NP4NTD: DISCOVERY OF NEW ANTIPARASITIC DRUG CANDIDATES FROM MICROBIAL NATURAL PRODUCTS

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Background and Aims

Leishmaniasis and American trypanosomiasis (Chagas disease) are neglected tropical diseases (NTDs) caused by the parasites *Leishmania* spp. and *Trypanosoma cruzi,* respectively, that lead to thousands of deaths worldwide every year. They are also emerging as a health problem in developed countries. New therapeutic solutions are required due to increasing resistance and undesirable side effects of existing treatments.[1,2] Natural products (NPs) possess a wide chemical diversity and historically have constituted a rich source of new bioactive molecules, with many drugs inspired by natural products used today in clinical practice.[3]

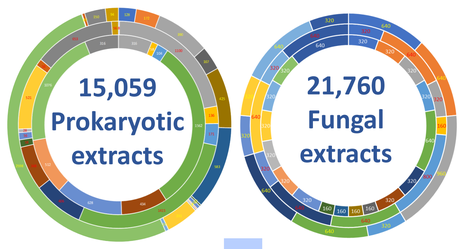
The main goals of the NP4NTD project are the discovery of new NP scaffolds with novel mechanisms of action (MoA) against *L. donovani* and *T. cruzi* and the development of a new parasite painting approach to assist the MoA characterization of NP drug candidates.

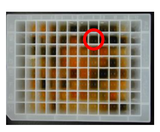
Methods

HIT DISCOVERY

WP1 (IPK & MEDINA)
NP HTS/HCS Intracellular *T. cruzi* & *L. donovani* + MS Dereplication

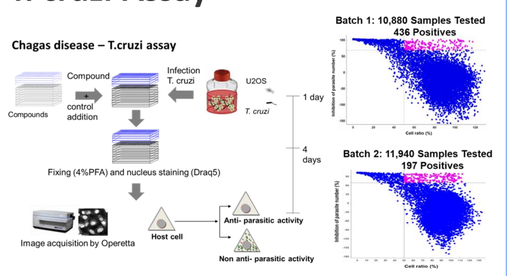
Diversity of extracts according to ecology, taxonomy & origin



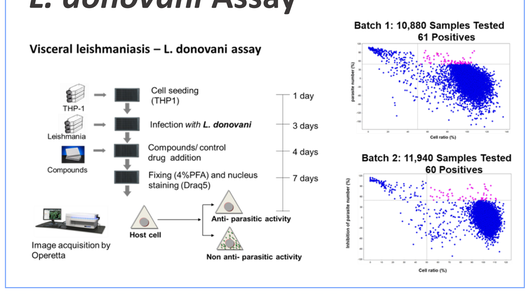


40K CRUDE EXTRACTS

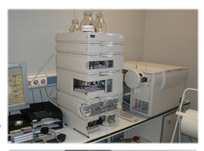
T. cruzi Assay



L. donovani Assay



HITS



LC/MS dereplication
MEDINA & DNP database

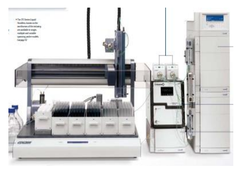
WP2 (MEDINA)
NP Bioassay-guided Isolation & Structure Elucidation

EXTRACTION

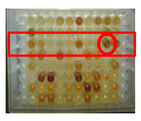
Regrowths

LOW PRESSURE CHROMATOGRAPHY

SEMI-PREPARATIVE HPLC FRACTIONATION



80 FRACTIONS



PREPARATIVE HPLC PURIFICATION

STRUCTURE ELUCIDATION (HRMS and NMR)

Activity confirmation

HIT PROFILING

WP3 (IPK & DND*i*)
Parasite & Cell Painting Assay Development

Assay development

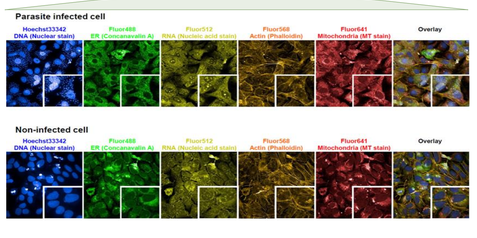
- Seeding
- Attachment
- Staining
- Image quality
- Stage of parasite

Compound perturbations to parasites & cells

Opera Phenix Plus HCS (Laser light sourced Confocal Microscope, 63x objective)

Reference compounds (Known MoA)

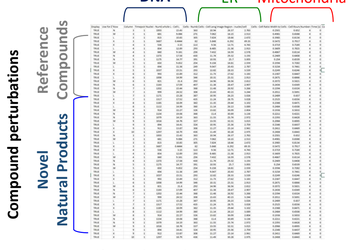
Novel compounds Natural Products



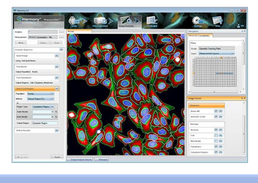
Parasites and cell image acquisition (HCS)

Image quantification (feature extraction)

