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Phenotypic screening on 'Pathogen Box' yield novel antiparasitic compounds in *Leishmania infantum*.

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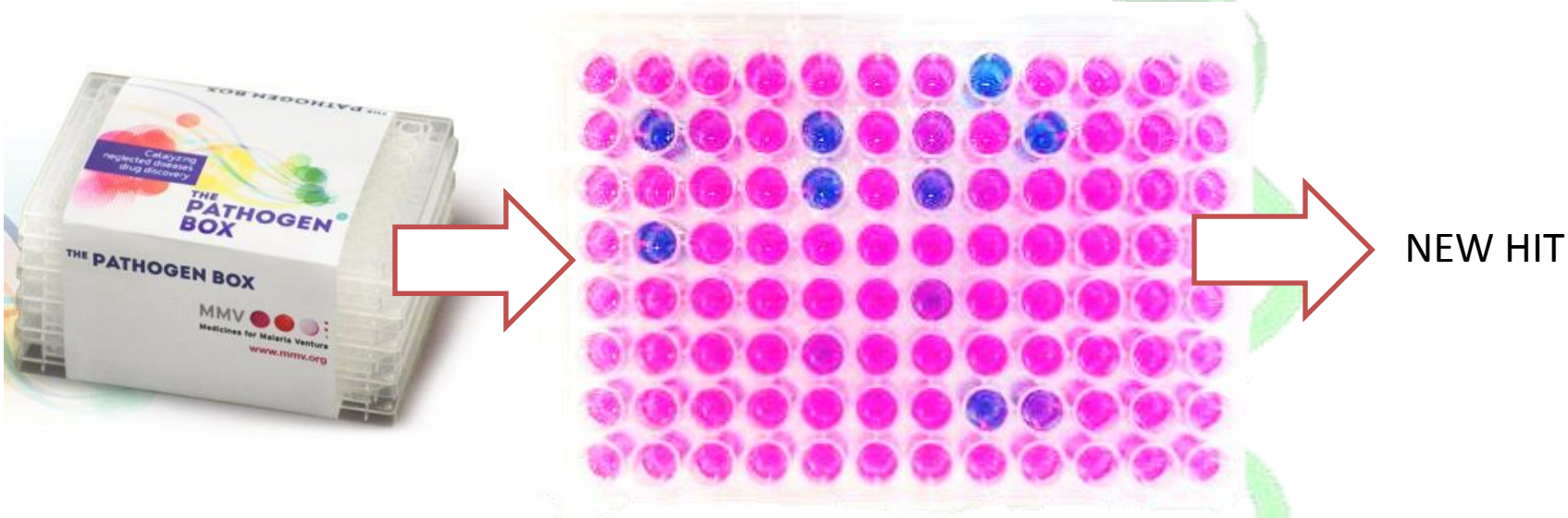
UNIVERSIDAD DE LA REPÚBLICA URUGUAY

Phenotypic screening on ‘Pathogen Box’ yield novel antiparasitic compounds in *Leishmania infantum*

Graphical Abstract

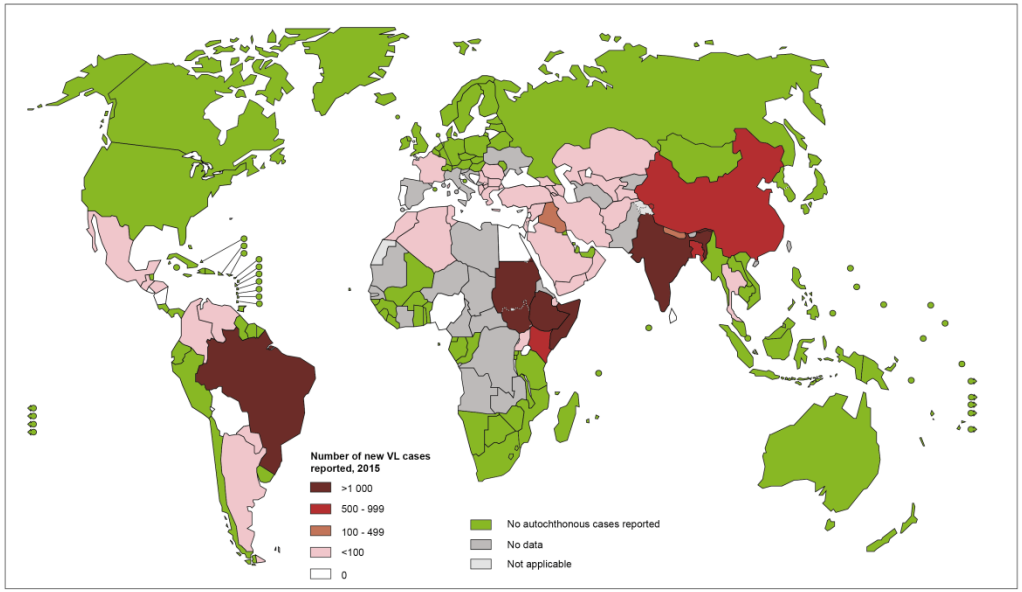


Abstract:
Leishmanioses are zoonotic diseases caused by intracellular protozoans of the genus Leishmania. Recent research has revealed the extensive distribution and expansion of canine leishmaniosis in large areas of the world, where the high prevalence of canine infection is associated with an increased risk of human disease. There are not specific pharmacologic treatments for canine leishmaniasis. The only way to manage the situation is the euthanasia of the infected dogs. The sacrifice of the dog was used to try to control the expansion of the infection since decades without success. Also there are a lot of other Animal species that can act as host for the disease, also with human contact. Then, to achieve a solution, we must develop a vaccine or a specific drug against for canine leishmaniasis. The Pathogen Box is a project led by Medicines for Malaria Venture (MMV, Switzerland; http://www.pathogenbox.org/) that aims to identify novel drugs with activity against diseases such as tuberculosis, malaria, toxoplasmosis, and dengue, among others. The box consists of 400 mostly novel synthetic chemicals that were initially selected from a set of ~4 million compounds due to their low toxicity for mammalian cells and activity against specific microbial pathogens. In fact, the compounds display cytotoxicity at levels that are thought to be reasonable for drug discovery programs. In this study, we screened the Pathogen Box compounds for antiparasitic activity against *Leishmania infantum* (reference strain and clinical insolates). This screen led to the discovery of a 5 novel hits to drug development and drug design.
Keywords: Pathogen Box, canine leishmaniosis, drug discovery, drug repositioning.

