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Novel 3-Nitro-1*H*-1,2,4-triazole-Based Aliphatic and Aromatic Amines as Anti-Chagasic Agents

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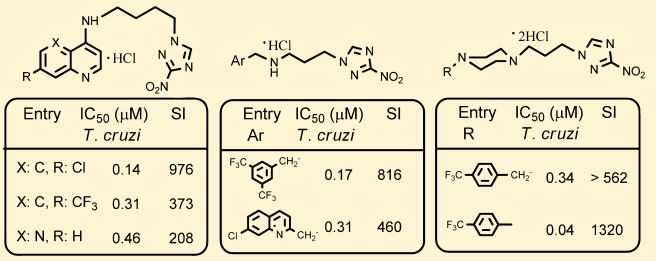
Supporting Information

ABSTRACT: A series of novel 2-nitro-1*H*-imidazole- and 3-nitro-1*H*-1,2,4-triazole-based aromatic and aliphatic amines were screened for antitrypanosomal activity and mammalian cytotoxicity by the Drugs for Neglected Diseases initiative (DNDi). Out of 42 compounds tested, 18 3-nitro-1,2,4-triazoles and one 2-nitroimidazole displayed significant growth inhibitory properties against *T. cruzi* amastigotes (IC_{50} ranging from 40 nM to 1.97 μM), without concomitant toxicity toward the host cells (L6 cells), having selectivity indices (SI) 44–1320. Most (16) of these active compounds were up to 33.8-fold more potent than the reference drug benznidazole, tested in parallel. Five novel 3-nitro-1,2,4-triazoles were active against bloodstream-form (BSF) *T. b. rhodesiense* trypomastigotes (IC_{50} at nM levels and SI 220–993). An NADH-dependent nitroreductase (TbNTR) plays a role in the antiparasitic activity because BSF *T. b. brucei* trypomastigotes with elevated TbNTR levels were hypersensitive to tested compounds. Therefore, a novel class of affordable 3-nitro-1,2,4-triazole-based compounds with antitrypanosomal activity has been identified.

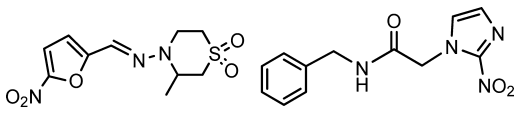


INTRODUCTION

The protozoan parasites *Trypanosoma cruzi* (*T. cruzi*), *Trypanosoma brucei* (*T. brucei*), and various *Leismania* species, also referred to as trypanosomatids, are the causative agents of Chagas disease, human African trypanosomiasis (HAT), and different forms of leishmaniasis, respectively. Over 10 million people are infected by *T. cruzi* and 50000 to 80000 by *T. b. gambiense* or *T. b. rhodesiense*, resulting in more than 40000 deaths per year.[1] Chagas disease is transmitted by blood sucking triatomine insects and occurs mainly in Latin America. Although over the past 20 years the number of incidences has declined, primarily due to vector control initiatives,[2] the number of cases in nonendemic regions such as the United States is on the rise.[3] Reasons for this rise include population migration, drug usage, and medical practices. With no immediate prospect for vaccines, chemotherapy is the only way to fight the parasite in the patient.

Currently, two nitroheterocycle prodrugs, nifurtimox (4-(5-nitrofurfurylidenamino)-3-methylthio-morpholine-1,1-dioxide) (Nfx) and benznidazole (*N*-benzyl-2-(2-nitro-1*H*-imidazol-1-yl)acetamide) (Bnz) (Chart 1), are used to treat Chagas disease.[4] However, their use is problematic as both can cause side effects and have limited efficacy while some strains are refractory to treatment.[5] In addition, the large quantities of medication required render it expensive, and the recommended long course of treatment is often not completed, resulting in the development of resistance. Therefore, the need for new drugs to treat this disease is urgent.

Chart 1



As with most nitroheterocyclic compounds, Nfx and Bnz both function as prodrugs and must undergo activation before mediating their cytotoxic effects. Initially it was proposed that the trypanocidal action of Nfx was due to its ability to induce oxidative stress within the parasite[5−7] and several trypanosomal flavoproteins have been shown to mediate the 1-electron reduction of this prodrug's conserved nitro-group that subsequently promotes formation of superoxide anions via a

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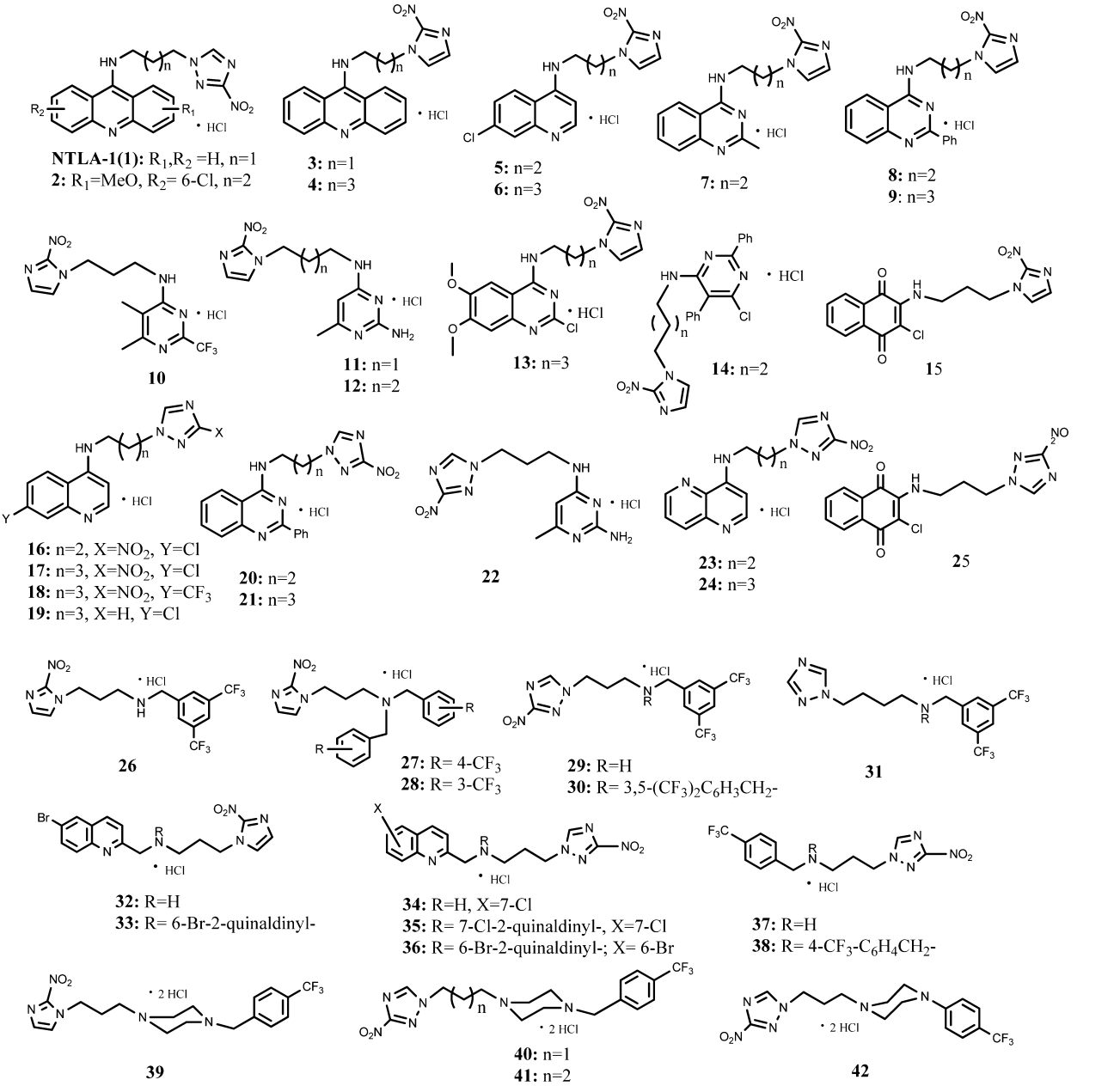
Chart 2



futile cycle.[7−9] However, although this reaction does occur in parasite cells, the available functional data suggests that it does not occur at levels that are toxic to the trypanosome.[10] Recently, an alternative reduction pathway has been elucidated involving the activity of a type I nitroreductase (NTR).[11] This enzyme can mediate a series of 2-electron reduction reactions of both Nfx and Bnz resulting in fragmentation of the heterocyclic ring and production of toxic metabolites.[10,12]

Recent reports about several new nitroheterocycles having trypanocidal activities with no or low toxicity,[13−18] in conjunction with the fact that the activation of nitroheterocyclic prodrugs can be catalyzed by the type I NTR, which is normally absent in most eukaryotes, with trypanosomes being a major exception, have led to a renewed interest in the use of these compounds as antiparasitic agents.

