STUDIES ON CEPHALOSPORIN ANTIBIOTICS

IV. SYNTHESIS, ANTIBACTERIAL ACTIVITY AND ORAL ABSORPTION OF NEW 3-(2-SUBSTITUTED-VINYLTHIO)-7β-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-(CARBOXYMETHOXYIMINO)ACETAMIDO]CEPHALOSPORINS

CHIHIRO YOKOO, MASAMI GOI, AKIRA ONODERA,
HIROSHI FUKUSHIMA and TAKATOSHI NAGATE

Research Center, Taisho Pharmaceutical Co., Ltd.,
1-403 Yoshino-cho, Omiya, Saitama 330, Japan

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A series of new 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(carboxymethoxyimino)acetamido]cephalosporins (**1**) having various substituted-vinylthio groups at the C-3 position of the cephem nucleus was synthesized and evaluated for antibacterial activity and oral absorption in rats in comparison with cefixime. Of these, the cephalosporins (**1a** and **1c**) with a lower alkoxycarbonylvinylthio group (Z-form) at the C-3 position showed a potent antibacterial activity against Gram-negative bacteria, improved activity against *Staphylococcus aureus* as well as good oral absorption in rats. The structure-activity relationships of **1** are also presented.

In the course of our studies on aminothiazole-oxime type cephalosporin antibiotics, so called third generation cephalosporins, possessing a hetero-atom attached directly to the C-3 position of the cephem nucleus, we have already found that 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(carboxymethoxyimino)acetamido]-cephalosporins with a substituted-alkylthio group, represented by **2a** as shown in Fig. 1, display potent antibacterial activities against Gram-positive and Gram-negative bacteria as well as good oral absorption in rats[1].

Since natural occurring carbapenem antibiotics such as *N*-acetyldehydrothienamycin[2] and PS-7[3] have been discovered, the substituent effect of the C-2 vinylthio moiety has been studied extensively[4]. However, only a little is known about the cephalosporin analogues having a vinylthio group at the C-3 position of cephem nucleus[5].

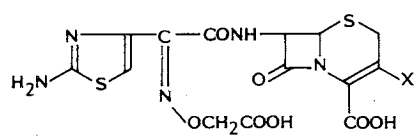
Thus, we were interested in studying the cephalosporin derivatives (**1**) (Fig. 1) having various vinylthio substituents at the C-3 position as an attempt to find new orally active cephalosporins.

This paper describes the synthesis, antibacterial activity and oral absorption in rats of **1**.

Chemistry

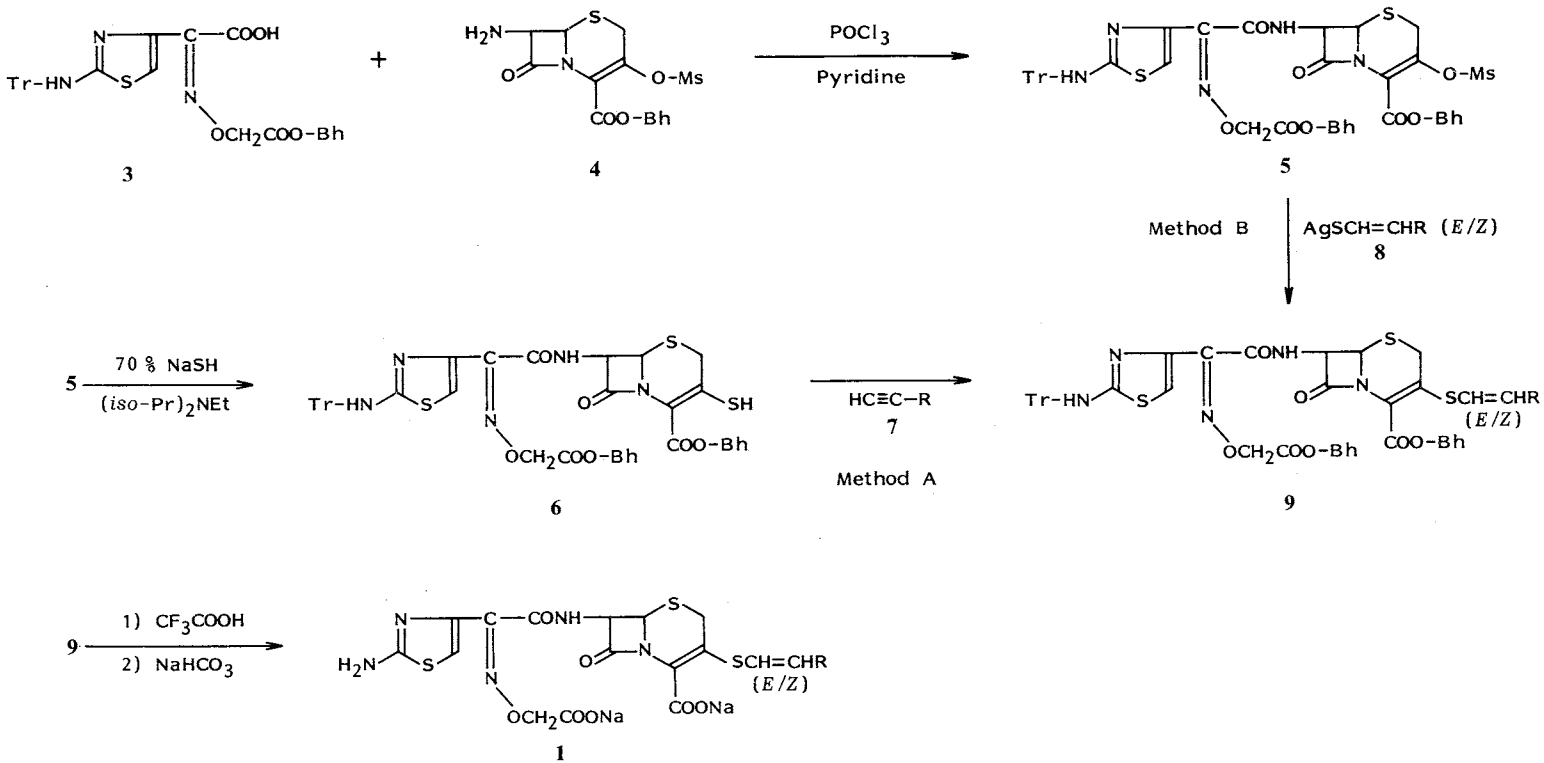
The new cephalosporins (**1a**～**1s**) were prepared by the synthetic route as shown in Scheme 1. Diphenylmethyl 7β-[(Z)-2-(2-tritylaminothiazol-4-yl)-2-(diphenylmethoxycarbonylmethoxyimino)-acetamido]-3-methanesulfonyloxy-3-cephem-4-carboxylate (**5**)[1], prepared from 2-aminothiazole-4-acetic acid derivative (**3**) and 7β-amino-3-methanesulfonyloxycephalosporanic acid diphenylmethyl (Bh) ester (**4**), was reacted with sodium hydrosulfide

