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Comparative Evaluation of a New β-Lactamase Inhibitor, YTR 830, Combined with Different β-Lactam Antibiotics against Bacteria Harboring Known β-Lactamases

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YTR 830, a new β-lactamase inhibitor, combined with amoxicillin or carbenicillin, showed a synergistic effect similar to that observed with clavulanic acid, and generally better than that with sulbactam, against strains harboring chromosome-encoded penicillinases and broad-spectrum β-lactamases or plasmid-determined β-lactamases. With ampicillin, YTR 830 showed the best synergistic activity of the inhibitors against *Proteus morganii*, *Citrobacter freundii*, and *Enterobacter cloacae* and their mutants with a derepressed chromosome-encoded cephalosporinase.

YTR 830 is a new derivative of penicillinate sulfone, which was previously shown to have a spectrum of activity similar to that of clavulanic acid when combined with amoxicillin against clinical isolates of *Staphylococcus aureus*, *Haemophilus influenzae*, and different *Enterobacteriaceae* (1). In this study we have compared YTR 830 with clavulanic acid and sulbactam, in combination with different β-lactam antibiotics, against a range of bacteria harboring known β-lactamases.

YTR 830 was obtained from Taiho Pharmaceuticals; ampicillin was from Bristol Laboratories; amoxicillin, carbenicillin, and sodium clavulanate were from Beecham Laboratories; cefotaxime was from Roussel-Uclaf; cefoperazone and sulbactam were from Pfizer Inc. We studied strains harboring known chromosome- or plasmid-mediated β-lactamases (2, 4). MICs (18 h) were determined by using the agar dilution method. Increasing concentrations of β-lactam antibiotics were added to Mueller-Hinton agar containing 8 μg of the inhibitor per ml, and about 10^4 CFU were deposited onto the surface of the agar plate with a Steers-type replicator device. The MICs of the different inhibitors were ≥32 μg/ml. Synergy or antagonism was defined as a fourfold or greater decrease or increase, respectively, in the MIC of the β-lactam antibiotic combined with the inhibitor. The K_i values for the inhibitors with different cephalosporinases (10 mU per assay) in crude extracts were determined by using cephalothin as the substrate (5, 10, 20, 60, and 100 μM). The concentration of inhibitors required to inhibit 50% of the beta-lactamase activity was measured after 5 min of preincubation with cephalothin as the substrate at 100 μM. Hydrolysis was measured in 10 mM phosphate buffer (pH 7) as A_{262} at 25°C with a double-beam spectrophotometer (model 550S) coupled to a 561 recorder (The Perkin Elmer Corp.).

When amoxicillin was combined with YTR 830 there was an 8- to 1,024-fold decrease in the MICs against the strains containing different chromosome-encoded penicillinases, broad-spectrum β-lactamases, or plasmid-mediated β-lactamases (Table 1). Against *Klebsiella oxytoca* 46, which possesses a broad-spectrum β-lactamase similar to those described by Hart and Percival (3) and which produced about 300-fold more enzyme than *K. oxytoca* 921, a synergistic effect was only seen with clavulanic acid. Amoxicillin showed a synergistic effect in combination with YTR 830 when different plasmid-mediated β-lactamases were present in *Escherichia coli* C1a, particularly TEM-1, OXA-2, OXA-3, SHV-1, and HMS-1 (Table 1). Synergy was also demonstrated when YTR 830 was combined with amoxicillin or carbenicillin against other enterobacterial species and *H. influenzae* producing TEM-1 (Table 1). Synergy was shown between carbenicillin and YTR 830 against *Pseudomonas aeruginosa* producing PSE-3 and PSE-4, but not in *P. aeruginosa* producing the most frequently found PSE-1 and PSE-2 β-lactamases (5). The synergistic activity of YTR 830 appeared therefore to be either equal to or slightly lower than that of clavulanic acid against these β-lactamase-producing strains, but was generally higher than that of sulbactam, particularly against strains producing the widely distributed TEM-1. The apparent better activity of clavulanic acid compared with YTR 830 against strains of *E. coli* producing TEM-2, OXA-1, and SHV-1 is not due to a better enzyme-inhibitory effect, since YTR 830 is at least as effective as clavulanic acid against most plasmid-mediated β-lactamases, including TEM-1, TEM-2, and SHV-1 (F. Moosden, J. D. Williams, and S. Yamabe, Program Abstr. 14th Int. Congr. Chemother. abstr. no. S13-13, 1985). The superiority of clavulanic acid may be related to the moderate synergistic effect caused by its intrinsic activity when combined with the other β-lactam antibiotics, as shown with certain β-lactamase-negative strains such as *E. coli* and *Salmonella anatum* (Table 1). Clavulanic acid may also have a better penetration rate than the other inhibitors.

