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Structure–Activity Relationships within a Series of Caspase Inhibitors: Effect of Leaving Group Modifications

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Abstract—Various aryloxy methyl ketones of the 1-naphthyloxyacetyl-Val-Asp backbone have been prepared. A systematic study of their structure–activity relationship (SAR) related to caspases 1, 3, 6, and 8 is reported. Highly potent irreversible broad-spectrum caspase inhibitors have been identified. Their efficacy in cellular models of cell death and inflammation are also discussed.

Caspase 1 (Interleukin-1β Converting Enzyme, ICE) is a cysteine protease localized primarily in monocytes. In addition to promoting the pro-inflammatory and immunoregulatory properties of IL-1β, caspase 1 and its homologues, are involved in the regulation of apoptosis.[1] These caspases (**c**ysteinyl **asp**artic prote**ases**) are thought to be associated with the regulation of apoptosis in degenerative diseases. Therefore, inhibitors of the ICE/ced-3 family of caspases may be useful therapeutic targets for the treatment of infectious diseases; respiratory diseases; inflammatory conditions, such as arthritis and hepatitis; reperfusion injury, such as myocardial infarction, stroke, and ischemic kidney disease; and neurodegenerative diseases, such as Parkinson's and Alzheimer's disease (Fig. 1).[2]

Caspase inhibitors represent a class of compounds useful for the control of the previously stated disease states.

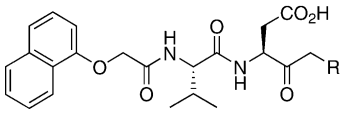

Figure 1.

The majority of the published research in the area of caspase inhibition has been focused on the use of caspase 1 inhibitors as anti-inflammatory agents.[3] Several peptide and peptidyl caspase inhibitors have been reported in the literature.[4] Previously, we were able to truncate known tetra- and tripeptide inhibitors to an acyl dipeptide of general structure **7**[5,6] (Scheme 1). Herein we describe modification and analysis of the terminal warhead functionality (the electrophilic moiety that covalently interacts with the active site of the enzyme) on our 1-naphthyloxyacetyl-Val-Asp inhibitor backbone (Table 1)[7,8] Our main focus was modification of the phenol leaving group of **3** to develop novel, potent, irreversible broad-spectrum caspase inhibitors. We examined cellular activity in the following assays: Con A,[9] a model of neuronal apoptosis involving cortical neurons exposed to concanavalin A; Monocyte,[10] an inflammation model measuring of IL-1β secretion from isolated human moncytes induced with Staph A; and Jurkat,[11] a Fas-induced model of apoptosis.

Preparation of the acyl dipeptide aryloxy methyl ketones is described in Scheme 1. Acyl dipeptide **5** was prepared from 1-naphthyloxyacetic acid and L-valinyl-L-asparyl-β-(*tert*-butyl ester) methyl ester[5] using ethyl-(dimethylaminopropyl)carbodiimide (EDAC)/1-hydroxybenzotriazole (HOBt) coupling conditions in CH_2Cl_2 followed by ester hydrolysis with lithium

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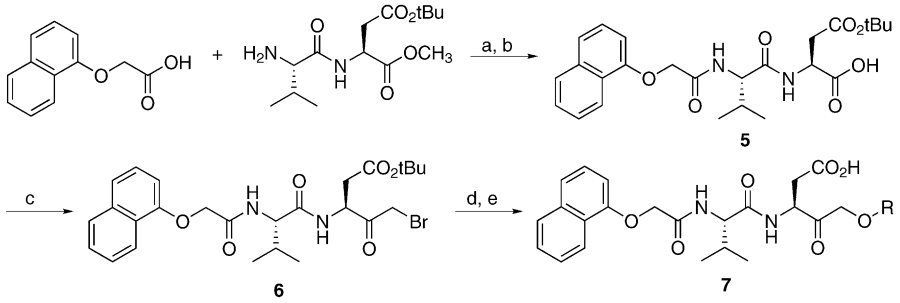

