INHIBITION OF GROWTH OF *Leishmania donovani* PROMASTIGOTES BY NEWLY SYNTHESIZED 1,3,4-THIADIAZOLE ANALOGS

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*Leishmania donovani*, the causative agent of visceral leishmaniasis, is transmitted by sand flies and replicates intracellularly in their mammalian host cells. The emergence of drug-resistant strains has hampered efforts to control the spread of the disease worldwide. Forty-four 1, 3, 4-thiadiazole derivatives and related compounds were tested *in vitro* for possible anti-leishmanial activity against the promastigotes of *L. donovani*. Micromolar concentrations of these agents were used to study the inhibition of multiplication of *L. donovani* promastigotes. Seven compounds were identified with potential antigrowth agents of the parasite. Compound 4a was the most active at 50 µM followed by compound 3a. These compounds could prove useful as a future alternative for the control of visceral leishmaniasis.

**Key words:** Thiadiazoles, *Leishmania donovani*, antiparasite, antileishmanial

**Introduction**

*Leishmania* parasites have a life cycle that includes an extracellular flagellated promastigote stage, and an obligatory intracellular, non-motile amastigote stage. The promastigotes are transmitted to mammalian hosts through the bite of sand flies and are rapidly transformed into non-flagellated amastigotes within mononuclear phagocytes.

Visceral leishmaniasis is one of the three clinical syndromes (visceral, cutaneous, and mucocutaneous leishmaniasis) produced by *Leishmania* infection. Visceral leishmaniasis is caused by 3 related species or subspecies (*Leishmania donovani*, *L.d. infantum*, *L.d. chagasi*), which make up the *L. donovani* complex. These protozoan parasites produce a systemic and life-threatening infection by infecting reticuloendothelial cells and macrophages in all organs. More than 90% of visceral leishmaniasis cases occur in Bangladesh, Brazil, India and Sudan. It is a zoonotic disease using different reservoirs and sand fly vectors in different parts of the world. Infection may be subclinical but clinical disease is fatal if untreated (1-3).

Currently, the treatment of choice for visceral leishmaniasis is pentavalent antimonial compounds in the form of sodium stibogluconate and N-methylglucamine antimoniate. Cases of visceral leishmaniasis may also be treated by other agents such as pentamidine and paromomycin. Recently, other new potentially powerful drugs such as liposomal amphotericin B have been introduced, and the advantage is that liposome-encapsulated drugs are more effective and less toxic. Several new compounds also show promising effects (4-7).

Within the last decade, the incidence of *Leishmania* infection has increased significantly due...
mainly to the high cost of drugs and emergence of immunosuppressive illnesses like AIDS (8-10). Drug resistance has been a major problem with about 25% of strains becoming resistant to antimonial treatment (11-14). These facts highlight the urgent need to develop new and more effective drugs to combat visceral leishmaniasis.

In our previous report (15), pyrazoloquinoline derivatives were evaluated as leishmanicidal agents and a few of these compounds showed good potential activity against _L. donovani_. Furthermore, several recent reports have pointed out to the importance of 1,3,4-thiadiazole derivatives as potential treatment against the cutaneous form of leishmaniasis caused by _L. major_ (16, 17). In this study, we tested a new family of 1, 3, 4-thiadiazoles and related compounds for their effect on the growth of _L. donovani_ promastigotes. Few compounds showed an inhibitory effect on growth of the parasite.

**Materials and Methods**

**Compounds:**

Forty-four 1,3,4-thiadiazole analogs were synthesized according to the method described by Chaaban _et al._ (18). Seven of these compounds were found to possess antileishmanial activity. The chemical structures of these seven compounds are presented in Fig. 1.

**Parasites:**

_Leishmania donovani_ strain DD8 was a kind gift from Dr. May Al-Jaser, Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia. The parasites were cultured in Medium 199 (Invitrogen, Bethesda, MD, USA) supplemented with 10% fetal bovine serum and penicillin and streptomycin added at a concentration of 100 IU/ml and 100 μg/ml respectively. All incubations were carried on at 26°C. Stock solutions of these compounds were made in dimethyl sulfoxide (DMSO) (Merck, Germany) and stored in the dark at -20°C.

