RESEARCH ARTICLE

REVISED Computer-aided drug design approaches applied to screen natural product's structural analogs targeting arginase in Leishmania spp [version 3; peer review: 1 approved, 2 approved with reservations]

Haruna Luz Barazorda-Ccahuana[1], Luis Daniel Goyzueta-Mamani[1,2], Mayron Antonio Candia Puma[1,3], Camila Simões de Freitas[4], Grasiele de Sousa Vieria Tavares[4], Daniela Pagliara Lage[4], Eduardo Antonio Ferraz Coelho[4,5], Miguel Angel Chávez-Fumagalli[1]

[1]Computational Biology and Chemistry Research Group, Vicerrectorado de Investigación, Universidad Catolica de Santa Maria de Arequipa, Arequipa, Peru
[2]Sustainable Innovative Biomaterials Department, Le Qara Research Center, Arequipa, Peru
[3]Universidad Católica de Santa María, Facultad de Ciencias Farmacéuticas, Bioquímicas y Biotecnológicas, Arequipa, Peru
[4]Universidade Federal de Minas Gerais, Programa de Pós-Graduação em Ciências da Saúde: Infectologia e Medicina Tropical, Faculdade de Medicina, Belo Horizonte, Minas Gerais, Brazil
[5]Universidade Federal de Minas Gerais, Departamento de Patologia Clínica, COLTEC, Belo Horizonte, Minas Gerais, Brazil

v3 **First published:** 24 Jan 2023, **12**:93
https://doi.org/10.12688/f1000research.129943.1
Second version: 24 May 2023, **12**:93
https://doi.org/10.12688/f1000research.129943.2
Latest published: 13 Jul 2023, **12**:93
https://doi.org/10.12688/f1000research.129943.3

Abstract

Introduction: Leishmaniasis is a disease with high mortality rates and approximately 1.5 million new cases each year. Despite the new approaches and advances to fight the disease, there are no effective therapies.

Methods: Hence, this study aims to screen for natural products' structural analogs as new drug candidates against leishmaniasis. We applied Computer-aided drug design (CADD) approaches, such as virtual screening, molecular docking, molecular dynamics simulation, molecular mechanics–generalized Born surface area (MM–GBSA) binding free estimation, and free energy perturbation (FEP) aiming to select structural analogs from natural products that have shown anti-leishmanial and anti-arginase activities and that could bind selectively against the *Leishmania* arginase enzyme.

Results: The compounds 2H-1-benzopyran, 3,4-dihydro-2-(2-methylphenyl)-(9CI), echioidinin, and malvidin showed good results against arginase targets from three parasite species and negative results for potential toxicities. The echioidinin and malvidin ligands

Open Peer Review

Approval Status ✓ ? ?

	1	2	3
version 3 (revision) 13 Jul 2023			
version 2 (revision) 24 May 2023	✓ view	? view	? view
version 1 24 Jan 2023	? view		

1. **Francisco Centeno**, Universidad de Extremadura, Badajoz, Spain

2. **Anupam Nath Jha**, Tezpur Central University, Tezpur, India

generated interactions in the active center at pH 2.0 conditions by MM-GBSA and FEP methods.
Conclusions: This work suggests the potential anti-leishmanial activity of the compounds and thus can be further *in vitro* and *in vivo* experimentally validated.

Keywords
leishmaniasis, Leishmania arginase, computer-aided drug design, molecular dynamics simulation, antiprotozoal agents; drug discovery

3. **Rubens L. Monte-Neto**, Fundação Oswaldo Cruz (FIOCRUZ), Belo Horizonte, Brazil
Alessandra Sousa, Faculdades Oswaldo Cruz, São Paulo, Brazil

Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the Cheminformatics gateway.

Corresponding author: Miguel Angel Chávez-Fumagalli (mchavezf@ucsm.edu.pe)

Author roles: Barazorda-Ccahuana HL: Conceptualization, Funding Acquisition, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Goyzueta-Mamani LD**: Supervision, Writing – Review & Editing; **Candia Puma MA**: Conceptualization, Supervision, Writing – Review & Editing; **Simões de Freitas C**: Conceptualization, Supervision, Writing – Review & Editing; **de Sousa Vieria Tavares G**: Conceptualization, Supervision, Writing – Review & Editing; **Pagliara Lage D**: Conceptualization, Supervision, Writing – Review & Editing; **Ferraz Coelho EA**: Conceptualization, Supervision, Writing – Review & Editing; **Chávez-Fumagalli MA**: Conceptualization, Funding Acquisition, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This research was funded by Universidad Católica de Santa María (grants 7309-CU-2020, 24150-R-2017, 27574-R-2020, and 28048-R-2021).
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Barazorda-Ccahuana HL, Goyzueta-Mamani LD, Candia Puma MA *et al.* **Computer-aided drug design approaches applied to screen natural product's structural analogs targeting arginase in Leishmania spp [version 3; peer review: 1 approved, 2 approved with reservations]** F1000Research 2023, **12**:93 https://doi.org/10.12688/f1000research.129943.3

First published: 24 Jan 2023, **12**:93 https://doi.org/10.12688/f1000research.129943.1

REVISED **Amendments from Version 2**

We have modified the current version of the manuscript attending the 2nd reviewer's suggestions. We have added information regarding the identity of the parasite and human targets at the results section.

Any further responses from the reviewers can be found at the end of the article

Introduction

Leishmaniasis is an ancient disease that has been described in archaic ceramics, statues, and writings, and in molecular findings from mummified human bodies and archaeological material.[1] The disease causes high morbidity and mortality worldwide, where about one billion people are at risk of infection across 98 countries, with over 1.5 million new cases and 20,000-40,000 deaths reported each year.[2,3] The increase in leishmaniasis incidence and prevalence is mainly attributed to several risk factors that are man-propelled,[4] whereas, in many regions, the transmission pattern shows expansion, with new territories affected by the disease.[5,6] Also, leishmaniasis has gained greater importance in HIV-infected patients as an opportunistic infection in areas where both pathogens are endemic.[7] Leishmaniasis is caused by the protozoan parasites of the genus *Leishmania* (Kinetoplastida: *Trypanosomatidae*), which has a digenetic life cycle that alternates between the midgut of sandflies and the phagolysosomes of mammalian macrophages.[8] When exposed to extreme environmental changes, such as low pH, the parasites respond to the acidification of their environment by changing the pattern of expression of several proteins.[9,10] About 21 parasite species can infect mammals and many of them cause human disease[11] and the clinical manifestations depend on both the parasite species and the hosts' immune response,[12] varying from a chronic, slow-to-heal disease known as tegumentary leishmaniasis (TL), to a potentially fatal form of the disease, namely, visceral leishmaniasis (VL), in which parasites disseminate to internal organs, such as the liver, spleen, and bone marrow.[13]

Despite significant progress, the development of a human vaccine remains hampered by significant gaps in the development pipeline[14]; and the treatment against disease has used drugs that cause side effects in the patients, such as myalgia, arthralgia, anorexia, fever, and urticaria, as well as toxicity in the liver, kidneys, and spleen.[15] Therefore, the necessity for cost-effective treatment which promotes the cure completely, with few side effects, low relapse rates, high effectiveness, and a reduction of toxicity remains.[16] The number of drugs derived from natural products (NPs) present in the total amount of drug launchings in the market over four decades represents a significant source of new pharmacological entities,[17] while a series of secondary plant-purified products has already been described with leishmanicidal potential.[18–21] Likewise, computer-aided drug design (CADD) can be defined as computational approaches that are used to discover, develop, and analyze drug and active molecules with similar biochemical properties,[22] and this has become crucial for screening of potential metabolite databases from natural sources that can be repurposed against diseases for faster, safer, and cheaper drug development.[23,24] The strategy of target-based drug discovery is used extensively by the pharmaceutical industry and has been applied to leishmaniasis.[25,26] However, *in silico* methods to identify new potential drugs to be applied against leishmaniasis present limitations, such as the dependency on the quality, accuracy, and completeness of the information present in databases.[27] The arginase (ARG) enzyme has recently obtained considerable attention since new studies have highlighted it as a potential therapeutic target in leishmaniasis.[28] ARG is the first enzyme of the polyamine pathway and catalyzes the conversion of L-arginine to L-ornithine and urea, down-regulating the polyamine pathway, affecting the parasite growth and infectivity.[29] The inhibition results in a lack of protection against reactive oxygen species (ROS), which damages *Leishmania*'s genetic material and ultimately leads it to die by apoptosis.[30] As a result, various NPs have demonstrated anti-arginase action,[31,32] and the majority of these NPs have also demonstrated a strong affinity against human ARG.[33] In the current study, we used CADD techniques, such as virtual screening, molecular docking, and molecular dynamics simulations, to identify structural analogs of NPs that have demonstrated anti-leishmanial and anti-ARG activities and that may bind specifically to the *Leishmania* ARG. Our goal was to identify a promising compound candidate that could be used in the treatment of leishmaniasis.

Methods

Data collection

The search for natural products with anti-leishmanial and anti-ARG activities was performed at the Nuclei of Bioassays, Ecophysiology, and Biosynthesis of Natural Products Database (NuBBEDB) online web server (version 2017) (http://nubbe.iq.unesp.br/portal/nubbe-search.html, accessed on 23 January 2022), which contains the information of more than 2,000 natural products and derivatives[34]; while the "anti-leishmanial property" was selected in the biological properties segment of the web server. The bibliographic data extraction, regarding the compounds found in NuBBEDB, was performed from the National Center for Biotechnology Information (NCBI) databases (https://www.ncbi.nlm.nih.gov/pubmed/, accessed on 07 February 2022); and the simplified molecular-input line-entry system (SMILES) was searched and retrieved from PubChem server (https://pubchem.ncbi.nlm.nih.gov/, accessed on 10 February 2022).[35] Likewise, the

physicochemical properties: total molecular weight (MW), octanol/water partition coefficient (iLOGP), number of H-bond acceptors (HBAs), number of H-bond donors (HBDs), and the topological polar surface area (TPSA), for each compound were calculated within the Osiris DataWarrior v5.2.01 software[36]; and, the rotatable bonds (RB); number of heavy atoms (NHA); and synthetic accessibility (SynAcce) were calculated within SwissADME server (http://www.swissadme.ch/index.php, accessed on 15 February 2022).[37]

Structural analogs search and virtual screening

The SMILES from the compounds were used for high throughput screening to investigate structural analogs by the SwissSimilarity server (http://www.swisssimilarity.ch/index.php, accessed on 01 March 2022)[38]; whereas the commercial class of compounds was selected and the Zinc-drug like compound library, which comprises 9'205'113 molecules, with the combined screening method, was chosen for the high throughput screening to achieve the best structural analogs. The zinc-drug like compound library selection allowed the screening of compounds in the subsequent commercially available chemical libraries: Enamine, ChemBridge, Maybridge, Asinex, AsisChem, Otava, SPECS, TimTec, Vitas, Life Chemicals, ChemDiv, and Innovapharm.[39] Threshold values for positivity were selected by default parameters. Also, the FASTA sequences of the ARG sequences from *L. infantum* (A4IB49), *L. mexicana* (Q6TUJ5), *L. braziliensis* (A4HMH0), and *Homo sapiens* (P05089) were retrieved from UniProt database (http://www.uniprot.org/, accessed on 03 March 2022) (RRID:SCR_002380), and subjected to automated modeling in SWISS-MODEL[40] (RRID:SCR_018123), whereas the best model was selected based on the GQME and QMEAN4 scores.

