ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1991, p. 922–928
0066-4804/91/050922-07$02.00/0
Copyright © 1991, American Society for Microbiology
Vol. 35, No. 5

In Vitro Activity and In Vivo Efficacy of a New Series of 9-Deoxo-12-Deoxy-9,12-Epoxyerythromycin A Derivatives

DWIGHT J. HARDY,†* ROBERT N. SWANSON, NATHAN L. SHIPKOWITZ, LESLIE A. FREIBERG, PAUL A. LARTEY, AND JACOB J. CLEMENT

Anti-Infective Research Division, Abbott Laboratories, Abbott Park, Illinois 60064-3500

Received 23 October 1990/Accepted 26 February 1991

Analogs of 9-deoxo-12-deoxy-9,12-epoxyerythromycin A with an epimeric hydroxy, amino, or ketone substitution at the 11 position of the macrolide ring and an amino or epimeric hydroxy substitution at the 4″ position of the cladinose sugar were synthesized in an attempt to produce acid-stable derivatives of erythromycin with improved bioavailability and activity against gram-negative bacteria. These modifications produced compounds with in vitro activities which were generally similar to that of erythromycin. In mice, however, selected analogs were more active than was erythromycin against staphylococci, streptococci, *Haemophilus influenzae*, and *Legionella pneumophila*. In mice, the 11-keto (A-63881), 11-epiamino (A-69334), 11-epiamino-4″-amino (A-71671), and 11-epiamino-4″-epiamino (A-73020) analogs achieved peak concentrations in serum and lung, serum half-lives, and/or areas under the serum curve which were greater than those of erythromycin. Improved pharmacokinetics, as compared with those of erythromycin, may explain the increased in vivo antibacterial activities of these compounds.

The conversion of erythromycin to its 6,9;9,12-spiroketal form by aqueous acid (19) or to its enol ether form by glacial acetic acid (12) results in markedly reduced antibacterial activity. The formation of these inactive forms of erythromycin involves the hydroxyl group at position 6 and the ketone at position 9 in the macrolide ring. Attempts to protect the erythromycin molecule from degradation by gastric acidity have included its formulation with an acid-protective coating designed for release at a higher pH in the intestine and its formulation as a water-insoluble salt. Chemical modifications of erythromycin A designed to eliminate the acid-sensitive structural features of carbons 6 and 9 have produced clarithromycin (3, 14), with a methyl substitution on the 6-hydroxyl; dirithromycin (8), with an alkylidene bridge between carbons 9 and 11 of erythromycylamine which is metabolized to erythromycylamine with an amino substitution on carbon 9; roxithromycin (2), with an oxime replacing the ketone at carbon 9; flurithromycin (18), with a fluorine substitution at carbon 8; and azithromycin (17), with a nitrogen atom inserted between the 9 and 10 positions of the macrolide ring and with the carbonyl at position 9 reduced to a methylene. The in vitro antibacterial activities of these compounds relative to each other and to erythromycin have been reported recently (8, 11).

9-Deoxo-12-deoxy-9,12-epoxyerythromycin A (A-69328) is a novel derivative of erythromycin in which the ketone at carbon 9 is removed and replaced with an oxygen bridge to carbon 12. Analogs of this compound with an epimeric hydroxy, amino, or ketone substitution at the 11 position of the macrolide ring have been synthesized from anhydroerythromycin A in an attempt to produce acid-stable derivatives of erythromycin with improved bioavailability and activity against gram-negative bacteria. In addition, the hydroxyl group at the 4″ position of the cladinose sugar has also been substituted with an amino group or epimerized (4, 5). In this paper, we present the in vitro and in vivo antibacterial activities of these compounds (Fig. 1) relative to erythromycin.

(This work was presented in part at the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy [9].)

MATERIALS AND METHODS

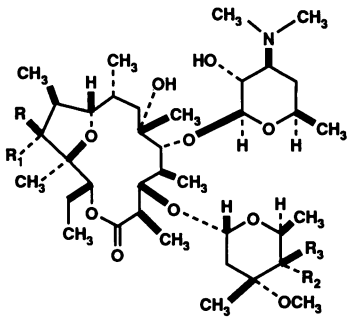
Bacterial strains. The organisms used in this study were clinical isolates from the Abbott Laboratories culture collection or were obtained from the American Type Culture Collection, Rockville, Md. All strains were identified by standard procedures and were maintained frozen at −60°C.

Antibacterial agents. All compounds, except ampicillin, were synthesized at Abbott Laboratories. For in vitro tests, all macrolides were dissolved in methanol. For administration to animals, macrolides were dissolved in phosphate-buffered saline (pH 7.2). Ampicillin was purchased from Parke-Davis (Morris Plains, N.J.) and was dissolved in sterile water.

