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Studies on Anti-MRSA Parenteral Cephalosporins

II. Synthesis and Antibacterial Activity of 7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetamido]-3-(substituted imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylates and Related Compounds

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In an effort to discover a novel cefozopran (CZOP) derivative having excellent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), we performed chemical modification of the alkoxyimino moiety and imidazo[1,2-*b*]pyridazinium group of CZOP. Among the prepared compounds, the cyclopentyloxyimino derivative 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-(3,6-diaminoimidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate (**20g**) showed the most potent anti-MRSA activity, reflecting its high affinity (IC_{50}=1.6 μg/ml) for penicillin binding protein 2′ (PBP2′), although its anti-MRSA activity was slightly inferior to that of vancomycin (VCM). In experimental systemic infection in mice, however, **20g** showed activity comparable to that of VCM against MRSA. In addition, **20g** showed activity similar or slightly inferior to that of CZOP against *Pseudomonas aeruginosa* both *in vitro* and *in vivo*. Considering its favorable antibacterial activity profile, **20g** was considered to be the most promising CZOP derivative for further studies.

In the course of our search for novel anti-MRSA (methicillin-resistant *Staphylococcus aureus*) cephalosporins, we have found that the anti-MRSA activity of cefozopran (CZOP) derivatives is affected by changes in the structure of the oxyimino moiety[1]. In addition, we have reported that incorporation of a certain amine-based substituent on the imidazo[1,2-*b*]pyridazinium group enhances the activity against MRSA.

Concerning the cephalosporins, it is generally accepted that chemical modification of the oxyimino moiety in the C-7 acyl group causes alteration of compound's antibacterial properties. As one successful example of such modification, it has been reported that introduction of a carboxy group into the oxyimino moiety dramatically enhances the activity against Gram-negative bacteria, especially *Pseudomonas aeruginosa*[2].

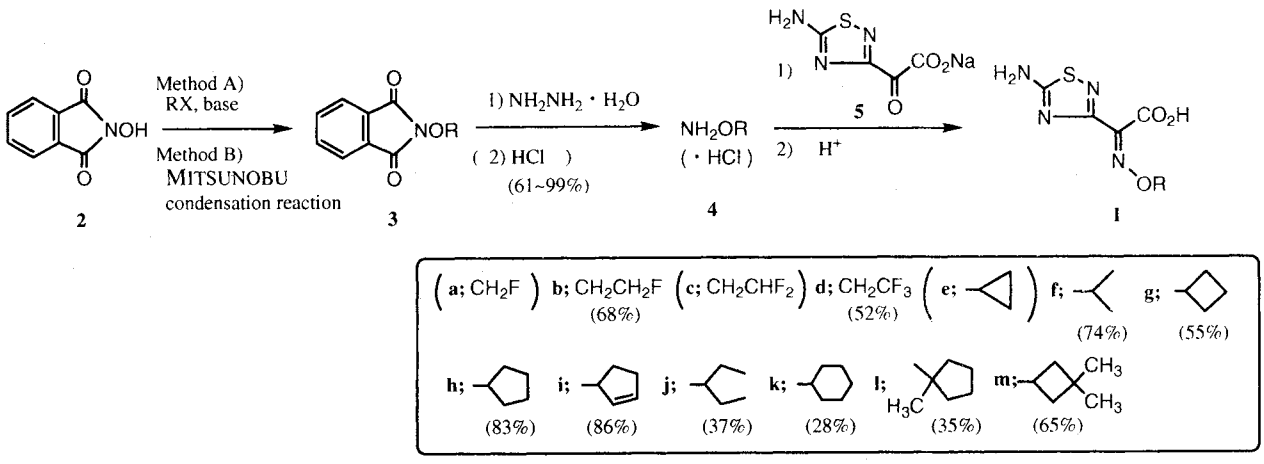
According to several recent reports[3~5], fluoromethoxyiminoacetyl derivatives exhibit activity against Gram-positive bacteria, including MRSA, superior to that of the corresponding methoxyiminoacetyl derivatives. Similarly, it was reported that an increase in the lipophilicity of the substituent in the oxyimino moiety contributes to the high anti-MRSA activity of these compounds[6~8].

Based on the recent advances described above, we tried to find CZOP derivatives with improved anti-MRSA activity by increasing the lipophilicity of the oxyimino moiety of CZOP in combination with the introduction of a substituent on the imidazo[1,2-*b*]pyridazinium group.

In this paper, synthesis and structure-activity relationships (SAR) of the CZOP derivatives bearing a

Dedicated to the memory of Sir EDWARD ABRAHAM.

Scheme 1.



2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-lipophilic alkoxyiminoacetyl group and a substituted imidazo[1,2-*b*]-pyridazinium group are described. Especially, SAR of the disubstituted imidazo[1,2-*b*]pyridazinium derivatives as compared with the monosubstituted derivatives are discussed.

Chemistry

Synthesis of 7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetamido] CZOP Derivatives **8a**~**8m**

The procedure for the preparation of 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetic acids **1** bearing a lipophilic alkyl substituent is illustrated in Scheme 1. Alkylation of *N*-hydroxyphthalimide (**2**) was achieved by treatment with alkylating agents in the presence of a base (Method A) or MITSUNOBU condensation with the corresponding alcohol (Method B). The obtained *N*-alkoxyphthalimide derivatives **3** were transformed into the corresponding *O*-alkylhydroxylamine derivatives **4** by reaction with hydrazine monohydrate. Some of these derivatives were isolated as the corresponding crystalline hydrochloride salts. *O*-(1-Methylcyclopentyl)hydroxylamine (**4l**) was prepared by the reported procedure[9].

Condensation of **4** with the sodium salt of α-ketoacid **5**, prepared by the reported procedure[1], followed by acidification gave the desired *Z*-isomer **1** as a single product in 28~86% yields.

The known oxyiminoacetic acid derivatives **1a**[10], **1c**[11] and **1e**[12] were obtained using the reported procedures, respectively.

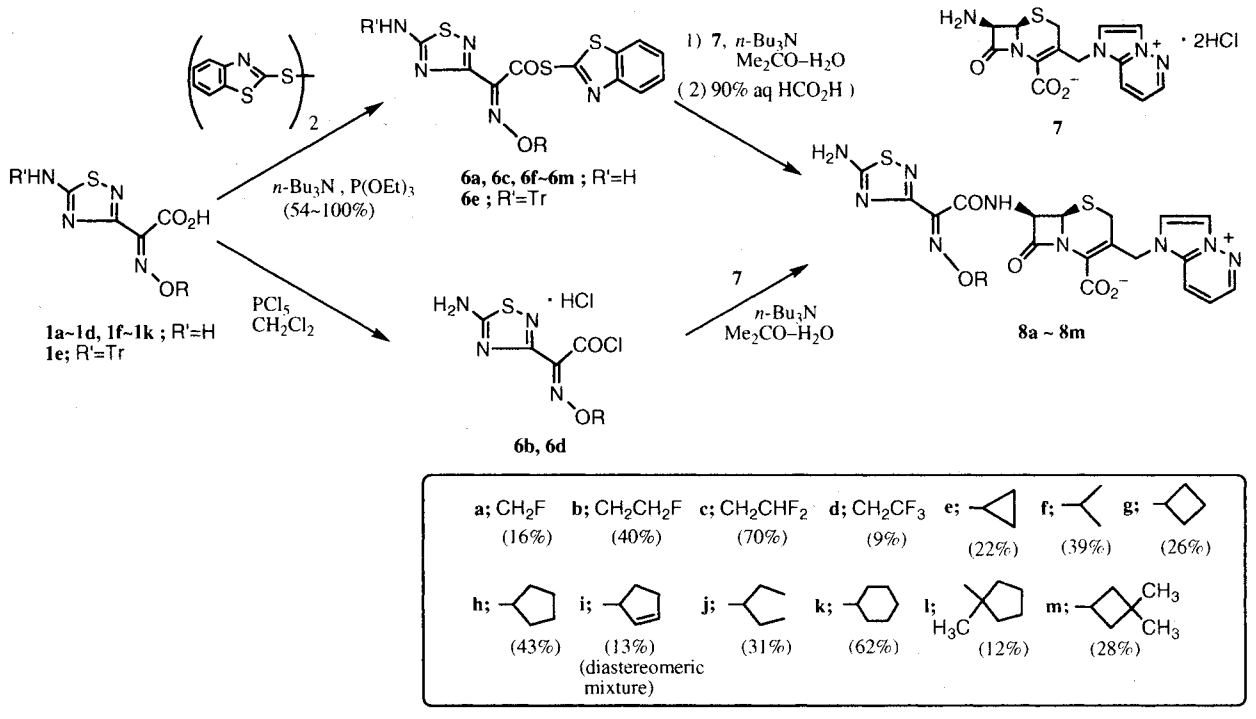
In order to examine the effect of the lipophilicity of the alkoxyimino substituent on the antibacterial activity, the obtained acids **1** were condensed with 7β-amino-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate dihydrochloride[13] (**7**), bearing the same C-3 substituent as that of CZOP by two methods (the thiolester and acid chloride methods), as shown in Scheme 2.

Treatment of the acids (**1a**, **1c** and **1e**~**1m**) with 2,2′-dibenzothiazolyl disulfide in the presence of tri-*n*-butylamine and triethylphosphite gave the corresponding *S*-(2-benzothiazolyl) 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminothioacetates (**6a**, **6c** and **6e**~**6m**) in 54~100% yields. By treatment with phosphorus pentachloride, the acids **1b** and **1d** were converted into the corresponding acid chloride **6b** and **6d**, respectively. Condensation of the acylating agents with **7** was performed in the presence of tri-*n*-butylamine under aqueous conditions to give the desired 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetamido]-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylates (**8a**~**8m**) in 9~70% yields. The CZOP derivative **8i**, having a (2-cyclopentenyl)oxyiminoacetyl group, was obtained as a diastereomeric mixture. In the case of **8e**, additional treatment with aqueous 90% formic acid was needed for removal of the trityl group.

