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Article

Contributions to the Development of New Substitution Patterns of Penicillins: Synthesis of New Penicillins Derivatives and Evaluation of their Porcine Pancreatic Elastase Inhibitory Activity

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São relatadas as sínteses dos sulfono-ésteres de 6α-cloropenicilanato **6a-c**, **12**, os sulfono-acetatos e sulfono-benzoatos de vários 6α-(sulfonil)oxipenicilanatos **15**, **18a**$_{1\text{-}3}$, **18b**$_{1\text{-}3}$. Quando testado como inibidor da elastase pancreática suína, o acetato de 3α-hidroximetilpenam **8a** se mostrou como o mais ativo quando comparado com os ésteres do ácido 3α-carboxíco **6a-c** e **12**. Os compostos com vários substituintes 6α-(sulfonil)oxi apresentaram atividade inibidora de elastase melhor que os correspondentes 6α-cloroderivados **6a-c** e **12**; entre estes, os compostos **18a**$_2$ e **18b**$_2$ eram bastante instáveis, mas os compostos **18a**$_1$, **18a**$_3$, **18b**$_1$, **18b**$_3$ apresentaram uma atividade razoável combinada com uma maior estabilidade.

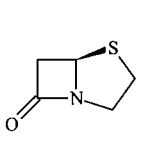
The synthesis of 6α-chloropenicillanate sulfone esters **6a-c**, **12**, the acetate and benzoate of 3α-hydroxymethyl-6α-chloropenam sulfones **8a-b** and pivaloyloxymethyl and benzyl esters of several 6α-(sulfonyl)oxypenicillanate sulfones **15**, **18a**$_1$-**a**$_3$, **18b**$_1$-**b**$_3$ are reported. When tested as inhibitors of porcine pancreatic elastase, the acetate of 3α-hydroxymethylpenam **8a** proved to be more active in comparison with the esters of 3α-carboxylic acid counterparts **6a-c** and **12**. Compounds with diverse 6α-(sulfonyl)oxy substituents showed elastase inhibitory activity improved over the corresponding 6α-chloro derivatives **6a-c** and **12**; among those, compounds **18a**$_2$ and **18b**$_2$ were rather unstable, but compounds **18a**$_1$, **18a**$_3$, **18b**$_1$, **18b**$_3$ combined fair activity with better stability.

Keywords: *elastase inhibitors, penicillins derivatives*

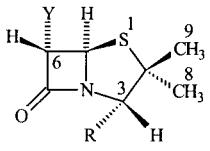
Introduction

The concept of structural modification at the C_3 and C_6 position of penam nucleus (**1**) is of current interest in the study of penicillins derivatives as elastase inhibitors.

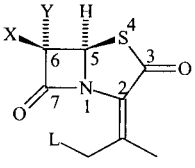
While abundant information on diverse substituion patterns of penicillin as antibiotic and β-lactamase inhibitors exist[1], the synthesis and applications of 6-substituted penicillanates (**2**) as elastase inhibitors[2] and 2'-Z-substituted anhydropenicillins (**3**) as carrier in the Antibody-Directed Enzyme Prodrug Therapy (ADEPT)[3], have been the subject of very few investigations.



1
Penam Nucleus



2



3
L= leaving group

Recent research from our laboratory has addressed these issues[2,4]. Human leukocyte elastase (HLE, EC 3.4.21.37) is a serine protease found in the azurophilic granules of polymorphonuclear leukocytes[5]. This enzyme has been the subject of extensive studies, both in terms of its biological role in numerous diseases[6] 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[1,5]. 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).

We have recently reported a preliminary account[2] of the structure-activity relationship (SAR) for C-6 substituted penicillin esters with either an α-(sulfonyl)oxy- or an α-chloro functionalities as inhibitors of Porcine Pancreatic Elastase (PPE, EC. 3.4.21.36) an enzyme related to HLE[7]. Here, we reports, in details, the synthesis, characterization and evaluation of PPE inhibitory activity of penicillin ester sulfones **2**.

[R = -CO_2-CH_2-$CO_2C(CH_3)_3$], -CO_2-CH_2-C_6H_5, -CO_2-$CH(CH_3)_2$, -$CO_2C(CH_3)_3$, -CH_2OCOCH_3 and -$CH_2OCOC_6H_5$) substituted at position 6 with a variety of α-oriented functionalities (Y = -Cl, -SO_3F, -SO_3CF_3, SO_3CH_3, and -SO_3-*p*-C_6H_4-CH_3).

Experimental

Infrared spectra (IR) were taken on a Bruker IFS 25 FT-IR spectrometer. Proton and carbon magnetic resonance spectra (^{1}H-NMR and ^{13}C-NMR) were taken on a Bruker AC 200 spectrometer. The signal assignments were normaly based upon signal multiplicities, chemical shift rules[8], DEPT (Distortionless Enhancement by Polarization Transfer), comparison with related compounds and, in some cases, heteronuclear correlated 2D spectra[9]. Melting points were taken on a Ernst Leitz melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was carried out with silica gel 60 F_{254} pre-coated aluminium sheets (Merck); flash column chromatography was performed using Merck silica gel 60 (230-400 mesh) according to the procedure developed by Still *et al.*[10]

Elastase (EC 3.4.21.36) was purchased from Sigma Chemical Co (Type III, from Porcine Pancreas). A stock solution (1 mg/mL) was prepared in sodium acetate 50 mM (pH 5.5), and frozen at -20 °C until used. N-Methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide (Sigma Chemical Co) was dissolved in dimethylsulfoxide (DMSO). Enzyme activity was assayed in potassium phosphate 100mM, pH 7 (final volume 3 mL). The reaction was started by addition of 10-50 μL of the enzyme stock solution. The release of *p*-nitroaniline was followed spectrophotometrically at 405 nm in a computer assisted LKB Ultrospec II Plus with a termostated cell holder (30 °C). The inhibitors were dissolved in DMSO. The maximal concentration of DMSO was 2% in the reaction medium. Control experiments were run in all the cases with equal amount of DMSO.

*Synthesis of benzyl 6α-chloropenicillanate sulfone (**6a**)*

To a solution of 6α-chloro-3α-chlorocarbonyl-2,2-dimethylpenam sulfone (**5**)[11] (129 mg, 0.45 mmol) in anhydrous chloroform (1 mL) was added dropwise benzyl alcohol (0.104 mL, 1.01 mmol) at room temperature and the resulting solution was stirred at the same temperature for 3 h. The reaction mixture was washed with water (2 x 0.5 mL) and the organic layer was dried (Na_2SO_4) and concentrated. The residue was chromatographed (hexane/AcOEt, 8.5:1.5) to give **6a** (100 mg, 62%) as a white solid; mp 75.5-76.0 °C (hexane/CH_2Cl_2); IR (KBr) 1805 (β-lactam), 1719 (ester), 1332 and 1118 cm^{-1} (sulfone); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.27 (s, 3H, α-CH_3), 1.56 (s, 3H, β-CH_3), 4.44 (s, 1H, 3-H), 4.63 (d, *J* = 1.51 Hz, 1H, 5-H), 5.15 (d, *J* = 1.51 Hz, 1H, 6-H), 5.20 (d, AB spin system, *J* = 11.90 Hz, 1 H, -O-C<u>H</u>$_2$-Ar), 5.30 (d, AB spin system, *J* = 11.90 Hz, 1 H, -O-C<u>H</u>$_2$-Ar), 7.39 (s, 5 H, Ar-<u>H</u>) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 16.45 (C-8), 19.73 (C-9), 55.35 (C-6), 62.98 (C-3), 63.12 (C-2), 66.29 (-O-<u>C</u>H_2-Ar) 69.10 (C-5), 128.75, 128.99 and 134.07 (Ar-<u>C</u>), 165.74 (C-7), 165.85 (C-<u>C</u>O_2-CH_2) ppm.

