Peptidyl α-Ketoheterocyclic Inhibitors of Human Neutrophil Elastase. 2.† Effect of Varying the Heterocyclic Ring on *in Vitro* Potency

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A series of peptidyl α-ketoheterocycles were synthesized and evaluated for their *in vitro* inhibition of human neutrophil elastase (HNE). Several heterocycles, including oxazoline and benzoxazole, afforded extremely potent inhibitors of HNE (**1p–r**) with nanomolar to subnanomolar K_i values. The structure–activity relationships revealed that for compounds with a K_i < 1000 nM potency tends to be positively correlated with the σ_I value of the heterocycle. Furthermore, the results in this study support the hypothesis that, in the covalent enzyme–inhibitor adduct, the azole nitrogen atom of the inhibitor heterocycle participates in a hydrogen-bonding interaction with the active-site His-57.

Introduction

Human neutrophil elastase (HNE)[1] is a serine proteinase which under nonpathological conditions functions as a major component of the inflammatory defense system. HNE has a broad substrate specificity with the ability to degrade a variety of diverse structural proteins. Under homeostatic conditions, the destructive potential of HNE is controlled by a number of endogenous inhibitors including α_1-proteinase inhibitor and secretory leukocyte proteinase inhibitor.[2] The balance between enzyme and inhibitor may be shifted in favor of the enzyme either by a genetic deficiency in the production of functional inhibitor such as occurs in familial emphysema or by overproduction of HNE due to chronic inflammation such as that caused by cigarette smoking.[3] This imbalance between HNE and its inhibitors is believed to play a role in either initiating or contributing to the pathological effects in pulmonary emphysema,[4] adult respiratory distress syndrome,[5] rheumatoid arthritis,[6] atheroschlerosis,[7] cystic fibrosis,[8] and other inflammatory disorders.[9] Emphysema is characterized by the degeneration of lung tissue due to the uncontrolled proteolysis by HNE, resulting in a decreased ability to exchange respiratory gasses between the blood and the lung. One approach to controlling the progression of emphysema is to supplement the elastase inhibitory capacity of the lung with low molecular weight inhibitors of HNE.[10] While a large number of synthetic HNE inhibitors have been developed,[11] only peptidyl trifluoromethyl ketones, peptidyl boronic acid esters, β-lactams, and pivaloyl acylating agents have emerged as leading candidates to demonstrate the utility of low molecular weight, synthetic proteinase inhibitors for the treatment of emphysema.[2]

Our research efforts have focused on the development of mechanism-based[12] inhibitors of elastase, in particular electrophilic ketones. We and others have identified a number of functional groups which activate the carbonyl of peptidyl ketones toward nucleophilic addition by the active-site Ser-195 hydroxyl group of HNE.[11] These have included the trifluoromethyl, difluoromethylene, halomethyl, ester, and keto groups. We envisioned that an aromatic heterocyclic ring positioned α to a ketone would also activate the carbonyl group toward nucleophilic addition by the active-site serine hydroxyl group. A perceived advantage of such α-ketoheterocycles **1** (Table 1) over other electrophilic ketones was that the wide variety of heterocycles which could be incorporated into a peptidyl ketone would allow the modulation of the physiochemical properties of the inhibitor. In this manner, both the *in vivo* activity as well as the *in vitro* potency could be finely tuned.

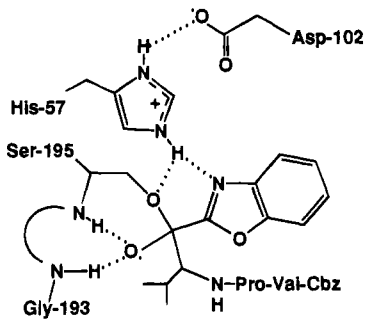

Figure 1. Covalent and hydrogen-bonding interactions between the peptidyl α-ketoheterocycle Cbz-Ala-Pro-Val-Box and the catalytic site of HNE.

However, an aromatic α-ketoheterocycle would be expected to exist in a conjugated, coplanar or nearly coplanar arrangement. In order to form a tetrahedral adduct with the enzyme active-site serine hydroxyl group, this resonance-stabilized system would have to be disrupted. Without a significant compensating binding interaction with the enzyme, the energy penalty for forming the tetrahedral enzyme–inhibitor adduct might be prohibitively large. Therefore, at the outset it was not clear whether an α-ketoheterocycle would be an effective mechanism-based inhibitor of serine proteinases.

Conceptually, we were intrigued by the possibility that incorporation of an appropriately positioned nitrogen atom within the heterocyclic ring might further stabilize the covalent complex by participating in a

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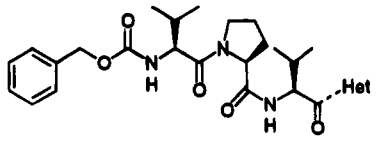
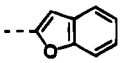
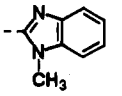
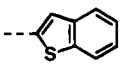
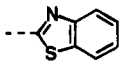
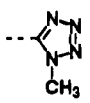
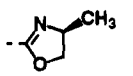
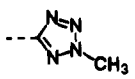
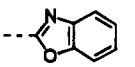
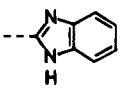
Table 1. *In Vitro* HNE Inhibitory Activities of Peptidyl α-Ketoheterocycles



1

Het Descriptor	Het in Compds 1, 3-5, 9	Compound	K_i (nM)[a]	Het Descriptor	Het in Compds 1, 3-5, 9	Compound	K_i (nM)[a]
a		1a	16 000 ± 6000	k		1k	80 000 ± 44 000
b		1b	3400 ± 1100	l		1l	12 000 ± 5000
c		1c	4300 ± 1000	m		1m	c
d		1d	b	n		1n	330 ± 140
e		1e	270 ± 50	o		1o	28 ± 3.3
f		1f	25 ± 4	p		1p	0.55 ± 0.13
g		1g	410 ± 67	q		1q	1.2 ± 0.2
h		1h	49 ± 10	r		1r	3.0 ± 0.5
i		1i	22 000 ± 10 000	s		1s	5600 ± 650
j		1j	87 000 ± 75 000				

[a] 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 as previously reported.[13] [b] K_i could not be accurately determined due to low solubility. [c] K_i varied with concentration.

hydrogen-bonding interaction with the protonated active-site His-57 (Figure 1). Upon formation of the tetrahedral intermediate within the enzyme active site, any resonance overlap between the carbonyl group and the heterocycle would be lost, thereby unmasking the full hydrogen-bonding potential of the heterocycle ring nitrogen atom. This is analogous to the mechanism of peptide bond hydrolysis wherein the scissile amide nitrogen atom, which is normally a hydrogen bond donor, is transformed into a hydrogen bond acceptor upon formation of the tetrahedral adduct and subsequently accepts a proton prior to release as part of the product amine. We speculated that a hydrogen-bonding interaction between the α-ketoheterocyclic inhibitors and His-57 would compensate for the disruption of the resonance-stabilized carbonyl system which occurs upon covalent bond formation with Ser-195.

