European Journal of Medicinal Chemistry xxx (2009) 1–8

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Second generation of 2*H*-benzimidazole 1,3-dioxide derivatives as anti-trypanosomatid agents: Synthesis, biological evaluation, and mode of action studies

Mariana Boiani [a], Lucía Boiani [a], Alicia Merlino [a], Paola Hernández [a], Agustina Chidichimo [b], Juan J. Cazzulo [b], Hugo Cerecetto [a,*], Mercedes González [a,*]

[a] *Departamento de Química Orgánica, Facultad de Ciencias-Facultad de Química, Universidad de la República, Igua 4225, 11400 Montevideo, Uruguay*
[b] *Instituto de Investigaciones Biotecnológicas, Universidad Nacional de General San Martín-CONICET, 1650 San Martín, Buenos Aires, Argentina*

ARTICLE INFO

Article history:
Received 2 May 2009
Received in revised form
3 June 2009
Accepted 4 June 2009
Available online xxx

Keywords:
2*H*-Benzimidazole 1,3-dioxide
Chagas disease
Leishmaniasis
Mitochondrial dehydrogenases

ABSTRACT

Exploring the influence of different substitution patterns of 2*H*-benzimidazole 1,3-dioxide derivatives (BzNO) we prepared fifteen new derivatives. Initially the BzNO were tested against *Trypanosoma cruzi* Tulahuen 2 strain epimastigote form rendering very potent anti-*T. cruzi* agents. Moreover, the BzNO were able to inhibit the growth of virulent and resistant to Benznidazole strains (CL Brener clone, Colombiana, and Y strains) and to *Leishmania braziliensis*. Interestingly, BzNO exhibited very high selectivity index and particularly the spiro-BzNO **13** provokes an important diminution of amastigotes in Vero cells. Besides, it was found a diminution of acetate and glycine as excreted metabolites but without increase of parasite glucose uptake indicating that the glycosome is probably not involucrate in the 2*H*-benzimidazole 1,3-dioxides mechanism of action.

1. 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. Diseases caused by *Trypanosomatidae*, which share a similar state regarding drug treatment, include Chagas' disease (*Trypanosoma cruzi*, *T. cruzi*) and leishmaniasis (*Leishmania spp.*) [1]. These trypanosomatids alone are responsible for an infected population of nearly 30 millions and more than 400 millions are at risk. *T. cruzi* presents three main morphological forms in a complex life cycle. The epimastigote form replicates within the crop and midgut of Chagas' disease vectors as, it is released with the insect excrements as the non-dividing highly infective metacyclic trypomastigotes that invade mammalian tissues via wounds provoked by blood sucking action. The parasite multiplies intracellularly as amastigote form which is released as the non-dividing bloodstream trypomastigote form that invades other tissues. Drugs currently used in the treatment of Chagas' disease are two nitroaromatic heterocycles, Nifurtimox (Nfx, Lampit®, recently discontinued by Bayer) and Benznidazole (Bnz, Rochagan®, Roche), introduced empirically over three decades ago [2]. Both drugs are active in the acute phase of the disease but its 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 [3].

The drugs of choice for the treatment of leishmaniasis are sodium stibolgluconate (Pentostam®), meglumine antimoniate (Glucantime®), pentamidine and liposomal amphotericin B, but these sometimes meet with failure [4]. Currently, WHO/TDR develops a research program with Miltefosine (Mtf), a very promising leishmanocidal drug [5]. These awful illnesses, associated to poverty, does not attract the pharmaceutical companies as a result of the lack of commercial motives; consequently, efforts to develop new and safer drugs have to be carried out mainly by academic institutions.

Extensive work, in the last two decades, has helped to understand the molecular basis of the antichagasic activity of Nfx and Bnz. Nfx mode of action is via the reduction of the nitro group to a nitroanion radical, and for Bnz it is postulated to act via intermediates that covalently modify bio-macromolecules [6]. Most frequent side effects of these drugs include anorexia, vomiting,

* Corresponding authors. Tel.: +598 2 525 86 18x216; fax: +598 2 525 07 49.
E-mail addresses: hcerecet@fq.edu.uy (H. Cerecetto), megonzal@fq.edu.uy (M. González).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.06.014

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 [2]. 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 [7]. The same could be said for drugs that produce reductive damage such as Bnz. Following this reasoning, we have been looking for less toxic and more selective antichagasic drugs by using an *N*-oxide moiety as the bioreductive group. Thus, benzofuroxan (Bfx) derivatives (benzo[1,2-*c*]1,2,5-oxadizole *N*-oxide) were described for the first time as *in vitro* anti-*T. cruzi* agents. These Bfx displayed activities similar or higher than the reference drugs Nfx and Bnz (i.e. **1**–**3**, Fig. 1) [8]. In order to study other *N*-oxide containing heterocycle we analyzed quinoxaline *N*,*N*′-dioxide system finding derivatives that displayed very good anti-*T. cruzi* activities, like compound **4** (Fig. 1) [9]. We also investigated different series of benzimidazole *N*-oxides [10] resulting 2*H*-benzimidazole 1,3-dioxide, BzNO (i.e. **5**–**7**, Fig. 1), the most *in vitro* active derivatives against to *T. cruzi* and *Leishmania spp*. Furthermore, these derivatives posses *in vivo* activity and add to Lipinski's rules of five, have adequate topological polar surface areas, and excellent solubility in water turning them into promising candidates for drug development [11].

In the context of our research on the 2*H*-benzimidazole 1,3-dioxides pharmacochemistry, new derivatives were designed in order to explore the biological effects of these structural changes in parent compounds **5**–**7**. The new BzNO were biological evaluated against different *T. cruzi* strains and against *Leishmania* protozoa. The new BzNO were obtained by modifications on positions 5 and 6 (series **a**, Fig. 1). Moieties present in parent compounds **1**–**4** where used to study its effects in the 2*H*-benzimidazole system. In addition, position 2 modifications were planned. Firstly, 2,2-dimethyl substituents are incorporated in a cyclic structure (series **b**, spiro derivatives, Fig. 1) and secondly, one of the 2-methyl substituent is changed by arylic or alkylic moiety (series **c**, Fig. 1). Additionally we studied some parasites biochemical changes promoted by 2*H*-benzimidazole 1,3-dioxides, i.e. effects on the mitochondrion dehydrogenase, on the glucose uptake, and on the excreted metabolites, trying to assess the mode of action.