Fig. 1. Structure of **1**, **2a** and cefixime.



1 X=−SCH=CHR (*E*/*Z*)
R=Ester, amide and nitrile
2a X=$-SCH_2CN$
Cefixime X=$-CH=CH_2$

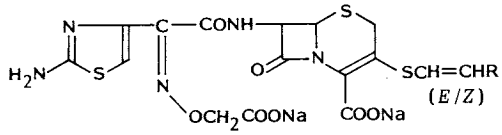
Scheme 1.



Ms=$-SO_2CH_3$, Tr=$-CPh_3$, Bh=$-CHPh_2$, R=ester, amide and nitrile.

in DMF in the presence of *N*,*N*-diisopropylethylamine to afford the 3-mercapto derivative (**6**). Subsequently, compound **6** was treated with various propiolic acid esters, propiolamides or propiolonitrile (**7**) at low temperature in the presence of *N*,*N*-diisopropylethylamine to give the 3-(2-substituted)vinylthiocephalosporins (**9**) as a mixture of *E* and *Z* isomers (*E*-*Z*, 1:6~8 by ^{1}H NMR spectrum) (Method A). The two geometric isomers could be separated by silica gel column chromatography, and the *E* and *Z* assignments of the vinyl moiety were based upon the observed coupling constant (J_{cis}=11 Hz, J_{trans}=15 Hz) in ^{1}H NMR spectra.

Table 1. *In vitro* antibacterial activity and peak serum level of **1a**~**1s**.



1

Compound No.	R	*E*/*Z*	MIC (μg/ml, 10^6 cfu/ml)[a] *S.a.*	*E.c.*	*K.p.*	*M.m.*	*S.m.*	Peak serum level (μg/ml)[b] po, 50 mg/kg rats (*n*=3)
1a	CO_2Me	*Z*	12.5	0.2	≦0.1	≦0.1	1.56	38.9
1b	CO_2Me	*E*	12.5	1.56	0.2	≦0.1	3.13	1.4
1c	CO_2Et	*Z*	6.25	0.2	≦0.1	≦0.1	0.39	37.1
1d	CO_2iPr	*Z*	12.5	0.39	≦0.1	≦0.1	0.39	8.0
1e	COO-⬡	*Z*	12.5	1.56	0.78	0.39	0.78	<1.0
1f	$CO_2CH_2CH=CH_2$	*Z*	6.25	0.39	≦0.1	≦0.1	0.39	11.5
1g	CO_2CH_2Ph	*Z*	6.25	0.78	0.2	≦0.1	0.39	<0.5
1h	CO_2Na	*Z*	100	3.13	0.2	0.39	6.25	NT
1i	$CONH_2$	*Z*	25	0.78	≦0.1	≦0.1	3.13	12.4
1j	CONHMe	*Z*	25	0.2	≦0.1	≦0.1	0.39	17.2
1k	CONHEt	*Z*	25	0.39	≦0.1	≦0.1	0.39	22.3
1l	CONH-⬡	*Z*	25	0.2	≦0.1	≦0.1	0.39	<0.1
1m	$CONHCH_2Ph$	*Z*	12.5	0.2	≦0.1	≦0.1	0.39	2.1
1n	$CONMe_2$	*Z*	25	0.2	≦0.1	≦0.1	0.2	8.0
1o	$CONEt_2$	*Z*	25	0.39	≦0.1	≦0.1	0.39	2.3
1p	CON⬠	*Z*	25	0.2	0.39	≦0.1	0.2	0.9
1q	CON⬡O	*Z*	25	0.2	≦0.1	≦0.1	0.39	1.5
1r	CON⬡	*Z*	12.5	0.2	≦0.1	≦0.1	0.39	2.1
1s	CN	*Z*	6.25	0.78	≦0.1	≦0.1	1.56	<4.0
2b	3-$SCH_2CH_2CO_2Me$[c]		50	0.78	≦0.1	≦0.1	1.56	2.4
2c	3-SCH_2CH_2CN[c]		12.5	0.78	≦0.1	≦0.1	0.78	14.7
Cefixime[c]			25	0.78	≦0.1	0.2	0.78	28.6

[a] The MICs were determined by a standard agar dilution method using Sensitive Test agar (Eiken, Japan).

[b] The peak serum levels were measured by a disc-plate method using *Escherichia coli* SC 507 or *Micrococcus luteus* NIHJ as the test organism.

[c] For compounds **2b**, **2c** and cefixime see refs 1 and 7, respectively.

NT: Not tested.

Abbreviations: *S.a.*; *Staphylococcus aureus* 209P JC-1, *E.c.*; *Escherichia coli* NIHJ JC-2, *K.p.*; *Klebsiella pneumoniae* IFO 3317, *M.m.*; *Morganella morganii* IID 602, *S.m.*; *Serratia marcescens* IID 618.

Compound **9** was also prepared from **5** by an alternative route using silver vinylthiolates (**8**)[5,6] (Method B) with retention of the configuration of the silver vinylthiolates[5].

Finally, the protecting groups in **9** were removed by conventional method using TFA and anisole to afford the desired cephalosporin derivatives (**1**).

