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Novel Antiprotozoal Products: Imidazole and Benzimidazole *N*-Oxide Derivatives and Related Compounds

The syntheses and biological evaluation of the first anti-protozoa imidazole *N*-oxide and benzimidazole *N*-oxide and their derivatives are reported. They were tested *in vitro* against two different protozoa, *Trypanosoma cruzi* and *Trichomonas vaginalis*. Derivative **7c**, ethyl-1-(*i*-butyloxycarbonyloxy)-6-nitrobenzimidazole-2-carboxylate, displayed activity on both protozoa. Lipophilicity and redox potential were experimentally determined in order to study the relationship with activity of the compounds. These properties are well related with the observed bioactivity. Imidazole and benzimidazole *N*-oxide derivatives are becoming leaders for further chemical modifications and advanced biological studies.

Keywords: Anti-protozoa compounds; Imidazole *N*-oxide; Structure-activity relationship

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Full Paper

Introduction

Parasitic diseases are among the most widespread of human diseases. In 1997, of a global total of 52.2 million deaths, 17.3 million were due to infectious and parasitic diseases, followed by circulatory diseases, cancer, and respiratory diseases [1]. In South America these constitute a major health and economic problem, where Chagas' disease occupies the third place in number of deaths per year, after malaria and schistosomiasis [2]. Commercially, only two drugs were available for the treatment of this disease: Nifurtimox (Nfx, actually discontinued) and Benznidazole (Bnz) (Figure 1). Unfortunately, they are not consistently effective and have serious side effects that explain the need for development of new selective chemotherapeutic solutions [3].

On the other hand trichomoniasis, an important sexually-transmitted protozoa disease, which is frequent in rich countries, also needs new effective agents. The treatment of trichomoniasis was revolutionized by metronidazole (Mtz) (Figure 1) [4]. The 5-nitroimidazole Mtz, a potent trichomononicidal agent under low oxygen concentrations conditions, [5] has shown important strain-resistance effects [6].

In previous works [7, 8] we found that some heterocycle *N*-oxide containing derivatives posses promising trypanocidal activity. In particular, compound **1** (Figure 1) has an IC_{50} for *Trypanosoma cruzi* (*T. cruzi*) epimastigote form (Tulahuen strain) similar to that found for Nfx. The well known *N*-oxide bio-reduction and it biological consequences [9, 10] were used to explain the trypanocidal activity. These facts induced us to investigate some related heterocycles (Figure 1). So, we have prepared some selected derivatives of imidazole *N*-oxide and benzimidazole *N*-oxide. We report here

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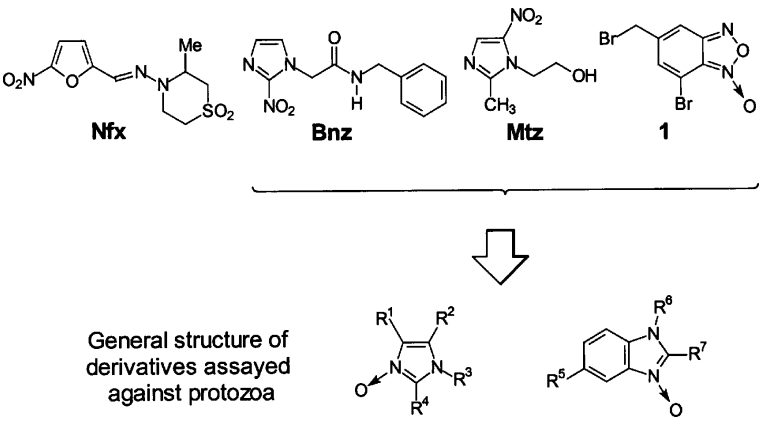


Figure 1. Designed structures from Nifurtimox (Nfx), Benznidazole (Bnz), Metronidazole (Mtz) and benzofuroxan (derivative **1**).

the synthesis, anti-protozoa (*T. cruzi* and *Trichomona vaginalis* (*T. vaginalis*)) activity, and the structure-activity studies of these series of compounds.

Results and discussion

Chemistry

Compounds **2a**–**f** were obtained through a simple chemical transformation as reported [11–14], or *via* cyclization of the corresponding a-substituted amine-oxime such as depicted in Scheme 1A. The a-substituted amine-oximes were obtained from the diacetyl mono-oxime and amines by reductive amination or *via* nucleophilic substitution from chloroacetone and *p*-toluidine (compound **II**, Scheme 1A). The imidazole system was generated by cyclization of the corresponding orthoester at reflux. Secondary products were identified as the result of condensation between cyclization intermediates and the orthoesters.

On the other hand, the basic cyclization of ethyl *N*-(2-nitro-4-substitutedphenyl)glycinate afforded the corresponding benzimidazole *N*-oxide **3a**–**c** (Scheme 1B) [15]. Derivatives **3d**–**e** were obtained by reaction between the corresponding benzofuroxan and nitroethane in basic medium [16]. In order to modify the bioactivity of derivative **3a**, it was transformed into compounds **4a**–**c**, **5**, **6** (Scheme 1B), and **7a**–**d** (Scheme 1C). Treatment of **3a** with excess of the corresponding alcohol (*i*-propanol, *n*-butanol and *n*-hexanol), in presence of *p*-toluenesulfonic acid (*p*-TsOH) and molecular sieves (4 Å), yielded derivatives **4a**–**c**. Compound **5**, was obtained in the same manner by reacting compound **3a** with *n*-butylamine in toluene. Compound **6** was prepared as it has previously been reported [15].

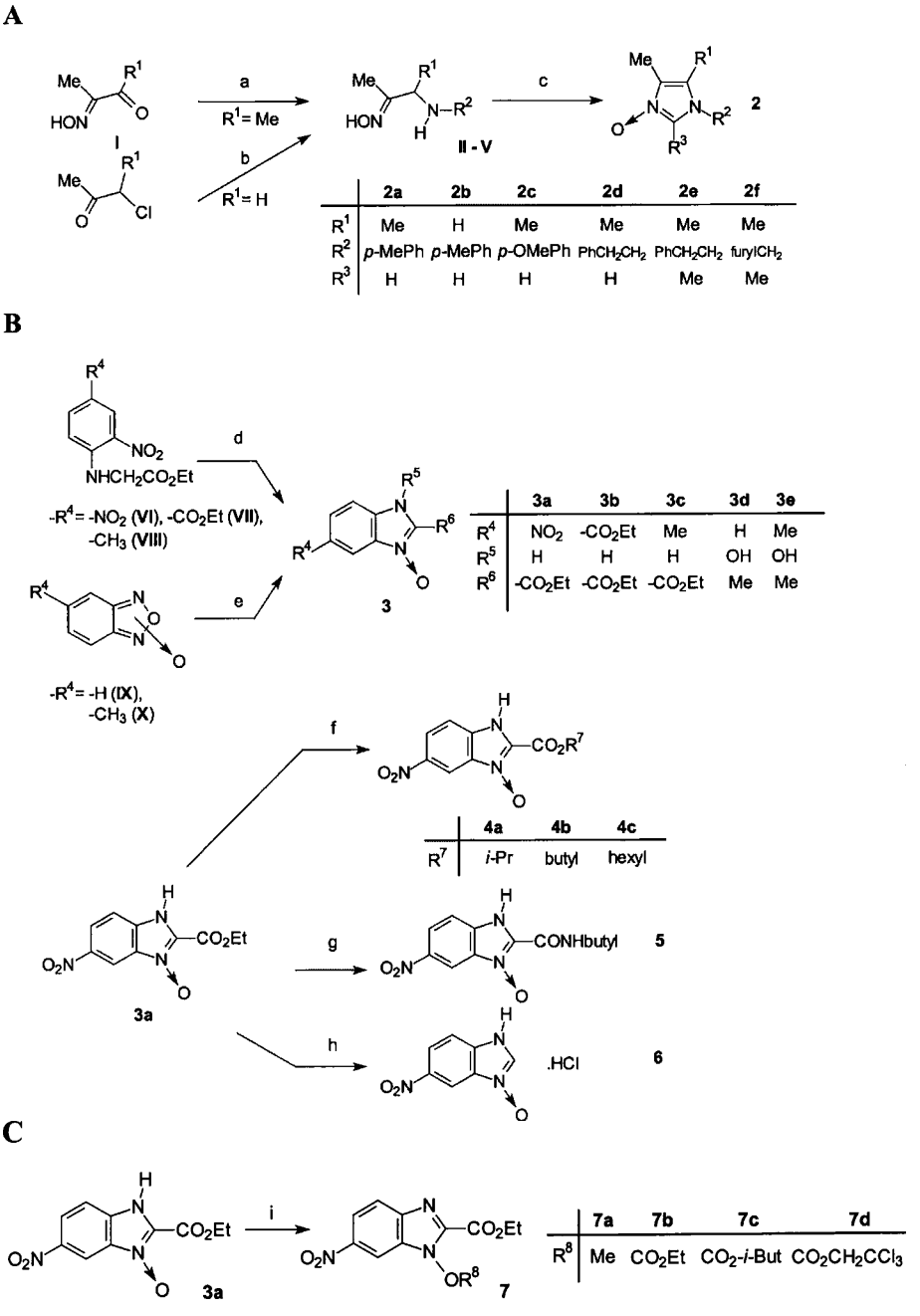
In order to obtain more apolar derivatives, compound **3a** was reacted with methyl iodide and different chloroformate derivatives. This procedure yielded derivatives **7a**–**d** as unique products of reaction (Scheme 1C). The course of reaction, *O*-alkylation process, was clearly evidenced by the **7a-d** NMR NOE-diff experiments (Figure 2A).

