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# Novel 3-Nitro-1*H*-1,2,4-triazole-Based Amides and Sulfonamides as Potential Antitrypanosomal Agents

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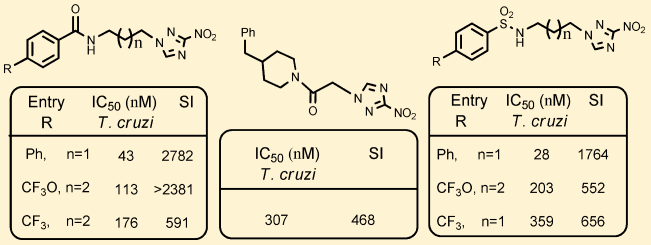
**ABSTRACT:** A series of novel 3-nitro-1*H*-1,2,4-triazole-based (and in some cases 2-nitro-1*H*-imidazole-based) amides and sulfonamides were characterized for their in vitro antitrypanosomal and antileishmanial activities as well as mammalian toxicity. Out of 36 compounds tested, 29 (mostly 3-nitro-1*H*-1,2,4-triazoles) displayed significant activity against *Trypanosoma cruzi* intracellular amastigotes ($IC_{50}$ ranging from 28 nM to 3.72 $\mu$M) without concomitant toxicity to L6 host cells (selectivity 66−2782). Twenty-three of these active compounds were more potent (up to 58-fold) than the reference drug benznidazole, tested in parallel. In addition, nine nitrotriazoles which were moderately active (0.5 $\mu M \leq IC_{50} < 6.0$ $\mu$M) against *Trypanosoma brucei rhodesiense* trypomastigotes were 5−31-fold more active against bloodstream-form *Trypanosoma brucei brucei* trypomastigotes engineered to overexpress reduced nicotinamide adenine dinucleotide dependent nitroreductase. Finally, three nitrotriazoles displayed a moderate activity against the axenic form of *Leishmania donovani*. Therefore, 3-nitro-1*H*-1,2,4-triazole-based amides and sulfonamides are potent antitrypanosomal agents.

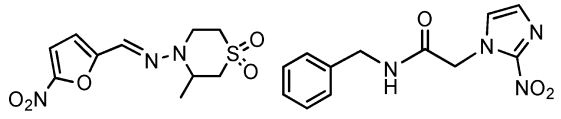



## ■ INTRODUCTION

The trypanosomatid protozoan parasites *Trypanosoma cruzi*, *Trypanosoma brucei*, and various *Leishmania* species are the causative agents of Chagas disease, human African trypanosomiasis (HAT), and different forms of leishmaniasis, respectively. Over 20 million people are infected by *T. cruzi*, *T. brucei*, and *Leishmania*, resulting in 100 000 deaths per year.[1] Chagas disease is transmitted by blood-sucking triatomine insects and occurs mainly in Latin America. Despite the fact that in the past 20 years the number of incidences for both Chagas and HAT has significantly declined, primarily due to vector control initiatives,[2] the number of cases in nonendemic regions such as the United States, Australia, Europe, and Japan 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, affordable, and safer drugs to treat this disease is urgent.

**Chart 1**




Most nitroheterocyclic compounds function as prodrugs and must undergo activation before mediation of their cytotoxic effects. Initially, it was proposed that the trypanocidal action of Nfx was due to its ability to induce oxidative stress through 1-electron reduction of its nitro group and the subsequent formation of superoxide anions via a futile cycle.[5−9] Several trypanosomal flavoproteins have been shown to mediate 1-electron reduction in vitro. However, more recent studies have shown that the above process does not occur to such a degree to cause toxicity to the parasites[10] and that a type I

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**Table 1. In Vitro Biological and Physical Properties of 3-Nitrotriazole-Based Amides/Sulfonamides**

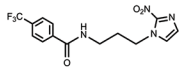
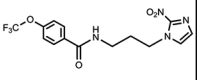
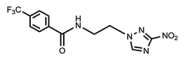
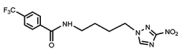
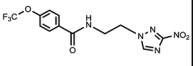
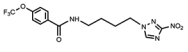
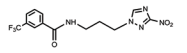
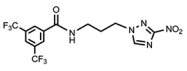
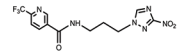
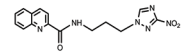
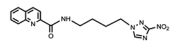
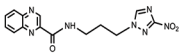
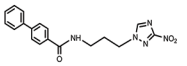
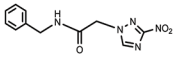
| Comp. | *T.b.rhod.*[a] $IC_{50}$ (μM) | SI | *T. cruzi*[b] $IC_{50}$ (μM) | SI | *L.don. ax.*[c] $IC_{50}$ (μM) | SI[d] | L6[e] $IC_{50}$ (μM) | $IC_{50}$ Bnz/ $IC_{50}$ Comp | logP | PSA (Å$^2$) | Chemical Structure |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Melars. | 0.012 | | | | | | | | | | Reference |
| Bnz | | | 1.562 | | | | | | | | Reference |
| Miltef. | | | | | 0.382 | | | | | | Reference |
| Podoph. | | | | | | | 0.022 | | | | Reference |
| **1** | 21.374 | | 6.053 | 29 | 13.77 | | 176.6 | 0.3 | 2.52 | 92.74 |  |
| **2** | 46.648 | | 3.715 | 74 | 36.03 | | 274 | 0.4 | 3.07 | 101.97 |  |
| **3** | 3.161 | | 0.438 | >625 | 33.44 | | >273.6 | 3.6 | 2.09 | 105.63 |  |
| **4** | 0.501 | 208 | 0.176 | 591 | 7.93 | | 104.3 | 8.9 | 2.66 | 105.63 |  |
| **5** | 1.986 | >131 | 0.73 | >357 | 29.25 | | >260.9 | 2.1 | 2.64 | 114.86 |  |
| **6** | 1.391 | >193 | 0.113 | >2381 | 12.98 | | >268 | 13.8 | 3.22 | 114.86 |  |
| **7** | 3.761 | | 0.353 | >826 | 37.32 | | >292 | 4.4 | 2.15 | 105.63 |  |
| **8** | 16.4 | 11.4 | 0.642 | 290.7 | 12.34 | | 186.6 | 2.4 | 3.03 | 105.63 |  |
| **9** | 3.546 | | 3.459 | >84 | 96.51 | | >291 | 0.5 | 1.32 | 118.52 |  |
| **10** | 3.22 | | 0.138 | 1579 | 19.94 | | 217.98 | 11.3 | 1.81 | 118.52 |  |
| **11** | 4 | | 0.132 | 691 | 13.41 | | 91.5 | 11.8 | 2.33 | 118.52 |  |
| **12** | 34.862 | | 0.807 | >379 | 62.69 | | >306 | 1.9 | 0.98 | 131.41 |  |
| **13** | 0.587 | 199 | 0.043 | 2782 | 8.37 | | 117 | 36.3 | 2.92 | 105.63 |  |
| **14** | 9.96 | | 3.383 | 102 | 28.12 | | 344.83 | 0.5 | 0.95 | 105.63 |  |



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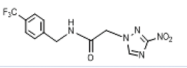
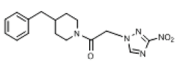
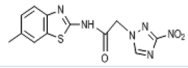
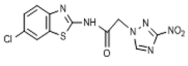
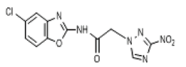
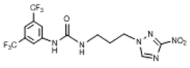
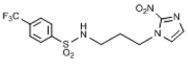
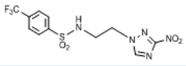
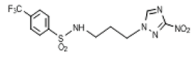
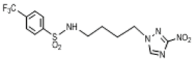
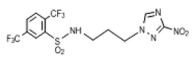
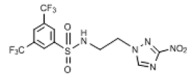
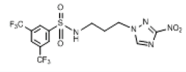
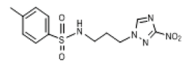
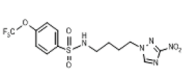
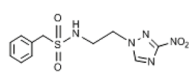
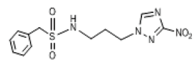
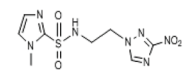
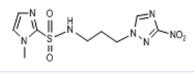
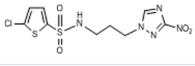
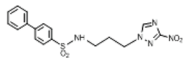
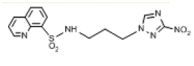
**Table 1. continued**

