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Synthesis and anti-trypanosomal activity of novel 5-nitro-2-furaldehyde and 5-nitrothiophene-2-carboxaldehyde semicarbazone derivatives

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Abstract

Several novel semicarbazones derivatives were prepared from 5-nitro-2-furaldehyde or 5-nitrothiophene-2-carboxaldehyde, and tested in vitro as potential anti-trypanosomal agents. The compounds were prepared in good to excellent yields in 2–3 steps from readily available starting materials. Some derivatives were found to be active against *Trypanosoma cruzi* with an activity similar to that of Nifurtimox.

Keywords: Semicarbazones; 5-Nitrofurfural and 5-nitrothiophene-2-carboxaldehyde derivatives; Anti-trypanosomal activity

1. Introduction

Trypanosoma cruzi is the causative agent of Chagas' disease which currently affects 16–18 million people leading to more than 45 000 deaths each year [1,2]. The disease occurs predominantly in Central and South America, however about 100 000 people in the USA are also infected, probably due to transfusion of blood products originating from South America [3]. The current chemotherapy against Chagas' disease is still very inadequate. The two principal drugs in use are Nifurtimox® (Nfx), whose mechanism of action involves the production of superoxide anion, which is highly toxic to the parasite [4], and Benznidazol®, which acts on the respiratory system of the parasite and also inhibits DNA synthesis [5]. Both drugs present undesirable side effects [6,7] and are yet inefficient to treat chronic Chagas' disease, which can be regarded as a disease with no cure.

To develop new drugs against this parasitic infection, research is often directed towards key differences between the metabolism of the mammalian host and parasite. In that sense, trypanothione metabolism can be regarded as a specific target in the case of trypanosomatids [8]. Trypanothione, N^1,N^8-bis(glutathionyl)spermidine [9], is a conjugate between glutathione and spermidine which does not occur in mammalian cells, and is critical for the survival of the parasite, where it mimics the function of glutathione in other cells [10]. Indeed, trypanosomatids contain no conventional glutathione reductase and instead glutathione disulfide is reduced by thiol-disulfide exchange with trypanothione. The trypanothione disulfide formed in this reaction is further reduced by a specific enzyme: trypanothione reductase [11]. In addition to maintenance of intracellular thiol concentrations, trypanothione is also used in the removal of peroxides and in trapping free radicals [12,13].

Recent crystallographic and our own computational studies of trypanothione reductase [14–18] provide some basis for the design of selective inhibitors which bind in the tripanothione-binding domain, e.g. inhibitors which bear a positive charge and/or a flexible side chain. Indeed, hydrazonoamine-substituted nitrofurans and quinones have been shown to inhibit trypanothione reductase more strongly than glutathione reductase [19]. However, the search for novel and selective inhibitors and 'subversive' substrates of trypanothione reductase, especially among previously described redox-active compounds, remains desirable [20].

In this paper we report on the synthesis of a series of 5-nitro-2-furaldehyde and 5-nitrothiophene-2-carboxaldehyde semicarbazones, in which the N^4-semicarbazone moiety is replaced by aliphatic, arylic and heterocyclic amines. These compounds could possibly mimic the spermidine part of try-

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panothione in different biochemical pathways and consequently show potential trypanocidal activity. The compounds were tested in vitro for their ability to inhibit growth of *Trypanosoma cruzi* epimastigote forms [21,22].

2. Chemistry

Fourteen new compounds, **3a–g** and **4a–g**, were prepared in three steps according to classical synthetic procedures as indicated in Scheme 1.

The carbamates **1a–g** were obtained by reaction of phenyl chloroformate with the corresponding amines in methylene dichloride and triethylamine (Table 1) The semicarbazides **2a–g** were synthesized by condensation of the starting carbamates **1a–g** with an excess of hydrazine monohydrate at 80–90°C (Table 1) [23].

The reaction of **2a–g** (except **2d**) with 5-nitro-2-furaldehyde diacetate in 50% sulfuric acid gave the compounds **3a–g** (except **3d**) in moderate to good yields [24]. Compound **3d** was prepared by acid catalyzed reaction (using *p*-toluenesulfonic acid) of semicarbazide **2d** with 5-nitro-2-furaldehyde in toluene, in moderate yield [22].