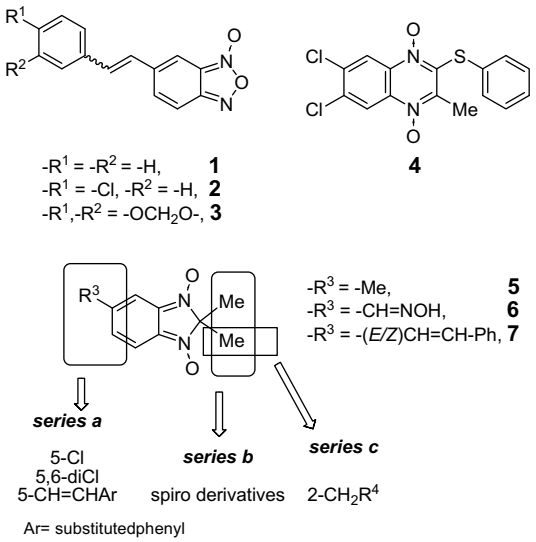


Fig. 1. *N*-Oxide derivatives previously described as *T. cruzi* growth inhibitors (**1**–**7**) and design of 2*H*-benzimidazole 1,3-dioxide second generation series **a** (**8**–**11**), series **b** (**12**–**17**) and series **c** (**18**–**22**).

2. Methods and results

2.1. Chemistry

2*H*-Benzimidazole 1,3-dioxide derivatives were obtained by a simple and efficient general approach using the reaction of the corresponding benzofuroxan analogues with nitroalkanes [12]. BzNO derivatives **8**–**22** were obtained in moderate to good yields by reaction of the benzofuroxan [8] with the corresponding nitroalkane in basic media at room temperature (Scheme 1). The corresponding benzofuroxan derivatives were prepared as previously described [8a,8h]. 2-Nitropropane and nitrocyclopentane are commercially available however 2-nitroheptane or 2-nitro-1-(substituted phenyl)propanes were prepared using nitroaldolic/reduction two-step procedure previously described [13]. Derivatives **8**–**22** are soluble in different organic solvents (hexane, chloroform, and acetone) as well as in water. All of the proposed structures were established by ^{1}H-, ^{13}C NMR (HMQC, HMBC) spectroscopy and MS. The purity was established by TLC and microanalysis.

2.2. Biological characterization

2.2.1. In vitro *anti*-T. cruzi *activity*

The new 2*H*-benzimidazole 1,3-dioxide derivatives were initially tested *in vitro* against the epimastigote form of *T. cruzi*, Tulahuen 2 strain. The existence of the epimastigote form of *T. cruzi* as an obligate mammalian intracellular stage has been revisited and confirmed [14]. 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) at day 5. The ID_{50} doses (50% inhibitory dose) were determined for all of them (Table 1). Nfx and Bnz were used as the reference trypanocidal drug. Parent compounds, **5**–**7**, and derivatives **8**, and **12**–**20**, were selected to study against the high virulent [15] CL Brener clone and the Nfx- and Bnz-partially resistant strains, Y and Colombiana strains [16]. In these last assays viability of *T. cruzi*, at day 5, was assessed colorimetrically using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay [17]. For each derivative, the percentage of cytotoxicity (PCyt) was initially determined at 25 μM as it is indicated in Experimental Section and then in a doses-response assay, between 1.0 and 50.0 μM, the ID_{50} concentration was calculated (Table 2).

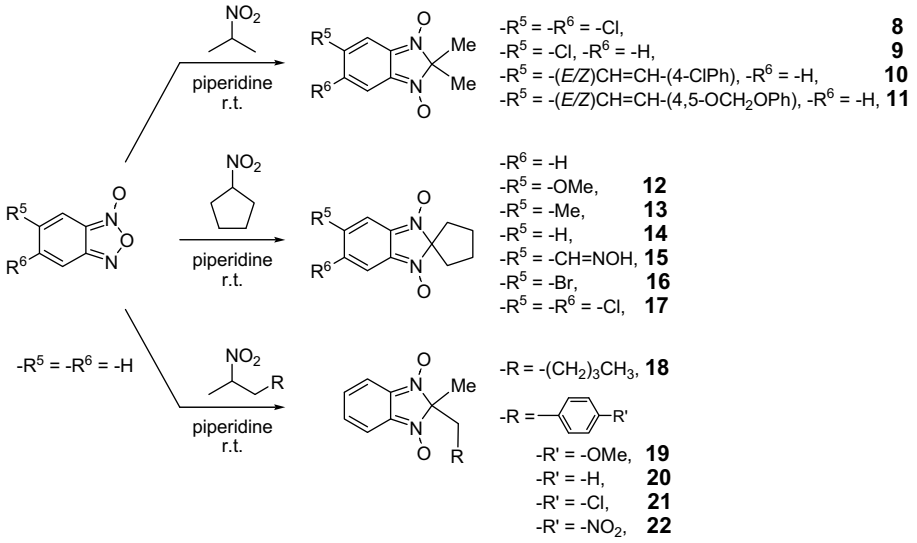
Parent compound **7** and derivatives **13**, and **16** were also evaluated, between 10.0 and 50.0 μM, against the bloodstream form of *T. cruzi* (trypomastigotes) of CL Brener clone during 2–26 h [11,18]. (Table 3). The lysis percentage was evaluated against a control without drug. Derivative **13**, lacking toxicity against Vero cells at its *T. cruzi*-epimastigote ID_{50}, was evaluated against the intracellular form of *T. cruzi* (amastigotes), CL Brener clone, at two doses at least 5 times lower than the ID_{50} value, 2.5 and 5.0 μM (Table 3) [19].

2.2.2. In vitro *leishmanocidal activity*

Selected 2*H*-benzimidazole 1,3-dioxides, **5**–**8**, and **12**–**20**, were studied as leishmanocidal agents. They were tested *in vitro* against promastigote form of *Leishmania braziliensis* (MHOM/BR/00/LTB300) strain. Viability of parasite, at day 5, was assessed colorimetrically using MTT assay [20]. For each derivative, the percentage of cytotoxicity was initially determined at 25 μM as it is indicated in Experimental Section and then in a doses-response assay, between 1.0 and 50.0 μM, the ID_{50} concentration was calculated (Table 2). Mtf was used as the reference leishmanocidal drug.

2.2.3. Unspecific cytotoxicity

Mammal cytotoxicity of the studied 2*H*-benzimidazole 1,3-dioxide derivatives was studied *in vitro* using J-774 mouse



Scheme 1. Synthetic procedures used to prepare the 2*H*-Benzimidazole 1,3-dioxide derivatives.

macrophages as the cellular model with doses between 1.0 and 400.0 μM, at least four times higher than the doses used for *T. cruzi* (25 μM) (Table 4) [8h]. The selectivity indexes were expressed as the ratio between ID_{50} in macrophages and ID_{50} in *T. cruzi* and in *Leishmania*.

2.3. Study of metabolic changes in the parasites

Parent compounds **5** and **7**, the spiro-derivatives **13** and **16**, the previous described quinoxaline dioxide **4** and Nfx were used to compare the intra-*T. cruzi* or intra-*Leishmania* changes as result of the action of these compounds. So on, the effect of these compounds on the mitochondrial dehydrogenase activities, the changes in the parasite glucose uptake, and the modifications in the excreted metabolites were evaluated.

