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Structure–activity relationships within a series of caspase inhibitors. Part 2: Heterocyclic warheads

Brett R. Ullman,[a,*] Teresa Aja,[a] Ning Chen,[a] Jose-Luis Diaz,[a] Xin Gu,[a] Julia Herrmann,[a] Vincent J. Kalish,[a] Donald S. Karanewsky,[a] Lalitha Kodandapani,[a] Joseph J. Krebs,[a] Steven D. Linton,[a] Steven P. Meduna,[a] Kip Nalley,[a] Edward D. Robinson,[a] Silvio P. Roggo,[b] Robert O. Sayers,[a] Albert Schmitz,[c] Robert J. Ternansky,[a] Kevin J. Tomaselli[a] and Joe C. Wu[a]

[a]*Idun Pharmaceuticals, Inc., 9380 Judicial Drive, San Diego, CA 92121, USA*
[b]*Discovery Technologies, Novartis Institutes for BioMedical Research, Novartis Pharma AG, 4002 Basel, Switzerland*
[c]*Neuroscience Research, Novartis Institutes for BioMedical Research, Novartis Pharma AG, 4002 Basel, Switzerland*

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Abstract—Various heterocyclic hetero-methyl ketones of the 1-naphthyloxyacetyl-Val-Asp backbone have been prepared. A study of their structure–activity relationship (SAR) related to caspase-1, -3, -6, and -8 is reported. Their efficacy in a cellular model of cell death is also discussed. Potent broad-spectrum caspase inhibitors have been identified.

Apoptosis (programmed cell death) is a highly regulated biological process involved in maintaining normal tissue homeostasis. Caspases (cysteinyl aspartate-specific proteases) play a critical role in the regulation of inflammation, apoptosis, and cytokine maturation.[1,2] Caspases can be classified into two subfamilies with distinct functions: apoptosis, caspase-2, 3, -6, -7, -8, -9, -10, and -12 and cytokine activation, caspses-1, -4, and -5.[3]

Therefore, inhibitors of the caspase family of cysteine proteases may be useful therapeutic agents for the treatment of inflammatory conditions such as arthritis and hepatitis; reperfusion injuries such as myocardial infarction and ischemic kidney disease; neurodegenerative diseases such as Huntington's, Parkinson's, and Alzheimer's disease; and for the treatment of stroke.[4] Such inhibitors may also be useful for the prolonging of organ viability for use in transplantation.[5]

Inhibitors of caspases represent a class of compounds useful for the control of the previously stated disease states. Early research in this area has been focused on the use of caspase-1 inhibitors as anti-inflammatory agents and more recently research has focused on pan caspase inhibitors and small molecule inhibitors of caspase-3.[6]

Previously, we described use and SAR of phenoxymethyl ketone warheads on a naphthyloxy acyl dipeptide backbone.[7] Herein, we describe modification and analysis of heteroaryl hetero-methyl ketones as the warhead functionality (the electrophilic moiety that covalently interacts with the active site of the enzyme) on the same naphthyloxy-Val-Asp backbone (Fig. 1). Cellular activity in the Jurkat (JFas) assay,[8] a Fas-induced model of apoptosis, is also discussed.

Preparation of the acyldipeptide aryloxy or arylthio methyl ketones is described in Scheme 1. Dipeptide **1** was prepared from 1-naphthyloxyacetic acid and L-valyl-L-asparyl-β-(*tert*-butyl ester)methyl ester **2**[9] using ethyl-(dimethylaminopropyl)carbodiimide (EDAC)/1-hydroxybenzotriazole (HOBt) coupling conditions in

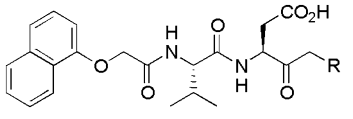

Figure 1.

* Corresponding author. Tel.: +858 646 8188, fax: +1 858 625 2677; e-mail: bullman@idun.com

