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Novel 3-nitro-1*H*-1,2,4-triazole-based piperazines and 2-amino-1,3-benzothiazoles as antichagasic agents

Maria V. Papadopoulos^{a,*}, William D. Bloomer^a, Howard S. Rosenzweig^b, Marcel Kaiser^{c,d}, Eric Chatelain^e, and Jean-Robert Ioset^e

^aNorthShore University HealthSystem, Evanston, IL, US ^bOakton Community College, Des Plaines, IL, US ^cSwiss Tropical and Public Health Institute, Parasite Chemotherapy, Basel, Switzerland ^dUniversity of Basel, Basel, Switzerland ^eDrugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland

Abstract

We have previously shown that 3-nitro-1*H*-1,2,4-triazole-based amines demonstrate significant trypanocidal activity, in particular against *T. cruzi*, the causative parasite of Chagas disease. In the present work we further expanded our research by evaluating *in vitro* the trypanocidal activity of nitrotriazole-based piperazines and nitrotriazole-based 2-amino-1,3-benzothiazoles to establish additional SARs. All nitrotriazole-based derivatives were active or moderately active against *T. cruzi*; however two of them did not fulfill the selectivity criteria. Five derivatives were active or moderately active against *T.b. rhodesiense* while one derivative was moderately active against *L. donovani*. Active compounds against *T. cruzi* demonstrated selectivity indexes (toxicity to host cells/toxicity to *T. cruzi* amastigotes) from 117-1725 and 12 of 13 compounds were up to 39-fold more potent than the reference compound benznidazole. Detailed SARs are discussed.

Keywords

nitrotriazoles; piperazines; benzothiazoles; Chagas disease; antitrypanosomal agents

1. Introduction

The trypanosomatid protozoan parasites *Trypanosoma cruzi* (*T. cruzi*), *Trypanosoma brucei* (*T. brucei*), and various *Leishmania* species are the causative agents of American trypanosomiasis (Chagas disease), human African trypanosomiasis (HAT) and various forms of leishmaniasis, respectively. Over 20 million people are infected by these parasites, resulting in 100,000 deaths per year [1]. Chagas disease, one of the most neglected diseases, is endemic mainly in Latin America and occurs in two phases: acute and chronic. The most

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*Correspondence to: Maria V. Papadopoulos, Ph.D., NorthShore University HealthSystem, Department of Radiation Medicine, 2650 Ridge Ave., Evanston IL, 60201, USA. Tel: (847)570-2262; Fax: (847)570-1878, mpapadopoulos@northshore.org.

‡**Abbreviations:** *T. cruzi*, *Trypanosoma cruzi*; *T. brucei*, *Trypanosoma brucei*; HAT, human African trypanosomiasis; Nfx, nifurtimox (4-(5-nitrofurfurylindenamino)-3-methylthio-morpholine-1,1-dioxide); Bnz, benznidazole (N-benzyl-2-(2-nitro-1*H*-imidazol-1-yl)acetamide); NTR, type I nitroreductase; TbNTR, *T. brucei* NTR; IC₅₀, concentration for 50% growth inhibition; SI, selectivity index; SARs, structure-activity relationships; TDR, Tropical diseases research (<http://www.who.int/tdr/en/>).

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important clinical manifestations of the chronic form of Chagas disease are heart insufficiency (Chagasic cardiopathy and arrhythmias, 90% of cases approximately) and gastrointestinal syndromes (megaesophagus and megacolon). It is estimated that around 100 million people are at risk of infection with *T. cruzi* in endemic areas in Latin America [2]. Despite the fact that the number of incidences has significantly declined in the past 20 years, primarily due to vector control initiatives, the number of cases in non-endemic regions (United States, Australia, Europe and Japan) is rising [3-5]. Reasons for this include population migration, illegal drug usage and medical practices. With no immediate prospect for vaccines, chemotherapy is the only current method to treat patients affected with Chagas disease.

The existing drugs for Chagas, nifurtimox (a nitrofurantoin; Nfx) and benznidazole (a 2-nitroimidazole; Bnz) (Chart 1) are old, have limited efficacy, significant side effects and some strains are refractory to treatment [6,7]. Recently, inhibitors of the sterol 14 - demethylase enzyme (CYP51), which is part of the ergosterol biosynthesis pathway, represent one form of new treatments under development as effective antichagasic agents [8], though this mechanism of action still remains to be clinically validated. Moreover, the high cost of these inhibitors may limit their use in poor countries where the disease is most prevalent [9]. Therefore, the need for new, affordable and safer drugs to treat Chagas disease is urgent.

We are interested in the synthesis and development of safer nitroheterocyclics as potential antichagasic agents [10,11]. Nitroheterocyclics function as prodrugs and must undergo activation before mediating their cytotoxic effects. It was demonstrated that an oxygen-insensitive, type I nitroreductase (NTR), present in trypanosomes and absent from most other eukaryotes, is responsible for Nfx and Bnz trypanocidal activity [12-14]. Via a series of 2 electron reduction reactions, this enzyme is involved in the production of toxic metabolites which can selectively kill trypanosomatids [15]. The NTR-mediated activation of nitroheterocyclic prodrugs, which occurs specifically in the trypanosomatids, has led to a renewed interest in the use of such compounds as antiparasitic agents [16-24].

