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# Antimicrobial Activity of Ro 23-9424, a Novel Ester-Linked Codrug of Fleroxacin and Desacetylcefotaxime

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**Ro 23-9424 is a novel ester-linked codrug of fleroxacin (Ro 23-6240; AM-833) and the cefotaxime metabolite desacetylcefotaxime. Its potency was determined against over 1,000 organisms and found to be intermediate between those of the two components. More than 99% of members of the family *Enterobacteriaceae* were inhibited by ≤8 μg of Ro 23-9424 per ml; its MIC for 50% of strains tested ranged from ≤0.06 to 1 μg/ml. Staphylococci, streptococci, *Branhamella catarrhalis*, *Corynebacterium jeikeium*, *Bacillus* spp., *Haemophilus influenzae*, *Listeria monocytogenes*, and the pathogenic *Neisseria* spp., including oxacillin-resistant *Staphylococcus aureus*, β-lactamase-producing strains, and penicillin-resistant pneumococci, were also inhibited by Ro 23-9424. *Pseudomonas aeruginosa*, *Enterococcus* spp., and *Bacteroides fragilis* group isolates were more refractory to Ro 23-9424 (the MIC for 90% of strains tested was ≥32 μg/ml). Overall, Ro 23-9424 inhibited 97% of the aerobic strains, compared with 90% for ceftazidime and 92% for cefoperazone. Ro 23-9424 was bactericidal, was relatively stable to inoculum effects on MICs at $10^7$ CFU/ml, and was determined to be highly active against organisms resistant to fluoroquinolones or ceftazidime. Preliminary quality control guidelines were determined, and a 30-μg disk concentration appears to be the most usable form.**

Compound Ro 23-9424 (Hoffmann-La Roche Inc.) is a novel ester-linked combination of fleroxacin (Ro 23-6240 or AM-833) and desacetylcefotaxime (Fig. 1) (G. Beskid, V. Fallat, E. R. Lipschitz, D. H. McGarry, R. Cleeland, K. Chan, D. D. Keith, and J. Unowsky, Antimicrob. Agents Chemother., in press; G. Beskid, J. Siebelist, C. M. McGarry, R. Cleeland, K. Chan, and D. D. Keith, submitted for publication; N. H. Georgopapadakou, A. Bertasso, K. K. Chan, J. S. Chapman, R. Cleeland, L. M. Cummings, B. A. Dix, and D. D. Keith, Antimicrob. Agents Chemother., in press). The fused drug is moderately stable to esterases after intravenous administration, having pharmacokinetics most similar to that of the cephalosporin component (J. G. Christenson, K. K. Chan, H. H. Farrish, I. H. Patel, and A. Specian, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 449, 1988). However, a consistently low level (1 μg/ml) of fleroxacin was found in various animal sera. This drug, therefore, differs from several clinically used esterified cephalosporins (8) or beta-lactams (14, 26) that are more rapidly modified by tissue or circulating esterases to their most active forms. In general, when two compounds linked by ester bonds, e.g., ampicillin and probenecid, ampicillin and mecillinam, or ampicillin and sulbactam, are administered, and in vivo concentrations of each component are equal without significant persistence of the esterified form (1, 2, 7, 9, 14, 26; B. G. Christensen and W. J. Leanza, U.S. patent 3,931,150, January 1976). Ro 23-9424 is excreted primarily intact by the kidneys and has a half-life of 75 min in primates.

The individual components of Ro 23-9424 are each very active against a number of gram-positive and gram-negative pathogens (4, 5, 10–12). In fact, as the antimicrobially active metabolite of cefotaxime (13), desacetylcefotaxime enhances cefotaxime activity against aerobic (5, 12) and anaerobic (10, 11) bacteria. Cefotaxime is converted to desacetylcefotaxime via the same esterase mechanism as the separation of the components of Ro 23-9424. Fleroxacin has a spectrum of activity comparable to those of other fluoroquinolones, such as norfloxacin and ofloxacin, but is slightly less active than ciprofloxacin, especially against gram-positive cocci (4, 10). These component antimicrobial agents produce a dual mode of action that is not unlike that of the compound reported by O'Callaghan et al. in 1976 (23). That cephalosporin-omadine combination was quite effective in vitro but failed as a therapeutic agent because of omadine-related toxicity (6, 23). Ten years later, Mobashery et al. (15, 17) described a cephalosporin-dipeptide ester that possessed in vitro activity against many strains of gram-positive and gram-negative species. Both of these cephem ester codrugs only produced an active second compound (noncephalosporin) after the cephalosporin beta-lactam ring was hydrolyzed by β-lactamases (3, 15–17, 23). Ro 23-9424 differs substantially from these previously studied codrugs because it is formed from two very active and therapeutically safe antimicrobial agents of two differing classes (H. A. Albrecht, G. Beskid, K.-K. Chan, J. Christensen, R. Cleeland, K. Deitcher, D. D. Keith, D. Pruess, J. Sepinwall, A. Specian, R. Then, M. Weigele, and K. West, 28th ICAAC, abstr. no. 441, 1988).

In this report we summarize the in vitro activity of Ro 23-9424 compared with those of fleroxacin, desacetylcefotaxime, a 1:1 ratio of fleroxacin and desacetylcefotaxime, cefotaxime, and five other broad-spectrum antimicrobial agents. Additional studies of Ro 23-9424 bactericidal activity, inoculum effects on Ro 23-9424 MICs, activity against drug-resistant organism populations (quinolone or ceftazidime resistant), and preliminary disk diffusion tests are also presented.

(These data were presented in part at the 26th ICAAC, Los Angeles, Calif., 1988 [abstr. no. 446].)

