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Short communication

Trypanocidal activity of mastoparan from *Polybia paulista* wasp venom by interaction with TcGAPDH

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ABSTRACT

Chagas disease, considered a neglected disease, is a parasitic infection caused by *Trypanosoma cruzi*, which is endemic throughout the world. Previously, the antimicrobial effect of Mastoparan (MP) from *Polybia paulista* wasp venom against bacteria was described. To continue the study, we report in this short communication the antimicrobial effect of MP against *Trypanosoma cruzi*. MP inhibits all *T. cruzi* developmental forms through the inhibition of *Tc*GAPDH suggested by the molecular docking. In conclusion, we suggest there is an antimicrobial effect also on *T. cruzi*.

1. Introduction

Chagas disease, caused by the *Trypanosoma cruzi* parasite, is endemic worldwide (WHO, 2010) and considered a neglected disease (WHO, 2012; Rassi et al., 2012). However, according to the World Health Organization (WHO), approximately 7.7–10 million individuals are infected.

Nifurtimox and benznidazole, drugs used to treat Chagas' disease, are ineffective in the chronic phase of the disease and have marked side effects (Cançado, 2002; Coura, 2007; Coura and Borges-Pereira, 2011; Araujo-Jorge, 2014). Therefore, there is an urgent need for new, safe and effective drugs.

The vital dependence on glycolysis as energy source makes the glycolytic enzymes of *T. cruzi* attractive targets for drug design. The inhibition of glyceraldehyde-3-phosphate dehydrogenase from *T. cruzi* (*Tc*GAPDH), important for the parasite's life cycle, has been identified as a molecular target to new drugs (Harris and Waters, 1976; Nowicki et al., 2008; Soares et al., 2013; Prokopczyk et al., 2014; Zinsser et al., 2014).

Several studies have sought to discover novel antiparasitic substances from animal venoms (Passero et al., 2007; Adade et al., 2011, 2013; 2014; Lima et al., 2016). The trypanocidal activity of Hymenoptera venoms and their compounds has been demonstrated (Adade et al., 2012, 2013; Lima et al., 2016). Wasp venom cationic peptides, namely mastoparan and its analogs, have shown very important potency as future antimicrobial agents (Dongol et al., 2016). The action of mastoparan from *Polybia paulista* wasp venom against bacteria (dos Santos Cabrera et al., 2008; Souza et al., 2009, 2011) has been previously demonstrated. To continue the study of the antimicrobial effect of mastoparan (MP) from *Polybia paulista* venom, we report in this short communication its action on *Trypanosoma cruzi* and performed molecular docking of MP in the

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active site of *Tc*GAPDH as a molecular target.

2. Materials and methods

2.1. Mastoparan peptide (MP) and T. cruzi strain

The mastoparan peptide (MP) isolated from *Polybia paulista* wasp venom with the primary sequence IDWLKLGKMVMDVL (Souza et al., 2004) was kindly provided by Dr. Mario Palma (Universidade de São Carlos, São Paulo, Brazil). For the experimental assays, final concentrations of 120, 60, 30, 15, 7.5, 3.75 and 1.87 μM were utilized, with sterile PBS being used as negative control (pH 7.4). *Trypanosoma cruzi* Y strain was isolated in São Paulo University Parasites Biochemical Laboratory and donated in epimastigote and trypomastigote forms.

2.2. Effect of MP on epimastigote forms of T. cruzi

Epimastigote forms of *T. cruzi* Y strain were plated in Liver Infusion Tryptose medium supplemented with antibiotics and 10% of FBS with different concentrations of MP and Benznidazole (Bz), incubated at 28 °C for 24, 48 and 72 h. Parasite growth inhibition was quantified in a Neubauer chamber (Abe et al., 2002; Gonçalves et al., 2002).

2.3. Effect of MP on trypomastigote forms of T. cruzi

The trypomastigote forms of *T. cruzi* obtained by infecting LLCMK2 cells, were incubated at 37 °C in an atmosphere with 5% CO_2 in DMEM medium (Vitrocell, São Paulo, Brazil) supplemented with antibiotics and 2% of FBS (Aparicio et al., 2004). Cells were incubated with different concentrations of MP and Bz for 24 h. Parasite growth inhibition was quantified in a Neubauer chamber (Abe et al., 2002; Gonçalves et al., 2002).

