Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 10, pp. 1337-1342, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00220-5

PYRIDONE-BASED PEPTIDOMIMETIC INHIBITORS OF INTERLEUKIN-1β-CONVERTING ENZYME (ICE)

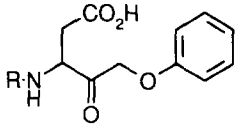
Graeme Semple[a]*, Doreen M. Ashworth[b], Graham R. Baker[b], Andrzej R. Batt[a], Andrew J. Baxter[a], David W.M. Benzies[a], Lucy H. Elliot[b], D. Michael Evans[a], Richard J. Franklin[a], Peter Hudson[a], Paul D. Jenkins[a], Gary R. Pitt[a], David P. Rooker[a], Andrew Sheppard[a], Michael Szelke[a], Satoshi Yamamoto[c] and Yasuo Isomura[c]

Departments of Medicinal Chemistry[a] and Biology[b], Ferring Research Institute, Chilworth Research Centre, 1, Venture Rd., Chilworth, Southampton, U.K., SO16 7NP.
Infectious Disease & Immunology Research Laboratories[c], Yamanouchi Institute for Drug Discovery Research, 21, Miyukigaoka, Tsukuba, Ibaraki 305, Japan.

Abstract
New potent, reversible inhibitors of recombinant human Interleukin-1β-converting enzyme (ICE, caspase-1) with significantly reduced peptide character are described. The compounds were designed by incorporation of pyridone and pyrimidone heterocyclic replacements for the P_2-P_3 amino acids of the native substrate and were optimised by manipulation of peripheral alkyl and aryl substituents.

Interleukin-1β-converting enzyme (ICE)[1] is an intracellular cysteine protease that activates the potent, pro-inflammatory cytokine IL-1β[2] by cleaving its inactive 31KDa precursor at the Asp^{116}-Ala^{117} site to produce the biologically active 17.5KDa form. ICE can cleave other sequences with an aspartic acid residue in the P_1 position and is now known to belong to a growing family of related enzymes, recently termed the caspases.[3] Several members of the family, including ICE (caspase-1), have also been implicated in the execution phase of apoptosis[4] making these enzymes interesting targets for the development of both anti-inflammatory and anti-apoptosis drugs.

