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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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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 anti-trypanosomal activity and mammalian cytotoxicity by the Drugs for Neglected Diseases initiative (DNDi). Out of 42 compounds tested, eighteen 3-nitro-1,2,4-triazoles and one 2-nitroimidazole displayed significant growth inhibitory properties against *T. cruzi* amastigotes (IC₅₀ ranging from 40 nM to 1.97 μM), without concomitant toxicity towards the host cells (L6 cells), having selectivity indices (SI) 44 to 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 (IC₅₀ at nM levels and SI 220 to 993). An NADH-dependent nitroreductase (TbNTR) plays a role in the anti-parasitic activity, since 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.

Keywords

nitrotriazoles; amines; *T. cruzi*; Chagas disease; antitrypanosomal agents

Introduction

The protozoan parasites *Trypanosoma cruzi* (*T. cruzi*)^a, *Trypanosoma brucei* (*T. brucei*), and various *Leishmania* 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 50,000 to 80,000 by *T.b. gambiense* or *T.b. rhodesiense*, resulting in more than 40,000 deaths per year.¹ Chagas disease is transmitted by blood sucking triatomine insects and occurs mainly

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^a**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; DNDi, Drugs for Neglected Diseases initiative; SI, selectivity index; SARs, structure-activity relationships; E_{1/2}, reduction potential; tet, tetracycline.

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 non-endemic 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-nitrofurfurylideneamino)-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, 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.

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 parasite⁵⁻⁷ 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 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 anti-cancer 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 IC₅₀ of 140 nM for the parasite and a selectivity index (SI = IC₅₀ for L6 cells/IC₅₀ 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 II,²⁰ demonstrated toxicity at 15 mg/kg given i.p. 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 on 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 amine¹⁹ with the appropriate chloro- or fluoro-aromatic chromophore²³ 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 K₂CO₃ (Scheme 1B). In most cases the monoalkylated product was the dominant one, since 1 equivalent of the required halide was used. Piperazine derivatives **39-42** were synthesized similarly, by nucleophilic attack of the appropriate, commercially available mono-substituted piperazine to the appropriate nitrotriazole- or nitroimidazole alkyl bromide²⁴ (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

Anti-proliferative 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 screens²⁵. From resultant dose response curves, IC₅₀ values in μ M were determined (Table 1). The criteria for activity were set as follows: For *T.b. rhodesiense*, compounds that gave 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'. Based on 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 6 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 IC₅₀ against L6 cells to IC₅₀ 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.

Based on the above, only 6 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 antichagasic 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* (IC₅₀ < 4 μ M) but not sufficiently specific (SI < 50), were exclusively

2-nitroimidazole 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 analog **15**.

To determine whether the nitro-group was important in the anti-parasitic 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-analogs **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 ($IC_{50} > 60 \mu M$) and the IC_{50} value was significantly increased (658- and 730-fold, respectively) compared to the nitro-containing analog (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 anti-parasitic activity of these compounds.

Analysis of aromatic amines

A closer look at the SARs for all **antichagasic** 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**) $\geq$ 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 analogs **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 analog **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-intercalation¹⁹ 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 analogs **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 analogs **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 antichagasic 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 analogs 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 antichagasic 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 nitrotriazole/imidazole ring and aromatic chromophore in the aromatic amines can not always predict the direction of changes in the antichagasic 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 analog **37**, violating twice the Lipinski rule of 5 (Table 2). However, its increased antichagasic 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 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 antichagasic activity and their SI corresponds inversely to their clogP value and toxicity (Table 1 and 2). All three analogs have similar activity with the *p*-trifluoromethylbenzylamine **37**, but the monoalkylated chloro-quinaldine analog **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 analogs **35** and **36** also violate the Lipinski rule of 5.

The piperazine systems (**40-42**) showed significant antichagasic activity *in vitro* (Table 1). However, the 1-phenyl-piperazine **42** showed about 10-fold increased activity (IC₅₀ 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 antichagasic activity in Table 2 have a polar surface area < 140 and >60 Å², which means good cell-membrane permeability and presumably absence of neurotoxicity since they can not cross the blood-brain barrier. In addition, all but compounds **8**, **20** and **21** (all 2-phenylquinazolines), demonstrate a better antichagasic activity (1.3-33.8 fold) than the reference compound benznidazole, tested in parallel. It appears that increased antichagasic 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 reductase²⁷, 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** (Fig. 1; supplemental material), 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 towards 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 sub-categories (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), 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 supplemental material). 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).

