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Inhibition of Human Leukocyte Elastase. 6.[1] Inhibition by 6-Substituted Penicillin Esters.

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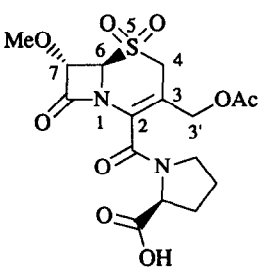
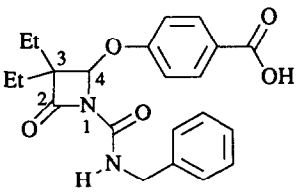
Abstract. Penicillin esters substituted at C-6 with a variety of functionalities are reported as human leukocyte elastase (HLE) inhibitors. The structure activity relations for the esters and C-6 derivatives are discussed and compared to the known cephalosporin structures.

Our current search for inhibitors of human leukocyte elastase (HLE) (EC 3.4.21.37), a serine protease associated with several pulmonary diseases,[2] has focused on the use of the β-lactam moiety to achieve potent, time-dependent, functionally irreversible inhibition. The use of cephalosporins was extensively investigated[2-6] and concluded with the selection of **L-658,758** (**1**) as a clinical candidate for development as a topical aerosol.[7] Subsequently, a study to use monocyclic β-lactams for topical administration was reported[8] and this work has now been extended to structures such as **2**[9] which have oral activity. In the preceding paper,[1] the use of penem benzyl esters **3** was disclosed and herein the penicillin structure **4** is discussed.[10] The structure activity relations (SAR) for the C-2 carboxyl and C-6 position were examined in detail and several similarities and distinctions were identified as compared to the cephalosporins. With the historically good oral bioavailability of the penicillin antibiotics compared to the cephalosporins, it was important for us to investigate as wide a variety of penicillin derivatives as possible.

Our synthetic strategy centered on the use of 6-aminopenicillanic acid (6-APA) (**4**), which also provided a chiral framework. Initially, the SAR was developed along the lines of the previous cephalosporin work for the C-2 esters and the C-6 position and then the C-6 SAR was expanded primarily utilizing the more active benzyl ester. These findings were finally applied to other C-2 carboxy derivatives as they were discovered in the cephalosporin series of HLE inhibitors as discussed in the following paper.[11]



L-658,758, 1



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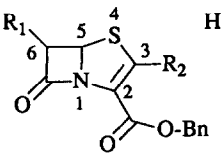

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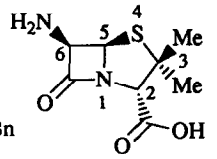

