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Acyl Dipeptides as Reversible Caspase Inhibitors. Part 2: Further Optimization

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Abstract—A new structural class of broad spectrum caspase inhibitors was optimized for its activity against caspases 1, 3, 6, 7, and 8. The most potent compound had low nanomolar broad spectrum activity, in particular, single digit nanomolar inhibitory activity against caspase 8.

The caspase family[1] of cysteine proteases play a key role in both cytokine maturation and apoptosis[2] (programmed cell death). These are a unique set of proteases having a aspartic acid specificity at P1. Caspase inhibitors 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. Other than publications on caspase-1 inhibitors,[3] there have been few reports in the literature on caspase inhibitors, broad spectrum or specific.

As discussed in our previous publication, truncation of the known peptide inhibitor, Ac-DEVD-H (**1**) resulted in a substantial loss of inhibitory activity (Table 1, Fig. 1).[4] Truncated compounds containing a more active P3 surrogate in the context of an acyl dipeptide inhibitor were then prepared by parallel synthesis, utilizing a resin bound Fmoc-Leu-Asp(O*t*Bu)-semicarbazone.[5] Compound **5** was identified as a broad spectrum caspase inhibitor. Herein we describe further optimization of this structural class of caspase inhibitors in order to obtain analogues with potent, broad spectrum activity.

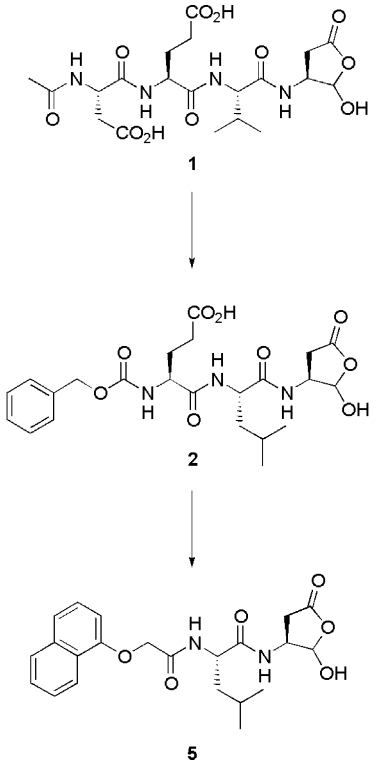

Figure 1. Tetra- and tripeptide inhibitors leading to an acylated dipeptide.

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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	2-Naphthyloxy acetyl-LD-H	10	0.94	18.56	8.87	10
5	1-Naphthyloxy acetyl-LD-H	0.570	0.135	0.940	1.81	0.770
6	Z-VD-H	5.85	1.75	10	6.81	11.96
7	Z-LD-H	18.82	3.47	14.03	21.9	50

[a]Assay conditions are described in ref 5.

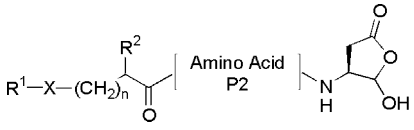


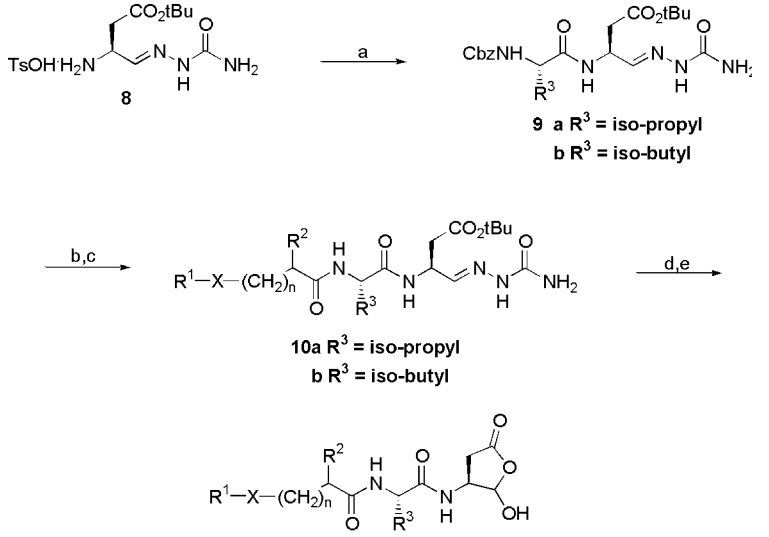
Figure 2.