2.4. Effect of MP on amastigote forms of T. cruzi

LLC-MK2 cells were seeded in 24-well plates containing glass coverslips (13-mm diameter) cultivated in DMEM supplemented with 10% FCS, and maintained at 37 °C in a 5% CO_2 atmosphere for 24 h. Cells were infected with trypomastigote forms (parasite: host cell ratio of 20:1) in DMEM medium containing 2% FCS. After 48 h of infection, the non-internalized parasites were removed and the cells were cultivated in 2% FCS DMEM medium with or without MP (IC50 of trypomastigotes: 10.62 μM) and Bz (IC50 of trypomastigotes: 282 μM). The coverslips were collected in after 48 h, washed with PBS, fixed in Bouin solution and stained with Giemsa (Adade et al., 2011; Lima et al., 2016). The percentage of infected cells and the number of intracellular amastigotes per 100 cells was determined by counting 300 cells in triplicate. The LLC-MK2 cells were donated by São Paulo University Parasites Biochemical Laboratory.

2.5. Flow cytometry analysis to assess parasite death

Epimastigote forms treated with MP (60 μM) incubated for 24 h were stained with FITC-conjugated to annexinV/7AAD according to the manufacturer's instructions (BD Pharmigen, California, USA). The population of AX-7AAD viable cells was evaluated. Mitochondrial transmembrane potential, Reactive oxygen species (ROS) were also performed. Epimastigote forms were treated with MP (60 μM) for 24 h and stained with Rhodamine 123 (10 μg/mL), DCFH-DA (20 mMol/L) for 6 h according to the manufacturer's instructions (Sigma–Aldrich™, St. Louis, USA). The results were established by determining the fold change (treated/non-treated cell ratio) of the geometric mean of fluorescence. At the end of each incubation period, the cells were washed and submitted to flow cytometry analysis. All data were collected in a FACSCalibur system and analyzed using the Cell Quest software (Becton-Dickinson, California, USA).

2.6. Molecular docking

We obtained the MP peptide three-dimensional structure starting from the primary sequence IDWLKLGKMVMDVL (Souza et al., 2004) in Avogadro 1.1.1 program (Hanwell et al., 2012). The geometric optimization of the MP structure was performed using semiempirical Hamiltonian PM7 in MOPAC2012 program (http://openmopac.net). The crystallographic structure of the *Tc*GAPDH-chalepin complex was obtained from RCSB Protein Data Bank (PDB code: 1K3T) elucidated by Pavão et al. (2002). To identify potential binding sites, molecular docking was carried out employing automated docking in a ZDOCK server (http://zdock.umassmed.edu/) with the ZDOCK 3.0.2 program (Pierce et al., 2014; Mintseris et al., 2007). The ligand used was the optimized MP peptide, and the target was the *Tc*GAPDH enzyme after the removal of the chalepin of the *Tc*GAPDH–chalepin complex. The best MP pose was selected based on the best-ranked conformation of the peptide in the enzyme, and used for the construction of *Tc*GAPDH-MP complex. The *Tc*GAPDH-chalepin complex was used to compare the position of the ligand in the binding site. Molecular graphics were performed using the UCSF Chimera 1.8 package (Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco) (Pettersen et al., 2004).

2.7. Statistical analysis

The statistical analysis was performed using the GraphPad Prism 5 program (GraphPad Software, San Diego, CA, USA). IC50 values were determined by nonlinear regression. Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's post-test. Significance was defined as $^{*}p < 0.05$.

3. Results and discussion

In the present study, the treatment of epimastigotes with MP resulted in dose-dependent growth inhibition, with an IC50/24 h treatment of 61.4 ± 4 μM, IC50/48 h of 32.5 ± 4 μM and IC50/72 h of 35.24 ± 6 μM, while BZ showed IC50/24 h treatment of 218 ± 15 μM, IC50/48 h of 61.1 ± 3 μM and IC50/72 h of 16.5 ± 1 μM for the Y strain of *T. cruzi*. The treatment of trypomastigote forms with MP also resulted in dose-dependent growth inhibition, with an IC50 of 5.31 ± 1 μM and IC50 of BZ of 282 ± 20 μM for the Y strain of *T. cruzi*.