Introduction

Leishmaniasis is a disease caused by a protozoa parasite from over 20 different *Leishmania* species and is transmitted to humans and other wild or domestic animals by the bite of infected female phlebotomine sandflies. The disease is spread worldwide affecting 98 countries in five continents, is categorized as one of the “most neglected tropical diseases” and is strongly associated with poverty and affects some of the poorest people on earth. Its spread is tightly linked to environmental changes such as deforestation, building of dams and urbanization.

Status of endemicity of visceral leishmaniasis worldwide, 2015



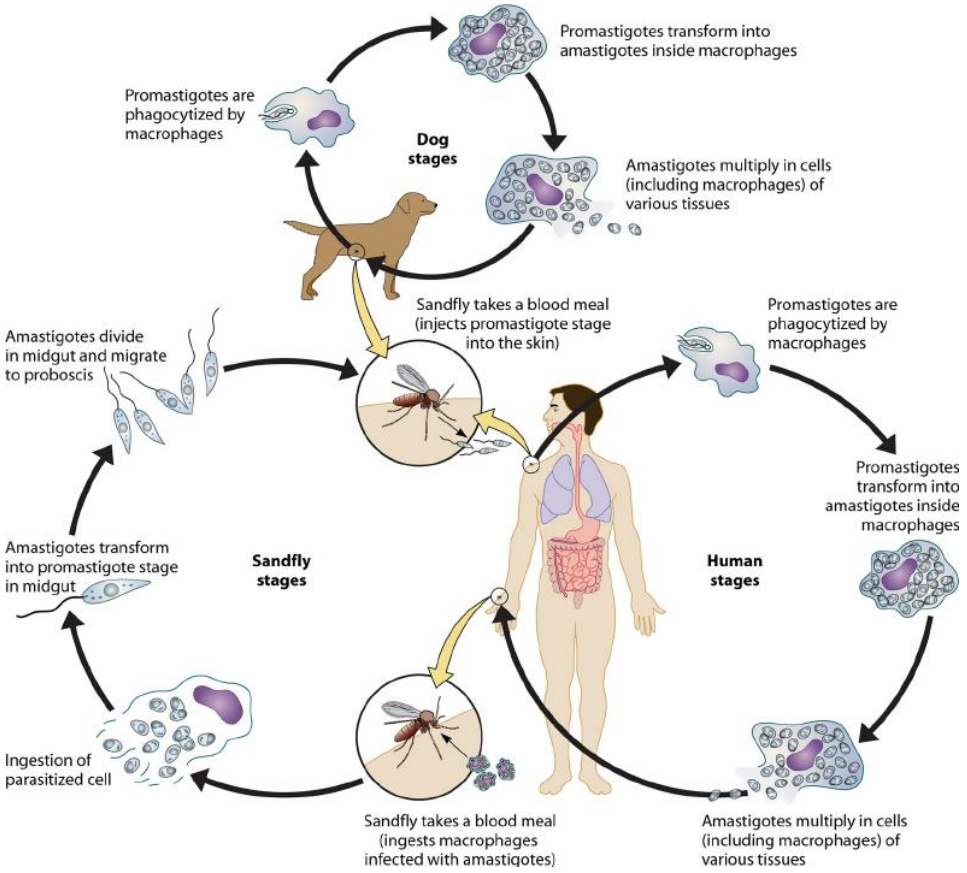
The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. © WHO 2017. All rights reserved

Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

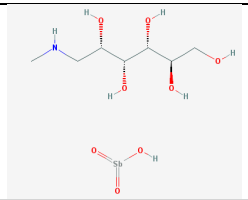
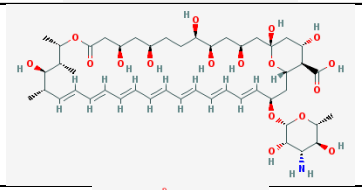
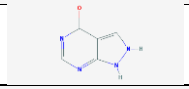
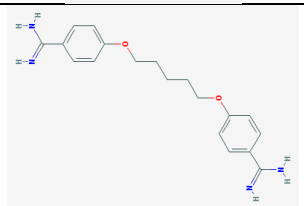
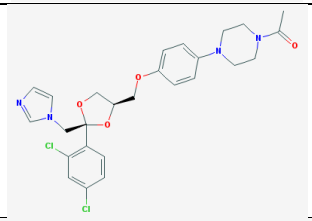
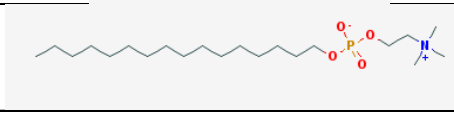
World Health Organization

Visceral leishmaniasis (VL) y one of the main clinical manifestations of the disease and is caused by *Leishmania infantum*, whose main vector is the dipteran *Lutzomya longipalpis*. Infected dogs are the main urban reservoir for zoonotic visceral leishmaniasis mostly due to the high rate of canine infection in endemic areas and intense parasitism in the skin, and are the most significant risk factor predisposing humans to infection.

Canine visceral leishmaniasis (CVL) is expanding in the American continent and a recent report of an outbreak in a northern locality of Salto in Uruguay, documents the southernmost case of the disease.