| Comp. | *T.b.rhod.*[a] $IC_{50}$ (μM) | SI | *T. cruzi*[b] $IC_{50}$ (μM) | SI | *L.don. ax.*[c] $IC_{50}$ (μM) | SI[d] | L6[e] $IC_{50}$ (μM) | $IC_{50}$ Bnz/ $IC_{50}$ Comp | logP | PSA (Å$^2$) | Chemical Structure |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **15** | 6.474 | | 0.970 | 133 | 10.39 | | 128.88 | 1.6 | 1.83 | 105.6 |  |
| **16** | 3.404 | | 0.307 | 468 | 51.37 | | 143.77 | 5.1 | 2.17 | 96.84 |  |
| **17** | 11.51 | 14.8 | 1.799 | 94.4 | 5.91 | 28.7 | 169.8 | 0.9 | 2.58 | 118.5 |  |
| **18** | 48.45 | 3.4 | 6.588 | 24.8 | 5.82 | 28 | 163.1 | 0.2 | 2.67 | 118.5 |  |
| **19** | 34.42 | <1 | 7.876 | 3.3 | 4.68 | 5.6 | 26.3 | 0.2 | 1.88 | 131.66 |  |
| **20** | 6.03 | 4 | 0.734 | 33 | 11.43 | | 24.18 | 2.1 | 3.08 | 117.66 |  |
| **21** | 27.51 | | 1.659 | 106 | 15.55 | | 175.99 | 0.9 | 2.27 | 109.81 |  |
| **22** | 2.79 | | 0.803 | 248.5 | 32.38 | | 199.5 | 1.9 | 1.84 | 122.70 |  |
| **23** | 0.504 | 467 | 0.359 | 656 | 13.09 | | 235.33 | 4.4 | 1.9 | 122.70 |  |
| **24** | 0.354 | 240 | 0.71 | 120 | 7.79 | | 84.91 | 2.2 | 2.42 | 122.70 |  |
| **25** | 10.313 | | 0.644 | 178 | 46.09 | | 114.77 | 2.4 | 2.78 | 122.70 |  |
| **26** | 36.7 | 3 | 1.677 | 66.2 | 33.26 | | 111.1 | 0.9 | 2.72 | 122.70 |  |
| **27** | 11.3 | 9.6 | 0.322 | 337.6 | 20.74 | | 108.7 | 4.9 | 2.78 | 122.70 |  |
| **28** | 2.54 | 121 | 0.412 | >746.8 | 38.15 | >8.1 | >307.7 | 3.8 | 1.54 | 122.70 |  |
| **29** | 0.477 | 234.9 | 0.203 | 551.7 | 7.8 | 14.4 | 112 | 7.7 | 2.97 | 131.93 |  |



dx.doi.org/10.1021/jm300508n | *J. Med. Chem.* 2012, 55, 5554−5565

Table 1. continued

| Comp. | *T.b.rhod.*[a] $IC_{50}$ (μM) | SI | *T. cruzi*[b] $IC_{50}$ (μM) | SI | *L.don. ax.*[c] $IC_{50}$ (μM) | SI[d] | L6[e] $IC_{50}$ (μM) | $IC_{50}$ Bnz/ $IC_{50}$ Comp | logP | PSA (Å$^2$) | Chemical Structure |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **30** | 8.39 | >38.3 | 6.463 | >48 | 112.86 | | >321.5 | 0.2 | 0.73 | 122.70 |  |
| **31** | 6.49 | >47.4 | 2.237 | >137.6 | 79.38 | | >307.7 | 0.7 | 0.79 | 122.70 |  |
| **32** | 35.88 | >9.3 | 83.39 | 4 | >332.2 | | >332.2 | 0.0 | -0.25 | 140.52 |  |
| **33** | 21.9 | >14.5 | 20.57 | >15 | 223.17 | | >317.5 | 0.1 | -0.19 | 140.52 |  |
| **34** | 1.99 | 122 | 0.438 | 556 | 33 | 7.4 | 243.5 | 3.6 | 1.74 | 122.70 |  |
| **35** | 1.049 | | 0.028 | 1764 | 7.54 | | 50 | 55.8 | 2.67 | 122.70 |  |
| **36** | 6.519 | | 0.4 | 519 | 32.87 | | 208 | 3.9 | 1.18 | 135.59 |  |
| | active | | moderately active | | | active but cytotoxic, low specificity | | | | | |

[a]*T.brucei rhodesiense*, strain STIB 900, trypomastigotes. [b]*T. cruzi*, strain Tulahuen C4, amastigotes. [c]Axenic *L. donovani*, strain MHOM-ET-67/L82, amastigotes. [d]SI is the ratio $IC_{50}$ in L6 cells/$IC_{50}$ in each parasite. [e]Cytotoxicity in L6 cells. Reference drugs: melarsoprol (Melars), benznidazole (Bnz), miltefosine (Miltef), podophylotoxin (podoph). The $IC_{50}$ value of each reference is the mean from 36 measurements in parallel with each compound (SD was 0.001, 0.011, and 0.005 for Melars, Bnz, and Miltef, respectively). PSA = polar surface area. All physical properties were predicted by using the Marvin Calculator (www.chemaxon.com).

nitroreductase (NTR)[11] is responsible for Nfx and Bnz trypanocidal activity. This enzyme mediates a series of 2-electron reduction reactions, resulting in the fragmentation of the heterocyclic ring and production of toxic metabolites.[10,12] The fact that the activation of nitroheterocyclic prodrugs can be catalyzed by the type I NTR, which is absent from most eukaryotes, with trypanosomes being a major exception, have led to a renewed interest in the use of such compounds[13−18] as antiparasitic agents.

We have recently reported[19] that 3-nitro-1*H*-1,2,4-triazole-based aromatic and aliphatic amines demonstrate excellent in vitro activity against intracellular *T. cruzi* amastigotes and in some cases activity against *Trypanosoma brucei rhodesiense* and *Trypansoma brucei brucei* parasites. We have also shown that 3-nitrotriazole-based amines are activated by type I nitroreductase and that bloodstream-form *T. brucei brucei* parasites over-expressing NTR are hypersensitive to these compounds. Moreover, these compounds were significantly less toxic in host cells compared to parasites and up to 34-fold more potent than the reference compound benznidazole.[19] Interestingly, the 3-nitrotiazole-based amines that were evaluated in the Ames test were found negative for mutagenicity, in contrast to their 2-nitroimidazole analogues (unpublished data). Treatment of *T. cruzi*-infected mice with one aromatic amine, NTLA-1,[19,20] given at just 2 (mg/kg)/day × 50 days, resulted in a rapid and persistent drop in peripheral parasite levels and in a fraction of cures.[21] 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.[21] Several other 3-nitrotriazole-based amines are currently being investigated in vivo for antichagasic activity. Encouraged by these results, we have expanded our investigation to the classes of 3-nitro-1*H*-1,2,4-triazole-based amides and sulfonamides. Here we describe the synthesis and in vitro evaluation of such compounds as antitrypanosomal agents.

## CHEMISTRY

The structures of all compounds are depicted in Table 1. Their synthesis is straightforward and based on well-established chemistry, outlined in Scheme 1. Compound **1** has been described before.[22] Amides **2**−**13** and sulfonamides **21**−**36** were synthesized at room temperature by nucleophilic substitution of the appropriate arylcarbonyl/arylsulfonyl chloride by the appropriate nitrotriazole/nitroimidazole alkylamine[23] in the presence of triethylamine (Scheme 1A). For compounds **3**, **5**, **22**, **26**, **30**, and **32** the hydrochloride salt of 2-(3-nitro-1*H*-1,2,4-triazolyl)ethylamine was used because the free amine was too water-soluble to be extracted by an organic solvent after its synthesis. Amides **14**−**19** were synthesized as depicted in Scheme 1B, according to the literature.[24] First, 3-nitro-1*H*-1,2,4-triazole was converted to its potassium salt by treatment with KOH in acetonitrile under mild heating, and then this mixture was added to a solution of the appropriate α-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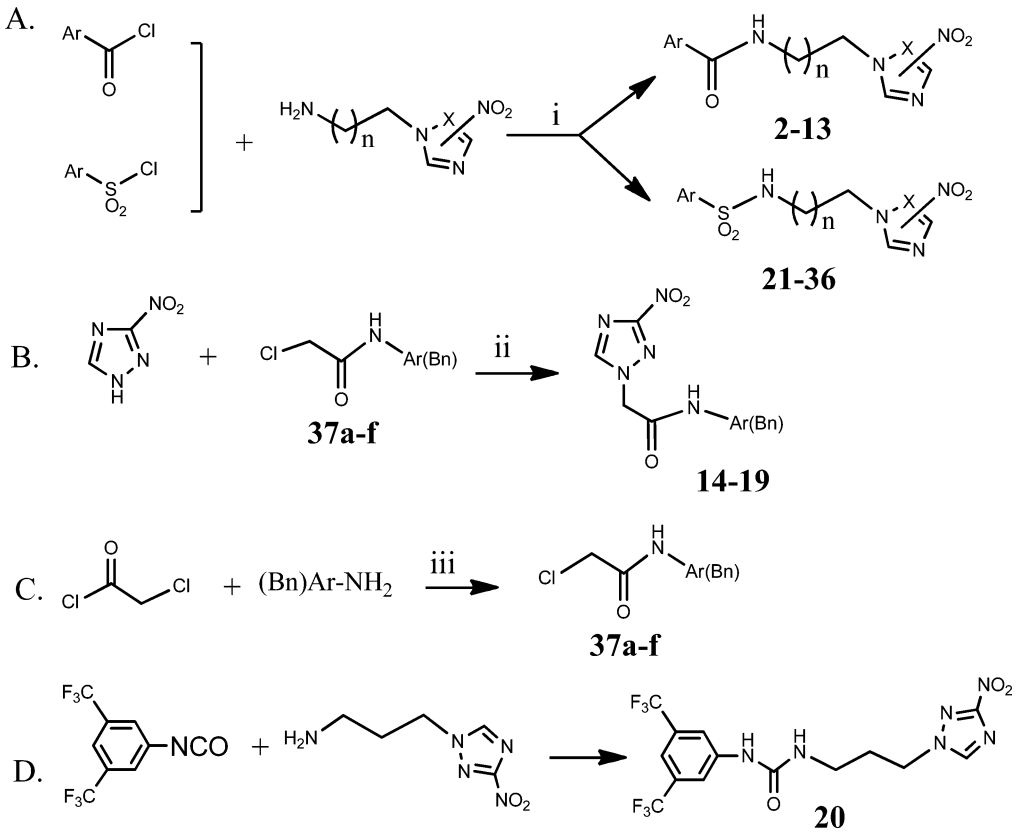
**Scheme 1**[a]




