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Dipeptidyl aspartyl fluoromethylketones as potent caspase inhibitors: Peptidomimetic replacement of the P_2 amino acid by 2-aminoaryl acids and other non-natural amino acids

Yan Wang, Shaojuan Jia, Ben Tseng, John Drewe and Sui Xiong Cai*

EpiCept Corporation, 6650 Nancy Ridge Drive, San Diego, CA 92121, USA

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Abstract—As a continuation of our SAR studies of dipeptidyl aspartyl-fmk as caspase inhibitors, we explored the replacement of the P_2 amino acid by a 2-aminoaryl acid or other non-natural amino acids. Several of these compounds, such as **6l** and **6p**, were found to have good activities with inhibition potencies of around 100 nM in a caspase-3 enzyme assay. EP1113, *Z*-Val-(2-aminobenzoyl)-Asp-fmk (**9b**), is identified as a potent broad-spectrum caspase inhibitor with IC_{50} values of 6–60 nM in different caspases. EP1113 also has good activity in a cell apoptosis protection assay.

Caspases are a family of structurally related cysteine proteases that cleave their substrates with strict substrate specificity for aspartic acid as the P_1 amino acid.[1] One member of the caspase family, represented by caspase-1, activates interleukin-1 and plays an important function in cytokine maturation and inflammation.[2] The other group of caspases, including caspase-3, -6, -7, -8, and -9, are key mediators of apoptosis via cleaving numerous important proteins leading to apoptotic cell death.[3] Excessive apoptosis has been established to play an important role in many pathological conditions, including liver diseases,[4] brain ischemia,[5] myocardial infarction,[6] and neurodegenerative disorders such as Huntington's disease and Alzheimer's disease.[7] Therefore the discovery and development of caspase inhibitors could result in novel anti-inflammatory and anti-apoptotic treatments for a variety of diseases.[8,9]

Many caspase inhibitors have been designed and synthesized.[10] These include peptide-based inhibitors,[11] peptidomimetic-based inhibitors such as those incorporated with a P_2–P_3 conformationally constrained dipeptide mimetic[12] and a thiazepine ring as P_2–P_3 mimetic,[13] aza-peptide,[14] as well as non-peptide inhibitors such as isatins discovered through screening of compound libraries.[15] Some of these are selective for specific caspases, including VX-740 which is a peptidomimetic, reversible, and a selective caspase-1 inhibitor.[16] Others are broad-spectrum caspase inhibitors, such as IDUN-6556 which is a dipeptide based, irreversible, and broad-spectrum caspase inhibitor.[17]

We have reported the discovery of the dipeptide-fmk EP1013 (MX1013, Cbz-Val-Asp-fmk) as a potent and broad-spectrum caspase inhibitor, with potent in vivo activities in several animal models of apoptosis.[18–21] Similar to IDUN-6556, MX1013 and other peptide-fmk molecules are competitive and irreversible inhibitors of caspase-3.[18] To maintain the preferred dipeptide scaffold of EP1013 but to reduce the peptide characteristics of the inhibitor, we have replaced the P_2 α-amino acid by an α-hydroxy acid, which led to the discovery of a series of potent caspase inhibitors including EP1153 (MX1153).[22] We also explored the replacement of the P_2 amino acid by non-natural amino acids such as 2-aminobenzoic acids (Chart 1). Herein we report the synthesis and evaluation of a group of non-natural amino acid derivatives as caspase inhibitors.

2-(Benzyloxycarbonylamino)benzoyl-Asp-fmk (**6a**) was prepared using procedures similar to the synthesis of dipeptide-fmk inhibitors as reported previously.[19] 2-(Benzyloxycarbonylamino)benzoic acid (**2a**) was prepared via reaction of 2-aminobenzoic acid (**1a**) with benzyl chloroformate in pyridine. Compound **2a** was

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* Corresponding author. Tel.: +1 858 202 4006; fax: +1 858 202 4000; e-mail: scai@epicept.com

