STUDIES ON CEPHALOSPORIN ANTIBIOTICS

VI. SYNTHESIS, ANTIBACTERIAL ACTIVITY AND ORAL EFFICACY IN MICE OF NEW 7β-[(Z)-2-(2-AMINOTHIAZOL-4-YL)-2-(HYDROXYIMINO)-ACETAMIDO]-3-(SUBSTITUTED ALKYLTHIO)CEPHALOSPORINS

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A series of new 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(hydroxyimino)acetamido]cephalosporins (**1**) having various substituted alkylthio groups at the C-3 position of the cephem nucleus were prepared and evaluated for antibacterial activity and oral absorption in rats. Of these, the cephalosporin with a cyanomethylthio group (**1a**) showed the greatest activity against *Staphylococcus aureus* and Gram-negative bacteria. Its pivaloyloxymethyl ester (**6a**), a representative prodrug, exhibited good *in vivo* efficacy in mice by oral administration. The structure-activity relationships of **1** are also presented.

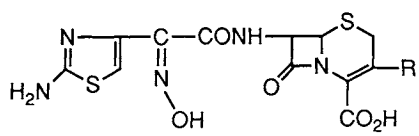
In the preceding paper[1], we reported the synthesis and biological properties of 2-aminothiazole-oxime type cephalosporin compounds having a (*Z*)-2-methoxycarbonylvinylthio group as the C-3 side chain, and demonstrated that the derivative **2** (Fig. 1) exhibited potent and broader antibacterial activities against Gram-positive and Gram-negative bacteria as well as good oral absorption in rats. However, the *in vivo* efficacy of **2** in mice was not sufficient, probably due to its extremely high binding rate (96%) to mouse serum protein. Therefore, we further studied the chemical modification of the C-3 substituent of **2** to find more promising oral cephalosporins.

Herein we describe the synthesis, *in vitro* antibacterial activity and oral absorption in rats of new cephalosporins (**1a**～**1g**) as well as the *in vivo* efficacy in mice of the most favorable compound **1a** in this series and its representative prodrug **6a** (4-pivaloyloxymethyl ester of **1a**).

Chemistry

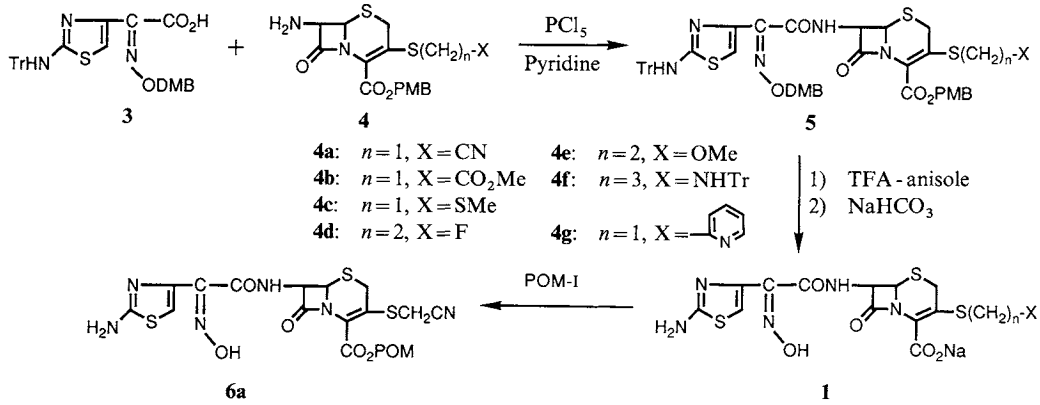
The new cephalosporins (**1a**～**1g**) listed in Table 1 were prepared by the synthetic route as shown in Scheme 1. 2-(2-Tritylaminothiazol-4-yl)-2-[(*Z*)-2,4-dimethoxybenzyloxyimino]acetic acid (**3**) was reacted with phosphorus pentachloride in dichloromethane in the presence of pyridine at low temperature to yield the acid chloride (not isolated). Then, a solution of *p*-methoxybenzyl 7β-amino-3-substituted alkylthio-3-cephem-4-carboxylate (**4**)[2] in dichloromethane was added to the acid chloride solution at low temperature to obtain the 7β-acylamino cephalosporins (**5**). Subsequently, the protecting groups in **5** were removed by a conventional method using trifluoroacetic acid and anisole to afford the desired cephalosporins (**1**). The

Fig. 1. Structure of **1**, **2** and FK482 (cefdinir).



