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MONOCYCLIC β-LACTAM INHIBITORS OF HUMAN LEUKOCYTE ELASTASE. STEREOSPECIFIC SYNTHESIS AND ACTIVITY OF 3,4-DISUBSTITUTED-2-AZETIDINONES.

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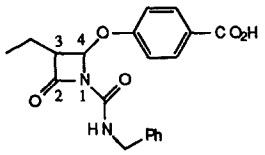
Abstract. The synthesis of the four stereoisomers of 3-ethyl-4-[(4-carboxyphenyl)oxy]-1-[[(phenylmethyl) amino]carbonyl]-2-azetidinone (**1**) starting from either D or L-aspartic acid is reported. The *trans* (3R,4R) isomer **7a,** prepared from L-aspartic acid had the most inhibitory activity against human leukocyte elastase (HLE). This monocyclic β-lactam was very resistant to hydrolysis and was found to be orally bioavailable in marmosets.

Human leukocyte elastase (HLE, EC 3.4.21.37), a serine protease present in the azurophilic granules of polymorphonuclear leukocytes, is believed to be responsible for tissue destruction in diseases such as emphysema, cystic fibrosis and arthritis.[1] It is hoped that inhibition of HLE may be therapeutically efficacious for these conditions. We have shown that cephalosporin sulfones are inhibitors of HLE[2] and further modifications of this lead resulted in highly potent and specific inhibitors of HLE which were devoid of antibiotic activity.[3] *In vivo*, some of these compounds were shown to be active when administered intratracheally; but they lacked systemic activity due to their hydrolytic instability.[4] In order to find systemically active compounds, we have evaluated other β-lactam structures for their activity against HLE. The results for the penem[5] and penicillin[6,7] ring systems are disclosed in the accompanying papers, and herein we report some work on a monocyclic β-lactam structure. In previous screening, the *trans* isomer of compound **1** had good activity against HLE ($k_{obs}/[I] = 2480$ $M^{-1}sec^{-1}$), but was inactive *in vivo* when administered intratracheally.[8] However, upon further investigation of these compounds, **1** was found to be very stable to hydrolysis at pH 8.[9] In order to better understand these preliminary observations and to find the most potent form of **1**, we decided to synthesize the individual isomers of **1**. The stereospecific synthesis of each isomer and its inhibitory activity against HLE is described in this paper.



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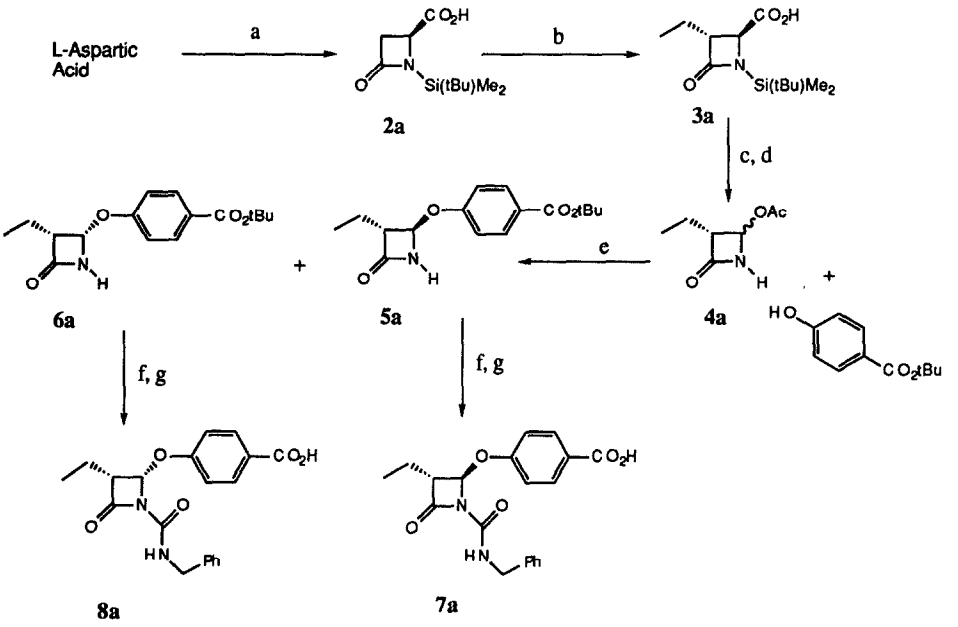
Since there are two asymmetric centers in **1**, four stereoisomers are possible. The synthesis of one pair of diastereomers is outlined in Scheme I. Starting from L-aspartic acid, 4-(S)-N-*t*-butyldimethylsilyl-4-carboxy-2-azetidinone (**2a**) was prepared in six steps by a literature procedure.[10] The dianion of **2a**, prepared by reaction with two equivalents of lithium diisopropylamide (LDA), was alkylated with ethyl iodide to give exclusively the *trans* (3R,4S) acid **3a** in 73% yield after crystallization from ether-hexane. This dianion is known to undergo stereospecifically *trans* aldol reaction without racemization at C-4.[11] Oxidative decarboxylation of **3a** with $Pb(OAc)_4$ and removal of the silyl protecting group with aqueous HCl gave an epimeric mixture of 4-acetyloxy-2-azetidinones **4a**, which was not purified. Reaction of **4a** with the sodium salt of *t*-butyl *p*-hydroxybenzoate in acetone-water gave an 8:1 mixture of *trans* (3R,4R) **5a** and *cis* (3R,4S) **6a** isomers (30-35% yield for three steps) which were separated by flash chromatography. The *trans* isomer **5a** was treated with benzyl isocyanate and the resulting urea was deesterified with cold trifluoroacetic acid to obtain in 80% yield, the (3R,4R) isomer **7a** as a white solid after chromatographic purification. Similarly, the *cis* isomer **6a** was converted to the (3R,4S) isomer **8a**. The other two stereoisomers **7b** (3S,4S) and **8b** (3S,4R) were prepared by an identical route starting with D-aspartic acid. The specific rotations for the four stereoisomers are listed in Table 1.

