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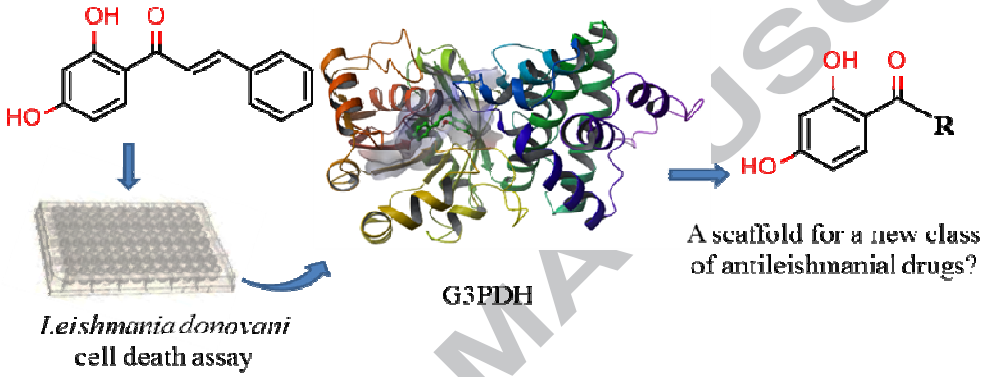

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The 2´,4´-dihydroxychalcone could be explored to develop new inhibitors against the glycerol-3-phosphate dehydrogenase from Leishmania species

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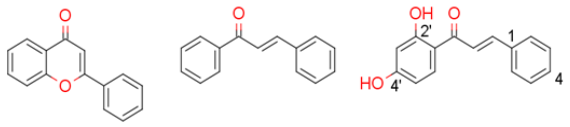
ABSTRACT

The enzyme glycerol-3-phosphate dehydrogenase (G3PDH) from Leishmania species is considered an attractive target to design new antileishmanial drugs and a previous in silico study reported on the importance of chalcones to achieve its inhibition. Here, we report the identification of a synthetic chalcone in our in vitro assays with promastigote cells from *Leishmania amazonensis*, its biological activity in animal models, and docking followed by molecular dynamics simulation to investigate the molecular interactions and structural patterns that are crucial to achieve the inhibition complex between this compound and G3PDH. A molecular fragment of this natural product derivative can provide new inhibitors with increased potency and selectivity.

The Leishmaniasis is a complex of diseases caused by a protozoan of the genus Leishmania, which is spread over 98 countries around the world and affects about 12 million people, endangering other 350 million.[1] It is one of the most neglected diseases due to the absence of interest from pharmaceutical industry for developing new drugs against it.[2] This parasitic disease has been classified into three groups, visceral, cutaneous, and mucocutaneous Leishmaniasis [3], which have different clinical manifestations and degree of morbidity and mortality.[4] Transmission is caused by the flying vector Phlebotomines[5], and two morphological forms are present during the life cycle of this parasite, promastigotes and amastigotes.[6] Promastigotes are injected into the mammalian host during the insect blood meal[7], and phagocytised by macrophages. After, they transform into amastigotes form that survive and multiply within the macrophage phagolysosome.[8] The current therapy of Leishmaniasis relies upon few drugs[9, 10] that show toxicity and/or inefficiency[5, 7], which evidence the high necessity of more efficacious drugs. In this sense, natural products can be a valuable alternative to provide huge diversity of chemical structures for biological screening campaigns against Leishmania species to develop new antileishmanial drugs.

We have identified a 2´,4´-dihydroxychalcone (DHC) inhibitor against promastigotesforms of *Leishmania amazonensis* during our cellular assays. Accordingly, chalcones have been reported that are promising inhibitors of glycerol-3-phosphate dehydrogenase[11], a glycosome enzyme that plays a critical role in the parasite to keep its essential metabolism, and it is considered as an attractive target to develop new antileishmanial drugs. The simple structure of chalcones and their ease of preparation make them an attractive scaffold to attach functional groups for enhancing their biological activities.[12] Basically, these molecules are open-chain flavonoids (figure 1) in which the two aromatic rings join by a three-carbon α, β-unsaturated carbonyl system.[13]



A B C

Figure 1. 2D basic structure of a flavonoid (A), a chalcone (B), and the leishmanicidal DHC identified in our screening assays with atoms numbered accordingly (C). Structures sketched with Marvin.[14]

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Based on these good results we extend the investigation with DHC and the G3PDH enzyme by docking and dynamics simulation to reveal the molecular interactions that could drive the formation of this inhibition complex. A deep understanding of such interactions is a crucial step to address the synthesis of new derivatives with increased affinity for G3PDH.

