STUDIES ON β-LACTAM ANTIBIOTICS

III. SYNTHESES AND ANTIBACTERIAL ACTIVITIES OF NEW 3-(1,3-DITHIOLAN-2-YL)CEPHALOSPORINS, YM-22508, YM-16457 AND THEIR PRODRUG-TYPE ESTERS

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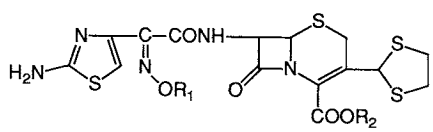
The syntheses and biological activities of new 7-β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-hydroxyiminoacetamido]-3-(1,3-dithiolan-2-yl)-3-cephem-4-carboxylic acid (YM-22508, **1a**), 7-β-[(*Z*)-2-(2-amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-(1,3-dithiolan-2-yl)-3-cephem-4-carboxylic acid (YM-16457, **1d**) and their prodrug-type esters are described. Among them, YM-22561 (**1c**), the 1-acetoxyethyl ester of **1a**, showed good *in vivo* efficacy in mice against infections of *Staphylococcus aureus* Smith, *Streptococcus pyogenes* S 23 and *Escherichia coli* NY-17 and a long plasma $T_{1/2}$ in mice.

After the discovery of cefuroxime axetil[1], a series of prodrug-type cephalosporin esters[2~6] bearing 2-aminothiazole-oxime moiety at the C-7 position of a cephem nucleus have been reported as orally active cephalosporins. They are mostly characterized by various functional groups at the C-3 position.

In the course of our research on cephalosporins possessing an unique C-3 side chain, we have synthesized new 3-(1,3-dithiolan-2-yl)cephalosporins, YM-22508 (**1a**) and YM-16457 (**1d**), which showed a broad antibacterial spectrum against Gram-positive and Gram-negative organisms. This paper describes the syntheses and antibacterial activities of the new cephalosporins (**1**) as shown in Fig. 1.

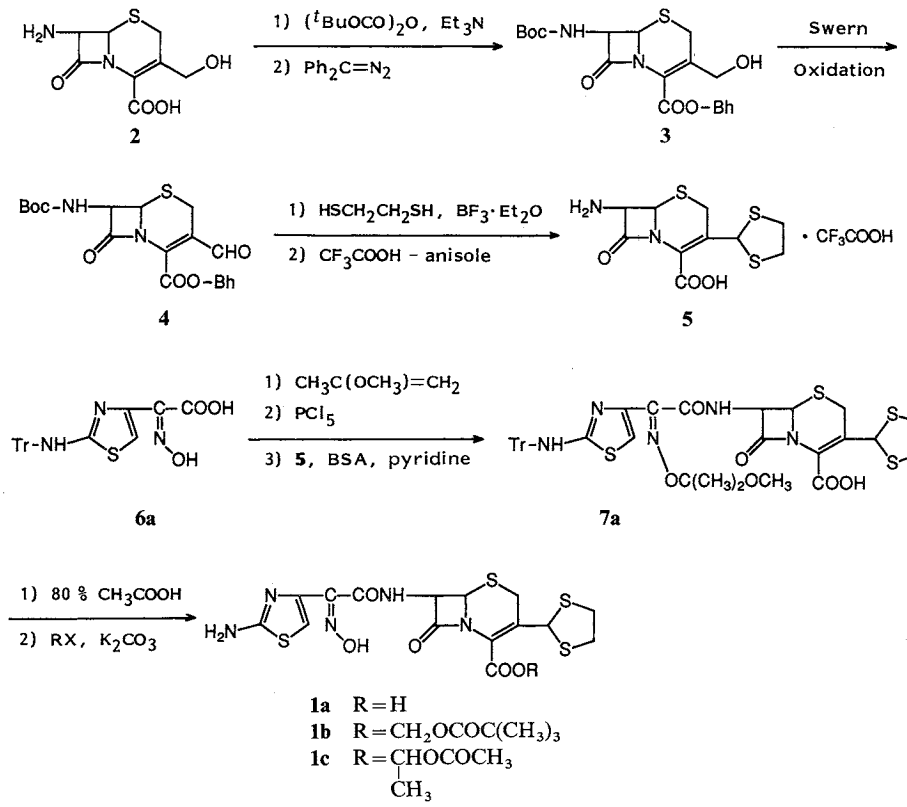
The new 3-(1,3-dithiolan-2-yl)cephalosporins (**1a**~**1c**) were prepared by the procedure shown in Scheme 1. Diphenylmethyl 7-*tert*-butoxycarbonylamino-3-hydroxymethyl-3-cephem-4-carboxylate (**3**)[7] was prepared as a crystalline material after stepwise protection of 7-amino-3-hydrxymethyl-3-cephem-4-carboxylic acid (**2**). Swern oxidation of **3** gave the aldehyde (**4**). The reaction of **4** with ethanedithiol catalyzed by $BF_3 \cdot Et_2O$ afforded the 3-(1,3-dithiolan-2-yl) compound (**5**) after deprotection by CF_3COOH-anisole. Compound **5** was acylated with (*Z*)-2-(2-tritylamino-4-thiazolyl)-2-(1-methoxy-1-methyl)ethoxyiminoacetic acid by the acid chloride method to afford compound **7a**. Removal of the pro-