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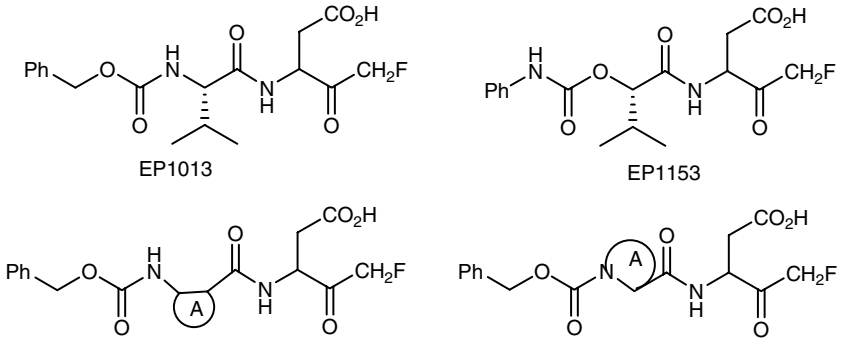
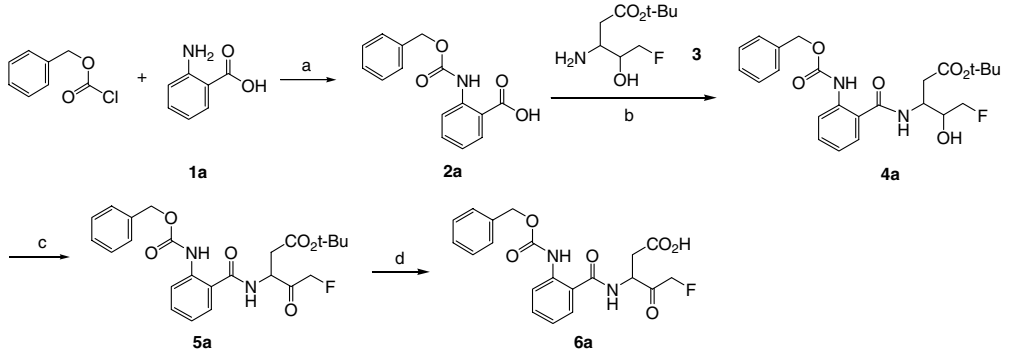


Chart 1.

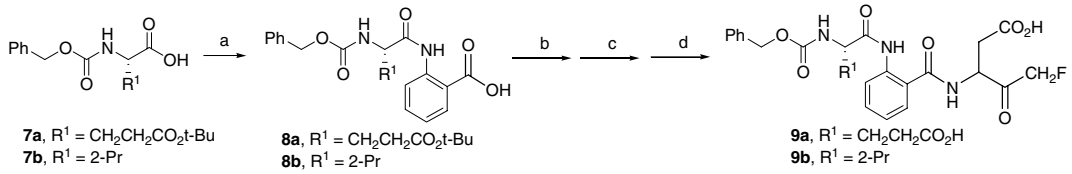
coupled with amine **3**[23] to give amide **4a**, which was oxidized by Dess–Martin reagent to produce the corresponding ketone **5a**. The *t*-Bu ester was then cleaved by TFA to give the free acid **6a** (Scheme 1). Other non-natural amino acid-based Asp-fmks **6b–6o** were prepared similarly from the corresponding substituted 2-aminobenzoic acids (**1b–1k**), 3-aminothiophene-2-carboxylic acid **1l**, *cis*- and *trans*-2-aminocyclohexanecarboxylic acids **1m** and **1n**, and *cis*-2-aminocyclopentanecarboxylic acid **1o.** Compounds **6p** and **6q** were prepared similarly using benzyloxycarbonyl protected (*S*)-azetidine-2-carboxylic acid **2p** and proline **2q**, respectively.

Compound **9a** was prepared in four steps similar to **6a** (Scheme 2). Benzyloxycarbonyl-Glu(O-*t*-Bu)-2-aminobenzoic acid **8a** was prepared from reaction of benzyloxycarbonyl-Glu(O-*t*-Bu)OH (**7a**) with isobutyl chloroformate and *N*-methylmorpholine in THF, followed by addition of 2-aminobenzoic acid. Compound **8a** was reacted with amine **3** to produce the amide, followed by oxidation to produce the ketone and deprotection to remove the two *t*-Bu groups to produce *Z*-Glu-(2-aminobenzoyl)-Asp-fmk (**9a**). *Z*-Val-(2-aminobenzoyl)-Asp-fmk was prepared similarly in four steps starting from benzyloxycarbonyl-Val-OH (**7b**).

