

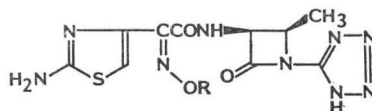
STUDIES ON MONOCYCLIC β -LACTAM ANTIBIOTICSIV. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF
(3*S*,4*R*)-3-[2-(2-AMINOTHIAZOL-4-YL)-(Z)-2-(O-SUBSTITUTED
OXYIMINO)ACETAMIDO]-4-METHYL-1-
(1*H*-TETRAZOL-5-YL)-2-AZETIDINONESCHOSAKU YOSHIDA*, KIYOSHI TANAKA, YOZO TODO, RIKIZO HATTORI,
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(Received for publication August 30, 1985)

The synthesis and *in vitro* activity of the 3-(*O*-substituted oxyiminoacetamido)-2-azetidiones (**IV**) possessing a tetrazole moiety at N-1 position are described. The introduction of lipophilic functions into the oxyimino moiety gave in some good activity against staphylococci, but decreased activity against Gram-negative bacteria. In contrast, the introduction of hydrophilic functions such as carboxycyclobutane resulted in strong activity against Gram-negative bacteria including *Pseudomonas aeruginosa*, and no or very small activity against the staphylococci.

In our previous papers,¹⁾ we have reported the antibacterial activity of monocyclic β -lactams with various substituents at N-1 position. Among them, *N*-(tetrazol-5-yl)azetid-2-one derivatives showed excellent activity. An extensive study on the effect of different substituents at C-3 and C-4, showed (3*S*,4*R*)-3-[2-(2-aminothiazol-4-yl)-(Z)-2-(*O*-substituted oxyimino)acetamido]-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidiones (Fig. 1) to have the highest activity. However, their antibacterial activities against Gram-positive bacteria are insufficient, and inferior to those of bicyclic β -lactam antibiotics. This is a common shortcoming among monocyclic β -lactam antibiotics. In order to overcome this problem, monocyclic β -lactam derivatives bearing various substituents in the oxyimino group were prepared, and studied.

Fig. 1.

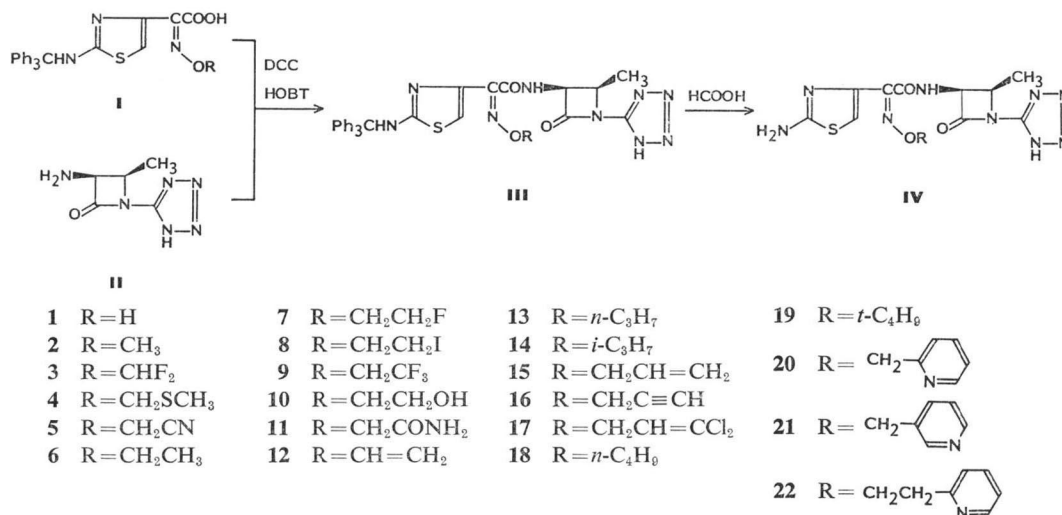


Chemistry

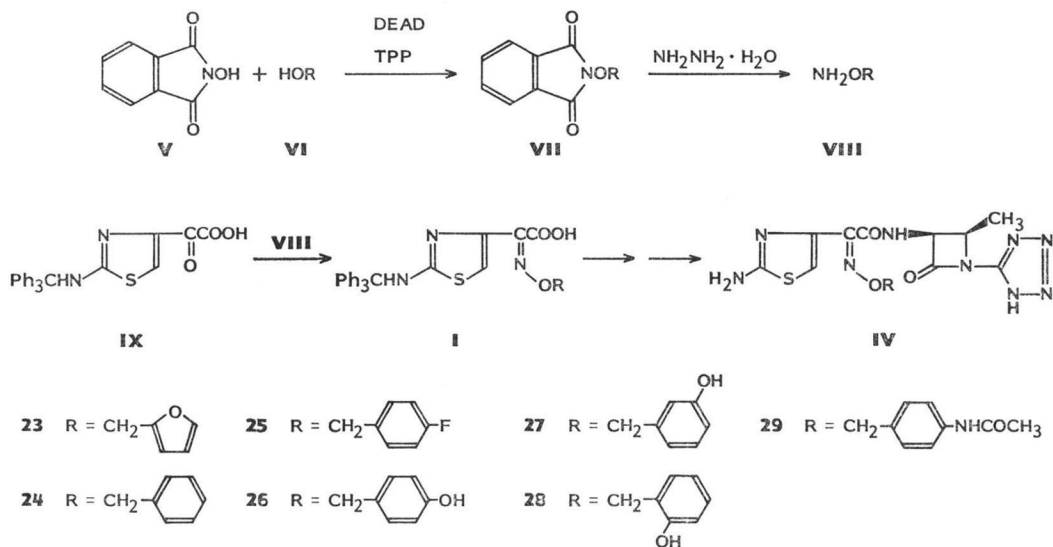
Four general synthetic methods were used for the preparation of (3*S*,4*R*)-3-[2-(2-aminothiazol-4-yl)-(Z)-2-(*O*-substituted oxyimino)acetamido]-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidiones (**IV**) (Schemes 1~4).

