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(2,3)-α-METHYLENEPENICILLANIC ACID SULFONE: SYNTHESIS AND β-LACTAMASE INHIBITING PROPERTIES

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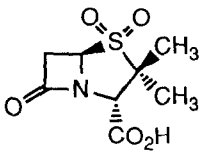
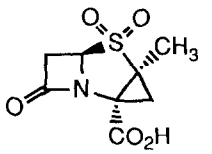
Abstract: The synthesis and β-lactamase inhibiting properties of 2,3-α-methylenepenicillanic acid sulfone (**3**) are described. The results presented are consistent with previous work indicating that β-lactamases recognize α-methylenepenams as cephalosporins.

Previous studies of the (2,3)-methylene analogs of penicillin G supported the proposal that the active conformation of penicillins is the open conformation (carboxy group pseudoequatorial) and that the position of the carboxy in space relative to the β-lactam carbonyl is an important determinant of the character of a β-lactam (cephalosporin-like or penicillin-like).[1,2,3] During these studies it was shown that the (2,3)-β-methylene analogs of penicillins generally had much poorer antibacterial activity than the penicillins themeselves. On the other hand, it was also shown that **2**, the (2,3)-β-methylene analog of sulbactam (**1**, a clinically useful β-lactamase inhibitor),[4] was comparable to sulbactam as an inhibitor of β-lactamases.



1



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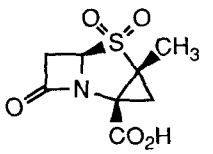
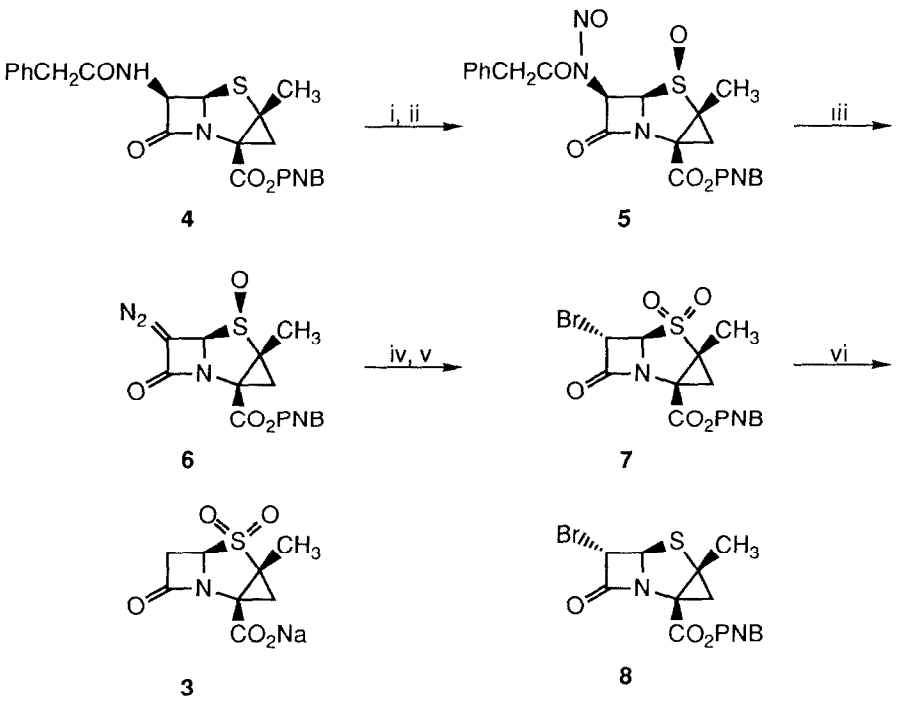


3

Since the position in space of the carboxy group relative to the β-lactam carbonyl in **3** is similar to the relative position of these groups in a cephalosporin,[1] we were particularly interested in determining its relative potency against a cephalosporinase and a penicillinase, and in comparing the results with the values for **1** and **2**. In light of the earlier results, we expected **3** to be more potent against cephalosporinases.

