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In vitro effects of the asymmetric peptidomimetic 157, containing L-tartaric acid core and valine/leucine substituents, on *Leishmania amazonensis* promastigotes and amastigotes

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ABSTRACT

The current treatments for leishmaniasis bump into several obstacles, including low efficacy, high costs, long monitoring, and several/severe side effects. Consequently, the search for promising compounds is a tangible need. Recently, we reported the anti-*Leishmania amazonensis* action of asymmetric peptidomimetic compounds containing tartaric acid as core, especially the **157** derivative that contains valine/leucine substituents in its structure. Herein, we decipher the multiple effects of **157** on the *L. amazonensis* physiology and on the interaction process with macrophages. The peptidomimetic **157** induced significant changes on the morphometric (internal granularity reduction as judged by flow cytometer) and on the ultrastructural (round-shaped parasites, presence of plasma membrane blebs and flagellum loss as visualized by scanning electron microscopy) aspects of treated promastigotes compared to untreated ones. The alteration on the plasma membrane permeability was confirmed by the passive incorporation of propidium iodide in **157**-treated promastigotes. In parallel, the low viability of promastigotes was also associated to the perturbation of mitochondrial transmembrane electric potential. These combined results demonstrated that **157** induced irreversible metabolic damages that led to *L. amazonensis* death. The pre-treatment of promastigotes with **157** inhibited the association index with macrophages in a typically dose-dependent manner. Additionally, **157** significantly reduced the number of intramacrophage amastigotes after 72 h of drug contact, presenting an IC_{50} value of 30.2 μM. Under our experimental conditions, **157** showed higher toxicity to promastigotes and amastigotes when compared to RAW cells, resulting in good selective indexes. Therefore, **157** can be considered as an interesting candidate for further optimization, since its synthesis is simple and cheap.

1. Introduction

Cutaneous, mucocutaneous and visceral leishmaniasis are neglected diseases caused by parasites belonging to the *Leishmania* genus in approximately 98 endemic countries worldwide [1,2]. The mucocutaneous and cutaneous forms have a wide clinical spectrum ranging from simple and localized to severe and diffuse lesions in skin and mucosa injuries. Visceral leishmaniasis causes the most severe illness, which can be fatal if not properly treated in time. The number of visceral cases is estimated in 200,000–400,000 annually, with

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10%–20% of mortality [1,2]. The current chemotherapy for all leishmaniasis manifestations is summarized in five drugs derived from natural and synthetic molecules: pentavalent antimonials, pentamidine, amphotericin B (AmpB), miltefosine and paromomycin [3]. Despite the high cost, these medicines have serious limitations, causing several and severe side effects, most of them requiring parenteral administration and long courses of treatment. Moreover, there is an increasing frequency of drug resistance and vaccination programs against leishmaniasis have not yet been successful developed [1,3].

The urgent need for chemotherapeutic formulations effective against *Leishmania* is obvious and imperative. In this context, the synthesis of new compounds with potential anti-*Leishmania* activity appears as a common goal for many research groups. We previously related the anti-*L. amazonensis* activity of some asymmetric tartaric acid derivatives [4,5]. The most powerful peptidomimetic compound, designated **157**, reduced the parasite viability in a dose-dependent manner, displaying IC_{50} value of 33.7 μM. Also, **157** blocked the aspartyl-type peptidase activity detected in promastigotes, indicating a possible target for this compound. Wondrously, the peptidomimetic reduced the leishmanial aspartyl peptidase activity in a nanomolar range (IC_{50} of 40 nM), while two classical aspartyl peptidase inhibitors, pepstatin A and diazo-acetyl-norleucinemetilester (DAN), presented IC_{50} values of 6.8 μM and 10.2 μM, respectively.

The compound **157** is a peptidiomimetic compound designed as an HIV aspartyl peptidase inhibitor. It is synthesized from L-tartaric acid, which makes it easy to be yielded and economically viable [4,6,7]. In view of the necessity of development of more selective, effective and low-cost compounds, the present work verified the influence of **157** on the (i) physiology of *L. amazonensis* promastigotes, (ii) interaction process of promastigotes with RAW macrophages and (iii) proliferation/viability of intramacrophage amastigotes.

