CEPHALOSPORINS HAVING A HETEROCYCLIC CATECHOL IN THE C3 SIDE CHAIN

II. IMPROVEMENT OF PHARMACOKINETIC PROFILE

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7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxy-(or hydroxy)-iminoacetamido]-3-[propen-1-yl]-cephalosporins having a variety of heterocyclic catechol in 3-position of the propenyl group were synthesized. Among them, 6,7-dihydroxyisoquinoline derivatives, **2a** and **2b**, showed very high and prolonged blood levels after intramuscular administration to mice and higher *in vivo* antibacterial activity than expected from their *in vitro* activity. The former cephalosporin (**2a**) gave well-balanced *in vitro* and *in vivo* antibacterial spectra including anti-methicillin-resistant *Staphylococcus* aureus (MRSA) activity. The latter cephalosporin (**2b**) also showed good *in vitro* and *in vivo* activities against Gram-positive bacteria, especially against *S. aureus* A15036, a strain of MRSA, the *in vivo* activity being comparable to vancomycin but was lacking in anti-pseudomonal activity.

During the course of exploring new cephalosporins having a heterocyclic catechol in the C3 side chain[1], we have found that certain derivatives showed high blood levels after intramuscular administration in mice. Since then, many derivatives have been synthesized and it was found that 6,7-dihydroxyisoquinoline derivatives of cephalosporins, **2a** and **2b**, showed extremely high blood levels. This report describes the synthesis and the relationship between the structures and blood levels of the cephalosporins, together with their *in vitro* and *in vivo* activities.

Synthesis

Scheme 1 shows the synthesis of 3-[(*E*)-3-heterocyclic catechol-substituted 1-propen-1-yl]cephalosporins. The 3-[(*E*)-iodo-1-propen-1-yl]cephalosporins **1a**[2] was coupled with 6,7-dihydroxyisoquinoline[3], 6,7-dihydroxyphthalazine[4], 5,6-dihydroxybenzimidazole[5] or 6-hydroxyisoquinoline[6] in DMF, followed by deblocking with trifluoroacetic acid to afford **2a**, **2e**, **2g** and **2i**, respectively. In a similar way, the iodide **1b**, which was synthesized by a similar method to **1a**, was coupled with the above heterocycles to afford **2b**, **2f**, **2h** and **2j**. Cephalosporins, **2c** and **2d** were prepared by coupling of the iodides, **1a** and **1b**, with 3,4-dihydroxypyridine[7] in the presence of *N,O*-bis(trimethylsilyl)acetamide in DMF, followed by deblocking.

In Vitro Antibacterial Activity

Table 1 summarizes *in vitro* activity of 11 cephalosporins synthesized in this study. Generally, the hydroxyimino derivatives, **2b**, **2d**, **2f**, **2h** and **2j** were more active against Gram-positive bacteria *Staphylococcus aureus* FDA 209P, *S. aureus* Smith, *S. aureus* A15036 (methicillin-resistant *S. aureus*;

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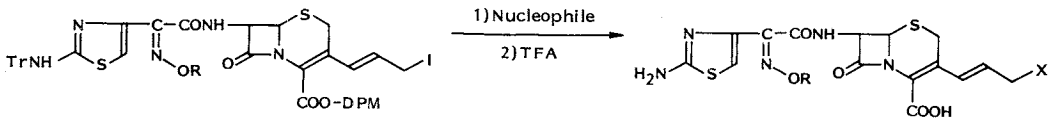
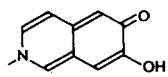
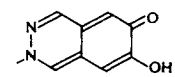
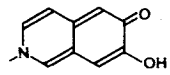
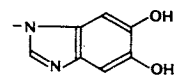
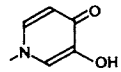
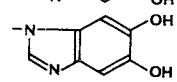
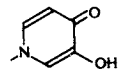
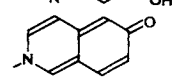
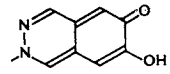
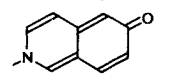
Scheme 1. Synthesis of cephalosporins.