1: R=−S(CH$_2$)$_n$−X
(n=1～3, X=various substituents)
2: R=−SCH=CHCO$_2$Me (*Z*)
FK482: R=−CH=CH$_2$

Scheme 1.



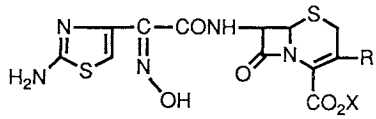
3 4 5

4a: $n=1$, X=CN **4e**: $n=2$, X=OMe
4b: $n=1$, X=CO_2Me **4f**: $n=3$, X=NHTr
4c: $n=1$, X=SMe
4d: $n=2$, X=F **4g**: $n=1$, X=

6a **1**

Tr=$-CPh_3$, DMB=2,4-dimethoxybenzyl, PMB=*p*-methoxy benzyl, POM=$-CH_2OCOC(CH_3)_3$, $n=1\sim3$, X=various substituents.

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



Compound			MIC (μg/ml, 10^6 cfu/ml)[a]					Peak serum level (μg/ml)[b] po, 50 mg/kg rats ($n=3$)
No.	R	X	*S.a.*	*E.c.*	*K.p.*	*M.m.*	*S.m.*	
1a	SCH_2CN	Na	0.10	0.20	0.05	0.10	1.56	3.0
1b	SCH_2CO_2Me	Na	0.39	0.20	0.20	0.39	1.56	4.3
1c	SCH_2SMe	Na	0.39	0.20	0.20	0.39	1.56	5.3
1d	SCH_2CH_2F	Na	0.78	0.20	0.39	0.39	3.13	5.5
1e	SCH_2CH_2OMe	Na	0.78	0.39	0.39	0.78	1.56	3.9
1f	$SCH_2CH_2CH_2NH_2$	Na	0.39	0.78	1.56	3.13	0.78	2.1
1g	SCH_2-(N)	Na	0.39	0.10	0.10	0.20	1.56	6.4
1h[c]	SCH_3	Na	0.78	0.78	0.39	3.13	3.13	2.6
FK482[c]	$CH=CH_2$	H	0.20	0.20	0.10	1.56	1.56	4.7
Cefixime[c]			25	0.10	0.025	0.20	0.20	19.9

[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 **1h**, FK482 and cefixime see ref 3 and 4, 5 and 6, respectively.
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.

pivaloyloxymethyl (POM) ester (**6a**), a typical prodrug of **1a**, was prepared from **1a** with pivaloyloxymethyl iodide in *N*,*N*-dimethylacetamide under ice-cooling.

Antibacterial Activity and Oral Absorption

The *in vitro* antibacterial activities of the new cephalosporins (**1a**~**1g**) against selected Gram-positive

and Gram-negative bacteria and their peak serum levels 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-methylthio analogue (**1h**)[3,4], cefdinir (FK482)[5] and cefixime[6] are listed at the bottom of Table 1.

Against *Staphylococcus aureus* 209P JC-1, these new cephalosporins exhibited fairly potent activity ranging from 0.1 to 0.78 μg/ml for the MICs. In particular, compound **1a** with a cyanomethylthio group at the C-3 position showed 2 to 250 times greater activity than FK482 and cefixime, respectively.

On the other hand, against Gram-negative bacteria, all these new cephalosporins except **1f** bearing an aminopropylthio group showed a potent antibacterial activity comparable to FK482, though their activities were somewhat less than that of cefixime. Interestingly, the antibacterial activity of new cephalosporins against *Morganella morganii* IID 602 were much greater than that of FK482.

It was also found that introduction of various substituted alkylthio groups into the C-3 position was liable to increase the antibacterial activity as compared with **1h** having unsubstituted alkylthio group.

