ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2010, p. 2188–2190 Vol. 54, No. 5
0066-4804/10/$12.00 doi:10.1128/AAC.01603-09

In Vitro Activity of a New Isothiazoloquinolone, ACH-702, against *Mycobacterium tuberculosis* and Other Mycobacteria[▽]

Carmen A. Molina-Torres,[1] Jorge Ocampo-Candiani,[1] Adrian Rendón,[2] Michael J. Pucci,[3] and Lucio Vera-Cabrera[1]*

Servicio de Dermatología, Hospital Universitario José E. González, Monterrey, Nuevo León, México[1]; Tuberculosis Clinic, CIPTIR, Hospital Universitario José E. González, Monterrey, Nuevo León, México[2]; and Achillion Pharmaceuticals, Inc., New Haven, Connecticut[3]

Received 11 November 2009/Returned for modification 11 December 2009/Accepted 2 March 2010

In this work, we describe the activity of ACH-702 against clinical isolates of *Mycobacterium tuberculosis* and six different nontuberculous mycobacteria. The MIC_{50} and MIC_{90} of both susceptible and drug-resistant *M. tuberculosis* strains tested were 0.0625 and 0.125 μg/ml, respectively. The MIC_{50} and MIC_{90} values for *Mycobacterium fortuitum* isolates were 0.0625 μg/ml in both cases; *Mycobacterium avium* complex isolates showed MIC_{50} and MIC_{90} values of 0.25 and 4 μg/ml, respectively.

Tuberculosis (TB) remains a public health problem, with 1.3 million deaths among HIV-negative incident cases of TB and an additional 456,000 deaths among HIV-positive incident TB cases in 2007 (33). The infection can be treated properly if an early diagnosis is made and the appropriate therapy is administered. However, due to multiple factors, multidrug-resistant strains of *M. tuberculosis* are becoming more numerous, increasing the urgency for alternative therapeutic options.

Recently, some compound analogs related to the quinolone class, the isothiazoloquinolones (ITQs), have been identified, and their chemical synthesis and preliminary biological profiling have been reported (13, 29). These ITQs target bacterial replication and are potent inhibitors of both DNA gyrase and topoisomerase IV (5). A lead ITQ compound, ACH-702, has demonstrated potent antibacterial activity against a number of medically relevant bacteria, including drug-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) (21, 28, 31). In the present work, we studied the antibacterial activity of ACH-702 against clinical isolates of *Mycobacterium tuberculosis*, including drug-resistant isolates, and additional isolates of nontuberculous mycobacteria (NTM).

Sixty clinical isolates of *M. tuberculosis* were tested, including nine isolates resistant to isoniazid and 20 clinical isolates resistant to both isoniazid and rifampin, with susceptibility assays to these drugs performed by the proportion method. The isolates were obtained from the Clínica de Tuberculosis (CIPTIR) from the Hospital Universitario José E. González in Monterrey, México. Also, a total of 30 NTM clinical isolates corresponding to *Mycobacterium fortuitum* ($n = 11$), *Mycobacterium chelonae* ($n = 2$), *Mycobacterium abscessus* ($n = 2$), *Mycobacterium intracellulare* ($n = 7$), *Mycobacterium avium* complex ($n = 3$), *Mycobacterium kansasii* ($n = 3$), and *Mycobacterium gordonae* ($n = 2$) were tested. All the atypical mycobacteria were identified by phenotypic characteristics and by using PCR-restriction fragment length polymorphism (RFLP) analysis of the *hsp65* gene as previously described (25).

ACH-702 was obtained from Achillion Pharmaceuticals, Inc., New Haven, CT. A stock solution (1 mg/ml) of ACH-702 was prepared in 100% dimethyl sulfoxide and diluted in 7H9GC broth (4.7 g of Middlebrook 7H9 broth base [Difco, Detroit, MI], 20 ml of 10% [vol/vol] glycerol, 1 g of Bacto Casitone [Difco], 880 ml of distilled water, 100 ml of oleic acid-albumin-dextrose-catalase [Becton Dickinson, MD]) (9). The final drug concentration range was 0.03 to 8 μg/ml. In order to determine the susceptibility of *M. tuberculosis* to ACH-702, the broth microdilution method with Alamar Blue was utilized (9). *M. tuberculosis* H37Rv was run as a susceptible-strain control; moxifloxacin was also tested for activity comparison.

