Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 1641–1653

BIOORGANIC & MEDICINAL CHEMISTRY

Novel Cephalosporin Derivatives Possessing a Bicyclic Heterocycle at the 3-Position. Part II: Synthesis and Antibacterial Activity of 3-(5-Methylthiazolo[4,5-*c*]pyridinium-2-yl)-thiomethylcephalosporin Derivatives and Related Compounds

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Received 3 April 1998; accepted 22 May 1998

Abstract—A series of cephalosporin derivatives with a thiazolopyridinium group at the 3-position was synthesized and evaluated for antibacterial activity. Some of these cephalosporin derivatives having a (5-alkylthiazolo[4,5-*c*]pyridinium-2-yl)thiomethyl group at the 3-position showed strong activity against Gram-positive and Gram-negative bacteria, including *Pseudomonas aeruginosa*. Among them, **5a** showed a good antibacterial spectrum in vitro, and also showed a similar or slightly superior activity to that of ceftazidime in vivo against *P. aeruginosa*.

Introduction

In a previous paper,[1] we reported that cephalosporin derivatives (**1** and **2**) bearing a benzothiazole or a thiazolopyridine group at the 3-position (Fig. 1) showed strong antibacterial activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), and had long-acting pharmacokinetic profiles. However, the activity of these compounds was relatively weak against Gram-negative bacteria, including *Pseudomonas aeruginosa*.

Recently, it was reported that cephalosporin derivatives with a quaternary ammonium moiety at the 3-position showed enhanced antibacterial activity against Gram-negative bacteria, including *P. aeruginosa*.[2–5] This led us to suppose that the quaternization of the thiazolopyridine group at the 3-position of our previous compounds might increase the antibacterial activity against Gram-negative bacteria. Here we describe the synthesis and antibacterial activity of 3-(5-methylthiazolo[4,5-*c*]pyridinium-2-yl)thiomethylcephalosporin derivatives and related compounds (Fig. 2).

Chemistry

Compounds **3a–3c, 3i, 4a–4c, 5a–5c, 6a–6c, 8a** and **9** were synthesized as shown in Scheme 1. Compound **10**[6] was acylated with (Z)-2-alkyloxyimino-2-(2-tritylaminothiazol-4-yl)acetic acid in the presence of phosphorus oxychloride ($POCl_3$) and pyridine to give **11–14**, respectively. The acylated products were converted into the sulfides **15a–15c, 16a–16c, 17a–17c** and **18a–18c** through the corresponding iodide intermediates by substitution with 2-mercaptothiazolo[4,5-*c*]pyridine (**a**), 2-mercaptothiazolo[5,4-*c*]pyridine (**b**) and 2-mercaptothiazolo[4,5-*b*]-pyridine (**c**), respectively. These sulfides were subjected to methylation with methyl iodide followed by deprotection using trifluoroacetic acid and anisole to afford the final compounds **3a–3c, 4a–4c, 5a–5c** and **6a–6c**, respectively. Compound **3i** was prepared from **15a** using tert-butyl bromoacetate instead of methyl iodide by means of a similar procedure to that described above. Compound **19**[1] was converted into **8a** in the same manner as described for the preparation of **3a**. Compound **9**

Key words: Cephalosporin; *Pseudomonas aeruginosa*; thiazolopyridinium group.
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0968-0896/98/$—see front matter
PII: S0968-0896(98)00103-5

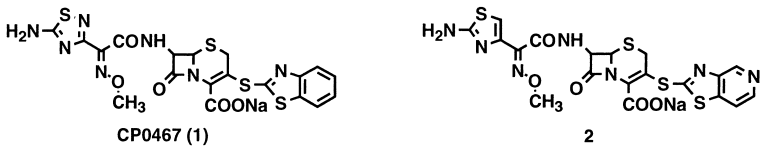


Figure 1. CP0467 (**1**) and the related compound (**2**).

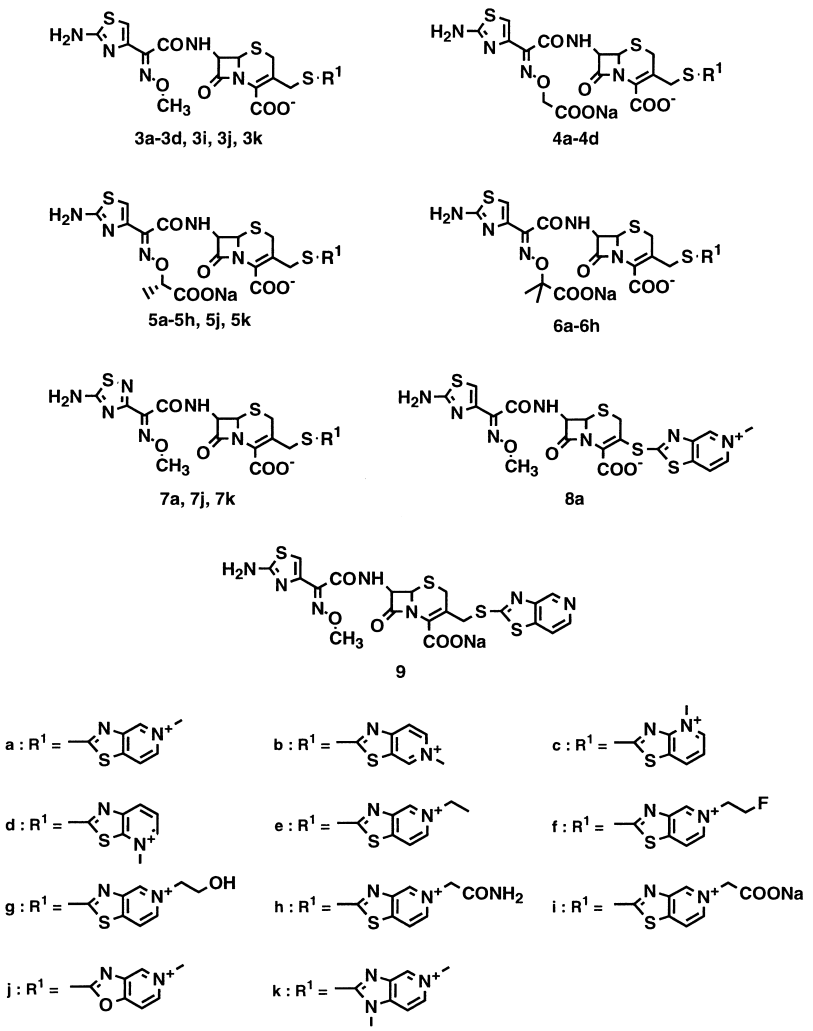
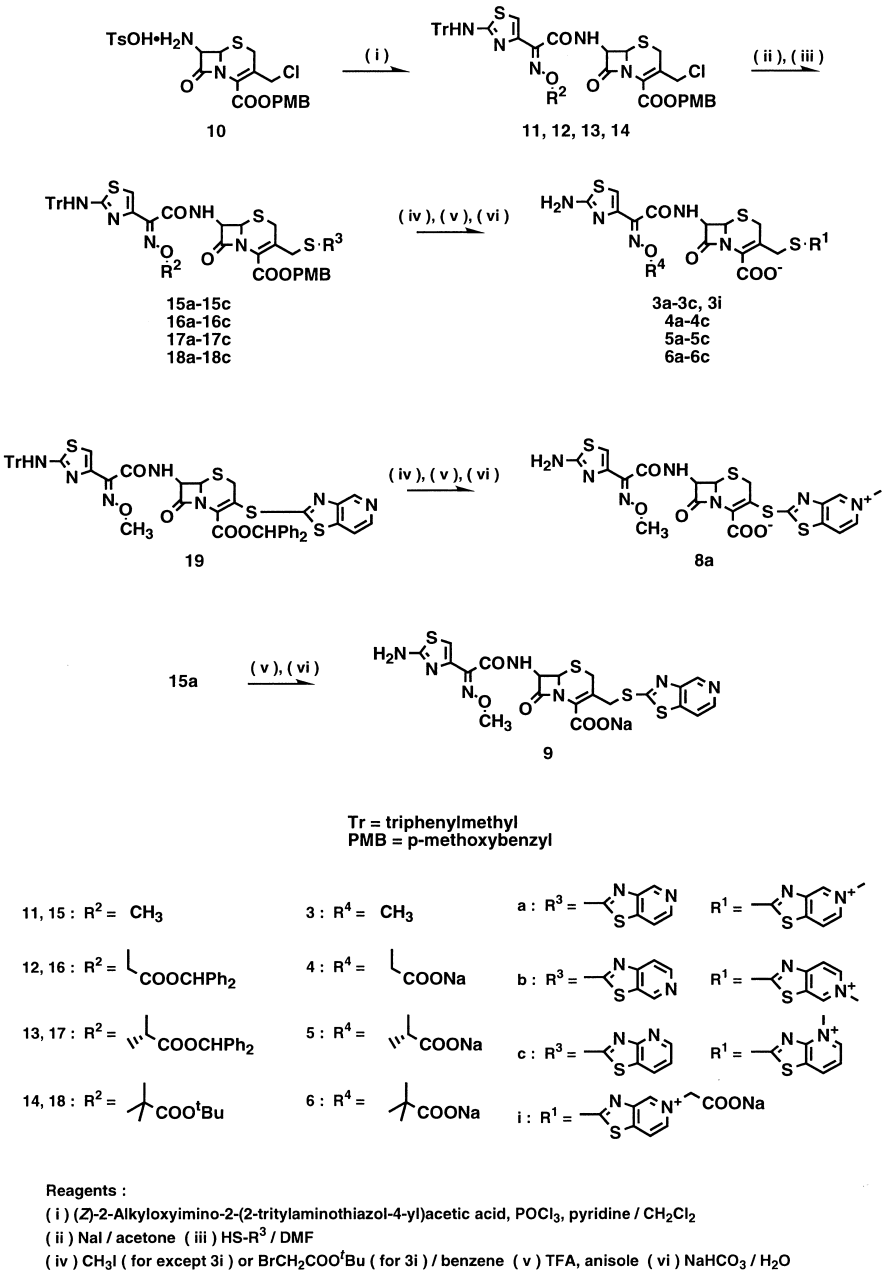


Figure 2. The series of cephalosporin derivatives.

was obtained by deprotection of **15a**. Contrary to expectation, we failed to obtain compound **3d** by a similar procedure to that described above. So the other compounds containing **3d** were prepared by using the quaternary ammonium thiols.

