17.5 KDa mature, biologically active cytokine, a key mediator of inflammation.² In addition, members of the ICE/CED-3 family of cysteine proteases,³ such as caspase-3 (CPP32, apopain, Yama),⁴ may play a key role in the regulation of programmed cell death (apoptosis).⁵ Of particular interest is the role of caspases in neuronal apoptosis.^{5b,f} Thus, inhibitors of the caspases may be of therapeutic value in the treatment of inflammatory and degenerative diseases such as rheumatoid arthritis, ALS, Alzheimer's Disease, and Parkinson's Disease.

Substrate specificity data¹ indicate that four residues to the N-terminal side of the scissile amide bond (P_4 - P_1) must be present for catalytic recognition by caspase-1 (ICE, Csp-1). Caspase-1 and its homologs are characterized by a strict requirement for aspartic acid at the P_1 position of its substrates. Substrate based inhibitor design has lead to the discovery of several potent C-terminal peptide aldehyde inhibitors⁶ of caspase-1 based on the pro-IL-1 β cleavage site TyrValHisAsp¹¹⁶-Ala¹¹⁷. The prototype Csp-1 inhibitor Ac-TyrValAlaAsp-H (**1**) has recently been co-crystallized with human Csp-1. Both the crystal structure⁷ and structure-activity studies^{6b,8} indicate that the P_2 amide nitrogen is not utilized in a hydrogen bonding interaction with the enzyme and that the backbone conformation of the bound inhibitor may allow for the introduction of conformational constraints from this amide nitrogen to either the P_2 or P_3 side chains. Also consistent with the lack of a hydrogen bond to the P_4 amide nitrogen observed in the crystal structure, we and others^{6bc} have found that the P_4 Ac-Tyr group can be replaced with either a carbobenzyloxy (i.e., **2**) or dihydrocinammoyl group with a only a modest loss in potency (see Table 1). The first examples of constrained analogs based on these observations were recently reported,⁹ in which a 5-aminopyrimidin-6-one (or pyridone) acetic acid moiety was used as a P_3 - P_2 dipeptide replacement. More recently, these studies were extended to series of pyridazinodiazepine peptidomimetics.¹⁰

