Synthesis and Biological Evaluation of a Series of Parenteral 3'-Quaternary Ammonium Cephalosporins[1]

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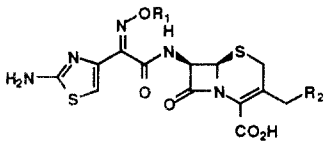
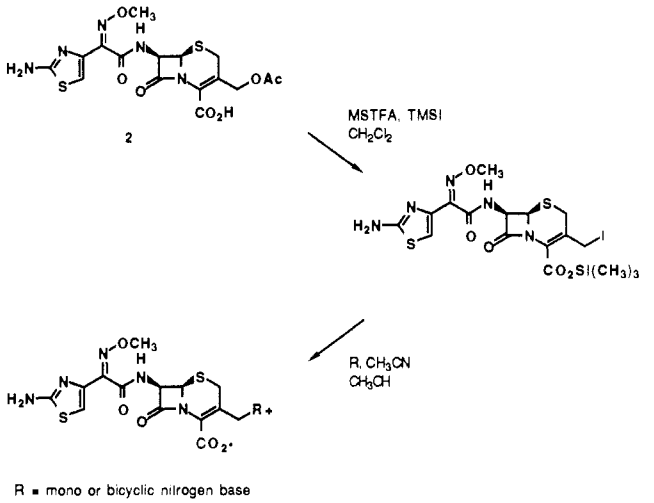
The preparation and biological evaluation of a series of 7β-[2-(2-aminothiazol-4-yl)-2(Z)-methoximinoacetamido]cephalosporins, substituted at the 3'-position with monocyclic or bicyclic nitrogen-containing heterocycles are described. The resulting family of parenteral compounds displays a broad spectrum of antibacterial activity. Some compounds exhibit a similar level of Gram-negative activity to that of the "third-generation" cephalosporins with increased staphylococcal activity. The in vitro and in vivo antimicrobial activity, structure–activity relationships, β-lactamase stability, and in vitro and in vivo pharmacological evaluations are presented.

The cephalosporin class of antimicrobial agents continues to be of clinical importance for the treatment of bacterial infection. A significant advancement in this area was the introduction of the 7β-[2-(2-aminothiazol-4-yl)-2(Z)-methoximinoacetamido] side chain.[2] Extended modification of this side chain in combination with alteration of the 3'-substituent of the cephem nucleus has led to the preparation and introduction of some highly potent broad-spectrum antibiotics. Ceftazidime (1), an aminothiazolyloxyimino derivative containing a 3'-pyridinium substituent, has been shown to be a "third-generation" antibacterial agent with excellent activity against a wide variety of Gram-positive and Gram-negative pathogens, including *Pseudomonas aeruginosa*.[3] In an effort to discover agents which maintain the excellent Gram-negative spectrum of ceftazidime while displaying increased Gram-positive activity, we have prepared a series of novel 3'-quaternary ammonium cephalosporins. This paper describes the full details of our efforts in this area, including the syntheses, structure–activity relationships, and pharmacological properties of these new agents.[4]



1: Ceftazidime $R_1 = C(CH_3)_2CO_2H$, R_2 = pyridinium
2: Cefotaxime $R_1 = CH_3$, $R_2 = OAc$

Scheme I



Chemistry

The quaternary cephalosporin derivatives were prepared according to the general method of Bonjouklian and Phillips, of these laboratories.[5] Silylation of cefotaxime (2) in methylene chloride with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide was followed by in situ formation of the 3'-iodide with trimethylsilyl iodide.[6] Excess trimethylsilyl iodide was destroyed by the addition of tetrahydrofuran, then the 3'-halogen was displaced by a nitrogen-containing heterocycle (R) in acetonitrile and the silylated cephem hydrolyzed by treatment with methanol (Scheme I).

(1) Preliminary accounts of this work have been presented: Bogard, S. J.; Brown, R. F.; Counter, F. T.; Ensminger, P. W.; Katner, A. S.; Kinnick, M. D.; Koehler, R. E.; Lunn, W. H. W.; Ott, J. L.; Preston, D. A.; Shadle, J. K.; Swartzendruber, J. K.; Turner, J. R.; Vasileff, R. T.; Webber, J. A. 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Las Vegas, NV, 1983; Abstract 995. Counter, F. T.; Eudaly, J. A.; Quay, J. F.; Ruffolo, R. R.; Stucky, J. F.; Wright, W. E. 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Las Vegas, NV, 1983; Abstract 996. Brown, R. F.; Counter, F. T.; Ensminger, P. W.; Katner, A. S.; Kinnick, M. D.; Kurz, K. D.; Morin, J. M., Jr.; Ott, J. L.; Preston, D. A.; Steinberg, M. I. 25th Interscience Conference on Antimicrobial Agents and Chemotherapy, Minneapolis, MN, 1985; Abstract 365.

(2) For a review, see: Duerckheimer, W.; Blumbach, J.; Lattrell, R.; Scheuenemann, K. H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 180.

(3) For a comprehensive review, see: Richards, D. M.; Brogden, R. N. *Drugs* **1985**, *29*, 105.

(4) After completion of this work a variety of cephalosporin analogues containing 3'-quaternary ammonium substituents have been prepared. For a review, see: Uri, J. V.; Burdash, N.; Bendas, C. M. *Acta Microbiol. Hung.* **1988**, *35*, 327. See also, Fujimoto, K.; Nakayama, E.; Muramatsu, S.; Miyauchi, M.; Ide, J.; Iwata, M.; Igarashi, I.; Misawa, H. *Sankyo Kenkyusho Nenpo* **1984**, *36*, 93. Lattrell, R.; Blumbach, J.; Duerckheimer, W.; Fehlhaber, H.-W.; Fleischman, K.; Kirrstetter, R.; Mencke, B.; Scheunemann, K.-H.; Schrinner, E.; Schwab, W.; Seeger, K.; Seibert, G.; Wieduwilt, M. *J. Antibiot.* **1988**, *41*, 1374 and references cited therein.

