SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF A NEW SERIES OF CEPHALOSPORINS, E1040 AND RELATED COMPOUNDS

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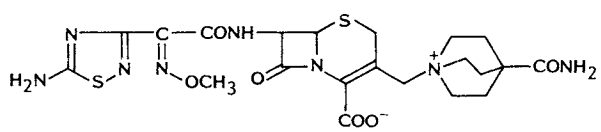
The synthesis and *in vitro* antibacterial activity of a series of 7-[(*Z*)-2-aminoaryl-2-oxyiminoacetamido]-3-ammoniomethyl-3-cephems are described. Variation of an oxyimino moiety with aminoaryl at the C-7 side chain and a quaternary ammonium moiety at the C-3 side chain were examined and structure-activity relationships were studied.

E1040, the 3-(4-carbamoylquinuclidinio)methyl derivative of the 7-α-methoxyimino series of aminothiadiazolyl cephalosporins, exhibited excellent activity against both Gram-positive and Gram-negative bacteria, particularly against *Pseudomonas aeruginosa*, and possessed high stability to β-lactamases.

Many of the recently marketed or currently developing injectable cephalosporins, such as ceftazidime (CAZ)[1~3], cefpirome[4] and cefepime[5~7], have the common structural feature of a quaternary ammoniomethyl group at the C-3 side chain and a 2-aminothiazolylacetamido group at the C-7 side chain with an α-oxyimino substitution. These compounds exhibit a high degree of activity against a broad range of Gram-positive and Gram-negative bacteria. Both CAZ and cefepime, so-called third generation cephalosporins, show a most potent antipseudomonal activity. However, infections caused by strains of *Pseudomonas aeruginosa* have recently been found clinically in compromised patients, so further improvement in activity against this bacteria is desirable.

Our aim was to prepare a new injectable cephalosporin which showed a broad antibacterial spectrum and potent antipseudomonal activity by chemical modification of the side chain. Therefore, with the idea of obtaining a compound possessing more potent antipseudomonal activity than CAZ and cefepime, we anticipated that introduction of a hydrophilic function, such as an aliphatic ammonium group at the C-3 side chain, could enhance activity against *P. aeruginosa*. This would be due to increasing the cell wall penetration, as compared to a pyridinium group. Quinuclidine derivatives were selected for their ease of synthesis and modification, and that the p*K*a[8,9] values of 4-substituted quinuclidines correlated moderately with their electron-withdrawing effect. We expected that the activation of the β-lactam ring due to this electron-withdrawing effect would increase antibacterial activity. We directed our main effort toward the modification of the quinuclidine ring in order to find new cephalosporins with better antipseudomonal activity.

Fig. 1. Structure of cefclidin (E1040, **25**).



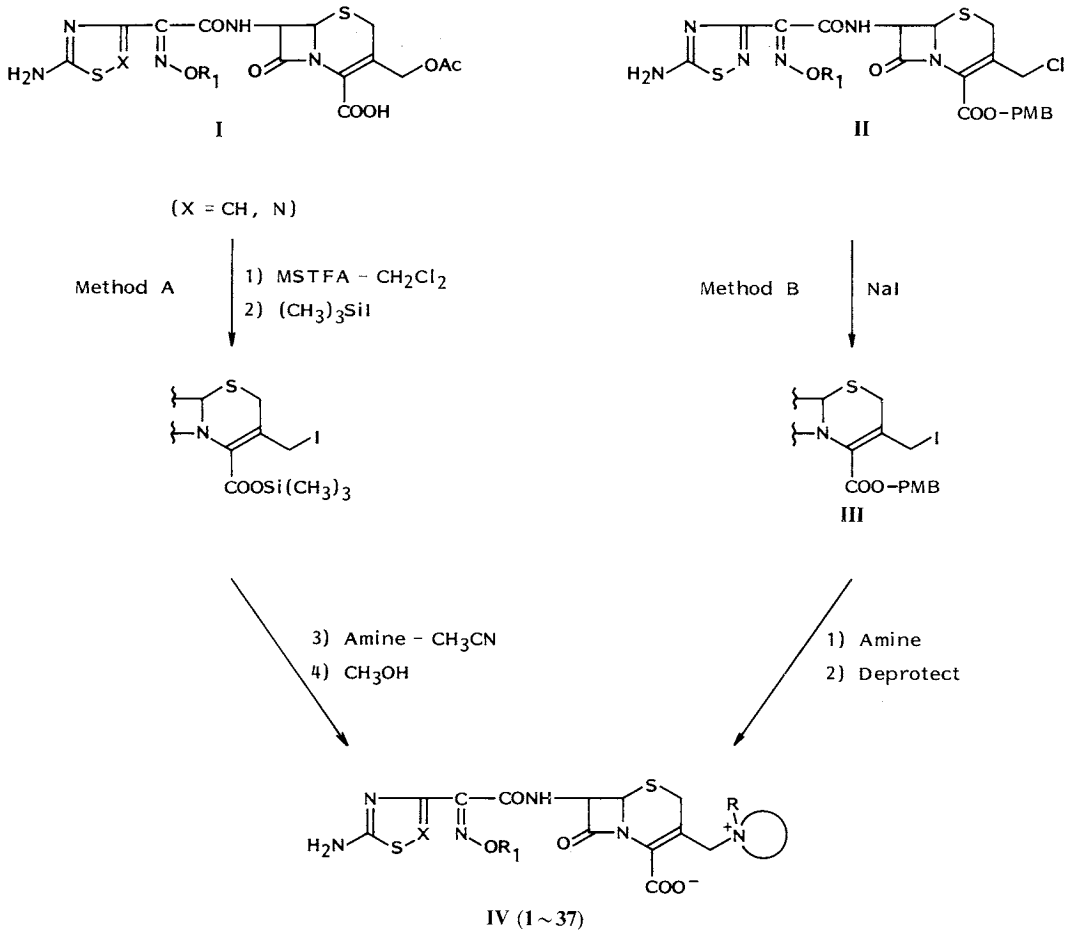
Among them, 7-[(*Z*)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetamido]-3-(4-carbamoylquinuclidinio)methyl-3-cephem-4-carboxylate, designated E1040 (**25**, Fig. 1), was found to be most promising in view of its antimicrobial spectrum and other biological properties. This paper describes the synthesis and the structure-activity relationships of E1040 and its analogs. Detail microbial evaluations of E1040 have been reported in a separate paper[10~12].

