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In Vitro Antituberculosis Activities of ACH-702, a Novel Isothiazoloquinolone, against Quinolone-Susceptible and Quinolone-Resistant Isolates[▽]

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ACH-702 is a new isothiazoloquinolone with potent *in vitro* and *in vivo* activities against important bacterial pathogens, including *Staphylococcus aureus*. In this study, ACH-702 was found to have promising *in vitro* antibacterial activity against *Mycobacterium tuberculosis*, with MICs of ≤1 μg/ml, comparable to that of the fluoroquinolone moxifloxacin for quinolone-susceptible isolates but superior to that for quinolone-resistant isolates. Biochemical assays involving *M. tuberculosis* gyrase enzymes indicated that ACH-702 had significantly improved inhibitory activity compared with fluoroquinolones.

Isoniazid (INH) and rifampin (RIF) are the current foundation for effective tuberculosis (TB) chemotherapy. New regimens are needed to treat tuberculosis, due to the increasing incidence of multidrug-resistant tuberculosis (MDRTB), which is defined by resistance to INH and RIF (2, 7, 19), and extensively drug-resistant tuberculosis (XDRTB), which is defined by MDR plus resistance to a fluoroquinolone and either kanamycin, amikacin, or capreomycin (4, 10, 13). Two newer quinolones, moxifloxacin (MXF) and gatifloxacin (GAT), are currently in phase III clinical trials for therapy of tuberculosis (3, 17). Isothiazoloquinolones (ITQs), a class of compounds structurally related to quinolones, have been found to have good *in vitro* and *in vivo* activities against several key bacterial pathogens such as *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA) isolates (11). Because of this potent, broad-spectrum antibacterial activity, the effectiveness of the lead ITQ, ACH-702, against *Mycobacterium tuberculosis* was tested. Initial studies in our laboratory demonstrated activity of ACH-702 (Fig. 1) against *M. tuberculosis* Erdman (ATCC 35801). The purpose of the present study was to evaluate the *in vitro* activities of ACH-702 against additional isolates of *M. tuberculosis*, particularly those with decreased susceptibility to quinolones, by using both MIC and target enzyme assays.

INH and RIF were purchased from Sigma Chemical Co. (St. Louis, MO). MXF was provided by Bayer Corporation (West Haven, CT). ACH-702 was provided by Achillion Pharmaceuticals (New Haven, CT). Each drug was dissolved in dimethyl sulfoxide at a final concentration of 1 mg/ml. Aliquots were frozen and stored at −20°C. Drugs were diluted in modified 7H10 broth (pH 6.6; 7H10 agar formulation with agar and malachite green omitted) with 10% OADC (oleic acid, albumin, dextrose, catalase) enrichment (BBL Microbiology Systems, Cockeysville, MD) and 0.05% Tween 80. Clinical *M. tuberculosis* isolates were obtained from the Clinical Microbiology Laboratory, University Hospital, Syracuse, NY (courtesy of Betty Ann Forbes) and from Jacques Grosset (Faculte de Medecine, Pitie-Salpetriere, Paris, France). ATCC 35801 (Erdman) was purchased from the ATCC (Manassas, VA). The quinolone-resistant *M. tuberculosis* mutant isolates 10, 11, and 12 were selected in our laboratory (Veterans Affairs Medical Center [VAMC], Syracuse, NY). *M. tuberculosis* isolate 11 was selected by plating *M. tuberculosis* Erdman on 7H10 agar with 10% OADC containing levofloxacin (LVX). Several colonies were selected, grown in 7H10 broth with 10% OADC, tested for LVX resistance, and frozen at −70°C. *M. tuberculosis* isolate 11 demonstrated a moderate level of LVX resistance. One LVX-resistant *M. tuberculosis* isolate was subsequently grown on 7H10 agar containing GAT to further enhance its quinolone resistance. Colonies were selected, grown in 7H10 broth, tested for LVX and GAT resistance, and frozen at −70°C. *M. tuberculosis* isolates 10 and 12 demonstrated slightly more quinolone resistance than the original LVX-resistant *M. tuberculosis*. All isolates were grown in modified 7H10 broth for 5 to 10 days on a rotary shaker at 37°C. The cultures were diluted to 100 Klett units (equivalent to 5×10^7 CFU/ml) (Photoelectric Colorimeter; Manostat Corp., New York, NY) and frozen in aliquots at −70°C until used.