The DHC (MW 240.26) was synthesized following the Claisen Schmidt condensation using the described protocol by Zeraik and collaborators[15], with minor modifications. Briefly, in a 200 mL vial, 2´,4´-dihydroxyacetophenone (20 mmol) and potassium hydroxide (0.8 mol) were dissolved in ethanol (80 mL), and the mixture was stirred at 5 ºC for 10 minutes followed by addition of benzaldehyde (20 mmol in 10 mL of ethanol). The reaction mixture was stirred at room temperature and monitored by TLC using hexane:ethyl acetate as the mobile phase (7:3). The reaction was quenched after 24 h by pouring the mixture into 500 mL of ice-cold water with stirring. A precipitate formed after quenching with cold water, which was then filtered. The crude product was purified by flash chromatography with hexane:ethyl acetate in increasing order of polarity. The compound was identified using 1H and 13C NMR spectral data obtained from a Varian DRX-500 spectrometer (11.7 T). Chemical shifts (δ) were expressed in ppm. Coupling constants (J) were expressed in Hz, and splitting patterns are described as follows: s = singlet; br s = broad singlet; d = doublet; t = triplet; m = multiplet; and dd = doublet of doublets. The white solid was obtained in 20% yield. 1H NMR (500 MHz, DMSO-d6):δ 8.12 (d; J = 9.0 Hz, H-6'), 7.93 (d; J = 15.5 Hz, H-β), 7.86 (dd; J = 7.0, 2.0 Hz, H-2 and H-6), 7.75 (d; J = 15.5 Hz, H- α), 7.43 (m, H-3 – H-5), 6.36 (dd; J = 9.0, 2.0, H-5'), 6.22 (d; J = 2.0 Hz, H-3');13C NMR (125 MHz, DMSO-d6): δ 190.7 (C-β'), 168.0 (C-4'), 166.1 (C-2'), 143.1 (C-β), 134.7 (C-1), 132.9, (C-6'), 130.5 (C-4), 128.9 (C-2, C-3, C-5, C-6), 121.5 (C-α), 112.3 (C-1'), 109.1 (C-5'), 102.7 (C-3').

Adult male Swiss albino mice (20–35 g) were used in the experiments. They were housed in single-sex cages under a 12 h light:12 h dark cycle (lights on at 06:00) in a controlled room temperature (22 °C), and free access to food and water. Groups of two animals were used in each test group. The experiments were performed after the protocol was approved by the local Institutional Ethics Committee (protocol number 53/2012), which follows the Legislation for the protection of animals as described by the Directives from the European Commission.

L. amazonensis promastigotes (MPRO/BR/1972/M1841-LV-79) freshly isolated from mice were kept at 28°C in liver infusion tryptose (LIT) supplemented with 10% fetal bovine serum (FBS), penicillin and streptomycin (Sigma-Aldrich®).

Flat-bottom plates (TPP®) were seeded with cultured promastigotes from L. amazonensis at the end of exponential growth phase (7 days) to achieve 8 x 106 parasites/mL per well. DHC was dissolved in DMSO, added to each well in dilution series ranging from 0.195 µg/mL to 100.0 µg/mL, and incubated at 28 °C for 72 h. The assays were carried out in triplicate. Leishmanicidal effect was assessed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) method modified accordingly.[16] Absorbance was read at 490 nm (Robonik®). The drug concentration corresponding to 50% of parasite growth inhibition was expressed as the inhibitory concentration (IC_{50}).

