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MICs and MBCs of Win 57273 against *Mycobacterium avium* and *M. tuberculosis*

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A new quinolone, Win 57273 [1-cyclopropyl-7-(2,6-dimethyl-4-pyridinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolonecarboxylic acid], synthesized by Sterling Research Group, was tested in vitro against *Mycobacterium tuberculosis* and *Mycobacterium avium* strains. The broth-determined MICs of this agent ranged from 1.0 to 4.0 μg/ml for *M. tuberculosis* strains and from 0.25 to 8.0 μg/ml for *M. avium* strains. A distinctive feature of this agent, in comparison with ofloxacin and ciprofloxacin, is its substantially greater activity at the low pHs. For *M. avium* strains, the MICs of Win 57273 were 2.0 μg/ml or less for 54.5% of strains at pH 6.8 and 85.5% of strains at pH 5.0. Win 57273 was more active than ciprofloxacin against *M. avium* strains, and this difference was very substantial for all *M. avium* strains at pH 5.0. Taking into account that the predominant locations of these organisms in vivo are within the phagosomes and phagolysosomes of macrophages, i.e., in acidic environments at pH 5.0 or lower, the greater activity of Win 57273 at low pH makes this quinolone especially promising for *M. avium* infection. The bactericidal activity of Win 57273 for *M. avium* strains was the same as that of ciprofloxacin, with MBCs from 4.0 to 16.0 μg/ml.

Evaluation of the in vitro activity of various quinolones led to the identification of the two, ofloxacin and ciprofloxacin, having the most potential against mycobacteria. The MICs of these two drugs were found to be within the same range when tested against *Mycobacterium tuberculosis* in liquid media (3, 14, 26), in agar plates (1, 5, 7, 14, 22, 23), and in Lowenstein-Jensen medium (27; R. D. Urbanczik, Abstr. XXVI Int. Union against Tuberculosis World Conf., A012, p. 13, 1986), but because of the differences in pharmacokinetics, the preference was given to ofloxacin. At the same time, ciprofloxacin was found to be more active than ofloxacin and other quinolones in experiments in vitro with *Mycobacterium avium* complex (4, 7–9, 14, 23, 25). The MICs of ofloxacin and ciprofloxacin were quite consistent in different studies, regardless of the type of media, solid or liquid, used in the test.

In our previous study (14), the MICs of ofloxacin found for 46 *M. avium* strains were over a wide range and were all greater than 2.0 μg/ml. The MICs of ciprofloxacin for these strains were also over a wide range, but for 13 of 46 strains (28%), they were 2.0 μg/ml or less. The same distribution of *M. avium* complex strains was found in another study (15), in which 30% of the cultures were susceptible to ≤2.0 μg of ciprofloxacin per ml. Ofloxacin and ciprofloxacin have been reported as having high bactericidal activities in vitro against *M. tuberculosis*, with MBC/MIC ratios of 2 and 4 (4, 9). We confirmed this in our previous study and also reported that these drugs are highly bactericidal for *M. avium* strains as well, with MBC/MIC ratios between 1 and 8 (14). The available information about MICs and MBCs of ofloxacin and ciprofloxacin for *M. tuberculosis* and *M. avium* and the fact that some quinolones are more active than others give potential to every new derivative of this class of drugs, especially against *M. avium*.

The aim of this study was to evaluate the in vitro activity of a new quinolone, Win 57273, in comparison with those of previously tested quinolones, especially ciprofloxacin, against *M. avium* and *M. tuberculosis*. Special attention was given to the evaluation of the activity at low pH (5.0), which is favorable for *M. avium* (2, 10, 16, 21) and is equivalent to the acidic environment within the phagosomes and phagolysosomes of macrophages (6, 19, 25), common sites for these organisms in vivo.

MATERIALS AND METHODS

Antimicrobial agents. Win 57273 was synthesized by Sterling Research Group (Rensselaer, N.Y.) and is 1-cyclopropyl-7-(2,6-dimethyl-4-pyridinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolonecarboxylic acid. Ofloxacin was obtained from Daiichi Pharmaceuticals (Tokyo, Japan), and ciprofloxacin was obtained from Miles Pharmaceuticals (West Haven, Conn.). The chemical structures of the three tested quinolones are shown in Fig. 1. Win 57273 was dissolved in 6 to 8 ml of 0.1 N NaOH and diluted to 50.0 ml with distilled water. Ciprofloxacin and ofloxacin were dissolved in distilled water. The drug solutions were filter sterilized, and doubling dilutions were made to obtain solutions of 1,280.0 to 2.5 μg/ml. Multiple samples of each concentration were stored at −70°C until needed for an experiment.

