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Activity of Novel Tryptophan Analogs against *Trypanosoma cruzi* and *Leishmania donovani*

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Drugs available for the treatment of Chagas disease and Leishmaniasis are grossly inadequate and have many drawbacks. Most of them are ineffective for the chronic form of the disease. The drugs available are expensive and most need parenteral administration. In addition, most of them are extremely toxic and resistance develops fast. There is an urgent need for new, safe and more effective drugs. As part of the continued search for novel antitrypanosomal drugs, the present study was aimed at the design and synthesis of novel tryptophan analogs which have a potential to inhibit essential trypanosomal enzymes. Some of these compounds have shown significant activity against *Trypanosoma brucei brucei in vitro*. In the present study, 17 of the most promising compounds were selected and tested for possible activity against the biochemically closely related protozoans *Trypanosoma cruzi* and *Leishmania donovani* using *in vitro* models. Seven compounds showed significant activity against *T. cruzi*, producing more than 50 % inhibition of multiplication at or below 30 µM concentrations. Four compounds also had significant activity against *L. donovani* promastigotes *in vitro*. These findings support the common observation that antiprotozoal drugs tend to exhibit a broad spectrum of activity among various protozoans.

Key words: Tryptophan analogs, novel drugs, *Trypanosoma cruzi*, *Leishmania donovani*,

INTRODUCTION

Chagas disease is caused by trypanozoons of the genus *Trypanosoma* of which *Trypanosoma cruzi* is the most significant agent. It is transmitted mainly by blood sucking bugs belonging to the family Reduviidae (subfamily Triatominae) [1-2]. Other routes of transmission are through blood transfusion which accounts for 1-18 % of all infections [3] and to a small extent by the transplacental route (1-2 %) [1].

Despite major efforts to eradicate it, Chagas disease is still prevalent in almost all countries in Central and Southern America [1,4]. Vaccine development is hampered by the problems of possible stimulation of autoimmunity [1, 5-6]. Only two drugs are established for the treatment of Chagas disease, nifurtimox and benznidazole. These drugs are useful for acute infections with *T. cruzi* but due to toxicity they are not useful for chronic disease [1].

Leishmaniasis in its various forms is caused by closely related dimorphic protozoa of the genus *Leishmania* (family Trypanosomatidae). [7-11]. The World Health Organization (WHO) estimates an annual incidence of 600,000 cases worldwide [12-13]. Recent reports of cases of HIV-visceral leishmaniasis (VL) co-infection has alarming implications for areas with endemic leishmaniasis especially sub-Saharan Africa, Brazil, and the Indian subcontinent, where the prevalence of HIV infection is increasing [14-16].

Currently, there are no vaccines for the prevention of leishmaniasis [17]. The main control strategy is case identification and treatment or, when feasible, vector and animal

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reservoir control. The drugs of choice today for visceral and cutaneous disease include the pentavalent antimonials like sodium stibogluconate, amphotericin B and pentamidine isethionate. These drugs have similar drawbacks to those used for Chagas disease [18-20]. Miltefosine is the only recently developed potential orally active drug for visceral leishmaniasis [4,18]. There is thus an urgent need for new safe, cheap and preferably orally active drugs for the two diseases. In the continuing search for drugs, the authors have designed and synthesized novel tryptophan analogs (Figure 1) which have a potential to inhibit essential trypanosomal enzymes. Some of these compounds have shown significant *in vitro* activity against *Trypanosoma brucei brucei* [21]. In consideration of the biochemical similarity between African and South American trypanosomes (*T. cruzi*) on one hand and *Leishmania* species on the other, the present study was designed to test the novel tryptophan analogs against *T. cruzi* and *L. donovani* using *in vitro* models [22].

MATERIALS AND METHODS

Test compounds

Seventeen novel tryptophan-acetylenic and/or dithiocarbamate compounds previously designed and synthesized as potential enzyme-activated inhibitors of putative pyridoxal and FAD-dependent enzymes in trypanosomes [21], were used in the study (Figure 1).