For NTM, MIC was determined as recommended by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical and Laboratory Standards) by a broth microdilution method in cation-adjusted Mueller-Hinton broth (CA-MHB) for rapidly growing mycobacteria (RGM) and in CA-MHB with the addition of an oleic acid-albumin-dextrose-catalase supplement (Becton Dickinson, Sparks, MD) at a final concentration of 10% for slowly growing mycobacteria (20). The final drug concentration range was 0.06 to 16 μg/ml. The MIC was determined for the RGM after 72 h of incubation at 30°C and for the slowly growing mycobacteria after 7 days of incubation at 35°C. For external controls, we utilized *Staphylococcus aureus* ATCC 29213.

In Table 1, we show the susceptibility of clinical isolates of *Mycobacterium tuberculosis* to ACH-702. All *M. tuberculosis* isolates were inhibited with ≤0.125 μg/ml, including the drug-resistant isolates. Moxifloxacin showed MIC_{50} and MIC_{90} values of 0.125 and 0.25 μg/ml, respectively (data not shown). ACH-702 displayed antibacterial activity equal or superior to that of other currently marketed quinolones against quinolone-susceptible *M. tuberculosis* clinical isolates and superior antibacterial activity against quinolone-resistant clinical isolates, including XDR strains as well as laboratory-selected

* Corresponding author. Mailing address: Servicio de Dermatología, Hospital Universitario José E. González, Madero y Gonzalitos, Col. Mitras Centro, Monterrey, NL CP 64460, Mexico. Phone: 5281-8348-0383. Fax: 5281-8348-4407. E-mail: luvera_99@yahoo.com.

▽ Published ahead of print on 15 March 2010.

TABLE 1. *In vitro* susceptibility of 60 clinical isolates of *M. tuberculosis* to ACH-702

M. tuberculosis isolates with phenotype (*n*):	MIC (μg/ml)[a]		
	Range	50%	90%
Susceptible to both isoniazid and rifampin (31)	≤0.03–0.12	0.06	0.12
Resistant to isoniazid or rifampin (9)	≤0.03–0.12	0.12	0.12
Resistant to both isoniazid and rifampin (20)	0.06–0.25	0.06	0.12

[a] 50% and 90%, MICs at which 50% and 90% of the *M. tuberculosis* strains are inhibited, respectively.

TABLE 2. *In vitro* susceptibility of clinical isolates of *M. avium* complex and *M. fortuitum* to ACH-702

Species	MIC (μg/ml)[a]		
	Range	50%	90%
M. avium complex (n = 10)	0.12–8	0.25	4
M. fortuitum (n = 11)	≤0.06–0.12	≤0.06	≤0.06

[a] 50% and 90%, MICs at which 50% and 90% of the *Mycobacterium* sp. strains are inhibited, respectively.

quinolone-resistant mutants (M. Pucci, M. Ackerman, J. Thanassi, C. Schoen, and M. Cynamon, submitted for publication).

The activity of ACH-702 against *M. avium* and *M. fortuitum* is presented in Table 2. The MIC value for two isolates of *M. chelonae* was 8 μg/ml; the MIC for two isolates of *M. abscessus* was 16 μg/ml. The range of MICs for *M. kansasii* (n = 3) was 0.12 to 16 μg/ml, and the MIC values for two *M. gordonae* isolates were 0.12 and 4 μg/ml. The MIC for quality control strains was determined with each lot of microtiter plates prepared, and it was ≤0.06 μg/ml for *S. aureus* ATCC 29213.

Fluoroquinolones are utilized as second-line anti-TB drugs (4, 19, 32). Moxifloxacin and gatifloxacin are being investigated in clinical trials, and they are candidates for shortening TB treatment since they have the lowest MICs (3, 10, 11) and greatest bactericidal activity (14, 16, 24). Quinolones have a long history of chemical modifications giving rise to new compounds with enhanced antibacterial activity. One example is the recently reported ITQ class (30, 31), an underexplored chemotype related to quinolones first described by Chu and coworkers at Abbott in the late 1980s (7, 8). ITQs have a substitution in the typical 3-carboxyl group by an isothiazolone ring and have demonstrated good antibacterial activity (28, 31). ACH-702 is an ITQ lead compound that has been studied against a number of bacterial pathogens, including MRSA, VRE, and *Nocardia brasiliensis*, with promising results (21, 27, 28, 31).

