CEPHALOSPORINS. VII

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NEW CEPHALOSPORINS BEARING A 2-IMINO-3-HYDROXYTHIAZOLINE (2-AMINOTHIAZOLE N-OXIDE) IN THE C-7 ACYLAMINO SIDE CHAIN

Ettore Perrone*, Marco Alpegiani, Franco Giudici,
Franco Buzzetti, Giuliano Nannini

Farmitalia Carlo Erba SpA, Ricerca e Sviluppo Chimico
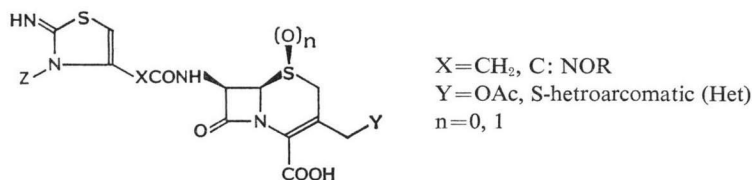Via C. Imbonati 24, 20159 Milan, Italy

Giuseppe Meinardi, Silvio Grasso, Alberta Bianchi
and Ivo de Carneri

Farmitalia Carlo Erba SpA, Ricerca e Sviluppo Biologico
Via C. Imbonati 24, 20159 Milan, Italy
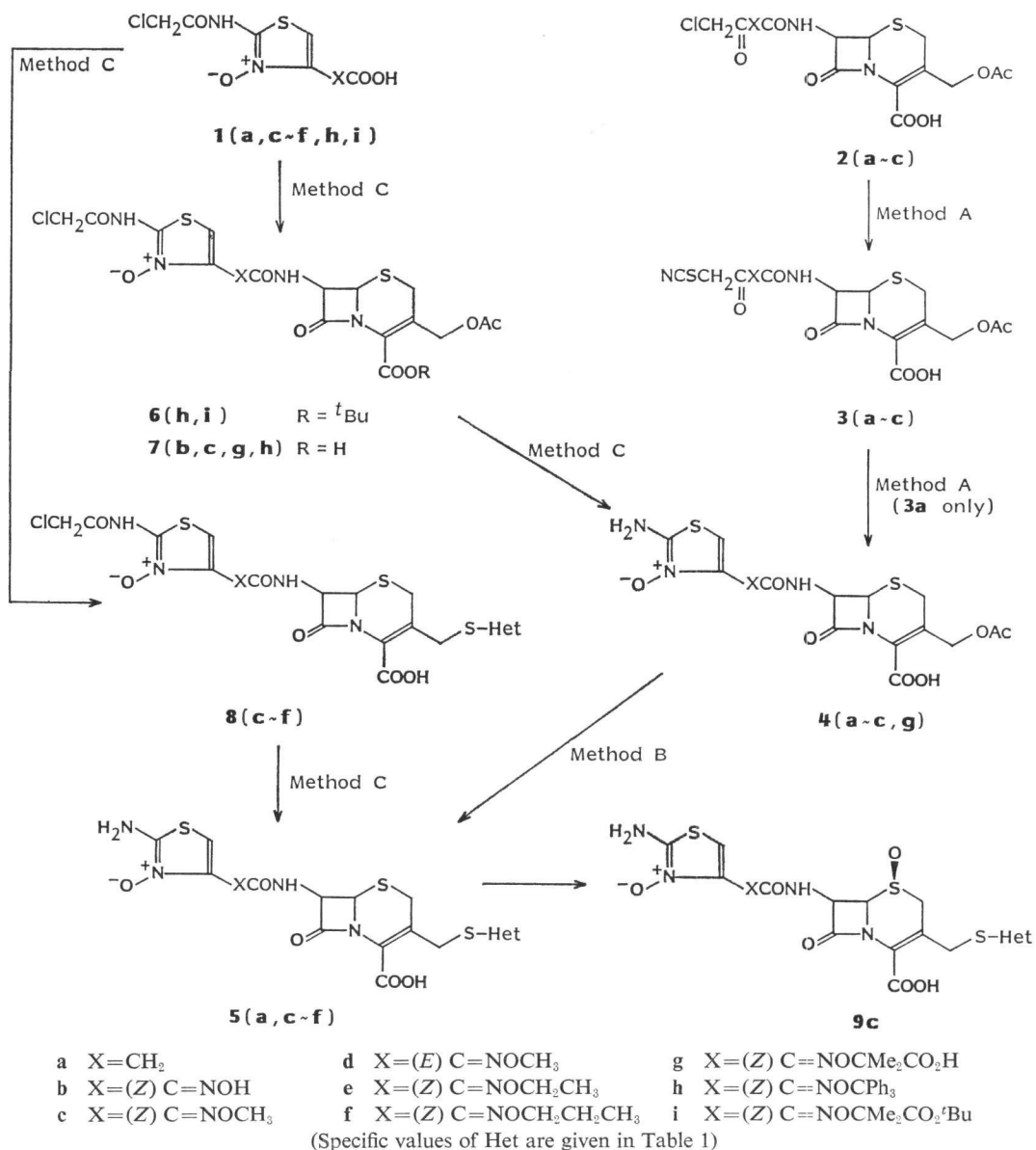
(Received for publication July 18, 1984)

Introduction of a hydroxyl group into the thiazole ring nitrogen of cephalosporins belonging to the cefotiam and cefotaxime families gave rise to products, better described by the tautomeric N-oxide form, which proved particularly active against Gram-negative bacteria. Cephems bearing a (Z)-alkoxyimino functionality are of special interest for broadness of spectrum; among them, 7β-[(Z)-2-(2-amino-4-thiazolyl-N-oxide)-2-methoxyiminoacetamido]-3-(tetrazolo-[1,5-b]pyridazin-6-yl)thiomethyl-3-cephem-4-carboxylic acid (5c-7, FCE 20635), in other ways similar to cefotaxime, showed useful levels of activity against cephalosporinase-producing strains resistant to the reference drug. Preliminary *in vivo* studies demonstrated the therapeutic efficacy of the new compound in the treatment of experimental systemic, subcutaneous and urinary tract infections in mice.

Exploitation of 2-aminothiazolylacetic acid derivatives as side-chain components of cephalosporins has triggered a major revolution in the design of new semisynthetic β-lactam antibiotics. The development of an impressive number of useful compounds, ranging from cefotiam[1] and cefotaxime[2] to the newer monobactams azthreonam[3] and AMA-1080[4], is just the emerging part of a vast research that has involved nearly every institution active in this area. In retrospect, it could be inferred that the 2-aminothiazole moiety has to be left untouched in order to retain antibacterial activity; 2-amino-5-halo derivatives are the sole substituted entries of some interest so far reported[5,6]. In this context the results of a programme we had started a few years ago, devoted to cephalosporins **Ia ~ c** formally derived from the

X=CH$_2$, C: NOR
Y=OAc, S-hetroarcomatic (Het)
n=0, 1

Ia   Z=OH
Ib   Z=O-Alkyl
Ic   Z=NH$_2$

Scheme 1.

a X=CH$_2$	d X=(E) C=NOCH$_3$	g X=(Z) C=NOCMe$_2$CO$_2$H
b X=(Z) C=NOH	e X=(Z) C=NOCH$_2$CH$_3$	h X=(Z) C=NOCPh$_3$
c X=(Z) C=NOCH$_3$	f X=(Z) C=NOCH$_2$CH$_2$CH$_3$	i X=(Z) C=NOCMe$_2$CO$_2$^{t}Bu

(Specific values of Het are given in Table 1)

classical structures upon introduction of heteroatomic functionalities on the aminothiazole ring nitrogen, are particularly significant; some of the new compounds, particularly members of the family represented by structure Ia, display potent and peculiar antimicrobial properties.

Chemistry

In principle, the thiazole portion of compounds Ia can be described either as a 2-imino-3-hydroxy-thiazoline or as a 2-aminothiazole N-oxide. Two synthetic approaches, i.e. sequential reaction of 4-chloroacetoacetates with potassium thiocyanate and hydroxylamine, or direct oxidation of suitably protected 2-aminothiazoles, were accordingly followed and their results compared. A close analysis of

the spectral properties of the compounds synthesized led us to propose the *N*-oxide structure as more representative, and such structure is therefore referred to throughout this paper in place of the 2-imino-3-hydroxythiazolinyl tautomers **Ia** first considered at the onset of our programme. Those studies, which include the preparation of the side-chain synthons **1** (**a**, **c** ~ **f**, **h**, **i**), are published elsewhere[7].

Acetamido Series (Ia, X=CH₂)

Although formally accessible from **1a** (Scheme 1), these analogs of cefotiam were more easily obtained by building up the thiazole *N*-oxide nucleus on the readily available 4-chloro-3-oxobutyramido cephem **2a**[1]. Thus, reaction of the latter with potassium thiocyanate afforded **3a**, which was in turn treated with hydroxylamine hydrochloride to give **4a** [Method A]. Displacement of acetate by the appropriate mercaptide according to a well-established procedure (acetone - water, 65°C, NaHCO₃) converted **4a** into **5a**, albeit in moderate yields [Method B].