**Assay for antileishmanial activity:**

After five days of incubation, the promastigote culture was centrifuged at 2000g for 10 min at room temperature. The pellet was suspended in Schneider’s insect medium (Sigma Chemical Company, St. Louis, MO, USA) supplemented with 10% fetal bovine serum. The number of promastigotes was adjusted to 1x10⁷ cell/ml. Ninty six-well plates (Nunc, Denmark) were inoculated with 50 μl/well of the parasite culture. Stock solutions of 1,3,4-thiadiazole compounds were dissolved in Scheiner’s medium and 50 μl of different concentrations were added to the culture in triplicate. Maximum concentration of DMSO in promastigote cultures (with or without the thiaidazole derivates) did not exceed 0.4%, which is within a safe limit for the parasites. After two days of incubation, wells in 96-well plates were examined microscopically for the motility of promastigotes. Growth of the parasites was monitored using tetrazolium (MTS) colorimetric assay purchased from Promega, Madison WI, USA (19). Plates were incubated at 37°C for 4-5 h and the absorbance was read at 490 nm wavelength according to manufacturer’s instructions. Amphoter cin B at a concentration of 50 μM was used in the test protocol as a control.

**Statistical Analysis:**

Absorbance values from the test and control wells were analyzed using two-sample t-test. All _p_-values ≤0.005 were considered significant.

**Results and Discussion**

Forty-four new 1,3,4-thiadiazole analogs were tested for their antileishmanial activity. These compounds were previously tested for their antimicrobial activity (unpublished results). Out of the forty-four compounds tested, only seven showed strong antileishmanial activity and their antiproliferative effects were comparable, at equimolar concentration, to that of the positive control, e.g. amphotericin B, a potent antileishmanial agent (Table 1).

Compounds 1a, 2a, 3a, 3b and 4a showed very promising antileishmanial effects, even at concentrations as low as 50 μM. Compound 4a was the most active at low concentration (50 μM). However, at higher concentrations, compound 3a showed the strongest activity against the growth of the parasite. Compounds 1a, 2a and 3b were also effective, especially at higher concentrations (i.e. 200 μM and 400 μM). Two compounds 1b and 1c also affected growth of the parasites at (400 μM) but at 50 μM, they were completely ineffective. The microscopic observation revealed that promastigotes were non-motile when incubated with the test compounds while the controls (with no compound) were actively motile.
Figure 1. Chemical structures of 1,3,4-thiadiazole derivatives and related compounds which showed activity against *L. donovani*. 
Table 1: Effect of 1,3,4-thiadiazole Analogs and related compounds on growth of *L. donovani* promastigotes.

<table>
<thead>
<tr>
<th></th>
<th>400 µM</th>
<th>200 µM</th>
<th>100 µM</th>
<th>50 µM</th>
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<tbody>
<tr>
<td>Control (Untreated)</td>
<td>0.859 ± 0.016</td>
<td></td>
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<td></td>
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<tr>
<td>Control (Amphotericin B-treated cells)</td>
<td>--</td>
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<td>--</td>
<td>0.381 ± 0.057</td>
</tr>
<tr>
<td>Compound 1a</td>
<td>0.330 ± 0.020</td>
<td>0.337 ± 0.010</td>
<td>0.430 ± 0.030</td>
<td>0.610 ± 0.070</td>
</tr>
<tr>
<td>Compound 1b</td>
<td>0.290 ± 0.020</td>
<td>0.380 ± 0.002</td>
<td>0.680 ± 0.04</td>
<td>1.060 ± 0.050**</td>
</tr>
<tr>
<td>Compound 1c</td>
<td>0.303 ± 0.003</td>
<td>0.402 ± 0.010</td>
<td>0.690 ± 0.040</td>
<td>1.070 ± 0.400**</td>
</tr>
<tr>
<td>Compound 2a</td>
<td>0.324 ± 0.004</td>
<td>0.360 ± 0.006</td>
<td>0.580 ± 0.010</td>
<td>0.630 ± 0.030</td>
</tr>
<tr>
<td>Compound 3a</td>
<td>0.260 ± 0.008</td>
<td>0.270 ± 0.003</td>
<td>0.340 ± 0.010</td>
<td>0.538 ± 0.050</td>
</tr>
<tr>
<td>Compound 3b</td>
<td>0.370 ± 0.014</td>
<td>0.330 ± 0.014</td>
<td>0.590 ± 0.070</td>
<td>0.670 ± 0.080</td>
</tr>
<tr>
<td>Compound 4a</td>
<td>0.449 ± 0.032</td>
<td>0.424 ± 0.031</td>
<td>0.426 ± 0.013</td>
<td>0.495 ± 0.050</td>
</tr>
</tbody>
</table>

Parasites were seeded in complete medium containing indicated concentration of each compound and the viability of cells was estimated using MTS assay. Each number represents the mean and standard deviation of three reading.