YTR 830 had a synergistic activity against a variety of strains with chromosome-encoded cephalosporinases (Table 2). It showed an activity similar to that of clavulanic acid and sulbactam against *Proteus vulgaris* and *Bacteroides fragilis*. Of particular interest was the strong synergistic effect observed with ampicillin and YTR 830 against the wild-type, low-level cephalosporinase-producing strains of *Proteus morganii*, *Citrobacter freundii*, and *Enterobacter cloacae*, whereas no synergy was demonstrated against *Serratia marcescens*. Against these strains ampicillin and sulbactam showed a lesser degree of synergy than ampicillin and YTR 830, and there was no synergy with clavulanic acid. The

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TABLE 1. MICs of amoxicillin and carbenicillin alone or in combination with 8 μg of clavulanic acid (Cla), sulbactam (Sul), or YTR 830 per ml for different strains producing chromosome- or plasmid-mediated β-lactamases

Strain	Plasmid introduced	β-Lactamase[a]	Antibiotic tested	MIC (μg/ml) with antibiotic alone or with indicated additive			
				Alone	+ Cla	+ Sul	+ YTR 830
Klebsiella pneumoniae 2222		C, IV	Amoxicillin	128	1	2	2
Klebsiella oxytoca 921		C, IV	Amoxicillin	128	0.5	16	1
Klebsiella oxytoca 46		C, IV	Amoxicillin	>2,048	32	>2,048	>2,048
Levinea malonatica		C, P	Amoxicillin	32	<0.5	<0.5	1
Escherichia coli C1a			Amoxicillin	2	0.5	1	2
	R6K	TEM-1	Amoxicillin	>2,048	1	512	4
	RP1	TEM-2	Amoxicillin	>2,048	2	>2,048	32
	RGN238	OXA-1	Amoxicillin	256	2	32	32
	R46	OXA-2	Amoxicillin	256	1	1	2
	R57b	OXA-3	Amoxicillin	64	0.5	0.5	1
	p453	SHV-1	Amoxicillin	>2,048	0.5	1,024	8
	R997	HMS-1	Amoxicillin	>2,048	2	>2,048	4
Salmonella anatum			Amoxicillin	2	0.5	2	2
	R6K	TEM-1	Amoxicillin	>2,048	0.5	64	2
Klebsiella pneumoniae 2222	R6K	TEM-1	Amoxicillin	>2,048	16	>2,048	32
Haemophilus influenzae 31	NC[b]	TEM-1	Amoxicillin	64	<0.5	<0.5	<0.5
Staphylococcus aureus 5353	NC	P	Amoxicillin	16	<0.5	<0.5	<0.5
Enterobacter cloacae 82	R6K	TEM-1	Carbenicillin	>2,048	16	>2,048	32
Proteus morganii 86	R6K	TEM-1	Carbenicillin	128	<0.5	1	<0.5
Citrobacter freundii 79	R6K	TEM-1	Carbenicillin	>2,048	16	2,048	16
Serratia marcescens 89	R6K	TEM-1	Carbenicillin	>2,048	16	128	8
Pseudomonas aeruginosa 38			Carbenicillin	8	32	8	8
	RPL11	PSE-1	Carbenicillin	>2,048	128	1,024	1,024
	R151	PSE-2	Carbenicillin	128	32	128	128
	Rms149	PSE-3	Carbenicillin	>2,048	32	32	32
	pMG19	PSE-4	Carbenicillin	>2,048	16	32	32

[a] C, chromosome encoded; IV, broad-spectrum β-lactamase according to the classification of Richmond and Sykes (6); P, penicillinase (4).
[b] NC, Plasmid present but not characterized.

