Synthesis and Antibacterial Activities of 2-Oxaisocephems

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A series of 2-oxaisocephems with a thio-substituted methyl group at the 3-position and a 2-aminothiazol-4-yl moiety at the 7-position was synthesized *via* benzyl 3-acetyloxymethyl-7-azido-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (2), derived from benzyl acetoacetate (1). The new 2-oxaisocephems were tested for antibacterial activities. Among them, the derivatives having a [2-(2-aminothiazol-4-yl)-2-(*Z*)-cyclopentyloxyimino]acetamido group at the 7-position characteristically showed potent activities against gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis* as compared with cefuzonam and cefmenoxime, which are third-generation cephalosporins.

Keywords 2-oxaisocephem; methicillin-resistant *Staphylococcus aureus*; antibacterial activity; *Enterococcus faecalis*; [2-(2-aminothiazol-4-yl)-2-(*Z*)-cyclopentyloxyimino]acetamido group; thio-substituted methyl group

Several investigators have examined 2-oxaisocephem derivatives,[1-3] but Doyle *et al.*[1] reported that 2-oxaisocephems lacked comprehensive antibacterial activity, and 2-oxaisocephem and cephalosporin nuclei with the same side-chain possessed about the same inherent activity.[1a] Later, Mastalerz *et al.* proposed the synthesis of orally absorbable 2-oxaisocephems which would be effective against gram-positive organisms.[2] However, these compounds mostly have the side-chains of the first-generation cephalosporins at the 7-position. We have been searching for compounds with more potent and broad-spectrum antibacterial activity.

The cephalosporin class of antibacterial agents continues to be of clinical importance for the treatment of infection. A significant advance in this area was the introduction of the [2-(2-aminothiazol-4-yl)-2-(*Z*)-methoxyimino]acetamido side-chain at the 7-position.[4] Extended modification of this side-chain in combination with alteration of the substituent at the 3-position of the cephalosporin nucleus has led to the preparation of some potent antibiotics. Cephalosporins with an aminothiazolyloxyiminoacetamido group at the 7-position have been introduced as third-generation antibacterial agents. We speculated that the introduction of side-chains of the third-generation cephalosporins into the 7-position of 2-oxaisocephems and alteration of the 3-substituents might enhance the activity and broaden the antibacterial spectrum. To discover agents which display a better gram-positive spectrum while maintaining gram-negative activity, we have prepared a series of novel 2-oxaisocephems. In particular, we wished to find compounds with improved antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis*.

We report herein the synthesis and antibacterial activity of new 2-oxaisocephem derivatives having a thio-