(5) Bonjouklian, R.; Phillips, M. L. *Tetrahedron Lett.* **1981**, *22*, 3915. See also: Lunn, W. H. W.; Shadle, J. K. U.S. Patent no. 4,336,253 1981; *Chem. Abstr.* **1982**, *97*, 182097c.

(6) If the entire reaction was run in acetonitrile as much as 50% of the product was isolated as δ-2-cephem. The use of methylene chloride in the formation of the 3'-iodomethyl cephem kept the presence of δ-2-cephem at less than 5% of the product.

0022-2623/90/1833-2114$02.50/0

Antibacterial Activity

Minimal inhibitory concentrations (MIC) of compounds **3–73** for an array of Gram-positive and Gram-negative bacterial species were determined by an agar dilution method[7] and are summarized in Table I. Ceftazidime (**1**), cefotaxime (**2**), and cefpirome (**74**)[8] are included for reference.

The pyridinium substituted compounds (**3–23**) were highly active against *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Serratia marcescens*. The parent compound (**3**) was superior to the substituted analogues against *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli*, and *Klebsiella pneumoniae*. Among the compounds containing a substituted pyridinium, those with an acidic functionality (COOH for **10**, **17**, and **20**; CONHOR for **11**, **12**, **13**, **18**, and **19**; or SO_3H for **16**) exhibited reduced potency against staphylococci. Against a constitutive high level chromosomal β-lactamase producer, *Enterobacter cloacae* 265A, all compounds within this series, except **3** and **23**, had MIC greater than 2 μg/mL. In contrast, all compounds, except **12** and **18**, had MIC less than 1 μg/mL against the inducible β-lactamase producing strain, EB5. The relationship between structure and anti-pseudomonal activity of these compounds was subtle and difficult to generalize on the basis of the compounds tested.

The two substituted pyrazinium derivatives (**24** and **25**) exhibited potency similar to **3** against most organisms, with the dimethylamino analogue **25** slightly more potent than the monomethyl analogue **24**.

The activities of the quinolinium and isoquinolinium derivatives (**26–34**) were generally equal to or more potent than **3** against staphylococci, while compounds **30** and **34** were less potent than the rest. With the exception of **34**, all had notable activity (MIC 2–8 μg/mL) against *K. pneumoniae* 1082E. Compound **29** was the most potent of these against the constitutive β-lactamase producer, *E. cloacae* 265A, while most compounds (except **26–28**) displayed reduced activity against *Pseudomonas aeruginosa*.

Among the four compounds having one or more nitrogens in the 5- or 6-membered ring fused to the pyridine (**35–38**), compound **36** was superior to compound **3** against most of the strains tested.

The furopyridinium derivatives (**39–42**) showed broad spectrum antibacterial activity similar to that of compound **3**. The furo[3,2-*c*]pyridinium isomer **42** was generally more potent than the other analogues.

The thienylpyridinium derivatives (**43–50**) were similar to compound **3** in activity and spectrum. However, substitution of an acidic function on the thiophene portion of the molecule (**48** and **49**) resulted in diminished *Staphylococcus* activity and a loss of activity against the constitutive chromosomal β-lactamase producer, *E. cloacae* 265A. Compounds substituted with thiazolopyridines at the 3'-position (**51–56**) exhibited activity against *P. aeruginosa* that varied inversely with the size of the substituent; diethylamino (**55**) and tertiary-butyl (**56**) substitution resulted in a pronounced loss of *Pseudomonas* activity relative to the others in this group. The imidazolopyridinium analogues containing an aryl substituent on the carbon of the imidazole (**64** and **65**) were less active on Gram-negatives in general, and particularly against *E. cloacae* 265A and *P. aeruginosa*.

(7) Kirst, H. A.; Wild, G. M.; Baltz, R. H.; Hamill, R. L.; Ott, J. L.; Counter, F. T.; Ose, E. E. *J. Antiobiot.* **1982**, *35*, 1675.
(8) Prous, J. R. *Drugs Future* **1988**, *13*, 369. Prous, J. R. *Drugs Future* **1987**, *12*, 389. Prous, J. R. *Drugs Future* **1986**, *11*, 324. Prous, J. R. *Drugs Future* **1984**, *9*, 252.

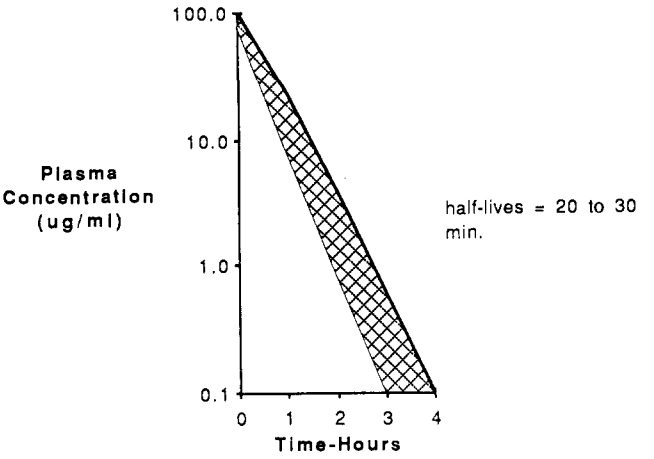


Figure 1. Range of plasma concentrations and half-lives in rats after intravenous administration of compounds **3**, **6**, **42**, **46**, and **59** at 20 mg/kg. Plasma concentrations of all members of this series tested fell within the shaded area on this graph. Plasma samples were assayed by a microbiological assay.

