# Novel Cephalosporins Having a Benzothiopyran Group
## 2. Synthesis and Biological Activity of Catecholic Benzothiopyran Group at the C-3 Side Chain

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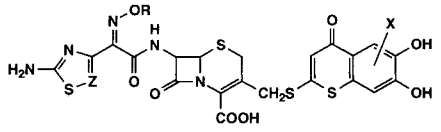
In the preceding paper, we described the synthesis and antibacterial activity of a new class of cephalosporins bearing a benzothiopyran-2-ylthiomethyl group as C-3 side chain[1]. These benzothiopyran cephalosporins exhibited broad and good antibacterial activity against both Gram-positive bacteria including *Enterococcus faecalis* and Gram-negative bacteria including *Pseudomonas aeruginosa*.

In recent years, it has been reported that cephalosporins having a catechol and hydroxypyridone group as bioisostere of catechol exhibit a potent antibacterial activity against Gram-negative bacteria, especially *P. aeruginosa*[2~5].

Our aim is to explore new cephalosporin antibiotics which possess more antibacterial activity against *P. aeruginosa* and more broad spectrum by chemical modification of benzothiopyran nucleus.

In this report, we describes the synthesis of cephalosporins (**I**) having catecholic benzothiopyran group and their biological effects.

Fig. 1.



I

## Chemistry

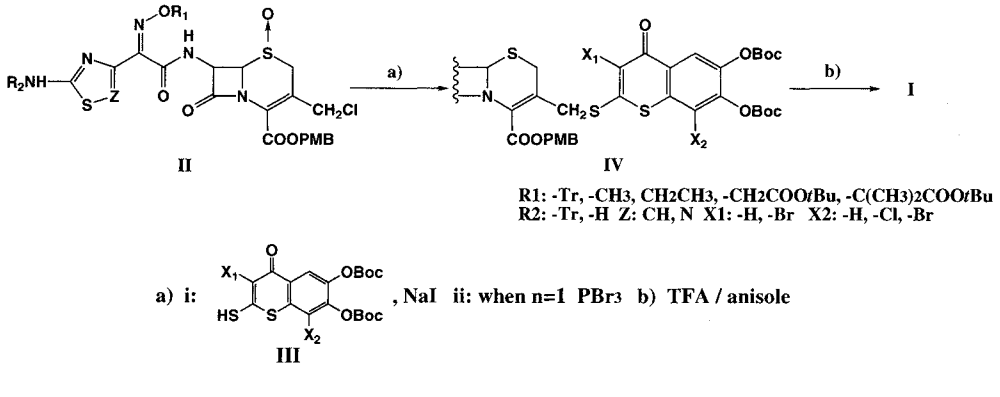
The synthetic routes employed for the new cephems are similar to those reported before[1] and the general procedure is shown in the scheme 1. Treatment of the sulfoxide (**II**) with the mercaptan (**III**) afforded the 3-substituted cephem. After reduction of sulfoxide by $PBr_3$, the protecting groups of **IV** were removed by treatment with TFA in the presence of anisole to give the desired novel cephalosporins. **3**: $^1$H NMR ($CD_3OD$) $\delta$ 3.42 (1H, d, $J$=18 Hz, 2-H), 3.74 (1H, d, $J$=17 Hz, 2-H), 4.05 (1H, d, $J$=14 Hz, 3-$CH_2$), 4.54 (2H, s, $CH_2COO$), 4.72 (1H, d, $J$=14 Hz, 3-$CH_2$), 5.07 (1H, d, $J$=5 Hz, 6-H), 5.74 (1H, d, $J$=5 Hz, 7-H), 6.87, 6.96, 7.05, 7.73 (1H each s); IR (KBr) cm$^{-1}$ 1760, 1660, 1590, 1520; SIMS $m/z$ 710 $(M+H)^+$ as disodium salt. The mercaptans (**III**) used in this work were prepared starting from vanilline derivatives as described in the patent literature[6].

## Biological Result and Discussion

Table 1 shows antibacterial activities (MICs) of the new cephalosporins. These compounds except the hydroxyimino analog (**1**) showed strong antibacterial activity against Gram-negative bacteria including *P. aeruginosa*. Recently, it has been reported that $\beta$-lactam antibiotics having catechol moiety exhibit potent anti-pseudomonal activity. This enhanced antibacterial activity has been concerned to be due to the ability of penetrating to the outer membrane of organisms such as *Escherichia coli via ton* B-dependent iron transport pathway[7,8]. The hydroxyimino analog **1** showed more potent antibacterial activity against Gram-positive bacteria but less activity against Gram-negative bacteria, especially glucose non-fermentative rods (*P. aeruginosa*, *P. cepacia* and *Xanthomonas maltophilia*), than the other alkoxyimino analogs (**2**~**6**).

