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In Vitro Activities of ICI 194008 and ICI 193428, Two New Cephem Antimicrobial Agents

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The in vitro activities of two new cephem antibiotics, ICI 193428 and ICI 194008, were compared with those of cefpirome, cefotaxime, ceftazidime, and piperacillin. Essentially all strains of the family *Enterobacteriaceae* were inhibited by both study drugs at concentrations of ≤4 μg/ml. Both new cephems were comparable to ceftazidime against *Pseudomonas aeruginosa* (MIC for 90% of strains, 8 μg/ml) and were the most active agents tested against *Pseudomonas maltophilia* (MIC for 90% of strains, 16 μg/ml).

ICI 194008 and ICI 193428 are new semisynthetic aminothiazolyl cephalosporins structurally resembling ceftazidime in having an isobutyric acid residue on the oxime group in the side chain at position 7 of the cephem nucleus but differing in their side chains at position 3 (Fig. 1). In the present study, we examined the in vitro activity of these two cephems in comparison with those of cefpirome, cefotaxime, ceftazidime, and piperacillin against approximately 500 clinical isolates of gram-positive and gram-negative bacteria and other strains specifically selected for resistance to β-lactam antibiotics.

Most bacterial strains used in this study were clinical isolates (from 1983 to 1985) collected primarily at the New England Deaconess Hospital, Boston, Mass.; some additional strains had been obtained previously from the Massachusetts General Hospital, Boston, and other sources (6, 7). In certain specified studies, multiply resistant strains of *Pseudomonas aeruginosa* from our collection, established over the past 5 to 10 years, were used. *P. aeruginosa* PU21 transconjugants containing specific plasmid-mediated β-lactamases were provided by G. Jacoby (Massachusetts General Hospital) and have been described previously (1, 5, 8).

Standard antimicrobial reference powders were obtained from the following sources: ICI 194008 and ICI 193428, Imperial Chemical Industries, Macclesfield, Cheshire, United Kingdom; cefpirome (HR 810) and cefotaxime, Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.; ceftazidime, Glaxo Laboratories, Fort Lauderdale, Fla.; imipenem, Merck Sharp & Dohme, Rahway, N.J.; and piperacillin, Lederle Laboratories, Pearl River, N.Y.

Susceptibility testing was performed by a standard agar dilution technique (10, 13, 15) with Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.). When streptococci were tested, Mueller-Hinton agar was supplemented with 5% sheep blood. For *Haemophilus influenzae* and *Neisseria gonorrhoeae*, chocolate agar supplemented with 1% IsoVitaleX (BBL) was used. Inocula of approximately 10^4 CFU were prepared from overnight cultures and applied with a 32-prong replicating device. In certain specified studies of inoculum effect, different inocula were used and are so specified. Plates were examined for growth after 20 h of incubation at 37°C. All plates were incubated in room air except for *N. gonorrhoeae* strains, which were incubated in 5% CO_2.

Antagonism of the activity of ICI 194008 and cefoperazone by cefoxitin against five strains each of *Enterobacter cloacae*, *P. aeruginosa*, and *Serratia marcescens* was evaluated by using a disk approximation technique (3, 9). Disks contained 30 μg of cefoxitin, 75 μg of cefoperazone, or 50 μg of ICI 194008. The truncation of the inhibitory zones of the test drugs (ICI 194008 or cefoperazone) in proximity to the cefoxitin disk was evaluated (9).

The stabilities of ICI 193428 and ICI 194008 in Mueller-Hinton broth after 24 h of incubation at 37°C and at room temperature were determined by microbiologic assay (2) with *Micrococcus luteus* as the indicator organism.

