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Molecular Docking for Development of Alternative Therapies against Leishmaniasis †

Guarimata Juan Diego [1], Alcívar Christian [1], Lavecchia Martin [2,*] and Poveda Ana [1,*]

[1] DNA Replication and Genome Instability Unit, Grupo de Investigación en Biodiversidad, Zoonosis y Salud Pública (GIBCIZ), Instituto de Investigación en Zoonosis-CIZ, Facultad de Ciencias Químicas, Universidad Central del Ecuador, Quito 170521, Ecuador; jdguarimata@uc.edu.ec (G.J.D.); cdalcivar@uce.edu.ec (A.C.)

[2] CEQUINOR (UNLP, CCT-CONICET La Plata, Associated with CIC), Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Boulevard 120 N° 1465, La Plata CP 1900, Argentina

* Correspondence: lavecchia@quimica.unlp.edu.ar (L.M.); apoveda@uce.edu.ec (P.A.); Tel.: +54-9-2214-95-7316 (L.M.); +593-99-987-1271 (P.A.)

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Abstract: Topoisomerases manage the topological state of DNA in essential processes such as transcription, DNA repair, or DNA replication, because of this, topoisomerases are biological targets in pathogenic microorganisms or malignant cells, in this study we aimed to identify potential inhibitory compounds against topoisomerases type II homology modeled of Leishmania mexicana via molecular docking. We screened 400 compounds provided by Medicines for Malaria Venture (MMV) in the Pandemic Response box. Here we identify the 20 best compounds against each topoisomerase type II of L. mexicana with the objective of identifying new alternatives to treat a neglected tropical disease such as leishmaniasis.

Keywords: *Leishmania mexicana*; topoisomerase; molecular docking; homology modeling; neglected tropical disease

1. Introduction

Leishmaniasis is a neglected tropical disease constituting a public health problem in the Americas due to its high incidence, morbidity, wide geographical distribution, variety of parasite species, and clinical forms, as well as a lack of adequate therapeutic and prevention measures [1]. Ecuador is an endemic area for cutaneous leishmaniasis caused by *L. mexicana* with around 900 annual cases [2]. Many of the drugs used to treat leishmaniasis have limited efficacy in advanced stages of the disease, are nonspecific, and/or are highly toxic [1]. Finding alternative drugs to effectively treat and control these diseases is therefore a priority.

The current increase in microbial resistance is a very serious public health problem that requires immediate attention from the scientific-multidisciplinary [3]. The Pandemic Response box (PRB) is a collection of 400 pre-synthesized compounds to facilitate drug discovery provided by Medicines for Malaria Venture (MMV) (www.mmv.org) [4]. The collection contains chemically characterized compounds that are freely available to the scientific community. Computational tools including molecular docking have allowed enormous progress in the discovery of new drugs by focusing experimental trials on promising compounds, improving efficiency in terms of time and money in the search for therapeutic alternatives [5]. Type II topoisomerases are enzymes that control changes in DNA topology by catalyzing a controlled break and resealing of DNA strands [6], interfering with the normal functioning of this type of enzyme becomes the mechanism of action of antibacterial drugs such as fluoroquinolones or anticancer drugs such as etoposide,

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leading to cell death [6,7]. *L. mexicana* has two Type II topoisomerases, one located in the nucleus and another in the kinetoplast, a giant and unique mitochondria characteristic of this parasites [8,9].

We report the 20 best candidates to inhibit each topoisomerases type II of *L. mexicana* through molecular docking carried out with software FRED (Chemgauss4), comparing beforehand the performance of three virtual screening methods (Autodock Vina, FRED and HYBRID (Chemgauss4)) to discern between decoys and active molecules in a molecular docking against human beta topoisomerase II (hTopII beta) (RCSB-PDB accession code: 3QX3). We implemented receiver operating characteristics (ROC) curves as a tool to compare the predictive power of the 3 virtual screening methods tested.