To evaluate the cytotoxicity, thioglycolate-stimulated mice were used to collect peritoneal macrophages. Murine peritoneal macrophages were seeded in 96 well flat-bottom plates (TPP®) at a density of 1 x 105 cells/well (100 µL/well) in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 25 mM HEPES and 2 mM L-glutamine, and incubated during 4 h at 37 °C in a 5% CO_2-air mixture. The medium was removed and a new one was added to the cells which were treated with different concentrations of DHC and pentamidine. Cells without drugs were used as negative control. After that, plates were incubated for 24 h at 37 °C in a 5% CO_2-air mixture. Subsequently, the MTT colorimetric assay was carried out as described above. Absorbance was read in a 96-well plate reader (Robonik®) at 540 nm. The drug concentration corresponding to 50% of cell growth inhibition was expressed as the cytotoxic concentration (CC_{50}).

Previous study reported that chalcones can exhibit strong interaction with G3PDH from Leishmania.[11] In that in silico investigation the crystal structures deposited in Protein Data Bank[17] with codes 1EVZ, 1M66, 1N1E and 1N1G were used, all of them with identical residue sequences. A rapid comparison of the aminoacid sequences of G3PDH from *L. mexicana*, *L. infantum*, *L. major*, *L. brasiliensis*, and *L. donovani* through an alignment procedure in the UniProt[18] (www.uniprot.org) corroborated the high conservation degree of their binding site (data not shown). These evidences prompted us to choose 1N1E[19] (1.90 Å of resolution) as the based target structure for our computational studies. The crystal structure of human G3PDH (PDB ID 1X0X)[20] was chosen for structural superposition and comparison. The steps of reconstruction, stereochemical corrections and energy minimization of the G3PDH structure 1N1E were performed by the KoBaMIN server.[21] The 3D structure of DHC was sketched through Maestro[22] and docking was performed with AutoDock v4.2 and AutoDock Tools v1.5.6[23] following the default steps. Briefly, merged non-polar hydrogens were set to DHC structure and in the modeled 1N1E, flexibility of the DHC was considered by the number of torsions set automatically, and Gasteiger charges were applied for both. The map of the region for docking was assigned through Autogrid considering the binding site of the adenosine analogue in the 1N1E structure. The grid was set to 70 Å in all dimensions taking the coordinates for the center as 29, 25 and 22 for x, y and z respectively. Rigid docking was carried out, using Lamarckian genetic algorithm as search parameter, keeping the other default options. After, we performed a structural comparison through a superposition of 1N1E with docked DHC and 1X0X to identify differences between the binding sites for useful subpockets to be explored for selectivity.

The best pose out of 10 conformations in terms of energy scoring was used as the initial complex to set up the system for molecular dynamics simulation with Desmond v3.8[24] with graphical user interface from Maestro.[22] The system was neutralized with adequate counter ions, NaCl in a concentration of 150 mM was added, and the complex of G3PDH-DHC was surrounded by single point charge (SPC) model of water molecules. The force field assigned for the system was OPLS-AA[25]in orthorhombic box shape with the distance of 10 Å from the complex to the box wall in all directions. The SHAKE algorithm was used for geometrical constrains of the molecules and bond lengths with hydrogens, and long-range electrostatic interactions treated by Smooth Particle Mesh Ewald while a cutoff radius of 9 Å was used to calculate short-range coulombic interactions. Minimization was carried out using steepest descent method until 1.0 kcal/mol/Å of convergence and equilibration following a multistep protocol. Basically, these were the steps: i) system relaxation with Brownian dynamics using NVT ensemble at 10 K with position restraints for protein and ligand, except for hydrogens; ii) simulation with NVT ensemble and Berendsen thermostat with temperature kept at 10 K; iii) simulation with NPT ensemble and Berendsen for both thermostat and barostat in

the same temperature with 1 atm of pressure, with position restraints excepting for hydrogens; iv) Solvation of the interaction site; v) simulation in NPT ensemble with Berendsen thermostat and barostat, at 300 K and pressure of 1 atm, keeping solution restraints, a fast relaxing constant of temperature, and a slow relaxing of pressure; vi) simulation in NPT ensemble, with the same thermostat and barostat at 300 K, pressure of 1 atm, a fast relaxing constant of temperature, and a normal relaxing constant of pressure. Then, simulation was run during 10 ns at 300 K and afterwards several analyses of the trajectory were performed to evaluate the time-dependent structural modifications and molecular interactions.

