CEPHALOSPORIN ANTIBIOTICS

I. SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF 3-THIAZOLIOMETHYL DERIVATIVES

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The synthesis and the structure-activity relationships of 3-thiazoliomethyl cephalosporins are described. In a series of these parenteral compounds, 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido group was found to be a favorable substituent for the C-7 position of the cephem nucleus. They showed potent antibacterial activity against both Gram-positive and Gram-negative bacteria including some β-lactamase producing species.

In the last decade, new types of cephalosporin antibiotics having an aminothiazol-oxime moiety at the C-7 position of the cephem nucleus have been developed[1~5]. They generally show a strong activity against a wide variety of microorganisms except Gram-positive bacteria such as *Staphylococcus aureus* and some β-lactamase producing species.

In a previous paper[6], we reported on the synthesis and the antibacterial activity of various types of 3-ammoniummethyl cephalosporin derivatives with a 2-aminoaryl-2-alkoxyiminoacetamido group in the C-7 position, among which the 3-thiazoliomethyl derivatives generally showed potent antibacterial activity against both Gram-positive and Gram-negative bacteria.

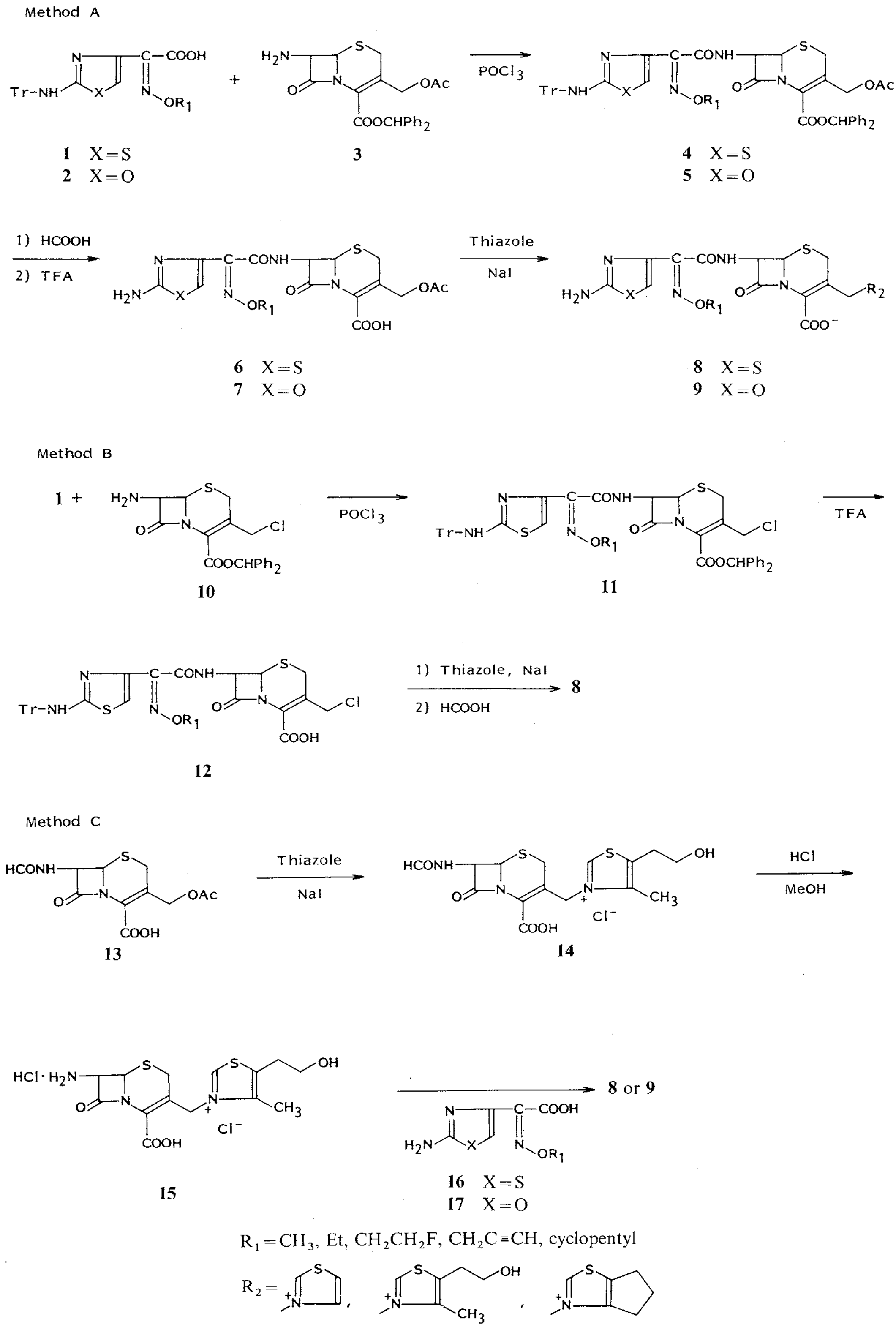
In this paper, in a further elaboration to optimize the antibacterial activity, 3-thiazoliomethyl cephalosporin derivatives with various types of acyl groups in the C-7 position are synthesized and their structure-activity relationships are discussed.

