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Molecular Docking and Evaluation of Antileishmania Activity of a Ruthenium Complex with Epiisopiloturine and Nitric Oxide

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Abstract

Leishmaniasis is an infectious disease that affects both animals and humans, caused by flagellated parasites belonging to the genus *Leishmania*. The disease is estimated to reach about 700,000 to 1 million people, causing the deaths of 20 to 30,000 individuals annually. Thus, the present study aims to perform molecular docking tests and evaluation of antileishmania activity *in vitro* of a ruthenium complex with epiisopiloturine and nitric oxide. *AutoDockTools*-1.5.6 *software* was used to perform molecular docking tests. Molecular targets were considered rigid, and Epiruno$_2$ considered flexible. The genetic algorithm Lamarckian (AGL) with global search and pseudo-Solis and Wets with local search were the methods adopted in the docking. The most promising results of molecular interaction were achieved in the targets Pteridine reductase and UDP-glucose Pyrophosphorylase with rates of −10.68 Kcal·mol^{-1} and −10.51 Kcal·mol^{-1}, respectively. This demonstrates that Epiruno$_2$ has molecular affinity with the targets of *L. major*. *In vitro* assays prove the antileishmania activity of

the complex in the face of promastigote forms with inhibition of growth, concluding through this study that the Epiruno$_2$ complex has antileishmania activity.

Keywords

Molecular Docking Simulation, Neglected Diseases, *Leishmania major*

1. Introduction

Leishmaniasis is a disease that affects more than 98 countries worldwide, with about 700,000 to 1 million new cases reported annually, and an annual rate of 20 to 30,000 deaths [1]. There are several ways for the disease to manifest clinically, and may present as cutaneous, mucocutaneous and visceral. Infection with *Leishmania major* (*L. major*) species has a chronic evolution that affects the structures of the nasopharyngeal epidermis and cartilage, either localized or diffuse [2]. The parasitic cycle results from the abundance of carbohydrates on the surface of *Leishmania*, which includes lipophosphoglycans, glycosylphosphatidylinositol lipid-anchored proteins and proteophosphoglycans [3]. These glycoproteins are part of the promastigote infectious glycocalyx, which is the most important process in host infectivity [4] and phlebotomine interaction [5].

After diagnosis, the patient undergoes treatment that depending on the infecting strain will be treated with some of the drugs available on the pharmaceutical market, they are: pentavalent antimonial; AmBisomew; liposomal; amphotericin B; miltefosine and diamidines, among others. All of these drugs are potentially toxic and have reduced efficacy in addition to adverse side effects. That in many cases, the patient chooses not to undergo treatment so that he does not suffer from side effects caused by drugs [6] [7].

Besides this problem, the pharmaceutical industries neglect investments in the search for new pharmacological agents that present high inhibition rates with new mechanisms of action and low toxicity. This lack of interest is related to market demand, as it is a neglected disease, that is, it affects only underdeveloped and developing countries, the sector has high risks of not making profits on their investments, because the population would not be able to afford it. With the costs of treatment, even the state would not be able to finance the services offered by the industries [8].

Thus, there is a clear need to search for new compounds with pharmacological potential and low toxicity by alternative methods that bring reliability in their results, speed and cost benefit. Thus, computational quantum chemistry presents itself as a promising alternative, using several computational tools that predict molecular properties related to a pharmacological potential. Using the laws of quantum chemistry and various programming techniques that are capable of predicting energy state, molecular structures, vibrational frequencies of atomic and molecular systems and molecular interaction between two molecules de-vel-oping virtual models saving time and materials that would be wasted on experiments in the field laboratory [9] [10].

In this sense, ruthenium (Ru) complexes have become attractive in pharmacological studies because they have low toxicity and are an excellent conductor of energy when dealing with a transition metal, where it plays an important role in the bioactive process of a compound in reaction with a target disease, having little energy loss in its path [11] [12]. The Ru complex with epiisopiloturin and nitric oxide ($Epiruno_2$) was synthesized by Rocha (2018) in anti-schistosoma mansoni studies, where the $Epiruno_2$ complex showed schistosomicidal activity *in sílico* and *ex vivo* studies. There was a 10-fold increase in the biological activity of Epiisopiloturin (EPI) when coupled with the Ru complex against Schistosoma parasites, eliminating 60% of male worms at a concentration of 50 μM within 72 hours, showing antiparasitic activity [13].

