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Novel 3-Nitro-1*H*-1,2,4-triazole-based Amides and Sulfonamides as Potential anti-Trypanosomal Agents

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Abstract

A series of novel 3-nitro-1*H*-1,2,4-triazole-(and in some cases 2-nitro-1*H*-imidazole)-based amides and sulfonamides were characterized for their *in vitro* anti-trypanosomal 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 *T. cruzi* intracellular amastigotes (IC₅₀ ranging from 28 nM to 3.72 μM) without concomitant toxicity to L6 host cells (selectivity 66 to 2782). Twenty three of these active compounds were more potent (up to 58 fold) than the reference drug benznidazole, tested in parallel. In addition, 9 nitrotriazoles which were moderately active (0.5 μM IC₅₀ < 6.0 μM) against *T. b. rhodesiense* trypomastigotes, were 5 to 31 fold more active against bloodstream-form *T. b. brucei* trypomastigotes engineered to overexpress NADH-dependent nitroreductase (TbNTR). Finally, 3 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 anti-trypanosomal agents.

Keywords

nitrotriazoles; amides; sulfonamides; *T. cruzi*; Chagas disease; anti-trypanosomal agents

INTRODUCTION

The trypanosomatids protozoan parasites *Trypanosoma cruzi* (*T. cruzi*), *Trypanosoma brucei* (*T. 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 of 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 non-endemic 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.

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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, affordable and safer drugs to treat this disease is urgent.

Most nitroheterocyclic compounds 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 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 parasites¹⁰ and that a type I 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 reported¹⁹ 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 *T. b. rhodesiense* and *T. b. brucei* parasites. We have also shown that 3-nitrotriazole-based amines are activated by type I nitroreductase and that blood stream form *T. b. brucei* parasites overexpressing 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-nitrotriazole-based amines that were evaluated in the Ames test, were found negative for mutagenicity, in contrast to their 2-nitroimidazole analogs (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 anti-trypanosomal agents.

CHEMISTRY

The structure of all compounds is depicted on 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 alkyl amine²³ in the presence of triethyl amine (Scheme 1A). For compounds **3**, **5**, **22**, **26**, **30** and **32** the hydrochloride salt of 2-(3-nitro-1*H*-1,2,4-triazole)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 literature²⁴. First, 3-nitro-1,2,4-triazole was converted to its potassium salt by treating with KOH in acetonitrile under mild heating and then this mixture was added to a solution of the appropriate α -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 dichloromethane²⁴ (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**, **30–32**) 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

Anti-Trypanosomal activity of nitrotriazole/nitroimidazole-based amides and sulfonamides

The *in vitro* growth inhibitory properties of all compounds against bloodstream form *T. b. rhodesiense* trypomastigotes, *T. cruzi* amastigotes (in infected L6 myoblasts), axenically cultured *L. donovani* amastigotes and rat skeletal myoblasts (L6 cells) were evaluated by using standard drug screens.²⁵ From resultant dose response curves, IC₅₀ values in μ 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. b. rhodesiense*, intracellular parasites. These criteria were established by the TDR's "compound screeners network", published in a review²⁶ and are 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'.

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. b. 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 IC₅₀ against L6 cells to IC₅₀ against each parasite, is also an important parameter and both IC₅₀ and SI values are used to rank compounds.²⁶ This SI must be 100 for *T. b. rhodesiense*, 50 for *T. cruzi* and 20 for *L. donovani* axenic amastigotes.