## MATERIALS AND METHODS

**Antimicrobial agents.** Ro 23-9424, desacetylcefotaxime (Ro 24-2414), and fleroxacin were obtained from Hoffmann-La Roche Inc., Nutley, N.J. All other comparison drugs, e.g., aztreonam, cefoperazone, cefotaxime, cefoxitin,

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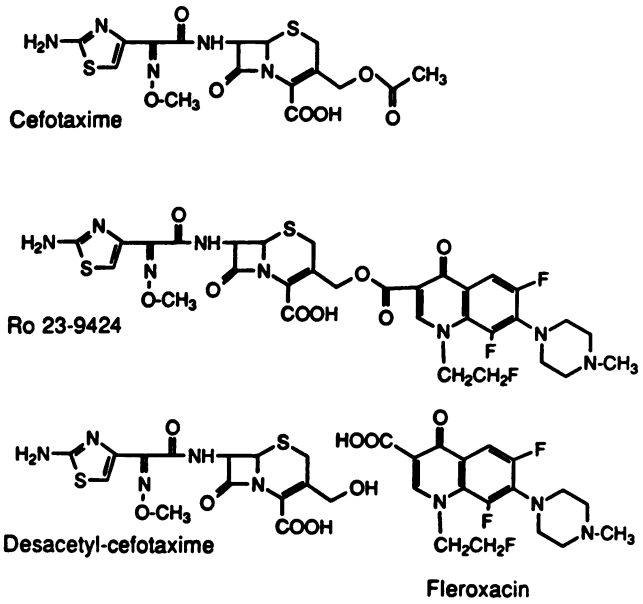


FIG. 1. Structures of cefotaxime and Ro 23-9424, including its hydrolysis components (desacetylcefotaxime and fleroxacin).

ceftazidime, ciprofloxacin, chloramphenicol, clindamycin, imipenem, metronidazole, penicillin, and tetracycline, were obtained from their principal U.S. manufacturers.

**Bacterial strains.** All tested strains were obtained from the stock culture collections of The Clinical Microbiology Institute and the Centers for Disease Control. A total of 1,004 isolates were tested (see Tables 1 to 3): 350 members of the family *Enterobacteriaceae*, 120 *Staphylococcus* spp. (20 resistant to oxacillin), 110 *Streptococcus* spp., 50 enterococci, 91 *Pseudomonas* spp., 59 *Haemophilus influenzae* (29 resistant to ampicillin), 58 *Neisseria gonorrhoeae* (33 resistant to penicillin, 6 without β-lactamase), 30 *Neisseria meningitidis*, 20 *Branhamella catarrhalis* (90% resistant to penicillins), 7 *Bacillus* spp. (all β-lactamase producers), 10 *Corynebacterium jeikeium*, 2 *Listeria monocytogenes*, 57 *Bacteroides fragilis* group strains, and 40 other nonenteric gram-negative bacilli (Tables 1 and 3).

In addition, we tested 60 isolates that were resistant or only moderately susceptible to ciprofloxacin (MIC, >1 μg/ml) or norfloxacin (MIC >4 μg/ml). Strains for which the ciprofloxacin and norfloxacin MICs were elevated (≥16-fold greater than MICs for the usual strains in the same species) were also included. Many of the test strains had resistances to other antimicrobial agents such as the newer cephalosporins, ceftazidime, and cefotaxime. These strains were analyzed separately.

A collection of 29 strains with well-characterized resistance mechanisms were tested for Ro 23-9424 bactericidal activity and inoculum effects on MICs. National Committee for Clinical Laboratory Standards (NCCLS)-recommended quality control organisms were used throughout these investigations as controls and in evaluations of possible Ro 23-9424 disk concentrations for further in vitro disk test development.

All cultures were taken from −60°C frozen storage and represent only three or four passages from original isolated specimens. This was especially true of strains possessing resistance mechanisms for which performance against control drugs has not significantly changed.

**Antimicrobial susceptibility testing methods.** All test procedures followed the recommendations of the NCCLS (18–22). Broth microdilution tests were most often used (19), except for the anaerobes and gonococci, for which the M11-A and agar dilution procedures were used, respectively (19, 20). The MICs for *Neisseria gonorrhoeae* were determined on GC agar base (BBL Microbiology Systems, Cockeysville, Md.), using IsoVitaleX with and without the cysteine component. The inoculum density used for the broth microdilution tests was approximately $5 \times 10^5$ CFU/ml, and the density for the agar dilution methods was $10^4$ CFU per spot (19) or $10^5$ CFU per spot when anaerobic bacteria were being tested. The Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) was supplemented with divalent cations.

MBCs were determined by the NCCLS M26-P method (21), with the rejection criteria of Pearson et al. (24). Duplicate 10-μg samples were subcultured onto drug-free blood agar plates from broth microdilution trays inoculated with a minimum of $5 \times 10^5$ CFU/ml. Inoculum controls were used for each tray to accurately assess the ≥99.9% kill endpoint and the selection of appropriate rejection criteria.

Disk diffusion tests with investigator-prepared 10- and 30-μg Ro 23-9424 disks were performed by NCCLS M2-A3 methods (18). At least 12 zone diameters were analyzed to determine the best candidate disk masses for further development of disk diffusion methods. Interpretive criteria for each drug tested and quality control limits were taken from the most recent NCCLS supplement (22).

