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In Vitro Activity of Ro 23-6240, a New Difluoroquinolone Derivative, Compared with That of Other Antimicrobial Agents

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Ro 23-6240 is a new difluorinated quinolone antimicrobial agent. Its in vitro activity against a wide range of bacteria was compared with those of other quinolones and β-lactams. Generally, members of the family *Enterobacteriaceae* were inhibited by low concentrations of Ro 23-6240 (MIC_{90} [MIC for 90% of isolates tested], ≤1 μg/ml). Ninety percent of *Staphylococcus aureus* (including methicillin-resistant strains) and *Neisseria gonorrhoeae* isolates were inhibited by 0.5 μg/ml. *Pseudomonas aeruginosa* (MIC_{90}, 2 μg/ml) and *Bacteroides fragilis* (MIC_{90}, 4 μg/ml) showed intermediate susceptibility, and *Streptococcus pneumoniae* (MIC_{90}, 8 μg/ml) was less susceptible. Strains resistant to nalidixic acid were less susceptible to all the quinolones tested. The protein binding of Ro 23-6240 (5 μg/ml) was 27%.

Ro 23-6240 [AM 833; 6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1 piperazinyl)-4-oxo-3-quinolinecarboxylic acid] is a new synthetic oral quinolone derivative containing two fluorine atoms on the quinolone nucleus. Preliminary human pharmacokinetic studies of the compound indicated that it has a longer serum half-life (11 h after a 1,000-mg dose; personal communication, I. Lenox-Smith) than other, newer quinolone antimicrobial agents. In this study, the activity of Ro 23-6240 was compared with that of three other quinolones (ciprofloxacin, norfloxacin, and ofloxacin), together with a number of β-lactam antibiotics, against a wide range of clinical isolates.

A total of 487 strains were examined, of which 439 were recent clinical isolates from Dudley Road Hospital. These included strains resistant to gentamicin and methicillin-resistant *Staphylococcus aureus*. The remaining 48 strains included β-lactamase producers and strains resistant to nalidixic acid. All strains were identified by conventional methods. The antimicrobial agents were obtained from their respective manufacturers: Ro 23-6240, Roche Products Ltd., Welwyn Garden City, United Kingdom; ciprofloxacin, Bayer U.K. Limited, Newbury, United Kingdom; norfloxacin, Merck Sharp & Dohme Ltd., Hoddesdon, United Kingdom; ofloxacin, Hoechst, Uxbridge, United Kingdom; cephalexin and cefuroxime, Glaxo Group Research Ltd., Greenford, United Kingdom; cefadroxil, Bristol-Myers Pharmaceuticals, Slough, United Kingdom; and amoxicillin, Beecham Pharmaceuticals, Brentford, United Kingdom.

The antimicrobial activities of the compounds were determined by an agar plate dilution method in which Iso-Sensitest agar (pH 7.2; Oxoid Ltd., Basingstoke, United Kingdom) was used throughout (except for *Bacteroides fragilis*). This medium was supplemented with 5% horse blood and 1% supplement C (Difco, East Molesley, United Kingdom) to support the growth of streptococci, *Haemophilus influenzae*, and *Neisseria* spp. Wilkins-Chalgren agar (Oxoid) was used to grow *B. fragilis*.

Inocula were prepared as follows. All strains of coliforms and staphylococci were grown overnight in nutrient broth, and streptococci (including pneumococci), *H. influenzae*, and *Neisseria* spp. were grown in brain heart infusion (plus supplement C) broth. Anaerobes were grown in Wilkins-Chalgren broth supplemented with 0.25% succinate for *B. fragilis* strains. All broths yielded viable counts of about 10^9 CFU/ml. Inocula were obtained by transferring 1 μl of an undiluted culture or a 1:100 dilution of the overnight culture to the surface of the antibiotic-containing media with a Denley multipoint inoculating device (Denley-Tech Ltd., Billingshurst, United Kingdom), yielding final inocula of 10^4 and 10^6 CFU. All drugs were tested simultaneously with the same inoculum. Incubation was for 18 h in air at 37°C except for streptococci, *H. influenzae*, and *Neisseria* spp., for which 6% CO_2 was added. The incubation (for 48 h) of *B. fragilis* was done in an anaerobic cabinet with an atmosphere of 80% nitrogen–10% carbon dioxide–10% hydrogen. Methicillin resistance in staphylococci was determined at 30°C and with the higher inoculum. The MIC of the antibiotic was defined as that concentration in agar at which there was an estimated reduction to less than 10 colonies (with the high inocula, a slight haze of growth was ignored).