Test strains. Fifty-five strains of *M. avium* identified by DNA-RNA hybridization (Gen Probe, San Diego, Calif.) and ten strains of *M. tuberculosis* were used in this study. $H_{37}Rv$ and nine drug-susceptible clinical isolates of *M. tuberculosis* and subcultures from transparent colonies of *M. avium* strains on 7H10 agar plates were subcultivated in 7H9 broth. When the turbidity of the broth cultures equalled that of a no. 1 McFarland standard, 1.0-ml aliquots of each culture were made and stored at −70°C.

MIC determination. For each strain of *M. avium*, two sets of BACTEC 7H12 broth vials were prepared. One set contained standard broth at pH 6.8; the other contained specially prepared broth (Becton Dickinson Diagnostic Instrument Systems, Towson, Md.) at pH 6.0. A phosphoric acid solution was added to the latter to lower the pH to 5.0 in order to simulate the acidic conditions within macrophages (6, 19, 25). For each *M. tuberculosis* strain, two sets

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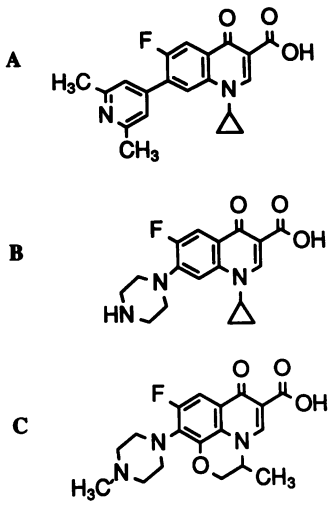

FIG. 1. Chemical structures of Win 57273 (A), ciprofloxacin (B), and ofloxacin (C). The structure of Win 57273 is adapted with the permission of the authors (M. Reuman, S. J. Daum, B. Singh, S. A. Coughlin, D. M. Sedlock, J. B. Rake, and G. Y. Lesher, Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1193, 1989).

of vials of 7H12 broth with pHs 6.8 and 6.0, respectively, were used. *M. tuberculosis* does not grow sufficiently at pHs lower than 6.0, and therefore we could not perform an MIC determination at pH 5.0. Win 57273, ciprofloxacin, and ofloxacin were added to obtain desired final concentrations within previously established ranges. In accordance with our previous studies (11), the following technique was used to achieve a final concentration of 10^4 to 10^5 CFU/ml in the drug-containing vials and in the undiluted drug-free controls. For *M. avium*, an inoculum of 0.1 ml of a 100-fold-diluted fresh BACTEC 7H12 broth culture that has a radiometric growth index (GI) of 999 was added to each 4.0-ml 7H12 vial designated as either a drug-containing test or a drug-free undiluted control. For *M. tuberculosis*, an undiluted fresh 7H12 broth culture that had a radiometric GI greater than 500 was used as an inoculum; 0.1 ml of this culture was added to the 4.0-ml drug-containing and drug-free vials. In addition, 1:100 drug-free controls were prepared for *M. avium* and for *M. tuberculosis* by adding 0.1 ml of 1:100-diluted inoculum, described above, per 4.0-ml 7H12 vial. The final concentration of bacteria in the 1:100 control was 10^2 to 10^3 CFU/ml, which represents 1% of the bacterial population in the drug-containing vials and undiluted drug-free controls.

All vials were incubated at 37°C, and the GI was read and recorded daily on the BACTEC 460-TB instrument. Incubation continued for no more than 8 days or until the GI of the 1:100 control for the *M. avium* strains had been greater than 10 for 3 consecutive days and was greater than 30 for the *M. tuberculosis* strains. The lowest concentration of a drug in whose presence the daily GI increase and the final GI reading were lower than those of the 1:100 control was considered to have inhibited more than 99% of the bacterial population and was designated the MIC. Our previous studies justified this approach for both *M. tuberculosis* and *M. avium* on the basis of comparison of the MICs determined by sampling and plating from the same broth culture and counting the number of CFU per milliliter (11–13).