We report herein the synthesis and *in vitro* activity of a novel class of HNE inhibitors, the peptidyl α-ketoheterocycles. Several compounds from this series are extremely potent inhibitors of HNE with K_i values in the nanomolar to subnanomolar range. The structure–activity relationships which have emerged support the hypothesis that binding interactions with both the enzyme active-site serine hydroxyl group and histidine imidazole ring are important for optimal *in vitro* activity. Previous work from our laboratories demonstrated that a 2-benzoxazole ring affords potent inhibitors of both HNE and porcine pancreatic elastase (PPE).[13] Peptidyl α-ketobenzoxazoles are particularly interesting with respect to their physical stability and *in vivo* potency, and the structure–activity relationships within a series of peptidyl α-ketobenzoxazoles will be reported separately.[14]

Table 2. Physicochemical Characteristics of Peptidyl α-Ketoheterocycles

compd[a]	method	yield[b] (%)	MS (M + 1)	formula	elemental analyses[c]
1a	A	66	508	$C_{29}H_{37}N_3O_5 \cdot 0.6H_2O$	C,H,N
1b	A	45	548	$C_{31}H_{37}N_3O_6$	C,H,N
1c	A	37	514	$C_{27}H_{35}N_3O_5S$	C,H,N
1d	A	38	564	$C_{31}H_{37}N_3O_5S$	C[d]H,N,S
1e	A	79	515	$C_{26}H_{34}N_4O_5S \cdot 0.5H_2O$	C,H,N
1f	B	52	565	$C_{30}H_{36}N_4O_5S$	C,H,N
1f	A	>100[e]	565	$C_{30}H_{36}N_4O_5S$	*f*
1g	A	45	514	$C_{25}H_{35}N_7O_5$	*f*
1h	A	43	514	$C_{25}H_{35}N_7O_5 \cdot 0.5H_2O$	C,H,N
1i	A	26	509	$C_{28}H_{36}N_4O_5 \cdot 0.25H_2O$	C,H,N
1j	A	45	553	$C_{30}H_{40}N_4O_6 \cdot 0.3H_2O$	C,H,N
1k	A	65	512	$C_{27}H_{37}N_5O_5 \cdot 0.5H_2O$	C,H,N
1l	A	56	562	$C_{31}H_{39}N_5O_5 \cdot 0.2H_2O$	C,H,N
1m	B	64	499	$C_{26}H_{34}N_4O_6 \cdot 0.5H_2O$	C,H,N
1n	B	67	575	$C_{32}H_{38}N_4O_6 \cdot 0.75H_2O$	C,H,N
1o	C	85	499	$C_{26}H_{34}N_4O_6$	C,H,N
1p	D	22	501	$C_{26}H_{36}N_4O_6 \cdot 0.75H_2O$	C,H,N
1q	D	59	*f*	$C_{27}H_{38}N_4O_6 \cdot 0.35H_2O$	C,H,N
1r	D	64	549	$C_{30}H_{36}N_4O_6 \cdot 0.2H_2O$	C,H,N
1s	D	55	548	$C_{30}H_{37}N_5O_5 \cdot 1.05H_2O$	C,H,N

[a] All compounds are greater than 90% of the diastereomer with the *S* configuration at the stereogenic center α to the ketone carbonyl group except **1g** which is approxamitely a 1:1 mixture of epimers at that center. [b] Yields are for the final step only. [c] All elemental analyses for the indicated elements were within ±0.4% of the calculated values unless oltherwise noted. [d] C: calculated, 66.09; found, 66.71. [e] Product was not purified. [f] Data not obtained.

Chemistry

Peptidyl α-ketoheterocycles were unknown prior to our investigations.[13] Three general synthetic approaches have been developed which allow access to a wide range of peptidyl α-ketoheterocycles whose *N*-terminal groups can be readily varied (Schemes 1–3). The method of preparation and physical properties for each compound are listed in Table 2. For all compounds except **1g**, 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 are drawn with the *S* configuration at this center. The epimeric ratio was determined from the integration of the ^{1}H NMR (DMSO-d_6/TFA) resonance for the P_1 α-proton. This resonance is base-line-separated in the two epimers. Several of the peptidyl α-ketoheterocycles have been evaluated for their stability toward epimerization. The least stable had an epimerization half-life of >3 h at pH 9/25 °C. Thus, even assuming a flat pH rate profile, no significant epimerization would be expected to occur during the time course of the kinetic assays. Compound **1g** was epimerized during synthesis. However, since the epimerized ketone intermediate(s) would be a mixture of enantiomers until the final coupling, the exact step(s) where the epimerization occurred is unknown.

Method A. A large number of aromatic heterocycles can be deprotonated at the α-position and treated with electrophiles to afford α-substituted heterocycles.[15] We have found that peptidyl *N*-methoxy-*N*-methyl amides react with lithiated heterocycles to afford directly peptidyl α-ketoheterocycles.[16] Thus, treatment of Cbz-Val-N(Me)OMe (**2**; Scheme 1) with the lithiated heterocycles **3a–l** (Table 1) afforded the α-ketoheterocycles **4a–l**. Heterocycles **3b–l** were lithiated by deprotonation with *n*-butyllithium, while lithiated **3a** was prepared by metal/halogen exchange between *n*-butyllithium and bromobenzene. The benzyloxycarbonyl group was removed with trifluoromethanesulfonic acid,[17] and the amino ketones **5a–l** were isolated following neutralization with sodium carbonate. Hydrogenolysis of the benzyloxycarbonyl group of **4** over palladium on carbon was less satisfactory in that it was accompanied by a substantial quantity of the carbinol resulting from reduction of the ketone carbonyl group. Interestingly, isolation of amino ketones **5a–l** as their free base was not accompanied by significant amounts of self-condensation, suggesting that the ketone carbonyl group of these inhibitors has only moderate electrophilicity.[18] WSCDI-mediated coupling of the amino ketones with the dipeptide acid **6** afforded the title peptidyl α-ketobenzoxazoles **1a–l**.

Methods B and C. A related approach was used in an attempt to prepare α-hydroxy-2-oxazoles such as **9o**. However, treatment of the 2-lithooxazoles **8a,b** (Scheme 2, method B) with aldehyde **7** afforded the regioisomeric α-hydroxy-5-oxazoles **9m,n**. The probable mechanism of this reaction is illustrated in Scheme 3. 2-Lithiooxazoles **13** are known to undergo ring opening to the isocyano enolate **14**, which may react with an electrophile on the enolate β-carbon to yield the intermediate aldehyde **15**.[19,20] Tautomerization of **15** to enolate **16** followed by ring closure would afford the 5-substituted oxazoles **17**.[21] Oxidation of the alcohols **9m,n** with Dess–Martin periodinane (DMP)[22] gave the corresponding α-ketoheterocycles **1m,n**.

