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Novel Cephalosporin Derivatives Possessing a Bicyclic Heterocycle at the 3-Position. Part I: Synthesis and Biological Activities of 3-(Benzothiazol-2-yl)thiocephalosporin Derivatives, CP0467 and Related Compounds

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Abstract—A series of cephalosporin derivatives with various bicyclic heterocycles at the C-3 position was synthesized and evaluated for antibacterial activity. Among them CP0467 (**3a**) showed excellent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) ($MIC_{90} = 6.25\,\mu g/mL$), and extremely high affinity for the penicillin binding protein 2′ of MRSA ($I_{50} = 0.49\,\mu g/mL$). Furthermore, **3a** showed a long-acting pharmacokinetic profile in mice ($AUC_{\infty} = 482.3\,\mu g/h/mL$ and $T_{1/2} = 1.9\,h$).

Introduction

Various new parenteral cephalosporin derivatives showing a broad antibacterial spectrum and high potency have been investigated.[1–4] Although they are generally effective against Gram-negative bacteria including *Pseudomonas aeruginosa*, their activity against Gram-positive bacteria is insufficient for clinical use. Consequently, new agents are still required to treat nosocomial and opportunistic infections caused by multiple drug-resistant Gram-positive bacteria, especially methicillin-resistant *Staphylococcus aureus* (MRSA). A few cephalosporin derivatives possessing enhanced antibacterial activity against Gram-positive bacteria have been reported,[3,5,6] but none has yet been developed as an anti-MRSA agent.

We have investigated hetero-bicyclic compounds having a thiazole ring[5] as the 3-substituent in the cephem ring with the aim of finding compounds with superior antibacterial activity against Gram-positive bacteria. In this paper, we describe the synthesis and the biological activities of 3-(benzothiazol-2-yl)thiocephalosporin derivatives, CP0467 (**3a**) and related compounds (Figure 1).[7]

Chemistry

The cephalosporin derivatives **1a**–**1h** were synthesized as shown in Scheme 1. Treatment of **4**[8,9] with *N*,*O*-bis (trimethylsilyl)acetamide, followed by reaction with (*Z*) 2-methoxyimino-2-(2-tritylaminothiazol-4-yl)acetic acid using Vilsmeier reagent gave the acylated product, which was treated with trifluoromethanesulfonic anhydride and pyridine to yield the enol triflate **5**.[10] Substitution of **5** with various sodium thiolates afforded mixtures of 3-cephem (**6a**–**6h**) and 2-cephem compounds (**7a**–**7h**). Without purification, treatment of each mixture with *m*-chloroperoxybenzoic acid (mCPBA) gave *S*-oxide compounds, which were reduced with phosphorus trichloride (PCl_3) to yield the 3-cephems **6a**–**6h**.[11] Removal of the protecting groups of **6a**–**6h** using trifluoroacetic acid and anisole gave the cephalosporin derivatives **1a**–**1h**.

The cephalosporin derivatives **2** and **3** were prepared as shown in Scheme 2. Compound **9** was prepared from **8**[12] in the same manner as described above. The phenylacetyl group of **9** was removed by treatment with phosphorus

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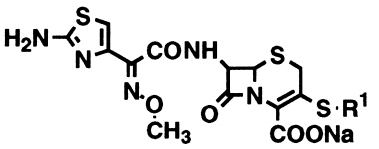
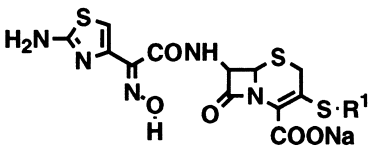
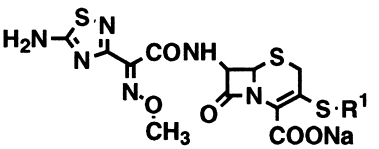


1a-1h



2a, 2b



3a (CP0467), 3b

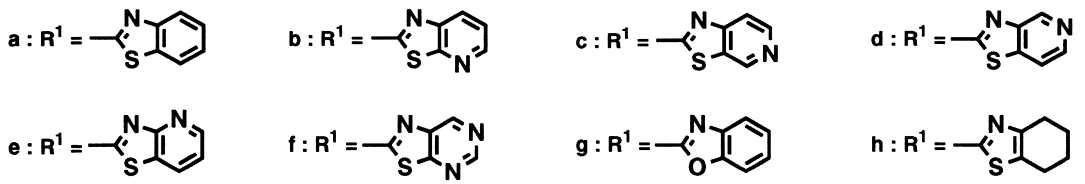


Figure 1. The series of cephalosporin derivatives.

pentachloride (PCl_5) and pyridine, followed by methanolysis and hydrolysis to afford the 7-aminocephem **10**.[13] Acylation of **10** with 2-(2-tritylaminothiazol-4-yl)-(*Z*)-2-trityloxyiminoacetic acid in the presence of phosphorus oxychloride ($POCl_3$) and pyridine yielded **11**. On the other hand, treatment of **10** with 2-(5-amino-1,2,4-thiadiazol-3-yl)-(*Z*)-2-methoxyiminoacetic acid in the presence of 1-hydroxybenzotriazole (HOBT) and dicyclohexylcarbodiimide (DCC) gave **12**. Removal of the protecting groups of **11** and **12** was performed by a similar procedure to that used in the synthesis of **1a**–**1h** to afford the final products **2** and **3**, respectively.

