Design, Synthesis, and Enzymatic Evaluation of N^1-Acyloxyalkyl- and N^1-Oxazolidin-2,4-dion-5-yl-Substituted β-lactams as Novel Inhibitors of Human Leukocyte Elastase

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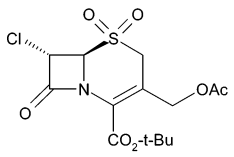
Human leukocyte elastase (HLE) is a serine protease that very efficiently degrades various tissue matrix proteins such as elastin. The imbalance between HLE and its endogenous inhibitors leads to excessive elastin proteolysis and is considered to be responsible for the onset of chronic obstructive pulmonary disease (COPD). A novel series of C-3-, C-4-, and N-1-substituted azetidin-2-ones were prepared as potential mechanism-based inhibitors of HLE to restore the protease/antiprotease imbalance. *N*-Acyloxyalkylazetidin-2-ones, **4**, and their carbamate counterparts, **5**, are weak HLE inhibitors, being 5 times less active than their bicyclic oxazolidin-2,4-dione-substituted analogues, **6**, containing an electron-withdrawing substituent at C-4. Compounds **6** containing a C-4 substituent exist as two diastereomeric pairs of enantiomers, each pair presenting similar inhibitory activity against HLE. Comparative docking experiments with the C-4-substituted oxazolidin-2,4-dione inhibitors **6** suggest that only the 4*R*,5′*S* and 4*S*,5′*S* diastereomers consistently interact with the β-lactam carbonyl carbon atom accessible to the serine hydroxyl oxygen.

Introduction

Human leukocyte (or neutrophil) elastase (HLE, EC 3.4.21.37) is a member of the chymotrypsin superfamily of serine proteases that very efficiently degrades various tissue matrix proteins such as elastin, when released from the azurophilic granules of polymorphonuclear leukocytes (neutrophils)[1] due to inflammatory stimuli and mediators.[2,3] In healthy individuals, the proteolytic activity of HLE is regulated by potent antiproteases such as α_1-antitrypsin and secretory leukocyte proteinase inhibitor. The imbalance between HLE and its endogenous inhibitors leads to excessive elastin proteolysis and destruction of connective tissues and is considered to be responsible for the onset of chronic obstructive pulmonary disease (COPD), which includes emphysema and chronic obstructive bronchitis.[4,5] Selective inhibitors of neutrophil elastase can restore the protease/antiprotease imbalance and thus are important candidates for the treatment of COPD and other inflammatory disorders such as rheumatoid arthritis and cystic fibrosis.[7−10]

β-Lactams are well-known serine protease inhibitors that acylate the nucleophilic serine residue of a wide range of enzymes,[11] including HLE.[8] Cephalosporin sulfones, for example, **1**, have been reported as one of the first β-lactam irreversible inhibitors of HLE.[12−15] Compound **1** and other cephalosporin sulfone analogues promote the acylation of the catalytic serine and alkylation of the histidine residue, probably via a mechanism-based inhibition pathway.[16] The need for improving oral bioavailability prompted the design of monocyclic β-lactams. Merck's pioneering work led to the development of several β-lactam inhibitors, for example, **2**, that contain a phenol leaving group at C-4,[17−19] two of them reaching clinical trials.[5] Recently, we reported that β-lactams **3** (LG = OCOR or OCONHR) (see Scheme 1) are time-dependent inhibitors of HLE.[20] These β-lactams were designed as potential mechanism-based inhibitors because the leaving group (LG) in **3** can be expelled following the initial Ser-195 attack at the β-lactam carbonyl atom to generate an electrophilic imine within the enzyme active site (Scheme 1). This reactive functionality has the potential of reacting with a second amino acid residue within the active site (e.g., His-57), leading to an inactivated enzyme through a double hit. In addition to a good leaving group, LG, compounds **3** contain two ethyl groups at C-3 required for molecular recognition by the enzyme and an electron-withdrawing substituent, EWG, at C-4 required for



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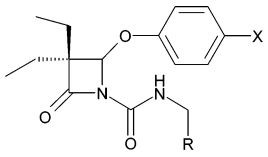

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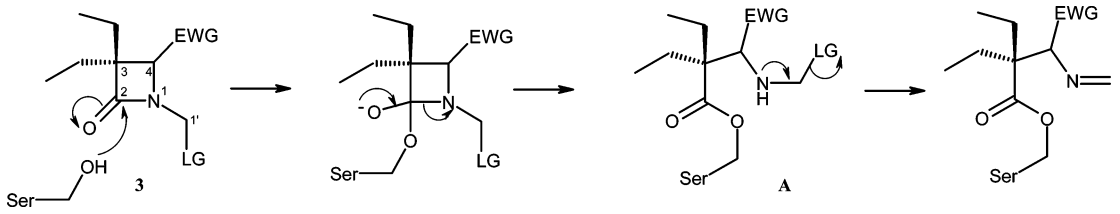
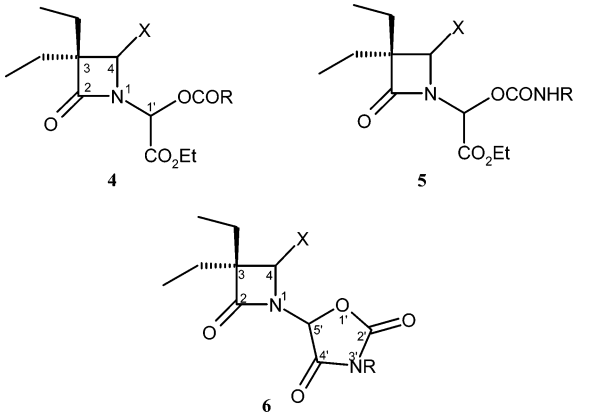
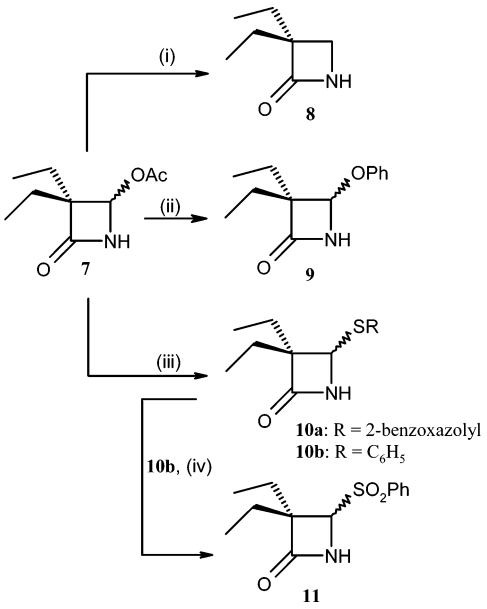
Scheme 1



Scheme 2



Scheme 3[a]



[a] (i) $NaBH_4$, EtOH; (ii) PhOH, NaOH, acetone; (iii) RSH, NaOH, acetone; (iv) MCPBA, DCM.

increasing the acylating power of the β-lactam.[20] In this paper, we report the design, synthesis, and in vitro HLE inhibitory activity of a novel series of acyloxyalkyl- (**4**), aminocarbonyloxymethyl- (**5**), and oxazolidin-2,4-dion-5-yl-substituted β-lactam analogues (**6**) (Scheme 2).