Fig. 1. New 3-(1,3-dithiolan-2-yl)cephalosporins.



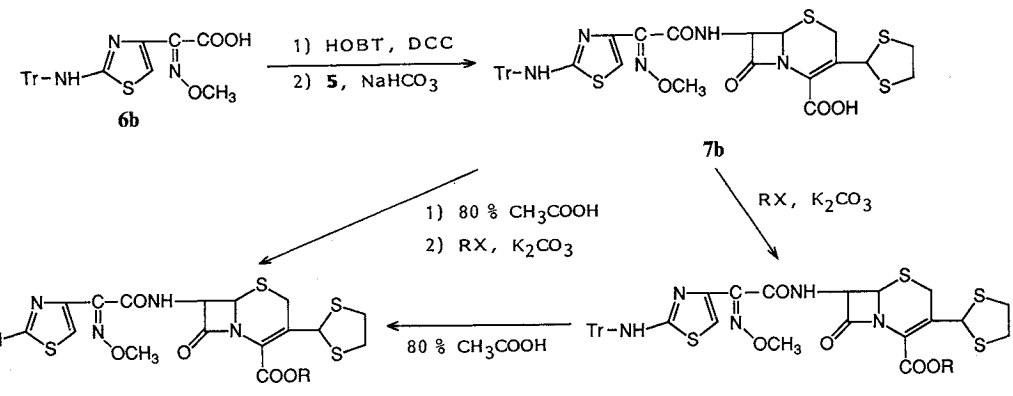
1a	$R_1=H$	$R_2=H$	**1d**	$R_1=CH_3$	$R_2=H$
1b	$R_1=H$	$R_2=CH_2OCOC(CH_3)_3$	**1e**	$R_1=CH_3$	$R_2=CH_2OCOC(CH_3)_3$
1c	$R_1=H$	$R_2=CHOCOCH_3$ (CH$_3$)	**1f**	$R_1=CH_3$	$R_2=CHOCOCH_3$ (CH$_3$)

Scheme 1.



1a R=H
1b R=$CH_2OCOC(CH_3)_3$
1c R=$CHOCOCH_3$ (CH_3)

Scheme 2.



1d R=H
1e R=$CH_2OCOC(CH_3)_3$
1f R=$CHOCOCH_3$ (CH_3)

esters (**1b** and **1c**). Similarly, the methoxime-analogues (**1d**~**1f**) were prepared as shown in Scheme 2.

Biological Evaluation

The MICs of the new cephalosporins (**1a** and **1d**) against selected Gram-positive and Gram-negative

Table 1. Comparative antibacterial activity (MIC, μg/ml) of the cepharosporins.