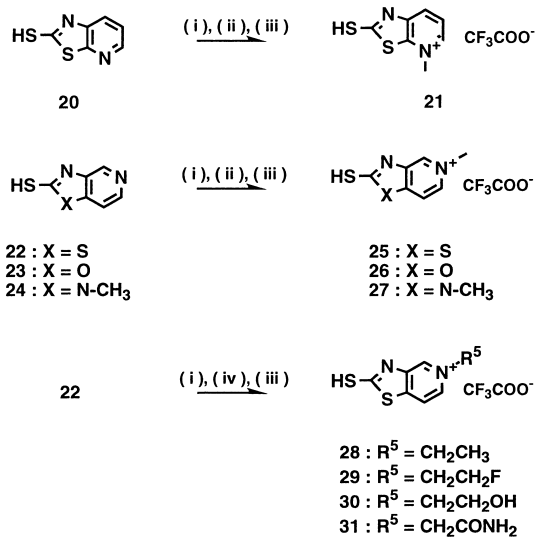
Heterocyclic compounds **21** and **25–31** were prepared as shown in Scheme 2. After the protection of the thiols **20** and **22–24** with diphenylmethyl bromide and triethylamine, the sulfides were methylated with methyl iodide to give quaternary ammonium sulfides. Removal of the diphenyimethyl group with trifluoroacetic acid and anisole yielded the quaternary ammonium thiols **21** and **25–27** as trifluoroacetates. Compounds **28–31** were also synthesized using the corresponding alkyl iodide for **28**, **30** and **31** or 2-fluoroethyl trifluoromethanesulfonate for **29**, instead of methyl iodide, by means of a similar procedure to that described above.



Scheme 1. Synthesis of the cephalosporin derivatives.

Compounds **3d, 3j, 3k, 4d, 5d–5h, 5j, 5k, 6d–6h, 7a, 7j** and **7k** were synthesized as shown in Scheme 3. Compounds **11–14** were converted into the sulfides **32d, 32j, 32k, 33d, 34d-34h, 34j, 34k** and **35d–35h** through the corresponding iodide intermediates by substitution with **21** and **25–31**, respectively. Removal of the protecting groups of these sulfides by treatment with trifluoroacetic acid and anisole gave the final compounds **3d, 3j, 3k, 4d, 5d–5h, 5j, 5k** and **6d–6h**.

Furthermore, acylation of **10** with 2-(5-amino-1,2,4-thiadiazol-3-yl)-(*Z*)-2-methoxyiminoacetyl chloride in the presence of *N,O*-bis(trimethylsilyl)acetamide gave **36**. Compound **36** was converted into the sulfides **37a, 37j** and **37k** through the corresponding iodide intermediates by substitution with **25–27**, respectively. Removal of the protecting group of these sulfides by treatment with trifluoroacetic acid and anisole afforded the cephalosporin derivatives **7a**, **7j** and **7k**.



Scheme 2. Synthesis of bicyclic heterocycles.

Results and Discussion

The antibacterial activity of compounds **2, 3a, 8a** and **9** is shown in Table 1. The quaternary ammonium derivative **8a** showed greater activity than **2** against Gram-negative bacteria except *Proteus vulgaris* and *P. aeruginosa*, whereas the activity of **8a** against Gram-positive bacteria, including MRSA, was decreased. The quaternary ammonium methyl derivative **3a** also showed greater activity than **9** against Gram-negative bacteria, while its activity against Gram-positive bacteria was similar to that of **9**. Furthermore, **3a** showed stronger activity than **2** and **8a** against Gram-positive and Gram-negative bacteria except MRSA and *Enterococcus faecalis*.

The antibacterial activity of compounds **3a–3d, 3i, 4a–4d, 5a–5d** and **6a–6d** is shown in Tables 2 and 3. Although **3a–3d** bearing the 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamide group at the 7-position showed potent activity against Gram-positive and Gram-negative bacteria, their activity against MRSA, *E. faecalis* and *P. aeruginosa* was weak. Compounds **4a–4d, 5a–5d** and **6a–6d** having a carboxyl group at the 7-position showed stronger activity against *P. aeruginosa* than **3a–3d**, but their activity against Gram-positive bacteria was decreased. On the other hand, introduction of a carboxyl group at the 3-position (**3i**) improved the activity against Gram-positive bacteria except MRSA and *E. faecalis* compared to the compounds containing a carboxyl group at the 7-position, but decreased the activity against *P. aeruginosa*. Furthermore, **3i** showed superior activity against Gram-negative bacteria and inferior activity against Gram-positive bacteria compared to **3a**. Among the compounds in Tables 2 and 3, **5a** showed potent activity against *P. aeruginosa*.

The antibacterial activity of compounds **5e–5h** and **6e–6h** is shown in Table 4. Their activity against Gram-positive and Gram-negative bacteria including *P. aeruginosa* was similar to that of **5a** and **6a**.

The antibacterial activity of compounds **3j, 3k, 5j, 5k, 7j** and **7k** is shown in Table 5. The oxazolo[4,5-*c*]pyridinium derivatives **3j, 5j** and **7j** showed inferior activity against Gram-positive bacteria compared to the corresponding thiazolo[4,5-*c*]pyridinium derivatives **3a, 5a** and **7a**. The imidazolo[4,5-*c*]pyridinium derivatives **3k, 5k** and **7k** showed similar activity to **3a, 5a** and **7a**.

As expected, the quaternary ammonium cephalosporin derivatives described in this report showed enhanced activity against Gram-negative bacteria. Some of them also showed potent activity against *P. aeruginosa*. Among them, **5a** showed a good antibacterial spectrum. Since the overall activity of **5a** was similar or slightly superior to that of ceftazidime,[7] which is in clinical use (Table 6), we selected **5a** for further evaluation.

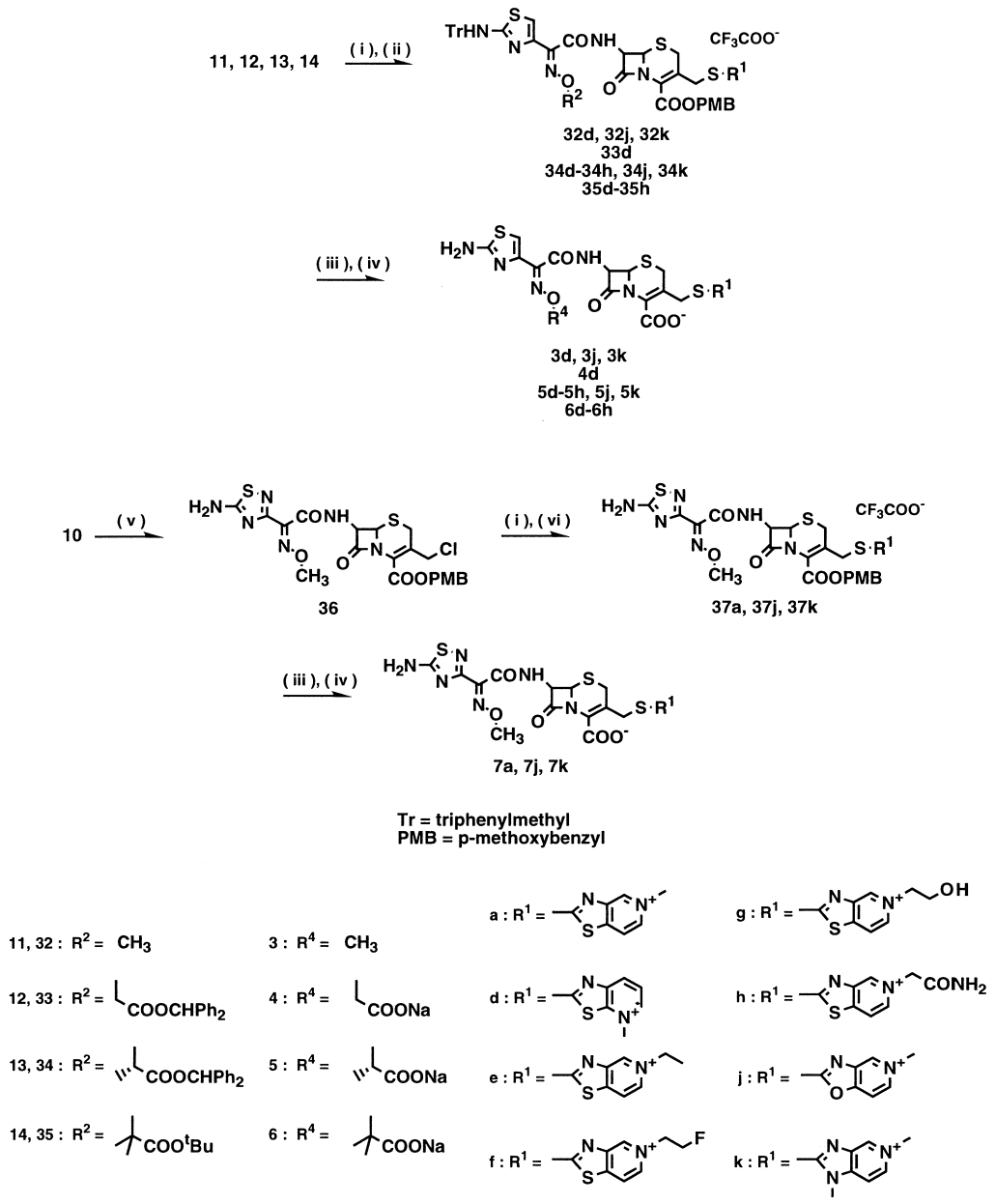
The pharmacokinetic profile of compound **5a** in mice was evaluated, and the result is shown in Table 7. This compound (**5a**) showed a similar or slightly superior pharmacokinetic profile to that of ceftazidime.