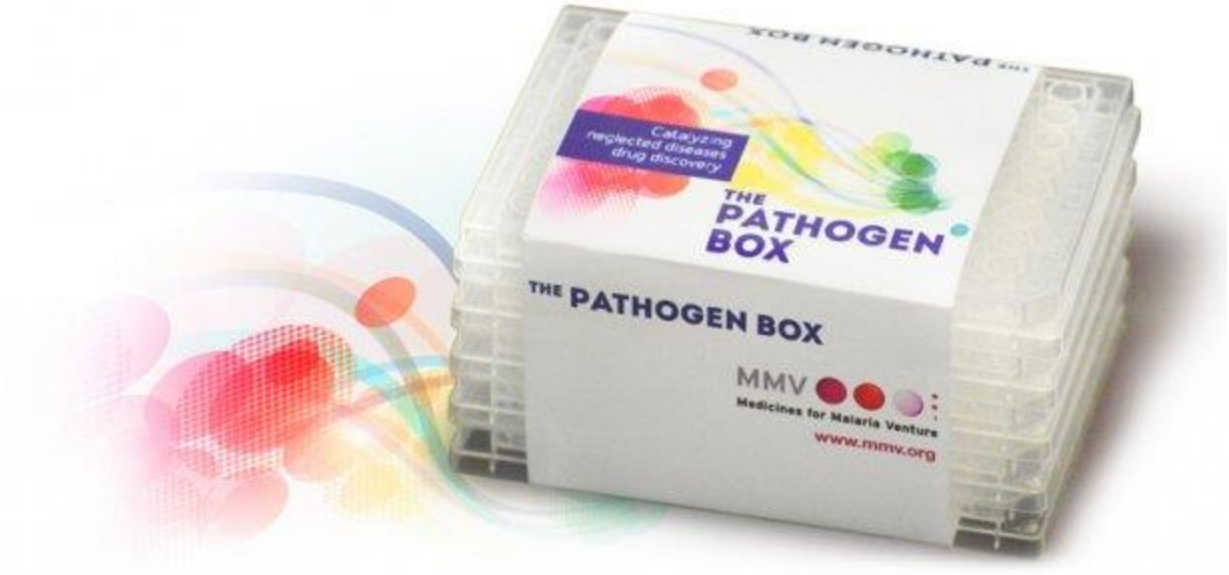


Further investment in drug development is imperative to fill the pipeline with novel compounds, as all of the current drugs have one or more drewbacks; 1) antimonials, still remain the first line of treatment in some endemic areas, are toxic drugs with frequent life-threatening adverse side effects which are potentiated by poor health of the patient.

Drug and treatments of CLV			
DRUG	**Structures**	**Dosage**	**Treatments**
Glucantime		**75-100mg/kg/day SC**	**4 to 6 weeks**
Amphotericin B		**0.5-0.8 mg/Kg IV**	**10 weeks**
Allopurinol		**20 mg/Kg day O**	**1 to 12 months**
Pentamidine		**4 mg/kg IM**	**5-7 weeks**
Ketoconazole		**7-25 mg/kg/day O**	**2-3 months**
Miltefosine		**2 mg/Kg/day O**	**4 weeks**

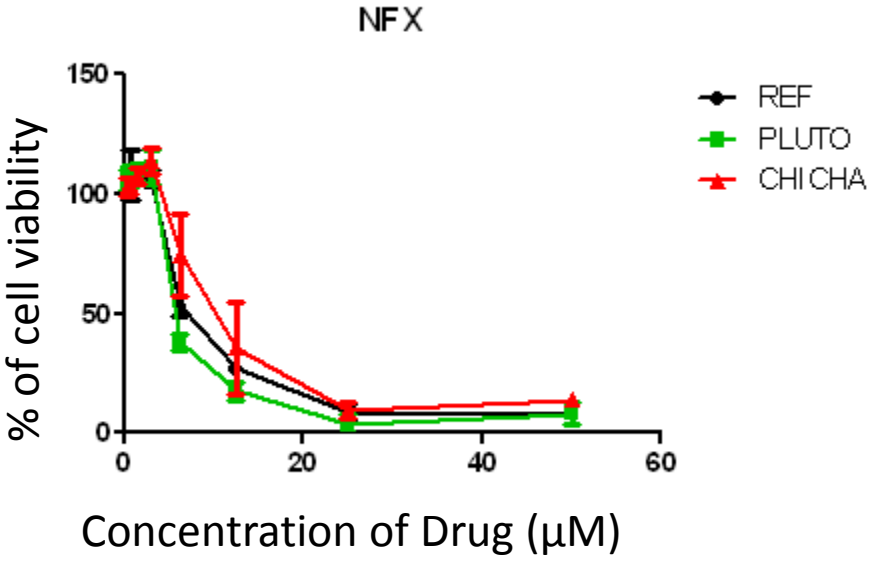
2) Conventional amphotericin B has replaced treatment in areas of India where treatment failure rates for antimonials reached > 60% mostly due to resistance. Moreover, this drug si costly and requires a complicated regime of administration. 3) Liposomal amphoterin B may be the best existing drug against VL and is the first line of treatment in Europe and US but has a high market price. 4) Miltefosine has shown efficacy with a cure rate of 82% and low toxicity rates, but some cases of parasite resistance have been reported.

