解　説

STRUCTURE-ACTIVITY RELATIONSHIPS IN VARIOUS COMPOUNDS DERIVED FROM CEFOTIAM LEADING TO CEFMENOXIME*1

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In the hope of improving the antibacterial activity of cefotiam, various kinds of new cephalosporin derivatives having 7β-[2-(2-aminothiazol-4-yl)acetamido]ceph-3-em-4-carboxylic acid nucleus were synthesized following a rational research strategy. Of these, 7β-[2-(2-aminothiazol-4-yl)-(Z)-2-methoxyiminoacetamido]cephalosporins were found to possess excellent activity against a variety of bacteria including β-lactamase-producing strains. An extensive study of structure-activity relationships and of resistance to β-lactamases led to the selection of cefmenoxime for further biological and subsequent clinical evaluation. A short history of the discovery including the related structure-activity relationships and the stability towards various β-lactamases is briefly given.

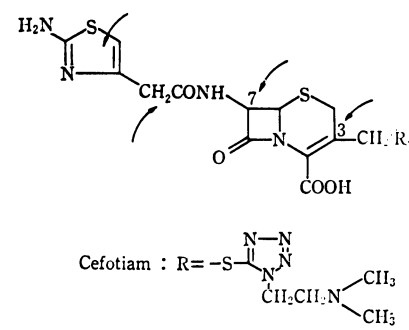
Since the discovery of cephalosporin C in 1955, a tremendous number of new derivatives have been synthesized to obtain a new cephalosporin which may fulfil various therapeutic demands. Some of them, especially those developed recently, have been conferred expanded antibacterial spectra and stability to various β-lactamases including penicillinases, cephalosporinases and oxyiminocephalosporin hydrolyzing enzyms (CXase)*2, and thus making a great contribution to the treatment of infectious diseases.

One of the most outstanding features in the flood of investigations for finding a desirable and potent cephalosporin derivative was the discovery made by one of the research groups in the Central Research Division, Takeda Chemical Industries, Ltd. (Numata et al., 1977 & 1978)[1,2], of the facile synthesis and the good biological properties of a series of cephalosporins bearing a 2-aminothiazolylacetamido group at the 7-position (Fig. 1) ; this being a result of an extensive synthetic effort based on the speculation (Morita et al., 1980)[3] that a new derivative with potent activity might be accessible.

Cefotiam, a representative member of the series, has been eventually developed and its good biological properties as well as high therapeutic value have been widely recognized by many scientists engaged in the field of chemotherapy.

Although cefotiam has potent and broad spectrum activity, some gram-negative bacteria, especially β-lactamase-producing strains, still remained resis-

Fig. 1 7β-[2-(2-Aminothiazol-4-yl)acetamido]-ceph-3-em-4-carboxylic acid nucleus and cefotiam

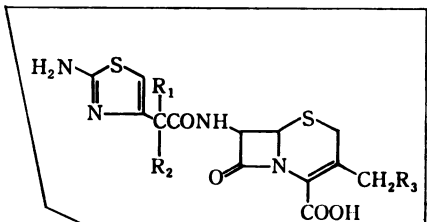


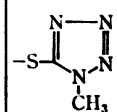
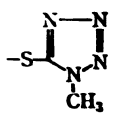
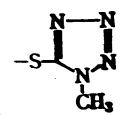
*1 Presented in part at the 13th International Congress of Chemotherapy, Vienna, August 29, 1983. Special session 4. 2/12.

*2 A proposed name for those β-lactamases which have been designated tentatively as cefuroximases (Mitsuhashi and Inoue, 1981).

