First Examples of Peptidomimetic Inhibitors of Interleukin-1β Converting Enzyme

Roland E. Dolle,*,† Catherine P. Prouty,‡ C. V. C. Prasad,§ Ewell Cook,⊥ Ashis Saha,∥ Tina Morgan Ross,∥ Joseph M. Salvino,▽ Carla T. Helaszek, and Mark A. Ator#

Sanofi Winthrop Inc., 1250 South Collegeville Road, P.O. Box 5000, Collegeville, Pennsylvania 19426

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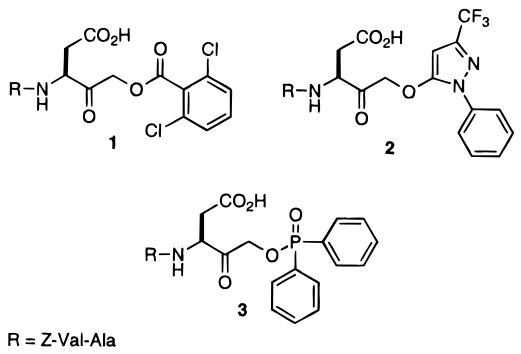
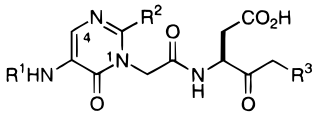
Interleukin-1β converting enzyme (ICE) is a cysteine protease found primarily in monocytic cells.[1] The enzyme cleaves precursor interleukin-1β (IL-1β) to generate biologically active mature IL-1β,[1,2] a cytokine which elicits an inflammatory response *in vivo*.[3] Orally active inhibitors of ICE having a high therapeutic index would be of importance in validating the enzyme as a therapeutic target for inflammation in a clinical setting.[3c] In a series of communications, we have described three novel classes of peptide-based ICE inhibitor.[4−6] Representative inhibitors possessing second-order rate constants $>10^5$ M^{-1} s^{-1} include Z-Val-Ala-AspCH_2DCB (**1**: DCB = (2,6-dichlorobenzoyl)oxy),[4] Z-Val-Ala-AspCH_2-PTP (**2**: PTP = (1-phenyl-3-(trifluoromethyl)pyrazol-5-yl)oxy),[5] and Z-Val-Ala-AspCH_2DPP (**3**: DPP = (diphenylphosphinyl)oxy).[6] Herein we present our initial work related to the design of peptidomimetic-based inhibitors of ICE.



Our starting point for peptidomimetic design was to discover a surrogate for the Val-Ala (P_3−P_2) dipeptide portion in inhibitors **1**−**3**. This design concept preserved the P_1 Asp residue, a critical recognition element for ICE,[3,4] and yet sufficed in removing much of the peptide character associated with the series (Figure 1). As a first approximation, we believed it essential that such a P_3−P_2 mimetic should contain the equivalent of the peptide P_3 carbonyl (P_3-CO) and P_3-NH. This ensured the integrity of the β-sheet hydrogen-bonding motif between the enzyme−inhibitor complex.[5,7] The importance of the P_3-NH (and P_1-NH) for optimal inhibitor potency was established via k_{obs}/[I] data obtained from an *N*-methyl scan of the Val-Ala-Asp backbone in **2**.[5] The 5-aminopyrimidin-6-one system was a mimetic which appeared to satisfy our design criteria (Figure 1). This mimetic was successfully used in the design of human leukocyte elastase inhibitors.[8,9] A series of novel inhibitors (**4**−**14**) incorporating the pyrimidinone unit was prepared and evaluated against ICE.

Table 1. Second-Order Rate Constants of Inactivation for Inhibitors **4**−**14** and Reference Peptides **1**−**3** against ICE



inhibitor no.	R^1	R^2	R^3	ICE[a] (k_{obs}/[I] (M^{-1} s^{-1}))
4	Z[b]	4-FPh	DCB[c]	268 000
5	Z	4-FPh	PTP[c]	157 000
6	Z	4-FPh	DPP[c]	145 000
7	Z	2-thienyl	DCB	272 000
8	Z	3-pyridinyl	PTP	145 000
9	Z	Me	PTP	95 000
10	$PhCH_2NHCO$	4-FPh	DCB	148 000
11	(2-furanyl)CO	4-FPh	DCB	46 000
12	$Me_2N(CH_2)_5CO$	4-FPh	DCB	90 000
13	$PhSO_2$	4-FPh	DCB	90 000
14	H	4-FPh	DCB	36 000
1	Z-Val-Ala-Asp-DCB			432 000[4]
2	Z-Val-Ala-Asp-PTP			280 000[5]
3	Z-Val-Ala-Asp-DPP			117 000[6]

[a] Assay as described in ref 4; standard error <10%. [b] Z = benzyloxycarbonyl. [c] DCB = (2,6-dichlorobenzoyl)oxy; PTP = (1-phenyl-3-(trifluoromethyl)pyrazol-5-yl)oxy; DPP = (diphenylphosphinyl)oxy.