Associated with the antiparasitic schistosomicidal effect presented by the $Epiruno_2$ complex in studies by Rocha (2018), we assume that the complex has antileishmania activity. Thus, the present study aims to perform molecular docking tests and evaluation of antileishmania activity *in vitro* of a ruthenium complex with epiisopiloturine and nitric oxide.

2. Materials and Methods

2.1. Molecular Docking

The 3D molecules of *L. major* targets were extracted from the PDB (*Protein data Bank*) database with codes 5g20 (Glycyl Peptide N-tetradecanoyltransferase); 5nzg (UDP-glucose Pyrophosphorylase); 5c7p (Nucleoside diphosphate kinase); 1e7w (Pteridine reductase); and 1ezr (*Nucleoside hydrolase*) [14] prepared for docking by removing mutant chains and all water molecules, ions and other groups using *Chimera v.*13.1 *software* [15] [16].

The three-dimensional molecular structure of the $Epiruno_2$ complex was designed using *Gauss View* 5.0 *software* [17] and optimized by DFT (Density Functional Theory) calculation using the B3lyp functional and the 6-311^{++}G (d, p) available in *Gaussian* 09 *W software* [18] [19].

The molecular docking process followed the protocol developed by Rocha and collaborators, with some modifications [20]. All molecular docking procedures were performed by *AutoDockTools*-1.5.6 *software* [21]. *L. major* targets and the $Epiruno_2$ complex were prepared for docking simulations, where targets were considered rigid and $Epiruno_2$ was considered flexible. Partial charges were calculated after the addition of all hydrogens. The nonpolar hydrogen atoms of the protein and binder were subsequently fused. A 60 × 60 × 60 point cubic box with a spacing of 0.375 Å between grid points was generated for the simulations. The molecular affinity grid centers were defined from the coordi-nates of the atoms of their respective active sites Asn376, Lys380, Gly91, Asn109 and Asp15, respectively.

The Lamarckian global search (LGA) genetic algorithm [22] and the pseudo-Solis and Wets [23] local search (LS) methods were applied in the search for molecular docking. The $Epiruno_2$ complex was subjected to 100 independent

runs of molecular coupling simulations [24]. The remaining parameters were set to default values.

Molecular docking analysis focused on the results that presented lower fitting conformation with lower G^{a}_{bind} energy, in addition to the interactions by hydrogen bridge and inhibition constant presented by the Epiruno$_2$ complex in the of the molecular targets of *L. major*.

2.2. *In Vitro* Trials on Promastigote forms MHOM/IL/80/Friedlin of *L. major*

For *in vitro* assays, the method adopted by Carneiro and collaborators was used, with some modifications [25]. The MHOM/IL/80/Friedlin promastigotes of *L. major* were donated by the Laboratory of Antileishmania Activity, located at the Research Core in Medicinal Plants of the Federal University of Piauí—UFPI and cultivated in Schneider media (Sigma, USA), supplemented with 10% bovine fetal serum (BFS) (Sigma, USA) and penicillin-streptomycin 10,000 IU/10mg (Sigma, USA) at 26˚C in a greenhouse of biological oxygen demand (BOD).

L. major promastigote forms MHOM/IL/80/Friedlin in log phase were seeded 1×10^6 parasites per well in a 96-well cell culture microplate containing supplemented Schneider medium and Epiruno$_2$ at serial concentrations of 800 to 6.25 μg/mL, respectively. Then the plates were incubated in a BOD greenhouse at 26˚C. After 48 h resazurine (1 mM) was added and the plate was re-infiltrated in the BOD incubator for another 6 h. Then the spectrophotometer reading was performed to obtain the optical density at 550 nm. Negative control was performed with Schneider medium at 0.2% DMSO and considered as 100% viability of the parasites. The amphotericin B (Amp-B) at a concentration of 2 μg/mL was used as a positive control to validate the experiment.

3. Results and Discussion

3.1. Molecular Docking

The evaluation criteria were defined by the results that showed lower cluster conformation with lower G^{a}_{bind} energy, besides the hydrogen bridge interactions and inhibition constant presented by the Epiruno$_2$ complex against the *L. major* molecular targets.

The molecular docking between the Epiruno$_2$ complex and the 1e7w protein obtained the lowest G^{a}_{bind} energy among all molecular couplings performed in this study, obtaining an energy of −10.68 Kcal·mol^{-1} and an inhibition constant of 14.8 nM (**Table 1**). This low G^{a}_{bind} energy indicates high molecular affinity of the complex with the target protein [26]. Thus, inhibiting its action would be to interrupt the disease development process, since the 1e7w enzyme has a function of reducing conjugated and unconjugated pterins, one example is biopterin and dihydrobiopterin (DHB), followed by 5, 6, 7, 8-tetrahydrobiopterin (THB) or DHF folate. It is the only protein known to reduce biopterin in Leishmania, proving to be essential for *in vivo* growth through genetic knockout studies [27].

