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Synthesis and Biological Properties of a Series of Optically Active 2-Oxaisocephems

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Abstract—A novel series of (6*S*, 7*S*)-3,7-disubstituted-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic acids **9a–o**, parenteral optically active 2-oxaisocephems, was synthesized, and *in vitro* and *in vivo* activities were determined against Gram-positive and Gram-negative bacteria. The 7-[2-(2-aminothiazol-4-yl)-2-(*Z*)-cyclopentyloxyimino]acetamido derivatives, **9g**, **9m** and **9o**, had enhanced antibacterial activity against Gram-positive organisms including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis* while maintaining Gram-negative activity. It is also significant that these compounds showed more potent activity against MRSA and *E. faecalis* isolates than cefuzonam (**10**) and flomoxef (**12**), which are the most popular third-generation antibiotics. The combination of the 7-[2-(aminothiazol-4-yl)-2-(*Z*)-cyclopentyloxyimino]acetamido group and 2-oxaisocephem nucleus contributes to the increased antibacterial activity against these clinical isolates. The 7-[2-(2-aminothiazol-4-yl)-2-(*Z*)-cyclopentyloxyimino]acetamido derivative **9g** provided good subcutaneous efficacy and exhibited more potent activity than cefmenoxime (**11**) against the systemic infection with *S. aureus* Smith in mice. The compound **9a** with a [2-(2-aminothiazol-4-yl)-2-(*Z*)-methoxyimino]acetamido group at the 7-position showed high *in vivo* efficacy on the experimental infection caused by *Escherichia coli* No. 29 in mice.

Introduction

The third-generation antibiotics such as cefuzonam (**10**), cefmenoxime (**11**), and flomoxef (**12**) have been clinically used as potent drugs around the world, because they have a broad spectrum of activity against both Gram-positive and Gram-negative bacteria. One of the most apparent defects of the third-generation antibacterial agents is a resistance against some clinically significant pathogens, i.e. methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis*. In particular, it has been reported that MRSA is a major pathogen in hospitals and has been associated with an increasing number of infections since 1961.[1] Therefore, the need for a novel effective agent for the treatment of bacterial infections such as MRSA has become extremely significant.

As part of our study to find more potent anti-infectives, we were interested in the construction of a clinically useful compound for the treatment of these types of infections. Our efforts have been directed toward the synthesis and evaluation of 2-oxaisocephem derivatives[2–19] with the advantage of potent activities against a variety of pathogenic organisms including MRSA and *E. faecalis*, since most third-generation antibiotics are not effective against them.

Recently, we reported the synthesis and antibacterial activity of a series of (6*SR*, 7*SR*)-3,7-disubstituted-8-

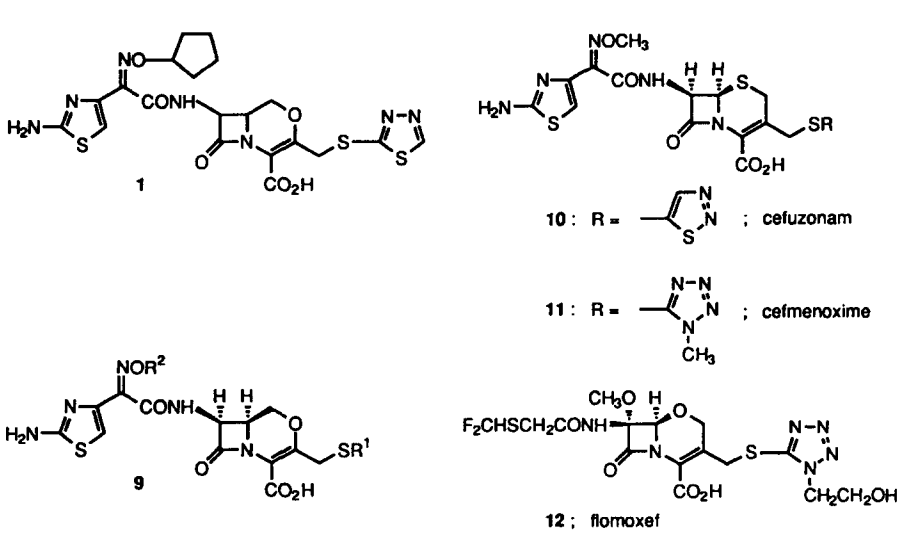


Chart 1.

