In Vitro Activities of Three Nonfluorinated Quinolones against Representative Bacterial Isolates

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In vitro susceptibility tests were performed to document the inhibitory activities of three nonfluorinated quinolone (NFQ) compounds (PGE 9262932, PGE 9509924, and PGE 4175997) compared to those of ciprofloxacin, levofloxacin, and trovafloxacin against 3,030 bacterial isolates. The spectra of the NFQ agents included most gram-positive species as well as quinolone-susceptible Enterobacteriaceae. Ciprofloxacin-resistant, methicillin-resistant Staphylococcus aureus strains were inhibited by the NFQ series at \( \leq 1.0 \) \( \mu \)g/ml. The NFQ compounds were not very active against Pseudomonas aeruginosa and most other nonfermentative gram-negative bacilli. Against other species, the potency of the NFQ agents was similar to that of trovafloxacin. Continued investigation of the NFQ compounds seems to be warranted.

Nalidixic acid was the first of a series of compounds belonging to the naphthylazine series; its use was limited to treatment of urinary tract infections. By replacing the nitrogen at position 8 with a carbon atom oxolinic acid was developed, and that was the first quinoline to be developed for clinical use. Against the Enterobacteriaceae, oxolinic acid was much more potent than its predecessors, but its clinical utility was still limited. The status of the quinolones was dramatically changed after the development of a series of quinolones with one or more fluorine atoms. The fluoroquinolones (FQs) could be administered parenterally or orally and they were at least 100-fold more active than their naphthylazine predecessors (1). More recently, a series of extended-spectrum FQs have been developed to obtain better activity against gram-positive species without substantial loss of activity against gram-negative species. Unfortunately, use of many FQs is limited because of the potential for adverse side effects (2, 3). Some FQs have been removed from the market because of more serious adverse events (3). Other FQ antimicrobial agents are now widely used for a variety of infections.

Scientists at Procter & Gamble Pharmaceuticals (Cincinnati, Ohio) have recently synthesized a series of nonfluorinated quinolones (NFQs) (B. Ledoussel, J. K. Almstead, S. M. Flaim, C. P. Gallagher, J. L. Gray, X. E. Hu, N. K. Kim, H. D. McKeever, C. J. Miley, T. L. Twinem, and S. X. Zheng, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F544, p. 303, 1999). These compounds maintain an extended spectrum of antibacterial activity even though they contain no fluorine at the 6 position of the quinolone nucleus. As part of the early in vitro screening tests, we evaluated the in vitro activities of three such NFQ compounds, compared to that of three FQs, against recent clinical isolates and appropriate stock cultures. This report summarizes the results of antibiotic dilution tests of 3,030 bacterial isolates.