Substitution on either, or both, of the adjacent carbons of the thiazole ring in compounds **67–73** improved the Gram-negative activity over that of the unsubstituted thiazolium compound **66**. This series was the most active against *Enterococcus faecalis* (MIC 16–64 μg/mL) and displayed excellent activity against the constitutive β-lactamase producer, *E. cloacae* 265A.

The quaternary cephalosporins were also tested for activity against a variety of anaerobic bacteria. These antibiotics were generally active against the Gram-positive species but less active against the Gram-negative species, including *Bacteroides fragilis*.

The in vitro effectiveness of the quaternary cephalosporin derivatives was mirrored by the excellent in vivo activity displayed by these derivatives in a mouse-protection model (Table II). The median effective doses (ED_{50}s) of the more active compounds tested were in the range of 0.02–10 mg/kg × 2.

A number of these derivatives were also tested for in vitro β-lactamase stability (Table III).[9] All derivatives tested were found to be quite stable to in vitro hydrolysis by a variety of β-lactamases.

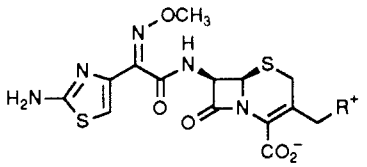
Pharmacokinetic Evaluation

Pharmacokinetic effects in rodents, dogs, and monkeys were studied for selected members of this series of 3'-quaternary ammonium cephalosporins. The pharmacokinetic properties (plasma concentration, half-life, plasma binding, and urinary excretion) of all members in this series were very similar.

In rats and mice, a number of derivatives representing several of the classes of 3'-quaternary substituents were examined and gave plasma levels typical of rapidly distributing, low plasma bound compounds with little or no renal secretion or reabsorption. Plasma concentrations of all the members of this series tested by intravenous administration to rats fell within a very narrow range, and the compounds exhibited half-lives of 20–30 min. A qualitatively similar behavior was seen in mice. These data illustrate the pharmacokinetic homogeneity of this series of compounds in rodents (Figure 1). All members that were tested not only exhibited similar plasma concentrations but also similarly high urinary excretion and low

(9) β-Lactamase studies were determined by the method of Mahoney, Koppel, and Turner: Mahoney, D. F.; Koppel, G. A.; Turner, J. R. *Antimicrob. Agents Chemother.* **1976**, *10*, 470.

Table I. Antibacterial Activity of 3′-Quaternary Ammonium Cephalosporins[a,b]



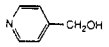
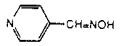
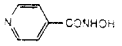
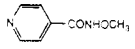
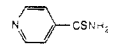
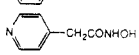
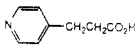
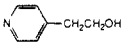
compd	strain / R	Staph. aureus Pen G sus.	Staph. aureus Pen G res.	Streptococcus pyog.	Streptococcus pneum.	Enteroc. faecium	E. coli EC14	E. coli TEM	Kleb. pn. X26	Kleb. pn. 1082E	Enterob. cloa. EB5	Enterob. cloa. 265A	Ps. aerug. X528	Ps. aerug. PS18	S. marces. SE3
1	Ceftazidime	8	32	0.125	0.125	>128	0.125	0.125	0.06	1	0.25	64	2	4	0.5
2	Cefotaxime	2	16	0.015	0.015	>128	0.03	0.03	0.008	1	0.25	32	32	64	0.25
3		1	2	0.03	0.03	>128	0.03	0.03	0.03	4	0.06	2	2	4	0.25
4		1	2	≤0.008	0.015	>128	0.06	0.06	0.06	16	0.25	8	2	4	0.06
5		2	4	0.015	0.03	>128	0.06	0.03	0.03	16	0.25	32	4	16	0.25
6		4	4	0.015	0.015	>128	0.015	0.03	0.015	16	0.5	>128	8	32	0.25
7		2	8	0.015	0.015	>128	0.03	0.03	0.03	4	0.125	16	1	4	0.06
8		4	8	0.06	0.06	>128	0.06	0.06	0.06	16	0.5	64	32	8	0.5
9		2	4	0.03	0.03	128	0.03	0.03	0.03	4	0.25	32	8	4	0.125
10		8	16	0.015	0.03	>128	0.03	0.25	0.03	32	0.5	128	4	16	0.25
11		4	4	0.03	0.03	>128	0.06	0.125	0.06	32	0.25	64	4	8	0.06
12		8	8	0.03	0.06	>128	0.06	0.125	0.06	32	4	>128	4	8	0.25
13		8	16	0.03	0.06	>128	0.03	0.125	0.06	16	0.5	128	16	16	0.5
14		1	2	0.015	0.015	>128	0.06	0.06	0.03	16	0.25	16	2	8	0.125
15		1	2	≤0.008	0.015	>128	0.03	0.03	0.03	8	0.125	8	1	2	0.06
16		4	8	0.06	0.06	>128	0.06	0.5	0.125	64	0.5	>128	8	32	0.25
17		8	16	0.03	0.03	>128	≤0.008	0.06	0.015	32	0.25	32	8	32	0.06
18		16	16	0.25	0.5	>128	0.25	0.5	0.25	32	2	128	32	64	1
19		4	8	0.015	0.03	>128	0.06	0.25	0.06	32	0.5	64	2	8	0.25
20		8	16	0.06	0.06	>128	0.03	0.03	0.03	8	0.25	64	8	16	0.25
21		2	4	0.015	0.03	>128	0.03	0.03	0.03	4	0.125	16	1	4	0.06
22		2	4	≤0.008	0.015	>128	0.125	0.06	0.03	8	5	16	16	64	0.5
23		1	2	≤0.008	0.015	128	0.03	0.03	0.03	4	0.125	2	4	16	0.125
24		4	8	0.03	0.03	>128	0.125	0.06	0.06	8	0.5	16	4	32	0.5
25		1	2	≤0.008	0.03	>128	0.06	0.06	0.03	8	0.125	8	4	8	0.125
26		0.5	1	0.015	0.015	128	0.03	0.015	0.015	2	0.125	4	2	4	0.125
27		1	1	≤0.008	0.015	128	0.015	0.03	0.015	2	0.06	8	2	4	0.06