In the amastigote assay, untreated infected cells exhibited a higher infection profile compared to MP-treated cells, with reduced numbers of amastigotes per 100 cells at 48 h of incubation, as well Bz (Fig. 1A).

It has been previously demonstrated that antimicrobial peptides show a high level of toxicity against both Gram-positive and Gram-negative bacteria, as well as fungi, viruses, metazoan parasites and even cancer cells (Zasloff, 2002; Hoskin and Ramamoorthy, 2008).

Antibacterial (dos Santos Cabrera et al., 2008; Souza et al., 2009, 2011), antifungal (Wang et al., 2014) and anticancer (De Azevedo et al., 2015) effects of mastoparans have been described. Recently, the antiparasitic effect of mastoparans and their analogues has been described against *Plasmodium falciparum* (Carter et al., 2013; Peatey et al., 2013). In this study, we demonstrated the trypanocidal effect of the MP on the all developmental forms of *T. cruzi* Y strain. Corroborating our findings, other authors have shown the trypanocidal effect of Hymenoptera venoms (Adade et al., 2012;

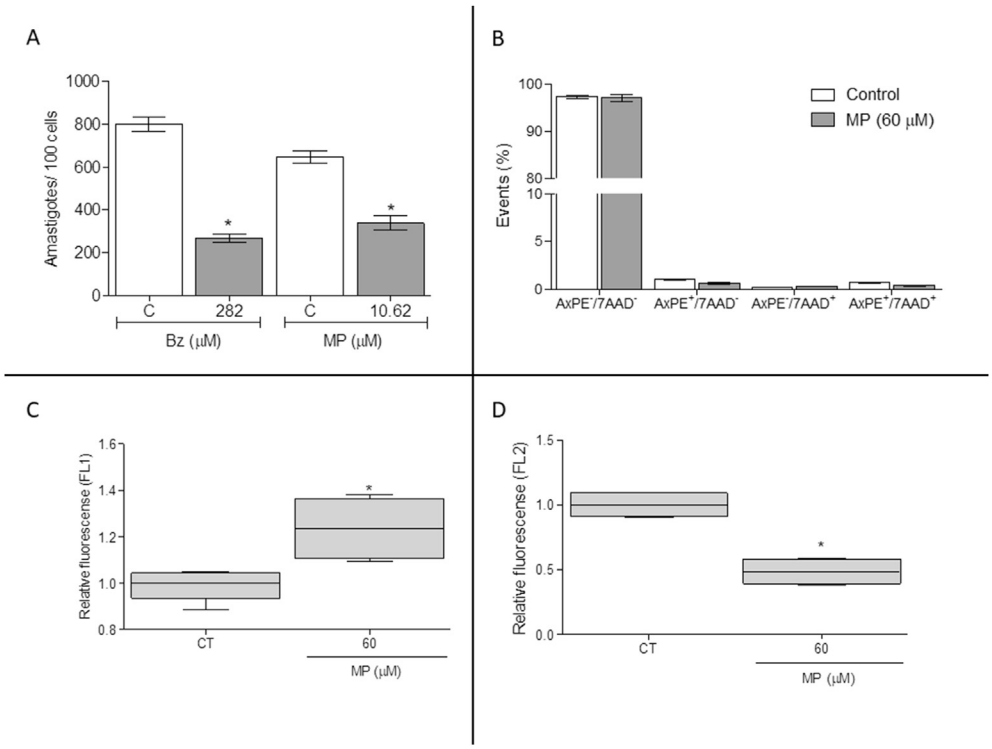

Fig. 1. Evaluation of trypanocidal effect of Mastoparan from *P. paulista* venom (MP). (A) Effect of MP and Benznidazole (Bz) on intracellular amastigotes in amastigote/100 cells at 48 h of incubation. (B) Cell death pathway evaluation using 7-AAD (7-aminoactinomycin D) and Annexin V-PE. **(C)** Relative cytoplasmic ROS production. **(D)** Transmembrane mitochondrial potential evaluation. All data were expressed as mean ± E.P.M (n = 3) and analyzed by ANOVA with Dunnett's post-test,*p < 0.05 compared to the corresponding control group.