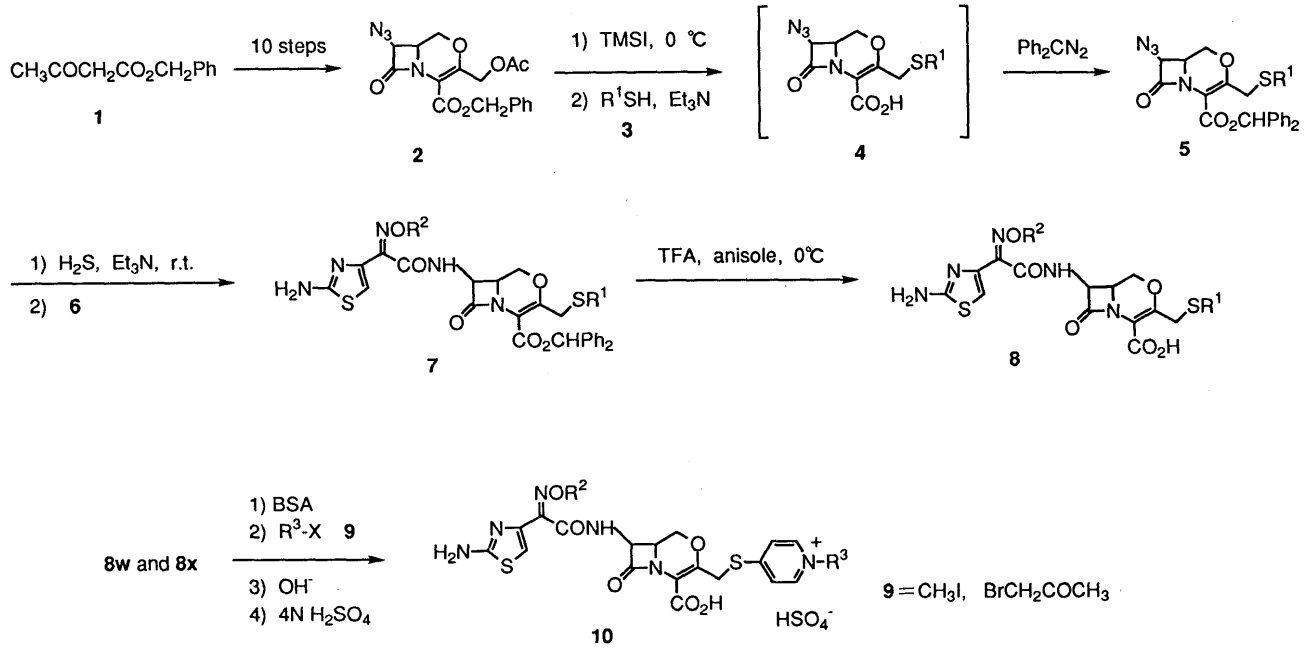


Chart I

substituted methyl group at the 3-position and the 2-aminothiazol-4-yl moiety at the 7-position.

Synthesis

First, the 3-thio-substituted compounds **5**, which are key intermediates in the synthesis of new 2-oxaisocephems (**8a**—**x** and **10a**—**d**), were prepared. The starting material **2** was synthesized in 10 steps from benzyl acetoacetate **1** according to Doyle's method.[1a,e] To introduce the thio-substituted methyl group at the 3-postition of 2-oxaisocephems, the use of trimethylsilyl iodide (TMSI)[5] was found to be efficient. Typical procedures to obtain **5** are as follows. A solution of the 3-acetyloxymethyl derivative **2** (10 g, 26.88 mmol) in anhydrous CH_2Cl_2 (150 ml) was treated with 2.2 eq of TMSI (11.82 g, 59.14 mmol) at 0 °C for 1 h. A solution of 2 eq of thiol compound **3** and triethylamine in dry CH_2Cl_2 (100 ml) was added, and the reaction mixture was stirred at room temperature for 2 h to give **4**. The crude **4**, without purification, was allowed to react with 2 eq of diphenyldiazomethane at room temperature for 2 h to afford **5** (65—76% yields). Compounds **3**, **4**, and **5** are shown in Table I.

Next, we wished to convert the 3-thio-substituted compounds **5** into the target compounds (**8a**—**x**). Reduction of **5** using hydrogen sulfide (H_2S) in the presence of triethylamine in CH_2Cl_2 at room temperature followed by *in situ* acylation with 1-hydroxybenzotriazole (HOBT) active esters **6** gave **7** having a 2-aminothiazol-4-yl moiety at the 7-position in good yields (70—75% yields). The HOBT active esters **6** are listed in Table II. The carboxylic acids used to prepare HOBT active esters were synthesized essentially according to the published method.[6] New 2-oxaisocephems **8** were easily derived from thus obtained **7**. To obtain new compounds, it was required to cleave the benzhydryl ester of **7**. When **7** was treated with trifluoroacetic acid (TFA) in the presence of anisole at 0 °C for 10 min, the benzhydryl ester was cleaved smoothly to afford **8** (60—65% yields). Treatment of **7** with TFA for a longer time often resulted in decomposition of products and difficulty of purification. The synthesized **7** and **8** are listed in Table III.

Furthermore, we synthesized 2-oxaisocephems having an (*N*-alkylpyridinium-4′-thio)methyl group at the 3-position from **8w** and **8x**. Compounds **8w** and **8x** were treated with 3 eq of *N*,*O*-bis(trimethylsilyl)acetamide (BSA) in *N*,*N*-dimethylformamide (DMF) at room temperature for 1 h, halides **9** were added, and the mixture was stirred at 0 °C-room temperature for 6 h to give **10a**—**d** (55—60% yields). Compounds **10** were isolated as hydrogensulfates. The synthesized **10** are listed in Table IV.