1a R=CH_3
1b R=Tr

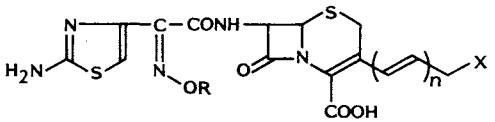
	R	X		R	X
2a	CH_3		**2f**	H	
2b	H		**2g**	CH_3	
2c	CH_3		**2h**	H	
2d	H		**2i**	CH_3	
2e	CH_3		**2j**	H	

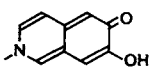
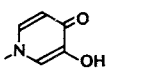
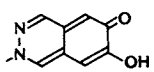
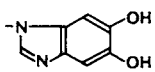
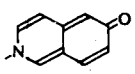
MRSA), *S. aureus* Sa-247 (MRSA), *Staphylococcus epidermidis* Si-22 and *Enterococcus faecalis* A9808 with the exception of the non-catecholic derivative **2i** against *S. epidermidis* Si-22 but were weaker against Gram-negative bacteria in comparison with their corresponding methoxyimino derivatives. Among the hydroxyimino derivatives, the 6,7-dihydroxyisoquinoline cephalosporin **2b** showed the best anti-Gram-positive activity especially against MRSAs though its activity against *Pseudomonas aeruginosa* was rather low. In the group of methoxyimino derivatives, **2a**, **2c**, **2e**, **2g**, **2i** and **3**[8], the non-catecholic cephalosporin (**2i**) was the most active against *S. aureus* FDA 209P, *S. aureus* Smith, *Micrococcus luteus* PCI 1001 and *Bacillus subtilis* PCI 219 while the catecholic derivatives, **2a** and **2e** which have 6,7-dihydroxy-isoquinoline and 6,7-dihydroxyphthalazine, respectively, in the C3 side chain, indicated fairly good *in vitro* antibacterial spectra.

In Vivo Antibacterial Activity and Blood Level in Mice

Among the above cephalosporins, three pairs of the hydroxyimino and methoxyimino derivatives (**2a** vs. **2b**, **2e** vs. **2f** and **2i** vs. **2j**) were evaluated for *in vivo* antibacterial activities against *S. aureus* Smith, *Escherichia coli* Juhl and *P. aeruginosa* A9843A infections in mice in comparison with ceftazidime (CAZ). As summarized in Table 2, structure-activity relationships between the hydroxyimino and methoxyimino derivatives were not demonstrated in their *in vitro* and *in vivo* activities especially against *E. coli* Juhl in terms of MIC/PD_{50} ratios. On the other hand, when compared with CAZ, **2a**, **2b**, **2e** and **2f** which have the heterocyclic catechols, 6,7-dihydroxyisoquinoline or 6,7-dihydroxyphthalazine, in the C3 side chain showed much higher *in vivo* efficacies against *S. aureus and E. coli* infections than expected from their *in vitro* activities with the exception of **2b** against *E. coli*, while non-catecholic derivatives **2i** and **2j** have lower MIC/PD_{50} ratios. Against *P. aeruginosa*, **2a** was twice less active *in vitro*, but on the contrary, it demonstrated twice more potent than CAZ with PD_{50} value of 1.6 mg/kg.