2. Material and methods

2.1. Chemicals

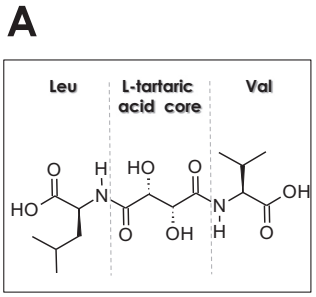
Schneider's insect medium, Dulbecco's modified Eagle's medium (DMEM), dimethylsulfoxide (DMSO), propidium iodide (PI), 7-hydroxy-3H-phenoxazin-3-one-10-oxide (Resazurin dye/AlamarBlueH), JC-1, carbonyl cyanide 4-(trifluoromethoxy)-phenylhydrazone (FCCP), ethyleneglycol tetraacetic acid (EGTA), medium constituents and buffer components were purchased from Sigma-Aldrich Chemical Co. (St Louis, USA). Fetal bovine serum (FBS) was obtained from Gibco Life Technology (New York, USA). The peptidomimetic compound, derived from L-tartaric acid (designated as **157**) (Fig. 1A), was synthesized as described by Resende and co-workers [4].

2.2. Parasite and macrophage cultivation

Promastigote forms of *L. amazonensis* strain PH8 (IFLA/BR/1967/PH8) were obtained from the *Leishmania* Collection of the Instituto Oswaldo Cruz (IOC), Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil (*Leishmania* Type Culture Collection – LTTC WDCM-731). The maintenance of the parasites occurred in Schneider's medium, pH 7.2, supplemented with 10% FBS at 26 °C. The RAW 264.7 murine macrophages (ATCC TIB-71) were maintained in DMEM supplemented with 10% FBS at 37 °C in an atmosphere containing 5% CO_2.

2.3. Treatment of L. amazonensis promastigotes

For the assays, promastigotes (10^5/mL) were maintained in Schneider's medium (10% FBS) and incubated at 26 °C for 72 h in the absence or presence of the 0.5-fold, 2-fold and 50% inhibitory concentration doses (IC_{50}/72 h) of the peptidomimetic compound **157.** Afterwards, promastigotes were washed in phosphate-buffered saline (PBS; 150 mM NaCl, 20 mM phosphate buffer, pH 7.2) and the viability



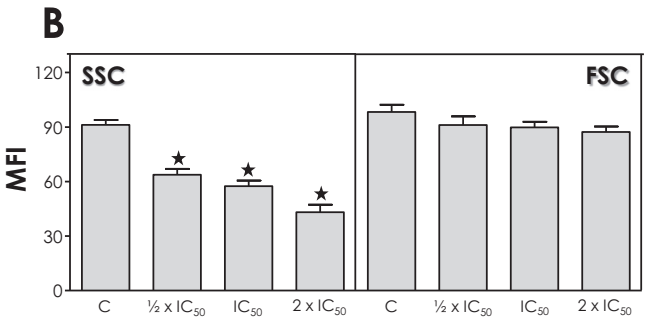


Fig. 1. Effects of 157 on *Leishmania amazonensis* morphology. (A) Chemical structure of the peptidomimetic compound **157**. (B) Untreated parasites and those treated with **157** were analyzed by flow cytometry in order to measure two morphometric parameters. Forward scatter (FSC) measurement is related to cell size and side scatter (SSC) measurement is related to the internal granularity and/or complexity of a cell that were expressed as mean of fluorescence intensity (MFI). Data shown are the mean ± standard deviation (SD) of three independent experiments performed in triplicate. The stars denote statistical differences compared to the control ($P < .01$).

was monitored by motility and lack of Trypan blue staining. Two control populations were used in all the experiments: untreated cells and promastigotes maintained in Schneider's medium (10% FBS) in the presence of DMSO, the drug vehicle, at a concentration corresponding to that used to prepare the highest drug concentration.

2.4. Morphometrics

Promastigotes were processed for flow cytometry in order to measure two morphometric parameters: size and granularity. Briefly, parasites were centrifuged (500 × *g*/10 min, 4 °C) and fixed in 0.4% paraformaldehyde for 30 min at room temperature. Then, each experimental population was acquired in a flow cytometer (FACSCalibur, USA) and mapped (n = 10.000) by using a two-parameter histogram of forward-angle light scatter (FSC) *versus* side scatter (SSC). FSC measurement is related to cell size and SSC to the internal granularity and/or complexity [8].

2.5. Scanning electron microscopy (SEM)

For scanning electron microscopy observation, promastigotes over coverslips were fixed and post-fixed after 72 h of drug treatment as described above and dehydrated in graded series of acetone (30–100%). Cells were dried by the critical point method, mounted on stubs, coated with gold (20–30 nm) and observed in a Jeol JSM 6490LV scanning electron microscope [9].

2.6. Plasma membrane integrity

Promastigotes were incubated with PI (1 mg/mL) for 5 min in PBS.

Then, cells were washed in PBS, harvested at 500 ×g/5 min and immediately analyzed in a in a flow cytometer (FACSCalibur, USA). Parasites permeabilized with 4% paraformaldehyde were used as non-viable cells (positive staining) in this set of experiment [8].