Antibacterial Activity and Oral Absorption

The *in vitro* antibacterial activities of the new cephalosporins (**1a**~**1s**) against selected Gram-positive and Gram-negative bacteria and their peak serum levels as a measure of gastro-intestinal absorption after oral administration (50 mg/kg) to rats are summarized in Table 1. For comparison, the MIC values and the peak serum levels of related 3-(substituted)alkylthio analogues (**2b** and **2c**)[1] and cefixime[7] are listed at the bottom of Table 1.

Against all the Gram-negative bacteria, these new cephalosporins having various ester, amide, carboxy and cyano groups in the vinylthio moiety at the C-3 position except **1b**, **1e** and **1h** exhibited a potent antibacterial activity comparable to cefixime.

On the other hand, against Gram-positive *Staphylococcus aureus* 209P JC-1, the cephalosporin derivatives **1c**, **1f**, **1g** and **1s** having the ethyl-, allyl- and benzyl ester group as well as cyano group, respectively, in the C-3 vinylthio moiety showed more potent activity than the others. Their activities were 4 times greater than that of cefixime.

When compared with the effect of geometric isomers of the C-3 substituent (**1a** vs. **1b**), the *Z*-isomer **1a** was more potent than the *E*-isomer **1b** against the Gram-negative bacteria.

It was also found that introduction of the vinylthio group into the C-3 position plays an important role to enhance the antibacterial activity against *S. aureus* (**1a**, **1b** vs. **2b**, and **1s** vs. **2c**), probably due to an increase of the lipophilicity of the molecule.

In the oral absorption study, compounds **1a** and **1c** having a lower alkyl ester such as methyl- and ethyl ester in the vinylthio moiety at the C-3 position displayed much better oral absorption than the other analogues, and their peak serum levels were higher than that of cefixime. Interestingly, the oral absorption of **1a** was much higher than those of the corresponding *E*-isomer **1b** and **2b**, which is the saturated analogue of the C-3 substituent of **1a**. These results shown in Table 1 indicate that the presence of the lower alkoxycarbonylvinylthio group (*Z*-form) at the 3-position of the cephalosporin is of importance on oral absorption as well as antibacterial activity.

The representative compound **1a** in this series possessing the potent *in vitro* antibacterial activity and good oral absorption in rats was then advanced to a preliminary *in vivo* efficacy trial by oral administration. As shown in Table 2, the derivative **1a** showed good *in vivo* efficacy against a systemic infection in mice induced by *Escherichia coli* TM-36, though the ED_{50} value of **1a** was about 2 times higher than that of cefixime.

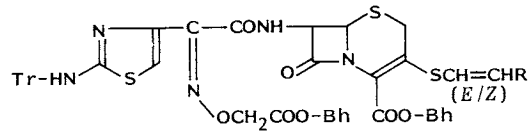
The combination of high potency, broad spectrum activity and good oral absorption in rats positions compound **1a** as a promising new oral cephalosporin agent.

Table 2. *In vivo* antibacterial activity of **1a** against systemic infections in mice induced by *Escherichia coli* TM-36.

Compound	ED_{50} (mg/kg)[a]	MIC (μg/ml)[b]
1a	3.09 (1.36~7.01)	0.2
Cefixime	1.52 (0.56~4.12)	0.78

Drugs were administered orally 1 hour after infection.
Infective challenge dose: 2.8×10^7 cfu/mouse, ip, (5% mucin).
Mouse: Male ICR strain, 4 weeks, 10 mice/groups.
[a] Probit method (95% confidence limits).
[b] Inoculum size: 10^6 cfu/ml.

Table 3. ^{1}H NMR, MS and IR spectral data of **9**.