In summary, we have established a convenient synthetic route to new 2-oxaisocephems from the 3-acetyloxymethyl derivative **2**, and this method allows us to introduce various substituents into the 3-position and the 7-position.

Biological Results

The compounds (**8a**—**x** and **10a**—**d**) prepared in this investigation were tested for *in vitro* antibacterial activities against gram-positive (*Staphylococcus aureus* FDA 209P, MRSA 57, and *Enterococcus faecalis*) and gram-negative (*Escherichia coli* NIHJ JC-2 and *Pseudomonas aeruginosa* ATCC 10145) bacteria. The minimum inhibitory concentrations (MIC, μg/ml; inoculum size, 10^6 cells/ml) against test organisms were determined by the twofold agar dilution method.[7] The results are summarized in Table VII. The antibacterial activity of cefuzonam and cefmenoxime as reference compounds is also presented.

Among the sixteen compounds (**8a**—**p**) with a (1,3,4-

TABLE I. Compounds **3**, **4**, and **5**

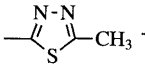
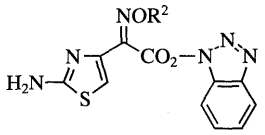
	a	**b**	**c**	**d**	**e**
R^1					

TABLE II. Compounds **6**



	a	**b**	**c**	**d**	**e**	**f**
R^2	CH_3	CH_2CH_3	CH_2SCH_3	CH_2CH_2Cl	CH_2CH_2F	CH_2 

	g	**h**	**i**	**j**	**k**	**l**
R^2	CH_2CN	$CH_2CH=CH_2$	$CH_2CH=CHCH_3$	$CH_2CH_2CH=CH_2$	$CH_2C\equiv CH$	CH_2Ph

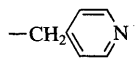
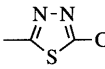
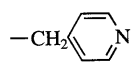
	m	**n**	**o**	**p**
R^2				

TABLE III. Compounds **7** and **8**

	a	b	c	d	e	f
R^1						
R^2	CH_3	CH_2CH_3	CH_2SCH_3	CH_2CH_2Cl	CH_2CH_2F	CH_2 

	g	h	i	j	k	l
R^1						
R^2	CH_2CN	$CH_2CH=CH_2$	$CH_2CH=CHCH_3$	$CH_2CH_2CH=CH_2$	$CH_2C\equiv CH$	CH_2Ph

	m	n	o	p	q	r
R^1						
R^2						CH_3

	s	t	u	v	w	x
R^1						
R^2	CH_2 		CH_3		CH_3	

TABLE IV. Compounds **10**

	a	b	c	d
R^2	CH_3		CH_3	
R^3	CH_3	CH_3	CH_2COCH_3	CH_2COCH_3

thiadiazol-2-yl)thiomethyl group at the 3-position, that with a [2-(2-aminothiazol-4-yl)-2-(*Z*)-cyclopentyloxyimino]acetamido group at the 7-position (**8o**) showed significantly increased activities against all the bacteria tested including MRSA and *E. faecalis*, which are resistant to most cephalosporins, as compared with cefuzonam and cefmenoxime. The replacement of the (1,3,4-thiadiazol-2-yl)thiomethyl group at the 3-position of **8o** by other thio-substituted methyl substituents (**8q**, **8t**, **8v**, and **8x**) also increased the activities against both gram-positive and gram-negative bacteria as compared with reference compounds. In particular, **8v** with a (1,2,4-thiadiazol-5-yl)thiomethyl group at the 3-position showed well-balanced and potent antibacterial activity against test organisms including MRSA and *E. faecalis*. But, substitution with a (2-methyl-1,3,4-thiadiazol-5-yl)thiomethyl or (4-pyridyl)thiomethyl group at the 3-position (**8q** and **8x**) caused a decrease in the activity against MRSA as compared with **8t** and **8v**. In the four compounds (**8a**, **8r**, **8u**, and **8w**) with a [2-(aminothiazol-4-yl)-2-(*Z*)-methoxyimino]acetamido group, which is often used as the side-chain of third-generation cephalosporins at the 7-position, a decrease of the activity against MRSA and *E. faecalis* was observed. Among the four 3-(*N*-alkylpyridinium-4′-thio)methyl compounds (**10a**—**d**), **10b** and **10d** derived from **8x** also possessed broader spectra of antibacterial activity than cefuzonam and cefmenoxime, and **10b** showed about the same *in vitro* activity as **8v**. Substitution of the 7-position by a [2-(2-aminothiazol-4-yl)-2-(*Z*)-methoxyimino]acetamido group (**10a** and **10c**) increased the activity against *E. coli*, but decreased the activity against MRSA and *E. faecalis*.

