*Book of Abstracts*

# São Paulo School of Advanced Sciences on Neglected Diseases Drug Discovery

## Focus on Kinetoplastids

CNPEM

➤ June 14th to 24th 2015, Campinas-SP | Brazil ◄

http://pages.cnpem.br/drugdiscovery-kinetoplastids/

FAPESP | LNBIO CNPEM

# CNPEM - Campus Map


- LNLS – Main Building
- LNLS – Storage Ring Building
- LNLS – Red Building
- LNLS – Magnets Building
- LNLS – Factory and Control Building
- CTBE
- LNBio
- LNNano
- Administration Building
- Warehouse
- Restaurant
- Guest House

*The lectures are going to be held in the LNLS Auditorium at the Storage Ring Building. The practical sessions are going to be held at the LNBio Building and at the CTBE Building.*

# Content

*The Brazilian Biosciences National Laboratory*

LNBIO

# Presentation

**CNPEM:** The Brazilian Center for Research in Energy and Materials (CNPEM) is a private nonprofit organization located in Campinas, Brazil, which is funded by the Ministry of Science, Technology & Innovation (MCTI). It is dedicated to cutting-edge research in materials, nanosciences, life sciences, physics, and chemistry through four National Laboratories: Synchrotron (LNLS), Biosciences (LNBio), Bioethanol (CTBE) and Nanotechnology (LNNano). The four laboratories are open facilities for external users and companies, in Brazil and abroad. They also have teams of researchers to provide support for projects, as well as to conduct joint research programs in biomass, green chemistry, drugs and cosmetics development, characterization of advanced materials, catalysts, etc.

**LNBio:** The Brazilian Biosciences National Laboratory (LNBio) is dedicated to cutting-edge research and innovation focused on biotechnology and drug discovery and development. LNBio activities are organized into four areas: Open Facilities; Innovation Core; Research in-house; Training and Education. This organizational strategy was designed to encourage the sharing of infrastructure and skills with the academic and industrial sectors. Thus, LNBio optimizes and directs its resources to Science, Technology and Innovation activities

**Sao Paulo School of Advanced Science on Neglected Diseases Drug Discovery – focus on Kinetoplastids (SPSAS-ND3):** SPSAS-ND3 will focus on multidisciplinary aspects of drug discovery applied to Chagas disease, African tripanosomiasis and leishmaniasis, introducing students to the basics of drug discovery science and also to cutting-edge research on the field. The course is structured in both lectures and practical activities covering high throughput and high content screening, structural biology and virtual screening, screening data analysis, medicinal chemistry, lead optimization, in vitro and in vivo ADME, and in vivo models for pharmacokinetics and efficacy studies.

**Thank you for coming, I hope you enjoy the Workshop.**

**Lucio Holanda Gondim de Freitas Junior**
Event Coordinator

**Dear Participants,**

It is with great pleasure that we welcome you to the **Sao Paulo School of Advanced Sciences on Neglected Diseases Drug Discovery – focus on kinetoplastids.** It offers an opportunity for students and professionals interested in neglected diseases and drug discovery to interact with some of the best professionals in the field. The selection process was highly competitive, with over 250 applications from around the world, and resulting in the selection of the top 90 candidates – a very diverse body of attendees, with participants originating from 36 different countries. We believe this diversity enriches greatly the experience of attending **SPSAS-ND3** and brings attention to the reality of a global health issue that are neglected diseases.

Our proposal is to bring together specialists from different fields of the drug discovery sciences and that have a successful history of advancing our knowledge on neglected diseases and translating the knowledge into new tools and molecules that will lead to new drugs. Translational, collaborative, and integrated multidisciplinary science is a key aspect of successful drug discovery programs and we hope to show the participants that this can only be achieved through collaboration with groups of complementary skills.

This course is the first of its kind in the world, and was made possible thanks to the **Sao Paulo state funding agency (FAPESP)**, which is providing the great majority of the financial resources, but also with a great help of the **Drugs for Neglected Diseases initiave (DNDi)**, which is providing support in varied forms. We also must thank **CNPEM** and its collaborators, who have been relentlessly working to make this course possible. A special thanks also goes for the 35 lecturers who are coming from near by, far and very far away and that have dedicated with great enthusiasm to the construction and execution of the course program.

Thank you for your participation and we hope that you enjoy and take home valuable knowledge and experiences from the Sao Paulo School of Advanced Sciences on Neglected Diseases Drug Discovery – focus on kinetoplastids.

**Lucio Freitas Junior**
On Behalf of the Organizing Committee

# Organizers & Partnerships

**Coordinator**
Lucio Holanda Gondim de Freitas Junior

**Local Committee**
Ildéria Santos
Maria Livia C. M. Ramos Gonçalves
Pâmela Machado

# Partnerships

**Funding**

FAPESP

**Organization**

LNBio CNPEM

**In partnership with**

DNDi

**Sponsors**

PerkinElmer

# Program


| June, 14th | |
|---|---|
| 13:00- 15:00 | **Lunch and Registration** |
| 15:00 – 16:00 | **Groups division**<br>Organizing Committee |
| 16:00-16:30 | **Coffee-break** |
| 16:30– 17:30 | **Welcoming and General Remarks** |
| **June, 15th** | |
| 08:20 – 08:45 | **Overview of SPSAS-ND3: dynamics and expected outcomes**<br>Dr. Lucio Freitas-Junior – LNBio/ Brazil |
| 08:45 – 09:30 | **Neglected Tropical Diseases: Needs and Current Drug Discovery Landscape**<br>Dr. Eric Chatelain – DNDi/ Switzerland |
| 09:30 – 10:00 | **Drug Discovery: The Pharmaceutical Industry Perspective**<br>Dr. Sheraz Gul – ScreeningPort Fraunhofer/ Germany |
| 10:00 – 10:45 | **Introduction to Drug Discovery/Design of Biochemical Assays for Drug Discovery Purposes – what can be achieved and learnings from past success and failures**<br>Dr. Sheraz Gul – ScreeningPort Fraunhofer/ Germany |
| 10:45 – 11:15 | **Coffee-break** |
| 11:15 – 12:00 | **Identification of metabolic targets in trypanosomatids**<br>Dr. Artur Cordeiro - LNBio/ Brazil |
| 12:00 – 12:45 | **Virtual Screening**<br>Dr. Rafael Guido – USP/ Brazil |
| 13:00 – 14:10 | **Lunch** |
| 14:10 – 14:40 | **Poster fixation** |
| 14:40 – 15:30 | **Biosafety in level II laboratory/ Instructions for practical activities**<br>Dr. Carolina Borsoi / Dr. Vanessa Fontana |
| 15:30 – 16:00 | **Official photography** |
| 16:00 – 16:50 | **Coffee-break** |


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|---|---|
| 16:50 – 17:10 | **Opening ceremony**<br>Dr. Kleber Franchini - LNBio/ Brazil |
| 17:10 – 17:30 | **Opening ceremony**<br>Eric Stobbaerts - DNDi Latin America |
| 17:30 – 17:50 | **Inaugural Class**<br>Prof. Michel Rabinovitch – Unifesp/Brazil |
| 17:50-19:50 | **Poster session I/ Opening cocktail: Brazilian evening**<br>*Supported by DNDi* |
| | **June, 16th** |
| 08:30 – 09:15 | **Screening jargon and terms**<br>Dr. Sheraz Gul – ScreeningPort Fraunhofer/ Germany |
| 09:15 – 10:00 | **HTS Assay Design**<br>Dr. Sheraz Gul – ScreeningPort Fraunhofer/ Germany |
| 10:00 - 10:45 | **Biochemistry of Kinetoplastids and Drug Discovery**<br>Prof. Sergio Schenkman - UNIFESP/ Brazil |
| 10:45 – 11:15 | **Coffee-break** |
| 11:15 – 12:00 | **Drug targets in Trypanosomes**<br>Prof. Joana Tavares – Universidade do Porto/ Portugal |
| 12:00 – 12:45 | **Current Chemotherapy for leishmaniasis: Drug Mechanism of Action**<br>Prof. Simon Croft - LSHTM/UK |
| 13:00 – 14:10 | **Lunch** |
| 14:10 – 16:10 | **Practice I – Biochemical Assays**<br>Module 1 – Protease assay development and validation- Dr. Sheraz Gul – Group A and B – Room 09 LNBio<br>Module 2 – High throughput binding screening using MicroScale Thermophoresis - Nanotemper – Group C and D –Room 22 LNBio<br>Module 3 – Using LabWare LIMS/ELN to catalogue Chemical Structures & ELN (Electronic Laboratory Notebook) Workshop - LabWare – Group E and F – Room 69 LNBio<br>Module 4 – Fluorescence intensity assay for Trypanosoma brucei viability using CLARIOStar – Photonics/ Dr. Bruno Pascoalino – Group G and H – Room 42 LNBio |
| 16:10 – 16:30 | **Coffee-break** |


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|---|---|
| 16:30 – 18:30 | **Practice I – Biochemical Assays**<br>Module 1 – Protease assay development and validation - Dr. Sheraz Gul – Group C and D - Room 09 LNBio<br>Module 2 – High throughput binding screening using MicroScale Thermophoresis - Nanotemper – Group E and F– Room 22 LNBio<br>Module 3 – Using LabWare LIMS/ELN to catalogue Chemical Structures & ELN (Electronic Laboratory Notebook) Workshop - LabWare – Group G and H – Room 69 LNBio<br>Module 4 – Fluorescence intensity assay for Trypanosoma brucei viability using CLARIOStar – Photonics/ Dr. Bruno Pascoalino – Group A and B – Room 42 LNBio |
| | **June, 17th** |
| 08:30 – 09:15 | **The Path to a Candidate: A multidisciplinary Effort and a Lot of Hurdles**<br>Dr. Eric Chatelain - DNDi/ Switzerland |
| 09:15 – 10:00 | **High Throughput and High Content Screening for Trypanosomes**<br>Prof. Vicky M Avery - Eskitis Institute/Griffith University/ Australia |
| 10:00 - 10:45 | **MiRNA screening for drug discovery**<br>Dr. Musa Mhlanga – University of Cape Town & CSIR & IMM/ South Africa |
| 10:45 – 11:15 | **Coffee-break** |
| 11:15 – 12:00 | **Integrative Drug Discovery Programs**<br>Dr. Sheraz Gul – ScreeningPort Fraunhofer/ Germany |
| 12:00 – 12:45 | **HCS Data Analysis and Reduction**<br>Dr. Kamyar Hadian - Helmholtz Zentrum München/ Germany |
| 13:00 – 14:10 | **Lunch** |
| 14:10 – 16:10 | **Practice I – Biochemical Assays**<br>Module 1 – Protease assay development and validation - Dr. Sheraz Gul – Group E and F- Room 09 LNBio<br>Module 2 – High throughput binding screening using MicroScale Thermophoresis - Nanotemper – Group G and H– Room 22 LNBio<br>Module 3 – Using LabWare LIMS/ELN to catalogue Chemical Structures & ELN (Electronic Laboratory Notebook) Workshop - Labware – Group A and B – Room 69 LNBio<br>Module 4 – Fluorescence intensity assay for Trypanosoma brucei viability using CLARIOStar – Photonics/ Dr. Bruno Pascoalino – Group C and D – Room 42 LNBio |
| 16:10 – 16:30 | **Coffee-break** |