Method A is commonly used in preparation of semi-synthetic cephalosporins. Easily obtainable ethyl 2-(2-(triphenylmethylamino)thiazol-4-yl)-(Z)-2-hydroxyiminoacetate²⁾ was reacted with alkyl halides and base (K_2CO_3 or *tert*-BuOK) in DMF to alkylate the hydroxyimino group. Subsequently, the ester group was hydrolyzed with aqueous sodium hydroxide and the corresponding acids (**I**) were coupled with the previously reported (3*S*,4*R*)-3-amino-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidione (**II**) by using dicyclohexylcarbodiimide-1-hydroxybenzotriazole (DCC-HOBT) to produce **III**. Removal of the triphenylmethyl group from **III** with 50% formic acid gave **IV**.

Scheme 1. Method A.



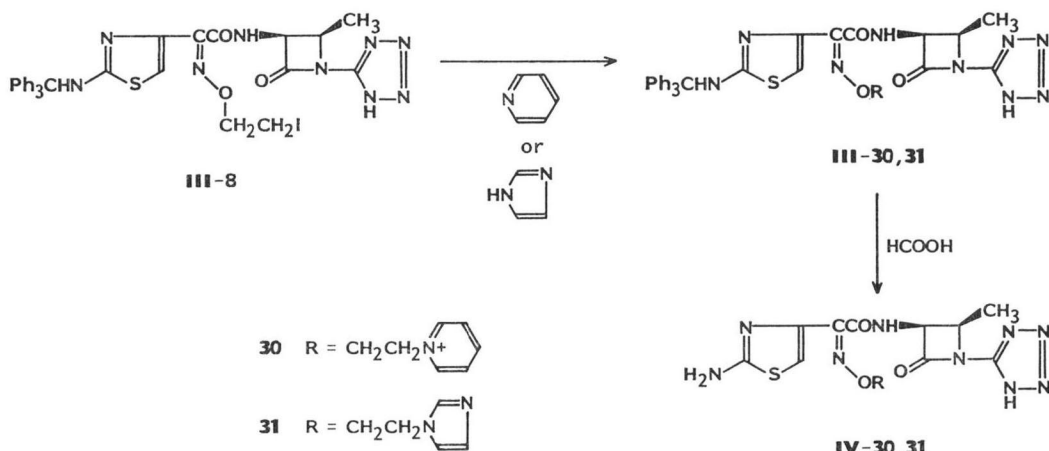
Scheme 2. Method B.



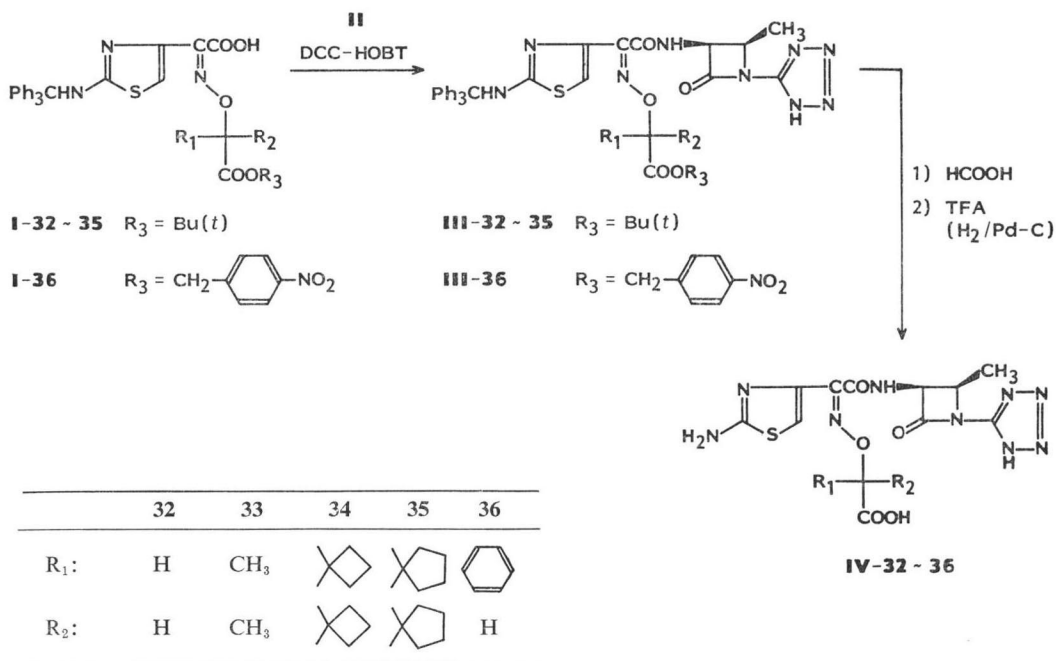
According to Method B (Scheme 2), substituted *O*-benzyl hydroxylamines (VIII) were reacted with 2-(2-(triphenylamino)thiazol-4-yl)glyoxylic acid (IX) in methanol to afford compounds I. Preparation of compounds IV was also performed in a similar manner as in Method A. Generally, *O*-benzylhydroxylamines having a hydroxy substituted benzyl group have been considered difficult to synthesize. However, they were successfully obtained from substituted benzylalcohols by a modified MITSUNOBU reaction³⁾ in which substituted benzylalcohols were reacted with *N*-hydroxyphthalimide (V) in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine (TPP), and then the obtained compounds VII were treated with hydrazine hydrate to afford the corresponding hydroxylamines (VIII).

According to Method C reported by HEYMES and VIGNAU⁴⁾ IV-8 obtained by condensing 2-iodo-

Scheme 3. Method C.



