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# Synthesis and in vitro antitrypanosomal activity of novel Nifurtimox analogues

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**Abstract**—Eight novel analogues of Nifurtimox, 4-[(5-nitrofurfurylidene)amino]-3-methylthiomorpholine-1,1-dioxide, containing alfa–beta unsaturated amides, were synthesized and evaluated for their in vitro activity against *Trypanosoma cruzi* epimastigotes. Four derivatives bearing a nitro group at the 5-position of the furan ring were the most active in inhibiting culture growth and provoking cell death, showing trypanocidal activity more than threefold the potency of Nifurtimox, our positive control. Two derivatives lacking a nitro group were less potent than the positive control. Active nitro derivatives very efficiently caused epimastigote death, which suggests a nitro reduction mechanism of action.


## 1. Introduction

The flagellate *Trypanosoma cruzi*, the etiological agent of Chagas disease or American trypanosomiasis, affects more than 18 million people in Latin America, leading to approximately 400,000 deaths per year. Since 1909 when this disease was initially characterized[1] several efforts were carried out in order to eradicate it, but in spite of the 75% reduction in the incidence of human cases observed in the South American countries (i.e., Argentine, Brazil, Chile, Paraguay, Uruguay and Venezuela) as a consequence of the successful vector control programs being set up,[2] Chagas disease still remains a regional health problem. Nifurtimox (a 5-nitrofuran derivative), Benznidazole (a 2-nitroimidazole acetamide) and Ketoconazole (a piperazine–imidazole–dioxolan), ameliorate acute *T. cruzi* infection[3] but exert serious side effects and little or no effect on the chronic phase of the disease,[4] probably due to the inherent characteristics of the host and the virulence and resistance of the parasite. Development of safer and more efficient therapeutic anti-*Trypanosoma cruzi* compounds continues to be a major goal in trypanocidal chemotherapy.

Several authors have reported that *T. cruzi* is particularly sensitive to compounds that can produce free radicals due to its lack of catalase and glutathione peroxidase, and proposed that the main mechanism by which Nifurtimox acts against *T. cruzi* is through the intracellular production of superoxide anion.[5]

Various series of 5-nitrofuran derivatives containing different heterocyclic moieties and alfa–beta unsaturated amides have been synthesized since their structural domains were associated with biological activities, such as trypanocidal, bactericidal, fungicidal and squistosomicidal.[6]

## 2. Chemistry

To prepare compounds **1**, **2**, **3**, **6** and **8**, 2-furylacrilic acid was treated with nitric acid in acetic anhydride, which led to 5-nitro-2-furylacrilic acid.[7] Reaction of this compound with thionyl chloride in benzene yielded the corresponding acid chloride, which by reaction with either isobutylamine, benzylamine, 4-chlorobenzylamine,

