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Bioorganic & Medicinal Chemistry Letters 15 (2005) 1173–1180
Bioorganic & Medicinal Chemistry Letters

Novel pyrazinone mono-amides as potent and reversible caspase-3 inhibitors

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Received 8 October 2004; revised 25 November 2004; accepted 2 December 2004
Available online 7 January 2005

Abstract—The iterative process for the discovery of a series of pyrazinone mono-amides as potent, selective and reversible non-peptide caspase-3 inhibitors (e.g., **M826** and **M867**) is reported. These compounds display potent anti apoptotic activities in a number of cell based systems in vitro as well as in several animal models in vivo.

1. Introduction

Caspases are a family of cysteine proteases with a specific requirement for an aspartate residue at the P_1 target cleavage sites. To date, 14 mammalian caspases have been identified and these enzymes are grouped into three subfamilies based on sequence homology and substrate specificity.[1] Group I (1, 4 and 5) caspases such as casp-1 (or ICE) play an important role in cytokine maturation and inflammation. Group II (3, 6 and 7) and group III (2, 8, 9 and 10) caspases are essential for apoptotic cell death. Casp-3, in particular, has been characterized as the dominant effector caspase involved in the proteolytic cleavage of a variety of protein substrates including cytoskeletal proteins, kinases and DNA repair enzymes during apoptosis[2] and recent studies have revealed the activation of casp-3 in many models of apoptosis.[3] On the other hand, knockout studies suggest a partial redundancy in the role of casp-3 and other caspases such as casp-7.[4] Thus the development of potent and selective casp-3 or dual casp-3/7 inhibitors has emerged as an attractive therapeutic approach.[5]

Recent reports indicated that caspase inhibitors such as z-VAD-fmk, z-VD-fmk, Boc-D-fmk and Ac-DEVD-fmk were effective in animal models of ischemic injury, burns, endotoximia, sepsis and neonatal hypoxia.[6] Most of these inhibitors, however, are irreversible pan-caspase inhibitors[7] and z-VAD-fmk is also reported to inhibit other cysteine proteases.[8] In order to assess the importance of casp-3 activation in apoptosis, potent, selective and reversible inhibitors are highly desirable. In this communication, we will describe the iterative process of discovering such inhibitors.

Reversible $P_{1'}$ aldehyde and ketone casp-3 inhibitors incorporating DxVD, the preferred tetrapeptide motif recognized by casp-3/7, have been studied previously.[9] These compounds generally lack cell potency due to limited cell permeability. For example, Ac-DEVD-phenylpropyl ketone (**1**) (the definition of P_4–P_3–P_2–P_1–$P_{1'}$ is shown in **1**)[10] was a potent inhibitor at rh-casp-3 (IC_{50}, 0.8 nM) yet its potency in preventing camptothecin induced DNA fragmentation (and thus cell death) in the neuronal precursor (NT2) cells was shifted dramatically (IC_{50}, 30 μM, Table 1).[9] Non peptide isatin casp-3/7 inhibitors were also described by Lee et al. but this class of compounds may have limited applications in vivo due to the highly reactive nature of the ketone carbonyl group toward nucleophiles.[11] We have reported

Keywords: Pyrazinone mono-amide; Caspase-3 inhibitors.
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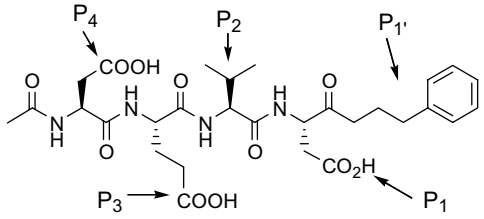
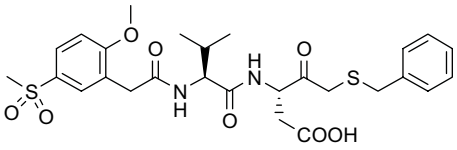
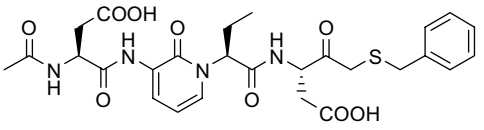
doi:10.1016/j.bmcl.2004.12.006



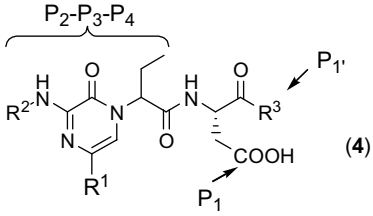
(1)



(2)



(3)



(4)

Table 1. Inhibitory activity (IC_{50}, μM) of compounds **4** against rh-casp-1,3,7 and 8, and in NT2 whole cells

