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SYNTHESIS AND PORCINE PANCREATIC ELASTASE INHIBITORY EVALUATION OF 6α-(SULFONYL)OXY- AND 6α-CHLOROPENICILLANATE SULFONE ESTERS AND 3α-(ACYLOXY)METHYL-6α-CHLOROPENAM SULFONES

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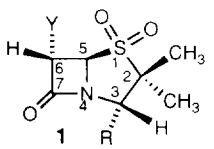
Abstract: The synthesis of 6α-chloropenicillanate sulfone esters **4a-c**, **9**, the acetate and benzoate of 3α-hydroxymethyl-6α-chloropenam sulfones **6a-b** and pivaloyloxymethyl and benzyl esters of several 6α-(sulfonyl)oxypenicillanate sulfones **12**, **15a1-a3**, **15b1-b3** are reported. When tested as inhibitors of porcine pancreatic elastase, the acetate of 3α-hydroxymethylpenam **6a** proved to be more active in comparison with the esters of 3α-carboxylic acid counterparts **4a-c** and **9**. Compounds with diverse 6α-(sulfonyl)oxy substituents showed elastase inhibitory activity improved over the corresponding 6α-chloro derivatives **4a-c** and **9**; among those, compounds **15a2** and **15b2** were rather unstable, but compounds **15a1**, **15a3**, **15b1**, **15b3** combined fair activity with better stability.

Human leukocyte elastase (HLE, EC 3.4.21.37) is a serine protease found in the azurophilic granules of polymorphonuclear leukocytes.[1] This enzyme has been the subject of extensive studies, both in terms of its biological role in numerous diseases [2] and in terms of the development of suitable therapeutic inhibitors to supplement the body's elastase inhibitory capacity and thereby shift the proposed proteinase/antiproteinase imbalance in pathogenic conditions.[1,3] The presence of a reactive catalytic-site hydroxyl group affords the opportunity for the development of inhibitors which will form a covalent adduct with the enzyme and thereby interfere with the mechanism of catalysis (i.e., mechanism-based inhibitors). This interest has led, over the last fifteen years, to the synthesis of a wide variety of inhibitors based on the β-lactam nucleus.[3]

We recently reported the synthesis of 6α-chloro-2,2-dimethyl-3α-(pivaloyloxy)methylpenam sulfone and 6α-chloro-2,2-dimethyl-3-*exo*-methylenepenam sulfone, as well as several benzyl and methyl 6α-substituted penicillanate sulfones.[4] These new penicillin derivatives were evaluated as elastase inhibitors using, as a model, porcine pancreatic elastase (PPE, EC. 3.4.21.36), an enzyme related to HLE.[5] We now report the synthesis and activity against PPE of penicillin ester sulfones **1** (R = CO_2Pom, CO_2Bn, $CO_2{}^i$Pr, $CO_2{}^t$Bu, CH_2OCOCH_3 and CH_2OCOPh) substituted at position 6 with a variety of α-oriented functionalities (Y = Cl, FSO_3-, F_3CSO_3-, H_3CSO_3-, and *p*-H_3C-C_6H_4-SO_3-).



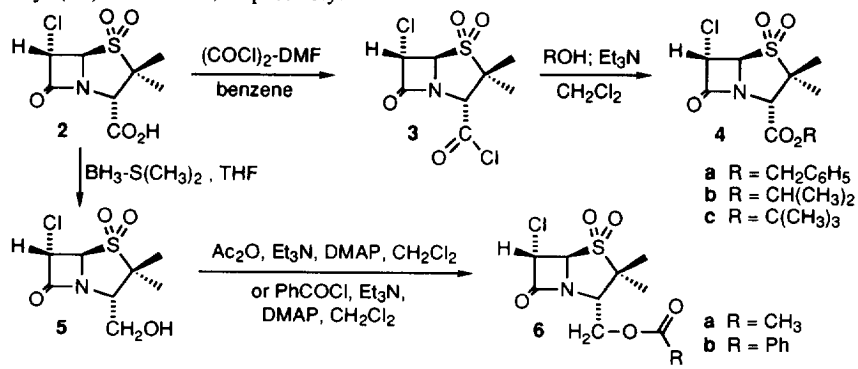
1

Chemistry[6]

Synthesis of penicillin ester sulfones. The synthesis of the benzyl, *iso*-propyl and *tert*-butyl 6α-chloropenicillanates sulfones **4a-c**, 6α-chloro-2,2-dimethyl-3α-(acetyl)oxymethyl-(**6a**), and 3α-(benzoyl)oxymethylpenam sulfones (**6b**) is shown in Scheme 1. The starting material was 6α-chloro-