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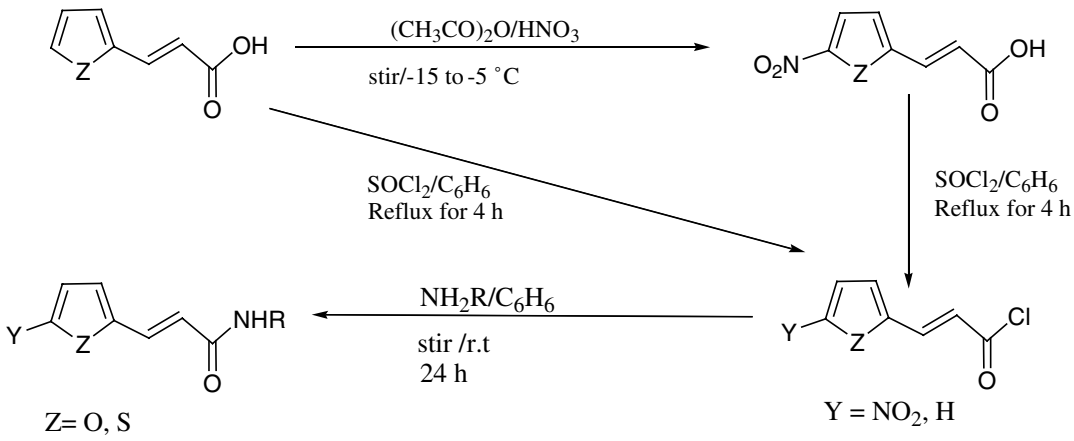
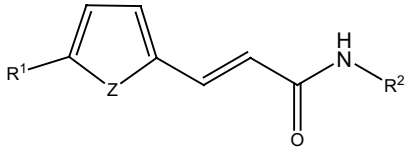
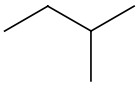
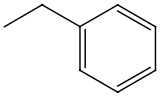
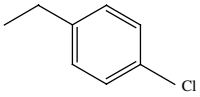
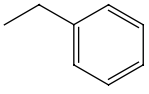
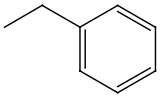
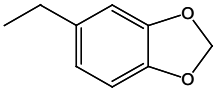
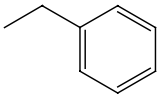
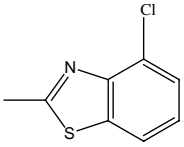



**Scheme 1.** General procedure for the chemical synthesis of the eight compounds.

piperonylamine or 4-chlorobenzothiazole, yielded compounds **1**, **2**, **3**, **6** and **8**, respectively. Compound **4** was elaborated through a two-step sequence: (a) reaction of furylacrilic acid with thionyl chloride and reaction

**Table 1.** Chemical structures of the synthesized compounds



| Comp. No. | Z | $R^1$ | $R^2$ | Yield (%) | Melting point (°C) | Name |
|---|---|---|---|---|---|---|
| 1[a] | O | $-NO_2$ |  | 67.5 | 124–125 | *N*-Isobutyl-3-(5-nitro-2-furyl)-2*E*-propenamide |
| 2[a] | O | $-NO_2$ |  | 89.0 | 177–178 | *N*-Benzyl-3-(5-nitro-2-furyl)-2*E*-propenamide |
| 3[a] | O | $-NO_2$ |  | 85.0 | 163–164 | *N*-(4-Chlorobenzyl)-3-(5-nitro-2-furyl)-2*E*-propenamide |
| 4 | O | –H |  | 94.0 | 146–147 | *N*-Benzyl-3-(2-furyl)-2*E*-propenamide |
| 5 | S | –H |  | 95.0 | 135–136 | *N*-Benzyl-3-(thiophen-2-yl)-2*E*-propenamide |
| 6[a] | O | $-NO_2$ |  | 88.5 | 147–148 | *N*-Piperonyl-3-(5-nitro-2-furyl)-2*E*-propenamide |
| 7 | S | $-NO_2$ |  | 91.3 | 153–154 | *N*-Benzyl-3-(5-nitrothiophen-2-yl)-2*E*-propenamide |
| 8 | O | $-NO_2$ |  | 82.0 | 186–187 | *N*-(4-Chloro-2-benzothiazole)-3-(5-nitro-2-furyl)-2*E*-propenamide |

[a] Most biologically potent compounds (see Table 2).

with benzylamine; compound **5** was prepared from 3-thiophen-2-yl-acrylic acid reacted with thionyl chloride in benzene, followed by treatment with benzylamine; whereas, compound **7** was synthesized by the reaction of 3-(5-nitro-thiophen-2-yl) acrylic acid with thionyl chloride in benzene, followed by treatment with benzylamine.[8] These general procedures are shown in Scheme 1.

