STUDIES ON β-LACTAM ANTIBIOTICS. VII†

EFFECT ON ANTIBACTERIAL ACTIVITY OF THE OXIME *O*-SUBSTITUENTS WITH VARIOUS FUNCTIONAL GROUPS IN THE 7β-[(*Z*)-2-(2-AMINO-4-THIAZOLYL)-2-OXYIMINOACETAMIDO]CEPHALOSPORINS

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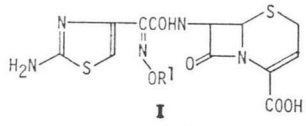
The synthesis and *in vitro* activity of the 7-[*O*-substituted oxyiminoacetamido]cephalosporins (**I**) without substitution at 3-position of a cephem nucleus are described. Effect of changing the oxime *O*-substituents (R^1) with various functional groups in the 7-acyl residue on antibacterial activity was examined. Against Gram-positive bacteria, cephems with hydrophilic functions in the R^1 moiety such as hydroxyethyl, aminoethyl and carboxymethyl groups showed decrease of the activity, while cephems with lipophilic functions such as cyanomethyl, methylthiomethyl and halogenoethyl groups exhibited increase of the activity. However, influence of the substituents (R^1) on activity against Gram-negative bacteria was observed to be relatively independent of the nature of their functional groups.

Recently, extensive studies have been undertaken on a new family of cephalosporins bearing the oxyiminoacetyl side chain at the 7-position of a cephem nucleus. Especially, cephalosporins with a (*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetyl group such as ceftizoxime,[1,2,3] cefotaxime,[4,5] cefmenoxime[6] and ceftriaxone[7] have been reported to possess excellent antibacterial activities.

In our previous papers,[2,8,8~10] we have reported the syntheses, chemical properties, antimicrobial activities, and structure-activity relationships of new 7-[oxyiminoacetamido]cephalosporins prepared during the course of our studies on ceftizoxime. In a series of our studies, we have reported the structure-activity relationships of 7β-[(*Z*)-2-alkoxyimino-2-(2-amino-4-thiazolyl)acetamido]-3-cephem-4-carboxylic acids[2,8] having a simple alkyl chain such as methyl, ethyl, propyl, butyl and hexyl in the oxime ether group. In our continuing investigation of the effect on the antimicrobial activity of changing the alkyl side chain in the *O*-substituted oxime, further studies on the structure-activity relationships of cephems (**I**) (Fig. 1) having a (*Z*)-2-(2-amino-4-thiazolyl)-2-substituted alkoxyiminoacetyl group at the 7-position of the cephem nucleus were undertaken.

Fig. 1



I

Ceftizoxime $R^1 = CH_3$

This paper describes the synthesis and *in vitro* activity of several kinds of cephems (**I**) having lipophilic functions such as cyanomethyl, methylthiomethyl and chloroethyl, or hydrophilic functions such as hydroxyethyl, aminoethyl and carboxymethyl groups in the oxime ether moiety (R^1).

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Chemistry

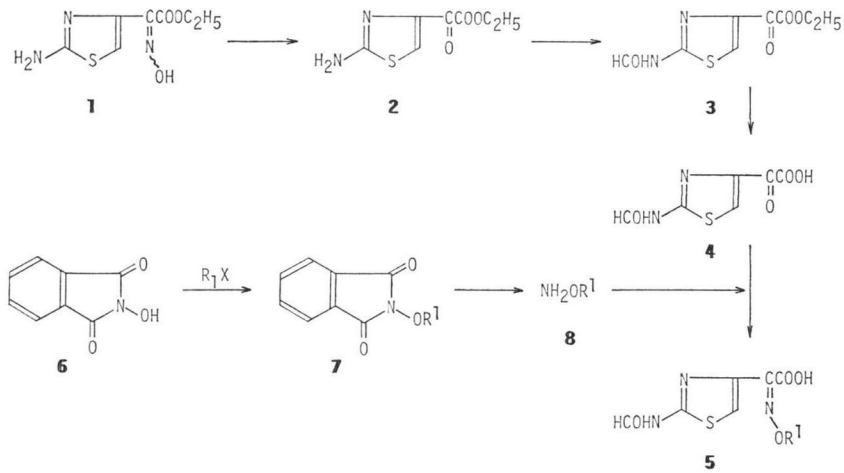
Two general synthetic methods[2,11,12] for the preparation of (*Z*)-2-(2-formamido-4-thiazolyl)-2-substituted alkoxyiminoacetic acids (**5**) are outlined in Schemes 1 (Method A) and 2 (Method B). Method A was generally found to be more applicable for the synthesis of various kinds of the acids (**5**).