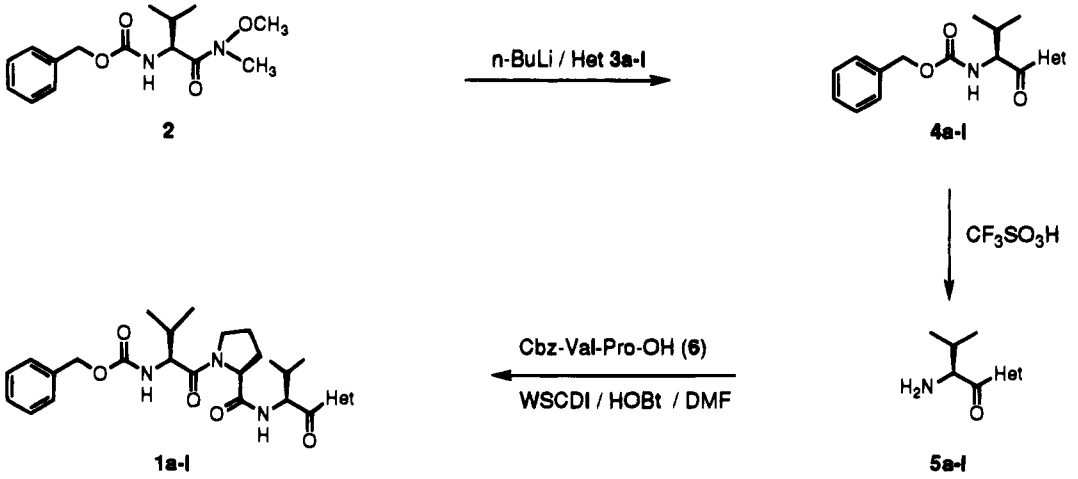
A solution to the regiochemical problem associated with alkylation of 2-lithiooxazoles has been developed by Dondoni.[23] Isocyano enolate **14** is *O*-silylated with trimethylsilyl chloride to give the silyl enol ether **18** which undergoes a base-promoted insertion reaction to give the 2-(trimethylsilyl)oxazole (**19**; Scheme 3). Treatment of aldehydes with **19** at elevated temperatures followed by desilylation affords the α-hydroxyoxazoles **20**. We have found that **19** can be prepared in a single, one-pot procedure. Following silylation of **14**, the low-boiling solvents were removed by fractional distillation; distillation of the higher boiling residue (130 °C/760 mmHg) induces cyclization, and **19** is isolated directly. Treatment of aldehyde **7** with **19** in refluxing toluene followed by acidic workup afforded the α-hydroxyoxazole **9o** which was oxidized with DMP to the α-keto-2-oxazole **1o** (Scheme 2, method C).

Method D. A different approach was necessary for the preparation of α-ketoheterocycles for which the metalated heterocycle is not readily accessible, such as the oxazoline (Scheme 2). 1,3-Azoles can be prepared by cyclization of vicinal amino alcohols, amino thiols, and diamines with imino ethers.[24] Thus, treatment of α-hydroxyimidate **11** with amino alcohols **12a–c**, diamine **12d**, or amino thiol **12f** afforded the peptidyl α-hydroxyheterocycles **9f,p–s**. Swern oxidation of alcohols **12p–r** afforded ketones **1p–r**, while treatment of alcohols **9f,s** with DMP afforded ketones **1f,s**.