On the basis of the above, only 9 compounds (**4–6**, **13**, **23**, **24**, **28**, **29** and **34**) were moderately active/active and selective against *T. b. rhodesiense*, whereas 30 compounds (83%), namely **1–17**, **21–31** and **34–36** were 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* have also 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 anti-trypanosomal agents and more toxic to the host cells than their 3-nitrotriazole analogs¹⁹, 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 anti-trypanosomal activity of these classes. However, it is apparent that all of these compounds were inactive against *T. b. rhodesiense*, and in general, they were less potent anti-chagasic agents than their closely related 3-nitrotriazoles or benznidazole (compare **1** with **3**, **4** and **7**; **2** with **5** and **6**; **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**; **5** with **6**). The same rule applies for the activity against *T. b. 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 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. b. 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. b. 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 lipophilicity of this compound and led to a decreased selectivity (Table 1). Going from the quinoline-2-carboxamide **10** to the quinoxaline analog **12**, we observe a decrease in the antichagasic activity and selectivity, and complete inactivity against *T. b. rhodesiense* (Table 1). A significant drop in logP value compared to **10** (Table 1) may be related to this inactivity. Finally, the 4-phenylbenzamide **13** was the most potent derivative against *T. cruzi*, with an IC₅₀ 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. b. 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 analog, benznidazole (Table 1), perhaps due to its decreased lipophilicity (logP 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 benzothiazole acetamides **17** and **18** and the benzoxazole acetamide **19** were less potent against *T. cruzi* amastigotes compared to benznidazole. Similarly, all three compounds were inactive against *T. b. rhodesiense* (Table 1). However, compounds **17–19** demonstrated a moderate anti-leishmanial 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* 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 logP values (Table 1).

Analysis of N-(3-nitrotriazole-alkyl) arene-sulfonamides

Evaluating sulfonamides **21–36**, it is observed that all but the methyl-imidazole sulfonamides **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 logP values and PSA > 140 Å, 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 analogs.

As in the case of N-(3-nitrotriazolyl-alkyl)benzamides, activity of N-(3-nitrotriazolyl-alkyl) benzene sulfonamides **22–24** against *T. b. rhodesiense*, proportionally increases with the length of the linker between the 3-nitrotriazole ring and sulfamido group (Table 1). The same rule, however, does not apply here for activity against *T. cruzi*, although it is clear that two methylene-linker corresponds 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**; **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**, **26** and **27**) resulted in inactivity against *T. b. rhodesiense*, independently of its position on the ring (**25**, **26**, **27**), with the linker length (**26**) being the most determinant parameter. 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. b. 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. b. rhodesiense* (Table 1). Exchanging the tolyl group in **28** with a benzyl group in **31** further decreased the logP value and resulted in lower activity and selectivity against both *T. cruzi* and *T. b. rhodesiense* (Table 1). Finally, shortening the linker of **31** by one methylene group in **30** significantly decreased the activity against *T. cruzi* and *T. b. rhodesiense* and resulted in unacceptable selectivity (Table 1). Interestingly, both benzyl sulfonamides **30** and **31** were less potent antichagasic agents than benznidazole.

As in the case of 4-phenylbenzamide **13**, the 4-phenylbenzene sulfonamide **35** was the most potent antichagasic compound in the series of sulfonamides, with an IC₅₀ of 28 nM (~56 times more potent than benznidazole) and selectivity of 1764. Sulfonamide **35** was more potent against *T. cruzi* than the analogous amide **13**, but less active than **13** against *T. b. rhodesiense*. In addition, increased toxicity of **35** to L6 host cells, independently of lipophilicity, resulted in decreased selectivity as compared to **13** (Table 1).

Replacing the phenyl ring with a chloro-thiophene in **34**, slightly decreased the potency against *T. cruzi* and had a more significant impact in selectivity due to an increase in toxicity (compare **28** with **34**). However, the activity against *T. b. rhodesiense* and selectivity of **34** was similar to that 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 **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).

The 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 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 sub-categories in Table 1 have been evaluated for anti-HAT activity against bloodstream form *T. b. brucei* (Table 2). With few exceptions (**3**, **13**, **20**, **35**), most compounds demonstrated a greater IC₅₀ value or were inactive against *T. b. brucei* compared to *T. b. rhodesiense* (Table 2). Compounds with an IC₅₀ > 5 µM against *T. b. brucei* were tested in a parasite line engineered to overexpress tetracycline-inducible TbNTR, in order 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 (non-induced/induced) ratio 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. b. brucei* (**6**, **23**, **28** 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 Fig. 1, all of the tested compounds were preferred substrates of the nitroreductase and there is, in general, a good correlation between enzymatic activity and activity against *T. b. rhodesiense*.

To exclude the possibility that these compounds may exert some of their anti-trypansomal 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 µM (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 of the 19 compounds have demonstrated significant antichagasic activity at low to intermediate nmolar 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 anti-leishmania drugs.

