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INHIBITION OF HUMAN LEUKOCYTE ELASTASE (HLE) BY NOVEL BICYCLIC β-LACTAMS

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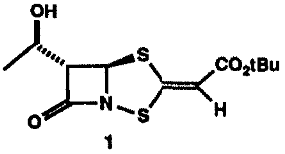
Abstract: Novel {3,2,0} bicyclic β-lactam sulfenamides and selected carbon isosteres are inhibitors of human leukocyte elastase. The comparative biological activity and hydrolytic stability of these compounds are described.

Recently we described the synthesis of a series of novel sulfenamide-containing β-lactams **1** and **2** which are potent inhibitors of human leukocyte elastase (HLE)[1]. The success of β-lactam antibacterials in inhibiting serine transpeptidases involved in bacterial cell wall biosynthesis prompted our evaluation of novel β-lactams **1** and **2** as inhibitors of HLE, a serine protease which has been implicated in the pathogenesis of degenerative disease states including pulmonary emphysema[2], rheumatoid arthritis[3], chronic bronchitis[4], cystic fibrosis[5], and respiratory distress syndrome[6]. Inhibition of HLE by β-lactams has precedent. Selected cephalosporin esters and amides, for example, function as potent irreversible HLE inhibitors [$K_i(M)=10^{-7}$; k_2/k_i or $k_{on}=161{,}000 M^{-1}s^{-1}$][7].



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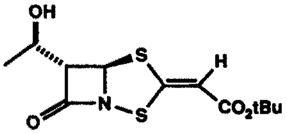

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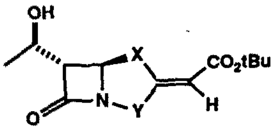
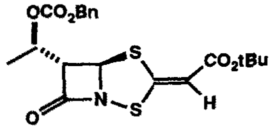
β-Lactam mediated elastase inactivation results from lactam ring cleavage by the active site serine. Given the biological activity and chemical reactivity of strained bicyclic β-lactams **1** and **2**, we initiated a synthetic program to evaluate isosteric changes in the bicyclic lactam framework of **1** and modification of the molecule's periphery. Our intent was to optimize elastase inhibition and at the same time reduce the susceptibility of the β-lactam moiety to hydrolysis. Herein, we report results of these efforts including the preparation of novel bicyclic β-lactams **3-5** along with β-lactam sulfenamides **6** and **7**. In addition, we describe a comparison of the hydrolytic stability and biological activity of these compounds which for comparative purposes were all prepared as their t-butyl esters.



3 X = C ; Y = S
4 X = C ; Y = C
5 X = S ; Y = C



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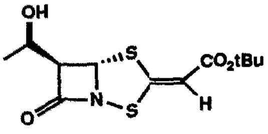