2. Materials and Methods

2.1. Sequence Retrieval, Homology Modeling, and Refinement

Both topoisomerases type II from *L. mexicana* were modeled due to the absence of X-ray crystallography of these proteins for the specie. The amino acid sequence of nuclear topoisomerase (nuclear TopII) and mitochondrial topoisomerase (mitochondrial TopII) retrieved from NCBI with the accession number "XP_003877071.1" and "XP_003873648.1" respectively, were selected for this study. Homology modeling was implemented via SWISS-MODEL (http://swissmodel.expasy.org) by extrapolating experimental information from related protein structures that serves as a template [10], in this case the best models were built from a *Saccharomyces cerevisiae* topoisomerase template (PDB accession code: 4gfh.1), with 46.20% of sequence identity for nuclear TopII and 32.72% of sequence identity for mitochondrial TopII, these models were saved in PDB format.

Before virtual screening, DNA chains, magnesium as co-factors, and Etoposide as ligand were added by extracting from hTopII beta (PDB accession code: 3QX3) for mitochondrial TopII and from human alpha topoisomerase (hTopII alpha) (PDB accession code: 5GWK) for nuclearTopII. A minimization was run with NAMD2 Version 2.14 to refine the modeled structures, using amber force field DNA.OL15 (DNA), FF14SB (protein) and GAFF (EVP). The topology was prepared with Leap, included in AmberTools22. The minimization consisted of a series of steps: hydrogen minimization, water minimization, side chain minimization, full structure minimization. All the minimization steps allowed the structure to relax and no atoms to overlap.

2.2. Binding Site Determination

To generate the grid box coordinates needed for virtual screening with Autodock Vina, a sequence alignment was made with the MultAlin tool (http://multalin.toulouse.inra.fr/multalin/multalin.html) [11], between the hTopIIbeta (NCBI accession code: NP_001317629.1) and the parasitic topoisomerases (accession codes above) that allowed the identification of homologous residues between these topoisomerases, with emphasis on catalytic residues present in hTopIIbeta (P819; Y821) as well as binding residues to etoposide (P501; L502; R503; E522; G776; E777; Q778; A779; M782; A816) [12,13]. To define the binding pocket for hTopIIbeta ADT from MGL tools-1.5.7 was used [14], and the next coordinates were generated: center_x = 30.41, center_y = 99.699, center_z = 43.198, size_x = 32, size_y = 34, size_z = 34. In the case of HYBRID and FRED, the active site was selected around Etoposide.

2.3. Receiver Operating Characteristics (ROC) Curve

To generate the ROC curves was necessary to have active compounds against hTopII beta, these compounds were obtained from the CHEMBL platform (https://www.ebi.ac.uk/chembl/) [15], where were selected compounds with a pChEMBL value of 5.5 to 8.26 [16]. The decoys needed for the ROC curve were generated with the active compounds via DUD-E (https://dude.docking.org/) [17], obtaining 50 decoys per active molecule. A molecular docking was made with 500 molecules, about 25 actives

molecules and 475 decoys. Three software were used (Autodock Vina 1.2.0 [18], HYBRID and FRED, the latter two from OpenEye's OEDOCKING 4.2.1.1 suite [19]) and ROC curves and ROC AUC were generated with Screening Explorer tools (http://stats.drugdesign.fr/) [20].

2.4. MMV Ligand Preparation and Molecular Docking

Once ROC curves were done, software FRED was used to do the molecular docking with etoposide, ligands of MMV, hTopII beta, hTopII alpha and topoisomerases II of *L. mexicana*, based on ROC AUC for this software. First, with the SMILES codes of all ligands (400) given by MMV and the SMILE code for etoposide taken from Pubchem (PubChem CID: 36462) (https://pubchem.ncbi.nlm.nih.gov/) [21], 3D coordinates, protonation of the molecules at pH 7.4 and minimization with a UFF force field were done with Obabel v3.1.1 [22], and with a subsequent transformation of all molecules to .pdbqt format. The Kollman charges of proteins of *L. mexicana* and human topoisomerases isoenzymes were added with MGL tools-1.5.7, and proteins were saved in format .pdbqt, finally, a docking molecular was made between the 401 molecules against each topoisomerase.