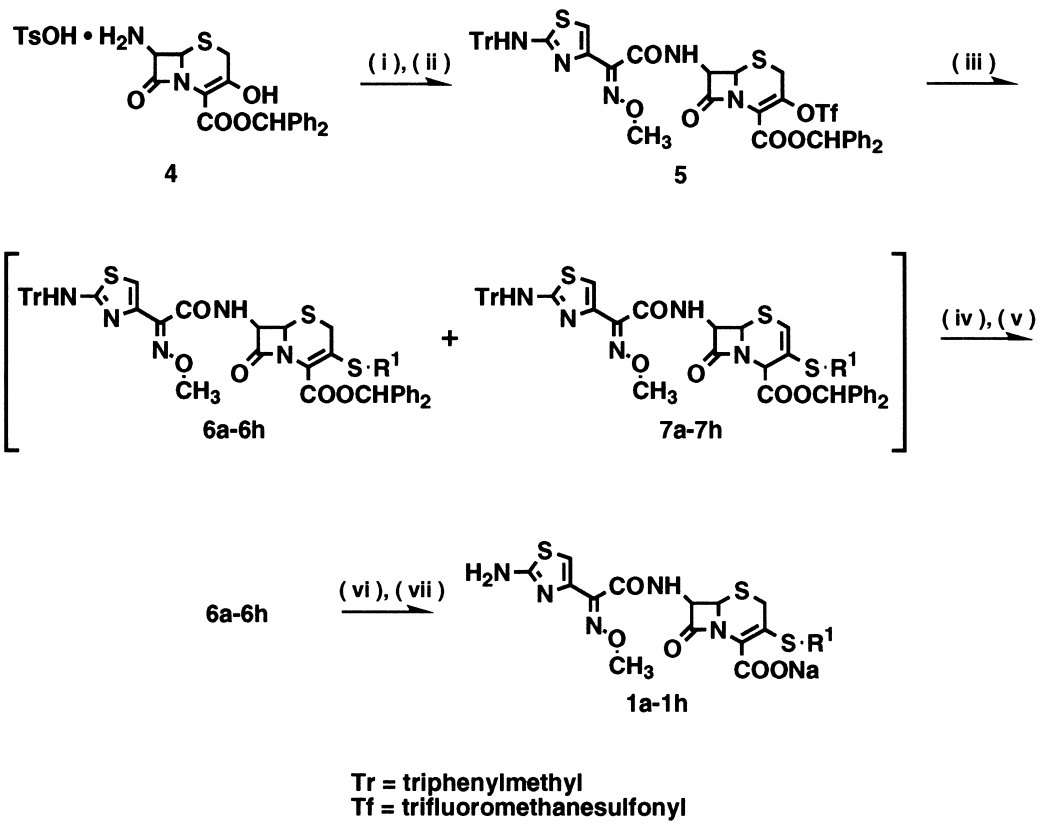
Results and Discussion

The antibacterial activity of the 3-(heterocycle)thiocephalosporin derivatives bearing a 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamide group at the 7-position (**1a**–**h**) is shown in Table 1. These compounds had strong or fairly strong antibacterial activity against Gram-positive bacteria, including MRSA. Among them, the benzothiazole derivative (**1a**), thiazolo[5,4-*b*]pyridine derivative (**1b**) and thiazolo[4,5-*c*]pyridine derivative (**1d**) exhibited strong antibacterial activity against MRSA. The benzoxazole derivative (**1g**), thiazolo[5,4-*d*]pyrimidine derivative (**1f**) and 4,5,6,7-tetrahydrobenzothiazole derivative (**1h**) were two to four times less active than **1a** against the two MRSA strains.

In an attempt to improve the antibacterial activity of **1a** and **1b**, the 2-(2-aminothiazol-4-yl)-(*Z*)-2-hydroxyiminoacetamide and 2-(5-amino-1,2,4-thiadiazol-3-yl)-(*Z*)-2-methoxyiminoacetamide groups were introduced at the 7-position instead of the 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamide group. The antibacterial activities of compounds **2** and **3** thus obtained are shown in Table 2. Compounds **2a** and **3a** showed stronger activity than **1a** against MRSA, but **2b** and **3b** were only as active or less active than **1b**, respectively. Among all the compounds, **2a** and **3a** (CP0467) showed the strongest antibacterial activity against MRSA.

The sensitivities of 25 clinical isolates of MRSA to **1a**, **2a** and **3a** were examined, and the results are shown in Figure 2 and Table 3. The MIC_{90} values of the three compounds were much lower than those of imipenem/cilastatin[14] and flomoxef.[15] Since these compounds showed such strong antibacterial activity against MRSA, they are considered to be promising candidates for clinical application.

The pharmacokinetic profiles in mice of **1a**, **1b**, **2a**, **2b**, **3a** and **3b** are summarized in Table 4. All of the



Reagents :
(i) *N,O*-bis(trimethylsilyl)acetamide, (*Z*)-2-methoxyimino-2-(2-tritylaminothiazol-4-yl)acetic acid, Vilsmeier reagent (DMF, trichloromethyl chloroformate), pyridine / CH_2Cl_2
(ii) Tf_2O, $^iPr_2NEt$ / CH_2Cl_2 (iii) NaS-R^1 / THF (iv) *m*CPBA / CH_2Cl_2
(v) PCl_3 / DMF (vi) TFA, anisole (vii) $NaHCO_3$ / H_2O

Scheme 1. Synthesis of the cephalosporin derivatives **1a–1h**.

compounds exhibited long acting pharmacokinetic profiles, with low urinary recoveries. Compounds **3a** and **3b** bearing the 2-(5-amino-1,2,4-thiadiazol-3-yl)-(*Z*)-2-methoxyiminoacetamide group at the 7-position showed both higher AUC_∞ and longer $T_{1/2}$ than **1a** and **1b** with the 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamide group, respectively. However, **2a** and **2b**, bearing the 2-(2-aminothiazol-4-yl)-(*Z*)-2-hydroxyiminoacetamide group, showed poorer pharmacokinetic profiles than **1a** and **1b**, respectively. Compound **3a** showed the best pharmacokinetic profile in mice.

