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# In Vitro and In Vivo Activities of a New Quinolone, WIN 57273, Possessing Potent Activity against Gram-Positive Bacteria

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**The antibacterial activity of a new 7-dimethylpyridinyl quinolone, WIN 57273, was assessed by using in vitro and in vivo models. Agar inclusion and broth dilution in vitro tests revealed broad-spectrum activity against gram-positive and selected gram-negative organisms, with the greatest potency observed against the staphylococci. The MIC for 90% of coagulase-positive strains tested ($MIC_{90}$) was ≤0.002 μg/ml; for the coagulase-negative strains the $MIC_{90}$ was 0.008 μg/ml. Against enterococci the $MIC_{90}$ was 0.06 μg/ml, with comparable activity observed against group A and group B streptococci as well as against the pneumococci. In general, the $MIC_{90}$s for the gram-negative bacteria were ≤1 μg/ml. Exceptions were *Serratia marcescens* ($MIC_{90}$, 16 μg/ml), *Citrobacter freundii* ($MIC_{90}$, 4 μg/ml), and *Pseudomonas aeruginosa* ($MIC_{90}$, 8 μg/ml). The greatest potency was observed against *Haemophilus* spp. and *Neisseria* spp., with $MIC_{90}$s of 0.06 and 0.016 μg/ml, respectively. Broad-spectrum activity was also observed against anaerobes, with $MIC_{90}$s ranging from 0.125 to 0.5 μg/ml among the species tested. The in vivo efficacy was determined by using a murine model by calculating the 50% protective doses against a lethal bacterial infection caused by strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Listeria monocytogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The staphylococci were most susceptible, with 50% protective doses for all strains ranging from 0.1 to 0.7 mg/kg. With the exception of the *Pseudomonas* infection, which was refractory to treatment, animals that were part of the other infection models responded to less than 10 mg/kg. Equivalent activity was seen with the subcutaneous or the oral route of drug administration. WIN 57273 was significantly more potent than ciprofloxacin in treating gram-positive bacterial infections (2- to 20-fold) but was significantly less effective at treating gram-negative bacterial infections (30- to 300-fold).**

During the past several years a renewed focus of laboratory and clinical research activities has been placed on quinolone antibacterial agents. Numerous new compounds have been described in the literature, with several new agents reaching general clinical use. While the most extensively studied and most successful candidate has been ciprofloxacin, the search has continued for further improvements in potency and pharmacokinetic profile. To complement the spectrum of activity exhibited by ciprofloxacin, with its excellent potency against gram-negative bacteria, a recent focus has been on compounds with improved activities against gram-positive bacteria and anaerobes. This is exemplified by compounds such as CI-934 (3, 9), A-60969 (T-3262) (1, 2), PD 117,596 (8), and PD 127,391 (6, 10).

Research efforts in our laboratories have centered on a study of the effects of a dimethylpyridinyl substitution at the 7 position of the quinolone ring. Previous studies have shown this modification to impart potent antistaphylococcal activity vis á vis contemporary quinolones (J. R. O'Connor, R. A. Dobson, R. B. Wagner, and G. Y. Lesher, Program Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 657, 1983). Further modifications of this compound have led to WIN 57273 [1-cyclopropyl-7-(2,6-dimethyl-4-pyridinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid], which incorporates the now recognized beneficial aspects of a 1-cyclopropyl substitution into this nucleus (Fig. 1).

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The study described here was conducted to examine and compare the antibacterial activity of WIN 57273 with those of reference compounds. The purpose was to define the spectrum of activity of the compound and confirm that this activity could be translated to an animal efficacy model.

## MATERIALS AND METHODS

**Antimicrobial agents.** Of the quinolones tested, WIN 57273 and amifloxacin were synthesized at the research laboratories of the Sterling Research Group (Rensselaer, N.Y.), ciprofloxacin was obtained from Miles Laboratories, Inc. (West Haven, Conn.), difloxacin was provided by Abbott Laboratories (North Chicago, Ill.), and ofloxacin was obtained from Hoechst-Roussel Pharmaceuticals Inc. (Somerville, N.J.). Additional compounds included oxacillin, vancomycin, gentamicin, penicillin G, ampicillin, and metronidazole, which were obtained from Sigma Chemical Co. (St. Louis, Mo.). Clindamycin was provided by The Upjohn Co. (Kalamazoo, Mich.), imipenem was provided by Merck Sharp & Dohme (West Point, Pa.), cefotaxime was provided by Hoechst-Roussel Pharmaceuticals Inc., ceftazidime was provided by Glaxo Inc. (Research Triangle Park, N.C.), and erythromycin was provided by Abbott Laboratories.