DHC was assayed in a culture of promastigote cells from *L. amazonensis* and its cytotoxic activity was evaluated with peritoneal macrophages. The half of the maximal inhibitory concentration (IC_{50}) and the cytotoxic concentration (CC50) values are given in table 1. The leishmanicidal activity of DHC was nearly 16 times higher than pentamidine, a drug that is used in the treatment of Leishmaniasis in South America[26], and it was about 200 times more selective for the parasite in comparison to the same control.

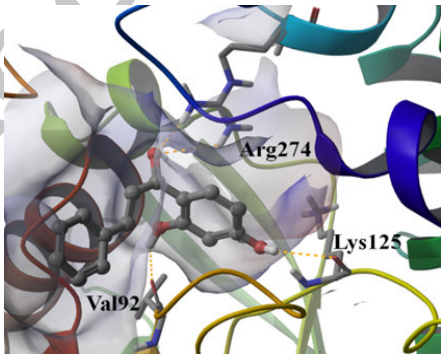
Table 1. Biological activity of DHC and Pentamidine against promastigotes and amastigotes of *L. amazonensis* (IC_{50}), macrophages (CC_{50}) and selectivity index (SI).

Compound	IC_{50} (µM) promastigotes	CC_{50} (µM) macrophages	SI
DHC	0.4 ± 0.1	416.7 ± 2.1	1041.7
Pentamidine	6.7 ±0.5	35.7±6.8	5.3

Selectivity index represented by the ratio between CC_{50} and IC_{50} for promastigotes.

Based on the good results from these biological assays and data previous reported we proceeded our investigation by applying docking and molecular dynamics simulation. As cells from *L. mexicana and L. amazonensis* show high morphological and a very similar mode of biochemical interaction with the host[27] we used the crystal structure of G3PDH from *L.mexicana* (PDB ID 1N1E) as the biochemical target for computational studies. As this is an essential enzyme in glycolysis pathway from which the parasite is highly dependent in a specific form of its life cycle, it is reasonable to propose the interaction between DHC and this biological target to explain the leishmanicidal effect observed.

The poses from the docking of DHC into the binding pocket of the 1N1E resulted in an estimative of the binding energy ranging from -6.68 to-8.39 kcal.mol^{-1}(figure 2).



BE = -8.39 LE = 0.47 nH-bonds = 3

Figure 2. Pose that retrieved best scoring from the DHC docked in the binding site of 1N1E. The residues and the H-bonds (yellow dashed lines) identified are highlighted, and also the pocket surface of DHC. BE: binding energy; LE: ligand efficiency; nH-bonds: number of H-bonds. Figure generated with Maestro.[22]

In the context of the drug design process the fragment-based approach has proved to be a very consistent and reward strategy to discover new leads, and Rule of Three (Ro3) is used largely as a guidance to enrich fragment libraries for this purpose.[28] As it can be seen from figure 2, important H-bonds contacts between DHC and the residues Val92, Lys125 and Arg274 were identified by docking, and the BE and LE indexes highlight that DHC is a promising fragment for the process of chemical optimization to generate new lead candidates.[29] Furthermore, the superposition of reconstructed 1N1E and 1X0X revealed differences in the residues surrounding the binding pocket used as receptor target for docking, which can be chemically explored for selectivity (figure 3).

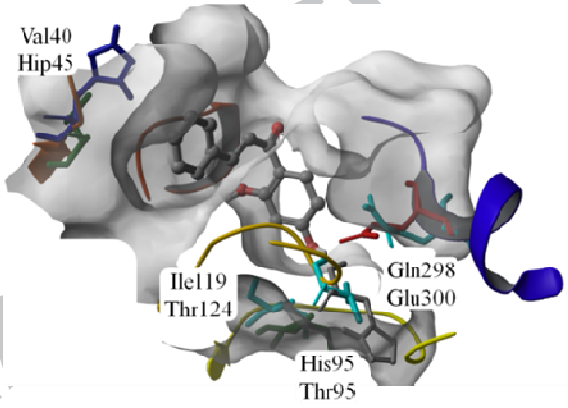
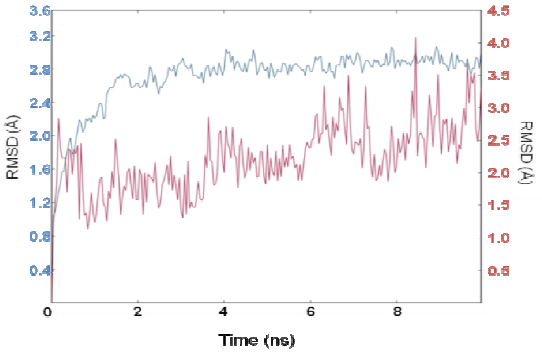


