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PEPTIDOMIMETIC AMINOMETHYLENE KETONE INHIBITORS OF INTERLEUKIN-1β-CONVERTING ENZYME (ICE)

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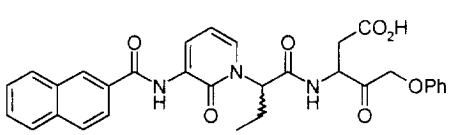
Abstract

Pyridone-based peptidomimetic inhibitors of recombinant human Interleukin-1β-converting enzyme (ICE, caspase-1) with an aminomethylene ketone activating group in the P_1' position are described. Several analogues with sub-nanomolar K_i's versus ICE and improved aqueous solubility are reported.

Interleukin-1β-converting enzyme (ICE), recently termed caspase-1,[1] is primarily responsible for the processing of the key inflammatory mediator IL-1β[2] from its inactive pro-form to the mature cytokine.[3] It thus represents an attractive target for the design of potential new anti-inflammatory compounds.

We have previously reported the design of potent peptidomimetic inhibitors of ICE (**1**) from the peptidic inhibitor **2**. This series of compounds incorporated an N-alkyl pyridone heterocycle in place of the central dipeptide unit and contained a phenyl keto ether moiety in the P_1' position.[4] The same or a closely related peptidomimetic backbone in combination with other C-terminal activated groups has also been shown by others to provide good inhibitors of ICE.[5]

Figure 1



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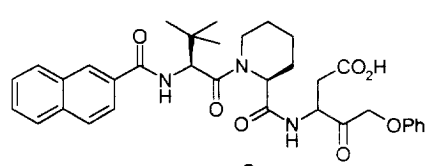

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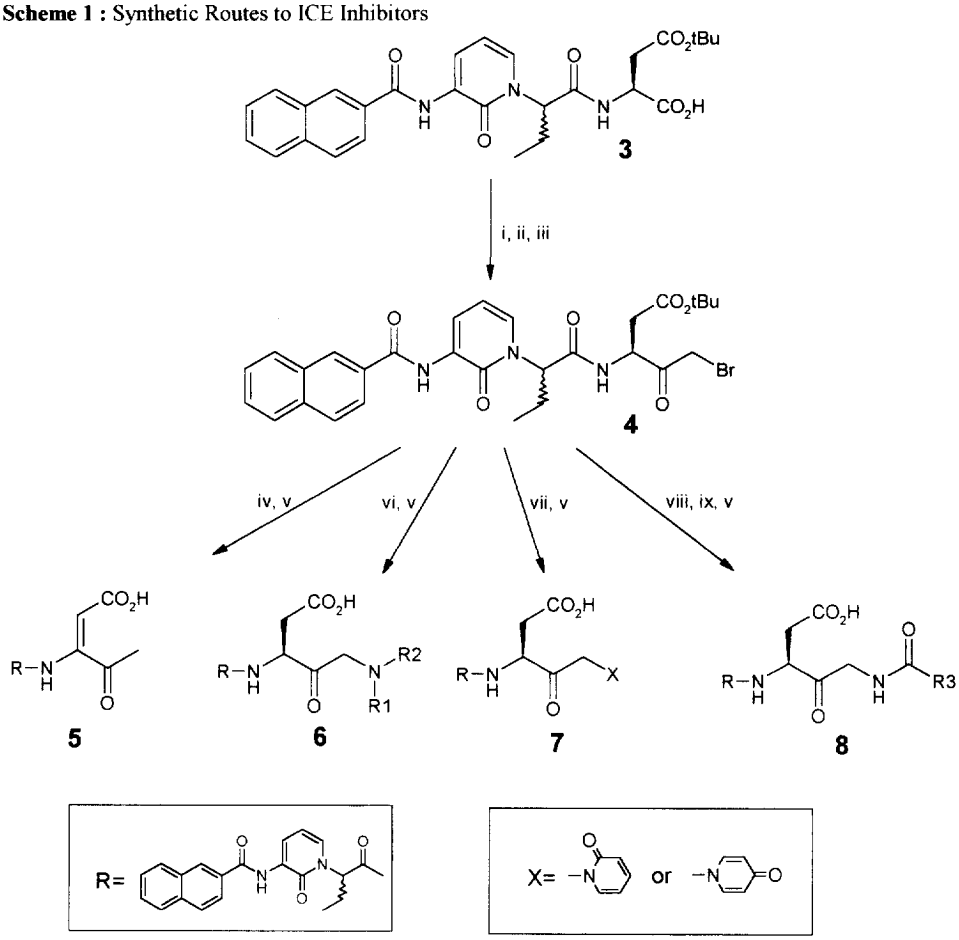
Aminomethylene ketones have been used as the enzyme active site targeting groups in the P_1' position both in tripeptide-sized molecules[6] and in much smaller aspartic acid derived compounds[7] to provide inhibitors of ICE. We now wish to report our recent studies on the combination of these two types of modification with the aim of improving both the activity and water solubility, and hence the general pharmacological profile, of our peptidomimetic lead structures.