Furthermore, the compounds were imported into Open Babel (RRID:SCR_014920) within the Python Prescription Virtual Screening Tool[41] and subjected to energy minimization. PyRx (RRID:SCR_018548) performs structure-based virtual screening applying molecular docking simulations using the AutoDock Vina tool[42] (RRID:SC_011958), whereas the drug targets were uploaded as macromolecules. For the analysis, the search space encompassed the whole of the modeled 3D models, and the molecular docking simulation was then run at an exhaustiveness of 8 and set to only output the lowest energy pose. The Osiris Data Warrior software was employed to calculate the potential tumorigenic, mutagenic, and reproductive effects, and irritant action of selected compounds predicted by comparison with a precompiled fragment library derived from the Registry of Toxic Effects of Chemical Substances (RTECS) database.[36]

Molecular dynamics simulation

Ligands preparation was based on the results from the virtual screening analysis; while the geometry optimization of these compounds was made in the Avogadro v. 1.2.0 program[43] (RRID:SCR_015983) and the ACPYPE (AnteChamber PYthon Parser interfacE)[44] server was employed to generate the topologies and parameters for molecular dynamics (MD) simulation. We determined the 3D structural conformation of *L. infantum* ARG by homology modeling with *L. mexicana* ARG (PDB ID: 4ITY) as a template in the SWISS-MODEL online server[40] and afterwards we determined the protonation/deprotonation states at pH 2.0 and pH 7.0 in the PDB2PQR.[45] Since ARG is a trimeric metalloprotein with three active sites binding to two manganese atoms (Mn^{+2}), we fixed the Mn^{+2} coordination with active site residues and a hydroxyl molecule (OH^{-1}), considering the following coordination: first Mn^{+2} with His114 (ND1), ASP137 (OD2), ASP141 (OD2), ASP243 (OD2) and the second Mn^{+2} with ASP137 (OD1), HIS139 (ND1), ASP243 (OD1) and ASP245 (OD2). The MD simulation was reproduced in GROMACS v. 2020[46] (RRID:SCR_014565), considering the AMBER99[47] force field. The systems were solvated with the TIP3P water model, and Na^{+1} or Cl^{-1} ions were added for neutralization. The box size was $12\times12\times12$ nm. Thus, the energy minimization was performed with the steep-descent algorithm with 20000 steps of calculation. The MD simulation was done in two steps; the first step was in the canonical ensemble (NVT) considering distance restraint of Mn^{+2} to the active site by 5 ns. The second step was the MD production in the isothermal-isobaric ensemble (NPT) with a time of 100 ns. The V-rescale[48] thermostat was used to regulate the temperature at 309.65 K and the Parrinello-Rahman barostat at a reference pressure of 1 bar. Molecular docking was done with the DockThor online server[49]; in the last frame, the molecular docking at two pH conditions was used as a receptor. A grid was considered in the active site of ARG (ChainA). The complex models with the best scores were chosen, and these were subsequently simulated in the isothermal-isobaric ensemble NPT for 100 ns. Gibbs free energy was calculated by the molecular mechanics-generalized Born surface area (MM-GBSA)[50] method in gmx_MMPBSA tool based on AMBER's MMPBSA.py, and AmberTools20[51] (RRID:SCR_014565) package was used. Additionally, to compare the binding free energy studies, we include the free energy perturbation (FEP) analysis where the Bennett acceptance ratio (BAR) calculates the free energy differences.[52] This analysis is achieved with the free energy implementation by the GROMACS tool.

Statistical analysis

Results were entered into Microsoft Excel (version 10.0, Microsoft Corporation, Redmond, WA, USA) spreadsheets and analyzed by GraphPad Prism version 9.4.0 for Windows, GraphPad Software, San Diego, California USA, (http://www.graphpad.com) (RRID:SCR_002798). To evaluate the correlation between the binding affinities of the compounds

against the protein targets, they were placed in a linear regression plot and analyzed by Pearson's correlation coefficient; differences were considered significant when $p<0.05$. Further, the selectivity score of binding affinities was calculated as described[53]; where a selectivity value >1 indicates a priority of the compounds to bind to the parasite ARG over the human target. Heatmaps were constructed in the R programming environment (version 4.0.3) using the "heatmap 2" function in the package "gplots".[54]

Results

Data collection and virtual screening

In this work, a search was performed in the NuBBEDB for NPs that had been described with anti-leishmanial and anti-ARG activities. The search in the database resulted in 33 NPs described with anti-leishmanial activity, whereas six of them had also been described as inhibitors of ARG activity. Startlingly, all the NPs selected were described in the same article, in which the compounds were isolated from *Byrsonima coccolobifolia* species and tested for *in vitro* anti-ARG activity.[55] Since no anti-leishmanial activity was reported in the article, a cross-reference search for each compound was performed in the PubMed database to validate the properties. Thereafter, the SMILES from quercetin (NuBBE_122), isoquercetin (NuBBE_123), quercitrin (NuBBE_161), (+)-syringaresinol (NuBBE_214), catechin (NuBBE_287) and (-)-epicatechin (NuBBE_866) were obtained from PubChem and submitted to physicochemical properties analysis related to an absorption, distribution, metabolism, and excretion (ADME) profile; Lipinski's rule of five (MW, iLOGP, HBAs and HBDs),[56] the quantitative estimate of drug-likeness (TPSA, RB, NHA and the number of alerts for undesirable substructures)[57] and the synthetic accessibility,[58] of the NPs are shown in Table 1.

To find structural analogs to the six NPs selected, a search of the SwissSimilarity server employing the commercial zinc-drug like compound library was performed, resulting in 400 analogs for each NP; however, the search comprised a high degree of redundancy between the analogs and a step in which duplicated compounds were excluded was executed, resulting in a total of 1499 unique compounds selected for virtual screening (Figure 1). The virtual screening results against *Leishmania infantum* and human ARG, which shown a 44% of sequence identity, are plotted in Figure 1A, where a positive linear relationship between the binding affinities of the compounds toward both targets is shown [Pearson r:0.931; r2:0.868]. Later, aiming to select compounds that showed higher affinity toward *L. infantum* ARG, the selectivity was calculated, and compounds with scores >1 were screened, resulting in 25 compounds selected (Figure 1A). Since *in vitro* evidence of inter-species differences in the susceptibility of parasites to anti-leishmanial drugs has been reported,[59] putative drug candidates must be active against several species of the parasite[60]; in this way, the selectivity of the compounds against *L. mexicana* and *L. braziliensis* ARG were also calculated and plotted in a heatmap; each compound's results showed differences in their affinities profile (Figure 1B). Also, to select potential nontoxic candidates, the tumorigenic, mutagenic and reproductive effects, as well as irritant action were assessed for the 25 compounds (Figure 1C). Thus, the compounds 2H-1-benzopyran, 3,4-dihydro-2-(2-methylphenyl)- (9CI) (ZINC39120134) (Figure 1D), echioidinin (ZINC14807307) (Figure 1E), and malvidin (ZINC897714) (Figure 1F) were selected for further analysis, since they showed favorable binding affinities against the three parasite species targets and negative results for potential toxicities.

Molecular dynamics simulations (MDS)

L. infantum ARG is an enzyme with trimeric conformation (ChainA, ChainB, and ChainC) and its structure showed stable behavior during a 100 ns of MDS performed at pH 2.0 and pH 7.0 (Figure 2). Here we included the metal ions (Mn^{+2}) and one hydroxyl molecule (OH^{-1}) for each active site, and it was observed that some regions lose their structural conformation at pH 2.0 conditions (green color). In addition, compared to ARG at pH 7.0, ARG at pH 2.0 exhibits large structural alterations and high variations per residue (see Figure 3A and 3B). In Figure 3C, the radius of gyration shows lower compaction of whole protein during the MDS at pH 7.0 than at pH 2.0. The report of the trajectory of each

Table 1. Natural compounds description selected in the NuBBE database.

NuBBE ID	PubChemID	Name	MW	iLOGP	TPSA	HBA	PAINS	Brenk	SynAcce
NuBBE_122	5280343	Quercetin	302.240	1.630	131.36	7	1	1	3.230
NuBBE_123	5378597	Isoquercetin	464.380	2.110	210.51	12	1	1	5.320
NuBBE_161	5353915	Quercitrin	448.380	1.270	190.28	11	1	1	5.280
NuBBE_214	100067	(+)-Syringaresinol	418.440	3.520	95.84	8	0	0	4.360
NuBBE_287	1203	Catechin	290.270	1.470	110.38	6	1	1	3.500
NuBBE_866	72276	(-)-epicatechin	290.270	1.470	110.38	6	1	1	3.500

MW: Molecular weight; iLOGP: octanol/water partition coefficient; TPSA: topological polar surface area; HBA: number of H-bond acceptors; HBD: Number of H-bond Donors; RB: rotatable bonds; NHA: number of heavy atoms; SynAcce: synthetic accessibility.

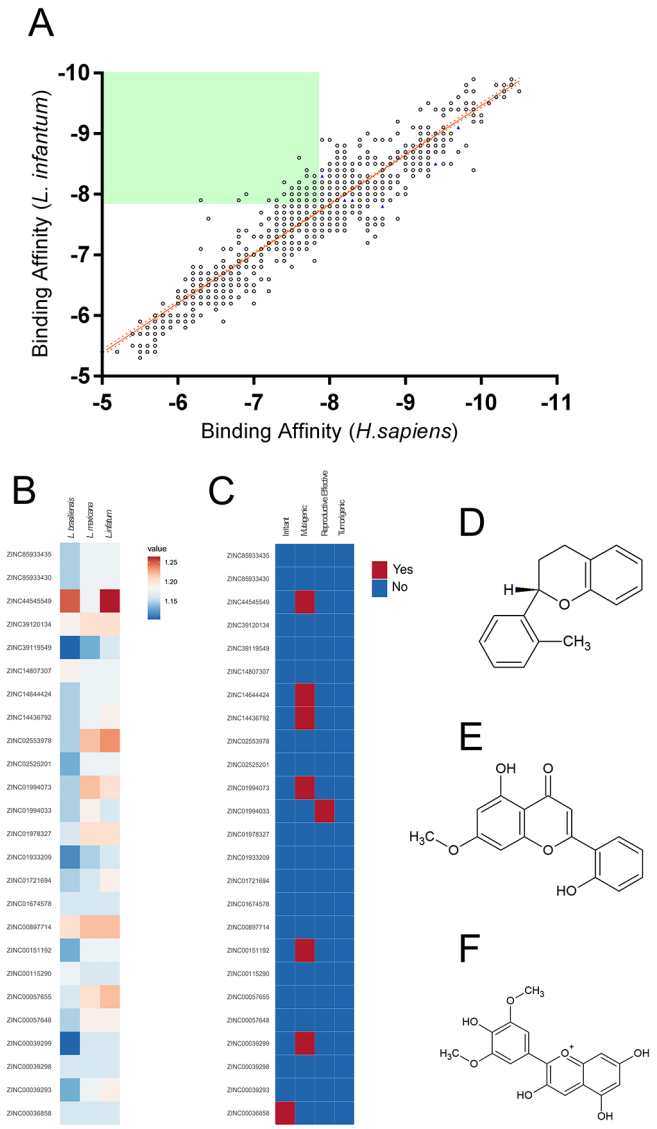

Figure 1. Virtual screening of the compounds selected from the NuBBE database. Binding affinities toward *L. infatum* and *H. sapiens* ARG targets were analyzed by linear regression and Pearson's correlation coefficient. Solid orange line: linear regression; dotted orange lines: 95% confidence intervals. The solid green square was calculated using the maximum binding affinities of the 6 NPs (A). Normalized binding affinities heatmap of 25 selected compounds on *L. infantum*, *L. mexicana*, and *L. braziliensis* against their human homolog (B). Binary heatmap showing positive (red) or negative (blue) predicted toxicities (C). Chemical structure of ZINC39120134 (D), ZINC14807307 (E), and ZINC897714 (F).

complex system (enzyme-ligand) and the protein without ligand is shown in Figure 4. Since the root-mean-squared deviation (RMSD) is a noteworthy analysis to verify the similarity between a protein-bound and not bound ligand.[61] The RMSD values in nm are presented that were taken from the ChainA of each protein in different pH conditions, whereas the enzyme-ligand systems presented greater conformational changes in the substrate-binding site (Figure 4A). Likewise, radius of gyration (RG) analysis verifies the compactness of protein structures, where the lowest RG demonstrates the tightest packing and high conformational stability.[62] The results showed that, at pH 2.0, low compactness and a large broadening of the macromolecules are reported (Figure 4B). Figure 4C shows the root-mean-squared fluctuation (RMSF) *per residue* of the backbone, where high fluctuations were shown from residue 50 to 100 in both systems. From the enzyme-ligand simulation results, we take each simulation's last frames (Figure 5). The compounds ZINC14807307 and ZINC897714 generate exciting interactions in the active center at the pH conditions evaluated and, at pH 7.0, hydrogen bonds are observed, which benefits enzyme-ligand coupling.