Firstly, the percentage of mitochondrial dehydrogenase activities (Pmdh) respect to untreated parasites control was assessed using the colorimetric MTT assay performed at very short times, no more than 240 min of parasite-derivative exposition. Using the method previously described for *Leishmania* parasite [21]. Fig. 2a shows the results for the studied compounds, **5**, **13** and **16** and Nfx and Bnz in the *T. cruzi* assay and Mtf for *Leishmania*. The glucose-uptake's changes were determined after 4 h of incubation of the parasite in phosphate buffer with glucose as energetic source and 20 μM doses of the studied compounds (**4**, **5**, **13**, **16**, and Nfx) [22]. Fig. 2b shows the results obtained in these assays. Finally, in order to study the changes in the biochemical pathways promoted by two of the parent compounds, **5** and **7**, and two of the active spiro-derivatives, **13** and **16**, we have studied the modifications in the excreted metabolites by ^{1}H NMR spectroscopy. This kind of studies has probed to be a useful tool in the mechanism of action elucidation [23]. For that, we compared the spectra of the cell-free medium of benzimidazoles-treated parasites with those of the untreated *T. cruzi*-free medium as control. In this study we have

Table 1
Biological characterization of 2*H*-benzimidazole 1,3-dioxide derivatives against Tulahuen 2 strain.

Comp.	ID_{50}[a] (μM)
5	5.1[b]
6	12.5[b]
7	3.4[b]
8	14.5
9	10.1
10	8.1
11	3.1
12	25.0
13	7.9
14	9.5
15	19.8
16	16.3
17	12.0
18	5.4
19	>50.0
20	>50.0
21	40.0
22	8.4
Nfx	7.7
Bnz	7.4

[a] The results are the means of three independent experiments with a SD less than 10% in all cases.
[b] From reference [11].

Table 2
Biological activity, measured as cellular viability, of selected 2*H*-benzimidazole 1,3-dioxide derivatives against different *T. cruzi* strains and *Leishmania* LTB300.

Comp.	ID_{50}[a,b] ($PCyt^{a,c,e}$)			
	CL Brener clone	Y strain	Colombiana strain	LTB300
5	(61.4)	3.3	(35.1)	30.4
6	>40.0 (46.6)	16.4	(21.7)	>50.0
7	8.5 (78.4)	6.0	(92.3)	3.5
8	(88.3)	9.9	(94.4)	10.1
12	(62.6)	4.9	(17.3)	>50.0
13	(56.5)	15.8	(59.3)	8.9
14	(83.7)	6.4	(94.8)	3.6
15	(65.6)	5.7	(29.0)	26.7
16	(91.5)	3.5	(83.8)	26.7
17	(67.1)	5.3	(60.9)	5.4
18	(88.2)	–[f]	(77.1)	>50.0
19	–	–	(30.8)	35.0
20	(47.0)	–	(18.6)	20.7
Nfx	4.9 (90.0)	9.7	3.4 (87.0)	–
Mtf	–	–	–	9.0

[a] The results are the means of three independent experiments with a SD less than 10% in all cases.
[b] In mM.
[c] Percentage of cytotoxicity.
[d] Doses = 25 μM.
[e] In %.
[f] "–": not studied.

Table 3
In Vitro Trypanocidal Activity against Trypomastigote and Amastigote Forms of Selected 2*H*-Benzimidazole 1,3-dioxide Derivatives.

Comp.	Trypomastigotes		
	Doses (μM)	Time (h)	% Lysis [a,b]
7	5.0	22	31
	10.0	2	3
		3	32
		22	84
		26	100
	25.0	19	64
	50.0	2	32
		19	100
13	10.0	2	1
		3	39
		22	25
		26	24
	25.0	19	24
	50.0	19	52
16	10.0	2	0
		3	35
		22	16
		26	16

Comp.	Amastigotes		
	PIC (%)[c]	NAC[d]	EI[e]
13[f]	34.8	6.1	211.3
13[g]	14.7	6.2	90.9
C[h]	32.3	9.3	300.2

[a] % Lysis = percentage of parasite lysis at the assayed doses.
[b] Results are the mean of three independent experiments with a SD less than 10% in all cases.
[c] PIC: percentage of amastigote-infected Vero cells (for details see Experimental Section).
[d] NAC: number of amastigotes per infected Vero cells (for details see Experimental Section).
[e] EI: endocytic index (for details see Experimental Section).
[f] At 2.5 μM.
[g] At 5.0 μM.
[h] C: control, untreated Vero cells infected with amastigotes.

Table 4
Cytotoxic effects against J-774 macrophages.

Series	Comp.	J-774 macrophages		SI[c]	
		Cyt[a,b]	ID_{50} (μM)[b]	TC[d]	Leish[e]
a	**6**	1.00	77.0	6.2	< 1.5
	8	0.87	100.0	6.9	9.9
	9	1.74	38.0	3.8	–
	10	1.48	52.0	6.4	–
	11	1.37	58.0	18.7	–
b	**12**	0.89	100.0	4.0	<2.0
	13	1.22	63.0	8.0	7.1
	14	0.75	>400.0	>42.1	>111.1
	15	0.82	130.0	6.6	4.9
	16	0.82	100.0	6.1	3.7
	17	1.65	39.0	3.3	7.2
c	**18**	0.01	233.0	43.1	<4.7
	19	0.32	>400.0	8.0	>11.4
	20	0.46	302.0	<6.0	14.6
	21	1.42	55.0	1.4	–
	22	0.47	122.0	14.5	–
Nfx		–	346.0	44.9	–
Mtf		–	136.0	–	15.1

[a] Cyt: Cytotoxicity is expressed respect to compound **6** at 100 μM doses.
[b] The results are the means of three independent experiments with a SD less than 10% in all cases.
[c] SI: selectivity indexes.
[d] $TC = ID_{50,\ macrophages}/ID_{50,Tulahuen\ 2}$.
[e] $Leish = ID_{50,\ macrophages}/ID_{50,\ LTB300}$.

focused mainly in the changes of the excreted salts of the carboxylic acids, lactate (Lac), acetate (Ace), pyruvate (Pyr), and succinate (Suc) and the aminoacids, alanine (Ala) and glycine (Gly), being these the most relevant modified metabolites in normal parasites (control-white column in Fig. 2c). It is well-known that *T. cruzi* sp. is not able to degrade carbohydrates completely producing a mixture of CO_2, mono- and dicarboxylic acids, which are mainly Suc, Ala and Ace [21,24]. It was observed, through enzymatic methods that under aerobic conditions epimastigotes in culture produced mainly Lac, Pyr, Ace and Suc [25]. The changes in the excreted end-products after the treatment of epimastigote form of *T. cruzi* Y strain with derivatives **5**, **7**, **13** and **16** are reported in Fig. 2c.

