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Original article

Synthesis and antitrypanosomal evaluation of *E*-isomers of 5-nitro-2-furaldehyde and 5-nitrothiophene-2-carboxaldehyde semicarbazone derivatives. Structure–activity relationships.

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Abstract – Several novel semicarbazone derivatives were prepared from 5-nitro-2-furaldehyde or 5-nitrothiophene-2-carboxaldehyde and semicarbazides bearing a spermidine-mimetic moiety. All derivatives presented the *E*-configuration, as determined by NMR-NOE experiments. These compounds were tested in vitro as potential antitrypanosomal agents, and some of them, together with the parent compounds, 5-nitro-2-furaldehyde and 5-nitrothiophene-2-carboxaldehyde semicarbazone derivatives, were also evaluated in vivo using infected mice. Structure–activity relationship studies were carried out using voltammetric response and lipophilic–hydrophilic balance as parameters. Two of the compounds (**1** and **3**) displayed the highest in vivo activity. A correlation was found between lipophilic–hydrophilic properties and trypanocidal activity, high R_M values being associated with low in vivo effects. © 2000 Éditions scientifiques et médicales Elsevier SAS

5-nitrofurfural and 5-nitrothiophene-2-carboxaldehyde semicarbazones / antitrypanosomal activity / in vivo evaluation

1. Introduction

Trypanosomiasis and leishmaniasis are major third-world diseases, with several millions of new infections presenting annually. *Trypanosoma cruzi* (*T. cruzi*) is the etiological agent of Chagas' disease [1, 2]. The current chemotherapy against this disease is still inadequate. The main drug in use is Nifurtimox® (Nfx), however, it has undesirable side effects [3, 4] and is yet inefficient to treat chronic Chagas' disease. A characteristic ESR signal corresponding to the nitro anion radical ($R–NO_2^{\cdot-}$) appears when Nfx is added to intact *T. cruzi* cells [5], suggesting that intracellular reduction of Nfx, followed by redox cycling yielding the superoxide anion, may be its major mode of action against *T. cruzi* [4–6]. To develop more selective and, consequently, less toxic drugs, research is often directed to exploit key differences between the metabolism of host and parasite. In the case of trypanosomatids, trypanothione metabolism can be regarded as a specific target [7]. Previous work [8–10] supported the idea of designing compounds which bear a positive charge and/or a flexible spermidine-like side chain capable of selectively inhibiting trypanothione reductase (TR). TR is a critical enzyme for the parasite, responsible for catalysing the reduction of trypanothione disulfide to trypanothione (N_1, N_8-bis(glutathionyl)-sper-

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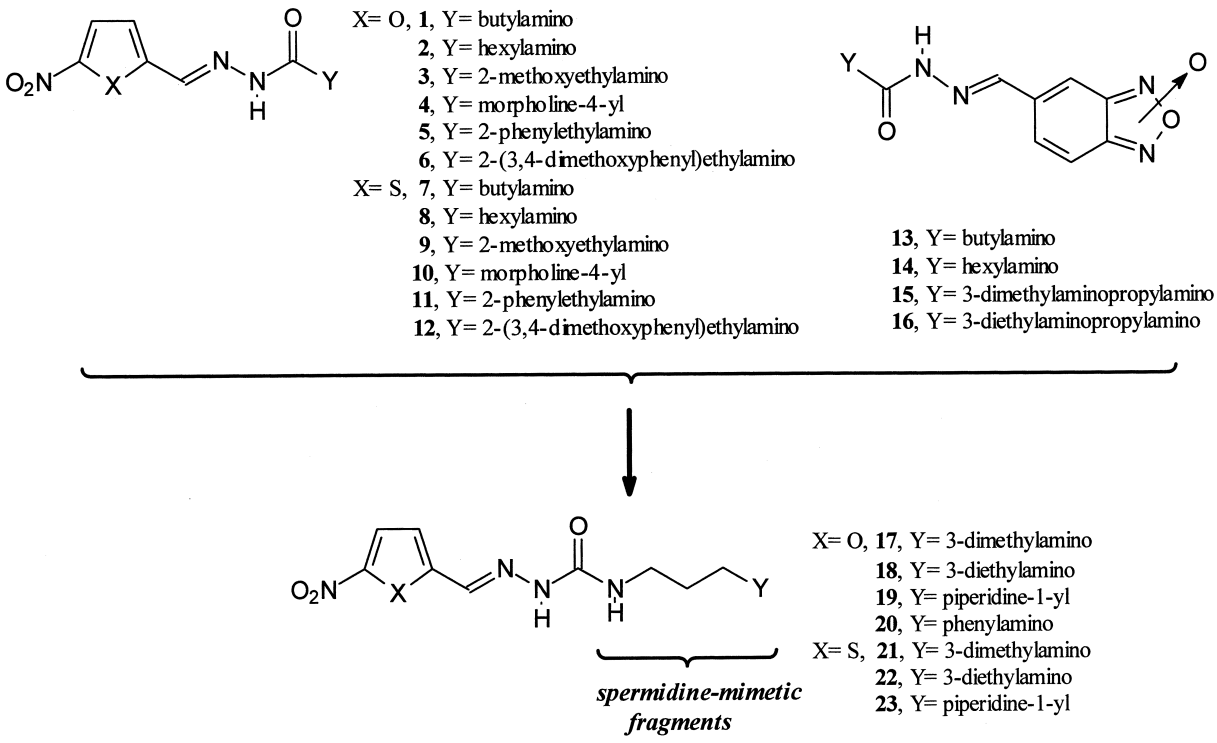

