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PHENYLALKYL KETONES AS POTENT REVERSIBLE INHIBITORS OF INTERLEUKIN-1β CONVERTING ENZYME

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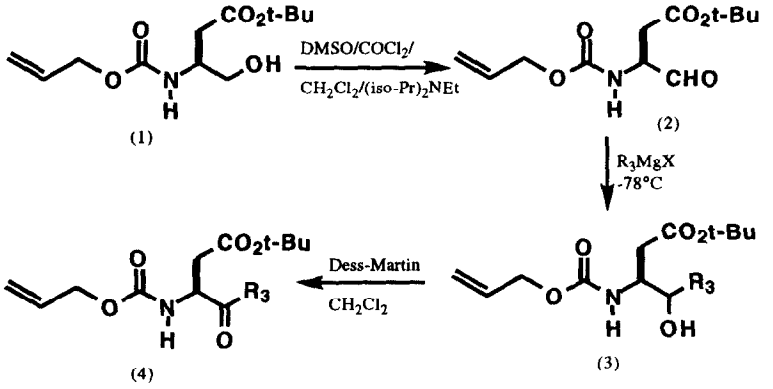
Abstract: Phenylalkyl ketones are potent reversible inhibitors of interleukin-1β converting enzyme (ICE). The extended alkyl chain ketones AcTyrValAlaAspCO$(CH_2)_n$Ph display increased potency over the simple aryl ketone. In particular, the tetrapeptide AcTyrValAlaAspCOPh has a K_i of 10μM while AcTyrValAlaAspCO$(CH_2)_5$Ph has a K_i of 18.5nM.

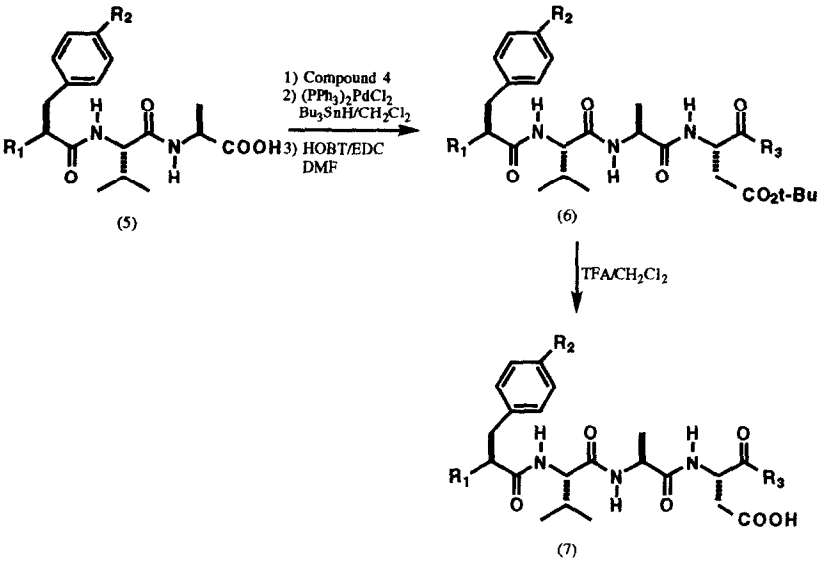
Interleukin-1β Converting Enzyme (ICE), a heterodimeric cysteine protease, cleaves the precursor form of IL-1β (pre-IL-1β) at Asp_{116}-Ala_{117} to produce the 17kDa active cytokine IL-1β.[1,2] Since IL-1β is implicated as a mediator in the pathogenesis of chronic and acute inflammatory diseases, the interruption of the processing of pre-IL-1β may prevent the progression and symptoms of IL-1 mediated diseases.[3] Substrate specificity studies suggest that human ICE prefers Tyr-Val-Ala-Asp to the N-terminal side of the scissile bond (P_4-P_1) in order to process the substrate. Additionally, there is evidence for a non-productive hydrophobic binding pocket in the P'_1-P'_2 region.[1] It has been reported that, peptidyl methylketones,[4,5] peptidyl aldehydes,[6] peptidyl nitriles[7] and peptidyl cyclopropenones[8] are reversible, competitive inhibitors of cysteine proteases and we have previously reported that the peptide aldehyde AcTyrValAlaAspCHO is a potent reversible inhibitor of ICE.[9] More recently, our laboratory has been active in developing new and novel types of ICE inhibitors and some of our approaches are described herein. Based on the existing evidence of a hydrophobic binding pocket in the P_1' to P_2' region, a number of C-terminal alkyl and phenylalkyl ketones of AcTyrValAlaAspCOR were prepared. We report herein the synthesis of alkyl and phenylalkyl ketones and their activity as potent competitive, reversible inhibitors of ICE.

Aspartic acid β-t-butylester was N-protected as allyloxycarbonyl and converted to the alcohol **1** as previously reported.[9] The alcohol **1** was subsequently converted to the aldehyde **2** by Swern oxidation.[10] and treatment of **2** with either commercially

available or freshly prepared Grignard reagent at -78°C afforded the corresponding alcohol **3** in >90% yield.[11] The alcohol was purified and then oxidized to the corresponding ketone **4** using the Dess-Martin[12] reagent in 92% yield.



R_3 = Me, Et, Ph, cyclohexyl, benzyl, phenylethyl, phenylpropyl, phenylbutyl, phenylpentyl.



Reaction of the ketone **4** with Bu_3SnH and a catalytic amount of $(PPh_3)_2PdCl_2$ in wet CH_2Cl_2 resulted in the removal of the alloc protecting group. Isolation and purification of the amino ketone was achieved, but in very low yield, presumably due to

its tendency to dimerize. This problem was circumvented by using the substrate peptide fragment AcTyrValAla or DHC.Val Ala **5** as a proton donor in the alloc deprotection followed by addition of peptide coupling reagents hydroxybenzotriazole (HOBT) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (DAEC) to effect the desired coupling to afford the tetrapeptide t-butyl ester **6** in 89% yield.[4] Treatment of these tetrapeptide esters with a 1:1 mixture of dichloromethane: trifluoroacetic acid provided the desired compounds **7** in quantitative yields.

A series of compounds which were made by the method listed above are shown in Table 1. The alkyl ketones (entry **7a-d**) in which the alkyl group was Me, Et, Ph, cyclohexyl showed poor activity against ICE. On the other hand, increasing the length of the tether (entries **7e-i**) from phenyl ketone (**7c**) (k_i>100μM) to phenylpentyl ketone (**7i**) (K_i=18.5nM) resulted in a dramatic improvement in the activity against ICE. Our results lend strong support to the idea that the C-terminus interacts with a hydrophobic binding pocket.

TABLE 1

Entry	R_1	R_2	R_3	K_i
7a	CH_3CONH	OH	Me	>10μM
7b	CH_3CONH	OH	Et	4.0μM
7c	CH_3CONH	OH	Ph	>100μM
7d	CH_3CONH	OH	Cyclohexyl	>100μM
7e	H	H	$PhCH_2$	3,100nM
7f	H	H	$Ph(CH_2)_2$	610nM
7g	H	H	$Ph(CH_2)_3$	100nM
7h	CH_3CONH	OH	$Ph(CH_2)_4$	42nM
7i	CH_3CONH	OH	$Ph(CH_2)_5$	18.5nM

These results demonstrate that tetrapeptide phenyl alkyl ketones can be prepared that are potent cysteine protease inhibitors. The study described herein has helped to define a hydrophobic binding region for inhibitors of this potentially important protease.

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