Finally, we examined the activity in vivo of compound **5a** against *P. aeruginosa*. As shown in Table 8, the therapeutic efficacy in systemic infection of **5a** in mice was similar or slightly superior to that of ceftazidime.

Further research on related compounds is in progress, with the aim of finding even more efficacious new drugs.

Experimental

General methods. ^{1}H NMR spectra were measured with a JEOL JNM-GSX 400 NMR spectrometer for 400 MHz or a Varian Gemini 300 NMR spectrometer for 300 MHz in $CDCl_3$, DMSO-d_6, or D_2O. TMS (0 ppm) in $CDCl_3$ and DMSO-d_6 or HDO (4.8 ppm) in D_2O was used as internal reference standard. IR spectra were recorded on a Shimadzu FT-IR 8100 spectrometer as KBr pellets. Mass spectra were obtained



Scheme 3. Synthesis of the series of cephalosporin derivatives.

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).

Table 1. Antibacterial activity of **2, 3a, 8a** and **9** (MIC, μg/mL)

Test organism	8a	3a	2	9
Staphylococcus aureus 209P JC-1	0.78	0.20	0.39	0.20
S. aureus M133[a]	6.25	50	3.13	25
S. aureus M126[a]	12.5	>100	6.25	>100
S. epidermidis ATCC 14990	0.78	0.20	0.39	0.20
Enterococcus hirae ATCC 8043	3.13	1.56	3.13	0.78
E. faecalis W-73	50	>100	25	100
Escherichia coli NIHJ JC-2	0.78	0.05	3.13	0.39
Klebsiella pneumoniae PCI602	0.39	0.05	1.56	0.39
Proteus vulgaris GN76	25	0.39	1.56	0.39
Morganella morganii 1510/S-1	0.78	<0.025	0.39	0.10
Citrobacter freundii GN346/16	3.13	0.10	6.25	0.39
Enterobacter cloacae G-0008	0.78	0.05	12.5	0.78
Serratia marcescens No. 1	12.5	0.05	>100	0.39
Pseudomonas aeruginosa E-2	>100	50	>100	50

[a]These strains are MRSA.

A 5 μL portion 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 3,000 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 (4.6ø×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 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.

Antibacterial activity in vivo. The antibacterial activity in vivo was tested using male mice (Jcl:ICR, 4-week old). Each of five mice in a group was challenged intraperitoneally with about 2.2×10^4 cfu of the bacterial suspension in 0.5 mL of saline containing 2.5% gastric mucin (Difco Laboratories). The animals were treated subcutaneously with test compounds 1 h after challenge. ED_{50} values (mg/mouse) were calculated by probit analysis from the number of survivals 7 days after infection.

Table 2. Antibacterial activity of **3a–3d, 3i** and **4a–4d** (MIC, μg/mL)

Test organism	3a	3b	3c	3d	4a	4b	4c	4d	3i
Staphylococcus aureus 209P JC-1	0.20	0.39	0.20	0.20	3.13	3.13	1.56	3.13	0.39
S. aureus M133[a]	50	25	1.56	6.25	50	50	50	50	100
S. aureus M126[a]	>100	100	>100	>100	>100	>100	>100	>100	>100
S. epidermidis ATCC14990	0.20	0.20	0.20	0.20	3.13	3.13	1.56	3.13	0.39
Enterococcus hirae ATCC8043	1.56	25	50	6.25	100	>100	>100	100	50
E. faecalis W-73	>100	100	25	100	>100	>100	>100	>100	>100
Escherichia coli NIHJ JC-2	0.05	0.10	0.10	0.05	0.05	0.05	0.05	0.05	<0.025
Klebsiella pneumoniae PCI602	0.05	0.05	0.05	0.05	<0.025	<0.025	<0.025	<0. 025	<0.025
Proteus vulgaris GN76	0.39	0.39	0.20	0.20	<0.025	<0.025	<0.025	0.05	0.05
Morganella morganii 1510/S-1	<0.025	0.05	0.10	<0.025	<0.025	<0.025	<0.025	0.05	<0.025
Citrobacter freundii GN346/16	0.10	0.10	0.10	0.10	0.20	0.20	0.10	0.20	0.05
Enterobacter cloacae G-0008	0.05	0.20	0.20	0.10	0.05	0.10	0.10	0.05	0.05
Serratia marcescens No.1	0.05	0.10	0.20	0.10	<0.025	<0.025	<0.025	<0.025	0.39
Pseudomonas aeruginosa E-2	50	100	25	100	12.5	25	12.5	50	100

[a]These strains are MRSA.

Table 3. Antibacterial activity of **5a–5d** and **6a–6d** (MIC, μg/mL)

Test organism	**5a**	**5b**	**5c**	**5d**	**6a**	**6b**	**6c**	**6d**
Staphylococcus aureus 209P JC-1	1.56	3.13	1.56	3.13	3.13	6.25	6.25	6.25
S. aureus M133[a]	12.5	100	100	50	50	100	> 100	100
S. aureus M126[a]	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
S. epidermidis ATCC14990	1.56	3.13	0.78	3.13	3.13	3.13	3.13	3.13
Enterococcus hirae ATCC8043	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
E. faecalis W-73	100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
Escherichia coli NIHJ JC-2	0.20	0.10	0.20	0.10	0.20	0.78	1.56	0.39
Klebsiella pneumoniae PCI602	0.05	0.10	0.05	0.05	0.20	0.20	0.39	0.20
Proteus vulgaris GN76	< 0.025	0.05	0.05	0.05	0.10	0.10	0.10	0.20
Morganella morganii 1510/S-1	0.05	0.10	0.05	0.10	0.05	0.10	0.10	0.10
Citrobacter freundii GN346/16	0.39	0.78	0.20	0.39	0.39	0.78	0.78	0.78
Enterobacter cloacae G-0008	0.20	0.20	0.20	0.20	0.20	0.78	0.78	0.39
Serratia marcescen No.1	0.05	0.05	0.05	0.05	0.05	0.10	0.20	0.10
Pseudomonas aeruginosa E-2	1.56	6.25	6.25	6.25	3.13	25	25	6.25

[a]These strains are MRSA.

Table 4. Antibacterial activity of **5a, 5e–5h, 6a** and **6e–6h** (MIC, μg/mL)

Test organism	**5a**	**5e**	**5f**	**5g**	**5h**	**6a**	**6e**	**6f**	**6g**	**6h**
Staphylococcus aureus 209P JC-1	1.56	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	1.56
S. aureus M133[a]	12.5	> 100	> 100	100	> 100	50	100	100	100	100
S. aureus M126[a]	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
S. epidermidis ATCC 14990	1.56	3.13	3.13	3.13	3.13	3.13	3.13	3.13	3.13	1.56
Enterococcus hirae ATCC8043	> 100	> 100	> 100	> 100	100	> 100	> 100	> 100	> 100	> 100
E. faecalis W-73	100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
Escherichia coli NIHJ JC-2	0.20	0.10	0.20	0.10	0.20	0.20	0.39	0.39	0.39	0.39
Klebsiella pneumoniae PCI602	0.05	0.05	0.05	0.05	0.10	0.20	0.10	0.20	0.20	0.20
Proteus vulgaris GN76	< 0.025	0.05	0.05	0.05	0.05	0.10	0.20	0.20	0.10	0.10
Morganella morganii 1510/S-1	0.05	0.05	0.10	0.05	0.10	0.05	0.10	0.10	0.10	0.10
Citrobacter freundii GN346/16	0.39	0.39	0.39	0.39	0.39	0.39	0.78	0.78	0.78	0.78
Enterobacter cloacae G-0008	0.20	0.10	0.20	0.20	0.20	0.20	0.39	0.39	0.39	0.78
Serratia marcescens No.1	0.05	< 0.025	< 0.025	< 0.025	0.05	0.05	0.05	0.05	0.05	0.10
Pseudomonas aeruginosa E-2	1.56	3.13	3.13	6.25	6.25	3.13	6.25	6.25	3.13	3.13

[a]These strains are MRSA.

Table 5. Antibacterial activity of **3a, 3j, 3k, 5a, 5j, 5k, 7a, 7j** and **7k** (MIC, μg/mL)

Test organism	**3a**	**3j**	**3k**	**5a**	**5j**	**5k**	**7a**	**7j**	**7k**
Staphylococcus aureus 209P JC-1	0.20	1.56	0.39	1.56	25	3.13	0.78	3.13	0.78
S. aureus M133[a]	50	> 100	12.5	12.5	> 100	100	12.5	100	6.25
S. aureus M126[a]	> 100	> 100	> 100	> 100	> 100	> 100	50	> 100	100
S. epidermidis ATCC 14990	0.20	1.56	0.39	1.56	25	3.13	0.78	3.13	1.56
Enterococcus hirae ATCC8043	1.56	100	3.13	> 100	> 100	100	3.13	> 100	50
E. faecalis W-73	> 100	> 100	100	100	> 100	> 100	100	> 100	50
Escherichia coli NIHJ JC-2	0.05	0.39	0.05	0.20	0.39	0.10	0.05	0.39	< 0.025
Klebsiella pneumoniae PCI602	0.05	0.10	< 0.025	0.05	0.20	0.05	0.05	0.39	< 0.025
Proteus vulgaris GN76	0.39	0.20	0.39	< 0.025	0.05	< 0.025	1.56	1.56	0.78
Morganella morganii 1510/S-1	< 0.025	0.20	< 0.025	0.05	0.39	< 0.025	0.10	0.78	< 0.025
Citrobacter freundii GN346/16	0.10	0.39	0.10	0.39	1.56	0.39	0.10	0.78	0.10
Enterbacter cloacae G-0008	0.05	0.78	0.05	0.20	0.78	0.20	0.10	0.78	0.05
Serratia marcescens No.1	0.05	0.39	0.05	0.05	0.20	< 0.025	0.10	0.78	0.05
Pseudomonas aeruginosa E-2	50	> 100	100	1.56	25	6.25	25	> 100	25

[a]These strains are MRSA.

