CEPHALOSPORIN ANTIBIOTICS

II†. SYNTHESIS AND BIOLOGICAL PROPERTIES OF CS-461 AND RELATED COMPOUNDS

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The synthesis, structure-activity relationships, and biological properties of 3-thiazoliomethyl cephalosporins are described. 7-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[5-(2-hydroxyethyl)-4-methylthiazoliomethyl]-3-cephem-4-carboxylate sulfate (CS-461) showed potent antibacterial activity against a wide variety of bacteria both *in vitro* and *in vivo*. Furthermore, CS-461 exhibited significantly low acute toxicity in mice.

In previous papers[1,2] we reported that 3-thiazoliomethyl cephalosporin derivatives possessed remarkable antibacterial activity against both Gram-positive and Gram-negative bacteria, including some β-lactamase producing species, and that in a study of the structure-activity relationships a combination of thiazoliomethyl groups on the C-3 and a 2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetyl group in the C-7 position of the cephem nucleus especially showed good activity.

Our further elaboration to optimize the antibacterial potency of the 3-thiazoliomethyl cephalosporin series has been made toward a chemical modification of the thiazole ring at the C-3′ position. In this paper we describe the synthesis, structure-activity relationships, and biological properties of 3-thiazoliomethyl cephalosporin derivatives having various kinds of substituents on the thiazole at the C-3′ position.

Chemistry

3-Thiazoliomethyl cephalosporins were prepared in a similar manner according to a previous paper[2], as shown in Scheme 1. 3-Acetoxymethyl cephalosporin (**1**) was converted directly into 3-thiazoliomethyl derivatives (**2**) by treatment with desired thiazoles in the presence of sodium iodide at 50 to 70°C. These compounds, obtained as amorphous solid, were rather unstable. This undesirable character was circumvented by formation of their sulfates (**3**), which were found to be stable enough in handling for biological evaluations.

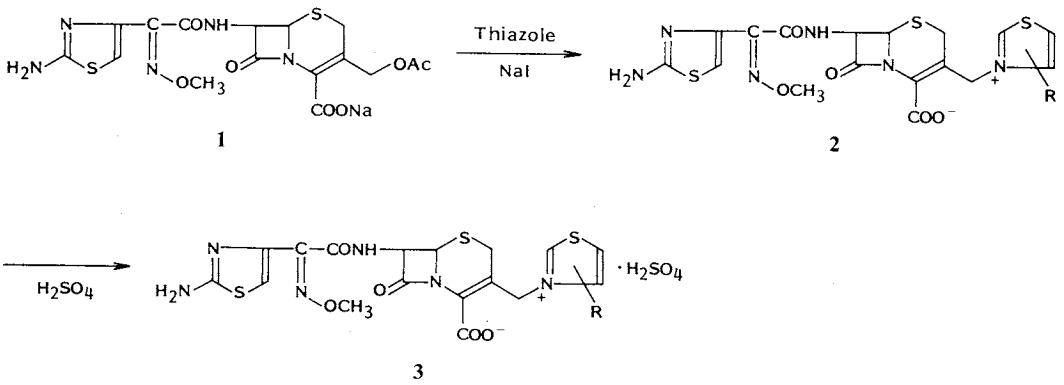
Thiazole derivatives employed for the C-3′ substituent were prepared according to the reported procedures[3~5], except for carbamate-type derivatives (**8** and **9**). The carbamates were prepared by a similar manner according to MURPHY's modification[6,7] as shown in Scheme 2.

Biological Results and Discussion

The antibacterial activity of the 3-thiazoliomethyl cephalosporins is shown in Table 1. As described

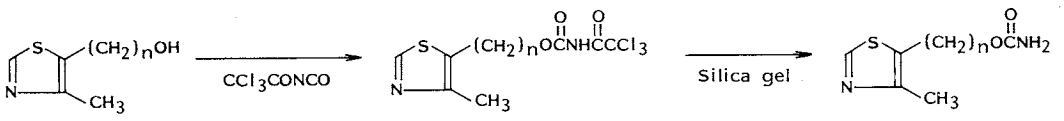
† Part I: See ref 2.