Lima et al., 2016), as well as of its antimicrobial peptides (Adade et al., 2013).

To evaluate the action mechanism, cytometry flow was also performed on a proliferative form, the epimastigote. Necrosis and apoptosis could be evaluated with staining of 7AAD/AX, overproduction of ROS and alterations on transmembrane mitochondrial potential (Vanden Berghe et al., 2013). Our findings did not show any increase of marked cells on 7AAD/AX (Fig. 1B), but showed increase on ROS (Fig. 1C) and reduction of transmembrane mitochondrial potential (Fig. 1D). So we investigated the interaction between MP and *Tc*GAPDH, since this enzyme is crucial to T. cruzi survival and it is responsible for many biological processes, including cell death regulation.

GAPDH is a simple, highly conserved enzyme required for glycolysis, the process of turning glucose into energy. Over the past 20 years, however, scientists have been uncovering additional roles of GAPDH, such as oxidative stress response, maintenance of DNA integrity, apoptosis, autophagy, histone gene regulation (Sirover, 2011).

The great dependence of trypanosomatids on glycolysis to obtain energy makes glycolytic enzymes attractive targets for drug design against Chagas' disease. *T. cruzi* glyceraldehyde-3-phosphate dehydrogenase (TcGAPDH) which catalyzes the oxidative phosphorylation of D-glyceraldehyde-3-phosphate (G3P) into 1,3-bisphosphoglycerate (1,3-BPG) coupled to reduction of nicotinamide adenine dinucleotide (NAD+) to NADH, is a key enzyme in the glycolytic pathway (Cheleski et al., 2010).

Recently, Prokopczyk et al. (2014), Soares et al. (2013) and Maluf et al. (2013) have investigated *Tc*GAPDH as a target for drug design. We used molecular docking analysis to suggest a mechanism of action of the peptide on the parasite through the study of the interaction between *Tc*GAPDH and the MP. The MP best-ranked pose in the docking simulation is shown in Fig. 2A, together with the chalepin pose, a known inhibitor of *Tc*GAPDH (obtained from crystallographic data of the *Tc*GAPDH–chalepin complex) for comparison (Pavão et al., 2002). We can observe that the docking pose of MP peptide is similar to that occupied by the chalepin molecule in the *Tc*GAPDH active site.

The region of the nearest neighbors between MP peptide and the atoms of amino acid residues of the binding site is shown in Fig. 2B. We considered residues with distances <3.5 Å from ligand atoms. According to Seidler (2013), Arg249, Asp210, Cis166, His194, Ser165, Thr197, 226 and Tyr333 residues play a significant role in the active enzyme center. These residues participate in the catalytic mechanism of *Tc*GAPDH oxidoreductase activity. We observed that the MP peptide and the *Tc*GAPDH can form three H-bonds: two involving Cis166 of TcGAPDH with Val13 and Asp12 of MP; and another involving Gly245 of TcGAPDH with Lys5 of MP. H-bonds are important in interactions between the ligand and the enzyme, especially with *Tc*GAPDH catalytic residues, such as the Cys166 (Seidler, 2013). Souza et al. (1998) suggest that the Asp210 residue may be involved in selective inhibition processes. For instance, chalepin inhibits *Tc*GAPDH, but does not inhibit the human GAPDH. The substitution of Asp210 (*T. cruzi*) by Leu194 (human) can produce less effective polar interactions, decreasing chalepin specificity for the human GAPDH (Pavão et al., 2002). Thus, the peptide's pose with the highest score obtained from the molecular docking with *Tc*GAPDH suggests the possibility of inhibition of this enzyme. This could be a mechanism for the biological action of the peptide on the parasite.

