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Design, Synthesis and Stability of *N*-Acyloxymethyl- and *N*-Aminocarbonyloxymethyl-2-azetidinones as Human Leukocyte Elastase Inhibitors

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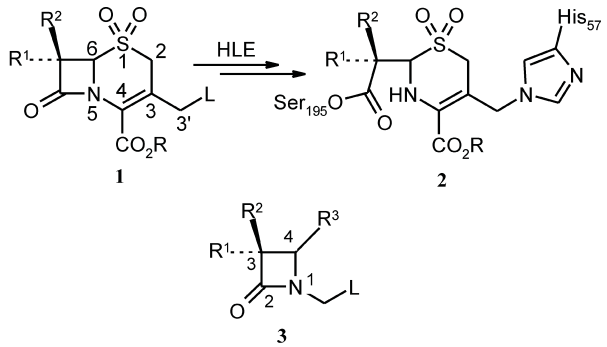
Abstract—A series of *N*-acyloxymethyl- and *N*-aminocarbonyloxymethyl derivatives of 2-azetidinones, **3**, with different substituent patterns at the β-lactam C-3 and C-4 positions, were designed as potential mechanism-based inhibitors for human leukocyte elastase and found to exhibit inhibitory potency and selectivity for the enzyme.

Human leukocyte elastase (HLE, EC 3.4.21.37) is a serine protease that has received great attention because it efficiently degrades various tissue matrix proteins such as elastin.[1] The imbalance between HLE and its endogenous inhibitors and the subsequent excessive elastin proteolysis has been implicated in acute and chronic inflammatory diseases of the lungs.[2,3] Thus, specific inhibitors of HLE capable of restoring the protease/antiprotease imbalance might be beneficial in such pathologies.[4,5]

Cephalosporin sulfones, e.g., **1**, are potent, mechanism-based irreversible HLE inhibitors.[6–8] A structural requirement of **1** is the presence of a good leaving group L (e.g., acetate) at the 3′-position, which is thought to provide an electrophilic center for the nucleophilic attack of histidine-57 following initial serine-195 attack at the β-lactam carbonyl atom (Scheme 1). We reasoned that simple β-lactams **3**, in which L is a good leaving group such as a carboxylic or carbamic acid, would also be capable of inhibiting HLE via a similar 'double-hit' mechanism. From our previous experience with the chemistry of *N*-acyloxymethylamides,[9,10] appending a leaving group with $pK_a > 4$ would be expected to increase substantially the stability of **3** in aqueous buffers and thus increase their ability to reach their target. We now report the synthesis and biochemical evaluation of C-3 and C-4 substituted *N*-acyloxymethyl and *N*-aminocarbonyloxymethyl-2-azetidinones.

Compounds **3** were prepared (Scheme 2) by known methods,[11] using the appropriate 4-acetoxy-2-azetidinones **4**[11b] as starting materials.

Compounds **3** were found to be time-dependent irreversible inhibitors of HLE, the inhibitory activity (Table 1)



Scheme 1.

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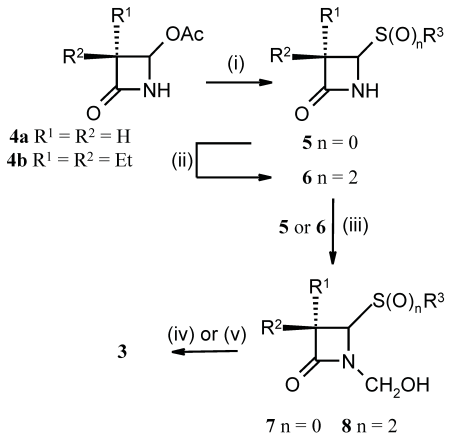


Scheme 2. Reagents and conditions: (i) R^3SH/NaOH/acetone; (ii) MCPBA; (iii) HCHO/aq K_2CO_3; (iv) RCOCl/Et_3N; RNCO/Et_3N.

