SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF 3-(2-IMIDAZOLYL)THIOMETHYL CEPHALOSPORINS

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The synthesis and structure-activity relationships of a series of 3-(2-imidazolyl)thiomethyl cephalosporins are described. Among the compounds, 7β-[2-(2-amino-1,3-thiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(4,5-dicarboxyimidazol-2-yl)thiomethyl]-3-cephem-4-carboxylic acid (**1**) exhibited potent activity against both Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa*. We also estimated lipophilicity of the compounds from the chromatographic log k' value of reversed-phase HPLC. The relationship between lipophilicity and biological activity showed that compound **1** had the most suitable lipophilicity.

While some so-called third generation cephalosporins are in clinical use, there still remain some problems in chemotherapy. For example, they are not very effective for chemotherapy in the compromised hosts.

In our previous papers[1], we have reported the synthesis and antibacterial activity of cefpimizole (AC-1370) and its analogues. It has high antibacterial activity, especially *in vivo*, because of stimulating activity on the host defense. This immunostimulatory activity is believed to be attributable to the imidazolyl group[2].

On the other hand, recently many cephalosporins with a (*Z*)-2-(alkoxyimino)-2-(2-amino-1,3-thiazol-4-yl)acetamido group, such as cefotaxime (CTX), cefmenoxime (CMX), *etc.*, have been reported to possess good antibacterial activity.

Attempts to create a cephalosporin which has both high antibacterial activity and immunostimulatory activity have stimulated the synthesis of cephalosporins which have a (*Z*)-2-(methoxyimino)-2-(2-amino-1,3-thiazol-4-yl)acetamido group at the 7-position and a 2-imidazolylthiomethyl group at the 3-position. In this paper we report the synthesis, antibacterial activity and structure-activity relationships of such cephalosporins.

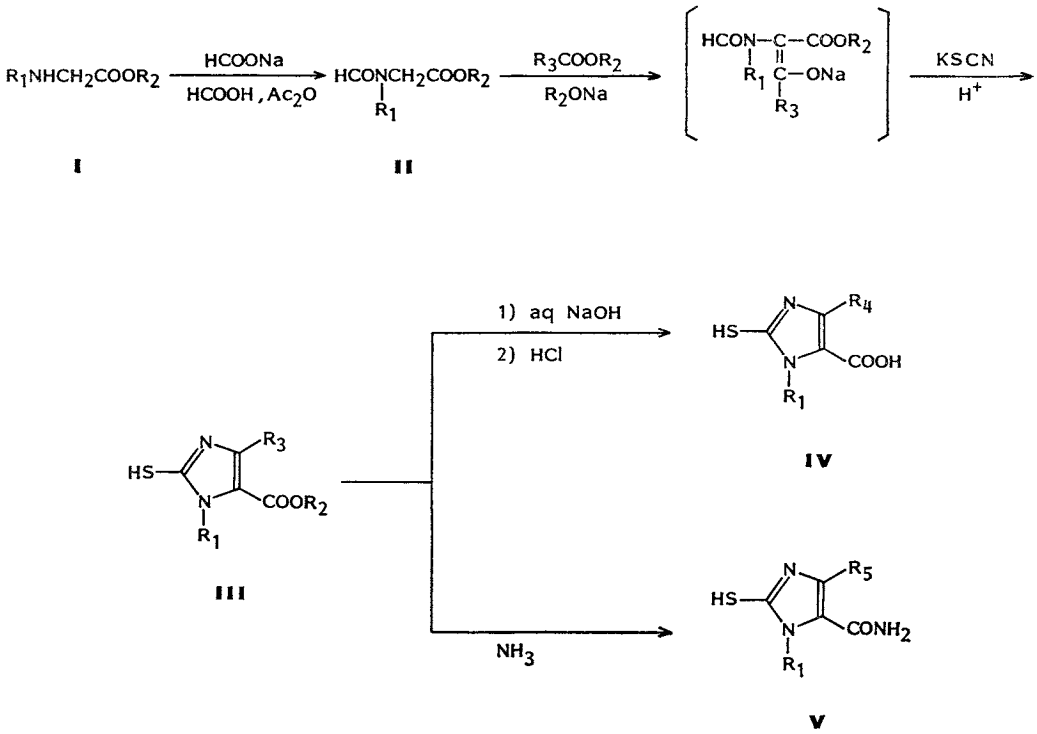
Chemistry

The necessary 3-position side chains, 2-mercaptoimidazoles, were prepared according to the previously reported procedure, as shown in Scheme 1[3~5]. Thus, *N*-alkyl glycine derivatives (**I**) were formylated and cyclized to imidazole compounds (**III**) which were then converted to compounds (**IV** and **V**). The imidazole derivatives (**III**~**V**) were then allowed to react with CTX in the usual manner to give the desired compounds **VI** (Scheme 2). The prepared cephalosporins are presented in Table 1.

