Chapter 16. Structure-Activity Relationships of "Non-Classical" β-Lactam Antibiotics

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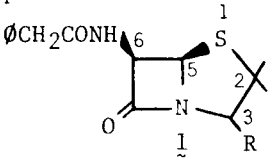
Introduction - Although examples of semi-synthetic penicillins 1 (R = COOH) and cephalosporins 2 (R = COOH) have been known for over twenty years, the thousands of semi-synthetic analogs which have been prepared by medicinal chemists are almost exclusively the C6(7)-amide type. The only other common variants are those produced at the C-3 methylene of cephalosporins by nucleophilic displacement of the naturally-occurring acetoxyl function. These simple derivatives or "classical" β-lactam antibiotics have been the subject of many reviews[1] and will not be considered here. Instead, attention will be directed to a discussion of the biological activities of those derivatives in which the bicyclic nucleus itself is altered either by the addition of a substituent[2] or by the synthesis[3,4] or isolation of a new nucleus itself. This report will attempt to highlight the unusual structural features of these "non-classical" analogs and detail the effects of these features on biological activity.

S-1 Substituents - It has long been supposed that oxidation of penicillins and cephalosporins to the sulfoxide or sulfone results in a marked loss of antibacterial activity.[1] In contrast to the penicillin sulfoxides and the S-cephalosporin sulfoxides, R-sulfoxides of various cephalosporins when compared to the parents show variable changes in biological activity.[5] However R-sulfoxides of cephalosporins are usually more active than the S-isomer *in vitro* and 7-phenylacetamidodeacetoxycephalosporanic acid R-sulfoxide has even higher antibacterial activity than the unoxidized parent.

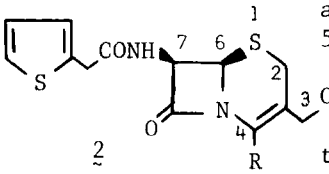
Carboxyl Variations - Early attempts to modify the C-3(4) carboxyl groups of penicillins and cephalosporins were largely discouraging.[1] The homopenicillins and cephalosporins 1b[6] and 2b[7] were obtained from 1a and 2a. The cephem aldehyde, 2g,[8] has been prepared from the 4-hydroxymethyl precursor *via* a Moffatt oxidation. The aldehyde was then converted to 2h-m. None of these derivatives are active at <25 μg/ml. All modifications, 1a,b and 2a-f were less active analogs, supporting the contention that an acidic function at C-3(4) is essential for activity. The importance of the position of the carboxyl function is indicated by the lack of bioactivity in 1b and 2b. It is of interest to note that the homopenicillins are powerful inducers of penicillinase formation. Replacement of the carboxyl function with a phosphonate[9] results in a less active compound than the analogous cephalosporin.



1a, R = $-COCHN_2$
1b, R = $-CH_2CO_2H$

One of the more interesting modifications of the penicillin molecule has been the conversion of the C-3 carboxyl group to that of a tetrazole. Two examples of 3-(5-tetrazolyl) penams have been reported to date. CP-35,587,[10] the better documented of the two analogs, is more potent than amoxicillin against *Klebsiella pneumoniae, Enterobacter, Serratia marcescens, Citrobacter, Providentia* and *Salmonella typhimurium*. Although less

ISBN 0-12-040513-X



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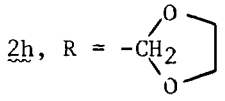
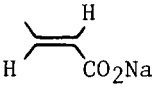
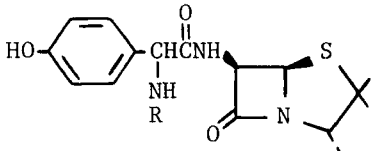
2a, R = $-COCHN_2$
2b, R = $-CH_2CO_2H$
2c, R = $-COCH_2Cl$
2d, R = $-COCH_2OAc$
2e, R = $-COCH_2OMe$
2f, R = $-CH_2OH$
2g, R = -CHO
2h, R = 
2i, R = -CH=NOH
2j, R = -CH=NOMe
2k, R = $-CH=NNHCONH_2$
2l, R = $-CH=NOCH_2CO_2H$
2m, R = 

