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Research paper

Phthalimido-thiazoles as building blocks and their effects on the growth and morphology of *Trypanosoma cruzi*

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ABSTRACT

Chagas disease is a parasitic infection caused by protozoan *Trypanosoma cruzi* that affects approximately 6–7 million people worldwide. Benznidazole is the only drug approved for treatment during the acute and asymptomatic chronic phases; however, its efficacy during the symptomatic chronic phase is controversial. The present work reports the synthesis and anti-*T. cruzi* activities of a novel series of phthalimido-thiazoles. Some of these compounds showed potent inhibition of the trypomastigote form of the parasite at low cytotoxicity concentrations in spleen cells, and the resulting structure-activity relationships are discussed. We also showed that phthalimido-thiazoles induced ultrastructural alterations on morphology, flagellum shortening, chromatin condensation, mitochondria swelling, reservosomes alterations and endoplasmic reticulum dilation. Together, these data revealed, for the first time, a novel series of phthalimido-thiazoles-structure-based compounds with potential effects against *T. cruzi* and lead-like characteristics against Chagas disease.

1. Introduction

Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). Approximately 6–7 million people are estimated to be infected worldwide, mostly in Latin America, where Chagas disease is endemic [1].

Despite the efforts of many investigators to research new anti-Chagas drugs, only one drug is currently used in therapy, benznidazole (Bdz) [2,3]. Current chemotherapy for Chagas disease is unsatisfactory due to the limited efficacy of Bdz, particularly during the chronic phase, with frequent side effects that can lead to discontinuation of treatment [4].

One pragmatic way to improve the quality of both the candidate drugs and screening collections is by improving the quality of the building blocks (reagents) that are used to synthesize them. Our strategic program focused on substructures and properties that are known to have imparted biological activity and good 'drug-like' properties previously. Among the chemical groups explored for anti-Chagas activity, thiazolyl hydrazones are noteworthy because of their wide biological, especially anti-parasitic, activities [5–8]. Caputo et al. have demonstrated trypanocidal activity for a series of 4- arylthiazolylhydrazones [9], which have broad and potent activities for all forms of the parasite.

Our efforts toward new antichagasic drugs since 2006 have led

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us to develop a variety of thiosemicarbazones and 1,3-thiazolyl hydrazones as trypanocidal agents [4,7,10–14]. In continuation of our search for bioactive molecules, we envisaged that the derivatization of the thiosemicarbazone group into a thiazole moiety would generate novel templates that are likely to exhibit anti-*T. cruzi* activity [4].

However, much effort has been invested to identify the key differences between drugs and other organic compounds. High-quality libraries are expected to exhibit drug-likeness to produce compounds with desirable pharmacokinetic and safety profiles. The phthalimide functional group has been used as an important tool in organic synthesis because it protects against unwanted reactions. Many research teams have used this nucleus as a building block to improve compound quality. In fact, phthalimide derivatives have shown a broad spectrum of pharmacological properties, such as analgesic [15], anticonvulsant [16], antitubercular [17,18], hypolipidaemic [18], anxiolytic [15], anti-inflammatory [15], antimicrobial [17,19,20] and antipsychotic [21].

For this reason, our research group has explored the pharmacological properties of phthalimide derivatives. As a result, bioactive prototypes were identified with potent anti-inflammatory [22], anti-proliferative [23], immunomodulatory [22,24,25], antitumor [23], antiangiogenic [26] and schistosomicidal properties [27], Indeed, Santiago et al. identified phthalimido-thiazole derivatives with potent schistosomicidal activities. The phthalimide **LpQM-45** caused significant ultrastructural changes, including destruction of the integument in both male and female worms [27], however, their antichagasic properties have not been explored.

Considering the promising results achieved by compounds bearing a thiazole ring and phthalimides nuclei, they were chosen as common pharmacophores that exist in diverse drug classes. In this way, we synthesized a set of molecules with phthalimide and thiazole nucleus. In this synthetic design of a substructure-based compound library, substituents around the phenyl ring attached at C4 in the thiazole ring (compounds **2b-n**) were explored. In addition, a spacer group between phthalimide and the thiazole ring was inserted and a phenyl group at N3 of the thiazole ring was also introduced (**6b-l**). To investigate the influence of the phthalimido moiety at the anti-*T. cruzi* activity, 26 new compounds were tested *in vitro* against the *T. cruzi* parasite epimastigote and trypomastigote forms. Ultrastructural studies and flow cytometry analysis were also investigated (Fig. 1).