2.7. Parasite metabolism assay

Resazurin dye/AlamarBlueH was employed for promastigote viability testing in sterile 96-well plates. After 72 h of incubation in the absence (control) or presence of **157**, 20 μL of resazurin (0.0125% in PBS) were added, and plates were incubated for a further 4 h. After incubation, cells were analyzed at a microplate reader (SpectraMax spectrofluorometer, Molecular Devices) using a pair of 590 nm and 544 nm as emission and excitation wavelengths, respectively. The viability was evaluated based on a comparison with untreated, control cells. Parasites were also treated with sodium azide (0.95 g/L) for 30 min in order to obtain non-viable cells to use as a positive control in the viability test.

2.8. Estimation of mitochondrial membrane potential (ΔΨm)

Promastigotes were incubated with 10 μg/ml JC-1 for 30 min in a reaction medium (125 mM sucrose, 65 mM KCl, 10 mM HEPES/K^+, pH 7.2, 2 mM Pi, 1 mM $MgCl_2$ and 500 μM EGTA) in 96-well opaque plates, with readings made every minute using a spectrofluorometer. As a positive control of the depolarization of the mitochondrial membrane, parasites were also incubated with 1 μM FCCP. After 30 min of reaction, 2 μM FCCP was additionally added to all systems (including control) in order to abolish the $\Delta\Psi m$. The relative $\Delta\Psi m$ value was obtained by calculating the ratio between the readings at 590 and 530 nm (590:530 ratio) [8].

2.9. Effects of 157 on L. amazonensis-macrophage interaction

To check the effects of **157** on the interaction process, the following system was adopted: promastigotes (10^5 cells) were incubated in DMEM with 10% FBS and treated for 1 h with different concentrations of **157** (0.5-fold, 2-fold and IC_{50} doses). To ensure the viability of the parasites in those conditions, promastigotes were submitted to the resazurin assay, as previously described. Then, untreated and **157**-treated promastigotes were washed and added to 24-well tissue plates to interact with RAW 264.7 cell lineage for 1 h in a cell ratio of 10:1 (parasites/macrophage) at 37 °C in a 5% CO_2 atmosphere. Subsequently, the non-adherent parasites were removed by washes with DMEM. The infected macrophages were fixed in Bouin and stained with Giemsa. The percentage of infected macrophages was determined by randomly counting at least 200 cells in each of triplicate cover slips in bright field microscopy. The association index was obtained by multiplying the percentage of infected macrophages by the number of amastigotes per infected macrophage.

2.10. Macrophage viability assays

The effect of **157** on the viability of RAW macrophages was evaluated by the neutral red incorporation assay [10]. Firstly, cells (5×10^5/mL) were allowed to adhere in 96-well plates in DMEM supplemented with 10% FBS for 24 h at 37 °C in a 5% CO_2 atmosphere. Cells were then incubated with single doses (time 0 h) of increasing concentrations of **157** (0 to 560 μM) for a period of 72 h. After this time, cells were incubated with 0.01% neutral red solution for 3 h at 37 °C in an atmosphere with 5% CO_2. Then, the medium was removed and the cells fixed with 4% formalin in PBS. The incorporation of the dye into the viable cells was eluted using a mixture of ethanol/acetic acid/water (50:1:49). The incorporated dye was determined by measuring the optical density of the fraction eluted at 490 nm in a spectrophotometer (ELx800TM, BioteK Instruments, Inc.). Concentrations of **157** capable of maintaining 95% of cellular viability were used in the subsequent interaction assay. Additionally, the 50% cytotoxic concentration (CC_{50}) for the cell lineage was determined by linear regression analysis after 72 h of treatment with the compound. The selectivity index (SI) was calculated by dividing the CC_{50} value of the mammalian cell by the IC_{50} values of promastigotes and amastigotes.

2.11. Effects of 157 on intramacrophage amastigotes

Promastigotes were added to 24-well tissue plates to infect RAW cells for 1 h, in a cell ratio of 10:1 (parasites/cell) at 37 °C in a 5% CO_2. After the incubation, free parasites were removed by washes with DMEM and fresh medium with 2% FBS was added. Then, infected macrophages were subjected to the treatment for 72 h with **157** at 0.5-fold, 2-fold and IC_{50} doses (calculated for promastigotes), and concentrations able to keep around 90% of host cell viability, as ensured by the cytotoxic assays. The percentage of infected macrophages and the association index were determined as also previously described. The IC_{50} for amastigotes was determined by linear regression analysis.

2.12. Statistical analysis

All the experiments were performed in triplicate, in three independent experimental sets. Data were analyzed statistically by means of one-way ANOVA using GraphPad Prism 6.0 computer software. *P* values of 0.01 or less were considered statistically significant.