Figure 1: SAR of Peptidic Phenyl Ketoethers

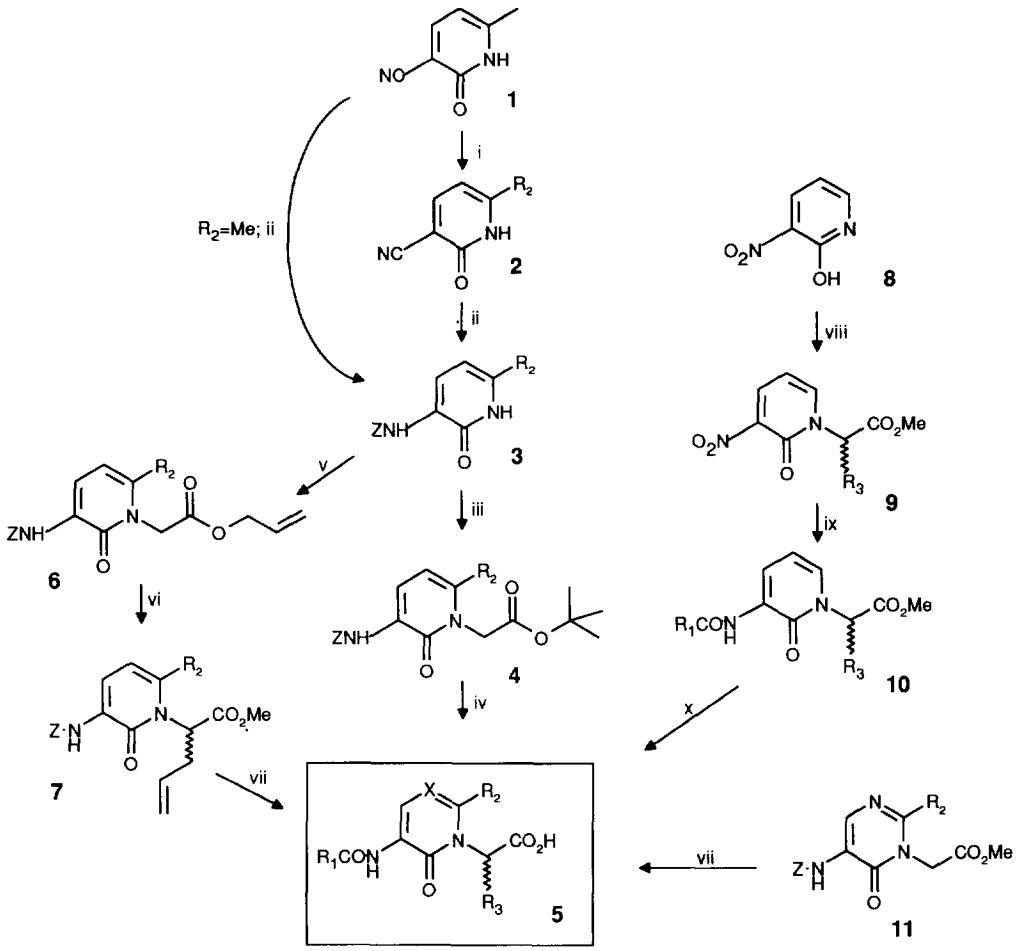


R=AcTyrValAla- K_i=1.1nM
R=2-NapCOValAla- K_i=0.15nM
R=2-NapCOValPro- K_i=0.20nM
R=2-NapCOValPic- K_i=0.15nM

(Pic= 2-carboxypiperidine)

* *e-mail: gsemple@msn.com*

Figure 2: Synthesis of Pyridone and Pyrimidone Building Blocks



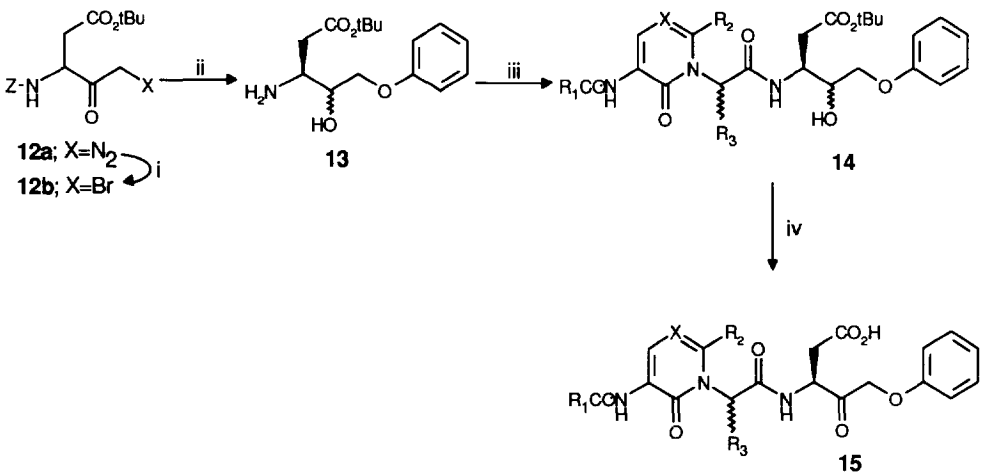
Reagents; (i) a) LDA; b) Alkyl halide; (ii) a) HBr, AcOH, Δ; b) NaOH; c) $(PhO)_2P(O)N_3$, BzOH, NEt_3, Δ; (iii) a) NaH, DMF; b) $BrCH_2CO_2{}^tBu$; (iv) a) H_2, 10% Pd/C; b) R_1COCl; c) TFA (v) NaH, DMF; b) $BrCH_2CO_2CH_2CH{=}CH_2$; (vi) a) LDA, $ZnCl_2$; b) $Pd(PPh_3)_4$; c) CH_2N_2 (vii) a) H_2, 10% Pd/C; b) R_1COCl; c) LiOH; (viii) a) NaH, DMF; b) $BrCH(R_3)CO_2Me$; (ix) a) H_2, 10% Pd/C; b) R_1COCl; (x) LiOH.