Table 6. Antibacterial activity of **5a** and ceftazidime (MIC, μg/mL)

Test organism	**5a**	**CAZ**[b]
Staphylococcus aureus 209P JC-1	1.56	3.13
S. aureus M133[a]	12.5	100
S. aureus M126[a]	> 100	> 100
S. epidermidis ATCC14990	1.56	3.13
Enterococcus hirae ATCC8043	> 100	> 100
E. faecalis W-73	100	> 100
Escherichia coli NIHJ JC-2	0.20	0.20
Klebsiella pneumoniae PCI602	0.05	0.10
Proteus vulgaris GN76	< 0.025	0.10
Morganella morganii 1510/S-1	0.05	0.10
Citrobacter freundii GN346/16	0.39	0.78
Enterobacter cloacae G-0008	0.20	0.20
Serratia marcescens No.1	0.05	0.05
Pseudomonas aeruginosa E-2	1.56	1.56

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

(6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyimino-acetamide]-3-(5-methylthiazolo[4,5-c]pyridinium-2-yl)thio-methyl-3-cephem-4-carboxylate (3a). To a solution of **10** (10.0 g, 18.5 mmol) in dry CH_2Cl_2 (200 mL) were added 2-(2-tritylaminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid (9.84 g, 22.2 mmol), pyridine (7.5 mL, 93 mmol) and phosphorus oxychloride (2.1 mL, 23 mmol) at −20 °C, and the mixture was stirred at the same temperature for 20 min. After addition of water (200 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 (200 mL). The combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography to afford **11** (12.79 g, 16.1 mmol, 87 %) as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 3.46 (1H, d, J = 18 Hz), 3.64 (1H, d, J = 18 Hz), 3.80 (3H, s), 4.07 (3H, s), 4.43 (1H, d, J = 14 Hz), 4.53 (1 H, d, J = 14 Hz), 5.03 (1H, d, J = 5 Hz), 5.22 (2H, s), 5.92 (1H, dd, J = 5 Hz, 9 Hz), 6.71 (1H, s), 6.82 (1H, d, J = 9 Hz), 6.89 (2H, d, J = 9 Hz), 7.01 (1H, s), 7.10–7.40 (17H, m).

Table 7. Pharmacokinetic profiles of **5a** and ceftazidime in mice

		5a	**CAZ**[a]
Serum level (μg/mL)	5 min	15.2	29.1
	15 min	32.6	21.4
	30 min	25.7	14.9
	1 h	6.7	1.4
	2 h	0.0	0.0
	AUC (μg h/mL)	22.0	14.6
	$T_{1/2}$ (h)	0.2	0.3
Urinary recovery	(%, 0–4 h)	70.3	63.8

[a]Ceftazidime.

Table 8. Therapeutic efficacy in systemic infections of **5a** and ceftazidime against *Pseudomonas aeruginosa* GN10362 in mice

	ED_{50} (μg/mouse)[b]	MIC (μg/mL)
5a	0.62	1.56
CAZ[a]	1.04	1.56

[a]Ceftazidime.
[b]Challenging dose: 2.2×10^4 cfu/mouse.

To a solution of **11** (500 mg, 0.629 mmol) in acetone (5 mL) was added sodium iodide (98 mg, 0.69 mmol), and the mixture was stirred at room temperature for 1 h. After addition of water (50 mL), the mixture was extracted with CH_2Cl_2 (50 mL×2). The combined organic layer was washed with 5% aqueous $Na_2S_2O_3$ (100 mL), dried over $MgSO_4$ and concentrated under reduced pressure to afford 3-iodomethylcephem compound.

To a solution of the 3-iodomethylcephem compound in DMF (5 mL) was added 2-mercaptothiazolo[4,5-*c*]pyridine (116 mg, 0.690 mmol), and the mixture was stirred at room temperature for 3.5 h. After addition of saturated aqueous NaCl (50 mL), the mixture was extracted with ethyl acetate (50 mL×2). The combined organic layer was washed with saturated aqueous NaCl (100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography to afford **15a** (443 mg, 0.478 mmol, 76%) as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 3.62 (1H, d, J = 18 Hz), 3.75 (1H, d, J = 18 Hz), 3.79 (3H, s), 4.05 (3H, s), 4.27 (1H, d, J = 13 Hz), 4.79 (1H, d, J = 13 Hz), 5.01 (1H, d, J = 5 Hz), 5.27 (2H, s), 5.91 (1H, dd, J = 5 Hz, 9 Hz), 6.72 (1H, s), 6.80 (1H, d, J = 9 Hz), 6.88 (2H, d, J = 9 Hz), 7.00 (1H, s), 7.15–7.30 (15H, m), 7.36 (2H, d, J = 9 Hz), 7.70 (1H, d, J = 6 Hz), 8.45 (1H, d, J = 6 Hz), 9.09 (1H, s).

To a solution of **15a** (443 mg, 0.478 mmol) in benzene (4 mL) was added methyl iodide (3.0 mL, 48 mmol), and the mixture was stirred at room temperature for 26 h. The mixture was concentrated under reduced pressure to give crude quaternary ammonium compound. To a solution of the crude compound in anisole (2.2 mL) was added trifluoroacetic acid (4.4 mL) at 0 °C. After the mixture was stirred at the same temperature for 1 h, the mixture was poured into diisopropyl ether (22 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 (40 mL) to afford **3a** (179 mg, 0.310 mmol, 65%) as an amorphous powder: ^{1}H NMR (D_2O) δ 3.50 (1H, d, J = 18 Hz), 3.90 (1H, d, J = 18 Hz), 4.03 (3H, s), 4.12 (1 H, d, J = 13 Hz), 4.52 (3H, s), 5.07 (1 H, d, J = 13 Hz), 5.16 (1H, d, J = 5 Hz), 5.80 (1H, d, J = 5 Hz), 7.03 (1H, s), 8.49 (1H, d, J = 6 Hz), 8.55 (1H, d, J = 6 Hz), 9.30 (1H, s); IR (KBr) cm^{-1} 1770, 1670, 1630, 1610, 1540, 1440, 1350, 1200, 1040, 1000, 970; FABMS m/z 578 [(M + H)$^+$]; FABHRMS calcd for $C_{21}H_{20}N_7O_5S_4$ [(M + H)$^+$]: 578.0409, found: 578.0411.

Compounds 12–14. They were prepared from **10** by a similar procedure as described for the preparation of **11**.

12: ^{1}H NMR ($CDCl_3$) δ 3.21 (1H, d, J = 18 Hz), 3.49 (1H, d, J = 18 Hz), 3.81 (3H, s), 4.36 (1H, d, J = 14 Hz), 4.60 (1H, d, J = 14 Hz), 4.91 (1H, d, J = 7 Hz), 4.96 (1H, d, J = 5 Hz), 5.02 (1H, d, J = 7 Hz), 5.23 (2H, s), 5.90 (1H, dd, J = 5 Hz, 9 Hz), 6.77 (1H, s), 6.89 (2H, d, J = 9 Hz), 6.96 (1H, s), 6.99 (1H, s), 7.10–7.40 (27H, m), 8.02 (1H, d, J = 9 Hz).

13: ^{1}H NMR ($CDCl_3$) δ 1.60 (3H, d, J = 7 Hz), 3.36 (1H, d, J = 18 Hz), 3.55 (1H, d, J = 18 Hz), 3.80 (3H, s), 4.42 (1H, d, J = 14 Hz), 4.59 (1H, d, J = 14 Hz), 4.95 (1H, d, J = 5 Hz), 5.17 (1H, q, J = 7 Hz), 5.18 (1H, d, J = 7 Hz), 5.26 (1H, d, J = 7 Hz), 5.91 (1H, dd, J = 5 Hz, 9 Hz), 6.73 (1H, s), 6.89 (2H, d, J = 9 Hz), 6.98 (1H, s), 7.10–7.40 (28H, m), 8.10 (1H, d, J = 9 Hz).

14: ^{1}H NMR ($CDCl_3$) δ 1.41 (9H, s), 1.58 (3H, s), 1.63 (3H, s), 3.44 (1H, d, J = 18 Hz), 3.61 (1H, d, J = 18 Hz), 3.81 (3H, s), 4.44 (1H, d, J = 14 Hz), 4.53 (1H, d, J = 14 Hz), 5.02 (1H, d, J = 5 Hz), 5.19 (1H, q, J = 7 Hz), 5.26 (1H, d, J = 7 Hz), 5.99 (1H, dd, J = 5 Hz, 9 Hz), 6.72 (1H, s), 6.86 (1H, s), 6.89 (2H, d, J = 9 Hz), 7.10–7.40 (17H, m), 8.22 (1H, d, J = 9 Hz).

Compounds 3b, 3c, 3i, 4a–4c, 5a–5c, 6a–6c and 8a. Compounds **3b, 3c, 4a–4c, 5a–5c** and **6a–6c** were prepared from **11–14** by a similar procedure as described for the preparation of **3a**, respectively. Compound **3i** was prepared from **15a** using tert-butyl bromoacetate instead of methyl iodide. Compound **8a** was prepared from **19** by a similar procedure as described for the preparation of **3a**. The analytical data of **3b, 3c, 3i, 4a–4c, 5a–5c, 6a–6c** and **8a** are shown in Tables 9–11.