MIC determinations. MICs were determined by a standard twofold dilution methodology in agar (15) with 10^4 CFU as the inoculum. Organisms for quality control were included in each test. *Staphylococcus aureus* and *Escherichia coli* were tested on Mueller-Hinton agar (pH 7.3). Mueller-Hinton agar supplemented with 5% (vol/vol) sheep blood was used for streptococci. *Haemophilus influenzae* was tested on haemophilus test medium (10a) and incubated for 20 to 24 h in ambient air. *Legionella pneumophila* was tested on buffered charcoal-yeast extract agar and was incubated for 20 to 24 h in ambient air. *Campylobacter jejuni* was tested on Mueller-Hinton agar (pH 8.0) and was incubated in a microaerophilic atmosphere in jars (Campy-Pak system; BBL Microbiology Systems, Cockeysville, Md.). *Neisseria gonorrhoeae* was tested on proteose no. 3 agar supplemented with 1% bovine hemoglobin and 1% (vol/vol) Kellogg supplement and was incubated in the presence of 5% CO_2 for 20 to 24 h. Anaerobic bacteria were tested on Wilkins-Chalgren agar

* Corresponding author.
† Present address: Department of Microbiology and Immunology, University of Rochester, Rochester, NY 14642.

Downloaded from http://aac.asm.org/ on September 15, 2016 by PENN STATE UNIV



ABBOTT NO.	R	R_1	R_2	R_3	Trivial Name
A-69328	H	OH	OH	H	11-hydroxy
A-63483	OH	H	OH	H	11-epihydroxy
A-63881	O	O	OH	H	11-keto
A-69334	NH_2	H	OH	H	11-epiamino
A-69369	OH	H	NH_2	H	11-epihydroxy-4"-amino
A-69370	OH	H	H	NH_2	11-epihydroxy-4"-epiamino
A-71671	NH_2	H	NH_2	H	11-epiamino-4"-amino
A-73020	NH_2	H	H	NH_2	11-epiamino-4"-epiamino

FIG. 1. Chemical structures of 9-deoxo-12-deoxy-9,12-epoxyerythromycin A (A-69328) and its analogs.

with 2×10^5 to 6×10^5 CFU as the inoculum (16). All plates were incubated at 35°C.

Effects of serum and pH on MICs of macrolides. The effects of 50% (vol/vol) serum and pHs 6.5, 7.3, and 8.0 on the MICs of macrolides were determined by a previously described twofold microdilution method (8). The pH of the serum was adjusted to 7.3 prior to testing to separate the effect of serum from that of pH. One strain each of *S. aureus*, *Streptococcus pyogenes*, and *H. influenzae* was tested in Mueller-Hinton broth, brain heart infusion broth, and haemophilus test medium, respectively.

Mouse protection tests. Female CF-1 mice weighing 20 to 25 g (Sasco, Inc., Oregon, Wis.) were injected intraperitoneally with bacterial suspensions containing 10 to 1,000 times the 50% lethal doses. Macrolides were administered orally or subcutaneously at 1 and 5 h postinfection (p.i.) to groups of 10 mice. Cumulative mortalities were recorded after 7 days p.i., and median 50% effective doses (ED_{50}s) were calculated by the trimmed logit method (7).

***H. influenzae* otitis media in gerbils.** A 5-h log-phase culture of *H. influenzae* ATCC 43095 was prepared in brain heart infusion broth supplemented with 4% Fildes enrichment (BBL) and 0.001% NAD (Sigma Chemical Co., St. Louis, Mo.). Female Mongolian gerbils weighing 40 to 50 g (Tumblebrook Farms, West Brookfield, Mass.) were anesthetized with ether and injected percutaneously in the superior posterior chamber of the left middle ear bulla with a 0.02-ml inoculum containing approximately 10^6 bacteria (6, 10). Immediately prior to treatment, middle ear aspirates from four gerbils were obtained as described below and cultured to document the level of infection at the onset of therapy. Compounds were administered orally by gavage in a 0.5-ml volume beginning 17 h p.i. and continuing three times daily for 2 days to groups of four animals. Eighteen hours after the final treatment, gerbils were euthanized with T-61 euthanasia solution (Hoechst Roussel, Sommerville, N.J.) and 0.02 ml of brain heart infusion broth was injected into the middle ear bulla through the tympanic membrane. Middle ear aspirates were collected before the needle was removed. The aspirates were diluted in brain heart infusion broth and cultured in duplicate on chocolate agar (BBL). Colonies were counted with an Artek colony counter (Artek Systems Corp., Farmingdale, N.Y.) after overnight incubation. Mean log CFU per middle ear were determined for each treatment group. The minimum number of bacteria detectable by this method was 100 CFU. Bacterial counts from the treated animals were compared with those from the untreated controls.