6-APA, 4

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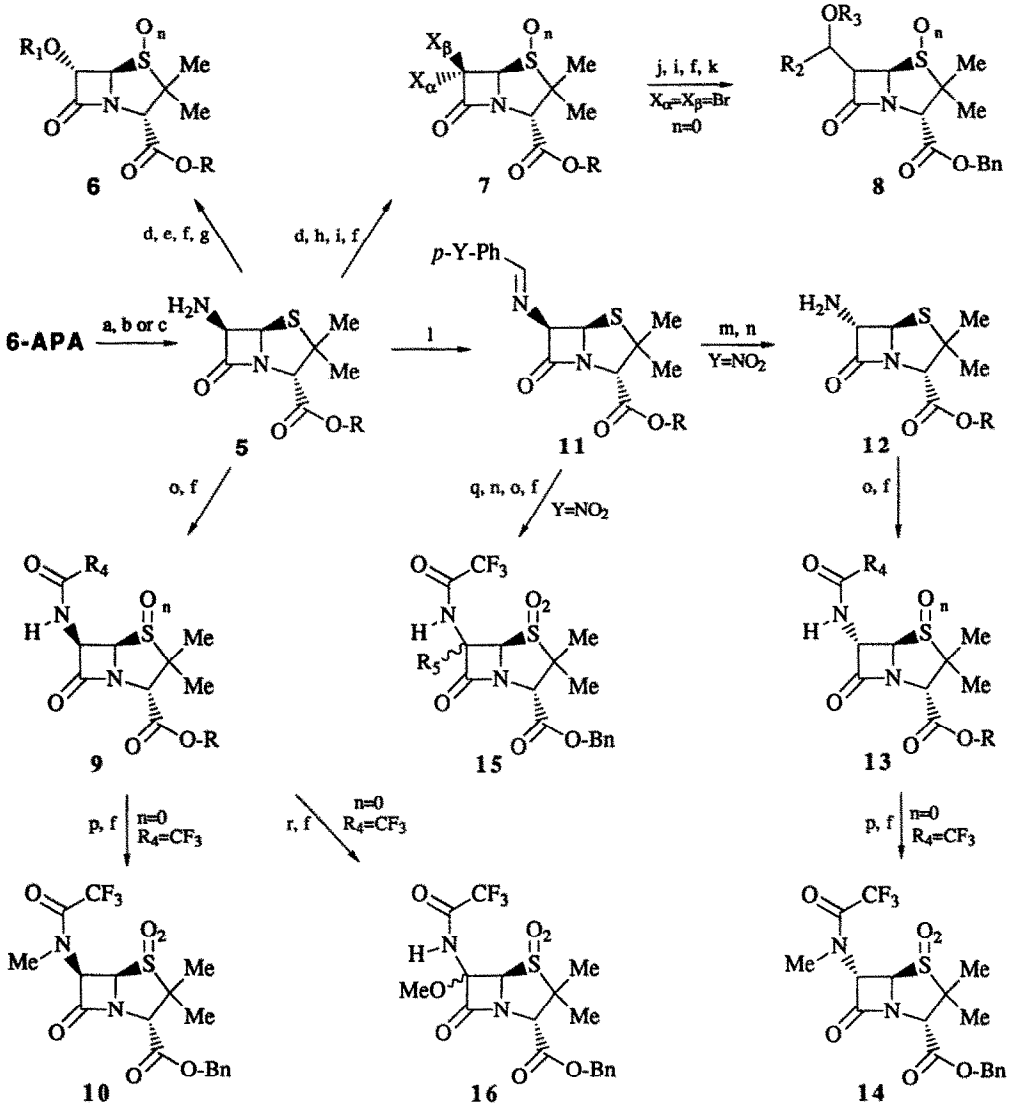
Scheme I



Reagents. (a) BnBr, Et_3N, acetone; (b) CH_2N_2, MeOH; (c) *t*-Butyl isourea, CH_2Cl_2; (d) $NaNO_2$, TsOH, CH_2Cl_2/H_2O, O °C; (e) TsOH, R_1OH or $HClO_4$, H_2O/acetone; (f) 1 or 2.2 eq. *m*-CPBA, CH_2Cl_2; (g) MeCOCl, $PhCH_2COCl$ or $PhOCH_2COCl$, pyridine, CH_2Cl_2, 0 °C; (h) HCl, HBr or Br_2, THF/H_2O; (i) Zn, Et_2O, NH_4OAc/H_2O; (j) MeMgBr, THF, -70 °C, then HCHO or MeCHO; (k) BnNCO, DMAP, CH_2Cl_2; (l) *p*-$NO_2C_6H_4CHO$, MePh, 3Å sieves; (m) Et_3N, $CHCl_3$; (n) Girard T, MeOH; (o) TFAA or Ac_2O, pyridine, CH_2Cl_2; (p) Me_2SO_4, K_2CO_3, acetone; (q) PhLi, THF, 0 °C, then MeI or EtI; (r) MeOLi, *t*-BuOCl, THF, -70 °C.