WIN 57273 and amifloxacin were initially dissolved in 0.1 N NaOH. The other compounds were dissolved in an appropriate diluent, as specified by the suppliers; all subsequent dilutions were made in distilled water. Final test

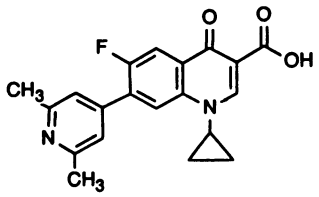

FIG. 1. Chemical structure of WIN 57273.

concentrations were made by twofold serial dilutions in either broth or agar as described below.

**Bacterial cultures.** All bacterial cultures were part of an in-house rotating culture collection and included isolates that were maintained as laboratory stock reference cultures, recent clinical isolates obtained from Albany Medical Center Hospital (Albany, N.Y.) and Bender Hygienic Laboratory (Albany, N.Y.), and a small group of cultures possessing resistance to the selected antibacterial reference agents used in this study. In this third group of organisms, we included three ciprofloxacin-resistant strains of *Staphylococcus aureus* that were kindly provided by G. Kaatz (Wayne State University, Detroit, Mich.) and H. Humbreys (Trinity College, Dublin, Ireland), two vancomycin-resistant strains of *Staphylococcus haemolyticus* obtained from R. Schwalbe (Albany Veteran Administration Medical Center, Albany, N.Y.), and one ciprofloxacin-resistant *Enterobacter cloacae* strain which was obtained from S. Chapman (Southmead Hospital, Bristol, England).

**MIC determinations.** A broth microdilution method (4) was used to quantitate activity against *Proteus*, *Salmonella*, and *Klebsiella* strains; all other cultures were tested by an agar dilution method (4, 5). The *Staphylococcus*, *Escherichia*, *Serratia*, *Enterobacter*, *Shigella*, *Citrobacter*, *Pseudomonas*, and *Acinetobacter* cultures were tested on Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.), but the oxacillin-resistant staphylococci were tested on Mueller-Hinton agar supplemented with 4% NaCl. All streptococci, enterococci, and the two *Listeria* strains were tested on Mueller-Hinton agar supplemented with 5% defibrinated sheep blood (Rockland Inc., Gilbertsville, Pa.). All *Haemophilus* and *Neisseria* species were tested on chocolate agar medium and incubated in an atmosphere of 10% $CO_2$. All anaerobes were tested on Wilkins-Chalgren agar (Difco Laboratories, Detroit, Mich.) and incubated in an atmosphere of 5% $CO_2$–10% $H_2$–85% $N_2$. Each culture inoculum was dispensed onto the surface of agar plates (MIC inoculum tray; Dynatech Laboratories, Inc., Alexandria, Va.) containing 30 ml of medium by using either a multiinoculating device (Quick Spense inoculator; Sandy Springs Instrument Co., Inc., Germantown, Md.) or a 96-pin handheld replicator (West Coast Scientific, Inc., Emeryville, Calif.). The inoculum was adjusted to deliver $10^4$ CFU per spot. The plates were incubated at 37°C for 18 to 24 h, but the anaerobes were incubated for 48 h.

We conducted the broth microdilution tests using cation-supplemented Mueller-Hinton broth (BBL). The inoculum was transferred to 96-well microdilution plates by using an MIC 2000 automated inoculator (MIC 2000; Dynatech Laboratories). The volume of medium per well was 100 μl; the final cell density was $10^5$ CFU/ml. The plates were incubated at 37°C for 18 to 24 h.

The MIC was defined as the lowest concentration of compound which completely inhibited growth (i.e., no discernible colonies on the agar plates and no turbidity on the broth plates). The concentration of compound which inhibited 50 and 90% ($MIC_{90}$) of the strains was calculated so that we could compare the antibacterial profiles of WIN 57273 with those of the reference agents.

**Murine infection models.** Female Swiss Webster mice (weight, 18 to 20 g; Taconic Farms, Germantown, N.Y.) were used to study the efficacy of WIN 57273 in the *Listeria* infection; for all other models we used female ICR mice (Blue Spruce Farms, Altamont, N.Y.). The culture and infectious doses were prepared as follows: *Staphylococcus aureus* Smith ($2 \times 10^5$ CFU/ml in 5% hog gastric mucin); *Staphylococcus aureus* 39881, methicillin resistant ($2 \times 10^9$ CFU/ml in 5% hog gastric mucin); *Staphylococcus epidermidis* 3089, methicillin resistant ($3 \times 10^9$ in 5% hog gastric mucin); *Streptococcus pneumoniae* ATCC 10813 ($1 \times 10^3$ CFU/ml in brain heart infusion broth); *Streptococcus pyogenes* ATCC 12384 ($2.5 \times 10^3$ CFU/ml in brain heart infusion broth plus 5% normal rabbit serum); *Escherichia coli* Vogel ($6 \times 10^7$ CFU/ml in saline); *Klebsiella pneumoniae* 39645 ($1 \times 10^3$ CFU/ml in saline); *Pseudomonas aeruginosa* MGH-2 ($1 \times 10^7$ CFU/ml in 5% hog gastric mucin); and *Listeria monocytogenes* SWRI 671 ($3 \times 10^6$ CFU/ml in saline). The infection was initiated by injecting 0.5 ml of the culture intraperitoneally; however, for *L. monocytogenes* 0.2 ml was injected intravenously.