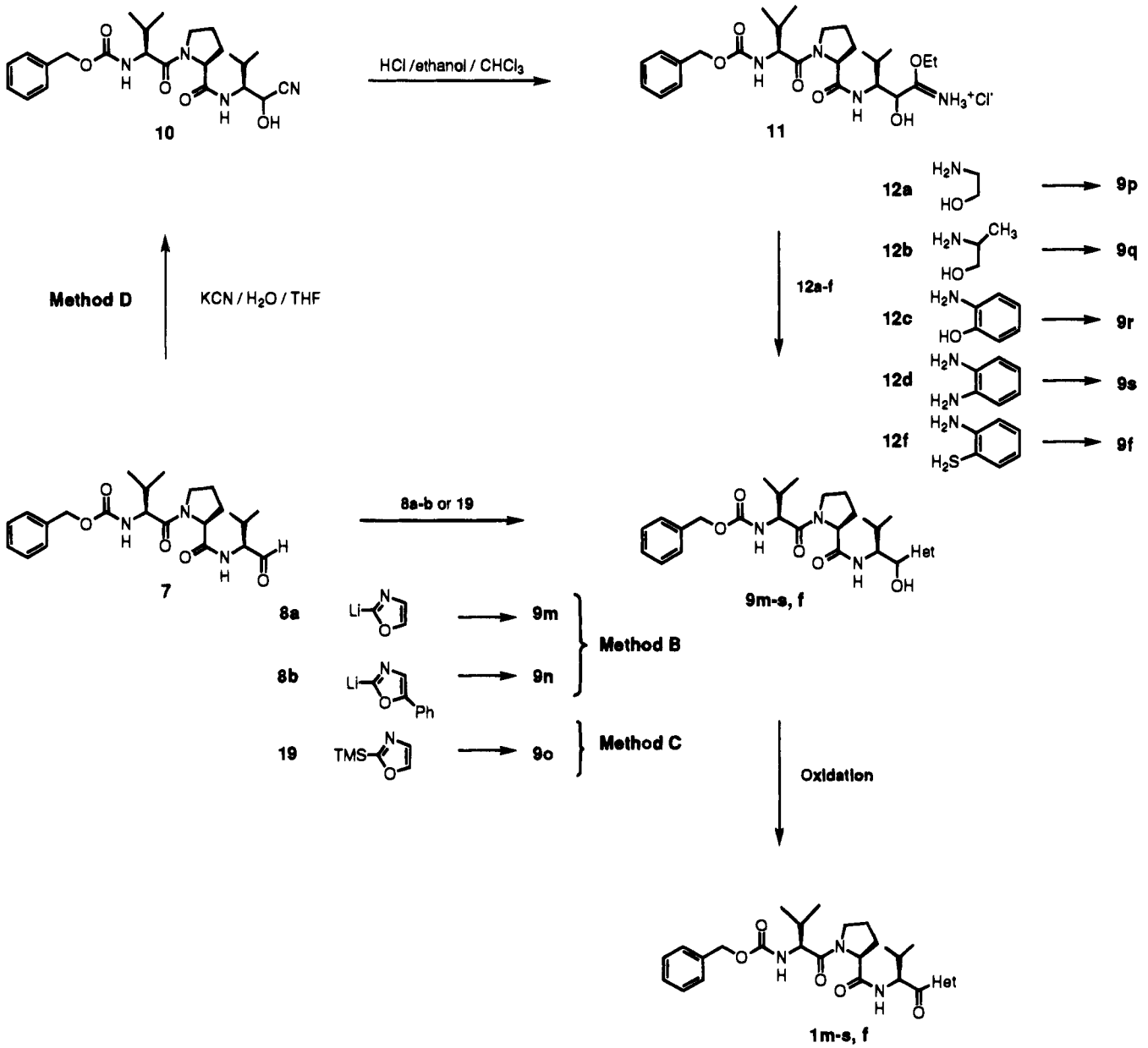
Results and Discussion

A variety of heterocycles were investigated for their ability to activate the carbonyl group of a peptidyl ketone toward nucleophilic addition by the hydroxyl group of Ser-195 of HNE. The selected heterocycles spanned a wide range of physical and electronic properties. The tripeptidyl backbone Cbz-Val-Pro-Val- was

Scheme 1. Method A for the Synthesis of Peptidyl α-Ketoheterocycles **1a–l**

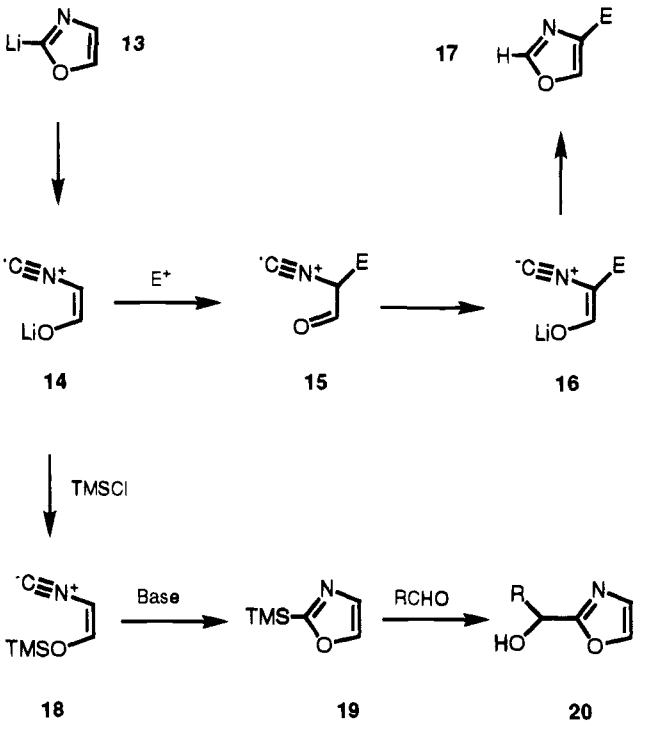


Scheme 2. Methods B, C, and D for the Synthesis of Peptidyl α-Ketoheterocycles **1f,m–s**



used as the ketone scaffold since it affords very potent inhibitors of HNE.[11] In addition, the similar tripeptide backbone Ac-Ala-Pro-Val- binds with an invariant topological complementarity to the extended binding pocket of PPE as observed in the X-ray crystal structures of the enzyme–inhibitor complexes between PPE

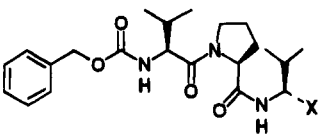
Scheme 3. Addition of Electrophiles to 2-Lithiooxazole

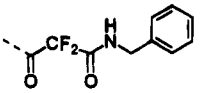
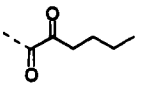
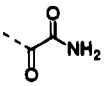
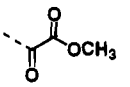
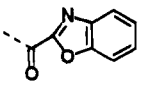
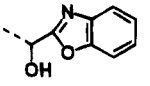
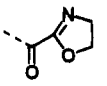


and a number of different electrophilic ketone inhibitors of both PPE and HNE.[13] Furthermore, one of these inhibitors, a peptidyl α,α-difluoro-β-keto amide, maintains this binding orientation even though its *C*-terminal end extends deeply into the S′ binding pocket of the enzyme. Thus, the tripeptidyl backbone of the various α-ketoheterocycles used in this study should bind to the enzyme in an identical manner. Therefore, any differences in the binding constants of the different α-ketoheterocycles can be attributed to the interaction of the heterocycle with the active site of the enzyme and not to an altered mode of binding of the peptide backbone. 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.[13] Except as noted in Table 1, all of the inhibitors studied were competitive, reversible inhibitors of HNE. None of these inhibitors displayed slow-binding inhibition as has been observed for certain peptidyl electrophilic ketone inhibitors of HNE such as trifluoromethyl ketones and α-keto esters.[11]

Several heterocycles afforded extremely potent inhibitors of HNE (Table 1). The 2-oxazoline **1p**, with a K_i of 5.5×10^{-10} M, is the most potent of the series. Indeed, the oxazoline ring is one of the most potent groups yet reported for activating peptidyl ketones. Comparison with other tripeptidyl electrophilic ketone inhibitors of HNE reveals that the ketooxazoline is more potent than the corresponding peptidyl aldehyde **7** and trifluoromethyl ketone **22** (Table 3). α-Ketobenzoxazole **1r** is also a very potent inhibitor of HNE with a K_i of 3.0×10^{-9} M. Supporting the proposed mechanism of inhibition, the α-hydroxybenzoxazole **9r** (Table 3), which cannot form a tetrahedral adduct with the enzyme, is not an effective inhibitor of HNE. Conclusive evidence for covalent attachment of the more potent inhibitors to the enzyme was obtained from the X-ray crystal structure of the complex between PPE and Ac-Ala-Pro-Val-2-benzoxazole.[13] In this complex, the ketone carbonyl carbon atom of the inhibitor was clearly covalently linked to the γ-oxygen atom of Ser-195.

Table 3. Kinetic Comparison of Electrophilic Carbonyl-Based Inhibitors of Human Neutrophil Elastase

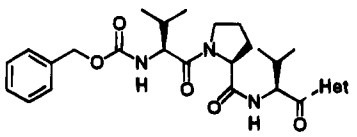


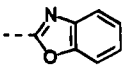
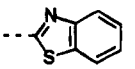
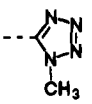
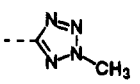
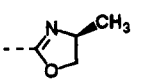
Compd	X	K_i (nM)
21		8000[a]
7		41[a]
22		1.6[b,e]
23		0.4[c]
24		1.6[c]
25		1.8[c]
26		0.6[d]
1r		3.0
9r		21 000[a]
1p		0.6

[a] Reference 13. [b] Reference 25. [c] Reference 26. [d] Reference 11. [e] Mixture of epimers at the stereogenic center α to the ketone carbonyl.