Figure 1. Chemical structures of the 'parent' compounds and the semicarbazone derivatives studied.

midine), which participates in oxygen-derived species detoxification [11–13].

We have previously reported [14–16] the synthesis and biological activity against *T. cruzi* epimastigote forms of a series of 5-nitrofurfural and 5-nitrothiophene-2-carboxaldehyde derivatives ('parent' compounds). Some of these compounds proved to generate nitro anion radicals, which were characterized using ESR spectroscopy [17, 18]. The derivatives **1** and **2** (*figure 1*) showed, in vitro, the best trypanocidal activity [15]. On the other hand, we have synthesized and evaluated compounds containing an *N*-oxide moiety as the potential pharmacophore of antitrypanosomal drugs. For these compounds, derivatives **13** and **14** (*figure 1*) showed the most interesting in vitro trypanocidal activity [19]. When, in these type of compounds, the alkylamino semicarbazide moiety was changed to an [(*N*, *N*-dialkylamino)alkyl]-amino group ('spermidine-mimetic' group, compounds **15** and **16**) a significant decrease in the in vitro activity was observed [19].

Since the nitrofurane and nitrothiophene groups presented a good profile of activity, we focused our efforts on developing a series of compounds containing the nitroheterocyclic group and a spermidine-mimetic moiety in a single molecule.

Herein, we report on the preparation and preliminary in vitro and in vivo evaluation of a series of 5-nitro-2-furaldehyde and 5-nitrothiophene-2-carboxaldehyde semicarbazone derivatives (**17**–**23**, *figure 1*) in which the N_4-semicarbazide moieties are spermidine-mimetic residues. We have also included preliminary in vivo characterization of parent compounds (**1**–**12**, *figure 1*). We also performed electrochemical measurements and relative lipophilicity determinations in order to study structure–activity relationships.

2. Chemistry

Compounds **17**–**23** were obtained as shown in *figure 2*. The spermidine-mimetic amines were prepared by treatment of simple amines (piperidine or aniline) with acrylonitrile [20, 21], followed by hydrogenation (Raney-Ni/Ac_2O) and hydrolysis for **III**, or reduction ($LiAlH_4$) for **IV** [22]. The semicarbazides reactants **V**–**VIII** were prepared according to a known procedure [15]. Thus,

