Experimental Parasitology 226–227 (2021) 108123

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

In vivo evaluation of anti-*Leishmania* activity of alkyltriazoles and alkylphosphocholines by oral route

Vanessa Silva Gontijo [a], Fabio Antônio Colombo [b], Patrícia Ferreira Espuri [c], Poliany Graziella de Freitas [d], Juliana Barbosa Nunes [e], Levy Bueno Alves [d], Márcia Paranho Veloso [d], Rosemeire Brondi Alves [a], Rossimiriam Pereira Freitas [a], Marcos José Marques [c,*]

[a] *Departamento de Química, ICEx, UFMG, Av. Pres. Antônio Carlos, 6627, Pampulha, Belo Horizonte, MG, 31270-901, Brazil*
[b] *Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas, Universidade Federal de Alfenas, Rua Gabriel Monteiro da Silva, 700- Centro, 37130-001, Alfenas, MG, Brazil*
[c] *Departamento de Patologia e Parasitologia, Instituto de Ciências Biomédicas, Universidade Federal de Alfenas, Rua Gabriel Monteiro da Silva, 700- Centro, 37130-001, Alfenas, MG, Brazil*
[d] *Laboratório de Modelagem Molecular e Simulação Computacional, MolMod-CS, Universidade Federal de Alfenas, Rua Gabriel Monteiro da Silva, 700- Centro, 37130-001, Alfenas, MG, Brazil*
[e] *Laboratório de Patologia de Moléstias Infecciosas, Departamento de Patologia, Faculdade de Medicina FMUSP, Universidade de São Paulo, Av. Dr. Arnaldo, 455. Cerqueira César, São Paulo, 01246-903, São Paulo, SP, Brazil*

ARTICLE INFO

Keywords:
Visceral leishmaniasis
In vivo activity
Alkylphosphocholine
Alkyltriazole
qPCR
Molecular docking

ABSTRACT

The failures in the treatment of leishmaniasis is an increasing problem around the world, especially related to resistance. Thus, we describe the synthesis and *in vivo* anti-*Leishmania* activity of alkylphosphocholine and alkyltriazoles; besides, their likely action mechanisms stem from some eventual inhibition of parasite enzymes using computational tools. These compounds were tested in an *in vivo* hamster model infected with *Leishmania Leishmania infantum chagasi*. Fifty days after parasite inoculation, the two compounds 12-azidedodecylphosphocholine (**3**) and 3-(1-(12-fluorododecyl)-1*H*-1,2,3-triazol-1-yl)propano-1-ol (**9**), were separately administered once a day as oral suspensions (25 and 12.5 mg/kg/day, respectively) during ten days, and their efficacy was compared to the reference compound pentavalent antimonial Glucantime (GLU). Compound **3** significantly reduced the number of parasites in the spleen (4.93×10^2 amastigotes/g) and liver (4.52×10^3 amastigotes/g). Compound **9** reduced the number of amastigotes in the spleen to 1.30×10^4 and 1.36×10^3 amastigotes/g in the liver. GLU was the most effective overall treatment (7.50×10^1 and 2.28×10^2 amastigotes/g in the spleen and liver, respectively). The high activity levels of these compounds *in vivo* may stem from their high *in vitro* leishmanicidal activity and lipophilicity. The *in silico* absorption, distribution, metabolism, and excretion studies also showed some anti-*Leishmania* potential. Compound **9** had more lipophilic characteristics than those of compound **3**. *In silico* studies of the nine enzymes of compounds **3** and **9** showed significant evidence of interactions with nicotimidase and tyrosine aminotransferase, demonstrating possible inhibition enzymes present in *L. (L.) infantum chagasi*. These compounds could be a promising template for developing a new class of leishmanicidal agents, by oral route, and deserve further investigation to explore different therapeutic regimens.

1. Introduction

Leishmaniasis is a complex infectious disease caused by protozoa in the genus *Leishmania*. This disease can cause a wide range of symptoms, such as single skin lesions that range from spontaneous healing to visceral form (VL), which can be fatal when untreated. Visceral leishmaniasis presents three clinical forms: asymptomatic, oligosymptomatic, and symptomatic. The symptomatic form was subdivided into acute, which can lead to death following abrupt onset and a duration of one to two months; subacute, which in turn manifests itself in children and can lead to death within a term of 5 months to one year when untreated (Anversa et al., 2018); and chronic, that has an extended

* Corresponding author.
E-mail addresses: vanessagontijo@yahoo.com.br (V.S. Gontijo), marcos.marques@unifal-mg.edu.br (M.J. Marques).

https://doi.org/10.1016/j.exppara.2021.108123
Received 2 June 2020; Received in revised form 4 June 2021; Accepted 5 June 2021
Available online 16 June 2021

course and a duration ranging from two to three years. This form is also called kalazar, and in some cases, it does not respond to chemotherapy treatment and evolves to the acute form (Pandey and Prajapati, 2018).

According to Moafi et al. (2019) some generations of canine vaccines have shown a protective immunity against *Leishmania* infection, besides one third that needs to be evaluated in other clinical trials. Although there is a proposal to establish a national vaccination policy against canine VL in Brazil, there is no solid scientific evidence that the transmission could be reduced in infected vaccinated dogs and, as a consequence, reduce the risk of *L. infantum* infection or visceral leishmaniasis in humans (Dantas-Torres et al., 2020). The treatment of the disease is restricted and has one or more complications associated with them, such as toxicity, resistance, high cost, need for long term treatment, or uncomfortable mode of administration (Michel et al., 2011; Reimão et al., 2012). Generally the treatment and control of VL are based on use of Meglumine antimoniate (Glucantime®, Sanofi-Aventis) and Sodium stibogluconate (Pentostam®, GlaxoSmithKline), which have limitations like the need for long-term administration, high toxicity, and unsuitability for use in pregnant patients (Michel et al., 2011). In 2006 (Croft and Engel, 2006), identified hexadecylphosphocholine (miltefosine), by repurposing strategy, as a lead antileishmanial compound, which had already been selected for clinical development for oral use in cancer treatment. Besides showed advantages related to the oral route of administration and had been used especially in the Indian subcontinent (Bhattacharya and Ouellette, 2018), MIL showed failures in the VL treatment (Charlton et al., 2018; da Silva et al., 2020). According to da Silva et al. (2020) it is attractive to develop analogous compounds from MIL to test regarding anti-*Leishmania* activity, because it is one of the strategies for drug development and control of leishmaniasis, as well as the synthesis and investigation of chemical molecules. These facts justify the improvements in the original molecule of MIL to overcome of VL treatment failures and the resistance from species variants of *L. infantum chagasi* and *L. donovani*. Research centers such as universities have a fundamental role in the development and establishment of study models for compounds with leishmanicidal potential *in vitro* and *in vitro* contexts (Alcântara et al., 2018), which would provide these necessary improvements to molecules such as miltefosine derivatives.

The development of new therapies against leishmaniasis has primarily been based on the screening of compounds with some potential for efficacy in growth and multiplication assays of the parasite, both *in vitro* and *in vivo*. Synthetic heterocyclic compounds containing 1,2,3-triazole rings have shown promising anti-*Leishmania* activity *in vitro*, and therefore, are important prototypes for the development of new drugs (Nandikolla et al., 2020; Pertino et al., 2020). Other authors have demonstrated the *in vitro* leishmanicidal activity of several compounds containing imidazole or triazole rings that act against promastigote forms of *Leishmania amazonensis* (Almeida-Souza et al., 2020; Holanda et al., 2020). Among the compounds tested, none show the same structure as the compound described here, which has two azoles with the difluoromethylene group that significantly inhibit parasite growth. According to the authors themselves, the results found in the *in vitro* tests make the compounds promising candidates for the development of an effective therapeutic agent (Colombo et al., 2017; Ferreira et al., 2007).