Peptide based inhibitors of ICE have been described based on the known P_4-P_1 cleavage sequence of pro-IL-1β (YVAD) coupled with some form of activated carbonyl derivative at the C-terminus, depending on whether reversible[5] or irreversible[6] inhibition is desired. We and others[7] have prepared peptidic compounds containing a C-terminal phenyl keto-ether moiety which we found to be potent, reversible inhibitors of the enzyme with K_i values in the nM or sub-nM range (Fig. 1).[8] Our studies in this series confirmed previous observations[6b] that the P_2 alanine could be replaced with a proline or

L-2-carboxypiperidine residue without a significant loss in potency. This lead us to investigate pyridone-based mimetics of the Val-Pro motif[9] in conjunction with the C-terminal phenyl keto-ether moiety described above in our quest for reversible inhibitors of ICE with reduced peptide character. We also prepared examples of the closely related pyrimidone analogues for comparison.[10]

The synthesis of the heterocyclic derivatives required as building blocks for preparation of peptidomimetic ICE inhibitors is outlined in Fig. 2. 6-Alkyl pyridones were prepared by alkylation of the lithium dianion of commercially available **1**. Hydrolysis of the nitrile group and Curtius rearrangement followed by trapping of the isocyanate with benzyl alcohol, gave the carbamates **3** as previously described.[11] N-Alkylation of the pyridone with t-butyl bromoacetate followed by modification of the N-terminal group where necessary and hydrolysis of the t-butyl ester, provided the required building blocks **5**. These were incorporated into the ICE inhibitors by water soluble carbodiimide (EDC) mediated coupling to **13** as outlined in Fig. 3. The bromoketone **12b** was prepared by treatment of the known[6a] diazoketone **12a** with HBr in EtOAc. Displacement of the bromide with phenoxide in DMF provided the phenyl keto-ether. Reduction of the ketone with sodium borohydride followed by hydrogenolysis of the Z

Figure 3: Synthesis of Pyridone and Pyrimidone Containing ICE Inhibitors



Reagents:(i) HBr/EtOAc, 0°C; (ii) a) NaH, PhOH, DMF; b) $NaBH_4$; c) H_2, 10%Pd/C; (iii) **5**, EDC, N-methylmorpholine; (iv) a) Dess-Martin Periodinane; b) TFA/DCM, 0°C.

Table 1: SAR of Pyridones and Pyrimidones (**15**, R_3=H) with Substituents on the Ring R_2-position

Compound No	R_1	R_2	X	hr-ICE Inhibition K_i (nM)[13]
15a	$PhCH_2O$	Me	CH	590 ± 140
15b	Ph-	Me	CH	530 ± 60
15c	2-Nap-	Me	CH	160 ± 40
15d	1-Nap-	Me	CH	95 ± 30
15e	1-Nap-	Et	CH	40 ± 8
15f	1-Nap-	nPr	CH	68 ± 16
15g	1-Nap-	nBu	CH	16 ± 5
15h	1-Nap-	nBu	N	46 ± 16
15i	1-Nap-	nHx	CH	40 ± 15
15j	1-Nap-	Ph	CH	25 ± 10
15k	1-Nap-	Ph	N	66 ± 14
15l	1-Nap-	CH_2Ph	CH	10 ± 3
15m	1-Nap-	H	CH	39 ± 11

protecting group gave the stable amino alcohol intermediate **13**, which could be readily stored and coupled to the required building block. The oxidation of the coupled product **14** with Dess-Martin periodinane and the subsequent removal of the aspartate protecting group by brief treatment with TFA provided the test compounds **15**.

Our prototypical peptidomimetic analogue **15a** (Table 1) exhibited reversible inhibition of recombinant human ICE with a K_i value of 590nM. Changing the N-terminal substituent to 1- or 2-napthoyl (**15c** and **15d**) gave a significant improvement in potency which correlated well with SAR observations made previously in our peptidic series. Extending the length of the alkyl group in the pyridone 6-position (R_2) gave an improvement in potency with an n-butyl substituent (**15g**) being the optimum size of those examined. As a direct comparison of the two types of heterocycle we prepared the corresponding 2-n-butyl pyrimidone compound **15h** from **11**, which in turn was prepared according to methods previously described.[9] The pyridone analogue **15g** was 2-3 times more active than the pyrimidone **15h**, a finding which was duplicated when we prepared the 6-phenyl pyridone derivative **15j** and its pyrimidone analogue **15k**.