Synthesis

Preparation of the 7-α-oxyimino derivatives having a quaternary ammonium group at the 3-side chain was performed according to the procedure in Scheme 1.

In Method A the acetoxy (**I**) was first silylated with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), followed by trimethylsilyl iodide[13,14] replacing the acetoxy, which was then quaternized with tertiary amine. The quaternary product was deblocked and purified with silica gel or ODS column to afford the desired Δ3-isomer of the final products in yields of 1~38%. Formation of considerable amounts

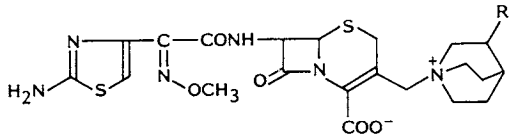
Scheme 1.



R_1 = a; CH_3, b; C_2H_5, c; cyclopropylmethyl, d; $CH(CH_3)_2COO$-*tert*-Bu for **II**, e; CH_2COO-*tert*-Bu for **II**.

PMB: *p*-Methoxybenzyl.

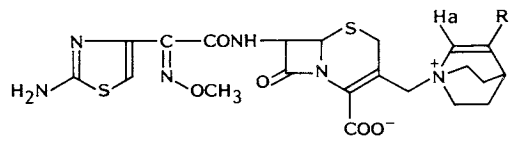
Table 1. Yield, IR and NMR data of 2-aminothiazolyl-α-methoxyimino derivatives.



Compound No.	R	Method of preparation (yield, %)	IR (Nujol) β-Lactam (cm^{-1})	^1H NMR (90 MHz, δ in D_2O, ppm)				
				Thiazole-H (1H, s)	OCH_3 (3H, s)	6-H (1H, d, J=5 Hz)	7-H (1H, d, J=5 Hz)	Quinuclidine and others
1	H	A (15)	1770	6.96	3.91	5.2	5.68	1.6~2.1 (7H, m), 3.1~3.4 (6H, m)
2[a]	OH	A (14)	1770	6.86	3.82	5.19	5.65	1.4~2.2 (5H, m)
3	Cl	A (3)	1760	6.95	3.90	5.05	5.65	1.8~2.4 (5H, m), 2.8~3.8 (6H, m)
4	=O	A (1)	—	7.00	3.95	5.10	5.65	1.2~1.6 (5H, m), 2.8~3.8 (6H, m)
5[a]	$OCONH_2$	A (4)	1760	6.85	3.85	5.05	5.60	1.0~1.6 (5H, m)
6[a]	$N^+H_3Cl^-$	A (6)	1783, 1760 (sh)	6.72	3.80	5.05	5.75	1.1~1.6 (5H, m)
7[a]	OCH_3	A (15)	1770	6.67	3.81	5.15	5.66	1.4~2.4 (5H, m)
8	$=CH_2$	A (15)	1770	7.15	4.12	5.46	6.00	2.2 (4H, m), 2.9 (1H, m), 3.4~4.3 (m)
9	$CH(OH)CH_3$	A (18)	1770	7.10	4.10	5.45	5.95	1.38 (3H, s), 1.8~2.61 (m), 2.9~4.4 (m)

[a] NMR spectrum was recorded in CD_3OD-D_2O.

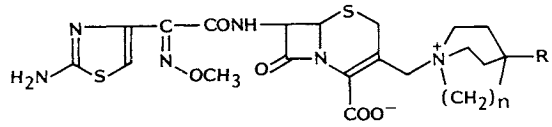
Table 2. Yield, IR and NMR data of 2-aminothiazolyl-α-methoxyimino derivatives.