Medication was initiated 0.5 h postinfection (p.i.) by the oral or the subcutaneous route. Animals with *Staphylococcus aureus* Smith, *Escherichia coli* Vogel, and *Klebsiella pneumoniae* infections were treated with only a single medication. The animals that made up all other models received multiple doses 0.5 and 4.5 h p.i. Additionally, animals with a *Pseudomonas aeruginosa* infection were treated with a third medication 7 h p.i., and *Listeria monocytogenes* infection were treated twice daily on days 0 to 4 p.i. Groups of 10 animals each were treated with each dose.

Survival was monitored for 7 days. For each compound and for each route of administration, 50% protective doses ($PD_{50}$s) with 95% confidence limits were calculated by Probit analysis . A comparison of $PD_{50}$s was done to compare treatments. Nonoverlapping confidence intervals were used as a criterion to determine significant differences among these treatments. In animals making up all of the acute infection models, the infectious dose was lethal for animals in the untreated groups within 48 h p.i.

In the *Listeria monocytogenes* model, the surviving animals were sacrificed and viable bacterial counts from the spleens of each group of animals were compared by a one-way analysis of variance and the Duncan multiple range test on the rank order of the $\log_{10}$ bacterial counts. Results for the treatment groups were compared with those for the ampicillin control group to assess treatment efficacy.

## RESULTS

**Antibacterial activity.** Data comparing the in vitro activities of WIN 57273 with those of the reference agents are shown in Table 1. Of significant note was the potency of WIN 57273 against all of the staphylococci tested, with MICs not exceeding 0.008 μg/ml for any of the cultures, including several strains with low resistances to ciprofloxacin. WIN 57273 was at least 64-fold more active against the coagulase-negative staphylococci when compared with the activities of any of the other reference agents tested. This difference was well over 100-fold against the coagulase-positive staphylococci.

The superiority of WIN 57273 over the reference quinolones was also observed against the streptococci, with the

TABLE 1. Comparative activities of WIN 572373 and reference compounds against bacterial cultures

