2*H*-Benzimidazole 1,3-Dioxide Derivatives: A New Family of Water-Soluble Anti-Trypanosomatid Agents†

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Received January 11, 2006

Three series of benzimidazole *N*-oxide derivatives were developed and were examined for their activity against trypanosomatid parasites (*Trypanosoma cruzi* and *Leishmania* spp.). 2*H*-Benzimidazole 1,3-dioxides displayed remarkable in vitro activities against both parasites, with derivatives **28**, **29**, and **32** being the most potent (IC_{50} < 5 μM) against the epimastigote form of *T. cruzi* and **28**, **33**, and **35** the most potent against the promastigote form of *Leishmania* spp. Unspecific cytotoxicity was evaluated using murine macrophages, and derivative **33** was not toxic at a concentration 30 times that of its IC_{50} against *T. cruzi* that was completely toxic for *Leishmania* spp., implying that the series of 2*H*-benzimidazole 1,3-dioxides is selective toward both trypanosomatid parasites. Derivatives **33** and **35** were submitted to an in vivo assay using an acute model of Chagas' disease and a short-term treatment (30 mg/kg/day orally administrated as aqueous solution, during 10 days). While in the control (untreated) and Benznidazole (50 mg/kg/day) groups survival fraction was 60.0% and 87.5%, respectively, none of the animals treated with derivatives **33** and **35** died. From the preliminary structure−activity relationship studies reduction potential and electrophilicity were found relevant to anti-*T. cruzi* activity. Active compounds are better electrophiles and more easily reduced than inactive ones.

Introduction

Parasitic diseases affect hundreds of millions people around the world, mainly in underdeveloped countries. Since parasitic protozoa are eukaryotic they share many common features with their mammalian host, making the development of effective and selective drugs a hard task. Despite the great effort that has been done in the discovery of unique targets that afford selectivity, many of the drugs used today have serious side effects. Diseases caused by *Trypanosomatidae*, which share a similar state regarding drug treatment, include Chagas' disease (*Trypanosoma cruzi*) and leishmaniasis (*Leishmania* spp.).[1] These trypanosomatids alone are responsible for an infected population of nearly 30 million and more than 400 million are at risk.[2] Drugs currently used in the treatment of Chagas' disease are two nitroaromatic heterocyles, Nifurtimox (4-(5-nitrofurfurylindenamino)-3-methylthiomorpholine-1,1-dioxide) (Nfx, recently discontinued by Bayer) and Benznidazole (*N*-benzyl-2-(2-nitro-1*H*-imidazol-1-yl)acetamide) (Bnz, Rochagan, Roche), introduced empirically over three decades ago.[3] Both drugs are active in the acute phase of the disease but efficacy is very low in the established chronic phase. What is more, differences in drug susceptibility among different *T. cruzi* strains lead to varied parasitological cure rates according to the geographical area. The drugs of choice for the treatment of leishmaniasis are sodium stibolgluconate (Pentostam), meglumine antimoniate (Glucantime), pentamidine (1,5-di-(4-amidinophenoxy)pentane) (Ptd) and liposomal amphotericin B, but these sometimes meet with failure.[4] Currently, WHO/TDR is developing a research program with Miltefosine (hexadecylphosphorylcholine), a very promising leishmanocidal drug, but new therapeutic alternatives should be found in order to increase the pharmaceutical arsenal.[5,6]

Extensive work, in the last two decades, has helped to understand the molecular basis of the antichagasic activity of both drugs currently used in clinic.[7,8] Nfx acts via the reduction of the nitro group to a nitroanion radical that in term reacts with oxygen to produce superoxide, a highly toxic metabolite, in a process known as redox cycling. The mechanism of action of Bnz also involves nitro reduction; but reduced intermediates act covalently, modifying biomacromolecules. Most frequent side effects of these drugs include anorexia, vomiting, peripheral polyneuropathy, and allergic dermopathy that are probably a result of oxidative or reductive damage to the host's tissue and are thus inextricably linked to its antiparasitic activity.[9]

However, it has been pointed out that drugs that produce oxidative stress by redox cycling may be selective, as long as they are selectively reduced by oxidoreductases that are unique to the parasite.[10] The same could be said for drugs that produce reductive damage such as Bnz. Following this reasoning, our group has been looking for less toxic and more selective antichagasic drugs by using an *N*-oxide moiety as the bioreductive group. Thus, benzofuroxan derivatives (benzo[1,2-*c*]1,2,5-oxadiazole *N*-oxide) were described for the first time

† Part of this research is presented in the Uruguayan patent of invention: Boiani, M.; Cerecetto, H.; González, M.; Merlino, A. Derivados de 1,3-dióxido de benzimidazol. Procedimiento de preparación y utilización (Benzimidazole 1,3-dioxide derivatives. Preparation procedure and utilization). UR Patent No. 29076, 2005.

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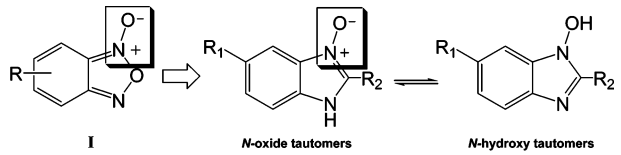
10.1021/jm0600343 CCC: $33.50
Published on Web 04/29/2006

Chart 1

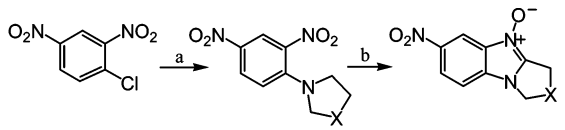


I — ***N*-oxide tautomers** — ***N*-hydroxy tautomers**

Benzofuroxan derivatives — **Benzimidazole *N*-oxide derivatives**

-R_1	-R_2	
-NO_2	-CO_2Et	**1**
-NO_2	-CO_2nBut	**2**
-NO_2	-CO_2iPr	**3**
-NO_2	-CO_2nHex	**4**
-NO_2	-CONHnBut	**5**
-NO_2	-H	**6**
-CH_3	-CO_2Et	**7**
-CO_2Et	-CO_2Et	**8**

Scheme 1[a]



-X- = -CH_2-	**9** (40%)*	**14** (46%)
-X- = -$[CH_2]_2$-	**10** (83%)	**15** (19%)
-X- = -CH_2-O-	**11** (80%)	**16** (21%)
-X- = -CH_2-S-	**12** (79%)	**17** (27%)
-X- = -CH_2-N(Ph)-	**13** (95%)	---

[a] Conditions: (a) corresponding amine (1 equiv), $NaHCO_3$ (1 equiv), EtOH, rt; (b) HCl, reflux. *Parentheses indicate the reaction yields.

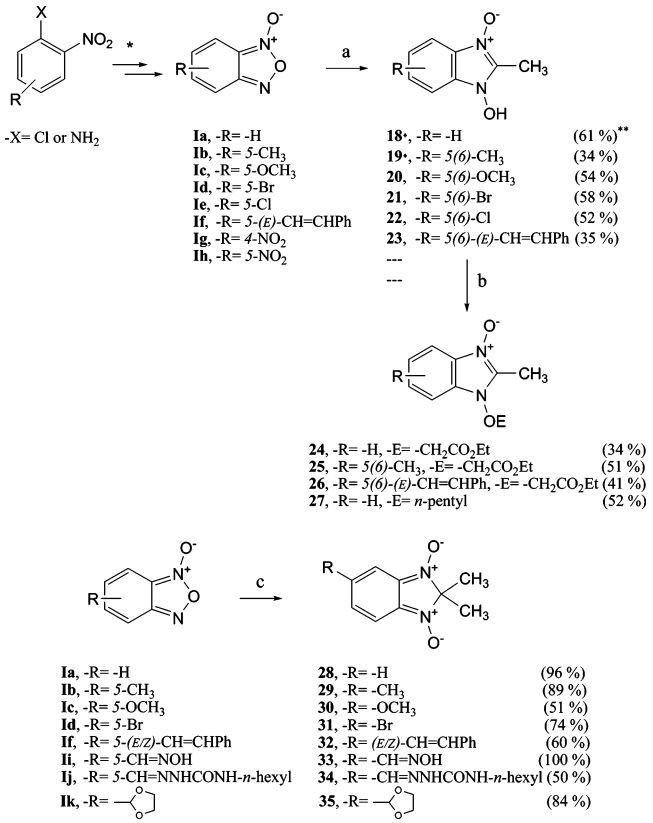
as anti-*T. cruzi* agents in vitro, displaying activities similar to or higher than the reference drugs (Chart 1).[11−17] To study other heterocyclic systems the first series of benzimidazole *N*-oxide derivatives was developed (Chart 1), but showed very low activity in vitro.[18] Since these derivatives were tautomeric isomers of *N*-hydroxybenzimidazoles, it can be argued that they were not "true" *N*-oxides.[19]

In the present work, three series of nontautomerizable benzimidazole *N*-oxides were developed and were examined for antiproliferative in vitro activity against *T. cruzi* (epimastigote form) and *Leishmania* spp. (promastigote form). The lytic effect on *T. cruzi* blood trypomastigotes was also studied in vitro. Compounds previously described against *T. cruzi* epimastigotes (Chart 1)[18] were evaluated against *T. cruzi* trypomastigotes and *Leishmania* spp. Unspecific mammal cytotoxicity of the most actives compounds was evaluated in vitro, and less toxic derivatives were submitted to in vivo biological evaluation using a murine model of acute Chagas' disease. Physicochemical properties that could be related to activity were determined, and preliminary structure−activity relationships are presented.