Compound 9. To a solution of **15a** (412 mg, 0.445 mmol) in anisole (2.0 mL) was added trifluoroacetic acid (4.1 mL) at 0 °C. After the mixture was stirred at the same temperature for 1 h, the mixture was poured into diisopropyl ether (20 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 (70 mL) to afford **9** (146 mg, 0.250 mmol, 56%) as an amorphous powder: ^{1}H NMR (D_2O) δ 3.40 (1H, d, J = 18 Hz), 3.72 (1H, d, J = 18 Hz), 3.95 (3H, s), 4.16 (1H, d, J = 13 Hz), 4.54 (1H, d, J = 13 Hz), 5.16 (1H, d, J = 5 Hz), 5.80 (1H, d, J = 5 Hz), 6.83 (1H, s), 7.70 (1H, d, J = 6 Hz), 8.18 (1H, d, J = 6 Hz), 8.66 (1H, s); IR (KBr) cm^{-1} 1770, 1670, 1600, 1540, 1410, 1390, 1350, 1040, 1000; FABMS m/z 564 [(M + H)$^+$]; FABHRMS calcd for $C_{20}H_{18}N_7O_5S_4$ [(M + H)$^+$]: 564.0252, found: 564.0254.

2-Mercapto-4-methylthiazolo[5,4-*b*]pyridinium trifluoroacetate (21). To a solution of **20** (500 mg, 2.97 mmol) in DMF (5 mL) were added diphenylmethyl bromide (881 mg, 3.56 mmol) and triethylamine (0.5 mL, 3.6 mmol) at 0 °C, and the mixture was stirred at 0 °C for 2.5 h. After addition of saturated aqueous NaCl (50 mL), the mixture was extracted with ethyl acetate (50 mL×2). The combined organic layer was washed with saturated aqueous NaCl (100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography to afford 2-diphenylmethylthiothiazolo[5,4-*b*]-pyridine (993 mg, 2.97 mmol, quant.): ^{1}H NMR ($CDCl_3$) δ 6.38 (1H, s), 7.10–7.60 (11H, m), 8.03 (1H, d, J = 8 Hz), 8.40 (1H, d, J = 5 Hz).

To a solution of above sulfide (927 mg, 2.77 mmol) in DMF (9 mL) was added methyl iodide (17.2 mL, 0.27 mol), and the mixture was stirred at 50 °C for 8 h. After addition of ethyl acetate (90 mL), the precipitates were collected by filtration to afford 2-diphenylmethylthio-4-methylthiazolo[5,4-*b*]pyridinium iodide (883 mg, 1.85 mmol, 67%): ^{1}H NMR (DMSO-d_6) δ 4.43 (3H, s), 6.59 (1H, s), 7.25–7.65 (10H, m), 8.10 (1H, dd, J = 6 Hz, 9 Hz), 8.87 (1H, d, J = 9 Hz), 9.03 (1H, d, J = 6 Hz).

To a solution of trifluoroacetic acid (7 mL) and anisole (3.5 mL) was added above quaternary ammonium iodide (700 mg, 1.47 mmol) at 0 °C. After the mixture was stirred at the same temperature for 1.5 h, the mixture was poured into diisopropyl ether (35 mL) under ice cooling. The precipitates were collected by filtration to afford **21** (435 mg, 1.47 mmol, quant.) ^{1}H NMR (DMSO-d_6) δ 4.30 (3H, s), 7.83 (1H, dd, J = 6 Hz, 9 Hz), 8.23 (1H, d, J = 9 Hz), 8.70 (1H, d, J = 6 Hz).

Compounds 25–31. Compounds **25–27** were prepared from **22–24** by a similar procedure as described for the preparation of **21**, respectively. Compounds **28, 30** and **31** were prepared from **22** using corresponding alkyl iodide instead of methyl iodide. Compound **29** was

Table 9. Analytical data of the compounds **3b, 3c, 3i–3k, 4a–4d** and **5a–5c**

3b: ^{1}H HMR (D_2O) δ 3.50 (1H, d, J = 18 Hz), 3.89 (1H, d, J = 18 Hz), 4.01 (3H, s), 4.21 (1H, d, J = 13 Hz), 4.44 (3H, s), 5.05 (1H, d, J = 13 Hz), 5.18 (1H, d J = 5 Hz), 5.80 (1H, d, J = 5 Hz), 7.03 (1H, s), 8.21 (1H, d, J = 7 Hz), 8.65 (1H, d, J = 7 Hz), 9.34 (1H, s); IR (KBr) cm^{-1} 1760, 1670, 1610, 1540, 1390, 1340, 1300, 1200, 1050; FABMS m/z 578 [(M + H)$^+$]; FABHRMS calcd for $C_{21}H_{20}N_7O_5S_4$ [(M + H)$^+$]: 578.0409, found: 578.0411.

3c: ^{1}H HMR (D_2O) δ 3.50 (1H, d, J = 18 Hz), 3.85 (1H, d, J = 18 Hz), 3.96 (3H, s), 4.24 (1H, d, J = 13 Hz), 4.53 (3H, s), 4.99 (1H, d, J = 13 Hz), 5.15 (1H, d, J = 5 Hz), 5.77 (1H, d, J = 5 Hz), 6.99 (1H, s), 7.75 (1H, t, J = 6 Hz), 8.68 (1H, d, J = 6 Hz), 8.86 (1H, d, J = 6 Hz); IR (KBr) cm^{-1} 1760, 1670, 1600, 1540, 1400, 1300, 1180, 1040, 900, 750; FABMS m/z 578 [(M + H)$^+$]; FABHRMS calcd for $C_{21}H_{20}N_7O_5S_4$ [(M + H)$^+$]: 578.0409, found: 578.0411.

3i: ^{1}H HMR (D_2O) δ 3.49 (1H, d, J = 18 Hz), 3.86 (1H, d, J = 18 Hz), 3.98 (3H, s), 4.15 (1H, d, J = 13 Hz), 4.96 (1H, d, J = 13 Hz), 5.14 (1H, d, J = 5 Hz), 5.32 (2H, s), 5.78 (1H, d, J = 5 Hz), 7.00 (1H, s), 8.51 (2H, s), 9.26 (1H, s); IR (KBr) cm^{-1} 1760, 1640, 1530, 1380, 1040; FABMS m/z 622 [(M + H)$^+$]; FABHRMS calcd for $C_{22}H_{20}N_7O_7S_4$ [(M + H)$^+$]: 622.0307, found: 622.0281.

3j: ^{1}H HMR (D_2O) δ 3.51 (1H, d, J = 18 Hz), 3.93 (1H, d, J = 18 Hz), 4.02 (3H, s), 4.14 (1H, d, J = 13 Hz), 4.50 (3H, s), 4.95 (1H, d, J = 13 Hz), 5.17 (1H, d, J = 5 Hz), 5.82 (1H, d, J = 5 Hz), 7.03 (1H, s), 8.15 (1H, d, J = 6 Hz), 8.72 (1H, d, J = 6 Hz), 9.19 (1H, s); IR (KBr) cm^{-1} 1760, 1600, 1550, 1380, 1280, 1100, 1050; FABMS m/z 562 [(M + H)$^+$]; FABHRMS calcd for $C_{21}H_{20}N_7O_6S_3$ [(M + H)$^+$]: 562.0638, found: 562.0662.

3k: ^{1}H HMR (D_2O) δ 3.49 (1H, d, J = 18 Hz), 3.84 (1H, d, J = 18 Hz), 3.86 (3H, s), 3.98 (3H, s), 4.16 (1H, d, J = 13 Hz), 4.40 (3H, s), 4.71 (1H, d, J = 13 Hz), 5.15 (1H, d, J = 5 Hz), 5.76 (1H, d, J = 5 Hz), 7.00 (1H, s), 7.92 (1H, d, J = 7 Hz), 8.41 (1H, d, J = 7 Hz), 9.00 (1H, s); IR (KBr) cm^{-1} 1760, 1640, 1530, 1380, 1100, 1040; FABMS m/z 575 [(M + H)$^+$]; FABHRMS calcd for $C_{22}H_{23}N_8O_5S_3$ [(M + H)$^+$]: 575.0954, found: 575.0959.

4a: ^{1}H HMR (D_2O) δ 3.47 (1H, d, J = 18 Hz), 3.85 (1H, d, J = 18 Hz), 4.02 (1H, d, J = 13 Hz), 4.48 (3H, s), 4.56 (2H, s), 5.01 (1H, d, J = 13 Hz), 5.12 (1H, d, J = 5 Hz), 5.77 (1H, d, J = 5 Hz), 7.01 (1H, s), 8.45 (1H, d, J = 6 Hz), 8.51 (1H, d, J = 6 Hz), 9.27 (1H, s); IR (KBr) cm^{-1} 1760, 1660, 1600, 1540, 1400, 1350, 1200, 1050, 1000; FABMS m/z 622 [(M + H)$^+$]; FABHRMS calcd for $C_{22}H_{20}N_7O_7S_4$ [(M + H)$^+$]: 622.0307, found: 622.0281.

4b: ^{1}H HMR (D_2O) δ 3.50 (1H, d, J = 18 Hz), 3.84 (1H, d, J = 18 Hz), 4.25 (1H, d, J = 13 Hz), 4.45 (3H, s), 4.59 (2H, s), 4.93 (1H, d, J = 13 Hz), 5.19 (1H, d, J = 5 Hz), 5.79 (1H, d, J = 5′Hz), 6.95 (1H, s), 8.18 (1H, d, J = 7 Hz), 8.64 (1H, d J = 7 Hz), 9.31 (1H, s); IR (KBr) cm-$^{-1}$ 1760, 1660, 1620, 1600, 1540, 1400, 1330, 1300, 1200, 1050; FABMS m/z 622 [(M + H)$^+$]; FABHRMS calcd for $C_{22}H_{20}N_7O_7S_4$ [(M + H)$^+$]: 622.0307, found: 622.0325.