3. Discussion

The development of a second generation of 2*H*-benzimidazole 1,3-dioxides, series **a**–**b** (Fig. 1), and its biological activity against epimastigote form of four different strains of *T. cruzi*, trypomastigote and amastigote form of one strain of *T. cruzi*, promastigote form of one strain of *L. braziliensis*, and murine macrophages was reported herein.

In the assays against Tulahuen 2 strain, some derivatives belonging to each one of the new series were most active than the references drugs Nfx and Bnz (for series **a** compounds **10** and **11**, for series **b** compounds **13** and **14**, for series **c** compounds **18** and **22**, Table 1). When profoundly biological studies were done with other *T. cruzi* strains (CL Brener clone, Y and Colombiana) and protozoa (*L. braziliensis*), relevant findings were assessed. In particular, parent compound **7** and BzNO **8**, **14**, and **16**–**18** showed better or similar activities than reference drugs, Nfx and Mtf (Table 2). Additionally, activities of parent compound **7** against the trypomastigote form of CL Brener clone was maintained, promoting near to 100% of lysis at 10 μM after 22 h of treatment (Table 3). In the same conditions, BzNO **13** and **16** were fewer actives against CL Brener clone trypomastigotes (Table 3). However, at the Vero cells non-toxic doses (5.0 μM) BzNO **13**, has an endocytic index, corresponding to the percentage of infected Vero cells multiplied by the average of number of intracellular amastigotes (EI, Table 3), more than three-time lower than untreated control. These results transform BzNO **13** in an excellent candidate for further biological studies. Regarding to the cytotoxicity against macrophages and comparing to parent compound **6** (the least toxic in our previous study [11]) it was found new derivatives with lesser toxicity, i.e. **8**, belonging to series **a**, **12**, **14**, **15**, and **16**, belonging to series **b**, **18**, **19**, **20**, and **22**, belonging to series **c** (Table 4).

In particular the least cytotoxic derivative was **14** with selectivity indexes higher than 40, for *T. cruzi*, and 100 for *Leishmania*. Derivative **19** with ID_{50} against J774 higher than 400.0 μM was also less cytotoxic against both protozoa.

In the metabolic changes studies, some relevant features could be observed. On the one hand, unlike quinoxaline dioxide **4** (Fig. 1) and Bnz, at the highest time, but like Nfx the studied 2*H*-benzimidazole 1,3-dioxides, **5**, **13**, and **16**, do not affect *T. cruzi* mitochondrial dehydrogenases (Fig. 2a, left). However, for *Leishmania* spiro-benzimidazoles **13** and **16**, unlike parent compound **5**, displayed some levels of mitochondrial dehydrogenases variation at the earlier studied times. These levels of inhibitions (compare to untreated control taken as 100%) do not reach the Mtf levels at the same doses (Fig. 2a, right). Similar results were observed in the *T. cruzi*-glucose-uptake experiments finding that spiro-benzimidazoles **13** and **16** promote some degree of glucose-levels diminution but in the case of derivative **5**, like Nfx, no changes was observed (Fig. 2b). Besides, 2*H*-benzimidazole 1,3-dioxides promoted, in general, a decrease of the end-metabolites concentrations respect to untreated parasites (Fig. 2c). The most notorious

Please cite this article in press as: M. Boiani et al., Second generation of 2*H*-benzimidazole 1,3-dioxide derivatives as..., European Journal of Medicinal Chemistry (2009), doi:10.1016/j.ejmech.2009.06.014

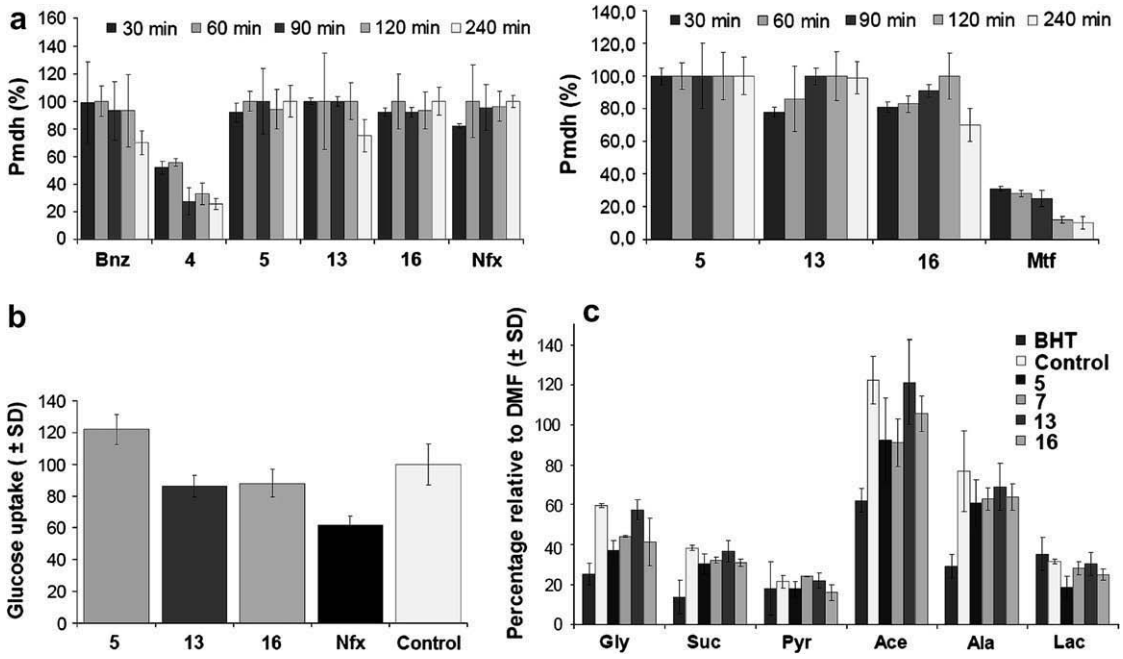


Fig. 2. (a) Mitochondrial dehydrogenases activities (%) compared to untreated control of *T. cruzi* epimastigote, Y strain, (for details, see Experimental Section). (left), or *Leishmania* promastigote, LTB300 strain (right), with time and compound (for details see Experimental Section). (b) Glucose uptake after 4 h exposure of parasite, *T. cruzi* epimastigote Y strain (for details see Experimental Section), of the studied compounds (**5**, **13**, **16**, and Nfx) and untreated. (c) Percentage of the end-products excreted in the medium after treatment of *T. cruzi* epimastigote Y strain with derivatives **5**, **7**, **13** and **16** compared to untreated epimastigotes (white column). Culture medium, BHI-Tryptose (BHT), without parasite is included as reference of the end-products basal levels (left grey column). Results are expressed as molar % relative to DMF, used as internal standard (for details, see Experimental Section). Results are presented as means ± SEM ($n = 3$).

concentration reductions were observed for Gly and Ace in the case of parent compounds **5** and **7**. Compound **5** does not decrease the amount of uptake-glucose however Ace level decreases showing the effect of this compound in some other biochemical pathway different to those belonging to glycosome [26].

