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In Vitro Activity of ACH-702, a New Isothiazoloquinolone, against *Nocardia brasiliensis* Compared with Econazole and the Carbapenems Imipenem and Meropenem Alone or in Combination with Clavulanic Acid[▽]

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The *in vitro* activities of ACH-702 and other antimicrobials against 30 *Nocardia brasiliensis* isolates were tested. The MIC_{50} (MIC for 50% of the strains tested) and MIC_{90} values of ACH-702 were 0.125 and 0.5 μg/ml. The same values for econazole were 2 and 4 μg/ml. The MIC_{50} and MIC_{90} values of imipenem and meropenem were 64 and >64 μg/ml and 2 and 8 μg/ml, respectively; the addition of clavulanic acid to the carbapenems had no effect.

Actinomycetoma caused by *Nocardia brasiliensis* is a localized but progressive infectious disease which affects the skin, subcutaneous tissue, and bones (21). Several antimicrobials, including sulfonamides, aminoglycosides, beta-lactams, etc., have been used in the therapy of actinomycetoma (3). However, in some cases a cure is not obtained, making it important to evaluate the *in vitro* and *in vivo* activities of new antimicrobials.

Quinolones have been widely used to treat infections by gram-negative and gram-positive bacteria (14); among them, moxifloxacin and gatifloxacin are active *in vitro* and *in vivo* against *N. brasiliensis* (6, 8, 18). ACH-702, (*R*)-7-[3-(1-amino-1-methyl-ethyl)-pyrrolidin-1-yl]-9-cyclopropyl-6-fluoro-8-methoxy-9H isothiazolo[5,4-b]quinoline-3,4-dione (Fig. 1), is a novel isothiazoloquinolone compound with good antibacterial activity against *Staphylococcus aureus* and enterococci, even against methicillin- and vancomycin-resistant isolates (16, 20). Its high activity against these gram-positive microorganisms raises the possibility that *Nocardia* spp., especially *N. brasiliensis* isolates, would also be susceptible to this new isothiazoloquinolone.

Imidazoles have been traditionally used against eukaryotic organisms since they interfere in the synthesis of ergosterol by inhibiting the cytochrome oxidase group of enzymes (CYP proteins); these enzymes were thought not to exist in prokaryotic organisms. However, sequencing in recent years of the genomes of many prokaryotes, including *Mycobacterium tuberculosis*, has shown the presence of CYP proteins in many of them, prompting testing of the activity of these compounds against species of *Mycobacterium* and *Nocardia*. Some of these compounds were active *in vitro*, particularly econazole (1, 2, 7). However, in one study (7), only one isolate of *N. brasiliensis* isolate was used, exhibiting an MIC of 1 μg/ml, making it necessary to extend the study using more clinical isolates.

Beta-lactams have been used widely to treat gram-positive infections. Because of the presence of potent beta-lactamases in nocardiae, their use against these microorganisms has been very limited (3, 21); amoxicillin-potassium clavulanate acid has been one of the most successfully used compounds to treat human cases of actinomycetoma (5). Imipenem has been used in clinical cases of *N. brasiliensis* infection in combination with other drugs, with good results observed (10). More recently, it has been observed that some carbapenems, such as imipenem and meropenem, are active against *M. tuberculosis* isolates, but only when combined with a beta-lactamase inhibitor, clavulanic acid (12).

In this work, we report the susceptibility of *N. brasiliensis* isolates to ACH-702, as well as other potentially active compounds, including econazole and carbapenems (imipenem and meropenem), alone or combined with clavulanic acid.

We studied 30 isolates from the collection of the Laboratorio Interdisciplinario de Investigación Dermatológica of the Servicio de Dermatología, Hospital Universitario, Universitario de Nuevo León, including *N. brasiliensis* HUJEG-1, which was utilized previously in other *in vitro* and *in vivo* assays (11, 17). All of the isolates tested came from human cases of actinomycetoma and were identified as *N. brasiliensis* by biochem-

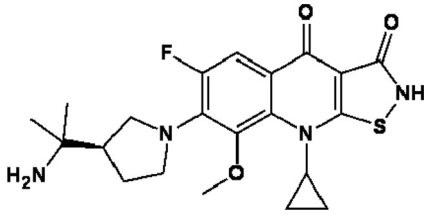

FIG. 1. Chemical structure of ACH-702.

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TABLE 1. *In vitro* activities of ACH-702 and other antimicrobials against *N. brasiliensis* isolated from human cases of actinomycetoma

Antimicrobial agent(s)	MIC (μg/ml)		
	Range	50% of strains tested	90% of strains tested
ACH-702	0.03–2	0.125	0.5
Econazole	1–8	2	4
Imipenem	1–>64	64	>64
Imipenem + clavulanic acid	1–>64	64	>64
Meropenem	1–>64	2	8
Meropenem + clavulanic acid	1–>64	2	8

ical methods and by nucleotide sequence analysis of a fragment of the 16S RNA gene of the small ribosomal unit as previously described (17).

