CEPHALOSPORINS HAVING A HETEROCYCLIC CATECHOL IN THE C3 SIDE CHAIN

I. ENHANCEMENT OF EFFICACY AGAINST GRAM-NEGATIVE BACTERIA

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Two groups of cephalosporins substituted with a variety of heterocyclic catechols in the C3 side chain were synthesized. One is a group of 3-(heterocyclic catechol-substituted methyl)cephalosporins and another is 3-[(*E*)-3-heterocyclic catechol-substituted 1-propen-1-yl]cephalosporins. Cephalosporins in the latter group showed higher *in vivo* efficacies than those in the former group having the same heterocyclic catechol, especially against *Pseudomonas aeruginosa* A9843A, although there was no significant difference between their *in vitro* activity. Among the latter group, the 5,6-dihydroxybenzimidazole derivative (**6e**) and the 2,6-dihydro-7-hydroxy-6-oxo-isoquinoline derivative (**6b**) showed much higher activity than ceftazidime (CAZ) and imipenem (IPM) against *P. aeruginosa* A9843A both in *in vitro* and *in vivo* studies.

In our previous paper[1], we reported synthesis of a variety of cephalosporins having a catechol moiety in the C3 side chain. Among them, 7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxy-imino)acetamido]cephalosporins having a catechol-substituted methyl and a catechol-substituted propenyl as C3 side chain showed excellent *in vitro* antibacterial activity especially against Gram-negative bacteria. In order to explore more potent derivatives, we synthesized both types of the cephalosporins substituted with a heterocyclic catechol in the C3 side chain. This report describes the synthesis of the above cephalosporins and their *in vitro* and *in vivo* structure-activity relationships, together with comparison of their activities with those of ceftazidime (CAZ) and imipenem (IPM).

Synthesis

3-(Heterocyclic Catechol-substituted Methyl)cephalosporins (Group A)

Scheme 1 illustrates the synthesis of 3-(heterocyclic catechol-substituted methyl)cephalosporins. In order to prevent the formation of undesirable Δ^2-isomer of cephalosporin, 3-iodomethylcephalosporins 1-oxide (**1**)[2] was employed in the coupling reactions with 6,7-dihydroxyphthalazine, which was prepared from 6,7-dimethoxyphthalazine[3]. The reaction product was reduced with phosphorus tribromide, followed by deblocking with trifluoroacetic acid to give **3a** after purification by column chromatography. In a similar way, coupling of **1** and 5,6-dihydroxybenzimidazole[4] afforded **3b**. The coupling of the iodide (**2**)[2] with 4,5-dihydroxypyridazine[5] in the presence of *N*,*O*-bis(trimethylsilyl)acetamide in dichloromethane afforded **3c** without generation of the its Δ^2-isomer after deblocking. Methylation of **4**, the intermediate for the preparation of **3b** with methyl iodide, followed by deblocking afforded the quaternary ammonium

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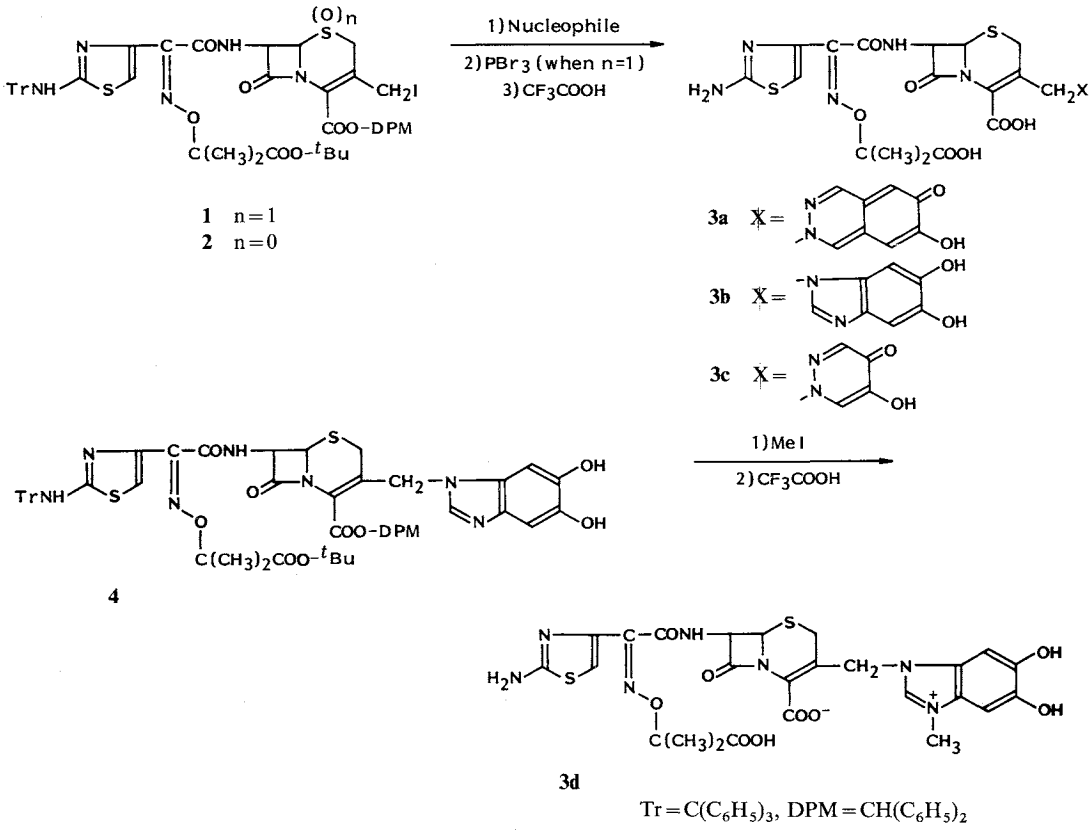
Scheme 1. Synthesis of cephalosporins having a heterocyclic catechol in the 3-methyl group.