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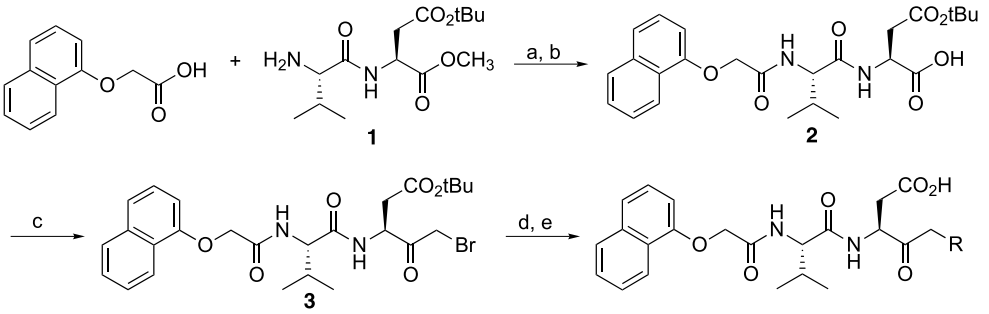
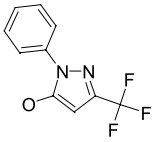
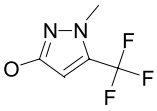
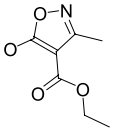
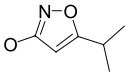
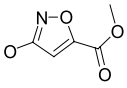
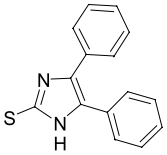
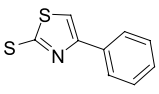


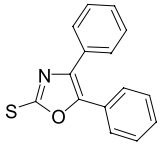
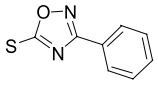
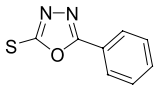
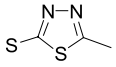
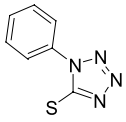
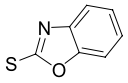
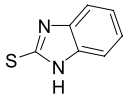
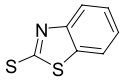
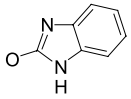
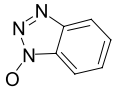
Scheme 1. Reagents and conditions: (a) EDAC (1.5 equiv.), HOBt (1.05 equiv.), *N*-methylmorpholine (NMM) (1 equiv.), CH_2Cl_2, 0 °C 2 h, 20 h, 83%; (b) LiOH (1 equiv.), dioxane/H_2O (3:1), 1 h, 99%; (c) (i) Isobutyl-chloroformate (1.05 equiv.), NMM (1.5 equiv.), THF, $^{-}$20 °C, 20 min, (ii) CH_2N_2,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

Table 1. Warhead analysis of five-membered heterocycles

Compound	R	Enzyme assays[a] k^3/K_i (M^{-1} s^{-1})				Cellular assay IC_{50} (μM)
		mCsp-1[b]	Csp-3	Csp-6	Csp-8	Jurkat
4		7,572	71,138	23,909	2,266	28
5		9,396	35,478	509	295	46
6		1,445	5,674	0[c]	1,141	>200
7		3,671	21,844	220	279	>200
8		42,463	143,850	5,804	6,344	71
9		173	850	22	0[c]	>200
10		1,201	11,546	570	212	>200
11		199	1,391	22	0[c]	>200
12		186	2,295	228	231	>200
13		217	2,731	0[c]	0[c]	>200

(*continued on next page*)

Table 1 (*continued*)

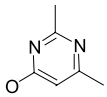
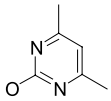
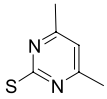
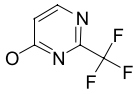
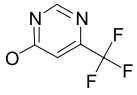
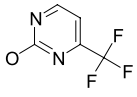
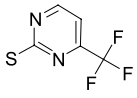
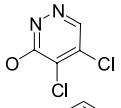
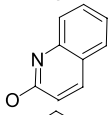
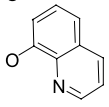
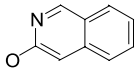
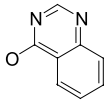
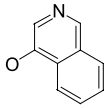
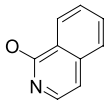
Compound	R	Enzyme assays[a] k^3/K_i (M^{-1} s^{-1})				Cellular assay IC_{50} (μM)
		mCsp-1[b]	Csp-3	Csp-6	Csp-8	Jurkat
14		200	2,010	0[c]	218	>200
15		1,191	38,119	975	3,059	102
16		280	4,798	110	276	150
17		115	401	17	0	>200
18		180	2,259	221	293	>200
19		5,477	5,239	83	0[c]	190
20		400	19,186	224	0[c]	153
21		3,629	34,433	2,150	6506	>200
22		0[c]	0[c]	0[c]	0[c]	>200
23		168	1,769	25	10	>200
24		202	3,569	0[c]	0[c]	>200
25		41	958	0[c]	2	>200
26		28,324	121,491	8,534	14,518	>200

[a] Assay conditions are described in Ref. 13.
[b] Murine caspase 1.
[c] Indicates reversible inhibition.

methylene chloride followed by ester hydrolysis with lithium hydroxide in dioxane/water (3:1). Bromomethyl ketone **3** was obtained by treatment of the mixed anhydride of **2** with diazomethane[10] followed by reaction of the diazo ketone with hydrogen bromide. Warheads were attached by treating **3** with the appropriate alcohol or thiol, sodium iodide, and potassium fluoride in DMF under the modified Finkelstein[11] conditions. Removal of the *t*-butyl ester protecting group with trifluoroacetic acid produced the target inhibitors, after trituration in diethyl ether.