Table I (Continued)

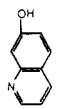
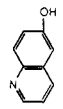
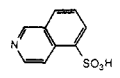
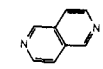
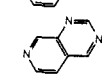
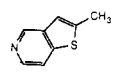
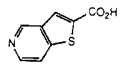
compd	strain / R	Staph. aureus Pen G sus.	Staph. aureus Pen G res.	Streptococcus pyog.	Streptococcus pneum.	Enteroc. faecium	E. coli EC14	E. coli TEM	Kleb. pn. X26	Kleb. pn. 1082E	Enterob. cloa. EB5	Enterob. cloa. 265A	Ps. aerug. X528	Ps. aerug. PS18	S. marces. SE3
28		1	2	≤0.008	0.015	128	0.015	0.015	0.015	2	0.06	8	2	2	0.06
29		1	2	≤0.008	≤0.008	>128	0.06	0.03	0.015	8	0.25	2	4	16	0.25
30		4	8	0.03	0.06	>128	0.06	0.125	0.03	4	0.25	8	16	32	0.5
31		1	2	≤0.008	≤0.008	128	0.03	0.03	≤0.008	4	0.5	16	8	8	0.5
32		2	2	0.015	0.03	128	0.03	0.06	0.03	8	0.5	32	8	8	0.5
33		1	2	≤0.008	0.05	128	0.015	0.03	≤0.008	8	0.125	16	8	8	0.25
34		4	8	0.015	0.015	>128	0.015	0.03	0.03	32	0.25	64	32	16	0.25
35		2	4	0.03	0.06	>128	0.06	0.125	0.06	4	0.25	8	4	8	0.5
36		1	1	≤0.008	≤0.008	32	≤0.008	≤0.008	≤0.008	2	0.03	2	2	2	0.03
37		4	32	0.06	0.03	128	0.25	0.125	0.125	16	1	16	32	64	1
38		1	8	≤0.008	0.015	>128	0.5	0.125	0.06	64	2	32	32	64	0.5
39		2	16	≤0.008	≤0.008	>128	≤0.008	0.06	≤0.008	4	0.125	8	4	16	0.125
40		0.5	4	0.06	0.06	32	0.06	0.06	0.06	8	0.5	4	4	8	0.25
41		1	8	≤0.008	0.015	>128	0.015	0.015	0.015	4	0.06	8	2	8	0.03
42		0.5	4	≤0.008	≤0.008	>128	0.015	0.03	0.015	4	0.06	4	1	2	0.03
43		1	2	0.03	0.03	128	0.03	0.06	0.03	4	0.125	4	4	8	0.06
44		0.5	4	0.015	0.03	128	0.06	0.06	0.03	1	0.25	4	2	4	0.125
45		1	4	≤0.008	≤0.008	>128	0.015	0.03	0.015	4	0.06	8	2	4	0.03
46		1	2	≤0.008	0.015	>128	0.015	0.03	0.015	2	0.06	4	2	4	0.125
47		0.5	4	≤0.008	≤0.008	>128	0.015	0.015	≤0.008	1	0.06	4	2	8	0.06
48		2	16	≤0.008	≤0.008	>128	≤0.008	0.03	≤0.008	4	0.06	32	2	8	0.06
49		2	16	0.03	0.015	>128	0.015	0.06	0.015	4	0.125	32	4	16	0.03

Table I (Continued)

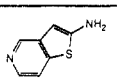
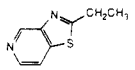
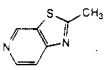
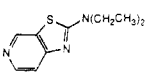
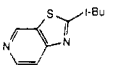
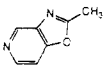
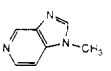
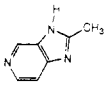
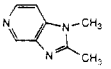
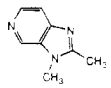
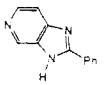
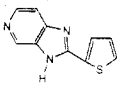
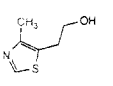
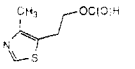
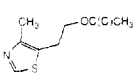
compd	R	Staph. aureus Pen G sus.	Staph. aureus Pen G res.	Streptococcus pyog.	Streptococcus pneum.	Enteroc. faecium	E. coli EC14	E. coli TEM	Kleb. pn. X26	Kleb. pn. 1082E	Enterob. cloa. EB5	Enterob. cloa. 265A	Ps. aerug. X528	Ps. aerug. PS18	S. marces. SE3
50		0.5	4	0.015	≤0.008	>128	0.015	0.03	≤0.008	2	0.06	8	1	4	0.06
51		1	8	0.015	0.015	128	0.015	0.03	0.015	4	0.06	8	1	4	0.03
52		2	2	0.008	0.015	128	0.015	0.03	0.03	2	0.06	2	4	8	0.125
53		1	4	0.008	0.008	128	0.015	0.015	0.008	2	0.06	4	4	16	0.03
54		1	4	0.015	0.03	128	0.03	0.03	0.03	1	0.125	16	8	8	0.25
55		0.5	0.5	0.008	0.008	128	0.06	0.015	0.008	2	0.25	2	16	128	0.5
56		1	4	0.015	0.015	128	0.25	0.06	0.03	4	1	16	32	128	2
57		1	8	0.015	0.015	128	0.03	0.03	0.015	4	0.125	8	4	8	0.06
58		2	2	0.008	0.008	128	0.008	0.008	0.008	8	0.03	8	2	8	0.125
59		2	2	0.008	0.03	128	0.03	0.03	0.03	8	0.125	8	2	8	0.125
60		2	16	0.015	0.008	128	0.015	0.015	0.015	8	0.06	8	1	4	0.03
61		2	8	0.008	0.008	128	0.008	0.008	0.008	2	0.06	8	2	8	0.03
62		2	16	0.008	0.008	128	0.008	0.008	0.008	2	0.03	4	4	8	0.03
63		2	16	0.015	0.015	128	0.015	0.015	0.015	2	0.06	4	2	8	0.06
64		2	8	0.008	0.008	128	0.125	0.06	0.015	2	0.5	32	64	128	1
65		2	8	0.008	0.008	128	0.25	0.03	0.015	4	0.5	32	64	128	1
66		4	8	0.125	0.125	64	0.125	0.125	0.125	8	0.5	32	32	16	0.25
67		2	4	0.03	0.03	32	0.06	0.06	0.06	8	0.25	8	16	32	0.25
68		2	4	0.03	0.06	32	0.06	0.03	0.06	4	0.25	4	16	32	0.25
69		2	4	0.03	0.03	16	0.06	0.03	0.03	4	0.125	4	8	16	0.125
70		1	2	0.015	0.015	16	0.03	0.03	0.015	4	0.06	2	8	16	0.06
71		2	4	0.015	0.015	32	0.03	0.03	0.015	8	0.125	2	32	64	0.125
72		2	4	0.06	0.06	32	0.06	0.06	0.06	4	0.25	4	8	32	0.125

