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Short communication

In vitro screening of 2-(1*H*-imidazol-1-yl)-1-phenylethanol derivatives as antiprotozoal agents and docking studies on *Trypanosoma cruzi* CYP51

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ABSTRACT

Sterol 14α-demethylase (CYP51) is a key enzyme involved in the survival and virulence of many parasite protozoa, such as *Trypanosoma* and *Leishmania* species, thus representing a valuable drug target for the treatment of Kinetoplastid diseases. A set of azole-based compounds selected from an in-house compound library was *in vitro* screened against different human protozoan parasites. Several compounds showed selective activity against *Trypanosoma cruzi*, with compound **7** being the most active ($IC_{50} = 40$ nM). Given the structural similarity between the compounds here reported and known CYP51 inhibitors, a molecular docking study was performed to assess their binding with protozoal target and to rationalize the biological activity data.

1. Introduction

Trypanosoma cruzi (*T. cruzi*) is an intracellular protozoan parasite responsible for Chagas disease (CD), considered one of the neglected diseases [1]. It is transmitted to the mammalian host primarily by infected faeces of blood-sucking triatomine bugs, also known as "kissing bugs". Other modes of transmission include infected blood transfusion, vertical transmission or infected organ transplantation [2]. CD occurs mainly in Latin America, however, the number of infections in the United States of America, Canada, many European and some Western Pacific countries continues to increase because of population mobility [3]. To date, CD treatment includes the nitro-derivatives benznidazole and nifurtimox [4]. Despite their significant activity in congenital and adult acute *T. cruzi* infections, their use presents major drawbacks, including long duration of therapy, poor efficacy in the chronic phase, different efficacy related to geographical area and several adverse reactions [5]. These facts motivate to continue the research and development of new more safe and effective drugs [6,7].

Sterol 14α-demethylase (CYP51) represents a main target to develop new drugs for the treatment of CD. This enzyme catalyzes the removal of 14α-methyl group of sterols, an essential step in sterol biosynthesis leading to the formation of cholesterol in vertebrates, ergosterol in fungi, and a variety of 24-alkylated ergosterol derivatives in plants and protozoa [8]. Unlike mammals that can accumulate cholesterol from diet, blocking of ergosterol production in fungi and protozoa is lethal: it affects cytokinesis, stops cell growth, and eventually leads to collapse of the cellular membrane [9]. Due to the similarity in sterol composition, several antifungal agents have been tested against *T. cruzi*. Among these, posaconazole and the ravuconazole prodrug E1224 have recently terminated Phase-2 clinical trials for treatment of chronic Chagas disease [10]. These studies have led to disappointing conclusions about their effectiveness in chronic CD, mainly due to poor pharmacokinetic properties. Moreover, the chemical synthesis of posaconazole and ravuconazole is coumbersome and very expensive. Nevertheless, research for inhibitors specifically designed for the CYP51 of *T. cruzi* ($CYP51_{Tc}$), less expensive and endowed with optimal

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pharmacokinetic properties, is still promising [11].

In previous studies [12,13], we identified promising imidazole derivatives with a high and selective *in vitro* activity against intracellular amastigotes of *T. cruzi* (Tulahuen C2C4). The most potent among them (**I**–**III**) showed IC_{50} values in the low nM range and are reported in Chart 1. These compounds were found to inhibit the *T. cruzi* sterol 14α-demethylase and, as demonstrated by the co-crystals, they fit into the deepest segment of the CYP51 cavity and disrupt the heme support from the protein moiety (compound **II**) or block the entrance into the CYP51 substrate access channel (compound **III**).

Here, we report the *in vitro* antiprotozoal activity evaluation of eight compounds selected from our in-house imidazole library; they are structurally correlated with already described **I**–**III** and some of them possess antifungal activity [14,15].

All selected molecules possess the three principal pharmacophoric features of CYP51 inhibitors: i) an iron chelating nitrogen containing heterocycle; ii) an hydrophobic group quite close to it; iii) a second hydrophobic group (Chart 1). Furthermore, we tried to rationalize the biological data by means of a molecular docking study based on known crystal structures of *T. cruzi* CYP51 ($CYP51_{Tc}$).

Compounds **1** and **2** (logP 5.963 and 5.662 respectively) were chosen to verify how the presence of a halogen atom (chlorine or fluorine) on the first hydrophobic group influences the antiparasitic activity in comparison to compound **I** (logP 5.566). Compound **3** (logP 7.236) was chosen in order to verify the effect of more bulky and hydrophobic group in the same position. In addition, we have chosen compounds **4**–**8** to evaluate how side chains with different size and polarity in the second hydrophobic group affect the activity. In particular, compounds **5** (logP 4.405) and **6** (logP 4.138) possess a more polar piperazine moiety instead of the aromatic ring closer to carbonyl linker (**5** and **6** *vs* **I**); compound **4** (logP 3.775) with respect to **5** and **6** contains a more polar furoylpiperazine moiety, present in some compounds with antitrypanosomal activity [16,17]. Finally, compounds **7** (logP 4.096) and **8** (logP 5.011) have been selected for their similitude with compound **III** (logP 8.155) but with more polar groups in the terminal portion of the side chain.