| Organism (no. of strains tested) | Compound | MIC (μg/ml)[a] | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| Coagulase-positive staphylococci | | | | |
| Methicillin susceptible (18) | WIN 57273 | ≤0.002–0.004 | ≤0.002 | ≤0.002 |
| | Ciprofloxacin | 0.25–2 | 1 | 1 |
| | Ofloxacin | 0.25–1 | 0.5 | 1 |
| | Amifloxacin | 0.5–8 | 1 | 2 |
| | Vancomycin | 0.5–1 | 0.5 | 1 |
| | Oxacillin | 0.25–4 | 0.5 | 4 |
| | Ceftazidime | 8–>32 | 16 | >32 |
| Methicillin resistant (15) | WIN 57273 | ≤0.002–0.004 | ≤0.002 | ≤0.002 |
| | Ciprofloxacin | 0.5–1 | 1 | 1 |
| | Ofloxacin | 0.5 | 0.5 | 0.5 |
| | Amifloxacin | 1–2 | 1 | 2 |
| | Vancomycin | 0.5–1 | 1 | 1 |
| | Oxacillin | >32 | >32 | >32 |
| | Ceftazidime | >32 | >32 | >32 |
| Ciprofloxacin resistant (3) | WIN 57273 | $\leq 0.002_3$[b] | | |
| | Ciprofloxacin | $4_2$, 8 | | |
| | Ofloxacin | $1_2$, 8 | | |
| | Amifloxacin | $8_2$, 16 | | |
| | Vancomycin | $0.5_2$, 1 | | |
| | Oxacillin | $0.5_2$, 1 | | |
| | Ceftazidime | 8, 16, >32 | | |
| Coagulase-negative staphylococci | | | | |
| Methicillin susceptible (38) | WIN 57273 | ≤0.002–0.008 | 0.004 | 0.008 |
| | Ciprofloxacin | 0.125–1 | 0.25 | 0.5 |
| | Ofloxacin | 0.25–1 | 0.5 | 0.5 |
| | Amifloxacin | 0.25–2 | 1 | 1 |
| | Vancomycin | 0.5–2 | 1 | 2 |
| | Oxacillin | 0.006–32 | 0.25 | 8 |
| | Ceftazidime | 4–>32 | 8 | 16 |
| Methicillin resistant (19) | WIN 57273 | ≤0.002–0.008 | 0.004 | 0.008 |
| | Ciprofloxacin | 0.125–0.5 | 0.5 | 0.5 |
| | Ofloxacin | 0.25–1 | 0.5 | 1 |
| | Amifloxacin | 0.5–1 | 1 | 1 |
| | Vancomycin | 0.5–2 | 1 | 2 |
| | Oxacillin | 8–>32 | 32 | >32 |
| | Cetazidime | 8–>32 | 32 | >32 |
| Vancomycin resistant (2) | WIN 57273 | ≤0.002, .004 | | |
| | Ciprofloxacin | $0.25_2$[b] | | |
| | Ofloxacin | $0.25_2$ | | |
| | Amifloxacin | 0.5, 1 | | |
| | Vancomycin | 2, 4 | | |
| | Oxacillin | $>32_2$ | | |
| | Ceftazidime | $>32_2$ | | |
| *Streptococcus pneumoniae* (21) | WIN 57273 | 0.016–0.06 | 0.03 | 0.03 |
| | Ciprofloxacin | 1–2 | 2 | 2 |
| | Ofloxacin | 1–4 | 2 | 2 |
| | Amifloxacin | 4–>32 | 16 | >32 |
| | Penicillin G | 0.008–0.03 | 0.016 | 0.016 |
| | Erythromycin | 0.03–0.06 | 0.03 | 0.06 |
| | Ceftazidime | 0.006–0.25 | 0.125 | 0.25 |
| Group A streptococci (18) | WIN 57273 | 0.06–0.125 | 0.06 | 0.125 |
| | Ciprofloxacin | 0.5–4 | 1 | 2 |
| | Ofloxacin | 1–8 | 2 | 4 |
| | Amifloxacin | 8–>32 | >32 | >32 |
| | Penicillin G | 0.008–0.006 | 0.016 | 0.03 |
| | Erythromycin | 0.03–0.125 | 0.06 | 0.125 |
| | Ceftazidime | 0.125–0.5 | 0.125 | 0.25 |

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TABLE 1—*Continued*

| Organism (no. of strains tested) | Compound | MIC (μg/ml)[a] | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| Group B streptococci (15) | WIN 57273 | 0.06–0.125 | 0.06 | 0.125 |
| | Ciprofloxacin | 1 | 1 | 1 |
| | Ofloxacin | 2–4 | 2 | 2 |
| | Amifloxacin | 32–>32 | >32 | >32 |
| | Penicillin G | 0.03–0.25 | 0.06 | 0.25 |
| | Erythromycin | 0.125 | 0.125 | 0.125 |
| | Ceftazidime | 0.25–2 | 0.5 | 1 |
| Group D enterococci (96) | WIN 57273 | 0.016–1 | 0.06 | 0.06 |
| | Ciprofloxacin | 1–8 | 2 | 4 |
| | Ofloxacin | 2–16 | 4 | 4 |
| | Amifloxacin | 1–>32 | 8 | 16 |
| | Vancomycin | 0.5–>32 | 2 | 4 |
| | Ampicillin | 0.5–>32 | 2 | 2 |
| | Gentamicin | 1–>32 | 16 | >32 |
| *Escherichia coli* (35) | WIN 57273 | 0.03–1 | 0.125 | 0.25 |
| | Ciprofloxacin | 0.016–0.125 | 0.003 | 0.03 |
| | Ofloxacin | 0.03–0.25 | 0.125 | 0.25 |
| | Amifloxacin | 0.06–0.5 | 0.06 | 0.125 |
| | Gentamicin | 0.25–4 | 1 | 2 |
| | Ampicillin | 8–>32 | >32 | >32 |
| | Cefotaxime | 0.06–16 | 0.125 | 1 |
| *Enterobacter cloacae* (29) | WIN 57273 | 0.125–1 | 0.25 | 1 |
| | Ciprofloxacin | 0.016–1 | 0.03 | 0.5 |
| | Ofloxacin | 0.06–2 | 0.25 | 1 |
| | Amifloxacin | 0.06–2 | 0.25 | 0.5 |
| | Gentamicin | 0.5–8 | 2 | 2 |
| | Ampicillin | >32 | >32 | >32 |
| | Cefotaxime | 0.25–>32 | 2 | 32 |
| Ciprofloxacin-resistant *Enterobacter cloacae* (1) | WIN 57273 | 32 | | |
| | Ciprofloxacin | 16 | | |
| | Ofloxacin | 32 | | |
| | Amifloxacin | >32 | | |
| | Gentamicin | 16 | | |
| | Ampicillin | >32 | | |
| | Cefotaxime | >32 | | |
| *Enterobacter aerogenes* (13) | WIN 57273 | 0.125–2 | 0.25 | 1 |
| | Ciprofloxacin | 0.03–0.125 | 0.06 | 0.125 |
| | Ofloxacin | 0.06–1 | 0.25 | 1 |
| | Amifloxacin | 0.06–1 | 0.25 | 0.25 |
| | Gentamicin | 0.5–2 | 2 | 2 |
| | Ampicillin | >32 | >32 | >32 |
| | Cefotaxime | 0.125–32 | 0.5 | >32 |
| *Shigella* species (11) | WIN 57273 | 0.03–16 | 0.125 | 0.5 |
| | Ciprofloxacin | 0.004–0.6 | 0.016 | 0.06 |
| | Ofloxacin | 0.06–0.5 | 0.25 | 0.25 |
| | Amifloxacin | 0.06–1 | 0.125 | 0.25 |
| | Gentamicin | 1–16 | 2 | 2 |
| | Ampicillin | >32 | >32 | >32 |
| | Cefotaxime | 0.25–16 | 0.5 | 8 |
| *Serratia marcescens* (31) | WIN 57273 | 0.5–32 | 2 | 16 |
| | Ciprofloxacin | 0.03–1 | 0.125 | 1 |
| | Ofloxacin | 0.125–4 | 0.5 | 4 |