Regarding all biological processes dependent of GAPDH, its inhibition could be related to ROS induction and mitochondrial disruption, resulting in energetic collapse. This can explain why MP could not cause lyses of membrane (7AAD staining) or induce

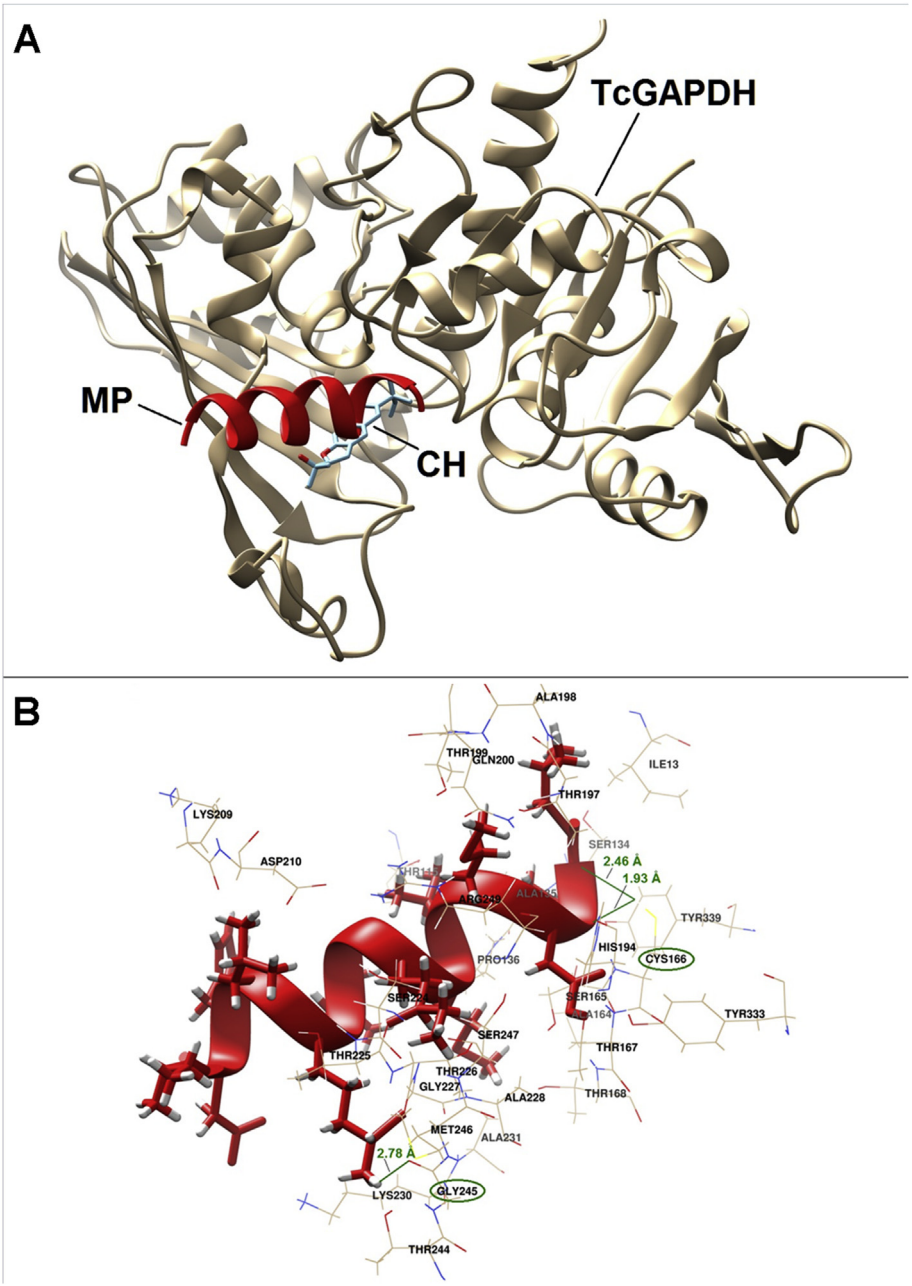

Fig. 2. Molecular docking of MP peptide with the highest score (red helix) in the *Tc*GAPDH enzyme. The chalepin (CH) pose obtained from crystallographic data of the *Tc*GAPDH–chalepin complex is also shown for comparison. Side chains of the residues of the peptide are not shown **(A)**. Nearest amino acids residues with contact distance <3.5 Å (wire) of MP peptide with the highest score (red helix and stick) in the binding site of TcGAPDH enzyme. Inter-molecular H-bonds (green lines) between the peptide and Cys166 and Gly245 residues (green ellipses) of the enzyme are shown. Lengths of H-bonds are also indicated. The Cys166 is an important catalytic residue that plays a significant role at the active center of the enzyme **(B)**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

externalization of PS (AX staining) on epimastigote forms on our assays.