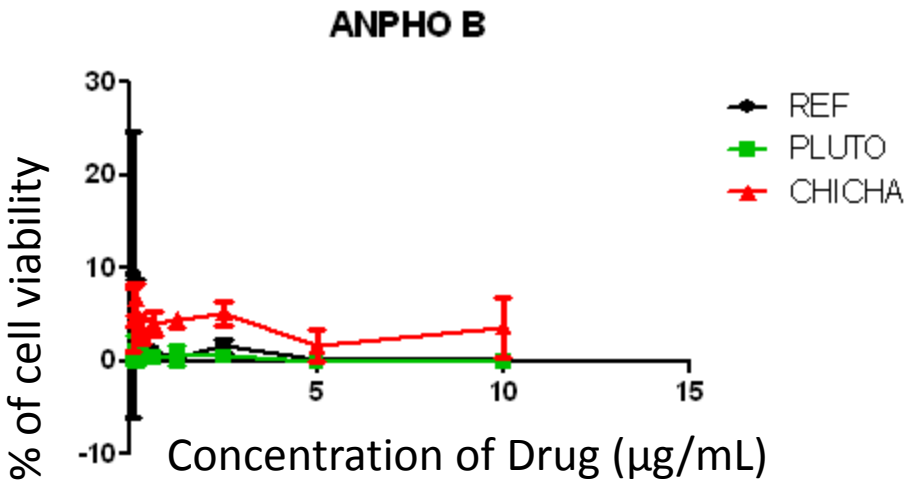
The Pathogen Box (PHB) is a project led by Medicines for Malaria Venture (MMV, Switzerland; http://www.pathogenbox.org/) that aims to identify novel drugs with activity against diseases such as tuberculosis, malaria, toxoplasmosis, and dengue, among others. The box consists of 400 mostly novel synthetic chemicals that were initially selected from a set of ~4 million compounds due to their low toxicity for mammalian cells and activity against specific microbial pathogens. In fact, the compounds display cytotoxicity at levels that are thought to be reasonable for drug discovery programs. The PHB project seeks to exploit hits through target identification and chemical optimization to deliver series available for robust drug discovery series ready for the take up by the community.



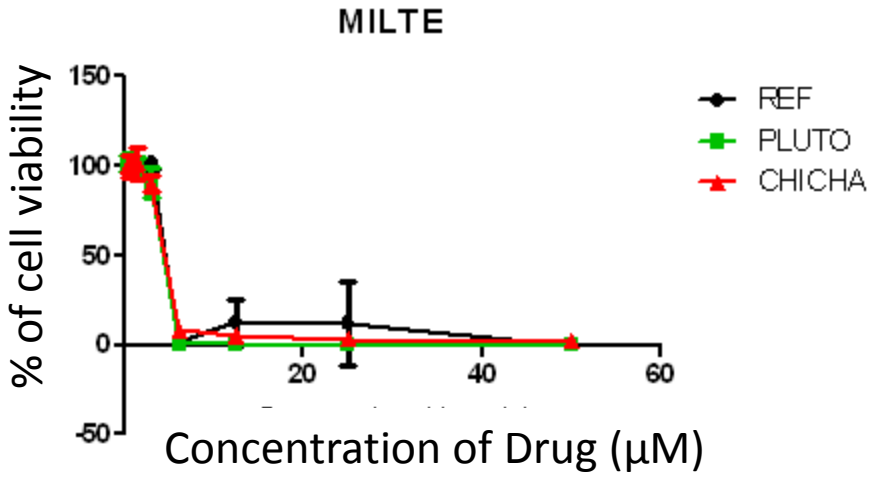
The aim of this work is to challenge different strains of leishmania (reference strain and local isolates) against the pathogen box, in order to identify new drugs against visceral leishmaniasis and also to compare the different behavior profiles among the strains.

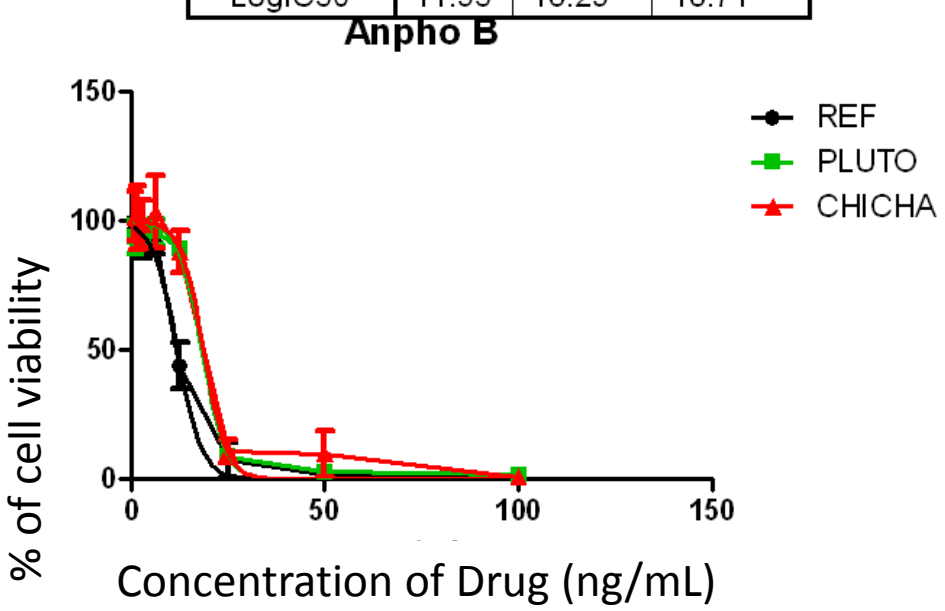
Results and discussion Behavior of the clinical insolate parasite with the reference drugs.



