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Research paper

Design, synthesis and *in vitro* trypanocidal and leishmanicidal activities of novel semicarbazone derivatives

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ABSTRACT

Trypanosomatids are protozoan parasites that cause various diseases in human, such as leishmaniasis, Chagas disease and sleeping sickness. The highly syntenic genomes of the trypanosomatid species lead the assumption that they can encode similar proteins, indicating the possibility to design new anti-trypanosomatid drugs with dual trypanosomicidal and leishmanicidal activities. In this work a series of compounds (**6a**–**h** and **7a**–**h**), containing a semicarbazone scaffold as a peptide mimetic framework, was designed and synthesized. From this series compound **7g** (LASSBio-1483) highlighted, showing dual *in vitro* trypanosomicidal and leishmanicidal activities, with potency similar to the standard drugs nifurtimox and pentamidine. This data, taken together with its good *in silico* druglikeness profile and its great chemical and plasma stability, make LASSBio-1483 (**7g**) a new antitrypanosomatid lead-candidate.

1. Introduction

Neglected diseases (DN) represent a set of parasitic illnesses that primarily affect poor people in developing countries. Those caused by Trypanosomatidae protozoans include Chagas disease and sleeping sickness, produced by *Trypanosoma* species, and leishmaniasis, caused by different species belonging to the genus *Leishmania* [1]. Affording to World Health Organization (WHO), trypanosomiasis and leishmaniasis are the most challenging among the neglected tropical diseases [2]. A comparative genomics of trypanosomatid parasitic protozoa revealed a conserved core proteome of about 6200 genes among *Leishmania major*, *Trypanosoma cruzi*, and *Trypanosoma brucei* [3]. The highly syntenic genomes of the trypanosomatid species lead the assumption that they can encode similar proteins and drugs designed against conserved core processes should have the advantage of being potentially useful against all three protozoa [4–7]. Among the possible drug targets in trypanosomatids, the peptidases or proteases have concerned attention due their many roles in highly specific functions to the parasites' life cycles [8–10]. Considering the ability of these enzymes to catalyze the hydrolysis of peptide bonds [11–13] compounds containing amide or amide-mimetic frameworks can be designed as proteolytic inhibitors with antitrypanosomatid activity, as exemplified by compounds **1**–**4** (Chart 1) [13–16]. In similar manner, the *ortho*-hydroxyphenyl group linked to the imine subunit of a hydrazone functional group is believed to be an interesting scaffold for cysteine proteases inhibition. This statement is based on a theoretical proposed mechanism involving the nucleophilic attack of sulfhydryl group of a cysteine-protease on a reactive *ortho*-quinonemethyde intermediate, generated from the

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tautomeric equilibrium of *ortho*-hydroxyarylaldehydehydrazone moiety (*e.g.* compound **5,** Chart 2) [17].

In order to design new peptide mimetic derivatives enclosing frameworks able to be recognized by trypanosomatids proteases, a series of semicarbazone derivatives (**6a**–**h** and **7a**–**h**) were planned by molecular modification on prototype **5** (LASSBio-1022) [18]. These modification were based on ring replacement between quinoxaline nucleus and 1,3-benzodioxole system (**a**, Chart 2); molecular simplification represented by elimination of methyl group (**b**, Chart 2); followed by aza-homologation strategy (**c**, Chart 2), converting the *N*-acylhydrazone subunit in a semicarbazone framework. The congeners series was further designed by classical isosterism replacement on 2-hydroxyphenyl subunit, varying the electronic nature of the monovalent group (**a-f**) and by isosteric ring replacement of phenyl group by a substituted furan system (**g**) and its phenylogous analogue (**h**) (Chart 2) [19]. In this paper we described the synthesis of the designed compounds **6a**–**h** and **7a**–**h** and their trypanosomicidal and leishmanicidal activities.

2. Results and discussion

2.1. Chemistry

Compounds **6a**–**h** and **7a**–**h** were synthesized in three linear steps from the amines **8** and **9**, obtained commercially (Scheme 1). In the first step the amines were condensed with phenyl chloroformate in chloroform at room temperature in order to furnish the carbamates **10** and **11** [20,21]. These compounds were treated with hydrazine monohydrate in ethanol to provide the semicarbazide derivatives **12** and **13** [22]. These key-intermediates were finally condensed with appropriated aldehydes [23], selected based on the design concept depicted in Chart 2, to obtain the semicarbazones **6a**–**h** and **7a**–**h** in good overall yields (Scheme 1). The chemical structure of the compounds **6a**–**h** and **7a**–**h** was elucidated by ^{1}H and ^{13}C NMR, IR and mass spectrometry. The analysis of the ^{1}H and ^{13}C NMR spectra of these compounds revealed the presence of only one signal relative to the hydrogen and carbon of imine double bond (N=CH), suggesting that all compounds were synthesized as a single diastereoisomer. The unequivocal characterization of the relative configuration of imine double bond (*E* or *Z*) was performed using X-ray diffraction study. However, considering the difficulty of getting compounds **6a**–**h** and **7a**–**h** in crystalline form, only derivative **7g** (LASSBio-1483), obtained as crystal solid, was used in X-ray experiment. As shown in Fig. 1, this experiment revealed that compound **7g** was obtained as diastereoisomer *E*. Based on these data and considering the similarity in chemical shifts of imine hydrogen in ^{1}H NMR spectra of compounds **6a**–**h** and **7a**–**h**, is reasonable to propose that all semicarbazone derivatives (**6a**–**h** and **7a**–**h**) were obtained with the same stereochemistry (N=CH; configuration *E*).