Table 1. Molecular affinity parameters of the Epiruno$_2$ complex with *L. major* targets.

Complex (Protein-ligand)	ΔG^a_{bind} (kcal·mol^{-1})	Kib (μM)	Number of independent docking runs	Number of conformations in the first ranked cluster	Amino acids that interact through hydrogen bondsc
Epiruno$_2$/1e7w	−10.68	14.8 nM	100	7	Asp232, Lys198, Ser111, Ser227
Epiruno$_2$/5nzg	−10.51	19.74 nM	100	6	Asp221, Gly220, Lys95
Epiruno$_2$/5g20	−9.65	83.81 nM	100	81	-
Epiruno$_2$/5c7p	−8.22	935.7 nM	100	43	Arg104, Asn114, Ser98
Epiruno$_2$/1ezr	−8.19	996.06 nM	100	4	Pro11, gln40

Note: Epiruno$_2$—ruthenium complex with epiisopiloturine and nitric oxide; 1e7w—Pteridine reductase; 5nzg—UDP-glucose Pyrophosphorylase; 5g20—Glycyl Peptide N-tetradecanoyltransferase; 5c7p—Nucleoside diphosphate kinase; and 1ezr—Nucleoside hydrolase. (Araújo *et al.*, 2020).

The most intense interactions between the target protein and the Epiruno$_2$ complex occur between the residues Asp232, Lys198, Ser111 and Ser227, places where the highest intermolecular forces act (**Figure 1**).

The Epiruno$_2$ complex also showed excellent molecular affinity results with the target protein 5nzg of *L. major*, obtaining a G^a_{bind} energy of −10.51 Kcal·mol^{-1} (**Table 1**) and formation of three hydrogen bridges located on amino acids Asp211, Gly220 and Lys95, where the most intense interactions between the complex and the target protein occur (**Figure 1**). This may be related to UGP (UDP—glucose pyrophosphorylase) catalyzing the synthesis of activated form glucose, UDP-Glc, uridine triphosphate (UTP) and glu-cose-1-phosphate (Glc-1p). Because the UDP-Glc reaction is critical in the production of carbohydrates such as cell surface glycans and other pathogen processes becoming an attractive target in interaction and molecular inhibition studies [28] [29] [30]. The resulting inhibition constant was 19.74 nM, presenting antileishmania inhibitory activity of the Epiruno$_2$ complex against target protein 5nzg (**Table 1**).

This result indicates that the Epiruno$_2$ complex has antileishmania inhibitory activity, since docking studies analyze the inhibitory action of coupled compounds at the active site of the target protein [26] even if there is a difference between *in sílico* and *in vitro* experiments, the results tend to differ, where *in sílico* studies by molecular docking pre-dict quickly and reliably if a compound has biological activity and experimental labora-tory studies validate their analyzes, complementing each other, providing technical via-bility in the results presented [31] [32].

The 5g20 protein also showed molecular affinity with the Epiruno$_2$ complex obtaining attractive G^a_{bind} energy in docking molecular affinity studies with −9.65 Kcal·mol^{-1} and an inhibition constant of 83.81 nM [26] (**Table 1**). This molecular interaction did not result in hydrogen bridges, unlike previous interactions between the Epiruno$_2$ complex with the 1e7w and 5nzg proteins that had 4 and 3 hydrogen bridges, respectively, however, the interactions in the Val374, Leu227 and His219 residues make intense interactions in the active site borders of the protein, in particular the interactions Val374 with O1 and Leu227 with O2 and

