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Research paper

Antitrypanosomal activity of 5-nitro-2-aminothiazole-based compounds

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ABSTRACT

A small series of 5-nitro-2-aminothiazole-based amides containing arylpiperazine-, biphenyl- or aryloxyphenyl groups in their core were synthesized and evaluated as antitrypanosomatid agents. All tested compounds were active or moderately active against *Trypanosoma cruzi* amastigotes in infected L6 cells and *Trypanosoma brucei brucei*, four of eleven compounds were moderately active against *Leishmania donovani* axenic parasites while none were deemed active against *T. brucei rhodesiense*. For the most active/moderately active compounds a moderate selectivity against each parasite was observed. There was good correlation between lipophilicity (clogP value) and antileishmanial activity or toxicity against L6 cells. Similarly, good correlation existed between clogP values and IC_{50} values against *T. cruzi* in structurally related subgroups of compounds. Three compounds were more potent as antichagasic agents than benznidazole but were not activated by the type I nitrorectusase (NTR).

1. Introduction

American trypanosomiasis (Chagas disease), human African trypanosomiasis (HAT or sleeping sickness) and leishmaniasis are considered neglected tropical diseases (NTD) and represent a severe global health problem [1,2]. It is estimated that together these three diseases, caused by protozoan parasitic infections, affect approximately 20 million people and are responsible for more than 110,000 deaths annually [2]. African trypanosomiasis is endemic in many sub-Saharan African countries and is caused by *Trypanosoma brucei rhodesiense* and *T. brucei gambiense*. Chagas disease, caused by *Trypanosoma cruzi*, is endemic in South and Central America but is now spreading worldwide, mainly due to human and vector migration [3,4]. Leishmaniasis, caused by more than 20 *Leishmania* species, occurs throughout tropical and sub-tropical regions and is now spreading worldwide as an HIV co-infection [5].

Treatment of these NTD is currently based on a series of problematic drugs. Thus, nifurtimox (Nfx) and benznidazole (Bnz), the two currently used medications for Chagas disease are associated with limited efficacy, severe toxicity and long treatment requirements [6,7]. Similarly, drugs used to treat HAT and leishmaniasis are highly toxic (e.g. melarsoprol, antimonials), may require i.v. administration (e.g. melarsoprol, suramin, DFMO, antimonials), can cause severe side effects, or are of high cost (e.g. DFMO, liposomal amphotericin B, miltefosine and paromomycin) [8–10]. Therefore, there is an urgent need for new effective, safe and affordable alternatives.

Although inhibitors of the fungal sterol 14α-demethylase

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; 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.

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enzyme (CYP51) and the orthologous enzyme *T. cruzi* CYP51 (TcCYP51) demonstrated promising efficacy against Chagas disease in preclinical studies [11–13], data from clinical trials using posaconazole or ravuconazole were disappointing [14,15]. Moreover, recent evidence indicates that nitroheterocyclics might be more efficacious trypanocidal agents than CYP51 inhibitors [16], and combinations of the two may offer even a better solution [17].

We have shown that several chemical classes of 3-nitro-1*H*-1,2,4-triazole-based compounds exhibit excellent antichagasic activity both *in vitro* and *in vivo* [18–25]. Furthermore, appreciable anti-HAT activity was also observed *in vitro* with several such analogs [18–25] whereas *in vitro* antileishmanial activity was demonstrated with a sub-class of 3-nitrotriazole- and 2-nitroimidazole-based aryloxyphenylamides [25]. Nitro-activation by an oxygen-insensitive type I nitroreductase (NTR), an enzyme located in the mitochondrion of trypanosomatids and absent from most other eukaryotes, is partially responsible for the trypanocidal activity of these and other nitroheterocyclic compounds [18,19,21–29]. More recently, we have synthesized 3-nitrotriazole-based rigid amides and carbinols which act as bifunctional agents; they exert their antitrypanosomal activity upon activation by type I NTRs and by inhibiting the parasite's CYP51 enzyme [23,25]. Interestingly, 3-nitrotriazole-based compounds are significantly more potent and less toxic than their 2-nitroimidazole-based counterparts [18–25,30].

Here we have expanded our research by investigating the role that another nitroheterocyclic ring, 5-nitro-2-aminothiazole, plays in antitrypanosomatid activity. Nitrothiazole- and nitrobenzothiazole-containing compounds exhibit antiparasitic, antibacterial, antifungal and antitubercular activities [31–34]. Therefore, we have synthesized and evaluated *in vitro* a small series of 5-nitro-2-aminothiazole-based compounds bearing moieties that were previously proven effective in the trypanocidal activity of 3-nitrotriazole-based agents.

2. Results and discussion

2.1. Chemistry

The synthesis of 5-nitro-2-aminothiazole-based compounds (Table 1) is straightforward and based on well-established chemistry, outlined in Scheme 1.

