

# Artículos Originales

## New agents with potential leishmanicidal activity identified by virtual screening of chemical databases

### Nuevos agentes con potencial actividad leishmanicida identificados mediante tamizaje virtual

#### New agents with potential leishmanicidal activity

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#### ABSTRACT

**Introduction and Objectives:** Leishmaniosis, a disease caused by a protozoan parasite, remains a serious public health problem threatening about 350 million people around the world, of which 12 million are believed to be currently infected (WHO 2010). To date, there are no vaccines against the species of parasites and the treatment is based only on chemotherapy with toxic-, expensive- and inefficient- drugs. There is an urgent need for better drugs against *Leishmania*, the etiological agent of the disease. The main anti-leishmanial drug used in Colombia is meglumineantimoniate [chemical name according to the International Union of Pure and Applied Chemistry (IUPAC): Hydroxy-dioxostiborane; (2R,3R,4R,5S)-6-methylamino-hexane-1,2,3,4,5-pentol, (C<sub>7</sub>H<sub>17</sub>NO<sub>3</sub>)], which is not efficient in the treatment of infections caused by *Leishmania braziliensis*, the most prevalent specie in the Caribbean coast of Colombia. **Methods:** We performed an *in silico* virtual screening of several datasets including ChemBridge and Pubchem. We virtually screened a total of 28.755 compounds against a 3D model of 6-phosphoglucono -lactonase (6-PGL) from *Leishmania braziliensis* to identify novel inhibitors. Molecular docking of databases was performed using the software Sybyl 8.0 and AutoDockVina. **Results:** The initial virtual screening using a structure-based method identified 10 compounds, which were later tested with AutodockVina and classified according to their docking scores. **Conclusions:** These novel and potential inhibitors constitute new drug candidates that must be biologically tested to define their value as an alternative chemotherapeutic agent in the treatment of these protozoan infections. *Salud UIS* 2013; 45 (1): 33-40

**Keywords:** leishmaniosis, virtual screening, therapeutics, molecular docking simulation, drug search.

**Evidence level:** III

#### RESUMEN

**Introducción y Objetivos:** Leishmaniosis, una enfermedad causada por un parásito protozoario, representa un serio problema de salud pública que amenaza a cerca de 350 millones de personas alrededor del mundo, de los cuales se cree que unos 12 millones se encuentran actualmente infectados (WHO 2010). A la fecha

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no existen vacunas contra las especies del parásito y el tratamiento está basado solo en la quimioterapia con medicamentos tóxicos, costosos, e ineficientes. Existe una necesidad urgente por mejores medicamentos contra *Leishmania*, el agente etiológico de la enfermedad. El principal medicamento en Colombia usado contra la leishmaniosis es el antimonio de meglumine [nombre químico según los parámetros de la International Union of Pure and Applied Chemistry (IUPAC): Hydroxy-dioxostiborane; (2R,3R,4R,5S)-6-methylaminohexane-1,2,3,4,5-pentol, (C<sub>7</sub>H<sub>17</sub>NO<sub>5</sub>)], el cual no es eficiente en el tratamiento de infecciones causadas por *Leishmania braziliensis*, la especie más prevalente en la costa Caribe de Colombia. **Métodos:** En este trabajo efectuamos un tamizaje virtual *in silico* de varias bases de datos incluyendo ChemBridge y Pubchem. Con el objetivo de identificar nuevos inhibidores, un total de 28.755 compuestos fueron tamizados virtualmente contra un modelo 3D de la enzima 6-phosphoglucono  $\gamma$ -lactonase (6-PGL) de *Leishmania braziliensis*. El acoplamiento molecular de las bases de datos se efectuó con el programa Sybyl 8.0 y AutoDock Vina. **Resultados:** mediante tamizaje virtual basado en la estructura se identificaron 10 compuestos, los cuales fueron posteriormente evaluados con AutoDockVina y clasificados de acuerdo a los puntajes de acoplamiento. **Conclusiones:** Estos nuevos potenciales inhibidores constituyen candidatos a medicamentos que deben ser evaluados biológicamente para definir su valor como alternativas quimioterapéuticas en el tratamiento de estas infecciones parasitarias. *Salud UIS* 2013; 45 (1): 33-40

**Palabras clave:** leishmaniosis, tamizaje virtual, terapéutico, biblioteca virtual, base de datos.

**Nivel de evidencia:** III

## INTRODUCTION

Leishmaniosis is a disease caused by protozoan parasites of the genus *Leishmania*<sup>1</sup>. It is considered by the World Health Organization (WHO) as a serious public health problem threatening about 350 million people in 88 countries around the world of which 12 million are believed to be currently infected<sup>2</sup>. Leishmaniosis has been classified as endemic in 98 countries or territories, including tropical and subtropical regions of Africa, Asia, Europe, North, Central and South America<sup>3</sup>. In Colombia, the disease is a growing problem, reported by the National Institute of Health with about 15 000 new cases in 2010<sup>4</sup>, and the appearing of new sources of transmission reflect an increase in the spread of *Leishmania (L.)* strains<sup>5</sup>.