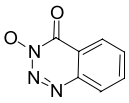
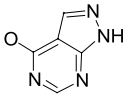
In our continuing focus on warhead optimization, we synthesized various heterocyclic hetero-methyl ketones.

Table 2. Warhead analysis six-membered heterocycles

Compound	R	Enzyme assays[a] k_3/K_i (M^{-1} s^{-1})				Cellular assay IC_{50} (μM)
		mCsp-1[b]	Csp-3	Csp-6	Csp-8	Jurkat
27		138	733	5	8	>200
28		0[c]	348	0[c]	0[c]	ND
29		0[c]	0[c]	0[c]	0[c]	>200
30		1,326	7,484	903	367	>200
31		10	283	0[c]	0[c]	ND
32		950	7,130	656	120	>200
33		24,834	113,766	5,147	3,199	37
34		479	1,752	156	0[c]	>200
35		0[c]	0[c]	0[c]	0[c]	>200
36		0[c]	0[c]	0[c]	0[c]	>200
37		152	1,763	8	0[c]	>200
38		67	979	0[c]	9	>200
39		1,261	4,359	140	39	>200
40		693	3,350	96	8	>200
41		83	343	0[c]	4	>200
42		0[c]	0[c]	0[c]	0[c]	166
43		360	1,307	462	177	106

(*continued on next page*)

Table 2 (*continued*)

Compound	R	Enzyme assays[a] k_3/K_i (M^{-1} s^{-1})				Cellular assay IC_{50} (μM)
		mCsp-1[b]	Csp-3	Csp-6	Csp-8	Jurkat
44		45,379	168,736	44,007	18,230	137
45		273	906	143	107	161

[a] Assay conditions are described in Ref. 13.
[b] Murine caspase 1.
[c] Indicates reversible inhibition.

Starting with the known ((1-phenyl-3-(trifluoromethyl)-pyrazol-5-yl)oxy) (or PTP) warhead (**4**),[12] we first investigated five-membered aromatic heterocycles, as well as benzofused five-membered heterocycles (Table 1). Changing the regiochemistry and substituent (phenyl to methyl, compounds **4** and **5**, respectively) of the pyrazole ring maintained activity in caspase-1 and -3, but resulted in reduced activity in caspase-6 and -8.

Interestingly, the isoxazoles (entries **6**, **7**, and **8**) showed good enzyme activity with a preference for ether substitution at the 3-position, which mimics the positioning of **5**. Compound **8** was highly active in the enzyme assays. However, there was little or no cellular potency associated with this series.

Thioethers (compounds **9**–**24**) exhibited some activity in caspase-3. The N-substituted heterocycles showed better activity than their unsubstituted analogues (**10** vs **9**, **18** vs **17**); this may be due to the charge on the ring. The positioning of the heteroatoms in the oxadiazole derivatives (**15**, **16**) exhibited a large effect on potency. The tetrazoles (**20**, **21**) exhibited good enzyme activity which was better than that of the triazole (**18**). The benzofused analogues exhibited very little activity with the exception of the benzotriazole (**26**), which was very active in the enzymes studied but inactive in the JFas model.

Next, we focused on the use of six-membered ring heterocycles (Table 2). The two pyridyl derivatives (**27**, **28**) were inactive. However, the pyrimidine series (compounds **29**–**36**) proved to be most interesting. These warheads not only preferred an electron withdrawing (trifluoromethyl) moiety, but also the appropriate regiochemistry to properly line up in the P′ pocket for activity. Compound (**33**) closely mimics that of **4** and yielded better enzyme activity with similar cellular activity. The benzofused analogues (**38**–**45**) of the six-membered heterocycle series showed very little activity, with the exception of the benzotriazinone (**44**) which corresponds with the results seen for the five-membered ring series and the benzotriazole (**26**).

In conclusion, we found mimicking the PTP moiety (**4**) led to several new active warheads. The most notable of these new warheads were the oxazoles (**7**, **8**) and the 2-trifluoromethyl pyrimidine (**33**), which are similar in structure to the pyrazole (**4**). The use of benzotriazole and benzotriazinone (**26** and **44**, respectively) also proved to be effective. In general, these inhibitors were found to be somewhat selective for caspase-3 in nature. Unfortunately, the cellular efficacy of the inhibitors was moderate. We also found that some of the heterocyclic warheads investigated proved to be reversible inhibitors of some or all of the enzymes studied. The use and optimization of these warheads will be the topic of future publications.

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