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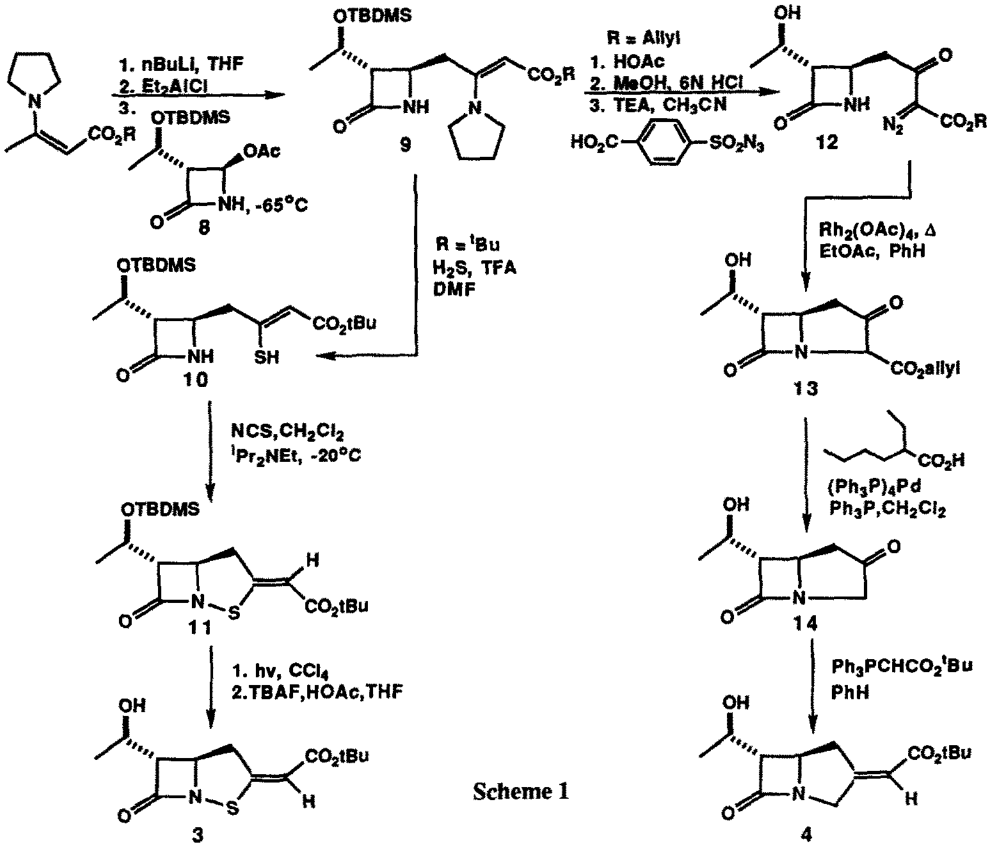
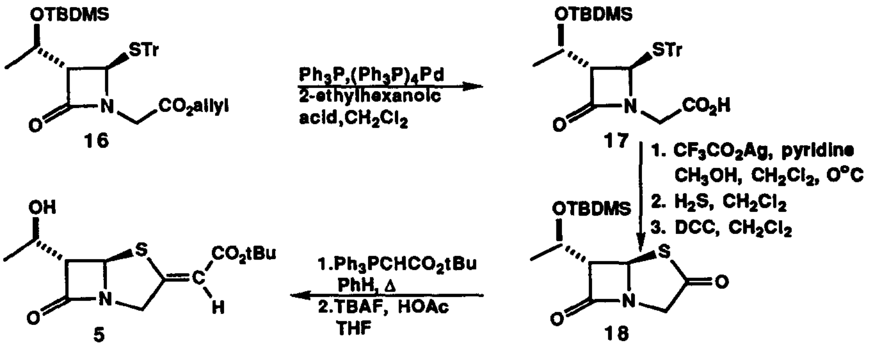
Enamino ester **9** served as a convenient synthon for the preparation of sulfenamide **3** and carbapenam **4** (Scheme 1) and was prepared by the known condensation of acetoxyazetidinone **8**[8] and tert-butyl 3-pyrrolidinocrotonate[9]. To access sulfenamide **3**, **9** (R=^{t}Bu) was converted to β-thioxo ester **10** (35%) (H_2S/DMF/TFA/0°C/10 min)[10]. Conditions (NCS/^{i}Pr_2EtN/CH_2Cl_2/-20°C)[1] developed for the preparation of **1** and **2**, provided bicyclic β-lactam sulfenamide **11** in 50% yield (accompanied by 5% of the other isomeric α,β-unsaturated ester)[11]. Olefin isomerization (hv,CCl_4) of **11** (35%) followed by t-butyldimethylsilyl ether cleavage (3 equiv 1M TBAF/THF/20 equiv HOAc/48 h) generated sulfenamide **3** in 88% yield.



Scheme 1

To access carbapenam **4**, enamino ester **9**[9] (R=allyl) was converted to carbapenam **13**. Accordingly, concomitant enamine hydrolysis/ t-butyldimethylsilyl ether cleavage (MeOH, 6N HCl) followed by diazo transfer (TEA, p-carboxybenzenesulfonyl azide, CH_3CN) afforded **12**[12] which was treated with rhodium acetate (PhH/EtOAc) to generate carbapenam allyl ester **13**. $(Ph_3P)_4Pd$ mediated ester exchange (2 equiv 2-ethylhexanoic acid/ CH_2Cl_2)[13] followed by decarboxylation of the resultant β-ketoacid yielded desired ketone **14**[14]. Wittig condensation of **14** with ($Ph_3PCHCO_2{}^tBu$/PhH) generated carbapenam **4** as a 1:1 mixture (38%) of isomers which could be separated by silica gel chromatography.