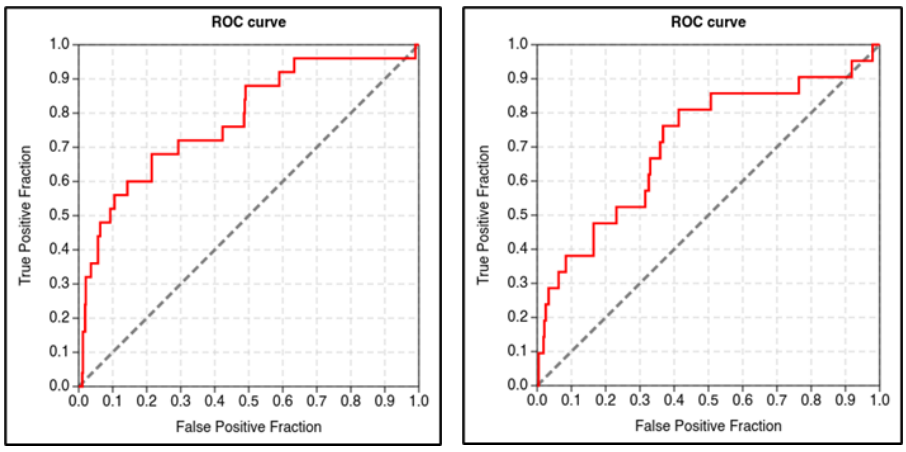
2.5. Interactions

The analysis of four docking results was carried out using Discovery Studio Visualizer v21.1.0.20298 [23].

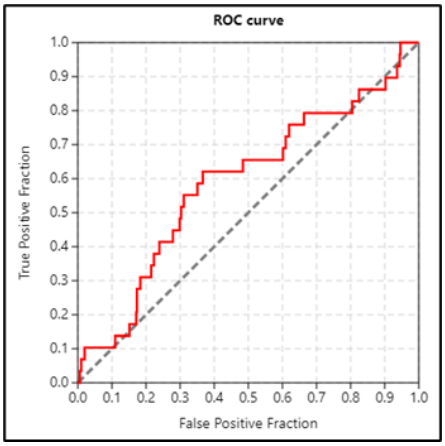
3. Results

In the alignment carried out between human and leishmania topoisomerases (not shown), the residues involved in binding with etoposide in hTopII beta (P501; L502; R503) are conserved in all topoisomerases, additionally, the residues also involved in binding with etoposide (G776; E777; Q778), the residue Q778 is only conserved in nuclear TopII of *L. mexicana*, however, is changed for a non-polar residue in hTopII alpha (M762) and changed for another non-polar residue in mitochondrial TopII (A732). Residues G776 and E777 are conserved in all topoisomerases. On the other hand, the catalytic residues of hTopIIbeta (P819; Y821) are conserved in all topoisomerases except in nuclearTopII, where residue P819 is changed to another non-polar residue G769.

Figure 1 shows the 3 ROC curves of the 3 virtual screening methods implemented (Autodock Vina, HYBRID and FRED) made with 475 decoys and 25 active molecules against hTopIIbeta.