Among the above compounds, **8v** and **10b** showed potent and broad-spectrum activities against test organisms including MRSA and *E. faecalis*, which cause a serious clinical problem in antibacterial chemotherapy.

Experimental

All the melting points were determined on a Yanaco MP-500D apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC250 instrument operating at 250 MHz. Chemical shifts are expressed in parts per million (ppm) on the δ scale from internal tetramethylsilane and coupling constants in Hz. Infrared (IR) spectra were measured for KBr pellets with a JASCO IR-810 infrared spectrophotometer.

Benzyl 3-Acetyloxymethyl-7-azido-8-oxo-1-aza-4-oxabicyclo[4.2.0]-oct-2-ene-2-carboxylate (2) This compound was prepared essentially as described by Doyle *et al.*[1a,e] in 10 steps from benzyl acetoacetate **1**.

TABLE V. Benzhydryl 7-Azido-8-oxo-3-substituted-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylates **5**

Compound	Yield (%)[a]	mp (°C)[b]	Formula	Analysis (%) Calcd			Found		
				C	H	N	C	H	N
5b	74	208—209	$C_{23}H_{18}N_6O_4S_2$	54.54	3.58	16.59	54.46	3.69	16.35
5c	70	205—206	$C_{23}H_{18}N_6O_4S_2$	54.54	3.58	16.59	54.33	3.56	16.41
5d	71	197—198	$C_{24}H_{20}N_6O_4S_2$	55.37	3.87	16.14	55.49	3.85	15.93
5e	65	192—193	$C_{26}H_{21}N_5O_4S$	62.51	4.24	14.02	62.41	4.22	13.80

a) Yield from **2**. *b*) Decomposition.

TABLE VI. Substituted 8-Oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic Acids **8** and **10**