The most important finding of the present study was the activity of MP against the intracellular amastigote form. Amastigotes develop and maintain the infections by *T. cruzi* and significantly low MP concentrations inhibit amastigote proliferation.

Several chemical reagents with specific biochemical targets are effective inhibitors of parasite attachment and/or invasion. Inhibition of ATP synthesis in *T. cruzi* with drugs that interfere with glycolysis profoundly affects the level of host cell invasion. The decrease in the invasive capacity results from the severe inhibition of parasite attachment to host cells, reducing amastigote development (Schenkman et al., 1991).

In conclusion, mastoparan isolated from *Polybia paulista* wasp venom showed an inhibitory effect on the epimastigote, trypomastigote and amastigote forms of *Trypanosoma cruzi* Y strain. Additionally, we suggest the interaction of MP with *Tc*GAPDH.

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References

Abe, F., Nagafuji, S., Yamauchi, T., Okabe, H., Maki, J., Higo, H., et al., 2002. Trypanocidal constituents in plants 1. Evaluation of some mexican plants for their trypanocidal activity and active constituents in guaco, roots of Aristolochia taliscana. Biol. Pharm. Bull. 25, 1188–1191.

Adade, C.M., Carvalho, A.L., Tomaz, M.A., Costa, T.F., Godinho, J.L., Melo, P.A., et al., 2014. Crovirin, a snake venom cysteine-rich secretory protein (CRISP) with promising activity against Trypanosomes and Leishmania. PLoS Negl. Trop. Dis. 8, e3252.

Adade, C.M., Chagas, G.S., Souto-Padrón, T., 2012. Apis mellifera venom induces different cell death pathways in Trypanosoma cruzi. Parasitology 139, 1444–1461.

Adade, C.M., Cons, B.L., Melo, P.A., Souto-Padrón, T., 2011. Effect of Crotalus viridis viridis snake venom on the ultrastructure and intracellular survival of Trypanosoma cruzi. Parasitology 138, 46–58.

Adade, C.M., Oliveira, I.R., Pais, J.A., Souto-Padrón, T., 2013. Melittin peptide kills Trypanosoma cruzi parasites by inducing different cell death pathways. Toxicon 69, 227–239.

Aparicio, I.M., Scharfstein, J., Lima, A.P.C.A., 2004. A new cruzipain-mediated pathway of human cell invasion by Trypanosoma cruzi requires trypomastigota membranes. Infect. Immun. 72, 5892–5902.

Araujo-Jorge, T., 2014. Doença de Chagas. https://www.agencia.fiocruz.br/doen%C3%A7a-de-chagas.

Cançado, J.R., 2002. Long term evaluation of etiological treatment of Chagas disease with Benznidazole. Rev. Inst. Med. Trop. São Paulo 44, 29–37.

Carter, V., Underhill, A., Baber, I., Sylla, L., Baby, M., Larget-Thiery, I., Zettor, A., Bourgouin, C., Langel, U., Faye, I., Otvos, L., Wade, J.D., Coulibaly, M.B., Traore, S.F., Tripet, F., Eggleston, P., Hurd, H., 2013. Killer bee molecules: antimicrobial peptides as effector molecules to target sporogonic stages of Plasmodium. PLoS Pathog. 9, e1003790.

Cheleski, J., Freitas, R.F., Wiggers, H.J., Rocha, J.R., de Araújo, A.P., Montanari, C.A., 2010. Expression, purification and kinetic characterization of His-tagged glyceraldehyde-3-phosphate dehydrogenase from Trypanosoma cruzi. Protein Expr. Purif. 76, 190–196.

Coura, J.R., 2007. Chagas disease: what is known and what is needed – a background article. Mem. Inst. Oswaldo Cruz 102, 113–122.

Coura, J.R., Borges-Pereira, J., 2011. Chronic phase of Chagas disease: why should it be treated? A comprehensive review. Mem. Inst. Oswaldo Cruz 106, 641–645.

de Azevedo, R.A., Figueiredo, C.R., Ferreira, A.K., Matsuo, A.L., Massaoka, M.H., Girola, N., Auada, A.V., Farias, C.F., Pasqualoto, K.F., Rodrigues, C.P., Barbuto, J.A., Levy, D., Bydlowski, S.P., de Sá-Junior, P.L., Travassos, L.R., Lebrun, I., 2015. Mastoparan induces apoptosis in B16F10-Nex2 melanoma cells via the intrinsic mitochondrial pathway and displays antitumor activity in vivo. Peptides 68, 113–119.