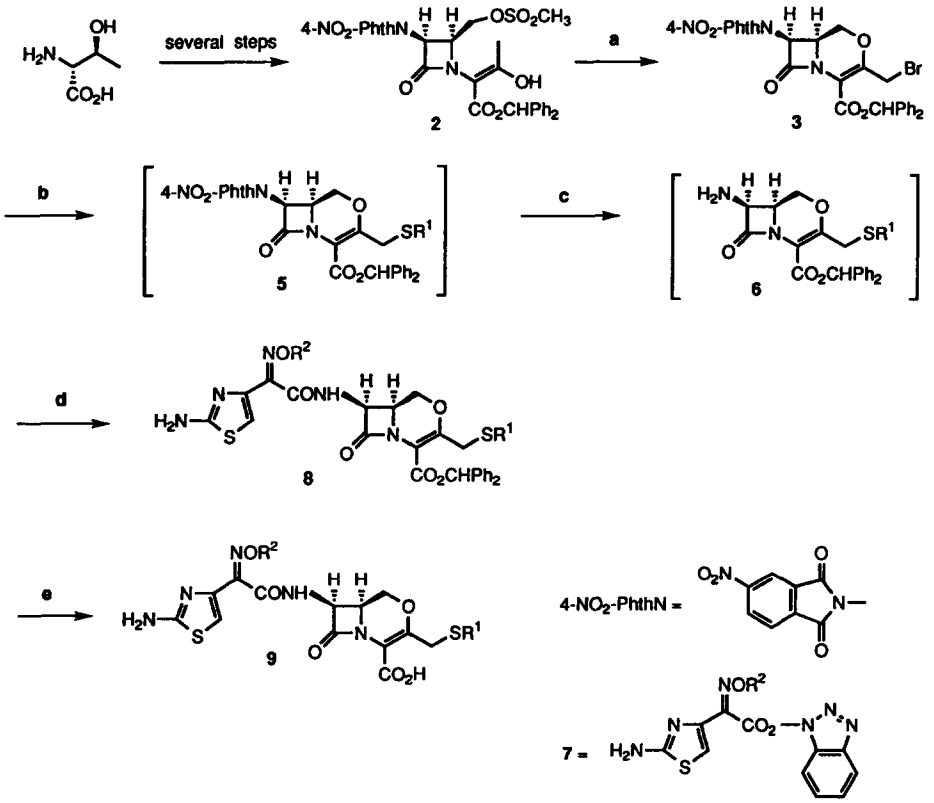
oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic acids, in which the 7-[2-(2-aminothiazol-4-yl)-2-(*Z*)-cyclopentyloxyimino]acetamido derivative such as **1** showed potent and broad-spectrum activity against test organisms including MRSA and *E. faecalis* as compared with **10** (cefuzonam) and **11** (cefmenoxime).[20] Along with this study, and in order to obtain compounds having more effective antibacterial activity than (6*SR*, 7*SR*) derivatives, we have recently presented the convenient synthetic method of 3,7-disubstituted optically active 2-oxaisocephems.[21,22] Therefore, we next focused on the structure of the optically active form **9** and attempted to synthesize a series of (6*S*, 7*S*)-3,7-disubstituted-2-oxaisocephems to reveal their structure–activity relationship.

We describe herein the synthesis and *in vitro* and *in vivo* antibacterial activities of optically active 2-oxaisocephems of a novel type of antibiotics, including their potency against MRSA and *E. faecalis*.

Chemistry

The synthetic route leading to the (6*S*, 7*S*)-3,7-disubstituted-2-oxaisocephems **9a–o** is summarized in Scheme 1. The important intermediate for **9a–o** is the 3-bromomethyl derivative **3** with 4-nitrophthalimido group at the 7-position readily derived from D-threonine in several steps.[21,22] Namely, the compound **3** was reacted with thiol derivatives **4** in the presence of triethylamine in DMF to afford **5** followed by deprotection of 4-nitrophthalimido group with methylhydrazine under mild conditions[22,23] to give amines **6**. These were next converted into **8** having 2-aminotriazol-4-yl moiety at the 7-position by *in situ* acylation with 1-hydroxybenzotriazole (HOBT) active esters **7** in CH_2Cl_2. One pot conversion of **3** into the intermediates **6** seems to proceed smoothly because of the enhanced reactivity of imido functionality of the 4-nitrophthaloyl protection at the 7-position of **3**. As described in our previous paper,[23] the yield of this deprotection step was poor when using the phthalimido-protecting group which is generally used to provide various primary amines. The carboxylic acids used to prepare HOBT active esters **7** were synthesized essentially according to the published method.[24]