(Oxyimino)acetamido Series (Ia, X=C: NOR)

Following the successful preparation of **4a**, the hydroxyiminoacetamido and methoxyiminoacetamidocephems **4b** and **4c** were first approached through method A. Although **2b** and **2c** readily reacted with potassium thiocyanate, further reaction with hydroxylamine hydrochloride to give the cyclized products **4b**, **4c** could not be achieved. Thus, we went back to the study of activation and coupling of the isolated side-chain synthon **1c** with 7-aminocephalosporanic acid (7-ACA) and its 3-heterocyclylthiomethyl derivatives. Different activating agents and coupling conditions were tested; our best results were obtained by silylating the aminocephem with bis(trimethylsilyl)acetamide in tetrahydrofuran or acetonitrile, adding the resultant solution to the reaction mixture from **1c** and dicyclohexylcarbodiimide (0.6 ~ 0.8 molar equivalents) in the same solvent. Removal of the amino protecting group in **7c** or **8c** completed the sequence [Method C]; alternatively (but less satisfactorily) **5c** could be prepared from **4c** and the appropriate thiol according to Method B. Similar procedures on the (*E*)-methoxime **1d** and the homologated (*Z*)-alkoximes **1e**, **f** afforded the cephems **5d** ~ **f**. Preparation of the hydroxyimino and 2-carboxy-2-propoxyimino representatives **4b**, **4g** required additional protection; suitable starting materials were, respectively, the chloroacetamido-trityloxy synthon **1h** and the chloroacetamido-*tert*-butoxycarbonyl analogue **1i**. Activation as above and coupling with 7-ACA *tert*-butyl ester gave the fully protected intermediates **6h**, **6i**, which were converted into the target compounds by conventional cleavage of the protecting groups. The cephem (1*S*)-sulfoxide **9c** was obtained after oxidation with *m*-chloroperbenzoic acid; the use of formic acid as a solvent allowed this reaction to be performed on the free amino acid **5c**.

Bacteriology

Materials and Methods

Antibiotic Spectrum

Minimal inhibitory concentrations (MICs) were determined by the standard two-fold serial dilution method in diagnostic sensitivity test agar (Oxoid); the plates were inoculated with 2×10^5 colony forming units using an automatic inoculator (Denley Tech. Ltd.).

Susceptibility Distribution of Bacteria Isolated from In-patients and Out-patients to FCE 20635

This study was carried on 180 strains, 64 of them isolated in Ospedale Maggiore (Milan) from urines of patients with a bacterial load of over 10^5 cells/ml; the other 116 were isolated from out-patients attending 17 clinics in Lombardy, Liguria and Piedmont. Organisms tested were: *Escherichia coli* (30 strains), *Klebsiella* spp. (30 strains), *Proteus mirabilis* (30 strains), indole positive *Proteus* (40 strains),

Enterobacter spp. (14 strains), *Citrobacter* spp. (2 strains), *Serratia* spp. (4 strains) and *Staphylococcus* spp. (30 strains). The antibacterial activity of FCE 20635 (compound **5c-7**) was evaluated by determining the MICs against these strains according to the above described method; cefotaxime, cefuroxime and cefazolin were assayed as comparison drugs.

Therapeutic Activity in Experimental Infections in Mice

Acute Systemic Infection: Male albino Iva: N.M.R.I. (Kiss) mice, $18 \sim 20$ g, were infected intraperitoneally with bacterial suspensions with or without gastric mucin, depending on the virulence of the strain, in amounts corresponding to the LD_{99}. Treatments were given subcutaneously immediately after the infection and 3 hours later according to a complete balanced block design, with random assignment of the drugs (2 groups of 6 or 7 mice per dose). The animals were kept under observation for 7 days; from the survival frequency the median effective doses (ED_{50}) were calculated by probit analysis[5]. In this experiment FCE 20635 was compared with cefotaxime and cefazolin.

Local Subcutaneous Infection: For this study male albino Iva: N.M.R.I. (Kiss) mice weighing about 25 g were used. A metal chamber was inserted under the skin of each mouse and was allowed to fill with a pathological fluid for 5 days. Then these chambers were injected with 0.05 ml of bacterial suspensions able to infect $99 \sim 100\%$ of the animals. Treatments were given subcutaneously immediately after infection and 6 hours later (5 or 15 mice per dose). Therapeutic activity was checked 24 hours later by counting the viable cells in chamber fluid. On the basis of the mean logs of the bacterial cell numbers found in animals treated with various doses of drug, a dose-response curve was constructed. From this curve the ED_{50}, *i.e.* the dose that reduced the log of the number of bacteria to 50% of the log for the untreated control mice, was obtained. In this model for local infection, FCE 20635 was tested in parallel with cefotaxime and cefoxitin against *Klebsiella aerogenes* 1082 E (inoculum 5×10^6 cells/mouse).

Urinary Tract Infection: Renal infections with *E. coli* S were obtained by injecting 2.5×10^5 cells per mouse into the left kidney of male albino Icem: CET (SPF Caw) mice weighing about 25 g. The drugs were given subcutaneously $\frac{1}{2}$ and 5 hours after infection and then twice a day for the next four days (total of 10 treatments). The efficacy of treatment was checked 72 hours after the last treatment, by counting the bacteria present in the bladders and kidneys of treated and untreated (control) mice. The dose-response curve was drawn as described for the local subcutaneous infection. In this model, FCE 20635 was studied in parallel with cefotaxime and cefuroxime.

Pharmacokinetics in Mice

Serum Levels: Serum levels of FCE 20635 and cefazolin were determined in Crl: CD-1(ICR)BR mice, after a single iv injection of 50 mg/kg. Groups of 8 mice (20 ± 1 g) were used at each sampling time. Serum antibiotic concentrations were determined by the agar plate diffusion method, using *Bacillus pumilus* NCTC 8241 as test microorganism and Folic TE (Difco) as assay medium. The standard curve and sample dilutions were prepared using sterile horse serum. Serum half-life was calculated by the least squares method in the therminal linear phase of the curve obtained by plotting log concentrations *versus* time.

Serum Protein Binding: Serum protein binding of FCE 20635 and cefazolin was determined by an ultrafiltration method at concentration of 40 μg/ml in fresh human, mouse and rat sera. Ultrafiltration was carried out by centrifugation in Centriflo membrane cones, type CF 50 (Amicon Corp). The antibiotic concentrations in the ultrafiltrate were determined microbiologically by the described agar plate diffusion method, with *B. pumilus* NCTC 8241.

Resistance to β-Lactamases

The resistance of FCE 20635 to β-lactamases was compared to that of cefotaxime and cephaloridine by the iodometric method of PERRET[9] as modified by JACK and RICHMOND[10]. For these substrates the maximum rate of hydrolysis (*Vmax*) was measured at 30°C in conditions of enzyme saturation (substrate concentrations: 6 mM in phosphate buffer, pH 5.9) using three different types of β-lactamases, produced by Gram-negative bacteria. The maximum hydrolysis rate of FCE 20635 and cefotaxime was quoted in relation to that of cephaloridine, taken arbitrarily as 100. The three β-lactamases employed

were produced by *E. coli* TEM (plasmid mediated β-lactamase), *K. aerogenes* 1082 E and *Enterobacter cloacae* P 99 (chromosomally mediated β-lactamases); they belong respectively to RICHMOND and SYKES classes III, IVc and Ia[11].
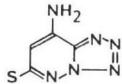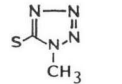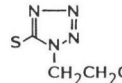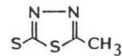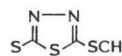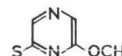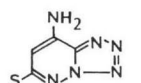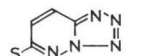
Results and Discussion

Structure - *In Vitro* Activity Relationships

In vitro assessment of antimicrobial properties against a number of bacterial strains was routinely performed following the synthesis of each compound; Table 1 summarizes the most representative results, where cefazolin and cefotaxime are taken as reference standards. The prototype of the new family, **4a**, displayed the antibacterial spectrum proper of a classical cephalosporin (cefazolin), with a marked shift in favor of activity against Gram-negative organisms. Replacement of the acetoxy group with 8-amino-6-mercaptotetrazolo[1,5-b]pyridazine, a thiol we had developed and employed with success under similar circumstances[12], resulted in a ten-fold increase of activity against most Gram-positive and Gram-negative bacteria, embodied in **5a-6**, a product of absolute interest at that time but lacking the β-lactamase stability shown by the newly emerging cephalosporins.

Investigation along this line was soon discontinued, as we were made aware of an interfering patent[13], and our attention focused on the oxyimino analogs **4b, 4c, 4g**. Inspection of data from Table 1 revealed some interesting trends, which only in part fitted the structure-activity relationships commonly accepted in the cefotaxime family. First, replacement of the α-methylene hydrogen atoms of the thiazolylacetyl side chain by a (*Z*)-methoxyimino group (**4c** vs. **4a**) produced an enhancement of potency against Enterobacteriaceae (but loss against staphylococci), while extending the antibacterial spectrum to cover activity against plasmid and chromosomally mediated β-lactamase producers. Second, the unsubstituted hydroxyimino derivative (**4b** vs. **4c**) gave, contrary to commonly accepted lipophilicity-activity correlations[14], improved results against staphylococci and less favorable performances against Gram-negative and β-lactamase-producing bacteria. The product bearing the oxime ether moiety characterizing ceftazidime fell short of our expectations (**4g** vs. **4c**), leading to a general decrease of activity (except for *Proteus* species), without the hoped-for improvement against *Pseudomonas aeruginosa*. In comparison with cefotaxime, the methoxime **4c** retained the outstanding activity of the reference drug against Gram-negative microorganisms, but suffered from substantial losses against Gram-positive bacteria. The most appealing feature of **4c** was, however, its superior activity against type Ia and IVc β-lactamase-producing strains; this particular antimicrobial spectrum was, at the time when compounds **4, 5** were first synthesized[15], unprecedented in the cephalosporin field. It was later, and much to our surprise, that a report[16] appeared describing the peculiar antibacterial spectrum of HR 109, the cefotaxime (1*S*)-sulfoxide. As a matter of fact this compound, synthesized in our laboratories for comparison purposes and included in Table 1, does strikingly reproduce the antimicrobial activity of **4c**. Interestingly, both **4c** and HR 109 are oxides of cefotaxime — the first an *N*-oxide and the second an *S*-oxide — and penetration properties into the outer membrane of bacteria, which are held responsible for the activity of the latter, could in principle, be similar.