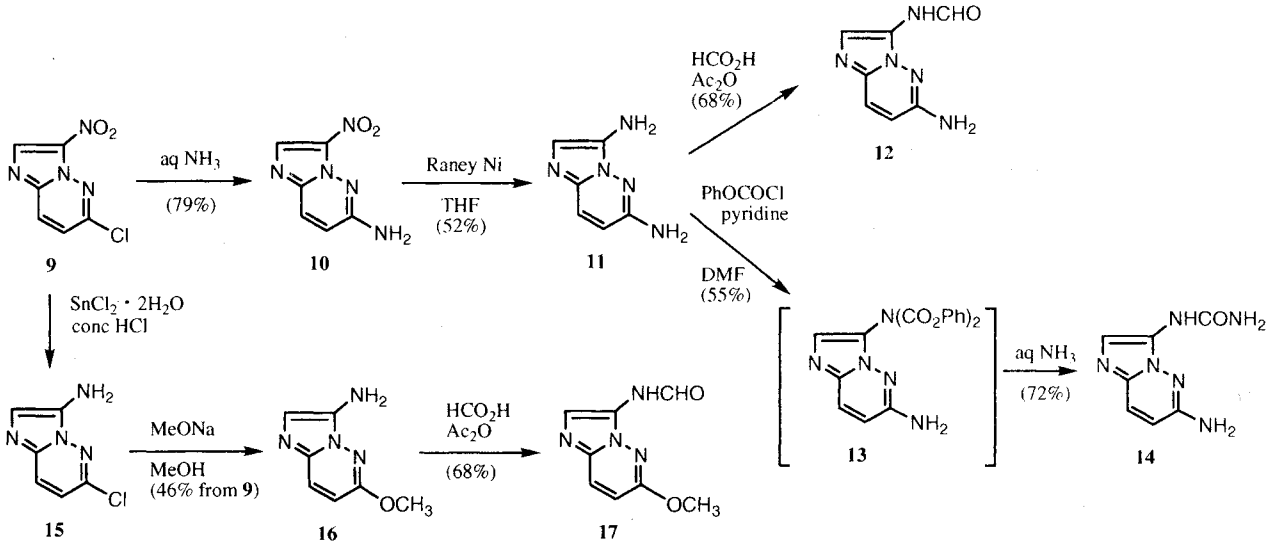
Synthesis of the 3,6-Disubstituted Imidazo[1,2-*b*]-pyridazine Derivatives

We focused our attention on modification of imidazo[1,2-*b*]pyridazine at both the C-3 and the C-6

Scheme 2.



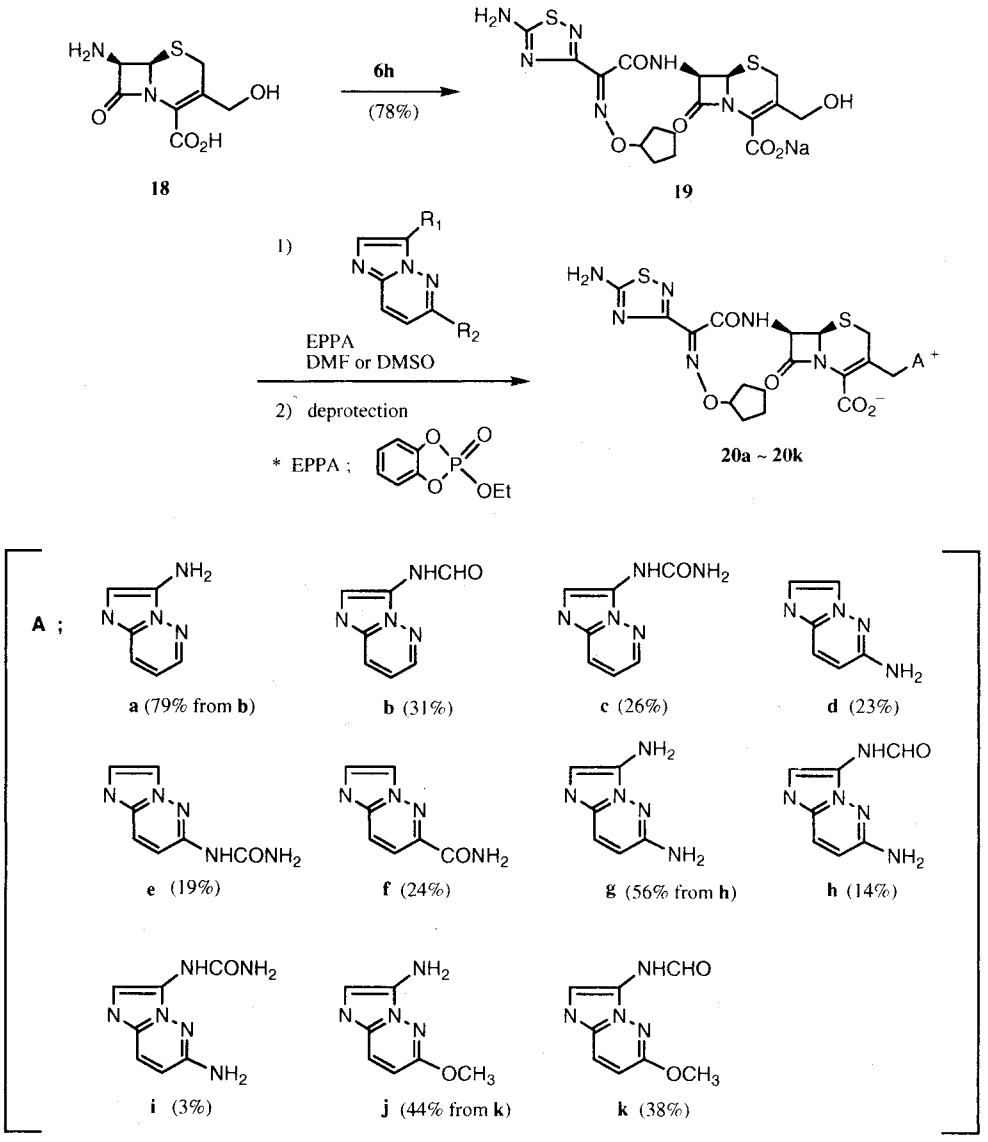
Scheme 3.



position and selected 6-chloro-3-nitroimidazo[1,2-*b*]pyridazine[14] (**9**) as the starting material (Scheme 3).

Nucleophilic substitution of the 6-chloro group with ammonia (79% yield) followed by reduction of the nitro group with Raney Ni (52% yield) afforded 3,6-diaminoimidazo[1,2-*b*]pyridazine (**11**). Formylation of **11** gave 6-amino-3-formylaminoimidazo[1,2-*b*]pyridazine (**12**) in 68% yield. Treatment of **11** with three equivalents of

Scheme 4.

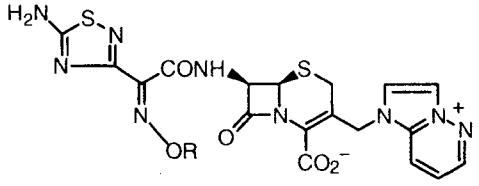


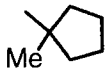
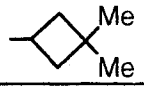
phenyl chloroformate gave the bisphenoxycarbonylated product in 55% yield, whose structure was speculated by ^{1}H NMR to be **13**. Following ammonia treatment of **13** afforded the ureido derivative **14** in 72% yield. Reduction of **9** with stannous chloride in conc HCl followed by treatment with sodium methoxide afforded 3-amino-6-methoxyimidazo[1,2-*b*]pyridazine (**16**) in 46% overall yield. Formylation of **16** gave 3-formylamino-6-methoxyimidazo[1,2-*b*]pyridazine (**17**) in 68% yield.

Synthesis of the C-3′ Modified Cephalosporin Derivatives

The general procedure for preparation of the C-3′ modified cephalosporin derivatives is shown in Scheme 4. The C-3 hydroxymethyl intermediate **19**, bearing a 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetyl group at the C-7 position, was prepared by acylation using the modified thiolester method (*vide supra*) in 78% yield. Introduction of the substituted imidazo[1,2-*b*]pyridazines at the C-3 position was accomplished by treatment with ethyl *o*-phenylenephosphate[15] (EPPA) in DMF or DMSO to give the desired derivatives **20a**~**20k** in 3~38% yields. The 3-amino

Table 1. Antibacterial activity (MIC, μg/ml) of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetamido]-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylates (**8a**~**8m**) and CZOP.