MBC determination. The definition of MBC most often used in clinical microbiology is that it is the lowest concentration of a drug that kills 99.9% of the bacterial population (18). We suggested previously (12) that 99% killing was more accurate and reproducible in mycobacteriology. To determine the MBC, duplicate 7H12 broth vials were inoculated the same way as for determining the MIC but were allowed to incubate until the GI in the *M. avium* cultures reached 20 to 80 and that in the *M. tuberculosis* cultures reached >500, indicating that the cultures contained 10^5 to 10^6 CFU/ml (12, 14). At this time, concentrations equivalent to 1-, 2-, 4-, 8-, 16-, 32-, and 64-fold the MICs were added to separate vials. Samples were taken from alternate vials and diluted 10^{-1} to 10^{-6}, depending on GI readings and drug concentrations. Dilutions were inoculated into each of duplicate 7H10 agar plates. At the end of each experiment, the 10^{-3} dilutions reduced the drug concentrations below the previously determined MIC. Plates seeded with such diluted samples that were taken from vials containing concentrations subsequently found to be the MBCs showed no growth. After 12 to 14 days of incubation at 37°C in a 5% CO_2 atmosphere, the colonies were counted and CFU per milliliter was calculated from plates inoculated with 10^{-1} dilutions made on the final day of observation. The fact that there was no growth by the 10^{-3}-diluted samples but that growth appeared on the plates seeded with 10^{-1} dilutions convinced us that there was no drug carry-over (20, 24). More technical details of MBC determination are given in our previous publications (12, 14).

TABLE 1. Broth-determined MICs of three quinolones for 10 *M. tuberculosis* strains

Strain	MIC (μg/ml) of indicated quinolone[a] at:					
	pH 6.8			pH 6.0		
	OFL	CIP	WIN	OFL	CIP	WIN
$H_{37}Rv$	1.0	1.0	4.0	2.0	2.0	1.0
1620-6	1.0	0.5	1.0	1.0	1.0	0.25
131-4	1.0	1.0	2.0	2.0	1.0	0.5
126-4	1.0	0.5	4.0	2.0	1.0	1.0
3105-5	2.0	1.0	4.0	2.0	1.0	1.0
1773-6	1.0	0.5	4.0	1.0	1.0	1.0
1024-8	0.5	0.5	2.0	2.0	1.0	0.5
1685-6	1.0	0.5	2.0	1.0	1.0	0.5
1717-6	2.0	0.5	4.0	1.0	1.0	0.5
483-8	0.5	0.5	2.0	1.0	0.5	0.5

[a] OFL, Ofloxacin; CIP, ciprofloxacin; WIN, Win 57273.

RESULTS

MICs for *M. tuberculosis*. In 7H12 broth at pH 6.8, the MICs of Win 57273 were higher than the MICs of ofloxacin and ciprofloxacin for all 10 tested strains (Table 1). Comparison at pH 6.0 showed that Win 57273 was more active than ofloxacin for 9 of 10 strains and more active than ciprofloxacin for 6 of 10 strains (Table 1). The MICs of Win 57273 at pH 6.0 were between 0.25 and 1.0 μg/ml.

MICs for *M. avium*. At both pHs, Win 57273 had the lowest MICs for 50 and 90% of the strains and ofloxacin had the highest, with ciprofloxacin in an intermediate position (Table 2). The distribution of these 55 strains by the degree of their susceptibility expressed in MICs also indicated that at both pHs, Win 57273 was the most active and ofloxacin was the least active among the three tested quinolones. At pH 6.8, the proportions of strains inhibited by concentrations of 1.0 μg/ml or less were 30.9% for Win 57273, 29.1% for ciprofloxacin, and 3.6% for ofloxacin. At pH 5.0, the differences in the activities of these drugs were more dra-

