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Potentiating Metronidazole Scaffold against Resistant Trichomonas: Design, Synthesis, Biology and 3D−QSAR Analysis†

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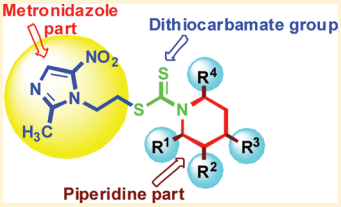
Supporting Information

ABSTRACT: Metronidazole (MTZ), the FDA-approved drug against *Trichomonas vaginalis* (TV), is being challenged seriously by drug resistance, while its inertness to sperm makes it ineffective as a vaginal contraceptive. Thirteen piperidine dithiocarbamate hybrids of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethane (**8**−**20**) were designed to potentiate the MTZ framework against drug resistance and sperm. New compounds were 1.2−12.1 times more effective against MTZ-susceptible and -resistant strains of TV. All of the compounds exhibited high safety toward cervical (HeLa) cells and *Lactobacillus*. Thirty-eight compounds were scrutinized by CoMFA and CoMSIA techniques of 3D quantitative structure−activity relationship. Good predictive r_{pred}^2 values for CoMFA and CoMSIA models reflected the robustness of the predictive ability. This was validated by designing five new analogues (**46**−**50**), which were potently microbicidal (3−10 and 10−20 times against MTZ-susceptible and -resistant TV, respectively) and spermicidal. This in vitro study may have significant clinical relevance, which could become evident in due course.



KEYWORDS: *dithiocarbamate, metronidazole, piperidine, antitrichomonas, microbicidal, contraception*

Diseases like sexually transmitted infections (STIs)[1] are insidious and diabolic and a global urgency[2] that cannot be overlooked. Each year, 340 million new cases of curable STIs are reported, among which trichomoniasis has the highest incidence, and the infected populations are at an elevated risk of contracting viral sexually transmitted diseases (STDs) including acquired immunodeficiency syndrome (AIDS).[3] On the other hand, of 208 million pregnancies that occurred globally in 2008, ∼41% were unintended,[4] which indicates that contraception remains an unmet need for many. As a direct consequence of this, ∼46 million abortions are performed annually, mostly in developing countries under unsafe conditions that result in the death of ∼70 000 women each year.[5] MTZ, the FDA-approved drug[6] against trichomoniasis, is nonspermicidal and hence cannot offer pregnancy protection when used vaginally. On the other hand, the emergence of resistance against MTZ has made the situation grimmer. It would be highly desirable to arrest *Trichomonas* along with sperm in the vagina as soon as it is transmitted in semen; therefore, there is a need to develop topically active medication against sperm and *Trichomonas vaginalis* (TV) (MTZ also has a poor cure rate when used vaginally[7]). The hybridization of two active ligands leading to a novel class of molecules with a better biological activity profile has been an approach in medicinal chemistry[8,9] whereby a single chemical entity can be made to modulate multiple targets simultaneously.[10] Thus, it was thought worthwhile to modify the structure of MTZ (**1**, Figure 1) without altering the orientation of its active sites (e.g., the 5-nitro group, which on being reduced to a nitro radical within the TV parasite exhibits trichomonacidal activity[11]). The antimicrobial activity[12] of dithiocarbamates (**2**, Figure 1) is well documented, and its incorporation into other chemical moieties has resulted in compounds with appreciable antitrichomonas activity (ATA).[13−15] On the other hand, the metabolism[11] of MTZ into 1-acetic acid metabolite results in loss of activity.

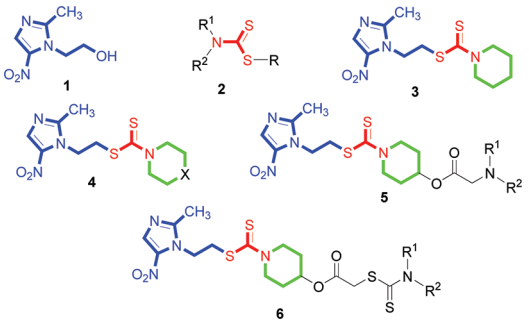


Figure 1. Structures of MTZ (**1**), dithiocarbamates (**2**), lead molecule of previous work (**3**), and general structures of the synthesized compounds (**4**−**6**).