The protein binding of Ro 23-6240 was determined by an ultrafiltrate technique by using a Centriflo cone (Amicon Corp., Lexington, Mass.) which excludes molecular weights of 50,000 or greater. The concentrations of Ro 23-6240 in human serum were 5 and 50 μg/ml. Carbon dioxide was bubbled through the ultrafiltrate to adjust the pH to 6.5, and the sample was assayed for Ro 23-6240 content against standards prepared in phosphate-buffered saline. The ultrafiltrate was assayed against standards prepared in phosphate-buffered saline (pH 6.5) with *Escherichia coli* SCH 12655 (Schering Corp., Bloomfield, N.J.) as the indicator organism.

The strains, the number of isolates examined, and activity with the 10^4 CFU inoculum are shown in Table 1. Only the activity of the quinolones is shown; the β-lactams are mentioned below when appropriate. Against members of the family *Enterobacteriaceae*, Ro 23-6240, ciprofloxacin, norfloxacin, and ofloxacin had generally similar degrees of activity; however, ciprofloxacin was somewhat more active than Ro 23-6240, except against *Serratia* spp., for which all four compounds were equally active. Generally, the activity of Ro 23-6240 was comparable to that of ofloxacin and norfloxacin. Sixteen strains of *Enterobacteriaceae* resistant to gentamicin (MIC, ≥4 μg/ml) were included. All the gentamicin-resistant strains were highly susceptible to Ro 23-6240 (MIC_{90} [MIC for 90% of isolates tested], <0.25

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TABLE 1. MICs inhibiting cumulative percentages of isolates

