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IN SILICO STUDIES TO EVALUATE INTERACTIONS BETWEEN KAURANE-TYPE DITERPENES AND THE DIHYDROFOLATE REDUCTASE – THYMILIDINE SYNTHASE OF THREE LEISHMANIA SPECIES.

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Abstract: Leishmaniasis is a group of Neglected Tropical Diseases (NTDs) which are present in around 88 countries on tropical and subtropical regions worldwide. The current treatments against these diseases present several problems such as low effectiveness, resistance, and high toxicity. More than 50% of new small molecules approved in U.S. are related with natural products, being these interesting alternatives very important in the search of new efficient and safety treatments for this group of diseases. Kauranes (diterpenes) have presented potential biological activities highlighting some molecules that have anticancer properties; however, this type of compounds has not yet been studied deeply against Leishmaniasis. In this study, hybrid homology models for an important target of *Leishmania*, dihydrofolate reductase – thymidylate synthase (DHFR-TS), were constructed using YASARA software. This test enzyme was related to three species, *L. panamensis*, *L. amazonensis* and *L. braziliensis*, whose incidence is responsible by causing Leishmaniasis in Central and South America. In parallel, a database of 360 kaurane-type structures was constructed and all 3D structures were minimized using a MM2 force field. Thus, molecular docking studies, using the entire database were performed using autodock/vina. Best docking scores resulted between 10.85 and 10.96 kcal/mol, involving RMSD values below 1, even better than pteridine-related compound used as control. Structural features were then identified to be crucial by multivariable analysis and used to establish a Structural- Activity relationship for this kaurane-type set. The compound 3α-cinnamoyloxy-*ent*-kaur-16-en- 19-oic acid exhibited the best docking result for *La* and *Lb*DHFR-TS and it also was part of the best five-ranked compounds for *Lp*DHFR-TS. Finally, using GROMACS package, molecular dynamics simulations were therefore performed to validate the docking findings through the physical movements and evolution of the enzyme-ligand complexes over the time.

Keywords: *Leishmania*, molecular docking, kauranes, *in-silico*, molecular dynamics

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1. Introduction

Since the fifties the search of safety therapies for the control and elimination of *Leishmania* parasites has been a great challenge for the researches. The current effective drugs based on antimonial compounds can kill the parasite but are highly toxic. These treatments against these diseases present additional problems such as low effectiveness and resistance of the parasite [1]. Looking for new alternative therapies, the use of natural products has emerged as important way to develop new leishmanicidal drugs. Kaurane-related diterpenes have presented potential biological activities highlighting some molecules that have anticancer properties [2]; however, this type of compounds has not yet been studied deeply against Leishmaniasis.

In this study, computational tools were used to evaluate the antileishmanial activity of a series of 360 kauranes, using hybrid models of dihydrofolate reductase – thymidylate synthase (DHFR-TS), an important target of *Leishmania* parasite. Models were built from sequences of three Leishmania species, *L. panamensis*, *L. amazonensis* and *L. braziliensis*, whose incidence is responsible by causing Leishmaniasis in Central and South America.

2. Results and Discussion

The entire minimized set of kauranes was tested in Autodock/vina. Results showed that 6.7%, 10.5% and 6.9% of these structures have higher affinity for *Lb*, *Lp*, and *La*DHFR-TS, respectively, compared to methotrexate, a potent inhibitor of *L. major* DHFR-TS [3]. Affinity values for the three best-docked compounds are observed in Table 1; in all cases the best-ranked kauranes had root mean square deviation (RMSD) values below 1, meanwhile methotrexate resulted in values above 3.

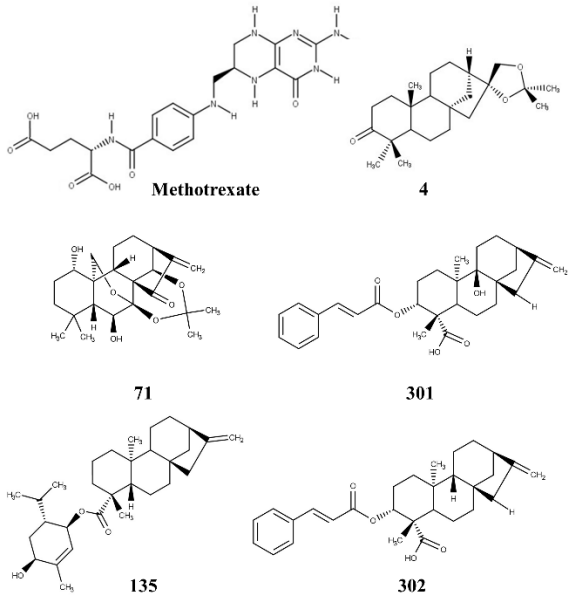

Figure 1. Kauranes with the highest affinity values for DHFR-TS *Leishmania* hybrid models.