ICI 194008 and ICI 193428 exhibited relatively comparable activities against all organisms tested, with ICI 193428 frequently more active than ICI 194008 by only a single dilution (Table 1). When tested against members of the family *Enterobacteriaceae*, both new cephems exhibited activity generally equivalent to that of ceftazidime but inferior to that of cefpirome. However, ICI 194008 and ICI 193428 were notably more active than ceftazidime against *E. cloacae*, *Enterobacter aerogenes*, and *Citrobacter freundii*, with MICs for 90% of the strains (MIC_{90}s) of all these species equal to or less than 4 μg/ml, compared with MIC_{90}s of 16 to 64 μg/ml for ceftazidime. ICI 194008 and ICI 193428 were the most active antibiotics tested against *Pseudomonas maltophilia*, inhibiting 90% of the strains at ≤16 μg/ml, compared with MIC_{90}s of >128 μg/ml for both cefpirome and ceftazidime. Against *P. aeruginosa*, ICI 194008, ICI 193428, and ceftazidime were the most active agents tested (MIC_{90}s, ≤8 μg/ml).

Against most gram-positive bacteria, ICI 194008 and ICI 193428 exhibited activities very similar to those of ceftazidime but inferior to those of cefotaxime and cefpirome. However, when tested against methicillin-susceptible strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*, ICI 194008 exhibited activity comparable to that of cefotaxime and significantly superior to that of ceftazidime, inhibiting 90% of such strains at concentrations of 4.0 and 16.0 μg/ml, respectively. Both new cephems were ineffective against enterococci, *Listeria monocytogenes*, penicillin-resistant pneumococci, and methicillin-resistant staphylococci.

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TABLE 1. Drugs and organisms tested

Organism (no. of isolates)	Drug	MIC (μg/ml)		
		Range	50%	90%
Escherichia coli (40)	ICI 194008	≤0.06–0.25	0.125	0.25
	ICI 193428	≤0.06–0.125	≤0.06	0.125
	Cefpirome	≤0.06–0.125	≤0.06	0.125
	Cefotaxime	≤0.06–0.125	≤0.06	0.125
	Ceftazidime	≤0.06–0.5	0.125	0.25
	Piperacillin	0.5–>128	2	64
Klebsiella pneumoniae (30)	ICI 194008	≤0.06–1	0.125	0.5
	ICI 193428	≤0.06–1	≤0.06	0.5
	Cefpirome	≤0.06–4	≤0.06	≤0.06
	Cefotaxime	≤0.06–2	≤0.06	0.125
	Ceftazidime	≤0.06–2	0.25	0.5
	Piperacillin	2–>128	8	64
Enterobacter cloacae (30)	ICI 194008	0.125–8	0.25	2
	ICI 193428	≤0.06–16	0.125	2
	Cefpirome	≤0.06–8	≤0.06	0.5
	Cefotaxime	0.125–>64	0.25	32
	Ceftazidime	0.125–>64	0.50	16
	Piperacillin	1.0–>128	4	16
Enterobacter aerogenes (20)	ICI 194008	0.125–8	0.25	4
	ICI 193428	≤0.06–16	0.125	4
	Cefpirome	≤0.06–1.0	≤0.06	0.25
	Cefotaxime	≤0.06–32	0.125	16
	Ceftazidime	0.125–64	0.25	32
	Piperacillin	1.0–128	4	64
Citrobacter freundii (20)	ICI 194008	0.125–4	0.5	4
	ICI 193428	≤0.06–16	0.125	4
	Cefpirome	≤0.06–4	≤0.06	1.0
	Cefotaxime	≤0.06–64	0.125	16
	Ceftazidime	0.125–>64	0.5	64
	Piperacillin	2–128	4	64
Serratia marcescens (40)	ICI 194008	0.25–8.0	0.5	0.5
	ICI 193428	≤0.06–16	0.25	0.5
	Cefpirome	≤0.06–16	0.125	0.25
	Cefotaxime	0.125–64	0.5	4
	Ceftazidime	0.25–16	0.25	0.5
	Piperacillin	2–128	4	16
Proteus mirabilis (20)	ICI 194008	≤0.06–0.25	0.125	0.125
	ICI 193428	≤0.06	≤0.06	≤0.06
	Cefpirome	≤0.06–0.25	0.125	0.25
	Cefotaxime	≤0.06	≤0.06	≤0.06
	Ceftazidime	≤0.06–0.125	≤0.06	0.125
	Piperacillin	0.5–>128	1.0	128
Proteus vulgaris (10)	ICI 194008	0.125–0.25	0.125	0.25
	ICI 193428	≤0.06–0.125	≤0.06	0.125
	Cefpirome	0.125–1.0	0.25	1.0
	Cefotaxime	≤0.06–0.5	≤0.06	0.5
	Ceftazidime	≤0.06–0.125	0.125	0.125
	Piperacillin	0.05–8	1.0	8
Morganella morganii (10)	ICI 194008	0.5–1.0	0.5	1.0
	ICI 193428	≤0.06–2	0.125	0.5
	Cefpirome	≤0.06–0.5	≤0.06	0.25
	Cefotaxime	≤0.06–8	0.5	2
	Ceftazidime	0.25–16	0.5	2
	Piperacillin	1.0–64	2	8
Pseudomonas aeruginosa (40)	ICI 194008	2–16	4	8
	ICI 193428	1.0–32	4	8
	Cefpirome	0.5–64	4	16
	Cefotaxime	8–>128	32	128
	Ceftazidime	0.5–64	2	8
	Piperacillin	1.0–>128	8	32
Pseudomonas cepacia (10)	ICI 194008	1.0–16	4	16
	ICI 193428	1.0–16	2	16
	Cefpirome	4–32	4	16
	Cefotaxime	2–16	2	16
	Ceftazidime	0.5–32	2	32
	Piperacillin	1.0–>128	16	>128