## RESULTS

**Activity against gram-negative aerobic organisms.** Table 1 summarizes the susceptibility testing results for Ro 23-9424, desacetylcefotaxime, cefotaxime, desacetylcefotaxime-cefotaxime (1:1 ratio), and fleroxacin against 627 gram-negative organisms. Ro 23-9424 had an activity against these strains that was intermediate between the activities of its hydrolysis components, desacetylcefotaxime (least active) and fleroxacin (most active). The Ro 23-9424 MICs against 90% of strains tested ($MIC_{90}$s) for members of the family *Enterobacteriaceae* ranged from ≤0.25 to 16 μg/ml. The highest Ro 23-9424 MICs were found for the *Serratia marcescens* strains (five isolates at 16 μg/ml) and *Enterobacter cloacae* strains (one isolate at 16 μg/ml). The desacetylcefotaxime MICs were slightly superior to those of cefuroxime and cefoxitin (data not shown) but not as potent as that of its parent compound, cefotaxime. Fleroxacin MICs against the enteric bacilli were usually lower, with $MIC_{90}$s indicating that the organisms were in the susceptible category (≤2 μg/ml) for all species except *Serratia* spp. ($MIC_{90}$, 8 μg/ml). The 350 members of the family *Enterobacteriaceae* that we tested were inhibited at the following rates: 87% by ≤8 μg of desacetylcefotaxime per ml, 94% by ≤8 μg of cefotaxime per ml, 95% by ≤2 μg of fleroxacin per ml, and 98% by ≤8 μg of Ro 23-9424 per ml (100% by ≤16 μg/ml). The 1:1 ratio of unfused desacetylcefotaxime and fleroxacin was equal in potency to the more active component and had the widest spectrum against the enteric bacilli; e.g., >99% of the strains were inhibited by ≤8/8 μg/ml.

*Branhamella catarrhalis*, *Haemophilus influenzae*, and the pathogenic *Neisseria* species were very susceptible to all tabulated drugs (Table 1). Generally, all MICs were ≤0.5 μg/ml. The production of β-lactamase by some of these organisms did not significantly affect the drug MIC; therefore, all strains within a species were listed together. The presence of cysteine in the IsoVitaleX supplement used for gonococcal tests elevated the Ro 23-9424 MICs for five strains from

TABLE 1. Antimicrobial activity of Ro 23-9424 and other drugs against 627 gram-negative organisms

