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Synthesis and in vitro leishmanicidal activity of 2-(5-nitro-2-furyl) and 2-(5-nitro-2-thienyl)-5-substituted-1,3,4-thiadiazoles

Alireza Foroumadi,[a] Shirin Pournourmohammadi,[a] Fatemeh Soltani,[a] Mitra Asgharian-Rezaee,[a] Shahriar Dabiri,[a] Arsalan Kharazmi[b] and Abbas Shafiee[c,*]

[a]*Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman 76351, Iran*
[b]*LICA Pharmaceuticals, Symbion Sciences Park, Copenhagen DK 2100, Denmark*
[c]*Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14174, Iran*

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Abstract—A series of 2-(5-nitro-2-furyl) and 2-(5-nitro-2-thienyl)-5-substituted-1,3,4-thiadiazoles (**5a**–**d** and **6a**–**j**) were synthesized and evaluated against *Leishmania major* promastigotes using ^{3}H-thymidine incorporation. Most of the compounds showed activity better than the reference drug sodium stibogluconate (Pentostam). The most active compound was **6c** (IC_{50} = 0.1 μM).

Leishmaniasis is a major and increasing health problem in many parts of the world, with about 350 million people living in areas of disease endemicity and about 2 million new cases each year.[1] About 500,000 of these are visceral leishmaniasis, which is nearly always fatal if left untreated.[2] Chemotherapy remains the most effective control measure for this disease. The treatment options for leishmaniasis are limited and involve the administration of pentavalent antimonial as first line and amphotericin B and pentamidine as second line drugs.[3,4] These drugs are expensive and potentially toxic and require long-term treatment. In addition the development of drug resistance by the pathogens especially in HIV leishmania co-infected patients, has aggravated public health risks.[5] The spread of drug resistance combined with other shortcoming of the available antileishmanial drugs emphasizes the importance of the development of new, effective and safe drugs against leishmaniasis.

A great number of natural and synthetic compounds have been tested in the recent years in antileishmanial assays.[6–8]

Also the use of 5-nitrofuran and 5-nitrothiophene as antibacterial and antiprotozoal is well established.[9,10] Although there is a large amount of experimental work on these heterocycles, they still remain an area of active research interest.[11,12] In this study we would like to report the synthesis and in vitro antileishmaniasis activities of some 2-(5-nitro-2-furyl) and 2-(5-nitro-2-thienyl)-5-substituted-1,3,4-thiadiazoles (**5a**–**d** and **6a**–**j**).

The 2-amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole **3a** was obtained by oxidative cyclization of 5-nitrofurancarboxaldehyde thiosemicarbazone **2a**. Diazotization of **3a** in hydrochloric acid in the presence of copper powder gave 2-chloro-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (**4a**).[13]

Reaction of compound **4a** with piperidine or morpholine in refluxing ethanol gave compounds **5a** or **b**, respectively, in good yields. Similarly, the reaction of compound **4a** with piperazine, *N*-methylpiperazine or *N*-phenylpiperazine gave the corresponding compounds **6a**–**c**, respectively.

Starting from 2-chloro-5-(5-nitro-2-thienyl)-1,3,4-thiadiazole[14] compounds **5c**,**d** and **6f**–**h** were prepared similarly.

Acetylation of **6a** or **f** with acetic anhydride gave acetylated compound **6d** and **i**, respectively. The reaction of **6a**

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* Corresponding author. Tel.: +98 21 6406757; fax: +98 21 6461178; e-mail: ashafiee@ams.ac.ir

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or **f** with benzoyl chloride yielded compounds **6e** or **j** in high yield. The purity of the synthesized compounds was confirmed by thin layer chromatography (TLC), using various solvents of different polarities (Scheme 1).