In collaboration with the Drugs for Neglected Diseases initiative (DNDi), we have found that 9-[(3-nitro-1*H*-1,2,4-triazolyl)-propylamino]acridine hydrochloride (NTLA-1 or NLA-6, **1**; Chart 2), a compound that was originally designed as a DNA-targeting anticancer agent[19,20] and which was screened against *T. cruzi*, *T. b. rhodesiense*, and *L. donovani*, was significantly and selectively active against *T. cruzi* amastigotes in infected L6 myoblasts, without showing toxicity for the host cells.[21] Thus, NTLA-1 demonstrated an IC_{50} of 140 nM for the parasite and a selectivity index (SI = IC_{50} for L6 cells/IC_{50} for *T. cruzi*) of 146.[21] NTLA-1, given at just 2 mg/kg/day for 50 days in mice infected with *T. cruzi*, in the acute phase of infection, resulted in a rapid and persistent drop in peripheral parasite levels and in a fraction of cures (20%).[22] 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.[22] However, NTLA-1, which inhibits topoisomerase I and

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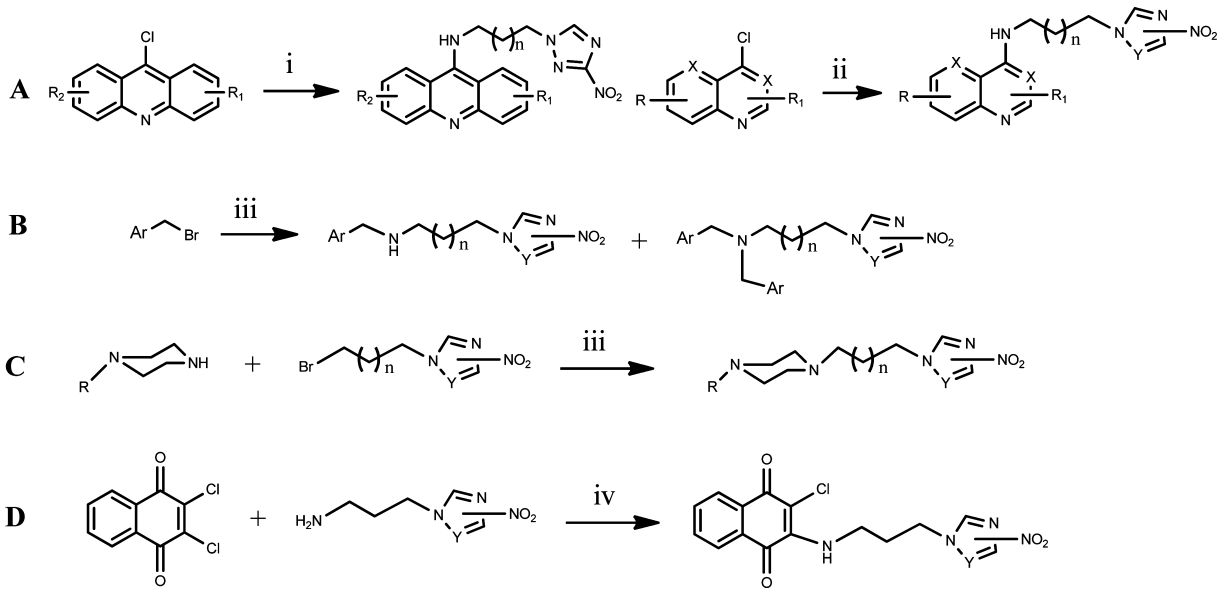
Scheme 1[a]



[a](i) EtOH, reflux, 12 h; (ii) propanol, reflux 12−30 h; (iii) K_2CO_3 (9 equiv), CH_3CN, RT, 48 h; (iv) CH_2Cl_2, RT. n = 1 or 2; X = C or N; Y = C (2-NO_2) or N(3-NO_2); other substituents vary.

II,[20] demonstrated toxicity at 15 mg/kg given ip for 30 days. Therefore, a more thorough investigation was initiated for the development of less toxic and more efficacious nitrotriazole- and nitroimidazole-based compounds as trypanocidal agents. Here we describe the synthesis and in vitro evaluation of 3-nitro-1*H*-1,2,4-triazole-based and 2-nitroimidazole-based aromatic and aliphatic amines as antiparasitic agents.

CHEMISTRY

The structure of all compounds is depicted in Chart 2. Their synthesis is straightforward and based on well-established chemistry, outlined in Scheme 1. Aromatic amines **1**−**14** and **16**−**24** were synthesized by coupling the appropriate nitrotriazole or nitroimidazole alkyl amine[19] with the appropriate chloro- or fluoro-aromatic chromophore[23] by nucleophilic aromatic substitution (Scheme 1A). The yields were in general moderate to good with the exception of **18**. Aliphatic secondary and tertiary amines **26**−**38** were synthesized via the same reaction by nucleophilic attack of the appropriate nitrotriazole or nitroimidazole alkyl amine to a chosen bromide in the presence of K_2CO_3 (Scheme 1B). In most cases, the monoalkylated product was the dominant one because 1 equiv of the required halide was used. Piperazine derivatives **39**−**42** were synthesized similarly, by nucleophilic attack of the appropriate, commercially available monosubstituted piperazine to the appropriate nitrotriazole or nitroimidazole alkyl bromide[24] (Scheme 1C). Finally, enamines **15** and **25** were synthesized from 2,3-dichloro-1,4-naphthoquinone and 2-nitroimidazole-propylamine or 3-nitro-1,2,4-triazole-propylamine, respectively, by nucleophilic substitution (Scheme 1D).

RESULTS AND DISCUSSION

Antiproliferative Effects of Nitrotriazole and Nitroimidazole Compounds. The in vitro growth inhibitory properties of all compounds 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) were evaluated by using standard drug screens.[25] From resultant dose−response curves, IC_{50} values in μM were determined (Table 1). The criteria for activity were set as follows: For *T. b. rhodesiense*, compounds that gave an IC_{50} < 0.5 μM, were designated as "active", while those yielding an IC_{50} = 0.5−6.0 μM or an IC_{50} > 6.0 μM were designated "moderately active" and "inactive", respectively. For *T. cruzi*, IC_{50} < 4.0 μM, "active"; IC_{50} = 4.0−60 μM, "moderately active"; IC_{50} > 60 μM, "inactive". For *L. donovani*, IC_{50} < 1 μM, "active"; IC_{50} = 1.0−6.0 μM, "moderately active"; IC_{50} > 6.0 μM, "inactive". On the basis of these criteria, all but compounds **19** and **31** were active or moderately active against *T. cruzi*, about 66% of all compounds were active or moderately active against *T. brucei rhodesiense*, and only six compounds (∼14%) were active or moderately active against *L. donovani* parasites. However, for a compound to be considered for further in vivo investigation, the growth inhibitory effect against the mammalian cell line L6 has to be evaluated from which a measure of a compound's cytotoxicity can be deduced. Thus, the selectivity index (SI), namely the ratio of IC_{50} against L6 cells to IC_{50} against each parasite, is also an important parameter. This SI must be ≥100 for *T. b. rhodesiense*, ≥50 for *T. cruzi*, and ≥20 for *L. donovani* axenic amastigotes.

On the basis of the above, only six compounds (**16**−**18**, **24**, **36**, **38**) were active and selective against *T. b. rhodesiense*, whereas 18 compounds (**1**, **2**, **16**−**18**, **20**, **23**, **24**, **27**, **29**, **34**−**38**, **40**−**42**) were active and selective against *T. cruzi* (Table 1). Only one compound, **38**, was active and selective against *L. donovani*. Therefore, the anti-Chagasic activity of these compounds is of the greatest interest based on the number of active molecules.

