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Novel Cephalosporins 2. Synthesis of 3-Heterocyclic-fused Thiopyranylthiovinyl Cephalosporins and Antibacterial Activity against Methicillin-resistant *Staphylococcus aureus* and Vancomycin-resistant *Enterococcus faecalis*

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In the previous paper[1], we reported the synthesis of novel cephalosporins having vinyl-thio linkage attached by benzothiopyrans at C-3 position. They showed good antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE). Especially HMRZ-4, fluoro derivative showed the highest activity among the new compounds and was comparable to that of TOC-50[2~4]. Encouraged by the favorable results from the benzothiopyrans as substituents of vinyl-thio linkage then we have focused our effort on the synthesis of heterocyclic-fused thiopyrans to obtain more effective compounds against MRSA and VRE.

In this paper, we describe the synthesis and antibacterial activity of these new 3-heterocyclic-fused thiopyranyl-thiovinyl cephalosporins represented by formula **I** (Fig. 1).

Chemistry

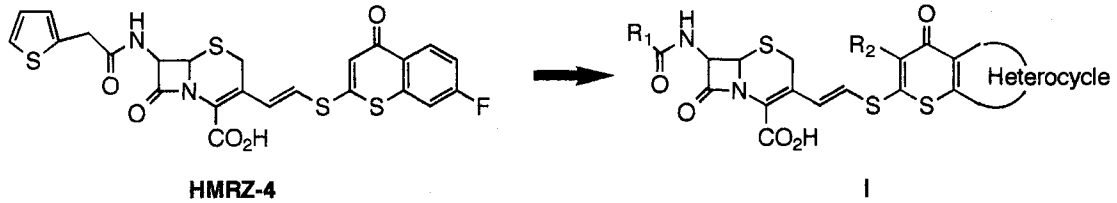
The synthetic routes employed for these cephems are similar to those reported in the previous paper[1]. The required heterocyclic-fused thiopyranylmercaptans (**III**) were all new except 2-mercapto-4-oxo-4*H*-1-thiopyrano[2,3-b]pyridine[5] used in the synthesis of compound **8**, and these were prepared by the method of DUNN[5] or ANDERSON-MCKAY[6].

The typical procedure is shown in the Scheme 1. Reaction of vinyltriflate (**II**), a small excess of mercaptan (**III**) and 0.8 equiv. of ethyl di-isopropylamine in *N,N*-dimethylformamide at room temperature afforded thiopyranylthiovinyl cephem (**IV**). 7-Protecting group of **IV** was removed by treatment with *p*-toluenesulfonic acid to give amine (**V**), which was coupled with thiophene-2-acetic acid (**VI**) using DCC to afford the 7-thienylacetamido cephem. This protected cephem was treated with TFA in the presence of anisole to give 7β-[2-(2-thienyl)acetamido]-3-[2-(7-oxo-7*H*-thieno[3,2-b]thiopyran-5-yl)thiovinyl]-3-cephem-4-carboxylic acid (**1**): ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.68 (1H, d, J=17.8 Hz), 3.77 (2H, s), 4.02 (1H, d, J=17.8 Hz), 5.17 (1H, d, J=5.0 Hz), 5.73 (1H, dd, J=5.0, 8.3 Hz) 6.94~6.97 (2H, m), 7.10 (1H, d, J=15.5 Hz), 7.11 (1H, s), 7.23 (1H, d, J=15.5 Hz), 7.37 (1H, dd, J=1.6, 5.0 Hz), 7.64 (1H, d, J=5.3 Hz), 8.27 (1H, d, J=5.3 Hz), 9.19 (1H, d, J=8.3 Hz); FAB-MS m/z 549 $(M+H)^+$.

