L-658,310, A NEW INJECTABLE CEPHALOSPORIN

I. *IN VITRO* ANTIBACTERIAL PROPERTIES†

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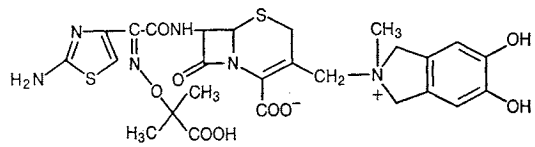
(Received for publication June 23, 1988)

The *in vitro* antibacterial spectrum of L-658,310, a new semisynthetic cephalosporin, was compared with ceftazidime, aztreonam and piperacillin against a wide variety of randomly selected human clinical isolates. The compound was found to be a broad spectrum bactericidal agent that was more potent than any of the comparison drugs against glucose non-fermenting bacteria. It has especially potent activity against *Pseudomonas aeruginosa* including multiply-resistant strains. The superior activity of L-658,310 against this group of organisms is attributed to the presence of the dihydroxy substituents on the 2-methylisoindoline moiety of the compound. L-658,310 is not cross-resistant with either imipenem, ceftazidime or piperacillin (representatives of three different classes of β-lactam compounds) against *P. aeruginosa*. The lack of cross-resistance with ceftazidime extends to other glucose non-fermenters and several strains of Enterobacteriaceae as well. The compound is active against bacteria known to possess either R-plasmid- or chromosomally-mediated β-lactamases.

L-658,310 (Fig. 1) is a novel injectable cephalosporin[1,2] synthesized at the Okazaki Research Laboratories of the Banyu Pharmaceutical Co., Ltd., Japan, an affiliate of Merck & Co., Inc., U.S.A. It is designated by Banyu as BO-1236. The compound has four functional groups, three of which are responsible for the outstanding microbiological activity of the compound. The *cis* alkoximino substituent on the α carbon atom of the 7-β-acylamino group is responsible for the stability of the compound to bacterial β-lactamases[3,4] and the *gem*-dimethyl substituents add the anti-*Pseudomonas* activity to the compound[4~6]. This activity is both enhanced and extended to other glucose non-fermenting bacteria by the dihydroxy substituents on the 2-methylisoindoline moiety, which by itself, especially in these organisms, is thought to facilitate the entry of the compound into the cell. The fourth group, the 3-methyl-1-methylpyrrolidium position of the molecule is thought to be responsible for the relatively long serum half-life[7~9] of the compound.

Fig. 1. Structure of L-658,310 (BO-1236).



Summarized in this paper are the *in vitro* antibacterial spectrum and associated antibacterial properties of L-658,310.

† Presented in part at the 27th Intersci. Conf. on Antimicrob. Agents Chemother.[1].

Materials and Methods

Antimicrobial Agents

The dehydroxy-, monohydroxy- and dihydroxyisoindoline (L-658,310) substituted cephems were synthesized at the Okazaki Research Laboratories, Banyu Pharmaceutical Co., Ltd., Japan. Other standard antimicrobial powders were supplied as follows: Ceftazidime, Glaxo Group Research Ltd.; aztreonam, E.R. Squibb & Sons, Inc.; piperacillin, Lederle Laboratories; imipenem, Merck Sharp & Dohme Research Laboratories; cefpirome, Hoechst-Roussel Pharmaceuticals Inc.; ceftriaxone, Hoffmann-La Roche Inc.; moxalactam, Eli Lilly and Company; cefoperazone, Pfizer International Inc.; cefepime, Bristol-Myers Co.; and cefsulodin, Abbott Laboratories.

Test Strains

Most of the bacterial strains used in this study were randomly selected human clinical isolates collected from various hospitals throughout the U.S.A. These organisms were maintained as suspensions in 15% skim milk or on Trypticase Soy agar (TSA; BBL) slants stored at −70°C. The numbers of these cultures are those of the Merck stock culture collection (MB) and the Clinical Laboratory Culture Collection (CL). The inocula for the susceptibility and bactericidal tests were broth dilutions or cell suspensions of fresh overnight cultures. The glucose non-fermenters were all grown on a G10 Gyrotory Shaker (New Brunswick Scientific Co., Inc., New Brunswick, NJ, U.S.A.) at a speed of 250 rpm; the other organisms were grown without shaking.