We have recently reported that 3-nitro-1*H*-1,2,4-triazole-based amines, amides and sulfonamides demonstrate excellent activity against *T. cruzi* amastigotes in infected L6 cells with no toxicity towards the host cells [10,11]. The IC₅₀ values of these compounds against the intracellular parasite ranged from low nM to less than 4 μ M and have selectivity indices ranging from 66 to 2682. In addition, several of these compounds were up to 56 fold more active than the reference drug benznidazole (Bnz), tested in parallel. We have also shown that such nitrotriazoles are activated by the type I NTR and that *T.b. brucei* parasites overexpressing the enzyme are hypersensitive to these compounds [10,11]. Interestingly, in preliminary *in vivo* studies, we found that treatment of *T. cruzi*-infected mice with one nitrotriazole-based aromatic amine, NTLA-1 [25], given at just 2 mg/kg/day $\times$ 50 days, resulted in a rapid and persistent drop in peripheral parasite levels and in a fraction of cures [26,27]. More importantly, there was an absolute correlation between treatment efficacy as determined parasitologically and the increase in the fraction of *T. cruzi*-specific CD8⁺ T cells with a T central memory phenotype in the peripheral blood of treated mice [26, 27]. More recently, representative nitrotriazoles of different chemical classes have been evaluated for *in vivo* efficacy by using a fast luminescence assay in mice infected with transgenic parasites that express luciferase [28]. Most of the tested compounds demonstrated *in vivo* antichagasic activity in this assay, and at least three of them were identified with superior efficacy to benznidazole [29]. In addition, several nitrotriazole-based derivatives were tested in the Ames test, and in contrast to their 2-nitroimidazole analogs, were not mutagenic, at least at non-toxic concentrations [29].

In the present study we have further investigated into two subclasses of 3-nitrotriazole-based amines as antitrypanosomal agents: piperazines and 2-amino-1,3-benzothiazoles. We have synthesized eleven novel piperazine-based derivatives and six 2-amino-1,3-benzothiazoles. For comparison purposes, and to confirm our previous findings, a small number of 2-nitroimidazole-based analogs were included in the above numbers. The synthesis and *in vitro* evaluation of the compounds as antitrypanosomal agents is described and SARs are discussed.

2. Results and Discussion

2.1. Chemistry

The structures of all compounds are depicted on Table 1. Their synthesis is straightforward and based on well-established chemistry, outlined in Scheme 1. Piperazine derivatives **1-14** were synthesized by nucleophilic attack of an *N*-monosubstituted piperazine to the appropriate nitro(triazole/imidazole)-alkylbromide [30] in the presence of K₂CO₃ as described before [10]. *N*-substituted-2-amino-1,3-benzothiazoles **15-20** were synthesized by coupling of the proper 2-chloro-1,3-benzothiazole with the appropriate nitro(triazole/imidazole)-alkylamine [31] by nucleophilic aromatic substitution (Scheme 1). In general the yields were moderate to good but if we account for the recovered unreacted starting materials, the yields are significantly greater than reported.

2.2. Biological Evaluation

The *in vitro* growth inhibitory properties of compounds **1-14** against *T. b. rhodesiense* bloodstream form trypomastigotes, *T. cruzi* amastigotes (in infected L6 myoblasts), axenically cultured *L. donovani* amastigotes and rat skeletal myoblasts (L6 cells) are shown in Table 1. IC₅₀ values in μ M were determined from resultant dose response curves (Table 1). The following criteria were used to interpret activity: for *T.b. rhodesiense*, compounds with an IC₅₀ < 0.5 μ M, were designated as 'active', while those yielding an IC₅₀ = 0.5-6.0 μ M or an IC₅₀ > 6.0 μ M were designated 'moderately active' and 'inactive', respectively. For *T. cruzi*, IC₅₀ < 4.0 μ M, 'active'; IC₅₀ = 4.0-60 μ M, 'moderately active'; IC₅₀ > 60 μ M, 'inactive'. For *L. donovani*, IC₅₀ < 1 μ M, 'active'; IC₅₀ = 1.0-6.0 μ M, 'moderately active'; IC₅₀ > 6.0 μ M, 'inactive'. In addition, to evaluate toxicity, growth inhibition of the mammalian L6 host cells has to be taken into account. Thus, the selectivity index (SI), namely the ratio of IC₅₀ against L6 cells to IC₅₀ against each parasite should be 100 for *T.b. rhodesiense*, 50 for *T. cruzi* and 20 for *L. donovani* axenic amastigotes. These criteria were established by the TDR's "compound screeners network", published in a review [32] and take into consideration the complex life cycles of the parasites and the fact that *T. cruzi* and *L. donovani* are (in contrast to *T.b. rhodesiense*) intracellular parasites.

2.2.1. Analysis of the antitrypanosomal activity of all tested compounds—

Based on the above, all but two (**6**, **12**) 3-nitrotriazole-based piperazines and benzothiazoles (87%) were active against *T. cruzi* and fulfilled the corresponding selectivity criteria (Table 1). In addition, most of them demonstrated antichagasic activity at nanomolar concentrations and a SI > 200 (**1-3**, **7-9**, **15-17**). The *p*-pyrido-piperazine **6** and the cyclopropylcarbonyl-piperazine **12** were moderately active against *T. cruzi* but also toxic towards the L6 host cells (SI<50). All 2-nitroimidazole based analogs were moderately active against *T. cruzi* but toxic to the host cells (**13**, **14**, **18-20**). Similar behavior has been observed before with other chemical classes of 2-nitroimidazole-based compounds [10].

With regard to *T. b. rhodesiense*, three 3-nitrotriazole-based analogs were active and selective (**7**, **15** and **17**) and two (**6** and **16**) were moderately active and selective. The *p*-trifluoromethylbenzyl-piperazine **2** was moderately active against *T. b. rhodesiense* and

marginally selective (SI =99). In addition, the 3-nitrotriazole-based analogs **1**, **3**, **11** and the 2-nitroimidazole-based compound **20** were moderately active towards *T. b. rhodesiense* but toxic to the host cells (Table 1).

Only one compound, the 3-nitrotriazole-based 6-chlorobenzothiazole **15** was moderately active towards *L. donovani*. In fact, this compound was found to be active towards all tested parasites.