active than amoxicillin against other strains, CP-35, 587 nevertheless is highly potent (1.56-12 μg/ml). The extension of spectrum is presumably due to the stability of β-lactamase conferred upon the compound by the tetrazolyl functionality. The extrapolation of the *in vitro* activities to *in vivo* efficacy in protecting mice against experimental infections has also been demonstrated. CP-38,118 is also stable to penicillinase and the extension of its spectrum to include anti-pseudomonal activity is claimed.[11] Thus, the substitution of a 3-(5-tetrazolyl) function for a carboxyl in the penicillin series seems compatible with retention of bioactivity while conferring increased β-lactamase stability to the penam nucleus.

C-5(6) Substituents - The bridgehead position has not been substituted extensively in penicillins or cephalosporins. Only one example in each case is known. Penicillin V has been substituted at C-5 by an α-phenyl group.[12] The compound shows only 1/1000 the activity of the parent compound. An α-methoxy group has been substituted at the C-6 position of cephalothin.[13] Again, the compound has a very low order of activity. The low activity of the 6α-methoxy cephalosporin derivative is not easily understood since 7α-methoxy cephalothin has excellent activity.[14]



CP-35,587: R = -H
CP-38,118: R = $-COCH_2NHCONH-\overset{\overset{NH}{\|}}{C}-NH_2$

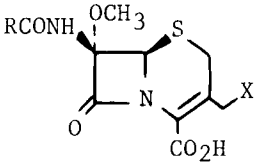
C-6(7) Substituents - The discovery of the naturally-occurring cephamycin[15] family of 7α-methoxycephalosporins has stimulated the synthesis of a large number of C-6(7) substituents in the penicillin and cephalosporin series. The primary interest in this class derives from their stability to β-lactamase. Substitution at C-7 in the cephalosporin series uniformly increases stability to these enzymes. Under conditions where >96% of cephalothin is destroyed by the β-lactamase from *E. coli* MB 2885, the following C-7 substituted analogs of cephalothin were destroyed to the indicated extent:[14]

TABLE I

7-Substituent	-H	$-OCH_3$	$-OCH_2CH_3$	$-SCH_3$	$-CH_2OH$
% Destruction	>96%	16%	0%	20%	0%

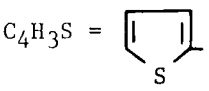
Disruption of the azetidinone by β-lactamase appears to be extremely sensitive to steric bulk in the 7α position. On the other hand, while the introduction of a 7α-methoxy group produces cephalosporin analogs with high antibacterial potency, there is a rapid decrease in activity as the size of the ether group is increased. Other groups, such as CHO, $COCH_3$, NHCOOEt, CH_3 and -CH(Ø)OH, are also devoid of useful activity and indeed these groups and even the 6α-methoxy group on penicillin does not lead to useful compounds. Thus, the utility of the 7α-methoxy group is due to its unique ability to confer stability of the cephalosporin molecule to β-lactamase while retain-

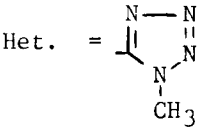
ing full antibacterial activity. It has long been clear that since the 7α-methoxycephalosporin class of β-lactam antibiotics possesses the attributes of the cephalosporins combined with β-lactamase stability, a family of new clinically useful antibacterials should result. Cefoxitin,[16] 3, is the most studied of this type. Cefoxitin, when compared to a classical cephalosporin, *e.g.*, cephalothin 2 (R = COOH), shows comparable safety, pharmacokinetics,[17] and inhibition of the cephalosporin sensitive organisms.[18] However, because of its β-lactamase stability,[19] 3 is also active against many cephalosporin-resistant gram-negative bacteria including the anaerobic organism, *Bacteroides fragilis*.[20]



Three other 7α-methoxycephalosporins have received considerable attention. They are 4 (CS-1170),[21] 5 (SKF 73678)[22] and 6 (SQ 14,359).[23] They share similar pharmacokinetics to cefoxitin and enhanced *in vitro* potency.

