# Synthesis and Biological Evaluation of a Series of New Parenteral Optically Active 3-[[(*N*-Alkylpyridinium-4′-yl)thio]methyl]-2-oxaisocephems

Hidetsugu Tsubouchi,*,† Koichi Tsuji,† Koichi Yasumura,‡ Makoto Matsumoto,† Takuya Shitsuta,† and Hiroshi Ishikawa†

*Microbiological Research Institute, Otsuka Pharmaceutical Company, Ltd., Kagasuno 463-10, Kawauchi-cho, Tokushima 771-01, Japan, and Fujii Memorial Research Institute, Otsuka Pharmaceutical Company, Ltd., Karasaki 1-11-1, Ohtsu 520-01, Japan*

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The preparation and biological evaluation of a series of 7-[2-(2-aminothiazol-4-yl)-2-(*Z*)-[(cyclopentyloxy)imino]acetamido] optically active 2-oxaisocephems, substituted at the 3-position with [(*N*-alkylpyridinium-4′-yl)thio]methyl groups, are described. The resulting family of parenteral compounds displays a broad spectrum of *in vitro* antibacterial activity. These compounds exhibit increased activity against Gram-positive organisms including methicillin-resistant *Staphylococcus aureus* and *Enterococcus faecalis* which are resistant to most cephalosporins with a similar level of Gram-negative activity to that of the third-generation antibiotics. *In vivo* efficacy of new antibacterial agents in this investigation is excellent against both Gram-positive and Gram-negative bacteria as compared with reference compounds. The *in vitro* and *in vivo* antimicrobial activity and the structure–activity relationships are presented.

Among β-lactam antibacterial agents, 2-oxaisocephems represent a new class of antibiotics.[1-18] Doyle *et al.* reported that 2-oxaisocephems had only partial antibacterial activity and 2-oxaisocephem and cephalosporin nuclei bearing the same side chain possessed about the same inherent activity.[1] Later, Mastalerz *et al.* presented the preparation of orally absorbable, optically active 2-oxaisocephems with potent effect against Gram-positive organisms.[10] However, these compounds primarily have the side chains of the first-generation cephalosporins at the 7-position. The cephalosporin class of antibiotics continues to play clinically an important role in the treatment of bacterial infections. An outstanding progress in the field of cephalosporins was the introduction of a 2-(2-aminothiazol-4-yl)-2-(*Z*)-(methoxyimino)acetamido side chain into the 7-position.[19] Further modification of this side chain together with alteration of the substituent of the 3-position of the cephalosporin nucleus has often succeeded in the preparation of some potent and broad-spectrum antibiotics. Cephalosporins, aminothiazolyl oxyimino derivatives containing various substituents at the 3-position, have been introduced as the third-generation antibacterial agents with potent activity against a wide variety of pathogens. We also have been investigating a search for compounds with more potent and broad-spectrum antibacterial activity from the standpoint that the introduction of side chains of the third-generation cephalosporins into the 7-position of the 2-oxaisocephem nucleus and the alteration of the 3-substituents might enhance the activity and broaden the antibacterial spectrum. In order to obtain agents which possess an increased Gram-positive spectrum while maintaining Gram-negative activity, we have prepared a series of new optically active 2-oxaisocephems. In particular, our efforts have been directed toward the synthesis and evaluation of 2-oxaisocephem derivatives having the advantage of interesting activity against a variety of pathogenic organisms including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis*. MRSA is *S. aureus* with acquired resistance to methicillin,[20] the antibiotic developed as a penicillin effective for resistant *S. aureus*. The need for a useful agent for the treatment of bacterial infections such as MRSA and *E. faecalis* has become particularly significant, since most cephalosporins are not effective against them. In our precedings papers,[21-23] we reported that 2-oxaisocephems with a 2-(2-aminothiazol-4-yl)-2-(*Z*)-[(cyclopentyloxy)imino]acetamido group at the 7-position and thio-substituted methyl groups at the 3-position possessed high *in vitro* potency against MRSA, and among them, 3-[(*N*-alkylpyridinium-4′-yl)thio]methyl derivatives showed excellent *in vivo* efficacy.[22] In our further investigation, we recently found that 3-[(*N*-alkylpyridinium-4′-yl)thio]methyl derivatives showed potent antibacterial activity against ampicillin-insensitive *Streptococcus pneumoniae* which will become a serious medical problem. This paper describes the full details of our efforts in this area, including the syntheses and the *in vitro* and *in vivo* antibacterial activities of these novel agents.