Polystyrene 96-well round-bottom microtiter plates (Corning Inc., Corning, NY) were prepared to contain 50 μl of modified 7H10 broth with serial dilutions of the drugs to be tested using a multichannel electronic pipetter. To each well, 50 μl of the appropriate mycobacterial cell suspension was added to yield a final concentration of about 6×10^4 CFU/ml (range for various isolates tested, 1.2×10^4 CFU/ml to 2.6×10^5 CFU/ml). Each isolate was tested in duplicate. The microtiter plates were covered with SealPlate adhesive sealing film (Excel Scientific, Wrightwood, CA) and incubated at 37°C in ambient air for about 21 days before the results were read. The MIC was defined as the lowest concentration of antimicrobial agent yielding no visible turbidity.

Expression and purification of *M. tuberculosis* wild-type and mutant GyrA protein were performed as previously described

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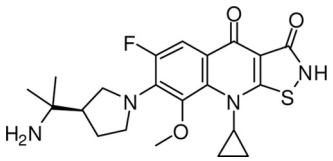

FIG. 1. Chemical structure of ACH-702.

for *Staphylococcus aureus* enzyme (5, 15). The design of *M. tuberculosis* mutant gyrase enzymes was based on earlier reports in the literature (1, 9, 14, 18). Specific *gyrA* mutations resulting in amino acid substitutions A90V and D94G were made by site-directed mutagenesis of the wild-type expression plasmid using a QuikChange XL mutagenesis kit (Stratagene, Agilent Technologies, La Jolla, CA). Verification of the expression plasmid sequence was done at SeqWright, Houston, TX. DNA gyrase proteins were reconstituted *in vitro* as the wild-type or mutant GyrA subunit plus the wild-type GyrB subunit at molar ratios of 2:3. Gyrase activity was measured by a supercoiling assay that monitored the ATP-dependent conversion of relaxed pBR322 DNA to the supercoiled form (Topogen, Inc., Port Orange, FL) (5). The supercoiled products were quantitated using ethidium bromide fluorescence following agarose gel electrophoresis. The 50% inhibitory concentration (IC_{50}) was defined as the concentration of compound that inhibits 50% of the catalytic activity. Compound potencies were compared with values determined for three marketed quinolones: ciprofloxacin (CIP), MXF, and GAT.

MIC assays were done for 17 clinical isolates and four laboratory strains (Erdman and *M. tuberculosis* isolates 10, 11, and 12) as shown in Table 1. The MICs of ACH-702 for 14 of 15 quinolone-susceptible isolates (MXF MIC, ≤0.06 μg/ml) were similar to those of MXF for the same isolates (6). An exception was *M. tuberculosis* isolate 8, with an ACH-702 MIC of 0.25 μg/ml compared to an MXF MIC of 0.06 μg/ml. The MICs of MXF for the six quinolone-resistant isolates ranged from 1 μg/ml to >8 μg/ml, compared to 0.125 μg/ml to 1 μg/ml for ACH-702, with the MICs for ACH-702 lower than those for MXF for each isolate. Of particular note are *M. tuberculosi*s isolates 10, 11, and 12, mutants selected in the laboratory, for which the MXF MICs were >8, 4, and 8 μg/ml, while the ACH-702 MICs were 0.125, 0.25, and 1 μg/ml, respectively. ACH-702 also had lower MICs for each of three XDR isolates tested (Table 1).

