Cyclopental quinolin-9-amines as potent inhibitors of *Leishmania donovani*, and *Leishmania infantum*: causative agents of Old and New Worlds visceral leishmaniasis

Bilal Zulfiqar[1] , Fabio Antonio Colombo[2], Sujatha Manthri[3] , Laura Alcantara[3] , Carolina Moraes[4,5], Lucio Freitas Junior[4,5], Manu De Rycker[3] , Marcos Jose Marques[2], Vicky M Avery[1]

[1]Discovery Biology, Griffith Institute for Drug Discovery, Griffith University, Nathan, Queensland, 4111, Australia.; [2] Universidade Federal de Alfenas - UNIFAL-MG, Brazil.: [3]Drug Discovery Unit, University of Dundee, Dundee, UK.; [4]Laboratório Nacional de Biociências, Centro Nacional de Pesquisa em Energia e Materiais, Campinas-SP, Brazil.; [5]Departamento de Microbiologia, Instituto de Ciências Biomédicas,, São Paulo-SP, Brazil

OVERVIEW:

Current treatment regimens for leishmaniasis often have poor toxicity profiles and resistance has begun to emerge against the standard of care therapies. There is an urgent need for new treatments, thus identification of new chemical entities is essential for translation into potential new drugs.

INTRODUCTION:

Leishmaniasis, caused by the trypanosomatid protozoan parasite, *Leishmania,* is endemic in 98 countries with more than 350 million people at risk of acquiring the disease. Based on the clinical manifestations, the disease can be characterized as cutaneous, muco-cutaneous or visceral leishmaniasis; the latter being fatal.

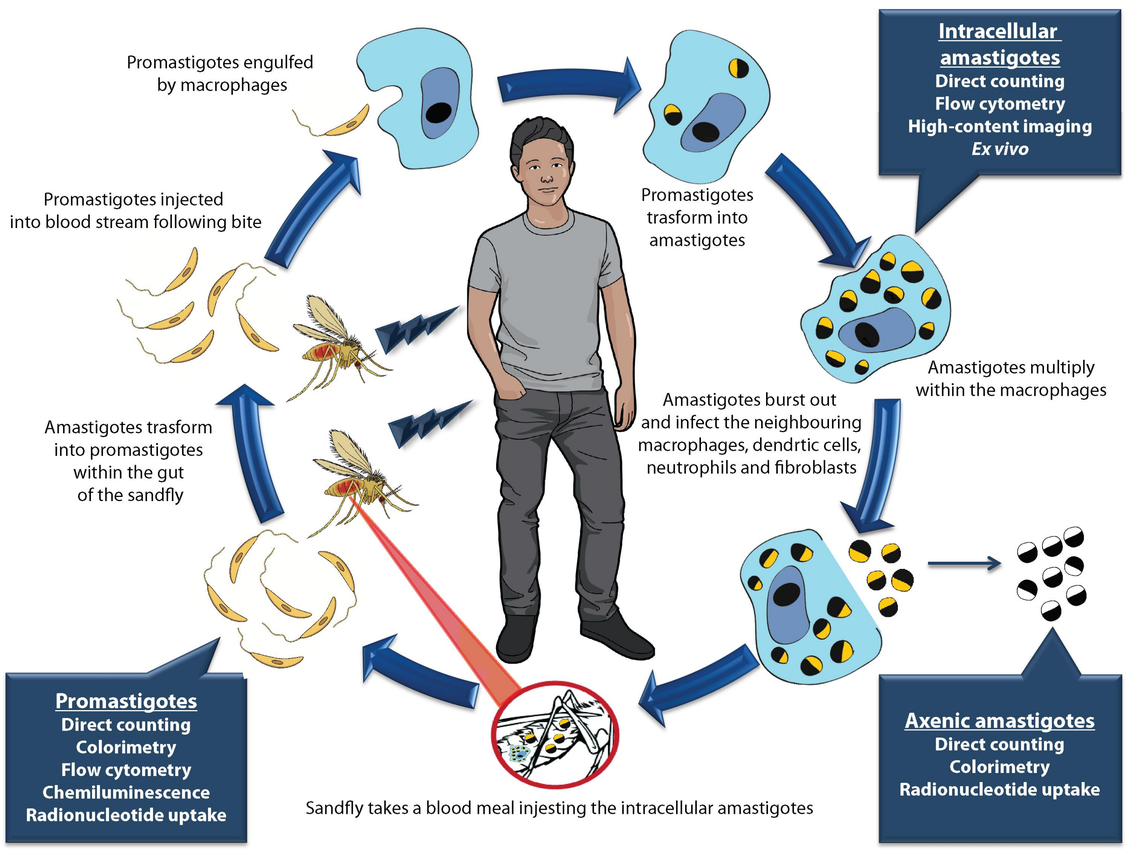
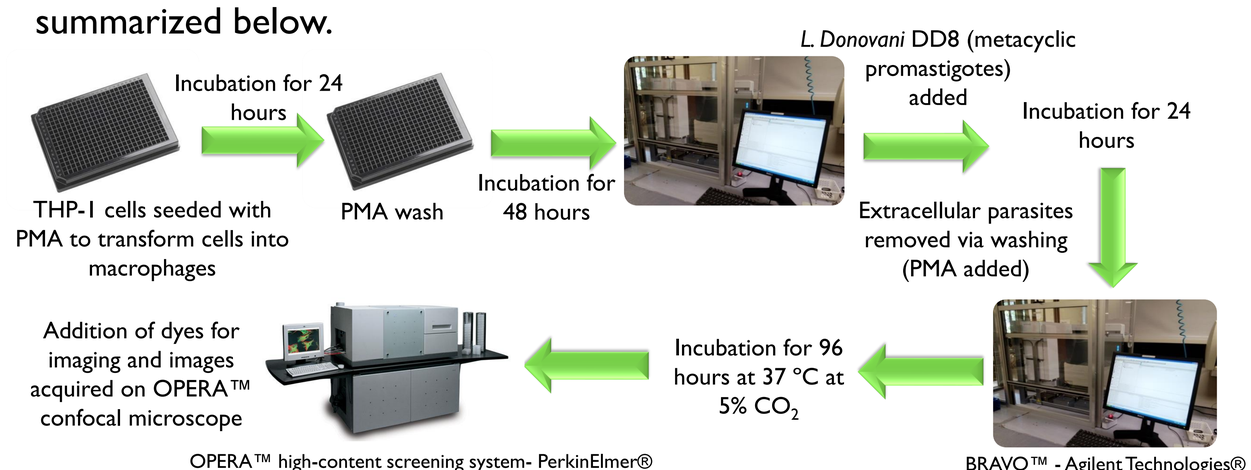