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



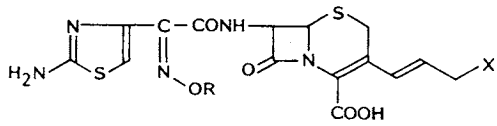
Organism	X												Ceftazidime
	R	CH_3	H	CH_3	CH_3	H	CH_3	H	CH_3	H	CH_3	H	
	n	1	1	0	1	1	1	1	1	1	1	1	
		2a	**2b**	**3**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**	**2i**	**2j**	
S.a. FDA 209P		1.6	0.4	3.1	3.1	0.8	3.1	1.6	6.3	3.1	0.8	0.4	12.5
S.a. Smith		1.6	0.4	3.1	1.6	0.8	1.6	0.8	6.3	1.6	0.4	0.4	12.5
S.a. A15036 (MRSA)		6.3	3.1	100	12.5	6.3	25	6.3	50	12.5	50	6.3	100
S.a. Sa-247 (MRSA)		>100	25	>100	>100	>100	>100	100	>100	100	>100	>100	>100
S.e. Si-22		6.3	3.1	12.5	6.3	6.3	12.5	6.3	50	12.5	6.3	12.5	50
E.fl. A9808		25	12.5	50	100	12.5	50	6.3	100	12.5	12.5	3.1	>100
E.fl. A24817		>100	100	>100	>100	>100	>100	100	>100	>100	>100	>100	>100
M.l. PCI 1001		0.2	0.2	0.1	0.2	0.8	0.2	0.4	0.8	0.8	0.05	0.1	1.6
B.s. PCI 219		0.8	0.8	0.8	0.4	0.8	0.4	0.8	3.1	1.6	0.2	0.4	12.5
E.c. Juhl		0.1	0.1	0.05	0.05	0.2	0.1	0.4	0.2	0.4	0.05	0.2	0.4
E.c. 255		0.2	0.4	0.2	0.2	3.1	0.4	1.6	0.8	0.8	1.6	1.6	50
K.p. PC 1602		<0.0063	0.025	<0.0063	<0.0063	0.1	<0.0063	0.1	0.05	0.1	0.013	0.05	0.2
P.m. IFO 3849		0.1	0.1	0.025	0.05	0.4	0.1	0.8	0.1	0.8	0.1	0.2	0.2
M.m. 1510		3.1	6.3	>100	6.3	25	6.3	25	25	25	6.3	12.5	25
P.r. Pr-63		0.1	3.1	0.2	0.1	1.6	0.1	3.1	0.8	3.1	0.1	0.2	0.1
S.m. Sm-238		50	>100	100	50	>100	50	>100	>100	>100	50	>100	>100
P.a. A9843A		12.5	>100	25	>100	>100	25	>100	6.3	6.3	100	>100	3.1
P.a. Pa-246		25	>100	50	>100	>100	25	>100	25	3.1	>100	>100	25

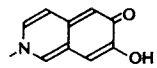
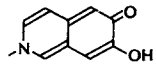
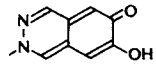
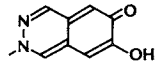
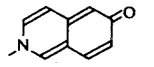
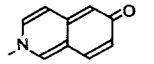
MIC (μg/ml)

Medium: Mueller-Hinton agar (pH 7.2), incubation; 37°C, 18 hours, inoculum size; 10^6 cells/ml.

Abbreviations: *S.a.*, *Staphylococcus aureus*; *S.e.*, *S. epidermidis*, *E. fl.*, *Enterococcus faecalis*; *M.l.*, *Micrococcus luteus*; *B.s.*, *Bacillus subtilis*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *P.m.*, *Proteus mirabilis*; *M.m.*, *Morganella morganii*; *P.r.*, *Providencia rettgeri*; *S.m.*, *Serratia marcescens*; *P.a.*, *Pseudomonas aeruginosa*.

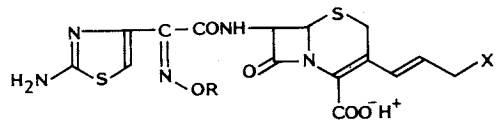
Table 2. *In vivo* antibacterial activity in mice.

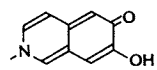
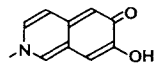
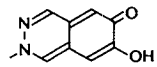
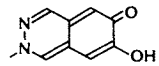
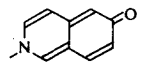
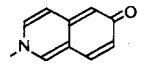


Compound	R	X	PD_{50} (mg/kg, im, bid[a])		
			S.a. Smith	*E.c.* Juhl	*P.a.* 9843A
2a	CH_3		0.05 (32)[b]	0.011 (9.1)	1.6 (7.8)
2b	H		0.03 (13)	0.036 (2.8)	>12.5
2e	CH_3		0.09 (18)	0.017 (5.9)	>12.5
2f	H		0.03 (27)	0.0076 (53)	>12.5
2i	CH_3		0.14 (2.9)	0.039 (1.3)	>12.5
2j	H		0.13 (3.1)	0.1 (2.0)	NT
Ceftazidime			1.8 (6.9)	0.13 (3.1)	3.1 (1.0)

Abbreviations: *S.a.*, *Staphylococcus aureus*; *E.c.*, *Escherichia coli*; *P.a.*, *Pseudomonas aeruginosa*.
[a] Administered at 0 and 2 hours postinfection.
[b] Figures in parentheses denote MIC/PD_{50} ratio.

Table 3. Blood levels after im administration in mice.



