NOTES

COMPARATIVE INHIBITION OF β-LACTAMASES BY CEPHAMYCIN ANTIBIOTICS

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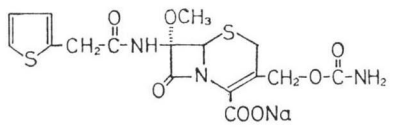
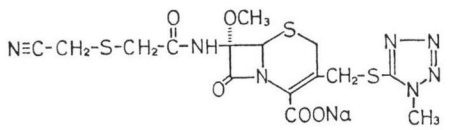
Cefoxitin (CFX), cefmetazole (CMZ) and YM09330[1], and 6059-S[2] have a 7α-methoxyl group (a unique structural feature of the cephamycin family) in the cephalosporin nucleus and 1-oxa cephalosporin nucleus, respectively (Fig. 1).

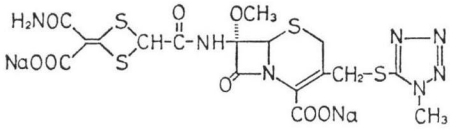
Fig. 1. Chemical structures of cefoxitin, cefmetazole, YM09330, and 6059-S.



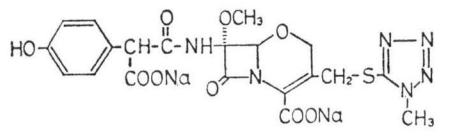
Cefoxitin



Cefmetazole



YM09330



6059-S

These compounds are resistant to hydrolysis by various bacterial β-lactamases. This resistance character also provides the antibiotic with a broad antibacterial spectrum, including activity against indole-positive *Proteus* strains, *Serratia* spp. and *Bacteroides* spp. (including *B. fragilis* and all its subspecies) as well as against all the bacterial pathogens normally sensitive to the cephalosporins. There is great interest in antibiotics that are resistant to β-lactamase hydrolysis and in compounds that are inhibitors of β-lactamases[3,4,5]. CFX and CMZ, cephamycin antibiotics, have been reported to be potent competitive inhibitors of some β-lactamases[6,7]. We wished to assess the β-lactamase-inhibiting activity of the compounds of the cephamycin family.

We have investigated the chromosomally-mediated β-lactamase-inhibiting activity of CFX, CMZ, YM09330 and 6059-S by determining the inhibitor constant (*Ki*) with cephalothin (CET) as a substrate. The plasmid-mediated penicillinase-inhibiting activity of CFX, CMZ, YM-09330 and 6059-S was investigated by determining the *Ki* with cephaloridine (CER) as a substrate. The maximal rate of reaction (*Vmax*) and the dissociation constant (*Km*) were derived from a LINEWEAVER-BURK plot by standard procedures. The minimal inhibitory concentrations (MICs) of the strains were determined by an agar dilution technique. For the *Enterobacteriaceae* and *Pseudomonas* strains, the test medium was MUELLER-HINTON agar (Nissui Pharm. Co., Ltd.). For *B. fragilis*, the test medium was GAM agar (Nissui Pharm. Co., Ltd.). Plates were inoculated with one loopful of 10^6 colony-forming units (CFU) per ml. Anaerobic incubation was for 18 hours at 37°C in a Gas-Pak jar. Chromosomally-mediated β-lactamases were extracted and purified from *Escherichia coli* GN5482, *Enterobacter cloacae* GN7471, *Citrobacter freundii* GN7391, *S. marcescens* GN10857, *P. aeruginosa* GN10362, *P. rettgeri* GN4430, *P. morganii* GN5407, *P. vulgaris* GN7919, *P. cepacia* GN 11164 and *B. fragilis* GN11477. Plasmid-mediated penicillinase type I (Rms212), type II (Rms213) and type III (Rte16)[8,9,10] were extracted and purified from the transconjugants of these plasmids in *E. coli* W3630. Plasmid-mediated

penicillinase type IV (Rms139)[11] was extracted and purified from the transconjugant of this plasmid in *P. aeruginosa* M1. Purified β-lactamases prepared by previously published methods[7,11,12] were used in some experiments. β-Lactamase assay was done by the spectrophotometric me-

Table 1. Comparative activity of cephamycin antibiotics against β-lactamase-producing organisms.

