Biochemical and inhibition studies on *Leishmania donovani* tyrosine aminotransferase

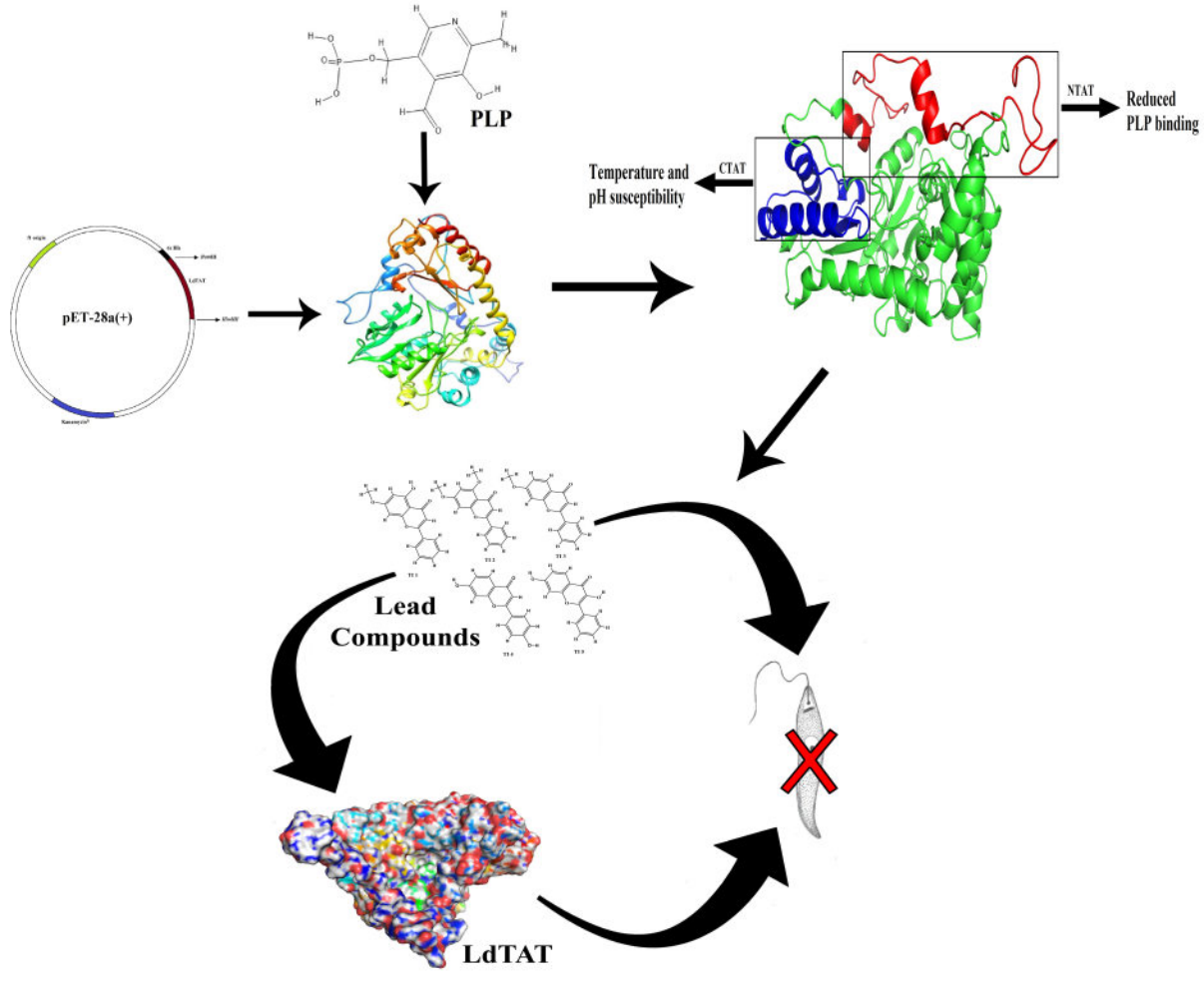
Santanu Sasidharan and Prakash Saudagar

Department of Biotechnology, National Institute of Technology, Warangal-506004, Telangana, India

Abstract

Leishmaniasis is a neglected tropical disease affecting millions worldwide every year. The treatment regimen currently includes miltefosine and liposomal amphotericin B. However, these drugs are toxic and non-economical. Considering these drawbacks and the dearth of drugs against *Leishmania*, Tyrosine aminotransferase (TAT), an enzyme that catalyzes the transamination of amino acids in *Leishmania,* was studied. The full-length TAT from *Leishmania donovani* LDP18 was cloned, expressed, and purified by affinity chromatography. Biochemical studies revealed the K_m and V_{max} values as 3.5 ± 0.9 mM and 11.7 ± 1.5 µM.min.µg^{-1} with a three-state folding mechanism. The recombinant TAT was catalytically active over a wide range of pH (3.5-10.5) and temperature (20-75 °C). Spectroscopic and computational studies found that the pH tolerance was due to the concerted action of the charges between active site and co-factor and structural folding. Moreover, the non-conserved N-terminal (NTAT) and conserved C-terminal domains (CTAT) of TAT were truncated, cloned, and purified to determine their roles in regulating the enzyme activity. NTAT, like TAT, was stable at extreme temperatures and pH conditions, whereas CTAT was relatively susceptible to these variations. The unfolding studies indicated that the full-length TAT and NTAT unfolded via a three-state mechanism, while the CTAT exhibited two-state folding. From this, N-terminal was determined to be responsible for the stabilization of the co-factor (PLP) in the active site while C-terminal conferred active site protection to extreme conditions. Further, a curated ZINC15 database containing 1,83,659 natural compounds was screened against the full-length TAT, and the top five compounds (TI1-TI5) were chosen based on their binding scores. Molecular dynamic simulations revealed the high-affinity interactions of TI1, TI3, TI4, and TI5 towards the active site residues. *In vitro* inhibition studies indicated K_i values of 0.9 ± 0.2 µM and 0.3 ± 0.1 µM for TI3 and TI4, respectively. Notably, TI3 and TI4 exhibited anti-leishmanial activity with IC50 values of 5.6 ± 1.72 µM and 9.6 ± 3.11 µM, respectively. We conclude that the lead inhibitors TI3 and TI4 offer huge potential as anti-leishmanial candidates and require further characterization in cell and animal systems to validate their potential as an anti-leishmanial drug.

Keywords: Leishmaniasis; Tyrosine aminotransferase; Anti-leishmanial; Biochemical characterization; Enzyme;


PLP
pET-28a(+)
NTAT
Reduced
PLP binding
CTAT
Temperature and
pH susceptibility
Lead
Compounds
LdTAT