Table I (Continued)

compd	strain R	Staph. aureus Pen G sus.	Staph. aureus Pen G res.	Streptococcus pyog.	Streptococcus pneum.	Enteroc. faecium	E. coli EC14	E. coli TEM	Kleb. pn. X26	Kleb. pn. 1082E	Enterob. cloa. EB5	Enterob. cloa. 265A	Ps. aerug. X528	Ps. aerug. PS18	S. marces. SE3
73		1	2	0.03	0.03	16	0.06	0.03	0.03	2	0.125	1	4	16	0.125
74[c]		1	1	0.008	0.015	8	0.015	0.03	0.015	4	0.06	0.5	2	8	0.125

[a] Agar dilution method using Mueller-Hinton agar and an inoculum of $\sim 10^4$ colony forming units applied to agar surfaces. [b] Data in the table are minimal inhibitory concentrations expressed as μg/mL. [c] Cefpirome.

Table II. In Vivo Efficacy of Selected Quaternary Cephalosporins against Experimental Infections in Mice[a]

compd	ED_{50} (mg/kg × 2) [MIC, μg/mL] *E. coli*	*S. aureus*	*Ps. aeruginosa*
3	0.07 [0.06]	0.8 [1]	14.4 [16]
4	0.14 [0.06]	1.6 [1]	>50 [8]
6	0.13 [0.015]	7.8 [4]	>50 [128]
7	0.04 [0.06]	1.5 [1]	19.6 [8]
8	0.82 [0.03]	2.5 [4]	>40 [8]
9	<0.3 [0.03]	1 [1]	39 [16]
10	<0.16 [0.03]	7.2 [8]	- [128]
11	<0.16 [0.03]	4 [2]	- [128]
12	<0.16 [0.03]	5.3 [4]	8.6 [8]
13	0.05 [0.03]	>10 [8]	31 [64]
14	0.09 [0.06]	1.4 [1]	20 [8]
15	<0.16 [0.03]	1.4 [1]	7.2 [8]
16	0.08 [0.06]	>10 [4]	41.6 [64]
17	<0.16 [0.03]	6.2 [8]	- [128]
18	<0.16 [0.06]	2.7 [2]	12 [64]
19	<0.16 [0.06]	2.5 [4]	- [128]
20	<0.03 [0.06]	1.4 [1]	20 [8]
21	0.07 [0.03]	3.3 [2]	8.6 [8]
22	0.5 [0.125]	2.5 [4]	11 [64]
24	0.09 [0.06]	1.4 [1]	17.4 [16]
26	0.04 [0.03]	0.4 [0.5]	9.5 [8]
27	0.04 [0.015]	0.4 [1]	9 [8]
28	<0.03 [0.03]	0.2 [0.5]	6 [2]
30	0.05 [0.06]	4 [4]	>50 [16]
31	0.04 [0.03]	1 [1]	29 [8]
33	<0.03 [0.015]	2 [1]	>50 [8]
42	<0.6 [0.015]	<0.6 [1]	18 [4]
43	0.05 [0.015]	0.8 [1]	13.5 [4]
44	0.04 [0.03]	0.6 [1]	9.3 [2]
45	0.02 [<0.008]	0.4 [1]	9.5 [4]
46	0.02 [<0.008]	0.4 [1]	6.3 [2]

[a] Experimental infections were produced by intraperitoneal injection with the challenge organisms suspended in 5% hog gastric mucin. The infections were lethal to all untreated mice. Treatment of infected mice was carried out at 1 and 5 h postinfection.

Table III. In Vitro Relative Hydrolysis Rates of Selected Cephalosporin Analogues by β-Lactamase[a]

compd	enzyme source *Enterobacter* (Ia)	*Pseudomonas* (Id)	*E. coli* (IIIa)	*Klebsiella* (IV)	*S. aureus*
9	0.2	0.1	0.1	1.2	6.9
11	0.4	0.1	0.5	10.4	3.4
25	0.1	0.1	0.5	1.5	6.1
26	0.1	0.1	0.2	0.8	8.4
27	0.3	0.1	0.2	2.8	9.3
28	1.1	0.6	0.7	6.8	0.2
43	0.1	0	0.5	1.5	9.6
44	0.2	0.2	0.2	0.7	6.2
45	0.7	0.4	0.2	6.9	7.1
46	0.6	0.3	0.3	3.4	7.8

[a] Hydrolysis of cephalosporins by β-lactamases was determined spectrophotometrically at 265 nm by using the candidate substrates exposed to partially purified cell-free preparations of the enzymes. Rates of hydrolysis are reported as percentages of the observed rate for cephaloridine.