Results and Discussion

Chemistry. Two general approaches were used to obtain benzimidazole *N*-oxide derivatives: cyclization of *o*-nitroanilines and reaction of benzofuroxan derivatives with nitroalkanes.[20] This made it possible to achieve diversity even with the synthesis of a small number of compounds. In the first approach, 1*H*-benzimidazole *N*-oxide **14**−**17** were synthesized through acid-catalyzed thermal cyclization of *o*-nitro-*t*-anilines in moderate yield (Scheme 1). Other conditions were assayed in order to improve this yield (use of organic acids, microwave irradiation) but without success. Although this description is quite old,[21] until now it is the only one known to obtain 1*H*-benzimidazole *N*-oxide derivatives substituted at nitrogen-1. The low yields observed could be attributed to low reactivity, since the starting anilines were recovered from the reaction mixture. In the case of aniline **13** no reaction was observed after 80 h. Synthesis of 1-hydroxy-1*H*-benzimidazole 3-oxide derivatives

Scheme 2[a]



-X= Cl or NH_2

Ia, -R= -H	**18**•, -R= -H	(61 %)**
Ib, -R= 5-CH_3	**19**•, -R= *5(6)*-CH_3	(34 %)
Ic, -R= 5-OCH_3	**20**, -R= *5(6)*-OCH_3	(54 %)
Id, -R= 5-Br	**21**, -R= *5(6)*-Br	(58 %)
Ie, -R= 5-Cl	**22**, -R= *5(6)*-Cl	(52 %)
If, -R= *5-(E)*-CH=CHPh	**23**, -R= *5(6)-(E)*-CH=CHPh	(35 %)
Ig, -R= *4*-NO_2	---	
Ih, -R= *5*-NO_2	---	

24, -R= -H, -E= -CH_2CO_2Et (34 %)
25, -R= *5(6)*-CH_3, -E= -CH_2CO_2Et (51 %)
26, -R= *5(6)-(E)*-CH=CHPh, -E= -CH_2CO_2Et (41 %)
27, -R= -H, -E= *n*-pentyl (52 %)

Ia, -R= -H	**28**, -R= -H	(96 %)
Ib, -R= 5-CH_3	**29**, -R= -CH_3	(89 %)
Ic, -R= 5-OCH_3	**30**, -R= -OCH_3	(51 %)
Id, -R= 5-Br	**31**, -R= -Br	(74 %)
If, -R= *5-(E/Z)*-CH=CHPh	**32**, -R= *(E/Z)*-CH=CHPh	(60 %)
Ii, -R= 5-CH=NOH	**33**, -R= -CH=NOH	(100 %)
Ij, -R= 5-CH=NNHCONH-*n*-hexyl	**34**, -R= -CH=NNHCONH-*n*-hexyl	(50 %)
Ik, -R=	**35**, -R=	(84 %)

[a] Conditions: (a) nitroethane (1 equiv), piperidine (1 equiv), THF, rt; (b) ethyl 2-bromoacetate or pentyl iodide (1.2 equiv), piperidine (1.2 equiv), DMSO, rt; (c) 2-nitropropane (1.2 equiv), piperidine, (1.2 equiv), THF, rt. *References 11, 12, 16, and 17. ◆Reference 18. **Parentheses indicate the reaction yields.

from benzofuroxans has already been described using different alkylating agents.[22,23] Among them nitroalkanes offer a simple and attractive alternative.[24] Benzimidazole derivatives **18**−**23** were easily obtained by reaction of the corresponding benzofuroxan with nitroethane in basic media at room temperature (Scheme 2). Benzofuroxans **Ia**−**k** were selected from our library of antiparasite agents as the starting material.[11,12,16,17] Benzofuroxans having a nitro moiety lead only to tarry products, even under milder conditions (ice-bath). Probably, the presence of other electrophilic atoms is responsible for the final complex mixture of reactions. Derivatives substituted at the benzene ring (**19**−**23**) are present as an isomeric mixture (5 and 6 isomer) due to the tautomeric equilibrium shown in Scheme 2. Derivatives **18**−**23** displayed a very low solubility in the different organic solvents assayed (acetone, methanol, DMSO), which could be assigned to their high polarity and the presence of a free hydroxyl moiety. Alkylation of this group using ethyl 2-bromoacetate or pentyl iodide lead to derivatives **24**−**27** (Scheme 2).[18,19] Also, these derivatives were obtained as chromatographically inseparable mixtures of positional isomers (5 and 6 isomers from NMR analysis). In contrast with their parent compounds (**18**−**23**), derivatives **24**−**27** displayed high solubility in organic solvents. In an analogous manner, reaction of the corresponding benzofuroxans with 2-nitropropane afforded the 2*H*-benzimidazole 1,3-dioxide derivatives **28**−**35** in very good yields (Scheme 2). Contrary to the solubility problems observed for derivatives **18**−**23**, derivatives **28**−**35** are soluble in different organic solvents (hexane, chloroform, and acetone) as well as in water.

Table 1. In Vitro Antiproliferative Activity (Epimastigote Form, Tulahuen 2 Strain)

compd	%GI[a,b]	compd	%GI[a,b]
14	0	**26**	8 (11)
15	0	**27**	0 (57)
16	0	**28**	87
17	0	**29**	85
18	0 (0)[c]	**30**	52
19	0 (0)	**31**	77
20	0 (24)	**32**	100
21	0 (18)	**33**	72
22	0 (8)	**34**	69
23	19 (15)	**35**	59
24	1 (12)	Nfx	93
25	25 (15)	Bnz	92

[a] %GI = percentage of growth inhibition at 25 μM of the compound. [b] Results are the mean of three different experiments with a SD less than 10% in all cases. [c] Values in parentheses are %GI at 100 μM.

Table 2. In Vitro Antiproliferative Activity of 2*H*-Benzimidazole 1,3-Dioxides (Epimastigote Form)

	IC_{50} (μM)		
compd	Tulahuen 2 strain	CL Brener strain	Y strain
28	4.3	9.0	14.0
29	5.1	13.5	13.0
30	28.5	25.0	17.0
31	8.3	12.0	13.8
32	3.4	6.0	5.0
33	12.5	14.5	11.8
35	22.7	20.0	14.0
Nfx	7.7	8.5	6.5
Bnz	8.5	3.4	3.8

All of the proposed structures were established by ^{1}H and ^{13}C NMR (HMQC, HMBC) spectroscopy and MS. The purity was established by TLC and microanalysis.

Biology. In Vitro Anti-Trypanosomatid Activity. As a first screening, the ability of benzimidazole derivatives to inhibit the growth of the epimastigote form of *T. cruzi* (Tulahuen 2 strain) was evaluated at 25 and/or 100 μM, and the IC_{50} was determined for the most active compounds (Tables 1 and 2). Parasites were grown in the presence of the compound for 5 days, and the percentage of growth inhibition (%GI) was determined against control (no drug added to the medium) as explained in Experimental Section.[25] Benzimidazoles **14**−**17**, "true" N^3-oxides, showed no activity at 25 μM, implying that the sole presence of the *N*-oxide moiety is not enough for the activity. A similar result could be seen with derivatives **18**−**27**, *N*-hydroxy and *N*-alkoxy benzimidazole *N*-oxides. While solubility problems could be responsible for the lack of activity of derivatives **18**−**23**, another explanation has to be found for the corresponding alkyl derivatives (**24**−**27**).