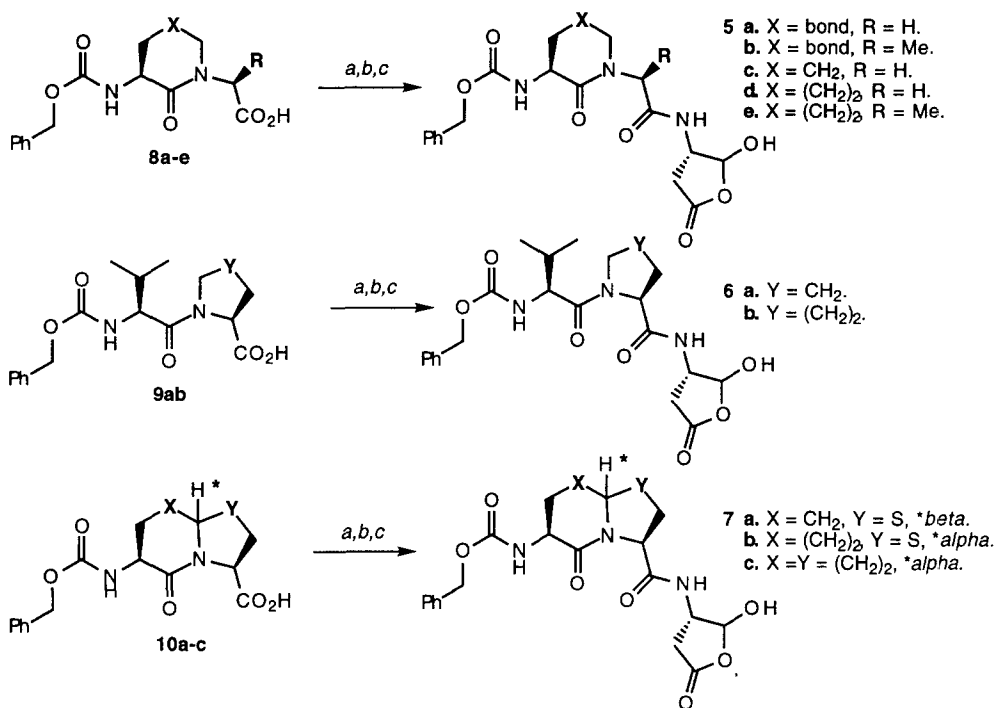
Scheme 1



Workers at Merck have also reported^{4a} a potent C-terminal aldehyde inhibitor of Csp-3 based on the cleavage site of poly(ADP-ribose) polymerase (PARP), a known intracellular substrate of the protease. This prototype Csp-3 inhibitor Ac-AspGluValAsp-H (4) is also a potent inhibitor of Csp-1 (see Table 1).^{1d} We have observed that inhibitors of the general structure Ac-AspX³X²Asp-H where X³ ≠ Glu (e.g., Ac-AspValAlaAsp-H, 3) retain significant potency against Csp-3 and selectivity vs. Csp-1. The Csp-3 co-crystal structure of 4¹¹ as well as a related fluoromethylketone¹² indicate an analogous hydrogen bonding pattern with the peptide backbone of the inhibitor to that observed in the case of Csp-1 and inhibitor 1. This suggests that a similar strategy made be applied to the design of conformationally constrained Csp-3 inhibitors. In this paper we report the results of a systematic study of a series of mono- and bicyclic conformationally constrained analogs of Cbz-ValAlaAsp-H (2) and Ac-AspValAlaAsp-H (3) in which the P₂ amide nitrogen has been "tied back" to either or both the P₃ and P₂ side chains (i.e., analogs 5-7). This has led to the discovery of novel, specific inhibitors of both Csp-1 and Csp-3. While a number of related conformationally constrained, irreversible inhibitors of Csp-1 have been reported,¹⁰ this paper is the first report of peptidomimetic inhibitors of Csp-3 and demonstrates a general strategy for the preparation of Csp-3 selective compounds.

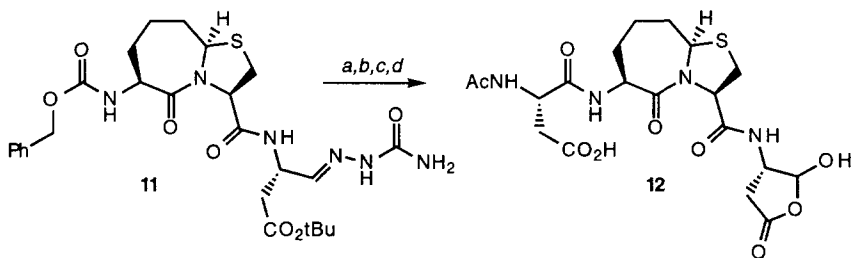
Synthesis

The use of conformationally constrained dipeptide mimics is well preceded in the design of inhibitors of angiotensin converting enzyme (ACE) and dual inhibitors of ACE/kidney neutral endopeptidase (KNEP).¹³ The required dipeptide mimics 8a-e and 10a-c were synthesized according to literature methods.¹⁴ Carboxylic acids 8a-e, 9a,b, and 10a-c were coupled to the aspartyl aldehyde synthon H-Asp(OtBu)-semicarbazone first reported^{6c} by Graybill et al. using ethyl-(dimethylaminopropyl)carbodiimide (EDAC)/1-hydroxybenzotriazole (HOBt) in either CH₂Cl₂ or CH₂Cl₂/DMF. Removal of the *t*-butyl ester and semicarbazone protecting groups by successive treatment with trifluoroacetic acid (TFA)/CH₂Cl₂/anisole (4/3/1) and 37% aqueous formaldehyde/acetic acid/methanol (1/1/3) gave, after either extractive workup or direct purification by reverse-phase chromatography on CHP-20 (divinylbenzene-polystyrene co-polymer supplied by Mitsubishi), the target aspartyl aldehydes 5, 6, and 7.

Scheme 2

(a) H-Asp(OtBu)-semicarbazone, EDAC, HOBt, CH₂Cl₂/DMF; (b) TFA/CH₂Cl₂/anisole (4/3/1);
 (c) 37% aq HCHO/AcOH/MeOH (1/1/3).

The *P₄* aspartic acid analog **12** was prepared from the protected intermediate **11** derived from carboxylic acid **10b**. Hydrogenolysis of the carbobenzyloxy group over Pd(OH)₂-C in methanol followed by EDAC-HOBt mediated coupling to Ac-Asp(OtBu)OH in DMF gave the target compound in fully protected form. Deprotection and purification as above gave aspartyl aldehyde **12**.