Compound No.	R	Method of preparation (yield, %)	IR (Nujol) β-Lactam (cm^{-1})	^1H NMR (90 MHz, δ in D_2O, ppm)					
				Thiazole-H (1H, s)	OCH_3 (3H, s)	6-H (1H, d, J=5 Hz)	7-H (1H, d, J=5 Hz)	Ha (1H, s)	Quinuclidine and others
10[a]	H	A (10)	1760	6.95	4.05	5.40	5.94	[b]	1.7~2.3 (4H, m)
11	CH_3	A (5)	1770	7.15	4.12	5.45	5.99	6.50	1.8~2.4 (4H, m), 2.10 (3H, s)
12[a]	$COOCH_3$	A (10)	1770	7.03	4.03	5.40	5.95	7.65	1.8~2.4 (4H, m), 3.92 (3H, s)
13	CH_2OH	A (5)	1770	7.13	4.10	5.45	5.98	6.65	1.8~2.4 (4H, m)
14	$C(CH_3)_2OH$	A (13)	1770	7.13	4.10	5.45	5.98	6.66	1.50 (6H, s), 1.6~2.4 (4H, m)
15	$CONH_2$	A (25)	1770	7.08	4.03	5.42	5.95	7.40	1.8~2.4 (4H, m)

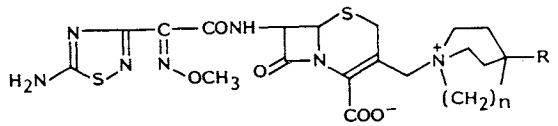
[a] NMR spectrum was recorded in CD_3OD-D_2O.
[b] 6.75~7.20 (2H, m).

Table 3. Yield, IR and NMR data of 2-aminothiazolyl-α-methoxyimino derivatives.



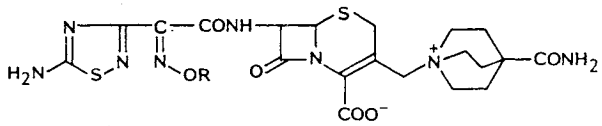
Compound No.	n	R	Method of preparation (yield, %)	IR (Nujol) β-Lactam (cm^{-1})	^1H NMR (90 MHz, δ in D_2O, ppm) Thiazole-H (1H, s)	OCH_3 (3H, s)	6-H (1H, d, J=6 Hz)	7-H (1H, d, J=6 Hz)	Others
16	2	$CONH_2$	A (18)	1770	7.12	4.10	5.45	5.97	2.3 (6H, m), 3.3~4.0 (m)
17	2	CH_2OH	A (2)	1770	7.15	4.12	5.47	5.97	1.96 (6H, m), 3.2~4.0 (m)
18	2	CN	A (5)	1770	7.12	4.08	5.44	5.96	2.52 (6H, m), 3.4~4.4 (m)
19	2	$CO_2C_2H_5$	A (9)	1770	7.10	4.06	5.44	5.96	1.35 (3H, t, J=8 Hz), 2.3 (6H, m), 3.2~4.4 (m)
20	2	OH	A (1)	1765	7.10	4.10	5.44	5.96	2.2 (6H, m), 3.4~4.0 (m)
21	1	OH	A (15)	1765	7.09	4.08	5.43	5.94	2.3 (6H, m), 3.2~4.4 (m)
22	2	SCH_3	B (5)	1770	7.14	4.12	5.48	6.00	2.21 (3H, s), 2.29 (6H, m), 3.2~4.4 (m)
23	1	H	A (4)	1760	7.10	4.10	5.44	5.95	2.3 (4H, m), 3.2~4.4 (m)
24	2	$CONHCH_3$	A (10)	1770	7.10	4.08	5.43	5.96	2.25 (6H, m), 2.83 (3H, s), 3.3~4.2 (m)

Table 4. Yield, IR and NMR data of aminothiadiazolyl-α-methoxyimino derivatives.



