1,2,5-Oxadiazole *N*-Oxide Derivatives and Related Compounds as Potential Antitrypanosomal Drugs: Structure−Activity Relationships

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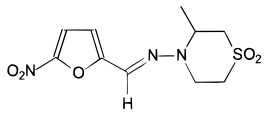
The syntheses of a new series of derivatives of 1,2,5-oxadiazole *N*-oxide, benzo[1,2-*c*]1,2,5-oxadiazole *N*-oxide, and quinoxaline di-*N*-oxide are described. In vitro antitrypanosomal activity of these compounds was tested against epimastigote forms of *Trypanosoma cruzi*. For the most effective drugs, derivatives **IIIe** and **IIIf**, the 50% inhibitory dose (ID_{50}) was determined as well as their cytotoxicity against mammalian fibroblasts. Electrochemical studies and ESR spectroscopy show that the highest activities observed are associated with the facile monoelectronation of the *N*-oxide moiety. Lipophilic−hydrophilic balance of the compounds could also play an important role in their effectiveness as antichagasic drugs.

Introduction

Trypanosoma cruzi (*T. cruzi*) is the etiologic agent of Chagas' disease.[1,2] Trypanosomiasis and leishmaniasis are major third-world diseases, with several millions of new infections presenting annually. About 100 000 people in the United States are also infected, probably due to transfusion of blood products originating from South America.[3] The current chemotherapy against Chagas' disease is still inadequate. The main drug in use is Nifurtimox (Nfx); however, it has undesirable side effects[4,5] and is yet inefficient to treat chronic Chagas' disease, which can be regarded as a disease with no cure. A characteristic ESR signal corresponding to the nitro anion radical ($R\text{-}NO_2^{\bullet-}$) appears when Nfx is added to intact *T. cruzi* cells,[6] suggesting that intracellular reduction of Nfx followed by redox cycling yielding superoxide anion may be its major mode of action against *T. cruzi*.[5−7]



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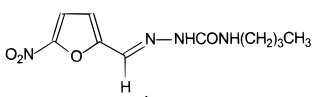

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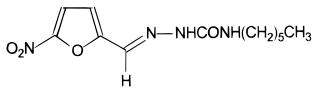

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We have previously reported[10,11] the synthesis and biological activity against *T. cruzi* epimastigote forms of a series of 5-nitrofurfural and 5-nitrothiophene-2-carboxaldehyde derivatives. These compounds proved to generate nitro anion radicals, which were characterized using ESR spectroscopy.[12,13] The derivatives **1** and **2** showed interesting in vitro trypanocidal activity but failed to be good trypanocidal agents in vivo because of their inherent toxicity (principally **2**).[14] The undesirable toxicity of **1** and **2** on host is probably due to the nitro moiety which acts as a nonselective redox-damaging function.

Similar to the nitro pharmacophore of antitrypanosomal drugs, the *N*-oxide moiety has proved to be responsible for the biological activity of numerous drugs (such as antitumoral, antibacterial) through the production of free radical species.[15,16] These data suggested the possibility of designing new antitrypanosomal compounds where the *N*-oxide moiety would be the pharmacophore that generates the radical toxic species. Herein we report on the preparation and biological evaluation of compounds derived from 1,2,5-oxadiazole *N*-oxide, benzo[1,2-*c*]1,2,5-oxadiazole *N*-oxide, quinoxaline 1,4-di-*N*-oxide, and some reduced analogues. We have also performed electrochemical measurements, ESR spectroscopy, and relative lipophilicity to study structure−activity relationships.

Our general strategy for the design of new structures was based on the conjunction of *N*-oxide systems and the semicarbazide moieties present in **1** and **2** (See Chart 1). Initially we chose 1,2,5-oxadiazole *N*-oxide as the heteroaromatic *N*-oxide system, due to the structural similarity with the nitrofuran heterocycle. Then we extended the studies over benzo[1,2-*c*]1,2,5-oxadiazole *N*-oxide and quinoxaline di-*N*-oxide. Subsequently, "spermidine-mimetic" residues substituted the N_4-semicarbazide moiety. Previous work[17−19] supported the idea of designing compounds which bear a positive charge and/or a flexible side chain "spermidine-like", capable of inhibiting trypanothione reductase (TR). TR is a critical enzyme for the parasite, responsible of catalyzing the reduction of trypanothione disulfide to trypanothione, which participates in free radical- and oxygen-derived species detoxification.[20−22] In addition,

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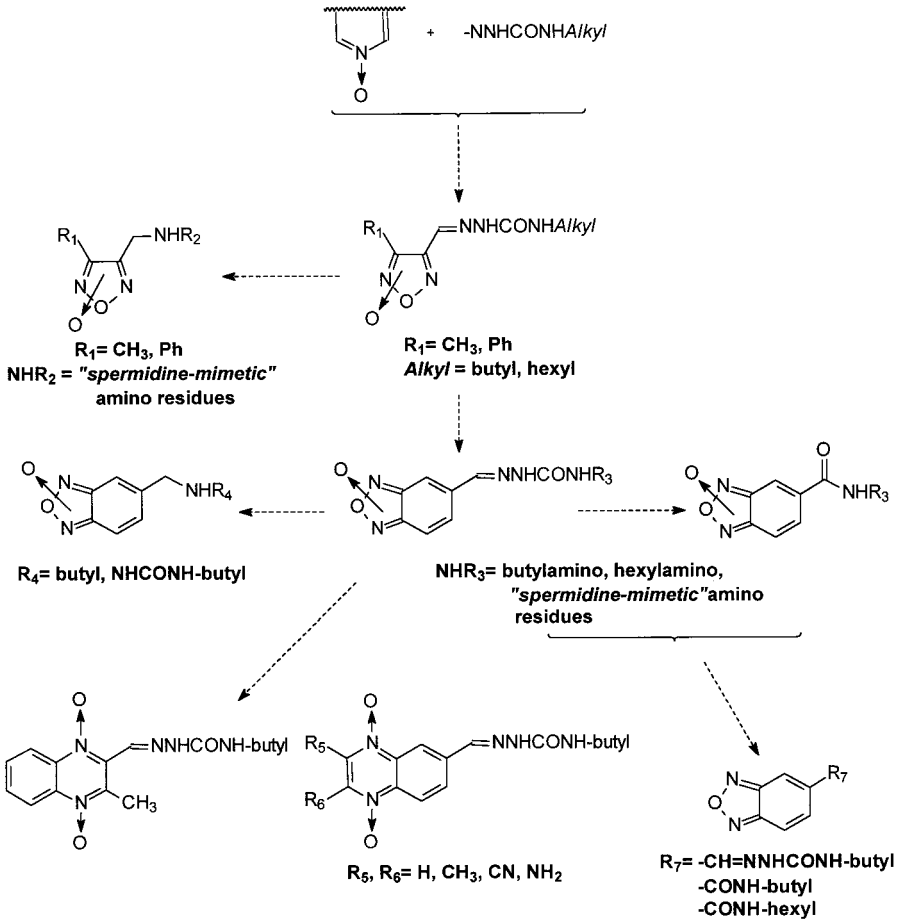
Chart 1



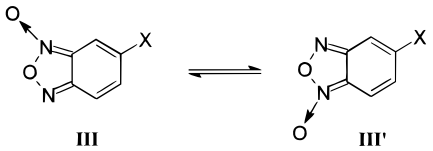
the more active derivatives were deoxygenated in order to corroborate the pharmacological action of the *N*-oxide group.

Chemistry

The 1,2,5-oxadiazole derivatives were obtained as shown in Scheme 1. Crotonaldehyde was treated with sodium nitrite in acetic acid to afford the basic system **3**, which was converted to the corresponding semicarbazone derivatives **Ia**,**b** in moderate yields.[11,23] Reductive amination of **3** with "spermidine-mimetic" amines gave **Ic**,**d**.[24] These two amines were prepared by treatment of simple amines (morpholine or piperidine) with acrylonitrile,[25] followed by hydrogenation (Pd/Ni/Ac_2O) and hydrolysis.[26] Reaction of cinnamyl alcohol with sodium nitrite in acetic acid afforded the basic system **4**.[27] Alcohol **4** was converted into the aldehyde **5** by oxidation with manganese dioxide.[27] This compound, treated with semicarbazides (**11**, **12**, see Scheme 1), produced the compounds **IIa**,**b** in moderate yields.[11] Also, treatment of **4** with thionyl chloride at room temperature gave the compound **6**, which was transformed into compounds **IIc**,**d** by reaction with the corresponding amines in boiling tetrahydrofuran.[28]