Ethyl 2-(2-amino-4-thiazolyl)glyoxalate (**2**), prepared by treatment of ethyl 2-(2-amino-4-thiazolyl)-2-(hydroxyimino)acetate[13] (**1**) with sodium hydrogen sulfite ($NaHSO_3$), was formylated with acetic anhydride (Ac_2O) - formic acid to give ethyl 2-(2-formamido-4-thiazolyl)glyoxalate (**3**) which was hydrolized to the corresponding acid (**4**). The acids (**5a**, **b**, **c**, **e**, **f**, **g**) were obtained by the reaction of **4** with a solution of the alkoxyamines (**8**) which were prepared by treatment of a substituted *N*-alkoxyphthalimide[14,15] (**7**) with 100% hydrazine hydrate.

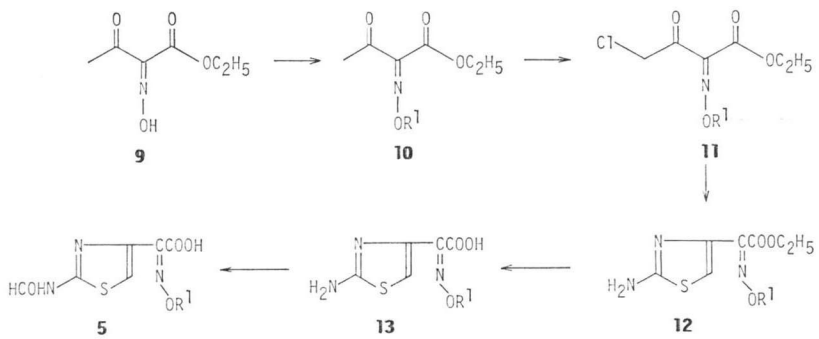
The procedure of Method B was satisfactorily employed only for the preparation of **5d** and **5h**.

Semisynthetic cephalosporins (**16**) were prepared by acylation of 7β-amino-3-cephem-4-carboxylic acid[16] (**14**) with the acids (**5**) followed by subsequent removal of the formyl group, as outlined in Scheme 3. Activation of the acid (**5**) with Vilsmeier reagent prepared from phosphoryl chloride ($POCl_3$) and dimethylformamide (DMF) was satisfactorily employed for the above acylation. The acylation of **14** was carried out in excellent yields (>90%) under non-aqueous conditions by trimethylsilylation using *N*-(trimethylsilyl)acetamide (MSA). Deprotection of the *N*-formamidocephem

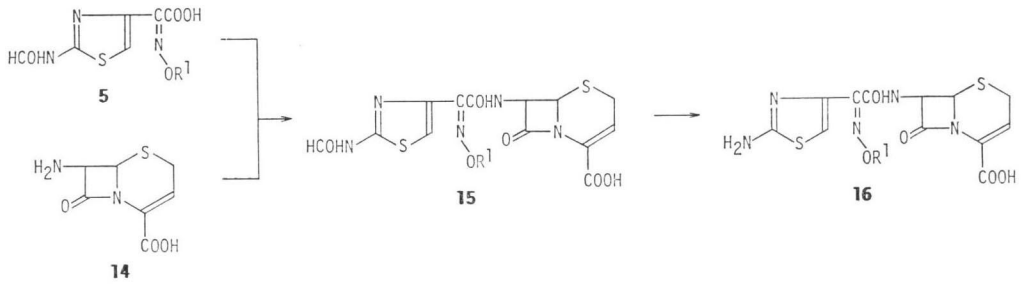
Scheme 1. Scheme for preparation of **5a**, **5b**, **5c**, **5e**, **5f** and **5g** (Method A).



