Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2969–2971

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Acyl Dipeptides as Reversible Caspase Inhibitors. Part 1: Initial Lead Optimization

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Received 31 January 2002; accepted 1 July 2002

Abstract—Parallel synthesis was used to explore the SAR of a peptidomimetic caspase inhibitor. The most potent compound had nanomolar activity against caspases 1, 3, 6, 7, and 8.
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Caspases[1] are a family of cysteine proteases with specificity for aspartic acid at the S1 subsite of the enzyme that are involved in both cytokine maturation and apoptosis.[2] Caspase-1 (interleukin-1β converting enzyme, ICE) is involved in the induction of inflammation by catalyzing the cleavage of the pro-form of IL-1β.[1c] Other caspases play a role in the regulation of apoptosis, either as apoptosis signaling molecules or as downstream effectors. Inhibition of caspases, either broad spectrum or caspase specific, could be of therapeutic value[3] in the treatment of inflammatory and degenerative diseases, such as rheumatoid arthritis, ALS, Alzheimer's disease, Parkinson's disease, stroke, and myocardial infarction. Up to now there have been few reports in the literature on caspase inhibitors, other than a significant body of work associated with caspase 1.[3]

Herein we describe a series of novel, potent, broad spectrum caspase inhibitors. Our starting point was the known peptide inhibitor, Ac-DEVD-H, which upon truncation to a Z-XXD-H tripeptide[4] retained potent, broad spectrum activity (Table 1, Fig. 1, compounds **2** and **3**). However, further truncation to a Z-XD-H dipeptide resulted in a significant loss in caspase inhibitory activity. Given this, we sought to prepare other truncated compounds containing a more active P3 surrogate in the context of an acyl dipeptide inhibitor. Using parallel synthesis, incorporating a resin bound

Table 1. Caspase activity of classical tetra- and tripeptides compared to acylated dipeptides

Compd		Caspase activity[a] IC_{50} (μM)				
		mCsp-1	Csp-3	Csp-6	Csp-7	Csp-8
1	Ac-DEVD-H	0.05	0.0035	0.01	0.01	0.08
2	Z-ELD-H	0.0065	0.0023	—	0.01	0.03
3	Z-FLD-H	0.043	0.137	2.53	0.68	8.7
4	Z-VD-H	5.85	1.75	10	6.81	11.96
5	Z-LD-H	18.82	3.47	14.03	21.9	50
6	2-Naphthyloxy acetyl-LD-H	10	0.94	18.56	8.87	10

[a]Assay conditions are described in ref 5.

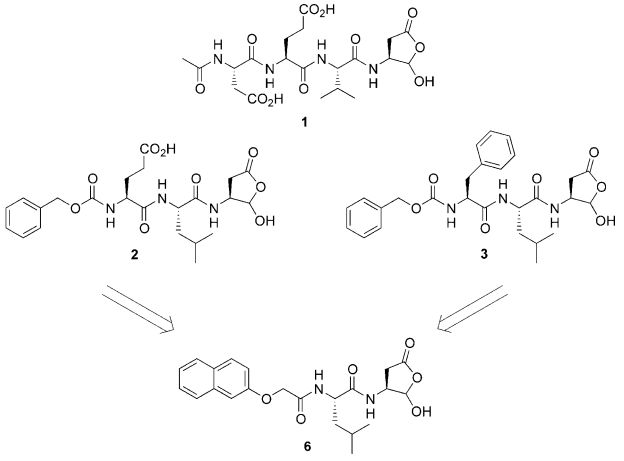

Figure 1. Tetra- and tripeptide inhibitors leading to an acylated dipeptide.

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PII: S0960-894X(02)00629-7

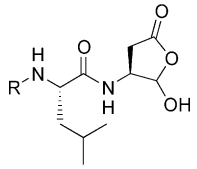


Figure 2.

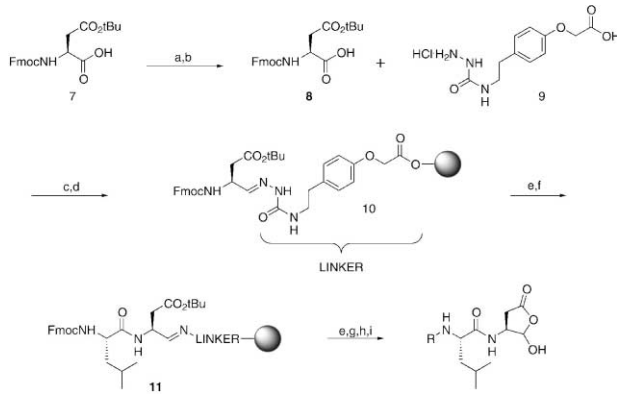
Fmoc-Leu-Asp(O*t*Bu)-semicarbazone,[5] we identified compound **6** as the basis for a more directed library of analogues.