***L. pneumophila* pneumonia in guinea pigs.** Male Hartley guinea pigs weighing 250 to 300 g (Sasco) were infected with *L. pneumophila*. In brief, *L. pneumophila* ATCC 33152 was grown on buffered charcoal-yeast extract agar and suspended in saline to a concentration of 10^6 to 10^7 CFU/ml. One-half milliliter of this suspension was intranasally instilled into both nostrils of ether-anesthetized guinea pigs. Compounds were administered intraperitoneally two times daily (8 h apart) for 2 days beginning 20 h p.i. Prior to treatment onset, the lungs from four animals were cultured as described below to determine premedication infection levels. Eighteen hours after the final treatment, animals were euthanized and the lungs were removed, homogenized in 10 ml of phosphate-buffered saline, and quantitatively cultured on buffered charcoal-yeast extract agar. The minimum number of bacteria detectable by this method was 100 CFU. Groups of four guinea pigs were used. One group of animals was untreated and served as controls. Mean log CFU from both lungs of animals in each treatment group were determined.

Pharmacokinetics in mice. Serum and tissue concentrations of macrolides in female CF-1 mice weighing 20 to 25 g were determined following a single oral or subcutaneous dose of 25 mg/kg. At 0.5, 1, 2, 3, 6, and 24 h after drug administration, blood and tissue samples were collected

Downloaded from http://aac.asm.org/ on September 15, 2016 by PENN STATE UNIV

TABLE 1. MICs of 9-deoxo-12-deoxy-9,12-epoxyerythromycin A and its analogs for selected strains

Taxon	MIC (μg/ml)								
	A-69328	A-63483	A-63881	A-69334	A-69369	A-69370	A-71671	A-73020	Erythromycin[a]
S. aureus ATCC 6538P	12.5	0.2	0.4	0.2	0.4	0.2	0.2	0.2	0.1–0.2
S. pyogenes EES61	0.8	0.02	≤0.05	≤0.005	0.02	≤0.005	0.01	≤0.005	0.01–0.02
S. pneumoniae ATCC 6303	ND[b]	0.015	0.06	0.03	ND	ND	0.008	0.015	0.015–0.03
E. coli Juhl	>100	25	100	>100	12.5	50	1.56	3.1	25–50
H. influenzae ATCC 10211	>64	1	4	2	0.5	0.5	2	1	0.5–2
L. pneumophila ATCC 33152	ND	1	0.5	1	ND	ND	4	4	0.5–1
N. gonorrhoeae F-28	ND	0.015	0.25	0.12	ND	ND	0.12	0.12	0.03–0.06
C. jejuni ATCC 29428	ND	0.12	0.06	0.06	ND	ND	0.03	ND	0.12–0.25

[a] Range of MICs from several experiments.
[b] ND, Not determined.

from groups of five mice. Lungs and spleens were homogenized in phosphate-buffered saline. Samples were assayed for bioactivity by a previously described disk agar diffusion procedure (1) with *Micrococcus luteus* 9341 as the assay organism.

RESULTS

MIC determinations. 9-Deoxo-12-deoxy-9,12-epoxyerythromycin A (A-69328) and its analogs were screened for in vitro activities against several gram-positive and gram-negative organisms. The parent compound was 16- to 256-fold less active than were its analogs and erythromycin against strains of staphylococci, streptococci, and *H. influenzae*. As compared with erythromycin, the 11-epiamino-4″-amino (A-71671) and 11-epiamino-4″-epiamino (A-73020) analogs were as active against staphylococci and streptococci, 16-fold more active against *E. coli*, 4- to 8-fold less active against *L. pneumophila*, and as active against other gram-negative organisms. The other compounds were generally as active as or less active than was erythromycin. MICs for representative strains are presented in Table 1.

The ranges of MICs, MICs for 50% of strains (MIC_{50}s), MIC_{90}s, and geometric mean MICs of compounds observed to have in vitro and in vivo activities and good pharmacokinetics in mice are presented in Table 2. The MICs of the 11-epiamino (A-69334), 11-epiamino-4″-amino (A-71671), and 11-epiamino-4″-epiamino (A-73020) analogs of 9-deoxo-12-deoxy-9,12-epoxyerythromycin A and erythromycin for *S. aureus* and streptococci were identical or only twofold different. Against gram-negative bacteria, however, inter- and intragenic differences in the in vitro activities of the compounds were observed. As compared with the MICs of erythromycin, the MICs of these compounds for *H. influenzae* were generally the same or only 2-fold different, while the MICs for *C. jejuni* were 4- to 16-fold lower. Although the MIC_{90}s of these compounds for *N. gonorrhoeae* were two- to fourfold lower than was that of erythromycin, the MIC_{50}s and geometric mean MICs were the same or only twofold different. Against *L. pneumophila*, these compounds were 2- to 16-fold less active than was erythromycin, while the 11-keto (A-63881) analog was as active as was erythromycin. The 11-epiamino-4″-amino (A-71671) analog was at least 10-fold more active than were erythromycin and the 11-epiamino (A-69334) analog against *E. coli*. Against gram-positive and gram-negative anaerobes, the 11-epiamino (A-69334) analog was as active as was erythromycin, while the 11-epiamino-4″-amino (A-71671) analog was more than 20-fold less active.