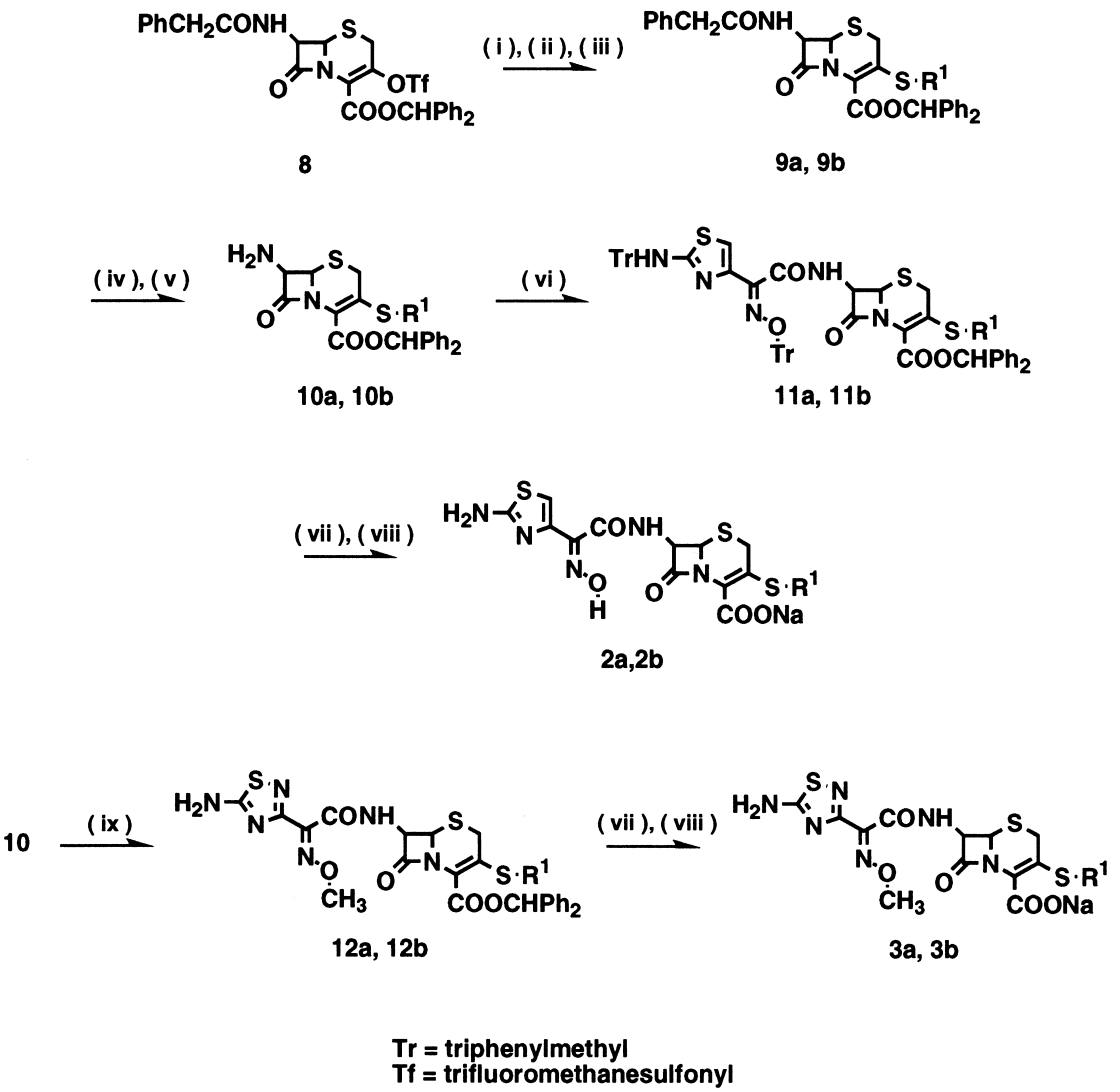
The binding affinity of **3a** (CP0467) for penicillin-binding protein 2′ (PBP2′) is shown in Table 5. The inhibitory concentration of **3a** was very much lower than that of imipenem/cilastatin or flomoxef. The strong antibacterial activity of **3a** against MRSA was considered to be due to the extremely high affinity of this compound for PBP2′ of MRSA.

Since **3a** (CP0467) showed such strong antibacterial activity against MRSA with an excellent pharmacokinetic profile, we have selected it for further evaluation. Further research on CP0467 and related compounds is in progress.

Experimental

General methods

^1H NMR spectra were measured with a JEOL JNM-GSX 400 NMR spectrometer for 400 MHz in $CDCl_3$ or DMSO-d_6 using TMS as an internal standard.



Reagents :
(i) NaS-R^1 / THF (ii) mCPBA / CH_2Cl_2 (iii) PCl_3 / DMF (iv) PCl_5, pyridine / CH_2Cl_2 (v) MeOH
(vi) 2-(2-tritylaminothiazol-4-yl)-(*Z*)-2-trityloxyiminoacetic acid, pyridine, $POCl_3$ / CH_2Cl_2
(vii) TFA, anisole (viii) $NaHCO_3$ / H_2O
(ix) 2-(5-amino-1,2,4-thiadiazol-3-yl)-(*Z*)-2-methoxyiminoacetic acid, HOBT, DCC / DMF

Scheme 2. Synthesis of the cephalosporin derivatives **2** and **3**.

IR spectra were recorded on a Shimadzu FT-IR 8100 spectrometer as KBr pellets. Mass spectra were obtained on a JEOL JMS-700 mass spectrometer for FABMS and FABHRMS. Silica gel flash column chromatography was performed on Wako-gel C-300 (Wako Chemical).