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|---|---|
| 16:30 – 18:30 | **Practice I – Biochemical Assays**<br>Module 1 – Protease assay development and validation - Dr. Sheraz Gul – Group G and H- Room 09 LNBio<br>Module 2 – High throughput binding screening using MicroScale Thermophoresis - Nanotemper – Group A and B– Room 22 LNBio<br>Module 3 – Using LabWare LIMS/ELN to catalogue Chemical Structures & ELN (Electronic Laboratory Notebook) Workshop - Labware – Group C and D – Room 69 LNBio<br>Module 4 – Fluorescence intensity assay for Trypanosoma brucei viability using CLARIOStar – Photonics/ Dr. Bruno Pascoalino – Group E and F – Room 42 LNBio |
| 18:30 – 20:30 | **Poster session II/ Pastel evening**<br>*Supported by DNDi* |
| | **June, 18th** |
| 08:30 – 09:15 | **Screening Databases, Hit Clustering and Prioritization**<br>Dr. Jean-Robert Ioset – DNDi/ Switzerland |
| 09:15 – 10:00 | **Host-Parasite Interactions at the Cellular Level**<br>Prof. Renato Mortara - UNIFESP/ Brazil |
| 10:00 - 10:45 | **Genetic Diversity of Kinetoplastids**<br>Prof. Bianca Zingales - USP/ Brazil |
| 10:45 – 11:15 | **Coffee-break** |
| 11:15 – 12:00 | **Secondary Assays for Hit-to-Lead and Lead Prioritization**<br>Dr. Carolina B. Moraes – LNBio/ Brazil |
| 12:00 – 12:45 | **Recent advances in HCS technologies**<br>Dr. Kamyar Hadian - Helmholtz Zentrum München/ Germany |
| 13:00 – 14:10 | **Lunch** |
| 14:10 – 16:10 | **Practice II – HCS/ Data Analysis**<br>Module 1 – High Content Screening in Neglected Diseases Research: the Basics – Dr.Kaliandra de Almeida Gonçalves/ Dr. Carolina Borsoi – Group A and B – Room 09 LNBio<br>Module 2 – Phenotypic Antiparasitic Screening Data Analysis and Visualization – Dr. Sheraz Gul/ Dr. Kamyar Hadian – Group C and D – Room 69 LNBio<br>Module 3 – Tools for HTS Data Analysis and Visualization - Dr. Jair L. Siqueira-Neto - Group E and F– Room 201 LNLS<br>Module 4 – Screening Hit Analysis and Clustering/Screening Data Sharing – Dr. Jean-Robert Ioset – Group G and H – Room 227 B CTBE |


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| 16:10 – 16:30 | **Coffee-break** |
| 16:30 – 18:30 | **Practice II – HCS/ Data Analysis**<br>Module 1 –High Content Screening in Neglected Diseases Research: the Basics – Dr. Kaliandra de Almeida Gonçalves/ Dr. Carolina Borsoi - Group C and D – Room 09 LNBio<br>Module 2 – Phenotypic Antiparasitic Screening Data Analysis and Visualization – Dr. Sheraz Gul/ Dr. Kamyar Hadian – Group E and F – Room 69 LNBio<br>Module 3 – Tools for HTS Data Analysis and Visualization - Dr. Jair L. Siqueira-Neto - Group G and H– Room 201 LNLS<br>Module 4 – Screening Hit Analysis and Clustering/Screening Data Sharing – Dr. Jean-Robert Ioset – Group A and B – Room 227 B CTBE |
| **June, 19th** | |
| 08:30 – 09:15 | **Medicinal Chemistry: An Introduction**<br>Dr. Tom Von Geldern/ USA |
| 09:15 – 10:00 | **Assessing Physicochemical and ADME Properties in Early Drug Discovery**<br>Prof. Susan Charman –Monash Univ/ Australia |
| 10:00 - 10:45 | **Issues Related to Drug Development in Kinetoplastid Diseases**<br>Prof. Simon Croft – LSHTM/UK |
| 10:45 – 11:15 | **Coffee-break** |
| 11:15 – 12:00 | **Pharmacokinetics Properties to Ensure Efficacy and Safety**<br>Prof. Susan Charman –Monash Univ/ Australia |
| 12:00 – 12:45 | **Compound Safety Profiling: in silico, in vitro and in vivo**<br>Dr. Tom Von Geldern/ USA |
| 13:00 – 14:10 | **Lunch** |
| 14:10 – 16:10 | **Practice II – HCS/ Data Analysis**<br>Module 1 –High Content Screening in Neglected Diseases Research: the Basics – Dr. Kaliandra de Almeida Gonçalves/ Dr. Carolina Borsoi – Group E and F– Room 09 LNBio<br>Module 2 – Phenotypic Antiparasitic Screening Data Analysis and Visualization – Dr. Sheraz Gul/ Dr. Kamyar Hadian – Group G, H, A and B – Room 69 LNBio<br>Module 3<br>Module 4 – Screening Hit Analysis and Clustering/Screening Data Sharing – Dr. Jean-Robert Ioset – Group C and D – Room 227 B CTBE |


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| 16:10 – 16:30 | **Coffee-break** |
| 16:30 – 18:30 | **Practice II – HCS/ Data Analysis**<br>Module 1 – High Content Screening in Neglected Diseases Research: the Basics – Dr. Kaliandra de Almeida Gonçalves/ Dr. Carolina Borsoi – Group G and H– Room 09 LNBio<br>Module 2<br>Module 3 - Tools for HTS Data Analysis and Visualization - Dr. Jair L. Siqueira-Neto - Group A, B, C and D– Room 69 LNBio<br>Module 4 - Screening Hit Analysis and Clustering/Screening Data Sharing – Dr. Jean-Robert Ioset – Group E and F – Room 227 B CTBE |
| 18:30 – 20:30 | **Poster session III/ Pizza evening**<br>*Supported by DNDi* |
| | **June, 20th** |
| 08:30 – 09:15 | **Think Medicinal Chemistry**<br>Dr. Gilles Courtemanche– Bioaster/ France |
| 09:15 – 10:00 | **Lead Optimization Program for Chagas disease: An Example**<br>Martine Keenan – Epichem/ Australia |
| 10:00 - 10:45 | **Building a National Consortium on Neglected Diseases Drug Discovery**<br>Dr. Lucio Freitas-Junior – LNBio/ Brazil |
| 10:45 – 11:15 | **Coffee-break** |
| 11:15 – 11:50 | **Synthesis and Biological Activity of Natural Products and Derivatives**<br>Prof. Dr. Ronaldo Pilli - UNICAMP/ Brazil |
| 11:50 – 12:25 | **New Synthetic Tools for the Construction of Molecules of Medicinal Value**<br>Prof. Dr. Carlos R.D. Correia – UNICAMP/ Brazil |
| 12:25 – 14:00 | **Lunch** |
| 14:00 – 14:35 | **Fluorescent Tags and Probes: Mechanisms of Actions and Dynamics in Live Cells of Bioactive Small Molecules**<br>Dr. Brenno Neto - UNB/ Brazil |
| 14:35 – 15:10 | **Hypervalent Iodine in Synthetic Organic Chemistry**<br>Dr. Luiz Fernando Silva Jr-USP/Brazil |
| 15:10 – 15:45 | **Developing a library of heterocycles to fight neglected diseases**<br>Dr. Flavio da Silva Emery- USP/Brazil |

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| 15:45 -16:15 | **Coffee-break** |
| 16:15 – 17:15 | **Round-table** |
| 17:30 – 22:00 | **Social gathering: Festa Junina (Typical Brazilian Festival)** |
| | **June, 21th** |
| 08:00 – 10:00 | **Practice III: In vitro and In vivo ADME/Medicinal Chemistry Problem Solving**<br>Module 1 – Hit to Lead - Dr. Gilles Courtemanche Group A and B – Room 111 B CTBE<br>Module 2 –- Lead Optimization – Dr. Martine Keenan/ Dr. Tom Von Geldern -Group C and D– Room 227 B CTBE<br>Module 3 – In vitro metabolic stability and metabolic profile – Daniel Lebre – Group E and F– Room 22 LNBio<br>Module 4 – Drug Candidates: Bioanalytical Validation and *In vivo* Pharmacokinetic Studies. Dr. Silvana A. Rocco/Raphael Morales - Group G and H– Room 69 LNBio |
| 10:00 – 10:15 | **Coffee-break** |
| 10:15 – 12:15 | **Practice III: In vitro and In vivo ADME/Medicinal Chemistry Problem Solving**<br>Module 1 – Hit to Lead - Dr. Gilles Courtemanche Group C and D– Room 111 B CTBE<br>Module 2 – Lead Optimization – Dr. Martine Keenan/ Dr. Tom Von Geldern – Group E and F– Room 227 B CTBE<br>Module 3 – In vitro metabolic stability and metabolic profile – Daniel Lebre - Group G and H– Room 22 LNBio<br>Module 4 – Drug Candidates: Bioanalytical Validation and *In vivo* Pharmacokinetic Studies.- Dr. Silvana A. Rocco/Raphael Morales – Group A and B– Room 69 LNBio |
| 12:15 – 13:45 | **Lunch** |
| 13:45 – 15:45 | **Practice III: In vitro and In vivo ADME/Medicinal Chemistry Problem Solving**<br>Module 1 – Hit to Lead - Dr. Gilles Courtemanche – Group E and F– Room 111 B CTBE<br>Module 2 – Lead Optimization – Dr. Martine Keenan/ Dr. Tom Von Geldern – Group G and H– Room 227 B CTBE<br>Module 3 – In vitro metabolic stability and metabolic profile – Daniel Lebre – Group A and B– Room 22 LNBio<br>Module 4 – Drug Candidates: Bioanalytical Validation and *In vivo* Pharmacokinetic Studies.- Dr. Silvana A. Rocco/Raphael Morales – Group C and D– Room 69 LNBio |
| 15:45 – 16:15 | **Coffee-break** |
| 16:15 – 18:15 | **Practice III: In vitro and In vivo ADME/Medicinal Chemistry Problem Solving** |


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| | Module 1 – Hit to Lead - Dr. Gilles Courtemanche - Group G and H– Room 111 B CTBE<br>Module 2 – Lead Optimization – Dr. Martine Keenan/ Dr. Tom Von Geldern – Group A and B– Room 227 B CTBE<br>Module 3 – In vitro metabolic stability and metabolic profile – Daniel Lebre – Group C and D– Room 22 LNBio<br>Module 4 – Drug Candidates: Bioanalytical Validation and *In vivo* Pharmacokinetic Studies.- Dr. Silvana A. Rocco/Raphael Morales – Group E and F– Room 69 LNBio |
| **June, 22th** | |
| 08:30 – 09:15 | **Drug Efficacy Models for Chagas Disease**<br>Prof. John Kelly – LSHTM/UK |
| 09:15 – 10:00 | **Animal Models of Leishmaniasis Applied to Drug Discovery**<br>Prof. Silvia Uliana - USP/ Brazil |
| 10:00 - 10:45 | **Drug Efficacy Models for Leishmaniasis and HAT**<br>Prof. Joana Tavares – Universidade do Porto/ Portugal |
| 10:45 – 11:15 | **Coffee-break** |
| 11:15 – 12:00 | **Pre-clinical Pharmacology: How to Take a Lead Compound to Clinical Studies**<br>Prof. João Batista Calixto - UFSC/ Brazil |
| 12:00 –12:45 | **Virulence Factors in Visceral Leishmaniasis**<br>Prof. Carlos Henrique Nery Costa - UFPI/ Brazil |
| 13:00 – 14:10 | **Lunch** |
| 14:10 –16:10 | **Practice IV: Animal models**<br>Module 1 – Chagas Disease: from bench to trench – Dr. Ana Torrecilhas – Group A and B – Room 09 LNBio<br>Module 2 – VL hamster model for Drug Efficacy – Dr. Andre Tempone - Group C and D - Room 22 LNBio<br>Module 3 – Bioluminescence imaging of Kinetoplastid infections – Dr. John Kelly/Dr. Joana Tavares – Group E and F - Room 09 LNBio<br>Module 4 – Guided tour – Group G and H – LNBio entrance |
| 16:10 –16:30 | **Coffee-break** |
| 16:30 –18:30 | **Practice IV: Animal models** |