Effect of serum on MICs. The MICs of the 11-epiamino (A-69334), 11-epiamino-4″-amino (A-71671), and 11-epiamino-4″-epiamino (A-73020) analogs and erythromycin for *S. aureus* CMX553 and *S. pyogenes* EES61 in the presence of 50% human or mouse serum were decreased two- to eightfold as compared with the MICs determined in broth without serum. The MICs for *H. influenzae* 1435 were not affected by serum.

Effect of pH on MICs. The MICs of the 11-epihydroxy (A-63483), 11-epiamino (A-69334), 11-epiamino-4″-amino (A-71671), and 11-epiamino-4″-epiamino (A-73020) analogs for *S. aureus* CMX553 and *S. pyogenes* EES61 were determined at pHs 6.5, 7.3, and 8.0. The MICs of these compounds and erythromycin at pH 6.5 were increased four- to eightfold as compared with the MICs at pH 7.3, while the MICs at pH 8.0 were decreased two- to fourfold.

Mouse protection tests. When administered orally, the 11-keto (A-63881) analog was approximately two times more active than was erythromycin against *S. aureus*, while the other analogs were generally as active as was erythromycin, except for the 11-epihydroxy-4″-amino (A-69369) analog, which was approximately two times less active (Table 3). Following subcutaneous injection, these compounds were approximately two to three times less active than was erythromycin against *S. aureus*. The 11-keto (A-63881) and 11-epiamino (A-69334) analogs were approximately four and two times more active, respectively, than was erythromycin when administered orally against *S. pyogenes*, while the other analogs were approximately as active as was erythromycin (Table 4). When administered subcutaneously, the 11-epiamino (A-69334) analog was approximately two times more active than was erythromycin against *S. pyogenes*, while the other analogs were as active as was erythromycin. When administered orally, the analogs were generally as effective as was erythromycin against *Streptococcus pneumoniae* (Table 5), except for the 11-epihydroxy-4″-epiamino (A-69370) analog, which was two times less active. Following subcutaneous administration, all analogs tested were comparable to erythromycin in activity.

Efficacy against *H. influenzae* in gerbils. When administered orally to gerbils, three analogs were more effective than was erythromycin in treating *H. influenzae* otitis media (Table 6). At 300 mg/kg/day, the 11-epiamino (A-69334) analog reduced bacterial counts in the middle ear to undetectable levels (>5.1 log CFU reduction), as compared with those in untreated controls. The 11-epiamino-4″-amino (A-71671) and 11-epiamino-4″-epiamino (A-73020) analogs reduced bacterial counts in the middle ear by approximately 4 log CFU, while erythromycin did not reduce bacterial counts in the middle ear.

Downloaded from http://aac.asm.org/ on September 15, 2016 by PENN STATE UNIV

TABLE 2. MICs of selected analogs of 9-deoxo-12-deoxy-9,12-epoxyerythromycin A

Taxon (no. of strains)	Compound	MIC (μg/ml)			
		Range	50%	90%	Geometric mean
S. aureus (15)[a]	A-69334	0.25–0.5	0.25	0.25	0.26
	A-71671	0.25	0.25	0.25	0.25
	A-73020	0.25	0.25	0.25	0.25
	Erythromycin	0.12–0.25	0.12	0.25	0.15
S. pyogenes (15)	A-69334	0.015–0.03	0.03	0.03	0.03
	A-71671	0.008–0.03	0.03	0.03	0.03
	A-73020	0.03–0.06	0.03	0.03	0.03
	Erythromycin	0.03	0.03	0.03	0.03
S. pneumoniae (14)	A-69334	0.015–0.03	0.03	0.03	0.03
	A-71671	0.015–0.03	0.015	0.03	0.02
	A-73020	0.03	0.03	0.03	0.03
	Erythromycin	0.015–0.03	0.03	0.03	0.03
H. influenzae (17)[b]	A-69334	0.12–8	2	4	1.66
	A-71671	0.25–4	1	2	0.82
	A-73020	0.12–4	1	2	1.16
	Erythromycin	0.12–4	2	2	1.36
H. influenzae (17)[b]	A-69369	0.06–2	0.5	1	0.52
	A-69370	0.12–2	0.5	1	0.61
	Erythromycin	0.12–2	1	1	0.81
L. pneumophila (11)	A-63881	0.5–1	0.5	0.5	0.53
	A-69334	1–2	1	2	1.2
	A-71671	2–>4	>4	>4	>2.92
	A-73020	2–16	4	16	5.48
	Erythromycin	0.25–1	0.5	1	0.58
C. jejuni (13)	A-69334	0.06–0.5	0.25	0.5	0.15
	A-71671	0.03–0.12	0.06	0.12	0.05
	A-73020	0.03–0.25	0.06	0.25	0.07
	Erythromycin	0.12–2	0.5	2	0.40
N. gonorrhoeae (12)[c]	A-69334	0.12–1	0.25	0.5	0.28
	A-71671	0.12–1	0.25	0.25	0.20
	A-73020	0.12–1	0.25	0.5	0.25
	Erythromycin	0.03–2	0.12	1	0.22
E. coli (10)	A-69334	8–64	32	64	25.99
	A-71671	1–8	2	4	2.64
	Erythromycin	8–128	64	128	39.40
Gram-positive anaerobes (12)[d]	A-69334	0.03–2	0.5	1	0.38
	A-71671	0.25–16	8	16	2.13
	Erythromycin	≤0.015–1	0.25	1	0.26
Gram-negative anaerobes (19)[e]	A-69334	0.06–4	2	4	0.89
	A-71671	0.25–>128	128	>128	>23.04
	Erythromycin	≤0.015–8	1	8	1.07