Antibacterial activity in vitro

Minimum inhibitory concentration (MIC) was determined by the agar plate dilution method. Test strains were subjected to seed culture using Sensitivity test broth (STB, Nissui Pharmaceutical). A 5 μL portion

Table 1. Antibacterial activity of **1a**–**h** and Flomoxef (MIC, μg/mL)

Test organism	1a	1b	1c	1d	1e	1f	1g	1h	FMOX[a]
S. aureus 209P JC-1	0.39	0.78	0.78	0.39	0.78	0.78	0.78	0.39	0.39
S. aureus M133[b]	3.13	3.13	6.25	3.13	6.25	6.25	12.5	6.25	3.13
S. aureus M126[b]	6.25	6.25	12.5	6.25	12.5	25	25	25	50
S. epidermidis ATCC14990	0.20	0.39	0.39	0.39	0.78	0.39	0.78	0.39	0.78
Enterococcus hirae ATCC8043	1.56	1.56	1.56	3.13	3.13	3.13	12.5	3.13	0.78
E. faecalis W-73	12.5	50	50	25	100	50	50	25	100
Escherichia coli NIHJ JC-2	3.13	1.56	3.13	3.13	1.56	3.13	1.56	1.56	0.10
Klebsiella pneumoniae PCI602	3.13	1.56	3.13	1.56	3.13	3.13	1.56	1.56	0.10
Proteus vulgaris GN76	3.13	1.56	1.56	1.56	1.56	3.13	> 100	0.78	0.78
Morganella morganii 1510/S-1	0.78	0.78	0.78	0.39	1.56	0.78	1.56	0.78	0.78
Citrobacter freundii GN346/16	3.13	3.13	6.25	6.25	3.13	6.25	3.13	1.56	0.39
Enterobacter cloacae G-0008	6.25	12.5	12.5	12.5	6.25	12.5	3.13	3.13	50
Serratia marcescens No.1	6.25	50	> 100	> 100	12.5	12.5	6.25	1.56	1.56
Pseudomonas aeruginosa E-2	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100

[a] Flomoxef.
[b] These strains are MRSA.

Table 2. Antibacterial activity of **2** and **3** (MIC, μg/mL)

Test organism	1a	2a	3a	1b	2b	3b
S. aureus 209P JC-1	0.39	0.20	0.39	0.78	0.20	0.78
S. aureus M133[a]	3.13	1.56	1.56	3.13	3.13	3.13
S. aureus M126[a]	6.25	3.13	3.13	6.25	6.25	12.5
S. epidermidis ATCC14990	0.20	0.20	0.39	0.39	0.20	0.39
Enterococcus hirae ATCC8043	3.13	0.78	1.56	1.56	3.13	6.25
E. faecalis W-73	50	1.56	6.25	50	6.25	25
Escherichia coli NIHJ JC-2	1.56	25	3.13	1.56	12.5	3.13
Klebsiella pneumoniae PCI602	3.13	25	3.13	1.56	12.5	3.13
Proteus vulgaris GN76	0.78	100	3.13	1.56	> 100	12.5
Morganella morganii 1510/S-1	1.56	3.13	1.56	0.78	1.56	0.78
Citrobacter freundii GN346/16	3.13	25	3.13	3.13	12.5	6.25
Enterobacter cloacae G-0008	6.25	> 100	12.5	12.5	100	6.25
Serratia marcescens No.1	6.25	> 100	12.5	50	100	12.5
Pseudomonas aeruginosa E-2	> 100	> 100	> 100	> 100	> 100	> 100

[a] These strains are MRSA.

of cell suspension of test strains having about 10^6 cfu/mL was inoculated and incubated at 37°C for 20 h. The MIC was then measured.

Pharmacokinetic test in mice

Test compound was subcutaneously administered at 25 mg/kg to 4-week old male Jcl:ICR mice. Blood was collected from the armpits of the mice at 5 min, 15 min, 30 min, 1 h and 2 h after the administration ($n = 1$). The collected blood was allowed to stand at 4°C for 2 h and centrifuged at 3000 rpm for 10 min to obtain the serum. To the serum was added an equivalent volume of methanol, and the resulting mixture stirred and centrifuged at 12 000 rpm for 3 min at 4°C. The supernatant was filtered through a filter having a pore size of 0.45 μm (Millipore) to give a serum sample. The concentration of the test compound in the serum sample was measured by the HPLC method. Pharmacokinetic parameters (AUC_∞ and $T_{1/2}$) was calculated by the Gauss-Newton method. The HPLC was performed as follows; column: Lichrosorb RP-18 (4.60×150 mm), flow rate: 0.7 mL/min, temperature: 40°C, detected by UV: 270 nm, developing solvent: CH_3CN, 10 mM aqueous CH_3COONH_4 system.

Subsequently, a test compound was subcutaneously administered to three mice in the same manner as described above. The three mice were put in a metabolic cage MM type (Sugiyamagen Co., Tokyo, Japan) and urine was collected at 0–4 h after the administration. The collected urine was filtered through a filter having a

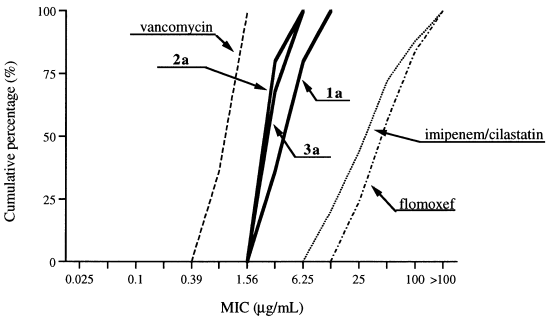


Figure 2. Sensitivities of MRSA (25 strains) to **1a**, **2a** and **3a**.

pore size of 0.45 μm (Millipore) to obtain an urine sample. The concentration of the test compound in the urine sample was measured by the HPLC method as described above, and recovery in the urine was calculated.

Binding affinity to PBP2′ of MRSA

The binding affinity to PBP2′ of MRSA were performed by the method described by Yokota.[16] PBP2′ was prepared from *Staphylococcus aureus* M-12 EHR.

Diphenylmethyl (6*R*,7*R*)-7-[(*Z*)-2-methoxyimino-2-(2-trityl-aminothiazol-4-yl)acetamide]-3-trifluoromethanesulfonyl-oxy-3-cephem-4-carboxylate (5). To a solution of DMF (1.121 g, 15.3 mmol) in dry CH_2Cl_2 (20 mL) was added trichloromethyl chloroformate (0.76 mL, 6.3 mmol) at 0°C and the mixture was stirred at the same temperature for 30 min to prepare Vilsmeier reagent. To the Vilsmeier reagent solution was added a suspension of (*Z*)-2-methoxyimino-2-(2-tritylaminothiazol-4-yl)-acetic acid (5.601 g, 12.6 mmol) in dry CH_2Cl_2 (20 mL) at −40°C, and the mixture was stirred at 0°C for 1 h to obtain an acid chloride solution.