Evaluation of Structure−Activity Relationships: Analysis of the Nitroheterocyclic Ring. As a large set of the compounds showed significant anti-*T. cruzi* activity, we were able to conduct a detailed structure−activity relationship. Analysis of the trypanocidal activity in relation to the nitroheterocyclic ring revealed that compounds (**3**−**9**, **13**, **21**, **27**, **30**) that were active against *T. cruzi* (IC_{50} < 4 μM) but not sufficiently specific (SI < 50) were exclusively 2-nitroimidazole

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Table 1. In Vitro Screening Data against Three Different Trypanosomatids

Compound	All values as μM							Comp.
	T.b.rhodesiense[a]		*T. cruzi*[b]		*L. donovani axen.*[c]		Cytotox. L6[d]	
	IC-50	SI	IC-50	SI	IC-50	SI	IC-50	Type[e]
1	0.611	34	0.140	147	35.37	1	20.52	Nitro-Trz
2	0.134	74	0.151	66	10.15	1	9.935	Nitro-Trz
3	0.996	0	0.996	0	9.09	0	0.308	Nitro-Im
4	0.601	3	0.926	2	14.77	0	1.675	Nitro-Im
5	1.397	18	1.041	24	54.35	0	24.95	Nitro-Im
6	1.872	36	3.508	19	39.06	2	66.83	Nitro-Im
7	3.455	9	2.097	15	68.29	0	31.56	Nitro-Im
8	3.922	22	1.973	45	10.11	9	87.82	Nitro-Im
9	2.756	12	3.628	9	6.17	5	32.46	Nitro-Im
10	15.16	>16	4.28	>55	20.05	>12	>236.5	Nitro-Im
11	10.271	>28	7.081	>41	>95.69	~3	>287.0	Nitro-Im
12	11.782	>23	41.82	>7	98.07	>3	>274.7	Nitro-Im
13	2.257	7	0.968	17	3.63	5	16.48	Nitro-Im
14	15.46	1	9.67	1	12.43	1	11.71	Nitro-Im
15	0.621	6	30.12	0	0.516	8	3.91	Nitro-Im
16	0.309	463	0.607	236	45.78	3	143.22	Nitro-Trz
17	0.193	708	0.14	976	64.49	2	136.6	Nitro-Trz
18	0.117	973	0.305	373	136.56	1	113.83	Nitro-Trz
19	21.82	>14	92.14	>3	>298.5	~1	>298.5	Trz
20	1.417	68	1.48	66	9.48	10	96.99	Nitro-Trz
21	0.562	137	1.74	44	8.69	9	77	Nitro-Trz
22	2.191	>131	33.7	>8	>95.39	~3	>286	Nitro-Trz
23	2.22	60	1.031	129	182.29	1	132.67	Nitro-Trz
24	0.435	220	0.46	208	>275.5	0	95.77	Nitro-Trz
25	0.882	8	15.6	0	6.53	1	7.16	Nitro-Trz
26	13.8	10	24.48	6	>69.36	~2	139.65	Nitro-Im
27	23.04	>7	3.77	>46	6.49	>27	>172.25	Nitro-Im
28	21.57	4	8.02	10	4.27	19	79.62	Nitro-Im
29	7.84	18	0.169	816	11.07	12	137.9	Nitro-Trz
30	8.17	10	1.96	40	4.87	16	78.79	Nitro-Trz
31	32.30	>7	123.40	>2	172.25	>1	>223.6	Trz
32	8.56	16	6.05	23	80.28	2	137.03	Nitro-Im
33	14.23	7	4.67	20	18.67	5	93.6	Nitro-Im
34	1.05	136	0.311	460	63.81	2	143.13	Nitro-Trz
35	0.917	78	0.358	200	9.58	7	71.62	Nitro-Trz
36	0.463	99	0.32	144	9.33	5	46	Nitro-Trz
37	3.61	34	0.32	383	158.7	1	122.41	Nitro-Trz
38	0.271	339	0.145	634	0.348	264	91.88	Nitro-Trz
39	15.30	>13	20.72	>9	>191.49	~1	>191.49	Nitro-Im
40	1.38	>140	0.34	>562	58.15	>3	>191.08	Nitro-Trz
41	1.2	99	0.412	287	44.45	3	118.37	Nitro-Trz
42	5.33	10	0.04	1320	25.92	2	52.79	Nitro-Trz
Melarsoprol	0.01*							
Benznidazole	1.35*							
Miltefosine	0.44*							

active,

moderate activity

active, but cytotoxic, low specificity

compounds have been previously synthesized.[19, 23, 29-31]

[a]STIB 900 trypomastigotes. [b]Tulahuen C4 amastigotes. [c]MHOM-ET-67/L82 amastigotes. [d]Cytotoxicity measurements. [e]Nitro-Trz, 3-nitro-1*H*-1,2,4-triazole; Nitro-Im, 2-nitro-1*H*-imidazole; Trz, not nitro triazole. *Median values from 43 measurements in parallel with each compound. SI = IC_{50} in L6 cells/IC_{50} in parasites.

derivatives except for compounds **21** and **30**. Similarly, moderately active compounds with low specificity against *T. cruzi* (**11**, **12**, **14**, **15**, **22**, **25**, **26**, **28**, **32**, **33**, **39**) were seen mainly in the 2-nitroimidazole series. In contrast, all 3-nitrotriazoles, with the exception of **22** and **25**, demonstrated significant in vitro anti-*T. cruzi* activity coupled with excellent selectivity (Table 1). In all cases where an active/moderately active trypanocidal effect was observed, irrespective of SI values, the 3-nitrotriazole derivatives (**1**, **16**, **17**, **20**, **21**, **29**, **38**) always had a greater effect (1.3−45 fold) on parasite growth as compared to their 2-nitroimidazole counterparts (**3**, **5**, **6**, **8**, **9**, **26**, **27**) and no toxicity: compare **1** with **3**, **16** with **5**, **17** with **6**, etc. (Table 1). Similar results are seen even with the moderately active and not specific 3-nitrotriazole **25** (a naphthoquinone derivative), which is 2-fold more potent than its 2-nitroimidazole analogue **15**.

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Table 2. Biological and Physical Properties of Analogues Active against *T. cruzi* Amastigotes

compd	*T. cruzi* IC_{50} (uM)	SI	Bzn/Comp[a]	clogP	pK_a	Lipinski rule of 5	PSA (A^2)
1	0.14	147	9.6	3.20	9.20	S	101.45
2	0.15	66	8.9	4.16	8.84	S	110.68
8	1.97	45	0.7	4.56	5.06	S	101.45
16	0.61	236	2.2	2.43	7.31	S	101.45
17	0.14	976	9.6	2.95	7.31	S	101.45
18	0.31	373	4.4	3.22	7.53	S	101.45
20	1.48	66	0.9	4.05	5.06	S	114.34
21	1.74	44	0.8	4.52	5.06	S	114.34
23	1.03	129	1.3	0.99	6.81	S	114.34
24	0.46	208	2.9	1.51	6.81	S	114.34
29	0.17	816	8.0	3.51	9.44	S	88.56
34	0.31	460	4.3	2.60	8.76	S	101.45
35	0.36	200	3.8	5.55	6.87	V(2)	105.55
36	0.32	144	4.2	5.88	6.87	V(2)	105.55
37	0.32	383	4.2	2.63	9.65	S	88.56
38	0.15	634	9.3	5.62	8.79	V(2)	79.77
40	0.34	>562	4.0	2.86	8.33	S	83.01
41	0.41	287	3.3	3.38	8.52	S	83.01
42	0.04	1320	33.8	3.03	7.85	S	83.01
Bnz	1.32[b]		1.0	1.32		S	92.74

[a]Bzn/Comp: IC_{50} of Bnz/IC_{50} of Comp. PSA: polar surface area. [b]Median values from 43 measurements in parallel with each compound. All physical properties were predicted by using the Marvin Calculator (www.chemaxon.com).

To determine whether the nitro-group was important in the antiparasitic activity of the triazoles, two non-nitro compounds (**19** and **31**) were synthesized and their growth inhibitory properties against *T. cruzi* compared with that of their nitro-analogues **17** and **29** (note: **31** has an extra methylene group as compared to **29**). In both cases, the removal of the nitro-group led to inactivity (IC_{50} > 60 μM) and the IC_{50} value was significantly increased (658- and 730-fold, respectively) compared to the nitro-containing analogue (Table 1). The anti-HAT activity of **19** and **31** was also reduced compared to that of **17** and **29** but to a lesser degree. Therefore, the nitro group present on the triazole ring is essential in mediating the antiparasitic activity of these compounds.