3. Results and discussion

The design of new compounds as analogues of current drugs is an interesting strategy in view of the necessity of alternative, more efficient and low-cost treatments for different diseases. In this context, some works have reported the synthesis of peptidomimetic compounds with tartaric acid backbones as the core of HIV-1 aspartyl peptidase inhibitors (HIV-PIs) [4,6,7,11–14]. HIV-PIs as nelfinavir, indinavir, saquinavir, ritonavir, amprenavir and lopinavir are drugs commonly used in a combined therapy in patients with acquired immune deficiency syndrome (AIDS) where those PIs target the viral aspartyl enzyme [15]. Moreover, HIV-PIs present a powerful effect against promastigotes and intramacrophage amastigotes of several species of *Leishmania* that cause cutaneous, mucocutaneous and visceral diseases (for more details, see the comprehensive review of Santos and coworkers [16]).

In a recent work of our group [5], we tested the effects of 6 asymmetric peptidomimetics, containing L-tartaric acid core, on both aspartyl peptidase and growth of *L. amazonensis* promastigotes. The peptidomimetics designated as **88**, **154** and **158** promoted a reduction of 50% on the leishmanial aspartyl peptidase activity at concentrations ranging from 40 to 85 μM, whereas the peptidomimetic **157** was by far the most effective, presenting IC_{50} of 40 nM. Particularly, it is noteworthy that while the inhibitory activity of **154** and **157** against the aspartyl peptidase is quite different, the promastigote proliferation inhibition activity of those compounds is very similar (> 60%), being the most effective compounds tested. The compounds **157** and **158** arrested the growth of *L. amazonensis* promastigotes in a dose-dependent manner, and the IC_{50} values after 72 h were 33.7 μM and 44.5 μM. Probably, this difference occurs due to the hydrolysis of **154** ester groups that convert compound **154** to **157** [5]. The peptidomimetic **157** was the most efficient compound able to arrest both aspartyl peptidase activity and parasite proliferation, and for this reason, selected for the present study.

Therefore, initially we analyzed two morphometric parameters of promastigotes under **157** exposure at the 0.5-fold, 2-fold and IC_{50} doses. After 72 h of treatment, **157** induced a remarkable decrease on the granularity/complexity parameter in a typical dose-dependent fashion, while no significant alterations were detected in the parasite