Otherwise, benzimidazole 1,3-dioxide derivatives, **28**−**35**, displayed very interesting activities, in the order of the reference drugs (Nfx and Bnz), and derivatives **28**, **29**, and **32** were even more potent against the Tulahuen 2 strain (Table 2). This family of derivatives was also evaluated against other *T. cruzi* strains, the susceptible CL Brener strain and the partially Nfx- and Bnz-resistant Y strain[26] (Table 2). Derivative **32**, with a phenylethenyl substituent, was the most potent, and derivative **30**, with a methoxy substituent, was the least potent for the three strains. Major differences were seen for derivatives **28** and **29**, possessing a proton and a methyl group, respectively.

Since transmission of *T. cruzi* by blood transfusion is becoming a source of concern in countries free of vectorial transmission, the need for new compounds for chemoprophylaxis has been pointed out.[27] Despite the high efficacy of gentian violet (GV), a phenylmethane dye currently used in the treatment of blood, there are some restrictions to its use. Here, the lytic effect on mouse blood trypomastigotes (CL Brener strain) was analyzed for some of the new and previously described[18] benzimidazole derivatives by incubation of the parasites with the compounds for 24 h at 4 °C (see Experimental Section) (Table 3).[28] However, at the dose assayed only derivative **8** showed moderate activity as compared to the reference drug. The "true" benzimidazole N^3-oxide **20** and the 1,3-dioxides **30**, **33**, and **35** were less active than derivative **8**.

Table 3. In Vitro Trypanocidal Activity (Trypomastigote Form)

compd	% lysis[a,b]
1	0
2	0
8	50
18	0
19	0
20	23
28	10
29	0
30	34
33	21
35	28
GV	100

[a] % lysis = percentage of parasite lysis at 250 μg/mL of the compound. [b] Results are the mean of three different experiments with a SD less than 10% in all cases.

Table 4. In Vitro Leishmanicidal Activity (Promastigote Form)[a]

compd	LPH8	L2259	L2903
1	+	+	+
8	+	+	+
18	+	+	++
19	+	+	+
20	+	+	+
28	+++	+++	+++
29	++	++	++
30	++	+	++
33	+++	+++	+++
35	+++	+++	+++
Ptd	+++	+++	+++

[a] Lysis after 72 h of treatment with 100 μg/mL of the compound. LPH8 = *L. amazonensis*; L2259 = *L. infantum*; L2903 = *L. braziliensis*. +++ = total lysis of the parasite, ++ = parcial lysis (70−90%), + = same growth as control but low motility.

Leishmanicidal activity was assayed in vitro in three different *Leishmania* species using a qualitative methodology (see footnote of Table 4).[29] Similar to what was seen for anti-epimastigotes of *T. cruzi* activity, benzimidazole 1,3-dioxides were the most active compounds, but they displayed a different pattern of responses (Table 4). Compound **29**, as active as **28** against *T. cruzi* epimastigotes (Tulahuen strain), showed lower activity against *Leishmania* spp. than **33** and **35**. Interestingly, compound **18**, an *N*-hydroxybenzimidazole *N*-oxide, showed an intermediate activity against *Leishmania braziliensis.* In general, important differences in susceptibility among *Leishmania* species were not seen.

Unspecific Cytotoxicity. Mammal cytotoxicity of the best trypanocidal derivatives, benzimidazole 1,3-dioxides, was evaluated in vitro at 100, 10, and 1 μg/mL, using J774 macrophages as the cellular model (Figure 1).[30] Considering an averaged molecular weight of 250 g/mol, these concentrations correspond to 400, 40, and 4 μM, respectively.

Derivative **33** was not toxic at any of the concentrations assayed, while derivative **35** was slightly toxic, near 50%, at the highest assayed concentration (400 μM). Derivatives **28**−**32** showed high toxicity at 100 μg/mL but moderate to low

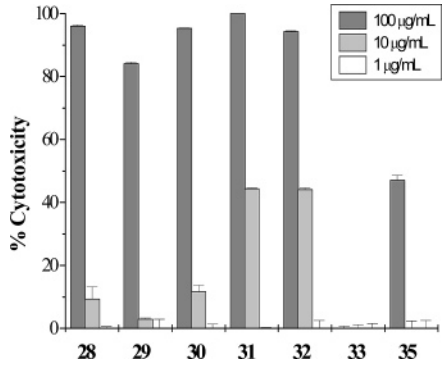


Figure 1. Cytotoxicity of benzimidazole 1,3-dioxide derivatives to J774 macrophages at different compound concentrations. Means of three test ± standard deviation.

toxicity, lower than 50%, at the intermediate concentration, approximately 40 μM, where they were highly toxic to *T. cruzi* epimastigotes. At the lower concentration assayed none of the compounds showed cytotoxicity, while derivatives **28**, **29**, and **32** remained toxic to the Tulahuen 2 *T. cruzi*. Remarkably, compound **33** showed no mammal cytotoxicity at a concentration that was almost 30 times that of its IC_{50} against *T. cruzi* epimastigotes and that was completely toxic for the three species of *Leishmania*.

In Vivo Anti-*T. cruzi* Evaluation.[31,32] The best in vitro anti-*T. cruzi* and the least toxic derivatives, compounds **33** and **35**, were evaluated in vivo in a murine model of acute Chagas' disease. In this preliminary study, male BALB/c mice were inoculated intraperitoneally with 1000 blood trypomastigotes and treatment began 10 days post-infection with oral administration of 30 mg/kg/day of each compound during 10 days. Since compounds presented good solubility in water, administration was done using a phosphate-buffered saline (PBS) solution. Groups treated, in the same manner, with Bnz (50 mg/kg/day) and PBS (control) were included. The level of parasitemia was determined weekly,[33] and serological tests were performed 30 and 60 days post-infection. The mortality was observed daily, and the survival fraction is shown in Figure 2. None of the animals treated with the benzimidazole 1,3-dioxide derivatives, **33** and **35**, died while in the control and Bnz groups; survival fraction was 60% and 87.5%, respectively. Since the experiment lasted 60 days it was not possible to obtain a negative serology. In the first serology, 30 days post-infection, the four groups had the same level (Wilcoxon test) of anti-*T. cruzi* antibodies; however, 60 days post-infection both groups treated with the benzimidazole 1,3-dioxide derivatives, **33** and **35**, showed a significantly ($p < 0.05$) lower level of anti-*T. cruzi* antibodies than control and Bnz groups (Figure 2). Overall, both benzimidazole derivatives showed a higher performance than the reference drug (Bnz) in this assay.

Structure−Activity Relationship Studies.[34] A total of 30 benzimidazole derivatives, including previously described compounds,[18] were used in a preliminary structure−activity relationship (SAR) study. For this, all the structures were optimized using a quantum mechanical methodology (AM1), and 10 molecular descriptors were obtained that were grouped into three categories: steric descriptors (surface area, molecular volume), electronic descriptors (module of dipolar moment, highest occupied molecular orbital energy (E_{HOMO}), lowest unoccupied molecular orbital energy (E_{LUMO}), molecular hardness, Mulliken's electronegativity), and hydrophilic−lipophilic descriptors (total energy in aqueous phase (E_{aq}), solvation energy (SM5.4 model),[35] logP (Ghose−Crippen)).

In a first approximation, compounds were divided in two classes according to their in vitro anti-*T. cruzi* activity (% GI, epimastigote form Tulahuen 2 strain), active compounds (% GI $\geq$ 50) and inactive compounds (% GI $<$ 50), and the difference in each variable between classes was analyzed using nonparametric tests.[36] At a 0.05 level all descriptors except steric ones and solvation energy were found significantly different between classes (data not shown). Active compounds are less polar (lower dipolar moment module, lower E_{aq} and higher logP) and softer electrophile (lower hardness, lower E_{LUMO}) than inactive compounds. This high electrophilic character could be related to the participation of these derivatives in a bioreduction process or in a reaction with biological nucleophiles. To further asses this possibility, the LUMO was studied using a density functional methodology (B3LYP/6-31*) for derivatives of the three series and benzofuroxan. In Figure 3 is shown the LUMO mapped onto a bond surface, indicating which regions of the molecule are most susceptible to nucleophilic attack.[19,35] Clearly, derivatives **14** and **18** are less electrophilic than the benzimidazole 1,3-dioxide **28**, which resembles benzofuroxan more. What is more, there is a marked difference in the contribution from the *N*-oxide moiety to the LUMO among the three series. While for derivatives **14** and **18** there is almost no contribution, the reverse happens for the di-*N*-oxide derivative **28** and benzofuroxan. This would be in agreement with the participation of this moiety in a bioreduction process.