Compd.	R	*S. a.*	MRSA1	MRSA2	MRSA3	*E.c.*	*E.cl.*	*S.m.*	*P.v.*	*P.a.*1	*P.a.*2
CZOP	CH_3	0.78	25	25	100	0.05	0.1	0.1	0.2	1.56	6.25
8a	CH_2F	0.78	12.5	25	50	0.025	0.1	0.05	0.1	1.56	6.25
8b	CH_2CH_2F	0.78	12.5	25	50	0.05	0.1	0.1	0.2	1.56	3.13
8c	CH_2CHF_2	0.78	25	50	50	0.1	0.78	0.2	0.39	3.13	6.25
8d	CH_2CF_3	0.78	12.5	25	50	0.2	1.56	0.39	1.56	6.25	12.5
8e		0.78	12.5	25	50	0.2	0.39	0.39	0.78	1.56	3.13
8f		0.78	12.5	25	25	0.2	1.56	0.39	1.56	3.13	3.13
8g		0.39	12.5	12.5	25	0.78	1.56	0.78	3.13	3.13	3.13
8h		0.39	6.25	12.5	12.5	1.56	3.13	3.13	3.13	3.13	3.13
8i		0.78	12.5	25	25	1.56	1.56	3.13	6.25	6.25	3.13
8j		0.39	6.25	12.5	25	6.25	25	25	25	12.5	12.5
8k		0.39	6.25	12.5	12.5	12.5	25	25	25	12.5	12.5
8l		0.78	12.5	12.5	25	12.5	25	50	25	12.5	12.5
8m		0.39	6.25	12.5	12.5	25	50	50	50	25	25

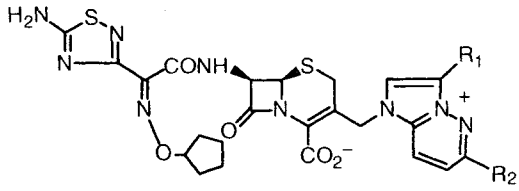
S. a., *Staphylococcus aureus* 308A-1; MRSA1, *S. aureus* J-108 ; MRSA2, *S. aureus* N133 ; MRSA3, *S. aureus* OFU4 ; *E.c.*, *Escherichia coli* NIHJ JC-2 ; *E. cl.*, *Enterobacter cloacae* GN5788 ; *S. m.*, *Serratia marcescens* IFO 12648 ; *P.v.*, *Proteus vulgaris* IFO 3988 ; *P.a.*1, *Pseudomonas aeruginosa* P9 ; *P.a.*2, *P. aeruginosa* U31.

derivatives **20a**, **20g** and **20j** were generated from the corresponding formylamino derivatives **20b**, **20h** and **20k** by HCl-methanol treatment in 44~79% yields.

Biological Results and Discussion

MICs of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetamido] CZOP derivatives **8a**~**8m** against Gram-positive and Gram-negative bacteria are shown in Table 1. As compared with CZOP, all compounds showed comparable or improved antibacterial activity against MRSA. Increasing the lipophilicity of the alkoxyimino group, as seen from a comparison of **8e** and **8k**, enhanced the anti-MRSA activity of the derivatives, but the activity against Gram-negative bacteria was decreased. A comparison of the antibacterial activity of **8e** and **8f** and of **8h** and **8j**, each set bearing the same number of carbon skeleton on the oxyimino group, revealed that a sterically demanding structure diminished the activity against Gram-negative bacteria. The derivatives with a bulky alkyl group might not be able to permeate the outer membrane of

Table 2. Antibacterial activity (MIC, μg/ml) of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-(substituted imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylates (**8h**, **20a**~**20k**) and CZOP.



Compd.	R_1	R_2	*S. a.*	MRSA1	MRSA2	MRSA3	*E.c.*	*E.cl.*	*S.m.*	*P.v.*	*P.a.1*	*P.a.2*
8h	H	H	0.39	6.25	12.5	12.5	1.56	3.13	3.13	3.13	3.13	3.13
20a	NH_2	H	0.2	3.13	6.25	6.25	0.78	3.13	3.13	3.13	3.13	6.25
20b	NHCHO	H	0.78	6.25	6.25	12.5	1.56	6.25	6.25	6.25	6.25	6.25
20c	$NHCONH_2$	H	0.78	6.25	12.5	25	1.56	3.13	3.13	6.25	3.13	6.25
20d	H	NH_2	0.2	3.13	6.25	12.5	0.78	3.13	3.13	1.56	3.13	6.25
20e	H	$NHCONH_2$	0.39	6.25	12.5	12.5	0.78	1.56	3.13	3.13	6.25	6.25
20f	H	$CONH_2$	0.78	6.25	12.5	12.5	1.56	6.25	6.25	6.25	6.25	6.25
20g	NH_2	NH_2	0.2	1.56	3.13	6.25	0.78	3.13	1.56	3.13	6.25	6.25
20h	NHCHO	NH_2	0.39	3.13	6.25	12.5	1.56	6.25	6.25	3.13	3.13	6.25
20i	$NHCONH_2$	NH_2	0.39	3.13	12.5	12.5	1.56	3.13	3.13	3.13	3.13	6.25
20j	NH_2	OCH_3	0.39	3.13	6.25	6.25	0.78	1.56	3.13	3.13	6.25	6.25
20k	NHCHO	OCH_3	0.78	3.13	6.25	12.5	1.56	3.13	3.13	3.13	3.13	6.25
CZOP			0.78	25	25	100	0.05	0.1	0.1	0.2	1.56	6.25

S. a., *Staphylococcus aureus* 308A-1; MRSA1, *S. aureus* J-108 ; MRSA2, *S. aureus* N133 ; MRSA3, *S. aureus* OFU4 ; *E.c.*, *Escherichia coli* NIHJ JC-2 ; *E. cl.*, *Enterobacter cloacae* GN5788 ; *S. m.*, *Serratia marcescens* IFO 12648 ; *P.v.*, *Proteus vulgaris* IFO 3988 ; *P.a.*1, *Pseudomonas aeruginosa* P9 ; *P.a.*2, *P. aeruginosa* U31.

Gram-negative bacteria. Among the derivatives in Table 1, **8h** was found to show the most well-balanced antibacterial activity. Therefore, we selected the cyclopentyloxyimino group as an optimal alkoxyimino moiety.

We next examined novel cyclopentyloxyimino derivatives bearing the substituent at the 3- and/or 6-position of the imidazo[1,2-*b*]pyridazinium group (Table 2). Out of the 3-substituted derivatives **20a**, **20b** and **20c**, the 3-amino derivative **20a** showed improved activity relative to the non-substituted compound **8h**, especially against *S. aureus* including MRSA. Similarly, among the 6-substituted derivatives **20d**, **20e** and **20f**, the 6-amino derivative **20d** had the most promising antibacterial spectrum profile. Acylamino groups such as formylamino, carbamoylamino and carbamoyl group slightly diminished the activity against some strains. The derivative **20g** with the amino substitution at the both C-3 and C-6 positions showed the most potent anti-MRSA activity. The 3-amino-6-methoxy derivative **20j** had an antibacterial spectrum similar to that of **20a**. These 3- and/or 6-amino derivatives, **20a**, **20d**, **20g** and **20j**, exhibited four to sixteen times more potent activity against MRSA than CZOP. In addition, their anti-*P. aeruginosa* activity was similar to that of CZOP.

Table 3. Affinity of **20a**, **20d**, **20g** and CZOP for PBP2′ of *S. aureus* N200P.

Compd.	IC_{50} (μg/ml)
20a	2.4
20d	2.9
20g	1.6
CZOP	92.0

The amino derivatives **20a**, **20d** and **20g** were selected for further evaluation. In order to clarify the mechanism responsible for the potent anti-MRSA activity of the derivatives, we measured their affinity for penicillin binding protein 2′ (PBP2′) of *S. aureus* N200P (Table 3). The affinity of the selected compounds was about fifty times as high as that of CZOP. Among the three derivatives, **20g**

Table 4. Protective effect of **20a**, **20d**, **20g**, CZOP and VCM against experimental systemic infection in mice.

Compd.	*S. aureus* 308A-1		*S. aureus* N133 (MRSA)		*P. aeruginosa* P9		*P. aeruginosa* TY5285	
	ED_{50}, mg/kg	MIC, μg/ml	ED_{50}, mg/kg	MIC, μg/ml	ED_{50}, mg/kg	MIC, μg/ml	ED_{50}, mg/kg	MIC, μg/ml
20a	0.50	0.2	4.41	6.25	2.26	3.13	8.37	12.5
20d	0.80	0.2	5.26	6.25	3.06	3.13	17.0	12.5
20g	0.50	0.2	4.16	3.13	3.45	6.25	11.6	25
CZOP	1.95	0.78	16.7	25	0.73	1.56	13.4	25
VCM	2.41	0.78	3.61	0.78	NT	>100	NT	>100

NT; not tested

showed the highest affinity (IC_{50}=1.6 μg/ml) for PBP2′. The affinity of the compounds for PBP2′ was reflected in their excellent anti-MRSA activity.

We next evaluated *in vivo* activity of the selected compounds using four strains: *S. aureus* 308A-1, *S. aureus* N133 (MRSA), *P. aeruginosa* P9 and *P. aeruginosa* TY5285 (Table 4). In experimental systemic infection in mice, the selected compounds **20a**, **20d** and **20g** showed three to four times more potent activity than the reference compound, CZOP, against infection caused by *S. aureus* including MRSA. Their *in vivo* anti-MRSA activity was comparable to that of vancomycin (VCM). The *in vivo* effect reflected their *in vitro* antibacterial activity. Against *P. aeruginosa* infection, **20a**, **20d** and **20g** showed activity comparable to or slightly less than that of CZOP.

In conclusion, cyclopentyloxyimino CZOP derivatives **20a**, **20d** and **20g** exhibited a well-balanced antibacterial activity spectrum in comparison with CZOP. Especially, 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-(3,6-diaminoimidazo-[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate (**20g**) showed the most potent anti-MRSA activity, which was ten times (*in vitro*) or four times (*in vivo*) superior to that of CZOP. The *in vivo* anti-MRSA activity of **20g** was comparable to that of VCM, though it was less potent than that of VCM *in vitro*. The derivative **20g** exhibited affinity for PBP2′ more than 50 times higher than that of CZOP. Because of its potent anti-MRSA activity and broad spectrum, **20g** was found to be the most promising successor of CZOP. Further evaluation as well as modification of **20g** is now on progress.

Experimental

MPs were determined with a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were taken on a Hitachi 215 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian gemini 200 (200 MHz) spectrometer using TMS as the internal standard. Column chromatography was carried out on Merck Kieselgel 60 (Art No. 7734) and Mitsubishi Chemical MCI gel CHP-20P.

Determination of *In Vitro* Antibacterial Activity

The MICs against selected strains of Gram-positive and Gram-negative bacteria were determined by the standard serial 2-fold agar dilution method with Mueller-Hinton agar as the test medium. The agar plates were inoculated with about 10^4 CFU of microorganisms per spot and were incubated overnight at 37°C.