Chemistry. A generalized synthetic approach to a variety of C-6 derivatives is shown in Scheme I.[12] The readily available benzyl ester of 6-APA (**5**, R = Bn)[13] was the primary starting material since the methyl ester (**5**, R = Me, was prepared from 6-APA with diazomethane in 6% yield) and the *t*-butyl ester (**5**, R = *t*-Bu, was prepared from 6-APA with *t*-butyl isourea in 60% yield) derivatives demonstrated considerably poorer inhibitory activity (see below). Diazotization of **5** with sodium nitrite[13] followed by tosic acid catalyzed decomposition in the presence of an alcohol afforded the corresponding ethers (**6**, R_1 = Me, Et, Bn; n = 0). Oxidation with 1 or 2 equivalents of *m*-chloroperbenzoic acid (*m*-CPBA) selectively afforded the sulfoxides (**6**, n = 1) or the sulfones (**6**, n = 2). Attempted preparation of the phenyl ether **6** (R_1 = Ph) failed to give the desired product but did afford the very potent tosylate **6** (R_1 = Ts). Reaction of the 6-diazo penam with perchloric acid in aqueous acetone gave the 6-hydroxy derivative **6** (R_1 = H),[13] which could be acylated to give esters (**6**, R_1 = MeCO, PhCO, $PhCH_2CO$). The diazo intermediate could also be reacted with hydrochloric or hydrobromic acid to yield the monohalides (**7**, X_α = Cl, Br; R_β = H).[13] Dehalogenation of the monobromide with zinc afforded the unsubstituted compound **7** ($X_\alpha = X_\beta$ = H). Reaction of the diazo intermediate with bromine also afforded the dibromide **7** ($X_\alpha = X_\beta$ = Br; n = 0). From this the bromo-enolate could be produced by metal-halogen exchange with methylmagnesium bromide and reacted with formaldehyde or acetaldehyde to give the hydroxyalkyl derivatives (**7**, X_α = $HOCH_2$, (R)- and (S)-MeCHOH; X_β = Br; n = 0). Zinc debromination and oxidation afforded a separable mixture of α and β hydroxyalkyls (**8**, R_2 = H, (R)- and (S)-Me; n = 2).[14] Acylation with benzyl isocyanate gave the carbamates (**8**, R_2 = H, (R)- and (S)-Me; R_3 = CONHBn; n = 2).

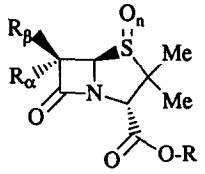
While the inhibitory activity of the cephalosporin nucleus was limited to small groups at C-7 (Cl > MeO > Et),[4] the SAR of the penicillin structure was much more flexible and introduction of larger groups was possible. In particular, the trifluoroacetamide was found to be a very potent pharmacophore, for which some β-derivatives even had reasonable activity. Acylation and oxidation of the 6-APA esters **5** afforded the β amides (**9**, R_4 = CF_3, Me, H; n = 2). Further alkylation of the trifluoroacetamide sulfide **9** (R_4 = CF_3; n = 0) to the tertiary amide was achieved with potassium carbonate and dimethyl sulfate in acetone. Oxidation with *m*-CPBA as usual led to the corresponding sulfone **10**. Epimerization of the 6-β-amino to the 6-α-amino was accomplished[15] *via* treatment of the *p*-nitrobenzyl imine **11** (Y = NO_2) with triethylamine. Separation of the epimeric mixture and regeneration of the amine with Girard T afforded the pure 6-*epi*-APA esters **12** and acylation as above gave the amides (**13**, R_4 = CF_3, Me, H; n = 0). However, subsequent oxidation of these amide derivatives failed to give any isolable product under these conditions with anything other than the trifluoroacetamide **13** (R_4 = CF_3). Alkylation and oxidation as above produced the tertiary amide **14**. The disubstituted ether-amide **16** was available from the trifluoroacetamide sulfide **9** (R_4 = CF_3; n = 0) *via* nitrogen chlorination/elimination with *t*-butyl hypochlorite and lithium methoxide.[16] Subsequent oxidation gave a separable mixture of the two diastereomers **16**. The alkyl-amide disubstitution could also be achieved by deprotonation of the imine **11** (Y = NO_2) with phenyl lithium and quenching with methyl or ethyl iodide.[15,17] Hydrolysis of the imine, acylation and oxidation as above gave a separable mixture of the disubstituted products **15** (R_5 = Me, Et; n = 2). Finally, the trifluoroethyl esters **6** (R = CH_2CF_3; R_1 = Me; n = 2) and **13** (R = CH_2CF_3; R_4 = CF_3; n = 2) were prepared and showed very interesting activity (see below). The initial trifluoroethyl 6-APA was prepared by protection of the 6-amino as an imine (**11**, Y = H, R = H) with benzaldehyde and esterification with DCC and trifluoroethanol. Hydrolysis of the imine with Girard T gave the trifluoroethyl ester **5** (R = CH_2CF_3) which was carried on as above.

