ANTI-*TRYPANOSOMA CRUZI* ACTIVITY OF THE MARINE MICROBIAL ALKALOID (R)-SALSOLINOL: CRUZIPAIN AS A POTENTIAL TARGET

MARIANA BABBERG ABIUZI[1,2], BEATRIZ ALVES DE ANDRADE[2], MYRON CHRISTODOULIDES[3], RAVI KANT[3], JOÃO HENRIQUE GHILARDI LAGO[4], ANDRE GUSTAVO TEMPONE[1]

[1]PATHOPHYSIOLOGY LABORATORY, INSTITUTO BUTANTAN, SÃO PAULO, BRAZIL.

[2]PROGRAMA DE PÓS-GRADUAÇÃO DA COORDENADORIA DE CONTROLE DE DOENÇAS, SES-SP, BRAZIL

[3]NEISSERIA RESEARCH LABORATORY, MOLECULAR MICROBIOLOGY, SCHOOL OF CLINICAL AND EXPERIMENTAL SCIENCES, UNIVERSITY OF SOUTHAMPTON, SOUTHAMPTON, UNITED KINGDOM

[4]CENTRE OF NATURAL SCIENCES AND HUMANITIES, FEDERAL UNIVERSITY OF ABC, SÃO PAULO, BRAZIL

Chagas disease is a potentially fatal disease with only one available drug in Brazil. Over the past 50 years, the marine environment has provided more than 20,000 inspiring small molecules for drug discovery studies, with a myriad of biological activities including parasites. In this work, we aimed to isolate, characterize and evaluate the anti-*Trypanosoma cruzi* activity of secondary metabolites from the marine bacteria *Bacillus altitudinis*. The microbial secondary metabolites were fractionated by HPLC and the chemical elucidation of compounds was performed by Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR) and HRLC/MS. The evaluation of the EC_{50} was determined in trypomastigotes and intracellular amastigotes and the mammalian cytotoxicity was carried out in human-derived monocytes (THP-1). *In silico* studies were performed for drug-like properties and protein-ligand interactions to assess the binding stability and molecular recognition of the active compound within cruzipain. The active compound was characterized as the alkaloid (R)-salsolinol (MW 260 Da), which showed activity against *T. cruzi* trypomastigotes and intracellular amastigotes, with EC_{50} values of 78 µM and 110 µM, respectively, without toxicity to THP-1 cells (CC_{50} >200 µM). The *in silico* pharmacokinetic predictions indicated a high gastrointestinal absorption and low blood-brain barrier permeability. Additionally, (R)-salsolinol showed no inhibitor characteristics for the five main P-450 cytochrome isoforms and it was approved by four pharma filters as an oral candidate. The interaction profile with cruzipain revealed that the key residues involved in binding were CYS25, TRP26, GLY66, LEU67, MET68, ASN69, VAL132, ALA133, VAL134, GLN156, LEU157, ASP158, HIS159, GLY160, GLU205, and SER207 as primary contributors. Targeting this enzyme with novel small-molecule inhibitors could offer a viable strategy for therapeutic intervention.

Supported by FAPESP 2024/16243-4, 2023/07414-7.

Keywords: Trypanosoma cruzi, therapy, natural products