Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2137–2140

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Nicotinyl Aspartyl Ketones as Inhibitors of Caspase-3

Elise Isabel,[a,*] W. Cameron Black,[a] Christopher I. Bayly,[a] Erich L. Grimm,[a] Marc K. Janes,[a] Daniel J. McKay,[a] Donald W. Nicholson,[a] Dita M. Rasper,[a] Johanne Renaud,[a] Sophie Roy,[a] John Tam,[a] Nancy A. Thornberry,[b] John P. Vaillancourt,[a] Steven Xanthoudakis[a] and Robert Zamboni[a]

[a]*Merck Frosst Centre for Therapeutic Research, Merck Frosst Canada & Co., PO Box 1005, Pointe-Claire-Dorval, Quebec, Canada H9R 4P8*

[b]*Merck Research Laboratories, Rahway, NJ 07065, USA*

Received 15 May 2002; accepted 8 April 2003

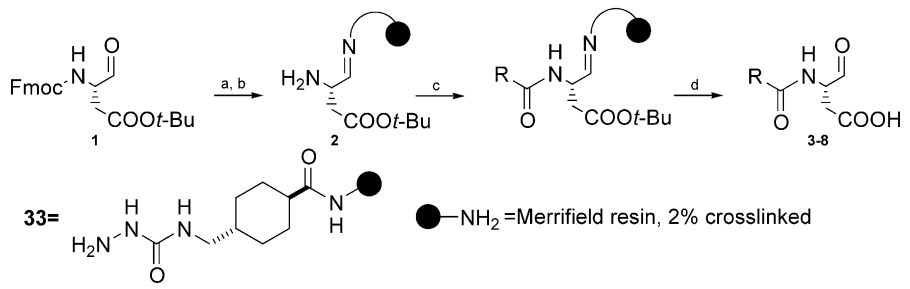
**Abstract**—Caspase-3 is a cysteinyl protease that mediates apoptotic cell death. Its inhibition may have an important impact in the treatment of several degenerative diseases. Since $P_1$ aspartic acid is a required element of recognition for this enzyme, a library of capped aspartyl aldehydes was synthesized using solid-phase chemistry. The 5-bromonicotinamide derivative of the aspartic acid aldehyde was identified to be an inhibitor of caspase-3. Substitution at the 5-position of the pyridine ring and conversion of the aldehyde to ketones led to a series of potent inhibitors of caspase-3.


The human caspases are a family of at least 11 cysteinyl-aspartate-specific proteinases that are central components in the molecular pathways that result in the apoptosis of cells.[1] These enzymes are divided into three groups. Group I caspases (1, 4, and 5) mediate cytokine maturation and are implicated in the inflammatory response. Group II caspases (2, 3 and 7) are the major effectors of cell death. Group III caspases (6, 8, 9, and 10) are upstream activator enzymes of the group II caspases. Caspase-14 is a heratinocyte specific caspase that has not yet been classified. Caspase-3 appears to be a critical participant in apoptosis in neurons. Prototype peptidyl inhibitors of caspase-3 have shown efficacy in models such as stroke, traumatic brain/spinal cord injury, hypoxic brain damage, and cardiac ischemia/reperfusion injury.[2] DEVD-CHO is a tetrapeptide inhibitor based on the preferred amino acid sequence recognized by caspase-3. The aspartic acid is an essential element of recognition of the enzyme and was used as the basis to develop new inhibitors. Here we report nicotinyl aspartyl ketones as potent and selective inhibitors of caspase-3.

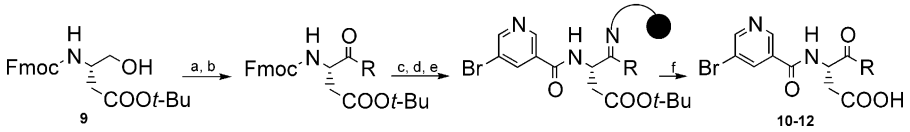
In order to generate libraries of aspartyl aldehydes, rapid analogues synthesis was performed using solid-phase chemistry (Scheme 1). Fmoc-aspartyl aldehyde **1** was reacted with a semi-carbazone linked resin **33**.[3,4] The Fmoc protecting group was then cleaved under standard conditions to afford the polymer **2**. The free amine was submitted to peptidic couplings with a wide variety of carboxylic acids. Reactions were routinely conducted on a 100-mg scale of resin. Subsequent treatment with 9:1 TFA/$H_2O$ led to cleavage from the resin with concomitant *t*-butyl ester removal to give final compounds with a good level of purity. Two aldehyde replacements were considered: alkyl and thioether ketones. Alkyl ketones were prepared from Fmoc aspartate alcohol **9** (Scheme 2), which were submitted to Swern oxidation followed by an in situ addition of Grignard reagents. Resulting alcohols were oxidized to ketones using the Dess–Martin periodinane. These ketones were then attached to resin **33**. The Fmoc protecting group was cleaved and the free amine was coupled to the 5-bromonicotinic acid. Cleavage from the resin with wet TFA afforded ketones such as **10**–**12**. Thioether ketones were synthesized using the Fmoc-aspartic acid β-*t*-butyl ester **13** (Scheme 3). After formation of the mixed anhydride, treatment with $CH_2N_2$ and addition of a 1:1 mixture of aqueous 48% HBr/$CH_3COOH$, the α-bromoketone **14** was obtained. As in