Antimicrobial Activity

Table 2 shows the comparative *in vitro* antimicrobial potencies of compounds in this series. The data show that some compounds in this series have high activity against various kinds of Gram-negative bacteria. Superior activity against glucose non-fermenting Gram-negative rods is seen including

Scheme 1.



R_1=H, CH_3, CH_2COOEt, CH_2COOH, CH_2Ph, Ph
R_2=CH_3, Et
R_3=H, $COOCH_3$, COOEt
R_4=H, COOH
R_5=H, $CONH_2$

Scheme 2.

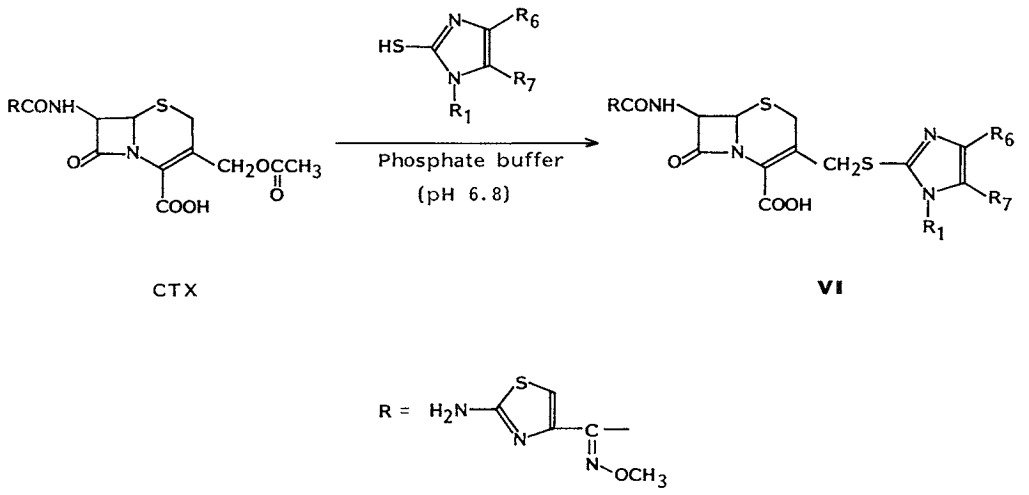
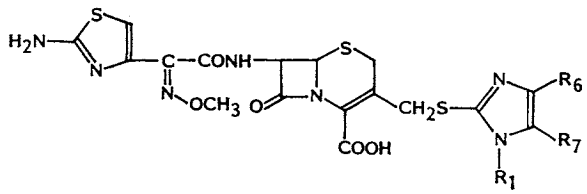


Table 1. Structures of 3-(2-imidazolyl)thiomethyl cephalosporins.



Compound	R_1	R_6	R_7	log k′
1	H	COOH	COOH	−0.359
2	CH_3	COOH	COOH	0.002
3	H	H	H	−0.152
4	H	H	COOH	−0.450
5	H	COOEt	COOEt	1.027
6	CH_2COOH	COOH	COOH	−0.757
7	CH_2Ph	COOH	COOH	0.917
8	Ph	COOH	COOH	0.743
9	H	$CONH_2$	$CONH_2$	0.099

Pseudomonas aeruginosa when compared to CTX and CMX. In addition, the data show the following relationships between structure and activity.

1: Compounds **3** and **4**, which have fewer carboxyl groups than compound **1**, showed parallel or slightly poorer activity compared with compound **1**. They showed however a significant decrease in potency against *P. aeruginosa*.

2: As shown by data for compound **2**, *N*-methylation of the imidazole ring resulted in parallel or slightly poorer activity against both Gram-positive and Gram-negative bacteria. Furthermore, the introduction of a carboxymethyl group, as seen in compound **6**, dramatically decreased the anti-staphylococcal activity, although the compound displayed equivalent antipseudomonal activity. On the other hand, compounds having bulky substituents at the N-atom of the imidazole ring (**7** and **8**) showed a significant decrease in activity against almost all species.

3: The data for compound **5** showed that esterification of the carboxyl groups on the imidazole ring to the corresponding ethyl esters drastically reduced the activity against Gram-negative bacteria, but the compounds exhibited parallel activity against *Staphylococcus aureus*. On the other hand, the change of carboxyl groups to carboxamido groups, as seen in compound **9**, resulted in a decreased activity against *P. aeruginosa* but an increased activity against *S. aureus*.

Summarizing the results obtained, compound **1** showed the best balanced spectrum of activity among all the compounds of this series. The structure-activity relationships described above might be explained by different lipophilicity of the compounds, as described below.

