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Preliminary studies of 3,4-dichloroaniline amides as antiparasitic agents: Structure–activity analysis of a compound library in vitro against *Trichomonas vaginalis*

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ABSTRACT

Trichomonas vaginalis, a human-infectious protozoan, can display resistance to treatment by metronidazole. A library of 3,4-dichloroaniline amides based on propanil, an herbicide, has been synthesized and screened to test susceptibility to these analogs. From this preliminary study, the most effective compound **15**, inhibits growth of the organism by 66% and 69% on the two strains tested, T1 and G3, respectively.

Trichomonas vaginalis is a flagellated, facultative anaerobic protozoan that causes the sexually transmitted disease trichomoniasis in humans. The current FDA-approved treatment for this disease is the compound metronidazole. The medication is typically prescribed as a 2 g single dosage to be taken orally. However, approximately 2.5-5% of all cases are resistant to metronidazole with this percentage increasing. Currently, the only way to treat resistant strains is to take a larger dose than the prescribed 2 g.[1] However, increased dosages can also lead to resistance to the medication as well.

The increasing number of resistant cases to metronidazole supports the need to find alternative forms of treatment. In this study, we tested the effect of propanil, an herbicide used commercially in the rice cultivation industry, against *T. vaginalis* cultured in vitro. In plants, propanil has been shown to inhibit the activity of 4-coumarate:CoA ligase. This enzyme is part of the phenylpropanoid pathway and plays a role in plant growth.[2] A homologue of 4-couma rate:CoA ligase is not present in the human genome. This observation would also support the high IC_{50} values of propanil (305 μM against human erythroid progenitor cells and greater than 500 μM against colony-forming unit-granulocyte/macrophage progenitor cells[3] (Table 1). A careful search of the *T. vaginalis* genome reveals a homologue of 4-coumarate:CoA ligase. This putative protein, based on sequence analysis, is an AMP-binding enzyme (Accession No. XP_001316928) and has 25% sequence identity to the homologue in *Oryza sativa*.

Propanil represents an attractive compound due to its low molecular weight and chemical simplicity. Analog synthesis to vary the acyl portion of the molecule can be accomplished by a nucleophilic acyl-substitution reaction between an acid chloride and 3,4-dichloroaniline. To this end, we have screened a library of 3,4-dichloroaniline amides to assess their effectiveness at inhibiting the growth of two laboratory strains of the organism, T1 and G3 (Fig. 1). The G3 strain is more virulent than the T1 strain; however, both strains are susceptible to treatment by metronidazole. The experimentally determined IC_{50} values for metronidazole on the T1 and G3 strains are 0.72 μM and 0.77 μM, respectively (Table 1). The compounds were screened for preliminary inhibitory activities.[4]

The library was screened against two strains of the organism to compare the inhibitory activity of the compounds, since variation in compound susceptibilities have been observed among different strains of the same protozoan species. If a compound has similar activity against multiple strains of an organism, it would make it more attractive for further development as a possible chemotherapeutic. The genomes of the T1 and G3 strains also have differences that might allow them to react differently to the same compound.

In this study, compounds are considered effective if they inhibit the growth at least 50%. Of the 21 compounds in the library, four compounds are shown to be effective at inhibiting the growth of the T1 strain at a concentration of 100 μM, while five are effective

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Table 1
Calculated IC_{50} values

Organism	Compound	IC_{50} value (μM)
Human, CFU-granulocyte/macrophage progenitor cells[2]	Propanil	>500.00
Human, erythroid progenitor cells[2]	Propanil	305.00
T. vaginalis, T1 strain	Propanil	119.87
T. vaginalis, G3 strain	Metronidazole	0.77
T. vaginalis, T1 strain	Metronidazole	0.72

against the G3 strain (Table 2). Compound **15** displayed the greatest inhibitory activity against both strains of *T. vaginalis* tested.

The mechanism of growth inhibition of *T. vaginalis* by the compounds from this library is currently unknown. For nearly half of these compounds, it appears that activity follows the relative electron deficiency of the aromatic acyl group.

Compounds that possess a strong electron-withdrawing group attached to the aromatic acyl group are able to inhibit the growth of the organism effectively. Compound **18**, which possesses an electron withdrawing group in the *meta* position on the aromatic acyl group, is able to inhibit the growth of the virulent strain more effectively than the T1 strain. Compound **15**, which possesses a trifluoromethyl group in the *meta* position, displays inhibition of both

Table 2
Inhibitory activity of compound library against two strains of *T. vaginalis* (T1 and G3) tested at 100 μM

Compounds	% Inhibition[a]	
	T1	G3
Propanil	29(±8)	20(±9)
1	62(±5)	30(±6)
2	46(±6)	28(±10)
3	12(±7)	40(±17)
4	59(±6)	46(±3)
5	39(±12)	49(±4)
6	22(±7)	13(±9)
7	49(±7)	37(±13)
8	64(±5)	24(±8)
9	34(±4)	10(±10)
10	36(±6)	28(±10)
Acetaminophen	17(±7)	14(±3)
11	0	19(±8)
12	22(±6)	51(±13)
13	25(±5)	39(±13)
14	44(±7)	40(±10)
15	**69(±5)**	**66(±8)**
16	29(±5)	55(±13)
17	29(±7)	61(±5)
18	19(±5)	70(±5)
19	38(±3)	46(±4)

[a] Values are means of 21 experiments; standard error of the mean is given in parentheses.