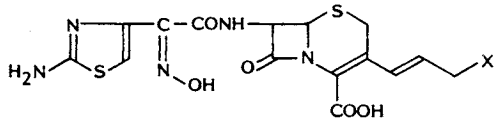
Compound	R	X	Blood levels (20 mg/kg, im)		
			Cmax (μg/ml)	$T_{1/2}$ (hours)	AUC (μg·hours/ml)
2a	CH_3		45	1.5	129
2b	H		55	1.6	161
2e	CH_3		26	0.5	22
2f	H		13	1.8	36
2i	CH_3		8	0.4	5
2j	H		7	0.3	4
Ceftriaxone			39	0.5	36
Ceftazidime			10	0.4	10

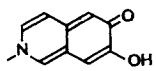
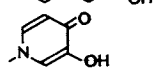
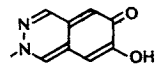
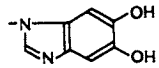
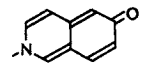
Since the catecholic derivatives demonstrated higher MIC/PD_{50} ratios than the non-catecholic derivatives and CAZ, their blood levels were comparatively determined after intramuscular administration of 20 mg/kg to mice. As shown in Table 3, AUC values of **2a**, **2b**, **2e** and **2f** were higher than those of **2i**, **2j** and CAZ. Especially the isoquinoline derivatives **2a** and **2b** showed unexpected high blood levels, which were much higher than those of the long-acting cephalosporin, ceftriaxone and gave approximately 100% urinary recovery of the dosed volume at 24 hours after administration (the data not shown). The results suggest that the excellent *in vivo* activity of the above catecholic derivatives may be, at least partly, due to their high AUC values. The methoxyimino derivative **3**, which has 6,7-dihydroxyisoquinoline similarly to **2a** without the propenyl group in C3 position, gave much lower AUC value than **2a** in different experiment. Therefore, in order to clarify the structure-activity relationships in more detail, structure requirements, possibility of active or inactive metabolite(s) and other biological properties, which are attributable to *in vivo* antibacterial efficacy and/or pharmacokinetic profile, of the above will be further examined.

In Vitro and *In Vivo* Anti-MRSA Activities

Since the hydroxyimino derivatives were active against MRSAs, *S. aureus* A15036 and/or *S. aureus* Sa-247 as described above, they were further evaluated for anti-MRSA activity *in vitro* against 29 strains of MRSA and *in vivo* against *S. aureus* A15036 infection in immunocompromized mice treated with cyclophosphamide. Table 4 indicates that among **2b**, **2d**, **2f**, **2h** and **2j**, *in vitro* anti-MRSA activity of **2b**

Table 4. *In vitro* and *in vivo* anti-MRSA activities.



Compound	X	*In vitro* anti-MRSA (29 strains) activity			*In vivo* anti-MRSA[a] activity
		Range	MIC_{50}[b] (μg/ml)	MIC_{90}[b] (μg/ml)	PD_{50} (mg/kg, im, bid)[c]
2b		1.6 ~ 100	12.5	50	0.58
2d		3.1 ~ >100	50	>100	1.2
2f		3.1 ~ 100	12.5	50	1.5
2h		6.3 ~ >100	50	100	2.5
2j		1.6 ~ >100	50	>100	0.90
Flomoxef		1.6 ~ >100	50	100	4.1
Imipenem		<0.05 ~ 100	12.5	50	2.9
Vancomycin		0.8 ~ 1.6	0.8	1.6	0.41

[a] *Staphylococcus aureus* A15036.
[b] Medium, Mueller-Hinton agar, inoculum size, 10^6 cells/ml; incubation, 32°C, 18 hours.
[c] Administered at 0 and 2 hours postinfection.

and **2f** was twice more active than that of flomoxef and the same as that of imipenem (IPM) but much less than that of vancomycin in terms of MIC_{50} and MIC_{90}. On the other hand, **2b** demonstrated 4 and 7 times more potent *in vivo* activity than IPM and flomoxef, respectively and similar to vancomycin in terms of PD_{50} values. Although the datum is not shown, the methoxyimino derivatives **2a** was also active against *S. aureus* A15036 infection comparable to IPM with PD_{50} value of 2.7 mg/kg.

