

Ultrastructural and Morphological Alterations Induced by **23-4-A-CC** on Promastigotes of *Leishmania amazonensis*

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Leishmania amazonensis is the principal protozoan that causes cutaneous leishmaniasis and affects approximately 12 million people worldwide. Current therapies available have variable efficacy and high toxicity. In this context, are necessary studies for the development of new alternatives of treatment for leishmaniasis [1][2]. In previous studies, our research group has already shown that the synthetic compound 3-carboxyethyl-1-phenyl-4-[(fluorophenyl)amino]methyl}-5-(4-nitrophenyl)-1H-pyrazole (**23-4-A-CC**) showed an IC₅₀ of 3.8 μM after 72 h of treatment. The aim of this study was to evaluate ultrastructural and morphological alterations in *L. amazonensis* promastigotes. For this, promastigotes treated with IC₅₀ of **23-4-A-CC** for 72 h, were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 24 h at 4 °C. For SEM, the parasites were dehydrated, critical point-dried in CO₂, sputter-coated with gold and observed using a FEI Scios. For TEM, parasites were postfixated, dehydrated, embedded in Polybed 812 resin, obtained ultrathin sections, contrasted with uranyl acetate and lead citrate, and observed in JEOL JEM 1400. To evaluate the appearance of lipids bodies, the parasites were probed with Nile red and observed in confocal microscopy Zeiss LSM 5 Pascal. Our results demonstrated that **23-4-A-CC** induced morphological and ultrastructural changes, such as mitochondrial swelling, presence of lipid storage bodies and abnormalities in flagellum. Our results support further studies and may open the way for the development of new chemotherapeutic agents against *L. amazonensis* [3].

References:

- [1] T.F. Stefanello et al., AAC, **58** (2014), p. 7112.
- [2] L.T. Tonin et al., Pharmacotherapy, **64** (2010), p. 386.
- [3] The authors acknowledge founding from CAPES, CNPq, COMCAP-UEM, FINEP and PRONEX/Fundação Araucária.

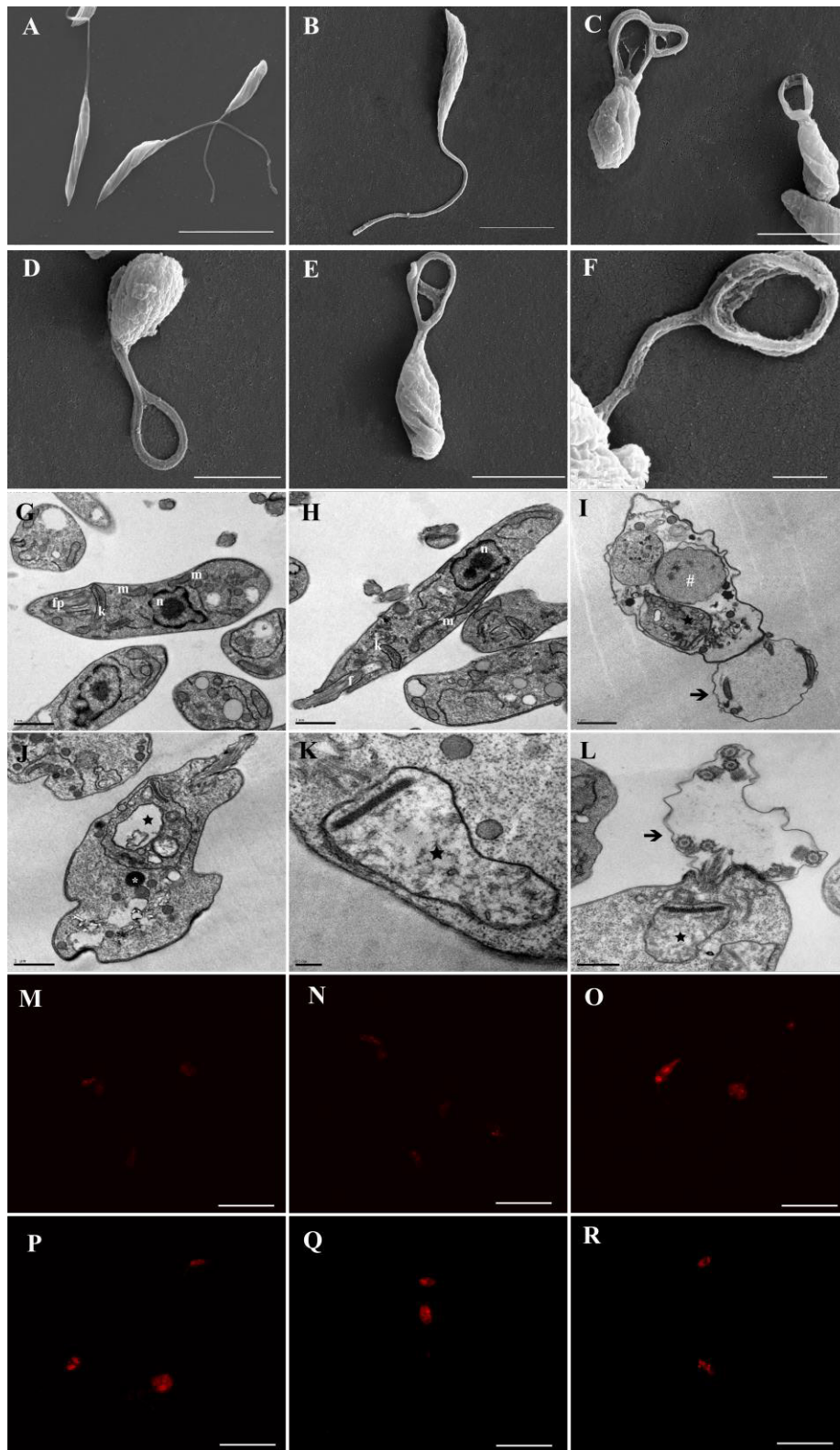


Figure 1. Morphological and ultrastructural alterations in promastigote forms of *Leishmania amazonensis* treated with 3.8 μ M of **23-4-A-CC** for 72 h. (A-F) SEM images. (G-L) TEM images. (M-R) Nile red confocal microscopy images. (A-B, G-H, M-N) Untreated promastigotes. (C-F, I-L, O-R) Promastigotes treated with 3.80 μ M of **23-4-A-CC**. (#) DNA disorganization in nuclei; (*) lipids bodies; (star) mitochondrial swelling in the kinetoplast region; (arrow) flagellum disorganization; (f) flagellum; (fp) flagellar pocket; (k) kinetoplast; (m) mitochondrion; (n) nucleus. Scale bars = 20 μ m in A, M-R; 5 μ m in B; 4 μ m in C, E; 3 μ m in D; 1 μ m in F-J; 0.5 μ m in K-L.