Antimicrobial Activity

The susceptibility of aerobic, facultative anaerobic, or obligate anaerobic bacteria was determined using an agar dilution method. Each compound was dissolved according to the manufacturer's instructions at a concentration of 1.28 mg/ml and subsequent 2-fold serial dilutions were made in sterile distilled water or in 0.07 M SORENSEN's phosphate buffer, pH 7.0. One ml of each antibiotic-containing solution was mixed with 9 ml of molten agar in 15×100 mm Petri dishes. Brain heart infusion agar (Difco), supplemented with yeast extract 5%, hemin 5 μg/ml, menadione 0.5 μg/ml and defibrinated sheep blood 5%, was employed for the strict anaerobes. Mueller-Hinton agar (M-H; Difco), supplemented with lysed horse blood 5% for the Streptococci or with bovine hemoglobin 1% and IsoVitaleX (BBL) 1% for the *Neisseria* and *Haemophilus*, was used for the fastidious organisms. TSA was employed for the remaining organisms. The antibiotic-agar plates were inoculated with the cultures using a Denley Multipoint Inoculator (Denley Instruments Ltd., Sussex, England) designed to deliver 1 μl directly onto the agar surface. A final inoculum of 10^6 cfu/spot was used for the strict anaerobes, while, for the other organisms, 10^4, 10^5 or 10^6 cfu/spot was used, depending on the experimental design. The strict anaerobes were incubated in a Forma Scientific anaerobic system, (Forma Scientific, Marietta, Ohio, U.S.A.) for 48 hours at 35°C. Anaerobic conditions were provided by an atmosphere of hydrogen 10%, carbon dioxide 8% and nitrogen 82%. All other organisms were incubated in ambient atmosphere for 20 hours at 35°C except for the *Neisseria*, which were incubated in a Forma Scientific water-jacketed incubator enriched with CO_2 5%. The MIC in each case was defined as the lowest concentration of antibiotic showing no distinct growth or less than five discrete colonies/spot.

Determination of Susceptibility to Antimicrobial Agents

Susceptibility of clinical isolates to selected antibacterial agents was determined by the NCCLS single, high concentration disk susceptibility method[10,11].

Bactericidal Activity

The MIC of each culture was first determined by a microdilution method in Trypticase Soy broth (TSB; BBL). Each compound was dissolved as previously described, diluted 1:10 in TSB and then sterilized by filtration through a 0.22-μm Millex disposable filter unit fitted onto a 10-ml glass syringe. Subsequent 2-fold serial dilutions were then made with TSB giving final test concentrations of 128 to 0.125 μg/ml. A Dynatech MIC-2000 Inoculator (Dynatech Laboratories, Inc., Alexandria, Virginia, U.S.A.) was employed to add 1.5 μl of an appropriately diluted test culture to each 50 μl dilution of

compound, thereby achieving a final cell density of approximately 1×10^5 cfu/ml in each test well. After incubation at 35°C for 20 hours, the test wells were examined for growth and the MIC was recorded as the lowest level of compound that prevented visible growth.

After the MIC was determined, the plates were shaken vigorously, using a Dynatech Micro-Shaker II, and 1.5 μl from each well was transferred to the surface of drug-free TSA plates, again using the MIC-2000 inoculator. These plates were then incubated at 35°C for 20 hours. The MBC was defined as the lowest concentration of antibiotic that permitted no growth or <3 discrete colonies on subculture, indicating that at least 98% of the initial inoculum had been killed.

Results and Discussion

Activity against Aerobic and Facultative Anaerobic Bacteria

L-658,310 is a broad spectrum β-lactam antibiotic (Fig. 1) that is more potent than either ceftazidime, aztreonam or piperacillin against glucose non-fermenting bacteria (Table 1). Many of the 48 strains of *Pseudomonas aeruginosa* tested were resistant to at least one compound belonging to the fluoroquinolone, aminoglycoside, or β-lactam classes of antibacterial agents. L-658,310 had an MIC of <4 μg/ml for 90% of these strains. Good potency was also demonstrated against *Acinetobacter anitratus* (MIC_{90} of 1 μg/ml), *Acinetobacter lwoffi* (MIC_{90} of 2 μg/ml) and *Pseudomonas maltophilia* (MIC_{90} of 16 μg/ml), organisms that are usually resistant to β-lactam antibiotics. In addition, exceptional activity of the compound was shown against *Pseudomonas cepacia* with an MIC_{90} of 0.25 μg/ml.