In the oral absorption study in rats, compound **1g** having the 2-pyridinylmethylthio group as C-3 substituent exhibited the highest concentrations in serum. However, the peak serum levels of all these new cephalosporins and FK482 having hydroxyimino group at the α position of the C-7 side chain were generally much lower than that of cefixime bearing a carboxymethoxyimino group at the same position.

Subsequently, the serum protein binding of **1a**, the most favorable compound in this series, and *in vivo* efficacy of **1a** and its prodrug **6a** were studied. As shown in Table 2, compound **1a** exhibited medium serum protein binding to mouse, dog and human serum, respectively, with no significant species difference.

Against systemic infections in mice induced by *Streptococcus pneumoniae* J-4, compound **1a** and its prodrug **6a** showed good efficacy by oral administration, and the ED_{50} value of **6a** was 2 to 3 times superior to those of FK482, cefaclor and **1a**.

Further studies on the various prodrugs of **1a** designed for improving bioavailability are now in progress.

Table 2. Serum protein binding rate of **1a**.

Compound	Binding rate (%)		
	Mouse	Dog	Human
1a	62.1	63.5	58.4
FK482	68.8	96.9	61.0
Cefaclor	16.9	32.8	35.7

Method: Ultrafiltration.
Drug concentration: 25 μg/ml.
Serum: 90% fresh serum.

Experimental

IR spectra were taken on a Perkin-Elmer 1760 IR spectrometer. ^1H NMR spectra were recorded on a Varian XL-200 NMR spectrometer using TMS or sodium trimethylsilyl propionate-d_4 (in D_2O) as an

Table 3. *In vivo* antibacterial activity of **1a** and **6a** against systemic infections in mice.

Organism	Challenge dose (cfu/mouse), ip	5% mucin	Compound	MIC (μg/ml)[a]	ED_{50} (mg/mouse)[b] po	Peak serum level (μg/ml)[c]
S. pneumoniae J-4	4.5×10^3	(−)	**1a**	0.025	1.71 (1.09～3.67)	1.2
			6a		0.52 (0.31～0.88)	6.2
			FK482	0.05	1.04 (0.73～1.43)	2.3
			Cefaclor	0.39	1.10 (0.65～2.31)	28.6

Drugs were administered orally 1 hour after infection.
Mouse: Male ICR strain, 4 weeks, 10 mice/group.
[a] Inoculum size: 10^6 cfu/ml.
[b] Probit method (95% confidence limits).
[c] po, 50 mg/kg, male ICR mice ($n=3$), nonfasting.

internal standard. Mass spectra (MS) were measured on a JMS-SX102 mass spectrometer. Chromatographic separations were done by using Wako Silica gel C-200 (100~200 mesh, Wako, Japan), Lobar column packed with LiChroprep Si 60 (40~63 μm, Merck) or Sephadex LH-20 (Pharmacia, Sweden). Analytical HPLC of **1** 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 1.5 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. 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[7].

Oral Absorption Study in Rat

Male SLC/Wistar rats ($n=3$) weighing 180~220 g were fasted overnight and orally dosed with 50 mg/kg of the test compounds in 5% gum arabic. 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.

Serum Protein Binding Study

The test compounds (25 μg in 0.1 ml saline) were diluted in fresh serum to a final concentration of 25 μg/ml and the solutions were then left at 37°C for 30 minutes, following which the bound fraction was separated from the free fraction using an Amicon Micropartition System centrifuged at 3,000 rpm for 15 minutes. Then, the compounds were assayed by the disc-plate method, as described above.

p-Methoxybenzyl 7β-[2-(2-Tritylaminothiazol-4-yl)-2-[(*Z*)-2,4-dimethoxybenzyloxyimino]acetamido]-3-cyanomethylthio-3-cephem-4-carboxylate (**5a**, R=SCH_2CN, Table 4)