Scheme 1. Reagents: (a) EDAC (1.5 equiv), HOBt (1.05 equiv), *N*-methylmorpholine (NMM) (1 equiv), CH_2Cl_2, 0 °C 2 h, 20 h, 83%; (b) LiOH (1 equiv), dioxane/H_2O (3:1), 1 h, 99%; (c) (i) isobutyl-chloroformate (1.05 equiv), NMM (1.5 equiv), THF, −20 °C, 20 min; (ii) CH_2N_2; (iii) HBr (50%, H_2O), Et_2O, 0 °C, 30 min, 71%; (d) KF (2.5 equiv), NaI (0.1 equiv), ROH (1 equiv), DMF, 40–80%; (e) TFA/CH_2Cl_2/anisole (4/3/1), 99%.

Table 1. Initial warhead analysis

Compd	R	Enzyme assays[a] k_3/K_i (M^{-1} s^{-1})				Cellular assays IC_{50} (μM)		
		mCsp-1[b]	Csp-3	Csp-6	Csp-8	Con A	Monocyte	Jurkat
1	F	129,000	207,000	36,842	71,667	3.05	0.95	0.24
2	OCO(2,6-Cl_2-Ph)	52,109	725,222	56,883	17,010	2.15	11.50	10.61
3	OPh	3630	19,152	2399	4258	38.00	11.17	13.95
4	$OP(O)(Ph)_2$	142,948	350,785	39,714	59,312	—	3.48	18.42

[a]Assay conditions are described in ref 17.
[b]Murine caspase 1.

hydroxide in dioxane/water (3:1). Bromomethyl ketone **6** was obtained by treatment of the mixed anhydride of **5** with diazomethane[12] followed by displacement of the azide with hydrobromic acid. Aryloxy methyl ketones were synthesized by treating **6** under modified Finkelstein[13] conditions with the appropriate alcohol in DMF. Removal of the *t*-butyl ester protecting group with trifluoroacetic acid (TFA)/CH_2Cl_2/anisole (4/3/1) gave, after trituration in diethyl ether, the target inhibitors of general structure **7** in overall yields of 23–46%.

Analogues of the phenyl group of **3** led to numerous potent irreversible broad-spectrum inhibitors (Table 2). Changing the phenyl to a naphthyl group (entries **8**, **9**) provided interesting results. Analogue **9**, the 2-naphthyl derivative, exhibited a decrease in potency in both cellular and enzyme assays. However, the 1-naphthyl analogue **8** provided a significant increase in activity in caspase 1 and 3 activity, as well as potent activity in the Con A cellular assay. It should be noted that both naphthyl derivatives are reversible inhibitors of caspases 6 and 8. The difference in binding affinities of these two derivatives for caspase 1 ($K_i = 0.070$ and 3.17 μM, **8** and **9**, respectively) and caspase 3 ($K_i = 0.0071$ and 0.09 μM, **8** and **9**, respectively) suggests the warhead pocket (P1′) may not be able to accommodate a linear bulky group. The 4-biphenyl derivative, **10**, had similar results to that of the 2-naphthyl derivative, **9** (caspase 1 $K_i = 2.68$ and 3.17 μM; caspase 3 $K_i = 0.1$ and 0.09 μM, **10** and **9**, respectively).

Substituted phenyl analogues (entries **11**–**17**) provided little or no improvement over the parent phenyl ether **3** in the enzymes studied, and also dramatically reduced the cellular activity. Similarly, the use of hetero-substituted (O, N) phenols (entries **18**–**25**) provided no improvement in potency, with the exception of the addition of a nitro group (entries **24**, **25**), which modestly increased cellular potency in the Con A enzyme assay. Although there is some selectivity expressed towards caspase 3, the substituted phenols examined here (entries **11**–**25**) do not allow for broad-spectrum activity. This may be due to differences in S_1' homology between the caspases.[14]