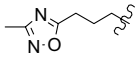
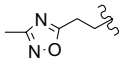
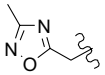
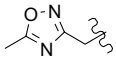
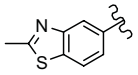
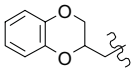
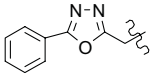
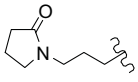
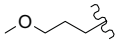
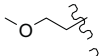
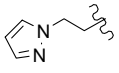
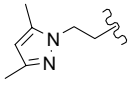
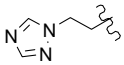
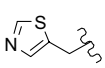
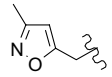
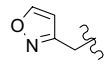
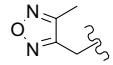
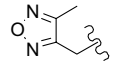
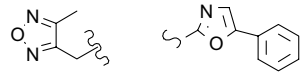
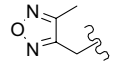
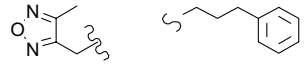
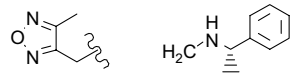
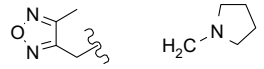
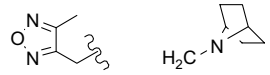
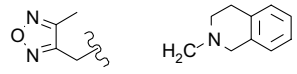
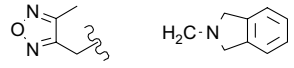
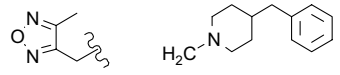
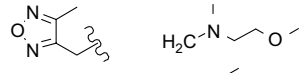
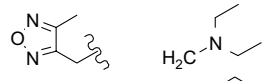
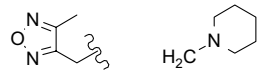
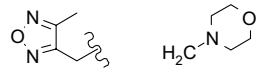
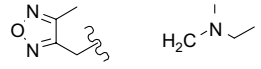
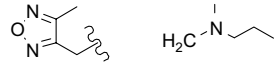
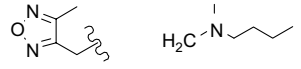
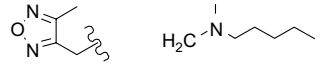
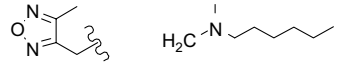
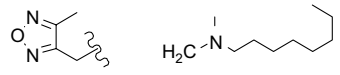
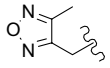
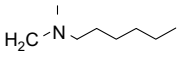
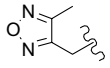
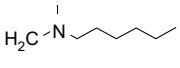
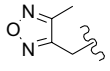
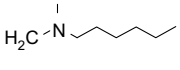
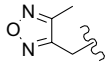
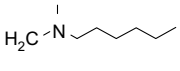
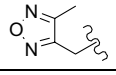
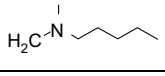
Compd	R^1	R^2	R^3	IC_{50} (μM)[a]				
				rh-csap-1	rh-casp-3	rh-casp-7	rh-casp-8	NT2
1	—	—	—	0.31	0.0008	0.008	0.016	30.0
2	—	—	—	1.60	0.0050	0.120	0.49	1.0
3	—	—	—	0.060	0.0003	0.010	0.22	5.2
4a	H		CH_2SBn	1.70	0.053	2.60	>10.0	7.10
4b	H		CH_2SBn	0.90	0.080	>10.0	9.10	15.30
4c	H		CH_2SBn	0.67	0.048	4.68	6.42	5.33
4d	H		CH_2SBn	1.08	0.072	>10.0	7.56	8.06
4e	H		CH_2SBn	0.22	0.088	2.42	2.73	37.9
4f	H		CH_2SBn	0.087	0.14	3.28	8.22	30.0
4g	H		CH_2SBn	0.98	0.31	>10.0	4.92	52.10
4h	H		CH_2SBn	0.065	0.036	0.81	2.09	4.56
4i	H		CH_2SBn	1.90	0.19	>10.0	9.92	50.0
4j	H		CH_2SBn	0.92	0.071	9.73	7.65	>10.0
4k	H		CH_2SBn	1.11	0.14	>10.0	>10.0	6.79
4l	H		CH_2SBn	0.99	0.082	>10.0	2.26	7.09
4m	H		CH_2SBn	1.82	0.16	>10.0	1.66	9.48
4n	H		CH_2SBn	1.93	0.018	9.71	>10.0	5.74
4o	H		CH_2SBn	0.13	0.026	1.13	1.75	3.08
4p	H		CH_2SBn	0.19	0.012	0.49	6.59	3.42

Table 1 (*continued*)