## Chemistry

The key intermediate for the preparation of new optically active 2-oxaisocephems was the 3-bromomethyl derivative **6**[22] with a 4-nitrophthalimido group at the 7-position readily derived from the enol derivative **3** which was obtained in five steps from D-threonine via **2**.[21] Tosylation of **3** with *p*-toluenesulfonyl chloride in the presence of *N*-methylpyrrolidine followed by addition of morpholine gave the enamine **4**. After workup, **5** was obtained by bromination of **4** with pyridine perbromide followed by hydrolysis. The thus obtained **5** was treated with $NaHCO_3$ to afford **6** in 51% yield from **3**. As described in our previous paper,[22] optically active 2-oxaisocephems with a [(*N*-alkylpyridinium-4′-yl)thio]methyl substituent at the 3-position and a 2-(2-aminothiazol-4-yl)-2-(*Z*)-[(cyclopentyloxy)imino]aceta-

† Microbiological Research Institute.
‡ Fujii Memorial Research Institute.
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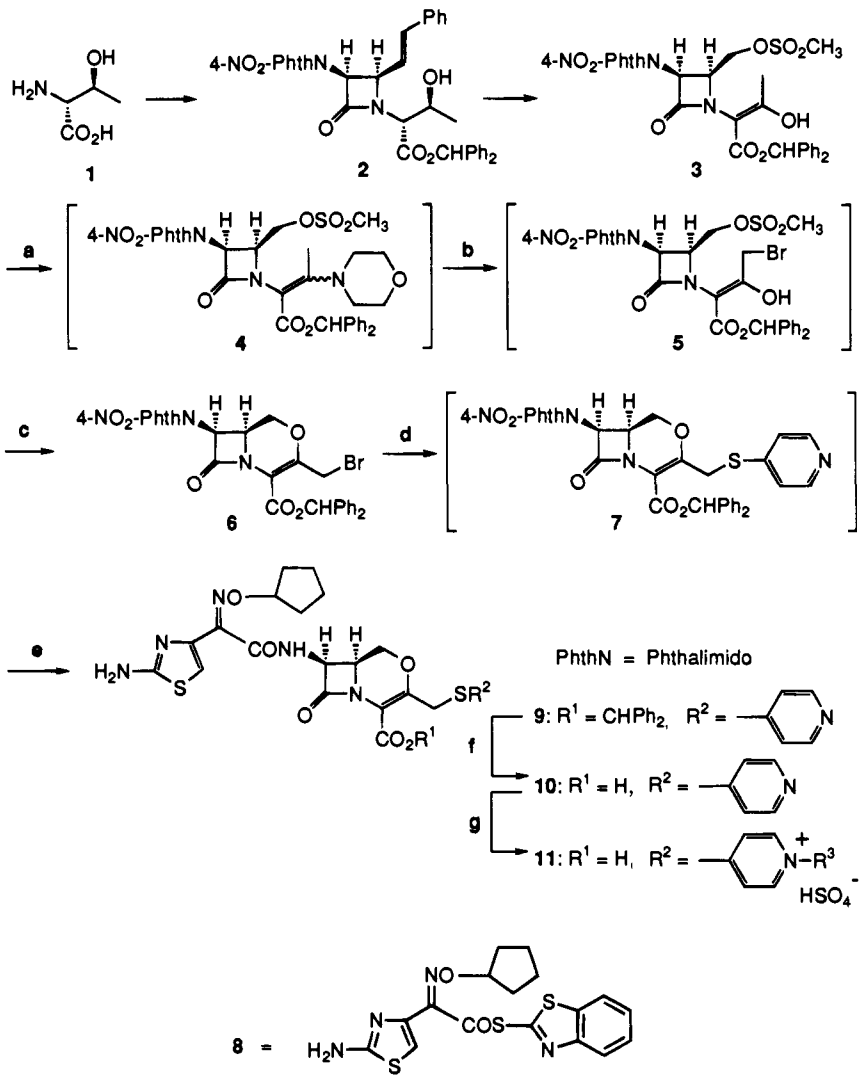
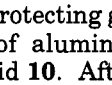
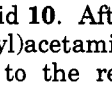
**Scheme 1**[a]




[a] (a) (1) *p*-Toluenesulfonyl chloride/*N*-methylpyrrolidine, (2) morpholine; (b) (1) pyridine perbromide, (2) $H^+$; (c) $NaHCO_3$; (d) 4-mercaptopyridine/$Et_3N$; (e) (1) $CH_3NHNH_2$, (2) AcOH, (3) **8**; (f) $AlCl_3$/anisole/$CH_3NO_2$; (g) (1) BSA, (2) halides.

mido group at the 7-position were found to have high *in vitro* potency and excellent *in vivo* efficacy. Therefore, we wished to convert **6** into the desired target compounds **11**. To introduce the 2-(2-aminothiazol-4-yl)-2-(*Z*)-[(cyclopentyloxy)imino]acetamido group into the 7-position, deprotection of the 4-nitrophthalimido group was required. Methylhydrazinolysis was proved to be efficient for the removal of this group.[24] After treating of the bromide **6** with 4-mercaptopyridine in the presence of triethylamine, methylhydrazine was added to the reaction mixture without isolation of **7**. The thus generated amine was allowed to react with 2-mercaptobenzothiazole active ester **8** to provide the benzhydryl ester **9** in 61% yield from **6**. Removal of the benzhydryl-protecting group of **9** could then be achieved by the use of aluminum trichloride[25] to obtain the carboxylic acid **10**. After **10** was treated with *N,O*-bis(trimethylsilyl)acetamide (BSA), bromides or iodides were added to the reaction mixture to afford new optically active 2-oxaisocephems **11**. Compounds **11** were isolated as hydrogen sulfates. The synthesized **11b–o** are shown in Table 1 (**11a**: $R^3 = CH_3$). Bromides to obtain **11c–e,h,n,o** were synthesized essentially according to Weaver's method[26] or by bromination of pinacolone.