Scheme 4. Method D.



ethoxyimino derivative (I-9) with II, was reacted with pyridine or imidazole to produce IV-30 or IV-31, respectively. For a further study of structure-activity relationship, the acyl moiety of ceftazidime and analogs' moiety were also synthesized (Scheme 4). Compounds (III-32~36) were prepared from their corresponding bromides and ethyl 2-(2-triphenylmethylaminothiazol-4-yl)-(Z)-2-hydroxyiminoacetate by reactions similar to those employed for the synthesis of I and III in Scheme 1. The removal of the *tert*-butyl group with trifluoroacetic acid (TFA) gave IV-32~36. In case of III-36, the *p*-nitrobenzyl group was easily removed by hydrogenolysis to afford IV-36. The chemical structures of the obtained compounds IV were verified by IR and NMR spectra data as shown in Table 5.

Antibacterial Activity

The minimum inhibitory concentrations (MICs) of the *N*-(tetrazol-5-yl)azetidin-2-ones (IV-1 ~ 36) against two staphylococci and several Gram-negative bacteria are shown in Tables 1 ~ 3. Aztreonam^{b)} was used as reference compound.

As the number of carbons of the substituent (R) on the oxyimino group in the 3-acyl chain increased, the activity against staphylococci increased somewhat, however, it appeared to decrease against Gram-negative bacteria, particularly *Serratia marcescens*, *Proteus mirabilis*. Similarly, the introduction of lipophilic substituent such as halogen, thiomethyl, or cyano groups increased the activity, sometimes several-fold, against the staphylococci, as compared with compounds having unsubstituted alkyl groups. α -Fluoroethoxyimino derivate (IV-7) possessed relatively broad spectrum of activity. The introduction of bulky group such as isopropyl and *tert*-butyl groups, however, decreased the activity against staphylococci.

Aromatic substituents on the oxyimino group resulted in increased activity against Gram-positive and decreased activity against Gram-negative bacteria (Table 2). The introduction of a fluorine and an acetamido group into the benzyl group, did not increase the activity against Gram-positive and Gram-negative bacteria. In contrast, the introduction of a hydroxyl group, especially in the *ortho* position, increased the activity against both types of bacteria. Pyridine and imidazole in place of the phenyl nucleus decreased the activity against staphylococci, but, increased against Gram-negative bacteria sometimes several-fold as compared with the benzyl derivative (IV-27).

Table 3 shows the structure-activity relationship of *N*-(tetrazol-5-yl)azetidin-2-ones bearing a

Table 1. Effect of the aliphatic substituent (R) on the *in vitro* antibacterial activity (MIC μ g/ml) of *N*-(tetrazol-5-yl)azetidin-2-ones (IV).

Compound No.	<i>S.e.</i> ^a IID 866	<i>S.a.</i> [*] F-137	<i>E.c.</i> NIHJ JC-2	<i>K.p.</i> Y-50	<i>E.cl.</i> IID 977	<i>S.m.</i> IID 620	<i>P.m.</i> T-111
IV-1	1.56	25	0.78	3.13	3.13	3.13	1.56
IV-2	12.5	100	0.2	≤ 0.1	0.2	0.39	≤ 0.1
IV-3	6.25	50	≤ 0.1	≤ 0.1	≤ 0.1	0.39	0.2
IV-4	6.25	50	0.39	0.39	0.78	1.56	0.78
IV-5	6.25	50	0.2	0.2	0.39	0.39	0.39
IV-6	12.5	50	≤ 0.1	≤ 0.1	0.2	0.39	0.2
IV-7	6.25	25	≤ 0.1	≤ 0.1	0.2	0.2	≤ 0.1
IV-8	0.39	3.13	0.78	0.78	0.78	1.56	0.78
IV-9	3.13	25	0.2	0.39	0.39	0.78	0.78
IV-10	25	200	≤ 0.1	≤ 0.1	0.78	0.39	0.2
IV-11	25	200	≤ 0.1	≤ 0.1	0.39	0.2	0.2
IV-12	12.5	50	≤ 0.1	0.2	0.2	0.39	≤ 0.1
IV-13	3.13	25	0.2	0.39	0.39	0.78	0.78
IV-14	25	>200	0.39	0.2	0.78	3.13	0.78
IV-15	6.25	25	0.39	0.39	0.39	1.56	0.78
IV-16	12.5	25	0.39	0.2	0.39	1.56	0.39
IV-17	0.39	0.78	1.56	3.13	3.13	12.5	12.5
IV-18	1.56	25	0.2	0.78	0.39	6.25	3.13
IV-19	100	>200	1.56	0.78	3.13	6.25	3.13
Aztreonam	>200	>200	0.2	≤ 0.1	3.13	≤ 0.1	≤ 0.1

^a Organisms included in the Table are: *S.e.*, *Staphylococcus epidermidis*; *S.a.*, *Staphylococcus aureus*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *E.cl.*, *Enterobacter cloacae*; *S.m.*, *Serratia marcescens*; *P.m.*, *Proteus mirabilis*.

^{*} Penicillinase producer.

Table 2. Effect of the aromatic substituent (R) on the *in vitro* antibacterial activity (MIC $\mu\text{g/ml}$) of *N*-(tetrazol-5-yl)azetid-2-ones (IV).

Compound No.	<i>S.e.</i> ^a IID 866	<i>S.a.</i> [*] F-137	<i>E.c.</i> NIHJ JC-2	<i>K.p.</i> Y-50	<i>E.cl.</i> IID 977	<i>S.m.</i> IID 620	<i>P.m.</i> T-111
IV-23	6.25	25	≤ 0.1	1.56	0.39	6.25	3.13
IV-24	0.39	3.13	0.78	6.25	3.13	50	6.25
IV-25	0.78	6.25	≤ 0.1	6.25	3.13	50	6.25
IV-26	3.13	12.5	0.39	1.56	0.78	6.25	3.13
IV-27	1.56	6.25	0.2	1.56	3.13	6.25	3.13
IV-28	0.39	0.78	1.56	3.13	3.13	12.5	3.13
IV-29	3.13	12.5	1.56	3.13	6.25	12.5	6.25
IV-20	0.78	12.5	≤ 0.1	0.78	0.78	12.5	6.25
IV-21	6.25	25	0.78	0.78	0.78	12.5	3.13
IV-22	25	200	0.78	0.78	3.13	6.25	3.13
IV-30	100	>200	0.78	0.78	1.56	6.25	3.13
IV-31	1.56	>200	0.39	0.2	0.78	0.78	1.56
Aztreonam	>200	>200	0.2	≤ 0.1	3.13	≤ 0.1	≤ 0.1

^a See the footnotes in the Table 1.