The incorporation of fluorine[15] was envisioned to increase cellular permeability and therefore increase activity as well as modify the electronics of the phenol without altering its size. Mono-substitution of the phenyl ring with fluorine (entries **26**–**28**) had little effect on enzyme activity and had a detrimental effect on the inhibitors activity in the Jurkat cellular assay. The substitution pattern of the difluorinated analogues proved to be important for cellular activity. The 2,6 analogue (entry **32**) maintained the enzymatic potency of the parent and provided an increase in the cellular assays (Con A and Jurkat). The trifluoro analogues exhibited similar results with the 2,3,6 and 2,4,6 analogues (entries **35** and **39**) giving the largest improvements in the enzyme and cellular assays. This led us to the tetra- and pentafluoro analogues (entries **40**, **41**), which proved to be potent caspase inhibitors with a moderate improvement in both the enzyme (up to 25-fold, caspase 6) and cellular assays studied. The use of chlorine appears to give similar results as with fluorine (entry **32** vs **42**).

In conclusion, we found that the size, lipophilicity and binding affinity[16] of the warhead play important roles in the overall inhibitory activity of these compounds, in terms of caspase potency and selectivity. The number and substitution pattern of fluorines on the phenol has an impact on the SAR. We also found that some of

Table 2. Phenol analogues

Compd	R	Enzyme Assays[a] k_3/K_i ($M^{-1}s^{-1}$)				Cellular Assays IC_{50} (μM)		
		mCsp-1[b]	Csp-3	Csp-6	Csp-8	Con A	Monocyte	Jurkat
3	OPh	3630	19152	2399	4258	38	11.17	13.95
8	O(1-Naphthyl)	354,241	1,958,905	0[c]	0[c]	2.65	19	>100
9	O(2-Naphthyl)	263	7521	0[c]	0[c]	>50	15.90	>100
10	O(4-Ph-Ph)	1554	9993	270	1311	>50	38.70	87.85
11	O(2-CF_3-Ph)	5631	19,960	494	75	>50	17.33	>100
12	O(3-CF_3-Ph)	2452	7976	0[c]	150	>50	16.61	>100
13	O(4-CF_3-Ph)	2582	13,240	77	196	>50	12.28	>100
14	O(3,5-di-CF_3-Ph)	783	5352	193	68	>50	—	>100
15	O(4-CN-Ph)	3725	10,207	46	48	>50	26.65	>100
16	O(4-PhCO-Ph)	1522	17,717	0[c]	21	—	—	>100
17	O(4-$CONH_2$-Ph)	3654	17,735	744	0[c]	>50	—	>100
18	O(3,4-OCOS-Ph)	2238	5889	81	130	—	25.65	>100
19	O(4-PhO-Ph)	1901	9524	370	668	>50	18.99	12.65
20	O(3-CF_3O-Ph)	1409	30,612	0[c]	359	>50	15.39	>100
21	O(4-CF_3O-Ph)	2365	10,478	776	2423	>50	—	29.05
22	O(4-(4′-CF_3-PhO)Ph)	1454	6931	625	1124	>50	24.88	9.3
23	O(3-NHAc-Ph)	612	4898	0[c]	0[c]	>50	—	>100
24	O(2-NO_2-Ph)	23,479	73,023	10721	1811	5.85	27.65	>100
25	O(4-NO_2-Ph)	6130	8403	117	137	13.5	22.81	>100
26	O(2-F-Ph)	6019	26,835	0[c]	233	15	15.73	>100
27	O(3-F-Ph)	2732	17,813	48	461	>50	11.96	>100
28	O(4-F-Ph)	9119	8561	0[c]	699	>50	14.26	>100
29	O(2,3-F_2-Ph)	14,780	28,827	1610	197	5.20	—	–
30	O(2,4-F_2-Ph)	8401	21,739	0[c]	628	>50	17.58	33.17
31	O(2,5-F_2-Ph)	12,123	49,024	1330	483	4.85	19.67	>100
32	O(2,6-F_2-Ph)	53,875	200,052	9979	9075	2.40	>100	2.04
33	O(3,4-F_2-Ph)	1624	6460	86	117	17.00	16.18	>100
34	O(3,5-F_2-Ph)	2168	9242	106	154	11.55	15.89	>100
35	O(2,3,6-F_3-Ph)	47,081	187,700	81,749	16,972	2.433	—	1.195
36	O(2,3,4-F_3-Ph)	11,905	35,802	253	769	>50	20.63	35.79
37	O(2,3,5-F_3-Ph)	14,739	60,109	1508	1469	3.75	11.15	30.00
38	O(2,4,5-F_3-Ph)	11,450	24,390	634	668	4.16	16.67	39.31
39	O(2,4,6-F_3-Ph)	20,681	114,035	5144	9076	2.35	14.87	6.84
40	O(2,3,5,6-F_4-Ph)	77,528	267,953	78,887	30,173	2.17	6.25	4.50
41	O(2,3,4,5,6-F_5-Ph)	14,675	56,615	13,576	8040	—	8.89	3.05
42	O(2,6-Cl_2-Ph)	25,259	178,629	87,715	2713	2.75	15.32	3.00
43	O(F_9-4-biphenyl)	6683	52,114	26,975	10,407	>50	40.02	20.00
44	O(2-F-3-CF_3-Ph)	7225	33,883	683	1027	3.70	18.18	50.42
45	O(2-F-4-NO_2-Ph)	12,271	36,477	1769	1851	6.20	15.78	30.33

