Recent Development of YTR 830 H : A Novel β-Lactamase Inhibitor

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I. Introduction

Since the discovery of penicillin (PC) by Fleming in 1929 and the first success in the clinical trial using crude preparation by the Oxford group in 1940, a long and fruitful development of β-lactam antibiotics is continuing up to the present. During the past 50 years, numerous antibiotics belonging to the PC-group and its analogous cephalosporin (CEP) group with high clinical usefulness have appeared and contributed to chemotherapy for infectious diseases.

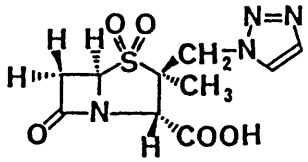
The antimicrobial activities have been more potentiated with more novel β-lactam antibiotics but the appearance of the resistance due to β-lactamase producing strains is one of the most serious problems in the chemotherapy.

The discovery of β-lactamase inhibitors, however, is demonstrating a new way to protect the antibiotics from hydrolysis by β-lactamases.

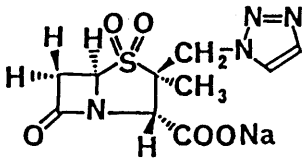
Two β-lactamase inhibitors, clavulanic acid (CVA) and sulbactam (SBT), are clinically used in combination with amoxicillin (AMPC) and ampicillin (ABPC), respectively, for infectious diseases with AMPC- and ABPC-resistant stains. Recently TAIHO Pharmaceutical Co. has developed a novel inhibitor, YTR 830 H which belongs to penicillanic acid sulfone as shown in **Figure 1**. YTR 830 H and YTR 830 which is sodium salt of YTR 830 H were designed by Yamabe to aim at being a more useful drug and has the unique triazolylmethyl group at 2β-position.

This communication describes recent development of YTR 830 H and YTR 830 and includes the toxicological, enzymological, bacteriological and mouse protection studies.

The synthetic study on YTR 830 H should refer to the recent reports by Micetich et al.



YTR 830 H
(M.W. 300.29)



YTR 830
(M.W. 322.27)

Figure 1 Chemical structures of YTR 830 H and YTR 830. (Both inhibitors show the same activity in aqeous solution on the equimolar bases but YTR 830 H is more suitable for the pharmaceutical processing due to its low hygroscopy)

Ⅱ. Ames test (Geno-toxicological study) by Case Western Reserve University group

Table 1 shows the results of the typical experiments on YTR 830 H as well as four control mutagens. All of the *Salmonella* tester strains behaved properly as evidenced by their expected responses with the appropriate control mutagens. Under these conditions, it can be concluded that YTR 830 H is completely non-mutagenic for the tester strains TA 98, TA 100 and TA 102 in the presence or absence of an exogenous activation mixture derived from hamster liver when tested up to levels of 333 μg per plate.

Table 1. Testing the mutagenicity of YTR 830 H in *Salmonella typhimurium*

		Revertants per plate					
		TA100**		TA98**		TA102**	
Addition	μg/plate	−S9	+HEA*	−S9	+HEA*	−S9	+HEA*
None		138±19	130±2	18±4		81±3	114±8
DMSO (Control)	0	143±20		13±4	29±3	81±9	
YTR830H	3.3	154±4	175±5	12±2	24±3	67±2	112±3
	10.0	159±10	199±30	16±6	22±5	63±7	104±10
	33.3	158±20	178±21	13±3	25±7	72±8	98±11
	100	144±8	184±13	17±3	23±4	66±13	97±1
	333	149±6	154±9	17±2	25±4	66±4	94±9
Sodium azide	10	491±19					
2-Aminoanthracene	1	109±14	606±8				
	10			26±4	1428±115	99±7	514±37
β-Propiolactone	0.01μl					695±74	
2-Nitrofluorene	100			1042±151			

*HEA : hepatic enzymes activation by S9
**TA98, TA100 and TA 102 were provided by Prof. B.N. Ames.

Ⅲ. Enzymological study by The London Hospital group

The 50% inhibitory concentrations (μg/ml) of YTR 830, CVA and SBT against a variety of the extracted β-lactamases with and without preincubation are shown in **Table 2**. It is apparent that the inhibitory activity of YTR 830 was much higher than that of SBT and certainly more potent than that of CVA.