Compd	R^1	R^2	R^3	IC_{50} (μM)[a]				
				rh-csap-1	rh-casp-3	rh-casp-7	rh-casp-8	NT2
4q	H		CH_2SBn	0.23	0.051	2.54	3.30	6.41
4r	H		CH_2SBn	0.40	0.046	5.17	2.90	8.56
4s	H		CH_2SBn	0.22	0.0079	0.73	2.46	1.39
4sa	H		H	0.15	0.078	1.00	0.99	3.56
4sb	H			0.46	0.063	0.75	9.57	8.39
4sc	*t*-Bu		Me	1.23	0.064	0.48	10.0	5.12
4sd	*t*-Bu			5.13	0.014	0.14	5.28	1.38
4se	H			0.064	0.014	1.04	9.26	1.20
4sf	H			0.29	0.033	0.41	6.22	0.84
4sg	H			0.88	0.023	0.26	9.22	1.45
4sh	H			0.013	0.013	0.083	7.38	1.25
4si	H			0.0087	0.014	0.13	4.29	1.52
4sj	H			0.047	0.056	0.84	6.02	1.62
4sk	H			1.75	0.10	1.01	>10.0	3.99
4sl	H			1.14	0.12	3.24	>10.0	3.90
4sm	*t*-Bu			0.62	0.021	0.13	>10.0	0.29
4sn	*t*-Bu			0.73	0.11	0.54	>10.0	8.62
4so	H			0.89	0.042	0.31	>10.0	2.64
4sp	H			0.056	0.026	0.54	>10.0	1.53
4sq	H			0.62	0.017	0.32	>10.0	1.42
4sr	H			0.031	0.015	0.13	>10.0	0.30
4ss	H			0.048	0.013	0.11	>10.0	0.30
4st	H			0.028	0.011	0.14	>10.0	0.13
4su	H			0.025	0.009	0.10	>10.0	0.15

(*continued on next page*)

Table 1 (*continued*)

Compd	R^1	R^2	R^3	IC_{50} (μM)[a]				
				rh-csap-1	rh-casp-3	rh-casp-7	rh-casp-8	NT2
4sv	Et			0.025	0.006	0.60	9.54	0.078
4sw	*i*-Pr			0.063	0.013	0.065	9.04	0.0048
4sx (M826)	*t*-Bu			0.067	0.006	0.042	6.48	0.021
4sy				0.11	0.016	0.087	>10.0	0.025
4sz (M867)	*t*-Bu			0.11	0.0001	0.036	5.95	0.0027

[a] Average of 2–8 titrations.

previously that truncating the tetrapeptide backbone to a dipeptide motif led to the discovery of inhibitors such as **2** with substantial improvement in whole cell potencies, suggesting the beneficial effect of removing polar and charged residues such as amides and carboxylates from these molecules.[12] We further observed that the P_2–P_3 could be replaced by a pyridone core, giving compounds such as **3** with good enzymatic activity and selectivity (Table 1). Yet these inhibitors had little advantage over tetrapeptide **1** in whole cell assays due to the number of polar residues present. We describe herein the successful replacement of the P_2–P_3–P_4 backbone with an amino pyrazinone template, resulting in the discovery of a series of non peptide casp-3 inhibitors (**4**) with dramatically improved anti apoptotic activities both in vitro and in vivo.