(**a**) (**b**)



(**c**)

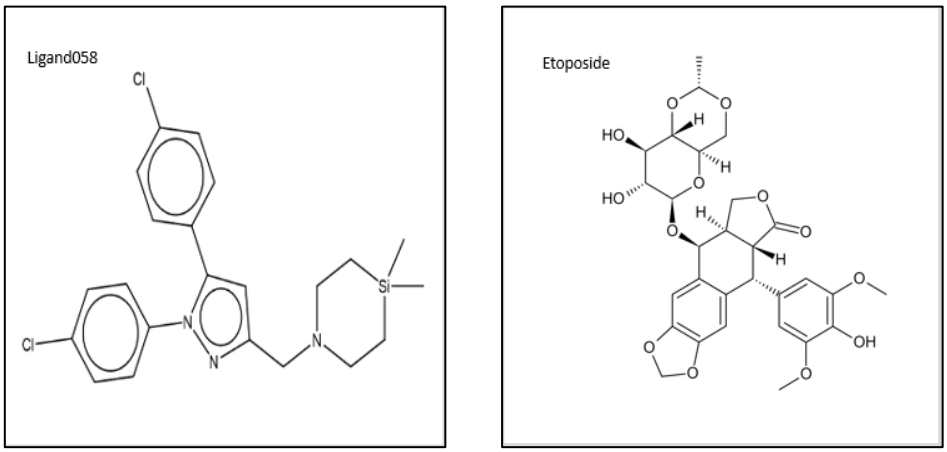
Figure 1. ROC curves for each docking software: (**a**) ROC curve for FRED software, ROC AUC: 0.780; (**b**) ROC curve for HYBRID software, ROC AUC: 0.710 ; (c) ROC curve for AutoDock Vina software, ROC AUC: 0.589.

Table 1 shows the 20 best free energy binding results of a molecular docking with software FRED (each topoisomerase II of *L. mexicana* and each human topoisomerase isoenzyme against MMV ligands). EVP was added to compare the binding energy between ligands and this molecule. The software FRED was selected for the virtual screening based on the predictive power detected with the ROC curve.

Table 1. Show 20 best binding energies between each topoisomerase evaluated and ligands of MMV, also shows the binding energy of etoposide with these topoisomerases.

Nuclear TopII *L. mexicana*		**Mitochondrial TopII** *L. mexicana*		**hTopII Alpha**		**hTopII Beta**	
Ligand ID	**Score**	**Ligand ID**	**Score**	**Ligand ID**	**Score**	**Ligand ID**	**Score**
058	−17.895	389	−19.766	046	−19.840	**Etoposide**	**−19.884**
363	−17.140	280	−18.509	180	−19.413	208	−19.522
091	−17.040	301	−18.232	170	−19.073	070	−19.056
389	−16.610	058	−18.069	108	−18.996	288	−19.026
050	−16.560	151	−18.059	190	−18.892	364	−18.824
313	−16.516	155	−17.960	290	−18.813	040	−18.700
296	−16.440	128	−17.949	363	−18.519	180	−18.478
191	−16.228	269	−17.916	373	−18.497	280	−18.428
192	−16.172	091	−17.845	280	−18.445	091	−18.411
354	−16.126	078	−17.775	040	−18.409	108	−18.347
016	−16.072	187	−17.567	288	−18.392	37	−18.151
280	−15.930	363	−17.448	**Etoposide**	**−18.381**	190	−18.059
326	−15.892	344	−17.422	078	−18.316	016	−17.991
134	−15.817	386	−17.391	016	−18.263	397	−17.951
204	−15.705	205	−17.366	389	−18.255	043	−17.875
376	−15.667	133	−17.317	023	−18.224	335	−17.870
133	−15.591	195	−17.313	091	−18.096	065	−17.826
370	−15.586	288	−17.302	333	−18.079	023	−17.794
288	−15.494	399	−17.177	070	−18.022	373	−17.713
048	−15.467	100	−17.158	138	−17.987	333	−17.674
Etoposide	**−13.252**	**Etoposide**	**−15.270**	168	−17.983	100	−17.588