Starting from an aspartyl aldehyde intermediate **8** originally reported by Graybill et al.,[6] either (*N*-benzyloxycarbonyl)leucine *N*-hydroxysuccinimide ester or (*N*-benzyloxycarbonyl)valine could be coupled[5] (see Scheme 1) to give the protected dipeptides **9ab**. After hydrogenolysis, the carboxylic acid of interest could be coupled[7–11] to give protected acyl dipeptides **10ab**. Hydrolysis of the semicarbazone[5,6] and *t*-butyl esters yielded the analogues listed in Table 2.

Table 2 examines the data for these optimized analogues. Extension of the naphthyloxy moiety deeper into the S4 pocket (**12**) did not improve potency. Analogues incorporating a P3 glutamic acid mimetic (**13** and **14**) showed a significant increase in activity against caspases 3, 6, 7, and 8 (the natural *S* isomer being the more potent), but a 5- to 10-fold loss of potency against caspase 1. Compounds **15**, **16**, and **17** show that caspase 3 and 8 activity can also be significantly increased by the proper placement of a carboxyl group on the naphthylene

Table 2. Caspase activity of optimized acyl dipeptide inhibitors

Compd	R^1	X	*n*	R^2	P2	Caspase activity IC_{50} (μM)				
						mCsp-1	Csp-3	Csp-6	Csp-7	Csp-8
5	1-Naphthyl	O	0	H	Leu	0.570	0.135	0.940	1.81	0.770
11	1-Naphthyl	O	0	H	Val	0.336	0.355	>10	2.10	1.20
12	1-Naphthyl	O	2	H	Val	0.25	4.4	4.6	37.03	19.59
13	1-Naphthyl	O	0	*R*-$(CH_2)_2CO_2H$	Leu	2.55	0.021	0.015	0.587	0.012
14	1-Naphthyl	O	0	*S*-$(CH_2)_2CO_2H$	Leu	4.86	0.0038	0.0035	0.130	0.031
15	1-CO_2H-2-naphthyl	O	0	H	Val	2.96	0.401	3.61	10.9	0.733
16	1-Naphthyl-2-CO_2H	O	0	H	Val	0.385	0.054	1.43	1.65	0.048
17	2-Naphthyl-3-CO_2H	O	0	H	Val	1.89	0.731	1.90	17.0	0.200
18	2-Naphthyl-3-$NHSO_2CF_3$	O	0	H	Val	2.18	1.92	3.03	43.66	1.42
19	1-Naphthyl-5-$NHSO_2CF_3$	O	0	H	Val	0.127	0.207	1.01	11.0	0.615
20	*1-Naphthyl*	*N*	*0*	*H*	*Leu*	*0.033*	*0.013*	*0.037*	*1.32*	*0.0076*
21	1-Naphthyl	N	0	CH_3	Leu	0.087	0.512	0.310	7.24	0.017
22	1-Naphthyl	N	0	H	Val	0.015	1.55	7.0	6.14	0.450
23	1,2-Diphenyl-ethyl	N	0	H	Leu	0.790	0.320	0.589	2.92	0.990
24	3-Phenoxy-phenyl	N	0	H	Leu	0.330	0.230	0.308	2.67	0.670



Scheme 1.[5] Reagents and conditions: (a) Val: Cbz-Val-OH (1 equiv), EDAC (1.5 equiv), HOBt (1.05 equiv), *N*-methylmorpholine (NMM) (1 equiv), CH_2Cl_2, 0 °C 2 h, 20 h, 93%; Leu: Cbz-Leu-OSu (1 equiv), TsOH·H-Asp(O*t*Bu)Sc (1.3 equiv), DIEA (1.4 equiv), CH_2Cl_2, 16 h; (b) H_2 (1 ATM), Pd/C 10%, EtOH, 1.5 h; (c) carboxylic acid (0.9–1 equiv), EDAC (1.5 equiv), HOBt (1.4 equiv), NMM (1.2 equiv), NMP/CH_2Cl_2 (1:1); (d) TFA, CH_2Cl_2, anisole 4/3/1, 4.5 h; (e) 37% aq HCHO, AcOH, THF, TFA 1/1//5/0.025, 4–16 h.