9

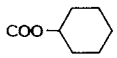
Compound No.	Compound R	*E*/*Z*	^{1}H NMR δ ($CDCl_3$)	MS (*m*/*z*)[a]	IR (KBr) cm^{-1}
9b	CO_2Me	*E*	3.27 (1H, *J*=18 Hz), 3.63 (1H, d, *J*=18 Hz), 3.73 (3H, s), 4.91 (1H, d, *J*=17 Hz), 5.05 (1H, d, *J*=17 Hz), 5.07 (1H, d, *J*=5 Hz), 5.93 (1H, d, *J*=15 Hz), 5.95 (1H, dd, *J*=5, 9 Hz), 6.80 (1H, s), 6.96 (2H, s), 7.01 (1H, br s), 7.20~7.48 (35H, m), 7.53 (1H, d, *J*=15 Hz), 8.11 (1H, d, *J*=9 Hz)	1,118	1,785 1,725 1,695
9c	CO_2Et	*Z*	1.32 (3H, t, *J*=7 Hz), 3.23 (1H, d, *J*=17 Hz), 3.61 (1H, d, *J*=17 Hz), 4.23 (2H, q, *J*=7 Hz), 4.89 (1H, d, *J*=17 Hz), 5.04 (1H, d, *J*=5 Hz), 5.06 (1H, d, *J*=17 Hz), 5.80 (1H, d, *J*=11 Hz), 5.94 (1H, dd, *J*=5, 9 Hz), 6.79 (1H, s), 6.80 (1H, d, *J*=11 Hz), 6.96 (1H, s), 7.01 (2H, s), 7.24~7.46 (35H, m), 8.12 (1H, d, *J*=9 Hz)	1,132	1,785 1,730 1,685
9d	CO_2iPr	*Z*	1.31 (6H, d, *J*=7 Hz), 3.24 (1H, d, *J*=18 Hz), 3.62 (1H, d, *J*=18 Hz), 4.90 (1H, d, *J*=17 Hz), 4.96~5.16 (1H, m), 5.04 (1H, d, *J*=5 Hz), 5.06 (1H, d, *J*=17 Hz), 5.79 (1H, d, *J*=10 Hz), 5.95 (1H, dd, *J*=5, 9 Hz), 6.78 (1H, d, *J*=10 Hz), 6.80 (1H, s), 6.97 (1H, s), 7.02 (1H, s), 7.04 (1H, br s), 7.24~7.46 (35H, m), 8.11 (1H, d, *J*=9 Hz)	1,146	1,785 1,730 1,685
9e		*Z*	1.26~1.98 (10H, m), 3.23 (1H, d, *J*=17 Hz), 3.61 (1H, d, *J*=17 Hz), 4.78~4.92 (1H, m), 4.89 (1H, d, *J*=17 Hz), 5.04 (1H, d, *J*=5 Hz), 5.06 (1H, d, *J*=17 Hz), 5.80 (1H, d, *J*=11 Hz), 5.94 (1H, dd, *J*=5, 9 Hz), 6.77 (1H, d, *J*=11 Hz), 6.80 (1H, s), 6.96 (1H, s), 7.00 (2H, s), 7.23~7.47 (35H, m), 8.13 (1H, d, *J*=9 Hz)	1,186	1,785 1,730 1,680
9f	$CO_2CH_2CH=CH_2$	*Z*	3.22 (1H, d, *J*=16 Hz), 3.61 (1H, d, *J*=16 Hz), 4.68 (2H, m), 4.89 (1H, d, *J*=17 Hz), 5.04 (1H, d, *J*=5 Hz), 5.06 (1H, d, *J*=17 Hz), 5.24~5.45 (2H, m), 5.83 (1H, d, *J*=11 Hz), 5.88~6.09 (2H, m), 6.80 (1H, s), 6.83 (1H, d, *J*=11 Hz), 6.96 (1H, s), 7.01 (2H, br s), 7.22~7.48 (35H, m), 8.11 (1H, d, *J*=9 Hz)	1,144	1,785 1,685
9g	CO_2CH_2Ph	*Z*	3.22 (1H, d, *J*=18 Hz), 3.61 (1H, *J*=18 Hz), 4.89 (1H, d, *J*=17 Hz), 5.03 (1H, d, *J*=5 Hz), 5.06 (1H, d, *J*=17 Hz), 5.22 (2H, s), 5.84 (1H, d, *J*=10 Hz), 5.94 (1H, dd, *J*=5, 9 Hz), 6.80 (1H, s), 6.83 (1H, d, *J*=10 Hz), 6.96 (1H, s), 7.00 (2H, s), 7.22~7.45 (40H, m), 8.11 (1H, d, *J*=9 Hz)	1,194	1,780 1,730 1,685
9h	CO_2CHPh_2	*Z*	3.20 (1H, d, *J*=17 Hz), 3.59 (1H, d, *J*=17 Hz), 4.88 (1H, d, *J*=17 Hz), 5.02 (1H, d, *J*=5 Hz), 5.05 (1H, d, *J*=17 Hz), 5.92 (1H, d, *J*=11 Hz), 5.94 (1H, dd, *J*=5, 9 Hz), 6.79 (1H, s), 6.85 (1H, d, *J*=11 Hz), 6.95 (1H, s), 6.98 (1H, s), 7.00 (2H, s), 7.24~7.42 (45H, m), 8.11 (1H, d, *J*=9 Hz)	1,270	1,785 1,730 1,685
9j	CONHMe	*Z*	2.87 (3H, d, *J*=5 Hz), 3.22 (1H, d, *J*=18 Hz), 3.60 (1H, d, *J*=18 Hz), 4.89 (1H, d, *J*=18 Hz), 5.01 (1H, d, *J*=5 Hz), 5.06 (1H, d, *J*=18 Hz), 5.52 (1H, q, *J*=5 Hz), 5.67 (1H, d, *J*=11 Hz), 5.92 (1H, dd, *J*=5, 9 Hz), 6.55 (1H, d, *J*=11 Hz), 6.80 (1H, s), 6.96 (1H, s), 7.00 (2H, s), 7.22~7.50 (35H, m), 8.07 (1H, d, *J*=9 Hz)	1,117	1,780 1,730 1,680

Table 3. (Continued)