[a]Reagents and conditions: (i) $Et_3N$ (2 equiv), $CH_2Cl_2$, room temperature, 12 h; $n$ = 1−3; X = C, 2-$NO_2$; X = N, 3-$NO_2$; when $n$ = 1, the HCl salt was used instead of the f ree amine with 4 equiv of $Et_3N$. (ii) KOH, $CH_3CN$, mild heating. (iii) $Et_3N$, $CH_2Cl_2$.

chloroacetamide **37a−f** in acetonitrile for a nucleophilic substitution, which occurred under refluxing conditions (8 h). The 2-chloro-*N*-arylacetamides **37a−f** were synthesized through nucleophilic acyl substitution of an appropriate arylamine with 2-chloroacetyl chloride in dry dichloromethane[24] (Scheme 1C). The yields of the final compounds in Table 1 were in general good to very good, with the exception of some compounds (**14**, **19**, **22**, **26**, **29**−**32**) with yields <50%. However, the yields are higher if they are calculated on the basis of recovered starting material, since on many occasions unreacted chloride was isolated from the reaction mixture. Finally, the urea **20** was formed by addition of 3-(3-nitro-1*H*-1,2,4-triazolyl)propylamine to 3,5-bis-(trifluoromethyl)phenyl isocyanate.

## RESULTS AND DISCUSSION

**Antitrypanosomal Activity of Nitrotriazole/Nitroimidazole-Based Amides and Sulfonamides.** The in vitro growth-inhibitory properties of all compounds against bloodstream-form *T. brucei rhodesiense* trypomastigotes, *T. cruzi* amastigotes (in infected L6 myoblasts), axenically cultured *Leishmania donovani* amastigotes, and rat skeletal myoblasts (L6 cells) were evaluated by using standard drug screens.[25] From resultant dose−response curves, $IC_{50}$ values ($\mu$M) were determined (Table 1). The criteria used for activity take into account the complex life cycles of the parasites and the fact that *T. cruzi* and *L. donovani* are, in contrast to *T. brucei rhodesiense*, intracellular parasites. These criteria were established by the TDR's (Special Programme for Research and Training in Tropical Diseases, World Health Organization) "compound screeners network", published in a review,[26] and are as follows: For *T. brucei rhodesiense*, compounds that gave an $IC_{50}$ < 0.5 $\mu$M were designated as "active", while those yielding an $IC_{50}$ = 0.5−6.0 $\mu$M or an $IC_{50}$ > 6.0 $\mu$M were designated "moderately active" and "inactive", respectively. For *T. cruzi*, compounds that gave an $IC_{50}$ < 4.0 $\mu$M were designated as active, those that gave an $IC_{50}$ = 4.0−60 $\mu$M as moderately active, and those with $IC_{50}$ > 60 $\mu$M as inactive. For *L. donovani*, compounds that yielded an $IC_{50}$ < 1 $\mu$M were designated as active, those with $IC_{50}$ = 1.0−6.0 $\mu$M as moderately active, and those that gave $IC_{50}$ > 6.0 $\mu$M as inactive.

On the basis of these criteria, all but compound **32** were active or moderately active against *T. cruzi*, 16 compounds (47%) were active or moderately active against *T. brucei rhodesiense*, and only 3 compounds (~8%) were moderately active against *L. donovani* parasites (Table 1). 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, and both $IC_{50}$ and SI values are used to rank compounds.[26] This SI must be ≥100 for *T. brucei rhodesiense*, ≥50 for *T. cruzi*, and ≥20 for *L. donovani* axenic amastigotes.

On the basis of the above information, only 9 compounds (**4**−**6**, **13**, **23**, **24**, **28**, **29**, and **34**) were moderately active/active and selective against *T. brucei rhodesiense*, whereas 30 compounds (83%), namely, **1**−**17**, **21**−**31**, and **34**−**36**, were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active (with the exception of **30**, which was moderately active) and selective against *T. cruzi* (Table 1). Compounds **17** and **18**, which were moderately active against *L. donovani*, also have an acceptable selectivity. Therefore, as in the case of 3-nitrotriazole-based amines,[19] the majority of these 3-nitrotriazole-based amides/sulfonamides act as antichagasic agents.

**Evaluation of SARs: Analysis of the Nitroheterocyclic Ring.** On the basis of our previous experience that the 2-nitroimidazole-based aromatic and aliphatic amines tend to be significantly less potent as antitrypanosomal agents and more toxic to the host cells than their 3-nitrotriazole analogues,[19] we focused more on the synthesis and evaluation of 3-nitrotriazole-based amides/sulfonamides. Therefore, only two 2-nitroimidazole-based amides (**1** and **2**) and one sulfonamide (**21**) were included. Because of the very limited number of such compounds, no solid conclusions can be obtained regarding the effect of the nitroheterocyclic ring on the antitrypanosomal activity of these classes. However, it is apparent that all of these compounds were inactive against *T. brucei rhodesiense*, and in general, they were less potent antichagasic agents than their closely related 3-nitrotriazoles or benznidazole (compare **1** with **3**, **4**, and **7**, **2** with **5** and **6**, and **21** with **23**) (Table 1).

**Analysis of Amides in Which the 3-Nitrotriazole Ring Is Linked through the Amino Group.** Comparing the antichagasic activity of the *N*-[(3-nitrotriazolyl)alkyl]-benzamides **3**−**8**, it is observed that activity increases with the length of the linker between the 3-nitrotriazole ring and amido group (Table 1; compare **3** with **4** and **7**, and **5** with **6**). The same rule applies for the activity against *T. brucei rhodesiense* as well. Replacing the trifluoromethyl group in **3** with the trifluoromethoxy group resulted in decreased activity and selectivity against *T. cruzi* in **5**; however, the opposite effect was observed in the case of compounds **4** and **6**. Interestingly, the more lipophilic **6** was slightly less toxic to L6 cells compared to the less lipophilic **5** and, because of its increased potency against *T. cruzi*, resulted in a very high selectivity of >2381. It is also worthy mentioning that the trifluoromethoxy group increased the lipophilicity to the same degree as two methylene groups (Table 1). However, this increased lipophilicity was not always translated to increased antichagasic or anti-HAT activity, and the length of the linker played a more important role. The addition of an extra trifluoromethyl group in the phenyl ring of **8** resulted also in decreased antichagasic activity and selectivity, as well as in inactivity against *T. brucei rhodesiense* (Table 1). Exchanging the phenyl group with a pyridino in **9** significantly decreased the activity and selectivity against *T. cruzi* but did not have any dramatic effect on the moderate activity against *T. brucei rhodesiense* (compare **7** with **9**).

Quinoline-2-carboxamides **10** and **11** demonstrated exceptional in vitro activity against *T. cruzi* and very good selectivity. The additional methylene in the linker of **11** naturally increased the lipophilicity of this compound and led to a decreased selectivity (Table 1). Going from the quinoline-2-carboxamide **10** to the quinoxaline analogue **12**, we observe a decrease in the antichagasic activity and selectivity and complete inactivity against *T. brucei rhodesiense* (Table 1). A significant drop in the log *P* value compared to that of **10** (Table 1) may be related to this inactivity. Finally, the 4-phenylbenzamide **13** was the most potent derivative against *T. cruzi*, with an $IC_{50}$ of 43 nM (36 times more potent than benznidazole) and selectivity of 2782, the highest selectivity observed in all compounds. Compound **13** was also moderately active against *T. brucei rhodesiense* (Table 1).

All the 3-nitrotriazole-based amides in which the nitrotriazole ring was linked through the amino group (**3**−**13**), with the exception of **9**, were 1.9−36-fold more potent than benznidazole against *T. cruzi* amastigotes (Table 1).

**Analysis of Amides in Which the 3-Nitrotriazole Ring Is Linked through the Carbonyl Group.** A small number of amides (**14**−**19**) in which the 3-nitrotriazole ring is linked through the carbonyl group were also synthesized for comparison with benznidazole. Compound **14** was 0.5-fold less potent against *T. cruzi* amastigotes than its 2-nitroimidazole-bearing analogue benznidazole (Table 1), perhaps due to its decreased lipophilicity (log *P* = 0.95 versus 1.32 for benznidazole). Indeed, the more lipophilic amides **15** and **16** were also more potent antichagasic agents than benznidazole (Table 1).

Interestingly, despite their relatively high lipophilicity, the benzothiazoleacetamides **17** and **18** and the benzoxazoleacetamide **19** were less potent against *T. cruzi* amastigotes compared to benznidazole. Similarly, all three compounds were inactive against *T. brucei rhodesiense* (Table 1). However, compounds **17**−**19** demonstrated a moderate antileishmanial activity and could be considered as initial scaffolds for further investigation for such drugs.

