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

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Abstract: A systematic study of interleukin-1β 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.

Introduction

Interleukin-1β converting enzyme (ICE, caspase-1)[1] catalyzes the cleavage of the biologically inactive 31KDa IL-1β precursor at Asp^{116}-Ala^{117} 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 data[1] 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 inhibitors[6] of caspase-1 based on the pro-IL-1β cleavage site $TyrValHisAsp^{116}$-Ala^{117}. The prototype Csp-1 inhibitor Ac-TyrValAlaAsp-H (**1**) has recently been co-crystallized with human Csp-1. Both the crystal structure[7] 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]

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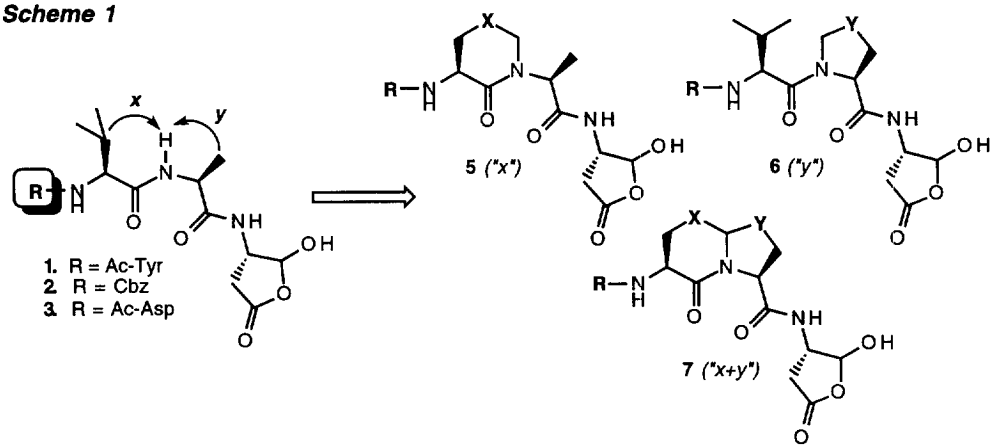
Scheme 1

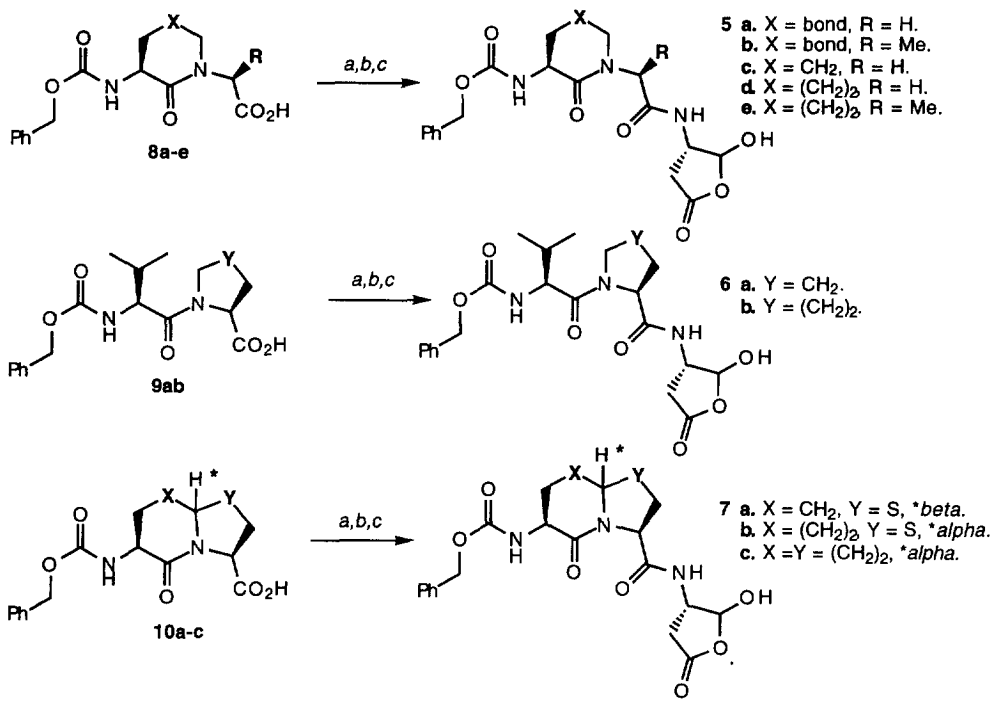


Workers at Merck have also reported[4a] a potent C-terminal aldehyde inhibitor of Csp-3 based on the cleavage site of poly(ADP-ribose) polymerase (PARP), a known intracellular substrate of the protease. This prototype Csp-3 inhibitor Ac-AspGluValAsp-H (**4**) is also a potent inhibitor of Csp-1 (see Table 1).[1d] We have observed that inhibitors of the general structure Ac-AspX^3X^2Asp-H where $X^3 \neq$ 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 **4**[11] as well as a related fluoromethylketone[12] 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 P_2 amide nitrogen has been "tied back" to either or both the P_3 and P_2 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 precedented 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 CH_2Cl_2 or CH_2Cl_2/DMF. Removal of the *t*-butyl ester and semicarbazone protecting groups by successive treatment with trifluoroacetic acid (TFA)/CH_2Cl_2/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**.

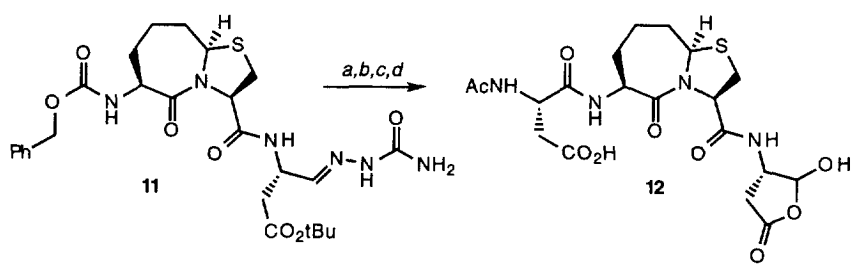
Scheme 2



(a) H-Asp(OtBu)-semicarbazone, EDAC, HOBt, CH_2Cl_2/DMF; (b) TFA/CH_2Cl_2/anisole (4/3/1); (c) 37% aq HCHO/AcOH/MeOH (1/1/3).

The P_4 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) H_2, 20% $Pd(OH)_2$-C, MeOH; (b) Ac-Asp(OtBu)OH, EDAC, HOBt, DMF; (c) TFA/CH_2Cl_2/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 activity[15] 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			
4	0.071	0.0012	**7a**	4.80	86.0
			7b	0.159	>10
5a	>10	>10	**7c**	0.036	>10
5b	3.70	>100			
5c	37.0	>100	**12**	10.4	0.018
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 inhibitors[8] of hCsp-1. We were surprised to find that both **6a** and **6b** showed enhanced hCsp-3 inhibitory activity[15] 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 μM while its fluoromethyl ketone counterpart (z-VAD-fmk) has Ki's against mCsp-1 and hCsp-8 of 0.015 μM and 0.018 μM, 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.

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