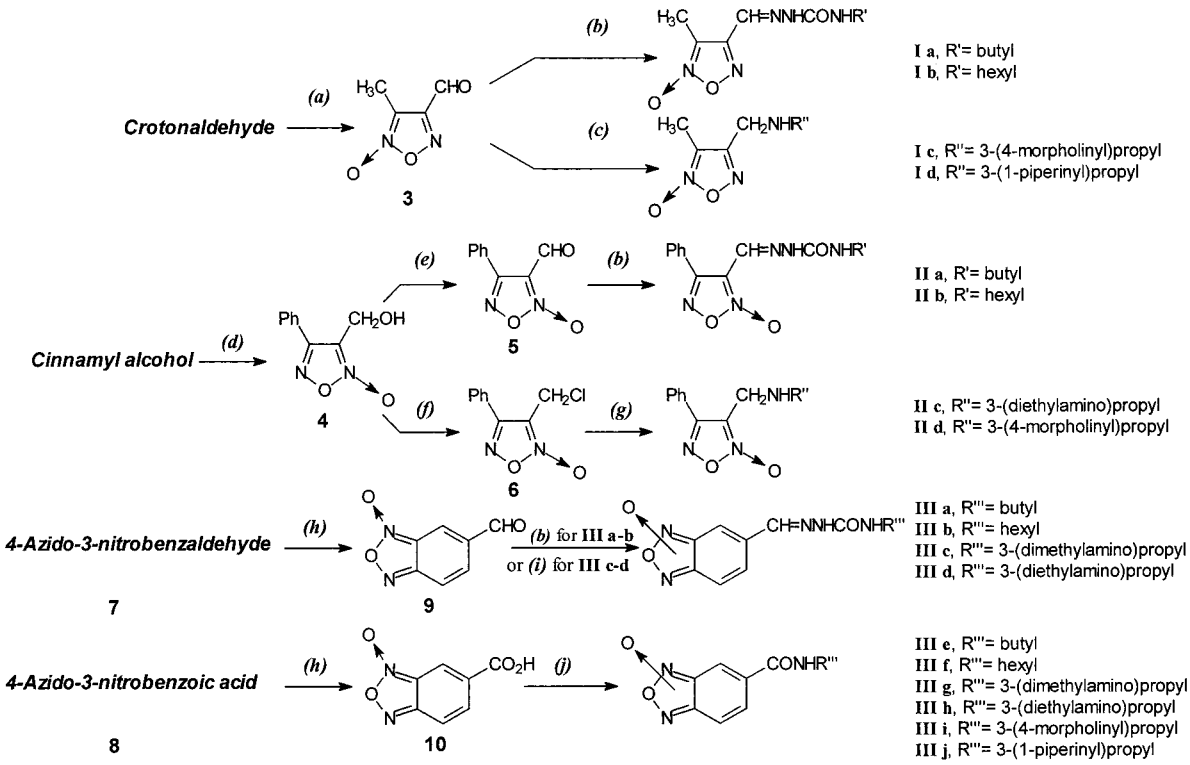
The benzo[1,2-*c*]1,2,5-oxadiazole system was prepared using the appropriate nitrophenyl azides **7** and **8**.[29−31] Cyclocondensation of these azides in boiling toluene yielded the heterocycles **9** and **10**.[32] The semicarbazones **IIIa**−**d** were obtained in variable yields (30−70%), using the corresponding semicarbazide reactants **13** and **14** (Scheme 1), which were prepared according to a known procedure.[11] Thus, **13** and **14** were obtained from commercially available 3-(dimethylamino)propylamine and 3-(diethylamino)propylamine by treatment with phenyl chloroformate to give the corresponding carbamates and further reaction with hydrazine. The amides **IIIe**−**j** were obtained from acid **10**, which was successively reacted with thionyl chloride at reflux and the corresponding amine.

The compounds **IIIa**−**j** (and also **Va**,**b**) exist as a mixture of isomers at room temperature (**III** and **III′**):



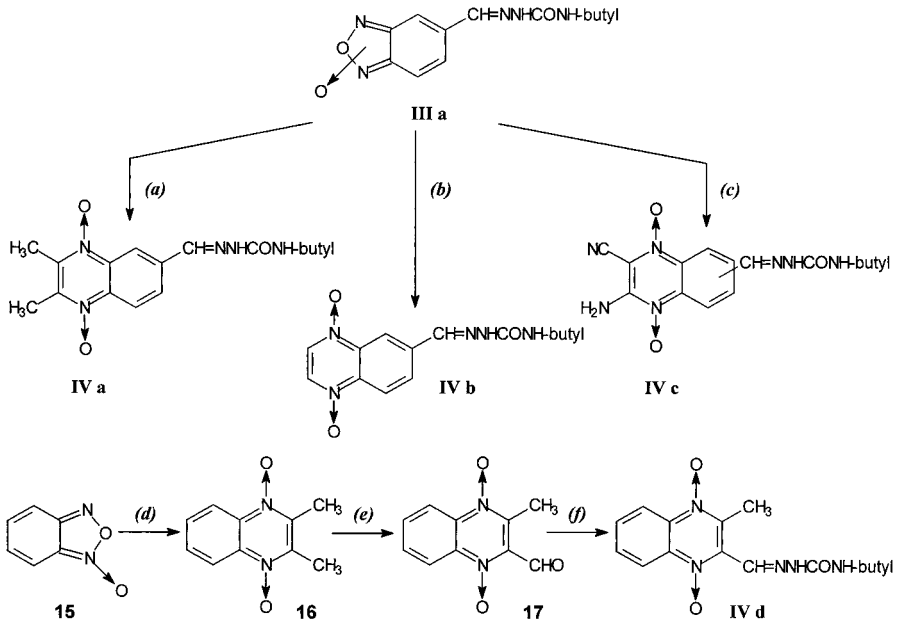
This phenomenon was observed through the corresponding NMR spectra (proton and carbon), which showed complex groups of signals in the aromatic zone (7.20−8.30 and 110−155 ppm, respectively) at room temperature. The spectra simplified at higher temperature, where one of these isomers predominated. Careful examination of coupling constants and chemical shifts indicated that the major isomer at high temperature is **III′**, which is the 1,5-disubstituted heterocycle (variable-temperature NMR data for derivative **IIIa** is available as Supporting Information).[33−35] Interestingly, the products **Ia**−**d** and **IIa**−**d** do not exist as isomeric mixtures, and they were obtained as simple products as confirmed by ^{13}C NMR and crystallographic studies.[28,36]

Scheme 1[a]



[a] Reaction conditions: (a) $NaNO_2$/AcOH/0−14 °C; (b) N_4-butylsemicarbazide (**11**) or N_4-hexylsemicarbazide (**12**)/*p*-TsOH/toluene/rt; (c) amine/$NaBH_3CN$/$ZnCl_2$/MeOH/rt; (d) $NaNO_2$/AcOH/rt; (e) MnO_2/$CHCl_3$/rt/1 day; (f) $SOCl_2$/rt/1 day; (g) amine/K_2CO_3/KI/THF/reflux; (h) toluene/reflux/3 h; (i) N_4-[3-(dimethylamino)propyl]semicarbazide (**13**) or N_4-[3-(diethylamino)propyl]semicarbazide (**14**)/*p*-TsOH/MeOH/reflux; (j) (i) $SOCl_2$/DMF/reflux/3 h, (ii) amine/Et_3N/rt/12 h.

Scheme 2[a]

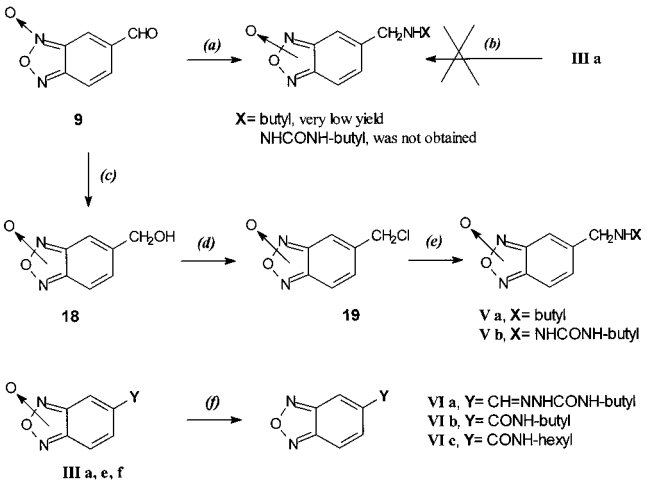


[a] Reaction conditions: (a) butanone/morpholine/rt; (b) vinyl acetate/Et_2NH/DMF/40 °C; (c) malononitrile/Et_3N/DMF/0 °C/rt; (d) butanone/morpholine/rt; (e) SeO_2/AcOEt/reflux; (f) **11**/*p*-TsOH/toluene−CH_2Cl_2/rt.

In another synthetic effort, we expanded the oxadiazole system using compound **IIIa** (Scheme 2), to obtain the quinoxaline 1,4-dioxide nucleus. When compound **IIIa** was reacted with butanone in morpholine as base, the dimethylquinoxaline **IVa** was obtained.[29] Reaction of **IIIa** with vinyl acetate in DMF at 40 °C produced the compound **IVb** in low yield (7%).[37] Beirut reaction of **IIIa** with malononitrile in the presence of triethylamine at low temperature gave the compound **IVc** in good yield as a mixture of 6- and 7-substituted isomers.[38−41] The compound **IVd** was obtained from the aldehyde **17**[29,42] in moderate yield.

Substituent effects in the benzo system were studied. The products **Va,b**, as the non-electron-withdrawing analogues of compounds **IIIe**,**a**, respectively, were prepared as delineated in Scheme 3. The direct reductive

Scheme 3[a]



[a] Reaction conditions: (a) butylamine or **11**/$NaCNBH_3$/Et_3N/MeOH/rt; (b) $NaCNBH_3$/Et_3N/DMF/reflux; (c) $NaBH_4$/MeOH/0 °C/rt; (d) $SOCl_2$/reflux; (e) butylamine or **11**/K_2CO_3/KI/THF/reflux; (f) triphenylphosphine/EtOH/reflux/1.5 h.

amination of aldehyde **9** produced a very low yield of **Va**. Product **Vb** was not observed in these conditions or by direct reduction of **IIIa** with sodium cyanoborohydride. Further, these compounds were obtained by nucleophilic substitutions of the chloride **19**.

The deoxygenated derivatives **VIa**−**c** were prepared by reaction of the corresponding *N*-oxides (**IIIa**,**e**,**f**) with triphenylphosphine in boiling ethanol (Scheme 3).[43,44] The reaction was difficult to follow by TLC (SiO_2, petroleum ether:ethyl acetate (30%)), because of the very close R_f values of reactants and products.

Results

Antitrypanosomal Activities. All the compounds were tested in vitro against *T. cruzi*. Epimastigote forms of *T. cruzi*, Tulahuen strain, were grown in axenic media as described in the Experimental Section. 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 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.[11,45] The percentage of inhibition, summarized in Table 1, was calculated as follows: percent (%) $=\{1 - [(A_p - A_{0p})/(A_c - A_{0c})]\} \times 100$, 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.

The ID_{50} concentration (50% inhibitory dose) was assessed for compounds presenting higher trypanocidal activity (**IIIe**,**f**, Nfx). Readings were done on day 5 of growth and were determined as the drug concentration required to reduce the absorbance to one-half of that of the control (without drug) (Figure 1A). The ID_{50} concentrations for compounds **IIIe**,**f** were 10 and 16 μM, respectively (compared to 2 μM for Nfx).

Cytotoxicity Assay. To evaluate the potential cytotoxicity of compounds **IIIe**,**f** against the host, mammalian fibroblasts were grown as described in the Experimental Section and exposed to 10 μM concentration of each compound. Cell viability was assessed by measuring absorbance at 570 nm due to MTT reduction to formazan after 48 h of culture, which was proved to be proportional to the number of viable cells present. Figure 1B shows the percentage of cell survival referred to the control culture. The percentage of cell survival in the presence of 10 μM **IIIe**,**f** or Nfx was 69, 58, or 75%, respectively.