| Organism (no. tested) | Antimicrobial agent[a] | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | 50% | 90% | Range |
| *Acinetobacter calcoaceticus* (30) | Ro 23-9424 | 1 | 2 | 1–4 |
| | dCTX/FLER | 0.25 | 0.5 | 0.12–1 |
| | dCTX | 8 | 16 | 1–>32 |
| | CTX | 8 | 16 | 4–32 |
| | FLER | 0.5 | 0.5 | 0.25–1 |
| *Branhamella catarrhalis* (20)[b] | Ro 23-9424 | 0.25 | 0.5 | 0.12–1 |
| | dCTX/FLER | 0.25 | 0.25 | 0.12–0.25 |
| | dCTX | 0.5 | 0.5 | 0.5 |
| | CTX | ≤0.06 | 0.12 | ≤0.06–0.5 |
| | FLER | 0.25 | 0.25 | 0.12–0.25 |
| *Haemophilus influenzae* (59)[c] | Ro 23-9424 | ≤0.06 | 0.25 | ≤0.06–0.25 |
| | dCTX/FLER | ≤0.06 | ≤0.06 | ≤0.06 |
| | dCTX | ≤0.06 | ≤0.12 | ≤0.06–0.25 |
| | CTX | ≤0.06 | ≤0.06 | ≤0.06–0.25 |
| | FLER | ≤0.06 | 0.12 | ≤0.06–0.25 |
| *Neisseria gonorrhoeae* (58)[d] | Ro 23-9424 | ≤0.06 | ≤0.06 | ≤0.06–0.12 |
| | Penicillin | 1 | >16 | ≤0.03–>16 |
| | Tetracycline | 0.5 | 2 | 0.12–2 |
| *Neisseria meningitidis* (30) | Ro 23-9424 | ≤0.06 | ≤0.06 | ≤0.06 |
| | dCTX/FLER | ≤0.06 | ≤0.06 | ≤0.06 |
| | dCTX | ≤0.06 | ≤0.06 | ≤0.06–0.12 |
| | CTX | ≤0.06 | ≤0.06 | ≤0.06 |
| | FLER | ≤0.06 | ≤0.06 | ≤0.06 |
| *Pseudomonas aeruginosa* (51) | Ro 23-9424 | 8 | 32 | 2–>32 |
| | dCTX/FLER | 2 | 8 | 0.5–>32 |
| | dCTX | >32 | >32 | >32 |
| | CTX | 16 | >32 | 4–>32 |
| | FLER | 2 | 8 | 0.5–>32 |
| *Xanthomonas maltophilia* (10) | Ro 23-9424 | 8 | 16 | 4–16 |
| | dCTX/FLER | 2 | 8 | 2–8 |
| | dCTX | >32 | >32 | >32 |
| | CTX | 32 | >32 | 4–>32 |
| | FLER | 2 | 8 | 2–8 |
| *Citrobacter diversus* (14) | Ro 23-9424 | 0.12 | 0.25 | 0.12–0.25 |
| | dCTX/FLER | ≤0.06 | ≤0.06 | ≤0.06 |
| | dCTX | 0.12 | 0.25 | 0.12–0.5 |
| | CTX | ≤0.06 | 0.12 | ≤0.06–0.12 |
| | FLER | ≤0.06 | ≤0.06 | ≤0.06 |
| *Citrobacter freundii* (12) | Ro 23-9424 | 0.5 | 2 | 0.25–2 |
| | dCTX/FLER | 0.12 | 0.5 | ≤0.06–0.5 |
| | dCTX | 2 | >32 | 0.12–>32 |
| | CTX | 0.25 | >32 | 0.12–>32 |
| | FLER | 0.12 | 0.5 | ≤0.06–0.5 |
| *Enterobacter aerogenes* (30) | Ro 23-9424 | 0.25 | 0.25 | 0.12–0.5 |
| | dCTX/FLER | 0.12 | 0.12 | ≤0.06–0.5 |
| | dCTX | 0.5 | 32 | 0.12–>32 |
| | CTX | 0.12 | 1 | ≤0.06–16 |
| | FLER | 0.12 | 0.12 | ≤0.06–0.5 |
| *Enterobacter agglomerans* (10) | Ro 23-9424 | 0.25 | 0.5 | 0.12–2 |
| | dCTX/FLER | ≤0.06 | 0.12 | ≤0.06–1 |
| | dCTX | 1 | 8 | 0.12–>32 |
| | CTX | 0.25 | 1 | 0.12–>32 |
| | FLER | ≤0.06 | 2 | ≤0.06–2 |
| *Enterobacter cloacae* (30) | Ro 23-9424 | 0.25 | 1 | 0.12–16 |
| | dCTX/FLER | ≤0.06 | 0.25 | ≤0.06–4 |
| | dCTX | 1 | 32 | 0.25–>32 |
| | CTX | 0.25 | 16 | ≤0.06–>32 |
| | FLER | ≤0.06 | 0.25 | ≤0.06–4 |
| *Escherichia coli* (30) | Ro 23-9424 | 0.12 | 0.25 | 0.12–4 |
| | dCTX/FLER | ≤0.06 | 0.12 | ≤0.06–1 |
| | dCTX | 0.25 | 1 | 0.12–>32 |
| | CTX | ≤0.06 | 0.12 | ≤0.06–>32 |
| | FLER | ≤0.06 | 0.12 | ≤0.06–4 |
| *Klebsiella pneumoniae* (24) | Ro 23-9424 | 0.12 | 0.5 | 0.12–0.5 |
| | dCTX/FLER | ≤0.06 | 0.25 | ≤0.06–0.5 |
| | dCTX | 0.12 | 0.5 | ≤0.06–1 |
| | CTX | ≤0.06 | 0.12 | ≤0.06–0.25 |
| | FLER | 0.12 | 1 | 0.12–16 |
| *Morganella morganii* (20) | Ro 23-9424 | 0.25 | 0.25 | 0.12–1 |
| | dCTX/FLER | ≤0.06 | ≤0.06 | ≤0.06–0.25 |
| | dCTX | 32 | >32 | 0.5–>32 |
| | CTX | 0.25 | 4 | ≤0.06–8 |
| | FLER | ≤0.06 | ≤0.06 | ≤0.06–0.25 |
| *Proteus mirabilis* (31) | Ro 23-9424 | 0.12 | 0.25 | ≤0.06–0.25 |
| | dCTX/FLER | ≤0.06 | ≤0.06 | ≤0.06 |
| | dCTX | ≤0.06 | ≤0.06 | ≤0.06 |
| | CTX | ≤0.06 | ≤0.06 | ≤0.06 |
| | FLER | 0.12 | 0.25 | 0.12–16 |
| *Proteus vulgaris* (20) | Ro 23-9424 | 0.25 | 0.5 | 0.12–0.5 |
| | dCTX/FLER | ≤0.06 | 0.12 | ≤0.06–0.25 |
| | dCTX | 0.25 | 1 | 0.12–>32 |
| | CTX | 0.12 | 0.25 | ≤0.06–4 |
| | FLER | 0.12 | 0.12 | ≤0.06–0.25 |
| *Providencia rettgeri* (20) | Ro 23-9424 | 0.12 | 0.25 | ≤0.06–1 |
| | dCTX/FLER | ≤0.06 | ≤0.06 | ≤0.06–0.12 |
| | dCTX | ≤0.06 | 0.12 | ≤0.06–1 |
| | CTX | ≤0.06 | 0.25 | ≤0.06–0.25 |
| | FLER | 0.12 | 0.5 | ≤0.06–4 |
| *Providencia stuartii* (31) | Ro 23-9424 | 0.25 | 1 | 0.12–2 |
| | dCTX/FLER | ≤0.06 | 0.25 | ≤0.06–1 |
| | dCTX | 0.12 | 2 | ≤0.06–4 |
| | CTX | 0.25 | 1 | ≤0.06–1 |
| | FLER | 0.25 | 2 | ≤0.06–8 |
| *Serratia* spp. (30) | Ro 23-9424 | 0.5 | 16 | 0.5–16 |
| | dCTX/FLER | 0.25 | 4 | 0.12–16 |
| | dCTX | 1 | >32 | 0.5–>32 |
| | CTX | 0.5 | 32 | 0.25–>32 |
| | FLER | 0.25 | 8 | 0.12–16 |
| *Yersinia enterocolitica* (10) | Ro 23-9424 | 0.25 | 0.25 | 0.12–0.25 |
| | dCTX/FLER | ≤0.06 | ≤0.06 | ≤0.06–0.12 |
| | dCTX | 1 | 8 | 0.25–8 |
| | CTX | 0.12 | 0.5 | ≤0.06–0.5 |
| | FLER | ≤0.06 | ≤0.06 | ≤0.06–0.12 |
| *Bacteroides fragilis* group (57)[e] | Ro 23-924 | 32 | >32 | 2–>32 |
| | Cefoxitin | 16 | >32 | 2–>32 |

[a] Abbreviations: dCTX, desacetylcefotaxime; CTX, cefotaxime; FLER, fleroxacin; dCTX/FLER, unfused equal concentrations of desacetylcefotaxime and fleroxacin.

[b] Eighteen strains were β-lactamase producers (10 Ravasio and 8 type 1908).

[c] Includes 29 β-lactamase-positive isolates.

[d] Includes 27 β-lactamase-positive isolates.

[e] Organisms included 6 *B. distasonis*, 43 *B. fragilis*, 3 *B. ovatus*, 3 *B. thetaiotaomicron*, and 2 *B. vulgaris*. All strains were susceptible to metronidazole (MIC, ≤8 μg/ml) and chloramphenicol (MIC, ≤16 μg/ml); 7% of isolates were resistant to clindamycin (MIC, >4 μg/ml).