Scheme 1 : Synthetic Routes to ICE Inhibitors

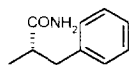
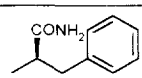
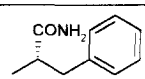


Reagents: i) $^tBuOCOCl$, NMM, THF ii) CH_2N_2, Et_2O iii) HBr, EtOAc iv) $ArNHR_1$, $NaHCO_3$, THF v) TFA vi) NHR_1R_2 , $NaHCO_3$, THF vii) pyridone, KF, DMF viii) NaN_3, DMF ix) a) H_2, 10% Pd on C, MeOH, HCl b) R_3COCl, DIEA

Compounds were prepared as shown in Scheme 1. Intermediate **3** was prepared by conventional coupling of the previously reported racemic 2-(3-(2-naphthoyl amino)-1-pyrid-2-one)-butanoic acid[4] to aspartic acid protected as the α-methyl, β-t-butyl diester derivative, followed by base hydrolysis of the methyl ester group. Conversion to the key bromoketone intermediate **4**, was accomplished by treatment

of the mixed anhydride derivative of **3** with diazomethane followed by brief treatment of the intermediate diazoketone with dry HBr. Aliphatic aminomethylene ketone derivatives **6** were obtained by treatment of **4** with the appropriate amine under basic conditions, followed by hydrolysis of the aspartate *t*-butyl ester moiety with TFA. Formation of aryl aminomethylene ketones was attempted in a similar way using a variety of basic conditions but the only product isolated was the previously observed α,β-unsaturated methyl ketone produced by base-catalysed dehydrobromination and rearrangement of **4**.[8] The deprotected compound **5** was tested in the ICE assay but found to be inactive.

Table 1: SAR of pyridone-based aminomethylene ketone inhibitors (**6a-6p**)

Compound No.	R1	R2	K_i vs hrICE[9] (nM)
6a	$PhCH_2$	H	3.05 ± 0.2
6b	$PhCH_2CH_2$	H	0.37 ± 0.04
6c	$PhCH_2CH_2CH_2$	H	1.15 ± 0.02
6d	$CH_3(CH_2)_5$	H	0.85 ± 0.02
6e	$CH_3(CH_2)_3$	H	0.76 ± 0.08
6f		H	78 ± 6.0
6g		H	25 ± 2.0
6h	$PhCH_2$	Me	58 ± 1.0
6i	$PhCH_2CH_2$	Me	4.8 ± 0.1
6j	Me	Me	22.8 ± 1.0
6k	$CH_3(CH_2)_3$	Me	3.1 ± 0.1
6l	$CH_3(CH_2)_3$	Et	2.3 ± 0.3
6m	$CH_3(CH_2)_5$	$CH_3(CH_2)_5$	2.39 ± 0.01
6n	$CH_3(CH_2)_5$	Me	1.79 ± 0.03
6o	$H_2NCO(CH_2)_3$	Me	37 ± 7
6p		Me	255 ± 25

The formation of compounds containing an unsaturated heterocyclic moiety β to the ketone was achieved by displacement of the bromoketone **4** with 2-pyridone or 4-hydroxypyridine in the presence of KF in DMF. This gave exclusively the N-alkylated products which were in turn deprotected to give compounds **7a** and **7b**.

N-Acyl derivatives **8** were obtained by treatment of **4** with sodium azide, followed by catalytic hydrogenation in the presence of one equivalent of HCl, to give the corresponding aminomethylene ketone. Immediate acylation with the requisite acid chloride provided the amido series **8**.

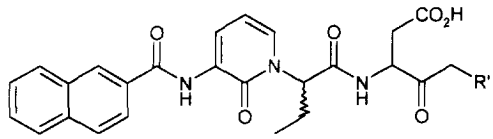
The SAR obtained for the secondary and tertiary aliphatic acyclic aminomethylene ketone series is shown in Table 1. The most potent analogue was found to be the phenethyl derivative **6b** which parallels the activity previously observed in a series of truncated aspartate based inhibitors.[7] Sub-nanomolar potencies were also obtained for straight chain secondary aliphatic amines, with the n-hexyl (**6d**) and n-butyl derivatives (**6e**) being essentially equiactive. All of the amine derivatives had enhanced water solubility over the corresponding phenyl keto-ether derivative **1** and the parent peptidic inhibitor **2**, reflecting their lower cLogP values.[10] However, a lack of long-term stability with secondary amine inhibitors was noted, leading to difficulties in the isolation and purification of these compounds and a reduction in activity over a period of a few days. Fortunately, the tertiary amine derivatives were also more water soluble than **1** but did not suffer from this lack of stability.