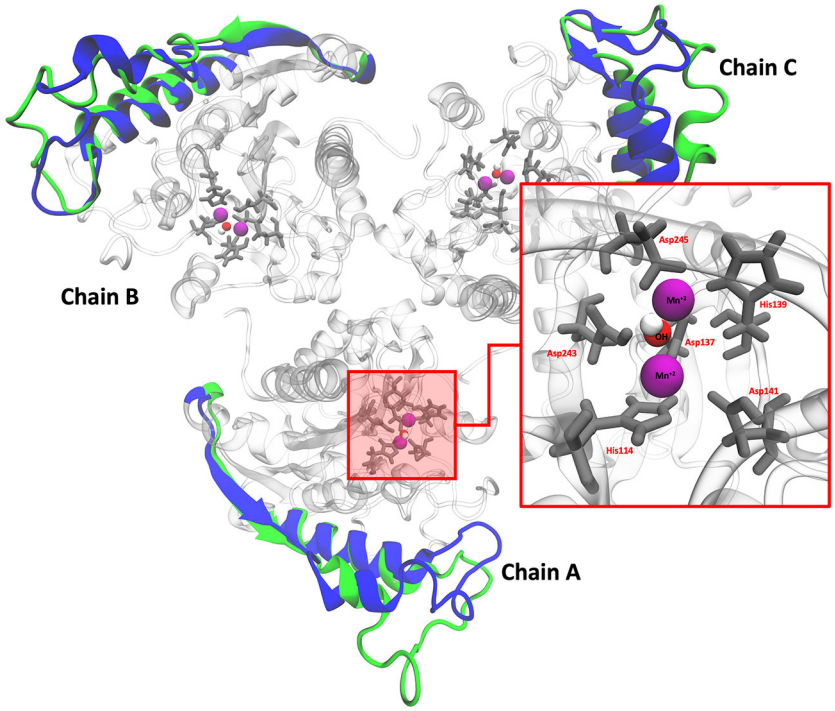

Figure 2. Structural conformation of ARG with its active site. Colors blue and green represent the cartoon representation of pH 2.0 and pH 7.0. The red box shows the active site of ARG.

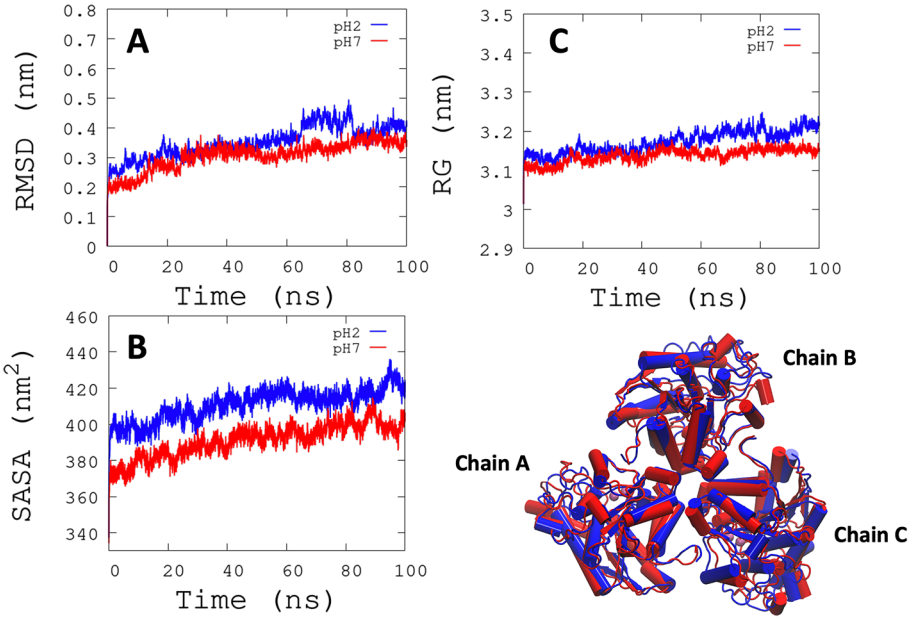

Figure 3. RMSD, SASA, and RG analysis. (A) RMSD is shown the conformational changes reported at pH 2.0. (B) SASA shows a greater solvent access surface area to ARG at pH 2.0 than at pH 7.0. (C) RG shows the same behavior as RMSD.

Binding free energy estimation

The binding free energy analysis of pH 2.0 and pH 7.0 from the frames of each simulation is shown in Table 2. The propitious energetic contribution with a binding free energy of -28.59 kcal/mol (ZINC897714/pH2) maximum and -14.07 kcal/mol (ZINC14807307/pH2) minimum were obtained. The estimated phase-gas binding free energy (ΔGgas)

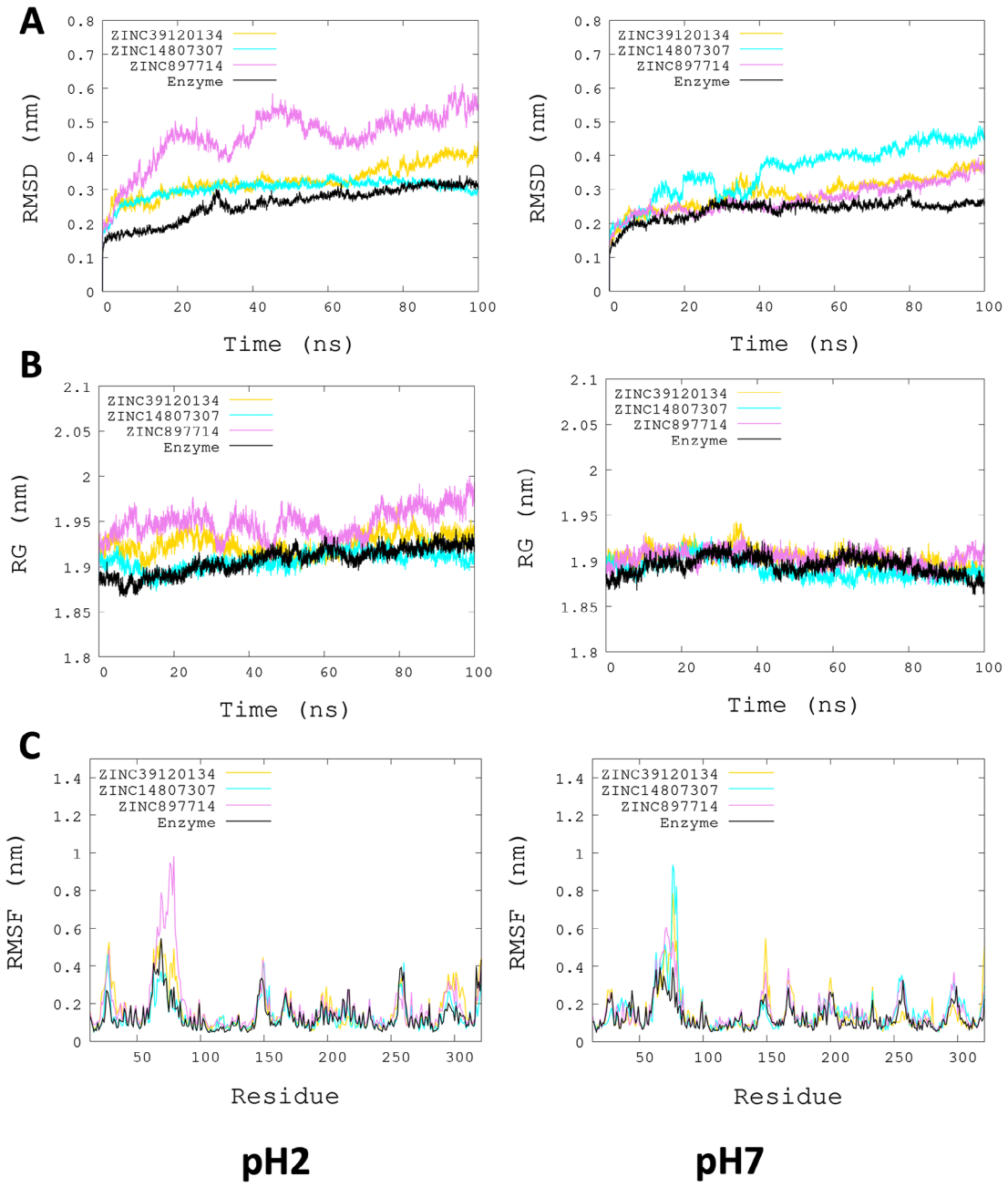

Figure 4. Plots of MD simulation of each complex. More significant conformational changes of ARG enzyme are shown at pH 2.0. (A) RMSD plot of ChainA concerning the whole protein. (B) RG analysis. (C) RMSF *per residue* of backbone.

provided the highest energy contributions for ZINC897714 in both pHs. Contrary, the van der Waals energies (ΔEvdW) provided the highest energy contributions at pH 2.0 in ZINC897714.

It is well understood that hydrophobic interactions favorably contribute to binding. The electrostatic energies (ΔEele) contributed positively to the binding enzyme-ligand, which the best energy was -24.76 kcal/mol (ZINC897714/pH2). Despite this, the solvation energies (ΔGsolv) offset the negative electrostatic interactions, thus unfavorably contributing to the binding of ZINC897714 to ARG in both pHs (ZINC897714/pH2 = 29.64 kcal/mol and ZINC897714/pH7 = 22.18 kcal/mol). These results show that the protonation states at a given pH can positively or negatively favor the enzyme-ligand binding, where it is expected that at a pH above 7.0 the enzyme-ligand binding can be increased.

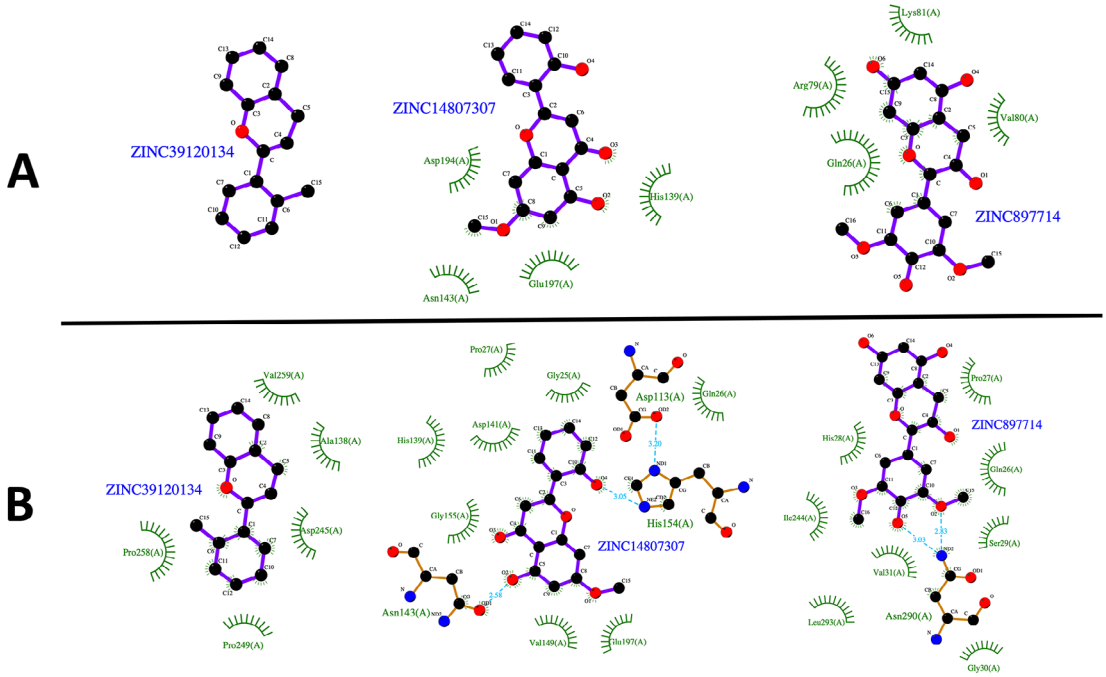

Figure 5. 2D representation of the last frame of each complex. (A) Last frame at pH 2.0. and (B) Last frame at pH 7.0. Green represents the hydrophobic interaction between enzyme-ligand, color sky blue represents the hydrogen bond interaction.