Table 1

compd	percentage growth inhibition (%)[a,b]	E_{pc} (V) vs SCE[c]	R_M
Nfx	100	−0.91[d]	−0.02[e]
IIIe	90	−1.14	0.14
IIIf	79	−1.19	0.52
IIIb	55	−1.07	0.69
IIIa	45	−1.07	0.41
IIIj	45	−1.12	1.44
IIIi	19	−1.17	1.37
IIIh	17	−1.19	1.67
IIIg	15	−1.29	1.47
IIIc	11	−1.02	1.28
IIId	8	−1.06	1.30
IIb	53	−1.49	0.82
IId	38	−1.10	0.74
IIc	22	ND[f]	ND
IIa	17	−1.37	0.58
Ib	3	−1.59	0.41
Ia	0	ND	ND
Ic	0	ND	ND
Id	0	ND	ND
IVb	23	ND	ND
IVc	17	−0.70	−0.01
IVd	6	ND	ND
IVa	0	−0.83	−0.09
Vb	55	ND	0.06
Va	21	−0.96	1.24
VIc	10		0.49
VIb	0		0.36
VIa	0		0.39

[a] Inhibition of epimastigotes growth, dose = 25 μM. [b] The results are the means of three different experiments with SD less than 10% in all cases. [c] First reduction step of *N*-oxides or nitro group of Nfx. [d] Peak potentials (∼±0.01 V) measured at a scan rate of 0.2 V/s. [e] The mean R_f values obtained were transformed into R_M (as described under Lipophilicity Studies). [f] Not determined.

Electrochemical Studies. The voltammetric response of *N*-oxide derivatives was determined in DMSO at a mercury dropping working electrode.[13,14,46] For the 1,2,5-oxadiazole *N*-oxide, a typical one reduction peak was observed during forward cathodic scan, which is linked to an oxidation peak on the reverse anodic scan. The benzo[1,2-*c*]1,2,5-oxadiazole *N*-oxide derivatives presented one to two reduction peaks, which were linked to oxidation peaks. For quinoxaline di-*N*-oxide three to four reduction peaks were observed during forward cathodic scan, some of which may be linked to oxidation peaks on the reverse anodic scan. Only the first reduction step was studied as a function of voltage sweep rate and switching potential. Table 1 summarizes E_{pc} (cathodic peak potential) for the first reduction step of *N*-oxide derivatives.

Lipophilicity Studies. Reverse-phase TLC experiments were performed on precoated TLC-C_{18} and eluted with acetone:water (50:50, v/v). The R_f values for the compounds were converted into R_M values using the relationship: $R_M = \log[(1/R_f) - 1]$.[47−51] Table 1 shows

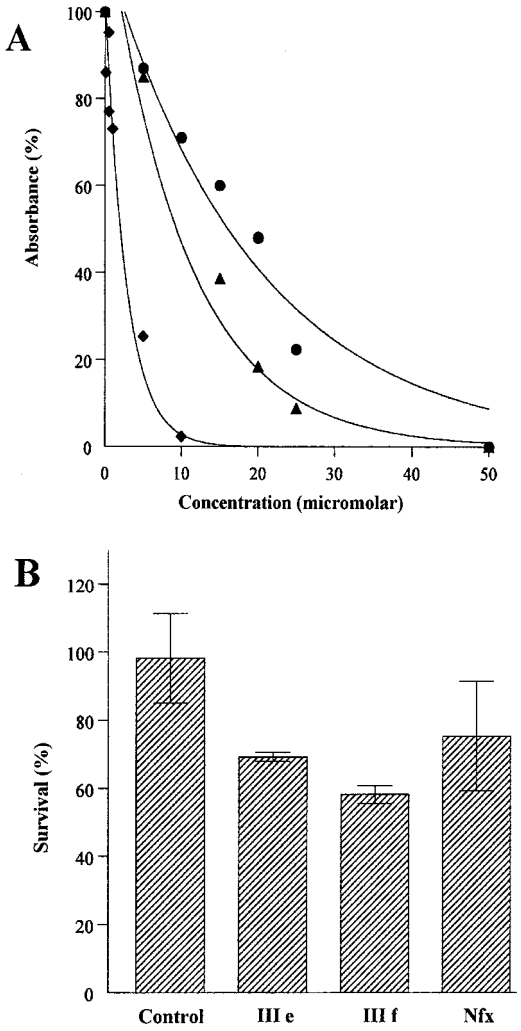


Figure 1. (A) Determination of ID_{50} values. Epimastigote growth was followed in the absence and presence of increasing concentrations of Nfx (◆), **IIIe** (▲), and **IIIf** (●), as described in the Experimental Section. (B) Estimation of cytotoxicity. Mammalian fibroblasts (V79-M8) were grown in the absence (control) and presence of 10 μM **IIIe**, **IIIf**, or Nfx. The number of viable cells was assesed by the MTT reduction method and the percentage of survival calculated as described in the Experimental Section.

the R_M values obtained for each compound, and Nfx was used as the reference drug.

ESR Studies. The ability of these *N*-oxide derivatives to yield free radicals in solution was investigated using ESR spectroscopy. One representative compound of each type of system was chosen: compounds **Ib**, **IIIb**,**d**, and **IVa**. The radicals were generated by electrolytical reduction in situ at room temperature in DMSO, applying the same cathodic potential obtained from the cyclic voltammetry experiments.[13,14] The half-life of the generated radicals was not determined, but they were stable enough to perform the corresponding experiments (10−30 min). The interpretation of the ESR spectra by means of a simulation process has led to the determination of the coupling constants for all magnetic nuclei.[46] The hyperfine patterns indicated that the unpaired electron is delocalized exclusively at the N−O function for compound **Ib** (oxadiazole system), but for the other heterocycles (**IIIb**,**d**, **IVa**) the radicals are able to delocalize as far as the semicarbazone system (Figure 2).

Discussion

Some of the *N*-oxide derivatives investigated showed antitrypanosomal activity similar to that of Nfx. At 25 μM, the 3-methyloxadiazole *N*-oxide derivatives (**Ia**−**d**) were not active; however, the 4-phenyl analogues (**IIa**−**d**) showed moderate activity. When we changed the heterocycle (benzo[1,2-*c*]1,2,5-oxadiazole instead of 1,2,5-oxadiazole), using the same N_4-semicarbazide moiety, compounds were twice as effective (**IIIa** vs **IIa**) or presented comparable activity (**IIIb** vs **IIb**). Within the benzoxadiazole series, when comparing compounds with identical side chain, the substitution of the linking residue from amide to semicarbazone was shown to be critical for antitrypanosomal activity (the amides **IIIe**,**h** were 2-fold more active than the semicarbazones **IIIa**,**d**). The change from alkylamino to [(*N*,*N*-dialkylamino)alkyl]amino group ("spermidine-mimetic" group) resulted in a significant decrease in the activity (the semicarbazones **IIIa**,**b** were 4-fold more active than **IIIc**,**d**, and the amides **IIIe**,**f** are 2-fold more active than **IIIg**−**j**). Also, the expansion of the heterocycle produced a notorious decrease in activity (compound **IIIa** showed an average 3-fold increase of activity compared to compounds **IVa**−**d**). The change of the semicarbazone moiety from the C-6 (compound **IVa**) to the C-2 (compound **IVd**) quinoxaline position did not result in a significant increase of activity. The non-electron-withdrawing analogues, compounds **Va**,**b**, maintain antitrypanosomal activity; however, compound **Va** was 4-fold less active than its electron-withdrawing (**IIIe**) counterpart. Interestingly, for the most active benzoxadiazoles, the expansion of the conjugation to the side chain did not result in an increase of activity (compare **Vb** with 55% of growth inhibition to **IIIa** with 45% of growth inhibition).

Importantly, the absence of the *N*-oxide moiety produced a total loss of activity, indicating the direct participation of this group on the mechanism for trypanosomal toxicity (compare compounds **IIIa**,**e**,**f** to compounds **VIa**−**c**, showing 45, 90, and 79% and 0, 0, and 10% growth inhibition, respectively).

The electrochemical studies show that the potential for the first reduction step of the *N*-oxide derivatives is similar for all compounds and more negative than the corresponding potential for Nfx. Using heterocycles capable of increasing radical stability (observed by ESR), the potential for the first reduction step becomes less negative, and therefore, the compounds are more readily reduced and are also more active (compare **Ib** to **IIIb**).

The lipophilic-hydrophilic properties, expressed as R_M values, may be related with the antitrypanosomal activities of the compounds. This can be seen, for example, in the R_M values for the active members of the benzoxadiazole series (semicarbazones **IIIa**,**b** and amides **IIIe**,**f**), more related to Nifurtimox's R_M than the less active members (semicarbazones **IIIc**,**d** and amides **IIIg**−**j**).

Compounds **IIIe**,**f** (with 90 and 79% growth inhibition, respectively) were the most effective drugs in inhibiting epimastigote growth (Table 1). The ID_{50} values obtained for **IIIe**,**f** were 10 and 16 μM, respectively, in the same range of that observed for Nfx (2 μM). In addition, the cytotoxicity of these compounds against mammalian fibroblasts is comparable to that of the