Molecular docking studies were performed to determine the best modes of binding of molecules that are present in the ligand-receptor interaction and their relative affinities, thus enabling the identification of the preferred position of compounds on the tested targets through their hydrogen bonds, ionic, hydrophobic interactions, and Van der Waals force, according to molecular weight, logP (O/W), CaCO-2 permeability, Lipinski rule, and oral absorption (Kumar and Kumar, 2019). In addition, *in silico* studies of ADME pharmacokinetic properties were carried out in order to predict the behavior of compounds after administration, supporting the identification of physicochemical parameters that affect their absorption, distribution, metabolism and excretion in the body (Chandrasekaran et al., 2018; Zhu et al., 2018). The oral bioavailability must be evaluated because the liver is an important organ of first-pass elimination. In addition to the liver, gastrointestinal epithelial cells also should be considered in this bioavailability (Chandra and Brouwer, 2004; Nakanishi and Tamai, 2015). Computational tools were used that had already been used for studies of other miltefosine analog compounds (Borsari et al., 2019; Eldehna et al., 2019; Hassan et al., 2019). Besides, these studies allow to evaluate the inhibition of *Leishmania* enzymes such as acyltransferases and cytochrome oxidases (Luque-Ortega and Rivas, 2007; Lux et al., 2000) stem from molecules that are present in the ligand-receptor interaction. This way, the present work shows the use of the 3-(1-(12-fluorododecyl)-1*H*-1,2,3-triazol-4-yl)propane-1-ol alkyltriazole compound and its pharmaceutical compositions in the treatment of leishmaniasis. The substance efficacy and its pharmaceutical composition have been shown for both *in vitro* and *in vivo* activity. This protocol adopts the oral route for administration of the pharmaceutical composition, aiming to reduce the discomfort the patient experiences during intramuscular and intravenous administration. The use of an oral route can further reduce toxic effects and, above all, allow patients undergo treatment without the need for hospital admission, which is necessary in conventional therapy. Compounds that have a higher partition coefficient have a higher affinity for the organic phase and tend to have a higher permeability rate for hydrophobic biomembranes. This quality presents a better bioavailability profile, which may result in an increase in their pharmacological effects on leishmanicidal activity (Barreiro and Fraga, 2001). In addition, the *in silico* pharmacology paradigm allows for a rich array of opportunities that will help accelerate the discovery of new targets and ultimately lead to the creation of compounds with predicted biological activity for these new targets (Ekins et al., 2007).

In the present work, we describe the synthesis of one alkylphosphocholine and other alkyltriazole compound with potential anti-*Leishmania* activity; besides their likely action mechanisms stem from some eventual inhibition of parasite enzymes using computational tools. In addition, these compounds were assayed *in vivo* in a hamster model with *Leishmania Leishmania infantum chagasi* infection.

2. Experimental

2.1. General

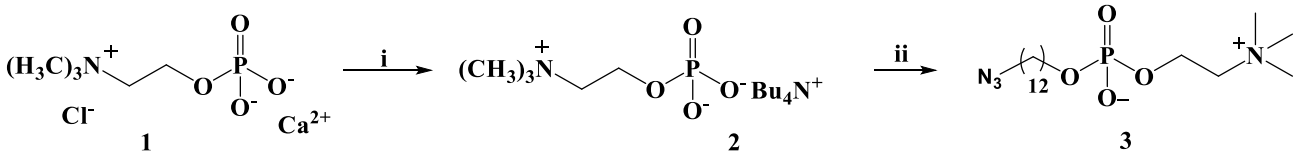
Reagents and solvents were purchased as reagent grade PA and Molecular Biology and were used without further purification. The progress of the reactions was monitored using TLC (Thin-layer chromatography) on Merck silica plates (GF254). Column chromatography was performed over silica gel 60, 70–230 mesh (Merck). Glucantime (GLU), PBS, *Tris*-HCl, EDTA, 0.5% SDS, 0.01% *N*-Lauroylsarcosine sodium salt, QIAamp DNA extraction Mini Kit (Qiagen), 100 μg/ml Proteinase K, dNTPs mix (10 mM), DTT (100 mM), KCl, $MgCl_2$, M-MLV RT enzyme, and agarose gel were purchased from Sigma Chemicals Co. (St. Louis, MO).

2.2. Synthesis

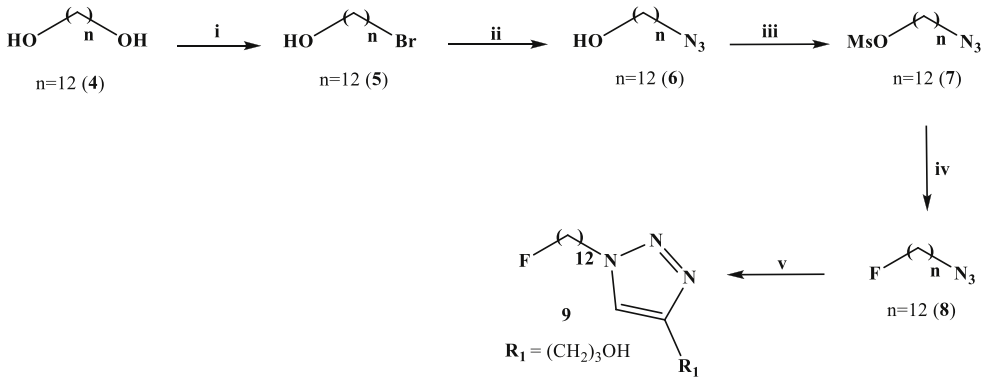
The following compounds were used in this study: 12-azidedodecylphosphocholine (**3**) and 3-(1-(12-fluorododecyl)-1*H*-1,2,3-triazol-1-yl) propano-1-ol (**9**) (Schemes 1 and 2). Their synthesis and chemical characterization were described previously (Gontijo et al, 2015a, 2015b).

2.3. Parasites and animals

L. (L.) infantum chagasi (strain MHOM/BR/1972/BH46) amastigotes were maintained by passaging in female golden hamsters (*Mesocricetus auratus*). Animals were kept in sterile absorbent material boxes with food and water *ad libitum* and in natural light/dark cycle. All experimental procedures involving animals were approved by the Research



Scheme 1. Reagents and conditions: (i) Oxalic acid, tetra-*n*-butylammonium hydroxide, pH 9; (ii) methanesulfonate alkylazide (**7**), acetonitrile, rt., 24 h and reflux, 3 h; 19%.



Scheme 2. Reagents and conditions: (i) HBr (48%), toluene, 110 °C, 24 h, 87%; (ii) NaN_3, DMSO, rt, 24 h, 85%; (iii) CH_2Cl_2, mesyl chloride, triethylamine, rt, 24 h, 90%; (iv) KF/18-crown-6, DMSO, 110 °C, 24 h, 49%; (v) NaAsc (20 mol%), $CuSO_4{\cdot}5H_2O$ (8 mol%), pent-4-yn-1-ol, CH_2Cl_2:H_2O (1:1), rt., 24 h, 46%.

Ethics Commission of the Federal University of Alfenas (project number 394/2012) and were performed according to the Guide for the Care and Use of Laboratory Animals (National Research Council Committee, 2011).

For the maintaining of the experimental infections, the spleen of infected animals was removed and macerated using a tissue grinder, and the number of amastigotes was determined as described previously (Stauber et al., 1958).

2.4. *In vivo testing of experimental compounds against Leishmania Leishmania infantum chagasi*

Female golden hamsters that had been recently weaned and weighed approximately 120 g were infected intraperitoneally with 1×10^7 amastigotes of *L. (L.) infantum chagasi* (MHOM/BR/1972/BH46) and maintained in sterile absorbent material boxes with water and food *ad libitum*. After 50 days of infection, animals were divided into four (4) groups (Compound **3**, Compound **9**, and Untreated group, n = 5/group, Glucantime n = 3) and subjected for 10 consecutive days to one of the following treatments: 0.5% carboxymethyl cellulose (CMC, vehicle) suspension, administered orally (untreated group, or UTG group), 25 mg/kg/day compound **3** and 12.5 mg/kg/day compound **9**, administered orally as suspensions in 0.5% carboxymethyl cellulose, and 50 mg/kg/day Glucantime (GLU) by intraperitoneal injection (GLU group). After 10 days of treatment, animals were sacrificed in a CO_2 chamber, and a sample of the spleen and the liver (approximately 20 mg) was removed, weighed, and used for total RNA extraction, as previously described (Reimão et al., 2011).