Compound	Yield (%)[a]	^{1}H-NMR δ (250 MHz, DMSO-d_6)
8b	62	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)
8c	60	2.20 (3H, s), 3.70—4.10 (2H, m), 4.40—4.70 (3H, m), 5.20 (2H, s), 5.74 (1H, dd, J=4.8, 8.4 Hz), 6.82 (1H, s), 7.30 (2H, s), 9.25 (1H, d, J=8.4 Hz), 9.51 (1H, s)
8d	61	3.71—4.13 (4H, m), 4.30 (2H, t, J=6.3 Hz), 4.45—4.65 (3H, m), 5.71 (1H, dd, J=4.8, 8.5 Hz), 6.83 (1H, s), 7.31 (2H, s), 9.25 (1H, d, J=8.5 Hz), 9.56 (1H, s)
8e	61	3.84—4.25 (2H, m), 4.50—4.94 (7H, m), 5.71 (1H, dd, J=4.7, 8.5 Hz), 6.84 (1H, s), 7.32 (2H, s), 9.28 (1H, d, J=8.5 Hz), 9.56 (1H, s)
8f	64	0.2—0.38 (2H, m), 0.50—0.63 (2H, m), 1.00—1.23 (1H, m), 3.80—4.08 (4H, m), 4.48—4.65 (3H, m), 5.67 (1H, dd, J=4.8, 8.5 Hz), 6.77 (1H, s), 7.22 (2H, s), 9.24 (1H, d, J=8.5 Hz), 9.57 (1H, s)
8g	62	3.77—4.20 (2H, m), 4.47—4.67 (3H, m), 5.05 (2H, s), 5.75 (1H, dd, J=4.8, 8.4 Hz), 6.95 (1H, s), 7.25 (2H, s), 9.44 (1H, d, J=8.4 Hz), 9.56 (1H, s)
8h	63	3.83—4.05 (2H, m), 4.43—4.70 (3H, m), 5.15—5.40 (3H, m), 5.70 (1H, dd, J=4.7, 8.4 Hz), 5.83—6.05 (2H, m), 6.79 (1H, s), 7.22 (2H, s), 9.23 (1H, d, J=8.4 Hz), 9.57 (1H, s)
8i	61	1.73—1.92 (3H, m), 3.73—4.17 (2H, m), 4.47—5.00 (5H, m), 5.43—5.60 (1H, m), 5.64—5.93 (2H, m), 6.81 (1H, s), 7.23 (2H, s), 9.19 (1H, d, J=8.5 Hz), 9.51 (1H, s)
8j	60	2.25—2.48 (2H, m), 3.51—4.51 (4H, m), 4.33—4.75 (3H, m), 4.92—5.27 (2H, m), 5.66—6.05 (2H, m), 6.85 (1H, s), 7.22 (2H, s), 9.27 (1H, d, J=8.5 Hz), 9.56 (1H, s)
8k	61	3.45—3.58 (1H, m), 3.70—4.10 (2H, m), 4.28—4.88 (5H, m), 5.69 (1H, dd, J=4.8, 8.4 Hz), 6.84 (1H, s), 7.23 (2H, s), 9.27 (1H, d, J=8.4 Hz), 9.52 (1H, s)
8l	65	3.71—4.55 (5H, m), 5.14 (2H, s), 5.69 (1H, dd, J=4.8, 8.5 Hz), 6.82 (1H, s), 7.16—7.44 (7H, m), 9.28 (1H, d, J=8.5 Hz), 9.57 (1H, s)
8m	62	3.78—4.17 (2H, m), 4.31—4.82 (3H, m), 5.38 (2H, s), 5.73 (1H, dd, J=4.7, 8.3 Hz), 6.79 (1H, s), 7.24 (2H, s), 7.71 (2H, d, J=6.3 Hz), 8.69 (2H, d, J=6.3 Hz), 9.33 (1H, d, J=8.3 Hz), 9.44 (1H, s)
8n	65	0.75—1.00 (6H, m), 1.45—1.78 (4H, m), 3.83—4.15 (3H, m), 4.43—4.65 (3H, m), 5.68 (1H, dd, J=4.7, 8.4 Hz), 6.77 (1H, s), 7.22 (2H, s), 9.21 (1H, d, J=8.4 Hz), 9.57 (1H, s)
8o	65	1.38—1.86 (8H, m), 3.84—4.06 (2H, m), 4.45—4.73 (4H, m), 5.66 (1H, dd, J=4.8, 8.5 Hz), 6.75 (1H, s), 7.22 (2H, s), 9.16 (1H, d, J=8.5 Hz), 9.57 (1H, s)