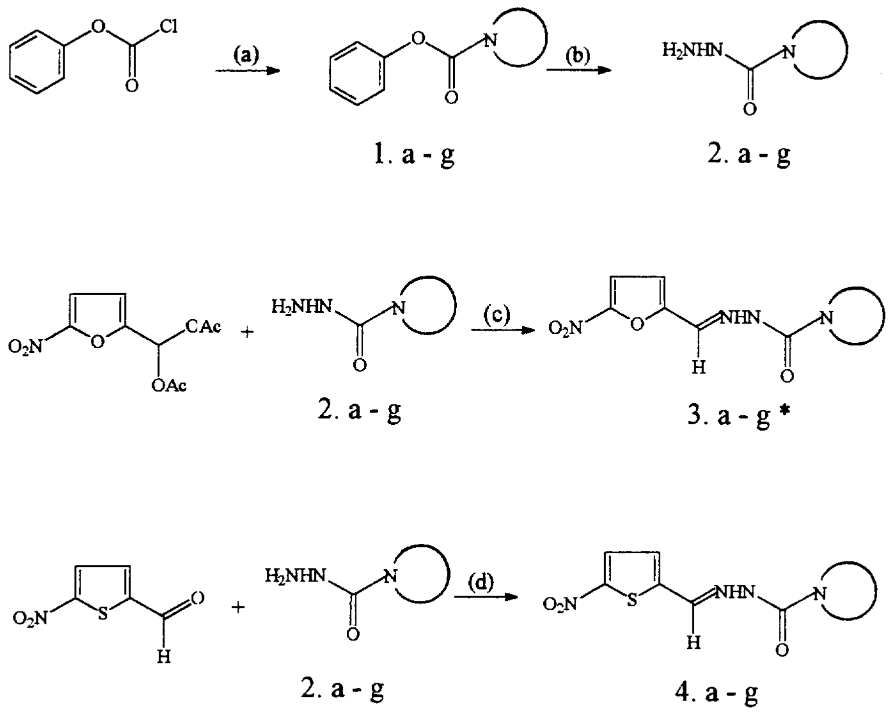
The thio derivatives **4a–g** were prepared by reaction of semicarbazides **2a–g** with 5-nitrothiophene-2-carboxaldehyde in toluene, catalyzed with *p*-toluenesulfonic acid [22]. The compounds **4a–g** were produced in high to moderate yield.

The new compounds **3a–g** and **4a–g** were identified by ^{1}H NMR, IR and MS (in some cases by HRMS) and their purity established by TLC, HPLC and microanalysis.

3. Chemical experimental

Phenyl chloroformate and the corresponding amines are commercially available and were distilled before use. 5-Nitro-2-furaldehyde diacetate, 5-nitro-2-furaldehyde and 5-nitrothiophene-2-carboxaldehyde are commercially available and were used without further purification. The solvents were used after being dried and distilled [25].

Melting points were determined using a Leitz microscope heating stage model 350 apparatus and are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorus pentoxide at 3–4 mm Hg, 24 h at room temperature) and performed on a Fisons EA 1108 CHNS-O analyzer, and were within ±0.4% of theoretical values. IR spectra were recorded on a Perkin-Elmer 1310 apparatus, using potassium bromide tablets for solid and oil products; the frequencies are expressed in cm^{-1}. ^{1}H NMR spectra were recorded on a Varian XL-100 (100 MHz) instrument, using tetramethylsilane as the internal reference and with the indicated solvent; the chemical shifts are reported in ppm. Mass spectra were recorded on a Shimadzu GS-MS QP 1100 EX instrument at 70 eV unless otherwise indicated. High resolution mass spectra were recorded on a VG ULTIMA instrument at 70 eV.