*Corresponding author. Tel.: +1-514-428-3655; fax: +1-514-428-4939; e-mail: elise_isabel@merck.com

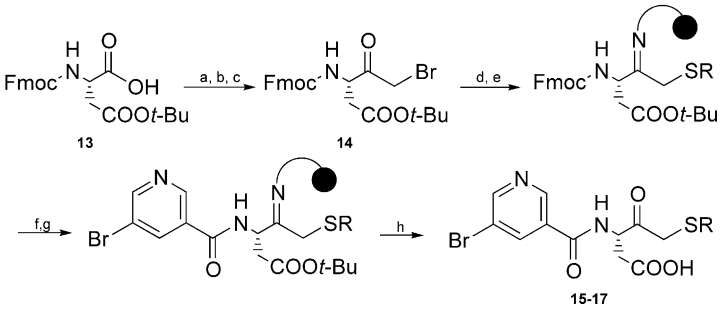
0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00390-1




**Scheme 1.** Synthesis of libraries of capped aldehydes: (a) **33**, AcOH, THF; (b) 20% piperidine/DMF; (c) RCOOH, HATU, DMF, $(i\text{Pr})_2$NEt; (d) 9:1 TFA/$H_2O$.



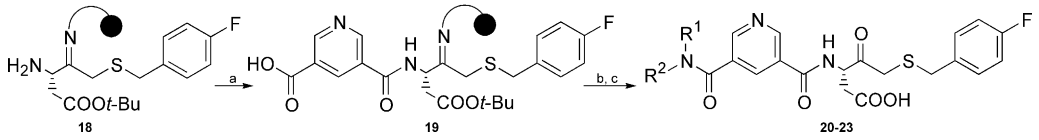

**Scheme 2.** Synthesis of alkyl ketones: (a) DMSO, $CH_2Cl_2$, $(COCl)_2$, $(i\text{Pr})_2$NEt, RMgBr; (b) Dess–Martin periodinane, $CH_2Cl_2$; (c) **33**, AcOH, THF; (d) 20% piperidine/DMF; (e) 5-bromonicotinic acid, HATU, DMF, $(i\text{Pr})_2$NEt; (f) 9:1 TFA/$H_2O$.



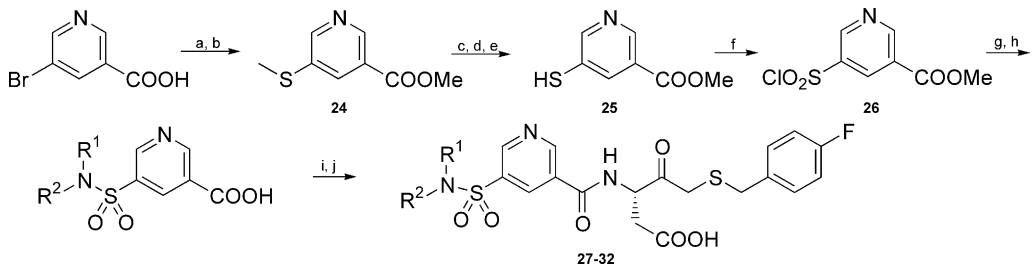

**Scheme 3.** Synthesis of thioether ketones: (a) *i*-BuOCOCl, NMM, $CH_2Cl_2$ (b) $CH_2N_2$, $Et_2O$; (c) HBr/AcOH; (d) **33**, AcOH, THF; (e) RSH, DMF, $(i\text{Pr})_2$NEt; (f) 20% piperidine/DMF; (g) 5-bromonicotinic acid, HATU, DMF, $(i\text{Pr})_2$NEt; (h) 9:1 TFA/$H_2O$.