Biological Results and Discussion. The activity of a limited number of the more potent penicillin derivatives and a few other selected compounds is given in Table 1. The HLE inhibitory activities were measured against Suc-Ala-Ala-Pro-Ala-*p*-NA hydrolysis[3] and reported as a nominal 2 minute IC_{50} value (μM), which is the concentration of inhibitor required to obtain a 50% reduction in the rate of hydrolysis of the peptide. Since some compounds appeared to be time-dependent on longer incubation, this value is only an estimate of the initial K_i value. A more accurate and informative second-order rate constant ($k_{obs}/[I]$, $M^{-1}sec^{-1}$) was determined for the latter amide series discussed in the following paper.[11]

From our previous work in the cephalosporin area, it was known that the 7-substituent occupies the S-1 specificity pocket of HLE and that small groups, such as chloro, ethyl and methoxy, were required while the unsubstituted compound was only a weakly competitive substrate. It became apparent early on that the penicillin nucleus SAR at C-6 was much less restrictive and a variety of larger groups could be accommodated, especially the trifluoroacetamide. This finding is in fact more reasonable in view of the preference for valine over alanine as the P-1 residue in peptide substrates.[18] Again, the sulfone was the most potent oxidation state for the sulfur, as indicated by the representative 6-acetoxy benzyl ester series **6g-i** (R = Bn; R_1 = Ac; n = 0, 1, 2) (IC_{50}=10, 5 and 0.2 μM, respectively). This is consistent with the proposed mechanism of action of β-lactamases by penicillin sulfone inhibitors[19] and suggests reaction of serine 195 of HLE with the β-lactam carbonyl, opening of the β-lactam to give an acyl enzyme intermediate and cleavage of the thiazolidine ring at the S-4/C-5 ring junction. For the 6α-methoxy sulfone esters, as well as all other cases, the benzyl ester **6c** was the most active, while the methyl ester **6a** had weak activity and the *t*-butyl ester **6b** was inactive (IC_{50} = 5, 20 and >20 μM, respectively). Interestingly, the trifluoroethyl esters with the 6α-methoxy (**6d**) and both 6α- and 6β-trifluoroacetamides (**9c** and **13c**) derivatives were equipotent to the benzyl esters **6c**, **9b** and **13b** (IC_{50} = 4 *vs* 5, 3 *vs* 2 and 0.1 *vs* 0.15 μM, respectively). However, since the benzyl ester was also the most readily available, most of the 6-substituent SAR was determined with this ester.

The unsubstituted (**7**, $X_\alpha = X_\beta$ = H), α-hydroxy (**7**, X_α = HO; X_β = H), α-bromo (**7**, X_α = Br; X_β = H) and dibromo (**7**, $X_\alpha = X_\beta$ = Br) sulfone benzyl esters were all inactive, (IC_{50} > 20 μM) while the 6α-chloro derivative **7a**, the most potent 7α substituent for the cephalosporins,[4] was only weakly active (IC_{50} = 14 μM). In the ether series, methoxy **6a** was less active than ethoxy **6e** (IC_{50} = 5 *vs* 2 μM) and the acetoxy **6i** was now found to be very active (IC_{50} = 0.2 μM). Unlike with the cephalosporins,[4] the benzyloxy derivative **6f** now had marginal activity (IC_{50} = 4 μM) but the benzoate was still inactive. As observed with the cephalosporins,[4] the larger phenyl- and phenoxyacetates **6j** and **6k** had some activity (IC_{50} = 4 and 5 μM, respectively). To follow up on these activities, some oxyalkyl derivatives were prepared, in particular the (R)-hydroxyethyl which is prominent in the penem and carbapenem antibiotics. While the alcohols were only marginally active (for example, **8a** was only 20 μM), some of the benzyl carbamate derivatives were quite active inhibitors and showed some profound stereochemical preferences. As expected, with the (R)-hydroxyethyls **8b** and **8c**, the 6α configuration was more potent than the 6β (IC_{50} = 0.7 *vs* 10 μM). However, for the (S)-hydroxyethyls **8d** and **8e** and the hydroxymethyls **8f** and **8g**, the observed activity was actually reversed with the 6β configuration being more potent (**8d** and **8e**, IC_{50} = 7 and 0.3 μM; **8f** and **8g**, IC_{50} = 4 and 0.7 μM). **8e** and **8g** were the most potent of the penicillins with a β configuration. Surprisingly, the tosylate by-product **6l** was the most potent group found for the 6α position, attributable at least in part to activation of the β-lactam ring since the analogous benzoate was inactive.

Table 1. Activity of selected penicillin ester derivative.