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 antichagasic 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 anti-parasitic activity against *T. cruzi*, both *T. brucei* sub-species and *L. major* with high selectivity, although this compound does violate two of the Lipinski's rule of 5.

Experimental

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 silicagel GF (1500 microns) plates (Analtech). All the amines were purified by preparative TLC chromatography on alumina plates ($\geq 95\%$ 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-fluoroquinoline²³ 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 Na₂SO₄. 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; ¹H NMR (500 MHz, CD₃OD) 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 C₂₀H₂₀ClN₆O₃ *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.); ¹H NMR (500 MHz, CD₃OD) d: 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 C₂₀H₁₉N₆O₂ *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%). ¹H NMR (500 MHz, CD₃OD) d: 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 C₂₁H₂₁N₆O₂ *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); ¹H NMR (500 MHz, CD₃OD) d: 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 Hz, 2H), 2.25 (s, 3H), 2.19 (m, 2H). HRESIMS calcd for C₁₁H₁₆N₇O₂ *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); ¹H NMR (500 MHz, CD₃OD) d: 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 C₁₂H₁₈N₇O₂ *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%). ¹H NMR (500 MHz, CDCl₃) d: 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 C₁₇H₂₀ClN₆O₄ *m/z* [M+H]⁺ 407.1229, found 407.1236.

6-Chloro-N-[4-(2-nitro-1*H*-imidazol-1-yl)butyl]-2,5-diphenylpyrimidin-4-amine hydrochloride (14)

pale white powder (72%): mp 79-81 °C; ¹H NMR (500 MHz, CD₃OD) d: 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 Hz, 2H), 1.91 (quintet, *J*=7.5 Hz, 2H), 1.71 (quintet, *J*=7.5 Hz, 2H). HRESIMS calcd for C₂₃H₂₂ClN₆O₂ *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; ¹H NMR (500 MHz, D₂O) d: 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 Hz, 2H), 2.48 (m, 2H). HRESIMS calcd for C₁₄H₁₄ClN₆O₂ *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); ¹H NMR (500 MHz, D₂O) d: 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 Hz, 2H), 2.07 (m, 2H), 1.77 (m, 2H). HRESIMS calcd for C₁₅H₁₆ClN₆O₂ *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%). ¹H NMR (500 MHz, CD₃OD) d: 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 C₁₆H₁₆F₃N₆O₂ *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; ¹H NMR (500 MHz, CDCl₃) d: 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 C₁₅H₁₇ClN₅O₂ *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); ¹H NMR (500 MHz, CD₃SOCD₃) d: 8.91 (s, 1H), 8.49 (br d, *J*=7.0 Hz, 1H), 8.33 (d, *J*=7.5 Hz, 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 C₁₉H₁₈N₇O₂ *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; ¹H NMR (500 MHz, CD₃SOCD₃) d: 8.90 (s, 1H), 8.57 (d, *J*=8.0 Hz, 1H), 8.39 (d, *J*=7.5 Hz, 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 C₂₀H₂₀N₇O₂ *m/z* [M+H]⁺ 390.1679, found 390.1681. Calculated analysis for C₂₀H₂₀ClN₇O₂: 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; ¹H NMR (500 MHz, CD₃OD) d: 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 C₁₀H₁₅N₈O₂ *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); ¹H NMR (500 MHz, CD₃OD) d: 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 C₁₃H₁₄N₇O₂ *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%). ¹H NMR (500 MHz, CD₃OD) d: 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 C₁₄H₁₆N₇O₂ *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; ¹H NMR (500 MHz, CD₃COCD₃) d: 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; 1H NMR (500 MHz, CD_3OD) d: 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.); 1H NMR (500 MHz, CD_3OD) d: 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; 1H NMR (500 MHz, CD_3OD) d: 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.); 1H NMR (400 MHz, CD_3OD) d: 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%). 1H NMR (400 MHz, CD_3OD) d: 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.); 1H NMR (500 MHz, CD_3OD) d: 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.); 1H NMR (500 MHz, CD_3OD) d: 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); ¹H NMR (500 MHz, CD₃OD) d: 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 C₂₅H₂₂Br₂N₇O₂ *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; ¹H NMR (500 MHz, CD₃OD) d: 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 C₁₃H₁₅F₃N₅O₂ *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; ¹H NMR (500 MHz, CD₃OD) d: 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 C₂₁H₂₀F₆N₅O₂ *m/z* [M+H]⁺ 488.1516 found 488.1513. Calculated analysis for C₂₁H₂₀F₆ClN₅O₂: 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; ¹H NMR (500 MHz, D₂O) d: 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 C₁₈H₂₃F₃N₅O₂ *m/z* [M+H]⁺ 398.1798, found 398.1803. Calculated analysis for C₁₈H₂₄F₃Cl₂N₅O₂: 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); ¹H NMR (500 MHz, D₂O) d: 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 C₁₇H₂₂F₃N₆O₂ *m/z* [M+H]⁺ 399.1751, found 399.1761. Calculated analysis for C₁₇H₂₃F₃Cl₂N₆O₂: 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); ¹H NMR (500 MHz, CD₃OD) d: 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 C₁₈H₂₄F₃N₆O₂ *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.); ¹H NMR (500 MHz, CD₃OD) d: 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 μ L of growth medium containing different concentrations of a nitrotriazole or nifurtimox. Where appropriate, induction of the TbNTR was carried out by adding tetracycline (1 μ g/mL). After incubation for 3 days at 37 °C, 20 μ L 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 before¹¹ 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 μ M) and NADH (100 μ M) and expressed as nmol NADH oxidized min⁻¹ mg⁻¹ of enzyme.

