Peptidyl α-Ketoheterocyclic Inhibitors of Human Neutrophil Elastase. 3.† *In Vitro* and *in Vivo* Potency of a Series of Peptidyl α-Ketobenzoxazoles

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A series of peptidyl α-ketobenzoxazoles were synthesized and evaluated for their *in vitro* and *in vivo* inhibition of human neutrophil elastase (HNE). These compounds inhibit HNE by forming both a covalent bond between the ketone carbonyl carbon atom and the hydroxyl group of Ser-195 and a hydrogen bond between the benzoxazole nitrogen atom and His-57. Appending to the parent benzoxazole ring a variety of substituents which spanned a range of physicochemical properties had only a modest effect on *in vitro* potency (K_i = 3–0.4 nM). This apparent lack of a significant effect is believed to result from the fact that any increased ketone carbonyl activation by the ring substituent is counter balanced by a corresponding decrease in the hydrogen-bonding ability of the benzoxazole nitrogen atom. In contrast to the results *in vitro*, maximizing *in vivo* activity was critically dependent upon the choice of the benzoxazole ring substituent. Several substituted peptidyl α-ketobenzoxazoles effectively inhibited HNE-induced lung injury when administered intratracheally 24 h prior to the enzyme.

Introduction

As part of our program aimed at developing therapies to treat chronic degenerative diseases associated with human neutrophil elastase (HNE),[1] we have investigated a number of peptidyl electrophilic ketone inhibitors of HNE including trifluoromethyl ketones, difluoromethylene ketones, α-keto amides, α-diketones, and α-keto esters.[2] Recently we have described a novel series of peptidyl ketones in which the ketone carbonyl group is activated by a heterocyclic ring.[3,4] Through a combination of the *in vitro* structure–activity relationship (SAR) and the X-ray crystal structure of the complex between a peptidyl α-ketobenzoxazole and porcine pancreatic elastase (PPE), it was demonstrated that several members of this class of compounds inhibit HNE by forming both a covalent adduct with the active-site Ser-195 and a hydrogen bond with the protonated His-57 (Figure 1). Tsutsumi et al. recently reported an extension of our earlier work to a series of peptidyl α-ketoheterocyclic inhibitors of prolyl endopeptidase.[5] The results of their studies provide additional support for the proposed mechanism of binding of this class of inhibitors.

HNE has been implicated in causing the proteolytic degradation of lung tissue characteristic of emphysema[6–9] and is believed to contribute to the mucous hypersecretion associated with cystic fibrosis.[10–12] One approach we have pursued for treating these pulmonary diseases is to supplement the elastase inhibitory capacity of the lung by intratracheal administration of low molecular weight inhibitors of HNE. Intratracheal administration of drugs offers several potential advantages over other routes of administration for the treatment of pulmonary diseases: the drug is delivered directly to the target organ, and therefore, only small quantities of drug may need to be administered; problems associated with absorption, distribution, and elimination are either minimized or eliminated; and intestinal and hepatic metabolism are avoided. A number of elastase inhibitors have been shown to be effective following intratracheal administration in various models of HNE-induced lung injury.[13] Trifluoromethyl ketones,[14–16] β-lactams,[17–19] and boronic acids[20–22] have been intensively studied, and they have emerged as leading candidates for aerosol administration in the clinic.

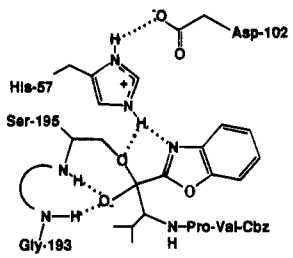

Figure 1. Covalent and hydrogen-bonding interactions between the peptidyl α-ketoheterocycle Cbz-Ala-Pro-Val-Box and the catalytic site of HNE.

In this report we describe the *in vitro* and *in vivo* SAR of a series of peptidyl α-ketobenzoxazoles. This particular series of α-ketoheterocycles was chosen for study since the α-ketobenzoxazoles possess the desired degree of chemical and physical stability as well as *in vitro* potency. By appropriate modification of the substituents on both the benzoxazole ring and the N-terminal amino group, several peptidyl α-ketobenzoxazoles were identified which displayed extremely good *in vivo* activity following intratracheal administration.

Chemistry

We previously reported a number of methodologies for preparing peptidyl α-ketoheterocycles.[4] All of the α-ketobenzoxazoles in the current study were prepared by a Pinner condensation between an appropriately substituted aminophenol and a cyanohydrin.[23] Several

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different strategies were used for incorporating the ketobenzoxazoles into the peptide backbone (methods A and B, Scheme 1) and for elaborating the N-terminus of the peptides (methods C–E, Scheme 2). The method of preparation and the physical properties for each compound are listed in Tables 1 and 2. Except as noted in these tables, the *S*:*R* ratio at the stereogenic carbon α to the P_1 amino group is greater than 9:1. Thus, all structures in the tables and schemes have been drawn with the *S* configuration at this center. The epimeric ratio was determined from the integration of the ^1H NMR (DMSO-d_6/TFA) resonance for the P_1 α-proton. Several of the peptidyl α-ketobenzoxazoles have been evaluated for their stability toward epimerization. The least stable compound had an epimerization half-life of >3 h at pH 9/25 °C. Thus, even assuming a flat pH/rate profile, the ketones would not be expected to undergo significant epimerization during the time course of the kinetic assays. Therefore, the reported K_i values reflect the potency of the P_1 *S* isomer and not the epimerized *RS* mixture. The peptidyl α-ketobenzoxazoles are generally stable toward epimerization, with little or no epimerization occurring during routine handling and chromatography. The extensive epimerization observed for some compounds resulted from epimerization of the aldehyde or ketone intermediates.

Method A. Treatment of the tripeptidyl aldehyde **1** with KCN afforded the cyanohydrin **2** (Scheme 1). Although KCN was used to prepare the cyanohydrin **2** employed in the current study, acetone cyanohydrin/TEA is also a very useful reagent for effecting this transformation. Cyanohydrin **2** was converted into the imidate **3** with ethanolic HCl. Due to its relative instability, imidate **3** was generally used immediately after its formation and condensed with aminophenols **4** to yield the α-hydroxymethyl heterocycles **5**. Oxidation of alcohols **5** with Dess–Martin periodinane (DMP)[24] or Swern, Collins, or Pfitzner–Moffatt oxidations afforded the α-ketobenzoxazoles **6**. Modification of the benzoxazole ring substituents could be accomplished either before oxidation to the ketone (**5d** to **5c**, **5j** to **5k**) or after oxidation (**6c** to **6d**). Method A is particularly useful for preparing a series of α-ketobenzoxazoles with the same peptide backbone but different ring substituents.

Method B. The monopeptidyl aldehyde **7** was converted into the cyanohydrin **8** using acetone cyanohydrin/TEA (Scheme 1). Treatment of **8** with ethanolic HCl afforded imidate **9**, which was condensed with 2-amino-4-cyanophenol (**4l**) to give the monopeptidyl alcohol **10**. Hydrogenolysis of the benzyloxycarbonyl group and coupling the resulting amine with Cbz-Val-Pro-OH afforded the tripeptidyl alcohol **5l**. Pfitzner–Moffatt oxidation gave the ketone **6l**. This method is more efficient than method A when it is desired to prepare a variety of inhibitors containing different tripeptide backbones while retaining the same heterocycle ring substituents.

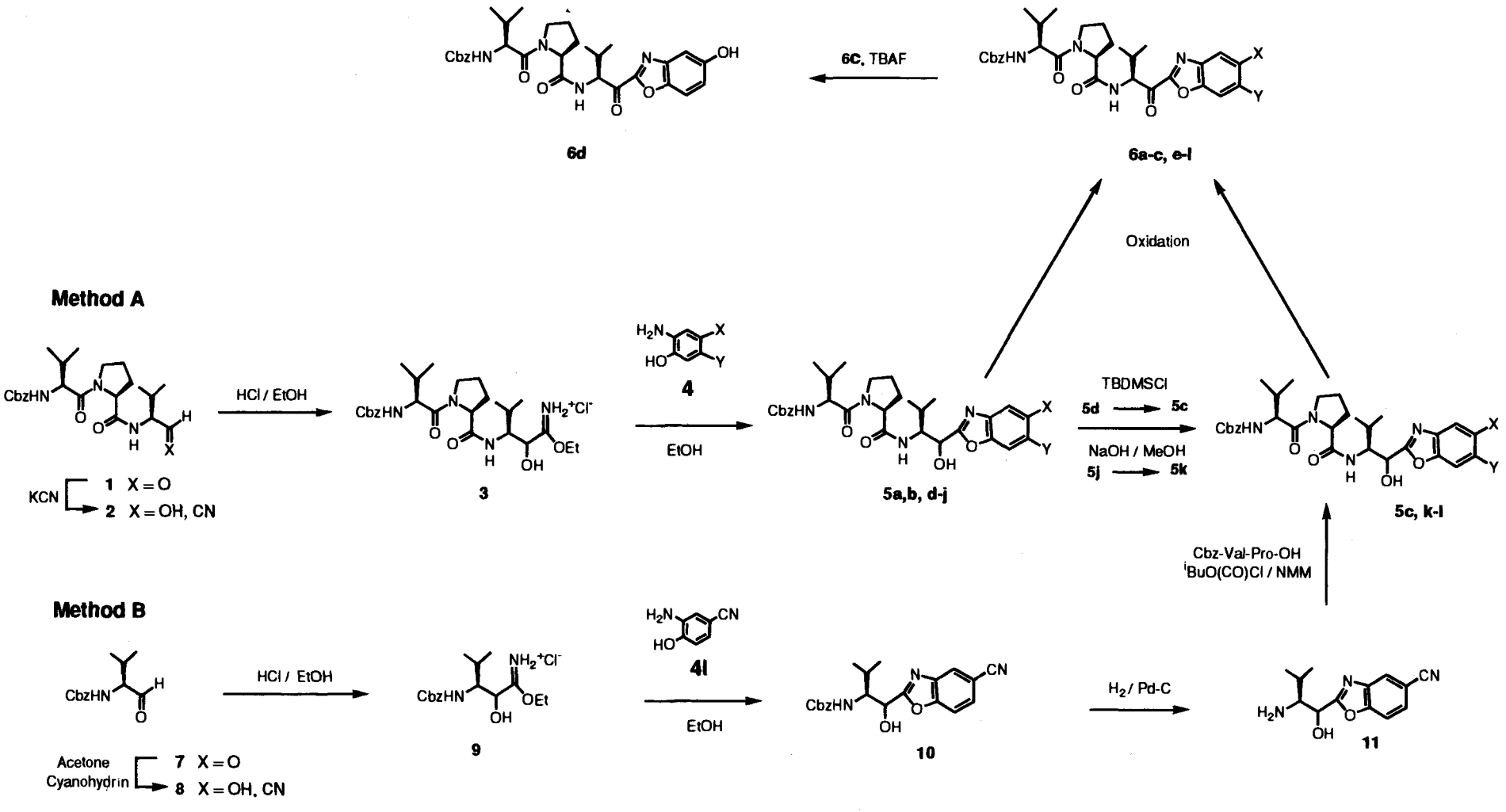
Methods C–E. Three different strategies were employed to construct the inhibitors with extended P_4 groups[25] (Scheme 2). Hydrogenolysis of the N-terminal benzyloxycarbonyl group of alcohols **5** followed by coupling with acids **13** afforded the P_4-extended α-hydroxymethyl heterocycles **14**. Oxidation of alcohols **14** with DMP or Collins reagent gave the target α-ketoheterocycles **15** (method C). Alternatively, ketones **6** were deprotected with trifluoromethanesulfonic acid[26] to yield the amino ketones **16** (Table 2), which were then coupled with acids **13** to give the P_4-extended ketones **15** (method D). While the carbonyl group of the amino α-ketobenzoxazoles **16** is sufficiently unreactive such that self-condensation is not a major problem, optimal yields are obtained only when **16** is used immediately after isolation. A third method for preparing the P_4-extended ketones is illustrated by the preparation of **15j** (method E). Trifluoroacetic acid deprotection of the *tert*-butyl ester of ketone **15b** afforded the terepthalic acid derivative **15c.** Coupling acid **15c** with methanesulfonamide afforded **15j**.