cephalosporin **3d**.

3-[(*E*)-3-Heterocyclic Catechol-substituted 1-Propen-1-yl]cephalosporins (Group B)

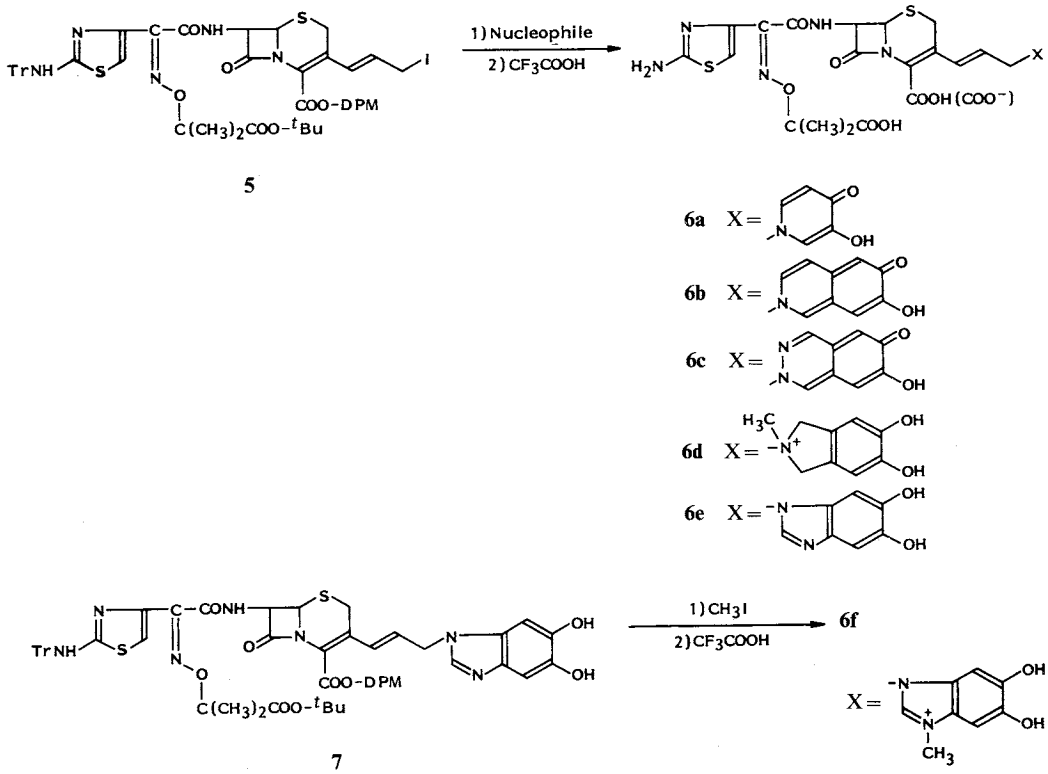
Scheme 2 shows the synthesis of 3-[(*E*)-3-heterocyclic catechol-substituted 1-propen-1-yl]cephalosporins. The (*E*)-3-iodopropenyl derivative (**5**) was synthesized by coupling of diphenylmethyl 7-amino-3-[(*Z*)-3-chloro-1-propen-1-yl]-3-cephem-carboxylate[6] and (*Z*)-2-(2-tritylaminothiazol-4-yl)-2-(1-methyl-1-*tert*-butoxycarbonylethoxyimino)acetic acid, followed by treatment with NaI in acetone by the method we reported[6]. Coupling of **5** with 3,4-hydroxypyridine[7] in DMF in the presence of *N,O*-bis(trimethylsilyl)acetamide, followed by deblocking gave **6a**. Reaction of the iodide (**5**) with 6,7-dihydroxyisoquinoline[8], 6,7-dihydroxyphthalazine and 5,6-dihydroxybenzimidazole in DMF, followed by deblocking with trifluoroacetic acid, gave the cephalosporins, **6b**, **6c** and **6e**, respectively. Coupling of **5** with 5,6-diacetoxy-2-methyl-2-isoindoline prepared from 5,6-dihydroxy-2-methyl-2-isoindoline[9], followed by deblocking afforded the 5,6-diacetoxy-2-methyl-2-isoindoline derivative, which were treated with acetyl esterase to afford **6d**. Quaternization of **7** with methyl iodide, followed by deblocking with trifluoroacetic acid, gave **6f**.