**Table 1.** 3-[[(*N*-Alkylpyridinium-4′-yl)thio]methyl]-2-oxaisocephems **11**

| compd no. | $R^3$ | formula | anal.[a] |
|---|---|---|---|
| **11b** | $CH_2CONH_2$ | $C_{25}H_{27}N_7O_7S_2{\cdot}H_2SO_4{\cdot}H_2O$ | C, H, N |
| **11c** | $CH_2CONHCH_3$ | $C_{26}H_{29}N_7O_7S_2{\cdot}H_2SO_4{\cdot}H_2O$ | C, H, N |
| **11d** | $CH_2CON(CH_3)_2$ | $C_{27}H_{31}N_7O_7S_2{\cdot}H_2SO_4{\cdot}H_2O$ | C, H, N |
| **11e** | $CH_2CONHC(CH_3)_3$ | $C_{29}H_{35}N_7O_7S_2{\cdot}H_2SO_4{\cdot}{}^1/_2H_2O$ | C, H, N |
| **11f** | $CH_2COCH_3$ | $C_{26}H_{28}N_6O_7S_2{\cdot}H_2SO_4{\cdot}2H_2O$ | C, H, N |
| **11g** | $CH_2COC_2H_5$ | $C_{27}H_{30}N_6O_7S_2{\cdot}H_2SO_4{\cdot}{}^5/_2H_2O$ | C, H, N |
| **11h** | $CH_2COC(CH_3)_3$ | $C_{29}H_{34}N_6O_7S_2{\cdot}H_2SO_4{\cdot}H_2O$ | C, H, N |
| **11i** | $CH_2COPh$ | $C_{31}H_{30}N_6O_7S_2{\cdot}H_2SO_4{\cdot}{}^1/_2H_2O$ | C, H, N |
| **11j** | $CH_2CH{=}CH_2$ | $C_{26}H_{28}N_6O_6S_2{\cdot}H_2SO_4{\cdot}H_2O$ | C, H, N |
| **11k** | $CH_2C{\equiv}CH$ | $C_{26}H_{26}N_6O_6S_2{\cdot}H_2SO_4{\cdot}{}^1/_2H_2O$ | C, H, N |
| **11l** | $CH_2CH{=}C(CH_3)_2$ | $C_{28}H_{32}N_6O_6S_2{\cdot}H_2SO_4{\cdot}{}^1/_2H_2O$ | C, H, N |
| **11m** | $CH_2CN$ | $C_{25}H_{25}N_7O_6S_2{\cdot}H_2SO_4{\cdot}{}^3/_2H_2O$ | C, H, N |
| **11n** |  | $C_{29}H_{33}N_7O_8S_2{\cdot}H_2SO_4{\cdot}2H_2O$ | C, H, N |
| **11o** |  | $C_{30}H_{35}N_7O_8S_2{\cdot}H_2SO_4{\cdot}2H_2O$ | C, H, N |

[a] Analytical results (C, H, N) are within ±0.4% of the calculated values unless otherwise noted.

## Antibacterial Activity

The compounds **11a–o** prepared in this investigation were tested for their *in vitro* antibacterial activity

**Table 2.** Antibacterial Activity of 3-[[(*N*-Alkylpyridinium-4′-yl)thio]methyl]-2-oxaisocephems 11 (MICs,[a] μg/mL)

| | Gram-positive organisms[b] | | | | Gram-negative organisms[b] | | | |
|---|---|---|---|---|---|---|---|---|
| compd | *S. au.* FDA 209P | MRSA 5038 | *E. f.* ATCC 21212 | *S. ep.* ATCC 12228 | *E. c.* NIHJ JC-2 | *K. pn.* NCTC 9632 | *S. m.* ATCC 12648 | *P. ae.* ATCC 10145 |
| **11a** | 0.05 | 3.13 | 1.56 | 0.05 | 0.39 | 0.2 | 1.56 | 6.25 |
| **11b** | 0.1 | 6.25 | 1.56 | 0.05 | 0.2 | 0.2 | 1.56 | 3.13 |
| **11c** | 0.2 | 6.25 | 1.56 | 0.2 | 0.78 | 0.39 | 1.56 | 12.5 |
| **11d** | 0.2 | 12.5 | 1.56 | 0.39 | 0.78 | 0.78 | 1.56 | 12.5 |
| **11e** | 0.2 | 12.5 | 1.56 | 0.39 | 1.56 | 1.56 | 3.13 | 25 |
| **11f** | 0.2 | 6.25 | 0.78 | 0.2 | 0.39 | 0.39 | 0.78 | 6.25 |
| **11g** | 0.2 | 6.25 | 1.56 | 0.2 | 0.39 | 0.39 | 1.56 | 6.25 |
| **11h** | 0.2 | 6.25 | 1.56 | 0.39 | 1.56 | 1.56 | 3.13 | 25 |
| **11i** | 0.2 | 12.5 | 1.56 | 0.39 | 1.56 | 1.56 | 3.13 | 25 |
| **11j** | 0.1 | 6.25 | 0.78 | 0.05 | 0.39 | 0.39 | 1.56 | 6.25 |
| **11k** | 0.2 | 6.25 | 1.56 | 0.1 | 0.39 | 0.39 | 1.56 | 6.25 |
| **11l** | 0.2 | 12.5 | 1.56 | 0.2 | 0.78 | 0.78 | 1.56 | 12.5 |
| **11m** | 0.2 | 12.5 | 1.56 | 0.2 | 0.39 | 0.39 | 1.56 | 6.25 |
| **11n** | 0.2 | 12.5 | 1.56 | 0.2 | 0.78 | 0.78 | 1.56 | 6.25 |
| **11o** | 0.2 | 25 | 3.13 | 0.39 | 1.56 | 0.78 | 3.13 | 12.5 |
| flomoxef | 0.39 | 50 | 100 | 0.39 | 0.1 | 0.1 | 0.78 | >100 |
| cefpirome | 0.39 | 100 | 25 | 0.39 | 0.05 | 0.05 | 0.2 | 6.25 |
| vancomycin | 0.78 | 0.78 | 0.78 | 3.13 | >100 | >100 | >100 | >100 |