	R	X
3,	$C_4H_3S-CH_2-$	$-OC-NH_2$ (O)
4,	$NCCH_2-SCH_2-$	-SHet.
5,	CF_3S-CH_2-	-SHet.
6,	C_4H_3S-CH-	-SHet.



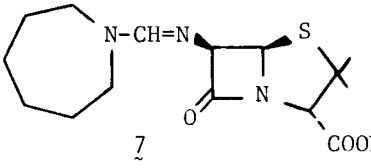


C-6(7) Side Chains Other than Those Derived from C-6(7) Amino Function - A number of penicillins and cephalosporins having side chains other than those derived from the C-6(7) amino group have been prepared.

Simple groups (Cl, Br, OH, CH_3) on penicillins[24] or cephalosporins[25] give compounds devoid of activity. The details of the activity of penicillin and cephalosporin analogs in which the nitrogen of the amide side chain has been replaced by O, CH_2 or S,[26] are not available but all show some antibacterial activity. Thus, though a β-amide is necessary for good activity, any carbonyl function isosteric with the amide seems to contribute positively toward antibiotic activity.

The 1-hydroxyethyl side chain is of considerable interest when it occurs on the C-6α position of the very active naturally-occurring thienamycin.[61] This side chain has been substituted on penicillins and cephalosporins at the C-6(7) α and β positions. The compounds obtained were markedly lower in activity than those with the amide side chain. Surprisingly, the C-6 β (1-hydroxyethyl) penicillin is slightly more active than the α isomer.[27]

β-Amidino Penicillins - These remarkable examples of non-amide side chain β-lactams have been reviewed recently[28] and will not be commented on further. Mecillinam (FL1060) 7 is used clinically.



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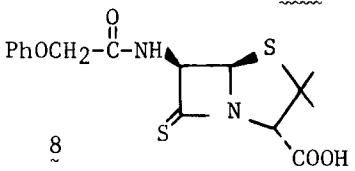
Substitution of the β-Lactam Carbonyl Oxygen - Compounds 8 and 9 in which the β-lactam carbonyl oxygen is replaced by a sulfur have been reported.[29] These compounds have from 1/20 to 1/1000 the activity of the parent compounds. This may be a reflection of the larger bond order and decreased reactivity of the 4-7 (5-8) C-N bond.

Nuclear Analogs of Cephalosporins - In 1971, Lowe and co-workers[30] described 10, a compound in which the sulfur atom of a cephalosporin was replaced by carbon. Compound 10 was inactive because it lacked the Δ^3 double bond necessary for activity in the cephalosporin system. The first nuclear analogs which had all the features of cephalothin (2, R = COOH) except that the sulfur atom was replaced by oxygen[31] and carbon[32] were prepared by total synthesis. Compounds 11a (R^1 = thienylacetyl; R^2 = H; R^3 = OAc; X = O) and 11b (R^1 = thienylacetyl; R^2 = H; R^3 = OAc; X = CH_2) were found to parallel cephalothin in their antibiotic spectrum with the oxygen analog 11a about twice as active and the carbon analog 11b 1/2 as active as cephalothin. This was the first demonstration of the fact that the sulfur atom was not essential for antibiotic activity in the cephalosporins. This has been followed by the total synthesis of oxa and carba derivatives of cefamandole, 11c (R^1 = mandelyl; R^2 = H; R^3 = 5-thiomethyltetrazolyl; X = O), 11d (R^1 = mandelyl; R^2 = H; R^3 = 5-thio-1-methyltetrazolyl; X = CH_2), and the 1-carba derivatives of cefoxitin,[33] 11e (R^1 = thienylacetyl; R^2 = OCH_3; R^3 = $OCONH_2$; X = CH_2). The activity of these compounds again compares favorably with the corresponding cephalosporin derivative.