Biological Activity

In Vitro Activity

Table 1 shows comparison of *in vitro* activity of the 3-(substituted methyl)cephalosporins (**3a**~**3d**,

Scheme 2. Synthesis of 3-propenyl cephalosporins having a heterocyclic catechol in the 3-position of propenyl group.

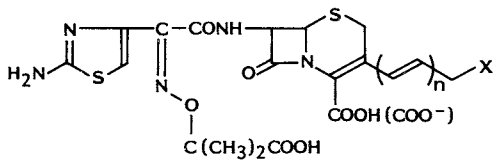


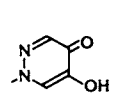
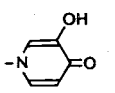
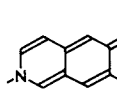
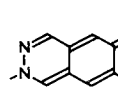
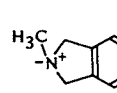
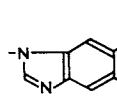
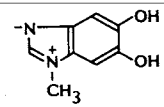
8[10], **9**[11] and **10**[12]; Group A) and the 3-(substituted propenyl)cephalosporins (**6a**~**6f**; Group B) against 14 organisms. On the whole, there was no significant difference in *in vitro* activity against Gram-negative bacteria between the compounds in Groups A and B, which have the same heterocyclic catechol to each other. The isoquinoline derivatives (**9** and **6b**), the phthalazine derivatives (**3a** and **6c**), the isoindoline derivatives (**10** and **6d**) and the *N*-methylbenzimidazole derivatives (**3d** and **6f**) showed broad antibacterial spectrum against Gram-negative bacteria, which include CAZ- or IPM-resistant organisms, *Escherichia coli* 255, *Proteus vulgaris* Pv-45, *Morganella morganii* 1510, *Enterobacter cloacae* El-83, *Serratia marcescens* Sm-238, *Citrobacter freundii* GN7391, *Pseudomonas aeruginosa* Pa-246, *Xanthomonas maltophilia* Pl-19 and *Pseudomonas cepacia* Pp-105, while the heteromonocyclic derivatives, **3c 8** and **6a** showed weak or no activity against the CAZ-resistant strains, *E. cloacae* El-83, *S. marcescens Sm*-238 and *C. freundii* GN7391. All the compounds showed much weak activity against Gram-positive bacteria. Against 16 clinical isolates of moderately to high CAZ-resistant *P. aeruginosa*, all the compounds were active with much better MIC_{50} and MIC_{90} than CAZ (Table 2). Among them, **9**, **6b**, **6c**, **3d** and **6f** were eight and two times as active as IPM in terms of MIC_{50} and MIC_{90}, respectively.

In Vivo Activity

Table 3 summarizes *in vivo* antibacterial activity of the compounds in Groups A and B against systemic infections in normal mice. Except the heteromonocyclic derivatives (**3c**, **8** and **6a**), all the cephalosporins

Table 1. *In vitro* activity of cephalosporins.



Organism X	MIC (μg/ml)														
															
n	0	0	1	0	1	0	1	0	1	0	1	0	1	CAZ	IPM
	3c	**8**	**6a**	**9**	**6b**	**3a**	**6c**	**10**	**6d**	**3b**	**6e**	**3d**	**6f**		
S.a. Smith	50	50	25	25	12.5	25	25	100	12.5	50	25	25	100	12.5	0.025
B.s. PCI 219	12.5	12.5	12.5	12.5	12.5	25	12.5	25	12.5	12.5	6.3	12.5	12.5	12.5	0.025
E.c. Juhl	0.4	0.4	0.4	0.4	0.1	0.4	0.2	0.8	0.4	0.8	0.4	0.2	0.2	0.4	0.2
E.c. 255	1.6	0.8	0.1	0.2	0.013	0.1	0.013	0.1	0.8	0.4	0.2	0.025	0.05	50	0.4
P.v. Pv-45	3.1	25	>100	12.5	12.5	12.5	12.5	12.5	100	6.3	>100	6.3	12.5	50	6.3
M.m. 1510	100	25	12.5	12.5	6.3	12.5	0.8	1.6	3.1	6.3	12.5	3.1	1.6	25	1.6
*E.cl.*El-83	>100	>100	>100	25	6.3	6.3	0.8	3.1	6.3	3.1	50	1.6	3.1	>100	6.3
S.m. Sm-238	>100	>100	25	25	6.3	6.3	1.6	25	6.3	>100	25	6.3	3.1	>100	6.3
C.f. GN7391	>100	>100	>100	50	25	25	25	12.5	6.3	>100	100	25	12.5	>100	1.6
P.a. A9843A	0.4	0.2	0.4	0.2	0.4	0.2	0.1	0.4	0.4	0.8	0.4	0.1	0.1	3.1	3.1
P.a. A20599	0.8	0.4	0.8	0.4	0.4	0.4	0.2	0.8	0.4	1.6	0.8	0.2	0.2	3.1	3.1
P.a. Pa-246	3.1	1.6	0.4	0.1	0.05	0.2	0.2	0.1	0.8	0.4	0.2	0.1	0.1	25	25
X.m. Pl-19	6.3	3.1	1.6	1.6	0.4	0.4	50	3.1	3.1	1.6	0.8	0.2	0.8	50	>100
P.c. Pp-105	12.5	1.6	1.6	0.8	3.1	0.8	1.6	3.1	3.1	6.3	6.3	6.3	3.1	25	25

Medium: Mueller-Hinton agar (pH 7.2), incubation; 32°C, 18 hours, inoculum size; 10^6 cells/ml.