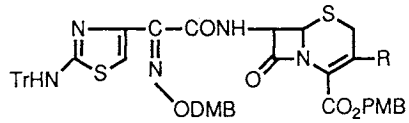
To a solution of 2-(2-tritylaminothiazol-4-yl)-2-[(*Z*)-2,4-dimethoxybenzyloxyimino]acetic acid **3** (500 mg, 0.86 mmol) in dry CH_2Cl_2 (20 ml) were added pyridine (210 mg, 3.0 equiv) and phosphorus pentachloride (180 mg, 1.0 equiv) successively at −15°C with stirring, and the reaction mixture was stirred for 15 minutes. Then, a solution of *p*-methoxybenzyl 7β-amino-3-cyanomethylthio-3-cephem-4-carboxylate **4a**[2] (260 mg, 0.77 equiv) in dry CH_2Cl_2 (1 ml) was added to the reaction mixture at −15°C and stirred for 20 minutes at −10~−5°C. After the reaction, 0.5% HCl (50 ml) was added to the reaction mixture and extracted with EtOAc (50 ml). The extract was washed with brine (30 ml), dried ($MgSO_4$) and evaporated *in vacuo*. The residue was chromatographed on silica gel (eluent; benzene-EtOAc, 5:1) to yield 320 mg (51%) of **5a** as a pale yellow powder: IR (KBr) cm^{-1} 2247 (nitrile), 1790 (β-lactam), 1730, 1680; ^1H NMR ($CDCl_3$) δ 3.32 (1H, d, $J=17$ Hz, 2-Hα), 3.35 and 3.61 (2H, ABq, $J=17$ Hz, SCH_2CN), 3.58 (1H, d, $J=17$ Hz, 2H-β), 3.73 (3H, s, OCH_3), 3.81 (6H, s, $OCH_3\times2$), 4.94 (1H, d, $J=5$ Hz, 6-H), 5.25 (2H, s, CO_2CH_2Ph), 5.25 and 5.35 (2H, ABq, $J=10$ Hz, $=NOCH_2$), 5.84 (1H, dd, $J=5$ and 9 Hz, 7-H), 6.40~6.49 (2H, m, aromatic H), 6.83 (1H, s, thiazole 5-H), 6.90 (2H, d, $J=9$ Hz, aromatic H), 7.00 (1H, br s, TrN*H*), 7.20~7.40 (19H, m, aromatic H and CONH); FAB-MS *m/z* 953 $(M+H)^+$.

Similarly, compounds **5b**~**5g** were prepared from **3** with various 7β-amino-3-(substituted alkylthio)cephalosporanic acid derivatives (**4b**~**4g**)[2] according to the procedure described for **5a**, and their spectral data are summarized in Table 4.

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

To a mixture of TFA (4 ml) and anisole (0.8 ml) was added compound **5a** (300 mg, 0.31 mmol) under ice-cooling, and the reaction mixture was stirred for 45 minutes at the same temperature and further 10 minutes at room temperature. Then, the reaction mixture was added dropwise into a mixture of Et_2O and *n*-hexane (1:2, 40 ml). The precipitated TFA salt of **1a** was collected by filtration and washed with a small

Table 4. ^{1}H NMR, MS and IR spectral data of **5**.



