ORALLY ABSORBABLE D-FORPHENICINOL-CEPHALOSPORINS

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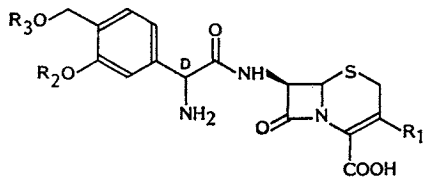
The synthesis and biological evaluation of D-forphenicinol-cephalosporins are described. 7-Amino-3-(substituted)-3-cephem-4-carboxylic acid esters were coupled with *N-tert*-butoxycarbonyl-D-forphenicinol derivatives. Most of cephalosporin analogs in this series had good antibacterial activities and were well absorbed from the gastrointestinal tract in mice. Among them, 7-(mono-*O*-methyl-D-forphenicinol)-3-propenyl analog showed the most significant result in biological evaluations.

In the clinical field, the demand for new oral cephalosporins has become progressively greater. Most of orally absorbable cephalosporins possess 7-arylglycyl amide residue in their structures. In our efforts to search for new cephalosporins with the improved activity and pharmacokinetic properties, we found that several cephalosporins acylated by D-forphenicinol[1] or its congeners at the C-7 position of cephem nucleus had good antibacterial activity and high gastrointestinal absorption in mice. Forphenicinol,[1] L-(3-hydroxy-4-hydroxymethylphenyl)glycine, has biologically attractive properties, most significantly as an immunomodifier.[2,3] A cephalosporin derivative acylated by forphenicinol at C-7 showed only poor antibacterial activities.

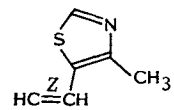
We describe here synthesis and biological evaluation of new orally absorbable cephalosporin derivatives, to the nucleus of which is attached D-forphenicinol or *O*-methyl-D-forphenicinol side chain at the C-7 position. The substituents at the C-3 position are selected from those of cephalexin,[4] cefpodoxime,[5] ME1207,[6] cefixime[7] and BMY-28100;[8] *i.e.*, methyl, methoxymethyl, 2-(4-methylthiazol-5-yl)ethenyl, vinyl and 1-propenyl groups (Fig. 1).

The synthetic route is shown in Scheme 1. 7-Aminocephalosporanic acid esters (**2a**~**2e**) were acylated with *N-tert*-butoxycarbonyl (Boc) derivative of D-forphenicinol (**3**) in DMF by the active