Next, the effects of substitution in the C-3 methyl group were investigated in an effort to restore the anti-staphylococcal activity of **4c** up to an acceptable level while further increasing the newly discovered advantages over cefotaxime. Replacement of the acetoxy with the 1-methyltetrazol-4-ylthio group (**5c-l** vs. **4c**) was rather disappointing, since the two-fold increase against Gram-positive bacteria and the three-fold increase against *E. cloacae* P99 thus gained were not enough to substantiate expectations of

Table 1. *In vitro* antibacterial activity of cephalosporins **Ia**.

Compound	X	Y	n	MIC (μg/ml)[a,b]											
				S.a.+	S.p.	E.c.	K.p.	E.ae.	S.s.	P.m.	P.v.	E.c.+	K.ae.+	E.cl.+	P.ae.
4a	CH₂	OCOCH₃	0	5.66	0.17	0.35	0.12	0.25	2	0.06	>128	0.5	90.5	>128	>256
5a-6	"	(4-amino-tetrazolo[1,5-b]pyridazin-6-yl)thio	"	0.57	0.015	0.04	0.015	0.12	0.17	0.008	90.5	0.17	>128	>128	181
4b	(Z)C: NOH	OCOCH₃	0	8	0.25	0.17	0.12	0.35	0.35	0.25	0.35	0.17	1.4	>128	256
4g	(Z)C:NOCMe₂CO₂H	OCOCH₃	"	>128	1.4	1	0.5	2	1	0.015	0.03	2	1	>128	90.5
4c	(Z)C: NOMe	OCOCH₃	"	32	0.12	0.08	0.011	0.12	0.17	0.03	0.03	0.06	0.17	90.5	90.5
5c-1	"	(1-methyl-1H-tetrazol-5-yl)thio	"	16	0.06	0.12	0.011	0.17	0.25	0.03	0.02	0.06	0.17	32	128
5c-2	"	(1-(2-cyanoethyl)-1H-tetrazol-5-yl)thio	"	16	0.06	0.25	0.011	0.25	0.7	0.03	0.02	0.12	0.17	32	181
5c-3	"	(5-methyl-1,3,4-thiadiazol-2-yl)thio	"	11.3	0.03	0.25	0.008	0.25	0.35	0.06	0.011	0.12	0.25	22.6	181
5c-4	"	(5-methylthio-1,3,4-thiadiazol-2-yl)thio	"	8	0.03	1	0.011	2	1	0.12	0.011	0.7	1	11.3	256
5c-5	"	(3-methoxypyridazin-6-yl)thio	"	8	0.02	1.4	0.04	1.4	1.4	0.35	0.02	0.7	1.4	64	>256
5c-6	"	(4-amino-tetrazolo[1,5-b]pyridazin-6-yl)thio	"	4	0.015	0.35	0.003	0.5	0.7	0.03	0.04	0.25	0.35	8	46
5c-7[c]	"	(tetrazolo[1,5-b]pyridazin-6-yl)thio	"	2.8	0.015	0.08	0.003	0.17	0.25	0.008	0.006	0.06	0.08	2.8	64

	R			S.a.+	S.p.	E.c.	K.p.	E.ae.	S.s.	P.m.	P.v.	E.c.+	K.ae.+	E.cl.+	P.ae.
5d-7	(*E*)C: NOMe	″	″	32	0.25	8	0.25	16	32	0.25	0.35	5.66	5.66	128	>256
5e-7	(*Z*)C: NOEt	″	″	2	0.008	0.17	0.004	0.5	0.5	0.015	0.002	0.25	0.12	4	32
5f-7	(*Z*)C: NO*n*Pr	″	″	1.4	0.008	0.5	0.006	1	0.7	0.17	0.002	0.5	0.5	8	16
8c-7[d]	(*Z*)C: NOMe	″	″	8	0.08	1	0.04	2	2.8	0.06	0.06	1	1.4	32	256
9c-7	(*Z*)C: NOMe	″	1	>128	0.5	0.12	0.04	0.25	0.5	0.015	0.12	0.25	0.17	1	>256
	Cefazolin			0.57	0.06	1.4	0.7	2	4	5.7	>128	8	>128	>128	>256
	Cefotaxime			2	0.006	0.12	0.004	0.12	0.12	0.008	0.003	0.06	5.7	>128	4
	HR 109			32	0.06	0.12	0.03	0.12	0.12	0.03	0.04	0.08	0.5	64	32

[a] Organisms included in this Table are: *S.a.*+, *Staphylococcus aureus* 39/2 (penicillinase producer); *S.p.*, *Streptococcus pyogenes* C203; *E.c.*, *Escherichia coli* G; *K.p.*, *Klebsiella pneumoniae* ATCC 10031; *E.ae.*, *Enterobacter aerogenes* ATCC 8308; *S.s.*, *Shigella sonnei* ATCC 11060; *P.m.*, *Proteus mirabilis* ATCC 9921; *P.v.*, *P. vulgaris* X20; *E.c.*+, *E. coli* TEM (producer of plasmid mediated β-lactamase); *K.ae.*+, *K. aerogenes* 1082E (producer of chromosomally mediated β-lactamase IVc); *E.cl.*+, *Enterobacter cloacae* P99 (producer of chromosomally mediated β-lactamase Ia); *P.ae.*, *Pseudomonas aeruginosa* ATCC 9027.

[b] Geometric mean values of two determinations.

[c] Code-named FCE 20635.

[d] *N*-(Chloroacetyl)derivative of **5c-7**.

Fig. 1. Susceptibility distribution curves for FCE 20635 and other cephalosporins of 30 strains of *Staphylococcus* sp. isolated from in-patients and out-patients.

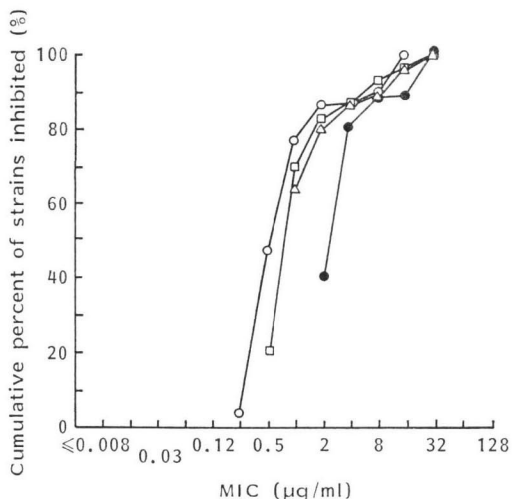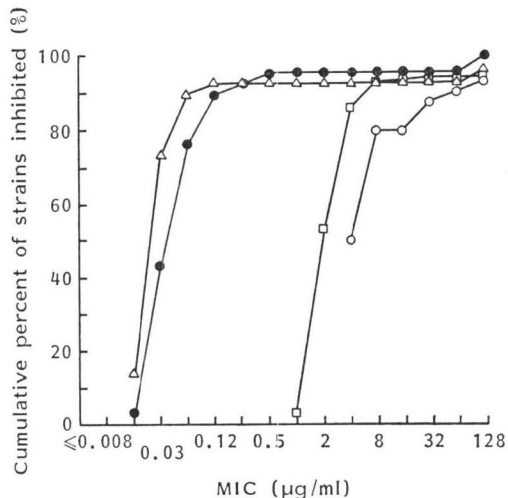● FCE 20635, △ cefotaxime, □ cefuroxime, ○ cefazolin.

Fig. 2. Susceptibility distribution curves for FCE 20635 and other cephalosporins of 30 strains of *P. mirabilis* isolated from in-patients and out-patients.

● FCE 20635, △ cefotaxime, □ cefuroxime, ○ cefazolin.

therapeutic usefulness.   More lipophilic thiols were of little avail (**5c-3～5**), rather threatening erosion of the brilliant properties of the parent compound against Enterobacteriaceae, but again activity increased by introduction of the tetrazolopyridazine groups[17] (**5c-6, 5c-7**).   In particular **5c-7**, code-named FCE 20635, proved reasonably effective against staphylococci and *E. cloacae* P99, at the same time displaying an outstanding level of activity against Gram-negative bacteria, *K. aerogenes* 1082 E included. Oxime geometry was an essential contributor to activity, the anti (*E*) isomer **5d** being two orders of magnitude less active against most bacterial strains.   Almost equally impressive was the loss of activity resulting from protection of the thiazole amino group (**8c-7** vs. **5c-7**): in both compounds, however, the β-lactamase stability was not apparently affected.   The relationship between activity and length of the oxime ether group followed a well-established pattern[14] but for *Pseudomonas aeruginosa*, were an unusual positive trend was observed.   Finally, stimulated by the previously suggested correlation with *S*-oxides of the cefotaxime family, we prepared the *S,N*-dioxide **9c**; although generally inferior to the parent compound, this product did show, as expected, a first-rate degree of activity when tested against the β-lactamase producing *Klebsiella* and *Enterobacter* strains.

Susceptibility Distribution of Bacterial Isolates to FCE 20635

Figs. 1～6 illustrate the susceptibility to FCE 20635 and cefotaxime, cefuroxime, cefazolin (cumulative percent of strains inhibited at different concentrations) of several bacterial isolates from in-patients and out-patients.   Apart that against *Staphylococcus* (Fig. 1), where cefazolin was the most active compound and FCE 20635 and cefotaxime were only moderately active, the latter two compounds surpassed the other reference drugs, with a trend towards greater potency in cefotaxime and towards wider spectrum in FCE 20635 (Figs. 2～5).

Thus on *P. mirabilis* (30 strains) FCE 20635 inhibited 29 out of the 30 strains tested at a concentration of 0.5 µg/ml; at this concentration cefuroxime and cefazolin did not inhibit any of the strains (Fig.

Fig. 3. Susceptibility distribution curves for FCE 20635 and other cephalosporins of 40 strains of indole-positive *Proteus* (17 *P. vulgaris*, 14 *P. morganii*, 9 *P. rettgeri*) isolated from in-patients and out-patients.