[a] Minimum inhibitory concentrations ($10^6$ cells/mL). [b] Definitions of organism abbreviations: *S. au.* = *S. aureus*, MRSA = methicillin-resistant *S. aureus*, *E. f.* = *E. faecalis*, *S. ep.* = *S. epidermidis*, *E. c.* = *E. coli*, *K. pn.* = *K. pneumoniae*, *S. m.* = *S. marcescens*, *P. ae.* = *P. aeruginosa*.

**Table 3.** *In Vitro* Antibacterial Activity (MICs, μg/mL) of Compound **11g** against Clinical Isolates of Methicillin-Resistant *S. aureus*, *E. faecalis*, and Ampicillin-Insensitive *S. pneumoniae*

| strains | compd | $MIC_{50}$[a] | $MIC_{80}$[b] | $MIC_{90}$[c] | MIC range[d] |
|---|---|---|---|---|---|
| MRSA[e] | **11g** | 6.25 | 6.25 | 6.25 | 0.78–6.25 |
| | flomoxef | 25 | 100 | 100 | 0.78–100 |
| | cefpirome | 50 | 100 | 100 | 3.13–100 |
| | vancomycin | 1.56 | 1.56 | 1.56 | 0.78–1.56 |
| *E. faecalis*[f] | **11g** | 3.13 | 3.13 | 6.25 | 0.78–6.25 |
| | flomoxef | >100 | >100 | >100 | 100–>100 |
| | cefpirome | 50 | 100 | 100 | 12.5–100 |
| ampicillin-insensitive *S. pneumoniae*[g] | **11g** | 0.025 | 0.05 | 0.05 | <0.006–0.05 |
| | flomoxef | 3.13 | 6.25 | 6.25 | 1.56–6.25 |
| | cefpirome | 0.39 | 0.78 | 0.78 | 0.2–0.78 |
| | ampicillin | 3.13 | 3.13 | 3.13 | 1.56–3.13 |

[a] The MIC value for 50% of isolates. [b] The MIC value for 80% of isolates. [c] The MIC value for 90% of isolates. [d] The range of MIC value for isolates. [e] 33 clinical isolates. [f] 27 clinical isolates. [g] 18 clinical isolates.

against Gram-positive (*S. aureus* FDA 209P, methicillin-resistant *S. aureus* (MRSA) 5038, *E. faecalis* ATCC 21212, and *Staphylococcus epidermidis* ATCC 12228) and Gram-negative (*Escherichia coli* NIHJ JC-2, *Klebsiella pneumoniae* NCTC 9632, *Serratia marcescens* ATCC 12648, and *Pseudomonas aeruginosa* ATCC 10145) bacteria. The minimum inhibitory concentrations (MICs: μg/mL, inoculum size: $10^6$ cells/mL) for an array of Gram-positive and Gram-negative bacterial species were determined by 2-fold agar dilution method.[27] The results are summarized in Table 2. The antibacterial activities of flomoxef,[28] cefpirome,[28] and vancomycin[28] as reference compounds are also presented. New optically active 2-oxaisocephems **11a,b,j** were highly active against *S. aureus* and *S. epidermidis*. Generally speaking, 3-[[(*N*-alkylpyridinium-4′-yl)thio]methyl]-2-oxaisocephems of this series were superior to reference compounds against staphylococci. Among these compounds containing 3-[(*N*-alkylpyridinium-4′-yl)thio]-methyl groups, those with methyl for **11a**, carbamoylmethyl for **11b**, (*N*-methylcarbamoyl)methyl for **11c**, acetonyl for **11f**, propionylmethyl for **11g**, (*tert*-butylcarbonyl)methyl for **11h**, allyl for **11j**, and propargyl for **11k** exhibited increased antibacterial activity against MRSA as compared with flomoxef and cefpirome. Because MRSA has been associated with an increasing number of infections in hospitals,[29–32] the potent activity of these compounds against it seems to be significant. In addition, the activity of **11f,j** against *E. faecalis* was equal to that of vancomycin, while most cephalosporins, including cefpirome and flomoxef, of the 1-oxacephem derivative are not effective against it. Although all new 2-oxaisocephems have lower MIC values against *E. faecalis* than flomoxef and cefpirome, **11o** was found to be slightly less potent against MRSA than **11a–n**. Vancomycin has potent antibacterial activities against Gram-positive organisms as described in Table 2, but it is not effective against Gram-negative bacteria at all. Against *E. coli* and *K. pneumoniae*, **11b** showed about the same activity as flomoxef. The compound **11b** also showed significantly the enhanced activity against *P. aeruginosa* as compared with flomoxef and vancomycin. And the activity of **11f** against *S. marcescens* was equal to that of flomoxef. All new 2-oxaisocephems were found to have potent antibacterial activities characteristically against Gram-positive organisms including MRSA and *E. faecalis* while maintaining Gram-negative activities. In particular, **11a,b,j** have more well-balanced potency and a broader spectrum of antibacterial activities than reference compounds.