Organism (no. of isolates) and antibiotic	MIC (μg/ml)		
	Range	MIC_{50}	MIC_{90}
Escherichia coli (49)			
Ciprofloxacin	0.008–0.5	0.015	0.25
Norfloxacin	0.03–0.5	0.06	1.0
Ofloxacin	0.03–1	0.06	0.5
Ro 23-6240	0.03–2	0.06	1.0
Klebsiella spp. (49)			
Ciprofloxacin	0.002–4	0.03	0.5
Norfloxacin	0.03–16	0.12	2.0
Ofloxacin	0.06–8	0.12	1.0
Ro 23-6240	0.06–8	0.06	1.0
Enterobacter spp. (23) (5 *E. aerogenes* and 18 *E. cloacae*)			
Ciprofloxacin	0.008–0.5	0.03	0.25
Norfloxacin	0.03–1	0.12	1.0
Ofloxacin	0.03–1	0.12	0.5
Ro 23-6240	0.03–1	0.12	0.5
Serratia spp. (10) (5 *S. marcescens*)			
Ciprofloxacin	0.03–1	0.06	0.12
Norfloxacin	0.06–2	0.12	0.12
Ofloxacin	0.12–0.25	0.12	0.25
Ro 23-6240	0.06–2	0.06	0.12
Citrobacter spp. (14) (8 *C. freundii* and 5 *C. diversus*)			
Ciprofloxacin	0.008–0.5	0.015	0.12
Norfloxacin	0.06–2	0.06	0.5
Ofloxacin	0.06–1	0.06	0.5
Ro 23-6240	0.06–1	0.06	0.5
Providencia stuartii (16)			
Ciprofloxacin	0.015–8	0.06	1
Norfloxacin	0.03–32	0.12	4
Ofloxacin	0.03–4	0.25	1
Ro 23-6240	0.06–4	0.12	0.5
Proteus mirabilis (50)			
Ciprofloxacin	0.015–0.5	0.03	0.12
Norfloxacin	0.06–1	0.06	0.25
Ofloxacin	0.06–2	0.12	0.5
Ro 23-6240	0.06–2	0.12	0.5
Proteus vulgaris (18)			
Ciprofloxacin	0.015–0.06	0.03	0.03
Norfloxacin	0.03–0.25	0.06	0.06
Ofloxacin	0.06–0.25	0.06	0.12
Ro 23-6240	0.06–0.25	0.06	0.12
Morganella morganii (26)			
Ciprofloxacin	0.008–0.25	0.015	0.03
Norfloxacin	0.03–0.25	0.06	0.06
Ofloxacin	0.03–1	0.06	0.25
Ro 23-6240	0.03–0.5	0.06	0.12
Acinetobacter anitratus (15)			
Ciprofloxacin	0.12–2	0.25	1
Norfloxacin	0.5–32	2	16
Ofloxacin	0.12–2	0.25	0.5
Ro 23-6240	0.12–2	0.25	0.5
Pseudomonas aeruginosa (47)			
Ciprofloxacin	0.008–2	0.12	0.5
Norfloxacin	0.03–16	0.5	2
Ofloxacin	0.03–4	0.5	2
Ro 23-6240	0.03–4	0.5	2
Staphylococcus aureus (19) (3 methicillin-resistant isolates)			
Ciprofloxacin	0.12–2	0.25	0.5
Norfloxacin	0.25–4	1	2
Ofloxacin	0.12–0.5	0.25	0.5
Ro 23-6240	0.25–1	0.5	0.5
Staphylococcus epidermidis (15)			
Ciprofloxacin	0.12–0.5	0.25	0.5
Norfloxacin	0.25–2	0.5	1
Ofloxacin	0.12–0.5	0.5	0.5
Ro 23-6240	0.25–1	0.5	1
Staphylococcus saprophyticus (15)			
Ciprofloxacin	0.25–0.5	0.25	0.5
Norfloxacin	1–4	2	2
Ofloxacin	0.5–1	1	1
Ro 23-6240	1–2	2	2
Streptococcus pneumoniae (16)			
Ciprofloxacin	0.5–2	1	2
Norfloxacin	2–8	4	8
Ofloxacin	1–8	2	2
Ro 23-6240	4–8	4	8
Haemophilus influenzae (28) (including 9 β-lactamase producers)			
Ciprofloxacin	0.008–0.03	0.015	0.015
Norfloxacin	0.03–4	0.06	0.12
Ofloxacin	0.03	0.03	0.03
Ro 23-6240	0.03–0.12	0.06	0.12
Neisseria gonorrhoeae (24) (5 β-lactamase producers)			
Ciprofloxacin	0.002–0.25	0.004	0.25
Norfloxacin	0.008–1	0.015	1
Ofloxacin	0.008–0.5	0.008	0.5
Ro 23-6240	0.008–0.5	0.015	0.5
Bacteroides fragilis (12)			
Ciprofloxacin	2–16	4	4
Norfloxacin	16–32	16	32
Ofloxacin	1–8	2	4
Ro 23-6240	2–16	4	4

μg/ml), except the *Providencia rettgeri* strain (MIC, 4 μg/ml), which possessed aminoglycoside-modifying enzymes aminoglycoside acetyltransferase-6′-III [AAC (6′)-III] and aminoglycoside phosphotransferase-3′ [APH (3′)] and was resistant to nalidixic acid (MIC, ≥128 μg/ml). A similar decrease in susceptibility by this strain was observed with ofloxacin but not with norfloxacin and ciprofloxacin. Twenty-four strains of *Enterobacteriaceae* were resistant to nalidixic acid (MIC, >128 μg/ml). All nalidixic acid-resistant strains were susceptible to Ro 23-6240 (MIC, ≤1 μg/ml) and the other quinolones, except one strain of *Klebsiella pneumoniae* which was susceptible to 4 μg of ciprofloxacin and 8 μg of Ro 23-6240 per ml. Generally, the four quinolones were less active against the strains of nalidixic acid-resistant bacteria studied (MIC, >128 μg/ml) than against nalidixic acid c-susceptible strains. The MIC_{50} (MIC for 50% of isolates tested) of Ro 23-6240 and ofloxacin was greater by