In order to prepare a directed library of aspartyl aldehyde inhibitors, we employed a strategy utilizing an aldehyde linked to solid phase via a semicarbazone was employed (Scheme 1).[5a,b] Commercially available Fmoc-Asp (O*t*Bu)-OH was converted to the corresponding aldehyde via the Weinreb amide, then condensed with an aminoethylphenoxyacetic acid semicarbazide linker.[5a] The Fmoc protected aspartyl semicarbazone β-*t*-butyl ester could now be coupled to aminomethylpolystyrene resin,[5b] the amine deprotected, and then coupled to Fmoc protected leucine. Upon Fmoc deprotection, the resin-bound dipeptide aldehyde was coupled to a collection of carboxylic acids. Hydrolysis of the *tert*-butyl ester and subsequent cleavage[4,5a] from the resin afforded the desired library of acyl dipeptide aldehyde inhibitors (Table 2, Fig. 2).

Table 2 lists a set of diverse analogues designed to probe the SAR in the S3 caspase subsite. Naphthoyl (**14** and **15**) and naphthyl acetyl (**16** and **17**) analogues showed no significant improvement in potency over analogue **6**. A notable improvement in broad spectrum caspase inhibitory activity was seen with compound **18**, implying that orientation of the naphthyl ring is critical to binding with respect to compound **6**. The carbon isostere of **18** (**19**) showed a 3-fold or greater loss of activity across the panel of casapase isozymes. Addition of a methyl group in the alpha position of the (aryloxy)acetyl moiety as in **21** also resulted in a loss in caspase inhibitory activity. The sulfur isosteres of analogues **18** and **6** (**23** and **24**) were interesting in that **23** showed caspase 3 selectivity, while the naphthyl positional isomer showed improved

Table 2. Caspase activity of inhibitors from a directed parallel solid-phase synthesis

Compd	R	Caspase activity IC_{50} (μM)				
		mCsp-1	Csp-3	Csp-6	Csp-7	Csp-8
13	4-Biphenyl acetyl	7.43	5.06	> 10	> 10	> 10
14	1-Naphthoyl	2.30	> 10	> 10	> 10	> 10
15	2-Naphthoyl	1.74	6.74	> 10	> 10	> 10
16	1-Naphthyl acetyl	3.24	0.853	—	7.27	—
17	2-Naphthyl acetyl	3.14	0.849	11.11	> 10	12.7
18	1-Naphthyloxy acetyl	0.570	0.135	0.940	1.81	0.770
6	2-Naphthyloxy acetyl	10	0.944	18.56	8.87	> 10
19	3-(1-Naphthyl)propionyl	1.86	1.59	4.18	8.77	12.2
20	3-(1-Naphthyl)acryloyl	0.650	1.39	5.05	9.72	13.4
21	2-(1-Naphthyloxy)propionyl	3.99	0.376	1.28	1.32	2.43
22	2-(6-Bromo-1-naphthyloxy)-propionyl	6.84	4.81	13.8	32.4	29.1
23	1-Naphthylmercapto acetyl	2.75	0.195	1.43	1.74	7.42
24	2-Naphthylmercapto acetyl	0.792	0.269	3.16	2.52	11.0
25	4(2-Naphthyl) butyryl	1.80	2.76	14.5	18.2	> 50
26	3-(1-Naphthoyl)propionyl	0.408	0.967	11.8	11.3	11.2
27	3-(2-Napthoyl)propionyl	0.543	1.42	10.3	7.43	5.23
28	3-(1-Naphthyloxy)propionyl	0.686	0.059	0.305	1.37	9.81
29	3-(2-Naphthyloxy)propionyl	1.32	0.910	5.90	9.65	15.2
30	3-(1-Naphthylmercapto)propionyl	0.563	0.412	2.72	3.60	16.3
31	3-(2-Naphthylmercapto)propionyl	0.611	0.837	1.62	5.89	15.0
32	2-Methyl-1-naphthyoxy acetyl	0.843	0.375	32.4	4.16	4.14
33	4-Methoxy-1-naphthyloxy acetyl	0.831	0.263	22.6	4.08	1.45
34	4-Chloro-1-naphthyloxy acetyl	0.429	0.231	12.0	3.38	1.69
35	2,4-Dichloro-1-naphthyloxyacetyl	0.141	0.357	21.4	3.61	3.04
36	1-Isoquinolinyloxy acetyl	44.2	1.57	> 50	34.7	> 50
37	4-Quinolinyloxy acetyl	35.3	0.232	> 50	4.57	> 50
38	5-Quinolinyloxy acetyl	5.25	0.412	> 50	3.85	4.02
39	5-Isoquinolinyloxy acetyl	5.14	0.407	42.7	3.48	3.64
40	8-Quinolinyloxy acetyl	13.7	0.147	12.5	1.51	2.24
41	Phenyl	> 10	3.4	> 50	> 10	> 10
42	3-Phenoxy propionyl	9.42	0.419	> 50	6.04	> 10
43	Phenoxy acetyl	> 10	3.40	> 50	> 10	> 10
44	2-Biphenoxy acetyl	0.636	0.095	0.717	2.02	1.71
45	3-Biphenoxy acetyl	1.10	0.311	14.5	3.75	3.86
46	4-Biphenoxy acetyl	1.90	0.763	20.5	12.0	7.53
47	(2-Benzyl)phenoxy acetyl	0.521	0.490	10.1	3.36	6.05
48	(4-Benzyl)phenoxy acetyl	1.80	0.346	18.9	4.41	4.72
49	(4-Phenoxy)phenoxy acetyl	2.21	0.545	21.2	6.82	9.28
50	(2-Benzyloxy)phenoxy acetyl	2.40	0.222	9.75	2.20	4.34
51	(4-Benzyloxy)phenoxy acetyl	2.51	0.57	33.4	7.25	8.60
52	(2-Cyclopentyl)phenoxy acetyl	0.538	0.197	3.37	1.49	1.86
53	(4-Cyclopentyl)phenoxy acetyl	2.20	0.319	51.2	5.23	5.90
54	[2-(1-Adamantyl)-4-methyl]phenoxy acetyl	1.43	0.474	5.86	2.79	3.87
55	4-(1-Adamantyl)-phenoxy acetyl	1.83	0.528	32.5	8.24	4.35
56	5,6,7,8-Tetrahydro-1-naphthyloxy acetyl	1.81	0.324	11.8	2.74	1.75
57	5,6,7,8-Tetrahydro-2-naphthyloxy acetyl	2.57	0.162	28.6	2.31	4.95