TABLE 2. Antimicrobial activity of Ro 23-9424 and other drugs against 278 gram-positive organisms

| Organism (no. tested) | Antimicrobial agent[a] | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | 50% | 90% | Range |
| *Corynebacterium jeikeium* (10) | Ro 23-9424 | 2 | 4 | 0.25–4 |
| | dCTX/FLER | 2 | 2 | 0.25–2 |
| | dCTX | 16 | >32 | 0.25–>32 |
| | CTX | 8 | >32 | 0.12–>32 |
| | FLER | 1 | 2 | 1–2 |
| *Enterococcus faecalis* (28) | Ro 23-9424 | 16 | 32 | 4–32 |
| | dCTX/FLER | 4 | 8 | 1–16 |
| | dCTX | >32 | >32 | 16–>32 |
| | CTX | >32 | >32 | >32 |
| | FLER | 4 | 8 | 1–8 |
| *Enterococcus faecium* (10) | Ro 23-9424 | 32 | >32 | 16–32 |
| | dCTX/FLER | 8 | 8 | 4–8 |
| | dCTX | >32 | >32 | >32 |
| | CTX | >32 | >32 | >32 |
| | FLER | 8 | 8 | 4–8 |
| *Staphylococcus aureus* | | | | |
| Oxacillin susceptible (50) | Ro 23-9424 | 1 | 2 | 0.5–2 |
| | dCTX/FLER | 0.5 | 0.5 | 0.12–1 |
| | dCTX | 8 | 8 | 4–16 |
| | CTX | 2 | 2 | 1–4 |
| | FLER | 0.5 | 0.5 | 0.12–1 |
| Oxacillin resistant (10) | Ro 23-9424 | 2 | 2 | 1–2 |
| | dCTX/FLER | 0.5 | 0.5 | 0.25–0.5 |
| | dCTX | >32 | >32 | >32 |
| | CTX | 32 | >32 | 8–>32 |
| | FLER | 0.5 | 1 | 0.25–1 |
| *Staphylococcus* spp. (coagulase-negative) | | | | |
| Oxacillin susceptible (50)[b] | Ro 23-9424 | 1 | 2 | 1–8 |
| | dCTX/FLER | 0.5 | 1 | 0.5–4 |
| | dCTX | 1 | 8 | 0.25–16 |
| | CTX | 1 | 2 | 0.5–4 |
| | FLER | 0.5 | 2 | 0.5–16 |
| Oxacillin resistant (10) | Ro 23-9424 | 1 | 2 | 0.5–2 |
| | dCTX/FLER | 0.5 | 0.5 | 0.25–1 |
| | dCTX | >32 | >32 | 0.5–>32 |
| | CTX | 16 | >32 | 2–>32 |
| | FLER | 0.5 | 1 | 0.25–1 |
| *Streptococcus pneumoniae* | | | | |
| Penicillin susceptible (20) | Ro 23-9424 | ≤0.06 | ≤0.06 | ≤0.06 |
| | dCTX/FLER | ≤0.06 | ≤0.06 | ≤0.06 |
| | dCTX | ≤0.06 | ≤0.06 | ≤0.06 |
| | CTX | ≤0.06 | ≤0.06 | ≤0.06 |
| | FLER | 8 | 8 | 4–16 |
| Penicillin resistant (10)[c] | Ro 23-9424 | 0.12 | 0.25 | ≤0.06–0.5 |
| | dCTX/FLER | 0.5 | 2 | 0.25–4 |
| | dCTX | 0.5 | 4 | 0.25–8 |
| | CTX | 0.25 | 0.5 | 0.12–2 |
| | FLER | 8 | 8 | 4–16 |
| Serogroup A (30) | Ro 23-9424 | ≤0.06 | ≤0.06 | ≤0.06 |
| | dCTX/FLER | ≤0.06 | ≤0.06 | ≤0.06–0.12 |
| | dCTX | ≤0.06 | ≤0.06 | ≤0.06–0.12 |
| | CTX | ≤0.06 | ≤0.06 | ≤0.06 |
| | FLER | 4 | 8 | 4–>32 |
| Serogroup B (30) | Ro 23-9424 | ≤0.06 | 0.12 | ≤0.06–0.25 |
| | dCTX/FLER | 0.12 | 0.25 | ≤0.06–0.5 |
| | dCTX | 0.12 | 0.25 | ≤0.06–0.5 |
| | CTX | ≤0.06 | ≤0.06 | ≤0.06–0.12 |
| | FLER | 8 | 8 | 4–16 |
| Serogroup C (10) | Ro 23-9424 | ≤0.06 | ≤0.06 | ≤0.06 |
| | dCTX/FLER | ≤0.06 | ≤0.06 | ≤0.06–0.12 |
| | dCTX | ≤0.06 | ≤0.06 | ≤0.06–0.12 |
| | CTX | ≤0.06 | ≤0.06 | ≤0.06 |
| | FLER | 4 | 4 | 4–8 |
| Serogroup G (10) | Ro 23-9424 | ≤0.06 | ≤0.06 | ≤0.06 |
| | dCTX/FLER | ≤0.06 | ≤0.06 | ≤0.06 |
| | dCTX | ≤0.06 | ≤0.06 | ≤0.06 |
| | CTX | ≤0.06 | ≤0.06 | ≤0.06 |
| | FLER | 4 | 4 | 2–8 |

[a] For abbreviations, see Table 1, footnote *a*.
[b] Includes 3 *S. hominis*, 29 *S. epidermidis*, 3 *S. capitis*, 4 *S. saprophyticus*, 4 *S. simulans*, and 7 *S. warneri*.
[c] Penicillin MICs, ≥0.12 μg/ml.

≤0.06 to 0.12 μg/ml. Similarly, penicillin MICs for 13 of the 58 *Neisseria gonorrhoeae* strains were higher when tested in the presence of a cysteine supplement. The gonococcal data shown in Table 1 were determined on agar without cysteine. The nonenteric gram-negative bacilli were not as susceptible to the five listed compounds. Ro 23-9424 $MIC_{90}$s for *Pseudomonas* spp. ranged from 16 to 32 μg/ml, but Ro 23-9424 was the most active (in terms of the percentage of strains inhibited) single drug tested at potential susceptibility breakpoint concentrations. Ro 23-9424 MICs for two *Achromobacter xylosoxidans* strains and three *Pseudomonas aeruginosa* strains were the highest recorded (>32 μg/ml); all of these strains were also resistant to fleroxacin and other quinolones. Again, the 1:1 free drug ratio of desacetylcefotaxime and fleroxacin was the most effective.