Analysis of Aromatic Amines. A closer look at the SARs for all **anti-Chagasic** compounds is given in Table 2. In the subclass of 3-nitrotriazole bearing aromatic amines (**1**, **2**, **16**–**18**, **20**, **21**, **23**, **24**), activity decreases in the following order: acridines (**1**, **2**) ≥ quinolines (**16**–**18**) > 1,5-naphthyridines (**23**, **24**) > quinazolines (**20**, **21**). The 2-nitroimidazole linked quinazoline derivative **8** demonstrates similar activity with the 3-nitrotriazole analogues **20** and **21**.

An extra methylene group in the linkage in compound **2** and the chloro-substituent in the acridine ring increased lipophilicity and toxicity, compared to its analogue **1**, but did not decrease activity. It is assumed that the acridine compounds **1** and **2** demonstrate increased toxicity and lack of sufficient selectivity due to DNA-intercalation[19] and topoisomerase I/II inhibition.[20] Thus, compound **1**, which was tested in vivo for Chagas, could not be given at sufficient doses for an extended period of time due to the observed toxicity.[22]

Comparing the quinoline analogues **16** and **17**, it is observed that increased lipophilicity in **17**, due to an extra methylene group in the linkage, slightly increased toxicity (Table 1); however, at the same time, activity was also increased, resulting in an improved SI (by a factor of 4) compared to **16**. Comparing the analogues **17** and **18**, it is observed that the replacement of chlorine in **17** with a trifluoromethyl group increases lipophilicity and toxicity in **18**, however the activity remains at low nM concentrations, slightly less than that in **17**, but still better than the one in **16**. All three quinoline compounds show excellent selectivity, significantly higher than the threshold of 50 we have set, and are candidates for in vivo studies.

Comparing the quinazoline systems **20** and **21** (Tables 1 and 2), it is observed that in this case an extra methylene group in the linkage of **21** did not improve the anti-Chagasic activity but increased lipophilicity and thus toxicity, lowering thus the SI from 66 to 44. Similar results, but significantly more prominent, can be seen with the 2-nitroimidazole-based quinazoline systems **8** and **9**, which are the corresponding analogues of **20** and **21**, respectively; in this case, **9** was totally inactive, whereas **8** is more comparable with **21** rather than **20** with regard to its anti-Chagasic activity and selectivity (Table 2).

Finally, in the case of the two naphthyridine compounds **23** and **24**, the beneficial effect of an extra methylene group in the linkage of **24** is reflected in its improved activity and selectivity, despite its increased toxicity (Tables 1 and 2).

It is worth mentioning that while alteration in the length of the linkage between the nitro-triazole/imidazole ring and aromatic chromophore in the aromatic amines can not always predict the direction of changes in the anti-Chagasic activity, it is clear in all cases (**2**, **17**, **18**, **21**, **24**) that four methylene groups in the linkage favor anti-HAT activity (Table 1).

Analysis of Aliphatic Amines. The 3-nitrotriazole-based benzylamines **29**, **37**, and **38** are all active against *T. cruzi* and demonstrate very good SI values (Table 2). The dibenzylated derivative **38** is significantly more lipophilic and thus more toxic than the monobenzylated analogue **37**, violating twice the Lipinski rule of 5 (Table 2). However, its increased anti-Chagasic activity balances out its toxicity, so it appears with a better SI value than **37** (Tables 1 and 2). Interestingly, **38** is the only compound active across all parasites tested (Table 1). Compound **29**, although more lipophilic (due to 2 trifluoromethyl groups) than **37**, appears less toxic, perhaps because

Table 3. The Effect of Type I Nitroreductase (TbNTR) on the Activity of Selected Compounds against Bloodstream-Form *T. brucei brucei* Parasites

	T. b. brucei[a]	TbNTR[b]	TbNTR[b]	ratio	
compd	IC_{50} (μM)	−tet	+tet	−tet/+tet	$E_{1/2}$[c] (V)
8	7.47 ± 0.71	7.58 ± 0.19	0.95 ± 0.11	8.00	−1.03
17	0.17 ± 0.04	0.44 ± 0.06	0.10 ± 0.04	4.00	−1.18
20	2.63 ± 0.25	4.48 ± 0.19	0.07 ± 0.02	64.00	−1.04
23	>10	nd	nd	nd	nd
29	7.83 ± 0.32	11.08 ± 2.50	0.76 ± 0.16	14.00	−1.07
38	0.21 ± 0.01	0.20 ± 0.01	0.10 ± 0.02	2.00	−1.06
40	>10	nd	nd	nd	nd
41	>10	nd	nd	nd	−1.04
42	2.30 ± 0.10	2.63 ± 0.12	0.21 ± 0.01	13	nd
Nifurtimox[d]		1.71 ± 0.06	0.13 ± 0.04	13	−0.88

[a]Bloodstream-form wild type *T. brucei brucei* parasites. [b]Bloodstream-form *T. b. brucei*, engineered to overexpress type I nitroreductase in the presence of tetracycline (tet). [c]Reduction potential of each compound was measured in DMSO (except for **17**, in CH_3CN) by cyclic voltammetry relative to Ag/AgCl. [d]The $E_{1/2}$ value is taken from ref 32.

the trifluoromethyl groups being in meta positions offer a better compound stability compared to **37**.

The 3-nitrotriazole-based quinaldinamines **34**, **35**, and **36** demonstrate similar anti-Chagasic activity, and their SI corresponds inversely to their clogP value and toxicity (Table 1 and 2). All three analogues have similar activity with the *p*-trifluoromethylbenzylamine **37**, but the monoalkylated chloroquinaldine analogue **34** demonstrates a superior SI value, presumably due to its decreased toxicity compared to **37**, despite the fact that both **34** and **37** have similar clogP values. As was expected, the dialkylated analogues **35** and **36** also violate the Lipinski rule of 5.

The piperazine systems (**40**−**42**) showed significant anti-Chagasic activity in vitro (Table 1). However, the 1-phenylpiperazine **42** showed about 10-fold increased activity (IC_{50} at low nM concentrations) compared to the 1-benzyl-piperazines **40** and **41**. Although the lipophilicity of **42** was between that of **40** and **41**, its toxicity was higher than both of them. Despite an increased toxicity (Table 1), the SI of **42** was 1320, the highest of all tested compounds, making **42** a good candidate for in vivo studies. Comparing the substituted benzylpiperazine derivatives **40** and **41**, it is observed that an extra methylene in **41**, in the linkage between 3-nitrotriazole and the piperazine ring, decreased potency and increased lipophilicity and toxicity, resulting in a lower SI value compared to **40** (Table 2).

It can be observed that all compounds with anti-Chagasic activity in Table 2 have a polar surface area <140 and >60 Å^2, which means good cell-membrane permeability and presumably absence of neurotoxicity because they can not cross the blood−brain barrier. In addition, all but compounds **8**, **20**, and **21** (all 2-phenylquinazolines) demonstrate a better anti-Chagasic activity (1.3−33.8-fold) than the reference compound benznidazole, tested in parallel. It appears that increased anti-Chagasic activity is observed with increased basicity in the amines of Table 2.

Evaluating the Mechanism of Action of Nitrotriazoles. As was mentioned earlier, nitroheterocyclic prodrugs must undergo enzyme-mediated activation within the pathogen to have cytotoxic effects. These enzymes are most likely nitroreductases, although other reducing enzymes specific to the parasite, such as trypanothione reductase[8,26] or NADH-fumarate reductase,[27] could be involved. Both Nfx and Bnz are activated by the NADH-dependent, oxygen insensitive, mitochondrially localized, bacterial-like, type I NTR, and down-regulation of this enzyme explains how resistance emerges.[10−12] Therefore, we investigated the role of recombinant *T. brucei* NTR (TbNTR) in the activation of selected nitrotriazoles and the nitroimidazole **8** (Figure 1, Supporting Information), as well as the susceptibility of bloodstream-form *T. brucei brucei*, engineered to overexpress tetracycline-inducible TbNTR, to these compounds (Table 3). The reduction potentials ($E_{1/2}$) of the active compounds toward bloodstream-form *T. b. brucei* were also measured by cyclic voltametry, to elucidate if there is any correlation between enzymatic activity and redox properties, and are shown in Table 3. Compounds from all subcategories (aromatic and aliphatic amines, as well as piperazinic derivatives) have been chosen for these studies.