Table 3. Effect of the carboxylic acid substituent (R) on the *in vitro* antibacterial activity (MIC $\mu\text{g/ml}$) of *N*-(tetrazol-5-yl)azetid-2-ones (IV).

Organisms ^a	IV-32	IV-33	IV-34	IV-35	IV-36	Aztreonam
<i>S.e.</i> IID 866	>200	>200	100	>200	50	>200
<i>S.a.</i> F-137*	>200	>200	>200	>200	>200	>200
<i>E.c.</i> NIHJ JC-2	0.39	0.2	0.2	0.39	6.25	0.2
<i>E.c.</i> TK-3*	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	3.13	≤ 0.1
<i>E.c.</i> GN 5482**	1.56	0.2	≤ 0.1	0.2	3.13	6.25
<i>K.p.</i> Y-50	0.2	0.2	≤ 0.1	0.39	3.13	≤ 0.1
<i>K.p.</i> Y-4*	0.2	0.39	0.2	0.39	6.25	≤ 0.1
<i>E.cl.</i> IID 977	1.56	0.78	0.2	0.78	12.5	3.13
<i>S.m.</i> IID 620	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	1.56	≤ 0.1
<i>S.m.</i> W-8**	0.39	0.78	0.2	0.39	3.13	6.25
<i>P.m.</i> T-111	≤ 0.1	≤ 0.1	≤ 0.1	0.2	3.13	≤ 0.1
<i>P.v.</i> GN 76**	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	0.78	≤ 0.1
<i>P.a.</i> IFO 3445	200	12.5	6.25	6.25	100	3.13
<i>P.a.</i> GN 918**	100	0.78	0.78	0.78	12.5	12.5

^a Organisms included in the Table are: *S.e.*, *Staphylococcus epidermidis*; *S.a.*, *Staphylococcus aureus*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *E.cl.*, *Enterobacter cloacae*; *S.m.*, *Serratia marcescens*; *P.m.*, *Proteus mirabilis*; *P.v.*, *Proteus vulgaris*; *P.a.*, *Pseudomonas aeruginosa*.

* Penicillinase producer. ** Cephalosporinase producer.

carboxyl group as substituent of the oxyimino group. All of them showed excellent activity against Gram-negative bacteria, and β -lactamase-producing resistant strains, but, had lost the activity against staphylococci to a great extent. The 1-carboxymethyl derivate (IV-32) did not give activity against *Pseudomonas aeruginosa* IFO 3445, but substituted analogs (IV-33~35) resulted in better activity against resistant strains than aztreonam. Compound IV-36 showed nearly the same activity as aztreonam.

Conclusion

The structure-activity relationship of various compounds, with different substituent (R) at the oxyimino group of (3*S*,4*R*)-3-[2-aminothiazol-4-yl)-(Z)-2-(*O*-substituted oxyimino)acetamido]-4-

methyl-1-(1*H*-tetrazol-5-yl)-2-azetidinones was investigated. One compound **IV-34** was found to show the same antibacterial activity as aztreonam. However, we could not find a compound, possessing good activity against both staphylococci and Gram-negative bacteria.

We intend to report on the introduction of further new substituents at the C-4 position in a forthcoming paper.

Experimental

Melting points are uncorrected. IR spectra were recorded on a Hitachi model 260-30 spectrophotometer. NMR spectra were recorded on a Hitachi R-24 (60 MHz) spectrometer using TMS as an internal standard. Organic solvents were dried over anhydrous MgSO₄, and all concentration and evaporation of solvents were carried out under reduced pressure. Column chromatography was carried out on Wako silica gel (C-200).

In Vitro Antibacterial Activity

Minimum inhibitory concentrations (MICs) were determined by the agar dilution method using heart infusion agar (Eiken) after incubation for 20 hours at 37°C and an inoculum size of about 10⁸ cfu.

Materials

I-1, 2, 6, 14, 15,²⁾ **I-3,**⁸⁾ **I-8,**⁴⁾ **I-12,**⁷⁾ **I-13, 18, 24,**⁸⁾ **I-16,**⁹⁾ **I-5, 11, 33,**¹⁰⁾ **I-34, 35,**¹¹⁾ **VIII-23**¹²⁾ and **IX**⁸⁾ were synthesized according to the literatures. **IV-1, 2, 6, 13, 14, 18, 19, 32, 33** were reported in the patent literature.¹³⁾

Table 4. Spectral and physical properties of compounds I.