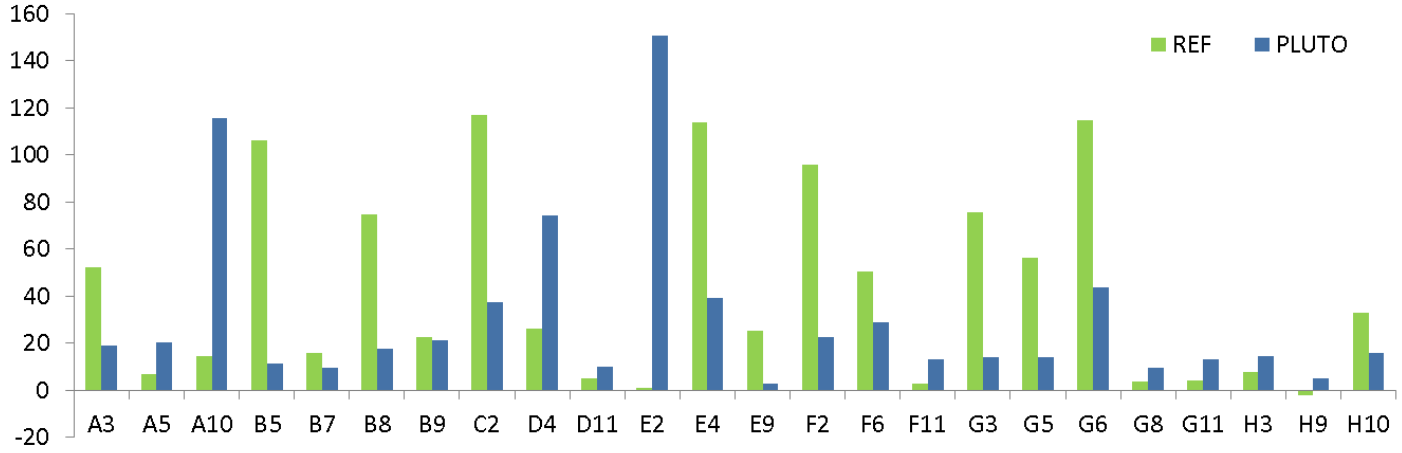


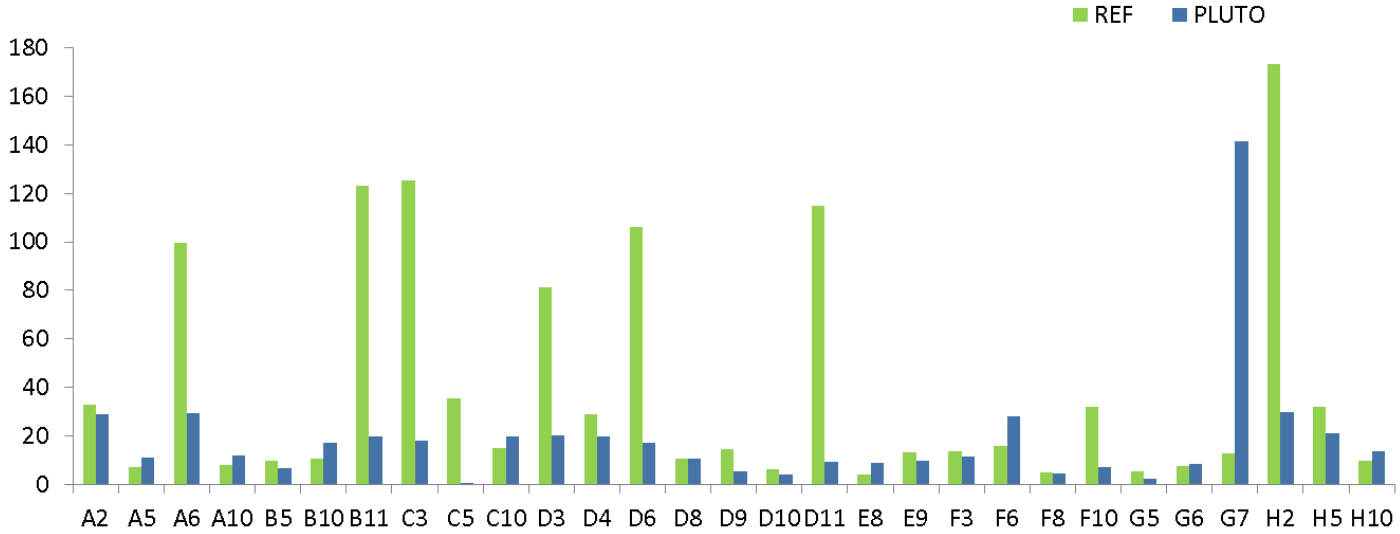
	REF	PLUTO	CHICHA
LogIC50	11.95	18.29	18.71





Behavior of the clinical insolate parasite with some compounds from the pBox.

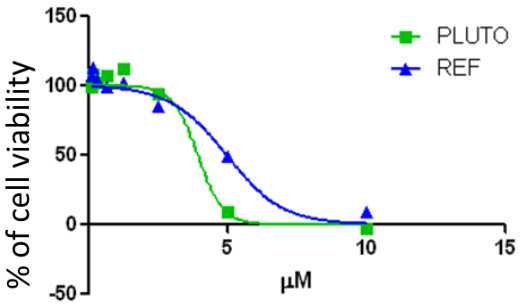




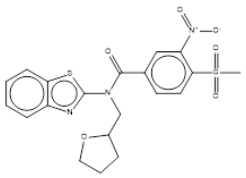
We observe high variability, between the clinical isolates an the reference strain. Also between the insolates from the same geographic area and isolation time.

Most of the active compound were reported previously with anti-leishmania activity, but we found 5 without reports in this parasite.