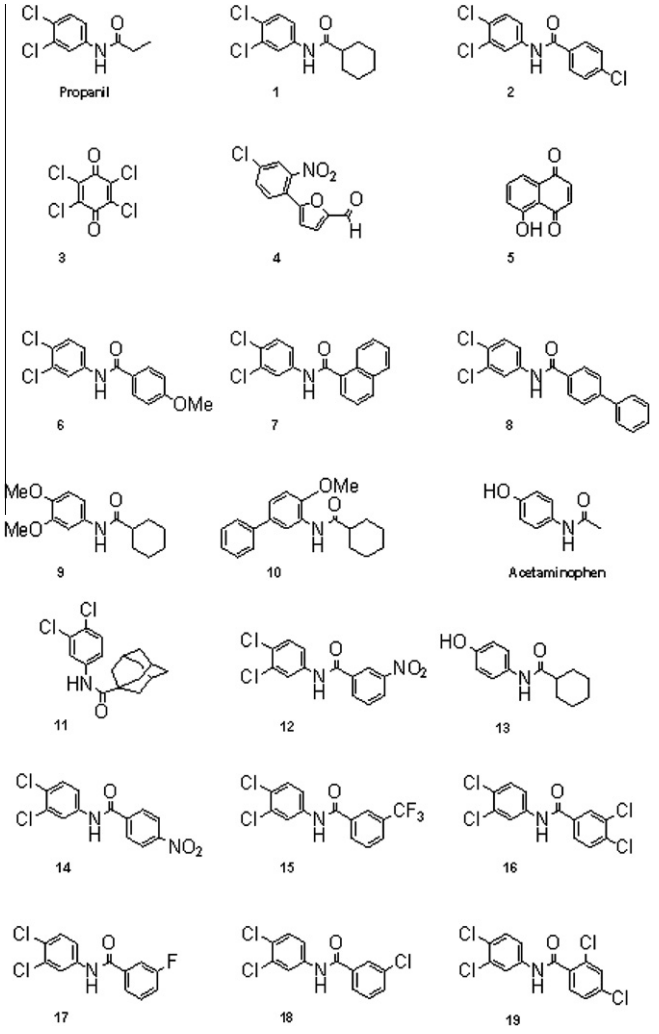


Figure 1. Library of 3,4-dichloroaniline amides and related compounds screened.

strains tested, and represents a potential avenue of further modification. This compound has IC_{50} values against *T. vaginalis* that are four times less than that of propanil against human cells[3] (Table 3).

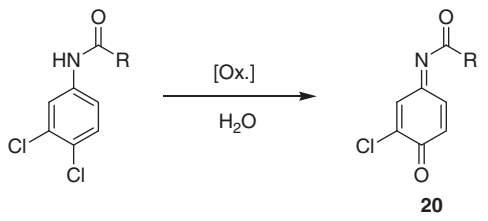
The 3,4-dichloroaniline amides that do not possess an aromatic acyl group have varied effects on the organism. Propanil, the parent compound for the majority of the library, has a marginal effect on the inhibition of the organism. Compound **1** which varies by only a cyclohexyl group can effectively inhibit 62% of the growth of the less virulent strain, but it is less effective on the G3 strain.

The cellular target for these compounds is uncertain. It has been shown that propanil may undergo oxidation catalyzed by iron(III) tetrasulfonatophthalocyanine.[5] Scheme 1 shows the possible products of such an oxidation. Previous studies indicate that iron complexes may catalyze the oxidation of propanil to electrophilic amidoquinone species similar to the acetaminophen toxic metabolite, and that acetaminophen and analog **13** have modest activity against *T. vaginalis* similar to that of propanil. It seems plausible

Table 3
IC_{50} determination for compound **15**

Compound	IC_{50} value[a] (μM)	
	T1	G3
15	77(±7)	90(±11)

[a] Values are derived from the mean of 21 experiments; standard error of the mean is given in parentheses.



Scheme 1. Proposed mechanism of oxidation of the 3,4-dichloroaniline amides.

that propanil and its analogs are oxidatively activated, perhaps by the oxidized form of a *T. vaginalis* thioredoxin reductase.

Although the compounds represented in this library are not as effective as metronidazole, they do exert an inhibitory effect. These preliminary findings suggest that these compounds might be good candidates for further development. Furthermore, the identification of the biological pathway(s) affected by these compounds may also help in the development of 3,4-dichloroaniline amides as antiparasitic agents.

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4. Cultures of two different strains of *T. vaginalis*, T1 and G3, were grown in 10 mL completed TYM Diamond's media in a 37 °C incubator for 24 h. One hundred millimolar stocks of the compounds, dissolved in DMSO, were screened against the T1 and G3 strains of *T. vaginalis*. Cells untreated and inoculated with 10 μL DMSO are used as controls. Ten microliter of 100 mM stocks of the compound library were inoculated against the various parasite strains for a final concentration of 100 μM. Results were calculated based off of counts utilized by a hemocytometer after 24 h.
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