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TABLE 1—*Continued*

| Organism (no. of strains tested) | Compound | MIC (μg/ml)[a] | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| | Amifloxacin | 0.25–8 | 0.5 | 4 |
| | Gentamicin | 0.5–>32 | 2 | 4 |
| | Ampicillin | >32 | >32 | >32 |
| | Cefotaxime | 0.5–>32 | 4 | 32 |
| *Citrobacter freundii* (27) | WIN 57273 | 0.125–8 | 0.5 | 4 |
| | Ciprofloxacin | 0.008–0.125 | 0.03 | 0.06 |
| | Ofloxacin | 0.06–1 | 0.25 | 0.5 |
| | Amifloxacin | 0.125–0.5 | 0.25 | 0.5 |
| | Gentamicin | 0.5–2 | 0.5 | 2 |
| | Ampicillin | >32 | >32 | >32 |
| | Cefotaxime | 0.25–>32 | 2 | >32 |
| *Proteus mirabilis* (35) | WIN 57273 | 0.125–1 | 0.5 | 1 |
| | Ciprofloxacin | 0.06–0.5 | 0.006 | 0.125 |
| | Ofloxacin | 0.125–1 | 0.25 | 0.5 |
| | Amifloxacin | 0.125–1 | 0.25 | 0.5 |
| | Gentamicin | 1–4 | 4 | 4 |
| *Salmonella* species (20) | WIN 57273 | 0.03–0.5 | 0.125 | 0.25 |
| | Ciprofloxacin | 0.03–0.06 | 0.06 | 0.06 |
| | Ofloxacin | 0.06–0.5 | 0.25 | 0.25 |
| | Amifloxacin | 0.125–0.25 | 0.25 | 0.25 |
| | Gentamicin | 0.5–4 | 1 | 4 |
| *Klebsiella pneumoniae* (29) | WIN 57273 | 0.125–1 | 0.25 | 0.5 |
| | Ciprofloxacin | 0.03–2 | 0.125 | 0.25 |
| | Ofloxacin | 0.125–2 | 0.5 | 1 |
| | Amifloxacin | 0.125–4 | 0.25 | 2 |
| | Gentamicin | 0.25–4 | 1 | 2 |
| *Pseudomonas aeruginosa* (32) | WIN 57273 | 1–>32 | 2 | 8 |
| | Ciprofloxacin | 0.125–2 | 0.25 | 1 |
| | Ofloxacin | 0.5–8 | 2 | 8 |
| | Amifloxacin | 0.5–8 | 2 | 4 |
| | Ceftazidime | 2–32 | 4 | 16 |
| | Imipenem | 1–32 | 2 | 8 |
| | Gentamicin | 1–32 | 8 | 32 |
| *Acinetobacter* species (23) | WIN 57273 | 0.06–1 | 0.125 | 0.25 |
| | Ciprofloxacin | 0.125–2 | 0.5 | 1 |
| | Ofloxacin | 0.25–8 | 0.5 | 2 |
| | Amifloxacin | 0.5–4 | 1 | 2 |
| | Ceftazidime | 4–>32 | 16 | >32 |
| | Imipenem | 0.125–2 | 0.25 | 0.5 |
| | Gentamicin | 1–>32 | 16 | >32 |
| *Haemophilus* species (22) | WIN 57273 | 0.004–0.125 | 0.008 | 0.06 |
| | Ciprofloxacin | 0.016–0.06 | 0.03 | 0.03 |
| | Ofloxacin | 0.03–0.25 | 0.06 | 0.06 |
| | Amifloxacin | 0.03–0.25 | 0.06 | 0.125 |
| | Ampicillin | 0.25–>32 | 0.5 | 4 |
| | Erythromycin | 4–16 | 8 | 16 |
| *Neisseria gonorrhoeae* (30) | WIN 57273 | ≤0.002–0.06 | 0.004 | 0.016 |
| | Ciprofloxacin | 0.008–0.25 | 0.03 | 0.06 |
| | Ofloxacin | 0.008–0.5 | 0.06 | 0.06 |
| | Amifloxacin | 0.03–4 | 0.06 | 0.125 |
| | Ampicillin | 0.03–>32 | 0.25 | >32 |
| | Erythromycin | 0.03–4 | 0.5 | 2 |