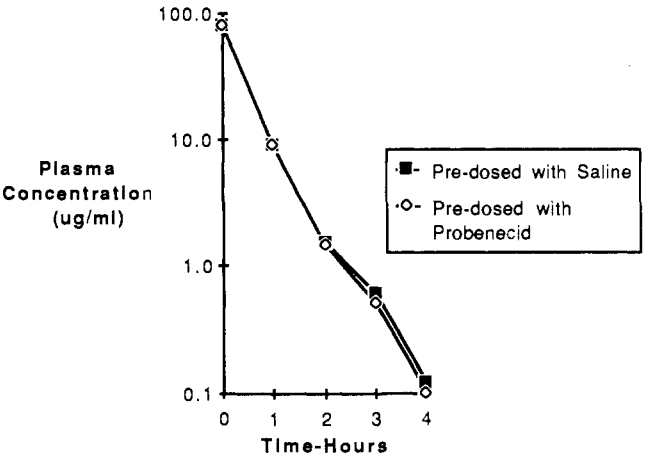


Figure 2. Effect of coadministered probenecid on the plasma concentration of compound **46** administered intravenously to rats at 20 mg/kg. No probenecid effect is seen with this compound, or compounds **3**, **6**, or **42**. Plasma samples were assayed by a microbiological assay.

plasma binding. A number of compounds were tested to examine the effect of coadministered probenecid on their plasma concentrations. No probenecid effect was observed with any of the compounds (Figure 2).

In dogs, after intravenous doses of 60 mg/kg, terminal half-lives of antibacterial activity ranged from 70–140 min. Urinary recovery was generally high accounting for 58–102% of the dose (Table IV).

In addition, analogue **59** was evaluated for pharmacokinetics versus ceftazidime and cefpirome in rhesus monkeys. The compounds were administered intravenously at 30 mg/kg to male rhesus monkeys. The plasma half-lives and urinary recoveries for all three compounds were similar (Table V).

Anticholinergic Response

Some, but not all, of these 3'-quaternary cephalosporins exhibited varying levels of weak anticholinergic activity in an in vitro model (Table VI).[10] Cephalosporins not containing a 3'-quaternary substituent, as well as ceftazidime, were inactive in the same test. The basic substituent at the 3'-position of the cephalosporin nucleus was shown to have a great influence on antimuscarinic potency. Compound **46**, with a thieno[3,2-*c*]pyridinium substituent, exhibited significantly more antimuscarinic activity than any other derivative tested. It is interesting to note that compound **44**, with an isomeric thieno[3,2-*b*]pyridinium substituent, was 50 times less potent than **46**. Similarly, ceftazidime (1) and compound **3** have the same 3'-position substitution, yet the latter is nine times more potent.

(10) O'Donnell, S. R.; Wanstall, J. C. *Br. J. Pharmacol.* **1977**, *60*, 255.

Table IV. Pharmacokinetics of Selected Cephalosporin Analogues in Dogs[a]

compd	no. of dogs	dose, mg/kg	% of dose in urine	terminal half-life, min
cephaloridine	3	60	102	140
3	2	60	94	100
40	2	60	78	100
42	2	60	94	80
46	1	30	58	90
46	2	15	55	105
47	2	60	64	100
48	2	60	98	90
59	3	20	–	70
59	3	10	–	76

[a] Overnight fasted, female mongrel dogs were fitted with a Foley retention catheter (French no. 14) in the urinary bladder. Antibiotic was administered as a bolus injection into the cephalic vein. Plasma and urine samples were collected before and during 24-h period following administration of the antibiotic. The plasma and urine samples were assayed by a microbiological assay.

Table V. Pharmacokinetics of Cephalosporin Analogues 1, **59**, and **74** in Rhesus Monkeys[a]

compd	no. of monkeys	dose, mg/kg	% of dose in urine	terminal half-life, min
ceftazidime (1)	4	30	64	56[b]
59	4	30	52	65
cefpirome (**74**)	4	30	61	68[c]

[a] Ceftazidime (1) was administered intravenously to male rhesus monkeys with plasma samples taken at 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, and 6 h after dosing, and urinary recoveries were determined 0–6 and 6–24 h after dosing. Compound **59** and cefpirome (**74**) were administered intravenously to male rhesus monkeys with plasma samples taken at 0.1, 0.15, 0.25, 0.5, 1, 2, 3, 4, 6, and 8 h after dosing with urinary recoveries determined 0–8 and 8–24 hours after dosing. The plasma and urine samples were assayed by a microbiological assay. [b] Lit. value, 49 min in cynomolgus monkeys, 10.0 mg/kg, im: Acred, P. *Infection* **1983**, *11*, suppl., S44. [c] Lit. value, 70 min in macaca monkeys, 10.0 mg/kg, iv: Klesel, N.; Seeger, K. *Infection* 1983, *11*, 318.

Clearly, the structural determinants for cholinergic blocking activity in this series are complex and probably cannot be related to a simple substitution in the 3′-position, but must include a consideration of the entire molecule.

Compounds with minimal anticholinergic activity (**44**, **59**, and **69**) were also tested for cardiac responses in conscious, unrestrained, spontaneously hypertensive rats.[11] None of these quaternary cephalosporins produced statistically significant changes in heart rate or mean arterial blood pressure after intravenous bolus administration of doses between 30–300 mg/kg. In contrast, compounds which exhibited significant anticholinergic activity in the in vitro guinea pig trachea model (**3** and **46**) caused a marked elevation of heart rate and mean arterial blood pressure in the same rat model after intravenous administration of doses as low as 30 mg/kg. Further, compounds **3** and **46** also caused tachycardia in conscious dogs[12] after intravenous administration of 30 mg/kg.