Compound No.	MP (°C, dec)	¹ H NMR (Solvent) δ (J =Hz)	IR $\nu_{C=O}^{KBr}$ (cm ⁻¹)
I-4	182~184	(DMSO- <i>d</i> ₆); 2.12 (3H, s), 3.80 (1H, br s), 5.12 (2H, s), 6.78 (1H, s), 7.40 (15H, m), 8.85 (1H, br s)	1730
I-7	140~142	(DMSO- <i>d</i> ₆); 3.80~4.30 (2H, m), 4.80~5.10 (2H, m), 5.40 (1H, br s), 6.85 (1H, s), 7.30 (15H, m), 8.77 (1H, br s)	1730
I-9	140~143	(DMSO- <i>d</i> ₆); 3.80~4.10 (3H, m), 6.83 (1H, s), 7.26 (15H, s), 8.72 (1H, br s)	1730
I-10	116~119	(DMSO- <i>d</i> ₆); 3.70 (2H, t, 6), 4.20 (2H, t, 6), 5.30 (2H, m), 6.98 (1H, s), 7.45 (15H, s), 8.90 (1H, br s)	1720
I-17	167~169	(DMSO- <i>d</i> ₆); 4.63 (2H, d, 6), 5.70 (1H, br s), 6.24 (1H, t, 6), 6.83 (1H, s), 7.23 (15H, s), 8.75 (1H, s)	1725 (sh), 1710
I-20	150~153	(DMSO- <i>d</i> ₆); 5.29 (2H, s), 5.58 (1H, br s), 6.95 (1H, s), 7.36 (17H, m), 7.80 (1H, m), 8.62 (1H, m), 8.85 (1H, br s)	1660 (sh), 1610
I-21	190~192	(DMSO- <i>d</i> ₆); 5.14 (2H, s), 5.92 (1H, br s), 6.91 (1H, s), 7.34 (16H, m), 7.80 (1H, m), 8.52 (2H, m), 8.84 (1H, br s)	1650
I-22	170~172	(DMSO- <i>d</i> ₆); 3.03 (2H, t, 6), 4.32 (2H, t, 6), 5.59 (1H, br s), 6.72 (1H, s), 7.14~7.69 (18H, m), 8.29~8.64 (2H, m)	1710 (sh), 1615
I-25	130~132	(CDCl ₃); 5.00 (2H, s), 6.39 (1H, s), 6.43~7.13 (20H, m), 8.40 (1H, br s)	1720
I-26	146~149	(CDCl ₃); 4.98 (2H, s), 6.55 (1H, s), 6.68 (2H, d, 8), 7.07 (2H, d, 8), 7.20 (16H, m), 7.92 (2H, m)	1720
I-27	145~147	(CDCl ₃); 5.09 (2H, s), 6.60 (1H, s), 6.52~7.10 (4H, m), 7.20 (16H, m), 8.92 (2H, m)	1725
I-28	149~150	(DMSO- <i>d</i> ₆); 5.10 (2H, s), 6.55~7.55 (22H, m), 8.70 (1H, br s)	1720
I-29	145~148	(DMSO- <i>d</i> ₆); 2.06 (3H, s), 5.05 (2H, s), 6.80 (1H, s), 7.25 (17H, m), 7.58 (2H, d, 8), 8.80 (2H, br s), 9.92 (1H, s)	1725, 1660
I-36	140~143	(CDCl ₃); 5.31 (2H, s), 5.99 (1H, s), 6.75 (1H, s), 6.97 (1H, br s), 7.22~7.64 (22H, m), 7.82 (1H, br s), 8.92 (2H, d, 9)	1775, 1740

Table 5. Spectral and physical properties of compounds IV.

Compound No.	MP (°C, dec)	¹ H NMR (DMSO- <i>d</i> ₆) δ (<i>J</i> =Hz)	IR $\nu_{\text{C=O}}$ ^{KBr} (cm ⁻¹)
IV-3	242~245	1.42 (3H, d, 6), 4.55 (1H, m), 5.55 (1H, m), 6.98 (1H, s), 7.09 (1H, t, 7), 7.30 (2H, br s), 9.65 (1H, d, 8)	1780, 1670
IV-4	233~236	1.45 (3H, d, 6), 2.19 (3H, s), 4.55 (1H, m), 5.20 (2H, s), 5.50 (1H, m), 6.80 (1H, s), 7.15 (2H, br s), 9.45 (1H, d, 8)	1770, 1650
IV-5	235~240	1.43 (3H, d, 6), 4.60 (1H, m), 5.07 (2H, s), 5.57 (1H, m), 6.95 (1H, s), 7.20 (3H, m), 9.63 (1H, d, 8)	1765, 1665
IV-7	218~220	1.45 (3H, d, 6), 3.90~4.40 (2H, m), 4.40~5.20 (3H, m), 5.50 (1H, m), 6.75 (1H, s), 7.25 (2H, br s), 9.40 (1H, d, 8)	1770, 1660
IV-8	210~213	1.54 (3H, d, 6), 3.46 (2H, t, 6), 4.30~4.80 (3H, m), 5.68 (1H, m), 6.97 (1H, s), 7.80 (2H, br s), 9.59 (1H, d, 8)	1765, 1660
IV-9	>250	1.40 (3H, d, 6), 4.4~5.0 (3H, m), 5.50 (1H, m), 6.85 (1H, s), 7.20 (2H, br s), 9.52 (2H, d, 8)	1770, 1660
IV-10	238~240	1.44 (3H, d, 6), 3.60 (2H, m), 4.07 (2H, m), 4.50 (1H, m), 5.45 (1H, m), 6.72 (1H, s), 6.92~8.62 (4H, m), 9.20 (1H, d, 8)	1765, 1665
IV-11	200~204	1.46 (3H, d, 6), 4.53~4.80 (3H, m), 5.73 (1H, m), 6.03 (2H, br s), 7.04 (1H, s), 7.30 (1H, br s), 7.63 (1H, br s), 9.98 (1H, d, 9)	1760, 1680, 1665
IV-12	235~240	1.50 (3H, d, 6), 4.20~4.72 (3H, m), 5.57 (1H, m), 6.95 (1H, m), 6.97 (1H, s), 7.40 (2H, br s), 8.40 (1H, br s), 9.67 (1H, d, 8)	1775, 1665
IV-15	238~240	1.40 (3H, d, 6), 4.30~4.80 (3H, m), 5.00~6.00 (4H, m), 6.73 (1H, s), 7.22 (2H, br s), 9.32 (1H, d, 8)	1770, 1660
IV-16	235~237	1.40 (3H, d, 6), 3.45 (1H, m), 4.30~4.80 (3H, m), 5.50 (1H, m), 6.85 (1H, s), 7.25 (2H, br s), 9.38 (1H, d, 8)	1770, 1660
IV-17	245~248	1.40 (3H, d, 6), 4.40~4.80 (3H, m), 5.50 (1H, m), 6.30 (1H, t, 6), 6.80 (1H, s), 7.40 (2H, br s), 9.38 (1H, d, 8)	1760, 1655
IV-20	170~173	1.41 (3H, d, 6), 4.67 (1H, m), 5.35 (2H, s), 5.53 (1H, m), 5.99 (2H, br s), 6.95 (1H, s), 7.32~8.09 (3H, m), 8.67 (1H, m), 9.80 (1H, d, 10)	1760, 1660
IV-21	165~168	1.39 (3H, d, 6), 4.70 (1H, m), 5.38 (2H, s), 5.65 (1H, m), 7.00 (1H, s), 7.20~7.70 (3H, m), 8.00 (1H, m), 8.80 (2H, m), 9.75 (1H, d, 9)	1760, 1660
IV-22	161~165	1.34 (3H, d, 6), 3.39 (2H, t, 6), 4.56 (3H, m), 5.50 (1H, m), 6.30 (2H, br s), 6.80 (1H, s), 7.72 (2H, m), 8.16 (1H, m), 8.70 (1H, m), 9.51 (1H, d, 8)	1765, 1665
IV-23	238~242	1.33 (3H, d, 6), 4.55 (1H, m), 5.10 (2H, s), 5.55 (1H, m), 6.50 (2H, s), 6.82 (1H, s), 7.30 (2H, br s), 7.70 (1H, s), 8.52 (1H, br s), 9.50 (1H, d, 8)	1760, 1655
IV-24	>250	1.35 (3H, d, 6), 4.60 (1H, m), 5.24 (2H, s), 5.65 (1H, m), 6.88 (1H, s), 7.42 (5H, s), 8.90 (2H, br s), 9.58 (1H, d, 8)	1760, 1650
IV-25	236~237	1.32 (3H, d, 6), 4.54 (1H, m), 5.16 (2H, s), 5.54 (1H, m), 6.80 (1H, s), 6.90~7.80 (6H, m), 9.50 (1H, d, 8), 10.20 (1H, br s)	1755, 1650
IV-26	190~192	1.42 (3H, d, 6), 4.62 (1H, m), 5.15 (2H, s), 5.65 (1H, m), 6.86 (2H, d, 8), 6.88 (1H, s), 7.30 (2H, d, 8), 7.47 (4H, m), 9.57 (1H, d, 8)	1760, 1655
IV-27	178~183	1.34 (3H, d, 6), 4.56 (1H, m), 5.10 (2H, s), 5.54 (1H, m), 6.80 (1H, s), 6.60~7.60 (7H, m), 7.90 (1H, br s), 9.50 (1H, d, 8)	1760, 1655
IV-28	213~214	1.34 (3H, d, 6), 4.55 (1H, m), 5.13 (2H, s), 5.52 (1H, m), 6.60~7.60 (6H, m), 9.10 (2H, br s), 9.45 (1H, d, 8)	1760, 1680