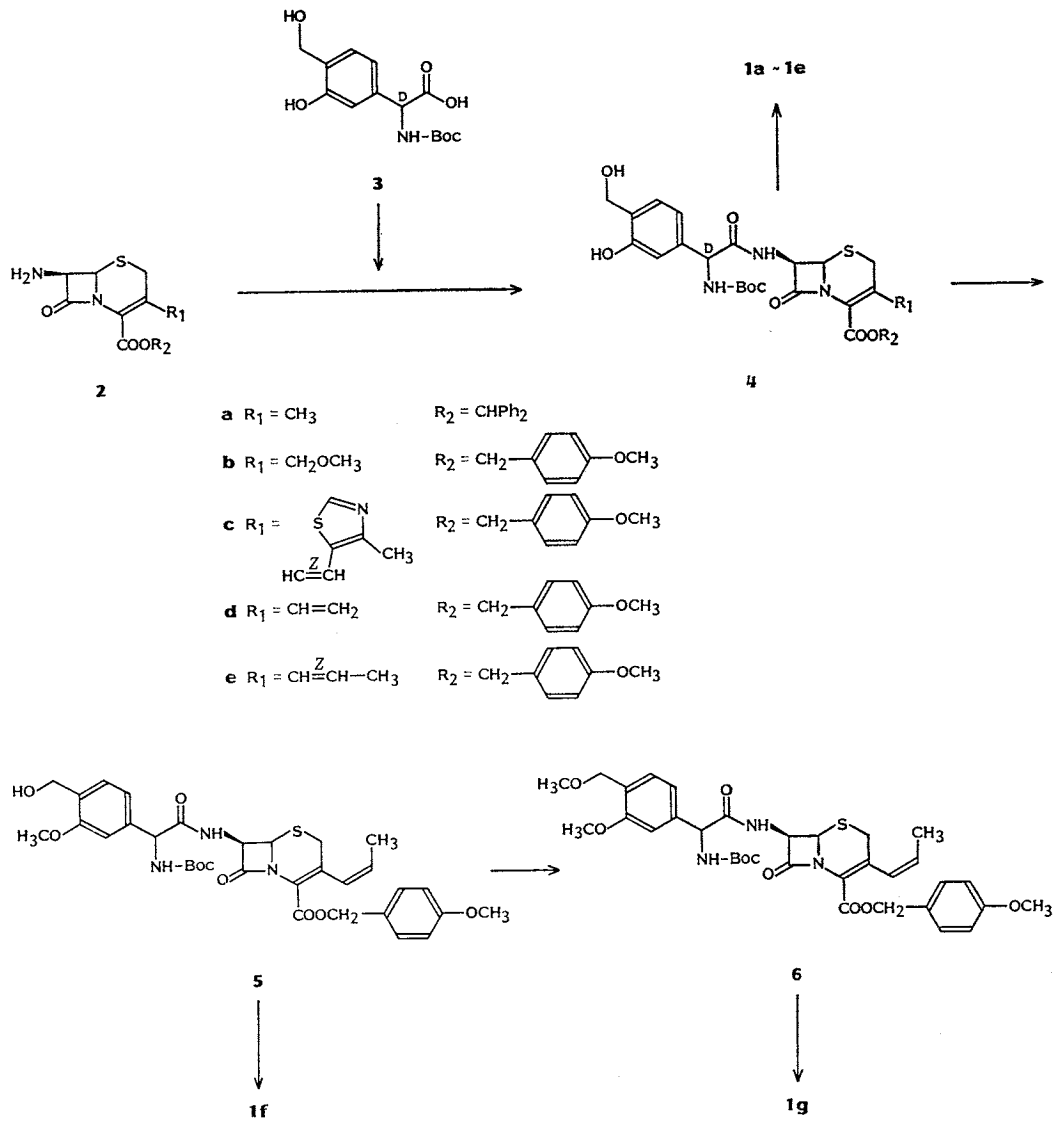
Fig. 1. Orally absorbable cephalosporin derivatives.



1

	R_1	R_2	R_3
1a	CH_3	H	H
1b	CH_2OCH_3	H	H
1c		H	H
1d	$CH{=}CH_2$	H	H
1e	$\overset{Z}{CH{=}CHCH_3}$	H	H
1f	$\overset{Z}{CH{=}CHCH_3}$	CH_3	H
1g	$\overset{Z}{CH{=}CHCH_3}$	CH_3	CH_3

Scheme 1.



ester method (HOBT-DCC method) to give corresponding compounds **4a**~**4e**. These were treated with a mixture of trifluoroacetic acid and anisole and subsequently with Amberlite IR-45 (OH) to afford **1a**~**1e**. By the similar route described above, the L-isomer of **1d** was synthesized from *N*-Boc-forphenicinol.

The phenolic hydroxyl group of **4e** was methylated with diazotrimethylsilylmethane in chloroform to give mono-*O*-methyl derivative **5**. No di-*O*-methyl derivative could be detected under this condition. The *N*-Boc group of **5** was removed to give **1f**. Further *O*-methylation of **5** with diazotrimethylsilylmethane in the presence of boron trifluoride etherate in chloroform gave di-*O*-methyl derivative **6**. Compound **1g** was afforded by deprotection of **6**.

Cephalosporin derivatives acylated with D-forphenicinol at the 7-amino group have been shown to possess significant antibacterial activity. As shown in Table 1, compounds **1e**, **1f** and **1g** sub-

Table 1. MICs (μg/ml).

