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SYNTHESIS OF A POTENT β-LACTAMASE INHIBITOR- 1,1-DIOXO-6-(2-PYRIDYL)METHYLENEPENICILLANIC ACID AND ITS REACTION WITH SODIUM METHOXIDE

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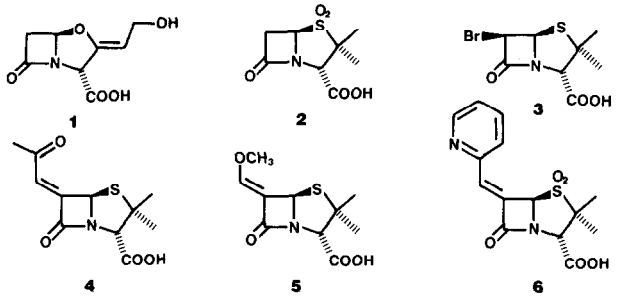
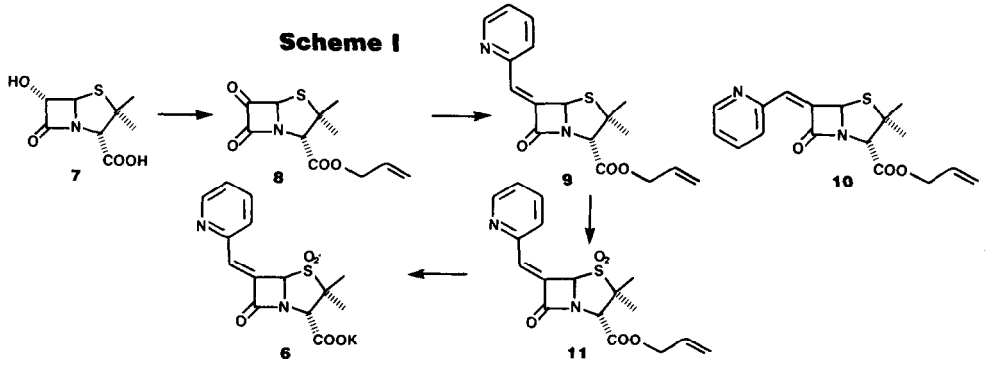
Summary: 1,1-Dioxo-6-(2-pyridyl)methylenepenicillanic acid (**6**) was prepared and found to be a potent β-lactamase inhibitor. Its reaction with sodium methoxide was studied to provide insight into its mechanism of enzyme inactivation.

Since the discovery of the first clinically important β-lactamase inhibitor clavulanic acid (**1**)[1], many new mechanism-based inactivators, for example **2-5** have appeared in the literature[2]. Mechanistic studies[3] indicated that a variety of pathways of enzyme inhibition are followed by these compounds. In the course of studying structural modification of sulbactam (**2**), we identified 6-(2-pyridyl)methylene penam sulfone (**6**) as an effective β-lactamase inhibitor. Here we report the synthesis of **6** and its reaction with sodium methoxide, which we believe to be relevant to its mechanism of enzyme inactivation.



The steps involved in the synthesis of **6** are illustrated in Scheme I. Allyl 6-oxopenicillanate (**8**) was prepared by oxidation of the allyl ester of **7**[4] using trifluroacetic anhydride and DMSO in the presence

Scheme I



of triethylamine at -78°C[5]. A 95% yield of oil **8** was obtained which was used without further purification. Compound **8** was treated with the Wittig reagent prepared from 2-picolyltriphenylphosphonium chloride and sodium amide in THF, at -78°C for 3 minutes to afford a mixture of Z isomer **9** and a trace amount of E isomer **10**. Compound **9** was isolated as an oil in 61% yield after silica gel column chromatography. Oxidation of **9** with m-chloroperbenzoic acid gave the corresponding crystalline sulfone **11** in 66% yield. Deallylation was achieved in quantitative yield by the method of Jeffrey and McCombie[6].

Compound **6** inactivated a variety of important β-lactamases (Table I). Because of its inhibitory activity, we were interested in the mechanism of enzyme inactivation. Earlier studies[8] have shown that the chemical reaction of these inhibitors with a good nucleophile, such as sodium methoxide, can be a good model for rationalizing the enzyme inhibition process. We, therefore, examined methanolysis of the allyl ester of **6** to provide information about the possible interaction between β-lactamase active site serine residues and **6**.

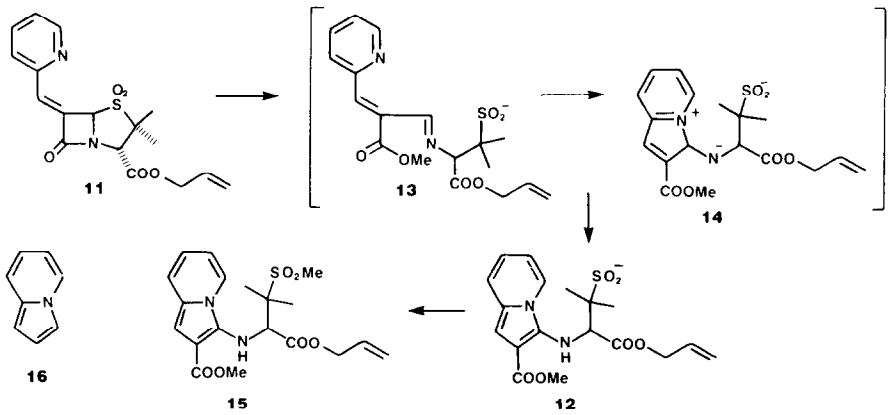
Table I. % Inhibition of substrate hydrolysis[7]

Source of β-Lactamase		[I] μM/[S] μM*	% Inhibition		
			6	clavulanic acid	17
S. aureus	01A400	8/32	90	85	0
E. coli	51A129	1/32	100	81	28