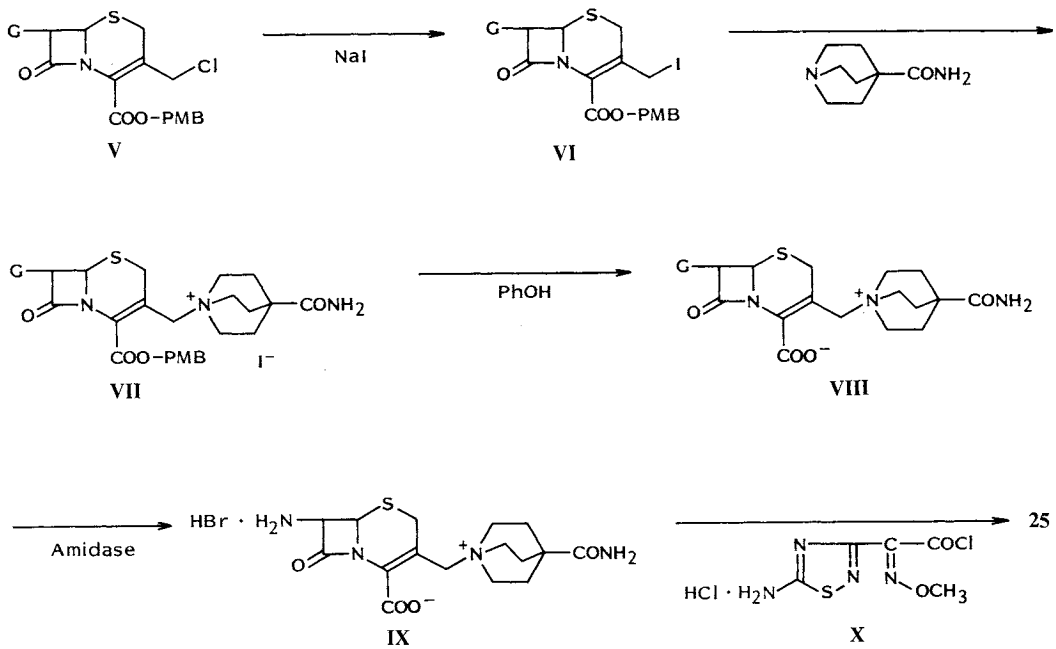
Compound No.	n	R	Method of preparation (yield, %)	IR (Nujol) β-Lactam (cm^{-1})	^1H NMR (90 MHz, δ in D_2O, ppm) OCH_3 (3H, s)	6-H (1H, d, J=6 Hz)	7-H (1H, d, J=6 Hz)	Others
25 (E1040)	2	$CONH_2$	A (37) B (38)	1775	4.16	5.43	5.97	2.30 (6H, m), 3.15~4.00 (m)
26	2	$CSNH_2$	B (4)	1770	4.20	5.47	6.01	2.44 (6H, m), 3.4~4.2 (m)
27	2	CH_2OH	A (1)	1770	4.19	5.44	5.99	1.96 (6H, m), 3.2~4.3 (m)
28	2	CH_2OCH_3	B (8)	1775	4.21	5.49	6.03	2.0 (6H, m), 3.3~4.3 (m)
29	2	OH	A (2)	1765	4.18	5.43	5.97	2.2 (6H, m), 3.3~4.0 (m)
30	1	OH	A (10)	1775	4.18	5.43	5.98	2.32 (4H, m), 3.3~4.0 (m)
31	2	CH_3	B (5)	1765	4.20	5.47	6.01	1.14 (3H, s), 1.94 (6H, m), 3.3~4.2 (m)
32	2	$NHCOCH_3$	B (17)	1765	4.20	5.47	6.01	2.07 (3H, s), 2.44 (6H, m), 3.4~4.3 (m)
33	2	SO_2CH_3	B (13)	1770	4.20	5.48	6.01	2.35 (6H, m), 2.76 (3H, s), 3.2~4.3 (m)

Table 5. Yield, IR and NMR data of alkoxyimino analogs (**34**~**37**).



Compound No.	R	Method of preparation (yield, %)	IR (Nujol) β-Lactam (cm^{-1})	^1H NMR (90 MHz, δ in D_2O, ppm) 6-H (1H, d, J=5~6 Hz)	7-H (1H, d, J=5~6 Hz)	Others
34	C_2H_5	A (17)	1770	5.46	6.00	1.45 (3H, t, J=8 Hz), 2.30 (6H, m), 3.2~4.20 (m), 4.46 (2H, q, J=8 Hz)
35	CH_2-cyclopropyl	B (10)	1770	5.47	6.02	0.3~0.9 (4H, m), 1.37 (1H, m), 2.3 (6H, m), 3.2~4.2 (m), 4.25 (2H, d, J=8 Hz)
36	CH_2COOH	A (6)	1765	5.44	6.00	2.3 (6H, m), 3.2~4.3 (m)
37	$C(CH_3)_2COOH$	A (16)	1770	5.47	6.01	1.65 (6H, s), 2.25 (6H, m), 3.3~4.2 (m)

Scheme 2.



of Δ2-isomer was observed during the quaternization step, lowering the yield of Δ3-isomer. It was necessary for the reaction to remain in an anhydrous condition.

In Method B the key intermediate (**III**)[7,15] was treated with tertiary amine to give the quaternary ammonium derivatives, which were deblocked and purified by ODS (a reversed phase silica gel) column. This way, formation of Δ2-isomer was suppressed when an adequate solvent and temperature were used. These derivatives are shown in Tables 1~5.

Another method shown in Scheme 2 was explored for bulk preparation of **25**. In contrast to the above

Table 6. *In vitro* antibacterial activity (MIC, μg/ml) of 2-aminothiazolyl-α-methoxyimino derivatives (**1**～**24**).