Table 5. (continued)

Compound No.	MP (°C, dec)	¹ H NMR (DMSO- <i>d</i> ₆) δ (<i>J</i> =Hz)	IR ν _{C=O} ^{KBr} (cm ⁻¹)
IV-29	224~226	1.32 (3H, d, 6), 2.05 (3H, s), 4.52 (1H, m), 5.10 (2H, s), 5.50 (1H, m), 5.75 (2H, br s), 6.80 (1H, s), 7.13 (1H, br s), 7.26 (2H, d, 8), 7.56 (2H, d, 8), 9.47 (1H, d, 8), 10.05 (1H, s)	1760, 1660
IV-30	128~132	1.31 (3H, d, 6), 4.55~5.30 (5H, m), 5.53 (1H, m), 7.00 (1H, s), 7.35 (2H, br s), 8.27 (2H, m), 8.75 (1H, m), 9.20 (2H, m), 9.62 (1H, d, 10)	1765, 1670
IV-31	201~205	1.30 (3H, d, 6), 4.45 (5H, m), 5.48 (1H, m), 6.28 (2H, br s), 6.88 (1H, s), 7.12~7.69 (2H, m), 8.28 (1H, s), 9.42 (1H, d, 8)	1755, 1660
IV-34	190~193	1.55 (3H, d, 6), 1.75~2.55 (6H, m), 4.72 (1H, m), 5.70 (1H, m), 7.02 (1H, s), 9.05 (3H, m), 9.68 (1H, d, 8)	1770, 1720, 1665
IV-35	200~205	1.44 (3H, d, 6), 1.55~2.11 (8H, m), 4.60 (1H, m), 5.00 (1H, br s), 5.41 (1H, m), 6.77 (1H, s), 7.30 (2H, br s), 9.37 (1H, d, 8)	1760, 1710, 1670
IV-36	200~204	1.30 (3H, d, 6), 4.55 (1H, m), 5.40~5.74 (2H, m), 6.95 (1H, s), 7.52 (5H, s), 8.00 (3H, m), 9.65 (1H, d, 9)	1770, 1670, 1630

Method A

General Procedure for 2-(2-Triphenylmethylaminothiazol-4-yl)-(Z)-2-(O-substituted oxyimino)-acetic Acids (**I-4**, **7**, **9**, **10**, **17**, **20~36**): To a solution of ethyl (Z)-2-hydroxyimino-2-(2-triphenylmethylaminothiazol-4-yl)acetate (5 g, 10.9 mmol) and K₂CO₃ (1.81 g, 13.1 mmol) or *tert*-BuOK (1.23 g, 11 mmol) at -30~-25°C in DMF (20 ml) was added alkyl halide (13~20 mmol) at room temp, and stirred at 30~70°C for 1.5~3 hours. The reaction mixture was poured into H₂O (150 ml), and extracted with EtOAc. The organic layer was washed successively with H₂O and brine, dried, and evaporated to give a residue, which was purified by column chromatography (toluene - EtOAc, 50:1), or triturated with Et₂O to afford the corresponding ethyl esters in 50~90%. To a suspension of the ester (7.3 mmol) in dioxane (25 ml) and EtOH (15 ml) was dropwise added 2 N NaOH aqueous solution (7.5 ml) at 50°C, and stirred at the same temp for 1 hour. The reaction mixture was cooled to 15°C under stirring to form precipitates, which were collected by filtration. The precipitates were suspended in H₂O (30 ml) and EtOAc (20 ml), and acidified to pH 2.0 with 2 N HCl, and stirred for 1 hour at room temp. The resulting precipitate was filtered, washed with H₂O, and dried over P₂O₅ to afford **I-4**, **7**, **9**, **10**, **17**, **20**, **21**, **30** in 60~70% (Table 4).