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|---|---|
| | Module 1 – Chagas Disease: from bench to trench – Dr. Ana Torrecilhas - Group C and D– Room 09 LNBio<br>Module 2 – VL hamster model for Drug Efficacy – Dr. Andre Tempone – Group E and F - Room 22 LNBio<br>Module 3 – Bioluminescence imaging of Kinetoplastid infections – Dr. John Kelly/Dr. Joana Tavares – Group G and H - Room 09 LNBio<br>Module 4 – Guided tour – Group A and B– LNBio entrance |
| **June, 23th** | |
| 08:30 – 12:45 | **CLOSING SESSION: Brazilian Pharma: how it can actively drive neglected diseases drug discovery?**<br>Chair: Prof. João Batista Calixto – UFSC/ Brazil |
| 08:30 – 08:45 | Prof. João Batista Calixto– UFSC/ Brazil |
| 08:45 – 09:15 | Dr. Kleber Franchini – Brazilian Biosciences National Laboratory (LNBio)/ Brazil |
| 09:15 - 09:45 | André Berto Gimenez – Brazilian Innovation Agency (FINEP)/ Brazil |
| 09:45 - 10:15 | Pedro Palmeira – Brazilian Development Bank (BNDES)/ Brazil |
| 10:15 - 10:45 | Simone Godoi – São Paulo Research Foundation (FAPESP)/ Brazil |
| 10:45 – 11:15 | **Coffee-break** |
| 11:15 – 11:45 | **Cristalia: A History of Research, Development and Innovation**<br>Prof. Dr. Spartaco Astolfi Filho – Cristalia/ Brazil |
| 11:45 – 12:15 | Eric Chatelain - DNDi/ Switzerland |
| 12:15 – 12:45 | **Round table** |
| 13:00 – 14:10 | **Lunch** |
| 14:10-16:10 | **Practice IV: Animal models**<br>Module 1 – Chagas Disease: from bench to trench – Dr. Ana Torrecilhas – Group E and F– Room 09 LNBio<br>Module 2 – VL hamster model for Drug Efficacy – Dr. Andre Tempone – Group G and H - Room 22 LNBio<br>Module 3 – Bioluminescence imaging of Kinetoplastid infections – Dr. John Kelly/Dr. Joana Tavares– Group A and B- Room 09 LNBio<br>Module 4 – Guided tour – Group C and D– LNBio entrance |


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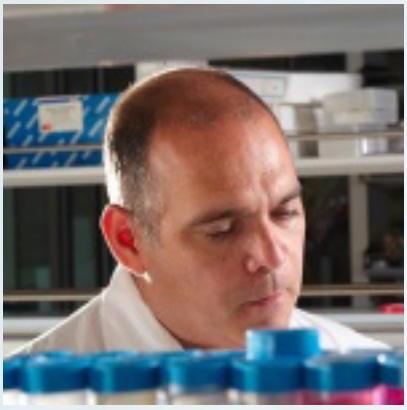
| 16:10 – 16:30 | **Coffee-break** |
| 16:30 – 18:30 | **Practice IV: Animal models**<br>Module 1 – Chagas Disease: from bench to trench – Dr. Ana Torrecilhas – Group G and H– Room 09 LNBio<br>Module 2 – VL hamster model for Drug Efficacy – Dr. Andre Tempone – Group A and B - Room 22 LNBio<br>Module 3 – Bioluminescence imaging of Kinetoplastid infections – Dr. John Kelly/Dr. Joana Tavares – Group C and D- Room 09 LNBio<br>Module 4 – Guided tour – Group E and F– LNBio entrance |
| 19:00 | **Closing dinner** |
| **June, 24th** | |
| 09:15 – 10:40 | **Feedback** |
| 10:40 – 11:00 | **Closing remarks** |

São Paulo School of Advanced Sciences on Neglected Diseases Drug Discovery

## Focus on Kinetoplastids

CNPEM

# *Speakers | Professional Profile*



# Lucio Freitas-Junior

**Event Coordinator**
**Chemical Biology and Screening Platform**
**Brazilian Biosciences National Laboratory – LNBio-CNPEM**
**Campinas, Brazil**
*http://lnbio.cnpem.br/freitasjunior/*

Lucio Freitas-Junior is a molecular parasitologist that has been working in the field of tropical diseases for the past 23 years. From 2009 to 2013 Dr. Freitas-Junior was the director of the Center for Neglected Diseases Drug Discovery (CND3) at Institut Pasteur Korea, where his group worked on assay development, high throughput screening and lead optimization for Leishmaniasis, Chagas disease, Malaria and Dengue. Among the work developed by the team led by Dr Freitas-Junior, the development of image-based high throughput/high content drug screening assay for visceral leishmaniasis was a breakthrough in the field because it allowed, for the first time, the screening of chemical compounds against the mammalian form of Leishmania donovani, the clinically relevant form of the parasite. This assay has been used to screen over 700,000 compounds for leishmaniasis, in collaboration with different Pharma partners and the Drugs for Neglected Diseases initiative, radically changing the drug discovery landscape for this devastating neglected disease. Since 2013 Dr. Freitas-Junior is at the National Center of Research on Energy and Materials, where he continues to develop translational research on drug discovery for neglected diseases, focusing on Chagas disease, Leishmaniasis, Dengue and Chikungunya. Lucio Freitas-Junior is also working on the implementation of a Brazilian Drug Discovery Consortium, to combine multidisciplinary academic expertise in different fields of the discovery sciences to produce drugs against these diseases and, by doing so, aiding on the development of a product-oriented drug discovery matrix in the Brazilian Academia.

# Spartaco Astolfi Filho

**Full Professor of Genetic Engineering and Biotechnology**
**Federal University of Amazonas, Manaus – AM, Brazil**
*http://buscatextual.cnpq.br/buscatextual/visualizacv.do?id=K4781849Y1*

1975: BSc in Molecular Biology, University of Brasilia, Brasilia, Brazil; 1978: MSc in Molecular Biology, University of Brasilia; 1987: PhD in Sciences – Genetic Engineering, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; 1998-1999: Pos-doc fellow at University of Manchester Institute of Science and Technology (UMIST), Manchester, UK. My main area of research refers to the development of new cloning and expression shuttle vectors and their use to develop technologies for production of enzymes and hormones, such as: amylases, cellulases, phosphatases, restriction enzymes, DNA polymerases, insulins and growth hormones. Another area of research and interest are genomic, transcriptomic and proteomic of Amazon species in order to discover new metabolic routes and genes of biotechnological value. In the last decade I participated in the creation and implementation of two graduate programs in biodiversity, conservation and biotechnology in Amazon, I was also director of the Multidisciplinary Support Center located in Amazon Federal University. My interaction with companies occurred mainly with Cristalia: Pharmaceutical and Chemical Inc., a prestigious Brazilian company, being consultant for installation of the industrial drug production plant whose technologies are based in genetic engineering and fermentation processes.

# Vicky Avery

**Chief Investigator & Head**
**Discovery Biology**
**Griffith University**
**Australia**
*http://www.discoverybiology.org/team/vicky-avery*

Professor Vicky Avery obtained her PhD in 1995 (Flinders University, SA), and was awarded an Australian NHMRC Postdoctoral Fellowship which was undertaken at the University of Adelaide. Prof Avery gained significant industry experience whilst working for Active Biotech AB, Sweden (1998-2004). Her positions included, Section Head, Protein Interaction and Drug Discovery; Scientific Project Leader to identify the molecular target of 'Laquinimod', a novel oral treatment for MS; Director, Biochemistry and Molecular Biology and Director, Business Development. She was responsible for the development of assays for FDA to assess efficacy of a cholera vaccine, and identification of compounds against CD80, which led to RhuDex®, an oral treatment for Rheumatoid Arthritis. As Head of Biology for the AstraZeneca /Griffith University collaboration, she was responsible for more than 50 HTS campaigns conducted between 2004 -2007. These spanned all disease areas and encompassed a diverse range of technologies. Professor Avery is currently the Chief Investigator & Head of Discovery Biology, and is responsible for setting the broad research directions of the group.

# João Batista Calixto

**Director**
**Center of Innovation and Preclinical Studies**
**Florianopolis, Brazil**
*http://www.cienp.org.br/en/*

João B. Calixto received degree in Biological Sciences in 1973 at the University of Brasilia and then concluded his Master Science in Pharmacology by Federal University of São Paulo, (EPM) in 1976 and this PhD in Pharmacology in 2004, at the University of São Paulo, Ribeirão Preto, Brazil. He has been professor of Pharmacology at the Federal University of Santa Catarina, Florianópilis, Brazil (1976-2013) and currently he is Director of the Center of Innovation and Pre-clinical Studies (CIEnP), located in Sapiens Parque, Florianópolis, SC. Professor Calixto published about 400 papers in distinguished international peer review journals in the field of Pharmacology, Neuroscience and Medicinal Plants. These papers have been cited more than 12,000 times. His major areas of interest are: pain, inflammation and medicinal plants. He has supervised more than 100 undergraduate students, 37 Master Science, 33 PhD and 22 post-doctoral students. He served as Editor and participates in the Editorial board of several international journals. He has actively acted as referee in more than 70 international scientific journals and most national and international agencies that support the scientific research. He received several awards and also served in many Brazilian committees that support research in Brazil. Professor Calixto has also actively participated in several research projects in partnership with Brazilian and international pharmaceutical companies. He has 23 patents (in Brazil and abroad) and has participated of the development of 03 products that are currently in the market in Brazil.

## Susan Charman

**Director of the Centre for Drug Candidate Optimisation Monash Institute of Pharmaceutical Sciences**
**Faculty of Pharmacy and Pharmaceutical Sciences**
**Monash University**
**Australia**
*http://www.monash.edu.au/pharm*

Susan Charman is Director of the Centre for Drug Candidate Optimisation, and Professor at the Monash Institute of Pharmaceutical Sciences. The theme of her research is understanding mechanistic relationships between physicochemical properties of drug candidates and their absorption, distribution, metabolism and elimination (ADME) characteristics. Optimisation of ADME properties is essential for new drug candidates to ensure safe and efficacious exposure profiles and convenient dosing regimens. She leads a group of 20 scientists that have established platforms to assess physicochemical, permeation, and protein binding properties, to profile metabolic stability and identify potential metabolites, to assess the potential for metabolic drug interactions, to characterize oral bioavailability and pharmacokinetic properties, and to establish clearance mechanisms. She has created a successful model for conducting collaborative, translational research within a university environment, and her group has contributed to programs that have progressed 22 new drug candidates into clinical trials. She is particularly interested in the lead optimisation of novel drug candidates for neglected and tropical diseases and has contributed to projects resulting in one approved antimalarial, two antimalarial candidates currently in clinical development and four in preclinical development. She has attracted significant funding from industry and competitive grants, has published over 125 manuscripts, and is co-inventor on 7 patent applications.