Parasite morphological features

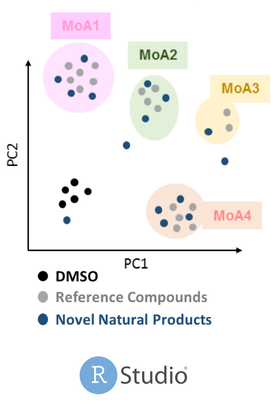


Shape, intensity, texture properties



Data analysis (MoA prediction)

Dimension reduction of features (>500)
Classification by MoA



Antiparasitic Candidates

WP4 (MEDINA & DND*i*)
MoA & ADMET profiling

- ***In vitro*** **activity/safety**
- **ADMET profiling**
- **PK/PD-*in vivo* efficacy/tox**

WP5 (DND*i*) Project Management

Results

30K CRUDE EXTRACTS

Active compounds

T. cruzi

15 pure compounds

DEREPLICATED: Antimycins A & A3, bottromycin A2, cordycepin, cycloheximide, oligomycin D, 9-methylstreptimidone, tunicamycin V, tambromycin A

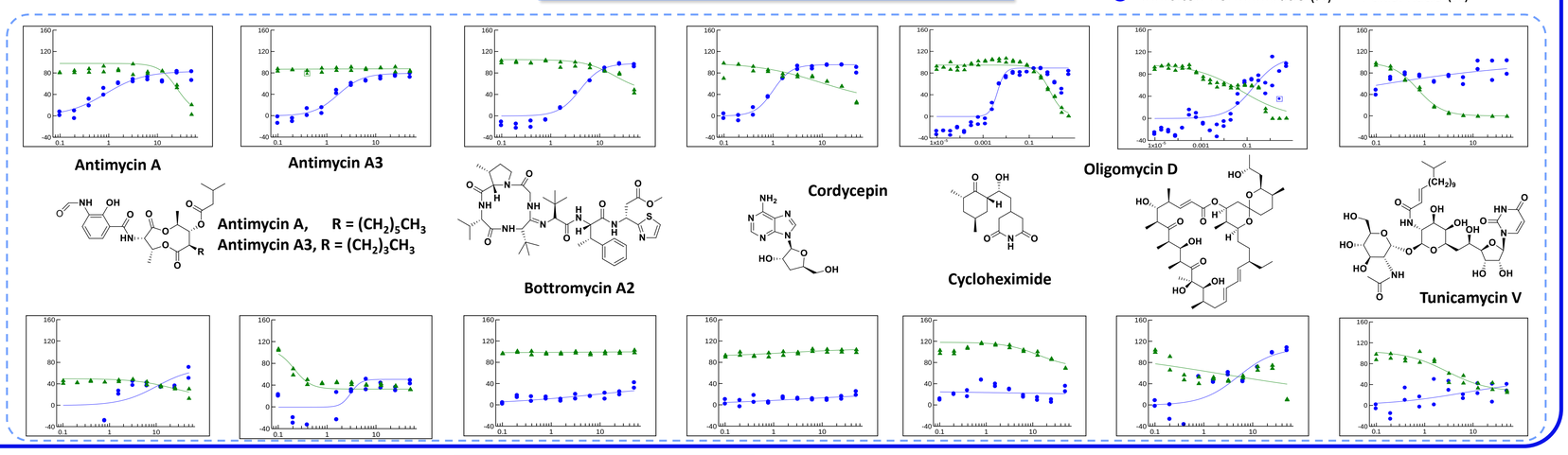
NEW: madurastatin H2, kronopolitides (A-E)

L. donovani

10 pure compounds

DEREPLICATED: Antimycin A & A3, bottromycin A2, cordycepin, cycloheximide, oligomycin D, leucinostatins B & B2 & actiphenol

Compounds selected for ADME testing



Conclusions

- **54 compounds identified from first 30 K extracts studied**, of which **6 are new natural products** (madurastatin H2 & kronopolitides A-E).
- **Seven *T. cruzi* active compounds** selected for further **MoA and ADME studies.**
- **Parasite and infected cell painting assays with *Leishmania donovani* and *Trypanosoma cruzi* were developed in 384 format** with multiple cell staining dyes for the non-adherent and small parasites using a **high-resolution HCS confocal instrument.**

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Acknowledgements

The project leading to these results has received funding from "la Caixa" Foundation under the project code HR20-00584.

We also thank to Department for International Development of UK government (DFID, UK) and FCDO, the Dutch Ministry of Foreign Affairs (DGIS, the Netherlands) and the Federal Ministry of Education and Research (BMBF) through KfW (Germany) for supporting via DND*i* the bioassays run at Institut Pasteur Korea.

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[1] J. H. No. Visceral leishmaniasis: Revisiting current treatments and approaches for future discoveries. *Acta Trop*. **2016**, 115, 113-123.
[2] J. A. Pérez-Molina, I. Molina. Chagas disease. *Lancet* **2018**, 391, 82-94.
[3] D. J. Newman, G. M. Cragg. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J. Nat. Prod.* **2020**, 83, 770–803.