Structure-Activity Relationships of 2-Thioisocephem against Methicillin-resistant *Staphylococcus aureus*

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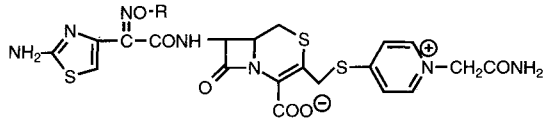
Recently, methicillin-resistant *Staphylococcus aureus* (MRSA), which is a major pathogen along with *Pseudomonas aeruginosa* which is resistant to most commercially available antibiotics, has been causing serious problems in hospitals[1,2]. Arbekacin, used in the clinical treatment of MRSA infections, has been reported to be ineffective against certain MRSA[3]. Probably the only chemotherapeutic drug to which MRSA-resistant strains have not yet arisen is vancomycin[4]. However, the slow cytokilling activity[5] and side effects[6] of vancomycin hinder its effective treatment toward MRSA infections[7]. Therefore, as it is critical to develop new cephem antibiotics with higher killing activity against MRSA and fewer side effects than vancomycin, we have been investigating how to obtain more effective antibiotics against MRSA that show a broad spectrum of antibacterial activity. To this end, we have synthesized 2-thioisocephem compounds with various substituents introduced into the 3- and 7-positions. In this communication, we report that anti-MRSA activity was increased, not unexpectedly, by combined modification of 2-thioisocephem with aminothiazol oxime-substituents at the C-7 position and linkage pattern at the C-3 position.

The derivatives of 2-thioisocephem (TOC-compounds) were obtained from Otsuka Chemical Co., Ltd. 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, *Esche-*

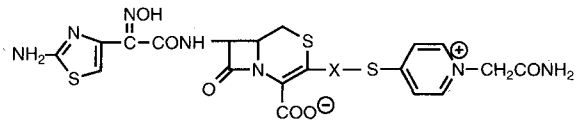
Table 1. Antibacterial activities of TOC-compounds (MIC, μg/ml).



Test organism	Compound: R group:	TOC-24 H	TOC-17 CH_3	TOC-15 $C(CH_3)_2$-COOH	TOC-18 ⬠
Staphylococcus aureus FDA 209 P		0.025	0.39	1.56	0.05
MRSA-70*		**3.13**	**25**	**>100**	**25**
Enterococcus faecalis ATCC-12968		6.25	50	>100	25
Escherichia coli NIHJ JC-2		0.1	0.2	0.39	0.78
Serratia marcescens IFO-12968		0.2	0.2	0.1	1.56
Pseudomonas aeruginosa E-2		50	100	25	25

* : MIC of methicillin against methicillin-resistant *Staphylococcus aureus* 70 was 50μg/ml.

Table 2. Antibacterial activities of TOC-compounds (MIC, μg/ml).



Test organism	Compound: X group:	TOC-24 ∨	TOC-29 ⩘	TOC-25 ⩘
Staphylococcus aureus FDA 209 P		0.025	0.05	0.1
MRSA-70*		**3.13**	**0.78**	**12.5**
Enterococcus faecalis ATCC-12968		6.25	1.56	12.5
Escherichia coli NIHJ JC-2		0.1	0.2	0.2
Serratia marcescens IFO-12968		0.2	1.56	0.39
Pseudomonas aeruginosa E-2		50	50	100

* : MIC of methicillin against methicillin-resistant *Staphylococcus aureus* 70 was 50μg/ml.

richia coli NIHJ JC-2, *Serratia marcescens* IFO-12968, and *Pseudomonas aeruginosa* E-2 were used as the standard test organisms in this study. 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.