4. Conclusions

In conclusion, we have developed and identified new 2*H*-benzimidazole 1,3-dioxides as excellent anti-trypanosomatid agents. Observing high selectivity indexes of this kind of compounds, one could propose it as a molecular lead for further structural modifications and further biological studies, especially *in vivo* evaluations.

5. Experimental

All starting materials were commercially available research-grade chemicals and used without further purification. All solvents were dried and distilled prior to use. All the reactions were carried out in a nitrogen atmosphere. Derivatives **5**–**7**, **19**–**22** and benzofuroxan reactants were prepared following synthetic procedures previously reported [8,11,13]. Melting points were determined with an electrothermal melting point apparatus (Electrothermal 9100) and were 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. Mass spectra were determined either on a MSD 5973 Hewlett-Packard or LC/MSD-Serie 100 Hewlett-Packard spectrometers using electronic impact (EI) or electrospray ionization (ESI), respectively. Microanalyses were performed on a Fisons EA 1108 CHNS-O instrument and were within ±0.4% of the calculated compositions. Column chromatography was carried out using Merck silica gel (60–230 mesh).

5.1. General procedure for the preparation of 2H-benzimidazole 1,3-dioxide derivatives

The corresponding benzofuroxan (50 mg), 2-nitropropane (1.2 eq.), piperidine (1.2 eq.) 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%)).

5.1.1. 5,6-Dichloro-2,2-dimethyl-2H-benzimidazole 1,3-dioxide (**8**)

Red solid (37%); mp 147.5–150.0 °C. ^{1}H NMR ($CDCl_3$) δ_H: 1.72 (s, 6H), 7.43 (s, 2H). ^{13}C NMR ($CDCl_3$) δ_C: 14.48 (CH_3), 110.00 (C-2), 116.44 (Ar, 2C), 135.00 (Ar, 2C), 137.06 (Ar, 2C). EI-MS, *m*/*z* (abundance, %): 246 (M^+, 40), 230 (28), 215 (27), 213 (10).

5.1.2. 5-Chloro-2,2-dimethyl-2H-benzimidazole 1,3-dioxide (**9**)

Brown solid (71%); mp 125.0 °C. ^{1}H NMR ($CDCl_3$) δ_H: 1.72 (s, 6H), 6.83 (dd, 1H, $J = 9.7$ Hz, $J = 1.5$ Hz), 7.22 (d, $J = 9.8$ Hz), 7.28 (1H). ^{13}C NMR ($CDCl_3$) δ_C: 24.65 (CH_3), 98.42 (C), 114.89 (Ar), 117.72 (Ar), 133.00 (Ar), 135.50 (Ar), 138.00 (Ar). EI-MS, *m*/*z* (abundance, %): 212 (M^+, 62), 196 (52), 181 (36).

5.1.3. (E/Z)-5-[(p-Chlorophenyl)ethenyl]-2,2-dimethyl-2H-benzimidazole 1,3-dioxide (**10**)

Red solid (14%); mp 94.0–95.0 °C (dec). ^{1}H NMR (acetone-d_6) δ_H: 1.60 (s, 3H), 1.63 (s, 3H), 6.61 (d, 0.5H, $J = 12.0$ Hz), 6.72 (d, 0.5H, $J = 9.6$ Hz), 6.82 (d, 0.5H, $J = 12.0$ Hz), 7.02 (d, 0.5H, $J = 9.6$ Hz), 7.08 (s, 0.5H),7.22-7.38 (m, 3H), 7.42-7.45 (m, 3.5H), 7.68 (d, 0.5H, $J = 8.5$ Hz). EI-MS, *m*/*z* (abundance, %): 314 (M^+, 37), 298 (82), 283 (68).

5.1.4. (E/Z)-2,2-Dimethyl-5-[(3,4-methylendioxyphenyl)ethenyl]-2H-benzimidazole 1,3-dioxide (**11**)

Red solid (19%); mp 110.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ_H: 1.74 (s, 6H), 6.02 (s, 2H), 6.77–6.84 (m, 2H), 6.97 (d, 1H, $J = 8.0$ Hz),

7.05–7.13 (m, 3H), 7.48–7.74 (m, 2H). ^{13}C NMR ($CDCl_3$) δ_C: 24.59 (CH_3), 97.86 (C-2), 101.84 (CH_2), 106.13 (Ar), 109.05 (Ar), 111.94 (Ar), 116.06 (Ar), 123.24 (Ar), 125.02 (CH), 128.90 (Ar), 131.00 (Ar), 132.35 (Ar), 133.00 (Ar), 136.06 (Ar), 136.99 (Ar), 140.24 (Ar), 148.92 (Ar). EI-MS, *m*/*z* (abundance, %): 324 (M^+, 25), 308 (98), 291 (100), 277 (83).

*5.1.5. 5-Methoxy-2*H*-benzimidazole-2-spirocyclopentane 1,3-dioxide (**12**)*

Red solid (62%); mp 143.0–145.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ_H: 2.13 (m, 4H), 2.36 (m, 4H), 3.84 (s, 3H), 6.39 (d, 1H, $J = 2.0$ Hz), 6.63 (dd, 1H), 7.15 (d, 1H, $J = 10.0$ Hz). ^{13}C NMR ($CDCl_3$) δ_C: 27.14 (CH_2, 2C), 39.74 (CH_2, 2C), 56.58 (CH_3), 90.59 (Ar), 105.84 (C), 114.00 (Ar), 116.78 (Ar), 118.00 (Ar), 128.80 (Ar), 162.35 (Ar). EI-MS, *m*/*z* (abundance, %): 234 (M^+, 100), 218 (12), 201 (8).

*5.1.6. 5-Methyl-2*H*-benzimidazole-2-spirocyclopentane 1,3-dioxide (**13**)*

Red solid (43%); mp 129.0–130.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ_H: 2.13 (m, 4H), 2.23 (s, 3H), 2.34 (m, 4H), 6.73 (d, 1H, 9.5 Hz), 6.97 (d, 1H, $J = 1.3$ Hz), 7.12 (d, 1H, $J = 9.5$ Hz). ^{13}C NMR ($CDCl_3$) δ_C: 22.36 (CH_3), 27.12 (CH_2, 2C), 40.00 (CH_2, 2C), 105.50 (C), 113.55 (Ar), 115.37 (Ar), 134.55 (Ar), 136.47 (Ar), 137.47 (Ar), 141.97 (Ar). EI-MS, *m*/*z* (abundance, %): 218 (M^+, 41), 202 (45), 185 (37).