# Eric Chatelain

**Head of Drug Discovery, DNDi**
**Geneva, Switzerland**
*http://www.dndi.org/*

Eric Chatelain has extensive Research and Drug development experience gained from the academia and the industry, as well as management experience, and is well connected with international groups working on Neglected Diseases. Eric Chatelain graduated with a PhD in Biochemistry in 1993 at the INSA Lyon, France. Following 5 years in the academia (ICRF, London, UK and FMI/Novartis, Basel, Switzerland) as a Research Fellow, he joined the pharmaceutical industry (Spirig Pharma AG, Switzerland) in 1999. Focus was on Dermatology R&D and Eric Chatelain led the Biopharmacy Research Lab (till 2003) before heading the Pre-clinical Department (till 2007). In that position, he gained extensive acquaintance with all the processes, workflows and interfaces involved in drug development; he was involved in project development and interacting with people of all disciplines at all levels. His activities led to major projects being taken on board for further clinical development. Since joining DNDi (Drugs for Neglected Diseases initiative, a not-for-profit R&D organisation, whose focus is to develop drugs/treatments for the most neglected diseases) in 2007, Eric's main role is the management of various projects/teams around the world working on the development of new drugs for neglected diseases. Until mid-2009 he was responsible for all screening/early discovery activities before joining the Chagas disease team and taking on responsibility for all Chagas discovery and preclinical projects. During that period he was responsible for the development for high-throughput screening assays (HTS) to assess compounds for their activity against Leishmania and T. cruzi, the pathogen involved in Chagas disease, and led DNDi global current effort in lead optimization for that disease with major and recognized institutions in Australia, Brazil, Europe and South Korea. In 2011 he played an essential role in restructuring of DNDi lead optimization programs and now co-leads the entire DNDi lead optimization effort.

## Simon L. Croft

**Professor**
**London School of Hygiene and Tropical Medicine**
**London, UK**
*http://www.lshtm.ac.uk/aboutus/people/croft.simon*

Simon Croft is Professor of Parasitology in the Faculty of Infectious and Tropical Diseases at the London School of Hygiene & Tropical Medicine (LSHTM). He has worked on the discovery and development of anti-infective drugs for over 30 years in academia, industry and with public-private partnerships (PPPs). His expertise and knowledge on antimicrobial and antiprotozoal chemotherapy was developed whilst working for the Wellcome Research Laboratories, Beckenham, UK and, following his return to academia, on projects funded by WHO, EU, MRC, Medicines for Malaria Venture (MMV) and the Gates Foundation. Simon's research has focussed on the identification and evaluation of novel drugs and formulations for the treatment of leishmaniasis, malaria, human African trypanosomiasis (sleeping sickness) and American trypanosomiasis (Chagas disease), including projects on miltefosine, AmBisome and topical paromomycin, all of which reached clinical trials for the treatment of leishmaniasis. His work on anti-malarials included several MMV supported discovery and pre-clinical projects. Current research interests include PK PD relationships, predictive models for drugs and vaccines, topical formulations and drug resistance. He works extensively with industry and PPPs on Neglected Infectious Diseases and with a network of collaborators in disease endemic countries. From 2004 to 2007 Simon was the first R & D Director of the Drugs for Neglected Diseases Initiative (DNDi), Geneva and from 2008 to 2014 he was Dean of Faculty at the LSHTM.

## Carlos Nery Costa

**Professor**
**Federal University of Piauí**
**Teresina, Brazil**
*http://www.ufpi.br/page.php?id=1*

My main area of interest is visceral leishmaniasis as model for the control of vector borne diseases, of systemic inflammation, and live vaccine development. We have been involved in projects on the finding of Leishmania infantum virulence factors, on host inflammatory response, on mathematical modelling and spatial analysis, on transmission to sandflies, and on landscape. Besides, we have collaborations on biomarkers of disease severity, biosensors, and clinical trials, all about visceral leishmaniasis. However, we also have been working on the diagnosis of neurocysticercosis, neurocryptococcosis and HIV infection.

# Artur Torres Cordeiro

**Principal Investigator**
**Brazilian Biosciences National Laboratory – LNBio-CNPEM**
**Campinas, Brazil**
*http://lnbio.cnpem.br/cordeiro/*

Graduated in biological science and PhD at the Physics Institute of São Carlos, from the University of São Paulo, in the area of protein crystallography and characterization protein-ligand complexes. Four years of post-doctoral experiences on target validation and early drug discovery against neglected diseases, two of those at the Research Unit for Tropical Diseases at De Duve Institute of Cellular Pathology, Brussels. At the present, is PI in the LNBio where coordinates distinct projects on target-based HTS and structure-based hit optimization.

## Carlos Roque Duarte Correia

**Professor**
**Institute of Chemistry**
**University of Campinas**
**Campinas, Brazil**
*http://www.correia-group.com*

Prof. Carlos RD Correia got his PhD degree in Chemistry from Stanford University, USA, in 1986 under the supervision of Professor Paul A. Wender. After completing his PhD, he returned to Brazil and started his independent career as an assistant professor at the Federal University of Rio de Janeiro, moving to the rank of full professor at the same University in 1990. In 1993, Dr. Correia moved with his research group to the State University of Campinas (UNICAMP), São Paulo/ Brazil, where he has been ever since. He is a former head of graduate studies at the Chemistry Institute of Unicamp, acting as Vice-Director of the institute from 2009 to 2010. Prof. Carlos Correia is a 1A class researcher of the Brazilian National Research Council, the recipient of the 3M "Non-Tenured Faculty Award" in 2000, the Unicamp "Zeferino Vaz Excellence Award" in 2013, and has been acting as international adviser for the International Foundation for Science (IFS), Sweden, since 1994. In 2014 he became Editor for South America for the journal "Letters of Organic Chemistry", and member of the board of the journal "Current Organocatalysis". Dr. Correia is currently the vice-chairman of the Organic Chemistry Department of the Chemistry Institute.

His main research interests include organic synthesis and the development of new synthetic methodologies involving palladium, aiming at the concise and practical synthesis of natural products, bioactive heterocyclic compounds, pharmaceuticals and biofunctional compounds.

# Gilles Courtemanche

**Antimicrobials Unit Director**
**Bioaster Technology Research Institute**
**Paris, France**
*http://www.bioaster.org/home.html*

Gilles Courtemanche got his PhD on Synthetic Chemistry (1991) at Pierre & Marie Curie University Paris, France. He has been working in the pharma industry for 20 years. First he took a position of Scientist and worked in Central Nervous System field where he participated in the discovery of the first CRF antagonists (1991-1994). Next, as a project leader at Synthelabo, he contributed in the nomination of several drug candidates for development phase in Urology, Gastroenterology and Metabolism fields. Then, he took of position of Group Leader in Medicinal Chemistry, where he managed a team of up to 30 medicinal chemists working in the field of Infectious Diseases. Finally, he took the responsibility of Collaborative Research on Neglected Tropical Diseases at Sanofi where he worked in collaboration with not for profit organizations like DNDi or MMV, and several international academic partners. He has filed more than 20 patent applications. Currently, he is working for Bioaster, a Technology Research Institute for Health, where he is heading the Antimicrobials Unit. His mission is to build public-private collaborative projects to overcome technical bottlenecks that impair the discovery or development or drugs against Infectious Diseases.

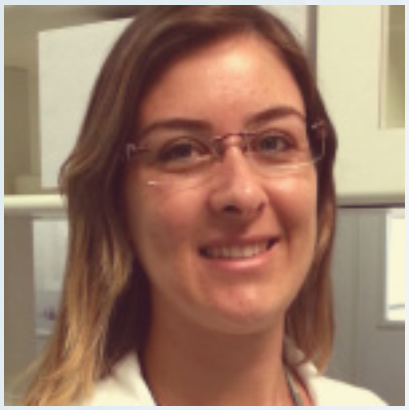
# Flavio da Silva Emery

**Professor**
**School of Pharmaceutical Sciences at Ribeirao Preto**
**University of Sao Paulo**
**Ribeirao Preto, Brazil**
*http://www.qhetem.com*

He completed his degree in pharmacy in 1998 at Universidade Federal do Rio de Janeiro (UFRJ); in 2005, he received a Ph.D. degree in Natural Product Chemistry from Núcleo de Pesquisa de Produtos Naturais (NPPN) at the UFRJ. Currently, is an associate professor at School of Pharmaceutical Sciences at Ribeirão Preto, Universidade de São Paulo.

He is a non-EU member of Chemistry and Molecular Sciences and Technologies (CMST) Co-operation in Science and Technology (COST) Action TD0905, Epigenetics: Bench to Bedside, a scientific activity in the European Union (EU), which aims at integration of the scientific and technological developments across European countries. Since 2013, he is the first secretary of the Brazilian Association of Pharmaceutical Science, and since 2014, he is an active member of IUPAC's subcommittee on Drug Discovery and Development.

He works in the fields of medicinal chemistry, chemical biology, and heterocycle synthesis, aiming to explore several targets for the discovery of novel bioactive compounds. His interested is mainly focused on heterocyclic chemistry, trying to develop new scaffolds as chemical or fluorescent probes. Regarding neglected diseases, he is focusing on epigenetic targets and DHODH, trying to improve the understanding of biochemical processes by using molecular tools and chemical probes to find new chemical space for drug discovery. He is currently coordinating a collaborative project with GSK (FAPESP/GSK call) targeting epigenetic to fight neglected diseases.



## Vanessa Fontana

**Researcher**
**Chemical Biology and Screening Platform**
**Brazilian Biosciences National Laboratory – LNBio CNPEM**
**Campinas, Brazil**
*http://lnbio.cnpem.br/*

Vanessa Fontana received her Bachelor of Pharmacy degree in 2006, Master degree in Genetics (2009) and PhD in Pharmacology (2012) at University of Sao Paulo, Ribeirao Preto, Brazil. During 2-years post-doc experience at University of Campinas (2012-2014), she had a short-term experience at Center for Pharmacogenomics – University of Florida (2013) on genome-wide association studies in hypertension. In 2014 she joined Drug Discovery and Development team at Brazilian Biosciences National Laboratory (LNBio – CNPEM) working on high-throughput screening applied for drug discovery for neglected diseases. Vanessa has experience in high-throughput genotyping, pharmacogenetics studies, clinical research, cell biology and high-content screening for drug discovery. She has 21 peer-reviewed articles on the fields of basic, clinical research and pharmacology.

# Tom von Geldern

**Pharma/biotech consultant**
**Embedded Consulting**
**Illinois, USA**

Tom von Geldern has been an independent consultant to the pharmaceutical and biotech industries since 2007, specializing in medicinal chemistry and discovery strategy and tactics. Prior to this, Dr. von Geldern spent over 20 years in the pharmaceutical industry, most recently serving as a Research Fellow and Senior Group Leader at Abbott Laboratories. In this capacity he led medicinal chemistry efforts resulting in the identification of clinical candidates in the areas of oncology, inflammation, cardiovascular and metabolic diseases. He is an author of 78 peer-reviewed articles, an inventor on 42 US patent applications, and has lectured by invitation on more than 40 occasions. Dr. von Geldern received S.B. degrees in Chemistry, Mathematics, and Biology from MIT, a Ph.D. in Chemistry from the University of California at Berkeley, and performed post-doctoral research at Stanford University.

# Kaliandra A. Gonçalves

**Cellular Assay Development & High Content Imaging Specialist**
**Biosciences National Laboratory (LNBio), Brazilian Center for Research in energy and materials (CNPEM) – Campinas SP, Brazil.**
*http://lnbio.cnpem.br/*

Kaliandra A. Gonçalves has PhD in Biochemistry and Molecular Biology (2011) at UNICAMP (State University of Campinas), Brazil. Post-doc experience in tumor metabolism (2011-2012) at Biosciences National Laboratory (LNBio), Brazilian Center for Research in Energy and Materials (CNPEM) – Campinas – Brazil. In 2011, a short training has performed at European Screening Port –Hamburg in Cellular imaging using Operetta High Content Screening System, Image analysis using Columbus Image Data Storage and Analysis System and Development of High-content assays for the identification and validation of new therapeutic targets. She has been publishing papers in indexed journals. Dra. Kaliandra has wide and broad skills in molecular biology, cell culture, bioassay development and validation, primary screening of small molecules and Image analysis using Columbus Image Data Storage and Analysis System.

