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STUDIES ON MONOBACTAMS
II. SYNTHESIS AND β-LACTAMASE INHIBITORY ACTIVITY OF 4α-METHYL-3-[(THIEN-2-YL)-METHYLENE]-2-AZETIDINONE-1-SULFONATE

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Two monobactam derivatives, potassium 4α-methyl-(3*E*)-[(thien-2-yl)methylene]-2-azetidinone-1-sulfonate and its (3*Z*)-isomer, were prepared and evaluated for their β-lactamase inhibitory activities. These compounds were devoid of β-lactamase inhibitory activity.

The apparently endless capacity of β-lactamases to develop the ability to degrade new β-lactams has led to the alternative strategy of seeking inhibitors to block their action. The discovery of clavulanic acid, sulbactam, and tazobactam has confirmed the success of this approach, but none inhibits the class C enzymes. Because of the widespread use of third-generation cephalosporins, resistance caused by chromosomally-mediated class C cephalosporinase is increasing rapidly and may pose a threat in the future. Aztreonam, the first monocyclic β-lactam antibiotic to be clinically used, has long been known to act as a competitive and progressive inhibitor of class C cephalosporinase [1-3]. Continuing efforts in our laboratory have focussed on the design and synthesis of novel β-lactamase inhibitors. Our interest in the monobactam area was in response to the β-lactamase inhibitory activity displayed by several monobactam derivatives [4, 5], including aztreonam [1] and carumonam [6]. Our earlier chemical modification of monobactam [7] revealed that 3-[(N-methyl-1,2,3-triazol-4-yl)methylene]-2-azetidinone-1-sulfonates having various substituents at the C-4 position generally lack the activity against β-lactamases. To correct this deficiency we explored new derivatives with the α-methyl group at the C-4 position. This paper deals with the synthesis and β-lactamase inhibitory activity of potassium 4α-methyl-(3*E*)-[(thien-2-yl)methylene]-2-azetidinone-1-sulfonate (I) and its (3*Z*)-isomer (II) as the representative compounds of this class.

CHEMISTRY

The starting material for the synthesis of these compounds was the previously described (3*S*)-trans-3-[(*tert*-butoxycarbonyl)amino]-4-methyl-2-azetidinone (III) [8]. The azetidinone derivative III was reacted with 2,2,2-trichloroethyl chloroformate under standard conditions to provide the adduct IV in good yield, m.p. 156-157°C. This material was then treated with TFA to remove the amine protecting group to afford the monobactam intermediate V in 97% yield. Then the β-lactam V was reacted with 2.5 N H_2SO_4, $NaNO_2$, and bromine in dichloromethane (Scheme 1) to give the dibromo derivative VI in 63% yield, m.p. 82-83°C. The dibromo derivative VI underwent metal halogen exchange with methylmagnesium bromide in THF at –78°C to give an enolate intermediate which, on quenching with thiophene-2-carboxaldehyde afforded an inseparable diastereomer mixture of hydroxy adducts VII. Acetylation of this mixture gave the acetyl derivative VIII. Reductive elimination using powdered zinc in DMF