4c: ^{1}H HMR (D_2O) δ 3.51 (1H, d, J = 18 Hz), 3.85 (1H, d, J = 18 Hz), 4.30 (1H, d, J = 13 Hz), 4.53 (3H, s), 4.59 (2H, s), 4.95 (1H, d, J = 13 Hz), 5.19 (1H, d, J = 5 Hz), 5.78 (1H, d, J = 5 Hz), 6.94 (1H, s), 7.76 (1H, t, J = 7 Hz), 8.69 (1H, d, J = 7 Hz), 8.88 (1H, d, J = 7 Hz); IR (KBr) cm^{-1} 1760, 1660, 1600, 1530, 1400, 1300, 1030; FABMS m/z 622 [(M + H)$^+$]; FABHRMS calcd for $C_{22}H_{20}N_7O_7S_4$ [(M + H)$^+$]: 622.0307, found: 622.0325.

4d: ^{1}H HMR (D_2O) δ 3.49 (1H, d, J = 18 Hz), 3.86 (1H, d, J = 18 Hz), 4.16 (1H, d, J = 13 Hz), 4.50 (3H, s), 4.56 (2H, s), 4.99 (1H, d, J = 13 Hz), 5.14 (1H, d, J = 5 Hz), 5.79 (1H, d, J = 5 Hz), 7.03 (1H, s), 8.01 (1H, t, J = 9 Hz), 8.76 (2H, d, J = 9 Hz); IR (KBr) cm^{-1} 1760, 1660, 1600, 1540, 1450, 1400, 1380, 1200, 1060, 1040, 1020, 980; FABMS m/z 622 [(M + H)$^+$]; FABHRMS calcd for $C_{22}H_{20}N_7O_7S_4$ [(M + H)$^+$]: 622.0307, found: 622.0325.

5a: ^{1}H HMR (D_2O) δ 1.46 (3H, d, J = 7 Hz), 3.48 (1H, d, J = 18 Hz), 3.85 (1H, d, J = 18 Hz), 4.12 (1H, d, J = 13 Hz), 4.49 (3H, s), 4.65 (1H, q, J = 7 Hz), 5.03 (1H, d, J = 13 Hz), 5.14 (1H, d, J = 5 Hz), 5.78 (1H, d, J = 5 Hz), 6.99 (1H, s), 8.46 (1H, d, J = 6 Hz), 8.52 (1H, d, J = 6 Hz), 9.28 (1H, s); IR (KBr) cm^{-1} 1760, 1650, 1600, 1540, 1350, 1200, 1100, 1040, 1000, 970; FABMS m/z 636 [(M + H)$^+$]; FABHRMS calcd for $C_{23}H_{22}N_7O_7S_4$ [(M + H)$^+$]: 636.0464, found: 636.0502.

5b: ^{1}H HMR (D_2O) δ 1.47 (3H, d, J = 7 Hz), 3.49 (1H, d, J = 18 Hz), 3.84 (1H, d, J = 18 Hz), 4.25 (1H, d, J = 13 Hz), 4.43 (3H, s), 4.68 (1H, q, J = 7 Hz), 4.94 (1H, d, J = 13 Hz), 5.19 (1H, d, J = 5 Hz), 5.79 (1H, d, J = 5 Hz), 6.93 (1H, s), 8.18 (1H, d, J = 7 Hz), 8.63 (1H, d, J = 7 Hz), 9.31 (1H, s); IR (KBr) cm^{-1} 1760, 1620, 1600, 1540, 1390, 1330, 1290, 1200, 1100, 1040; FABMS m/z 636 [(M + H)$^+$]; FABHRMS calcd for $C_{23}H_{22}N_7O_7S_4$ [(M + H)$^+$]: 636.0464, found: 636.0500.

5c: ^{1}H HMR (D_2O) δ 1.45 (3H, d, J = 7 Hz), 3.53 (1H, d, J = 18 Hz), 3.85 (1H, d, J = 18 Hz), 4.30 (1H, d, J = 13 Hz), 4.55 (3H, s), 4.65 (1H, q, J = 7 Hz), 4.99 (1H, d, J = 13 Hz), 5.19 (1H, d, J = 5 Hz), 5.81 (1H, d, J = 5 Hz), 6.98 (1H, s), 7.77 (1H, t, J = 7 Hz), 8.70 (1H, d, J = 7 Hz), 8.89 (1H, d, J = 7 Hz); IR (KBr) cm^{-1} 1760, 1600, 1540, 1400, 1300, 1040, 900, 750; FABMS m/z 636 [(M + H)$^+$]; FABHRMS calcd for $C_{23}H_{22}N_7O_7S_4$ [(M + H)$^+$]: 636.0464, found: 636.0502.

prepared from **22** using 2-fluoroethyl trifluoromethanesulfonate instead of methyl iodide.

25: ^{1}H NMR (DMSO-d_6) δ 4.40 (3H, s), 8.47 (1H, d, J = 6 Hz), 8.66 (1H, d, J = 6 Hz), 9.29 (1H, s).

26: ^{1}H NMR (DMSO-d_6) δ 4.11 (3H, s), 7.46 (1H, d, J = 7 Hz), 8.31 (1H, d, J = 7 Hz), 8.57 (1H, s).

27: ^{1}H NMR (DMSO-d_6) δ 3.76 (3H, s), 4.30 (3H, s), 8.04 (1H, d, J = 7 Hz), 8.69 (1H, d, J = 7 Hz), 8.98 (1H, s).

Table 10. Analytical data of the compounds **5d–5h, 5j, 5k** and **6a–6d**

5d: ^{1}H HMR (D_2O) δ 1.45 (3H, d, J = 7 Hz), 3.49 (1H, d, J = 18 Hz), 3.86 (1H, d, J = 18 Hz), 4.15 (1H, d, J = 13 Hz), 4.50 (3H, s), 4.65 (1H, q, J = 7 Hz), 4.99 (1H, d, J = 13 Hz), 5.14 (1H, d, J = 5 Hz), 5.79 (1H, d, J = 5 Hz), 7.01 (1H, s), 8.01 (1H, dd, J = 7 Hz, 9 Hz), 8.76 (1H, d, J = 7 Hz), 8.76 (1H, d, J = 9 Hz); IR (KBr) cm^{-1} 1760, 1660, 1600, 1540, 1450, 1400, 1380, 1280, 1260, 1040, 980; FABMS m/z 636 [$(M+H)^+$]; FABHRMS cared for $C_{23}H_{22}N_7O_7S_4$ [$(M+H)^+$]: 636.0464, found: 636.0500.

5e: ^{1}H NMR (D_2O) δ 1.45 (3H, d, J = 7 Hz), 1.68 (3H, t, J = 7 Hz), 3.47 (1H, d, J = 18 Hz), 3.85 (1H, d, J = 18 Hz), 4.11 (1H, d, J = 13 Hz), 4.64 (1H, q, J = 7 Hz), 4.74 (2H, q, J = 7 Hz), 5.04 (1H, d, J = 13 Hz), 5.13 (1H, d, J = 5 Hz), 5.79 (1H, d, J = 5 Hz), 7.00 (1H, s), 8.48 (1H, d, J = 7 Hz), 8.59 (1H, d, J = 7 Hz), 9.35 (1H, s); IR (KBr) cm^{-1} 1760, 1660, 1600, 1530, 1440, 1390, 1350, 1100, 1030, 1000; FABMS m/z 650 [$(M+H)^+$]; FABHRMS calcd for $C_{24}H_{24}N_7O_7S_4$ [$(M+H)^+$]: 650.0620, found: 650.0615.

5f: ^{1}H NMR (D_2O) δ 1.46 (3H, d, J = 7 Hz), 3.48 (1H, d, J = 18 Hz), 3.86 (1H, d, J = 18 Hz), 4.12 (1H, d, J = 13 Hz), 4.65 (1H, q, J = 7 Hz), 4.90–5.15 (6H, m), 5.79 (1H, d, J = 5 Hz), 7.01 (1H, s), 8.53 (1H, d, J = 7 Hz), 8.62 (1H, d, J = 7 Hz), 9.38 (1H, s); IR (KBr) cm^{-1} 1760, 1670, 1600, 1540, 1430, 1400, 1450, 1040, 1000; FABMS m/z 668 [$(M+H)^+$]; FABHRMS calcd for $C_{24}H_{23}FN_7O_7S_4$ [$(M+H)^+$]: 668.0526, found: 668.0513.

5g: ^{1}H NMR (D_2O) δ 1.46 (3H, d, J = 7 Hz), 3.48 (1H, d, J = 18 Hz), 3.86 (1H, d, J = 18 Hz), 4.05–4.20 (3H, m), 4.65 (1H, q, J = 7 Hz), 4.80–4.90 (2H, m), 5.05 (1H, d, J = 13 Hz), 5.14 (1H, d, J = 5 Hz), 5.79 (1H, d, J = 5 Hz), 7.02 (1H, s), 8.51 (1H, d, J = 7 Hz), 8.60 (1H, d, J = 7 Hz), 9.34 (1H, s); IR (KBr) cm^{-1} 1760, 1670, 1600, 1540, 1440, 1400, 1350, 1040, 1000; FABMS m/z 666 [$(M+H)^+$]; FABHRMS calcd for $C_{24}H_{24}N_7O_8S_4$ [$(M+H)^+$]: 666.0569, found: 666.0579.

5h: ^{1}H NMR (D_2O) δ 1.46 (3H, d, J = 7 Hz), 3.48 (1H, d, J = 18 Hz), 3.86 (1H, d, J = 18 Hz), 4.12 (1H, d, J = 13 Hz), 4.65 (1 H, q, J = 7 Hz), 5.06 (1H, d, J = 13 Hz), 5.13 (1H, d, J = 5 Hz), 5.60 (2H, s), 5.79 (1H, d, J = 5 Hz), 7.01 (1H, s), 8.54 (2H, s), 9.30 (1H, s); IR (KBr) cm^{-1} 1760, 1700, 1600, 1530, 1400, 1360, 1040, 1000; FABMS m/z 679 [$(M+H)^+$]; FABHRMS calcd for $C_{24}H_{23}N_8O_8S_4$ [$(M+H)^+$]: 664.0777, found: 664.0773.