the case of alkyl ketones, the α-bromoketone **14** was attached to the resin **33**. The bromide was displaced by a variety of thiols. The Fmoc group was removed and the free amine was coupled to the 5-bromonicotinic acid. Compounds such as **15–17** were cleaved from the resin when treated with wet TFA. Modifications to the pyridine ring were carried out as follows. Pyridine 5-carboxamides like **20–23** were readily available by first treating **18** with 3,5-pyridinecarboxylic acid under standard conditions to afford **19** (Scheme 4). This acid was used to synthesize a variety of amides under standard conditions. Sulfonyl chloride **26** was synthesized in six steps starting from 5-bromonicotinic acid (Scheme 5). Esterification followed by bromide displacement by NaSMe[5] afforded thioether **24**. Oxidation to the sulfoxide using MMPP, and Pummerer rearrangement with TFAA[6] provided thiol **25** after treatment with $Et_3N$ in MeOH. The crude material was treated with chlorine in AcOH[7] for a few min to afford the sulfonyl chloride **26**. It was reacted with different amines to obtain a wide range of sulfonamides. Methyl esters were cleaved to the corresponding carboxylic acids and were coupled to the intermediate **18** under standard conditions. Final compounds[8] such as **27–32** were cleaved from the resin as previously described.

Tetrapeptide inhibitors of caspase-3 show poor cell penetration. The purpose of this work was to identify a cell-penetrant family of inhibitors. The first library revealed that introduction of a nicotinic acid (**6**) was offering a 2-fold improvement over a benzoic acid derivative (**4**) when tested against caspase-3[9] (Table 1). Others isomers of the nicotinic acid were also synthesized and were shown to be less potent. Introduction of a bromine atom at the 5-position provided a promising lead for the replacement of the tetrapeptide (**8**). It was found later by X-ray crystallography that the 3-pyridyl nitrogen interacts strongly with the enzyme. To take advantage of the $P_1'$ subsite and to increase inhibitor stability, aldehydes were replaced by a variety of

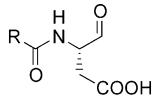


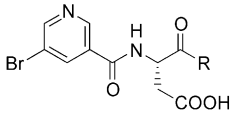

**Scheme 4.** Synthesis of 5-amido-substituted pyridines: (a) 3,5-Pyridinecarboxylic acid, HATU, DMF, $(i\text{Pr})_2$NEt; (b) $R^1R^2$NH, HATU, DMF, $(i\text{Pr})_2$NEt; (c) 9:1 TFA/$H_2O$.



**Scheme 5.** Synthesis of 5-sulfonamido-substituted pyridines: (a) MeOH, $H_2SO_4$ (cat.); (b) MeSNa, DMF, 80 C; (c) MMPP, $CH_2Cl_2$, MeOH; (d) TFAA, $CH_2Cl_2$; (e) $Et_3N$, MeOH; (f) $Cl_2$, AcOH; (g) $R^1R^2NH$, $CH_2Cl_2$; (h) LiOH, MeOH, $H_2O$; (i) **18**, HATU, DMF, $(i\text{Pr})_2\text{NEt}$; (j) 9:1 TFA/$H_2O$.

**Table 1.** Enzyme inhibition of aldehydes and ketones



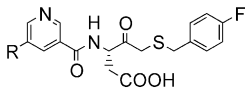


| Compd | R | Rh-Caspase-3 $IC_{50}$ (μM) | Compd | R | Rh-Caspase-3 $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| **3** | AcDEV | 0.027 | **10** | $CH_2Ph$ | 63.7 |
| **4** | Ph | 23 | **11** | $(CH_2)_3Ph$ | 13.7 |
| **5** | 2-Pyridyl | 55 | **12** | $(CH_2)_5Ph$ | 96.9 |
| **6** | 3-Pyridyl | 10 | **15** | $CH_2SPh$ | 11.7 |
| **7** | 4-Pyridyl | >200 | **16** | $CH_2SCH_2Ph$ | 1.1 |
| **8** | 5-Bromo-3-pyridyl | 6 | **17** | $CH_2SCH_2$-Ph-4-F | 1.2 |

ketones. As shown in Table 1, a three-carbon chain length connecting the carbonyl and an aromatic ring was optimal. Introduction of a sulfur atom to replace the central carbon in the chain gave a compound with an $IC_{50}$ of 1.1 μM (**16**). Substitution around the phenyl ring did not provide any improvement in potency. Having in hand a suitable ketone warhead, introduction of an amide group at the 5-position on the pyridine ring allowed us to obtain compounds with sub-micromolar activity. SAR of this series suggested that lipophilic, disubstituted amides at the 5-position gave a boost in potency relative to the bromide (Table 2). Compound **23** has an $IC_{50}$ of 130 nM against caspase-3. Compounds having an intrinsic activity better than 1 μM were also tested in the NT2 whole cell assay.[10,11] In that assay too, lipophilic groups seemed to be favored. However there was a large shift between the $IC_{50}$ value in the enzyme assay and the whole cell assay. Postulating that this shift may be due to the presence of the amide bond, we next examined sulfonamides in this position. The SAR of the sulfonamide family is comparable to that of the amides with the added advantage of improved whole cell potency (Table 2). Lipophilic groups increased the potency. Surprisingly, the best compound in the whole cell assay (**30**) incorporated a small cyclopropylamine. Using this optimized piece on the left side, the ketone moiety was revisited (Table 3). Both aldehyde **30** and aminoketone **34** showed a large shift in the NT2 cell assay. However, the phenolic ether **35** demonstrated an improved potency in this assay. Finally, the acyoxymethyl ketone **36**, which gives an irreversible inhibitor against caspase-3, had an $IC_{50}$ of 1.3 μM in the cell assay.