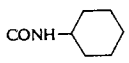
Compound No.	R	*E*/*Z*	^{1}H NMR δ ($CDCl_3$)	MS (*m*/*z*)[a]	IR (KBr) cm^{-1}
9k	CONHEt	*Z*	1.23 (3H, t, *J*=7 Hz), 3.23 (1H, d, *J*=18 Hz), 3.39 (2H, dq, *J*=7.7 Hz), 3.61 (1H, d, *J*=18 Hz), 4.89 (1H, d, *J*=18 Hz), 5.03 (1H, d, *J*=5 Hz), 5.06 (1H, d, *J*=18 Hz), 5.43 (1H, t, *J*=7 Hz), 5.93 (1H, dd, *J*=5, 9 Hz), 6.56 (1H, d, *J*=11 Hz), 6.81 (1H, s), 6.97 (1H, s), 7.02 (2H, s), 7.20~7.50 (35H, m), 8.03 (1H, d, *J*=9 Hz)	1,131	1,780 1,730 1,680
9l		*Z*	1.06~2.08 (10H, m), 3.22 (1H, d, *J*=17 Hz), 3.60 (1H, d, *J*=17 Hz), 3.85 (1H, m), 4.89 (1H, d, *J*=18 Hz), 5.01 (1H, d, *J*=5 Hz), 5.05 (1H, d, *J*=18 Hz), 5.35 (1H, d, *J*=8 Hz), 5.63 (1H, d, *J*=11 Hz), 5.93 (1H, dd, *J*=5, 9 Hz), 6.54 (1H, d, *J*=11 Hz), 6.80 (1H, s), 6.96 (1H, s), 7.00 (2H, s), 7.22~7.50 (35H, m), 8.07 (1H, d, *J*=9 Hz)	1,185	1,780 1,730 1,680
9m	$CONHCH_2Ph$	*Z*	3.24 (1H, d, *J*=18 Hz), 3.61 (1H, d, *J*=18 Hz), 4.52 (2H, d, *J*=6 Hz), 4.89 (1H, d, *J*=17 Hz), 5.01 (1H, d, *J*=5 Hz), 5.06 (1H, d, *J*=17 Hz), 5.69 (1H, d, *J*=11 Hz), 5.80 (1H, t, *J*=6 Hz), 5.91 (1H, dd, *J*=5, 9 Hz), 6.61 (1H, d, *J*=11 Hz), 6.80 (1H, s), 6.96 (1H, s), 7.01 (2H, s), 7.02~7.50 (40H, m), 8.07 (1H, d, *J*=9 Hz)	1,193	1,780 1,730 1,680
9n	$CONMe_2$	*Z*	3.00 (3H, s), 3.02 (3H, s), 3.24 (1H, d, *J*=17 Hz), 3.62 (1H, d, *J*=17 Hz), 4.89 (1H, d, *J*=17 Hz), 5.03 (1H, d, *J*=5 Hz), 5.06 (1H, d, *J*=17 Hz), 5.94 (1H, dd, *J*=5, 9 Hz), 6.07 (1H, d, *J*=10 Hz), 6.63 (1H, d, *J*=10 Hz), 6.80 (1H, s), 6.96 (1H, s), 7.01 (2H, br s), 7.24~7.48 (35H, m), 8.03 (1H, d, *J*=9 Hz)	1,131	1,780 1,720 1,620
9o	$CONEt_2$	*Z*	1.17 (3H, t, *J*=7 Hz), 1.18 (3H, t, *J*=7 Hz), 3.25 (1H, d, *J*=17 Hz), 3.30 (2H, q, *J*=7 Hz), 3.38~3.53 (2H, m), 3.62 (1H, d, *J*=17 Hz), 4.88 (1H, d, *J*=17 Hz), 5.03 (1H, d, *J*=5 Hz), 5.06 (1H, d, *J*=17 Hz), 5.93 (1H, dd, *J*=5, 9 Hz), 6.03 (1H, d, *J*=10 Hz), 6.61 (1H, d, *J*=10 Hz), 6.80 (1H, s), 6.96 (1H, s), 7.00 (2H, s), 7.22~7.46 (35H, m), 8.04 (1H, d, *J*=9 Hz)	1,159	1,780 1,730 1,680
9p		*Z*	1.81~2.07 (4H, m), 3.22 (1H, d, *J*=18 Hz), 3.31~3.61 (4H, m), 3.61 (1H, d, *J*=18 Hz), 4.88 (1H, d, *J*=18 Hz), 5.02 (1H, d, *J*=5 Hz), 5.05 (1H, d, *J*=17 Hz), 5.90 (1H, d, *J*=10 Hz), 5.94 (1H, dd, *J*=5, 9 Hz), 6.59 (1H, d, *J*=10 Hz), 6.80 (1H, s), 6.96 (1H, s), 7.01 (2H, br s), 7.20~7.48 (35H, m), 8.04 (1H, d, *J*=9 Hz)	1,157	1,780 1,730 1,680
9q		*Z*	3.25 (1H, d, *J*=17 Hz), 3.34~3.78 (8H, m), 3.62 (1H, d, *J*=17 Hz), 4.90 (1H, d, *J*=17 Hz), 5.04 (1H, d, *J*=5 Hz), 5.06 (1H, d, *J*=17 Hz), 5.94 (1H, dd, *J*=5, 9 Hz), 6.05 (1H, d, *J*=10 Hz), 6.71 (1H, d, *J*=10 Hz), 6.80 (1H, s), 6.96 (1H, s), 7.01 (2H, br s), 7.20~7.48 (35H, m), 8.05 (1H, d, *J*=9 Hz)	1,173	1,780 1,725 1,680
9r		*Z*	2.14~2.26 (2H, m), 3.24 (1H, d, *J*=17 Hz), 3.47~3.58 (1H, m), 3.62 (1H, d, *J*=17 Hz), 3.72~3.80 (1H, m), 3.88~3.96 (1H, m), 4.09~4.16 (1H, m), 4.88 (1H, d, *J*=17 Hz), 5.02 (1H, d, *J*=5 Hz), 5.05 (1H, d, *J*=5 Hz), 5.64~5.89 (2H, m), 5.93 (1H, dd, *J*=5, 9 Hz), 5.99~6.18 (1H, m), 6.65 (1H, d, *J*=10 Hz), 6.80 (1H, s), 6.96 (1H, s), 7.00 (2H, br s), 7.22~7.46 (35H, m), 8.04 (1H, d, *J*=9 Hz)	1,169	1,785 1,730 1,685

Table 3. (Continued)

Compound No.	R	E/Z	^{1}H NMR δ ($CDCl_3$)	MS (m/z)[a]	IR (KBr) cm^{-1}
9s	CN	Z	3.19 (1H, d, J=17 Hz), 3.62 (1H, d, J=17 Hz), 4.88 (1H, d, J=17 Hz), 5.06 (1H, d, J=17 Hz), 5.07 (1H, d, J=5 Hz), 5.32 (1H, d, J=10 Hz), 5.95 (1H, dd, J=5, 9 Hz), 6.80 (1H, s), 6.91 (1H, d, J=10 Hz), 6.96 (1H, s), 7.00 (2H, br s), 7.20~7.50 (35H, m), 8.25 (1H, d, J=9 Hz)	1,085	1,780 1,730 1,680

[a] FAB, $(M+H)^+$.

Experimental

IR spectra were taken on a Jasco DS-701G IR spectrometer. ^{1}H NMR spectra were recorded on a Varian XL-200 NMR spectrometer using TMS or sodium trimethylsilyl propionate-d_4 (in D_2O) as an internal standard. Mass spectra (MS) were measured on a Jeol JMX-DX303 or JMS-SX102 mass spectrometer. Most chromatographic separations were done by using Wako Silica gel C-200 (100~200 mesh, Wako, Japan) or Sephadex LH-20 (Pharmacia, Sweden). Analytical HPLC was performed on a TSK gel LS-410 column (5 μm, 150×4.6 mm, i.d., Tosoh, Japan) eluted with 35% aq acetonitrile containing tetra-*n*-amylammonium bromide (10 mmol) and ammonium acetate (10 mmol), flow rate 1.0 ml/minute at ambient temperature monitoring UV absorbance at 290 nm.

In Vitro and *In Vivo* Antibacterial Activities

MICs were determined by the 2-fold agar dilution method using Sensitive Test agar (Eiken, Japan) after incubation at 37°C for 18 hours with an inoculum size 10^6 cfu/ml. Mouse protecting experiments were conducted by use of male ICR mice (n=10) infected intraperitoneally with 0.5 ml of a bacterial suspension containing 100% or more minimal lethal doses. Hog gastric mucin (5% w/v) was added to the suspension before injection. The test drugs in 5% gum arabic were administered orally 1 hour after the infection. Mortality of the animals was recorded daily over a period of 7 days and the ED_{50} values were calculated by the method of probit[8].