Figure 1: Life cycle of Leishmaniasis. Reproduced from Zulfiqar, B *et al* 2017. *Drug discovery today*, 22(10), 1516-1531.

METHODS:

The intracellular amastigote assay involves multiple steps over an 8 day period summarized below.



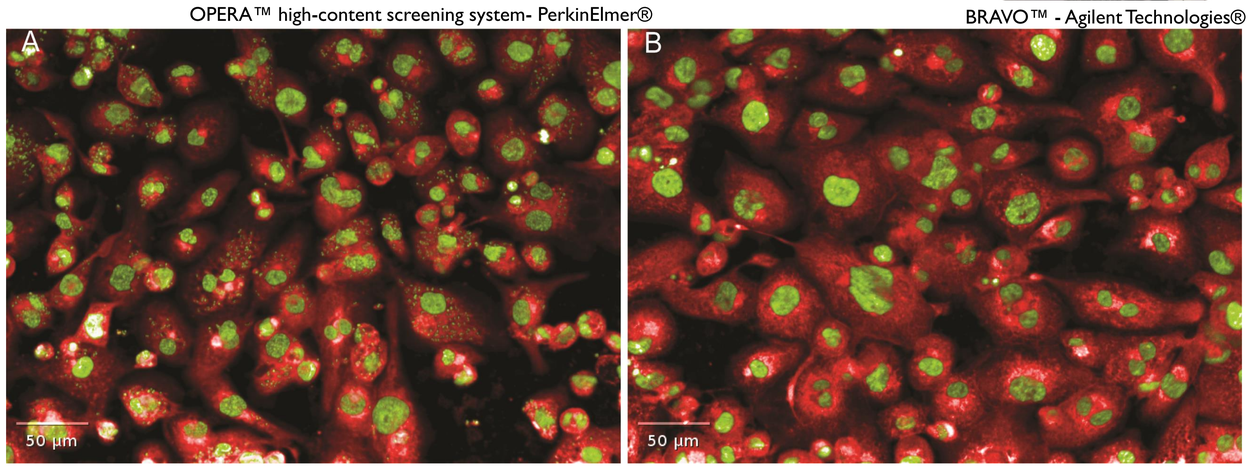


Figure 2: Controls for intracellular amastigote assay (A) Negative control with 0.4% DMSO (B) Positive control with 1 µM Amphotericin B. The cytoplasm is stained with CellMask™ deep red and the nucleus of the host cell and the parasite is stained by Sybr green.

RESULTS:

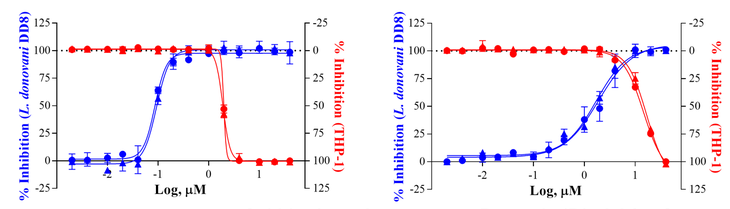


Figure 3: The dose response curves of (A) Amphotericin B and (B) Miltefosine against *L. donovani* DD8 intracellular amastigotes. The IC_{50} values of Amphotericin and Miltefosine were 0.20 ± 0.02 and 2.54 ± 0.57 µM, respectively. These values are comparable to previously reported values.

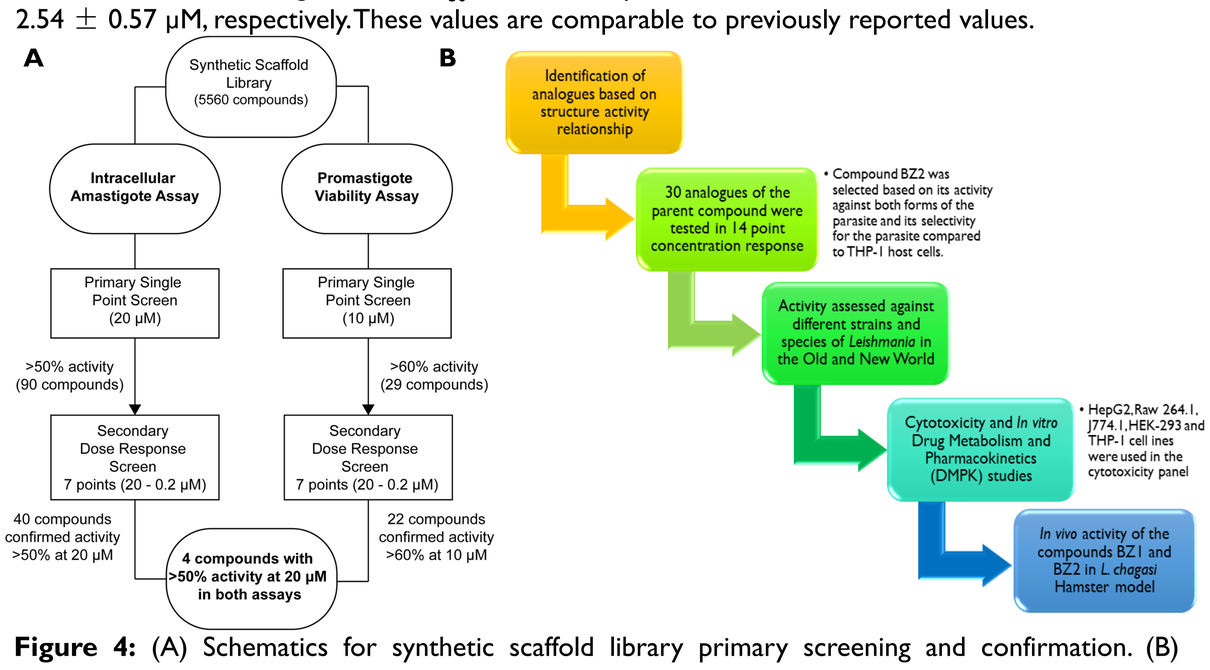


Figure 4: (A) Schematics for synthetic scaffold library primary screening and confirmation. (B) Schematics for analogues screening and downstream processing in drug development.

