PLANNING OF NEW INHIBITORS OF *Tc*CYP51 USING *DE NOVO* APPROACH

Flores Junior, L.A.P. [1]*; Lima, C.H.S.[2] ; Dias, L.R.S.[1]**
[1]*Universidade Federal Fluminense/Faculdade de Farmácia, R. Mario Viana 523, Niterói, RJ, Brazil*
[2]*Universidade Federal do Rio de Janeiro/Instituto de Química, Av. Athos da Silveira Ramos 149, Rio de Janeiro, RJ, Brazil*
**lapfjunior@id.uff.br* ***lrsdias@id.uff.br*

Introduction

Chagas disease is a severe public health problem since millions of people are being infected worldwide, mainly in Latin America, where this disease is endemic [1]. The etiological agent of the disease is the protozoan *Trypanosoma cruzi* (*T. cruzi*), and the pharmacological treatment is based on the drugs nifurtimox and benznidazole. These drugs are most effective in the early stages of infection and have several side effects [2,3]. In the search for new drug candidates, the sterol 14α-demethylase cytochrome P450 (CYP51) of *T. cruzi* (*Tc*CYP51) is a potential therapeutic target due to its role in the ergosterol biosynthesis, which is essential for the parasite's survival [4]. Among the drug search strategies for new treatments, those focused on biological targets of the etiologic agent seem promising, particularly the CYP51 enzyme [5]. This work focused on the design of new *Tc*CYP51 inhibitors using the *de novo* method.

Material and Methods

We constructed a library of chemical structure fragments using *Tc*CYP51 inhibitors selected from the literature [6]. Open Babel 2.2.2 program was used to construct the inhibitor's 3D structures and their optimization [7]. We used the BRICS fragmentation method to construct a library of fragments from the selected inhibitors. We reduced the number of fragments using the rule of three as follows: molecular mass (MM< 300 Da), clogP (< 3), and the number of hydrogen bond acceptors and donors (HBA and HBD < 3). With the aim of structural optimization of compounds with anti-*T. cruzi* activity, we inserted compounds containing pyrazole-pyridine nuclei into the fragment library. The AutoGrow 4.0.2 program was used to design new *Tc*CYP51 inhibitors, and the fragment library was generated based on the structure of *Tc*CYP51 [8]. The protocol used in the AutoGrow 4.0.2 program consisted of the follow range of parameters: number of generations (1-10), number of mutations and crossovers (0-1000), and population (100-10000). Molecular docking studies were performed in GOLD 2020.1 using the ChemScore scoring function and molecular interactions analysis in the Discovery Studio program [9].

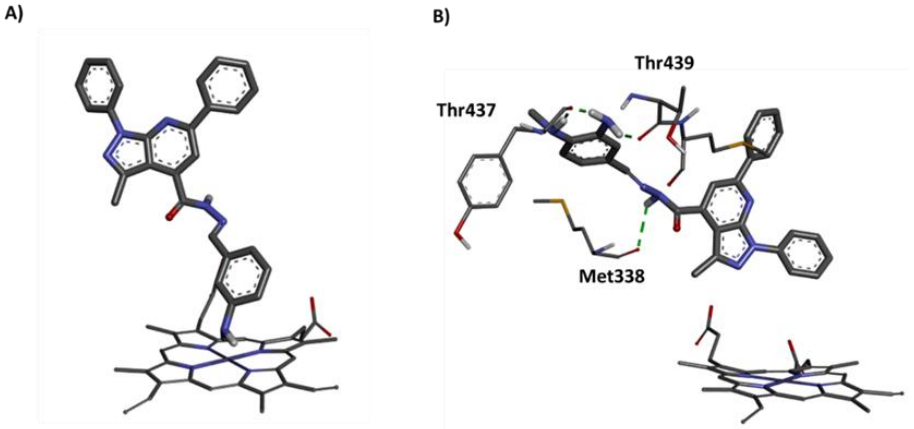
Results and Discussion

We selected 583 *Tc*CYP51 inhibitor compounds from the literature containing several nitrogenous heterocyclics. They were subsequently subjected to the BRICS fragmentation method, generating 8196 fragments. This fragmentation mode allows the breaking of carbon and heteroatom bonds (C-X), leading to the generation of reactive fragments, which is essential for the Autogrow program. Considering the physicochemical parameters and applying the rule of three, we reduced the fragment set to 3061. The developed fragment library presents a wide structural diversity for good planning of new compounds [10]. Ninety-six molecular structures were generated in the Autogrow software. However, we kept in the structure set only 39, which were structurally different. Among these proposal structures, 12 contain the pyrazole-pyridine core present in the fragment library to optimize the compounds inserted in the library. To improve the interaction with the *Tc*CYP51 enzyme, the designed compounds presented either the modification of functional groups or the insertion of fragments.

The molecular docking score analysis shows that 2 of the 39 proposal structures had relevant scores (> 52.3), indicating they are potential *Tc*CYP51 inhibitors [9]. These two proposal structures presented different poses (**Fig. 1**). The docking pose of compound 1 (**Fig.1A**) shows it is close to the Fe atom from

the cofactor but did not present hydrogen bond interactions. On the other side, the docking pose of compound 2 (**Fig.1B**) shows it distant from the cofactor but interacting with residues Met338, Thr439, and Thr437 by hydrogen bonding.

Fig. 1. Intermolecular interactions of the proposed inhibitor structures that show the best docking scores.



Conclusion

We constructed a library of structurally diverse fragments using the inhibitors selected from the literature to design new inhibitors. From this library, we generated in the Autogrow software 39 chemical structures with potential activity against *Tc*CYP51. Our results showed that two proposed inhibitors (1 and 2) performed relevant interactions with *Tc*CYP51 in molecular docking simulation. These results reinforce the *de novo* method as a relevant tool for planning new bioactive compounds and optimizing compounds for a specific target.

Acknowledgments

The authors thank for the support of the sponsors FAPERJ (E-26/210.068/2021 E-26/210.915/2021) and CAPES (Finance Code 001).

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