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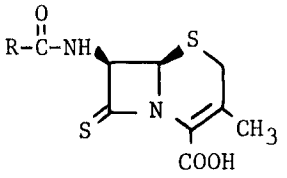
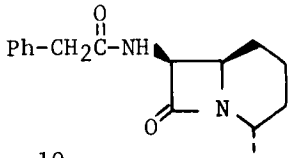


9a, R = $PhOCH_2$
b, R = $PhCH_2$



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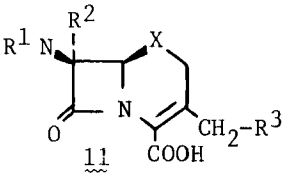

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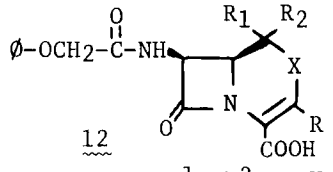


12

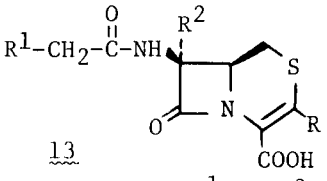
	R	R^1	R^2	X
a,	H	H	H	O
b,	CH_3	H	H	O
c,	$CH_2\emptyset$	H	H	O
d,	$CH_2CH_2\emptyset$	H	H	O
e,	CH_3	CH_3	H	O
f,	CH_3	H	CH_3	O
g,	CH_3	- O -		O
h,	H	H	H	$N\text{-}CH_3$
i,	H	H	H	$N\text{-}\overset{O}{\overset{\|}{C}}\text{-}OC_2H_5$

0-2-Isocephems - Compounds 12a to 12d, obtained *via* total synthesis by Doyle and co-workers,[34] show activity which is somewhat better than or comparable to the corresponding cephalosporins. 12e and 12f, in which the 1-CH_2 group is substituted by an α-methyl or β-methyl, have also been synthesized.[35] These are reported to have antibacterial activity but the details are not reported. A very interesting compound, 12g, in which the 1-CH_2 is replaced by a carbonyl has also been made.[36] This lactone acid, 12g, is unstable and the instability is due to opening of the highly strained and reactive β-lactam carbonyl (whose IR frequency appears at 1800 cm^{-1}) and not due to hydrolysis of the lactone functionality. 12g appears to be too unstable to be a useful antibiotic.

N-2-Isocephems - Doyle and co-workers have also reported the synthesis of N-2-isocephems, 12h and 12i.[37] Compound 12h is relatively unstable and also has a low order of antibiotic activity. However, 12i is more stable and is active as an antibiotic but the details of the activity have not been reported.

2-Isocephems - Two groups have reported the synthesis of 2-isocephem compounds, 13. Com-

pounds 13 are reported as quite active.[38,39] The activity of 13a has been compared with the corresponding cephalosporin.[39] Its gram-positive activity is poorer but it shows better gram-negative activity. The 7α-methyl-2-isocephem, 13e, has been reported by Lowe,[40] which like the 7α-methyl cephalosporins,[41] also shows very low antibacterial activity. Thus a 7α-methyl group is detrimental to activity in both nuclei. Transposition of the hetero atom to position 2 gives rise to compounds whose antibiotic activity shows some difference in their gram-positive and gram-negative spectra, but the change in spectrum or overall activity is not drastically different from the corresponding cephalosporins. This finding is commensurate with the small changes in the reactivity of the β-lactam caused by shifting of the hetero atom or replacement of the sulfur by oxygen.

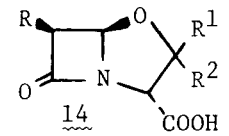


13

	R	R^1	R^2
a,	H	2-thienyl	H
b,	H	ØO-	H
c,	CH_3	ØO-	H
d,	CH_2OAc	ØO-	I
e,	H	C_6H_5	CH_3

Compounds with 3 Hetero Atoms in the 6-Membered Ring of Cephalosporins - A number of compounds of this type have recently been reported. They lack Δ^3 double bond and show very little or no activity.[42,43]

1-Oxapenams - Penicillins in which the sulfur atom of a penicillin nucleus is substituted by the smaller oxygen atom have been reported.[44,45] The compounds 14a, b, and c are considerably less active than the corresponding penicillins. This is surprising since fusion of the β-lactam ring to the oxazolidine ring should give a more strained, more reactive β-lactam than the β-lactam of a penicillin. The C-3 epimer of 14c is unstable.[45]