5j: ^{1}H NMR (D_2O) δ 1.46 (3H, d, J = 7 Hz), 3.49 (1H, d, J = 18 Hz), 3.88 (1H, d, J = 18 Hz), 4.18 (1H, d, J = 13 Hz), 4.47 (3H, s), 4.66 (1H, q, J = 7 Hz), 4.69 (1H, d, J = 13 Hz), 5.17 (1H, d, J = 5 Hz), 5.79 (1H, d, J = 5 Hz), 6.97 (1H, s), 8.12 (1H, d, J = 6 Hz), 8.69 (1H, d, J = 6 Hz), 9.15 (1H, s); IR (KBr) cm^{-1} 1760, 1600, 1540, 1380, 1280, 1100, 1040; FABMS m/z 620 [$(M+H)^+$]; FABHRMS calcd for $C_{23}H_{22}N_7O_8S_3$ [$(M+H)^+$]: 620.0692, found: 620.0710.

5k: ^{1}H NMR (D_2O) δ 1.45 (3H, d, J = 7 Hz), 3.49 (1H, d, J = 18 Hz), 3.83 (1H, d, J = 18 Hz), 3.86 (3H, s), 4.18 (1H, d, J = 13 Hz), 4.40 (3H, s), 4.65 (1H, q, J = 7 Hz), 4.69 (1H, d, J = 13 Hz), 5.16 (1H, d, J = 5 Hz), 5.78 (1H, d, J = 5 Hz), 6.99 (1H, s), 7.91 (1H, d, J = 7 Hz), 8.41 (1H, d, J = 7 Hz), 8.99 (1H, s); IR (KBr) cm^{-1} 1760, 1700, 1600, 1530, 1400, 1350, 1300, 1100, 1030; FAMBS m/z 633 [$(M+H)^+$]; FABHRMS calcd for $C_{24}H_{25}N_8O_7S_3$ [$(M+H)^+$]: 633.1008, found: 633.0982.

6a: ^{1}H NMR (D_2O) δ 1.48 (3H, s), 1.50 (3H, s), 3.48 (1H, d, J = 18 Hz), 3.85 (1H, d, J = 18 Hz), 4.10 (1H, d, J = 13 Hz), 4.48 (3H, s), 5.04 (1H, d, J = 13 Hz), 5.13 (1H, d, J = 5 Hz), 5.77 (1H, d, J = 5 Hz), 6.96 (1H, s), 8.46 (1H, d, J = 6 Hz), 8.51 (1H, d, J = 6 Hz), 9.27 (1H, s); IR (KBr) cm^{-1} 1760, 1600, 1540, 1440, 1400, 1350, 1200, 1150, 1000, 960; FABMS m/z 650 [$(M+H)^+$]; FABHRMS calcd for $C_{24}H_{24}N_7O_7S_4$ [$(M+H)^+$]: 650.0620, found: 650.0615.

6b: ^{1}H NMR (D_2O) δ 1.50 (3H, s), 1.52 (3H, s), 3.50 (1H, d, J = 18 Hz), 3.85 (1H, d, J = 18 Hz), 4.25 (1H, d, J = 13 Hz), 4.43 (3H, s), 4.97 (1H, d, J = 13 Hz), 5.18 (1H, d, J = 5 Hz), 5.80 (1H, d, J = 5 Hz), 6.93 (1H, s), 8.19 (1H, d, J = 7 Hz), 8.64 (1H, d, J = 7 Hz), 9.31 (1H, s); IR (KBr) cm^{-1} 1760, 1610, 1600, 1540, 1400, 1300, 1200, 1010; FABMS m/z 650 [$(M+H)^+$]; FABHRMS calcd for $C_{24}H_{24}N_7O_7S_4$ [$(M+H)^+$]: 650.0620, found: 650.0615.

6c: ^{1}H NMR (D_2O) δ 1.54 (3H, s), 1.56 (3H, s), 3.56 (1H, d, J = 18 Hz), 3.90 (1H, d, J = 18 Hz), 4.31 (1H, d, J = 13 Hz), 4.58 (3H, s), 5.03 (1H, d, J = 13 Hz), 5.21 (1H, d, J = 5 Hz), 5.83 (1H, d, J = 5 Hz), 7.02 (1H, s), 7.80 (1H, t, J = 7 Hz), 8.72 (1H, d, J = 7 Hz), 8.92 (1H, d, J = 7 Hz); IR (KBr) cm^{-1} 1760, 1600, 1540, 1400, 1300, 1200, 1030, 980, 800, 750; FABMS m/z 650 [$(M+H)^+$]; FABHRMS calcd for $C_{24}H_{24}N_7O_7S_4$ [$(M+H)^+$]: 650.0620, found: 650.0652.

6d: ^{1}H NMR (D_2O) δ 1.49 (3H, s), 1.50 (3H, s), 3.50 (1H, d, J = 18 Hz), 3.87 (1H, d, J = 18 Hz), 4.16 (1H, d, J = 13 Hz), 4.51 (3H, s), 5.00 (1H, d, J = 13 Hz), 5.15 (1H, d, J = 5 Hz), 5.79 (1H, d, J = 5 Hz), 6.98 (1H, s), 8.02 (1H, t, J = 9 Hz), 8.77 (2H, d, J = 9 Hz); IR (KBr) cm^{-1} 1760, 1660, 1600, 1540, 1450, 1400, 1370, 1200, 1150, 1010, 980, 960; FABMS m/z 650 [$(M+H)^+$]; FABHRMS calcd for $C_{24}H_{24}N_7O_7S_4$ [$(M+H)^+$]: 650.0620, found: 650.0615

28: ^{1}H NMR (DMSO-d_6) δ 1.55 (3H, t, J = 7 Hz), 4.65 (1H, q, J = 7 Hz), 8.40 (1H, d, J = 6 Hz), 8.74 (1H, d, J = 6 Hz), 9.09 (1H, s).

29: ^{1}H NMR (DMSO-d_6) δ 4.84 (1H, t, J = 4 Hz), 4.93 (1H, t, J = 4 Hz), 5.01 (2H, m), 8.34 (1H, d, J = 6 Hz), 8.64 (1H, d, J = 6 Hz), 8.88 (1H, s).

30: ^{1}H NMR (DMSO-d_6) δ 3.86 (2H, t, J = 4 Hz), 4.67 (2H, t, J = 4 Hz), 8.45 (1H, d, J = 6 Hz), 8.68 (1H, d, J = 6 Hz), 9.09 (1H, s).

31: ^{1}H NMR (DMSO-d_6) 5.41 (2H, s), 8.33 (1H, d, J = 6 Hz), 8.59 (1H, d, J = 6 Hz), 8.84 (1H, s).

Table 11. Analytical data of the compounds **6e–6h, 7j, 7k** and **8a**

6e: ^{1}H NMR (D_2O) δ 1.48 (3H, s), 1.49 (3H, s), 1.69 (3H, t, J = 7 Hz), 3.48 (1H, d, J = 18 Hz), 3.85 (1H, d, J = 18 Hz), 4.12 (1H, d, J = 13 Hz), 4.74 (2H, q, J = 7 Hz), 5.03 (1H, d, J = 13 Hz), 5.13 (1H, d, J = 5 Hz), 5.77 (1H, d, J = 5 Hz), 6.96 (1H, s), 8.48 (1H, d, J = 6 Hz), 8.60 (1H, d, J = 6 Hz), 9.35 (1H, s); IR (KBr) cm^{-1} 1760, 1660, 1600, 1640, 1470, 1440, 1400, 1350, 1200, 1150, 1000; FABMS m/z 664 [(M + H)$^+$]; FABHRMS calcd for $C_{25}H_{26}N_7O_7S_4$ [(M + H)$^+$]: 664.0777, found: 664.0773.

6f: ^{1}H NMR (D_2O) δ 1.48 (3H, s), 1.50 (3H, s), 3.47 (1H, d, J = 18 Hz), 3.86 (1H, d, J = 18 Hz), 4.12 (1H, d, J = 13 Hz), 4.90–5.20 (6H, m), 5.78 (1H, d, J = 5 Hz), 6.98 (1H, s), 8.53 (1H, d, J = 6 Hz), 8.62 (1H, d, J = 6 Hz), 9.38 (1H, s); IR (KBr) cm^{-1} 1760, 1660, 1600, 1530, 1470, 1440, 1400, 1350, 1200, 1150, 1100, 1050, 990; FABMS m/z 682 [(M + H)$^+$]; FABHRMS calcd for $C_{25}H_{25}FN_7O_7S_4$ [(M + H)$^+$]: 682.0682, found: 682.0678.

6g: ^{1}H NMR (D_2O) δ 1.49 (3H, s), 1.50 (3H, s), 3.49 (1H, d, J = 18 Hz), 3.87 (1H, d, J = 18 Hz), 4.05–4.15 (3H, m), 4.85–4.95 (2H, m), 5.06 (1H, d, J = 13 Hz), 5.14 (1H, d, J = 5 Hz), 5.78 (1H, d, J = 5 Hz), 6.98 (1H, s), 8.52 (1H, d, J = 6 Hz), 8.60 (1H, d, J = 6 Hz), 9.35 (1H, s); IR (KBr) cm^{-1} 1760, 1660, 1600, 1530, 1470, 1440, 1400, 1350, 1200, 1150, 990; FABMS m/z 680 [(M + H)$^+$]; FABHRMS calcd for $C_{25}H_{26}N_7O_8S_4$ [(M + H)$^+$]: 680.0725, found: 680.0737.