Results and Discussion

***In Vitro* SAR.** We have previously shown that a number of peptidyl α-ketoheterocycles, such as the benzoxazole **6a**, are potent inhibitors of HNE.[4] The peptidyl α-ketobenzoxazoles were selected for further investigation since they possessed superior physical stability as well as excellent *in vitro* potency. As a means of fine tuning the activity of this series, we explored the effects that varying the benzoxazole ring substituent had on both *in vitro* and *in vivo* activity. Initially, a series of tripeptides with an N-terminal benzyloxycarbonyl group were evaluated (Table 1). The peptide backbone -Val-Pro-Val- was used since it imparts selectivity for HNE versus other proteinases and affords potent inhibitors. The binding constants were derived from the inhibition of the HNE-catalyzed hydrolysis of MeO-Suc-Ala-Ala-Pro-Val-*p*Na as previously reported.[3] All of the inhibitors studied were reversible inhibitors of HNE. The nature of the inhibition was determined for several compounds and found to be competitive. None of these inhibitors were hydrated in aqueous DMSO, nor did they display slow-binding inhibition. These observations as consistent with previous studies which demonstrated that certain slow-binding peptidyl electrophilic ketone inhibitors of HNE such as trifluoromethyl ketones and α-keto esters were hydrated in aqueous DMSO.[2]

When we initiated these studies it was anticipated that increasing the electron-withdrawing ability of the entire ring system would increase the electrophilicity of the ketone carbonyl group and thereby increase potency both by activating the ketone carbonyl toward nucleophilic addition of Ser-195 and by stabilizing the resulting covalent adduct. This hypothesis was supported by our previous study which demonstrated that the *in vitro* potency of a series of peptidyl α-ketoheterocycles tended to be positively correlated with the σ_I of the heterocycle.[4] Unexpectedly, varying the benzoxazole ring substituent had only a modest effect on *in vitro* potency: only a 10-fold difference in inhibition constants was observed between the most and least potent inhibitors in a series of compounds incorporating a number of ring substituents (Table 1). The ring substituents used were selected because they spanned a wide range of electron-donating and -withdrawing abilities. With the exception of **6f**, all substituents were incorporated into the 5-position of the benzoxazole ring. The 5-position was initially explored due to the ready availability of the requisite starting materials. While the SAR could be quite different for other regioisomers, we feel this is unlikely and did not extensively investigate regioisomer

Scheme 1. Methods A and B for the Synthesis of Peptidyl α-Ketobenzoxazoles **6**



Scheme 2. Methods C–E for the Synthesis of P_4-Extended Peptidyl α-Ketobenzoxazoles **15**

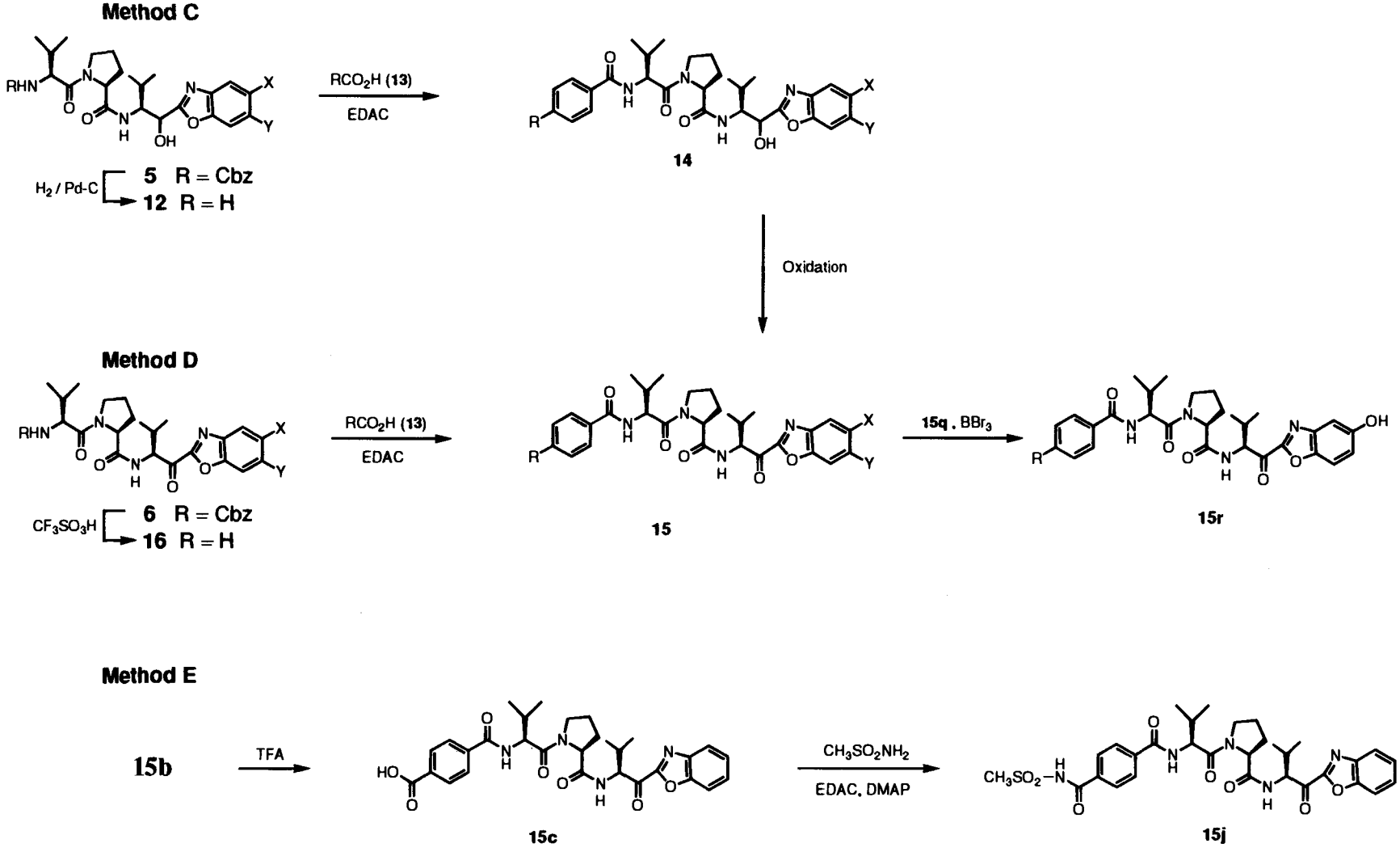
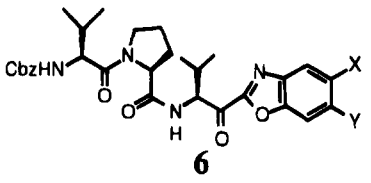


Table 1. Physicochemical Properties and *in Vitro* Activity of α-Ketobenzoxazoles **6**



