6-β-(Trifluoromethane sulfonyl)-amido-penicillanic acid sulfone

A potent inhibitor for β-lactamases

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1. INTRODUCTION

β-Lactamases have attracted considerable attention in recent years in view of their ability to confer upon microorganisms resistance to penicillins [1]. It is not surprising to find the emphasis being placed on the development of potent inhibitors of these enzymes. The finding that clavulanic acid, a naturally occurring β-lactam, could inactivate β-lactamase [2–4] provided an impetus for the development of penicillin analogs capable of serving as suicide substrates. Such compounds include: 6-β-bromopenicillanic acid [5,6], penicillanic acid sulfone [7–9], 6-α-chloropenicillanic acid sulfone [10] and quinacillin sulfone [11]. The presence of a sufficiently acidic hydrogen at C_6 and/or a good leaving substituent at C_5 (features favoring facile elimination across the C_6–C_5 bond of the molecule) render penams as potent suicide substrates for β-lactamases. Here, the synthesis of 6-β-(trifluoromethane sulfonyl)-amido-penicillanic acid sulfone, IV, was accomplished so as to incorporate both of the above mentioned features in the penicillin molecule. Compound IV proved to be a more potent inhibitor of β-lactamases than penicillanic acid sulfone and quinacillin sulfone.

2. EXPERIMENTAL

The steps involved in the synthesis of IV are illustrated in fig.1. Treatment of 6-β-aminopenicillanic acid benzyl ester (I) with one equivalent of triflic anhydride in the presence of triethylamine in methylene chloride at – 78°C yeilded monotriflamide (II). Oxidation of II with potassium permanganate in acetic acid [12] resulted in the formation of the sulfone (III). The benzyl ester, III was converted to the corresponding free acid, IV, by catalytic hydrogenation over 10% palladium on carbon in methanol.

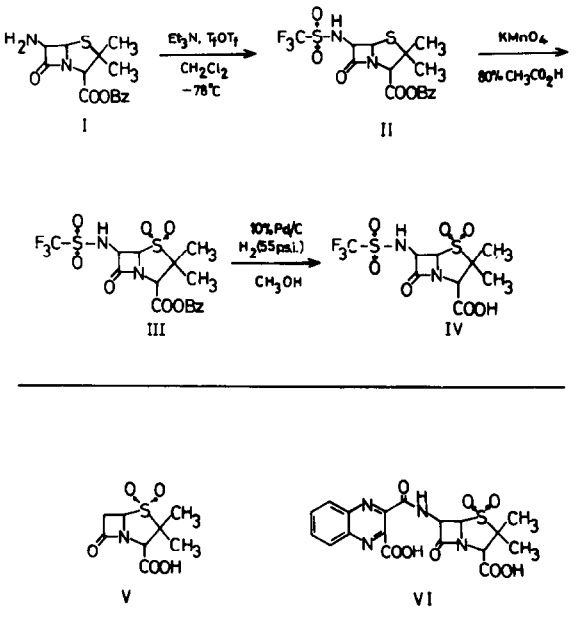

Fig.1. Procedure for the synthesis of 6-β-(trifluoromethane sulfonyl)-amido-penicillanic acid sulfone, IV (TfOTf = triflic anhydride); penicillanic acid sulfone, V; quinacillin sulfone, VI.

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IV:
^{1}H NMR (D_2O, HDO = 4.65) 1.32 (s, 3, –C–CH_3), 1.47 (s, 3, –C–CH_3), 4.20 (s, 1, –NC<u>H</u>COO^-), 4.97 (d, 1, J = 4 Hz, –NCHC<u>H</u>SO_2–), 5.26 (d, 1, J = 4 Hz, –NC<u>H</u>$CHSO_2$–);
^{19}F NMR (D_2O, external standard CF_3COOH set at 0.00 ppm) 3.00 (s, CF_3SO_2–);
IR(NUJOL) 1815 (β-lactam).

The ability of IV to inactivate homogeneous preparations of *B. cereus* 569/H β-lactamase I [13] and of *E. coli* RTEM β-lactamase [2] was investigated by incubating the enzyme (0.6–1.7 μM) with the compound, the final concentration of which was adjusted to achieve the desired molar excess over that of the protein. The progress of the reaction was monitored by periodic measurements of enzymatic activity using benzyl penicillin (1 mM) as substrate [14]. In the assessment of the pH-dependence of the interaction, the buffers employed were 100 mM acetate between pH 4.80–5.5 and 100 mM phosphate at pH > 5.5.