Chemistry

The 3-thiazoliomethyl cephalosporins were prepared by the procedures illustrated in the following scheme. Method A, which comprised the conversion of 3-acetoxymethyl group into 3-thiazoliomethyl moiety, was well suited for efficient preparation of various 3-thiazoliomethyl cephalosporins on a small scale to allow testing of their antibacterial activity. Acylation of the 7-aminocephalosporanate (**3**) with 2-aminothiazolyl or 2-aminooxazolyl-2-alkoxyiminoacetic acid[6] (**1** or **2**) was efficiently achieved with phosphoryl chloride under mild conditions. Subsequent deprotection of the acylated products (**4** and **5**) was carried out by successive treatment with formic acid in methanol for the trityl group and with trifluoroacetic acid (TFA) for the diphenylmethyl ester group to afford 7-(2-aminoaryl-2-alkoxyiminoacetamido)cephalosporins (**6** and **7**). The 3-acetoxy group of the resulting acids (**6** and **7**) was directly converted into a 3-thiazolio moiety with a corresponding thiazole in the presence of sodium iodide.

For water-insoluble thiazoles, method A resulted only in poor yields. Therefore, method B was alternatively employed, in which the reaction of the acid (**12**) with a desired thiazole was carried out in an aprotic solvent such as *N*,*N*-dimethylacetamide below room temperature to afford the desired compound **8**.

Scheme 1.


Method A
POCl3
1 X=S
2 X=O
3
4 X=S
5 X=O
1) HCOOH
2) TFA
Thiazole
NaI
6 X=S
7 X=O
8 X=S
9 X=O
Method B
POCl3
10
11
TFA
12
1) Thiazole, NaI
2) HCOOH
8
Method C
Thiazole
NaI
13
14
HCl
MeOH
15
8 or 9
16 X=S
17 X=O
R1=CH3, Et, CH2CH2F, CH2C≡CH, cyclopentyl
R2=

Method C, which was suitable for a relatively large-scale laboratory syntheses, gave a fairly good yield in each step, especially in the formation of 3-thiazoliomethyl derivatives, in contrast to methods A and B. 7-Formamidocephalosporanic acid (**13**) was converted into 3-thiazoliomethyl derivative (**14**) according to method A. The formyl group of **14** was removed by treatment with hydrochloric acid in methanol to give the 7-aminocephalosporanate (**15**), which was successively acylated with carboxylic acid (**16** or **17**) by an active ester method using 1-hydroxybenzotriazole and dicyclohexylcarbodiimide without protection of the amino group of the carboxylic acids (**16** and **17**).

The aminothiadiazolyl cephalosporins (**18** and **19**) were prepared in a similar manner according to the reported procedure[7,8].

Biological Results and Discussion

The comparative antibacterial activity of 7-(2-aminothiazolyl-2-alkoxyiminoacetamido)- and 7-(2-aminooxazolyl-2-alkoxyiminoacetamido)-3-thiazoliomethyl cephalosporin antibiotics is shown in Table 1. Although aminooxazoyl derivatives showed fairly good activity against the tested organisms, aminothiazolyl derivatives exhibited excellent activity; the latter ones possessed higher activity than the former ones against Gram-negative bacteria. Concerning the C-3 substituent of the cephem nucleus, each type of hydrophilic (**8c** and **9c**), lipophilic (**8d** and **9d**) and unsubstituted (**8a**, **8b**, **9a** and **9b**) thiazolio derivatives revealed similar results in antibacterial activity.

The comparative antibacterial activity of aminothiazolyl and aminothiadiazolyl cephalosporins is shown in Table 2. The aminothiazolyl compound **8f** bearing an unsubstituted thiazolio group in the C-3 position showed potent activity comparable to the aminothiadiazolyl derivative (**19**) against various kinds of microorganisms. However, the compound **8e** (CS-461) having a 5-(2-hydroxyethyl)-4-methylthiazolio group on the C-3 position exhibited higher activity than the aminothiadiazolyl derivative (**18**). These aminothiadiazolyl derivatives tended to have higher antipseudomonal activity than the aminothiazolyl derivatives[8].