Relationships between Lipophilicity and Activity

It is known that lipophilicity is one of the most important characteristics in determining the structure-activity relationships of cephalosporins. Among some lipophilic indexes, log k′ values obtained from retention times on reversed-phase HPLC are known to be reliable and useful[6~9]. Therefore we estimated the lipophilicity of the compounds described in this paper from log k′ values[10~13], which were calculated from formula (1):

$$\log k' = \log [t_r/t_0 - 1] \quad \cdots\cdots (1)$$

Table 2. Comparative *in vitro* activity (MIC, μg/ml) of the cephalosporins in Table 1.

Organisms	1	2	3	4	5	6	7	8	9	CTX	CMX
Staphylococcus aureus 209P JC-1	3.13	6.25	3.13	25	3.13	100	6.25	12.5	0.78	0.78	0.78
Escherichia coli TG 25	0.78	1.56	0.20	1.56	12.5	25	50	25	0.78	0.39	0.20
Klebsiella pneumoniae GN69	<0.10	<0.10	<0.10	<0.10	1.56	0.39	1.56	0.78	0.20	<0.10	<0.10
Enterobacter cloacae TH 35	0.20	0.39	0.39	0.39	6.25	3.13	6.25	1.56	0.78	0.20	0.20
Proteus vulgaris GN4412	3.13	1.56	3.13	12.5	25	0.78	12.5	12.5	6.25	6.25	3.13
P. mirabilis TG 7	1.56	0.39	6.25	6.25	50	1.56	1.56	6.25	6.25	6.25	0.78
Serratia marcescens GN629	0.39	0.39	0.78	0.78	12.5	0.78	6.25	3.13	1.56	0.39	0.20
Pseudomonas aeruginosa IFO 3445	1.56	6.25	25	25	>100	3.13	12.5	100	25	3.13	6.25
P. aeruginosa TH 10	0.78	3.13	25	3.13	100	3.13	6.25	25	25	3.13	6.25
P. cepacia GN8972	0.78	3.13	1.56	3.13	12.5	6.25	3.13	3.13	—	1.56	3.13
Alcaligenes faecalis TG 2	<0.10	0.20	3.13	0.39	25	0.78	3.13	6.25	0.78	0.39	1.56

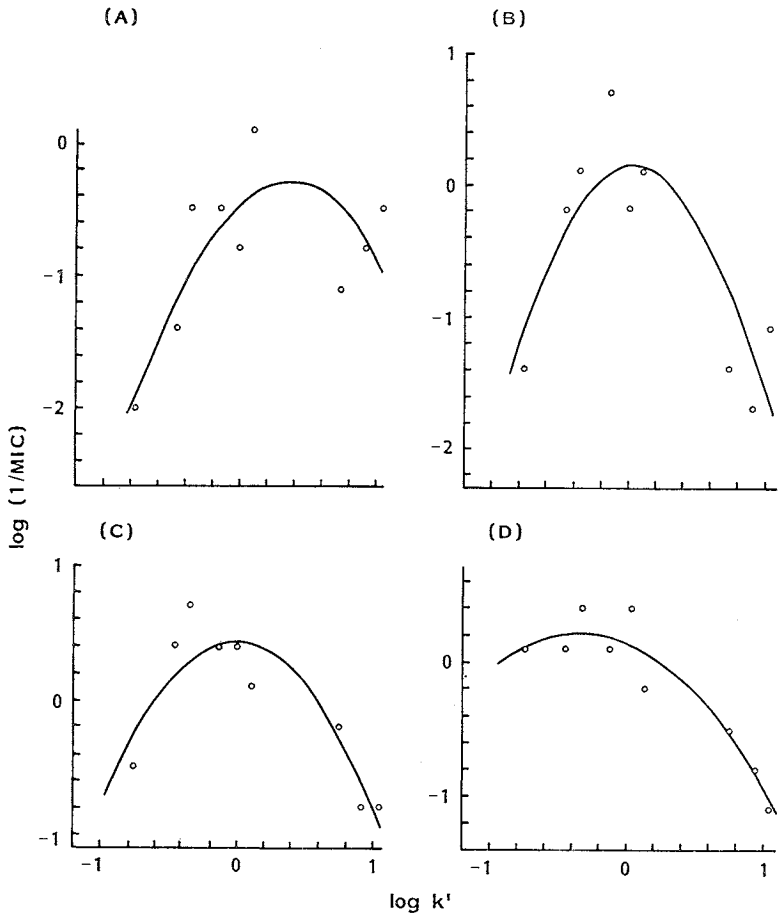
CTX, Cefotaxime; CMX, cefmenoxime.

Table 3. Regression analysis of the cephalosporins on *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter cloacae* and *Serratia marcescens*.

Organisms	Equation	r[a]	SD
S. aureus 209P JC-1	$\log(1/MIC) = -1.35(\log k')^2 + 0.914 \log k' - 0.430$	0.777	0.440
E. coli TG 25	$\log(1/MIC) = -1.96(\log k')^2 + 0.127 \log k' + 0.159$	0.852	0.512
E. cloacae TH 35	$\log(1/MIC) = -1.27(\log k')^2 - 0.043 \log k' + 0.454$	0.915	0.262
S. marcescens GN629	$\log(1/MIC) = -0.667(\log k')^2 - 0.462 \log k' + 0.146$	0.951	0.190

[a] r: Multiple correlation coefficient.

