DEVELOPMENT OF A PROMASTIGOTE RESAZURIN BASED VIABILITY ASSAY IN 384-WELL FORMAT FOR HIGH THROUGHPUT SCREENING OF *LEISHMANIA DONOVANI* DD8.

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Introduction

Leishmaniasis is characterized as a parasitic disease caused by the trypanosomatid protozoan termed *Leishmania*. Leishmaniasis is endemic in 88 countries with increased cases of morbidity and mortality emerging each day. Presently, 12 million people are infected and around 350 million people are constantly at risk of acquiring this disease. According to clinical manifestations leishmaniasis can be characterized as cutaneous, muco-cutaneous or visceral leishmaniasis; the latter being fatal. The mode of transmission of this disease is via the bite of a sandfly, genus Phlebotomus (old world) and Lutzomyia (new world). The life cycle of *Leishmania* exists between the sand fly (promastigote form) and the mammalian host (amastigote form). Although leishmaniasis is treatable, it faces challenges largely due to emerging resistance and extensive toxicity for current drugs. There is considerable need for assays which are cost effective, robust, automated for ideal therapeutic candidate selection.

Aim

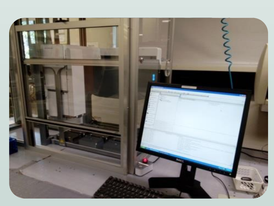
The development, optimization and execution of a resazurin based high throughput assay to identify new chemical scaffolds against *Leishmania donovani* DD8 promastigotes.

Methods

The *leishmania* promastigote assay involves multiple steps over a 4 day period, summarized below:

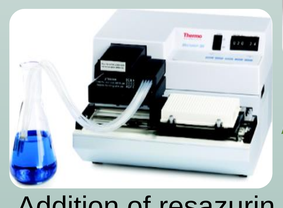
Culturing of *Leishmania donovani* DD8 (mid log phase parasites)

Parasites seeded 5x10^5 parasites/mL in 384 well plate



Addition of Drugs & Controls

Incubation at 27 ºC for 68 hours



Addition of resazurin

Source: Multilabel Reader Multidrop™ 384 Reagent Dispenser

Incubation for additional 4 hours at 27 ºC

Reading on Envision

Source: EnVision® PerkinElmer

Results

1- Determination of optimal parasite number and resazurin concentration

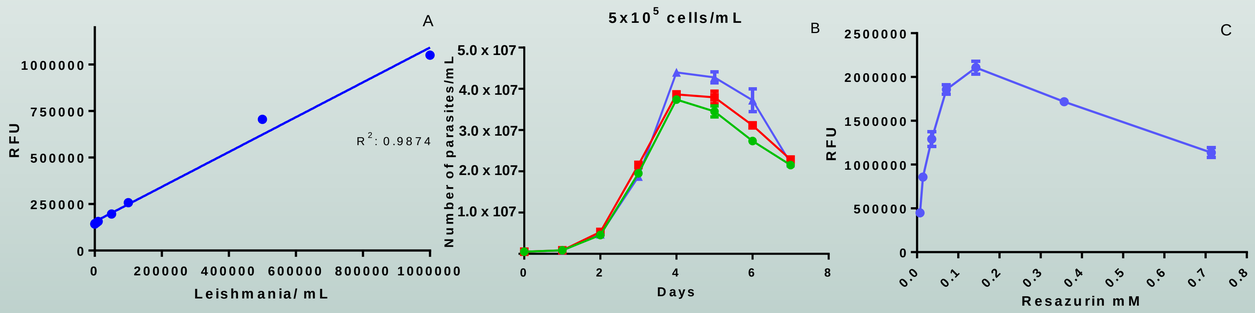


Figure 1: (A) The linear correlation of the parasite number with the signal intensity in RFU (relative fluorescence units), (B) 5x10^5 parasites/mL promastigotes in a 384 well plate following 7 days incubation at 27 ºC, (C) Represents the optimal resazurin concentration in mM at which maximum signal was achieved.

2- Establishing reference drug activity

A panel of reference compounds currently used for the treatment of leishmaniasis were screened for the established promastigote resazurin based viability assay.