L-658,310 was also more potent than any of the comparison drugs against the strains of *Enterobacter aerogenes* (MIC_{90} of 1 μg/ml), *Enterobacter cloacae* (MIC_{90} of 4 μg/ml) and *Citrobacter freundii* (MIC_{90} of 1 μg/ml) tested. Against the other Enterobacteriaceae, however, the MIC_{90} for L-658,310, which was never more than 2 μg/ml, was within 2-fold of that found for ceftazidime. Both L-658,310 and ceftazidime were more active than aztreonam against strains of *Klebsiella oxytoca* and *Serratia marcescens*, but aztreonam was the most active agent against the other strains of Enterobacteriaceae. Both ceftazidime and aztreonam (MIC_{90}s of 0.125 μg/ml) were slightly more active than L-658,310 (MIC_{90} of 0.5 μg/ml) against ampicillin-resistant *Haemophilus influenzae*, while ceftazidime was the only agent that was more effective than L-658,310 against *Neisseria gonorrhoeae* (respective MIC_{90}s of $\leq$0.008 and 0.063 μg/ml). Piperacillin was the least potent antibiotic against all of these Gram-negative organisms except for the ampicillin-susceptible strains of *H. influenzae*, against which, it was the most potent.

Although L-658,310 has activity against a variety of Gram-positive bacteria, it has a narrower spectrum and is less potent against these strains than against the Gram-negative organisms. Good activity was demonstrated for the compound against *Streptococcus agalactiae*, *Streptococcus pneumoniae* and *Streptococcus pyogenes* (MIC_{90}s of 8, 8 and 1 μg/ml, respectively); however, it had generally poor activity against methicillin-susceptible strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* (MIC_{90}s of 32 μg/ml). Thus, L-658,310 differs from aztreonam, which has little or no activity against these pathogens. As with other cephalosporin antibiotics, L-658,310 has no activity against *Enterococcus faecalis* at 128 μg/ml, but activity has been demonstrated at 32 to 128 μg/ml against methicillin-resistant Staphylococci (data not shown).

Activity Against Obligate Anaerobic Bacteria

L-658,310 has a spectrum of activity and degree of potency similar to that of ceftazidime against the clinical isolates of obligate anaerobic bacteria tested (Table 2). Both compounds exhibited good

Table 1. Comparative *in vitro* activity of L-658,310 against aerobic and facultative anaerobic clinical isolates.

Organism (No.)	Antibiotic	Range	MIC (μg/ml)[a]	
			50%	90%
Acinetobacter anitratus (10)	L-658,310	0.25~4	0.5	1
	Ceftazidime	2~32	4	8
	Aztreonam	8~32	32	32
	Piperacillin	8~>128	16	>128
A. lwoffi (10)	L-658,310	0.063~4	0.5	2
	Ceftazidime	0.125~64	1	8
	Aztreonam	0.125~>128	8	64
	Piperacillin	1~>128	8	64
Citrobacter diversus (10)	L-658,310	0.5~1	1	1
	Ceftazidime	0.125~0.5	0.25	0.5
	Aztreonam	0.063~0.125	0.06	0.125
	Piperacillin	32~64	64	64
C. freundii (22)	L-658,310	0.063~2	0.25	1
	Ceftazidime	0.125~64	0.5	16
	Aztreonam	0.03~128	0.125	16
	Piperacillin	2~>128	4	>128
Enterobacter aerogenes (13)	L-658,310	0.063~16	0.25	1
	Ceftazidime	0.03~64	0.25	64
	Aztreonam	0.03~16	0.125	16
	Piperacillin	0.25~>128	4	>128
E. cloacae (10)	L-658,310	0.5~4	2	4
	Ceftazidime	0.25~128	1	64
	Aztreonam	0.063~32	0.25	32
	Piperacillin	2~>128	4	>128
Escherichia coli ampicillin-susceptible (7)	L-658,310	0.063~1		0.5
	Ceftazidime	0.125~0.5		0.25
	Aztreonam	0.063~0.125		0.125
	Piperacillin	1~4		2
E. coli ampicillin-resistant (13)	L-658,310	0.063~1	0.25	0.5
	Ceftazidime	0.125~1	0.25	0.25
	Aztreonam	0.063~0.25	0.125	0.25
	Piperacillin	32~>128	>128	>128
Haemophilus influenzae ampicillin-susceptible (18)	L-658,310	0.015~0.25	0.125	0.125
	Ceftazidime	≤0.008~0.25	0.03	0.125
	Aztreonam	≤0.008~0.06	≤0.008	0.03
	Piperacillin	≤0.008~0.03	≤0.008	≤0.008
H. influenzae ampicillin-resistant (12)[b]	L-658,310	0.015~1	0.03	0.5
	Ceftazidime	≤0.008~0.5	0.015	0.125
	Aztreonam	≤0.008~0.5	≤0.008	0.125
	Piperacillin	≤0.008~128	32	64
Klebsiella oxytoca (10)	L-658,310	0.125~1	0.25	1
	Ceftazidime	0.063~1	0.25	1
	Aztreonam	0.063~32	0.5	8
	Piperacillin	8~>128	16	>128
K. pneumoniae (20)	L-658,310	0.063~1	0.25	1
	Ceftazidime	0.125~0.5	0.25	0.5
	Aztreonam	0.03~0.12	0.063	0.125
	Piperacillin	4~>128	8	32
Morganella morganii (20)	L-658,310	0.03~1	0.25	1
	Ceftazidime	0.063~16	0.25	1
	Aztreonam	0.03~1	0.063	0.25
	Piperacillin	0.5~>128	4	64
Neisseria gonorrhoeae penicillin-susceptible and -resistant (9)	L-658,310	≤0.008~0.06		0.063
	Ceftazidime	≤0.008~0.12		≤0.008
	Aztreonam	≤0.008~0.12		0.063
	Piperacillin	≤0.008~16		0.5
Proteus mirabilis (10)	L-658,310	0.125~0.5	0.25	0.5
	Ceftazidime	0.063~0.25	0.125	0.25
	Aztreonam	≤0.03~0.06	≤0.03	≤0.03
	Piperacillin	0.25~2	1	2