The unsubstituted pyridone derivative **15m** was prepared from 2-hydroxy-3-nitropyridine (**8**) as outlined in Fig. 2. We observed that this compound was only around 2-fold less potent than our optimal 6-substituted pyridone which lead us to question whether the substituent in the 6-position was in the correct place to make a useful contribution towards binding to the enzyme, or whether it was simply restricting the conformations available to those which were closer to the conformation found in the natural

substrate. We reasoned that if the latter were true, such a restricting effect may also be achieved by replacing the pyridone 6-substituent (R_2) with a substituent α to the amide carbonyl (R_3). The building blocks for such analogues were prepared by alkylation of **8** with the appropriate methyl-2-bromoacetate derivative followed by reduction of the nitro group and acylation with the required N-terminal blocking group as outlined in Fig.2. All the compounds were prepared as epimeric mixtures at R_3. As can be seen in Table 2, when R_3 was a simple alkyl group we obtained at least a 5-fold improvement in potency over the unsubstituted analogue **15m**. The optimal straight chain substituent was ethyl, giving compounds **15q** and **15r** with K_i values in the nM range. The phenyl analogues **15x**, **15y** and the allyl derivative **15w** were essentially equiactive with **15r** whereas the β-branched isopropyl analogue **15t**, gave a significant decrease in potency.

Table 2: SAR of Pyridones (**15**, X=CH) with Substituents in the R_3 Position.

Compound No	R_1	R_2	R_3	hr-ICE Inhibition K_i (nM)[13]
15m	1-Nap-	H	H	39 ± 11
15n	2-Nap-	H	H	45 ± 7
15o	1-Nap-	H	Me	7 ± 2
15p	2-Nap-	H	Me	14 ± 5
15q	1-Nap-	H	Et	2.2 ± 0.6
15r	2-Nap-	H	Et	1.4 ± 0.4
15s	1-Nap-	H	nPr	1.8 ± 0.8
15t	1-Nap-	H	iPr	21 ± 2
15u	1-Nap-	H	nBu	5.3 ± 1.0
15v	2-Nap-	H	$-CH_2OMe$	7.1 ± 1.6
15w	2-Nap-	H	allyl	1.5 ± 0.15
15x	1-Nap-	H	Ph	1.8 ± 0.4
15y	2-Nap-	H	Ph	1.3 ± 0.6
15z	2-Nap-	H	2-Pyr	2.0 ± 0.3
15aa	2-Nap-	Me	nPr	2200
15bb	1-Nap-	Ph	nPr	7200

Finally we incorporated substituents in both of the positions described above by ester enolate Claisen-like rearrangement of the requisite allyl ester **6**,[12] followed by esterification with diazomethane for ease of purification, to give the α-allyl substituted compound **7** as outlined in Fig. 2. Reduction of the allyl group to an n-propyl group was achieved during removal of the Z protecting group prior to the standard N-acylation procedure. However it can be seen (**15aa**, **15bb**) that having two such groups in the same molecule had a highly detrimental effect on enzyme inhibition. We would speculate that the two groups may either be competing for the same binding site (probably S_2) in the enzyme or the combination may

simply be forcing the key binding groups into unfavourable positions and hence the molecule out of its bioactive conformation.

In conclusion, we have identified potent, reversible inhibitors of ICE with significantly reduced peptide character, by incorporation of heterocyclic replacements for the Val-Pro motif followed by adding further peripheral alkyl substituents. We are currently investigating the behaviour of these compounds in cell-based assays, including measuring their ability to inhibit the release of mature IL-1β from LPS stimulated cell lines. These results and the effects of these inhibitors *in vivo* will be reported in due course.