Scheme 1. *Except compound **3d** (see Section 3). (a) Amine/Et$_3$N/CH$_2$Cl$_2$/room temperature; (b) NH$_2$NH$_2$·H$_2$O/80–90°C; (c) H$_2$SO$_4$ (50%)/room temperature; (d) *p*-TsOH/toluene/room temperature. Amine: a, butylamine; b, hexylamine; c, morpholine; d, 1-methylpiperazine; e, 2-methoxyethylamine; f, 2-phenylethylamine; g, 2-(3,4-dimethoxyphenyl)ethylamine.

Table 1
Intermediate carbamates and semicarbazides

Product	Yield (%), aspect	IR (KBr) ν_{max} (cm^{-1})	^{1}H NMR (CDCl$_3$) δ (ppm)
1a	97, oil	3300, 1720, 1200	0.95 (t,3H); 1.50 (m,4H); 3.30 (q,2H); 5.60 (bs,1H); 7.10–7.50 (m,5H)
1b	81, solid (28–30°C)	3340, 1725, 1210	0.95 (t,3H); 1.40 (m,8H); 3.25 (q,2H); 5.20 (bs,1H); 7.15–7.60 (m,5H)
1c	95, solid (48–50°C)	1715, 1200, 1160	3.50–3.80 (m,8H); 7.10–7.50 (m,5H)
1d	78, oil	1715, 1200	2.40 (s,3H); 2.50 (m,4H); 3.68 (m,4H); 7.10–7.52 (m,5H)
1e	64, oil	3310, 1725, 1200	3.35 (s,3H); 3.50 (m,4H); 5.50 (bs,1H); 7.10–7.50 (m,5H)
1f	94, oil	3320, 1710, 1220	2.90 (t,2H); 3.60 (q,2H); 5.10 (bs,1H); 7.10–7.50 (m,10H)
1g	98, solid (95–97°C)	3360, 1730, 1205	2.92 (t,2H); 3.65 (q,2H); 3.90 (s,6H); 5.70 (bs,1H); 6.70–6.90 (m,3H); 7.10–7.50 (m,5H)
2a	66, oil	3400, 3210, 1660	0.95 (t,3H); 1.20–1.60 (m,4H); 3.40 (q,2H); 6.20 (bs,2H); 7.70 (bs,2H)
2b	66, solid (55–58°C)	3350, 3150, 1670	0.98 (t,3H); 1.45 (m,8H); 3.20 (q,2H); 6.30 (bs,2H); 8.00 (bs,2H)
2c	95, oil	3400, 3230, 1650	3.30–3.45 (m,4H); 3.55–3.70 (m,4H); 8.00 (bs,3H)
2d	41, oil	3430, 3180, 1660	2.40 (s,3H); 2.55 (m,4H); 3.10 (m,4H); 6.00 (bs,1H); 9.10 (bs,2H)
2e	84, oil	3415, 3200, 1675	3.38 (s,3H); 3.52 (m,4H); 6.45 (bs,2H); 7.82 (bs,2H)
2f	89, oil	3310, 3150, 1645	2.95 (t,2H); 3.60 (q,2H); 6.30 (bs,2H); 7.30 (s,5H); 7.75 (bs,2H)
2g	90, solid (103–105°C)	3390, 3240, 1670	2.92 (t,2H); 3.66 (q,2H); 3.88 (s,6H); 6.20 (bs,2H); 6.70–6.93 (m,3H); 9.20 (bs,2H)

*3.1. General procedure for the preparation of carbamates **1a–g***

Phenyl chloroformate (1 equiv.) was added to a stirred and cooled (0°C) solution of the corresponding amine (1 equiv.) and triethylamine (1 equiv.), using methylene dichloride as solvent. After addition, the mixture was stirred at room temperature for 45 min. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and the solvent was evaporated to give the products **1a–g** (Table 1), which were pure enough to be used in the next preparation without further purification.

*3.2. General procedure for the preparation of semicarbazides **2a–g***

The corresponding carbamate (**1a–g**) (1 equiv.) and hydrazine monohydrate (1.2 equiv.) were stirred at 80–90°C for 12 h. The mixture was treated with carbon tetrachloride (three times) and evaporated in vacuo. The residue was purified by chromatography (SiO_2, MeOH in CH_2Cl_2 (0–10%)) to give **2a–g** (Table 1).