Organisms	YM-22508 (**1a**)	YM-16457 (**1d**)	CXM	CCL	CFTM
Staphylococcus aureus FDA 209P JC-1	0.39	1.56	1.56	0.78	3.13
S. epidermidis IID 866	0.2	0.39	0.2	0.78	1.56
Streptococcus pyogenes Cook	0.013	≦0.006	0.1	0.2	0.1
Escherichia coli O-1	0.39	0.2	0.78	0.78	0.1
Klebsiella pneumoniae ATCC 10031	0.78	0.025	0.1	0.39	0.025
Serratia marcescens IID 620	6.25	0.2	12.5	>100	0.39
Proteus vulgaris OXK US	0.05	0.013	0.025	0.2	≦0.006
P. mirabilis IFM OM-9	0.2	0.013	0.1	0.39	0.013
Providencia rettgeri IFO 3850	0.025	0.025	0.1	0.78	0.013
Morganella morganii Kono	0.78	0.2	12.5	100	0.1

CXM: Cefuroxime, CCL: cefaclor, CFTM: cefteram.

Table 2. Therapeutic efficacy in experimental infections in mice.

Compound	Route of administration	*Staphylococcus aureus* Smith[a]		*Escherichia coli* NY-17[b]		*Streptococcus pyogenes* S 23[c]	
		MIC (μg/ml)	ED_{50} (mg/kg)	MIC	ED_{50}	MIC	ED_{50}
YM-22508 (**1a**)	sc	0.39	0.69	0.78	<2.5	0.013	0.09
YM-22561 (**1c**)	po		3.1		6.1		0.39
YM-16457 (**1d**)	sc	1.56	10.2	0.78	1.1		
1e	po		17.1		3.4		
1f	po		29.6		5.1		
CXM	sc	0.78	0.85	1.56	9.1	0.025	0.06
CXM-AX	po		1.3		8.4		0.12
CCL	po	1.56	0.024	0.78	1.1	0.39	<0.36
CFTM-PI	po	3.13	7.5	0.2	1.1	≦0.006	<0.36
CFIX	po	12.5	67.8	0.05	1.1	0.2	1.4

[a] 3.1×10^6 cfu/mouse, [b] 3.6×10^3 cfu/mouse, [c] 2.0×10^5 cfu/mouse.
CXM: Cefuroxime, CXM-AX: cefuroxime axetil, CCL: cefaclor, CFTM-PI: cefteram pivoxil, CFIX: cefixime.

bacteria are listed in Table 1[8]. For comparison, the MIC values of cefuroxime, cefaclor and cefteram are also listed at the right side of the Table 1. It is clearly shown that compound **1d** has the most balanced spectrum among all the compounds, and the activities of compound **1a** against the Gram-positive test strains were eight times as strong as that of cefteram.

The *in vivo* antibacterial activities of 3-(1,3-dithiolan-2-yl) derivatives (**1a**, **1c**～**1f**) against experimental infections with *Staphylococcus aureus* Smith, *Escherichia coli* NY-17 and *Streptococcus pyogenes* S 23 are shown in Table 2[8]. Although methoxime-type compounds (**1d**～**1f**) had poor activities against the infection with *S. aureus* Smith contrary to its MIC value, oxime-type compounds (**1a** and **1c**) showed satisfactory results by subcutaneous and oral administration, respectively.

The urinary and biliary recovery rates in rats by oral administration of the compounds (**1a**～**1f**) are listed in Table 3. The prodrug esters of the methoxime derivative (**1e** and **1f**) were recovered in bile much more than in urine, while the esters of oxime derivative (**1b** and **1c**) showed an opposite tendency. Regarding the 1-acetoxyethyl esters (**1c**, **1f** and cefuroxime axetil), they showed similar recovery rates in total.

Finally, we investigated the concentration of **1c** in plasma after oral administration to mice[9]. As

Table 3. Urinary and biliary excretion in rats.