fourfold and that of ciprofloxacin and norfloxacin was greater by eightfold against nalidixic acid-resistant strains. Seventeen strains of *Enterobacteriaceae* producing β-lactamases of Richmond and Sykes groups I, III, IV, and V (2) were as susceptible to the four quinolones as strains of the same species without such enzymes. Against *Pseudomonas aeruginosa*, ciprofloxacin was the most active agent, with four times the activity of Ro 23-6240, norfloxacin, and ofloxacin. Four strains resistant to gentamicin and cefotaxime appeared to be as susceptible to Ro 23-6240 (and the other quinolones) as the gentamicin-susceptible strains. The activity of Ro 23-6240 against *S. aureus* was similar to that of ciprofloxacin and ofloxacin and fourfold greater than that of norfloxacin. Three methicillin-resistant strains of *S. aureus* were as susceptible to Ro 23-6240 and the other quinolones as the methicillin-susceptible strains. Against *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*, ciprofloxacin and ofloxacin were the most active agents, with at least twofold more activity than Ro 23-6240 and norfloxacin. Group D streptococci were susceptible to 4 to 8 μg of Ro 23-6240 per ml (data not shown). *Streptococcus pneumoniae* had MICs of 4 to 8 μg/ml, and viridans group streptococci (18 strains) had MICs of 4 to 16 μg/ml (data not shown). All the quinolones were highly active against *Haemophilus influenzae* (including β-lactamase producers). Ro 23-6240 was eightfold less active than ciprofloxacin but had a greater degree of activity against *H. influenzae* than cefuroxime (MIC_{90}, 2 μg/ml) and amoxicillin (MIC_{90}, 8 μg/ml).

Against *Neisseria gonorrhoeae*, Ro 23-6240 (MIC_{90}, 0.5 μg/ml) was at least twofold less active than ciprofloxacin. Norfloxacin was the least active quinolone against *B. fragilis*, Ro 23-6240, ciprofloxacin, and ofloxacin being equal in activity.

An increase in inoculum to 10^6 CFU was accompanied by at most a twofold rise in the MIC_{90}s of Ro 23-6240 and norfloxacin, but ciprofloxacin and ofloxacin exhibited an even smaller inoculum effect. In the case of the β-lactams studied, increases in the MIC were seen if the strains were β-lactamase producers and the β-lactam was susceptible to that enzyme. The protein binding of Ro 23-6240 was 27% at 5 μg/ml and 24.5% at 50 μg/ml.

Although a difluorinated compound, Ro 23-6240 appeared to have a degree of activity similar to that of the monofluorinated compounds we studied. Other difluorinated compounds under investigation are NY-198 (T. Hirose, E. Okezaki, H. Kato, Y. Ito, M. Inoue, and S. Mitsuhashi, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 567, 1985), and CI-934 (1). Ro 23-6240 had marked antipseudomonal activity, as does the former, but lacked the high activity against *S. pneumoniae* (and other streptococci) exhibited by the latter.

In general terms, the activity of Ro 23-6240 was similar to that in preliminary published information (N. X. Chin and H. C. Neu, 25th ICAAC, abstr. no. 569, 1985), except that we found the antipseudomonal activity to be about fourfold greater than that reported by Chin and Neu. Like them, we demonstrated no cross resistance between this agent and other groups of chemotherapeutic agents (β-lactams and aminoglycosides), with the exception of the quinolones. Strains resistant to nalidixic acid were less susceptible to all the quinolones we studied. Cross resistance between quinolones has been well documented (3, 4). In assessing the clinical relevance of the in vitro data, it is reasonable to examine the susceptibilities in terms of the levels in serum achieved in humans. Preliminary pharmacokinetic data (Roche Products Ltd.) suggest that after an oral dose of 1,000 mg the peak level in serum is 9 to 11 μg/ml and the elimination half-life is 10 to 12 h. This suggests that most systemic infections caused by members of the family *Enterobacteriaceae*, staphylococci, and *H. influenzae* should be amenable to treatment. It is also probable that infections caused by *P. aeruginosa* will respond to oral therapy. The MIC_{90} for *S. pneumoniae* (8 μg/ml) suggests that infections caused by this organism should not be treated with Ro 23-6240 until clinical evidence of efficacy is available. The superior pharmacokinetic potential of this compound might bring infections caused by *B. fragilis* within the therapeutic orbit of this quinolone. Hence, Ro 23-6240 could be an important addition to this range of antimicrobial agents.

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