Table 2. I_{50}-values (μg/ml) of three inhibitors against a variety of β-lactamases.

Type/Source of β-lactamase	CVA		SBT		YTR-830	
	10Min	0 Min	10Min	0 Min	10Min	0 Min
TEM-1	< 0.01	0.91	1.25	5.4	0.04	0.14
TEM-2	0.06	1.1	3.2	5.4	0.09	0.18
SHV-1	< 0.01	1.32	4.7	22.0	0.26	0.90
K1	0.03	0.92	1.9	>50.0	0.07	0.38
K. pneumoniae K14 1961E	0.02	0.56	1.9	>50.0	0.09	0.60
C. intermedius 2046E	0.51	4.2	2.6	33.0	0.01	0.19
E. coli D31 1541E	>50.0	>50.0	5.5	>50.0	15.0	>50.0

10Min=Preincubation time of inhibitor and enzyme.
0 Min=Inhibitor, enzyme and substrate assayed without preincubation.

Ⅳ. Enzymological study by University of Oxford group

The inhibitory activity of YTR 830 was determined on three completely purified β-lactamases. Whether YTR 830 could be hydrolyzed as their substrate was also measured.

Pseudomonas aeruginosa 1822 S/H β-lactamase. Conditions :

8.3 μM enzyme was incubated with 5 mM YTR 830 at 30°C for 10 min in 20 mM TEA, pH 8. Remaining activity was measured in a pH-stat using 5 mM CEP C and pH 8.

Also similar conditions were used but with 1 mM and 50 μM YTR 830.

	% activity remaining
5 mM	0
1 mM	0
50 μM	14.8

B. cereus 569/H/9 β-lactamase Ⅰ. Conditions :

1.5 μM enzyme was incubated with 5 mM YTR 830 in 0.1 M succinate, pH 6 at 30°C for 10 min. Remaining activity measured in pH-stat using 5 mM PCG and pH 7, gave 61% inactivation with no detectable hydrolysis of YTR 830.

B. cereus 569/H/9 β-lactamase Ⅱ. Conditions :

0.1 mg/ml βⅡ was incubated 5 mM YTR 830 in 0.1 M succinate, pH 6 at 30°C for 10 min. Remaining activity was measured in a pH-stat using 5 mM PCG and pH 7.In this case no inactivation was observed. Hydrolysis of YTR 830 by Ⅱ was observed although very slowly.

YTR 830 was also looked at competitively using 5 mM and 10 mM CEP C with 50 and 100 μM YTR 830. The reaction was followed in a pH-stat at pH 8 after the addition of *Pseudomonas* enzyme (8.3 mM in pH-stat). This showed it to be a good competitive inactivator. YTR 830 was also incubated, at various concentrations with 12 μM *Pveudomonas* enzyme in 20 mM TEA, pH 8 for 10 min at 30°C.

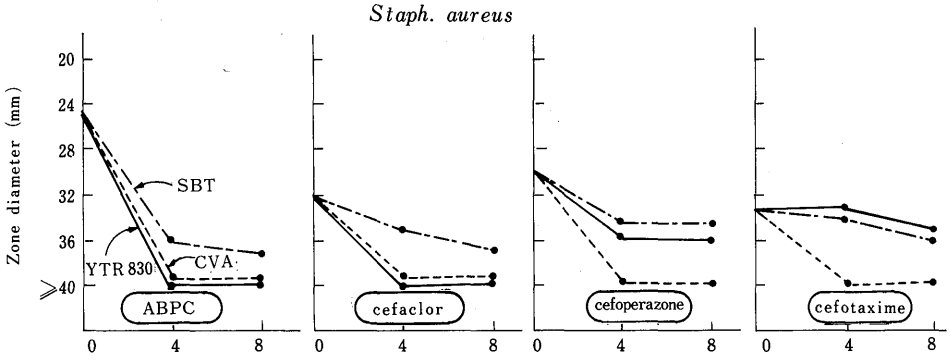
Remaining activity was measured as previously described. This indicated that about 20 molar excess of YTR 830 over enzyme were required to achieve total inactivation.