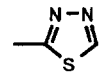
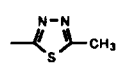
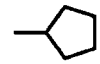
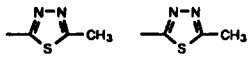
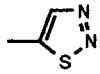
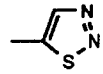
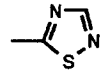
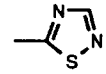
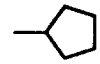
New optically active 2-oxaisocephems **9** were easily derived from **8** thus obtained. It was necessary to cleave the benzhydryl-protecting group of **8** to obtain the new target compounds **9**. When **8** was treated with trifluoroacetic acid (TFA) in the presence of anisole at 0 °C for 10 min, the protecting group was removed smoothly to afford **9**. The color of the reaction solution often became dark by treatment of **8** with TFA for a longer time, which caused difficulty in the purification of products. Therefore, it seems that a satisfactory result is achieved by shorter reaction time in this deprotection step. All compounds **9a–o** synthesized in this investigation are listed in Table 1.



Scheme 1.

a (1) *p*-Toluenesulfonyl chloride/*N*-methylpyrrolidine (2) morpholine (3) pyridine perbromide (4) H^+ (5) $NaHCO_3$. **b** R^1SH 4/Et_3N/DMF. **c** (1) CH_3NHNH_2 (2) AcOH. **d** HOBT active esters 7/CH_2Cl_2. **e** TFA/anisole.

Table 1. (6*S*, 7*S*)-3,7-Disubstituted-8-oxo-1-aza-4-oxabicyclo[4.2.0]-oct-2-ene-2-carboxylic acids **9a–o**

compd.	**9a**	**9b**	**9c**	**9d**	**9e**	**9f**	**9g**	**9h**
R^1								
R^2	CH_3	CH_2CH_3		$CH_2CH{=}CH_2$	CH_2CN	CH_2 		CH_3
compd.	**9i**	**9j**	**9k**	**9l**	**9m**	**9n**	**9o**	
R^1								
R^2			CH_3	CH_2 		CH_3		

In summary, we have demonstrated that new optically active 2-oxaisocephem derivatives were readily prepared from the important intermediate **3** and this method made it possible to introduce various substituents into the 3- and the 7-positions.

Results and Discussion

The compounds **9a–o** synthesized in this investigation were examined for *in vitro* antibacterial activity against Gram-positive (*S. aureus* FDA 209P, MRSA 57, *Staphylococcus epidermidis* ATCC 12228, *E. faecalis* ATCC 21212) and Gram-negative (*E. coli* NIHJ JC-2, *Klebsiella pneumoniae* NCTC 9632, *Serratia marcescens* ATCC 12648, *Pseudomonas aeruginosa* ATCC 10145) bacterial species. The minimum inhibitory concentrations (MICs: μg mL^{-1}, inoculum size: 10^6 cells mL^{-1}) were determined by a twofold agar dilution method.[25] The results are summarized in Table 2. The antibacterial activity of cefuzonam (**10**), cefmenoxime (**11**) and flomoxef (**12**) as reference compounds are also presented.