The structure of these new compounds was confirmed by IR (recorded in a FTIR Perkin–Elmer 1605 spectrometer), RMN (recorded in a Varian 300 MHz $^{1}H$ and 750 MHz $^{13}C$ spectrometer. Chemical shifts are reported in parts per million, relative to tetramethylsilane. Splitting patterns are designated as s, for singlet, d, doublet and t, triplet), low resolution mass spectra, MS, were recorded in a JOEL SX-102 A spectrometer in electron-impact mode and UV spectra were recorded in a UV/vis Perkin–Elmer Lamda 2 spectrometer.[9] Chemical structure, yield, melting point and name of each compound are described in Table 1.

## 3. Biological evaluation

Trypanocidal activity was assayed on epimastigote forms of *T. cruzi* Y strain, cultured at 28 °C in liver infusion tryptose medium (LIT), supplemented with 10% heat inactivated (56° for 30 min) fetal calf serum. Parasites in logarithmic growth phase (from an initial culture with $2 \times 10^6$ epimastigotes/mL) were incubated with increasing concentrations of the test compounds (1, 10 and 100 μg/mL) in dimethylsulfoxide (1% final concentration) for 24, 48, 72 and 96 h. Parasite viability and growth response were determined every 24 h in an inverted microscopy, a Spectronic 20 D+ spectrophotometer at 580 nm, and a Neubauer chamber. Morphology was analyzed in fixed preparations stained with Giemsa. All assays were carried out in triplicate. The activity was calculated as growth inhibition % and the data express the mean of three experiments (Table 2).

## 4. Discussion and conclusions

In clinical practice, trypanocidal drugs must act upon *T. cruzi* infective stages (trypomastigote and amastigote). Nevertheless, in this study we used the epimastigote non-infective form, since culture and handle in the laboratory are easier and safer. Moreover, replicative, non-infective cells have been used in several other studies for screening natural and synthetic compounds with potential trypanocidal activity, nitrofurans included. Agents showing biological activity against *T. cruzi* epimastigotes have proved to be also efficient against the infective forms (5).

Trypanocidal activities, reported in Table 2, show that the most potent derivatives are compounds **6**, **1**, **2** and **3**, all of them containing a 5-nitro furan.

**Table 2.** Biological activity of compounds **1–8** reported as percentage of growth inhibition (Data are the mean of three different experiments.)

| | 24 h | | | 48 h | | | 72 h | | | 96 h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 μg/mL (~4 μM) | 10 μg/mL (~40 μM) | 100 μg/mL (~400 μM) | 1 μg/mL (~4 μM) | 10 μg/mL (~40 μM) | 100 μg/mL (~400 μM) | 1 μg/mL (~4 μM) | 10 μg/mL (~40 μM) | 100 μg/mL (~400 μM) | 1 μg/mL (~4 μM) | 10 μg/mL (~40 μM) | 100 μg/mL (~400 μM) |
| c.c | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DMSO | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nifurtimox | 17.39 | 100 | 100 | 20 | 100 | 100 | 19.64 | 100 | 100 | 13.24 | 100 | 100 |
| **1** | 47.83 | 100 | 100 | 65 | 100 | 100 | 69.64 | 100 | 100 | 72.06 | 100 | 100 |
| **2** | 47.83 | 100 | 100 | 65 | 100 | 100 | 71.43 | 100 | 100 | 72.06 | 100 | 100 |
| **3** | 39.13 | 100 | 100 | 60 | 100 | 100 | 67.83 | 100 | 100 | 70.59 | 100 | 100 |
| **4** | 8.7 | 21.74 | 39.13 | 5 | 32.5 | 60 | 5.36 | 28.57 | 67.86 | 1.47 | 19.12 | 66.18 |
| **5** | 17.39 | 17.39 | 39.13 | 15 | 22.5 | 47.5 | 10.72 | 19.64 | 55.36 | 7.35 | 13.24 | 57.35 |
| **6** | 52.17 | 100 | 100 | 67.5 | 100 | 100 | 71.43 | 100 | 100 | 72..06 | 100 | 100 |
| **7** | 30.44 | 30.44 | 100 | 25 | 47.5 | 100 | 25 | 53.57 | 100 | 13.24 | 55.88 | 100 |
| **8** | 26.09 | 21.74 | 100 | 22.5 | 42.5 | 100 | 23.22 | 50 | 100 | 11.77 | 50 | 100 |