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 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 dry dichloromethane and added dropwise to a solution of 3-nitro-1*H*-1,2,4-triazolyl-alkylamine²³ (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)-propylamine²³ (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-triazole)ethylamine (instead of the free amine) and 4 eq 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-1,2,4-triazole (0.9–1.0 mmol) was stirred under an inert atmosphere and exclusion of moisture with 1.2 eq of KOH in acetonitrile under mild heating (ca. 40 °C) and then this suspension was slowly added to an acetonitrile solution of the appropriate α -chloroacetamide²⁴ (**37a–f**).

α -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 α -chloroacetyl chloride (3.07 mmol), according to the literature²⁴.

N-[3-(2-Nitro-1H-imidazol-1-yl)propyl]-4-(trifluoromethoxy)benzamide (2)—Off white powder (54%): mp 68–70 °C; ¹H NMR (500 MHz, CDCl₃) δ : 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 C₁₄H₁₄F₃N₄O₄ and C₁₄H₁₃F₃N₄NaO₄ m/z [M+H]⁺ and [M+Na]⁺ 359.0962, 381.0781, found 359.0962, 381.0784.

N-[2-(3-Nitro-1H-1,2,4-triazol-1-yl)ethyl]-4-(trifluoromethyl)benzamide (3)—White powder (65%): mp 155–157 °C; ¹H NMR (500 MHz, CDCl₃) δ : 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 C₁₂H₁₁F₃N₅O₃ m/z [M+H]⁺ 330.0809, found 330.0815.

N-[4-(3-Nitro-1H-1,2,4-triazol-1-yl)butyl]-4-(trifluoromethyl)benzamide (4)—White powder (62%): mp 78–79 °C; ¹H NMR (500 MHz, CD₃COCD₃) δ : 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 C₁₄H₁₅F₃N₅O₃ m/z [M+H]⁺ 358.1122, found 358.1131.

N-[2-(3-Nitro-1H-1,2,4-triazol-1-yl)ethyl]-4-(trifluoromethoxy)benzamide (5)—White powder (65%): mp 108–109 °C; ¹H NMR (500 MHz, CD₃COCD₃) δ : 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 C₁₂H₁₁F₃N₅O₄ m/z [M+H]⁺ 346.0758, found 346.0765.

N-[4-(3-Nitro-1H-1,2,4-triazol-1-yl)butyl]-4-(trifluoromethoxy)benzamide (6)—White powder (72%): mp 64–65 °C; ¹H NMR (500 MHz, CDCl₃) δ : 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 C₁₄H₁₅F₃N₅O₄ m/z [M+H]⁺ 374.1071, found 374.1075.

N-[3-(3-Nitro-1H-1,2,4-triazol-1-yl)propyl]-3-(trifluoromethyl)benzamide (7)—White powder (70%): mp 81–83 °C; ¹H NMR (500 MHz, CDCl₃) δ : 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 C₁₃H₁₃F₃N₅O₃ m/z [M+H]⁺ 344.0965, found 344.0969.

N-[3-(3-Nitro-1H-1,2,4-triazol-1-yl)propyl]-3,5-bis(trifluoromethyl)benzamide (8)—White powder (83%): mp 152–153 °C; ¹H NMR (500 MHz, CD₃COCD₃) δ : 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 C₁₄H₁₂F₆N₅O₃ m/z [M+H]⁺ 412.0839 found 412.0844.

N-[3-(3-Nitro-1H-1,2,4-triazol-1-yl)propyl]-6-(trifluoromethyl)pyridine-3-carboxamide (9)—White powder (71%): mp 92–94 °C; ¹H NMR (500 MHz, CDCl₃) δ : 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 C₁₂H₁₂F₃N₆O₃ and C₁₂H₁₁F₃N₆NaO₃ m/z [M+H]⁺ and [M+Na]⁺ 345.0917,

367.0737, found 345.0929, 367.0745. Calculated analysis 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.

N-[3-(3-Nitro-1H-1,2,4-triazol-1-yl)propyl]quinoline-2-carboxamide (10)—Off white powder (70%): mp 135–137 °C; 1H NMR (500 MHz, CD_3OD) δ : 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.

N-[4-(3-Nitro-1H-1,2,4-triazol-1-yl)butyl]quinoline-2-carboxamide (11)—Off white powder (67%): mp 124–126 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 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.