Figure 3. Superposition of the active sites from 1N1E and 1X0X. DHC is showed positioned in the center with respect to its docking onto 1N1E. Surface denotes the binding pocket of DHC and differences in terms of the residues from the binding sites are highlighted. The residues Val40, His95, Ile119 and Gln298 in 1X0X are substituted by His45, Thr95, Thr124 and Glu300 in 1N1E respectively. Hip: protonated Histidine. Residues colored by properties. Gray: hydrophobic; cyan: hydrophilic; red: anionic; blue: protonated. Figure generated with Maestro.[22]

Investigating the dynamics between DHC and G3PDH is fundamental for revealing the structural pattern and chemical groups that respond for the intermolecular recognition. Then, we investigated such complex by running 10 ns of MD simulation in an attempt to identify the time-dependent events that can dictate the formation of the DHC-G3PDH complex identified by docking. The results are shown in figure 4.



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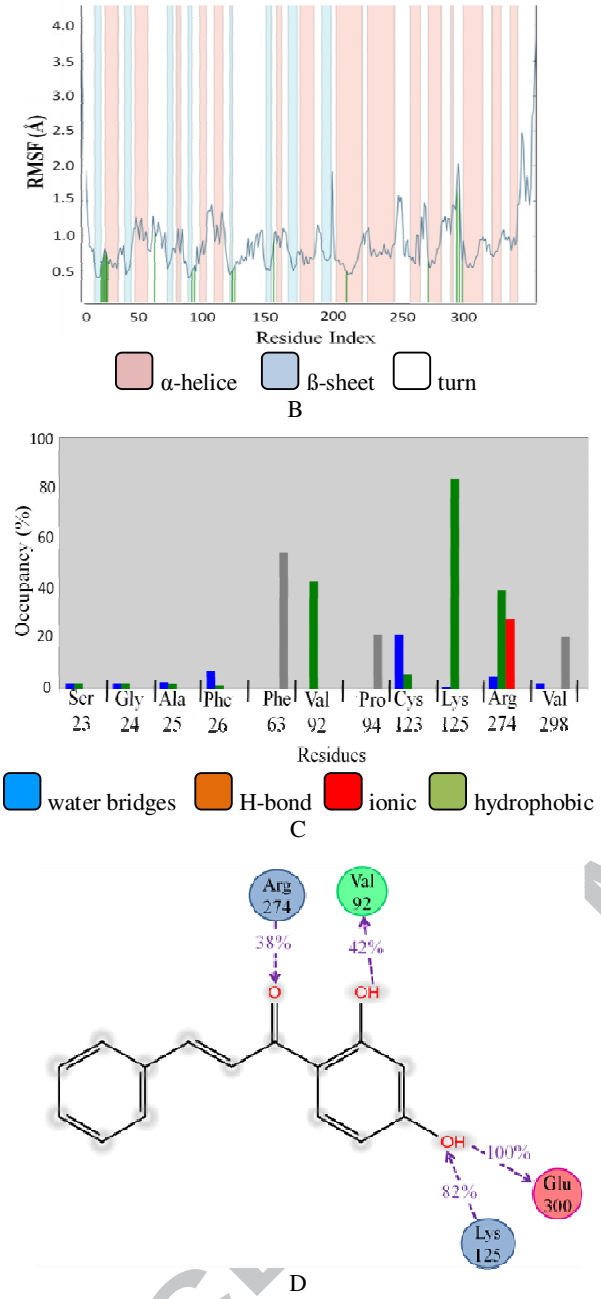

Figure 4. Dynamics of interactions between DHC and G3PDH. A) Root Mean Square Deviation (RMSD) from DHC-G3PDH complex observed for the trajectory during 10 ns of simulation; B) Root Mean Square Fluctuation (RMSF) from the G3PDH C-alphas and numbered residues interacting with DHC (green lines); D) Bar plot of main interactions between DHC and G3PDH binding pocket during the simulation; D) Representative interaction of the main H-bonds detected with an interactions cutoff of 35%. Figures generated with Maestro.[22]