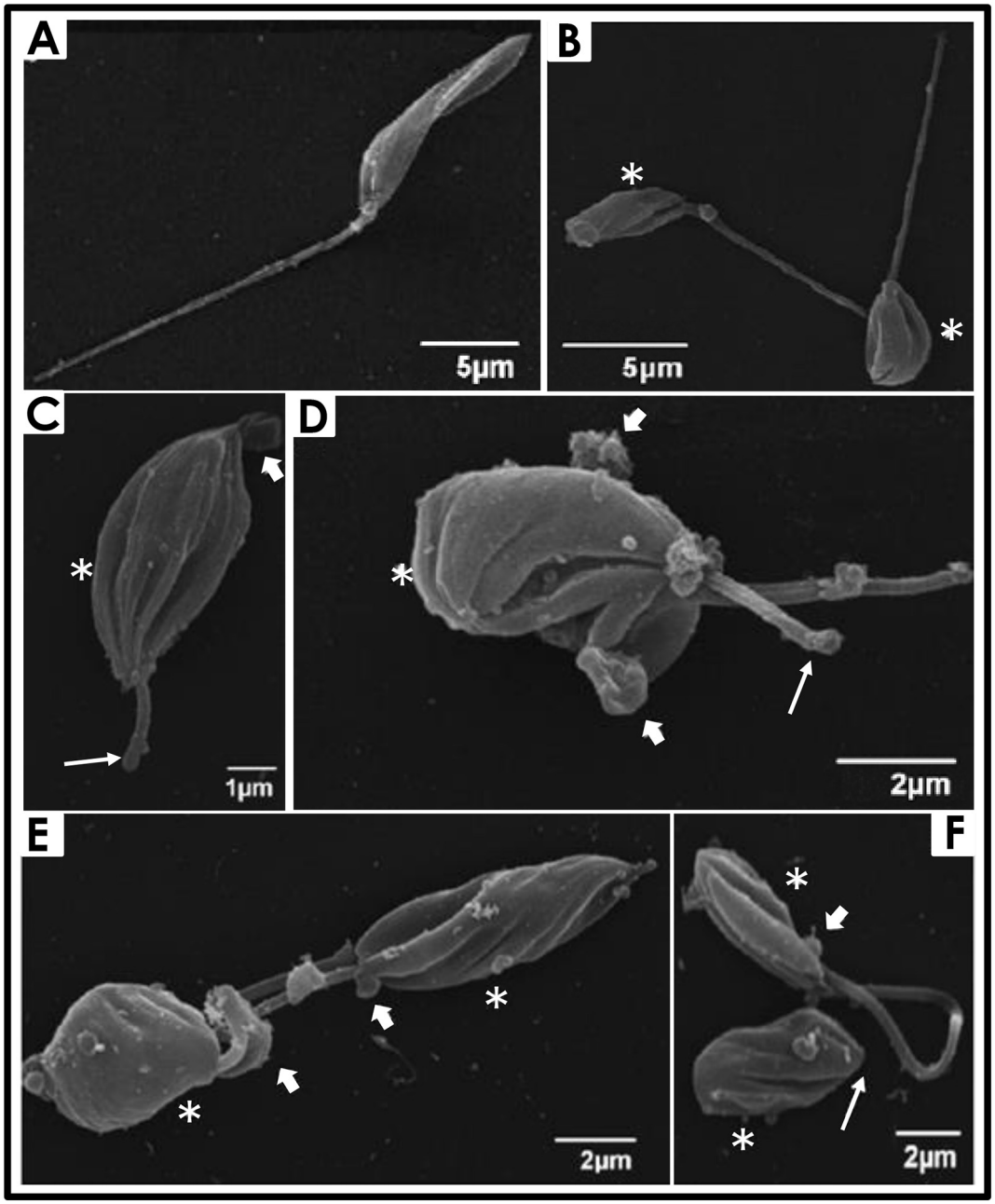


Fig. 2. Effects of 157 on the ultrastructure of *Leishmania amazonensis* promastigotes. Scanning electron microscopy images of promastigotes treated with the ½ × IC_{50} (b), IC_{50} (c and d) and 2 × IC_{50} (e and f) doses of **157** show parasites displaying round in shape (asterisks), blebs of the plasma membrane (large arrows) and shortening or loss of flagellum (white arrow). Control cells (a) display the typical promastigotes morphology. The images are a representative set of three independent experiments.

global size (Fig. 1B). Also, we decided to investigate the effects of this compound on the ultrastructure of **157**-treated parasites. In SEM assay, the control, non-treated parasites retained their normal features, like a stable cell surface, the typical elongated shape and long flagellum at all time points (Fig. 2A). Promastigotes treated with **157** exhibited shrinking/rounding up responses in all doses tested (Fig. 2B-F). In addition, many cells treated with the IC_{50} and 2 × IC_{50} doses were bizarrely shaped, sometimes presenting membrane protrusions resembling surface blebs and also the loss of flagellum, consistent with the decrease in their viability (Fig. 2C-F). Similar alterations were achieved in *Leishmania* and *Trypanosoma cruzi* parasites under distinct aspartyl peptidase inhibitors such as the classic ones, pepstatin A and DAN, and the HIV-PIs [9,17–20].

The surface alterations observed by SEM indicate possible alterations in the plasma membrane of promastigotes. Indeed, mostly of **157**-treated parasites (> 85%) presented PI-labeling, indicating that the compound affected plasma membrane integrity (Fig. 3**-left panel**). In addition, a quantitative analysis confirmed that the permeability of the plasma membrane was increased in a significant dose-dependent way (Fig. 3**-right panel**). These alterations are very common in parasites exposed to different classes of natural and synthetic compounds [21–24]. The increased membrane permeabilization can lead to parasite to death as a consequence of the osmotic imbalance, as commonly observed in parasites passing through necrotic mechanisms [25–27]. Similar alterations were also observed in *T. cruzi* epimastigote and trypomastigote forms when treated with the HIV-PIs nelfinavir and lopinavir [8,28].

In our study, the low parasite viability was also confirmed by the

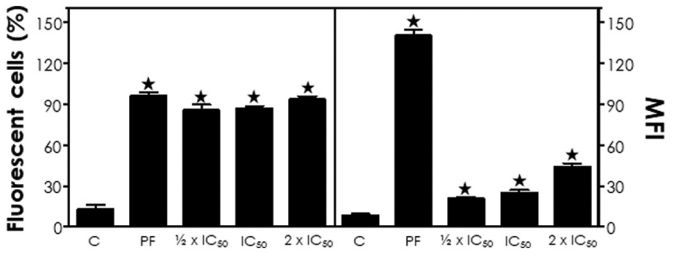


Fig. 3. Effects of 157 on the fine membrane structure of *Leishmania amazonensis* promastigotes. Flow cytometry analysis of the incorporation of PI in promastigotes untreated (control, C) and treated with different concentrations (½ × IC_{50}, IC_{50} and 2 × IC_{50} values) of **157** for 72 h. The results in the left panel express the percentage of fluorescent cells and in the right panel the mean of the fluorescence intensity (MFI) of each population. Parasites fixed with 4% paraformaldehyde (PF) were used as non-viable cells (PI-positive staining). Data shown are the mean ± standard deviation (SD) of three independent experiments performed in triplicate. The stars represent significant statistical differences compared to the respective negative control ($P < .01$).