Scheme 2. Scheme for preparation of **5d** and **5h** (Method B).



Scheme 3. Scheme for preparation of **16**.



(**15**) proceeded smoothly at room temperature in a methanolic solution containing conc. hydrochloric acid to give **16** in good yields (70~90%). Both *N*-formyl and *N-tert*-butoxycarbonyl groups in **15g** were simultaneously deprotected under the above mild conditions to give **16j** in 92% yield. However, deprotecting the *tert*-butyl ester group in **15f** necessitated more acidic conditions such as trifluoroacetic acid (for details, see Experimental).

The structures of **15** and **16** were confirmed on the basis of IR and NMR spectral data as shown in Tables 3 and 4.

Microbiology

The minimum inhibitory concentrations (MIC) of the cephems (**16**) against several Gram-positive and Gram-negative bacteria are shown in Table 5. For comparison, the MIC values of ceftizoxime (**I**: $R^1=CH_3$) are listed at the bottom of the Table.

The cephems (**16a**, **16b**, **16d**) with a lipophilic substituent such as methylthiomethyl, 2,2,2-trifluoroethyl, or 2-chloroethyl in the *O*-alkyl group (R^1) exhibited two to four fold higher antibacterial activity against *Staphylococcus aureus* 6 than ceftizoxime. However, the cephems (**16i**, **16j**, **16k**) with a hydrophilic function such as aminoethyl, hydroxyethyl, or carboxymethyl groups were found less active against *Staphylococcus aureus* 6, showing MIC values up to 25 μg/ml. Interestingly, the MIC value of **16e** (formally derived from **16i** by esterification of the carboxylic acid in the 7-acyl group) for the Gram-positive strain fell to 6.25 μg/ml.

On the other hand, all these cephems (**16**) were found to be about two to sixty-four times less active than ceftizoxime against all the Gram-negative bacteria.

The activity of the cephem (**16c**) with a cyanomethyl group was similar to that of ceftizoxime, but was slightly decreased against the Gram-negative bacteria.

Table 1. Substituted *N*-alkoxyphthalimides (**7**).

	R^1	X	Base	Yield	mp
a	CH_2SCH_3	Cl	Et_3N	75.7%	96~ 97°C (EtOAc - Et_2O)
b	CH_2CF_3	OSO_2- 	*t*-BuOK	42.8%	100~102°C (iPE)
c	CH_2CN	Cl	Et_3N	84.1%	152~153°C (EtOAc)
e	$CH_2COOC_2H_5$	Cl	Et_3N	84.1%	95~ 96°C (EtOAc - Et_2O)
f	$CH_2COOC_4H_9{}^t$	Cl	Et_3N	88.9%	145~146°C (EtOAc)

Table 2. (*Z*)-2-(2-Formamido-4-thiazolyl)-2-(alkoxyimino)acetic acids (**5a**~**h**).

	R^1	Method	mp (°C, dec.)	Yield (%)	NMR (DMSO-d_6, δ) HCONH, 1H, br.s	HCO, 1H, s	Ring proton, 1H, s	R^1	IR ν_{max}^{nujol} (cm^{-1})
a	CH_2SCH_3	A	157	70.1	12.73	8.57	7.61	5.31 (2H, s), 2.24 (3H, s)	1700
b	CH_2CF_3	A	141	53.8	12.60	8.57	7.65	4.83 (2H, q, J=8.5 Hz)	1700
c	CH_2CN	A	121	65.4	12.68	8.62	7.73	5.20 (2H, s)	1680
d	CH_2CH_2Cl	B	115	80.2	12.63	8.56	7.60	4.40 (2H, t, J=6 Hz), 3.87 (2H, t, J=6 Hz)	1740, 1690
e	$CH_2COOC_2H_5$	A	152	47.8	12.67	8.54	7.56	4.73 (2H, s), 4.16 (2H, q, J=7 Hz), 1.23 (3H, t, J=7 Hz)	1740, 1670
f	$CH_2COOC_4H_9^t$	A	117	68.5	12.67	8.57	7.56	4.66 (2H, s), 1.46 (9H, s)	1750, 1690
g	$CH_2CH_2NHBoc^t$	A	145	77.3	12.64	8.50	7.33	6.70 (1H, br.s), 3.97 (2H, m), 3.20 (2H, m), 1.37 (9H, s)	1680
h	CH_2CH_2OCOH	B	115	56.3	12.70	8.54	7.58	8.26 (1H, s), 4.38 (4H, m)	1690