Test organisms

Trypanosoma cruzi trypomastigotes (MHOM/BR/OO/Y) obtained from rat (L6) myoblast cultures and *L. donovani* amastigotes **(**MHOM/ET/67/L82**)** obtained from hamster spleen were used in the screens.

Activity of target compounds against *T. cruzi*

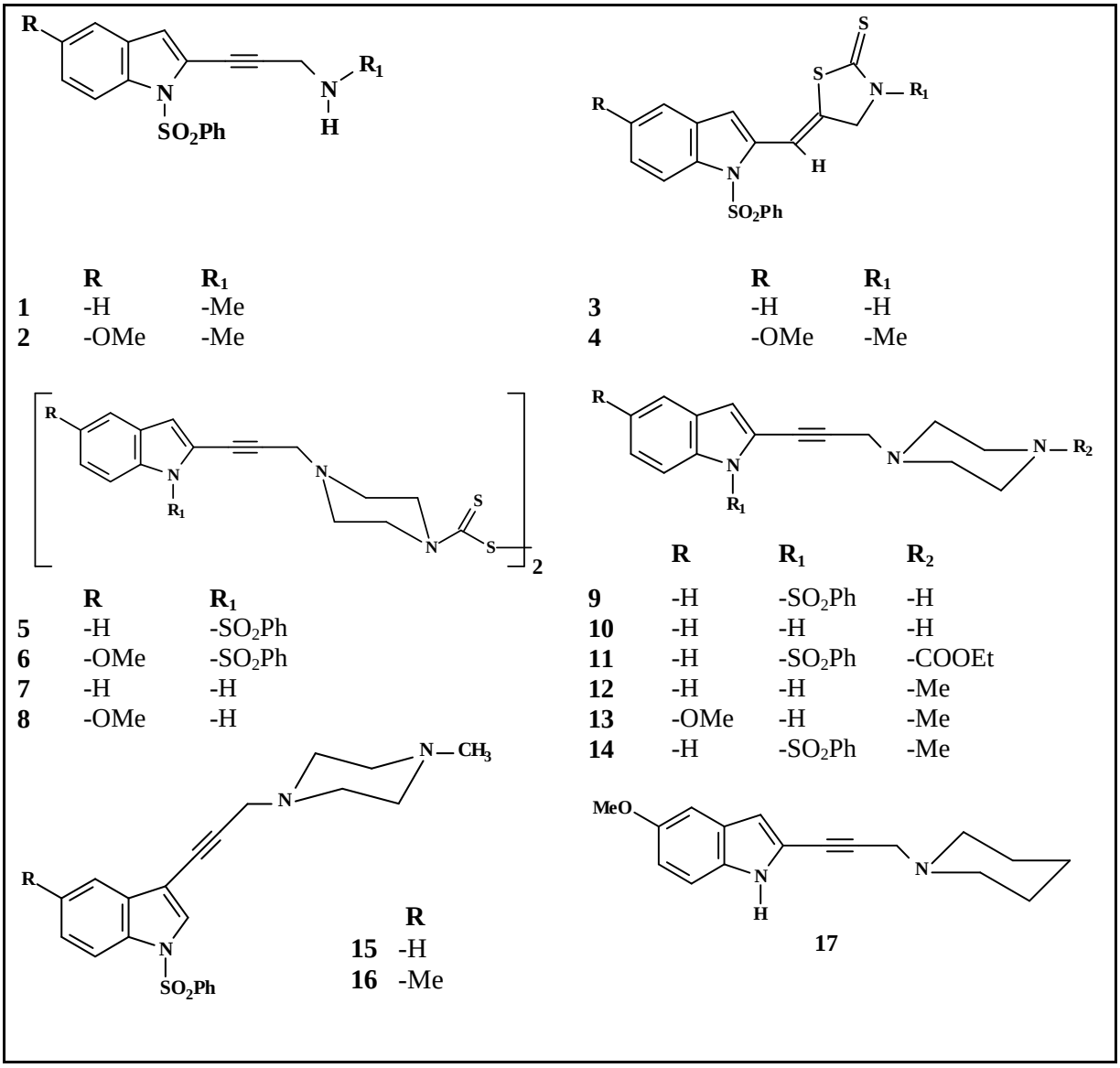
An *in vitro* model, previously developed [22], was used to test the novel compounds against *T. cruzi* and *L. donovani*. *Trypanosoma cruzi* trypomastigotes were used to infect mouse peritoneal macrophages in medium containing compounds **1-17,** each at 90, 30, 10, and 3 µM concentrations and incubated for 72 h at 37 °C. The experiments were carried out in quadruplicate and activity was determined by calculating the average percentage of uninfected macrophages in treated cultures. This was indicative of the ability of the test compounds to protect macrophages from infection. One hundred percent infection was reached in media containing no test drugs. Nifurtimox was used as the positive control in all cases. The ED_{50} (concentration which protects 50 % of the macrophages from infection) was calculated for the most active compounds [22].