To further expand the class of amides, we have evaluated one urea (**20**). Although urea **20** was similarly active against *T. cruzi* compared with the analogous amide **8**, it was significantly more toxic, resulting in an unacceptable selectivity of 33 (Table 1). Lipophilicity alone could not account for the toxicity of **20**, since both **8** and **20** have similar log *P* values (Table 1).

**Analysis of *N*-[(3-Nitrotriazolyl)alkyl]-arenesulfonamides.** Evaluating sulfonamides **21**−**36**, it is observed that all but the methylimidazolesulfonamides **32** and **33** were potent antichagasic agents. Looking at Table 1, it is apparent that compounds **32** and **33** were the only ones with negative log *P* values and PSA (polar surface area) > 140 $Å^2$, indicative of poor penetration through cell membranes.

The 2-nitroimidazole-based sulfonamide **21** was a more potent antichagasic agent than the analogous amides **1** and **2**, but still slightly less active than the reference drug benznidazole (Table 1). These results imply that perhaps further evaluation of 2-nitroimidazole-based sulfonamides as antichagasic agents is worthwhile. However, as was mentioned previously, both 2-nitroimidazole-based amides and sulfonamides were not effective anti-HAT agents compared to their 3-nitrotriazole-based analogues.

As in the case of *N*-[(3-nitrotriazolyl)alkyl]benzamides, the activity of *N*-[(3-nitrotriazolyl)alkyl]benzenesulfonamides **22**−**24** against *T. brucei rhodesiense* proportionally increases with the length of the linker between the 3-nitrotriazole ring and the sulfamido group (Table 1). The same rule, however, does not apply here for activity against *T. cruzi*, although it is clear that two methylene linkers correspond to the lowest activity (Table 1).

In general, sulfonamides were slightly less potent antichagasic agents compared to their analogous amides (compare **22** with **3**, **24** with **4** and **29** with **6**). However, sulfonamides **27** and **35** were more potent than amides **8** and **13**, respectively, against *T. cruzi* (Table 1). A second trifluoromethyl group on the phenyl ring (**25**−**27**) resulted in inactivity against *T. brucei rhodesiense*, independently of its position on the ring (**25**−**27**), with the linker length (**26**) being the most determinant parameter.


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**Table 2. Effect of TbNTR Expression on the Activity of Selected Compounds against Bloodstream-Form *T. brucei brucei* Parasites**

| ID No | *T.b. rhod.*[a] $IC_{50}$ (μM) | *T.b. brucei*[b] $IC_{50}$ (μM) | TbNTR[c] -tet | TbNTR[c] +tet | Ratio -tet/+tet |
|---|---|---|---|---|---|
| **3** | 3.16 | 1.3 ± 0.4 | 1.26 ± 0.27 | 0.09 ± 0.02 | 14 |
| **4** | 0.50 | 0.9 ± 0.1 | 1.30 ± 0.28 | 0.13 ± 0.01 | 10 |
| **6** | 1.39 | 3.6 ± 0.7 | 1.05 ± 0.05 | 0.10 ± 0.00 | 11 |
| **7** | 3.76 | > 10 | nd[d] | nd | nd |
| **8** | 16.4 | >10 | nd | nd | nd |
| **9** | 3.55 | 7.9 ± 0.2 | nd | nd | nd |
| **10** | 3.22 | > 10 | nd | nd | nd |
| **11** | 4.00 | > 10 | nd | nd | nd |
| **12** | 34.86 | > 10 | nd | nd | nd |
| **13** | 0.59 | 0.3 ± 0.0 | 0.28 ± 0.02 | 0.05 ± 0.01 | 6 |
| **14** | 9.96 | > 10 | nd | nd | nd |
| **15** | 6.47 | 8.5 ± 0.2 | nd | nd | nd |
| **16** | 3.40 | > 10 | nd | nd | nd |
| **20** | 6.03 | 1.0 ± 0.0 | 0.81 + 0.07 | 0.18 + 0.02 | 5 |
| **22** | 2.79 | > 10 | nd | nd | nd |
| **23** | 0.50 | 3.4 ± 0.6 | 7.83 ± 0.50 | 0.25 ± 0.01 | 31 |
| **25** | 10.31 | > 10 | nd | nd | nd |
| **26** | 36.7 | 7.9 ± 0.1 | nd | nd | nd |
| **27** | 11.3 | 6.6 ± 0.1 | nd | nd | nd |
| **28** | 2.54 | 4.0 ± 0.3 | 5.63 + 2.40 | 0.24 + 0.02 | 23 |
| **34** | 1.99 | 2.3 ± 0.1 | 4.34 ± 0.05 | 0.23 + 0.01 | 19 |
| **35** | 1.05 | 0.5 ± 0.0 | 0.44 ± 0.03 | 0.07 ± 0.01 | 6 |
| **36** | 6.52 | > 10 | nd | nd | nd |
| **Nfx**[e] | | | 1.71 ± 0.06 | 0.13 ± 0.04 | 13 |
| **Bnz**[f] | | | 21.80 ± 1.00 | 2.20 ± 0.30 | 10 |
| **Melars**[g] | | | 0.0034 ± 0.0001 | 0.0034 ± 0.0001 | 1 |

[a]STIB 900 trypomastigotes. [b]Bloodstream-form wild-type *T. brucei brucei* (Lister 427, clone 221a) parasites. [c]Bloodstream-form *T. brucei brucei* parasites, engineered to overexpress type I nitroreductase in the presence (+tet) or absence (−tet) of tetracycline. [d]Not determined. [e]Nifurtimox (positive control). [f]Benznidazole (positive control). [g]Melarsoprol (negative control).

However, the effect of the second trifluoromethyl group on the antichagasic activity of sulfonamides was not clear (Table 1). Replacing the trifluoromethyl group in **24** with a trifluoromethoxy group in **29** increased the activity and selectivity against *T. cruzi* but slightly reduced the activity and selectivity against *T. brucei rhodesiense*. Membrane permeability issues, due to a greater PSA value in **29**, may be the reason for this slight reduction in anti-HAT activity (Table 1).

Replacing the trifluoromethyl group in **23** with a methyl group in **28** resulted in slightly decreased activity and slightly increased selectivity against *T. cruzi*, perhaps due to a slight decrease in lipophilicity (Table 1). However, this slight decrease in lipophilicity of **28** had a more dramatic decrease in both activity and selectivity against *T. brucei rhodesiense* (Table 1). Exchanging the tolyl group in **28** with a benzyl group in **31** further decreased the log *P* value and resulted in lower activity and selectivity against both *T. cruzi* and *T. brucei rhodesiense* (Table 1). Finally, shortening the linker of **31** by one methylene group in **30** significantly decreased the activity against *T. cruzi* and *T. brucei rhodesiense* and resulted in unacceptable selectivity (Table1). Interestingly, both benzenesulfonamides **30** and **31** were less potent antichagasic agents than benznidazole.

As in the case of 4-phenylbenzamide **13**, the 4-phenylbenzenesulfonamide **35** was the most potent antichagasic compound in the series of sulfonamides, with an $IC_{50}$ of 28 nM (~56 times more potent than benznidazole) and a selectivity of 1764. Sulfonamide **35** was more potent against *T. cruzi* than the analogous amide **13**, but less active than **13** against *T. brucei rhodesiense*. In addition, increased toxicity of **35** to L6 host cells, independently of lipophilicity, resulted in decreased selectivity as compared to that of **13** (Table 1).

Replacing the phenyl ring with a chlorothiophene in **34** slightly decreased the potency against *T. cruzi* and had a more significant impact on selectivity due to an increase in toxicity (compare **28** with **34**). However, the activity against *T. brucei rhodesiense* and selectivity of **34** were similar to those of **28** (Table 1). Replacing the benzene ring with an 8-quinoline in **36** did not affect the antichagasic potency but resulted in increased toxicity and decreased selectivity as compared to those of **28**. The decreased anti-HAT activity of **36** compared to **28** may be related to a decreased lipophilicity and an increased PSA value (Table 1).

**Involvement of Type I Nitroreductase in the Activation of 3-Nitrotriazole-Based Amides/Sulfonamides.** Nitroheterocyclic prodrugs must undergo enzyme-mediated activation within the pathogen to have cytotoxic effects, a reaction catalyzed by nitroreductases. Both Nfx and Bnz are activated by the reduced nicotinamide adenine dinucleotide (NADH) dependent, oxygen-insensitive, mitochondrially localized, bacterial-like, type I NTR, and down-regulation of this enzyme resulted in resistance to these compounds.[10−12]

Several compounds from all subcategories in Table 1 have been evaluated for anti-HAT activity against bloodstream-form *T. brucei brucei* (Table 2). With few exceptions (**3**, **13**, **20**, **35**), most compounds demonstrated a greater $IC_{50}$ value or were inactive against *T. brucei brucei* compared to *T. brucei rhodesiense* (Table 2). Compounds with an $IC_{50} \leq 5$ μM against *T. brucei brucei* were tested in a parasite line engineered to overexpress tetracycline-inducible TbNTR to examine the involvement of this enzyme in their activation (Table 2).