The RMSD graph of the backbone (figure 4.A) highlights a fluctuation of about 3 Å of the protein backbone in comparison to the initial complex until a more stable conformation of this system is reached, which occurs at around 3 ns, thus reflecting that G3PDH exhibits small changes in its conformation with exception of the random N- and C-terminal portions. DHC showed conformational changing (RMSD) up to 1 Å as the simulation proceeds, indicating that DHC fills in the pocket and shows compatible flexibility to follow the dynamics of the binding site as simulation proceeds. MD uncovered the formation of additional H-bonds with other residues, in particular between the 4´-hydroxy group and Glu300, providing a clue to emphasize the need of an H-bond donor in this position to assure high affinity for the binding site of G3PDH, which was not identified by docking. This is a relevant structural pattern and distinguished molecular behavior of DHC as its structure can follow the conformations adopted by the binding site of G3PDH, thus allowing complementary of the interactions with other residues also buried in the binding pocket. DHC structure shows both flexibility and strategic groups to switch interactions with various residues in additional hot spots as the structure of the protein moves ahead and that can operate as a paramount feature of the good DHC affinity for G3PDH. Then, a combination of factors like strategic positions of groups for H-bond interactions, compatible volume to a buried binding pocket and reasonable flexibility of DHC collaborate for promoting the complex formation with G3PDH. The pattern of DHC interaction with G3PDH by the MD simulation indicated a prevalence of H-bond with the residues Val92, Lys125, Arg274, and Glu300 equal or more than 40% of the simulation time. The more important hydrophobic contact was with Phe63 located at the entrance of the binding site, and together with others hydrophobic residues in this region could reinforce the DHC interaction by decreasing the solvent accessibility and avoiding the return of DHC to the aqueous phase. Ionic interaction was identified with Arg274, which persisted about 30% of the trajectory. The H-bonds with Glu300 (100% of the trajectory), Lys125 (more than 80%), Val92 (more than 40%) together with the multiple interactions with Arg274 were the prevalent hydrophilic forces. Table 2 lists the residues and the type of interactions they kept with DHC.

Table 2. Main residues that interacted with DHC and type of interactions observed during the MD simulation.

Residue	Type of interaction	Chemical group in DHC	Occupancy (%)
Ser23	Hb	Phenyl ring	2
Gly24	Hb (m, d)	C=O	2
Ala25	Hb (m, d)	C=O	2
Phe26	Hb (m, d)	C=O, -OH (2´)	1
Phe63	h (s)	Phenyl ring	54
Val92	Hb (m, a)	-OH (2´)	43
Pro94	h	dihydroxyphenyl ring	21
Cys123	Hb (m, a)	-OH (2´)	27
Lys125	Hb (m, d)	-OH (4´)	84
Arg274	Hb (s, d), i	C=O	72
Val298	Hb (m, d)	C=O	23
Glu300	Hb (s, a)	-OH (4´)	100

wb: water-bridge; Hb: H-bond; i: ionic; h: hydrophobic; m: main chain; s: side chain; d: donor; a: acceptor: C=O: carbonyl oxygen; -OH: hydroxyl group numbered accordingly.

The MD simulation provided a substantial evidence of the hot spots in the binding pocket of G3PDH with respect the DHC compound. The side chains of Arg274 and Glu300 as well as the NH of main chain from Lys125 provided the stronger hydrophilic interactions, and they can be considered as key residues for H-bond interactions with DHC. On the other hand, the residues

Val92, Cys123, and Val298 can provide additional H-bonds to also explore for more potent DHC ligands. The carbonyl group attached to and the dihydroxyphenyl ring is likely to hold the moiety that keeps the effective interaction with the binding site, and thus it represents the lowest structural fragment that should be retained in the structure of new derivatives to assure its inhibitory activity against G3PDH. Low occupancy observed with other residues also contributes transiently to enforce the complex, and can be important to the design of new inhibitors. These findings open up a new route to address the development of even other classes of G3PDH inhibitors starting from a carbonyl dihydroxyphenyl ring moiety. Additionally, the side chains of the residues His45, Thr95, Thr124 and Glu300 that are equivalent to Val40, His95, Ile119 and Gln298 respectively in the human G3PDH provide important subpockets for the exploration of more selective inhibitors.