Otherwise, **4** (5.00 g, 9.02 mmol) was dissolved in dry CH_2Cl_2 (200 mL) at 0°C, followed by addition of *N*,*O*-bis(trimethylsilyl)acetamide (6.7 mL, 27 mmol) and stirred at room temperature for 1 h. To the mixture were added pyridine (1.6 mL, 20 mmol) and the above acid chloride solution at −40°C and the mixture was stirred at the same temperature for 10 min. After addition of water (200 mL), the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (200 mL). The combined organic layer was washed with water (200 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography to afford diphenylmethyl 3-hydroxy-7-[(*Z*)-2-methoxyimino-2-(2-tritylaminothiazol-4-yl)acetamide]-3-cephem-4-carboxylate (5.003 g, 6.19 mmol, 69%) as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 3.34 (1H, d, J = 18 Hz), 3.55 (1H, d, J = 18 Hz), 4.09 (3H, s), 5.12 (1H, d, J = 5 Hz), 5.83 (1 H, dd, J = 5 Hz, 9 Hz), 6.79 (1H, s), 6.90 (1H, s), 7.20–7.40 (27H, m), 11.22 (1H, s).

To a solution of the above 3-hydroxy compound (3.572 g, 4.42 mmol) in dry CH_2Cl_2 (36 mL) were added *N*,*N*-diisopropylethylamine (0.85 mL, 4.9 mmol) and trifluoromethanesulfonic anhydride (1.372 g, 4.86 mmol) at −60°C and the mixture was stirred at the same

Table 3. Antibacterial activity of **1a**, **2a** and **3a** against MRSA (25 strains)

	MIC (μg/mL)		
	Range	MIC_{50}	MIC_{90}
1a	1.56–12.5	6.25	12.5
2a	1.56–6.25	3.13	6.25
3a	1.56–6.25	3.13	6.25
Imipenem/cilastatin	6.25–>100	50	>100
Flomoxef	12.5–>100	50	>100
Vancomycin	0.39–1.56	1.56	1.56

Table 5. Inhibitory concentration of **3a** against PBP2′ of MRSA

	I_{50}(μg/mL)	MIC(μg/mL)
3a	0.49	3.13
Imipenem/cilastatin	>400	100
Flomoxef	>400	>100

Strain: *Staphylococcus aureus* M-12 EHR.

Table 4. Pharmacokinetic profiles of **1**, **2** and **3** in mice

		1a	**2a**	**3a**	**1b**	**2b**	**3b**
Serum level	5 min	68.9	62.1	61.8	46.1	41.9	61.8
(μg/mL)	15 min	117.2	129.5	154.6	78.4	63.8	92.5
	30 min	111.1	102.0	130.1	58.4	42.6	82.6
	1 h	80.8	58.1	103.6	37.3	17.5	59.0
	2 h	58.3	30.6	79.9	12.5	10.3	24.6
	AUC_∞(μg/h/mL)	324.9	203.0	482.3	97.1	60.7	150.4
	$T_{1/2}$(h)	1.7	0.7	1.9	0.6	0.4	0.9
Urinary recovery	(%, 0–4 h)	0.9	0.0	6.4	11.1	0.0	9.5

temperature for 30 min. After addition of water (36 mL), the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (36 mL). The combined organic layer was washed with water (36 mL×3), dried over $MgSO_4$ and evaporated to give **5** (3.806 g, 4.05 mmol, 92%) as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 3.46 (1H, d, J = 18 Hz), 3.81 (1H, d, J = 18 Hz), 4.08 (3H, s), 5.15 (1H, d, J = 5 Hz), 5.95 (1H, dd, J = 5 Hz, 9 Hz), 6.73 (1H, s), 7.00 (1H, s), 7.20–7.40 (27H,m).

Sodium (6*R*,7*R*)-7-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamidel-3-(thiazolo[5,4-c]pyridin-2-yl)thio-3-cephem-4-carboxylate (1c). To a solution of 2-mercaptothiazolo[5,4-*c*]pyridine (196 mg, 1.17 mmol) in dry THF (10 mL) was added sodium hydride (47 mg of a 60% suspension, 1.2 mmol) at 0°C and the mixture was stirred at room temperature for 1 h to give the sodium thiolate solution.

To a solution of **5** (1.00 g, 1.06 mmol) in dry THF (10 mL) was added the sodium thiolate solution at 0°C and the mixture was stirred at room temperature for 1 h. After addition of 20% aqueous NaCl (50 mL), the mixture was extracted with ethyl acetate (50 mL×2). The combined organic layer was washed with 20% aqueous NaCl (50 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography to afford the mixture of **6c** and **7c** (507 mg, 0.529 mmol, 50%)

To the mixture of **6c** and **7c** (507 mg, 0.529 mmol) in CH_2Cl_2 (5 mL) was added a solution of *m*-chloroperoxybenzoic acid (183 mg, 1.06 mmol) in CH_2Cl_2 (5 mL) at 0°C and the mixture was stirred at the same temperature for 15 min. The reaction mixture was washed with 5% aqueous $Na_2S_2O_3$ (10 mL) and saturated aqueous $NaHCO_3$ (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. To a solution of the residue in dry DMF (5 mL) was added phosphorus trichloride (51 μL, 0.58 mmol) at 0°C, and the mixture was stirred at the same temperature. After addition of ethyl acetate (50 mL) and 20% aqueous NaCl (50 mL), the organic layer was separated and the aqueous layer was extracted with ethyl acetate (50 mL). The combined organic layer was washed with 20% aqueous NaCl (100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography to give **6c** (187 mg, 0.195 mmol, 37%) as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 3.58 (1H, d, J = 18 Hz), 3.97 (1H, d, J = 18 Hz), 4.08 (3H, s), 5.23 (1H, d, J = 5 Hz), 6.05 (1H, dd, J = 5 Hz, 9 Hz), 6.75 (1H, s), 6.97 (1H, s), 7.15–7.35 (27H, m), 7.25 (1H, d, J = 5 Hz), 8.60 (1H, d, J = 5 Hz), 9.03 (1 H, s).