It is observed that parasites overexpressing tetracycline-inducible TbNTR were more susceptible to all such compounds (**3**, **4**, **6**, **13**, **20**, **23**, **28**, **34**, and **35**) as compared to wild-type parasites, with −tet/+tet (noninduced/induced) ratios ranging from 5 to 31 (Table 2). This implies that the major growth-inhibitory activity of these compounds is via type I NTR activation. It is also observed in Table 2 that the least active compounds against wild-type *T. brucei brucei* (**6**, **23**, **28**,


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and **34**) showed a greater −tet/+tet ratio than the most active compounds **13** and **35**.

Selected compounds from Table 1 were tested as substrates of purified type I TbNTR. As shown in Figure 1, all of the

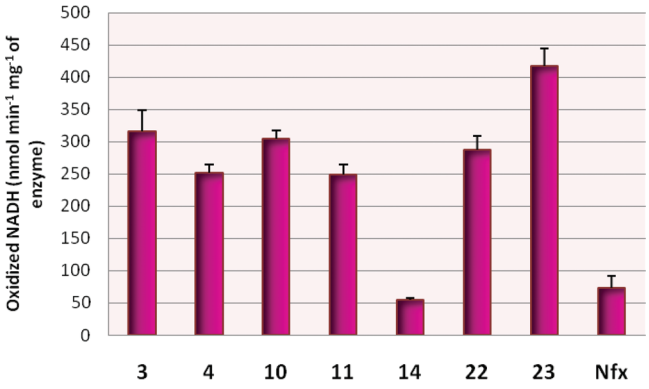


**Figure 1.** Activity of recombinant TbNTR toward different amides/sulfonamides and Nfx.

tested compounds were preferred substrates of the nitroreductase and there is, in general, a good correlation between enzymatic activity and activity against *T. brucei rhodesiense*.

To exclude the possibility that these compounds may exert some of their antitrypanosomal activity via trypanothione reductase (TR) inhibition,[8,27] we have tested selected compounds (**3**, **6**, **10**, **15**, **16**, **21**, **23**) against this enzyme. None of the compounds showed an inhibitory activity against TR at concentrations <100 $\mu$M (unpublished results, private communication with Dr. Mary O'Sullivan, Canisius College, Buffalo, NY).

## CONCLUSIONS

From the above Results and Discussion, it is concluded that, like the 3-nitrotriazole-based aromatic and aliphatic amines, 3-nitrotriazole-based amides and sulfonamides exert exceptional in vitro antichagasic and anti-HAT activities. All tested compounds satisfy the Lipinski rule of 5, and at least 19 of them (**3**−**8**, **10**−**13**, **16**, **22**, **23**, **27**−**29**, **34**−**36**) have been identified (Table 1) as potential candidates for in vivo studies in *T. cruzi*-infected mice. All 19 compounds have demonstrated significant antichagasic activity at low to intermediate nanomolar concentrations and selectivity >200. In addition, all of them were 2−56-fold more potent as antichagasic agents than benznidazole (Table 1). Compounds **4**, **13**, **23**, **24**, and **29** also deserve further in vivo investigation as anti-HAT agents, whereas compounds **17**−**19** should be used as initial scaffolds for further investigation of antileishmania drugs.

## EXPERIMENTAL SECTION

All starting materials and solvents were purchased from Sigma-Aldrich (Milwaukee, WI), were of research-grade quality, and were 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 $CDCl_3$. HRESIMS (high-resolution electrospray ionization mass spectrometry) spectra were obtained on an Agilent 6210 LC-TOF mass spectrometer at 11 000 resolution. Thin-layer chromatography (TLC) 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 $\mu$m) or silicagel GF (1500 $\mu$m) plates (Analtech). All of the amides/sulfonamides were purified by preparative TLC chromatography on silicagel GF plates (≥95% purity). The results from elemental analysis for C, H, and N were within 0.4 of the theoretical value.

The synthesis of compound **1** has been described before.[22]

**General Synthetic Procedure of Arylamides/Sulfonamides and Urea 20.** For compounds **2**−**13** and **21**−**36**, the appropriate commercially available arylcarbonyl/arylsulfonyl chloride (1.24 mmol) was dissolved in 2−3 mL of dry dichloromethane and added dropwise to a solution of (3-nitro-1*H*-1,2,4-triazolyl)alkylamine[23] (1.24 mmol) and triethylamine (2.48 mmol) in 6−8 mL of dry dichloromethane at room temperature and under an inert atmosphere. In three cases (**1**, **2**, **21**), 3-(2-nitro-1*H*-imidazolyl)propylamine[23] (1.24 mmol) was used. The reaction mixture was worked up after 12 h of stirring at room temperature. For compounds **3**, **5**, **22**, **26**, **30**, and **32** the hydrochloride salt of 2-(3-nitro-1*H*-1,2,4-triazolyl)ethylamine (instead of the free amine) and 4 equiv of triethylamine were used. In this case, the reaction mixture was a suspension and the yields of the final product were not very good.

For urea **20**, the commercially available 3,5-bis(trifluoromethyl)-phenyl isocyanate (1.1 mmol) was added dropwise to a dichloromethane solution of (3-nitro-1*H*-1,2,4-triazolyl)propylamine (1.1 mmol) at room temperature and under an inert atmosphere. The urea was formed immediately at 100% yield as a white precipitate.

For amides **14**−**19**, 3-nitro-1*H*-1,2,4-triazole (0.9−1.0 mmol) was stirred under an inert atmosphere and exclusion of moisture with 1.2 equiv of KOH in acetonitrile under mild heating (ca. 40 °C), and then this suspension was slowly added to an acetonitrile solution of the appropriate $\alpha$-chloroacetamide[24] **37a**−**f**.

$\alpha$-Chloroacetamides **37b**−**f** were synthesized at room temperature by adding a dichloromethane solution of an appropriate amine (2.79 mmol) and triethylamine (3.07 mmol) to a dichloromethane solution of $\alpha$-chloroacetyl chloride (3.07 mmol), according to the literature.[24]

**Data for *N*-[3-(2-nitro-1*H*-imidazol-1-yl)propyl]-4-(trifluoromethoxy)benzamide (2):** off-white powder (54%); mp 68−70 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.84 (d, $J$ = 9.0 Hz, 2H), 7.31 (br s, 2H), 7.30 (s, 1H), 7.18 (s, 1H), 6.40 (br s, 1H), 4.54 (t, $J$ = 7.0 Hz, 2H), 3.57 (m, 2H), 2.21 (m, 2H); HRESIMS calcd for $C_{14}H_{14}F_3N_4O_4$ and $C_{14}H_{13}F_3N_4NaO_4$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 359.0962, 381.0781, found 359.0962, 381.0784.

**Data for *N*-[2-(3-nitro-1*H*-1,2,4-triazol-1-yl)ethyl]-4-(trifluoromethyl)benzamide (3):** white powder (65%); mp 155−157 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.24 (s, 1H), 7.86 (br s, 1H), 7.72 (d, $J$ = 8.0 Hz, 2H), 7.46 (d, $J$ = 8.0 Hz, 2H), 4.43 (t, $J$ = 5.0 Hz, 2H), 3.76 (m, 2H); HRESIMS calcd for $C_{12}H_{11}F_3N_5O_3$ $m/z$ $[M + H]^+$ 330.0809, found 330.0815.

**Data for *N*-[4-(3-nitro-1*H*-1,2,4-triazol-1-yl)butyl]-4-(trifluoromethyl)benzamide (4):** white powder (62%); mp 78−79 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.69 (s, 1H), 8.07 (d, $J$ = 8.0 Hz, 2H), 8.04 (br s, 1H), 7.81 (d, $J$ = 8.0 Hz, 2H), 4.49 (t, $J$ = 7.0 Hz, 2H), 3.49 (m, 2H), 2.06 (m, 2H), 1.69 (m, 2H); HRESIMS calcd for $C_{14}H_{15}F_3N_5O_3$ $m/z$ $[M + H]^+$ 358.1122, found 358.1131.

**Data for *N*-[2-(3-nitro-1*H*-1,2,4-triazol-1-yl)ethyl]-4-(trifluoromethoxy)benzamide (5):** white powder (65%); mp 108−109 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.70 (s, 1H), 8.17 (br s, 1H), 7.95 (d, $J$ = 8.5 Hz, 2H), 7.41 (d, $J$ = 8.5 Hz, 2H), 4.65 (t, $J$ = 5.5 Hz, 2H), 3.93 (t, $J$ = 5.5 Hz, 2H); HRESIMS calcd for $C_{12}H_{11}F_3N_5O_4$ $m/z$ $[M + H]^+$ 346.0758, found 346.0765.

**Data for *N*-[4-(3-nitro-1*H*-1,2,4-triazol-1-yl)butyl]-4-(trifluoromethoxy)benzamide (6):** white powder (72%); mp 64−65 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.26 (s, 1H), 7.82 (d, $J$ = 8.5 Hz, 2H), 7.27 (d, $J$ = 8.0 Hz, 2H), 6.44 (br s, 1H), 4.39 (t, $J$ = 7.0 Hz, 2H), 3.53 (m, 2H), 2.06 (quintet, $J$ = 7.0 Hz, 2H), 1.69 (quintet, $J$ = 7.0 Hz, 2H); HRESIMS calcd for $C_{14}H_{15}F_3N_5O_4$ $m/z$ $[M + H]^+$ 374.1071, found 374.1075.