The synthesis of **3** is outlined in Scheme 1. Oxidation of the (2,3)-α-methylenepenam **4**[1,5] with m-chloroperbenzoic acid, followed by reaction with N_2O_4 at -12° C gave the nitroso derivative **5** in 76% yield. Subsequent treatment of **5** with 4-dimethylaminopyridine produced the diazo compound **6** in 73% yield. Bromination and oxidation to yield **7** (26%) was accomplished by treatment of **6** with 1 equivalent of HBr in EtOAc at -17° C, followed by reaction with excess $KMnO_4$. Use of more than 1 equivalent of HBr during the bromination resulted in concomitant sulfoxide reduction to form **8**. The best yields were achieved following the protocol described in Scheme 1. Finally, debromination and ester deprotection of **7** was achieved in 65% overall yield by hydrogenolysis over 10% Pd/C in the presence of $NaHCO_3$ to give the sodium salt of **3**.[6]



Scheme 1. (i) m-CPBA, 0° C, CH_2Cl_2 (85%); (ii) N_2O_4, AcONa, -12° C, 3 hrs, CH_2Cl_2 (89%); (iii) 4-dimethylaminopyridine, -12° C, CH_2Cl_2 (73%); (iv) HBr, -17° C, AcOEt (48%); (v) $KMnO_4$, $AcOH/H_2O$ (55%); (vi) 10% Pd/C, H_2 (55 psi), $NaHCO_3$, H_2O/AcOEt (65%).

For enzymatic studies, β-lactamases were purified from *E. cloacae* P99 and a TEM-1-producing strain of *E. coli* essentially by published methods.[7,8,9] Purified *Staphylococcus aureus* penicillinase was obtained from the Centre for Applied Microbiology and Research (Porton Down, England). β-Lactamase assays were conducted at 30° C in 40 mM sodium phosphate buffer, pH 7.0 using 100 μM nitrocefin as substrate. Various concentrations of inhibitors were assayed to determine the concentrations required for 50% inhibition (IC_{50}) of nitrocefin hydrolysis, which was determined at 486 nm. Reactions were initiated by addition of substrate after ten minutes pre-incubation of enzyme and inhibitor. Under these conditions, the *E. cloacae* enzyme may be considered to be primarily a cephalosporinase, while the *S. aureus* enzyme is primarily a penicillinase and the TEM-1 enzyme hydrolyzes both classes of β-lactams at comparable rates.[10]

Table 1

	IC_{50} (μM)		
β-Lactamase	Sulbactam (**1**)[11]	**2**[11]	**3**
E. cloacae P99	20	322	12
E. coli TEM-1	1.9	2.5	406
S. aureus PC1	6.8	14	548

As shown in Table 1, the (2,3)-α-methylene compound, **3**, was a better inhibitor of the *E. cloacae* P99 cephalosporinase than either sulbactam or the β-methylene compound, but it was a much poorer inhibitor of the TEM-1 enzyme and the *S. aureus* penicillinase. These results are consistent with previous work indicating that the penicillinase recognizes β-methylenepenams as penicillins, but recognizes α-methylenepenams more as cephalosporins.[2] In the present study, both the *S. aureus* and *E. cloacae* β-lactamases clearly distinguished between the α- and β-methylene sulbactam analogs, apparently recognizing the β-methylene analog, **2**, as a penam and the α-methylene analog, **3**, as a cephem.

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References and Notes

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6. **5**, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.29, 1.81 (AB, 2H, J = 8.4 Hz), 1.76 (s, 3H), 4.50 (d, 1H, J = 1.9 Hz), 4.50, 4.54 (AB, 2H, J = 14 Hz), 5.39 (s, 2H), 6.11 (d, 1H, J = 1.9 Hz), 7.33 (s, 5H), 7.63 (d, 2H, J = 8.7 Hz), 8.22 (d, 2H, J = 8.7 Hz).
7, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.78 (s, 3H), 1.86, 2.03 (AB, 2H, J = 8.8 Hz), 4.38 (d, 1H, J = 1.2 Hz), 5.05 (d, 1H, J = 1.2 Hz), 5.40 (s, 2H), 7.64 (d, 2H J = 8.8 Hz), 8.26 (d, 2H, J = 8.8 Hz).
3, ^{1}H NMR (D_2O, 400 MHz): δ 1.69, 2.13 (AB, 2H, J = 8.6 Hz), 1.76 (s, 3H), 3.29 (d, 1H, J = 16.6 Hz), 3.64 (dd, 1H, J = 16.6 and 4.7 Hz), 4.77 (m, 1H).
8, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.64, 1.74 (AB, 2H J = 7.7 Hz), 1.73 (s, 3H), 4.73 (s, 2H), 5.39 (s, 2H), 7.67 (d, 2H, J = 8.8 Hz), 8.25 (d, 2H, J = 8.8 Hz).
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11. Compounds **1** and **2** were prepared as previously described.[3]