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	in 4–**6**, **12**, and **16**							
compd[a]	X	Y	K_i (nM)[b]	method	oxidation protocal	yield (%)	MS (M + 1)	formula[c]
6a	H	H	3.0 ± 0.5	A	Swern	64	549	$C_{30}H_{36}N_4O_6 \cdot 0.65H_2O$
6b	OMe	H	1.9 ± 1.4	A	DMP	91	579	$C_{31}H_{38}N_4O_7$
6c	$CH_2OTBDMS$	H	0.43 ± 0.1	A	DMP	90	693	$C_{37}H_{52}N_4O_7Si \cdot 0.25H_2O$
6d	CH_2OH	H	2.4 ± 0.6	A[d]		53	579	$C_{31}H_{38}N_4O_7 \cdot 1.0H_{20}$
6e	Cl	H	1.1 ± 0.2	A	DMP	72	583	$C_{30}H_{35}N_4O_6Cl \cdot 0.4H_2O$
6f	H	CO_2Me	0.55 ± 0.1	A	DMP	86	607	$C_{32}H_{38}N_4O_8 \cdot 0.5H_2O$
6g	CO_2Me	H	0.37 ± 0.1	A	DMP	90	607	$C_{32}H_{38}N_4O_8 \cdot 0.5H_2O$
6h	CO_2H	H	4.2 ± 0.5	A	DMP	18	593	$C_{31}H_{36}N_4O_8 \cdot 1.0H_2O \cdot 0.5C_6H_{14}$
6i	CO_2NH_2	H	1.0 ± 0.1	A[e]	CrO_3	29	592	$C_{31}H_{37}N_5O_7 \cdot 0.6H_2O$[f]
6j	CH_2CO_2Me	H	1.1 ± 0.1	A	EDAC/DMSO	60	621	$C_{33}H_{40}N_4O_8$
6k	CH_2CO_2H	H	4.4 ± 0.8	A[e]	CrO_3	21	607	$C_{32}H_{38}N_4O_8 \cdot 1.0H_2O$
6l	CN	H	1.8 ± 0.4	B[e]	EDAC/DMSO	17	574	$C_{31}H_{35}N_5O_6 \cdot 0.5H_2O$

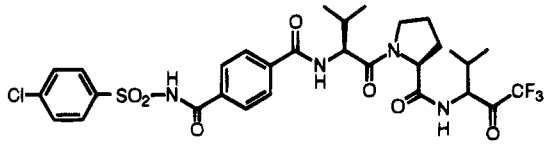
[a] All compounds are greater than 90% of the diastereomer with the *S* configuration at the stereogenic center α to the ketone carbonyl group (P_1) unless otherwise indicated. [b] All inhibition constants in this report were determined for the inhibition of HNE-catalyzed hydrolysis of the synthetic substrate MeO-Suc-Ala-Ala-Pro-Val-*p*Na. [c] All elemental analysis for C, H, and N agree within ±0.4% of calculated values unless otherwise noted. [d] Epimeric ratio at P_1 is 1:1 *S:R*. [e] Epimeric ratio at P_1 is 4:1 *S:R*. [f] N: calcd 11.62; found, 11.08.

effects both due to the similarity between the K_i values for **6f,g** and due to the general lack of effect for any of the 5-position substituents on *in vitro* potency. Indeed, the majority of compounds had K_i values in a very narrow range of 1–4 nM. Even the nitrile-substituted compound **6l**, with the most electron-withdrawing substituent, had a K_i in this same range (K_i = 1.8 nM). Equally surprising was the finding that inserting a methylene between the heterocyclic ring and a carboxyl group, a change which should dramatically alter the inductive and resonance effects of the heterocycle, had virtually no effect on *in vitro* potency (**6h** vs **6k**). It might be possible to account for this lack of effect of the ring substituents if the ketone carbonyl group of these inhibitors was either partially or completely hydrated. Because the theoretical effects of ketone hydration are multifacited, exactly how the presence of hydration would manifest itself within the current SAR is not obvious. However, since none of the inhibitors in this study are hydrated in aqueous media, hydration of the ketone carbonyl does not provide insight into the unexpected lack of effect that benzoxazole ring substituents have on *in vitro* potency.

In all likelihood, increasing the electron-withdrawing ability of the benzoxazole ring substituent does increase the electrophilicity of the ketone carbonyl carbon atom and increases the strength of the covalent bond formed with Oγ of Ser-195. This effect should, therefore, increase the stability of the adduct and lower the K_i value. However, previous studies of peptidyl α-keto-heterocycles demonstrated that the ability of the heterocycle nitrogen atom to form a hydrogen-bonding interaction with His-57 was an important, if not dominant, factor in determining relative potency (Figure 1).[3–5] Since any increased electron withdrawal by the benzoxazole substituent would also be expected to decrease the hydrogen bond-accepting ability of the azole nitrogen atom, it should also decrease the stability of the complex and lead to an increase in K_i. Thus, these opposing effects of the substituent on carbonyl activation and hydrogen bond-accepting ability tend to cancel one another. We believe this is the most probable explanation for the lack of a more profound effect of the ring substituent on *in vitro* potency observed in the current study. Significantly, these results lend further support to the importance of the proposed hydrogen-bonding interaction between the inhibitor and His-57.

***In Vivo* SAR.** Our focus while investigating the α-ketobenzoxazoles was the identification of compounds for the treatment of pulmonary diseases by aerosol administration of low molecular weight inhibitors of HNE. The *in vivo* model we used to evaluate our inhibitors was based on the finding that a hemorrhagic lesion forms in the lungs of hamsters 24 h following administration of an intratracheal dose of HNE. This lesion is characterized by an increase in red blood cells as a result of hemorrhage, an increase in white blood cells as part of the inflammatory response, and an increase in lung weight relative to body weight as a result of edema. Test compounds were evaluated by administering them either admixed with the enzyme or at some time point prior to administration of the enzyme. This latter protocol affords an indication of the test compound's residence time in the lung or its duration of action. This model has been termed the **a**cute **l**ung **i**njury **m**odel or ALIM.

Previous studies from our laboratories had shown that in a series of peptidyl trifluoromethyl ketones (TFMKs) acidic N-terminal groups were necessary for obtaining any significant activity in the ALIM when the inhibitors were dosed prior to the enzyme.[27,28] Although no compounds with simple N-terminal carboxylic acids had K_i values less than 1 nM, a number were shown to be effective when predosed up to 4 h prior to HNE. However, these compounds did not significantly reduce the HNE-induced lesion when dosed 6 h prior to the enzyme. Efforts to improve the duration of action for the peptidyl trifluoromethyl ketones led to the discovery of acylsulfonamide and sulfonylurea N-terminal groups. It was rationalized that the acidic acylsulfonamides (R-SO_2-NHCO-C_6H_5-R) and sulfonylureas (R-SO_2-NHC(O)-NH-C_6H_5-R) could serve as acidic replacements for the



K_i = 0.5 nM
Duration of action in ALIM 36 h

Figure 2. ICI 200,880.

carboxylic acids, thereby giving reasonable *in vivo* activity in the predose paradigm. In addition, the flanking substituents would help present a lipophilic surface to the S_4-S_5 subsites of the enzyme, thereby improving the inhibitor binding constant. The combination of these two effects was anticipated to afford inhibitors with a superior duration of activity *in vivo*. This proved to be the case and led to the identification of the clinical candidate ICI 200,880 (Figure 2) which had a K_i of 0.5 nM and a duration of activity in the acute lung injury model of 36 h.[14,15]

Similar findings have been demonstrated for the peptidyl α-ketobenzoxazoles. All of the inhibitors in Table 1 contain nonacidic N-terminal groups, and none were active in the ALIM when dosed 6 h prior to HNE. Similarly, the inhibitors in Table 3 containing nonacidic N-terminal groups (**15a,b,d,e**) showed no meaningful *in vivo* activity when predosed at 6 h. The carboxylic acid derivative **15c** was also inactive at 6 h. On the other hand, all of the sulfonylureas (**15f–h**) and acylsulfonamides (**15j–t**) we investigated were active when predosed at 6 h. While the sulfonylureas displayed a similar level of activity to the acylsulfonamides (**15g** vs **15m**), they were found to be unstable and decomposed slowly in organic solvents. This instability was reflected in the inability to obtain a molecular ion for the sulfonylureas upon mass spectral analysis (Table 2). Therefore, the sulfonylureas were not investigated in depth.

As observed with the peptidyl trifluoromethyl ketones, the (chlorophenyl)terepthaloyl acylsulfonamide was the acylsulfonamide N-terminal group that afforded the highest degree of protection in the acute lung injury model (compare **15n,o** vs **15i**, **15m** vs **15j–l**). While in the TFMK series the (chlorophenyl)terepthaloyl acylsulfonamide analog had a duration of activity of 36 h (ICI 200,880, Figure 2), when combined with the unsubstituted α-ketobenzoxazole **15m**, this N-terminal group afforded a maximum duration of activity of only 6 h. However, one of the general design concepts behind the development of the peptidyl α-ketoheterocycles was that varying the substituent on the heterocycle would allow modulation of the physicochemical properties of the inhibitor and, as a consequence, modification of *in vitro* and/or *in vivo* activity. As detailed above, ring substituents had only a modest effect on *in vitro* activity. In contrast, the choice of ring substituent greatly influenced the duration of action *in vivo* (Table 3).

Thus, a 5-methoxy (**15q**) benzoxazole ring substituent afforded an inhibitor which was less active than the unsubstituted heterocycle **15m**, while a 5- or 6-methoxycarbonyl (**15n,o**), 5-hydroxy (**15r**), 5-hydroxymethyl (**15s**), or 5-aminocarbonyl (**15t**) yielded more active inhibitors. Three of these inhibitors displayed a duration of action of 24 h (**15r–t**). The most active inhibitor, **15t**, contained the 5-aminocarbonyl substituent. Compound **15t** was extremely selective for HNE, showing little or no activity against other hydrolytic enzymes except the closely related serine proteinase porcine pancreatic elastase (Table 4). In addition, **15t** was a crystalline solid which had good aqueous solubility (10 mg/mL, pH 7.2, PBS) and stability ($t_{1/2}$ for decomposition = 18 d, 25 °C, pH 9, sodium borate buffer). Because of its excellent biological and physicochemical profiles, **15t** was selected for further preclinical evaluation.