Table 2. MM-GBSA binding free energy estimation average values.

Energy component	ZINC39120134		ZINC14807307		ZINC897714	
	pH2	pH7	pH2	pH7	pH2	pH7
ΔEvdW	-20.22	-23.65	-20.41	-23.62	-33.46	-32.06
ΔEele	-2.08	-3.93	-4.41	-5.24	-24.76	-18.38
ΔEgb	10.37	12.63	13.26	14.98	33.90	26.00
ΔEsurf	-2.62	-3.02	-2.51	-3.15	-4.26	-3.83
ΔGgas	-22.30	-27.58	-24.83	-28.86	-58.22	-50.44
ΔGsolv	7.75	9.62	10.75	11.02	29.64	22.18
ΔGTotal	-14.55	-17.96	-14.07	-17.04	-28.59	-28.26

ΔEvdW = Van Der Waals energy; ΔEele = electrostatic energy; ΔEgb = electrostatic contributionfree energy calculated by generalized Born; ΔGgas = estimated phase-gas binding free energy; ΔGsolv = estimates binding free energy solvent; ΔG TOTAL = estimated binding free energy. Values of energy in kcal/mol.

In an attempt to improve the enzyme-ligand binding energy analysis, the FEP approach was used, which estimates the difference in free energy between two states (*A* state and *B* state) by slowly change from one state to another. *A* state corresponds to the initial state of free energy and *B* state corresponds to the final state. This study sampled 20 microstates with a time of 20 ns for each microstate; the results are presented in Table 3. Herein, it is observed that, at both pHs,

Table 3. FEP and MM-GBSA average values of ΔG TOTAL in kcal/mol.

Compound	FEP $_{pH2}$	FEP $_{pH7}$	MM-GBSA $_{pH2}$	MM-GBSA $_{pH7}$
ZINC39120134	3.38	4.77	-14.55	-17.96
ZINC14807307	-6.27	-4.08	-14.07	-17.04
ZINC897714	-2.80	-0.29	–28.59	-28.26

the best compounds occurred in the following order: ZINC14807307 > ZINC897714 > ZINC39120134. On the other hand, the compounds ZINC14807307 and ZINC897714 are shown to be stable at pH 2.0 conditions.

Discussion

The World Health Organization (WHO) considers leishmaniasis to be one of the major neglected global diseases and responsible for millions of disability-adjusted life years (DALYs), representing one of the top burdens among the neglected tropical diseases.[63] Worldwide, 13 countries have a high burden of VL (Bangladesh, China, Ethiopia, Georgia, India, Kenya, Nepal, Paraguay, Somalia, South Sudan, Spain, Sudan, and Uganda), and 11 have a high burden of TL (Afghanistan, Algeria, Colombia, Iran, Morocco, Pakistan, Peru, Saudi Arabia, Syrian Arab Republic, Tunisia, and Turkey), while Brazil has a high burden of both clinical forms.[64] Thus, TL treatment choice is based on the clinical presentation and infecting species, while any person with VL signs and symptoms and a verified diagnosis warrants chemotherapy.[65] The range of currently available drugs for treating leishmaniasis is relatively small and it includes repurposed molecules, such as amphotericin B, miltefosine, and paromomycin; while few new drug candidates reached clinical trials in the last decades.[66,67] For these reasons, the investigation of new therapies has been very active recently, and a wide range of compounds have been identified as potential hits and leads.[68] The unique and vast chemical diversity of NPs places them as a major component of the biologically relevant chemical space,[69] while NP classes like alkaloids, coumarins, flavonoids, lignans, neolignans, quinones, and terpenoids have demonstrated anti-leishmanial activity.[70] Several of these that target *Leishmania* ARG have been investigated for their potential as new drug candidates, although quercetin,[71–73] catechin, (-)-epicatechin, (+)-syringaresinol, isoquercetin, quercitrin, resveratrol, and cinnamic acid derivatives had shown *in vitro* efficacy.[31,33,74] Additionally, certain NPs had demonstrated favorable *in vivo* effectivity, including epigallocatechin gallate,[75] gallic acid,[76] rosmarinic acid,[77] and quercetin.[78,79] The equilibrium between biological activity and pharmacological qualities is one of several aspects, nevertheless, that restricts the translation of NPs into commercial drugs.[80,81] *In silico* based drug repositioning potential for discovering new applications for existing drugs and for developing new drugs in pharmaceutical research and the industry has gained importance[82,83]; whereas, in the chemical structure and molecule information approach, the structural similarity is incorporated with molecular activity and other biological information to identify new associations.[84]

The present work aimed to apply CADD approaches to select analogs to NPs with known anti-leishmanial and anti-ARG activities; although results of the quercetin analogs, the anthocyanin malvidin (ZINC897714; PubChem CID: 159287), and the flavone echioidinin (ZINC14807307; PubChem CID: 15559079) showed favorable binding affinity to *L. infantum*, *L. mexicana*, and *L. braziliensis* ARG and no predicted toxicity. Besides that, in the ARG super-family, the active site is conserved in all organisms, which includes the coordination of divalent metal Mn^{2+},[85] and differences between the parasite and its human homolog have been described,[86,87] highlighting the possibility to target selectively the parasite enzyme. However, recently, cinnamides[88] and 1-phenyl-1H-pyrazolo[3,4-d] pyrimidine synthetic derivatives[89] have been described as potential selective inhibitors of parasite ARG and have shown *in vitro* anti-leishmanial activity. A major bottleneck of drug discovery for leishmaniasis was aimed at the *in silico* workflow proposed, which is that compounds must show activity in the acidic environment of the phagolysosome[90]; thus, the analyzed compounds in this work showed stable enzyme-ligand interaction and favorable binding free energy at pH 2.0 in MDS analysis. However, when taking into consideration the target product profile (TPP), proposed by the Drugs for Neglected Diseases initiative (DNDi), which includes regard for the oral route of administration for new candidates,[91] both ADME profiles showed the potential for oral route administration and high bioavailability, but only malvidin results have been ratified by experimental studies published elsewhere.[92–94] Furthermore, malvidin has shown the potential to be an antioxidant, anti-hypertensive, anti-inflammatory, anti-obesity, anti-osteoarthritis, anti-proliferative, and anticancer drug candidate,[95–99] whereas to the best of our knowledge no research has been published studying the potential pharmacological activity of echioidinin. Anthocyanins are commonly found in many plants, while the most common types are cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin, which are distributed in fruits and vegetables in 50%, 12%, 12%, 12%, 7%, and 7% proportions, respectively.[100] These molecules are more stable at a lower pH solution, and in such conditions the flavylium cation formed enables the anthocyanin to be highly soluble in water.[101] The physicochemical properties offered by anthocyanins should be considered of interest for anti-leishmanial drug discovery since the parasite is adapted to live in parasitophorous vacuoles of infected macrophages in mammalian hosts, where it survives, proliferates, and is responsible for the development of the active disease.[102] Recently, the anthocyanidin profile of *Arrabidaea chica* has been examined and its anti-leishmanial activity analyzed,[103] and carajurin (PubChem CID: 44257040) showed the highest activity against the intracellular parasites, altering all parameters of *in vitro* infection.[104] Additionally, it has been shown that carajurin leads to a decrease in the mitochondrial membrane potential, an increase in ROS production, and cell death by late apoptosis in *L. amazonensis*.[105] Furthermore, flavones showing anti-leishmanial potential have been described in the literature,[106] whereas apigenin (PubChem CID: 5280443) and luteolin (PubChem CID: 5280445) have shown the potential of inhibiting the growth of *L. amazonensis*.[107]

Limitations of the present study should be also mentioned, such as the protein dynamics and complex stabilities with MDS lasting within nanoseconds scales (0-100 ns), while most structural dynamics and biological activities of proteins occur within timescales of microseconds and milliseconds.[108] Even so, complex dynamics and interactions between enzymes and ligands have been reported using nanosecond timescales.[109,110] Additionally, the work did not include *in vitro* or *in vivo* validation. It is important to note that anti-leishmanial *in vitro* assays have drawbacks, including metabolic differences between the amastigote and promastigote stages,[111] variations in drug effectiveness and susceptibility among parasites isolated from patients,[112] and a variety of biochemical pathways linked to drug-resistant phenotypes in the parasite,[113,114] which can lead to false positive results. Additionally, numerous animal models are used in the validation tests for VL and TL drug candidates; however, due to insufficient translation to human disease, their predictive value is frequently low. Furthermore, reliable main models for VL are frequently employed, including Syrian golden hamsters and BALB/c mice,[115,116] while there are no validated animal models for TL since different species experience varied clinical symptoms, and current models lack human characteristics such as pathophysiology, symptomatology, and treatment response.[117]

Conclusion

In the first screening, this work identified three substances with natural products structural analogs with potential effects against *Leishmania* ARG using *in silico* analysis from the available data and research of natural products found in databases. The substances were: ZINC39120134 (3,4-dihydro-2-(2-methylphenyl)-(9CI)), ZINC14807307 (echioidinin) and ZINC897714 (malvidin), where the most suitable compounds were ZINC14807307 and ZINC897714, showing favorable binding affinity to *L. infantum*, *L. mexicana*, and *L. braziliensis* ARG, no potential toxicity and stability at pH 2.0; important factors due to the acidic environment of the phagolysosomes of mammalian hosts. Taking into consideration that the oral bioavailability of malvidin has experimental data published and that its pharmacological potential has been widely studied, the results presented in this work warrant further *in vitro* and *in vivo* studies using malvidin to confirm its potential as a drug candidate against leishmaniasis.

Data availability

Underlying data

Figshare. Supplementary material. https://figshare.com/articles/dataset/Supplementary_material_xlsx/21867822.[118]

This project contains the following underlying data:

- Table S1. (Compounds obtained by chemical similarity against the natural products analyzed)
- Table S2. (Virtual screening results of the compounds selected against *L. infatum* and *H. sapiens*arginase enzymes)
- Table S3. (Virtual screening results of the compounds selected against *L. braziliensis* and *L. mexicana* arginase enzymes)
- Table S4. (Toxicity prediction of the selected compounds)

References

1. Tuon FF, Neto VA, Amato VS: **Leishmania: origin, evolution and future since the precambrian.** *FEMS Immunol. Med. Microbiol.* 2008; **54**(2): 158–166.
PubMed Abstract | Publisher Full Text

2. Alvar J, Vélez ID, Bern C, *et al.*: **Leishmaniasis worldwide and global estimates of its incidence.** *PLoS One.* 2012; **7**(5): e35671.
PubMed Abstract | Publisher Full Text | Free Full Text

3. Hotez PJ: **The rise of leishmaniasis in the twenty-first century.** *Trans. R. Soc. Trop. Med. Hyg.* 2018; **112**(9): 421–422.
PubMed Abstract | Publisher Full Text

4. Oryan A, Akbari M: **Worldwide risk factors in leishmaniasis.** *Asian Pac. J. Trop. Med.* 2016; **9**(10): 925–932.
Publisher Full Text

5. Curtin JM, Aronson NE: **Leishmaniasis in the united states: Emerging issues in a region of low endemicity.** *Microorganisms.* 2021; **9**(3): 578.
PubMed Abstract | Publisher Full Text | Free Full Text

6. Ready PD: **Leishmaniasis emergence in europe.** *Eurosurveillance.* 2010; **15**(10): 19505.
PubMed Abstract | Publisher Full Text

7. Lindoso JAL, Cunha MA, Queiroz IT, *et al.*: **Leishmaniasis–hiv coinfection: current challenges.** *Hiv/aids (Auckland, NZ).* 2016; **8**: 147–156.
Publisher Full Text

8. Dostálová A, Volf P: **Leishmania development in sand flies: parasite-vector interactions overview.** *Parasit. Vectors.* 2012; **5**(1): 1–12.