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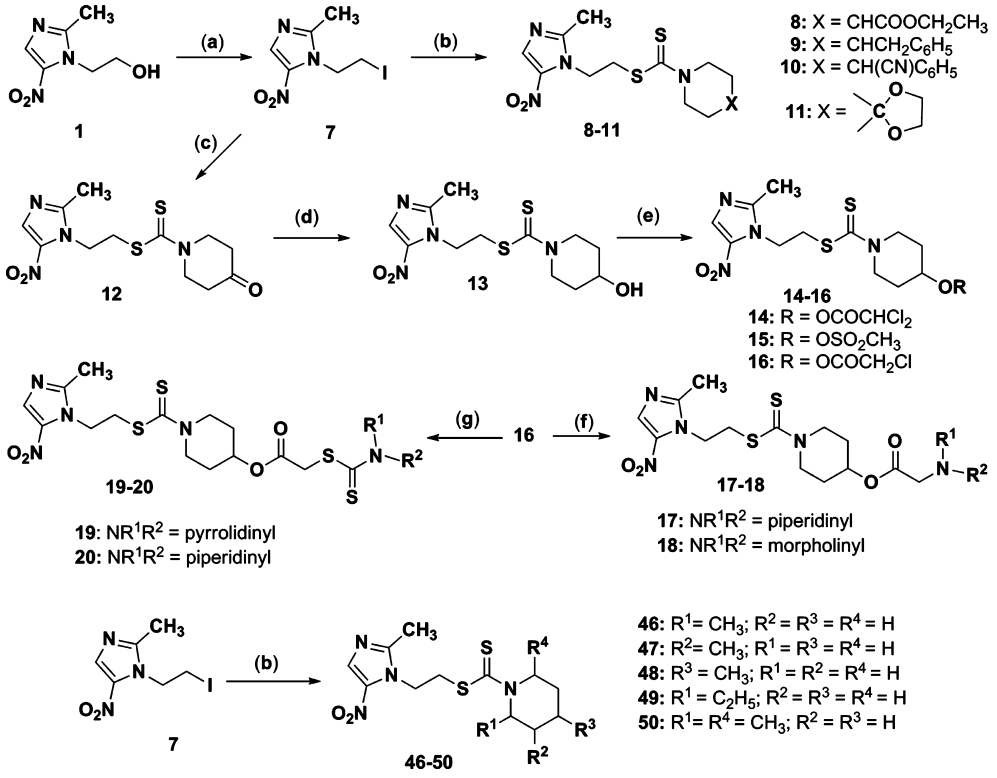
Scheme 1[a]



[a]Reagents and conditions: (a) Imidazole, $(C_6H_5)_3P$, I_2, toluene, reflux, 4 h. (b) Substituted piperidine, CS_2, neat, 3−5 h. (c) Piperidin-4-one monohydrate hydrochloride, Na_2CO_3, CS_2, CH_3CN, 0−5 °C to room temperature, 5 h. (d) $NaBH_4$, MeOH, 0−5 °C, 2 h. (e) $ClCOCHCl_2$/ $ClSO_2CH_3$/ $ClCOCH_2Cl$, CH_2Cl_2, $(C_2H_5)_3N$, 0−5 °C. (f) HNR^1R^2, $(C_2H_5)_3N$, toluene, reflux. (g) $NaSCSNR^1R^2$, H_2O−MeOH, 60 °C.