Firstly, 2-thioisocephems with aminothiazol-oxime substituents introduced by use of *N*-carbamoyl-pyridinyl-thiomethylene were prepared because, in general, oxime substituents are well known to be relatively stable β-lactamase and to show a good antibacterial activity. As shown in Table 1, when hydrogen (TOC-24), methyl (TOC-17), α,α-dimethyl-caroboxymethyl (TOC-01), and cyclopentyl (TOC-18) were used as *R* substituents, the anti-MRSA-70 activity was 3.13, 25, >100, and 25 μg/ml, respectively. TOC-24, possessing the hydroxyimino-aminothiazol group, thus showed the most effective activity against MRSA 70. On the basis of these results, TOC-24, -25, and -29 were prepared by introducing a methylene, propylene, and vinyl group, respectively, for linking thiopyridinum-*N*-carbamoylmethyl as the C-3 side chain to 2-thioisocephem having hydroxyimino-aminothiazol at the C-7 position. As shown in Table 2, anti-MRSA-70 activities of these compounds were 3.13, 12.5 and 0.78 μg/ml, respectively. TOC-29, bearing *N*-carbamoylmethyl-pyridinumthiovinyl at the C-3 position and hydroxyimino-aminothiazol at the C-7 position, showed the best activity against MRSA-70 in the comparison with the other compounds. In addition, as seen in Table 3, TOC-29 showed an excellent activity against clinically isolated MRSA-70 compared with the reference compound flomoxef. The MIC-80, which is that concentration of drug required to inhibit 80% of the growth of the strains tested, of TOC-24, TOC-25, and TOC-29 against clinically isolated MRSA was 100, >100, and 6.25 μg/ml, respectively (Fig. 1). Reports investigating the activity against MRSA of 2-thioisocephems possessing various linkage groups at the C-3 position have not appeared heretofore. Presently we found out that anti-MRSA activity was influenced by the linkage pattern at the C-3 position, with the strength of anti-MRSA activity decreasing in the order of was the vinyl, methylene, and propylene type. Finally, we tried again the modification with aminothiazol-oximesubstituents on 2-thioisocephem with the *N*-carbamoylpyridinyl-thiovinyl substituent at the C-3 position. Hydrogen (TOC-29), methyl (TOC-8), α,α-dimethyl-carboxymethyl (TOC-15), and cyclopentyl (TOC-02) as oximesubstituents were introduced (Table 3). Antibacterial activities of TOC-29, TOC-8, TOC-15, and TOC-02 against MRSA 70 were 0.78, 25, >100, and 25 μg/ml, respectively (Table 3). These results taken together show that the anti-MRSA activity was strongly enhanced by the nature of a linker at the C-3 position and by that of an aminothiazol-oximesubstituent at the C-7 position. This research approach shows great promise in the search for novel cephems with anti-MRSA activity.

Antibacterial activities of TOC-compounds except for TOC-01, and TOC-15 showed similar good activities

Fig. 1. Antibacterial activities of TOC-compounds, methicillin, and flomoxef against methicillin-resistant *Staphylococcus aureus*. (27 strains)

△ Flomoxef, ▽ methicillin, ◆ TOC-25, ● TOC-29, ■ TOC-24.

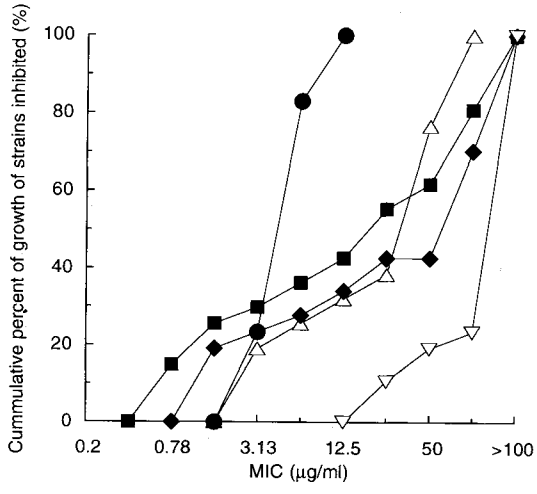
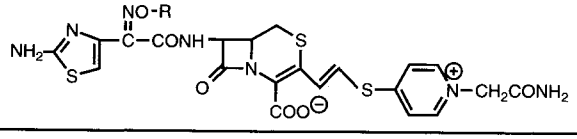


Table 3. Antibacterial activities of TOC-compounds (MIC, μg/ml).