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a, R = $C_6H_5CH_2C(=O)-NH$; $R^1 = R^2 = H$

b, R = $C_6H_5OCH_2C(=O)-NH$; $R^1 = R^2 = H$

c, R = $C_6H_5OCH_2C(=O)-NH$; $R^1 = R^2 = CH_3$

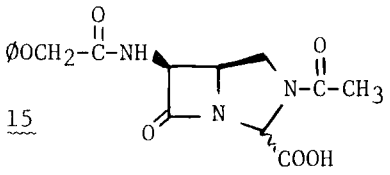
N-2-Isopenam - Though the 1-aza penicillin nucleus has not been reported, the 2-aza-penicillin 15 has been reported recently.[46] This compound (mixture of C-3 epimers) is unstable in aqueous solution but shows low antibacterial activity against *B. subtilis*, *S. aureus* and *Shigella paradysenteriae*. The IR of the benzyl ester of 15 shows a β-lactam absorption at 1795 cm^{-1} indicating a highly strained β-lactam which should be more reactive. This is reflected in the instability of 15 but not in its bioactivity.

Carbapenam - A nuclear analog of penicillin in which the sulfur is replaced by carbon has been reported.[47] Compound 16 is reported as unstable.

2-Penem Nucleus - Woodward and co-workers[48] have reported the synthesis of 17. These have antibacterial activity but the details are not available. The β-lactam carbonyl of the *t*-butyl ester of 17 (R = H) appears at 1805 cm^{-1} indicating a highly strained, reactive β-lactam. Whether this implies that it is relatively unstable or has very high antibiotic activity is not reported.

1-Oxa-2-Penem Nucleus - The oxygen analog of the penem nucleus, 17, has been

recently synthesized.[49] 18 is unstable as the ester. The free acid has not been reported. In the case of the saturated nuclear analogs of penicillins, replacement of the ring sulfur by smaller atoms seems to give unstable compounds. In spite of the more strained β-lactams in these systems, the antibiotic activity is lower, contrary to expectation. It would appear that a certain amount of chemical stability is essential for good activity. Analogs of the penicillin nuclei which have a double bond in the 5-membered ring are extremely interesting. Though compound 18 is probably unstable, compound 17 and thienamycin and its epimers are relatively stable and thienamycin shows excellent antibiotic activity.



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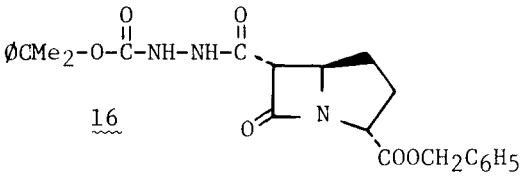

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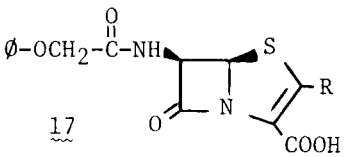
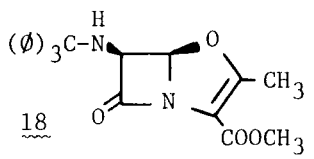


17

R = H, CH_3, CH_2(cyclopropyl), $C_6H_4NO_2$(p)

