5-Nitroimidazole Drugs Effective against Metronidazole-Resistant
Trichomonas vaginalis and Giardia duodenalis

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Metronidazole (Mz)-resistant Giardia and Trichomonas were inhibited by 1 of 30 new 5-nitroimidazole drugs. Another five drugs were effective against some but not all of the Mz-resistant parasites. This study provides the incentive for the continued design of 5-nitroimidazole drugs to bypass cross-resistance among established 5-nitroimidazole antiparasitic drugs.

Metronidazole (Mz) and a related 5-nitroimidazole, tinidazole, are the only drugs recommended for the treatment of trichomoniasis and are the most-prescribed drugs for the treatment of giardiasis. However, clinical resistance to these drugs has been well documented; and in the event of overt clinical resistance to Mz in trichomonads, there is no alternative for treatment, when one keeps in mind the documented cross-resistance between the currently used 5-nitroimidazole drugs and their worldwide availability (7, 8, 18, 23). Some success has been obtained with quinacrine and albendazole in combination (4, 11, 18). Thus, Mz is not activated to its toxic radical sufficiently low to activate Mz, while no such electron couple is present in the mammalian host (9). In the laboratory we see a threefold down-regulation of PFOR activity in Mz-resistant (Mz') Giardia duodenalis (14), and in highly Mz' Trichomonas vaginalis the activity of the hydrogenosome organelle is down-regulated such that there is no detectable PFOR orFd expression (4, 11, 18). Thus, Mz is not activated to its toxic radical state in these cells. On the other hand, it is well documented that clinically Mz' T. vaginalis strains do not have down-regulated hydrogenosomes, and the mechanism of Mz resistance in these cells is not understood (8).

Previously, we showed that some 5-nitroimidazole derivatives were significantly more effective antiprotozoal agents (based on in vitro molar drug concentrations) than Mz against Mz-susceptible (Mz") parasites but were not as effective against Mz' parasites (17). Given the impetus for the development of 5-nitroimidazole drugs that vary markedly in their efficacies (both positively and negatively), we tested 30 new 5-nitroimidazoles in our anaerobic drug susceptibility screening assay (16) for their efficacies against T. vaginalis and G. duodenalis, with the focus on laboratory-derived Mz' (Mz") lines and clinical isolates derived from patients with treatment failures.

Parasites were cultured axenically in anaerobic TYI-S-33 (6), which was modified as described previously (16). Mz" G. duodenalis isolates WB-1B, BRIS/87/HEPU/713 (713), and BRIS/83/HEPU/106 (106) were tested along with their respective Mz' lines WB-M3, 713-M3, and 106-2ID10 (13, 16). Mz" T. vaginalis isolate BRIS/92/HEPU/F1623 (F1623) and the highly Mz" line derived from it, F1623-M1 (16), were used throughout. BRIS/92/HEPU/7268 (B7268) (16) and DUR/03/FMUN/36 (DUR36) were the T. vaginalis Mz' clinical (Mz") isolates used. Breakpoints for susceptibility versus resistance to Mz were previously described as an MIC of 3.2 μM (with a maximum of 6.3 μM and minimum of 1.6 μM) for Mz" T. vaginalis and 6.3 μM (with a minimum of 3.2 μM and maximum of 12.5 μM in a few cases) for Mz" G. duodenalis (16). The minimum MIC among all assays for Mz" parasites was 25 μM (16). For the purposes of screening new 5-nitroimidazoles, we have directly compared their MICs with those for Mz.

All new 5-nitroimidazole compounds (Fig. 1) were identified by spectral data, purified by chromatography on silica gel columns, and recrystallized from appropriate solvents. Their purities were checked with appropriate controls by thin-layer chromatography and elemental analysis (C, H, N). The purity was always over 99.6%. The synthesis of compounds 1 to 9, 10 to 16, 17 to 19, 20 to 23, and 24 to 28 were as described in references 2, 1, 20, 21, and 22, respectively. The reaction of compound 28 with 2-nitropropane or nitrocyclohexane anion led to the C-alkylation products 29 and 30, respectively, by the SRN1 mechanism.

Of the 30 compounds tested, compounds 11, 12, 13, 24, 26, and 30 demonstrated MICs against all three Mz" G. duodenalis isolates of ≥100 μM (Mz MIC ≤ 10 μM) (data not shown).
FIG. 1. Structures of the 5-nitroimidazole drugs used in this study compared with that of Mz. MW, molecular weight.
TABLE 1. Comparison of MICs of the six most effective new 5-nitroimidazole drugs against $M_z^G$, $G. duodenalis$ and $T. vaginalis$ lines and isolates