Surprisingly, no other heterocycles afforded inhibitors with binding constants less than 10^{-8} M. The principles underpinning the design of the α-ketoheterocycles suggested that the electron-withdrawing ability of the heterocycle would be the dominant property affecting the potency of the inhibitors. It was anticipated, therefore, that the degree of inhibition would be positively correlated with the heterocycle's electron-withdrawing ability. This is clearly not the case (Tables 1 and 4). The keto-2-benzoxazole **1r** (σ_I for Het **r** = 0.41)[27] is 5-fold less potent than the keto-2-oxazoline **1p** even though the oxazole has a much smaller electron-withdrawing effect (σ_I for Het **p** = 0.32). Even more revealing, the keto-2-tetrazole **1h,** which possesses a very large σ_I (σ_I for Het **h** = 0.49), is almost 100-fold less potent than keto-2-oxazoline **1p**. It is evident that properties other than the electron-withdrawing ability of the heterocycles contribute to the relative potency within this series of peptidyl α-ketoheterocycles.

Table 4. Comparison of K_i vs σ_I for Potent Peptidyl α-Ketoheterocycles



Compd	Het	σ_I[a]	K_i (nM)
1r		0.41	3.0
1o		0.38	28
1f		0.37	25
1e		0.34	270
1n		0.13	330
1g		0.49	410
1h		0.49	49
1p		0.32	0.55
1q		0.32	1.2

[a] Reference 27.

In an attempt to understand the factors responsible for potency in this diverse set of compounds, we have divided the heterocyclic inhibitors into two groups on the basis of their K_i. The first group contains inhibitors with heterocycles such as imidazole (**1k**), benzimidazole (**1l,s**), thiophene (**1c**), benzofuran (**1b**), and phenyl (**1a**) which have K_i values greater than about 1000 nM. These compounds are believed *not* to form a covalent adduct with the hydroxyl group of the active-site Ser-195 but to rely exclusively upon noncovalent interactions between the peptide backbone and the extended binding pocket of the enzyme for their activity. The value of 1000 nM as the cutoff for covalent bond formation was derived from the observation that the peptidyl carbinols **9r** (K_i = 16 000 nM) and Cbz-Val-Pro-NH-CH(*i*-Pr)CH(OH)CF_3 (K_i = 80 000 nM)[25] have K_i values >1000 and are incapable of forming covalent adducts. The fact that methyl ketone Cbz-Val-Pro-Val-CH_3 (**22**) has a K_i only slightly lower than the above alcohols (8000) suggests that it also fails to form a covalent adduct with the enzyme. The enhanced potency of the methyl ketone relative to the alcohols probably results from the ability of the polarized ketone carbonyl oxygen atom to participate in additional binding interactions with the NH groups of Ser-195 and Gly-193 in the oxyanion hole. We speculate that the heterocycles of inhibitors with K_i values of greater than about 1000 either do not have a large enough inductive electron-withdrawing ability or have too large a resonance electron-donating component, such that nucleophilic addition to the carbonyl is energetically disfavored.

In the second group of α-ketoheterocycles are inhibitors which have K_i values of less than 1000 nM and are almost certainly forming covalent adducts with the enzyme. Within this subset of inhibitors, potency tends to be positively correlated with the hetereocycle's σ_I (Table 4). Thus, potency decreases with decreasing σ_I within the group benzoxazole (**1r**), oxazole (**1o**), benzthiazole (**1f**), and thiazole (**1e**). However, there are two heterocycles which are obvious outliers in this analysis: the oxazolines **1p,q** and the tetrazoles **1g,h**. The tetrazole is the most electron-withdrawing heterocycle investigated (σ_I = 0.49) and, on the basis of our σ_I argument, would be expected to be the most potent inhibitor of the series. However, the tetrazoles used in this study both contain a pendant methyl group. Table 1 reveals that heterocycles containing a ring substituent have a larger K_i than the corresponding unsubstituted heterocycles (e.g., **1l** vs **1s**; **1q** vs **1p**; **1j** vs **1i**). Apparently, ring substituents experience a negative steric interaction within the active site. This negative interaction can be relieved somewhat by moving the substituent to a more extended position on the ring, resulting in improved activity (**1h** vs **1g**). However, the methyl group in **1h** may still cause a decrease in potency (compare **1q** vs **1p**), and thus the intrinsic ketone-activating ability of the tetrazole ring cannot be determined from the examples used in this study.

The other outlier in Table 4 is the oxazoline **1p**, which despite having one of the smaller σ_I values is the most potent α-ketoheterocycle we have prepared. An argument based solely on σ_I would predict this compound to be a poor inhibitor. However, the nitrogen atom of the 2-oxazoline is the best hydrogen bond acceptor of those heterocyclic inhibitors which form a covalent adduct with the enzyme.[28] Thus, if a hydrogen bond between the azole nitrogen atom of the heterocyclic ring of the α-ketoheterocycles contributes significant binding energy to the enzyme–inhibitor complex, the α-ketooxazoline would be predicted to be one of the most potent compounds within this class of inhibitors, consistent with the observed kinetic results.

Further experimental evidence for the proposed binding mechanism of this series was obtained from the X-ray crystal structure of an α-ketobenzoxazole bound to PPE.[13] In this complex, the azole nitrogen atom was observed to possess the appropriate geometry and distance (2.80 Å) from the nitrogen atom of His-57 to be participating in a strong hydrogen-bonding interaction. Thus the combination of experimental SAR and X-ray crystallographic data confirms that our proposed design goals have been achieved with some of the peptidyl α-ketoheterocycles investigated in this study.

Summary

We have discovered a unique series of human neutrophil elastase inhibitors which contain a heterocyclic ring at the *C*-terminus of a peptide. A number of the peptidyl α-ketoheterocycles, most notably the α-ketoox-

azolines **1p,q** and the α-ketobenzoxazole **1r**, are extremely potent inhibitors of HNE which equal or surpass the activity of other comparable electrophilic ketones. While it would be naive to expect complete rationalization of the variance in activity of such a diverse structural group, a correlation appears to exist between inhibitor activity and σ_I of the heterocycle. Additionally, the data from this study combined with the previous crystal structure study[13] confirm the importance of the hydrogen bond between the inhibitor heterocyclic ring and the imidazole ring of His-57 in the covalent enzyme–inhibitor complex. This is the only reported example of fully reversible inhibitors[29] which have been shown, both kinetically and crystallographically, to simultaneously bind to both Ser-195 and His-57 of serine proteinases. On the basis of the concepts underpinning the design and development of the peptidyl α-ketoheterocycles, this class of compounds should find broad application as inhibitors of other serine proteinases.[30]