Ro 23-9424 results were compared with results for cefoxitin tested against 57 strains of the *Bacteroides fragilis* group (Table 1). Metronidazole and chloramphenicol were uniformly effective against these strains, but 7% of these organisms were resistant to clindamycin (>4 μg/ml; data not shown). Ro 23-9424 was only moderately active, with an MIC mode at 32 μg/ml. One-third of the strains were cefoxitin resistant by NCCLS criteria, with an MIC mode of 16 μg/ml.

**Activity against gram-positive strains.** The activity of Ro 23-9424 was generally comparable to that of desacetylcefotaxime and fleroxacin against the gram-positive cocci (Table 2). Ro 23-9424 was usually equal in potency to its most active component drug when tested against the *Staphylococcus* spp. and the streptococci. All but one Ro 23-9424 MIC were ≤2 μg/ml for the 200 staphylococci, including 20 oxacillin-resistant strains. The *Streptococcus* strains were 8- to 16-fold more susceptible to Ro 23-9424 than to fleroxacin (MICs for 100% of strains [$MIC_{100}$], ≤0.06 to 0.5 μg/ml). MICs of Ro 23-9424 and comparison cephalosporins for the *Streptococcus pneumoniae* isolates resistant to penicillin were also higher (≤0.06 to 0.5 μg/ml) than those for the penicillin-susceptible strains (≤0.06 μg/ml). This antipneumococcal activity of Ro 23-9424 was superior to that of either component alone. All drugs tested were less effective against the four enterococcal species (Ro 23-9424 MICs for 50% of

TABLE 3. In vitro activity of Ro 23-9424 against 99 additional strains from more than 20 species

| Organism (no. tested) | MIC ($\mu$g/ml)[a] |
|---|---|
| *Achromobacter xylosoxidans* | $8_2$, $>32_2$ |
| *Aeromonas hydrophila* | $\leq 0.06_1$, $0.12_2$, $2_1$ |
| *Bacillus* spp. | $1_2$, $2_5$ |
| *Cedecae lapagei* | $0.12_1$, $0.25_1$ |
| *Citrobacter amalonaticus* | $0.25_1$, $1_2$ |
| *Enterobacter sakazakii* | $0.12_1$, $0.5_1$, $1_1$ |
| *Enterococcus durans* | $\leq 0.06_1$, $0.5_2$, $4_1$, $8_3$ |
| *Enterococcus hirae* | $0.5_1$, $2_1$, $4_1$, $8_1$, $16_1$ |
| *Flavobacterium* spp. | $4_1$, $8_1$ |
| *Hafnia alvei* | $0.25_3$ |
| *Klebsiella oxytoca* | $\leq 0.06_2$, $0.12_2$, $0.25_1$ |
| *Listeria monocytogenes* | $4_2$ |
| *Providencia alcalifaciens* | $\leq 0.06_2$, $0.12_1$ |
| *Pseudomonas cepacia* | $0.5_1$, $1_1$, $2_1$, $4_1$, $8_2$, $16_1$ |
| *Pseudomonas fluorescens* | $2_1$, $4_2$, $8_3$, $16_1$, $32_1$ |
| *Pseudomonas putida* | $8_4$, $16_1$ |
| *Pseudomonas stutzeri* | $0.5_2$, $1_2$, $8_1$ |
| *Pseudomonas acidovorans* | $0.5_3$, $1_2$ |
| *Salmonella enteritidis* | $0.25_6$, $0.5_3$ |
| *Serratia liquefaciens* | $0.12_1$ |
| *Shigella* spp. | $\leq 0.06_6$, $0.12_3$ |

[a] The subscript is the number of isolates for each MIC result.

strains tested [$MIC_{50}$s], 4 to 32 $\mu$g/ml), although some *Enterococcus durans* and *Enterococcus hirae* strains may be susceptible (see Table 3). Group JK *Corynebacterium* strains were significantly inhibited by fleroxacin ($MIC_{50}$, 1 $\mu$g/ml) and Ro 23-9424 ($MIC_{50}$, 2 $\mu$g/ml) but not by cefotaxime or desacetylcefotaxime.

**Activity against infrequently isolated species.** The Ro 23-9424 MICs for 99 additional organisms are given in Table 3. Ro 23-9424 was very active against the 38 enteric bacilli representing nine species ($MIC_{100}$, 1 $\mu$g/ml). Ro 23-9424 MICs were $\geq$32 $\mu$g/ml for only *Achromobacter xylosoxidans* (two strains) and *Pseudomonas fluorescens* (one strain).

**Activity against all aerobic organisms.** The five antimicrobial agents listed in Tables 1 and 2 plus aztreonam, cefoperazone, ceftazidime, ciprofloxacin, and imipenem were tested against all the aerobic species we used (945 strains). Ro 23-9424 had a spectrum of inhibition ($\leq$8 $\mu$g/ml) identical to that of the broadest-spectrum agents, ciprofloxacin (97%) and imipenem (97%). For the 647 gram-negative strains, aztreonam was the least effective, with 11% of strains resistant, compared with resistance rates of 2, 3, and 5% for Ro 23-9424 (MICs, $\geq$32 $\mu$g/ml), ceftazidime (MICs, $\geq$32 $\mu$g/ml), and cefotaxime (MICs, $\geq$64 $\mu$g/ml), respectively. Ceftazidime was the least active agent against the gram-positive strains, exhibiting 27% resistance compared with only 7% for cefoperazone. The rank order of spectrum of activity against all organisms was (percentage of resistant strains in parentheses) as follows: ceftazidime (10.4%) < cefotaxime (9.5%) < cefoperazone (8.5%) < Ro 23-9424 (3.3%) < ciprofloxacin (3.2%) < imipenem (2.8%).