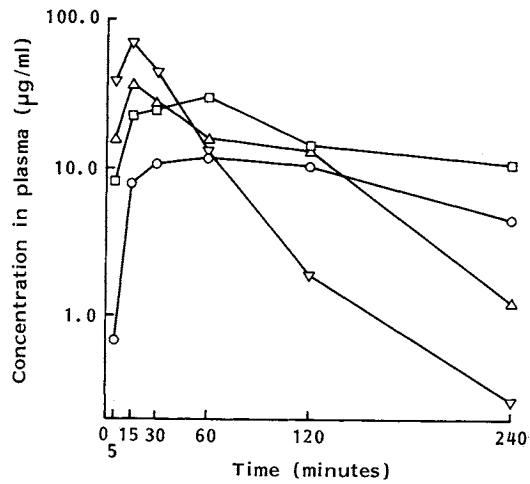
Compound	Urinary recovery[a] (%)	Biliary recovery[a] (%)	Total (%)
YM-22508 (**1a**)	4.5	4.4	8.9
1b	6.9	3.4	10.3
YM-22561 (**1c**)	14.1	8.5	22.6
YM-16457 (**1d**)	2.2	4.8	7.0
1e	4.5	21.5	26.0
1f	7.1	25.5	32.6
CXM-AX	24.9	1.2	26.1
CFIX	20.7	28.4	49.1

[a] After 50 mg/kg po.
CXM-AX: Cefuroxime axetil, CFIX: cefixime.

Fig. 2. Concentration of cepharospolins in plasma after po administration of 50 mg/kg to mice.

□ **1c**, ○ cefixime (CFIX), △ cefteram pivoxil (CFTM-PI), ▽ cefuroxime axetil (CXM-AX).

	Cmax (μg/ml)	AUC (μg·hour/ml)	$T_{1/2}$ (minutes)
1c	29.9	69.3	116.0
CFIX	11.7	34.6	60.9
CFTM-PI	37.7	52.9	50.4
CXM-AX	67.2	47.1	15.4



shown in Fig. 2, the plasma $T_{1/2}$ of **1c** was 116 minutes, which was markedly longer than any other comparative cephalosporin derivatives such as cefixime, cefteram pivoxil and cefuroxime axetil.

Experimental

MP's were determined with a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were taken on a Hitachi 270-30 spectrophotometer. ^{1}H NMR spectra were recorded at 90 MHz on a Jeol EX-90 and at 100 MHz on a Jeol EX-100 NMR spectrometer using TMS as an internal standard. MS was measured on a Jeol JMS DX-300 mass spectrometer. For column chromatography, silica gel (Kieselgel 60, Merck) was used.

Diphenylmethyl 7-*tert*-Butoxycarbonylamino-3-hydroxymethyl-3-cephem-4-carboxylate (**3**)

To a solution of 7-amino-3-hydroxymethyl-3-cephem-4-carboxylic acid (**2**, 54.0 g, 0.23 mol) and Et_3N (60 ml) in dioxane-water (1 : 1, 420 ml) was added di-*tert*-butyl dicarbonate (90.8 g, 0.42 mol) at room temperature. After being stirred at 30°C for 48 hours, dioxane was evaporated under reduced pressure. The residual aqueous layer was washed with EtOAc (210 ml × 2) and acidified (pH 3.6) with 2 N HCl. The resulting solid was collected by filtration and washed with water (100 ml). The solid was then dried over P_2O_5 under reduced pressure to give 62.4 g of 7-*tert*-butoxycarbonylamino-3-hydroxymethyl-3-cephem-4-carboxylic acid. This acid was dissolved in CH_2Cl_2 (650 ml) and esterified with 50% diphenyldiazomethane solution in CH_2Cl_2 (200 ml) at 20°C for 1 hour. The reaction mixture was concentrated under reduced pressure and the residue was crystallized by adding benzene-EtOAc (3 : 1, 120 ml). Filtration and washing of the resulting crystalline solid gave 68.5 g (60%) of **3**: MP 168~169°C; IR ν_{max} (cm^{-1}) 1755, 1720, 1690; ^{1}H NMR (DMSO-d_6) δ 1.40 (9H, s, *tert*-Bu), 3.58 (2H, br s, 2-H), 4.25 (2H, d, J=5 Hz, CH_2OH), 5.09 (1H, d, J=4 Hz, 6-H), 5.48 (1H, dd, J=4 and 8 Hz, 7-H), 6.88 (1H, s, $CHPh_2$), 7.10~7.60 (10H, m, phenyl), 8.01 (1H, d, J=8 Hz, CONH); FAB-MS m/z 497 $(M+1)^+$.