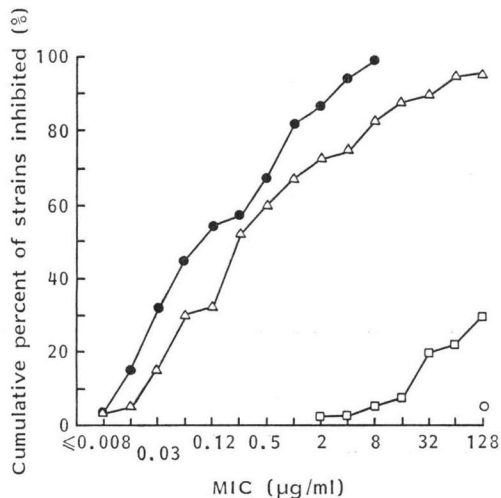● FCE 20635, △ cefotaxime, □ cefuroxime, ○ cefazolin.

Fig. 4. Susceptibility distribution curves for FCE 20635 and other cephalosporins of 14 strains of *Enterobacter*, 2 strains of *Citrobacter* and 4 strains of *Serratia* isolated from in-patients and out-patients.

● FCE 20635, △ cefotaxime, □ cefuroxime, ○ cefazolin.
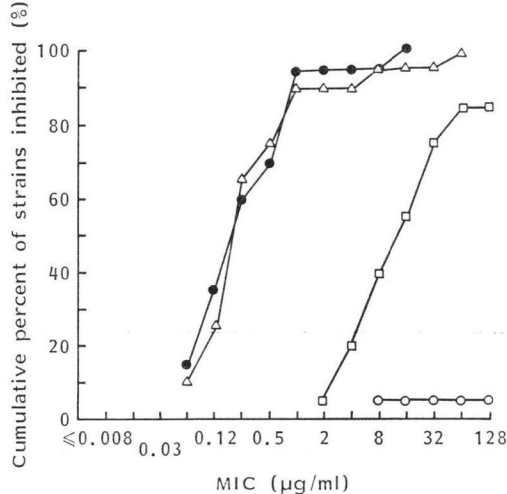
Fig. 5. Susceptibility distribution curves for FCE 20635 and other cephalosporins of 30 strains of *Klebsiella* sp. isolated from in-patients and out-patients.

● FCE 20635, △ cefotaxime, □ cefuroxime, ○ cefazolin.
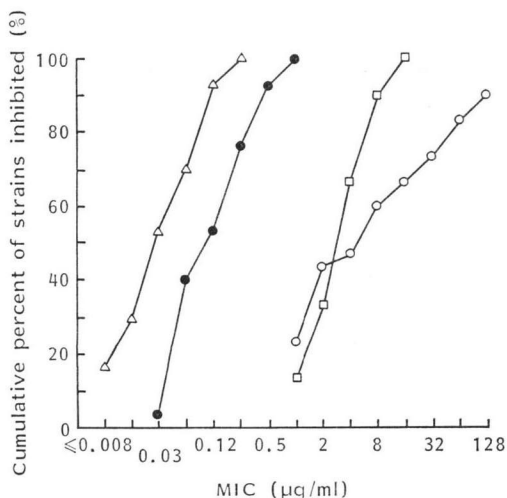
Fig. 6. Susceptibility distribution curves for FCE 20635 and other cephalosporins of 30 strains of *E. coli* isolated from in-patients and out-patients.

● FCE 20635, △ cefotaxime, □ cefuroxime, ○ cefazolin.
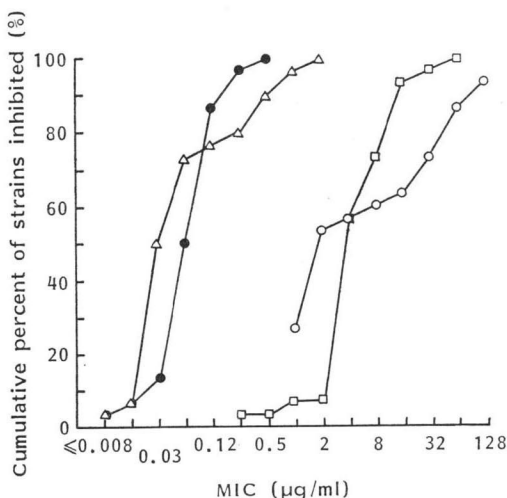
2). On indole-positive *Proteus* normally resistant to older cephalosporins, FCE 20635 inhibited 87.5% of the strains at 2 μg/ml. Cefotaxime was less active inhibiting 72.5% of these strains at this concentration. All the strains were inhibited by FCE 20635 at 8 μg/ml, whereas 5 strains showed moderate re-

Table 2.   Therapeutic activities of FCE 20635, cefotaxime and cefazolin in acute systemic infections in mice.

Organisms	ED_{50} mg/kg (fiducial limits for $P=0.95$)		
	FCE 20635	Cefotaxime	Cefazolin
S. aureus Smith	3.43	0.8	0.11
	$(2.34 \sim 5.03)$	$(0.52 \sim 1.35)$	$(0.07 \sim 0.17)$
S. pyogenes C 203	0.41	0.1	0.79
	$(0.33 \sim 0.48)$	$(0.06 \sim 0.20)$	$(0.58 \sim 1.08)$
E. coli G	0.19	0.2	9.32
	$(0.14 \sim 0.24)$	$(0.12 \sim 0.34)$	$(6.97 \sim 12.47)$
P. mirabilis ATCC 9921	0.06	0.14	14.82
	$(0.03 \sim 0.12)$	$(0.04 \sim 0.46)$	$(12.47 \sim 17.81)$
Salmonella typhi Watson	0.53	0.43	16.03
	$(0.32 \sim 0.90)$	$(0.29 \sim 0.62)$	$(10.23 \sim 25.14)$
K. pneumoniae ATCC 10031	0.13	0.19	15.46
	$(0.05 \sim 0.28)$	$(0.05 \sim 0.76)$	$(9.5 \sim 25.15)$
E. coli G R^+ TEM	0.39	0.57	83.1
	$(0.21 \sim 0.71)$	$(0.33 \sim 0.99)$	$(58.41 \sim 131.75)$

Table 3.   Therapeutic activities of FCE 20635, cefotaxime and cefoxitin in local subcutaneous infection in the mouse with β-lactamase producing K. aerogenes 1082 E (RICHMOND type IVc).

Drug	Dose (mg/kg)	No. mice	Mean log No. bacteria/ml	ED_{50}* (mg/kg)
FCE 20635	25	5	6.34	
	100	5	5.70	160
	400	5	3.54	
Cefotaxime	25	5	8.54	
	100	5	7.84	>400
	400	5	6.35	
Cefoxitin	25	5	7.12	
	100	5	4.39	85
	400	5	2.53	
Untreated controls	—	5	9.64	—

* ED_{50}=dose needed to reduce the logarithm of the number of bacteria to 50% of that for the controls.

sistance to cefotaxime (MIC between 16 and 64 μg/ml), and 2 strong resistance (MIC $\geq$ 128 μg/ml, Fig. 3).   Against *Enterobacter*, *Citrobacter* and *Serratia*, FCE 20635 showed the same activity of cefotaxime.   Nineteen of the 20 strains were inhibited by 1 μg/ml of FCE 20635, and the same concentration of cefotaxime inhibited 18.   Cefuroxime was much less active, and cefazolin showed no activity (Fig. 4).   Similarly, against *E. coli* the highest percentages of inhibitions were reached at lower concentrations of FCE 20635 than of cefotaxime (Fig. 5).   Against *Klebsiella*, FCE 20635 was slightly inferior to cefotaxime, but nonetheless the totality of the 30 strains tested were inhibited at a concentration of 1 μg/ml (Fig. 6).

Mouse Protection Tests

The therapeutic effectiveness of FCE 20635 was demonstrated against experimental systemic, subcutaneous and urinary tract infections in the mouse (Tables 2~4).

In acute systemic infections (Table 2), FCE 20635 was found on the whole to have the same activity

Table 4. Therapeutic activities of FCE 20635, cefotaxime and cefuroxime in experimental urinary tract infection in the mouse: renal infection with *E. coli* S.

Drug	Dose (mg/kg)	No. mice	Mean log No. bacterial/ml (K+B)*/2	ED_{50}** (mg/kg)
FCE 20635	0.01	12	3.90	0.12
	0.1	12	2.91	
	1	12	0.77	
	10	12	0.13	
Cefotaxime	0.01	12	4.10	0.28
	0.1	12	2.89	
	1	12	2.17	
	10	12	0.39	
Cefuroxime	0.1	12	5.23	7
	1	12	5.40	
	10	12	2.39	
	100	12	0.12	
Untreated controls	—	12	5.86	—

* (K+B)=Kidney+Bladder

** ED_{50}=dose needed to reduce the logarithm of the number of bacteria to 50% of that for the controls.

Fig. 7. Serum levels of FCE 20635 and cefazolin in mice, Iva: N.M.R.I. (Kiss), after iv injection of 50 mg/kg.
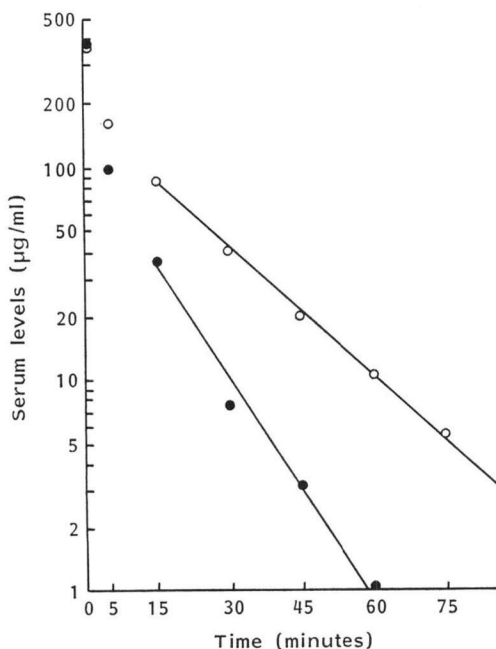
○ Cefazolin t$\frac{1}{2}$ 15 minutes, ● FCE 20635 t$\frac{1}{2}$ 9 minutes.