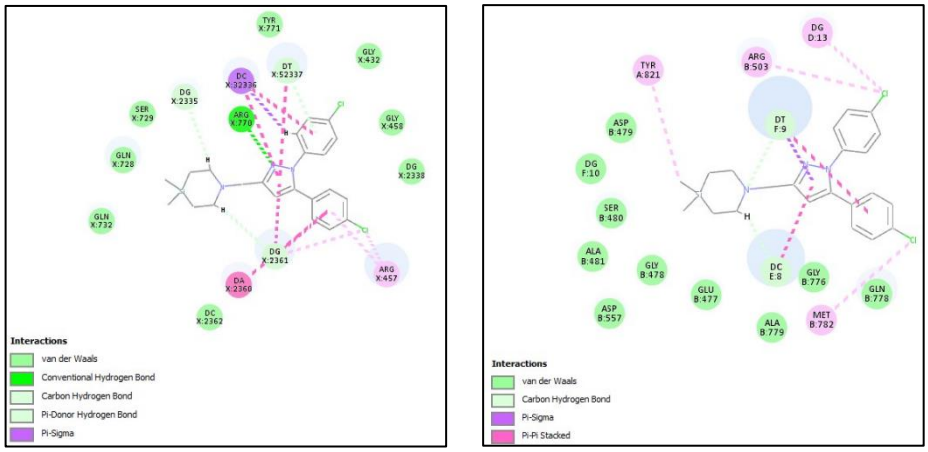
Figure 2 shows the chemical structure of ligand058, which exhibits binding to nuclearTopII and mitochondrialTopII of *L. mexicana* but not with human topoisomerases, also shows the chemical structure of etoposide to compare.



(**a**) (**b**)

Figure 2. Chemical structure of molecules: (**a**) Ligand058 only selective for type II *L. mexicana* topoisomerases; (**b**) Etoposide.

Figure 3 shows the different forms of binding between Ligand058 and nuclearTopII or hTopIIbeta.



(**a**) (**b**)

Figure 3. Binding between nuclearTopII and hTopIIbeta with Ligand058: (**a**) nuclear TopII/ 058; (**b**) hTopII beta/058.

4. Discussion

This study reports an application of molecular docking to discover the 25 best compounds in all those given by the MMV pandemic box as new possible TopII *L. mexicana* inhibitors. The implemented receiver ROC curves shown in Figure 1 demonstrated that FRED software have best capacity to distinguish between active and inactive compounds to screen databases with accuracy among software tested, which allows the identification of candidates in a time and cost-effective manner, however this doesn't imply that experimental confirmation of hits will occur [18].

Table 1 shows that the 25 best compounds selective only for *L. mexicana* topoisomerases have higher binding energy than etoposide, perhaps due to the presence of imidazole,

pyridine, pyrimidine, benzimidazole and piperidine rings among the ligands, unlike etoposide, shown in Figure 2. These rings allowed hydrogen bonds with key residues such as Arg770 as an example with 058 in Figure 3, this is possibly due because nitrogen heterocycles rich in electrons exhibit a notable capacity to easily accept or donate electrons, allowing them to engage in a wide range of weak interactions, enabling them to readily attach to various therapeutic targets [24,25]. The ring skeletons mentioned above would be excellent pharmacophores for developing TopII inhibitors for targeted leishmaniasis therapy. On the other hand, halogens, especially chlorine and fluorine, encounter in most of the best ligands (Figure 2) have a beneficial impact on the biological characteristics of molecules through halogen bonding, this bonding has been identified as one of the mechanisms through which chlorine and fluorine modify the biological effects of molecules [26].

By reviewing the scores obtained for etoposide and the ligands against the evaluated topoisomerases, it is expected that etoposide will serve as a drug to validate the computational method in vitro way since human topoisomerases should be more sensitive to etoposide than topoisomerases from *L. mexicana* Based on this, tests are being done in the laboratory with human monocytes (THP-1) [27], and with *L. mexicana* (bel 21) [28], as a starting point to test the found hits and complete our objective of identifying new alternatives to treat a neglected tropical disease such as leishmaniasis.

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