Fig. 1. The relationship between log k′ value of 3-(2-imidazolyl)thiomethyl cephalosporins and their activity against *Staphylococcus aureus* 209P JC-1 (A), *Escherichia coli* TG 25 (B), *Enterobacter cloacae* TH 35 (C) and *Serratia marcescens* GN629 (D).



where t_r is the retention time of the compound, t_0 is the retention time of mobile phase and k′ is called the capacity factor. The log k′ values obtained are shown in Table 1.

The equations were calculated from log k′ and 1/MIC by means of multiple regression analysis and are reported in Table 3. A parabolic relationship between lipophilic character and biological activity against *S. aureus*, *Escherichia coli*, *Enterobacter cloacae* and *Serratia marcescens* is seen, as illustrated in Fig. 1. These also show that the optimum log k′ value against *S. aureus* is higher than

that against Gram-negative bacteria. This agrees with the results of BIAGI *et al.*[6] and YOSHIMOTO and WATANABE[14]. We found that compound **6** was too hydrophilic and compounds **5**, **7** and **8** are too lipophilic. On the other hand compounds **1**~**4** and **9**, especially compound **1**, have suitable lipophilicity, resulting in well balanced activity of compound **1** against both Gram-positive and Gram-negative bacteria.

Experimental

IR spectra were measured on a Shimadzu IR-430 or Digilab STS-15E spectrometer. NMR spectra were recorded on a Varian EM-390 (90 MHz) or Jeol GX-400 (400 MHz) using TMS, sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 (TSP) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard. Mass spectra were recorded on a Jeol DX-300.

Side Chain Synthesis

Side chain, 2-mercaptoimidazoles, were prepared according to the reported methods[3~5]. Examples illustrating the preparation of new compounds will hereinafter be described.

Diethyl *N*-Formyliminodiacetate (**II**, R_1=CH_2COOEt, R_2=Et)

Formic acid (33 g, 0.72 mol) was added dropwise to diethyl iminodiacetate (1.89 g, 0.1 mol). To this solution, acetic anhydride (30 g, 0.3 mol) was added dropwise, and the mixture was gradually warmed to 80°C and stirred for 1.5 hours. After concentration, the residue was chromatographed by using silica gel (EtOAc - hexane, 1 : 1) to give diethyl *N*-formyliminodiacetate (18.5 g, 85.0%) as an oil: IR (Nujol) cm^{-1} 1720, 1680; ^1H NMR (D_2O) δ 1.30 (6H, t), 4.15 (8H, m), 8.10 (1H, s); electron impact (EI)-MS (high resolution) calcd for $C_9H_{15}NO_5$: 217.09512, found: 217.09341.

Diethyl 1-Ethoxycarbonylmethyl-2-mercaptoimidazole-4,5-dicarboxylate (**III**, R_1=CH_2COOEt, R_2=Et, R_3=COOEt)

To a suspension of sodium ethoxide (purity 95%, 2.15 g, 0.03 mol) in dry diethyl ether (20 ml), diethyl oxalate (4.4 g, 0.03 mol) was added dropwise. After 20 minutes, diethyl *N*-formyliminodiacetate (6.5 g, 0.03 mol) was added to the mixture. After being stirred for 2.5 hours, the mixture was allowed to stand for 3 days. To this mixture ice water was added, and the ether layer was separated. The aqueous layer was cooled in an ice bath, and potassium thiocyanate (4.07 g, 0.042 mol) and 12 N HCl (6 ml) were added. After dissolving the mixture, ether was evaporated. The remaining aqueous solution was stirred at 50~60°C for 7 hours and let stand overnight in a refrigerator. An insoluble precipitate was collected by filtration, washed with 1 N HCl and water, and dried under reduced pressure. It was suspended in ether and EtOAc (2 : 1) and the insoluble material was removed by filtration. The filtrate was concentrated to give diethyl 1-ethoxycarbonylmethyl-2-mercaptoimidazole-4,5-dicarboxylate (1.2 g, 12.1%) as yellowish powder: MP 103~104°C; IR (Nujol) cm^{-1} 1760, 1730, 1710; ^1H NMR (DMSO-d_6) δ 1.25 (9H, m), 4.20 (6H, m), 5.08 (2H, s); field desorption (FD)-MS *m/z* 330 (M: $C_{13}H_{18}N_2O_6S$).

Anal Calcd for $C_{13}H_{18}N_2O_6S$: C 47.27, H 5.49, N 8.48.
Found: C 47.04, H 5.74, N 8.18.