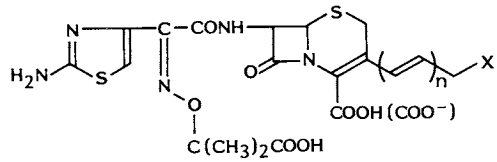
Abbreviations: *S.a.*, *Staphylococcus aureus*; *B.s.*, *Bacillus subtilis*; *E.c.*, *Escherichia coli*; *P.v.*, *Proteus vulgaris*; *M.m.*, *Morganella morganii*; *E.cl.*, *Enterobacter cloacae*; *S.m.*, *Serratia marcescens*; *C.f.*, *Citrobacter freundii*; *P.a.*, *Pseudomonas aeruginosa*; *X.m.*, *Xanthomonas maltophilia*; *P.c.*, *Pseudomonas cepacia*.

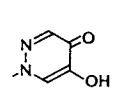
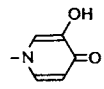
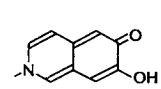
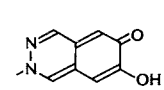
Table 2. *In vitro* activity of cephalosporins against CAZ-resistant *Pseudomonas aeruginosa* (16 strains).

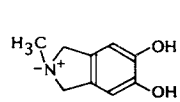
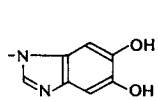
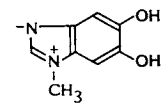
Compound	MIC (μg/ml)			Compound	MIC (μg/ml)		
	Range	MIC_{50}	MIC_{90}		Range	MIC_{50}	MIC_{90}
8	0.2 ~ 6.3	3.1	6.3	**6d**	0.2 ~ 3.1	0.8	1.6
6a	0.1 ~ 6.3	0.4	3.1	**3b**	0.1 ~ 6.3	0.4	1.6
9	≤0.05~ 1.6	0.2	0.8	**6e**	0.4 ~ 6.3	0.8	6.3
6b	0.05~ 3.1	0.2	0.8	**3d**	0.05~ 3.1	0.2	0.8
3a	0.1 ~ 1.6	0.8	1.6	**6f**	0.05~ 3.1	0.2	0.8
6c	0.1 ~ 3.1	0.2	0.8	CAZ	6.3 ~100	25	100
10	0.1 ~ 3.1	0.2	1.6	IPM	0.2 ~ 3.1	1.6	1.6

MIC_{50} and MIC_{90} denote MICs for 50% and 90% of strains tested, respectively.

Table 3. *In vivo* activity of cephalosporins.



Organism	X	PD_{50}* (mg/kg, single im, mice)						
								
	n	0	0	1	0	1	0	1
		3c	**8**	**6a**	**9**	**6b**	**3a**	**6c**
S. aureus Smith		>8.5	>8.5	3.4	1.8	0.98	7.4	1.0
E. coli Juhl		1.2	0.50	0.19	0.078	0.019	0.18	0.059
P. aeruginosa A9843A		>8.5	>8.0	>9.0	0.45	0.17	1.4	0.43

Organism	X	PD_{50}* (mg/kg, single im, mice)							
									
	n	0	1	0	1	0	1	CAZ	IPM
		10	**6d**	**3b**	**6e**	**3d**	**6f**		
S. aureus Smith		6.3	0.36	>10	1.1	8.3	2.9	4.5	0.38
E. coli Juhl		0.14	0.0081	0.068	0.14	0.029	0.05	0.27	0.033
P. aeruginosa A9843A		2.9	2.0	0.72	0.091	1.8	0.23	11	0.54

* The 50% protective dose.

showed better *in vivo* activity against *P. aeruginosa* A9843A than CAZ. Against *Staphylococcus aureus* Smith and *E. coli* Juhl, the Group B-cephalosporins showed better *in vivo* activity than the corresponding Group A-cephalosporins. Among the Group B-cephalosporins, the isoquinoline derivative, **6b** and the

Table 4. *In vivo* activity of **6b** in mice.

Organism		PD_{50} (mg/kg*., im, $n=5$)		
	Mice	**6b**	CAZ	IPM
Pseudomonas aeruginosa Pa-246	A	0.47	>100	1.3
Pseudomonas cepacia Pp-105	B	16	>100	38
Xanthomonas maltophilia Pl-19	B	22	>100	>100

*: Administered at 0 and 2 hours after bacterial challenge.
A: Determined in normal mice.
B: Determined in mice immunocompromized with cyclophosphamide.