*Synthesis of iso-propyl 6α-chloropenicillanate sulfone (**6b**)*

To a solution of acid chloride **5** [obtaining from the corresponding acid **4**[11] (48.6 mg, 0.18 mmol)] in anhydrous CH_2Cl_2 (1 mL) was added succesively *iso*-propyl alcohol (0.027 mL, 0.35 mmol) and a solution of triethylamine (0.049 mL, 0.35 mmol) in anhydrous CH_2Cl_2 (1 mL) and the mixture was stirred at room temperature for 3 h. After removing the solvent *in vacuo*, the crude material was chromatographed (hexane/AcOEt, 9:1) to give **6b** (6.1 mg, 11% from **4**); IR (KBr) 1810 (β-lactam), 1752 (ester), 1330 and 1118 cm^{-1} (sulfone); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.32 [d, *J* = 6.25 Hz, 3H, -CH(C<u>H</u>$_3$)$_2$], 1.33 [d, *J* = 6.25 Hz, 3H, -CH(C<u>H</u>$_3$)$_2$], 1.43 (s, 3H, α-CH_3), 1.61 (s, 3H, β-CH_3), 4.39 (s, 1H, 3-H), 4.66 (d, *J* = 1.50 Hz, 1H, 6-H), 5.14 [sept, *J* = 6.25 Hz, 1H, -C<u>H</u>(CH$_3$)$_2$], 5.16 (d, *J* = 1.50 Hz, 1H, 5-H) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 18.74 (C-8), 19.88 (C-9), 21.65 [-CH(<u>C</u>H$_3$)$_2$], 55.42 (C-6), 63.10 (C-2), 63.16 (C-3), 69.27 (C-5), 71.02 [-<u>C</u>H(CH$_3$)$_2$], 165.32 (C-7), 165.88 (C-<u>C</u>O_2-CH) ppm.

*Synthesis of tert-butyl 6α-chloropenicillanate sulfone (**6c**)*

According to a procedure similar to that described above for the preparation of **6b**, compound **6c** was obtained

after purification by column chromatography ($CHCl_3/Et_2O$: 9.6:0.4) as a white solid (26% yield from **4**); mp 147.0-151.0 °C; IR (KBr) 1794 (β-lactam), 1742 (ester), 1332 and 1116 cm^{-1} (sulfone); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.45 (s, 3H, α-CH_3), 1.53 [s, 9H, -C(CH_3)$_3$], 1.59 (3H, β-CH_3), 4.33 (s, 1H, 3-H), 4.64 (d, J = 1.51 Hz, 1H, 5-H), 5.15 (d, J = 1.51 Hz, 1H, 6-H), ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 18.92 (C-8), 19.80 (C-9), 27.90 [-C(CH_3)$_3$], 55.46 (C-6), 63.15 (C-2), 63.55 (C-3), 69.37 (C-5), 84.62 [-C(CH_3)$_3$], 164.71 (C-7), 165.90 (C-CO_2-C) ppm.

*General procedure for the selective oxidation of penicillanate sulfide (-S-) into penicillanate sulfone (-SO_2-). Synthesis of (pivaloyloxy)methyl 6α-chloropenicillanate sulfone (**12**)*

To a solution of (pivaloyloxy)methyl (Pom) 6α-chloropenicillanate **11**[12] (57 mg, 0.163 mmol) in a mixture of acetic acid/water (8.5:1.5) (6.4 mL) was added powdered potassium permanganate (51.5 mg, 0.326 mmol) and the mixture was stirred for 1h. The reaction mixture was then quenched with drops of H_2O_2 until disappearance of color, diluted with CH_2Cl_2 (6 mL) and water (6 mL). The layers were separated and the aqueous layer extracted with CH_2Cl_2 (3 x 6 mL). The combined organic layers were washed with 5% aqueous sodium bicarbonate solution (3 x 6 mL) and water (1 x 6 mL), dried (Na_2SO_4) and the solvent removed under reduced pressure to yield **12**[12] (57 mg, 92%) as white crystals; mp 117.5-118.5 °C, lit.[12] 118.0-120.0 °C; ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.23 [s, 9H, -C(CH_3)$_3$], 1.43 (s, 3H, α-CH_3), 1.59 (s, 3H, β-CH_3), 4.46 (s, 1H, 3-H) 4.68 (d, J = 1.53 Hz, 1H, 5-H), 5.18 (d, J = 1.53 Hz, 1H, 6-H), 5.74 (d, AB spin system J = 5.45 Hz, 1H, -O-CH_2-O), 5.96 (d, AB spin system J = 5.45 Hz, 1H, -O-CH_2-O) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 18.13 (C-8), 19.72 (C-9), 26.65 [C(CH_3)$_3$], 38.64 [C(CH_3)$_3$)], 55.32 (C-6), 62.67 (C-3), 62.89 (C-2), 68.96 (C-5), 80.42 (-O-CH_2-O), 164.71 (C-7), 165.84 (-C-CO_2-CH_2), 176.63 [O-CO-C(CH_3)$_3$] ppm.

*Synthesis of 6α-chloro-2,2-dimethyl-3α-(acetyl) oxymethylpenam sulfone (**8a**)*

To a stirred solution of alcohol **7**[13] (23 mg, 0.091 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (cat. amount) in CH_2Cl_2 (1.5 mL) was added dropwise succesively triethylamine (0.019 mL, 0.136 mmol) and acetic anhydride (0.013 mL, 0.136 mmol) at room temperature and the mixture was stirred for 1 h. at the same temperature. Then was diluted with CH_2Cl_2 (5 mL) and washed succesively with a saturated ammonium chloride solution (2 x 4 mL) and brine (1 x 4 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give a colorless residual oil which was chromatographed on silica gel (hexane/AcOEt, 8:2) to give **8a** (17 mg, 64%) as white crystals; mp 119.5-121.5 °C; IR (KBr) 1806 (β-lactam), 1740 (ester), 1316 and 1132 cm^{-1} (sulfone); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.46 (s, 3H, α-CH_3), 1.50 (s, 3H, β-CH_3), 2.14 (s, 3H, O-COCH_3), 4.06 (dd, ABX spin system, J_{vic1} = 9.39 Hz, J_{vic2} = 3.58 Hz, 1H, 3-H), 4.15 (dd, ABX spin system, J_{gem} = 10.97 Hz, J_{vic2} = 3.58 Hz, 1H, -CH_2-O-), 4.19 (dd, ABX spin system, J_{gem} = 10.97 Hz, J_{vic1} = 9.39 Hz, 1H, -CH_2-O-), 4.54 (d. J = 1.59 Hz, 1H, 5-H), 5.14 (d, J = 1.59 Hz, 1H, 6-H), ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 18.80 (C-8), 18.83 (C-9), 20.58 (O-COCH_3), 55.43 (C-6), 60.50 (C-3), 61.51 (C-CH_2-O), 62.90 (C-2), 69.18 (C-5), 166.69 (C-7), 170.18 (-O-CO-CH_3) ppm.

*Synthesis of 6α-chloro-2,2-dimethyl-3α-(benzoyl) oxymethylpenam sulfone (**8b**)*

The procedure for the preparation of **8b** from alcohol **7**[13], DMAP and benzoyl chloride was similar to that described for **8a**. The stirred reaction mixture, initially at 0 °C was allowed to warm at room temperature overnight (14 h). Then the mixture was quenched with methanol (3 drops) diluted with CH_2Cl_2 (3 mL) and washed with saturated aqueous sodium bicarbonate solution (2 x 3 mL). The organic layer was dried (Na_2SO_4) and concentrated to a pale yellow oil. Flash chromatography of the crude product with chloroform as eluting solvent, afforded **8b** as white needles (yield 62%); mp 172.0-173.5 °C; IR (KBr) 1796 (β-lactam), 1718 (ester), 1318 and 1128 cm^{-1} (sulfone); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.53 (s, 3H, α-CH_3), 1.55 (s, 3H, β-CH_3), 4.19-4.47 (m, 3H, 3-H and C-CH_2-O-), 4.59 (d, J = 1.56 Hz, 1H, 6-H), 5.19 (d, J = 1.56 Hz, 1H, 5-H), 7.44-7.66 (m. 3H, Ar-H). 8.08-8.13 (m, 2H, Ar-H) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 18.63 (C-8), 19.02 (C-9), 55.30 (C-6), 60.56 (C-3), 62.23 (C-CH_2-O), 62.84 (C-2), 69.03 (C-5), 128.54, 128.75, 129.75 and 133.56 (Ar-C), 165.86 (C-7), 166.69 (-O-CO-Ph) ppm.