In designing the target inhibitors, we took into consideration the electronic factors that can enhance the reactivity of β-lactams. Strong evidence has been put forward suggesting that the driving force for β-lactam reactivity toward nucleophiles lies on the leaving group ability of the amine formed from the decomposition of the tetrahedral intermediate, rather than on the strain energy in the four-membered ring or on the reduced amide resonance.[21] For the β-lactams **3**, a C-4 electron-withdrawing substituent such as SO_2Ph and the acyloxymethyl moiety reduce the pK_a of the amine leaving group (**A**, Scheme 1) to ca. 2,[22] a value in the region of those reported for other reactive β-lactam inhibitors (e.g., cephalosporin antibiotics[23]). To further decrease the pK_a of the amine leaving group and thus improve the enzyme inhibitory activity, we decided to incorporate the electron-withdrawing ethoxycarbonyl substituent at the N^1-alkyl group of the azetidin-2-one ring, that is, **4** (Scheme 2). Indeed, the calculated pK_a values for the amine leaving group, which result fom the β-lactam ring opening of **4**, range from ca. 0.7 (X = OPh) to −0.5 (X = SO_2Ph).[24] Similar, although slightly higher, pK_a values can be calculated for the amine leaving group resulting from the β-lactam ring opening of carbamate analogues **5** and their cyclic counterparts **6**. The oxazolidin-2,4-dione derivatives **6** were also selected as potential inhibitors of HLE because they are likely to be the active form of the carbamates **5**, since α-carbamoyl esters cyclize rapidily to the corresponding oxazolidin-2,4-diones.[25,26]

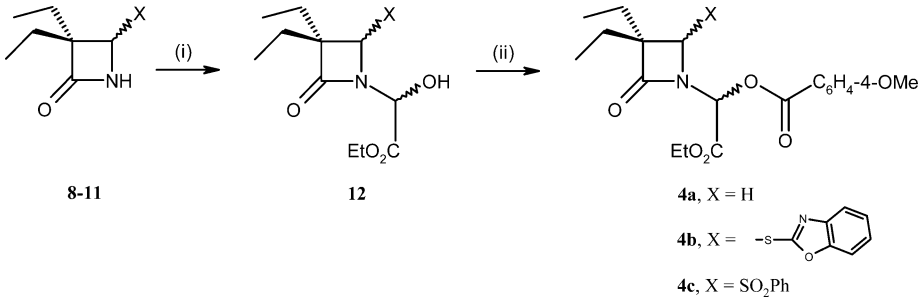
Results and Discussion

Chemistry. The synthetic pathway to compounds **4**−**6** used 4-acetoxy-3,3-diethylazetidin-2-one (**7**)[17] as the starting material to give direct access to the differently C-4-substituted azetidin-2-one key intermediates **8**−**10** (Scheme 3). Reduction of compound **7** with $NaBH_4$ in ethanol at 0 °C gave 3,3-diethylazetidin-2-one, **8**, in 30% yield. Reaction of **7** with phenol,[17] thiophenol,[27] or 2-mercaptobenzoxazole and sodium hydroxide in acetone at room temperature gave the corresponding C-4-substituted β-lactams **9**, **10a**, and **10b**, respectively. Treatment of thioether **10b** with 3-chloroperbenzoic acid (MCPBA) yielded the sulfone **11** (Scheme 3).

The acyloxyalkyl- (**4**), aminocarbonyloxymethyl- (**5**), and oxazolidin-2,4-dione-5-yl-substituted β-lactams (**6**) were all prepared from the corresponding alcohols **12** (Schemes 4 and 5). Condensation of the appropriate azetidin-2-one **8**−**11** with ethyl glyoxalate in toluene or benzene[28] afforded a complex reaction mixture from which the alcohols **12** were isolated in very poor yield. In contrast, when compounds **8**−**11** were refluxed with ethyl glyoxalate, K_2CO_3, and tetrabutylammonium bromide (TBAB) in DCM, the alcohols **12** were obtained in 80−85% yield. The reaction of the C-4-substituted azetidin-2-ones **9**−**11** with ethyl glyoxalate generates a new chiral center; thus, two diastereomeric pairs for **12b**−**d** (X = 2-thiobenzoxazole, OPh, and SO_2Ph, respectively) were obtained in ratios of ca. 1:1, as determined by ^1H NMR analysis of the crude mixtures.

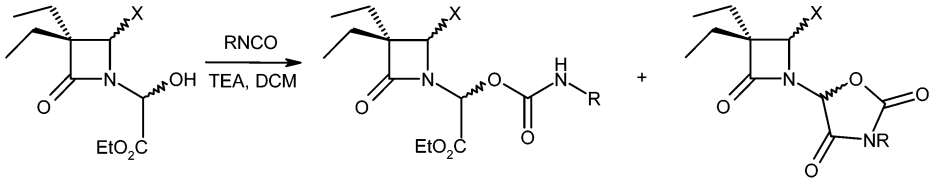
The esters **4** were prepared by reacting the appropriate diastereomeric alcohol mixture **12** with 4-methoxybenzoyl chloride in DCM containing triethylamine. A characteristic feature of the ^1H NMR spectrum of **4a** (X = H, R = 4-$MeOC_6H_4$) is the resonance of the C-1′ OC*H*N group, which appears as a singlet at 6.5 ppm.

Scheme 4[a]



[a] (i) EtO_2CCHO, TBAB, K_2CO_3, DCM, reflux 1.5 h; (ii) 4-$MeOC_6H_4COCl$, TEA, DCM.

Scheme 5



5a, 6a: R = CH_2Ph, X = H

5b, **6b**: R = 4-MeC_6H_4, X =

5c, **6c**: R = CH_2Ph, X = OPh

5d, **6d**: R = CH_2Ph, X = SO_2Ph

5e, **6e**: R = 4-$MeOC_6H_4NCO$, X = SO_2Ph

5f, **6f**: R = 2-$MeO_2CC_6H_4NCO$, X = SO_2Ph

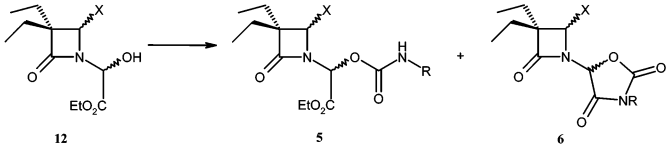
The C-4 proton signal of **4a** appears as two doublets with a geminal coupling constant of ^2J = 6.0 Hz, reflecting the diastereotopic nature of the methylene protons. Moreover, the ester CH_2O signal appears as two doublets of quartets, also with a geminal coupling constant (^2J = 10.7 Hz). For the derivative **4b** (X = 2-thiobenzoxazole, R = 4-$MeOC_6H_4$), two diastereomeric pairs of enantiomers were to be expected and their formation was confirmed by the doubling of some of the ^{13}C NMR signals: CH_2CH_3, C-2, C-4, C-5, CH_2CH_3O, and CH_3O. Compound **4b** was isolated as a 1:1 mixture of diastereomers according to the ^1H NMR analysis. Similarly, two diastereomeric pairs of enantiomers are to be expected for compound **4c** (X = SO_2Ph, R = 4-$MeOC_6H_4$), but in this case, it proved possible to isolate a sample of one of the diastereomeric pairs in a pure state by column chromatography; the other pair was always contaminated.

When the C-4-unsubstituted alcohol **12a** was reacted with benzylisocyanate in DCM and triethylamine (1 mol equiv), the isolated product was the corresponding oxazolidine-2,4-dione **6a** (X = H, R = Bz) with no traces of the carbamate counterpart (Table 1, entry 1). The oxazolidine-2,4-dione **6b** (X = 2-thiobenzoxazole, R = C_6H_4-4-Me), derived from the racemic alcohol **12b**, was isolated as a 2:1 mixture of two coeluting diastereomeric pairs of enantiomers (Table 1, entry 2), as determined by analysis of the pairs of singlets at δ 5.8−5.9 ppm (C-5′H) and 4.7−4.8 ppm (C-4H). For the oxazolidine-2,4-diones **6c**−**e**, each diastereomeric pair of enantiomers was easily separated by column chromatography. The structure and absolute stereochemistry for the crystalline minor diastereomeric pair of **6d** was confirmed by X-ray crystallography.[29]

Interestingly, during the purification process for **6c**, the carbamate counterpart **5c** (X = OPh, LG = $OCONHCH_2Ph$) was also isolated (Table 1, entry 4). Unfortunately, although both diastereomeric pairs of enantiomers for **5c** were observed in the ^1H NMR spectrum of the reaction mixture, only one pair was isolated as a pure fraction. In contrast, when the sterically hindered 2-methoxycarbonylphenylisocyanate reacted with the alcohol **12d**, only the corresponding carbamate **5f** was isolated as separable diastereomeric pairs of enantiomers (Table 1, entry 6). These results suggest that carbamates are inially formed in the reaction mixture but then cyclize rapidly to form the corresponding oxazolidine-2,4-diones. Indeed, carbamates have been postulated as intermediates in the synthesis of oxazolidine-2,4-diones, particularly those containing *N*-aryl substituents, from 2-hydroxy esters and isocyanates.[25,26] In the present case, the formation of oxazolidine-2,4-diones **6** is apparently not affected by the pK_a of the corresponding carbamate or by the size of the substituent at the β-lactam C-4 position.