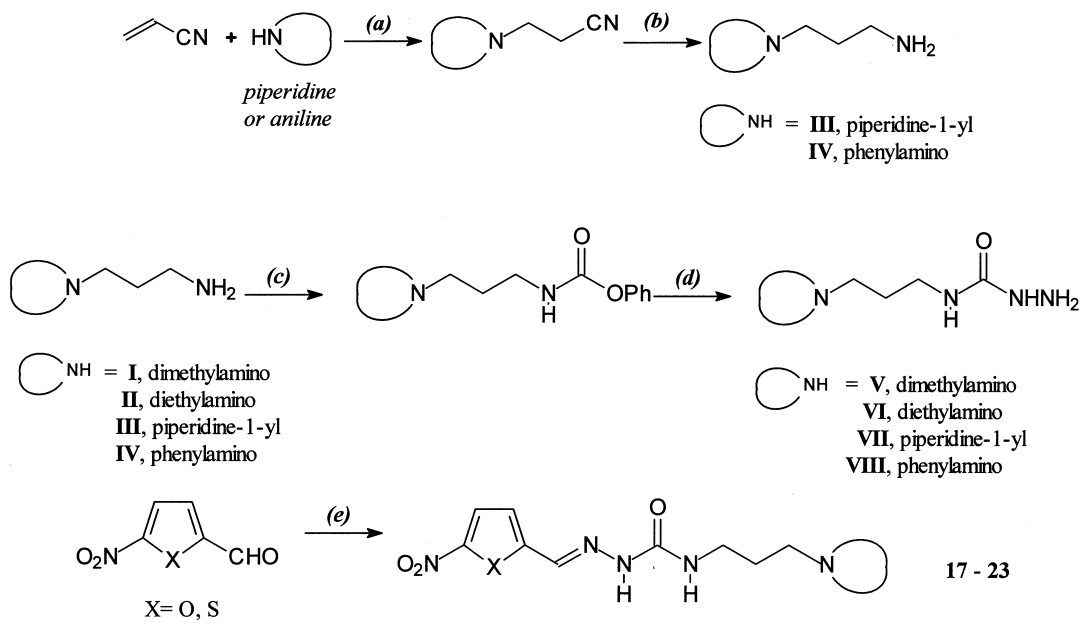

Figure 2. Scheme for the synthesis of compounds **17–23**.
Conditions: a) Reflux/6 h, for piperidine; p-TsOH/reflux/48 h, for aniline; b) 1. Raney-Ni-Ac_2O/50 °C/50 psi, 2. $H_3O^{+,}$ for **III**; $LiAlH_4/Et_2O$/r.t., for **IV**; c) Phenyl chloroformate/Et_3N/CH_2Cl_2/r.t.; d) $NH_2NH_2 \cdot H_2O$/80–90 °C; e) **V–VIII**/p-TsOH/MeOH/r.t.

V–VIII were obtained from commercially available 3-(dimethylamino)propylamine, 3-(diethylamino)propylamine or **III** or **IV**, by treatment with phenyl chloroformate to give the corresponding carbamates, and further reaction with hydrazine hydrate. Reaction of **V–VIII** with 5-nitro-2-furaldehyde in methanol, catalysed with *p*-toluenesulfonic acid, gave the compounds **17–20** in moderate yields. 5-Nitrothiophene-2-carboxaldehyde was treated with semicarbazides **V–VII** to produce the thio derivatives **21–23** in moderate yields. All new compounds were identified by ^{1}H-NMR, IR and MS and their purity established by TLC and microanalysis. Compounds **19** and **23** were purified as the corresponding hydrochlorides.

The stereochemical assignment as *E*-isomers was performed through NOE difference spectroscopy. For example, on irradiation at the frequency of the NH semicarbazone proton (10.94 ppm) in **21**, a positive NOE was observed for the signals at 8.08 (ylidenic proton) and 7.25 ppm (NH) (*figure 3*). Selected NOE data for compounds **1**, **21** and **23** are available as supplementary material. The positive NOE observed for the iminic proton allowed us to assign the stereochemistry around the double bond as *E*.

3. Pharmacology

3.1. In vitro activities

All new compounds (**17–23**) were tested in vitro against *T. cruzi* [23]. Epimastigote forms of *T. cruzi*, Tulahuen strain, were grown in axenic media as described in the Experimental protocols. The compounds were incorporated into the media at 25 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

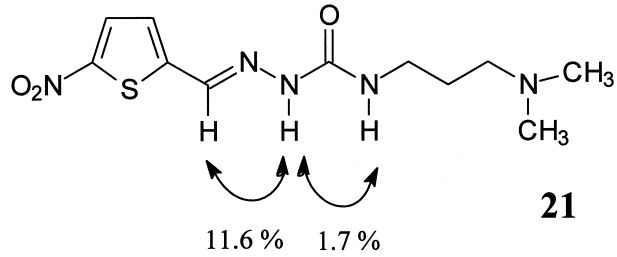

Figure 3. Positive NOE effects observed in compound **21**.

Table I. Biological and physico-chemical properties of 5-nitrofurane derivatives.