Experimental Section

General. Analytical samples were homogeneous by thin-layer chromatography (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 parts per million relative to Me_4Si as internal standard. 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 Group Analytical Department on a Perkin-Elmer 241 elemental analyzer and are within ±0.4% of theory for the formulae given. Analytical TLC was conducted on prelayered silica gel GHLF plates (Analtech, Newark, DE). Visualization of the plates was accomplished by 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 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 volume: volume ratios. Solutions were evaporated under reduced pressure on a rotary evaporator. Most starting materials were commercially available. Cbz-Val-Pro-Val-H (**7**) was prepared as previously reported.[32] Four of the heterocycles employed in the preparation of ketones **1** by method A were not commercially available and were prepared by literature methods: both methyl tetrazoles,[33] *N*-methylbenzimidazole,[34] and 3-ethoxypyridine.[35]

Method A (Scheme 1)

General Procedure for the Preparation of Ketones 1a–l. (*S*)-(Benzyloxycarbonyl)-L-valyl-*N*-[1-[(2-thiazolyl)-carbonyl]-2-methylpropyl]-L-prolinamide (1e). WSCDI (0.760 g, 3.96 mmol) was added to a solution of amino ketone **5e** (0.730 g, 3.96 mmol), acid **6** (1.38 g, 3.96 mmol), and HOBt monohydrate (1.07 g, 7.92 mmol) in dichloromethane (25 mL). The reaction mixture was stirred at room temperature for 15 h, diluted with dichloromethane, washed with saturated $NaHCO_3$ and HCl (10%), dried (Na_2SO_4), and evaporated. Purification by flash chromatography on silica gel eluting with hexanes/ethyl acetate (1:1) afforded **1e** (1.61 g, 79%) as a solid foam: TLC R_f = 0.30, hexanes/ethyl acetate (1:1); MS (DCI) m/z = 515 (M + 1, base), 407, 331, 282, 91; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.90–0.98 (12H, m), 1.67–2.14 (5H, m), 2.28–2.45 (1H, m), 3.50–3.64 (1H, m), 3.74–3.84 (1H, m), 4.07 (1H, d, J = 6.8 Hz), 4.50–4.63 (1H, br m), 5.03 (1H, d, J = 11 Hz), 5.10 (1H, d, J = 11 Hz), 5.41 (1H, d, J = 4.7 Hz), 7.30–7.40 (5H, m), 8.19 (1H, d, J = 2.5 Hz), 8.23 (1H, d, J = 2.5 Hz). Anal. ($C_{26}H_{34}N_4O_5S$·0.5H_2O) C, H, N.

N^{α}-(Benzyloxycarbonyl)-*N*-methoxy-*N*-methyl-L-valinamide (2). HOBt monohydrate (13.4 g, 99.5 mmol) and *N*-methylmorpholine (11.5 mL, 105 mmol) were added to a −10 °C solution of WSCDI (19.1 g, 99.5 mmol) in dichloromethane (500 mL). A solution of *N*-(benzyloxycarbonyl)-L-valine (25.0 g, 99.5 mmol) in dichloromethane (200 mL) was added dropwise to the reaction mixture which was then allowed to warm to ambient temperature, stirred for 0.5 h, and cooled to 10 °C. A mixture of *N*-methyl-*O*-methylhydroxylamine hydrochloride (9.70 g, 99.4 mmol) and *N*-methylmorpholine (11.5 mL, 105 mmol) in dichloromethane (150 mL) was added dropwise, and the resulting mixture was warmed to ambient temperature and stirred for 14 h. The reaction mixture was evaporated and partitioned between water and ethyl acetate. The organic layer was washed with HCl (10%), saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated to afford **2** (26.2 g, 95%) as a gum which solidified upon standing in the freezer: TLC R_f = 0.57, chloroform/methanol (40:1); MS (DCI) m/z = 295 (M + 1), 234, 187, 162, 152, 119; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.84 (3H, d, J = 6.9 Hz), 0.88 (3H, d, J = 6.8 Hz), 1.80–2.03 (1H, m), 3.11 (3H, s), 3.73 (3H, s), 4.34 (1H, t, J = 8 Hz), 5.02 (2H, s), 7.30–7.45 (5H, m), 7.50 (1H, d, J = 8.7 Hz).

General Procedure for the Preparation of Z-Val-Het 4a–l. (*S*)-1-(2-Benzothiazolyl)-2-[(benzyloxycarbonyl)-amino]-3-methyl-1-butanone (4e). To a −35 °C solution of thiazole (1.23 mL, 1.73 mmol) in dry THF (40 mL) was added *n*-butyllithium (6.60 mL, 2.18 M, 14.4 mmol) over 3 min, and the dark brown mixture was stirred for an additional 10 min at −30 to −25 °C. A solution of **2** (1.70 g, 5.78 mmol) in dry THF (15 mL) was added rapidly over 1 min and the mixture stirred for 15 min at −30 °C. The reaction mixture was poured into saturated NH_4Cl and extracted with ethyl acetate. The combined organic extracts were washed with saturated $NaHCO_3$, dried (Na_2SO_4), and evaporated. Purification by flash chromatography on silica gel eluting with hexanes/ethyl acetate (2:1) afforded **4e** (1.81 g, 98%) as a yellow oil: TLC R_f = 0.45, hexanes/ethyl acetate (2:1); MS (DCI) m/z = 319 (M + 1), 275; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.89 (3H, d, J = 6.9 Hz), 0.98 (3H, d, J = 6.8 Hz), 2.28–2.50 (1H, m), 5.06 (2H, s), 5.26 (1H, d, J = 5.7 Hz), 7.30–7.40 (5H, m), 8.20 (1H, d, J = 2.8 Hz), 8.24 (1H, d, J = 2.8 Hz).

General Procedure for the Preparation of H-Val-Het 5a–l. (*S*)-2-Amino-1-(2-thiazolyl)-3-methyl-1-butanone (5e). Trifluoromethanesulfonic acid (2.50 mL, 28.3 mmol) was added to a solution of **4e** (1.80 g, 5.65 mmol) in dichloromethane (30 mL) at room temperature. The resulting mixture was stirred for 5 min, diluted with dichloromethane, and extracted with water. The aqueous extracts were brought to pH 8 with saturated $NaHCO_3$ and extracted with dichloromethane. The organic extracts were dried (Na_2SO_4) and evaporated to afford **5e** (0.78 g, 75%) as a yellow oil: TLC R_f = 0.70, chloroform/methanol (10:1); MS (DCI) m/z = 185 (M + 1, base), 167, 140. Compounds **5** are unstable and were used immediately in the subsequent reaction to form 1.