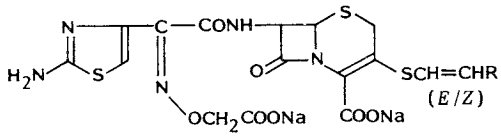
Oral Absorption Study

Male SLC/Wister rats (n=3) weighing 180~220 g were fasted overnight and orally dosed with 50 mg/kg of the test compounds. Serum samples were collected at 0.5, 1, 2 and 4 hours, respectively, after dosing. Serum levels of the test compounds were measured by the disc-plate method using *Escherichia coli* SC 507 or *Micrococcus luteus* NIHJ as a test organism and Sensitive Test agar as the test medium.

Diphenylmethyl 7β-[2-(2-Tritylaminothiazol-4-yl)-2-[(*Z*)-diphenylmethoxycarbonylmethoxyimino]-acetamido]-3-mercapto-3-cephem-4-carboxylate (**6**)

To a solution of the 3-methanesulfonyloxycephalosporanic acid Bh ester derivative **5**[1] (2.0 g, 1.83 mmol) in DMF (20 ml) were added 70% sodium hydrosulfide (164 mg, 1.1 equiv) and *N*,*N*-diisopropylethylamine (284 mg, 1.2 equiv) dissolved in DMF (8 ml) at −10°C. After being stirred for 30 minutes at the same temperature, water (100 ml) was added to the reaction mixture, and washed with Et_2O (20 ml). The aqueous layer was then adjusted to pH 2 with 5% HCl and extracted with EtOAc (100 ml). The extract was washed with brine (100 ml), dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on Sephadex LH-20 (eluent; acetone) to give 660 mg (70%) of **6** as a pale yellow powder: IR (KBr) cm^{-1} 1785 (β-lactam), 1733, 1687; ^{1}H NMR (DMSO-d_6) δ 3.40 (1H, br s, SH), 3.73 (2H, br s, 2-Hα and β), 4.85 (2H, br s, $=NOCH_2$), 5.26 (1H, d, J=5 Hz, 6-H), 5.68 (1H, dd, J=5 and 8 Hz, 7-H), 6.85 (1H, s, thiazole 5-H), 6.86 (1H, s, $CHPh_2$), 6.90 (1H, s, $CHPh_2$), 7.16~7.61 (35H, m, aromatic H), 8.93 (1H, br s, TrN*H*), 9.58 (1H, d, J=8 Hz, CONH); FAB-MS m/z 1,034 $(M+H)^+$.

Table 4. ^{1}H NMR and IR spectral data of **1**.