*Synthesis of (pivaloyloxy)methyl 6α-[(fluorosulfonyl)oxy]-penicillanate sulfone (**15**)*

(Pivaloyloxy)methyl 6α-[(fluorosulfonyl)oxy]penicillanate **14**,[14] was oxidized according the same procedure as **12** to give **15** as a white solid (57% yield); mp 74.0-76.0 °C; IR (KBr) 1816 (β-lactam), 1776, 1748 (ester), 1318 and 1112 cm^{-1} (sulfone); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.23 [s, 9H, -C(CH_3)$_3$], 1.46 (s, 3H, α-CH_3), 1.61 (s, 3H, β-CH_3), 4.49 (s, 1H, 3-H), 4.89 (d, J = 1.46 Hz, 1H, 5-H), 5.76 (d, AB spin system, J = 5.41 Hz, 1H, -O-CH_2-O), 5.89 (d, J = 1.46 Hz, 1H, 6-H), 5.97 (d, AB spin system, J = 5.41 Hz, 1H, -O-CH_2-O) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 18.12 (C-8), 19.75 (C-9), 26.71 [C(CH_3)$_3$], 38.73 [C(CH_3)$_3$], 62.93 (C-5), 63.31 (C-2),

66.57 (C-3), 80.64 (O-$\underline{C}H_2$-O), 81.48 (C-6), 161.39 (C-7), 164.28 (-O-$\underline{C}O$-CH_2), 176.69 [O-$\underline{C}O$-$C(CH_3)_3$] ppm.

*Synthesis of benzyl 6α-[(methanesulfonyl)oxy]penicillanate (**17a**$_1$)*

To a solution of benzyl 6α-hydroxypenicillanate **16a**[15] (32.2 mg, 0.11 mmol) in anhydrous CH_2Cl_2 (2 mL) at 0 °C was added dropwise succesively triethylamine (22 μL, 0.16 mmol) and mesyl chloride (12 μL, 0.16 mmol). The mixture was stirred at 0 °C for 1 h, poured into saturated ammonium chloride solution and the layers separated. The organic layer was washed with saturated ammonium chloride solution (2 x 1 mL) and then stirred for 30 min with water (2 mL) at 0 °C. Finally, phases were separated and the organic one was dried (Na_2SO_4), filtered and concentrated to afford **17a**$_1$ (32.5 mg, 80%) as a white solid; mp 95.0-97.0 °C, lit.[16] 98.0-99.0 °C; IR (KBr) 1784 (β-lactam), 1744, (ester), 1362, 1176 and 954 cm^{-1} (mesylate); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.40 (s, 3H, α-CH_3), 1.56 (s, 3H, β-CH_3), 3.18 (s, 3H, -SO_2-$\underline{C}H_3$) 4.54 (s, 1H, 3-H), 5.19 (d, AB spin system J = 12.65 Hz, 1H, -O-$C\underline{H}_2$-Ar), 5.21 (d, AB spin system J = 12.65 Hz, 1H, -O-$C\underline{H}_2$-Ar), 5.39 (d, J = 1.38 Hz, 1H, 5-H), 5.47 (d, J = 1.38 Hz, 1H, 6-H), 7.37 (s, 5H, Ar-$\underline{H}$) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 25.23 (C-8), 33.69 (C-9), 38.79 (-SO_2-$\underline{C}H_3$), 64.22 (C-2), 67.48 (-O-$\underline{C}H_2$-Ar), 68.87 (C-3), 69.18 (C-5), 85.26 (C-6), 128.53, 128.60, 128,66 and 134.45 (Ar-$\underline{C}$), 164.93 (C-7), 166.45 (C-$\underline{C}O_2$-CH_2) ppm.

*Synthesis of (pivaloyloxy)methyl 6α-[(methanesulfonyl) oxy]penicillanate (**17b**$_1$)*

According to a similar procedure to that used for the synthesis of **17a**$_1$, compound **16b** was converted into **17b**$_1$ (89% yield); IR (film) 1788 (β-lactam), 1758, (ester), 1370, 1180, 1112 and 964 cm^{-1} (mesylate); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.23 (s, 9H, [-$C(C\underline{H}_3)_3$], 1.50 (s, 3H, α-CH_3), 1.59 (s, 3H, β-CH_3), 3.21 (s, 3H, -SO_2-$\underline{C}H_3$), 4.55 (s, 1H, 3-H), 5.41 and 5.48 (d, J = 1.36, 2H, 5-H and 6-H), 5.81 (d, AB spin system J = 5.52 Hz, 1H, -O-$C\underline{H}_2$-O), 5.85 (d, AB spin system J = 5.52 Hz, 1H, -O-$C\underline{H}_2$-O) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 25.31 (C-8), 26.78 [-$C(\underline{C}H_3)_3$], 33.57 (C-9), 38.71 [-$\underline{C}(CH_3)_3$], 38.88 (-SO_2-$\underline{C}H_3$), 64.15 (C-2), 68.90 (C-3 and C-5), 79.75 (-O-$\underline{C}H_2$-O), 85.19 (C-6), 164.99 (C-7), 165.43 (C-$\underline{C}O_2$-CH_2-), 176.70 [-CH_2-O-$\underline{C}O$-$C(CH_3)_3$] ppm.

*Synthesis of benzyl 6α-[(trifluoromethanesulfonyl) oxy] penicillanate (**17a**$_2$)*

To a stirred solution of benzyl 6α-hydroxypenicillanate **16a**[15] (49.6 mg, 0.16 mmol) and triethylamine (34 μL, 0.24 mmol) in anhydrous CH_2Cl_2 (1 mL) at 0 °C was added dropwise during 5 min. a solution of trifluoromethanesulfonic anhydride (41 μL, 0.24 mmol) in anhydrous CH_2Cl_2 (1 mL) also at 0 °C. The reaction solution was stirred at 0 °C for 10 min. and then a mixture of water:dichloromethane (1:1, 2 mL) was added. After separation, the organic layer was washed with water (1 mL), dried (Na_2SO_4), filtered and concentrated to afford a brown oil. Flash chromatography of the crude product with hexane/AcOEt (9.2:0.8) as eluting solvent, afforded **17a**$_2$ as a colorless oil (63.0 mg, 89%); IR (KBr) 1797 (β-lactam), 1746 (ester), 1425, 1246, 1212, 1141 and 957 cm^{-1} (triflate); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.40 (s, 3H, α-CH_3), 1.57 (s, 3H, β-CH_3), 4.57 (s, 1H, 3-H), 5.21 (d, AB spin system J = 11.83 Hz, 1H, -O-$C\underline{H}_2$-Ar), 5.21 (d, AB spin system J = 11.83 Hz, 1H, -O-$C\underline{H}_2$-Ar), 5.50 (m, 2H, 5-H and 6-H), 7.37 (s, 5H, Ar-$\underline{H}$) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 25.13 (C-8), 33.86 (C-9), 64.51 (C-2), 67.64 (-O-$\underline{C}H_2$-Ar), 68.88 (C-3), 69.30 (C-5), 88.73 (C-6), 118.26 (q, J = 319 Hz, CF_3), 128.61, 128.75, and 134.35 (Ar-$\underline{C}$), 162.03 (C-7), 166.21 (C-$\underline{C}O_2$-CH_2) ppm.

*Synthesis of (pivaloyloxy)methyl 6α-[(trifluoromethanesulfonyl)oxy]penicillanate (**17b**$_2$)*

According to a similar procedure to that used for the synthesis of **17a**$_2$, compound **16b** was converted into **17b**$_2$ (85% yield); IR (film) 1805 (β-lactam), 1790 and 1785 cm^{-1} (ester); ^{1}H-NMR (80.13 MHz; $CDCl_3$; standard Me_4Si) δ 1.22 (s, 9H, [-$C(C\underline{H}_3)_3$], 1.51 (s, 3H, α-CH_3), 1.59 (s, 3H, β-CH_3), 4.59 (s, 1H, 3-H), 5.52 (m, 2H, 5-H and 6-H), 5.83 (s, 2H, -O-$C\underline{H}_2$-O) ppm; ^{13}C-NMR (20.15 MHz; $CDCl_3$; standard $CDCl_3$) δ 25.14 (C-8), 26.68 [-$C(\underline{C}H_3)_3$], 33.69 (C-9), 38.63 [-$\underline{C}(CH_3)_3$], 64.36 (C-2), 68.82 (C-5), 69.03 (C-3), 79.77 (-O-$\underline{C}H_2$-O), 88.64 (C-6), 118.24 (q, J = 324 Hz, -CF_3), 161.98 (C-7), 165.12 (C-$\underline{C}O_2$-CH_2-), 176.54 [-CH_2-O-$\underline{C}O$-$C(CH_3)_3$] ppm.