9. Zilberstein D, Shapira M: **The role of ph and temperature in the development of leishmania parasites.** *Annu. Rev. Microbiol.* 1994; **48**: 449–470.
Publisher Full Text

10. Magalhaes RDM, Duarte MC, Mattos EC, *et al.*: **Identification of differentially expressed proteins from leishmania amazonensis associated with the loss of virulence of the parasites.** *PLoS Negl. Trop. Dis.* 2014; **8**(4): e2764.
PubMed Abstract | Publisher Full Text | Free Full Text

11. Akhoundi M, Kuhls K, Cannet A, *et al.*: **A historical overview of the classification, evolution, and dispersion of leishmania parasites and sandflies.** *PLoS Negl. Trop. Dis.* 2016; **10**(3): e0004349.
PubMed Abstract | Publisher Full Text | Free Full Text

12. Kaye P, Scott P: **Leishmaniasis: complexity at the host–pathogen interface.** *Nat. Rev. Microbiol.* 2011; **9**(8): 604–615.
PubMed Abstract | Publisher Full Text

13. Burza S, Croft S, Boelaert M: **Leishmaniasis.** *Lancet.* 2018; **392**(10151): 951–970.
Publisher Full Text

14. Kaye PM, Mohan S, Mantel C, *et al.*: **Overcoming roadblocks in the development of vaccines for leishmaniasis.** *Expert Rev. Vaccines.* 2021; **20**(11): 1419–1430.
PubMed Abstract | Publisher Full Text

15. Lindoso JAL, Costa JML, Queiroz IT, *et al.*: **Review of the current treatments for leishmaniases.** *Res. Rep. Trop. Med.* 2012; **3**: 69.
Publisher Full Text

16. Chakravarty J, Sundar S: **Current and emerging medications for the treatment of leishmaniasis.** *Expert. Opin. Pharmacother.* 2019; **20**(10): 1251–1265.
Publisher Full Text

17. Newman DJ, Cragg GM: **Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019.** *J. Nat. Prod.* 2020; **83**(3): 770–803.
PubMed Abstract | Publisher Full Text

18. Ribeiro TG, Nascimento AM, Henriques BO, *et al.*: **Antileishmanial activity of standardized fractions of stryphnodendron obovatum (barbatimão) extract and constituent compounds.** *J. Ethnopharmacol.* 2015; **165**: 238–242.
PubMed Abstract | Publisher Full Text

19. Lage PS, Rocha PH, de Andrade A, *et al.*: **Strychnos pseudoquina and its purified compounds present an effective in vitro antileishmanial activity.** *Evid. Based Complement. Alternat. Med.* 2013; **2013**: 1–9.
PubMed Abstract | Publisher Full Text | Free Full Text

20. Ribeiro TG, Chávez-Fumagalli MA, Valadares DG, *et al.*: **Antileishmanial activity and cytotoxicity of brazilian plants.** *Exp. Parasitol.* 2014; **143**: 60–68.
PubMed Abstract | Publisher Full Text

21. Lage PS, Chávez-Fumagalli MA, Mesquita JT, *et al.*: **Antileishmanial activity and evaluation of the mechanism of action of strychnobiflavone flavonoid isolated from strychnos pseudoquina against leishmania infantum.** *Parasitol. Res.* 2015; **114**(12): 4625–4635.
PubMed Abstract | Publisher Full Text

22. Sabe VT, Ntombela T, Jhamba LA, *et al.*: **Current trends in computer aided drug design and a highlight of drugs discovered via computational techniques: A review.** *Eur. J. Med. Chem.* 2021; **224**: 113705.
PubMed Abstract | Publisher Full Text

23. Goyzueta-Mamani LD, Barazorda-Ccahuana HL, Mena-Ulecia K, *et al.*: **Antiviral activity of metabolites from peruvian plants against sars-cov-2: an in silico approach.** *Molecules.* 2021; **26**(13): 3882.
PubMed Abstract | Publisher Full Text | Free Full Text

24. Goyzueta-Mamani LD, Barazorda-Ccahuana HL, Chávez-Fumagalli MA, *et al.*: **In silico analysis of metabolites from peruvian native plants as potential therapeutics against alzheimer's disease.** *Molecules.* 2022; **27**(3): 918.
PubMed Abstract | Publisher Full Text | Free Full Text

25. Durieu E, Prina E, Leclercq O, *et al.*: **From drug screening to target deconvolution: a target-based drug discovery pipeline using leishmania casein kinase 1 isoform 2 to identify compounds with antileishmanial activity.** *Antimicrob. Agents Chemother.* 2016; **60**(5): 2822–2833.
PubMed Abstract | Publisher Full Text | Free Full Text

26. Bell AS, Mills JE, Williams GP, *et al.*: **Selective inhibitors of protozoan protein n-myristoyltransferases as starting points for tropical disease medicinal chemistry programs.** *PLoS Negl. Trop. Dis.* 2012; **6**(4): e1625.
PubMed Abstract | Publisher Full Text | Free Full Text

27. Chavez-Fumagalli MA, Lage DP, Tavares GSV, *et al.*: **In silico leishmania proteome mining applied to identify drug target potential to be used to treat against visceral and tegumentary leishmaniasis.** *J. Mol. Graph. Model.* 2019; **87**: 89–97.
PubMed Abstract | Publisher Full Text

28. Raj S, Sasidharan S, Balaji SN, *et al.*: **An overview of biochemically characterized drug targets in metabolic pathways of leishmania parasite.** *Parasitol. Res.* 2020; **119**(7): 2025–2037.
PubMed Abstract | Publisher Full Text

29. Roberts SC, Tancer MJ, Polinsky MR, *et al.*: **Arginase plays a pivotal role in polyamine precursor metabolism in leishmania: characterization of gene deletion mutants.** *J. Biol. Chem.* 2004; **279**(22): 23668–23678.
PubMed Abstract | Publisher Full Text

30. Pessenda G, da Silva JS: **Arginase and its mechanisms in leishmania persistence.** *Parasite Immunol.* 2020; **42**(7): e12722.
PubMed Abstract | Publisher Full Text

31. Roberto E, da Silva C, do Carmo Maquiaveli C, *et al.*: **The leishmanicidal flavonols quercetin and quercitrin target leishmania (leishmania) amazonensis arginase.** *Exp. Parasitol.* 2012; **130**(3): 183–188.
Publisher Full Text

32. Wulsten IF, Costa-Silva TA, Mesquita JT, *et al.*: **Investigation of the anti-leishmania (leishmania) infantum activity of some natural sesquiterpene lactones.** *Molecules.* 2017; **22**(5): 685.
PubMed Abstract | Publisher Full Text | Free Full Text

33. Carter NS, Stamper BD, Elbarbry F, *et al.*: **Natural products that target the arginase in leishmania parasites hold therapeutic promise.** *Microorganisms.* 2021; **9**(2): 267.
PubMed Abstract | Publisher Full Text | Free Full Text

34. Pilon AC, Valli M, Dametto AC, *et al.*: **Nubbedb: an updated database to uncover chemical and biological information from brazilian biodiversity.** *Sci. Rep.* 2017; **7**(1): 1–12.

35. Kim S, Chen J, Cheng T, *et al.*: **Pubchem 2019 update: Improved access to chemical data, 2019.**10.
Reference Source

36. Sander T, Freyss J, von Korff M, *et al.*: **Datawarrior: an open-source program for chemistry aware data visualization and analysis.** *J. Chem. Inf. Model.* 2015; **55**(2): 460–473.
PubMed Abstract | Publisher Full Text

37. Daina A, Michielin O, Zoete V: **Swissadme: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules.** *Sci. Rep.* 2017; **7**(1): 1–13.

38. Bragina ME, Daina A, Perez MAS, *et al.*: **The swisssimilarity 2021 web tool: Novel chemical libraries and additional methods for an enhanced ligand-based virtual screening experience.** *Int. J. Mol. Sci.* 2022; **23**(2): 811.
PubMed Abstract | Publisher Full Text | Free Full Text

39. Irwin JJ, Tang KG, Young J, *et al.*: **Zinc20–a free ultralarge-scale chemical database for ligand discovery.** *J. Chem. Inf. Model.* 2020; **60**(12): 6065–6073.
PubMed Abstract | Publisher Full Text | Free Full Text

40. Biasini M, Bienert S, Waterhouse A, *et al.*: **Swiss-model: modelling protein tertiary and quaternary structure using evolutionary information.** *Nucleic Acids Res.* 2014; **42**(W1): W252–W258.
PubMed Abstract | Publisher Full Text | Free Full Text

41. Dallakyan S, Olson AJ: **Small-molecule library screening by docking with pyrx.** *Chemical biology.* Springer; 2015; pages 243–250.
PubMed Abstract | Publisher Full Text

42. Trott O, Olson AJ: **Software news and update autodock vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading.** *J. Comput. Chem.* 2009; **31**(2): 455–461.
Publisher Full Text

43. Hanwell MD, Curtis DE, Lonie DC, *et al.*: **Avogadro: an advanced semantic chemical editor, visualization, and analysis platform.** *J. Cheminformatics.* 2012; **4**(1): 1–17.

44. Alan W, da Silva S, Vranken WF: **Acpype-antechamber python parser interface.** *BMC. Res. Notes.* 2012; **5**(1): 1–8.
Publisher Full Text

45. Dolinsky TJ, Nielsen JE, Andrew McCammon J, *et al.*: **Pdb2pqr: an automated pipeline for the setup of poisson–boltzmann electrostatics calculations.** *Nucleic Acids Res.* 2004; **32**(suppl_2): W665–W667.
PubMed Abstract | Publisher Full Text | Free Full Text

46. Van Der Spoel D, Lindahl E, Hess B, *et al.*: **Gromacs: fast, flexible, and free.** *J. Comput. Chem.* 2005; **26**(16): 1701–1718.
PubMed Abstract | Publisher Full Text

47. Case DA, Metin Aktulga H, Belfon K, *et al.*: *Amber 2021*. San Francisco: University of California; 2021.

48. Bussi G, Donadio D, Parrinello M: **Canonical sampling through velocity rescaling.** *J. Chem. Phys.* 2007; **126**(1): 014101.
PubMed Abstract | Publisher Full Text

49. Santos KB, Guedes IA, Karl ALM, *et al.*: **Highly flexible ligand docking: benchmarking of the dockthor program on the leads-pep protein–peptide data set.** *J. Chem. Inf. Model.* 2020; **60**(2): 667–683.
PubMed Abstract | Publisher Full Text

50. Miller BR III, McGee TD Jr, Swails JM, *et al.*: **Mmpbsa. py: an efficient program for end-state free energy calculations.** *J. Chem. Theory Comput.* 2012; **8**(9): 3314–3321.

51. Valdés-Tresanco MS, Valdés-Tresanco ME, Valiente PA, *et al.*: **gmx_mmpbsa: a new tool to perform end-state free energy calculations with gromacs.** *J. Chem. Theory Comput.* 2021; **17**(10): 6281–6291.
PubMed Abstract | Publisher Full Text

52. Bennett CH: **Efficient estimation of free energy differences from monte carlo data.** *J. Comput. Phys.* 1976; **22**(2): 245–268.
Publisher Full Text

53. Wang Z, Li Y, Ai C, *et al.*: **In silico prediction of estrogen receptor subtype binding affinity and selectivity using statistical methods and molecular docking with 2-arylnaphthalenes and 2-arylquinolines.** *Int. J. Mol. Sci.* 2010; **11**(9): 3434–3458.
PubMed Abstract | Publisher Full Text | Free Full Text

54. Warnes GR, Bolker B, Bonebakker L, *et al.*: **Package "gplots": Various r programming tools for plotting data. r packag. version 2.17. 0.** 2016; 1–68.