With regard to anti-HAT activity, it is observed that compounds **17** and **38** that were very active against *T. b. rhodesiense* (Table 1) and were similarly active against bloodstream-form *T. b. brucei* (Table 3), whereas compounds that were inactive (**8**, **29**, **42**) or moderately active (**20**, **23**, **40**, **41**) against *T.b. rhodesiense* (Table 1) were in general more inactive against bloodstream-form *T. b. brucei* (Table 3). With regard to the tetracycline (+tet)-inducible TbNTR overexpression system, it is observed that parasites induced to overexpress TbNTR are more susceptible to all nitrotriazoles/nitroimidazole tested, with compounds being moderately active against bloodstream-form *T. b. brucei* showing a greater difference than the most active **17** and **38**. As a general rule of thumb, if a −tet/+tet ratio is >5, then it is assumed that the major growth inhibitory activity of a compound is via NTR activation. For compounds with a −tet/+tet ratio <5, alternative systems may be involved or the NTR generated reduction products are extremely trypanocidal.

There was no correlation between trypanocidal activity and enzymatic activity (see Supporting Information). Furthermore, no conclusive data were obtained by comparing the enzymatic activity with reduction potentials ($E_{1/2}$), although there was a trend suggesting an increasing activity at more negative $E_{1/2}$ values, values that possibly lie outside the normal range of mammalian redox systems. If this is true, then the mutagenic potential of these compounds may be low,[28] something that has been confirmed in limited Ames studies with **16**, **20**, and **29** (data not shown).

CONCLUSION

In conclusion, nine nitrotriazole-based compounds (**16**−**18**, **24**, **29**, **34**, **40**−**42**) have been identified from Table 2 as potential candidates for in vivo studies in *T. cruzi* infected mice and further development against Chagas. All of them have demonstrated significant anti-Chagasic activity at low to intermediate nmolar concentrations, SI values of ≥200, and satisfy the Lipinski's rule of 5. In addition, compound **38** may also warrant additional attention as it displays significant antiparasitic activity against *T. cruzi*, both *T. brucei* subspecies and *L. major* with high selectivity, although this compound does violate two of the Lipinski's rule of 5.

EXPERIMENTAL SECTION

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 Me_4Si or to the corresponding protonated solvent if the solvent was not $CDCl_3$. 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/UV_{254} or polygram silica gel G/UV_{254} coated plates (0.2 mm, Analtech, Newark, DE). Chromatography was carried out on preparative TLC alumina GF (1000 μm) or silicagel GF (1500 μm) plates (Analtech). All the amines were purified by preparative TLC chromatography on alumina plates (≥95% purity). The results from elemental analysis for C, H, and N were within 0.4 of the theoretical value.

The synthesis of compounds **1**, **3**−**7**, **10**, and **15** has been described before.[19,23,29−31] Compounds **2** and **25** were synthesized in a similar manner with **1**[19] and **15**,[31] respectively.

General Synthetic Procedure of Aromatic Amines. For compounds **8**−**14**, **16**−**24**: The appropriate chloro-aromatic starting material (commercially available in most cases) (1.24 mmol) was coupled with 2-nitro-1*H*-imidazolyl-alkylamine (1.24 mmol)[19] or 3-nitro-1,2,4-triazolyl-alkylamine (1.24 mmol)[19] by refluxing in absolute propanol (7−10 mL) for 12 −30 h. In the case of compounds **16**, **17**, and **19**, the 4,7-dichloroquinoline was first converted to 7-chloro-4-fluoroquinoline[23] before coupling. In the case of compound **19**, 4-(1*H*-1,2,4-triazol-1-yl)butylamine was first synthesized as in ref 19, to be then coupled with 7-chloro-4-fluoroquinoline. In the case of compound **18**, 4-fluoro-7-trifluoromethyl quinoline could not be synthesized from the corresponding 4-chloro-7-trifluoromethylquinoline. In most cases the hydrochloride salt of the final product was precipitated upon cooling of the reaction mixture and separated by filtration. In some cases, the free amine of the desired product was isolated by preparative TLC on alumina, dissolved in ethyl acetate and converted to its HCl salt by treating with 1 M HCl in diethyl ether. In the case of compounds **23** and **24**, the starting material 4-chloro-1,5-naphthyridine was synthesized in 4 steps as described previously.[29]

General Synthetic Procedure of Mono- and Dialkylated Aliphatic Amines 26−38. The appropriate bromide (1.035 mmol) was added dropwise (15 min) to a solution of 2-nitro-1*H*-imidazolyl-alkylamine (1.035 mmol) or 3-nitro-1*H*-1,2,4-triazolyl-alkylamine (1.035 mmol)[19] in the presence of potassium carbonate (9.52 mmol) in dry acetonitrile (15 mL), and the reaction mixture was stirred under a nitrogen atmosphere at room temperature for 48 h. In the case of **31**, 4-(1*H*-1,2,4-triazol-1-yl)butylamine was used. The reaction mixture was then filtered, the solids were washed with acetonitrile, the organic filtrate was evaporated, and the residue extracted from water−chloroform. The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was evaporated, and the residue was separated by preparative TLC on alumina plates with ethyl acetate:petroleum ether mixture. Monoalkylated and dialkylated products were obtained in the same reaction at varying ratios for each case. The separated products were dissolved in ethyl acetate and converted to their HCl salts by treating with HCl gas in dry ether (1 M solution).

Piperazine derivatives (**39**−**42**) were synthesized from the commercially available appropriate monoalkylated piperazines (1.44 mmol) and the appropriate 2-nitro-1*H*-imidazolyl-alkylbromide or 3-nitro-1*H*-1,2,4-triazolyl-alkylbromide (1.485 mmol)[24] in the presence of potassium carbonate (13.24 mmol) in dry acetonitrile (25 mL) as above.

6-Chloro-2-methoxy-*N*-[4-(3-nitro-1*H*-1,2,4-triazol-1-yl)-butyl]acridin-9-amine Hydrochloride (2). Yellow powder (35%): mp 214−216 °C. ^{1}H NMR (500 MHz, CD_3OD) δ: 8.58 (s, 1H), 8.44 (d, J = 9.5 Hz, 1H), 7.78 (m, 3H), 7.68 (dd, J = 8.0, 2.0 Hz, 1H), 7.50 (d, J = 9.0 Hz, 1H), 4.40 (t, J = 6.5 Hz, 2H), 4.18 (t, J = 7.0, 2H), 4.01 (s, 3H), 2.11 (m, 2H), 1.99 (m, 2H). HRESIMS calcd for $C_{20}H_{20}ClN_6O_3$ m/z $[M + H]^+$ 427.1286, found 427.1286.

***N*-[3-(2-Nitro-1*H*-imidazol-1-yl)propyl]-2-phenylquinazolin-4-amine Hydrochloride (8).** Off-white powder (44%): mp 174−176 °C (dec). ^{1}H NMR (500 MHz, CD_3OD) δ: 8.34 (d, J = 8.5 Hz, 1H), 8.27 (d, J = 7.5 Hz, 2H), 8.07 (t, J = 7.5 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H), 7.81−7.76 (m, 2H), 7.69 (t, J = 8.0 Hz, 2H), 7.59 (s,1H), 7.13 (s, 1H), 4.68 (t, J = 7.0 Hz, 2H), 4.04 (t, J = 7.0 Hz, 2H), 2.45 (quintet, J = 7.0 Hz, 2H). HRESIMS calcd for $C_{20}H_{19}N_6O_2$ m/z $[M + H]^+$ 375.1570, found 375.1569.

***N*-[4-(2-Nitro-1*H*-imidazol-1-yl)butyl]-2-phenylquinazolin-4-amine Hydrochloride (9).** Off-white powder (51%). ^{1}H NMR (500 MHz, CD_3OD) δ: 8.34−8.30 (m, 3H), 8.05 (t, J = 7.5 Hz, 1H), 7.96 (d, J = 8.5 Hz, 1H), 7.80−7.75 (m, 2H), 7.69 (t, J = 8.0 Hz, 2H), 7.49 (s,1H), 7.08 (s, 1H), 4.55 (t, J = 7.0 Hz, 2H), 3.99 (t, J = 7.0 Hz, 2H), 2.06 (quintet, J = 7.0 Hz, 2H), 1.93 (quintet, J = 7.0 Hz, 2H). HRESIMS calcd for $C_{21}H_{21}N_6O_2$ m/z $[M + H]^+$ 389.1721, found 389.1729.