The activity of these compounds to inhibit human recombinant caspase-3 was determined using a standard fluorometric assay,[18,24] and the results are summarized in Table 1. Compound **6a**, with a 2-aminobenzoic acid as P_2, had good activity with an IC_{50} value of 200 nM for caspase-3. Compounds **6b–6d**, with a Me group substituted in different positions of the phenyl ring, except 5-Me analog **6c**, were 3- to 4-fold less active than **6a**, indicating that substitution at those positions is not preferred. The 3,5-dimethyl analog **6e** was the least active one among the Me-substituted analogs, confirming the above observation. Similarly, the Cl-substituted analogs **6f–6i** were all less active than **6a**. Interestingly, the 5-substituted analogs **6c**, **6g**, and **6j** all were only slightly less active than **6a**, indicating that substitution at the 5-position is more tolerated. In comparison,

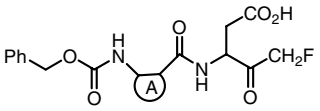


Scheme 1. Reagents: (a) pyridine; (b) **3**, EDCI, HOBT, DMAP, THF; (c) Dess–Martin, dichloromethane; (d) TFA, dichloromethane.



Scheme 2. Reagents: (a) 1—isobutyl chloroformate, *N*-methylmorpholine/THF; 2—anthranilic acid; (b) **3**, EDCI, HOBT, DMAP, THF; (c) Dess–Martin, dichloromethane; (d) TFA, dichloromethane.

Table 1. Caspase-3 inhibition activity of 2-amino-benzamides and related compounds



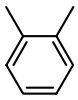
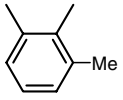
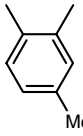
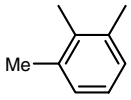
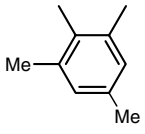
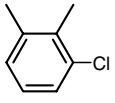
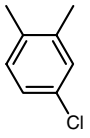
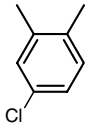
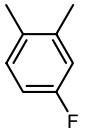
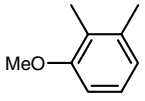
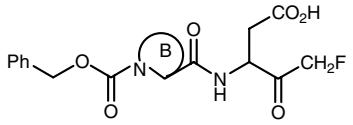
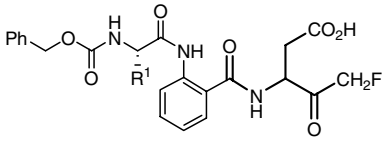
Entry	A	Caspase-3 inhibition[a] IC_{50}[b] (μM)
6a		0.20
6b		0.86
6c		0.26
6d		0.72
6e		1.7
6f		0.45
6g		0.32
6i		0.55
6j		0.27
6k		2.5
6l		0.10

Table 1 (*continued*)

Entry	A	Caspase-3 inhibition IC_{50} (μM)
6m		0.25
6n		1.9
6o		0.21



	B	Caspase-3 inhibition IC_{50} (μM)
6p		0.10
6q		0.41



	R^1	Caspase-3 inhibition IC_{50} (μM)
9a	$CH_2CH_2CO_2H$	0.34
9b	2-Pr	0.033
EP1013	NA[c]	0.030

[a] IC_{50} is determined as described in Ref. 18.
[b] Enzyme IC_{50} results are expressed as ±20% or less.
[c] NA, not applied, please see Chart 1 for the structure of EP1013.

substitution at the 3-position, such as those in compounds **6d**, **6e**, and **6k**, led to a 3- to 12-fold reduction in activity, indicating that substitution at the 3-position is the least tolerated, probably due to unfavorable interaction with the enzyme. Interestingly, replacing the 6-membered benzene ring by a 5-membered thiophene ring led to **6l**, which had an IC_{50} value of 100 nM and was 2-fold more potent than **6a**.

We then explored the replacement of the aromatic ring with a saturated ring. Compound **6m**, with a *cis*-substituted cyclohexane ring, was about as active as **6a**. Interestingly, the corresponding *trans*-isomer **6n** was almost 8-fold less active than **6m**. The *cis*-cyclopentane analog **6o** was about as active as **6m**. The (*S*)-azetidine-2-carboxylic acid derivative **6p** was found to be highly active

showing an IC_{50} value of 100 nM, 4-fold more potent than the corresponding 5-membered ring analog *Z*-Pro-Asp-fmk **6q**.

Since it is known that glutamate is the preferred P_3 amino acid for caspase-3,[25] we explored the incorporation of Glu to the N-terminal of **6a** and prepared **9a**. However, **9a** was found to be about 2-fold less active than **6a**, suggesting that the Glu side chain did not fit into the S_3 pocket of caspase-3. Interestingly, compound **9b**, with a Val added to the N-terminal of **6a**, was found to be highly active with an IC_{50} value of 33 nM, 6-fold more potent than **6a** and as active as the dipeptide EP1013.