55. Freitas LR, de Sousa S, Ramalho D, *et al.*: **Isolation of arginase inhibitors from the bioactivity-guided fractionation of byrsonima coccolobifolia leaves and stems.** *J. Nat. Prod.* 2014; **77**(2): 392–396.
Publisher Full Text

56. Chagas CM, Moss S, Alisaraie L: **Drug metabolites and their effects on the development of adverse reactions: Revisiting lipinski's rule of five.** *Int. J. Pharm.* 2018; **549**(1-2): 133–149.
PubMed Abstract | Publisher Full Text

57. Richard Bickerton G, Paolini GV, Besnard J, *et al.*: **Quantifying the chemical beauty of drugs.** *Nat. Chem.* 2012; **4**(2): 90–98.
PubMed Abstract | Publisher Full Text | Free Full Text

58. Ertl P, Schuffenhauer A: **Estimation of synthetic accessibility score of drug-like molecules based on molecular complexity and fragment contributions.** *J. Cheminformatics.* 2009; **1**(1): 1–11.
Publisher Full Text

59. Fernández OL, Diaz-Toro Y, Ovalle C, *et al.*: **Miltefosine and antimonial drug susceptibility of leishmania viannia species and populations in regions of high transmission in colombia.** *PLoS Negl. Trop. Dis.* 2014; **8**(5): e2871.
PubMed Abstract | Publisher Full Text | Free Full Text

60. Baek K-H, Piel L, Rosazza T, *et al.*: **Infectivity and drug susceptibility profiling of different leishmania-host cell combinations.** *Pathogens.* 2020; **9**(5): 393.
PubMed Abstract | Publisher Full Text | Free Full Text

61. Coutsias EA, Wester MJ: **Rmsd and symmetry.** *J. Comput. Chem.* 2019; **40**(15): 1496–1508.
PubMed Abstract | Publisher Full Text

62. Yu Lobanov M, Bogatyreva NS, Galzitskaya OV: **Radius of gyration as an indicator of protein structure compactness.** *Mol. Biol.* 2008; **42**(4): 623–628.
Publisher Full Text

63. Vos T, Lim SS, Abbafati C, *et al.*: **Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the global burden of disease study 2019.** *Lancet.* 2020; **396**(10258): 1204–1222.
PubMed Abstract | Publisher Full Text | Free Full Text

64. Torres-Guerrero E, Quintanilla-Cedillo MR, Ruiz-Esmenjaud J, *et al.*: **Leishmaniasis: a review.** *F1000Res.* 2017; **6**: 750.
PubMed Abstract | Publisher Full Text | Free Full Text

65. Mann S, Frasca K, Scherrer S, *et al.*: **A review of leishmaniasis: current knowledge and future directions.** *Curr. Trop. Med. Rep.* 2021; **8**(2): 121–132.
PubMed Abstract | Publisher Full Text | Free Full Text

66. Braga SS: **Multi-target drugs active against leishmaniasis: A paradigm of drug repurposing.** *Eur. J. Med. Chem.* 2019; **183**: 111660.
PubMed Abstract | Publisher Full Text

67. De Rycker M, Baragaña B, Duce SL, *et al.*: **Challenges and recent progress in drug discovery for tropical diseases.** *Nature.* 2018; **559**(7715): 498–506.
PubMed Abstract | Publisher Full Text | Free Full Text

68. Olías-Molero AI, de la Fuente C, Cuquerella M, *et al.*: **Antileishmanial drug discovery and development: Time to reset the model?** *Microorganisms.* 2021; **9**(12): 2500.
PubMed Abstract | Publisher Full Text | Free Full Text

69. Rosén J, Gottfries J, Muresan S, *et al.*: **Novel chemical space exploration via natural products.** *J. Med. Chem.* 2009; **52**(7): 1953–1962.
PubMed Abstract | Publisher Full Text | Free Full Text

70. Gervazoni LFO, Barcellos GB, Ferreira-Paes T, *et al.*: **Use of natural products in leishmaniasis chemotherapy: an overview.** *Front. Chem.* 2020; **8**: 1031.
Publisher Full Text

71. Fonseca-Silva F, Inacio JDF, Canto-Cavalheiro MM, *et al.*: **Reactive oxygen species production by quercetin causes the death of leishmania amazonensis intracellular amastigotes.** *J. Nat. Prod.* 2013; **76**(8): 1505–1508.
PubMed Abstract | Publisher Full Text

72. Vila-Nova NS, Morais SM, Falcão MJC, *et al.*: **Leishmanicidal and cholinesterase inhibiting activities of phenolic compounds of dimorphandra gardneriana and platymiscium floribundum, native plants from caatinga biome.** *Pesqui. Vet. Bras.* 2012; **32**: 1164–1168.
Publisher Full Text

73. Cataneo AHD, Tomiotto-Pellissier F, Miranda-Sapla MM, *et al.*: **Quercetin promotes antipromastigote effect by increasing the ros production and anti-amastigote by upregulating nrf2/ho-1 expression, affecting iron availability.** *Biomed. Pharmacother.* 2019; **113**: 108745.
PubMed Abstract | Publisher Full Text

74. Costa RS, Souza OP, Filho OD, *et al.*: **In vitro antileishmanial and antitrypanosomal activity of compounds isolated from the roots of zanthoxylum tingoassuiba.** *Revista Brasileira de Farmacognosia.* 2018; **28**: 551–558.
Publisher Full Text

75. Sosa AM, Álvarez AM, Bracamonte E, *et al.*: **Efficacy of topical treatment with (-)-epigallocatechin gallate, a green tea catechin, in mice with cutaneous leishmaniasis.** *Molecules.* 2020; **25**(7): 1741.
PubMed Abstract | Publisher Full Text | Free Full Text

76. de Moraes Alves MM, Arcanjo DDR, Figueiredo KA, *et al.*: **Gallic and ellagic acids are promising adjuvants to conventional amphotericin b for the treatment of cutaneous leishmaniasis.** *Antimicrob. Agents Chemother.* 2020; **64**(12): e00807–e00820.
Publisher Full Text

77. Montrieux E, Perera WH, García M, *et al.*: **In vitro and in vivo activity of major constituents from pluchea carolinensis against leishmania amazonensis.** *Parasitol. Res.* 2014; **113**(8): 2925–2932.
PubMed Abstract | Publisher Full Text

78. Sousa-Batista AJ, Poletto FS, Philipon CIMS, *et al.*: **Lipid-core nanocapsules increase the oral efficacy of quercetin in cutaneous leishmaniasis.** *Parasitology.* 2017; **144**(13): 1769–1774.
PubMed Abstract | Publisher Full Text

79. Tasdemir D, Kaiser M, Brun R, *et al.*: **Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: in vitro, in vivo, structure-activity relationship, and quantitative structure-activity relationship studies.** *Antimicrob. Agents Chemother.* 2006; **50**(4): 1352–1364.
PubMed Abstract | Publisher Full Text | Free Full Text

80. Atanasov AG, Zotchev SB, Dirsch VM, *et al.*: **Natural products in drug discovery: advances and opportunities.** *Nat. Rev. Drug Discov.* 2021; **20**(3): 200–216.
PubMed Abstract | Publisher Full Text | Free Full Text

81. Batiha GE-S, Beshbishy AM, Ikram M, *et al.*: **The pharmacological activity, biochemical properties, and pharmacokinetics of the major natural polyphenolic flavonoid: Quercetin.** *Foods.* 2020; **9**(3): 374.
PubMed Abstract | Publisher Full Text | Free Full Text

82. Mullins JGL: **Drug repurposing in silico screening platforms.** *Biochem. Soc. Trans.* 2022; **50**(2): 747–758.
PubMed Abstract | Publisher Full Text

83. Pushpakom S, Iorio F, Eyers PA, *et al.*: **Drug repurposing: progress, challenges and recommendations.** *Nat. Rev. Drug Discov.* 2019; **18**(1): 41–58.
PubMed Abstract | Publisher Full Text

84. Jarada TN, Rokne JG, Alhajj R: **A review of computational drug repositioning: strategies, approaches, opportunities, challenges, and directions.** *J. Cheminformatics.* 2020; **12**(1): 1–23.

85. Perozich J, Hempel J, Morris SM Jr: **Roles of conserved residues in the arginase family.** *Biochim. Biophys. Acta.* 1998; **1382**(1): 23–37.
PubMed Abstract | Publisher Full Text

86. da Silva ER, Castilho TM, Pioker FC, *et al.*: **Genomic organisation and transcription characterisation of the gene encoding leishmania (leishmania) amazonensis arginase and its protein structure prediction.** *Int. J. Parasitol.* 2002; **32**(6): 727–737.
PubMed Abstract | Publisher Full Text

87. D'Antonio EL, Ullman B, Roberts SC, *et al.*: **Crystal structure of arginase from leishmania mexicana and implications for the inhibition of polyamine biosynthesis in parasitic infections.** *Arch. Biochem. Biophys.* 2013; **535**(2): 163–176.
PubMed Abstract | Publisher Full Text | Free Full Text

88. Roberto E, da Silva J, Alfredo dos Santos Simone Come A, *et al.*: **Cinnamides target leishmania amazonensis arginase selectively.** *Molecules.* 2020; **25**(22): 5271.
Publisher Full Text

89. Feitosa LM, da Silva ER, Hoelz LVB, *et al.*: **New pyrazolopyrimidine derivatives as leishmania amazonensis arginase inhibitors.** *Bioorg. Med. Chem.* 2019; **27**(14): 3061–3069.
PubMed Abstract | Publisher Full Text

90. Alcântara LM, Ferreira TCS, Gadelha FR, *et al.*: **Challenges in drug discovery targeting tritryp diseases with an emphasis on leishmaniasis.** *Int. J. Parasitol. Drugs Drug Resist.* 2018; **8**(3): 430–439.
PubMed Abstract | Publisher Full Text

91. Uliana SRB, Trinconi CT, Coelho AC: **Chemotherapy of leishmaniasis: present challenges.** *Parasitology.* 2018; **145**(4): 464–480.
Publisher Full Text

92. Eker ME, Aaby K, Budic-Leto I, *et al.*: **A review of factors affecting anthocyanin bioavailability: Possible implications for the inter-individual variability.** *Foods.* 2019; **9**(1): 2.
PubMed Abstract | Publisher Full Text | Free Full Text

93. Fagundes FL, Pereira QC, Zarricueta ML, *et al.*: **Malvidin protects against and repairs peptic ulcers in mice by alleviating oxidative stress and inflammation.** *Nutrients.* 2021; **13**(10): 3312.
PubMed Abstract | Publisher Full Text | Free Full Text

94. Bub A, Watzl B, Heeb D, *et al.*: **Malvidin-3-glucoside bioavailability in humans after ingestion of red wine, dealcoholized red wine and red grape juice.** *Eur. J. Nutr.* 2001; **40**(3): 113–120.
PubMed Abstract | Publisher Full Text

95. Ma Y, Li Y, Zhang H, *et al.*: **Malvidin induces hepatic stellate cell apoptosis via the endoplasmic reticulum stress pathway and mitochondrial pathway.** *Food Sci. Nutr.* 2020; **8**(9): 5095–5106.
PubMed Abstract | Publisher Full Text | Free Full Text

96. Saulite L, Jekabsons K, Klavins M, *et al.*: **Effects of malvidin, cyanidin and delphinidin on human adipose mesenchymal stem cell differentiation into adipocytes, chondrocytes and osteocytes.** *Phytomedicine.* 2019; **53**: 86–95.
PubMed Abstract | Publisher Full Text

97. Huang W-Y, Liu Y-M, Wang J, *et al.*: **Anti-inflammatory effect of the blueberry anthocyanins malvidin-3-glucoside and malvidin-3-galactoside in endothelial cells.** *Molecules.* 2014; **19**(8): 12827–12841.
PubMed Abstract | Publisher Full Text | Free Full Text

98. Baba AB, Nivetha R, Chattopadhyay I, *et al.*: **Blueberry and malvidin inhibit cell cycle progression and induce mitochondrial-mediated apoptosis by abrogating the jak/stat-3 signalling pathway.** *Food Chem. Toxicol.* 2017; **109**: 534–543.
PubMed Abstract | Publisher Full Text