** Not significant
All other values are statistically significant compared to the untreated control when calculated at a 95 % confidence level. $P$ value ≤ 0.005

Some 1,3,4-thiadiazole derivatives have received recognition because they have been shown to possess potential antimicrobial and antiviral agents (20-22). A few of these compounds have been described to be very potent in inhibiting reverse transcriptase in HIV-1 (23).

It is evident that there is a growing interest in 1,3,4-thiadiazole derivatives as potential antileishmanial compounds. Foroumadi et al. showed that thiadiazole derivatives have leishmanicidal activity against *L. major* (24, 25). Also, da Silva et al. tested a group of similar compounds against *L. amazonensis* and observed effective killing of the parasites (26). The effect observed could be related to modulation and/or inhibition of G protein-coupled receptors in the parasite as it has been shown that some thiadiazole analogs are strong modulators of such cell molecules (27). *Leishmania* was shown to possess G protein-coupled receptors similar to those found in mammalian cells (28, 29). One possible explanation for the inhibitory effect of these compounds on the parasite may be attributed to their interference with the redox potential in the cells (30). There are several similar available compounds shown to be effective against the growth of *Trypanosoma cruzi* (31) and *T. brucei* (32). It was suggested that these compounds might exert antiproliferative effects through production of nitro radical anions (32). However, we would like to suggest that there could be other mechanisms by which these thiadiazole analogs work, since compounds described in this study do not have nitro groups. The antileishmanial effect of these compounds could be attributed to the functional groups which are capable of formation of free radicals such as 1,4-2,6-dimethoxyphenyl group (i.e. compounds 1a, 1b, 1c and 2a) or 1,4-dihydroxyphenyl group (i.e. compounds 3a, 3b, and 4a). These compounds could permeate through the cell plasma membrane and damage nucleic acids and/or proteins inside the cell (33). Alternatively, they might damage proteins or other molecules essential for the growth of the parasite located at the extracellular space of the plasma membrane such as adenosine receptors (34-35). Furthermore, 1,3,4-thiadiazole analogs might exert their effect through modification of sulphydryl groups of cysteine residues in some essential enzymes and other important proteins as they have been
shown to be strong sulphhydryl modifying agents (36).

In conclusion, 1,3,4-thiadiazole derivatives have been shown to possess a promising antileishmanial effect in vitro. However, these compounds have to be thoroughly appraised for their acute toxicity and genotoxicity. Furthermore, their effect in vivo in experimental animals should be performed to draw a meaningful conclusion. Both lines of experiments are underway in our laboratory.

References


 степيط نمو الليشمانيات الدونوفانية المشيدة بواسطة مضادات 1، 3، 4-ثياديزاول مشيدة حديثًا

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ملخص البحث

تنقل الليشمانيات الدونوفانية، المسيلة لداء الليشمانيات الحنوية، بواسطة دبابة الرمل وتكاثر داخل خلايا مضيفة من الدميات. ولقد أظهر ظهور سلالات مقاومة للدواء الجهود المبذولة لمكافحة انتشار المرض في جنوب باندل العالم. وقد تم عمل الاختبار العملي لأربعة وأربعين مركباً من مشغقات 1، 3، 4-ثياديزاول ومركبات وفق الشصلة بها لفاعليتها المضادة لليشمانيات المميتة ضد مشيدات الليشمانية الدونوفانية (مرحلة من دورة حياة الطفيليات). وتم استخدام تراكيز ميكرومولاية من هذه المركبات لدراسة تثبيت تانك عادات المشيدات. وتم التعرف على سوية مركبات لها فاعلية واحدة مضادة نمو الطفيل. وكان المركب 4a أكثر فاعلية عند تركيز 50 ميكرومولا تلو المركب 3a. وربما أثبتت هذه المركبات فائدة كبدائل مستقبلية لمكافحة داء الليشمانيات الحنوية.

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