PHB-A11

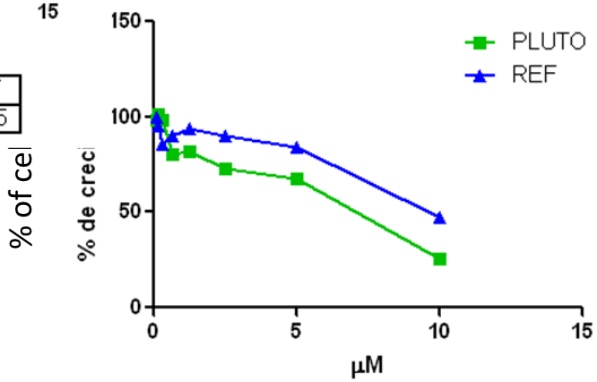


	PLUTO	REF
LogIC50	3.922	4.965



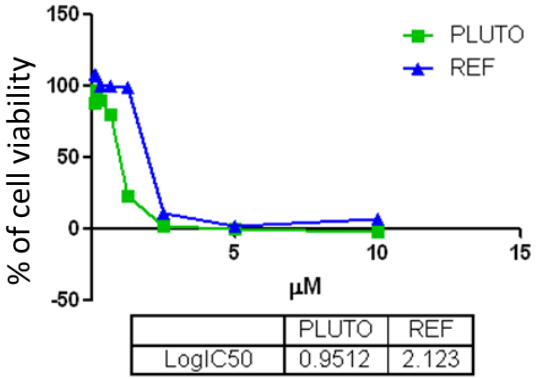
MMV688761
SCHISTOSOMIASIS

PHB-G10



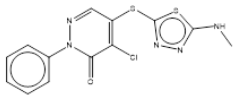
	PLUTO	REF
LogIC50	6.791	9.807

PHB-C11

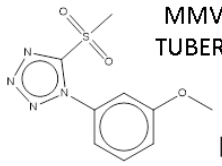


	PLUTO	REF
LogIC50	0.9512	2.123

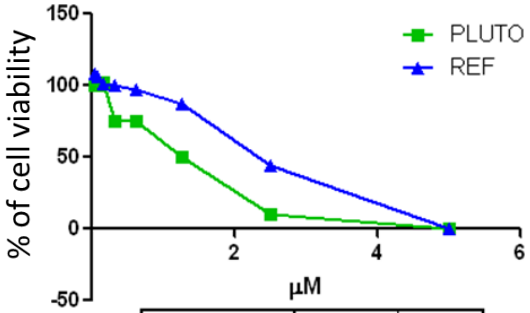
MMV688763
SCHISTOSOMIASIS



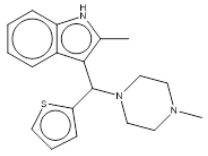
MMV272144
TUBERCULOSIS



PHE-F8

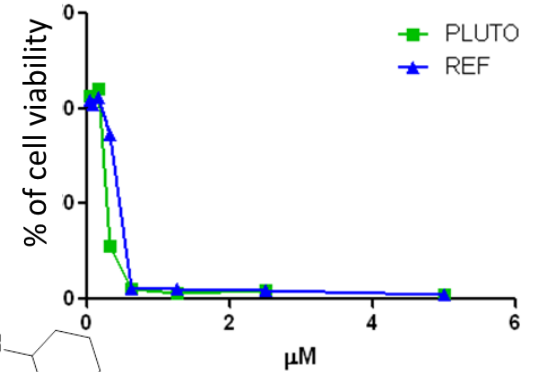


	PLUTO	REF
LogIC50	1.237	2.369



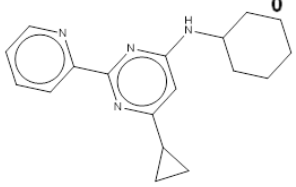
MMV688768
SCHISTOSOMIASIS

PHE-G4



	PLUTO	REF
LogIC50	~ 0.3032	0.4309

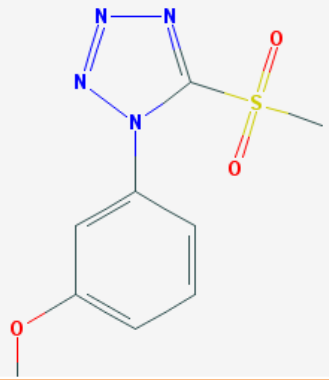
MMV021013
TUBERCULOSIS



HIT PROFILE

PART I: Oral toxicity prediction results for input compound

MMV272144



Predicted LD50: 1750mg/kg

Predicted Toxicity Class: 4

1 2 3 4 5 6

Average similarity: 46.55%

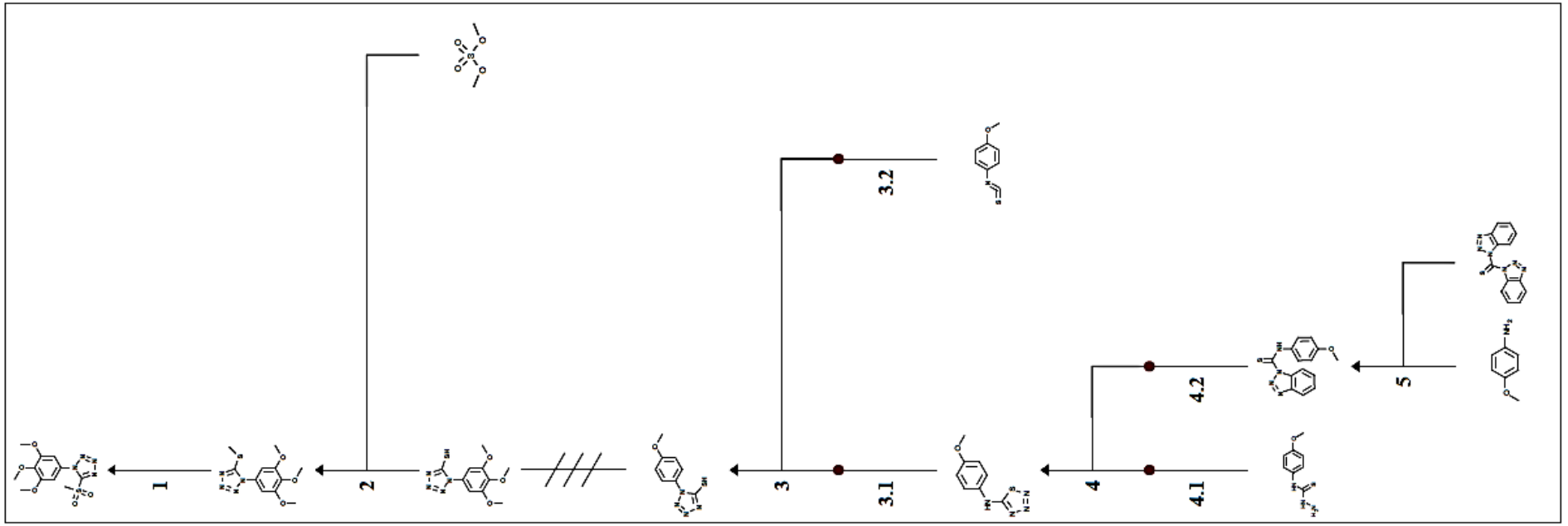
Prediction accuracy: 54.26%

20% 40% 60% 80%

Name	name:FAILED
Molweight	254.27
Number of hydrogen bond acceptors	6
Number of hydrogen bond donors	0
Number of atoms	17
Number of bonds	18
Number of rings	2
Number of rotable bonds	3
Total charge	0
Molecular Polar Surface Area	95.35