Comparing the antichagasic activity of the tested compounds with that of Bnz, we observed that all but three 3-nitrotriazole-based analogs (**4**, **6** and **12**) (80%) were significantly more potent (2- to 39-fold) than Bnz (Table 1). All 2-nitroimidazole-based analogs were less potent than Bnz with the exception of **20** which was slightly more potent than Bnz (Table 1).

2.2.2. SAR analysis of the antichagasic and anti-HAT activity of nitro(triazole/imidazole)-based piperazines—A closer look at the SARs of nitrotriazole-based piperazines with regard to their antichagasic activity (Table 1) suggests that the most potent compounds were 1-phenyl piperazines, substituted in the *para* or *meta* position with electron withdrawing groups. Thus, *p*-chloro-(**7**) or *p*-trifluoromethyl-substituted 1-phenyl piperazines (**3**) were more active against *T. cruzi* compared to their *p*-methyl (**8**) or *p*-methoxy- (**9**) analogues. Indeed, the Hammett substituent constants of $-CF_3$, *p*-Cl-, *m*-Cl-, CH_3 - and MeO - groups are 0.54, 0.23, 0.37, -0.17 and -0.27, respectively [33]. Plotting the *T. cruzi* IC₅₀ values of these compounds versus the constants, we find a good correlation ($R^2=0.985$) between antichagasic activity and electronegativity (plot is not shown). Compound **10** with the electron withdrawing cyano group in the *ortho* position of the phenyl group demonstrated a significantly greater IC₅₀ against *T. cruzi* compared to **3** and **7**. It is well known that Hammett analysis cannot include ortho-substitution because such substitution introduces steric effects. Thus, it is speculated that since the cyano-group, besides its excellent inductive properties can also function as a hydrogen bond acceptor, it could cause some unfavorable interactions with type I Tc-NTR. Substituted 1-phenyl piperazines were significantly more potent (6-10 fold) against *T. cruzi* than their 1-benzyl analogs (compare **1** and **2** to **3**, and **11** to **7**). With regard to anti-HAT activity, both 1-phenyl- and 1-benzylpiperazines substituted with electron withdrawing groups in the *para*-position were similarly active against *T. b. rhodesiense*, however only the phenyl analog **7** fulfilled the selectivity criteria (Table 1).

Antichagasic activity was reduced significantly when the 1-phenyl group was replaced with a nitrogen(s) containing heteroaryl ring (**4-6**). The position of the nitrogen(s) in the ring had an effect on the antichagasic activity of 1-pyridino-/1-pyrimidino-piperazines. Thus, one N in the 2-position (**5**) resulted in an IC₅₀ against *T. cruzi* of 848 nM, very similar to that of 1-(2-cyanophenyl)piperazine **10**, whereas two N atoms in 2 and 6 positions in **4** decreased the activity by 2-fold compared to **5**. When N was in the 4 position (**6**) the antichagasic activity was reduced by more than 10-fold compared to **5**. Compound **6** was the only one among the heteroarylpiperazines tested to show moderate activity against *T.b. rhodesiense* (Table 1). It is not clear why several 3-nitrotriazole-based piperazines (e.g. heteroaryl-piperazines but not only) showed so great a difference between their antichagasic and anti-HAT activity. Differences in the active site of Tc- and Tb-nitroreductases could account for differences in activation, if we assume that NTRs are the only enzymes that activate this type of compounds in the parasites. These issues are going to be clarified in future studies, in collaboration with the Queen Mary University of London.

Antichagasic activity was significantly reduced when the 1-phenyl group was replaced with an 1-alkylcarbonyl-group (**12**).

Comparing the 2-nitroimidazole-based *p*-methoxyphenyl-piperazine **13** with its 3-nitrotriazole counterpart **9**, we observed that the antichagasic and anti-HAT activity of **13** was reduced about 62- and 2-fold, respectively, compared to **9**. Similarly, comparing the 2-nitroimidazole-based benzylpiperazine **14** with its 3-nitrotriazole-based counterpart **11**, the antichagasic and anti-HAT activity of **14** was reduced about 33- and 14-fold, respectively, compared to **11**.

2.2.3. SAR analysis of the antichagasic and anti-HAT activity of nitro(triazole/imidazole)-based benzothiazoles—

Chloro-substitution in the 6 position of the benzothiazole ring increased the activity against all parasites of either nitrotriazole- or nitroimidazole-based analogs (compare **15** and **17** to **16**; **18** and **20** to **19** in Table 1). Increasing the linker between the nitro-bearing ring and 2-aminobenzothiazole resulted in an increase in the antichagasic and anti-HAT activity of the 2-nitroimidazole-based analogs but a decrease in their 3-nitrotriazole-based counterparts (compare **15** to **17** and **18** to **20** in Table 1).

2.2.4. Correlation between the physical properties and antitrypanosomal activity of tested compounds—

The calculated physical properties of all tested compounds are listed in Table 2. All compounds fulfill the Lipinski rule of 5 [34]. Plotting the *T. cruzi* IC₅₀ values of all active nitrotriazole-based piperazines *versus* their clogP values, we observe that a good correlation exists ($R^2 = 0.97$), following an exponential relationship (Fig. 1). Thus, lipophilicity seems to play an important role in the antichagasic activity of 3-nitrotriazole-based piperazines and clogP values from 2.5-3.5 show the best antichagasic activity. No such correlation exists however for the corresponding anti-HAT activity of these compounds.

Plotting the *T. cruzi* IC₅₀ values of all active nitrotriazole-based piperazines *versus* their polar surface area (PSA) we observe that, although there is not a good linear correlation ($R^2 = 0.78$), antichagasic activity is decreased with increasing PSA and compounds with the best *T. cruzi* IC₅₀ values have a PSA value between 80 and 85 Å² (Fig. 2).