There are no vaccines against these parasites and the approaches to control the vector are not effective. The main line of defense against the disease is chemotherapy which is not efficient against the different species of the parasite, and patients commonly suffer toxic side effects, including heart, kidney, liver and gastrointestinal failure; worsened by the long time drug administration and the increasing parasite resistance. In addition, Meglumine antimoniate [chemical name according to the International Union of Pure and Applied Chemistry (IUPAC): Hydroxy-dioxostiborane; (2R,3R,4R,5S)-6-methylaminohexane-1,2,3,4,5-pentol, (C<sub>7</sub>H<sub>17</sub>NO<sub>5</sub>)], the first line of treatment currently used in Colombia<sup>6</sup>, has led to progressive rise in treatment failure<sup>7-10</sup>. On the other hand, Miltefosine, a drug effective against infections caused by *L. panamensis*, is not effective against

infections produced by *L. braziliensis* (cure rates below 50%)<sup>11</sup>, which is the most prevalent specie reported in the Caribbean region of the country<sup>12</sup>. Thus, there is an urgent need for new drugs against this parasite. Despite this situation, leishmaniosis is still a neglected tropical disease (NTDs)<sup>13</sup> prevalent in developing countries and poor populations<sup>14</sup>. Therefore, implementation of rational and cost effective approaches to treat this health problem is required. In this regard, virtual screening, and in particular receptor-based virtual screening, has emerged as a reliable, inexpensive method for identifying leads<sup>15</sup>, and the *in silico* screening of chemical databases to search for new compounds against *Leishmania* would allow us to identify specific inhibitors of target molecules of the parasite.

The current study describes a rational scheme in the quest for new *Leishmania* targets, through the study of its biochemical pathways, and shows the potential of structure based virtual screening to identify novel compounds with inhibitory activity against essential target enzymes from the parasite using computer aided molecular docking of databases<sup>16,17</sup>.

## MATERIAL AND METHODS

### Selection of target proteins

To carry out virtual screening using molecular docking, we first selected the most appropriate target protein of the parasite, which met the following selection criteria: the putative target should be involved in a biochemical pathway essential for survival of the parasite cell; it

should have low homology to its counterpart in the human host; and it may have a suitable three dimensional model in Protein Data Bank (PDB).

Potential targets were identified through a systematic search of relevant scientific literature on *Leishmania* metabolism indexed in MEDLINE database and retrieved through PubMed search engine, using key words “*Leishmania* metabolism”, “*Leishmania* drug targets” and “*Leishmania* metabolic pathways”.

Homology of each selected protein to their human counterpart was determined with the PSI-BLAST program from the European Bioinformatics Institute (EMBL-EBI).

### Modeling the three dimensional structure of selected targets

A search for the three dimensional structure of each protein on Protein Data Bank (PDB) was performed. For proteins lacking a defined three-dimensional structure, experimental models were developed with integrated SYBYL8.0 Orchestra, and the servers I-TASSER<sup>18</sup>, EsyPred3d<sup>19</sup> and 3D-JigSaw<sup>20</sup>.

In order to select the best model, the 3D structure of each potential target was assessed by ProQ-Protein Quality Predictor Server, SWISS-MODEL and SAVES, a meta-server for analysis and verification of models.

### Selection of compound datasets

Diverse chemical datasets used in the study were obtained from ChemBridge Corporation portfolio and PubChem database, containing 20,000 and 8,755 compounds, respectively. Molecules from PubChem were selected based on their structural relationship with the natural ligands of selected protein targets from *Leishmania*. SDF files available online and containing two dimensional coordinates of the compounds were downloaded for each collection, and a Sybyl database was built.