Benzimidazole *N*-oxide derivatives **3a**–**e**, **4a-c**, **5**, and **6** are present, in solution, as a mixture of tautomers **XI** and **XI'** [15, 17] (Figure 2B). This fact was clearly observed in the ^{1}H NMR spectra and was confirmed chemically with the exclusive formation of compounds **7a**–**d** from **3a**.

The structures of all derivatives were confirmed by NMR (^{1}H, ^{13}C, and HETCOR experiments, *i.e.* HMQC and HMBC) and MS, and their purity established by TLC and microanalysis.

Biological studies

The existence of the epimastigote form as an obligate mammalian intracellular stage has been revisited [18, 19] and confirmed recently [20]. For this reason, the compounds were tested *in vitro* against epimastigote forms of *T. cruzi* (Tulahuen strain) at two different concentrations, 25 μM and 100 μM, as indicated in Experimental [7]. The percentage of inhibition was calculated as follows: % = $\{1-[(A_p-A_{0p})/(A_c-A_{0c})]\} \times 100$, where $A_p = A_{600}$ of the culture containing the drug at day 5; $A_{0p} = A_{600}$ of the culture containing the drug just after addition of the inocula (day 0); $A_c = A_{600}$ of the culture in the absence of any drug (control) at day 5;



Scheme 1. A. Preparation of 1-substituted imidazole N^3-oxide derivatives: (a) 1. $R^2NH_2/ZnCl_2$/MeOH/molecular sieves 4 Å/rt, 2. $NaCNBH_3$; (b) *p*-toluidine/K_2CO_3/KI/acetone/reflux.; (c) $R^3C(OR')_3$ (R' = Me or Et) (solvent)/*p*-TsOH/reflux. **B.** Preparation of the benzimidazole *N*-oxide derivatives: (d) piperidine/EtOH/reflux for **3a**; EtONa/EtOH/rt for **3b** and **3c**; (e) nitroethane/piperidine/THF/rt; (f) R^7OH (solvent)/*p*-TsOH/ molecular sieves 4Å/reflux; (g) butylamine (1 equiv.)/toluene/*p*-TsOH/molecular sieves 4Å/ reflux; (h) HCl (c)/reflux. **c)** Preparation of derivatives **7a-d**: (i) R^8X, $NaHCO_3$ (for 7a)/acetone/rt.

$A_{0c} = A_{600}$ in the absence of the drug at day 0. Table 1 shows the percentage of inhibition for the evaluated derivatives.

Trichomonacidal activity *in vitro* was developed using recently isolated strain JH31A no. 4 of *T. vaginalis* at 1 μg/mL, 10 μg/mL, and 100 μg/mL as indicated in

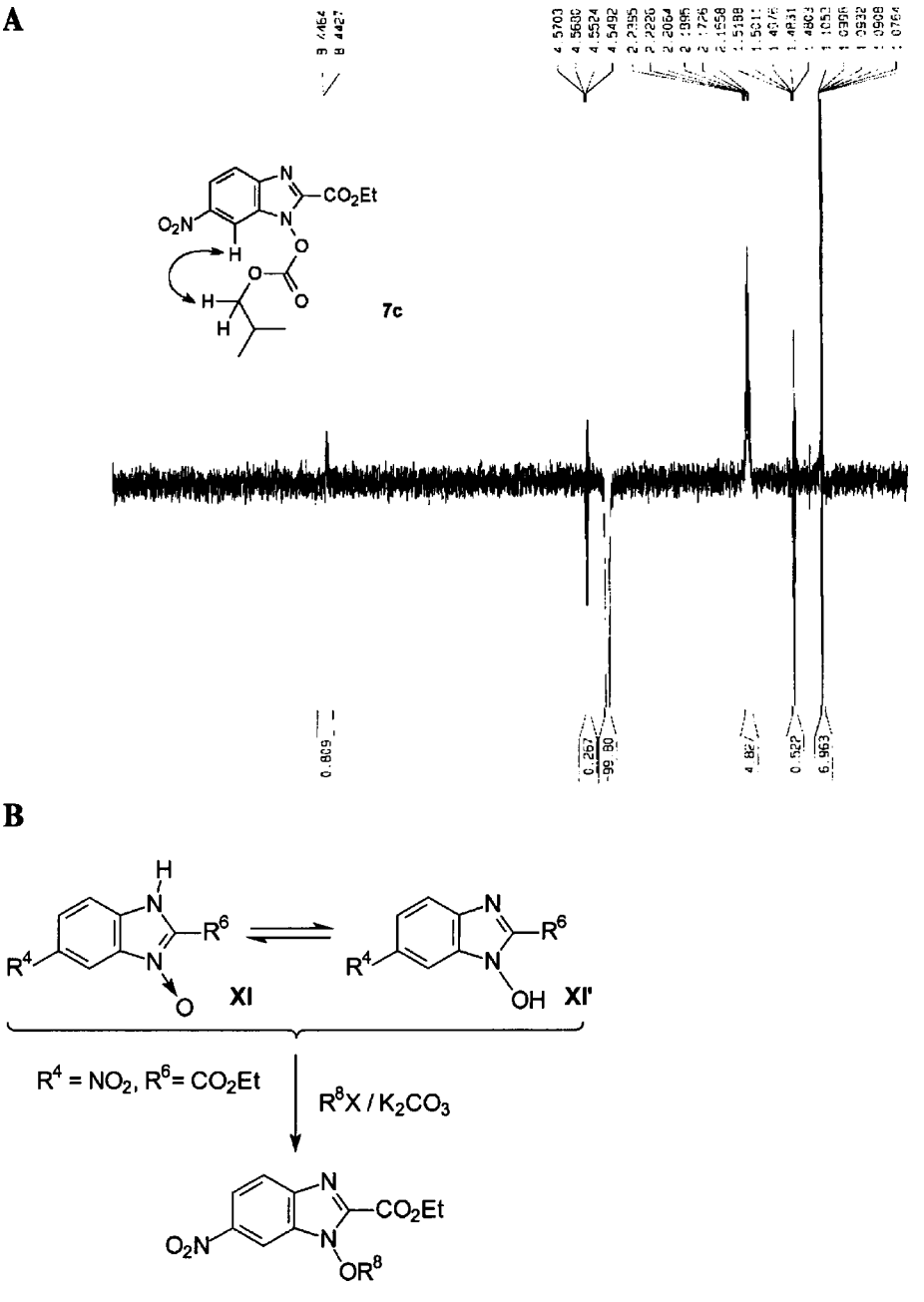


Figure 2. A. Tautomeric forms of benzimidazole *N*-oxide derivatives. **B.** NOE-diff experiments of derivative **7c** confirming the *O*-acylation process involved in the reaction between derivative **3a** and electrophile reactants.