## Rafael Victorio Carvalho Guido

**Professor**
**Physics Institute of Sao Paulo – University of Sao Paulo**
**Sao Carlos, Brazil**
*http://www.ifsc.usp.br/english/*

Dr. Guido is Assistant professor 2 at the Physics Institute of São Carlos (IFSC) of the University of São Paulo (USP). He received his PhD in biomolecular physics from the University of São Paulo and the University of Marburg in 2008, and completed two years of postdoctoral work in medicinal chemistry and structural biology at the University of São Paulo. He's specialist in structure-based drug design and in the integration of computational and experimental methods. His research is focused on the structural elucidation of relevant molecular targets and the application of this knowledge toward the discovery and development of new drugs and agrochemicals for human and plant diseases. He's the vice-chair of the Division of Medicinal Chemistry of the Brazilian Chemical Society (SBQ) and a CNPq research fellow – 2 level. He's also an associate investigator of the National Institute of Structural Biotechnology and Medicinal Chemistry in Infectious Diseases and the Center for Innovation in Biodiversity and Drug Discovery.

# Sheraz Gul

**Assay Development and Screening**
**Fraunhofer Institute for Molecular Biology and Applied Ecology**
**ScreeningPort Fraunhofer-IME SP**
**Hamburg, Germany**
*http://screeningport.com/about-us/team*

Sheraz Gul is currently in the Assay Development and Screening department of the Fraunhofer-IME SP where he focuses on small molecule drug discovery across many disease areas. He manages a group that is responsible for developing assays that are subsequently utilized in High Throughput Screening campaigns, as well as validation of Hit compounds and this includes determining their mechanism of action. Prior to this, he worked for GlaxoSmithKline for 7 years where he developed biochemical and cellular assays for High Throughput Screening as well and Hit compound characterization. He has a PhD and 5 years post-doctoral research experience all from the University of London. He has co-authored numerous papers, book chapters and the Enzyme Assays: Essential Data Handbook. In addition, he has been appointed to the editorial board of the European Pharmaceutical Review. Sheraz Gul has experience in progressing small molecule drug discovery programs from the target identification stage through to Lead compound selection. His contributions to these activities have led to multiple publications in peer-reviewed journals, talks at scientific conferences, the identification of multiple Lead series and two development Candidates. Currently, he is also work-package lead in the EU-FP7 projects that focus on neglected parasitic diseases (NMTrypI and PDE4NPD).

## Kamyar Hadian

**Head of Assay Development and Screening Platform**
**HelmholtzZentrum München**
**German Research Center for Environmental Health (GmbH)**
**Institute of Molecular Toxicology and Pharmacology**
**Germany**
*http://www.helmholtz-muenchen.de/adsp*

Dr. Kamyar Hadian is currently the Head of the 'Assay Development and Screening Platform' at the HelmholtzZentrum München in Munich/Germany. He has in-depth experience in designing biochemical High-Throughput Screening (HTS) assays as well as phenotypic cell-based assays for High-Content Screening (HCS) in various disease areas including Cancer, Diabetes, Virology, Immunology and more. His current research focus is targeting protein-protein interactions in various cellular signaling processes. Before that, Kamyar studied Biology at the Technical University of Munich (TUM) and gained his PhD at the HelmholtzZentrum München/Ludwig Maximilians University (LMU) in the field of Virology (HIV research). After a short PostDoc period in the field of NF-kB signaling, he was appointed the Head of 'Assay Development and Screening Platform' at the HelmholtzZentrum München.

## Luiz Fernando Silva Jr

**Associate Professor**
**Institute of Chemistry**
**University of Sao Paulo**
**Sao Paulo, Brazi**
*http://www.iq.usp.br/luizfsjr*

Luiz F. Silva, Jr. was born in São Paulo, Brazil, in 1971. He studied chemistry at University of São Paulo (USP), where he received his B.Sc. He got his Ph.D. in 1999 at USP under the supervision of Prof. H. Ferraz. He then worked one year as a postdoctoral research associate with Professor Gary A. Molander, at the University of Pennsylvania. He returned to Brazil to work as a postdoctoral research associate in the group of Professor Ferraz. In April/2002, he accepted an appointment at Institute of Chemistry of USP, as Assistant Professor. In January/2008, he became Associate Professor. He is Secretary General of Brazilian Chemical Society (2014-2016). His research interests are focused on total synthesis of natural products and on reactions promoted by hypervalent iodine.

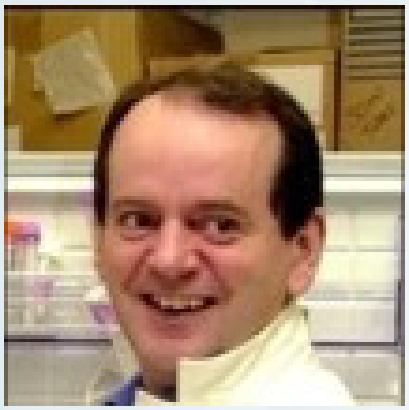
## Martine Keenan

**Head of Drug Discovery**
**Epichem**
**Murdoch, Australia**
*http://www.epichem.com.au/index.php*

Dr Martine Keenan, Epichem's Head of Drug Discovery, is a creative synthetic and medicinal chemist with a commitment to high quality drug discovery and has over 15 years professional experience in the pharmaceutical industry. She obtained her BSc from Kings' College in London, completing a PhD in natural product synthesis at the same institution before moving to Germany to take up a post-doctoral position developing chiral catalysts for asymmetric hydrogenation.

Returning to the UK in 1998, Martine took up a medicinal chemistry position in the neuroscience division of international pharmaceutical company Eli Lilly and remained there for eight years working on a variety of projects. She made a significant contribution to Lilly's fledgling nicotinic platform leading project teams designing and synthesising novel compounds as potential new treatments for CNS indications.

A move to Perth, Australia enabled her to join Epichem in 2008 to lead the medicinal chemistry team working on the development of novel drugs for protozoan parasitic diseases as part of an international consortium funded by a leading R&D Non-Government Organisation. She was appointed Head of Drug Discovery in 2009 and has been working to expand Epichem's R&D portfolio as well managing contract medicinal chemistry projects for an expanding list of clients. She has extensive expertise in all phases of preclinical discovery including design and synthesis of novel analogues, computer-aided drug design, compound optimisation towards drug candidates and successful delivery of project milestones.



# John M. Kelly

**Head of Department of Pathogen Molecular Biology**
**London School of Hygiene and Tropical Medicine**
**London UK**
*http://www.lshtm.ac.uk/aboutus/people/kelly.john*

John Kelly has been Professor of Molecular Biology at the London School of Hygiene and Tropical Medicine since 2005 and Head of the Pathogen Molecular Biology Department since 2010. The Kelly group were the first to report the transfection of the American trypanosome Trypanosoma cruzi and have a long record in the development genetic tools for this parasite. He has published widely in the areas of trypanosome biochemistry, chromosome structure and function, and the mechanisms of drug action and resistance. A major focus of his current research, funded by the Wellcome Trust, British Heart Foundation and the Drugs for Neglected Diseases Initiative is the development and optimisation in vivo imaging techniques applicable to T. cruzi, as tools for assessing drug efficacy and disease pathology. He has published more than 100 peer-reviewed papers, has served on expert review panels for the Wellcome Trust, and is an external adviser for TriTrypDB, the trypanosomatid community genome database.

# Daniel Lebre

**Scientific Director**
**Center for Applied Mass Spectrometry**
**São Paulo, Brazil**
*http://www.cemsalab.com.br/*

Graduated in Chemistry – Oswaldo Cruz University (1996) and master's degree in Nuclear Technology (Applications) by the Institute of Nuclear and Energy Research (2000) (IPEN/USP). Professional skills in chemistry, with emphasis on analytical chemistry, mainly in the following areas: mass spectrometry, liquid chromatography, ADME, analytical method validation. Since 2009 is a partner and Scientific Director of CEMSA – Center for Applied Mass Spectrometry Ltd and responsible for research and development projects: coordinating projects funded by FAPESP (PIPE PIPE I and II) and FUNCET. With experience of over 14 years in mass spectrometry, he worked for nine years at AB Sciex in Brazil and Canada. During his experience in Canada he was responsible for LC/MS applications for small molecules in the pharmaceutical, food, beverage, forensics and environmental. He was responsible for sample collection, generation and interpretation of data, but mostly by the development of analytical methodologies for new MS technologies. As professor, we has disseminated the knowledge and the application of mass spectrometry. He is a member and vice president of the Brazilian Society of Mass Spectrometry.

# Jean-Robert Ioset

**Discovery Manager**
**Drugs for Neglected Diseases initiative – DNDi**
**Geneva, Switzerland**
*http://www.dndi.org/*

Dr. Jean-Robert Ioset joined DNDi in 2005. He is currently the Discovery Manager responsible for the management of the global early discovery portfolio and the supervision and coordination of the DNDi screening network (4 centres and 8 FTE) – through these centres, his objective is to deliver novel lead series to the DNDi preclinical programs. Dr. Ioset also plays an active role in the Department of Communication, Advocacy and Fundraising by providing key information related to all discovery activities. He recommends the organization on the early discovery strategy – a position he's held since 2009. Prior to being the Discovery Manager, Dr. Ioset worked as a consultant for DNDi responsible for coordinating early discovery activities. In his past role, he supported the R&D team with data management, provided advice on R&D projects related to natural products, and coordinated the Pan-Asian Screening Network research network. Prior to joining DNDi, Dr. Ioset was a scientific collaborator and lecturer at the School of Pharmacy of the University of Geneva and the University of Lausanne. His academic research focused on the discovery of new antiprotozoal drugs from plants. He also supervised several PhD, Master and undergraduate students. Before completing a post-doctoral fellowship at the London School of Hygiene and Tropical Medicine (Prof. Simon Croft), he earned a diploma in Public Health and Tropical Medicine from Humboldt University in Berlin. Dr. Ioset is a pharmacist by training, with a PhD in Plant Chemistry from the University of Lausanne. He authored more than 40 publications in the field of natural product chemistry, anti-infective drug discovery and counterfeit drugs.

# Carolina Borsoi Moraes

**Principal Investigator**
**Brazilian Biosciences National Laboratory – LNBio-CNPEM**
**Campinas,Brazil**
*http://lnbio.cnpem.br/moraes/*

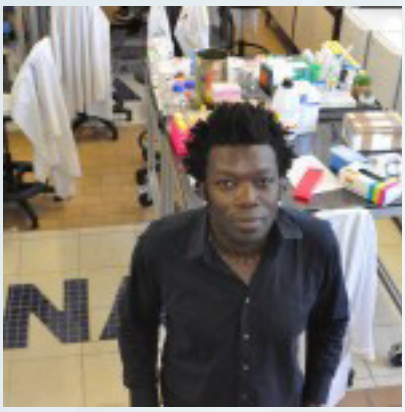
Carolina Borsoi Moraes obtained a PhD in 2009 on Microbiology and Immunology from a joint program between the Institut Pasteur Korea and the Federal University of São Paulo. From 2010 to 2012 she had a postdoctoral position at the Center for Neglected Diseases Drug Discovery (CND3) in Institut Pasteur Korea, coordinating the HCS activities (assay development for lead optimization) for Chagas disease drug discovery.

The research of Dr. Moraes' group focuses on drug discovery for Chagas disease, using high content screening (HCS) assays that permit the triage of chemical compounds for determination of activity against Trypanosoma cruzi, the protozoan parasite that causes Chagas disease. These assays are amenable to automation and have been used in high throughput mode to screen chemical libraries. Additionally, these assays are used to provide routine testing and guide hit prioritization and chemical optimization of hit compounds selectivity/antiparasitic properties during structure-activity relationship (SAR) studies, a process also known as lead optimization. A major goal is developing and improving in vitro and in vivo assays that will translate human disease into experimental models applicable to drug discovery programs, ultimately leading to the discovery of novel chemotherapeutic agents for Chagas disease, while generating knowledge about Chagas disease pathogenesis.