2.2. X-ray diffraction analyses

Fig. 1 is a structure representation of **7g** crystallized in the $P2_1/c$ space group**.** Table 1 present its main crystallographic data. The geometric features were studied with the software MOGUL [24] and this analysis showed that all bond lengths and angles were in agreement with the expected statistical values when compared with similar fragments of structures deposited in Cambridge Structural Database (CSD) [25]. The least–square plane through the non-hydrogen atoms of the 5-nitro-2-furaldehyde semicarbazone moiety shows a high planarity (r.m.s = 0.0358). This molecular moiety forms an angle of 10.78(6)^{o} with that one through the benzoic ring (r.m.s = 0.0281).

Information about intermolecular geometry of **7g** and the details of all hydrogen bond contacts involved in its networks can be found in the Supplementary Material (Fig. 29 and Table 1S)

2.3. Cytotoxic studies

Before starting the evaluation of the trypanosomicidal and leishmanicidal activities of semicarbazones **6a**–**h** and **7a**–**h**, the eventual cytotoxic profile of these compounds against mammalian cells was investigated by MTT assay [26]. In this study murine macrophages cell line J774.A1 was treated with compounds **6a**–**h** and **7a**–**h** at serial concentrations (0.1–100 μM) and the half maximal inhibitory concentration (IC_{50}) was determined as illustrated in Table 2. Only compounds **7b** and **7e** showed cytotoxic activity to mammalian cell with IC_{50} = 71.2 and 35.7 μM, respectively (Table 2).

2.4. Trypanosomicidal activity

Semicarbazone derivatives **6a**–**h** and **7a**–**h** were evaluated *in vitro* against epimastigote forms of *Trypanosoma cruzi*, Tulahuen 2 strain, discrete typing unit, DTU, Tc VI [27] in a screening concentration of 100 μM. Compounds which presented an inhibition superior than 50% at 100 μM were selected to determine their IC_{50} values, and their ability to inhibit the parasite growth was tested in comparison to the standard drug nifurtimox [28]. As shown in Table 2, compounds **7a** (IC_{50} = 21 μM), **7g** (IC_{50} = 11.9 μM), **6d** (IC_{50} = 8.5 μM) and **6g** (IC_{50} = 11.5 μM) presented the better trypanosomicidal profile being equipotent to the standard drug nifurtimox (IC_{50} = 7.7 μM).

2.5. Leishmanicidal activity

The ability of compounds **6a**–**h** and **7a**–**h** to inhibit the growth of promastigotes forms of *L. major* were investigated, using pentamidine as standard [29]. Compounds with IC_{50} values <100 μM were selected to study their cytotoxic activity against amastigostes forms of *L. major*. As exemplified in Table 2, all compounds containing the 1,3-benzodioxole system (**6a**–**h**) were inactive as leishmanicide. In contrast, compounds **7c**, **7d**, **7f**, **7g** and **7h** showed cytotoxic activity against promastigotes of *L. major*, although with potency inferior than pentamidine. Among these compounds only the semicarbazones **7d** (IC_{50} = 74.0 μM), **7g** (IC_{50} = 1.5 μM) and **7h** (IC_{50} = 0.6 μM) were active against amastigote forms of *L. major*; being compounds **7g** and **7h** more potent than pentamidine (IC_{50} = 17.1 μM).

Considering the aim of identify a new antitrypanosomatid, the analysis of the results depicted in Table 2, allowed the selection of compound **7g** (LASSBio-1483) as a dual trypanosomicidal and leishmanicidal agent. Therefore, the *in silico* prediction of physicochemical, ADME and toxicity properties of LASSBio-1483 (**7g**) were calculated using the ACD/Labs Percepta Platform (License# 56950) and the results were compared to those obtained for nifurtimox and pentamidine.

As demonstrated in Table 3, the druglikeness of compound **7g** was very similar to nifurtimox and different from pentamidine, with no violations of Lipinsky's rule of 5 [30]. Regardless of the poor solubility (predicted in buffer at pH of 6.5), compound **7g** (LASSBio-1483) was showed to be highly permeable based on predicted permeability across Caco-2 monolayers (P_e) and human intestinal absorption (HIA) test. These results were similar to nifurtimox and opposite to pentamidine, that was demonstrated to be a poorly permeable drug ($P_e \leq 1$ * 10-6 cm/s and HIA < 30%) with zero oral bioavailability (F = 0%, Table 3). LASSBio-1483 (**7g**) was expected to have an oral bioavailability (F) of 39% (Table 3). The drug safety profile of these compounds was also projected using Program ACD/