References and Notes

1) Thornberry, N.A.; Bull, H.G.; Calaycay, J.R.; Chapman, K.T.; Howard, A.D.; Kosturas, M.J.; Millar, D.K.; Molineaux, S.M.; Weidner, J.R.; Aunins, J.; Elliston, K.O.; Ayala, J.M.; Casano, F.J.; Chin, J.; Ding, G. J.-F.; Egger, L.A.; Gaffney, E.P.; Limjuco, G.; Palyha, O.C.; Raju, S.M.; Rolando, A.M.; Salley, J.P.; Yamin, T.-T.; Lee, T.D.; Shively, J.E.; MacCross M.; Mumford, R.A.; Schmidt, J.A.; Tocci, M.J. *Nature* **1992**, *356*, 768-774.
2) IL-1β has been implicated in the pathogenesis of several inflammatory disorders. Dinarello, C.A. *Blood* **1991**, *77*, 1627-1652.
3) Alnemri, E.S.; Livingston, D.J.; Nicholson. D.W.; Salvesen, G.; Thornberry, N.A.; Wong, W.W.; Yuan, J-Y. *Cell* **1996**, *87*, 171.
4) Steller, H. *Science* **1995**, *267*, 1445.
5) a) Chapman, K.T. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 613-618. b) Mjalli, A.M.M.; Chapman, K.T.; MacCross, M.; Thornberry, N.A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2689-2695.
6) a) Dolle, R.E.; Hoyer, D.; Prasad, C.V.C.; Schmidt, S.J.; Helaszek, C.T.; Miller, R.E.; Ator, M.A. *J. Med. Chem.* **1994**, *37*, 563-564. b) Dolle, R.E.; Singh, J; Rinker, J.; Hoyer, D.; Prasad, C.V.C.; Graybill T.L.; Salvino, J.M.; Helaszek, C.T.; Miller, R.E.; Ator, M.A. *J. Med. Chem.* **1994**, *37*, 3863-3866. c) Dolle, R.E.; Singh, J.; Whipple, D.; Osifo, I.K.; Speier, G.; Graybill T.L.; Gregory, J.S.; Harris, A.L.; Helaszek, C.T.; Miller, R.E.; Ator, M.A. *J. Med. Chem.* **1995**, *38*, 220-222.
7) Mjalli, A.M.M.; Zhao, J.J.; Chapman, K.T.; Thornberry, N.A.; Peterson, E.P.; MacCross, M.; Hagmann, W.K. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1409-1414.
8) Irreversible inhibition was characterised by the lack of substrate turnover following pre-incubation of enzyme and inhibitor. With all the compounds described herein as reversible inhibitors, the progress curves were independent of the order of addition of enzyme or inhibitor to the substrate/buffer mixture.
9) Pyridones have been used previously as Val-Pro mimetics in the design of inhibitors of human leukocyte elastase see: Brown, F.J.; Andisik, D.W.; Bernstein, P.R.; Bryant, C.B.; Ceccarelli, C.; Damewood, J.R.Jr.; Edwards, P.D.; Earley, R.A.; Feeney, S.; Green, R.C.; Gomes, B.C.; Kosmider, B.J.; Krell, R.D.; Shaw, A.; Steelman, G.B.; Thomas, R.M.; Vacek, E.P.; Veale, C.A.; Tuthill, P.A.; Warner, P.; Williams, J.C.; Wolanin, D.J.; Woolson, S.A. *J. Med. Chem.* **1994**, *37*, 1259-1261.
10) Irreversible pyrimidone-based inhibitors of ICE have been described recently: Dolle, R.E.; Prouty, C.P.; Prasad, C.V.C.; Cook, E.; Saha, A.; Ross, T.M.; Salvino, J.M.; Helaszek, C.T.; Ator, M.A. *J. Med. Chem.* **1996**, *39*, 2438-2440.
11) Damewood, J.R.Jr.; Edwards, P.D.; Feeney, S.; Gomes, B.C.; Steelman, G.B.; Tuthill, P.A.; Williams, J.C.; Warner, P.; Woolson, S.A.; Wolanin, D.J.; Veale, C.A. *J. Med. Chem.* **1994**, *37*, 3303-3312.
12) Kazmaier, U. *J. Org. Chem.* **1994**, *59*, 6667-6670.
13) Inhibition assays using recombinant human ICE (hrICE) were performed according to the methods described in ref. 1 except that AcTyrValAlaAspAFC (AFC = 7-amino-4-trifluoromethylcoumarin) was used in place of AcTyrValAlaAspAMC (AMC = 7-amino-4-methylcoumarin) as the fluorogenic substrate. This substrate had excitation and emission wavelengths at 395nm and 515nm respectively. K_i values were determined using standard Dixon Plots and are ± S.E.M.

(*Received in Belgium* 21 *February* 1997; *accepted* 17 *April* 1997)