To a solution of **6c** (187 mg, 0.195 mmol) in anisole (0.9 mL) was added trifluoroacetic acid (1.8 mL) at 0°C. After the mixture was stirred at the same temperature for 30 min, the mixture was poured into diisopropyl ether (9 mL) under ice cooling. The precipitates were collected by filtration. The suspension of the precipitates in water (3 mL) was adjusted to pH 8.0 with saturated aqueous $NaHCO_3$ and purified by Diaion HP-20 (Mitsubishi chemical) column chromatography (20 mL) to afford **1c** (75 mg, 0.13 mmol, 67%) as an amorphous powder: ^{1}H NMR (DMSO-d_6) δ 3.42 (1H, d, J = 18 Hz), 3.85 (3H, s), 3.96 (1H, d, J = 18 Hz), 5.20 (1H, d, J = 5 Hz), 5.71 (1H, dd, J = 5 Hz, 9 Hz), 6.73 (1H, s), 7.18 (2H, s), 7.75 (1H, d, J = 4 Hz), 8.51 (1H, d, J = 4 Hz), 9.16 (1H, s), 9.70 (1H, d, J = 9 Hz); IR (KBr) cm^{-1} 1770, 1670(sh), 1610, 1540, 1400, 1390, 1350, 1050, 1010; FABMS m/z 572 [$(M+Na)^+$]; FABHRMS calcd for $C_{19}H_{15}N_7O_5S_4Na$ [$(M+Na)^+$]: 571.9916, found: 571.9913.

Compounds 1a, **1b**, **1d**–**1h**. They were prepared from **5** by a similar procedure as described for the preparation of **1c**.

1a: ^{1}H NMR (DMSO-d_6) δ 3.40 (1H, d, J = 17 Hz), 3.86 (3H, s), 3.94 (1H, d, J = 17 Hz), 5.18 (1H, d, J = 5 Hz), 5.70 (1H, dd, J = 5 Hz, 9 Hz), 6.73 (1H, s), 7.16 (2H, s), 7.32 (1H, t, J = 8 Hz), 7.42 (1H, t, J = 8 Hz), 7.80 (1H, d, J = 8 Hz), 7.92 (1H, d, J = 8 Hz), 9.71 (1H, d, J = 9 Hz); IR (KBr) cm^{-1} 1770, 1670, 1610, 1540, 1450, 1420, 1390, 1350, 1050, 1000, 760; FABMS m/z 571 [$(M+Na)^+$]; FABHRMS calcd for $C_{20}H_{16}N_6O_5S_4Na$ [$(M+Na)^+$]: 570.9963, found: 570.9985.

1b: ^{1}H NMR (DMSO-d_6) δ 3.42 (1H, d, J = 18 Hz), 3.86 (3H, s), 3.96 (1H, d, J = 18 Hz), 5.21 (1H, d, J = 5 Hz), 5.72 (1H, dd, J = 5 Hz, 9 Hz), 6.73 (1H, s), 7.16 (2H, s), 7.46 (1H, dd, J = 8 Hz, 4 Hz), 8.13 (1H, d, J = 8 Hz), 8.45 (1H, d, J = 4 Hz), 9.70 (1H, d, J = 9 Hz); IR (KBr) cm^{-1} 1770, 1670, 1610, 1540, 1440, 1420, 1390, 1350, 1050, 800; FABMS m/z 572 [$(M+Na)^+$]; FABHRMS calcd for $C_{19}H_{15}N_7O_5S_4Na$ [$(M+Na)^+$]: 571.9916. found: 571.9913.

1d: ^{1}H NMR (DMSO-d_6) δ 3.40 (1H, d, J = 18 Hz), 3.85 (3H, s), 3.94 (1H, d, J = 18 Hz), 5.19 (1H, d, J = 5 Hz), 5.71 (1H, dd, J = 5 Hz, 9 Hz), 6.73 (1H, s), 7.19 (2H, s), 8.03 (1H, d, J = 4 Hz), 8.40 (1H, d, J = 8 Hz), 9.03 (1H, s), 9.79 (1H, d, J = 9 Hz); IR (KBr) cm^{-1} 1770, 1670(sh), 1610, 1540, 1440, 1420, 1390, 1350, 1050, 1010(sh); FABMS m/z 572 [$(M+Na)^+$]; FABHRMS calcd for $C_{19}H_{15}N_7O_5S_4Na$ [$(M+Na)^+$]: 571.9916, found: 571.9913.

1e: ^{1}H NMR (DMSO-d_6) δ 3.42 (1H, d, J = 18 Hz), 3.87 (3H, s), 3.96 (1H, d, J = 18 Hz), 5.20 (1H, d, J = 5 Hz), 5.72 (1H, dd, J = 5 Hz, 9 Hz), 6.73 (1H, s), 7.16 (2H, s), 7.30 (1H, dd, J = 8 Hz, 4 Hz), 8.40 (1H, d, J = 8 Hz), 8.53

(1H, d, $J=4$ Hz), 9.71 (1H, d, $J=9$ Hz); IR (KBr) cm^{-1} 1770, 1670, 1610, 1540, 1450, 1390, 1350, 1050; FABMS m/z 572 [$(M+Na)^+$], FABHRMS calcd for $C_{19}H_{15}N_7O_5S_4Na$ [$(M+Na)^+$]: 571.9916, found: 571.9913.