being expressed by the bimolecular rate constant k_{obs}/[I] determined by Kitz and Wilson's pre-incubation method.[12,13] The irreversible nature of the inactivation was shown when no significant reactivation of enzyme activity was detected upon dialyzing HLE against pH 7.2 HEPES buffer for 24 h. Independently, the native enzyme itself was found to retain activity after dialysis. Inhibition toward pancreatic porcine elastase (PPE) was also evaluated,[14] but none of the compounds studied inactivated this enzyme at [I]/[E] = 200. The compounds **3** displayed variable inhibitory potencies towards HLE, the unsubstituted prototype **3a** being a fair inhibitor (Table 1). Unfortunately, compound **3b** could not be evaluated under the assay conditions due to solubility problems. For comparison, data for two cephalosporins is also presented in Table 1. Inspection of the data in Table 1, allows the following conclusions to be drawn.

Substitution at C-3

Substitution at this position generally leads to more potent inhibitors. Incorporation of two ethyl groups at C-3 increases inhibition up to ca. 4-fold (compounds **3c** and **3g**). This finding is in agreement with the known S_1 subsite specificity of HLE toward small hydrophobic substituents with three or four carbon atoms[15] and is identical to the reported preference of HLE toward C-3 substituted *N*-acyl-2-azetidinones.[11,16]

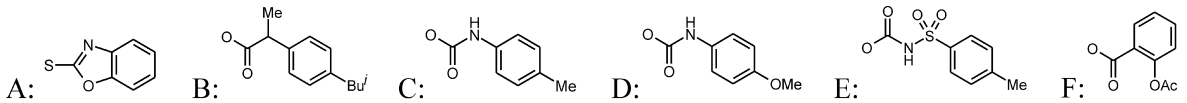
Substitution at C-4

The phenyl sulfones **3c** and **3g** are more potent inhibitors of HLE than the corresponding sulfides, **3d** and **3h**, respectively. This result compares to the superior HLE inhibitory activity displayed by cephalosporin sulfones **1** relative to their sufide counterparts.[6,7] However, there is not an absolute requirement for a sulfone at C-4 to achieve HLE inhibition, as shown by the inhibition displayed by the sulfides **3e** and **3f** as well as by the 2-fold greater inhibitory potency of **3a** over its sulfone-containing analogue **3c**. The superior activity displayed by the 4-phenylsulfone and 4-(2′-thiobenzoxazolyl) derivatives can be ascribed to the electronegativity of these substituents, which is likely to increase the reactivity of the β-lactam carbonyl group. Alternatively, both sulfone and 2-mercaptobenzoxazole may function as leaving groups after the β-lactam ring opening (Scheme 3, path B). A similar mechanism, in which a sulfone serves as a leaving group, has been suggested for the β-lactamase inhibition by sulbactam.[17] Indeed, it was recently reported that the use of 2-mercaptobenzoxazole and related sulfides as leaving groups in the 1,2,5-thiadiazolidin-3-

Table 1. Second-order rate constants, k_{obs}/[I], for the inhibition of HLE and PPE by *N*-acyloxymethyl- and *N*-aminocarbonloxymethyl-2-azetidinones **3** at 25 °C, and half-lives in pH 7.4 phosphate buffer at 37 °C