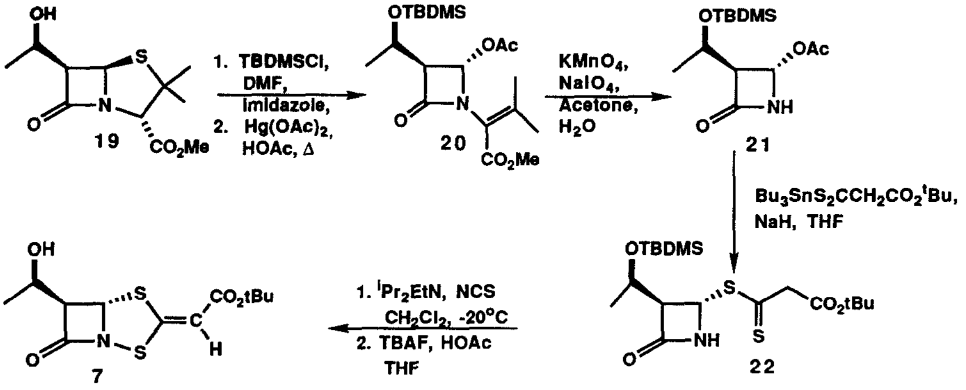
An analogous Wittig-based strategy involving thiolactone **18** was designed to generate penam **5** (Scheme 2). S-Tritylazetidinone **16**[15], available in a two step sequence from acetoxyazetidinone **8**, was allowed to react with $(Ph_3P)_4Pd$(1 equiv 2-ethylhexanoic acid/CH_2Cl_2/Ph_3P) to produce azetidinone acid **17** (64%). Removal of the S-trityl protecting group (CF_3CO_2Ag/pyridine/CH_3OH/CH_2Cl_2;then H_2S gas) yielded the corresponding thiol-acid which was treated with DCC to afford the desired thiolactone **18** (36%). Wittig condensation ($Ph_3PCHCO_2{}^tBu$/PhH) of **18** followed by t-butyldimethylsilyl ether cleavage utilizing standard conditions provided **5** as a 1:1 mixture (33%) of isomeric olefins. These isomers were separated by silica gel chromatography.



Scheme 2

To shed additional light on the key structural features responsible for HLE inhibition within the β-lactam series, β-lactam sulfenamides **6** and **7** were targeted. The first target, carbonate **6**, was selected to probe the effect of increased steric congestion on the alpha face of the β-lactam ring and was generated by treatment of **1** with benzylchloroformate (DMAP/CH_2Cl_2).

Sulfenamide **7** was selected to assess the importance of the absolute stereochemistry of the bicyclic nucleus to HLE inhibition. To generate **7**, penicillin-derived alcohol **19**[16] was protected (TBDMSCl/imidazole/DMF) and treated with mercuric acetate (HOAc) to give azetidinone **20** which could be converted in high yield to acetoxyazetidinone **21** upon standard olefin oxidation/oxalamide hydrolysis ($KMnO_4$/$NaIO_4$/acetone/H_2O) conditions[17]. The three step procedure[1] employed for the preparation of **1** was utilized for the conversion of **21** to sulfenamide **7** (Scheme 3).



Scheme 3

In order to evaluate the possible correlation of β-lactam reactivity with elastase inhibition, the following experiments were performed. Alkaline hydrolysis rates were measured on compounds **1** - **7** by dissolving the compounds in acetonitrile to a concentration of 1 mM and dilution into a cuvette containing 0.1 N NaOH to a final concentration of 10 μM. Hydrolysis of the β-lactam ring was monitored by measuring the absorbance spectrum of each compound before and after hydroxide addition and choosing the optimum wavelength to follow ring cleavage. Inhibition of human leukocyte elastase by compounds **1** - **7** was measured as described in Copp *et al.*[18,19]. Compounds which showed inhibition were found to be time-dependent inhibitors[20,21] and were fit to the equation y=Ae-kt + B + Ct by non linear least squares regression[18,22], in which the steady state (final) rate equals C, the initial rate is -Ak + C, and the observed rate constant is k.