Test organism	**1a**	**1b**	**1c**	**1d**	L-Isomer of **1d**	**1e**	**1f**	**1g**	CCL
Staphylococcus aureus FDA 209P JC-1	3.13	1.56	3.13	1.56	6.25	1.56	1.56	0.78	1.56
S. aureus Terajima	0.20	0.20	0.20	0.20	0.78	0.10	0.10	0.10	0.10
S. aureus MS353	3.13	1.56	6.25	1.56	6.25	1.56	0.78	1.56	1.56
Bacillus subtilis ATCC 6633	0.78	0.20	0.39	0.05	0.78	0.20	0.05	0.05	0.10
Escherichia coli NIHJ JC-2	12.5	12.5	25	6.25	50	6.25	6.25	25	1.56
Klebsiella pneumoniae PCI 602	6.25	6.25	3.13	3.13	25	0.78	0.78	1.56	0.39
Salmonella typhi 901	6.25	6.25	6.25	3.13	25	0.78	1.56	12.5	0.39
S. paratyphi 1015	12.5	12.5	3.13	3.13	25	1.56	0.78	3.13	0.78
S. schottmuelleri 8006	12.5	6.25	3.13	3.13	25	1.56	0.39	1.56	0.78
S. enteritidis G 14	50	6.25	6.25	3.13	12.5	0.78	0.78	6.25	0.39
Morganella morganii IFO 3848	>100	50	1.56	50	>100	1.56	3.13	1.56	25
Providencia rettgeri IFO 3850	12.5	25	25	50	>100	1.56	3.13	12.5	12.5
Proteus vulgaris OX19	100	100	25	25	100	25	50	100	12.5
Pseudomonas aeruginosa IFO 3445	>100	>100	>100	>100	>100	>100	>100	>100	>100
Rms212/*E. coli* K-12 W3630[a]	12.5	50	>100	12.5	50	12.5	12.5	100	3.13
Rms213/*E. coli* K-12 W3630[a]	12.5	12.5	25	12.5	50	6.25	25	12.5	3.13
Rte16/*E. coli* K-12 W3630[a]	12.5	25	25	12.5	50	12.5	3.13	25	1.56
K. oxytoca GN10560[b]	>100	>100	>100	>100	>100	>100	>100	>100	>100

[a] PCase. [b] CXase.

Table 2. Urinary recovery in mice.

Compound	Recovery (5 hours, %)
1a	65.1
1b	48.3
1c	13.0
1d	59.6
1e	58.8
1f	62.3
1g	32.9
CCL	56.0

stituted with 1-propenyl group at C-3 in the cephem nucleus have stronger activity than others having another substituent at C-3. Particularly **1f**, mono-*O*-methyl-D-forphenicinol derivative, shows the strongest antibacterial activity. The urinary recoveries in mice after oral administration of **1a**~**1g** and cefaclor (CCL) are shown in Table 2. Recoveries of compounds **1a**, **1d**, **1e** and **1f** in urine are higher than that of CCL. Blood levels in mice after oral administration of **1a**~**1f** and CCL are shown in Table 3. Compounds **1a**, **1d**, **1e** and **1f** have higher blood level than that of CCL. Marked therapeutic efficacies of **1e** and **1f** against *Staphylococcus aureus* Smith in systemically infected mice were shown after oral administration; ED_{50} of **1e**: 0.56 mg/kg and **1f**: 0.33 mg/kg.

Experimental

General Methods

MP's were determined with a Yanagimoto micro melting point apparatus and were uncorrected. Mass spectra were obtained on a Hitachi M-80H mass spectrometer. ^1H NMR spectra (400 MHz) were measured with a Jeol JNM-GX 400 spectrometer in D_2O (internal standard).

7-[(*R*)-2-Amino-2-(3-hydroxy-4-hydroxymethylphenyl)acetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylic Acid (**1e**)

To a solution of 4-methoxybenzyl 7-amino-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylate[7] (**2e**, 270 mg), *N*-Boc-D-forphenicinol[1] (**3**, 227 mg) in DMF (4 ml) was added HOBT (112 mg) and DCC (170 mg) at 5°C. The solution was stirred for 2 hours at room temperature. After the evaporation to remove DMF, ethyl acetate (50 ml) was added to the residue. The insoluble material was filtered. The ethyl acetate solution was washed with water and aqueous sodium chloride solution, dried over anhydrous sodium sulfate and concentrated to give a solid. The solid was chromatographed on a silica gel column (chloroform - methanol, 97 : 3) to afford **4e** (325 mg), mp 104~112°C (dec), secondary ion (SI)-MS *m/z* 639 (MH^+). Compound **4e** (100 mg) was treated with trifluoroacetic acid (1 ml) in the presence of anisole (0.1 ml) at 5°C for 1 hour. Isopropyl ether was added to this solution. After evaporation, the residue was triturated with isopropyl ether and diethyl ether. The obtained solid was dissolved in water (10 mg/ml) and the insoluble material was filtered. The aqueous solution was adjusted to pH 5~6 with Amberlite IR-45 (OH). After removal of resin, the filtrate was

Table 3. Blood level in mice.