[a]Assay conditions are described in ref 17.
[b]Murine caspase 1.
[c]Indicates Reversible Inhibition.

these compounds were reversible inhibitors of caspases 6 and/or 8. Also, the incorporation of fluorine onto an inhibitor (entries **43**, **44**, and **45** compared to **10**, **12**, and **25**, respectively) can increase their cellular potency. Optimization of the phenol portion of the warhead provided several potent broad-spectrum irreversible caspase inhibitors with low micromolar activity in the cellular models studied. The optimization of other warheads will be the topic of future publications.

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References and Notes

1. (a) Yuan, J.; Shaham, J.; Ledoux, S.; Ellis, H. M.; Horvitz, H. R. *Cell* **1993**, *75*, 641. (b) Miura, M.; Zhu, H.; Rotello, R.; Hartwieg, E. A.; Yuan, J. *Cell* **1993**, *75*, 653. (c) Nett-Giordalisi, M. A.; Berson, D. R.; Chaplin, D. D. *J. Cell. Biochem.* **1993**, *17B*, 117.
2. (a) Marx, J.; Baringa, M. *Science* **1993**, *259*, 760. (b) Gagliardini, V.; Fernandez, P.-A.; Lee, R. K. K.; Drexler, H. C. A.; Rotello, R. J.; Fishman, M. C.; Yuan, J. *Science* **1994**, *263*, 826.
3. Talanian, R. V.; Brady, K. D.; Cryns, V. L. *J. Med. Chem.* **2000**, *43*, 3351.
4. (a) Lee, D.; Long, S. A.; Murray, J. H.; Adams, J. L.; Nutall, M. E.; Nadeau, D. P.; Kikly, K.; Winkler, J. D.; Sung, C.-M.; Ryan, M. D.; Levy, M. A.; Keller, P. M.; DeWolf, W. E. *J. Med. Chem.* **2000**, *44*, 2015. (b) Guo, Z.-M.; Xian, M.; Zhang, W.; McGill, A.; Wang, P. G. *Bioorg. Med. Chem.* **2001**, *9*, 99. (c) Shahripour, A. B.; Plummer, M. S.; Lunney, E. A.; Sawyer, T. K.; Stankovic, C. J.; Connolly, M. K.; Rubin, J. R.; Walker, N. P. C.; Brady, K. D.; Allen, H. J.; Talanian, R. V.; Wong, W. W.; Humblet, C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2779. (d) Shahripour, A. B.; Plummer, M. S.; Lunney, E. A.; Albrecht, H. P.; Hays, S. J.; Kostlan, C. R.; Sawyer, T. K.; Walker, N. P. C.; Brady, K. D.; Allen, H. J.; Talanian, R. V.; Wong, W. W.; Humblet, C. *Bioorg. Med. Chem. Lett.* **2002**, *10*, 31. (e) For a review of the caspase inhibitor patent literature, see: Ashwell, S. *Expert Opin. Ther.*