Therefore, to prevent this, compounds were synthesized by substituting the hydroxyl group of ethyl side chain in MTZ scaffold with various dithiocarbamates containing 4-substituted piperidine moiety, as reported by us earlier (**3**, Figure 1).[14] Various substitutions in piperidine moiety were explored (**4** and **5**; Figure 1) to improve the lipophilicity (and hence bioavailability in target cells through enhanced cell permeability), and an additional dithiocarbamate group was introduced to augment the biological activity further (**6**, Figure 1). Dithiocarbamates are capable of reversing the MTZ resistance[16] through their antioxidant property[17] and exhibit potent spermicidal activity through interaction with sulfhydryl groups present over sperm.[18] Spermicidal property could be considered as a highly complementary effect since TV is transmitted along with spermatozoa that cause unwanted pregnancies. The normal human vagina is naturally protected against STDs by its low pH, which is growth inhibitory for several pathogenic organisms, including *Trichomonas*.[19] Infections normally occur when the vaginal pH is disturbed, especially during the deposition of alkaline semen containing sperm and STD pathogen(s); therefore, agents capable of inactivating both are highly desirable. The new compounds were evaluated in vitro against susceptible as well as MTZ-resistant strains of TV, and their structure−activity relationship (SAR) was studied by three-dimensional quantitative structure−activity relationship (3D QSAR). The comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) techniques were used by fitting the parameters of the model to the activity data. To validate our proposed models and the predictions done by QSAR, new molecules were designed and evaluated for their biological activities.

Because the synthesized compounds are intended to be used vaginally and dually active agents are the need of the present time,[20] these compounds were also evaluated for spermicidal effect against human sperm and for safety toward cervicovaginal epithelium and microflora.

Compounds **8**−**11** (Scheme 1) were synthesized in one pot directly from iodo derivative (**7**), substituted piperidine, and carbon disulfide under solvent free condition by reported method.[21] Similarly, compound **12** was prepared directly from **7**, piperidone monohydrate hydrochloride, sodium carbonate, and carbon disulfide in an acetonitrile−water mixture (2:1), which was then reduced by using sodium borohydride to hydroxyl compound **13**. Compound **13** was dichloroacetylated, mesylated, and chloroacetylated to provide compounds **14**, **15**, and **16**, respectively, by using usual reaction conditions. Substitution of chloro group of **16** by cyclic amine in toluene provided compounds **17** and **18** (Scheme 1),[15] whereas substitution by sodium salt of carbodithioic acid in methanol−water mixture (1:1) at 60 °C gave compounds **19** and **20** in good yield.

2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl-4-substituted piperidine-1-carbodithioates (**8**−**20**) were found to be active against MTZ-susceptible as well as MTZ-resistant strains of TV (Table 1). Eleven compounds (**8**, **10**−**15**, and **17**−**20**) were 3.6−8.6 times more active as compared to MTZ, while compound **9** was equipotent and **16** was 4 times less active against susceptible strain of TV. On the other hand, all of the 13 compounds showed 1.2−12.1 times more efficacy against

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Table 1. In Vitro Antitrichomonas, Spermicidal Activities, and Safety Data of the Compounds 8−20

	MIC μmol			IC_{50} (μg/mL)	
compd	ATA[a] [susceptible]	ATA[b] [resistant]	SI[c] (at 1%) (%)	cytotoxicity against HeLa	compatibility with *Lactobacillus*[d]
8	3.24	64.8	100	510	>1000
9	12.4	49.5	100	>1000	>1000
10	1.68	24.1	<100	>1000	>1000
11	1.90	26.9	<100	>1000	>1000
12	1.89	30.5	<100	>1000	380
13	1.41	30.3	100	930	350
14	1.53	56.7	100	>1000	740
15	12.4	61.3	100	>1000	>1000
16	49.3	246	100	930	590
17	1.37	54.9	<100	760	>1000
18	1.36	54.7	<100	840	>1000
19	2.41	96.7	<100	>1000	>1000
20	2.35	37.7	<100	980	>1000
MTZ	11.7	292	<100	>1000	>1000
N-9			100	33.0	33.0

[a]ATA, antitrichomonas activity against MTZ-susceptible clinical isolate of *T. vaginalis*. [b]ATA, antitrichomonas activity against MTZ-resistant (ATCC 50143) strain of *T. vaginalis*. [c]SI, sperm immobilization (normal human spermatozoa). [d]*Lactobacillus jensenii* (ATCC 25258).

