NEW DRUGS FOR NEGLECTED DISEASES: A JOINT EFFORT TO ADDRESS UNMET NEEDS IN THE *LEISHMANIASIS* AND CHAGAS DISEASE EARLY DISCOVERY PIPELINES

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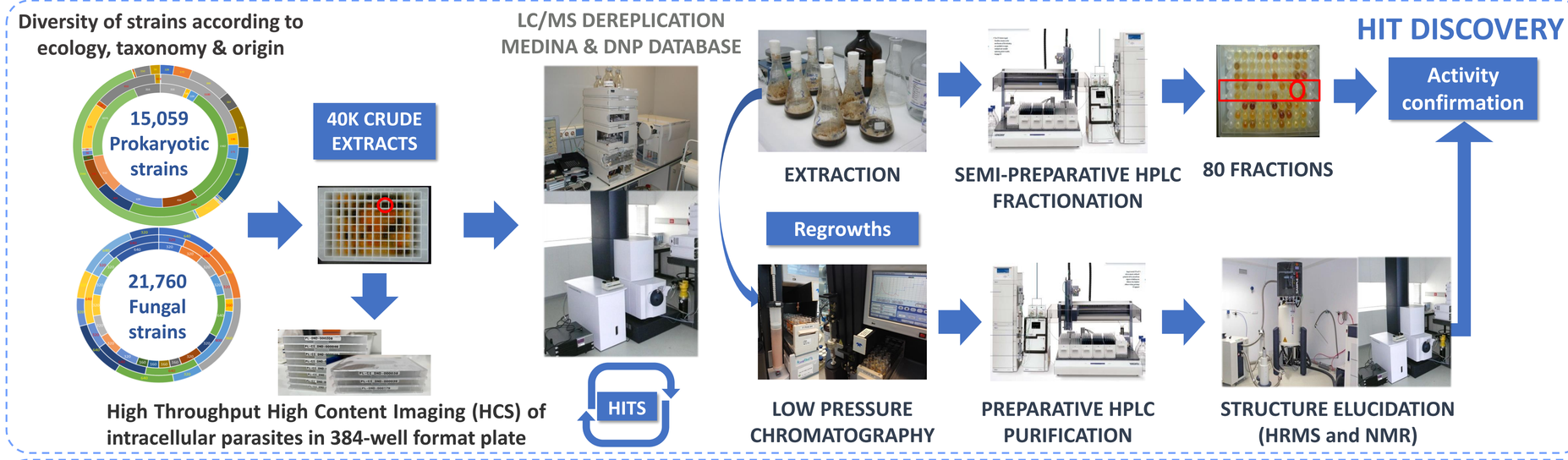
Introduction

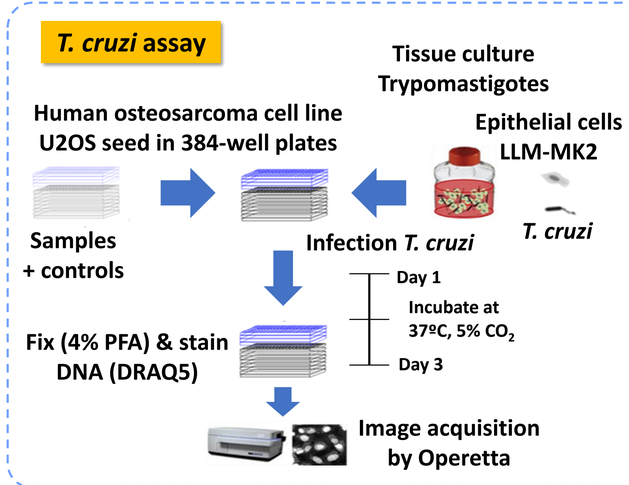
Leishmaniasis and American Trypanosomiasis (Chagas disease) are two neglected tropical diseases caused by the parasites *Leishmania* spp. and *Trypanosoma cruzi*, respectively. They cause thousands of deaths worldwide, and in recent years have also emerged as a health concern in developed countries. New therapeutic solutions are required due to increasing resistance and adverse effects of existing treatments[1,2]. Natural products (NPs) have been historically a rich reservoir of new bioactive compounds, and the origin of new drugs used today in clinical practice[3].

Aims

- The **discovery of new NP scaffolds** with novel mechanisms of action (MoA) against *Leishmania donovani* and *Trypanosoma cruzi.*
- The application of a **High Throughput High Content Imaging Platform** for testing NPs in 384-well format plates to discover new drugs for neglected diseases .