As another approach, a principal components analysis (PCA) was performed. PCA is widely used to simplify large data sets in a way that patterns, and relationships can be readily recognized and understood. The underlying purpose of this technique is the dimension reduction. What is more important, since biological activity is not used in the analysis, it is an unsupervised methodology.[37] Different sets of variables were analyzed for a good discrimination between active and inactive classes. When only electronic (dipolar moment module, E_{HOMO}, E_{LUMO}, hardness, electronegativity) and hydrophilic−lipophilic variables (E_{aq}, logP) were included, two principal components were obtained with an eigenvalue greater than 1. Discrimination between classes was achieved in the first component where active compounds were situated in a narrow range as is shown in Figure 4. The first principal component (PC), which explains 62% of the total variance, consists mainly of electronic descriptors E_{HOMO}, hardness, and electronegativity, pointing again to the relevance of these variables to the anti-epimastigote-*T. cruzi* activity. The second PC has contributions from the dipolar moment module, E_{LUMO}, and logP. Overall, PCA results agree with results from nonparametric tests.

Due to the importance of the lipophilicity and electrophilicity to the anti-*T. cruzi* activity, these parameters were studied experimentally. Lipophilicity of compounds was determined by reverse phase TLC experiments on precoated TLC-C_{18}. The R_f values were converted into R_M values via the following relationship: $R_M = \log [(1/R_f) - 1]$.[38,39] To avoid interassays differences, Nfx was used as an internal reference and ΔR_M parameter was calculated as the difference in R_M between Nfx and the studied compound. Hence, derivatives more polar than Nfx would yield negative ΔR_M values, while less polar derivatives would yield positive ones (Table 5). The redox properties of the compounds, related to electrophilicity, were studied using cyclic voltametry. Reduction potential for the *N*-oxide moiety (E_{pc}) was determined in DMF with a platinum working electrode (Table 5).[15,40,41] All derivatives displayed comparable voltammetric behavior in DMF, showing one to two reduction peaks and in some cases the anodic counterparts. In

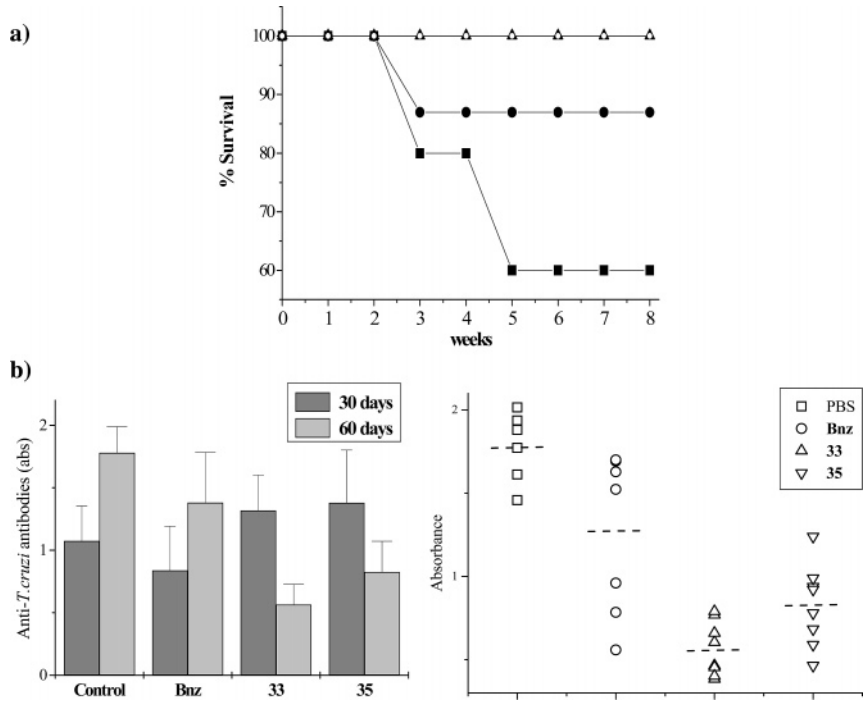


Figure 2. (a) Survival expressed as percentage of living animals. Group treated with 50 mg/kg/d of Bnz (●), group treated with 30 mg/kg/d of **33** (▲), group treated with 30 mg/kg/d of **35** (□), and control group (■). (b) Left: Level of anti-*T.* cruzi antibodies expressed in absorbance units (abs) for the four studied groups. Right: Dispersion diagrams of antibody levels in control (treated with PBS solution) animals and those receiving Bnz, **33**, and **35** treatments at 60 days post-infection.

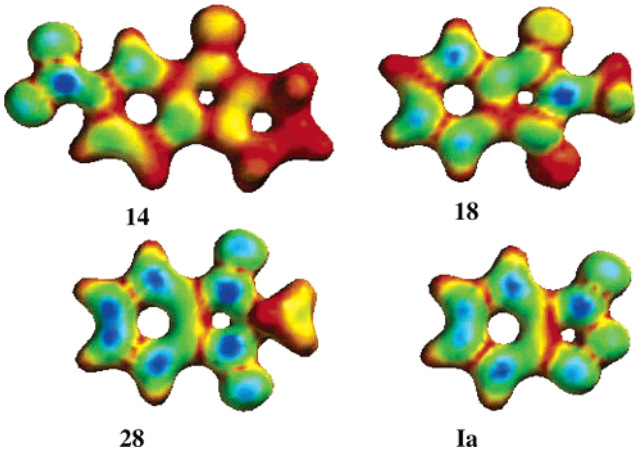


Figure 3. LUMO mapped onto a bond surface for derivatives of the three studied benzimidazole series, 1*H*-benzimidazole 3-oxide (**14**), 1-hydroxy-1*H*-benzimidazole 3-oxide (**18**), 2*H*-benzimidazole 1,3-dioxide (**28**), and benzofuroxan (**Ia**). Blue areas correspond to regions of the molecule most susceptible to nucleophilic attack.

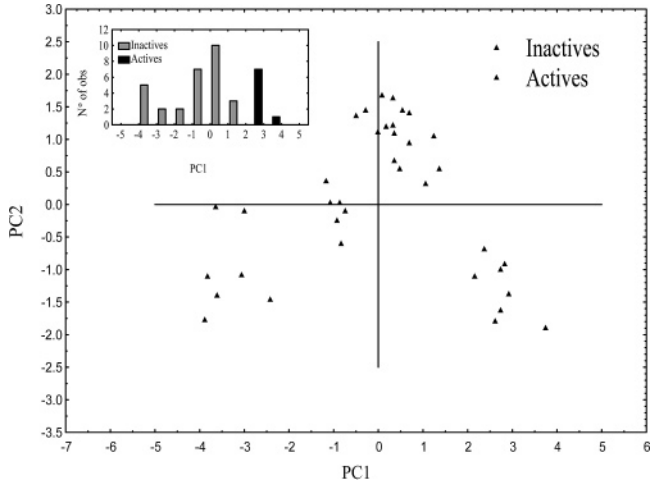


Figure 4. Principal component 1: eigenvalue = 4.3, explained variability = 62%, variable composition = E_{HOMO}, hardness, electronegativity. Principal component 2: eigenvalue = 1.3, explained variability = 19%, variable composition = dipole, E_{LUMO}, logP. Inset: Frequency histogram of PC1 coordinates.

every case an irreversible *N*-oxide reduction process was verified ($I_{pa}/I_{pc} < 1$).

Lipophilicity, expressed as ΔR_M values, showed no significant differences between active and inactive compounds. In general, benzimidazole derivatives displayed an adequate lipophilicity, similar to that of Nfx. E_{cp}, however, was significantly highest for active compounds, indicating that active derivatives are more easily reduced than inactive ones. In Figure 5a series of voltamograms corresponding to derivatives **28**, **30**, and **32** are shown.