Quinolone-resistant isolates of *M. tuberculosis* are becoming more prevalent in both high-burden and low-burden countries; therefore, both MXF and GAT may have limited functional usefulness in the future. ACH-702, a new isothiazoloquinolone antibacterial compound, has promising *in vitro* activities against *M. tuberculosis* which are comparable to those of MXF against quinolone-susceptible strains. However, this compound distinguishes itself from MXF by maintaining its activity against quinolone-resistant isolates. This finding is consistent with its antibacterial activity against quinolone-resistant isolates of other bacteria (12). This increased activity is apparently due to excellent inhibition of bacterial gyrase by compounds in this class, as shown by its approximately 3-fold-lower micromolar IC_{50}s for *M. tuberculosis* wild-type gyrase enzyme than those of MXF and GAT and approximately 20-fold-lower IC_{50}s than those of LVX (Table 2). Moreover, these differences in IC_{50}s between ACH-702 and MXF, GAT, and LVX were higher when mutant gyrase enzymes were assayed (Table 2). When the mutations resulting in A90V and D94G, two of the more common GyrA amino acid substitutions found in resistant *M. tuberculosis* (1, 9, 18), were introduced via site-directed mutagenesis into the *gyrA* gene, the IC_{50}s increased for all of the compounds. However, for the A90V mutant enzyme, ACH-702 displayed a 6-fold-lower IC_{50} than those of MXF and GAT. For the D94G mutant enzyme, ACH-702 displayed 5-fold- and 7-fold-lower IC_{50}s than GAT and MXF,

TABLE 1. *In vitro* activities of ACH-702 against *M. tuberculosis* isolates

Isolate	Comment[a]	MIC (μg/ml)			
		INH	RIF	MXF	ACH-702
Erdman[b]	ATCC	0.06	0.008	0.06	0.06
1	WT	0.03	0.004	0.03	0.06
2	WT	0.06	≤0.001	0.03	0.03
3	WT	0.06	0.004	0.03	0.06
4	WT	0.06	0.008	0.06	0.06
5	WT	0.125	0.015	0.06	0.06
6	WT	0.06	0.008	0.03	0.03
7	WT	0.06	0.004	0.06	0.06
8	SM^r	0.125	0.008	0.06	0.25
9	MDR[c]	4	64	0.06	0.06
10	Quin^r	0.03	0.004	>8	0.125
11	Quin^r	0.03	0.03	4	0.25
12	Quin^r	0.03	0.004	8	1
13	Beijing strain	0.125	0.002	0.03	0.06
14	WT	0.06	0.002	0.03	0.06
15	INH^r	>8	0.002	0.06	0.06
16	XDR	1	8	1	0.5
17	XDR	1	8	2	1
18	XDR	2	8	1	0.125
19	MDR	2	64	0.03	0.03
20	MDR	2	16	0.03	0.03

[a] WT, pansusceptible clinical isolate. Quin^r indicates quinolone-resistant isolate selected in the laboratory at VAMC; all others (except Erdman) are clinical strains. Sequenced mutations for isolate 10 were D94Y S95T, and those for isolate 11 were G88C S95T. Abbreviations: INH, isoniazid; RIF, rifampin; MXF, moxifloxacin; SM^r, streptomycin resistant; MDR, multidrug drug resistant; XDR, extensively drug resistant.

[b] ATCC 35801 (pansusceptible); all MICs for this strain are the average of 8 assays.

[c] Streptomycin MIC, 32 μg/ml (versus 0.125 to 0.25 μg/ml for susceptible strains).

TABLE 2. Inhibition of *M. tuberculosis* wild-type and mutant gyrase catalytic activities by ACH-702 and quinolones

Compound	Gyrase IC_{50} (μM)[a]		
	Wild type	Gyr A90V[b]	Gyr D94G[c]
ACH-702	2.9	4.6	7.9
Moxifloxacin	9.2	31	59
Gatifloxacin	9.4	28	44
Levofloxacin	52	>300	>300

[a] Inhibition of DNA gyrase supercoiling activity; assays were run two to four times in duplicate.

[b] GyrA mutation A90V in the quinolone resistance-determining region (QRDR) (8).

[c] GyrA mutation D94G in the QRDR (8).

respectively. These differences probably account for the retention of MICs of ≤ 1 μg/ml for quinolone-resistant clinical isolates. Similar results have been previously observed in assays using staphylococcal gyrase and topoisomerase IV enzymes (16). Enzyme inhibition analyses against additional gyrase enzymes from quinolone-resistant clinical isolates of *Mycobacterium tuberculosis* are in progress. Further optimization of this series of isothiazoloquinolones may yield analogs with even better potency against such quinolone-resistant isolates. In light of the *in vitro* enzyme inhibition and antibacterial activity of ACH-702 observed against MXF-resistant isolates, this compound should be evaluated further against quinolone-resistant and pansusceptible *M. tuberculosis* isolates to ascertain *in vivo* efficacy.

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