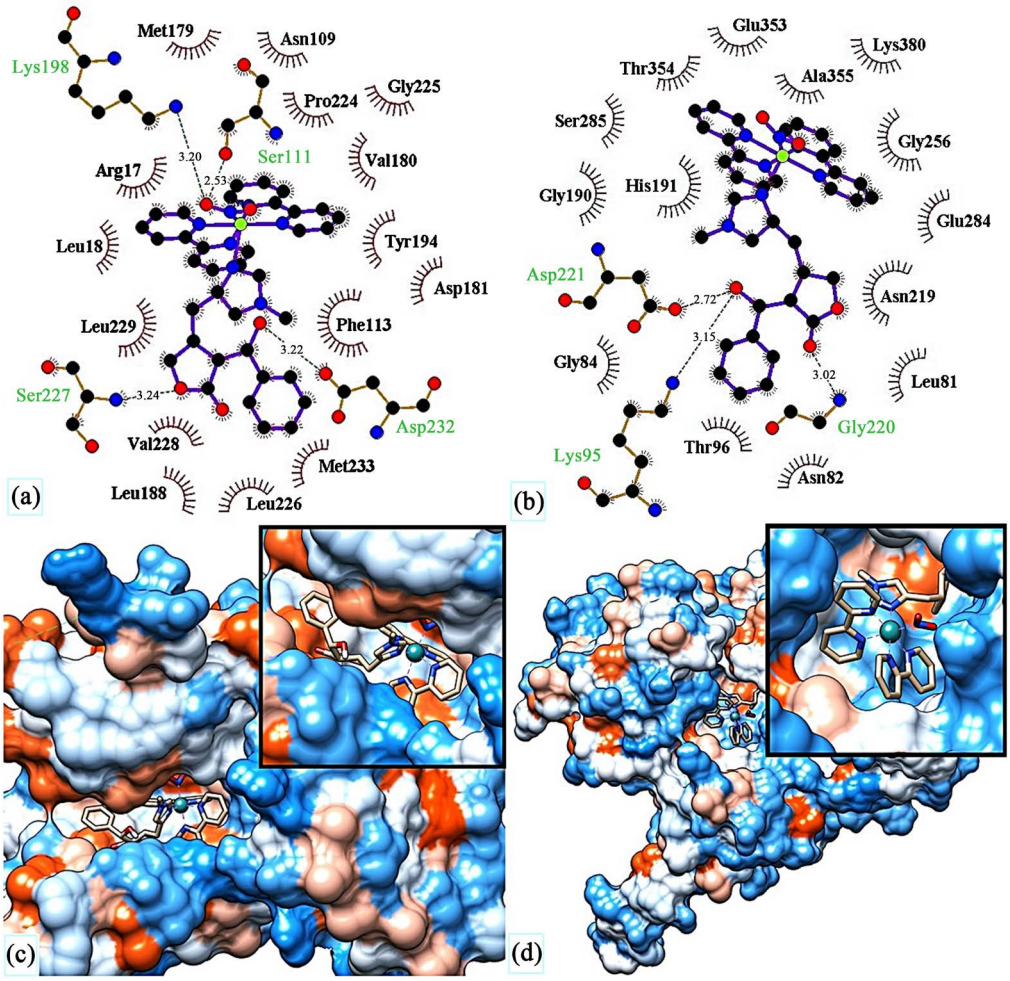

Figure 1. Molecular docking between Epiruno$_2$ complex and 1e7w and 5nzg target proteins. (a) Docking at target protein active site 1e7w; (b) molecular interaction between Epiruno$_2$ and target protein 1e7w; (c) Docking at 5nzg target protein active site; (d) molecular interaction between Epiruno$_2$ and 5nzg target protein.

both residues with C8 (**Figure 2**). These interactions at the edges of the active site make the Epiruno$_2$ complex have a very promising high inhibitory action, and the tertiary structure is part of recognition elements that facilitate the molecular interactions between protein and ligand, in this case the Epiruno$_2$ complex [33] [34].

Molecular docking between the 5c7p protein and the Epiruno$_2$ complex formed three hydrogen bridges at amino acids Arg104, Asn114 and Ser98 (**Figure 2**) and showed G^{a}_{bind} energy of −8.22 Kcal·mol^{-1} and an inhibition constant of 935.7 nM (**Table 1**). These results are promising against this indispensable protein for the maintenance of intracellular nucleoside triphosphate (NTP) levels [35]. They carry the γ-phosphoryl group from an NTP to a nucleoside diphosphate (NDP) through a functional scheme called ping-pong involving the covalent intermediate phosphohistidine. Eukaryotic NDKs are composed of 15 to 18 KDA subunits with similarities in their general structures and a conserved active site [36] [37].