2. Chemistry

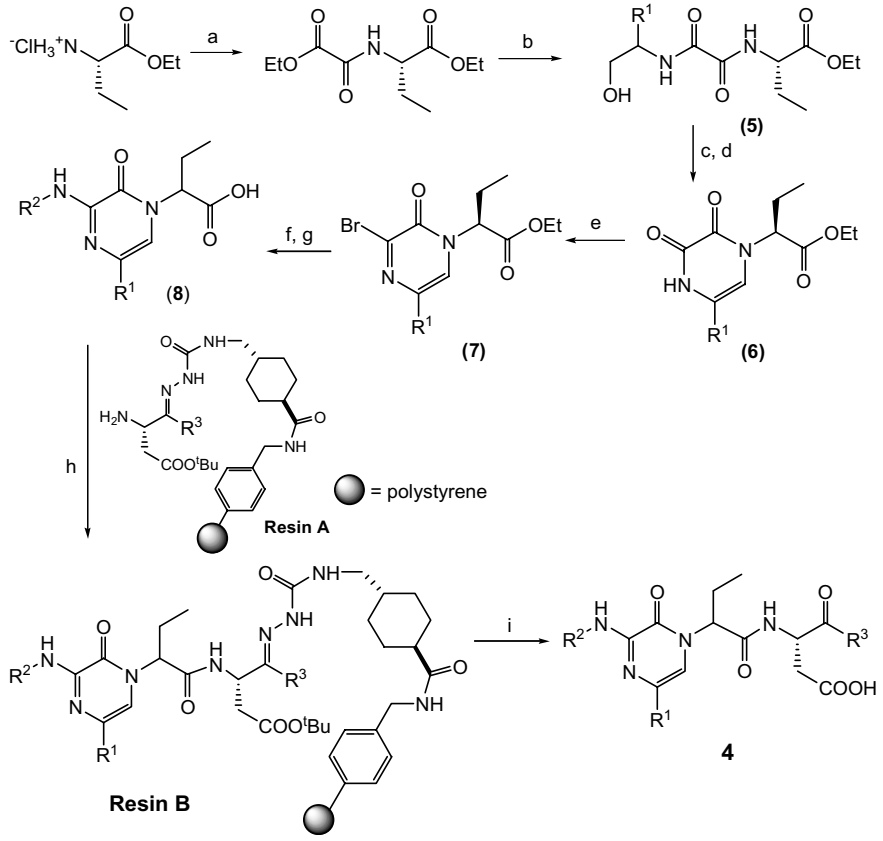
The major advantage of the pyrazinone core over a pyridone in **3** is the chemical stability of the α-amino moiety.[13] In addition, many variants of the acid template **8** are easily attainable from readily available materials according to modified literature procedures as illustrated in Scheme 1.[14] Reaction of ethyl (*S*)-(+)-2-aminobutyrate hydrochloride with ethyl oxalyl chloride followed by replacing the ethyl ester with an amino alcohol $R^1CH(NH_2)CH_2OH$ gave the oxalyl diamide **5** in high yield. The alcohol in **5** was oxidized using the Dess–Martin periodinane and the aldehyde thus obtained was treated with TFA/TFAA in acetic acid at 80–120 °C, giving the cyclic product **6** in 60–80% yield in four steps. The ester **6** was converted to bromide **7** using $POBr_3$ in dichloroethane at 60 °C. Displacing the bromide with amine R^2 NH_2 followed by hydrolysis of the ethyl ester gave acid **8** with partial racemization (20–25%). Acid **8** was then coupled to the amino group on resin-**A** using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′*N*′-tetramethyl-uronium hexafluorophosphate (HATU) in the presence of Hünig's base in DMF as reported previously.[9,12] Treating the resultant resin **B** with 90% TFA in water gave the desired inhibitors **4** with good purity (>90% by ^1H NMR analysis) as a mixture of diastereomers. These compounds were screened directly, without further purification, against four representative recombinant human caspases (e.g., rh-casp-1,3,7,8), as well as in the NT2 cells against camptothecin induced apoptosis (DNA fragmentation by ELISA[9]), results are summarized in Table 1.

The strategy to make $P_{1'}$ aminomethyl ketones is outlined in Scheme 2 since this class of compounds could not be obtained by the solid phase protocol shown in Scheme 1. Thus, acid **8** was reacted with (*S*)(−)-β-*t*-butyl aspartic acid methyl ester in the presence of HATU and Hünig's base in DMF and the resultant methyl ester was hydrolyzed to the corresponding acid using LiOH. The acid was then treated with isobutyl chloroformate (IBCF) in the presence of N-methylmorpholine (NMM) and the mixed anhydride thus formed was reacted with an excess amount of CH_2N_2 in ether. The resulting diazoketone intermediate was treated directly with a solution of HBr (45%) in acetic acid to yield bromomethyl ketone **9**.[15] Reaction of ketone **9** with an amine in THF gave the corresponding amino derivatives with complete isomerization of the α-chiral center. Treating the *t*-butyl ester with either TFA or HCl(g) in CH_2Cl_2 gave aminomethyl ketone salt **4s** as a mixture of diastereomers. The in vitro profiles of these inhibitors are summarized in Table 1 (compds **4se**–**sz**).