# Renato Arruda Mortara

**Professor**
**Federal University of São Paulo**
**São Paulo, Brazil**
*http://www.ecb.epm.br/~ramortara/*

Renato Mortara got his PhD in Cell Biology (1986) at the MRC-Laboratory of Molecular Biology, Cambridge, UK. He took a position at the Parasitology Division of Escola Paulista de Medicina, UNIFESP, São Paulo, Brazil, where he is presently Full Associate Professor. He is mainly interested in the mechanisms of mammalian cell infection by the protozoan Trypanosoma cruzi in single or mixed infections with other pathogens. Important tools of his study include electron and confocal microscopy. He has been in charge of the first confocal microscope facility at EMP-UNIFESP since 1996 that allowed him to collaborate with several research groups in Brazil and abroad. Since 1996, he is a researcher of the top 1A level from the Brazilian Nacional Research Council, CNPq. He has 159 publications in indexed journals, supervised 15 MSc and PhD theses, and is a member of the Brazilian Society for Protozoology.



# Musa M. Mhlanga

**Gene Expression & Biophysics**
**Professor & Research group leader**
**University of Cape Town Dept. of Integrative Biomedical Sciences &**
**CSIR & IMM (Lisboa)**

Musa M. Mhlanga (USA citizen), American-born male cell biologist, holds a PhD in cell biology & molecular genetics from New York University School of Medicine (2003). He began his PhD at the Rockefeller University in the laboratory of David Ho where he worked on spectral genotyping of human alleles. He then went on to work on the development of in vitro and in vivo applications of molecular beacons for their use in visualizing RNA in living cells with Fred Russell Kramer and Sanjay Tyagi at New York University School of Medicine. Upon completion of his doctoral work he was awarded a U.S. National Science Foundation post-doctoral fellowship at the Institut Pasteur in Paris, France to work in the laboratory of nuclear cell biology. There he worked on RNA transport and single molecule visualization and tracking of RNA in living cells. In late 2008 he moved his lab to South Africa to join the Council of Scientific and Industrial Research as the Research Leader of the Synthetic Biology Emerging Research Area. He heads the Laboratory for Gene Expression & Biophysics and holds a joint appointment to the Institute of Molecular Medicine in Lisbon, Portugal. His laboratory now at the University of Cape Town Medical school works on gene regulation, host-pathogen interactions, single molecule imaging of gene expression and the development of cell-based visual high-throughput biology techniques for screening in basic and clinical biology.

# Brenno Amaro Da Silveira Neto

**Laboratory of Medicinal & Technological Chemistry**
**University of Brasília (UnB), Brasília, Brazil**
**ISI Web of Science: Research ID ISI = I-4579-2012**
**SCOPUS: AU-ID (16029224200)**
*http://orcid.org/0000-0003-3783-9283*

Dr. Brenno A. D. Neto has conducted his independent research career since the end of 2006 when he finished his Ph.D. defending his Thesis. He is Professor of Organic and Medicinal Chemistry at the University of Brasilia (Chemistry Institute) and presently he manages his own research group. Nowadays, he is the Coordinator of the Laboratory of Medicinal and Technological Chemistry group. Currently, his research group comprises 8 Ph.D. students, 2 M.Sc. students and 7 undergraduate students. Already, 4 Ph.D., 7 M.Sc. and several undergraduate students have successfully graduated under his guidance. He published (independent publications) more than 50 articles after he finished his Ph.D. (all indexed at ISI and Scopus, and only a few as a collaborator) underpinning his research independence. Among his outputs, there are 4 reviews which underline the importance of his independent work.
Dr. Neto was also the Guest Editor of a Special Issue (hot topic) held in Current Organic Chemistry (2013, vol. 17, n. 3) thus showing the impact of his own work in the field of catalysis. He also works as a referee for about 35 international journals and has received some national and international awards such as:
BMOS/RSC Young Investigator Award 2013
Honourable Mention – Best Brazilian Thesis in Chemistry 2013. Category: Advisor.
Petrobras Inventor Award for the best-filed patent in 2008.
Best Thesis from the Graduate Program from the Chemistry Institute at the Federal University of Rio Grande do Sul (UFRGS), 2006.

# Jair Lage de Siqueira Neto

**Assistant Adjunct Professor**
**University of California, San Diego**
**La Jolla, USA**
*http://pharmacy.ucsd.edu/faculty/bios/neto.shtml*

Jair Siqueira-Neto has a Bachelor degree in Biology and PhD in Genetics & Molecular Biology from Unicamp, where he graduated in 2007 studying the telomere biology of Leishmania parasites. After, he went to South Korea for a post-doc at the recently founded Institut Pasteur Korea, where he pioneered the development of High-Content High-Throughput Screening assays for drug discovery against leishmaniasis. As Staff Scientist and later Team Leader he was involved in drug discovery programs against the parasites Leishmania and Trypanosoma cruzi, working in projects with the Drugs for Neglected Diseases Initiative (DNDi) collaborating with a number of pharmaceutical companies including Pfizer, GlaxoSmithKline, Sanofi, Merck, Astra Zeneca, among others. In 2013 he moved to the Silicon Valley as the Kinetoplastid Core Director at the Center for Discovery and Innovation in Parasitic Diseases (CDIPD), University of California San Francisco – USA. He led projects and collaborations to accelerate the development of new chemotherapies for the kinetoplastid-caused parasitic diseases: leishmaniasis, Chagas disease and Human African Trypanosomiasis. In the summer of 2014 he was hired as Assist. Adj. Professor at the Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego where he is currently involved in the construction of a Screening Platform to foster drug discovery and development for neglected and rare diseases with low economical interest from the major pharmas.

# Ronaldo Aloise Pilli

**Professor**
**Institute of Chemistry**
**University of Campinas**
**Campinas, Brazil**
*http://www.iqm.unicamp.br/en/institucional-departamentos/ronaldo-aloise-pilli*

Ronaldo A. Pilli obtained a PhD in Organic Chemistry at UNICAMP (Campinas, Brazil) in 1981, followed by a postdoctoral research period at the University of California, Berkeley. R. Pilli returned to Institute of Chemistry – UNICAMP as a professor and implemented a research line in the area of stereoselective synthesis of natural products and drugs. R. Pilli contributed decisively to the implementation of competences in organic synthesis in the country, guiding generations of researchers in this area. His research group has given important contributions to the synthesis of natural products using methods of asymmetric synthesis. He received several awards and honors, and worked as a visitor researcher to the Georg-August University (Göttingen, Germany) in 1994, and at Warwick University (UK, 2005). He served as director Institute of Chemistry (2006-2009) and as UNICAMP Research Dean (2009-2013). Currently, R. Pilli works on natural products chemistry for drug discovery. More often than not, a natural product itself does not possess all the potency, selectivity, and pharmacokinetic properties required to render it a clinically useful drug. Additionally, there is always concern about the supply of enough amounts in order to secure pre-clinical and clinical investigations. Chemical synthesis and biotechnology are two approaches that have stood to the task of taking hit compounds to the lead stage and, eventually, to clinical use. In this presentation, general aspects of the drug development pipeline will be presented as well as results from our laboratory on the investigation of the chemical synthesis and biological activity of a focused library of synthetic compounds modelled on the structure of natural compounds isolated from brazilian biodiversity.

## Silvana A. Rocco

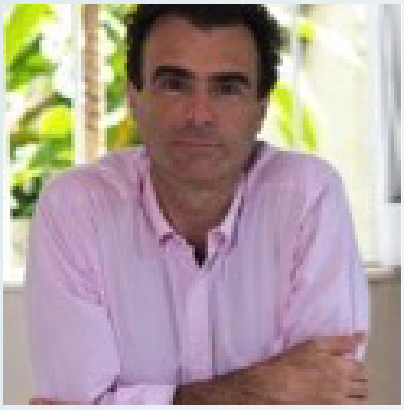
**Researcher**
**Nuclear Magnetic Resonance Laboratory**
**Brazilian Biosciences National Laboratory – LNBio-CNPEM**
*http://lnbio.cnpem.br/*

Silvana A. Rocco is a graduate in Chemistry from the State University of Maringá. She got her Master's degree (1998) and PhD (2002) on Synthetic Organic Chemistry at the State University of Campinas. From 2000 to 2001, she had a valuable experience at University College London and GlaxoSmithKline Medicine Research Centre (Stevenage) when she joined Dr. Michael H. Abraham´s Group. During her trainee, she worked on experimental and calculated partition coefficient (logP) determinations, using a library of quinazoline compounds. She has post-doc experience at Department of Internal Medicine, School of Medicine at the State University of Campinas. Currently she is a researcher in the NMR Group at the Brazilian Biosciences National Laboratory. Dr. Silvana´s research interests are focused in organic chemistry, with emphasis on the synthesis of nitrogen heterocyclic compounds, mainly in the following topics: quinazoline derivatives; NMR studies; validation of HPLC methods (HPLC-UV or HPLC/MS/MS) for measuring compounds in biological fluids; pharmacokinetic studies and drug discovery. She has jointed research activities with Dr. Kleber G. Franchini, Dr. Ana Carolina Zeri and Dr. Gonçalo A. Guimarães Pereira groups and Cristália pharmaceutical Company.

# Sérgio Schenkman

**Full Professor and member of the Brazilian Academy of Sciences**
**Federal University of São Paulo**
**São Paulo, Brazil**
*http://www.ecb.epm.br/~sschenkman/*

Our laboratory investigates mechanisms involved in growth control and differentiation of protozoan parasites of the Trypanosoma genus. We have studied the signaling events involved in translation initiation and replication control. These include protein phosphorylation and acetylation by protein kinases and acetylases/deacetylases. We have demonstrated that phosphorylation of the eukaryotic initiation factor 2 (eIF2a) is required for nutritional stress-induced differentiation of Trypanosoma cruzi, the agent of Chagas' disease. We have shown that heme is as a key molecule involved in activation of an endosomal eIF2alpha kinase that phosphorylates eIF2a. Trypanosomes also have four different Target of Rapamycin (TOR) kinases that in eukaryotes control cellular growth. We have found that one of the TOR kinases, containing a unique PDZ domain, is required for survival in hyperosmotic conditions in Trypanosoma brucei, the agent of African Trypanosomiasis. We have also detected variable histone acetylation in differentiated forms and during the T. cruzi division cycle. We found that histone acetylation is related to chromatin assembly during the replication stages of Trypanosomes. In addition, different levels of acetylation were found in cytosolic and mitochondrial proteins, as revealed by proteomic analysis, in different stages of T. cruzi and T. brucei. Taken as a whole, these studies will help to understand how this group of organisms adapt and survive different environmental conditions, which is an essential step in developing novel anti-parasitic drugs.



## Eric Stobbaerts

**Head of Regional Office, DNDi Latin America**

Having joined DNDi in March 2009 as Policy & Advocacy Coordinator of the Chagas Campaign on the 100th anniversary of the disease discovery, Eric Stobbaerts became the Head of DNDi Latin American office in January 2010.
Under his direction, DNDi Latin America has developed two new treatments for neglected patients in the region and currently leads the regional office's activities with focus on research and development (R&D) for Chagas disease and visceral and cutaneous Leishmaniasis. Other areas of major interest are Advocacy, Access, Fundraising and Communications. DNDi Latin America aims to expand the social mission of the organization on the continent, with cutting-edge scientific research and the empowerment of regional partners.
In the last 20 years, Eric took part of Médecins Sans Frontières (MSF) as a Coordinator and a Head of Mission in several contexts (Lebanon, Afghanistan-Pakistan, Iraq, former Yugoslavia, Indonesia, Egypt and the MENA region). In 1998, he takes over the executive direction of the organization in Spain, one of MSF's 5 Operational Centers. From 2004 onwards, he consulted for various NGOs in London and more recently headed the delegate office of MSF in Brazil. Eric has an MBA from the University of Geneva after completing studies in Economics and Public Affairs at the University of Louvain.

