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Evaluation of in-vitro leishmanicidal activity of hydrazones of thiophene carboxaldehydes against promastigotes of *Leishmania infantum* and *Leishmania tropica*

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Abstract—The lack of leishmanicidal drugs has prompted the synthesis and testing of new hydrazones of thiophene carboxaldehydes against three *Leishmania* strains. The compounds were obtained by condensation of appropriate hydrazines with thiophene 2-carboxaldehyde (series *1*), thiophene 3-carboxaldehyde (series *2*), and 5-nitrothiophene-2-carboxaldehyde (series *3*). Leishmanicidal activity was assessed against promastigotes of *Leishmania* strains, grown in-vitro in nutrient broth medium complemented with fresh rabbit blood. The minimal inhibitory concentrations were evaluated against pentamidine, as a reference drug. Several compounds exhibited significant leishmanicidal activity, the best being ten times more active than pentamidine.

Leishmaniasis is a major infectious disease for which there are few effective treatments (D'Arcy & Harron 1983). Derivatives of heterocyclic aldehydes (Dodd et al 1989) have been found to have potent cytotoxic effects against various *Leishmania* strains, and we have previously demonstrated the antiparasitic activity of thiophene carboxaldehydes (Maldonado et al 1986). We now report on the preparation of various hydrazones of those heterocyclic aldehydes and their activity against promastigotes of three *Leishmania* strains.

Materials and methods

Chemical synthesis. The derivatives belonged to three series: hydrazones of thiophene carboxaldehydes (series *1* and *2*) and 5-nitrothiophene-2-carboxaldehyde (series *3*) (Table 1). They were prepared by a standard procedure in which equimolecular quantities of the hydrazine and the aldehyde were refluxed in methanol. After completion of the reaction, the precipitated hydrazone was filtered and recrystallized from the appropriate solvent. The new compounds were identified by IR and H NMR spectra and their purity established by controls on thin-layer chromatography and microanalyses.

Leishmania strains. The *Leishmania* strains were *Leishmania infantum*–strain 1 (MCAN/FR/74/LPMA 57. WHO)–strain 2 (MCAN/FR/73/LPMA 56. WHO) and one strain of *L. tropica* (MHOM/FR/65/LPMA 59. WHO)

Isolation. Leishmania infantum was originally isolated from ganglia of dogs in Marseille and *Leishmania tropica* from a human case of cutaneous leishmaniasis with numerous typical ulcerations. These isolates containing numerous *Leishmania* amastigotes were cultivated in NNN (Novy, Mac Neal, Nicolle) (Nicolle 1908) and Tobie (Tobie 1958) media where they were transformed into promastigote forms.

Experimental techniques. After 5 to 10 subcultures, the parasites were adapted to these media, after which they could be cultivated in sterile liquid nutrient broth medium (Nutrient Broth (Oxoid) 13 g, sodium chloride 3 g, distilled water 1L) complemented by fresh rabbit blood (Ranque 1966) for screening the compounds.

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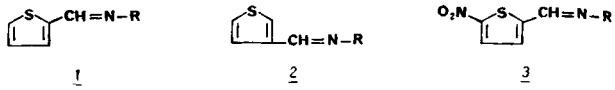
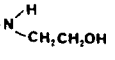
Table 1. List of the synthesized compounds.



1 2 3

1	2	3	R	R NH_2
	2a	3a		N-aminopiperidine
1b	2b	3b		N-aminomorpholine
1c	2c	3c		3-aminorhodanine
		3d		2-hydrazinoimidazoline
		3e		N-aminohomopiperidine
1f	2f	3f		2-hydrazinopyridine
		3g		2-hydroxyéthylhydrazine
1h		3h		1-amino 4-méthylpiperazine

concentrations of 100; 50; 25; 10; 5; 1; 0·5 $\mu g\ mL^{-1}$. Each strain and each concentration, was tested in triplicate. The minimal inhibitory concentrations (MIC) of the compounds were determined after the parasites had been seven days in culture by checking for the presence or absence of promastigotes microscopically ($\times 40$). The absence of promastigotes in the tubes was confirmed by retroculture. If the parasites did not recover that concentration of a compound was considered leishmanicidal. The MIC for each compound was then compared with that of pentamidine.

Results

All the derivatives tested had some activity against the three strains of *Leishmania*. This activity was weak for the series *1* and *2* (MIC $\geqslant 100\ \mu g\ mL^{-1}$), but in series *3*, the derivative *3d* (MIC $= 10\ \mu g\ mL^{-1}$) was half as active as pentamidine (MIC $= 5\ \mu g\ mL^{-1}$), *3g* (MIC $= 5\ \mu g\ mL^{-1}$) was as active and *3c* (MIC $= 0{\cdot}5\ \mu g\ mL^{-1}$) was ten times more active than pentamidine (Table 2).

Table 2. Leishmanicidal activity of tested compounds in series *3* (compounds of series *1* and *2* had MICs of $> 100\ \mu g\ mL^{-1}$).

	MIC ($\mu g\ mL^{-1}$)		
Compounds	*L. infantum* strain 1	*L. infantum* strain 2	*L. tropica*
3a	> 100	> 100	> 100
3b	25	25	25
3c	0, 5	0, 5	0, 5
3d	10	10	10
3e	> 100	> 100	> 100
3f	> 100	> 100	> 100
3g	5	5	5
3h	50	100	50
Pentamidine	5	5	5

Discussion

That only the compounds of series *3* showed enhanced leishmanicidal activity furthers interest in this chemical series and, like derivatives of carboxaldehydethiophene (Gasquet et al 1989), they require a nitro group. Hydrazones of thiophene carboxaldehydes not substituted by a nitro group were inactive. The most potent compound was the hydrazone derivative *3c* bearing a moiety of 2-oxo-5-thiono-1,3-thiazolidine.

Since hydrazones of 5-nitrothiophene 2-carboxaldehyde have leishmanicidal activity, other derivatives such as semicarbazones and thiosemicarbazones may prove of interest. This in-vitro study requires in-vivo confirmation of the possible protozoocidal activity of these new molecules.

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