Ⅴ. Bacteriological study by Saint-Joseph Hospital group

The synergistic activity of YTR 830 was tested in combination with ABPC (ABPC-YTR) on stains containing different β-lactamases and compared with CVA (ABPC-CVA) and SBT (ABPC-SBT). Typical experimental data are shown in **Figure 2**.

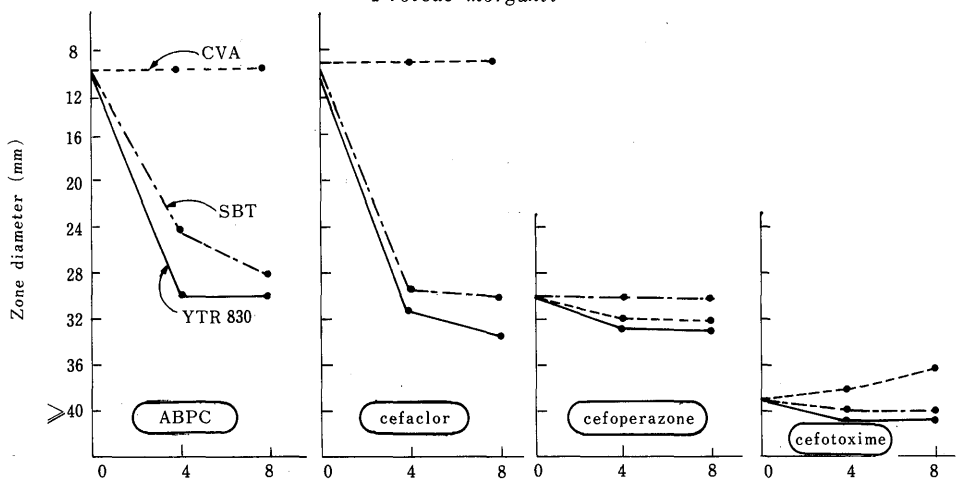
From comparison of the size of inhibition zone by YTR 830 on the agar-plate with those by CVA and SBT, it is concluded that ABPC-YTR demonstrated a synergistic effect on *E. coli* Ci contaning TEM 1, TEM 2, OXA 1, 2, 3, HMS 1 and SHV 1 plasmid mediated β-lactamases. ABPC-YTR was generally more active than ABPC-SBT (particularly on TEM 1, HMS 1 and SHV 1) and almost as effective as ABPC-CVA except on TEM 2 and OXA 1. On *Klebsiella pneumoniae* containing the common Ⅳ -β-lactamase, ABPC-YTR was as effective as ABPC-CVA. ABPC-YTR was more effective than ABPC-SBT and ABPC-CVA on strains with a natural chromosomally encoded cephalosporinase (CEPase) such as *Enterobacter cloacae, Serratia marcescens* and particularly *Proteus morganii*. On β-lactamase-producing *Staph. aurers, Haemophilus* and *Bacteroides fragilis*, ABPC-YTR had a synergistic effect similar to the other combinations.

Staph. aureus



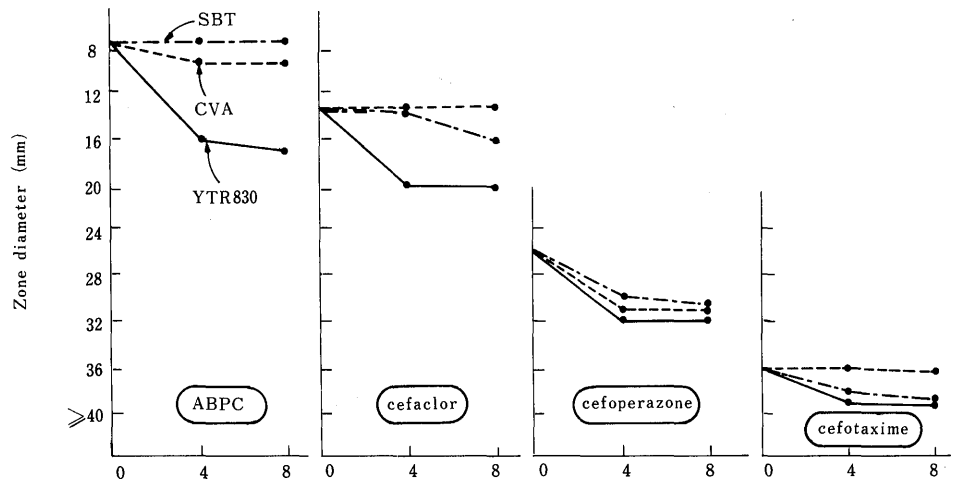
Concentration of inhibitor (μg/ml)