Selected compounds were tested in the HeLa cell apoptosis protection assay,[18] which measures the protecting effects of caspase inhibitors against apoptosis induced by TNF-α. The viability of the cells was quantified by calcein AM uptake, and the concentration of inhibitor that provided 50% of cell protection is summarized in Table 2. Compound **6a** was found to provide 50% cell protection at a concentration of 4000 nM. Compound **9b** was the most active one, provided 50% protection at a concentration of 450 nM, approaching that of EP1013.

Compound **9b** (EP1113) was then tested against other caspases and the results are summarized in Table 3. Compound **9b** was found to have high activity in caspase-1, -3, -6, -7, -8, and -9, with IC_{50} values between 6 and 61 nM. Therefore similar to EP1013 (Table 3),[19] compound **9b** is a broad-spectrum caspase inhibitor.

Table 2. Cell apoptosis protection activity and caspase-3 inhibiting activity of 2-amino-benzamides and related compounds

Entry	Caspase-3 IC_{50}[a] (μM)	50% Cell protection[b,c] (μM)
6a	0.20	4
6l	0.10	>2
6p	0.10	1.7
6q	0.41	>2
9b	0.033	0.45
EP1013[d]	0.030	0.25

[a] Data from Table 1.
[b] Concentration of inhibitor that provided 50% of cell protection is determined as described in Ref. 18.
[c] Cell protection IC_{50} results are expressed as ±30% or less.
[d] Data from Ref. 19.

Table 3. Inhibiting activity of compound **9b** (EP1113) against different caspases in comparison with EP1013

Caspases	IC_{50} (μM)[a]	
	9b[b]	EP1013[c]
Caspase-1	0.012	0.020
Caspase-3	0.033	0.030
Caspase-6	0.061	0.018
Caspase-7	0.011	0.007
Caspase-8	0.006	0.007
Caspase-9	0.006	0.005

[a] Enzyme IC_{50} results are expressed as ±20% or less.
[b] IC_{50} is determined as described in Ref. 18.
[c] Data from Ref. 19.

In conclusion, we have designed and synthesized a group of novel peptidomimetic-based caspase inhibitors by replacement of the P_2 amino acid with non-natural amino acid such as 2-aminobenzoic acid. Several of these compounds were found to have good activity with IC_{50} values in the sub 100 nM range. Interestingly, incorporation of Val at the P_3 position led to compound **9b** which is a potent and broad-spectrum caspase inhibitor, with IC_{50} values of 6–61 nM for caspase-1, -3, -6, -7, -8, and -9. Compound **9b** is also highly active in the cell apoptosis protection assays. Although peptidomimetics such as **9b** might be more stable in vivo than peptide-based caspase inhibitors, additional in vitro and in vivo studies are needed to determine whether **9b** has any true advantages over dipeptide caspase inhibitors such as EP1013 and is suitable for future development.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.030.