Table 5. Blood levels and urinary recovery of **6b** at 20 mg/kg, im in mce.

Compound	Blood level* ($n=5$)			Urinary recovery (%, $n=3$)
	Cmax (μg/ml)	$T_{1/2}$ (hour)	AUC (μg·hours/ml)	0~24 hours
6b	13	0.80	17	97
CAZ	10	0.39	10	100

* Determined by bioassay.

benzimidazole derivative, **6e** showed the best activity against both *S. aureus* and *P. aeruginosa*. Their activity was much higher than CAZ and IPM against *P. aeruginosa*. Table 4 shows *in vivo* activity of **6b** against other CAZ-highly resistant strains. Against *P. aeruginosa* Pa-246 and *P. cepacia* Pp-105, **6b** was, similar to the results in *in vitro* experiments, over two times more active than IPM. Against *X. maltophilia* Pl-19, **6b** showed PD_{50} of 22 mg/kg, while neither IPM nor CAZ showed activity at 100 mg/kg.

Pharmacokinetics

Since **6b** demonstrated much higher *in vivo* activity than expected from its *in vitro* activity, blood levels and urinary recovery after intramuscular administration of 20 mg/kg in normal mice were determined in comparison with CAZ (Table 5). There was no significant difference in the urinary recoveries at 24 hours between **6b** and CAZ, being 97 and 100% doses, respectively but the former showed approximately twice higher AUC and longer half life than the latter.

In summary, 3-[(*E*)-3-heterocyclic-substituted 1-propen-1-yl]cephalosporins (Group B) showed higher therapeutic efficacies in bacterial infections than the corresponding 3-(heterocyclic catechol-substituted methyl)cephalosporins (Group A), although there was no significant difference between their *in vitro* activity. Among the compounds in Group B, the 5,6-dihydroxybenzimidazol derivative (**6e**) and the 2,6-dihydro-7-hydroxy-6-oxo-isoquinoline derivative (**6b**) showed much higher *in vitro* and *in vivo* activities than CAZ and IPM against *P. aeruginosa* A9843A. Additionally **6b** was more active than IPM against *P. aeruginosa* Pa-246, *P. cepacia* Pp-105 and *X. maltophilia* Pl-19 infections, where CAZ was inactive, and showed improved pharmacokinetic profile.

Experimental

MP's were determined using a Yanagimoto micro hot-stage apparatus and were uncorrected. UV spectra were recorded on a Shimadzu UV-200 spectrophotometer. NMR spectra were recorded on a JEOL

GX-400 (400 MHz). Mass spectra were recorded on a JEOL JMS-AX505H (FAB) mass spectrometer.

Syntheses of Cephalosporins

[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(heterocyclic Catechol-substituted Methyl)-3-cephem-4-carboxylic Acids

Cephalosporin **3a**

A mixture of **1** (538 mg), 6,7-dihydroxyphthalazine hydrobromide (158 mg, prepared by heating 6,7-dimethoxyphthalazine in hydrobromic acid) and triethylamine (0.1 ml, 0.7 mmol) in DMF (1.5 ml) was stirred for 3 hours at room temperature. The mixture was diluted with EtOAc, washed with aqueous sodium thiosulfate and water and concentrated *in vacuo*. The residue was chromatographed on a column of silica gel and the column was eluted with chloroform containing 5% of methanol. The fractions containing the product were combined and concentrated to give 532 mg of the 3-phthalazinylmethyl cephem ester 1-oxide (FAB-MS *m/z* 1,110 $(M+H)^+$). To a solution of the above compound (510 mg) in DMF (1 ml) was added phosphorus tribromide (0.051 ml) at $-10°C$. The mixture was stirred for 2 hours at the same temperature, diluted with ethyl acetate, washed with water and concentrated *in vacuo*. Chromatography of the residue on a column of silica gel and elution with chloroform containing 1% of methanol afforded 149 mg of the 3-phthalazinylmethyl cephem ester (FAB-MS *m/z* 1,094 $(M+H)^+$). The cephem ester (149 mg) was treated with trifluoroacetic acid (TFA, 1 ml) at room temperature for one hour and diluted with isopropyl ether to precipitate the crude product, which was chromatographed on a column of reversed phase silica gel (Prep C_{18}, 100 g). The column was eluted with water containing 10% of acetonitrile. The fractions were monitored by HPLC and desired fractions were combined. Concentration *in vacuo* and freeze-drying of the aqueous residue afforded 28 mg (overall yield 10%) of the product. MP 150°C (gradual dec); UV λ_{max} (pH 7 buffer) nm (ε) 250 (sh, 29,000), 270 (36,600); ^{1}H NMR ($D_2O+NaHCO_3$) δ 1.47 (3H, s, CH_3), 1.48 (3H, s, CH_3), 3.44 (1H, d, $J=18$ Hz, 2-H), 3.60 (1H, d, $J=18$ Hz, 2-H), 5.25 (1H, d, $J=5$ Hz, 6-H), 5.35 (1H, d, $J=15$ Hz, 3-CH_2), 5.43 (1H, d, $J=15$ Hz, 3-CH_2), 5.86 (1H, d, $J=5$ Hz, 7-H), 6.98 (1H, s, thiazole-H), 7.08, 7.38, 8.95 and 9.25 (1H each s, phthalazine-H); FAB-MS *m/z* 630 $(M+H)^+$.