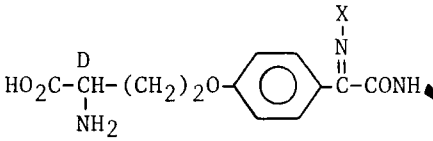


18

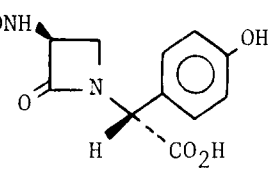
New, Naturally-Occurring β-Lactam Antibiotics - Nocardicin A and B - The first of a series of structurally remarkable β-lactam antibiotics was reported by the Fujisawa group. Nocardicin A and B[50] have been assigned structures 19 and 20 respectively. These new antibiotics were isolated from *Nocardia uniformis* subsp. *tsuyamaniasis*[51] and were surprisingly active when one recalls the lack of potency[52] exhibited by 21. It should be noted that these workers ascribe the name 3-aminonocardicinic acid (3-ANA) to the nucleus, 22, by analogy to the penicillanic and cephalosporanic acid nomenclature. Nocardicin A is stable to the action of several β-lactamases (except from *Proteus vulgaris*), but demonstrates poor gram-positive activity (MIC's of 50 and 800 against *B. subtilis* and *S. aureus*, respectively). However, some quite low MIC's against gram-negative organisms have been observed, *e.g.*, *Sh. sonnei*, 12.5; *Sal. typhimurium*, 25; *A. lwoffi*, 3.13; *Pr. mirabilis*, 1.56; *Pr. vulgaris*, 1.56; *Pr. rettgeri*, 3.13; *Pr. inconstans*, 12.5; *Ps. aeruginosa*, 12.5; *N. gonorrhoeae*, 1.56 and *N. meningitidis*, 1.56. Other gram-negative MIC's ranged from 100-800 μg/ml. In addition to its novel structure, perhaps the other most surprising feature is the unexpectedly high *in vivo* activity which is observed. This enhanced potency may be related to the observed increase in *in vitro* bactericidal activity in the presence of rabbit polymorphonuclear leukocytes.[53] Nocardicin A is more effective than carbenicillin in some gram-negative animal infections.[54] No cross resistance is seen between Nocardicin A and other β-lactam antibiotics and higher serum levels than carbenicillin are observed.[55]

Clavulanic Acid - Clavulanic acid 23[56] is the second in a structurally remarkable series of naturally-occurring β-lactam antibiotics of novel structure isolated during the last few years. Its antibacterial activity has been characterized as "weak," but it is a potent β-lactamase inhibitor.[57] Levels of 1-10 μg/ml, when added to penicillinase-labile penicillins, *e.g.*, ampicillin or amoxicillin, can protect the antibiotics and extend their

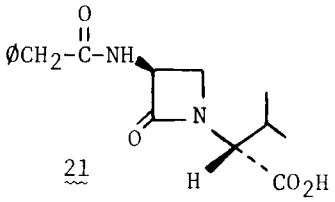
spectrum to include resistant strains of *Staphylococcus*, *Klebsiella*, *Proteus* and *E. coli*.[58] I_{50}s (μg/ml) of 0.06, .03, .03 and .08 are reported for β-lactamases isolated from these organisms. Table II shows the *in vitro* protection afforded by clavulanic acid to ampicillin in the presence of these β-lactamases. Clavulanic acid is a progressive and irreversible inhibitor of these enzymes. 25a-c were prepared by total synthesis and reported as moderate inhibitors, while 25d is a potent inhibitor.



19, X = -syn(Z)OH
20, X = -anti(E)OH



21



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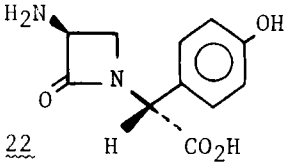

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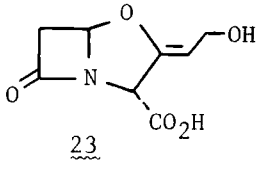

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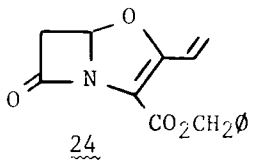

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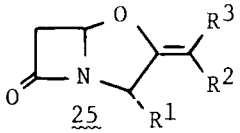


a, R^1 = H; R^2 = $COOCH_3$; R^3 = H
b, R^1 = H; R^2 = H; R^3 = $COOCH_3$
c, R^1 = H, R^2 = $COOCH_3$; R^3 = Cl
d, R^1 = COONa; R^2 = H; R^3 = Ø

The mode of action of clavulanic acid is similar to other β-lactam antibiotics since it binds to the same enzyme site as benzylpenicillin.[59] Clavulanic acid has been converted to the novel 1-oxapenem system 24,[60] but no bioactivities are reported.