Compound No.	*S.a.* 209P JC-1	*S.a.* JS1	*E.c.* NIHJ JC-2	*K.p.* IID875	*E.cl.* E10005	*E.cl.* GN7471	*S.m.* IID620	*P.v.* E08001	*P.a.* PAO1	*P.a.* E03110
1	3.13	>100	0.10	0.10	0.39	0.39	0.39	0.39	6.25	50
2	1.56	100	0.10	0.05	0.10	0.20	≤0.025	0.20	3.13	3.13
3	25	NT	0.78	0.20	3.13	12.5	3.13	0.78	>100	>100
4	6.25	12.5	0.39	0.20	0.78	3.13	1.56	0.78	25	>100
5	50	100	3.13	0.78	25	>100	6.25	3.13	>100	>100
6	25	>100	3.13	1.56	12.5	>100	12.5	6.25	>100	>100
7	0.78	>100	0.10	0.10	0.20	0.20	≤0.05	0.10	6.25	50
8	0.78	50	0.20	0.10	0.20	0.39	0.05	0.20	6.25	25
9	1.56	50	0.20	0.10	0.20	0.39	0.10	0.20	3.13	25
10	0.78	50	0.05	0.05	0.10	0.20	0.05	0.10	3.13	25
11	0.78	50	0.10	0.10	0.20	0.20	0.05	0.39	3.13	50
12	3.13	>100	0.20	0.20	0.39	1.56	0.10	0.78	12.5	50
13	1.56	>100	0.20	0.10	0.20	0.39	0.05	0.39	3.13	25
14	1.56	>100	0.39	0.20	0.39	0.39	0.10	0.78	6.25	50
15	1.56	100	0.10	0.10	0.10	0.39	<0.025	0.20	3.13	25
16	1.56	>100	0.05	0.05	0.10	0.20	0.05	0.20	1.56	12.5
17	1.56	>100	0.10	0.10	0.10	0.20	0.05	0.20	3.13	25
18	1.56	100	0.10	0.10	0.20	0.20	0.10	0.39	3.13	12.5
19	1.56	50	0.20	0.20	0.39	0.39	0.20	0.39	12.5	50
20	1.56	50	0.10	0.05	0.10	0.10	0.05	0.20	3.13	12.5
21	0.78	>100	0.10	0.10	0.20	0.39	0.10	0.39	3.13	25
22	0.78	25	0.10	0.05	0.10	0.78	0.10	0.20	3.13	12.5
23	0.78	50	0.10	0.05	0.10	0.20	0.05	0.20	3.13	25
24	3.13	>100	0.10	0.05	0.10	0.20	0.05	0.20	3.13	25
Ceftazidime	6.25	>100	0.20	0.20	0.39	12.5	0.20	0.10	1.56	6.25

Abbreviations: *S.a.*, *Staphylococcus aureus*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *E.cl.*, *Enterobacter cloacae*; *S.m.*, *Serratia marcescens*; *P.v.*, *Proteus vulgaris*; *P.a.*, *Pseudomonas aeruginosa*.
NT: Not tested.

described methods, this procedure comprised initial introduction of 3-side chain and subsequent formation of 7-side chain by 7-*N*-acylation. The 3-chloromethyl derivative (**V**)[16] was converted to the 3-iodomethyl derivative (**VI**), and subsequently treated with 4-carbamoylquinuclidine at 0°C. The precipitated quaternary product (**VII**) was deblocked with phenol giving (**VIII**) followed by removal of the side-chain with amidase to yield (**IX**), which was slightly contaminated with its Δ2-isomer. Acylation of derivative (**IX**) with the acyl chloride of the C-7 side chain acid, smoothly proceeded to afford E1040 as crystals in 38% overall yield from derivative (**V**). Modification in the methoxyimino moiety of **25** was also accomplished by Method B using an appropriate 3-iodomethyl (**III**) derivative.

Antimicrobial Activity

The antibacterial activities (MICs) were determined by the 2-fold serial dilution method using Mueller-Hinton agar against cephalosporin-sensitive and cephalosporin-resistant strains of bacteria.

In vitro antibacterial activities of 24 derivatives with the aminothiazolyl-α-methoxyimino group are compared with that of CAZ in Table 6.

Among the compounds having 3-substituted quinuclidine and 3-substituted dehydroquinoclidine groups (**1**～**15**) at the C-3 side chain of the cephem nucleus, **2** and **10** exhibited potent activity against *Staphylococcus aureus* and Gram-negative bacteria, except against *P. aeruginosa*, as compared to CAZ. Compound **10** exhibited higher activity against all organisms in comparison to **1**. It was speculated that

Table 7. *In vitro* antibacterial activity (MIC, μg/ml) of aminothiadiazolyl-α-methoxyimino derivatives (**25**～**33**).