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**Data for *N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-3-(trifluoromethyl)benzamide (7):** white powder (70%); mp 81−83 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.45 (s, 1H), 8.05 (s, 1H), 7.98 (d, $J$ = 7.5 Hz, 1H), 7.81 (d, $J$ = 8.0 Hz, 1H), 6.63 (t, $J$ = 8.0 Hz, 1H), 6.55 (br s, 1H), 4.43 (t, $J$ = 6.5 Hz, 2H), 3.58 (m, 2H), 2.32 (quintet, $J$ = 6.5 Hz, 2H); HRESIMS calcd for $C_{13}H_{13}F_3N_5O_3$ $m/z$ $[M + H]^+$ 344.0965, found 344.0969.

**Data for *N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-3,5-bis-(trifluoromethyl)benzamide (8):** white powder (83%); mp 152−153 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.72 (s, 1H), 8.52 (s, 2H), 8.46 (br s, 1H), 8.25 (s, 1H), 4.57 (t, $J$ = 7.0 Hz, 2H), 3.59 (q, $J$ = 6.5 Hz, 2H), 2.35 (quintet, $J$ = 7.0 Hz, 2H); HRESIMS calcd for $C_{14}H_{12}F_6N_5O_3$ $m/z$ $[M + H]^+$ 412.0839 found 412.0844.

**Data for *N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-6-(trifluoromethyl)pyridine-3-carboxamide (9):** white powder (71%); mp 92−94 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 9.09 (s, 1H), 8.39 (s, 1H), 8.33 (d, $J$ = 8.5 Hz, 1H), 7.82 (d, $J$ = 8.0 Hz, 1H), 6.73 (br s, 1H), 4.43 (t, $J$ = 6.5 Hz, 2H), 3.60 (q, $J$ = 6.5 Hz, 2H), 3.42 (quintet, $J$ = 6.5 Hz, 2H); HRESIMS calcd for $C_{12}H_{12}F_3N_6O_3$ and $C_{12}H_{11}F_3N_6NaO_3$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 345.0917, 367.0737, found 345.0929, 367.0745. Anal. Calcd for $C_{12}H_{11}F_3N_6O_3$: C, 41.87; H, 3.22; N, 24.41. Found: C, 41.93; H, 3.38; N, 24.17.

**Data for *N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]quinoline-2-carboxamide (10):** off-white powder (70%); mp 135−137 °C; $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 8.68 (s, 1H), 8.46 (d, $J$ = 8.5 Hz, 1H), 8.17 (t, $J$ = 9.0 Hz, 2H), 8.0 (d, $J$ = 8.5 Hz, 1H), 7.83 (t, $J$ = 8.5 Hz, 1H), 7.69 (t, $J$ = 8.0 Hz, 1H), 4.46 (t, $J$ = 8.0 Hz, 2H), 3.58 (t, $J$ = 6.5 Hz, 2H), 2.34 (quintet, $J$ = 6.5 Hz, 2H); HRESIMS calcd for $C_{15}H_{15}N_6O_3$ and $C_{15}H_{14}N_6NaO_3$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 327.1200, 349.1020, found 327.1209, 349.1026.

**Data for *N*-[4-(3-nitro-1*H*-1,2,4-triazol-1-yl)butyl]quinoline-2-carboxamide (11):** off-white powder (67%); mp 124−126 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.75 (br s, 1H), 8.70 (s, 1H), 8.52 (d, $J$ = 8.5 Hz, 1H), 8.24 (d, $J$ = 8.5 Hz, 1H), 8.06 (t, $J$ = 9.5 Hz, 2H), 7.84 (t, $J$ = 7.0 Hz, 1H), 7.70 (t, $J$ = 7.0 Hz, 1H), 4.52 (t, $J$ = 7.0 Hz, 2H), 3.58 (q, $J$ = 6.5 Hz, 2H), 2.09 (m, 2H),1.76 (quintet, $J$ = 7.0 Hz, 2H); HRESIMS calcd for $C_{16}H_{17}N_6O_3$ $m/z$ $[M + H]^+$ 341.1357, found 341.1369.

**Data for *N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-quinoxaline-2-carboxamide (12):** off-white powder (66%); mp 143−144 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 9.67 (s, 1H), 8.47 (s, 1H), 8.22 (d, $J$ = 8.0 Hz, 1H), 8.21 (br s, 1H), 8.12 (d, $J$ = 7.5 Hz, 1H), 7.90 (m, 2H), 4.45 (t, $J$ = 6.5 Hz, 2H), 3.66 (q, $J$ = 6.5 Hz, 2H), 2.39 (quintet, $J$ = 6.5 Hz, 2H); HRESIMS calcd for $C_{14}H_{14}N_7O_3$ $m/z$ $[M + H]^+$ 328.1153, found 328.1166. Calculated analysis for $C_{14}H_{13}N_7O_3$: C, 51.38; H, 4.0; N, 29.96. Found: C, 51.29; H, 4.17; N, 29.68.

**Data for *N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-4-phenylbenzamide (13):** white powder (96%); mp 177−179 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.48 (s, 1H), 7.85 (d, $J$ = 8.5 Hz, 2H), 7.69 (d, $J$ = 7.0 Hz, 2H), 7.48 (t, $J$ = 7.5 Hz, 2H), 7.40 (t, $J$ = 7.5 Hz, 1H), 6.49 (br t, 1H), 4.43 (t, $J$ = 6.5 Hz, 2H), 3.57 (q, $J$ = 6.5 Hz, 2H), 2.30 (quintet, $J$ = 6.5 Hz, 2H); HRESIMS calcd for $C_{18}H_{18}N_5O_3$ and $C_{18}H_{17}N_5NaO_3$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 352.1404, 374.1224, found 352.1406, 374.1222. Anal. Calcd for $C_{18}H_{17}N_5O_3$: C, 61.53; H, 4.88; N, 19.93. Found: C, 61.79; H, 4.96; N, 19.58.

**Data for *N*-benzyl-2-(3-nitro-1*H*-1,2,4-triazol-1-yl)acetamide (14):** off-white powder (40%); mp 103−106 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.39 (s, 1H), 7.38−7.28 (m, 5H), 6.26 (br s, 1H), 4.98 (s, 2H), 4.50 (d, $J$ = 5.5 Hz, 2H); HRESIMS calcd for $C_{11}H_{12}N_5O_3$ $m/z$ $[M + H]^+$ 262.0935, found 262.0935.

**Data for 2-(3-nitro-1*H*-1,2,4-triazol-1-yl)-*N*-{[4-(trifluoromethyl)phenyl]methyl}acetamide (15):** white microcrystal (78%); mp 168−170 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.37 (s, 1H), 7.62 (d, $J$ = 8.0 Hz, 2H), 7.40 (d, $J$ = 8.0 Hz, 2H), 6.35 (br s, 1H), 4.99 (s, 2H), 4.56 (d, $J$ = 6.0 Hz, 2H); HRESIMS calcd for $C_{12}H_{11}F_3N_5O_3$ and $C_{12}H_{10}F_3N_5NaO_3$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 330.0809, 352.0628, found 330.0814, 352.0632.

**Data for 1-(4-benzylpiperidin-1-yl)-2-(3-nitro-1*H*-1,2,4-triazol-1-yl)ethan-1-one (16):** white powder (84%); mp 129−131 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.57 (s, 1H), 7.31−7.18 (m, 5H), 5.47 (dt, $J$ = 19.0, 16.5 Hz, 2H), 4.44 (d, $J$ = 13 Hz, 1H), 3.97 (d, $J$ = 13.5 Hz, 1H), 3.16 (t, $J$ = 13.5 Hz, 1H), 2.64 (t, $J$ = 13.0 Hz, 1H), 2.60 (d, $J$ = 7.0 Hz, 2H), 1.88 (m, 1H), 1.76 (d, $J$ = 13.0 Hz, 1H), 1.69 (d, $J$ = 13.0 Hz, 1H), 1.36−1.32 (dq, $J$ = 12.5, 4.5 Hz, 1H), 1.16−1.13 (dq, $J$ = 12.0, 4.0 Hz, 1H); HRESIMS calcd for $C_{16}H_{20}N_5O_3$ $m/z$ $[M + H]^+$ 330.1561, found 330.1576. Anal. Calcd for $C_{16}H_{19}N_5O_3$: C, 58.35; H, 5.82; N, 21.26. Found: C, 58.27; H, 5.83; N, 21.30.

**Data for *N*-(6-methyl-1,3-benzothiazol-2-yl)-2-(3-nitro-1*H*-1,2,4-triazol-1-yl)acetamide (17):** off-white powder (59%); mp 230 °C dec; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.81 (s, 1H), 7.74 (s, 1H), 7.63 (d, $J$ = 8.0 Hz, 1H), 7.28 (d, $J$ = 8.0 Hz, 1H), 5.68 (s, 2H), 2.44 (s, 3H); HRESIMS calcd for $C_{12}H_{11}N_6O_3S$ $m/z$ $[M + H]^+$ 319.0608, found 319.0617.