1-Carboxymethyl-2-mercaptoimidazole-4,5-dicarboxylic Acid (**IV**, R_1=CH_2COOH, R_4=COOH)

A solution of diethyl 1-ethoxycarbonylmethyl-2-mercaptoimidazole-4,5-dicarboxylate (1.05 g, 3.2 mmol) in 6 N NaOH (4.5 ml) was stirred for 1 hour at 80°C. After cooling, the solution was treated with active carbon and then filtered. To the filtrate concentrated HCl (3.5 ml) was added under cooling. The solution was concentrated to 10 ml and cooled to give 0.37 g (47.4%) of 1-carboxymethyl-2-mercaptoimidazole-4,5-dicarboxylic acid as a yellowish powder: MP 254°C (dec); IR (Nujol) 1730 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.18 (2H, s); fast atom bombardment (FAB)-MS *m/z* 247 (M+H, M: $C_7H_6N_2O_6S$), 269 (M+H, M: $C_7H_5N_2O_6SNa$), 291 (M+H, M: $C_7H_4N_2O_6SNa_2$), 313 (M+H, M: $C_7H_3N_2O_6SNa_3$).

Anal Calcd for $C_7H_5N_2O_6SNa \cdot 0.5H_2O$: C 30.33, H 2.18, N 10.11, Na 9.17.
Found: C 30.50, H 2.16, N 10.16, Na 8.8.

2-Mercaptoimidazole-4,5-dicarboxamide (V, R_1=H, R_5=$CONH_2$)

Diethyl 2-mercaptoimidazole-4,5-dicarboxylate (7.0 g, 28.9 mmol) was dissolved in liquid ammonia and was allowed to react for 2 hours at 80~90°C at 44 kg/cm^2 pressure in an autoclave. The reaction mixture was dissolved in ammonia water and evaporated to remove ammonia. After treatment with active carbon, the solution was concentrated. The resulting precipitate was collected by filtration and dried to give 4.0 g (74.4%) of 2-mercaptoimidazole-4,5-dicarboxamide: MP >300°C; IR (KBr) 1690 cm^{-1} ($CONH_2$); FD-MS *m/z* 186 (M: $C_5H_6N_4O_2S$).

Anal Calcd for $C_5H_6N_4O_2S \cdot 0.3H_2O$: C 31.35, H 3.47, N 29.24.
Found: C 31.18, H 3.21, N 28.96.

N-Benzyl-*N*-formylglycine Methyl Ester (II, R_1=benzyl, R_2=CH_3)

To a solution of *N*-benzylglycine methyl ester (21.1 g, 0.118 mol) in formic acid (63 ml), acetic anhydride (42 ml) was added at 0°C. The solution was stirred for 1.5 hours at 80~90°C and concentrated. The residue was chromatographed by using silica gel (EtOAc - hexane, 1:1) to give *N*-benzyl-*N*-formylglycine methyl ester 22.4 g (91.8%) as an oil: IR (neat) cm^{-1} 1740 ($COOCH_3$), 1670 (NCHO); ^{1}H NMR ($CDCl_3$) δ 3.54 (3H, s, OCH_3), 3.79, 3.86 (2H, each s, CH_2CO), 4.42, 4.50 (2H, each s, CH_2Ph), 7.20 (5H, m, phenyl), 8.09, 8.26 (1H, each s, CHO); EI-MS (high resolution) calcd for $C_{11}H_{13}NO_3$: 207.08962, found: 207.09204.

1-Benzyl-2-mercaptoimidazole-4,5-dicarboxylic Acid Dimethyl Ester (III, R_1=benzyl, R_2=CH_3, R_3=$COOCH_3$)

To a suspension of sodium methoxide (10.1 g) in dry ether (105 ml), dimethyl oxalate (22.1 g, 0.187 ml) was added at 0°C with stirring. To this solution *N*-benzyl-*N*-formylglycine methyl ester (31.2 g, 0.151 mol) was added. The mixture was allowed to stand overnight and diluted with water (150 ml). The aqueous layer was separated. Sodium thiocyanate (21.3 g) and 12 N HCl (36 ml) were added to the aqueous layer. After removing the remaining ether by evaporation, the remaining aqueous solution was stirred for 6 hours at 50~60°C and stored overnight in a refrigerator. The resulting precipitate was collected by filtration, washed with a small amount of cold water and dried under vacuum to give 1-benzyl-2-mercaptoimidazole-4,5-dicarboxylic acid dimethyl ester 22.0 g (47.6%): MP 164~165°C; IR (Nujol) cm^{-1} 1730, 1710 ($COOCH_3$); ^{1}H NMR (DMSO-d_6) δ 3.66 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 5.40 (2H, s, CH_2), 7.20 (5H, m, phenyl); FAB-MS *m/z* 307 (M+H, M: $C_{14}H_{14}N_2O_4S$).