Experimental [21]. Results are expressed as percentages of growth inhibition (cytostatic activity) respective to controls, as follows: % C.A. = 100-[(G.I.$_{experimental}$/G.I.$_{control}$) × 100], where G.I. (growth indices) is the ratio between the number of parasites at 24 (or 48 h) and the number of parasites at 0 h. When this ratio (G.I.) is <1, the values so calculated represent percentages of reduction (% R), because in these cases, the number of parasites is reduced with respect to initial population, and the activity is cytocidal. Table 1 shows the cytostatic or cytocidal activity in brackets for the evaluated derivatives. Table 2 shows the percentage of reduction of *T. vaginalis* growth at 48 h for some selected derivatives.

Table 1. *In vitro* activity of developed compounds on *T. cruzi* and *T.vaginalis*.

Compound	*T. cruzi*[#] % Inh.[†]		*T. vaginalis*[#] % C.A.[‡]		
	100 μM	25 μM	100 μg/mL	10 μg/mL	1 μg/mL
2a	26.0	na	48.2	32.4	27.5
2b	11.0	na	46.6	27.5	20.9
2c	5.0	na	34.3	11.1	0.0
2d	19.0	na	ND	ND	ND
2e	19.0	na	ND	ND	ND
2f	31.0	na	20.2	17.2	7.1
3a	5.0	na	56.0	13.0	6.0
3b	19.0	na	40.2	30.8	19.7
3c	35.0	na	51.5	38.4	9.1
3d	5.0	na	ND	ND	ND
3e	5.0	na	ND	ND	ND
4a	39.0	na	42.6	24.1	15.9
4b	34.0	5.0	57.1	12.4	16.3
4c	61.0	na	47.5	17.2	7.1
5	38.0	9.0	59.6	19.2	6.7
6	ND	na	23.2	13.6	8.1
7a	5.0	na	47.9	32.5	19.7
7b	34.0	5.0	71.3	14.4	0.0
7c	61.0	9.0	(87.5)	17.3	9.6
7d	19.0	10.0	43.3	22.1	15.4
Nfx	100.0	93.0	–	–	–
Mtz	–	–	(100.0)	(100.0)	(92.1)

[#] Values are means of two experiments. [†] % Inh. – percentage of inhibition of *T. cruzi* growth at the indicated doses at day 5. [‡] % C.A. – percentage of inhibition of *T. vaginalis* growth at the indicated doses at 24 h. na – not active, (% Inh. lower than 4.0%). ND – not determined. In brackets – percentage of reduction (% R).

Structure-activity relationships

The anti-protozoa activity of (benz)imidazole derivatives developed can be related to their lipophilicity, in general the derivatives including lipophilic moieties in their structures resulted the more active compounds. Thus, compound **3a**, an ethyl ester, was in general less active than **4b**–**c** and **5**, compounds bearing a lipophilic-butyl and -hexyl chains, and **7b**–**d** derivatives, with a NH function blocked with a $NOCO_2R$ moiety, were in general more active than **3a** bearing an NH group. Also, the *N*-oxide bio-reduction can be related with the observed activity such as the nitro group in Nfx or Mtz. Perhaps, in the present case the reduction potentials of the imidazole and benzimidazole *N*-oxides are not adequate to allow the electronation process and the subsequent biological damage. In order to explain the biological response observed we determined these physicochemical properties potentially related with the activity.

Table 2. Percentage of reduction of *T. vaginalis* growth at the indicated doses after 48 h contact with the listed compounds.

Compound	Dose[#]		
	100 μg/mL	10 μg/mL	1 μg/mL
2a	16.7	4.3	1.5
2b	12.2	0.0	0.0
3a	17.0	7.0	2.8
3c	53.0	33.7	11.0
4c	0.0	0.0	0.0
5	48.1	12.3	5.5
7b	0.0	0.0	0.0
7c	75.3	16.6	11.5
Mtz	100.0	100.0	92.1

[#] Values are means of two experiments.

Lipophilicity studies [7, 22, 23]

Reversed-phase TLC experiments were performed for all the derivatives on precoated TLC-C_{18} and eluted with acetone:water (50:50, v/v). The R_f values were converted into R_M values *via* the relationship: $R_M = \log [(1/R_f)-1]$. Nfx, the bio-assays reference compound, was used as inter-assays reference. Table 3 summarizes ΔR_M, defined as ΔR_M = Nfx R_M – derivative R_M, for each derivative.

Electrochemical studies [7, 24–26]

The redox properties of *N*-oxide derivatives **2a**, **2c**–**f** and **3a**–**c**, **4a**, **4c** and **5** were studied using the cyclic voltammetry methodology. The voltammetric responses of derivatives were comparable in DMF at a platinum working electrode. Typically one or two well-defined reduction peaks were observed during forward cathodic scan. The first wave for all studied compounds corresponds to a reversible one-electron transfer. The reverse scan showed the anodic counterpart of the reduction waves. The breadth of the cathodic wave at its half intensity has a relatively constant value of 60 mV. The ratio ipa/ipc has a value near to one, so the one-electron reduction process is reversible. Table 3 summarizes E_{pc} for the first reduction step of each (benz)imidazole *N*-oxide derivative studied.

Table 3. Physicochemical data and bioactivity of imidazole *N*-oxide derivatives.

Compound	ΔR_M[†,‡]	E_{pc} (V)[†,#]	Log_{10} (% Inh.)[§]	Log_{10} (% C.A.)[††]
2a	−0.09	−1.27	1.41	1.68
2b	−0.02	−	1.04	1.67
2c	−0.04	−1.47	0.70	1.54
2d	−0.98	−1.45	1.28	ND[‡‡]
2e	−1.11	−1.31	1.28	ND
2f	−0.97	−1.50	1.49	1.31
3a	0.07	−1.40	0.70	1.75
3b	0.07	−1.49	1.28	1.60
3c	0.19	−1.25	1.54	1.71
3d	0.49	LS[§§]	0.70	ND
3e	−0.09	LS	0.70	ND
4a	0.13	−1.24	1.59	1.63
4b	−0.13	ND	1.53	1.76
4c	−0.49	−1.19	1.79	1.68
5	−0.31	−1.22	1.58	1.78
6	0.07	LS	ND	1.37
7a	−0.17	−	0.7	1.68
7b	−0.14	−	1.53	1.85
7c	−0.23	−	1.79	2.00
7d	−0.03	−	1.28	1.64

[†] Values are means of two experiments. [‡] ΔR_M − Nifurtimox's R_M − derivative' R_M. [#] Peak potentials (±0.01 V) measured at a scan rate of 0.2 V/s. [§] % Inh. − percentage of inhibition of *T. cruzi* growth at 100 μM at day 5. [††] % C.A. − percentage of inhibition of *T. vaginalis* growth at 100 μg/mL at 24 h. [‡‡] ND − not determined. [§§] LS − The low solubility did not permit to determine the redox potential in non-aqueous medium.