Table 1. (Continued)

Organism (No.)	Antibiotic	Range	MIC (μg/ml)[a]	
			50%	90%
P. vulgaris (10)	L-658,310	0.063~0.5	0.25	0.25
	Ceftazidime	0.03~0.25	0.125	0.125
	Aztreonam	≤0.03~0.12	≤0.03	≤0.03
	Piperacillin	0.5~8	1	4
Providencia rettgeri (12)	L-658,310	0.063~0.5	0.063	0.25
	Ceftazidime	0.03~0.5	0.063	0.5
	Aztreonam	≤0.008~0.25	0.015	0.063
	Piperacillin	0.25~4	0.5	4
P. stuartii (20)	L-658,310	0.125~2	0.25	1
	Ceftazidime	0.125~2	0.5	1
	Aztreonam	≤0.008~0.25	0.03	0.063
	Piperacillin	1~>128	8	>128
Pseudomonas aeruginosa (48)	L-658,310	0.063~8	1	4
	Ceftazidime	0.5~>128	2	32
	Aztreonam	0.125~128	4	32
	Piperacillin	2~>128	16	>128
P. cepacia (15)	L-658,310	≤0.03~0.25	0.063	0.25
	Ceftazidime	1~8	2	4
	Aztreonam	8~128	32	64
	Piperacillin	4~128	8	64
P. maltophilia (20)	L-658,310	0.25~32	2	16
	Ceftazidime	2~>128	32	128
	Aztreonam	8~>128	128	>128
	Piperacillin	16~>128	>128	>128
Salmonella sp. (10)	L-658,310	0.03~0.5	0.063	0.125
	Ceftazidime	0.125~2	0.25	0.25
	Aztreonam	0.063~0.25	0.063	0.125
	Piperacillin	2~>128	2	32
Serratia marcescens (20)	L-658,310	0.125~4	1	2
	Ceftazidime	0.125~2	1	2
	Aztreonam	0.125~16	1	4
	Piperacillin	2~>128	>128	>128
Shigella sp. (10)	L-658,310	0.125~1	0.25	0.5
	Ceftazidime	0.063~2	0.125	0.5
	Aztreonam	0.03~2	0.063	0.125
	Piperacillin	1~>128	2	>128
Staphylococcus aureus methicillin-susceptible (10)	L-658,310	16~64	32	32
	Ceftazidime	4~16	8	8
	Aztreonam	>128	>128	>128
	Piperacillin	0.5~64	4	64
S. epidermidis methicillin-susceptible (10)	L-658,310	16~64	32	32
	Ceftazidime	2~16	4	8
	Aztreonam	>128	>128	>128
	Piperacillin	0.25~32	2	16
Streptococcus agalactiae Group B (11)	L-658,310	2~8	4	8
	Ceftazidime	0.125~0.25	0.25	0.5
	Aztreonam	>128	>128	>128
	Piperacillin	0.125~0.25	0.125	0.25
S. pneumoniae penicillin-susceptible and -resistant (24)	L-658,310	0.25~16	0.5	8
	Ceftazidime	≤0.008~8	0.063	1
	Aztreonam	2~>128	32	>128
	Piperacillin	≤0.008~4	≤0.008	0.25
S. pyogenes Group A (20)	L-658,310	0.5~1	0.5	1
	Ceftazidime	0.015~0.063	0.063	0.063
	Aztreonam	8~32	16	16
	Piperacillin	≤0.008~0.03	0.03	0.03

[a] Agar dilution method; 10^5 cfu/spot; 50 and 90%, MIC of the antibiotic that inhibited 50 and 90%, respectively, of the isolates.

[b] Includes three β-lactamase negative strains.

Table 2. Comparative *in vitro* activity of L-658,310 against selected obligate anaerobic clinical isolates.