[a] Methicillin-susceptible strains.
[b] Includes eight type b (three β-lactamase-positive) and nine non-type b strains. Results from two experiments are presented separately.
[c] Includes 2 β-lactamase-positive and 10 β-lactamase-negative strains.
[d] Includes three strains of *Clostridium perfringens*, two strains of *C. difficile*, and one strain each of *C. ramosum*, *Peptostreptococcus asaccharolyticus*, *P. magnus*, *P. micros*, *P. anaerobius*, *Peptostreptococcus* species, and *Propionibacterium acnes*.
[e] Includes six strains of *Bacteroides fragilis*, three strains of *B.thetaiotaomicron*, two strains each of *B. loescheii* and *B. vulgatus*, and one strain each of *B. disiens*, *B. bivius*, *B. melaninogenicus*, *Fusobacterium nucleatum*, *Fusobacterium* species, and *Veillonella parvula*.

Downloaded from http://aac.asm.org/ on September 15, 2016 by PENN STATE UNIV

TABLE 3. Activity of analogs of 9-deoxo-12-deoxy-9,12-epoxyerythromycin A against *S. aureus* NCTC 10649 in mouse protection tests[a]

Compound	MIC (μg/ml)	Route[b]	ED_{50}[c] of:	
			Analog	Erythromycin A
A-63483	0.12	Oral	55.0 (40.8–74.2)	95.8 (61.4–149.5)
		s.c.	22.6 (14.3–35.6)	12.9 (7.8–21.5)
A-63881	0.12	Oral	25.2 (12.3–51.7)	58.1 (35.0–96.6)
		s.c.	22.6 (14.3–35.6)	8.3 (5.7–12.0)
A-69334	0.25	Oral	75.4 (52.1–109.1)	123.2 (78.0–194.6)
		s.c.	20.7 (13.6–31.5)	11.6 (6.9–19.3)
A-69369	ND[d]	Oral	>250	91.8 (52.0–162.0)
		s.c.	30.1 (19.0–47.5)	10.9 (7.2–16.6)
A-69370	ND	Oral	126.2 (74.5–213.9)	91.8 (52.0–162.0)
		s.c.	39.6 (25.3–62.0)	10.9 (7.2–16.6)
A-71671	0.25	Oral	183.8 (118.2–285.7)	170.5 (102.7–282.9)
		s.c.	33.4 (21.2–52.7)	15.5 (9.0–26.8)

[a] Mice were intraperitoneally infected with 3.2×10^5 to 1.3×10^6 CFU of *S. aureus* NCTC 10649.
[b] Compounds were administered orally or subcutaneously (s.c.) 1 and 5 h p.i.
[c] Reported in milligrams per kilogram per day (95% confidence interval). ED_{50}s of test compounds and erythromycin A from the same experiments are given.
[d] ND, Not determined.

TABLE 5. Activity of analogs of 9-deoxo-12-deoxy-9,12-epoxyerythromycin A against *S. pneumoniae* ATCC 6303 in mouse protection tests[a]