<table>
<thead>
<tr>
<th>Drug</th>
<th>$M_z^G$ Giardia</th>
<th>Trichomonas</th>
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<tr>
<td></td>
<td>WB-M3</td>
<td>713-M3</td>
</tr>
<tr>
<td>Mz</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>1</td>
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<td>8</td>
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<td>7</td>
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<td>3</td>
<td>3.1</td>
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*a* drugs are listed in the order of their efficacies.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (µM)</th>
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<tr>
<td>14</td>
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<tr>
<td>18</td>
<td>1</td>
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<td>25</td>
<td>5</td>
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$M_z$ Giardia WB-M3 and 713-M3 (MICs ≥ 50 µM for one or both lines) (data not shown). Compounds 3, 7, 8, 14, 17, and 18 were ≥10-fold more inhibitory than Mz against all $M_z^d$ Giardia (Table 1). In addition, compound 14 had the same MICs of 1 µM (Table 1 and data not shown) against both Mz and $M_z^d$ Giardia parasites. Of these six most effective antigiardial drugs, compound 14 was 16- to 100-fold more inhibitory than Mz against all $M_z^d$ parasites (Table 1); compounds 17 and 18 were effective against $M_z^e$ T. vaginalis isolates (fivefold or greater more inhibitory than Mz) but not against $M_z^d$ T. vaginalis (MICs = 50 and 25 µM, respectively) (Table 1); and compounds 3, 7, and 8 were effective only against $T. vaginalis$ $M_z^e$ isolate DUR36 (10- to 16-fold more inhibitory than Mz) (Table 1). The significance of the data presented in Table 1 is the low in vitro MICs for six 5-nitroimidazole compounds against some or all of the $M_z^e$ parasites tested. No other 5-nitroimidazole compounds tested so far were inhibitory to the Mz parasites used in this study.

The most effective drug overall, compound 14, differs in structure from compound 16 (Fig. 1) by the remote substituents of the side chain attached at the 2 position on the nitroimidazole ring, notably, a nitro group in compound 14 but not in compound 16, which showed no inhibition of two $M_z^G$ G. duodenalis isolates (MICs = 100 µM). Other compounds with remote nitro groups (compounds 20, 21, 22, 23, 29, and 30; Fig. 1) did not inhibit Mz parasites (MICs ≥ 100 µM). We also tested a number of quinoxaline and 3,4-diphenylfuran derivatives with and without nitro groups (unpublished synthesis data) and $p$-nitrobenzyl derivatives (3), none of which were effective antiprotozoal agents (data not shown).

Of the highly effective antitrichomonal 5-nitroimidazoles, compound 17 was selected for extended studies, due to its inhibition of $M_z^e$ T. vaginalis (Table 1) and its ease of preparation. We measured the anaerobic MICs (16) of compound 17 and Mz against 31 T. vaginalis clinical isolates collected in South Africa. All Mz isolates were susceptible to compound 17, with at least fourfold lower MICs than that of Mz (data not shown). Four isolates demonstrated some level of Mz resistance (MICs for Mz of 12.5, 12.5, 50, and 100 µM, respectively) but had MICs with compound 17 of 1.6, 3.1, 6.3, and 6.3 µM, respectively. The most highly Mz isolate was DUR36 (Table 1). Compounds 14, 17, and 18 therefore provide precedence for the design of new 5-nitroimidazole antitrichomonal drugs. It has been claimed that the eradication of trichomoniasis may well be the single most cost-effective step in human immunodeficiency virus infection incidence reduction (5, 10, 12), and for this reason the development of drugs for the treatment of cases of $M_z^e$ T. vaginalis has been a focus of our work (8, 16).

Previously, we reported that among a 5-nitroimidazole series of compounds, the most antimicrobial and antiparasitic compounds showed a greater resonance conjugation in the molecular structure (17, 19). For this study, we have synthesized under mild conditions and in good yields highly conjugated 5-nitroimidazole derivatives using electron transfer reactions ($SN_2$, $LD-SNR_1$, bis-$SNR_1$, and $ER_1$ [1, 2, 20, 21, 22]). By increasing the conjugated system, we have developed highly active compounds, especially the C-alkylation products obtained by LD-$SNR_1$, compounds 8 and 14. The present study again demonstrates the importance of the 5-nitroimidazole side chain in antiparasitic activity (e.g., compounds 3 and 7 are $E$ and $Z$ isomers, and the differences between the antiprotozoal activities of compounds 14 and 16 and between compounds 18 and 19). These dramatic differences may relate to solubility and membrane permeability. The positive influence of bromine (compound 17), a sulfonyl group (compounds 3 and 7), or a dioxole nucleus (compound 18) were noted, but deprotection of the remote dioxole ring in compound 18 resulted in the formation of the diphenol compound 19, which was detrimental to protozoocidal activity.

These data suggest that the 5-nitro group is highly active in compound 14, but it does not explain why this drug is so active against $M_z^G$ T. vaginalis, which supposedly does not have the ability to reduce Mz (4). We have previously identified alternative 2 oxoacid oxidoreductase (OR) activity in $M_z^G$ T. vaginalis (4), and it is possible that these alternative ORs are responsible for the activation of compound 14 to its toxic radical state.

There are examples in the literature of patients who have failed to respond to all antiangiardial or antitrichomonal treatments (7, 23). Here we have shown that at least three different 5-nitroimidazole compounds, including one that is readily synthesized, inhibited highly $M_z^G$ G. duodenalis and $M_z^e$ T. vaginalis isolates. The clinical application of such drugs may well overcome the documented cross-resistance among the 5-nitroimidazole drugs, and the lead compounds described here provide the basis from which new 5-nitroimidazole drugs clinically active against $M_z$ Trichomonas and Giardia can be derived.
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REFERENCES

15. Reference deleted.