Table 5. Serum protein binding of FCE 20635 and cefazolin (% of drug bound).

Antibiotic	Man	Mouse	Rat
FCE 20635	77.8	60	74.8
Cefazolin	90	72	92

as cefotaxime against Gram-negative bacteria and from 10 to 600 times the activity of cefazolin. On the β-lactamase producing strain, *E. coli* carrying R6K plasmid, FCE 20635 was slightly more active than cefotaxime. On *S. aureus* Smith and *Streptococcus pyogenes* C 203, cefotaxime was about 4 times more active than FCE 20635.

In local infection with *K. aerogenes* 1082 E (Table 3), FCE 20635 confirmed the *in vitro* findings, being efficacious at considerably lower doses than those required for cefotaxime (cefoxitin was here slightly more active than FCE 20635).

In kidney infections due to *E. coli* (Table 4), FCE 20635 was approximately twice as active as cefotaxime. Both drugs were considerably more effective than cefuroxime.

Pharmacokinetics

Serum levels of FCE 20635 in the mouse are shown in Fig. 7. The serum levels obtained with cefazolin on animals of the same strains and in the same experimental conditions are reported for com-

parison. The serum half-life of FCE 20635 (9 minutes) was approximately 2/3 of that of cefazolin, a well known long acting cephalosporin.

Binding of FCE 20635 to serum proteins was lower than that of cefazolin in all the species studied (Table 5).

Resistance to β-Lactamases

Table 6 shows that the resistance of FCE 20635 to crude extracts of β-lactamases is of the same order as that of cefotaxime. Thus, the superior efficacy of the former compound against living cells of *K. aerogenes* 1082E and *E. cloacae* P99 must be ascribed to other factors, such as better penetration properties or the ability to escape the "trapping mechanism"[15] which inactivates cefotaxime.

Table 6. Relative rates of hydrolysis (*Vmax*) of FCE 20635, cefotaxime and cephaloridine by β-lactamases from Gram-negative bacteria.

Organism producing β-lactamase	Class of β-lactamase*	Cephaloridine	FCE 20635	Cefotaxime
E. coli TEM	III	100	0.7	0.3
K. aerogenes 1082 E	IVc	100	4.7	5.8
E. cloacae P 99	Ia	100	0.2	0.2

*   According to RICHMOND and SYKES (1973).

In summary, the family of cephalosporins of the present study display interesting features, in part reminiscent of those possessed by the (1*S*)-oxide of cefotaxime; these properties can be found at their best in compound **5c-7** (FCE 20635), which exhibits a very remarkable activity against Gram-negative bacteria, including many cefotaxime-resistant strains, producers of cephalosporinases. Further investigations are encouraged by the pharmacokinetic profile of the product and preliminary *in vivo* studies.

Experimental

NMR spectra were recorded at 60 MHz (Hitachi-Perkin Elmer R24b instrument) or at 90 MHz (Bruker HX-90 instrument); chemical shifts are reported in parts per million (δ) relative to Me$_4$Si.   IR spectra were taken on a Perkin Elmer spectrophotometer (model 125).   Melting points are uncorrected.

Method A
7β-[2-(2-Amino-4-thiazolyl-*N*-oxide)acetamido]-3-acetoxymethyl-3-cephem-4-carboxylic Acid (**4a**)

A solution of 7β-(4-chloro-3-oxobutyramido)cephalosporanic acid (**2a**[1]) (1.8 g, 5 mmol) in DMF (5 ml) was stirred overnight with potassium thiocyanate (0.5 g, 5.14 mmol), after which time complete conversion into the thiocyano derivative **3a** had occurred (TLC monitoring).   Finely powdered hydroxylamine hydrochloride (0.35 g, 5 mmol) was then added and the mixture stirred for 24 hours at room temp.   The reaction mixture was poured with stirring into cold H$_2$O, thus obtaining the separation of a gum, which was collected and dissolved in demineralized H$_2$O containing NaHCO$_3$ (1.26 g, 15 mmol). This solution was passed through a reverse-phase column (LiChroprep RP-18, Merck), eluting with H$_2$O, to afford an aqueous solution of the sodium salt of the title product.   The free acid **4a** was obtained as a white-off precipitate after concentration and treatment with 1 M HCl; 0.98 g (46%), mp 235°C (dec); IR (KBr) 1765, 1725, 1670, 1620, 1380 cm^{-1}; NMR (90 MHz, DMSO-d_6) δ 2.05 (3H, s, COCH$_3$), 3.38 (2H, ABq, *J*=18 Hz, 2-H), 3.60 (2H, s, CH$_2$CO), 4.85 (2H, ABq, *J*=12 Hz, 3-CH$_2$), 5.0 (1H, d, *J*=5 Hz, 6-H), 5.60 (1H, dd, *J*=5 and 8 Hz, 7-H), 6.7 (1H, s, 5-H on thiazole ring), 8.2 (2H, br s, NH$_2$), 10.4 (1H, br d, *J*=8 Hz, CONH).

Anal Calcd for C$_{15}$H$_{16}$N$_4$O$_7$S$_2$:   C 42.05, H 3.76, N 13.07, S 14.97.
Found: C 42.11, H 4.00, N 12.78, S 14.76.

Method B

7β-[2-(2-Amino-4-thiazolyl-N-oxide)acetamido]-3-(8-aminotetrazolo[1,5-b]pyridazin-6-yl)thiomethyl-3-cephem-4-carboxylic Acid (5a-6)

8-Amino-6-mercaptotetrazolo[1,5-b]pyridazine (0.123 g, 0.74 mmol) was added to a solution obtained from the acetoxymethylcephem 4a (0.45 g, 1.05 mmol), distilled H_2O (25 ml) and $NaHCO_3$ (0.15 g, 1.78 mmol). The mixture was heated at 65°C for 4 hours, then cooled to 5°C and treated under stirring with 1 M HCl. The precipitate was collected, washed with H_2O and then with a small amount of EtOH. The obtained solid was repeatedly triturated in an Me_2CO - H_2O solution (7: 1) until freed from the starting heterocyclyl thiol and some faster-running impurities (TLC monitoring, Merck Kieselgel 60 plates, $CHCl_3$ - MeOH - HCOOH, 16: 7: 3 as eluants), thus obtaining the title compound, 0.35 g (64%); mp 235°C. For an analytical sample, further purification through reverse-phase chromatography of the sodium salt, as indicated for 4a, was required. IR (KBr) 1760, 1670, 1620, 1380 cm^{-1}; NMR (60 MHz, DMSO-d_6) δ 3.65 (2H, s, CH_2CO), 3.80 (2H, ABq, 2-H), 4.40 (2H, ABq, $J=14$ Hz, 3-CH_2), 5.10 (1H, d, $J=5$ Hz, 6-H), 5.71 (1H, dd, $J=5$ and 8 Hz, 7-H), 6.42 (1H, s, 7-H on pyridazine ring), 6.70 (1H, s, 5-H on thiazole ring), 8.0 (2H, br s, NH_2 on pyridazine ring).

Anal Calcd for $C_{17}H_{16}N_{10}O_5S_3 \cdot \frac{1}{2}H_2O$: C 37.42, H 3.14, N 25.67, S 17.63.
Found: C 37.31, H 3.32, N 25.33, S 17.24.

7β-[(Z)-2-(2-Amino-4-thiazolyl-N-oxide)-2-methoxyiminoacetamido]-3-(1-methyl-1H-tetrazol-5-yl)-thiomethyl-3-cephem-4-carboxylic Acid (5c-1)

By a similar procedure to that used for compound 5a, 5c-1 was obtained (32%) from the 3-acetoxymethylcephem 4c and 5-mercapto-1-methyltetrazole; mp 205~210°C (dec). TLC on silica gel gave a single spot with $CHCl_3$ - MeOH - HCOOH - H_2O (140: 70: 20: 10), Rf 0.61. IR (KBr) 1780, 1670, 1625, 1530, 1040 cm^{-1}; NMR (90 MHz, DMSO-d_6) δ 3.67 (2H, ABq, $J=18.3$ Hz, 2-H), 3.98 (6H, s, NCH_3 and OCH_3), 4.33 (2H, ABq, $J=13.8$ Hz, 3-CH_2), 5.09 (1H, d, $J=4.8$ Hz, 6-H), 5.75 (1H, dd, $J=4.8$ and 8.5 Hz, 7-H), 7.06 (1H, s, 5-H on thiazole ring), 11.10 (1H, br d, $J=8.5$ Hz, CONH).

Anal Calcd for $C_{16}H_{17}N_9O_6S_3$: C 36.43, H 3.25, N 23.89, S 18.23.
Found: C 36.71, H 3.53, N 23.59, S 17.97.