Scheme 1. Reagents and conditions: (a) *N,O*-dimethylhydroxylamine·HCl (1.2 equiv), HOBt (1.2 equiv), EDAC (1.2 equiv), *N*-methylmorpholine (1.2 equiv), THF, 0 °C, 2 h, 16 h. 98%; (b) 1.0 M LAH in ether (0.5 equiv), ether, 0 °C, 1 h; (c) **9** (1.03 equiv), NaOAc (1.3 equiv), ethanol, 0 °C 3 h, 16 h, 69%; (d) aminomethylpolystyrene resin, pyBOP (1.5 equiv), DIEA (diisopropylethylamine) (3 equiv), THF/*N*-methylpyrolidinone (NMP) (1:1), 3 h; (e) piperidine, DMF (1:4), 1 h; (f) Fmoc-Leu-OH (2 equiv), pyBOP (3 equiv), DIEA (6 equiv), THF/NMP (1:1), 2.5 h; (g) carboxylic acid (3.75 equiv), pyBOP (3 equiv 0.25 M pyBOP in NMP), DIEA (6.25 equiv 0.5 M DIEA in NMP), 16 h; (h) TFA, CH_2Cl_2, anisole 4/3/1, 6 h; (i) 37% aq HCHO, AcOH, THF, TFA 1/1/5/0.025, 4 h, 30–88%.

caspase 1 activity (**24**). However, neither analogue had potencies against caspases 6 or 8 comparable to that of **18**. Further chain extension to analogue **25** and its naphthoyl derivatives (**26** and **27**) did not improve broad spectrum activity, although good caspase 1 inhibitory activity was seen with **26** and **27**. The homologues of **18** and **6** (**28** and **29**) have almost the same respective activities, except that **28** is more potent than **18** against caspase 3, but not as potent as **18** against caspase 8. The sulfur isosteres of **28** and **29** (**30** and **31**) had similar activity as **28** and **29**, except **30** was less potent against caspase 6 and **31** was more potent against caspase 1. Substituted naphthyloxy acetyl analogues showed good activity against caspases 1 and 3, but were not broad spectrum inhibitors. Quinolinyloxy and isoquinolinyloxy analogues were inactive as broad spectrum inhibitors, but showed caspase 3 selectivity. Some phenoxy and 5,6,7,8-tetrahydro-naphthyloxy acetyl analogues followed this same trend. Among this series of analogues, the (1-naphthyloxy)acetic acid, **18**, is the only inhibitor to have nanomolar activity against caspases 1, 3, 6 and 8.

It has been shown that the peptide AcDEVD-H inhibitor can be truncated to a novel aryloxyacetyl dipeptide, and retain nanomolar, broad spectrum caspase inhibitory activity. Further work has been done on this new structural class of caspase inhibitors and will be the subject of future publications.

Acknowledgements

The authors wish to thank Alfred Spada, Judy Dryden, and Silvio Roggo (Novartis Pharma, Ltd.) for their contributions in preparing this manuscript.

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