Determination of *In Vivo* Antibacterial Activity

Bacterial strains were cultured overnight at 37°C in brain heart infusion broth, suspended in 5% mucin and inoculated intraperitoneally into ICR male mice. Compounds were administered subcutaneously immediately after the bacterial challenge. The 50% effective dose (ED_{50}) was calculated from the survival rate recorded on day 5 after infection.

Determination of Affinity for Penicillin Binding Protein 2′

Membrane was prepared from *S. aureus* N200P cells grown to the late exponential phase in trypticase soy broth and incubated with [^{14}C]benzylpenicillin. PBPs were separated by SDS-polyacrylamide gel electrophoresis and detected by fluorography. Binding affinity of each antibiotic for PBP2′ was assessed by a competition assay in which the membrane was incubated with dilutions of the antibiotic at 30°C for 10 minutes before being labeled with [^{14}C]benzylpenicillin for 10 minutes. Binding affinity was expressed in terms of the concentration required to prevent

[^{14}C]benzylpenicillin binding by 50% (IC_{50}).

N-Cyclopentyloxyphthalimide (**3h**) (Method A)

Potassium carbonate (34.5 g, 250 mmol) was added to a solution of *N*-hydroxyphthalimide (**2**, 16.3 g, 100 mmol) in DMSO (150 ml), and the mixture was stirred at room temperature for 5 minutes. To the mixture was added cyclopentylbromide (16 ml, 150 mmol), and the mixture was stirred at 80°C for 3 hours. After ice-cooling, the reaction mixture was poured into cooled water (500 ml). The resulting crystals were collected by filtration, washed with water (100 ml) and *n*-hexane (50 ml) and dried under a vacuum to give **3h** (22.8 g, 99%): MP 82~83°C; ^{1}H NMR ($CDCl_3$) δ 1.5~2.0 (8H, m, cyclopentyl), 4.83 (1H, m, cyclopentyl), 7.86 (4H, s, Ph).

The phthalimides **3b**, **3d**, **3f**, **3g**, **3i** and **3k** were prepared by a method similar to that used for the preparation of **3h**. The yields, analytical data and the reagents used were as follows.

3b (32.4 g, 79%) was obtained from the reaction of 1-bromo-2-fluoroethane (197 mmol) with **2** (196 mmol) in the presence of *N*-ethyldiisopropylamine (213 mmol) in DMF (250 ml): MP 102~103°C; *Anal* Calcd for $C_{10}H_8FNO_3$: C 57.42, H 3.85, N 6.70. Found: C 57.15, H 3.68, N 6.79.; ^{1}H NMR ($CDCl_3$) δ 4.50 (2H, dt, J=3.8 and 32 Hz, OCH_2), 4.81 (2H, dt, J=3.8 and 48 Hz, CH_2F), 7.83 (4H, m, Ph).

3d (52 g, 41%) was obtained from the reaction of 2,2,2-trifluoroethyl trifluoromethanesulfonate (532 mmol) with **2** (520 mmol) in the presence of *N*-ethyldiisopropylamine (1.06 mol) in CH_2Cl_2 (600 ml): MP 105~106°C; *Anal* Calcd for $C_{10}H_6F_3NO_3$: C 48.99, H 2.47, N 5.71. Found: C 49.04, H 2.56, N 5.79.; ^{1}H NMR ($CDCl_3$) δ 4.56 (2H, q, J=8.0 Hz, OCH_2), 7.84 (4H, m, Ph).

3f (24.6 g, 96%) was obtained from the reaction of 2-bromopropane (250 mmol) with **2** (125 mmol) in the presence of potassium carbonate (125 mmol) in DMSO (350 ml): MP 52~53°C; *Anal* Calcd for $C_{11}H_{11}NO_3$: C 64.38, H 5.40, N 6.83. Found: C 64.44, H 5.38, N 6.90.; ^{1}H NMR ($CDCl_3$) δ 1.28 (6H, d, J=6.2 Hz, CH_3), 4.44 (1H, m, CH), 7.86 (4H, s, Ph).

3g (9.1 g, 69%) was obtained from the reaction of cyclobutylbromide (67 mmol) with **2** (61 mmol) in the presence of potassium carbonate (36 mmol) in DMSO (200 ml): MP 104~105°C; *Anal* Calcd for $C_{12}H_{11}NO_3$: C 66.35, H 5.10, N 6.45. Found: C 66.19, H 5.15, N 6.44.; ^{1}H NMR ($CDCl_3$) δ 1.4~1.9 (2H, m, cyclobutyl), 2.2~2.5 (4H, m, cyclobutyl), 4.78 (1H, m, cyclobutyl), 7.7~7.9 (4H, m, Ph).

3i (79.1 g, 97%) was obtained from the reaction of 1-chloro-2-cyclopentene (440 mmol) with **2** (360 mmol) in the presence of triethylamine (660 mmol) in acetonitrile (370 ml): MP 96~98°C; ^{1}H NMR ($CDCl_3$) δ 2.0~2.8 (4H, m, cyclopentenyl), 5.42, 5.96, 6.25 (each 1H, m, cyclopentenyl), 7.7~7.9 (4H, m, Ph).

3k (33.1 g, 67%) was obtained from the reaction of cyclohexylbromide (800 mmol) with **2** (200 mmol) in the presence of potassium carbonate (400 mmol) and 18-crown-6 (20 mmol) in DMSO (500 ml): MP 121~124°C; ^{1}H NMR ($CDCl_3$) δ 1.2~2.1 (10H, m, cyclohexyl), 4.24 (1H, m, cyclohexyl), 7.7~7.9 (4H, m, Ph).

N-(3,3-Dimethylcyclobutyl)oxyphthalimide (**3m**) (Method B)

Under ice-cooling, a solution of diethyl azodicarboxylate (19.6 ml, 124 mmol) in THF (20 ml) was added dropwise to a mixture of **2** (16.2 g, 99 mmol), 3,3-dimethylcyclobutanol[16] (8.3 g, 83 mmol) and triphenylphosphine (32.6 g, 124 mmol) in THF (480 ml), and the mixture was stirred at room temperature for 16 hours. The reaction mixture was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (400 g: eluents=*n*-hexane~*n*-hexane - EtOAc=6 : 1) to give a solid residue, which was crystallized from *n*-hexane to give **3m** (16.1 g, 79%) as crystals: MP 106~107°C; *Anal* Calcd for $C_{14}H_{15}NO_3$: C 68.56, H 6.16, N 5.71. Found: C 68.52, H 6.44, N 5.72.; ^{1}H NMR ($CDCl_3$) δ 1.10 (3H, s, CH_3), 1.23 (3H, s, CH_3), 2.16 (4H, d, J=7.2 Hz, CH_2), 4.82 (1H, m, CH), 7.80 (4H, m, Ph).

The phthalimide **3j** was prepared by a similar method to that used for the preparation of **3m**. The yield, analytical data and the used reagents were as shown below.

3j (28.0 g, 50%) was obtained from the reaction of 3-pentanol (240 mmol) with **2** (240 mmol) in the presence of diethyl azodicarboxylate (264 mmol) and triphenylphosphine (240 mmol) in THF (800 ml): ^{1}H NMR ($CDCl_3$) δ 1.05 (6H, t, J=7.2 Hz, CH_3), 1.73 (4H, m, CH_2), 4.15 (1H, m, CH), 7.7~7.9 (4H, m, Ph).

O-Cyclobutylhydroxylamine Hydrochloride (**4g**)

Hydrazine monohydrate (3.72 ml, 77 mmol) was added dropwise to a solution of **3g** (8.33 g, 38 mmol) in a mixture of CH_2Cl_2 (200 ml) and MeOH (20 ml), and the mixture was stirred at room temperature for 2 hours. The resulting precipitate was filtered off, and the filtrate was washed with 5 N aqueous ammonia (200 ml). The aqueous layer was extracted twice with CH_2Cl_2 (200 ml). The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure. To the concentrate was added a mixture of conc HCl (6.38 ml) and EtOH (50 ml), and the solution was evaporated under

reduced pressure. Treatment of the residual solid with diethyl ether (100 ml) gave **4g** (4.23 g, 89%) as crystals: MP 134~136°C; ^{1}H NMR (DMSO-d_6) δ 1.7~1.9 (2H, m, cyclobutyl), 1.9~2.2 (4H, m, cyclobutyl), 4.64 (1H, m, cyclobutyl), 11.1 (3H, br s, NH_3).

The *O*-alkylhydroxylamines **4b**, **4d**, **4f**, **4j**, **4k** and **4m** were prepared by a method similar to that used for the preparation of **4g**. The yields and analytical data were as follows.

4b (70%): MP 190~193°C; *Anal* Calcd for C_2H_7ClFNO: C 20.80, H 6.11, N 12.13. Found: C 20.65, H 5.93, N 12.30.; ^{1}H NMR (DMSO-d_6) δ 4.20, 4.34, 4.55, 4.78 (each 1H, m, CH_2CH_2F), 10.78 (3H, br s, NH_3).

4d (61%): MP 273~275°C; ^{1}H NMR (DMSO-d_6) δ 4.70 (2H, q, CH_2), 8.20 (3H, br s, NH_3).

4f (88%): MP 87~88°C; *Anal* Calcd for $C_3H_{10}ClNO$: C 32.30, H 9.03, N 12.55. Found: C 32.59, H 8.88, N 12.70.; ^{1}H NMR (DMSO-d_6) δ 1.23 (6H, d, J=6.2 Hz, CH_3), 4.35 (1H, m, CH), 10.01 (3H, br s, NH_3).

4j (60%): MP 56~58°C; ^{1}H NMR (DMSO-d_6) δ 0.86 (6H, t, J=7.2 Hz, CH_3), 1.59 (4H, m, CH_2), 3.99 (1H, m, CH), 11.04 (3H, br s, NH_3).