Scheme 3

(a) H₂, 20% Pd(OH)₂-C, MeOH; (b) Ac-Asp(OtBu)OH, EDAC, HOBt, DMF;
 (c) TFA/CH₂Cl₂/anisole (4/3/1); (d) 37% aq HCHO/AcOH/MeOH (1/1/3).

Results and Discussion

The 5 and 6-membered P_2 -NH to P_3 side chain constrained analogs **5a,c** demonstrated greatly reduced inhibitory activity¹⁵ against the murine Csp-1 relative to the parent linear acyl tripeptide analog **2**. However, the situation is somewhat improved when the constraint is relaxed to a 7-membered ring (i.e., **5d**). The potency of azepinone **5d** can be further enhanced by re-introduction of the alanine methyl side chain present in the parent acyl tripeptide. The resulting analog **5e** demonstrates Csp-1 inhibitory activity within a factor of three of the parent compound **2**. Enhanced Csp-1 binding affinity with analogues incorporating the P_2 alanine side chain relative to their unsubstituted counterparts has also been observed in a related series of pyridone-based inhibitors.^{9b}

Table 1. Inhibitory activity of constrained analogs against mCsp-1 and hCsp-3

Compd	mCsp-1 IC ₅₀ (μM)	hCsp-3 IC ₅₀ (μM)	Compd	mCsp-1 IC ₅₀ (μM)	hCsp-3 IC ₅₀ (μM)
1	0.0046	15.0	6a	0.087	14.0
2	0.064	47.0	6b	0.095	3.49
3	0.523	0.074	7a	4.80	86.0
4	0.071	0.0012	7b	0.159	>10
5a	>10	>10	7c	0.036	>10
5b	3.70	>100	12	10.4	0.018
5c	37.0	>100			
5d	1.68	53.0			
5e	0.186	>10			

Assays were carried out as described in ref 15 utilizing Ac-TryValAlaAsp-amc and Ac-AspGluValAsp-amc as substrates for mCsp-1 and hCsp-3, respectively.

In contrast, both the 5 and 6-membered P_2 -NH to P_2 constrained analogs **6a,b** show activity comparable to that of the parent acyl tripeptide against mCsp-1. This observation is consistent with results obtained by the Sanofi-Winthrop group in a related series of heteroaryloxy methyl ketone irreversible inhibitors⁸ of hCsp-1. We were surprised to find that both **6a** and **6b** showed enhanced hCsp-3 inhibitory activity¹⁵ relative to the parent linear tripeptide **2** although all of the analogs with a carbobenzyloxy group at P_4 were much less potent inhibitors of hCsp-3 than they were of mCsp-1.

Consistent with results obtained with the pyrrolidone-based inhibitor **5c**, the 6,5-bicyclic inhibitor **7a** was a poor inhibitor of mCsp-1. However, as was expected on the basis of results obtained with inhibitors **5e** and **6a,b**, both the 7,5-bicyclic and 7,6-bicyclic inhibitors **7b,c** showed excellent mCsp-1 inhibitory activity. In the case of **7c**, mCsp-1 inhibitory potency exceeded that of the parent unconstrained analog **2**. In addition, both **7b** and **7c** were highly selective inhibitors of mCsp-1 vs. hCsp-3. This result suggests that the backbone conformation of **7c** may be very close to the optimal enzyme bound conformation of the linear peptide aldehydes **1** and **2**.

As was expected based on results obtained in the linear acyl tripeptide series discussed above, replacement of the carbobenzyloxy group of the selective mCsp-1 inhibitor **7b** with an Ac-Asp residue resulted in a potent, highly selective inhibitor of hCsp-3. In fact, **12** was considerably more hCsp-3 selective (580-fold) than its unconstrained counterpart **3** (7-fold). Thus, it appears that the introduction of the conformational constraint

present in the bicyclic dipeptide mimetic of **12**, in conjunction with a charged residue at P_4 , results in a overall poor fit with the active site of mCsp-1 while retaining excellent binding affinity for hCsp-3 (compare **4** and **12**). Thus, the combination of conformational constraint and negative charge at P_4 may represent a general strategy for the preparation of highly selective inhibitors of this biologically important target. At present, all of the hCsp-3 inhibitors reported in the literature are also potent inhibitors of other caspase family members. Indeed, as the most hCsp-3 selective inhibitor have been reported to date, **12** may of considerable value in dissecting the role of hCsp-3 in the cell death pathway. We have also found that the irreversible Csp-1 inhibitors reported in the literature lose significant Csp-1 selectivity relative to their reversible counterparts making them useless in determining the role of a specific caspase in a biological process. For example, aldehyde **2** has an IC_{50} against hCsp-8 of 2.96 μ M while its fluoromethyl ketone counterpart (z-VAD-fmk) has K_i 's against mCsp-1 and hCsp-8 of 0.015 μ M and 0.018 μ M, respectively. This further emphasizes the need for selective, reversible caspase inhibitors such as **7c** and **12** for these studies. The extension of these findings to other peptidomimetic scaffolds will be the subject of future publications.