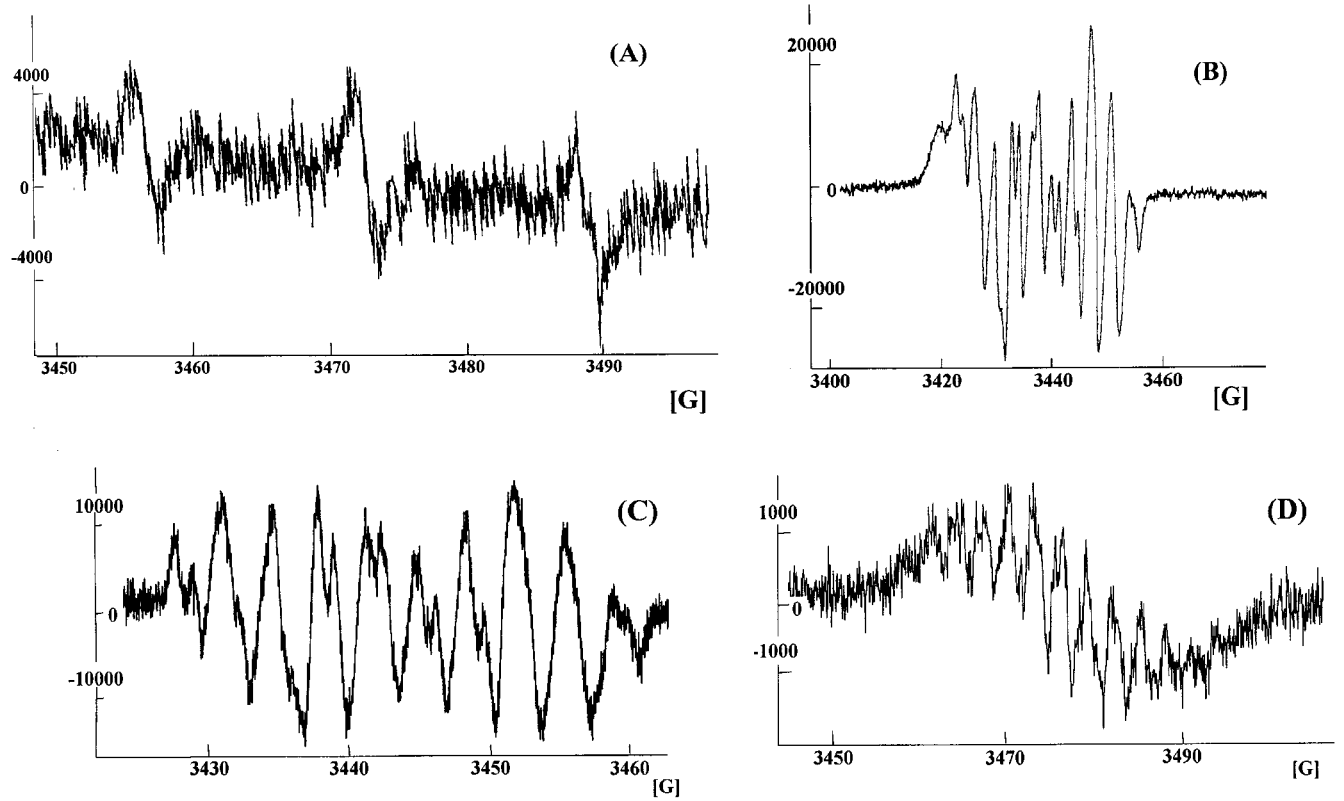


Figure 2. ESR spectra (electrochemical generation) of compounds **Ib** (A) (from ref 46), **IIIb** (B), **IIId** (C), and **IVa** (D).

reference drug. The ratio percentage of mammalian cell survival (%)/ID_{50} (μM) could be considered as an index of drug effectiveness. Although the ratio values obtained for **IIIe**,**f** (7 and 4, respectively) are lower than the one observed for the reference drug Nfx (36), they are high enough to be considered as potential antichagasic drugs. It is worth mentioning that only one cell line was studied here in order to assess the cytotoxicity of these new compounds against the host, and the susceptibility of other cell types could be different.

In summary, our results show that these novel *N*-oxide derivatives have potential trypanocidal activity, and the mechanism of antitrypanosomal action is through *N*-oxide radical formation. In vivo studies to investigate the ability of these drugs to decrease the parasitemia of infected mice are currently underway.

Conclusions

A new series of 1,2,5-oxadiazole *N*-oxides, benzo[1,2-*c*]1,2,5-oxadiazole *N*-oxides, and quinoxaline di-*N*-oxides was synthesized using a facile methodology. The preliminary antitrypanosomal studies of these derivatives showed that some of them exhibit significant activity in vitro. The physicochemical properties studied indicate that the redox potential of the monoelectronation of the *N*-oxide moiety and the lipophilic−hydrophilic balance play an important role in the biological activity. Our studies indicate that the *N*-oxide group of these compounds is the pharmacophore and that stabilization of the free radical species generated by reduction in the biological medium is related to their antitrypanosomal activity.

To our knowledge this is the first time that *N*-oxide-containing structures are reported as trypanocidal compounds.

Experimental Section

Chemistry. All starting materials were commercially available research-grade chemicals and used without further purification. The compounds **3**−**12**, **15**−**17**, **Ia**,**b**, and **IIc** were prepared according to the literature.[11,23,27−30,52,53] All solvents were dried and distilled prior to use.[54] All the reactions were carried out in a nitrogen atmosphere. The typical workup 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 phosphorus pentoxide at 3−4 mmHg, 24 h at room temperature), were performed on a Fisons EA 1108 CHNS−O analyzer, and were within ±0.4% of theoretical values. Infrared 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}. ^1H and ^{13}C NMR spectra were recorded on a Varian XL-100 (at 100 MHz) instrument, Varian Gemini 300 (at 300 and 75.5 MHz) instrument, and Bruker DPX-400 (at 400 and 100 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. High-resolution mass spectra were recorded on a VG ULTIMA instrument at 70 eV.

Preparation of Amines Ic,d. General Procedure. A mixture of aldehyde **3** (1 equiv), the corresponding amines (1 equiv), $NaCNBH_3$ (1 equiv), $ZnCl_2$ (0.5 equiv), and MeOH was stirred at room temperature until the carbonyl compound was not present (Al_2O_3, 1% MeOH in CH_2Cl_2). After the workup process the residue was purified as indicated.

4-[3-(4-Morpholinyl)propylaminomethyl]-3-methyl-1,2,5-oxadiazole N_2-oxide dihydrochloride, Ic: purified by column chromatography (Al_2O_3, CH_2Cl_2:MeOH (0−5%)), colorless oil (44%). The oil was dissolved in MeOH and treated with MeOH saturated in hydrogen chloride. The dihydrochloride was crystallized from Et_2O:EtOAc:EtOH: white needles (5%); mp 198.5−200.5 °C; IR ν_{max} 2959, 2463, 1618, 1393, 1260 cm^{-1}; ^1H NMR (MeOH-d_4−D_2O, 400 MHz) δ 2.25 (s, 3H), 2.33 (quint, J = 7.9 Hz, 2H), 3.34 (m, 6H), 3.50 (t, J = 7.1 Hz, 2H), 4.00

(m, 4H), 4.52 (s, 2H); ^{13}C NMR (MeOH-d_4−D_2O, 100 MHz) δ 6.70, 20.67, 41.36, 45.21, 52.36, 54.02, 63.96, 112.43, 151.90; MS *m/z* (abundance) 199 ($M^{+\cdot}$ − CH_2═$CHOCH_2$, 0.5%), 183 (0.6%). Anal. ($C_{11}H_{20}N_4O_3$·2HCl) C, H, N.

4-[3-(1-Piperinyl)propylaminomethyl]-3-methyl-1,2,5-oxadiazole N_2-oxide, Id: purified by column chromatography (Al_2O_3, CH_2Cl_2:MeOH (0−1%)); colorless oil (33%); IR ν_{max} 3332, 2948, 1611, 1383 cm^{-1}; ^{1}H NMR (acetone-d_6−DMSO-d_6, 100 MHz) δ 1.65 (m, 2H), 1.95 (m, 6H), 2.30 (s, 3H), 2.98 (t, J = 7.0 Hz, 2H), 3.20 (m, 6H), 4.15 (s, 2H), 4.62 (bs, 1H); MS *m/z* (abundance) 255 ($M^{+\cdot}$ + H, 0.2%), 237 (0.6%).

Preparation of Semicarbazones IIa,b, IIIa,b, and IVd. General Procedure. A mixture of aldehyde **5**, **9**, or **17** (1 equiv), the corresponding semicarbazide (1 equiv), *p*-TsOH (catalytic amounts), and toluene (toluene−CH_2Cl_2 for **IVd**) as solvent was stirred at room temperature until the carbonyl compound was not present (SiO_2, 1% MeOH in CH_2Cl_2). After the workup process the residue was purified as indicated.

4-Butyl-1-[(4-phenyl-1,2,5-oxadiazol-3-yl N_2-oxide)-methylidene]semicarbazide, IIa: purified by crystallization from EtOH; pale yellow needles (49%); mp 137.5−139.5 °C; IR ν_{max} 3400, 2940, 2860, 1685, 1590, 1355 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.91 (t, J = 7.1 Hz, 3H), 1.24 (sextet, J = 7.9 Hz, 2H), 1.36 (quint, J = 6.9 Hz, 2H), 3.14 (q, J = 6.3 Hz, 2H), 5.42 (t, J = 5.5 Hz, 1H), 7.55 (m, 3H), 7.67 (m, 2H), 7.70 (s, 1H), 10.85 (s, 1H); ^{13}C NMR-DEPT ($CDCl_3$, 75.5 MHz) δ 13.72 (CH_3−C), 19.83 (C−CH_2−C), 31.93 (C−CH_2−C), 39.38 (CH_2−NHCO), 112.62 (C═N^+−O^-), 124.70 (CH−Ar), 126.66 (C−Ar), 128.60 (CH−Ar), 128.87 (CH−Ar), 130.95 (CH−Ar), 155.35 (C═O), 155.84 (C═N); MS *m/z* (abundance) 303 ($M^{+\cdot}$, 0.2%), 287 (0.2%). Anal. ($C_{14}H_{17}N_5O_3$) C, H, N.

4-Hexyl-1-[(4-phenyl-1,2,5-oxadiazol-3-yl N_2-oxide)-methylidene]semicarbazide, IIb: purified by column chromatography (SiO_2, CH_2Cl_2:MeOH (0−5%)) and then crystallized from petroleum ether:EtOAc; yellow needles (43%); mp 115.5−118.0 °C; IR ν_{max} 3380, 3320, 2920, 2850, 1675, 1355 cm^{-1}; ^{1}H NMR ($CDCl_3$, 100 MHz) δ 0.90 (t, J = 8.0 Hz, 3H), 1.30−1.60 (m, 8H), 3.18 (q, J = 6.0 Hz, 2H), 5.48 (t, J = 6.0 Hz, 1H), 7.60 (m, 3H), 7.70 (m+s, 3H), 9.72 (bs, 1H); MS *m/z* (abundance) 301 ($M^{+\cdot}$ − CH_3CH_3, 14.3%), 271 (1.8%). Anal. ($C_{16}H_{21}N_5O_3$) C, H, N.