Cyclic Voltammetry

Reduction potentials ($E_{1/2}$) were measured by cyclic voltammetry and evaluated relative to the Ag/AgCl reference electrode. Supporting electrolyte was 0.1M 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/sec.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Stuart K, Brun R, Croft S, Fairlamb A, Gürtler RE, McKerrow J, Reed S, Tarleton R. Kinetoplastids: related protozoan pathogens, different diseases. *J Clin Invest.* 2008; 118:1301–1310. [PubMed: 18382742]
2. Report of the Scientific Working Group on Chagas Disease, WHO/TDR, 2005.
3. (a) Urbina J. Chemotherapy of Chagas disease. *Curr Pharm Des.* 2002; 8:287–295. [PubMed: 11860367] (b) Moncayo A, Silveira AC. Current epidemiological trends for Chagas disease in Latin

- America and future challenges in epidemiology, surveillance and healthy policy. *Memorias do Instituto Oswaldo Cruz*. 2009; 104 1:17–30. [PubMed: 19753454]
4. (a) Murta SM, Gazzinelli RT, Brener Z, Romanha AJ. Molecular characterization of susceptible and naturally resistant strains of *Trypanosoma cruzi* to benznidazole and nifurtimox. *Mol Biochem Parasitol*. 1998; 93:203–214. [PubMed: 9662705] (b) Rodriques Coura J, de Castro SL. A critical review on Chagas disease chemotherapy. *Memorias do Instituto Oswaldo Cruz*. 2002; 97:3–24. [PubMed: 11992141]
 5. Docampo R, Moreno SNJ. Free radical metabolism of antiparasitic agents. *Fed Proc*. 1986; 45:2471–2476. [PubMed: 3017765]
 6. Docampo R. Sensitivity of parasite to free radical damage by antiparasitic drugs. *Chem Biol Interact*. 1990; 73:1–27. [PubMed: 2406032]
 7. Viode C, Bettache N, Cenas N, Krauth-Siegel RL, Chauviere G, Bakalara N, Perie J. Enzymatic reduction studies of nitroheterocycles. *Biochem Pharmacol*. 1999; 57(5):549–557. [PubMed: 9952319]
 8. Blumenstiel K, Schoneck R, Yardley V, Croft SL, Krauth-Siegel RL. Nitrofurans as common subversive substrates of *Trypanosoma cruzi* lipoamide dehydrogenase and trypanothione reductase. *Biochem Pharmacol*. 1999; 58(11):1791–1799. [PubMed: 10571254]
 9. Turrens JF. Oxidative stress and antioxidant defenses: a target for the treatment of diseases caused by parasitic protozoa. *Mol Aspects Med*. 2004; 25:211–220. [PubMed: 15051329]
 10. Hall BS, Bot C, Wilkinson SR. Nifurtimox activation by trypanosomal type I nitroreductases generates cytotoxic nitrile metabolites. *J Biol Chem*. 2011; 286(15):13088–13095. [PubMed: 21345801]
 11. Wilkinson SR, Taylor MC, Horn D, Kelly JM, Cheeseman I. A mechanism for cross-resistance to nifurtimox and benznidazole in trypanosomes. *PNAS*. 2008; 105(13):5022–5027. [PubMed: 18367671]
 12. Wilkinson SR, Bot C, Kelly JM, Hall BS. Trypanocidal activity of nitroaromatic prodrugs: current treatments and future perspectives. *Curr Top Med Chem*. 2011; 11:2072–2084. [PubMed: 21619510]
 13. Baliani A, Gerpe A, Aran VJ, Torres de Ortiz S, Serna E, Vera de Bilbao N, Sanabria L, Yaluff G, Nakayama H, Rojas de Arias A, Maya JD, Morello JA, Cerecetto H, Gonzalez M. Design and synthesis of a series of melamine-based nitroheterocycles with activity against trypanosomatid parasites. *J Med Chem*. 2005; 48:5570–5579. [PubMed: 16107157]
 14. Rodriguez J, Aran VJ, Boiani L, Olea-Azar C, Lavaggi ML, Gonzalez M, Cerecetto H, Maya JD, Carrasco-Pozo C, Cosoy HS. New potent 5-nitroindazole derivatives as inhibitors of *Trypanosoma cruzi* growth: Synthesis, biological evaluation, and mechanism of action studies. *Bioorg Med Chem*. 2009; 17:8186–8196. [PubMed: 19900812]
 15. Boiani L, Gerpe A, Aran VJ, Torres de Ortiz S, Serna E, Vera de Bilbao N, Sanabria L, Yaluff G, Nakayama H, Rojas de Arias A, Maya JD, Morello JA, Cerecetto H, Gonzalez M. *In vitro* and *in vivo* antitrypanosomatid activity of 5-nitroindazoles. *Eur J Med Chem*. 2009; 44:1034–1040. [PubMed: 18706738]
 16. Hall BS, Wu X, Hu L, Wilkinson SR. Exploiting the Drug-Activating Properties of a Novel Trypanosomal Nitroreductase. *Antimicrob Agents Chemother*. 2010; 54:1193–1199. [PubMed: 20028822]
 17. Bot C, Hall BS, Bashir N, Taylor MC, Helsby NA, Wilkinson SR. Trypanocidal activity of aziridinyl nitrobenzamide prodrugs. *Antimicrob Agents Chemother*. 2010; 54(10):4246–4252. [PubMed: 20679506]
 18. Hu L, Wu X, Han J, Chen L, Vass SO, Browne P, Hall BS, Bot C, Gobalakrishnapillai V, Searle PF, Knox RJ, Wilkinson SR. Synthesis and structure-activity relationships of nitrobenzyl phosphoramidate mustards as nitroreductase-activated prodrugs. *Bioorg Med Chem Lett*. 2011; 21(13):3986–3991. [PubMed: 21620697]
 19. Papadopoulou MV, Bloomer WD. Nitroheterocyclic-linked acridines as DNA-targeting bioreductive agents. *Drugs of the Future*. 1993; 18:231–238.