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TABLE 1—*Continued*

Organism (no. of isolates)	Drug	MIC (μg/ml)		
		Range	50%	90%
Pseudomonas maltophilia (10)	ICI 194008	1.0–32	4	16
	ICI 193428	2–16	4	16
	Cefpirome	128–>128	>128	>128
	Cefotaxime	64–>128	128	>128
	Ceftazidime	4–>128	128	>128
	Piperacillin	>128	>128	>128
Aeromonas hydrophila (10)	ICI 194008	0.25–2	0.5	1.0
	ICI 193428	0.125–4	0.25	1.0
	Cefpirome	≤0.06–0.5	≤0.06	0.25
	Cefotaxime	≤0.06–1.0	≤0.06	1.0
	Ceftazidime	0.125–1.0	0.125	1.0
	Piperacillin	1.0–64	2	4
Acinetobacter calcoaceticus subsp. *anitratus* (10)	ICI 194008	4–64	16	32
	ICI 193428	4–>64	32	64
	Cefpirome	1.0–>64	4	64
	Cefotaxime	8–>64	16	64
	Ceftazidime	2–>64	16	64
	Piperacillin	16–128	32	128
Haemophilus influenzae, β-lactamase negative (10)	ICI 194008	≤0.06	≤0.06	≤0.06
	ICI 193428	≤0.06–0.125	≤0.06	≤0.06
	Cefpirome	≤0.06–0.25	≤0.06	≤0.06
	Cefotaxime	≤0.06	≤0.06	≤0.06
	Ceftazidime	≤0.06–0.25	≤0.06	0.125
	Piperacillin	≤0.06–0.125	≤0.06	0.125
Haemophilus influenzae, β-lactamase positive (10)	ICI 194008	≤0.06	≤0.06	≤0.06
	ICI 193428	≤0.06	≤0.06	≤0.06
	Cefpirome	≤0.06–0.125	0.125	0.125
	Cefotaxime	≤0.06	≤0.06	≤0.06
	Ceftazidime	≤0.06–0.125	≤0.06	0.125
	Piperacillin	64–>128	128	>128
Neisseria gonorrhoeae, β-lactamase positive (10)	ICI 194008	≤0.06–0.125	≤0.06	≤0.06
	ICI 193428	≤0.06	≤0.06	≤0.06
	Cefpirome	≤0.06	≤0.06	≤0.06
	Cefotaxime	≤0.06	≤0.06	≤0.06
	Ceftazidime	≤0.06	≤0.06	≤0.06
	Piperacillin	0.125–128	2	32
Staphylococcus aureus, methicillin susceptible (15)	ICI 194008	2–8	2	4
	ICI 193428	2–8	4	4
	Cefpirome	1.0–2	1.0	2
	Cefotaxime	2–4	2	4
	Ceftazidime	8–32	16	16
	Piperacillin	1.0–32	8	32
Staphylococcus epidermidis, methicillin susceptible (14)	ICI 194008	1.0–>64	4	16
	ICI 193428	2–>64	4	64
	Cefpirome	0.128–8	0.5	4
	Cefotaxime	0.25–>64	2	16
	Ceftazidime	2–64	8	32
	Piperacillin	0.125–32	2	32
Streptococcus pyogenes (10)	ICI 194008	≤0.06–0.125	0.125	0.125
	ICI 193428	≤0.06–0.125	0.125	0.125
	Cefpirome	≤0.06	≤0.06	≤0.06
	Cefotaxime	≤0.06	≤0.06	≤0.06
	Ceftazidime	0.125–0.25	0.125	0.125
	Piperacillin	≤0.06–0.125	≤0.06	≤0.125
Streptococcus agalactiae (10)	ICI 194008	0.5–2	0.5	0.5
	ICI 193428	0.25–2	0.25	0.25
	Cefpirome	≤0.06–0.125	≤0.06	≤0.06
	Cefotaxime	≤0.06–0.25	≤0.06	0.25
	Ceftazidime	0.5–2	0.5	1.0
	Piperacillin	0.125–2	0.125	0.125
Group G and C streptococci (9)	ICI 194008	0.125–0.5		
	ICI 193428	0.125–0.5		
	Cefpirome	≤0.06		
	Cefotaxime	≤0.06		
	Ceftazidime	0.25–0.5		
	Piperacillin	≤0.06–0.125		