### Protein and ligand preparation

The protein chosen in our study was the 6-phosphogluconolactonase (6-PGL) from *L. braziliensis*. The three dimensional structure of 6-PGL from *Leishmania braziliensis* (PDB ID: 3CH7) resolved by X-ray diffraction at 2.29 Å was downloaded from the Protein Data Bank. The protein was edited, converted to mol2 format and processed for docking procedure using the Sybyl docking interface. Water molecules

were removed before docking simulation, the protocol was built through the automatic mode and confirmed by alignment with the already determined active site of 6-PGL from *Trypanosoma brucei* (PDB ID: 3E7F). For the Vina based docking, 6-PGL was processed with the Autodock tools (ADT) software, water molecules were removed, polar hydrogen atoms added and Kollman charges assigned.

The ligand datasets in SDF format were converted to a Sybyl database in mol2 format and each compound was minimized by Sybyl energy minimizer using the Tripos force field and Gasteiger-Hückel charges. The maximum number of interactions was set to 100 and the minimization was terminated by the Powell method, when the energy gradient of 0.05 kcal/mol/Å was reached.

The analysis by AutodockVina required the conversion of the ligands and protein to the PDBQT format, which is the input format for AutoDockVina calculations.

### Structure Based Virtual screening

Virtual screening of 28,755 compounds against the 3D structure of 6-PGL was performed with the Surflex-Dock application coupled in Sybyl 8.0. The ligands were later classified according to the total score and the best docked compounds (Total Score > 8) were selected for testing using the AutoDockVina software. The selected ligands were docked to a rigid receptor limited by the coordinates of the grid, which was defined using the ADT application and the exhaustiveness of the docking was set to nine. Each compound was docked individually. The best dockings were manually inspected and their interactions with the receptor were described.

## RESULTS AND DISCUSSION

### Selection of target proteins

A rational approach to search and design new anti-leishmanial compounds must select a suitable target protein, which should be essential for the parasite survival and as different as possible from its human counterpart. A systematic review of the literature searching for potential target molecules involved in *Leishmania* metabolism identified five enzymes as potential targets for drugs. These biochemical targets are involved in glycolysis (phosphofructokinase and pyruvate kinase), the pentose phosphate pathway (6-phosphogluconolactonase), nucleotide metabolism (dihydrofolatereductase) and antioxidants (tryparedoxin peroxidase), all of them considered essential for the survival of *Leishmania*<sup>21,22</sup>.

Enzymes involved in glycolysis appear to be appropriate therapeutic targets because they are essential for the amastigote form of the parasite. In addition, there is a huge evolutionary distance between *Leishmania* and *Homo sapiens* that make the parasite proteins, involved in this pathway, structurally different from their mammalian equivalents<sup>23</sup>. The phosphofructokinase (PFK), an enzyme present in the amastigote form of *Leishmania*, participates in this biochemical pathway, catalyzing the phosphorylation of fructose-6-phosphate (F6P) to produce fructose 1,6-bisphosphate<sup>24</sup> and is structurally different from the human PFK, a feature useful for the designing of specific inhibitors of its biological function. On the other hand, pyruvate kinase (PyK) catalyzes the conversion of phosphoenol pyruvate (PEP) to pyruvate to produce ATP. This enzyme is allosterically regulated by fructose 2,6-bisphosphate, which induces the conversion to the active state of the protein<sup>22</sup>. Studies that compared the PyK crystal structure of the parasite with the PyK of other organisms have identified important differences between these enzymes at the level of its effector site revealing unique regulatory properties for the parasite enzyme, which could be a potential target for drugs<sup>25,26</sup>.

The trypanothione metabolism is another biochemical pathway with potential targets; the enzyme trypanothione peroxidase (TryP) is a key molecule in the defense mechanism against oxidative stress<sup>27</sup>. This enzyme is responsible for the removal of reactive oxygen intermediates such as superoxide anion (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), peroxynitrite (ONOO<sup>-</sup>) and hydroxyl radical (OH<sup>-</sup>) produced during

the process of cellular respiration. Furthermore, studies with mutants of *L. donovani* and *L. major* lacking a functional trypanothione reductase have established that this enzyme is essential for parasite survival<sup>28</sup>. Another important enzyme that protects *Leishmania* from toxic radicals is the 6-Phosphogluconolactonase from the pentose phosphate pathway. It converts 6-phosphogluconolactone to 6-phosphogluconate maintaining a source of NADPH, which serves to protect against oxidative stress and generates carbohydrate intermediates used in nucleotide and other biosynthetic pathways. Furthermore, the loss of this enzyme has been shown to be toxic for the parasite, making it a suitable target for chemotherapy<sup>29</sup>.