3. Results and discussion

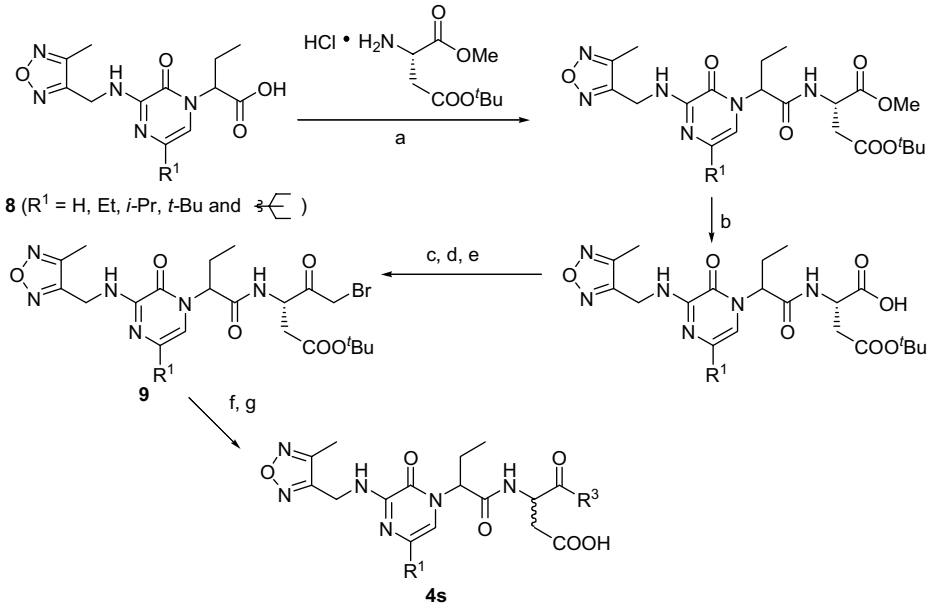
3.1. Optimization at R^2

We will concentrate our current discussion on compounds bearing an ethyl at P_2 since it was the optimal group discovered (unpublished results), similar to the observation by Semple and co-workers for casp-1 inhibitors bearing a P_2–P_3 pyridone template.[16] Molecular modeling suggested, and an subsequent X-ray structure of the rh-casp-3 complex with **M826** substantiated, that the amino-pyrazinone template in compounds **4** was superimposable with the P_2–P_3 of the tetrapeptide inhib-



Scheme 1. (a) Ethyl oxalyl chloride, Et_3N, DCM, 0 °C; (b) $R^1CH(NH_2)CH_2OH$, Et_3N, EtOAc, 80 °C; (c) Dess–Martin periodinane, DCM, rt; (d) TFA/TFAA, AcOH, 80–120 °C; (e) $POBr_3$, $ClCH_2CH_2Cl$, 60 °C; (f) R^2NH_2, EtOAc, 80 °C; (g) LiOH, THF, 0 °C; (h) resin **A**, HATU, *i*-Pr_2 NEt, DMF, rt; (i) TFA/H_2O (9:1 v/v), rt.

itor Ac-DEVD-PPK (**1**).[17] We further speculated that a suitable R^2 group could potentially form beneficial interactions with the Phe381b and Asn342 residues at the S_4 pocket of the casp-3 enzyme due to its proximity. We initially explored non-charged heterocycles such as an 1,2,4-oxadiazole (compds **4a**–**d**) since a dipeptide



Scheme 2. (a) HATU, *i*Pr_2 NEt, DMF; (b) LiOH (1 equiv), THF/H_2O (10:1), then AcOH; (c) IBCF, NMM, THF, −78 → −20 °C; (d) excess CH_2N_2/ether, −20 °C–rt; (e) 45%HBr/AcOH, 0 °C; (f) amine, TEA, THF, 0 °C; (g) HCl, or TFA, DCM.

casp-3 inhibitor bearing this group was found to form excellent polar interactions in the S_4 pocket of casp-3.[18] The effect of the chain length and position of substitution were first investigated and we observed that the intrinsic activity was not affected significantly. Puzzled by these findings, we systematically replaced the Br in **7** with a variety of readily available amines and observed that more bulky and lipophilic amines generally gave compounds with poorer selectivity against rh-casp-1 (compds **4e**–**h**) while more polar and smaller amino residues afforded compounds (**4i**–**s**) with better activity and selectivity against rh-casp-3. This is in agreement with the fact that the casp-3 enzyme has a more polar and restricted S_4 pocket at the active site.[17,19] Among all amines examined, three derivatives bearing a triazolylethylamino (**4n**), a thiazolylmethylamino (compds **2o**,**p**) or a (4-methyl-1,2,5-oxadiazol-3-yl)methylamino (furazanmethylamino) (**4s**) derivatives exhibited the best potency and selectivity against rh-casp-3. The neutral furazan analog **4s**, in particular, showed excellent intrinsic activity ($IC_{50} \sim 8$ nM), good selectivity (>25 fold) and good whole cell activity (IC_{50}, 1.4 μM). This group was thus employed as a starting point for further SAR optimization at other positions as discussed herein.

3.2. Optimization at $P_{1'}$

As illustrated in Table 1, $P_{1'}$ substitutions had a dramatic influence on the activity, especially whole cell potency of these inhibitors. As previously observed with tetrapeptide[9] and dipeptide[12] casp-3 inhibitors, an aldehyde (**4sa**), phenyloxazolyl ketone (**4sb**), methyl ketone (**4sc**) or phenylpropyl ketone (**4sd**) replacements resulted in reversible inhibitors with significant loss of potency comparing to the benzylthiomethyl ketone **4s**. The $P_{1'}$ amino substitution, on the other hand, generally gave inhibitors with superior whole cell activity. Secondary aminomethyl ketones such as **4se** were chemically unstable as reported by Semple and co-workers for casp-1 inhibitors and thus not vigorously explored.[20] Incorporation of cyclic amino groups gave compounds **4sf**–**sj** and **4sm** with moderate intrinsic and whole cell activity. Incorporation of polar residues such as the ones in **4sk** and **4sn** was highly detrimental, especially for whole cell activity. Further systematic investigation revealed that an N-methyl-N-alkyl side chain was preferable for obtaining compounds with good whole cell potency, and that the N-methyl-N-hexyl amino group was the optimal moiety. It is of interest to observe that, on going from an N-dimethylamino to an N-methyl-N-hexyl group (compds **4so**–**st**), the intrinsic potency against rh-casp-3 did not increase dramatically (4-fold) while the whole cell activity improved by more than 30 fold, suggesting that compounds bearing longer N-alkyl side chains (thus higher lipophilicity) had improved cell permeability. The introduction of a longer chain such as an N-octyl groups did not further enhance either the intrinsic or the whole cell activity.