4-Butyl-1-[(benzo[1,2-*c*]1,2,5-oxadiazol-5(6)-yl N_1-oxide)methylidene]semicarbazide, IIIa: purified by crystallization from EtOH; yellow needles (52%); mp 227.0−229.0 °C; IR ν_{max} 3400, 2930, 2860, 1675, 1610, 1350 cm^{-1}; ^{1}H NMR (DMSO-d_6−D_2O, 300 MHz) δ 0.89 (t, J = 7.1 Hz, 3H), 1.28 (sextet, J = 7.3 Hz, 2H), 1.46 (quint, J = 7.1 Hz, 2H), 3.13 (q, J = 6.8 Hz, 2H), 7.50−7.90 (m+s, 4H); ^{13}C NMR (DMSO-d_6−D_2O, 75.5 MHz) δ 13.78, 19.58, 32.17, 38.78, 110.77, 117.67, 122.14, 131.60, 135.71, 136.55, 136.61, 155.15, 155.22; MS *m/z* (abundance) 277 ($M^{+\cdot}$, 4.1%), 261 (0.4%). Anal. ($C_{12}H_{15}N_5O_3$) C, H, N.

4-Hexyl-1-[(benzo[1,2-*c*]1,2,5-oxadiazol-5(6)-yl N_1-oxide)methylidene]semicarbazide, IIIb: purified by crystallization from petroleum ether:EtOAc; pale orange needles (30%); mp 140.0−142.0 °C; IR ν_{max} 3400, 3090, 2930, 1680, 1610, 1355 cm^{-1}; ^{1}H NMR ($CDCl_3$, 100 MHz) δ 0.90 (t, J = 5.5 Hz, 3H), 1.38 (m, 4H), 1.60 (m, 4H), 3.40 (q, J = 6.0 Hz, 2H), 6.10 (m, 1H), 7.40−8.10 (m, 4H), 9.85 (bs, 1H); HRMS 305.1481 ($M^{+\cdot}$, 7.6%) (MW theoret 305.1488); HPLC (SiO_2, acetonitrile, μ = 3.3 mL/min, λ = 254 nm) t_R = 2.5 min. Anal. ($C_{14}H_{19}N_5O_3$) C, H, N.

4-Butyl-1-[(3-methylquinoxalin-2-yl N_1,N_4-dioxide) methylidene]semicarbazide, IVd: purified by column chromatography (Al_2O_3, CH_2Cl_2:MeOH (0−1%)) and then crystallized from petroleum ether:EtOAc:EtOH; yellow solid (28%); mp 235.0−237.0 °C; IR ν_{max} 3420, 2930, 2870, 1690, 1600, 1350 cm^{-1}; ^{1}H NMR (DMSO-d_6−D_2O, 100 MHz) δ 0.91 (t, J = 7.0 Hz, 3H), 1.45 (m, 4H), 2.86 (s, 3H), 3.78 (m, 2H), 7.95 (dd, J_1 = 7.0 Hz, J_2 = 8.0 Hz, 2H), 8.49 (s, 1H), 8.52 (d, J = 8.0 Hz, 1H), 8.58 (d, J = 8.0 Hz, 1H); MS *m/z* (abundance) 317 ($M^{+\cdot}$, 7.3%), 300 (21.6%). Anal. ($C_{15}H_{19}N_5O_3$) C, H, N.

Preparation of Semicarbazones IIIc,d. General Procedure. A mixture of **9** (1 equiv), the corresponding semicarbazide (1 equiv), *p*-TsOH (catalytic amounts), and MeOH as solvent was placed in a flask fitted with a Soxhlet extractor containing Linde 3A molecular sieves, to which was attached a condenser. The mixture was heated at reflux until **9** was not present (SiO_2, 1% MeOH in CH_2Cl_2). After the workup process the residue was purified as indicated.

4-[3-(Dimethylamino)propyl]-1-[(benzo[1,2-*c*]1,2,5-oxadiazol-5(6)-yl N_1-oxide)methylidene]semicarbazide, IIIc: purified by column chromatography (Al_2O_3, CH_2Cl_2:MeOH (0−10%)); colorless oil (60%); IR ν_{max} 3350, 3250, 1676, 1537, 1350 cm^{-1}; ^{1}H NMR (MeOH-d_4−D_2O, 100 MHz) δ 1.78 (quint, J = 6.0 Hz, 2H), 2.28 (s, 6H), 2.45 (m, 2H), 3.34 (m, 2H), 7.50−8.20 (m, 4H); MS *m/z* (abundance) 306 ($M^{+\cdot}$, 1.1%), 290 (1.4%).

4-[3-(Diethylamino)propyl]-1-[(benzo[1,2-*c*]1,2,5-oxadiazol-5(6)-yl N_1-oxide)methylidene]semicarbazide, IIId: purified by column chromatography (Al_2O_3, CH_2Cl_2:MeOH (0−10%)); colorless oil (70%); IR ν_{max} 3300, 3220, 1674, 1532, 1375, 1348 cm^{-1}; ^{1}H NMR (MeOH-d_4−D_2O, 100 MHz) δ 1.04 (t, J = 7.0 Hz, 6H), 1.80 (quint, J = 7.0 Hz, 2H), 2.60 (m, 6H), 3.38 (m, 2H), 7.50−8.30 (m, 4H); MS *m/z* (abundance) 334 ($M^{+\cdot}$, 0.1%), 318 (0.5%).

General Procedure for the Preparation of Amines IIc,d. A mixture of **6** (1 equiv), the corresponding amine (1 equiv), K_2CO_3 (1 equiv), KI (0.1 equiv), and THF as solvent was heated at reflux until **6** was not present (SiO_2, 10% EtOAc in petroleum ether). After the workup process the residue was purified as indicated.

3-[3-(Diethylamino)propylaminomethyl]-4-phenyl-1,2,5-oxadiazole N_2-Oxide Dihydrochloride, IIc. The base[28] obtained as an oil was dissolved in CH_2Cl_2 and treated with Et_2O saturated in hydrogen chloride. The hydrochloride was crystallized from Et_2O:EtOAc:MeOH, to give white needles (15%): mp 135.0−137.0 °C. Anal. ($C_{16}H_{24}N_4O_2$·2HCl·H_2O) C, H, N.

3-[3-(4-Morpholinyl)propylaminomethyl]-4-phenyl-1,2,5-oxadiazole N_2-oxide, IId: purified by column chromatography (Al_2O_3, CH_2Cl_2:MeOH (0−10%)); colorless oil (75%); IR ν_{max} 3300, 3050, 2945, 2850, 2810, 1595, 1115 cm^{-1}; ^{1}H NMR ($CDCl_3$, 100 MHz) δ 1.92 (quint, J = 6.0 Hz, 2H), 2.68 (m, 6H), 2.93 (t, J = 6.0 Hz, 2H), 3.95 (m, 4H), 4.14 (s, 2H), 7.20 (bs, 1H), 7.80 (m, 3H), 8.10 (m, 2H); MS *m/z* (abundance) 319 ($M^{+\cdot}$ + H, 0.2%), 301 (0.7%).

General Procedure for the Preparation of Amides IIIe−j. A mixture of acid **10** (1 equiv), $SOCl_2$ (10 equiv), and DMF (catalytic amounts) was heated at reflux for 3 h. The excess $SOCl_2$ was eliminated by distillation at reduced pressure, and the mixture was cooled to 0 °C. After addition of a mixture of the corresponding amine (1 equiv), Et_3N (2 equiv), and dry CH_2Cl_2 as solvent, the reaction was stirred at room temperature for 12 h. After the workup process the residue was purified as indicated.

5(6)-(Butylcarbamoyl)benzo[1,2-*c*]1,2,5-oxadiazole N_1-oxide, IIIe: purified by column chromatography (Al_2O_3, petroleum ether:EtOAc (0−20%)) and then crystallized from EtOH; yellow needles (30%); mp 164.0−166.0 °C; IR ν_{max} 3300, 3070, 2930, 2850, 1640, 1610, 1310 cm^{-1}; ^{1}H NMR ($CDCl_3$, 100 MHz) δ 0.98 (t, J = 6.0 Hz, 3H), 1.30−1.80 (m, 4H), 3.50 (q, J = 5.0 Hz, 2H), 6.30 (bs, 1H), 7.56 (d, J = 9.0 Hz, 1H), 7.74 (d, J = 9.0 Hz, 1H), 7.80 (s, 1H); MS *m/z* (abundance) 235 ($M^{+\cdot}$, 34.2%), 219 (9.5%). Anal. ($C_{11}H_{13}N_3O_3$) C, H, N.

5(6)-(Hexylcarbamoyl)benzo[1,2-*c*]1,2,5-oxadiazole N_1-oxide, IIIf: purified by column chromatography (Al_2O_3, petroleum ether:EtOAc (0−20%)) and then crystallized from petroleum ether:EtOAc; pale yellow needles (19%); mp 115.0−117.0 °C; IR ν_{max} 3270, 3070, 2920, 2840, 1635, 1610, 1310 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.93 (t, J = 6.8 Hz, 3H), 1.38 (m, 6H), 1.66 (quint, J = 7.2 Hz, 2H), 3.49 (q, J = 7.1 Hz, 2H), 6.18 (bs, 1H), 7.50−8.10 (m, 3H); MS *m/z* (abundance) 263 ($M^{+\cdot}$, 16.0%), 247 (15.0%). Anal. ($C_{13}H_{17}N_3O_3$) C, H, N.

5(6)-[3-(Dimethylamino)propylcarbamoyl]benzo[1,2-*c*]1,2,5-oxadiazole N_1-oxide, IIIg: purified by column chromatography (Al_2O_3, CH_2Cl_2); colorless oil (34%); IR ν_{max} 3300, 3090, 2960, 2880, 1660, 1540, 1300 cm^{-1}; ^{1}H NMR ($CDCl_3$, 100 MHz) δ 1.80 (quint, J = 6.0 Hz, 2H), 2.36 (s, 6H), 2.58 (t, J =

6.0 Hz, 2H), 3.58 (q, J = 6.0 Hz, 2H), 7.45−8.00 (m, 3H), 9.20 (bs, 1H); MS m/z (abundance) 264 ($M^{+\cdot}$, 1.1%), 248 (0.6%).

5(6)-[3-(Diethylamino)propylcarbamoyl]benzo[1,2-*c*]1,2,5-oxadiazole N_1-oxide, IIIh: purified by column chromatography (Al_2O_3, CH_2Cl_2); colorless oil (24%); IR ν_{max} 3300, 3050, 2960, 2880, 1650, 1535, 1290 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 1.10 (t, J = 7.0 Hz, 6H), 1.75 (quint, J = 6.5 Hz, 2H), 2.60 (q+m, 6H), 3.58 (q, J = 6.0 Hz, 2H), 7.30−8.30 (m, 3H), 9.50 (bs, 1H); MS m/z (abundance) 292 ($M^{+\cdot}$, 0.3%), 276 (0.8%).