No quantitative relationship could be found between biological activity (IC_{50} against *T. cruzi*) and experimental or theoretical descriptors for benzimidazole 1,3-dioxide derivatives, which could be assigned to the small range of IC_{50} values. In general, all dioxide derivatives were active against the three strains of *T. cruzi*, with derivative **30** being the least potent and derivative **32** the most potent. What is more, no correlation between mammal cytotoxicity and parasite toxicity was observed. Derivative **30** is more toxic to macrophages than derivatives **28**, **29**, **33**, and **35**, whereas it is the least active against *T. cruzi* epimastigotes and *Leishmania* promastigotes. Besides, mammal cytotoxicity seems to be correlated to lipophilicity; the most mammal cytotoxic agents (i.e., **32** and **31**) are the most lypophilic ones, while no statistical correlation between this property (ΔR_M) and parasite toxicity was found.

Conclusions

Three series of benzimidazole *N*-oxide derivatives were developed using simple chemical methodologies. Among them,

Table 5. Physicochemical Data for Benzimidazole *N*-Oxide Derivatives

compd	ΔR_M[a]	E_{cp} vs Ag/AgCl[b] (mV)	compd	ΔR_M[a]	E_{cp} vs Ag/AgCl[b] (mV)
1	0.07[c]	−1400[c]	**17**	nd	−1085
2	0.07[c]	−1490[c]	**28**	−0.29	−940
3	0.13[c]	−1240[c]	**29**	−0.38	−1000
4	−0.49[c]	−1190[c]	**30**	−0.25	−1030
5	−0.31[c]	−1220[c]	**31**	−0.18	−937
6	0.07[c]	nd[d]	**32**	0.09	−880
7	0.19[c]	−1250[c]	**33**	−0.55	−830
8	0.07[c]	−1490[c]	**34**	0.18	nd
14	0.34	−1159	**35**	−0.38	−860

[a] $\Delta R_M = R_{M,Nfx} - R_{M,studied\ compound}$. [b] Cathodic peak potential for *N*-oxide moiety (±10 mV) measured at a scan rate of 500 mV/s. [c] Reference 18. [c] nd: not determined.

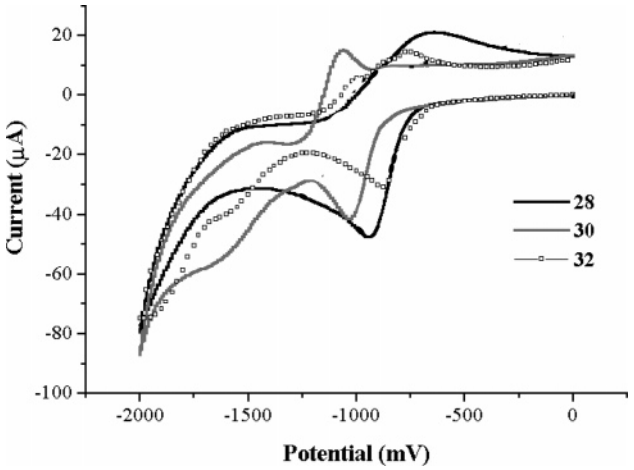


Figure 5. Representative voltamogram of benzimidazole *N*-oxide derivatives belonging to the active (**28**, **30**, and **32**) series.

benzimidazole 1,3-dioxide derivatives displayed remarkable activities against *T. cruzi* epimastigotes and *Leishmania* promastigotes, whereas nontautomerizable benzimidazole N^3-oxide and *N*-hydroxy/*N*-alkoxy benzimidazole *N*-oxide derivatives showed no activity. In general, benzimidazole 1,3-dioxide derivatives were selective toward trypanosomatids since parasite toxicity was not associated with mammal cytotoxicity in macrophages. For instance, derivative **33** (with a hydroxyimino substituent) was not toxic at the highest concentration assayed (more than 30 times the IC_{50} against *T. cruzi*). Due to their therapeutic index, compounds **33** and **35** were evaluated in vivo in an acute model of Chagas' disease, where both derivatives were able to rescue mice from death and lowered anti-*T. cruzi* antibodies with only 10 doses in a short-term scheme. Structure−activity relationship studies pointed to the relevance of the reduction potential and electrophilicity to anti-*T. cruzi* activity. Active compounds are more electrophilic and more easily reduced than inactive ones. These results are in agreement with the participation of these derivatives in a bioreduction process.

To further consider the potential use of these derivatives as drugs they should present an adequate pharmacokinetics, which was assessed by using Lipinski's rules[42] and the topological polar surface area (TPSA).[43,44] Lipinski has described desired ranges for certain properties thought to be important for drug bioavailability and absorption. Besides, the topological polar surface area is correlated with various types of drug transport properties including intestinal absorption, blood−brain barrier penetration, and Caco-2 cell permeability. Compliance of these criteria for all active derivatives along with their high solubility in water turn them into promising candidates for drug development (Table 6).

Table 6. Assessment of Lipinski's Rules and TPSA Values for Benzimidazole 1,3-Dioxide Derivatives

	miLogP[a]	HBD[b]	HBA[b]	mol wt	criteria met	TPSA
rule	<5	5	10	<500	at least 3	−
28	1.40	0	4	178.19	all	57.51
29	1.73	0	4	192.22	all	57.51
30	1.35	0	5	208.22	all	66.74
31	2.16	0	4	257.09	all	57.51
32	4.29	0	4	280.33	all	57.51
33	1.69	1	6	221.21	all	90.10
34	3.82	0	8	347.42	all	111.00
35	0.88	0	6	250.25	all	75.97
Nfx	−0.35	0	8	287.30	all	108.70

In summary, a new family of anti-trypanosomatid agents was developed that displays interesting activities both in vitro and in vivo. A lead compound was identified, namely 2,2-dimethyl-5-(hydroxyimino)methyl-2*H*-benzimidazole 1,3-dioxide, **33**, that looks promising as a short-time treatment antichagasic agent, i.e., for treating acute infected children. Preliminary studies of its mechanism of action and in vivo anti-*Leishmania* assays are currently in progress.

Experimental Section

Compounds **1**, **2**, **8**, **18**, and **19** and benzofuroxans **Ia**−**k** were prepared according to literature procedures.[11,12,16−18] Melting points were determined with a LEITZ heating stage Model 350 melting point apparatus and are uncorrected. Proton and carbon NMR spectra were recorded on a Bruker DPX-400 spectrometer. The chemical shifts values are expressed in ppm relative to tetramethylsilane as internal standard: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet. Mass spectra were determined in a SHIMADZU GC-MS QP 1100 EX using electronic impact at 70 eV. Microanalyses were performed in a Fisons EA 1108 CHNS-O equipment and were within ±0.4% of the calculated compositions. Column chromatography was carried out using Merck silica gel (60−230 mesh). Most chemical and solvents were analytical grade and used without further purification.

General Procedure for the Preparation of Derivatives 14−17. General Procedure for the Preparation of *o*-Nitro-*t*-anilines 9−13. 2,4-Dinitrochlorobenzene (0.5 g, 2.5 mmol), the corresponding amine (1 equiv), and $NaHCO_3$ (0.2 g, 2.5 mmol) were dissolved in anhydrous EtOH (30 mL). The solution was stirred for 12 h at room temperature, and the precipitate was filtered and washed with water. After dried in vacuo the *o*-nitro-*t*-anilines were used in the next step without further purification. All the *o*-nitro-*t*-anilines were characterized by ^{1}H NMR, and their structures are coincident with the spectral data obtained.

General Procedure for the Cyclization of *o*-Nitro-*t*-anilines. The corresponding *o*-nitro-*t*-anilines (100 mg) was dissolved in concentrated HCl (10 mL) and heated at reflux as is indicated below. After the reaction mixture had cooled to room temperature, aqueous bicarbonate solution saturated was added until basic pH and the solution extracted with ethyl acetate. The organic layer was discarded, and water was removed by evaporation in vacuo. The residue was washed with excess of acetone. Evaporation of acetone in vacuo gave the desired product.

2,3-Dihydro-6-nitro-1*H*-pyrrolo[1,2-*a*]benzimidazole 4-Oxide (14). Reaction time: 15 h; light-brown solid. ^{1}H NMR (acetone-d_6) δ_H: 2.91 (m, 2H), 3.27 (t, 2H, J = 7.4 Hz), 4.46 (t, 2H, J = 7.0 Hz), 7.75 (d, 1H, J = 9.0 Hz), 8.24 (d, 1H, J = 9.0 Hz), 8.58 (s, 1H). ^{13}C NMR (acetone-d_6) δ_C: 22.12 (CH_2), 26.24 (CH_2), 45.76 (CH_2), 110.62 (Ar), 112.37 (Ar), 119.76 (Ar), 130.29 (Ar), 137.68 (Ar), 143.01 (Ar), 151.03 (Ar); *m*/*z* (%): 219 ($M^{+\bullet}$, 17), 203 (100), 173 (50), 157 (42). Anal. ($C_{10}H_9N_3O_3$) C, H, N.