Compound	MIC (μg/ml)	Route[b]	ED_{50}[c] of:	
			Analog	Erythromycin A
A-63483	0.03	Oral	31.3 (18.0–54.6)	18.7 (11.9–29.6)
		s.c.	1.6 (0.9–3.0)	1.0 (0.5–2.1)
A-63881	0.06	Oral	23.7 (12.2–46.4)	28.2 (15.4–51.4)
		s.c.	1.1 (0.2–5.8)	0.5 (0.2–1.4)
A-69334	0.03	Oral	10.0 (6.3–15.8)	12.6 (4.5–35.3)
		s.c.	0.7 (0.4–1.3)	1.2 (0.7–1.9)
A-69369	ND[d]	Oral	37.3 (25.0–55.6)	33.6 (19.9–56.7)
		s.c.	4.0 (1.9–8.4)	2.1 (1.2–3.9)
A-69370	ND	Oral	84.8 (53.8–133.7)	33.6 (19.9–56.7)
		s.c.	4.0 (1.9–8.4)	2.1 (1.2–3.9)
A-71671	0.008	Oral	16.0 (8.7–29.6)	27.4 (13.8–54.6)
		s.c.	0.9 (0.5–1.4)	1.2 (0.9–1.8)
A-73020	0.015	Oral	37.5 (29.3–48.1)	23.6 (11.9–46.8)
		s.c.	2.4 (1.2–5.0)	1.5 (0.8–2.7)

[a] Mice were intraperitoneally infected with 3.2×10^2 to 6.3×10^3 CFU of *S. pneumoniae* ATCC 6303.
[b] See Table 3, footnote *b*.
[c] See Table 3, footnote *c*.
[d] See Table 3, footnote *d*.

Efficacy against *L. pneumophila* in guinea pigs. When administered intraperitoneally at 25 mg/kg/day to guinea pigs with *L. pneumophila* infections, the 11-epiamino (A-69334) analog reduced bacterial counts in the lungs by 3.7 log CFU, as compared with those in untreated controls (Table 7). Erythromycin at 25 mg/kg/day reduced bacterial counts by 1.7 log CFU. At 6.2 mg/kg/day, the 11-epiamino (A-69334) analog reduced bacterial counts by 1.0 log CFU, while erythromycin reduced bacterial counts by 0.4 log CFU.

Pharmacokinetics in mice. Peak concentrations in mouse serum after a single oral or subcutaneous dose of 25 mg/kg,

TABLE 4. Activity of analogs of 9-deoxo-12-deoxy-9,12-epoxyerythromycin A against *S. pyogenes* C203 in mouse protection tests[a]

Compound	MIC (μg/ml)	Route[b]	ED_{50}[c] of:	
			Analog	Erythromycin A
A-63483	0.06	Oral	38.7 (23.3–64.4)	53.8 (19.2–150.8)
		s.c.	6.8 (4.1–11.3)	8.1 (4.5–14.3)
A-63881	0.03	Oral	12.0 (6.9–20.8)	51.5 (32.1–82.6)
		s.c.	1.7 (0.6–4.3)	1.8 (0.9–3.9)
A-69334	0.03	Oral	28.9 (16.8–49.6)	53.3 (26.1–109.1)
		s.c.	2.5 (1.1–5.7)	6.4 (2.2–18.1)
A-69369	ND[d]	Oral	47.9 (30.7–74.8)	64.6 (22.7–183.5)
		s.c.	5.0 (3.2–7.9)	7.3 (3.5–15.0)
A-69370	ND	Oral	51.5 (32.1–82.6)	64.6 (22.7–183.5)
		s.c.	9.3 (3.1–27.4)	7.3 (3.5–15.0)
A-71671	0.03	Oral	43.1 (21.5–86.5)	41.1 (20.6–81.8)
		s.c.	1.1 (0.7–1.7)	1.6 (1.0–2.4)

[a] Mice were intraperitoneally infected with 4.0×10^2 to 3.2×10^3 CFU of *S. pyogenes* C203.
[b] See Table 3, footnote *b*.
[c] See Table 3, footnote *c*.
[d] See Table 3, footnote *d*.

TABLE 6. Activity of analogs of 9-deoxo-12-deoxy-9,12-epoxyerythromycin A against *H. influenzae* ATCC 43095 otitis media in gerbils[a]

Compound	MIC (μg ml)	Dose[b] (mg/kg/day)	Log CFU/middle ear (geometric mean ± SEM)
A-69334	2	300	<2.0
		75	7.0 ± 0.1
		18.7	7.1 ± 0.1
A-71671	1	300	3.0 ± 1.3
		75	7.1 ± 0.1
		18.7	7.1 ± 0.1
A-73020	2	300	2.9 ± 0.7
		75	7.4 ± 0.1
		18.7	7.1 ± 0.1
Erythromycin	1	300	7.1 ± 0.1
		75	6.8 ± 0.1
		18.7	6.8 ± 0.1
Ampicillin	0.25	100	<2.0
		25	4.7 ± 1.1
		6.2	7.3 ± 0.1
None (untreated controls)			7.1 ± 0.1

[a] Gerbils had 5.1 to 5.4 log CFU per middle ear at the start of treatment.
[b] Compounds were administered orally at 17, 21, 25, 41, 45, and 49 h p.i.