In summary, we have identified a 2´,4´-dihydroxychalcone as an important leishmanicidal compound against. Previous studies indicated high affinity of chalcones for G3PDH and results from biological assays evidenced the potency and selectivity of DHC against promastigote cells from *L. amazonenesis*, which supported the investigation of the mechanism by which DHC could inhibit G3PDH. Docking studies retrieved good values of binding estimation which corroborates that this essential glycolytic enzyme could be a target of such compound. MD simulation highlighted the interactions that could dictate the molecular behavior from G3PDH-DHC complex, providing the molecular knowledge for the design of new analogue inhibitors with increased potency and selectivity for G3PDH of Leishmania species. Additionally, the simulation data revealed that DHC is very suitable to employ for chemical optimization to generate a new antileishmanial lead. The better IC50, CC50, and selectivity profile in comparison to the drug pentamidine – which is in clinical use – is also a substantial evidence of its features that establishes the DHC structure as a very useful ligand with increased chances to provide a new chemotherapeutic agent to treat Leishmaniasis. Furthermore, the carbonyl dihydroxyphenyl ring can be considered as a very relevant substructure to be included in a fragment-based pipeline for discovering new inhibitor classes of G3PDH from Leishmania species.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

References and Notes

[1] A.K. Haldar, P. Sen, S. Roy, Use of Antimony in the Treatment of Leishmaniasis: Current Status and Future Directions, Molecular Biology International, 2011 (2011) 23.
[2] J.N. Burrows, R.L. Elliott, T. Kaneko, C.E. Mowbray, D. Waterson, The role of modern drug discovery in the fight against neglected and tropical diseases, MedChemComm, 5 (2014) 688-700.
[3] C.A. Philips, C.R. Kalal, K.N.C. Kumar, C. Bihari, S.K. Sarin, Simultaneous Occurrence of Ocular, Disseminated Mucocutaneous, and Multivisceral Involvement of Leishmaniasis, Case Reports in Infectious Diseases, 2014 (2014) 4.
[4] N. Singh, M. Kumar, R.K. Singh, Leishmaniasis: Current status of available drugs and new potential drug targets, Asian Pacific Journal of Tropical Medicine, 5 (2012) 485-497.
[5] P. Bhargava, R. Singh, Developments in Diagnosis and Antileishmanial Drugs, Interdisciplinary Perspectives on Infectious Diseases, 2012 (2012) 13.
[6] P.A. Bates, Transmission of Leishmania metacyclic promastigotes by phlebotomine sand flies, International Journal for Parasitology, 37 (2007) 1097-1106.
[7] L.H. Freitas-Junior, E. Chatelain, H.A. Kim, J.L. Siqueira-Neto, Visceral leishmaniasis treatment: What do we have, what do we need and how to deliver it?, International Journal for Parasitology: Drugs and Drug Resistance, 2 (2012) 11-19.
[8] F. Chappuis, S. Sundar, A. Hailu, H. Ghalib, S. Rijal, R.W. Peeling, J. Alvar, M. Boelaert, Visceral leishmaniasis: what are the needs for diagnosis, treatment and control?, Nat Rev Micro, 5 (2007) 873-882.
[9] G. Kaur, B. Rajput, Comparative Analysis of the Omics Technologies Used to Study Antimonial, Amphotericin B, and Pentamidine Resistance in Leishmania, Journal of Parasitology Research, 2014 (2014) 11.
[10] M. Ouellette, J. Drummelsmith, B. Papadopoulou, Leishmaniasis: drugs in the clinic, resistance and new developments, Drug Resistance Updates, 7 (2004) 257-266.
