Discovery of novel *Leishmania major* Pteridine Reductase 1 inhibitors

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Palavras-chave: *Themorfluor®, Pterdine Reductase 1, Thiazolidinone.*

Introduction

According to WHO, Leishmaniasis is the second most important disease caused by protozoans. However, the available therapeutic arsenal for its treatment is limited and has low efficacy and safety profile[1,2]. Aiming at circumvent this dilemma, key enzymes of the folate metabolism have been targeted. However, Dihydrofolate Reductase–Thymidylate Synthase (DHFR-TS) inhibitors are ineffective against *Leishmania major* due to an alternative folate pathway regulated by Pteridine Reductase (PTR1). Thus, identification of novel PTR1 inhibitors poses as an essential step towards leishmanicidal drug development. As PTR1 is a NADPH dependent enzyme and thiazolidinone ring has been proposed as a purine ring bioisosteric replacement[3], 20 thiazolidinone derivatives (Figure 1) were synthesized and assayed against PTR1.

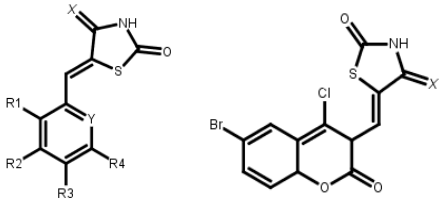

Figure 1: General scaffolds of thiazolidinone derivatives.

Results and Discussion

In vitro evaluation of PTR1 can be carried out at 340 nm (NADPH consumption through time), but most thiazolidinone derivatives absorb within this bandwidth. Therefore, we resorted to Differential Scanning Fluorimetry, also known as Thermofluor® assay that probes the effects of molecules/additives over the protein unfolding temperature (Tm)[4]. Thermodynamics dictates that bound-protein is more stable than unbound-ones. As a consequence, inhibitors should produce a positive ΔTm, whereas chaotropic agents shall reduce Tm (negative ΔTm). Accordingly, 6 thiazolidinone derivatives lead to thermal shift higher than 1ºC at 50μM (Figure 2). Furthermore, bioactive molecules were evaluated in dose-response assays (Figure 3). Although the saturation curve can be used to calculate Kd/Ki values, Thermofluor® does not correctly describes ΔH and TΔS parameters. Thus, this value (Th-19, Kd= 142 ± 52.67 μM) should be considered as an approximation only.

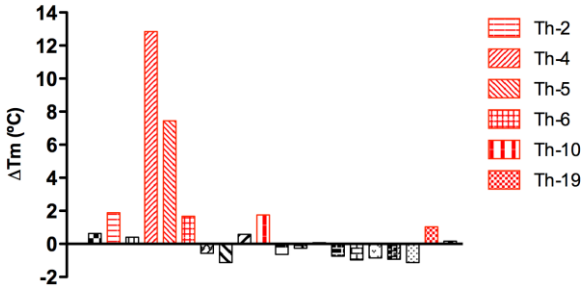

Figure 2: PTR1 thermal shift (ΔTm) in the presence of thiazolidinione derivatives. DMSO was employed as reference (ΔTm=0).

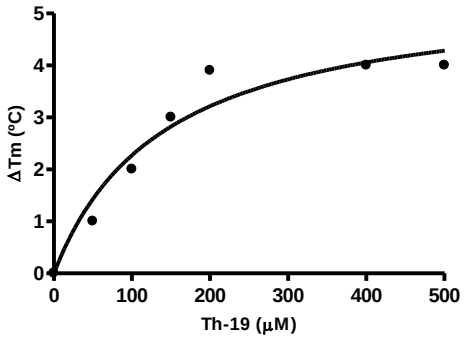

Figure 3: Concentration-dependent stabilization of PTR1 by Th-19.

Preliminary SAR analysis suggests that oxygen at position X increases affinity towards PTR1, in comparison to sulfur. Likewise, electron withdrawing moieties at R1 and R4 have a positive effect over the biological property.

Conclusion

Themofluor® assay was essential not only to identify a novel chemical class of PTR1 inhibitors, but also to shed some light on these inhibitors SAR.

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