Inhibition of HLE. All β-lactam derivatives **4**−**6** inhibited HLE in a time-dependent manner, suggesting that these compounds behave as irreversible inhibitors. The irreversible nature of the inactivation was shown in a routine assay, when no reactivation of enzyme activity was detected even after 4 days of dialysis at 25 °C. The inhibitory potency of compounds **4**−**6** against HLE was assessed using Kitz and Wilson's incubation method.[30] Accordingly, the inactivation of HLE may be represented by the minimal reaction depicted in eq 1, where E·I represents the noncovalent enzyme−inhibitor complex, E∼I is the covalently bound complex, K_I represents the dissociation constant of the E·I complex,

Table 1. Products and Corresponding Yields for the Reactions of Alcohol Intermediates **12** with Isocyanates



12 **5** **6**

	Reactants			Products						
Entry	**12**	X	Isocyanate		Yield/%	Diastereomeric ratio		Yield/%	Diastereomeric ratio	R
1	**12a**	H	$C_6H_5CH_2NCO$	**5a**	0	--	**6a**	33	--	$C_6H_5CH_2$
2	**12b**		4-MeC_6H_4NCO	**5b**	0	--	**6b**	63	2.3:1[a]	4-MeC_6H_4
3	**12c**	OC_6H_5	$C_6H_5CH_2NCO$	**5c**	17[c]	ND	**6c**	43	1.5:1[b]	$C_6H_5CH_2$
4	**12d**	$SO_2C_6H_5$	$C_6H_5CH_2NCO$	**5d**	0	--	**6d**	40	2:1[b]	$C_6H_5CH_2$
5	**12d**	$SO_2C_6H_5$	4-$MeOC_6H_4NCO$	**5e**	0	--	**6e**	50	2:1[b]	4-$MeOC_6H_4$
6	**12d**	$SO_2C_6H_5$	2-$MeO_2CC_6H_4NCO$	**5f**	19	1.2:1[b]	**6f**	0	--	2-$MeO_2CC_6H_4$

[a] From ^{1}H NMR analysis. [b] From yields of isolated diastereomeric pairs. [c] Only one diastereomeric pair was isolated.

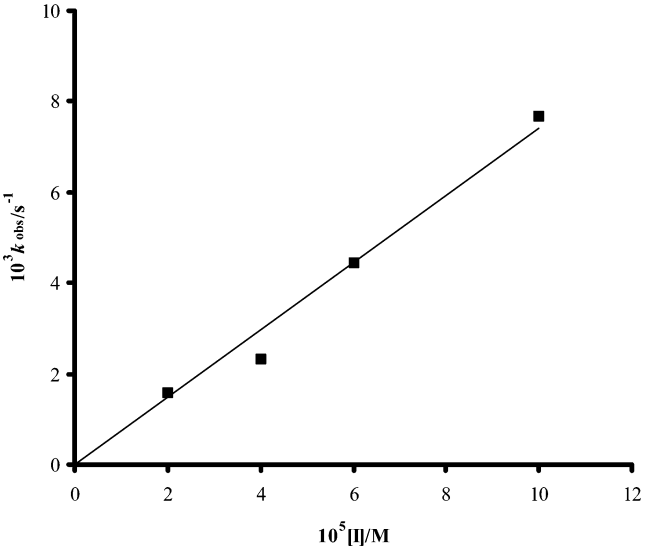


Figure 1. Plot of the first-order rate constant, k_{obs}, for the inactivation of HLE by compound **6b** versus the inhibitor concentration.

and k_{inact} is the first-order rate constant for the chemical inactivation step. The values of k_{obs} were determined from plots of $\ln(v/v_0)$ versus incubation time. Due to solubility problems with some of the inhibitors, we were forced to work in the linear region corresponding to eq 2 (Figure 1). In these conditions, eq 2 simplifies to eq 3, and thus the second-order rate constants for inactivation were expressed as k_{obs}/[I]. The corresponding values for compounds **4**−**6** are presented in Table 2.

$$E + I \overset{K_I}{\rightleftharpoons} E{\cdot}I \xrightarrow{k_{inact}} E{\sim}I \quad (1)$$

$$k_{obs} = \frac{k_{inact}[I]}{K_I + [I]} \quad (2)$$

$$\frac{k_{inact}}{K_I} = \frac{k_{obs}}{[I]} \quad (3)$$

The data presented in Table 2 reveal some interesting features. First, it can be observed that *N*-(oxazolidin-2,4-dion-5-yl)azetidin-2-ones **6** are more potent than their ester, **4**, and carbamate, **5**, analogues. For example, the oxazolidin-2,4-dione **6b** is ca. 6 times more potent than its ester counterpart **4b**, while the major diastereomeric pair of **6d** is ca. 3 times more active than **4c**. Moreover, introducing an ortho substituent in the carbamate moiety, that is, **5f**, leads to complete loss of activity for the minor diastereomer, while the major displays only marginal activity. Second, oxazolidin-2,4-diones **6** containing an electron-withdrawing substituent at C-4 of the β-lactam ring display higher inhibitory activity (**6c**,**d** versus **6a**), although there is no obvious correlation between the k_{obs}/[I] values and the Taft σ^* parameter. An electron-withdrawing substituent at C-4 is likely to increase the rate of nucleophilic attack of Ser-195 hydroxyl at the β-lactam carbonyl group or to increase the rate of decomposition of the tetrahedral intermediate by decreasing the pK_a of the corresponding amine leaving group. In contrast, C-4 electron-withdrawing substituents have a minimal effect on the inhibitory activity exerted by the weak inhibitors **4a**−**c**. Third, there is an apparent lack of stereospecificity in the inhibition by oxazolidin-2,4-dione deriva-

Table 2. Second-Order Rate Constants, k_{obs}/[I], for the Inhibition of HLE by Compounds **4**−**6**

	k_{obs}/[I]/M^{-1} s^{-1}	
compound	major diastereomeric pair of enantiomers	minor diastereomeric pair of enantiomers
4a	10.7	
4b	12.9	*a*
4c	24.4[b]	
5c	38.2	*a*
5f	26.6	*c*
6a	23.9	
6b	80.0[b]	
6c	115.3	144.2
6d	68.5	51.0
6e	43.2	49.7
2[d]	1500	
3[e]	100.3	

[a] Not determined; single pair of enantiomers. [b] Two pairs of enantiomers. [c] No inhibition at 20 μM. [d] **2**, X = CO_2H, R = Ph from ref 17. [e] **3**, EWG = SO_2Ph, LG = $OCONHC_6H_4$-4-OMe from ref 20.