TABLE 2. MICs of different β-lactam antibiotics alone or in combination with 8 μg of clavulanic acid (Cla), sulbactam (Sul), or YTR 830 per ml against gram-negative bacteria producing chromosome-encoded cephalosporinases

Strain	Antibiotic tested	MIC (μg/ml) with antibiotic alone or with indicated additive			
		Alone	+ Cla	+ Sul	+ YTR 830
Bacteroides fragilis 42	Amoxicillin	32	≤0.5	≤0.5	≤0.5
Proteus vulgaris 486	Amoxicillin	256	4	4	2
S. marcescens 89	Ampicillin	128	128	128	128
P. morganii 86	Ampicillin	256	256	8	1
	Carbenicillin	≤0.5	≤0.5	≤0.5	≤0.5
	Cefoperazone	0.5	2	0.25	0.25
	Cefotaxime	0.015	2	≤0.003	≤0.003
C. freundii 79	Ampicillin	128	64	4	2
	Carbenicillin	4	4	4	4
	Cefoperazone	0.25	0.25	0.12	0.25
	Cefotaxime	0.25	0.25	0.06	0.06
E. cloacae 82	Ampicillin	256	128	64	8
	Carbenicillin	4	4	4	4
	Cefoperazone	0.25	1	0.25	0.25
	Cefotaxime	0.25	2	0.12	0.12
P. morganii 86	Ampicillin	512	256	16	1
(+ Cpase, 65-fold)[a]	Carbenicillin	4	4	2	≤0.5
	Cefoperazone	4	16	0.5	0.25
	Cefotaxime	4	8	0.03	≤0.003
C. freundii 79 (+Cpase,	Ampicillin	2,048	512	512	64
192-fold)	Carbenicillin	256	128	128	64
	Cefoperazone	32	32	32	8
	Cefotaxime	128	64	64	16
E. cloacae 82 (+ Cpase,	Ampicillin	1,024	512	64	64
170-fold)	Carbenicillin	128	64	64	64
	Cefoperazone	8	16	8	0.5
	Cefotaxime	16	16	8	4

[a] Cpase, Derepressed cephalosporinase; values indicate the fold increase compared with parental strains.

TABLE 3. K_i and concentration required for 50% inhibition (I_{50}) of different cephalosporinases by clavulanic acid (Cla), sulbactam (Sul), and YTR 830

Cephalosporinase source	K_i (μM)			I_{50} (μM)		
	Cla	Sul	YTR 830	Cla	Sul	YTR 830
P. morganii 86	180	34	2.3	150	0.4	0.08
C. freundii 79	250	64	13	40	3.1	0.35
E. cloacae 82	>1,000	380	48	45	6.5	1
S. marcescens 89	>1,000	120	229	200	9	13

synergy of ampicillin plus YTR 830 against *P. morganii*, *C. freundii*, and *E. cloacae* was 4- to 16-fold greater than that of amoxicillin plus YTR 830, but the reason for this effect is unclear since the activities of ampicillin and amoxicillin alone were very similar. Against the mutants with derepressed cephalosporinases, ampicillin, carbenicillin, cefoperazone, and cefatoxime had a synergistic effect when combined with YTR 830. The greatest effect was shown against *P. morganii*, and the lowest MICs were obtained when YTR 830 was combined with cefoperazone or cefotaxime. However, except for the *P. morganii* mutant, none of the MICs decreased to those for the low producers. The K_is and 50% inhibitory doses of YTR 830 (Table 3) were 5- to 10-fold lower than those of sulbactam for the different cephalosporinases tested, except for that of *S. marcescens*, which had the lowest affinity for YTR 830. This latter result could explain the lack of synergy of YTR 830 with ampicillin against this strain. The 50% inhibitory doses for YTR 830 and sulbactam after 5 min of preincubation were 20- to 50-fold lower than those without preincubation (data not shown), indicating that a significant and progressive inactivation of the cephalosporinases occurred with these inhibitors, as previously shown for sulbactam (7).

In the species harboring an inducible chromosome-encoded cephalosporinase, no antagonism between YTR 830 or sulbactam and the different β-lactam antibiotics tested was found. In contrast, clavulanic acid showed an antagonism when combined either with carbenicillin against *P. aeruginosa* 38 (Table 1) or with cefoperazone or cefotaxime against the low-level cephalosporinase producers of *P. morganii* and *E. cloacae* (Table 2). Such antagonism could be due to the induction of the cephalosporinase by clavulanic acid.

Our results show that YTR 830 is a β-lactamase inhibitor with a broad spectrum of activity. This includes chromosome-encoded penicillinases and broad-spectrum β-lactamases, a variety of plasmid-mediated β-lactamases, in particular TEM-1, and different chromosome-encoded cephalosporinases within the *Enterobacteriaceae*. In contrast to clavulanic acid, YTR 830 showed no antagonism when tested in combination with different β-lactam antibiotics against species harboring an inducible β-lactamase. Its usefulness for therapy should be considered.

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