*3.3. General procedure for the preparation of semicarbazones **3a**, **3b**, **3c**, **3e**, **3f** and **3g***

To a stirred mixture of 5-nitro-2-furaldehyde diacetate (2.00 mmol) in 50% sulfuric acid (8.6 ml), **2a–g** (2.60 mmol) were added in three portions. The suspension was stirred at room temperature until the nitro compound was not present (SiO_2, 20% AcOEt in petroleum ether). The mixture was then poured into water-ice (50 ml) and the resulting solid was filtered and washed with a saturated solution of sodium bicarbonate. The precipitated nitrofuran semicarbazone derivative (**3a–g**) was recrystallized from a suitable solvent.

*3.4. Preparation of semicarbazone **3d***

To a stirred suspension of semicarbazide **2d** (2.13 mmol) in toluene (5.0 ml), a mixture of 5-nitro-2-furaldehyde (2.13 mmol) and *p*-toluenesulfonic acid (catalytic amounts) in toluene (10.0 ml) was added. The suspension was stirred for 10 h at room temperature. The mixture was washed with 5% aqueous sodium bicarbonate (3 × 10 ml) and then with brine (2 × 10 ml). The organic layer was dried over anhydrous sodium sulfate and rotary evaporated to afford an oil, which was chromatographed (SiO_2, AcOEt in petroleum ether (0–30%)) to give semicarbazone **3d** (60%).

*3.5. General procedure for the preparation of semicarbazones **4a–g***

To a stirred mixture of 5-nitrothiophene-2-carboxaldehyde (2.00 mmol) and *p*-toluenesulfonic acid (catalytic amounts) in toluene (10.0 ml), **2a–g** (2.00 mmol) were added in three portions. The suspension was stirred at room temperature until the nitro compound was not present (SiO_2, 20% AcOEt in petroleum ether). The mixture was washed with 5% aqueous sodium bicarbonate (3 × 10 ml) and then with brine (2 × 10 ml). The organic layer was dried over anhydrous sodium sulfate and rotary evaporated to afford the nitrothiophene semicarbazone derivative (**4a–g**) as a solid residue, which was recrystallized from a suitable solvent. The product **4d** was purified by chromatography (SiO_2, AcOEt in petroleum ether (0–30%)) and recrystallized from a chloroform:toluene mixture.

*3.5.1. 4-Butyl-1-(5-nitrofurfurylidene)semicarbazide (**3a**)*

Yellow needles (water:acetone). Yield: 80%; m.p.: 182.0–184.0°C. IR: ν_{max} 3390, 3110, 1690, 1675, 1575, 1540, 810. ^{1}H NMR (CDCl$_3$): δ 0.98 (t, J = 8.0 Hz, 3H), 1.60 (m, 4H), 3.40 (q, J = 8.0 Hz, 2H), 6.20 (m, 1H), 6.80 (d, J = 4.0 Hz, 1H), 7.39 (d, J = 4.0 Hz, 1H), 7.70 (s, 1H), 9.80 (bs, 1H).

MS (70 eV): 254 ($M^{\cdot +}$, 4%). *Anal.* ($C_{10}H_{14}N_4O_4\cdot 2/5H_2O$): C, H, N.

*3.5.2. 4-Butyl-1-(5-nitrothienylidene)semicarbazide (**4a**)*

Orange needles (toluene). Yield: 85%; m.p.: 175.0–176.0°C. IR: ν_{max} 3380, 3110, 1675, 1555, 1530, 800. ^1H NMR ($CDCl_3$): δ 0.98 (t, J = 6.0 Hz, 3H), 1.45–1.70 (m, 4H), 3.38 (q, J = 5.0 Hz, 2H), 6.04 (m, 1H), 7.08 (d, J = 3.0 Hz, 1H), 7.85 (d + s, 2H), 9.74 (bs, 1H); MS (70 eV): 270 ($M^{\cdot +}$, 4%). *Anal.* ($C_{10}H_{14}N_4O_3S$): C, H, N.