Compound	Blood level (μg/ml)				
	0.25	0.5	1	2	4 (hours)
1a	10.0	12.5	7.0	ND	ND
1b	5.0	5.3	3.8	1.0	ND
1c	2.2	2.7	0.81	ND	ND
1d	10.0	11.2	1.7	0.50	ND
1e	9.5	11.5	6.4	1.4	0.58
1f	5.7	10.0	6.7	1.3	1.3
CCL	6.1	3.5	1.9	ND	ND

ND: Not determined.

concentrated to give a solid of **1e** (47 mg): MP >175°C (dec); SI-MS *m/z* 420 (MH^+); ^1H NMR δ 1.59 (3H, dd, *J*=1.5 and 7 Hz, =CHCH_3), 3.20 (1H, d, *J*=18 Hz, 2-H), 3.47 (1H, d, *J*=18 Hz, 2-H), 4.69 (2H, s, ArCH_2OH), 5.14 (1H, d, *J*=4.5 Hz, 6-H), 5.15 (1H, s, ArC*H*(NH_2)CO), 5.69 (1H, dq, *J*=7 and 12 Hz, =C*H*CH_3), 5.71 (1H, d, *J*=4.5 Hz, 7-H), 5.87 (1H, br d, *J*=12 Hz, C*H*=$CHCH_3$), 7.01 (1H, d, *J*=1.5 Hz, Ar), 7.07 (1H, dd, *J*=1.5 and 8 Hz, Ar), 7.42 (1H, d, *J*=8 Hz, Ar).

7-[(*R*)-2-Amino-2-(3-hydroxy-4-hydroxymethylphenyl)acetamido]-3-methyl-3-cephem-4-carboxylic Acid (**1a**)

Compound **1a** was prepared from diphenylmethyl 7-amino-3-methyl-3-cephem-4-carboxylate[9] by the similar method to **1e**.

MP >180°C (dec); SI-MS *m/z* 394 (MH^+); ^1H NMR δ 1.86 (3H, s, CH_3), 3.07 (1H, d, *J*=18 Hz, 2-H), 3.49 (1H, d, *J*=18 Hz, 2-H), 4.68 (2H, s, ArCH_2OH), 5.05 (1H, d, *J*=4.5 Hz, 6-H), 5.12 (1H, s, ArC*H*(NH_2)CO), 5.65 (1H, d, *J*=4.5 Hz, 7-H), 7.0 (1H, d, *J*=1.5 Hz, Ar), 7.06 (1H, dd, *J*=1.5 and 8 Hz, Ar), 7.42 (1H, d, *J*=8 Hz, Ar).

7-[(*R*)-2-Amino-2-(3-hydroxy-4-hydroxymethylphenyl)acetamido]-3-methoxymethyl-3-cephem-4-carboxylic Acid (**1b**)

Compound **1b** was prepared from 4-methoxybenzyl 7-amino-3-methoxymethyl-3-cephem-4-carboxylate[10] by the similar method to **1e**.

MP >172°C (dec); SI-MS *m/z* 424 (MH^+); ^1H NMR δ 3.22 (1H, d, *J*=18 Hz, 2-H), 3.25 (3H, s, OCH_3), 3.50 (1H, d, *J*=18 Hz, 2-H), 4.14 (2H, ABq, *J*=12 Hz, C$H_2$$OCH_3$), 4.69 (2H, s, ArC$H_2$OH), 5.11 (1H, d, *J*=4.5 Hz, 6-H), 5.14 (1H, s, ArC*H*(NH_2)CO), 5.72 (1H, d, *J*=4.5 Hz, 7-H), 7.0 (1H, d, *J*=1.5 Hz, Ar), 7.06 (1H, dd, *J*=1.5 and 8 Hz, Ar), 7.43 (1H, d, *J*=8 Hz, Ar).