2,3,4-Trihydro-7-nitro-1*H*-pyrido[1,2-*a*]benzimidazole 5-Oxide (15). Reaction time: 70 h; light-brown solid. ^{1}H NMR (acetone-d_6) δ_H: 2.09 (m, 2H), 2.22 (m, 2H), 3.11 (m, 2H), 4.37 (t, 2H, J = 5.6 Hz), 7.82 (d, 1H, J = 9.0 Hz), 8.27 (d, 1H, J = 9.0 Hz), 8.62 (s, 1H). ^{13}C NMR (acetone-d_6) δ_C: 18.70 (CH_2), 22.14 (CH_2), 23.12

(CH_2), 43.59 (CH_2), 110.01 (Ar), 111.72 (Ar), 119.57 (Ar), 133.50 (Ar), 140.47 (Ar), 145.05 (Ar), 154.51 (Ar). *m*/*z* (%): 233 ($M^{+\bullet}$, 38), 217 (100), 204 (11), 187 (36). Anal. ($C_{11}H_{11}N_3O_3$) C, H, N.

2,4-Dihydro-7-nitro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazole 5-Oxide (16). Reaction time: 22 h; yellow solid; mp 166.7 °C (dec). ^{1}H NMR (acetone-d_6) δ_H: 4.33 (m, 2H), 4.44 (m, 2H), 5.11 (s, 2H), 7.87 (d, 1H, J = 9.0 Hz), 8.30 (dd, 1H, J_1 = 9.0 Hz, J_2 = 2.0 Hz), 8.59 (d, 1H, J = 2.0 Hz). ^{13}C NMR (acetone-d_6) δ_C: 42.97 (CH_2), 61.43 (CH_2), 63.84 (CH_2), 110.04 (Ar), 112.14 (Ar), 120.17 (Ar), 132.01 (Ar), 14.03 (Ar), 145.10 (Ar), 149.89 (Ar). *m*/*z* (%): 236 ($M^{+\bullet}$ + H, 100), 220 (4), 206 (17). Anal. ($C_{10}H_9N_3O_4$) C, H, N.

2,4-Dihydro-7-nitro-1*H*-[1,4]thiazino[4,3-*a*]benzimidazole 5-Oxide (17). Reaction time: 36 h; **y**ellow solid. ^{1}H NMR (acetone-d_6) δ_H: 3.63 (m, 4H), 4.03 (m, 2H), 7.50 (d, 1H, J = 9.0 Hz), 8.33 (dd, 1H, J_1 = 9.0 Hz, J_2 = 3.0 Hz), 8.64 (d, 1H, J = 3.0 Hz). *m*/*z* (%): 252 ($M^{+\bullet}$ + H, 100), 236 (5), 222 (37). Anal. ($C_{10}H_9N_3O_3S$) C, H, N, S.

General Procedure for the Preparation of 1-Hydroxybenzimidazole 3-Oxide Derivatives 20−23. The corresponding benzofuroxan (**Ia**−**f**, 50 mg), nitroethane (1 equiv), piperidine (1 equiv), and THF (5.0 mL) were stirred for 12 h at room temperature. The product was filtered and crystallized from methanol.

1-Hydroxy-5(6)-methoxy-2-methyl-1*H*-benzimidazole 3-Oxide (20). As mixture of positional isomers (50:50); white solid; mp 198.6−199.8 °C (dec).^{1}H NMR (CD_3OD) δ_H: 2.67 (s, 3H), 3.90 (two s, 3H), 7.08 (dd, 1H, J_1 = 9.0 Hz, J_2 = 2.0 Hz), 7.17 (d, 1H, J = 2.0 Hz), 7.62 (d, 1H, J = 9.0 Hz). ^{13}C NMR (CD_3OD) δ_C: 9.07 (CH_3), 55.46 (CH_3), 92.77 (Ar), 112.11 (Ar), 115.57 (Ar), 122.50 (Ar), 128.86 (Ar), 137.50 (Ar), 158.86 (Ar). *m*/*z* (%): 194 ($M^{+\bullet}$, 17), 193 (25), 178 (100), 162 (83). Anal. ($C_9H_{10}N_2O_3$) C, H, N.

5(6)-Bromo-1-hydroxy-2-methyl-1*H*-benzimidazole 3-Oxide (21). White solid; mp 211.7−211.9 °C (dec). Due to solubility problems it was not possible to obtain NMR data. *m*/*z* (%):243 ($M^{+\bullet}$, 6), 226 (100), 212 (39). Anal. ($C_8H_7BrN_2O_2$) C, H, N.

5(6)-Chloro-1-hydroxy-2-methyl-1*H*-benzimidazole 3-Oxide (22). White solid; mp 212.7 °C (dec). ^{1}H NMR (DMSO-d_6) δ_H: 2.70 (s, 3H), 7.46 (dd, 1H, J_1 = 9.0 Hz, J_2 = 2.0 Hz), 7.72 (d, 1H, J = 9.0 Hz), 7.76 (d, 1H, J = 2.0 Hz). *m*/*z* (%): 198 ($M^{+\bullet}$, 4), 197 (15), 182 (100), 165 (45). Anal. ($C_8H_7ClN_2O_2$) C, H, N.

(*E*)-1-Hydroxy-2-methyl-5(6)-(2-phenylethenyl)-1*H*-benzimidazole 3-Oxide (23). White solid; mp 217.4 °C (dec). Due to solubility problems it was not possible to obtain NMR data. *m*/*z* (%): 266 ($M^{+\bullet}$, 4), 251 (15), 250 (77), 233 (100). Anal. ($C_{16}H_{14}N_2O_2$) C, H, N.

General Procedure for the Alkylation of 1-Hydroxybenzimidazole 3-Oxide Derivatives. Synthesis of Derivatives 24−27. The corresponding benzimidazole (**18**, **19**, or **23**, 50 mg) and the alkylating agent (ethyl 2-bromoacetate or pentyl iodide, 1.2 equiv) were dissolved in dimethyl sulfoxide (3 mL). Piperidine (1.2 equiv) was added, and the reaction mixture was stirred at room temperature until the solution became clear. After addition of water the solution was extracted with ethyl acetate. The organic layer was dried and evaporated in vacuo. The product was purified by column chromatography (SiO_2, hexane:ethyl acetate (0 to 30%)).

1-(Ethyloxycarbonylmethyloxy)-2-methyl-1*H*-benzimidazole 3-Oxide (24). Oil. ^{1}H NMR ($CDCl_3$) δ_H: 1.32 (t, 3H, J = 7.0 Hz), 2.91 (s, 3H), 4.30 (q, 2H, J = 7.0 Hz), 4.95 (s, 2H), 7.38−7.42 (m, 2H), 7.60 (d, 1H, J = 7.0 Hz), 7.83 (d, 1H, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$) δ_C: 12.26 (CH_3), 14.42 (CH_3), 62.64 (CH_2), 74.47 (CH_2), 109.59 (Ar), 118.41 (Ar), 125.08 (two carbons, Ar), 129.04 (Ar), 134.50 (Ar), 149.00 (Ar), 167.01 (CO). *m*/*z* (%): 250 ($M^{+\bullet}$, 2), 234 (1), 205 (10). Anal. ($C_{12}H_{14}N_2O_4$) C, H, N.

1-(Ethyloxycarbonylmethyloxy)-2,5(6)-dimethyl-1*H*-benzimidazole 3-Oxide (25). As mixture of positional isomers (57:43). Oil. ^{1}H NMR ($CDCl_3$) δ_H: 1.24−1.32 (m, 3H), 2.46 (s, 1.3H), 2.49 (s, 1.7H), 2.66 (s, 3H), 4.26−4.31 (m, 2H), 4.81−4.82 (two s, 2H), 7.05−7.10 (m, 1H), 7.26 (s, 0.57H), 7.36 (d, 0.43H, J = 8.0 Hz), 7.43 (s, 0.43H), 7.52 (d, 0.57H, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$) δ_C: 13.00 (CH_3), 14.45 (two carbons, CH_3), 21.83 (CH_3), 22.07 (CH_3), 62.23 (two carbons, CH_2), 74.25 (two carbons, CH_2), 108.32 (Ar), 108.59 (Ar), 119.63 (Ar), 119.91 (Ar), 124.44 (Ar), 124.63 (Ar), 128.80 (Ar), 130.86 (Ar), 132.66 (Ar), 133.36 (Ar), 136.73 (Ar), 138.97 (Ar), 147.90 (Ar), 148.35 (Ar), 167.07 (CO). *m*/*z* (%): 264 ($M^{+\bullet}$, 3), 248 (5), 219 (7). Anal. ($C_{13}H_{16}N_2O_4$) C, H, N.