8p	65	1.12—1.98 (10H, m), 3.85—4.13 (3H, m), 4.43—4.63 (3H, m), 5.67 (1H, dd, J=4.8, 8.5 Hz), 6.74 (1H, s), 7.21 (2H, s), 9.17 (1H, d, J=8.5 Hz), 9.57 (1H, s)
8q	60	1.40—1.87 (8H, m), 2.69 (3H, s), 3.83—4.12 (2H, m), 4.43—4.75 (4H, m), 5.65 (1H, dd, J=4.8, 8.5 Hz), 6.75 (1H, s), 7.22 (2H, s), 9.16 (1H, d, J=8.5 Hz)
8r	62	3.75—4.02 (5H, m), 4.30 (1H, d, J=14 Hz), 4.45 (1H, d, J=14 Hz), 4.61 (1H, dd, J=3, 11.1 Hz), 5.73 (1H, dd, J=4.8, 9 Hz), 6.81 (1H, s), 7.25 (2H, s), 8.90 (1H, s), 9.24 (1H, d, J=9 Hz)
8s	63	0.18—0.39 (2H, m), 0.51—0.64 (2H, m), 0.92—1.26 (1H, m), 3.63—4.08 (4H, m), 4.25 (1H, d, J=14 Hz), 4.53 (1H, d, J=14 Hz), 4.62 (1H, dd, J=3.1, 11.2 Hz), 5.67 (1H, dd, J=4.7, 8.4 Hz), 6.75 (1H, s), 7.17 (2H, s), 8.85 (1H, s), 9.18 (1H, d, J=8.4 Hz)
8t	65	1.35—1.90 (8H, m), 3.85—4.10 (2H, m), 4.25 (1H, d, J=14.2 Hz), 4.45—4.72 (3H, m), 5.66 (1H, dd, J=4.8, 8.4 Hz), 6.75 (1H, s), 7.24 (2H, s), 8.90 (1H, s), 9.16 (1H, d, J=8.4 Hz)
8u	61	3.80—4.03 (5H, m), 4.44—4.70 (3H, m), 5.72 (1H, dd, J=4.8, 8.4 Hz), 6.79 (1H, s), 7.21 (2H, s), 8.73 (1H, s), 9.23 (1H, d, J=8.4 Hz)
8v	65	1.38—1.90 (8H, m), 3.80—4.05 (2H, m), 4.48—4.70 (4H, m), 5.66 (1H, dd, J=4.8, 8.4 Hz), 6.74 (1H, s), 7.23 (2H, s), 8.73 (1H, s), 9.14 (1H, d, J=8.4 Hz)
8w	60	3.85—4.19 (5H, m), 4.25 (1H, d, J=14 Hz), 4.40 (1H, d, J=14 Hz), 4.48 (1H, dd, J=3, 10.3 Hz), 5.85 (1H, dd, J=4.6, 8.3 Hz), 6.69 (1H, s), 7.32 (2H, d, J=6.2 Hz), 8.36 (2H, d, J=6.2 Hz), 9.14 (1H, d, J=8.3 Hz)
8x	60	1.40—1.88 (8H, m), 3.83—4.08 (2H, m), 4.28 (1H, d, J=14.1 Hz), 4.41 (1H, d, J=14.1 Hz), 4.49 (1H, dd, J=2.8, 10 Hz), 4.59—4.76 (1H, m), 5.63 (1H, dd, J=4.4, 8.3 Hz), 6.74 (1H, s), 7.38 (2H, d, J=6.2 Hz), 8.38 (2H, d, J=6.2 Hz), 9.12 (1H, d, J=8.3 Hz)
10b	60	1.40—1.85 (8H, m), 3.85—4.05 (2H, m), 4.19 (3H, s), 4.45—4.75 (4H, m), 5.65 (1H, dd, J=4.4, 8.2 Hz), 6.78 (1H, s), 8.02 (2H, d, J=7.1 Hz), 8.70 (2H, d, J=7.1 Hz), 9.20 (1H, d, J=8.2 Hz)
10c	55	2.27 (3H, s), 3.78—4.21 (4H, m), 4.45—4.75 (4H, m), 5.50 (2H, s), 5.64 (1H, dd, J=4.5, 8.3 Hz), 6.75 (1H, s), 8.09 (2H, d, J=7.1 Hz), 8.52 (2H, d, J=7.1 Hz), 9.15 (1H, d, J=8.3 Hz)
10d	58	1.40—1.83 (8H, m), 2.29 (3H, s), 3.94—4.10 (2H, m), 4.41—4.77 (4H, m), 5.56 (2H, s), 5.66 (1H, dd, J=4.5, 8.3 Hz), 6.76 (1H, s), 8.10 (2H, d, J=7.1 Hz), 8.52 (2H, d, J=7.1 Hz), 9.14 (1H, d, J=8.3 Hz)