A convergent approach to the synthesis of pyrimidines **4**−**14** is delineated in Scheme 1. The approach involved the coupling of pyrimidinecarboxylic acids **15**−**18** with the aspartylamines **19**−**21** using standard amide bond coupling reagents. Pyrimidinecarboxylic acids **15**−**18** were prepared by the method described by Bernstein.[8a,b] Amine hydrochlorides **19**−**21** were prepared from corresponding benzyl carbamates as described previously.[5,6,10] The preparation of **4** serves to represent the general synthesis of inhibitors **4**−**9**. Thus, amine **19** and *N*-methylmorpholine (NMM; 1.0 equiv each) were added to a solution of acid **15** (1.1 equiv) containing ethyl chloroformate and NMM (1.1 equiv each) in THF at −20 °C. After the reaction mixture was stirred for 1 h at 0 °C, ester **22** was isolated using a standard purification protocol (R_f (silica gel) = 0.3 (EtOAc)). Exposure of **22** to trifluoroacetic acid (25% v/v TFA−CH_2Cl_2) resulted in the hydrolysis of the *tert*-butyl ester function, yielding **4** in 60% overall yield for the two steps.[12] For compounds **10**−**14**, ester **22** was subjected to catalytic hydrogenation in ethanol (10% Pd/C, 0.2 M solution of **22** in absolute ethanol containing 2 equiv of 6 M aqueous HCl, 1 atm of H_2, 3 h, 25 °C) to furnish amine **23** in >95% yield. This material (without purification) was reacted with either benzyl isocyanate (1.0 equiv of **23**, 1.1 equiv each of $PhCH_2NCO$ and diisopropylethylamine (DIEA), CH_2Cl_2, 1 h, −10 °C), 2-furoic acid (1.0 equiv of **23**, 2-furoic acid, BOP,[11] and DIEA (1.1 equiv each), DMF, 12 h, 25 °C), 6-(dimethylamino)-

† Present address: Pharmacopeia, Inc. 101 College Rd. E., Princeton, NJ 08540.
‡ Present address: R. W. Johnson Pharmaceutical Research Institute, Route 202, P.O. Box 300, Raritan, NJ 08869.
§ Present address: Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492.
⊥ Present address: Pfizer Central Research, Eastern Point Road, Groton, CT 06340.
∥ Present address: Janssen Research Foundation, Spring House, PA 19477.
▽ Present address: Rhone-Paulenc Rorer, 500 Arcola Rd, Collegeville, PA 19426.
Present address: Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380.

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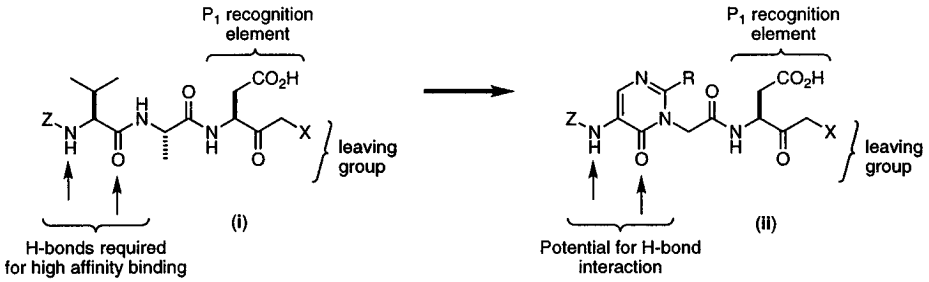
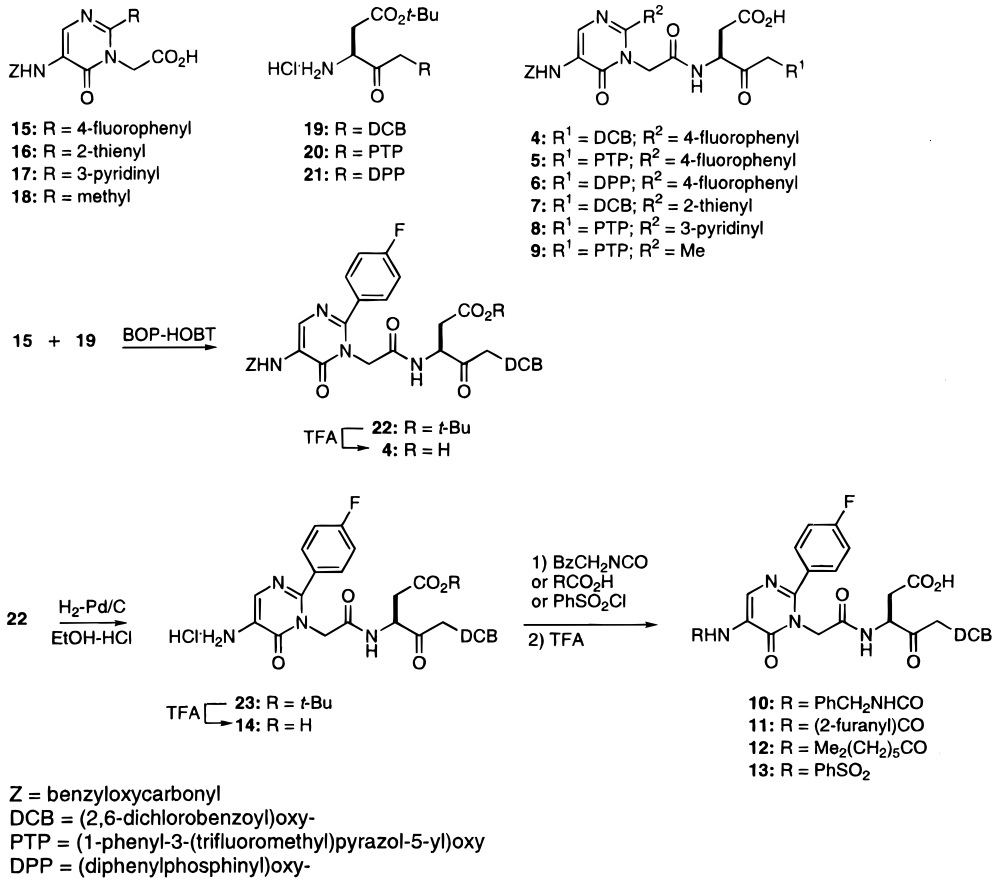