Compound	In vitro bioassays[a,b]		In vivo bioassays[a]				
	Dose (μM)	Percentage of growth inhibition (%)	Growth inhibition, middle of treatment (%), (day)	Growth inhibition, last day of treatment (%), (day)	Percentage of survival, day 28	E_{pc}, V vs. SCE[c]	R_M
1	5	51[d]	63, (14)	94, (21)	100	–0.86	0.43
2	5	62[d]	26, (14)	–22, (21)	60	–0.83	1.06
3	10	50[d]	90, (14)	99, (20)	100	–0.86	0.07
4	10	2[d]	4, (14)	57, (20)	60	–0.85	0.03
5	10	16[d]	85, (15)	94, (21)	80	–0.87	0.68
6	10	23[d]	21, (15)	86, (21)	40	–0.86	0.52
7	10	3[d]	44, (14)	64, (21)	60	–0.79	0.75
8	10	4[d]	4, (14)	–14, (21)	20	–0.78	1.38
9	10	0[d]	42, (15)	82, (21)	60	–0.78	0.35
10	10	0[d]	–41, (14)	44, (20)	20	–0.78	0.33
11	10	3[d]	–24, (15)	–[e]	0	–0.79	1.06
12	10	0[d]	38, (14)	50, (21)	40	–0.79	0.95
17	25	6	–14, (17)	–125, (23)	80	–0.88	0.63
	10	1					
18	25	8	32, (17)	29, (23)	80	ND[f]	0.33
	10	1					
19	25	27	ND	ND		ND	0.59
20	25	97	ND	ND	–	–0.84	0.92
	5	28					
21	25	6	ND	ND	–	ND	0.70
	10	1					
23	25	33	ND	ND	–	–0.78	0.72
Nfx	5	67	100, (14)	100, (20)	100	–0.91	0.16
Bzl	–	ND	99, (15)	100, (21)	95	ND	ND
Control	–	–	0	0	65	–	–

[a]See Experimental protocols for the method of determination and calculation. [b]The results are the mean of three different experiments with a SD less than 10% in all cases. [c]First reduction step of nitro group. Peak potentials (~ ± 0.01 V) measured at a scan rate of 0.2 V/s. [d]From reference [15]. [e]The animals treated did not survive. [f]Not determined.

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 [15, 19]. The percentage of inhibition, summarized in *table I*, 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; $A_{0p} = A_{600}$ 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; $A_{0c} = A_{600}$ in the absence of the drug at day 0.

3.2. In vivo activities

The parent compounds **1**–**12**, **17** and **18** were tested in vivo, using mice infected with *T. cruzi* (Tulahuen strain). Swiss mice were inoculated with the trypomastigote form (day zero) as described in the Experimental protocols. Ten days after infection the compounds were given in 10 daily doses (66 mg/kg body weight/day) and parasitaemia was measured in blood on days 10, 15, 20 and 25 after infection. Percentages of *T. cruzi* growth inhibition were calculated with respect to controls receiving only the vehicle. Nfx or Benznidazole® (Bzl) were used as the reference trypanocidal drugs. Parasitaemia in blood was determined by measuring the number of parasites per 100 microscopic fields. These results for two typical experiments are shown in *figure 4*.

4. Electrochemical studies

The voltammetric response of semicarbazones **1**–**12**, **17**, **20** and **23** was determined in DMSO at a mercury dropping working electrode [17, 18, 24]. The derivatives presented two reduction peaks during forward cathodic scan, which were linked to oxidation peaks. Only the first reduction step was studied as a function of voltage sweep rate and switching potential. *Table I* summarizes *E*pc (cathodic peak potential) for the first reduction step of the nitro moiety.