Conclusions

The imidazole *N*-oxide derivatives (compounds **2**) proved to be the least actives against the protozoa *T. cruzi* and *T. vaginalis* of the developed compounds, in the assayed conditions.

Some activity was displayed at the highest tested concentration (100 μM), against *T. cruzi*, however not comparable to Nfx. The latter were more cytostatic against *T. vaginalis*, **2a**, **2b** showing reduction in the growth of parasite at the lowest assayed concentration (1 μg/mL) at 24 h of contact. However, this activity was not maintained for 48 h (see Table 2).

The benzimidazole derivatives, in the assayed conditions, showed more activity against protozoa than the imidazole counterparts.

Derivatives **3c**, **4a**−**4c**, **5**, **7b** and **7c** displayed good activity against *T. cruzi* at 100 mM, whereas compound **7c,** bearing a lipophilic substituent in the NO-moiety was one of the best. Against *T. vaginalis*, benzimidazole derivatives showed high activity at 100 μg/mL and 24 h of contact which was maintained up to 48 h for some derivatives (**3c**, **5**, and **7c**, Table 2). Further, compound **7c** showed trichomonacidal activity at 100 μg/mL and 24 h of contact (Table 1, result in brackets).

Lipophilic-hydrophilic properties can be related to the observed anti-protozoa activity of (benz)imidazole derivatives. Analyzing the correlation between the determined ΔR_M and the activities, we did not obtain a statistically valid correlation. However, we observed a parabolic or bilinear [27] tendency between the anti-protozoa activities of derivatives (as expressed in Table 3) and ΔR_M (Figure 3). Derivatives **4c** and **7c** showed the best anti-*T. cruzi* activity, appearing at the maximum of the curve activity *vs* ΔR_M (Figure 3A). In the same manner derivatives **7c** and **7b** presented the top of the curve showing trichomonacidal activity *vs* ΔR_M (Figure 3B).

The *N*-oxide bio-reduction capacity of these derivatives, expressed as E_{pc}, can be related with the observed activity. The reduction potential of the imidazole and benzimidazole *N*-oxide derivatives were more negative, so the mono-electronation processes

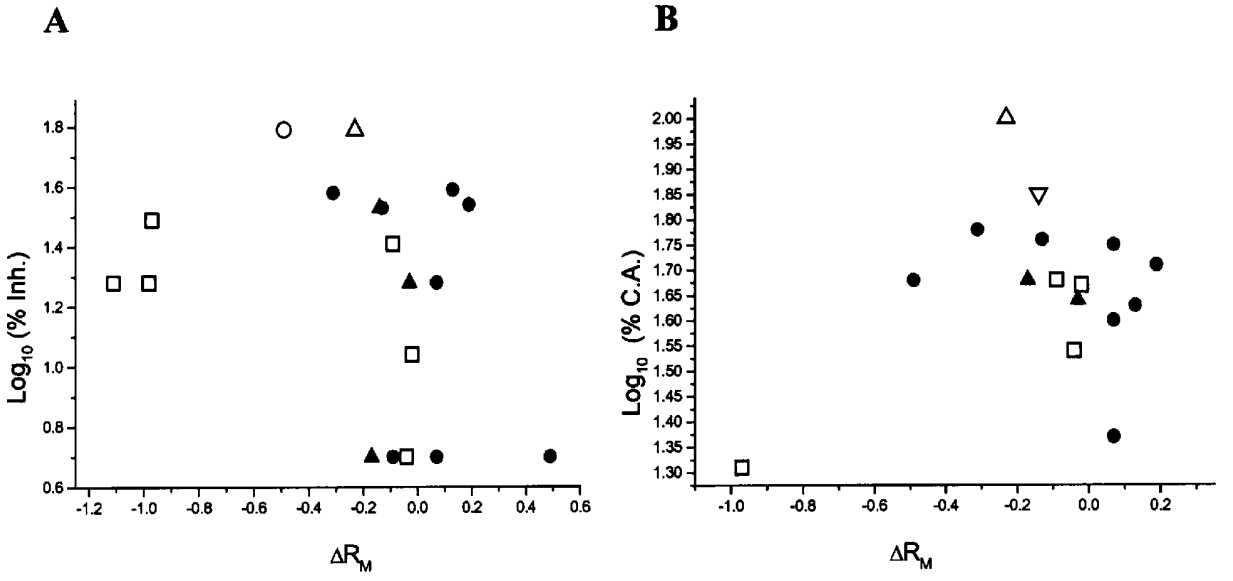


Figure 3. Lipophilicity *vs* anti-protozoa activity of studied compounds. Imidazole *N*-oxide derivatives (□), benzimidazole *N*-oxide derivatives (l, ○ derivative **4c**), *O*-substituted benzimidazole *N*-oxide derivatives (▲, △ derivative **7c**, ▽ derivative **7b**). **A.** ΔR_M *vs* trypanocidal *in vitro* activity. **B.** ΔR_M *vs* trichomonacidal *in vitro* activity.

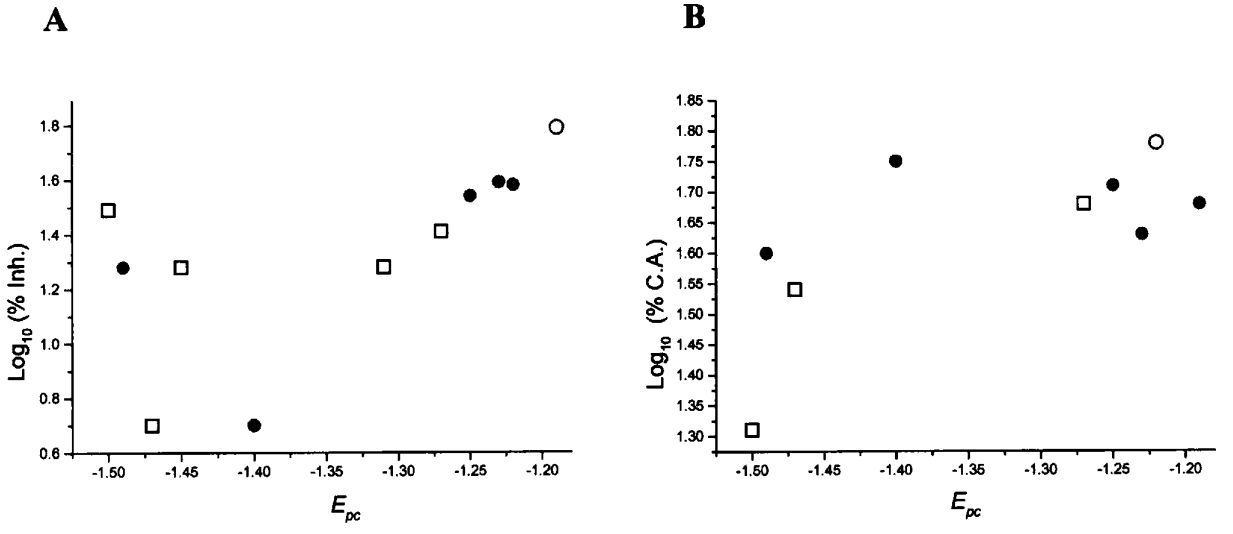
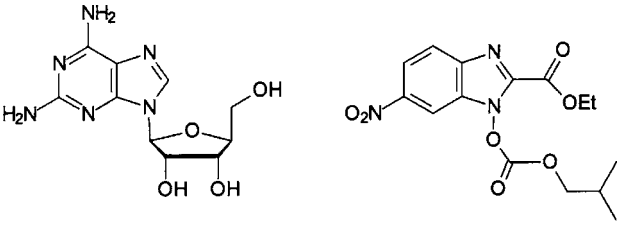


Figure 4. Electrochemical properties *vs* anti-protozoa activity of studied compounds. Imidazole *N*-oxide derivatives (□), benzimidazole *N*-oxide derivatives (l, ○ derivative **4c**). **A.** E_{pc} *vs* trypanocidal *in vitro* activity. **B.** E_{pc} *vs* trichomonacidal *in vitro* activity.

are less favourable than for the reference compounds Nfx and Mtz [24, 26, 28]. Statistic correlation between E_{pc} and bioactivities were not obtained but a clear tendency was observed (Figure 4). Compounds bearing more negative first reduction potentials showed poor activity against both protozoa.