(*E*)-1-(Ethyloxycarbonylmethyloxy)-2-methyl-5(6)-(2-phenylethenyl)-1*H*-benzimidazole 3-Oxide (26). As mixture of positional isomers; oil. ^{1}H NMR ($CDCl_3$) δ_H: 1.33 (m, 3H), 2.71 (s, 3H), 4.33 (m, 2H), 4.88 (s, 2H), 7.09−7.22 (m, 1.5H), 7.38 (m, 2H),, 7.50 (m, 1H), 7.54 (m, 3H), 7.61 (s, 1H), 7.71−7.72 (m, 1.5H). ^{13}C NMR ($CDCl_3$) δ_C: 13.33 (CH_3), 14.47 (CH_3), 62.34 (CH_2), 74.42 (CH_2), 106.56 (CH=), 109.01 (CH=), 118.05 (Ar), 120.18 (Ar), 123.20 (Ar), 126.79 (Ar), 127.95 (Ar), 128.70 (Ar), 131.24 (Ar), 132−138 (four carbons, Ar), 168.02 (CO). *m*/ *z* (%): 352 ($M^{+\bullet}$, 6), 336 (1), 307 (12). Anal. ($C_{20}H_{20}N_2O_4$) C, H, N.

2-Methyl-1-pentyloxy-1*H*-benzimidazole 3-Oxide (27). Oil. ^{1}H NMR ($CDCl_3$) δ_H: 0.88 (t, 3H, J = 7.2 Hz), 1.34 (m, 2H), 1.44 (m, 2H), 1.77 (m, 2H), 2.59 (s, 3H), 4.20 (t, 2H, J = 6.6 Hz), 7.19 (m, 2H), 7.32 (d, 1H, J = 7.8 Hz), 7.57 (d, 1H, J = 7.4 Hz). ^{13}C NMR ($CDCl_3$) δ_C: 12.56 (CH_3), 14.25 (CH_3), 22.79 (CH_2), 28.32 (two carbons, CH_2), 79.61 (CH_2), 108.86 (Ar), 119.18 (Ar), 123.39 (Ar), 123.66 (Ar), 130.48 (Ar), 136.91 (Ar), 148.08 (Ar). *m*/*z* (%): 234 ($M^{+\bullet}$, 2), 219 (11), 218 (55), 148 (56), 131 (22). Anal. ($C_{13}H_{18}N_2O_2$) C, H, N.

General Procedure for the Preparation of Benzimidazole 1,3-Dioxide Derivatives 28−35. The corresponding benzofuroxan (**Ia**−**d**, **If**, **Ii**−**k**, 50 mg), 2-nitropropane (1.2 equiv), piperidine (1.2 equiv), and THF (5.0 mL) were stirred at room temperature until the benzofuroxan was not present. The solvent was evaporated in vacuo and the product purified by column chromatography (SiO_2, hexane:ethyl acetate (0−50%)).

2,2-Dimethyl-2*H*-benzimidazole 1,3-Dioxide (28). Red solid; mp 122.6−123.4 °C. ^{1}H NMR ($CDCl_3$) δ_H: 1.72 (s, 6H), 6.90 (m, 2H), 7.22 (m, 2H). ^{13}C NMR ($CDCl_3$) δ_C: 24.59 (CH_3), 97.40 (C-2), 116.14 (Ar), 131.20 (Ar), 136.91 (Ar). *m*/*z* (%): 178 ($M^{+\bullet}$, 58), 163 (18), 147 (4). Anal. ($C_9H_{10}N_2O_2$) C, H, N.

2,2,5-Trimethyl-2*H*-benzimidazole 1,3-Dioxide (29). Red solid; mp 116.4−117.7 °C. ^{1}H NMR ($CDCl_3$) δ_H: 1.70 (s, 6H), 2.23 (s, 3H), 6.74 (d, 1H, J = 9.5 Hz), 7.00 (s, 1H), 7.15 (d, 1H, J = 9.5 Hz). ^{13}C NMR ($CDCl_3$) δ_C: 22.39 (CH_3), 24.54 (CH_3), 97.52 (C-2), 113.73 (Ar), 115.58 (Ar), 134.81 (Ar), 136.01 (two carbons, Ar), 142.30 (Ar). *m*/*z* (%): 192 ($M^{+\bullet}$, 95), 176 (35%), 161 (23). Anal. ($C_{10}H_{12}N_2O_2$) C, H, N.

5-Methoxy-2,2-dimethyl-2*H*-benzimidazole 1,3-Dioxide (30). Deep red solid; mp 142.4−143.2 °C. ^{1}H NMR ($CDCl_3$) δ_H: 1.73 (s, 6H), 3.85 (s, 3H), 6.41 (s, 1H), 6.65 (d, 1H, J = 10.0 Hz), 7.19 (d, 1H, J = 10.0 Hz). ^{13}C NMR ($CDCl_3$) δ_C: 24.47 (CH_3), 56.62 (CH_3), 90.68 (Ar), 97.90 (C-2), 116.99 (Ar), 129.10 (Ar), 132.52 (two carbons, Ar), 162.52 (Ar). *m*/*z* (%): 208 ($M^{+\bullet}$, 100), 192 (90), 177 (63). Anal. ($C_{10}H_{12}N_2O_3$) C, H, N.

5-Bromo-2,2-dimethyl-2*H*-benzimidazole 1,3-Dioxide (31). Red solid; mp 134.5−135.3 °C. ^{1}H NMR ($CDCl_3$) δ_H: 1.71 (s, 6H), 6.96 (d, 1H, J = 9.0 Hz), 7.12 (d, 1H, J = 10.0 Hz), 7.50 (s, 1H). ^{13}C NMR ($CDCl_3$) δ_C: 24.61 (CH_3), 98.25 (C-2), 117.11 (Ar), 118.35 (Ar), 126.34 (Ar), 135.11 (Ar), 136.30 (two carbons, Ar). *m*/*z* (%): 258 ($M^{+\bullet}$ + 2, 6), 256 ($M^{+\bullet}$, 8), 242 (26), 240 (38). Anal. ($C_9H_9N_2O_2$) C, H, N.

(*E*/*Z*)-2,2-Dimethyl-5-(2-phenylethenyl)-2*H*-benzimidazole 1,3-Dioxide (32). As mixture of geometric isomers *E*/*Z* (2:8); red solid; mp 117.8−118.9 °C. ^{1}H NMR ($CDCl_3$) δ_H: 1.72 (bs, 6H), 6.41−7.51 (m, 10H). ^{13}C NMR ($CDCl_3$) δ_C: 24.58 (CH_3), 97.76 (C-2), 97.92 (C-2), 112.57−140.84 (CH= and Ar-carbons). *m*/*z* (%): 280 ($M^{+\bullet}$, 88), 264 (100), 249 (51). Anal. ($C_{17}H_{16}N_2O_2$) C, H, N.

2,2-Dimethyl-5-(hydroxyimino)methyl-2*H*-benzimidazole 1,3-Dioxide (33). Red solid; mp 177.5−177.6 °C (dec). ^{1}H NMR (CD_3OD) δ_H: 1.70 (s, 6H), 4.51 (s, 1H, OH), 7.25 (m, 2H), 7.55 (d, 1H, J = 10.0 Hz), 8.03 (s, 1H). ^{13}C NMR (CD_3OD) δ_C: 23.36 (CH_3), 96.27 (C-2), 113.90 (Ar), 115.54 (Ar), 129.62 (Ar), 137.5 (two carbons, Ar), 138.0 (Ar), 146.97 (CH=). *m*/*z* (%): 221 ($M^{+\bullet}$, 100), 205 (16), 190 (8). Anal. ($C_{10}H_{11}N_3O_3$) C, H, N.