2.5. *RNA extraction and cDNA synthesis*

The RNA extraction and the cDNA synthesis were performed as previously described (Reimão et al., 2011). Fragments of liver and spleen (~20 mg, weighed using sterile and disposable surgical material) removed from treated hamsters were placed in sterile microfuge tubes and frozen immediately at −80 °C in a storage buffer (RNA*later*). RNA extraction was performed 24 h after fragment removal, using the RNeasy Mini Kit (Qiagen), according to the manufacturer's instructions. RNA samples were frozen immediately after extraction. For reverse-transcription into cDNA, 1 μL of dNTPs mix (10 mM) and 1 μL of random primers (3 μg/μL) were added to 11 μL of RNA sample, and samples were incubated in a thermal cycler for approximately 5 min at 65 °C. Then, tubes were placed on ice for 20 s, and 2 μL of DTT (100 mM) and 4 μL 5x buffer (Tris-HCl 250 mM, pH 8.3, containing 375 mM KCl, 15 mM $MgCl_2$) were added, and samples were incubated again in the thermal cycler for 20 s at 37 °C. Finally, 1 μL (200 U/μL) M-MLV RT enzyme was added, and samples were incubated for 50 min for cDNA synthesis. The purity of the cDNA sample was confirmed by measuring the absorbance at 260/280 in a NanoDrop ND2000, and sample integrity was verified by agarose gel electrophoresis and PCR. Samples were frozen at −20 °C for subsequent use in qPCR.

2.6. *Parasite load estimation by Linj31 quantitative PCR (qPCR)*

Quantitative real time PCR (qPCR) was performed using the TaqMan® probe 5′CCT CCT TGG ACT TTG C3' (double-labeled with FAM at the 5′-end and a non-fluorescent quencher at the 3′-end), and the primers LINJ31F (5′CCG CGT GCC TGT CG3′) and LINJ31R (5′CCC ACA CAA GGA GCG ACT3′), which amplify *L. (L.) infantum* hypothetical protein (partial mRNA; GeneBank accession number LinJ31.1310); besides qPCR was performed as previously described (Colombo et al., 2011). Reactions were performed in a StepOne™ Real-Time PCR System. (Thermo Fisher Scientific), and reaction mixtures contained 3 μL of DNA or cDNA samples (or control samples, see below), 10 μL of 2x TaqMan Universal PCR Master Mix, 1 μL of a mixture of forward (LINJ31F) and reverse (LINJ31R) primers (at a concentration of 18 μM), and 5 μM of the labeled TaqMan® probe, with a final volume of 20 μL. For negative and positive controls, water or DNA extract from *L. (L.) infantum chagasi* (MHOM/BR/1972/BH46) were added, respectively. The following PCR conditions were used: one step of 50 °C for 2 min, followed by one step of 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

2.7. *Statistical analysis*

The number of parasites per gram of spleen or liver tissue was

calculated based on the linear regression data from a standard curve made with promastigote parasites (Colombo et al., 2011). Statistical analysis was performed using a Student's t-test with Mann-Whitney (unpaired, two-tailed) to tests for significance ($p < 0.05$).

2.8. Molecular docking study

The reverse molecular docking study for the nine crystal structures (Table 1) was performed using the Glide dock-XP software. *Leishmania infantum* genome enzymes recovered from Protein Data Bank (Berman et al., 2000; Degtyarenko et al., 2008; Schrödinger Release 2019-1, 2019) were selected according to data available in the literature for verification of residues at the active site and were previously prepared using the Protein Preparation Wizard protocol (Schrödinger Release 2019-1, 2019). This protocol optimizes and minimizes the enzymes under the protonation state of pH 7.0. The substrate of each enzyme was obtained from the ChEBI base (Degtyarenko et al., 2008). The selected ligands were submitted to the Macromodel minimization protocol [30] using water as the solvent. The docking process was performed using the Induced Fit Docking (IFD) protocol (Schrödinger Release 2019-1, 2019), and the active site of the enzymes was defined by the centroids of their respective catalytic residues. All calculations were performed with the OPLS3 force field, and the box area was automatically generated. Finally, the poses were ranked using the Glide score function to represent the pharmacodynamic aptitude between protein-ligand interaction. The resulting three-dimensional complexes were visualized through the Maestro 11.2 interface (Schrödinger Release 2019-1, 2019). The ADME *in silico* properties of the proposed binders were determined using the Schrödinger Suite QikProp (Schrödinger Release 2019-1, 2019).

3. Results and discussion

Recently, we have described the synthesis of alkylphosphocholine **3** and alkyltriazoles **9** which have potent activity against promastigote (**3**, IC_{50} (μM) = 25.56 ± 1.89; **9**, IC_{50} (μM) = 37.06 ± 0.86) and amastigote (**3**, IC_{50} (μM) = 3.81 ± 0.1; **9**, IC_{50} (μM) = 15.05 ± 4.03) forms *Leishmania (L.) amazonensis in vitro*. In addition, these compounds were found to be the least toxic to human macrophages with a CC_{50} of 128.62 (**3**) and 162.81 μM (**9**), besides, it proved to be more selective (SI = 33.76 and 9.83 to **3** and **9**, respectively) than the standard drugs, such as pentamidine (CC_{50} = 11.22 μM, SI = 1.69) and amphotericin B (CC_{50} = 27.10 μM, SI = 4.44) [26].

Here, we evaluated the anti-parasitic effect of alkylphosphocholine **3** and alkyltriazoles **9** *in vivo* in hamsters with established infection (50 days post-inoculation) of *L. (L.) infantum chagasi*. For sensitive and accurate quantification of parasites in infected tissues, we used a quantitative reverse transcription PCR (qRT-PCR) assay to detect the LINJ31 marker (Linj31-qPCR), which has been successfully used for the quantification of live amastigotes of *Leishmania (L.) infantum chagasi* (Colombo et al., 2011; Reimão et al., 2011). The use of RNA as a sample, rather than DNA, minimizes the detection of residual DNA from dead parasites (Borsari et al., 2019; Colombo et al., 2015). Similar qPCR-based tests have allowed for effective quantification of parasite genetic material from the spleen and liver of infected hamsters [5,28] and yielded reliable estimates of the parasite burden per gram of tissue, while minimizing laboratory contamination, since tubes remain closed during the entire reaction time (Reimão et al., 2011; Reithinger and Dujardin, 2007).

Table 1
Crystalline structures obtained from Protein Data Bank with their respective crystallographic resolutions and binding sites.

PDB code	Resolution (Å)	Binding site	REF.
4P4M	1,91	D194, D196, D271, R273	Mejia et al. (2014)
4IX8	2,35	Y109, Y178, N225, Y256	Moreno et al. (2014a)
4AGS	2,30	V55, P56, E70, C240	Fyfe et al. (2012)
3QW3	1,70	D21, K84, I88, P199	French et al. (2011)
2P18	1,80	H78, D80, H139, H210	Silva et al. (2008)
5OL0	1,99	H144, V188, F189, F190	Ronin et al. (2018)
3R2J	2,68	D32, H75, K117, C161	Gazanion et al. (2011)
2JK6	2,95	C52, C57, T335, H461	Baiocco et al. (2009)
3L4D	2,75	Y102, L207, F289, C422	Hargrove et al. (2011)

To confirm that the system allows linear detection of parasites in infected tissue samples, we produced a standard curve using serial dilutions of DNA from cultured promastigotes of the standard strain of *L. (L.) infantum chagasi* (MHOM/BR/1972/BH46). The standard curve showed robust linearity and reproducibility (R^2 = 0.9925, slope = −3.139, and P value < 0.0001), with a strong correlation between Ct values and the number of parasites used to prepare DNA samples. We also determined that the detection limit of the system corresponded to a Ct value of 41.52.

To evaluate the anti-parasitic effect of compounds **3** and **9**, hamsters with established leishmanial infection (50 days after parasite inoculation) were treated for ten days with 25 and 12.5 mg/kg/day, respectively, of each test compound in oral suspension, and the spleen and liver of the infected animals were collected for RNA extraction, cDNA generation by reverse transcription, and qPCR analysis (qRT-PCR). The number of amastigotes per gram of spleen or liver tissue was then calculated based on the linear regression parameters obtained from the standard curve (Fig. 1).