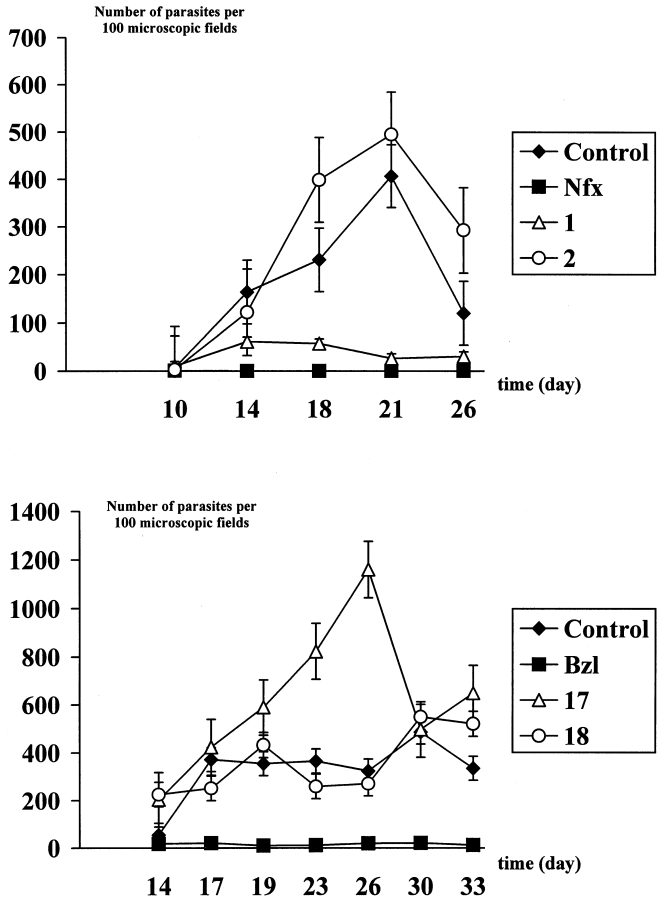

Figure 4. In vivo biological results for compounds **1**, **2**, **17**, **18** and reference drugs (Nfx and Bzl).

5. Lipophilicity studies

Reverse-phase TLC experiments were performed on precoated TLC-C_{18} and eluted with methanol:water (50:50, v/v) [19, 24]. The R_f values for compounds **1**–**12**, **17**–**21** and **23** were converted into R_M values using the relationship: $R_M = \log [(1/R_f) - 1]$ [19, 24–29]. *Table I* shows the R_M values obtained for each new compound and Nfx, used as the reference drug.

6. Results and discussion

As shown in *table I*, the use of spermidine-mimetic residues in the nitrofurane or nitrothiophene radical-producing moieties resulted in a significant decrease in the in vitro activity. At 10 μM, compounds **17**, **18** and **21** were not able to inhibit parasite growth. However, the parent compounds (**1**–**3**), were 50–100-fold more active [15]. Only compound **20** displays some interesting in vitro activity at 25 μM; however **20** was 2-fold less active than **1**–**2** at 5 μM.

Given the poor activity displayed by the spermidine-mimetic compounds **17** and **18** in both in vitro and in vivo assays, and considering the low activity of all this type of derivative in the in vitro assay, it was decided not to submit the whole series of spermidine-mimetic derivatives to the in vivo test.

In vivo bioassays showed that some of the 5-nitrofurane derivatives could be considered trypanocidal drugs. Compounds **1** (*figure 4*) and **3** have shown to be the best in vivo inhibitors, with the best survival percentage (mice treated with derivative **3** survived more than 35 days). They were also good inhibitors of epimastigote growth at low concentrations. However, compound **2**, that displayed the highest in vitro activity, did not show any *T. cruzi* growth inhibition in vivo, suggesting a poor bioavailability.

The electrochemical studies show that the potential for the first reduction step of the nitro moiety is similar for all 5-nitrofurane derivatives (–0.83 to –0.88 V) and for all 5-nitrothiophene derivatives (–0.78 to –0.79 V), and less negative than the corresponding potential for Nfx (–0.91 V). Therefore, the difference in biological activities observed can not be attributed to difference in the reduction potential capacities of the compounds since all of them are potentially good reducers.

We were unable to find correlations between in vitro activity and the lipophilic–hydrophilic properties (as R_M values). Perhaps this parameter appears as a key property for an in vivo active drug. The null in vivo activities of **2**, **8** and **11** can be related with their R_M values higher than 1.06 (1.06, 1.38 and 1.06, respectively). The R_M values for the more in vivo active and less toxic members (**3**, **1** and **5**) are closer to Nifurtimox's R_M (0.07, 0.43, 0.68 and 0.16, respectively). A secondary effect caused by high lipophilicity could be expected in which the effect of drugs enhance the parasite growth. A reasonable explanation for this fact on parasitaemia must involve a lessening of the host defensive mechanisms. Immunosupression can not be ruled out. The same effect was previously reported for another set of lipophilic drugs tested by us [30]. Since the products were administered orally, the absorption, distribution, metabolism and excretion in the mouse could be different for each of the compounds. So the lipophilicity could play an important role in the pharmacokinetic properties of these kinds of compound.