4k (99%): MP 189~192°C; *Anal* Calcd for $C_6H_{13}ClNO$: C 47.84, H 8.70, N 9.30. Found: C 47.76, H 8.84, N 9.18.; ^{1}H NMR (DMSO-d_6) δ 1.1~2.0 (10H, m, cyclohexyl), 4.01 (1H, m, cyclohexyl), 10.75 (3H, br s, NH_3).

4m (80%): MP 173~175°C; *Anal* Calcd for $C_6H_{14}ClNO$: C 47.53, H 9.31, N 9.24. Found: C 47.17, H 9.26, N 9.57.; ^{1}H NMR (DMSO-d_6) δ 1.08 (3H, s, CH_3), 1.13 (3H, s, CH_3), 1.85, 2.14 (each 2H, m, CH_2), 4.65 (1H, m, CH), 10.93 (3H, br s, NH_3).

O-Cyclopentylhydroxylamine (**4h**)

Hydrazine monohydrate (9.23 ml, 190 mmol) was added dropwise to a solution of **3h** (22 g, 95 mmol) in a mixture of CH_2Cl_2 (100 ml) and MeOH (12 ml), and the mixture was stirred at room temperature for 4 hours. The resulting precipitate was filtered off, and the filtrate was washed with 5 N aqueous ammonia (50 ml). The aqueous layer was extracted twice with CH_2Cl_2 (100 ml). The combined extract was washed with brine (50 ml). The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give **4h** as a pale yellow oil (8.9 g, 93%): ^{1}H NMR ($CDCl_3$) δ 1.4~1.8 (8H, m, cyclopentyl), 4.17 (1H, m, cyclopentyl), 5.31 (2H, br s, NH_2).

O-(2-Cyclopentenyl)hydroxylamine (**4i**)

Hydrazine monohydrate (1.84 ml, 38 mmol) was added dropwise to a suspension of **3i** (9.17 g, 40 mmol) in EtOH (50 ml), and the mixture was stirred at room temperature for 15 minutes. The resulting precipitate was filtered off, and the filtrate containing **4i** was used for the next reaction without further purification.

2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetic Acid (**1h**)

To a stirred suspension of 3-(5-amino-1,2,4-thiadiazol-3-yl)coumarin[1] (14.7 g, 60 mmol) in EtOH (120 ml), 1 N NaOH (120 ml) was added, and the mixture was stirred at 40°C for 1 hour to afford a clear solution. Under ice-cooling, ethyl chloroformate (6 ml, 63 mmol) was added to the reaction mixture, and the mixture was stirred at 5°C for 5 minutes. CH_2Cl_2 (180 ml) and 1 N HCl (60 ml) were added to the mixture successively, and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (120 ml), and the combined extract was cooled at −78°C. An excess amount of gaseous O_3 was bubbled into the solution under cooling at −78°C for 3 hours. After the excess O_3 was removed by N_2 bubbling, sodium acetate (4.92 g, 60 mmol) and dimethylsulfide (30 ml, 408 mmol) were added to the mixture successively, and the mixture was stirred below 0°C for 15 minutes. The resulting sodium salt was then extracted twice with water (120 ml, 60 ml), and the combined aqueous layer was washed with EtOAc (100 ml). The separated aqueous layer was used in the next reaction without further purification. To the aqueous solution was added **4h** (6.7 g, 66 mmol). After the pH of the aqueous solution was adjusted to 5.0 with 1 N NaOH, the mixture was stirred overnight at room temperature, and then 1 N HCl (80 ml) was added to the mixture which was then extracted three times with EtOAc (400 ml). The combined organic layer was washed with brine (300 ml), dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. Treatment of the residual solid with EtOAc (30 ml) gave **1h** (12.8 g, 83%) as crystals: MP 142~143°C; IR (KBr) cm^{-1} 3300, 2950, 1720, 1640, 1520; ^{1}H NMR (DMSO-d_6) δ 1.3~1.9 (8H, m, cyclopentyl), 4.74 (1H, m, cyclopentyl), 8.17 (2H, br s, NH_2).

The 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetic acids **1b**, **1d**, **1f**, **1g** and **1i**~**1m** were prepared by a method similar to that used for the preparation of **1h**. The yields and analytical data were as follows.

1b (68%): MP 176~178°C; IR (KBr) cm^{-1} 3400, 3130, 1720, 1620, 1540; ^{1}H NMR (DMSO-d_6) δ 4.38 (2H, dt, J=3 and 26 Hz, OCH_2), 4.65 (2H, dt, J=3 and 48 Hz, CH_2F), 8.17 (2H, br s, NH_2).

1d (52%): MP 130~135°C; *Anal* Calcd for $C_6H_5F_3N_4O_3S\cdot 0.2H_2O$: C 26.32, H 1.99, N 20.46. Found: C 26.69, H 1.97, N 20.07.; ^{1}H NMR (DMSO-d_6) δ 4.86 (2H,

q, J=9 Hz, OCH_2), 8.31 (2H, br s, NH_2).

1f (74%): MP 152~154°C; ^{1}H NMR (DMSO-d_6) δ 1.23 (6H, d, J=6.2 Hz, CH_3), 4.40 (1H, m, CH), 8.18 (2H, br s, NH_2).

1g (55%): MP 94~96°C; ^{1}H NMR (DMSO-d_6) δ 1.4~2.3 (6H, m, cyclobutyl), 4.71 (1H, m, cyclobutyl), 8.16 (2H, br s, NH_2).

1i (86%): ^{1}H NMR (DMSO-d_6) δ 1.7~2.5 (4H, m, cyclopentenyl), 5.36, 5.87, 6.15 (each 1H, m, cyclopentenyl), 8.18 (2H, br s, NH_2).

1j (37%): MP 136~137°C; ^{1}H NMR (DMSO-d_6) δ 0.87 (6H, d, J=7.4 Hz, CH_3), 1.58 (4H, m, CH_2), 4.02 (1H, m, CH), 8.18 (2H, br s, NH_2).

1k (28%): ^{1}H NMR (DMSO-d_6) δ 1.2~1.9 (10H, m, cyclohexyl), 4.17 (1H, m, cyclohexyl), 8.17 (2H, br s, NH_2).

1l (35%): ^{1}H NMR (DMSO-d_6) δ 1.40 (3H, s, CH_3), 1.4~2.0 (8H, m, cyclopentyl), 8.12 (2H, br s, NH_2).

1m (65%): ^{1}H NMR (DMSO-d_6) δ 1.10, 1.13 (each 3H, s, CH_3), 1.86, 2.15 (each 2H, s, cyclobutyl), 4.71 (1H, m, cyclobutyl), 8.16 (2H, br s, NH_2).

S-(2-Benzothiazolyl) 2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminothioacetate (**6h**)

Under cooling at −10°C, triethylphosphite (5 ml, 29.2 mmol) was added to a mixture of **1h** (5 g, 19.5 mmol), 2,2′-dibenzothiazolyl disulfide (8.43 g, 25.4 mmol) and tri-*n*-butylamine (9.29 ml, 39 mmol) in acetonitrile (110 ml). The reaction mixture was stirred at −10°C for 2 hours. The resulting precipitate was collected by filtration, washed with cooled acetonitrile (15 ml) and dried under a vacuum to give **6h** (4.25 g, 54%): *Anal* Calcd for $C_{16}H_{15}N_5O_2S_3$: C 47.39, H 3.73, N 17.27. Found: C 47.62, H 3.75, N 17.37; IR (KBr) cm^{-1} 3400, 3140, 2950, 1720, 1610, 1530, 1450; ^{1}H NMR (DMSO-d_6) δ 1.5~2.0 (8H, m, cyclopentyl), 4.86 (1H, m, cyclopentyl), 7.59 (2H, m, Ph), 8.07, 8.21 (each 1H, m, Ph), 8.30 (2H, br s, NH_2).

The *S*-(2-benzothiazolyl) 2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminothioacetates **6a**, **6c**, **6e**~**6g** and **6i**~**6m** were prepared by a method similar to that used for the preparation of **6h**. The yields and analytical data were as follows.

6a (77%): ^{1}H NMR (DMSO-d_6) δ 5.69 (2H, d, J=54 Hz, CH_2F), 7.5~8.3 (4H, m, Ph), 8.37 (2H, br s, NH_2).

6f (73%): ^{1}H NMR (DMSO-d_6) δ 1.28 (6H, d, J=6.4 Hz, CH_3), 4.50 (1H, m, CH), 7.58 (2H, m, Ph), 8.07, 8.21 (each 1H, m, Ph), 8.30 (2H, br s, NH_2).

6g (77%): ^{1}H NMR (DMSO-d_6) δ 1.5~2.3 (6H, m, cyclobutyl), 4.84 (1H, m, cyclobutyl), 7.58 (2H, m, Ph), 8.07, 8.22 (each 1H, m, Ph), 8.31 (2H, br s, NH_2).

6j (quant.): ^{1}H NMR (DMSO-d_6) δ 0.89 (6H, t, J=7.2 Hz, CH_3), 1.64 (4H, m, CH_2), 4.15 (1H, m, CH), 7.60 (2H, m, Ph), 8.06, 8.22 (each 1H, m, Ph), 8.32 (2H, br s, NH_2).

6k (60%): *Anal* Calcd for $C_{17}H_{17}N_5O_2S_3$: C 48.67, H 4.08, N 16.69. Found: C 48.89, H 4.39, N 16.48.; ^{1}H NMR ($CDCl_3$) δ 1.2~2.0 (10H, m, cyclohexyl), 4.45 (1H, m, cyclohexyl), 6.41 (2H, br s, NH_2), 7.52 (2H, m, Ph), 7.94, 8.07 (each 1H, m, Ph).

6m (96%): ^{1}H NMR (DMSO-d_6) δ 1.12, 1.15 (each 3H, s, CH_3), 1.89, 2.17 (each 2H, m, CH_2), 4.87 (1H, m, CH), 7.59 (2H, m, Ph), 8.07, 8.22 (each 1H, m, Ph), 8.30 (2H, br s, NH_2).