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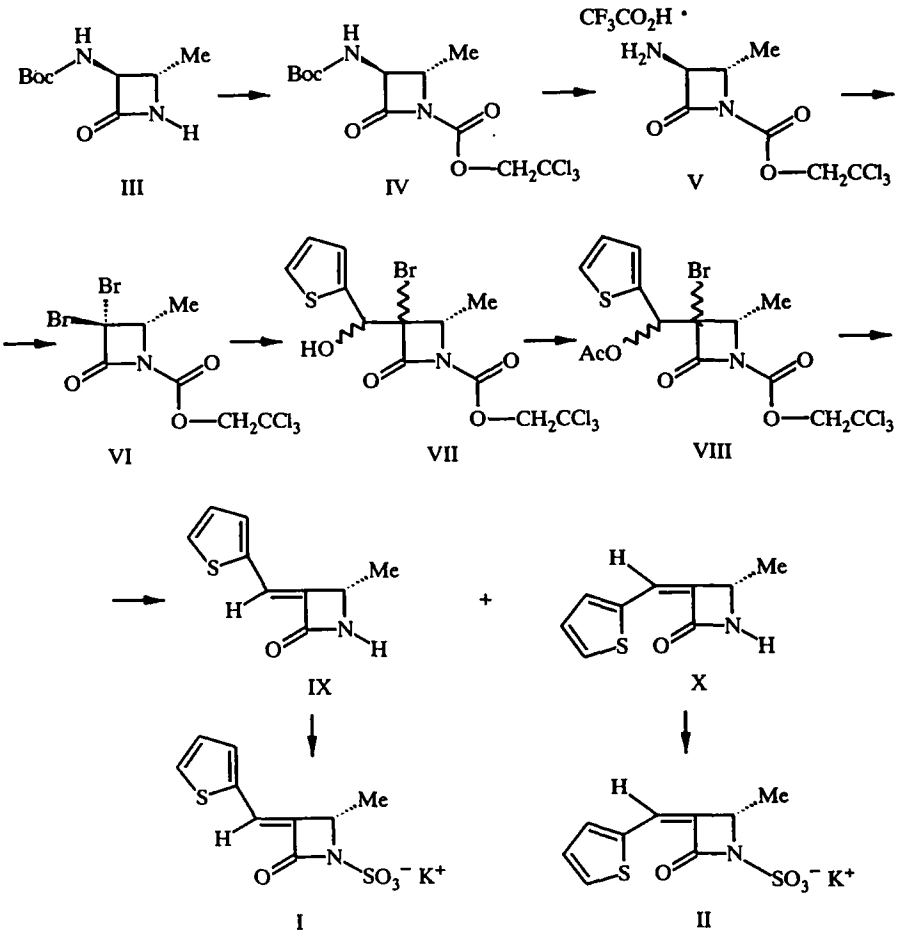
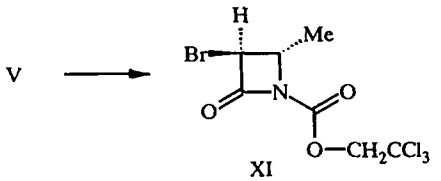
Scheme 1



in the presence of glacial acetic acid afforded a 3.5:1 mixture of the (*E*)- and (*Z*)-thienylmethylene azetidinone derivatives IX and X respectively, which were separated by silica gel column chromatography (Scheme 1).

The configurations of the geometric isomers were assigned by ^{1}H NMR spectroscopy on the basis of the anisotropic deshielding effect of the β-lactam carbonyl on the vinylic proton. The vinylic proton of the (*E*)-isomer IX appeared at δ 7.08, downfield from that of the (*Z*)-isomer X, which appeared at δ 6.69 (solvent DMSO-D6). These derivatives were then treated with DMF-SO_3 complex [9] in dichloromethane. At this point the products were converted to their potassium salts by treatment with $KHCO_3$ solution and purified by reverse phase preparative TLC (CH_3CN—H_2O, 7:1). In this way the (3*E*)-isomer I was obtained in 60% yield and the (3*Z*)-isomer II in 30% yield.

Alternatively, the amino β-lactam V reacted with 2.5 N H_2SO_4, $NaNO_2$, and potassium bromide in 95% ethanol to give the (3*S*)-bromo derivative XI in 22% yield. The β-orientation of the bromo group was evident from the coupling constant of the 3-H and 4-H J = 2.6 Hz.



RESULTS AND DISCUSSION

The β-lactamase inhibitory properties (IC_{50}) of the target monobactams I and II were determined against cell free β-lactamase (cephalosporinase) from *E. cloacae* and *P. aeruginosa* species by spectrophotometrically measuring the hydrolysis of the substrate (cephaloridine) in the presence and absence of the β-lactamase inhibitors. The IC_{50} values for both compounds were >10 μg/ml against the cephalosporinases from *E. cloacae* and *P. aeruginosa*, indicating lack of activity.

The minimum inhibitory concentrations (MIC) of ceftazidime (CAZ) in combination with the monobactams I and II were determined against a series of β-lactamase-producing microorganisms. The bacteria were cultivated in Mueller Hinton Broth (Difco) and diluted to 10^7 cfu/ml, and were then inoculated into the same medium containing ceftazidime and the prepared monobactams in a specific concentration and incubated at 37°C for 20 h. The growth of the microorganisms was observed to determine the MIC for rendering the inoculated medium free from turbidity. None of the compounds showed synergy in combination with CAZ; MICs against all organisms tested were >32 μg/ml.