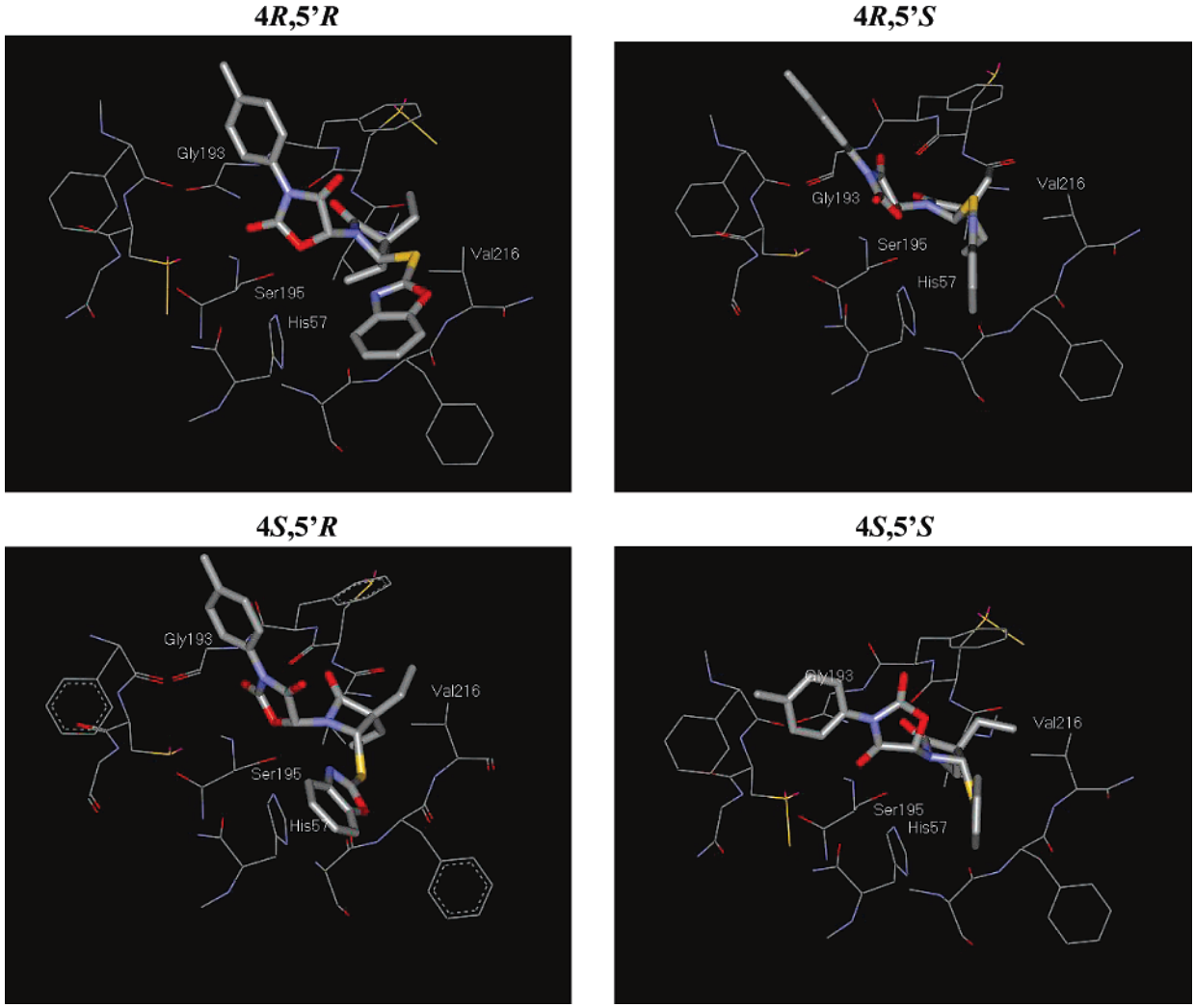


Figure 2. Molecular docking of diastereomeric pairs of **6b** into the active site of HLE (see text for details of docking procedure).

tives **6**, as suggested by the relatively small differences in the k_{obs}/[I] values for each of the diastereomeric pairs of enantiomers **6c**−**e**. This lack of stereospecificity might suggest that the enzyme−inhibitor complex for any of the stereoisomers is able to provide an appropriate stereochemical orientation and distance between the Ser-195 hydroxyl group and the β-lactam carbonyl carbon atom. Nucleophilic attack at a carbonyl carbon atom can take place within a cone of 30° on each side of the carbonyl plane,[31] and thus there are a number of possible productive orientations between the enzyme and inhibitor reactive sites. Another possible explanation for the lack of stereospecificity observed for **6c**−**e** is that the tetrahedral transition states resulting from the nucleophilic attack of the Ser-195 residue at the β-lactam carbonyl group are equally stabilized. It has been suggested that the stereospecificity in enzymatic reactions is determined in the transition-state rather than in the enzyme−inhibitor complexes.[32,33]

In contrast to the oxazolidin-2,4-diones **6**, compounds **4** and **5** are less active than the previously reported counterparts **3** (Table 2). Since the amines generated from β-lactam ring opening of **4**, **5**, and **6** have similar leaving group abilities, such loss of activity most likely reflects poorer enzyme molecular recognition of **4** and **5**.

Molecular Modeling. To understand the trends observed in the enzyme assays, the molecular interactions between the more potent oxazolidin-2,4-dione inhibitors **6** and HLE were studied using the program Sybyl 6.8. [34] By use of the crystal structure of **6d** as a guide, the lowest energy conformer of each possible enantiomer and diastereomer of **6a**−**d** (see Experimental Section) was positioned into the HLE binding cavity according to the model proposed by Shah et al. for *N*-acyl β-lactams,[17] in which the inhibitor is bound to the active site with (1) the hydroxyl group of Ser-195 in a position to interact with the β-lactam carbonyl carbon atom, (2) the β-lactam carbonyl oxygen atom pointing toward the oxyanion hole defined by the backbone of Ser-195 and Gly-193, and (3) the 3,3-diethyl group occupying the S_1 pocket. Finally, the geometry of each isomer docked in the HLE active site was further energy minimized as described in the Experimental section.

The docking study with the C-4-unsubstituted inhibitor **6a** revealed that the interaction of the corresponding 5′*S* (see Scheme 2 for numbering of **6**) enantiomer with HLE presents the β-lactam carbonyl carbon atom as slightly more accessible to the Ser-195 hydroxyl oxygen when compared with its 5′*R* isomer (3.1 versus 3.3 Å). For those inhibitors containing a substituent at C-4 of the β-lactam ring, the interaction with the HLE active

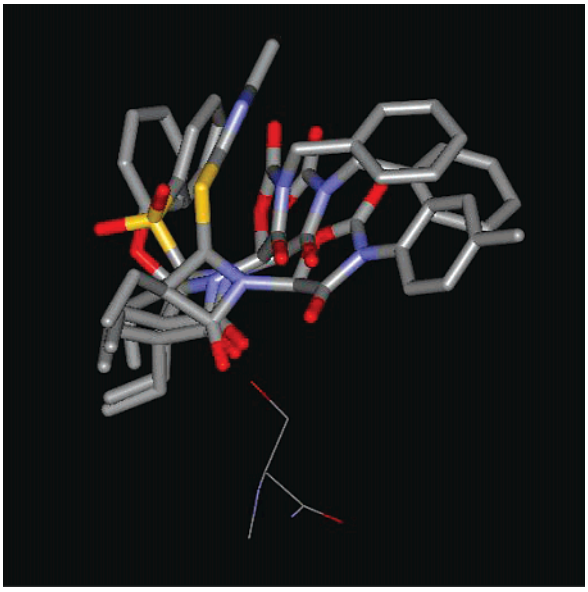


Figure 3. Superimposition of the (4*R*,5′*S*) diastereomers for compounds **6b**−**d**. For simplicity, only Ser-195 is shown.

site varies for each of the four diastereomers (4*R*,5′*R*; 4*S*,5′*S*; 4*R*,5′*S*, and 4*S*,5′*R*). Figure 2 shows all isomers of **6b** docked into the HLE active site in the conformation corresponding to that predicted to lead to the most favorable energy, in which the two ethyl groups at C-3 are accommodated within the S_1 subsite. However, only diasteromers 4*R*,5′*S* and 4*S*,5′*S* interact in a manner that seems to force the β-lactam ring deeper inside the active site, orienting the β-lactam carbonyl carbon atom close to Ser-195 hydroxyl oxygen (d < 3Å). This is due, in part, to enhanced van der Waals contacts between the C-4 benzoxazolyl moiety with Leu-99 and His-57 and additional stabilizing interactions between the *N*-aryl ring in the oxazolidin-2,4-dione moiety and the Phe-41 and Cys-42 hydrophobic residues. Interestingly, for those inhibitors with a phenoxy or phenyl sulfone substituent at C-4 (**6c** and **6d**, respectively), a similar pattern of interaction with the elastase active site was observed. Figure 3 compares the relative docked positions of the 4*R*,5′*S* diastereomers of **6b**−**d** in the HLE active site. There is a close superposition of the β-lactam rings of **6b**−**d**. Despite the slight relative displacement of C-4 and N-1 substituents, the distance between the β-lactam carbonyl carbon atom and the Ser-195 hydroxyl oxygen atom is not significantly affected: 2.9, 2.6, and 2.7 Å for the 4*R*,5′*S* isomers of **6b**, **6c,** and **6d**, respectively. This result is consistent with only a 2-fold difference in the inhibitory activities of **6b**−**d**. In contrast, the distance between the β-lactam carbonyl carbon atom and the Ser-195 hydroxyl oxygen atom in compounds **4** is consistently higher (3.0−3.2 Å) than those observed with **6** or those previously reported for their counterparts **3**, which might explain their lower activity.