*Synthesis of benzyl 6α-[(p-toluenesulfonyl)oxy]penicillanate (**17a**$_3$)*

To a stirred solution of benzyl 6α-hydroxypenicillanate **16a**[15] (47.2 mg, 0.15 mmol) in anhydrous CH_2Cl_2 (1 mL) at room temperature was added dropwise a solution of tosyl chloride (58.6 mg, 0.31 mmol) in anhydrous CH_2Cl_2 (1 mL) and then pyridine (37 μL., 0.46 mmol). The mixture was stirred at room temperature for 65 h.and then quenched with aqueous saturated ammonium chloride solution (1.5 mL), diluted with CH_2Cl_2 (4 mL) and stirred for another 15 min at room temperature. Extractive workup (aqueous saturated NH_4Cl solution) followed by purification by column chromatography (hexane/AcOEt, 9:1) gave **17a**$_3$ (49.6 mg, 70%) as a colorless oil; IR (KBr) 1792 (β-lactam), 1744 (ester), 1376, 1192, and 1178 cm^{-1} (tosylate); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.35 (s, 3H, α-CH_3), 1.51 (s, 3H, β-CH_3), 2.46 (s, 3H, Ar-$C\underline{H}_3$), 4.48 (s, 1H,

3-H), 5.15 (d, AB spin system J = 13.44 Hz, 1H, -O-C$\underline{H}_2$-Ar), 5.18 (d, AB spin system J = 13.44 Hz, 1H, -O-C$\underline{H}_2$-Ar), 5.23 (m, 2H, 5-H and 6-H), 7.36 (s, 5H, Ar-$\underline{H}$), 7.38 (d, J = 8.5 Hz, 2H, -SO_2-$C_6\underline{H}_4$-CH_3), 7.82 (d, J = 8.5 Hz, 2H, -SO_2-$C_6\underline{H}_4$-CH_3) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 21.64 (Ar-$\underline{C}H_3$), 25.26 (C-8), 33.86 (C-9), 64.17 (C-2), 67.46 (-O-$\underline{C}H_2$-Ar), 69.00 (C-3), 69.18 (C-5), 85.69 (C-6), 128.11, 130.09, 132.16 and 145.86 (-SO_2-$\underline{C}_6H_4$-CH_3), 128.55, 128.61, 128,68 and 134.46 (Ar-$\underline{C}$), 164.42 (C-7), 166.57 (C-$\underline{C}O_2$-CH_2) ppm.

*Synthesis of (pivaloyloxy)methyl 6α-[(p-toluenesulfonyl)oxy]penicillanate (**17b**$_3$)*

According to a similar procedure to that used for the synthesis of **17a**$_3$, compound **16b** was converted into **17b**$_3$ (60% yield); IR (film) 1794 (β-lactam), 1758 (ester), 1376, 1192, and 1180 cm^{-1} (tosylate); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.21 (s, 9H, [-C($\underline{C}H_3$)$_3$], 1.45 (s, 3H, α-CH_3), 1.54 (s, 3H, β-CH_3), 2.48 (s, 3H, Ar-$\underline{C}H_3$), 4.49 (s, 1H, 3-H), 5.23 and 5.25 (d, AB spin system J = 1.50 Hz, 2H, 5-H and 6-H), 5.78 (d, AB spin system J = 5.59 Hz, 1H, -O-C$\underline{H}_2$-O), 5.81 (d, AB spin system J = 5.59 Hz, 1H, -O-C$\underline{H}_2$-O), 7.39 (d, J = 8.21 Hz, 2H, -SO_2-$C_6\underline{H}_4$-CH_3), 7.83 (d, J = 8.21 Hz, 2H, -SO_2-$C_6\underline{H}_4$-CH_3) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 21.87 (Ar-$\underline{C}H_3$), 25.66 (C-8), 27.15 [-C-$\underline{C}H_3$)$_3$], 33.92 (C-9), 38.87 [-$\underline{C}$(CH_3)$_3$], 64.21 (C-2), 69.19 (C-3 and C-5), 79.88 (-O-$\underline{C}H_2$-O), 85.91 (C-6), 128.17, 130.08, 145.51 and 145.94 (-SO_2-$\underline{C}_6H_4$-CH_3), 164.72 (C-7), 165.63 (C-$\underline{C}O_2$-CH_2), 176.87 [-CH_2-O-$\underline{C}$O-C(CH_3)$_3$] ppm.

*Synthesis of benzyl 6α-[(methanesulfonyl)oxy]penicillanate sulfone (**18a**$_1$)*

Penicillanate sulfone **18a**$_1$ was prepared according to the general procedure (82% yield); mp 114.0-116.0 °C; IR (film) 1806 (β-lactam), 1756, (ester), 1376, 1184, and 954 (mesylate), 1324 and 1120 cm^{-1} (sulfone); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.28 (s, 3H, α-CH_3), 1.55 (s, 3H, β-CH_3), 3.21 (s, 3H, -SO_2-$\underline{C}H_3$) 4.44 (s, 1H, 3-H), 4.81 (d, J = 1.47 Hz, 1H, 5-H), 5.21 (d, AB spin system J = 11.49 Hz, 1H, -O-C$\underline{H}_2$-Ar), 5.30 (d, AB spin system J = 11.49 Hz, 1H, -O-C$\underline{H}_2$-Ar), 5.75 (d, J = 1.47 Hz, 1H, 6-H), 7.38 (s, 5H, Ar-$\underline{H}$) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 18.28 (C-8), 19.70 (C-9), 38.84 (-SO_2-$\underline{C}H_3$), 62.88 (C-3), 63.38 (C-2), 67.45 (C-5), 68.41 (-O-$\underline{C}H_2$-Ar), 77.66 (C-6), 128.60, 128.80, 129.06, and 134.05 (Ar-$\underline{C}$),164.60 (C-7), 165.65 (C-$\underline{C}O_2$-CH_2-) ppm.

*Synthesis of (pivaloyloxy)methyl 6α-[(methanesulfonyl)oxy]penicillanate sulfone (**18b**$_1$)*

Penicillanate sulfone **18b**$_1$ was prepared according to the general procedure (85% yield), as a white solid; mp 141.0-143.0 °C; IR (film) 1810 (β-lactam), 1778, 1754, (ester), 1372, 1178, 1112, 966 (mesylate), 1320 and 1160 cm^{-1} (sulfone); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.23 (s, 9H, [-C($\underline{C}H_3$)$_3$], 1.44 (s, 3H, α-CH_3), 1.59 (s, 3H, β-CH_3), 3.23 (s, 3H, -SO_2-$\underline{C}H_3$) 4.45 (s, 1H, 3-H), 4.85 (d, J = 1.42, 1H, 5-H), 5.76 (d, AB spin system J = 6.50 Hz, 1H, -O-C$\underline{H}_2$-O), 5.77 (d, J = 1.42 Hz, 1H 6-H), 5.96 (d, AB spin system J = 6.50 Hz, 1H, -O-C$\underline{H}_2$-O) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 18.01 (C-8), 19.73 (C-9), 26.71 [-C($\underline{C}H_3$)$_3$], 38.71 [-$\underline{C}$(CH_3)$_3$], 38.82 (-SO_2-$\underline{C}H_3$), 62.64 (C-5), 63.18 (C-2), 67.37 (C-3), 77.61 (C-6), 80.52 (-O-$\underline{C}H_2$-O), 164.57 (C-7), 164.64 (C-$\underline{C}O_2$-CH_2-), 176.66 [-CH_2-O-$\underline{C}$O-C(CH_3)$_3$] ppm.