**Data for *N*-(6-chloro-1,3-benzothiazol-2-yl)-2-(3-nitro-1*H*-1,2,4-triazol-1-yl)acetamide (18):** off-white powder (58%); mp 245−248 °C dec; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.81 (s, 1H), 8.06 (s, 1H), 7.74 (d, $J$ = 8.5 Hz, 1H), 7.04 (dd, $J$ = 8.5, 2.0 Hz, 1H), 5.71 (s, 2H); HRESIMS calcd for $C_{11}H_8ClN_6O_3S$ $m/z$ $[M + H]^+$ 339.0062, 341.0034, found 339.0072, 341.0045.

**Data for *N*-(5-chloro-1,3-benzoxazol-2-yl)-2-(3-nitro-1*H*-1,2,4-triazol-1-yl)acetamide (19):** off-white powder (45%); mp 208−210 °C dec; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.76 (s, 1H), 7.60 (s, 1H), 7.59 (d, $J$ = 9.0 Hz, 1H), 7.34 (dd, $J$ = 8.5, 2.0 Hz, 1H), 5.80 (s, 1H); HRESIMS calcd for $C_{11}H_6ClN_6O_4$ $m/z$ $[M - H]^-$ 321.0145, 323.0119, found 321.0147, 323.0143.

**Data for 1-[3,5-bis(trifluoromethyl)phenyl]-3-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]urea (20):** white powder (95%); mp 151−152 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.71 (s, 1H), 8.68 (br s, 1H), 8.15 (s, 2H), 7.54 (s, 1H), 6.31 (br s, 1H), 4.51 (t, $J$ = 6.5 Hz, 2H), 3.35 (m, 2H), 2.21 (quintet, $J$ = 6.5 Hz, 2H); HRESIMS calcd for $C_{14}H_{13}F_6N_6O_3$ $m/z$ $[M + H]^+$ 427.0948, found 427.0954.

**Data for *N*-[3-(2-nitro-1*H*-imidazol-1-yl)propyl]-4-(trifluoromethyl)benzene-1-sulfonamide (21):** white powder (56%); mp 129−131 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.99 (d, $J$ = 8.0 Hz, 2H), 7.82 (d, $J$ = 8.5 Hz, 2H), 7.24 (s, 1H), 7.19 (s, 1H), 4.77 (br t, 1H), 4.57 (t, $J$ = 7.0 Hz, 2H), 3.06 (q, $J$ = 6.5 Hz, 2H), 2.12 (quintet, $J$ = 6.5 Hz, 2H); HRESIMS calcd for $C_{13}H_{14}F_3N_4O_4S$ and $C_{13}H_{13}F_3N_4NaO_4S$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 379.0682, 401.0502 found 379.0685, 401.0506.

**Data for *N*-[2-(3-nitro-1*H*-1,2,4-triazol-1-yl)ethyl]-4-(trifluoromethyl)benzene-1-sulfonamide (22):** white powder (35%); mp 155−156 °C; $^1$H NMR (500 MHz, $CDCl_3$ + several drops of $CD_3COCD_3$) $\delta$ 8.51 (s, 1H), 7.99 (d, $J$ = 8.5 Hz, 2H), 7.82 (d, $J$ = 8.5 Hz, 2H), 7.04 (br s, 1H), 4.56 (t, $J$ = 6.0 Hz, 2H), 3.56 (m, 2H); HRESIMS calcd for $C_{11}H_{11}F_3N_5O_4S$ $m/z$ $[M + H]^+$ 366.0478, found 366.0481.

**Data for *N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-4-(trifluoromethyl)benzene-1-sulfonamide (23):** white powder (88%); mp 67−68 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.36 (s, 1H), 7.97 (d, $J$ = 8.5 Hz, 2H), 7.81 (d, $J$ = 8.5 Hz, 2H), 5.01 (br t, 1H), 4.51 (t, $J$ = 6.5 Hz, 2H), 3.03 (q, $J$ = 6.5 Hz, 2H), 2.23 (quintet, $J$ = 6.0 Hz, 2H); HRESIMS calcd for $C_{12}H_{13}F_3N_5O_4S$ $m/z$ $[M + H]^+$ 380.0635, found 380.0635.

**Data for *N*-[4-(3-nitro-1*H*-1,2,4-triazol-1-yl)butyl]-4-(trifluoromethyl)benzene-1-sulfonamide (24):** white powder (49%); mp 83−85 °C; $^1$H NMR (500 MHz, $CD_3OD$) $\delta$ 8.59 (s, 1H), 8.03 (d, $J$ = 8.0 Hz, 2H), 7.89 (d, $J$ = 8.0 Hz, 2H), 4.32 (t, $J$ = 7.0 Hz, 2H), 2.94 (t, $J$ = 6.5 Hz, 2H), 1.96 (quintet, $J$ = 7.5 Hz, 2H), 1.51 (quintet, $J$ = 7.5 Hz, 2H); HRESIMS calcd for $C_{13}H_{15}F_3N_5O_4S$ $m/z$ $[M + H]^+$ 394.0791, found 394.0796.

**Data for *N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-2,5-bis-(trifluoromethyl)benzene-1-sulfonamide (25):** white powder (85%); mp 131−133 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.42 (s, 1H), 8.31 (s, 1H), 8.06 (d, $J$ = 8.0 Hz, 1H), 8.00 (d, $J$ = 8.0 Hz, 2H), 5.10 (br s, 1H), 4.49 (t, $J$ = 6.5 Hz, 2H), 3.07 (m, 2H), 2.25 (quintet, $J$ = 6.5 Hz, 2H); HRESIMS calcd for $C_{13}H_{12}F_6N_5O_4S$ and $C_{13}H_{11}F_6N_5NaO_4S$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 448.0509, 470.0328, found 448.0496, 470.0310.

**Data for *N*-[2-(3-nitro-1*H*-1,2,4-triazol-1-yl)ethyl]-3,5-bis-(trifluoromethyl)benzene-1-sulfonamide (26):** white powder (40%); mp 164−165 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.67


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(s, 1H), 8.40 (s, 2H), 8.37 (s, 1H), 4.60 (t, $J$ = 5.5 Hz, 2H), 3.67 (t, $J$ = 5.5 Hz, 2H); HRESIMS calcd for $C_{12}H_{10}F_6N_5O_4S$ and $C_{12}H_9F_6N_5NaO_4S$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 434.0352, 456.0172, found 434.0358, 456.0178.

**Data for *N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-3,5-bis-(trifluoromethyl)benzene-1-sulfonamide (27):** white microcrystals (62%); mp 132−134 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.64 (s, 1H), 8.41 (s, 2H), 8.38 (s, 1H), 7.15 (br s, 1H), 4.53 (t, $J$ = 7.0 Hz, 2H), 3.15 (t, $J$ = 6.5 Hz, 2H), 2.22 (quintet, $J$ = 7.0 Hz, 2H); HRESIMS calcd for $C_{13}H_{12}F_6N_5O_4S$ $m/z$ $[M + H]^+$ 448.0509, found 448,0495.

**Data for 4-methyl-*N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)-propyl]benzene-1-sulfonamide (28):** white microcrystals (81%); mp 122−124 °C; $^1$H NMR (500 MHz, $CD_3COD$) $\delta$ 8.57 (s, 1H), 7.70 (d, $J$ = 8.5 Hz, 2H), 7.37 (d, $J$ = 8.5 Hz, 2H), 4.41 (t, $J$ = 6.5 Hz, 2H), 3.31 (t, $J$ = 6.5 Hz, 2H), 2.42 (s, 3H), 2.08 (quintet, $J$ = 6.5 Hz, 2H); HRESIMS calcd for $C_{12}H_{16}N_5O_4S$ and $C_{12}H_{15}N_5NaO_4S$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 326.0918, 348.0737, found 326.0917, 348.0734. Anal. Calcd for $C_{12}H_{15}N_5O_4S$: C, 44.30; H, 4.65; N, 21.53; S, 9.85. Found: C, 44.51; H, 4.81; N, 21.22; S, 9.89.

**Data for *N*-[4-(3-nitro-1*H*-1,2,4-triazol-1-yl)butyl]-4-(trifluoromethoxy)benzene-1-sulfonamide (29):** white powder (42%); mp 66−68 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.64 (s, 1H), 7.99 (d, $J$ = 8.5 Hz, 2H), 7.55 (d, $J$ = 8.0 Hz, 2H), 6.68 (br s, 1H), 4.42 (t, $J$ = 7.0 Hz, 2H), 3.01 (t, $J$ = 6.5 Hz, 2H), 2.01 (m, 2H), 1.59 (quintet, $J$ = 6.5 Hz, 2H); HRESIMS calcd for $C_{13}H_{15}F_3N_5O_5S$ $m/z$ $[M + H]^+$ 410.0741, found 410.0744.

**Data for *N*-[2-(3-nitro-1*H*-1,2,4-triazol-1-yl)ethyl]-1-phenyl-methanesulfonamide (30):** white powder (31%); mp 165−166 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.62 (s, 1H), 7.42−7.35 (m, 5H), 6.42 (br s, 1H), 4.50 (t, $J$ = 5.5 Hz, 2H), 4.37 (s, 2H), 3.58 (t, $J$ = 5.5 Hz, 2H); HRESIMS calcd for $C_{11}H_{14}N_5O_4S$ and $C_{11}H_{13}N_5NaO_4S$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 312.0761, 334.0580, found 312.0773, 334.0594.