Cbz-Val-Pro-OH (6). HOBt monohydrate (484 g, 3.58 mmol) was added to a 0–5 °C solution of Cbz-Val-OH (450 g, 1.79 mmol) in dry DMF (3.0 L). The mixture was stirred for 30 min, and a slurry of L-proline methyl ester hydrochloride (296 g, 1.79 mmol) and TEA (1.87 g, 1.84 mmol) in DMF (1.5 L) was added and the mixture cooled to 0 °C. A solution of WSCDI (378 g, 1.97 mol) in DMF (500 mL) was added, and the mixture was stirred at 0–5 °C for 3 h, warmed gradually to room temperature, and stirred for 65 h. The resulting mixture was filtered and evaporated. The residue was partitioned between ether and 1 N HCl and the precipitate which formed removed by filtration. The ethereal layer was separated, washed with water, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. The crude ester was purified by flash chromatography on silica gel eluting with a gradient of dichloromethane and methanol/dichloromethane (1:99, 2.5:97.5, 5:95). The impure fractions from the first column were flash chromatographed on silica gel eluting with a gradient of dichloromethane/hexanes (1:3), dichloromethane, and methanol/dichloromethane (2:98). The purified material from each

chromatography was combined to afford Cbz-Val-Pro-Val-OMe (597 g, 92%) as a colorless oil: TLC R_f = 0.45–0.60, methanol/dichloromethane (5:95); MS (DCI) m/z = 363 (M + 1, base), 319, 255, 130, 91; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.90 (3H, d, J = 6.9 Hz), 0.90 (3H, d, J = 6.9 Hz), 1.72–2.00 (4H, m), 2.07–2.28 (1H, m), 3.60 (3H, s), 3.5–3.67 (1H, m), 3.74–3.91 (1H, m), 4.05 (1H, t, J = 8.6 Hz), 4.33 (1H, dd, J = 8.6, 4.3 Hz), 4.99 (1H, ABq, J = 12 Hz), 5.04 (1H, ABq, J = 12 Hz), 7.30–7.40 (5H, m), 7.52 (1H, d, J = 8.4 Hz).

NaOH (1.80 L, 1 N, 1.80 mol) was added to a solution of Cbz-Val-Pro-OMe (595 g, 1.64 mol) in methanol (4.8 L). The resulting solution was stirred for 18 h and the methanol evaporated. The aqueous mixture was acidified (pH 2) with 1 N HCl and extracted with dichloromethane. The combined organic extracts were washed with brine, dried (Na_2SO_4), and evaporated to afford acid **6** (520 g, 91%) as a white solid: TLC R_f = 0.0–0.1, chloroform/methanol (95:5); MS (DCI) m/z = 349 (M + 1, base), 305, 241, 206, 116, 91; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.89 (3H, d, J = 6.7 Hz), 0.92 (3H, d, J = 6.7 Hz), 1.67–2.05 (4H, m), 2.07–2.24 (1H, m), 3.50–3.67 (1H, m), 3.74–3.88 (1H, m), 4.23 (1H, dd, J = 4.9, 8.8 Hz), 5.00 (1H, ABq, J = 9.9 Hz), 5.02 (1H, ABq, J = 9.9 Hz), 7.24–7.41 (5H, m), 7.49 (1H, d, J = 8.4).

Method B (Scheme 2)

General Procedure for the Preparation of Ketones 1m,n. (*S*)-(Benzyloxycarbonyl)-L-valyl-*N*-[1-[(5-oxazolyl)-carbonyl]-2-methylpropyl]-L-prolinamide (1m). Trifluoroacetic acid (0.081 mL, 120 mg, 1.05 mmol) was added to a suspension of **9m** (132 mg, 0.26 mmol) and DMP (447 mg, 1.05 mmol) in dichloromethane (5 mL), and the resulting solution was stirred at room temperature for 16 h. The reaction mixture was dissolved in ethyl acetate, 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 (65:35) afforded **1m** (0.083 g, 64%) as a white solid: TLC R_f = 0.39, hexanes/acetone (3:2); MS (DCI) m/z = 499 (M + 1, base), 391, 331, 266; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.94 (12H, m), 1.67–2.37 (6H, m), 3.81 (1H, m), 3.78 (1H, m), 4.08 (1H, d, J = 7.9 Hz), 4.55 (1H, m), 5.12 (3H, m), 7.35 (5H, m), 8.53 (1H, s), 8.99 (1H, m). Anal. ($C_{26}H_{34}N_4O_6$·0.5H_2O) C, H, N.

General Procedure for the Preparation of 9m,n. (Benzyloxycarbonyl)-L-valyl-*N*-[1-[(5-oxazolyl)hydroxymethyl]-2-methylpropyl]-L-prolinamide (9m). *n*-Butyllithium (0.560 mL, 2.60 M, 1.45 mmol) was added to a −78 °C solution of oxazole (0.100 g, 1.45 mmol) in THF (3 mL). The solution was stirred for 30 min, cannulated into a −78 °C solution of aldehyde **7** (625 mg, 1.45 mmol) in THF (6 mL), stirred for an additional 1 h at −78 °C, warmed to room temperature, and evaporated. Purification by flash chromatography on silica gel eluting with a chloroform/methanol gradient (100:0, 99:1, 98:2) afforded **9m** (152 mg, 21%) as a solid: TLC R_f = 0.35, chloroform/methanol (95:5); MS (DCI) m/z = 501 (M + 1, base), 484, 483, 383, 91; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.82–0.98 (12H, m), 1.60–2.17 (6H, m), 3.37–4.76 (6H, m), 5.04 (2H, m), 7.36 (5H, m), 7.80 (1H, m), 8.27 (1H, m). Anal. ($C_{26}H_{36}N_4O_6$·1.25H_2O) C, H, N.

Method C (Scheme 2)

(*S*)-(Benzyloxycarbonyl)-L-valyl-*N*-[1-[(2-oxazolyl)carbonyl]-2-methylpropyl]-L-prolinamide (1o). *tert*-Butyl alcohol was added to a solution of **9o** (4.40 g, 8.80 mmol) and DMP (15.0 g, 35.2 mmol) in dichloromethane (150 mL) and the mixture stirred for 16 h. The resulting suspension was partitioned between ethyl acetate and saturated $Na_2S_2O_3$/saturated $NaHCO_3$ (1:1). The ethyl acetate layer was washed with saturated $Na_2S_2O_3$/saturated $NaHCO_3$ (1:1), saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography on silica gel eluting with hexanes/acetone (65:35) afforded a solid which was dissolved in ethyl acetate, washed with saturated $Na_2S_2O_3$/saturated $NaHCO_3$ (1:1), saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated to give **1o** (3.74 g, 85%) as a white foam: TLC R_f = 0.32, hexanes/acetone (3:2); MS (DCI) m/z = 499 (M + 1, base), 266; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.93 (12H, m), 1.70–2.15 (5H, m), 2.31 (1H, heptet, J = 6.4 Hz), 3.56 (1H, m), 3.75 (1H, m), 4.07 (1H, d, J = 8.3 Hz), 4.55 (1H, m), 5.04 (2H, m), 5.19 (1H, d, J = 5.7 Hz), 7.37 (5H, m), 7.58 (1H, s), 8.41 (1H, s). Anal. ($C_{26}H_{34}N_4O_6$) C, H, N.

