Kinetic assay standardization and *in vitro* screening of *L. chagasi* Pteridine reductase (LcPTR1) inhibitors

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Keywords: *PTR1,purification, kinetic assay, Thiazolidinone.*

Abstract

PTR1 is crucial to leishmanial survival. However, no inhibitor of *L. chagasi* PTR1 has been reported so far. Our molecular cloning and expression/purification efforts afforded the development of a standardized kinetic assay that allowed us to identify a novel series of inhibitors.

Introduction

Although Leishmania parasites are auxothophs for purine and pteridine, classical folate inhibitors have poor efficacy against this parasite.[1] The escape mechanism depends on the activity of pteridine reductase (PTR1) and for that reason several inhibitors of *L. major* PTR1 (LmPTR1) have been described.[2] On the other hand, few studies have been carried out with *L. chagasi* PTR1 (90% identity to LmPTR1 according to MULTALIGN). We have shown that thiazolidinone derivatives inhibit LmPTR1 (Figure 1) but their effect over LcPTR1 was not investigated.

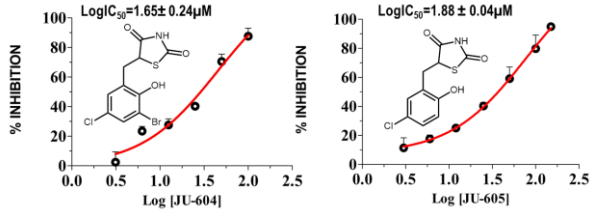

Figure 1. Inhibitory profile of thiazolidinone derivatives against *L. major* PTR1

Herein we report not only the molecular cloning of *ptr1* gene from *L. chagasi* into *E. coli* (BL21-DE3), the soluble expression of this enzyme and its purification protocol, but also the results from screening assays under balanced conditions.

Results and Discussion

Ligase independent cloning (LIC) strategy was employed to insert *ptr1* gene into pET-M11, pET-NUSa and pET-TRX expression vectors. Initial screening of expression conditions, under different temperatures (18ºC, 25ºC, 37ºC) and IPTG concentrations (0,1-1,0 mM), with *E. coli* BL21-DE3 have shown that high amounts of soluble protein are obtained at 25 ºC with 0.5 mM IPTG in pET-M11. As this vector adds a HIS-tag to the N-terminal of LcPTR1, the enzyme was purified by affinity chromatography (Ni-sepharose). The purified protein was eluted with 400 mM imidazole and then the tag was removed with TEV protease (1:10). This strategy affords 8-10 mg/L of LcPTR1 that shows a single peak in size exclusion chromatography. Kinetic assays under steady state conditions and high cofactor (NADPH= 100 μM) concentrations shows that biopterin KM=24.5 μM and VMAX= 0.15. Conversely, the KM and VMAX for NADPH (35.3 μM and 0.20 respectively) was determined at high substrate concentration (biopterin= 100 μM) (Figure 2).

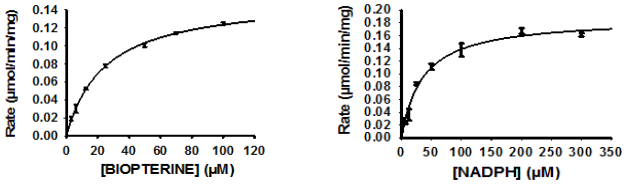

Figure 2. Michellis-Menten plots for *Lc*PTR1.

Kinetic assays reveal that thiazolidinone derivatives JU604 (IC_{50}= 23.29 μM ± 4.43) and JU605 (IC_{50}=24.45 μM ± 4.13) are more potent against LcPTR1 than against *L. major* PTR1.

Conclusions

Although the efforts reported in this work lead to the identification of micromolar inhibitors of LcPTR1, further investigation is required to explain their flat SAR against their macromolecular target and prove if their inhibition mechanism is the same observed for LmPTR1.

Aknowlegments

CNPq, FAPESB and RVC GUIDO for LIC cloning assistance

[1] WHO. Tecnical Report Series. Control of the leishmaniasis: report of a meeting of the WHO.Expert Committee on the Control of Leishmaniases, Geneva, 2010.

[2] Wang J, Leblanc E, Chang CF, et al. Pterin and folate reduction by the Leishmania tarentolae H locus short-chain dehydrogenase/ reductase PTR1. Arch Biochem Biophys 1997;342:197–202.