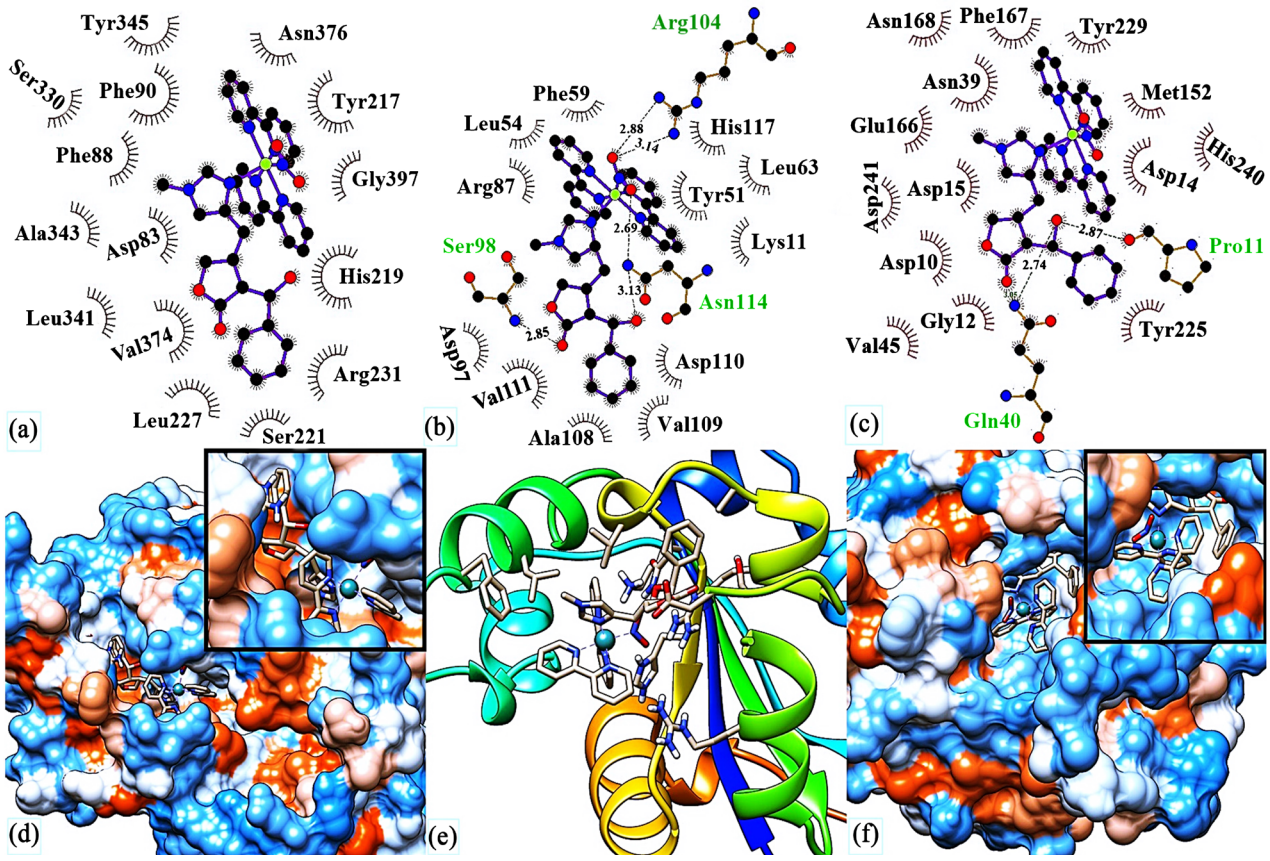

Figure 2. Molecular docking between the Epiruno$_2$ complex and the 5g20, 5c7p and 1ezr proteins. (a) Docking at active site of protein 5g20; (b) molecular interaction between 5g20 protein and Epiruno$_2$ complex; (c) molecular interaction between 5c7p protein and Epiruno$_2$ complex; (d) Docking at active site of protein 1ezr; (e) molecular interaction between 1ezr protein and Epiruno$_2$ complex.

The 1ezr protein can be identified in free extracts of Leishmania cells, is also present in several parasitic protozoa. It is a protein useful in parasitic infections, acting as a catalyst for the hydrolysis of both purine and pyrimidine nucleosides [38] [39], where its inhibition is fundamental for the treatment of *L. major*. In this sense, the Epiruno$_2$ complex presented interesting interaction and molecular affinity results, obtaining a G^{a}_{bind} energy of −8.19 Kcal.mol^{-1} and an inhibition constant of 996.06 nM [26] (**Table 1**). The most intense interactions between the complex and the protein occur at residues Pro11 and Gln40, the two hydrogen bonds formed (**Figure 2**).