In the untreated group (UTG, vehicle-treated), the average number of parasites was 4.67 and 5.92 × 10^5 per gram of tissue in the spleen and liver, respectively. This confirmed that these animals had an established infection with *L. (L.) infantum chagasi*. Treatment with compound **3** significantly reduced the number of amastigotes in the spleen to 4.93 × 10^2/g and in the liver to 4.52 × 10^3/g, when compared with the respective UTG ($p < 0.05$) (Fig. 1). The same was observed with compound **9**, as it reduced the number of amastigotes in the spleen of infected hamsters to 1.30 × 10^4/g ($p < 0.05$ vs. UTG) and led to a decrease in parasite numbers in the liver to 1.36 × 10^3/g, when compared with the UTG group (Fig. 1).

To check the validity of current anti-leishmanial treatment, we also treated one group of infected animals with glucantime (meglumine antimoniate) as a reference compound, since this pentavalent antimonial is currently the first-line of treatment against VL (Croft et al., 2005). In a previous study, Pinto et al. (2014) used a similar methodology to

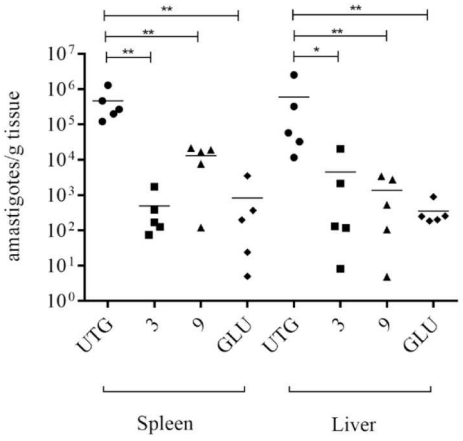


Fig. 1. Quantification of parasite burden in the spleen and liver from hamsters infected with *Leishmania (L.) infantum chagasi*, as quantified by quantitative reverse transcription PCR (qRT-PCR) for the detection of the Linj31marker. The numbers of parasites (amastigotes) per gram of tissue were calculated based on a qPCR standard curve using promastigotes. UTG, untreated group (vehicle-treated), GLU, animals treated with Glucantime (50 mg/kg/day); compound **3** (25 mg/kg/day), compound **9** (12.5 mg/kg/day). Data are represented group median and individual values for each animal (*$p < 0.05$; **$p < 0.01$; Mann-Whitney test).

evaluate the leishmanicidal activity of H1 histamine receptor antagonists using drugs that belong to therapeutic classes, such as antiallergics and anxiolytics. Unlike our study, this study used Miltefosine as the standard drug in experimental treatment. Da Silva et al. (2020) used glucantime and miltefosine as positive control to the *in vivo* assay however the derivative of alkyltriazole showed a better result in relation to miltefosine when the liver was evaluated.

Glucantime given intraperitoneally at 50 mg/kg/day was the most effective treatment for reducing the parasite burden in infected animals, and treatment with this drug led to a reduction in the number of amastigotes to 7.50×10^1 and 2.28×10^2 in the spleen and liver, respectively (Fig. 1). Glucantime was administered intraperitoneally, while the test compounds **3** and **9** were administered orally. Theoretically, soluble compounds are completely absorbed in the intravenous route, but this absorption rate does not apply to other mechanisms of drug administration. Thus, different administration routes can show different effects, because the absorbed dose can be very different from the administered dose. The nature of the compounds can lead different absorption rates as well, as most compounds are more quickly absorbed in solution than in undissolved suspensions. Furthermore, the biotransformation process after oral administration is distinct from the other routes due the first-pass effect in the intestines (Ning et al., 2015) as well as in the liver (Chandra and Brouwer, 2004; Nakanishi and Tamai, 2015). This difference in administration route may have contributed to the increased efficacy of glucantime relative to the other compounds, and the possibility remains that the anti-parasitic effect of compounds **3** and **9** would improve by increasing the concentration of compounds or changing the therapeutic scheme.

Therefore, to analyze the ADME pharmacokinetic properties of compounds **3** and **9**, an *in silico* study was performed to analyze their pharmaceutically relevant physicochemical parameters. Four descriptors were analyzed: octanol-water partition coefficient (logP O/W), Lipinski's rule of five, Caco-2 permeability, and oral absorption (Table 2). The compounds did not violate any of the Lipinski rules, presenting logP $\leq$5, molecular mass <500 g/mol, hydrogen acceptors $\leq$10, and hydrogen donors $\leq$5 (Jadhav P.B., Yadav A.R., 2015). Compounds that violate more than one of these criteria are not considered good candidates for orally active drugs. In addition, they had significant human oral absorption percentages, which may be associated with higher permeability of compound **9,** compared to **3,** and may be a factor in its greater bioavailability in the liver, which allows for efficient performance in this organ than in the spleen. Because compound **3** is a zwitterionic type, and is thus less lipophilic, it promotes greater difficulty in transposing plasma barriers and therefore expresses less leishmanicidal activity than compound **9,** which is not a zwitterionic type and has high lipophilicity. Thus, it is possible to establish a correlation between the structural characteristics of each compound and the ADME data with the observed leishmanicidal activity (Wang et al., 2015), according to the results observed in the *in vivo* liver assay (Fig. 1). In addition, the *in vitro* results (Gontijo et al., 2015a) showed that compound **3** had more activity in promastigote form whereas compound **9** was more effective in amastigote form. Probably, the explanation is related to different lipophilicity of these compounds. It is also possible that the higher leishmanicidal potential observed in front of the spleen is associated with immune stimulation of alkylphosphocoline (Sharikadze and Gongadze, 2006) that could explain the activity from compound **3** against amastigote forms of *Leishmania (L.) infantum chagasi* (Table 3).

Still within context computational analysis molecular docking studies were performed to elucidate the molecular interactions formed between compounds **3** and **9** and the existing three-dimensional structures of *Leishmania infantum* target enzymes (Table 4). Some of these targets from *Leishmania* such as acyltransferases and cytochrome oxidases (Luque-Ortega and Rivas, 2007; Lux et al., 2000) can be used to explain the mechanism of action from these compounds. The evaluation of an eventual inhibition of parasite enzymes may be done *in silico* analysis using molecules that are present in the ligand-receptor interaction. In this way following enzyme crystal structures were obtained from the PBD database for reverse docking: beta polymerase (PDB ID: 4P4M), tyrosine aminotransferase (PDB ID: 4IX8), glutathione transferase (PDB ID: 4AGS), orotate phosphoribosyltransferase (PDB ID: 3QW3), Glyoxalase II (PDB ID: 2P18), Deacetylase (PDB ID: 5OL0), Nicotinamidase (PDB ID: 3R2J), Trypanothione Reductase (PDB ID: 2JK6), and Sterol 14α-demethylase (PDB ID: 3L4D). Some of these enzymes (nicotinamidase, glutathione transferase, trypanothione reductase, and tyrosine aminotransferase) have been selected as targets in docking for previous studies (Chávez-Fumagalli et al., 2017; Moreno et al., 2014b; Ogungbe and Setzer, 2013). The remaining enzymes were reported as targets as specified in the references in Table 1. The pharmacodynamic profiles of the molecules tested were evaluated based on the energetic interactions between the enzymes and their respective natural substrates. Compound **9** reached an interaction energy close to that of the substrate for glutathione transferase and deacetylase enzymes. Compound **3** also showed an interaction energy close to that of the glyoxalase II substrate. However, compounds **3** and **9** showed significant results related to binding affinity at the active site of the enzymes tyrosine aminotransferase and nicotinamidase. Compound **3** showed better binding energy against Tyrosine aminotransferase (-6.039 kcal mol^{-1}) and against nicotinamidase (-13.83 kcal mol^{-1}), in relation to the energies obtained with the respective substrates of each enzyme. While compound **9** showed the binding energies of -4.352 and -11.523 kcal mol^{-1} against tyrosine aminotransferase and nicotinamidase, respectively. The two compounds docked well into the active pocket against these enzymes. The interactions of these enzymes with the molecules showed hydrogen bonds, salt bridges, and π interactions. The glide score energies obtained from molecular docking are shown in Table 2. The interactions of the best anchored complexes are shown in Figs. 2 and 3. Tyrosine aminotransferase and nicotinamidase carry out key role in the cellular metabolism and energy production in trypanosomatids because they are crucial for NAD + production (Gazanion et al., 2011) and to metabolize tyrosine in the cell, besides transfer the amino group to a co-substrate (Sasidharan and Saudagar, 2020), respectively.