(Benzyloxycarbonyl)-L-valyl-*N*-[1-[(2-oxazolyl)hydroxymethyl]-2-methylpropyl]-L-prolinamide (9o). A solution of aldehyde **7** (7.40 g, 17.1 mmol) and 2-(trimethylsilyl)-oxazole (**19**; 4.84 g, 34.3 mmol) in toluene (10 mL) was heated at 80 °C for 24 h and at 60 °C for 14 h and evaporated. The residue was dissolved in THF (50 mL), treated with 1 N HCl (5 mL), and stirred for 30 min. The mixture was dissolved 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 hexanes/acetone (3:7) afforded **9o** (4.57 g, 53%) as a white solid: TLC R_f = 0.31, chloroform/methanol (95:5); MS (DCI) m/z = 501 (M + 1, base), 483, 393; ^{1}H NMR (250 MHz, DMSO-d_6/TFA) δ 0.90 (12H, m), 1.52–2.27 (6H, m), 3.53 (1H, m), 3.73 (1H, m), 3.83–4.60 (3H, m), 4.97 (3H, m), 7.34 (5H, m), 8.00 (0.3H, s), 8.08 (0.7H, m), 7.18 (0.3H, s), 7.32 (0.7H, s). Anal. ($C_{26}H_{36}N_4O_6$) C, H, N.

2-(Trimethylsilyl)oxazole (19). 19 was prepared by a modification of the procedure in ref 21. *n*-Butyllithium (28.5 mL, 2.54 M, 72.4 mmol) was added to a −78 °C solution of oxazole (5.0 g, 72.4 mmol) in Et_2O (150 mL). The resulting solution was stirred at −78 °C for 30 min followed by the addition of trimethylsilyl chloride (9.19 mL, 7.86 g, 72.4 mmol) and warmed to room temperature. The reaction mixture was distilled, and the fraction with a boiling point of 130 °C at atmospheric pressure was collected to afford **19** (5.17 g, 51%) as a colorless liquid: MS (DCI) m/z = 142 (M + 1, base), 91, 73.

Method D (Scheme 2)

General Procedure for the Preparation of Ketones 1p–r. (*S*)-(Benzyloxycarbonyl)-L-valyl-*N*-[1-[(2-benzoxazolyl)carbonyl]-2-methylpropyl]-L-prolinamide (1r). 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 **9r** (170 mg, 0.31 mmol) was added in dichloromethane (5 mL) and the resulting slurry stirred at −40 °C for 1 h. TEA (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 **1r** (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); HPLC, t_R = 6.84 min, FR = 2 mL/min, water/acetonitrile (1:1); MS (DCI) m/z = 549 (M + 1); ^{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 Hz), 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. ($C_{30}H_{36}N_4O_6$·0.2H_2O) C, H, N.

General Procedure for the Preparation of 1f,s. (*S*)-(Benzyloxycarbonyl)-L-valyl-*N*-[1-[(2-benzimidazolyl)-carbonyl]-2-methylpropyl]-L-prolinamide (1s). *tert*-Butyl alcohol (161 mg, 0.205 mL, 21.8 mmol) was added to a solution of **9s** (300 mg, 0.545 mmol) and DMP (926 mg, 2.18 mmol) in dichloromethane (12 mL), and the mixture was stirred for 16 h. The resulting suspension was partitioned between ethyl acetate and saturated $Na_2S_2O_3$/saturated $NaHCO_3$ (1:1). The ethyl acetate layer was washed with saturated $Na_2S_2O_3$/saturated $NaHCO_3$ (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 (92.5:7.5) afforded a solid which was rechromatographed eluting with chloroform/methanol (99:1) to give **1s** (163 mg, 55%) as a white solid: TLC R_f = 0.50, chloroform/methanol (95:5); MS (DCI) m/z = 548 (M + 1, base), 203, 91; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.91 (12H, m), 1.67–2.10 (5H, m), 2.43 (1H, m), 3.55 (1H, m), 3.75 (1H, m), 3.73 (1H, m), 4.02 (1H, t, J = 9.0 Hz), 5.01 (2H, m), 5.48 (1H, m), 7.35 (7H, m), 7.47 (1H, d, J = 8.4 Hz), 8.23 (1H, d, J = 7.8 Hz), 7.6–8.0

(2H, br m, NH), 13.4 (1H, br m, NH). Anal. ($C_{30}H_{37}N_5O_5 \cdot 1.05H_2O$) C, H, N.

General Procedure for the Preparation of Alcohols 9p,q. (Benzyloxycarbonyl)-L-valyl-N-[1-[(2-oxazolinyl)-hydroxymethyl]-2-methylpropyl]-L-prolinamide (9p). A suspension of imidate **11** (2.00 g, 3.70 mmol; prepared from compound **10** as described below under compound **9r**) in dichloromethane (15 mL) was treated with 2-aminoethanol (452 mg, 7.39 mmol) and triethylamine (1.03 mL, 7.39 mmol) and the resulting homogeneous solution stirred at ambient temperature for 16 h. The reaction mixture was diluted with ethyl acetate, washed with 1 N NaOH and brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography on silica gel eluting with methanol/chloroform (3:97, 600 mL) gave **9p** (259 mg, 14%) as a white solid: TLC $R_f = 0.32$, methanol/chloroform (3:97); MS (DCI) $m/z = 503$ (M + 1, base); ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.87 (12H, m), 1.75–1.96 (6H, m), 3.64 (4H, m), 3.90–4.40 (4H, m), 5.00 (2H, m), 7.34 (7H, m).

General Procedure for the Preparation of Alcohols 9f,r,s. (Benzyloxycarbonyl)-L-valyl-N-[1-[(2-benzoxazolyl)-hydroxymethyl]-2-methylpropyl]-L-prolinamide (9r). 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 a solution of nitrile **10** (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 **11** taken up in ethanol (5 mL) and treated with *o*-aminophenol (**12c**; 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 **9r** (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.

(Benzyloxycarbonyl)-L-valyl-N-[1-(cyanohydroxymethyl)-2-methylpropyl]-L-prolinamide (10). A solution of Cbz-Val-Pro-Val-H (**7**;[32] 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 **10** (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).

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(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; WSCDI, water soluble carbodiimide, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole monohydrate; TFA, trifluoroacetic acid; DMF, dimethylformamide; DMP, Dess–Martin periodinane, 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one; MeO, methoxy; Suc, succinyl; *p*Na, *p*-nitroanilide; Het, heterocycle; FR, flow rate.

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