*In vitro* antibacterial activity of compound **11g** against MRSA, *E. faecalis*, and ampicillin-insensitive *S. pneumoniae* strains isolated clinically is shown in Table 3. Data for flomoxef, cefpirome, vancomycin, and ampicillin[28] are also included for comparison purpose. The antibacterial activity of **11g** against MRSA isolates was

**Table 4.** *In Vivo* Efficacy of Selected 3-[[(*N*-Alkylpyridinium-4′-yl)thio]methyl]-2-oxaisocephems 11 against Experimental Infections in Mice[a] in Comparison with Flomoxef, Cefpirome, Vancomycin, and Ampicillin

| test organism | compd | MIC (μg/mL) | challenge dose (cells/mouse) | $ED_{50}$ (mg/kg)[b] | 95% confidence limits (mg/kg) |
|---|---|---|---|---|---|
| *S. aureus* Smith | **11a** | 0.2 | $1.32 \times 10^7$ | 0.08 | 0.04–0.12 |
| | **11c** | 0.2 | | 0.18 | 0.11–0.31 |
| | **11f** | 0.2 | | 0.18 | 0.10–0.30 |
| | **11g** | 0.2 | | 0.22 | 0.11–0.37 |
| | flomoxef | 0.39 | | 0.53 | 0.17–0.87 |
| | cefpirome | 0.39 | | 1.72 | 1.29–2.40 |
| | vancomycin | 0.78 | | 3.74 | 2.58–5.12 |
| MRSA 5038 | **11a** | 3.13 | $1.00 \times 10^7$ | 3.40 | 0.29–4.73 |
| | **11c** | 6.25 | | 2.27 | 0.66–4.45 |
| | **11f** | 6.25 | | 2.76 | 1.50–4.55 |
| | vancomycin | 1.56 | | 2.70 | 1.90–4.00 |
| MRSA 5120 | **11g** | 6.25 | $7.60 \times 10^7$ | 1.00 | 0.60–1.57 |
| | flomoxef | 25 | | 8.52 | 4.41–17.26 |
| | cefpirome | 50 | | 27.70 | 12.92–105.43 |
| | vancomycin | 0.78 | | 11.49 | 7.12–17.25 |
| MRSA 5129 | **11a** | 6.25 | $1.38 \times 10^8$ | 3.91 | 0.92–9.15 |
| | vancomycin | 0.78 | | 18.68 | 9.38–27.38 |
| *E. faecalis* C 0063 | **11g** | 0.78 | $7.00 \times 10^7$ | 5.23 | 3.88–7.31 |
| | flomoxef | 100 | | >200 | |
| | cefpirome | 12.5 | | >100 | |
| *S. pneumoniae* C 0096 | **11g** | <0.0015 | $1.60 \times 10^2$ | 0.56 | 0.31–0.90 |
| | flomoxef | 0.2 | | 18.60 | 10.08–63.74 |
| | ampicillin | 3.13 | | 8.23 | 5.47–12.83 |
| *E. coli* No. 29 | **11a** | 0.39 | $1.35 \times 10^6$ | 0.09 | 0.05–0.12 |
| | **11g** | 0.39 | | 0.15 | 0.12–0.19 |
| | flomoxef | 0.1 | | 0.43 | 0.31–0.57 |
| | cefpirome | 0.025 | | 0.64 | 0.46–0.86 |

[a] Experimental infections were produced by intraperitoneal injection with the challenge organisms suspended in 5% gastric mucin. The infections were lethal to all untreated mice. [b] Dose required to prevent death in 50% of animals (subcutaneous administration).

slightly less potent than that of vancomycin but significantly superior to that of flomoxef and cefpirome, the third-generation antibiotics. In addition, compound **11g** showed excellent activity against *E. faecalis* and ampicillin-insensitive *S. pneumoniae* isolates as compared with reference compounds. These data indicate that the 3-[(*N*-alkylpyridinium-4′-yl)thio]methyl group in combination with the 7-(2-aminothiazol-4-yl) moiety contributes to the enhancement of the activity against clinical MRSA, *E. faecalis*, and ampicillin-insensitive *S. pneumoniae* isolates.