ACH-702 was kindly donated by Achillion Pharmaceuticals, Inc., New Haven, CT. Econazole and clavulanic acid were purchased from Sigma Chemical Co. (St. Louis, Mo.). Imipenem and meropenem were obtained from commercial sources.

The broth microdilution method used was based on the CLSI M24-A document (15) and has been previously described (18). Briefly, ground colonies were suspended in 1 ml of saline solution and diluted with cation-adjusted Mueller-Hinton broth until the turbidity matched that of a 0.5 McFarland standard. This suspension was diluted to obtain a solution with a final concentration of 1×10^4 to 5×10^4 CFU per well in 0.1 ml. This solution then was added to microplate wells (Microtest Primaria; Becton Dickinson and Co., Franklin Lakes, NJ) containing an equal volume of broth with serial dilutions of the drugs to be tested. As a growth control, we similarly inoculated a well containing cation-adjusted Mueller-Hinton broth without drug. After 3 days of incubation at 35°C, the plates were read and the MIC was determined as the lowest concentration of drug that totally inhibited nocardial growth. As external controls, we used *Escherichia coli* ATCC 25922 and *S. aureus* ATCC 29213. Econazole, imipenem, and meropenem were tested at concentrations of 64 to 0.25 μg/ml. The lowest concentration of ACH-702 used was 0.03 μg/ml. To test the effect of the beta-lactamase inhibitor on carbapenem activity, 0.25 μg/ml clavulanic acid was added to all of the carbapenems (12).

The MICs of ACH-702 and the other antimicrobial agents tested for the 30 clinical isolates of *N. brasiliensis* tested are summarized in Table 1. The MIC_{50} (MIC for 50% of the strains tested) and MIC_{90} values for ACH-702 were 0.125 and 0.5 μg/ml. The same values for econazole were 2 and 4 μg/ml. The MIC_{50} and MIC_{90} values for imipenem were 64 and >64 μg/ml, respectively. Only seven isolates had an MIC of <2 μg/ml. For meropenem, the values were 2 and 8 μg/ml. For this compound, 16 out of 30 isolates tested exhibited an MIC value of ≤2 μg/ml. The addition of clavulanic acid to the carbapenems did not change the MIC values significantly.

Although imidazoles have been traditionally used against eukaryotic organisms, recently it has been observed that actinobacteria, including mycobacteria and nocardiae, are also susceptible to this class of antimicrobials (2, 7). In our work, we observed that most (90%) of the isolates exhibited an MIC of ≤2 μg/ml for econazole. In mice, econazole given orally at 3.3 mg/kg, is rapidly metabolized and achieves plasma concentrations (maximum concentration of drug in plasma, 0.23 μg/ml; time to maximum concentration of drug in plasma, 1 h) below this MIC value (2). Given these parameters, we believe that econazole will not work effectively in the experimental mouse model. However, new imidazole compounds are being launched in the market and it is possible that in the future more potent drugs or other delivery methods would open up the possibility of therapy of actinomycetoma caused by *N. brasiliensis* using this class of antimicrobials.

Beta-lactams have been of limited use in the treatment of *N. brasiliensis* infections. Recently, it has been reported that carbapenems plus clavulanic acid have shown some activity against *M. tuberculosis* isolates (12). In our work, we did not find any difference when clavulanic acid was added, with MIC_{50} and MIC_{90} values remaining the same. At best, imipenem or meropenem can be effective in human patients with isolates exhibiting MIC values of ≤2 μg/ml, since the levels of these drugs remain over 2 μg/ml for 3 to 4 h in humans after the intravenous administration of 1,000 mg (9). Given the observed differences in susceptibility among clinical isolates, it would be important to perform susceptibility tests prior to the initiation of therapy with these agents. Also, it will be important to assess the antibacterial activity of future beta-lactams that have the desirable advantage of oral administration for the treatment of *Nocardia* infections.

Quinolones, including ofloxacin, ciprofloxacin, and moxifloxacin, have been used in the therapy in infections caused by *Nocardia* spp. (4, 13, 22). However *N. brasiliensis* is a more resistant species and only newer compounds such as gatifloxacin, garenoxacin, and moxifloxacin have shown excellent *in vitro* and *in vivo* effects against this microorganism (3, 6, 8, 18). Of these quinolones, only moxifloxacin is available on the market, but its use has not been reported for actinomycetoma patients. ACH-702 showed MIC_{50} and MIC_{90} values (0.125 and 0.5 μg/ml) lower than those of gatifloxacin (0.5 and 2 μg/ml) and moxifloxacin (0.5 and 2 μg/ml) (18), which suggests that this may be a promising compound to treat *N. brasiliensis* infections, although it will be important to test its intracellular (19) and *in vivo* activities against this bacterium.

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