# Joana Tavares

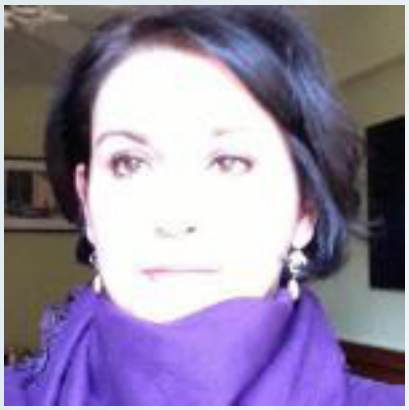
**Researcher**
**Institute for Molecular and Cell Biology**
**University of Porto, Portugal**
*https://www.ibmc.up.pt/research/research-fellows*

Joana Tavares graduated in Pharmaceutical Sciences in 2003 and received in 2008 a PhD degree in Biochemistry by the University of Porto. From 2009 to 2013 she conducted post-doctoral research at Pasteur Institute in Paris. She was from 2003 to 2007 Instructor at the Biochemistry department of the Faculty Pharmacy University of Porto and from 2007 to 2011 Invited Assistant of Immunology. J. Tavares has been research associate at the Institute for Molecular and Cell Biology (IBMC), University of Porto, since 2013. She has been working with protozoan parasites responsible for important human diseases such as Leishmania, Trypanosoma brucei and Plasmodium. She has contributed to the identification of potential drug targets in Trypanosomatids and to the search for target specific inhibitors. More recently, she became an expert on live imaging, including intravital confocal microscopy to dissect in mice the mechanisms used by parasites to overcome host defenses.

## André Gustavo Tempone

**Adolfo Lutz Institute**
**São Paulo, Brazil**
*http://www.ial.sp.gov.br*

Scientific Researcher VI at Adolfo Lutz Institute (Secretary of Health of São Paulo State). Graduated in Pharmacy (1997), master degree (1999), PhD degree (2003) in Parasitology (Parasite-Host Relationships) at University of Sao Paulo, with part of his PhD at the London School of Hygiene and Tropical Medicine (2001). He coordinates the Laboratory of Applied Toxinology in Antiparasitic Drugs since 2004 and since 2009, is the vice-director of the Department of Parasitology and Mycology. He works with Drug Discovery for Leishmaniasis and Chagas disease, from drug repositioning to natural products, preclinical studies, mechanism of action of lead compounds and targeting delivery of drugs by liposomes.



## Ana Claudia Trocoli Torrecilhas

**Professor**
**Federal University of São Paulo**
**São Paulo, Brazil**
*http://www2.unifesp.br/home_diadema/busca_info_docente.php?id=9170541*

During my post-doctoral training, I studied the role of these vesicles on the in vivo T. cruzi infection in the murine model. Our data suggest that, similar to what was observed in vitro, the vesicles generate a strong inflammatory response in vivo. We demonstrated that these membranes increase inflammatory reaction and produced more parasites in the tissues and a severe heart pathology. This severe pathology was due to an increase of T CD4+ and T CD8+ cells with a decrease of iNOS producing. In a previous work, we showed that vesicles released by infective forms of Trypanosoma cruzi, the agent causative of Chagas' disease, modulate the inflammatory responses and the infection in cultured cells and animals. Our group showed also have shown that virulent strains of T. cruzi release more vesicles in vitro when compared to less virulent isolates of the parasite. Our project aims initially to characterize the composition and quantity of vesicles of different isolates of the parasite and then correlate these with the infectivity in cultured cells and experimental animal models. As the vesicles have an immunomodulatory effect, parallel studies will evaluate the role of these vesicles in a model of infection by virus ou protozoa. This work will help to demonstrate the role of these vesicles as immunomodulatory factors and verify whether these factors also act in other models of infection.

## Silvia Reni Bortolin Uliana

**Assistant Professor at Department of Parasitology**
**Biomedical Sciences Institute, University of São Paulo**
**São Paulo – Brazil**
*http://lattes.cnpq.br/5238500995853873*

After graduating in Medicine at the University of São Paulo in 1982, Silvia Uliana joined the Infectious Diseases Program a t Hospital das Clínicas – USP and obtained the title of Specialist in Infectious Diseases in 1985. She did her MSc at the Medicine School – USP (1988-1990) and the PhD at the Biomedical Sciences Institute – USP (1990-1993). She was a Post-Doc at Imperial College of Science, Technology and Medicine, under the supervision of Dr. Deborah Smith (1995-1996). In 1986 she joined the Department of Infectious Diseases at Hospital das Clínicas – FMUSP as a physician and, in 1989, became an Assistant at the Parasitology Department, Biomedical Sciences Institute, USP, where she is now an Associate professor. Silvia's primary research focus at present is leishmaniasis chemotherapy including evaluation of available drugs, combined therapy and discovery of new therapeutics using a "repurposing" approach.

# Bianca Zingales

**Professor**
**Department of Biochemistry**
**Institute of Chemistry**
**University of São Paulo**
**São Paulo, Brazil**
*http://www2.iq.usp.br/docente/bszodnas/*

Bianca Zingales is Professor of Biochemistry at the University of São Paulo. Since 1985, Zingales has focused her research on the Molecular Epidemiology of Trypanosoma cruzi. Her laboratory contributed to the definition that T. cruzi is partitioned into six genetic lineages (TcI-TcVI), which have distinct eco-epidemiological characteristics. A major focus of her current research, funded by the FAPESP and CNPq, is the characterization of one ABCG transporter potentially involved in therapeutic failures to benznidazole and the screening of candidates for Chagas disease treatment. Zingales has been a consultant of TDR since 1992. She was appointed by WHO as a Member of the Expert Advisory Panel of Trypanosomiasis (2000-2009) and as Co-chair of the Disease Reference Group on Chagas disease, Leishmaniasis and Human African Trypanosomiasis (2009-2012). Since 2011, Zingales integrates the Chagas Research Platform of DNDi. She has published more than 110 peer-reviewed papers. Recently, recommendations have been issued on which strains representing T. cruzi diversity should be selected for drug discovery for Chagas disease.

São Paulo School of Advanced Sciences on
Neglected Diseases Drug Discovery

Focus on Kinetoplastids

CNPEM

# *Attendees*

## Alberta Serwah Anning

Country of Birth: Ghana
University of Cape Coast, Ghana
Supervisor: PROF. JOHNSON N. BOAMPONG & DR. ELVIS O. AMEYAW

Abstract: IN VITRO ANTI-LEISHMANIAL ACTIVITY OF SOME SELECTED LOCAL MEDICINAL PLANTS IN GHANA

## Aline Araujo Zuma

Country of Birth: Brazil
Universidade Federal do Rio de Janeiro, Brazil
Supervisor: Maria Cristina Machado Motta

Abstract: Inhibition of Trypanosoma cruzi histone ethyltransferases affects parasite proliferation, cell cycle and ultrastructure

## Alok Kumar Singh

Country of Birth: India
Jawaharlal Nehru University, India
Supervisor: Prof. Rentala Madhubala

Abstract: A proteomic based approach to gain insight into reprogramming of THP-1 cells exposed to Leishmania donovani over an early temporal window

## Anna Frieda Fesser

Country of Birth: Germany
University of Basel, Switzerland
Supervisor: Pascal Mäser, Marcel Kaiser

Abstract: Stage-specific reporter gene expression in Trypanosoma cruzi

## Bilal Zulfiqar

Country of Birth: Pakistan
The Eskitis Institute for Drug Discovery, Australia
Supervisor: Prof. Vicky Avery

Abstract: Development of a promastigote resazurin based viability assay in 384-well format for high throughput screening of Leishmania donovani DD8.

## Carolina Bioni Garcia Teles

Country of Birth: Brazil
Fundação Instituto Oswaldo Cruz (FIOCRUZ / RO), Brazil
Supervisor: Dr. Luís Marcelo Aranha Camargo

Abstract: ACTIVITY OF SOME COMPOUNDS ISOLATED FROM COMBRETUM LEPROSUM IN THE INFECTIVITY AND INTRACELLULAR DEVELOPMENT OF L. AMAZONENSIS IN MURINE MACROPHAGES

## Cathia Cecilia Coronel Molas

Country of Birth: Paraguay
CEDIC/FIOCRUZ/FOCEM/COF 0311, Paraguay-Brazil
Supervisor: Dra. Ma. Celeste Veja

Abstract: Cribado farmacologico sobre T. cruzi y Leishmania sp

## Celestin Nzanzu Mudogo

Country of Birth: Democratic Republic of Congo
University of Tübingen, Germany
Supervisor: Prof. Dr. Michael Duszenko

Abstract: EXPRESSION, REFOLDING AND PURIFICATION OF A PUTATIVE CYCLOOXYGENASE LIKE-ENZYME FROM TRYPANOSOMA BRUCEI FOR STRUCTURAL STUDIES

## Cláudia Jassica Gonçalves Moreno

Country of Birth: Cabo Verde
Institute of hygiene and tropical medicine of Lisbon, Portugal
Supervisor: Dr. Marcelo Silva

Abstract: ESTABLISHMENT OF MURINE MODEL FOR MODULATION OF TOXICITY OF BENZNIDAZOLE DURING TREATMENT OF CHAGAS DISEASE (TRYPANOSOMA CRUZI).

## Cristiane França Da Silva

Country of Birth: Brazil
Instituto Oswaldo Cruz, Brazil
Supervisor: Maria de Nazaré Correia Soeiro

Abstract: In vitro and in vivo activity of the chloroaryl-substituted imidazole viniconazole against Trypanosoma cruzi

## Daniel N.A Tagoe

Country of Birth: Ghana
Glasgow, United Kingdom
Supervisor: Harry P. de Koning

Abstract:INVESTIGATING THE DOWNSTREAM EFFECTORS OF CAMP SIGNALLING AS POTENTIAL DRUGGABLE TARGETS IN TRYPANOSOMA BRUCEI

## Daniel Paape

Country of Birth: Germany
Glasgow, United Kingdom
Supervisor: Richard McCulloch & Jeremy C. Mottram

Abstract:Dissecting the kinome of T. brucei: RIT-seq of cell cycle sorted T. brucei identifies kinases involved in the regulation of nuclear DNA replication

## Daniela Ribeiro Alves

Country of Birth: Brazil
University of State of Ceará, Brazil
Supervisor: Selene Maia de Morais

Abstract: Assessment of Primary and Secondary Metabolites From Fungi and Vegetals as Leishmanicidal Drugs

## Darlan da Silva Candido

Country of Birth: Brazil
University of São Paulo, Brazil
Supervisor: Ludmila Rodrigues Pinto Ferreira

Abstract: MARINE NATURAL PRODUCTS AS CANDIDATES TO TREAT CHAGAS' DISEASE: A HIGH-THROUGHPUT SCREENING APPROACH

## Denise da Gama Jaén Batista

Country of Birth: Panamá
Instituto Oswaldo Cruz , Brazil
Supervisor: Maria de Nazaré C. Soeiro and Marcello A. Barcinski

Abstract: Copper(II)–fluoroquinolone complexes: biological activity against Trypanosoma cruzi

## Diego Benítez

Country of Birth: Uruguay
Institut Pasteur de Montevideo, Uruguay
Supervisor: Marcelo Comini

Abstract: OPTIMIZATION OF PAULLONE SCAFFOLD AS ANTI-TRYPANOSOMATID AGENT BY TARGET-BASED AND PHENOTYPIC DRUG DISCOVERY APPROCHES

## Edubiel Arturo Alpizar Sosa

Country of Birth: Mexico
Institut U. Glasgow, Scotland
Supervisor: Marcelo Comini

Abstract: EFFECT OF A DRUG CANDIDATE FORMULATED INTO NANOPARTICLES IN LEISHMANIA MEXICANA

## Elany Barbosa Silva

Country of Birth: Brazil
Universidade Federal de Minas Gerais, Brazil
Supervisor: Rafaela Ferreira

Abstract: OPTIMIZATION AND BIOLOGICAL EVALUATION OF A CLASS OF COMPETITIVE INHIBITORS OF THE PARASITIC ENZYMES CRUZAIN AND RHODESAIN

## Elias Matias Laurentino

Country of Birth: Venezuela
Federal University of Goias, Brazil
Supervisor: Carolina Horta Andrade

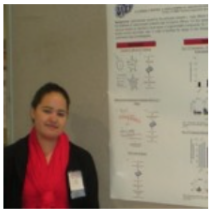
Abstract: MOLECULAR MODELING STUDY AND PHARMACOPHORE MAPPING OF CYP51 ENZYME FOR DESIGN NEW LEISHMANICIDE AGENTS

## Ender Javier Quintero Troconis

Country of Birth: Venezuela
Universidad de Los Andes, Venezuela
Supervisor: Juan Luis, Concepción and Paul AM, Michels

Abstract: Molecular and biochemical characterization of Aldose 1-Epimerase and Glucose 6-phosphate 1-Epimerase from Trypanosoma cruzi.