**Activity against drug-resistant strains.** Sixty organisms with more limited susceptibility to ciprofloxacin and norfloxacin (Table 4, footnote *b*) were tested against Ro 23-9424 and its hydrolysis components. Of these strains, 45 and 48% were susceptible to desacetylcefotaxime and fleroxacin, respectively. However, Ro 23-9424 inhibited 65 and 85% of the strains $\leq$8 and $\leq$16 $\mu$g/ml, respectively.

A total of 19 ceftazidime-resistant (MIC, >16 $\mu$g/ml) gram-negative bacilli were identified in the test organism collection. Ro 23-9424 inhibited 95% of these organisms at $\leq$16 $\mu$g/ml, compared with 68% for fleroxacin ($\leq$2 $\mu$g/ml) and 5% for desacetylcefotaxime ($\leq$8 $\mu$g/ml; data not shown).

**Bactericidal activity and inoculum effects.** Table 5 shows the MIC and MBC results of testing 29 strains with known resistance mechanisms. Minimal inoculum effects in the form of Ro 23-9424 MIC increases were identified over the range of $10^4$ to $10^7$ CFU/ml. MBCs determined with a minimum inoculum of $10^5$ to $10^6$ CFU/ml produced Ro 23-9424 results at or only one dilution step above the MIC for 17 of 18 organisms. An eightfold increase was found for the *Serratia marcescens* strain (type I cephalosporinase). No elevation in Ro 23-9424 MICs or MBCs at an inoculum of $10^7$ CFU/ml was observed for the type IVc enzyme-producing strains of *K. oxytoca*. These strains have been found to be more resistant to aminothiazolyl-methoxyimino cephalosporins at high inocula (>$10^6$ CFU/ml), and the MBCs of the drugs may be elevated. These findings indicate a possible $\beta$-lactamase stability of Ro 23-9424 and were also consistent with the reported twofold-higher enzyme stability of desacetylcefotaxime than cefotaxime (25). The fused compound also appeared to be bactericidal against the *Enterococcus faecalis* strain (MIC and MBC, 16 $\mu$g/ml).

**Preliminary disk diffusion tests.** NCCLS-recommended quality control strains were processed concurrently with these studies, and investigator-prepared 10- and 30-$\mu$g Ro 23-9424 disks were also tested. The zones of inhibition around the 30-$\mu$g disks were 3 to 4 mm larger than those observed around the 10-$\mu$g disks. If a Ro 23-9424 susceptibility breakpoint of $\leq$8 or $\leq$16 $\mu$g/ml were selected and if *Pseudomonas* spp. such as *P. aeruginosa* ATCC 27853 were considered Ro 23-9424 susceptible, a disk concentration of $\geq$30 $\mu$g would have to be used. Mean Ro 23-9414 MICs for the four recommended NCCLS strains were determined as 0.12 $\mu$g/ml for *Escherichia coli* ATCC 25922, 10.6 $\mu$g/ml for *P. aeruginosa* ATCC 27853, 1.29 $\mu$g/ml for *Staphylococcus aureus* ATCC 29213, and 15.0 $\mu$g/ml for *Enterococcus faecalis* ATCC 29212.

## DISCUSSION

The Hoffmann-La Roche compound Ro 23-9424 appears to be a unique, somewhat stable linkage of the quinolone fleroxacin and the cephalosporin desacetylcefotaxime (4, 5, 10–13, 25; Beskid et al., in press; Beskid et al., submitted; Georgopapadakou et al., in press). Its antimicrobial activity

TABLE 4. Activity of Ro 23-9424, fleroxacin, and desacetylcefotaxime against 60 organisms[a] previously found to be more resistant to fluoroquinolones[b]

| Antimicrobial agent | Cumulative % inhibited at MIC ($\mu$g/ml) of: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $\leq$0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 |
| Desacetylcefotaxime | 23 | 27 | 28 | 30 | 38 | 45 | 47 | 52 |
| Fleroxacin | 5 | 10 | 25 | 48 | 63 | 83 | 95 | 98 |
| Ro 23-9424 | 7 | 22 | 33 | 40 | 52 | 65 | 85 | 93 |

[a] These 60 strains included 4 *Acinetobacter* spp., 1 *Aeromonas* spp., 4 *Achromobacter xylosoxidans*, 1 *Enterobacter aerogenes*, 3 *Enterobacter agglomerans*, 1 *Enterobacter cloacae*, 2 *Escherichia coli*, 4 *Klebsiella pneumoniae*, 7 *Pseudomonas aeruginosa*, 4 *Pseudomonas cepacia*, 10 *Pseudomonas maltophilia*, 1 *Pseudomonas stutzeri*, 1 *Proteus mirabilis*, 2 *Providencia rettgeri*, 1 *Providencia alcalifaciens*, 8 *Providencia stuartii*, and 6 *Serratia marcescens*.

[b] For these strains, ciprofloxacin MICs were >1 $\mu$g/ml and/or norfloxacin MICs were >4 $\mu$g/ml. Some strains were included because the ciprofloxacin or norfloxacin MICs were $\geq$16-fold higher than those for other strains within the same species, although they were susceptible by current breakpoint criteria (22).