[11] I.V. Ogungbe, W.R. Erwin, W.N. Setzer, Antileishmanial phytochemical phenolics: Molecular docking to potential protein targets, Journal of Molecular Graphics and Modelling, 48 (2014) 105-117.
[12] R. Romagnoli, P.G. Baraldi, M.D. Carrion, O. Cruz-Lopez, C.L. Cara, J. Balzarini, E. Hamel, A. Canella, E. Fabbri, R. Gambari, G. Basso, G. Viola, Hybrid α-bromoacryloylamido chalcones. Design, synthesis and biological evaluation, Bioorganic & Medicinal Chemistry Letters, 19 (2009) 2022-2028.
[13] Z. Nowakowska, A review of anti-infective and anti-inflammatory chalcones, European Journal of Medicinal Chemistry, 42 (2007) 125-137.
[14] Marvin 5.7.1, ChemAxon, www.chemaxon.com.
[15] V.F.X. M.L. Zeraik, L.O. Regasini, L.A. Dutra, D.H.S. Silva, L.M. Fonseca, D. Coelho, S.A.S. Machado, V.S. Bolzani, 4´-Aminochalcones as novel inhibitors of the chlorinating activity of myeloperoxidase., Current Medicinal Chemistry, 19 (2012) 5405-5413.
[16] V.A.F.F.M. Santos, L.O. Regasini, C.R. Nogueira, G.D. Passerini, I. Martinez, V.S. Bolzani, M.A.S. Graminha, R.M.B. Cicarelli, M. Furlan, Antiprotozoal Sesquiterpene Pyridine Alkaloids from Maytenus ilicifolia, Journal of Natural Products, 75 (2012) 991-995.
[17] F.C. Bernstein, T.F. Koetzle, G.J.B. Williams, E.F. Meyer, M.D. Brice, J.R. Rodgers, O. Kennard, T. Shimanouchi, M. Tasumi, The Protein Data Bank, European Journal of Biochemistry, 80 (1977) 319-324.
[18] T.U. Consortium, Reorganizing the protein space at the Universal Protein Resource (UniProt), Nucleic Acids Research, 40 (2012) D71-D75.
[19] J. Choe, D. Guerra, P.A.M. Michels, W.G.J. Hol, Leishmania mexicana Glycerol-3-phosphate Dehydrogenase Showed Conformational Changes Upon Binding a Bi-substrate Adduct, Journal of Molecular Biology, 329 (2003) 335-349.
[20] X. Ou, C. Ji, X. Han, X. Zhao, X. Li, Y. Mao, L.-L. Wong, M. Bartlam, Z. Rao, Crystal Structures of Human Glycerol 3-phosphate Dehydrogenase 1 (GPD1), Journal of Molecular Biology, 357 (2006) 858-869.
[21] J.P.G.L.M. Rodrigues, M. Levitt, G. Chopra, KoBaMIN: a knowledge-based minimization web server for protein structure refinement, Nucleic Acids Research, 40 (2012) W323-W328.
[22] v. Schrödinger Release 2014-2: Maestro, Schrödinger, LLC, New York, NY, 2014.
[23] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, AutoDock4 and AutoDockTools4:

Automated docking with selective receptor flexibility, Journal of Computational Chemistry, 30 (2009) 2785-2791.
[24] v. Schrödinger Release 2014-2: Desmond Molecular Dynamics System, D. E. Shaw Research, New York, NY, 2014. Maestro-Desmond Interoperability Tools, version 3.8, Schrödinger, New York, NY, 2014.
[25] G.A. Kaminski, R.A. Friesner, J. Tirado-Rives, W.L. Jorgensen, Evaluation and Reparametrization of the OPLS-AA Force Field for Proteins via Comparison with Accurate Quantum Chemical Calculations on Peptides†, The Journal of Physical Chemistry B, 105 (2001) 6474-6487.
[26] S.L. Croft, P. Olliaro, Leishmaniasis chemotherapy—challenges and opportunities, Clinical Microbiology and Infection, 17 (2011) 1478-1483.
[27] L. Soong, Subversion and utilization of host innate defense by Leishmania amazonensis, Frontiers in Immunology, 3 (2012).
[28] H. Jhoti, G. Williams, D.C. Rees, C.W. Murray, The 'rule of three' for fragment-based drug discovery: where are we now?, Nat Rev Drug Discov, 12 (2013) 644-644.
[29] R.A.E. Carr, M. Congreve, C.W. Murray, D.C. Rees, Fragment-based lead discovery: leads by design, Drug Discovery Today, 10 (2005) 987-992.