8 **7c**

Figure 5. Trypanosomatidae glycolysis inhibitor **8** and derivative **7c**.

Statistically significant multiple correlations between physicochemical properties studied and the anti-protozoa activities were not obtainable.

On the other hand, some of the more active derivatives developed do not have the *N*-oxide moiety. Derivatives **7b** and **7c** showed high activity toward both assayed protozoa, thus probably the lipophilic properties could be related with the bio-response. The increment in lipophilicities of derivatives **7a**–**d** (compare their ΔR_M with that of derivative **3a**, Table 3) allow them access to the core of protozoa where the carbonate moiety is hydrolyzed and they are transformed to the corresponding *N*-oxide. Furthermore, the structures of derivatives **7a**–**7d** have the structure of adenosine derivatives developed as trypanosomatidae glycolysis inhibitors [29-31] (i.e. **8**, Figure 5). This mechanism of action could be the form how compounds **7a**–**d** display their activities. Further studies of enzymatic *O*-substituted benzimidazole *N*-oxide derivatives' inhibition should be performed.

These compounds are interesting leading substances for further chemical modifications and biological studies. In fact, other studies allowing the elucidation of the reaction mechanisms of these drugs as well as schedules for toxicity determinations are currently in progress.

Acknowledgments

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Experimental

Chemistry

All starting materials were commercially available research-grade chemicals and used without further purification. The compounds **I**, **VI**, **IX**, **X**, **2a**, **3a**, **3d**, **6**, and 4-chloro-3-nitrobenzoic acid were prepared according to literature procedures [13, 15, 16, 32–37]. All solvents were dried and distilled prior to use. All the reactions were carried out in a nitrogen atmosphere. The typical work-up included washing with brine and drying the organic layer with magnesium sulphate before concentration. Melting points were determined using a Leitz Microscope Heating Stage Model 350 apparatus (Wetzlar, Germany) and are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorous pentoxide at 3-4 mm Hg, 24 h at room temperature) and performed on a Fisons EA 1108 CHNS-O analyzer (Valencia, USA). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 (at 400 MHz and 100 MHz, (Rehinstetten, Germany)) instrument, with tetramethylsilane as the internal reference and in the indicated solvent; the chemical shifts are reported in ppm. Mass spectra were recorded on a Shimadzu GC-MS QP 1100 EX instrument (Kyoto, Japan) at 70 eV.

1-(4-Methylphenylamino)propanone oxime ***II***

A mixture of chloroacetone (2.0 g, 21 mmol), *p*-toluidine (2.3 g, 21 mmol), K_2CO_3 (2.9 g, 21 mmol), KI (catalytic amounts) in acetone (50 mL) was heated at reflux during 10 h. The mixture was concentrated *in vacuo* and treated with Et_2O. After the work-up process the residue, 1.4 g (40%), was used in the next step without purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.22 (s, 6H), 3.97 (s, 2H), 4.43 (bs, 1H), 6.52 (d, J = 8.3 Hz, 2H), 6.98 (d, J = 8.3 Hz, 2H). The corresponding α-amine carbonyl derivative (0.16 g, 1 mmol), $NH_2OH.HCl$ (0.07 g, 1 mmol), and anhydrous AcONa (0.13 g, 1 mmol) in methanol (5 mL) were heated at reflux during 1 h and then stirred at room temperature for 12 h. The mixture was concentrated *in vacuo* and treated with EtOAc. After the work-up process the residue was used without further purification (0.12 g, 70%).

A mixture of **I** (1.0 equiv.), the corresponding amine (1.5 equiv.), $ZnCl_2$ (catalytic amounts), methanol as solvent and molecular sieve (4 Å) was stirred for 24 h at room temperature. Then $NaCNBH_3$ (1.0 equiv) was added and agitation was continued for 48 h. The mixture was concentrated *in vacuo* and treated with EtOAc. After the work-up process the residue was purified by chromatographic column (SiO_2, EtOAc:petroleum ether (1:1)).

General procedure for the synthesis of α*-substituted amine-oxime* ***III***–***V***

3-(4-Methoxyphenylamino)-2-butanone oxime ***III***

Oil, 0.66 g (32%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.38 (d, J = 6.8 Hz, 3H), 1.84 (s, 3H), 3.40 (bs, 1H), 3.75 (s, 3H), 4.07 (q, J = 6.8 Hz, 1H), 6.68 (m, 2H), 6.77 (m, 2H), 9.92 (bs, 1H).

3-(2-Phenylethylamino)-2-butanone oxime ***IV***

Oil, 0.98 g (48%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.22 (d, J = 6.7 Hz, 3H), 1.78 (s, 3H), 2.80 (m, 4H), 3.45 (q, J = 6.7 Hz, 1H), 7.22 (m, 3H), 7.28 (m, 2H).

3-(2-Furylmethylamino)-2-butanone oxime ***V***

Oil, 0.86 g (46%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.21 (d, *J* = 6.8 Hz, 3H), 1.84 (s, 3H), 3.43 (q, *J* = 6.8 Hz, 1H), 3.69 (s, 2H), 5.74 (bs, 2H), 6.17 (m, 1H), 6.29 (m, 1H), 7.34 (m, 1H), 10 (bs, 2H).

General Procedure for the Synthesis of Imidazole N-Oxide ***2b*** *–* ***2f***

A mixture of oximes **II-V**, *p*-TsOH (catalytic amounts) and the corresponding orthoester as solvent was heated at reflux until the oxime was not present (SiO_2, MeOH:EtOAc (4:6)). The mixture was concentrated *in vacuo* and treated with EtOAc. After the work-up process the residue was purified as indicate.

4-Methyl-1-(4-methylphenyl)imidazole N^3-oxide ***2b***

Chromatographic column (SiO_2, EtOAc:MeOH (0 to 40%)); beige solid (52%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.32 (s, 3H), 2.40 (s, 3H), 6.89 (s, 1H), 7.20 (d, *J* = 8.5 Hz, 2H), 7.29 (d, *J* = 8.5 Hz, 2H), 8.17 (s, 1H). ^{13}C RMN (HMQC, HMBC) ($CDCl_3$, 100 MHz) δ (ppm): 8.50, 21.34, 112.76, 121.04, 124.44, 131.12, 132.26, 135.00, 138.78. MS *m/z* (rel. int.%): 188 ($M^{\bullet+}$, 40.0), 172 (8.0). mp(°C) 202.0 – 203.0. *Anal.* Calcd for $C_{11}H_{12}N_2O$: C, 70.19; H, 6.43; N, 14.88. Found: C, 70.02; H, 6.27; N, 14.48.

