NOTES

# Novel Cephalosporins 1. Synthesis of 3-Benzothiopyranylthiovinyl Cephalosporins and Antibacterial Activity against Methicillin-resistant *Staphylococcus aureus* and Vancomycin-resistant *Enterococcus faecalis*

HIROAKI YAMAZAKI[†,*], YOSHIO TSUCHIDA[†], HIDEKI SATOH[†], SEIICHIRO KAWASHIMA[†], HIDEAKI HANAKI[††] and KEIICHI HIRAMATSU[††]

[†] Research Laboratory, Zenyaku Kogyo Co., Ltd., 2-33-7, Ohizumimachi, Nerima-ku, Tokyo 178-0062, Japan
[††] Department of Bacteriology, Juntendo University, 2-1-1, Hongo, Bunkyo-ku, Tokyo 1133-8421, Japan

(Received for publication November 17, 1999)

Nosocomial infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) or vancomycin-resistant Enterococci (VRE) has become a great deal of recent concern. Although a number of cephalosporins had been synthesized in the past, few of them was effective against these resistant bacteria. Recently, TOC-39 and TOC-50[1~3] bearing vinyl-thio linkage attached by substituted pyridines at C-3 were reported their high antibacterial activity against MRSA and *E. faecalis*. However, toxicological feature of the compounds prevented them from further development. Therefore, we have conducted explorative studies to find novel cephalosporin derivatives having favorable profiles with considerable activities against MRSA and VRE mainly by varying the heterocycles.

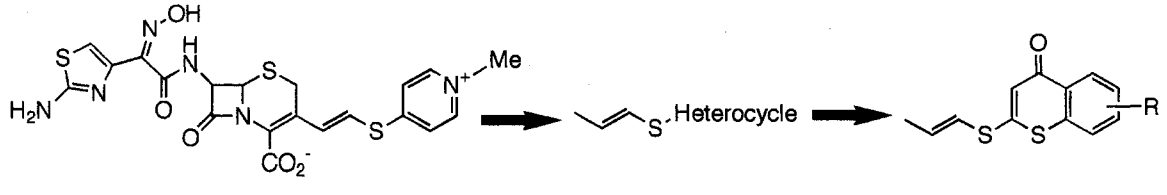
In the meanwhile, OBI[4] reported that methylthio linked benzothiopyrans were useful heterocycles to enhance the antibacterial activity.

In this present communication, we describe that anti-MRSA and anti-*E. faecalis* activities of new cephalosporins having vinyl-thio linkage attached by various 4-oxo-4*H*-1-benzothiopyrans at C-3 side chain (Fig. 1).