**Data for *N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-1-phenyl-methanesulfonamide (31):** white microcrystals (45%); mp 104−106 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.62 (s, 1H), 7.43−7.35 (m, 5H), 6.25 (br s, 1H), 4.49 (t, $J$ = 7.0 Hz, 2H), 4.35 (s, 2H), 3.13 (m, 2H), 2.17 (quintet, $J$ = 7.0 Hz, 2H); HRESIMS calcd for $C_{12}H_{16}N_5O_4S$ and $C_{12}H_{15}N_5NaO_4S$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 326.0918, 348.0737, found 326.0923, 348.0737.

**Data for 1-methyl-*N*-[2-(3-nitro-1*H*-1,2,4-triazol-1-yl)ethyl]-1*H*-imidazole-2-sulfonamide (32):** white powder (24%); mp 170−172 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.72 (s, 1H), 7.45 (br s, 1H), 7.30 (s, 1H), 7.02 (s, 1H), 4.63 (t, $J$ = 6.0 Hz, 2H), 3.90 (s, 3H), 3.79 (t, $J$ = 6.0 Hz, 2H); HRESIMS calcd for $C_8H_{12}N_7O_4S$ and $C_8H_{11}N_7NaO_4S$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 302.0666, 324.0485, found 302.0664, 324.0480.

**Data for 1-methyl-*N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)-propyl]-1*H*-imidazole-2-sulfonamide (33):** white powder (61%); mp 106−109 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.68 (s, 1H), 7.28 (s, 1H), 6.99 (s, 1H), 4.57 (t, $J$ = 7.0 Hz, 2H), 3.92 (s, 3H), 3.28 (t, $J$ = 6.5 Hz, 2H). 2.25 (quintet, $J$ = 7.0 Hz, 2H); HRESIMS calcd for $C_9H_{14}N_7O_4S$ and $C_9H_{13}N_7NaO_4S$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 316.0822, 338.0642, found 316.0832, 338.0649. Anal. Calcd for $C_9H_{13}N_7O_4S$: C, 34.28; H, 4.16; N, 31.10; S, 10.17. Found: C, 34.32; H, 4.27; N, 30.83; S, 9.85.

**Data for 5-chloro-*N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-thiophene-2-sulfonamide (34):** white powder (75%); mp 104−105 °C; $^1$H NMR (500 MHz, $CD_3COCD_3$) $\delta$ 8.65 (s, 1H), 7.47 (d, $J$ = 4.0 Hz, 1H), 7.16 (d, $J$ = 4.0 Hz, 1H), 7.01 (br s, 1H), 4.53 (t, $J$ = 7.0 Hz, 2H), 3.13 (t, $J$ = 6.5 Hz, 2H), 2.23 (quintet, $J$ = 6.5 Hz, 2H); HRESIMS calcd for $C_9H_{11}ClN_5O_4S_2$ and $C_9H_{10}ClN_5NaO_4S_2$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 351.9935, 373.9755, found 351.9930, 353.9899, 373.9751, 375.9721.

**Data for *N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]-4-phenyl-benzene-1-sulfonamide (35):** white powder (60%); mp 132−133 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.36 (s, 1H), 7.88 (d, $J$ = 8.5 Hz, 2H), 7.73 (d, $J$ = 8.0 Hz, 2H), 7.59 (d, $J$ = 7.0 Hz, 2H), 7.49 (t, $J$ = 7.5 Hz, 2H), 7.43 (t, $J$ = 7.0 Hz, 1H), 4.76 (t, $J$ = 6.0 Hz, 1H), 4.51 (t, $J$ = 6.5 Hz, 2H), 2.80 (q, $J$ = 6.5 Hz, 2H), 2.21 (quintet, $J$ = 6.5 Hz, 2H); HRESIMS calcd for $C_{17}H_{18}N_5O_4S$ and $C_{17}H_{17}N_5NaO_4S$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 388.1074, 410.0893, found 388.1070, 410.0887.

**Data for *N*-[3-(3-nitro-1*H*-1,2,4-triazol-1-yl)propyl]quinoline-8-sulfonamide (36):** off-white powder (63%); mp 142−143 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 9.04 (d, $J$ = 4.0 Hz, 1H), 8.40 (s, 2H), 8.32 (d, $J$ = 8.0 Hz, 1H), 8.10 (d, $J$ = 8.5 Hz, 1H), 7.68 (t, $J$ = 7.5 Hz, 1H), 7.62−7.59 (m, 1H), 6.61 (br t, $J$ = 6.0 Hz, 1H), 4.55 (t, $J$ = 6.0 Hz, 2H), 2.80 (m, 2H), 2.18 (m, 2H); HRESIMS calcd for $C_{14}H_{15}N_6O_4S$ and $C_{14}H_{14}N_6NaO_4S$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 363.0870, 385.0689, found 363.0883, 385.0680.

***N*-Benzyl-2-chloroacetamide (37a).** This was commercially available from Aldrich.

**Data for 2-chloro-*N*-{[4-(trifluoromethyl)pheny]methyl}-acetamide (37b):** pink-white crystallic powder[28] (89%); mp 87−88 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.62 (d, J=7.5 Hz, 2H), 7.42 (d, $J$ = 8.0 Hz, 2H), 6.98 (br s, 1H), 4.57 (d, $J$ = 6.0 Hz, 2H), 4.14 (s, 2H); HRESIMS calcd for $C_{10}H_{10}ClF_3NO$ $m/z$ $[M + H]^+$ 252.0398, 254.0370, found 252.0407, 254.0378.

**Data for 1-(4-benzylpiperidin-1-yl)-2-chloroethan-1-one (37c):** yellow oil[29] (91%); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.32−7.14 (m, 5H), 4.55 (d, $J$=13.0 Hz, 1H), 4.07 (m, 2H), 3.83 (d, $J$ = 13.5 Hz, 1H), 3.05 (t, $J$ = 13.0 Hz, 1H), 2.61−2.55 (m, 3H), 1.81−1.74 (m, 3H), 1.20−1.29 (m, 2H); HRESIMS calcd for $C_{14}H_{19}ClNO$ $m/z$ $[M + H]^+$ 252.1150, 254.1124, found 252.1161, 254.1134.

**Data for 2-chloro-*N*-(6-methyl-1,3-benzothiazol-2-yl)-acetamide (37d):** off-white crystallic powder[30] (100%); mp 190−191 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 9.74 (br s, 1H), 7.70 (d, $J$ = 8.5 Hz, 1H), 7.63 (s, 1H), 7.28 (d, $J$ = 8.5 Hz, 1H), 4.31 (s, 2H), 2.49 (s, 3H); HRESIMS calcd for $C_{10}H_{10}ClN_2OS$ $m/z$ $[M + H]^+$ 241.0197, 243.0168, found 241.0194, 243.0163.

**Data for 2-chloro-*N*-(6-chloro-1,3-benzothiazol-2-yl)-acetamide (37e):** white microcrystallic powder[30,31] (73%); mp 203−204 °C dec; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 9.71 (br s, 1H), 7.82 (s, 1H), 7.73 (d, $J$ = 9.0 Hz, 1H), 7.43 (dd, $J$ = 10.5, 6.5 Hz, 1H), 4.33 (s, 2H); HRESIMS calcd for $C_9H_7Cl_2N_2OS$ $m/z$ $[M + H]^+$ 260.9651, 262.9621, found 260.9663, 262.9630.

**Data for 2-chloro-*N*-(5-chloro-1,3-benzoxazol-2-yl)-acetamide (37f):** light brown powder (70%); mp 168−170 °C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 9.38 (br s, 1H), 7.80−7.30 (m, 3H), 4.38 (s, 2H); HRESIMS calcd for $C_9H_7Cl_2N_2O_2$ and $C_9H_6Cl_2N_2NaO_2$ $m/z$ $[M + H]^+$ and $[M + Na]^+$ 244.9879 and 266.9699, found 244.9871 and 266.9700.

**In Vitro Biological Evaluation.** In vitro activity against *T. cruzi*, *T. brucei rhodesiense*, and *L. donovani* axenic amastigotes was determined and cytotoxicity assessment using L6 cells (rat skeletal myoblasts) performed using a 96-well plate format as previously described.[25] Data were analyzed with the graphic program Softmax Pro (Molecular Devices, Sunnyvale, CA), 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 $1 \times 10^3$ $mL^{-1}$ in 200 $\mu$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 $\mu$g/mL). After incubation for 3 days at 37 °C, 20 $\mu$L of Alamar blue was added to each well, and the plates were 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 $\mu$M) and NADH (100 $\mu$M) and expressed as nanomoles of NADH oxidized per minute per milligram of enzyme.

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

*T. cruzi*, *Trypanosoma cruzi*; *T. brucei*, *Trypanosoma brucei*; HAT, human African trypanosomiasis; Nfx, nifurtimox (4-(5-nitrofurfurylidenamino]-3-methylthiomorpholine 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; tet, tetracycline

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