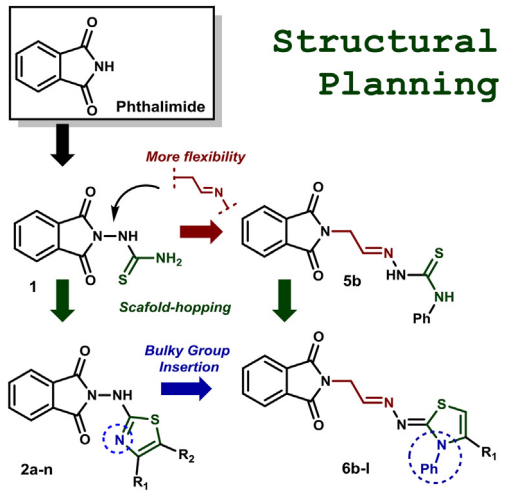

Fig. 1. Structural planning of the proposed compounds.

2. Results and discussion

2.1. Chemistry

Initially, 14 phthalimido-thiazoles (**2a-n**) were synthesized in a two-step reaction, following the procedures reported by Pessoa et al. [25]. Firstly, a reaction of phthalic anhydride with thiosemicarbazide, in DMF under reflux for 4 h, with a catalytic amount of DMAP, led us to compound **1**. The synthesis of the series **2a-n** was performed via Hantsch cyclization between compound **1** and the appropriate α-halogenated ketone (1,3-dichloroacetone for compound **2a**), under ultrasound irradiation, at room temperature (rt) for 1 h. This reaction condition led to average yields from 36 to 65%. To synthesize the series **6a-l**, was followed the reaction protocol reported by Cardoso et al. [23]. The desired compounds **6a-l** were obtained by the reaction of Intermediate **5b** (or **5a**, for compound **6a**) with the appropriate α-halogenated ketone (1,3-dichloroacetone for compound **6a**), via Hantsch cyclization, leading good yields (46–82%) (Scheme 1). All the synthesized compounds were well characterized by infrared (IR), nuclear magnetic resonance (^{1}H, ^{13}C NMR), mass spectroscopy (ESI-TOF) and, in case of compounds **2e**, **2f** and **2g**, by single crystal X-ray diffraction analysis (Fig. 2).

The ^{1}H NMR spectra of some compounds showed that phthalimido-thiazoles **6a-l** are composed by diastereomers. Next, we aimed to define the configuration of the major isomer by crystallographic analysis. However, we did not succeed in crystallizing phthalimido-thiazoles **6a-l** suitable for X-ray analysis. Based on previous crystallized compounds by our group, we suggest that the major isomer formed present the *E-Z* configuration (Fig. 3). Indeed, hydrazine double-bond C2=N2 is commonly assigned as *E* configuration [4,23,28]. Concerning the exocyclic double-bond N3=C3, we suggest that the predominant configuration is in *Z*-configuration [7,29]. Besides, a representative ^{1}H-NMR spectrum of compound **6i** is presented in Supplementary Material.

2.2. Anti-T. cruzi evaluation

Initially, compounds **2a-n** were planned to improve the trypanocidal activity and cytotoxic tolerance with the cyclization of phthalimido-thiosemicarbazone to the phthalimido-thiazole ring. From the results, it was observed in most of the cases that new phthalimido-thiazoles showed high cytotoxic activity in spleen cells. In opposition, only compounds **2i** and **2j** showed low cytotoxicity.

Concerning trypanocidal activity for epimastigotes, it is observed that 16 compounds (of 28) present better potency than Benznidazole (Table 1). Among series **2a-n**, compound **2i** was the most active, among the series and the entire work. The most active compound in series **6** is **6k**, a 3-NO_2 derivative, presenting an IC_{50} of 6.0 μM. Observing compounds with withdrawer substituents (**2e-h**, **2k-n**), compound 2,4-dichloro substituted (**2m**) was the most active. It analogue disubstituted 3,4-dichloro (**2l**) present lower trypanocidal activity and high toxicity for BALB/c mice spleen cells, denoting that the orientation of the substituents is important for the activity. Observing bulk substituted compounds (phenyl, 2-naphthyl and 4-biphenylyl), in series **2a-n**, a relationship of LogP and trypanocidal activity (Fig. 4) is observed, being compound **2i** the most active of this sub-series.

The trend of bulky substituents (LogP) observed for series **2a-n** is not observed for series **6a-l**, being compound **6k** (3-NO_2) the most active of the series **6a-l**.

When comparing the trypanocidal activity against the trypomastigote form of the series **2a-n** of phthalimido-thiazole derivatives, compound **2j** was found to be the most potent of this sub-