Microorganism	Strain No.	MIC (μg/ml)[a]			
		L-658,310	Ceftazidime	Aztreonam	Piperacillin
Bacteroides fragilis	CL 279	8	16	64	4
	CL 280	8	16	64	2
	CL 283	4	4	16	1
	CL 285	4	4	64	1
	CL 287	4	4	64	2
B. distasonis	CL 233	>128	>128	>128	>128
	CL 234	>128	>128	>128	128
	CL 247	64	32	>128	8
	CL 286	>128	>128	>128	>128
B. thetaiotaomicron	CL 271	>128	>128	64	16
	CL 274	>128	>128	>128	32
	CL 276	>128	>128	>128	128
	CL 290	>128	32	>128	32
B. vulgatus	CL 244	>128	128	128	16
	CL 245	64	32	2	8
	CL 246	128	64	32	8
	CL 258	64	64	4	4
	CL 275	32	16	2	8
Clostridium butyricum	CL 123	>128	>128	>128	0.063
C. difficile	CL 218	>128	>128	>128	4
	CL 219	>128	>128	>128	4
	CL 220	>128	>128	>128	2
	CL 221	>128	>128	>128	2
	CL 222	32	32	>128	8
C. innocuum	CL 293	16	128	>128	1
C. perfringens	CL 151	2	1	>128	≤0.063
	CL 152	0.5	1	>128	≤0.063
	CL 153	2	1	>128	≤0.063
	CL 155	1	4	>128	0.125
	CL 156	1	2	>128	0.125
	CL 253	0.5	2	>128	0.125
	CL 254	1	1	>128	0.125
	CL 255	0.5	2	>128	0.125
	CL 256	0.5	2	>128	0.125
C. tertium	CL 129	>128	32	128	2
	CL 132	32	>128	>128	2
Peptostreptococcus anaerobius	CL 107	8	2	128	0.125
Peptococcus prevotii	CL 101	16	4	>128	0.5
	CL 106	32	8	>128	0.063
	CL 112	16	4	>128	0.125

[a] Agar dilution assay; 10^6 cfu/spot; supplemented Brain Heart Infusion agar.

activity against strains of *Bacteroides fragilis*, *Clostridium perfringens* and several of the anaerobic cocci with an MIC range of 0.5 to 32 μg/ml for L-658,310 and an MIC range of 1 to 16 μg/ml for ceftazidime. However, little or no activity was observed for either of these compounds against most of the other anaerobic bacteria. In contrast, piperacillin was found to be a very potent antibacterial agent (range of MICs, <0.063 to 16 μg/ml) against all of the anaerobes, except for a few strains of *Bacteroides*. Aztreonam had little or no activity against any of these organisms except one strain of *B. fragilis* (MIC, 16 μg/ml) and three strains of *Bacteroides vulgatus* (range of MICs, 2 to 4 μg/ml).

Table 3. Bactericidal activity of L-658,310, imipenem and ceftazidime for resistant clinical strains of *Pseudomonas aeruginosa*.

Strain No.	Resistant to[a]	MIC (μg/ml)[b]			MBC (μg/ml)[c]		
		L-658,310	IPM	CAZ	L-658,310	IPM	CAZ
CL 1440	GM	1.0	4.0	1.0	1.0	8.0	1.0
CL 1999	AN, CB, GM	4.0	4.0	2.0	4.0	4.0	2.0
CL 2049	GM, NN	0.5	2.0	1.0	1.0	2.0	2.0
CL 2411	AN, GM	8.0	1.0	2.0	8.0	4.0	2.0
CL 2850	AN, CB, GM, IPM, PIP	1.0	16.0	4.0	2.0	32.0	4.0
CL 2979	AN, CB, CAZ, GM, IPM	1.0	32.0	32.0	1.0	32.0	32.0
CL 3015	AN, CB, GM, IPM, MOX, PIP	4.0	16.0	16.0	8.0	32.0	16.0
CL 3092	CB, CAZ, IPM, MOX, PIP	4.0	16.0	64.0	4.0	16.0	128.0

[a] Resistance determined by NCCLS standard disk diffusion assay[10,11].

[b] MIC: Microdilution broth method; 10^5 cfu/ml; Trypticase Soy broth.

[c] MBC: Lowest concentration at which 98% of the initial inoculum was killed.

AN: Amikacin, CAZ: ceftazidime, CB: carbenicillin, GM: gentamicin, IPM: imipenem, MOX: moxalactam, NN: tobramycin, PIP: piperacillin.