*Synthesis of benzyl 6α-[(trifluoromethanesulfonyl)oxy]penicillanate sulfone (**18a**$_2$)*

Penicillanate sulfone **18a**$_2$ was prepared according to the general procedure (60% yield); IR (film) 1810 (β-lactam), 1758, (ester), 1432, 1248, 1226, 1140, 952 (triflate), 1328 and 1120 cm^{-1} (sulfone); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.28 (s, 3H, α-CH_3), 1.56 (s, 3H, β-CH_3), 4.47 (s, 1H, 3-H), 4.81 (d, J = 1.48 Hz, 1H, 5-H), 5.21 (d, AB spin system J = 11.83 Hz, 1H, -O-C$\underline{H}_2$-Ar), 5.31 (d, AB spin system J = 11.83 Hz, 1H, -O-C$\underline{H}_2$-Ar), 5.91 (d, J = 1.48 Hz, 1H, 6-H), 7.39 (s, 5H, Ar-$\underline{H}$.) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 18.38 (C-8), 19.68 (C-9), 63.08 (C-3), 63.48 (C-2), 67.31 (C-5), 68.58 (-O-$\underline{C}H_2$-Ar), 81.07 (C-6), 118.24 (q, J = 319 Hz, -CF_3), 128.82, 128.86, 129.15 and 133.91 (Ar-$\underline{C}$), 161.85 (C-7), 165.35 (C-$\underline{C}O_2$-CH_2-) ppm.

*Synthesis of (pivaloyloxy)methyl 6α-[(trifluoromethanesulfonyl)oxy]penicillanate sulfone (**18b**$_2$)*

Penicillanate sulfone **18b**$_2$ was prepared according to the general procedure (60% yield); IR (film) 1818 (β-lactam), 1758, (ester), 1430, 1248, 1212, 1140, 956 (triflate), 1330 and 1114 cm^{-1} (sulfone); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.23 (s, 9H, [-C($\underline{C}H_3$)$_3$], 1.45 (s, 3H, α-CH_3), 1.60 (s, 3H, β-CH_3), 4.47 (s, 1H, 3-H), 4.84 (d, J = 1.48 Hz, 1H, 5-H), 5.75 (d, AB spin system J = 5.46 Hz, 1H, -O-C$\underline{H}_2$-O), 5.93 (m, 1H, 6-H), 5.97 (d, AB spin system J = 5.46 Hz, 1H, -O-C$\underline{H}_2$-O) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 18.16 (C-8), 19.77 (C-9), 26.73 [-C($\underline{C}H_3$)$_3$], 38.75 [-$\underline{C}$(CH_3)$_3$], 62.91 (C-5), 63.32 (C-2), 67.25 (C-3), 80.64 (-O-$\underline{C}H_2$-O), 81.05 (C-6), 161.81 (C-7), 164.34 (C-$\underline{C}O_2$-CH_2-), 176.72 [-CH_2-O-$\underline{C}$O-C(CH_3)$_3$] ppm.

*Synthesis of benzyl 6α-[(p-toluenesulfonyl)oxy]penicillanate sulfone (**18a**$_3$)*

Penicillanate sulfone **18a**$_3$ was prepared according to the general procedure (96% yield); IR (KBr) 1808 (β-lac-

tam), 1756 (ester), 1382, 1192, 1180 (tosylate), 1326 and 1118 cm^{-1} (sulfone); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.23 (s, 3H, α-CH_3), 1.51 (s, 3H, β-CH_3), 2.48 (s, 3H, Ar-CH_3), 4.38 (s, 1H, 3-H), 4.63 (d, J = 1.55 Hz, 1H, 5-H), 5.18 (d, AB spin system J = 12.00 Hz, 1H, -O-CH_2-Ar), 5.28 (d, AB spin system J = 12.0 Hz, 1H, -O-CH_2-Ar), 5.52 (d, J = 1.55 Hz, 1H, 6-H), 7.38 (s, 5H, Ar-H), 7.42 (d, J = 8.5 Hz, 2H. -SO_2-C_6H_4-CH_3), 7.84 (d, J = 8.5 Hz, 2H. -SO_2-C_6H_4-CH_3) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 18.81 (C-8), 19.94 (C-9), 21.93 (Ar-CH_3), 63.08 (C-3), 64.52 (C-2), 67.81 (C-5), 68.46 (-O-CH_2-Ar), 78.17 (C-6), 128.34 and 134.22 (Ar-C), 128.89, 130.47, 131.25 and 146.51 (-SO_2-C_6H_4-CH_3), 164.15 (C-7), 165.79 (-C-CO_2-CH_2) ppm.

*Synthesis of (pivaloyloxy)methyl 6α-[(p-toluenesulfonyl) oxy]penicillanate sulfone (**18b$_3$**)*

Penicillanate sulfone **18b$_3$** was prepared according to the general procedure (88% yield); IR (film) 1812 (β-lactam), 1758 (ester), 1382, 1194, 1180 (tosylate), 1328 and 1112 cm^{-1} (sulfone); ^{1}H-NMR (200 MHz; $CDCl_3$; standard Me_4Si) δ 1.22 [s, 9H, -C(CH_3)$_3$], 1.39 (s, 3H, α-CH_3), 1.54 (s, 3H, β-CH_3), 2.48 (s, 3H, Ar-CH_3), 4.38 (s, 1H, 3-H), 4.65 (d, J = 1.48 Hz, 1H, 5-H), 5.53 (d, J = 1.48 Hz, 1H, 5-H), 5.73 (d, AB spin system J = 5.51 Hz, 1H, -O-CH_2-O), 5.93 (d, AB spin system J = 5.51 Hz, 1H, -O-CH_2-O), 7.42 (d, J = 8.41 Hz, 2H, -SO_2-C_6H_4-CH_3), 7.85 (d, J = 8.21 Hz, 2H, -SO_2-C_6H_4-CH_3) ppm; ^{13}C-NMR (50 MHz; $CDCl_3$; standard $CDCl_3$) δ 18.19 (C-8), 19.72 (C-9), 21.67 (Ar-CH_3), 26.71 [-C-(CH_3)$_3$], 38.71 [-C(CH_3)$_3$], 62.71 (C-5), 63.11 (C-2), 67.45 (C-3), 78.01 (C-6), 80.49 (-O-CH_2-O), 128.29, 130.38, 131.04 and 146.49 (-SO_2-C_6H_4-CH_3), 164.02 (C-7), 164.65 (-C-CO_2-CH_2), 176.67 [-CH_2-O-CO-C(CH_3)$_3$] ppm.

Results and Discussion

Synthesis of penicillin ester sulfones

The synthesis of the benzyl, *iso*-propyl and *tert*-butyl 6α-chloropenicillanates sulfones **6a-c**, 6α-chloro-2,2-dimethyl-3α-(acetyl)oxymethyl-(**8a**), and 3α-(benzoyl)-oxymethylpenam sulfones (**8b**) is shown in Scheme 1. The starting material was 6α-chloro-penicillanic acid sulfone (**4**)[17]. Conversion of **4** into the 6α-chloro-2,2-dimethyl-3α-chlorocarbonylpenam sulfone (**5**) in 95% isolated yield was accomplished by oxalyl chloride and dimethylformamide[11] in benzene at room temperature.