CONCLUSION

The enzymological and synergy data indicate that the prepared monobactams I and II are completely devoid of β-lactamase inhibitory activity. Although the introduction of a thienylmethylene group at position 6 of the penem skeleton [10] and the introduction of similar 6-(heterocyclyl)methylene groups in the penam sulfone nucleus [11] provided potent β-lactamase inhibitors, a monobactam skeleton having the thienylmethylene group at position 3, like I and II, lacks β-lactamase inhibitory activity, thus suggesting the necessity of the oxyimino substituted amido side chain at position 3 of aztreonam for β-lactamase inhibitory activity.

EXPERIMENTAL

Melting points were determined on a Thomas—Hoover melting point apparatus and are uncorrected. The ^{1}H-NMR spectra (δ ppm) were obtained in DMSO-D_6 with TMS as an internal standard on a Bruker AC-200-F (200 MHz) spectrometer.

Analytical results were determined by the Department of Chemistry, University of Alberta. All column chromatographic purifications were accomplished on silica gel 60 (E Merck, 230-400 mesh) with the appropriate solvent gradients.

3(*S*)-[*tert*-Butoxycarbonyl)amino]-4-(*R*)-methyl-1-(2,2,2-trichloroethoxycarbonyl)-2-azetidinone (IV). To a stirred and ice-cooled solution of compound III [8] (28.0 g, 139.83 mmoles) in dichloromethane (150 ml) was added triethylamine (16.98 g, 167.8 mmoles) followed by dropwise addition of a solution of 2,2,2-trichloroethyl chloroformate (32.59 g, 153.82 mmoles) in dichloromethane (20 ml). After the addition was over, the cooling bath was removed and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with water and the organic layer was separated out, washed with brine, dried (Na_2SO_4), and concentrated to give a gummy residue (55.3 g). To the residue, a mixture of ether (250 ml) and hexane (450 ml) was added and stirred at –70°C for 30 min. The precipitated solid was collected by filtration (29.2 g). Purification of the mother liquor over a silica gel column afforded an additional amount of the product (2.33 g). Total weight 31.53 g, 60% yield. An analytical sample was obtained by crystallization (ethyl acetate–hexane); m.p. 156-157°C, decomp. ^{1}H-NMR (DMSO-D_6): 1.39 (9H, s,); 1.49 (3H, d, J = 6.2 Hz); 4.07-4.18 (1H, m,); 4.34 (1H, dd, J = 3.5 and 8.0 Hz); 5.00 (2H, ABq, J = 12.3 Hz); 7.68 (1H, d, J = 8.0 Hz). Found, %: C 38.01; H 4.63; N 7.30. $C_{12}H_{17}Cl_3N_2O_5$. Calculated, %: C 38.37; H 4.56; N 7.46.

3-(*S*)-Amino-4-(*R*)-methyl-1-(2,2,2-trichloroethoxycarbonyl)-2-azetidinone trifluoroacetate salt (V). To a solution of Boc-protected azetidinone IV (25.0 g, 66.55 mmoles) in dichloromethane (48 ml) at –5°C was added anisole (48 ml), followed by TFA (360 ml), and the mixture was stirred at –5°C for 3.5 h. After removal of the solvent under reduced pressure, the gummy residue was triturated with a mixture of hexane—ether (1:2) under cooling (–78°C). The precipitated solid was collected by filtration (25.2 g, 97% yield). This product was used in the next step without

further purification. ^{1}H-NMR (DMSO-D$_6$): 1.55 (3H, d, J = 6.3 Hz); 4.16-4.24 (1H, m,); 4.44 (1H, d, J = 3.2 Hz); 5.04 (2H, ABq, J = 12.2 Hz); 8.50-9.80 (3H, br, s,).

3,3-Dibromo-4-(S)-methyl-1-(2,2,2-trichloroethoxycarbonyl)-2-azetidinone (VI). To 300 ml of dichloromethane, cooled to ~5 °C, were added with stirring bromine (16.2 ml), 2.5 N H_2SO_4 (159 ml), and $NaNO_2$ (14.5 g). The ammonium salt V (40.90 g, 0.105 mole) was added portionwise over 15 min maintaining the temperature of the reaction mixture between 4-10°C. The resulting dark red solution was stirred at 5°C for an additional 1 h. A solution of 1 M $Na_2S_2O_3$ was added dropwise until the bromine color was discharged. The organic layer was separated out and the aqueous layer was extracted with dichloromethane. The combined dichloromethane extracts were washed with brine, dried (Na_2SO_4), and concentrated under vacuum to give a yellow gum which was purified by silica gel column chromatography (ethyl acetate—hexane, 1:3) to afford the compound VI as a solid (27.8 g, 63% yield). Analytical sample was obtained by recrystallization from diethyl ether hexane to give a crystalline solid m.p. 82-83°C. ^{1}H-NMR (DMSO-D$_6$): 1.56 (3H, d, J = 6.3 Hz); 4.79 (1H, q, J = 6.3 Hz); 5.02 (2H, ABq, J = 12.2 Hz). Found, %: C 20.36; H 1.26; N 3.34. $C_7H_6Cl_3Br_2NO_3$. Calculated, %: C 20.10; H 1.45; N 3.35.