Downloaded from http://aac.asm.org/ on September 15, 2016 by PENN STATE UNIV

TABLE 7. Activity of analogs of 9-deoxo-12-deoxy-9,12-epoxyerythromycin A against *L. pneumophila* ATCC 33152 pneumonia in guinea pigs[a]

Compound	MIC (μg/ml)	Dose[b] (mg/kg/day)	Log CFU[c] (geometric mean ± SEM)
A-69334	1	25	5.0 ± 1.2
		6.2	7.7 ± 0.2
		1.6	8.2 ± 0.1
Erythromycin	1	25	7.0 ± 0.6
		6.2	8.3 ± 0.3
		1.6	8.5 ± 0.1
None (untreated controls)			8.7± 0.1

[a] Guinea pigs had 7.5 log CFU in lungs at the start of treatment.
[b] Compounds were administered intraperitoneally at 20, 28, 44, and 52 h p.i.
[c] CFU from both lungs.

respectively, were as follows: 11-epihydroxy (A-63483), 0.3 and 1.6 μg/ml; 11-keto (A-63881), 0.9 and 2.4 μg/ml; 11-epiamino (A-69334), 0.5 and 3.0 μg/ml; 11-epiamino-4″-amino (A-71671), 0.2 and 2.9 μg/ml; 11-epiamino-4″-epiamino (A-73020), 0.3 and 4.6 μg/ml; and erythromycin, 0.2 and 3.0 μg/ml. The serum half-lives of these compounds, when calculable, were at least as long as was that of erythromycin after oral dosing. Following subcutaneous administration, the serum half-lives of these compounds were comparable to that of erythromycin, except for those of A-71671 and A-73020, which were 9 to 10 times longer than was that of erythromycin.

The peak concentrations in lungs and spleen, respectively, following a single oral dose of 25 mg/kg were as follows: A-69334, 1.4 and 1.8 μg/g; A-71671, 0.3 and <0.1 μg/g; A-73020, 0.5 and 0.1 μg/g; and erythromycin, <0.1 and 8.1 μg/g.

DISCUSSION

9-Deoxo-12-deoxy-9,12-epoxyerythromycin A (A-69328), an acid-stable derivative of erythromycin A, has been modified at its 11 and 4″ positions to explore the structure-activity relationships of these functional groups. These modifications have resulted in compounds with in vitro activities similar to those of erythromycin against *S. aureus* and streptococci. In vivo, however, the 11-keto (A-63881) analog was two to four times more active than was erythromycin against *S. aureus* and *S. pyogenes*, while the 11-epiamino (A-69334) analog was approximately two times more active than was erythromycin against *S. pyogenes*.

The in vitro activities of these compounds against gram-negative organisms were variable and may be related to the individual abilities of the compounds to cross the different barriers to penetration presented by generic differences in the chemical compositions of gram-negative cell outer membranes. Only against *C. jejuni* were the in vitro activities of several analogs significantly improved over that of erythromycin. Although the MICs of the 11-epiamino (A-69334), 11-epiamino-4″-amino (A-71671), and 11-epiamino-4″-epiamino (A-73020) analogs for *H. influenzae* were the same as or twofold higher than that of erythromycin, these compounds were more effective in treating gerbils with *H. influenzae* otitis media than was erythromycin. Although the MICs of the 11-epiamino (A-69334) analog and erythromycin for *L. pneumophila* were the same, the 11-epiamino (A-69334) analog was more active in treating legionellosis in guinea pigs. The increased in vivo activities of these compounds as compared with that of erythromycin can be explained, in part, by their improved pharmacokinetics. In mice, the 11-keto (A-63881) and 11-epiamino (A-69334) analogs reached much higher peak levels in serum than did erythromycin following oral administration, and the 11-epiamino-4″-amino (A-71671) and 11-epiamino-4″-epiamino (A-73020) analogs had serum half-lives longer than that of erythromycin following subcutaneous administration. The pharmacokinetics of analogs of 9-deoxo-12-deoxy-9,12-epoxyerythromycin A in dogs and primates have also been shown to be superior to those of erythromycin (13).

Although the 9,12-epoxy bridge in these compounds prevents acid-induced conversion to inactive enol ether and 6,9;9,12-spiroketal forms, several sites subject to ionization are present. Ionization of these molecules in the presence of acid may alter their ability to penetrate into the bacterial cell and may explain their reduced in vitro activities at an acidic pH.

In conclusion, it has been shown that 9-deoxo-12-deoxy-9,12-epoxyerythromycin A (A-69328) can be modified to produce new compounds with activities against gram-positive and gram-negative organisms and pharmacokinetics which are superior to those of erythromycin. The results of this study suggest that these or related compounds may have potential for the development of an acid-stable macrolide with improved bioavailability and gram-negative activity, as compared with those of erythromycin.