Figure 1. Design features for the pyrimidinone mimetic (**ii**) versus the tripeptide-based inhibitor (**i**).

Scheme 1. Convergent-Based Synthesis of the Peptidomimetics **4**−**14** (Absolute Stereochemistry Is as Shown)



hexanoic acid[13] (coupled to **23** using the mixed-anhydride method as described above) or benzenesulfonyl chloride (1.0 equiv of **23**, 3.5 equiv of $PhSO_2Cl$, 4 equiv of DIEA, 0.2 equiv of 4-(*N*,*N*-dimethylamino)pyridine, CH_2Cl_2, 72 h, 25 °C) to afford **10**−**13** following treatment with TFA in CH_2Cl_2. Direct exposure of **23** to TFA (neat) furnished **14** in quantitative yield.

Enzyme inhibition data obtained for **4**−**14** is presented in Table 1. The second-order rate constant of inactivation for the DCB analog **4** against ICE is 268 000 M^{-1} s^{-1}, while inactivation rates of *ca.* 150 000 M^{-1} s^{-1} are obtained for the PTP and DPP analogs **5** and **6**. Direct comparison of the potency of agents **4**−**6** to their tripeptide congeners **1**−**3** demonstrates that the pyrimidine is an effective P_3−P_2 (Val-Ala) mimetic.[14] Inhibitors **4** and **5** come within 2-fold of the potency of **1** and **2**, with a slight increase in potency observed for pyrimidine **6** versus tripeptide **3**.

Substitution of the fluorophenyl ring in inhibitors **4** and **5** with the 2-thienyl and 3-pyridinyl aromatic functionalities provided inhibitors **7** and **8**, equipotent to **4** and **5**. A modest reduction in potency is seen upon replacing the aryl ring with a methyl group (**7** → **9**). These data suggest the C(2)-pyrimidine substituents may not play a salient role in enzyme affinity. This contention is supported by molecular modeling studies (data not shown) wherein the C(2) substituent overlays with the solvent-exposed P_2 side chain in the peptide inhibitors.[7]

Within the pyrimidine-based DCB class of inhibitor, modification of the N-terminal benzyloxycarbonyl group (Z group) was carried out. In examples **10**−**12**, the Z group was exchanged with hydrophilic N-termini (e.g., benzylurea, furanoyl, and 6-(dimethylamino)hexanoyl groups). These groups were introduced to enhance the solubility of the inhibitors, but they suffer from an overall loss (2−5-fold) in potency as compared to the parent inhibitor **4**. Attenuation in potency is observed for inhibitors **13** and **14** when the Z group is replaced with phenylsulfonyl or is eliminated entirely. The nascent structure−activity relationship of this limited series (**10**−**14**) indicates a preference for hydrophobic N-terminal groups.

In summary, the first examples of potent peptidomi-

metic inhibitors of ICE have been described. The design strategy we pursued sought to retain the P_1 aspartic acid residue and critical hydrogen-bonding functionality (P_1- and P_3-NH) associated with peptides **1**−**3**. Pyrimidinone-based inhibitors **4**−**14** embody these design elements (Figure 1). In addition, we have outlined a convergent approach to the synthesis of mimetics **4**−**14**. This approach utilizes readily available aspartylamine cassettes **19**−**21** in coupling with carboxylic acid partners **15**−**18**. As will be reported separately, the convergent-based inhibitor synthesis permitted us to "mix and match" **19**−**21** with virtually any carboxylic or sulfonic acid (including other peptidomimetics), providing an expedient route to analog generation.

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