Lack of a nitro group as in derivatives **4** and **5**, drastically decreased trypanocidal response, confirming the key role played by this portion of the molecule for the trypanocidal activity. Nevertheless, it is necessary to point out that the element at the 2-furil position is also important, since derivatives **7** and **8** also bear the 5-nitro group and, even they show trypanocidal activity, this is not as high as that showed by derivatives **1**, **2**, **3** and **6**.

The heterocycle in compound **7** is a thiophene, instead of a furan, and although $R^2$ is the same as in compound **2** its activity was diminished, the only change of O by S reduced the activity.

Compounds **2** and **4** have the same $R^2$ substituent, but compound **4** has not nitro group, therefore its activity was lower.

Compounds **2** and **3** had a very similar biological activity even though the $R^2$ substituent in compound **3** is a chlorine; it appears that the addition of this element had no influence in activity. This also applies for compounds **1** and **2**, where **1** has an aliphatic chain, while **2** has an aromatic substituent, and both showed similar biological activities.

The data show that the nitro derivatives, analogues to Nifurtimox synthesized by an easy, cheap route, obtained at a high yield, represent useful, novel leads for the development of new and effective drugs for American Trypanosomiasis treatment. Indeed, these nitro derivatives appear to be more potent that the commercial analogue Nifurtimox, widely used as trypanocidal agent.

Since active nitro compounds are highly citotoxic, one could assume that these kind of molecules perturb physiological cellular functions of the parasite, mainly those related to its redox system,[5] since Trypanosomatides lack an efficient reduction system; therefore, this class of free radicals-producing compounds must be effective to combat the parasite and probably less harmful against the host, which is entailed with an effective redox system to detoxicate reactive radicals.

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8. Elliott, M.; Farnaham, A. W. *Pestic. Sci.* **1987**, *18*, 191.
9. *N*-Isobutyl-3-(5-nitro-2-furyl)-2*E*-propenamide (**1**). Ethyl acetate (68%) mp 124–125 °C IR $\nu$ $cm^{-1}$ 3270 (N–H), 1664.7 (C=O), 1516.8, 1348.6 ($NO_2$), 979.4 (C=C *trans*); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.4, 6.67 (dd, 2H, $J$ = 15.38, C=C *trans*); 7.34,6.69 (dd, 2H, $J$ = 3.7, furane); 6.11 (br s, 1H, –NH); 3.24 (t, 2H, CH–); 1.85 (m, 1H, –CH–); 0.95 (d, 6H, 2($CH_3$)). UV ($\lambda_{max}$ nm) 347.8. MS *m*/*z* 238$M^+$.
*N*-Benzyl-3-(5-nitro-2-furyl)-2*E*-propenamide (**2**). Ethyl acetate (89%) mp 177–178 °C. IR $\nu$ $cm^{-1}$ 3250.33 (N–H), 1666.55 (C=O), 1513.09, 1356 ($NO_2$), 969.97 (C=C *trans*); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.44, 6.66 (dd, 2H, $J$ = 15.5, C=C *trans*); 7.32, 6.68 (dd, 2H, $J$ = 3.7, furane); 7.31 (m, 5H, aromatic); 6.11 (br s, 1H, –NH); 4.57 (d, 2H, $CH_2$) UV ($\lambda_{max}$ nm) 355.65. MS *m*/*z* 272$M^+$.
*N*-(4-Chlorobenzyl)-3-(5-nitro-2-furyl)-2*E*-propenamide (**3**). Ethyl acetate (85%) mp 163–164 °C. IR $\nu$ $cm^{-1}$ 3254.13 (N–H), 1654.25 (C=O), 1520.7, 1346.38 ($NO_2$), $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.37, 6.66 (dd, 2H, $J$ = 15.3, C=C *trans*); 7.27, 6.65 (dd, 2H, $J$ = 3.7 furane); 7.26 (m, 4H, aromatic); 6.55 (br s, 1H, –NH); 4.50 (d, 2H,–$CH_2$) UV ($\lambda_{max}$ nm) 347.66 MS *m*/*z* 306$M^+$.
*N*-Benzyl-3-(2-furyl)-2*E*-propenamide (**4**). Ethyl acetate (94%) mp146–147 °C IR $\nu$ $cm^{-1}$ 3257.64 (N–H), 1654.85 (C=O), 974.18 (C=C *trans*); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.46, 6.32 (dd, 2H, $J$ = 15.35, C=C *trans*); 7.29 (m, 5H, aromatic); 7.30, 6.54, 6.41 (3d, 3H, furane); 6.0 (br s, 1H, –NH); 4.54 (d, 2H,–$CH_2$) UV ($\lambda_{max}$ nm) 398.31 MS *m*/*z* 227 $M^+$.
*N*-Benzyl-3-(2-thiol)-2*E*-propenamide (**5**). Ethyl acetate (95%) mp 135–136 °C IR $\nu$ $cm^{-1}$ 3247.48 (N–H), 1648.16 (C=O), 973.62 (C=C *trans*) $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.46, 6.32 (dd, 2H, $J$ = 16.15, C=C *trans*); 7.29 (m, 5H, aromatic); 7.75, 7.25, 7.10 (3d, 3H, thiophene);6.18 (br s, 1H, –NH); 4.55 (d, 2H, –$CH_2$) UV ($\lambda_{max}$ nm) 306.37 MS *m*/*z* 243 $M^+$.
*N*-Piperonil-3-(5-nitro-2-furyl)-2*E*-propenamide (**6**). Ethyl acetate (88.5%) mp 147–148 °C IR $\nu$ $cm^{-1}$ 3273.31 (N–H),