Proteus morganii



Concentration of inhibitor (μg/ml)

Serratia marcescens



Concentration of inhibitor (μg/ml)

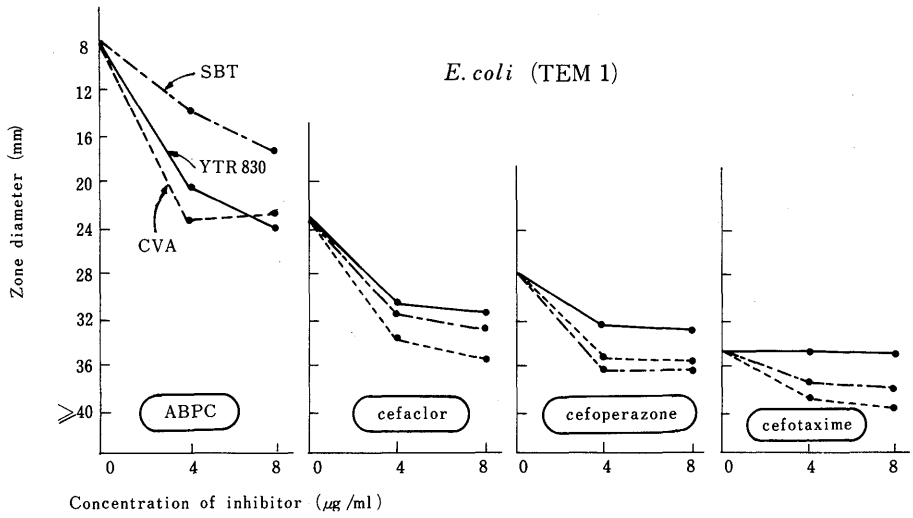


Figure 2. Comparison of synergistic activity of YTR830, CVA and SBT combined with ampicillin, cefaclor, cefoperazone or cefotaxime in *Staph. aureus, Proteus morganii, Serratia marcescens and E. coli* (TEM1)

Thus YTR 830 showed a broad spectrum inhibition of β-lactamases different from CVA and SBT. Similar superiority of YTR 830 was observed in combinations with cefaclor, cefoperazone and cefotaxime.

Ⅵ. Bacteriological study by Case Western Reserve University group

As shown in **Table 3**, YTR 830 and SBT were effective in reducing the MICs against *Citrobacter* species, which may carry a TEM 1 enzyme or a chromosomal enzyme. The poor activity of CVA, a potent TEM 1 inhibitor, suggests that many of our test strains possess the chromosomal enzyme. In summary, YTR 830 is synergistic with AMPC presumably by inhibiting bacterial β-lactamases.

The spectrum of synergistic acitivity of this compound includes AMPC-resistant strains of *Staph. aureus, H. influenzae, E. coli, K. pneumoniae, Providencia, Proteus, Morganella,* and *Citrobacter.* In comparison with SBT and CVA, YTR 830 appears to have a broader spectrum of activity.

Ⅶ. Mouse protection study by Case Western Reserve University group

Unlike CVA, YTR 830 H has no intrinsic antibacterial activity against *Staph. aureus.* The synergistic activities of YTR 830 H or CVA combined with AMPC against a penicillinase producing isolate of *Staph. aureus* (ATCC 29213) and *Proteus mirabilis* were determined *in vitro* and *in viro.*

The MICs of AMPC alone or combined with YTR 830 or CVA against *S. aureus* were determined by broth dilution. The MIC for AMPC was 32 μg/ml ; combined with 8 or 16 μg/ml of YTR 830 or CVA, the MIC of AMPC was $<1\mu$g/ml.