Conclusions

The members of this series of 3′-quaternary ammonium 7β-[2-(2-aminothiazol-4-yl)-2(*Z*)-methoximinoacetamido]cephalosporins represent a new class of antibiotic agents with enhanced microbiological spectra and potency. These derivatives display the excellent Gram-negative activity of the "third-generation" cephalosporins, activity against *P. aeruginosa*, high β-lactamase stability, a satisfactory pharmacokinetic profile, and excellent activity against *Staphylococcus* species. Some members of this class may deserve to be characterized as "fourth-generation" cephalosporins and may become important future agents for the treatment of clinical infections.

Table VI. In Vitro Relative Anticholinergic Activity of Selected Cephalosporin Analogues in Isolated Guinea Pig Trachea

compd (*n*) (10^{-3} M)	dose ratio[a]	pA_2[b]	RAP[g]
46[e] (6)	23.67 ± 3.20	4.36	100
3[d] (6)	3.04 ± 0.31	3.31	9
52[d] (6)	2.68 ± 0.22	3.22	7
54[d] (6)	2.05 ± 0.12	3.02	5
cefpirome (6)	1.76 ± 0.12	2.91	4
63[c] (6)	1.57 ± 0.04	2.75	2
44[c] (6)	1.50 ± 0.15	2.71	2
48[c] (5)	1.52 ± 0.25	2.71	2
ceftazidime[c] (7)	1.33 ± 0.21	2.46	1
62[c] (6)	1.31 ± 0.08	2.43	1
69[c] (7)	1.27 ± 0.24	2.44	1
59[c] (6)	1.26 ± 0.12	2.23	1
60[c] (6)	0.79 ± 0.15	–	1
Kreb's (8)	0.87 ± 0.09	–	–

[a] Relative shift in log dose–response curve in presence and absence of antagonist (10^{-3} M). [b] Negative log of the molar concentration of an antagonist that causes a 2-fold rightward shift in the dose–response curve. [c] Not significantly different from Kreb's ($P \geq 0.05$).[f] [d] Significantly different from ceftazidime ($P < 0.05$).[f] [e] Significantly different from all other tested compounds. [f] Statistical analysis performed with use of Tukey's multiple comparison test based on $\log_2$ transformation of mean dose ratios. [g] Relative anticholinergic potential. The anticholinergic activity of compound **46** was arbitrarily set at a value of 100 with the anticholinergic activity of all other cephalosporins expressed in relation to this value (i.e., relative anticholinergic potential). Using this scale atropine has a value of approximately 500 000.

Experimental Section

General Methods. Reagents were used as supplied unless otherwise noted. Reactions were run under an atmosphere of dry nitrogen or argon unless otherwise noted. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Jeol FX-90Q, General Electric QE-300, Bruker WM-270, Varian HA-100, or Varian T-60 spectrometer. Chemical shifts are reported in parts per million (δ) downfield relative to tetramethylsilane or DDS as an internal standard. Infrared (IR) spectra were determined on a Nicolet MX-1 FT-IR, optical rotations on a Perkin-Elmer 241 spectrophotometer, and ultraviolet (UV) spectra on a Cray 219 instrument. Mass spectral data (MS) were obtained on either a CEC-21-110 or a Varian MAT-731 spectrometer. Elemental analyses were performed by the Lilly Research Laboratories Department of Physical Chemistry. Satisfactory spectral data were obtained for all new compounds. Satisfactory elemental analyses (±0.4%) were obtained for all crystalline derivatives.

General Procedure for the Preparation of Quaternary Cephalosporins. 7β-[2-(2-Aminothiazol-4-yl)-2(*Z*)-methoximinoacetamido]cephalosporanic acid (Cefotaxime, **2**) (910 mg, 2.0 mmol) was suspended in methylene chloride (5 mL) under nitrogen atmosphere. *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (1.24 mL, 7 mmol) was added and the mixture was warmed briefly to 40 °C. The resulting homogeneous solution of cephem trimethylsilyl ester was cooled to room temperature and trimethylsilyl iodide (0.77 mL, 5.4 mmol) was added. The solution was stirred for 0.5 h, then the solvent was evaporated in vacuo to afford the 3′-iodomethyl cephem as a viscous oil. This oil was dissolved in acetonitrile (3 mL), and tetrahydrofuran (0.73 mL, 9 mmol) was added to destroy any excess TMSI. The appropriate nitrogen-containing heterocycle (2 mmol) was dissolved in acetonitrile (10 mL) and added in one portion to the iodomethyl cephem solution. The reaction was stirred at room temperature

(11) Okamoto, K.; Yamori, Y.; Ooshima, A.; Park, C.; Haebara, H.; Matsumoto, M.; Tanaka, T.; Okuda, T.; Hazama, F.; Kyogoku, M. *Spontaneous hypertension, its pathogenesis and complications*; Okamoto, K., Ed.; Igaku Shoin Ltd.: Tokyo, 1972; p 1.

(12) Bopp, R. J.; Quay, J. F.; Morris, R. M.; Stucky, J. F.; Miner, D. J. *J. Pharm. Sci.* **1985**, *74*, 846.

for 3 h, and then poured into 5% methanol–95% acetone (100 mL) to hydrolyze the trimethylsilyl groups. The crude product, isolated as the hydrogen iodide salt, was filtered and dried.

The crude HI salt was dissolved in aqueous sodium bicarbonate and chromatographed on either C-18 reverse-phase silica or HP-20 resin, eluting with mixtures of 2–20% acetonitrile, 1% acetic acid, and water. Product elution was monitored by analytical reverse-phase HPLC. The product-containing fractions were partially concentrated and lyophilized to yield pure products as amorphous colorless solids.