great decrease in resazurin reduction after **157** treatment, mainly with the IC_{50} and 2 × IC_{50} doses of the drug (Fig. 4A). This result indicated that the compound **157** induced loss of viability in a concentration-dependent manner also by affecting the parasite metabolism. However, further studies are necessary to determine the targets involved. In this sense, incubation with the fluorochrome JC-1 revealed an important mitochondrial depolarization of promastigotes treated with **157** at concentrations of IC_{50} and 2 × IC_{50} doses (Fig. 4B). For a proper comparison, parasites were also incubated with the classical inhibitor of the mitochondrial function, FCCP, a standard protonophore uncoupler that dissipates the mitochondrial electrochemical H^+ gradient. At 34 min of reaction, FCCP reduced the $\Delta\Psi m$ in 59.7%, while **157** induced mitochondrial depolarization by *ca.* 40.3% when used at the IC_{50} dose and *ca.* 48.4% at 2 × IC_{50} dose (Fig. 4C). After 34 min of reaction, 2 μM FCCP were added to all the systems in order to completely collapse the $\Delta\Psi m$ (Fig. 4C). *Leishmania* parasites, as well as *T. cruzi* and *T. brucei*, disposes of only one mitochondrion, which has a fundamental role in the overall metabolism of the parasite [29]. So, undoubtedly, the $\Delta\Psi m$ loss findings promoted by **157** support the belief that the compound imbalance the correct mitochondrial functionality that could strongly contribute to protozoal death. In line with our current findings, it has also been demonstrated that the HIV-PI nelfinavir promotes a severe loss of $\Delta\Psi m$ in *L. donovani* amastigotes and *T. cruzi* epimastigotes and trypomastigotes. In *T. cruzi* parasites, the same occurred in the treatment with lopinavir, another HIV-PI [8,28,30].

Since it is clear that **157** reduces drastically the viability of promastigotes, it becomes important to verify the effects of this peptidomimetic compound in two processes extremely relevant for the parasite in leishmaniasis. One of them is the interaction with phagocyte cells, as macrophages, the first step for infection development. The second one is the development and replication of intracellular amastigotes that are the forms responsible for maintaining and spreading the infections in vertebrate hosts. Therefore, in this set of experiments, promastigotes were firstly pre-treated for 1 h with **157** at ½ × IC_{50}, IC_{50} and 2 × IC_{50} doses before interaction with RAW murine macrophages. In comparison to control, **157** only at the highest concentrations reduced the association index with RAW cells. The adhesion was impaired by around 41% and 63% when parasites were treated with IC_{50} and 2 × IC_{50} doses, respectively (Fig. 5A). It should be pointed out that these inhibition profiles were not caused by a decrease in promastigote viability, as judged by the resazurin reduction assay (Fig. 5A-**inset**). Taken together, the interference in the early steps of cell infection may happen due to the direct, non-specific toxic effects of **157** or due to the non-specific modulation of the expression of surface parasite molecules [31]. In another report, Santos and co-workers [18] demonstrated that the interaction process between mouse macrophages and *L. amazonensis*