In this work, we observed that both susceptible and drug-resistant *M. tuberculosis* isolates were inhibited with MICs of ≤0.25 μg/ml ACH-702 for all 60 isolates tested. Comparing these data with previously published *in vitro* data for quinolones like ciprofloxacin, gatifloxacin, ofloxacin, garenoxacin, and levofloxacin, it appears that ACH-702 may have antibacterial activity superior to that of these representative quinolones (1, 22, 10). In our work, we also tested moxifloxacin as a quinolone control and obtained MIC values in agreement with previously reported results.

In addition, the incidence of diseases caused by NTM appears to be increasing worldwide, particularly due to its association with HIV AIDS (12). Management of NTM infections is difficult in most cases due to intrinsic resistance to many antimycobacterial agents. The fluoroquinolones have been shown to be active *in vitro* against many mycobacterial species, such as *M. fortuitum*, and some strains of *M. kansasii* and the *M. avium-intracellulare* (MAI) complex (2, 15, 18, 23, 26). Ciprofloxacin, ofloxacin, gatifloxacin, and sparfloxacin are the best studied of these agents to date and are among the most active of this group against NTM (2, 6, 17, 18, 26). Some of them are recommended in the prophylaxis and treatment against NTM disease (12), but many strains of MAI are resistant to fluoroquinolones. Although for ACH-702 is not yet possible to establish breakpoints, all the NTM species tested were inhibited with ≤16 μg/ml, obtaining the best results for *M. fortuitum* isolates with both MIC_{50} and MIC_{90} values of 0.0625 μg/ml. Depending on the pharmacokinetics in humans, these MIC values, particularly those of *M. fortuitum*, raise the possibility that at least some of these NTM may be susceptible *in vivo* as well.

In conclusion, based on *in vitro* data, the ITQ ACH-702 appears to be a promising drug against mycobacterial disease, although it will be important to test it in animal models in order to determine its possible usefulness in the treatment of human infections. We believe that this improved antibacterial activity is due to a more potent inhibition of the mycobacterial gyrase enzyme than that with quinolones, and this hypothesis is supported by recent biochemical assay data (M. Pucci et al., submitted for publication).

REFERENCES

1. **Akcali, S., S. Surucuoglu, C. Cicek, and B. Ozbakkaloglu.** 2005. In vitro activity of ciprofloxacin, ofloxacin and levofloxacin against *Mycobacterium tuberculosis*. Ann. Saudi Med. **25:**409–412.
2. **Alcaide, F., L. Calatayud, M. Santín, and R. Martín.** 2004. Comparative in vitro activities of linezolid, telithromycin, clarithromycin, levofloxacin, moxifloxacin, and four conventional antimycobacterial drugs against *Mycobacterium kansasii*. Antimicrob. Agents Chemother. **48:**4562–4565.
3. **Alvirez-Freites, E. J., J. L. Carter, and M. H. Cynamon.** 2002. In vitro and in vivo activities of gatifloxacin against *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **46:**1022–1025.
4. **Blumberg, H. M., W. J. Burman, R. E. Chaisson, C. L. Daley, S. C. Etkind, L. N. Friedman, P. Fujiwara, M. Grzemska, P. C. Hopewell, M. D. Iseman, R. M. Jasmer, V. Koppaka, R. I. Menzies, R. J. O'Brien, R. R. Reves, L. B. Reichman, P. M. Simone, J. R. Starke, and A. A. Vernon.** 2003. American Thoracic Society/Centers for Dis. Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. Am. J. Respir. Crit. Care Med. **167:**603–662.
5. **Brighty, K. E., and T. D. Gootz.** 2000. Chemistry and mechanism of action of the quinolone antibacterials, p. 34–82. *In* V. T. Andriole (ed.), The quinolones, 3rd ed. Academic, San Diego, CA.
6. **Brown-Elliott, B. A., R. J. Wallace, Jr., C. J. Crist, L. Mann, and R. W. Wilson.** 2002. Comparison of in vitro activities of gatifloxacin and ciprofloxacin against four taxa of rapidly growing mycobacteria. Antimicrob. Agents Chemother. **46:**3283–3285.
7. **Chu, D. T. W., P. B. Fernandes, A. K. Claiborne, L. L. Shen, and A. G. Pernet.** 1989. Structure-activity relationships in quinolone antibacterials: replacement of the 3-carboxylic acid group, p. 37–45. *In* P. B. Fernandes (ed.), Quinolones. J. R. Prous, Barcelona, Spain.
8. **Chu, D. T. W., P. B. Fernandes, A. K. Claiborne, L. Shen, and A. G. Pernet.** 1988. Structure-activity relationships in quinolone antibacterials: design, synthesis and biological activities of novel isothiazoloquinolones. Drugs Exp. Clin. Res. **14:**379–383.
9. **Franzblau, S. G., R. S. Witzig, J. C. McLaughlin, P. Torres, G. Madico, A. Hernandez, M. T. Degnan, M. B. Cook, V. K. Quenzer, R. M. Ferguson, and R. H. Gilman.** 1998. Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. J. Clin. Microbiol. **36:**362–366.
10. **Fung-Tomc, J., B. Minassian, B. Kolek, T. Washo, E. Huczko, and D. Bonner.**