Bactericidal Activity

The bactericidal activity of L-658,310 was compared with that of imipenem and ceftazidime against eight strains of *P. aeruginosa* selected for their resistance to either one or two aminoglycosides or combinations of β-lactams and aminoglycosides. The results of this experiment are presented in Table 3. The MBC/MIC ratio for L-658,310 was never more than two, even against the multiply-resistant organisms. Although imipenem and ceftazidime were also bactericidal, their respective MICs and MBCs were generally higher than for L-658,310 against the more resistant strains (CL 2850, CL 2979, CL 3015 and CL 3092). Thus, these organisms were susceptible to L-658,310 (respective MICs of 1, 1, 4 and 4 μg/ml), but are resistant to either or both ceftazidime (MIC of $\geq$32 μg/ml) and imipenem (MIC of $\geq$16 μg/ml).

The bactericidal activity of L-658,310 against *P. aeruginosa* suggests that the compound has a high affinity for the penicillin binding proteins in the cell membrane of this species[12]. Microscopic examination of these cells after exposure to subinhibitory concentrations of L-658,310 revealed a predominance of long chains of elongated bacilli (not shown). The results indicate that the compound binds preferentially to PBP-3 in this organism[12]. Other studies have also determined that L-658,310 binds preferentially to PBP-3 in *Escherichia coli* K-12 (Dr. P. J. CASSIDY; unpublished data).

Activity against Resistant Clinical Isolates

The activity of L-658,310 was compared with that of nine other β-lactam antibiotics (Table 4) against fifteen strains of *P. aeruginosa* selected for their resistance to either imipenem or combinations of other antibiotics. L-658,310 was the most active agent, having a geometric mean MIC (G-MIC) of 2.41 μg/ml. Using this criterion, it was more potent than either ceftazidime or imipenem by almost 6-fold and was more potent than either cefsulodin or cefepime by 7- and 8-fold, respectively.

In another study, the results of which are presented in Table 5, L-658,310 was not cross-resistant with imipenem, ceftazidime, or piperacillin when tested against strains of *P. aeruginosa* resistant to these agents. The study included thirteen isogenic pairs of organisms representing both imipenem-resistant and susceptible strains. There were virtually no differences between the G-MICs for L-658,310 against organisms resistant or susceptible to the other comparison drugs. In contrast, the

Table 4. Comparative activity of L-658,310 and selected β-lactams against resistant strains of *Pseudomonas aeruginosa*.

Strain No.	MIC (μg/ml)[a]										Resistant to[b]
	L-658,310	ATM	PIP	CAZ	CEF	CPO	IPM	CFS	CFP	MOX	
CL 725	4	16	128	4	128	128	2	8	16	32	AN, CBPC, GM, NN, IPM, PIP
CL 2014	4	16	>128	4	64	64	4	16	128	32	AN, CBPC, GM, NN, PIP
CL 2448	8	64	>128	32	32	128	16	32	>128	>128	AN, CBPC, GM, PIP, IPM
CL 2450	4	64	>128	128	64	128	2	64	>128	>128	CBPC, CAZ, PIP
CL 2533	4	64	32	8	8	8	16	16	64	>128	AN, CBPC, GM, IPM
CL 2720	2	16	16	4	4	8	16	4	16	64	IPM
CL 2820	4	8	8	2	2	4	16	4	16	32	IPM
CL 2850	4	16	>128	4	32	16	32	128	>128	64	AN, CBPC, GM, IPM, PIP
CL 2860	0.5	128	>128	>128	32	64	16	32	>128	>128	AN, CBPC, GM, CAZ, IPM, PIP
CL 2950	0.5	16	8	1	4	8	16	2	8	16	IPM, GM
CL 2979	1	128	>128	64	32	128	64	64	>128	>128	AN, CBPC, GM, IPM, CAZ
CL 2997	0.5	8	8	8	8	16	32	4	16	32	AN, GM, MOX, IPM, PIP
CL 3015	4	16	128	16	16	32	32	8	128	128	AN, CBPC, GM, MOX, IPM, PIP
CL 3020	8	64	>128	64	32	64	8	32	>128	>128	CBPC, CAZ, MOX, PIP
CL 3092	2	64	>128	128	32	128	32	32	>128	>128	IPM, MOX, CAZ, CBPC, PIP

[a] Agar dilution assay; multipoint inoculator; 10^5 cfu/spot; Trypticase Soy agar.

[b] Resistance determined by NCCLS standard disk diffusion assay[10,11].

ATM: Aztreonam, PIP: piperacillin, IPM: imipenem, CFS: cefsulodin, AN: amikacin, CBPC: carbenicillin, CAZ: ceftazidime, GM: gentamicin, NN: tobramycin, MOX: moxalactam, CFP: cefoperazone, CPO: cefpirome, CEF: cefepime.