Table 3. NMR spectral data of **15a**~**h**.

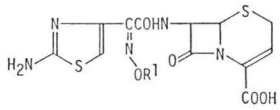
	R^1	NMR (DMSO-d_6, δ) HCONH, 1H, br.s	CONH, 1H, d, J=8 Hz	HCO, 1H, s	Ring, proton, 1H, s	C_3-H 1H	C_7-H 1H, dd, J=5, 8 Hz	C_6-H 1H, d, J=5 Hz	C_2-CH_2 2H	R^1
a	CH_2SCH_3	12.62	9.87	8.53	7.59	6 48 (t, J=4 Hz)	5.88	5.14	3.60 (s)	5.29 (2H, s), 2.21 (3H, s)
b	CH_2CF_3	12.67	9.83	8.57	7.52	6.53 (t, J=4 Hz)	5.92	5.17	3.67 (s)	4.78 (2H, q, J=8.5 Hz)
c	CH_2CN	12.67	9.91	8.56	7.56	6.52 (br.s)	5.87	5.15	3.62 (s)	5.08 (2H, s)
d	CH_2CH_2Cl	12.72	9.68	8.53	7.50	6.52 (t, J=4 Hz)	5.90	5.16	3.62 (br.s)	4.37 (2H, t, J=6 Hz), 3.86 (2H, t, J=6 Hz)
e	$CH_2COOC_2H_5$	12.58	9.62	8.50	7.43	6.48 (s)	5.87	5.13	3.61 (br.s)	4.73 (2H, s), 4.15 (2H, q, J=7 Hz), 1.23 (3H, t, J=7 Hz)
f	$CH_2COOC_4H_9^t$	12.62	9.57	8.50	7.42	6.48 (br.s)	5.87	5.12	3.63 (br.s)	4.62 (2H, s), 1.44 (9H, s)
g	$CH_2CH_2NHBoc^t$	12.63	9.50	8.50	7.37	6.45 (t, J=4 Hz)	5.83	5.10	3.57 (br.s)	4.07 (2H, m), 3.22 (2H, m), 1.33 (9H, s)
h	CH_2CH_2OCOH	12.60	9.66	8.50	7.58	6.48 (br.s)	5.86	5.12	3.60 (br.s)	4.36 (4H, s), 7.43 (1H, s), 8.22 (1H, s)