Compound	R^1	R^2	R^3	L	k_{obs}/[I], $M^{-1}s^{-1}$ HLE	k_{obs}/[I], $M^{-1}s^{-1}$ PPE	Stability $t_{1/2}$/h
3a	H	H	H	$OCOC_6H_5$	35.5	NI[a]	1.3
3b	H	H	H	B	ND	ND[b]	ND
3c	H	H	$SO_2C_6H_5$	$OCOC_6H_5$	18.7	NI	978
3d	H	H	SC_6H_5	$OCOC_6H_5$	NI	ND	ND
3e	H	H	A	$OCOC_6H_5$	43.2	NI	ND
3f	Et	Et	A	$OCOC_6H_5$	52.8	NI	316
3g	Et	Et	$SO_2C_6H_5$	$OCOC_6H_5$	67.5	NI	2995
3h	Et	Et	SC_6H_5	$OCOC_6H_5$	2.2	ND	ND
3i	Et	Et	$SO_2C_6H_5$	$OCONHC_6H_5$	41.9	NI	ND
3j	Et	Et	$SO_2C_6H_5$	C	60.9	NI	ND
3k	Et	Et	$SO_2C_6H_5$	D	100.3	NI	> 5000
3l	Et	Et	$SO_2C_6H_5$	E	32.3	ND	ND
8b	Et	Et	$SO_2C_6H_5$	OH	NI	ND	ND
9	H	H	H	OH	NI	ND	ND
1a[7a]	MeO	H	—	OAc	19000	—	–
1b[7b]	MeO	H	—	F	0.11 μM[c]	—	19

[a]No inhibition.
[b]Not determined.
[c]IC_{50}.



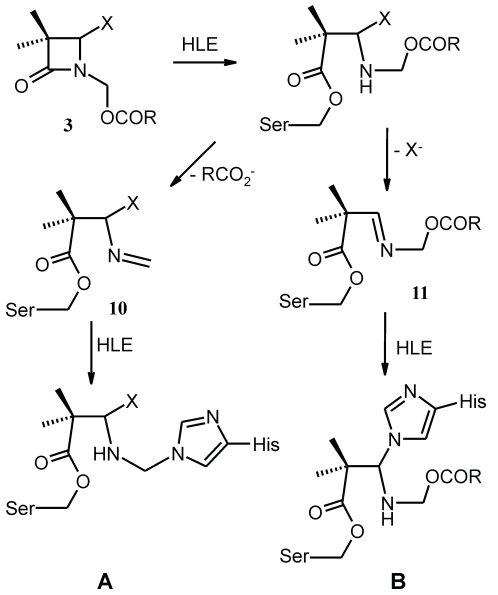
one 1,1-dioxide scaffold resulted in highly potent HLE inhibitors.[18]

Nature of the Leaving Group L

Both carboxylate and carbamic acid leaving groups lead to active compounds, the most active compound being the 4-methoxyphenylcarbamic acid derivative **3k**. Use of a poor leaving group, L = OH, (**8b** and **9**) leads to the loss of inhibitory activity. Modification of the leaving group by using an ionizable functionality to increase solubility reduced inhibitory potency (compare compounds **3j** and **3l**, the latter of which has pK_a ca. 3[19]).

Stability

The stability study carried out in pH 7.4 phosphate buffer reveals that reactivity is mainly dependent on the C-4 substituent (Table 1). Indeed, while the unsubstituted compound **3a** is rapidly hydrolyzed with $t_{1/2}$ of ca. 80 min, its 4-phenylsulfone analogue **3c** presents a 750-fold increase in stability. Introducing the C3 substituents leads to further increase in stability (**3c** vs **3g**). Carbamate leaving groups lead to improved stability, when compared to a carboxylate leaving group (**3g** vs **3k**).



Scheme 3.

Molecular Modeling

The molecular interactions between the inhibitors **3** and HLE (PDB code 1hne) were studied with the program GOLD.[20–22] Docking results show that the presence of ethyl groups at C3 consistently restricts the degrees of freedom of ligand binding as compared to the non-substituted analogues (compounds **3d**/**3h**, **3c**/**3g** and **3e**/**3f**), and helps anchor the ligand in the S_1 subsite forcing the carbonyl C2 into close vicinity of the Ser195 hydroxyl oxygen (d < 3Å).