Pat. **2001**, *11*, 1593. (f) Han, B. H.; Xu, D.; Choi, J.; Yongxin, H.; Xanthoudakis, S.; Roy, S.; Tam, J.; Vaillancourt, J.; Colucci, J.; Siman, R.; Giroux, A.; Robertson, G. S.; Zamboni, R.; Nicholson, D. W.; Holtzman, D. M. *J. Biol. Chem*. **2002**, *277*, 30128. (g) Choong, I. C.; Lew, W.; Lee, D.; Pham, P.; Burdett, M. T.; Lam, J. W.; Wiesmann, C.; Luong, T. N.; Fahr, B.; DeLano, W. L.; McDowell, R. S.; Allen, D. A.; Erlanson, D. A.; Gordon, E. M.; O'Brien, T. *J. Med. Chem*. **2002**, *45*, 5005.

5. Karanewsky, D. S.; Kalish, V. J.; Robinson, E. D.; Ullman, B. R. US Patent 6,242,422 B1, 2001.

6. Linton, S. D.; Karanewsky, D. S.; Chen, N.; Guo, X.; Jahangiri, K. G.; Kalish, V. J.; Meduna, S. P.; Robinson, E. D.; Ullman, B. R.; Wu, J.; Nalley, K.; Smidt, R.; Diaz, J.-L.; Aja, T.; Herrmann, J.; Tomaselli, K. *J. Bioorg. Med. Chem. Lett*. **2002**, *12*, 2973.

7. (a) 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. (b) 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.

8. (a) Krantz, A.; Copp, L. J.; Coles, P. J.; Smith, R. A.; Heard, S. B. *Biochemistry* **1991**, *30*, 4678. (b) Thornberry, N. A.; Peterson, E. P.; Zhao, J. J.; Howard, A. D.; Griffin, P. R.; Chapman, K. T. *Biochemistry* **1994**, *33*, 3934. (c) Wagner, B. M.; Smith, R. A.; Coles, P. J.; Copp, L. J.; Ernest, M. J.; Krantz, A. *J. Med. Chem*. **1994**, *37*, 1833.

9. Deckwerth, T. L.; Adams, L. M.; Wiessner, C.; Allegrini, P. R.; Rudin, M.; Sauter, A.; Hengerer, B.; Sayer, R. O.; Rovelli, G.; Aja, T.; May, R.; Nalley, K.; Karanewsky, D. S.; Wu, J. C.; Roggo, S.; Schmidt, A.; Contreras, P. C.; Tomaselli, K. J. *Drug Devel. Res*. **2001**, *52*, 579.

10. (a) For similar assays see: Bleeker, M. W. P.; Netea, M. G.; Kullberg, B. J.; van der Ven-Jongekrijgg, J.; van der Meer, J. W. M. *Immunology* **1997**, *91*, 548. (b) Hamon, Y.; Luciani, M.-F.; Becq, F.; Verrier, B.; Rubartelli, A.; Chimini, G. *Blood* **1997**, *90*, 2911.

11. Armstrong, R. C.; Aja, T.; Xiang, J.; Gaur, S.; Krebs, J. F.; Hoang, K.; Bai, X.; Korsmeyer, S. J.; Karanewsky, D. S.; Fritz, L. C.; Tomaselli, K. J. *J. Biol. Chem*. **1996**, *271*, 16850.

12. Arndt, F. *Org. Syn*. **1943**, *2*, 165 (Coll. Vol.).

13. March, J. In *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 4th ed.; John Wiley & Sons: New York, 1992; p 430, and references therein.

14. Stennicke, H. R.; Renatus, M.; Meldal, M.; Salvesen, G. S. *Biochem. J*. **2000**, *350*, 563.

15. Park, B. K.; Kitteringham, N. R.; O'Neill, P. M. *Ann. Rev. Pharmacol. Toxicol*. **2000**, *41*, 443.

16. Thornberry, N. A.; Peterson, E. P.; Zhao, J. J.; Howard, A. D.; Griffin, P. R.; Chapman, K. T. *Biochemistry* **1994**, *33*, 3934.

17. Wu, J. C.; Fritz, L. C. *Methods Companion Methods Enzymol*. **1999**, *17*, 320.