Table 1 Antibacterial activities of 2-hydroxy- and 2-alkyl-acyl derivatives

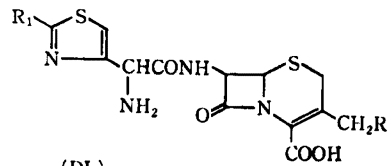
(MIC : μg/ml)



Organism		No.	cefotiam	cefazolin	1	2	3	4	5
		R_1			OH	OH	OH	CH_3	CH_3
		R_2			H	H	H	H	CH_3
		R_3			 (DL)	$OCONH_2$ (DL)	$OCOCH_3$ (DL)	 (DL)	
S. aureus 1840		PCase	1.56	0.78	1.56	3.13	3.13	3.13	50
E. coli T-7		PCase	3.13	100	1.56	3.13	6.25	25	>100
S. marcescens TN24		PCase CSase	100	>100	25	100	>100	12.5	>100
P. vulgaris GN4413		CXase	>100	>100	>100	>100	>100	>100	>100
E. cloacae TN1282		CSase	100	>100	6.25	50	100	100	>100

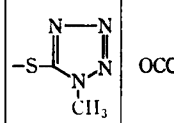
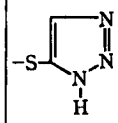
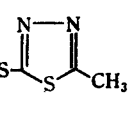
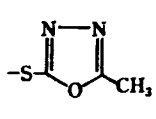
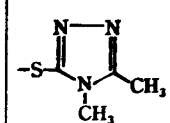
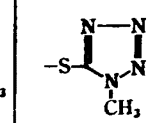
The MICs reported in this paper were determined by a standard agar dilution method in Trypticase soy agar (BBL).
The inoculum size; one loopful (about 5μl) of bacterial suspension (10^8CFU/ml).
PCase, penicillinase; CSase, cephalosporinase; CXase, oxyiminocephalosporin hydolyzing enzyme.

Table 2 Antibacterial activity of 2-aminoacyl derivatives



(DL)

(MIC : μg/ml)

Compound / Organism	No.	cefotiam	cefazolin	6	7	8	9	10	11	12	13
	R			$OCOCH_3$		$OCONH_2$					
	R_1			NH_2	NH_2	NH_2	NH_2	NH_2	NH_2	NH_2	CH_3
S. aureus 1840		1.56	0.78	6.25	12.5	6.25	3.13	6.25	25	12.5	25
E. coli T-7		3.13	100	3.13	3.13	6.25	3.13	6.25	25	3.13	100
S. marcescens TN24		100	>100	100	50	>100	50	>100	>100	100	>100
P. vulgaris GN4413		>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
E. cloacae TN1282		100	>100	50	25	>100	25	100	>100	25	>100

tant. Therefore, considering possible future situation in infectious diseases, Takeda research group had continued to explore, by chemical modification on this unique structure, a better member of the series with enhanced resistance to β-lactamases and hence improved antibacterial activity especially against β-lactamase-producing strains.

Our rational strategy was to take advantage of various specific substituents appeared in those cephalosporin derivatives which had been reported to be, more or less, active against β-lactamase-producing strains. One approach was an introduction of a substituent(s) such as an amino, hydroxy, methyl or methoxyimino to the methylene group in the 7-acyl moiety. The other was an introduction of a methoxy group to the 7α-position. Variation of a substituent on the thiazole and cephem rings was additionally planned (Fig. 1).

A number of compounds involving 2-hydroxy-, 2-alkyl-, 2-amino- and 2-alkoxyimino-acyl derivatives and 7α-methoxy derivatives were synthesized along this strategy. Most of the newly synthesized compounds exhibited high *in vitro* antibacterial activity comparable or superior to that of unsubstituted 7-acyl derivatives.

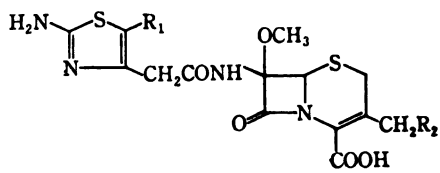
Table 1 shows *in vitro* antibacterial activity of 2-hydroxy- and 2-alkyl-acyl derivatives against several β-lactamase-producing strains (OCHIAI et al., 1980)[6]. It is apparent that the compound 1 has improved activity against *Serratia marcescens* and *Enterobacter cloacae*. A slight increase in activity is observed in the other compounds against *Serratia marcescens* (4) and *Enterobacter cloacae* (2), but these compounds have no enhanced activity against *Proteus vulgaris*. The dimethyl derivative (5) showed a definite loss of activity.