Cephalosporin **3b**

This cephalosporin was prepared by coupling of **1** and 5,6-dihydroxybenzimidazole, followed by deblocking under conditions similar to the synthesis of **3a**. Overall yield 10%; MP 200°C (gradual dec); UV λ_{max} (pH 7 buffer) nm (ε) 256 (18,300), 262 (17,700), 295 (13,200); ^{1}H NMR ($D_2O+NaHCO_3$) δ 1.47 (3H, s, CH_3), 1.49 (3H, s, CH_3), 3.17 (1H, d, $J=18$ Hz, 2-H), 3.26 (1H, d, $J=18$ Hz, 2-H), 5.10 (1H, d, $J=15$ Hz, 3-CH_2), 5.17 (1H, d, $J=5$ Hz, 6-H), 5.28 (1H, d, $J=15$ Hz, 3-CH_2), 5.82 (1H, d, $J=5$ Hz, 7-H), 6.98 (1H, s, thiazole-H), 7.17, 7.22 and 8.35 (1H each s, benzimidazole-H); FAB-MS *m/z* 618 $(M+H)^+$.

Cephalosporin **3c**

N,O-Bis(trimethylsilyl)acetamide (BSA, 0.5 ml) was added to a suspension of 4,5-dihydroxypyridazine hydrobromide (86 mg, 0.45 mmol) in dichloromethane (2 ml). To the solution was added **2** (315 mg) and the mixture was stirred for one hour at room temperature. After evaporation, the residue was chromatographed on a column of silica gel and elution with chloroform containing 2~5% of MeOH gave the pyridazine derivative (401 mg), which was treated with TFA (3 ml) and anisole (3 ml) for one hour at room temperature. The mixture was concentrated under reduced pressure and the residue was triturated with isopropyl ether to afford crude product, which was purified by a similar procedure to the purification of **3a** to give 55 mg (overall yield 30%) of the product. MP >150°C (dec); UV λ_{max} (pH 7 buffer) nm (ε) 267 (19,100); ^{1}H NMR ($D_2O+NaHCO_3$) δ 1.50 (3H, s, CH_3), 1.51 (3H, s, CH_3), 3.35 (1H, d, $J=18$ Hz, 2-H), 3.56 (1H, d, $J=18$ Hz, 2-H), 5.11 (1H, d, $J=14$ Hz, 3-CH_2), 5.15 (1H, d, $J=14$ Hz, 3-CH_2), 5.86 (1H, d, $J=5$ Hz, 6-H), 7.02 (1H, s, thiazole-H), 8.07, 8.42 (1H each s, pyridazine-H); FAB-MS *m/z* 580 $(M+H)^+$.

Cephalosporin **3d**

The intermediate **4** (620 mg) for the synthesis of **3b** was dissolved in methyl iodide (8 ml). The mixture was stirred for 8 hours at room temperature and concentrated *in vacuo*. To the residue was added TFA

(3 ml) and anisole (0.3 ml) and the mixture was stirred for 1 hour at room temperature. After concentration under reduced pressure, the residue was triturated with isopropyl ether to give crude product, which was purified by a similar procedure to the purification of **3a** to give 40 mg (overall yield 10%) of the product. MP 180°C (gradual dec); UV λ_{max} (pH 7 buffer) nm (ε) 255 (sh, 17,300), 297 (14,200); ^{1}H NMR ($D_2O+NaHCO_3$) δ 1.46 (3H, s, CH_3), 1.47 (3H, s, CH_3), 3.20 (1H, d, J=18 Hz, 2-H), 3.48 (1H, d, J=18 Hz, 2-H), 3.95 (3H, s, benzimidazole-CH_3), 5.18 (1H, d, J=15 Hz, 3-CH_2), 5.22 (1H, d, J=5 Hz, 6-H), 5.26 (1H, d, J=15 Hz, 3-CH_2), 5.83 (1H, d, J=5 Hz, 7-H), 6.95 (1H, s, thiazole-H), 7.11, 7.18 and 8.92 (1H, each s, benzimidazole-H); FAB-MS m/z 632 $(M+H)^+$.