*3.5.3. 4-Hexyl-1-(5-nitrofurfurylidene)semicarbazide (**3b**)*

Yellow needles (water:acetone). Yield: 70%; m.p.: 95.0–97.0°C. IR: ν_{max} 3410, 3120, 1675, 1520, 810. ^1H NMR (acetone-d_6): δ 0.90 (t, J = 5.0 Hz, 3H), 1.20–1.70 (m, 8H), 3.25 (q, J = 6.0 Hz, 2H), 6.78 (t, J = 6.0 Hz, 1H), 7.05 (d, J = 4.0 Hz, 1H), 7.55 (d, J = 4.0 Hz, 1H), 7.90 (s, 1H), 9.95 (bs, 1H). HRMS (70 eV): 282.1328 ($M^{\cdot +}$, 9%) (MW theor.: 282.1328). *Anal.* ($C_{12}H_{18}N_4O_4$) C, H, N.

*3.5.4. 4-Hexyl-1-(5-nitrothienylidene)semicarbazide (**4b**)*

Yellow needles (toluene). Yield: 89%; m.p.: 171.0–173.0°C. IR: ν_{max} 3375, 3100, 1668, 1560, 1528, 800. ^1H NMR ($CDCl_3$): δ 0.93 (t, J = 6.0 Hz, 3H), 1.25–1.55 (m, 4H), 1.55–1.80 (m, 2H), 3.40 (q, J = 6.0 Hz, 2H), 6.12 (t, J = 6.0 Hz, 1H), 7.13 (d, J = 4.0 Hz, 1H), 7.89 (m, 2H), 10.90 (bs, 1H). HRMS (70 eV): 298.1092 ($M^{\cdot +}$, 5%) (MW theor.: 298.1100). *Anal.* ($C_{12}H_{18}N_4O_3S$): C, H, N.

*3.5.5. 2′-(5-Nitrofurfurylidene)-1-(tetrahydro-1,4-oxazin-4-yl)methanehydrazide (**3c**)*

Yellow–green solid (toluene:petroleum ether). Yield: 60%; m.p.: 186.5–189.0°C. IR: ν_{max} 3240, 1645, 1240, 810. ^1H NMR ($CDCl_3$): δ 3.40–3.55 (m, 4H), 3.55–3.70 (m, 6H), 6.87 (d, J = 3.5 Hz, 1H), 7.28 (d, J = 3.5 Hz, 1H), 7.96 (s, 1H), 9.62 (bs, 1H). HRMS (70 eV): 268.0801 ($M^{\cdot +}$, 8%) (MW theor.: 268.0808). *Anal.* ($C_{10}H_{12}N_4O_5$): C, H, N.

*3.5.6. 2′-(5-Nitrothienylidene)-1-(tetrahydro-1,4-oxazin-4-yl)methanehydrazide (**4c**)*

Red–brown solid (toluene:petroleum ether). Yield: 70%; m.p.: 194.0–195.0°C. IR: ν_{max} 3200, 1635, 1560, 1530, 815. ^1H NMR (acetone-d_6): δ 3.45–3.55 (m, 4H), 3.62–3.72 (m, 4H), 7.30 (d, J = 4.0 Hz, 1H), 7.96 (d, J = 4.0 Hz, 1H), 8.48 (s, 1H), 9.90 (bs, 1H). HRMS (70 eV): 284.0582 ($M^{\cdot +}$, 7%) (MW theor.: 284.0579). *Anal.* ($C_{10}H_{12}N_4O_4S$): C, H, N.

*3.5.7. 1-(4-Methylhexahydropyrazin-1-yl)-2′-(5-nitrofurfurylidene)methanehydrazide (**3d**)*

Oil. Yield: 60%. IR: ν_{max} 3300, 1650, 805. ^1H NMR (D_2O:methanol-d_4): δ 2.44 (s, 3H), 2.62 (m, 4H), 3.62 (m, 4H), 7.14 (d, J = 4.0 Hz, 1H), 7.54 (d, J = 4.0 Hz, 1H), 8.03 (s, 1H). MS (20 eV): 181 ($M^{\cdot +}$ − 100, 6%).