N-[3-(3-Nitro-1H-1,2,4-triazol-1-yl)propyl]quinoxaline-2-carboxamide (12)—Off white powder (66%): mp 143–144 °C; 1H NMR (500 MHz, $CDCl_3$) δ : 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.

N-[3-(3-Nitro-1H-1,2,4-triazol-1-yl)propyl]-4-phenylbenzamide (13)—White powder (96%): mp 177–179 °C; 1H NMR (500 MHz, $CDCl_3$) δ : 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. Calculated analysis 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.

N-Benzyl-2-(3-nitro-1H-1,2,4-triazol-1-yl)acetamide (14)—Off white powder (40%): mp 103–106 °C; 1H NMR (500 MHz, $CDCl_3$) δ : 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.

2-(3-Nitro-1H-1,2,4-triazol-1-yl)-N-[[4-(trifluoromethyl)phenyl]methyl]acetamide (15)—White microcrystal (78%): mp 168–170 °C; 1H NMR (500 MHz, $CDCl_3$) δ : 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.

1-(4-Benzylpiperidin-1-yl)-2-(3-nitro-1H-1,2,4-triazol-1-yl)ethan-1-one (16)—White powder (84%): mp 129–131 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 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. Calculated analysis 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.

N-(6-methyl-1,3-benzothiazol-2-yl)-2-(3-nitro-1H-1,2,4-triazol-1-yl)acetamide (17)—Off white powder (59%): mp 230 °C (dec); ¹H NMR (500 MHz, CD₃COCD₃) δ: 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 C₁₂H₁₁N₆O₃S *m/z* [M+H]⁺ 319.0608, found 319.0617.

N-(6-chloro-1,3-benzothiazol-2-yl)-2-(3-nitro-1H-1,2,4-triazol-1-yl)acetamide (18)—Off white powder (58%): mp 245–248 °C (dec); ¹H NMR (500 MHz, CD₃COCD₃) δ: 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 C₁₁H₈ClN₆O₃S *m/z* [M+H]⁺ 339.0062, 341.0034, found 339.0072, 341.0045.

N-(5-chloro-1,3-benzoxazol-2-yl)-2-(3-nitro-1H-1,2,4-triazol-1-yl)acetamide (19)—Off white powder (45%): mp 208–210 °C (dec); ¹H NMR (500 MHz, CD₃COCD₃) δ: 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 C₁₁H₆ClN₆O₄ *m/z* [M–H][–] 321.0145, 323.0119, found 321.0147, 323.0143.

1-[3,5-bis(trifluoromethyl)phenyl]-3-[3-(3-Nitro-1H-1,2,4-triazol-1-yl)propyl]urea (20)—White powder (95%): mp 151–152 °C; ¹H NMR (500 MHz, CD₃COCD₃) δ: 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 C₁₄H₁₃F₆N₆O₃ *m/z* [M+H]⁺ 427.0948, found 427.0954.

N-[3-(2-Nitro-1H-imidazol-1-yl)propyl]-4-(trifluoromethyl)benzene-1-sulfonamide (21)—White powder (56%): mp 129–131 °C; ¹H NMR (500 MHz, CDCl₃) δ: 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 C₁₃H₁₄F₃N₄O₄S and C₁₃H₁₃F₃N₄NaO₄S *m/z* [M+H]⁺, [M+Na]⁺ 379.0682, 401.0502 found 379.0685, 401.0506.

N-[2-(3-Nitro-1H-1,2,4-triazol-1-yl)ethyl]-4-(trifluoromethyl)benzene-1-sulfonamide (22)—White powder (35%): mp 155–156 °C; ¹H NMR (500 MHz, CDCl₃ + several drops of CD₃COCD₃) δ: 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 C₁₁H₁₁F₃N₅O₄S *m/z* [M+H]⁺ 366.0478, found 366.0481.

N-[3-(3-Nitro-1H-1,2,4-triazol-1-yl)propyl]-4-(trifluoromethyl) benzene-1-sulfonamide (23)—White powder (88%): mp 67–68 °C; ¹H NMR (500 MHz, CDCl₃) δ: 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 C₁₂H₁₃F₃N₅O₄S *m/z* [M+H]⁺ 380.0635, found 380.0635.