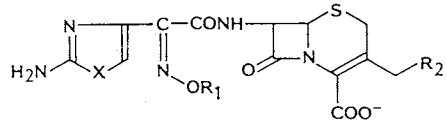
In order to examine antibacterial activity in detail, the effect of various oxime substituents of the C-7 acyl moiety was investigated. The results are shown in Table 3. Most of these compounds except **8h** showed remarkable activity against various microorganisms. Among these compounds, the compound **8e** (CS-461) bearing a methoxyimino substituent possessed the most prominent activity against both Gram-positive and Gram-negative bacteria including some β-lactamase producing species. Both compounds **8b** and **8c** having 2-fluoroethoxyimino residue also showed excellent activity. The compounds **8h** and **8i** bearing cyclopentyloxyimino and hydroxyimino residue exhibited relatively high activity against *S. aureus*, but were less active against Gram-negative bacteria than the methoxyimino derivatives.

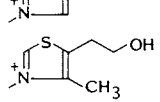
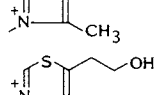
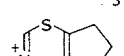
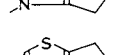
According to these results, the 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetyl group, as seen in CS-461, was found to be the most favorable residue for the C-7 acyl moiety of 3-thiazoliomethyl cephalosporin derivatives.

Experimental

IR spectra were recorded on a Jasco A302 spectrometer. ^{1}H NMR spectra were determined on a Varian EM-360 (60 MHz) or a Jeol GX-270 (270 MHz) spectrometer using TMS or 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard. The mp's were determined using a Yanagimoto micro-melting point apparatus and are uncorrected.

Table 1. Comparative activity (MIC, μg/ml)[a] of aminothiazolyl and aminooxazolyl cephalosporins.

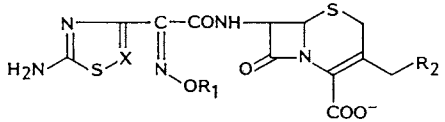


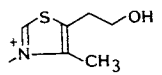
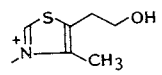
Compound	X	R_1	R_2	*S.a.*[b]	*S.a.* (R)	*E.c.*	*E.c.*(R)	*K.p.*	*K.p.* (R)	*E.cl.*	*S.m.*	*P.v.*	*M.m.*	*P.a.*
8a	S	CH_3		0.4	0.8	0.05	0.1	0.1	0.8	0.1	0.02	0.05	0.8	12.5
9a	O	CH_3		0.4	1.5	0.8	6.2	0.8	3.1	0.8	0.4	0.4	6.2	200
8b	S	CH_2CH_2F		0.4	0.8	0.05	0.1	0.05	0.8	0.1	≦0.01	≦0.01	0.4	6.2
9b	O	CH_2CH_2F		0.2	0.8	0.4	1.5	0.4	3.1	0.8	0.2	0.2	6.2	25
8c	S	CH_2CH_2F		0.2	0.8	0.05	0.4	0.05	0.8	0.1	0.05	0.05	0.2	6.2
9c	O	CH_2CH_2F		0.4	0.8	0.2	1.5	0.4	3.1	0.4	0.4	0.1	1.5	12.5
8d	S	CH_3		0.4	0.8	0.05	0.1	0.05	0.8	0.05	≦0.01	0.02	0.1	6.2
9d	O	CH_3		0.2	0.8	0.4	1.5	0.4	3.1	0.4	0.2	0.2	1.5	100

[a] Agar dilution method: Nutrient agar; 10^7 cfu/ml.

[b] *S.a.*: *Staphylococcus aureus* 209P JC-1, *S.a.* (R): *S. aureus* 56, *E.c.*: *Escherichia coli* NIHJ JC-2, *E.c.* (R): *E. coli* 609, *K.p.*: *Klebsiella pneumoniae* 806, *K.p.* (R): *K. pneumoniae* 846, *E. cl.*: *Enterobacter cloacae* 963, *S.m.*: *Serratia marcescens* 1184, *P.v.*: *Proteus vulgaris* 1420, *M.m.*: *Morganella morganii* 1510, *P.a.*: *Pseudomonas aeruginosa* 1001. (R) means β-lactamase producing strains.

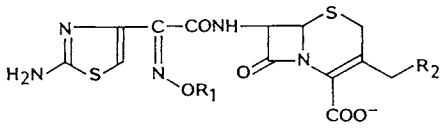
Table 2. Comparative activity (MIC, μg/ml)[a] of aminothiazolyl and aminothiadiazolyl cephalosporins.