IC50 (µM)	Target **(previously reported Biological activities)
1.2/2.4	*Leishmania infantum* (clinical insolate/reference strain)*our results
Not reported	*Leishmania major* promastigote HTS**
15.4	cell division cycle 42 (GTP binding protein, 25kDa) [*Homo sapiens*]**
10	neuropeptide S receptor isoform A [*Homo sapiens*]**
12.5	aldehyde dehydrogenase 1 family, member A1 [*Homo sapiens*]**
1.3	Sphingosine-1-phosphate receptor 4 [*Homo sapiens*]**
1.8	Fluorescence Cell-Based Retest of *C. albicans* Growth in the Presence of Fluconazole**
7.3/1.1	recombinase A [*Mycobacterium tuberculosis* H37Rv]**
6.6	Hsf1 protein [*Mus musculus*]**
3.1	replicative DNA helicase [*Mycobacterium tuberculosis* H37Rv]**

Synthesis plan

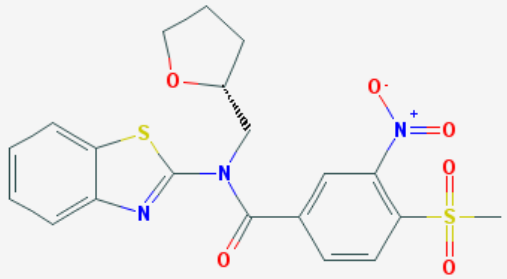


1-Substituted-5-[(3,5-dinitrobenzyl)sulfanyl]-1H-tetrazoles and their isosteric analogs: A new class of selective antitubercular agents active against drug-susceptible and multidrug-resistant mycobacteria.

Karabanovich, Galina; Roh, Jaroslav; Smutný, Tomáš; Němeček, Jan; Vicherek, Petr; Stolaříková, Jiřina; Vejsová, Marcela; Dufková, Ida; Vávrová, Kateřina; Pávek, Petr; Klimešová, Věra; Hrabálek, Alexandr
European Journal of Medicinal Chemistry, **2014** , vol. 82, p. 324 - 340

PART I: Oral toxicity prediction results for input compound

MMV688761



Predicted LD50: 1000mg/kg

Predicted Toxicity Class: 4

1 2 3 4 5 6

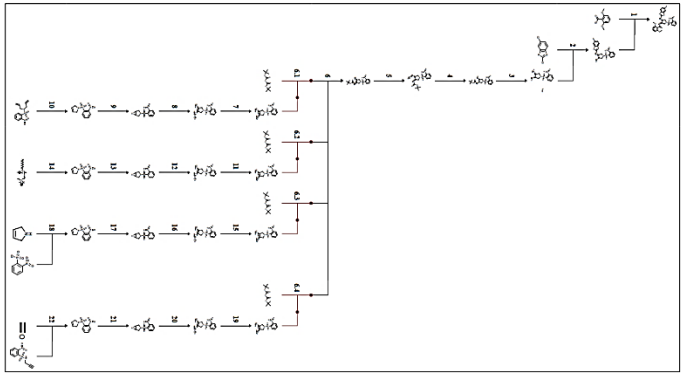
Average similarity: 53.08%

Prediction accuracy: 67.38%

20% 40% 60% 80%

Name	name:FAILED
Molweight	461.51
Number of hydrogen bond acceptors	7
Number of hydrogen bond donors	0
Number of atoms	31
Number of bonds	34
Number of rings	4
Number of rotable bonds	7
Total charge	0
Molecular Polar Surface Area	159.01

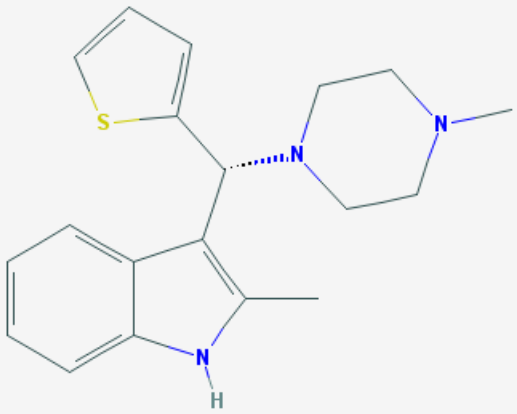
IC50 (μM)	Target **(previously reported Biological activities)
3,9/4,9	*Leishmania infantum* (clinical insolate/reference strain)*our results



Synthesis plan multiple steps, no report by Reaxys, 1 in PubChem without biological reports.

PART I: Oral toxicity prediction results for input compound

MMV688768

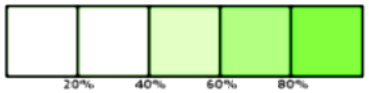


Predicted LD50: 400mg/kg

Predicted Toxicity Class: 4

Average similarity: 49.22%

Prediction accuracy: 54.26%



Name	name:FAILED
Molweight	325.47
Number of hydrogen bond acceptors	2
Number of hydrogen bond donors	1
Number of atoms	24
Number of bonds	27
Number of rings	4
Number of rotable bonds	3
Total charge	0
Molecular Polar Surface Area	50.51

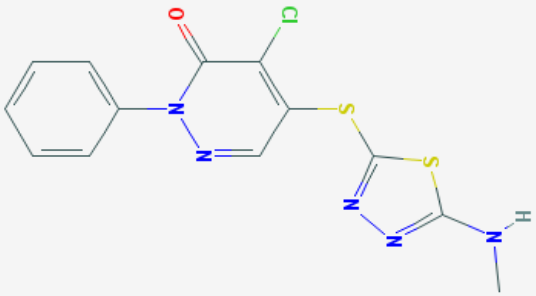
IC50 (µM)	Target **(previously reported Biological activities)
6.8/9.8	***Leishmania infantum*** **(clinical insolate/reference strain)*our results**
3.1/32*	MIC *Candida albicans* Biofilm Inhibitors/Human 535 hepatocellular carcinoma (HepG2) cell line.
1.5/4.70**	*T. brucei brucei /P. falciparum* ABS activity

* Vila T, Lopez-Ribot JL. Screening the Pathogen Box for Identification of Candida albicans Biofilm Inhibitors. Antimicrob Agents Chemother. 2016 Dec 27;61(1). pii: e02006-16. doi: 10.1128/AAC.02006-16. Print 2017 Jan.