Finally, in the same group of compounds, a correlation exists between pK_a values and antichagasic activity (Fig. 3) following a second degree polynomial relationship ($R^2 = 0.93$). 3-Nitrotriazole-based piperazines with a pK_a around 8 demonstrate the best antichagasic activity. No correlation exists between pK_a values and antichagasic activity for the 3-nitrotriazole-based 2-aminobenzothiazoles, perhaps because they demonstrate an acidic rather than a basic character.

In general, with regard to nitro(triazole/imidazole)based benzothiazoles, both antichagasic and anti-HAT activity increased with increasing lipophilicity (Table 2), but this was more apparent with 2-nitroimidazole-based benzothiazoles.

Oral administration and good intestinal permeability are critical parameters for antichagasic drugs. It was recently shown that Partition coefficients (logD and logP) and molecular surface area (PSA) are potential predictors of the intestinal permeability of drugs [34]. According to this study, the logD value at pH 6 (logD₆) more accurately predicts intestinal permeability compared to other parameters, and a logD₆ > -0.42 (the logD₆ value of labetalol) is associated with high permeability [35]. As can be seen in Table 2, most of the active compounds demonstrate logD₆ values > -0.42 and thus they may demonstrate good intestinal permeability. However, ADME studies are necessary for reliable predictions with regard to absorption, bioavailability and metabolic stability of these compounds.

Using the Osiris Property Explorer software (<http://www.organic-chemistry.org/prog/peo/>) we calculated values that indicate the drug-likeness of the compounds **1-20** (Table 2). The drug-likeness value is based on a list of about 5300 distinct substructure fragments with associated druglikeness scores. The distribution of drug-likeness values calculated from 15000 Fluka compounds and from 3300 traded drugs showed that most traded drugs demonstrate drug-likeness values in the range of -2 to 5 whereas the big majority of Fluka chemicals accounts for negative values. Based on the drug-likeness values, all tested compounds contain predominantly fragments which are frequently present in commercial drugs. However, other parameters (e.g. lipophilicity, toxicity, bioavailability, etc.) will determine if a compound qualifies as an acceptable pharmaceutical. Our previous studies with several nitrotriazole-based sulfonamides and amides showed promising results with regard to *in vivo* efficacy, stability, bioavailability and lack of mutagenicity in the Ames test (in contrast to 2-nitroimidazole analogs) [29] and make us optimistic.

As mentioned earlier, all nitrotriazole-based compounds described in our previous work were very good substrates for the type I NTR [10, 11]. Indeed, the activity of recombinant, purified his-tagged *T. b. brucei* nitroreductase (TbNTR) using compounds **1**, **2** and **3** as substrates, was 420 15, 364 13 and 788 54 nmol NADH oxidized min⁻¹mg⁻¹, respectively, compared to 74 18 and 218 4 nmol NADH oxidized min⁻¹mg⁻¹ for nifurtimox and benznidazole, respectively [10, 11]. Since the enzymatic activity of **1-3** correlates with their antichagasic activity, we anticipate that the rest of the compounds in Table 1 most likely are also substrates of type I NTR. Since the precise nature of the active site of TcNTR and TbNTR still remains unclear, modeling studies are prohibited.

In conclusion, several analogs of 3-nitrotriazole-based piperazines and benzothiazoles (Table 1) have been identified as potential candidates for *in vivo* studies in *T. cruzi* infected mice and further development as antichagasic drugs. All of them demonstrate significant activity against *T. cruzi* amastigotes at low to intermediate nmolar concentrations, have SI values of 200, satisfy the Lipinski's rule of 5 and demonstrate drug-like characteristics. In addition, compounds **7**, **15** and **17** are good candidates for further evaluation *in vivo* as anti-HAT agents. However, initial ADMET studies are necessary for better lead optimization and selection for *in vivo* testing.

3. Experimental

3.1. Chemistry

All starting materials and solvents were purchased from Sigma-Aldrich (Milwaukee, WI), were of research-grade quality and used without further purification. Solvents used were anhydrous and the reactions were carried out under a nitrogen atmosphere and exclusion of moisture. Melting points were determined by using a Mel-Temp II Laboratory Devices apparatus (Holliston, MA) and are uncorrected. Elemental Analyses were obtained by Midwest Microlab, LLC (Indianapolis, IN). Proton NMR spectra were obtained on a Varian Inova-500 or a Bruker Avance-III-500 spectrometer at 500 MHz and are referenced to Me₄Si or to the corresponding protonated solvent, if the solvent was not CDCl₃. High-resolution electrospray ionization (HRESIMS) mass spectra were obtained on a Agilent 6210 LC-TOF mass spectrometer at 11000 resolution. Thin-layer chromatography was carried out on aluminum oxide N/UV₂₅₄ or polygram silica gel G/UV₂₅₄ coated plates (0.2 mm, Analtech, Newark, DE). Chromatography was carried out on preparative TLC alumina GF (1000 microns) or silica gel GF (1500 microns) plates (Analtech). All the amines were purified by preparative TLC chromatography on alumina plates (> 95% purity). The results from elemental analysis for C, H, Cl and N were within 0.4 of the theoretical value for piperazines **1-3**[10]; from these data and the similar synthetic preparation of the novel

piperazine derivatives, it was concluded that all (but piperazine **12**) were the dihydrochloride salts.