Table 1. Best-ranked kauranes in molecular docking for the DHFR-TS *Leishmania* models. Methotrexate is used as control.

***Lb*DHFR-TS**		
Molecule	**Affinity (kcal/mol)**	**RMSD**
302	-10.90	0.6
4	-10.70	0.1
135	-10.50	0.2
Control	-9.80	4.0
***Lp*DHFR-TS**		
4	-10.96	0.5
71	-10.92	0.5
302	-10.84	1.7
Control	-9.67	3.9
***La*DHFR-TS**		
302	-10.85	0.6
4	-10.68	0.1
301	-10.55	0.9
Control	-9.67	3.4

Structure **302,** 3α-cinnamoyloxy-*ent*-kaur-16-en-19-oic acid exhibited the best docking result for *La* and *Lb*DHFR-TS and it also was part of the five best-ranked compounds for *Lp*DHFR-TS. This compound is present in species of the *Wedelia* genus [4,5]. Figure 2 shows the complex between the best pose of **302** with each one of the three DHFR-TS hybrid models constructed in this study. For *Lb* and *La* species (Figure 2a and 2c) similar intermolecular interactions were observed, highlighting the hydrogen bond formed between Val156 and the carboxylic group at C-19. A

different spatial conformation is observed between kaurane **302** and *Lp* DHFR-TS (Figure 2b), whose carboxylic group at C-19 interacts through hydrogen bonds with Thr83 and Ser86. Additionally, Ile30 and Ile39 exhibit steric interactions with the cinnamic group of the kaurane. This type of interaction is not observed in *Lb* and *La* species.

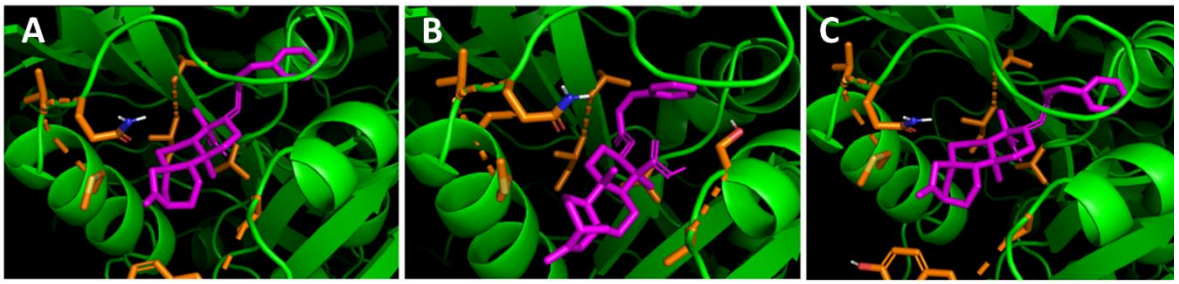

Figure 2. Best pose of 3α-cinnamoyloxy-ent-kaur-16-en- 19-oic acid (**302,** purple) in the active site of A) *La* B) *Lp* and C) *Lb* DHFR-TS (green). Flexible aminoacids are marked in orange.

Multivariate analysis was performed using Orthogonal Analysis of Partial Least Squares (OPLS), using a 1+2 model, to relate the affinity values with some structural features of kauranes. Thus, on evaluating those features as dependent variable as a function of the docking affinity on the three *Leshmania* DFHR -TS, it was clearly observed that structural features (such as the presence of aromatic rings and unsaturations) have more contribution to the latent variables of the system analyzed (Figure 3). In this way, the affinity observed in docking calculations for compounds **301** and **302** (Figure 3, red dots) is therefore correlated to their structural features, being the kaurane **302** the most interactive one within the active site of *L. braziliensis* and *L. amazonensis*. These two molecules are characterized by the presence of a cinnamic group into their structures. From these studies, it can be identified that behavior of the models of *Lb* and *La*DHFR-TS are very similar, while *Lp*DHFR-TS exhibited affinity more related to kauranes having unsaturations into structures.

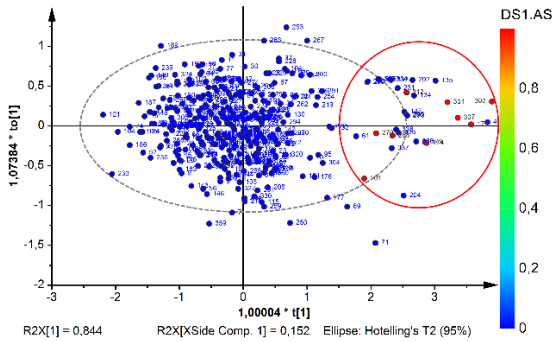

Figure 3. Score plot showing the relationship between docking affinity in *La*DHFR-TS with some kaurane structural features.