4,5-Dimethyl-1-(4-methoxyphenyl)imidazole N^3-oxide ***2c***

Chromatographic column (SiO_2, EtOAc:MeOH (0 to 40%)); beige oil (20%). ^{1}H NMR (methanol-d_4, 400 MHz) δ (ppm): 2.11 (s, 3H), 2.24 (s, 3H), 3.88 (s, 3H), 7.11 (d, *J* = 6.8 Hz, 2H), 7.36 (d, *J* = 6.8 Hz, 2H), 8.32 (s, 1H). ^{13}C NMR (HMQC, HMBC) (methanol-d_4, 100 MHz) δ (ppm): 6.13, 8.04, 55.18, 115.03, 123.70, 126.30, 127.57, 127.74, 129.09, 161.08. MS *m/z* (rel. int.%): 218 (M^+, 100.0), 202 (49.0), 148 (72.0), 107 (15.0). *Anal.* Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84. Found: C, 65.89; H, 6.38; N, 12.50.

4,5-Dimethyl-1-(2-phenylethyl)imidazole N^3-oxide ***2d***

Chromatographic column (SiO_2, EtOAc:MeOH (0 to 40%)); brown oil (6%). ^{1}H NMR (methanol-d_4, 400 MHz) δ (ppm): 2.04 (s, 3H), 2.18 (s, 3H), 3.00 (t, *J* = 7.3 Hz, 2H), 4.05 (t, *J* = 7.3 Hz, 2H), 7.28 (m, 5H), 7.36 (s, 1H). ^{13}C NMR (HMQC, HMBC) (methanol-d_4, 100 MHz) δ (ppm): 8.56, 12.60, 37.95, 56.14, 122.50, 127.44, 129.04, 129.07, 132.50, 134.81, 137.70. MS *m/z* (rel. int.%): 200 ($M^{+\cdot}$ -16, 100.0), 138 (28.0), 111 (42.0), 105 (47.0). *Anal.* Calcd for $C_{13}H_{16}N_2O$: C, 72.19; H, 7.46; N, 12.95. Found: C, 71.98; H, 7.29; N, 12.72.

2,4,5-Trimethyl-1-(2-phenylethyl)imidazole N^3-oxide ***2e***

Chromatographic column (SiO_2, EtOAc:MeOH (0–40%)); brown oil (54%). ^{1}H NMR (methanol-d_4, 400 MHz) δ (ppm): 2.00 (s, 3H), 2.10 (s, 6H), 2.87 (t, *J* = 8.0 Hz, 2H), 3.91 (t, *J* = 8.0 Hz, 2H), 7.03 (d, *J* = 7.4 Hz, 2H), 7.26 (m, 3H). ^{13}C NMR (HMQC, HMBC) (methanol-d_4, 100 MHz) δ (ppm): 9.10, 12.71, 13.26, 37.33, 45.80, 121.78, 127.30, 129.13, 129.19, 131.50, 138.07, 142.59. MS *m/z* (rel. int.%): 214 ($M^{+\cdot}$ -16, 98.0), 123 (100.0), 104 (31.0), 82 (21.0). *Anal.* Calcd for $C_{14}H_{18}N_2O$: C, 73.01; H, 7.88; N, 12.16. Found: C, 72.66; H, 7.57; N, 11.95.

2,4,5-Trimethyl-1-(furylmethyl)imidazole N^3-oxide ***2f***

Chromatographic column (SiO_2, EtOAc:MeOH (0 to 40%)); orange oil (42%). ^{1}H NMR (methanol-d_4, 400 MHz) δ (ppm): 2.11 (s, 3H), 2.13 (s, 3H), 2.39 (s, 3H), 4.87 (s, 2H), 6.10 (d, *J* = 3.4 Hz, 1H), 6.29 (d, *J* = 3.4 Hz, 1H), 7.33 (d, *J* = 3.4 Hz, 1H). ^{13}C NMR (HMQC, HMBC) (methanol-d_4, 100 MHz) δ (ppm): 9.17, 12.71, 13.52, 41.07, 107.99, 110.78, 122.23, 131.58, 142.96, 143.03, 150.17. MS *m/z* (rel. int.%): 190 ($M^{+\cdot}$ -16, 18.0), 109 (6.0), 81 (100.0). *Anal.* Calcd for $C_{11}H_{14}N_2O_2$: C, 64.06; H, 6.84; N, 13.58. Found: C, 63.70; H, 6.90; N, 13.22.

Diethyl 3-oxide-1H-benzimidazole-2,5-dicarboxylate ***3b***

Ethyl 4-chloro-3-nitrobenzoate: A mixture of 4-chloro-3-nitrobenzoic acid (4.15 g, 20.6 mmol), H_2SO_4 (c) (0.3 mL, 6.2 mmol) and EtOH (20 mL) was heated at reflux for 4 h. The mixture was concentrated in vacuo and the solid residue was washed with water (4.85 g, 100%).

Ethyl N-(4-ethoxycarbonyl-2-nitrophenyl)glycinate ***VII***

A mixture of ethyl 4-chloro-3-nitrobenzoate (4.00 g, 17.5 mmol), ethyl glycinate hydrochloride (2.45 g, 17.5 mmol) and $NaHCO_3$ (2.93 g, 34.9 mmol) in EtOH (20 mL) was heated at reflux during 6 h. The mixture was concentrated in vacuo and treated with EtOAc/H_2O. The aqueous phase was acidified and treated with EtOAc. After the work-up process the residue was crystallized from EtOH (0.5 g, 10%).

Diethyl 3-oxide-1H-benzimidazole-2,5-dicarboxylate ***3b***

Sodium (40.4 mg, 1.8 mmol) was dissolved in EtOH (10 mL) and then **VII** (200 mg, 0.67 mmol) was added. The mixture was stirred at room temperature for 12 h. Then it was concentrated in vacuo and treated with Et_2O/H_2O. The aqueous phase was acidified. The solid was filtered and crystallized from EtOH (148 mg, 79%). ^{1}H NMR ($CDCl_3$, 400 MHz) d (ppm) (main tautomer): 1.46 (t, *J* = 7.1 Hz, 3H), 1.54 (t, *J* = 7.1 Hz, 3H), 4.46 (q, *J* = 7.1 Hz, 2H), 4.61 (q, *J* = 7.1 Hz, 2H), 7.86 (d, *J* = 8.7 Hz, 1H), 8.08 (d, *J* = 8.7 Hz, 1H), 8.39 (s, 1H), 11.00 (bs, 1H). ^{13}C RMN (HMQC, HMBC) ($CDCl_3$, 100 MHz) d (ppm) (main tautomer): 14.50, 14.74, 61.75, 64.14, 113.17, 122.04, 125.49, 128.51, 130.00, 134.66, 140.26, 162.55, 166.57. MS *m/z* (rel. int.%): 278 ($M^{\cdot+}$, 0.1), 206 (59.0), 178 (25.0), 161 (100.0). mp(°C) 189.9 – 190.0. Anal. Calcd for $C_{13}H_{14}N_2O_5$: C, 56.11; H, 5.07; N, 10.07. Found: C, 56.02; H, 5.20; N, 9.87.

Ethyl 5-methyl-3-oxide-1H-benzimidazole-2-carboxylate ***3c***

Ethyl N-(4-methyl-2-nitrophenyl)glycinate ***VIII***

A mixture of 4-methyl-2-nitroaniline (1.00 g, 6.6 mmol), ethyl 2-bromoacetate (0.73 mL, 6.6 mmol) and $NaHCO_3$ (1.10 g, 13.2 mmol) in acetone (10 mL) was heated at reflux during 6 d. The mixture was concentrated in vacuo and treated with EtOAc/H_2O. After the work-up process the residue was purified by chromatography (SiO_2, petroleum ether:EtOAc (9:1)). Orange solid (0.48 g, 30%).