the MTZ-resistant strain of TV. Interestingly, the least active compound (**16**) was comparable to MTZ in its activity against the resistant strain. Six compounds (**8**, **9**, and **13**−**16**) completely immobilized human sperm at 1% concentration, while the rest were inactive (Table 1). The results of spermicidal activity indicated that a tetrahedral carbon at position 4 of piperidine with smaller groups (**8**, **9**, and **13**−**16**) was preferred over a longer chain at position 4 of the piperidine moiety (**10**, **11**, and **17**−**20**) and trigonal carbon (**12**). All of the compounds exhibited a high safety profile in comparison to nonoxynol-9 (N-9, the OTC spermicide for vaginal application) toward both HeLa cells and *Lactobacillus* with IC_{50} values ranging from 350 to >1000 μg/mL (Table 1). MTZ is reported to inhibit *Lactobacillus* growth partially at 1000−4000 μg/mL and totally at ≥5000 μg/mL but was found to have no effect at concentrations of 500−1000 μg/mL.[22]

With a view to further optimize the biological activities, these nitroimidazolyl-4-substituted piperidine-1-carbodithioates (**8**−**20**) along with 25 compounds (**21**−**45**) reported[14] earlier by us were investigated by 3D-QSAR techniques.

In the present QSAR study, CoMFA and CoMSIA models were derived from the 34 ligands in the training set (Table S1 in the Supporting Information). In comparison to CoMFA, the CoMSIA methodology provides a more detailed three-dimensional information in terms of hydrophobic and hydrogen bond interactions. Using CoMSIA analysis, we achieved comparatively good correlation for combination of four molecular descriptors: steric, electrostatic, hydrophobic, and hydrogen bond acceptor fields. Cross-validated coefficient (leave-one-out) q^2 of 0.692 with five optimal number of component and 0.603 with four optimal number of component and noncross-validated r^2 of 0.925 with standard error of estimate (SEE) of 0.148 and 0.882 with SEE of 0.183 were obtained for CoMFA and CoMSIA models, respectively. Good predictive r_{pred}^2 values for the test set (Table S2 in the Supporting Information) of 0.679 and 0.732 for CoMFA and CoMSIA models, respectively, reflect the predictive ability of CoMFA and CoMSIA models. The statistical results are summarized in Table S3 in the Supporting Information. Figure S2 (Supporting Information) shows the plot of experimental versus predicted values for CoMFA and CoMSIA models. These results indicate that 3D QSAR models could be used reliably to design new antitrichomonas agents. Figure S3a,b (Supporting Information) obtained from CoMFA and CoMSIA models is comparable in terms of steric and electrostatic requirements. It seems from Figure S3a in the Supporting Information that the presence of an optimal size of the steric group around piperidine ring plays an important role in ATA. Although CoMFA and CoMSIA models disfavor the presence of more bulky groups around the piperidine ring after a certain distance, some flexible molecules with bulkier groups like **11**, **17**, **18**, **33**, and **34** are found to be very active, as these tend to fold into a smaller size during the energy minimization. Figure S3c (Supporting Information) obtained from CoMSIA contour analyses provides additional information in terms of the effect of hydrophobic and hydrogen bond acceptor substituents on ATA for compound **38**.

On the basis of the structural guidelines derived from CoMFA and CoMSIA models and their contour maps, a set of five compounds (**46**−**50**) were designed, and their activities were predicted (Table 2). We synthesized compounds (**46**−**50**) having methyl and ethyl groups as substituents around the piperidine ring due to less bulkyness nature of these group, according to Scheme 1.

Table 2. Predicted and Experimental ATA by CoMFA and CoMSIA and in vitro Antitrichomonas, Spermicidal Activities, and Safety Data of the Compounds 46−50

	PA[a] [susceptible]			MIC μmol			IC_{50} (μg/mL)	
compd	CoMFA	CoMSIA	EA[b] [susceptible]	ATA[c] [susceptible]	ATA[d] [resistant]	SI[e] (at 1.0%) (%)	cytotoxicity against HeLa	compatibility with *Lactobacillus*[f]
46	5.752	5.870	5.72	1.90	15.2	100	170	>1000
47	5.716	5.719	5.72	1.90	15.2	100	170	>1000
48	5.623	5.615	5.99	1.03	14.3	100	190	>1000
49	6.839	5.905	5.44	3.65	14.6	100	94.0	>1000
50	6.721	5.895	5.74	1.83	29.2	100	130	>1000
MTZ				11.7	292	<100	>1000	>1000
N-9						100	33.0	33.0