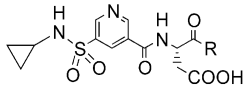
Libraries of capped aspartic acid aldehydes allowed the identification of the 5-bromonicotinic acid as a lead for a non-peptidic caspase-3 inhibitor. Optimization of the P1′ substituent with thioethers afforded a suitable replacement for the original aldehyde. Substitution of the pyridine ring at the 5-position with amides and sulfonamides improved the potency in the intrinsic

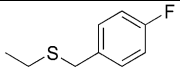
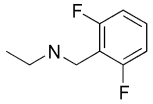
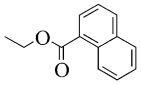
**Table 2.** Biological activity of amides and sulfonamides



| Compd | R | Rh-Caspase-3 $IC_{50}$ (μM) | NT2 cell $IC_{50}$ (μM) | Compd | R | Rh-Caspase-3 $IC_{50}$ (μM) | NT2 cell $IC_{50}$ (μM) |
|---|---|---|---|---|---|---|---|
| **3** | AcDEVCHO | 0.027 | >100 | **28** | $Et_2NSO_2$ | 0.17 | 12 |
| **20** | $Et_2NCO$ | 0.62 | >100 | **29** | $(n\text{-Pr})_2NSO_2$ | 0.17 | 25 |
| **21** | $Pr_2NCO$ | 0.41 | >100 | **30** | $c$-$PrNSO_2$ | 0.40 | 8 |
| **22** | $(i\text{Pr})\text{EtNCO}$ | 0.25 | 16 | **31** | $c$-$PrMeNSO_2$ | 0.27 | 22 |
| **23** | $(i\text{Bu})_2NCO$ | 0.13 | 39 | **32** | $BnNSO_2$ | 0.34 | 59 |
| **27** | $Me_2NSO_2$ | 0.30 | 21 | | | | |

**Table 3.** Biological activity of ketones



| Compd | R | Rh-Caspase-3 $IC_{50}$, (μM) | NT2 cell $IC_{50}$, (μM) |
|---|---|---|---|
| **30** |  | 0.4 | 8 |
| **33** | H | 1.07 | 57 |
| **34** |  | 0.52 | 53 |
| **35** |  | 0.39 | 4.3 |
| **36** |  | 0.13 | 1.3 |

assay. Sulfonamides proved to be superior for the cell assay. Introduction of an acyloxymethyl ketone at the P1′ position gave a potent irreversible inhibitor, with a potency of 1.3 μM in the NT2 cell assay.

## References and Notes

1. Nicholson, D. W. *Cell Death Diff*. **1999**, *6*, 1028.
2. Nicholson, D. W. *Nature* **2000**, *407*, 810 and references therein.
3. Grimm, E. L. Unpublished results.
4. Murphy, A. M.; Dagnino, R., Jr.; Vallar, P. L.; Trippe, A. J.; Sherman, S. L.; Lumpkin, R. H.; Tamura, S. Y.; Webb, T. R. *J. Am. Chem. Soc*. **1992**, *114*, 3156.
5. Boussad, N.; Trefouel, T.; Dupas, G.; Bourguignon, J.; Queguiner, G. *Phosphorus Sulfur* **1992**, *66*, 127.
6. Young, R. N.; Gauthier, J.-Y.; Coombs, W. *Tetrahedron Lett*. **1984**, *25*, 1753.
7. Kharasch, N.; Bruice, T. C. *J. Am. Chem. Soc*. **1951**, *73*, 3240.
8. All final compounds were purified by flash chromatography and characterized by $^1$H NMR.
9. Roy, S.; Bayly, C. I.; Gareau, Y.; Houtzager, V. M.; Kargan, S.; Keen, S. L.; Rowland, K.; Seiden, I. M.; Thornberry, N. A.; Nicholson, D. W. *Proc. Natl. Acad. Sci. U.S.A*. **2001**, *98*, 6132.
10. Tawa, P.; Tam, J.; Cassady, R.; Nicholson, D. W.; Xanthoudakis, S. *Cell Death Diff*. **2001**, *8*, 30.
11. NT2 cells were plated in 96-well plates. The following day, camptothecin 5 μg/mL and ± different concentrations of the inhibitors were added for 5 h. Cells were then harvested, lysed and analyzed by cell death ELISA following manufacturer kit's instructions (Roche Molecular Biochemicals) measuring DNA fragmentation.