5

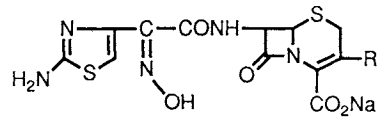
Compound No.	R	^{1}H NMR δ ($CDCl_3$)	MS[a] (m/z)	IR (KBr) cm^{-1}
5b	SCH_2CO_2Me	3.20 (1H, d, J=17 Hz), 3.40 (1H, d, J=15 Hz), 3.52 (1H, d, J=15 Hz), 3.54 (1H, d, J=17 Hz), 3.71 (3H, s), 7.73 (3H, s), 3.79 (3H, s), 3.80 (3H, s), 4.92 (1H, d, J=5 Hz), 5.19 (1H, d, J=12 Hz), 5.25 (1H, d, J=10 Hz), 5.26 (1H, d, J=12 Hz), 5.34 (1H, d, J=10 Hz), 5.77 (1H, dd, J=5, 9 Hz), 6.39~6.47 (2H, m), 6.84 (1H, s), 6.88 (2H, d, J=9 Hz), 6.98 (1H, br s), 7.20~7.40 (19H, m)	986	1785, 1735, 1685
5c	SCH_2SMe	2.17 (3H, s), 3.25 (1H, d, J=17 Hz), 3.55 (1H, d, J=17 Hz), 3.73 (1H, d, J=13 Hz), 3.73 (3H, s), 3.80 (6H, s), 3.86 (1H, d, J=13 Hz), 4.93 (1H, d, J=5 Hz), 5.19 (1H, d, J=12 Hz), 5.24 (1H, d, J=11 Hz), 5.26 (1H, d, J=12 Hz), 5.34 (1H, d, J=11 Hz), 5.75 (1H, dd, J=5, 9 Hz), 6.39~6.47 (2H, m), 6.84 (1H, s), 6.88 (2H, d, J=9 Hz), 6.98 (1H, br s), 7.22~7.38 (19H, m)	974	1785, 1685, 1615
5d	SCH_2CH_2F	2.92~3.11 (2H, m), 3.13 (1H, d, J=17 Hz), 3.45 (1H, d, J=17 Hz), 3.74 (3H, s), 3.81 (3H, s), 3.82 (3H, s), 4.37~4.47 (1H, m), 4.60~4.70 (1H, m), 4.93 (1H, d, J=5 Hz), 5.21 (1H, d, J=11 Hz), 5.26 (1H, d, J=10 Hz), 5.28 (1H, d, J=11 Hz), 5.36 (1H, d, J=10 Hz), 5.76 (1H, dd, J=5, 9 Hz), 6.38~6.50 (2H, m), 6.87 (1H, s), 6.91 (2H, d, J=9 Hz), 7.01 (1H, br s), 7.23~7.40 (19H, m)	960	1785, 1730, 1680
5e	SCH_2CH_2OMe	2.86~2.95 (2H, m), 3.13 (1H, d, J=18 Hz), 3.32 (3H, s), 3.45 (1H, d, J=18 Hz), 3.47~3.55 (2H, m), 3.74 (3H, s), 3.79 (3H, s), 3.80 (3H, s), 4.92 (1H, d, J=5 Hz), 5.17 (1H, d, J=12 Hz), 5.24 (1H, d, J=12 Hz), 5.25 (1H, d, J=10 Hz), 5.34 (1H, d, J=10 Hz), 5.70 (1H, dd, J=5, 9 Hz), 6.37~6.46 (2H, m), 6.85 (1H, s), 6.88 (2H, d, J=9 Hz), 6.98 (1H, br s), 7.20~7.38 (19H, m)	972	1785, 1685, 1615
5f	$S(CH_2)_3NHTr$	1.63~1.83 (2H, m), 2.23 (2H, t, J=7 Hz), 2.81 (2H, t, J=7 Hz), 3.05 (1H, d, J=17 Hz), 3.35 (1H, d, J=17 Hz), 3.72 (6H, s), 3.78 (3H, s), 4.92 (1H, d, J=5 Hz), 5.16 (1H, d, J=11 Hz), 5.23 (1H, d, J=11 Hz), 5.25 (1H, d, J=10 Hz), 5.35 (1H, d, J=10 Hz), 5.68 (1H, dd, J=5, 9 Hz), 6.33~6.45 (2H, m), 6.87 (1H, s), 6.87 (1H, d, J=9 Hz), 7.00 (1H, br s), 7.15~7.50 (35H, m)	1,213	1785, 1685, 1615
5g	SCH_2-	3.18 (1H, d, J=17 Hz), 3.57 (1H, d, J=17 Hz), 3.73 (3H, s), 3.78 (6H, s), 4.01 (1H, d, J=14 Hz), 4.13 (1H, d, J=14 Hz), 4.83 (1H, d, J=5 Hz), 5.15 (1H, d, J=12 Hz), 5.23 (1H, d, J=12 Hz), 5.25 (1H, d, J=10 Hz), 5.33 (1H, d, J=10 Hz), 5.69 (1H, dd, J=5, 9 Hz), 6.39~6.47 (2H, m), 6.83 (1H, s), 6.87 (2H, d, J=9 Hz), 6.98 (1H, br s), 7.16 (1H, dd, J=5, 8 Hz), 7.20~7.40 (20H, m), 7.61 (1H, dt, J=8, 2 Hz), 8.48 (1H, d, J=5 Hz)	1,005	1785, 1690, 1615