20. Rosenzweig HS, Papadopoulou MV, Bloomer WD. Interaction of strong DNA-intercalating bioreductive compounds with topoisomerases I and II. *Oncol Res.* 2005; 15:219–231. [PubMed: 17822282]
21. Papadopoulou, MV.; Bourdin, B.; Bloomer, WD.; Brun, R.; Kaiser, M.; Torreele, E. Novel nitroaromatic heterocycles as potential anti-trypanosomal drugs. Keystone Symposium on Molecular and Cellular Biology: “Drug Discovery for Protozoan Parasites”; Breckenridge, CO. March 22-26, 2009; Proceedings
22. Bustamante, JM.; Evans, A.; Papadopoulou, MV.; Tarleton, R. Use of CD8+ T central memory characteristics as immunologic evidence for treatment efficacy in mice infected with *Trypanosoma cruzi*. 12th Woods Hole Immunoparasitology Meeting; Woods Hole, Massachusetts. April 27-29, 2008; (b) Canavaci AMC, Bustamante JM, Padilla AM, Brandan CMP, Simpson LJ, Xu D, Boehlke CL, Tarleton RL. In vitro and in vivo high-throughput assays for the testing of anti-trypanosoma cruzi compounds. *Plos Neglected Tropical Diseases.* 2010; 4(7):e740. [PubMed: 20644616]
23. Papadopoulou MV, Ji M, Rao MK, Bloomer WD. 4-[3-(2-Nitro-1-imidazolyl)-propylamino]-7-chloroquinoline hydrochloride (NLCQ-1), a novel bioreductive compound as a hypoxia-selective cytotoxin. *Oncol Res.* 2000; 12:185–192. [PubMed: 11341468]
24. Cowan DSM, Panicucci R, McClelland RA, Rauth AM. Targeting radiosensitizers to DNA by attachment of an intercalating group: Nitroimidazole linked phenanthridines. *Radiat Res.* 1991; 127:81–89. [PubMed: 2068275]
25. Orhan I, Sener B, Kaiser M, Brun R, Tasdemir D. Inhibitory activity of marine sponge-derived natural products against parasitic protozoa. *Mar Drugs.* 2010; 8:47–58. [PubMed: 20161970]
26. Bonse S, Santelli-Rouvier C, Barbe J, Krauth-Siegel RL. Inhibition of *Trypanosoma cruzi* trypanothione reductase by acridines: Kinetic studies and structure-activity relationships. *J Med Chem.* 1999; 42:5448–5454. [PubMed: 10639286]
27. Turrens JF, Watts BP Jr, Zhong L, Docampo R. Inhibition of *Trypanosoma cruzi* and *T. b. brucei* NADH fumarate reductase by benzimidazole and antihelminthic imidazole derivatives. *Mol Biochem Parasitol.* 1996; 82:125–129. [PubMed: 8943158]
28. Barry CE, Boshoff HIM, Dowd CS. Prospects for clinical introduction of nitroimidazole antibiotics for the treatment of tuberculosis. *Curr Pharmaceut Design.* 2004; 10:3239–3262.
29. Papadopoulou MV, Bloomer WD. Nitroimidazole-based bioreductive compounds bearing a quinazoline or a naphthyridine chromophore. *Anti-Cancer Drugs.* 2009; 20(6):493–502. [PubMed: 19430289]
30. Papadopoulou MV, Ji M, Bloomer WD. Novel Fluorinated Hypoxia-targeted Compounds as Non-invasive Probes for Measuring Tumor-hypoxia by ¹⁹F-Magnetic Resonance Spectroscopy (¹⁹F-MRS). *Anticancer Res.* 2006; 26(5):3253–3258. [PubMed: 17094437]
31. Papadopoulou MV, Bloomer WD. NLNQ-1, a 2-[3-(2-nitro-1-imidazolyl)-propylamino]-3-chloro-1,4-naphthoquinone as a hypoxia-selective cytotoxin and radiosensitizer. *In Vivo.* 2008; 22:285–288. [PubMed: 18610737]
32. Olea-Azar C, Rigol C, Mendizabal F, Morello A, Maya JD, Moncada C, Cabrera E, Di Maio R, Gonzalez M, Cerecetto H. ESR Spin Trapping Studies of Free Radicals Generated from Nitrofurant Derivative Analogues of Nifurtimox by Electrochemical and *Trypanosoma cruzi* Reduction. *Free Radical Res.* 2003; 37(9):993–1001. [PubMed: 14670007]