Ethyl 5-methyl-3-oxide-1H-benzimidazole-2-carboxylate ***3c***

Sodium (88.1 mg, 3.8 mmol) was dissolved in EtOH (7.3 mL) and then **VIII** (176.2 mg, 0.74 mmol) in DMF (0.44 mL) was added. The mixture was stirred at room temperature during 4 h. Then it was concentrated in vacuo and treated with Et_2O/H_2O. The aqueous phase was acidified and extracted with EtOAc. After the work up the organic phase was purified by chromatography (SiO_2, petroleum ether:EtOAc (8:2)). Red solid (28.5 mg, 18%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) (main tautomer): 1.49 (t, J=6.9 Hz, 3H), 2.50 (s, 3H), 4.55 (q,

J=6.0 Hz, 2H), 7.19 (d, J=8.0 Hz, 1H), 7.55 (d+s, J=7.6 Hz, 2H), 9.40 (bs, 1H). ^{13}C RMN (HMQC, HMBC) ($CDCl_3$, 100 MHz) δ (ppm) (main tautomer): 14.57, 22.05, 64.20, 113.04, 126.81, 126.97, 128.30, 128.93, 138.36, 147.52, 153.02. mp(°C) 221.0 – 222.0. Anal. Calcd for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 59.73; H, 5.33; N, 12.83.

1-Hydroxy-2,5-dimethyl-3-oxide-benzimidazole ***3e***

Compound **X** (1.0 equiv.) was dissolved in THF (7.3 mL) and then nitromethane (1.2 equiv.) and piperidine (1.2 equiv.) were added. The mixture was stirred at room temperature during 12 h. Then the solid was collected by filtration and crystallized from MeOH. White solid (34%). It was not possible to acquire NMR spectra due to the insolubility of **3e** in the assayed solvents ($CDCl_3$, CD_3OD, DMSO-d_6, and D_2O). MS *m/z* (rel. int.%): 178 ($M^{\cdot+}$, 11.0), 177 (32.0), 145 (32.0), 131 (8.0). mp(°C) 224.6 – 225.6 (d). Anal. Calcd for $C_9H_{10}N_2O_2$: C, 60.66; H, 5.66; N, 15.72. Found: C, 61.00; H, 6.02; N, 15.89.

General procedure for the synthesis of derivatives ***4a–4c***

A mixture of **3a**, catalytic amounts of *p*-TsOH, molecular sieves (4 Å) and the corresponding alcohol (*i*-PrOH, *n*-butanol, and *n*-hexanol) as solvent was heated at reflux during the time indicate below. The solvent was distilled *in vacuo*. After the work-up the residue was purified by chromatography (SiO_2, petroleum ether:EtOAc).

Isopropyl 5-nitro-3-oxide-1H-benzimidazole-2-carboxylate ***4a***

Time reflux: 4 h. Brown solid (72%). ^{1}H NMR (DMSO-d_6:D_2O, 400 MHz) d (ppm) (main tautomer): 1.39 (d, *J* = 6.2 Hz, 6H), 5.27 (h, *J* = 6.2 Hz, 1H), 7.96 (d, *J* = 9.0 Hz, 1H), 8.17 (dd, J_1 = 9.0 Hz, J_2 = 2.2 Hz, 1H), 8.40 (d, *J* = 2.2 Hz, 1H). ^{13}C NMR (HMQC, HMBC) ($CDCl_3$, 100 MHz) δ (ppm) (main tautomer): 22.35, 71.08, 108.03, 119.16, 122.84, 132.84, 141.49, 143.12, 145.46, 157.81. MS *m/z* (rel. int.%): 265 ($M^{\cdot+}$, 20.3), 249 (4.2), 223 (100.0), 205 (80.8). mp(°C) 172.8 – 173.4. *Anal.* Calcd for $C_{11}H_{11}N_3O_5$: C, 49.81; H, 4.18; N, 15.84. Found: C, 49.55; H, 3.99; N, 15.60.

n-Butyl 5-nitro-3-oxide-1H-benzimidazole-2-carboxylate ***4b***

Time reflux: 1 h. Yellow solid (68%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) (main tautomer): 1.03 (t, *J* = 7.4 Hz, 3H), 1.55 (m, 2H), 1.91 (m, 2H), 4.61 (t, *J* = 6.8 Hz, 2H), 7.96 (d, *J* = 9.1 Hz, 1H), 8.27 (dd, J_1 = 9.1 Hz, J_2=2.2 Hz, 1H), 8.62 (d, *J* = 2.2 Hz, 1H), 9.00 (bs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) (main tautomer): 13.97, 19.39, 30.77, 68.37, 108.02, 119.65, 123.10, 129.30, 136.00, 140.87, 146.08, 162.50. MS *m/z* (rel. int.%): 279 ($M^{\cdot+}$, 14.0), 262 (59.0), 223 (75.0), 205 (100.0). mp(°C) 189.2 – 190.0. *Anal.* Calcd for $C_{12}H_{13}N_3O_5$: C, 51.61; H, 4.69; N, 15.05. Found: C, 51.43; H, 4.35; N, 14.78.

n-Hexyl 5-nitro-3-oxide-1H-benzimidazole-2-carboxylate ***4c***

Time reflux: 3 h. Brown solid (61%). ^{1}H NMR (DMSO-d_6: D_2O, 400 MHz) d (ppm) (main tautomer): 0.86 (t, *J* = 7.0 Hz, 3H), 1.25 (m, 2H), 1.30 (m, 2H), 1.41 (m, 2H), 1.74 (quint, *J* = 7.0 Hz, 2H), 4.38 (t, *J* = 6.6 Hz, 2H), 7.96 (d, *J* = 9.0 Hz, 1H), 8.16 (dd, J_1 = 9.0 Hz, J_2 = 2.2 Hz, 1H), 8.40 (d, *J* = 2.1 Hz, 1H). MS *m/z* (rel. int.%): 307 (M^+, 4.5), 291 (18.6), 290 (100.0), 277 (20.8). mp(°C) 160.2 – 161.8. *Anal.* Calcd for $C_{14}H_{17}N_3O_5$: C, 54.72; H, 5.58; N, 13.67. Found: C, 54.31; H, 5.23; N, 13.44.

N-(n-Butyl) 5-nitro-3-oxide-1H-benzimidazole-2-carboxamide ***5***

A mixture of **3a** (50 mg, 0.20 mmol), *n*-butylamine (0.02 mL, 0.20 mmol), *p*-TsOH (catalytic amounts), molecular sieves (4 Å) and toluene (5.0 mL) as solvent was heated at reflux for 12 h. After the work-up, the organic phase was purified by chromatography (SiO_2, petroleum ether: EtOAc (9:1)). Brown solid (10.5 mg, 19%). Ratio of tautomers 5:5′ in $CDCl_3$ at 30°C was 55:45. Tautomer 5: ^{1}H NMR ($CDCl_3$, 30°C) δ (ppm): 1.02 (t, J=7.3 Hz, 3H), 1.51 (sext, *J* = 7.5 Hz, 2H), 1.75 (quint, J=7.0 Hz, 2H), 3.62 (q, *J* = 7.0 Hz, 2H), 7.68 (d, *J* = 9.0 Hz, 1H), 7.74 (bs, 1H), 8.33 (dd, J_1 = 9.0 Hz, J_2 = 2.0 Hz, 1H), 8.74 (d, J=2.0 Hz, 1H), 12.07 (bs, 1H); ^{13}C NMR ($CDCl_3$, 30 sC) δ (ppm): 14.06, 20.49, 31.91, 40.12, 112.86, 117.87, 120.76, 138.61, 142.62, 144.94, 148.68, 158.91. Tautomer 5′: ^{1}H NMR ($CDCl_3$, 30 sC) δ (ppm): 1.02 (t, *J* = 7.3 Hz, 3H), 1.51 (sext, J=7.5 Hz, 2H), 1.73 (quint, *J* = 7.0 Hz, 2H), 3.60 (q, *J* = 7.0 Hz, 2H), 7.74 (bs, 1H), 7.88 (d, *J* = 9.0 Hz, 1H), 8.27 (dd, J_1 = 9.0 Hz, J_2 = 2.2 Hz, 1H), 8.57 (d, *J* = 2.0 Hz, 1H), 12.31 (bs, 1H); ^{13}C NMR ($CDCl_3$, 30°C) δ (ppm): 14.06, 20.49, 31.91, 40.12, 109.81, 119.24, 121.21, 131.00, 145.53, 147.30, 149.45, 158.91. MS *m/z* (rel. int.%): 278 (M^+, 22.0), 262 (20.2), 261 (72.2), 249 (11.4), 235 (18.5). mp(°C) 206.7 – 207.6. Anal. Calcd for $C_{12}H_{14}N_4O_4$: C, 51.80; H, 5.07; N, 20.13. Found: C, 51.48; H, 5.00; N, 19.98.