6h: ^{1}H NMR (D_2O) δ 1.48 (3H, s), 1.50 (3H, s), 3.48 (1H, d, J = 18 Hz), 3.86 (1H, d, J = 18 Hz), 4.12 (1H, d, J = 13 Hz), 5.06 (1H, d, J = 13 Hz), 5.13 (1H, d, J = 5 Hz), 5.60 (2H, s), 5.78 (1H, d, J = 5 Hz), 6.98 (1H, s), 8.54 (2H, s), 9.31 (1H, s); IR (KBr) cm^{-1} 1760, 1700, 1600, 1530, 1440, 1400, 1350, 1200, 1150, 1100, 1000; FABMS m/z 693 [(M + H)$^+$]; FABHRMS calcd for $C_{25}H_{25}N_8O_8S_4$ [(M + H)$^+$]: 693.0678, found: 693.0688.

7j: ^{1}H NMR (D_2O) δ 3.48 (1H, d, J = 18 Hz), 3.90 (1H, d, J = 18 Hz), 4.01 (1H, d, J = 14 Hz), 4.08 (3H, s), 4.93 (1H, d, J = 14 Hz), 5.13 (1H, d, J = 5 Hz), 5.82 (1H, d, J = 5 Hz), 8.13 (1H, d, J = 6 Hz), 8.71 (1H, d, J = 6 Hz), 9.17 (1 H, s); IR (KBr) cm^{-1} 1760, 1600, 1520, 1400, 1290, 1100, 1050; FABMS m/z 563 [(M + H)$^+$]; FABHRMS calcd for $C_{20}H_{19}N_8O_6S_3$ [(M + H)$^+$]: 563.0590, found: 563.0559.

7k: ^{1}H NMR (D_2O) δ 3.48 (1H, d, J = 18 Hz), 3.84 (1H, d, J = 18 Hz), 3.86 (3H, s), 4.07 (3H, s), 4.15 (1H, d, J = 13 Hz), 4.40 (3H, s), 4.72 (1H, d, J = 13 Hz), 5.15 (1H, d, J = 5 Hz), 5.79 (1H, d, J = 5 Hz), 7.92 (1H, d, J = 7 Hz), 8.41 (1H, d, J = 7 Hz), 9.00 (1H, s); IR (KBr) cm^{-1} 1760, 1680, 1650, 1600, 1520, 1400, 1350, 1300, 1100, 1050; FABMS m/z 576 [(M + H)$^+$]; FABHRMS calcd for $C_{21}H_{22}N_9O_5S_3$ [(M + H)$^+$]: 576.0906, found: 576.0911.

8a: ^{1}H NMR (D_2O) δ 3.65 (1H, d, J = 18 Hz), 4.02 (3H, s), 4.11 (1H, d, J = 18 Hz), 4.48 (3H, s), 5.48 (1H, d, J = 5 Hz), 5.93 (1H, d, J = 5 Hz), 7.02 (1H, s), 8.49 (1H, d, J = 6 Hz), 8.54 (1H, d, J = 6 Hz), 9.29 (1H, s); IR (KBr) cm^{-1} 1770, 1670(sh), 1610, 1540, 1440, 1390, 1350, 1300, 1250, 1050; FABMS m/z 564 [(M + H)$^+$]; FABHRMS calcd for $C_{20}H_{18}N_7O_5S_4$ [(M + H)$^+$]: 564.0252, found: 564.0273.

(6*R*, 7*R*)-7-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyimino-acetamide]-3-(4-methylthiazolo[5,4-b]pyridinium-2-yl)thio-methyl]-3-cephem-4-carboxylate (3d). To a solution of **11** (238 mg, 0.300 mmol) in acetone (3 mL) was added sodium iodide (47 mg, 0.33 mmol), and the mixture was stirred at room temperature for 1 h. After addition of water (10 mL), the mixture was extracted with CH_2Cl_2 (10 mL×2). The combined organic layer was washed with 5% aqueous $Na_2S_2O_3$ (20 mL), dried over $MgSO_4$ and concentrated under reduced pressure to afford 3-iodomethylcephem compound.

To a solution of 3-iodomethylcephem compound in DMF (3 mL) was added **21** (107 mg, 0.361 mmol), and the mixture was stirred at room temperature for 3.5 h. After addition of saturated aqueous NaCl (50 mL), the mixture was extracted with ethyl acetate (50 mL×2). The combined organic layer was washed with saturated aqueous NaCl (100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by LH-20 (Pharmacia) column chromatography to give **32d** (227 mg, 0.215 mmol, 72%) as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 3.52 (1H, d, J = 18 Hz), 3.77 (1H, d, J = 18 Hz), 3.80 (3H, s), 4.05 (3H, s), 4.27 (1H, d, J = 13 Hz), 4.77 (3H, s), 4.86 (1H, d, J = 13 Hz), 5.07 (1H, d, J = 5 Hz), 5.21 (1H, d, J = 12 Hz), 5.33 (1H, d, J = 12 Hz), 5.90 (1H, dd, J = 5 Hz, 9 Hz), 6.67 (1H, s), 6.82 (1H, d, J = 9 Hz), 6.88 (2H, d, J = 9 Hz), 7.05 (1H, s), 7.20–7.30 (15H, m), 7.36 (2H, d, J = 9 Hz), 8.97 (1H, dd, J = 7 Hz, 9 Hz), 8.37 (1H, d, J = 9 Hz), 9.74 (1H, d, J = 7 Hz).

To a solution of **32d** (227 mg, 0.215 mmol) in anisole (2.2 mL) was added trifluoroacetic acid (4.4 mL) at 0 °C, and the mixture was stirred at the same temperature. After 1 h, the mixture was poured into diisopropyl ether (22 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 (15 mL) to afford **3d** (65 mg, 0.11 mmol, 52%) as an amorphous powder: ^{1}H NMR (D_2O) δ 3.49 (1H, d, J = 18 Hz), 3.89 (1H, d, J = 18 Hz), 3.99 (3H, s), 4.14 (1 H, d, J = 13 Hz), 4.51 (3H, s), 5.02 (1H, d, J = 13 Hz), 5.14 (1H, d, J = 5 Hz), 5.77 (1H, d, J = 5 Hz), 7.00 (1H, s), 8.02 (1H, dd, J = 7 Hz, 9 Hz), 8.77 (2H, d, J = 7 Hz); IR (KBr) cm^{-1} 1760, 1670, 1600, 1540, 1450, 1380, 1040, 980, 950;

FABMS *m/z* 578 [(M + H)$^+$]; FABHRMS calcd for $C_{21}H_{20}N_7O_5S_4$ [(M + H)$^+$]: 578.0409, found: 578.0383.

Compounds 3j, 3k, 4d, 5d–5h, 5j, 5k and 6d–6h. They were prepared from **11–14** by a similar procedure as described for the preparation of **3d**, respectively. The analytical data of **3j, 3k, 4d, 5d–5h, 5j, 5k** and **6d–6h** are shown in Tables 9–11.

(6*R*, 7*R*)-7-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-(*Z*)-2-methoxyiminoacetamide]-3-(5-methylthiazolo[4,5-*c*]pyridinium-2-yl)thiomethyl-3-cephem-4-carboxylate (7a). To a suspended solution of 2-(5-amino-1,2,4-thiadiazol-3-yl)-(*Z*)-2-methoxyiminoacetic acid (2.13 g, 10.5 mmol) in dry CH_2Cl_2 (50 mL) was added phosphorous pentachloride (2.1 g, 10 mmol) at −40 °C, and the mixture was stirred at −5 °C for 3 h to afford the acid chloride solution. To a suspended solution of **10** (5.4 g, 10 mmol) in dry CH_2Cl_2 (50 mL) were added *N,O*-bis(trimethylsilyl)acetamide (12.5 mL, 50 mmol) at room temperature, then above acid chloride solution at −40 °C, and the mixture was stirred at −10 °C for 30 min. After addition of water (100 mL), the mixture was stirred at room temperature for 30 min, and extracted with ethyl acetate (400 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (400 mL) and saturated aqueous NaCl (400 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography to give **36** (2.11 g, 3.82 mmol, 38%) as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 3.48 (1H, d, *J* = 18 Hz), 3.68 (1H, d, *J* = 18 Hz), 3.81 (3H, s), 4.11 (3H, s), 4.39 (1H, d, *J* = 13 Hz), 4.60 (1H, d, *J* = 13 Hz), 5.07 (1H, d, *J* = 5 Hz), 5.23 (2H, s), 6.12 (1H, dd, *J* = 5 Hz, 9 Hz), 6.52 (2H, s), 6.90 (2H, d, *J* = 9 Hz), 7.32 (2H, d, *J* = 9 Hz), 8.39 (1H, d, *J* = 9 Hz).

Compound **7a** was prepared from **36** by a similar procedure as described for the preparation of **3d** from **32d**. **7a**: ^{1}H NMR (D_2O) δ 3.46 (1H, d, *J* = 18 Hz), 3.88 (1H, d, *J* = 18 Hz), 4.07 (1H, d, *J* = 14 Hz), 4.08 (3H, s), 4.49 (3H, s), 5.07 (1H, d, *J* = 14 Hz), 5.12 (1H, d, *J* = 5 Hz), 5.80 (1H, d, *J* = 5 Hz), 8.47 (1H, d, *J* = 6 Hz), 8.52 (1H, d, *J* = 6 Hz), 9.28 (1H, s); IR (KBr) cm^{-1} 1760, 1670, 1620, 1520, 1450, 1350, 1050, 1000, 960; FABMS *m/z* 579 [(M + H)$^+$]; FABHRMS calcd for $C_{20}H_{19}N_8O_5S_4$ [(M + H)$^+$]: 579.0361, found: 579.0353.

Compounds 7j and 7k. They were prepared from **36** by a similar procedure as described for the preparation of **3d** from **32d**. The analytical data of **7j** and **7k** are shown in Table 11.

Acknowledgements

The authors wish to thank Dr K. Atsumi for valuable discussions, and Dr S. Kondo (Institute of Microbial Chemistry) for encouragement. We are grateful to Mr T. Hara, Mrs A. Miyata, Mrs K. Tohyama, Miss M. Iida, and Mr S. Sakakibara, for the biological study, and to Miss S. Miki and Miss Y. Fukurose for measurement of mass spectra.

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