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Antimicrobial susceptibility tests were performed exactly as described in the National Committee for Clinical Laboratory Standards M7-A5 and M11-A4 documents (4, 5). Agar dilution methods were used for testing Haemophilus influenzae, Neisseria gonorrhoeae, and anaerobic bacteria. All other species were tested by the broth microdilution procedure using cation-adjusted Mueller-Hinton broth. Lysed horse blood (3 to 5%) was added to the broth when testing streptococci. The NFQ compounds that were provided by Procter & Gamble Pharmaceuticals were designated as PGE 9262932, PGE 9509924, and PGE 4175997. The chemical structures of these compounds are shown in Fig. 1. The three FQ comparator drugs that we tested were obtained from their respective U.S. manufacturers. Standard quality control strains were included with each batch of tests; the control strains were those appropriate for the species being studied on each test day. The microorganisms that were selected for this study were all clinical isolates originally obtained over the past 10 years from a variety of medical centers throughout North America. Isolates were selected to represent a wide variety of bacterial species and to include antibiotic-resistant strains, when available. The species identi-
| Microorganism (no. of isolates tested) and antimicrobial agent | MIC (µg/ml) | 50%  | 90%  |
|---------------------------------------------------------------|------------|------|------|
| **Staphylococcus aureus**                                     |            |      |      |
| Cipr (231)                                                   |            |      |      |
| PGE 9262932                                                  | 0.008–0.06 | ≤0.016 | 0.03 |
| PGE 9509924                                                  | 0.008–0.12 | 0.016 | 0.03 |
| PGE 4175997                                                  | 0.008–0.06 | 0.016 | 0.03 |
| Trovafloxacin                                                | 0.03–0.5   | 0.12 | 0.25 |
| Levofloxacin                                                 | 0.03–1.0   | 0.25 | 0.5  |
| Ciprofloxacin                                                | 0.12–16    | 2.0  | 16   |
| Levofloxacin                                                 | 4.0–16     | >16  | >16  |
| Ciprofloxacin                                                | 0.008–0.06 | 0.016 | 0.03 |
| **Streptococcus pneumoniae (366)**                           |            |      |      |
| PGE 9262932                                                  | 0.008–0.06 | ≤0.016 | 0.03 |
| PGE 9509924                                                  | 0.008–0.12 | 0.016 | 0.03 |
| PGE 4175997                                                  | 0.008–0.12 | 0.016 | 0.03 |
| Trovafloxacin                                                | 0.03–1.0   | 0.12 | 0.12 |
| Levofloxacin                                                 | 0.25–8.0   | 1.0  | 1.0  |
| Ciprofloxacin                                                | 0.5–16     | 1.0  | 2.0  |
| **Streptococcus viridans group (60)**                         |            |      |      |
| PGE 9262932                                                  | 0.008–0.12 | 0.016 | 0.03 |
| PGE 9509924                                                  | 0.016–0.25 | 0.06 | 0.12 |
| PGE 4175997                                                  | 0.016–0.25 | 0.03 | 0.06 |
| Trovafloxacin                                                | 0.06–8.0   | 0.12 | 0.25 |
| Levofloxacin                                                 | 0.25–8.0   | 1.0  | 2.0  |
| Ciprofloxacin                                                | 0.25–16    | 1.0  | 1.0  |
| **Streptococcus agalactiae (77)**                            |            |      |      |
| PGE 9262932                                                  | 0.008–0.06 | ≤0.016 | 0.016 |
| PGE 9509924                                                  | 0.03–0.12  | 0.06 | 0.06 |
| PGE 4175997                                                  | 0.016–0.06 | 0.03 | 0.06 |
| Trovafloxacin                                                | 0.12–0.5   | 0.25 | 0.25 |
| Levofloxacin                                                 | 0.5–2.0    | 0.5  | 1.0  |
| Ciprofloxacin                                                | 0.25–2.0   | 0.5  | 1.0  |
| **Streptococcus pyogenes (90)**                              |            |      |      |
| PGE 9262932                                                  | 0.008–0.06 | ≤0.008 | ≤0.008 |
| PGE 9509924                                                  | 0.03–0.06  | 0.03 | 0.06 |

*Includes 215 methicillin-susceptible and 16 methicillin-resistant strains.
*b* Excludes one ciprofloxacin-intermediate strain of methicillin-resistant *S. aureus*.
*c* Includes 11 methicillin-susceptible and 114 methicillin-resistant strains.
*d* Includes 148 methicillin-susceptible and 61 methicillin-resistant strains.
*e* Includes 12 ciprofloxacin-intermediate (MIC, 2.0 µg/ml) strains.
*f* Includes 10 methicillin-susceptible and 115 methicillin-resistant strains.
*g* Includes 132 penicillin-intermediate, 84 penicillin-intermediate, and 150 penicillin-resistant strains.
*h* MIC₉₀'s were not calculated for species with fewer than 10 strains.
*i* Includes 19 *C. perfringens*, 1 *C. tertium*, and 1 *C. histolyticum* isolates.
The NFQ compounds have been shown to be capable of rapidly killing methicillin-resistant Staphylococcus aureus strains (S. Roychoudhury, K. M. Makin, E. J. McIntosh, H. D. McKeever, T. L. Twinem, P. M. Koenigs, and C. E. Catrenich, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F547, p. 304, 1999).

The NFQ compounds and trovafloxacin were especially potent against Streptococcus pneumoniae and other streptococci. Ciprofloxacin and levofloxacin were less active. Resistance to the NFQ compounds is currently uncommon among these pathogens and they all appear to be active, but the NFQ compounds are 2 to 32 times more potent.