Six week old mice were inoculated intraperitoneally with 10 times thef LD_{50} of *S. aureus* and lethality was recorded at 24 hours. All drugs were administered orally immediately after inocula-

Table 3. Comparative synergistic activities of YTR830 CVA and SBT on the aminopenicillin MIC

Strain	Drug and addition	Inhibitory activity (μg of aminopenicillin per ml)*	
		MIC_{50}	MIC_{90}
S. aureus methicillin resistant ($n=5$)	AMPC	32	64
	+CVA	4	8
	+YTR830	8	16
	ABPC	32	32
	+SBT	8	16
E. coli ($n=21$)	AMPC	>128	>128
	+CVA	2	8
	+YTR830	4	32
	ABPC	>128	>128
	+SBT	8	>128
Klebsiella spp. ($n=25$)	AMPC	64	>128
	+CVA	0.5	1
	+YTR830	1	4
	ABPC	32	128
	+Sulbactam	1	8
Proteus, Providencia. and *Morganella* spp. ($n=25$)	AMPC	>128	>128
	+CVA	64	>128
	+YTR830	4	32
	ABPC	>128	>128
	+SBT	8	>128
Citrobacter diversus. ($n=8$)	AMPC	64	128
	+CVA	2	2
	+YTR830	1	2
	ABPC	32	32
	+SBT	1	2
H. influenzae β-lactamase producing. ($n=14$)	AMPC	32	64
	+CVA	<0.25	<0.25
	+YTR830	<0.25	<0.25
	ABPC	32	64
	+SBT	<0.25	<0.25

*MIC_{50} anb MIC_{90} are the MICs required for 50 and 90% inhibition, respectively.

tion. The dosage of YTR 830 or CVA protecting 50% of the mice (PD_{50}) was determined at 3 dosages of AMPC. The highest AMPC dosage used was 1/2 of the minimal protective dose.

The PD_{50} of AMPC was 737 mg/kg ; AMPC provided no protection at dosages less than 400 mg/kg. At a dosage of 1. 7 mM/kg, CVA alone provided 100% protection ; YTR 830 provided no protection as a single agent at this dosage. The effect of YTR 830 or CVA combined with AMPC is shown in **Table 4**.

The difference in PD_{50} between YTR 830 and CVA combined with AMPC is probably due to the intrinsic activity of CVA against S. aureus. This interpretation as well as the superiority

Table 4. Comparison of mouse protection activities of YTR830 H and CVA combined with AMPC against *S. aureus* infection and *Proteus mirabilis* infection.

PD_{50} (μM/kg) against *S. aureus*			PD_{50} (μM/kg) against *Proteus mirabilis*		
AMPC (mg/kg)	YTR830H	CVA	AMPC (mg/kg)	YTR830H	CVA
200	150	74	300	7	21
100	444	384	200	14	33
50	2,482	1,125	100	2,690	4,060

of YTR 830 H to CVA was confirmed when the test organism was changed to *Proteus mirabilis* as shown in **Table 4**.

For the strain of *Proteus* used, the MIC of AMPC greater than 512 μg/ml. CVA and YTR 830 yielded AMPC MICs of 2. The PD_{50}'s for YTR 830 and CVA combined with 100 mg/kg of AMPC were 14 μM/kg and 33 μM/kg, respectively. Neither CVA nor YTR 830 provided any protection in the dosages used. As a result, it is clear that YTR 830 is superior to CVA against *Proneus* infection.

Ⅷ. Binding to PBPs and morphological changes by The London Hospital group

Penicillin binding proteins (PBPs) studies were carried out according to Spratt, B. G. (Eur. J. Biochem. *72*, 341-352, 1977). Growth and morphological changes were monitored at MIC of the inhibitors.

YTR 830 had the highest affinity for PBP-2 as did CVA whereas SBT bound to PBP-3. Cells which were subjected to YTR 830 and CVA formed spheroplasts whereas filament formation occurred with SBT.

Fifty-four strains of *Enterobacter, Klebsiella, Proteus, Citrobacter* and *Morganella* were examined for induction capacity by CVA, SBT, YTR 830, ABPC and cefoxitin. Cefoxitin proved to be the most potent inducer affecting most of the strains of *Enterobacter* and *Morganella*. CVA induced 30% of all strains studied. SBT and YTR 830 did not show induction of beta-lactamases.