The *in vitro* effectiveness of new 2-oxaisocephem derivatives was mirrored by their excellent *in vivo* efficacy displayed by these derivatives in a mouse-protection model. Efficacy in systemic infection due to *S. aureus* Smith, clinically isolated MRSA 5038 (low-resistant), MRSA 5120 (high-resistant), and MRSA 5129 (high-resistant), *E. faecalis* C 0063, *S. pneumoniae* C 0096 (insensitive bacteria to ampicillin), and *E. coli* No. 29 in mice of several selected compounds (**11a,c,f,g**, flomoxef, cefpirome, vancomycin, and ampicillin) is shown in Table 4. One hour after intraperitoneal infection, several doses of each compound were subcutaneously administered to mice. Efficacy of each compound was expressed as 50% effective dose values ($ED_{50}$) which were calculated from survivals on the seventh day after infection by the probit method. The *in vivo* efficacy of new 2-oxaisocephems on the experimental infection due to *S. aureus* Smith and *E. coli* No. 29 was apparently greater than that of flomoxef, cefpirome, or vancomycin. Compound **11g** showed higher subcutaneous efficacy on the systemic infection caused by *E. faecalis* in mice than flomoxef and cefpirome. Similarly, **11g** exhibited 16 times more excellent *in vivo* efficacy than ampicillin on the experimental infection caused by *S. pneumoniae* C 0096 which is an insensitive bacteria to ampicillin and will bring about a clinical serious problem in antibacterial chemotherapy in near future. Vancomycin is widely used clinically as an anti-MRSA agent. Even though vancomycin has lower MIC values against MRSA than **11a,c,f,g**, *in vivo* potency of **11a,c,f** on systemic infection caused by low-resistant MRSA 5038 is about the same as that of vancomycin. In addition, *in vivo* efficacy of **11a** or **11g** on the experimental infection due to high-resistant MRSA 5120 and 5129 was superior to that of vancomycin. As a result of this study, it was found that the above 2-oxaisocephem derivatives with a [(*N*-alkylpyridinium-4′-yl)thio]methyl group at the 3-position and a 2-(2-aminothiazol-4-yl)-2-(*Z*)-[(cyclopentyloxy)imino]acetamido group at the 7-position are potent antibacterial agents and possess excellent *in vivo* efficacy as compared with flomoxef, cefpirome, vancomycin, and ampicillin as reference compounds.

## Experimental Section

**General Methods.** Reagents were used as supplied unless otherwise noted. All the melting points were determined on a Yanaco MP-500D apparatus and are uncorrected. Proton nuclear magnetic resonance ($^1H$ NMR) spectra were recorded on a BRUKER AC250 instrument operating at 250 MHz. Chemical shifts are reported in parts per million (ppm) on the $\delta$ scale downfield relative to tetramethylsilane as an internal standard and coupling constants in hertz (Hz). Infrared (IR) spectra were measured for KBr pellets with a JASCO IR-810 infrared spectrophotometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. Satisfactory spectral data were obtained for all new compounds. Satisfactory elemental analyses (±0.3%) were obtained for all crystalline derivatives.

**(3S,4*R*)-1-[(*R*)-1-[(Benzhydryloxy)carbonyl]-(*S*)-2-hydroxypropyl]-3-(4-nitrophthalimido)-4-styrylazetidin-2-one (2).** This compound was prepared essentially as described in our preceding paper:[21] mp 222.5–223 °C; $[\alpha]^{28}_D$ −93.3° (*c* 0.12, $CHCl_3$). Anal. ($C_{36}H_{29}N_3O_8$) C, H, N.

**(3S,4S)-1-[1-[(Benzhydryloxy)carbonyl]-2-hydroxypropenyl]-4-[(mesyloxy)methyl]-3-(4-nitrophthalimido)azetidin-2-one (3)**. This compound was also obtained as described in our previous paper.[21]