Organism	Enzyme* activity (Units/mg protein)	Inducible or constitutive	MIC (μg/ml) of following antibiotics:**					
			CET	APC	CFX	CMZ	YM09330	6059-S
E. coli GN5482	0.2	C	25	100	25	6.25	0.78	0.20
E. cloacae GN7471	4.3	C	>800	800	200	400	12.5	0.78
C. freundii GN7391	6.0	I	>800	>800	800	400	200	12.5
S. marcescens GN10857	0.8	I	>800	>800	200	200	25	6.25
P. aeruginosa GN10362	0.16	I	>800	>800	>800	>800	400	12.5
P. rettgeri GN4430	0.9	I	50	12.5	3.13	0.78	0.05	0.025
P. morganii GN5407	0.5	I	>800	200	12.5	6.25	0.78	0.20
P. vulgaris GN7919	3.2	I	800	>800	3.13	3.13	0.39	0.20
P. cepacia GN11164	1.2	I	>800	800	50	25	1.56	3.13
B. fragilis GN11477	0.5	C	400	800	12.5	12.5	6.25	6.25
Rms 212/*E. coli* W3630	3.5	C	6.25	400	3.13	0.78	0.20	0.20
Rms 213/*E. coli* W3630	0.11	C	3.13	100	1.56	0.78	0.10	0.10
Rte 16/*E. coli* W3630	0.18	C	3.13	200	3.13	0.39	0.20	0.20
Rms 139/*P. aeruginosa* M1	5.9	C	>800	>800	800	>800	25	1.56

* Crude β-lactamase preparations were used.
** CET, Cephalothin; APC, ampicillin; CFX, cefoxitin; CMZ, cefmetazole.

Table 2. Kinetic constants for β-lactamase hydrolysis of cephalothin or cephaloridine in the presence of cephamycin antibiotics.

β-Lactamase source	*Km* (μM)	*Ki* (μM)			
	Cephalothin	Cefoxitin	Cefmetazole	YM09330	6059-S
E. coli GN5482	63	0.11	0.57	0.22	0.86
E. cloacae GN7471	105	0.50	0.64	0.22	0.81
C. freundii GN7391	16	0.33	0.19	0.09	0.07
S. marcescens GN10857	44	0.15	0.39	0.44	13.0
P. aeruginosa GN10362	71	0.23	0.10	0.15	0.39
P. rettgeri GN4430	105	0.34	4.31	3.64	102
P. morganii GN5407	20	0.22	0.40	0.11	0.16
P. vulgaris GN7919	57	12.4	5.22	13.3	107
P. cepacia GN11164	71	—**	—	—	—
B. fragilis GN11477	100	0.50	0.20	0.20	0.10
Rms 212/*E. coli* W3630 (PCase type I)	400*	—	—	—	—
Rms 213/*E. coli* W3630 (PCase type II)	333*	245	116	8.53	16.7
Rte 16/*E. coli* W3630 (PCase type III)	81*	16.7	2.74	1.02	27.8
Rms 139/*P. aeruginosa* M1 (PCase type IV)	111*	—	—	—	—

* Cephaloridine as a substrate.
** Not inhibited.

thod, as described previously[13,14].

Table 1 shows the susceptibility of β-lactamase-producing organisms to CET, ampicillin (APC), CFX, CMZ, YM09330 and 6059-S in terms of their MIC. CFX, CMZ, YM09330 and 6059-S inhibited those bacteria which were resistant to CET and APC as a result of β-lactamase instability. No significant hydrolysis of cephamycin antibiotics could be detected by the spectrophotometric assay.