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TABLE 1—*Continued*

| Organism (no. of strains tested) | Compound | MIC (μg/ml)[a] | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *Listeria monocytogenes* (2) | WIN 57273 | $0.125_2$[b] | | |
| | Ciprofloxacin | 2, 4 | | |
| | Ofloxacin | $2_2$ | | |
| | Amifloxacin | $8_2$ | | |
| | Penicillin G | $0.5_2$ | | |
| *Bacteroides* species (29) | WIN 57273 | 0.125–0.5 | 0.25 | 0.5 |
| | Ciprofloxacin | 4–>32 | 16 | 32 |
| | Ofloxacin | 2–32 | 8 | 16 |
| | Amifloxacin | 16–>32 | >32 | >32 |
| | Difloxacin | 1–16 | 4 | 8 |
| | Metronidazole | 0.25–1 | 0.5 | 0.5 |
| | Clindamycin | 0.25–4 | 1 | 4 |
| *Clostridium* species (21) | WIN 57273 | 0.004–0.5 | 0.03 | 0.25 |
| | Ciprofloxacin | 0.25–16 | 0.5 | 4 |
| | Ofloxacin | 0.25–16 | 1 | 4 |
| | Amifloxacin | 0.5–>32 | 1 | 32 |
| | Difloxacin | 0.25–8 | 0.5 | 2 |
| | Metronidazole | 0.03–2 | 0.5 | 1 |
| | Clindamycin | 0.03–16 | 0.5 | 16 |
| *Peptostreptococcus* species (14) | WIN 57273 | 0.016–0.25 | 0.03 | 0.125 |
| | Ciprofloxacin | 0.125–16 | 1 | 4 |
| | Ofloxacin | 0.25–32 | 1 | 8 |
| | Amifloxacin | 1–>32 | 8 | 32 |
| | Difloxacin | 0.5–8 | 1 | 2 |
| | Metronidazole | 0.125–1 | 0.25 | 1 |
| | Clindamycin | 0.03–1 | 0.06 | 0.5 |

[a] 50% and 90%, MIC for 50 and 90% of strains tested, respectively.
[b] The inferior number is the number of isolates with the indicated MIC.

$MIC_{90}$s ranging from 0.03 to 0.125 μg/ml. WIN 57273 was 32-fold more active against the enterococci than any of the reference agents tested.

Testing of WIN 57273 against members of the family *Enterobacteriaceae* revealed, in general, that it had lower activity than it did against the gram-positive cocci; however, low $MIC_{90}$s (≤1 μg/ml) were recorded for *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella* spp., and *Salmonella* spp. WIN 57273 was poorly active, however, against *Serratia marcescens* and *Citrobacter freundii*. Ciprofloxacin was the most active compound against these two organisms.

WIN 57273 exhibited a mixed profile against additional gram-negative bacteria. Activity of less than or equal to 0.25 μg/ml was seen against *Acinetobacter*, *Haemophilus*, and *Neisseria* spp., with the greatest potency observed against *Neisseria gonorrhoeae* ($MIC_{90}$, 0.016 μg/ml). The activity of WIN 57273 against these three groups of organisms was equal to or better than those of all of the reference agents tested. *Pseudomonas aeruginosa*, however, was much less susceptible to WIN 57273; the $MIC_{90}$ was only 8 μg/ml.

The spectrum of anaerobes tested included gram-negative and gram-positive rods and cocci. WIN 57273 exhibited a broad spectrum of activity against all strains tested. Its activity was at least eightfold superior compared with those of all reference agents except metronidazole, with which it was equipotent against *Bacteroides* spp.

