Structure-activity Relationships of Cephalosporin Derivatives against Methicillin-resistant *Staphylococcus aureus* and *Enterococcus faecalis*

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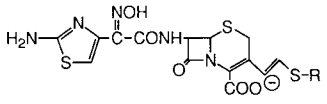
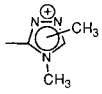
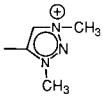
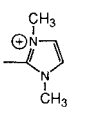
Over the past few years, a number of cephalosporin antibiotics having a broad spectrum and stability to β-lactamase have been developed[1]. These drugs have a heterocyclic group at the C-3 position for broad-spectrum activity and an alkoxyimino-aminothiazol group at the C-7 position for stability to β-lactamase. But, these drugs do not show strong antibacterial activity against Gram-positive bacteria, especially methicillin-resistant *Staphylococcus aureus* (MRSA)[2~4] and *Enterococcus faecalis*, against which almost all cephem antibiotics are ineffective. Therefore, we investigated the activity of various cephalosporin derivatives toward MRSA and *E. faecalis*. We already reported that hydroxyimino-aminothiazol at the C-7 position of 2-thioisocephem having a vinyl-thio linkage at C-3 showed strong activity against MRSA and *E. faecalis*[5] and that the same on 1-thiocephem showed the strongest activity against MRSA and *E. faecalis*[6~8]. In this present communication, we describe that the anti-MRSA and anti-*E. faecalis* activities of 1-thiocephem having hydroxy-imino-aminothiazol at the C-7 position and the vinyl-thio linkage at C-3 were changed by varying the C-3 side chain.

Table 1. Investigation of C-3 side chain.



MIC (μg/ml)

Compound No.	C-3 side chain R	*S.a*	MRSA *MIC_{80}	*E.f*	*E.c*	*S.m*	*P.a*
1		0.2	**12.5**	0.39	0.1	0.78	>50
2		0.1	**12.5**	0.2	0.025	0.2	>100
3		0.2	**12.5**	0.39	0.05	0.2	>100
4		0.1	**12.5**	0.78	0.05	0.2	>100

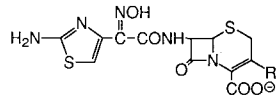
Abbreviations: *S.a.*, *Staphylococcus aureus* FDA 209P; MRSA, methicillin resistant *Staphylococcus aureus*; *E.f.*, *Enterococcus faecalis* ATCC-12968; *E.c.*, *Esherichia coli* NIHJ JC-2; *S.m.*, *Serratia marcescens* IFO-12648; *P.a.*, *Pseudomonas aeruginosa* E-2.
* MIC_{80}: MIC for 80% of clinically isolated MRSA (45 strains).

Biological Results and Discussion

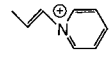
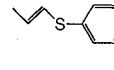
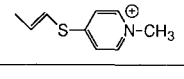
Minimum inhibitory concentration (MIC) was determined by the 2-fold serial agar dilution method with approximately 10^6 CFU/ml of test organism after incubation for 18 hours at 37°C on Muller-Hinton agar (Difco). *S. aureus* FDA 209 P, *Enterococcus faecalis* ATCC-12968, *Escherichia coli* NIHJ JC-2, *Serratia marcescens* IFO-12968, and *Pseudomonas aeruginosa* E-2 were used as the standard test organisms in this study. The MRSA strains used were originally isolated from clinical specimens in Japan in 1992. The MIC of these strains against methicillin was over 12.5 μg/ml.

All synthesized compounds in this study showed good antibacterial activity against Gram-positive and Gram-negative bacteria except *Pseudomonas aeruginosa* (Tables 1~6). Against *P. aeruginosa*, these compounds were ineffective. Table 1 summarizes the antibacterial activities of 1-thiocephem having hydroxyimino-aminothiazol at the C-7 position, the vinyl-thio linkage at C-3, and various heterocyclic C-3 side chains. The MIC_{80}, which is the concentration required to inhibit 80% of the strains tested, was 12.5 μg/ml for all heterocyclic compounds tested against MRSA. The MIC_{80}'s of these compounds against MRSA were 4-fold lower than the MIC_{80} of flomoxef. Against *E. faecalis* ATCC-21212, against which most of the previously developed cephalosporins were ineffective, these synthesized compounds exhibited

Table 2. Investigation of linkage style and side chain at C-3 position.



