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Original article

In vitro antiparasitic activity of new thiosemicarbazones in strains of *Trypanosoma cruzi*

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ABSTRACT

In this study thiosemicarbazones derivatives of 5-[(trifluoromethyl)phenylthio]-2-furaldehyde were synthesized and evaluated in terms of their efficiency in challenging the growth of epimastigote forms of *Trypanosoma cruzi*, the etiological agent of Chagas' disease. A number of compounds were synthesized from 5-bromo-2-furfuraldehyde using nucleophilic aromatic substitution, with a series of trifluoromethyl thiolates, followed by condensation reactions with thiosemicarbazide. Their molecular structures were determined by ^{1}H, ^{13}C and ^{19}F NMR, MS and IR spectroscopy. When tested with *T. cruzi*, they showed a stronger reaction, similar to nifurtimox and benznidazole, with the 5-[nitro-4-(trifluoromethyl)phenyltio]-2-furaldehyde thiosemicarbazone (compound **4**) showing the highest antiparasitic activity. This improved activity may be explained due to the nitro group present in the molecule, which potentiates its activity. The thiosemicarbazone derivatives in this study showed no apoptosis in platelets or monocytes, nor did they induce platelet activation. The trypanocidal activity of these substances represents a good starting point for a medicinal chemistry program aimed at therapy for Chagas' disease.

1. Introduction

Parasitic diseases represent a huge health problem worldwide, in particular, tropical diseases, which are found to a greater extent, in economically vulnerable sectors of society. The flagellate *Trypanosoma cruzi* causes Chagas' disease, which currently has very limited treatments available, therefore, it is important to identify new drugs for infected patients. At present, the drugs in use for this disease are Nifurtimox (Nfx) and Benznidazole (Benz) [1]. Due to the high affinity to ribonucleoside-diphosphate reductase, telomerase or the activity of nitric oxide synthase [2], various investigations have focused on the search for molecules with semicarbazones and thiosemicarbazones moieties in order to identify their role in inhibiting enzymes, or in interacting with DNA. Different classes of compounds have been studied in the context of their reaction to *T. cruzi*, and a molecular structure-activity relationship has been suggested as an important parameter amongst the steric and electrostatic effects. In addition to the lipophilicity of the compounds and, in the case of nitro derivatives, they are known for their reduction capability to form superoxide anion, and, under specific conditions, their ability to inactivate the enzyme trypanothione reductase [2–4].

Thiosemicarbazones are known to possess antibacterial, antiparasitic, antifungal [5,6] and anti-viral properties [7]. Aryl [8,9], 4-N-(2-methoxy styryl) [10] and carbamate nitrofurans [11], thiosemicarbazones, and their metal complexes with copper or zinc [12,13], ruthenium (II) [14], and platinum [15] show antitrypanosomal activity.

The aim of the study was to identify trypanocidal properties of thiosemicarbazones obtained from 5-[(trifluoromethyl)

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Abbreviations

Benz	benznidazole
CD61	integrin β_3 or gpIIIa
CD62 P	P-selectin
DMSO	dimethyl sulfoxide
VIOLE	gentian violet
LC_{50}	lethal concentration, 50%
Nfx	nifurtimox
PL	punta lobos
QRO	Querétaro
Compounds **1**–**5**	thiosemicarbazone derivatives
TPO	thrombopoietin
TXA2	thromboxane A2

phenylthio]-2-furaldehyde, which have a different position on the benzene ring to *ortho, meta* and *para,* the trifluoromethyl group. These compounds were tested using an *in vitro T. cruzi* assay and the LC_{50} was also determined [16]. The characterization of compounds was performed using instrumental physicochemical techniques, permitting the identification of the derivatives. After completing this test of apoptosis, the viability of monocytes, platelets and their activation was evaluated.

2. Results and discussion

2.1. Trypanocidal activity

Five thiosemicarbazones derivatives in total, labelled compounds **1** to **5**, were synthetized and analyzed. All compounds were tested *in vitro* to measure antiparasitic activity, using two different *T. cruzi* strains, 'Punta Lobos' and 'Queretaro', with Benznidazole (Benz), Nifurtimox (Nfx) and gentian violet (VIOLE) as trypanocidal controls.

The LC_{50} was determined by the controls in both isolates. In 'Punta Lobos', 1.2 μg/mL of the LC_{50} was obtained by adding Nfx, 4.0 μg/mL of the LC_{50}, was obtained by adding Benz, and 15.8 μg/mL of the LC_{50} was obtained by adding VIOLE. In contrast, in 'Querétaro', 8.5 μg/mL of the LC_{50} was obtained by adding Nfx, 6.5 μg/mL of the LC_{50}, was obtained by adding Benz, and 6.2 μg/mL of the LC_{50} was obtained by adding VIOLE.