**Duffy S, et al Screening the Medicines for Malaria Venture Pathogen Box across Multiple Pathogens Reclassifies Starting Points for Open-Source Drug Discovery. Antimicrob Agents Chemother. 2017 Aug 24;61(9). pii: e00379-17. doi: 10.1128/AAC.00379-17. Print 2017 Sep.

PART I: Oral toxicity prediction results for input compound

MMV688763



Predicted LD50: 2520mg/kg

Predicted Toxicity Class: 5

1 2 3 4 5 6

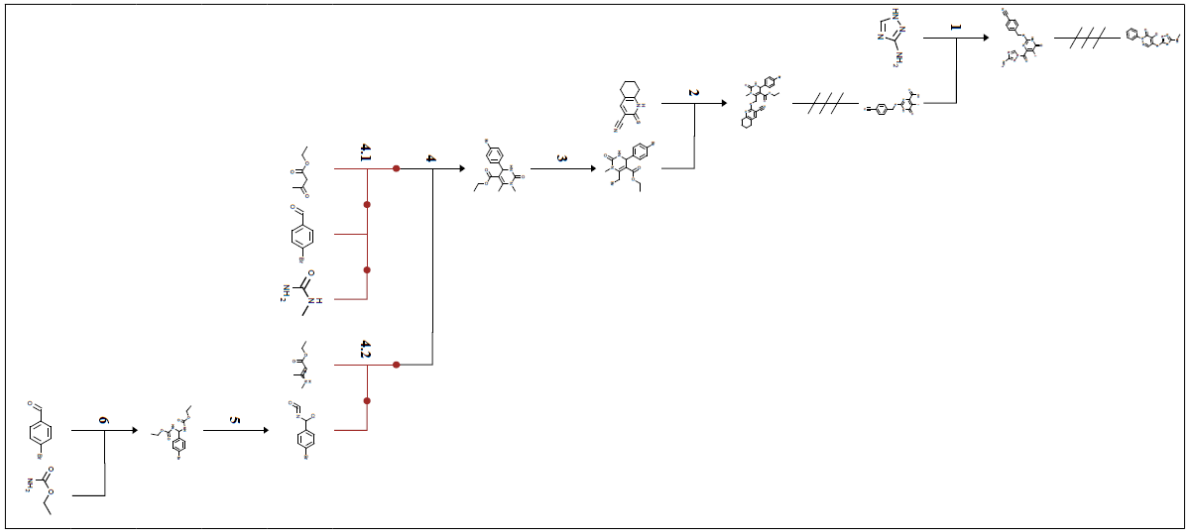
Average similarity: 48.16%

Prediction accuracy: 54.26%

20% 40% 60% 80%

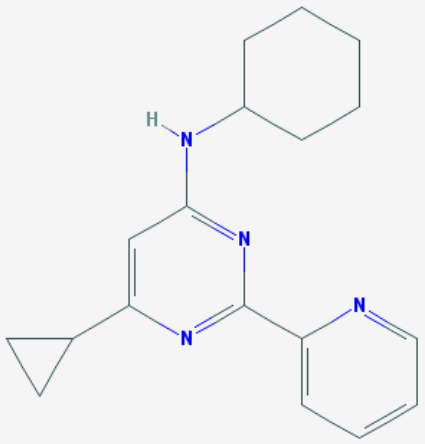
Name	name:FAILED
Molweight	351.83
Number of hydrogen bond acceptors	5
Number of hydrogen bond donors	0
Number of atoms	22
Number of bonds	24
Number of rings	3
Number of rotable bonds	4
Total charge	0
Molecular Polar Surface Area	126.24

IC50 (µM)	Target **(previously reported Biological activities)
0.9/2.1	***Leishmania infantum*** **(clinical insolate/reference strain)*our results**
nd	Potential Nrf2 Activators



PART I: Oral toxicity prediction results for input compound

MMV021013



Predicted LD50: 300mg/kg

Predicted Toxicity Class: 3

1 2 3 4 5 6

Average similarity: 44.38%

Prediction accuracy: 54.26%

20% 40% 60% 80%

Name	name:FAILED
Molweight	294.39
Number of hydrogen bond acceptors	4
Number of hydrogen bond donors	0
Number of atoms	22
Number of bonds	25
Number of rings	4
Number of rotable bonds	4
Total charge	0
Molecular Polar Surface Area	50.7

IC50 (µM)	Target **(previously reported Biological activities)
0.3/0.4	***Leishmania infantum*** **(clinical insolate/reference strain)*our results**
0.8/400*	***Leishmania donovani*** **(amastigotes)/HepG2 human cell line**
0.7**	***P. falciparum***
1.7/3.5***	***T. cruzi/T. brucei brucei***

* Peña I, ET AL. New compound sets identified from high throughput phenotypic screening against three kinetoplastid parasites: an open resource. Sci Rep. 2015 Mar 5;5:8771. doi: 10.1038/srep08771.

**Ballell L Fueling open-source drug discovery: 177 small-molecule leads against tuberculosis. ChemMedChem. 2013 Feb;8(2):313-21. doi: 10.1002/cmdc.201200428. Epub 2013 Jan 10.

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Conclusions

We test 400 molecules from the pathogen Box library *in vitro* in promastigotes of 3 different strains of *L. infantum*. This screen led to the discovery of a 5 novel hits to drug development and drug design, 3 of them without report in *Leishmania spp*.

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