In Vitro Activities of Cloxyquin (5-Chloroquinolin-8-ol) against 
*Mycobacterium tuberculosis*\

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The in vitro activities of cloxyquin (5-chloroquinolin-8-ol) against 150 clinical isolates of *Mycobacterium tuberculosis* were studied. The MICs ranged from 0.062 to 0.25 μg/ml. The MIC50 and MIC90 were 0.125 and 0.25 μg/ml, respectively. These indicate that cloxyquin exhibited good antituberculosis activity, even for multidrug-resistant isolates.

Current first-line drugs for treatment of tuberculosis consist of only 5 agents, i.e., isoniazid (INH), rifampin (RIF), ethambutol (EMB), pyrazinamide (PZA), and streptomycin (STR). Resistance to the first-line drugs, especially RIF and INH, usually causes treatment failure and necessitates the use of the second-line drugs with a prolonged period of therapy. Even with that, treatment frequently fails. New antituberculous agents, especially the ones with novel mechanisms of action are urgently required.

Bihalogenated 8-hydroxyquinolines (quinolin-8-ols) are a group of known drugs with antiamebic activities and were widely used to treat intestinal infection. The commonly used ones include broxyquinoline, clioquinol chlorquinaldol, and iodoquinol (4, 6). They also exhibit antibacterial and antifungal activities (1, 14).

Herewith, we report the antituberculosis activities of a monohalogenated 8-hydroxyquinoline, cloxyquin (5-chloroquinolin-8-ol), against 150 clinical *Mycobacterium tuberculosis* isolates, including multidrug-resistant strains. Cloxyquin (Fig. 1) was known to possess activities against bacteria, fungi, and protozoa (3, 10, 11, 12), but the antimycobacterial activity has never been documented.

A total of 159 strains of *M. tuberculosis*, including 9 reference strains (H37Rv ATCC 27294, H37Ra ATCC 25177, H37Rv-PAS-R ATCC 35821 [p-aminosalicylic acid resistant], H37Rv-CS-R ATCC 35826 [cycloserine resistant], H37Rv-KM-R ATCC 35827 [kanamycin resistant], H37Rv-PZA-R ATCC 35828 [pyrazinamide resistant], H37Rv-TAC-R ATCC 35829 [thiacetazone resistant], H37Rv-ETA-R ATCC 35830 [ethionamide resistant], and H37Rv-EMB-R ATCC 35837 [ethambutol resistant]) and 150 isolates from pulmonary and extrapulmonary patients in Ramathibodi Hospital, Bangkok, Thailand, including 100 sensitive strains, 20 drug-resistant strains (7, 3, 3, and 12 isolates resistant to INH, RIF, EMB, and STR, respectively), and 30 multidrug-resistant (MDR) strains (7 isolates resistant to INH and RIF, 3 isolates additionally resistant to EMB, 13 isolates additionally resistant to STR, and 7 isolates additionally resistant to EMB and STR), were investigated. The MICs of cloxyquin (Sigma Chemical Co., St. Louis, MO) for all *M. tuberculosis* isolates were determined duplicated by microplate Alamar blue assay (8), which has been showed to correlate well (>90%) with the BACTEC and proportional methods (2, 8, 16, 19). Briefly, cloxyquin was prepared in dimethyl sulfoxide (Sigma) and subsequently diluted twofold in 100 μl of Middlebrook 7H9GC in clear flat-bottom, 96-well microplates. A mycobacterial suspension was prepared in 0.04% Tween 80 and diluted with sterile distilled water to a turbidity of the McFarland no. 1. The suspension was then diluted 1:50 with 7H9GC, and 100 μl was added to the wells. The highest final concentration of dimethyl sulfoxide was 0.156% (vol/vol). The plates were incubated at 37°C for 7 days; 12.5 μl of 20% Tween 80 and 20 μl of Alamar blue (SeroTec Ltd., Oxford, United Kingdom) were added to all wells. Growth of the organisms was determined after reincubation at 37°C for 16 to 24 h by visual determination of a color change from blue to pink. The MIC was defined as the lowest concentration which prevented the color change. RIF and INH (Sigma) were included as controls.

The MICs of 8-hydroxyquinoline, cloxyquin, clioquinol, chlorquinaldol, and broxyquinoline against *M. tuberculosis* H37Ra were 0.125, 0.125, 6.25, 0.38, and 6.25 μg/ml, respectively. This suggested that 8-hydroxyquinoline and its derivatives are fairly active against *M. tuberculosis*. To elucidate more on their potentials, MICs of cloxyquin were further studied. The MICs of cloxyquin for the 9 reference strains ranged from

![FIG. 1. Chemical structure of cloxyquin.](http://aac.asm.org/)

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from established, as environmental factors, such as B12 deficiency, are also likely to be important. Nevertheless, recently, the interest in clioquinol has been increased due to its favorable effects on Alzheimer’s disease (5, 7). In conclusion, the excellent in vitro activity (even for MDR tuberculosis) of clioquinol against \textit{M. tuberculosis} deserves further investigation.

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### REFERENCES


### TABLE 1. MICs of cloxyquin for clinical isolates of \textit{M. tuberculosis}a

<table>
<thead>
<tr>
<th>\textit{M. tuberculosis} strain type (n)</th>
<th>No. (%) of isolates for which MIC (g/ml) is:</th>
<th>MICso (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.062</td>
<td>0.125</td>
</tr>
<tr>
<td>Drug sensitive (100)</td>
<td>15 (15)</td>
<td>74 (74)</td>
</tr>
<tr>
<td>Drug resistant (20)</td>
<td>2 (10)</td>
<td>13 (65)</td>
</tr>
<tr>
<td>MDR (30)</td>
<td>5 (16.7)</td>
<td>24 (80)</td>
</tr>
<tr>
<td>Total (150)</td>
<td>22 (14.7)</td>
<td>111 (74)</td>
</tr>
</tbody>
</table>

a MICso are 0.125 g/ml for all groups.