References and Notes

- (a) Sleath, P. R.; Hendrickson, R. C.; Kronheim, S. R.; March, C. J.; Black, R. A. *J. Biol. Chem.* **1990**, 265, 14526. (b) Howard, A. D.; Kostura, M. J.; Thornberry, N. A.; Ding, G. J. F.; Limjoco, G.; Weidner, J.; Salley, J. P.; Hogquist, K. A.; Chaplin, D. D.; Mumford, R. A.; Schmidt, J. A.; Tocci, M. J. *J. Immunol.* **1991**, 147, 2964. (c) Thornberry, N. A.; Bull, H. G.; Calaycay, 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.; Limjunco, G.; Palyha, O. C.; Raju, S. M.; Rolando, A. M.; Salley, J. P.; Yamin, T.-T.; Lee, T. D.; Shively, J. E.; MacCross, M.; Mumford, R. A.; Schmidt, J. A.; Tocci, M. J. *Nature* **1992**, 356, 768. (d) Margolin, N.; Raybuck, S. A.; Wilson, K. P.; Chen, W.; Fox, T.; Gu, Y.; Livingston, D. J. *J. Biol. Chem.* **1997**, 272, 7223.
- For a review on IL-1, see: Dinarello, C. A.; Wolff, S. M. *N. Engl. J. Med.* **1993**, 328, 106.
- For nomenclature of ICE homologs, see: Alnemri, E. S.; Livingston, D. J.; Nicholson, D. W.; Salvesen, G.; Thornberry, N. A.; Wong, W. W.; Yuan, J.-Y. *Cell* **1996**, 87, 171.
- (a) Nicholson, D. W.; Ali, A.; Thornberry, N. A.; Vaillancourt, J. P.; Ding, C. K.; Gallant, M.; Gareau, Y.; Griffin, P. R.; Labelle, L.; Lazenby, Y. A.; Munday, N. A.; Raju, S. M.; Smulson, M. E.; Yamin, T.-T.; Yu, V. L.; Miller, D. K. *Nature* **1995**, 376, 37-43. (b) Tewari, M.; Quan, L. T.; O'Rourke, K.; Desnoyers, S.; Zeng, Z.; Beidler, D. R.; Poirier, G. G.; Savesen, G. S.; Dixit, V. M. *Cell* **1995**, 81, 801.
- (a) Yuan, J.; Shaham, S.; Ledoux, S.; Ellis, H. M.; Horvitz, H. R. *Cell* **1993**, 75, 641-652. (b) Armstrong, R. C.; Aja, T. J.; Hoang, K. D.; Gaur, S.; Bai, X.; Alnemri, E. S.; Litwack, G.; Karanewsky, D. S.; Fritz, L. C.; Tomaselli, K. J. *J. Neuroscience* **1997**, 15, 553. (c) Villa, P.; Kaufmann, S. H.; Earnshaw, W. C. *TIBS* **1997**, 22, 388. (d) Miller, D. K. *Immunology* **1997**, 9, 35. (e) Schwartz, L. M.; Milligan, C. E. *Trends Neurosci.* **1996**, 19, 555. (f) Kuide, K.; Lippke, J. A.; Ku, G.; Harding, M. W.; Livingston, D. J.; Su, M. S.-S.; Flavell, R. A. *Science* **1995**, 267, 2000. (g) Steller, H. *Science* **1995**, 267, 1445.
- (a) Chapman, K. T. *Bioorg. Med. Chem. Lett.* **1992**, 2, 613. (b) Mullican, M. D.; Lauffer, D. J.; Gillespie, R. J.; Matharu, S. S.; Kay, D.; Porritt, G. M.; Evans, P. L.; Golec, J. M.; Murcko, M. A.; Luong, Y.-P.; Raybuck, S. A.; Livingston, D. J. *Bioorg. Med. Chem. Lett.* **1994**, 4, 2359. (c) Graybill, T. L.; Dolle, R. E.; Helaszek, C. T.; Miller, R. E.; Ator, M.A. *Int. J. Peptide Protein Res.* **1994**, 44, 173.