Table 4. NMR and IR spectral data of **16a~k.**

	NMR (DMSO-d_6, δ)								IR ν_{max}^{Nujol} (cm^{-1})	
R^1	CONH, 1H, d, J=8 Hz	NH_2, 2H, br.s	Ring proton, 1H, s	C_3-H, 1H	C_7-H, 1H, dd, J=5, 8 Hz	C_6-H, 1H, d, J=5 Hz	C_2-CH_2, 2H	R^1	β-Lactam	CONH
a CH_2SCH_3	9.63	7.23	6.78	6.48 (t, J=4 Hz)	5.83	5.12	3.59 (d, J=4 Hz)	5.28 (2H, s), 2.20 (3H, s)	1770	1655
b CH_2CF_3	9.80	7.28	6.87	6.52 (d, J=4 Hz)	5.87	5.13	3.60 (br.s)	4.70 (2H, q, J=8.5 Hz)	1780	1660
c CH_2CN	9.79	7.32	6.88	6.48 (br.s)	5.82	5.12	3.60 (br.s)	5.01 (2H, s)	1790	1660
d CH_2CH_2Cl	9.58	7.24	6.78	6.47 (br.s)	5.83	5.10	3.60 (s)	4.30 (2H, t, J=6 Hz), 3.80 (2H, t, J=6 Hz)	1780	1660
e $CH_2COOC_2H_5$	9.43	7.23	6.78	6.47 (br.s)	5.83	5.10	3.59 (br.s)	4.66 (2H, s), 4.17 (2H, q, J= 7 Hz), 1.21 (3H, t, J=7 Hz)	1775	1660
f $CH_2COOC_4H_9{}^t$	9.43	7.23	6.78	6.47 (br.s)	5.82	5.10	3.63 (d, J=4 Hz)	4.57 (2H, s), 1.43 (9H, s)	1785	1660
i CH_2COOH	9.57	7.33	6.82	6.47 (t, J=4 Hz)	5.86	5.13	3.64 (br.s)	4.64 (2H, s)	1775	1670
j $CH_2CH_2NH_2 \cdot HCl$	9.86	8.37*	7.04*	6.52 (t, J=5 Hz)	5.82	5.12	3.65 (br.s)	4.40 (2H, m), 3.33 (2H, m)	1770	1660
k CH_2CH_2OH	9.48	7.22	6.75	6.48 (br.s)	5.83	5.17	3.63 (br.s)	4.10 (2H, m), 3.63 (2H, m)	1775	1665

* HCl salt.

Table 5. Antimicrobial activity of cephalosporins (**16**).



Compounds	R^1	MIC (μg/ml)						
		Staphylococcus aureus 6	*Escherichia coli* 32		*Klebsiella pneumoniae* 20	*Proteus mirabilis* 18	*Proteus vulgaris* 1	*Pseudomonas aeruginosa* NCTC10490
a	CH_2SCH_3	3.13	0.78	0.20	0.05	0.05	0.05	6.25
b	CH_2CF_3	3.13	1.56	0.20	0.39	0.20	0.10	3.13
c	CH_2CN	6.25	0.39	0.20	0.05	$\leqq$0.025	0.05	25
d	CH_2CH_2Cl	1.56	0.39	0.39	0.78	0.20	0.10	12.5
e	$CH_2COOC_2H_5$	6.25	0.39	0.39	0.10	0.05	0.05	50
i	CH_2COOH	25	0.39	0.78	0.20	$\leqq$0.025	0.39	12.5
j	$CH_2CH_2NH_2 \cdot HCl$	$>$100	0.20	0.39	0.20	0.20	3.13	400
k	CH_2CH_2OH	25	0.20	0.20	0.05	0.10	0.05	12.5
	CH_3 (Ceftizoxime)	6.25	0.10	0.10	$\leqq$0.025	$\leqq$0.025	$\leqq$0.025	0.39

[a] Cephalosporinase producer.

Experimental

Melting points were detemined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded at 60 MHz on a JNM-PMX 60 NMR spcetrometer and at 100 MHz on a JEOL-MH 100 NMR spectrometer using Me_4Si as an internal standard. IR spectra were taken on a Hitachi 260–10 spectrophotometer or a Shimadzu IR-420 spectrophotometer.

Preparation of Substituted *N*-Alkoxyphthalimides (**7a, b, c, e, f**)

To a mixture of *N*-hydroxyphthalimide (**6**) (0.1 mol) and Et_3N (or *t*-BuOK) (0.11 mol) in DMF (100~200 ml) was added a substituted alkylhalide (or a substituted alkyltosylate) (0.1 mol) at room temperature and the mixture was stirred at 30~150°C for 1~6 hours. The reaction mixture was poured into H_2O. The precipitate was filtered, washed with H_2O, and dried (P_2O_5) to afford the corresponding substituted *N*-alkoxyphthalimide (**7**). Results of **7** are summarized in Table 1.