2000. In vitro antibacterial spectrum of a new broad-spectrum 8-methoxy fluoroquinolone, gatifloxacin. J. Antimicrob. Chemother. **45:**437–446.
11. **Gillespie, S. H., and O. Billington.** 1999. Activity of moxifloxacin against mycobacteria. J. Antimicrob. Chemother. **44:**393–395.
12. **Griffith, D. E., T. Aksamit, B. A. Brown-Elliott, A. Catanzaro, C. Daley, F. Gordin, S. M. Holland, R. Horsburgh, G. Huitt, M. F. Iademarco, M. Iseman, K. Olivier, S. Ruoss, C. F. von Reyn, R. J. Wallace, Jr., and K. Winthrop.** 2007. American Thoracic Society. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am. J. Respir. Crit. Care Med. **175:**367–416.
13. **Hashimoto, A., G. C. G. Pais, Q. Wang, E. Lucien, C. D. Incarvito, M. Deshpande, B. J. Bradbury, and J. A. Wiles.** 2007. Practical synthesis and molecular structure of a potent broad-spectrum antibacterial isothiazoloquinolone. Org. Process Res. Dev. **11:**389–398.
14. **Hu, Y., A. R. M. Coates, and D. A. Mitchison.** 2003. Sterilizing activities of fluoroquinolones against rifampin-tolerant populations of *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **47:**653–657.
15. **Jacobs, M. R.** 2004. Fluoroquinolones as chemotherapeutics against mycobacterial infections. Curr. Pharm. Des. **10:**3213–3220.
16. **Ji, B., N. Lounis, C. Maslo, C. Truffot-Pernot, P. Bonnafous, and J. Grosset.** 1998. In vitro and in vivo activities of moxifloxacin and clinafloxacin against *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **42:**2066–2069.
17. **Kohno, Y., H. Ohno, Y. Miyazaki, Y. Higashiyama, K. Yanagihara, Y. Hirakata, K. Fukushima, and S. Kohno.** 2007. In vitro and in vivo activities of novel fluoroquinolones alone and in combination with clarithromycin against clinically isolated *Mycobacterium avium* complex strains in Japan. Antimicrob. Agents Chemother. **51:**4071–4076.
18. **Leysen, D. C., A. Haemers, and S. R. Pattyn.** 1989. Mycobacteria and the new quinolones. Antimicrob. Agents Chemother. **33:**1–5.
19. **Mwinga, A., and F. P. Bernard.** 2004. Prospects for new tuberculosis treatment in Africa. Trop. Med. Int. Health **9:**827–832.
20. **National Committee for Clinical Laboratory Standards.** 2003. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard. NCCLS document M24-A. National Committee for Clinical Laboratory Standards, Wayne, PA.
21. **Pucci, M. J., J. Cheng, S. D. Podos, C. L. Thoma, J. A. Thanassi, D. D. Buechter, G. Mushtaq, G. A. Vigliotti, B. J. Bradbury, and M. Deshpande.** 2007. In vitro and in vivo antibacterial activities of heteroaryl isothiazolones against resistant gram-positive pathogens. Antimicrob. Agents Chemother. **51:**1259–1267.
22. **Rastogi, N., V. Labrousse, and K. S. Goh.** 1996. In vitro activities of fourteen antimicrobial agents against drug susceptible and resistant clinical isolates of *Mycobacterium tuberculosis* and comparative intracellular activities against the virulent H37Rv strain in human macrophages. Curr. Microbiol. **33:**167–175.
23. **Rodriguez Diaz, J. C., M. Lopez, M. Ruiz, and G. Royo.** 2003. In vitro activity of new fluoroquinolones and linezolid against non-tuberculous mycobacteria. Int. J. Antimicrob. Agents **21:**585–588.