5(6)-[3-(4-Morpholinyl)propylcarbamoyl]benzo[1,2-*c*]1,2,5-oxadiazole N_1-oxide, IIIi: purified by column chromatography (Al_2O_3, petroleum ether:CH_2Cl_2 (50−100%)); colorless oil (39%); IR ν_{max} 3310, 3090, 2970, 2870, 1650, 1595, 1535, 1310 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 1.83 (quint, J = 7.0 Hz, 2H), 2.55 (m, 6H), 3.57 (q, J = 5.5 Hz, 2H), 3.71 (m, 4H), 7.30−8.30 (m, 3H), 8.46 (bs, 1H); MS m/z (abundance) 306 ($M^{+\cdot}$, 0.5%), 290 (0.4%).

5(6)-[3-(1-Piperinyl)propylcarbamoyl]benzo[1,2-*c*]1,2,5-oxadiazole N_1-oxide, IIIj: purified by column chromatography (Al_2O_3, petroleum ether:CH_2Cl_2 (50−100%)); colorless oil (26%); IR ν_{max} 3300, 2930, 1645, 1530, 1285 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.51 (m, 2H), 1.61 (quint, J = 5.5 Hz, 4H); 1.80 (quint, J = 5.5 Hz, 2H), 2.49 (m, 4H), 2.58 (t, J = 5.3 Hz, 2H), 3.57 (t, J = 5.3 Hz, 2H), 7.35−8.30 (m, 3H), 9.29 (bs, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.80, 24.46, 26.39, 42.15, 55.15, 59.82, 114.65, 115.85, 117.03, 124.24, 128.91, 129.71, 131.07, 132.22, 132.39, 135.18, 146.21, 149.36, 164.16, 164.69; MS m/z (abundance) 288 ($M^{+\cdot}$ − 16, 1.4%).

4-Butyl-1-[(2,3-dimethylquinoxalin-6-yl N_1,N_4-dioxide)-methylidene]semicarbazide, IVa. A solution of **IIIa** (0.1 g, 0.36 mmol) in morpholine (0.9 mL) was stirred for 10 min at 0 °C. Butanone (0.1 mL) was then added, and the resulting solution was stirred for 18 h at room temperature. After concentration of the solution under reduced pressure, the crude oil was precipitated with Et_2O: purified by crystallization from EtOAc:acetone; yellow needles (40%); mp 238.0−240.0 °C; IR ν_{max} 3340, 3230, 2960, 2920, 2860, 1675, 1530, 1315 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 0.98 (t, J = 7.0 Hz, 3H), 1.60 (m, 4H), 2.76 (s, 3H), 2.78 (s, 3H), 3.40 (q, J = 6.0 Hz, 2H), 6.20 (t, J = 6.0 Hz, 1H), 8.06 (s, 1H), 8.15 (dd, J_1 = 2.0 Hz, J_2 = 9.0 Hz, 1H), 8.62 (d, J = 9.0 Hz, 1H), 8.71 (d, J = 2.0 Hz, 1H), 9.80 (bs, 1H); MS m/z (abundance) 331 ($M^{+\cdot}$, 7.2%), 315 (3.0%). Anal. ($C_{16}H_{21}N_5O_3 \cdot H_2O$) C, H, N.

4-Butyl-1-[(quinoxalin-6-yl N_1,N_4-dioxide)methylidene]semicarbazide, IVb. To a solution of **IIIa** (0.2 g, 0.72 mmol) and Et_2NH (0.07 mL) in DMF (0.5 mL) was added vinyl acetate (0.13 mL, 1.44 mmol) during 10 min at 0 °C. The reaction mixture was stirred for 24 h at 40 °C. Vinyl acetate (1.3 mL, 14.4 mmol) and Et_2NH (1.0 mL) were then added, and the heating was continued at 40 °C during 48 h. The solvent was removed in vacuo and the oily residue coevaporated with toluene (3 × 10 mL). The crude product was purified by column chromatography (SiO_2, CH_2Cl_2): colorless oil (7%); IR ν_{max} 3300, 2950, 2920, 2860, 1675, 1530, 1350 cm^{-1}; ^1H NMR (MeOH-d_4−D_2O, 100 MHz) δ 0.98 (t, J = 7.0 Hz, 3H), 1.50 (m, 4H), 3.34 (q, J = 6.0 Hz, 2H), 7.98 (s, 1H), 8.03 (dd, J_1 = 2.0 Hz, J_2 = 8.0 Hz, 1H), 8.31 (d, J = 2.0 Hz, 1H), 8.44 (d, J = 8.0 Hz, 1H), 8.48 (d, J = 2.0 Hz, 1H), 8.51 (d, J = 2.0 Hz, 1H); MS m/z (abundance) 301 ($M^{+\cdot}$ - 2, 2.7%), 285 (2.9%).

1-[(2(3)-Amino-3(2)-cyanoquinoxalin-6-yl N_1,N_4-dioxide)methylidene]-4-butylsemicarbazide, IVc. A mixture of **IIIa** (0.2 g, 0.72 mmol) and malononitrile (0.05 mL, 0.76 mmol) was stirred for 10 min at 0 °C. Over the cooled suspension was added a solution of Et_3N (1 drop) in DMF (0.22 mL). The mixture was allowed to stand at room temperature over 24 h and filtered off. The solid product was washed with Et_2O, boiling MeOH, boiling DMF, and boiling H_2O to give a red solid (67%): mp (the mixture) > 320 °C; IR ν_{max} 3390, 3280, 2930, 2860, 2230, 1685, 1605, 1525, 1340 cm^{-1}; ^1H NMR (DMSO-d_6−D_2O, 100 MHz) δ 0.97 (t, J = 7.0 Hz, 3H), 1.45 (m, 4H), 3.27 (m, 2H), 8.11 (d, J = 8.0 Hz, 0.4H), 8.14 (d, J = 8.0 Hz, 0.6H), 8.29 (s, 0.4H), 8.33 (s, 0.6H), 8.38 (s, 0.6H), 8.42 (s, 0.4H), 8.58 (d, J = 8.0 Hz, 0.6H), 8.61 (d, J = 8.0 Hz, 0.4H); MS m/z (abundance) 343 ($M^{+\cdot}$, 1.2%), 327 (6.6%). Anal. ($C_{15}H_{17}N_7O_3 \cdot H_2O$) C, H, N.

5(6)-(Hydroxymethyl)benzo[1,2-*c*]1,2,5-oxadiazole N_1-Oxide, 18. A solution of **9** (0.4 g, 2.4 mmol) in MeOH (5.0 mL) was stirred at 0 °C. $NaBH_4$ (90 mg, 2.4 mmol) was then added, and the resulting solution was stirred for 3 h at room temperature. The solvent was removed in vacuo, and the residue was purified by column chromatography (SiO_2, petroleum ether:EtOAc (10−30%)): colorless oil (76%); IR ν_{max} 3380, 3100, 2920, 2860, 1620, 1590, 1530, 1350, 1045 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.25 (bs, 1H), 4.70 + 4.73 (two singlets, 2H), 7.00−7.84 (m, 3H); MS m/z (abundance) 166 ($M^{+\cdot}$, 100.0%), 150 (4.0%).

5(6)-(Chloromethyl)benzo[1,2-*c*]1,2,5-oxadiazole N_1-Oxide, 19. A mixture of **18** (0.3 g, 1.8 mmol) and $SOCl_2$ (2.0 mL, 26.4 mmol) was stirred at reflux during 8 h. The mixture was poured onto ice, and the aqueous mixture was extracted with EtOAc (3 × 30 mL). The combined extracts were dried and evaporated in vacuo. The residue was purified by column chromatography (SiO_2, petroleum ether:EtOAc (0−5%)): colorless oil (52%); IR ν_{max} 3090, 2960, 1620, 1590, 1530 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.58 (s, 2H), 7.20−7.60 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 45.33, 112.25, 114.28, 116.96, 119.48, 130.35, 133.41, 139.26, 142.61, 152.49; MS m/z (abundance) 184 ($M^{+\cdot}$, 85%), 168 (4.0%).

General Procedure for the Preparation of Compounds Va,b. A mixture of **19** (1 equiv), butylamine or **11** (1 equiv), K_2CO_3 (1 equiv), KI (0.1 equiv), and THF as solvent was heated at reflux until **19** was not present (SiO_2, 5% EtOAc in petroleum ether). After the workup process the residue was purified as indicated.

5(6)-(Butylaminomethyl)benzo[1,2-*c*]1,2,5-oxadiazole N_1-oxide, Va: purified by column chromatography (SiO_2, petroleum ether:EtOAc (20−70%)); colorless oil (38%); IR ν_{max} 3330, 2960, 2930, 2860, 1530, 1350 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.91 (t, J = 7.2 Hz, 3H), 1.37 (sex, J = 7.7 Hz, 2H), 1.47 (quint, J = 7.1 Hz, 2H), 1.64 (bs, 1H), 2.61 (q, J = 7.2 Hz, 2H), 3.86 (s, 2H), 7.44−7.87 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.32, 20.76, 32.55, 49.53, 52.83, 113.25, 116.42, 125.16, 125.37, 131.96, 132.97, 133.64, 141.96, 145.37; MS m/z (abundance) 221 ($M^{+\cdot}$, 1.5%), 205 (1.2%).

4-Butyl-1-[(benzo[1,2-*c*]1,2,5-oxadiazol-5(6)-yl N_1-oxide)methyl]semicarbazide, Vb: purified by column chromatography (SiO_2, CH_2Cl_2:MeOH (0−5%)) and then crystallized from petroleum ether:EtOAc; yellow needles (25%); mp 149.0−151.0 °C; IR ν_{max} 3390, 3250, 3070, 2960, 2870, 1645, 1620, 1025 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 0.88 (t, J = 7.3 Hz, 3H), 1.26 (sex, J = 7.6 Hz, 2H), 1.39 (quint, J = 7.0 Hz, 2H), 3.12 (q, J = 6.4 Hz, 2H), 4.06 (s, 2H), 4.98 (bs, 1H), 6.22 (bs, 1H), 6.60 (bs, 1H), 7.50−8.00 (m, 3H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 13.55, 20.08, 32.90, 39.15, 55.86, 114.50, 116.12, 126.00, 131.89, 135.00, 143.10, 159.32; MS m/z (abundance) 279 ($M^{+\cdot}$, 2.2%), 263 (2.6%). Anal. ($C_{12}H_{17}N_5O_3$) C, H, N.