Method C

7β-[(Z)-2-(2-Chloroacetamido-4-thiazolyl-N-oxide)-2-methoxyiminoacetamido]-3-(tetrazolo[1,5-b]-pyridazin-6-yl)thiomethyl-3-cephem-4-carboxylic Acid (8c-7)

A suspension of 4-[(Z)-1-carboxy-1-methoxyimino]methyl-2-chloroacetamidothiazole-N-oxide (1c) (5.9 g, 20 mmol) in THF (295 ml) was stirred for 1 hour (ice bath) in the presence of dicyclohexylcarbodiimide (2.47 g, 12 mmol). In a separate vessel, 7β-amino-3-(tetrazolo[1,5-b]pyridazin-6-yl)thiomethyl-3-cephem-4-carboxylic acid (3.65 g, 10 mmol) was silylated by treatment with N,O-bis(trimethylsilyl)-acetamide (4.07 g, 20 mmol) in a mixture of THF (83 ml) and acetonitrile (55 ml) at 55°C for 30 minutes. The clear solution thus obtained was added to the suspension containing the crude symmetrical anhydride of the side chain acid at −10°C. The resulting mixture was let rise to room temp and filtered after 90 minutes from any suspended material. Evaporation of the solvent left a solid, which was sequentially washed with EtOH (50 ml) and $CHCl_3$ (2×100 ml), and then dissolved in a 2: 1 Me_2CO - MeOH mixture (165 ml). After filtration from a small aliquot of unreacted 7-aminocephem, the solution was concentrated to small volume, thus causing the separation of 8c-7, 5.12 g (80%) as light-brown crystals; mp 225°C (dec); TLC on silica gel gave a single spot with $CHCl_3$ - MeOH - HCOOH (160: 20: 20), Rf 0.43; IR (KBr) 1775, 1675, 1630, 1540, 1440, 1355 cm^{-1}; NMR (90 MHz, DMSO-d_6) δ 3.72 (2H, ABq, $J=19$ Hz, 2-H), 3.97 (3H, s, OCH_3), 4.45 (2H, s, $ClCH_2$), 4.66 (2H, ABq, $J=13.8$ Hz, 3-CH_2), 5.13 (1H, d, $J=4.8$ Hz, 6-H), 5.76 (1H, dd, $J=4.8$ and 8.5 Hz, 7-H), 7.50 (1H, s, 5-H on thiazole ring), 7.70 (1H, d, $J=9.6$ Hz, 8-H on tetrazolopyridazine ring), 8.57 (1H, d, $J=9.6$ Hz, 7-H on tetrazolopyridazine ring), 9.9 (1H, br s, $ClCH_2CONH$), 9.93 (1H, br d, $J=8.5$ Hz, 7-NHCO).

Anal Calcd for $C_{20}H_{17}ClN_{10}O_7S_3 \cdot H_2O$: C 36.45, H 2.91, N 21.27, Cl 5.38.
Found: C 36.33, H 2.92, N 20.98, Cl 5.43.

7β-[(Z)-2-(2-Amino-4-thiazolyl-N-oxide)-2-methoxyiminoacetamido]-3-(tetrazolo[1,5-b]pyridazin-6-yl)thiomethyl-3-cephem-4-carboxylic Acid (5c-7)

Thiourea (1.06 g, 14 mmol) was added to a suspension of the chloroacetamido compound **8c-7** (8.96 g, 14 mmol) in dimethylacetamide (28 ml), and the mixture was stirred for 150 minutes at 20~25°C. EtOAc (125 ml) was then added to precipitate a solid which was collected, washed with fresh EtOAc, and dissolved in 1% aq NaHCO$_3$ (310 ml). This solution was brought to pH 5.3 by dropwise addition of 23% HCl (approx 2 ml), filtered from some impurities, treated with Amberlite XAD-7 ion-exchange resin (22 g) under stirring, filtered after 30 minutes, and finally made acidic (pH 2.3) with 23% HCl under ice cooling. The precipitate was collected, sequentially washed with H$_2$O, EtOH, ether, and dried to give 1.77 g (22.4%) of the title compound as a pale-cream powder; mp 225°C (dec); TLC on silica gel gave a single spot with CHCl$_3$ - MeOH - HCOOH (160: 50: 20), Rf 0.36; IR (KBr) 1770, 1670, 1535, 1040 cm^{-1}; NMR (90 MHz, DMSO-d_6) δ 3.67 (2H, ABq, J=18.3 Hz, 2-H), 3.94 (3H, s, OCH$_3$), 4.40 (2H, ABq, J=13.6 Hz, 3-CH$_2$), 5.10 (1H, d, J=4.8 Hz, 6-H), 5.75 (1H, dd, J=4.8 and 8.5 Hz, 7-H), 7.05 (1H, s, 5-H on thiazole ring), 7.70 (1H, d, J=9.5 Hz, 8-H on tetrazolopyridazine ring), 8.54 (1H, d, J=9.5 Hz, 7-H on tetrazolopyridazine ring), 11.42 (1H, br d, J=8.5 Hz, CONH).

Anal Calcd for C$_{15}$H$_{16}$N$_{10}$O$_6$S$_3$·H$_2$O: C 37.10, H 3.11, N 24.05, S 16.50.
    Found: C 37.09, H 2.98, N 23.81, S 16.31.

By similar procedures **4c, 5c-2** ~ **5c-6, 5e** and **5f** were prepared through their *N*-chloroacetyl precursors **6c, 8c-2** ~ **8c-6, 8e** and **8f**. Data for each compound are as follows:

7β-[(Z)-2-(2-Amino-4-thiazolyl-*N*-oxide)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic Acid (**4c**)

MP 220~230°C (dec); TLC on silica gel gave a single spot with CHCl$_3$ - MeOH - HCOOH (160: 70: 30), Rf 0.25; IR (KBr) 1770, 1720, 1670, 1630, 1530 cm^{-1}; NMR (60 MHz, DMSO-d_6) δ 2.05 (3H, s, COCH$_3$), 3.4 (2H, ABq, J=18 Hz, 2-H), 3.97 (3H, s, OCH$_3$), 4.8 (2H, ABq, J=13 Hz, 3-CH$_2$), 7.1 (1H, s, 5-H on thiazole ring).

Anal Calcd for C$_{16}$H$_{17}$N$_5$O$_6$S$_2$:   C 40.75, H 3.63, N 14.85.
    Found: C 40.51, H 3.70, N 14.58.

7β-[(Z)-2-(2-Amino-4-thiazolyl-*N*-oxide)-2-methoxyiminoacetamido]-3-[1-(2-cyanoethyl)-1*H*-tetrazol-5-yl]thiomethyl-3-cephem-4-carboxylic Acid (**5c-2**)

MP 170~175°C (dec); TLC on silica gel gave a single spot with CHCl$_3$ - MeOH - HCOOH - H$_2$O (140: 70: 20: 10), Rf 0.43; IR (KBr) 2240, 1775, 1670, 1625, 1040 cm^{-1}; NMR (90 MHz, DMSO-d_6) δ 3.17 (2H, t, J=6.5 Hz, CH$_2$CN), 3.73 (2H, ABq, J=18.3 Hz, 2-H), 3.98 (3H, s, OCH$_3$), 4.36 (2H, ABq, 3-CH$_2$), 4.60 (2H, t, J=6.5 Hz, NCH$_2$CH$_2$CN), 5.11 (1H, d, J=4.8 Hz, 6-H), 5.76 (1H, dd, J=4.8 and 8.5 Hz, 7-H), 7.11 (1H, s, 5-H on thiazole ring), 10.53 (1H, br d, CONH).

Anal Calcd for C$_{18}$H$_{18}$N$_{10}$O$_6$S$_3$·H$_2$O: C 36.95, H 3.45, N 23.94, S 16.44.
    Found: C 37.07, H 3.53, N 23.58, S 16.08.

7β-[(Z)-2-(2-Amino-4-thiazolyl-*N*-oxide)-2-methoxyiminoacetamido]-3-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl-3-cephem-4-carboxylic Acid (**5c-3**)

MP 185~190°C (dec); TLC on silica gel gave a single spot with CHCl$_3$ - MeOH - HCOOH (160: 70: 30), Rf 0.62; IR (KBr) 1780, 1680, 1625, 1040 cm^{-1}; NMR (90 MHz, DMSO-d_6) δ 2.72 (3H, s, CH$_3$), 3.78 (2H, ABq, J=18.3 Hz, 2-H), 3.99 (3H, s, OCH$_3$), 4.40 (2H, ABq, J=13.8 Hz, 3-CH$_2$), 5.11 (1H, d, J=4.8 Hz, 6-H), 5.76 (1H, dd, J=4.8 and 8.5 Hz, 7-H), 7.09 (1H, s, 5-H on thiazole ring), 10.34 (1H, br d, J=8.5 Hz, CONH).

Anal Calcd for C$_{17}$H$_{17}$N$_7$O$_6$S$_4$·$\frac{1}{2}$H$_2$O: C 36.94, H 3.34, N 17.74.
    Found: C 36.76, H 3.49, N 17.42.

7β-[(Z)-2-(2-Amino-4-thiazolyl-*N*-oxide)-2-methoxyiminoacetamido]-3-(5-methylthio-1,3,4-thiadiazol-2-yl)thiomethyl-3-cephem-4-carboxylic Acid (**5c-4**)

MP 205~210°C (dec); TLC on silica gel gave a single spot with CHCl$_3$ - MeOH - HCOOH (160: 40: 20), Rf 0.36; IR (KBr) 1765, 1665, 1620, 1530, 1380, 1040 cm^{-1}; NMR (60 MHz, DMSO-d_6) δ 2.76 (3H, s, SCH$_3$), 3.67 (2H, ABq, 2-H), 4.04 (3H, s, OCH$_3$), 4.4 (2H, ABq, 3-CH$_2$), 5.06 (1H, d, J=4.8 Hz, 6-H), 5.82 (1H, dd, J=4.8 and 8.5 Hz, 7-H), 7.06 (1H, s, 5-H on thiazole ring), 10.76 (1H, br d, J=8.5 Hz, CONH).

Anal Calcd for $C_{17}H_{17}N_7O_6S_5 \cdot H_2O$:   C 34.39, H 3.22, N 16.51, S 27.00.
          Found: C 34.48, H 3.15, N 16.20, S 26.76.