In summary, among the cephalosporins synthesized in this study, the methoxyimino derivative **2a**, which has 6,7-dihydroxyisoquinoline in the C3 side chain, showed very high blood levels and fairly good and well-balanced *in vitro* and *in vivo* antibacterial spectra including anti-MRSA activity.

Experimental

MPs were determined using a Yanagimoto micro hot-stage apparatus and were not corrected. 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

Cephalosporin **2a**

To an ice-cooled solution of 6,7-dihydroxyisoquinoline hydrobromide (290 mg) and triethylamine (110 mg) in DMF (6 ml) was added the iodide **1a** (1.05 g). The mixture was stirred under cooling for one hour and poured into a stirred aqueous solution of sodium thiosulfate (5%, 100 ml) to afford the precipitate, which was collected by filtration, washed with water, dried and dissolved in dichloromethane (2 ml) containing anisole (1 ml). The mixture was treated with trifluoroacetic acid (8 ml) under ice-cooling and stirred for one hour at room temperature. After concentration *in vacuo*, the residue was triturated with isopropyl ether to afford the crude product, which was dissolved in water (2 ml) containing sodium bicarbonate (100 mg) and chromatographed on a column of reverse phase silica gel (Waters, Prep C_{18} 125 Å, 90 ml). The column was eluted with water and then 20% aqueous methanol. The fractionated eluates were monitored by HPLC. The fractions containing the desired product were combined, acidified with 2 N-HCl and passed through a column of Diaion HP-20 (40 ml). The column was eluted with water and then 50% aqueous methanol. Concentration of the methanolic fraction afforded 67 mg of **2a**.

Cephalosporins **2b**, **2e**, **2f**, **2g**, **2h**, **2i** and **2j** were prepared by coupling of **2a** or **2b** with an appropriate heterocycle by a similar procedure to the preparation of **2a**. The overall yields, MPs and spectral data are summarized below.

2a: Overall yield, 9%; MP>187°C (dec.); UV λ_{max} (pH 7 buffer) nm (ε) 245 (35,800), 255 (36,100), 285 (33,200), 356 (18,100); ^{1}H NMR ($D_2O+NaHCO_3$) δ 3.65 (2H, br s, 2-H), 3.99 (3H, s, OCH_3), 5.06 (2H, br s, 3-CH=CHCH_2), 5.26 (1H, d, J=5 Hz, 6-H), 5.82 (1H, d, J=5 Hz, 7-H), 6.07~6.15 (1H, m, 3-CH=CH), 6.86 (1H, d, J=16 Hz, 3-CH=CH), 6.90 (1H, s, isoquinoline-H), 7.01 (1H, s, thiazole-H), 7.29 (1H, s, isoquinoline-H), 7.60 (1H, d, J=7 Hz, isoquinoline-H), 7.81 (1H, d, J=7 Hz, isoquinoline-H), 8.87 (1H, s, isoquinoline-H); FAB-MS m/z 583. $(M+H)^+$.

2b: Overall yield 8%; MP>175°C (dec.); UV λ_{max} (pH 7 buffer) nm (ε) 254 (33,500), 282 (30,700), 357 (17,500); ^{1}H NMR ($D_2O+NaHCO_3$) δ 3.65 (2H, ABq, 2-H), 5.02 (2H, br d, J=7 Hz, 3-CH=CHCH_2), 5.27 (1H, d, J=5 Hz, 6-H), 5.85 (1H, d, J=5 Hz, 7-H), 6.10 (1H, dt, J=16 and 7 Hz, 3-CH=CH), 6.86 (1H, d, J=16 Hz, 3-CH=CH), 6.91 (1H, s, isoquinoline-H), 6.98 (1H, s, thiazole-H), 7.29 (1H, s, isoquinoline-H), 7.60 (1H, d, J=7 Hz, isoquinoline-H), 7.81 (1H, d, J=7 Hz, isoquinoline-H), 8.68 (1H, br s, isoquinoline-H); FAB-MS m/z 569 $(M+H)^+$.