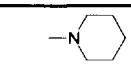
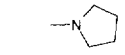
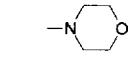
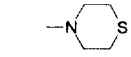
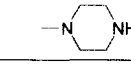
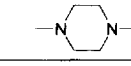
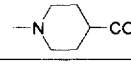
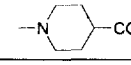
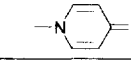
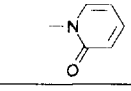
In contrast to the SAR observed with truncated analogues where a drastic loss in activity was seen following N-methylation,[7] the N-methylated derivatives **6h** and **6i** were only one order of magnitude less potent than the corresponding secondary amine analogues **6a** and **6b**. It is probable then, that the much larger N-terminal group of our inhibitors makes an overriding contribution to the binding of the compounds to the enzyme active site. In this tertiary amino series, the n-butyl and n-hexyl derivatives **6k** and **6n** were more active than the phenylethyl analogue. Chain extension of the R_2 group provided compounds **6l** and **6m** which maintained potent enzyme inhibitory activity.[11] Incorporation of primary amide groups into the aliphatic chains of compounds in this series (**6f, 6g, 6o,** and **6p**) had a deleterious effect on both activity and solubility.

As can be seen in Table 2, the incorporation of simple saturated 5- and 6-membered cyclic amines provided compounds which retained reasonable activity with the piperidyl analogue **6q** being slightly more potent than the pyrrolidyl derivative **6r**. However, substitution of the ring or incorporation of further heteroatoms gave no improvement in potency with only the 4-ethoxycarbonyl compound **6x** retaining a K_i comparable to that of **6q**. Heteroaryl methylene ketones **7a** and **7b,** also failed to show significant activity against the enzyme.

Acylation of the aminomethylene moiety to give the amide series **8a-8d** resulted in a significant decrease in potency and solubility compared to the alkyl amino series, indicating that a basic amino group is essential to obtain the desired profile.

Table 2: SAR of pyridone-based aminomethylene ketones (**6q - 8**).



Compound No.	R'	K_i vs hrICE[9] (nM)
6q		4.0 ± 0.2
6r		7.3 ± 0.3
6s		45 ± 2.5
6t		11.25 ± 0.75
6u		410 ± 20
6v		62.5 ± 9.5
6w		11.6 ± 0.8
6x		8.0 ± 1.0
7a		395 ± 25
7b		1650
8a	-NHCOPh	57.5 ± 7.5
8b	-NHCO$(CH_2)_4CH_3$	34.3 ± 2.8
8c	-NHCOCH_3	117 ± 23
8d	-NHCO$(CH_2)_2CH_3$	81 ± 7

In summary, we have described a series of novel aminomethylene ketone compounds analogous of our earlier phenyl keto-ether derivatives which maintain potent inhibition of ICE activity and show improved solubility in aqueous solution. We are currently investigating whether this change in the

physicochemical properties of our ICE inhibitors series will manifest itself in enhanced activity over our lead compounds **1** and **2** in *in vivo* models of inflammation.

References and Notes

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7) Mjalli, A.M.M.; Chapman, K.T.; Zhao, J.J.; Thornberry, N.A.; Peterson, E.P.; MacCross, M.; *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1605-1608.
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9) Inhibition assays using human recombinant ICE (hrICE) were performed according to the methods described in ref. 3 except that AcTyrValAlaAspAFC (AFC=7-amino-6-trifluoromethylcoumarin) was used in place of AcTyrValAlaAspAMC (AMC=7-amino-6-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.
10) Calculated (using cLogP for Windows 2.0.0 BioByte Corp.) and measured (using Sirius instruments PCA101) LogP values and aqueous solubility data for selected representative compounds are as follows:-

Compound	calc. LogP	measured LogP	Solubility†
1	3.68	4.26	<0.2mg/mL
2	5.53	4.73	0.46mg/mL
6n	2.30	n.d.	0.9mg/mL
6e	0.91	n.d.	>1.2mg/mL

† Approximate solubility in 0.1% MeCN/0.1M Tris buffer at pH 7.4

11) Irreversible inhibition was characterised by the lack of substrate turnover following pre-incubation of enzyme and inhibitor for up to 20 min prior to addition of substrate. All the compounds described herein were reversible inhibitors and hence the progress curves were independent of the order of addition of enzyme or inhibitor to the substrate/buffer mixture.