Table 1: A comparative analysis of compound activity against old world and new world intracellular species causing visceral leishmaniasis.

	Old World			Old World		New World	
Compounds and Reference Drugs	*Leishmania donovani* (MHOM/IN/80/DD8)			*Leishmania donovani* (MHOM/SD/62/1S-CL2D, LdBOB)		*Leishmania infantum (chagasi)* (MHOM/BR/1972/BH46)	
	IC_{50} (µM) [Mean ± SD]	Selectivity		IC_{50} (µM) [Mean ± SD]	Selectivity	IC_{50} (µM) [Mean ± SD]	Selectivity
		HEK-293 cells	THP-1 cells		THP-1 cells		THP-1 cells
BZ1	0.59 ± 0.13	>33.72	>33.89	4.40 ± 0.12	>11.76	6.30 ± 0.14	>15.65
BZ2	0.40 ± 0.38	>24.11	>49.12	4.26 ± 0.24	>11.49	5.45 ±1.06	10.73
Amphotericin B	0.20 ± 0.02	47.61	10.24	-	-	1.5 ± 0.01	14.74
Miltefosine	2.54 ± 0.57	15.74	7.87	-	-	0.80 ± 0.02	62.72

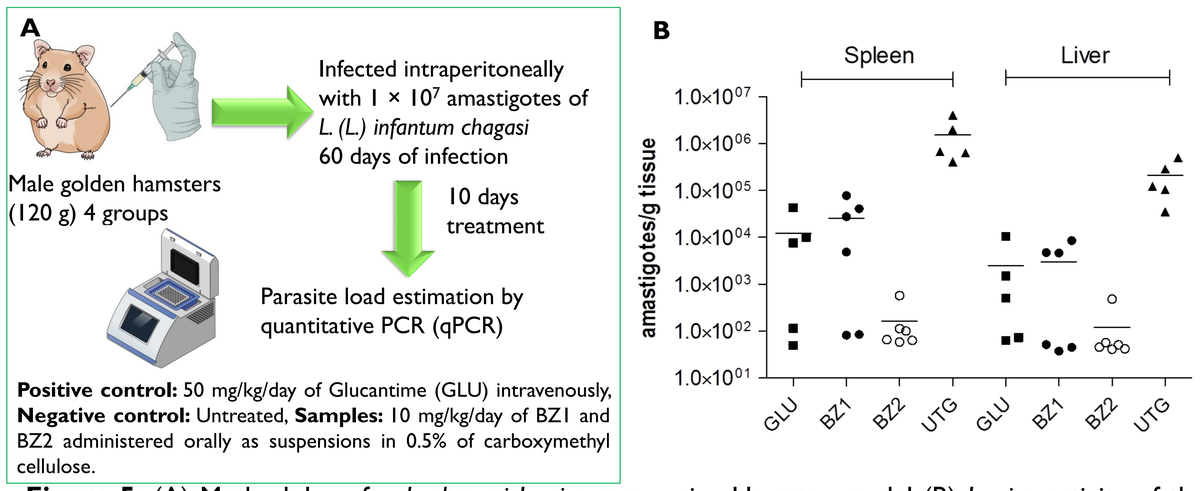


Figure 5: (A) Methodology for *L. chagasi In vivo* assay using Hamster model (B) *In vivo* activity of the compounds BZ1 and BZ2 in *L. chagasi* Hamster model. GLU: Glutamine, UTG: Untreated control.

CONCLUSION:

From a library of 5560 structurally diverse synthetic compounds, two compounds (BZ1 and BZ2) active against both Old world and New world *Leishmania* parasites responsible for visceral leishmaniasis were identified (Table 1). *In vivo* studies have illustrated that the activity observed *in vitro* is translated *in vivo,* with outstanding results. This data suggests that BZ-1 and BZ-2 have potential for further development. Thus, we present here hit compounds fulfilling the requirements for urgently needed starting points for the development of novel lead series for future anti-leishmanial therapeutics.