TABLE 5. Ro 23-9424 bactericidal activity and effects of inoculum concentrations on MIC results for 29 strains having defined antimicrobial resistances

| β-Lactamase or resistance mechanism | Host species | Ro 23-9424 MIC (μg/ml) at inoculum (CFU/ml) of: | | | Ro 23-9424 MBC (μg/ml) at $5 \times 10^5$ CFU/ml |
|---|---|---|---|---|---|
| | | $10^4$ | $5 \times 10^5$ | $10^7$ | |
| | *Escherichia coli* | 0.12 | 0.25 | 2 | 0.25 |
| | *Klebsiella oxytoca* | ≤0.06 | 0.12 | 0.25 | 0.12 |
| | *Pseudomonas aeruginosa* | 4 | 16 | 32 | 32 |
| Ia | *Citrobacter freundii* | 0.25 | 0.25 | 1 | 0.25 |
| Ia | *Enterobacter aerogenes* | 0.12 | 0.25 | 0.5 | 0.25 |
| Ia (P99) | *Enterobacter cloacae* | 0.25 | 0.5 | 0.5 | 0.5 |
| Ia | *Enterobacter cloacae* | 0.12 | 0.5 | 1 | 0.5 |
| I | *Serratia marcescens* | 0.25 | 0.25 | 0.5 | 2 |
| IIIa (TEM-1) | *Escherichia coli* | 0.12 | 0.12 | 0.25 | 0.25 |
| IIIb (TEM-2) | *Escherichia coli* | ND[a] | ≤0.06 | ND | ND |
| IVc (K1) | *Klebsiella oxytoca* | 0.25 | 0.5 | 0.5 | 0.5 |
| IVc (K14) | *Klebsiella oxytoca* | 0.25 | 0.5 | 1 | 0.5 |
| IVc, V | *Klebsiella pneumoniae* | ND | 0.25 | ND | ND |
| V (CARB-1) | *Pseudomonas aeruginosa* | ND | 4 | ND | ND |
| V (CARB-2) | *Pseudomonas aeruginosa* | 4 | 8 | 16 | 16 |
| V (CARB-4) | *Pseudomonas aeruginosa* | ND | 4 | ND | ND |
| V (OXA-1) | *Escherichia coli* | ND | 0.5 | ND | ND |
| V (OXA-2) | *Escherichia coli* | 0.25 | 0.25 | 1 | 0.25 |
| V (OXA-3) | *Escherichia coli* | ND | 0.12 | ND | ND |
| V (OXA-4) | *Pseudomonas aeruginosa* | ND | 4 | ND | ND |
| V (HMS-1) | *Escherichia coli* | ND | 0.25 | ND | ND |
| V (SHV-1) | *Escherichia coli* | ND | 0.12 | ND | ND |
| V | *Escherichia coli* | ND | 0.25 | ND | ND |
| V | *Escherichia coli* | ND | 0.12 | ND | ND |
| Penicillinase | *Staphylococcus aureus* | 1 | 2 | 4 | 2 |
| Penicillinase | *Staphylococcus aureus* | 1 | 1 | 2 | 2 |
| PBP[b] | *Staphylococcus aureus* | 1 | 2 | 4 | 2 |
| PBP[c] | *Streptococcus pneumoniae* | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 |
| | *Enterococcus faecalis* | 8 | 16 | >32 | 16 |

[a] ND, Not determined.
[b] Oxacillin resistant by altered penicillin-binding proteins (PBPs).
[c] Penicillin resistant (MIC, 0.5 μg/ml) by altered penicillin-binding proteins (PBPs).

generally produced MICs between those recorded for the two hydrolysis components. The potentially favorable antimicrobial interaction was rarely observed for gram-negative organisms but was the rule for the penicillin-resistant pneumococci. The Ro 23-9424 spectrum of activity will be dependent upon its pharmacokinetic features, reportedly most similar to that of cefotaxime in primates (Christenson et al., 28th ICAAC). Its interaction with organism penicillin-binding proteins also most resembles that of cefotaxime, after which the fleroxacin component is freed to exert a DNA gyrase inhibitor action (Georgopapadakou et al., in press). In addition, levels of fleroxacin in serum in animal pharmacokinetic studies have been reported at approximately 1 μg/ml, thus indicating minimal esterase action on Ro 23-9424. β-Lactamase hydrolysis of the desacetylcefotaxime component will also result in free fleroxacin and inactive cephalosporin components (Georgopapadakou et al., in press).

If the cefotaxime-like pharmacokinetics are substantiated in humans, along with associated low, nontoxic fleroxacin levels, an Ro 23-9424 susceptibility breakpoint MIC of ≤8 or ≤16 μg/ml would be possible and consistent with results for other cephalosporins (22). At these breakpoint concentrations, the Ro 23-9424 spectrum includes virtually all clinically relevant gram-positive and gram-negative aerobes, including strains resistant to quinolones or newer cephalosporins (Table 3). This degree of activity was most comparable to that of ciprofloxacin and imipenem. In vivo activity has been proven by animal model experiments with fluoroquinolone-resistant and cefotaxime-resistant organisms (Beskid et al., submitted). Ro 23-9424 activity was only moderate (MIC mode, 32 μg/ml) against *Bacteroides fragilis* group anaerobes with increased resistance to cefoxitin.

Ro 23-9424 appears to be bactericidal and did not show a significant inoculum effect. When *Neisseria gonorrhoeae* strains are being tested, medium supplemented with cysteine may inactivate Ro 23-9424, resulting in slightly higher MICs (0.12 μg/ml). We recommend that such supplements be avoided. Preliminary disk diffusion tests with NCCLS-recommended quality control strains favor a disk potency consistent with that of other cephalosporins, e.g., 30 μg (22).

This fusion product of two potent compounds seems quite different from other clinically utilized ester-linked drugs (1, 2, 7, 16) or uses of an ester to improve the gastrointestinal absorption of a drug (8, 10, 14). Unlike previously reported co- or prodrugs, Ro 23-9424 seems best applied by parenteral routes, and its activity does not rely entirely on active free components (1, 2, 7, 14; Christensen and Leanza, patent) or their potentially favorable interaction (1, 7, 9). The greater esterase stability of the Ro 23-9424 structure could allow higher, possibly nontoxic quinolone concentrations in the bacterial periplasmic space following Ro 23-9424–penicillin-binding protein interactions (Georgopapadakou et al., in press). Mutual prodrugs such as sultamicillin have been successful as orally administered antimicrobial agent combinations (1). If similar findings are confirmed in Ro 23-9424 human trials, this fused codrug will be a valuable addition to the chemotherapy of infections caused by either cephalosporin- or fluoroquinolone-resistant pathogens.

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