Preparation of *tert*-Butoxycarbonylaminoethoxyphthalimide (**7h**)

To a solution of *N*-hydroxyphthalimide (**6**) (3.36 g, 20.6 mmol), *tert*-butoxycarbonylaminoethanol (3.40 g, 21.1 mmol) and triphenylphosphine (5.93 g, 22.6 mmol) in THF (100 ml) was added diethyl azodicarboxylate (3.82 g, 21.9 mmol) at room temperature, and the resulting mixture was stirred at this temperature for 24 hours. The reaction mixture was evaporated *in vacuo* and the residue was subjected on silica gel column chromatography. The column was eluted with $CHCl_3$-EtOAc (13: 10, v/v). The fractions were collected and evaporated *in vacuo* to give the residue, which was recrystallized from EtOAc-diisopropyl ether (iPE) to afford 5.15 g (81.6%) of **7h**; mp 114~115°C.

Preparation of Ethyl 2-(2-Amino-4-thiazolyl)glyoxalate (**2**)

A mixture of ethyl 2-(2-amino-4-thiazolyl)-2-(hydroxyimino)acetate (**1**) (3.7 g, 17.7 mmol) and $NaHSO_3$ (7.6 g) in 50% aqueous EtOH (100 ml) was stirred at 65~70°C for 20 hours. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in H_2O (80 ml). The solution was washed with Et_2O, and the aqueous layer was saturated with NaCl. The resulting solution was extracted with EtOAc and dried ($MgSO_4$). The solvent was evaporated to give the residue which was recrystallized from EtOAc-Et_2O to afford 1.78 g (52.0%) of ethyl 2-(2-amino-4-thiazolyl)glyoxalate (**2**); mp 147~149°C; IR (Nujol) 3230, 3100, 1725, 1665, 1610 cm^{-1}; NMR (DMSO-d_6) δ 1.33 (3H, t, J= 7 Hz), 4.35 (2H, q, J=7 Hz), 7.91 (1H, s).

Preparation of Ethyl 2-(2-Formamido-4-thiazolyl)glyoxalate (**3**)

A mixture of Ac_2O (20.5 g, 0.2 mol) and HCOOH (9.0 g, 0.2 mol) was stirred at 45~50°C for

an hour. To the mixture was added **2** (10 g, 0.05 mol) at 15°C, and the reaction mixture was stirred at room temperature for 2 hours. To the resulting mixture was added iPE (300 ml) under stirring. The precipitate was filtered, washed with iPE, and dried (P_2O_5) to afford 9.0 g (78.9%) of **3**; mp 229°C (AcOH); IR (Nujol) 3140, 1725, 1675 cm^{-1}; NMR (DMSO-d_6) δ 1.34 (3H, t, J=7 Hz), 4.38 (2H, q, J=7 Hz), 8.56 (1H, s), 8.63 (1H, s), 12.36 (1H, br.s).

Preparation of 2-(2-Formamido-4-thiazolyl)glyoxalic Acid (**4**)

To a suspension of **3** (6.0 g, 13 mmol) in MeOH (36 ml) was dropwise added NaOH aqueous solution (36 ml, 33 mmol) at 20~30°C, and the mixture was stirred at this temperature for 1.5 hours. The reaction mixture was dissolved in ice-water (260 ml) and the solution acidified to pH 1.5 with 17.5% HCl. The acidified solution was stirred under ice-cooling for an hour. The precipitate was filtered, washed with H_2O and MeOH, and dried (P_2O_5) to give 4.7 g (90.5%) of **4**; mp 300°C (MeOH-H_2O); IR (Nujol) 3130, 1730, 1690, 1640 cm^{-1}; NMR (DMSO-d_6) δ 8.28 (1H, s), 8.56 (1H, s), 12.70 (1H, m).