Table 5. Lack of cross-resistance[a] between L-658,310 and imipenem, ceftazidime or piperacillin in *Pseudomonas aeruginosa*.

Susceptibility to comparison drug	No. of strains tested	G-MIC (μg/ml)[b]	
		L-658,310	Comparison drug
IPM^S	25	$\leq$0.52	1.03
IPM^R	13	$\leq$0.52	17.68
CAZ^S	24	$\leq$0.71	1.58
CAZ^R	8	$\leq$0.99	>91.20
PIP^S	23	0.96	6.25
PIP^R	15	$\leq$0.99	>128.00

[a] Organisms were classified as imipenem (IPM)-resistant if the MIC was $\geq$16 μg/ml; ceftazidime (CAZ) resistant if the MIC was $\geq$32 μg/ml; and piperacillin (PIP)-resistant if the MIC was $\geq$128 μg/ml.
[b] G-MIC: Geometric mean of the MIC; agar dilution method.
^{S}: Sensitive, ^{R}: resistant.

Table 6. Lack of cross-resistance[a] between L-658,310 and ceftazidime in other glucose non-fermenters and Enterobacteriaceae.

Organism	Strain No.	MIC (μg/ml)[b]	
		L-658,310	Ceftazidime
Acinetobacter anitratus	CL 993	4	32
A. lwoffi	CL 2269	0.5	64
Pseudomonas maltophilia	CL 2225	0.25	64
	CL 2226	1	32
	CL 2228	4	64
	CL 2231	4	128
	CL 2233	2	32
	CL 2999	2	32
	CL 3182	2	64
	CL 3184	4	32
	CL 3195	4	128
Citrobacter freundii	CL 1076	1	64
Enterobacter aerogenes	CL 404	16	64
	CL 1414	1	64
E. cloacae	CL 2787	2	32
	CL 2793	4	64
	CL 3263	4	128

[a] Organisms were classified as ceftazidime- or L-658,310-resistant if the MIC was $\geq$32 μg/ml.
[b] Agar dilution method; 10^5 cfu/spot; Trypticase Soy agar.

G-MIC for imipenem was 17-fold higher against the resistant strains, for ceftazidime, >57-fold higher and for piperacillin, >20-fold higher.

The lack of cross-resistance with ceftazidime was also determined with strains of other glucose non-fermenters and selected Enterobacteriaceae (Table 6). Against these clinical isolates, the MICs for ceftazidime ranged from 32 to 128 μg/ml, while L-658,310 had MICs that ranged from 0.25 to 16 μg/ml. Thus, the MICs for L-658,310 were 4- to 256-fold lower than for ceftazidime against each of these resistant pathogens.

Resistance to Hydrolysis

Although both L-658,310 and ceftazidime are very resistant to hydrolysis by β-lactamases of most Gram-negative bacteria[3,4], ceftazidime has been shown to be slightly more susceptible than L-658,310

Table 7. Comparative activity of L-658,310 and ceftazidime against a β-lactamase (+) strain of *Enterobacter cloacae* and its spontaneous β-lactamase (−) mutant.

Organism	Strain No.	MIC (μg/ml)[a]	
		L-658,310	Ceftazidime
Enterobacter cloacae P99 (+)	MB 2646	4	64
E. cloacae P99 (−)	MB 2647	0.5	0.25

[a] Agar dilution method; 10^5 cfu/spot; Trypticase Soy agar.

Table 8. Effect of inoculum size on the antibacterial activity of L-658,310 against β-lactamase (+) strains of bacteria.

Organism	Strain No.	MIC (μg/ml)[a]	
		10^4 cfu/spot	10^6 cfu/spot
Escherichia coli TEM 2 (+)[b]	MB 4351	0.063	0.25
Enterobacter cloacae P99 (+)[c]	MB 2646	2	4
E. aerogenes[c]	MB 2828	0.125	0.5
Klebsiella oxytoca K1 (+)[c]	MB 4354	0.125	0.5
Proteus vulgaris[c]	MB 2829	0.0156	0.063
Morganella morganii[c]	MB 2833	0.125	0.5
Pseudomonas aeruginosa RPL11 (+)[d]	MB 3350	0.5	1
P. aeruginosa[c]	MB 2835	0.125	0.125
	MB 4279	1	2
Serratia marcescens[c]	MB 2840	0.25	1

[a] Agar dilution method; Trypticase Soy agar; the MIC given is the mode or midpoint of 4 separate assays.
[b] R-plasmid-mediated β-lactamase producer.
[c] Chromosomally-mediated β-lactamase producer.
[d] RPL11 plasmid codes for gentamicin, carbenicillin, streptomycin, tetracycline, sulfa and chloramphenicol resistance[14].