7β-[(Z)-2-(2-Amino-4-thiazolyl-*N*-oxide)-2-methoxyiminoacetamido]-3-(6-methoxypyrazin-2-yl)-thiomethyl-3-cephem-4-carboxylic Acid (**5c-5**)

MP 170~180°C (dec); TLC on silica gel gave a single spot with CHCl$_3$ - MeOH - HCOOH (160:50: 20), Rf 0.47; IR (KBr) 1775, 1680 br, 1630 cm^{-1}; NMR (90 MHz, DMSO-d_6) δ 3.66 (2H, ABq, $J=$18 Hz, 2-H), 3.98 (3H, s, NOCH$_3$), 4.00 (3H, s, OCH$_3$ on pyrazine ring), 4.35 (2H, ABq, $J=$13.8 Hz, 3-CH$_2$), 5.11 (1H, d, $J=$4.8 Hz, 6-H), 5.74 (1H, dd, $J=$4.8 and 8.5 Hz, 7-H), 7.10 (1H, s, 5-H on thiazole ring), 7.97 and 8.13 (each 1H, s, 5-H and 3-H on pyrazine ring), 10.12 (1H, br d, $J=$8.5 Hz, CONH).
          Anal Calcd for $C_{19}H_{19}N_7O_7S_3 \cdot H_2O$:   C 39.92, H 3.70, N 17.15.
          Found: C 39.81, H 3.87, N 16.72.

7β-[(Z)-2-(2-Amino-4-thiazolyl-*N*-oxide)-2-methoxyiminoacetamido]-3-(8-aminotetrazolo[1,5-b]-pyridazin-6-yl)thiomethyl-3-cephem-4-carboxylic Acid (**5c-6**)

MP 230°C; IR (KBr) 1760, 1630, 1380 cm^{-1}; NMR (60 MHz, DMSO-d_6) δ 3.7 (2H, ABq, 2-H), 4.0 (3H, s, OCH$_3$), 4.40 (2H, ABq, $J=$14 Hz, 3-CH$_2$), 5.10 (1H, d, $J=$5 Hz, 6-H), 5.80 (1H, dd, $J=$5 and 8 Hz, 7-H), 6.45 (1H, s, 7-H on pyridazine ring), 7.1 (1H, s, 5-H on thiazole ring), 10.15 (1H, br d, $J=$8 Hz, CONH).
          Anal Calcd for $C_{15}H_{17}N_{11}O_6S_3 \cdot \frac{1}{2}H_2O$:   C 36.72, H 3.08, N 26.18.
          Found: C 36.57, H 2.95, N 25.72.

7β-[(E)-2-(2-Amino-4-thiazolyl-*N*-oxide)-2-methoxyiminoacetamido]-3-(tetrazolo[1,5-b]pyridazin-6-yl)thiomethyl-3-cephem-4-carboxylic Acid (**5d-7**)

MP 210~220°C (dec); TLC on silica gel gave a single spot with CHCl$_3$ - MeOH - HCOOH (160: 50: 20), Rf 0.32; IR (Nujol) 1775, 1670, 1625 cm^{-1}; NMR (90 MHz, DMSO-d_6) δ 3.68 (2H, ABq, $J=$18.3 Hz, 2-H), 3.98 (3H, s, OCH$_3$), 4.44 (2H, ABq, $J=$13.6 Hz, 3-CH$_2$), 5.12 (1H, d, $J=$4.5 Hz, 6-H), 5.76 (1H, dd, $J=$4.5 and 8.5 Hz, 7-H), 7.07 (1H, s, 5-H on thiazole ring), 7.72 (1H, d, $J=$9.5 Hz, 8-H on tetrazolopyridazine ring), 8.56 (1H, d, $J=$9.5 Hz, 7-H on tetrazolopyridazine ring), 10.67 (1H, d, $J=$8.5 Hz, CONH).
          Anal Calcd for $C_{15}H_{16}N_{10}O_6S_3 \cdot H_2O$:   C 37.10, H 3.11, N 24.05.
          Found: C 36.81, H 3.07, N 23.77.

7β-[(Z)-2-(2-Amino-4-thiazolyl-*N*-oxide)-2-ethoxyiminoacetamido]-3-(tetrazolo[1,5-b]pyridazin-6-yl)thiomethyl-3-cephem-4-carboxylic Acid (**5e-7**)

MP 205~210°C (dec); TLC on silica gel gave a single spot with CHCl$_3$ - MeOH - HCOOH (160: 50: 20), Rf 0.37; IR (KBr) 1770, 1670, 1630, 1540, 1450, 1360 cm^{-1}; NMR (90 MHz, DMSO-d_6) δ 1.24 (3H, t, CH$_2$C*H*$_3$), 3.72 (2H, ABq, 2-H), 4.22 (2H, q, OC*H*$_2$CH$_3$), 4.45 (2H, ABq, 3-CH$_2$), 5.17 (1H, d, $J=$5 Hz, 6-H), 5.82 (1H, dd, $J=$5 and 8 Hz, 7H), 7.13 (1H, s, 5-H on thiazole ring), 7.80 (1H, d, $J=$9 Hz, 8-H on tetrazolopyridazine ring), 8.64 (1H, d, $J=$9 Hz, 7-H on tetrazolopyridazine ring), 11.13 (1H, br d, CONH$_2$).
          Anal Calcd for $C_{19}H_{18}N_{10}O_6S_3$:   C 39.43, H 3.13, N 24.21.
          Found: C 39.11, H 3.30, N 23.95.

7β-[(Z)-2-(2-Amino-4-thiazolyl-*N*-oxide)-2-(propoxyimino)acetamido]-3-(tetrazolo[1,5-b]pyridazin-6-yl)thiomethyl-3-cephem-4-carboxylic Acid (**5f-7**)

MP 200~205°C (dec); IR (KBr) 1770, 1675, 1630, 1540, 1360 cm^{-1}; NMR (90 MHz, DMSO-d_6) δ 0.91 (3H, t, CH$_2$CH$_2$C*H*$_3$), 1.70 (2H, sextet, CH$_2$C*H*$_2$CH$_3$), 3.72 (2H, ABq, 2-H), 4.20 (2H, t, OC*H*$_2$-CH$_2$CH$_3$), 4.46 (2H, ABq, 3-CH$_2$), 5.15 (1H, d, $J=$5 Hz, 6-H), 5.80 (1H, dd, $J=$5 and 8.5 Hz, 7-H), 7.06 (1H, s, 5-H on thiazole ring), 7.74 (1H, d, 8-H on tetrazolopyridazine ring), 8.55 (1H, d, 7-H on tetrazolopyridazine ring), 10.90 (1H, d, $J=$8.5 Hz, CONH).
          Anal Calcd for $C_{20}H_{20}N_{10}O_6S_3$:   C 40.53, H 3.40, N 23.63.
          Found: C 40.17, H 3.42, N 23.21.

7β-[(Z)-2-(2-Amino-4-thiazolyl-*N*-oxide)-2-hydroxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic Acid Hydrochloride (**4b**)

A mixture of 4-[(Z)-1-carboxy-1-trityloxyimino]methyl-2-chloroacetamidothiazole-N-oxide (**1h**) (0.8 g, 1.53 mmol), *tert*-butyl 7-aminocephalosporanate (0.8 g, 2.43 mmol) and dicyclohexylcarbodiimide (0.38 g, 1.84 mmol) was stirred in an ice bath for 3 hours. The separated dicyclohexylurea (0.27 g) was filtered off, the solution was concentrated *in vacuo*, the residue was taken up in EtOAc and washed in sequence with 4% aq NaHCO₃, 0.1 M HCl and brine. Removal of the solvent left a residue which was triturated in ethyl ether (3 × 70 ml). The undissolved material was discarded and the ethereal extracts were evaporated to give 1.05 g (82%) of the *tert*-butyl cephalosporanate **6h** as a powder. Without further purification, this material was dissolved in trifluoroacetic acid (3 ml) and kept at 15°C for 30 minutes. Addition of isopropyl ether caused the separation of the N,O-bis protected cephalosporanic acid **7h**, which was collected, washed with ether, dissolved in THF (7 ml) and heated at 50°C for 30 minutes in the presence of 50% aq HCOOH (3 ml). After concentration *in vacuo*, ethyl ether was added to precipitate the crude detritylated compound **7b**, 0.6 g. A solution of this product in dry dimethylacetamide (2 ml) was stirred with thiourea (0.07 g) at room temp for 2 hours, after which time EtOAc was added to separate a brownish powder, which was collected and triturated with fresh amounts of EtOAc (2 times). The isolated solid, 0.32 g, was triturated in a MeOH - Me₂CO mixture (50+50 ml); the undissolved material was discarded and the solution, after partial evaporation, kept overnight in the refrigerator, thus obtaining the precipitation of the title product **4b**, in part as the hydrochloride salt; 0.28 g (37% overall). TLC on silica gel gave a single spot with CHCl₃ - MeOH - HCOOH (160: 60: 30), Rf 0.40; IR (KBr) 1775, 1725, 1675, 1630 cm⁻¹; NMR (60 MHz, DMSO-d₆) δ 2.05 (3H, s, OCOCH₃), 3.55 (2H, ABq, 2-H), 4.90 (2H, ABq, 3-CH₂), 5.15 (1H, d, J=5 Hz, 6-H), 5.85 (1H, dd, J=5 and 8 Hz, 7-H), 7.1 (1H, s, 5-H on thiazole ring).

　　Anal Calcd for C₁₅H₁₅N₅O₅S₂·½HCl:　C 37.87, H 3.28, N 14.72, Cl 3.72.
　　　　　　Found:　　　　　　　　　　　　C 37.95, H 3.36, N 14.53, Cl 3.85.