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

Abbreviations: DMB; 2,4-dimethoxybenzyl, PMB; *p*-methoxybenzyl, Tr; trityl.

amount of the mixed solvent mentioned above. Subsequently, the TFA salt (150 mg) was dissolved in water (5 ml) with $NaHCO_3$ (79 mg, 0.94 mmol) and chromatographed on Sephadex LH-20 column (eluent; H_2O), and then lyophilized to afford 108 mg (78%) of **1a** as an amorphous solid: IR (KBr) cm^{-1} 2250

Table 5. ^{1}H NMR and IR spectral data of **1**.



1

Compound No.	R	^{1}H NMR δ (DMSO-d_6 or D_2O)	IR (KBr)[a] cm^{-1}
1b	SCH_2CO_2Me	(D_2O) 3.50 (1H, d, J=17 Hz), 3.50 (1H, d, J=15 Hz), 3.66 (1H, d, J=15 Hz), 3.85 (1H, d, J=17 Hz), 5.27 (1H, d, J=5 Hz), 5.85 (1H, d, J=5 Hz), 7.00 (1H, s)	1765
1c	SCH_2SMe	2.15 (3H, s), 3.48 (1H, d, J=17 Hz), 3.68 (1H, d, J=17 Hz), 3.85 (1H, d, J=13 Hz), 3.97 (1H, d, J=13 Hz), 5.00 (1H, d, J=5 Hz), 5.60 (1H, dd, J=5, 8 Hz), 6.65 (1H, s), 7.16 (2H, br s), 9.42 (1H, d, J=8 Hz), 11.42 (1H, s)	1760
1d	SCH_2CH_2F	(D_2O) 3.03 (1H, dt, J=2, 7 Hz), 3.15 (1H, dt, J=2, 7 Hz), 3.57 (1H, d, J=17 Hz), 3.87 (1H, d, J=17 Hz), 4.51 (1H, t, J=7 Hz), 4.74 (1H, t, J=7 Hz), 5.28 (1H, d, J=5 Hz), 5.84 (1H, d, J=5 Hz), 7.02 (1H, s)	1765
1e	SCH_2CH_2OMe	2.82 (2H, t, J=7 Hz), 3.23 (3H, s), 3.30 (1H, d, J=17 Hz), 3.44 (2H, t, J=7 Hz), 3.64 (1H, d, J=17 Hz), 5.02 (1H, d, J=7 Hz), 5.58 (1H, dd, J=5, 8 Hz), 6.65 (1H, s), 7.14 (2H, br s), 9.42 (1H, d, J=8 Hz), 11.45 (1H, s)	1765
1f	$S(CH_2)_3NH_2$	(D_2O) 1.85~2.01 (2H, m), 2.85 (2H, t, J=7 Hz), 3.13 (2H, t, J=7 Hz), 3.55 (1H, d, J=17 Hz), 3.84 (1H, d, J=17 Hz), 5.30 (1H, d, J=5 Hz), 5.84 (1H, d, J=5 Hz), 7.02 (1H, s)	1760
1g	SCH_2-(pyridyl)	3.35 (1H, d, J=17 Hz), 3.60 (1H, d, J=17 Hz), 4.07 (2H, s), 4.96 (1H, d, J=5 Hz), 5.60 (1H, dd, J=5, 8 Hz), 5.88 (1H, d, J=5 Hz), 6.65 (1H, s), 7.14 (2H, s), 7.24 (1H, dd, J=5, 8 Hz), 7.44 (1H, d, J=8 Hz), 7.74 (1H, dt, J=8, 2 Hz), 8.47 (1H, d, J=5 Hz), 9.41 (1H, d, J=8 Hz), 11.45 (1H, s)	1760

[a] β-Lactam.