Diphenylmethyl 7-*tert*-Butoxycarbonylamino-3-formyl-3-cephem-4-carboxylate (**4**)

A solution of DMSO (1.76 g, 24.1 mmol) in CH_2Cl_2 (2 ml) was added dropwise to a solution of oxalyl chloride (1.53 g, 12.1 mmol) in CH_2Cl_2 (10 ml) at −70~−60°C and the mixture stirred for 15 minutes. A solution of **3** (5.00 g, 10.1 mmol) in CH_2Cl_2 (50 ml) was added dropwise at the same temperature. After being stirred for 15 minutes, a solution of Et_3N (2.44 g, 24.1 mmol) in CH_2Cl_2 (3.5 ml) was added at

$-70°C$ over a period of 5 minutes. 2 N HCl (12.7 ml) was added and the reaction mixture was diluted with CH_2Cl_2 (30 ml), washed with water, dried over anhydrous $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluted with benzene - EtOAc (19 : 1) to give 3.62 g (73%) of **4**: MP 174~176°C; IR ν_{max} (cm^{-1}) 1785, 1720, 1690; ^1H NMR (DMSO-d_6) δ 1.40 (9H, s, *tert*-Bu), 3.69 (2H, ABq, $J=18$ Hz, 2-H), 5.25 (1H, d, $J=5$ Hz, 6-H), 5.75 (1H, dd, $J=5$ and 9 Hz, 7-H), 7.08 (1H, s, C*H*Ph_2), 7.20~7.60 (10H, m, phenyl), 8.14 (1H, d, $J=9$ Hz, CONH), 9.46 (1H, s, CHO); FAB-MS *m/z* 495 $(M+1)^+$.

7-Amino-3-(1,3-dithiolan-2-yl)-3-cephem-4-carboxylic Acid Trifluoroacetate (**5**)

$BF_3 \cdot Et_2O$ (16.8 ml) was added to a mixture of **4** (49.5 g, 0.10 mol) and 1,2-ethanedithiol (19.0 g, 0.20 mol) in CH_2Cl_2 (500 ml) at $-40°C$. After being stirred at -40~$-30°C$ for 90 minutes, the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with benzene - EtOAc (9 : 1). The concentrated eluate was converted to a powdery solid by triturating with *n*-hexane - Et_2O (10 : 1) to give 49.1 g (86%) of the desired 3-(1,3-dithiolan-2-yl) derivative. To an ice-cooled mixture of the above compound (60.0 g, 0.11 mol) and anisole (12 ml) in CH_2Cl_2 (50 ml) was added TFA (50 ml) below 20°C. The reaction mixture was stirred at 15~20°C for 1 hour and concentrated *in vacuo*. The residual oil was triturated with Et_2O (500 ml) to give 39.1 g (89%) of **5**: IR ν_{max} (cm^{-1}) 3360~3420, 1790; ^1H NMR (DMSO-d_6) δ 4.30~4.60 (4H, m, SCH_2CH_2S), 4.70 (2H, m, 2-H), 4.80~5.20 (2H, m, 6-H and 7-H), 5.96 (1H, s, SCHS); FAB-MS *m/z* 305 $(M+1)^+$.