*3.5.8. 1-(4-Methylhexahydropyrazin-1-yl)-2′-(5-nitrothienylidene)methanehydrazide (**4d**)*

Red–brown solid (chloroform:toluene). Yield: 40%; m.p.: 175.0–177.0°C. IR: ν_{max} 3250, 1680, 810. ^1H NMR (D_2O:methanol-d_4): δ 2.37 (s, 3H), 2.53 (m, 4H), 3.58 (m, 4H), 7.25 (d, J = 4.0 Hz, 1H), 7.92 (d, J = 4.0 Hz, 1H), 8.20 (s, 1H). MS (20 eV): 197 ($M^{\cdot +}$ − 100, 7%). *Anal.* ($C_{11}H_{15}N_5O_3S$): C, H, N.

*3.5.9. 4-(2-Methoxyethyl)-1-(5-nitrofurfurylidene)-semicarbazide (**3e**)*

Yellow–brown needles (ethanol:toluene). Yield: 88%; m.p.: 135.0–136.5°C. IR: ν_{max} 3408, 3115, 1690, 1520, 805. ^1H NMR (D_2O:acetone-d_6): δ 3.34 (s, 3H), 3.48 (m, 4H), 7.08 (d, J = 4.0 Hz, 1H), 7.58 (d, J = 4.0 Hz, 1H), 7.93 (s, 1H). HRMS (70 eV): 256.0810 ($M^{\cdot +}$, 6%) (MW theor.: 256.0808). *Anal.* ($C_9H_{12}N_4O_5\cdot 1/2H_2O$): C, H, N.

*3.5.10. 4-(2-Methoxyethyl)-1-(5-nitrothienylidene)-semicarbazide (**4e**)*

Yellow needles (water:ethanol). Yield: 70%; m.p.: 187.0–188.0°C. IR: ν_{max} 3400, 3100, 1670, 1545, 1525, 810. ^1H NMR (D_2O:acetone-d_6): δ 3.32 (s, 3H), 3.44–3.50 (m, 4H), 7.33 (d, J = 4.2 Hz, 1H), 7.95 (d, J = 4.2 Hz, 1H), 8.14 (s, 1H). HRMS (70 eV): 272.0575 ($M^{\cdot +}$, 4%) (MW theor.: 272.0579). *Anal.* ($C_9H_{12}N_4O_4S\cdot 1/4H_2O$): C, H, N.

*3.5.11. 1-(5-Nitrofurfurylidene)-4-(2-phenylethyl)-semicarbazide (**3f**)*

Brown needles (ethanol). Yield: 60%; m.p.: 206.0–208.0°C. IR: ν_{max} 3410, 3380, 1670, 1530, 805. ^1H NMR ($CDCl_3$): δ 2.90 (t, J = 7.0 Hz, 2H), 3.62 (q, J = 7.0 Hz, 2H), 6.20 (bs, 1H), 6.72 (d, J = 4.0 Hz, 1H), 7.26 (m, 5H), 7.35 (d, J = 4.0 Hz, 1H), 7.60 (s, 1H), 9.40 (bs, 1H). MS (70 eV): 302 ($M^{\cdot +}$, 3%). *Anal.* ($C_{14}H_{14}N_4O_4$): C, H, N.

*3.5.12. 1-(5-nitrothienylidene)-4-(2-phenylethyl)-semicarbazide (**4f**)*

Brown needles (acetone). Yield: 65%; m.p.: 218.0–221.0°C. IR: ν_{max} 3380, 1668, 1555, 1525, 815. ^1H NMR (CH_3CN-d_3:$CDCl_3$): δ 2.95 (t, J = 6.0 Hz, 2H), 3.64 (q, J = 6.0 Hz, 2H), 6.22 (t, J = 6.0 Hz, 1H), 7.11 (d, J = 5.0 Hz, 1H), 7.34 (m, 5H), 7.88 (d + s, 2H), 9.58 (bs, 1H). MS (70 eV): 318 ($M^{\cdot +}$, 9%). *Anal.* ($C_{14}H_{14}N_4O_3S$): C, H, N.