TABLE 2. MICs of three quinolones for 55 *M. avium* strains

Drug	pH	% of strains at MIC (μg/ml)								MIC (μg/ml)[a]	
		0.25	0.5	1.0	2.0	4.0	8.0	16.0	>16.0	50%	90%
Win 57273	6.8	1.8	9.1	20.0	23.6	38.2	7.3	0	0	1.80	3.85
Ciprofloxacin	6.8	3.6	5.5	20.0	12.7	30.9	21.8	5.5	0	2.55	7.40
Ofloxacin	6.8	0	0	3.6	3.6	23.7	18.2	30.9	20.0	8.20	>16.0
Win 57273	5.0	16.3	25.5	20.0	23.7	12.7	1.8	0	0	0.70	3.0
Ciprofloxacin	5.0	0	1.80	16.4	10.9	18.1	30.9	16.4	5.5	4.35	13.9
Ofloxacin	5.0	0	0	0	1.8	18.2	9.1	14.5	56.4	>16.0	>16.0

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.

matic, since only Win 57273 showed higher activity at this low pH than at pH 6.8 and since the proportions of strains inhibited by 1.0 μg/ml or less at pH 5.0 were 61.8% for Win 57273, 18.2% for ciprofloxacin, and none for ofloxacin (Table 2). Since ofloxacin was the least active among the three tested drugs, we have compared the MICs of only ciprofloxacin and Win 57273 for each tested strain (Fig. 2). This comparison at pH 6.8 showed that the MICs of Win 57273 were slightly higher for only 5 strains, the MICs of both drugs were the same for 22 strains, and the MICs of Win 57273 were higher for the remaining 28 strains. At pH 5.0, the difference was more dramatic. The MICs of Win 57273 were lower for all tested strains, and this difference was 4- to 16-fold for 41 of the 55 tested strains.

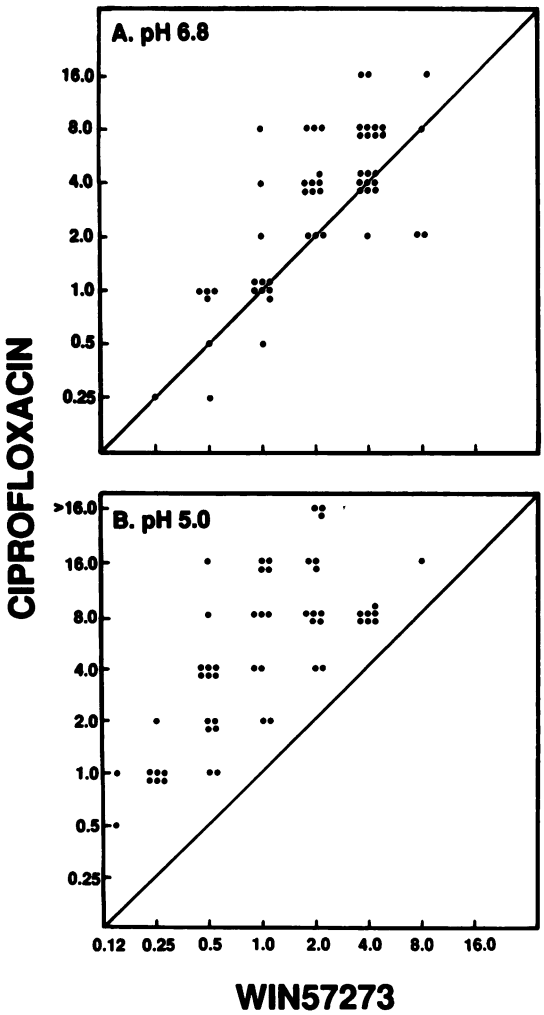

FIG. 2. MICs of Win 57273 and ciprofloxacin for the same strains of *M. avium* at pHs 6.8 (A) and 5.0 (B).

MBCs for *M. tuberculosis*. The MBCs of Win 57273 were determined for three *M. tuberculosis* strains and were 4.0 to 16.0 μg/ml at pH 6.8 and 1.0 to 4.0 μg/ml at pH 6.0 (Table 3). Comparison with MICs for these three strains (Table 1) gives MBC/MIC ratios ranging from 2 to 8 at pH 6.8 and 2 to 4 at pH 6.0.