**Benzhydryl (6S,7S)-3-(Bromomethyl)-7-(4-nitrophthalimodo)-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (6)**. To a mixture of **3** (6.7 g, 10.5 mmol) and *p*-toluenesulfonyl chloride (2.21 g, 11.6 mmol) in $CH_2Cl_2$ (100 mL) was added *N*-methylpyrrolidine (990 mg, 11.6 mmol) at 0 °C dropwise. After stirring for 1 h, morpholine (3.66 g, 42 mmol) was added at −15 °C dropwise to the reaction mixture, which was stirred for 1.5 h. After this, the mixture was washed with water (50 mL × 4) and brine (50 mL), dried over $MgSO_4$, and filtered, and the filtrate was concentrated under reduced pressure to afford the crude **4**. The residue was dissolved in THF (100 mL). To this solution was added pyridine perbromide (2.51 g, 10.5 mmol) at −30 °C. Then 4 N aqueous sulfuric acid solution (70 mL) was added to the reaction mixture, which was stirred for 3 h at room temperature. The mixture was diluted with AcOEt (150 mL), washed with water (75 mL × 5), dried over $MgSO_4$, and filtered, and the filtrate was evaporated under reduced pressure to give the crude **5**. To a solution of this residue in acetone (70 mL) and water (35 mL) was added $NaHCO_3$ (882 mg, 10.5 mmol), and the mixture was stirred at room temperature for 1 h. The resulting precipitates were collected by filtration, washed with water, and recrystallized from $CH_2Cl_2$−hexane to give **6** (3.3 g, 51%) as pale yellow needles: mp 187−188.5 °C; $[\alpha]^{27}_D$ −35.4° (*c* 0.226, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 3.94−4.08 (1H, m), 4.30−4.50 (2H, m), 4.55 (1H, dd, *J* = 4.0, 10.3 Hz), 4.72 (1H, d, *J* = 10.5 Hz), 5.97 (1H, d, *J* = 5.4 Hz), 6.97 (1H, s), 7.20−7.60 (10H, m), 8.11 (1H, d, *J* = 8.1 Hz), 8.67 (1H, dd, *J* = 2.0, 8.1 Hz), 8.71 (1H, d, *J* = 2.0 Hz); IR ($cm^{-1}$) 1800, 1790, 1730, 1700, 1620, 1540, 1380, 1340. Anal. ($C_{29}H_{20}BrN_3O_8$) C, H, N.

**Benzhydryl (6S,7S)-7-[2-(2-Aminothiazol-4-yl)-2-[(Z)-(cyclopentyloxy)imino]acetamido]-8-oxo-3-[[(4′-pyridyl)thio]methyl]-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (9)**. To a solution of **6** (1 g, 1.62 mmol) and 4-mercaptopyridine (180 mg, 1.62 mmol) in DMF (8 mL) was added triethylamine (164 mg, 1.62 mmol) at 0 °C dropwise. After stirring for 30 min, methylhydrazine (82 mg, 1.8 mmol) was added at −50 °C to the reaction mixture without isolation of **7**, which was stirred for 30 min. Then AcOH (0.4 mL) was added to the solution, which was allowed to warm to room temperature and stirred for 2 h. The thus obtained mixture was diluted with AcOEt (20 mL) and washed with 5% aqueous $NaHCO_3$ solution (50 mL). The aqueous layer was extracted with additional AcOEt (20 mL). The organic extracts were combined, washed with aqueous 5% $NaHCO_3$ solution (30 mL × 4) and brine (30 mL), dried over $Na_2SO_4$, and filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in $CH_2Cl_2$ (20 mL). Then, 2-mercaptobenzothiazole active ester **8** (567 mg, 1.62 mmol) was added to the solution, which was stirred at room temperature overnight. The mixture was washed with 5% aqueous $NaHCO_3$ solution (10 mL × 2) and water (10 mL × 2) and extracted with 0.2 N HCl (10 mL × 2). The combined aqueous extracts were washed with AcOEt (10 mL × 3), and the pH of this aqueous solution was adjusted to 6 with 5% aqueous $NaHCO_3$ solution. Then the mixture was extracted with $CH_2Cl_2$ (15 mL × 2). The combined organic extracts were dried over $Na_2SO_4$ and filtered, and the filtrate was evaporated under reduced pressure. The residue was purified by silica gel column chromatography to afford **9** (650 mg, 61%) as a pale yellow powder: $^1H$ NMR ($CDCl_3$) δ 1.42−1.87 (8H, m), 3.96−4.11 (2H, m), 4.30 (2H, s), 4.64 (1H, dd, *J* = 2.8, 10.0 Hz), 4.75−4.85 (1H, m), 5.67 (1H, dd, *J* = 4.7, 6.3 Hz), 6.74 (1H, s), 6.94 (1H, s), 7.14 (2H, dd, *J* = 1.6, 4.7 Hz), 7.26−7.55 (10H, m), 8.06 (1H, d, *J* = 6.3 Hz), 8.29 (2H, dd, *J* = 1.6, 4.7 Hz); IR ($cm^{-1}$) 3450, 1790, 1760, 1720, 1670. Anal. ($C_{36}H_{34}N_6O_6S_2 \cdot {}^1/_2H_2O$) C, H, N.