1-[(2,2-Dimethyl-1,3-dioxide-2*H*-benzimidazol-5-yl)methylene]-4-hexyl Semicarbazide (34). Red solid. ^{1}H NMR ($CDCl_3$) δ_H: 0.92 (bs, 3H), 1.36 (bs, 4H), 1.60 (bs, 4H), 1.74 (s, 6H), 3.37 (q, 2H), 6.05 (t, 1H), 7.22 (s, 1H), 7.26 (s, 1H), 7.40 (d, 1H, J = 10.0 Hz), 7.58 (s, 1H), 9.22 (bs). ^{13}C NMR ($CDCl_3$) δ_C: 14.38 (CH_3, CH_2), 22.95 (CH_2), 24.63 (CH_3), 30.55 (CH_2), 31.89 (CH_2), 40.43 (CH_2), 98.40 (C-2), 114.65 (Ar), 116.44 (Ar), 128.54 (Ar), 136.23 (Ar), 136.71 (Ar), 137.25 (CH═), 155.87 (CO). *m*/*z* (%): 347 ($M^{+\bullet}$, 2), 331 (15), 318 (4). Anal. ($C_{17}H_{25}N_5O_3$) C, H, N.

2,2-Dimethyl-5-(1,3-dioxolan-2-yl)-2*H*-benzimidazole 1,3-Dioxide (35). Red solid; mp 101.1−101.6 °C (dec). ^{1}H NMR ($CDCl_3$) δ_H: 1.71 (s, 6H), 4.06 (m, 4H), 5.63 (s, 1H), 7.03 (d, 1H, J = 8.5 Hz), 7.24 (d, 1H, J = 9.6 Hz), 7.34 (s, 1H). ^{13}C NMR ($CDCl_3$) δ_C: 24.62 (CH_3), 65.88 (CH_2), 98.08 (C-2), 102.42 (CH), 113.86 (Ar), 116.44 (Ar), 130.02 (Ar), 136.90 (Ar), 137.00 (Ar), 142.05 (Ar). *m*/*z* 250 ($M^{+\bullet}$, 100%), 234 (52%), 219 (18%), 203 (5%). Anal. ($C_{12}H_{14}N_2O_4$) C, H, N.

In Vitro Anti-*T. cruzi* Activity (Epimastigote Form). *Trypanosoma cruzi* epimastigotes (Tulahuen 2 strain, Brener strain, Y strain) were grown at 28 °C in an axenic medium (BHI-tryptose) complemented with 5% fetal calf serum. Cells from 5-day-old culture (stationary phase) were inoculated to 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 every day. Before inoculation, the medium was supplemented with the indicated amount of the studied compound from a stock solution in DMSO. The final concentration of DMSO in the culture medium never exceeded 0.4%, and the control was run in the presence of 0.4% DMSO and in the absence of compound. No effect on epimatigotes growth was observed by the presence of up to 1% DMSO in the culture medium. The percentage of inhibition was calculated as follows: $\% = \{1 - [(A_p - A_{0p})/(A_c - A_{0c})]\}100$, where $A_p = A_{600}$ of the culture containing the drug at day 5; $A_{0p} = A_{600}$ of the culture containing the compound just after the addition of the inocula (day 0); $A_c = A_{600}$ of the culture in the absence of any compound (control) at day 5; $A_{0c} = A_{600}$ in the absence of the compound at day 0. To determine IC_{50} values, 50% inhibitory concentrations, parasite growth was followed in the absence (control) and presence of increasing concentrations of the corresponding compound. At day 5, the absorbance of the culture was measured and related to the control. The IC_{50} value was taken as the concentration of compound needed to reduce the absorbance ratio to 50%.

In Vitro Anti-*T. cruzi* Activity (Trypomastigote Form). *Trypanosoma cruzi* trypomastigotes (CL Brener strain) were obtained from infected mice blood. Compounds were prepared in DMSO, and PBS was used to obtain a final concentration of 250 μg/mL. Blood containing 10^6 parasites and the compound at a 90/10 ratio were incubated in 96-well flat bottom microplates for 24 h at 4 °C. Parasites were counted by the method of Brener using an OLYMPUS BH-2 microscope. Gentian violet was included as a positive control, and PBS was used as negative control.

In Vitro Anti-*Leishmania* Activity (Promatigote Form). *Leishmania amazonensis* (PH8), *Leishmania infantum* (2259), and *Leishmania braziliensis* (2903) promastigotes were grown at 28 °C in Schneider's medium supplemented with 20% (v/v) fetal calf serum. Parasites were seeded (10^7 parasites/mL) in 96-well flat bottom microplates, and compounds were prepared in a minimun amount of DMSO or Tween 80 using Schneider's medium to reach a final concentration of 100 μg/mL. After 72 h of incubation at 28 °C, parasites were observed using an inverted microscope OLYMPUS IMT-2 and compared to control (no compound added).

Cytotoxicity to Macrophages. J774 Macrophages were seeded (70.000 cells/well) in 96-well flat bottom microplates (Nunclon) with 200 μL of RPMI 1640 medium supplemented with 20% heat-inactivated fetal calf serum. Cells were allowed to attach for 24 h in a humidified 5% CO_2/95% air atmosphere at 37 °C. Then, cells were exposed to the compounds (100, 10, and 1 μg/mL) for 48 h. Afterward, the cells were washed with PBS and incubated (37 °C) with MTT 0.4 mg/mL for 60 min. Then, formazan was dissolved with DMSO (100 μL), and optical densities were measured. Each concentration was assayed three times, and six growth controls were used in each test. Cytotoxicity percentages (%*C*) were determined as follows: $\%C = [100 - (ODd - ODdm)/(ODc - ODcm)] \times 100$, where ODd is the mean of OD_{595} of wells with macrophages and different concentrations of the compounds; ODdm is the mean of OD_{595} of wells with different compounds concentration in the medium; ODc is the growth control, and ODcm is the mean of OD_{595} of wells with only medium.

In Vivo Anti-*T. cruzi* Activity (Acute Model). BALB/c male mice (30 days old, 25−30 g) were infected by intraperitoneal injection of 10^3 blood trypomastigotes. One group of 10 animals was used as control (PBS), and three groups of eight animals were treated with the two compounds and Bnz, respectively. First parasitemia was done 5 days post-infection and the treatment begun 5 days after. Compounds were administered orally, as aqueous solution in PBS, at 30 mg/kg/day for benzimidazole derivatives and 50 mg/kg/day for Bnz, during 10 days. The level of parasitemia was checked weekly by counting in a Neubauer chamber the number of parasites in 5 μL of blood drawn from the tail of the mice and diluted 1:10 in ammonium chloride. Serology was analyzed using ELISA (Chagas' test)[45] at 30 days post-infection and 60 days post-infection.

Molecular Descriptors. Quantum-chemical semiempirical AM1 calculations were performed for the lowest energy conformation of the compounds using the Spartan'04 suite of programs.[46] The compounds were built with standard bond lengths and angles using the Spartan'04 1.0.1 version, and the geometry of each molecule was fully optimized by applying the semiempirical AM1 method in gas phase from the most stable conformer obtained using molecular mechanics (MMFF) methods. The calculated variables were total surface area, molecular volume, Ghose− Crippen LogP,[47] dipole moment, E_{HOMO}, E_{LUMO}, molecular hardness, Mulliken's electronegativity, total energy in aqueous phase, and solvation energy using Cramer−Truhlar SM5.4 model.[48] Analysis of molecular orbital distributions was done using a density functional methodology (B3LYP/6-31G*)[49,50] on structures optimized at the same level of theory.

Cyclic Voltammetry. Experiments were carried out using a BAS-Epsilon EC (Bioanalytical Systems, Inc., West Lafayette, USA) instrument in a BAS C3 cell in DMF (Aldrich, Milwaukee, USA, spectroscopy grade) with tetrabutylammonium perchlorate (Fluka, Buchs, Switzerland) (ca. 0.1 mol/mL) as the supporting electrolyte and purged with nitrogen at room temperature. A three-electrode cell configuration was used, with a platinum working electrode, platinum wire auxiliary electrode, and a Ag/AgCl reference electrode. Voltage scan rates ranged from 0.05 to 1.00 V/s.

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

Acknowledgment. Financial support from CSIC (Uruguay) and Fondo Clemente Estable #5060 (Uruguay) is acknowledged. We thank AMSUD-PASTEUR network for a scholarship to L.B. and PEDECIBA for a scholarship to M.B.

Supporting Information Available: Results from elemental analysis of 2*H*-benzimidazole 1,3-dioxide derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0600343