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THE EFFECT OF N-ACYL SUBSTITUENTS ON THE STABILITY OF MONOCYCLIC β-LACTAM INHIBITORS OF HUMAN LEUKOCYTE ELASTASE

William K. Hagmann†*, Kevan R. Thompson†, Shrenik K. Shah†, Paul E. Finke†,
Bonnie M. Ashe¶, Hazel Weston¶, Alan L. Maycock¶∞, James B. Doherty†

†Department of Medicinal Chemical Research, ¶Department of Enzymology
Merck Research Laboratories
Rahway, New Jersey 07065, USA

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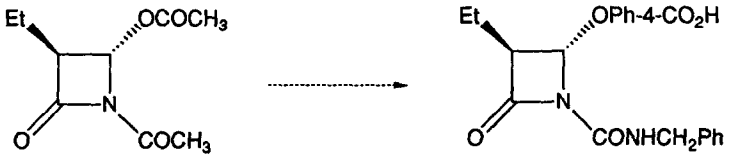
Abstract - Substituted monocyclic β-lactam have recently been reported as inhibitors of human leukocyte elastase (HLE). Simple N-acetyl-2-azetidinone lead structures were found to undergo N-deacylation as well as β-lactam ring opening. The development of the N-carbamoyl-2-azetidinone nucleus was crucial to the stability of these compounds for effective oral bioavailability.

We recently reported the inhibition by a series of substituted monocyclic β-lactams[1] of human leukocyte elastase (HLE), a serine proteinase found in the azurophilic granules of polymorphonuclear leukocytes (PMN's) and implicated in the degradation of connective tissues in inflammatory diseases.[2-6] Initial reports from our laboratories document the progression of our efforts in exploiting the β-lactam nuclei from the cephalosporins through simple monocyclic 2-azetidinones for inhibition of HLE.[7-14] We had developed compounds with good selectivity and potency for HLE that had demonstrable *in vivo* activities when administered locally, eg. intratracheally or subcutaneously. However, few compounds had sufficient stability in plasma and none were orally bioavailable. Herein, we describe the development of N-acyl-2-azetidinones with the enhanced hydrolytic stability that led to the discovery of orally absorbed elastase inhibitors such as **5**.

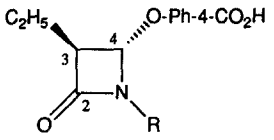


I **5**

Our earliest inhibitor lead in the monocyclic β-lactams, (±)-*trans*-4-acetoxy-1-acetyl-3-ethyl-2-azetidinone (**I**) [15], was rapidly hydrolyzed ($t_{1/2}$ ~ 2 hrs) under mild conditions (pH = 8.0, 25°C). The 4-acetoxy substituent was thought to be a likely source of hydrolytic instability and was replaced with the phenolic ether acid as in **1** (Table 1) which had a half life of disappearance of ~ 9 hours under the same conditions. HPLC analysis of the products of hydrolysis of **1** revealed the appearance of two subsequently identified products: the deacylated β-lactam **2** and 4-hydroxybenzoic acid. The N–H compound **2** was found to be significantly more stable under the

To whom correspondence and reprint requests should be sent
∞ Present address: Sterling Research Group, Malvern, PA 19355

Table 1. Stability [a] and Elastase Inhibition of N-Substituted (±) 2-Azetidinones.



Compd. No.	R	$t_{1/2}$[b] (hr ± SD)	Inhibition of Human Leukocyte Elastase (± SD) [c]	
			$k_{obs}/[I]$ ($M^{-1}sec^{-1}$)	K_i (μM)
1	$COCH_3$	8.6 (0.5)	6680 (120)	
2	H	31.8 (2.5)		290 (30)
3	$CO_2C_2H_5$	29.7 (1.6)	6350 (1150)	
4	$CONHCH_3$	>> 80		16.5 (1.1)
5	$CONHCH_2Ph$	>> 80	2480 (100)	

[a] MOPS (0.42 M), pH = 8.0 @ 25°C. [b] Calculated from first order disappearance of compound: $A_t = A_0/2^{t/h}$ where A_t = HPLC area counts at time t ($A_0 = A_t$ at time 0); t = time in hours; h = half life in hours ($t_{1/2}$). [c] Values for compounds which exhibited time dependent inhibition ($k_{obs}/[I]$) were determined by the method described in Reference 9. K_i values were obtained from initial velocities.