**(6S,7S)-7-[2-(2-Aminothiazol-4-yl)-2-[(Z)-(cyclopentyloxy)imino]acetamido]-8-oxo-3-[[(4′-pyridyl)thio]methyl]-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (10)**. To a mixture of **9** (70 g, 98.5 mmol) and anisole (63.9 g, 590.9 mmol) in $CH_2Cl_2$ (1 L) was added a solution of aluminum trichloride (46 g, 262.4 mmol) in nitromethane (80 mL) at 0 °C. After vigorous stirring for 1 h at room temperature, the reaction mixture was poured into ice−water (6 L), and concentrated HCl (750 mL) was added to the mixture. The mixture was washed with AcOEt (3 L × 3), and the aqueous layer was separated. The pH of the aqueous solution was adjusted to 8 with $NaHCO_3$, and the resulting insoluble substances were filtered off through Celite. The pH of the filtrate was adjusted to 6 with 10% HCl, and the aqueous solution was subjected to chromatography on Diaion HP-20 using $CH_3CN-H_2O$ mixtures as solvent. After combining the appropriate fractions and evaporation under reduced pressure to remove $CH_3CN$, freeze-drying gave **10** (48.5 g, 90%) as a pale yellow powder: $^1H$ NMR (DMSO-$d_6$) δ 1.40−1.88 (8H, m), 3.85−4.10 (2H, m), 4.27 (1H, d, *J* = 14.1 Hz), 4.42 (1H, d, *J* = 14.1 Hz), 4.49 (1H, dd, *J* = 2.8, 10.0 Hz), 4.60−4.75 (1H, m), 5.63 (1H, dd, *J* = 4.5, 8.3 Hz), 6.74 (1H, s), 7.38 (2H, d, *J* = 6.2 Hz), 8.38 (2H, d, *J* = 6.2 Hz), 9.13 (1H, d, *J* = 8.3 Hz); IR ($cm^{-1}$) 3300, 1770, 1710, 1650, 1620. Anal. ($C_{23}H_{24}N_6O_6S_2 \cdot H_2O$) C, H, N.

**(6S,7S)-7-[2-(2-Aminothiazol-4-yl)-2-[(Z)-(cyclopentyloxy)imino]acetamido]-3-[[(1′-methylpyridinium-4′-yl)thio]methyl]-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Hydrogen Sulfate (11a)**. To a solution of **10** (54.4 g, 89.2 mmol) in DMF (243 mL) was added *N,O*-bis(trimethylsilyl)acetamide (54.4 g, 267.5 mmol) at 0 °C dropwise, and the mixture was stirred at room temperature for 1h. Then methyl iodide (38 g, 267.7 mmol) was added to the mixture. After stirring at room temperature for 6 h, *i*-PrOH (1 L) was added to the solution. The resulting precipitates were collected by filtration, washed with *i*-PrOH, and dissolved in 5% aqueous $NaHCO_3$ solution (5 L), and insoluble substances were filtered off. The pH of the filtrate was adjusted to 6 with 10% HCl. The aqueous solution was subjected to chromatography on Diaion HP-20 using $CH_3CN-H_2O$ mixtures as solvent. The appropriate fractions were combined and evaporated under reduced pressure to remove $CH_3CN$; 4 N $H_2SO_4$ (89.2 mL) was added in an ice bath to the resulting aqueous solution, which was stirred for 30 min. The resultant precipitates were collected by filtration and washed with ice−water to give **11a** (37.5 g, 64%) as a pale yellow powder: $^1H$ NMR (DMSO-$d_6$) δ 1.40−1.85 (8H, m), 3.87−4.05 (2H, m), 4.19 (3H, s), 4.47−4.73 (4H, m), 5.65 (1H, dd, *J* = 4.4, 8.2 Hz), 6.79 (1H, s), 8.03 (2H, d, *J* = 7.0 Hz), 9.19 (1H, d, *J* = 8.2 Hz); IR ($cm^{-1}$) 3330, 1780, 1700, 1680, 1640. Anal. ($C_{24}H_{26}N_6O_6S_2 \cdot H_2SO_4 \cdot 2H_2O$) C, H, N.

Compounds **11b−o** were obtained by the same procedure as described for **11a**.

***In Vitro* Antibacterial Activity**. Minimum inhibitory concentrations (MICs) were determined by the 2-fold agar dilution method[27] with Müller-Hinton agar (Difco Laboratories, Detroit, MI). The overnight broth cultures were diluted to approximately $10^6$ CFU/mL with fresh broth, and an inoculum of $10^4$ CFU/spot was applied to agar plates containing graded concentrations of each compound with an incubating apparatus (microplanter; Sakuma Seisakusyo, Tokyo, Japan). After incubation at 37 °C for 18 h, the MIC was defined as the minimum drug concentration which completely inhibited the growth of bacteria.

***In Vivo* Antibacterial Activity**. *In vivo* activities were determined against systemic infections caused by Gran-positive and Gram-negative pathogens. Male ICR strain mice weighting approximately 20 g, in groups of 10, were used for each dosage group. Mice were challenged intraperitoneally with 0.5 mL of approximately 10−100 times the 50% lethal doses ($LD_{50}$) of the respective pathogens. The bacterial suspensions, which were prepared by overnight cultures at 37 °C on tryptic soy broth (Difco) for *S. aureus*, *E. faecalis*, and MRSA and Müller-Hinton broth for *E. coli* and *S. pneumoniae*, were suspended in the same fresh broth of overnight culture containing 5% gastric mucin. One hour after infection, various doses of each compound were subcutaneously administered to mice. The number of mice surviving at each dose was counted on the seventh day after infection, and the 50% effective dose values ($ED_{50}$) were calculated by the probit method.

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