General Procedure for the Preparation VIa−c. A mixture of the corresponding N-oxide **IIIa**,**e**,**f** (1 equiv), Ph_3P (1 equiv), and EtOH as solvent was heated at reflux for 1.5 h. The EtOH was eliminated at reduced pressure. After the workup process the residue was purified as indicated.

1-[(Benzo[1,2-*c*]1,2,5-oxadiazol-5-yl)methylidene]-4-butylsemicarbazide, VIa: purified by column chromatography (SiO_2, petroleum ether:EtOAc (0−20%)) and then crystallized from petroleum ether:EtOAc: yellow needles (50%); mp 202.0−205.0 °C; IR ν_{max} 3420, 3090, 2950, 2860, 1675, 1550, 1350 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 1.02 (t, J = 7.0 Hz, 3H), 1.60 (m, 4H), 3.45 (q, J = 6.0 Hz, 2H), 6.15 (bt, J = 5.5 Hz, 1H), 7.75 (dd, J_1 = 1.0 Hz, J_2 = 8.5 Hz, 1H), 7.89 (bs, 2H), 8.00 (dd, J_1 = 1.0 Hz, J_2 = 9.0 Hz, 1H), 9.80 (bs, 1H); MS m/z (abundance) 261 ($M^{+\cdot}$, 4.0%), 232 (0.1%). Anal. ($C_{12}H_{15}N_5O_2$) C, H, N.

5-(Butylcarbamoyl)benzo[1,2-*c*]1,2,5-oxadiazole, VIb: purified by column chromatography (Al_2O_3, petroleum ether: Et_2O (10−30%)) and then crystallized from petroleum ether: EtOAc: white needles (49%); mp 205.0−207.0 °C; IR ν_{max} 3290, 3090, 2950, 2860, 1630, 1560 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz)

δ 0.97 (t, J = 7.0 Hz, 3H), 1.35−1.75 (m, 4H), 3.52 (q, J = 6.0 Hz, 2H), 6.62 (bs, 1H), 7.83 (dd, J_1 = 1.0 Hz, J_2 = 7.0 Hz, 1H), 7.93 (dd, J_1 = 1.0 Hz, J_2 = 7.0 Hz, 1H), 8.21 (t, J = 1.0 Hz, 1H); MS *m/z* (abundance) 219 ($M^{+\cdot}$, 18.1%), 204 (1.6%). Anal. ($C_{11}H_{13}N_3O_2$) C, H, N.

5-(Hexylcarbamoyl)benzo[1,2-*c*]1,2,5-oxadiazole, VIc: purified by column chromatography (Al_2O_3, petroleum ether: Et_2O (10−50%)) and then crystallized from petroleum ether: EtOAc: white needles (65%); mp 93.0−95.0 °C; IR ν_{max} 3280, 3070, 2920, 2850, 1625, 1550, 1270 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 0.92 (t, J = 6.0 Hz, 3H), 1.37 (m, 6H), 1.68 (m, 2H), 3.53 (q, J = 6.0 Hz, 2H), 6.28 (bs, 1H), 7.84 (dd, J_1 = 2.0 Hz, J_2 = 8.0 Hz, 1H), 7.95 (dd, J_1 = 2.0 Hz, J_2 = 8.0 Hz, 1H), 8.18 (t, J = 2.0 Hz, 1H); MS *m/z* (abundance) 247 ($M^{+\cdot}$, 15.5%), 218 (9.7%). Anal. ($C_{13}H_{17}N_3O_2$) C, H, N.

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. Voltages scan rates ranged from 0.1 to 0.5 V/s.

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

ESR Measurement. The radicals were generated by electrolytical reduction in situ at room temperature. ESR spectra were recorder in the *X* band (9.85 GHz) on a Bruker ECS 106 spectrometer, using a rectangular cavity with a 50-kHz field modulation, in DMSO (Aldrich, spectroscopy grade). The hyperfine splitting constants were estimated to be accurate within 0.05 G.

Antitrypanosomal Bioassays. *T. cruzi* epimastigotes (Tulahuen 2 strain) were grown at 28 °C in an axenic medium (BHI-tryptose) as previously described,[45] 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 give an initial concentration of 1×10^6 cells/mL. Cell growth was followed by measuring everyday the absorbance of the culture at 600 nm. 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 epimastigotes growth was observed by the presence of up to 1% DMSO in the culture media.

To determine ID_{50} values, 50% inhibitory doses, parasite growth was followed in the absence (control) and presence of increasing concentrations of the corresponding drug. At day 5, the absorbance of the culture was measured and related to the control. The ID_{50} value was taken as the concentration of drug needed to reduce the absorbance ratio to 50%.

Cytotoxicity Assays. Chinese hamster lung fibroblasts (V79-M8) were grown at 37 °C in Dubelcco's modified Eagle's medium (DMEM), pH 7.0, supplemented with 10% fetal calf serum, 472 U/mL penicillin, and 100 μg/mL streptomycin. The cells were cultured in humidified air plus 5% CO_2. Cells were plated in 96-well plates (4×10^3 cells/well) and allowed to proliferate for 16 h (∼2 doubling times). At this time point the drugs were added and incubated for 24 h. Then, cells were washed, new media were supplied, and after 4 h MTT (5 mg/mL) was added. Plates were wrapped in aluminum foil and further incubated for 4 h. Medium and MTT were removed from wells, and DMSO in glycine buffer (0.1 M glycine, 0.1 M NaCl, pH 10.5) was added to dissolve the formazan crystals. Absorbance was measured at 570 nm immediately. For this assay, compounds were supplemented from stock solutions in ethanol. Final concentration of ethanol in culture media never exceeded 0.14%, and control was run in the presence of 0.14% ethanol and in the absence of any drug.

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Supporting Information Available: Variable-temperature ^1H NMR data for **IIIa**. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Brener, Z. Present Status of Chemotherapy and Chemoprophylasis of Human Trypanosomiasis in the Western Hemisphere. *Pharmacol. Ther.* **1979**, *7*, 71−78.
(2) Messeder, J. C.; Tinoco, L. W.; Figueroa-Villar, J. D.; Souza, E. M.; Santa Rita, R.; de Castro, S. L. Aromatic Guanyl Hydrazones: Synthesis, Structural and in vitro Activity Against *Trypanosoma cruzi*. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 3079−3084.
(3) Schirmer, R. H.; Müller, J. G.; Krauth-Siegel, R. L. Disulfide-reductase Inhibitors as Chemotherapeutic Agents; The Design of Drugs for Trypanosomiasis and Malaria. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 141−145.
(4) Morello, A. The Biochemistry of the Mode of Action of Drugs and the Detoxification Mechanism in *Trypanosoma cruzi*. *Comp. Biochem. Physiol.* **1988**, *90C*, 1−12.
(5) Nagel, R. Genotoxicity Studies with Two Antichagasic Drugs. *Mutat. Res.* **1987**, *191*, 17−20.
(6) Docampo, R.; Stoppani, A. O. M. Generation of Oxygen-reduction Derivatives Induced by Nifurtimox and other Nitrocompounds in *Trypanosoma cruzi*. *Medicina* **1980**, *40* (Suppl. 1), 10−16.
(7) Docampo, R.; Moreno, S. N. J. In *Free Radicals in Biology: Free Radical Intermediates in the Trypanocidal Action of Drugs and Phagocytic Cells*; Pryor, W. A., Ed.; Academic Press: New York, 1984; Vol. VI, pp 243−288.
(8) Docampo, R.; Moreno, S. N. J. In *Oxygen Radicals in Chemistry and Biology: Free Radical Intermediates in the Trypanocidal Action of Drugs and Phagocytic Cells*; Bors, W., Ed.; 1984; pp 749−751.
(9) Docampo, R.; Moreno, S. N. J.; Stoppani, A. O. M.; Leon, W.; Cruz, F. S.; Villalta, F.; Muniz, R. P. A. Mechanism of Nifurtimox Toxicity in Different forms of *Trypanosoma cruzi*. *Biol. Pharmacol.* **1981**, *30*, 1947−1951.
(10) Cerecetto, H.; Di Maio, R.; González, M.; Seoane, G. Preparation of Enamines from the Condensation of Glycine Esters with Nitro-Heterocyclic Aldehydes. *Heterocycles* **1997**, *45* (10), 2023−2031.
(11) Cerecetto, H.; Di Maio, R.; Ibarruri, G.; Seoane, G.; Denicola, A.; Peluffo, G.; Quijano, C.; Paulino, M. Synthesis and Antitrypanosomal Activity of Novel 5-Nitro-2-furaldehyde and 5-Nitrothiophene-2-carboxaldehyde Semicarbazone Derivatives. *Farmaco* **1998**, *53* (2), 89−94.
(12) Olea-Azar, C.; Atria, A. M.; Mendizabal, F.; Di Maio, R.; Seoane, G.; Cerecetto, H. Cyclic Voltammetry and Electron Paramagnetic Resonance Studies of Some Analogues of Nifurtimox. *Spectrosc. Lett.* **1998**, *31* (1), 99−109.
(13) Olea-Azar, C.; Atria, A. M.; Mendizabal, F.; Di Maio, R.; Seoane, G.; Cerecetto, H. Electron Spin Resonance and Cyclic Voltammetry Studies of Nitrofurane and Nitrothiophene Analogues of Nifurtimox. *Spectrosc. Lett.* **1998**, in press.
(14) Paulino, M.; Iribarne, F.; Hansz, M.; Vega, M.; Seoane, G.; Cerecetto, H.; Di Maio, R.; Caracelli, I.; Zukerman-Schpector, J.; Olea, C.; Stoppani, A. O. M.; Basombrío, M. A.; Fairlamb, A. H.; Tapia, O. Unpublished results.
(15) Cahill, A.; White, I. N. Reductive Activation of *N*-oxides to Cause DNA Strand Breakage in Cell Lines *in vitro*. *Biochem. Soc. Trans.* **1991**, *19* (2), 1275.
(16) Brown, J. M. Redox Activation of Benzotriazine *N*-oxides: Mechanisms and Potential as Anticancer Drugs. In *Selective Activation of Drugs by Redox Processes*; Breccia, A., Adams, G. E., Fielden, E. M., Wardman, P., Eds.; Plenum Press: Fermo, Italy; pp 137−148.
(17) Henderson, G. B.; Ulrich, P.; Fairlamb, A. H.; Rosenberg, I.; Pereira, M.; Sela, M.; Cerami, A. "Subversive" Substrates for the Enzyme Trypanothione Disulfide Reductase: Alternative Approach to Chemotherapy of Chagas Disease. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5374−5378.