Figure 4 shows a model of the tetracoordinate intermediate of the 4*R*,5′*S* **6b** isomer, which corresponds to the first intermediate in the reaction coordinate leading to the inactivation of the Michaelis−Menten inhibitor−elastase complex. This structure was obtained by creating a covalent bond between the side chain oxygen atom of Ser-195 and the carbonyl carbon atom of the β-lactam

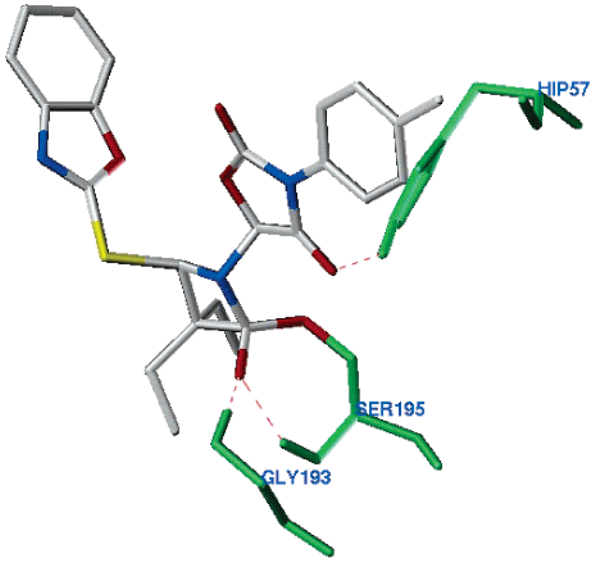


Figure 4. Model of the putative tetrahedral intermediate formed from the attack of Ser-195 hydroxyl group at the β-lactam carbonyl carbon atom of the (4*R*,5′*S*) diastereomer of **6b**, stabilized by the active site oxoanion hole. The energy minimization for this complex was done as described in the text for the Michaelis−Menten complexes.

ring of the **6b** isomer, followed by energy minimization of the complex. This tetracoordinate intermediate is stabilized in the "oxy-anion" pocket, defined by hydrogen bonding with the Gly-193 and Ser-195 NH sites, in a similar way to that reported for the tetracoordinate intermediate derived from the Merck *N*-acyl β-lactams. In addition, the presence of the oxazolidin-2,4-dione moiety enables a further stabilization of the intermediate by hydrogen bonding to the NH of His-57. The same type of stabilization by Gly-193 and Ser-195 NH's was observed for the tetrahedral intermediates derived from the remaining compounds **6**.

Conclusion

N-(Oxazolidin-2,4-dion-5-yl)azetidin-2-ones, **6**, are novel time-dependent irreversible inhibitors of HLE that were rationally designed on the basis of both the active-site topology and the catalytic mechanism for the enzyme reaction with substrates. The inhibitory potency displayed by **6** can be ascribed, in part, to the effect of the oxazolidin-2,4-dione moiety on the leaving group ability of the amine formed from the decomposition of the tetrahedral intermediate resulting from the nucleophilic attack of Ser-195 hydroxyl group at the β-lactam carbonyl carbon. In agreement with structure−activity relationships for other monocyclic β-lactams, we found that the most active inhibitors **6** contain an electron-withdrawing substituent at C-4. Compounds **6** containing a C-4 substituent exist as two diastereomeric pairs of enantiomers. Interestingly, for each inhibitor, both diastereomeric pairs of enantiomers exhibit similar inhibitory activity against HLE. One possible explanation for this apparent lack of stereospecificity is that the corresponding enzyme−inhibitor complexes might provide the proper stereochemical orientation and distance between the Ser-195 hydroxyl group and the β-lactam carbonyl carbon atom. Comparative docking experiments with the C-4-substituted *N*-(oxazolidin-2,4-dion-5-yl)azetidin-2-one inhibitors **6** suggest that only

the 4*R*,5′*S* and 4*S*,5′*S* diastereomers consistently interact with the β-lactam carbonyl carbon atom accessible to the serine hydroxyl oxygen. This finding suggests that a stereospecific synthesis of these diastereomers alone should improve inhibitor potency.

Experimental Section

Abbreviations. brs, broad singlet; Bzl, benzyl; COPD, chronic obstructive pulmonary disease; d, doublet; DCM, dichloromethane; dd, double doublet; dt, double triplet; HLE, human leukocyte elastase; MCPBA, *meta*-chloroperbenzoic acid; ppm, parts per million; q, quartet; s, singlet; t, triplet; TBAB, tetrabutylammonium bromide; TEA, triethylamine; THF, tetrahydrofuran; TLC, thin-layer chromatography; TMS, tetramethylsilane.

General. Melting points were determined using a Kofler camera Bock Monoscope M and are uncorrected. The IR spetra were recorded on a Nicolet Impact 400 FTIR spectrophotometer, and only the most significant absorption bands are reported. The ^{1}H and ^{13}C NMR spectra were recorded on a JEOL LA 300 spectrometer (300 MHz) in $CDCl_3$ solutions unless otherwise stated; chemical shifts, δ, are expressed in ppm in reference to Me_4Si (TMS). Low-resolution mass spectra were recorded using either VG Mass Lab 20-250 EI-MS or VG Quattro LCMS instruments. Elemental analyses were performed by Medac Ltd, Brunel Science Centre, Cooper's Hill Lane, Englefield Green, Egham TW20 0JZ, U.K. Enzyme assays were carried out using either Perkin-Elmer Lambda 20 or Shimadzu UV-2100PC spectrophotometers. Solvents and buffer materials for enzyme kinetics were of analytical reagent grade and bought from Merck (Germany) or Sigma-Aldrich (Spain). DCM was dried with $CaCl_2$ and stored with preactivated molecular sieves (4 Å). Both thin-layer chromatography (TLC) aluminum foil plates covered with silica 60 F254 (0.25 mm) and silica gel 60 (70−230 mesh ASTM) for preparative column chromatography were also purchased from Merck. HLE was obtained from Calbiochem. The HLE substrate, *N*-methoxysuccinyl-L-alanyl-L-alanyl-L-prolyl-L-valyl-*p*-nitroanilide (MeOSuc-Ala-Ala-Pro-Val-pNA) was obtained from Sigma-Aldrich. The 4-acetoxy-3,3-diethylazetidin-2-one starting material, **7**, was prepared as reported in the literature.[17]

3,3-Diethylazetidin-2-one (8). To a solution of 4-acetoxy-3,3-diethylazetidin-2-one, **7** (1 g, 5.4 mmol), in absolute ethanol (5 mL) at 0 °C, $NaBH_4$ (0.22 g, 5.9 mmol) was added, and the resulting suspension was kept at 0 °C for 1 h. Amberlite acid resin (5 g) was added to the suspension, and the reaction mixture stirred for a further 30 min. The reaction mixture was then filtered, and the solvent was evaporated. The resulting residue was washed with ethyl acetate (200 mL) and filtered. The solid in the filter was washed with DCM (20 mL). The organic solutions were combined, the solvents were removed, and the residue was purified by chromatography on silica gel using DCM−ethyl acetate (7:3) to give **8** as a colorless oil (28%); ν_{max} (film) 3266, 2967, 1743, 1460 cm^{-1}; δ ^{1}H NMR 0.92 (6H, t, J = 7.5 Hz), 1.62 (4H, q, J = 7.5 Hz), 3.02 (2H, s), 5.64 (1H, brs); δ ^{13}C NMR 8.97, 25.50, 45.31, 61.80, 174.30; ESI-MS *m/z* 128 (MH^+).