**In vivo efficacy.** The efficacy of WIN 57273 in the mouse systemic infection model, in general, followed predictions which could be made from the in vitro activity. The greatest efficacy was observed in the treatment of *Staphylococcus* infections, with $PD_{50}$s being as low as 0.08 mg/kg by subcutaneous (s.c.) administration (Table 2). Also of special note was the efficacy of WIN 57273 when the oral (p.o.) and s.c.

TABLE 2. In vivo efficacy of WIN 57273 against staphylococcal infections in mice

| Culture | Compound | Route of drug administration | $PD_{50}$ (mg/kg per dose [95% confidence interval]) |
|---|---|---|---|
| *Staphylococcus aureus* Smith | WIN 57273 | s.c. | 0.08 (0.05–0.12) |
| | | p.o. | 0.22 (0.17–0.31) |
| | Ciprofloxacin | s.c. | 1.0 (0.8–1.49) |
| | Ofloxacin | s.c. | 2.6 (2.0–3.3) |
| | Penicillin G | s.c. | 0.50 (0.37–0.62) |
| *Staphylococcus aureus* 39881[a] | WIN 57273 | s.c. | 0.18 (0.12–0.26) |
| | | p.o. | 0.30 (0.24–0.39) |
| | Ciprofloxacin | s.c. | >3.1 |
| | Ofloxacin | s.c. | 2.7 (2.0–3.6) |
| | Methicillin | s.c. | >100 |
| | Vancomycin | s.c. | 7.3 (4.2–9.6) |
| *Staphylococcus epidermidis*[a] | WIN 57273 | s.c. | 0.25 (0.18–0.35) |
| | | p.o. | 0.66 (0.46–0.97) |
| | Methicillin | s.c. | >100 |
| | Vancomycin | s.c. | 14.5 (11.4–17.1) |

[a] Methicillin-resistant cultures.

TABLE 3. Comparative in vivo efficacy of WIN 57273 against streptococcal infections in mice

| Culture | Compound | Route of drug administration | $PD_{50}$ (mg/kg per dose [95% confidence interval]) |
|---|---|---|---|
| *Streptococcus pneumoniae* ATCC 10813 | WIN 57273 | s.c. | 9.5 (7.2–12.8) |
| | | p.o. | 10.3 (7.5–13.0) |
| | Ciprofloxacin | s.c. | 85.3 (70.3–118.3) |
| | | p.o. | >250 |
| | Ofloxacin | s.c. | 59.8 (41.4–86.7) |
| | | p.o. | 89.2 (79.3–100.9) |
| | Penicillin G | s.c. | 1.8 (1.3–2.4) |
| *Streptococcus pyogenes* C203 | WIN 57273 | s.c. | 2.2 (1.8–2.6) |
| | | p.o | 2.4 (1.8–3.1) |
| | Ciprofloxacin | s.c. | 4.4 (3.5–5.7) |
| | Ofloxacin | s.c. | 9.7 (7.2–11.9) |
| | Penicillin G | s.c. | 0.05 (0.04–0.07) |

routes of drug administration were compared. Treatment of animals in this and all other models by either the p.o. or s.c. route yielded very similar results, implying that WIN 57273 has close to 100% bioavailability, at least in the murine model.

Other gram-positive cocci tested included *Streptococcus pneumoniae* and *Streptococcus pyogenes*. Animals infected with these organisms were also effectively treated with WIN 57273 (Table 3). While the efficacy of WIN 57273 was not on a par with that of the penicillin G reference agent, comparison with other quinolone compounds indicated that WIN 57273 had a severalfold superior activity.

Activity against the gram-negative bacterial infections was demonstrated in the *Escherichia coli* and *Klebsiella pneumoniae* models but not in the *Pseudomonas aeruginosa* model (Table 4). The $PD_{50}$s, which ranged from 3 to 4 mg/kg against *Escherichia coli* and *Klebsiella pneumoniae*, revealed that WIN 57273 has a lower efficacy than those of the reference compounds, although its activity was comparable to that of amifloxacin. Very weak activity was measured against a *Pseudomonas* infection, showing a clear distinction between WIN 57273 and, for example, ciprofloxacin.

In testing its efficacy against a *Listeria monocytogenes* infection, WIN 57273 was examined both for its activity in protecting the animal from lethal infection and in reducing the levels of the intracellular parasite, as estimated by viable counts taken from the spleens of infected animals. The calculated $PD_{50}$s of WIN 57273 were not different from those of the ampicillin reference, but they were significantly better than those of either ciprofloxacin or ofloxacin (Table 5). Comparison of the bactericidal activity of WIN 57273 against the invading *Listeria monocytogenes* revealed that it was superior to ampicillin at equal doses. No bacteria could be recovered from over half of the animals treated with WIN 57273; no case of tissue sterilization was observed with ampicillin treatment.