Substitution at C4 greatly enhances the van der Waals contacts with Leu99 and His57. Moreover, the presence of a sulfone (compounds **3c** and **3g** compared to **3d** and **3h**, respectively) enables a further stabilization of the complex by hydrogen bonding to the NH of Val216 (2.5 Å) (Fig. 1). The larger benzoxazolyl moiety of **3f** seems to force the β-lactam ring deeper inside the active site, reducing the distance between C2 and Ser195 hydroxyl oxygen. Comparative docking experiments with the C4 substituted inhibitors (e.g., **3k**) indicate that only *R*-enantiomers consistently interact with the β-lactam carbonyl carbon atom accessible to the serine hydroxyl oxygen. This finding suggests that a stereospecific synthesis of the *R*-enantiomers alone should improve inhibitor potency.

Interestingly, our docking results with the intermediate **11** do not support the hypothetical mechanism in which the substituents at C4 would function as the leaving group (Scheme 3, path B). In fact, all docked configurations of intermediate **11** show that the electron deficient C4 atom becomes sterically inaccessible to the

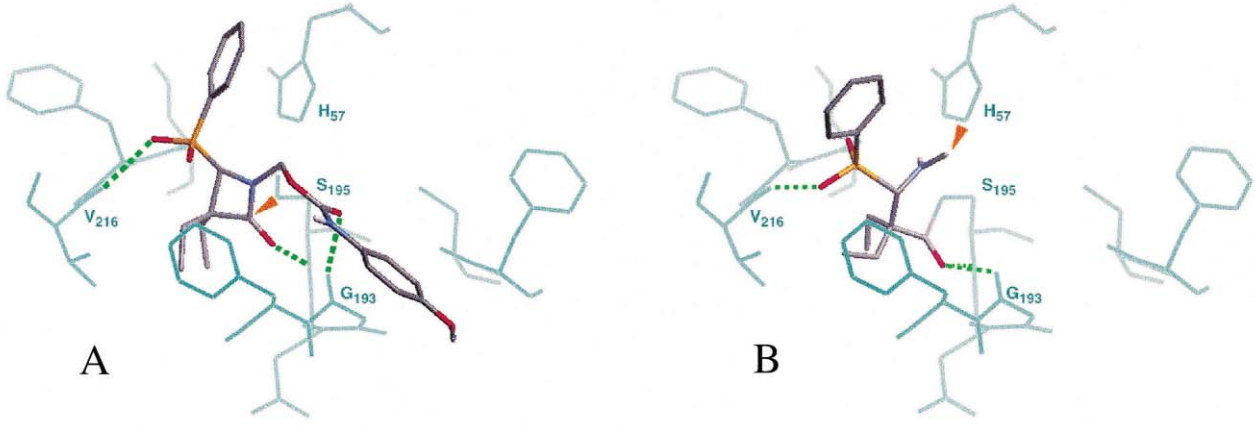


Figure 1. Molecular docking of inhibitors into the active site of HLE (see text for details of docking procedure). A: Inhibitor **3k** interacting with active site by van der Waals contacts and hydrogen bonding (green dashed lines). B: Best scoring docked configuration of intermediate **10**, covalently bound to Ser195. Orange arrows show close contacts between enzyme nucleophiles and electron deficient atoms of the ligand.

His57 nitrogen atom. In contrast, the alternative mechanism involving the intermediate **10** (Scheme 3, path A) appears to be compatible with our docking experiments. Figure 1B shows this intermediate bound to the active site Ser195 and with the methylene group facing the NE2 atom of His57 (d = 2.7 Å).

In conclusion, *N*-acyloxymethyl- and *N*-aminocarbonyloxymethyl-2-azetidinones **3** are time-dependent irreversible inhibitors of HLE. Derivatives **3** containing either a phenyl sulfone or a 2′-mercaptobenzoxazolyl substituent at C-4 and a *gem*-diethyl at C-3 were the most potent inhibitors. The results are consistent with, though do not prove, the mechanism A depicted in Scheme 3, with substituents at C-4 enhancing the reactivity of the β-lactam carbonyl. Ongoing studies aimed at optimizing inhibitory potency and elucidating the mechanism of action of these compounds are currently in progress.

Acknowledgements

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