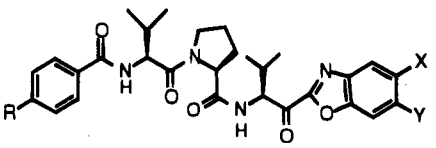
Summary

The peptidyl α-ketoheterocycles were developed from the hypothesis that a heterocycle would activate a ketone carbonyl group toward nucleophilic addition by the hydroxyl group of the active-site Ser-195 of HNE. In addition, it was theorized that both the *in vivo* and *in vitro* activity of the inhibitors could be modulated by varying the substituent on the heterocyclic ring. These design concepts have been realized with the peptidyl α-ketobenzoxazoles. Previously it was shown that several heterocycles, including benzoxazole, afforded potent *in vitro* inhibitors of HNE.[3,4] In the current study, a series of peptidyl α-ketobenzoxazoles was explored in depth. The α-ketobenzoxazoles were chosen for this study due to their superior stability profile and potency. Variation of the benzoxazole ring substituent had a significant but relatively modest effect on *in vitro* potency. When an appropriate acidic group is appended to the N-terminus of the peptide backbone, intratracheally administered α-ketobenzoxazoles effectively inhibit HNE-induced lung injury. In contrast to its effect on in *vitro* activity, varying the benzoxazole ring substituent had a profound effect on *in vivo* activity. These results demonstrate that maximizing *in vivo* activity following intratracheal administration is primarily dependent on a subtle balance of physicochemical properties and only secondarily on *in vitro* potency. In the case of peptidyl α-ketoheterocycles, the desired physicochemical profile can be obtained by modifying both the N-terminal group and the benzoxazole ring substituent. Compound **15t**, containing a 5-aminocarbonyl group, is effective in the acute lung injury model when administered intratracheally 24 h prior to HNE and is the leading candidate from this class of inhibitors for aerosol administration.

Experimental Section

General. Analytical samples were homogeneous by TLC and afforded spectroscopic results consistent with the assigned structures. Proton NMR spectra were obtained using either a Bruker WM-250 or AM-300 spectrometer. Chemical shifts are reported in ppm relative to Me_4Si as internal standard. Some shifts are reported with fractional protons as a result of the compounds existing as a mixture of diastereomers. Mass spectra (MS) were recorded on a Kratos MS-80 instrument operating in the chemical ionization (DCI) mode (only peaks ≥ 10% of the base peak are reported). Elemental analyses for carbon, hydrogen, and nitrogen were determined by the ZENECA Pharmaceuticals Analytical Department on a Perkin-Elmer 241 elemental analyzer and are within ±0.4% of theory for the formulae given. Analytical thin-layer chromatography (TLC) was conducted on prelayered silica gel GHLF plates (Analtech, Newark, DE). Visualization of the plates was accomplished using UV light, phosphomolybdic acid–ethanol, and/or iodoplatinate charring. Analytical high-pressure liquid chromatography (HPLC) was conducted on a Zorbax ODS analytical column (4.6 mm × 25 cm) with a Beckman Liquid

Table 2. Physicochemical Properties of P_4-Extended Peptidyl α-Ketobenzoxazoles **15**



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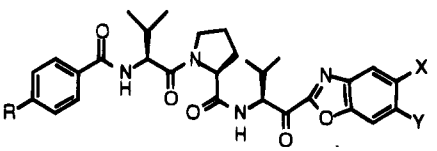
compd[a]	R in 13–15	in 14 and 15 X	in 14 and 15 Y	method	oxidation protocal	yield (%)	MS (M + 1)	formula
15a	MeO_2C	H	H	C[b]	DMP	73	577	$C_{31}H_{38}N_4O_7 \cdot 0.45H_2O$
15b	$^tBuO_2C$	H	H	C	DMP	81	619	$C_{34}H_{42}N_4O_7 \cdot 0.4H_2O$
15c	HO_2C	H	H	C		96	563	$C_{30}H_{34}N_4O_7 \cdot 0.75H_2O \cdot 0.2Na$
15d	H_2NSO_2	H	H	C	DMP	39	598	$C_{29}H_{35}N_5O_7S \cdot 1.5H_2O \cdot 0.8AcOH$
15e	H_2NSO_2	CO_2Me	H	D		89	656	$C_{31}H_{37}N_5O_9S \cdot 2.0H_2O \cdot 1.1AcOH$
15f	$PhSO_2NHC(O)NH$	H	H	C	DMP	25	no M+1	$C_{36}H_{40}N_6O_8S \cdot 1.0H_2O \cdot 2.0AcOH$
15g	4-$ClPhSO_2NHC(O)NH$	H	H	C	DMP	23	no M+1	$C_{36}H_{39}N_6O_8SCl \cdot 1.0AcOH$
15h	4-$ClPhSO_2NHC(O)NH$	CO_2Me	H	D		69	no M+1	$C_{36}H_{41}N_6O_{10}SCl \cdot 3.7H_2O \cdot 0.7AcOH$
15i	CF_3SO_2NH	CO_2Me	H	D[b]		38	724	$C_{32}H_{36}F_3N_{509}S \cdot 0.6H_2O \cdot 0.8AcOH$
15j	$CH_3SO_2NHC(O)$	H	H	E[b]		33	640	$C_{31}H_{37}N_5O_8S \cdot 0.8AcOH$
15k	$^iPrSO_2NHC(O)$	H	H	C	DMP	61	668	$C_{33}H_{41}N_5O_8S \cdot 0.85H_2O$[d]
15l	$PhSO_2NHC(O)$	H	H	C	DMP	57	702	$C_{36}H_{39}N_5O_8S \cdot 1.0H_2O \cdot 75Na$
15m	4-$ClPhSO_2NHC(O)$	H	H	C	DMP	61	736	$C_{36}H_{38}N_5O_8ClS \cdot 1.9H_2O$
15n	4-$ClPhSO_2NHC(O)$	H	CO_2Me	D		22	794	$C_{38}H_{40}N_5O_{10}ClS \cdot 1.0H_2O \cdot 1.5AcOH$
15o	4-$ClPhSO_2NHC(O)$	CO_2Me	H	D		52	794	$C_{38}H_{40}N_5O_{10}ClS \cdot 1.0H_2O \cdot 1.0AcOH$
15p	4-$ClPhSO_2NHC(O)$	CO_2H	H	C	DMP	18	780	$C_{37}H_{38}N_5O_{10}ClS \cdot 0.5H_2O \cdot 1.5AcOH$
15q	4-$ClPhSO_2NHC(O)$	OMe	H	C	DMP	74	766	$C_{37}H_{40}N_5O_9ClS \cdot 1.0H_2O \cdot 1.1AcOH$
15q	4-$ClPhSO_2NHC(O)$	OMe	H	D[c]		62	766	$C_{37}H_{40}N_5O_9ClS \cdot 0.25H_2O \cdot 0.75AcOH$
15r	4-$ClPhSO_2NHC(O)$	OH	H	D		35	752	$C_{36}H_{38}N_5O_9ClS \cdot 1.5H_2O \cdot 1.5AcOH$
15s	4-$ClPhSO_2NHC(O)$	CH_2OH	H	D[b]		32	766	$C_{37}H_{40}N_5O_9ClS \cdot 1.0H_2O \cdot 1.0AcOH$
15t	4-$ClPhSO_2NHC(O)$	$C(O)NH_2$	H	C	CrO_3	26	779	$C_{37}H_{39}N_6O_9ClS \cdot 1.5H_2O$

[a] See footnotes in Table 1. [b] Epimeric ratio at P_1 is 7:3 *S:R*. [c] Epimeric ratio at P_1 is 4:1 *S:R*. [d] N: calcd, 10.25; found, 9.67.

Table 3. *In Vitro* and *in Vivo* Activity of P_4-Extended Peptidyl α-Ketobenzoxazoles **15**



15

compd	R	X	Y	K_i (nM)	compd dose (μmol)	dose interval[a]	Lw/Bw[b]	cells[b] red	cells[b] white
15a	MeO_2C	H	H	2.3 ± 0.4	0.3	6	−12 (8)	17 (7)	−5 (7)
15b	$^tBuO_2C$	H	H	0.34 ± 0.1	0.15	6	41* (14)	29 (14)	−14 (14)
15c	HO_2C	H	H	11 ± 1.3	0.3	6	25 (8)	49* (7)	−1 (7)
15d	H_2NSO_2	H	H	1.3 ± 0.2	0.3	6	0 (8)	−44 (6)	−42 (7)
15e	H_2NSO_2	CO_2Me	H	0.35 ± 0.1	0.3	6	15 (8)	−32 (8)	30 (8)
15f	$PhSO_2NHC(O)NH$	H	H	0.6 ± 0.2	0.3	6	63* (8)	62* (8)	31 (8)
15g	4-$ClPhSO_2NHC(O)NH$	H	H	0.2 ± 0.05	0.12[c]	6	62* (7)	68* (8)	82* (7)
15h	4-$ClPhSO_2NHC(O)NH$	CO_2Me	H	0.06 ± 0.02	0.3	18	48 (9)	13 (9)	−31 (9)
15i	CF_3SO_2NH	CO_2Me	H	0.3 ± 0.07	0.3	6	71* (8)	82* (7)	77* (8)
15j	$CH_3SO_2NHC(O)$	H	H	4.6 ± 0.6	0.3	6	87* (7)	84* (6)	78* (6)
15k	$^iPrSO_2NHC(O)$	H	H	1.5 ± 0.3	0.3	6	68* (6)	60* (5)	70 (5)
15l	$PhSO_2NHC(O)$	H	H	0.5 ± 0.1	0.12[c]	6	29* (7)	58* (7)	64* (7)
15m	4-$ClPhSO_2NHC(O)$	H	H	0.33 ± 0.07	0.12[c]	6	69* (7)	80* (6)	84* (7)
15n	4-$ClPhSO_2NHC(O)$	H	CO_2Me	0.18 ± 0.05	0.3	18	73* (10)	76* (10)	72* (10)
15o	4-$ClPhSO_2NHC(O)$	CO_2Me	H	0.1 ± 0.02	0.3	18	67* (10)	71* (10)	−32 (10)
15p	4-$ClPhSO_2NHC(O)$	CO_2H	H	0.65 ± 0.06	0.3	6	63* (7)	94* (6)	66* (6)
15q	4-$ClPhSO_2NHC(O)$	OMe	H	0.66 ± 0.2	0.3	6	48 (8)	21 (8)	24 (8)
15r	4-$ClPhSO_2NHC(O)$	OH	H	0.81 ± 0.1	0.3	24	38* (9)	57* (9)	40* (9)
15s	4-$ClPhSO_2NHC(O)$	CH_2OH	H	0.66 ± 0.1	0.3	24	30* (8)	46* (8)	39* (8)
15t	4-$ClPhSO_2NHC(O)$	$C(O)NH_2$	H	0.2 ± .04	0.3	24	54* (7)	64* (7)	53* (7)

[a] Dose interval reported is the longest interval at which significant inhibition of the HNE-induced lesion was observed. Shortest dose interval used was 6 h. Dose of HNE used was 400 μg/hamster. [b] Values for wet lung weight (Lw) relative to body weight (Bw), red cells, and white blood cells are the percent reduction relative to HNE and saline controls: 100% indicates identical with saline control; 0% indicates identical with HNE control; a negative value indicates parameter was worse than HNE control. An asterisk (*) indicates value is statistically significant relative to control ($p = 0.05$). Number in parentheses is number of animals used in experiment. [c] No significant inhibition was observed at a dose of 0.3 μmol and a dose interval of 12 h.