Table 2. *In vitro* antibacterial activity (MICs[a], μg mL^{-1}) of the compounds **9a–o**

Compd.	Gram-positive bacteria[b]				Gram-negative bacteria[b]			
	Sa	MRSA	Se	Ef	Ec	Kp	Sm	Pa
9a	0.39	>100	0.2	25	0.1	0.1	0.39	12.5
9b	0.78	>100	0.39	25	0.1	0.1	0.39	12.5
9c	0.2	12.5	0.2	6.25	0.39	0.78	0.39	6.25
9d	0.39	50	0.2	12.5	0.2	0.2	0.78	12.5
9e	0.78	100	0.2	50	0.1	0.2	0.39	12.5
9f	0.39	25	0.2	12.5	0.39	0.39	0.78	6.25
9g	0.2	6.25	0.1	3.13	0.2	0.1	0.78	6.25
9h	0.39	>100	0.2	50	0.1	0.2	0.39	12.5
9i	0.2	12.5	0.2	6.25	0.78	0.78	0.78	6.25
9j	0.39	12.5	0.2	3.13	0.78	0.39	0.78	6.25
9k	0.2	>100	0.2	25	0.1	0.1	0.39	12.5
9l	0.2	25	0.2	6.25	0.39	0.2	0.78	6.25
9m	0.2	3.13	0.1	1.56	0.39	0.39	0.78	6.25
9n	0.2	50	0.1	25	0.1	0.1	0.39	6.25
9o	0.2	3.13	0.1	1.56	0.39	0.2	0.78	6.25
cefuzonam	0.39	100	0.1	100	0.1	0.024	0.05	25
cefmenoxime	1.56	>100	0.1	>100	0.1	0.012	0.1	25
flomoxef	0.39	50	0.39	100	0.1	0.1	0.78	>100

[a]Minimum inhibitory concentrations. [b]Definitions of organism abbreviations: Sa = *Staphylococcus aureus* FDE 209P, MRSA = methicillin-resistant *Staphylococcus aureus* 57, Se = *Staphylococcus epidermidis* ATCC 12228, Ef = *Enterococcus faecalis* ATCC 21212, Ec = *Escherichia coli* NIHJ JC-2, Kp = *Klebsiella pneumoniae* NCTC 9632, Sm = *Serratia marcescens* ATCC 12648, Pa = *Pseudomonas aeruginosa* ATCC 10145.

Among seven compounds (**9a–g**) with a (1,3,4-thiadiazol-2-yl)thiomethyl group at the 3-position, the one having a [2-(2-aminothiazol-4-yl)-2-(*Z*)-cyclopentyloxyimino]acetamido group at the 7-position (**9g**) showed apparently enhanced activity against Gram-positive bacteria including MRSA *and E. faecalis* as compared with reference compounds. The replacement of the (1,3,4-thiadiazol-2-yl)thiomethyl group at the 3-position of **9g** by other thio-substituted methyl substituents (**9j**, **9m**, and **9o**) similarly increased the activities against MRSA and *E. faecalis*. The derivatives containing a [2-(2-aminothiazol-4-yl)-2-(*Z*)-isopropyloxyimino]acetamido side-chain at the 7-position (**9c** and **9i**) also showed about the same activities against Gram-positive bacteria tested as **9j**. On the other hand, in the compounds with a [2-(2-aminothiazol-4-yl)-2-(*Z*)-methoxyimino]acetamido group (**9a**, **9h**, **9k** and **9n**), which is often used as the side-chain at the 7-position of third-generation cephalosporins, a decrease of the activity against MRSA and *E. faecalis* was observed. Against Gram-negative bacteria (except for *P. aeruginosa*), the introduction of [2-(2-aminothiazol-4-yl)-2-(*Z*)-methoxyimino]acetamido group into the 7-position gave a good result. Namely, the compounds **9a**, **9h**, **9k** and **9n** exhibited about the same activity against Gram-negative organisms as flomoxef (**12**). Although 7-[2-(2-aminothiazol-4-yl)-2-(*Z*)-cyclopentyloxyimino]acetamido derivatives (**9g**, **9j**, **9m**, and **9o**) showed slightly less potent activity against Gram-negative bacteria than flomoxef (**12**), it seems that they also maintain the Gram-negative activity.

Consequently, we found that the use of the 7-[2-(2-aminothiazol-4-yl)-2-(*Z*)-cyclopentyloxyimino]acetamido side-chain enhanced Gram-positive *in vitro* antibacteial activity and all compounds prepared maintained Gram-negative activity.