MIC (μg/ml)

Compound No.	C-3 side chain R	*S.a*	*MRSA MIC_{80}	*E.f*	*E.c*	*S.m*	*P.a*
5		0.2	**12.5**	0.1	0.05	0.1	>100
6		0.2	**12.5**	0.39	0.1	1.56	>100
7		0.1	**1.56**	0.1	0.0125	0.05	>100

Abbreviation: See footnote for Table 1.

high activity compared with other general cephalosporins.

Table 2 shows the effects on the MIC of the linkage style at the C-3 position, and the effects on the MIC of pyridine and pyridinium at the C-3 side chain on 1-thiocephem with hydroxyimino-aminothiazol at the C-7 position. The MIC_{80} of compounds **5** and **6** against MRSA was the same (12.5 μg/ml). But, that of compound **7** against MRSA was 1.56 μg/ml; *i.e.*, this compound was more 8-fold active than compounds **5** and **6**, and 32-fold or more active than the reference compounds (Table 6). Against *E. faecalis* ATCC-21212, The MIC's of compounds **5**, **6**, and **7** were 0.1, 0.39, and 0.1 μg/ml, respectively. Especially, compounds **5** and **7** showed great activity against *E. faecalis*. Thus, compound **7** displayed the strongest activity against both MRSA and *E. faecalis*, indicating that the vinyl-thio-pyridinium at C-3 was necessary for good activity against both types of bacteria.

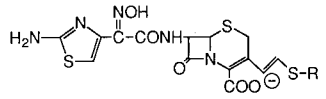
Next, using compound **7**, we investigated the effect of changing the site on pyridinium for the vinyl-thiol linkage (Table 3). Against MRSA, MIC_{80}'s of compounds **7**, **8**, and **9**, in which the respective binding site of pyridinium was at C-4, C-3, and C-2 were 1.56, 1.56, and 3.13 μg/ml, respectively. The antibacterial activity of compounds **7**, **8**, and **9** against *E. faecalis* ATCC-21212 was the same (MIC = 0.1 μg/ml). From the results of Table 3, we concluded that the binding site of pyridinium for the best activity against both MRSA and *E. faecalis* was at either the C-4 or C-3 of pyridinium.

Table 4 shows the antibacterial activity of carboxylic acid derivatives of pyridinium in compound **7**. MIC_{80}'s of compound **10** and other compounds against MRSA were 25 and 12.5 μg/ml, respectively. Thus, the antibacterial activities of carboxylic acid derivatives of pyridinium against MRSA and *E. faecalis* were lower than those of the compounds shown in Table 3.

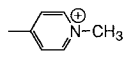
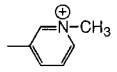
Table 5 shows the effect of pyridyl-N-alkyls as C-3 side chains against MRSA and *E. faecalis*. The MIC_{80}'s of compounds **7**, **15**, **16** and **17** against MRSA were 1.56, 3.13, 3.13 , 3.13 μg/ml, respectively. Compounds **15**, **16**, and **17**, which were more lipophilic than compound **7**, showed lower activity than compound **7** against MRSA. These results might indicate that lipophilicity is not related to anti-MRSA activity. Against *E. faecalis*, compounds **15** and **17** showed almost as strong activity as compound **7**. Compound **16** was not tested against *E. faecalis*.

Finally, we investigated the other pyridyl-N-alkyls shown in Table 6. MIC_{80}'s of N-methyl (compound **7**),

Table 3. Investigation of C-3 side chain.

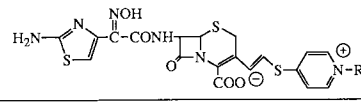


MIC (μg/ml)

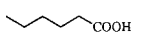
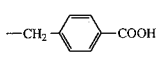
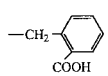
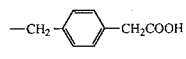
Compound No.	C-3 side chain R	*S.a*	*MRSA MIC_{80}*	*E.f*	*E.c*	*S.m*	*P.a*
7		0.1	**1.56**	0.1	0.0125	0.05	>100
8		0.05	**1.56**	0.1	0.025	0.05	>100
9		0.1	**3.13**	0.1	0.0125	0.1	>100

Abbreviations: See footnote for Table 1.