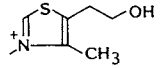
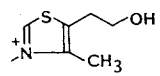
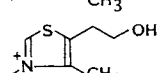


Compound	X	R_1	R_2	*S.a.*[b]	*S.a.* (R)	*E.c.*	*E.c.* (R)	*K.p.*	*K.p.* (R)	*E.cl.*	*S.m.*	*P.v.*	*M.m.*	*P.a.*
8e (CS-461)	CH	CH_3		0.1	0.8	≦0.01	0.2	≦0.01	0.2	≦0.01	≦0.01	≦0.01	0.1	3.1
18	N	CH_3		0.4	1.5	0.05	0.4	0.1	0.4	0.1	0.05	0.2	0.8	0.8
8f	CH	CH_2CH_3		0.8	1.5	0.2	0.4	0.2	0.8	0.2	0.05	0.02	0.8	3.1
19	N	CH_2CH_3		0.4	1.5	0.05	0.2	0.1	0.4	0.1	0.05	0.1	3.1	1.5

[a] Agar dilution method: Nutrient agar; 10^7 cfu/ml.
[b] Abbreviations mean the same strains as in Table 1.

Table 3. Antibacterial activity (MIC, μg/ml)[a] of aminothiazolyl cephalosporins.



Compound	R_1	R_2	*S.a.*[b]	*S.a.* (R)	*E.c.*	*E.c.* (R)	*K.p.*	*K.p.* (R)	*E.cl.*	*S.m.*	*P.v.*	*M.m.*	*P.a.*
8a	CH_3		0.4	0.8	0.05	0.1	0.1	0.8	0.1	0.02	0.05	0.8	12.5
8f	CH_2CH_3		0.8	1.5	0.2	0.4	0.2	0.8	0.2	0.05	0.02	0.8	3.1
8b	CH_2CH_2F		0.4	0.8	0.05	0.1	0.05	0.8	0.1	$\leqq$0.01	$\leqq$0.01	0.4	6.2
8g	$CH_2C{\equiv}CH$		0.4	0.8	0.2	0.4	0.2	0.8	0.4	0.05	0.02	1.5	6.2
8h			0.1	0.8	1.5	3.1	3.1	1.5	1.5	1.5	$\leqq$0.01	3.1	6.2
8i	H		0.02	0.2	0.2	1.5	0.4	0.8	0.4	0.1	0.4	0.2	100
8e (CS-461)	CH_3		0.1	0.8	$\leqq$0.01	0.2	$\leqq$0.01	0.2	$\leqq$0.01	$\leqq$0.01	$\leqq$0.01	0.1	3.1
8c	CH_2CH_2F		0.2	0.8	0.05	0.4	0.05	0.8	0.1	0.05	0.05	0.2	6.2

[a] Agar dilution method: Nutrient agar; 10^7 cfu/ml.
[b] Abbreviations mean the same strains as in Table 1.

Diphenylmethyl 7-[2-(2-Tritylaminooxazol-4-yl)-(*Z*)-2-methoxyiminoacetamino]-3-acetoxymethyl-3-cephem-4-carboxylate (**5**)

To a soluton of diphenylmethyl 7-amino-3-acetoxymethyl-3-cephem-4-carboxylate (**3**: 7.02 g), 2-(2-tritylaminooxazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid (**2**: 7.20 g) and *N*,*N*-diethylaniline (5.03 g) in dichloromethane (100 ml) was added dropwise phosphoryl chloride (2.58 g) at $-10°C$. After stirring for 30 minutes, the solvent was removed *in vacuo* and the residue was taken-up into EtOAc and 5% HCl. The separated organic layer was washed successively with 5% HCl, water, 5% $NaHCO_3$ and brine. The solution was dried ($MgSO_4$) and concd *in vacuo* and then the residue was chromatographed on a silica gel column (EtOAc-cyclohexane, 1:1) to give **5** (9.55 g, 70.4%). ^1H NMR (60 MHz, $CDCl_3$) δ 2.00 (3H, s, acetyl), 3.45 (2H, s, 2-CH_2), 4.08 (3H, s, OCH_3), 4.74 and 5.05 (2H, ABq, $J=14$ Hz, 3'-CH_2), 4.98 (1H, d, $J=5$ Hz, 6-CH), 5.87 (1H, dd, $J=5$ and 9 Hz, 7-CH), 6.35 (1H, s, N*H*Tr), 6.94 (1H, s, C*H*Ph_2), 7.0~7.5 (26H, m, Ph×5 and oxazole ring-H), 7.76 (1H, d, $J=9$ Hz, NHCO).