The *Ki* values of CFX, CMZ, YM09330 and 6059-S for inhibition of hydrolysis of CET or CER by various β-lactamases are shown in Table 2. CFX, CMZ and YM09330 had a high affinity for the purified enzymes from *E. coli, E. cloacae, C. freundii S. marcescens, P. aeruginosa, P. rettgeri, P. morganii* and *B. fragilis*, as indicated by their low *Ki* values, and were powerful inhibitors of the action of the enzyme, although CFX, CMZ and YM09330 were less effective inhibitors of the enzyme from *P. vulgaris* than they were of the enzymes from the above organisms. 6059-S also had a high affinity to the purified enzymes from *E. coli, E. cloacae, C. freundii, P. aeruginosa, P. morganii* and *B. fragilis*, but the *Ki* values of this compound for the β-lactamases from *S. marcescens, P. rettgeri* and *P. vulgaris* were higher than those of CFX, CMZ and YM09330. The *Ki* values of 6059-S for the enzymes from *S. marcescens, P. rettgeri* and *P. vulgaris* were 13.0, 102 and 107 μM, respectively. KURIYAMA and coworkers[7] reported that CFX and CMZ were potent competitive inhibitors of the purified β-lactamase from *P. morganii*. DARLAND and BIRNBAUM[8] also reported that CFX was not a substrate for the enzyme from *B. fragilis* and was an excellent competitive inhibitor. In our investigation, newly introduced cephamycins such as YM09330 and 6059-S also exhibited orders of inhibitory activity in a competitive manner as high as CFX and CMZ against *E. coli, E. cloacae, C. freundii, S. marcescens, P. aeruginosa, P. rettgeri, P. morganii, P. vulgaris* and *B. fragilis*. However, 6059-S was somewhat different from CFX, CMZ and YM09330 in the case of *S. marcescens, P. rettgeri* and *P. vulgaris*. On the other hand, β-lactamases purified from *P. cepacia* and plasmid-mediated penicillinase types I and IV were not inhibited by CFX, CMZ, YM09330 and 6059-S. In the case of plasmid-mediated penicillinase type II, the *Ki* values for CFX, CMZ, YM09330 and 6059-S were approximately 245, 116, 8.53 and 16.7 μM, respectively. In the case of plasmid-mediated penicillinase type III, the *Ki* values for CFX, CMZ, YM09330 and 6059-S were approximately 16.7, 2.74, 1.02 and 27.8 μM, respectively. YM09330 was the best competitive inhibitor of penicillinase types II and III among the compounds of the cephamycin family.

In marked contrast to the cephalosporins tested (data not shown), the compounds of the cephamycin family were not hydrolyzed by the β-lactamases from various bacteria. Not only are these compounds stable in solutions containing purified β-lactamases, but they have also been shown to be inhibitors of the enzymes except for *P. cepacia* β-lactamase and plasmid-mediated penicillinase types I and IV. They are competitive inhibitors of the enzymes as well. Studies in our laboratory have also proved that clavulanic acid (CVA) and CP-45899 were effective inhibitors of *P. vulgaris, P. cepacia* and *B. fragilis* cephalosporinase and plasmid-mediated penicillinase types I, II, III and IV (unpublished data). However, cephalosporinases from *E. coli, E. cloacae, C. freundii, S. marcescens, P. aeruginosa, P. rettgeri* and *P. morganii* were not inhibited by CVA and CP-45899. On the other hand, certain β-lactamases of Gram-negative bacilli are inhibited by β-lactam antibiotics such as cloxacillin or methicillin[5]. The degree of inhibition has therefore been used in the classification of β-lactamases[15]. In this investigation, cephamycin antibiotics were found to possess not only antibacterial activity against β-lactamase-producing organisms, but also inhibitory activity against a number of types of β-lactamases. The inhibitory activity of cephamycin antibiotics might contribute to the characterization of β-lactamases.

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