3.1.1. General Synthetic Procedure of 3-Nitrotriazole/2-Nitroimidazole-based Piperazines—Piperazine derivatives **1-3** are known [10]. Piperazine derivatives **4-14** were synthesized from the commercially available appropriate monoalkylated piperazines (1.44 mmol) and 2-nitro-1*H*-imidazolyl-propylbromide or 3-nitro-1*H*-1,2,4-triazolyl-propylbromide (1.485 mmol) [30] in the presence of potassium carbonate (13.24 mmol) in dry acetonitrile (25 mL) as described before [10]. The reaction mixture was stirred under a nitrogen atmosphere at room temperature for 48 h, then filtered to remove the inorganic salts. The organic filtrate was evaporated and the residue extracted from water-chloroform. The organic layer was separated and dried over anhydrous Na₂SO₄. After filtration, the solvent was evaporated and the residue purified by preparative TLC on alumina plates with ethyl acetate: petroleum ether mixture. The desired product was dissolved in ethyl acetate and converted to its HCl salt by treating with HCl gas in dry ether (1 M solution).

3.1.1.1. 2-[4-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]piperazin-1-yl]pyrimidine dihydrochloride (4**):** Light yellow powder (75%): mp > 210 °C (dec); ¹H NMR (500 MHz, D₂O) δ: 8.65 (s, 1H), 8.44 (dd, *J*=5.0, 2.5 Hz, 2H), 6.86 (m, 1H), 4.52 (t, *J*=6.5 Hz, 2H), 3.80-3.50 (br m, 10H), 2.48 (quintet, *J*=8.0 Hz, 2H). HRESIMS calcd for C₁₃H₁₉N₈O₂*m/z* [M+H]⁺ 319.1625, found 319.1627.

3.1.1.2. 1-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-4-(pyridin-2-yl)piperazine dihydrochloride (5**):** The free amine formed yellow crystals and the HCl salt was a pale yellow powder (40%): mp 69-71 °C (free amine); ¹H NMR of the 2HCl salt (500 MHz, D₂O) δ: 8.66 (s, 1H), 8.12 (t, *J*=9.0 Hz, 1H), 8.01 (d, *J*=6.5 Hz, 1H), 7.36 (d, *J*=9.0 Hz, 1H), 7.14 (t, *J*=7.0 Hz, 1H), 4.54 (t, *J*=6.5 Hz, 2H), 4.00 (br s, 4H), 3.60 (br s, 4H), 3.39 (m, 2H), 2.49 (quintet, *J*=8.0 Hz, 2H). HRESIMS calcd for C₁₄H₂₀N₇O₂*m/z* [M+H]⁺ 318.1673, found 318.1675.

3.1.1.3. 1-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-4-(pyridin-4-yl)piperazine (6**):** White crystals (35%). mp 116-118 °C (dec, free amine); ¹H NMR (500 MHz, CD₃OD) δ: 8.63 (s, 1H), 8.16 (d, *J*=6.5 Hz, 2H), 6.91 (d, *J*=7.0 Hz, 2H), 4.42 (t, *J*=6.5 Hz, 2H), 3.41 (t, *J*=5.0 Hz, 4H), 2.63 (t, *J*=5.0 Hz, 4H), 2.47 (t, *J*=7.5 Hz, 2H), 2.18 (quintet, *J*=7.5 Hz, 2H). HRESIMS calcd for C₁₄H₂₀N₇O₂*m/z* [M+H]⁺ 318.1673, found 318.1676.

3.1.1.4. 1-(3,4-dichlorophenyl)-4-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]piperazine dihydrochloride (7**):** Off white powder (50%): mp 204-206 °C (dec); ¹H NMR (500 MHz, D₂O) δ: 8.66 (s, 1H), 7.45 (d, *J*=9.0 Hz, 1H), 7.24 (d, *J*=2.5 Hz, 1H), 6.98 (dd, *J*=9.0, 3.0 Hz, 1H), 4.52 (t, *J*=6.5 Hz, 2H), 3.84 (br d, 2H), 3.71 (br d, 2H), 3.34 (m, 2H), 3.25 (br d, 2H), 3.15 (br d, 2H), 2.47 (quintet, *J*=8.0 Hz, 2H). HRESIMS calcd for C₁₅H₁₉Cl₂N₆O₂ and C₁₅H₁₈Cl₂N₆NaO₂*m/z* [M+H]⁺ and [M+Na]⁺, respectively 385.0941, 387.0916 and 407.0761, 409.0734 found 385.0945, 387.0917 and 407.0757, 409.0728, respectively.

3.1.1.5. 1-(4-methylphenyl)-4-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]piperazine dihydrochloride (8**):** Light yellow powder (40%): mp 185-190 °C (dec); ¹H NMR (500 MHz, D₂O) δ: 8.65 (s, 1H), 7.24 (d, *J*=8.5 Hz, 2H), 7.05 (d, *J*=8.5 Hz, 2H), 4.52 (t, *J*=6.5 Hz, 2H), 3.70-3.30 (br s, 8H), 3.34 (m, 2H), 2.46 (m, 2H). HRESIMS calcd for C₁₈H₂₅N₃O₃*m/z* [M+H]⁺ 331.1890, found 331.1885.

3.1.1.6. 1-(4-methoxyphenyl)-4-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]piperazine dihydrochloride (9**):** White microcrystalline powder (45%): mp 210 °C (dec); ¹H NMR

(500 MHz, D₂O) δ : 8.66 (s, 1H), 7.14 (d, J =9.0 Hz, 2H), 7.02 (d, J =9.0 Hz, 2H), 4.53 (t, J =6.5 Hz, 2H), 3.82 (s, 3H), 3.60-3.40 (br m, 8H), 3.36 (m, 2H), 2.48 (quintet, J =8.5 Hz, 2H). HRESIMS calcd for C₁₆H₂₃N₆O₃ m/z [M+H]⁺ 347.1826, found 347.1828.