7. Conclusions

A new series of semicarbazone derivatives was synthesized from 5-nitro-2-furaldehyde or 5-nitrothiophene-2-carboxaldehyde and semicarbazides bearing a spermidine-mimetic moiety. The incorporation of the spermidine-mimetic group was intended to increase the selectivity of these compounds to the critical parasite TR. However, the antitrypanosomal assays (in vitro and in vivo) of these derivatives showed that they were less effective than the parent compounds. Structure–activity relationship studies of these type of compounds indicate that the lipophilic–hydrophilic balance could play an important role in the in vivo activity. All active compounds have low redox potential for the monoelectronation of the nitro group. However, we cannot assure this being a sufficient condition for activity since other compounds of the series with similar redox potentials displayed no activity.

Recently, two proteins involved in the trypanothione-dependent hydroperoxide metabolism have been purified in *Crithidia fasciculata* [31, 32], tryparedoxin and tryparedoxin peroxidase. These proteins function in concert to reduce hydroperoxides at the expense of reduced trypanothione and therefore represent a more promising target for drug development than inhibition of trypanothione reductase itself.

8. Experimental protocols

8.1. Chemistry

All starting materials were commercially available research-grade chemicals and used without further purification. The compounds **1–12**, and **III–VIII** were prepared according to literature [15, 19]. All solvents were dried and distilled prior to use [33]. All the reactions were carried out in a nitrogen atmosphere. The typical work-up included washing with brine and drying the organic layer with sodium sulfate. 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 phosphorous pentoxide at 3–4 mm Hg, 24 h at room temperature) and performed on a Fisons EA 1108 CHNS-O analyser. Analyses indicated by the symbols of the elements were within ± 0.4% of theoretical values. Infrared spectra were recorder 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 (at 100 MHz) instrument, or DPX-Bruker 400 (at 400 MHz) instrument, with tetramethylsilane as the internal reference and in the indicated solvent; the chemical shifts are reported in ppm. Mass spectra were recorded on a Shimadzu GC-MS QP 1100 EX instrument at 70 eV.

8.1.2. General procedure for compounds **17**–**23**

A mixture of aldehyde (5-nitro-2-furaldehyde or 5-nitrothiophene-2-carboxaldehyde) (1 equiv.), the corresponding semicarbazide (1 equiv.), *p*-TsOH (catalytic amounts) and MeOH as solvent was stirred at room temperature until the carbonyl compound was not present (SiO_2, 1% MeOH in CH_2Cl_2). After the work-up process the residue was purified as indicated.

8.1.2.1. 4-(3-Dimethylamino)propyl-1-(5-nitrofurfurylidene)semicarbazide **17**

Purified by column chromatography (Al_2O_3, CH_2Cl_2:MeOH (0–10%)), brown/orange solid (35%); m.p. 161.0–163.0 °C; IR ν_{max} 3 450, 2 920, 1 670, 1 520, 1 320, 810 cm^{-1}. ^{1}H-NMR (methanol-d_4:D_2O (9:1), 400 MHz): δ 1.78 (q, *J* = 7.2 Hz, 2H), 2.31 (s, 6H), 2.45 (t, *J* = 7.2 Hz, 2H), 3.35 (m, 2H), 7.03 (d, *J* = 3.8 Hz, 1H), 7.54 (d, *J* = 3.8 Hz, 1H), 7.79 (s, 1H); MS, m/z (abundance): 283 ($M^{\cdot+}$, 1.2%), 58 ($CH_2=N^+(CH_3)_2$, 100.0%). Anal. ($C_{11}H_{17}N_5O_4$) C, H, N.

8.1.2.2. 4-(3-Diethylamino)propyl-1-(5-nitrofurfurylidene)semicarbazide **18**

Purified by column chromatography (Al_2O_3, CH_2Cl_2:MeOH (0–10%)), orange/yellow solid (48%); m.p. 135.0–137.0 °C; IR ν_{max} 3 340, 1 650, 1 630, 1 560 cm^{-1}. ^{1}H-NMR (methanol-d_4:D_2O (9:1), 100 MHz): δ 1.04 (t, *J* = 6.0 Hz, 6H), 1.64 (m, 2H), 2.54 (m, 6H), 3.30 (m, 2H), 7.00 (d, *J* = 4.0 Hz, 1H), 7.50 (d, *J* = 4.0 Hz, 1H), 7.74 (s, 1H); MS, m/z (abundance): 311 ($M^{\cdot+}$, 1.0%), 282 ($M^{\cdot+}$–29, 3.0%), 86 ($CH_2=N^+(CH_2CH_3)_2$, 100.0%). Anal. ($C_{13}H_{21}N_5O_4$) C, H, N.