Ⅸ. Concluding remarks with general profile study by Taiho Pharmaceutical Co. group

YTR 830 H, a novel β-lactamase inhibitor belongs to penicillanic acid sulfone and has the triazolylmethyl group at 2β-position. This structural feature leads to its unique enzymological, bacteriological and pharmacological activities in comparison with those of SBT and CVA. YTR 830 Hwas more stable than CVA in aqueous solution and its LD_{50} (i. v.) was over 5, 000 mg/kg in mice.

YTR 830 H was 4-40 times as potent as SBT in the tested β-lactamases including cephalosporinase (CEPase) and inhibited CEPase from *Proteus morganii* on which CVA had no effect.

YTR 830 H showed marked synergism with aminopenicillins and ureidopenicillins in many β-lactamase producing clinical isolates : *S. aurers, E. coli, K. pneumoniae, Proteus, Enterobacter, Citrobacter* and *Serratia,* as shown in **Figure 3**. The synergism of YTR 830 H with AMPC was equal to CVA and better than SBT in *E. coli* and *K. pneumoniae*. YTR 830 H was more potent than SBT and CVA in indole negative and positive *Proteus* as shown in **Figure 4**.

As excellent mouse protection effects of YTR 830 H with aminopenicillins and ureidopenicillins were obtained against the highly PCs-resistant stains of *E. coli*. Both the oral and subcutaneous administrations of YTR 830 H were effective in combination with AMPC.

From a variety of studies as described in the present review on YTR 830 H, we conclude that its future development as a potent β-lactamase inhobitor should be warranted.