3.1.1.7. 2-{4-[3-(3-nitro-1H-1,2,4-triazol-1-yl)propyl]piperazin-1-yl}benzonitrile dihydro-chloride (10): Off white powder (55%): mp 168-170 °C (dec); ¹H NMR (500 MHz, D₂O) δ : 8.67 (s, 1H), 7.76 (dd, J =8.0, 1.5 Hz, 1H), 7.67 (t, J =7.5 Hz, 1H), 7.26 (m, 2H), 4.55 (t, J =6.5 Hz, 2H), 3.80-3.30 (br m, 8H), 3.38 (t, J =8.0 Hz, 2H), 2.49 (quintet, J =8.0 Hz, 2H). HRESIMS calcd for C₁₆H₂₀N₇O₂ m/z [M+H]⁺ 342.1673, found 342.1680.

3.1.1.8. 1-[(3,4-dichlorophenyl)methyl]-4-[3-(3-nitro-1H-1,2,4-triazol-1-yl)propyl]piperazine dihydrochloride (11): Fine white powder (75%): mp > 220 °C; ¹H NMR (500 MHz, D₂O) δ : 8.65 (s, 1H), 7.68 (d, J =2.0 Hz, 1H), 7.66 (d, J =8.0 Hz, 1H), 7.41 (dd, J =8.0, 2.0 Hz, 1H), 4.51 (t, J =7.0 Hz, 2H), 4.35 (s, 2H), 3.60-3.45 (br m, 8H), 3.32 (m, 2H), 2.42 (quintet, J =8.0 Hz, 2H). HRESIMS calcd for C₁₆H₂₁Cl₂N₆O₂ m/z [M+H]⁺ 399.1098, 401.1071, found 399.1112, 401.1086.

3.1.1.9. 1-cyclopropanecarbonyl-4-[3-(3-nitro-1H-1,2,4-triazol-1-yl)propyl]piperazine hydrochloride (12): White powder (68%): mp 210 °C (dec); ¹H NMR (500 MHz, D₂O) δ : 8.66 (s, 1H), 4.52 (t, J =6.5 Hz, 2H), 3.80-3.00 (m, 10H), 2.47 (m, 2H), 1.97 (m, 1H), 0.92 (quintet, J =2.5 Hz, 2H), 0.89 (quintet, J =2.5 Hz, 2H). HRESIMS calcd for C₁₃H₂₁N₆O₃ and C₁₃H₂₀N₆NaO₃ m/z [M+H]⁺ and [M+Na]⁺ 309.1670 and 331.1489, found 309.1664 and 331.1490.

3.1.1.10. 1-(4-methoxyphenyl)-4-[3-(2-nitro-1H-imidazol-1-yl)propyl]piperazine dihydro-chloride (13): Off white powder (55%): mp 165-169 °C (dec); ¹H NMR (500 MHz, D₂O) δ : 7.54 (s, 1H), 7.27 (s, 1H), 7.18 (br s, 2H), 7.07 (br s, 2H), 4.62 (t, J =7.5 Hz, 2H), 3.86 (s, 3H), 3.62-3.38 (br m, 10H), 2.45 (quintet, J =8.5 Hz, 2H). HRESIMS calcd for C₁₇H₂₄N₅O₂ and C₁₇H₂₃N₅NaO₂ m/z [M+H]⁺ and [M+Na]⁺ 346.1874 and 368.1693, found 346.1871 and 368.1690.

3.1.1.11. 1-[(3,4-dichlorophenyl)methyl]-4-[3-(2-nitro-1H-imidazol-1-yl)propyl]piperazine dihydrochloride (14): White powder (75%): mp 185-188 °C (dec); ¹H NMR (500 MHz, D₂O) δ : 7.72 (d, J =2.0 Hz, 1H), 7.69 (d, J =8.0 Hz, 1H), 7.50 (s, 1H), 7.44 (dd, J =8.5, 2.0 Hz, 1H), 7.23 (s, 1H), 4.59 (t, J =7.0 Hz, 2H), 4.47 (s, 2H), 3.65 (br s, 8H), 3.41 (m, 2H), 2.40 (quintet, J =7.5 Hz, 2H). HRESIMS calcd for C₁₇H₂₂Cl₂N₅O₂ m/z [M+H]⁺ 398.1145, 400.1119, found 398.1142, 400.1115.

3.1.2. General Synthetic Procedure of 2-Amino-substituted 1,3-Benzothiazoles (15-20)—The appropriate commercially available 2-chloro-1,3-benzothiazole (1.24 mmol), was coupled with 2-nitro-1H-imidazolyl-alkylamine (1.24 mmol) [31] or 3-nitro-1H-1,2,4-triazolyl-alkylamine (1.24 mmol) [31] by refluxing in absolute 1-propanol (7-10 ml) for 16-24 h in the presence of 5 fold excess triethylamine. In most cases the desired product was precipitated upon cooling of the reaction mixture and then extracted from ethyl acetate-water. The organic layer was dried over Na₂SO₄ and upon evaporation gave the final product. Additional product was separated from the reaction mixture by preparative TLC on alumina by using ethyl acetate as eluent, occasionally containing 1% MeOH.

3.1.2.1. 6-chloro-N-[3-(3-nitro-1H-1,2,4-triazol-1-yl)propyl]-1,3-benzothiazol-2-amine (15): Orange powder (63%): mp 193-195 °C ¹H NMR (500 MHz, CD₃COCD₃) δ : 8.71 (s, 1H), 7.70 (d, J =2.5 Hz, 1H), 7.39 (d, J =8.5 Hz, 1H), 7.35 (br s, 1H), 7.25 (dd, J =8.5, 2.5 Hz,

1H), 4.57 (t, $J=7.0$ Hz, 2H), 3.62 (t, $J=6.0$ Hz, 2H), 2.39 (quintet, $J=6.5$ Hz, 2H). HRESIMS calcd for $C_{12}H_{12}ClN_6O_2S$ m/z $[M+H]^+$ 339.0425, 341.0398, found 339.0427, 341.0395.