7-[(*R*)-2-Amino-2-(3-hydroxy-4-hydroxymethylphenyl)acetamido]-3-[(*Z*)-1-(2-(4-methylthiazol-5-yl))ethenyl]-3-cephem-4-carboxylic Acid (**1c**)

Compound **1c** was prepared from 4-methoxybenzyl 7-amino-3-[(*Z*)-1-(2-(4-methylthiazol-5-yl))-ethenyl]-3-cephem-4-carboxylate[6] by the similar method to **1e**.

MP >220°C (dec); SI-MS *m/z* 503 (MH^+); ^1H NMR δ 2.37 (3H, s, CH_3), 3.16 (1H, d, *J*=18.5 Hz, 2-H), 3.46 (1H, d, *J*=18.5 Hz, 2-H), 4.67 (2H, s, ArCH_2OH), 5.12 (1H, s, ArC*H*(NH_2)CO), 5.24 (1H, d, *J*=5 Hz, 6-H), 5.74 (1H, d, *J*=5 Hz, 7-H), 6.27 and 6.64 (each 1H, d, *J*=12 Hz, *cis*-olefin protons), 7.0 (1H, d, *J*=2 Hz, Ar), 7.06 (1H, dd, *J*=2 and 8 Hz, Ar), 7.41 (1H, d, *J*=8 Hz, Ar), 9.77 (1H, s, thiazole ring proton).

7-[(*R*)-2-Amino-2-(3-hydroxy-4-hydroxymethylphenyl)acetamido]-3-vinyl-3-cephem-4-carboxylic Acid (**1d**)

Compound **1d** was prepared from 4-methoxybenzyl 7-amino-3-vinyl-3-cephem-4-carboxylate[7] by the similar method to **1e**.

MP >184°C (dec); SI-MS *m/z* 406 (MH^+); ^1H NMR δ 3.40 (1H, d, *J*=18 Hz, 2-H), 3.47 (1H, d, *J*=18 Hz, 2-H), 4.69 (2H, s, ArCH_2OH), 5.12 (1H, d, *J*=5 Hz, 6-H), 5.14 (1H, s, ArC*H*(NH_2)CO), 5.24 (1H, d, *J*=11 Hz, CH=CH_2), 5.38 (1H, d, *J*=18 Hz, CH=CH_2), 5.71 (1H, d, *J*=5 Hz, 7-H), 6.70 (1H, dd, *J*=11 and 18 Hz, C*H*=CH_2), 7.0 (1H, d, *J*=1.5 Hz, Ar), 7.07 (1H, dd, *J*=1.5 and 8 Hz, Ar), 7.43 (1H, d, *J*=8 Hz, Ar).

7-[(*S*)-2-Amino-2-(3-hydroxy-4-hydroxymethylphenyl)acetamido]-3-vinyl-3-cephem-4-carboxylic Acid

This compound was prepared from 4-methoxybenzyl 7-amino-3-vinyl-3-cephem-4-carboxylate[7] and *N*-Boc-forphenicinol[11] by the similar method to **1e**.

MP >200°C (dec); SI-MS *m/z* 406 (MH^+); ^1H NMR δ 3.59 (1H, d, *J*=18 Hz, 2-H), 3.70 (1H, d, *J*=18 Hz, 2-H), 4.69 (2H, s, ArCH_2OH), 5.13 (1H, s, ArC*H*(NH_2)CO), 5.15 (1H, d, *J*=5 Hz, 6-H), 5.27 (1H, d, *J*=11 Hz, CH=CH_2), 5.43 (1H, d, *J*=18 Hz, CH=CH_2), 5.50 (1H, d, *J*=5 Hz, 7-H), 6.73 (1H, dd, *J*=11 and 18 Hz, C*H*=CH_2), 7.01 (1H, d, *J*=1.5 Hz, Ar), 7.06 (1H, dd, *J*=1.5 and 8 Hz, Ar), 7.43 (1H, d, *J*=8 Hz, Ar).