7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido)acetamido]-3-[(*E*)-3-heterocyclic Catechol-substituted-1-propen-1-yl]-3-cephem-4-carboxylic Acid

Cephalosporin **6a**

To a suspension of 3,4-dihydroxypyridine (70 mg) in DMF (2 ml) was added BSA (0.38 ml) and the mixture was cooled in an ice-water bath. The iodide **5** (606 mg) was added. After the mixture was stirred for 30 minutes under ice-cooling, it was poured dropwise to a stirred solution of 5% sodium thiosulfate (100 ml) and the precipitate (597 mg) was collected by filtration. To an ice-cooled mixture of the precipitate and anisole (0.5 ml) in dichloromethane (1.5 ml) was added TFA (3 ml). The mixture was stirred for 1 hour at room temperature and diluted with isopropyl ether (50 ml). The precipitate was collected by filtration and dissolved in water by addition of $NaHCO_3$ (150 mg). The mixture was chromatographed on a column of reversed phase silica gel (Waters, Prep C_{18}, 70 ml). The column was eluted with water and the eluate was monitored by HPLC at 254 nm. The desired fractions were combined and acidified to pH 3 with 2 N HCl. The mixture was chromatographed on a column of HP-20 (30 ml). The column was eluted with water and then 50% aqueous MeOH. The methanolic fraction was concentrated *in vacuo* and freeze-dried to give 62 mg (overall yield 17%) of the product as an amorphous powder. MP 162°C (gradual dec); UV λ_{max} (pH 7 buffer) nm (ε) 293 (34,500); ^{1}H NMR ($D_2O+NaHCO_3$) δ 1.45 (3H, s, CH_3), 1.51 (3H, s, CH_3), 3.65 (2H, ABq, 2-H), 5.27 (1H, d, J=5 Hz, 6-H), 5.84 (1H, d, J=5 Hz, 7-H), 6.04 (1H, dt, J=7 and 16 Hz, 3-CH=C*H*), 6.60 (1H, d, J=7 Hz, pyridine-H), 6.73 (1H, d, J=16 Hz, 3-C*H*=CH), 7.00 (1H, s, thiazole-H), 7.67 (1H, d, J=2 Hz, pyridine-H), 7.70 (1H, dd, J=2 and 7 Hz, pyridine-H); FAB-MS m/z 605 $(M+H)^+$.

Cephalosporin **6b**

To an ice-cooled solution of 6,7-dihydroxyisoquinoline (100 mg) in DMF (4 ml) was added **5** (600 mg). The mixture was stirred under cooling for 30 minutes and poured into a chilled solution of aqueous $Na_2S_2O_3$ (5%, 100 ml) to afford precipitate, which was collected by filtration, washed with water and dried. The precipitate was dissolved in dichloromethane (1 ml) containing anisole (0.5 ml). TFA (5 ml) was added under ice-cooling. The mixture was stirred for 1 hour, concentrated *in vacuo* and diluted with isopropyl ether to afford the crude product, which was collected by filtration and purified by a similar procedure to that described in the preparation of **6a**. Overall yield 20%; MP 220°C (gradual dec); UV λ_{max} (pH 7 buffer) nm (ε) 255 (33,100), 285 (30,100), 357 (16,200); ^{1}H NMR ($D_2O+NaHCO_3$) δ 1.48 (3H, s, CH_3), 1.50 (3H, s, CH_3), 3.65 (2H, ABq, 2-H), 5.07 (2H, br d, 3-CH=CHCH_2), 5.26 (1H, d, J=5 Hz, 6-H), 5.84 (1H, d, J=5 Hz, 7-H), 6.11 (1H, m, 3-CH=C*H*), 6.85 (1H, d, J=16 Hz, 3-C*H*=CH), 6.92 (1H, s, isoquinoline-H), 7.00 (1H, s, thiazole-H), 7.29 (1H, s, isoquinoline-H), 7.62 (1H, d, J=7 Hz, isoquinoline-H), 7.82 (1H, d, J=7 Hz, isoquinoline-H), 8.69 (1H, s, isoquinoline-H); FAB-MS m/z 655 $(M+H)^+$.

Cephalosporin **6c**

This compound was prepared by coupling of **5** with 6,7-dihydroxyphthalazine by a procedure similar to the preparation of **6b**. Overall yield 31%; MP 170°C (gradual dec); UV λ_{max} (pH 7 buffer) nm (ε) 250 (29,700, sh), 267 (38,100), 287 (37,300); ^{1}H NMR ($D_2O+NaHCO_3$) δ 1.49 (3H, s, CH_3), 1.51 (3H, s, CH_3), 3.64 (1H, d, J=17 Hz, 2-H), 3.70 (1H, d, J=17 Hz, 2-H), 5.22 (2H, br s, 3-CH=CHCH_2), 5.27 (1H, d, J=5 Hz, 6-H), 5.83 (1H, d, J=5 Hz, 7-H), 6.1~6.2 (1H, m, 3-CH=C*H*), 6.88 (d, J=16 Hz, 3-C*H*=CH), 6.99 (1H, s, thiazole-H), 7.05, 7.27, 8.91 and 9.12 (1H, each s, phthalazine-H); FAB-MS m/z 656 $(M+H)^+$.