References and notes

1. Timmer, J. C.; Salvesen, G. S. *Cell Death Differ.* **2007**, *14*, 66.
2. Cornelis, S.; Kersse, K.; Festjens, N.; Lamkanfi, M.; Vandenabeele, P. *Curr. Pharm. Des.* **2007**, *13*, 365.
3. Kumar, S. *Cell Death Differ.* **2007**, *14*, 32.
4. Linton, S. D.; Aja, T.; Armstrong, R. A.; Bai, X.; Chen, L. S.; Chen, N.; Ching, B.; Contreras, P.; Diaz, J. L.; Fisher, C. D.; Fritz, L. C.; Gladstone, P.; Groessl, T.; Gu, X.; Herrmann, J.; Hirakawa, B. P.; Hoglen, N. C.; Jahangiri, K. G.; Kalish, V. J.; Karanewsky, D. S.; Kodandapani, L.; Krebs, J.; McQuiston, J.; Meduna, S. P.; Nalley, K.; Robinson, E. D.; Sayers, R. O.; Sebring, K.; Spada, A. P.; Ternansky, R. J.; Tomaselli, K. J.; Ullman, B. R.; Valentino, K. L.; Weeks, S.; Winn, D.; Wu, J. C.; Yeo, P.; Zhang, C. Z. *J. Med. Chem.* **2005**, *48*, 6779.
5. Han, B. H.; Xu, D.; Choi, J.; Han, Y.; Xanthoudakis, S. R.; 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*, 30126.
6. Haunstetter, A.; Izumo, S. *Circ. Res.* **1998**, *82*, 1111.
7. Wellington, C. L.; Hayden, M. R. *Clin. Genet.* **2000**, *57*, 1.
8. Talanian, R. V.; Brady, K. D.; Cryns, V. L. *J. Med. Chem.* **2000**, *43*, 3351.
9. Callus, B. A.; Vaux, D. L. *Cell Death Differ.* **2007**, *14*, 73.
10. Linton, S. D. *Curr. Top. Med. Chem.* **2005**, *5*, 1697.
11. (a) Dolle, R. E.; Hoyer, D.; Prasad, C. V.; Schmidt, S. J.; Helaszek, C. T.; Miller, R. E.; Ator, M. A. *J. Med. Chem.* **1994**, *37*, 563; (b) Linton, S. D.; Karanewsky, D. S.; Ternansky, R. J.; Wu, J. C.; Pham, B.; Kodandapani, L.; Smidt, R.; Diaz, J.-L.; Fritz, L. C.; Tomaselli, K. J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2969; (c) Han, Y.; Giroux, A.; Grimm, E. L.; Aspiotis, R.; Francoeur, S.; Bayly, C. I.; Mckay, D. J.; Roy, S.; Xanthoudakis, S.; Vaillancourt, J. P.; Rasper, D. M.; Tam, J.; Tawa, P.; Thornberry, N. A.; Paterson, E. P.; Garcia-Calvo, M.;

Becker, J. W.; Rotonda, J.; Nicholson, D. W.; Zamboni, R. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 805.
12. Karanewsky, D. S.; Bai, X.; Linton, S. D.; Krebs, J. F.; Wu, J.; Pham, B.; Tomaselli, K. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2757.
13. Ellis, C. D.; Oppong, K. A.; Laufersweiler, M. C.; O'Neil, S. V.; Soper, D. L.; Wang, Y.; Wos, J. A.; Fancher, A. N.; Lu, W.; Suchanek, M. K.; Wang, R. L.; De, B.; Demuth, T. P., Jr. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4728.
14. Ekici, O. D.; Li, Z. Z.; Campbell, A. J.; James, K. E.; Asgian, J. L.; Mikolajczyk, J.; Salvesen, G. S.; Ganesan, R.; Jelakovic, S.; Grutter, M. G.; Powers, J. C. *J. Med. Chem.* **2006**, *49*, 5728.
15. Lee, D.; Long, S. A.; Murray, J. H.; Adams, J. L.; Nuttall, 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., Jr. *J. Med. Chem.* **2001**, *43*, 2015.
16. Siegmund, B.; Zeitz, M. *IDrugs* **2003**, *6*, 154.
17. Hoglen, N. C.; Chen, L. S.; Fisher, C. D.; Hirakawa, B. P.; Groessl, T.; Contreras, P. C. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 634.
18. Yang, W.; Guastella, J.; Huang, J.-C.; Wang, Y.; Zhang, L.; Xue, D.; Tran, M.; Woodward, R.; Kasibhatla, S.; Tseng, B.; Drewe, J.; Cai, S. X. *Br. J. Pharmacol.* **2003**, *140*, 402.
19. Wang, Y.; Huang, J.-C.; Zhou, Z.-L.; Yang, W.; Guastella, J.; Drewe, J.; Cai, S. X. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1269.
20. Jaeschke, H.; Farhood, A.; Cai, S. X.; Tseng, B. Y.; Bajt, M. L. *Toxicol. Appl. Pharm.* **2000**, *169*, 77.
21. Cai, S. X.; Guan, L.; Jia, S.; Wang, Y.; Yang, W.; Tseng, B.; Drewe, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5295.
22. Wang, Y.; Guan, L.; Jia, S.; Tseng, B.; Drewe, J.; Cai, S. X. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1379.
23. Revesz, L.; Briswalter, C.; Heng, R.; Leutwiler, A.; Mueller, R.; Wuethrich, H. J. *Tetrahedron Lett.* **1994**, *35*, 9693.
24. Thornberry, N. A.; Peterson, E. P.; Zhao, J. J.; Howard, A. D.; Griffin, P. R.; Chapman, K. T. *Biochemistry* **1994**, *33*, 3934.
25. Thornberry, N. A. *Chem. Biol.* **1998**, *5*, R97.