The aminothiadiazolyl series of methoxyimino and ethoxyimino analogs (**5** and **6**) showed an excellent antibacterial activity against Gram-negative and Gram-positive bacteria.

Scheme 1.



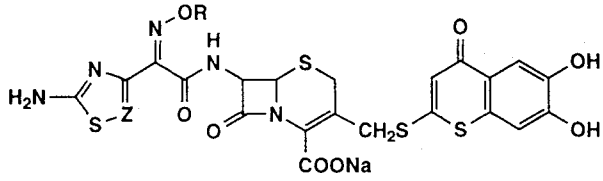
II IV I

R1: -Tr, -CH3, CH2CH3, -CH2COOtBu, -C(CH3)2COOtBu
R2: -Tr, -H Z: CH, N X1: -H, -Br X2: -H, -Cl, -Br

a) i: , NaI ii: when n=1 PBr3 b) TFA / anisole

III

Table 1. *In vitro* antibacterial activity (MIC, μg/ml) of novel catecholic cephalosporins.



| Z | CH | | | | N | | CAZ |
|---|---|---|---|---|---|---|---|
| R | -H | -CH3 | -CH2COOH | -CMe2COOH | -CH3 | -CH2CH3 | |
| Compound No. | 1 | 2 | 3 | 4 | 5 | 6 | |
| *S. a.* 209P | 0.39 | 3.13 | 6.25 | 6.25 | 1.56 | 3.13 | 3.13 |
| *S. p.* IID692 | 0.025 | 0.20 | 0.39 | 0.39 | 0.05 | 0.10 | 0.10 |
| *E. f.* IID682 | 25 | >100 | >100 | >100 | 50 | 100 | >100 |
| *E. c.* NIHJ JC-2 | 0.025 | 0.05 | ≤0.0063 | 0.0125 | 0.0125 | 0.025 | 0.20 |
| *P. v.* IFO3167 | 0.39 | 0.39 | ≤0.0063 | ≤0.0063 | 0.05 | 0.0125 | 0.025 |
| *K. p.* KY6445 | 0.0125 | 0.0125 | ≤0.0063 | ≤0.0063 | ≤0.0063 | ≤0.0063 | 0.39 |
| *P. a.* V-1 | 0.78 | 0.20 | 0.025 | 0.025 | 0.05 | 0.05 | 0.39 |
| *P. a.* IID1210 | 25 | 0.78 | 0.10 | 0.20 | 0.20 | 0.39 | 3.13 |
| *P. c.* GIFU518 | 25 | 0.20 | 0.05 | ≤0.0063 | 0.10 | 0.10 | 3.13 |
| *X. m.* GIFU2491 | 100 | 100 | 25 | 3.13 | 6.25 | 6.25 | 50 |

Abbreviations: *S.a., Staphylococcus aureus*; *S.p., Streptococcus pyogenes*; *E.f., Enterococcus faecalis*; *E.c., Escherichia coli*; *P.v., Proteus vulgaris*; *K.p., Klebsiella pneumoniae*; *P.a., Pseudomonas aeruginosa*; *P.c., Pseudomonas cepacia*; *X.m., Xanthomonas maltophilia.*

Table 2. Therapeutic efficacy of novel cephalosporins andCAZ in systematic infection of mice.

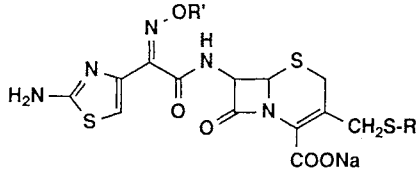
| Test organism | Challenge dose (cfu/mouse) | Compound | ED50 (mg/kg) |
|---|---|---|---|
| *Pseudomonas aeruginosa* IID1210 | $1.3 \times 10^5$ ( +5% mucin ) | 2 | 33 |
| | | 3 | 14 |
| | | 4 | 19 |
| | | CAZ | 53 |

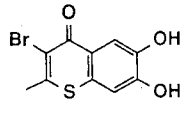
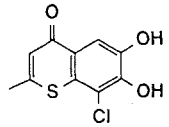
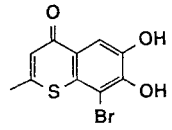
In Gram-negative bacteria, increasing hydrophilicity of cephalosporins has been well known to be effective mean for increasing their membrane permeability[9]. Based on these findings, we also made a change of alkoxyimino group to carboxyalkoxy group at 7-position of catecholic benzothiopyran cephalosporins. Compounds **3** and **4** exhibited several fold more potent against Gram-negative bacteria than **2**. These excellent antibacterial activity of **3** and **4** might be accounted for their high membrane permeability.