Crystallization Procedure. Extensive efforts were carried out to purify these compounds. Crystallization of some the cephalosporins as the dimethylacetamide solvate and the sulfate salt was achieved. The crude HI salt was stirred with water at room tempeature for 1 h and insoluble material was removed by filtration. While the pH was continuously monitored, the clear red filtrate was treated with small portions of IRA-68 (OH– form) resin until the pH rose to 7.5. Dowex-50 (H+ form) resin was added as needed to maintain the pH at 7.5. On stirring the red color gradually disappeared over the period of 0.5 to 2 h. Stirring for longer periods of time led to reduced yields since the product may have begun to adhere to the resins. The resins were removed by filtration and the filtrate was lyophilized to yield the product as an amorphous white solid. This solid was suspended in dimethylacetamide (DMAC) and just enough water was added to induce solution. Small portions of amorphous product were added until the solution became cloudy and large crystals of DMAC solvate separated on standing. These crystals were dissolved in 1 N H_2SO_4 and the solution was partially concentrated, yielding the crystalline sulfate salt.[13] Both crystalline forms exhibited birefringence when examined under a polarizing microscope.

Relative Anticholinergic Potential Assay. Male albino quinea pigs (Hartley) were sacrificed. The trachea was cleaned of fat and connective tissue, separated at its juncture with the main bronchus and cut into helical strips approximately 2-mm wide. Two strips were prepared from each animal. One end was attached via a threaded loop to a stationary hook and submerged in a 10-mL organ bath, and the other end was attached by a thread to a force displacement transducer (Grass FT03). The Kreb's solution contained the following ions: NaCl, 118 mM; KCl, 4.7 mM; $MgCl_2$, 0.54 mM; $CaCl_2$, 2.5 mM; NaH_2PO_4, 1 mM; $NaHCO_3$, 25 mM; glucose, 11 mM. The tissues were maintained at a temperature of 37.5 °C and aerated with a mixture of 95% oxygen and 5% carbon dioxide to maintain the pH at 7.4. Two grams of resting tension was maintained for 1–2 h after which carbachol (10^{-5} M) was added to cause a maximum contraction. The tissue was then washed every 5 min with fresh solution for 30 min or until the tissue completely relaxed. A stepwise, cumulative log dose–response curve to carbachol was obtained by increasing the bath concentration 3-fold at each dose. Each addition of carbachol was made only after a previous dose had reached a steady-state level. Following the achievement of a maximal contractile response, the tissue was washed and allowed to return to baseline and remain there for at least 1 h. The antagonist was then added to a concentration of 1 mM, and 30 min later, the dose–response curve to carbachol was repeated. Control tissues received the same volume of Kreb's buffer as the test tissues. All antagonists were dissolved in appropriate volumes of buffer and were prepared immediately before the experiment. pA_2 values were determined according to the equation: $A'/A = 1 + B/K_B$.[14] In preliminary studies, atropine yielded a pA_2 value of 9.57 ± 0.23 ($K_B = 2.85 \times 10^{-10}$ M).

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Registry No. **2**, 63527-52-6; **3**, 65243-49-4; **4**, 95055-24-6; **5**, 127399-99-9; **6**, 84956-96-7; **7**, 98604-23-0; **8**, 127400-00-4; **9**, 86301-76-0; **10**, 98604-24-1; **11**, 83421-26-5; **12**, 83421-22-1; **13**, 127400-01-5; **14**, 127400-02-6; **15**, 127470-65-9; **16**, 127400-03-7; **17**, 84957-18-6; **18**, 83421-25-4; **19**, 83421-28-7; **20**, 127400-04-8; **21**, 127400-05-9; **22**, 127400-06-0; **23**, 84957-30-2; **24**, 86400-44-4; **25**, 86400-46-6; **26**, 92737-95-6; **27**, 86070-93-1; **28**, 127400-07-1; **29**, 127400-08-2; **30**, 127400-09-3; **31**, 127400-10-6; **32**, 127400-11-7; **33**, 86070-99-7; **34**, 86070-94-2; **35**, 127400-12-8; **36**, 127400-13-9; **37**, 127445-39-0; **38**, 127400-14-0; **39**, 86236-50-2; **40**, 86236-49-9; **41**, 86236-51-3; **42**, 86236-48-8; **43**, 86236-40-0; **44**, 86236-41-1; **45**, 86236-42-2; **46**, 86236-43-3; **47**, 86236-45-5; **48**, 86236-46-6; **49**, 127400-15-1; **50**, 127400-16-2; **51**, 89786-48-1; **52**, 89786-58-3; **53**, 98383-08-5; **54**, 114498-63-4; **55**, 114498-54-3; **56**, 114498-53-2; **57**, 114498-62-3; **58**, 98382-98-0; **59**, 98401-29-7; **60**, 98383-00-7; **61**, 98383-01-8; **62**, 98383-02-9; **63**, 98383-03-0; **64**, 98383-05-2; **65**, 98383-04-1; **66**, 97900-10-2; **67**, 97900-11-3; **68**, 127400-17-3; **69**, 98265-10-2; **70**, 127400-18-4; **71**, 118939-66-5; **72**, 98288-66-5; **73**, 98265-05-5; β-lactamase, 9073-60-3; 7β-[2-(2-aminothiazol-4-yl)-2-(*Z*)-methoximinoacetamide]-3-iodomethyl-2-[(trimethylsilyloxy)carbonyl]cephalosporin, 83421-24-3.

Supplementary Material Available: ^1H NMR for compounds **3–73** (9 pages). Ordering information is given on any current masthead.

(13) The sulfate salt of **59** was determined to be the monosulfate, dihydrate. Lilly Research Laboratories, unpublished findings.

(14) Tallarida, R. J.; Jacob, L. S. *The Dose–Response Relation in Pharmacology*; Springer-Verlag: New York, 1979.