99. Mazewski C, Liang K, de Mejia EG: **Comparison of the effect of chemical composition of anthocyanin-rich plant extracts on colon cancer cell proliferation and their potential mechanism of action using in vitro, in silico, and biochemical assays.** *Food Chem.* 2018; **242**: 378–388.
PubMed Abstract | Publisher Full Text

100. Castañeda-Ovando A, Pacheco-Hernández ML, Páez-Hernández ME, *et al.*: **Chemical studies of anthocyanins: A review.** *Food Chem.* 2009; **113**(4): 859–871.
Publisher Full Text

101. Khoo HE, Azlan A, Tang ST, *et al.*: **Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits.** *Food Nutr. Res.* 2017; **61**(1): 1361779.
PubMed Abstract | Publisher Full Text | Free Full Text

102. Burchmore RJS, Barrett MP: **Life in vacuoles–nutrient acquisition by leishmania amastigotes.** *Int. J. Parasitol.* 2001; **31**(12): 1311–1320.
PubMed Abstract | Publisher Full Text

103. Moragas-Tellis CJ, Almeida-Souza F, do Socorro dos Santos Chagas M, *et al.*: **The influence of anthocyanidin profile on antileishmanial activity of arrabidaea chica morphotypes.** *Molecules.* 2020; **25**(15): 3547.
PubMed Abstract | Publisher Full Text | Free Full Text

104. Silva-Silva JV, Moragas-Tellis CJ, Chagas MSS, *et al.*: **Carajurin: A anthocyanidin from arrabidaea chica as a potential biological marker of antileishmanial activity.** *Biomed. Pharmacother.* 2021a; **141**: 111910.
PubMed Abstract | Publisher Full Text

105. Silva-Silva JV, Moragas-Tellis CJ, Chagas MSS, *et al.*: **Carajurin induces apoptosis in leishmania amazonensis promastigotes through reactive oxygen species production and mitochondrial dysfunction.** *Pharmaceuticals.* 2022; **15**(3): 331.
PubMed Abstract | Publisher Full Text | Free Full Text

106. Sasidharan S, Saudagar P: **Flavones reversibly inhibit leishmania donovani tyrosine aminotransferase by binding to the catalytic pocket: An integrated in silico-in vitro approach.** *Int. J. Biol. Macromol.* 2020; **164**: 2987–3004.
PubMed Abstract | Publisher Full Text

107. Silva-Silva JV, Moragas-Tellis CJ, do Socorro dos Santos Chagas M, *et al.*: **Antileishmanial activity of flavones-rich fraction from arrabidaea chica verlot (bignoniaceae).** *Front. Pharmacol.* 2021b; **12**: 703985.
PubMed Abstract | Publisher Full Text | Free Full Text

108. Brust R, Lukacs A, Haigney A, *et al.*: **Proteins in action: Femtosecond to millisecond structural dynamics of a photoactive flavoprotein.** *J. Am. Chem. Soc.* 2013; **135**(43): 16168–16174.
PubMed Abstract | Publisher Full Text | Free Full Text

109. Hünenberger PH, Mark AE, Van Gunsteren WF: **Fluctuation and cross-correlation analysis of protein motions observed in nanosecond molecular dynamics simulations.** *J. Mol. Biol.* 1995; **252**(4): 492–503.
PubMed Abstract | Publisher Full Text

110. Charlier C, Khan SN, Marquardsen T, *et al.*: **Nanosecond time scale motions in proteins revealed by high-resolution nmr relaxometry.** *J. Am. Chem. Soc.* 2013; **135**(49): 18665–18672.
PubMed Abstract | Publisher Full Text | Free Full Text

111. Siqueira-Neto JL, Moon S, Jang J, *et al.*: **An image-based high-content screening assay for compounds targeting intracellular leishmania donovani amastigotes in human macrophages.** *PLoS Negl. Trop. Dis.* 2012; **6**(6): e1671.
PubMed Abstract | Publisher Full Text | Free Full Text

112. Rugani JN, Quaresma PF, Gontijo CF, *et al.*: **Intraspecies susceptibility of leishmania (viannia) braziliensis to antileishmanial drugs: antimony resistance in human isolates from atypical lesions.** *Biomed. Pharmacother.* 2018; **108**: 1170–1180.
PubMed Abstract | Publisher Full Text

113. Andrade JM, Gonçalves LO, Liarte DB, *et al.*: **Comparative transcriptomic analysis of antimony resistant and susceptible leishmania infantum lines.** *Parasit. Vectors.* 2020; **13**(1): 1–15.
Publisher Full Text

114. Maes L, Beyers J, Mondelaers A, *et al.*: **In vitro 'time-to-kill' assay to assess the cidal activity dynamics of current reference drugs against leishmania donovani and leishmania infantum.** *J. Antimicrob. Chemother.* 2017; **72**(2): 428–430.
PubMed Abstract | Publisher Full Text

115. Oliveira DM, Mariana Amália F, Costa MA, *et al.*: **Evaluation of parasitological and immunological parameters of leishmania chagasi infection in balb/c mice using different doses and routes of inoculation of parasites.** *Parasitol. Res.* 2012; **110**(3): 1277–1285.
PubMed Abstract | Publisher Full Text

116. Garg R, Dube A: **Animal models for vaccine studies for visceral leishmaniasis.** *Indian J. Med. Res.* 2006; **123**(3): 439–454.
PubMed Abstract

117. Mears ER, Modabber F, Don R, *et al.*: **A review: the current in vivo models for the discovery and utility of new anti-leishmanial drugs targeting cutaneous leishmaniasis.** *PLoS Negl. Trop. Dis.* 2015; **9**(9): e0003889.
PubMed Abstract | Publisher Full Text | Free Full Text

118. Barazorda-Ccahuana HL, Goyzueta-Mamani LD, Candia-Puma MA, *et al.*: **Computer-aided drug design approaches applied to screen natural product's structural analogs targeting arginase in Leishmania spp. Figshare. Supplementary material.** 2023.
Reference Source

Open Peer Review

Current Peer Review Status: ✔ ? ?

Version 2

Reviewer Report 05 July 2023

https://doi.org/10.5256/f1000research.148092.r178761

?

Rubens L. Monte-Neto
[1] Biotechnology Applied to Pathogens (BAP), Instituto René Rachou, Fundação Oswaldo Cruz (FIOCRUZ), Belo Horizonte, Brazil
[2] Biotechnology Applied to Pathogens (BAP), Instituto René Rachou, Fundação Oswaldo Cruz (FIOCRUZ), Belo Horizonte, Brazil
Alessandra Sousa
[1] Biotechnology Applied to Pathogens (BAP), Instituto René Rachou, Faculdades Oswaldo Cruz, São Paulo, State of São Paulo, Brazil
[2] Biotechnology Applied to Pathogens (BAP), Instituto René Rachou, Faculdades Oswaldo Cruz, São Paulo, State of São Paulo, Brazil

Authors used computer-aided tools to better understand anti-leishmanial activity of natural products-based analogs toward arginase binding. Based on 33 ant-leishmanial NPs, authors selected 6 compounds able to inhibit ARG; selected 1499 analogs and filtered 25 compounds by selectivity and bonding affinity towards ARG from Leishmania. Three out of 25 analogs were further investigated and authors affirm they are good hit to be validated. The rational is quite clear and the manuscript well organized. However, several points must be addressed in order to improve impact and paper quality

Comments

- Authors initially selected 33 anti-leishmanial natural products on NuBBE database. More than one year after their access I've got 33 selected NPs with anti-leishmanial properties, which I think is very limited. Some of anti-leishmanial NPs I know, were not listed there. This must be highlighted as one of the study limtations. Several other molecules are missing which would improve manuscript impact.

- Page 4 (1st paragraph): I suggest the authors to rerun ADME analysis on pkCSM tool, considering ADMET https://biosig.lab.uq.edu.au/pkcsm/

- Page 4 (2nd paragraph): I understand that SWISS-MODEL can be a very useful tool. However

some limitations could impact on assertiveness. Considering our recent advances on modelling, I encourage authors to consider AlphaFold and after comparing with Swiss-model results highlight any study limitations or confirm that swiss-model was enough to reach the main goal. https://www.nature.com/articles/s41586-021-03819-2

- Page 4 (3rd paragraph): "The Osiris Data Warrior software... and reproductive effects" In order to improve toxicity prediction, not only based in a fragment library, I strongly encourage authors to perform analysis using graph-based tools. Please find below a link were several of these tools could be used. For example cardioToxCSM, embryoToxCSM, pdCSM-PPI, toxCSM, mCSM-lig... https://biosig.lab.uq.edu.au/tools

- Page 4 (4th paragraph): Why authors considered pH 2.0 (acidic), once the parasitophorous vacuole is around pH 4.7-5.2? I suggest that authors consider more realistic pH inspired by real measures of Leishmania PV https://pubmed.ncbi.nlm.nih.gov/1689700/#:~:text=We%20found%20statistically%20different%20mean,t ; https://link.springer.com/article/10.1007/s40495-020-00209-6

- Page 5 (3rd paragraph): "...all compounds with scores... in 25 compounds selected" Please provide a table showing all selectivities (>1) to the selected compounds. Fig 1B is showing binding affinities but we need to see the relationship with selectivity

- Page 5 (3rd paragraph): "Thus the compounds.. potential toxicities" This is partially true. When carefully analyzing Fig 1B, I agree with the authors that compounds ZINC897714 and ZINC39120134 showed acceptable binding affinities among the three testes Lesihamnia species. However, compound ZINC14807307 presented divergent binding affinities when comparing the three tested species. Additionally, compound ZINC44545549 presented the bests binding affinities - with comparable BA considering ZINC..7307 for L. mexicana - although it was not included in further analysis. Based on that I suggest to authors to consider ZINC44545549 for the pipeline.

- Page 5 (4th paragraph): "... it was observed..(green color)" What about pH 4-5, which match with Leishmania PV

- Fig 1: Instead of predicting only 4 toxicity parameters, authors are encouraged to run pkCSM (graph-based ADMET prediction experimentally curated) and predict:

  ABSORPTION
  - water solubility
  - Caco2 permeability
  - Instestinal abssorption
  - skin permeability
  - P-glycoprotein substrate
  - P-glycoprotein I inhibition
  - P-glycoprotein II inhibition
  DISTRIBUTION
  - VDss (human)
  - Fraction unbound (human)
  - BBB permeability

- CNS permeability
METABOLISM
- CYP2D6 substrate
- CYP3A4 substrate
- CYP1A2 inhibitor
- CYP2C19 inhibitor
- CYP2C9 inhibitor
- CYP2D6 inhibitor
- CYP3A4 inhibitor
EXCRETION
- Total celarance
- Renal OCT2 substrate
TOXICITY
- AMES toxicity
- Max tolerated dose (human)
- hERG I inhibitor
- hERG II inhibitor
- Oral Rat acute toxicity (LD50)
- Oral rat chronic toxicity (LOAEL)
- Hepatotoxicity
- Skin sensitization
- T. pyriformis toxicity
- Minnow toxicity

All parameters can be show as binary heatmap (as in Fig 1B) including hierarchical clustering to better visualization

- Page 10 (1st paragraph): "ZINC14807307...pH 2.0 conditions" Please consider ZINC44545549 for this conclusion!