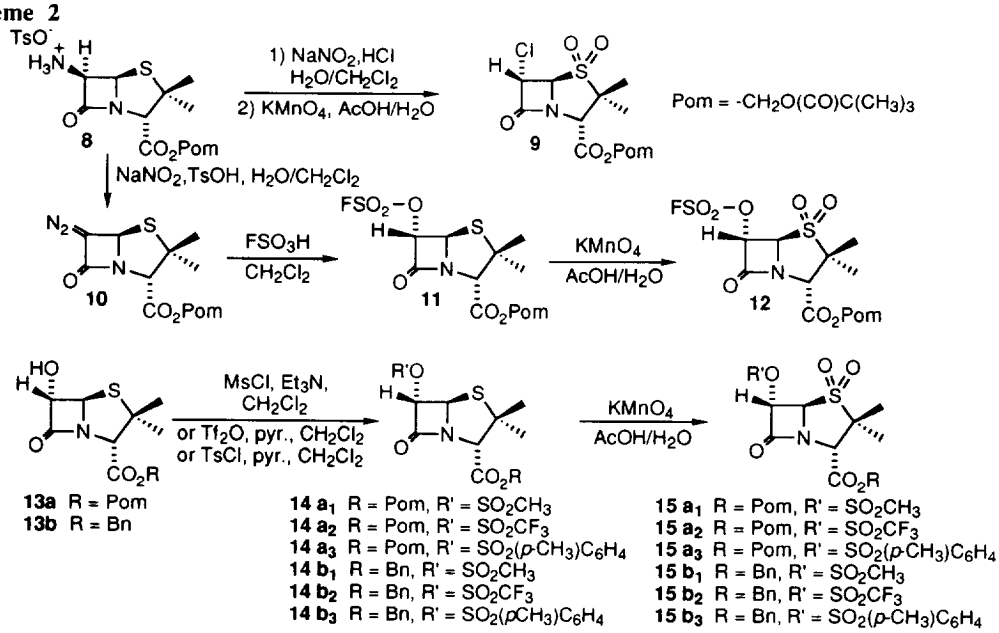
penicillanic acid sulfone (**2**).[7] Conversion of **2** into the 6α-chloro-2,2-dimethyl-3α-chlorocarbonylpenam sulfone (**3**) in 95% isolated yield was accomplished by oxalyl chloride and dimethylformamide[8] in benzene at room temperature. Subsequent treatment of **3** with the appropriate alcohol (benzyl, *iso*-propyl and *tert*-butyl) afforded the esters **4a-c**. Alternatively, reduction of **2** with borane-methyl sulfide complex[9] afforded the alcohol **5** which was then treated with acetic anhydride or benzoyl chloride to give the corresponding acetyl (**6a**) and benzoyl (**6b**) derivatives, respectively.

Scheme 1



The synthesis of (pivaloyloxy)methyl (Pom) 6α-chloropenicillanate sulfone (**9**), was performed by diazotization-hydrochlorination of ester **8** using the methodology reported by McMillan and Stoodley,[10] and subsequent oxidation (Scheme 2).

Scheme 2



Synthesis of 6α-(sulfonyl)oxypenicillanates. We have found that the fluorosulfonyl group can be conveniently and stereospecifically introduced in the 6α orientation by a single-step procedure in a reasonable yield (63%) by treatment of Pom 6-diazopenicillanate (**10**) with fluorosulfonic acid in methylene chloride;[11] oxidation gave the corresponding sulfone (**12**) (Scheme 2).

The preparation of benzyl 6α-hydroxypenicillanate (**13b**) has been described by Sheehan *et al.*[12]. The synthesis of Pom 6α-hydroxypenicillanate (**13a**) from **10**, was done following that procedure. These carboxylic esters reacted with mesyl chloride, tosyl chloride or trifluoromethanesulfonic anhydride to give the corresponding Pom and benzyl 6α-methanesulfonyl (**14a$_1$** and **14b$_1$**), 6α-trifluoromethanesulfonyl (**14a$_2$** and **14b$_2$**) and 6α-*p*-toluenesulfonyl (**14a$_3$** and **14b$_3$**) derivatives in good yield (70 to 90%). Oxidation gave the corresponding sulfones in very good yields (**15a$_{1-3}$, 15b$_{1-3}$**). The preparation of Pom 6α-(trifluoromethanesulfonyl)oxypenicillanate (**14a$_2$**) was previously reported by us[13] using a different methodology.