moiety of **5**. Among the carboxy-substituted naphthoxy acetyl derivatives studied, the 2-CO_2H, 1-naphthoxy acetyl analogue **16** showed the largest improvement in caspase 3 and 8 inhibitory activity. Use of a trifluoromethyl sulfonamide as a carboxylic acid isostere (**18**) gave a compound (**17**) with a caspase inhibitory profile similar to its carboxylic acid counterpart. This substituent in the 5-position gained some broad spectrum activity, but was not as potent as compound **16**. The aminonaphthalene analogue of compound **5** (**20**) showed the best overall broad spectrum activity, with excellent potency against caspase 8. The valine analogue, **22**, showed caspase 1 and 8 selectivity, more than was seen with the Leu and Val analogues of the napthyloxy series, **5** and **11**. Other phenyl based derivatives showed good broad spectrum activity, but were not as potent as **20**. Results from our modeling studies suggest that the aryl ether oxygen of compound **5** may occupy the space occupied by the P3 amide-NH of tetrapeptide inhibitors. An X-ray structure of Ac-DVAD-fmk bound to caspase 3 reveals that this N–H forms a hydrogen bond with the carbonyl oxygen of Arg-207.[12] Therefore, the enhanced potency of compound **20** could be due to increased binding of the inhibitor due to this additional hydrogen bonding interaction (Fig. 2).

It has been shown that using solid-phase, parallel synthesis[4] followed by a focused SAR study that AcDEVD-H can be truncated to an arylamino-acyl dipeptide and retain good broad spectrum caspase inhibitory activity. Further work in this area will be the subject of future publications.

Acknowledgements

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7. Compound **12** was prepared on solid phase employing the same method in Scheme 1, using Fmoc-Valine and 4-(1-naphthyloxy)butyric acid.[5]
8. The carboxylic acid used to make compound **13** was prepared from D-glutamic acid in the following manner:[5] (a) KBr (3 equiv), 2.5 N H_2SO_4, $NaNO_2$ (1.7 equiv), 0 °C 1 h, 45 min; (b) CH_2N_2 (excess generated from 1-methyl-3-nitro-1-nitrosoguanidine and 40% KOH), Et_2O, 0 °C, 51% over 2 steps; (c) 1-naphthol (1.1 equiv), K_2CO_3 (1.5 equiv), DMF, 3.5 h 92%; (d) H_2 (1 atm), Pd/C 10%, methanol, 1.5 h; (e) 2,4,6-trichlorobenzoyl chloride (1.3 equiv), triethylamine (TEA) (1.6 equiv), THF, 18 h; (f) *t*-butanol, dimethylaminopyridine (DMAP), CH_2Cl_2, 3.5 h, 82% overall; (g) 1 N LiOH (1.3 equiv), 3/1 1,4-dioxane/H_2O, 0 °C 30 min, 1.25 h, used as crude material in coupling. The carboxylic acid used to make compound **14** was prepared from L-glutamic acid following the preceding method.
9. The carboxylic acids used to make compounds **15**, **16**, and **17** were prepared from 1-carbomethoxy-2-naphthol, 2-carbomethoxy-1-naphthol, and 3-carbomethoxy-2-naphthol, respectively, in the following manner:[5] (a) *t*-butyl bromoacetate (1 equiv), K_2CO_3 (3 equiv), DMF, 18 h; (b) TFA/H_2O (9/1), anisole, CH_2Cl_2, 16 h. The carbomethoxy groups were hydrolyzed (1 N LiOH (1.1 equiv) in 3/1 1,4-dioxane/ H_2O, 4 h) after the peptide coupling and before the last 2 deprotection steps.
10. The carboxylic acids used to make compounds **18** and **19** were prepared from 3-amino-2-naphthol and 5-amino-1-naphthol, respectively, in the following manner:[5] Compound **18**: (a) *t*-butyl bromoacetate (1 equiv), K_2CO_3 (3 equiv), DMF, 18 h, used crude; (b) trifluoromethanesulfonic anhydride (1.2 equiv), TEA (1.2 equiv), CH_2Cl_2, −78 °C 30 min, 1 h, 72% overall; (c) TFA/H_2O (9/1), anisole, CH_2Cl_2, 16 h, 92%. Compound **19**: (a) methyl bromoacetate (1.2 equiv), K_2CO_3 (3 equiv), DMF, 18 h, used crude; (b) trifluoromethanesulfonic anhydride (1.2 equiv), TEA (1.2 equiv), CH_2Cl_2, −78 °C 30 min, 1 h, used crude; (c) 1 N LiOH (2.2 equiv), 16 h, 73% overall.
11. The carboxylic acids used to make compounds **20** and **21** were prepared in the following manner:[5] (a) Compound **20**: 1-aminonaphthalene, methylbromoacetate (1.6 equiv), TEA (1.1 equiv), DMF, 60 h, used crude; Compound **21**: 1-aminonaphthalene, ethyl 2-bromopropionoate (1.1 equiv), TEA (1.1 equiv), DMF, 60 °C 18 h, used crude; (b) 1 N LiOH (1–1.2 equiv), 1,4-dioxane, 1.5 h. Overall yields: compound **20**: 51%, compound **21**: 70%.
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