In vitro antibacterial activity of the compounds **9g**, **9m** and **9o** against some strains isolated clinically (*S. aureus*, MRSA, *E. faecalis*, *E. coli* and *P. aeruginosa*) is shown in Table 3. Data for cefuzonam (**10**) and flomoxef (**12**) are also included for comparison purposes. The derivatives **9g**, **9m** and **9o** evidently showed higher activity against *S. aureus* and *P. aeruginosa* isolates than reference compounds. In addition, **9g** and **9m** significantly displayed potent *in vitro* activity against *E. faecalis* isolates. Against *E. coli* isolates, the activity of **9g**, **9m** and **9o** was slightly less potent than that of flomoxef (**12**), but seems to be equal to that of cefuzonam (**10**). Recently, infections caused by MRSA strains have brought about a serious clinical problem in antibacterial chemotherapy.[26–29] The activity of the compounds **9g**, **9m** and **9o** against clinically isolated MRSA strains is similarly shown in Table 3, indicating that cefuzonam- and flomoxef-resistant MRSA strains have emerged. These three compounds showed excellent antibacterial activity against MRSA isolates including cefuzonam- and flomoxef-resistant MRSA strains, suggesting that the combination of the 7-[2-(2-aminothiazol-4-yl)-2-(*Z*)-cyclopentyloxyimino]-acetamido group and 2-oxaisocephem nucleus is efficient for increasing the activity against clinical MRSA isolates.

Efficacy (subcutaneous administration) of the selected compounds **9a**, **9g**, cefmenoxime (**11**) and flomoxef (**12**) in systemic infections caused by *S. aureus* Smith, *E. coli* No. 29, and MRSA 5038 in mice is shown in Table 4. Efficacy of each compound was expressed as fifty percent effective dose value (ED_{50}) calculated by the probit method. The derivatives **9a** and **9g** showed good subcutaneous efficacy on the experimental infection caused by *S. aureus* Smith as compared with **11**. The efficacy of **9g** was slightly greater than that of **9a**. *In vivo* potency of **9a** against *E. coli* No. 29 was superior to that of **9g**, but slightly less potent than that of **11**. Consequently, the *in vivo* efficacy of **9a** and **9g** against *S. aureus* and *E. coli* was the same level as we expected from their *in vitro* antibacterial activities. On the other hand, the efficacy of **9g** against MRSA was somewhat less potent contrary to our expectation.

In conclusion, the optically active 2-oxaisocephems having [2-(2-aminothiazol-4-yl)-2-(*Z*)-cyclopentyloxyimino]acetamido group at the 7-position synthesized in this investigation were effective anti-bacterial agents against Gram-positive bacteria including MRSA and *E. faecalis* strains, while maintaining Gram-negative activity. However, subcutaneous efficacy against experimental infection caused by MRSA 5038 was not necessarily satisfactory. Further modification of the 7-substituent in combination with alteration of the side-chain at the 3-position may result in enhanced *in vivo* efficacy. We are now searching for more effective optically active 2-oxaisocephem antibiotics.

Experimental

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 (^{1}H NMR) spectra were recorded on a Bruker AC250 instrument operating at 250 MHz. Chemical shifts are reported in parts per million (ppm) on the δ scale downfield relative to tetramethylsilane as an internal standard and coupling constants in 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.

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

Benzhydryl-(6S,7S)-3-bromomethyl-7-(4-nitrophthalimido)-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (3). To a mixture of **2** (6.7 g, 10.5 mmol) and *p*-toluenesulfonyl chloride (2.21 g, 11.6 mmol) in CH_2Cl_2

Table 3. *In vitro* antibacterial activity (MICs, μg mL^{-1}) of **9g**, **9m** and **9o** against clinical isolates

strains	compd.	MIC_{50}[c]	MIC_{80}[d]	MIC range[e]
S. aureus[a]	9g	0.78	1.56	0.1 - 6.25
	9m	0.78	1.56	0.05 - 3.13
	9o	1.56	3.13	0.05 - 6.25
	cefuzonam	1.56	25	0.2 - 100
	flomoxef	0.78	6.25	0.1 - 100
MRSA[a]	9g	3.13	6.25	0.39 - 12.5
	9m	3.13	6.25	0.39 - 6.25
	9o	3.13	6.25	0.39 - 12.5
	cefuzonam	100	>100	0.78 - >100
	flomoxef	25	50	0.39 - >100
E. faecalis[a]	9g	6.25	6.25	1.56 - 25
	9m	3.13	3.13	0.78 - 12.5
	cefuzonam	100	100	25 - >100
	flomoxef	>100	>100	>100
E. coli[a]	9g	0.39	0.39	≤0.025 - 3.13
	9m	0.39	0.78	≤0.025 - 6.25
	9o	0.39	0.39	≤0.025 - 3.13
	cefuzonam	0.05	0.39	≤0.025 - 1.56
	flomoxef	0.05	0.1	≤0.025 - 0.78
P. aeruginosa[b]	9g	6.25	12.5	3.13 - 25
	9m	12.5	12.5	6.25 - 25
	9o	6.25	12.5	3.13 - 25
	cefuzonam	50	50	12.5 - 100
	flomoxef	>100	>100	>100