3-(*S*)-Bromo-4-(*R*)-methyl-1-(2,2,2-trichloroethoxycarbonyl)-2-azetidinone (XI). To a 50 ml three-necked round-bottomed flask equipped with a magnetic stirrer, a thermometer, and containing the ammonium salt V (1.42 g, 3.65 mmoles), cooled to ~5 °C, was added dropwise an ice-cooled solution of 2.5 N H_2SO_4 (15 ml). Then KBr (2.19 g, 18.41 mmoles) was added, followed by dropwise addition of 95% ethanol (12 ml) over 10 min, keeping the temperature below 4°C. A solution of $NaNO_2$ (387 mg) in water (3 ml) was added dropwise over 5 min to the reaction mixture. The reaction was stirred at ice bath temperature for 3.5 h, and the ethanol was evaporated off under vacuum. The resulting mixture was extracted with dichloromethane and the extract was washed with brine, dried (Na_2SO_4), and concentrated to give a yellow gum (855 mg). Purification by silica gel column chromatography (ethyl acetate—hexane, 1:5) afforded the compound XI as a viscous oil (270 mg, 22% yield). ^{1}H-NMR (DMSO-D$_6$): 1.56 (3H; d, J = 6.2 Hz); 4.29 (1H, dq, J = 2.6 Hz and 6.3 Hz); 5.01 (2H, ABq, J = 12.2 Hz); 5.13 (1H, d, J = 2.6 Hz). Found, %: C 24.56; H 2.02; N 4.20. $C_7H_7Cl_3BrNO_3$. Calculated, %: C 24.77; H 2.08; N 4.13.

3-Bromo-3-[1-hydroxy-1-(thien-2-yl)methyl]-4-(R)-methyl-1-(2,2,2-trichloroethoxycarbonyl)-2-azetidinone (VII). To a solution of the dibromo derivative VI (1.44 g, 3.44 mmoles) in dry THF (25 ml) under N_2 at -78°C was added dropwise a solution of CH_3MgBr (1.49 ml, 4.47 mmoles, 3 M solution in diethyl ether), and the resulting golden yellow solution was stirred for 15 min. To this solution, a solution of 2-thiophenecarboxaldehyde in dry THF was added dropwise and the mixture was stirred at -78°C for an additional 25 min. The reaction was quenched by addition of 2 ml of saturated aqueous ammonium chloride, extracted with EtOAc, and the organic layer was washed with water, brine, dried (Na_2SO_4), and concentrated to give a yellow gum. Purification by silica gel column chromatography (ethyl acetate—hexane, 1:3) gave the compound VII, as a yellow gum (904 mg, 58% yield). Found, %: C 32.01; H 2.31; N 3.20. $C_{12}H_{11}BrCl_3NO_4S$. Calculated, %: C 31.92; H 2.46; N 3.10. The product was obtained as a mixture of isomers and was utilized in the next step without further separation.

1-(2,2,2-Trichloroethyloxycarbonyl)-3-[1-acetoxy-1-(thiophen-2-yl)methyl]-3-bromo-4-(*R*)-methyl-2-azetidinone (VIII). To an ice-cooled solution of compound VII (858 mg, 1.90 mmol) in dry THF (20 ml) was added pyridine (1.69 ml, 20.90 mmol) and stirred for 10 min; acetic anhydride (1.79 ml, 19.0 mmol) was added and the reaction mixture was stirred at room temp. for 21 h. To the reaction mixture, ice water (50 ml) was added, stirred for 1 h, and extracted with dichloromethane. The organic layer was washed with a solution of 10% HCl, water, brine, dried (Na_2SO_4), and concentrated under vacuum to give the product VIII as a yellow viscous oil (901 mg, 96% yield). The product was used in the next step without further purification. Found, %: C 24.56; H 2.02; N 4.20. $C_7H_7Cl_3BrNO_3$. Calculated, %: C 24.77; H 2.08; N 4.13.