1-Benzyl-2-mercaptoimidazole-4,5-dicarboxylic Acid (IV, R_1=benzyl, R_4=COOH)

1-Benzyl-2-mercaptoimidazole-4,5-dicarboxylic acid dimethyl ester (17.5 g, 57.2 mmol) was added to a solution of 6 N NaOH (44.5 ml). The mixture was stirred for 1 hour at 110°C. After the resulting precipitate was filtered off, water (32 ml) and concentrated HCl (25.3 ml) were added to the filtrate. The solution was let stand overnight in a refrigerator. A generated precipitate was collected by filtration, washed with water and dried under vacuum. It was recrystallized from water and dried under vacuum to give 14.6 g (91.8%) of 1-benzyl-2-mercaptoimidazole-4,5-dicarboxylic acid: IR (Nujol) 1710 cm^{-1} (COOH); ^{1}H NMR (DMSO-d_6) δ 5.53 (2H, s, CH_2), 7.09 (5H, m, phenyl); FAB-MS *m/z* 279 (M+H, M: $C_{12}H_{10}N_2O_4S$).

General Procedure for Displacement at the 3-Position with Substituted 2-Mercaptoimidazole (**1~9**)

To a solution of cefotaxime (1 mmol) and substituted 2-mercaptoimidazole compound (1 mmol) in a pH 6.8 phosphate buffer (10 ml), 2 N NaOH was added to adjust the pH to 6.8. The solution was stirred at 60°C for 8 hours. During the reaction, 2 N NaOH was added to adjust the pH to 6.8. The mixture was cooled and chromatographed on Amberlite XAD-2 (200 ml). The elution was carried out with water and aqueous MeOH. The eluates containing the product were combined, concentrated to remove MeOH *in vacuo*, and lyophilized to give compounds **1~9** in 10~60% yield.

Spectral data of compounds **1~9** will hereinafter be described.

7β-[2-(2-Amino-1,3-thiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(4,5-dicarboxyimidazol-2-yl)-thiomethyl]-3-cephem-4-carboxylic Acid (Sodium Salt) (**1**)

IR (Nujol) 1760 cm^{-1} (β-lactam); ^1H NMR (DMSO-d_6) δ 3.62 (2H, m, 2-H_2), 3.80 (3H, s, =$NOCH_3$), 4.70 (2H, m, 3-CH_2), 5.03 (1H, d, 6-H), 5.55 (1H, d, 7-H), 6.70 (1H, s, thiazole), 7.20 (2H, br s, NH_2), 9.50 (1H, br d, CONH); FAB-MS *m/z* 606 (M+H, M: $C_{19}H_{16}N_7NaO_9S_3$), 628 (M+H, M: $C_{19}H_{15}N_7Na_2O_9S_3$), 650 (M+H, M: $C_{19}H_{14}N_7Na_3O_9S_3$).

7β-[2-(2-Amino-1,3-thiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-4,5-dicarboxyimidazol-2-yl)thiomethyl]-3-cephem-4-carboxylic Acid (Sodium Salt) (**2**)

IR (Nujol) 1760 cm^{-1} (β-lactam); ^1H NMR (DMSO-d_6) δ 3.30 (2H, m, 2-H_2), 3.80 (6H, br s, =$NOCH_3$+imidazole N-CH_3), 4.40 (2H, q, 3-CH_2), 4.94 (1H, d, 6-H), 5.52 (1H, m, 7-H), 6.70 (1H, s, thiazole), 7.20 (2H, br s, NH_2), 9.45 (1H, br d, CONH); FAB-MS *m/z* 620 (M+H, M: $C_{20}H_{18}N_7NaO_9S_3$), 642 (M+H, M: $C_{20}H_{17}N_7Na_2O_9S_3$), 664 (M+H, M: $C_{20}H_{16}N_7Na_3O_9S_3$).

7β-[2-(2-Amino-1,3-thiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(imidazol-2-yl)thiomethyl]-3-cephem-4-carboxylic Acid (Sodium Salt) (**3**)

IR (Nujol) 1760 cm^{-1} (β-lactam); ^1H NMR (D_2O) δ 3.63 (2H, br s, 2-H_2), 3.93 (3H, s, =$NOCH_3$), 4.40 (2H, m, 3-CH_2), 5.16 (1H, d, *J*=6 Hz, 6-H), 5.72 (1H, d, *J*=6 Hz, 7-H), 6.90 (1H, s, thiazole), 7.33 (2H, s, imidazole); FAB-MS *m/z* 496 (M+H, M: $C_{17}H_{17}N_7O_5S_3$), 518 (M+H, M: $C_{17}H_{16}N_7NaO_5S_3$).