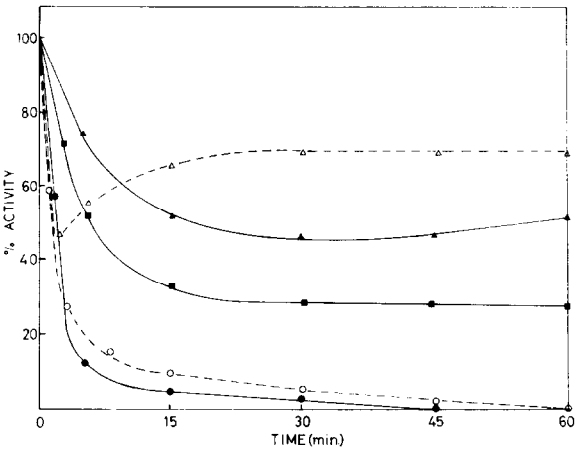

Fig.2. Inactivation of β-lactamase by penam sulfones at 25°C (pH 6.8). *B. cereus* 569/H β-lactamase I: enzyme, 1.67 μM; inhibitor, 1.7 mM; (●—●) IV; (▲—▲) V; (■—■) VI, *E. coli* RTEM β-lactamase: enzyme, 2.5 μM; inhibitor concentrations as shown; (○—○) IV (25 μM); (△—△) V (500 μM).

3. RESULTS AND DISCUSSION

Initial experiments performed with *B. cereus* 569/H β-lactamase I revealed a diminution in inhibitory activity of IV with the increase in the pH of the reaction medium. The observed first-order rate constants of inactivation at pH values of 6.8, 5.0 and 4.0 using a 10-fold molar excess of IV over that of the enzyme were 7.6 × 10^{-5} s^{-1}, 1.0 × 10^{-3} s^{-1} and 1.2 × 10^{-2} s^{-1}, respectively. Consequently, complete inactivation of the enzyme at low pH could be achieved at inhibitor concentrations significantly lower than those required at high pH-values of the reaction medium. Thus, total inhibition of *B. cereus* 569/H β-lactamase I could be achieved by treatment for 30 min with 5- and 120-fold molar excess of IV at pH 4.8 and 6.8, respectively. Experiments with *E. coli* RTEM β-lactamase revealed a pH-dependence of inactivation similar to that noted above, with total inhibition being accomplished upon treatment of the enzyme with 5- and 60-fold molar excess of IV at pH 4.8 and 6.8, respectively.

The potency of IV as an inhibitor of β-lactamases was compared with that of other penam sulfones, such as penicillanic acid sulfone, V (obtained from Bristol Laboratories) and quinacillin sulfone, VI, prepared by the permanganate oxidation [12] of quinacillin (a generous gift from Boots Co. Ltd, Nottingham). The rate constants of inactivation of *B. cereus* 569/H β-lactamase I by V and VI, 2.64 × 10^{-4} s^{-1} and 1.2 × 10^{-2} s^{-1}, respectively, were found to be independent of pH between 4.8–7.0. Complete inactivation of the enzyme required ~ 20 000 and ~ 1300 molar excess of V and VI, respectively. The results of a study of the relative effectiveness of IV, V and VI as inhibitors of *B. cereus* β-lactamase I performed at pH 6.8 using a 1000-fold molar excess of each of the compounds over that of the enzyme are shown in fig.2. Under these conditions, V and VI effected a rapid diminution in enzymatic activity to ~ 50% and 30%, respectively, of the initial value. No further loss of activity was observed upon prolonged incubation. In contrast, IV caused virtually complete and irreversible inactivation of the enzyme. Thus, even at pH 6.8 when its potency as a β-lactamase inhibitor is relatively low (compared to that at pH 4.8), IV is superior to V and VI in the inactivation of the enzyme.

In the interaction of *E. coli* RTEM β-lactamase with V and VI, the recorded values for the inactivation rate constants were 2.64 × 10^{-4} s^{-1} and < 1.5 × 10^{-2} s^{-1}, respectively [9,11]. Complete

inactivation of the enzyme required ~ 7000 and 400 molar excess of V and VI, respectively. A comparison of the effect of IV to that of V on *E. coli* RTEM enzyme is presented in fig. 2. Treatment of the enzyme with 120-fold molar excess of V, at pH 6.8 resulted in rapid diminution in enzymatic activity (to ~ 50% of the initial value) followed by a slow reversal of inhibition leading to partial regeneration of the enzymatic activity. In contrast, IV at a 60-fold molar excess caused complete and irreversible inactivation of the enzyme.

The above observations indicate that the anti-β-lactamase activity of IV is superior to that exhibited by the 2 penam sulfones cited above. The enhanced acidity of the C_6 α-proton as well as the presence of a good leaving group at C_5 of IV renders the molecule more effective as an inhibitor of β-lactamases than penicillanic acid sulfone and quinacillin sulfone which are endowed with only the latter feature. The pH-dependence of inactivation by IV could be a reflection of the dissociation of an essential functional group either in the enzyme or in the inhibitor. However, the former possibility appears remote in view of the absence of such pH-dependence in the action of V and VI cited above. Since the sulfonamide hydrogen of triflamide derivatives can undergo dissociation with 3.0–8.0 [15], such a phenomenon occurring in IV may provide the basis for the decline in its ability to inactivate the enzyme at neutral pH. Implicit in this proposal is the assumed inability of the dianionic species of IV (formed by the dissociation of both the triflamide and carboxyl functions) to interact favorably with the enzyme.

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