Chromatography 340 instrument. Flash chromatography was conducted on Kieselgel 60, 230–400 mesh (E. Merck, Darmstadt, West Germany). Solvents were either reagent or HPLC grade. Reactions were run at ambient temperature and under a nitrogen atmosphere unless otherwise noted. Solvent mixtures are expressed as vol:vol ratios. Solutions were evaporated under reduced pressure on a rotary evaporator. Most starting materials were commercially available. Noncommer-

Table 4. Enzyme Selectivity for **15t**

enzyme	K_i
human neutrophil elastase	0.2 nM
porcine pancreatic elastase	26 nM
chymotrypsin	92 μM
trypsin	>50 μM
thrombin	>50 μM
acetyl cholinesterase	>2 mM
papain	>200 μM
angiotensin-converting enzyme	>200 μM
cathepsin G	>2 mM

cially available aminophenols **4** and N-terminal acids **13** were prepared as described in ref 29.

Method A. [(Benzyloxycarbonyl)-L-valyl]-*N*-[1-(cyanohydroxymethyl)-2-methylpropyl]-L-prolinamide (2). A solution of Cbz-Val-Pro-Val-H (**1**)[30] (12.8 g, 29.7 mmol) in THF (128 mL) and water (154 mL) was treated with solid KCN (7.74 g, 119 mmol). The resulting mixture was stirred for 4.5 h and then partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were washed with saturated $NaHCO_3$ and brine, dried [10% (w/w) K_2CO_3/Na_2SO_4], and evaporated to afford crude **2** (14.0 g) which was used without further purification: TLC $R_f = 0.17$, acetone/hexanes (1:3); MS (DCI) $m/z = 432$ (M − HCN + 1, base); ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.91 (12H, m), 1.76−2.00 (6H, m), 3.60 (1H, m), 3.72 (1H, m), 4.02 (1H, m), 4.36−4.72 (2H, m), 5.02 (2H, m), 6.58 (1H, m), 7.35 (6H, m), 7.83 (0.5H, d, $J = 9.5$ Hz), 7.95 (0.5H, d, $J = 9.5$ Hz).

General Procedure for the Preparation of Alcohols 5a,b,d−j: [(Benzyloxycarbonyl)-L-valyl]-*N*-[1-[(2-benzoxazolyl)hydroxymethyl]-2-methylpropyl]-L-prolinamide (5a). A solution of anhydrous ethanol (1.22 mL, 20.7 mmol) in chloroform (2 mL) at 0 °C was treated with acetyl chloride (1.24 mL, 17.4 mmol) followed by the addition of nitrile **2** (500 mg, 1.09 mmol) in chloroform (3 mL). The mixture was allowed to warm to ambient temperature and stirred for 16 h. The solvent was evaporated and the crude imidate **3** taken up in ethanol (5 mL) and treated with *o*-aminophenol (**4a**) (119 mg, 1.09 mmol). The mixture was heated at 60 °C for 4 h, diluted with ethyl acetate, washed with 1 N NaOH and brine, dried [10% (w/w) K_2CO_3/Na_2SO_4], and evaporated. Purification by flash chromatography on silica gel eluting with THF/hexanes (35:65, 2.1 L) gave **5a** (209 mg, 35%) as a white solid: TLC $R_f = 0.21$, chloroform/methanol (95:5); MS (DCI) $m/z = 551$ (M + 1, base); ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.84−1.05 (12H, m), 1.52 (3H, m), 1.90 (2.5H, m), 2.28 (0.5H, m), 3.41 (1H, m), 3.62 (1H, m), 3.86−4.38 (3H, m), 4.75 (0.5H, d, $J = 8.6$ Hz), 5.03 (2.5H, m), 7.36 (7H, m), 7.66 (2H, m). Anal. ($C_{30}H_{38}N_4O_6$) C, H, N.

(1*S*)-[(Benzyloxycarbonyl)-L-valyl]-*N*-[1-[[5-[[(*tert*-butyldimethylsilyl)oxy]methyl]benzoxazol-2-yl]hydroxymethyl]-2-methylpropyl]-L-prolinamide (5c). A solution of **5d** (736 mg, 1.26 mmol, prepared according to the procedure for **5a**), DMAP (7.8 mg, 0.058 mmol), TEA (0.370 mL, 268 mg, 2.65 mmol), and *tert*-butyldimethylsilyl chloride (380 mg, 2.54 mmol) in dichloromethane (10 mL) was stirred at room temperature for 16 h. The mixture was diluted with ethyl acetate, washed successively with 1 N HCl, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography on silica gel eluting with acetone/hexanes (1:3) gave **5c** (715 mg, 82%) as a light yellow solid: TLC $R_f = 0.46$, acetone/hexanes (2:3); MS (DCI) $m/z = 695$ (M + 1, base), 679, 587, 563, 455; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.041 (3H, s), 0.051 (3H, s), 0.81−1.00 (12H, m), 1.30−2.23 (6H, m), 3.37 (1H, m), 3.60 (1H, m), 3.85 (1H, d, $J = 8.4$ Hz), 3.95 (1H, d, $J = 7.7$ Hz), 4.30 (2H, m), 4.58 (1H, s), 4.78 (1H, s), 5.01 (2H, m), 7.31 (6H, m), 7.57 (2H, m). Anal. ($C_{37}H_{54}N_4O_7Si·0.25H_2O$) C, H, N.

(1*S*)-[(Benzyloxycarbonyl)-L-valyl]-*N*-[1-[[5-(carboxymethyl)benzoxazol-2-yl]hydroxymethyl]-2-methylpropyl]-L-prolinamide (5k). A solution of **5j** (450 mg, 0.723 mmol, prepared according to the procedure for **5a**) in methanol (7.25 mL) was treated with 1 N NaOH (2.17 mL, 2.17 mmol) and stirred at room temperature for 3 h. The solution was evaporated and the residue partitioned between ethyl acetate and 1 N HCl. The ethyl acetate layer was washed successively with 1 N HCl and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography on silica gel eluting with dichloromethane/methanol/acetic acid (95:5:1) gave **5k** (380 mg, 86%) as a white foam: TLC $R_f = 0.13$, dichloromethane/methanol/acetic acid (95:5:1); MS (DCI) $m/z = 609$ (M + 1, base), 591, 501, 348, 197, 168, 115, 91; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.83−1.03 (12H, m), 1.36−1.74 (3H, m), 1.81−1.98 (2.5H, m), 2.26 (0.5H, m), 3.47 (1H, m), 3.63 (1H, m), 3.71 (2H, s), 3.96 (1.5H, m), 4.29 (1.5H, m), 4.69 (0.5H, d, $J = 9.9$ Hz) 5.05 (2.5 H, m), 7.25−7.36 (6H, m), 7.60 (2H, m). Anal. ($C_{32}H_{40}N_4O_8·0.15CH_3CO_2H$) C, H, N.

General Procedure for the Preparation of Ketones 6 by Swern Oxidation of Alcohols 5: (*S*)-[(Benzyloxycarbonyl)-L-valyl]-*N*-[1-[(2-benzoxazolyl)carbonyl]-2-methylpropyl]-L-prolinamide (6a). A solution of oxalyl chloride (0.27 mL, 3.1 mmol) in dichloromethane (10 mL) at −40 °C was treated with DMSO (0.44 mL, 6.2 mmol) and stirred for 15 min. Alcohol **5a** (170 mg, 0.31 mmol) was added in dichloromethane (5 mL) and the resulting slurry stirred at −40 °C for 1 h. Triethylamine (2.2 mL, 15 mmol) was added and the mixture allowed to warm to ambient temperature and stirred an additional 3 h. The mixture was diluted with ethyl acetate, washed successively with 5% aqueous NaOCl and brine, dried [10% (w/w) K_2CO_3/Na_2SO_4], and evaporated. Purification by flash chromatography on silica gel eluting with acetone/hexanes (1:4) gave **6a** (108 mg, 64%) as a white solid and as a 9:1 mixture of diastereomers epimeric at the carbon α to the ketone carbonyl group: TLC $R_f = 0.36$, THF/hexanes (35:65); MS (DCI) $m/z = 549$ (M + 1, base), 124; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.88−1.03 (12H, m), 1.84 (5H, m), 2.43 (1H, m), 3.59 (1H, m), 3.74 (1H, m), 4.06 (1H, d, $J = 8.3$ Hz), 4.57 (1H, m), 5.05 (1H, ABq, $J = 3.8$ Hz), 5.12 (1H, ABq, $J = 3.8$ Hz), 5.31 (0.9H, d, $J = 5.7$ Hz), 5.37 (0.1H, d, $J = 5.0$ Hz), 7.37 (5H, br s), 7.55 (1H, t, $J = 7.6$), 7.62 (1H, t, $J = 8.1$ Hz), 7.89 (1H, d, $J = 8.1$ Hz), 8.01 (1H, d, $J = 7.6$ Hz). Anal. ($C_{30}H_{36}N_4O_6·0.65H_2O$) C, H, N.