7. Wilson, K. P.; Black, J. F.; Thompson, 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.
8. 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.
9. (a) Dolle, R. E.; Prouty, C. P.; Prasad, C. V. C.; Cook, E.; Saha, A.; Ross, T. M.; Salvino, J. M.; Helaszek, C. T.; Ator, M. A. *J. Med. Chem.* **1996**, *39*, 2438. (b) Golec, J. M.; Mullican, M. D.; Murcko, M. A.; Wilson, K. P.; Kay, D.; Jones, S. D.; Murdoch, R.; Bemis, G. W.; Raybuck, S. A.; Luong, Y.-P.; Livingston, D. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2181. (c) Semple, G.; Ashworth, D. M.; Baker, G. R.; Batt, A. R.; Baxter, A. J.; Benzies, D. W.; Elliot, L. H.; Evans, M.; Franklin, R. J.; Hudson, P.; Jenkins, P. D.; Pitt, G. R.; Rooker, D. P.; Sheppard, A.; Szelke, M.; Yamamoto, S.; Isomura, Y. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1337.
10. Dolle, R. E.; Prasad, C. V. C.; Prouty, C. P.; Salvino, J. M.; Awad, M. M. A.; Schmidt, S. J.; Hoyer, D.; Ross, T. M.; Graybill, T. L.; Speier, G. J.; Uhl, J.; Miller, B. E. Helaszek, C. T.; Ator, M. A. *J. Med. Chem.* **1997**, *40*, 1941.
11. Rotonda, J.; Nicholson, D. W.; Fazil, K. M.; Gallant, M.; Gareau, Y.; Labelle, M.; Peterson, E. P.; Rasper, D. M.; Ruel, R.; Vaillancourt, J. P.; Thornberry, N. A.; Becker, J. W. *Nat. Struct. Biol.* **1996**, *3*, 619.
12. Mittl, P. R. E.; Di Marco, S.; Krebs, J. F.; Bai, X.; Karanewsky, D. S.; Priestle, J. P.; Tomaselle, K. J.; Grutter, M. G. *J. Biol. Chem.* **1997**, *272*, 6539.
13. For example, see: Robl, J. A.; Sun, C.-Q.; Stevenson, J.; Ryono, D. E.; Simpkins, L. M.; Cimarusti, M. P.; Dejneka, T.; Slusarchyk, W. A.; Chao, S.; Stratton, L.; Misra, R. N.; Bednarz, M. S.; Assad, M. M.; Cheng, H. S.; Abboa-Offei, B. E.; Smith, P. L.; Mathers, P. D.; Fox, M.; Schaeffer, T. R.; Seymour, A. A.; Trippodo, N. C. *J. Med. Chem.* **1997**, *40*, 1570 and references therein.
14. For the synthesis of **8a-d**, see: Freidinger, Perlow, D. S.; Veber, D. F. *J. Org. Chem.* **1982**, *47*, 104. For the synthesis of **8d-e**, see Robl, J. A.; Cimarusti, M. P.; Simpkins, L. M.; Weller, H. N.; Pan, Y. Y.; Malley, M.; DiMarco, J. D. *J. Am. Chem. Soc.* **1994**, *116*, 2348. For the synthesis of **10a**, see: Etzkorn, F. A.; Guo, T.; Lipton, M. A.; Goldberg, S. D.; Bartlett, P. A. *J. Am. Chem. Soc.* **1994**, *116*, 10412. For the synthesis of **10b**, see: Thorsett, E. D.; *Actual. Chim. Ther.* **1987**, *13*, 257. For the synthesis of **10c**, see: Robl, J. A. *Tetrahedron Lett.* **1994**, *35*, 393.
15. Fluorescence enzyme assays utilizing recombinant mICE and hCPP32 enzymes were carried out essentially according to Thornberry (ref 1c) and Nicholson (ref 4c), respectively. The substrate is Ac-TryValAlaAsp-(7-amino-4-methylcoumarin) for the mICE assay and Ac-AspGluValAsp-(7-amino-4-methylcoumarin) for the hCPP32 assay. Enzyme reactions were run in ICE buffer (25 mM HEPES, 1 mM EDTA, 0.1% CHAPS, 10% sucrose, pH 7.5) containing 2 mM DTT at room temperature in duplicate.