## Chemistry

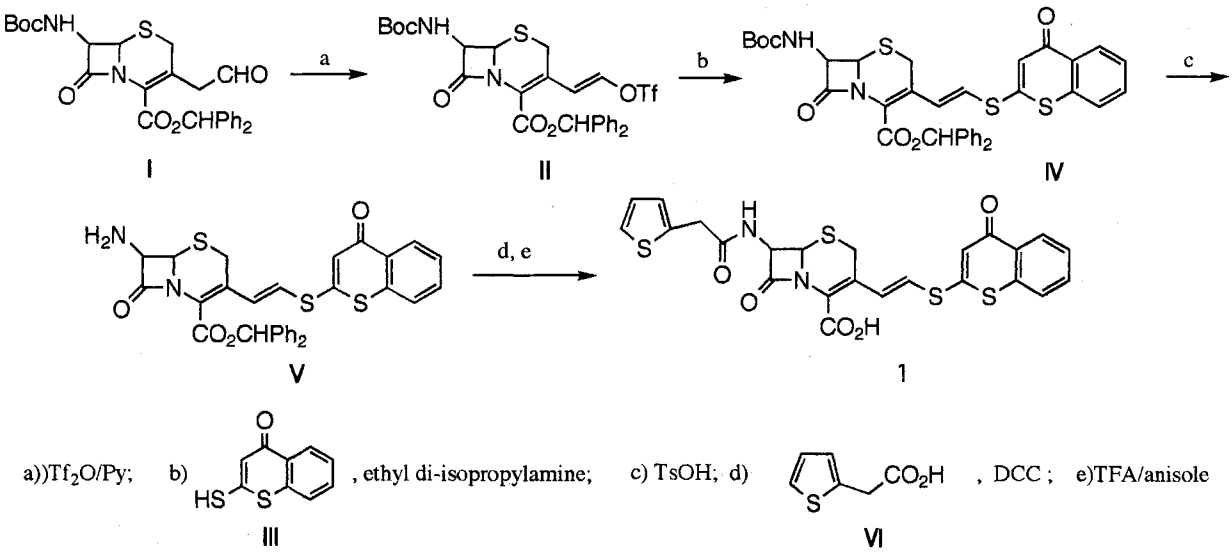
The typical procedure is shown in Scheme 1. Aldehyde (**I**) prepared from 7-aminodesacetoxycephalosporanic acid (7-ADCA) was treated with 1.5 equiv. of triflic anhydride in the presence of pyridine at −35°C to give (*E*)-vinyltriflate (**II**) which was the key intermediate in this procedure. Reaction of **II**, mercaptobenzothiopyran (**III**)[5] and 0.8 equiv. of ethyl di-isopropylamine in *N*,*N*-dimethylformamide at room temperature afforded 3-benzothiopyranylthiovinyl 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-substituted cephem. This protected cephem was treated with TFA in the presence of anisole to give the desired cephem (**1**): $^1$H NMR (270 MHz, DMSO-$d_6$) $\delta$ 3.69 (1H, ABq, $J$=17.5 Hz), 4.05 (1H, ABq, $J$=17.5 Hz), 5.18 (1H, d, $J$=5.0 Hz), 5.73 (1H, dd, $J$=5.0, 8.2 Hz), 6.94 (2H, m), 7.08 (1H, s), 7.14 (1H, d, $J$=15.0 Hz), 7.26 (1H, d, $J$=15.0 Hz), 7.35 (1H, dd, $J$=2.0, 4.6 Hz), 7.61 (1H, dt, $J$=1.0, 8.0 Hz), 7.69 (1H, dt, $J$=1.0, 8.0 Hz), 7.82 (1H,

Fig. 1.




TOC-50

Scheme 1.




dd, $J$=1.0, 8.0 Hz), 8.25 (1H, dd, $J$=1.0, 8.0 Hz), 9.20 (1H, d, $J$=8.2 Hz), 13.80 (1H, br); FAB-MS $m/z$ 543 $(M+H)^+$.

## 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 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 summarizes the antibacterial activities of 7$\beta$-[2-(thienyl)acetamido]-3-[2-(4-oxo-4*H*-1-benzothiopyran-2-yl)thiovinyl]-3-cephem-4-carboxylic acids. All these compounds were highly active against MRSA and *Enterococcus faecalis* including VRE. But these were ineffective against *E. coli* and *P. aeruginosa*. Among these compounds, fluoro derivatives (**2** and **3**) were highly active against VRE. Especially compound **2** showed the strongest activity against MRSA and VRE.

The antibacterial activity of compounds **7**, **9**, **10** and **12** against VRE was lower than compound **2**. These data suggested that the introduction of bulky or hydrophilic substituents in the benzothiopyran ring resulted in the reduction of the antibacterial activity against VRE.

Table 2 showed the effect of the side chain at C-7 of cephalosporin. The hydroxyimino derivatives (**13** and **14**) were 4- to 16-fold more active against MRSA than corresponding alkoxyimino derivatives (**15** and **16**). These compounds **15** and **16** were not active against VRE, especially, compound **16** was not active against these resistant bacteria.