2e: Overall yield 18%; MP>140°C (dec.); UV λ_{max} (pH 7 buffer) nm (ε) 250 (27,900, sh), 268 (35,600), 288 (35,400); ^{1}H NMR ($D_2O+NaHCO_3$) δ 3.67 (2H, br s, 2-H), 3.98 (3H, s, OCH_3), 5.22 (2H, br s, 3-CH=CHCH_2), 5.25 (1H, d, J=5 Hz, 6-H), 5.81 (1H, d, J=5 Hz, 7-H), 6.1~6.2 (1H, m, 3-CH=CH), 6.90 (1H, d, J=16 Hz, 3-CH=CH), 6.98 (1H, s, thiazole-H), 7.03, 7.25, 8.88 and 9.08 (1H each s, phthalazine-H); FAB-MS m/z 584 $(M+H)^+$.

2f: Overall yield 17%; MP>140°C (dec.), UV λ_{max} (pH 7 buffer) nm (ε) 267 (36,300), 285 (34,800);

^{1}H NMR (D_2O+$NaHCO_3$) δ 3.54 (2H, br s, 2-H), 5.19 (2H, br s, 3-CH=CHCH_2), 5.27 (1H, d, J=5 Hz, 6-H), 5.84 (1H, d, J=5 Hz, 7-H), 6.1~6.2 (1H, m, 3-CH=CH), 6.88 (1H, d, J=16 Hz, 3-CH=CH), 6.95 (1H, s, thiazole-H), 7.14, 7.24, 8.88 and 9.08 (1H each s, phthalazine-H); FAB-MS m/z 570 $(M+H)^+$.

2g: Overall yield 15%; MP>190°C (dec.); UV λ_{max} (pH 7 buffer) nm (ε) 294 (31,300); ^{1}H NMR (DMSO-d_6) δ 3.82 (3H, s, OCH_3), 4.85 (2H, m, 3-CH=CHCH_2), 5.13 (1H, d, J=5 Hz, 6-H), 5.72 (1H, dd, J=5 and 9 Hz, 7-H), 6.11 (1H, m, 3-CH=CH), 6.73 (1H, s, aromatic proton), 6.86 (1H, d, J=16 Hz, 3-CH=CH), 6.94 (1H, s, aromatic proton), 7.21 (1H, s, aromatic proton), 7.88 (1H, s, aromatic proton), 8.70 (1H, s, OH), 8.85 (1H, s, OH), 9.61 (1H, d, J=9 Hz, 7-NH); FAB-MS 572 $(M+H)^+$.

2h: Overall yield 13%; MP>170°C (dec.); UV λ_{max} (pH 7 buffer) nm (ε) 264 (sh, 4,500), 294 (22,500); ^{1}H NMR (D_2O+$NaHCO_3$) δ 3.56 (2H, br s, 2-H), 4.86 (2H, br, 3-CH=CHCH_2), 5.22 (1H, d, J=5 Hz, 6-H), 5.82 (1H, d, J=5 Hz, 7-H), 5.9~6.0 (1H, m, 3-CH=CH), 6.68 (1H, d, J=16 Hz, 3-CH=CH), 6.96, 6.99, 7.16 and 7.98 (1H each s, aromatic protons); FAB-MS m/z 558 $(M+H)^+$.

2i: Overall yield 7%; MP>166°C (dec.); UV λ_{max} (pH 7 buffer) nm (ε) 231 (34,800), 270 (27,800), 290 (24,600), 320 (11,300), 358 (12,400); ^{1}H NMR (D_2O+$NaHCO_3$) δ 3.69 (2H, br s, 2-H), 4.02 (3H, s, OCH_3), 5.10 (2H, br d, J=7 Hz, 3-CH=CHCH_2), 5.29 (1H, d, J=5 Hz, 6-H), 5.85 (1H, d, J=5 Hz, 7-H), 6.1~6.2 (1H, m, 3-CH=CH), 6.90 (1H, d, J=16 Hz, 3-CH=CH), 6.90 (1H, d, J=2 Hz, isoquinoline-H), 7.04 (1H, s, thiazole-H), 7.21 (1H, dd, J=2 and 9 Hz, isoquinoline-H), 7.67 (1H, d, J=7 Hz, isoquinoline-H), 7.89 (1H, br d, J=7 Hz, isoquinoline-H), 7.99 (1H, d, J=9 Hz, isoquinoline-H), 8.89 (1H, br s, isoquinoline-H); FAB-MS m/z 567 $(M+H)^+$.