As other derivatives **6c**, **6e**, **6i** and **6l** were not precipitated, their crude oil was obtained by concentration and used for the next reaction.

2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-(2-fluoroethoxy)-iminoacetyl Chloride Hydrochloride (**6b**)

Under cooling at −20°C, phosphorus pentachloride (4.58 g, 22 mmol) was added portionwise to a suspension of **1b** (4.68 g, 20 mmol) in CH_2Cl_2 (60 ml), and the mixture was stirred at −20~−5°C for 2 hours. To the reaction mixture was added diisopropyl ether (80 ml), and the mixture was stirred at −5~5°C for 30 minutes. The resulting crystals were collected by filtration, washed with diisopropyl ether (20 ml) and dried under a vacuum to give **6b** (4.55 g, 79%): MP 112~115°C; IR (KBr) cm^{-1} 3200, 1790, 1640, 1460.

2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-(2,2,2-trifluoroethoxy)iminoacetyl chloride hydrochloride (**6d**) was prepared by a method similar to that used for the preparation of **6b**, and used for the next acylation step without crystallization.

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-(2-fluoroethoxy)iminoacetamido]-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate (**8b**)

Under cooling at −10°C, **6b** (100 mg, 0.35 mmol) was added all at once to a solution of 7β-amino-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate dihydrochloride[13] (**7**, purity=44%, 163 mg, 0.42 mmol) in a mixture of Me_2CO (6 ml) and H_2O (6 ml) containing tri-*n*-butylamine (0.42 ml, 1.75 mmol), and the mixture was stirred at −10~5°C for 2 hours. The reaction mixture was concentrated under reduced pressure. The concentrate was purified by silica gel column chromatography (40 g: eluents=Me_2CO~70% aq Me_2CO). The eluted fractions were concentrated under reduced pressure. The concentrate was further purified by MCI gel CHP-20P column

chromatography (100 ml: eluents=H_2O~10% aq EtOH). The fractions eluted with 10% aq EtOH were concentrated under reduced pressure, and the concentrate was lyophilized to give **8b** (75 mg, 40%). The analytical results are shown in Table 5 and Table 6.

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-(2,2,2-trifluoroethoxy)iminoacetamido]-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate (**8d**) was prepared by a method similar to that used for the preparation of **8b** in 9% yield. The analytical results are shown in Table 5 and Table 6.

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopropoxyiminoacetamido]-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate (**8e**)

Under cooling at −10°C, triethylphosphite (0.22 ml, 1.28 mmol) was added to a mixture of 2-(5-tritylamino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopropoxyiminoacetic acid[12] (**1e**, 0.4 g, 0.85 mmol), 2,2′-dibenzothiazolyl disulfide (0.37 g, 1.1 mmol) and tri-*n*-butylamine (0.4 ml, 1.7 mmol) in acetonitrile (9 ml). The reaction mixture was stirred at −10°C for 1.5 hours and concentrated under reduced pressure. Under ice-cooling, the concentrate dissolved in THF (2 ml) was added to a solution of **7** (purity=44%, 0.77 g, 1.0 mmol) in a mixture of Me_2CO (5 ml) and H_2O (5 ml) containing tri-*n*-butylamine (0.97 ml, 4.1 mmol). The reaction mixture was stirred at room temperature for 5 hours and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (50 g: eluents=Me_2CO~70% aq Me_2CO). The eluted fractions were evaporated under reduced pressure. Under ice-cooling, the residue was suspended in CH_2Cl_2 (10 ml). To the suspension was added 90% aq formic acid (10 ml), and the reaction mixture was stirred at room temperature for 5 hours. After being concentrated under reduced pressure, the concentrate was purified by MCI gel CHP-20P column chromatography (100 ml: eluents=H_2O~15% aq EtOH). The fractions eluted with 15% aq EtOH were concentrated under reduced pressure, and the concentrate was lyophilized to give **8e** (102 mg, 22%). The analytical results are shown in Table 5 and Table 6.

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-(imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate (**8h**)

Under ice-cooling, **6h** (200 mg, 0.51 mmol) was added to a solution of **7** (purity=44%, 0.39 g, 0.51 mmol) in a mixture of Me_2CO (3 ml) and H_2O (3 ml) containing tri-*n*-butylamine (0.524 ml, 2.2 mmol), and the mixture was stirred at room temperature for 4 hours. The reaction mixture was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (50 g: eluents=Me_2CO~70% aq Me_2CO). The eluted fractions were concentrated under reduced pressure. The concentrate was further purified by MCI gel CHP-20P column chromatography (100 ml: eluents=H_2O~25% aq EtOH). The fractions eluted with 25% aq EtOH were concentrated under reduced pressure, and the concentrate was lyophilized to give **8h** (126 mg, 43%). The analytical results are shown in Table 5 and Table 6.

The cephalosporin derivatives **8a**, **8c**, **8f**, **8g** and **8i**~**m** were prepared by a method similar to that used for the preparation of **8h**. The yields were as follows: **8a** (16%); **8c** (70%); **8f** (39%); **8g** (26%); **8i** (13%); **8j** (31%); **8k** (62%); **8l** (12%); **8m** (28%). The analytical results are shown in Table 5 and Table 6.

6-Amino-3-nitroimidazo[1,2-*b*]pyridazine (**10**)

A solution of 6-chloro-3-nitroimidazo[1,2-*b*]pyridazine[14] (**9**, 10.5 g, 52.9 mmol) in a mixture of 25% aq ammonia (100 ml) and THF (100 ml) was heated at 120°C in a stainless sealed tube for 7 hours. After cooling, the resulting crystals were collected by filtration, washed with H_2O (30 ml), EtOH (20 ml) and diethyl ether (50 ml) successively and dried under a vacuum to give **10** (7.5 g, 79%): MP 250°C (dec.); ^{1}H NMR (DMSO-d_6) δ 6.99, 7.97 (each 1H, d, J=9.4 Hz, C_7-H and C_8-H), 7.02 (2H, br s, NH_2), 8.43 (1H, s, C_2-H).

3,6-Diaminoimidazo[1,2-*b*]pyridazine (**11**)

Under a hydrogen atmosphere (2~3 atm), a solution of **10** (5.13 g, 28.6 mmol) was stirred in the presence of commercially available Raney nickel (5 g) at room temperature for 2.5 hours. The reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. Treatment of the residue with diethyl ether (50 ml) gave **11** (3.18 g, 74%): MP 200°C (dec.); ^{1}H NMR (DMSO-d_6) δ 4.83 (2H, br s, NH_2), 6.06 (2H, br s, NH_2), 6.13, 7.47 (each 1H, d, J=9.6 Hz, C_7-H and C_8-H), 6.68 (1H, s, C_2-H).

6-Amino-3-formylaminoimidazo[1,2-*b*]pyridazine (**12**)

A mixture of acetic anhydride (1.79 ml, 19 mmol) and formic acid (0.86 ml, 22.8 mmol) was stirred at room temperature for 1 hour. The mixture was diluted with DMF (10 ml). Under ice-cooling, to the mixture was added dropwise a solution of **11** (2.02 g, 13.5 mmol) in DMF (40 ml). The reaction mixture was stirred at room temperature for 1 hour. The mixture was evaporated under reduced pressure. To the residue was added EtOH (10 ml), and the resulting crystals were collected by filtration,

washed with diethyl ether (20 ml) and dried under a vacuum to give **12** (1.46 g, 61%): MP 260°C (dec.); ^{1}H NMR (DMSO-d_6) δ 6.29 (2H, br s, NH_2), 6.60, 7.67 (each 1H, d, J=9.4 Hz, C_7-H and C_8-H), 7.49 (1H, s, C_2-H), 8.32 (1H, s, CHO), 10.34 (1H, br s, NH).

6-Amino-3-bis(phenoxycarbonyl)aminoimidazo[1,2-*b*]pyridazine (**13**)

Under ice-cooling, phenyl chloroformate (7.53 ml, 60 mmol) was added dropwise to a mixture of **11** (2.98 g, 20 mmol) and pyridine (8.09 ml, 100 mmol) in DMF (70 ml), and the mixture was stirred at 5°C for 15 minutes. The reaction mixture was diluted with EtOAc (500 ml), and the mixture was washed with H_2O (700 ml). The organic layer was washed with 10% aq citric acid (200 ml), H_2O (300 ml) and brine (300 ml) successively, dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (60 g: eluent=CH_2Cl_2~CH_2Cl_2-MeOH=20:1) to give **13** (4.30 g, 55%): *Anal* Calcd for $C_{20}H_{15}N_5O_4$: C 61.69, H 3.88, N 17.99. Found: C 61.40, H 3.85, N 18.23.; ^{1}H NMR ($CDCl_3$) δ 4.66 (2H, br s, NH_2), 6.61, 7.75 (each 1H, d, J=9.6 Hz, C_7-H and C_8-H), 7.10~7.39 (10H, m, Ph), 7.73 (1H, s, C_2-H).

6-Amino-3-ureidoimidazo[1,2-*b*]pyridazine (**14**)

To a solution of **13** (3.94 g, 10 mmol) in THF (40 ml) was added 25% aq ammonia (40 ml), and the mixture was stirred at room temperature for 10 minutes. The reaction mixture was concentrated under reduced pressure. The concentrate was diluted with H_2O (10 ml), and the resulting crystals were collected by filtration, washed with diisopropyl ether (30 ml) and dried under a vacuum to give **14** (1.40 g, 72%): MP 245°C (dec.); ^{1}H NMR (DMSO-d_6) δ 6.18 (2H, br s, NH_2), 6.19 (2H, br s, NH_2), 6.52, 7.61 (each 1H, d, J=9.6 Hz, C_7-H and C_8-H), 7.30 (1H, s, C_2-H), 8.32 (1H, br s, NH).