7-[2-(2-Aminooxazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic Acid (**7**)

A suspension of **5** (9.55 g) in formic acid (40 ml) and MeOH (67 ml) was stirred at 50°C for 1 hour and the resulting solution was concd *in vacuo*. The residue was dissolved in EtOAc and the solution was washed with 5% $NaHCO_3$ and brine successively, then dried ($MgSO_4$). The solvent was removed *in vacuo* and the residue was chromatographed on a silica gel column (EtOAc-cyclohexane, 2:1~5:1) to give diphenylmethyl 7-[2-(2-aminooxazol-4-yl)-(*Z*)-2-methoxyiminoacetamido)-3-acetoxymethyl-3-cephem-4-carboxylate (6.13 g, 89.9%). ^1H NMR (60 MHz, $CDCl_3$) δ 2.02 (3H, s, acetyl), 3.49 (2H, s, 2-CH_2), 4.06 (3H, s, OCH_3), 4.78 and 5.10 (2H, ABq, $J=14$ Hz, 3'-CH_2), 5.08 (1H, d, $J=5$ Hz, 6-CH), 5.30 (2H, s, NH_2), 6.04 (1H, dd, $J=5$ and 9 Hz, 7-CH), 7.01 (1H, s, C*H*Ph_2), 7.40 (10H, s, Ph×2), 7.48 (1H, s, oxazole ring-H), 8.62 (1H, d, $J=9$ Hz, NHCO).

To a solution of the intermediate (6.13 g) mentioned above in dichloromethane (10 ml) and anisole (10 ml) was added TFA (15 ml) and the solution was stirred for 30 minutes at room temperature. After evaporation *in vacuo*, the oily residue was triturated with isopropyl ether to give **7** as a TFA salt (5.20 g, 93.4%). This crude product was used for the next reaction without further purification.

7-[2-(2-Aminooxazol-4-yl)-(*Z*)-2-methoxyiminoacetamino)]-3-thiazoliomethyl-3-cephem-4-carboxylate (**9a**)

A mixture of **7** (TFA salt, 553 mg), $NaHCO_3$ (185 mg), NaI (1.5 g), thiazole (426 mg) and water (1.7 ml) was stirred at 50°C for 4.5 hours. The resulting solution was diluted with acetonitrile (5 ml) and the solution was chromatographed on a silica gel column (acetonitrile-water, 3:1~2:1) to give **9a** (36.1 mg, 7.8%). IR (KBr) cm^{-1} 1768 (β-lactam C=O). ^1H NMR data are listed in Table 5.

The compounds **8c**, **8f**, **8g**, **8h** and **9d** were prepared by a similar manner as that mentioned above and the ^1H NMR data are listed in Tables 4 and 5.

Diphenylmethyl 7-[2-(2-Tritylaminothiazol-4-yl)-(*Z*)-2-(2-fluoroethyl)oxyiminoacetamido]-3-chloromethyl-3-cephem-4-carboxylate (**11**)

To a solution of diphenylmethyl 7-amino-3-chloromethyl-3-cephem-4-carboxylate (**10**: HCl salt, 4.51 g), *N*,*N*-diethylaniline (3.13 g) and sodium salt of 2-(2-tritylaminothiazol-4-yl)-(*Z*)-2-(2-fluoroethyl)oxyiminoacetic acid (**1**: 5.22 g) in dichloromethane (120 ml) was added dropwise phosphoryl chloride (1.61 g) at $-15°C$. After stirring for 40 minutes at -5 to $-10°C$, the solvent was evaporated *in vacuo* and EtOAc was added to the residue. The resulting mixture was washed successively with dil HCl, water, 5% $NaHCO_3$ and brine. The solution was dried ($MgSO_4$) and concd *in vacuo*. The residue was then chromatographed on a silica gel column (EtOAc-cyclohexane, 2:3) to give **11** (6.11 g, 70.1%). ^1H NMR (60 MHz, $CDCl_3$) δ 3.37 and 3.58 (2H, ABq, $J=18$ Hz, 2-CH_2), 4.1~5.1 (4H, m, CH_2CH_2F), 4.33 (2H, s, 3'-CH_2), 4.96 (1H, d, $J=4.5$ Hz, 6-CH), 5.84 (1H, dd, $J=4.5$ and 9 Hz, 7-CH), 6.66 (1H, s, thiazole ring-H), 6.81 (1H, d, $J=9$ Hz, NHCO), 6.87 (1H, s, C*H*Ph_2), 7.0~7.4 (26H, m, TrNH and C*H*Ph_2).

Table 4. ^{1}H NMR (60 MHz) data of aminothiazolyl cephalosporins.