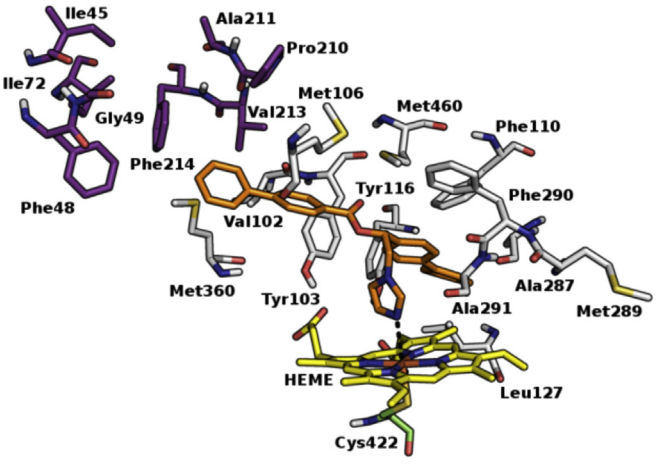

Fig. 1. Compound (***R***)-**3** docking binding pose (orange stick). The hydrophobic cavity surrounding the heme prosthetic group is shown in gray stick and the HC2 in purple stick. Cys422 coordinating the heme iron is represented in light green stick and the heme prosthetic group in yellow stick. Coordination at the heme iron is represented as black dotted lines. Atoms are colored according to their atom types and non-polar hydrogen atoms are omitted. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Results and discussion

2.1. Chemistry

Compounds **1**, **2**, **4**–**8** were previously synthesized and evaluated for their antifungal activities [14,15]. The new compound **3** was prepared as previously reported for compounds **1** and **2** [15]. In brief, 1-(biphenyl-4-yl)-2-(1*H*-imidazol-1-yl)ethanol was activated as alcoholate using NaH in dry CH_3CN and then the biphenyl-4-carbonyl chloride was added (Scheme 1).

The synthesis of **4**–**8** was accomplished by the activation of the −OH group of 2-(1*H*-imidazol-1-yl)-1-phenylethanol with triphosgene in dry CH_3CN to obtain the desired chloroformate; then TEA and the selected amines were added to the chloroformate stirring overnight (Scheme 1) [14].

Commercially available amines were used to synthesize compounds **4**–**6**; otherwise the side chains of **7** and **8** were prepared as described in Scheme 2. The synthesis of the amines **11 and 12** started by condensation of 1-fluoro-4-nitrobenzene with the appropriate commercial piperazine refluxed for 2 h in CH_3CN. The obtained nitro compounds (**9** and **10**) were reduced to the corresponding amines (**11** and **12**) with 50 psi of H_2 for 4 h in a Parr apparatus, using Pd/C (5%) as catalyst. All the selected compounds contain a chiral carbon atom and were obtained as racemic mixtures.

In order to evaluate if the pure enantiomers possess different biological activity and if this can be related to the absolute configuration, we synthesized some of the selected compounds in the enantiomerically pure form. The pure enantiomers of esters **1** and **2** and carbamates **5**, **6** and **8** were obtained as described for racemates from enantiomerically pure *R* or *S* 1-(4-fluorophenyl)-2-(1*H*-imidazol-1-yl)ethanol and *R* or *S* 1-(4-chlorophenyl)-2-(1*H*-imidazol-1-yl)ethanol, as previously reported [12].

2.2. Antiparasitic activity

All compounds were *in vitro* tested *vs* amastigote stage of *T. cruzi*, Tulahuen CL2, β-galactosidase strain (nifurtimox-sensitive). In addition the screening was extended to include the activity of compounds **1**–**8** towards the other human protozoan parasites *Leishmania infantum* (MHOM/MA-BE/67 amastigotes), *T. brucei* (Squib-427 strain, suramin-sensitive) and *Plasmodium falciparum* (Chloroquine-resistant K1-strain). The *in vitro* screening was carried out according to the previously described procedures [18]. The results are reported in Table 1. All the racemic azole derivatives show an anti-*T. cruzi* activity with IC_{50} values ranging from 0.04 to 5.84 μM. In particular, **1**, **2**, **5**–**8** were more active than benznidazole used as reference drug. The most active compound **7** showed IC_{50} value of 40 nM and it proved to be approximately fifty times more active than benznidazole and eight times less active than azole reference compound, posaconazole (Table 1).

The antiparasitic activity and cytotoxicity data show that the imidazole derivatives **1**–**8** are highly selective towards *T. cruzi*. In particular, compound **7** possess the highest activity (*T. cruzi* IC_{50} 0.04 μM) and the lowest cytotoxicity (MRC-5 IC_{50} > 64 μM and a SI > 1600), making it an excellent candidate for a new series of anti-Chagas imidazole derivatives.

Compounds **1**, **3** and **5** presented an antileishamial activity similar to miltefosine but they showed a cytotoxicity on MRC-5 cell line of the same magnitude, thus indicating the lack of selectivity towards *L. infantum*.

Furthermore, compound **2** showed antiplasmodial activity (IC_{50} 0.6 μM), approximately twice than the reference drug chloroquine, and good selectivity against *P. falciparum*, with low cytotoxicity vs MRC-5 cell (MRC-5 IC_{50} 7.8 μM).