to hydrolysis by type II penicillinase from *E. coli* W3630[2] and, to a lesser extent by oxyiminocephalosporin-hydrolyzing enzyme type II from *P. maltophilia* GN12873[2]. When the MICs for L-658,310 and ceftazidime were compared (Table 7) against a β-lactamase producing strain of *E. cloacae* P99 (+), a constitutive producer of large quantities of β-lactamase[13], and a spontaneous mutant P99 (−)[13], the MIC of L-658,310 for the P99 (+) strain was only increased 8-fold. The increase for ceftazidime, however, was 256-fold. This suggests that the activity of L-658,310 against some ceftazidime-resistant organisms may be due to its increased resistance to bacterial β-lactamases.

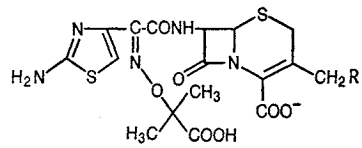
Effect of Inoculum Size on Activity in β-Lactamase Producing Organisms

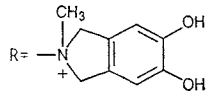
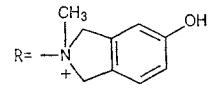
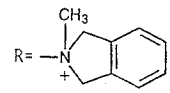
The activity of L-658,310 was determined against laboratory strains known to possess either R-plasmid- or chromosomally-mediated β-lactamases at inocula of 10^4 and 10^6 cfu/spot (Table 8). When tested against either group of organisms, the MIC for L-658,310 was never more than 4-fold higher against the 10^6 cfu/spot inoculum than against the 10^4 cfu/spot inoculum. Thus, even when exposed to large quantities of β-lactamase produced by large numbers of bacteria, L-658,310 was still a very potent antibacterial agent.

Comparative Activity of 2-Methylisoindoline Analogues

When the *in vitro* antibacterial activity of L-658,310 was compared with either its monohydroxy or its dehydroxy analogue against a variety of glucose non-fermenters (Table 9), a significant loss in

Table 9. Antibacterial activity of L-658,310 (5,6-dihydroxy-) and its 5-monohydroxy- and dehydroxy-2-methylisoindoline analogues against glucose non-fermenters.



Organism	Strain No.	MIC (μg/ml)[a] 2-Methylisoindoline substituent		
		5,6-Dihydroxy 	5-Monohydroxy 	Dehydroxy 
Acinetobacter anitratus	CL 805	1	64	128
	CL 1102	8	>128	>128
	CL 2234	0.5	32	32
A. lwoffi	CL 104	4	128	128
	CL 948	1	128	128
	CL 1491	0.015	8	8
	CL 2269	4	>128	>128
Pseudomonas aeruginosa	CL 2824	0.5	8	8
	CL 2835	0.25	32	32
	CL 2845	1	8	16
	CL 2965	0.25	2	2
	CL 2966	0.5	8	8
	CL 2968	4	8	32
	CL 2970	4	16	32
	CL 2971	0.063	16	16
	CL 2975	0.25	16	32
P. cepacia	CL 2210	≤0.008	4	8
	CL 2212	0.031	8	8
	CL 2215	≤0.008	4	8
	CL 2216	≤0.008	4	8
P. maltophilia	CL 2225	0.063	8	4
	CL 2226	1	32	32
	CL 2233	0.25	8	8
	CL 2367	0.063	2	4
	CL 2409	0.125	4	8

[a] Agar dilution assay; Trypticase Soy agar; 10^5 cfu/spot.

potency was seen with the analogues. Against Staphylococci or Enterobacteriaceae (data not shown), however, the MICs for the analogues were never more than 4-fold higher than for L-658,310. Thus, we have tentatively ascribed the potent activity of L-658,310 against glucose non-fermenters to the dihydroxy substituents on the aromatic ring system.

In conclusion, L-658,310 is a new broad-spectrum, semisynthetic cephalosporin with outstanding activity against glucose non-fermenters. This is due to the presence of the hydroxyl groups on position 5 and 6 on the aromatic ring of the 3-position side chain. The antibiotic also has good activity against Enterobacteriaceae, *H. influenzae* and non-enteric Streptococci. Activity against Staphylococci, however, is generally poor. In addition, the compound has activity against some, but not all anaerobic organisms. The lack of cross-resistance demonstrated between L-658,310 and

other β-lactam antibiotics is thought to be due to its stability to both R-plasmid- and chromosomally-mediated β-lactamases and/or the entry of the compound into the cell *via* a novel pathway.

Acknowledgment

The authors wish to express their appreciation to Mrs. Eileen Miller for preparing the manuscript.

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