General Procedure for the Preparation of Ketones 6 by Dess−Martin Periodinane Oxidation of Alcohols 5: (*S*)-[(Benzyloxycarbonyl)-L-valyl]-*N*-[1-[[5-[[(*tert*-butyldimethylsilyl)oxy]methyl]benzoxazol-2-yl]carbonyl]-2-methylpropyl]-L-prolinamide (6c). *tert*-Butyl alcohol (0.068 mL, 0.72 mmol) was added to a suspension of **5c** (500 mg, 0.72 mmol) and DMP (1.22 mg, 2.88 mmol) in dichloromethane (5 mL), and the resulting solution was stirred at room temperature for 16 h. The reaction mixture was partitioned between ethyl acetate and saturated $NaHCO_3$/saturated $Na_2S_2O_3$ (1:1), washed with two portions of saturated $NaHCO_3$/saturated $Na_2S_2O_3$ (1:1), saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography on silica gel eluting with hexanes/acetone (3:1) afforded **6c** (448 mg, 90%) as a white foam: TLC $R_f = 0.54$, hexanes/acetone (3:2); MS (DCI) $m/z = 693$ (M + 1, base), 677, 635, 585, 460; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.11 (6H, s), 0.94 (21H, m), 1.67−2.07 (5H, m), 2.33 (1H, m), 3.48 (1H, m), 3.63 (1H, m), 4.01 (1H, t, $J = 5.8$ Hz), 4.47 (1H, m), 4.87 (2H, s), 5.01 (2H, m), 5.24 (1H, m), 7.35 (5H, m), 7.45 (1H, d, $J = 7.0$ Hz), 7.56 (1H, m), 7.87 (2H, m), 8.42 (1H, d, $J = 5.8$ Hz). Anal. ($C_{37}H_{52}N_4O_7Si·0.25H_2O$) C, H, N.

General Procedure for the Preparation of Ketones 6 by Collins Oxidation of Alcohols 5: (*S*)-[(Benzyloxycarbonyl)-L-valyl]-*N*-[1-[[5-(aminocarbonyl)benzoxazol-2-yl]-carbonyl]-2-methylpropyl]-L-prolinamide (6i). Pyridine (0.780 mL, 767 mg, 9.70 mmol) was added to a solution of CrO_3 (485 mg, 4.85 mmol) in dichloromethane (50 mL) and the resulting homogeneous mixture stirred at room temperature for 30 min. Diatomaceous earth (1.0 g) was added and the mixture stirred for 15 min followed by the addition of alcohol **5i** (480 mg, 0.81 mmol) in dichloromethane (10 mL). The reaction mixture was stirred at room temperature for 2 h and filtered through a thin pad of silica gel, the pad was washed with methanol/chloroform (1:9), and the filtrate was evaporated. The residue was purified by flash chromatography on silica gel eluting with hexanes/acetone (1:1) to afford **6i** (139

mg, 29%) as a white foam which was a 4:1 (*S*:*R*) mixture of diastereomers epimeric at the carbon α to the ketone carbonyl group: TLC R_f = 0.36, methanol/chloroform (1:9); MS (DCI) *m/z* = 592 (M + 1, base), 484, 359, 91; ^{1}H NMR (250 MHz, DMSO-*d*$_6$/TFA) δ 0.87–1.07 (12H, m), 1.63–2.13 (5H, m), 2.41 (1H, heptet, *J* = 6.31 Hz), 3.58 (1H, m), 3.74 (1H, m), 4.05 (1H, d, *J* = 8.3 Hz), 5.02 (1H, ABq, *J* = 12.6 Hz), 5.06 (1H, ABq, *J* = 12.6 Hz), 5.29 (0.75H, d, *J* = 5.7 Hz), 5.35 (0.25H, d, *J* = 5.2 Hz), 7.35 (5H, m), 7.36 (5H, br s), 7.49 (1H, d, *J* = 8.6), 8.20 (1H, dd, *J* = 8.9, 1.7 Hz), 8.54 (1H, s). Anal. ($C_{31}H_{37}N_5O_7$·0.6H_2O) C, H; N: calcd, 11.62; found, 11.08.

General Procedure for the Preparation of Ketones 6 by Pfitzner–Moffatt Oxidation of Alcohols 5: (*S*)-[(Benzyloxycarbonyl)-L-valyl]-*N*-[1-[(5-cyanobenzoxazol-2-yl)-carbonyl]-2-methylpropyl]-L-prolinamide (6l). Dichloroacetic acid (0.300 mL, 3.61 mmol) was added over 2 min to a suspension of alcohol **5l** (520 mg, 0.903 mmol) and EDAC (1.73 g, 9.03 mmol) in DMSO/toluene (18 mL, 1:1) and the resulting homogeneous solution stirred at room temperature for 16 h. The solvents were evaporated under high vacuum (<500 mT). The residue was dissolved in chloroform, washed successively with 1 N HCl, 1 N NaOH, and brine, dried (Na_2SO_4), and evaporated. Purification by flash chromatography on silica gel eluting with a gradient of hexanes/ethyl acetate (100:0, 4:1, 2:30) followed by a second silica gel chromatography eluting with hexanes/acetone (5:1) afforded **6l** (90 mg, 17%) as a white foam which was a 4:1 (*S*:*R*) mixture of diastereomers epimeric at the carbon α to the ketone carbonyl group: TLC R_f = 0.75, methanol/dichloromethane (2:98); MS (DCI) *m/z* = 574 (M + 1, base), 466, 341, 331, 145, 107, 99, 91; ^{1}H NMR (250 MHz, DMSO-*d*$_6$/TFA) δ 0.90 (12H, m), 1.67–2.12 (5H, m), 2.45 (1H, m), 3.55 (1H, m), 3.71 (1H, m), 4.03 (0.8H, d, *J* = 8.2 Hz), 4.19 (0.2H, d, *J* = 4.6 Hz), 4.53 (1H, s), 5.03 (2H, m), 5.22 (0.2H, d, *J* = 5.5 Hz), 5.27 (0.8H, d, *J* = 5.4 Hz), 7.36 (5H, m), 8.04–8.15 (2H, m), 8.66 (1H, s). Anal. ($C_{31}H_{35}N_5O_6$·0.5H_2O) C, H, N.

(*SR*)-[(Benzyloxycarbonyl)-L-valyl]-*N*-[1-[[5-(hydroxymethyl)benzoxazol-2-yl]carbonyl]-2-methylpropyl]-L-prolinamide (6d). Tetra-*n*-butylammonium fluoride (1.20 mL, 1.00 N, 1.20 mmol) was added to a room temperature solution of silyl ether **6c** (418 mg, 0.6 mmol) in THF (5 mL). After 10 min the resulting red solution was stored at 0 °C for 16 h. The solution was taken up in ethyl acetate, washed with 1 N HCl, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography on silica gel eluting with acetone/hexanes (35:65) followed by a second chromatography eluting with chloroform/methanol (97.5:2.5) afforded **6d** (183 mg, 53%) as a white solid which was a 1:1 mixture of diastereomers epimeric at the carbon α to the ketone carbonyl group: TLC R_f = 0.52, acetone/hexanes (3:2); MS (DCI) *m/z* = 579 (M + 1), 561, 331, 225, 197, 91 (base); ^{1}H NMR (250 MHz, DMSO-*d*$_6$/TFA) δ 0.95 (12H, m), 1.67–2.33 (5H, m), 2.41 (1H, heptet, *J* = 6.9 Hz), 3.74 (1H, m), 4.06 (1H, d, *J* = 8.3 Hz), 4.57 (1H, m), 4.68 (2H, s), 5.05 (2H, m), 5.31 (0.5H, d, *J* = 5.6 Hz), 5.38 (0.5H, d, *J* = 5.2 Hz), 7.37 (5H, br s), 7.61 (1H, d, *J* = 8.7 Hz), 7.83 (1H, d, *J* = 8.7 Hz), 7.93 (1H, s). Anal. ($C_{31}H_{36}N_4O_7$·1.0H_2O) C, H, N.

Method B. Cbz-valinal Cyanohydrin (8). Triethylamine (24.0 mL, 17.4 g, 172 mmol) was added to a solution of aldehyde **7**[30] (67.2 g, 286 mmol) and acetone cyanohydrin (79.0 mL, 73.6 g, 858 mmol) in dichloromethane (900 mL), and the mixture was stirred at room temperature for 4 h. The solvents were evaporated, and the residue was taken up in ether, washed with water (5×) and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography on silica gel eluting with hexanes/acetone (2:1) afforded **8** (55.5 g, 74%) as an oil: TLC R_f = 0.57, hexanes/acetone (2:1); MS (DCI) *m/z* = 263 (M + 1), 236, 192, 146, 119, 91 (base); ^{1}H NMR (250 MHz, DMSO-*d*$_6$/TFA) δ 0.86 (6H, m), 1.92 (1H, m), 3.55 (0.5H, m), 3.75 (0.5H, m), 4.35 (0.5H, d, *J* = 9.7 Hz), 4.65 (0.5H, d, *J* = 4.4 Hz), 5.10 (2H, s), 7.36 (5H, m).