[a]27 Clinical isolates. [b]39 Clinical isolates. [c]The MIC value for 50% of isolates. [d]The MIC value for 80% of isolates. [e]The range of MIC value for isolates.

Table 4. Subcutaneous efficacy of **9a** and **9g** on systemic infections in mice in comparison with cefmenoxime and flomoxef

organisms	compd.	MIC (μg / mL)	ED_{50} (mg / kg)[a]	95% confidence limits (mg / kg)
S. aureus Smith	9a	0.78	1.07	0.78 - 1.59
	9g	0.39	0.75	0.51 - 1.02
	cefmenoxime	1.56	2.00	1.24 - 2.76
E. coli No.29	9a	0.024	0.08	0.06 - 0.11
	9g	0.2	0.75	0.54 - 1.13
	cefmenoxime	0.024	0.03	0.02 - 0.04
MRSA 5038	9g	6.25	30.26	21.13 - 42.91
	flomoxef	6.25	19.24	12.52 - 29.09

[a]Dose required to prevent death in 50% of animals

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 time, the mixture was washed with water (50 mL × 4) and brine (50 mL), dried over $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure. 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 $MgSO_4$, filtered, and the filtrate was evaporated under reduced pressure. 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 **3** (3.3 g, 51%) as pale yellow needles, mp 187–188.5 °C. $[\alpha]_D^{27}$ −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. calcd for $C_{29}H_{20}BrN_3O_8$: C, 56.33; H, 3.26; N, 6.79. Found: C, 56.25; H, 3.15; N, 6.80.

Benzhydryl-(6S,7S)-7-[2-(2-aminothiazol-4-yl)-2-[(Z)-methoxyimino]acetamido]-8-oxo-3-[(1,3,4-thiadiazol-2-yl)thiomethyl]-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (8a). To a solution of **3** (5 g, 8.09 mmol) and 2-mercapto-1,3,4-thiadiazole (956 mg, 8.09 mmol) in DMF (40 mL) was added triethylamine (818 mg, 8.09 mmol) at 0 °C dropwise. After stirring for 30 min, methylhydrazine (900 mg, 8.89 mmol) was added at −50 °C to the reaction mixture, without isolation of **5**, which was stirred for 30 min. Then AcOH (2 mL) was added to the solution, which was allowed to warm to room temperature and stirred for 2 h. The mixture thus obtained was diluted with AcOEt (100 mL) and washed with 5% aqueous $NaHCO_3$ solution (250 mL). The aqueous layer was extracted with additional AcOEt (100 mL). The organic extracts were combined, washed with aqueous 5% $NaHCO_3$ solution (150 mL × 4) and brine (150 mL), dried over Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 mL). To this solution was added the HOBT active ester **7** prepared from the corresponding carboxylic acid (1.63 g, 8.09 mmol), *N,N'*-dicyclohexylcarbodiimide (DCC) (1.67 g, 8.09 mmol), and 1-hydroxybenzotriazole (HOBT) (1.09 g, 8.09 mmol) in CH_2Cl_2 (50 mL), and this mixture was stirred at room temperature for 10 h. The solvent was removed under reduced pressure and the residue was purifled by silica gel column chromatography (eluent: CH_2Cl_2:AcOEt, 6:1) to give **8a** (3.43 g, 64%) as a pale yellow powder. ^1H NMR ($CDCl_3$) δ: 3.82–4.20 (5H, *m*), 4.22 (1H, *d*, *J* = 13.7 Hz), 4.31 (1H, *d*, *J* = 13.7 Hz), 4.69 (1H, *dd*, *J* = 3.7, 11.1 Hz), 5.65 (1H, *dd*, *J* = 4.8, 6.5 Hz), 6.85 (1H, *s*), 6.91 (1H, *s*), 7.22–7.60 (10H, *m*), 9.01 (1H, *s*). IR (cm^{-1}): 3340, 1780, 1700, 1670, 1610, 1530.