Activity of target compounds against *L. donovani*

Leishmania donovani amastigotes were used to infect mouse peritoneal macrophages in chamber slides. Infected macrophages were subjected to the test compounds **1-17** at 90, 30, 10 and 3 µM concentrations in quadruplicate cultures for 7 days at 37 °C. Drug activity was expressed as the average percentage of infected mouse peritoneal macrophages cleared of amastigotes in treated cultures at the various concentrations used. This was a measure of the ability of the compounds to kill the intracellular parasites. Macrophages in cultures containing no test drugs were not cleared of the parasites during the 7 days of incubation. Sodium stibogluconate was used as a positive control in all tests. The ED_{50} was calculated for the most promising compounds [22].

RESULTS AND DISCUSSION

The results obtained are summarized in Tables 1 and 2. Seventeen compounds, **1-17,** were tested for in *vitro* activity against T. *cruzi* and *L. donovani*. Compounds **5**, **6**, **9**, **10**, **15**, **16** and **17** showed significant activity against *T. cruzi*, inhibiting the infection of macrophages by more than 50 % at a concentration of 30 µM or lower. Compound **9** was the most active giving a 75 % inhibition at 10 µM. It was however found to be toxic to macrophages above 30 µM. Compound **12** and **13** protected the macrophages only at 90 µM with 100 % inhibition. All the tested compounds were less active than the standard drug nifurtimox (ED_{50} 2.21µM).



	R	R_1
1	-H	-Me
2	-OMe	-Me

	R	R_1
3	-H	-H
4	-OMe	-Me

	R	R_1
5	-H	$-SO_2Ph$
6	-OMe	$-SO_2Ph$
7	-H	-H
8	-OMe	-H

	R	R_1	R_2
9	-H	$-SO_2Ph$	-H
10	-H	-H	-H
11	-H	$-SO_2Ph$	-COOEt
12	-H	-H	-Me
13	-OMe	-H	-Me
14	-H	$-SO_2Ph$	-Me

	R
15	-H
16	-Me

Figure 1: Chemical structures of Novel Tryptophan analogs tested for activity against *L. donovani* and *T. cruzi*.

Compounds **7, 8, 13** and **17** inhibited the multiplication of *L. donovani* amastigotes by about 50 % at 30 µM with most compounds being toxic to macrophages at higher concentrations. Compounds **13** and **17** were the most active giving approximately 100 % inhibition at 30 µM. Compound **12** gave 100 % inhibition at 90 µM, but was inactive at lower concentrations. Compound **2** was weaker, producing a 50 % inhibition at concentrations above 30 µM (ED_{50} = 63.86 µM). Compound **1**, which differs from compound **2** only by a 5-methoxy group, had no significant activity up to a concentration 90 µM. The test compounds were much weaker than the standard drug sodium stibogluconate (ED_{50} = 3.82 µM).

Most of the compounds exhibited toxicity to macrophages at concentrations 0≥30µM. This would suggest that a common structural feature may be responsible for the toxicity. It is highly probable that the acetylenic propargyl group

gives rise to allenes which are generally cytotoxic [23]. Preparation and testing of the partially reduced (allenic) and fully reduced (propylic) analogs of these compounds should give more insight into the mechanism of toxicity to macrophages.