General Procedure for the Synthesis of C-4-Substituted 3,3-Diethylazetidin-2-ones 9−11. A solution of the appropriate arylthiol (1,3-benzoxazole-2-thiol or benzenethiol) or phenol (11.9 mmol) in acetone (12 mL) and 1 M NaOH (14 mmol, 14 mL) was stirred for 10−20 min at room temperature. 4-Acetoxy-3,3-diethylazetidin-2-one, **7** (10.8 mmol), in acetone (9 mL) was added, and the resulting mixture was stirred at room temperature, reaction progress being monitored by TLC. The solvent was evaporated under reduced pressure, and the resulting water layer was extracted with diethyl ether (2 × 70 mL). The organic layers were washed with water (60 mL) and brine (60 mL), dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel or crystallization. Data for compounds **9−11** can be found in the Supporting Information.

General Procedure for *N*-(1-Ethoxycarbonyl-1-hydroxy)methylazetidin-2-ones 12. To a stirred solution of **8−11** (1.45 mmol) in dry DCM (1.6 mL) was added ethyl glyoxalate (4.35 mmol, 0.87 mL of 50% solution), K_2CO_3 (0.16 g, 1.16 mmol), and tetrabutylammonium bromide (TBAB, 0.187 g, 0.58 mmol). This mixture was refluxed during 80−120 min and followed by TLC. The excess of ethyl glyoxalate solution and DCM was evaporated, and the residue was chromatographed on a small amount of silica gel and DCM−ethyl acetate 80:20 as eluant to remove the K_2CO_3 and TBAB. The products so obtained were used in the subsequent reaction without further purification. Data for compounds **12** can be found in the Supporting Information.

General Synthesis of *N*-{[1-(4-Methoxybenzoyl)oxy-1-ethoxycarbonyl]methyl}azetidin-2-ones 4. To a cold solution of the crude **12** (1.2 mmol) in dry DCM (2 mL) was added 4-methoxybenzoyl chloride (0.248 g, 1.45 mmol) and triethylamine (1.45 mmol). After 20 min, the mixture was allowed to reach room temperature and was stirred for a further 2 h. The residue obtained after evaporation of the DCM was purified by sequential column chromatography: in a first column, mixtures of diethyl ether−light petroleum were used as eluants, and in a second column, DCM followed by DCM−ethyl acetate (98:2) were used as eluants.

3,3-Diethyl-*N*-{[1-(4-methoxybenzoyl)oxy-1-ethoxycarbonyl]methyl}azetidin-2-one (4a). Yellow crystals (27%); mp 52−53 °C; ν_{max} (film) 2969, 1757, 1720, 1606, 1257, 1086 cm^{-1}; δ ^{1}H NMR 0.97 (3H, t, J = 7.5 Hz), 1.00 (3H, t, J = 7.5 Hz), 1.31 (3H, t, J = 7.2 Hz), 1.65−1.78 (4H, m), 3.32 (1H, d, J = 5.9 Hz), 3.34 (1H, d, J = 6.0 Hz), 3.87 (3H, s), 4.30 (2H, 2 × dq, J = 7.2, 10.8 Hz), 6.48 (1H, s), 6.94 (2H, d, J = 9.0 Hz), 8.01 (2H, d, J = 9.0 Hz); δ ^{13}C NMR 8.66, 8.70, 14.05, 25.31, 47.98, 55.47, 60.67, 62.40, 71.17, 113.77, 120.99, 132.10, 163.95, 164.87, 165.07, 172.72; ESI-MS *m/z* 363 (MH^+); Anal. ($C_{19}H_{25}NO_6$) C, H, N.

4-(1,3-Benzoxazol-2-ylthio)-3,3-diethyl-*N*-{[1-(4-methoxybenzoyl)oxy-1-ethoxycarbonyl]methyl}azetidin-2-one (4b). The two diastereomers (52:48 mixture) coeluted in the two columns; light yellow crystals (65%); mp 35−40 °C; ν_{max} (film) 2972, 1789, 1756, 1721, 1358, 1259, 1081 cm^{-1}; δ ^{1}H NMR 0.85 and 0.86 (3H, 2 × t, J = 7.5 Hz), 1.04 and 1.31 (3H, 2 × t, J = 7.2 Hz), 1.18 and 1.19 (3H, 2 × t, J = 7.4 Hz), 1.46−2.14 (4H, m), 3.60−4.41 2H, m), 3.80 and 3.85 (3H, 2 × s), 6.39 and 6.53 (1H, 2 × s), 6.55 and 6.60 (1H, 2 × s), 6.66−7.92 (8H, m); δ ^{13}C NMR 8.18, 8.95, 13.71, 14.24, 20.57, 20.81, 24.39, 24.58, 55.64, 62.67, 63,39, 66.84, 66.93, 72.02, 72.70, 73.16, 73.36, 110.48, 110.67, 112.43, 112.84, 113.67, 114.02, 124.39, 124.58, 124.97, 125.04, 132.01, 132.29, 146.90, 163.85, 164.34, 172.22, 180.43; ESI-MS m/*z* 513 (MH^+); Anal. ($C_{26}H_{28}N_2O_7S$) C, H, N.

3,3-Diethyl-*N*-{[(1-(4-methoxybenzoyl)oxy-1-ethoxycarbonyl]methyl}-4-(phenylsulfonyl)azetidin-2-one (4c). Only one major diastereomer was isolated; white crystals (24%); mp 110−112 °C; ν_{max} (film) 2974, 1788, 1724, 1327, 1606, 1257, 1083 cm^{-1}; δ ^{1}H NMR 0.91 (3H, t, J = 7.4 Hz), 1.07 (3H, t, J = 7.4 Hz), 1.24 (3H, t, J = 7.2 Hz), 1.72 (1H, dq, J = 7.3, 14.8 Hz), 1.84−1.99 (2H, m), 2.43 (1H, dq, J = 7.5, 14.9 Hz), 3.88 (3H, s), 4.12 (2H, 2 × dq, J = 7.2, 10.8 Hz), 4.88 (1H, s), 6.42 (1H, s), 6.97 (2H, d, J = 9.0 Hz), 7.55 (2H, t, J = 7.4 Hz), 7.68 (1H, t, J = 7.4 Hz), 8.00 (2H, d, J = 7.4 Hz), 8.07 (2H, d, J = 9.0 Hz); δ ^{13}C NMR 8.41, 8.83, 13.90, 20.45, 24.51, 55.48, 62.85, 67.73, 71.10, 77.26, 113.86, 120.85, 129.03, 129.36, 132.33, 134.45, 138.45, 164.44, 164.05, 170.85; ESI-MS *m/z* 504 $(MH)^+$; Anal. ($C_{25}H_{29}NO_8S$) C, H, N.

Reaction of *N*-[(1-Ethoxycarbonyl-1-hydroxy)methyl]-azetidin-2-ones 12 with Isocyanates. The desired isocyanate (0.53−0.61 mmol) and triethylamine (0.51 mmol) were added to a solution of crude **12** (0.51 mmol) in distilled DCM (1.8 mL). The mixture was stirred at room temperature for 60−90 min. The residue obtained after evaporation of DCM and triethylamine was purified by column chromatography as described above for compounds **4**.

***N*-(3-Benzyloxazolidin-2,4-dion-5-yl)-3,3-diethylazetidin-2-one (6a).** Light yellow crystals (33%); mp 54−56 °C; ν_{max} (film) 2969, 1821, 1751, 1440, 1379, 1345 cm^{-1}; δ ^{1}H NMR 0.95 (3H, t, J = 7.4 Hz), 0.96 (3H, t, J = 7.4 Hz), 1.61−1.76 (4H,

m), 3.05 (1H, d, J = 5.1 Hz), 3.07 (1H, d, J = 5.1 Hz), 4.67 (1H, d, J = 14.5 Hz), 4.73 (1H, d, J = 14.5 Hz), 6.02 (1H, s), 7.29−7.40 (5H, m); δ ^{13}C NMR 8.83, 25.32, 25.36, 46.91, 44.41, 62.29, 78.42, 128.87, 128.93, 129.15, 134.38, 153.77, 166.41, 173.40; ESI-MS m/z 334 $(MNH_4)^+$; Anal. ($C_{17}H_{20}N_2O_4$) C, H, N.