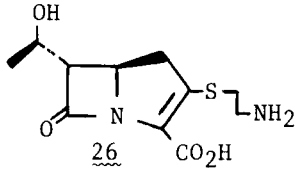
Thienamycin - Thienamycin[61] (26) is the final and perhaps most remarkable of the naturally-occurring β-lactam antibiotics of novel structure found during the past few years. Although its properties are only described in abstracts,[62] it appears to be the most potent and broadest spectrum β-lactam anti-

TABLE II

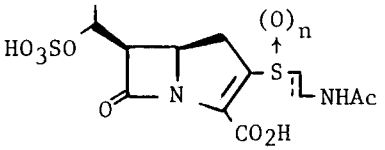
	MIC (μg/ml)			
	S. aureus	*K. aerogenes*	*Pr. mirabilis*	*E. coli*
Na Clavulanate alone	15	31	62-125	31
Ampicillin alone	500	250	>2000	>2000
Ampicillin + 5 μg/ml Na Clavulanate	.02	.1	8	4

biotic reported. MIC's and, with few exceptions, MBC's of ≤10 μg/ml were reported for both gram-positive and gram-negative bacteria including *Pseudomonas*, *Serratia* and anaerobic *Bacteroides* species. 26 is fully effective against β-lactamase-producing strains at the same levels. Presumably the 2-carbon chain of the 6α-hydroxyethyl side chain provides β-lactamase stability while the hydroxyl can hydrogen bond to the same site as the amide side chain of classical β-lactams. Thienamycin is not absorbed orally, but is effective s.c. at levels of 0.005-0.2 mg/kg for gram-positive infections

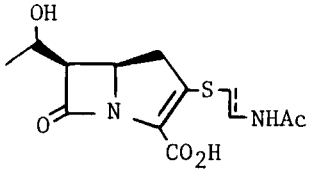
and 2-10 mg/kg for gram-negative ones. Against *Ps. aeruginosa*, thienamycin is active at levels one-quarter those for gentamicin. Thienamycin also binds to the same protein-binding site as benzylpenicillin.[59] Several "epi" thienamycins have been reported.[64] 27, 28 and 29 are "powerful" inhibitors of β-lactamases and "potent" antibacterials.[63] 27 (also known as MM13902) is more potent than 28 (MM 4550) inhibiting a wide range of bacteria at concentrations less than 5 μg/ml. 30-33 have also been isolated.[65] All are cell-wall biosynthesis inhibitors, but while some have equal or greater activity against the *Enterobacteriaceae* than thienamycin, none are as active against other organisms and up to a ten-fold difference is observed. Wide variation in β-lactamase stability is also observed.



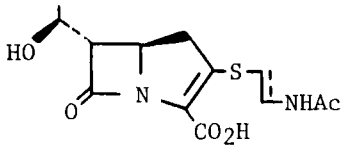
26



27, n = 0 (side chain unsaturated)
28, n = 1 (side chain unsaturated)
29, n = 0 (side chain saturated)



30 (side chain saturated)
31 (side chain unsaturated)



32 (side chain saturated)
33 (side chain unsaturated)

Conclusion - The previous efforts of medicinal chemists to vary the C-6(7) β-amide side chains of penicillins and cephalosporins, as well as the C-3 position of cephalosporins remains an important part of medicinal chemical lore and have provided many different clinically useful antibiotics. The exact medical position of the "non-classical" β-lactam antibiotics remains unknown, for only two of these new-generation antibiotics, cefoxitin and mecillinam have been approved in any country. One conclusion, however, has become abundantly clear. Virtually all of the "classical" structure-activity relationships of β-lactam antibiotics,[34,66] must be revised. The S-1 sulfur atom may be modified (α-sulfoxide) or replaced (by oxygen or methylene) with full retention of activity. Similarly, the carboxyl group may be replaced by an isosteric acidic function (tetrazolyl) with not only retention of activity but with the added feature of β-lactamase stability. Cephalosporanic acids may be substituted with a methoxyl function conferring β-lactamase stability upon the molecule. The penicillin and cephalosporanic acid nuclei, previously considered essential for activity, may be replaced by other bicyclic nuclei (thienamycin) and by a monocyclic nucleus (Nocardicin A). Finally, the C-6(7) amide side chain may be absent while retaining full or even enhanced potency (thienamycin). Indeed when these new structural features, all of which are consistent with full biological activity, are considered, the only essential common feature remains the azetidinone ring system itself.

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