Human Leukocytes Elastase Inhibition
Examination of Carbon Isosteres of Sulfenamide 1

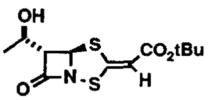
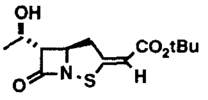
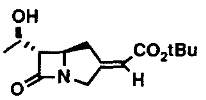
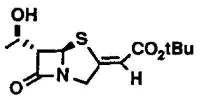
				
Compound	1	3	4	5
IR (β-Lactam) cm^{-1} (CHCl3)	1802	1782	1755	1781
Alkaline Hydrolysis k$_{OH^-}$ (M^{-1}sec^{-1})	12.0±0.6	20±1	—	0.08
K$_i$ (μM)	1.5±0.3	3.2±0.2	>100	4±1
k$_{on}$ (M^{-1}sec^{-1})	1000	130	—	170
k$_{off}$ (sec^{-1}) x 10^{-3}	1.4	0.4	—	0.67

Table 1

As shown in Table 1, replacement of either of the sulfur atoms of **1** (β-lactams **3** and **5**) does not result in significant erosion of elastase inhibition. However, carbon atom replacement of both sulfur atoms in the form of carbapenam **4**, destroys biological activity (HLE inhibition: K_i (μM) > 100).

β-Lactam reactivity (as measured by alkaline hydrolysis rate)[23] does not necessarily predict the rate of elastase inhibition as is exemplified by comparison of compounds **1** and **5** (Table 1), which shows that hydrolysis of the β-lactam ring is much slower for **5** than **1** (k_{OH^-} ($M^{-1}sec^{-1}$) of 0.08 vs. 12) while HLE inhibition is comparable. Thus, increasing β-lactam ring reactivity does not translate to greater potency (presumably by increasing k_{on}), suggesting that the rate determining step in elastase inhibition is not nucleophilic attack on the β-lactam carbonyl group.

Human Leukocytes Elastase Inhibition
Probing the Effects of Substitution and Ring Stereochemistry

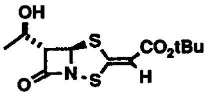
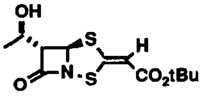
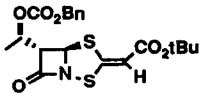
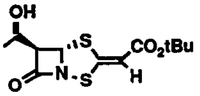
				
Compound	**1**	**2**	**6**	**7**
IR (β-Lactam) cm^{-1} ($CHCl_3$)	1802	1796	1806	1800
Alkaline Hydrolysis k_{OH^-} ($M^{-1}sec^{-1}$)	12.0±0.6	3.0±0.2	0.04±0.01	10.3±0.4
K_i (μM)	1.5±0.3	12±2	0.4±0.1	>100
k_{on} ($M^{-1}sec^{-1}$)	1000	150	970	—
k_{off} (sec^{-1}) x 10^{-3}	1.4	2.0	0.4	—

Table 2

As anticipated, the absolute stereochemistry of the bicyclic sulfenamide β-lactam framework is a key determinant for elastase inhibition (cf. K_i (μM) of 1.5 vs. >100 for **1** vs. **7** (Table 2)). Steric congestion on the alpha face of the β-lactam ring, in the form of carbonate **6**, increases elastase inhibition (cf. K_i (μM) of 1.5 vs. 0.4 for **1** vs. **6**) while reducing the susceptibility of the β-lactam ring to base hydrolysis (cf. k_{OH^-} ($M^{-1}sec^{-1}$) of 12 vs. 0.04 for **1** vs. **6**). The structural flexibility these molecules offer for fine tuning biological activity underscores their promise for the design of potent HLE (serine protease) inhibitors.

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