### Modeling the three dimensional structure of selected targets

The only enzyme reported in the PDB with a three-dimensional structure defined by X-ray diffraction was the 6-Phosphogluconolactonase. For the other enzymes, it was necessary to build their three-dimensional models from the amino acid sequence of each protein through different modeling programs (I-TASSER, 3D-JigSaw and Orchestra, included in Sybyl 8.0). After evaluation of each model by the meta server SAVS (Structural Analysis and Verification Server), the most suitable model was selected for docking analysis. Considering the quality of the three-dimensional model retrieved from the PDB and the three basic characteristics that a target must satisfy (**Table 1**), the protein and model chosen was the 6-PGL from *L. braziliensis*.

**Table 1.** Evaluation of the protein target models through multiple servers.

Target	Source: L. braziliensis (Method)	Meta server Evaluation			Identity with human homologue
		Pro-Q*	PROCHECK†	Verify_3D‡	
Pyruvate kinase (PyrK)	PREDICTION ESYMPRED	5.521	86.8% core 12.2% allow 0.2% disall	95.10% Pass	48.5%
6-phosphoglucono lactonase (6-PGL)	X-RAY DIFFRACTION	7.616	93.2% core 6.4% allow 0.0% disall	96.25% Pass	30%
Phosphofructokinase (PFK)	PREDICTION ESYMPRED	6.144	94.6% core 4.9% allow 0.3% disall	83.48% Pass	33.6%
Trypanothione peroxidase (TRYP1)	PREDICTION ESYMPRED	4.542	94.6% core 5.4% allow 0.0% disall	88.27% Pass	59.9%
Dihydrofolate Reductase-Thymidylate synthase (DHFR-TS)	PREDICTION ESYMPRED	5.887	73.0% core 23.0% allow 0.9% disall	79.89% Fail	60.6%

\* ProQLGscore: an score >1.5 is a fairly good model; >2.5, very good model; >4, extremely good model<sup>30</sup>.

† Procheck description of the Ramachandran Plot for each model, where “core” means the percentage of residues in the most favored region, “allow” is the percentage of residues in additional allowed regions and “disall” represent residues in disallowed regions.

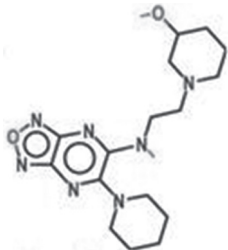
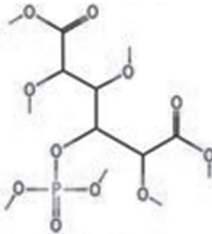
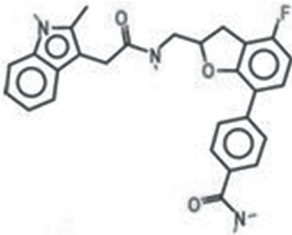
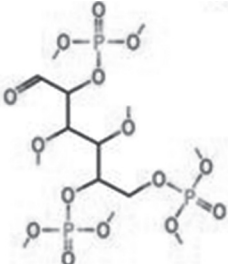
‡ Verify 3D score shows the percentage of residues having an averaged 3D-1D score > 0.2<sup>31,32</sup>

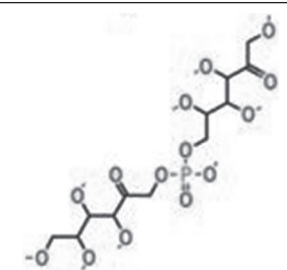
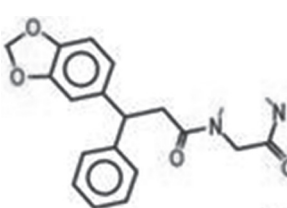
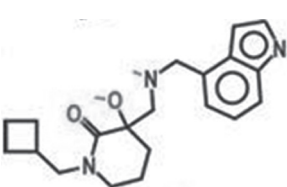
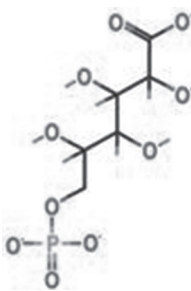
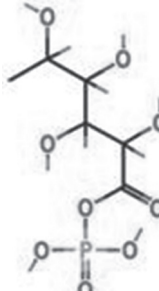
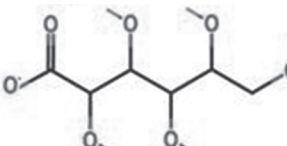
### Virtual Screening