(*Z*)-3-(1,3-Dithiolan-2-yl)-7-[2-(1-methoxy-1-methyl)ethoxyimino-2-(2-tritylamino-4-thiazolyl)-acetamido]-3-cephem-4-carboxylic Acid (**7a**)

2-Methoxypropene (3.6 ml) was added to a suspension of (*Z*)-2-hydroxyimino-2-(2-tritylamino-4-thiazolyl)acetic acid (**6a**, 5.39 g, 12.5 mmol) in CH_2Cl_2 (105 ml) at 10°C. The reaction mixture was stirred at room temperature for 30 minutes and concentrated under reduced pressure to dryness. The residue was dissolved in CH_2Cl_2 (75 ml). PCl_5 (2.74 g, 13.2 mmol) was then added at $-25°C$ and the mixture was stirred at -20~$-15°C$ for 20 minutes to give a solution of (*Z*)-2-(1-methoxy-1-methyl)ethoxyimino-2-(2-tritylamino-4-thiazolyl)acetyl chloride.

To a suspension of **5** (3.50 g, 8.4 mmol) in CH_2Cl_2 (70 ml) was added bis(trimethylsilyl)acetamide (4.13 ml) at 10°C. After being stirred at room temperature for 15 minutes, pyridine (3.38 ml) and the solution of the previously prepared acid chloride were successively added at $-65°C$. The reaction mixture was stirred at -40~$-35°C$ for 30 minutes, poured into saturated aqueous KH_2PO_4 (350 ml) and extracted with CH_2Cl_2 (100 ml × 2). The combined organic extracts were washed with saturated aqueous KH_2PO_4 (50 ml), dried over anhydrous $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel eluted with $CHCl_3$ - 2-PrOH - formic acid (100 : 3 : 0.3) to give 2.63 g (40%) of **7a**: IR ν_{max} (cm^{-1}) 3415, 1790, 1685; ^1H NMR (DMSO-d_6) δ 1.19 (6H, s, $2CH_3$), 3.12 (3H, s, OCH_3), 3.18~3.48 (4H, m, SCH_2CH_2S), 3.69 (2H, s, 2-H), 5.18 (1H, d, $J=6$ Hz, 6-H), 5.68 (1H, dd, $J=6$ and 8 Hz, 7-H), 5.99 (1H, s, SCHS), 6.72 (1H, s, thiazole H), 7.16~7.52 (15H, m, phenyl), 8.84 (1H, br s, NH), 9.47 (1H, d, $J=8$ Hz, CONH); FAB-MS *m/z* 788 $(M+1)^+$.

(*Z*)-3-(1,3-Dithiolan-2-yl)-7-[2-methoxyimino-2-(2-tritylamino-4-thiazolyl)acetamido]-3-cephem-4-carboxylic Acid (**7b**)

A mixture of (*Z*)-2-methoxyimino-2-(2-tritylamino-4-thiazolyl)acetic acid (**6b**, 531 mg, 1.2 mmol), 1-hydroxybenzotriazole (162 mg, 1.2 mmol) and dicyclohexylcarbodiimide (250 mg, 1.2 mmol) in dioxane (10 ml) was stirred at room temperature for 30 minutes. The reaction mixture was filtered to give a solution of the corresponding activated ester. The filtrate was added to a solution of **5** (304 mg, 0.7 mmol) and $NaHCO_3$ (178 mg, 2.1 mmol) in water (4 ml). After being stirred at room temperature overnight, the mixture was concentrated under reduced pressure. The residue was treated with saturated aqueous $NaHCO_3$ (5 ml) and washed with EtOAc (20 ml × 2). The aqueous layer was then extracted with methyl ethyl ketone after acidification (pH 1) with 2 N HCl. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and evaporated. The residue was triturated with 2-Pr_2O (15 ml) to give 444 mg (84%) of **7b**: IR ν_{max} (cm^{-1}) 3360~3240, 1775, 1665; ^1H NMR (DMSO-d_6) δ 3.10~3.44 (4H, m, SCH_2CH_2S), 3.64 (2H, m, 2-H), 3.70 (3H, s, OCH_3), 5.14 (1H, d, $J=6$ Hz, 6-H), 5.61 (1H, dd, $J=6$ and 8 Hz, 7-H), 5.93 (1H, s,

SCHS), 6.68 (1H, s, thiazole-H), 7.10~7.40 (15H, m, phenyl), 8.75 (1H, s, NH), 9.46 (1H, d, J=8 Hz, CONH); FAB-MS m/z 730 $(M+1)^+$.