Scheme 1.



1 2 3

Scheme 2.

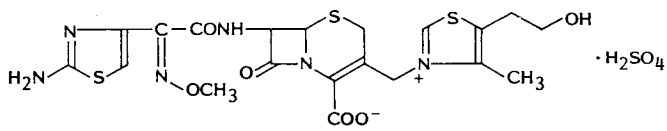


4 (n=1)
5 (n=2)

6 (n=1)
7 (n=2)

8 (n=1)
9 (n=2)

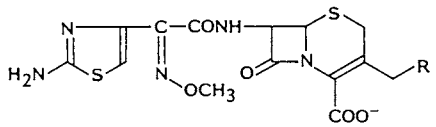
Fig. 1. Structure of CS-461.

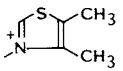
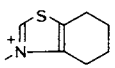
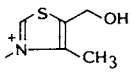
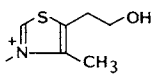
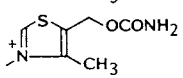
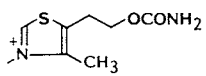
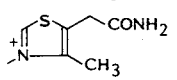
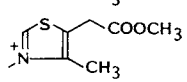
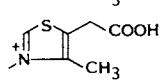


in previous papers[1,2], the compound **2a** having an unsubstituted thiazole and the compounds **2b** and **2c** having lipophilic substituents such as 4,5-dimethyl and 4,5-cyclohexeno groups, respectively, on the thiazole at the C-3′ position of the cephem nucleus showed potent antibacterial activity. On the other hand, CS-461 (Fig. 1) with a hydrophilic hydroxyethyl substituent on the thiazole (**5**), a constituent of thiamine, also revealed remarkable activity against both Gram-positive and Gram-negative bacteria.

Accordingly, chemical modification of the hydroxyethyl group in **5** was examined in detail. Degradation of one carbon atom of the hydroxyethyl substituent decreased the antibacterial activity; the compound **3a** having a hydroxymethyl substituent instead of the hydroxyethyl group in CS-461 gave diminished activity. The carbamate derivative (**2d**) of **3a** was also less active than the carbamate (**2e**) of CS-461. Compounds having an oxidized 5-substituent on the thiazole of CS-461 showed no improvement in activity; **3d** having a carboxyl group exhibited diminished activity against both Gram-positive and Gram-negative bacteria, however, **3b** and **3c** having an amide and an ester group, respectively, showed moderate activity comparable to that of ceftazidime, but less than CS-461. Among these compounds, the carbamate derivative **2e** showed strong and well-balanced antibacterial activity against various kinds of microorganisms including *Pseudomonas aeruginosa*. However, **2e** showed slightly less activity than CS-461 and the lipophilic derivative **2c**.

Table 1. Antibacterial activity (MIC, μg/ml)[a] of 3-thiazoliomethyl cephalosporins.

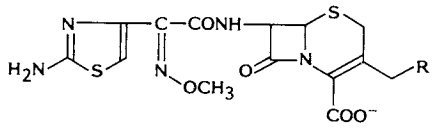


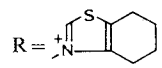
Compound	R	*S.a.*[b]	*S.a.* (R)	*E.c.*	*E.c.* (R)	*K.p.*	*K.p.* (R)	*E.cl.*	*S.m.*	*P.v.*	*M.m.*	*P.a.*
2a		0.4	0.8	0.05	0.1	0.1	0.8	0.1	0.02	0.05	0.8	12.5
2b		0.4	0.8	0.05	0.1	0.1	0.8	0.1	≦0.01	0.05	0.2	6.2
2c		0.2	0.4	0.02	0.05	0.05	0.8	0.05	≦0.01	0.02	0.02	6.2
3a		0.8	1.5	0.05	0.4	0.1	0.8	0.1	0.02	0.05	0.2	12.5
CS-461		0.1	0.8	≦0.01	0.2	≦0.01	0.2	≦0.01	≦0.01	≦0.01	0.1	3.1
2d		0.8	3.1	0.1	0.4	0.1	1.5	0.2	0.1	0.1	0.4	12.5
2e		0.4	1.5	0.05	0.2	0.1	0.8	0.1	0.02	0.05	0.1	3.1
3b		0.2	0.8	0.05	0.4	0.05	0.4	0.05	≦0.01	0.02	0.2	12.5
3c		0.2	0.8	0.05	0.4	0.05	0.4	0.05	≦0.01	≦0.01	0.2	12.5
3d		1.5	3.1	0.02	3.1	0.05	0.4	0.05	≦0.01	0.02	1.5	50
Ceftazidime		3.1	6.2	0.2	0.4	0.1	0.2	0.2	0.02	0.02	3.1	1.5