The precursor alkylchloride **1** as well as compound **8** were formed by nucleophilic substitution of 2-chloroacetyl chloride and [1,1′-biphenyl]-4-carbonyl chloride, respectively, by 5-nitro-2-aminothiazole, in the presence of triethylamine. Amides **2**–**7** were obtained by nucleophilic substitution of alkylchloride **1** by an appropriate piperazine at room temperature and in the presence of triethylamine. Finally, amides **9**–**12** were prepared by nucleophilic substitution of alkylchloride **1** by the potassium salt of an appropriate phenol in DMF, by heating for 3–4 h at 60 °C. Efforts were made to improve the yield of amides **9**–**12** by changing the solvent to anhydrous DMSO or CH_3CN without any positive results. All final compounds and intermediates were characterized by ^1H NMR (500 or 400 MHz) and HRMS.

2.2. Biological evaluation

2.2.1. Antiparasitic activity and cytotoxicity

Compounds in Table 1 were screened for antiparasitic activity against three trypanosomatids: *T. cruzi*, *T. b. rhodesiense* and *Leishmania donovani*. 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, used as host cells for *T. cruzi* amastigotes, in order to calculate a selectivity index for each parasite ($SI = IC_{50L6}/IC_{50parasite}$) (Table 1). Antiparasitic activity was evaluated according to the following criteria: an IC_{50} of <4.0 μM, between 4.0 and 60 μM or >60 μM, designates 'active', 'moderately active' or 'inactive' compounds, respectively, against *T. cruzi* amastigotes; for blood stream form (BSF) *T. b. rhodesiense*, IC_{50} values of <0.5 μM, between 0.5 and 6.0 μM or >6.0 μM identify 'active', 'moderately active' or 'inactive' compounds, respectively; finally, for *L. donovani* amastigotes, IC_{50} of <1 μM, between 1.0 and 6.0 μM or >6.0 μM, provides 'active', 'moderately active' or 'inactive' compounds, respectively [35].

According to the criteria set above, all tested compounds in Table 1 were active or moderately active antichagasic agents (green or light green, respectively). Four compounds (**6, 9, 10** and **12**) were moderately active (light green) against *L. donovani* parasites whereas no compound demonstrated antiparasitic activity against *T. b. rhodesiense*. Moreover, all compounds showed PSA values > 100 $Å^2$, which makes them highly unlikely to be capable of penetrating the blood–brain barrier and demonstrate anti-HAT activity *in vivo*.

Several analogs (**3, 5**–**9**) demonstrated IC_{50} values < 50 μM against L6 host cells, presumably due to their high lipophilicity (Table 1), resulting in low selectivity indices. However, even compounds with IC_{50} values > 50 μM against L6 cells demonstrated a less than ideal SI, which is desired to be ≥ 50 for *T. cruzi* and ≥20 for *L. donovani* [35].

2.2.2. SAR analysis of antichagasic activity

The compounds in Table 1 were synthesized having in mind 3-nitro-1,2,4-triazole-based analogs with known substantial trypanocidal properties, described previously by this group [20,23–25]. Taking a closer look at the piperazine derivatives **2**–**7**, we observe that these yielded IC_{50} values against *T. cruzi* parasites ranging from 0.571 to 9.31 μM; thus they are 1.1- to 9-fold less potent than the corresponding 3-nitrotriazole-based analogs (IC_{50} values 0.169–4.64 μM) [24]. Similarly, the aryl/aryloxy-derivatives **8**–**12** were only moderately active antichagasic agents, compared to 3-nitrotriazole-based aryloxyphenylamides which demonstrate *T. cruzi* IC_{50} values at low nM concentrations [23,25]. Therefore, clearly 5-nitro-2-aminothiazole-based amides are less potent antichagasic agents than their 3-nitrotriazole-based analogs.

Another general observation is that the 5-nitro-2-aminothiazole-based amides are significantly more lipophilic (Table 1) than their 3-nitrotriazole-based counterparts with the latter having clogP values between −0.198 and 3.1. In addition, 5-nitro-2-aminothiazole-based amides demonstrate higher PSA values than their 3-nitrotriazole-based analogs (the latter having PSA values less than 116 [24]), which may negatively affect cell permeation [36]. These features may contribute to the higher toxicity of the nitroaminothiazoles in L6 cells and their reduced potency against the parasites (Table 1).

There was an excellent correlation between antichagasic activity and lipophilicity ($R^2 = 0.979$) in the piperazine-amide subgroup of compounds **3**–**7** (which were active against *T. cruzi*) as shown in Fig. 1. Thus, the piperazine amide **6** with the highest clogP value (4.51) was the most active compound against *T. cruzi*, demonstrating an IC_{50} of 571 nM, 3.86-fold more active than Bnz (Table 1). Compound **6**, however, was about 8-fold less active than its 3-nitrotriazole-based analog, in which the piperazinic ring is directly connected with the carbonyl (piperazide) and the nitrotriazole ring is connected with the carbonyl through a methylene group [24]. SAR follows the same rules observed in the 3-nitrotriazole-based piperazines and piperazides [20,24]. Therefore, dichlorophenylpiperazine **5** was a slightly better antichagasic agent than trifluoromethylphenylpiperazine **3**, the latter