7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-hydroxyiminoacetamido]-3-(1,3-dithiolan-2-yl)-3-cephem-4-carboxylic Acid (**1a**)

To a solution of **7a** (9.01 g, 11.4 mmol) in CH_2Cl_2 (65 ml) was added 80% acetic acid (260 ml) and the mixture was stirred at 35~40°C for 1 hour. After removal of solvent under reduced pressure, EtOH (200 ml × 2) was added and evaporated. The residue was triturated with Et_2O (485 ml) to give 1.31 g (92%) of **1a**: IR ν_{max} (cm^{-1}) 3450, 1770, 1670; ^1H NMR (DMSO-d_6) δ 3.20~3.57 (4H, m, SCH_2CH_2S), 3.70 (2H, s, 2-H), 5.20 (1H, d, J=6 Hz, 6-H), 5.75 (1H, dd, J=6 and 8 Hz, 7-H), 6.01 (1H, s, SCHS), 6.68 (1H, s, thiazole-H), 7.12 (2H, br s, NH_2), 9.45 (1H, d, J=8 Hz, CONH); FAB-MS m/z 474 $(M+1)^+$.

7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-(1,3-dithiolan-2-yl)-3-cephem-4-carboxylic acid (**1d**) was similarly prepared from **7b** using the same procedure described for **1a** (yield, 64%): IR ν_{max} (cm^{-1}) 3320, 1770, 1660; ^1H NMR (DMSO-d_6) δ 3.20~3.60 (4H, m, SCH_2CH_2S), 3.71 (2H, s, 2-H), 3.85 (3H, s, OCH_3), 5.22 (1H, d, J=6 Hz, 6-H), 5.76 (1H, dd, J=6 and 8 Hz, 7-H), 5.99 (1H, s, SCHS), 6.79 (1H, s, thiazole-H), 9.65 (1H, d, J=8 Hz, CONH); FAB-MS m/z 488 $(M+1)^+$.

1-Pivaloyloxymethyl 7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-hydroxyiminoacetamido]-3-(1,3-dithiolan-2-yl)-3-cephem-4-carboxylate (**1b**)

A solution of pivaloyloxymethyl chloride (40 mg, 0.3 mmol) in DMF (1 ml) was added to a solution of **1a** (113 mg, 0.2 mmol) and K_2CO_3 (18 mg, 0.1 mmol) in DMF (5 ml) at −10°C. The mixture was then stirred at −10~2°C for 20 hours. After removal of solvent under reduced pressure, Et_2O (5 ml) was added to the residue. The resulting powder was filtered and purified by column chromatography eluted with $CHCl_3$ - MeOH - formic acid (90 : 10 : 2) to give 18 mg (13%) of **1b**: IR ν_{max}(cm^{-1}) 3370, 1790, 1750, 1680; ^1H NMR (DMSO-d_6) δ 1.20 (9H, s, *tert*-Bu), 3.50~3.52 (4H, m, SCH_2CH_2S), 3.77 (2H, ABq, J=15 Hz, 2-H), 5.26 (1H, d, J=5 Hz, 6-H), 5.81~5.84 (3H, m, $COOCH_2O$ and SCHS), 5.95 (1H, d, J=5 Hz, 7-H), 6.69 (1H, s, thiazole-H), 9.46 (1H, d, J=5 Hz, CONH); FAB-MS m/z 588 $(M+1)^+$.

The other prodrug-type esters (**1c**, **1e**, **1f**) were similarly prepared from **1a** or **1d** using the same procedure described for **1b**.