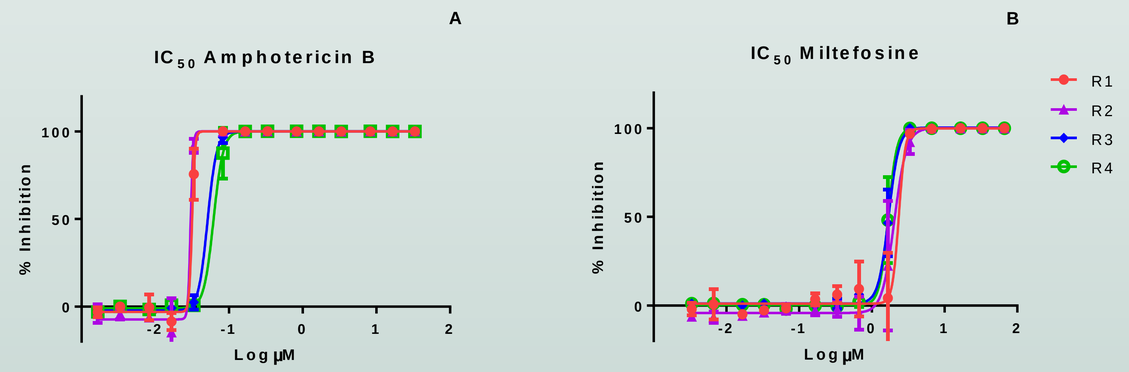


Figure 3: The dose response curves of (A) Amphotericin B and (B) Miltefosine against *L.donovani* DD8 promastigote. The IC$_{50}$ values of Amphotericin 0.056 ± 0.007 µM and Miltefosine 1.944 ± 0.317 µM are comparable to previously reported values.

3- Assay validation

Assay reproducibility was assessed with statistical parameters including Z', Signal to Background (S/B), Signal to Noise (S/N) and coefficient of variance (% CV).

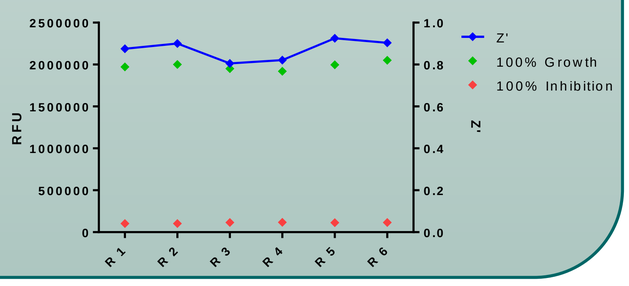
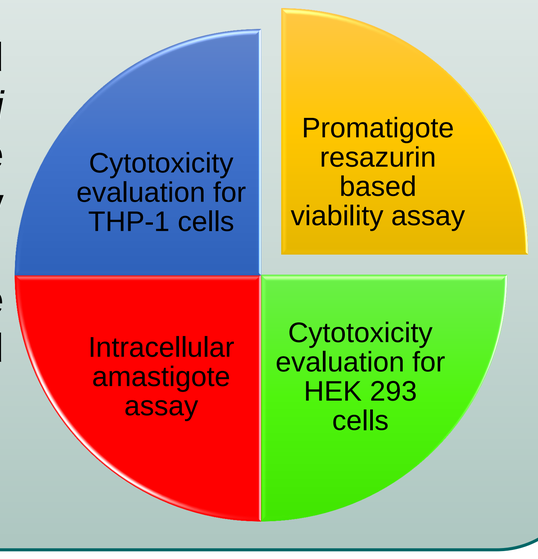


Figure 4: The average Z' value for the assay is 0.87 ± 0.04. The negative control is 0.4% DMSO and the positive control is 8.33 % DMSO final assay concentration.

Pilot screening and cross comparison with intracellular amastigote assay

Screening of a 5000 compound library against the L. *donovani* DD8 promastigote assay and the intracellular amastigote assay (Jones & Shelper) is in process. Cytotoxicity evaluation of the active hits will be performed against HEK293 and THP-1 cells.



Conclusion and future directions

An assay for high throughput screening of *Leishmania donovavi* DD8 promastigotes stage has been successfully developed monitoring parasite viability by measuring metabolical activity of parasites.

In future a cross comparison will be carried out between the compound efficacy on different strains from the old and new world based on genetic variability and susceptibility.

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