The *in vitro* antibacterial activity of new 2-aminoacyl derivatives (OCHIAI et al., 1980)[8] is exemplified in Table 2.

The activity profile showed that some of the compounds (7, 9, 12) possess improved activity especially against *Enterobacter cloacae*. A similar trend in activity against *Proteus vulgaris* to that of 2-hydroxy- and 2-alkyl-acyl derivatives is observed in these 2-aminoacyl derivatives. Replacement of the annular amino group by a methyl group (13) caused loss of activity.

The 7α-methoxy derivatives (14, 15) showed (OCHIAI et al., 1980)[9] enhanced activity against *Serratia marcescens* and *Proteus vulgaris* when compared with cefotiam, and compound 14 is comparable to cefoxitin (Table 3). The 7α-methoxy compounds have, in general, clearly improved activity against *Proteus vulgaris* but less activity against *Enterobacter cloacae* in sharp contrast to the 2-hydroxy- and 2-alkyl-acyl derivatives.

Table 3 Antibacterial activity of 7α-methoxy derivatives



(MIC : μg/ml)

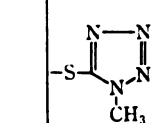
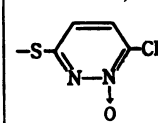
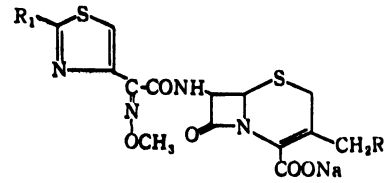
Compound / Organism	No.	cefotiam	cefazolin	cefoxitin	14	15	16	17
	R_1				H	H	H	Cl
	R_2					$OCOCH_3$		$OCOCH_3$
S. aureus 1840	PCase	1.56	0.78	3.13	3.13	6.25	12.5	3.13
E. coli T-7	PCase	3.13	100	25	6.25	6.25	55	25
S. marcescens TN24	PCase CSase	100	>100	6.25	6.25	12.5	100	50
P. vulgaris GN4413	CXase	>100	>100	12.5	6.25	25	25	50
E. cloacae TN1282	CSase	100	>100	>100	>100	>100	>100	>100

Table 4 Antibacterial activity of methoxyiminoacetyl derivatives



(MIC : μg/ml)

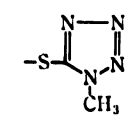
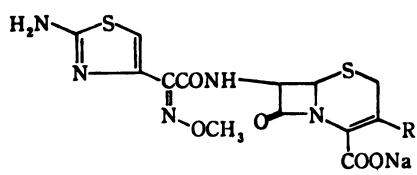
Compound / Organism	No.	18	19	20	21	22	23	24	25	cefotiam
	R_1	NH_2		NH_2		NH_2		CH_3		
	R_2	$OCOCH_3$		$OCONH_2$				$OCONH_2$		
	Config.	*Z*	*E*	*Z*	*E*	*Z*	*E*	*Z*	*E*	
S. aureus 1840		3.13	25	3.13	100	3.13	50	6.25	12.5	1.56
E. coli T-7		0.78	12.5	0.39	25	0.78	50	25	50	3.13
S. marcescens TN24		0.78	3.13	0.20	6.25	0.20	3.13	12.5	25	100
P. vulgaris GN4413		0.78	25	1.56	>100	0.39	>100	100	100	>100
E. cloacae TN1282		6.25	25	6.25	25	1.56	50	50	>100	100

Table 5 Effect of oxyimino group on antibacterial activity



(MIC : μg/ml)