1f: ^1H NMR (DMSO-d_6) δ 3.41 (1H, d, $J=18$ Hz), 3.85 (3H, s), 3.96 (1H, d, $J=18$ Hz), 5.22 (1H, d, $J=5$ Hz), 5.74 (1H, dd, $J=5$ Hz, 9 Hz), 6.73 (1H, s), 7.17 (2H, s), 9.00 (1H, s), 9.17 (1H, s), 9.72 (1H, d, $J=9$ Hz); IR (KBr) cm^{-1} 1770, 1670(sh), 1610, 1530, 1430, 1370, 1050, 1000; FABMS m/z 573 [$(M+Na)^+$]; FABHRMS calcd for $C_{18}H_{14}N_8O_5S_4Na$ [$(M+Na)^+$]: 572.9868, found: 572.9890.

1g: ^1H NMR (DMSO-d_6) δ 3.44 (1H, d, $J=18$ Hz), 3.86 (3H, s), 4. 00 (1H, d, $J=18$ Hz), 5.19 (1H, d, $J=5$ Hz), 5.70 (1H, dd, $J=5$ Hz, 9 Hz), 6.73 (1H, s), 7.15 (2H, s), 7.31 (2H, m), 7.60 (2H, m), 9.63 (1H, d, $J=9$ Hz); IR (KBr) cm^{-1} 1770, 1670, 1610, 1540, 1500, 1450, 1390, 1350, 1050, 750; FABMS m/z 555 [$(M+Na)^+$]; FABHRMS calcd for $C_{20}H_{16}N_6O_6S_3Na$ [$(M+Na)^+$]: 555.0191, found: 555.0210.

1h: ^1H NMR (DMSO-d_6) δ 1.77 (4H, m), 2.60–2.75 (4H, m), 3.24 (1H, d, $J=18$ Hz), 3.65 (1H, d, $J=18$ Hz), 3.83 (3H, s), 5.08 (1H, d, $J=5$ Hz), 5.62 (1H, dd, $J=5$ Hz, 9 Hz), 6.71 (1H, s), 7.17 (2H, s), 9.60 (1H, d, $J=9$ Hz); IR (KBr) cm^{-1} 1770, 1670,1610, 1540,1390, 1350, 1050, 1000; FABMS m/z 575 [$(M+Na)^+$]; FABHRMS calcd for $C_{20}H_{20}N_6O_5S_4Na$ [$(M+Na)^+$]: 575.0276, found: 575.0280.

Sodium (6*R*, 7*R*)-7-[2-(2-aminothiazol-4-yl)-(*Z*)-2-hydroxyiminoacetamide]-3-(benzothiazol-2-yl)thio-3-cephem-4-carboxylate (2a). The compound **9a** was prepared from **8** in 43% yield by a similar procedure as described for the preparation of **6c**.

9a: ^1H NMR ($CDCl_3$) δ 3.56 (1H, d, $J=18$ Hz), 3.64 (2H, ABq, $J=17$ Hz), 3.86 (1H, d, $J=18$ Hz), 5.07 (1H, d, $J=5$ Hz), 5.91 (1H, dd, $J=5$ Hz, 9 Hz), 6.13 (1H, d, $J=9$ Hz), 6.97 (1H, s), 7.20–7.50 (17H, m), 7.78 (1H, d, $J=7$ Hz), 7.95 (1H, d, $J=7$ Hz).

To a solution of **9a** (1.152 g, 1.17 mmol) in dry CH_2Cl_2 (12 mL) were added pyridine (0.33 mL, 4.1 mmol) and phosphorus pentachloride (553 mg, 2.66 mmol) at −15°C and the mixture was stirred at −5°C for 1 h. To the mixture was added methanol (4.3 mL, 0.11 mol) at −20°C and the solution was stirred at −5°C for 3 h. After addition of water (12 mL), the mixture was stirred at −5°C for 1 h and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (12 mL) and the combined organic layer was washed with saturated aqueous $NaHCO_3$. Then the organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography to afford **10a** (775 mg, 1.42 mmol, 80%) as an amorphous powder: ^1H NMR ($CDCl_3$) δ 1.78 (2H, br-s), 3.57 (1H, d, $J=18$ Hz), 3.88 (1H, d, $J=18$ Hz), 4.83 (1H, d, $J=5$ Hz), 5.06 (1H, d, $J=5$ Hz), 7.00 (1H, s), 7.15–7.50 (12H, m), 7.78 (1H, d, $J=7$ Hz), 7.93 (1H, d, $J=7$ Hz).

To a solution of **10a** (350 mg, 0.58 mmol) in dry CH_2C1_2 (7 mL) were added 2-(2-tritylaminothiazol-4-yl)-(*Z*)-2-trityloxyiminoacetic acid (531 mg, 0.790 mmol), pyridine (0.21 mL, 2.6 mmol), then phosphorus oxychloride (74 μL, 0.79 mmol) at −20°C, and the mixture was stirred at the same temperature for 20 min. After addition of water (7 mL), the mixture was stirred at room temperature for 1 h. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (7 mL). After the combined organic layer was dried over $MgSO_4$ and evaporated under reduced pressure, the residue was purified by silica gel flash column chromatography to give **11a** (566 mg, 0.477 mmol, 73%) as an amorphous powder: ^1H NMR ($CDCl_3$) δ 3.36 (1H, d, $J=18$ Hz), 3.81 (1H, d, $J=18$ Hz), 5.18 (1H, d, $J=5$ Hz), 6.12 (1 H, dd, $J=5$ Hz, 9 Hz), 6.43 (1H, s), 7.00 (1H, s), 7.20–7.50 (42H, m), 7.74 (1H, d, $J=8$ Hz), 7.93 (1H, d, $J=8$ Hz).

The compound **2a** was prepared from **11a** in 12% yield by a similar procedure as described for the preparation of **1c** from **6c**.