General Procedure for the Acylation of (3*S*,4*R*)-3-Amino-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidione (**II**): To a solution of **II** (0.29 g, 1.73 mmol) in DMF (5 ml) was added Et₃N (0.24 ml) (1.73 mmol) under ice-cooling. 2-(2-Triphenylmethylaminothiazol-4-yl)-(Z)-2-(O-substituted alkoxyimino)-acetic acid (**I**) (1.64 mmol), 1-hydroxybenzotriazole hydrate (0.27 g, 2 mmol), Molecular sieves 4A (1 g) and DCC (0.42 g, 2 mmol) were added to the solution at the same temp, and stirred for 2~5 hours at room temp. The precipitate was filtered off and the filtrate evaporated. To the residue was added EtOAc (15 ml) and H₂O (15 ml), and adjusted to pH 2.0 with 2 N HCl. The organic layer was washed with brine, dried, and evaporated to give a residue, which was purified by column chromatography (toluene - EtOAc, 5:1) to afford **III** in 50~70%.

General Procedure for Deprotection of **III**: Compound **III** (0.75 mmol) was dissolved in THF (5 ml) and 50% aqueous HCOOH (11 ml), and kept at 40~50°C for 1 hour. The solvent was evaporated to give a residue, which was dissolved in EtOAc (10 ml) and H₂O (10 ml). The mixture was adjusted to pH 7.0 with saturated NaHCO₃ solution. The separated aqueous layer was acidified to pH 2.5 with 2 N HCl, and extracted with EtOAc. The organic layer was washed with brine, dried, and evaporated to give a residue, which was triturated with a mixture of EtOAc (1 ml) and isopropyl ether (10 ml) to afford **IV** as a white powder in 60~80%. The obtained compound were supplied for determination of MIC without further purification. Data on **IV** are summarized in Table 5.

Table 6. Yield, spectral and physical properties of compounds VII.

Compound No.	Yield (%)	MP (°C)	¹ H NMR (DMSO- <i>d</i> ₆) δ (<i>J</i> =Hz)	IR $\nu_{\text{C=O}}^{\text{KBr}}$ (cm ⁻¹)
VII-25	58.6	158~159	5.16 (2H, s), 7.02~7.68 (4H, m), 7.82 (4H, s)	1775, 1730
VII-26	20.1	180~183	5.07 (2H, s), 6.81 (2H, d, 8), 7.35 (2H, d, 8), 7.87 (4H, s), 9.67 (1H, br s)	1780, 1705
VII-27	67	178~179	5.10 (2H, s), 6.72~7.32 (4H, m), 7.81 (4H, s), 9.54 (1H, s)	1780, 1720
VII-28	15.2	166~168	5.12 (2H, s), 6.60~7.40 (5H, m), 7.85 (4H, s)	1780, 1730
VII-29	65.5	214~215	1.98 (3H, s), 4.99 (2H, s), 7.22 (2H, d, 9), 7.47 (2H, d, 9), 7.62 (4H, s), 9.55 (1H, s)	1780, 1725

Method B

General Procedure for *N*-(Substituted benzyloxy)phthalimide (VII): To a solution of *N*-hydroxyphthalimide (V) (10 g, 61 mmol), substituted benzylalcohol (61 mmol) and TPP (16 g, 61 mmol) in THF (300 ml) was added DEAD (9.7 ml, 61 mmol) in THF (50 ml) at room temp over 30 minutes, and stirred at the same temp for further 30 minutes. The reaction mixture was evaporated to give a residue, which was treated with toluene (150 ml). The solid was filtered off and the filtrate evaporated. The residue was triturated with a mixture of EtOAc (10 ml) and Et₂O (100 ml) to afford VII as a white powder in 15~65%. Data on VII are summarized in Table 6.

General Procedure for 2-(2-Triphenylmethylaminothiazol-4-yl)-(Z)-2-(*O*-substituted oxyimino)-acetic Acids (I-25~29) from IX: A solution of *N*-(substituted benzyloxy)phthalimide (VII) (8 mmol) in EtOH (10 ml) was treated with 100% hydrazine hydrate 0.39 ml (8 mmol) at room temp, and stirred for 1 hour. The reaction mixture was poured into H₂O (10 ml) and Et₂O (30 ml), and adjusted to pH 11 with 28% NH₄OH. The resulting mixture was saturated with NaCl and extracted three times with Et₂O (20 ml). The organic layer was washed with brine, dried, and evaporated to give a residue, which was dissolved in MeOH (70 ml). 2-(2-Triphenylmethylaminothiazol-4-yl)glyoxalic acid (IX)⁸⁾ (1.67 g, 4 mmol) was added, and stirred at room temp for 2 hours. The solid was filtered off and the filtrate was evaporated to give a residue, which was triturated with EtOAc to afford I-25~29, which were transformed into IV-25~29 by the route described in Method A without further purification. Data on I are summarized in Table 4.