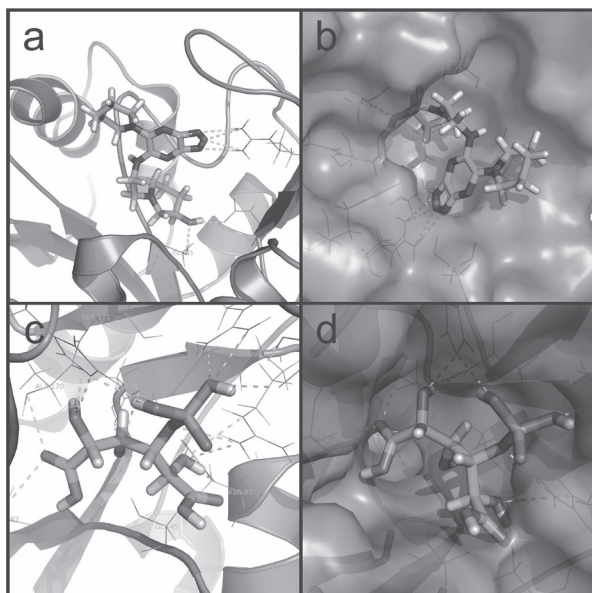
Structure-based virtual screening was carried out to predict the binding affinity of 28,755 molecules from a diverse chemical library, in order to identify novel drug-like inhibitors of 6-PGL from *Leishmania*. This method involved computational docking of ligands into an active site of a receptor using complex algorithms, followed by scoring and ranking of these compounds to identify potential leads. Compared to ligand- or pharmacophore- based method, the receptor-based method of screening is a more flexible approach that allows the identification of structurally novel ligands that may have similar interactions to those of known ligands, or may have different interactions with other parts of the binding site<sup>33</sup>.

The first screening, performed with Sybyl 8.0 program, provided a set of 11 candidate molecules, which bound to the receptor with the higher affinities (Total Score > 8) (**Table 2**). These ligands were later docked to the 6-PGL through the AutodockVina program to validate the binding affinities and to sort them according to their docking scores (**Table 2**). Compounds listed in this table could be considered as potential leads on the light of virtual docking, but should be tested *in vitro* or *in vivo* assays to confirm their real potential as anti-leishmanial drugs. **Figure 1** shows the molecular docking of compound L1 with the active site of 6-PGL from *Leishmania*; the ligand is maintained in the active site by a set of intermolecular forces that include polar interactions and hydrogen bonds.

**Table 2.** Compounds ranked according to Sybyl and AutoDock Vina Scores.

Compound	Sybyl 8.0 Total Score	AutoDock Vina Score (kcal/mol)	Structure
L1	8.33	-7.8	
L2	9.05	-7.3	
L3	8.02	-7.2	
L4	10.17	-7.1	

Compound	Sybyl 8.0 Total Score	AutoDock Vina Score (kcal/mol)	Structure
L5	10.44	-7.0	
L6	11.32	-7.0	
L7	8.12	-6.8	
L8	10.02	-6.6	
L9	8.11	-6.5	
L10	9.79	-6.5	



**Figure 1.** Docking-predicted poses and interactions between compounds L1 (upper panels) and L2 (bottom panels) with the active site of the 6-PGL protein from *L. braziliensis*. Polar interactions and hydrogen bonds are shown as yellow dotted lines; ligands are represented in stick and residues of the active site as lines labeled in orange with the 3 letters amino acid code. The remaining of the protein is shown as cartoon representation (left panels) and the protein surface is shown in orange (right panels).

## CONCLUSIONS

Structure-based virtual screening allowed us to identify a set of potential anti-leishmanial compounds through a computer-aided drug discovery approach; we screened *in silico* a diverse and large library of 28755 compounds against 6-PGL protein, a vital enzyme for *Leishmania* survival. Ligands bounded with higher affinity to the receptor protein were subjected to docking validation and ranked through the AutodockVina docking score function.

We consider that compounds identified represent candidates for testing as potential therapeutic agents against leishmaniasis. However, the real leishmanicidal activity of these compounds should be biologically evaluated before they can be proposed as anti-leishmanial drugs. Nevertheless, by this computer-aided screening we have identified from a large chemical database, the leads with the higher likelihood of binding to the target protein to elicit an inhibitory activity, which would reduce the cost of biological testing.

## ETHICAL CONSIDERATIONS

The authors declare that this manuscript was developed within the current regulations

## CONFLICTS OF INTEREST

The authors declare that no conflict of interest exists in the development of this research

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