Among the enterococci, resistance to vancomycin is now a major clinical concern in many institutions. Most of our vancomycin-resistant strains were relatively resistant to ciprofloxacin, levofloxacin, and trovafloxacin. The NFQs showed some activity against such strains, although they were more active against vancomycin-susceptible strains. The NFQ compounds were active against most Enterococcus faecalis strains, which were rarely resistant to vancomycin. Enterococcus faecium strains were commonly resistant to vancomycin and they were

### TABLE 2. In vitro activities of six quinolone compounds against 647 gram-negative species other than the Enterobacteriaceae

| Microorganism | MIC (μg/ml) | 50% | 90% |
|---------------|------------|-----|-----|
| Pseudomonas aeruginosa | | | |
| Cip (241)* | | | |
| PGE 9262932 | 0.12–16 | 1.0 | 4.0 |
| PGE 9509924 | 0.12–8.0 | 1.0 | 4.0 |
| PGE 4175997 | 0.25–16 | 4.0 | 8.0 |
| Trovafloxacin | 0.06–8.0 | 0.5 | 2.0 |
| Levofloxacin | 0.06–4.0 | 0.5 | 4.0 |
| Ciprofloxacin | 0.03–1.0 | 0.12 | 1.0 |
| Cip (72)* | | | |
| PGE 9262932 | 2.0–>16 | 16 | >16 |
| PGE 9509924 | 2.0–16 | 16 | >16 |
| PGE 4175997 | 4.0–16 | >16 | >16 |
| Trovafloxacin | 0.5–16 | >16 | >16 |
| Levofloxacin | 1.0–16 | >16 | >16 |
| Ciprofloxacin | 4.0–16 | 8.0 | >16 |
| Pseudomonas fluorescens/putida group (31) | | | |
| PGE 9262932 | 0.12–8.0 | 1.0 | 8.0 |
| PGE 9509924 | 0.12–8.0 | 1.0 | 8.0 |
| PGE 4175997 | 0.5–16 | 2.0 | 8.0 |
| Trovafloxacin | 0.06–4.0 | 0.5 | 1.0 |
| Levofloxacin | 0.06–16 | 0.5 | 2.0 |
| Ciprofloxacin | 0.06–16 | 0.12 | 4.0 |
| Burkholderia cepacia (23) | | | |
| PGE 9262932 | 0.06–>16 | 8.0 | >16 |
| PGE 9509924 | 0.06–>16 | 4.0 | >16 |
| PGE 4175997 | 0.25–16 | 16 | >16 |
| Trovafloxacin | 0.016–16 | 2.0 | >16 |
| Levofloxacin | ≤0.008–16 | 2.0 | >16 |
| Ciprofloxacin | ≤0.008–16 | 0.12 | >16 |
| Stenotrophomonas maltophilia (54) | | | |
| PGE 9262932 | 0.25–16 | 4.0 | 16 |
| PGE 9509924 | 0.25–16 | 2.0 | 16 |
| PGE 4175997 | 0.5–16 | 4.0 | >16 |
| Trovafloxacin | 0.06–16 | 1.0 | 4.0 |
| Levofloxacin | 0.25–16 | 1.0 | 8.0 |
| Ciprofloxacin | 0.5–16 | 4.0 | 16 |