General Procedure for the synthesis of derivatives ***7a–7d***

A mixture of **3a** (50.0 mg, 0.21 mmol), the corresponding electrophile reactants (methyl iodide, ethyl chloroformate, *i*-butyl chloroformate, 2,2,2-trichloroethyl chloroformate, 1.0 equiv.), $NaHCO_3$ (only when CH_3I was used as electrophile) (2.0 equiv.) and acetone (3.0 mL) as solvent, was stirred at room temperature for the time indicated below. The solvent was distilled *in vacuo*. After the work-up the residue was purified by chromatography (SiO_2, petroleum ether:EtOAc).

Ethyl 1-methoxy-6-nitrobenzimidazole-2-carboxylate ***7a***

Time: 7 d. Yellow solid (100%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.53 (t, J=7.0 Hz, 3H), 4.38 (s, 3H), 4.59 (q, *J* = 7.1 Hz, 2H), 7.97 (d, *J* = 9.0 Hz, 1H), 8.27 (d, *J* = 9.0 Hz, 1H), 8.52 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 14.57, 63.47, 68.08,107.10, 119.80, 123.31, 131.00, 134.00, 141.50, 146.00, 158.00. MS *m/z* (rel. int.%): 265 (M^+, 100.0), 248 (3.0), 190 (62.0). mp(°C) 155.0 – 156.0. *Anal.* Calcd for $C_{11}H_{11}N_3O_5$: C, 49.81; H, 4.18; N, 15.84. Found: C, 50.02; H, 4.52; N, 15.55.

Ethyl 1-ethyloxycarbonyloxy-6-nitrobenzimidazole-2-carboxylate ***7b***

Time: 30 min. Yellow solid (30%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.49 (t, *J* = 7.1 Hz, 3H), 1.53 (t, *J* = 7.1 Hz, 3H), 4.56 (q, J=7.1 Hz, 4H), 8.02 (d, *J* = 9.0 Hz, 1H), 8.31 (dd, J_1= 9.0 Hz, J_2 = 2.1 Hz, 1H), 8.44 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 14.47, 14.50, 63.69, 68.86,106.88, 120.14, 123.50, 131.31, 133.00, 140.85, 146.74, 152.69, 157.24. MS m/z (rel. int.%): 323 (M^+, 1.0), 307 (0.2), 279 (5.0), 251 (53.0). mp(°C) 133.3 – 134.3. Anal. Calcd for $C_{13}H_{13}N_3O_7$: C, 48.30; H, 4.05; N, 13.00. Found: C, 47.98; H, 3.92; N, 12.78.

Ethyl 1-(i-butyloxycarbonyloxy)-6-nitrobenzimidazole-2-carboxylate ***7c***

Time: 60 min. Yellow solid (74%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.08 (d, *J* = 6.7 Hz, 3H), 1.50 (t, *J* = 7.1 Hz, 3H), 2.19 (m, *J* = 6.7 Hz, 1H), 4.28 (d, *J* = 6.6 Hz, 2H), 4.56 (q,

J = 7.1 Hz, 2H), 8.03 (d, *J* = 9.0 Hz, 1H), 8.31 (dd, J_1 = 9.0 Hz, J_2 = 2.1 Hz, 1H), 8.44 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 14.54, 19.04, 28.27, 63.69, 78.44, 106.85, 120.13, 123.52, 131.33, 140.91, 146.75, 148.88, 152.85, 157.23. MS *m/z* (rel. int.%): 351 (M^+, 0.1), 336 (9.0), 251 (100.0). mp(°C) 112.5 – 113.0. *Anal.* Calcd for $C_{15}H_{17}N_3O_7$: C, 51.28; H, 4.88; N, 11.96. Found: C, 50.99; H, 4.65; N, 11.65.

Ethyl 1-(2,2,2-trichloroethyloxycarbonyloxy)-6-nitrobenzimidazole-2-carboxylate ***7d***

Time: 10 min. Yellow solid (76%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.51 (t, *J* = 7.2 Hz, 3H), 4.57 (q, *J* = 7.2 Hz, 2H), 5.05 (s, 2H), 8.06 (d, *J* = 9.0 Hz, 1H), 8.35 (dd, J_1 = 9.0 Hz, J_2 = 2.1 Hz, 1H), 8.45 (d, *J* = 2.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 14.51, 63.88, 79.34, 93.34, 106.65, 120.43, 123.73, 131.07, 140.54, 140.78, 146.95, 152.20, 157.18. mp(°C) 121.3 – 121.8. *Anal.* Calcd for $C_{13}H_{10}Cl_3N_3O_7$: C, 36.60; H, 2.36; N, 9.85. Found: C, 36.20; H, 2.15; N, 9.52.

Biology

Anti-trypanosomal bioassays

T. cruzi epimastigotes (Tulahuen 2 strain) were grown at 28°C in an axenic medium (BHI-Tryptose), complemented with 10% foetal calf serum. Cells from a 5-day old culture were inoculated into 50 mL of fresh culture medium to give an initial concentration of $1{\cdot}10^6$ cells/mL. Cell growth was followed by measuring the absorbance of the culture at 600 nm daily for 11 d. Before inoculation, the media was supplemented with 25 μM or 100 μM of compounds from a stock solution in DMSO and their ability to inhibit growth of the parasite was evaluated in comparison to the control (no drug added to the media). The final concentration of DMSO in the culture media never exceeded 0.4% and the control was run in the presence of 0.4% DMSO and in the absence of any drug. No effect on epimastigotes growth was observed by the presence of up to 1% DMSO in the culture media. Nfx was used as reference drug.

Anti-trichomonas bioassays

Trichomonacidal *in vitro* activity was developed using recently isolated strain JH31A no. 4 of *T. vaginalis* in TYM medium (Trypticase-Yeast extract-Maltose) supplemented with 10% of heat-inactivated equine serum at 37°C with 5% CO_2. The assays were carried out using glass tubes containing 100,000 protozoa/mL in a final volume of 2 mL. Compounds were added to the cultures 6 h after seeding. For each concentration assayed, there were three experimental and six growth controls. Viable protozoa were assessed 24 and 48 h after incubation in the presence of the compounds by counting in an haemocytometer. Mtz (Rhone Poulenc, Courbevoie, France) was used as reference drug.

Electrochemical method

Voltammetric responses for derivatives were obtained by cyclic voltammetry. Experiments were carried out using a BAS-Epsilon EC (Bioanalytical Systems, Inc., West Lafayette, USA) instrument in a BAS C3 cell, in *N,N*-dimethylformamide (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, a platinum wire auxiliary electrode, and a saturated calomel reference electrode. Voltage scan rates ranged from 0.05-1.0 V/s.

Lipophilicity studies

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, dried and the spots were located under UV light. The R_f values were averaged from two to three determinations, and converted into R_M.

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