2j: Overall yield 5%; MP>176°C (dec.); UV λ_{max} (pH 7 buffer) nm (ε) 230 (29,100), 270 (25,300), 290 (20,400), 330 (9,400), 360 (11,600); ^{1}H NMR (D_2O+$NaHCO_3$) δ 3.66 (2H, br s, 2-H), 5.08 (2H, br d, J=7 Hz, 3-CH=CHCH_2), 5.28 (1H, d, J=5 Hz, 6-H), 5.86 (1H, d, J=5 Hz, 7-H), 6.07~6.15 (1H, dt, J=15 and 7 Hz, 3-CH=CH), 6.87 (1H, d, J=2 Hz, isoquinoline-H), 6.87 (1H, d, J=15 Hz, 3-CH=CH), 6.99 (1H, s, thiazole-H), 7.18 (1H, dd, J=2 and 9 Hz, isoquinoline-H), 7.64 (1H, d, J=7 Hz, isoquinoline-H), 7.87 (1H, dd, J=1.5 and 7 Hz, isoquinoline-H), 7.97 (1H, d, J=9 Hz, isoquinoline-H), 8.86 (1H, br s, isoquinoline-H); FAB-MS m/z 553 $(M+H)^+$.

Cephalosporin **2c**

To a suspension of 3,4-dihydroxypyridine (150 mg) in DMF (3 ml) was added *N*,*O*-bis(trimethylsilyl)-acetamide (0.75 ml) and the resulting solution was cooled in an ice-water bath. To the chilled solution was added **1a** (606 mg) and the mixture was stirred for 30 minutes under ice-cooling. The mixture was poured into a stirred solution of 3% aqueous sodium thiosulfate (100 ml). The product was collected by filtration, deblocked and purified by a procedure similar to the preparation of **2a**. Cephalosporin **2d** was prepared by the similar procedure from **1b**. The overall yields, MPs and spectra data are summarized below.

2c: Overall yield 25%; MP>182°C (dec.); UV λ_{max} (pH 7 buffer) nm (ε) 293 (37,400); ^{1}H NMR (D_2O+$NaHCO_3$) δ 3.65 (2H, ABq, 2-H), 4.00 (3H, s, OCH_3), 5.25 (1H, d, J=5 Hz, 6-H), 5.82 (1H, d, J=5 Hz, 7-H), 6.03 (1H, dt, J=7 and 16 Hz, 3-CH=CH), 6.65 (1H, d, J=7 Hz, pyridine-H), 6.72 (1H, d, J=16 Hz, 3-CH=CH), 7.03 (1H, s, thiazole-H), 7.70 (1H, d, J=2 Hz, pyridine-H), 7.73 (1H, dd, J=2 and 7 Hz, pyridine-H); FAB-MS m/z 533 $(M+H)^+$.

2d: Overall yield 11%; MP>163°C (dec.); UV λ_{max} (pH 7 buffer) nm (ε) 293 (33,500); ^{1}H NMR (D_2O+$NaHCO_3$) δ 3.65 (2H, ABq, 2-H), 4.74 (2H, d, J=7 Hz, 3-CH=CHCH_2), 5.28 (1H, d, J=5 Hz, 6-H), 5.86 (1H, d, J=5 Hz, 7-H), 6.04 (1H, dt, J=7 and 16 Hz, 3-CH=CH), 6.59 (1H, d, J=7 Hz, pyridine-H), 6.73 (1H, d, J=16 Hz, 3-CH=CH), 7.00 (1H, s, thiazol-H), 7.66 (1H, d, J=2 Hz, pyridine-H), 7.70 (1H, dd, J=2 and 7 Hz, pyridine-H); FAB-MS m/z 519 $(M+H)^+$.

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 a inoculum size of 10^6 cfu/ml except for MRSA at 32°C. *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 twice immediately and 2 hours after the bacterial challenge to group of 5 mice at each dose level. In *S. aureus* A15036 (MRSA) infection,

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[10], from survival rate recorded on 4 days after the bacterial challenge. For the determination of blood levels, 20 mg/kg of test compound was administered im to a group of 5 male ddY mice. Blood samples were collected from the orbital sinuses and assayed by the paper disc method using *E. coli* NIHJ as a test organism. The area under the drug concentration curve (AUC, $\mu g \cdot$hours/ml) and half life ($T_{1/2}$, hours) were calculated by a nonlinear least squares program[11].

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