	DMSO-d_6, δ ppm, J in Hz
8b	2.9~3.8 (2H, m, 2-CH_2), 3.8~5.1 (4H, m, CH_2CH_2F), 5.00 (1H, d, J=5, 6-CH), 4.9~5.9 (2H, m, 3'-CH_2), 5.61 (1H, dd, J=5, 7.5, 7-CH), 6.62 (1H, s, thiazole ring-H), 7.19 (2H, br s, NH_2), 8.25 (1H, br s, thiazolio ring 5-H), 8.80 (1H, br s, thiazolio ring 4-H), 9.44 (1H, d, J=7.5, NHCO), 10.45 (1H br s, thiazolio ring 2-H)
8c[a]	2.46 (3H, s, CH_3), 3.00 (2H, t, J=5.6, CH_2CH_2OH), 3.12, 3.40 (2H, ABq, J=17.6, 2-CH_2), 3.64 (2H, t, J=5.6, CH_2CH_2OH), 4.27 (2H, dt, J=4.0, 29.8, CH_2CH_2F), 4.63 (2H, dt, J=4.0, 47.9, CH_2CH_2F), 5.05 (1H, d, J=4.9, 6-CH), 5.27 (2H, s, 3'-CH_2), 5.67 (1H, dd, J=4.9, 7.8, 7-CH), 6.75 (1H, s, thiazole ring-H), 7.23 (2H, s, NH_2), 9.57 (1H, d, J=7.8, NHCO), 10.29 (1H, s, thiazolio ring-H)
8d	2.0~3.5 (6H, m, $-CH_2CH_2CH_2-$), 3.0~3.8 (2H, m, 2-CH_2), 3.80 (3H, s, OCH_3), 5.00 (1H, d, J=5, 6-CH), 4.8~5.8 (2H, m, 3'-CH_2), 5.60 (1H, dd, J=5, 7.5, 7-CH), 6.60 (1H, s, thiazole ring-H), 7.05 (2H, br s, NH_2), 9.43 (1H, d, J=7.5, NHCO), 10.10 (1H, br s, thiazolio ring-H)
8f[b]	1.30 (3H, t, J=7, CH_2CH_3), 3.23, 3.75 (2H, ABq, J=18, 2-CH_2), 4.26 (2H, q, J=7, CH_2CH_3), 5.25 (1H, d, J=5, 6-CH), 5.46 (2H, br s, 3'-CH_2), 5.87 (1H, d, J=5, 7-CH), 6.87 (1H, s, thiazole ring-H), 8.21 (1H, d, J=4, thiazolio ring 5-H), 8.49 (1H, d, J=4, thiazolio ring 4-H)
8g	2.9~3.8 (2H, m, 2-CH_2), 3.1~3.5 (1H, m, C≡CH), 4.65 (2H, br s, $CH_2C\equiv C$), 5.00 (1H, d, J=5, 6-CH), 5.0~6.0 (2H, m, 3'-CH_2), 5.60 (1H, dd, J=5, 8, 7-CH), 6.65 (1H, s, thiazole ring-H), 7.10 (2H, br s, NH_2), 8.21 (1H, d, J=4, thiazolio ring 5-H), 8.82 (1H, d, J=4, thiazolio ring 4-H), 9.52 (1H, d, J=8, NHCO), 10.48 (1H, br s, thiazolio ring 2-H)
8h[b]	1.1~2.2 (8H, m, cyclopentyl), 3.30, 3.82 (2H, ABq, J=18, 2-CH_2), 4.4~5.0 (1H, m, OCH), 5.33 (1H, d, J=4.5, 6-CH), 5.49 (2H, br s, 3'-CH_2), 5.89 (1H, d, J=4.5, 7-CH), 6.95 (1H, s, thiazole ring-H), 8.22 (1H, d, J=3.5, thiazolio ring 5-H), 8.50 (1H, d, J=3.5, thiazolio ring 4-H)
8i	2.50 (2H, s, CH_3), 3.14 (2H, t, J=6, CH_2CH_2OH), 3.0~4.0 (2H, m, 2-CH_2), 3.68 (2H, t, J=6, CH_2CH_2OH), 5.05 (1H, d, J=5, 6-CH), 5.42 (2H, br s, 3'-CH_2), 5.64 (1H, dd, J=5, 8, 7-CH), 6.30 (1H, s, thiazole ring-H), 6.95 (2H, br s, NH_2), 8.92 (1H, d, J=8, NHCO), 10.40 (1H, br s, thiazolio ring-H)

[a] Measured at 270 MHz.
[b] Measured in D_2O.

Table 5. ^{1}H NMR (60 MHz) data of aminooxazolyl cephalosporins.