MBCs for *M. avium*. The MBCs of Win 57273 for four tested strains were from 2.0 to 16.0 μg/ml at pH 6.8 and were 1 doubling dilution lower at pH 5.0 for three of four strains (Table 4), and MBC/MIC ratios ranged from 2 to 16 at both pHs. For the same strains, the MBCs of ofloxacin at pH 6.8 were from 16.0 to 256.0 μg/ml and MBCs of ciprofloxacin ranged from 4.0 to 32.0 μg/ml (14) (Table 4). At pH 5.0, the MBCs of these two quinolones were either the same as or greater than those for Win 57273 in seven of eight experiments (Table 4).

DISCUSSION

Previously, we reported (14) that ciprofloxacin was more active than ofloxacin against *M. avium* in vitro. In the current study, we found that the new quinolone, Win 57273, was more active than ciprofloxacin against most of the same organisms. A unique feature of this new quinolone is that it is more active at low pH, specifically at pH 5.0, which is similar to the pH environment within the phagosomes and phagolysosomes of macrophages (6, 19, 25). This fact makes Win 57273 especially attractive for further evaluation as a drug with potential for treatment of *M. avium* disease. At the same time, Win 57273 did not appear to have an obvious advantage over ofloxacin and ciprofloxacin when the MICs of these three drugs were compared in tests against 10 *M. tuberculosis* strains.

The use of quinolones in chemotherapy of mycobacterial infections started with the report about the use of ofloxacin in chemotherapy of tuberculosis (29). The authors used this drug to treat 19 patients with advanced cavitary disease who had failed for many years to respond to chemotherapy with conventional drugs. Of these patients, 14 showed a substantial decrease in the number of *M. tuberculosis* in their sputum but did not convert to negative. The latter fact is not a surprising outcome, taking into account the history of their disease and the fact that their treatment with ofloxacin was essentially a monotherapy, which is usually doomed to failure. Despite these aggravating circumstances, the five remaining patients did convert to negative, which fact was considered by the authors of the paper as evidence that

TABLE 3. Bactericidal activity of Win 57273 for *M. tuberculosis* strains

Strain	Bactericidal activity at:							
	pH 6.8				pH 6.0			
	No. of CFU/ml		MBC (μg/ml)	MBC/MIC ratio	No. of CFU/ml		MBC (μg/ml)	MBC/MIC ratio
	When drug was added	At the end of observation			When drug was added	At the end of observation		
$H_{37}Rv$	8×10^4	1.2×10^2	16.0	4	8.8×10^4	3×10^1	4.0	4
1620-6	1.61×10^5	$<10^1$	8.0	8	1.6×10^5	$<10^1$	2.0	4
131-4	5.6×10^4	3×10^1	4.0	2	8.0×10^4	3×10^1	1.0	2

ofloxacin is indeed an active antituberculosis drug. Clinical efficacy of ofloxacin and other quinolones in the chemotherapy of *M. avium* disease is not known.

Because of limited information, it is hard to justify the drug susceptibility breakpoints on the basis of comparison of in vitro test results and the response of the patient to chemotherapy. Nevertheless, two facts can be considered. (i) *M. tuberculosis* clinical isolates are known to be very uniform in the degree of their susceptibility to all known antituberculosis drugs, if they have not been exposed to them previously. (ii) The data on pharmacokinetics in humans are given below. The MICs of both ofloxacin and ciprofloxacin were indeed within a very narrow range for *M. tuberculosis* strains and did not exceed 2.0 μg/ml regardless of the techniques used (1, 3, 5, 7, 8, 9, 14, 22, 23, 26, 28). The peak level of ofloxacin in serum in humans was originally reported to be between 2.0 and 3.0 μg/ml (29) but was assumed to be even higher for both ofloxacin and ciprofloxacin in subsequent studies which were analyzed in our previous publication (14). This, along with the data above on MICs for *M. tuberculosis* strains, was the basis for our suggestion to use 2.0 μg/ml as a breakpoint to classify mycobacteria as susceptible. Data on human pharmacokinetics of Win 57273 are not yet available, but preliminary subhuman primate pharmacokinetic data suggest that peak levels in serum of 2 to 4 μg/ml may be achieved in humans (J. Rake, personal communication).

The National Committee for Clinical Laboratory Standards has suggested the following interpretive criteria for MICs of ciprofloxacin for bacteria that grow aerobically: susceptible, ≤1.0 μg/ml; moderately susceptible, 2.0 μg/ml; and resistant, ≥4.0 μg/ml (see updated Table 2 in reference 17). For urinary tract infections, an MIC of ≤4.0 is considered susceptible. There is no recommendation for ofloxacin.