Table 1: *In vitro* activity of test compounds against *T. cruzi*.

	Percentage Inhibition				
Compound	**90 µM**	**30 µM**	**10 µM**	**3 µM**	**ED_{50}, µM**
1	-	0	0	0	-
2	-	19.4			
3	-	T	32.8	-	
4	0	0	0	0	-
5	-	63.6	34.5	0	
6	-	T	51.5	2.5	-
7	-	47.1	0	-	
8	-	47.1	16.8	-	
9	T	T	75.7	0	
10	-	74.0	0	0	
11	T	25.9	-	-	-
12	100	35.7	-	-	-
13	98.8	32.8	-	-	49.7
14	T	0	-	-	-
15	100	98.8	-		
16	100	59	-	-	27.6
17	100	57.5	-	-	42.9

T = toxic to macrophages; - = not tested; Nifurtimox ED_{50} = 2.21 µM

Table 2: *In vitro* activity of test compounds against *L donovani.*

	Percentage Inhibition				
Compound	**90 µM**	**30 µM**	**10 µM**	**3 µM**	**ED_{50}, µM**
1	1.1	0	0	0	-
2	58.2	39.7	1.5	0	63.9
3	-	0	0	0	-
4	8.9	0	0	0	-
5	T	0	0	0	
6	T	0	0	0	
7	T	59.4	16.7	0	
8	T	48.5	0	0	
9	T	T	10.5	0	-
10	T	11.5	0	0	
11	39.1	0	0	0	-
12	100	0	-	-	-
13	T	96.8	-	-	-
14	T	T	-	-	-
15	T	T	-		
16	T	T	-	-	
17	T	100	-	-	-

T = toxic to macrophages; - = not tested; Sodium stibogluconate ED_{50} = 3.82 µM

There were slight differences in the spectrum of activity of the tested compounds against the two organisms. For example, compound **7** was more active against *L donovani* as compared to *T. cruzi* while compound **8** which has a methoxy

group at position 5 of the indole ring had a similar activity against both organisms. Compounds **15** and **16** were very active against *T. cruzi* but were devoid of activity against *L. donovani*. Compound **17** was found to be very active against both *T. cruzi* and *L. donovani*.

From the results of this study, a limited structure-activity relationship can be drawn. The indole 5-methoxy analogs appear to be more active than 5-unsubstituted analogs for antileishmanial activity. Consequently, compound **2** is 50 times more active against *L. donovani* compared to compound **1.** However, at 30µM, compound **15** is slightly more active against *T. cruzi* than compound **16** which has a 5-methoxy group.

The piperazinyl and piperidinyl derivatives were found to be generally more active against both *T. cruzi* and *L. donovani* than the propargyl series, while the piperazinyl dithiocarbamates were more active against *T. Cruzi* than *L. donovani*. Piperazinyl N-unsubstitution of compounds conferred increased activity against *T. cruzi*.

The benzenesulphonyl group tends to decrease activity against both *T. cruzi* and *L. donovani* as demonstrated with compounds **12** and **14** while the indole ring 3-substituted compounds (**15** and **16**) appear more active than the 2- substituted analogs (**14**) against *T. cruzi*. However compounds **15** and **16** had no activity against *L. donovani.* Substitution at position 2 appears to confer activity against both *T. cruzi* and *L. donovani* (compound **17).**

The thiazolidine thiones (**3** and **4**), whether indole 5-methoxy substituted or not, showed no significant activity against both *T. cruzi* and *L. donovani*. These compounds have been shown to possess no antitrypanosomal activity in previous studies [21].

CONCLUSION

This study has demonstrated that some of the novel tryptophan analogs which showed significant antitrypanosomal activity against *T. brucei brucei* are also active against *L. donovani* and *T. cruzi* and thus can be further developed as potential future drugs for the two diseases. This underscores the general observation that antiprotozoal agents tend to be broad spectrum across various protozoan species. Toxicity to macrophages at higher concentrations needs to be further explored.

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