4-(1,3-Benzoxazol-2-ylthio)-3,3-diethyl-*N*-[3-(4-methylphenyl)oxazolidin-2,4-dion-5-yl]azetidin-2-one (6b). The two diastereomers (70:30 mixture) coeluted in two consecutive columns; light yellow crystals (63%); mp 89−96 °C; ν_{max} (film) 2973, 1831, 1788, 1758, 1408, 1356 cm^{-1}; δ ^{1}H NMR 0.83 and 0.83 (3H, 2 × t, J = 7.5 Hz), 1.15 and 1.20 (3H, 2 × t, J = 7.3 Hz), 1.42−2.07 (4H, m), 2.33 and 2.35 (3H, 2 × s), 5.59 and 5.76 (1H, 2 × s), 6.50 and 6.53 (1H, 2 × s), 7.16−7.84 (8H, m); δ ^{13}C NMR 7.94, 8.02, 8.62, 8.66, 20.65, 21.16, 21.34, 24.11, 24.46, 67.50, 67.94, 72.40, 72.75, 78.00, 78.62, 110.71, 110.79, 112.50, 112.70, 124.99, 125.04, 125.31, 125.65, 125.74, 127.34, 127.44, 128.20, 130.13, 130.18, 130.22, 139.90, 146.71, 146.85, 152.15, 164.96, 171.49, 179.96; ESI-MS *m/z* 466 (MH^+); Anal. ($C_{24}H_{23}N_3O_5S$) C, H, N.

***N*-[(1-Benzylaminocarbonyloxy-1-ethoxycarbonyl)-methyl]-3,3-diethyl-4-phenoxyazetidin-2-one (5c).** Only one diastereomer was isolated; colorless oil (17%); ν_{max} (film) 3365, 2971, 1781, 1763, 1736, 1493, 1224 cm^{-1}; δ ^{1}H NMR 1.00 (3H, t, J = 7.6 Hz), 1.03 (3H, t, J = 7.6 Hz), 1.26 (3H, t, J = 7.2 Hz), 1.68 (1H, dq, J = 7.3, 14.7 Hz), 1.75−1.85 (2H, m), 1.92 (1H, dq, J = 7.4, 14.9 Hz), 4.13−4.44 (2H, m), 4.34 (1H, d, J = 15 Hz), 4.41 (1H, d, J = 15 Hz), 5.23 (1H, t, J = 5.6 Hz), 5.50 (1H, s), 6.33 (1H, s), 6.86 (2H, d, J = 7.9 Hz), 7.03 (1H, t, J = 7.4 Hz), 7.26−7.36 (7H, m); δ ^{13}C NMR 8.84, 9.24, 14.12, 22.21, 23.97, 45.38, 62.61, 65.44, 71.73, 87.09, 116.74, 122.97, 127.81, 127.90, 128.93, 129.94, 158.83, 154.09, 165.19, 170.85; ESI-MS *m/z* (%) 455 $(MH)^+$; Anal. ($C_{25}H_{30}N_2O_6$) C, H, N.

***N*-(3-Benzyloxazolidin-2,4-dion-5-yl)-3,3-diethyl-4-phenoxyazetidin-2-one (6c).** Major diastereomer; white crystals (26%); mp 88−90 °C; ν_{max} (film) 2971, 1824, 1783, 1752, 1440, 1376, 1344, 1223 cm^{-1}; δ ^{1}H NMR 1.03 (3H, t, J = 7.5 Hz), 1.71 (1H, dq, J = 7.3, 14.8 Hz), 1.78−1.88 (2H, m), 1.95 (1H, dq, J = 7.4, 15 Hz), 4.43 (2H, s), 5.54 (1H, s), 5.88 (1H, s), 6.83 (2H, d, J = 7.5 Hz), 7.06 (1H, t, J = 7.5 Hz), 7.16−7.34 (7H, m); δ ^{13}C NMR 8.79, 9.13, 21.76, 23.95, 44.36, 65.95, 77.65, 87.06, 116.98, 123.63, 128.75, 128.83, 129.05, 130.28, 134.03, 156.12, 153.38, 166.06, 171.44; ESI-MS *m/z* 409 (MH^+). Anal. ($C_{23}H_{24}N_2O_5$) C, H, N. Minor diastereomer; light yellow oil (17%); ν_{max} (film) 2970, 1834, 1781, 1753, 1492, 1376, 1345, 1223 cm^{-1}; δ ^{1}H NMR 1.02 (3H, t, J = 7.5 Hz), 1.03 (3H, t, J = 7.5 Hz), 1.72 (1H, dq, J = 7.4, 14.8 Hz), 1.78−1.88 (2H, m), 1.96 (1H, dq, J = 7.4, 15.1 Hz), 4.36 (1H, d, J = 14.5 Hz), 4.44 (1H, d, J = 14.5 Hz), 5.57 (1H, s), 5.88 (1H, s), 6.82 (2H, d, J = 7.5 Hz), 7.05 (1H, t, J = 7.5 Hz), 7.18−7.31 (7H, m); δ ^{13}C NMR 8.56, 8.88, 21.66, 23.64, 44.00, 65.47, 76.92, 87.74, 116.14, 116.75, 123.24, 128.73, 128.83, 130.07, 133.83, 156.05, 153.34, 166.06, 171.23; ESI-MS *m/z* 409 (MH^+); Anal. ($C_{23}H_{24}N_2O_5$) C, H, N.

***N*-(3-Benzyloxazolidin-2,4-dion-5-yl)-3,3-diethyl-4-(phenylsulfonyl)azetidin-2-one (6d).** Major diastereomer; white crystals (27%); mp 54−56 °C; ν_{max} (film) 2973, 1827, 1789, 1751, 1443, 1334, 1155 cm^{-1}; δ ^{1}H NMR 0.70 (3H, t, J = 7.4 Hz), 0.89 (3H, t, J = 7.5 Hz), 1.49 (1H, dq, J = 7.3, 15 Hz), 1.59 (1H, dq, J = 7.4, 14.9 Hz), 1.78 (1H, dq, J = 7.4, 14.9 Hz), 2.04 (1H, dq, J = 7.3, 14.8 Hz), 4.54 (1H, d, J = 14.5 Hz), 4.55 (1H, s), 4.60 (1H, d, J = 14.3 Hz), 5.60 (1H, s), 7.23−7.85 (10H, m); δ ^{13}C NMR 8.70, 9.28, 20.31, 25.33, 45.15, 68.13, 78.05, 78.37, 129.23, 129.48, 129.68, 129.88, 130.37, 134.47, 135.59, 137.37, 153.80, 166.46, 172.11; ESI-MS m/z 475 (MNH_4^+); Anal. ($C_{23}H_{24}N_2O_6S$) C, H, N.

Minor diastereomer; white crystals (13%); mp 170−173 °C; ν_{max} (film) 2973, 1826, 1788, 1752, 1443, 1333, 1155 cm^{-1}; δ ^{1}H NMR 0.76 (3H, t, J = 7.4 Hz), 0.93 (3H, t, J = 7.5 Hz), 1.62 (1H, dq, J = 7.3, 14.8 Hz), 1.77 (2H, m), 2.24 (1H, dq, J = 7.3, 14.6 Hz), 4.53 (1H, d, J = 15.4 Hz), 4.64 (1H, d, J = 15.2 Hz), 4.93 (1H, s), 5.17 (1H, s), 7.25−7.96 (10H, m); δ ^{13}C NMR (CD_3CN) 8.06, 8.49, 20.14, 24.77, 44.19, 67.50, 77.36, 79.62, 128.59, 129.12, 129.45, 130.38, 135.12, 135.63, 138.08, 154.15, 166.41, 171.88; ESI-MS *m/z* 475 (MNH_4^+). Anal. ($C_{23}H_{24}N_2O_6S$) C, H, N.