3.1.2.2. N-[3-(3-nitro-1H-1,2,4-triazol-1-yl)propyl]-1,3-benzothiazol-2-amine (16): Light orange powder (45%): mp 155-156 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 8.73 (s, 1H), 7.63 (d, $J=7.5$ Hz, 1H), 7.43 (d, $J=8.0$ Hz, 1H), 7.26 (t, $J=8.5$ Hz, 1H), 7.22 (br s, 1H), 7.05 (t, $J=8.5$ Hz, 1H), 4.58 (t, $J=7.0$ Hz, 2H), 3.62 (t, $J=6.0$ Hz, 2H), 2.39 (quintet, $J=7.0$ Hz, 2H). HRESIMS calcd for $C_{12}H_{13}N_6O_2S$ m/z $[M+H]^+$ 305.0815, found 305.0814.

3.1.2.3. 6-chloro-N-[4-(3-nitro-1H-1,2,4-triazol-1-yl)butyl]-1,3-benzothiazol-2-amine (17): Bright yellow crystals (75%): mp 116-118 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 8.68 (s, 1H), 7.68 (d, $J=2.5$ Hz, 1H), 7.38 (d, $J=8.5$ Hz, 1H), 7.25 (br s, 1H), 7.25 (dd, $J=8.5$, 2.0 Hz, 1H), 4.50 (t, $J=7.0$ Hz, 2H), 3.56 (t, $J=5.5$ Hz, 2H), 2.10 (quintet, $J=7.5$ Hz, 2H), 1.78 (quintet, $J=7.5$ Hz, 2H). HRESIMS calcd for $C_{13}H_{14}ClN_6O_2S$ m/z $[M+H]^+$ 353.0582, 355.0555, found 353.0574, 355.0549.

3.1.2.4. 6-chloro-N-[3-(2-nitro-1H-imidazol-1-yl)propyl]-1,3-benzothiazol-2-amine (18): Light yellow powder (60%): mp 147-149 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 7.69 (d, $J=1.5$ Hz, 1H), 7.64 (s, 1H), 7.40 (d, $J=8.5$ Hz, 1H), 7.33 (br s, 1H), 7.25 (dd, $J=8.5$, 2.0 Hz, 1H), 7.13 (s, 1H), 4.66 (t, $J=7.5$ Hz, 2H), 3.61 (m, 2H), 2.33 (quintet, $J=7.0$ Hz, 2H). HRESIMS calcd for $C_{13}H_{13}ClN_5O_2S$ and $C_{13}H_{12}ClN_5NaO_2S$ m/z $[M+H]^+$ and $[M+Na]^+$ 338.0473, 340.0446 and 360.0292, 362.0265, found 338.0470, 340.0444 and 360.0290, 362.0265.

3.1.2.5. N-[3-(2-nitro-1H-imidazol-1-yl)propyl]-1,3-benzothiazol-2-amine (19): Bright yellow powder (35%): mp 124-126 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 7.66 (s, 1H), 7.63 (d, $J=7.5$ Hz, 1H), 7.43 (d, $J=8.5$ Hz, 1H), 7.25 (t, $J=8.5$ Hz, 1H), 7.18 (br s, 1H), 7.05 (t, $J=7.5$ Hz, 1H), 4.66 (t, $J=7.0$, 2H), 3.61 (m, 2H), 2.33 (quintet, $J=7.0$ Hz, 2H). HRESIMS calcd for $C_{13}H_{14}N_5O_2S$ and $C_{13}H_{13}N_5NaO_2S$ m/z $[M+H]^+$ and $[M+Na]^+$ 304.0863 and 326.0682, found 304.0861 and 326.0683.

3.1.2.6. 6-chloro-N-[4-(2-nitro-1H-imidazol-1-yl)butyl]-1,3-benzothiazol-2-amine (20): Light yellow powder (52%): mp 137-139 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 7.68 (d, $J=2.0$ Hz, 1H), 7.57 (s, 1H), 7.38 (d, $J=8.5$ Hz, 1H), 7.25 (br s, 1H), 7.24 (dd, $J=8.5$, 2.5 Hz, 1H), 7.11 (s, 1H), 4.58 (t, $J=7.0$ Hz, 2H), 3.56 (m, 2H), 2.03 (quintet, $J=7.5$ Hz, 2H), 1.78 (quintet, $J=7.5$ Hz, 2H). HRESIMS calcd for $C_{14}H_{15}ClN_5O_2S$ and $C_{14}H_{14}ClN_5NaO_2S$ m/z $[M+H]^+$ and $[M+Na]^+$ 352.0629, 354.0603 and 374.0449, 376.0422, found 352.0621, 354.0594 and 374.0436, 376.0412.

3.2. In vitro biological evaluation

In vitro activity against *T. cruzi*, *Trypanosoma b. rhodesiense*, *Leishmania donovani* axenic amastigotes and cytotoxicity assessment using L6 cells (rat skeletal myoblasts) was determined using a 96-well plate format as previously described [36]. Data were analyzed with the graphic program Softmax Pro (Molecular Devices, Sunnyvale, CA, USA), which calculated IC_{50} values by linear regression from the sigmoidal dose inhibition curves.

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Fig. 1. Correlation between lipophilicity and antichagasic activity in active 3-nitrotriazole-based piperazines.

Fig. 2. Correlation between polar surface area (PSA) in Å² and antichagasic activity in active 3-nitrotriazole-based piperazines.

Fig. 3. Correlation between *pKa* values and antichagasic activity in active 3-nitrotriazole-based piperazines.