- Page 10 (3rd paragraph): "...showed favorable.. no predicted toxicity" Toxicity prediction in the work is very poor and must be reinvestigated. Selected compounds (25) showed the highest binding affinity around 1.25 (Fig. 2B). Authors must provide what it represents in terms of selectivity. Reference antileishmanial candidates such as miltefosine and amphotericin B, show SI of hundreds. This is a study limitation and additional experimental validation are required to make predictions trustable

Minor

- Page 3 (2nd paragraph): "...been described with leishmanicidal..." Please confirm that in references 18-21 we found indeed leishmanicidal agents, otherwise, replace the term leishmanicidal by anti-leishmanial

- Page 3 (2nd paragraph): "..apoptosis." Please replace by programmed cell death.. https://pubmed.ncbi.nlm.nih.gov/23202528/

- Fig 1: Font size within Fig 1 should be reduced. They are too big compared to the other fonts

- First paragraph of discussion is introduction and should be removed, since the main theoretical basis was already provided in introduction section

This review was initially made by one of our PhD candidates, in which I agree with the highlighted points and selected the major concerns for the final version of this review

References

1. Pires DE, Blundell TL, Ascher DB: pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures.*J Med Chem*. 2015; **58** (9): 4066-72 PubMed Abstract | Publisher Full Text
2. Jumper J, Evans R, Pritzel A, Green T, et al.: Highly accurate protein structure prediction with AlphaFold.*Nature*. 2021; **596** (7873): 583-589 PubMed Abstract | Publisher Full Text
3. Antoine JC, Prina E, Jouanne C, Bongrand P: Parasitophorous vacuoles of Leishmania amazonensis-infected macrophages maintain an acidic pH.*Infect Immun*. 1990; **58** (3): 779-87 PubMed Abstract | Publisher Full Text
4. Kumar A: Survival Strategies of Leishmania Parasite: Too Many Questions and Few Answers. *Current Pharmacology Reports*. 2020; **6** (2): 25-27 Publisher Full Text
5. Proto WR, Coombs GH, Mottram JC: Cell death in parasitic protozoa: regulated or incidental?. *Nat Rev Microbiol*. 2013; **11** (1): 58-66 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular parasitology, anti-leishmanial compounds, pharmacology

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Reviewer Report 04 July 2023

https://doi.org/10.5256/f1000research.148092.r178758

?

Anupam Nath Jha
[1] Tezpur Central University, Tezpur, India
[2] Tezpur Central University, Tezpur, India

Authors have tried drug repurposing for Leishmaniasis and targeted arginase of *L. infantum*. There are some questions to be answered by authors:

1. Which pdb structure (pdb id) of human L-arginine was selected (https://www.uniprot.org/uniprotkb/P05089/entry#structure) for docking?

2. What is the sequence & structure level similarity between human and *L. infantum* ARG proteins? Give the justification of selection for ARG protein as target for drug discovery based on that similarity.

3. Any reason for selection of two pH values (2 & 7) and also how have they changed the pH during simulation. Are there any experimental data / results to support these pH values?

4. Fig 3 (A) and 4 (A) – rmsd of same protein is different or they represent different simulations / protein structure? Kindly explain.

5. How are the resultant compounds better than ones shown in Garcia *et al.*, (2019)[1] and Carter *et al.,* (2021)[2]?

Minor points: authors can check Kumari *et al.,* (2023)[4] and Saha & Nath Jha (2023)[4], for workflow and *Leishmania* related updates.

References

1. Garcia AR, Oliveira DMP, Claudia F Amaral A, Jesus JB, et al.: Leishmania infantum arginase: biochemical characterization and inhibition by naturally occurring phenolic substances.*J Enzyme Inhib Med Chem*. 2019; **34** (1): 1100-1109 PubMed Abstract | Publisher Full Text
2. Carter NS, Stamper BD, Elbarbry F, Nguyen V, et al.: Natural Products That Target the Arginase in Leishmania Parasites Hold Therapeutic Promise.*Microorganisms*. 2021; **9** (2). PubMed Abstract | Publisher Full Text
3. Kumari I, Lakhanpal D, Swargam S, Nath Jha A: Leishmaniasis: Omics Approaches to Understand its Biology from Molecule to Cell Level.*Curr Protein Pept Sci*. 2023; **24** (3): 229-239 PubMed Abstract | Publisher Full Text
4. Saha D, Nath Jha A: Computational multi-target approach to target essential enzymes of Leishmania donovani using comparative molecular dynamic simulations and MMPBSA analysis. *Phytochem Anal*. 2023. PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Computational Biophysics, Molecular Dynamics simulations, Drug design and discovery

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 10 Jul 2023

Miguel Angel Chavez-Fumagalli

1) Authors have tried drug repurposing for Leishmaniasis and targeted arginase of L. infantum. There are some questions to be answered by authors:
ANSWER: I appreciate your evaluation and helpful criticism. To enhance the manuscript, we have taken into consideration your suggestions.

2) Which pdb structure (pdb id) of human L-arginine was selected https://www.uniprot.org/uniprotkb/P05089/entry#structure) for docking?
ANSWER: I appreciate your analysis. As mentioned in the methods section, we obtained the Homo sapiens ARG's FASTA sequence, carried out automated modeling in Swiss-Model, and chose the top models.

3) What is the sequence & structure level similarity between human and L. infantum ARG proteins? Give the justification of selection for ARG protein as target for drug discovery based on that similarity.
ANSWER: I value what you pointed out. We addressed this crucial issue raised by the reviewer in the text's results sections by adding comments. While the discussion and introduction sections of the work have advocated for the choice of ARG as a target.

4) Any reason for selection of two pH values (2 & 7) and also how have they changed the pH during simulation. Are there any experimental data / results to support these pH values?

ANSWER: I value what you pointed out. Our research focused on a significant obstacle to the development of leishmaniasis drugs: the requirement that drugs work in the acidic environment of the phagolysosome. This important information is included in the introduction and discussion portion of the article. The pH ranges selected were intended to compare variations in the target dynamics and complex stabilities under the two settings.

5) Fig 3 (A) and 4 (A) – rmsd of same protein is different or they represent different simulations / protein structure? Kindly explain.
ANSWER: I appreciate your perceptive observation. Figure 4 contrasts the target dynamics and complex stabilities at pH 2 or 7 (with the two molecules and without), lasting within nanoseconds. Figure 3 depicts the target at both pHs without binding a molecule.

6) How are the resultant compounds better than ones shown in Garcia et al., (2019)1 and Carter et al., (2021)2?
ANSWER: I appreciate your astute observation. Compared to the results obtained for most of the chemicals mentioned in the cited studies, our findings are, nonetheless, preliminary. Since 2000, numerous in vitro, in vivo, and silico studies have been conducted to assess natural product's potential as an antileishmanial substance. as a single module, using nanocarriers and delivery systems [Alanazi AD, Ben Said M. Plant Bioactive Ingredients in Delivery Systems and Nanocarriers for the Treatment of Leishmaniasis: An Evidence-Based Review. Iran J Parasitol. 2022 Oct-Dec;17(4):458-472. doi: 10.18502/ijpa.v17i4.11272. PMID: 36694570; PMCID: PMC9825702.]. The approach outlined in this study aims to use a logical search for novel candidate molecules in which structural similarities to compounds that have been extensively investigated may result in novel molecular activities. While choosing substances that showed the potential for oral absorption and were selective against the parasite target. Malvidin performed the best in this case, however, it should first be studied in vitro and in vivo in order to meet the criteria for a candidate.

7) Minor points: authors can check Kumari et al., (2023)4 and Saha & Nath Jha (2023)4, for workflow and Leishmania related updates.
ANSWER: We value your highly regarded suggestion. I will definitely consider it in future studies.

Competing Interests: No competing interests

Reviewer Report 31 May 2023

https://doi.org/10.5256/f1000research.148092.r175026

Francisco Centeno
[1] Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura, Badajoz, Extremadura, Spain
[2] Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura, Badajoz, Extremadura, Spain

I have no further comments to make.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics, Arginase, Protein structure

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 11 April 2023

https://doi.org/10.5256/f1000research.142667.r165815

?

Francisco Centeno

[1] Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura, Badajoz, Extremadura, Spain
[2] Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura, Badajoz, Extremadura, Spain
[3] Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Ciencias, Universidad de Extremadura, Badajoz, Extremadura, Spain

The authors of this study employed various CADD techniques to screen structural analogs of natural products that could potentially serve as new therapeutic agents against leishmaniasis. The study is well-oriented and well-planned, particularly about how the screening of candidate molecules has been carried out from various compound libraries. This last point seems to be the best and most consistent aspect of the work in its current format.

However, all the work on simulating the molecular dynamics of the interaction between the ligands and the arginase of *L. infantum* is based on the structure determined in Swiss-Model, using the structure of the arginase of *L. mexicana* as a template. Since the entire study relies on this structure determination being accurate, it is crucial that sufficient structural data be incorporated into this work to convincingly demonstrate that the structure determination obtained in silico is the best possible.

In this regard, some issues also arise that should be addressed:

1. Have any refinements been made to the structure initially obtained from Swiss-Model to improve it?

2. Why has only the arginase from *L. infantum* been chosen for dynamic simulation studies, and not the arginases from *L. brasiliensis* and/or *L. mexicana*, whose structures are known? It would be interesting to compare the dynamic data obtained for *L. infantum* with those obtained for *L. mexicana*, whose structure is known.

Another important point that limits the work presented, in which I agree with the authors, is that the time scales obtained for the stabilities of the arginase-ligand complexes are surprisingly short. Although the authors discussed this in the discussion section, their explanation seems implausible to me. To clarify this point, I suggest comparing the stability times of the complexes with those obtained with the physiological ligand, arginine, and comparing the arginine complexes with human and *L. mexicana* arginases, both of which are proteins with known structures.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics, Arginase, Protein structure

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 11 May 2023

Miguel Angel Chavez-Fumagalli

REVIEWER 1
The authors of this study employed various CADD techniques to screen structural analogs of natural products that could potentially serve as new therapeutic agents against leishmaniasis. The study is well-oriented and well-planned, particularly about how the screening of candidate molecules has been carried out from various compound libraries. This last point seems to be the best and most consistent aspect of the work in its current format.
ANSWER: Thank you for your assessment and constructive criticism. We have included all the recommendations described below to improve the manuscript.

However, all the work on simulating the molecular dynamics of the interaction between the ligands and the arginase of L. infantum is based on the structure determined in Swiss-Model, using the structure of the arginase of L. mexicana as a template. Since the entire study relies on this structure determination being accurate, it is crucial that sufficient structural data be incorporated into this work to convincingly demonstrate that the structure determination obtained in silico is the best possible.
ANSWER: I appreciate your observation. We made comments in the methods sections of the text to address this crucial point brought up by the reviewer.

In this regard, some issues also arise that should be addressed:
Have any refinements been made to the structure initially obtained from Swiss-Model to improve it?
ANSWER: Thanks for your assessment. As stated in the methods section we performed an automated modeling analysis and selected the best models for each target.

Why has only the arginase from L. infantum been chosen for dynamic simulation studies, and not the arginases from L. brasiliensis and/or L. mexicana, whose structures are known? It would be interesting to compare the dynamic data obtained for L. infantum with those obtained for L. mexicana, whose structure is known.
ANSWER: Thank you for your keen observation. We chose the ARG from L. infatum

since it is the species that causes leishmaniasis, it has the most severe form of the disease, and if VL is confirmed, therapy against it is required. While many of the natural compounds included in this study bind to highly conserved residues in Leishmania ARG, according to docking experiments conducted by numerous labs. In fact, a lot of them seem to use the same residues regardless of their structural classification [da Silva, Edson Roberto, et al. "Cinnamic acids derived compounds with antileishmanial activity target Leishmania amazonensis arginase." Chemical Biology & Drug Design 93.2 (2019): 139-146.]. Additionally, we restricted our research to New World Leishmaniasis because, as far as we are aware, no other crystal structures outside L. mexicana ARG have been deposited.

Another important point that limits the work presented, in which I agree with the authors, is that the time scales obtained for the stabilities of the arginase-ligand complexes are surprisingly short. Although the authors discussed this in the discussion section, their explanation seems implausible to me. To clarify this point, I suggest comparing the stability times of the complexes with those obtained with the physiological ligand, arginine, and comparing the arginine complexes with human and L. mexicana arginases, both of which are proteins with known structures.
ANSWER: I appreciate your observation. We made comments in the methods and results sections of the text to address this crucial point brought up by the reviewer. We carried out the MDS at 100 ns under the same conditions as those mentioned earlier in the manuscript. Since the parasite replicates in the macrophages, stability at low pH is the requirement that a medication must meet, as stated in the article. Several of the natural products that were employed as models for the selection of the structural analogs have demonstrated intracellular action in vitro.

Competing Interests: No competing interests.