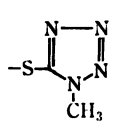
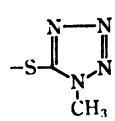
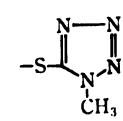
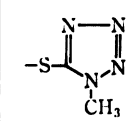
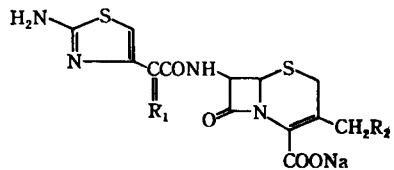
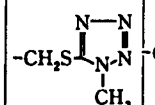
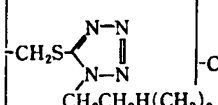
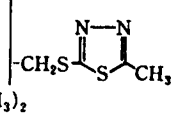
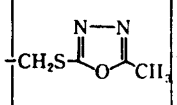
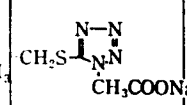
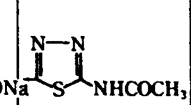
Compound / Organism	No.	22	26	27	28	29
	R_1	$N{=}OCH_3$	$N{=}OC_2H_5$	$N{=}OC_3H_7(i)$	$N{=}OCH_2COONa$	
	R_2					$OCOCH_3$
S. aureus 1840		3.13	3.13	3.13	50	12.5
E. coli T-7		0.78	1.56	6.25	0.39	0.78
S. marcescens TN24		0.20	3.13	3.13	0.78	3.13
P. vulgaris GN4413		0.39	1.56	12.5	0.025	0.78
E. cloacae TN1282		1.56	3.13	6.25	0.78	1.56

Table 6 Effect of 3-substituent on antibacterial activity



(MIC : μg/ml)

Compound No. / Organism	22	30	31	32	33	34	35	36	37	38
R_1							$-CH_3$	$-CH_2OCOCH_2COCH_3$	$-OCH_3$	$-CH{=}CHCOOC_2H_5$
S. aureus 1840	3.13	3.13	1.56	3.13	25	6.25	100	12.5	100	25
E. coli T-7	0.78	1.56	1.56	0.78	1.56	0.78	3.13	0.78	6.25	25
S. marcescens TN24	0.20	0.78	0.78	0.39	0.78	0.78	0.78	0.78	1.56	6.25
P. vulgaris GN4413	0.39	1.56	1.56	3.13	0.025	1.56	1.56	1.56	12.5	50
E. cloacae TN1282	1.56	6.25	3.13	12.5	>100	0.78	50	6.25	100	3.13

chlorination of the thiazole ring dose not seem to give favourable effect on the activity.

A remarkable improvement in activity was observed (OCHIAI et al., 1977 & 1981)[7,8] in 2-methoxyiminoacyl derivatives with *Z*-configuration (*syn*) at the methoxyimino moiety(Table 4). From Table 4 it is apparent that *Z*-isomers (**18, 20, 22**) exhibit excellent activity against all the β-lactamase-producing strains listed. Substitution of the annular amino group by a methyl group (**24, 25**) caused marked decrease in activity, thus indicating the importance of the amino group for the excel lent antibacterial activity.

Table 5 indicates the effect of changes in the oxyimino group on the activity. Simple homologation of the methoxyimino group (**26, 27**) caused a decrease in activity especially against gram -negative bacteria. Contrary to this, compound **28** which has a carboxymethyl function exhibited a remarkable increase in activity against all the gram -negative bacteria listed, but less activity against *Staphylococcus aureus*. A similar tendency was observed with a nitrone compound (**29**).

From these structure-activity relationships it appears that the combination of annular amino and *Z*-methoxyimino groups is one of the most promising structural features. The effects of substituent variation at the 3-position on the cephalosporin having this 7-acyl group are shown in Table 6.