*5.1.7. 2*H*-Benzimidazole-2-spirocyclopentane 1,3-dioxide (**14**)*

Red solid (67%); mp 123.0–125.0 °C. ^{1}H NMR ($CDCl_3$) δ_H: 2.14 (m, 4H), 2.34 (m, 4H), 6.88 (dd, 2H, $J_1 = 8.0$ Hz, $J_2 = 2.9$ Hz), 7.19 (dd, 2H). ^{13}C NMR ($CDCl_3$) δ_C: 27.10 (CH_2, 2C), 40.20 (CH_2, 2C), 105,40 (C), 115.90 (Ar, 2C), 130.90 (Ar, 2C), 137.80 (Ar, 2C). EI-MS, *m*/*z* (abundance, %): 204 (M^+, 32), 188 (30), 172 (25).

*5.1.8. 5-(Hydroxyimino)methyl-2*H*-benzimidazole-2-spirocyclopentane 1,3-dioxide (**15**)*

Red solid (68%); mp 157.0–158.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ_H: 2.14 (m, 4H), 2.37 (m, 4H), 7.20 (s, 1H), 7.23 (s, 1H), 7.35 (d, 1H, $J = 9.6$ Hz), 7.98 (s, 1H), 8.05 (bs, OH). ^{13}C NMR ($CDCl_3$) δ_C: 27.18 (CH_2, 2C), 40.26 (CH_2, 2C), 106.38 (C), 115.40 (Ar), 116.33 (Ar), 128.42 (Ar), 135.58 (Ar), 137.07 (Ar), 137.08 (Ar), 148.60 (CH). EI-MS, *m*/*z* (abundance, %): 247 (M^+, 100), 231(67), 214 (24).

*5.1.9. 5-Bromo-2*H*-benzimidazole-2-spirocyclopentane 1,3-dioxide (**16**)*

Red solid (57%); mp 145.0–147.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ_H: 2.13 (m, 4H), 2.34 (m, 4H), 6.95 (d, 1H, $J = 8.0$ Hz), 7.09 (d, 1H, $J = 8.0$ Hz), 7.47 (s, 1H). ^{13}C NMR ($CDCl_3$) δ_C: 27.10 (CH_2, 2C), 40.35 (CH_2, 2C), 106.22 (C), 116.89 (Ar), 118.14 (Ar), 126.00 (Ar), 134.80 (Ar), 136.6 (Ar). EI-MS, *m*/*z* (abundance, %): 282 (M^+, 17), 283 (M^+, 3), 266 (30), 267 (20), 249 (28), 250 (14).

*5.1.10. 5,6-Dichloro-2*H*-benzimidazole-2-spirocyclopentane 1,3-dioxide (**17**)*

Red solid (50%); mp 150.0 °C (dec). ^{1}H NMR ($CDCl_3$) δ_H: 2.12 (m, 4H), 2.32 (m, 4H), 7.38 (d, 2H, $J = 9.0$ Hz). ^{13}C NMR ($CDCl_3$) δ_C: 27.07 (CH_2, 2C), 40.44 (CH_2, 2C), 106.64 (C), 116.23 (Ar, 2C), 135.07 (Ar, 2C), 136.84 (Ar, 2C). EI-MS, *m*/*z* (abundance, %): 272 (M^+, 97), 256 (20), 239 (Ar, 2C).

*5.1.11. 2-Methyl-2-pentyl-2*H*-benzimidazole 1,3-dioxide (**18**)*

Red oil (70%). ^{1}H NMR ($CDCl_3$) δ_H: 0.82 (t, 3H, $J = 6.8$ Hz), 1.03 (m, 2H), 1.23 (m, 4H), 1.68 (s, 3H), 2.11 (t, 2H, $J = 8.2$ Hz), 6.89 (dd, 2H, $J_1 = 7.2$ Hz, $J_2 = 2.9$ Hz), 7.21 (dd, 2H, $J_1 = 7.2$ Hz, $J_2 = 2.9$ Hz). ^{13}C NMR ($CDCl_3$) δ_C: 14.21 (CH_3), 21.72 (CH_2), 22.58 (CH_2), 24.66 (CH_3), 31.24 (CH_2), 37.61 (CH_2), 100.26 (C-2), 115.84 (Ar), 131.16 (Ar), 137.91 (Ar). EI-MS, *m*/*z* (abundance, %): 218 (M^+-16, 20), 189 (40), 161 (64), 147 (100). IR (KBr) ν: 2359, 1614, 1586, 1412, 1381, 1312, 1098, 746, 530.

5.2. Biology

5.2.1. Anti-trypanosomatid in vitro *evaluation*

*5.2.1.1. Anti-*T. cruzi in vitro *test using epimastigotes of Tulahuen 2 strain. T. cruzi* epimastigotes (Tulahuen 2 strain) were grown at 28 °C in an axenic medium (BHI-Tryptose) as previously described [18,27], supplemented with 5% fetal bovine serum (FBS). 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 were supplemented with the indicated amount of the drug from a stock solution in DMSO. The final concentration of DMSO in the culture media never exceeded 0.4% and the control was run in the presence of 0.4% DMSO and in the absence of any drug. No effect on epimastigote growth was observed by the presence of up to 1% DMSO in the culture media. The percentage of inhibition (PGI) was calculated as follows: $PGI\ (\%) = \{1-[(Ap-A0p)/(Ac-A0c)]\} \times 100$, where $Ap = A_{600}$ of the culture containing the drug at day 5; $A0p = A_{600}$ of the culture containing the drug just after addition of the inocula (day 0); $Ac = A_{600}$ of the culture in the absence of any drug (control) at day 5; $A0c = A_{600}$ in the absence of the drug at day 0. To determine ID_{50} values, 50% inhibitory concentrations, 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%.

*5.2.1.2. Anti-*T. cruzi in vitro *test using trypomastigotes and amastigotes of the CL Brener clone.* [19] Vero cell cultures infected with cell culture trypomastigotes and incubated at 37 °C in humidified air with 5% CO_2 for 5–7 days. After that time, the culture medium was collected and centrifuged at 4000 g for 10 min, and the trypomastigote-containing pellet was left at 37 °C for 4 h, to allow the trypomastigotes to swim off. The remaining pellet was discarded, and this supernatant was used for the experiments. After trypomastigotes exposure to different doses and in different periods of the studied BzNO the percentage of lysis was determined in reference to untreated parasites. The percentage of lysis was determined using Neubauer counting chamber.

Vero cells (4×10^7/L) were cultured at 37 °C in modified Eagle's medium containing 3% (v/v) FBS, in 24-well plates containing glass coverslips. After 24 h the cultures were inoculated with CL Brener clone cell culture trypomastigotes (5×10^9/L) with or without compound **13**. After 24 h, the medium containing the non-internalized parasites, was removed; fresh medium, with or without compound **13**, was added and the infected cells were incubated for 72 h and stained with May-Grunwald-Giemsa. The percentage of infected cells and the number of intracellular parasites were estimated by observing 500 cells in a Nikon Eclipse E400 microscope. The results are expressed as the endocytic index (product of the percent of infected cells and the number of amastigotes per cell).