A further difference was observed with the addition of compounds **1** and **3**. With these compounds the LC_{50} in 'Punta Lobos' showed less trypanocidal activity than in 'Querétaro', but was less effective than the LC_{50} in the controls. In 'Querétaro' a LC_{50} of 7.8 μg/mL showed a higher level of trypanocidal activity than in 'Punta Lobos' with the addition of compound **2**, having a lower LC_{50} than Nfx in this strain.

Compounds **4** and **5** were compared, having LCs_{50} of 3.4 and 4.6 μg/mL respectively, in 'Querétaro', and LCs_{50} of 3.2 and 5.1 μg/mL respectively, in 'Punta Lobos'. Compound **4** was more effective than Benz and VIOLE in both strains, 3.2 vs 4.0 μg/mL in 'Punta Lobos', and 3.4 vs 6.2 μg/mL, and was more effective in comparison to Nfx, but only, in the 'Queretaro' strain.

Therefore, it can be assumed that compounds **1**–**3**, and **5** had lesser trypanocidal efficiency than compound **4**. In none of these compounds, did the different positions of the trifluoromethyl groups, have a significant effect over trypanocidal activity. Neither, did the presence of a perfluorinated benzene ring, determine increasing activity. However, compound **4** contained a nitro functional group, which is found in several anti-parasitic drugs, has a positions in the trifluoromethyl group and when present in a nitro compound, there was a marginal difference in activity within the strains, which may be significant, as shown in Table 1.

2.2. Monocyte, platelet viability and apoptosis in the presence of thiosemicarbazones

With the invasion of cells, the first barrier encountered by *T. cruzi* corresponds to a monocyte – macrophage cell line [17]. Apoptosis in monocytes with compounds had no affect on cell viability, as was observed in the mean % of live cells. Fig. 1.

Some thiosemicarbazone derivatives act as agonists towards the Thrombopoietin (TPO) receptor in platelets [18]. However, in this study, platelets from healthy subjects were used for the purpose of having similar cell types, and prove the ability of thiosemicarbazone derivatives to act on their own mechanisms for apoptosis [19].

It was observed that phosphatidylserine is translocated out from the cell's surface when apoptosis begins. Therefore, exposure of platelets to the five compounds induces no changes in basal expression levels of annexin, as shown in Fig. 2.

2.3. Platelet activation in the presence of thiosemicarbazones

In consideration of the fact that parasite-derived thromboxane A2 (TXA2) participates in the rate of parasite proliferation [20], it appears that platelets modulate cell differentiation just before the infectious stage [21] of Chaga's disease, and that some semicarbazone compounds cause antithrombotic activity [22].

Inducing platelet activation of compounds was ineffective overall, except in the case of Benz. It is suggested that further studies of compounds currently in use for the treatment of Chaga's disease are needed to identify platelet activation, as shown in Fig. 3.

3. Experimental section

3.1. Chemistry

All chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA), Meyers Chemical Inc. (Tonawanda, New York, USA) and J.T.Baker (Center Valley, PA USA), and solvents were distilled before use. Synthesized compounds were characterized using IR, ^{1}H, ^{13}C, ^{19}F NMR and MS. Infrared spectra were obtained with a FT-IR "SpectrumOne" Perkin–Elmer spectrophotometer. Nuclear magnetic resonance spectra were recorded with a 200 MHz Varian Gemini spectrometer, and ^{1}H and ^{13}C NMR solvent residue signals

Table 1
Trypanosoma cruzi strains in Punta Lobos (PL) and Querétaro (Qro) tested with the compounds for LC_{50} (μg/mL).

Compound	Punta lobos LC_{50} μg/mL (μM/mL)	Querétaro LC_{50} μg/mL (μM/mL)
1	11.4 (0.0330)	14(0.0405)
2	17.8 (0.0515)	7.8(0.0225)
3	8.3 (0.0240)	29.5 (0.0854)
4	3.2 (8.19×10^{-3})	3.4 (8.7×10^{-3})
5	5.1 (0.0122)	4.6 (2.95×10^{-5})
Benz	4.0 (0.0153)	6.5 (0.0249)
Nfx	1.2 (4.17×10^{-3})	8.5 (0.0290)
VIOLE	15.8 (0.0380)	6.2 (0.0151)

Compound **1**: 5-(2-(trifluoromethyl)phenylthio]-2-furaldehyde thiosemicarbazone, Compound **2**: 5-[3-(trifluoromethyl)phenylthio]-2-furaldehyde thiosemicarbazone, Compound **3**: 5-[4-(trifluoromethyl)phenylthio]-2-furaldehyde thiosemicarbazone, Compound **4**: 5-[2-nitro-4-(trifluoromethyl)phenylthio]-2-furaldehyde thiosemicarbazone, Compound **5**: 5-[(2,3,5,6-tetrafluoro-4(trifluoromethyl]phenylthio]-2-furaldehyde thiosemicarbazone, Benz: benznidazole, Nfx: nifurtimox, VIOLE: gentian violet.