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Research paper

Nitrotriazole-based acetamides and propanamides with broad spectrum antitrypanosomal activity

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ABSTRACT

3-Nitro-1*H*-1,2,4-triazole-based acetamides bearing a biphenyl- or a phenoxyphenyl moiety have shown remarkable antichagasic activity both *in vitro* and in an acute murine model, as well as substantial *in vitro* antileishmanial activity but lacked activity against human African trypanosomiasis. We have shown now that by inserting a methylene group in the linkage to obtain the corresponding propanamides, both antichagasic and in particular anti-human African trypanosomiasis potency was increased. Therefore, IC_{50} values at low nM concentrations against both *T. cruzi* and *T. b. rhodesiense,* along with huge selectivity indices were obtained. Although several propanamides were active against *Leishmania donovani*, they were slightly less potent than their corresponding acetamides. There was a good correlation between lipophilicity (clogP value) and trypanocidal activity, for all new compounds. Type I nitroreductase, an enzyme absent from the human host, played a role in the activation of the new compounds, which may function as prodrugs. Antichagasic activity *in vivo* was also demonstrated with representative propanamides.

1. Introduction

American trypanosomiasis (Chagas disease), human African trypanosomiasis (HAT disease or sleeping sickness) and Leismaniasis are parasitic infections. They are considered neglected tropical diseases (NTD) because they constitute a major health problem in particularly poor countries around the world [1]. HAT disease (caused by *Trypanosoma brucei rhodesiense* and *T. b. gambiense*) is endemic throughout sub-Saharan Africa while Chagas disease (caused by *T. cruzi*) affects populations in South and Central America. In contrast, leishmaniasis (caused by *Leishmania* species) is prevalent in many sub-tropical and tropical regions of the world, recently expanding in non-tropical regions as HIV/AIDS co-infection [2]. These insect transmitted diseases affect more than 20 million people and are responsible for more than 110,000 deaths per year [3]. HAT disease ranks high on the list of NTD because it is fatal if untreated and the treatment options are limited. The incidence of *T. cruzi* infection has significantly declined recently, due to implementation of vector control initiatives, however, the number of cases in non-endemic sites (United States, Australia, Europe and Japan) is rising, primarily due to human and vector migration and contaminated blood transfusions [4–6].

The treatment of neglected diseases is based on drugs with serious limitations. Thus, nifurtimox (Nfx) and benznidazole (Bnz), the two currently used medications for Chagas disease (Fig. 1) are associated with limited efficacy, severe toxicity and long treatment requirements [7,8]. Similarly, drugs used to treat HAT and

Abbreviations: NTD, Neglected tropical diseases; *T. brucei*, *Trypanosoma brucei*; HAT, human African trypanosomiasis; *T. cruzi*, *Trypanosoma cruzi*; Bnz, benznidazole (*N*-benzyl-2-(2-nitro-1*H*-imidazol-1-yl)acetamide); Nfx, nifurtimox (4-(5-nitrofurfurylindenamino)-3-methylthio-morpholine-1,1-dioxide); NTR, type I nitroreductase; TcNTR, *T. cruzi* NTR; TbNTR, *T. brucei* NTR; CYP51, sterol 14α-demethylase enzyme; TcCYP51, *T. cruzi* CYP51; IC_{50}, concentration for 50% growth inhibition; SI, selectivity index; SAR, structure-activity relationships; TDR, Tropical Diseases Research (http://www.who.int/tdr/en/).

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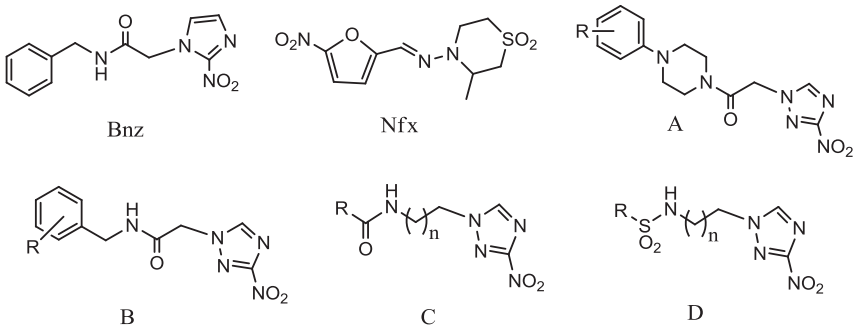

Fig. 1. The chemical structure of Bnz, Nfx and the general structure of representative classes of 3-nitrotriazole-based trypanocidal compounds (A: piperazides, B & C: amides, and D: sulfonamides).

leishmaniasis are highly toxic (e.g. melarsoprol, antimonials), or require i.v. administration (e.g. melarsoprol, suramin, DFMO, antimonials) resulting in severe side effects, or are of high cost (e.g. DFMO, liposomal amphotericin B, miltefosine and paromomycin) [9–11]. Therefore, new effective, safe and affordable drugs are urgently needed for the treatment of these neglected diseases.

