Pyrazinamide (PZA) is a first-line agent for the treatment of tuberculosis (1, 4) and an essential element of experimental preventive therapy regimens (6, 9). PZA appears to function as a prodrug of pyrazinic acid (PA) and is converted to PA intracellularly. The biochemical basis for the antituberculous activity of PA has not been established (7).

It is known that the majority of Mycobacterium tuberculosis isolates resistant to PZA in vitro have low levels of pyrazinamidase activity, as do Mycobacterium bovis isolates (8, 10–12). PZA-susceptible and -resistant isolates are generally susceptible to PA in vitro, but PA is not active in vivo (5). A series of esters of PA and 5-substituted PA have been found to have enhanced in vitro activity against both PZA-susceptible and -resistant M. tuberculosis as well as against PZA-resistant M. bovis, Mycobacterium kansasii, and Mycobacterium avium isolates (2, 3). The aim of this study was to evaluate the in vitro activity of 5-chloro-PZA (5-Cl PZA) and 5-Cl PA against various mycobacterial isolates, including PZA-resistant M. tuberculosis.

PZA was obtained from Sigma Chemical Company, St. Louis, Mo. PA was obtained from Aldrich Chemical Company, Milwaukee, Wis. 5-Cl PZA and 5-Cl PA were synthesized from 5-chloropyrazinyl chloride. 5-Cl PZA was obtained as follows: to 30 ml of NH4OH, 3.55 g (20 mmol) of 5-chloropyrazinyl chloride in 25 ml of dry tetrahydrofuran was added at 0°C over a 30-min period. After the addition was complete, the reaction mixture was stirred for another 30 min. The reaction mixture was diluted with 30 ml of ether, and the precipitated was filtered. The filtrate was washed with 30 ml of ether, and the filtrate was separated. The aqueous layer was extracted twice with 20 ml of ether each time, and the combined organic layer was separated. The aqueous layer was extracted twice with 20 ml of ether each time, and the combined organic layer was adjusted to pH 5.8 with 1 N KOH prior to sterilization. Serial twofold dilutions of each compound were made in modified 7H10 broth (concentrations ranged from 2.048 to 0.5 μg/ml).

Strains of M. tuberculosis (ATCC 27294, ATCC 35801, and ATCC 35828), M. bovis (ATCC 35720 and ATCC 27289), Mycobacterium smegmatis (ATCC 19420), and Mycobacterium fortuitum (ATCC 49403) were obtained from the American Type Culture Collection, Rockville, Md. Isolates of PZA-resistant M. tuberculosis were kindly provided by Salman Siddiqi (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.). M. avium strain 101 (serotype 1) was provided by Lowell Young (Kuzell Institute for Arthritis and Infectious Diseases, California Pacific Medical Center Research Institute, San Francisco, Calif.). M. avium ATCC 49601 (serotype 1) is a clinical isolate from a patient with AIDS at State University of New York Health Science Center, Syracuse, N.Y. M. kansasii strain S was a clinical isolate from a patient at the Veterans Affairs Medical Center, Syracuse, N.Y.

Mycobacteria were grown in modified 7H10 broth, pH 6.6, with 10% OADC enrichment and 0.05% Tween 80 (13). Cell suspensions were diluted in modified 7H10 broth, pH 5.8, to yield 1 Klett unit of M. tuberculosis, M. bovis, and M. smegmatis per ml and 0.1 Klett unit of M. avium, M. kansasii, and M. fortuitum per ml (Klett-Summerson colorimeter; Klett Manufacturing, Brooklyn, N.Y.) or approximately 5 x 10^4 CFU/ml. A 0.1-ml volume of culture suspension was added to each tube containing drug in 1.9 ml of modified 7H10 broth, pH 5.8, yielding a final inoculum of approximately 2.5 x 10^4 CFU/ml.

Susceptibility testing was performed with modified 7H10 broth, pH 5.8, because some isolates of M. tuberculosis grow poorly at pH 5.6, the standard pH used for susceptibility testing in agar. Inoculum size was determined by titration and counting from duplicate 7H10 agar plates (BBL Microbiology Systems, Cockeysville, Md.). A tube without drug was included for each isolate as a positive control. Tubes were incubated on a rotary shaker (190 rpm) at 37°C for 24 h to 2 weeks. The MIC was defined as the lowest concentration of drug that yielded no visible turbidity.

The broth dilution MICs of PZA, 5-Cl PZA, PA, and 5-Cl PA for the M. tuberculosis isolates (n = 7) are shown in Table 1. The MIC ranges of PZA and 5-Cl PZA were from 32 to >2,048 μg/ml and from 8 to 32 μg/ml, respectively. The MIC ranges of PA and 5-Cl PA were from 16 to 64 μg/ml and from 8 to 32 μg/ml, respectively. The MICs of 5-Cl PZA and PA for M. tuberculosis are more favorable than those of PZA and 5-Cl PA. PZA-resistant isolates retain susceptibility in vitro to 5-Cl PZA, PA, and 5-Cl PA, suggesting that 5-Cl PZA can circumvent the requirement for activation by mycobacte-
The MICs of pyrazinamide analogs for various mycobacteria are lower than those of 5-Cl PA, PZA, or PA. The activity against the poor activity of 5-Cl PA is significantly less effective than PA against the same organisms.

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REFERENCES