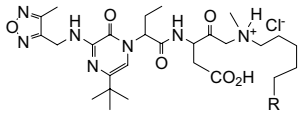
3.3. Optimization at R^1

Synergy between R^1 and $P_{1'}$. As shown in Table 1, compounds incorporating alkyl groups at R^1 (compds **4sv**–**sy**) exhibited similar intrinsic activity as the corresponding H analog **4st**. However, the whole cell activity increased gradually on going from H, Et, *i*-Pr to *t*-Bu, and the *t*-Bu group was the optimal moiety. Further increase in size by incorporating a bulky group such as that in **4sy** did not give further enhancement in whole cell activity. Overall, compounds bearing a *t*-Bu at R^1 and an N-methyl-N-pentyl (**4sz**, **M867**) or an N-methyl-N-hexyl (**4sx**, **M826**) group at $P_{1'}$ displayed the best in vitro profiles which are summarized in Figure 1. Both compounds showed sub-nanomolar binding affinity (K_i) of 0.7 nM against rh-casp-3, and were reversible and selective against other caspases. Both compounds were highly effective against camptothecin induced cell death in the NT2 cells[9] (IC_{50}, 20–30 nM), etoposide induced DNA fragmentation (thus cell death)[21] in mice cerebellar granule neurons (IC_{50}, 50–60 nM) and in rat cortical neurons (IC_{50}, 60–100 nM), cycloheximide induced cell death (cell death ELISA measuring DNA fragmentation) in white blood cells in the absence of plasma proteins (IC_{50}, 40–50 nM) and in the presence of plasma proteins (IC_{50}, 700–1200 nM). These compounds represent the most potent and selective reversible caspase-3 inhibitors discovered thus far.

In summary, we have described the discovery of potent, selective, reversible and non peptidic casp-3 inhibitors such as **M826** and **M867** that demonstrated excellent anti

Enzymatic activity against different caspases, IC_{50} (μM)

Cmpd	casp-1	casp-2	casp-3 (*Ki*)	casp-4	casp-5	casp-6	casp-7	casp-8	casp-9	casp-10
M826	0.05	>10	0.005[a] (0.0007)	0.3	0.2	1.7	0.013	2.1	nt[b]	17.7
M867	0.11	>10	0.0014 (0.0007)	0.5	0.81	3.44	0.09	4.03	nt[b]	29.5



M826, R = Me
M867, R = H

Whole Cell IC_{50} (μM)

Cmpd	NT2	mCGN[c]	rCort[d]	hWBC(-)[e]	hWBC(+)[f]
M826	0.021	0.05	0.09	0.05	1.2
M867	0.027	0.06	0.06	0.04	0.7

Figure 1. Full in vitro profile of **M826** and **M867**. [a]non linear behavior observed at lower inhibitor concentration; [b]not tested; [c]IC_{50} in preventing etoposide induced apoptosis in mice cerebellar granule neurons; [d] IC_{50} in inhibiting etoposide induced apoptosis in rat cortical neurons; [e]IC_{50} in inhibiting cycloheximide induced cell death in human white blood cells in the absence of plasma proteins; [f]in the presence of plasma proteins.

apoptotic activities in vitro. **M826** and **M867** were also found to be highly effective in inhibiting casp-3 activity and thus cell death in several in vivo models. For example, a single icv bolus of **M826** (10 μg) in P7 neonatal rats in the Levine model of hypoxia resulted in near complete inhibition of casp-3 activity for 48 h as measured by Ac-DEVD-AMC cleavage activity of the tissue homogenate and α II-spectrin cleavage activity as measured by ELISA.[22] This dosing regime also resulted in significant protection of cell death in the same model. The same compound also displayed neuronal protective effect in the malonate induced ischemic injury in rats.[23] **M867**, when given as continuous infusion, showed a dose dependent inhibition of casp-3 activity with an ED_{50} of ~0.5 mg/kg/h as measured by α II-spectrin cleavage in the thymocytes in a rat model of sepsis induced by cecal ligation and perforation.[24] At an infusion dose of 2 mg/kg/h, α II-spectrin cleavage was almost completely blocked, yet the level of inhibition of DNA fragmentation was significantly lower (50%). Higher doses of **M867** (4–5 mg/kg/h) were required to abolish DNA fragmentation in the same model, suggesting a high and persistent blockage of casp-3 active site might be needed for therapeutic benefit since DNA fragmentation was mostly likely sine qua non for cell survival. Further studies using a casp-3 active site probe confirmed that higher fractional inhibition (65–75%) was needed to block DNA fragmentation by 50%.[25] These findings, for the first time, demonstrated the potential therapeutic benefit and challenges of using a selective and reversible casp-3 inhibitor in treating acute injuries.