7β-[(Z)-2-(2-Amino-4-thiazolyl-N-oxide)-2-(2-carboxy-2-propoxyimino)acetamido]-3-acetoxy-methyl-3-cephem-4-carboxylic Acid (4g)

Dicyclohexylcarbodiimide (237 mg, 1.15 mmol) was added to a solution of 4-[(Z)-1-(2-*tert*-butoxy-carbonyl-2-propoxyimino)]methyl-2-chloroacetamidothiazole-N-oxide (**1i**) (600 mg, 1.42 mmol) and *tert*-butyl 7-aminocephalosporanate (377 mg, 1.15 mmol). After 90 minutes, the solvent was removed and replaced with EtOAc. The precipitate (dicyclohexylurea, 205 mg, 80%) was discarded and the solution was washed with aq NaHCO₃, dried and evaporated. Silica gel chromatography (EtOAc - cyclohexane, with a gradient in isopropanol) afforded 320 mg (38%) of the fully protected cephem **6i** as an amorphous solid. The product obtained was stirred for 30 minutes at room temp in neat trifluoroacetic acid (3 ml), after which time the crude dicarboxylic acid **7g** was precipitated by adding isopropyl ether under stirring at 0°C. The isolated solid was stirred for 2.5 hours at room temp in a solution of thiourea (31 mg, 0.31 mmol) in dimethylacetamide (1 ml). Addition of EtOAc caused the separation of the title product **4g**, in part as its hydrochloride, as a pale brown powder (130 mg, 20% overall). Preparation of an analytical sample required reverse-phase chromatography (LiChroprep RP-18, Merck) of a solution of this material in aq NaHCO₃, concentration of the appropriate fractions and final treatment with HCl.

MP 220~230°C (dec); TLC on silica gel gave a single spot with CHCl₃ - MeOH - HCOOH (160: 60: 30), Rf 0.64; IR (KBr) 1770, 1735, 1670, 1620 cm⁻¹; NMR (60 MHz, DMSO-d₆) δ 1.46 (6H, s, Me₂), 2.04 (3H, s, COCH₃), 3.60 (2H, ABq, 2-H), 4.95 (2H, ABq, 3-CH₂), 5.1 (1H, d, J=5 Hz, 6-H), 5.8 (1H, dd, J=5 and 8.5 Hz, 7-H), 7.0 (1H, s, 5-H on thiazole ring).

　　Anal Calcd for C₁₉H₂₁N₅O₁₀S₂·H₂O:　C 40.64, H 4.13, N 12.47.
　　　　　　Found:　　　　　　　　　　　　C 40.93, H 4.03, N 12.19.

7β-[(Z)-2-(2-Amino-4-thiazolyl-N-oxide)-2-methoxyiminoacetamido]-3-(tetrazolo[1,5-b]pyridazin-6-yl)thiomethyl-3-cephem-4-carboxylic Acid (1S)-oxide (9c-7)

The sulfide **5c-7** (290 mg, 0.51 mmol) in an ice-cold mixture of MeOH (0.7 ml) and HCOOH (2 ml) was treated dropwise with a solution of 85% *m*-chloroperoxybenzoic acid (104 mg, 0.51 mmol) in THF (1 ml). After 30 minutes, EtOH (10 ml) was slowly added, thus obtaining the precipitation of the title product **9c-7** as a white powder; 220 mg (74%); mp 220°C (dec); TLC on silica gel gave a

single spot with CHCl$_3$ - MeOH - HCOOH - H$_2$O (140: 75: 20: 20), Rf 0.33; IR (KBr) 1780, 1725, 1670 cm^{-1}; NMR (90 MHz, DMSO-d_6) δ 3.92 (2H, ABq, 2-H), 3.98 (3H, s, OCH$_3$), 4.50 (2H, ABq, J=13.7 Hz, S-CH$_2$), 4.96 (1H, d, J=4.8 Hz, 6-H), 5.91 (1H, dd, J=4.8 and 8.5 Hz, 7-H), 7.05 (1H, s, 5-H on thiazole ring), 7.70 (1H, d, J=9.6 Hz, 8-H on tetrazolopyridazine ring), 8.54 (1H, d, J=9.6 Hz, 7-H on tetrazolopyridazine ring), 9.49 (1H, br d, J=8.5 Hz, CONH).

Anal Calcd for C$_{18}$H$_{16}$N$_{10}$O$_7$S$_3$·2.5H$_2$O: C 34.76, H 3.38, N 22.52.
Found: C 34.51, H 3.45, N 22.17.

References

1) NUMATA, M.; I. MINAMIDA, M. YAMAOKA, M. SHIRAISHI, T. MIYAWAKI, H. AKIMOTO, K. NAITO & M. KIDA: A new cephalosporin. SCE-963: 7-[2-(2-Aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethylamino-ethyl)-1*H*-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylic acid. Chemistry and structure-activity relationships. J. Antibiotics 31: 1262~1271, 1978

2) BUCOURT, R.; R. HEYMES, A. LUTZ, L. PENASSE & J. PERRONNET: Cephalosporines a chaines amino-2-thiazolyl-4-acetyles. Influence de la presence et de la configuration d'une groupe oxyimino sur l'activité antibacterienne. Tetrahedron 34: 2233~2243, 1978

3) SYKES, R. B.; D. P. BONNER, K. BUSH & N. H. GEORGOPAPADAKOU: Azthreonam (SQ 26,776), a synthetic monobactam specifically active against aerobic Gram-negative bacteria. Antimicrob. Agents Chemother. 21: 85~92, 1982

4) KISHIMOTO, S.; M. SENDAI, S. HASHIGUCHI, M. TOMIMOTO, Y. SATOH, T. MATSUO, M. KONDO & M. OCHIAI: Synthesis of sulfazecin-type 2-azetidinones with a carbon substituent at the 4-position. J. Antibiotics 36: 1421~1424, 1983

5) BLUMBACH, J.; W. DUERCKHEIMER & K. SEEGER: Cephalosporin analogs. Ger. Offen. 3,006,888, Sept. 10, 1981 [Chem. Abstr. 96: 6475n, 1982]

6) BLUMBACH, J.; K. FLEISCHMANN, R. KIRRSTETTER, W. KLESEL, R. LATTRELL, B.C. ROSS, E. SCHRINNER & W. SCHWAB: Synthesis and structure-activity relationships in the series of HR-810. Variation of the amino-thiazole moiety. 13th International Congress of Chemotherapy, PS 4.2/11–4, Vienna, 1983

7) PERRONE, E.; M. ALPEGIANI, F. GIUDICI, A. BEDESCHI, R. PELLIZZATO & G. NANNINI: Ring nitrogen functionalization of 2-aminothiazol-4-acetic acid derivatives. I. Synthesis and properties of novel 2-amino-thiazole N-oxides. J. Heterocyclic Chem., in press.

8) FINNEY, D. J.: Probit Analysis. 2nd. Ed., 65, Cambridge University Press, 1982

9) PERRET, C. J.: Iodometric assay of penicillanase. Nature (London) 174: 1012~1013, 1954

10) JACK, G. W. & M. H. RICHMOND: A comparative study of eight distinct β-lactamases synthesized by Gram-negative bacteria. J. Gen. Microbiol. 61: 43~61, 1970

11) RICHMOND, M. H. & R. B. SYKES: The β-lactamases of Gram-negative bacteria and their possible physiological role. *In* Advances in Microbial Physiology. *Ed.*, A. H. ROSE & D. W. TEMPEST, Vol. 9, pp. 31~88, Academic Press, London and N. Y., 1983

12) NANNINI, G.; E. PERRONE, D. SEVERINO, F. CASABUONA, A. BEDESCHI, F. BUZZETTI, P. N. GIRALDI, G. MEINARDI, G. MONTI, A. CERIANI & I. DE CARNERI: Cephalosporins. III. Synthesis and structure-activity relationships of 7-vinylenethioacetamido cephalosporins with a tetrazolo-pyridazine at the 3-position. J. Antibiotics 34: 1456~1468, 1981

13) NUMATA, M.; M. YAMAOKA, I. MINAMIDA, M. SHIRAISHI & T. NISHIMURA: Cephalosporin derivatives. Ger. Offen. 2,714,419 Oct. 13, 1977 [Chem. Abstr. 88: 22962j, 1978]

14) TAKAYA, T.; H. TAKASUGI, T. MASUGI, H. KOCHI & H. NAKANO: Studies on β-lactam antibiotics. IV. Structure-activity relationships of 7β-[(Z)-2-alkoxyimino-2-(2-amino-4-thiazolyl)acetamido]-3-cephem-4-carboxylic acids. J. Antibiotics 34: 1357~1359, 1981

15) PERRONE, E.; G. NANNINI, M. ALPEGIANI, F. GIUDICI & G. MEINARDI: N-Substituted thiazolyl derivatives of oxy-imino-substituted cephalosporins, useful as anti-bacterial agents. U. S. 4,358,448, Nov. 9, 1982

16) DURCKHEIMER, W.; N. KLESEL, M. LIMBERT, E. SCHRINNER, K. SEEGER & H. SELIGER: HR 109, a highly active cephalosporin (*S*)-sulphoxide. *In* Recent Advances in the Chemistry of β-Lactam Antibiotics. *Ed.*, J. ELKS, pp. 46~56, Chemical Society (Special Publication No. 38), London, 1981

17) ALPEGIANI, M.; F. CASABUONA, R. GIORGI, G. NANNINI, E. PERRONE, G. MEINARDI, A. BIANCHI & G. MONTI: Cephalosporins. VI. Synthesis and structure-activity relationships of new 7β-[(Z)-2-alkoxyimino-2-(2-aminothiazol-4-yl)acetamido]cephalosporins with a tetrazolopyridazine at the 3-position. J. Antibiotics 36: 1013~1019, 1983

18) THEN, R. L. & T. ANGEHRN: Trapping of nonhydrolyzable cephalosporins by cephalosporinases in *Entero-bacter cloacae* and *Pseudomonas aeruginosa* as a possible resistance mechanism. Antimicrob. Agents Chemother. 21: 711~717, 1982