We have demonstrated that various chemical classes of 3-nitro-1*H*-1,2,4-triazole-based compounds, including aliphatic/aromatic amines, amides, sulfonamides, carbinols, piperazines and piperazides (some of them shown in Fig. 1) exhibit excellent antichagasic activity both *in vitro* and *in vivo,* with several analogs also showing appreciable *anti-T. b. rhodesiense* activity *in vitro*. [12–18] Futhermore, 3-nitrotriazole-based compounds are significantly more potent and less toxic than their 2-nitroimidazole-based counterparts [12–19]. Nfx, Bnz and other nitroheterocyclics work as prodrugs, needing enzymatic activation to exert their trypanocidal activity [20–23]. We have previously shown that 3-nitrotriazole-based compounds are excellent substrates of a type I nitroreductase (NTR), an oxygen-insensitive nitroreductase present in the mitochondrion of trypanosomatids and absent from most other eukaryotes [20–23] and that part of the trypanocidal activity of these compounds depends on the parasite's expression of type I NTR [12,13,15–18,24].

Despite the failure of the antifungal drug posaconazole to treat chronic Chagas disease in clinical trials [25], there is still a great interest in developing more specific inhibitors of *T. cruzi* CYP51 (TcCYP51), the orthologous enzyme of the fungal sterol 14α-demethylase enzyme (CYP51) [26–28]. Sterol 14α-demethylase is crucial for the formation of viable membranes and the regulation of metabolic processes such as cell growth and division, not only in fungi but also in trypanosomatids [29–32]. Since the triazole ring plays a significant role in CYP51 inhibition [31], we have previously evaluated 3-nitrotriazole-based amides with a linear, rigid core, as well as 3-nitrotriazole-based carbinols (fluconazole analogs) as bifunctional agents; such compounds can act as substrates for type I NTR in addition to being inhibitors of TcCYP51 [17]. These bifunctional compounds demonstrated remarkable antichagasic activity both *in vitro* and in an acute murine model [17]. A subclass of such bifunctional antitrypanosomal agents was 3-nitrotriazole-based aryloxyphenylacetamides, in which the 3-nitrotriazole ring is separated from the amidic carbonyl by one methylene-group [24]. 3-Nitrotriazole-based aryloxyphenylacetamides, besides being very potent antichagasic agents *in vitro* and *in vivo*, demonstrated also remarkable *in vitro* activity against *L. donovani* axenic amastigotes, something that was not seen before with other 3-nitrotriazole-based derivatives [24]. However, these acetamides were only moderately active against *T. b. rhodesiense*, with poor selectivity for this parasite, despite the fact that they were excellent substrates of TbNTR [24].

Therefore, in the present work we tried to further optimize the class of 3-nitrotriazole-based aryloxyphenylamides with the goal to increase their anti-HAT activity. This was obtained via linkage elongation between the nitrotriazole ring and the amidic carbonyl by inserting one additional methylene group. Thus, novel 3-nitrotriazole-based propanamides were synthesized and screened for antitrypanosomal activity. The novel propanamides were then compared side by side with the corresponding acetamides.

2. Results and discussion

2.1. Chemistry

The structure of the twelve novel compounds is shown in Table 1, together with the structure of previously synthesized analogs (in orange), for comparison purposes. The synthesis of the new compounds in Table 1 is straightforward and based on well-established chemistry, outlined in Scheme 1. Thus, acetamides **2, 3, 13** and **15** were obtained by nucleophilic substitution of the appropriate chloroacetamide **1a-c** with the potassium salt of 3-nitro-1,2,4-triazole (and in the case of **3** the potassium salt of 2-nitroimidazole) under refluxing conditions. Similarly, propanamides **6**–**12** and **14** were obtained by nucleophilic substitution of the appropriate bromopropanamide **4a-h** with the potassium salt of 3-nitro-1,2,4-triazole under refluxing conditions. During the synthesis of bromopropanamides **4a-h,** the acrylamides **5a-h** were also formed as β-elimination byproducts, which however were not isolated due to a similar R_f value they share on TLC with bromopropanamides **4a-h.** Fortunately, the acrylamides **5a-h** not only did not prevent the next step but in fact they furnished as starting materials for the formation of the final propanamides through Michael addition. The final compounds were obtained in 45–78% yield.

Chloroacetamides **1a-c** and bromopropanamides **4a-h** were prepared from appropriate, commercially available arylamines and chloroacetyl chloride or 3-bromopropanoyl chloride, respectively, in the presence of triethylamine, at room temperature.

2.2. Biological evaluation

2.2.1. Antiparasitic activity

Compounds in Table 1 were screened for anti-parasitic activity against three trypanosomatids: *T. cruzi*, *T. b. rhodesiense* and *Leishmania donovani* and compared with previously made analogs shown in orange and designated with **a**. The concentration of compound that inhibits parasite growth by 50% (IC_{50}) was calculated from dose response curves for each parasite (Table 1). In addition, compounds were tested for toxicity in L6 rat skeletal myoblasts, the host cells for *T. cruzi* amastigotes, in order to