* Excludes 20 ciprofloxacin-intermediate (MIC, 2.0 μg/ml) strains.
* Includes seven strains with elevated quinolone MICs; they were ciprofloxacin intermediate (MIC, 0.12 to 0.25 μg/ml).
* Includes 55 B. fragilis, 16 B. thetaiotaomicron, 9 B. uniformis, 5 B. vulgans, and 1 B. distasonis isolates.
| Microorganism (no. of isolates tested) and antimicrobial agent | MIC (µg/ml) | Microorganism (no. of isolates tested) and antimicrobial agent | MIC (µg/ml) |
|---------------------------------------------------------------|-------------|---------------------------------------------------------------|-------------|
| **Escherichia coli** (102)                                   |             | **Proteus vulgaris** (44)                                      |             |
| PGE 926932                                                   | 0.03–16     | PGE 926932                                                   | 0.06–2.0    |
| PGE 9509924                                                  | 0.03–16     | PGE 9509924                                                  | 0.06–2.0    |
| PGE 4175997                                                  | 0.06–16     | PGE 4175997                                                  | 0.12–4.0    |
| Trovafloxacin                                                | ≤0.008–16   | Trovafloxacin                                                | 0.016–4.0   |
| Levofloxacin                                                 | 0.016–16    | Levofloxacin                                                 | 0.06–0.12   |
| Ciprofloxacin                                                | 0.016–16    | Ciprofloxacin                                                | 0.016–0.06  |
| **Citrobacter freundii** (25)                                |             | **Morganella morganii** (40)                                   |             |
| PGE 926932                                                   | 0.06–8.0    | PGE 926932                                                   | 0.06–16     |
| PGE 9509924                                                  | 0.12–16     | PGE 9509924                                                  | 0.06–16     |
| PGE 4175997                                                  | 0.25–16     | PGE 4175997                                                  | 0.12–16     |
| Trovafloxacin                                                | 0.016–16    | Trovafloxacin                                                | 0.03–16     |
| Levofloxacin                                                 | 0.016–8.0   | Levofloxacin                                                 | 0.016–8.0   |
| Ciprofloxacin                                                | 0.016–16    | Ciprofloxacin                                                | 0.016–0.06  |
| **Enterobacter aerogenes** (72)                               |             | **Providencia rettgeri** (28)                                  |             |
| PGE 926932                                                   | 0.06–8.0    | PGE 926932                                                   | 0.12–16     |
| PGE 9509924                                                  | 0.12–16     | PGE 9509924                                                  | 0.12–16     |
| PGE 4175997                                                  | 0.25–16     | PGE 4175997                                                  | 0.25–16     |
| Trovafloxacin                                                | 0.016–16    | Trovafloxacin                                                | 0.03–16     |
| Levofloxacin                                                 | 0.03–8.0    | Levofloxacin                                                 | 0.03–8.0    |
| Ciprofloxacin                                                | 0.016–16    | Ciprofloxacin                                                | 0.016–4.0   |
| **Enterobacter cloacae** (70)                                 |             | **Providencia stuartii** (36)                                  |             |
| PGE 926932                                                   | 0.06–16     | PGE 926932                                                   | 0.06–16     |
| PGE 9509924                                                  | 0.016–16    | PGE 9509924                                                  | 0.12–16     |
| PGE 4175997                                                  | 0.12–16     | PGE 4175997                                                  | 0.25–16     |
| Trovafloxacin                                                | ≤0.008–16   | Trovafloxacin                                                | 0.016–16    |
| Levofloxacin                                                 | 0.016–16    | Levofloxacin                                                 | 0.03–16     |
| Ciprofloxacin                                                | ≤0.008–16   | Ciprofloxacin                                                | 0.016–0.06  |
| **Klebsiella pneumoniae** (122)                               |             | **Shigella spp.** (20)*                                      |             |
| PGE 926932                                                   | 0.03–16     | PGE 926932                                                   | 0.03–12     |
| PGE 9509924                                                  | 0.016–16    | PGE 9509924                                                  | 0.06–0.25   |
| PGE 4175997                                                  | 0.12–16     | PGE 4175997                                                  | 0.03–0.25   |
| Trovafloxacin                                                | 0.016–16    | Trovafloxacin                                                | 0.008–0.16  |
| Levofloxacin                                                 | 0.016–16    | Levofloxacin                                                 | 0.016–0.16  |
| Ciprofloxacin                                                | ≤0.008–16   | Ciprofloxacin                                                | ≤0.016–0.06 |
| **Klebsiella oxytoca** (50)                                   |             | **Salmonella enteritidis** (20)                                |             |
| PGE 926932                                                   | 0.12–8.0    | PGE 926932                                                   | 0.12–0.25   |
| PGE 9509924                                                  | 0.12–8.0    | PGE 9509924                                                  | 0.25–0.25   |
| PGE 4175997                                                  | 0.25–16     | PGE 4175997                                                  | 0.25–0.25   |
| Trovafloxacin                                                | 0.016–4.0   | Trovafloxacin                                                | 0.03–0.16   |
| Levofloxacin                                                 | 0.03–2.0    | Levofloxacin                                                 | 0.03–0.16   |
| Ciprofloxacin                                                | 0.016–2.0   | Ciprofloxacin                                                | 0.016–0.16  |
| **Serratia marcescens** (120)                                 |             | **All Enterobacteriaceae**                                    |             |
| PGE 926932                                                   | 0.06–16     | Cipr (816)*                                                   | 0.03–4.0    |
| PGE 9509924                                                  | 0.12–16     | PGE 9509924                                                  | 0.016–16    |
| PGE 4175997                                                  | 0.25–16     | PGE 4175997                                                  | 0.03–16     |
| Trovafloxacin                                                | 0.03–16     | Trovafloxacin                                                | 0.008–16    |
| Levofloxacin                                                 | 0.03–16     | Levofloxacin                                                 | 0.016–4.0   |
| Ciprofloxacin                                                | 0.016–16    | Ciprofloxacin                                                | 0.016–0.5   |
| **Proteus mirabilis** (120)                                   |             |                                                               |             |
| PGE 926932                                                   | 0.12–16     | PGE 926932                                                   | 4.0–4.0     |
| PGE 9509924                                                  | 0.12–16     | PGE 9509924                                                  | 4.0–4.0     |
| PGE 4175997                                                  | 0.25–16     | PGE 4175997                                                  | 8.0–16      |
| Trovafloxacin                                                | 0.016–16    | Trovafloxacin                                                | 1.0–16      |
| Levofloxacin                                                 | 0.016–16    | Levofloxacin                                                 | 2.0–16      |
| Ciprofloxacin                                                | 0.016–16    | Ciprofloxacin                                                | 4.0–16      |