5.2.1.3. Viability of CL Brener, Y or Colombiana strains of T. cruzi *and LTB300 strain of* L. braziliensis. *T. cruzi* epimastigotes (CL Brener, or Y strains) were grown as it is indicated in Section 5.2.1.1. *L. braziliensis* (MHOM/BR/00/LTB300 strain) promastigotes were grown at 28 °C in an axenic-RPMI medium supplemented with 5% FBS as previously described [20]. Cell-culture plates consisting of 24 wells were filled at 1 mL/well with the corresponding parasite strain

culture during its exponential growth in the corresponding medium. BHI-Tryptose medium supplemented with 5% FBS. Different doses of studied compounds dissolved in DMSO were added and maintained for 5 days. Afterwards, the cells were washed with PBS and incubated (37 °C) with 0.4 mg/mL MTT (Sigma) for 3 h. Then, formazan was dissolved with DMSO (180 μL), and optical densities were measured. Each concentration was assayed three times, and six growth controls were used in each test. Cytotoxicity percentages (PCyt (%)) were determined as follows: $PCyt = [100-(ODd-ODdm)/(ODc-ODcm)] \times 100$, where ODd is the mean of OD595 of wells with parasites and different concentrations of the compounds, ODdm is the mean of OD595 of wells with different compound concentrations in the medium, ODc is the growth control, and ODcm is the mean of OD595 of wells with medium only. The ID_{50} value was taken as the concentration of drug needed to reduce the absorbance ratio to 50%.

5.2.2. Study of metabolic changes in the parasites

5.2.2.1. Mitochondrial dehydrogenase activities. Mitochondrial dehydrogenase activities were measured in 24-well plates [28]. One million of *T. cruzi* epimastigotes (Y strain) in 500 μL medium were seeded in each well and 20 μM of studied compounds was added. Two wells with untreated parasites were maintained as controls corresponding to the given time of treatment (30–240 min). The cultures were incubated at 28 °C. At the different time incubations, the epimastigotes were counted and the colorimetric MTT dye-reduction assay was performed, the tetrazolium salt being converted into purple formazan by mitochondria. Fifty μL of a solution containing 5 mg/mL of MTT in PBS was added to each well and plates were incubated for an additional 4 h. The reaction was stopped by addition of 500 μL of acidic isopropanol (0.4 mL HCl 10 N in 100 mL isopropanol). The absorbance was measured at 570 nm. Under our conditions, compounds did not interfere with the reaction mixture. Percentage of mitochondrial dehydrogenase activities (Pmdh (%)) was determined using untreated parasites-activities as 100%.

5.2.2.2. Glucose-uptake's changes. T. cruzi epimastigotes (Y strain) 80×10^6 parasites/mL after washing twice with PBS-glucose were resuspended in PBS-glucose (1 mM) and transferred 800 μL to 24-well cell-culture plate. Then, compounds were added dissolved in DMSO, never exceed (8 μL), and incubated for 4 h. Afterwards, centrifugation at 1500 g (3 min) gave the parasite-free supernatant that was treated with 500 μL Benedicts reagents at reflux during 1 h. The glucose concentration was determined measuring at 700 nm the $CuSO_4$ concentration using a calibration curve.

5.2.2.3. ^1H NMR study of the excreted metabolites. [23,27,28] For the spectroscopic studies, 5 mL of a 2-day-treated *T. cruzi* (Y strain) with each studied compound (5 μM) was centrifuged at 1500 g for 10 min at 4 °C. The pellet was discarded, and the parasite-free supernatant was stored at −20 °C until used. Before the measurement, about 0.1 mL of DMF (10 mM) as internal standard and 0.1 mL of D_2O were added to 0.3 mL of the supernatant. The spectra were registered with water suppression in 5 mm NMR sample tubes. The chemical displacements used to identify the respective metabolites were confirmed by both adding each analyzed metabolite to the studied supernatant and by the study of a control solution with 4 μg/mL of each metabolites in buffer phosphate, pH = 7.4.

Acknowledgements

MB, LB, and AM thanks to PEDECIBA (Uruguay) for her scholarships. We thank IUBMB for a fellowship to LB. JJC is a member of the Research Career of the Argentinean National Research Council (CONICET), and AC has a fellowship for the Universidad Nacional de General San Martin.