7β-[2-(2-Amino-1,3-thiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(5-carboxyimidazol-2-yl)-thiomethyl]-3-cephem-4-carboxylic Acid (Sodium Salt) (**4**)

IR (Nujol) 1750 cm^{-1} (β-lactam); ^1H NMR (DMSO-d_6) δ 3.34 (1H, d, *J*=18 Hz, 2-H_2), 3.69 (1H, d, *J*=18 Hz, 2-H_2), 3.87 (1H, d, *J*=14 Hz, 3-CH_2), 3.96 (3H, s, =$NOCH_3$), 4.12 (1H, d, *J*=14 Hz, 3-CH_2), 5.16 (1H, d, *J*=5 Hz, 6-H), 5.74 (1H, d, *J*=5 Hz, 7-H), 7.01 (1H, s, thiazole), 7.50 (1H, s, imidazole); FAB-MS *m/z* 562 (M+H, M: $C_{18}H_{16}N_7NaO_7S_3$), 584 (M+H, M: $C_{18}H_{15}N_7Na_2O_7S_3$), 606 (M+H, M: $C_{18}H_{14}N_7Na_3O_7S_3$).

7β-[2-(2-Amino-1,3-thiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(4,5-diethoxycarbonylimidazol-2-yl)thiomethyl]-3-cephem-4-carboxylic Acid (Sodium Salt) (**5**)

IR (Nujol) 1760 cm^{-1} (β-lactam); ^1H NMR (DMSO-d_6) δ 1.23 (6H, t, CH_3), 3.50 (2H, m, 2-H_2), 3.73 (3H, s, =$NOCH_3$), 4.13 (4H, q, OCH_2), 4.73 (2H, m, 3-CH_2), 4.89 (1H, d, 6-H), 5.50 (1H, m, 7-H), 6.63 (1H, s, thiazole), 7.08 (2H, br s, NH_2), 9.48 (1H, br d, CONH); FAB-MS *m/z* 662 (M+H, M: $C_{23}H_{24}N_7NaO_9S_3$), 684 (M+H, M: $C_{23}H_{23}N_7Na_2O_9S_3$), 706 (M+H, M: $C_{23}H_{22}N_7Na_3O_9S_3$).

7β-[2-(2-Amino-1,3-thiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-carboxymethyl-4,5-dicarboxyimidazol-2-yl)thiomethyl]-3-cephem-4-carboxylic Acid (Sodium Salt) (**6**)

IR (Nujol) 1760 cm^{-1} (β-lactam); ^1H NMR (DMSO-d_6) δ 3.40 (2H, m, 2-H_2), 3.82 (3H, s, =$NOCH_3$), 4.32 (2H, q, 3-CH_2), 4.88 (2H, s, NCH_2COO), 4.97 (1H, d, 6-H), 5.56 (1H, m, 7-H), 6.71 (1H, s, thiazole), 7.20 (2H, br s, NH_2), 9.48 (1H, br d, CONH); FAB-MS *m/z* 686 (M+H, M: $C_{21}H_{17}N_7Na_2O_{11}S_3$), 708 (M+H, M: $C_{21}H_{16}N_7Na_3O_{11}S_3$), 730 (M+H, M: $C_{21}H_{15}N_7Na_4O_{11}S_3$).

7β-[2-(2-Amino-1,3-thiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-benzyl-4,5-dicarboxyimidazol-2-yl)thiomethyl]-3-cephem-4-carboxylic Acid (Sodium Salt) (**7**)

IR (Nujol) 1760 cm^{-1} (β-lactam); ^1H NMR (D_2O) δ 3.20 (2H, m, 2-H_2), 3.94 (3H, s, =$NOCH_3$), 4.03 (2H, m, 3-CH_2), 4.94 (1H, d, 6-H), 5.60 (1H, m, 7-H), 5.63 (2H, s, benzyl), 6.89 (1H, s, thiazole), 7.12 (5H, m, phenyl); FAB-MS *m/z* 696 (M+H, M: $C_{26}H_{22}N_7NaO_9S_3$), 718 (M+H, M: $C_{26}H_{21}N_7Na_2O_9S_3$), 740 (M+H, M: $C_{26}H_{20}N_7Na_3O_9S_3$).

7β-[2-(2-Amino-1,3-thiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-phenyl-4,5-dicarboxyimidazol-2-yl)thiomethyl]-3-cephem-4-carboxylic Acid (Sodium Salt) (**8**)

IR (Nujol) 1760 cm^{-1} (β-lactam); ^1H NMR (DMSO-d_6) δ 3.26 (2H, m, 2-H_2), 4.59 (3H, m, 3-CH_2 and 6-H), 5.53 (1H, m, 7-H), 6.72 (1H, s, thiazole), 7.50 (5H, m, phenyl); FAB-MS *m/z* 660 (M+H, M: $C_{25}H_{21}N_7O_9S_3$), 682 (M+H, M: $C_{25}H_{20}N_7NaO_9S_3$).