(nitrile), 1760 (β-lactam), 1675, 1615; ^{1}H NMR (D_2O) δ 3.65 (1H, d, J=17 Hz, 2-Hα), 3.71 and 3.82 (2H, ABq, J=17 Hz, SCH_2CN), 3.97 (1H, d, J=17 Hz, 2-Hβ), 5.33 (1H, d, J=5 Hz, 6-H), 5.88 (1H, d, J=5 Hz, 7-H), 7.02 (1H, s, thiazole 5-H); HPLC analysis: 97% purity.

According to the method for **1a**, compounds **1b**~**1g** were prepared from **5b**~**5g** and their spectral data are summarized in Table 5. The purities of **5b**~**5g** were approximately 94~96% by HPLC assay.

Pivaloyloxymethyl 7β-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-hydroxyimino]acetamido]-3-cyanomethylthio-3-cephem-4-carboxylate (**6a**)

To a solution of **1a** (1.50 g, 3.25 mmol) in dry *N*,*N*-dimethylacetamide (20 ml) was added pivaloyloxymethyl iodide (1.10 g, 1.4 equiv) under ice-cooling, and stirred for 40 minutes at the same temperature. After the reaction, EtOAc (100 ml) was added to the reaction mixture and washed with brine (50 ml × 2), dried ($MgSO_4$) and evaporated *in vacuo*. The residue was chromatographed on Sephadex LH-20 column (eluent; MeOH-$CHCl_3$, 3:2) to yield 1.14 g (63%) of **6a** as a pale yellow powder: IR (KBr) cm^{-1} 2250 (nitrile), 1785 (β-lactam), 1755, 1675; ^{1}H NMR (DMSO-d_6) δ 1.17 (9H, s, CH_3 × 3), 3.89 (2H, br s, 2-Hα and 2-Hβ), 4.20 (2H, br s, SCH_2CN), 5.27 (1H, d, J=5 Hz, 6-H), 5.70 and 5.92 (2H, ABq, J=6 Hz, CO_2CH_2O), 5.72 (1H, dd, J=5 and 9 Hz, 7-H), 6.69 (1H, s, thiazole 5-H), 7.15 (2H, br s, NH_2), 9.53 (1H, d, J=9 Hz, CONH), 11.34 (1H, s, =NOH); FAB-MS m/z 555 $(M+H)^+$.

References

1) YOKOO, C.; A. ONODERA, H. FUKUSHIMA, K. NUMATA & T. NAGATE: Studies on cephalosporin antibiotics. V. Synthesis, antibacterial activity and oral absorption of new 3-[(*Z*)-2-methoxycarbonylvinylthio]-7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(oxyimino)acetamido]cephalosporins. J. Antibiotics 45: 932~939, 1992

2) YOKOO, C.; A. ONODERA, H. FUKUSIMA, Y. WATANABE & K. SOTA (Taisho): Cephalosporin derivatives. U.S. 4,921,953, May 1, 1990
3) SAKAGAMI, K.; T. WATANABE, S. FUKATSU, H. NITTA, M. HATANAKA & T. ISHIMARU: Synthetic cephalosporins. V. Synthesis and antibacterial activity of 3-alkylthio-7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(*O*-substituted oxyimino)-acetamido]cephalosporins and related compounds. Yakugaku Zasshi (Japanese) 109: 913～925, 1989
4) INAMOTO, Y.; K. SAKANE, K. KAMIMURA & T. TAKAYA: Studies on FK482 (Cefdinir). IV. Synthesis and structure-activity relationships of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-substituted cephalosporin derivatives. Yakugaku Zasshi (Japanese) 110: 908～915, 1990
5) INAMOTO, Y.; T. CHIBA, T. KAMIMURA & T. TAKAYA: FK482, a new orally active cephalosporin. Synthesis and biological properties. J. Antibiotics 41: 828～830, 1988
6) YAMANAKA, H.; T. CHIBA, K. KAWABATA, H. TAKASUGI, T. MASUGI & T. TAKAYA: Studies on β-lactam antibiotics. IX. Synthesis and biological activity of a new orally active cephalosporin, cefixime (FK027). J. Antibiotics 38: 1738～1751, 1985
7) FINNEY, D. J.: The maximum likelihood solution. *In* Probit Analysis. 2nd Ed. *Ed.*, D. J. FINNEY, pp. 48～64, Cambridge University Press, 1952