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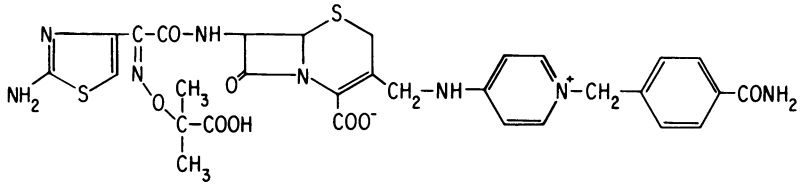
TABLE 1—*Continued*

Organism (no. of isolates)	Drug	MIC (μg/ml)		
		Range	50%	90%
Viridans group streptococci, penicillin susceptible (20)	ICI 194008	0.06–2	0.5	2
	ICI 193428	≤0.03–4	2	4
	Cefpirome	≤0.03–0.06	0.06	0.06
	Cefotaxime	≤0.03–0.25	0.125	0.125
	Ceftazidime	0.06–4	1.0	4
	Piperacillin	≤0.03–1.0	0.125	0.25
Viridans group streptococci, penicillin resistant (6)	ICI 194008	4–32		
	ICI 193428	4–32		
	Cefpirome	0.25–2		
	Cefotaxime	0.5–8		
	Ceftazidime	4–32		
	Piperacillin	2–32		
Streptococcus pneumoniae, pencillin resistant (10)	ICI 194008	8–>64	>64	>64
	ICI 193428	8–>64	>64	>64
	Cefpirome	2–64	8	64
	Cefotaxime	0.5–32	8	8
	Ceftazidime	8–>64	64	>64
	Piperacillin	2–32	4	16
Enterococcus faecalis (20)	ICI 194008	>64	>64	>64
	ICI 193428	>64	>64	>64
	Cefpirome	4–32	16	32
	Cefotaxime	32–>64	>64	>64
	Ceftazidime	32–>64	>64	>64
	Piperacillin	2–8	4	4
Enterococcus faecium (10)	ICI 194008	>64	>64	>64
	ICI 193428	>64	>64	>64
	Cefpirome	>64	>64	>64
	Cefotaxime	>64	>64	>64
	Ceftazidime	>64	>64	>64
	Piperacillin	32–128	64	64
Streptococcus avium (10)	ICI 194008	>64	>64	>64
	ICI 193428	>64	>64	>64
	Cefpirome	4–>64	8	32
	Cefotaxime	4–>64	8	32
	Ceftazidime	32–>64	64	>64
	Piperacillin	16–128	16	32
Listeria monocytogenes (10)	ICI 194008	32–>64	>64	>64
	ICI 193428	16–>64	>64	>64
	Cefpirome	1.0–>64	64	>64
	Cefotaxime	2–>64	>64	>64
	Ceftazidime	32–>64	>64	>64
	Piperacillin	4–8	4	8