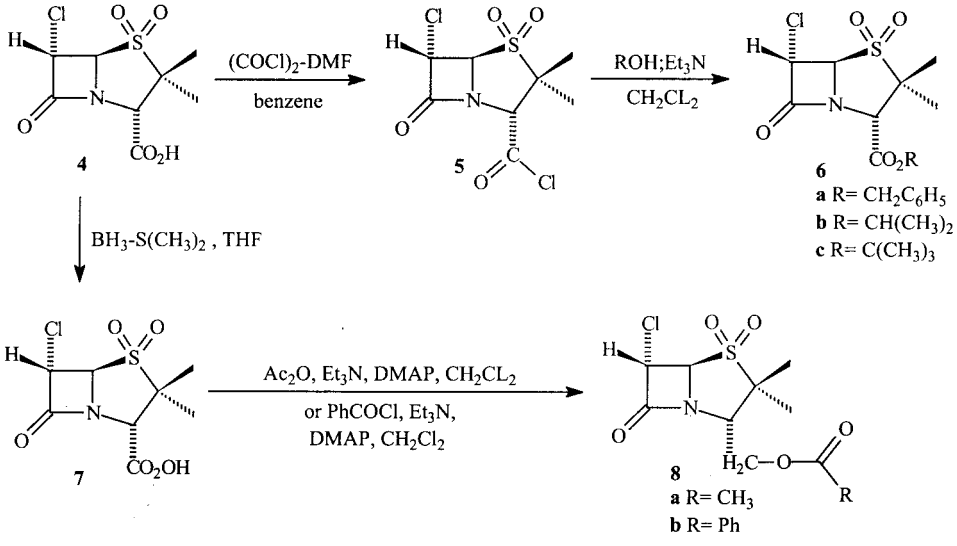
Subsequent treatment of **5** with the appropriate alcohol (benzyl, *iso*-propyl and *tert*-butyl) afforded the esters **6a-c**. Alternatively, reduction of **4** with borane-methyl sulfide complex[13] afforded the alcohol **7** which was then treated with acetic anhydride or benzoyl chloride to give the corresponding acetyl (**8a**) and benzoyl (**8b**) derivatives, respectively.

The synthesis of (pivaloyloxy)methyl (Pom) 6α-chloropenicillanate sulfone (**12**), was performed by diazotization-hydrochlorination of ester **10** using the methodology reported by McMillan and Stoodley[18], and subsequent oxidation (Scheme 2).

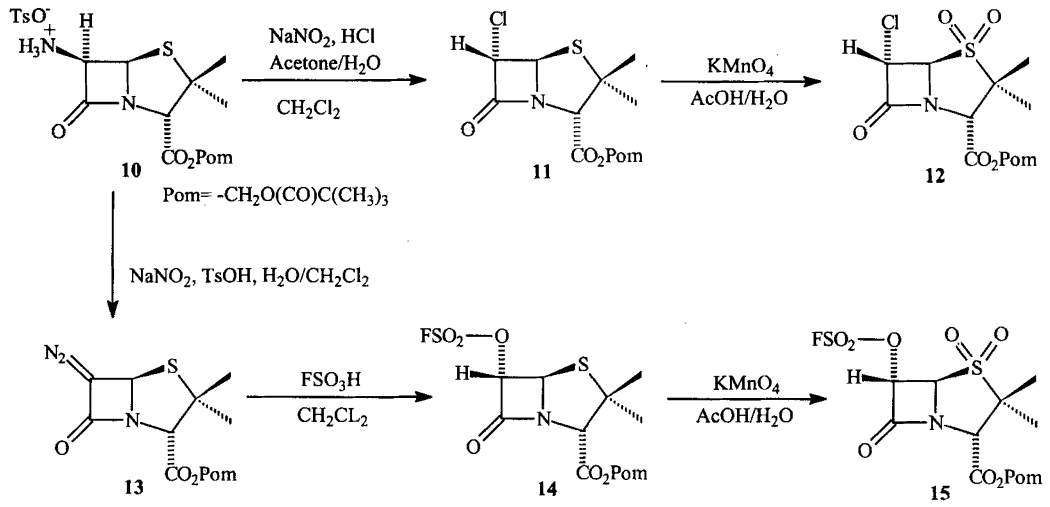
Synthesis of 6α-(sulfonyl)oxypenicillanates

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 (**13**) with fluorosulfonic acid in methylene chloride[14]; oxidation gave the corresponding sulfone (**15**) (Scheme 2).

The preparation of benzyl 6α-hydroxypenicillanate (**16b**) has been described by Sheehan *et al.*[15] The synthesis



Scheme 1.



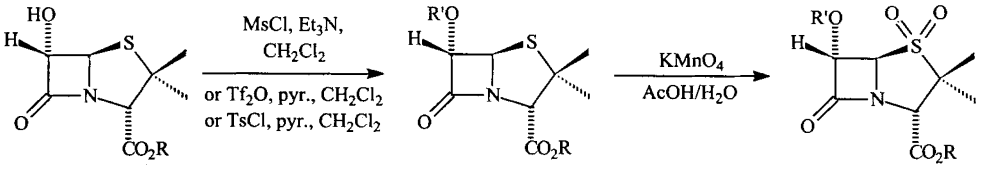
Scheme 2.

of Pom 6α-hydroxypenicillanate (**16a**) from **13**, was done following that procedure (Scheme 3). These carboxylic esters reacted with mesyl chloride, tosyl chloride or trifluoromethanesulfonic anhydride to give the corresponding benzyl and Pom 6α-methanesulfonyl (**17a**$_1$ and **17b**$_1$), 6α-trifluoromethanesulfonyl (**17a**$_2$ and **17b**$_2$) and 6α-*p*-toluenesulfonyl (**17a**$_3$ and **17b**$_3$) derivatives in good yield should be 70 to 90%.

Oxidation gave the corresponding sulfones in very good yields (**18a**$_{1\text{-}3}$, **18b**$_{1\text{-}3}$). The preparation of Pom 6α-(trifluoromethanesulfonyl)oxypenicillanate (**17b**$_2$) was previously reported by us[19] using a different methodology.

Inhibition Studies

In Table 1 are shown the IC_{50} values obtained for the inhibition of PPE by the novel compounds under study. For comparison purposes, values obtained with previously studied compounds (**19** and **20**)[20] are also included. The *tert*-butyl, *iso*-propyl (**6b,c**) as well as the methyl (**19**) esters were only weakly active. On the other hand, IC_{50} values obtained with Pom double ester (**12**) and benzyl ester (**6a**) were at least five times smaller than those obtained with the branched and alkyl unbranched esters. Pivaloyl (**20**)[20] and acetyl (**8a**) esters of 3α-hy-

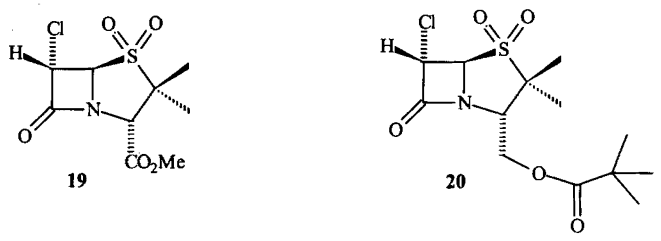


16a R= Bn
16b R= Pom

Bn= -CH_2Ph
Ms= -SO_2CH_3
Tf= -SO_2CF_3
Ts= -$SO_2(p\text{-}CH_3)C_6H_4$

17a$_1$ R= Bn, R'= Ms
17a$_2$ R= Bn, R'= Tf
17a$_3$ R= Bn, R'= Ts
17b$_1$ R= Pom, R'= Ms
17b$_2$ R=Pom, R'= Tf
17b$_3$ R=Pom, R'= Ts

18a$_1$ R= Bn, R'= Ms
18a$_2$ R= Bn, R'= Tf
18a$_3$ R= Bn, R'= Ts
18b$_1$ R= Pom, R'= Ms
18b$_2$ R=Pom, R'= Tf
18b$_3$ R=Pom, R'= Ts



19 **20**

Scheme 3.

Table 1. *In vitro* inhibition of PPE by novel penicillin derivatives.

Compound	Inhibition [IC_{50} (μM)]	
	instantaneous	10 min preincubation
6a[a]	205 ± 40	180 ± 45
6b	1160 ± 60	1210 ± 220
6c	1300 ± 230	1200 ± 140
8a	57 ± 6	68 ± 5
8b	N.D.[c]	
12	280 ± 90	220 ± 60
15	16.7 ± 3.1	--[e]
19[b]	950 (44 ± 3%)[d]	950 (40 ± 5%)[d]
20[b]	15 ± 2	20 ± 5
18a$_1$	2.2 ± 0.2	0.13 ± 0.01
18a$_2$	0.68 ± 0.09	--[e]
18a$_3$[a]	23 ± 6	0.15 ± 0.04
18b$_1$	4.3 ± 0.4	0.54 ± 0.08
18b$_2$	1.0 ± 0.1	--[e]
18b$_3$[a]	2.1 ± 0.2	0.49 ± 0.15

[a] Compounds **6a** and **18a$_3$** were previously reported by Thompson *et al.*[21a] with IC_{50} values against HLE of 16 and 0.05 μM, respectively[b]. These compounds were previously reported by us.[20] [c] Not determined due to insolubility of the compound in the reaction medium [d] Maximun [I] used in the assays; mean inhibition and its standard error obtained are shown in parenthesis[e]. IC_{50} values with preincubation were not determined due to instability of the compounds in the reaction medium.

droxymethyl-6α-chloropenam sulfones were even more potent than the Pom double esters and benzyl esters. None of these compounds are good candidates for a mechanism-based inhibition since: i) it could not be observed a significant decrease in IC_{50} values by preincubation of the enzyme with the inhibitor; and ii) it was not apparent a time-dependent inhibition.