The amino group of compound **3** forms three π-cation bonds with amino acid residues F37 and W89, and the azide group forms two salt bridges with residues D168 and K171. In addition, K117 is involved in a salt bridge near the Zn^{2+} ion, and the phosphate group forms a hydrogen bond with W89. Compound **9** forms four hydrogen bonds with D32, V156, A157 and C161. Attracted by Zn^{2+}, 1,2,3-triazole makes twoπ-π stacking connections with W89 and one with H92. Thus, both compounds can interact within the binding pocket formed by the catalytic

Table 2
ADME properties of compounds (**3**) and (**9**). The recommended values allow comparing the compounds with 95% of known drugs. Donor HB = hydrogen donor, Accept HB = hydrogen accept.

Compound	Donor HB	Accept HB	logP O/W	Solute molecular weight	Rule of five	Caco-2 permeability (nn/sec)	% Human oral absorption
3	2	2	3.311	394.493	0	50	77
9	1	4.2	4.949	313.458	0	1122	100
Recommended values banner	0–06	2–20	−2 - 6.5	130.0–725.0	Máx 4	<25 poor >500 great	>80% is high <25% is poor

Table 3
Molecular docking interaction energy. In the order of the list, the subtracts used to obtain the reference glide score were: 2′,3′-dideoxy-thymidine-5′-triphosphate (CHEBI: 41846), L-tyrosine (CHEBI: 17895), glutathione (CHEBI: 16856), orotidine 5′-phosphate (CHEBI: 15842), (R) -S-lactoylglutathione (CHEBI: 15694), N6-acetyl-L-lysine (CHEBI: 17752), nicotinamide (CHEBI: 17154), flavin adenine dinucleotide (CHEBI: 16238) and obtusifoliol (CHEBI: 17791).

PDB ID	Resolution (Å)	Protein	Glide gscore (Kcal.mol^{-1})		
			Substrate	Compound (3)	Compound (9)
4P4M	*1.91*	Beta *polymerase*	−12.768	−10.03	−9.961
4IX8	***2.35***	**Tyrosine *aminotransferase***	**−4.255**	**−6.039**	**−4.352**
4AGS	*2.30*	Glutathione *transferase*	−6.828	−5.476	−6.548
3QW3	*1.70*	Orotate *phosphoribosyltransferase*	−10.138	−3.623	−6.269
2P18	*1.80*	Glyoxalase *II*	−8.189	−8.05	−5.213
5OL0	*1.99*	Deacetylase	−11.271	−6.628	−8.574
3R2J	***2.68***	**Nicotinamidase**	**−7.411**	**−13.83**	**−11.523**
2JK6	*2.95*	Trypanothione *reductase*	−11.519	−5.284	−5.428
3L4D	*2.75*	Sterol *14α-desmethylase*	−12.475	−8.398	−9.335

Table 4
Interaction energy and description of hydrogen bonds of compounds (**3**) and (**9**) with the enzymes nicotimidase and tyrosine aminotransferase.

PDB code	Compound	Glide score (Kcal.mol^{-1})	Hydrogens bonds		
			Atom of ligand	Aminoacids	Distance (Å)
3R2J	(**3**)	−13.830	O	TRP89	1.99
	(**9**)	−11.523	O	CYS161	2.19
			O	ALA157	1.79
			H	VAL156	2.50
			H	ASP32	2.30
	Nicotinamide	−7.411	N	CYS161	2.09
			H	ASP124	1.99
			O	TRP89	1.97
4IX8	(**3**)	−6.039	O	ARG422	1.85
			O	ASN225	2.03
			O	PHE175	2.74
	(**9**)	−4.352	O	GLY79	1.90
	L-tyrosine	−4.255	H	ASN58	1.91
			H	ASN58	2.63
			O	ARG422	2.14
			O	ARG422	2.04
			O	ASN225	2.27

triad C161, K117, and D32, making them potential nicotinamidase inhibitors through competitive inhibition.

The phosphate group of compound **3** showed two hydrogen bonds with residues N225 and R422, a salt bridge involving R422, and the formation of an H-aromatic bond with F175. The azide group is stabilized *via* Van der Waals hydrophobic interactions by V155 and Y185. Compound **9** forms a hydrogen bond with G79 and a π-cation bond with R422. At the end of the carbon chain, the fluorine element is exposed to the solvent without any molecular interaction. Both compounds act on the enzyme's active site, making them potential drug candidates for tyrosine aminotransferase inhibition. Based on the docking study, it is hypothesized that these compounds can have the potential to act as anti-*leishmania* on these targets. Nicotinamidase inhibitors can infer the production of NAD^+ (lag/early log phases of growth) and the subsequent reduction of NAD^+ (stationary phase), impairing the growth and establishment of parasite infection (Gazanion et al., 2011). In tyrosine aminotransferase, inhibitors can interfere with the catabolism of aromatic amino acids, a highly active pathway in trypanosomatids (Moreno et al., 2014a). Docking results may help to underestimate possible mechanisms of action of the reported compounds.

4. Conclusion

In summary, the two compounds evaluated, 12-azidedodecylphosphocholine (**3**) and 3-(1-(12-fluorododecyl)-1*H*-1,2,3-triazol-1-yl)propano-1-ol (**9**), by oral route, showed higher levels of activity against *L. (L.) infantum chagasi in vivo* relative to that of UTG. This result correlates with the *in vitro* leishmanicidal activity of these two compounds and may be improved by changing the concentration of compounds in each dose, changing the therapeutic regimen by increasing the number of treatment days, treating two or more times a day, or a combination of these tactics. The pentavalent antimonial Glucantime was the most

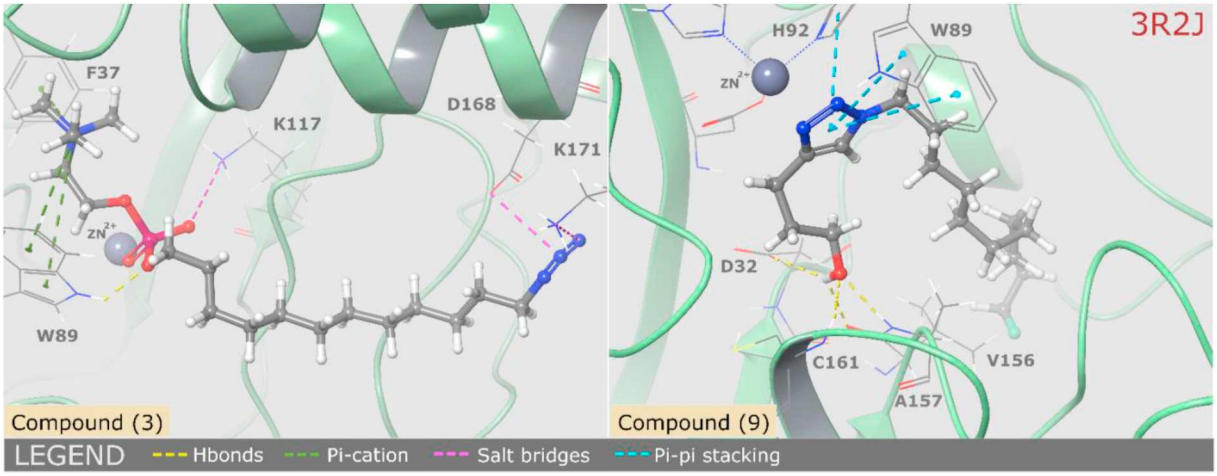


Fig. 2. Possession of interactions of compounds (**3**) and (**9**) with nicotinamidase. Atoms are represented by colors as follows: gray for carbon, white for hydrogen, blue for nitrogen, purple for phosphorus, red for oxygen and light green for fluorine. The amino acid residues that interacted with the compounds are labeled and interactions are represented as dashed lines as indicated in the image caption.