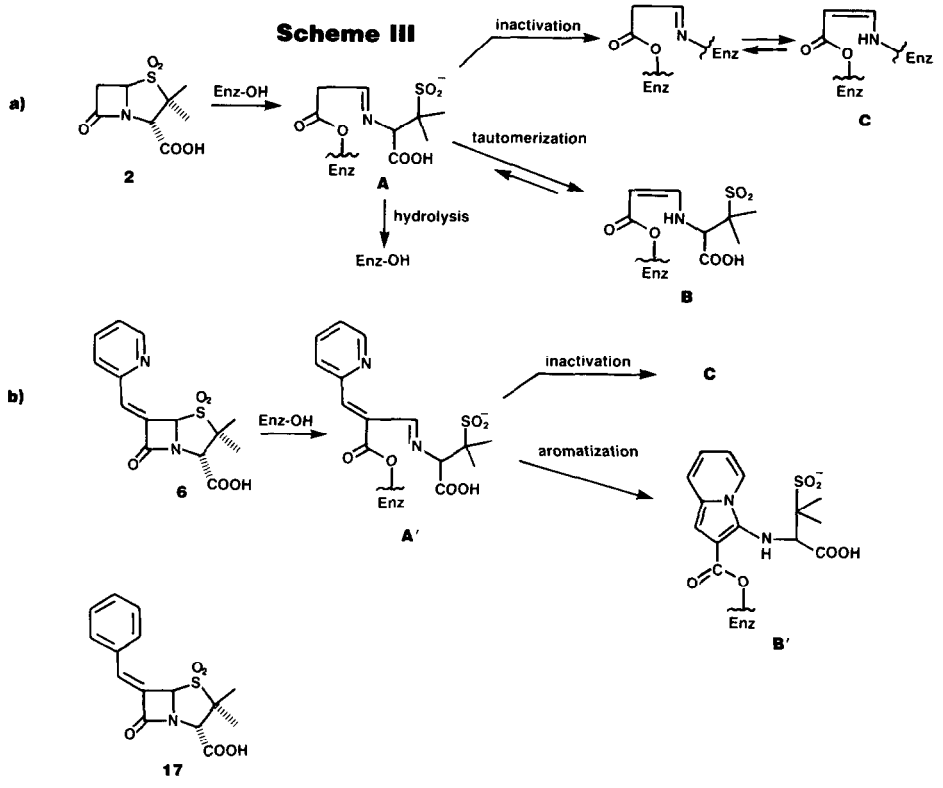
* [I] = inhibitor concentration
[S] = ampicillin concentration

Compound **11** was treated with 1 eq. of sodium methoxide in methanol at r.t. for 10 minutes to give a yellow solid **12** in 85% yield. The structure of **12** was assigned based on comparison of ^{1}HNMR, ^{13}CNMR, and UV spectra[9a] with the spectra of the basic skeleton, pyrrocoline **16**[10]. In addition, methylsulfone **15** was obtained by methylation of **12** with methyl iodide. The mass spectrum, ^{1}HNMR and UV spectra of **15** were also recorded[9b]. The transformation of **11** to **12** seems likely to involve intermediate **13** and **14** (see Scheme II).

Scheme II



Knowles, *et al.*[3c], have provided convincing evidence that sulbactam inhibition of the RTEM β-lactamase proceeds through stages **A**, **B**, and **C** (Scheme III). On the basis of the methanolysis of **11**, we propose a possible mechanistic pathway by which **6** might inactivate β-lactamase, involving intermediates **A′**, **B′**, and **C′**. In the case of sulbactam, intermediate **B** can regenerate active enzyme via tautomerization to **A**. On the other hand, aromatic **B′** derived from **6** cannot return to **A′** and apparently is more resistant to hydrolysis than **B**, making **6** a more effective β-lactamase inhibitor. Further support for the proposed mechanism is evident in the poorer inhibitory activity of phenyl analog, **17**[11], which cannot form a heterocyclic intermediate corresponding to **B′** (see Table I).



In conclusion, based on the methanolysis study, we propose that compound **6** proceeds through a novel mechanism of enzyme inhibition. The introduction of a (2-pyridyl)methylene group at C_6 of sulbactam leads to increased potency of inhibitory activity.

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9. a) Physical data of **12**: ^{1}HNMR($CDCl_3$) δ1.0(s,3H), 1.25(s,3H), 3.7(s,3H), 4.2(t,2H), 4.3 (d,J = 8Hz,1H), 4.9(m,2H), 5.6(m,1H), 6.0(d,J = 8Hz,1H; D_2O exchangeable), 6.3(s,1H), 6.3-6.5(m,2H), 7.1(d,1H), 7.9(d,1H) ppm; ^{13}CNMR($CDCl_3$) δ16.9, 17.5, 51.5, 57.7, 63.6, 65.2, 98.0, 108.2, 111.0, 117.0, 118.2, 119.9, 122.0, 127.5, 131.6, 132.3, 166.8, 171.5 ppm; UV(MeOH) $\epsilon = 2.537 \times 10^4$ at 243 nm. b) Physical data of **15**: ^{1}HNMR($CDCl_3$) δ1.5(s,3H), 1.8(s,3H), 3.3(s,3H,SO_2Me), 3.9(s,3H), 4.4(d,2H), 4.7(d,J = 12Hz,1H), 5.0-5.2(m,2H), 5.5-5.7(m,1H), 6.3(d,J = 12Hz,1H,NH), 6.5-6.7(m,3H), 7.3(d,1H), 8.2(d,1H)ppm; UV(MeOH) $\epsilon = 2.460 \times 10^4$ at 240 nm; MS408 (M+, 29%), 381, 328, 287, 229, 189 (100%), 157.

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