General Preparation of (*Z*)-2-(2-Formamido-4-thiazolyl)-2-(substituted alkoxyimino)acetic Acid (**5**)

Method A:

To a solution of substituted *N*-alkoxyphthalimide (**7**) (0.12 mol) in THF (90~120 ml) was added a solution of 100% hydrazine hydrate (0.12 mol) in MeOH (15~20 ml) at room temperature during 1~2 hours. To the above mixture was dropwise added 15% HCl (0.12 mol) under ice-cooling, and the resulting mixture was stirred at this temperature for 20~30 minutes. The insoluble material was filtered off, and the filtrate was adjusted to pH 7.0 with 10% NaOH solution. To the neutral solution was added **4** (0.1 mol), and the mixture was stirred at room temperature for 1~3 hours while keeping the pH value between 4.5~5.5 with saturated $NaHCO_3$ solution. The above mixture was adjusted to pH 7.0 with 10% NaOH solution and then washed with EtOAc. The aqueous solution was acidified to pH 1.5 with 10% HCl and the acidified solution was extracted with EtOAc. The EtOAc layer was washed with brine and dried ($MgSO_4$). The solvent was evaporated *in vacuo* to give the residue which was recrystallized from EtOAc-Et_2O to afford **5**.

Method B:

i) Preparation of Ethyl 2-(Substituted alkoxyimino)-3-oxobutylate (**10**): To a mixture of ethyl 2-hydroxyimino-3-oxobutylate (**9**) (0.4 mol) and K_2CO_3 (0.5 mol) in DMF (200~300 ml) was added substituted alkyl halide (0.4 mol) at room temperature and the mixture was stirred at 20~50°C for 3~10 hours. The reaction mixture was filtered. The filtrate was poured into H_2O (1~1.5 liters) and extracted with EtOAc. The EtOAc layer was washed successively with 10% K_2CO_3 solution and brine. The organic layer was dried ($MgSO_4$) and evaporated *in vacuo* to afford oily **10**, which was used in the following reaction without further purification.

ii) Preparation of Ethyl 2-(Substituted alkoxyimino)-3-oxo-4-chlorobutylate (**11**): To a solution of **10** (0.4 mol) in AcOH (90~120 ml) was added SO_2Cl_2 (0.4 mol) at room temperature, and the mixture was stirred at 25~40°C for 5~10 hours. The reaction mixture was poured into H_2O (500 ml) and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated $NaHCO_3$ solution and brine, and then dried ($MgSO_4$). The solvent was evaporated *in vacuo* to afford oily **11**.

iii) Preparation of Ethyl (*Z*)-2-(2-Amino-4-thiazolyl)-2-(substituted alkoxyimino)acetate (**12**): To a suspension of thiourea (0.3 mol) and NaOAc (0.3 mol) in 50% aqueous EtOH (400~600 ml) was added **11** (0.1 mol), and the mixture was stirred at 40~45°C for 2~5 hours. The reaction mixture was adjusted to pH 6.5~7.0 with 20% Na_2CO_3 solution under ice-cooling, and the resulting mixture was stirred at this temperature for 30 minutes. The precipitate was collected by filtration, washed with H_2O and iPE, and dried (P_2O_5) to afford **12**.

iv) Preparation of (*Z*)-2-(2-Amino-4-thiazolyl)-2-(substituted alkoxyimino)acetic Acid (**13**): To a solution of **12** (0.1 mol) in MeOH (80~100 ml) and THF (30~50 ml) was added 1 N NaOH solution (0.2~0.3 mol) at room temperature, and the mixture was stirred at 25~40°C for 2~5 hours. The resulting solution was evaporated *in vacuo* and the residue was dissolved in H_2O. The aqueous solution was adjusted to pH 3~3.3 with 10% HCl under ice-cooling, and the resulting mixture was

stirred at this temperature for 30 minutes. The precipitate was collected by filtration, washed with H_2O, and dried (P_2O_5) to afford **13**.

v) Preparation of (*Z*)-2-(2-Formamido-4-thiazolyl)-2-(substituted alkoxyimino)acetic Acid (**5**): A mixture of Ac_2O (0.4 mol) and HCOOH (0.4 mol) was stirred at 45~50°C for an hour. To the mixture was added **13** (0.1 mol) at 15°C, and the reaction mixture was stirred at room temperature for 2~4 hours. To the above mixture was added *n*-hexane under stirring. The resulting precipitate was filtered, washed with *n*-hexane, and dried (P_2O_5) to afford **5**. The crude **5** was recrystallized from EtOAc - Et_2O to afford pure **5**.