Compound No.	*S.a.* 209P JC-1	*S.a.* JS1	*E.c.* NIHJ JC-2	*K.p.* IID875	*E.cl.* E10005	*E.cl.* GN7471	*S.m.* IID620	*P.v.* E08001	*P.a.* PAO1	*P.a.* E03110
25 (E1040)	3.13	25	0.10	0.10	0.10	0.20	0.10	0.20	0.39	1.56
26	3.13	50	0.10	0.10	0.20	0.78	0.20	0.78	1.56	6.25
27	1.56	50	0.10	0.10	0.20	0.20	0.20	0.39	0.78	6.25
28	1.56	50	0.05	0.10	0.10	0.39	0.10	0.39	1.56	25
29	3.13	50	0.20	0.39	0.39	0.39	0.39	0.78	1.56	6.25
30	0.78	50	0.10	0.10	0.20	0.39	0.20	0.78	0.78	6.25
31	0.78	50	0.05	0.10	0.20	0.39	0.20	0.39	0.78	6.25
32	3.13	25	0.10	0.10	0.20	0.78	0.20	0.78	0.78	6.25
33	3.13	25	0.10	0.10	0.20	0.39	0.20	0.78	0.78	6.25

Abbreviations: See Table 6.

Table 8. *In vitro* antibacterial activity (MIC, μg/ml) of alkoxyimino analogs (**34**～**37**).

Compound No.	*S.a.* 209P JC-1	*S.a.* JS1	*E.c.* NIHJ JC-2	*K.p.* IID875	*E.cl.* E10005	*E.cl.* GN7471	*S.m.* IID620	*P.v.* E08001	*P.a.* PAO1	*P.a.* E03110
34	3.13	25	0.10	0.10	0.20	0.20	0.20	0.39	0.78	6.25
35	1.56	50	1.56	0.78	1.56	3.13	3.13	3.13	3.13	12.5
36	50	>100	0.10	0.10	0.20	0.39	0.10	0.39	0.78	3.13
37	100	>100	0.20	0.20	0.20	0.78	0.39	0.39	1.56	6.25

Abbreviations: See Table 6.

the difference in the p*K*a of quinuclidine (p*K*a 11.12) and dehydroquinuclidine (p*K*a 9.88) influences the electron-withdrawing effect.

Most of the compounds having 4-substituted quinuclidine and 1-azabicyclo[2.2.1]heptane groups (**16**～**24**) at the C-3 side chain showed a well-balanced antibacterial spectrum and potent activity against *P. aeruginosa*. Compound **16** exhibited somewhat higher activity in comparison to compound **15**, mainly against *P. aeruginosa*. This could be due to increasing cell wall penetration with increasing hydrophilicity.

However, no relationship between the p*K*a value of 4-substituted quinuclidines and activity were observed as indicated by comparison of the MICs of 4-hydroxymethylquinuclidine (**17**, p*K*a 10.46) and 4-cyanoquinuclidine (**18**, p*K*a 8.08), and no remarkable difference between 4-hydroxyquinuclidine (**20**) and 4-hydroxy-1-azabicyclo[2,2,1]heptane (**21**) were also observed on the broadness of antibacterial spectrum.

It was recognized that most of the quinuclidine derivatives with an aminothiazolyl-α-methoxyimino group exhibited more activity than CAZ, except against *P. aeruginosa*, as seen in Table 6.

In vitro activities of 4-substituted quinuclidine derivatives and 4-hydroxy-1-azabicyclo[2.2.1]heptane with aminothiadiazolyl-α-methoxyimino groups (**25**～**33**) are shown in Table 7. Most of these derivatives were more active than CAZ against all organisms, including *P. aeruginosa*. Compound **25** showed the highest activity against *P. aeruginosa*, which was 4-fold more active than CAZ. Compound **25**, designated E1040, was selected as a lead compound in this series in view of its well-balanced activity profile.

The MICs of four analogs of **25**, in which the methoxyimino moiety at the C-7 side chain of **25** was modified, are shown in Table 8. The ethoxyimino analog (**34**) and cyclopropylmethyl analog (**35**) was 2- to 16-fold less active than **25** against Gram-negative organisms, including *P. aeruginosa*. The carboxylic analogs (**36**, **37**) were 2- to 32-fold less active than **25** not only against most bacteria, but also against

P. aeruginosa.

In summary, it was observed that introduction of 4-substituted quinuclidinium group at the C-3 side chain and 5-aminothiadiazolyl-α-methoxyimino group at the C-7 side chain of the cephem nucleus lead to increase antibacterial activity. The effect of the substituted group of quinuclidine on antipseudomonal activity may be due to hydrophilicity rather than electron-withdrawing effect (p*K*a).

Experimental

IR spectra were recorded on a Hitachi 260-30 or a Shimadzu FTIR-4300 and NMR spectra were recorded on a Jeol 90 Q or on a Varian UNITY 400. FAB-MS were recorded using a Jeol JHS-HX100 mass spectrometer with glycerol as liquid matrix.