It is apparent that all the compounds with a heterocyclyl group either *via* thiomethyl group or directly attached to the cephem ring (**20, 30~33** or **34**) exceed those with aliphatic substituents, and **22** is the most potent compound.

The extensive study of structure-activity relationships thus far described which is briefly summarized in Table 7 led (OCHIAI et al., 1978; GOTO et al., 1980)[9,10] to the selection of 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-1 *H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (**22**), cefmenoxime (Fig. 2), for further biological evaluation.

Cefmenoxime possesses high resistance to various β-lactamases as shown in Table 8 and excellent *in vivo* activity as well as good pharmacokinetic properties (TSUCHIYA et al., 1980)[11].

The extensive study of structure-activity relationships of a number of new cephalosporin deriva-

Fig. 2 Cefmenoxime

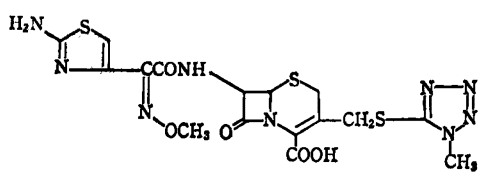
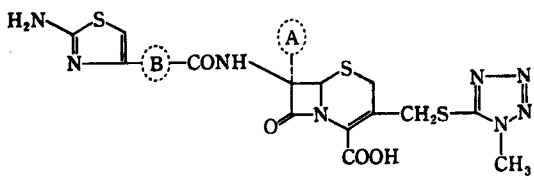


Table 7 Antibacterial activity of 2-aminothiazolylacetamidocephalosporin derivatives against β-lactamase-producing strains



(MIC : μg/ml)

Compound	Ⓐ	H	H	H	H	H	OCH_3	H
	Ⓑ	-CH(H)-	-CH(OH)-	-CH(CH_3)-	-C(CH_3)$_2$-	-CH(NH_2)-	-CH(H)-	-C(=N-OCH_3)-
Organism		39	(DL) 1	(DL) 4	5	(DL) 7	14	(*Z*) 22
S. aureus 1840	PCase	0.78	1.56	3.13	50	12.5	3.13	3.13
E. coli T-7	PCase	6.25	1.56	25	>100	3.13	6.25	0.78
S. marcescens TN24	PCase CSase	100	25	12.5	>100	50	6.25	0.20
P. vulgaris GN4413	CXase	>100	>100	>100	>100	>100	6.25	0.39
E. cloacae TN1282	CSase	>100	6.25	100	>100	25	>100	1.56

Table 8 Relative rate of hydrolysis of cefmenoxime and related compounds

Source of enzyme / Substrate	*S. aureus* 1840 (PCase)	*E. coli* T-7 (PCase)	*S. marcescens* TN24 (PCase)	*S. marcescens* TN24 (CSase)	*P. vulgaris* GN4413 (CXase)	*E. cloacae* TN1282 (CSase)
Cephaloridine	0.14	23.6	22.4	100	100	100
Penicillin G	100	100	100	11.4	16.7	28.5
Cefazolin	0.18	3.08	4.22	266	467	67.0
Cefotiam	0.02	1.53	1.77	41.9	174	41.4
Cefoxitin	<0.01	<0.01	<0.01	<0.1	<0.01	0.31
Cefuroxime	0.05	0.08	0.08	0.6	232	0.13
Cefmenoxime	<0.01	0.08	0.07	<0.1	44.9	0.08

In percent of hydrolysis of penicillin G or cephaloridine.

tives bearing 7β-[2-(2-aminothiazol-4-yl)acetamido] group, which were synthesized through rational chemical modification on the basic structure of cefotiam, led to the selection of cefmenoxime for further biological and subsequent clinical evaluation. Cefmenoxime which has been proved to be stable to various β-lactamases was eventually developed as a new parenteral cephalosporin characterized by excellent and broad spectrum antibacterial activity and high stability to β-lactamases (MITSUHASHI & INOUE, 1981)[12].

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