General Procedure for Acylation of 7β-Amino-3-cephem-4-carboxylic Acid (**14**)

To a solution of DMF (11.1 mmol) in THF (20~30 ml) was dropwise added $POCl_3$ (11.1 mmol) at −10~0°C under stirring, and the mixture was stirred at this temperature for 20~30 minutes to prepare Vilsmeier reagent. To the above mixture was added *N*-formyl acid (**5**) (10 mmol) under ice-cooling, and the reaction mixture was stirred at this temperature for 30 minutes to produce an activated acid solution of **5**. To a solution of 7β-amino-3-cephem-4-carboxylic acid (**14**) (10 mmol) and MSA (60 mmol) in EtOAc (30 ml) was added the above activated acid solution at −20°C, and the reaction mixture was stirred at −20~0°C for an hour. To the reaction mixture was added a mixture of EtOAc and H_2O, and the EtOAc layer was separated. After H_2O was added to the EtOAc layer, the mixture was adjusted to pH 7.5 with saturated $NaHCO_3$ solution. The separated aqueous layer was acidified to pH 2.0 with 10% HCl, and the acidified solution was extracted with EtOAc. The EtOAc layer was washed with brine and dried ($MgSO_4$). The solvent was evaporated and the residue was triturated with Et_2O to afford a *N*-protected cephalosporin derivative (**15**).

General Procedure for Deformylation of **15**

To a mixture of **15** (8 mmol) in MeOH (30~40 ml) and THF (10~30 ml) was added conc. HCl (24~32 mmol) at room temperature, and the mixture was stirred at this temperature for 2~5 hours. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in saturated $NaHCO_3$ solution. The solution was washed with EtOAc, and the aqueous layer was acidified to pH 2.5~3.0 with 10% HCl under ice-cooling. The resulting precipitate was filtered, washed with cold H_2O, and dried (P_2O_5) to afford **16**.

Preparation of 7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamido]-3-cephem-4-carboxylic Acid (**16i**)

To a mixture of **16f** (4.0 g, 8.27 mmol) and anisole (4 ml) in CH_2Cl_2 (8 ml) was added trifluoroacetic acid (16 ml) under ice-cooling, and the mixture was stirred at room temperature for an hour. The resulting mixture was dropwise added to iPE (150 ml) under stirring to form a precipitate which was collected by filtration. The precipitate was dissolved in aqueous $NaHCO_3$ solution and the solution was washed with EtOAc. The solution was acidified to pH 2.2 with 10% HCl under ice-cooling. The resulting precipitate was filtered and dried (P_2O_5) to give 2.4 g (68.0%) of **16i**.

Preparation of 7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-(2-aminoethoxyimino)acetamido]-3-cephem-4-carboxylic Acid Dihydrochloride (**16j**)

To a solution of **15g** (0.7 g, 1.29 mmol) in MeOH (7 ml) was added conc. HCl (0.48 g), and the mixture was stirred at room temperature for 4 hours. The reaction mixture was evaporated *in vacuo* and the residue dissolved in MeOH (10 ml). To the methanolic solution was added iPE (20 ml) under stirring. The resulting precipitate was filtered, washed with MeOH - iPE (1 : 1), and dried (P_2O_5) to give 0.58 g (92.3%) of **16j**.

Antibiotic Susceptibility

All the *in vitro* antibacterial activity data are given as the minimum inhibitory concentraion (MIC) in μg/ml required to prevent growth of the bacterial culture. MIC's were determined by the agar dilution method using heart infusion agar (Difco) after incubation at 37°C for 20 hours, the inoculum size being about 10^8 C.F.U./ml. *Escherichia coli* 28 is a cephalosporin-resistant strain.

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