ACKNOWLEDGMENTS

We acknowledge Jill M. Beyer, Dena M. Hensey, Kenneth Jarvis, Michael Mitten, and Nancy Ramer for technical assistance.

REFERENCES

1. **Bell, S. C., J. W. Hamman, and W. E. Grundy.** 1969. Micromethod for assaying serum levels of erythromycin. Appl. Microbiol. **17**:88–92.
2. **Chantot, J.-F., A. Bryskier, and J.-C. Gasc.** 1986. Antibacterial activity of roxithromycin: a laboratory evaluation. J. Antibiot. **39**:660–668.
3. **Fernandes, P. B., R. Bailer, R. Swanson, C. W. Hanson, E. McDonald, N. Ramer, D. Hardy, N. Shipkowitz, R. R. Bower, and E. Gade.** 1986. In vitro and in vivo evaluation of A-56268 (TE-031), a new macrolide. Antimicrob. Agents Chemother. **30**:865–873.
4. **Freiburg, L. A., C. M. Edwards, D. J. Bacino, L. L. Klein, R. Stephens, S. Spanton, and K. Kim.** 1989. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1028.
5. **Freiburg, L. A., C. M. Edwards, D. J. Bacino, L. Seif, P. A. Lartey, and D. Whittern.** 1989. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1029.
6. **Fulghum, R. S., J. E. Brinn, A. M. Smith, H. J. Danile III, and P. J. Loesche.** 1982. Experimental otitis media in gerbils and chinchillas with *Streptococcus pneumoniae*, *Haemophilus influenzae*, and other aerobic and anaerobic bacteria. Infect. Immun. **36**:802–810.
7. **Hamilton, M. A., R. C. Russo, and R. V. Thurston.** 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. **11**:714–719.
8. **Hardy, D. J., D. M. Hensey, J. M. Beyer, C. Vojtko, E. J. McDonald, and P. B. Fernandes.** 1988. Comparative in vitro activities of new 14-, 15-, and 16-membered macrolides. Antimicrob. Agents Chemother. **32**:1710–1719.
9. **Hardy, D. J., R. N. Swanson, L. A. Freiberg, P. A. Lartey, and J. J. Clement.** 1989. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1030.

Downloaded from http://aac.asm.org/ on September 15, 2016 by PENN STATE UNIV

10. **Hardy, D. J., R. N. Swanson, R. A. Rode, K. Marsh, N. L. Shipkowitz, and J. J. Clement.** 1990. Enhancement of the in vitro and in vivo activities of clarithromycin against *Haemophilus influenzae* by 14-hydroxy-clarithromycin, its major metabolite in humans. Antimicrob. Agents Chemother. **34**:1407–1413.

10a. **Jorgensen, J. H., J. S. Redding, L. A. Maher, and A. W. Howell.** 1987. Improved medium for antimicrobial susceptibility testing of *Haemophilus influenzae*. J. Clin. Microbiol. **25**:2105–2113.

11. **Kirst, H. A., and G. D. Sides.** 1989. New directions for macrolide antibiotics: structural modifications and in vitro activity. Antimicrob. Agents Chemother. **33**:1413–1418.

12. **Kurath, P., P. H. Jones, R. S. Egan, and T. J. Perun.** 1971. Acid degradation of erythromycin A and erythromycin B. Experientia **27**:362.

13. **Marsh, K. C., S. C. Quigley, S. Culbertson, L. A. Freiberg, D. Bacino, C. M. Edwards, D. McDaniel, and P. Lartey.** 1989. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1031.

14. **Morimoto, S., Y. Takahashi, Y. Watanabe, and S. Omura.** 1984. Chemical modification of erythromycins. I. Syntheses and antibacterial activity of 6-O-methyl-erythromycin A. J. Antibiot. **37**:187–189.

15. **National Committee for Clinical Laboratory Standards.** 1985. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.

16. **National Committee for Clinical Laboratory Standards.** 1985. Reference agar dilution procedure for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.

17. **Retsema, J., A. Girard, W. Schelkly, M. Manousos, M. Anderson, G. Bright, R. Borovoy, L. Brennan, and R. Mason.** 1987. Spectrum and mode of action of azithromycin (CP-62,993), a new 15-membered-ring macrolide with improved potency against gram-negative organisms. Antimicrob. Agents Chemother. **31**:1939–1947.

18. **Toscano, L., and G. Fiorielo.** 1983. New fluorinated erythromycins obtained by mutasynthesis. J. Antibiot. **36**:1439–1450.

19. **Wiley, P. F., K. Gerzon, E. H. Flynn, U. V. Sigal, Jr., O. Weaver, U. C. Quarck, R. R. Chauvette, and R. Mordian.** 1957. Erythromycin. X. Structure of erythromycin. J. Am. Chem. Soc. **79**:6062–6070.

Downloaded from http://aac.asm.org/ on September 15, 2016 by PENN STATE UNIV