[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. Protective effect of 3-thiazoliomethyl cephalosporins against experimental infection in mice.



Organism	ED_{50}[a]/(MIC[b])		
	2c	CS-461	CAZ
	R = 	R =	
Staphylococcus aureus Smith	2.5	1.6 (0.78)	12.2 (6.25)
S. aureus 560[c]	2.0	2.9 (0.78)	20.1 (6.25)
Escherichia coli 704	0.1	0.3 (0.025)	0.63 (0.10)
E. coli 609[c]	2.4	1.8 (0.10)	20.8 (0.39)
Klebsiella pneumoniae 866	0.2	0.5 (0.05)	0.77 (0.05)

[a] 50% effective subcutaneous dose (mg/kg).
[b] Agar dilution method; Mueller-Hinton agar; 10^6 cfu/ml.
[c] β-Lactamase producing strain.
CAZ: Ceftazidime.

In order to clarify the characteristics of the 3-thiazoliomethyl cephalosporins, further biological properties were investigated. The protective effect against experimental infection in mice of **2c** having a lipophilic substituent and CS-461 having a hydrophilic one on the thiazole at the C-3′ position are shown in Table 2. Both **2c** and CS-461 showed good protection against Gram-positive and Gram-negative bacteria especially against *Staphylococcus aureus*, and were not distinguished from each other in antibacterial potency.

In an acute toxicity test, however, differences were observed between hydrophilic (CS-461) and lipophilic (**2b** and **2c**) compounds. When the lipophilic compounds **2b** and **2c** were administered at the dose of 2,000 mg/kg intravenously in mice, some adverse symptoms such as irregular respiration, creeping, and sedation were noted. In contrast to these results, no adverse symptoms and no deaths were observed with less lipophilic **2a** and hydrophilic CS-461 when they were administered up to 3,000 mg/kg. According to these observations, it was found that there was tendency toward the enhancement of toxicity in proportion to the lipophilicity of these compounds.

Conclusion

CS-461 showed potent and well-balanced antibacterial activity against Gram-positive and Gram-negative bacteria including some β-lactamase producing species both *in vitro* and *in vivo*. Furthermore, CS-461 exhibited significantly low acute toxicity in mice. Consequently, CS-461, found to be the best compound of this series, was selected as a clinical candidate.

Experimental

IR spectra were recorded on a Nicolet NIC-60SX spectrometer. ^{1}H NMR spectra were determined on a Jeol GX-270 (270 MHz) or a Varian EM-360 (60 MHz) spectrometer using TMS as an internal standard. The mp's were determined using a Yanagimoto micro-melting point apparatus and are uncorrected.

(4-Methylthiazol-5-yl)methyl Trichloroacetylcarbamate (**6**)

To a solution of 5-hydroxymethyl-4-methylthiazole[4] (**4**, 1.0 g) in dry dichloromethane (7 ml) was added dropwise trichloroacetyl isocyanate (1.6 g) with ice-cooling. After stirring for 5 minutes, isopropyl ether (20 ml) was added to the mixture and the resulting precipitate was collected by filtration to afford **6** (2.33 g, 95.3%). MP 177~178.5°C (EtOAc). ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.53 (3H, s, CH_3), 5.46 (2H, s, CH_2), 8.66 (1H, br s, NH), 8.81 (1H, s, thiazole ring-H).