Scheme 1.

Chart 1.

Chart 2.

Table 1
***In vitro* screening data against three different trypanosomatids**

Compound	All values as μM									
	<i>T.b. rhodesiense</i> ^a		<i>T. cruzi</i> ^b		<i>L. donovani axen.</i> ^c		Cytotox. <i>Le</i> ^d		Comp. Type ^e	
	IC-50	SI	IC-50	SI	IC-50	SI	IC-50	SI		
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	

All values as μM									
Compound	<i>T.b.rhodesiense</i> ^a		<i>T. cruzi</i> ^b		<i>L. donovani axen.</i> ^c		Cytotox. <i>L6</i> ^d		Comp. Type ^e
	IC-50	SI	IC-50	SI	IC-50	SI	IC-50	SI	
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 [*]		1.35 [*]						
Benznidazole									
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 = IC₅₀ in L6 cells / IC₅₀ in parasites.

Table 2
Biological and physical properties of analogs active against *T. cruzi* amastigotes

Compound	<i>T. cruzi</i> IC ₅₀ (μM)	SI	Bzn/Comp ^a	clogP	pKa	Lipinski Rule of 5	PSA (Å 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	1.35*		1.0	1.32		S	92.74

^a Bzn/Comp: IC₅₀ of Bnz / IC₅₀ of Comp. PSA: Polar surface area.

* Median values from 43 measurements in parallel with each compound. All physical properties were predicted by using the Marvin Calculator (www.chemaxon.com).

Table 3

The effect of type I Nitroreductase (TbNTR) on the activity of selected compounds against bloodstream-form *T. brucei brucei* parasites.

ID No	<i>T.b. brucei</i> ^a	TbNTR ^b	TbNTR ^b	Ratio	E _{1/2} ^c
	IC-50 (μM)	-tet	+tet	-tet/+tet	(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 over-express type I nitroreductase in the presence of tetracycline (tet).

^c Reduction potential of each compound was measured in DMSO (except for **17**, in CH₃CN) by cyclic voltammetry relative to Ag/AgCl.

^d The E_{1/2} value is taken from ref. [32].