Table 4. Investigation of C-3 side chain.

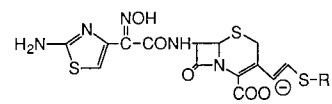


MIC (μg/ml)

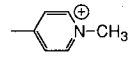
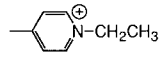
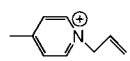
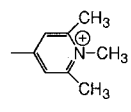
Compound No.	C-3 side chain R	*S.a*	*MRSA MIC_{80}*	*E.f*	*E.c*	*S.m*	*P.a*
10	$-CH_2COOH$	0.78	**25**	N.T.	0.025	0.2	>100
11		0.78	**12.5**	0.39	<0.006	0.1	>50
12		0.39	**12.5**	0.39	0.0125	0.1	>50
13		0.78	**12.5**	0.78	0.025	0.2	>100
14		0.78	**12.5**	0.78	0.05	6.25	>100

Abbreviations: N.T. = not tested; see footnote in Table 1 for meaning of other abbreviations.

Table 5. Investigation of C-3 side chain.

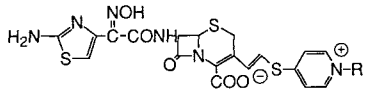


MIC (μg/ml)

Compound No.	C-3 side chain R	*S.a*	*MRSA MIC_{80}*	*E.f*	*E.c*	*S.m*	*P.a*
7		0.1	**1.56**	0.1	0.0125	0.05	>100
15		0.1	**3.13**	0.2	0.0125	0.1	>100
16		0.1	**3.13**	N.T.	0.0125	0.1	>100
17		0.1	**3.13**	0.1	0.025	0.2	>100

Abbreviations: N.T. = not tested; see footnote in Table 1 for meaning of other abbreviations.

Table 6. Investigation of C-3 side chain.



MIC (μg/ml)

Compound No.	C-3 side chain R	*S.a*	*MRSA MIC_{80}	*E.f*	*E.c*	*S.m*	*P.a*
7	$-CH_3$	0.1	**1.56**	0.1	0.0125	0.05	>100
18	$-CH_2CONH_2$	0.1	**1.56**	0.1	0.0125	0.05	>100
19	$-CH_2CH_2OH$	0.1	**3.13**	0.2	<0.006	0.1	>100
20	$-CH_2COCH_3$	0.1	**3.13**	0.2	0.0125	0.05	>100
Cefmetazole		0.78	**50**	>100	0.78	3.13	>100
Flomoxef		0.39	**50**	>100	0.2	0.2	>100
Cefpirome		0.2	**100**	25	0.05	0.05	6.25

Abbreviations: See footnote for Table 1.

N-carbamoylmethyl (compound **18**), N-hydroxyethyl (compound **19**), N-acetoxymethyl (compound **20**), cefmetazole, flomoxef, and cefpirome against MRSA were 1.56, 1.56, 3.13, 3.13, 50, 50, and 100 μg/ml, respectively. The N-methyl and N-carbamoylmethyl compounds showed the same and stronger activity against MRSA than compounds **19** and **20**[6~8]. Compounds **19** and **20** were at least 16-fold more active than cefmetazole, flomoxef, and cefpirome against MRSA. Against *E. faecalis* ATCC-21212, these compounds showed the almost same strong activity, and were at least 125-fold more active than the reference compounds.

Of the compounds we synthesized, compounds **7**, **8**, and **18** showed the strongest activity against both MRSA and *E. faecalis*. These results indicate that the vinyl-thio linkage style at the C-3 position and the C-3 side chain of N-methyl- or N-carbamoylmethyl-pyridinum linked at the 3- or 4-position of pyridinum on 1-thiocephem with hydroxyimino-aminothiazol at the C-7 position yields the greatest anti-MRSA and anti-*E. faecalis* activity.

We hope that these data will lead to the development of even more efficacious anti-MRSA and anti-*E. faecalis* drug in the future.

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