7-[(*R*)-2-Amino-2-(4-hydroxymethyl-3-methoxyphenyl)acetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylic Acid (**1f**)

Compound **4e** (90 mg) was methylated in chloroform with 10% solution of diazotrimethylsilylmethane in hexane (1.5 ml) at room temperature for 2.5 hours. After the removal of solvent, the residue was chromatographed on preparative silica gel plates. Mono-*O*-methyl derivative (**5**, 40 mg) was obtained as a colorless solid, mp 155~157°C. Compound **5** (13 mg) was treated with trifluoroacetic acid and anisole to give **1f** (6 mg) by a similar procedure to a preparation of **1e**: MP >167°C (dec); SI-MS *m/z* 434 (MH^+); ^1H NMR δ 1.58 (3H, dd, J=1.5 and 7 Hz, =CHCH_3), 3.19 (1H, d, J=18 Hz, 2-H), 3.46 (1H, d, J=18 Hz, 2-H), 3.93 (3H, s, ArOCH_3), 4.67 (2H, s, ArCH_2OH), 5.12 (1H, s, ArCH(NH_2)CO), 5.14 (1H, d, J=4.5 Hz, 6-H), 5.69 (1H, dq, J=7 and 12 Hz, =CHCH_3), 5.72 (1H, d, J=4.5 Hz, 7-H), 5.87 (1H, br d, J=12 Hz, CH=CHCH_3), 7.12 (1H, s, Ar), 7.13 (1H, d, J=8 Hz, Ar), 7.44 (1H, d, J=8 Hz, Ar).

7-[(*R*)-2-Amino-2-(3-methoxy-4-methoxymethylphenyl)acetamido]-3-[(*Z*)-1-propenyl]-3-cephem-4-carboxylic Acid (**1g**)

To a solution of **5** (38 mg) in dichloromethane (1 ml) was added 10% hexane solution of diazotrimethylsilylmethane (1 ml) and a catalytic amount of boron trifluoride etherate. The resulting solution was stirred for 10 hours at room temperature. After evaporation, di-*O*-methyl derivative (**6**) was obtained by preparative silica gel plates as a colorless solid (16 mg). Compound **6** (15 mg) was converted into **1g** (8 mg) with trifluoroacetic acid and anisole by the procedure mentioned above: MP >172°C (dec); SI-MS *m/z* 448 (MH^+); ^1H NMR δ 1.59 (3H, dd, J=1.5 and 7 Hz, =CHCH_3), 3.18 (1H, d, J=18 Hz, 2-H), 3.40 (3H, s, ArCH_2OCH_3), 3.45 (1H, d, J=18 Hz, 2-H), 3.91 (3H, s, ArOCH_3), 4.53 (2H, s, ArCH_2OCH_3), 5.13 (1H, s, ArCH(NH_2)CO), 5.13 (1H, d, J=5 Hz, 6-H), 5.69 (1H, dq, J=7 and 12 Hz, =CHCH_3), 5.71 (1H, d, J=5 Hz, 7-H), 5.87 (1H, br d, J=12 Hz, CH=CHCH_3), 7.13 (1H, d, J=8 Hz, Ar), 7.15 (1H, s, Ar), 7.44 (1H, d, J=8 Hz, Ar).

Antibacterial Activity

According to the method of Japan Society of Chemotherapy, MICs were determined in Mueller-Hinton agar medium by the standard 2-fold dilution method after incubation at 37°C for 18 hours.

Blood Level and Urinary Recovery in Mice

Two male ICR mice (4-week old) were given by oral administration (15 mg/kg). Blood samples were collected at 0.25, 0.50, 1, 2, 4 and 6 hour(s) and the antibiotic activities were determined by paper disc method using *Bacillus subtilis* PCI 219 as an assay organism. Urinary specimens were collected at 0~5 hours.

Therapeutic Efficacy in Mice

Male ICR mice (4-week old) were infected intraperitoneally with 53.1 times of the median lethal dose of *Staphylococcus aureus* Smith (1.7×10^7 cfu/mouse). Five mice at each dose level were individually given orally at 1 hour after the bacterial challenge. The 50% effective dose (ED_{50}) was calculated by the method of LITCHFIELD and WILCOXON[11], from survival rate recorded on 7 days.

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