N-[4-(3-Nitro-1H-1,2,4-triazol-1-yl)butyl]-4-(trifluoromethyl) benzene-1-sulfonamide (24)—White powder (49%): mp 83–85 °C; ¹H NMR (500 MHz, CD₃OD) δ: 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 C₁₃H₁₅F₃N₅O₄S *m/z* [M+H]⁺ 394.0791, found 394.0796.

N-[3-(3-Nitro-1H-1,2,4-triazol-1-yl)propyl]-2,5-bis(trifluoromethyl)benzene-1-sulfonamide (25)—White powder (85%): mp 131–133 °C; ¹H NMR (500 MHz, CDCl₃) δ: 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.

N-[2-(3-nitro-1H-1,2,4-triazol-1-yl)ethyl]-3,5-bis(trifluoromethyl)benzene-1-sulfonamide (26)—White powder (40%): mp 164–165 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 8.67 (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.

N-[3-(3-nitro-1H-1,2,4-triazol-1-yl)propyl]-3,5-bis(trifluoromethyl)benzene-1-sulfonamide (27)—White microcrystals (62%): mp 132–134 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 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.

4-Methyl-N-[3-(3-nitro-1H-1,2,4-triazol-1-yl)propyl]benzene-1-sulfonamide (28)—White microcrystals (81%): mp 122–124 °C; 1H NMR (500 MHz, CD_3COD) δ : 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. Calculated analysis 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.

N-[4-(3-nitro-1H-1,2,4-triazol-1-yl)butyl]-4-(trifluoromethoxy)benzene-1-sulfonamide (29)—White powder (42%): mp 66–68 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 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.

N-[2-(3-Nitro-1H-1,2,4-triazol-1-yl)ethyl]-1-phenylmethanesulfonamide (30)—White powder (31%): mp 165–166 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 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.

N-[3-(3-Nitro-1H-1,2,4-triazol-1-yl)propyl]-1-phenylmethanesulfonamide (31)—White microcrystals (45%): mp 104–106 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 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.

1-Methyl-N-[2-(3-nitro-1H-1,2,4-triazol-1-yl)ethyl]-1H-imidazole-2-sulfonamide (32)—White powder (24%): mp 170–172 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 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.

1-Methyl-N-[3-(3-nitro-1H-1,2,4-triazol-1-yl)propyl]-1H-imidazole-2-sulfonamide (33)—White powder (61%): mp 106–109 °C; 1H NMR (500 MHz, CD_3COCD_3) δ : 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. Calculated analysis

for C₉H₁₃N₇O₄S: 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.

5-Chloro-N-[3-(3-nitro-1H-1,2,4-triazol-1-yl)propyl]thiophene-2-sulfonamide

(34)—White powder (75%): mp 104–105 °C; ¹H NMR (500 MHz, CD₃COCD₃) δ: 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 C₉H₁₁ClN₅O₄S₂ and C₉H₁₀ClN₅NaO₄S₂ *m/z* [M+H]⁺ and [M+Na]⁺ 351.9935, 373.9755, found 351.9930, 353.9899, 373.9751, 375.9721.

N-[3-(3-Nitro-1H-1,2,4-triazol-1-yl)propyl]-4-phenylbenzene-1-sulfonamide (35)

—White powder (60%): mp 132–133 °C; ¹H NMR (500 MHz, CDCl₃) δ: 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 C₁₇H₁₈N₅O₄S and C₁₇H₁₇N₅NaO₄S *m/z* [M+H]⁺ and [M+Na]⁺ 388.1074, 410.0893, found 388.1070, 410.0887.

N-[3-(3-Nitro-1H-1,2,4-triazol-1-yl)propyl]quinoline-8-sulfonamide (36)—Off

white powder (63%): mp 142–143 °C; ¹H NMR (500 MHz, CDCl₃) δ: 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 C₁₄H₁₅N₆O₄S and C₁₄H₁₄N₆NaO₄S *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 by Aldrich.

2-Chloro-N-[[4-(trifluoromethyl)phenyl]methyl]acetamide (37b)—Pink-white

crystalline powder²⁸ (89%): mp 87–88 °C; ¹H NMR (500 MHz, CDCl₃) δ: 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 C₁₀H₁₀ClF₃NO *m/z* [M+H]⁺ 252.0398, 254.0370, found 252.0407, 254.0378.