Anal Calcd for $C_8H_7Cl_3N_2O_3S$: C 30.26, H 2.22, Cl 33.49, N 8.82, S 10.10.
Found: C 30.49, H 2.20, Cl 33.36, N 8.88, S 10.21.

(4-Methylthiazol-5-yl)methyl Carbamate (**8**)

A mixture of **6** (1.0 g) and silica gel (Kieselgel 60, Merck, 6.0 g) in THF (7 ml) and MeOH (14 ml) was stirred overnight at 40°C. The mixture was filtered over Celite and the filtrate was evaporated *in vacuo*. The residue was taken up into EtOAc and washed with brine. The organic layer was dried ($MgSO_4$) and evaporated *in vacuo* to afford **8** (529 mg, 99.1%). MP 103~105°C (EtOAc). ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.50 (3H, s, CH_3), 5.05 (2H, br s, NH_2), 5.23 (2H, s, CH_2), 8.72 (1H, s, thiazole ring-H).

Anal Calcd for $C_6H_8N_2O_2S$: C 41.85, H 4.68, N 16.27, S 18.62.
Found: C 42.13, H 4.62, N 16.27, S 18.79.

2-(4-Methylthiazol-5-yl)ethyl Carbamate[8] (**9**)

9 was prepared by a similar manner as that mentioned above resulting in excellent yield from **5**. ^{1}H NMR (60 MHz, acetone-d_6) δ 2.38 (3H, s, CH_3), 3.08 (2H, t, J=6.5 Hz, $ArCH_2$), 4.17 (2H, t, J=6.5 Hz, CH_2O), 5.73 (2H, br s, NH_2), 8.69 (1H, s, thiazole ring-H).

7-[2-(2-Aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-(5-carbamoylmethyl-4-methylthiazoliomethyl)-3-cephem-4-carboxylate (**2f**)

A mixture of sodium 7-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylate (**1**, 10.0 g), 4-methylthiazole-5-acetamide[4] (9.37 g), NaI (15.0 g) and water (20 ml) was stirred at 60°C for 8 hours. After cooling to room temperature, CH_3CN (60 ml) was added to the

Table 3. ^{1}H NMR (270 MHz) data of 3-thiazoliomethyl cephalosporins.