6-Methyl-4-*N*-[3-(2-nitro-1*H*-imidazol-1-yl)propyl]-pyrimidin-2,4-diamine Hydrochloride (11). Orange solid (34%): mp 178 °C (dec). ^{1}H NMR (500 MHz, CD_3OD) δ: 7.53 (s, 1H), 7.17 (s, 1H), 5.89 (s, 1H), 4.55 (t, J = 7.0 Hz, 2H), 3.52 (t, J = 6.5, 2H), 2.25 (s, 3H), 2.19 (m, 2H). HRESIMS calcd for $C_{11}H_{16}N_7O_2$ m/z $[M + H]^+$ 278.1366, found 278.1364.

6-Methyl-4-*N*-[4-(2-nitro-1*H*-imidazol-1-yl)butyl]-pyrimidin-2,4-diamine Hydrochloride (12). Off-white powder (45%): mp 225−226 °C (dec). ^{1}H NMR (500 MHz, CD_3OD) δ: 7.50 (s, 1H), 7.15 (s, 1H), 5.86 (s, 1H), 4.51 (t, J = 7.5 Hz, 2H), 3.49 (t, J = 6.5 Hz, 2H), 2.23 (s, 3H), 1.92 (quintet, J = 7.5 Hz, 2H), 1.66 (quintet, J = 7.5 Hz, 2H). HRESIMS calcd for $C_{12}H_{18}N_7O_2$ m/z $[M + H]^+$ 292.1522, found 292.1530.

2-Chloro-6,7-dimethoxy-*N*-[4-(2-nitro-1*H*-imidazol-1-yl)-butyl]quinazolin-4-amine (13). Light-yellowish powder (21%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.23 (s, 1H), 7.17 (s, 1H), 7.14 (s, 1H), 6.91 (s, 1H), 5.93 (br t, 1H), 4.54 (t, J = 7.5 Hz, 2H), 4.00 (s, 3H), 3.98 (s, 3H), 3.80−3.76 (m, 2H), 2.00 (quintet, J = 7.5 Hz, 2H), 1.82 (quintet, J = 7.5 Hz, 2H). HRESIMS calcd for $C_{17}H_{20}ClN_6O_4$ m/z $[M + H]^+$ 407.1229, found 407.1236.

6-Chloro-*N*-[4-(2-nitro-1*H*-imidazol-1-yl)butyl]-2,5-diphenyl-pyrimidin-4-amine Hydrochloride (14). Pale-white powder (72%): mp 79−81 °C. ^{1}H NMR (500 MHz, CD_3OD) δ: 8.21 (d, J = 7.5 Hz, 2H), 7.68−7.56 (m, 6H), 7.46 (s, 1H), 7.39 (d, J = 7.5 Hz, 2H), 7.08 (s, 1H), 4.49 (t, J = 7.0 Hz, 2H), 3.66 (t, J = 7.0, 2H), 1.91 (quintet, J = 7.5 Hz, 2H), 1.71 (quintet, J = 7.5 Hz, 2H). HRESIMS calcd for $C_{23}H_{22}ClN_6O_2$ m/z $[M + H]^+$ 449.1487, found 449.1488.

7-Chloro-*N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]quinolin-4-amine Hydrochloride (16). White powder (84%): mp 240−242 °C. ^{1}H NMR (500 MHz, D_2O) δ: 8.61 (s, 1H), 8.28 (d, J = 7.0 Hz, 1H), 7.89 (d, J = 9.0 Hz, 1H), 7.82 (s, 1H), 7.57 (d, J = 9.0 Hz, 1H), 6.76 (d, J = 7.0 Hz, 1H), 4.55 (t, J = 6.5 Hz, 2H), 3.74 (t, J = 6.5, 2H), 2.48 (m, 2H). HRESIMS calcd for $C_{14}H_{14}ClN_6O_2$ m/z $[M + H]^+$ 333.0867, found 333.0866.

7-Chloro-*N*-[4-(3-nitro-1*H*-1,2,4-triazol-1-yl)butyl]quinolin-4-amine Hydrochloride (17). White powder (67%): mp 210−220 °C

(dec). ^{1}H NMR (500 MHz, D_2O) δ: 8.58 (s, 1H), 8.20 (d, J = 7.0 Hz, 1H), 8.03 (d, J = 9.0 Hz, 1H), 7.78 (s, 1H), 7.68 (d, J = 9.0 Hz, 1H), 6.68 (d, J = 7.0 Hz, 1H), 4.39 (t, J = 6.5 Hz, 2H), 3.58 (t, J = 7.0, 2H), 2.07 (m, 2H), 1.77 (m, 2H). HRESIMS calcd for $C_{15}H_{16}ClN_6O_2$ m/z $[M + H]^+$ 347.1023, found 347.1019.

***N*-[4-(3-Nitro-1*H*-1,2,4-triazol-1-yl)butyl]-7-(trifluoromethyl)-quinolin-4-amine Hydrochloride (18).** White powder (14%). ^{1}H NMR (500 MHz, CD_3OD) δ: 8.64 (s, 1H), 8.57 (d, J = 8.5 Hz, 1H), 8.50 (d, J = 7.0 Hz, 1H), 8.16 (s, 1H), 7.95 (d, J = 8.5 Hz, 1H), 4.44 (t, J = 6.5 Hz, 2H), 3.69 (t, J = 7.0 Hz, 2H), 2.13 (m, 2H), 1.86 (m, 2H). HRESIMS calcd for $C_{16}H_{16}F_3N_6O_2$ m/z $[M + H]^+$ 381.1281, found 381.1286.

7-Chloro-*N*-[4-(1*H*-1,2,4-triazol-1-yl)butyl]quinolin-4-amine (19). White powder (43%): mp 125−127 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.54 (d, J = 5.30 Hz, 1H), 8.10 (s,1H), 8.01 (s, 1H), 7.96 (d, J = 2.1 Hz,1H), 7.73 (d, J = 9.0, 1H), 7.37 (dd, J = 8.9, 2.1 Hz, 1H), 6.39 (d, J = 5.4 Hz, 1H), 5.32 (br s, 1H), 4.30 (t, J = 6.8 Hz, 2H), 3.37 (m, 2H), 2.10 (m, 2H), 1.79 (m, 2H). HRESIMS calcd for $C_{15}H_{17}ClN_5O_2$ m/z $[M + H]^+$ 302.1167, found 302.1169.

***N*-[3-(3-Nitro-1*H*-1,2,4-triazol-1-yl)propyl]-2-phenyl-quinazolin-4-amine Hydrochloride (20).** Yellow powder (71%): mp 246−248 °C (dec). ^{1}H NMR (500 MHz, CD_3SOCD_3) δ: 8.91 (s, 1H), 8.49 (br d, J = 7.0 Hz,1H), 8.33 (d, J = 7.5 2H), 8.08 (br s, 1H), 8.03 (br s, 1H), 7.75 (br s, 2H), 7.65 (br t, J = 7.0 Hz, 2H), 4.52 (t, J = 6.5 Hz, 2H), 3.90 (br m, 2H), 2.38 (t, J = 6.5 Hz, 2H). HRESIMS calcd for $C_{19}H_{18}N_7O_2$ m/z $[M + H]^+$ 376.1522, found 376.1523.

***N*-[4-(3-Nitro-1*H*-1,2,4-triazol-1-yl)butyl]-2-phenyl-quinazolin-4-amine Hydrochloride (21).** Yellow powder (69%): mp >250 °C. ^{1}H NMR (500 MHz, CD_3SOCD_3) δ: 8.90 (s, 1H), 8.57 (d, J = 8.0 Hz,1H), 8.39 (d, J = 7.5 2H), 8.16 (d, J = 8.0 Hz, 1H), 8.04 (t, J = 7.5 Hz, 1H), 7.76 (t, J=7.0, 2H), 7.68 (t, J = 7.5 Hz, 2H), 4.41 (t, J = 7.0 Hz, 2H), 3.86 (br q, J = 6.0 Hz, 2H), 2.012 (quintet, J = 7.5 Hz, 2H), 1.78 (quintet, J = 7,0 Hz, 2H). HRESIMS calcd for $C_{20}H_{20}N_7O_2$ m/z $[M + H]^+$ 390.1679, found 390.1681. Calculated analysis for $C_{20}H_{20}ClN_7O_2$: C, 56.41; H, 4.73; N, 23.02; Cl, 8.33. Found: C, 56.06; H, 5.01; N, 22.84; Cl, 9.06.