1

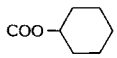
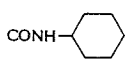
Compound No.	Compound R	*E*/*Z*	^{1}H NMR δ (D_2O)	IR (KBr)[a] cm^{-1}
1b	CO_2Me	*E*	3.51 (1H, d, *J*=17 Hz), 3.74 (3H, s), 3.92 (1H, d, *J*=17 Hz), 4.60 (2H, s), 5.37 (1H, d, *J*=5 Hz), 5.85 (1H, d, *J*=15 Hz), 5.91 (1H, d, *J*=5 Hz), 7.08 (1H, s), 7.72 (1H, d, *J*=15 Hz)	1,765
1c	CO_2Et	*Z*	1.30 (3H, t, *J*=7 Hz), 3.58 (1H, d, *J*=17 Hz), 3.98 (1H, d, *J*=17 Hz), 4.25 (2H, q, *J*=7 Hz), 4.60 (2H, s), 5.33 (1H, d, *J*=5 Hz), 5.90 (1H, d, *J*=5 Hz), 6.05 (1H, d, *J*=11 Hz), 7.08 (1H, s), 7.35 (1H, d, *J*=11 Hz)	1,760
1d	CO_2iPr	*Z*	1.28 (6H, d, *J*=7 Hz), 3.58 (1H, d, *J*=18 Hz), 3.97 (1H, d, *J*=18 Hz), 4.60 (2H, s), 5.06 (1H, m), 5.31 (1H, d, *J*=5 Hz), 5.88 (1H, d, *J*=5 Hz), 6.00 (1H, d, *J*=10 Hz), 7.07 (1H, s), 7.32 (1H, d, *J*=10 Hz)	1,760
1e		*Z*	1.20~1.96 (10H, m), 3.58 (1H, d, *J*=17 Hz), 3.97 (1H, d, *J*=17 Hz), 4.60 (2H, s), 4.78~4.90 (1H, m), 5.32 (1H, d, *J*=5 Hz), 5.88 (1H, d, *J*=5 Hz), 6.02 (1H, d, *J*=11 Hz), 7.08 (1H, s), 7.34 (1H, d, *J*=11 Hz)	1,760
1f	$CO_2CH_2CH=CH_2$	*Z*	3.60 (1H, d, *J*=17 Hz), 3.99 (1H, d, *J*=17 Hz), 4.60 (2H, s), 4.70 (2H, m), 5.28~5.46 (2H, m), 5.33 (1H, d, *J*=5 Hz), 5.90 (1H, d, *J*=5 Hz), 5.91~6.07 (1H, m), 6.09 (1H, d, *J*=11 Hz), 7.08 (1H, s), 7.39 (1H, d, *J*=11 Hz)	1,760
1g	CO_2CH_2Ph	*Z*	3.52 (1H, d, *J*=17 Hz), 3.92 (1H, d, *J*=17 Hz), 4.60 (2H, s), 5.23 (2H, s), 5.29 (1H, d, *J*=5 Hz), 5.87 (1H, d, *J*=5 Hz), 6.05 (1H, d, *J*=11 Hz), 7.05 (1H, s), 7.35 (1H, d, *J*=11 Hz), 7.46 (5H, br s)	1,760
1h	CO_2Na	*Z*	3.59 (1H, d, *J*=17 Hz), 3.96 (1H, d, *J*=17 Hz), 4.60 (2H, s), 5.31 (1H, d, *J*=5 Hz), 5.88 (1H, d, *J*=5 Hz), 5.96 (1H, d, *J*=10 Hz), 6.88 (1H, d, *J*=10 Hz), 7.08 (1H, s)	1,755
1i	$CONH_2$	*Z*	3.57 (1H, d, *J*=18 Hz), 3.96 (1H, d, *J*=18 Hz), 4.60 (2H, s), 5.31 (1H, d, *J*=5 Hz), 5.88 (1H, d, *J*=5 Hz), 6.11 (1H, d, *J*=11 Hz), 7.08 (1H, s), 7.10 (1H, d, *J*=11 Hz)	1,755
1j	CONHMe	*Z*	2.78 (3H, s), 3.58 (1H, d, *J*=18 Hz), 3.96 (1H, d, *J*=18 Hz), 4.60 (2H, s), 5.31 (1H, d, *J*=5 Hz), 5.89 (1H, d, *J*=5 Hz), 6.08 (1H, d, *J*=11 Hz), 6.99 (1H, d, *J*=11 Hz), 7.08 (1H, s)	1,760
1k	CONHEt	*Z*	1.12 (3H, t, *J*=7 Hz), 3.27 (2H, q, *J*=7 Hz), 3.58 (1H, d, *J*=18 Hz), 3.96 (1H, d, *J*=18 Hz), 4.61 (2H, s), 5.31 (1H, d, *J*=5 Hz), 5.89 (1H, d, *J*=5 Hz), 6.05 (1H, d, *J*=11 Hz), 6.99 (1H, d, *J*=11 Hz), 7.09 (1H, s)	1,760
1l		*Z*	1.06~1.60 (5H, m), 1.60~1.92 (5H, m), 3.57 (1H, d, *J*=18 Hz), 3.65 (1H, m), 3.96 (1H, d, *J*=18 Hz), 4.61 (2H, s), 5.31 (1H, d, *J*=5 Hz), 5.89 (1H, d, *J*=5 Hz), 6.04 (1H, d, *J*=11 Hz), 6.99 (1H, d, *J*=11 Hz), 7.08 (1H, s)	1,760
1m	$CONHCH_2Ph$	*Z*	3.55 (1H, d, *J*=17 Hz), 3.94 (1H, d, *J*=17 Hz), 4.45 (2H, s), 4.61 (2H, s), 5.30 (1H, d, *J*=5 Hz), 5.88 (1H, d, *J*=5 Hz), 6.13 (1H, d, *J*=11 Hz), 7.05 (1H, d, *J*=11 Hz), 7.08 (1H, s), 7.30~7.50 (5H, m)	1,760
1n	$CONMe_2$	*Z*	1.99 (3H, s), 2.12 (3H, s), 3.58 (1H, d, *J*=17 Hz), 3.96 (1H, d, *J*=17 Hz), 4.61 (2H, s), 5.32 (1H, d, *J*=5 Hz), 5.89 (1H, d, *J*=5 Hz), 6.48 (1H, d, *J*=10 Hz), 7.09 (1H, d, *J*=10 Hz), 7.10 (1H, s)	1,755
1o	$CONEt_2$	*Z*	1.16 (3H, t, *J*=7 Hz), 1.22 (3H, t, *J*=7 Hz), 3.45 (2H, q, *J*=7 Hz), 3.49 (2H, q, *J*=7 Hz), 3.60 (1H, d, *J*=17 Hz), 3.98 (1H, d, *J*=17 Hz), 4.63 (2H, s), 5.33 (1H, d, *J*=5 Hz), 5.91 (1H, d, *J*=5 Hz), 6.48 (1H, d, *J*=10 Hz), 7.10 (1H, s), 7.12 (1H, d, *J*=10 Hz)	1,755

Table 4. (Continued)

Compound No.	R	E/Z	^{1}H NMR δ (D_2O)	IR (KBr)[a] cm^{-1}
1p		Z	1.80~2.08 (4H, m), 3.40~3.66 (4H, m), 3.58 (1H, d, J=17 Hz), 3.96 (1H, d, J=17 Hz), 4.60 (2H, s), 5.31 (1H, d, J=5 Hz), 5.88 (1H, d, J=5 Hz), 6.32 (1H, d, J=10 Hz), 7.08 (1H, d, J=10 Hz), 7.08 (1H, s)	1,755
1q		Z	3.57 (1H, d, J=17 Hz), 3.58~3.82 (8H, m), 3.95 (1H, d, J=17 Hz), 4.80 (2H, s), 5.31 (1H, d, J=5 Hz), 5.88 (1H, d, J=5 Hz), 6.47 (1H, d, J=10 Hz), 7.08 (1H, s), 7.15 (1H, d, J=10 Hz)	1,760
1r		Z	2.14~2.32 (2H, m), 3.58 (1H, d, J=17 Hz), 3.61~3.75 (2H, m), 3.96 (1H, d, J=17 Hz), 4.01~4.16 (2H, m), 4.60 (2H, s), 5.32 (1H, d, J=5 Hz), 5.69~6.04 (2H, m), 6.40~6.59 (1H, m), 7.08 (1H, s), 7.12 (1H, d, J=10 Hz)	1,755
1s	CN	Z	3.57 (1H, d, J=17 Hz), 3.98 (1H, d, J=17 Hz), 4.60 (2H, s), 5.33 (1H, d, J=5 Hz), 5.65 (1H, d, J=10 Hz), 5.90 (1H, d, J=5 Hz), 7.07 (1H, s), 7.46 (1H, d, J=10 Hz)	1,755

[a] β-Lactam.