Materials and Methods





Data analysis

Columbus™ software
Positive control *T. cruzi*: Benznidazole
Positive control *L. donovani*: Amphotericin B
Negative control for both parasites: DMSO

% Inhibition of infection = [(Mean Min - Tested value)/ (Mean Min - Mean Max)] x 100
% Inhibition of Parasite Number = [(Mean Min – Tested value)/ (Mean Min - Mean Max)] x 100
% Cell ratio = (Tested value / Mean Min) x 100

Tested value: Infected cells/Total cells (infection ratio)
Mean Max: reference drug EC_{100} treated controls
Mean Min: infection with 0.5% DMSO

HTS ASSAYS

Results

High throughput screening

44,475 samples screened

	LC/MS dereplication	Dose response curves (DRC) hits	Medium-scale regrowth & activity confirmation	Bioassay-guided isolation	
T. cruzi	991 hits (2.2%)	181 DRC hits	107 hits prioritized	61 HPLC chromatographed	19 pure compounds
	Alteramides, antimycins, azalomycins, bafilomycins, cordycepin, cycloheximides, cyclosporins, lobophorins, mithramycin A, and oligomycin D among others				
L. donovani	270 hits (0.6%)	16 DRC hits	13 hits prioritized	11 HPLC chromatographed	11 pure compounds
	Alteramides, antimycins, azalomycins, bafilomycins, blastmycin, cytochalasins, grixorisin, nigericin, salbomycin, and secalonic acid among others				

Compounds activity expressed as IC_{50} (Half maximal inhibitory concentration)

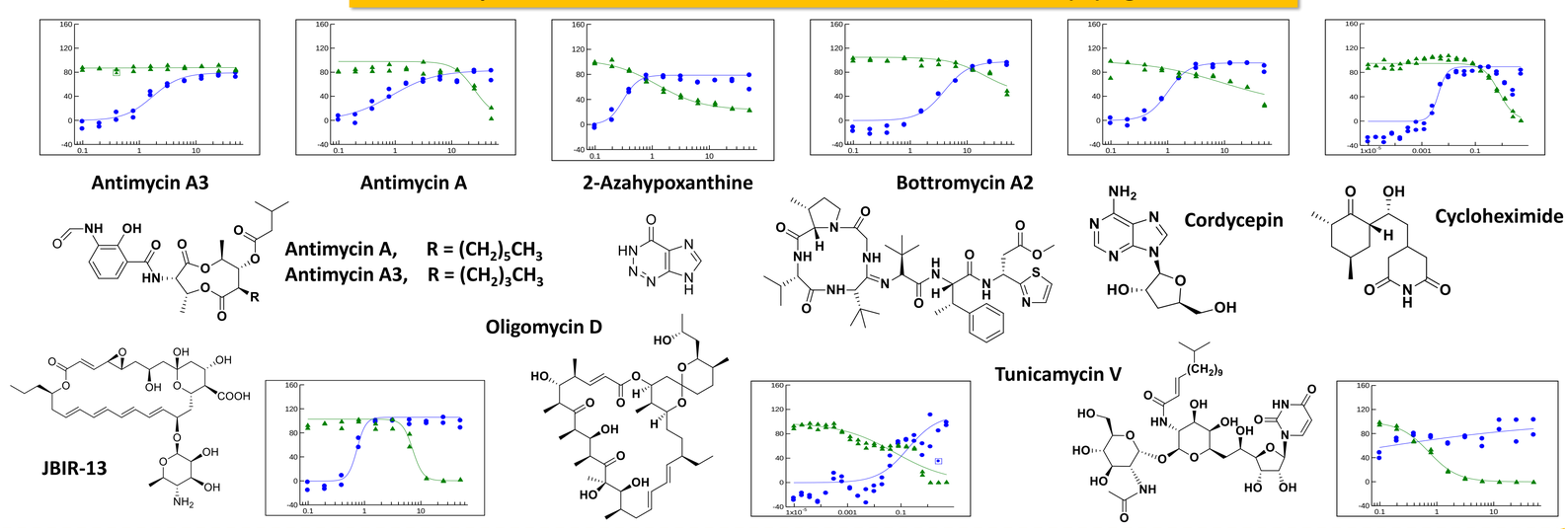
% Inhibition of Infection: Antiparasitic parameter (%) that measures the number of host cells non-infected with parasite in their cytoplasmic region.
Cell ratio (%): cytotoxicity parameter that count the number of host cells.

