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 with Mz in cases of giardiasis treatment failures (23). On the positive side, a great deal of flexibility is offered by the side chains attached to the imidazole ring structure that bear the all important nitro group (17).

The mechanisms of Mz resistance in *Giardia* and *Trichomonas* have been well studied in laboratory-induced resistance (18). It occurs by down-regulation of pathways, especially the enzyme pyruvate:ferredoxin oxidoreductase (PFOR) and ferredoxin (Fd) pathway, that activate Mz to its toxic radical state. The PFOR-Fd couple has an electron potential 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\(^r\)) *Giardia duodenalis* (14), and in highly Mz\(^r\) *Trichomonas vaginalis* the activity of the hydrogenosome organelle is down-regulated such that there is no detectable PFOR or Fd 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\(^r\) *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\(^s\)) parasites but were not as effective against Mz\(^r\) 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\(^r\) (Mz\(^r\)) 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\(^r\) *G. duodenalis* isolates WB-1B, BRIS/87/HEPU/713 (713), and BRIS/83/HEPU/106 (106) were tested along with their respective Mz\(^s\) lines WB-M3, 713-M3, and 106-2ID\(^{10}\) (13, 16). Mz\(^r\) *T. vaginalis* isolate BRIS/92/HEPU/F1623 (F1623) and the highly Mz\(^r\) line derived from it, F1623-M1 (16), were used throughout. BRIS/92/HEPU/T7268 (B7268) (16) and DUR/03/FMUN/36 (DUR36) were the *T. vaginalis* Mz\(^r\) clinical (Mz\(^r\)) 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\(^r\) *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\(^r\) *G. duodenalis* (16). The minimum MIC among all assays for Mz\(^r\) 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 SR\(_{N1}\) mechanism.

Of the 30 compounds tested, compounds 11, 12, 13, 24, 26, and 30 demonstrated MICs against all three Mz\(^r\) *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.
Compartments 6, 10, 15, 16, 19, 20, 21, 22, 23, 28, and 29 had higher MICs than Mz against one or more of the Mz \textsuperscript{r} isolates tested (data not shown). As a result, these drugs were not considered further, except where stated below.

Compounds 1, 2, 3, 4, 5, 7, 8, 9, 14, 17, 18, 25, and 27 demonstrated MICs equal to or less than that of Mz (≤10 μM) for all Mz \textsuperscript{r} Giardia and Trichomonas isolates tested and were subsequently assessed against all the Mz \textsuperscript{d} and Mz \textsuperscript{e} parasites described above. Of these compounds, compounds 1, 2, 4, 5, 9, 14, 17, 18, and 27 were similarly as ineffective as Mz against Mz \textsuperscript{d} 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 Mz \textsuperscript{d} Giardia (Table 1). In addition, compound 14 had the same MICs of 1 μM (Table 1 and data not shown) against both Mz \textsuperscript{r} and Mz \textsuperscript{d} Giardia parasites. Of these six most effective antigiardial drugs, compound 14 was 16- to 100-fold more inhibitory than Mz against all Mz \textsuperscript{r} parasites (Table 1); compounds 17 and 18 were effective against Mz \textsuperscript{e} T. vaginalis isolates (fivefold or greater more inhibitory than Mz) but not against Mz \textsuperscript{d} T. vaginalis (MICs = 50 and 25 μM, respectively) (Table 1); and compounds 3, 7, and 8 were effective only against T. vaginalis Mz \textsuperscript{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 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 \textsuperscript{r} 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 Mz \textsuperscript{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 (Si\textsubscript{N}H\textsubscript{2}, LD-Si\textsubscript{N}H\textsubscript{1}, bis-Si\textsubscript{N}H\textsubscript{1}, and E\textsubscript{H}C\textsubscript{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-Si\textsubscript{N}H\textsubscript{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 antiprotzoal 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 Mz \textsuperscript{d} T. vaginalis, which supposedly does not have the ability to reduce Mz (4). We have previously identified alternative 2 oxoacid oxidoreductase (OR) activity in Mz \textsuperscript{d} 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 antigiardial 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 Mz \textsuperscript{d} G. duodenalis and Mz \textsuperscript{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 Mz \textsuperscript{e} T. vaginalis and Giardia can be derived.

\begin{table}
\centering
\caption{Comparison of MICs of the six most effective new 5-nitroimidazole compounds against Mz, G. duodenalis and T. vaginalis lines and isolates.}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\textbf{Drug} & \textbf{MIC (μM)} & \textbf{Trichomonas} & & & & & \\
& & & \textbf{Mz} & \textbf{Giardia} & \textbf{Mz} & \textbf{Trichomonas} & \\
& & & \textbf{Mz\textsuperscript{d} F1623-M\textsuperscript{1}} & \textbf{B7268} & \textbf{Mz\textsuperscript{e} DUR36} & & \\
\hline
Mz & 50 & 100 & 50 & 50 & 50 & 100 & \\
14 & 1 & 1 & 1 & 1 & 3.1 & 1.6 & \\
18 & 1 & 0.5 & 1 & 25 & 6.3 & 6.3 & \\
17 & 5 & 10 & 5 & 50 & 10 & 6.3 & \\
8 & 3.1 & 5 & ND & 50 & 50 & 6.3 & \\
7 & 3.1 & 5 & ND & 50 & 50 & 6.3 & \\
3 & 3.1 & 1 & ND & 50 & 50 & 10 & \\
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REFERENCES

15. Reference deleted.