8.1.2.3. 1-(5-Nitrofurfurylidene)-4-(3-piperidine-1-yl)-propylsemicarbazide hydrochloride **19**

Purified by column chromatography (SiO_2, CH_2Cl_2:MeOH (0–5%)), yellow oil (60%). The oil was dissolved in MeOH and treated with Et_2O saturated in hydrogen chloride. The hydrochloride was crystallized from Et_2O:MeOH, orange needles (37%); m.p. 185.0–187.5 °C; IR ν_{max} 3 460, 1 650, 1 558, 820 cm^{-1}. ^{1}H-NMR (methanol-d_4:D_2O (9:1), 400 MHz): δ 1.54 (m, 1H), 1.83 (m, 3H), 2.01 (m, 4H), 2.95 (m, 2H), 3.17 (m, 2H), 3.42 (t, *J* = 6.0 Hz, 2H), 3.56 (m, 2H), 7.06 (d, *J* = 4.0 Hz, 1H), 7.55 (d, *J* = 4.0 Hz, 1H), 7.82 (s, 1H); MS, m/z (abundance): 323 ($M^{\cdot+}$, 0.6%), 169

($O=C^+NH(CH_2)_3N(CH_2)_5$, 22.0%), 98 ($CH_2=N^+(CH_2)_5$, 100.0%). Anal. ($C_{14}H_{21}N_5O_4 \cdot HCl \cdot 2/3H_2O$) C, H, N.

8.1.2.4. 1-(5-Nitrofurfurylidene)-4-(3-phenylaminopropyl)semicarbazide **20**

Purified by column chromatography (SiO_2, CH_2Cl_2:MeOH (0–10%)), brown solid (45%); m.p. 182.0–184.0 °C; IR ν_{max} 3 475, 1 660, 1 560, 1 500, 800 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 100 MHz): δ 1.64 (m, 2H), 3.30 (m, 2H), 3.80 (m, 2H), 7.12 (d, *J* = 4.0 Hz, 1H), 7.24–7.54 (m, 5H), 7.76 (d, *J* = 4.0 Hz, 1H), 7.82 (s, 1H); MS, m/z (abundance): 177 ($O=C^+NH(CH_2)_3NHPh$, 100.0%), 181 ($O_2N(C_4H_2O)CH=NN=C=O]^+$, 5.0%), 77 ($C_6H_6^+$, 27.0%). Anal. ($C_{15}H_{17}N_5O_4$) C, H, N.

8.1.2.5. 4-(3-Dimethylamino)propyl-1-(5-nitrothenylidene)semicarbazide **21**

Purified by crystallization from MeOH:EtOAc:Et_2O, orange needles (39%); m.p. 141.0–143.0 °C; IR ν_{max} 3 380, 3 110, 2 950, 1 670, 1 550, 810 cm^{-1}. ^{1}H-NMR (methanol-d_4:D_2O (9:1), 100 MHz): δ 1.80 (m, 2H), 2.45 (s, 6H), 2.80 (t, *J* = 6.0 Hz, 2H), 3.44 (t, *J* = 6.0Hz, 2H), 7.32 (d, *J* = 4.0 Hz, 1H), 7.98 (d, *J* = 4.0 Hz, 1H), 8.06 (s, 1H); MS, m/z (abundance): 299 ($M\cdot^+$, 3.0%), 58 ($CH_2=N^+(CH_3)_2$, 100.0%). Anal. ($C_{11}H_{17}N_5O_3S$) C, H, N, S.

8.1.2.6. 4-(3-Diethylamino)propyl-1-(5-nitrothenylidene)semicarbazide **22**

Purified by column chromatography (SiO_2, CH_2Cl_2:MeOH (0–5%)), orange oil (45%); IR ν_{max} 3 390, 3 120, 2 980, 1 660, 1 540, 810 cm^{-1}. ^{1}H-NMR (acetone-d_6, 100 MHz): δ 1.10 (t, *J* = 6.0 Hz, 6H), 1.80 (m, 2H), 2.70 (m, 6H), 3.40 (m, 2H), 7.38 (d, *J* = 4.0 Hz, 1H), 7.96 (d, *J* = 4.0 Hz, 1H), 8.20 (s, 1H); MS, m/z (abundance): 327 ($M\cdot^+$, 1.0%), 298 ($M\cdot^+$-29, 4.0%), 86 ($CH_2=N^+(CH_2CH_3)_2$, 100.0%). Anal. ($C_{11}H_{17}N_5O_3S \cdot 1/2H_2O$) C, H, N, S.