X axis: Compound (µM)
Y axis: % Inhibition/Cell ratio (%)
▲ Cell ratio (%)
● % Inhibition of infection

T. cruzi active compounds	% Inhibition of infection IC_{50} (µM)	Cell ratio (%) CC_{50} (µM)
Antimycin A3	2.29	>50
Antimycin A	1.23	25.70
2-Azahypoxanthine	0.36	2.35
Bottromycin A2	4.01	47.65
Brocazine C	6.40	14.75
Cordycepin	1.04	26.49
Cylocheximide	0.0045	0.64
Echoside C	10.39	>50
Fe-coprogen	>50	>50
Fusarin A	5.08	18.08
JBIR-13	0.71	7.24
Madurastatin H2	>50	>50
9-Methylstreptimidone	0.84	>50
Oligomycin D	0.058	0.36
Tambromycin A	35.62	>50
Tunicamycin V	0.12	0.71
Kronopolitide A	0.10	0.33
kronopolitide B	0.15	0.46
Kronopolitide C	0.58	2.09

Selected for MoA & ADME test

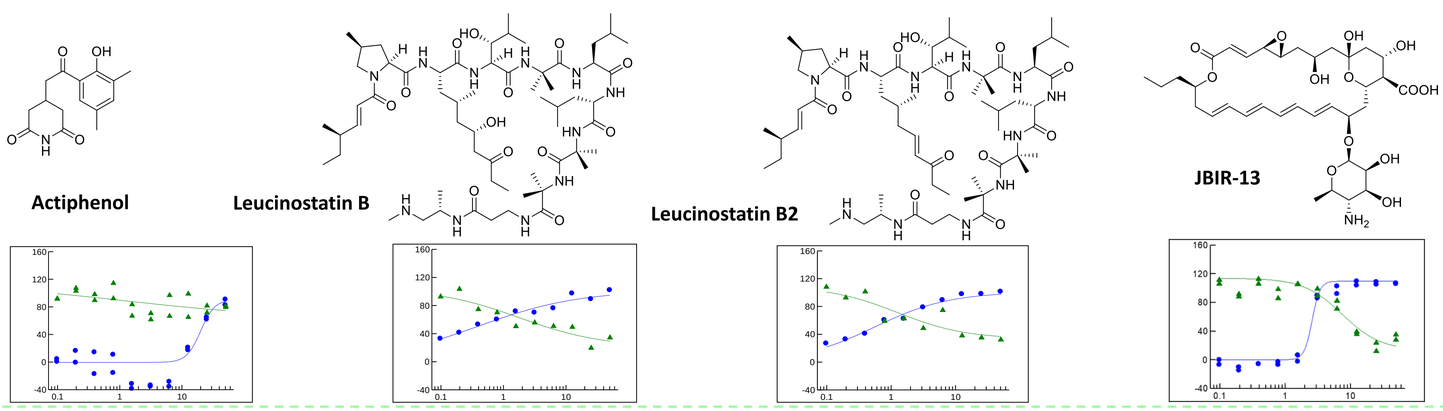
Dose response curves of % Inhibition of infection/Cell ratio (%) againt *T. cruzi*



L. donovani active compounds	% Inhibition of infection IC_{50} (µM)	Cell ratio (%) CC_{50} (µM)
Actiphenol	20.78	>50
Cytochalasin E	>50	>50
5-hydroxy-3,6-dimethoxy-2-methyl-5-(2-oxopropyl)-2H-naphtho[1,2-b]oxet-8(5H)-one	2.59	5.95
6-(1-Hydroxyethyl)-2,7-dimethoxyjuglone	2.51	5.81
JBIR-13	2.53	10.90
Leucinostatin B	0.34	0.64
Leucinostatin B2	0.66	1.17
9-Methylstreptimidone	>50	>50
Nigericin	7.01	13.40
kronopolitide B	3.74	2.68
Kronopolitide E	1.73	2.29

Selected for MoA & ADME test

Dose response curves of % Inhibition of infection/Cell ratio (%) againt *L. donovani*



Conclusions

- **142** and **60 compounds** were **dereplicated** in **active** samples against ***T. cruzi*** and ***L. donovani***, respectively. **Nineteen** showed **significative activity** against ***T. cruzi*** and **eleven** against ***L. donovani***. **Nine *T. cruzi* and four *L. donovani* active compounds** were selected for further **MoA and ADME studies.**
- **Six new compounds** from **a new family of compounds** were identified so far (**kronopolitides**).
- **The MoA of the active compounds** will be explored by parasite cell painting using a **high content imaging system.**

References

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