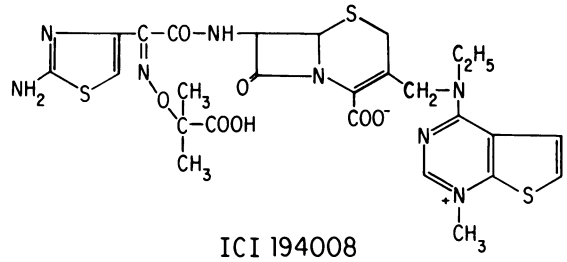


FIG. 1. Chemical structures of ICI 193428 and ICI 194008.

TABLE 2. Comparative activity of ICI 194008 against strains of *P. aeruginosa* isogenic except for the production of specific plasmid-mediated β-lactamases

β-Lactamase	MIC (μg/ml)			
	ICI 194008	Piperacillin	Ceftazidime	Imipenem
None	4	8	2	8
TEM-1	8	>128	4	4
TEM-2	4	>128	4	8
OXA-1	4	128	2	8
OXA-2	16	128	32	8
OXA-3	16	>128	32	8
PSE-1	4	>128	4	4
PSE-2	4	64	2	8
PSE-3	8	128	4	8
PSE-4	8	>128	4	8

Activities against β-lactamase-producing transconjugants of *P. aeruginosa* PU21 are presented in Table 2. In comparison with its activity against the plasmid-free parent strain, the activity of ICI 194008 was adversely affected only by the presence of OXA-2 and OXA-3 enzymes. This effect was similar to but of a lesser degree than that observed with ceftazidime. The activity of ICI 193428 was not tested against these strains. Neither drug exhibited a significant inoculum effect when tested at 10^4 and 10^6 CFU, except that there was a fourfold rise only in the MIC_{90} of ICI 193428 against *P. aeruginosa*.

ICI 194008 was not antagonized by cefoxitin against any strains of *E. cloacae*, *P. aeruginosa*, or *Serratia marcescens*. In contrast, the activity of cefoperazone was consistently antagonized by cefoxitin against these same strains.

The bioactivity of ICI 193428 did not decrease significantly after 24 h of incubation in Mueller-Hinton broth at either 37°C or room temperature. The bioactivity of ICI 194008 decreased by approximately 20% after 24 h of incubation in Mueller-Hinton broth at 37°C but did not decrease after 24 h of incubation at room temperature.

The in vitro activities of ICI 194008 and ICI 193428 against a broad range of gram-negative organisms closely resembled those of ceftazidime. The two new cephalosporins were significantly more active than ceftazidime, though they were less active than cefpirome against *Enterobacter* species, *Morganella morganii*, and *C. freundii*, species which commonly produce inducible chromosomal β-lactamases (4, 14). This superior activity of the new compounds against these strains and the lack of antagonism of the activity of ICI 194008 by cefoxitin, a potent inducer of chromosomal β-lactamase (12) suggest that the new drugs may be resistant to hydrolysis by such enzymes. However, specific hydrolysis studies need to be performed to determine this.

Both of the new cephems exhibited marked activity against *P. maltophilia*, which is often resistant to a broad range of other β-lactam antibiotics by virtue of a broad-spectrum cephalosporinase common in this organism (11). Given the potential importance of *P. maltophilia* as a multiply resistant nosocomial pathogen (16), additional studies to confirm the activities of the new antibiotics against this species and to characterize the stability of these compounds to hydrolysis by β-lactamases produced by these organisms would be of interest.

In summary, the two new cephalosporins studied here were found to have activities comparable to those of ceftazidime, a drug that has already proved to be of significant clinical use. Superior activities against *Enterobacter* species, *C. freundii*, and *P. maltophilia* may prove to be notable advantages of these drugs. The results of this study would support further in vitro and in vivo evaluation of one or both of these new cephems.

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