3-Quaternary Cephalosporins (**IV** in Scheme 1)

Introduction of the quaternary ammonium group in the C-3 side chain was accomplished by quaternization of the iodide derivatives (from acetoxy derivatives **I** with TMSI in Method A, **III** in Method B) with tertiary amine. The preparation procedures of E1040 by Methods A and B (Scheme 1) are described as representative examples for 3-quaternary cephalosporins prepared in this study. The physical data of methoxyimino (**1**～**33**), and alkoxyimino (**34**～**37**) derivatives are listed in Tables 1～5, respectively.

7β-[(*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetamido]-3-(4-carbamoylquinuclidinio)-methyl-3-cephem-4-carboxylate (**25**)

Method A: 7β-[(*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid (**Ia** 5.4 g, 11.8 mmol) was suspended in CH_2Cl_2 (54 ml), followed by an addition of MSTFA (7.2 ml, 39 mmol). The resulting mixture was stirred at room temperature for 1 hour. After ice cooling, iodotrimethylsilane (2.2 ml, 15.4 mmol) was added to the solution, and the resulting mixture was stirred at room temperature for 15 minutes. The reaction mixture was concentrated under reduced pressure. The silylated drivative was dissolved in CH_3CN (54 ml), followed by addition of THF (1.4 ml, 17.7 mmol). To the thus-obtained solution was added 4-carbamoylquinuclidine (1.6 g, 10.4 mmol) in three portions over a period of 50 minutes at 0°C, and the resulting mixture was stirred for 50 minutes at same temperature. MeOH (0.5 ml) was then added to the reaction mixture, and was stirred for 15 minutes. The resulting precipitate was collected by filtration and then washed with CH_3CN. The precipitate dissolved in 30% aq EtOH. Subsequent to its concentration under reduced pressure, the residue was dissolved in a 7:1 mixed solvent of Me_2CO and water. The resulting solution was purified by silica gel column chromatography to afford **25** (2.4 g, 37%).

Method B: *p*-Methoxybenzyl 7β-[(*Z*)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetamido]-3-iodomethyl-3-cephem-4-carboxylate (**IIIa** 1 g, 1.7 mmol) was dissolved in a mixed solution of ethyl acetate (50 ml) and CH_3CN (10 ml). After the whole was ice cooled, a solution of ethyl acetate (9.8 ml) and MeOH (2.4 ml) of 4-carbamoylquinuclidine (206 mg, 1.3 mmol) was added dropwise over 30 minutes at 0°C thereto, and the mixture was stirred for 30 minutes. The resulting precipitate was collected by filtration, followed by washing with ethyl acetate to obtain *p*-methoxybenzyl 7β-[(*Z*)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetamido]-3-(4-carbamoylquinuclidinio)methyl-3-cephem-4-carboxylate iodide (1.19 g, 84%). This compound (1.19 g) was suspended in anisole (6 ml). After ice cooling TFA (6 ml) was added dropwise thereto. The mixture was stirred for 1 hour at 0°C. The reaction mixture was dropped in diisopropyl ether (50 ml) at 0°C, and resulting precipitate was collected by filtration. The precipitate dissolved in water (20 ml). The solution was adjusted to pH 5 by the addition of sodium acetate. The mixture was subjected to a reversed phase silica gel column chromatography (eluent; water - 5% MeOH solution) for purification, to afford **25** (350 mg, 38%).

p-Methoxybenzyl 7-Phenylacetamido-3-(4-carbamoylquinuclidinio)methyl-3-cephem-4-carboxylate Iodide (**VII**)

To a solution of NaI (18 g, 120 mmol) in Me_2CO (200 ml) was added **V** (Otsuka Chemical Co., Ltd.)

(40 g, 82 mmol) under stirring. The mixture was stirred for 1 hour at room temperature and then poured in EtOAc (1 liter), washed consecutively with 10% aq $Na_2S_2O_3$ (200 ml × 2) and sat. NaCl (200 ml), dried over anhydrous $MgSO_4$. A part of the filtrate was evaporated to dryness to afford the 3-iodomethyl compound **VI**, which was unstable when kept at room temperature.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.43 and 3.70 (2H, ABq, J=18 Hz, 2-H_2), 3.63 (2H, d, J=8 Hz, CH_2CONH), 3.80 (3H, s, $C_6H_4OCH_3$), 4.29 and 4.35 (2H, ABq, J=9 Hz, 3'-H_2), 4.90 (1H, d, J=5 Hz, 6-H), 5.21 (2H, s, $CH_2C_6H_4$), 5.76 (1H, dd, J=5 and 9 Hz, 7-H), 6.12 (1H, d, J=9 Hz, CONH), 6.88 (2H, ABq, J=9 Hz, C_6H_4), 7.25~7.38 (7H, m). A major part of the filtrate obtained was used for the next step without isolation of **VI**. To the chilled (0°C) and stirred filtrate was added dioxane (200 ml), and then dropwise over a period of 3 hours a solution of 4-carbamoylquinuclidine (12.0 g, 78 mmol) in EtOAc (220 ml) - MeOH (80 ml). The resulting precipitates were collected by filtration, washed with EtOAc - dioxane - AcOH (8 : 2 : 0.1, 500 ml), Et_2O (500 ml) and dried *in vacuo* to give 48.2 g (80%) of **VII**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.93 (6H, m, quinuclidine H), 3.57 and 3.88 (2H, ABq, J=17 Hz, 2-H_2), 3.76 (3H, s, OCH_3), 4.11 and 4.28 (2H, ABq, J=14 Hz, 3'-H_2), 5.19 and 5.37 (2H, ABq, J=11 Hz, $CH_2C_6H_4$), 5.20 (1H, d, J=5 Hz, 6-H), 5.82 (1H, dd, J=5 and 9 Hz, 7-H), 6.97 and 7.38 (2H, ABq, J=9 Hz, C_6H_4), 7.16~7.32 (9H, m, $C_6H_5CH_2$ and $CONH_2$), 9.15 (1H, d, J=9 Hz, CONH).