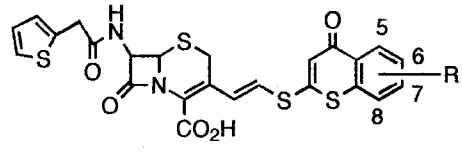
In Table 1, fluoro derivative (**2**) was 2-fold and 4-fold more active than unsubstituted benzothiopyran derivative (**1**) against MRSA and VRE respectively. But in comparison between hydroxyimino analogs (**13** and **14**), the antibacterial activities of them against MRSA were the same but unsubstituted benzothiopyran derivative (**13**) was 4-fold more active against VRE than fluoro derivative (**14**).

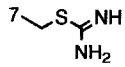
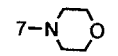
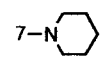
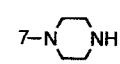
Table 3 showed the comparison of antibacterial activities between other heterocycles attached to C-3 vinyl-thio linkage. Benzopyranyl and benzoxazolyl derivatives (**17** and **18**) were less active against MRSA and VRE respectively than compound **13** and benzene derivative (**19**) did not show activity against these resistant bacteria.

This result exhibited that the benzothiopyran was more useful heterocycle attached to C-3 vinyl-thio linkage than other heterocycles.

In these compounds, the anti-MRSA and anti-*E. faecalis* activities of compound HMRZ-4 (**2**) were higher than those of flomoxef (FMOX) and was comparable to those of TOC-50. These results indicate that both benzothiopyran moiety

Table 1. *In vitro* antibacterial activity (MIC, $\mu$g/ml) of 7-thienylacetamido-3-benzothiopyranyl-thiovinyl derivatives.

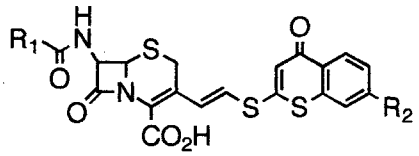


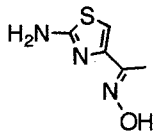
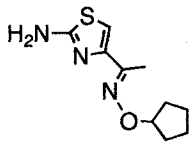
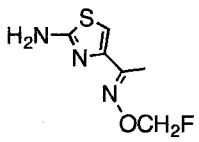
| Compound No. | R | MRSA *$MIC_{80}$ | *S. a.* FDA 209P | ***E. f.* (VRE) NCTC-12201** | *E. f.* ATCC-21212 | *E. c.* NIHJ JC-2 | *P. a.* 46001 |
|---|---|---|---|---|---|---|---|
| 1 | H | 3.13 | 0.20 | 3.13 | 1.56 | >100 | >100 |
| 2 | 7-F | 1.56 | 0.05 | 0.78 | 0.39 | >100 | >100 |
| 3 | 6,7,8-F | 3.13 | 0.025 | 0.78 | 0.39 | >100 | >100 |
| 4 | 7-Cl | 3.13 | 0.10 | 1.56 | 0.78 | >100 | >100 |
| 5 | 5-$CF_3$ | 6.25 | 0.05 | 1.56 | 0.39 | 100 | 100 |
| 6 | 7-$CF_3$ | 6.25 | 0.05 | 3.13 | 0.39 | >100 | >100 |
| 7 | 7-OH | 6.25 | 0.20 | 12.50 | 3.13 | >100 | >100 |
| 8 | 7-OMe | 3.13 | 0.10 | 1.50 | 0.78 | 100 | 100 |
| 9 |  | 3.13 | 0.025 | 6.25 | 0.78 | >100 | >100 |
| 10 |  | 6.25 | 0.05 | 6.25 | 3.13 | >100 | 100 |
| 11 |  | 3.13 | 0.10 | 3.13 | 0.78 | >100 | >100 |
| 12 |  | 3.13 | 0.006 | 6.25 | 0.78 | >100 | >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).