[a]PA, predicted ATA. [b]EA, experimental ATA against susceptible strain of TV. [c]ATA, antitrichomonas activity against MTZ-susceptible strain of TV. [d]ATA, antitrichomonas activity against MTZ-resistant (ATCC 50143) strain of TV. [e]SI, sperm immobilization. [f]*Lactobacillus jensenii* (ATCC 25258).

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The biological assay demonstrated that the newly designed compounds possessed potent ATA comparable to the predicted ATA (Table 2). In comparison to the standard drug MTZ, the activity increased by 3−10-fold against susceptible strain and 10−20 times against the resistant strain. All of these compounds (**46**−**50**) irreversibly immobilized the human sperm at 1% concentration and exhibited a high degree of safety toward cervical epithelium (HeLa) and normal vaginal microflora (*Lactobacillus*). Among them, 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl-4-methylpiperidine-1-carbodithioate (**48**) was the most active compound and that was 10 and 20 times more potent than MTZ against susceptible and resistant strains of TV, respectively. It also irreversibly immobilized 100% human sperm at 1% (MEC) in ∼30 s and exhibited a high degree of safety against HeLa cells and *Lactobacillus*.

The hybridization of MTZ framework with dithiocarbamate group led to the design of nitroimidazolyl-4-substituted piperidine-1-carbodithioates (**8**−**20**) with highly enhanced ATA that could defeat the infection's resistance to MTZ and complementary spermicidal activity. The 3D QSAR analysis of these compounds along with 25 compounds (**21**−**45**) reported earlier[14] by means of CoMFA and CoMSIA models and their contour maps was found to have good predictive abilities, r_{pred}^2 = 0.679 and 0.732 obtained from CoMFA and CoMSIA, respectively. 3D QSAR models could describe the steric, electrostatic, hydrophobic, and hydrogen bond acceptor requirements for the compounds to be active against trichomonas species. Analyses reveal that steric field at the position around the piperidine ring has a major influence on the ATA. A yellow contour, present nearby this group on the other side of the green contour, recommends not putting sterically increasing bulk in this direction (Figure S3a,b in the Supporting Information). The QSAR analysis resulted in the design of five new compounds (**46**−**50**) with highly improved ATA without forfeiting the complementary spermicidal activity. The augmented microbicidal activity and desirable sperm immobilizing capacity of these nitroimidazolyl carbodithioates (**8**−**20** and **46**−**50**) might be attributable to their additional capability to interact[18] with free thiol groups, which play an important role in the survival of predominantly anaerobic cells like spermatozoa[23] and TV.[24] The nucleophilic character of the sulfur atom and the unique redox properties of the thiol group make it a key residue for enzyme catalysis, protein folding, and redox signaling and regulation,[25] which are important for cellular energy metabolism, motility, and subsistence. The undoing of MTZ resistance (Table 2) may again be attributed to the redox properties of dithiocarbamate group[17] since the reduction[26,27] of the nitro group of MTZ to cytotoxic nitro radical is inhibited in resistant TV.[28,29]

This study highlights a novel method for potentiating the MTZ framework against susceptible and resistant TV strains. The incorporation of spermicidal activity into these improved drug candidates may have serious implications in their possible use as topical prophylactic contraceptives. The good statistical correlation indicated that 3D QSAR analysis along with contour mapping could reasonably be extended to the design of novel lead compounds incorporating essential pharmacophores that are closely associated with desired biological responses. Further lead optimization may result into a potent dual active microbicidal spermicide.

■ ASSOCIATED CONTENT

Supporting Information

Tables of training, test set data, and statistical results; plot of experimental vs predicted values for CoMFA and CoMSIA models; contour analysis from CoMFA and CoMSIA models; experimental procedure; ^{1}H, ^{13}C NMR; and HRMS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.
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