TABLE 4. Comparative in vivo efficacy of WIN 57273 against gram-negative infections in mice

| Culture | Compound | Route of drug administration | $PD_{50}$ (mg/kg per dose [95% confidence interval]) |
|---|---|---|---|
| *Escherichia coli* Vogel | WIN 57273 | s.c. | 3.1 (2.4–4.2) |
| | | p.o. | 3.8 (2.8–5.2) |
| | Ciprofloxacin | s.c. | 0.09 (0.06–0.14) |
| | Gentamicin | s.c. | 0.50 (0.37–0.61) |
| *Klebsiella pneumoniae* 39645 | WIN 57273 | s.c. | 4.4 (2.8–6.4) |
| | | p.o. | 3.2 (1.0–5.7) |
| | Ciprofloxacin | s.c. | 0.15 (0.09–0.28) |
| | Ofloxacin | s.c. | 0.84 (0.52–1.21) |
| | Gentamicin | s.c. | 0.35 (0.25–0.49) |
| *Pseudomonas aeruginosa* MGH-2 | WIN 57273 | s.c. | 160 (114–406) |
| | Ciprofloxacin | s.c. | 0.59 (0.45–0.77) |
| | Ofloxacin | s.c. | 3.9 (2.9–5.4) |
| | Gentamicin | s.c. | 6.9 (5.3–9.8) |

TABLE 5. Comparative in vivo efficacy of WIN 57273 administered s.c. against a *Listeria monocytogenes* infection in mice

| Compound | Dose (mg/kg) | Mean $\log_{10}$ spleen count ± SEM | $PD_{50}$ (mg/kg per dose [95% confidence interval]) |
|---|---|---|---|
| WIN 57273 | 6.25 | <3.11 ± 0.37[a] | 8.1 (6.6–10.9) |
| | 12.5 | <3.12 ± 0.33[a] | |
| Ampicillin | 6.25 | 4.86 ± 0.04 | 6.3 (4.5–9.5) |
| | 12.5 | 5.10 ± 0.39 | |
| Ciprofloxacin | | No survivors | >50 |
| Ofloxacin | | No survivors | >50 |

[a] Statistically significant difference from ampicillin controls ($P < 0.05$).

## DISCUSSION

Continued research activities centered on improving the antimicrobial spectrum of new quinolones have yielded some major advances and have generated considerable excitement in microbiological, chemical, and clinical research. To date, the most successful and best-studied compound has been ciprofloxacin, a potent and broad-spectrum quinolone that is effective both orally and parenterally against a variety of gram-negative and gram-positive bacterial infections (7). New agents have been sought, however, to improve the pharmacokinetics and to expand the microbiological spectrum by achieving greater potency against gram-positive pathogens. It is toward this end that we have been conducting research on WIN 57273.

Examination of the in vitro antibacterial activity of WIN 57273 demonstrated that it was an extremely potent agent against all of the staphylococci tested. Additionally, the activity was not confined to staphylococci. All of the gram-positive bacteria were susceptible to this compound in vitro at concentrations of ≤0.25 μg/ml. Also, several of the gram-negative pathogens exhibited sufficient susceptibilities for them to be considered candidates for potential clinical treatment.

The animal studies reported here verified the efficacy of WIN 57273, and equivalent activities were seen, when the compound was administered by the p.o. and s.c. routes. Additional studies to define the pharmacokinetics and pharmacodynamics of WIN 57273 are planned to determine the true clinical potential of this compound both as an oral and parenteral drug against systemic gram-positive bacterial infections; however, we were encouraged by the general uniformity of the in vivo results.

The results of the animal efficacy tests were in general agreement with expectations from the in vitro spectrum; i.e., the greatest potency was seen against *Staphylococcus* infec-

tions and the poorest activity was observed against *Pseudomonas* infections, infections caused by the most and least susceptible organisms, respectively, based on in vitro results. The activity against the staphylococcal infections was at least 1 order of magnitude better than that seen in any of the other models. Of special interest was the efficacy seen in the *Listeria* infection model. Since this is an intracellular infection, reduction of the viable counts from spleens indicated good penetration of WIN 57273 into the infected cells. Confirmation of this finding could lead to use of this compound in the general treatment of intracellular infections.

We showed that WIN 57273 possesses sufficiently novel activity to warrant its possible use in future clinical trials; additional studies with other organisms and infection models need to be conducted to explore fully the potential of this compound.

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