2a: ^1H NMR (DMSO-d_6) δ 3.32 (1H, d, $J=17$ Hz), 3.92 (1H, d, $J=17$ Hz), 5.18 (1H, d, $J=5$ Hz), 5.73 (1H, dd, $J=5$ Hz, 9 Hz), 6.65 (1H, s), 7.07 (2H, s), 7.31 (1H, t, $J=9$ Hz), 7.41 (1H, t, $J=9$ Hz), 7.80 (1H, d, $J=9$ Hz), 7.92 (1H, d, $J=9$ Hz), 9.55 (1H, d, $J=9$ Hz), 11.29 (1H, s); IR (KBr) cm^{-1} 1770, 1610, 1540, 1450, 1420, 1390, 1350, 750; FABMS m/z 557 [$(M+Na)^+$]; FABHRMS calcd for $C_{19}H_{14}N_6O_5S_4Na$ [$(M+Na)^+$]: 556.9807, found: 556.9813.

Sodium (6*R*, 7*R*)-7-[2-(2-aminothiazol-4-yl)-(*Z*)-2-hydroxyiminoacetamide]-3-(thiazolo[5,4-b]pyridin-2-yl)thio-3-cephem-4-carboxylate (2b). The compound **2b** was prepared from **8** in 10% yield by a similar procedure as described for the preparation of **2a**.

2b: ^1H NMR (DMSO-d_6) δ 3.40 (1H, d, $J=18$ Hz), 3.95 (1H, d, $J=18$ Hz), 5.22 (1H, d, $J=5$ Hz), 5.74 (1H, dd, $J=5$ Hz, 9 Hz), 6.65 (1H, s), 7.09 (2H, s), 7.46 (1H, dd, $J=8$ Hz, 4 Hz), 8.14 (1H, d, $J=8$ Hz), 8.45 (1H, d, $J=4$ Hz), 9.56 (1H, d, $J=9$ Hz), 11.29 (1H, s); IR (KBr) cm^{-1} 1770, 1610, 1540, 1440, 1390, 1350, 1300, 1050, 1020, 1000, 800, 750; FABMS m/z 558 [$(M+Na)^+$]; FABHRMS calcd for $C_{18}H_{13}N_7O_5S_4Na$ [$(M+Na)^+$]: 557.9759, found: 557.9733.

Sodium (6*R*, 7*R*)-7-[2-(5-amino-1,2,4-thiadiazol-3-yl)-(*Z*)-2-methoxyiminoacetamide]-3-(benzothiazol-2-yl)thio-3-cephem-4-carboxylate (3a). To a solution of 2-(5-amino-1,2,4-thiadiazol-3-yl)-(*Z*)-2-methoxyiminoacetic acid (136 mg, 0.673 mmol) in dry DMF (2 mL) were added *N*-hydroxybenzotriazole (91 mg, 0.67 mmol) and dicyclohexylcarbodiimide (139 mg, 0.674 mmol) and the mixture was stirred at room temperature for 1.5 h. After addition of a solution of **10a** (239 mg, 0.450 mmol) in dry DMF (3 mL), the mixture was stirred at room temperature for 6 h. The precipitates were filtered off and 20% aqueous NaCl (50 mL) was added to the filtrate. The mixture was extracted with ethyl acetate (50 mL×2) and the extract was washed with 20% aqueous NaCl (100 mL), then dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography to give **12a** (324 mg, 0.450 mmol, quant.) as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 3.55 (1H, d, J = 18 Hz), 3.94 (1H, d, J = 18 Hz), 4.05 (3H, s), 5.22 (1H, d, J = 5 Hz), 6.18 (1H, dd, J = 5 Hz, 9 Hz), 6.34 (2H, s), 6.99 (1H, s), 7.15–7.50 (12H, m), 7.79 (1H, d, J = 8 Hz), 7.95 (1H, d, J = 8 Hz), 8.13 (1H, d, J = 9 Hz).

The compound **3a** was prepared from **12a** in 64% yield by a similar procedure as described for the preparation of **1c** from **6c**.

3a: ^{1}H NMR (DMSO-d_6) δ 3.37 (1H, d, J = 18 Hz), 3.90 (1H, d, J = 18 Hz), 3.92 (3H, s), 5.17 (1H, d, J = 5 Hz), 5.72 (1H, dd, J = 5 Hz, 9 Hz), 7.32 (1H, t, J = 8 Hz), 7.42 (1H, t, J = 8 Hz), 7.80 (1H, d, J = 5 Hz), 7.94 (1H, d, J = 8 Hz), 8.10 (2H, s), 9.65 (1H, d, J = 9 Hz); IR (KBr) cm^{-1} 1770, 1670, 1610, 1530, 1450, 1420, 1410, 1390, 1350, 1050, 1010, 750; FABMS m/z 572 [(M + Na)$^+$]; FABHRMS calcd for $C_{19}H_{15}N_7O_5S_4Na$ [(M + Na)$^+$]: 571.9916, found: 571.9913.

Sodium (6*R*,7*R*)-7-[2-(5-amino-1,2,4-thiadiazol-3-yl)-(*Z*)-2-methoxyiminoacetamide]-3-(thiazolo[5,4-*b*]pyridin-2-yl) thio-3-cephem-4-carboxylate (3b). The compound **3b** was prepared from **8** in 17% yield by a similar procedure as described for the preparation of **3a**.

3b: ^{1}H NMR (DMSO-d_6) δ 3.40 (1H, d, J = 18 Hz), 3.93 (3H, s), 3.96 (1H, d, J = 18 Hz), 5.20 (1H, d, J = 5 Hz), 5.75 (1H, dd, J = 5 Hz, 9 Hz), 7.47 (1H, dd, J = 8 Hz, 4 Hz), 8.10 (2H, s), 8.13 (1H, d, J = 8 Hz), 8.45 (1H, d, J = 4 Hz). 9.68 (1H, d, J = 9 Hz); IR (KBr) cm^{-1} 1770, 1670, 1610, 1530, 1390, 1350, 1050, 1010; FABMS m/z 573 [(M + Na)$^+$]; FABHRMS calcd for $C_{18}H_{14}N_8O_5S_4Na$ [(M + Na)$^+$]: 572.9868, found: 572.9853.

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