	DMSO-d_6, δ ppm, J in Hz
9a	2.9~3.8 (2H, m, 2-CH_2), 3.82 (3H, s, OCH_3), 5.03 (1H, d, J=4, 6-CH), 5.0~5.8 (2H, m, 3'-CH_2), 5.62 (1H, dd, J=4, 8, 7-CH), 6.80 (2H, br s, NH_2), 7.43 (1H, s, oxazole ring-H), 8.33 (1H, br s, thiazole ring 5-H), 8.96 (1H, br s, thiazole ring 4-H), 9.51 (1H, d, J=8, NHCO), 10.55 (1H, br s, thiazole ring 2-H)
9c[a]	2.46 (3H, s, CH_3), 3.00 (2H, t, J=5.6, CH_2CH_2OH), 3.11, 3.40 (2H, ABq, J=17.6, 2-CH_2), 3.64 (2H, t, J=5.6, CH_2CH_2OH), 4.27 (2H, dt, J=3.9, 29.8, CH_2CH_2F), 4.63 (2H, dt, J=3.9, 47.9, CH_2CH_2F), 5.04 (1H, d, J=4.9, 6-CH), 5.27 (2H, s, 3'-CH_2), 5.64 (1H, dd, J=4.9, 7.8, 7-CH), 6.88 (2H, s, NH_2), 7.51 (1H, s, oxazole ring-H), 9.57 (1H, d, J=7.8, NHCO), 10.31 (1H, s, thiazole ring-H)
9d	2.5~3.8 (8H, m, 2-CH_2 and $-CH_2CH_2CH_2-$), 3.86 (3H, s, OCH_3), 5.06 (1H, d, J=4.5, 6-CH), 5.0~5.8 (2H, m, 3'-CH_2), 5.61 (1H, dd, J=4.5, 8, 7-CH), 6.80 (2H, br s, NH_2), 7.46 (1H, s, oxazole ring-H), 9.57 (1H, d, J=8, NHCO), 10.34 (1H, s, thiazole ring-H)

[a] Measured at 270 MHz.

7-[2-(2-Tritylaminothiazol-4-yl)-(*Z*)-2-(2-fluoroethyl)oxyiminoacetamido]-3-chloromethyl-3-cephem-4-carboxylic Acid (**12**)

A solution of **11** (1.5 g) in anisole (5 ml) and TFA (5 ml) was stirred for 30 minutes at room temperature and evaporated *in vacuo*. The resulting oily residue was triturated with isopropyl ether to give **12** (1.03 g). The crude product was used for the next reaction without purification.

7-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-(2-fluoroethyl)oxyiminoacetamido]-3-thiazoliomethyl-3-cephem-4-carboxylate (**8b**)

To a solution of **12** (0.7 g) in *N*,*N*-dimethylacetamide (2 ml) was added thiazole (0.5 ml) and the solution

was stirred for 30 minutes at room temperature. To the solution was added isopropyl ether and the resulting precipitate was collected by filtration. The residue was chromatographed on a silica gel column (acetonitrile - water) to give 7-[2-(2-tritylaminothiazol-4-yl)-(*Z*)-2-(2-fluoroethyl)oxyiminoacetamido]-3-thiazoliomethyl-3-cephem-4-carboxylate. This intermediate was dissolved in water (0.5 ml) and formic acid (2 ml) and the solution was stirred at 40°C for 1 hour. After evaporation, the residue was chromatographed on a silica gel column (acetonitrile - water, 4 : 1 ~ 3 : 1) to give **8b** (22.5 mg, 6.1%). ^{1}H NMR (Table 4).

The compound **8d** was prepared by a similar manner as that mentioned above and the ^{1}H NMR data are listed in Table 4.

7-Formamido-3-[5-(2-hydroxyethyl)-4-methylthiazoliomethyl]-3-cephem-4-carboxylate Hydrochloride (**14**)

To a mixture of $NaHCO_3$ (10.5 g), 5-(2-hydroxyethyl)-4-methylthiazole (71.6 g) and water (50 ml) was addded slowly 7-formamido-3-acetoxymethyl-3-cephem-4-carboxylic acid (**13**: 50.0 g) and the mixture was stirred for 20 minutes at room temperature. To the resulting solution was added NaI (124.8 g) and the mixture was stirred at 70°C for 2 hours. The solution was washed with hot EtOAc (300 ml × 2) and MeOH (50 ml) was added to the residue. To the solution was added a solution of conc HCl (24.3 ml) in MeOH (50 ml) under ice cooling. After stirring for 5 minutes, the insoluble solid was removed by quick filtration. To the filtrate was added MeOH (200 ml) and then the solution was allowed to stand for 3 hours on an ice bath. The precipitated crystals were filtered and washed with cold MeOH and ether successively to afford **14** (45.97 g, 63.0%). MP 144°C (dec); IR (KBr) cm^{-1} 1780 (β-lactam C=O); ^{1}H NMR (60 MHz, DMSO-d_6) δ 2.39 (3H, s, CH_3), 3.01 (2H, t, J=6 Hz, CH_2CH_2OH), 3.47 (2H, s, 2-CH_2), 3.64 (2H, t, J=6 Hz, CH_2CH_2OH), 5.13 (1H, d, J=4.5 Hz, 6-CH), 5.51 (2H, s, 3′-CH_2), 5.78 (1H, dd, J=4.5 and 8.5 Hz, 7-CH), 8.11 (1H, s, HCO), 9.05 (1H, d, J=8.5 Hz, NHCO), 10.16 (1H, s, thiazolio ring-H).