8.1.2.7. 1-(5-Nitrothenylidene)-4-(3-piperidine-1-yl)-propylsemicarbazide hydrochloride **23**

Purified by column chromatography (SiO_2, CH_2Cl_2:MeOH (0–5%)), orange oil (58%). The oil was dissolved in MeOH and treated with Et_2O saturated in hydrogen chloride. The hydrochloride was crystallized from Et_2O:MeOH, orange/brown needles (34%); m.p. 122.0–124.5 °C; IR ν_{max} 3 500, 1 640, 1 560, 1 500, 810 cm^{-1}. ^{1}H-NMR (methanol-d_4:D_2O (9:1), 400 MHz): δ 1.53 (m, 1H), 1.82 (m, 3H), 2.00 (m, 4H), 2.95 (m, 2H), 3.16 (m, 2H), 3.42 (t, *J* = 6.5 Hz, 2H), 3.56 (m, 2H), 7.30 (d, *J* = 4.0 Hz, 1H), 7.95 (d, *J* = 4.0 Hz, 1H), 8.04 (s, 1H); MS, m/z (abundance): 339 ($M\cdot^+$, 5.0%), 169 ($O=C^+NH(CH_2)_3N(CH_2)_5$, 18.0%), 98 ($CH_2=N^+(CH_2)_5$, 100.0%). Anal. ($C_{14}H_{21}N_5O_3S \cdot HCl \cdot H_2O$) C, H, N, S.

8.2. Biology

In vitro trypanocidal activity against *T. cruzi* epimastigotes was evaluated using established procedures [23]. *Trypanosoma cruzi* (Tulahuen 2 strain) were grown at 28 °C in an axenic medium (BHI-Tryptose), complemented with 10% foetal calf serum. Cells from a 10-day-old culture (stationary phase) were inoculated into 50 mL of fresh culture medium to give an initial concentration of 1×10^6 cells/mL. Cell growth was followed by measuring the absorbance of the culture at 600 nm everyday. Before inoculation, the media was supplemented with the indicated amount of the drug from a stock solution in 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 epimastigote growth was observed in the presence of up to 1% DMSO in the culture media.

In vivo trypanocidal activity was evaluated using infected mice. Two sets of Swiss mice (Swiss strain, male, 30–45 days old, 5 in each group) were inoculated intraperitionally with 1 000 trypomastigotes (Tulahuen strain) from blood of infected mice. Ten days thereafter, when the parasitaemia was increasing, the animals were given the compound (66 mg/kg body weight/day) for 10 days (from the 10th to the 20th day). The compounds, suspended in carboxy methyl cellulose, were administered by oral route using a gastric catheter. The animals were slightly anaesthetized with Et_2O during the dosage. Animals given the vehicle as above were used as controls. After 10, 15, 20 and 25 days of infection, parasitaemia was counted (number of parasites per 100 microscopic fields). The survival rates (percentages of live mice) were calculated for the most extended period (Day 28).

8.3. Electrochemical method

Voltammetric responses for the compounds were obtained by cyclic voltammetry. Experiments were carried out in DMSO (Aldrich, spectroscopy grade) with 0.1 M tetrabutylammonium perchlorate (Fluka) as the supporting electrolyte and purged with nitrogen at room temperature. Typically 10–12 mg of compound was used in a cell volume of ≈ 40 mL. A mercury-dropping electrode was used as the working electrode, a platinum wire as the auxiliary electrode and saturated calomel as the reference electrode. Voltammograms were obtained using a Weenking POS 88 instrument with a Kipp Zenen BD93 recorder. Voltage scan rates ranged from 0.1–0.5 V/s.

8.4. Lipophilicity studies

Reverse-phase TLC experiments were performed on precoated TLC plates SIL RP-18W/UV_{254} (Macherey-Nagel) and eluted with MeOH (Aldrich, HPLC grade): H_2O (distilled) (50:50, v/v). The plates were developed in a closed chromatographic tank, dried, and the spots were located under UV light.

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