Molecular dynamics (MD) simulation between the best docking pose of **302** and *La*DHFR-TS was performed in order to validate the docking results. Initially, RMSD values (Figure 4a) were recorded to evaluate the structural stability of the protein interacting with **302**. The results indicate that after 25000 ps the complex is reaching its stability with minimal variation, remaining almost constant in 0.3 Å. Subsequently, root mean square fluctuations (RMSF) values were determined (Figure 4b) to evaluate the flexibility and secondary structure of DHFR-TS when interacts with **302.** The highest fluctuations values are observed in three regions of the protein: 1) Glu117 have a value above 0.6 nm, 2) between aminoacids 310 to 330 and 3) from residue 187 to 191; this last region is the closer sequence to the active site of *La*DHFR-TS. Analyzing the 3D-structure of the complex, it is observed that these three-fluctuating regions correspond to loops.

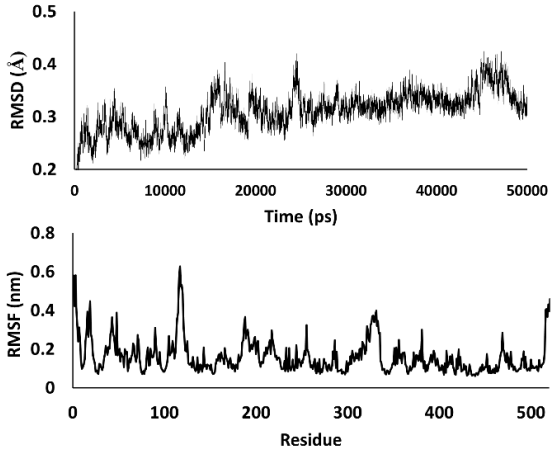

Figure 4. Results for A) RMSD and B) RMSF from MD simulations of the best pose of structure **302** with *La* DHFR-TS.

3. Materials and Methods

Hybrid models of dihydrofolate reductase – thymidylate synthase (DHFR-TS) were constructed using YASARA software (YASARA (18.4.24) Vienna, Austria: YASARA Biosciences GmbH; 2018), using the FASTA sequences of *Lb*DHFR-TS, *Lp*DHFR-TS and *La*DHFR-TS which were obtained from UNIPROT database (https://www.uniprot.org/).
A database of 360 kaurane-type structures was constructed using Marvin sketch (version 18.16.0 (2018), a calculation module developed by ChemAxon, http://www.chemaxon.com/). All the 3D structures were minimized using a MM2 force field using Chem3D 16.0 (Perkin Elmer Informatics) and the minimized structures were finally saved in PDB format using SPARTAN 2014. Molecular docking studies with the flexible amino acids of the active site of DHFR-TS, using the entire *in-house* database, were performed in autodock/vina plugin for Pymol (1.3r2). Docking calculations were then performed between the minimized ligand through a cube (dimensions 24 × 24 × 24 Å, grid spacing 0.375 Å) located in the geometric center. Each calculation was performed in 10 replicates. Methotrexate was used as control. The 2D-Residual interaction diagrams were visualized on Molegro Virtual Docker 6.0. Preliminary multivariable analysis of the docking results, Principal Components Analysis (PCA) and Partial Least Squares (PLS) were performed using SIMCA 14.0 (Umetrics Inc., Sweden). Molecular dynamics (MD) simulations were performed during 50 ns in Gromacs 5.0.5 on Ubuntu 12.04, installed on a Dell 7820 workstation. The best pose of 3α-cinnamoyloxy-*ent*-kaur-16-en-19-oic acid obtained from molecular docking and a hybrid model structure were used for MD calculations. Hydrogen atoms and charges (AM1-BCC charge scheme) were added to the ligand in UCSF Chimera. Protein topologies were obtained in Gromacs using the Amber 99SB force field, and the TIP3P water model was implemented. Solvation was performed in a triclinic box using a margin distance of 1.0 nm. The systems were energy-minimized by 2000 steps of the steepest descent method. NVT equilibration at 310 K for 50 ps [6].

4. Conclusions

This computational study allowed the preliminary evaluation of a set of 360 kauranes looking for new antileishmanial agents. The study comprised hybrid models of DHFR-TS, for three Leishmania species, which have high incidence in Central and South America. The best docked results for the three models showed a relationship between the affinity values obtained from the molecular docking with some structural features of kauranes, such as the presence of aromatic rings as well as unsaturations. Compound **302,** 3α-cinnamoyloxy-ent-kaur-16-en- 19-oic acid, presented the best docking results for *Lb* and *La*DHFR-TS and also have one of the highest affinity values in *Lp* DHFR-TS, being an interesting compound for further studies. MD simulations between best pose of **302** and *La*DHFR-TS, allowed to validate the docking results through the analysis of physical movements and evolution, which indicates good stability of the enzyme:lingand complex over the time and perturbations of enzyme loops regions.

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Author Contributions
CH and DM built database and performed all calculations; CH, EC and AF wrote the paper. All authors read and approved the final manuscript.

Conflicts of Interest
The authors declare no conflict of interest

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