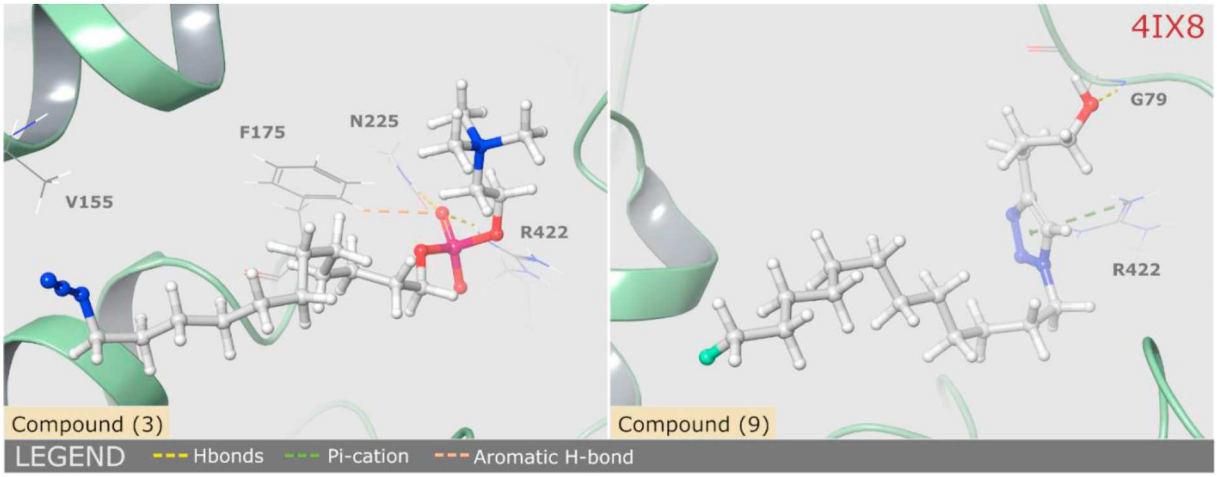


Fig. 3. Interactions of compounds (**3**) and (**9**) with tyrosine aminotransferase. Atoms are represented by colors as follows: gray for carbon, white for hydrogen, blue for nitrogen, purple for phosphorus, red for oxygen and light green for fluorine. The amino acid residues that interacted with the compounds are labeled and interactions are represented as dashed lines as indicated in the image caption.

effective in the treatment of hamsters infected with *L. (L.) infantum chagasi*. *In silico* studies of the nine enzymes of compounds **3** and **9** showed significant evidence of interactions with nicotimidase and tyrosine aminotransferase, demonstrating possible inhibition enzymes present in *L. (L.) infantum chagasi.* These compounds could be a promising template for developing a new class of leishmanicidal agents and deserve further investigation to explore different therapeutic regimens. Additional, immunological and pharmacological assays should clarify the mode of action against *Leishmania*.

Ethics approval and consent to participate

Not applicable.

Funding

This work was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG CBB APQ 02149/12), by scholarships from UNIFAL-MG, CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPEMIG and CAPES awarded to RAR and ISR, FAC, JBN.

Author statement

1) Vanessa S. Gontijo: Methodology, investigation, Writing - Original Draft;
2) Fábio A. Colombo: Software, formal analysis, Data Curation, Supervision;
3) Patrícia Ferreira Espuri: Software, formal analysis, Data Curation, Supervision
4) Poliany Graziella de Freitas: Software, methodology;
5) Juliana B. Nunes: Software, methodology, Data Curation, Writing - Original Draft, visualization;
6) Levy Bueno Alves: Software, methodology;
7) Márcia Paranho Veloso: Methodology, validation;
8) Rossimiriam P. de Freitas: Conceptualization, resources, project administration;
9) Rosemeire B. Alves: Validation, Data Curation;
10) Marcos J. Marques: Conceptualization, resources, Provision of study materials, Writing - Original Draft, Writing - Review & Editing, visualization, Supervision, project administration, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest, financial or otherwise.

Acknowledgements

We thank Dr. André Gustavo Tempone (Adolfo Lutz Institute) for providing Glucantime, and Dr. Juliana Quero Reimão (UNIFESP) for technical support. Conflict of Interest: The authors declare that they have no conflict of interest. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

List of abbreviations

ADME	Absorption, Distribution, Metabolism and Excretion
CH_2Cl_2	Dichloromethane
$CuSO_4{\cdot}5H_2O$	copper sulfate pentahydrate
DMSO	Dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylenediamine tetraacetic acid
GLU	Glucantime
HBr	Bromidic acid
IFD	Induced Fit Docking
KCl	potassium chloride
KF	potassium fluoride
logP O/W	octanol-water partition coefficient
logP	partition coefficient
$MgCl_2$	Magnesium chloride
NaAsc	sodium ascorbate
NaN_3	Sodium azide
PBS	sodium perborate
PDB	Protein Data Bank
pH	hydrogenionic potential
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
rt	room temperature
SDS	sodium dodecyl sulfate
UTG	vehicle-treated
TLC	Thin-layer chromatography
VL	Visceral Leishmaniasis

References

Alcântara, L.M., Ferreira, T.C.S., Gadelha, F.R., Miguel, D.C., 2018. Challenges in drug discovery targeting TriTryp diseases with an emphasis on leishmaniasis. Int. J. Parasitol. Drugs Drug Resist. https://doi.org/10.1016/j.ijpddr.2018.09.006.

Almeida-Souza, F., da Silva, V.D., Silva, G.X., Taniwaki, N.N., Hardoim, D. de J., Buarque, C.D., Abreu-Silva, A.L., Calabrese, K. da S., 2020. 1,4-disubstituted-1,2,3-triazole compounds induce ultrastructural alterations in leishmania amazonensis promastigote: an in vitro antileishmanial and in silico pharmacokinetic study. Int. J. Mol. Sci. 21, 1–20. https://doi.org/10.3390/ijms21186839.

Anversa, L.s., Tiburcio, M.G.S., Richini-Pereira, V. nia B., Ramirez, L.E., 2018. Human leishmaniasis in Brazil: a general review. Rev. Assoc. Med. Bras. https://doi.org/10.1590/1806-9282.64.03.281.

Baiocco, P., Colotti, G., Franceschini, S., Ilari, A., 2009. Molecular basis of antimony treatment in Leishmaniasis. J. Med. Chem. 52, 2603–2612. https://doi.org/10.1021/jm900185q.

Barreiro, E.J., Fraga, C.A.M., 2001. Química medicinal: as bases moleculares da ação dos fármacos, first ed. Porto Alegre.

Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., Bourne, P.E., 2000. The protein Data Bank. Nucleic Acids Res. https://doi.org/10.1093/nar/28.1.235.

Bhattacharya, A., Ouellette, M., 2018. New insights with miltefosine unresponsiveness in Brazilian Leishmania infantum isolates. EBioMedicine. https://doi.org/10.1016/j.ebiom.2018.10.016.

Borsari, C., Jiménez-Antón, M.D., Eick, J., Bifeld, E., Torrado, J.J., Olías-Molero, A.I., Corral, M.J., Santarem, N., Baptista, C., Severi, L., Gul, S., Wolf, M., Kuzikov, M., Ellinger, B., Reinshagen, J., Witt, G., Linciano, P., Tait, A., Costantino, L., Luciani, R., Tejera Nevado, P., Zander-Dinse, D., Franco, C.H., Ferrari, S., Moraes, C.B., Cordeiro-da-Silva, A., Ponterini, G., Clos, J., Alunda, J.M., Costi, M.P., 2019. Discovery of a benzothiophene-flavonol halting miltefosine and antimonial drug resistance in Leishmania parasites through the application of medicinal chemistry, screening and genomics. Eur. J. Med. Chem. 183, 111676. https://doi.org/10.1016/j.ejmech.2019.111676.

Chandra, P., Brouwer, K.L.R., 2004. The complexities of hepatic drug transport: current knowledge and emerging concepts. Pharm. Res. (N. Y.). https://doi.org/10.1023/B:PHAM.0000026420.79421.8f.

Chandrasekaran, B., Abed, S.N., Al-Attraqchi, O., Kuche, K., Tekade, R.K., 2018. Computer-aided prediction of pharmacokinetic (ADMET) properties. In: Dosage Form Design Parameters. Elsevier, pp. 731–755. https://doi.org/10.1016/B978-0-12-814421-3.00021-X.

Charlton, R.L., Rossi-Bergmann, B., Denny, P.W., Steel, P.G., 2018. Repurposing as a strategy for the discovery of new anti-leishmanials: the-state-of-the-art. Parasitology. https://doi.org/10.1017/S0031182017000993.