In vitro PPE inhibition[5]

The *in vitro* activity of the compounds in Table I were evaluated for their ability to inhibit PPE-catalyzed hydrolysis of the substrate MeO-Suc-Ala-Ala-Pro-Val-pNA. As expected, based on similar results in the cephem[14] and penam[15] series using HLE, the *tert*-butyl, *iso*-propyl (**4b,c**) as well as the methyl (**16**) esters were only weakly active. Use of a Pom double ester (**9**) and benzyl ester (**4a**) provided a greater than five fold increase in potency, as measured by their IC_{50} values, over the branched and unbranched alkyl esters. The series of pivaloyl (**17**)[4] and acetyl (**6a**) esters of 3α-hydroxymethyl-6α-chloropenam sulfones were more potent than the (pivaloyloxy)methyl double esters and benzyl esters.

Based on previous reports by Thompson *et al.* on HLE inhibition by penicillin esters,[15] we decided to study the introduction of different 6α-(sulfonyl)oxy substituents in an attempt to improve activity. The 6α-CF_3SO_3- derivatives (**15a$_2$** and **15b$_2$**) exhibited the lowest IC_{50} values obtained without preincubation. However, such compounds and the FSO_3- derivative **12** were so unstable that the preincubation assay could not be run (see Table I). Conversely, CH_3SO_3- and (*p*-CH_3)$C_6H_4SO_3$- substituents gave compounds **15a$_1$**, **15a$_3$**, **15b$_1$**, and **15b$_3$** that exhibited rather low instantaneous IC_{50} values and have better stability, showing a clearly progressive inhibition. The IC_{50} values with preincubation for these compounds were in such cases from four to fifteen times lower than those without preincubation. Therefore, it is rather likely that compounds **15a$_1$**, **15a$_3$**, **15b$_1$**, and **15b$_3$** behave as mechanism-based inhibitors. Interestingly, the results described suggest some parallelism beetwen PPE and HLE, i.e. the replacement of 6α-chloro (**4a**) by 6α-TsO (**15b$_3$**) caused a significative inprovement in both PPE (205→23 μM) and HLE[15a] (14→0.05 μM) inhibition.

Conclusion

We have extended the scope of structural requirements at C-3α and C-6α of the penam sulfones as inhibitors of PPE.[4] It is noteworthy that the esters of 3α-hydroxymethyl-6α-chloropenam sulfones (**6a** and **17**) markedly improve the inhibitory activity in comparison with the corresponding esters of 3α-carboxylic acid-6α-chloropenam sulfones **4a-c** and **9**. On the other hand, introduction of electron withdrawing 6α-(sulfonyl)oxy substituents in the penam nucleus allowed us to compare the effects that these (sulfonyl)oxy have on PPE activity in relation to the known compound **15b$_3$**. The SAR study indicated (see Table I) that compounds **15a$_2$** and **15b$_2$** are the most potent in this series. However, the less potent compounds **15a$_1$**, **15a$_3$**, **15b$_1$** and **15b$_3$** were shown to have better stability. Studies are underway to structurally modify these classes of compounds at C-3α and C-6α to improve their potency and address their chemical stability and the results of these investigations will be the subject of future publications.

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Table I

Compound	IC_{50} (μM)[a] Without Preincubation	With 10 min. Preincubation
4a[b]	205±40	180±45
4b	1160±60	1210±220
4c	1300±230	1200±140
Methyl 6α-chloropenicillanate sulfone (**16**)[c]	950 (44±3%)[d]	950 (40±5%)[d]
9	280±90	220±60
6a	57±6	68±5
6b	N.D.[e]	
6α-Chloro-2,2-dimethyl-3α-(pivaloyloxy)methylpenam sulfone (**17**)[c]	15±2	20±5
12	16.7±3.1	(f)
15a$_1$	4.3±0.4	0.54±0.08
15a$_2$	1.0±0.1	(f)
15a$_3$	2.1±0.2	0.49±0.15
15b$_1$	2.2±0.2	0.13±0.01
15b$_2$	0.68±0.09	(f)
15b$_3$[b]	23±6	0.15±0.04

[a]For methodology, see Ref. 4; IC_{50} and standard error values were estimated by non-linear least squares regression fitting the inhibition obtained at different [I] to the equation: **inhibition=maximal inhibition [I] / (IC_{50} + [I])**. [b]Compounds **4a** and **15b$_3$** were previously reported by Thompson *et al.*[15a] with IC_{50} values against HLE of 14 and 0.05 μM, respectively.[c]These compound were previously reported.[4] [d]Maximun [I] used in the assays; mean inhibition and its standard error obtained are shown in parenthesis. [e]Not determined due to insolubility of the compound in the reaction medium. [f]IC_{50} values with preincubation were not determined due to instability of the compounds in the reaction medium.

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