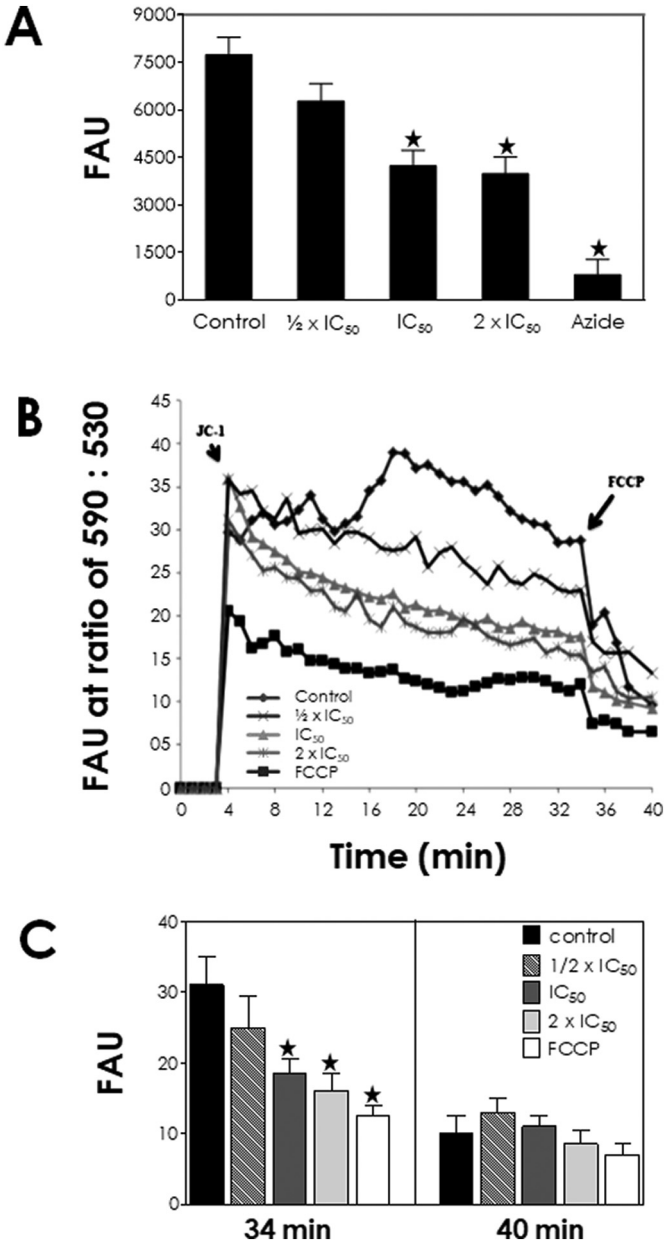


Fig. 4. Effects of 157 on *Leishmania. amazonensis* promastigotes metabolism. (A) Parasites were treated with different concentrations of the compound and after 72 h, the levels of resazurin reduction were estimated in comparison to control. Parasites were also treated with sodium azide to obtain non-viable cells. (B) The $\Delta\Psi m$ analysis of control and parasites treated with **157** at ½ × IC_{50}, IC_{50} and 2 × IC_{50} values was performed using the JC-1 fluorochrome during 30 min, after which the uncoupler FCCP at 2 μM (arrows at the time point of 34 min) was added in all the systems to collapse mitochondrial potential. As a positive control of the depolarization of the mitochondrial membrane, the reaction was also evaluated in the presence of FCCP (1 μM) from the beginning of the experiment. (C) Respective comparisons of the $\Delta\Psi m$ values shown in (B) before (34 min) and after (40 min) the addition of the uncoupler FCCP. The results are expressed as fluorescence arbitrary units (FAU). Data shown are the mean ± standard deviation (SD) of three independent experiments performed in triplicate. The stars represent significant statistical differences compared to the respective controls ($P < .01$).

promastigotes was blocked when the parasites were pre-treated with nelfinavir, lopinavir and amprenavir at concentrations above 50 μM. At lower concentrations, both nelfinavir and lopinavir also significantly reduced the association index of *T. cruzi* trypomastigotes with RAW macrophages [32].