In an attempt to improve activity we decided to prepare new analogues by introducing different 6α-(sulfonyl)oxy substituents, following previously reported results of Thompson *et al.* on HLE inhibition of penicillin esters[21]. The lowest IC_{50} values without preincubation were obtained with the 6α-CF_3SO_3- derivatives (**18a$_2$** and **18b$_2$**). Such compounds, as well as the FSO_3- derivative **15** were so unstable that IC_{50} values with preincubation could not be determined (see Table 1). On the other hand, compounds **18a$_1$**, **18a$_3$**, **18b$_1$**, and **18b$_3$** (containing CH_3SO_3- or (*p*-CH_3)$C_6H_4SO_3$- substituents at C-6) have better stability and exhibited rather low instantaneous IC_{50} values. The IC_{50} values obtained for these compounds when they were preincubated with the enzyme for 10 min, were four to fifteen times lower than those obtained without preincubation. Moreover, they showed a clearly time-dependent inhibition. Therefore, we decided to study thoroughly the kinetic behavior of compound **18a$_1$**.

A procedure similar to that reported by others was used[22]. Fig. 1A shows a typical assay where it can be clearly seen the time-dependency of the inhibition. From similar assays, carried out at different inhibitor and substrate con-

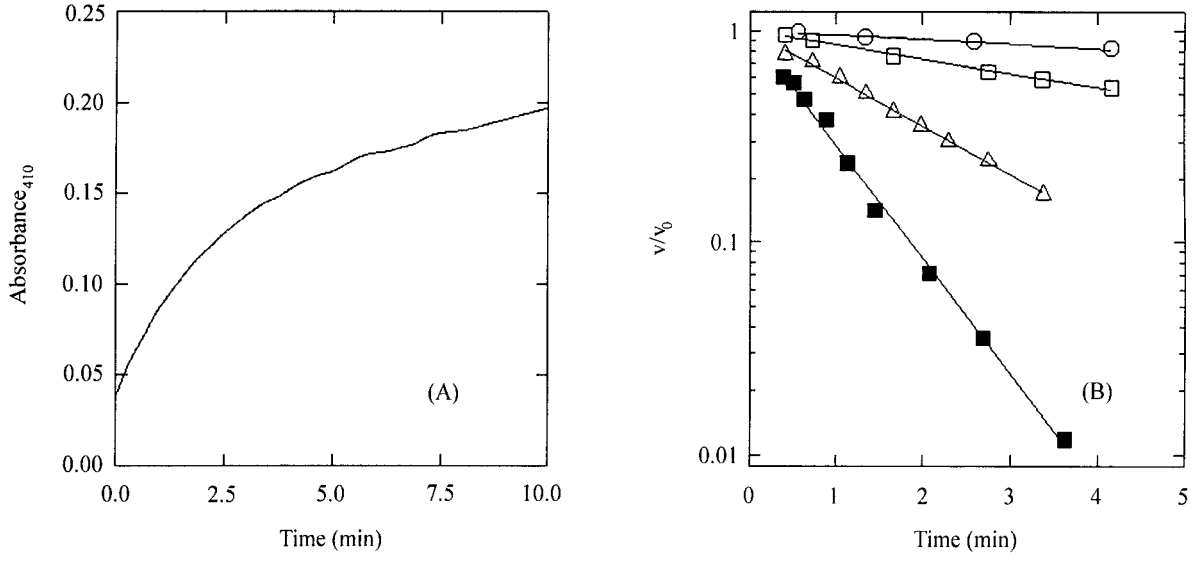
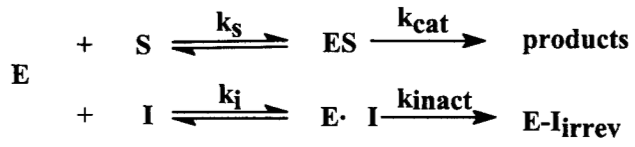


Figure 1. Time-dependent inhibition of PPE *in vitro* activity by compound 18a$_1$. (A) The enzyme activity was measured as indicated under Experimental. The line is the reaction time-course obtained with 590 μM substrate and 2.95 μM inhibitor. The reaction was started by the addition of 17 μg of PPE. A control run carried out in the absence of the inhibitor yielded a linear progress curve at least during 10 min. (B) The slopes at different reaction times of progress curves similar to that shown in Fig. 1(A) were determined by means of a Reaction Rate Software from LKB. The points indicate the residual activity (v/v_0) estimated at different reaction times and at different inhibitor concentrations: 0.49 (O), 0.99 (□), 2.95 (Δ) and 8.72 μM (■). Substrate concentration was 590 μM. The reactions were started by the addition of 17 - 42 μg PPE. The solid lines were obtained by least-square regression analysis to a single exponential decay, from which the pseudo-first order reaction constants (k_{obs}) and their standard deviations could be also estimated.

centrations, the values of velocity at different reaction times could be estimated. In all cases, the decrease in the enzymatic velocity could be fitted to a single exponential decay and the pseudo-first order reaction constant (k_{obs}) and the initial velocity (v_0) could be estimated. In Fig. 1B a semilogarithmic plot of v/v_0 vs time is shown to illustrate the kinetics of enzyme inactivation.

The k_{obs} values depended on $[I](K_m/[S]_0+K_m)$ ([I]') according to a rectangular hyperbola (Fig. 2). From the fitting of the experimental k_{obs} values to such a rectangular hyperbola, the values of K_i (7.4 ± 1.4 μM) and k_{inact} (2.5 ± 0.3 min^{-1}) could be also estimated (see Scheme 4). Therefore, the kinetic behavior of compound **18a**$_1$ is consistent with that of a mechanism-based inhibitor.

As we mentioned above compound **18b**$_2$ was rather unstable. It decomposed rapidly in water solutions ($t_{1/2}$ < 0.5 min). In Fig. 3A it is shown a typical enzymatic assay. After the addition of the enzyme, a linear increase of absorbance was obtained. When the compound **18b**$_2$ was added (after 9 min of reaction) a strong inhibition was instaneously produced which was followed by a slow reactivation of the enzyme. The instantaneous inhibition corresponded well to a competitive one with a K_i = 0.3 μM. We



Scheme 4.

estimated from the curve in Fig. 3A, the reaction velocities at different reaction times. The data obtained are shown in Fig. 3B.