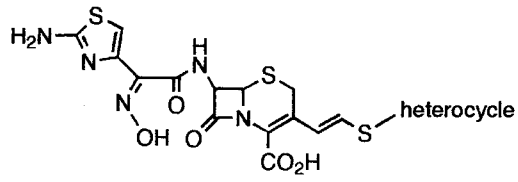
Table 2. *In vitro* antibacterial activity (MIC, $\mu$g/ml) of 7-(2-aminothiazol-4-yl-2-alkoxyiminoacetamido-3-benzothiopyranyl-thiovinyl derivatives.

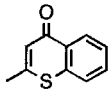
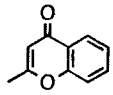
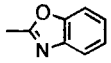


| Compound No. | $R_1$ | $R_2$ | **MRSA *$MIC_{80}$** | *S. a.* FDA 209P | ***E. f.* (VRE) NCTC-12201** | *E. f.* ATCC-21212 | *E. c.* NIHJ JC-2 | *P. a.* 46001 |
|---|---|---|---|---|---|---|---|---|
| 13 |  | H | 3.13 | 0.10 | 1.56 | 0.20 | 1.56 | >100 |
| 14 | | F | 3.13 | 0.39 | 6.25 | 0.78 | 6.25 | >100 |
| 15 |  | H | 12.5 | 0.78 | 25 | 1.56 | 1.56 | 25 |
| 16 |  | H | >50 | 1.56 | >100 | 6.25 | 3.13 | >100 |
| TOC-50 | | | 1.56 | 0.10 | 1.56 | 0.20 | 0.013 | >100 |
| FMOX | | | 100 | 0.20 | 100 | 100 | 0.05 | >100 |

Abbreviations: See footnote in Table 1.

Table 3. Antibacterial activity comparison of compounds with different heterocycles attached to C-3 vinyl-thio linkage.




| Compound No. | 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 |
|---|---|---|---|---|---|---|---|
| 13 |  | **3.13** | 0.10 | **1.56** | 0.20 | 1.56 | >100 |
| 17 |  | 6.25 | 0.05 | 25 | 0.39 | 12.5 | >100 |
| 18 |  | 12.5 | 0.20 | 12.5 | 1.56 | 6.25 | >100 |
| 19 |  | 25 | 0.39 | 50 | 6.25 | 6.25 | >100 |

Abbreviations: See footnote in Table 1.

and vinyl-thio linkage are key factors to have favorable profiles against MRSA and VRE.

## References


1) HANAKI, H.; H. AKAGI, C. SHIMIZU, A. HYODO, N. UNEMI, M. YASUI & Y. HARA: Active new cephalosporin, TOC-39, TOC-50, against multi-resistant MRSA. Program Abstr. 33rd Intersci. Conf. Antimicrob. Agents Chemother., (abstr 889), 1993
2) HIRAMATSU, K.; K. ASADA, E, TATEDA-SUSUKI & K. KUWAHARA-ARAI: TOC-39 and TOC-50, a novel class of cephalosporins having a high binding affinity to the MRSA-specific penicillin-binding protein. PBP2′. Program Abstr. 33rd Intersci. Conf. Antimicrob. Agents Chemother., (abstr 888), 1993
3) HANAKI, H.; H. AKAGI, S. NOMURA, N. UNEMI & K. HIRAMATSU: Structure-activity relationships of cephalosporin derivatives against methicillin-resistant *Staphylococcus aureus* and *Enterococcus faecalis*. J. Antibiotics 49: 402~404, 1996
4) OBI, K.; T. SAITO, H. FUKUDA, K. HIRAI & S. SUZUE: Novel cephalosporins having a benzothiopyran group. 1. Synthesis and antibacterial activity of cephalosporin derivatives characterized by novel C-3 substituents of benzothiopyran. J. Antibiotics 48: 274~277, 1995
5) ANDERSON-MCKAY, J. E. & A. J. LIEPA: The synthesis of 4-hydroxydithiocoumarins; A case of unusual tautomer stability. Aust. J. Chem. 40: 1179~1190, 1987