6-Methyl-4-*N*-[3-(2-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-pyrimidin-2,4-diamine Hydrochloride (22). Off-white powder (58%): mp 204−206 °C. ^{1}H NMR (500 MHz, CD_3OD) δ: 8.60 (s, 1H), 5.85 (s, 1H), 4.42 (t, J = 6.5 Hz, 2H), 3.53 (t, J = 6.5, 2H), 2.28 (m, 2H), 2.24 (s, 3H). HRESIMS calcd for $C_{10}H_{15}N_8O_2$ m/z $[M + H]^+$ 279.1318, found 279.1319.

***N*-[3-(3-Nitro-1*H*-1,2,4-triazol-1-yl)propyl]-1,5-naphthyridin-4-amine Hydrochloride (23).** Yellowish powder (50%): mp 215−217 °C (dec). ^{1}H NMR (500 MHz, CD_3OD) δ: 8.94 (d, J = 3.5 Hz,1H), 8.65 (s, 1H), 8.47 (d, J = 7.0 Hz, 1H), 8.27 (d, J = 8.5 Hz,1H), 7.95 (dd, J = 8.5, 4.5 Hz, 1H), 7.09 (d, J = 7.0 Hz, 1H), 4.54 (t, J = 6.5 Hz, 2H), 3.80 (t, J = 7.0, 2H), 2.48 (m, 2H). HRESIMS calcd for $C_{13}H_{14}N_7O_2$ m/z $[M + H]^+$ 300.1209, found 300.1206.

***N*-[4-(3-Nitro-1*H*-1,2,4-triazol-1-yl)butyl]-1,5-naphthyridin-4-amine Hydrochloride (24).** Off-white powder (61%). ^{1}H NMR (500 MHz, CD_3OD) δ: 8.97 (d, J = 4.0 Hz,1H), 8.64 (s, 1H), 8.43 (d, J = 7.0 Hz, 1H), 8.26 (d, J = 8.5 Hz,1H), 7.95 (dd, J = 8.5, 4.5 Hz, 1H), 7.06 (d, J = 7.5 Hz, 1H), 4.44 (t, J = 7.0 Hz, 2H), 3.72 (t, J = 7.0, 2H), 2.12 (m, 2H), 1.85 (m, 2H). HRESIMS calcd for $C_{14}H_{16}N_7O_2$ m/z $[M + H]^+$ 314.1360, found 314.1362.

2-Chloro-3-{[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]amino}-1,4-dihydronaphthalene-1,4-dione (25). Dark-red powder (74%): mp 137−138 °C. ^{1}H NMR (500 MHz, CD_3COCD_3) δ: 8.69 (s, 1H), 8.06 (d, J = 8.0 Hz,1H), 8.02 (d, J = 8.5 Hz, 1H), 7.84 (t, J = 8.0 Hz,1H), 7.75 (t, J = 8.0 Hz, 1H), 6.96 (br s, 1H), 4.61 (t, J = 7.0 Hz, 2H), 4.04 (t, J = 7.0 Hz, 2H), 2.44 (m, 2H). HRESIMS calcd for $C_{15}H_{13}ClN_5O_4$ m/z $[M + H]^+$ 362.0651, 364.0627, found 362.0654, 364.0632.

{[3,5-Bis(trifluoromethyl)phenyl]methyl}[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]amine Hydrochloride (29). White powder (60−64%): mp 140−142 °C. ^{1}H NMR (500 MHz, CD_3OD) δ: 8.68 (s, 1H), 8.20 (s, 2H), 8.12 (s, 1H), 4.52 (m, 2H), 4.44 (s, 2H), 3.27 (t, J = 8.0 Hz, 2H), 2.40 (m, 2H). HRESIMS calcd for $C_{14}H_{14}F_6N_5O_2$ m/z $[M + H]^+$ 398.1052, found 398.1054.

Bis({[3,5-bis(trifluoromethyl)phenyl]methyl})[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]amine Hydrochloride (30). White powder (8.5%): mp 138−140 °C (dec). ^{1}H NMR (500 MHz, CD_3OD) δ: 8.61 (s, 1H), 8.06 (s, 4H), 8.00 (s, 2H), 4.60−4.48 (br m, 6H), 3.29 (br m, 2H), 2.54 (br m, 2H). HRESIMS calcd for $C_{23}H_{18}F_{12}N_5O_2$ m/z $[M + H]^+$ 624.1263 found 624.1279.

{[3,5-Bis(trifluoromethyl)phenyl]methyl}[4-(1*H*-1,2,4-triazol-1-yl)butyl]amine Hydrochloride (31). White powder (37%): mp 120−123 °C. ^{1}H NMR (500 MHz, CD_3OD) δ: 9.00 (s, 1H), 8.33 (s, 1H), 8.19 (s, 2H), 8.12 (s, 1H), 4.41 (br s, 4H), 3.17 (br t, J = 5.8 Hz, 2H), 2.03 (m, 2H), 1.76 (m, 2H). HRESIMS calcd for $C_{15}H_{17}F_6N_4$ m/z $[M + H]^+$ 367.1352, found 367.1338.

[(6-Bromoquinolin-2-yl)methyl][3-(2-nitro-1*H*-imidazol-1-yl)propyl]amine Hydrochloride (32). White powder (22%): mp 158−160 °C (dec). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.33 (d, J = 8.4 Hz, 1H), 8.19 (s, 1H), 7.99 (d, J = 9.6 Hz, 1H), 7.89 (d, J = 8.8 Hz, 1H), 7.53 (s, 1H), 7.52 (d, J = 9.6 Hz, 1H), 7.17 (s, 1H), 4.61 (t, J = 7.2 Hz, 2H), 4.59 (s, 2H), 3.34 (br t, 2H), 2.41 (m, 2H). HRESIMS calcd for $C_{16}H_{17}BrN_5O_2$ m/z $[M + H]^+$ 390.0566, 392.0545 found 390.0569. 392.0551.

Bis[(6-bromoquinolin-2-yl)methyl][3-(2-nitro-1*H*-imidazol-1-yl)propyl]amine Hydrochloride (33). Pinkish powder (18%). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.36 (d, J = 8.4, 2H), 8.2 (s, 2H), 7.91 (s, 4H), 7.57 (d, J = 8.4 Hz, 2H), 7.47 (s, 1H), 7.09 (s, 1H), 4.95 (s, 4H), 4.63 (t, J = 7.6 Hz, 2H), 3.66 (t, J = 8.0 Hz, 2H), 2.60 (m, 2H). HRESIMS calcd for $C_{26}H_{23}Br_2N_6O_2$ m/z $[M + H]^+$ 609.0249, 611.0229, 613.0208, found 609.0251, 611.0233, 613.0210.

[(7-Chloroquinolin-2-yl)methyl][3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]amine Hydrochloride (34). Beige powder (29%): mp 135 °C (dec). ^{1}H NMR (500 MHz, CD_3OD) δ: 8.68 (s, 1H), 8.42 (d, J = 8.5 Hz, 1H), 8.12 (s, 1H), 7.99 (d, J = 9.0 Hz, 1H), 7.64 (d, J = 8.5 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 4.64 (s, 2H), 4.55 (t, J = 6.5 Hz, 2H), 3.35 (t, J = 8.0 Hz, 2H), 2.50 (m, 2H). HRESIMS calcd for $C_{15}H_{16}ClN_6O_2$ m/z $[M + H]^+$ 347.1018, 349.0994, found 347.1003. 349.0985.

Bis[(7-chloroquinolin-2-yl)methyl][3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]amine Hydrochloride (35). Off-white powder (17%): mp 104−106 °C (dec). ^{1}H NMR (500 MHz, CD_3OD) δ: 8.61 (s, 1H), 8.55 (d, J = 8.5 Hz, 2H), 8.14 (s, 2H), 8.03 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 8.0 Hz, 4H), 4.91 (s, 4H), 4.52 (t, J = 6.5 Hz, 2H), 3.56 (br t, 2H), 2.04 (m, 2H). HRESIMS calcd for $C_{25}H_{22}Cl_2N_7O_2$ m/z $[M + H]^+$ 522.1212, found 522.1216.