Taking into account all considerations quoted above, there are two options for interpretation of the MICs found in this study of three quinolones. One is to consider MICs of ≤1.0 μg/ml susceptible and 2.0 μg/ml moderately susceptible. Another, as we suggested previously (14), is to consider *M. tuberculosis* and *M. avium* strains susceptible if the MIC is 2.0 μg/ml or less.

If ≤1.0 μg/ml were taken as a breakpoint for susceptible strains, then at pH 5.0, no *M. avium* strains could be classified as susceptible to ofloxacin, 18.2% could be classified as susceptible to ciprofloxacin, and 61.8% could be classified as susceptible to Win 57273. If ≤2.0 μg/ml were taken as a breakpoint, then the percentage of *M. avium* strains classified as susceptible would be 1.8% for ofloxacin, 29.1% for ciprofloxacin, and 85.5% for Win 57273. By either criterion, Win 57273 was more active than the two other quinolones for a larger number of *M. avium* strains. This difference was especially dramatic when the test was performed at pH 5.0, but even at pH 6.8, Win 57273 was more active than the two other tested drugs.

We previously found (14) that ofloxacin and ciprofloxacin were more bactericidal than any other antimicrobial agent tested to date against *M. avium*. The MBC/MIC ratios of these two quinolones for *M. avium* were close to their MBC/MIC ratios for *M. tuberculosis* and ranged from 1 to 8 for ciprofloxacin and 2 to 16 for ofloxacin. In the present study, Win 57273 also showed high bactericidal activity against both *M. tuberculosis* and *M. avium*. The MBCs of this drug against *M. tuberculosis*, 4.0 to 8.0 μg/ml, were slightly higher than the MBCs of ofloxacin (2.0 μg/ml) and ciprofloxacin (2.0 μg/ml) reported previously (14). For *M. avium*, the MBCs of Win 57273 were within the same range as those of ciprofloxacin (4.0 to 16.0 μg/ml) and were lower than the MBCs of ofloxacin (16.0 to 256.0 μg/ml) for the

TABLE 4. Bactericidal activity of Win 57273 and two other quinolones against *M. avium* strains

pH and strain	Win 57273				Ciprofloxacin				Ofloxacin			
	No. of CFU/ml		MBC (μg/ml)	MBC/MIC ratio	No. of CFU/ml		MBC (μg/ml)	MBC/MIC ratio	No. of CFU/ml		MBC (μg/ml)	MBC/MIC ratio
	When drug was added	At the end of observation			When drug was added	At the end of observation			When drug was added	At the end of observation		
5.0												
453	1.62×10^5	$<10^3$	2.0	4	2.05×10^5	$<10^1$	4.0	4	2.05×10^5	$<10^1$	16.0	4
3337	1.83×10^5	7×10^2	8.0	8	1.13×10^5	$<10^1$	32.0	4	1.13×10^5	$<10^1$	128.0	2
3349	1.24×10^5	$<10^3$	8.0	4	1.97×10^5	$<10^2$	128.0	4	1.97×10^5	1.01×10^5	>256.0	≥4
3350	1.27×10^5	$<10^3$	2.0	2	1.72×10^5	5×10^1	8.0	4	1.72×10^5	3.5×10^2	64.0	4
6.8												
453	1.42×10^5	$<10^3$	4.0	4	4.84×10^5	$<10^1$	8.0	8	4.84×10^5	$<10^1$	16.0	4
3337	1.31×10^5	$<10^3$	8.0	8	3.4×10^5	4×10^1	16.0	2	3.47×10^5	$<10^2$	64.0	4
3349	2.4×10^5	2×10^3	8.0	2	2.02×10^5	10^2	32.0	2	2.02×10^5	3×10^1	256.0	4
3350	9.4×10^4	$<10^3$	4.0	4	3.6×10^5	10^2	4.0	1	3.6×10^5	$<10^3$	64.0	4

same four strains. Though for *M. avium* the bactericidal activities of Win 57273 and ciprofloxacin were about the same, the higher inhibitory activity of the new quinolone makes it the most promising within this class of antimicrobial agents.

ACKNOWLEDGMENTS

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