1-(4-benzylpiperidin-1-yl)-2-chloroethan-1-one (37c)—Yellowish oil²⁹ (91%): ¹H

NMR (500 MHz, CDCl₃) δ: 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 C₁₄H₁₉ClNO *m/z* [M+H]⁺ 252.1150, 254.1124, found 252.1161, 254.1134.

2-Chloro-N-(6-methyl-1,3-benzothiazol-2-yl)acetamide (37d)—Off white crystalline

powder³⁰ (100%): mp 190–191 °C; ¹H NMR (500 MHz, CDCl₃) δ: 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 C₁₀H₁₀ClN₂OS *m/z* [M+H]⁺ 241.0197, 243.0168, found 241.0194, 243.0163.

2-Chloro-N-(6-chloro-1,3-benzothiazol-2-yl)acetamide (37e)—White

microcrystalline powder^{30,31} (73%): mp 203–204 °C (dec); ¹H NMR (500 MHz, CDCl₃) δ: 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 C₉H₇Cl₂N₂OS *m/z* [M+H]⁺ 260.9651, 262.9621, found 260.9663, 262.9630.

2-Chloro-N-(5-chloro-1,3-benzoxazol-2-yl)acetamide (37f)—Light brownish

powder (70%): mp 168–170 °C; ¹H NMR (500 MHz, CDCl₃) δ: 9.38 (br s, 1H), 7.80–7.30 (m, 3H), 4.38 (s, 2H). HRESIMS calcd for C₉H₇Cl₂N₂O₂ and C₉H₆Cl₂N₂NaO₂ *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. 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 IC₅₀ values by linear regression from the sigmoidal dose inhibition curves.

***In vitro* *T. brucei* antiproliferating assays and susceptibility studies**

T. b. brucei bloodstream form parasites were seeded at 1×10^3 ml⁻¹ in 200 µL of growth medium containing different concentrations of a nitrotriazole or nifurtimox. Where appropriate, induction of the TbNTR was carried out by adding tetracycline (1 µg/mL). After incubation for 3 days at 37 °C, 20 µL of Alamar blue was added to each well and the plates incubated for a further 16 h. The cell density of each culture was determined as described before¹¹ and the IC₅₀ established.

Enzymatic activity studies

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

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ABBREVIATIONS USED

<i>T. cruzi</i>	<i>Trypanosoma cruzi</i>
<i>T. brucei</i>	<i>Trypanosoma brucei</i>
HAT	human African trypanosomiasis
Nfx	nifurtimox (4-(5-nitrofurfurylindenamino)-3-methylthio-morpholine-1,1-dioxide)
Bnz	benznidazole (N-benzyl-2(2-nitro-1 <i>H</i> -imidazol-1-yl)acetamide)
NTR	type I nitroreductase
TbNTR	<i>T. brucei</i> NTR
DNDi	Drugs for Neglected Diseases initiative
SI	selectivity index
SARs	structure-activity relationships
tet	tetracycline

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Fig. 1.
Activity of recombinant TbNTR toward different amides/sulfonamides and nifurtimox (Nfx).

Scheme 1.

i) Et₃N (2 eq), CH₂Cl₂, RT, 12 h; n = 1–3; X = C, 2-NO₂; X = N, 3-NO₂; when n = 1, the HCl salt was used instead of the free amine with 4 eq of Et₃N.

ii) KOH, CH₃CN, mild heating. iii) Et₃N, CH₂Cl₂.

Chart 1.

Table 1

In vitro biological and physical properties of 3-nitrotriazole-based amides/sulfonamides.