1653.41 (C═O), 1511.88, 1343.51 ($NO_2$), $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.37, 6.66 (dd, 2H, $J$ = 15.3, C═C *trans*); 7.27, 6.65 (m, 2H, $J$ = 3.7 furane); 7.45, 6.80 (dd, 2H, $J$ = 3.0 aromatic); and 6.10 (s, 1H, aromatic); 6.55 (br s, 1H, –NH); 5.95 (s, 2H, $-CH_2$); 4.45 (d, 2H, $-CH_2$); UV ($\lambda_{max}$ nm) 348.72 MS *m*/*z* 316 $M^+$.

*N*-Benzyl-3-(5-nitro-2-thiol)-2*E*-propenamide (**7**). Ethyl acetate (91.3%) mp 153–154 °C IR $v$ $cm^{-1}$ 3257.18 (N–H), 1664.91 (C═O), 1532.17, 1341.00 ($NO_2$); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.46, 6.45 (dd, 2H, $J$ = 15.3, C═C *trans*); 7.80, 7.05 (m, 2H, $J$ = 3.4 thiol); 7.28 (m, 5H, aromatic); 6.10 (br s, 1H, –NH); 4.50 (d, 2H, $-CH_2$) UV ($\lambda_{max}$ nm) 357.74 MS *m*/*z* 288 $M^+$.

*N*-(4-Chloro-2-benzothiazole)-3-(5-nitro-2-furyl)-2*E*-propenamide (**8**). Ethyl acetate (82%) mp 186–187 °C. IR $v$ $cm^{-1}$ 3466.57 (N–H), 1646.00 (C═O), 1536.57, 1348.37 ($NO_2$), $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.46, 6.45 (dd, 2H, $J$ = 16.3, C═C *trans*); 7.30 (m, 3H, aromatic); 7.40, 6.95 (m, 2H, $J$ = 3.7 furane); 6.05 (br s, 1H, –NH); UV ($\lambda_{max}$ nm) 266.76 MS *m*/*z* 349 $M^+$.