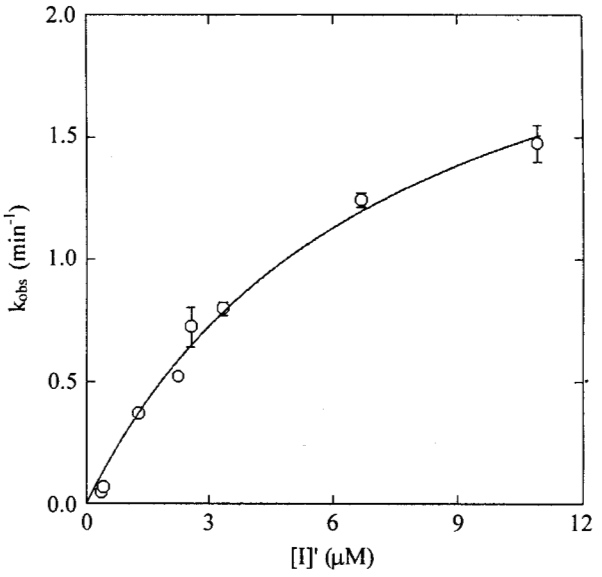
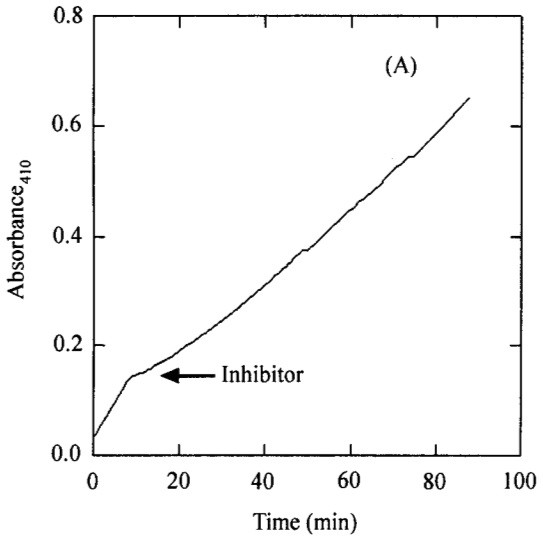


Figure 2. Estimation of K_i and k_i for the reaction between compound 18a$_1$ with PPE. The values of k_{obs} (O) obtained as indicated in Fig. 1-B at different inhibitor and substrate concentrations were plotted against [I]' which is equal to $[I](K_M/(K_M+[S]_0))$. The error bars represent the standard deviations. The solid line indicate the fitting of the estimated k_{obs} values to the following equation: $k_{obs} = k_{inact}[I]'/(K_i+[I]')$. The fitting was carried out by non-linear regression analysis using a least-square algorithm



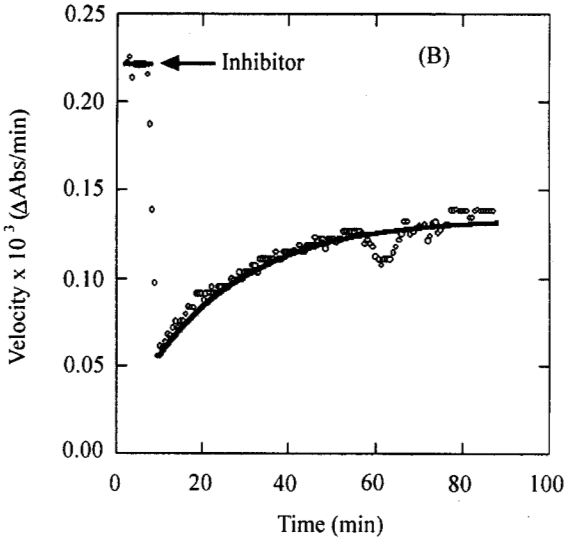


Figure 3. Slow reactivation of PPE inhibited by the unstable compound 18b$_2$. (A) The assay conditions are similar than those described under Experimental. Substrate concentration was 850 μM. The reaction was started by the addition of 0.8 μg PPE. After 9 min 1 μM inhibitor was added. A control experiment was run without inhibitor. The time course of the reaction was under that conditions linear up to 100 min. (B) The points (O) represent the values of velocity calculated at different reaction times from the progress curve in Fig. 3(A). Such values have been calculated as $\Delta Abs/\Delta t$ every 30 s and processed by a smooth procedure. The solid line drawn before the addition of the inhibitor (time < 9 min) coresponds to the uninhibited reaction. The solid line drawn after the addition of the inhibitor (time > 9 min) represents the fitting of the estimated reaction velocities values to an exponential increase to an asimptote (see equation in the text). The fitting was carried out by non-linear regression analysis using a least-square algorithm.

A constant velocity was obtained till the inhibitor was added. Afterwards a fast drop in the reaction velocity could be observed. This almost instantaneous decrease in velocity was followed by a slow increase that could be fitted to the following equation:

$$(\text{velocity})_{t>9\text{ min}} = (\text{velocity})_{t=9\text{ min}} + [(\text{velocity})_{t=\infty} - (\text{velocity})_{t=9\text{ min}}](1 - e^{-k(t-9\text{ min})})$$

The estimated velocity at $t = \infty$, when the [I] is zero due to its unstability, was only 65% of the velocity of the uninhibited reaction (see Table 2) indicating that complete reactivation could be achieved. Hence, in spite of the fact that compound **18b$_2$** is rather unstable, it is capable of inhibiting the enzyme in an irreversible manner. Therefore, it is also likely that compound **18b$_2$** behaves as a mechanism-based inhibitor.

Two alternative mechanisms for the reaction of PPE with **18b$_2$**, that take into account the results described above, are shown in Scheme 5.

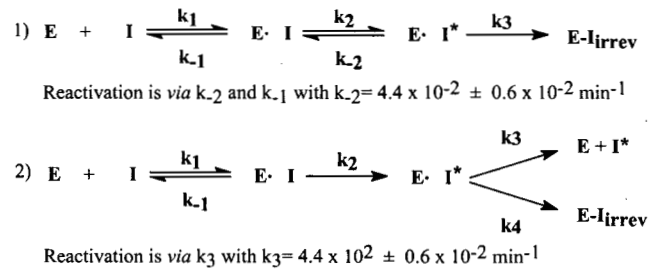
Conclusion

The structure-activity relationships studies around the substituents at C-3α and C-6α positions of the penam sulfones **6a-c**, **8a-b**, **12**, **15**, **18a$_1$-a$_3$**, and **18b$_1$-b$_3$** as inhibitors of PPE has been extended[20] and have demonstrated that these compounds may be fruitfully exploited in designing new elastase inhibitors.

Table 2. Instantaneous inhibition of PPE by **18b$_2$** and slow reactivation due to its unstability.

Reaction time	Velocity (ΔAbs/min)[a]	Inhibition (%)
< 9 min	13.0×10^{-3}	-
9 min	$3.3 \times 10^{-3} \pm 0.3 \times 10^{-3}$	75
> 9 min	$3.3 \times 10^{-3} \pm 5.1\ (1 - e^{-k(t-9\text{ min})})$	variable
∞	$8.4 \times 10^{-3} \pm 0.6 \times 10^{-3}$	35

[a] The values of velocity at time ≤ 9 min were obtained by non-linear regression analysis of the data in Figure 3B analyzed as indicated in the legend of the same figure and in the text. The value at t < 9 min was the mean of the values obtained at shorter times.



Scheme 5.

It is noteworthy that the esters of 3α-hydroxymethyl-6α-chloropenam sulfones (**8a** and **20**) markedly improve the inhibitory activity in comparison with the corresponding esters of 3α-carboxylic acid-6α-chloropenam sulfones **6a-c** and **12**. 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 **18a$_3$**. The SAR study indicated (see Table I) that compounds **18a$_2$** and **18b$_2$** are the most potent in this series. However, the less potent compounds **18a$_1$**, **18a$_3$**, **18b$_1$** and **18b$_3$** were shown to have better stability.

SAR studies accumulated to this date indicate that the essential structural requirements for good elastase inhibitory activity for penicillin derivatives must contain: (i) the electrofilic carbonyl group of β-lactam ring, (ii) the sulfone moiety, (iii) an electron withdrawing group at C-6α position, as well as (iv) a neutral substituent at C-3α position. (See Fig. 4).

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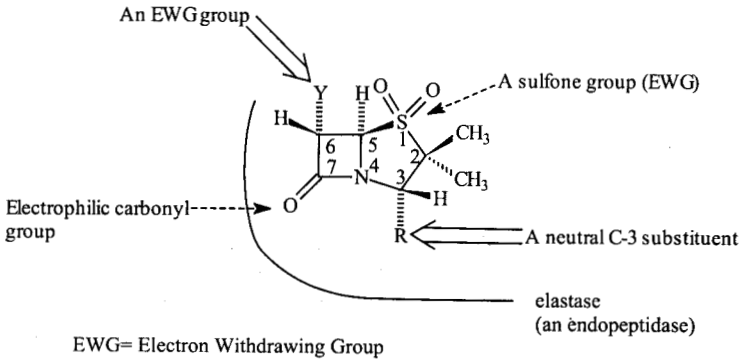

Figure 4. Structural requirements for good elastase inhibitory activity of penicillins derivatives.

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