Comp.	<i>T. br. rhod.</i> IC ₅₀ (μM)	SI	<i>T. cruzi</i> ^b IC ₅₀ (μM)	SI	L _{don. ax. c} IC ₅₀ (μM)	Std	L ₆ ^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	102
3	3.161		0.438	>625	33.44		>273.6	3.6	2.09	105.6
4	0.501	208	0.176	591	7.93		104.3	8.9	2.66	105.6
5	1.986	>131	0.73	>357	29.25		>260.9	2.1	2.64	114.9
6	1.391	>193	0.113	>2381	12.98		>268	13.8	3.22	114.9
7	3.761		0.353	>826	37.32		>292	4.4	2.15	105.6

Comp.	<i>T.b.rhod.</i> ^a		SI	<i>T. cruzi</i> ^b		SI	<i>L.don. ax.</i> ^c		Sf ^d	L6 ^e		IC ₅₀ Bnz/IC ₅₀ Comp	logP		PSA (Å ²)	Chemical Structure
	IC ₅₀ (μM)			IC ₅₀ (μM)			IC ₅₀ (μM)									
8	16.4	11.4		0.642	290.7		12.34			186.6	2.4	3.03	105.6			
				3.459	>84		96.51			>291	11.3	1.81				
9	3.546			0.138	1579		19.94			217.98	11.8	2.33	118.5			
				0.132	691		13.41			91.5	11.8	2.33	118.5			
10	3.22			0.807	>379		62.69			>306	1.9	0.98	131.4			
				0.043	2782		8.37			117	36.3	2.92	105.6			
11	4			3.383	102		28.12			344.83	0.5	0.95	105.6			
				0.970	133		10.39			128.88	1.6	1.83	105.6			
12	34.862	0.587	199	0.307	468		51.37			143.77	5.1	2.17	96.84			

Comp.	<i>T.b.rhod.</i> ^a		SI	<i>T. cruzi</i> ^b		SI	<i>L.don. ax.</i> ^c		SI ^d	<i>L6</i> ^e		IC ₅₀ Bnz/IC ₅₀ Comp	logP	PSA		Chemical Structure
	IC ₅₀ (μM)			IC ₅₀ (μM)			IC ₅₀ (μM)			IC ₅₀ (μM)				(Å ²)		
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

Comp.	<i>T.b.rhod.</i> ^a		SI	<i>T. cruzi</i> ^b		SI	<i>L.don. ax. c</i>		SI ^d	<i>L6^e</i>		IC ₅₀ Bnz/IC ₅₀ Comp	logP	PSA (Å ²)	Chemical Structure
	IC ₅₀ (μM)			IC ₅₀ (μM)			IC ₅₀ (μM)								
25	10.313		178	0.644		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	>307.7	3.8	1.54	122.70
29	0.477	234.9	0.203	551.7	7.8	112	7.7	2.97	131.93
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

Comp.	<i>T.b.rhod.</i> ^a		SI	<i>T. cruzi</i> ^b	SI	<i>L.don. ax.</i> ^c	SI ^d	L6 ^e		IC ₅₀ Bnz/IC ₅₀ Comp	logP		PSA (Å ²)	Chemical Structure
	IC ₅₀ (μM)	IC ₅₀ (μM)						IC ₅₀ (μM)	IC ₅₀ (μM)					
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.b. rhodesiense*, strain STIB 900 trypomastigotes;

^b *T. cruzi*, strain Tulahuen C4 amastigotes;

^c *L. donovani axenic*, strain MHOM-ET-67/L82 amastigotes;

^d SI is the ratio: IC₅₀ in L6 cells/IC₅₀ in each parasite.

^e Cytotoxicity in L6 cells. Reference drugs: Melarsoprol (Melars), Benznidazole (Bnz), Miltefosine (Miltef), Podophyllotoxin (Podoph). The IC₅₀ 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).

Table 2

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

ID No	<i>T.b. rhod.</i> ^a	<i>T.b. brucei</i> ^b	TbNTR ^c		Ratio
	IC ₅₀ (μM)	IC ₅₀ (μM)	-tet	+tet	-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
Buz ^f			21.80 ± 1.00	2.20 ± 0.30	10

ID No	<i>T.b. rhod.</i> ^a		<i>T.b. brucei</i> ^b	TbNTR ^c		Ratio	
	IC ₅₀ (μM)	IC ₅₀ (μM)	IC ₅₀ (μM)	-tet	+tet	-tet/+tet	
Melars ^g				0.0034 ± 0.0001	0.0034 ± 0.0001		1

^a STIB 900 trypomastigotes;

^b bloodstream form wild type *T. b. brucei* (Lister 427; clone 221a) parasites;

^c bloodstream form *T. b. 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).