1-Acetoxyethyl 7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-hydroxyiminoacetamido]-3-(1,3-dithiolan-2-yl)-3-cephem-4-carboxylate (**1c**)

IR ν_{max}(cm^{-1}) 3360, 1780, 1675; ^1H NMR (DMSO-d_6) δ 1.45 (1.5H, d, J=6 Hz, $CHCH_3$), 1.47 (1.5H, d, J=6 Hz, $CHCH_3$), 2.06 (3H, s, $COCH_3$), 3.15~3.46 (4H, m, SCH_2CH_2S), 3.70 (2H, s, 2-H), 5.17 (0.5H, d, J=3 Hz, 6-H), 5.22 (0.5H, d, J=3 Hz, 6-H), 5.63~5.90 (2H, m, 7-H and SCHS), 6.63 (1H, s, thiazole-H), 6.90 (1H, m, $CHCH_3$), 7.09 (2H, br s, NH_2), 9.43 (1H, d, J=8 Hz, CONH); FAB-MS m/z 560 $(M+1)^+$. The diastereoisomer ratio of the acetoxyethyl esters is 1 : 1 indicated by ^1H NMR spectrum.

1-Pivaloyloxymethyl 7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-(1,3-dithiolan-2-yl)-3-cephem-4-carboxylate (**1e**)

IR ν_{max} (cm^{-1}) 3330~3290, 1775, 1745, 1670; ^1H NMR (DMSO-d_6) δ 1.15 (9H, s, *tert*-Bu), 3.10~3.60 (4H, m, SCH_2CH_2S), 3.75 (2H, s, 2-H), 3.84 (3H, s, OCH_3), 5.24 (1H, d, J=5 Hz, 6-H), 5.64~6.00 (4H, m, $COOCH_2O$, SCHS and 7-H), 6.77 (1H, s, thiazole-H), 9.64 (1H, d, J=8 Hz, CONH); FAB-MS m/z 602 $(M+1)^+$.

1-Acetoxyethyl 7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-(1,3-dithiolan-2-yl)-3-cephem-4-carboxylate (**1f**)

IR ν_{max} (cm^{-1}) 2950, 1780, 1660; ^1H NMR (DMSO-d_6) δ 1.43 (1.5H, d, J=6 Hz, $CHCH_3$), 1.45 (1.5H, d, J=6 Hz, $CHCH_3$), 2.06 (3H, s, $COCH_3$), 3.10~3.60 (4H, m, SCH_2CH_2S), 3.60~3.90 (2H, m, 2-H), 3.82 (3H, s, OCH_3), 5.15 (0.5H, d, J=3 Hz, 6-H), 5.20 (0.5H, d, J=3 Hz, 6-H), 5.60~5.80 (2H, m, 7-H and SCHS), 6.68 (1H, s, thiazole-H), 6.72~7.00 (1H, m, $CHCH_3$), 9.56 (1H, d, J=8 Hz, CONH); FAB-MS m/z 574 $(M+1)^+$. Thd diastereoisomer ratio of the acetoxyethyl esters is 1 : 1 indicated by ^1H NMR spectrum.

Determination of Antibacterial Activity

MICs (μg/ml) were determined by the standard 2-fold agar dilution method in Mueller-Hinton agar after incubation at 37°C for 18 hours with an inoculum size of 10^6 cfu/ml.

Antibacterial activity *in vivo* was tested using male mice (ICR, 4~5 weeks old, $n=6$). Mice were individually given an antibiotic suspension in 0.5% methyl cellulose subcutaneously or orally 2 hours after the bacterial challenge. ED_{50} values (mg/kg) were calculated by the probit method for the number of mice surviving 7 days after infection.

Urinary and biliary excretion were tested using male rats (SD, 7 weeks old, $n=3$). The test compounds were administered orally to rats at a dose of 50 mg/kg. Urinary and biliary recovery rates (%) were calculated from the urinary and biliary concentrations of drugs at 0 to 24 hours after administration. Concentrations were determined by bioassay (agar well method) using *E. coli* NIHJ as a test organism.

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