Dongol, Y., Dhananjaya, B.L., Shrestha, R.K., Aryal, G., 2016. Wasp venom toxins as a potential therapeutic agent. Protein Pept. Lett. 23, 688–698.

dos Santos Cabrera, M.P., Costa, S.T., de Souza, B.M., Palma, M.S., Ruggiero, J.R., Ruggiero Neto, J., 2008. Selectivity in the mechanism of action of antimicrobial mastoparan peptide Polybia-MP1. Eur. Biophys. J. 37, 879–891.

Gonçalves, A.R., Soares, M.J., de Souza, W., DaMatta, R.A., Alves, E.W., 2002. Ultrastructural alterations and growth inhibition of Trypanosoma cruzi and Leishmania major induced by Bothrops jararaca venom. Parasitol. Res. 88, 598–602.

Hanwell, M.D., Curtis, D.E., Lonie, D.C., Vandermeersch, T., Zurek, E., Hutchison, G.R., 2012. Avogadro: An advanced semantic chemical editor, visualization, and analysis platform. J. Cheminformatics 4, 17.

Harris, J.I., Waters, M., 1976. In: Boyer, P.D. (Ed.), The Enzymes, vol. 13. Academic Press, New York, pp. 1–49.

Hoskin, D.W., Ramamoorthy, A., 2008. Studies on anticancer activities of antimicrobial peptides. Biochim. Biophys. Acta Biomembr. 1778, 357–375.

Lima, D.B., Sousa, P.L., Torres, A.F., Rodrigues, K.A., Mello, C.P., Menezes, R.R., Tessarolo, L.D., Quinet, Y.P., de Oliveira, M.R., Martins, A.M., 2016. Antiparasitic effect of Dinoponera quadriceps giant ant venom. Toxicon 120, 128–132.

Maluf, F.V., Andricopulo, A.D., Oliva, G., Guido, R.V., 2013. A pharmacophore-based virtual screening approach for the discovery of Trypanosoma cruzi GAPDH inhibitors. Future Med. Chem. 5, 2019–2035.

Mintseris, J., Pierce, B., Wiehe, K., Anderson, R., Chen, R., Weng, Z., 2007. Integrating statistical pair potentials into protein complex prediction. Proteins 69, 511–520.

Nowicki, M.W., Tulloch, L.B., Worralll, L., Mcnae, I.W., Hannaert, V., Michels, P.A.M., Fothergill-Gilmore, L.A., Walkinshaw, M.D., Turner, N.J., 2008. Design, synthesis and trypanocidal activity of lead compounds based on inhibitors of parasite glycolysis. Bioorg. Med. Chem. 16, 5050–5061.

Passero, L.F., Tomokane, T.Y., Corbett, C.E., Laurenti, M.D., Toyama, M.H., 2007. Comparative studies of the anti-leishmanial activity of three Crotalus durissus ssp. venoms. Parasitol. Res. 101, 1365–1371.

Pavão, F., Castilho, M.S., Pupo, M.T., Dias, R.L.A., Correa, A.G., Fernandes, J.B., Silva, M.F.G.F., Mafezoli, J., Vieira, P.C., Oliva, G., 2002. Structure of Trypanosoma cruzi glycosomal glyceraldehyde-3-phosphate dehydrogenase complexed with chalepin, a natural product inhibitor, at 1.95 Å resolution. FEBS Lett. 520, 13–17.

Peatey, C.L., Dixon, M.W., Gardiner, D.L., Trenholme, K.R., 2013. Temporal evaluation of commitment to sexual development in Plasmodium falciparum. Malar. J. 12, 134.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., Ferrin, T.E., 2004. UCSF Chimera -a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612.

Pierce, B.G., Wiehe, K., Hwang, H., Kim, B.H., Vreven, T., Weng, Z., 2014. ZDOCK server: interactive docking prediction of protein-protein complexes and symmetric multimers. Bioinformatics 30, 1771–1773.