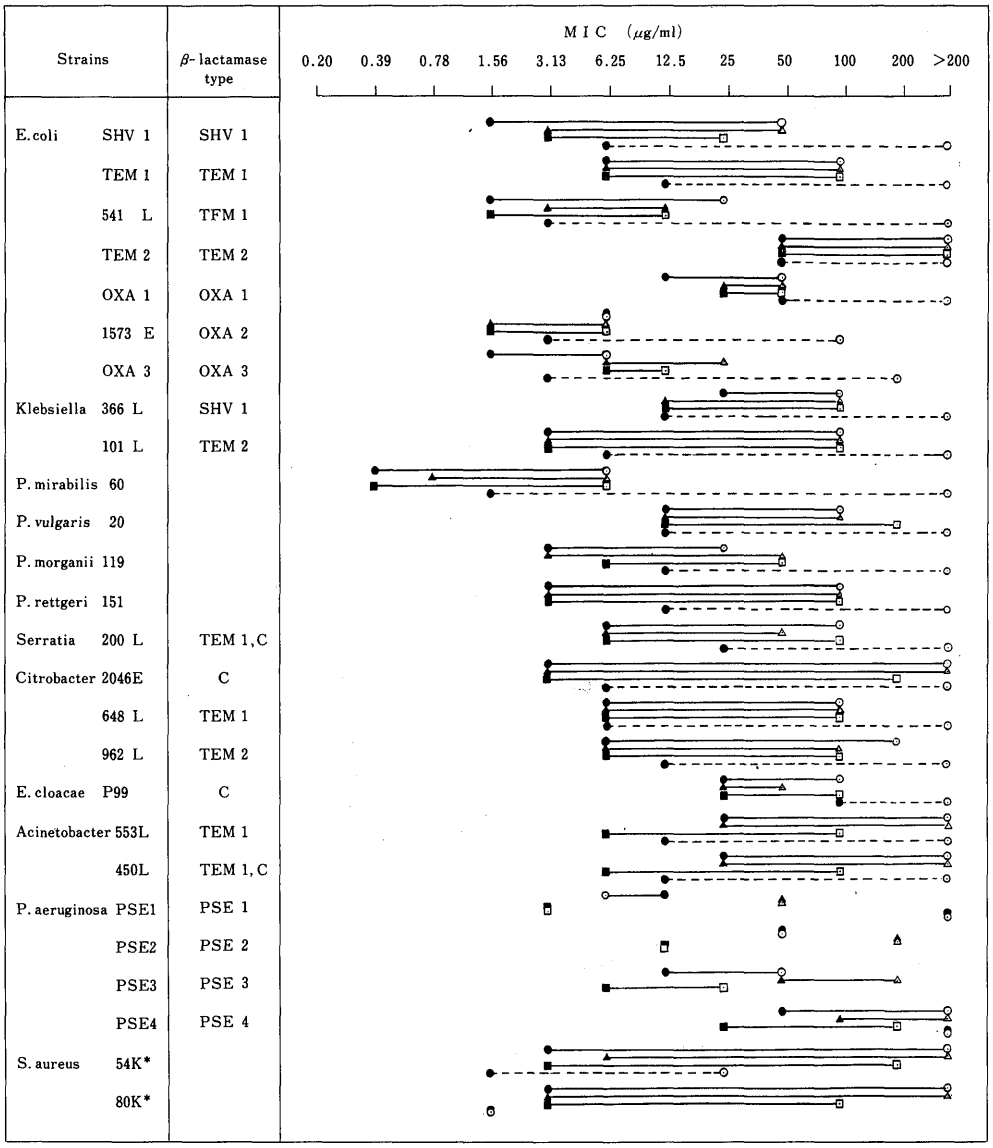


Figure 3. Typical synergism of YTR830 with AMPC and three amino-ureidopenicillins against a variety of PC-resistant strains.
Piperacillin (4 : 1) ●←—○
Mezlocillin (4 : 1) ▲←—△
Apalcillin (4 : 1) ■←—□
AMPC (2 : 1) ●←---○

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(2) Saint-Joseph Hospital group ; Prof. J. F. Acar, Drs. M. D. Kitzis & L. Gutmann

(3) The University of Oxford group ; Sir E. P. Abraham & Dr. S. G. Waley

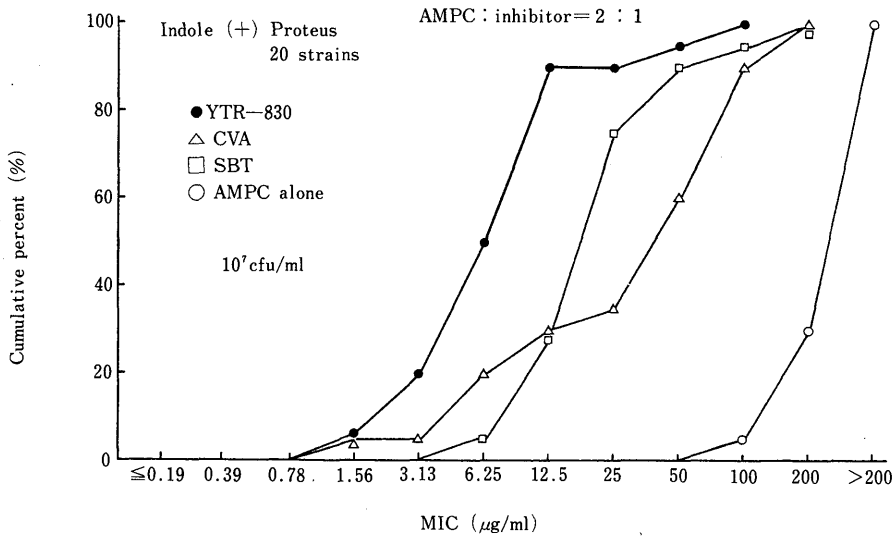

Figure 4. Sensitivity distribution of clinical isolates of Indole (+) *Proteus* to AMPC combined with YTR 830H, CVA or SBT

(4) University of Alberta group ; Prof. R. G. Michetich, Drs. T-W. Hall, S. N. Maiti & P. Spevak

(5) Case Western Reserve University group ; (1) Ass. Profs. S. C. Aronoff, M. R. Jacobs, Drs. S. Johenning & P. H. Labrozzi : (2) Prof. H. S. Rosenkranz, Dr. E. C. McCoy & Prof. S. Yamabe (Chemioterapia **4**, No. 3, 1985)

(6) Taiho Pharmaceutical Research Institute group ; Director K. Harima, Drs. N. Ishida, A. Hyodo, C. Hanehara, Y. Miyake & Y. Kawaguchi

The detailed experimental methods and results should refer to the Abstracts of The 14th International Congress of Chemotherapy, Kyoto, June 23-28, 1985.

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