Chávez-Fumagalli, M.A., Schneider, M.S., Lage, D.P., Machado-de-Ávila, R.A., Coelho, E.A.F., 2017. An in silico functional annotation and screening of potential drug targets derived from Leishmania spp. hypothetical proteins identified by immunoproteomics. Exp. Parasitol. 176, 66–74. https://doi.org/10.1016/j.exppara.2017.03.005.

Colombo, F.A., Azara Reis, R., Barbosa Nunes, J., Ferreira Dias, D., dos Santos, M.H., Viegas Junior, C., Marques, M.J., 2017. In vivo evaluation of leishmanicidal activity of benzophenone derivatives by qPCR. Med. Chem. 890–893. https://doi.org/10.4172/2161-0444.1000448, 07.

Colombo, F.A., Odorizzi, R.M.F.N., Laurenti, M.D., Galati, E.A.B., Canavez, F., Pereira-Chioccola, V.L., 2011. Detection of Leishmania (Leishmania) infantum RNA in fleas and ticks collected from naturally infected dogs. Parasitol. Res. 109, 267–274. https://doi.org/10.1007/s00436-010-2247-6.

Colombo, F.A., Pereira-Chioccola, V.L., Meira, C.D.S., Motoie, G., Gava, R., Hiramoto, R.M., de Almeida, M.E., da Silva, A.J., Cutolo, A.A., Menz, I., 2015. Performance of a real time PCR for leishmaniasis diagnosis using a L. (L.) infantum hypothetical protein as target in canine samples. Exp. Parasitol. 157, 156–162. https://doi.org/10.1016/j.exppara.2015.08.014.

Croft, S.L., Barrett, M.P., Urbina, J.A., 2005. Chemotherapy of trypanosomiases and leishmaniasis. Trends Parasitol. https://doi.org/10.1016/j.pt.2005.08.026.

Croft, S.L., Engel, J., 2006. Miltefosine - discovery of the antileishmanial activity of phospholipid derivatives. Trans. R. Soc. Trop. Med. Hyg. 100 https://doi.org/10.1016/j.trstmh.2006.03.009.

da Silva, J.C., Nunes, J.B., Gontijo, V.S., Malaquias, L.C.C., de Freitas, R.P., Alves, R.B., Colombo, F.A., Laurenti, M.D., Marques, M.J., 2020. LEISHMANICIDAL ACTIVITY in vivo OF A MILTEFOSINE DERIVATIVE IN Mesocricetus auratus. Acta Trop. 209, 105539. https://doi.org/10.1016/j.actatropica.2020.105539.

Dantas-Torres, F., Nogueira, F. dos S., Menz, I., Tabanez, P., da Silva, S.M., Ribeiro, V.M., Miró, G., Cardoso, L., Petersen, C., Baneth, G., Oliva, G., Solano-Gallego, L., Ferrer, L., Pennisi, M.G., Bourdeau, P., Maia, C., Otranto, D., Gradoni, L., Courtenay, O., Costa, C.H.N., 2020. Vaccination against canine leishmaniasis in Brazil. Int. J. Parasitol. 50, 171–176. https://doi.org/10.1016/j.ijpara.2020.01.001.

Degtyarenko, K., De matos, P., Ennis, M., Hastings, J., Zbinden, M., Mcnaught, A., Alcántara, R., Darsow, M., Guedj, M., Ashburner, M., 2008. ChEBI: a database and ontology for chemical entities of biological interest. Nucleic Acids Res. 36 https://doi.org/10.1093/nar/gkm791.

Ekins, S., Mestres, J., Testa, B., 2007. In silico pharmacology for drug discovery: applications to targets and beyond. Br. J. Pharmacol. https://doi.org/10.1038/sj.bjp.0707306.

Eldehna, W.M., Almahli, H., Ibrahim, T.M., Fares, M., Al-Warhi, T., Boeckler, F.M., Bekhit, A.A., Abdel-Aziz, H.A., 2019. Synthesis, in vitro biological evaluation and in silico studies of certain arylnicotinic acids conjugated with aryl (thio)semicarbazides as a novel class of anti-leishmanial agents. Eur. J. Med. Chem. 179, 335–346. https://doi.org/10.1016/j.ejmech.2019.06.051.

Ferreira, S.B., Costa, M.S., Boechat, N., Bezerra, R.J.S.S., Genestra, M.S., Canto-Cavalheiro, M.M., Kover, W.B., Ferreira, V.F., 2007. Synthesis and evaluation of new difluoromethyl azoles as antileishmanial agents. Eur. J. Med. Chem. 42, 1388–1395. https://doi.org/10.1016/j.ejmech.2007.02.020.

French, J.B., Yates, P.A., Soysa, D.R., Boitz, J.M., Carter, N.S., Chang, B., Ullman, B., Ealick, S.E., 2011. The Leishmania donovani UMP synthase is essential for promastigote viability and has an unusual tetrameric structure that exhibits substrate-controlled oligomerization. J. Biol. Chem. 286, 20930–20941. https://doi.org/10.1074/jbc.M111.228213.

Fyfe, P.K., Westrop, G.D., Silva, A.M., Coombs, G.H., Hunter, W.N., 2012. Leishmania TDR1 structure, a unique trimeric glutathione transferase capable of deglutathionylation and antimonial prodrug activation. Proc. Natl. Acad. Sci. U.S.A. 109, 11693–11698. https://doi.org/10.1073/pnas.1202593109.

Gazanion, E., Garcia, D., Silvestre, R., Gérard, C., Guichou, J.F., Labesse, G., Seveno, M., Cordeiro-Da-Silva, A., Ouaissi, A., Sereno, D., Vergnes, B., 2011. The Leishmania nicotinamidase is essential for NAD + production and parasite proliferation. Mol. Microbiol. 82, 21–38. https://doi.org/10.1111/j.1365-2958.2011.07799.x.

Gontijo, V.S., Espuri, P.F., Alves, R.B., De Camargos, L.F., Dos Santos, F.V., De Souza Judice, W.A., Marques, M.J., Freitas, R.P., 2015a. Leishmanicidal, antiproteolytic, and mutagenic evaluation of alkyltriazoles and alkylphosphocholines. Eur. J. Med. Chem. 101, 24–33. https://doi.org/10.1016/j.ejmech.2015.06.005.

Gontijo, V.S., Oliveira, M.É., Resende, R.J., Fonseca, A.L., Nunes, R.R., Júnior, M.C., Taranto, A.G., Torres, N.M.P.O., Viana, G.H.R., Silva, L.M., Alves, R.B., Varotti, F.P., Freitas, R.P., 2015b. Long-chain alkyltriazoles as antitumor agents: synthesis, physicochemical properties, and biological and computational evaluation. Med. Chem. Res. 24, 430–441. https://doi.org/10.1007/s00044-014-1137-3.

Hargrove, T.Y., Wawrzak, Z., Liu, J., Nes, W.D., Waterman, M.R., Lepesheva, G.I., 2011. Substrate preferences and catalytic parameters determined by structural characteristics of sterol 14α-demethylase (CYP51) from Leishmania infantum. J. Biol. Chem. 286, 26838–26848. https://doi.org/10.1074/jbc.M111.237099.

Hassan, A.H.E., Park, H.R., Yoon, Y.M., Kim, H.I., Yoo, S.Y., Lee, K.W., Lee, Y.S., 2019. Antiproliferative 3-deoxysphingomyelin analogs: design, synthesis, biological evaluation and molecular docking of pyrrolidine-based 3-deoxysphingomyelin analogs as anticancer agents. Bioorg. Chem. 84, 444–455. https://doi.org/10.1016/j.bioorg.2018.11.040.

Holanda, V.N., Silva, W.V. da, Nascimento, P.H. do, Silva, S.R.B., Filho, P.E.C, Assis, S.P. de O., Silva, C.A. da, Oliveira, R.N. de, Figueiredo, R.C.B.Q. de, Lima, V.L. de M., 2020. Antileishmanial activity of 4-phenyl-1-[2-(phthalimido-2-yl)ethyl]-1H-1,2,3-triazole (PT4) derivative on Leishmania amazonensis and Leishmania braziliensis: in silico ADMET, in vitro activity, docking and molecular dynamic simulations. Bioorg. Chem. 105 https://doi.org/10.1016/j.bioorg.2020.104437.