References

[1] World Health Organization, Thirteenth Program Report, UNDP/World Bank/World Health Organization Program for Research and Training in Tropical Diseases, World Health Organization, Geneva, 1997.
[2] H. Cerecetto, M. González, Curr. Top. Med. Chem. 2 (2002) 1187–1213.
[3] Report of the Scientific Working Group on Chagas Disease, WHO/TDR, 2005.
[4] J. Berman, Curr. Opin. Infect. Dis. 16 (2003) 397–401.
[5] (a) P.J. Guerin, P. Olliaro, S. Sundar, M. Boelaert, S.L. Croft, P. Desjeux, K. Wasunna, A.D.M. Bryceson, Lancet Infect. Dis. 2 (2002) 494–501;
(b) E. Rosenthal, P. Marty, J. Postgrad. Med. 49 (2003) 61–68.
[6] (a) R. Docampo, S.N.J. Moreno, Fed. Proc. 45 (1986) 2471–2476;
(b) R. Docampo, Chem. Biol. Interact. 73 (1990) 1–27.
[7] J.F. Turrens, Mol. Aspects Med. 25 (2004) 211–220.
[8] (a) H. Cerecetto, R. Di Maio, M. González, M. Risso, P. Saenz, G. Seoane, A. Denicola, G. Peluffo, C. Quijano, C. Olea-Azar, J. Med. Chem. 42 (1999) 1941–1950;
(b) G. Aguirre, H. Cerecetto, R. Di Maio, M. González, W. Porcal, G. Seoane, A. Denicola, M.A. Ortega, I. Aldana, A. Monge-Vega, Arch. Pharmacol. 335 (2002) 15–21;
(c) C. Olea-Azar, C. Rigol, L. Opazo, A. Morello, J.D. Maya, Y. Repetto, G. Aguirre, H. Cerecetto, R. Di Maio, M. González, W. Porcal, J. Chil. Chem. Soc. 48 (2003) 77–79;
(d) C. Olea-Azar, C. Rigol, F. Mendizábal, R. Briones, H. Cerecetto, R. Di Maio, M. González, W. Porcal, M. Risso, Spectrochim. Acta Part A 59 (2003) 69–74;
(e) C. Olea-Azar, C. Rigol, F. Mendizábal, H. Cerecetto, R. Di Maio, M. González, W. Porcal, A. Morello, Y. Repetto, J.D. Maya, Lett. Drugs Des. Dev. 2 (2005) 294–301;
(f) G. Aguirre, L. Boiani, H. Cerecetto, R. Di Maio, M. González, W. Porcal, L. Thomson, V. Tórtora, A. Denicola, M. Möller, Bioorg. Med. Chem. 13 (2005) 6324–6335;
(g) G. Aguirre, L. Boiani, M. Boiani, H. Cerecetto, R. Di Maio, M. González, W. Porcal, A. Denicola, O.E. Piro, E.E. Castellano, C.M.R. Sant'Anna, E.J. Barreiro, Bioorg. Med. Chem. 13 (2005) 6336–6346;
(h) W. Porcal, P. Hernández, G. Aguirre, L. Boiani, M. Boiani, A. Merlino, A. Ferreira, R. Di Maio, A. Castro, M. González, H. Cerecetto, Bioorg. Med. Chem. 15 (2007) 2768–2781;
(i) W. Porcal, P. Hernández, M. Boiani, G. Aguirre, L. Boiani, A. Chidichimo, J.J. Cazzulo, N.E. Campillo, J.A. Paez, A. Castro, R.L. Krauth-Siegel, C. Davies, M.A. Basombrío, M. González, H. Cerecetto, J. Med. Chem. 29 (2007) 6004–6015.
[9] G. Aguirre, H. Cerecetto, R. Di Maio, M. González, M.E. Alfaro, A. Jaso, B. Zarranz, M.A. Ortega, I. Aldana, A. Monge-Vega, Bioorg. Med. Chem. Lett. 16 (2004) 3835–3839.
[10] G. Aguirre, M. Boiani, H. Cerecetto, A. Gerpe, M. González, Y.F. Sainz, A. Denicola, C. Ochoa de Ocáriz, J.J. Nogal, D. Montero, J.A. Escario, Arch. Pharm. 337 (2004) 259–270.
[11] M. Boiani, L. Boiani, A. Denicola, S. Torres de Ortiz, E. Serna, N. Vera de Bilbao, L. Sanabria, G. Yaluff, H. Nakayama, A. Rojas de Arias, C. Vega, M. Rolan, A. Gómez-Barrio, H. Cerecetto, M. González, J. Med. Chem. 49 (2006) 3215–3220.
[12] (a) M. Boiani, M. González, Mini Rev. Med. Chem. 5 (2005) 409–424;
(b) M. El-Haj, J. Org. Chem. (1972) 2519–2520.
[13] A. Merlino, M. Boiani, H. Cerecetto, M. González, Spectrochim. Acta A Mol. Biomol. Spectrosc. 67 (2007) 540–549.
[14] (a) J.F. Faucher, T. Baltz, K.G. Petry, Parasitol. Res. 81 (1995) 441–443;
(b) M. Almeida-de-Faria, E. Freymuller, W. Colli, M.J. Alves, Exp. Parasitol. 92 (1999) 263–274.
[15] (a) N. Yoshida, Parasitol. Int. 57 (2008) 105–109;
(b) B. Zingales, M.E.S. Pereira, K.A. Almeida, E.S. Umezawa, N.S. Nehme, R.P. Oliveira, A. Macedo, R.P. Souto, Mem. Inst. Oswaldo Cruz 92 (1997) 811–814.
[16] (a) L.S. Filardi, Z. Brener, Trans. R Soc. Trop. Med. Hyg. 81 (1987) 755–759;
(b) J. Molina, Z. Brener, A.J. Romanha, J.A. Urbina, J. Antimicrob. Chemother. 46 (2000) 137–140.
[17] S. Muelas-Serrano, J.J. Nogal-Ruiz, A. Gómez-Barrio, Parasitol. Res. 86 (2000) 999–1002.
[18] A. Gerpe, G. Aguirre, L. Boiani, H. Cerecetto, M. González, C. Olea-Azar, C. Rigol, J.D. Maya, A. Morello, O.E. Piro, V.J. Arán, A. Azqueta, A.L. López de Ceráin, A. Monge, M.A. Rojas, G. Yaluff, Bioorg. Med. Chem. 14 (2006) 3467–3480.
[19] A.S. Bernacchi, B. Franke de Cazzulo, J.A. Castro, J.J. Cazzulo, Acta Pharmacol. Sin. 23 (2002) 399–404.
[20] (a) D. Sereno, J.L. Lemesre, Parasitol. Res. 83 (1997) 401–403;
(b) A. Dutta, S. Bandyopadhyay, C. Mandal, M. Chatterjee, Parasitol. Int. 54 (2005) 119–122.
[21] M. Maarouf, Y. De Kouchkovsky, S. Brown, P.X. Petit, M. Robert-Gero, Exp. Cell Res. 232 (1997) 339–348.
[22] B.H. Ter Kuile, F.R. Opperdoes, J. Biol. Chem. 266 (1991) 857–862.
[23] (a) C.M. Mesa-Valle, J. Castilla-Calvente, M. Sanchez-Moreno, V. Moraleda-Lindez, J. Barbe, A. Osuna, Antimicrob. Agents Chemother. 40 (1996) 684–690;

(b) M. Sánchez-Moreno, C. Fernández-Becerra, J. Castilla, A. Osuna, FEMS Microbiol. Lett. 133 (1995) 119–125;
(c) C. Fernandez-Ramos, F. Luque, C. Fernández- Becerra, A. Osuna, S.I. Jankevicius, V. Jankevicius, M.J. Rosales, M. Sanchez-Moreno, FEMS Microbiol. Lett. 170 (1999) 343–348;
(d) M.C. Caterina, I.A. Perillo, L. Boiani, H. Pezaroglo, H. Cerecetto, M. González, A. Salerno, Bioorg. Med. Chem. 16 (2008) 2226–2234.

[24] P. Penin, M. Sánchez-Moreno, J.A. de Diego, Comp. Biochem. Physiol. 120A (1998) 571–574.
[25] J.J.B. Cannata, J.J. Cazzulo, Comp. Biochem. Physiol. 79B (1984) 297–308.
[26] A.G.M. Tielens, J.J. Van Hellemond, Parasitol. Today 14 (1998) 265–271.
[27] A. Caligiani, D. Acquotti, G. Palla, V. Bocchi, Anal. Chim. Acta 585 (2007) 110–119.
[28] L. Boiani, G. Aguirre, M. González, H. Cerecetto, A. Chidichimo, J.J. Cazzulo, M. Bertinaria, S. Guglielmo, Bioorg. Med. Chem. 16 (2008) 7900–7907.

Please cite this article in press as: M. Boiani et al., Second generation of 2*H*-benzimidazole 1,3-dioxide derivatives as..., European Journal of Medicinal Chemistry (2009), doi:10.1016/j.ejmech.2009.06.014