Scheme 1.

i) K_2CO_3 (9 eq), CH_3CN , RT, 48 h; ii) 1-Propanol, reflux 16-24 h. R: varies (see Table 1);
 R_1 : H or 6-Cl; n: 1 or 2; X=C, 2- NO_2 ; X=N, 3- NO_2

Chart 1.

Table 1
In vitro screening data against three different trypanosomatids

ID No	<i>T.b. rhod.</i> ^a		SI	<i>T. cruzi</i> ^b		SI	<i>L. don. axen.</i> ^c		SI	Cytotox. L6		Bnz/Comp.	Chemical Structure
	IC-50 μ M			IC-50 μ M			IC-50 μ M			IC-50 μ M			
1	1.38	>14		0.34	>562		58.15	>3		>191.1	4.6
2	1.2	99		0.412	287		44.45	3		118.4	3.8
3	5.33	10		0.04	1320		25.92	2		52.79	39.2
4	49.6	5.2		1.678	152		>100	1		>100	0.9
5	35.65	4.3		0.848	180		>315	<1		152.68	1.9
6	1.34	114.6		8.8	17.5		>315	<1		153.9	0.2
7	0.231	180.2		0.048	868		11.35	3.7		41.7	32.7
8	23.85	5.1		0.419	289		173.45	<1		121.34	3.7
9	35.56	3.5		0.556	224.5		>238.66	<1		124.8	2.8

ID No	<i>T.b.rhod.</i> ^a	SI	<i>T. cruzi</i> ^b	SI	<i>L.don. axen.</i> ^c	SI	Cytotox. L6	Bnz/Comp.	Chemical Structure
	IC-50 μ M		IC-50 μ M		IC-50 μ M		IC-50 μ M		
10	10.46	10.8	0.828	137	180.2	<1	113.3	2.5	
11	0.78	42.9	0.286	117	23.9	1.4	33.5	7.1	
12	15.62	13	4.7	43.1	>290	<1	202.9	0.3	
13	62.4	2.2	34.4	4	186.4	<1	137.1	0.1	
14	10.74	7.4	9.4	8.4	149.5	<1	79.2	0.2	
15	0.204	500	0.059	1725	4.49	22.7	101.9	26.6	
16	0.954	170	0.566	287	20	8.1	162.5	3.6	
17	0.355	125.6	0.142	314	4.1	10.9	44.54	14.4	
18	7.61	6.5	3.23	15.3	4.27	11.6	49.5	0.6	
19	52.5	3.4	4.88	36.1	11.4	15.4	176	0.4	

ID No	<i>T.b.rhod.</i> ^a	SI	<i>T. cruzi</i> ^b	SI	<i>L.don. axen.</i> ^c	SI	Cytotox. L6 IC-50 μ M	Bnz/Comp.	Chemical Structure
	IC-50 μ M		IC-50 μ M		IC-50 μ M				
20	3.87	11.1	1.75	24.6	7.3	5.5	43	1.2

^a STIB 900 trypomastigotes;

^b Tulahuen C4 amastigotes;

^c MHOM-ET-67/L82 amastigotes; Cytotoxicity was measured in L6 cells. SI = IC₅₀ in L6 cells / IC₅₀ in each type of parasites. The reference compounds were: Melarsoprol (*T. b. rhodesiense*; IC₅₀ = 0.011 0.004), Benzimidazole (Bnz; *T. cruzi*; IC₅₀ = 1.85 0.33) and Miltefosine (*L. donovani*; IC₅₀ = 0.378 0.011). Bnz/comp.: The ratio of IC₅₀ values of Benzimidazole and each tested compound against *T. cruzi*. The IC₅₀ values are the means of two independent assays and varies less than $\pm$ 50%. Green color indicates active compounds, light red, moderately active, light blue, toxic and no color, inactive. Compounds **1-3** have been described before [10].

Table 2

Calculated physical properties of compounds listed in Table 1.

ID No	clogP	logD ₆ ^a	pKa	LR5 ^b	PSA ^c Å ²	Drug like Prediction	ID No	clogP	logD ₆ ^a	pKa	LR5 ^b	PSA ^c Å ²	Druglike Prediction
1	2.86	0.56	8.33	S ^d	83.01	0.64	11	3.19	1.08	8.12	S	83.01	7.61
2	3.38	0.90	8.52	S	83.01	-0.83	12	0.27	-0.86	7.09	S	100.08	9.86
3	3.03	0.89	8	S	83.01	-1.74	13	2.36	0.21	8.17	S	79.35	5.39
4	0.91	-0.77	7.38	S	108.79	9.24	14	3.56	1.46	8.11	S	70.12	7.61
5	1.53	-0.45	7.63	S	95.9	8.84	15	3.10	3.10	3.30	S	101.45	0.21
6	0.93	-1.97	8.74	S	95.9	7.18	16	2.49	2.49	3.36	S	101.45	-0.5
7	3.36	1.33	7.74	S	83.01	6.29	17	3.61	3.61	3.3	S	101.45	-1.03
8	2.66	0.42	8.27	S	83.01	5.68	18	3.47	3.46	3.3	S	88.56	0.21
9	1.99	-0.17	8.18	S	92.24	5.39	19	2.86	2.86	3.36	S	88.56	-0.5
10	2.01	0.14	7.57	S	106.8	3.99	20	3.98	3.98	3.3	S	88.56	-1.03

^a logP value at pH 6.

^b Lipinski rule of 5.

^c PSA: Polar surface area.

^d Satisfied. All physical properties were predicted by using the Marvin Calculator (www.chemaxon.com). Compounds **1-14** are piperazines, whereas compounds **15-20** are benzothiazoles. Drug-likeness prediction was calculated by using the Osiris Property Explorer (<http://www.organic-chemistry.org/prop/peco/>). Most traded drugs demonstrate drug-likeness values in the range of -2 to 5.