(18) Hunter, W. N.; Bailey, S.; Habash, J.; Harrop, S. J.; Helliwell, J. R.; Aboyage-Kwarteng, T.; Smith, K.; Fairlamb, A. H. Active Site of Trypanothione Reductase: A Target for Rational Drug Design. *J. Mol. Biol.* **1992**, *227*, 322−333.
(19) Chan, C.; Yin, H.; Garforth, J.; McKie, J. H.; Jaouhari, R.; Speers, P.; Douglas, K. T.; Rock, P. J.; Yardley, V.; Croft, S. L.; Fairlamb, A. H. Phenotiazine Inhibitors of Trypanothione Reductase as Potential Antitrypanosomal and Antileishmanial Drugs. *J. Med. Chem.* **1998**, *41*, 148−156.
(20) Fairlamb, A. H.; Blackburn, P.; Ulrich, P.; Chait, B. T.; Cerami, A. Trypanothione: a Novel Bis(glutathionyl)spermidine Cofactor for Glutathione Reductase in Trypanosomatids. *Science* **1985**, *227*, 1485−1487.
(21) Fairlamb, A. H.; Cerami, A. Metabolism and Functions of Trypanothione in the Kinetoplastida. *Annu. Rev. Microbiol.* **1992**, *46*, 695−729.
(22) Lantwin, C. B.; Schlichting, I.; Kabsch, W.; Pai, E. F.; Krauth-Siegel, R. L. The Structure of *Trypanosoma cruzi* Trypanothione Reductase in the Oxidized and NADPH Reduced State. *Proteins* **1994**, *18*, 161−173.
(23) Fruttero, R.; Ferrarotti, B.; Serafino, A.; Di Stilo, A.; Gasco, A. Unsymmetrically Substituted Furoxans. Part 11[1]. Methylfuroxancarbaldehydes. *J. Heterocycl. Chem.* **1989**, *26*, 1345−1347.
(24) Monge, A.; Aldana, I.; Cerecetto, H.; Rivero, A. Synthesis of New Oxazolidine, Oxazolidin-2-one and Perhydro-1,4-oxazine Derivatives of Arylethanolamine as Potential β_3-Adrenoceptor Agonists. *J. Heterocycl. Chem.* **1995**, *32*, 1429−1432.
(25) Israel, M.; Samour-Rosenfield, J.; Modest, E. J. Analogues of Spermine and Spermidine. I. Synthesis of Polymethylenepolyamines by Reduction of Cyanoethylated α,ω-Alkylenediamines. *J. Med. Chem.* **1964**, *1* (7), 710−716.
(26) Gould, F. E.; Johnson, G. S.; Ferris, A. F. The Hydrogenation of Nitriles to Primary Amines. *J. Org. Chem.* **1960**, *25*, 1658−1660.
(27) Gasco, A. M.; Fruttero, R.; Sorba, G.; Gasco, A. Phenylfuroxancarbaldehydes and Related Compounds. *Liebigs Ann. Chem.* **1991**, 1211−1213.
(28) Monge, A.; López de Ceráin, A.; Ezpeleta, O.; Cerecetto, H.; Dias, E.; Di Maio, R.; González, M.; Onetto, S.; Seoane, G.; Suescun, L.; Mariezcurrena, R. Synthesis and Biological Evaluation of 1,2,5-Oxadiazole *N*-oxide Derivatives as Hypoxia-Selective Cytotoxins. *Pharmazie* **1998**, in press.
(29) Edwards, M. L.; Bambury, R. E. 2,3-Dimethylquinoxaline-6-carboxaldehyde 1,4-Dioxide. *J. Heterocycl. Chem.* **1975**, *12* (5), 835−836.
(30) Ghosh, P. B.; Whitehouse, M. W. Potential Antileukemic and Immunosuppressive Drugs. Preparation and in Vitro Pharmacological Activity of some Benzo-2,1,3-oxadiazoles (Benzofurazans) and their *N*-Oxides (Benzofuroxans). *J. Med. Chem.* **1968**, *11*, 305−310.
(31) Haddadin, M. J.; Issidorides, C. H. Application of Benzofurazan Oxide to the Synthesis of Heteroaromatic *N*-Oxides. *Heterocycles* **1976**, *4* (4), 767−816.
(32) Monge, A.; Palop, J. A.; López de Ceráin, A.; Senador, V.; Martínez-Crespo, F. J.; Sáinz, Y.; Narro, S.; García, E.; de Miguel, C.; González, M.; Hamilton, E.; Barker, A. J.; Clarke, E. D.; Greenhow, D. T. Hypoxia-Selective Agents Derived from Quinoxaline 1,4-Di-*N*-oxides. *J. Med. Chem.* **1995**, *38*, 1786−1792.
(33) Harris, R. K.; Katritzky, A. R.; Oksne, S.; Bailey, A. S.; Paterson, W. G. *N*-Oxides and Related Compounds. Part XIX. Proton Resonance Spectra and the Structure of Benzofuroxan and its Nitro-derivatives. *J. Chem. Soc.* **1963**, 197−203.
(34) Boulton, A. J.; Katritzky, A. R.; Sewell, M. J.; Wallis, B. *N*-Oxides and Related Compounds. XXXI. The Nuclear Magnetic Resonance Spectra and Tautomerism of some Substituted Benzofuroxans. *J. Chem. Soc. B* **1967**, *9*, 914−917.
(35) Boulton, A. J.; Middleton, D. Furazans and Furazan Oxides. V. Tropono[4,5-*c*]-, Thieno[2,3-*c*]-, and Biphenyleno[2,3-*c*]furazan Oxides. *J. Org. Chem.* **1974**, *39* (20), 2956−2962.
(36) Calvino, R.; Fruttero, R.; Gasco, A.; Mortarini, V.; Aime, S. Unsymmetrically Substituted Furoxans. VII (1). A ^{13}C NMR Study of a Series of Isomeric Pairs of Furoxans and the Structure of Two Isomeric Chloro-phenyl-furoxans. *J. Heterocycl. Chem.* **1982**, *19*, 427−430.
(37) Diel, P. J.; Schmid, W. Ger. Offen. 2,344,314; *Chem. Abstr.* **1974**, *80*, 146201q.
(38) Ley, K.; Seng, F. Synthesis Using Benzofuroxanes. *Synthesis* **1975**, 415−422.
(39) Albin, A.; Pietra, S. *Heterocycles N-oxides*; CRC Press: Boca Raton, FL, 1991.
(40) Cheeseman, G. W. H.; Cookson, R. F. *Condensed Pyrazines*; J. Wiley and Sons, Inc.: New York, 1979.
(41) Tennant, G.; Mason, J. C. Synthesis of 1-Hidroxyquinoxalin-2(1H)-one 4-Oxides. *J. Chem. Soc. D* **1971**, 586−587.
(42) Johnston, J. D. U.S. Pat. 3,344,022; *Chem. Abstr.* **1967**, *67*, 111452b.
(43) Howard, E.; Olszewski, W. F. The Reaction of Triphenylphosphine with Some Aromatic Amine Oxides. *J. Am. Chem. Soc.* **1959**, *81*, 1483−14847.
(44) Boyer, J. H.; Ellzey, S. E. Deoxygenation of Aromatic *o*-Dinitroso Derivatives by Phosphines. *J. Org. Chem.* **1961**, *26*, 4684−4685.
(45) Denicola, A.; Rubbo, H.; Rodríguez, D.; Radi, R. Peroxynitrite-mediated Cytotoxicity to *Trypanosoma cruzi*. *Arch. Biochem. Biophys.* **1993**, *304*, 279−286.
(46) Monge, A.; López de Ceráin, A.; Ezpeleta, O.; Cerecetto, H.; Dias, E.; Di Maio, R.; González, M.; Onetto, S.; Risso, M.; Seoane, G.; Zinola, F.; Olea-Azar, C. 1,2,5-Oxadiazole *N*-oxide Derivatives as Hypoxia-selective Cytotoxins. Structure−activity Relationships. *Pharmazie* **1998**, in press.
(47) Cain, B. F.; Atwell, G. J.; Seelye, R. N. Potential Antitumour Agents. *J. Med. Chem.* **1969**, *12*, 199−206.
(48) Cain, B. F.; Atwell, G. J.; Denny, W. A. Potential Antitumour Agents. 16. 4′-(Acridin-9-ylamino)methanesulfonanilides. *J. Med. Chem.* **1975**, *18* (11), 1110−1117.
(49) Tsantili-Kakoulidou, A.; Antoniadou-Vyza, A. Hydrophobicity Studies on Diadamantyl Derivatives. Comparison Between Calculated Partition Coefficients and Experimental Lipophilicity Indices Determined by Reversed-Phase High-Performance Chromatography and Thin-Layer Chromatography. *J. Chromatogr.* **1988**, *445*, 317−326.
(50) Denny, W. A.; Atwell, G. J.; Roberts, P. B.; Anderson, R. F.; Boyd, M.; Lock, C. J. L.; Wilson, W. R. Hypoxia-Selective Antitumor Agents. 6. 4-(Alkylamino)nitroquinolines: A New Class of Hypoxia-Selective Cytotoxins. *J. Med. Chem.* **1992**, *35*, 4832−4841.
(51) Chowdhury, H.; Saxena, V. S.; Walia, S. Synthesis and Insect Growth Regulatory Activity of Alkoxy-Substituted Benzaldoxime Ethers. *J. Agric. Food Chem.* **1998**, *46*, 731−736.
(52) Smith, P. A. S.; Boyer, J. H. In *Organic Syntheses*; Norman Rabjohn, Ed.; John Wiley & Sons Inc.: New York, 1963; Collect. Vol. 4, pp 75−78.
(53) Ley, K.; Seng, F.; Eholzer, U.; Nast, R.; Schubart, R. New Syntheses of Quinoxaline and Phenazine Di-*N*-Oxides. *Angew. Chem., Int. Ed.* **1969**, *8* (8), 596−597.
(54) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*; Pergamon Press: England, 1988.

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