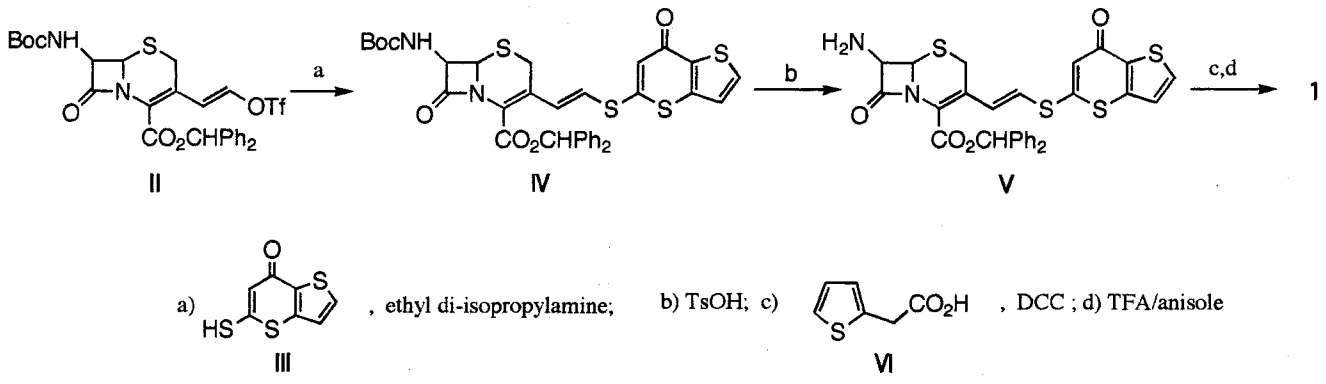
Biological Results and Discussion

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 Mueller-Hinton agar (Difco). *S. aureus* FDA209P, *Enterococcus faecalis* ATCC-21212, *Enterococcus faecalis* NCTC-12201(VRE), *Escherichia coli* NIHJ JC-2 and *Pseudomonas aeruginosa* 46001 were

Fig. 1.



Scheme 1.



used as standard test organisms in this study. The MRSA (27 strains) used in this study were isolated from clinical specimens in Juntendo University Hospital in Japan.

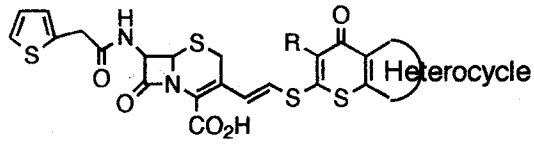
Table 1 shows the antibacterial activities of 7-thienyl-acetamido-3-heterocyclic-fused thiopyran derivatives. All these compounds except compound **3** were highly active against MRSA and VRE. These compounds were ineffective against Gram-negative bacteria such as *E. coli* and *P. aeruginosa* while, hydroxyimino aminothiazole derivatives such as compounds **10** and **11** showed activity against *E. coli* as indicated in Table 2. Hence, 7-substituent is supposed to be a key factor to govern the activity against *E. coli*. Among these compounds, thiophene-fused derivatives (**1**, **2** and **6**) were 2-fold more active against MRSA than HMRZ-4 and TOC-50, especially the bromo-substituted compound **2** was fairly active against VRE. By reason that compound **3** was not active against both MRSA and VRE, it was suggested that the introduction of bulky substituents at C-3 position of thiopyran ring resulted in the reduction of the antibacterial activity against resistant bacteria. In comparison between thiophene-fused derivative (**1**) and furan-fused derivative (**5**) on the activity, compound **1** was 4-fold more active against MRSA. The anti-MRSA and anti-VRE activities of pyridine- or pyrazine-fused derivatives (**8** and **9**) were moderate in comparison with compound **1**, but the anti-*Staphylococcus aureus* activity was superior in these compounds.

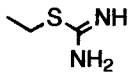
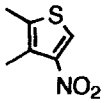
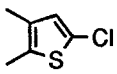
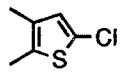
Table 2 shows the effect of the side chain (R) at C-7 position of cephem on the antibacterial activity against MRSA and VRE. Hydroxyimino aminothiazole derivative (**10**) reduced the activity against MRSA and VRE in comparison with thiophene derivative (**1**). But chloro-substituted aminothiazole derivative (**11**) showed an excellent antibacterial activity against MRSA and VRE. It seems that introduction of chloro substituent on aminothiazole is very effective to enhance the activity against these resistant bacteria.