a) **8b**—**x**: yields from **7b**—**x** respectively, **10b** and **10d**: yields from **8x**, **10c**: yield from **8w**.

TABLE VII. *In Vitro* Antibacterial Activity (MIC, μg/ml)

Compound	*S. aureus* FDA 209P	MRSA 57	*E. faecalis*	*E. coli* NIHJ JC-2	*P. aeruginosa* ATCC 10145
8a	0.78	>100	50	0.2	12 5
8b	1.56	>100	50	0.78	25
8c	1.56	>100	25	1.56	25
8d	0.78	>100	12.5	0.78	12.5
8e	1.56	>100	25	0.2	12.5
8f	0.78	>100	25	0.39	12.5
8g	1.56	>100	50	0.2	12.5
8h	0.78	100	12.5	0.39	12.5
8i	0.39	100	12.5	0.78	12.5
8j	0.39	100	12.5	1.56	12.5
8k	1.56	>100	25	0.39	12.5
8l	0.39	100	12.5	0.78	12.5
8m	0.78	100	25	0.78	12.5
8n	0.39	25	12.5	0.78	12.5
8o	0.39	12.5	6.25	0.39	12.5
8p	0.39	12.5	6.25	1.56	12.5
8q	0.39	25	6.25	0.78	12.5
8r	0.2	>100	25	0.2	25
8s	0.39	50	6.25	0.39	6.25
8t	0.39	6.25	3.13	0.78	12.5
8u	0.39	50	25	0.2	6.25
8v	0.2	3.13	1.56	0.39	6.25
8w	0.39	>100	100	0.2	>100
8x	0.2	25	3.13	0.78	12.5
10a	0.2	100	25	<0.025	12.5
10b	0.1	6.25	1.56	0.39	6.25
10c	0.39	100	50	<0.025	25
10d	0.39	12.5	3.13	0.39	12.5
Cefuzonam	0.39	100	100	0.1	25
Cefmenoxime	1.56	>100	>100	0.1	25

Benzhydryl 7-Azido-8-oxo-3-[(1,3,4-thiadiazol-2-yl)thiomethyl]-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (5a) Trimethylsilyl iodide (11.82 g, 59.14 mmol) was added dropwise to a stirred solution of **2** (10 g, 26.86 mmol) in CH_2Cl_2 (150 ml) at 0 °C. The mixture was stirred for 1 h, then a solution of 2-mercapto-1,3,4-thiadiazole **3a** (6.35 g, 53.72 mmol) and triethylamine (5.46 g, 53.72 mmol) in CH_2Cl_2 (100 ml) was added dropwise over a period of 30 min. The reaction mixture was stirred at room temperature for 2 h, and MeOH (50 ml) was added. The mixture was evaporated under reduced pressure. After treatment of the residue with Et_2O, the resultant crude **4a** (R^1 = (1,3,4-thiadiazol-2-yl)thiomethyl) was collected by filtration. Diphenyldiazomethane (10.43 g, 53.72 mmol) was added to a solution of this crude **4a** in tetrahydrofuran (THF) (100 ml) and MeOH (100 ml), and the mixture was stirred at room temperature for 2 h. The solvent was evaporated off under reduced pressure. The residue was purified by silica gel column chromatography (eluent, CH_2Cl_2/AcOEt = 6/1) and recrystallized from AcOEt/*n*-hexane to give **5a** (10.34 g, 76%) as colorless needles, mp 196—197 °C (dec.). NMR ($CDCl_3$) δ: 3.72—3.85 (1H, m), 4.00 (1H, dd, J=9.5, 11.1 Hz), 4.47 (1H, d, J=13.7 Hz), 4.59 (1H, dd, J=3.7, 11.1 Hz), 4.76 (1H, d, J=13.7 Hz), 5.24 (1H, d, J=5.1 Hz), 6.89 (1H, s), 7.24—7.60 (10H, m), 8.95 (1H, s). IR cm^{-1}: 2090, 1780, 1710, 1610. *Anal.* Calcd for $C_{23}H_{18}N_6O_4S_2$: C, 54.54: H, 3.58; N, 16.59. Found: C, 54.83; H, 3.52; N, 16.29.

Compounds **5b**—**e** were obtained by the same procedure as described for **5a**; yields, melting points, and elemental analysis data are given in Table V.