References and notes

1. (a) Nicholson, D. W. *Cell Death Differ.* **1999**, *6*, 1028; (b) Shi, Y. *Mol. Cell.* **2002**, *9*, 459; (c) Thornberry, N. A.; Rano, T. A.; Peterson, E. P.; Rasper, D. M.; Timkey, T.; Garcia-Calvo, M.; Houtzager, V. M.; Nordstrom, P. A.; Rpy, S.; Champman, K. T.; Nicholson, D. W. *J. Biol. Chem.* **1997**, *272*, 11907.
2. Slee, E. A.; Adrain, C.; Martin, S. J. *J. Biol. Chem.* **2001**, *276*, 7320.
3. Porter, A. G.; Janicke, R. U. *Cell Death Differ.* **1999**, *6*, 99.
4. Kuida, K.; Zheng, T. S.; Na, S.; Kuan, C.; Yang, D.; Karasuyama, H.; Rakic, P.; Flavell, R. H. *Nature* **1996**, *384*, 368.
5. Talanian, R. V.; Brady, K. D.; Cryns, V. L. *J. Med. Chem.* **2000**, *43*, 3351.
6. (a) Hotchkiss, R. S.; Chang, K. C.; Swanson, P. E.; Tinsley, K. W.; Hui, J. J.; Klender, P.; Xantoudakis, S.; Roy, S.; Black, C.; Grimm, E.; Han, Y.; Nicholson, D. W.; Karl, I. E. *Nature Immun.* **2000**, *1*, 496, and references cited therein; (b) Cheng, Y.; Deshmukh, M.; D'Costa, A.; Demaro, J. A.; Gidday, J. M.; Shah, A.; Sun, Y.; Jacquin, M. F.; Johnson, E. M.; Holtzman, D. M. *J. Clin. Invest.* **1998**, *101*, 1992; (c) 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.
7. (a) Wang, Y.; Huang, J.-C.; Zhou, Z.-L.; Yang, W.; Guastella, J.; Cai, S. X. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1269, and references cited therein; (b) Cai, S. X.; Guan, L.; Jia, S.; Wang, Y.; Yang, W.; Tseng, B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1269.
8. Schotte, P.; Declercq, W.; van Huffel, S.; Vandenabeele, P.; Beyaert, R. *FEBS Lett.* **1999**, *442*, 117.
9. Grimm, E.; Roy, B.; Aspiotis, R.; Bayly, C. J.; Nicholson, D. W.; Rasper, D. M.; Renaud, J.; Roy, S.; Tam, J.; Tawa, P.; Vaillancourt, J. P.; Xanthoudakis, S.; Zamboni, R. *Bioorg. Med. Chem.* **2004**, *12*, 845.
10. Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.
11. Lee, D.; Long, S. A.; Adams, J. L.; Chan, G.; Vaidya, K. S.; Francis, T. A.; Kirly, K.; Winkler, J. D.; Sung, C.-M.; Debouck, C.; Richardson, S.; Levy, M. A.; DeWolf, W. E., Jr.; Keller, P. M.; Tomaszek, T.; Head, M. S.; Dominic Ryan, M.; Curtis Haltiwanger, R.; Liang, P.-H.; Janson, C. A.; McDevitt, P. J.; Johanson, K.; Concha, N. O.; Chan, W.; Abdel-Meguid, S. S.; Badger, A. M.; Lark, M. W.; Nadeau, D. P.; Suva, L. J.; Gowen, M.; Nuttall, M. E. *J. Biol. Chem.* **2000**, *275*, 16007.
12. Han, Y.; Giroux, A.; Grimm, E.; 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.; Becher, J. W.; Rotonda, J.; Nicholson, D. W.; Zamboni, R. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 805.
13. Sanderson, P. E. J.; Lyle, T. A.; Cutrona, K. J.; Dyer, D. L.; Dorsey, B. D.; McDonough, C. M.; Naylor-Olson, A. M.; Chen, I.-W.; Chen, Z.; Cook, J. J.; Cooper, C. M.; Gardell, S. J.; Jare, T. R.; Krueger, J. A.; Dale Lewis, S.; Lin, J. H.; Lucas, B. J., Jr.; Lyle, E. A.; Lynch, J. J., Jr.; Stranieri, M. T.; Vastag, K.; Yan, Y.; Shafer, J. A.; Vacca, J. P. *J. Med. Chem.* **1998**, *41*, 4466.
14. Fleitz, F. J.; Lyle, T. A.; Zheng, N.; Armstrong, J. D., III; Volante, R. P. *Synth. Commun.* **2000**, *30*, 3171.
15. 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.
16. Semple, G.; Ashworth, D. M.; Baker, G. R.; Batt, A. R.; Baxter, A. J.; Benzies, D. W. M.; Elliot, L. H.; Michael Evans, D.; Franklin, R. J.; Hudson, P.; Jenkins, P. D.; Pitt, G. R.; Rooker, D. P.; Sheppard, A. S.; Szelke, M.; Yamamoto, S.; Isomura, Y. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1337.
17. Becker, J. W.; Rotonda, J.; Soisson, S. M.; Aspiotis, R.; Bayly, C. I.; Francoeur, S.; Gallant, M.; Margarita, G.-C.; Giroux, A.; Grimm, E.; Han, Y.; McKay, D.; Nicholson, D. W.; Paterson, E.; Renaud, J.; Roy, S.; Thornberry, N.; Zamboni, R. *J. Med. Chem.* **2004**, *47*, 2466, and references cited therein.
18. Aspiotis, R.; Bayly, C. I.; Becker, J.; Ethier, D.; Francoeur, S.; Margarita, G.-C.; Giroux, A.; Grimm, E.; Han, Y.; McKay, D. J.; Nicholson, D. W.; Paterson, E.; Rasper, D.; Renaud, J.; Retonda, J.; Roy, S.; Tam, J.; Tawa, P.; Thornberry, N.; Vaillancourt, J.; Xanthoudakis, S.; Zamboni, R.; *Abstracts of Papers*, 224th ACS National Meeting, Boston, MA, USA, August 18–22, 2002.
19. (a) Rotonda, J.; Nicholson, D. W.; Fazil, K. M.; Gallant, M.; Gareau, Y.; Labelle, M.; Peterson, E. P.; Rasper, D. M.; Ruel, R.; Vaillancourt, J. P.; Thornberry, N. A.; Becher, J. W. *Nat. Struct. Biol.* **1996**, *3*, 619; (b) Talanian, R. V.; Quinlan, C.; Trautz, S.; Hackett, M. C.; Mankovich, J. A.; Banach, D.; Ghayur, T.; Brady, K. D.; Wong, W. W. *J. Biol. Chem.* **1997**, *272*, 9677; (c) Chéreau, D.; Kodandapani, L.; Tomaselli, K. J.; Spada, A. P.; Wu, J. C. *Biochemistry* **2003**, *42*, 4151.
20. Semple, G.; Ashworth, D. M.; Batt, A. R.; Baxter, A. J.; Benzies, D. W. M.; Elliot, L. H.; Michael Evans, D.; Franklin, R. J.; Hudson, P.; Jenkins, P. D.; Pitt, G. R.; Rooker, D. P.; Yamamoto, S.; Isomura, Y. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1337.