Scheme I. Stereospecific Synthesis of 3-alkyl-4-phenoxy-2-azetidinones.

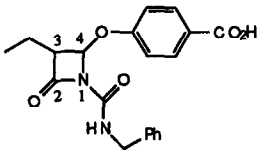


Reagents: (a) See ref. 10; (b) 2 eq. LDA/ THF, ethyl iodide (73%); (c) $Pb(OAc)_4$/ HOAc; (d) HCl/ H_2O/ THF; (e) NaOH/ H_2O/ acetone (30-35% for 3 steps); (f) Benzyl isocyanate/ Et_3N/ DMAP/ CH_2Cl_2 (100%); (g) CF_3CO_2H/ anisole (80%).

The HLE inhibitory activity of each isomer was measured by following the hydrolysis of the substrate Suc-Ala-Ala-Pro-Ala-pNA for 15 min and is also reported in Table 1. For compounds exhibiting time-dependent

inhibition, a second order rate constant, $k_{obs}/[I]$, was calculated. A K_i value was determined from the initial velocity for **8a** because it did not show time-dependent inhibition.[12] The *trans* (3R ,4R) isomer **7a** was the most potent of the four and it was 10 times more active than its enantiomer **7b**. In the (3S) series both the *cis* and *trans* isomers **8b** and **7b** were equipotent and time-dependent. The *cis* isomer of **7a**, **8a** appeared to be only a competitive inhibitor.

Table 1. Elastase Inhibitory Activity of 3-Ethyl-2-azetidinones.



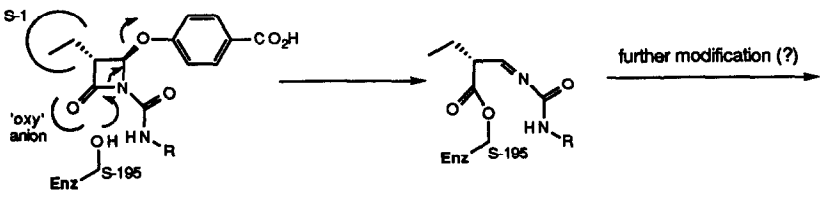
Compound	Stereochemistry		$[\alpha]_D$[a]	k_{obs}/I[b] $M^{-1}sec^{-1}$ (SD)
1	Trans		Racemic	2480 (100)
7a	Trans	3R,4R	+ 43.4 °	3842 (71)
8a	Cis	3R,4S	- 37.7 °	K_i = 5.6 (0.2) μM[c]
7b	Trans	3S,4S	- 42.9 °	424 (105)
8b	Cis	3S,4R	+ 37.3 °	383 (154)

[a] $[\alpha]_D$ for an approximate concentration of 0.25 g/ 100 mL of $CHCl_3$ at room temperature.

[b] See ref. 12 for methodology. [c] Time-dependent inhibition was not observed.

These results are consistent with our previously discussed cephalosporin model[4] (Figure 1) in which the compound is bound in the active site of HLE with the hydroxyl of serine 195 (Ser-195) interacting with the β-lactam carbonyl, the oxygen pointing towards the 'oxy' anion hole and the 3R ethyl group occupying the S-1 specificity pocket. Opening of the β-lactam ring by the hydroxyl group of Ser-195 followed by the loss of the phenol leaving group, results in an acyl-enzyme intermediate. Further reaction such as an addition of a nucleophile to the resultant imine, may provide additional stabilization to the initially formed acyl-enzyme. The poorer activities for the 3S isomers, **7b** and **8b**, may result from a lack of interaction with the S-1 binding pocket. The (3R,4S) isomer **8a** may be less active because the phenol in the 4S configuration interferes in the initial binding with HLE, and it prevents formation of a stable acyl-enzyme.

Figure 1. Mechanism of Inhibition



Since the HLE activity of **7a** was comparable to **L-658,758** ($k_{obs}/[I]$ = 3800 $M^{-1}sec^{-1}$), a cephalosporin sulfone selected for development as an aerosol,[4] the stability of **7a** was studied in more detail. It was not only stable in buffer at pH 7.4, (half-life = 330 ± 80 h *vs* 48 ± 3 h for **L-658,758**), but also in the presence of an additional nucleophile, 7-aminocaproic acid (0.5 M at pH 7.4), the half-life of degradation for **7a** was 48 ± 3 h compared to 1.5 ± 0.1 h for **L-658,758**. On incubation in dog or marmoset blood overnight at 25 °C, **7a** could still be detected. Even more important was the observation that **7a** was orally absorbed. Thus, after an oral dose of 10 mg/kg of **7a** to two marmosets, 5 μg/mL of **7a** was found in the blood after 20 min. However, it disappeared rapidly and no drug could be detected after 100 min. These results validated our hypothesis that increased stability can lead to oral absorption, and also suggested that further modification of **7a** might enhance the *in vivo* stability. In this endeavor, the observation that **8b**, with a 3S stereochemistry, was in fact a time-dependent inhibitor of HLE, prompted us to prepare 3,3-dialkyl compounds, which lead to more potent, orally efficacious compounds.[13] One compound of this class was selected for extensive evaluation and its activity in a number of biological systems will be reported in the very near future.[14]

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