Bis[(6-bromoquinolin-2-yl)methyl][3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]amine Hydrochloride (36). Off-white powder (16%): mp 128−130 °C (dec). ^{1}H NMR (500 MHz, CD_3OD) δ: 8.60 (s, 1H), 8.41 (d, J = 8.5 Hz, 2H), 8.22 (s, 2H), 7.92 (br s, 4H), 7.63 (d, J = 8.5 Hz, 2H), 4.95 (s, 4H), 4.52 (t, J = 6.5 Hz, 2H), 3.64 (t, J = 8.0 Hz, 2H), 2.62 (m, 2H). HRESIMS calcd for $C_{25}H_{22}Br_2N_7O_2$ m/z $[M + H]^+$ 611.0235, 612.0181, 613.0215 found 611.0254, 612.0233, 613.0230.

[3-(3-Nitro-1*H*-1,2,4-triazol-1-yl)propyl]({[4-(trifluoromethyl)phenyl]methyl})amine Hydrochloride (37). White powder (12%): mp 127−128 °C. ^{1}H NMR (500 MHz, CD_3OD) δ: 8.65 (s, 1H), 7.79 (d, J = 8.5 Hz, 2H), 7.71 (d, J = 8.0 Hz, 2H), 4.50 (t, J = 6.5 Hz, 2H), 4.33 (s, 2H), 3.22 (t, J = 8.0 Hz, 2H), 2.38 (m, 2H). HRESIMS calcd for $C_{13}H_{15}F_3N_5O_2$ m/z $[M + H]^+$ 330.1172 found 330.1179.

[3-(3-Nitro-1*H*-1,2,4-triazol-1-yl)propyl]bis({[4-(trifluoromethyl)phenyl]methyl})amine Hydrochloride (38). White powder (26%): mp 184−186 °C. ^{1}H NMR (500 MHz, CD_3OD) δ: 8.59 (s, 1H), 7.78 (d, J = 8.0 Hz, 4H), 7.70 (d, J = 7.5 Hz, 4H), 4.53 (br s, 4H), 4.44 (t, J = 6.0 Hz, 2H), 3.25 (br s, 2H), 2.52 (br m, 2H). HRESIMS calcd for $C_{21}H_{20}F_6N_5O_2$ m/z $[M + H]^+$ 488.1516 found 488.1513. Calculated analysis for $C_{21}H_{20}F_6ClN_5O_2$: C, 48.13; H, 3.85; N, 13.37; Cl, 6.77. Found: C, 48.21; H, 3.93; N, 13.29; Cl, 6.88.

1-[3-(2-Nitro-1*H*-imidazol-1-yl)propyl]-4-{[4-(trifluoromethyl)phenyl]methyl}piperazine Dihydrochloride (39). White powder (67%): mp 185−187 °C. ^{1}H NMR (500 MHz, D_2O) δ: 7.85 (d, J = 7.5 Hz, 2H), 7.70 (d, J = 7.5 Hz, 2H), 7.49 (s, 1H), 7.22 (s, 1H), 4.58 (br t, J = 7.0 Hz, 2H), 4.53 (s, 2H), 3.63 (br s, 8H), 3.34 (br s, 2H), 2.37 (br s, 2H). HRESIMS calcd for

$C_{18}H_{23}F_3N_5O_2$ m/z $[M + H]^+$ 398.1798, found 398.1803. Calculated analysis for $C_{18}H_{24}F_3Cl_2N_5O_2$: C, 45.95; H, 5.15; N,14.89; Cl, 15.08. Found: C, 45.85; H, 5.05; N, 14.59; Cl, 15.12.

1-[3-(3-Nitro-1*H*-1,2,4-triazol-1-yl)propyl]-4-{[4-(trifluoromethyl)phenyl]methyl}piperazine Dihydrochloride (40). White powder (74%): mp 233−235 °C (dec). ^{1}H NMR (500 MHz, D_2O) δ: 8.65 (s, 1H), 7.84 (d, J = 8.0 Hz, 2H), 7.67 (d, J = 8.0 Hz, 2H), 4.51 (t, J = 6.0 Hz, 2H), 4.39 (s, 2H), 3.48 (br s, 8H), 3.27 (t, J = 8.0 Hz, 2H), 2.41 (m, 2H). HRESIMS calcd for $C_{17}H_{22}F_3N_6O_2$ m/z $[M + H]^+$ 399.1751, found 399.1761. Calculated analysis for $C_{17}H_{23}F_3Cl_2N_6O_2$: C, 43.30; H, 4.92; N,17.83; Cl, 15.05. Found: C, 43.26; H, 4.91; N, 17.71; Cl, 15.42.

1-[4-(3-Nitro-1*H*-1,2,4-triazol-1-yl)butyl]-4-{[4-(trifluoromethyl)phenyl]methyl}piperazine Dihydrochloride (41). White powder (16%): mp 223−225 °C (dec). ^{1}H NMR (500 MHz, CD_3OD) δ: 8.67 (s, 1H), 7.80 (s, 4H), 4.44 (t, J = 7.0 Hz, 2H), 4.41 (s, 2H), 3.80−3.40 (br m, 10H), 2.06 (quintet, J = 7.5 Hz, 2H), 1.86 (m, 2H). HRESIMS calcd for $C_{18}H_{24}F_3N_6O_2$ m/z $[M + H]^+$ 413.1907, found 413.1909.

1-[3-(3-Nitro-1*H*-1,2,4-triazol-1-yl)propyl]-4-[4-(trifluoromethyl)phenyl]piperazine Dihydrochloride (42). White powder: mp 225 °C (dec). ^{1}H NMR (500 MHz, CD_3OD) δ: 8.68 (s, 1H), 7.56 (d, J = 8.5 Hz, 2H), 7.14 (d, J = 8.5 Hz, 2H), 4.52 (t, J = 6.5 Hz, 2H), 4.04 (d, J = 13 Hz, 2H), 3.72 (d, J = 11.5 Hz, 2H), 3.35 (t, J = 8.0 Hz, 2H), 3.27−3.20 (m, 4H), 2.48 (quintet, J = 6.5 Hz, 2H). HRESIMS calcd for $C_{16}H_{20}F_3N_6O_2$ m/z $[M + H]^+$ 385.1595, found 385.1606; calcd for $C_{16}H_{19}F_3N_6NaO_2$ m/z $[M + Na]^+$ 407.1414, found 407.1419. Calculated analysis for $C_{16}H_{21}F_3Cl_2N_6O_2$: C, 42.01; H, 4.63; N, 18.38; Cl, 15.51. Found: C, 42.29; H, 4.68; N, 18.79; Cl, 15.39.

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.[25] 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.

In Vitro *T. brucei brucei* Antiproliferating Assays and Susceptibility Studies. *T. brucei brucei* bloodstream-form parasites were seeded at 1×10^3 ml^{-1} in 200 μL of growth medium containing different concentrations of a nitrotriazole or nifurtimox. Where appropriate, induction of the TbNTR was carried out by adding tetracycline (1 μg/mL). After incubation for 3 days at 37 °C, 20 μL of Alamar blue was added to each well and the plates incubated for a further 16 h. The cell density of each culture was determined as described before[11] and the IC_{50} established.

Enzymatic Activity Studies. Recombinant TbNTR was prepared and assayed as previously described.[16] The activity of purified his-tagged TbNTR was assessed spectrophotometrically at 340 nm using various nitrotriazole substrates (100 μM) and NADH (100 μM) and expressed as nmol NADH oxidized min^{-1} mg^{-1} of enzyme.

Cyclic Voltametry. Reduction potentials ($E_{1/2}$) were measured by cyclic voltametry and evaluated relative to the Ag/AgCl reference electrode. Supporting electrolyte was 0.1 M of tetrabutyl ammonium hexafluorophosphate (TBAPF6), 98% purity from Sigma Aldrich. The working electrode was carbon mesh and the counter electrode Pt wire. The typical scan rate was 100 mV/s.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Evaluating the mechanism of action of nitrotriazoles. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

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; DNDi, Drugs for Neglected Diseases initiative; SI, selectivity index; SARs, structure−activity relationships; $E_{1/2}$, reduction potential; tet, tetracycline

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