Anal Calcd for $C_{15}H_{18}ClN_3O_5S_2 \cdot H_2O$: C 41.14, H 4.60, N 9.60, S 14.64.
Found: C 40.93, H 4.53, N 9.49, S 14.92.

7-Amino-3-[5-(2-hydroxyethyl)-4-methylthiazoliomethyl]-3-cephem-4-carboxylate Dihydrochloride (**15**)

A suspension of **14** (194.45 g) in MeOH (1,900 ml) and conc HCl (111 ml) was stirred at 40°C for 1.5 hours and cooled to room temperature. The insoluble solid was filtered off and the filtrate was evaporated *in vacuo*. The resulting crystalline residue was collected by filtration with a small amount of cold MeOH and washed with ether to give **15** (137.31 g, 72.0%). MP 159°C (dec); ^{1}H NMR (270 MHz, DMSO-d_6) δ 2.39 (3H, s, CH_3), 3.03 (2H, t, J=5.4 Hz, CH_2CH_2OH), 3.45 and 3.60 (2H, ABq, J=17.6 Hz, 2-CH_2), 3.65 (2H, t, J=5.4 Hz, CH_2CH_2OH), 5.28 (2H, s, 6-CH and 7-CH), 5.57 (2H, s, 3′-CH_2), 10.08 (1H, s, thiazolio ring-H).

7-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[5-(2-hydroxyethyl)-4-methylthiazoliomethyl]-3-cephem-4-carboxylate Sulfate (**8e**: CS-461)

To a solution of 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetic acid (**16**: 38.2 g) and 1-hydroxybenzotriazole hydrate (29.1 g) in DMF (380 ml) was added dicyclohexylcarbodiimide (39.2 g) below 15°C and the mixture was stirred for 1.5 hours at room temperature. A precipitate was filtered off and to the filtrate was added a solution of **15** (68.0 g) and $NaHCO_3$ (26.6 g) in DMF (150 ml) and water (190 ml). The resulting mixture was then stirred at 40°C for 20 minutes and cooled on an ice bath. To the solution was added 95% H_2SO_4 (16.35 g) slowly and a small amount of insoluble solid was filtered off after stirring for 10 minutes. The filtrate was evaporated *in vacuo* and the residue was dissolved in water (80 ml) and EtOH (150 ml). After standing for 30 minutes under ice cooling, the resulting precipitate was collected by filtration to give the crude product (108 g). The product was dissolved in water (350 ml) and the pH was adjusted to about 7 by adding $NaHCO_3$. After filtration over Celite, the filtrate was chromatographed on a reversed phase silica gel (LiChroprep RP-8, Merck) column (5% ~ 7% acetonitrile). The combined fraction was evaporated *in vacuo* and the residue (52.64 g) was dissolved in water (145 ml) and EtOH (145 ml). To the solution was added 95% H_2SO_4 (10.03 g) slowly under ice cooling and then an additional amount of EtOH (145 ml) was added to the solution. After standing for 2.5 hours on an ice bath, the resulting colorless crystals were collected by filtration to afford **8e** (43.81 g, 42.2%). MP 196°C (dec); IR

(KBr) cm^{-1} 1788 (β-lactam C=O); ^1H NMR (270 MHz, DMSO-d_6) δ 2.39 (3H, s, CH_3) 3.02 (2H, t, $J=5.6$ Hz, CH_2CH_2OH), 3.38 (2H, s, 2-CH_2), 3.65 (2H, t, $J=5.6$ Hz, CH_2CH_2OH), 3.82 (3H, s, OCH_3), 5.18 (1H, d, $J=4.9$ Hz, 6-CH), 5.43 and 5.49 (2H, ABq, $J=18$ Hz, 3'-CH_2), 5.85 (1H, dd, $J=4.9$ and 8.3 Hz, 7-CH), 6.72 (1H, s, thiazole ring-H), 7.21 (2H, s, NH_2), 9.61 (1H, d, $J=8.3$ Hz, NHCO), 9.90 (1H, s, thiazolio ring-H).

Anal Calcd for $C_{20}H_{24}N_6O_{10}S_4 \cdot H_2O$: C 36.69, H 4.00, N 12.84, S 19.59.
Found: C 36.60, H 3.78, N 13.01, S 19.45.

The compound **9c** was prepared by a similar manner as that mentioned above and the ^1H NMR data are listed in Table 5.

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