Diphenylmethyl 7β-[2-(2-Tritylaminothiazol-4-yl)-2-[(*Z*)-diphenylmethoxycarbonylmethoxyimino]-acetamido]-3-[(*Z*)-2-methoxycarbonylvinylthio]-3-cephem-4-carboxylate (**9a**) (Method A)

To a solution of the 3-mercaptocephalosporanic acid Bh ester **6** (500 mg, 0.48 mmol) in CH_3CN (8 ml) were added methyl propiolate (61 mg, 1.5 equiv) and *N*,*N*-diisopropylethylamine (32 mg, 0.5 equiv) at −20°C, and the reaction mixture was stirred for 1 hour at −20~−10°C. Then, 0.5% HCl (20 ml) was added to the reaction mixture and extracted with EtOAc (50 ml). The extract was washed with brine (50 ml), dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (eluent; *n*-hexane-EtOAc, 3:2) to yield 300 mg (56%) of **9a** and 47 mg (9%) of **9b**, the *E*-isomer of the C-3 substituent of **9a** as a yellow powder, respectively. **9a**: IR (KBr) cm^{-1} 1785 (β-lactam), 1735, 1690; ^{1}H NMR ($CDCl_3$) δ 3.22 (1H, d, J=17 Hz, 2-Hα), 3.61 (1H, d, J=17 Hz, 2-Hβ), 3.77 (3H, s, CO_2CH_3), 4.89 and 5.07 (2H, ABq, J=17 Hz, $=NOCH_2$), 5.04 (1H, d, J=5 Hz, 6-H), 5.81 (1H, d, J=11 Hz, $=CHCO_2CH_3$), 5.95 (1H, dd, J=5 and 9 Hz, 7-H), 6.80 (1H, s, thiazole 5-H), 6.81 (1H, d, J=11 Hz, SCH=), 6.97 (1H, s, $CHPh_2$), 7.02 (2H, s, TrNH and $CHPh_2$), 7.23~7.48 (35H, m, aromatic H), 8.11 (1H, d, J=9 Hz, CONH); FAB-MS *m/z* 1,118 $(M+H)^+$.

Compounds **9c**~**9h** and **9s** were similarly prepared from **6** with propiolic acid esters or propiolonitrile according to the procedure described for **9a**. Moreover, compounds **9n**~**9r** were also synthesized similarly with propiolamides, but a mixture of $CHCl_3$-MeOH (1:2) was used as the reaction solvent and the reaction was done at 5~20°C.

Diphenylmethyl 7β-[2-(2-Tritylaminothiazol-4-yl)-2-[(*Z*)-diphenylmethoxycarbonylmethoxyimino]-acetamido]-3-[(*Z*)-2-carbamoylvinylthio]-3-cephem-4-carboxylate (**9i**) (Method B)

To a suspension of silver (*Z*)-carbamoylvinylthiolate **8i** (115 mg, 0.55 mmol) in CH_3CN (6 ml) was added NaI (330 mg, 4.0 equiv) with stirring at room temperature. Then, a solution of compound **5** (500 mg, 0.83 equiv) in CH_3CN (4 ml) was added to the reaction mixture at 0°C, and stirred for 30 minutes at the same temperature. After the reaction, insoluble materials were removed by filtration and water (30 ml) was added to the filtrate, and extracted with EtOAc (50 ml). The extract was washed with brine (50 ml), and then dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (eluent; CH_2Cl_2) to afford 378 mg (75%) of **9i** as a pale yellow powder: IR (KBr) cm^{-1} 1780 (β-lactam), 1730, 1650; ^{1}H NMR ($CDCl_3$) δ 3.22 (1H, d, J=18 Hz, 2-Hα), 3.60 (1H, d, J=18 Hz, 2-Hβ), 4.90 and 5.06 (2H, ABq, J=17 Hz, $=NOCH_2$), 5.03 (1H, d, J=5 Hz, 6-H), 5.50 (2H, brs, NH_2), 5.74 (1H, d, J=11 Hz, $=CHCONH_2$), 5.93 (1H, dd, J=5 and 9 Hz, 7-H), 6.67 (1H, d, J=11 Hz, SCH=), 6.80 (1H, s, thiazole 5-H), 6.96 (1H, s, $CHPh_2$), 7.00 (1H, s, $CHPh_2$), 7.04 (1H, s, TrNH), 7.20~7.50 (35H, m, aromatic H), 8.07 (1H, d, J=9 Hz, CONH); FAB-MS *m/z* 1,103 $(M+H)^+$.

Similarly, compounds **9b** and **9j**~**9m** were prepared from **5** with a corresponding silver (*E*)- or (*Z*)-vinylthiolate **8** according to the procedure described for **9i**.

The spectral data of **9b**~**9h** and **9j**~**9s** prepared by the Method A or B are summarized in Table 3.

Sodium 7β-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-carboxymethoxyimino]acetamido]-3-[(*Z*)-2-methoxycarbonylvinylthio]-3-cephem-4-carboxylate (**1a**)

To a mixture of TFA (5 ml) and anisole (1 ml) was added **9a** (360 mg, 0.32 mmol) under ice-cooling, and stirred for 40 minutes at the same temperature. Then, the reaction mixture was added dropwise to a mixture of Et_2O and *n*-hexane (1 : 2, 40 ml). The precipitated TFA salt of the desired product was collected by filtration. Subsequently, the TFA salt (230 mg) was dissolved in water (5 ml) with $NaHCO_3$ (82 mg, 3.0 equiv) and chromatographed on Sephadex LH-20 column (eluent; H_2O), and then lyophilized to afford 180 mg (93%) of **1a** as an amorphous solid: IR (KBr) cm^{-1} 1760 (β-lactam), 1675; ^1H NMR (D_2O) δ 3.58 (1H, d, J=17 Hz, 2-Hα), 3.77 (3H, s, CO_2CH_3), 3.98 (1H, d, J=17 Hz, 2-Hβ), 4.60 (2H, s, =$NOCH_2$), 5.32 (1H, d, J=5 Hz, 6-H), 5.89 (1H, d, J=5 Hz, 7-H), 6.06 (1H, d, J=11 Hz, =$CHCO_2CH_3$), 7.08 (1H, s, thiazole 5-H), 7.35 (1H, d, J=11 Hz, SCH=); HPLC analysis: 95% purity.

Compounds **1b**~**1s** were similarly prepared from **9b**~**9s** according to the procedure for **1a**, and their spectral data are listed in Table 4. The purities of **1b**~**1s** were 94~96% by HPLC analysis.

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