*3.5.13. 4-[2-(3,4-Dimethoxyphenyl)ethyl]-1-(5-nitrofurfurylidene)semicarbazide (**3g**)*

Yellow–green solid (water:ethanol). Yield: 70%; m.p.: 156.0–158.0°C. IR: ν_{max} 3410, 3150, 1668, 1525, 1505, 805. ^1H NMR ($CDCl_3$): δ 2.90 (t, J = 6.0 Hz, 2H), 3.65 (q, J = 6.0 Hz, 2H), 3.92 (s, 6H), 6.30 (bs, 1H), 6.70–6.93 (m, 4H), 7.40 (d, J = 4.0 Hz, 1H), 7.70 (s, 1H), 10.00 (bs, 1H). MS (70 eV): 362 ($M^{\cdot +}$, 8%). *Anal.* ($C_{16}H_{18}N_4O_6\cdot 1/2H_2O$): C, H, N.

3.5.14. 4-[2-(3,4-Dimethoxyphenyl)ethyl]-1-(5-nitrothienylidene)semicarbazide (4g)

Yellow–green needles (water:acetone). Yield: 80%; m.p.: 243.0–245.0°C. IR: ν_{max} 3520, 3440, 1715, 840. ^{1}H NMR (DMSO-d_6): δ 2.78 (t, J = 6.0 Hz, 2H), 3.45 (m, 2H), 3.78 (s, 6H), 6.70–6.90 (m, 4H), 7.46 (d, J = 4.0 Hz, 1H), 8.10 (d + s, 2H), 10.85 (s, 1H). HRMS (70 eV): 378.1003 (M^+, 13%) (MW theor.: 378.0998). *Anal.* ($C_{16}H_{18}N_4O_5S$): C, H, N.

4. Pharmacology

The new compounds **3a–g** (except **3d**) and **4a–g** were tested in vitro against *Trypanosoma cruzi*. Epimastigote forms of *T. cruzi* (Tulahuen strain) were grown in axenic media as described in Section 5. The compounds were incorporated into the media at different concentrations (20, 15, 10 and 5 μM) and their ability to inhibit growth of the parasite was evaluated in comparison to the control (no drug added to the media). Nfx was used as the reference trypanocidal drug. Growth of the parasite was followed for 11 days by measuring the increase in absorbance at 600 nm, which was proved to be proportional to the number of cells present. Fig. 1 shows the inhibition of growth by micromolar concentrations of compounds **3a** and **4a**. Compound **3a** achieved almost 100% inhibition at 5 μM concentration, showing a trypanocidal activity similar to that of the reference drug, Nfx. However, the analogue sulfur derivative, compound **4a**, had to be present at 10 times higher concentrations to significantly inhibit parasite growth with respect to the control. In the same way, all the compounds synthesized (**3a–3g** and **4a–4g**) were tested and the results are summarized in Table 2. The percentage of inhibition reported in Table 2 was calculated as follows:

$$\% = \{1 - [(A_p - A_{0p})/(A_c - A_{0c})]\} \times 100$$

where $A_p = A_{600}$ of the culture containing the drug at day 5 or 7; $A_{0p} = A_{600}$ of the culture containing the drug right after addition of the inocula (day 0); $A_c = A_{600}$ of the culture in the absence of any drug (control) at day 5 or 7; $A_{0c} = A_{600}$ in the absence of the drug at day 0.

5. Pharmacological experimental

As a primary screening for trypanocidal activity of the new series of compounds synthesized, inhibition of epimastigote growth was assessed. *Trypanosoma cruzi* epimastigotes (Tulahuen 2 strain) were grown at 28°C in an axenic medium (BHI-Tryptose) as previously described [26], complemented with 10% fetal calf serum. Cells from a 10-day old culture (stationary phase) were inoculated into 50 ml of fresh culture medium to make an initial concentration of 1×10^6 cells/ml. Cell growth was followed by measuring everyday the absorbance at 600 nm of the culture. Before inoculation, the media were supplemented with the indicated amount of the drug from a stock solution in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the culture media never exceeded 0.4% and the control was run in the presence of 0.4% DMSO and in the absence of any drug. No effect on epimastigotes growth was observed by the presence of up to 1% DMSO in the culture media.