Compounds **8b–o** were obtained by the same procedures as described for **8a**.

7-[2-(2-Aminothiazol-4-yl)-2-[(Z)-methoxyimino]acetamido]-8-oxo-3-[(1,3,4-thiadiazol-2-yl)thiomethyl]-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (9a). A stirred suspension of **8a** (1.5 g, 2.26 mmol) in anisole (6 mL) was treated with trifluoroacetic acid (15 mL) at 0 °C. Vigorous stirring was maintained for 10 min, after which time Et_2O (120 mL) was added to the solution. The resulting precipitates were collected by filtration, and washed with AcOEt. The precipitates were dissolved in 5% aqueous $NaHCO_3$ solution (150 mL) and insoluble substances were filtered off. The filtrate was adjusted to pH 4 with 10% HCl, and subjected to chromatography on Diaion HP-20 with CH_3CN–H_2O mixtures. After combining the appropriate fractions and evaporation under reduced pressure to remove CH_3CN, freeze-drying gave **9a** (731 mg, 65%) as a white powder. ^1H NMR (DMSO-d_6) δ: 3.74–4.07 (5H, *m*), 4.45–4.65 (3H, *m*), 5.71 (1H, *dd*, *J* = 4.8, 9.1 Hz), 6.85 (1H, *s*), 7.30 (2H, *s*), 9.25 (1H, *d*, *J* = 9.1 Hz), 9.58 (1H, *s*). IR (cm^{-1}): 3400, 1760, 1750, 1700, 1670.

Compounds **9b–o** were obtained by the same procedure as described for **9a**; yields and NMR data are given in Table 5.

In vitro *antibacterial activity*

Minimum inhibitory concentrations (MICs) were determined by the twofold agar dilution method[25] with Muller–Hinton agar (Difco Laboratories, Detroit, MI, U.S.A.). The overnight broth cultures were diluted to approximately 10^6 CFU mL^{-1} with fresh broth, and an inoculum of 10^4 CFU per 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 Gram-positive and Gram-negative pathogens. Male ICR strain mice weighing approximately 20 g, in groups of ten, were used for each dosage group. Mice were challenged intraperitoneally with 0.5 mL of approximately 10 to 100 times the fifty per cent lethal doses (LD_{50}) of the respective pathogens. The bacteria suspension, which was prepared by overnight cultures at 37 °C on Tryptic soy broth (Difco) for *S. aureus* and MRSA, and Muller–Hinton broth for *E. coli*, 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 fifty per cent effective dose values (ED_{50}) were calculated by the probit method.

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Table 5. (6*S*, 7*S*)-3,7-Disubstituted-8-oxo-1-aza-4-oxabicyclo[4.2.0]-oct-2-ene-2-carboxylic acids **9b–o**