Compound no.	Rα	Rβ	n	R	IC50, μM[a]
6a	MeO-	H-	2	-Me	20
6b	MeO-	H-	2	-*t*-Bu	>20
6c	MeO-	H-	2	-Bn	5
6d	MeO-	H-	2	$-CH_2CF_3$	4
6e	EtO-	H-	2	-Bn-	2
6f	BnO-	H-	2	-Bn-	4
6g	AcO-	H-	0	-Bn	10
6h	AcO-	H-	1-(α)[b]	-Bn	5
6i	AcO-	H-	2	-Bn	0.2
6j	$PhCH_2CO_2$-	H-	2	-Bn	4
6k	$PhOCH_2CO_2$-	H-	2	-Bn	5
6l	TsO-	H-	2	-Bn	0.05
7a	Cl-	H-	2	-Bn	14
8a	(R)-HOCH(Me)-	H-	2	-Bn	20
8b	(R)-$BnNHCO_2CH$(Me)-	H-	2	-Bn	0.7
8c	H-	(R)-$BnNHCO_2CH$(Me)-	2	-Bn	10
8d	(S)-$BnNHCO_2CH$(Me)-	H-	2	-Bn	7
8e	H-	(S)-$BnNHCO_2CH$(Me)-	2	-Bn	0.3
8f	$BnNHCO_2CH_2$-	H-	2	-Bn	4
8g	H-	$BnNHCO_2CH_2$-	2	-Bn	0.7
9a	H-	CF_3CONH-	0	-Bn	12
9b	H-	CF_3CONH-	2	-Bn	2
9c	H-	CF_3CONH-	2	$-CH_2CF_3$	3
10	H-	CF_3CONMe-	2	-Bn	0.7
13a	CF_3CONH-	H-	0	-Bn	4
13b	CF_3CONH-	H-	2	-Bn	0.15
13c	CF_3CONH-	H-	2	$-CH_2CF_3$	0.1
14	CF_3CONMe-	H-	2	-Bn	0.2
15a	Me-	CF_3CONH-	2	-Bn	>20
15b	Et-	CF_3CONH-	2	-Bn	3
16a	CF_3CON-	MeO-	2	-Bn	0.8
16b	MeO-	CF_3CONH-	2	-Bn	9

[a]See ref. 3 for methodology. The values are a result of a single experiment and the deviation on repetition was less than a factor of 2. [b]This was the major sulfoxide isomer. The minor β-sulfoxide was not isolated in this case, others were less active or inactive.[11]

Consistent with the acetate series above, the 6α-trifluoroacetamides **13a** and **13b**, as well as all other series tested (data not shown), showed the same order of activity for the sulfur oxidation states and **13b** was equipotent with the acetate **6i** (IC_{50}=0.15 and 0.2 μM). Since the acetamides would be expected to have much better pharmacokinetics *in vivo*, this group was the most extensively investigated later.[11] This group was also unique in that the other α-amides could not be isolated when oxidation to the sulfone was attempted with *m*-CPBA. Although less active than the α analogues **13a** and **13b** and unexpected from previous work, the 6β-trifluoroacetamide derivatives **9a** and **9b** had moderate activity (IC_{50} = 12 and 2 μM). The trifluoroacetamide **9b** was again unique in that the 6β-acetamide **9** (R_4 = Me, n=2) and formamide **9** (R_4 = H, n=2) were both

inactive. On further alkylation, the tertiary amides **10** and **14** maintained their potencies. C-6 disubstitution, as with the 6-α-alkyl derivatives **15a** and **15b**, resulted in loss of activity for methyl and unchanged activity for the ethyl analogue. Although having diminished inhibitory activity compared to **13b**, the fact that the trifluoroacetamido binds best when in the α configuration is again seen with the methoxy disubstitution analogues, the β-methoxy **16a** being 10-fold more potent than the α-methoxy **16b** (IC_{50} = 0.8 and 9 μM).

Conclusion. The HLE inhibitory utility of the penicillin nucleus has been demonstrated and the SAR for the C-6 position was shown to be much broader than for the corresponding C-7 position of the cephalosporins. This finding offers the possibility of β and / or disubstitution on a β-lactam moiety and thus the flexibility of the β-lactam framework might be extended. This conclusion was further investigated with the penicillin structure[11] and has since been validated in the monocyclic series with the synthesis of 3,3-dialkyl derivatives such as **2** which are highly potent, orally active HLE inhibitors.[9,20]

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