21. Nakajima, M.; Kashiwagi, K.; Ohta, J.; Furukawa, S.; Hayashi, K.; Kawashima, T.; Hayashi, Y. *Brain Res.* **1994**, *641*, 350.
22. Han, B. H.; Xu, D.; Choi, J.; Han, Y.; 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.
23. Toulmond, S.; Tang, K.; Bureau, Y.; Ashdown, H.; Degen, S.; O'Donnell, R.; Tam, J.; Han, Y.; Colucci, J.; Giroux, A.; Zhu, Y.; Boucher, M.; Pikounis, B.; Xanthoudakis, S.; Roy, S.; Rigby, M.; Zamboni, R.; Robertson, G. S.; Ng, G. Y. K.; Nicholson, D. W.; Flückiger, J.-P. *Br. J. Pharmacol.* **2004**, *141*, 689.
24. Méthot, N.; Huang, J.; Coulombe, N.; Vaillancourt, J. P.; Rasper, D.; Tam, J.; Han, Y.; Colucci, J.; Zamboni, R.; Xanthoudakis, S.; Toulmond, S.; Nicholson, D. W.; Roy, S. *J. Exp. Med.* **2004**, *199*, 199.
25. Méthot, N.; Vaillancourt, J. P.; Huang, J. Q.; Colucci, J.; Han, Y.; Menard, S.; Zamboni, R.; Toulmond, S.; Nicholson, D. W.; Roy, S. *J. Biol. Chem.* **2004**, *279*, 27905.