3,3-Diethyl-*N*-[3-(4-methoxyphenyl)oxazolidin-2,4-dion-5-yl]-4-(phenylsulfonyl)azetidin-2-one (6e). Major diastereomer; recrystallized from acetone−light petroleum; white crystals (33%); mp 139−141 °C; ν_{max} (film) 2974, 1826, 1755, 1789, 1515, 1368, 1333, 1254 cm^{-1}; δ ^{1}H NMR 0.76 (3H, t, J = 7.4 Hz), 0.97 (3H, t, J = 7.5 Hz), 1.49 (1H, dq, J = 7.3, 15 Hz), 1.68 (1H, dq, J = 7.3, 14.8 Hz), 1.85 (1H, dq, J = 7.3, 14.8 Hz), 2.00 (1H, dq, J = 7.3, 14.8 Hz), 3.84 (3H, s), 4.72 (1H, s), 6.20 (1H, s), 7.00 (2H, d, J = 9.1 Hz), 7.37 (2H, d, J = 9.1 Hz), 7.63 (2H, t, J = 7.4 Hz), 7.74 (1H, t, J = 7.4 Hz), 8.02 (2H, d, J = 7.4 Hz); δ ^{13}C NMR 7.98, 8.61, 19.65, 24.68, 55.56, 67.14, 77.16, 78.14, 114.73, 122.92, 127.45, 129.43, 129.75, 135.11, 135.53, 152.59, 160.18, 165.48, 171.53; ESI-MS *m/z* 490 (MNH_4^+); Anal. ($C_{23}H_{24}N_2O_7S$) C, H, N. Minor diastereomer; white crystals (17%); mp 89−91 °C; ν_{max} (film) 2971, 1824, 1785, 1757, 1515, 1331, 1303, 1254 cm^{-1}; δ ^{1}H NMR 0.82 (3H, t, J = 7.4 Hz), 1.0 (3H, t, J = 7.5 Hz), 1.63−1.75 (2H, m), 1.89 (1H, dq, J = 7.3, 14.7 Hz), 2.22 (1H, dq, J = 7.3, 14.8 Hz), 3.81 (3H, s), 4.69 (1H, s), 5.58 (1H, s), 6.98 (2H, d, J = 9.2 Hz), 7.37 (2H, d, J = 9.2 Hz), 7.66 (2H, t, J = 7.3 Hz), 7.76 (1H, t, J = 7.3 Hz), 8.06 (2H, d, J = 7.3 Hz); δ ^{13}C NMR 8.48, 9.05, 20.17, 24.93, 55.95, 67.41, 78.15, 78.89, 115.15, 123.33, 127.90, 129.76, 130.25, 135.56, 137.41, 160.74, 166.3, 172.22; ESI-MS *m/z* (%) 490 (MNH_4^+); Anal. ($C_{23}H_{24}N_2O_7S$) C, H, N.

3,3-Diethyl-*N*-[1-ethoxycarbonyl-1-(2-methoxycarbonylphenyl)amino-carbonyloxy]-methyl-4-(phenylsulfonyl)azetidin-2-one (5f). Major diastereomer; recrystallized from diethyl ether−light petroleum; white crystals (11%); mp 117−119 °C; ν_{max} (film) 3264, 2974, 1790, 1747, 1693, 1532, 1269, 1205 cm^{-1}; δ ^{1}H NMR 1.05 (3H, t, J = 7.3 Hz), 1.09 (3H, t, J = 7.4 Hz), 1.13 (3H, t, J = 7.2 Hz), 1.79 (1H, dq, J = 7.3, 14.7 Hz), 1.98 (1H, dq, J = 7.3, 14.6 Hz), 2.10 (1H, dq, J = 7.3, 14.9 Hz), 2.48 (1H, dq, J = 7.3, 14.8 Hz), 3.96 (3H, s), 3.86 (1H, dq, J = 7.2, 10.6 Hz), 4.01 (1H, dq, J = 7.2, 10.5 Hz), 4.93 (1H, s), 6.45 (1H, s), 7.05−8.41 (9H, m), 10.85 (1H, brs); δ ^{13}C NMR 8.66, 8.91, 13.87, 20.59, 24.59, 52.45, 62.79, 68.48, 70.55, 77.42, 114.93, 119.03, 122.32, 129.28, 129.40, 130.90, 134.25, 134.74, 138.36, 140.87, 150.78, 164.10, 168.65, 170.97; ESI-MS *m/z* 564 (MNH_4^+); Anal. ($C_{26}H_{30}N_2O_9S$) C, H, N.

Minor diastereomer; white crystals (8%); mp 115−117 °C; ν_{max} (film) 3263, 2973, 1789, 1748, 1531, 1452, 1268, 1206 cm^{-1}; δ ^{1}H NMR 0.89 (3H, t, J = 7.3 Hz), 1.05 (3H, t, J = 7.5 Hz), 1.26 (3H, t, J = 7.2 Hz), 1.69 (1H, dq, J = 7.5, 15.1 Hz), 1.81−1.97 (2H, m), 2.38 (1H, dq, J = 7.3, 14.7 Hz), 3.95 (3H, s), 4.24 (2H, 2 × dq, J = 7.2, 9.9 Hz), 4.63 (1H, s), 5.66 (1H, s), 7.05−8.33 (9H, m), 10.75 (1H, brs); δ ^{13}C NMR 8.35, 8.73, 13.90, 19.98, 24.39, 52.43, 62.92, 67.30, 74.01, 78.51, 115.19, 118.97, 122.35, 128.81, 129.61, 130.95, 134.57, 134.62, 138.05, 140.69, 150.77, 164.14, 168.25, 170.97; ESI-MS m/z 564 (MNH_4^+); Anal. ($C_{26}H_{30}N_2O_9S$) C, H, N.

HLE Inhibition Studies. Assays were performed at 25 °C in 0.1 M pH 7.2 HEPES buffer. The substrate used was MeO−Suc-Ala-Ala-Pro-Val-*p*-NA, and stock solutions were made up in DMSO. Enzyme activity was monitored by following the appearance of the *p*-nitroaniline product at 410 nm. Reactions were started by addition of inhibitor stock solution in DMSO (20 μL) to the incubation solution containing HLE (10 μL from a 20 μM stock solution in pH 5.0 acetate buffer) and HEPES buffer (970 μL). At various time intervals the HLE activity was assayed by diluting 100 μL aliquots of the incubation solution into 880 μL of 0.1 M pH 7.2 HEPES buffer and 20 μL of the substrate 50 mM stock solution. Initial rates were monitored over a period of 60 s, and the gradients of the slopes obtained were used as a measure of enzyme activity. In all cases, assays of control incubations were performed at the same time as inhibitor incubations. Pseudo-first-order rate constants of inactivation, k_{obs}, were obtained from plots of % activity (v/v_0) against time, where v is the initial rate at time t and v_0 is the initial rate of the control incubation. In the same experimental conditions, the K_m value for the hydrolysis of

MeO−Suc-Ala-Ala-Pro-Val-*p*-NA by HLE is 0.16 ± 0.05 mM, which compares favorably with the value of 0.14 mM reported by Nakajima et al.[35]

Molecular Modeling. The structures of all enantiomers and diastereomers of compounds **6a**−**d** were energy- minimized using the MMFF94s force field.[36] The HLE structure used (PDB code 1HNE[37]) was obtained by deletion from the active site of the ligand (methoxysuccinyl-Ala-Ala-Pro-Ala chloromethyl ketone) present in the crystal structure and prepared using WHATIF[38] (for addition of hydrogen atoms and optimization of hydrogen bonds) and the Biopolymer module of Sybyl. Histidine protonation states were assigned according to their surrounding environment. Inhibitors were modeled in the active site using the model proposed by Shah et al. for *N*-acyl β-lactams,[17] and energy minimization of the complex was performed on a region comprising all residues in the protein within a distance of 6 Å from the atoms of the ligand using the MMFF94s force field.[36] All minimization procedures were performed using a simplex initial optimization, gradient termination (0.001 kcal·mol^{-1}·Å^{-1}), and a distance-dependent dielectric function.

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Supporting Information Available: Synthesis procedure for compound **11**, all spectroscopic data for compounds **9**−**12**, and the crystal data for the minor diastereomeric pair of enantiomers of **6d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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numbers CCDC262210. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (Fax +44(0)-1223-336033 or e-mail deposit@ccdc.cam.ac.uk).

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