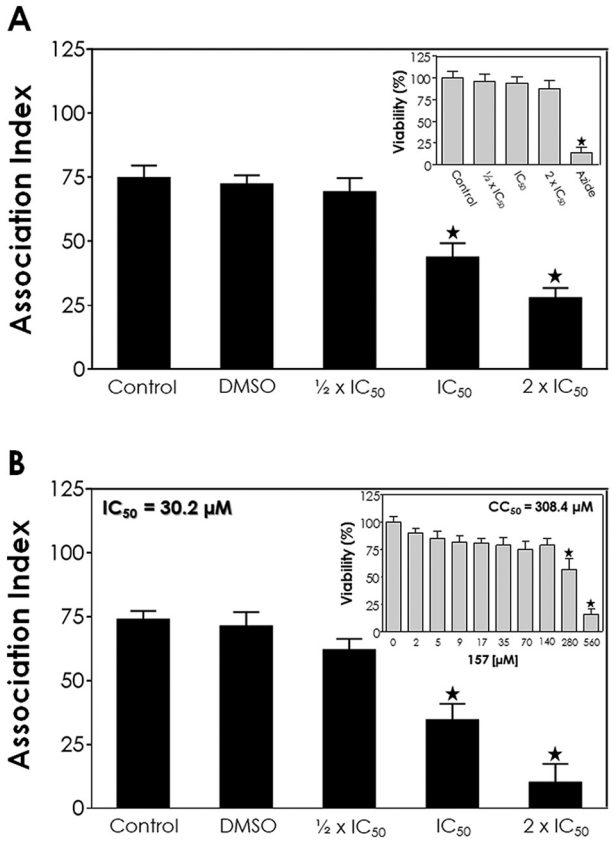


Fig. 5. Effects of the pre-treatment of *Leishmania amazonensis* promastigotes with 157 on the interaction with RAW macrophages and susceptibility of intracellular amastigotes. (A) Parasites (10^5/mL) were pre-treated or not (control) with different concentrations of **157** for 1 h. After this period, parasites were washed to remove the compound and then allowed to interact with RAW cells for 1 h in a cell ratio of 10:1 (parasites per cell). The inset confirm that all concentrations used for **157** have no effect on promastigotes viability, as accessed by the resazurin assay. (B) Susceptibility of intracellular amastigotes to **157**. Initially, RAW macrophages were previously infected with promastigotes at a ratio 10:1 (parasites/cell) for 1 h at 37 °C. After this period, systems were washed to remove the non-internalized parasites and then treated or not (control) with the compound **157** at different concentrations for 72 h. The concentrations employed did not display toxic effects to the RAW cells, as demonstrated by MTT assay in the inset. The IC_{50} and CC_{50} values of **157** for intracellular amastigotes and RAW macrophages, respectively, were determined after 72 h, as indicated. The association index in all systems were determined by light microscopy, counting at least 200 cells in each triplicate coverslip. Data shown are the mean ± standard deviation (SD) of three independent experiments performed in triplicate. The stars represent the significant differences in relation to control ($P < .01$).

After checking the effects of **157** in the early steps of parasite infection, we decided to investigate the effects of the compound on intramacrophage replicative amastigotes. For that, the cytotoxicity on RAW macrophages was primarily determined. In 72 h-treated RAW cultures, a significant deleterious effect was only observed with concentrations higher than 140 µM. The 50% cytotoxicity concentration dose (CC_{50}/72 h) was calculated as 308.4 µM (Fig. 5B-**inset**). These concentrations are much higher than the highest concentration desired to use ($2 \times IC_{50}$ = 67.4 µM) in the treatment of infected macrophages. For comparison, 157 is less toxic for macrophages than HIV-PIs, which present deleterious effects in concentrations above 12.5 µM for RAW macrophages [32] and 25 µM for mouse peritoneal macrophages [18].

After establishing the secure doses of **157**, infected RAW systems were treated once with different concentrations of the peptidomimetic ($½ \times IC_{50}$, IC_{50} and $2 \times IC_{50}$ doses for promastigotes), and then followed for 72 h. In infected cultures, a clear dose-dependent effect of **157** on the infection rate was observed, for which the IC_{50} value for the intracellular amastigotes was calculated as 30.2 µM (Fig. 5B). The highest dose used ($2 \times IC_{50}$) promoted an incredible reduction of 86.3% in the association index. Collectively, under our experimental conditions, the compound **157** showed higher toxicity to *L. amazonensis* promastigotes (IC_{50} value of 33.7 µM) and amastigotes (IC_{50} value of 30.2 µM) compared to RAW macrophages (CC_{50} value of 308.4 µM), resulting in good similar SI values: 9.15 and 10.23, respectively.

Trudel and co-workers [33] demonstrated that nelfinavir, ritonavir and saquinavir decreased the intracellular survival of *Leishmania* amastigotes in THP-1 macrophages and human monocyte-derived macrophages. In the study of Santos and co-workers [18], the authors verified that nelfinavir, lopinavir and amprenavir notably reduced the number of *Leishmania* parasites inside mouse macrophages. Nelfinavir and lopinavir are also capable of reducing drastically the number of *T. cruzi* amastigotes inside RAW cells [32]. The effects of **157** on the intracellular parasites can be related to non-specific toxic effects (acting on unknown targets) and/or to the inhibition of aspartyl type peptidases, as recently published by our group [5]. In this line, Mishra and co-workers [34] developed potent peptidomimetic inhibitors targeting specifically four vacuolar plasmepsins of *Plasmodium falciparum*, aspartyl peptidases involved in hemoglobin catabolism inside the acidic food vacuole in the intraerythrocytic stage [35]. The peptidomimetic compounds KNI-10743 and KNI-10333 exhibited nanomolar inhibition against the *Plasmodium* plasmepsins, blocking hemoglobin degradation inside the food vacuole and also possessing significant antimalarial activity.

4. Conclusion

Together, our results indicate that **157** affects the viability of promastigotes and amastigotes of *L. amazonensis*, presenting low toxicity to the host cells. The peptidomimetic **157** has the advantage to be a simple molecule in its structure, being easily synthesized in few steps with low cost; thus, it can be considered as an interesting candidate for further optimization to use against *Leishmania* as well as other neglected pathogenic parasites.

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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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