(*S*)-1-(5-Cyanobenzoxazol-2-yl)-2-[(benzyloxycarbonyl)amino]-3-methyl-1-hydroxybutane (10). Acetyl chloride (203 mL, 224 g, 2.86 mol) was added dropwise over 45 min to a 0 °C solution of chloroform (190 mL) and anhydrous ethanol (185 mL, 3.14 mol). When the addition was complete, cyanohydrin **8** (25.0 g, 95.3 mmol) in chloroform (190 mL) was added and the solution stirred at 0 °C for 2 h. The reaction mixture was divided into five equal portions, each of which was evaporated to afford crude **9**. Each portion was used separately in different benzoxazole-forming reactions. A solution of the crude imidate **9** and aminophenol **4l** (2.56 g, 19.1 mmol) in ethanol (100 mL) was heated at reflux for 6 h and stirred at room temperature for 72 h. The mixture was evaporated, the residue partitioned between ethyl acetate and water, and the ethyl acetate layer washed with 0.5 N NaOH, 0.5 N HCl, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and evaporated. Purification by flash chromatography on silica gel eluting with dichloromethane/methanol (98:2) afforded **10** (1.08 g, 15% from **8**) as a white solid: TLC R_f = 0.61, chloroform/methanol (98:2); MS (DCI) *m/z* = 280 (M + 1, base), 203, 119, 91; ^{1}H NMR (250 MHz, DMSO-*d*$_6$/TFA) δ 0.91 (3H, d), 1.01 (1H, d), 1.85 (0.7H, heptet), 2.28 (0.3H, heptet), 3.74 (0.7H, m), 4.03 (0.3H, m), 4.74–5.09 (3H, m), 7.18 (5H, m), 7.89 (2.5H, m), 8.29 (0.5H, s).

(*S*)-1-(5-Cyanobenzoxazol-2-yl)-2-amino-3-methyl-1-hydroxybutane (11). A suspension of **10** (1.00 g, 4.08 mmol) and 10% palladium on carbon (400 mg) in ethanol (40 mL) was hydrogenated at 50 psi for 16 h. The reaction mixture was filtered through diatomaceous earth and evaporated to afford 11 (680 mg, >100% yield) which was used without further purification: TLC R_f = 0.11, chloroform/methanol/NH_4-OH (95:5:1); MS (DCI) *m/z* = 246 (M + 1, base), 203, 135, 72; ^{1}H NMR (250 MHz, DMSO-*d*$_6$/TFA) δ 0.98 (6H, m), 1.01 (1H, d), 1.83–2.10 (1H, m), 3.41 (0.3H, m), 3.55 (0.7H, m), 4.39 (1H, m), 5.12 (0.3H, d, *J* = 5.8 Hz), 5.22 (0.7H, d, *J* = 5.2 Hz), 7.98 (2.5H, m), 8.43 (0.5H, m).

(1*S*)-[(Benzyloxycarbonyl)-L-valyl]-*N*-[1-[(5-cyanobenzoxazol-2-yl)hydroxymethyl]-2-methylpropyl]-L-prolinamide (5l). *N*-Methylmorpholine (0.320 mL, 294 mg, 2.89 mmol) was added dropwise to a −20 °C solution of Cbz-Val-Pro-OH[30] (918 mg, 2.63 mmol) in THF (25 mL). Isobutyl chloroformate (0.360 mL, 379 mg, 2.76 mmol) was added dropwise and the resulting suspension stirred at −20 °C for 30 min followed by the addition of amino alcohol 11 (646 mg, 2.63 mmol) in DMF (10 mL). The resulting mixture was allowed to warm to room temperature and stirred for 16 h, and the solvents were evaporated. The crude material was purified by flash chromatography on silica gel eluting with dichloromethane/methanol (98:2). A second chromatography eluting with ether/hexanes (95:5) afforded alcohol **5l** (614 mg, 40%) as a white solid: TLC R_f = 0.63, dichloromethane/methanol (95:5); MS (DCI) *m/z* = 576 (M + 1), 506, 349, 305, 206, 116, 107, 91 (base), 79; ^{1}H NMR (250 MHz, DMSO-*d*$_6$/TFA) δ 0.97 (12H, m), 1.75–203 (5H, m), 2.18 (1H, m), 3.40 (1H, m), 3.61 (1H, m), 3.86 (1H, m), 3.97 (0.5H, d, *J* = 8.0 Hz), 4.08 (0.5H, d, *J* = 8.5 Hz), 4.23 (1H, m), 4.79 (0.5H, d, *J* = 5.9 Hz), 5.00–5.14 (2.5H, m), 7.36 (5H, m), 7.89 (2.5H, m), 8.31 (0.5H, d, *J* = 8.8 Hz).

Method C. General Procedure for the Preparation of Amines 12 by Hydrogenation of 5: L-Valyl-*N*-[1-[(2-benzoxazolyl)hydroxymethyl]-2-methylpropyl]-L-prolinamide (12a). A suspension of **5a** (1.00 g, 1.82 mmol) and 10% palladium on carbon (150 mg, 50% water-wet) in ethanol (50 mL) was hydrogenated at 50 psi for 6 h, an additional charge of palladium on carbon (200 mg) was added, and the mixture was hydrogenated for an additional 3 h. The reaction mixture was filtered through diatomaceous earth and evaporated to afford **12a** (726 mg, 96% yield) which was used without further purification: TLC R_f = 0.0–0.15, chloroform/methanol (95:5); MS (DCI) *m/z* = 417 (M + 1, base), 399, 318.

General Procedure for Preparation of Alcohols 14: (1*S*)-[[4-[(1,1-Dimethylethoxy)carbonyl]benzoyl]-L-valyl]-*N*-[1-[(2-benzoxazolyl)hydroxymethyl]-2-methylpropyl]-L-prolinamide (14b). EDAC (510 mg, 2.64 mmol) was added to a solution of amine **12a** (1.00 g, 2.40 mmol), terepthalic acid mono-*tert*-butyl ester[31] (560 mg, 2.50 mmol), and HOBt (720 mg, 5.30 mmol) in THF (5 mL) at 0 °C. The resulting solution was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was partitioned between ethyl acetate and water and the organic phase washed successively with 1 N HCl, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and

evaporated. Purification by flash chromatography on silica gel eluting with acetone/hexanes (2:3) afforded **14b** (1.08 g, 73%) as a solid: TLC $R_f = 0.70$, hexanes/acetone (55:45); MS (DCI) $m/z = 621$ (M + 1), 565, 318; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.91 (12H, m), 1.55 (9H, s), 1.45–2.23 (6H, m), 2.28–2.45 (1H, m), 3.45–4.0 (3H, m), 4.31 (2H, m), 4.62 (0.4H, m), 5.03 (0.6H, m), 6.05 (0.6H, m), 6.31 (0.4H, m), 7.35 (2H, m), 7.66 (3H, m), 7.94 (4H, m), 8.7 (1H, m). Anal. ($C_{34}H_{44}N_4O_7 \cdot 0.4H_2O$) C, H, N.

General Procedure for the Preparation of Ketones 15 by Dess–Martin Periodinane Oxidation of Alcohols 14: (*S*)-[[4-[(1,1-Dimethylethoxy)carbonyl]benzoyl]-L-valyl]-*N*-[1-[(2-benzoxazolyl)carbonyl]-2-methylpropyl]-L-prolinamide (15b). *tert*-Butyl alcohol (0.030 mL, 0.32 mmol) was added to a suspension of **14b** (200 mg, 0.320 mmol) and DMP (410 mg, 0.97 mmol) in dichloromethane (5 mL), and the resulting solution was stirred at room temperature for 16 h. The reaction mixture was partitioned between ethyl acetate and saturated $NaHCO_3$/saturated $Na_2S_2O_3$ (1:1), and the organic phase was washed with two portions of saturated $NaHCO_3$/saturated $Na_2S_2O_3$ (1:1), saturated $NaHCO_3$, and brine, dried [10% (w/w) K_2CO_3/Na_2SO_4], and evaporated. Purification by flash chromatography on silica gel eluting with hexanes/acetone (3:1) afforded **15b** (161 mg, 80%) as a white foam: TLC $R_f = 0.35$, hexanes/acetone (3:1); MS (DCI) $m/z = 619$ (M + 1), 317, 316, 204, 115; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.95 (12H, m), 1.56 (9H, s), 1.86 (3H, m), 2.09 (2H, m), 2.38 (1H, m), 3.61 (1H, m), 3.88 (1H, m), 4.44 (1H, t, $J = 8.8$ Hz), 4.51 (1H, m), 5.29 (1H, d, $J = 6.1$ Hz), 7.56 (1H, t, $J = 7.5$ Hz), 7.66 (1H, t, $J = 7.5$ Hz), 7.94 (6H, m), 8.43 (1H, d, $J = 7.0$ Hz), 8.71 (1H, d, $J = 8.0$ Hz). Anal. ($C_{34}H_{42}N_4O_7 \cdot 0.4H_2O$) C, H, N.

General Procedure for the Preparation of Ketones 15 by Collins Oxidation of Alcohols 14: (*S*)-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]benzoyl]-L-valyl]-*N*-[1-[[5-(aminocarbonyl)benzoxazol-2-yl]carbonyl]-2-methylpropyl]-L-prolinamide (15t). Pyridine (7.65 mL, 7.48 g, 94.0 mmol) was added to a solution of CrO_3 (4.72 g, 47.2 mmol) in dichloromethane (100 mL) and the resulting homogeneous mixture stirred at room temperature for 5 min. Diatomaceous earth (100 mL) was added and the mixture stirred for 30 min followed by the addition of alcohol **14t** (3.07 g, 3.93 mmol) in DMF (30 mL). The reaction mixture was stirred at room temperature for 16 h and filtered through a thin pad of silica gel, the pad was washed with methanol followed by DMF, and the combined filtrates were evaporated. The residue was purified by flash chromatography on silica gel eluting with dichloromethane/methanol/acetic acid (95:5:1) followed by a second, identical chromatography to afford **15t** (2.01 g, 66%) as a gray solid which was homogeneous by TLC. This material was further purified to remove color by crystallization from chloroform (200 mL) and toluene (30 mL) to afford **15t** (785 mg, 26%) as a white solid: TLC $R_f = 0.83$, chloroform/methanol/acetic acid (90:10:1); MS (DCI) $m/z = 779$ (M + 1), 761, 421, 341 (base), 244, 192, 163; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 1.00 (12H, m), 1.60–1.94 (3H, m), 2.04–2.18 (2H, m), 2.42 (1H, heptet, $J = 6.6$ Hz), 3.60 (1H, m), 3.92 (1H, m), 4.47 (1H, d, $J = 9.3$ Hz), 4.54 (1H, m), 5.33 (1H, d, $J = 5.6$ Hz), 7.75 (2H, d, $J = 8.7$ Hz), 8.00 (7H, m), 8.20 (1H, dd, $J = 9.0$, 1.2 Hz), 8.56 (1H, s). Anal. ($C_{37}H_{39}N_6O_9ClS \cdot 1.5H_2O$) C, H, N.