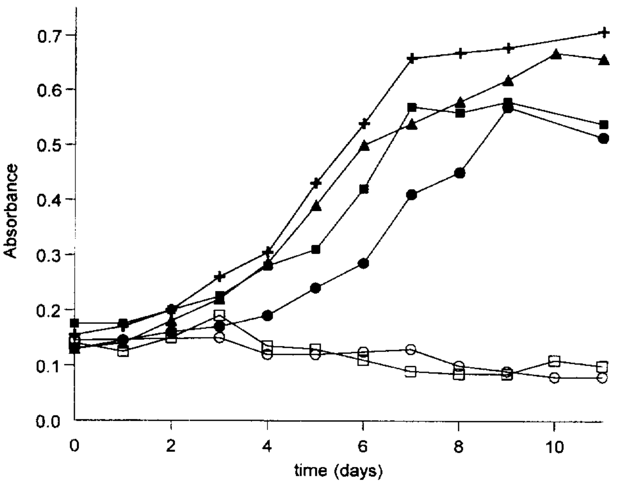


Fig. 1. Effect of compound **3a**, **4a** and Nfx on the growth of *T. cruzi* epimastigotes. At day 0, the parasite cells (1×10^6 cells/ml) were suspended in culture media containing: 0.4% DMSO, no drug (+), 5 μM Nfx (●), 5 μM compound **3a** (■), 15 μM compound **4a** (▲), 10 μM Nfx (○), and 10 μM compound **3a** (□). Growth was followed by measuring absorbance at 600 nm everyday. The results are the means of three different experiments with an SD less than 10% in all cases.

Table 2
Trypanocidal activity of semicarbazone derivatives

Compound	Conc. (μM)	Percentage of growth inhibition [a] (%)	
		Day 5	Day 7
3a	5	51	22
4a	10	3	13
3b	5	62	37
4b	10	4	0
3c	10	2	6
4c	10	0	9
4d	10	0	6
3e	10	50	47
4e	10	0	1
3f	10	16	15
4f	10	3	9
3g	10	23	9
4g	10	0	6
Nfx	5	67	42

[a] Percentage of growth inhibition was calculated as described in Section 4.

6. Results and discussion

Fourteen new semicarbazone derivatives were prepared using a three step synthetic route from 5-nitro-2-furaldehyde and 5-nitrothiophene-2-carboxaldehyde. All compounds were purified by recrystallization, except compound **3d** which was an oil.

As shown in Table 2, only nitrofurfurylidene derivatives (**3a–3g**) showed interesting in vitro trypanocidal activity. The corresponding nitrothienylidene derivatives (**4a–4g**) were significantly less active (see Table 2 and Fig. 1).

Semicarbazones containing a ring in their aminic portion showed very poor activity. This applies for both homocyclic (**3f**, **4f** and **3g**, **4g**) and heterocyclic (**3c**, **4c** and **4d**) rings. Lipophilicity does not play an important role, as can be seen from the activities of **3f** and **3g**.

All nitrofurfurylidene semicarbazones having an acyclic aminic residue displayed good activity. According to Table 2, compounds **3a**, **3b** and **3e** are the best potential inhibitors, the highest activity corresponding to the semicarbazone derivative **3b**. The aminic residue of both **3a** and **3e** contains a four-atom chain (**a** = butyl, **e** = 2-methoxyethyl), whereas the residue for **3b** contains a six-atom chain (**b** = hexyl). This suggests that, for this set of compounds, the activity is more dependent on the chain length than on the electronic effects brought about by the presence of heteroatoms.

Compared to semicarbazones **3a** and **3e**, the structure of **3b** is more like the structure of trypanothione because of the length of the hexyl residue, which better corresponds to the spermidine moiety (4-azaoctane-1,8-diamine). Consequently, **3b** should display higher activity than the semicarbazones with shorter chain **3a** and **3e**, which is indeed the observed case.

Studies of the toxicity of these compounds towards mammalian cells are currently underway, as well as in vivo studies on trypanocidal activity. In addition, electrochemical and EPR measurements to determine the ability to form free radicals are in progress.

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