7-Phenylacetamido-3-(4-carbamoylquinuclidinio)methyl-3-cephem-4-carboxylate (**VIII**)

To phenol (20 ml) warmed to 45~50°C, was added conc HCl (10 μl) and **VII** (10 g, 14 mmol) under stirring. The mixture was stirred at the same temperature for 2 hours. After cooled, to viscosity solution was added Me_2CO (200 ml), IPE (100 ml). The resulting precipitates were collected by filtration, washed Me_2CO, IPE and *in vacuo* to give 5.62 g (85%) of **VIII**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.99 (6H, m, quinuclidine H), 3.87 (1H, ABq, J=17 Hz, 2-H_2), 4.00 and 4.71 (2H, ABq, J=14 Hz, 3'-H_2), 5.14 (1H, d, J=5 Hz, 6-H), 5.68 (1H, dd, J=5 and 8 Hz, 7-H), 7.15~7.32 (9H, m, $C_6H_5CH_2$ and $CONH_2$), 9.12 (1H, d, J=8 Hz, CONH). FAB-MS m/z 485 (M+H).

7-Amino-3-(4-carbamoylquinuclidinio)methyl-3-cephem-4-carboxylate Hydrobromide (**IX**)

Compound **VIII** (15.0 g, 31 mmol) was added to a solution of AcONa (5.6 g, 68 mmol) in H_2O (150 ml) at 25~30°C, the mixture was adjusted to pH 8.0 by the addition of 14% aq NH_4OH. Then a Carrier-Fixed benzylpenicillin amidase (Baehringer Mamheim-Yamanouchi Co., Ltd.) (15 g) was added to the solution. The reaction mixture was stirred for 3 hours at 25~30°C. During the period of the reaction was kept to pH 8.0 with 5.6% NH_4OH. The insoluble solid was filtered off, and washed with H_2O (75 ml). The filtrate was acidified about <pH 2 with 48% hydrobromic acid, and added MeOH (225 ml). The resulting precipitate was collected by filtration, washed with MeOH and dried *in vacuo* to give **IX** (10.4 g, 75%). ^{1}H NMR (400 MHz, D_2O) δ 2.08 (6H, m, quinuclidine H), 3.33~3.45 (7H, m), 3.79 (1H, ABq, J=17 Hz, 2-H_2), 3.82 and 4.46 (2H, ABq, J=14 Hz, 3'-H_2), 5.02 (1H, d, J=5 Hz), 5.24 (1H, d, J=5 Hz). FAB-MS m/z 367 (M+H).

7β-[(*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-methoxyiminoacetamido]-3-(4-carbamoylquinuclidinio)-methyl-3-cephem-4-carboxylate (**25**)

Compound **IX** (10 g, 22.4 mmol) was suspended in H_2O - MeOH (1 : 6) (350 ml), followed by addition of AcONa (9.2 g, 111.18 mmol). The resulting solution was warmed to 25~30°C. Then **X**[17,18] (5.75 g, 22.4 mmol) was added to the solution under stirring. The reaction mixture was stirred for 3 hours at 25~30°C. After MeOH (150 ml) was added to the reaction mixture, cooled to 0°C. The resulting precipitate was collected by filtration, washed with chilled 90% aq MeOH (100 ml), MeOH (100 ml), Et_2O (200 ml), dried to give **25** (9.2 g, 75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.98 (6H, m, quinuclidine H), 3.75 (1H, ABq, J=17 Hz, 2-H_2), 3.78 and 4.91 (2H, ABq, J=14 Hz, 3'-H_2), 3.91 (3H, s, OCH_3), 5.09 (1H, d, J=5 Hz, 6-H), 5.67 (1H, dd, J=5 and 8 Hz, 7-H), 7.12 and 7.29 (each 1H, each s, $CONH_2$), 8.11 (2H, s, NH_2), 9.50 (1H, d, J=8 Hz, CONH). FAB-MS m/z 551 (M+H). IR (KBr) cm^{-1} 3423, 1774, 1670, 1618, 1528, 1396, 1354, 1042.

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