	DMSO-d_6, δ ppm, J in Hz
2d	2.47 (3H, s, CH_3), 3.41, 3.54 (2H, ABq, J=17.8, 2-CH_2), 3.90 (3H, s, OCH_3), 5.21 (1H, d, J=4.9, 6-CH), 5.25 (2H, s, CH_2O), 5.51, 5.58 (2H, ABq, J=15.9, 3′-CH_2), 5.85 (1H, dd, J=4.9, 7.8, 7-CH), 6.84 (1H, s, thiazole ring-H), 6.95 (2H, br s, NH_2), 7.71~9.25 (2H, br, $CONH_2$), 9.77 (1H, d, J=7.8, NHCO), 10.26 (1H, s, thiazolio ring-H)
2e	2.39 (3H, s, CH_3), 2.22 (2H, t, J=5.9, CH_2CH_2O), 3.31, 3.48 (2H, ABq, J=17.8, 2-CH_2), 4.11 (2H, t, J=5.9, CH_2CH_2O), 5.21 (1H, d, J=4.9, 6-CH), 5.52, 5.58 (2H, ABq, J=15.6, 3′-CH_2), 5.86 (1H, dd, J=4.9, 7.8, 7-CH), 6.60 (2H, br s, NH_2), 6.83 (1H, s, thiazole ring-H), 7.56~8.87 (2H, br, $CONH_2$), 9.75 (1H, d, J=7.8, NHCO), 10.21 (1H, s, thiazolio ring-H)
3a	2.36 (3H, s, CH_3), 3.39 (2H, s, 2-CH_2), 3.83 (3H, s, OCH_3), 4.75 (2H, s, CH_2OH), 5.18 (1H, d, J=4.9, 6-CH), 5.46 (2H, s, 3′-CH_2), 5.85 (1H, dd, J=4.9, 7.8, 7-CH), 6.27 (1H, br s, OH), 6.73 (1H, s, thiazole ring-H), 7.22 (2H, s, NH_2), 9.62 (1H, d, J=7.8, NHCO), 9.96 (1H, s, thiazolio ring-H)
3b	2.37 (3H, s, CH_3), 3.37 (2H, s, 2-CH_2), 3.83 (3H, s, OCH_3), 3.87 (2H, s, CH_2CO), 5.17 (1H, d, J=4.9, 6-CH), 5.42, 5.49 (2H, ABq, J=17.1, 3′-CH_2), 5.85 (1H, dd, J=4.9, 7.8, 7-CH), 6.73 (1H, s, thiazole ring-H), 7.22 (2H, s, NH_2), 7.45~7.82 (2H, br, $CONH_2$), 9.62 (1H, d, J=7.8, NHCO), 9.91 (1H, s, thiazolio ring-H)
3c	2.39 (3H, s, CH_3), 3.34, 3.42 (2H, ABq, J=21.6, 2-CH_2), 3.72 (3H, s, $COOCH_3$), 3.83 (3H, s, OCH_3), 4.20 (2H, s, CH_2CO), 5.19 (1H, d, J=4.9, 6-CH), 5.44, 5.50 (2H, ABq, J=16.2, 3′-CH_2), 5.85 (1H, dd, J=4.9, 7.8, 7-CH), 6.73 (1H, s, thiazole ring-H), 7.23 (2H, s, NH_2), 9.63 (1H, d, J=7.8, NHCO), 9.97 (1H, s, thiazolio ring-H)
3d	2.40 (3H, s, CH_3), 3.29, 3.39 (2H, ABq, J=19.3, 2-CH_2), 3.82 (3H, s, OCH_3), 4.06 (2H, s, CH_2CO), 5.13 (1H, d, J=4.9, 6-CH), 5.38, 5.44 (2H, ABq, J=16.6, 3′-CH_2), 5.78 (1H, dd, J=4.9, 7.8, 7-CH), 6.72 (1H, s, thiazole ring-H), 7.21 (2H, s, NH_2), 9.59 (1H, d, J=7.8, NHCO), 10.05 (1H, s, thiazolio ring-H)

resulting solution. The resulting mixture was chromatographed on a silica gel (Kieselgel 60, Merck) column (CH_3CN - H_2O, 7 : 1 ~ 3 : 1) to give a crude product. This product was chromatographed again on a reversed phase silica gel (LiChroprep RP-8, Merck) column (5% CH_3CN) to afford **2f** (2.90 g, 26.3%).

2d and **2e** were prepared by a similar manner as that mentioned above and the ^1H NMR data are listed in Table 3.

2f Sulfate (3b)

To a solution of **2f** (0.46 g) in water (2 ml) was added 1 N H_2SO_4 (1.66 ml) and the solution was allowed to stand for 1 hour on an ice bath after addition of EtOH (2 ml). The resulting crystals were collected by filtration to afford **3b** (0.38 g, 70.0%). MP 192°C (dec). IR (KBr) cm^{-1} 1791 (β-lactam C=O). ^1H NMR (Table 3).

Anal Calcd for $C_{20}H_{23}N_7O_{10}S_4 \cdot H_2O$: C 35.98, H 3.77, N 14.68, S 19.21.
Found: C 35.40, H 3.81, N 14.52, S 19.22.

3a, **3c**, and **3d** were prepared by a similar manner as that mentioned above and the ^1H NMR data are listed in Table 3.

Therapeutic Effect on Systemic Mouse Infections

Overnight cultures of organisms grown at 37°C in Trypto-soy broth (Eiken-Chemical Co., Ltd., Tokyo, Japan) were diluted according to their virulence. The diluted cultures, if necessary, were mixed with the same amount of 10% gastric mucin (Tokyokasei-Kogyo Co., Ltd., Tokyo, Japan). Ten male *dd*Y mice in each group were infected intraperitoneally with 0.2 ml portions of those bacterial mixtures. β-Lactam antibiotics were administered subcutaneously at 0 and 4 hours after infection. The ED_{50} of mice were calculated by the probit method according to the survival rate after 5 days.

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