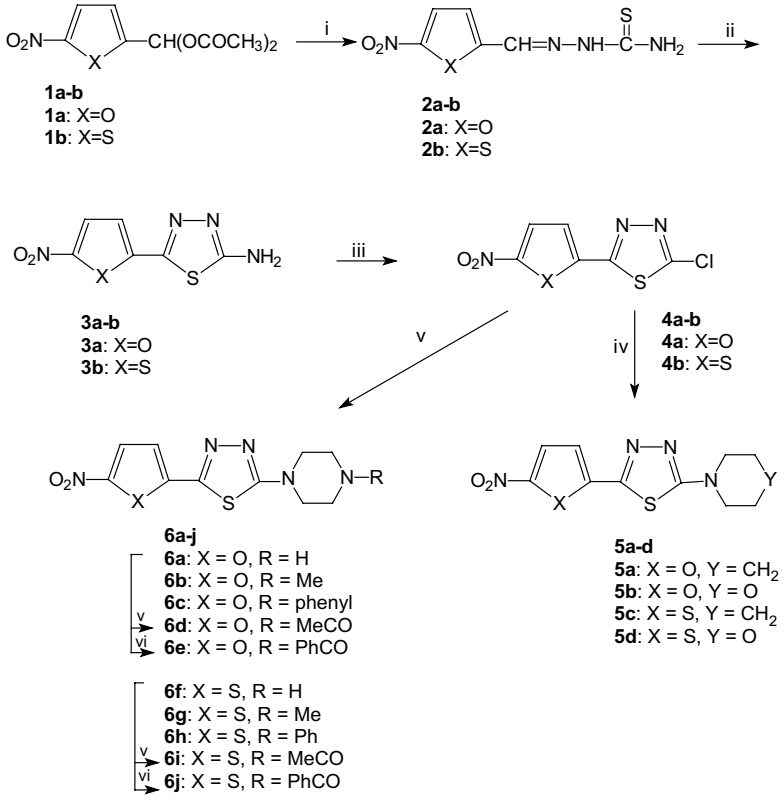
The in vitro efficacy of the synthesized compounds on promastigotes of *L. major* (ATCC J 774, HB-197) were assessed by a previously described method.[15] Promastigotes (3×10^6) were cultured in medium 199 containing 10% heat-inactivated fetal calf serum. Incubation and growth of the parasite were carried out at 26 °C. Promastigotes were harvested on day four of the culture and used. The culture of parasite was diluted with the fresh medium to a final concentration of 5×10^6 parasites/mL. The compounds to be checked were dissolved in DMSO (15 mM) and further diluted to appropriate concentrations in culture medium as given in the Table 1. In a 96-well micro titre plate, 160 μL of the promastigotes suspension was added to 40 μL of various concentrations of each compound, medium alone, or pentavalent antimonial Pentostam as positive control. The cultures were incubated for 18 h and ^{3}H-thymidine incorporation was measured and IC_{50} values of compounds possessing antileishmanial activity were calculated (Table 1).

To ensure that the solvent had no effect on parasite growth a control test was performed with test medium DMSO at the same dilutions as used in the experiment. As it could be seen from Table 1, it is clear that the nitrofuran derivatives (**5a**,**b** and **6a**–**e**) have greater leishmanicidal activities than their corresponding nitrothiophene derivatives (**5c**,**d** and **6f**–**j**). However, a slight difference in inhibitory concentration was noted for the morpholine analogues **5b** and **d** (IC_{50} = 5.2 and 10.6 μM, respectively).

In fact, compound **5d** bearing a morpholine ring was found to be the most active compound in nitrothiophene series.

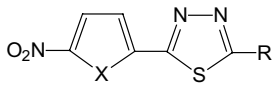
Despite to the good leishmanicidal activity of nitrofuran derivatives having piperidine (**5a**) or piperazine (**6a**) (IC_{50} = 3.2 and 8.9 μM, respectively), the corresponding nitrothiophene analogues (**5c** and **6f**) were inactive. If the hydrogen of piperazine ring in compound **6a**, is replaced with a methyl (**6b**), phenyl (**6c**), acetyl (**6d**), or benzoyl (**6e**), the activity is increased in the following order: **6c** > **e** > **d** > **b** > **a**. The nitrofuran analogue containing *N*-phenylpiperazine group (**6c**), was found to be the most active compound in this series (IC_{50} = 0.1 μM).

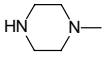
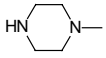
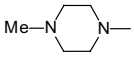
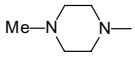
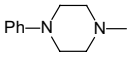
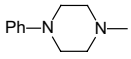
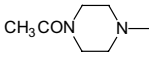
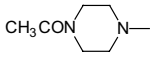
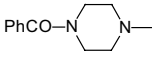
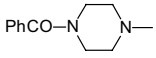
In conclusion it appears that 5-nitrofuran moiety might be responsible for leishmanicidal activity along with substituted piperazine ring attached to 1,3,4-thiadiazole ring system. In addition, most of compounds were much more active than the reference drug Pentostam. Furthermore, these compounds could represent new lead compounds for further pharmacomodulation.



Scheme 1. Reagents and conditions: (i) thiosemicarbazide, EtOH, HCl, reflux, 1 h; (ii) $NH_4Fe(SO_4)_2 \cdot 12H_2O$, H_2O, reflux, 16 h; (iii) $NaNO_2$, HCl, Cu, 0 °C → rt, 3 h; (iv) piperidine or morpholine, EtOH, reflux, 1 h; (v) piperazine or *N*-methylpiperazine or *N*-phenylpiperazine, EtOH, reflux, 1 h; (vi) Ac_2O, AcOH, reflux, 0.5 h; (vii) PhCOCl, pyridine, rt, 20 h.

Table 1. Effect of synthesized compounds on the growth of *Leishmania major* promastigotes



Compound	X	R	IC_{50} (μM)	Compound	X	R	IC_{50} (μM)
5a	O		3.2	**5c**	S		>150
5b	O		5.2	**5d**	S		10.6
6a	O		8.9	**6f**	S		>150
6b	O		7.3	**6g**	S		102.8
6c	O		0.1	**6h**	S		105.5
6d	O		1.1	**6i**	S		48.5
6e	O		0.5	**6j**	S		11.1
Pentostam			243.3				

Acknowledgments

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