Test organism	Compound: R group:	TOC-29 H	TOC-8 CH_3	TOC-15 $C(CH_3)_2$-COOH	TOC-02 	Flomoxef
Staphylococcus aureus FDA 209 P		0.05	0.1	3.13	0.1	0.39
MRSA-70*		**0.78**	**25**	**>100**	**25**	**50**
Enterococcus faecalis ATCC-12968		1.56	50	>100	6.25	100
Escherichia coli NIHJ JC-2		0.2	0.1	0.2	1.56	0.1
Serratia marcescens IFO-12968		1.56	0.39	0.78	6.25	0.39
Pseudomonas aeruginosa E-2		>100	>100	50	50	>100

* : MIC of methicillin against methicillin-resistant *Staphylococcus aureus* 70 was 50μg/ml.

against *S. aureus* FDA 209 P as a standard strain, as shown in Tables 1, 2, and 3. However, these activities were not related to the anti-MRSA activities. Accordingly, we speculate that the anti-MRSA activities might be caused by high affinity of TOC-compounds for penicillin-binding protein 2′ (PBP2′), since the key player in MRSA resistance is known to be PBP2′[8,9]. TOC-29 exhibiting potent activity against MRSA-70 showed further a good activity against *E. faecalis* ATCC-12968, which became spontaneously resistant to cephem antibiotics (Table 2). Antibacterial activity of TOC-compounds against *E. faecalis* ATCC-12968 paralleled their anti-MRSA activity (Tables 1～3). Furthermore, TOC-compounds possessed a good activity against Gram-negative bacteria except for *P. aeruginosa* E-2.

Very recently in another series of these studies, we also investigated the activity against MRSA of the nucleus derivatives 1-thiocephem, 2-thioisocephem, and 2-oxaisocephem. We found out that 1-thiocephem, designated TOC-39, 7-[2-hydroxyimino-2-(aminothiazol-4-yl)acetoamide]-3-[2-(1-carbamoylmethyl-4-pyridino)-thiovinyl]-3-cephem-4-carboxylate, showed the most potent activity (MIC_{90}, 3.13 μg/ml) against highly resistant MRSA (MIC_{90} against methicillin, >100 μg/ml) from clinical specimens. The *in vitro* and *in vivo* evaluations of TOC-39 will be published elsewhere in comparison with those of vancomycin and reference antibiotics.

Acknowledgment

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References

1) TONIN, E. & A. TOMASZ: β-Lactam-specific resistant mutants of *Staphylococcus aureus*. Antimicrob. Agents Chemother. 30: 577～583, 1986
2) MAPLE, P. A. C.; J. M. T. HAMILTON-MILLER & W. BRUMFITT: World-wide antibiotic-resistance in methicillin-resistant *Staphylococcus aureus*. Lancet i: 537～540, 1989
3) SUZUKI, T.; K. FUJITA & Y. NAGAMACHI: Emergence of arbekacin resistant strains among methicillin-resistant *Staphylococcus aureus*. Jap. J. Antibiotics 47: 47～49, 1994
4) SORRELL, T. C.; D. R. PACKHAN, S. SHANBER, M. GOLDES & R. MUNRO: Vancomycin therapy for methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. 97: 344～350, 1982
5) FOSTER, J. K.; J. R. LENTINO, R. STRODTMAN & D. VINCEN: Comparison of *in vitro* activity of quinolone antibiotics and vancomycin against gentamycin- and methicillin-resistant *Staphylococcus aureus* by time-killing studies. Antimicrob. Agents Chemother. 30: 823～827, 1986
6) FARBER, B. E. & R. C. MOELLERING, Jr.: Retrospective study of the toxicity of preparation of vancomycin from 1974 to 1981. Antimicrob. Agents Chemother. 23: 138～141, 1983
7) LEVINE, D. P.; B. S. FROMM & B. R. REDDY: Slow response to vancomycin or vancomycin plus refampicin in methicillin-resistant *Staphylococcus aureus* endocarditis. Ann. Intern. Med. 115: 674～680, 1991
8) HARTMAN, B. J. & A. TOMASZ: Low-affinity penicillin-binding protein associated with beta-lactam resistance in *Staphylococcus aureus*. J. Bacteriol. 158: 513～516, 1984
9) HAYES, M. V.; N. A. C. CURIS, A. W. WYKE & J. B. WARD: Decreased affinity of a penicillin-binding protein for beta-lactam antibiotics in a clinical isolate of *Staphylococcus aureus* resistant to methicillin. FEMS Lett. 298: 133～136, 1981