3-Amino-6-methoxyimidazo[1,2-*b*]pyridazine (**16**)

To a solution of 3-amino-6-chloroimidazo[1,2-*b*]-pyridazine[1] (**15**, 3.88 g, 23 mmol) in MeOH (90 ml), a 28% sodium methoxide MeOH solution (6.17 ml, 32 mmol) was added, and the reaction mixture was refluxed for 24 hours. After cooling, the resulting precipitate was filtered off, and the filtrate was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (50 g: eluent=CH_2Cl_2~CH_2Cl_2-MeOH=75:1) to give **16** (2.57 g, 68%): MP 90~92°C; ^{1}H NMR ($CDCl_3$) δ 3.97 (2H, br s, NH_2), 4.02 (3H, s, OCH_3), 6.48, 7.65 (each 1H, d, J=9.4 Hz, C_7-H and C_8-H), 7.08 (1H, s, C_2-H).

3-Formylamino-6-methoxyimidazo[1,2-*b*]pyridazine (**17**)

A mixture of acetic anhydride (7.5 ml, 80 mmol) and formic acid (4 ml, 106 mmol) was stirred at room temperature for 30 minutes. To the mixture was added **16** (2.22 g, 13.5 mmol), and the mixture was stirred at room temperature for 1.5 hours. The reaction mixture was concentrated under reduced pressure. To the concentrate was added saturated aq $NaHCO_3$ (10 ml). The resulting crystals were collected by filtration, washed with H_2O (10 ml) and diisopropyl ether (20 ml) successively and dried under a vacuum to give **17** (1.78 g, 68%): MP 210~211°C; *Anal* Calcd for $C_8H_8N_4O_2$: C 50.00, H 4.20, N 29.15. Found: C 49.93, H 4.26, N 29.25.; ^{1}H NMR (DMSO-d_6) δ 4.02 (3H, s, OCH_3), 6.81, 7.96 (each 1H, d, J=9.8 Hz, C_7-H and C_8-H), 7.73 (1H, s, C_2-H), 8.43 (1H, s, CHO), 10.72 (1H, br s, NH).

Sodium 7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-hydroxymethyl-3-cephem-4-carboxylate (**19**)

Under ice-cooling, 7β-amino-3-hydroxymethyl-3-cephem-4-carboxylic acid (**18**, 2.89 g, 12.6 mmol) was suspended in a mixture of H_2O (40 ml) and THF (50 ml). The pH of the mixture was adjusted to 8.0 with 1 N NaOH to afford a solution. To the solution was added **6h** (4.24 g, 10.5 mmol), and the mixture was stirred at room temperature for 16 hours. The reaction mixture was washed with EtOAc (30 ml), and the aqueous layer was concentrated under reduced pressure. The concentrate was purified by MCI gel CHP-20P column chromatography (600 ml: eluents=H_2O~5% aq EtOH). The fractions eluted with 5% aq EtOH were concentrated under reduced pressure. The concentrate was lyophilized to give **19** (4.0 g, 78%): *Anal* Calcd for $C_{17}H_{19}N_6O_6S_2Na\cdot 2.0H_2O$: C 38.78, H 4.40, N 15.96. Found: C 38.66, H 4.19, N 15.62.; IR (KBr) cm^{-1} 3400, 1760, 1670, 1600, 1520, 1400; ^{1}H NMR (DMSO-d_6) δ 1.4~2.0 (8H, m, cyclopentyl), 3.22, 3.47 (2H, ABq, J=18 Hz, C_2-H), 3.74, 4.16 (2H, ABq, J=12.4 Hz, C_3-CH_2), 4.73 (1H, m, cyclopentyl), 4.92 (1H, d, J=4.8 Hz, C_8-H), 5.55 (1H, dd, J=4.8 and 8.4 Hz, C_7-H), 6.20 (1H, br s, OH), 8.14 (2H, br s, NH_2), 9.39 (1H, d, J=8.4 Hz, C_7-NH).

3-(6-Amino-3-formylaminoimidazo[1,2-*b*]pyridazinium-1-yl)methyl-7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-cephem-4-carboxylate (**20h**)

A solution of ethyl *o*-phenylenephosphate[15] (1.5 g, 7.5

Table 5. IR and analytical data for 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetamido]-3-(substituted imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylates (**8a**~**8m**, **20a**~**20k**).

Compd. No.	Formula	*Anal* Calcd (%) C	H	N	Found (%) C	H	N	IR (KBr, cm^{-1})		
8a	$C_{19}H_{16}FN_9O_5S_2 \cdot 1.5H_2O$	40.71	3.42	22.49	40.64	3.77	22.65	1770	1670	1610
8b	$C_{20}H_{18}FN_9O_5S_2 \cdot 3.0H_2O$	39.90	3.99	20.95	40.13	3.79	20.72	1780	1680	1620
8c	$C_{20}H_{17}F_2N_9O_5S_2 \cdot 3.0H_2O$	38.77	3.74	20.35	39.02	3.84	20.31	1770	1680	1610
8d	$C_{20}H_{16}F_3N_9O_5S_2 \cdot 2.0H_2O$	38.77	3.25	20.35	38.72	3.41	20.65	1770	1680	1610
8e	$C_{21}H_{19}N_9O_5S_2 \cdot 4.2H_2O$	40.86	4.48	20.42	41.19	4.42	20.20	1760	1670	1610
8f	$C_{21}H_{21}N_9O_5S_2 \cdot 3.0H_2O$	42.20	4.55	21.09	42.15	4.36	21.23	1770	1670	1610
8g	$C_{22}H_{21}N_9O_5S_2 \cdot 4.0H_2O$	42.10	4.66	20.08	41.92	4.70	19.83	1760	1670	1605
8h	$C_{23}H_{23}N_9O_5S_2 \cdot 2.0H_2O$	45.61	4.49	20.81	45.56	4.68	20.83	1770	1670	1610
8i	$C_{23}H_{21}N_9O_5S_2 \cdot 3.5H_2O$	43.80	4.48	19.99	43.72	4.08	19.66	1770	1670	1610
8j	$C_{23}H_{25}N_9O_5S_2 \cdot 3.0H_2O$	44.15	4.99	20.15	44.21	5.01	20.36	1770	1665	1610
8k	$C_{24}H_{25}N_9O_5S_2 \cdot 2.5H_2O$	45.85	4.81	20.05	45.91	4.66	20.36	1770	1670	1610
8l	$C_{24}H_{25}N_9O_5S_2 \cdot 4.5H_2O$	43.37	5.16	18.96	43.36	5.14	19.10	1770	1670	1610
8m	$C_{24}H_{25}N_9O_5S_2 \cdot 2.5H_2O$	45.85	4.81	20.05	46.02	4.79	19.91	1770	1670	1610
20a	$C_{23}H_{24}N_{10}O_5S_2 \cdot 2.0H_2O$	44.51	4.55	22.57	44.34	4.70	22.73	1770	1660	1610
20b	$C_{24}H_{24}N_{10}O_6S_2 \cdot 2.5H_2O$	43.86	4.44	21.30	43.89	4.42	21.36	1780	1680	1605
20c	$C_{24}H_{25}N_{11}O_6S_2 \cdot 4.0H_2O$	41.20	4.75	22.02	41.22	4.74	21.73	1770	1680	1605
20d	$C_{23}H_{24}N_{10}O_5S_2 \cdot 3.0H_2O$	43.25	4.73	21.93	43.05	4.65	22.04	1770	1680	1610
20e	$C_{24}H_{25}N_{11}O_6S_2 \cdot 4.5H_2O$	40.67	4.84	21.74	40.61	4.64	21.43	1770	1700	1600
20f	$C_{24}H_{24}N_{10}O_6S_2 \cdot 4.5H_2O$	41.55	4.79	20.19	41.79	4.60	20.40	1770	1690	1610
20g	$C_{23}H_{25}N_{11}O_5S_2 \cdot 3.0H_2O$	42.26	4.78	23.57	42.30	4.93	23.30	1760	1650	1610
20h	$C_{24}H_{25}N_{11}O_6S_2 \cdot 4.0H_2O$	41.20	4.75	22.02	41.21	4.77	21.70	1760	1670	1605
20i	$C_{24}H_{26}N_{12}O_6S_2 \cdot 5.5H_2O$	38.86	5.03	22.66	39.05	4.92	22.51	1760	1670	1610
20j	$C_{24}H_{26}N_{10}O_6S_2 \cdot 3.0H_2O$	43.11	4.82	20.95	42.88	4.89	20.75	1770	1660	1610
20k	$C_{25}H_{26}N_{10}O_7S_2 \cdot 2.5H_2O$	43.66	4.54	20.37	43.24	4.91	20.11	1770	1680	1610

mol) in DMSO (2 ml) was added to a mixture of **12** (400 mg, 2.26 mmol) and **19** (736 mg, 1.6 mmol) in DMSO (12 ml). The mixture was stirred at room temperature for 4 hours. The reaction mixture was diluted with diethyl ether (160 ml). After being stirred at room temperature for 30 minutes, the mixture was allowed to stand. The resulting upper layer was removed by decantation. The residual oil was purified by silica gel column chromatography (60 g: eluents=Me_2CO~70% aq Me_2CO). The eluted fractions were concentrated under reduced pressure. The concentrate was further purified by MCI gel CHP-20P column chromatography (100 ml: eluents=H_2O~25% aq EtOH). The fractions eluted with 25% aq EtOH were concentrated under reduced pressure. The concentrate was lyophilized to give **20h** (400 mg, 42%). The analytical results are shown in Table 5 and Table 6.

The cephalosporin derivatives **20b**~**20f**, **20i** and **20k** were prepared by a method similar to that used for the preparation of **20h**. The yields were as follows: **20b** (31%); **20c** (26%); **20d** (23%); **20e** (19%); **20f** (24%); **20i** (3%); **20k** (38%). The analytical results are shown in Table 5 and Table 6.