7β-[2-(2-Amino-1,3-thiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(4,5-diaminocarbonylimidazol-2-yl)thiomethyl]-3-cephem-4-carboxylic Acid (Sodium Salt) (**9**)

IR (Nujol) 1760 cm^{-1} (β-lactam); ^1H NMR (DMSO-d_6) δ 3.76 (3H, s, $=NOCH_3$), 4.61 (2H, m, 3-CH_2), 4.96 (1H, br d, 6-H), 5.53 (1H, m, 7-H), 6.66 (1H, s, thiazole), 7.13 (4H, br s, $CONH_2$), 9.43 (1H, br d, CONH); FAB-MS *m/z* 582 (M: $C_{19}H_{18}N_9O_7S_3$), 604 (M+H, M: $C_{19}H_{18}N_9NaO_7S_3$), 626 (M+H, M: $C_{19}H_{17}N_9Na_2O_7S_3$).

Reversed-phase HPLC

A Model 635 Hitachi liquid chromatograph was used in this study. The chromatographic column (4.6 i.d.×150 mm) used was constructed of stainless steel and packed with Unisil Q C 18 (ODS; Gasukuro Kogyo Inc., Japan). Warm water was run through the jacket to maintain the column temperature at 40°C.

The mobile phase used was pH 5.5 phosphate buffer - MeOH (7:3) containing sodium chloride (0.4 mol/liter). All the samples were dissolved in the mobile phase to give about a 0.05~0.1% solution and 20 μl of this was injected into the HPLC column by means of a Rheodyne 7125 Syringe Loading Sample Injector, and solutes were detected by UV (260 nm). The flow rate of the mobile phase was set at 0.5 ml/minute. Capacity factor values were evaluated from the formula (**1**).

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References

1) YASUDA, N.; H. IWAGAMI, E. NAKANISHI, T. NAKAMIYA, Y. SASAKI & T. MURATA: Synthesis and antibacterial activity of 6- and 7-[2-(5-carboxyimidazole-4-carboxamido)phenylacetamido]penicillins and cephalosporins. J. Antibiotics 36: 242~249, 1983
2) KAMEOKA, Y.; S. MUTSUURA, S. YOSHIMOTO, M. TAMURA & E. TSUBURA: Studies on the enhancement of antimicrobial defence activities of AC-1370 and its derivatives. Chemotherapy (Tokyo) 32 (Suppl. 9): 103~107, 1984
3) JONES, R. G.: Studies on imidazoles. II. The synthesis of 5-imidazolecarboxylates from glycine and substituted glycine esters. J. Am. Chem. Soc. 71: 644~647, 1949
4) JONES, R. G.: Studies on imidazoles. V. The S-alkylation of 2-mercaptoimidazoles with esters. J. Am. Chem. Soc. 74: 1084~1085, 1952
5) FUGGER, J.; J. M. TIEN & I. M. HUNSBERGER: The preparation of substituted hydrazines. I. Alkylhydrazines *via* alkylsydnones. J. Am. Chem. Soc. 77: 1843~1848, 1955
6) BIAGI, G. L.; M. C. GUERRA, A. M. BARBARO & M. F. GAMBA: Influence of lipophilic character on the antibacterial activity of cephalosporins and penicillins. J. Med. Chem. 13: 511~516, 1970
7) YAMANA, T.; A. TSUJI, E. MIYAMOTO & O. KUBO: Novel method for determination of partition coefficients of penicillins and cephalosporins by high-pressure liquid chromatography. J. Pharm. Sci. 66: 747~749, 1977
8) KALISZAN, R.: Chromatography in studies of quantitative structure-activity relationships. J. Chromatogr. 220: 71~83, 1981
9) OKUMURA, T.: Application of thin-layer chromatography to reversed-phase liquid chromatographic separation of cephalosporin compounds. Bunseki Kagaku 26: 848~855, 1977
10) KARGER, B. L.: The relationship of theory to practice in high-speed liquid chromatography. *In* Modern Practice of Liquid Chromatography. *Ed.*, J. J. KIRKLAND, pp. 3~51, Wiley-Interscience, New York, 1971
11) NAKAMURA, K.; Y. MORIKAWA & I. MATSUMOTO: Studies on high speed liquid chromatography in cosmetic analysis. VII. Yukagaku 29 (Japan): 501~505, 1980
12) NAKAMURA, K.; Y. MORIKAWA & I. MATSUMOTO: Rapid analysis of ionic and nonionic surfactant homologs by high performance liquid chromatography. J. Am. Oil Chem. Soc. 58: 72~77, 1981
13) WANG, P.-H. & E. J. LIEN: Effects of different buffer species on partition coefficients of drugs used in quantitative structure-activity relationships. J. Pharm. Sci. 69: 662~668, 1980
14) YOSHIMOTO, M. & H. WATANABE: Quantitative structure-activity relationships of β-lactam antibiotics. Kagaku no Ryouiki, Zoukan (Japan) 136: 151~176, 1982