Table 2 shows *in vivo* anti-pseudomonal activity of catecholic benzothiopyran cephalosporins. Their activity was higher than that of ceftazidime (CAZ). Among the compounds, the carboxymethoxy derivative **3** showed the best *in vivo* activity against *P. aeruginosa.*

On the other hand, **4** exhibited more potent activity

Table 3. *In vitro* antibacterial activity (MIC, μg/ml) of novel catecholic cephalosporins having a halogen.



| R |  | |  | |  | | |
|---|---|---|---|---|---|---|---|
| R' | -CH3 | -CH2COOH | -CH3 | -CH2COOH | -CH3 | -CH2COOH | CAZ |
| Compound No. | 7 | 8 | 9 | 10 | 11 | 12 | |
| *S. a.* 209P | 0.78 | 12.5 | 0.78 | 6.25 | 1.56 | 6.25 | 3.13 |
| *S. p.* IID692 | 0.10 | 0.78 | 0.05 | 0.78 | 0.10 | 0.39 | 0.10 |
| *E. f.* IID682 | 50 | >100 | 50 | >100 | 50 | >100 | >100 |
| *E. c.* NIHJ JC-2 | 0.05 | 0.05 | 0.025 | 0.025 | 0.05 | 0.025 | 0.20 |
| *P. v.* IFO3167 | 0.10 | 0.025 | 0.10 | 0.0125 | 0.10 | ≤0.0063 | 0.025 |
| *K. p.* KY6445 | 0.0125 | ≤0.0063 | ≤0.0063 | ≤0.0063 | 0.0125 | ≤0.0063 | 0.39 |
| *P. a.* V-1 | 0.10 | 0.10 | 0.05 | 0.05 | 0.10 | 0.05 | 0.39 |
| *P. a.* IID1210 | 0.20 | 0.10 | 0.20 | 0.10 | 0.20 | 0.10 | 3.13 |
| *P. c.* GIFU518 | 0.10 | 0.05 | 0.20 | 0.10 | 0.20 | 0.10 | 3.13 |
| *X. m.* GIFU2491 | 25 | 50 | 6.25 | 12.5 | 6.25 | 6.25 | 50 |

Abbreviations: See footnote in Table 1.

against *P. cepacia* and *X. maltophilia* in comparison to **3**. It appears that the difference of the hydrophobicity between carboxymethoxy group (**3**) and 1-carboxy-1-methylethoxy group (**4**) on the imino moiety of C-7 side chain influences the antibacterial activity[10]. These results imply us that presence of the carboxylic group and hydrophobicity of the substituent around the cephalosporin nucleus is important for antibacterial activity, especially against *X. maltophilia*. Therefore, we intended to introduce a halogen as hydrophobic function to the catecholic benzothiopyran moiety of **2** and **3**. In the case of a presence of halogen at the adjacent to the hydroxy group of catecholic benzothiopyran, it would be expected to cause an increase in stability to catechol-*O*-methyl transferase (COMT)[11].

Table 3 shows *in vitro* antibacterial activity of the halogenated benzothiopyran derivatives. As we would expect, the halogenated catecholic benzothiopyran derivatives were 2- to 16-fold more active than unhalogenated catecholic derivatives (**2** or **3**) against *X. maltophilia*. The introduction of halogen to 8-position at benzothiopyran moiety (**9**～**12**) were more effective than that to 3-position (**7** and **8**). In addition, methoxyimino derivatives **7**, **9** and **11** showed slightly higher activity against Gram-positive bacteria than the carboxymethoxy derivatives **8**, **10** and **12**. On the other hand, disappointingly, *in vivo* activity of the halogenated benzothiopyran cephalosporins were inferior to that of the corresponding unhalogenated cephalosporins. This suggests that *in vivo* efficacy of cephalospolin having a catechol function may be related to other factors besides the metabolism.

In conclusion, we found that AM-1647 (**3**) showed potent antibacterial activity against Gram-negative bacteria including *P. aeruginosa*.

## Determination of *In Vivo* Antibacterial Activity

The test compounds were administered subcutaneously one hour after challenged. Untreated and treated groups at each dose were compound of 5 mice each. The 50% effective dose ($ED_{50}$) were calculated by the least square method on the basis of the number of survivors at 7 days after infection.

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