Cephalosporin **6d**

A solution of 5,6-diacetoxy-2-methyl-2-isoindoline (60 mg) in ether (5 ml) was added to a cooled solution of **5** (137 mg) in ether (5 ml) and the mixture was stirred for 30 minutes at room temperature. The precipitated quaternized product was collected by filtration (44.6 mg), which was treated with TFA (1 ml) at room temperature for one hour. Concentration of the mixture *in vacuo* and trituration of the residue with isopropyl ether, followed by chromatography on a reversed phase silica gel afforded the diacetoxy-**6d**. To a solution of the diacetoxy-**6d** in pH 7.0 phosphate buffer (20 ml) was added acetylesterase (Sigma, 1 ml) and the mixture was adjusted to pH 7.1 by addition of $NaHCO_3$. The mixture was stirred for 2 hours at room temperature, then acidified and chromatographed on a column of HP-20 (60 ml). The column was eluted with water and then 40% MeOH. The methanolic fraction was concentrated under reduced pressure and freeze-dried to give 13.1 mg of the product. Overall yield 5%; MP 168°C (gradual dec); UV λ_{max} (pH 7 buffer) nm (ε) 293 (25,800); ^{1}H NMR (CD_3OD) δ 1.52 (3H, s, CH_3), 1.54 (3H, s, CH_3), 3.24 (3H, s, NCH_3), 3.43 (2H, ABq, 2-H), 4.18 (2H, m, 3-CH=CHCH_2), 4.61~4.8 (4H, m, isoindoline-CH_2), 5.18 (1H, d, J=5 Hz, 6-H), 5.78 (1H, m, 3-CH=CH), 5.82 (1H, d, J=5 Hz, 7-H), 6.82, 6.84 (1H, each s, isoindoline-H), 6.94 (1H, s, thiazole-H), 6.95 (1H, d, J=16 Hz, 3-CH=CH): FAB-MS m/z 659 $(M+H)^+$.

Cephalosporin **6e**

This compound was preparation by coupling of **5** with 5,6-dihydroxybenzimidazole by a procedure similar to the preparation of **6b**. Overall yield 14%; MP 185°C (gradual dec); UV λ_{max} (pH 7 buffer) nm (ε) 294 (33,200); ^{1}H NMR ($D_2O+NaHCO_3$) δ 1.49 (3H, s, CH_3), 1.50 (3H, s, CH_3), 3.58 (1H, d, J=18 Hz, 2-H), 3.64 (1H, d, J=18 Hz, 2-H), 4.92 (2H, br d, J=8.0 Hz, 3-CH=CHCH_2), 5.23 (1H, d, J=5 Hz, 6-H), 5.82 (1H, d, J=5 Hz, 7-H), 5.97~6.07 (1H, m, 3-CH=CH), 6.71 (1H, d, J=16 Hz, 3-CH=CH), 6.99 (1H, s, thiazole-H), 7.04, 7.19, 8.03 (1H each s, benzimidazole-H); FAB-MS m/z 644 $(M+H)^+$.

Cephalosporin **6f**

This compound was synthesized from **7**, the intermediate for synthesis of **6e**, and methyl iodide by a procedure similar to the preparation of **3d**. Overall yield 10%; MP 180°C (gradual dec); UV λ_{max} (pH 7 buffer) nm (ε) 296 (33,000); ^{1}H NMR ($D_2O+NaHCO_3$) δ 1.49 (3H, s, CH_3), 1.51 (3H, s, CH_3), 3.62 (1H, d, J=18 Hz, 2-H), 3.67 (1H, d, J=18 Hz, 2-H), 3.94 (3H, s, benzimidazole-CH_3), 5.05 (2H, m, 3-CH=CHCH_2), 5.26 (1H, d, J=5 Hz, 6-H), 5.83 (1H, d, J=5 Hz, 7-H), 6.06 (1H, m, 3-CH=CH), 6.81 (1H, d, J=16 Hz, 3-CH=CH), 6.99 (1H, s, thiazole-H), 7.08 (2H, s, benzimidazole-H), 8.83 (1H, s, benzimidazole-H); FAB-MS m/z 658 $(M+H)^+$.

Biological Evaluation

MICs were determined by the 2-fold serial agar dilution method using Mueller-Hinton agar (pH 7.2) after incubation at 37°C for 18 hours with an inoculum size of 10^6 cfu/ml. *In vivo* antibacterial activity was determined in experimental systemic infections in male ddY mice weighing 22~25 g. Mice were challenged ip with 100 times the median lethal dose of the pathogen in 5% suspension of hog gastric mucin. Test compounds were administered im once immediately or twice immediately and 2 hours after the bacterial challenge to groups of 5 mice at each dose level. In the *P. cepacia* Pp-105 and *X. maltophilia* Pl-19 infections, mice pretreated ip with 200 mg/kg of cyclophosphamide 4 days prior to the bacterial challenge were used. The 50% protective dose (PD_{50}) was calculated by the method of VAN DER WAERDEN[13], from survival rate recorded on 4 days after the bacterial challenge. For the determination of blood and urine levels, 20 mg/kg of test compound was administered im to a group of 5 and 3 male ddY mice, respectively. Blood samples were collected from the orbital sinuses and urine samples were by use of metabolic cages for mice. Both samples were assayed by the paper disc method using *E. coli* NIHJ as a test organism. The area under the drug concentration curve (AUC, μg·hours/ml) and half life ($T_{1/2}$, hours) were calculated by a nonlinear least squares program[14].

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