Method D. General Procedure for the Preparation of Ketones 15 by Trifluoromethanesulfonic Acid Deprotection of Ketones 6: (*S*)-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]benzoyl]-L-valyl]-*N*-[1-[(5-methoxybenzoxazol-2-yl)carbonyl]-2-methylpropyl]-L-prolinamide (15q). Trifluoromethanesulfonic acid (0.382 mL, 649 mg, 4.32 mmol) was added to a solution of ketone **6b** (500 mg, 0.86 mmol) in dichloromethane (5 mL). The resulting dark brown mixture was stirred for 15 min, and the solvents were evaporated to afford crude amino ketone **16b**. To a solution of **16b** in THF (25 mL) were added DMAP (847 mg, 6.28 mmol), EDAC (184 mg, 0.946 mmol), and 4-[[[(4-chlorophenyl)-sulfonyl]amino]carbonyl]benzoic acid[30] (313 mg, 0.86 mmol), and the mixture was stirred for 16 h. The reaction mixture was taken up in ethyl acetate, washed successively with 1 N HCl and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography on silica gel eluting with acetone/hexanes/acetic acid (50:50:1) followed by a second chromatography eluting with chloroform/methanol/acetic acid (99:1:1) afforded **15q** (413 mg, 62%) as an off-white solid which was a 4:1 (*S:R*) mixture of diastereomers epimeric at the carbon α to the ketone carbonyl group: TLC $R_f = 0.47$, hexanes/acetone/acetic acid (55:45:1); MS (DCI) $m/z = 766$ (M + 1), 748, 423, 421, 377, 346, 328 (base), 327, 326; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.97 (12H, m), 1.72–2.23 (5H, m), 2.41 (1H, heptet, $J = 6.0$ Hz), 3.63 (1H, m), 3.88 (3H, s), 3.90 (1H, m), 4.45 (1H, m), 4.56 (1H, m), 5.32 (0.8H, d, $J = 5.7$ Hz), 5.41 (0.2H, d, $J = 5.4$ Hz), 7.25 (1H, dd, $J = 11.6$, 2.4 Hz), 7.53 (1H, d, $J = 2.4$ Hz), 7.76 (3H, m), 8.03 (6H, m). Anal. ($C_{37}H_{40}N_5O_9ClS \cdot 0.25H_2O \cdot 0.75CH_3CO_2H$) C, H, N.

(*S*)-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]-benzoyl]-L-valyl]-*N*-[1-[(5-hydroxybenzoxazol-2-yl)carbonyl]-2-methylpropyl]-L-prolinamide (15r). Boron tribromide (3.55 mL, 1.0 M in dichloromethane, 3.55 mmol) was added to a 0 °C solution of methoxy ether **15q** (680 mg, 0.89 mmol, single diastereomer) in dichloromethane (5 mL) and the solution allowed to warm to room temperature. After 2 h, an additional amount of BBr_3 (3.55 mL) was added, and the reaction mixture was stirred for 3 h, dissolved in ethyl acetate, washed successively with 1 N HCl and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography on silica gel eluting with chloroform/methanol/acetic acid (98:2:1) afforded **15r** (236 mg, 35%) as an off-white solid after drying under vacuum at 40 °C: TLC $R_f = 0.28$, chloroform/methanol/acetic acid (95:5:1); MS (DCI) $m/z = 752$ (M + 1), 734, 421, 377, 342, 314 (base); ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.99 (12H, m), 1.76–2.27 (5H, m), 2.36 (1H, heptet, $J = 6.8$ Hz), 3.60 (1H, m), 3.95 (1H, m), 4.46 (1H, m), 4.47 (1H, d, $J = 9.4$ Hz), 5.21 (1H, d, $J = 5.9$ Hz), 7.12 (1H, dd, $J = 9.0$, 2.4 Hz), 7.28 (1H, d, $J = 2.4$ Hz), 7.71 (3H, m), 8.00 (6H, m). Anal. ($C_{36}H_{38}N_5O_9ClS \cdot 1.5H_2O \cdot 1.5CH_3CO_2H$) C, H, N.

Method E. [(4-Carboxybenzoyl)-L-valyl]-*N*-[1-[(2-benzoxazolyl)carbonyl]-2-methylpropyl]-L-prolinamide (15c). A solution of ester **15b** (770 mg, 1.26 mmol) in trifluoroacetic acid (5 mL) was stirred at room temperature for 1.5 h and evaporated. The residue was taken up in ether and the resulting solution evaporated. This process was repeated six times and the residue placed under vacuum (<500 mT) for 48 h. The resulting solid foam was purified by flash chromatography on silica gel eluting with hexanes/acetone/acetic acid (60:40:1) to afford **15c** (680 mg, 96%) as a yellow solid: TLC $R_f = 0.23$, hexanes/acetone/acetic acid (60:40:1); MS (DCI) $m/z = 563$ (M + 1), 317, 316 (base), 315, 298, 297, 248, 245, 220, 204, 149, 148, 129, 120, 119, 91; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.94 (12H, m), 1.67–2.23 (5H, m), 2.40 (1H, m), 3.60 (1H, m), 3.90 (1H, m), 4.50 (2H, m), 5.35 (1H, t, $J = 5.0$ Hz), 7.55 (1H, t, $J = 7.5$ Hz), 7.66 (1H, t, $J = 8.3$Hz), 7.92 (1H, d, $J = 7.1$ Hz), 7.99 (5H, m), 8.40 (1H, d, $J = 5.0$ Hz), 8.75 (1H, d, $J = 7.0$ Hz), 13.2 (1H, s). Anal. ($C_{30}H_{34}N_4O_7 \cdot 0.75H_2O \cdot 0.2Na$) C, H, N.

[[4-[(Methylsufonyl)amino]carbonyl]benzoyl]-L-valyl]-*N*-[1-[(2-benzoxazolyl)carbonyl]-2-methylpropyl]-L-prolinamide (15j). EDAC (78 mg, 0.40 mmol) was added to a solution of acid **15c** (200 mg, 0.36 mmol), methanesulfonamide (37 mg, 0.38 mmol), and DMAP (50 mg, 0.41 mmol) in dichloromethane (2 mL). The resulting solution was stirred at room temperature for 16 h and evaporated. The residue was taken up in ethyl acetate, washed successively with 1 N HCl and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography eluting with ether/ethyl acetate/acetic acid (80:20:1) afforded **15j** (74.2 mg, 33%) as a solid which was a 7:3 (*S:R*) mixture of diastereomers epimeric at the carbon α to the ketone carbonyl group: TLC $R_f = 0.10$, ether/acetic acid (100:1); MS (DCI) $m/z = 640$ (M + 1), 622, 326, 325, 316, 299, 298, 297, 281, 204, 201; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.95 (12H, m), 1.63–2.23 (5H, m), 2.45 (1H, m), 3.60 (1H, m), 3.38 (3H, s), 3.90 (1H, m), 4.50 (2H, m), 5.40 (1H, m), 7.56 (1H, t, $J = 7.5$ Hz), 7.66 (1H, t, $J = 7.5$ Hz), 7.91 (1H, d, $J = 8.1$ Hz), 8.00 (5H, m), 8.50 (1H, m), 8.74 (1H, m). Anal. ($C_{31}H_{37}N_5O_8S \cdot 0.8CH_3CO_2H$) C, H, N.

***In Vivo* Assay: Acute Lung Injury Model.** Male Syrian hamsters (90–110 g) were anesthetized with brevital sodium (30 mg/kg, ip) and the tracheas surgically exposed. A dose of HNE (400 μg) in phosphate-buffered saline (0.3 mL, 0.01 M) was injected into the exposed trachea via a 0.5 in., 23 gauge needle. The incision was closed with stainless steel surgical staples, and the animals were allowed to recover. Twenty-four hours after the injection of HNE, the animals were killed with an overdose of pentobarbital sodium. The lungs and heart were resected and the lungs and trachea carefully cleaned of extraneous material. Following measurement of wet lung weight, the tracheas were cannulated and lavaged three times with PBS (2 mL). The recovered lavages were pooled for each animal, and the volume was recorded. Total red and white cells were determined using a Coulter counter. The data are expressed as lung wt/100 g of body weight and total cells recovered (white or red, cells/mL × volume recovered). The values for wet lung weights, total lavageable red cells, and total lavageable white cells are elevated in a dose-dependent manner following administration of HNE. Test compounds were evaluated in this model for their ability to reduce this effect of HNE when they are administered either admixed with the enzyme or at various times prior to administration of HNE.

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References

(1) Abbreviations: HNE, human neutrophil elastase; PPE, porcine pancreatic elastase; Box, 2-benzoxazolyl; Ac, acetyl; Cbz, benzyloxycarbonyl; TFMK, trifluoromethyl ketone; TEA, triethylamine; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; EDAC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole monohydrate; TFA, trifluoroacetic acid; DMF, dimethyl formamide; DMP, Dess–Martin periodinane, 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one; DMAP, (dimethylamino)pyridine; MeO, methoxy; Suc, succinyl; *p*Na, *p*-nitroanilide; PBS, phosphate-buffered sailine; ip, intraperitoneally; it, intratracheally; ALIM, acute lung injury model.

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