24. **Shandil, R. K., R. Jayaram, P. Kaur, S. Gaonkar, B. L. Suresh, B. N. Mahesh, R. Jayashree, V. Nandi, S. Bharath, and V. Balasubramanian.** 2007. Moxifloxacin, ofloxacin, sparfloxacin, and ciprofloxacin against *Mycobacterium tuberculosis*: evaluation of in vitro and pharmacodynamic indices that best predict in vivo efficacy. Antimicrob. Agents Chemother. **51:**576–582.
25. **Telenti, A., F. Marchesi, M. Balz, F. Bally, E. C. Böttger, and T. Bodmer.** 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clin. Microbiol. **31:**175–178.
26. **Tomioka, H., C. Sano, K. Sato, and T. Shimizu.** 2002. Antimicrobial activities of clarithromycin, gatifloxacin and sitafloxacin, in combination with various antimycobacterial drugs against extracellular and intramacrophage *Mycobacterium avium* complex. Int. J. Antimicrob. Agents. **19:**139–145.
27. **Vera-Cabrera, L., M. J. Pucci, C. A. Molina-Torres, P. Rivera, O. Welsh, and J. Ocampo-Candiani.** 2009. In vitro activity of ACH 702 against *Nocardia brasiliensis*, abstr. F1-1974. Abstr. 49th Intersci. Conf. Antimicrob. Agents Chemother.
28. **Wang, Q., E. Lucien, A. Hashimoto, G. C. G. Pais, D. M. Nelson, Y. Song, J. A. Thanassi, C. W. Marlor, C. L. Thoma, J. Cheng, S. D. Podos, Y. Ou, M. Deshpande, M. J. Pucci, D. D. Buechter, B. J. Bradbury, and J. A. Wiles.** 2007. Isothiazoloquinolones with enhanced antistaphylococcal activities against multidrug-resistant strains: effects of structural modifications at the 6-, 7-, and 8-positions. J. Med. Chem. **50:**199–210.
29. **Wiles, J. A., A. Hashimoto, J. A. Thanassi, J. Cheng, C. D. Incarvito, M. Deshpande, M. J. Pucci, and B. J. Bradbury.** 2006. Isothiazolopyridones: synthesis, structure, and biological activity of a new class of antibacterial agents. J. Med. Chem. **49:**39–42.
30. **Wiles, J. A., Q. Wang, E. Lucien, A. Hashimoto, Y. Song, J. Cheng, C. W. Marlor, Y. Ou, S. D. Podos, J. A. Thanassi, C. L. Thoma, M. Deshpande, M. J. Pucci, and B. J. Bradbury.** 2006. Isothiazoloquinolones containing functionalized aromatic hydrocarbons at the 7-position: synthesis and in vitro activity of a series of potent antibacterial agents with diminished cytotoxicity in human cells. Bioorg. Med. Chem. Lett. **16:**1272–1276.
31. **Wiles, J. A., Y. Song, Q. Wang, E. Lucien, A. Hashimoto, J. Cheng, C. W. Marlor, Y. Ou, S. D. Podos, J. A. Thanassi, C. L. Thoma, M. Deshpande, M. J. Pucci, and B. J. Bradbury.** 2006. Biological evaluation of isothiazoloquinolones containing aromatic heterocycles at the 7-position: in vitro activity of a series of potent antibacterial agents that are effective against methicillin-resistant *Staphylococcus aureus*. Bioorg. Med. Chem. Lett. **16:**1277–1281.
32. **World Health Organization.** 2003. Treatment of tuberculosis. Guidelines for national programmes, 3rd ed. World Health Organization, Geneva, Switzerland.
33. **World Health Organization.** 2009. Global tuberculosis control: epidemiology, strategy, financing. WHO report 2009. World Health Organization, Geneva, Switzerland.