Compd.	Yield (%)[a]	^{1}H NMR δ (250 MHz, DMSO-d_6)
9b	65	1.24 (3H, t, J = 6.3 Hz), 3.90-4.30 (4H, m), 4.42-4.63 (3H, m), 5.70 (1H, dd, J = 4.8, 8.4 Hz), 6.78 (1H, s), 7.25 (2H, s), 9.20 (1H, d, J = 8.4 Hz), 9.57 (1H, s).
9c	65	1.19 (6H, d, J = 6.3 Hz), 3.80-4.07 (2H, m), 4.23-4.65 (4H, m), 5.67 (1H, dd, J = 4.8, 8.6 Hz), 6.75 (1H, s), 7.24 (2H, s), 9.18 (1H, d, J = 8.6 Hz), 9.57 (1H, s).
9d	58	3.85-4.05 (2H, m), 4.45-4.70 (3H, m), 5.15-5.40 (3H, m), 5.70 (1H, dd, J = 4.7, 8.4 Hz), 5.85-6.05 (2H, m), 6.80 (1H, s), 7.23 (2H, s), 9.25 (1H, d, J = 8.4 Hz), 9.57 (1H, s).
9e	55	3.78-4.20 (2H, m), 4.47-4.65 (3H, m), 5.06 (2H, s), 5.76 (1H, dd, J = 4.8, 8.4 Hz), 6.95 (1H, s), 7.24 (2H, s), 9.45 (1H, d, J = 8.4 Hz), 9.55 (1H, s).
9f	51	0.2-0.35 (2H, m), 0.50-0.65 (2H, m), 1.00-1.23 (1H, m), 3.81-4.10 (4H, m), 4.47-4.65 (3H, m), 5.67 (1H, dd, J = 4.8, 8.5 Hz), 6.77 (1H, s), 7.22 (2H, s), 9.22 (1H, d, J = 8.5 Hz), 9.56 (1H, s).
9g	67	1.40-1.88 (8H, m), 3.84-4.07 (2H, m), 4.45-4.73 (4H, m), 5.65 (1H, dd, J = 4.8, 8.5 Hz), 6.74 (1H, s), 7.25 (2H, s), 9.16 (1H, d, J = 8.5 Hz), 9.57 (1H, s).
9h	63	2.70 (3H, s), 3.80-4.12 (5H, m), 4.44-4.63 (3H, m), 5.70 (1H, dd, J = 4.8, 9.0 Hz), 6.83 (1H, s), 7.25 (2H, s), 9.23 (1H, d, J = 9.0 Hz).
9i	64	1.19 (6H, d, J = 6.2 Hz), 2.69 (3H, s), 3.80-4.10 (2H, m), 4.27-4.63 (4H, m), 5.70 (1H, dd, J = 4.8, 8.6 Hz), 6.76 (1H, s), 7.24 (2H, s), 9.18 (1H, d, J = 8.6 Hz).
9j	66	1.40-1.87 (8H, m), 2.70 (3H, s), 3.83-4.12 (2H, m), 4.42-4.75 (4H, m), 5.67 (1H, dd, J = 4.8, 8.4 Hz), 6.80 (1H, s), 7.30 (2H, s), 9.18 (1H, d, J = 8.4 Hz).
9k	63	3.75-4.00 (5H, m), 4.31 (1H, d, J = 14 Hz), 4.45 (1H, d, J = 14 Hz), 4.62 (1H, dd, J = 3, 11.1 Hz), 5.73 (1H, dd, J = 4.8, 9 Hz), 6.80 (1H, s), 7.25 (2H, s), 8.90 (1H, s), 9.24 (1H, d, J = 9 Hz).
9l	52	0.2-0.35 (2H, m), 0.51-0.64 (2H, m), 1.00-1.21 (1H, m), 3.63-4.08 (4H, m), 4.25 (1H, d, J = 14 Hz), 4.53 (1H, d, J = 14Hz), 4.62 (1H, dd, J = 3.1, 11.2 Hz), 5.65 (1H, dd, J = 4.7, 8.4 Hz), 6.76 (1H, s), 7.20 (2H, s), 8.85 (1H, s), 9.18 (1H, d, J = 8.4 Hz).
9m	65	1.35-1.85 (8H, m), 3.85-4.11 (2H, m), 4.25 (1H, d, J = 14.2 Hz), 4.45-4.72 (3H, m), 5.67 (1H, dd, J = 4.8, 8.4 Hz), 6.76 (1H, s), 7.27 (2H, s), 8.90 (1H, s), 9.16 (1H, d, J = 8.4 Hz).
9n	63	3.80-4.02 (5H, m), 4.45-4.70 (3H, m), 5.73 (1H, dd, J = 4.8, 8.4 Hz), 6.80 (1H, s), 7.25 (2H, s), 8.73 (1H, s), 9.24 (1H, d, J = 8.4 Hz).
9o	66	1.35-1.90 (8H, m), 3.80-4.05 (2H, m), 4.48-4.70 (4H, m), 5.68 (1H, dd, J = 4.8, 8.4 Hz), 6.76 (1H, s), 7.25 (2H, s), 8.73 (1H, s), 9.14 (1H, d, J = 8.4 Hz).

[a] **9b–o**: Yields from **8b–o** respectively.

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