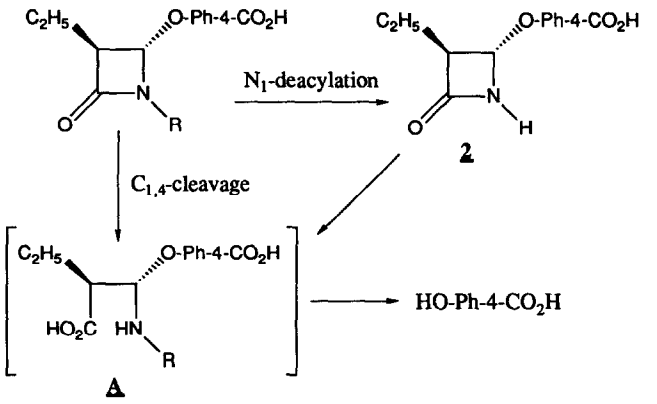
hydrolysis conditions than **1** ($t_{1/2}$ ~ 32 hrs) and also gave 4-hydroxybenzoic acid as a degradation product.

A simple mechanism for these results is outlined in Scheme 1. The 2-azetidinone (R = $COCH_3$, **1**) can undergo β-lactam ring opening ($C_{1,4}$-cleavage) to a possible intermediate **A** from which 4-hydroxybenzoic acid could be produced by collapse of the aminal or by elimination. Elimination of 4-hydroxybenzoic acid might also be concerted with ring opening without invoking the intermediacy of **A**. The deacylated product **2** would arise by hydrolysis of the N_1-$COCH_3$ bond. Hydrolysis of the β-lactam ring in **2** might occur analogous to **1**, albeit more slowly or **2** may disappear by base catalyzed N_1-C_4 elimination of 4-hydroxybenzoic acid.

It was reasoned that elimination of the N_1-deacylation process (**1** → **2**) might give a more stable compound. The ethoxycarbonyl group is less electron withdrawing than an acetyl group and should be less susceptible to direct hydrolytic attack. Indeed, **3** (R = $CO_2C_2H_5$) was more stable to the hydrolysis conditions than **1** and did not yield **2** as a hydrolysis product as determined by HPLC analysis. The only product identified on HPLC was 4-hydroxybenzoic acid. The ethoxycarbonyl group figured prominently in our efforts to develop a locally acting inhibitor of HLE with these types of compounds.[1] The effect of an N_1-carbamoyl group was even more dramatic. The methyl carbamoyl analog **4** (R = $CONHCH_3$) was still mostly intact after 5 days, exhibiting less than 30% degradation in that time.

A proposed mechanism of inhibition of HLE by these compounds invokes acylation of Ser^{195} at the active site of the enzyme[15] and therefore the chemical reactivity of the β-lactam ring will determine both the rate of nonspecific hydrolysis as well as acylation of the enzyme. Of course, this ignores specific interactions of other substituents with the enzyme that may facilitate ring opening by inducing further ring strain or stabilizing intermediates. Compounds **1** and **3** are equipotent inhibitors of HLE (Table 1). The half life for hydrolysis of **1** is a combination of ring opening and N_1-deacylation, so it is not be reasonable to compare nonspecific hydrolysis

Scheme I



rates and enzyme inhibition for these two compounds. On the other hand, **3** and **4** are a better comparison since nonspecific hydrolysis of these two compounds is most likely only a function of the rate of β-lactam ring opening. The carbamoyl compound **4** is very stable and does not exhibit time-dependent inhibition of HLE under our assay conditions. This could be the result of the unreactivity of the β-lactam ring.

As noted above, specific interactions of HLE with other substituents on the inhibitors might be expected to influence binding and reactivity of the β-lactam ring. Replacement of the methyl carbamoyl group in **4** with the benzyl carbamoyl group in **5** did not have much effect on the rate of nonspecific hydrolysis. Yet, compound **5** is a reasonably potent time-dependent inhibitor of HLE. The benzyl group has possibly found an additional specific interaction with the enzyme that influences the ability of Ser^{195} at the active site to efficiently open the β-lactam ring. It is also possible that this substituent stabilizes the acylated serine intermediate preventing turnover to active enzyme.

Thus, we were able to reduce the rates of nonspecific hydrolysis of our initial 2-azetidinone inhibitors and enhance their stability while retaining most of the inhibitory potencies of our lead compounds. However, even compounds such as **5**, which provided the first hints of oral bioavailability[16], were deemed too unstable and not potent enough as inhibitors for desired therapeutic efficacy. Incorporation of other substituents around the nucleus provided the greater stability and potency needed for an effective orally active agent.

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