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Effect of *N*-Formimidoyl Thienamycin (MK0787) on β-Lactamases and Activity Against β-Lactamase-Producing Strains

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N-Formimidoyl thienamycin (MK0787) was active against β-lactamase-producing strains and was an effective inhibitor of β-lactamases extracted and purified from these strains except for plasmid-mediated penicillinase type IV.

N-Formimidoyl thienamycin (MK0787) (Fig. 1) is a crystalline derivative of the novel β-lactam antibiotic thienamycin (7, 9) showing improved stability in the solid state and in solution. The antibacterial spectrum of the compound is considerably broader than that of known penicillins and cephalosporins (H. Kropp, J. G. Sundelof, J. S. Kahan, F. M. Kahan, and J. Birnbaum, Program Abstr. 11th Int. Congr. Chemother. and 19th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 231, 1979).

The activity of this new compound was tested against 10 β-lactamase-producing *Enterobacteriaceae* strains, 3 β-lactamase-producing *Pseudomonas* strains, and 1 β-lactamase-producing *Bacteroides* strain, and, in addition, the β-lactamase-inhibiting activity of MK0787 was compared with that of clavulanic acid by determining the kinetic constants for the inhibition of hydrolysis (K_i) of cephaloridine. The minimal inhibitory concentrations of the strains were determined by an agar dilution technique. For the *Enterobacteriaceae* and *Pseudomonas* strains, the test medium was Mueller-Hinton agar (Nissui Pharmaceutical Co., Ltd.). For *Bacteroides fragilis*, the test medium was GAM agar (Nissui Pharmaceutical Co., Ltd.). Plates were inoculated with 5 μl of 10^6 colony-forming units per ml. Chromosomally mediated β-lactamases were extracted and purified from *Escherichia coli* GN5482, *Enterobacter cloacae* GN7471, *Citrobacter freundii* GN7391, *Serratia marcescens* GN10857, *Pseudomonas aeruginosa* GN10362, *Proteus rettgeri* GN4430, *Proteus morganii* GN5407, *Proteus vulgaris* GN7919, *Pseudomonas cepacia* GN11164, and *B. fragilis* GN11477. Plasmid-mediated penicillinase type I (Rms 212), type II (Rms 213), and type III (Rte 16) (6, 11, 12) were extracted and purified from the transconjugants of these plasmids in *E. coli* W3630. Plasmid-mediated penicillinase type IV (Rms 139) (5) was extracted and purified from the transconjugants of this plasmid in *P. aeruginosa* M1. Purified β-lactamases prepared by published methods (1, 5, 10) were used in some experiments. The β-lactamase assay was done by the spectrophotometric method, as described previously (4, 8).

Table 1 summarizes the susceptibility testing results with MK0787 and 14 β-lactamase-producing strains. MK0787 proved to be remarkably active against β-lactamase-producing organisms with minimal inhibitory concentrations ranging from 0.2 to 1.56 μg/ml. The K_i values of MK0787

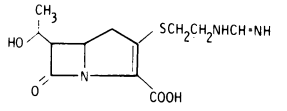

FIG. 1. *Chemical structure of N-formimidoyl thienamycin.*

and clavulanic acid that inhibit hydrolysis of cephaloridine by various β-lactamases are shown in Table 2. Clavulanic acid (2, 3) has been shown to be an effective inhibitor of certain β-lactamases. In this investigation, clavulanic acid also exhibited inhibitory activity in a competitive manner against *P. vulgaris*, *P. cepacia*, and *B. fragilis* β-lactamases and plasmid-mediated penicillinase types I, II, III, and IV. On the other hand, MK0787 has been found to be a competitive inhibitor of β-lactamases except for plasmid-mediated penicillinase type IV. In the cases of *E. coli*, *E. cloacae*, *C. freundii*, *S. marcescens*, *P. morganii*, *P. rettgeri*, and *P. vulgaris*, the K_i values of MK0787 for these β-lactamases were almost the same. MK0787 had the highest affinity to *B. fragilis* β-lactamase, as indicated by its having the lowest K_i value, but the K_i values of MK0787 for the β-lactamases from *P. aeruginosa* and *P. cepacia* were two orders of magnitude higher than the K_i value for *B. fragilis* β-

TABLE 1. *Minimal inhibitory concentrations of MK0787 against β-lactamase-producing organisms*

Organism	Enzyme activity[a] (U/mg of protein)	Susceptibility (μg/ml)	
		Cefazolin	MK0787
E. coli GN5482	0.2	12.5	0.39
E. cloacae GN7471	4.3	800	0.20
C. freundii GN7391	6.0	>800	0.78
S. marcescens GN10857	0.8	>800	1.56
P. aeruginosa GN10362	0.16	>800	1.56
P. rettgeri GN4430	0.9	6.25	0.39
P. morganii GN5407	0.5	200	1.56
P. vulgaris GN7919	3.2	800	0.39
P. cepacia GN11164	1.2	>800	0.20
B. fragilis GN11477	0.5	400	1.56
Rms 212/*E. coli* W3630	3.5	400[b]	0.20
Rms 213/*E. coli* W3630	0.11	100[b]	0.20
Rte 16/*E. coli* W3630	0.18	200[b]	0.78
Rms 139/*P. aeruginosa* M1	5.9	>800[b]	1.56

[a] Crude β-lactamase preparations were used.
[b] Minimal inhibitory concentration of ampicillin.

TABLE 2. *K_i values of MK0787 and clavulanic acid*

β-Lactamase source	K_i (μM)	
	MK0787	Clavulanic acid
E. coli GN5482	0.84	—[a]
E. cloacae GN7471	0.95	—
C. freundii GN7391	0.78	—
S. marcescens GN10857	0.39	—
P. aeruginosa GN10362	3.13	—
P. rettgeri GN4430	0.68	—
P. morganii GN5407	0.95	—
P. vulgaris GN7919	0.40	1.07
P. cepacia GN11164	2.55	1.72
B. fragilis GN11477	0.22	0.2
Rms 212/*E. coli* W3630 (PCase[b] type I)	1.42	0.47
Rms 213/*E. coli* W3630 (PCase type II)	1.02	18.3
Rte 16/*E. coli* W3630 (PCase type III)	0.03	21.7
Rms 139/*P. aeruginosa* M1 (PCase type IV)	—	2.5

[a] —, Not inhibited.
[b] PCase, Penicillinase.

lactamase. In the cases of plasmid-mediated penicillinase types I, II, and III, the K_i values of MK0787 were 1.42, 1.02, and 0.03 μM, compared with 0.47, 18.3, and 21.7 μM for clavulanic acid, respectively. A K_i value for MK0787 could not be determined for the plasmid-mediated penicillinase type IV, with a K_i value of 2.5 μM for clavulanic acid.

In summary, MK0787 was very active against β-lactamase-producing organisms. It was also an effective inhibitor of β-lactamases.

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