Prokopczyk, I.M., Ribeiro, J.F., Sartori, G.R., Sesti-Costa, R., Silva, J.S., Freitas, R.F., Leitão, A., Montanari, C.A., 2014. Integration of methods in cheminformatics and biocalorimetry for the design of trypanosomatid enzyme inhibitors. Future Med. Chem. 6, 17–33.

Rassi, J.R.A., Rassi, A., Marcondes De Rezende, J., 2012. American trypanosomiasis (Chagas disease). Infect. Dis. Clin. North. Am. 26, 275–291.

Schenkman, S., Robbins, E.S., Nussenzweig, V., 1991. Attachment of Trypanosoma cruzi to mammalian cells requires parasite energy, and invasion can be independent of the target cell cytoskeleton. Infect. Immun. 59, 645–654.

Seidler, N.W., 2013. GAPDH: Biological Properties and Diversity. Springer, New York, pp. 21–24.

Sirover, M.A., 2011. On the functional diversity of glyceraldehyde-3-phosphate dehydrogenase: biochemical mechanisms and regulatory control. Biochim. Biophys. Acta 1810 (8), 741–751.

Soares, F.A., Sesti-Costa, R., Silva, J.S., Souza, M.C.B.V., Ferreira, V.F., Santos, F.C., Monteiro, P.A.U., Leitão, A., Montanari, C.A., 2013. Molecular design, synthesis and biological evaluation of 1,4-dihydro-4-oxoquinoline ribonucleosides as TcGAPDH inhibitors with trypanocidal activity. Bioorg. Med. Chem. Lett. 23, 4597–4601.

Souza, B.M., da Silva, A.V., Resende, V.M., Arcuri, H.A., Dos Santos Cabrera, M.P., Ruggiero Neto, J., Palma, M.S., 2009. Characterization of two novel polyfunctional mastoparan peptides from the venom of the social wasp Polybia paulista. Peptides 30, 1387–1395.

Souza, B.M., Dos Santos Cabrera, M.P., Neto, J.R., Palma, M.S., 2011. Investigating the effect of different positioning of lysine residues along the peptide chain of mastoparans for their secondary structures and biological activities. Amino Acids 40, 77–90.

Souza, B.M., Marques, M.R., Tomazela, D.M., Eberlin, M.N., Mendes, M.A., Palma, M.S., 2004. Mass spectrometric characterization of two novel inflammatory peptides from the venom of the social wasp Polybia paulista. Rapid Commun. Mass Spectrom. 18, 1095–1102.

Souza, D.H.F., Garratt, R.C., Araújo, A.P.U., Guimaraes, B.G., Jesus, W.D.P., Michels, P.A.M., Hannaert, V., Oliva, G., 1998. Trypanosoma cruzi glycosomal glyceraldehyde-3-phosphate dehydrogenase: structure, catalytic mechanism and targeted inhibitor design. FEBS Lett. 424, 131–135.

Vanden Berghe, T., Grootjans, S., Goossens, V., Dondelinger, Y., Krysko, D.V., Takahashi, N., Vandenabeele, P., 2013. Determination of apoptotic and necroticcell death in vitro and in vivo. Methods 61, 117–129.

Wang, K., Yan, J., Dang, W., Xie, J., Yan, B., Yan, W., Sun, M., Zhang, B., Ma, M., Zhao, Y., Jia, F., Zhu, R., Chen, W., Wang, R., 2014. Dual antifungal properties of cationic antimicrobial peptides polybia-MPI: membrane integrity disruption and inhibition of biofilm formation. Peptides 56, 222–229.

Who - World Health Organization, 2010. First Who Report on Neglected Tropical Diseases: Working to Overcome the Global Impact of Neglected Tropical Diseases, p. 184.

Who - World Health Organization, 2012. Accelerating Work to Overcome the Global Impact of Neglected Tropical Diseases: a Roadmap for Implementation, p. 22.

Zasloff, M., 2002. Antimicrobial peptides of multicellular organisms. Nature 415, 389–395.

Zinsser, V.L., Hoey, E.M., Trudgett, A., Timson, D.J., 2014. Biochemical characterisation of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) from the liver fluke, Fasciola hepatica. Biochim. Biophys. Acta 44, 744–749.