3.2. *In Vitro* Trials on Promastigote Forms MHOM/IL/80/Friedlin of *L. major*

In these trials we evaluated the leishmanicidal effects of the Epiruno$_2$ complex against *L. major* promastigote MHOM/IL/80/Friedlin parasites. The Epiruno$_2$ complex showed 50.53% inhibition of promastigote growth at a concentration of 800 µg/mL (**Figure 3**), a significant reduction by analyzing the half maximal inhibitory concentration (CI-50) (**Table 2**) showing antileishmania activity, confirming the results presented *in sílico* tests by molecular docking. However, these

values are not considered clinically relevant, according to Santos *et al.* [40], which defines in their studies that only IC-50 lower than 500 µg/mL can be considered therapeutically relevant.

It is observed that the results presented in molecular docking analyzes were more promising than the results presented *in vitro* assays. This may be related to the topological polar surface area (TPSA), which uses functional groups obtained from a structural database, avoiding calculations of the ligand's three-dimensional (3D) structures, in this case the Epiruno$_2$ complex or the confirmation of which conformation. Since this biological method is relevant, this method is used in 2D structures for 14 sets of diverse pharmacological activity data. This methodology is promising for classic 2D descriptors such as calculated LogP (ClogP) and calculated molar refractivity (CMR) in the 2D-QSAR literature [41].

The discovery of new antileishmania chemical compounds has long been realized from the isolation of plant extracts. There are already several extracts and compounds that have proven antileishmania activity on promastigote and amastigote forms of through *in vitro* assays [42] [43] [44]. Despite several microbiological studies, several analyzes of new compounds extracted from natural and synthetic resources are still needed, as the search for new pharmacological potential leishmanicide has been important, since the drugs in the pharmacological market have high toxicity and reduced efficacy [6] [45].

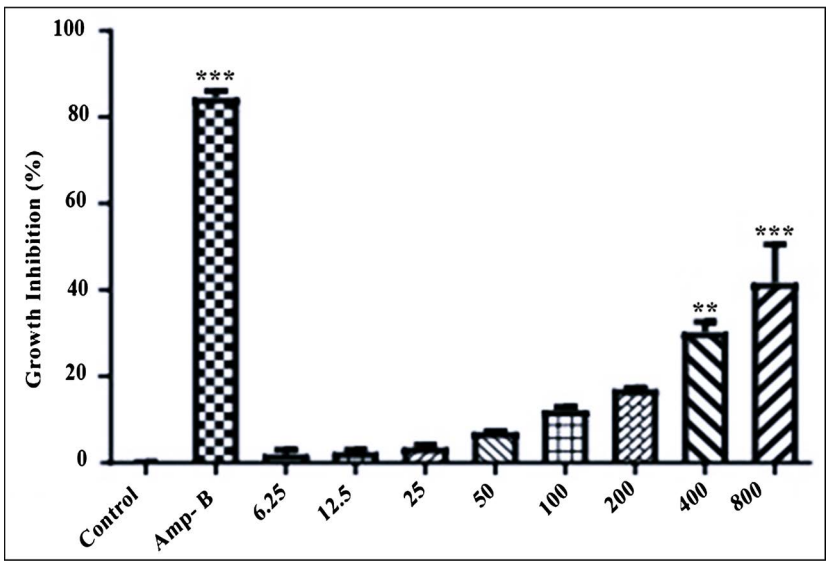

Figure 3. IC-50 of the Epiruno$_2$ complex against *L. major* promastigotes.

Table 2. IC-50 values of *L. major* promastigotes in the presence of the Epiruno$_2$ complex.

	Epiruno$_2$ complex
L. major	MHOM/IL/80/Friedlin
IC-50	800 µg/mL

Note: IC-50 – half maximal inhibitory concentration (Araújo *et al.*, 2020).

4. Conclusions

The Epiruno$_2$ complex presented antileishamania activity both *in sílico* studies by molecular docking and *in vitro* study. Its best molecular affinity parameter presented in docking studies was for target proteins 1e7w and 5nzg with G^{a}_{bind} energies −10.68 Kcal·mol^{-1} and −10.51 Kcal·mol^{-1}, respectively. In addition to these two targets, it was found that the complex has molecular affinity for the other molecular targets of *L. major* analyzed in this study.

In vitro assays proved the antileishmania activity of the complex against *L. major* promastigotes MHOM/IL/80/Friedlin with significant growth inhibitions. However, the values are not considered clinically relevant, concluding from *in sílico* and *in vitro* studies that the Epiruno$_2$ complex has antiparasitic activity that can be tested on other Leishmania targets such as *L. amazonensis* and *L. Chagasi* and also in other pathogens.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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