Benzhydryl 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 (7a) A stirred mixture of **5a** (5 g, 9.87 mmol) and triethylamine (2 g, 19.74 mmol) in CH_2Cl_2 (100 ml) was cooled in an ice bath and saturated with H_2S. While stirring, gas evolution occurred. The solution was stirred at room temperature for 1 h, washed with 5% aqueous $NaHCO_3$ solution (50 ml × 3) and brine (50 ml), dried over Na_2SO_4, and filtered. To this filtrate was added the HOBT active ester **6a** prepared from the corresponding carboxylic acid (1.99 g, 9.87 mmol), *N,N'*-dicyclohexylcarbodiimide (DCC) (2.04 g, 9.87 mmol), and HOBT (1.33 g, 9.87 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 purified by silica gel column chromatography (eluent, CH_2Cl_2/AcOEt = 6/1) to give **7a** (4.72 g, 72%) as a pale yellow powder. NMR ($CDCl_3$) δ: 3.80—4.20 (5H, m), 4.23 (1H, d, J=13.7 Hz), 4.31 (1H, d, J=13.7 Hz), 4.68 (1H, dd, J=3.7, 11.1 Hz), 5.65 (1H, dd, J=4.8, 6.5 Hz), 6.84 (1H, s), 6.90 (1H, s), 7.20—7.60 (10H, m), 9.01 (1H, s). IR cm^{-1}: 3340, 1780, 1700, 1670, 1610, 1530.

Compounds **7b**—**x** were obtained by the same procedures as described for **7a**.

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 (8a) A stirred suspension of **7a** (2 g, 3.01 mmol) in anisole (4 ml) was treated with trifluoroacetic acid (10 ml) at 0 °C. Vigorous stirring was maintained for 10 min, after which time Et_2O (80 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 (100 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 **8a** (944 mg, 63%) as a white powder. NMR (DMSO-d_6) δ: 3.75—4.08 (5H, m), 4.45—4.65 (3H, m), 5.72 (1H, dd, J=4.7, 9.1 Hz), 6.83 (1H, s), 7.31 (2H, s), 9.25 (1H, d, J=9.1 Hz), 9.58 (1H, s). IR cm^{-1}: 3400, 1760, 1750, 1700, 1670.

Compounds **8b**—**x** were obtained by the same procedure as described for **8a**; yields and NMR data are given in Table VI.

7-[2-(2-Aminothiazol-4-yl)-2-[(*Z*)-methoxyimino]acetamido]-3-[(1-methylpyridinium-4-yl)thiomethyl]-8-oxo-1-aza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid Hydrogensulfate (10a) *N,O*-Bis(trimethylsilyl)-acetamide (1.24 g, 6.12 mmol) was added dropwise to a solution of **8x** (1 g, 2.04 mmol) in DMF (4 ml) at 0 °C, and the mixture was stirred at room temperature for 1 h. Then methyl iodide (868 mg, 6.12 mmol) was added. Stirring was continued at room temperature for 6 h, and iso-PrOH (20 ml) and Et_2O (20 ml) were added to the solution. The resulting precipitates were collected by filtration, washed with iso-PrOH and Et_2O, and dissolved in 5% aqueous $NaHCO_3$ solution (100 ml), and insoluble substances were filtered off. The filtrate was adjusted to pH 6 with 10% HCl, and subjected to chromatography on Diaion HP-20 using CH_3CN–H_2O mixtures. The appropriate fractions were combined and evaporated under reduced pressure to remove CH_3CN. Then 4 N H_2SO_4 (2 ml) was added to the residual aqueous solution in an ice bath, and the mixture was stirred for 30 min. The resultant precipitates were collected by filtration to give **10a** (700 mg, 57%) as a white powder. NMR (DMSO-d_6) δ: 3.85—4.13 (5H, m), 4.21 (3H, m), 4.45—4.73 (3H, m), 5.64 (1H, dd, J=4.5, 8.4 Hz), 6.78 (1H, s), 8.02 (2H, d, J=7.1 Hz), 8.71 (2H, d, J=7.1 Hz), 9.21 (1H, d, J=8.4 Hz). IR cm^{-1}: 3300, 1780, 1750, 1700, 1680.

Compounds **10b**—**d** were obtained by the same procedure as described for **10a**; yields and NMR data are given in Table VI.

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