* Includes six S. boydii, five S. dysenteriae, six S. flexneri, and three S. sonnei isolates.

b Excludes 10 ciprofloxacin-intermediate (MIC, 2.0 µg/ml) strains: one C. freundii, one E. aerogenes, two K. pneumoniae, two K. oxytoca, one P. mirabilis, and three S. marcescens strains.
often resistant to the FQ agents; the NFQ compounds were also less potent against these strains. The nine strains of *Clostridium difficile* were inhibited by 1.0 to 2.0 μg of trovafloxacin or of the NFQ compounds per ml, but ciprofloxacin and levofloxacin were four to eight times less active. The 21 other *Clostridium* spp. and the nine *Peptostreptococcus* spp. were also inhibited by the NFQ compounds at concentrations of ≤1.0 μg/ml. On the other hand, all six study drugs showed less activity against members of the *Bacteroides* fragilis group (Table 2).

Against the *Enterobacteriaceae*, levofloxacin and trovafloxacin were 2- to 16-fold more active than the NFQ compounds (Table 2). Ciprofloxacin was the most potent agent that we evaluated, but a few ciprofloxacin-resistant strains were found among the recent clinical isolates included in this study. Ciprofloxacin resistance was observed with 15 *Providencia* spp., 4 *Morganella morganii*, 6 *Proteus mirabilis*, 8 *Serratia marcescens*, 6 *Klebsiella pneumoniae*, 3 *Enterobacter* spp., and 1 of the *Escherichia coli* isolates. Among those 43 isolates, 13 had high-level resistance to ciprofloxacin (MIC, ≥16 μg/ml) and the other isolates were only relatively resistant to ciprofloxacin (MIC, 4.0 or 8.0 μg/ml). The 43 ciprofloxacin-resistant strains showed cross-resistance to the other quinolones studied (Table 3). Against the 72 ciprofloxacin-resistant *Pseudomonas aeruginosa* isolates that were included in this series, the NFQ compounds had little activity (Table 2). Against quinolone-susceptible *P. aeruginosa*, *Pseudomonas fluorescens*, and *Pseudomonas putida*, ciprofloxacin and trovafloxacin were 2 to 16 times more potent than the NFQs.

*H. influenzae*, *Moraxella catarrhalis*, and *N. gonorrhoeae* are three other gram-negative species that were uniformly susceptible to the FQs; the NFQs were also active at low concentrations (Table 2).

All three nonfluorinated quinolones are potentially useful antimicrobial agents. The NFQ agents demonstrated broad spectrums of antibacterial activity that are similar to that of trovafloxacin. They are more active than trovafloxacin against gram-positive pathogens, somewhat less active against the *Enterobacteriaceae*, and have little activity against *Pseudomonas* spp. The absence of fluorine atoms at position 6 of the quinoline nucleus in these molecules might reduce the frequency of toxic side effects but that remains to be demonstrated. Clearly, the antibacterial activity of the quinolone compounds is not necessarily related to the fluorine component alone. Providing that appropriate pharmacokinetic properties (N. L. Mallalieu, D. H. Ellis, P. H. Zoutendam, M. Gavin, M. K. Dirr, M. J. Martin, and B. Ledoussal, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F550, p. 305, 1999) can be achieved in humans and that adverse events are minimized (2, 3), these agents might fill a valuable role in today’s ever-increasing concerns about infections due to antibiotic-resistant bacteria.

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