Method C

i) Preparation of (3*S*,4*R*)-3-[(*Z*)-2-(2-Iodoethoxyimino)-2-(2-triphenylmethylaminothiazol-4-yl)-acetamido]-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidinone - Dichloromethane Complex (III-8): To a solution of II (0.86 g, 5.1 mmol) in DMF (15 ml) was added Et₃N (0.72 ml, 5.17 mmol) under ice-cooling. 2-(2-Iodoethoxyimino)-2-(2-triphenylmethylaminothiazol-4-yl)acetic acid (I-8) (3.0 g, 5.14 mmol), 1-hydroxybenzothiazole hydrate (0.79 g, 5.85 mmol), Molecular sieves 4A (3 g) and DCC (1.2 g, 5.82 mmol) were added to the solution at the same temp, and stirred for 3 hours at room temp. The solid was filtered off and the filtrate evaporated to give a residue which was treated with EtOAc (50 ml) and H₂O (50 ml), and adjusted to pH 2.0 with 2 *N* HCl. The organic layer was washed successively with H₂O and brine, dried, and evaporated to give a residue, which was purified by column chromatography (CHCl₃ - MeOH, 100: 1) to afford a yellow amorphous powder. This was dissolved in CH₂Cl₂ (30 ml) and stirred for 2 hours to afford III-8 as a white powder, 1.7 g (40%). MP 170~173°C (dec); IR $\nu_{\text{C=O}}^{\text{KBr}}$ 1780, 1670 cm⁻¹; NMR (DMSO-*d*₆) δ 1.43 (3H, d, *J*=6.0 Hz), 3.36 (2H, t, *J*=6.0 Hz), 4.32 (2H, t, *J*=6.0 Hz), 4.55 (1H, m), 5.57 (1H, m), 5.77 (2H, s), 6.24 (1H, br s), 6.88 (1H, s), 7.37 (15H, s), 8.80 (1H, br s), 9.44 (1H, d, *J*=8.0 Hz).

ii) Preparation of (3*S*,4*R*)-3-[(*Z*)-2-[2-(Imidazol-1-yl)ethoxyimino]-2-(2-triphenylmethylaminothiazol-4-yl)acetamido]-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidinone (III-31 and IV-31): A solution of (3*S*,4*R*)-3-[(*Z*)-2-(2-iodoethoxyimino)-2-(2-triphenylmethylaminothiazol-4-yl)acetamido]-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidinone - dichloromethane complex (III-8) (0.7 g, 0.87 mmol) in DMF (2 ml) was treated with imidazole (0.87 g, 12.8 mmol) at room temp, and stirred for 48 hours at 30~35°C. The

reaction mixture was poured into isopropyl ether (30 ml). The solvent was removed by decantation to give an oily residue, which was dissolved in EtOAc (10 ml) and H₂O (10 ml), and adjusted to pH 3.8 with 2 N HCl. The separated aqueous layer was saturated with NaCl and extracted twice with THF (20 ml). The combined organic layers were dried, and evaporated to give a residue, which was triturated with THF (10 ml) to afford **III-31** as a white powder, 0.2 g (35%). **III-31** was transformed into **IV-31** by the route described in Method A.

iii) Preparation of (3*S*,4*R*)-4-Methyl-3-[(*Z*)-2-(2-pyridinium ethoxyimino)-2-(2-triphenylmethylaminothiazol-4-yl)acetamido]-1-(1*H*-tetrazol-5-yl)-2-azetidinone, HI Salt (**III-30** and **IV-31**): Compound **III-8** (1 g, 1.24 mmol) was dissolved in pyridine (5 ml) at room temp, and kept for 3 days. The reaction mixture was evaporated to give a residue, which was purified by column chromatography (CHCl₃ - MeOH, 10: 1) to afford **III-30**, 0.45 g (45.5%). **III-30** was transformed into **IV-30** by the route described in Method A.

Method D

i) Preparation of (3*S*,4*R*)-3-{2-(2-Aminothiazol-4-yl)-(Z)-2-[(1-carboxycyclobutan-1-yl)oxyimino]acetamido}-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidinone (**IV-34**) and Cyclopentane Analog (**IV-35**): To a solution of (3*S*,4*R*)-3-[2-(2-aminothiazol-4-yl)-(Z)-2-(1-*tert*-butoxycarbonylcyclobutan-1-yl)oxyimino]acetamido-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidinone (prepared from **I-34** by the Method A) (0.2 g, 0.42 mmol) in CH₂Cl₂ (10 ml) was added TFA (10 ml) under ice-cooling, and stirred for 2.5 hours at room temp. It was evaporated to give a residue, which was dissolved in EtOAc (10 ml) and H₂O (10 ml), and adjusted to pH 7.0 with saturated NaHCO₃ solution. The separated aqueous layer was adjusted to pH 2.0 with 2 N HCl, and washed with EtOAc (10 ml). The aqueous layer was saturated with NaCl and extracted twice with THF (10 ml). The combined organic layers were washed with brine (10 ml), dried, and evaporated to give a residue, which was triturated with Et₂O (30 ml) to afford **IV-34** as a white powder (0.14 g, 79.1%). Using above procedure, **IV-35** was similarly synthesized.

ii) Preparation of (3*S*,4*R*)-3-{2-(2-Aminothiazol-4-yl)-(Z)-2-[(*d,l*)-1-carboxy-1-benzyloxyimino]acetamido}-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidinone (**VI-36**): A suspension of (3*S*,4*R*)-3-{2-(2-aminothiazol-4-yl)-(Z)-2-[(*d,l*)-(4-nitrobenzyloxycarbonyl)benzyloxyimino]acetamido}-4-methyl-1-(1*H*-tetrazol-5-yl)-2-azetidinone (prepared from **I-36** by Method A) (0.52 g, 0.86 mmol), NaHCO₃ (0.072 g, 0.86 mmol) and 5% Pd-C (0.52 g) in H₂O (40 ml) and EtOAc (20 ml) was stirred for 30 minutes at room temp under a hydrogen atmosphere. The solid was filtered off and the separated aqueous layer adjusted to pH 2.0 with 6 N HCl. The solution was saturated with NaCl and extracted twice with THF (20 ml). The combined organic layers were washed with brine (10 ml), dried, and evaporated to give a residue, which was triturated with Et₂O (10 ml) to afford **IV-36** as a white powder, 0.25 g (62%). Results of **IV-34**~**36** are summarized in Table 5.

Acknowledgment

The authors wish to thank Mr. T. YASUDA and co-workers for providing the biological data and Mr. T. MAEDA and co-workers for microanalyses and spectral measurements. Thanks are also due to Mr. T. YAMAFUJI and Mrs. S. KISHIMOTO for their skillful experimental work.

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