7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-cyclopentyloxyiminoacetamido]-3-(3,6-diaminoimidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylate (**20g**)

Under ice-cooling, 1 N HCl (4 ml) was added to a mixture of **20h** (130 mg, 0.21 mmol) in MeOH (8 ml), and the mixture was stirred at room temperature for 15 hours. After the reaction mixture was concentrated under reduced pressure, the concentrate was purified by MCI gel CHP-20P column chromatography (100 ml: eluents=H_2O~25% aq EtOH). The fractions eluted with 25% aq EtOH were concentrated under reduced pressure. The concentrate was lyophilized to give **20g** (70 mg, 56%). The analytical results are shown in Table 5 and Table 6.

The cephalosporin derivatives **20a** and **20j** were prepared by a method similar to that used for the preparation of **20g**. The yields were 79% (**20a**) and 44% (**20j**), respectively. The analytical results are shown in Table 5 and Table 6.

Table 6. ^{1}H NMR spectral data for 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-alkoxyiminoacetamido]-3-(substituted imidazo[1,2-*b*]pyridazinium-1-yl)methyl-3-cephem-4-carboxylates (**8a**~**8m**, **20a**~**20k**).

Compd. No.	Chemical shift (*J*=Hz) (DMSO-d_6, δ)							
	Cephem nuclei					Thia(dia)zole		Imidazo[1,2-*b*]pyridazinium
	C_2-H ABq(17)	C_3-CH_2 ABq(14)	C_6-H d(5)	C_7-H dd(5&8)	C_7-NH d(8)	NH_2 br s	N-OR	
8a	3.02 3.51	5.24 5.49	5.00	5.64	9.65	8.20	5.72(2H,d,56Hz)	7.95 (1H,dd,*J*=10&4Hz), 8.76 (2H,s), 9.04 (1H,d,*J*=4Hz), 9.34 (1H,d,*J*=10Hz).
8b	3.02 3.42	5.27 5.49	5.00	5.65	9.54	8.14	4.32(2H,dt,28&4Hz) 4.62(2H,dt,48&4Hz)	7.96 (1H,dd,*J*=10&4Hz), 8.77 (2H,m), 9.05 (1H,d,*J*=4Hz), 9.35 (1H,d,*J*=10Hz).
8c*	3.19 3.58	5.35 5.42	5.23	5.85 (d)	–	–	4.53(2H,dt,14&4Hz) 6.16(1H,tt,54&4Hz)	7.90 (1H,dd,*J*=10&5Hz), 8.25, 8.44 (each1H,d,*J*=2Hz), 8.66 (1H,d,*J*=10Hz), 8.94 (1H,d,*J*=4Hz).
8d	3.01 3.43	5.24 5.49	4.99	5.65	9.66	8.19	4.70(2H,q,9Hz)	7.95 (1H,dd,*J*=9&4Hz), 8.76 (2H,m), 9.05 (1H,d,*J*=4Hz), 9.36 (1H,d,*J*=9Hz).
8e	3.05 3.38	5.27 5.49	4.98	5.62	9.44	8.11	0.5~0.8(4H,m) 4.03(1H,m)	7.95 (1H,dd,*J*=9&5Hz), 8.75 (2H,s), 9.04 (1H,d,*J*=5Hz), 9.31 (1H,d,*J*=9Hz).
8f	3.05 3.32	5.27 5.48	5.00	5.67	9.46	8.15	1.21(6H,d,6Hz) 4.34(1H,m)	7.97 (1H,dd,*J*=9&4Hz), 8.77 (2H,s), 9.06 (1H,d,*J*=4Hz), 9.33 (1H,d,*J*=9Hz).
8g	3.06 3.51	5.29 5.49	5.00	5.66	9.51	8.14	1.4~2.3(6H,m) 4.68(1H,m)	7.96 (1H,dd,*J*=9&5Hz), 8.77 (2H,s), 9.05 (1H,d,*J*=5Hz), 9.34 (1H,d,*J*=9Hz).
8h	3.04 3.36	5.28 5.46	4.98	5.63	9.44	8.12	1.4~1.9(8H,m) 4.67(1H,m)	7.95 (1H,dd,*J*=9&5Hz), 8.75 (2H,m), 9.04 (1H,d,*J*=5Hz), 9.31 (1H,d,*J*=9Hz).
8i	3.04 3.30	5.30 5.50	4.98	5.64	9.47	8.14	1.8~2.5(4H,m),5.40, 5.86,6.07(each1H,m)	7.97 (1H,dd,*J*=10&5Hz), 8.77 (2H,s), 9.04 (1H,d,*J*=5Hz), 9.34 (1H,d,*J*=10Hz).
8j	3.06 3.53	5.30 5.47	4.99	5.68	9.44	8.13	0.84(6H,t,7Hz), 1.56 (4H,m), 3.95(1H,m)	7.96 (1H,dd,*J*=9&5Hz), 8.76 (2H,s), 9.05(1H,d,*J*=5Hz), 9.30 (1H,d,*J*=9Hz).
8k	3.05 3.30	5.27 5.46	4.99	5.66	9.45	8.11	1.1~1.9(10H,m) 4.06(1H,m)	7.95 (1H,dd,*J*=9&5Hz), 8.73,8.76 (each1H,d,*J*=2Hz), 9.05 (1H,d,*J*=5Hz), 9.31 (1H,d,*J*=9Hz).
8l	3.05 3.49	5.28 5.48	4.99	5.65	9.39	8.14	1.3~2.1(8H,m) 1.34(3H,s)	7.95 (1H,dd,*J*=9&4Hz), 8.76 (2H,m), 9.05 (1H,d,*J*=4Hz), 9.32 (1H,d,*J*=9Hz).
8m	3.05 3.31	5.33 5.49	5.01	5.66	9.51	8.14	1.8~2.3(4H,m) 1.05,1.08(each3H,s)	7.96 (1H,dd,*J*=10&4Hz), 8.77 (2H,s), 9.06 (1H,d,*J*=4Hz), 9.35 (1H,d,*J*=10Hz).
20a	3.02 3.47	5.20 5.36	4.99	5.64	9.45	8.14	1.4~1.9(8H,m) 4.68(1H,m)	6.46 (2H,br s), 7.64 (1H,dd,*J*=9&4Hz), 7.86 (1H,s), 8.90 (1H,d,*J*=4Hz), 8.98 (1H,d,*J*=9Hz).
20b	3.06 3.35	5.31 5.53	4.98	5.64	9.47	8.13	1.4~1.9(8H,m) 4.67(1H,m)	7.92 (1H,dd,*J*=9&4Hz), 8.52, 8.83 (each1H,s), 9.09 (1H,d,*J*=4Hz), 9.34 (1H,d,*J*=9Hz).
20c	3.04 3.39	5.31 5.46	4.98	5.64	9.47	8.11	1.3~1.9(8H,m) 4.67(1H,m)	6.75 (2H,br s), 7.83 (1H,dd,*J*=10&4Hz), 8.46 (1H,s), 9.03 (1H,d,*J*=4Hz), 9.28 (1H,d,*J*=10Hz), 9.94 (1H,br s).
20d	3.17 3.33	5.15 5.31	4.97	5.61	9.43	8.12	1.4~1.9(8H,m) 4.68(1H,m)	7.20 (2H,br s), 7.21 (1H,d,*J*=10Hz), 8.17 (1H,d,*J*=2Hz), 8.32 (1H,d,*J*=2Hz), 8.77 (1H,d,*J*=10Hz).
20e	3.04 3.42	5.20 5.39	5.00	5.64	9.45	8.11	1.4~1.9(8H,m) 4.68(1H,m)	7.06 (2H,br s), 8.17 (1H,d,*J*=10Hz), 8.48 (1H,d,*J*=2Hz), 8.55 (1H,d,*J*=2Hz), 9.05 (1H,d,*J*=10Hz), 10.36 (1H,br s).
20f	3.04 3.43	5.31 5.50	5.00	5.65	9.44	8.11	1.4~1.9(8H,m) 4.68(1H,m)	8.21, 8.54 (each1H,br s), 8.36 (1H,d,*J*=10Hz), 8.69, 8.92 (each1H,d,*J*=2Hz), 9.39 (1H,d,*J*=10Hz).
20g	2.99 3.38	5.07 5.22	4.98	5.62	9.43	8.13	1.4~1.9(8H,m) 4.70(1H,m)	5.77, 7.00 (each2H,br s), 7.00 (1H,d,*J*=10Hz), 7.47 (1H,s), 8.52 (1H,d,*J*=10Hz).
20h	3.07 3.39	5.19 5.32	4.98	5.64	9.46	8.12	1.4~1.9(8H,m) 4.69(1H,m)	7.19 (2H,br s), 7.20 (1H,d,*J*=10Hz), 8.42 (1H,s), 8.44 (1H,s), 8.77 (1H,d,*J*=10Hz).
20i	3.05 3.38	5.17 5.35	4.98	5.63	9.46	8.12	1.4~1.9(8H,m) 4.68(1H,m)	6.64, 7.07 (each2H,br s), 7.13 (1H,d,*J*=10Hz), 8.09 (1H,s), 8.73 (1H,d,*J*=10Hz), 9.20 (1H,br s).
20j	2.98 3.39	5.09 5.24	4.98	5.62	9.42	8.11	1.4~1.9(8H,m) 4.67(1H,m)	4.08 (3H,s), 6.29 (2H,br s), 7.32 (1H,d,*J*=10Hz), 7.71 (1H,s), 8.86 (1H,d,*J*=10Hz).
20k	3.03 3.39	5.22 5.48	4.98	5.64	9.47	8.12	1.4~1.9(8H,m) 4.67(1H,m)	4.14 (3H,s), 7.60 (1H,d,*J*=10Hz), 8.56 (1H,s), 8.66 (1H,s), 9.19 (1H,d,*J*=10Hz).

* measured in D_2O.

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