4-(*R*)-Methyl-(3*E*)-[(thien-2-yl)methylene]-2-azetidinone (IX) and 4-(*R*)-Methyl-(3*Z*)-[(thien-2-yl)methylene]-2-azetidinone (X). A solution of the bromoacetate VIII (840 mg, 1.702 mmol) in DMF (10 ml) was cooled to 0°C and glacial acetic acid (779 µl, 13.62 mmol) was added. Freshly activated zinc powder (929 mg) was then added to the reaction and the mixture was stirred at ice temperature for 5 min and 2 h at room temp. The reaction mixture was diluted with ethyl acetate, filtered through a bed of Celite. The filtrate was diluted with water (180 ml) and extracted thoroughly with ethyl acetate. The ethyl acetate extracts were combined and washed successively with water, $NaHCO_3$, and brine; then dried (Na_2SO_4) and concentrated to give a solid. The crude product was purified over a silica gel column. Fast eluting component (hexane—ethyl acetate, 2:1) was the (3Z)-isomer X (48 mg, 16%). ^{1}H-NMR (DMSO-D$_6$): 1.31 (3H, d, J = 6.1 Hz); 4.10 (1H, m); 6.69 (1H, s); 7.09 (1H, dd, J = 3.3 Hz and 5.1 Hz); 7.61 (1H, d, J = 5.1 Hz); 7.71 (1H, d, J = 3.3 Hz); 8.64 (1H, br. s). Found, %: C 59.98; H 5.01; N 7.78. C_9H_9NOS. Calculated, %: C 60.33; H 5.06; N 7.82.

The next eluting component (hexane—ethyl acetate, 1:1) was the (3*E*)-isomer IX (169 mg, 55%). ^{1}H-NMR (DMSO-D_6): 1.43 (3H, d, J = 6.0 Hz); 4.36 (1H, m); 7.08 (1H, s); 7.14 (1H, dd, J = 3.6 Hz and 5.1 Hz); 7.34 (1H, d, J = 3.5 Hz); 7.72 (1H, d, J = 5.1 Hz); 8.63 (1H, br. s). Found, %: C 60.60; H 4.95; N 7.71. C_9H_9NOS. Calculated, %: C 60.33; H 5.06; N 7.82.

Potassium 4-(*R*)-methyl-(3*E*)-[(thien-2-yl)methylene]-2-azetidinone-1-sulfonate (I). To an ice-cooled solution of compound IX (90 mg, 0.502 mmol) in dichloromethane (8 ml) was added DMF·SO_3 complex (92 mg, 0.6025 mmol), and the reaction mixture was stirred at ice temperature for 1 h. Evaporation of the solvent under reduced pressure gave a foam. The foam was taken in water (2 ml), and a solution of $KHCO_3$ (65 mg dissolved in 5 ml of water) was added and stirred at room temperature for 15 min. The solution was concentrated under vacuum to a small volume and purified by reverse phase preparative TLC (acetonitrile—water, 7:1). After freeze-drying the potassium salt was obtained as a fluffy white solid (89 mg, 60% yield). ^{1}H-NMR (DMSO-D_6): 1.54 (3H, d, J = 6.1 Hz); 4.47 (1H, m); 7.16 (1H, dd, J = 3.6 Hz and 5.0 Hz); 7.20 (1H, s); 7.39 (1H, d, J = 3.4 Hz); 7.75 (1H, d, J = 5.1 Hz). Found, %: C 36.40; H 2.74; N 4.69. $C_9H_8KNO_4S_2$. Calculated, %: C 36.35; H 2.71; N 4.71.

Potassium 4-(R)-Methyl-(3*Z*)-[(thien-2-yl)methylene]-2-azetidinone-1-sulfonate (II). The compound II was prepared in 30% yield in a similar manner from X. ^{1}H-NMR (DMSO-D_6): 1.40 (3H, d, J = 6.1 Hz); 4.22 (1H, m); 6.80 (1H, s); 7.12 (1H, dd, J = 3.7 Hz and 5.1 Hz); 7.66 (1H, d, J = 5.1 Hz); 7.70 (1H, d, J = 3.6 Hz). Found, FAB [M+1]: 298. $C_9H_8KNO_4S_2$. Calculated, M: 297.

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