Jadhav, P.B., Yadav, A.R.G.M., 2015. Concept of drug likeness in pharmaceutical research. Int. J. Pharm. Biol. Sci. 6, 142–154.

Kumar, Shivani, Kumar, Suresh, 2019. Molecular docking: a structure-based approach for drug repurposing. In: In Silico Drug Design. Elsevier, pp. 161–189. https://doi.org/10.1016/b978-0-12-816125-8.00006-7.

Luque-Ortega, J.R., Rivas, L., 2007. Miltefosine (hexadecylphosphocholine) inhibits cytochrome c oxidase in leishmania donovani promastigotes. Antimicrob. Agents Chemother. 51, 1327–1332. https://doi.org/10.1128/AAC.01415-06.

Lux, H., Heise, N., Klenner, T., Hart, D., Opperdoes, F.R., 2000. Ether-lipid (alkyl-phospholipid) metabolism and the mechanism of action of ether-lipid analogues in Leishmania. Mol. Biochem. Parasitol. 111, 1–14. https://doi.org/10.1016/S0166-6851(00)00278-4.

Mejia, E., Burak, M., Alonso, A., Larraga, V., Kunkel, T.A., Bebenek, K., Garcia-Diaz, M., 2014. Structures of the Leishmania infantum polymerase beta. DNA Repair 18, 1–9. https://doi.org/10.1016/j.dnarep.2014.03.001.

Michel, G., Pomares, C., Ferrua, B., Marty, P., 2011. Importance of worldwide asymptomatic carriers of Leishmania infantum (L. chagasi) in human. Acta Trop. https://doi.org/10.1016/j.actatropica.2011.05.012.

Moafi, M., Sherkat, R., Taleban, R., Rezvan, H., 2019. Leishmania vaccines entered in clinical trials: a review of literature. Int. J. Prev. Med. https://doi.org/10.4103/ijpvm.IJPVM_116_18.

Moreno, M.A., Abramov, A., Abendroth, J., Alonso, A., Zhang, S., Alcolea, P.J., Edwards, T., Lorimer, D., Myler, P.J., Larraga, V., 2014a. Structure of tyrosine aminotransferase from Leishmania infantum. Acta Crystallogr. Sect. FStructural Biol. Commun. 70, 583–587. https://doi.org/10.1107/S2053230X14007845.

Moreno, M.A., Alonso, A., Alcolea, P.J., Abramov, A., de Lacoba, M.G., Abendroth, J., Zhang, S., Edwards, T., Lorimer, D., Myler, P.J., Larraga, V., 2014b. Tyrosine aminotransferase from Leishmania infantum: a new drug target candidate. Int. J. Parasitol. Drugs Drug Resist. 4, 347–354. https://doi.org/10.1016/j.ijpddr.2014.06.001.

Nakanishi, T., Tamai, I., 2015. Interaction of drug or food with drug transporters in intestine and liver. Curr. Drug Metabol. 16, 753–764. https://doi.org/10.2174/1389200216091201113537.

Nandikolla, A., Srinivasarao, S., Karan Kumar, B., Murugesan, S., Aggarwal, H., Major, L.L., Smith, T.K., Chandra Sekhar, K.V.G., 2020. Synthesis, study of antileishmanial and antitrypanosomal activity of imidazo pyridine fused triazole analogues. RSC Adv. 10, 38328–38343. https://doi.org/10.1039/d0ra07881f.

National Research Council Committee, 2011. Guide for the Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals. National Academies Press, Washington, D.C. https://doi.org/10.17226/12910.

Ning, Z.H., Long, S., Zhou, Y.Y., Peng, Z.Y., Sun, Y.N., Chen, S.W., Su, L.M., Zhao, Y.H., 2015. Effect of exposure routes on the relationships of lethal toxicity to rats from

oral, intravenous, intraperitoneal and intramuscular routes. Regul. Toxicol. Pharmacol. 73, 613–619. https://doi.org/10.1016/j.yrtph.2015.09.008.
Ogungbe, I.V., Setzer, W.N., 2013. In-silico Leishmania target selectivity of antiparasitic terpenoids. Molecules 18, 7761–7847. https://doi.org/10.3390/molecules18077761.
Pandey, R.K., Prajapati, V.K., 2018. Exploring sand fly salivary proteins to design multi-epitope subunit vaccine to fight against visceral leishmaniasis. J. Cell. Biochem. https://doi.org/10.1002/jcb.26719.
Pertino, M.W., Alexander, F., Schmeda-Hirschmann, G., Vega, C., Rolón, M., Coronel, C., Rojas de Arias, A., López, K.Leal, Carranza-Rosales, P., Valdez, E.Viveros, 2020. Synthesis, trypanocidal and anti-leishmania activity of new triazole-lapachol and nor-lapachol hybrids. Bioorg. Chem. 103 https://doi.org/10.1016/j.bioorg.2020.104122.
Pinto, E.G., da Costa-Silva, T.A., Tempone, A.G., 2014. Histamine H1-receptor antagonists against Leishmania (L.) infantum: an in vitro and in vivo evaluation using phosphatidylserine-liposomes. Acta Trop. 137, 206–210. https://doi.org/10.1016/j.actatropica.2014.05.017.
Reimão, J.Q., Colombo, F.A., Pereira-Chioccola, V.L., Tempone, A.G., 2012. Effectiveness of liposomal buparvaquone in an experimental hamster model of Leishmania (L.) infantum chagasi. Exp. Parasitol. 130, 195–199. https://doi.org/10.1016/j.exppara.2012.01.010.
Reimão, J.Q., Colombo, F.A., Pereira-Chioccola, V.L., Tempone, A.G., 2011. In vitro and experimental therapeutic studies of the calcium channel blocker bepridil: detection of viable Leishmania (L.) chagasi by real-time PCR. Exp. Parasitol. 128, 111–115. https://doi.org/10.1016/j.exppara.2011.02.021.
Reithinger, R., Dujardin, J.-C.C., 2007. Molecular diagnosis of leishmaniasis: current status and future applications. J. Clin. Microbiol. 45, 21–25. https://doi.org/10.1128/JCM.02029-06.
Ronin, C., Costa, D.M., Tavares, J., Faria, J., Ciesielski, F., Ciapetti, P., Smith, T.K., MacDougall, J., Cordeiro-Da-Silva, A., Pemberton, I.K., 2018. The crystal structure of the Leishmania infantum Silent Information Regulator 2 related protein 1: implications to protein function and drug design. PloS One 13. https://doi.org/10.1371/journal.pone.0193602.
Sasidharan, S., Saudagar, P., 2020. Mapping N- and C-terminals of Leishmania donovani tyrosine aminotransferase by gene truncation strategy: a functional study using in vitro and in silico approaches. Sci. Rep. 10 https://doi.org/10.1038/s41598-020-69512-y.
Schrödinger Release 2019-1, 2019. Protein Preparation Wizard.
Sharikadze, V.V., Gongadze, N.V., 2006. The modulatory role of phospholipids, phosphorylcholine ethers and alkylphosphocholines in signal transduction. Georgian Med. News 130, 91–97.
Silva, M.S., Barata, L., Ferreira, A.E.N., Romão, S., Tomás, A.M., Freire, A.P., Cordeiro, C., 2008. Catalysis and structural properties of Leishmania infantum glyoxalase II: trypanothione specificity and phylogeny. Biochemistry 47, 195–204. https://doi.org/10.1021/bi700989m.
Stauber, L.A., Franchino, E.M., Grun, J., 1958. An eight-day method for screening compounds against leishmania donovani in the golden hamster. J. Eukaryot. Microbiol. 5, 269–273. https://doi.org/10.1111/j.1550-7408.1958.tb02565.x.
Wang, Y., Xing, J., Xu, Y., Zhou, N., Peng, J., Xiong, Z., Liu, X., Luo, X., Luo, C., Chen, K., Zheng, M., Jiang, H., 2015. In silico ADME/T modelling for rational drug design. Q. Rev. Biophys. https://doi.org/10.1017/S0033583515000190.
Zhu, L., Zhao, J., Zhang, Y., Zhou, W., Yin, L., Wang, Y., Fan, Y., Chen, Y., Liu, H., 2018. ADME properties evaluation in drug discovery: in silico prediction of blood–brain partitioning. Mol. Divers. 22, 979–990. https://doi.org/10.1007/s11030-018-9866-8.