Table 3 shows the effect of the linkage attached to thiopyrane at C-3 position of cephem. Vinyl-thio derivative (**1**) was 32-fold more active against MRSA and 16-fold more active against VRE than thiomethyl derivative (**12**). Compound **1** and **12** were both active against *S. aureus*. It seems that the ability of electron transfer on linkage between cephem ring and external heterocyclic ring is very important for good activity against resistant Gram-positive bacteria.

In conclusion we found that the introduction of heterocyclic-fused thiopyrane linked vinylthio at C-3 position of cephem enhanced the anti-MRSA and anti-VRE activities. Among these compounds, we obtained HMRZ-23 (**1**), HMRZ-40 (**2**), HMRZ-24 (**6**) and HMRZ-62 (**11**) having higher anti-MRSA and anti-VRE activity than those of flomoxef (FMOX) and TOC-50.

Table 1. *In vitro* antibacterial activity (MIC, μg/ml) of 7-thienylacetamido-3-heterocyclic-fused thiopyran derivatives.

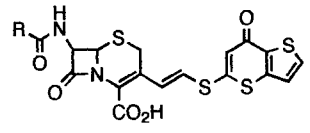


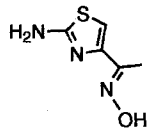
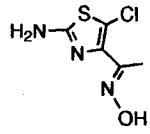
Compound No.	R	heterocycle	MRSA * MIC_{80}	*S. a.* FDA 209P	*E. f.* (VRE) NCTC-12201	*E. f.* ATCC-21212	*E. c.* NIHJ JC-2	*P. a.* 46001
1	H		**0.78**	0.025	**1.56**	0.78	100	>100
2	Br		**0.78**	0.013	**0.78**	0.39	>100	>100
3			25	0.20	25	1.56	>50	>50
4	H		1.56	0.025	1.56	0.78	>100	>100
5	H		3.13	0.025	3.13	0.39	50	>100
6	H		**0.78**	0.025	**1.56**	0.20	>100	>100
7			1.56	0.10	3.13	0.39	>100	>100
8	H		3.13	<0.006	6.25	0.39	>100	100
9	H		1.56	<0.006	3.13	0.39	>100	>100
	HMRZ-4		1.56	0.05	0.78	0.39	>100	100
	TOC-50		1.56	0.10	1.56	0.20	0.013	>100
	FMOX		100	0.20	100	100	0.05	>100

Abbreviation: *S.a., Staphylococcus aureus;* MRSA, *methicillin resistant Staphylococcus aureus; E.f., Enterococcus faecalis;* VRE, *vancomycin resistant enterococci; E.c., Escherichia coli; P.a., Pseudomonas aeruginosa.*

*MIC_{80}:MIC for 80% of clinically isolated MRSA(27strains)

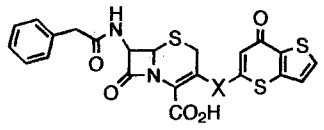
Table 2. *In vitro* antibacterial activity (MIC, μg/ml) of 7-substituted-3-thienothiopyranyl-thiovinyl derivatives.



Compound No.	R	MRSA *MIC_{80}	*S. a.* FDA 209P	***E. f.* (VRE) NCTC-12201**	*E. f.* ATCC-21212	*E. c.* NIHJ JC-2	*P. a.* 46001
1		0.78	0.025	1.56	0.78	100	>100
10		1.56	0.10	3.13	0.10	1.56	100
11		**0.39**	0.05	**0.78**	0.10	3.13	>100

Abbreviations: See footnote in Table 1.

Table 3. Effect of the linkage attached to thienothiopyrane.



Compound No.	X	MRSA *MIC_{80}	*S. a.* FDA 209P	***E. f.* (VRE) NCTC-12201**	*E. f.* ATCC-21212	*E. c.* NIHJ JC-2	*P. a.* 46001
1		**0.78**	0.025	**1.56**	0.78	100	>100
12		25	0.05	25	12.5	>50	>50

Abbreviations: See footnote in Table 1.

References

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