## Eva Adriana Iniguez



Country of Birth: Mexico
University of Texas at El Paso, USA
Supervisor: Dr. Rosa A. Maldonado

Abstract: RUTHENIUM-CLOTRIMAZOLE COMPLEXES AS POTENT CHEMOTHERAPEUTIC AGENTS AGAINST LEISHMANIA MAJOR.

## Fabiana Santana Celes

Country of Birth: Brazil
Centro de Pesquisas Gonçalo Moniz, FIOCRUZ-BA, Brazil
Supervisor: Dr. Camila Indiani de Oliveira

Abstract: Bacterial cellulose bio-curatives containing DETC for the topical treatment of cutaneous leishmaniasis caused by Leishmania braziliensis

## Fabrício Castro Machado

Country of Birth: Brazil
Universidade Federal de São Paulo, Brazil
Supervisor: Sergio Schenkman

Abstract: Effects of disubstituted urea compounds in Trypanosoma cruzi

## Felipe Andres Monsalve Marin

Country of Birth: Colombia
University of São Paulo, Brazil
Supervisor: Gabriel Padilla Maldonado

Abstract: Production of statins and antiparasitic molecules by fungi isolated from sugar cane plants of Brazil using solid state fermentation

## Fernanda Aparecida Heleno Batista

Country of Birth: Brazil
University of São Paulo, Brazil
Supervisor: Júlio Cesar Borges

Abstract: Identification of Molecules with Selective Inhibitory Action against Hsp90 of protozoa parasites: a Thermodynamic and Comparative Approach.

## Francisco Olmo Arévalo

Country of Birth: Spain
Universidad de Granada, Spain
Supervisor: Manuel Sánchez Moreno

Abstract: A SERIES OF TETRADENTATE POLYAMINES HAVE POTENTIAL ACTIVITY IN VITRO AND IN VIVO AS INHIBITORS OF SEVERAL SPECIES OF TRYPANOSOMATIDS.

## Guillermo Arango Duque

INRS-Institut Armand-Frappier
Centre for Host-Parasite Interactions, Canada
Supervisor: Albert DESCOTEAUX

Abstract: Leishmania promastigotes induce cytokine secretion in macrophages through the degradation of Synaptotagmin XI

## Happyness Jeremia

Country of Birth: TANZANIA
Sokoine University of Agriculture, Tanzania
Supervisor: Prof.GERALD MISINZO & Dr. WILLIAM MAKUNDE

Abstract: Current epidemiology of Bancroftian filariasis and clinical disease presentation in rural and urban settings during elimination process in Tanzania

## Jean Henrique da Silva Rodrigues

Country of Birth: Brazil
Universidade Estadual de Maringá, Brazil
Supervisor: Dr. Celso Vataru Nakamura

Abstract: THE NOVEL MOLECULE 3-CHLORO-6-METHOXY-2-(METHYLSULFONYL) QUINOXALINE KILLS TRYPANOSOMES BY A MIXED CELL DEATH PATHWAY

## João Henrique Coelho Campos

Country of Birth: Brazil
Federal University of Sao Paulo, Brazil
Supervisor: Ana Claudia Trocoli Torrecilhas

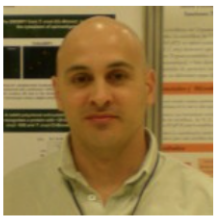
Abstract: The splenocyte cellular immune responses from chronic mice after stimulation with trypanosoma cruzi vesicles;

## Joseane Lima Prado Godinho

Country of Birth: Brazil
Universidade Federal do Rio de Janeiro, Brazil
Supervisor: Wanderley de Souza

Abstract: TC95, a drug candidate against
Leishmania sp.: antiproliferative effects and mechanisms of action

## Juan Carlos Ramirez Gomez



Country of Birth: Cuba
Instituto de Ingeniería Genética y Biología Molecular, Argentina
Supervisor: Alejandro Schijman

Abstract: TC95, a drug candidate against
Leishmania sp.: antiproliferative effects and mechanisms of action

## Julia Medeiros Souza

Country of Birth: Brazil
University of Franca, Brazil
Supervisor: Prof. Dra. Lizandra Guidi Magalhães

Abstract: Screening and in vitro evaluation of
leishmanicidal activity of plant extracts against the
parasite Leishmania amazonensis

## Juliana Maria Lima

Country of Birth: Brazil
Universidade de São Paulo, Brazil
Supervisor: Carmen Lúcia Cardoso

Abstract: 2D LC System for on-flow Ligands
Screening by Zonal Biochromatography Approach
for Nucleoside Diphosphate Kinase b from Leishmania major

## Katerina Doleckova

Country of Birth: CZECH REPUBLIC
HEBREW UNIVERSITY OF JERUSALEM, ISRAEL
Supervisor: DR. DANA REICHMANN

Abstract: ROLE OF REDOX-REGULATED INTRINSICALLY
DISORDERED CHAPERONE HSP33 IN THE OXIDATIVE
STRESS DEFENSE SYSTEM OF TRYPANOSOMA BRUCEI

### Kofi Dadzie Kwofie

Country of Birth: Ghana
University of Ghana, Legon, Ghana
Supervisor: Dr. Irene Ayi and Dr. Mitsuko Suzuki

Abstract: Novel compounds isolated from CVP005B crude extract show strong anti-trypanosomal activity in-vitro

### Lauve Rachel Tchokouaha Yamthe

Country of Birth: Cameroon
University of Yaoundé 1, Cameroon
Supervisor: Fabrice Fekam Boyom

Abstract: Antileishmanial and cytotoxicity activities of three annonaceae plants from Cameroon

### Luiz César Borro

Country of Birth: Brazil
University of Campinas, Brazil
Supervisor: Goran Nesic

Abstract: Ranking optimization in virtual screening using physical-chemical and structural descriptors of the nano-environment for protein-ligand interactions

### Luiza Almeida Figueiredo

Country of Birth: Brazil
University of Minas Gerais, Brazil
Supervisor: Ricardo Toshio Fujiwara

Abstract: Search for molecular targets for therapeutic control of infection by trypanosomatids

### Marcelo Santos da Silva

Country of Birth: Brazil
Butantan Institute, Brazil
Supervisor: Dra. Maria Carolina Quartin Barbosa Elias Sabbaga

Abstract: Modulation of Trypanosoma brucei Orc1/Cdc6 by ATP binding and hydrolysis

### Marta Oliveira

Country of Birth: Portugal
Centre of Marine Sciences, University of Algarve, Portugal
Supervisor: Luísa Custódio and Luísa Barreira

Abstract: Unlocking the antileishmanial potential of halophyte plants from the Algarve coast, Portugal

## Milena Menegazzo Miranda

Country of Birth: Brazil
State University of Londrina, Brazil
Supervisor: Dr. Wander Rogério Pavanelli

Abstract: ACTIONS OF FLAVONOIDS TRANS CHALCONE, HESPERIDIN METHYL-CHALCONE AND QUERCETIN IN EXPERIMENTAL LEISHMANIASIS

## Naseer Ali Shah

Country of Birth: Pakistan
COMSATS Institute of Technology, Islamabad, Pakistan
Supervisor: Dr. Muhammad Rashid Khan

Abstract: Phytochemical, Antileishmanial and Cytotoxic evaluation of Artemisia scoparia and Fraxinus xanthoxyloides, collected from District Islamabad

## Noura Neffati

Country of Birth: Tunisia
Institut Pasteur de Tunis, Université Tunis El Manar, Tunisia
Supervisor: Pr. Med Habib KAROUI

Abstract: Natural plant based-extracts, a potential source to fight against cutaneous Leishmaniasis

## Rajeev Kumar Pandey

Country of Birth: India
National Institute of Immunology, New Delhi, India
Supervisor: Dr. Chandrima Shaha

Abstract: Role of Bcl-2 family proteins during Leishmania donovani Infection

## Sandip Mukherjee

Country of Birth: India
CSIR-Indian Institute of Chemical Biology, India
Supervisor: Dr Syamal Roy

Abstract: NEW USE OF OLD DRUG: IMIPRAMINE AND LIPOSOMAL IMIPRAMINE IS AN EFFECTIVE DRUG AGAINST BOTH ANTIMONY SENSITIVE AND RESISTANT LEISHMANIA DONOVANI CLINICAL ISOLATES IN EXPERIMENTAL INFECTION

## Sanjana Mehrotra

Country of Birth: India
Guru Nanak Dev University, India
Supervisor: Not applicable, Independent position

Abstract: In vitro Antileishmanial Drug Susceptibility of Clinical Isolates from Patients with Indian Visceral Leishmaniasis—Status of Newly Introduced Drugs

## Sergio Sifontes-Rodríguez

Country of Birth: Cuba
Universidad Central "Martha Abreu" de Las Villas, Cuba
Supervisor: Dr. José Antonio Escario/Dr. Miguel Angel Cabrera

Abstract: Rational Discovery of Drugs for the Treatment of Leishmaniasis & Chagas' Disease.

## Thais Alves da Costa Silva

Country of Birth: Brazil
Universidade Estadual Paulista, "Julio de Mesquita Filho", Brazil
Supervisor: Marcia A. S. Graminha

Abstract: In vitro and in vivo activity of palladium compound on Leishmania infantum

## Thais Alves da Costa Silva

Country of Birth: Brazil
Instituto Adolfo Lutz, Brazil
Supervisor: André Gustavo Tempone Cardoso

Abstract: AMITRIPTYLINE AND CYCLOBENZAPRINE ARE EFFECTIVE IN VITRO ANTILESHMANIALS AND SUPPRESS PRO-INFLAMMATORY CYTOKINES

## Thaisa Lucas Sandri

Country of Birth: Brazil
FEDERAL UNIVERSITY OF PARANÁ, Brazil
Supervisor: Iara José de Messias Reason

Abstract: MOLECULAR EPIDEMIOLOGY OF TRYPANOSOMA CRUZI AND ITS CORRELATION WITH IMMUNOLOGICAL RESPONSE (OR INFLAMMATORY MARKERS) OF CHRONIC CHAGASIC PATIENTS IN PARANÁ STATE

## Tiago Rodrigues Ferreira

Country of Birth: Brazil
Ribeirao Preto Medical School, University of Sao Paulo, Brazil
Supervisor: Angela Kaysel Cruz

Abstract: Study of the effects of PRMT7-catalyzed methylation on the function and expression of the RNA-binding protein Alba20 in Leishmania major

## Vijay Kumar Prajapati

Country of Birth: India
Central University of Rajasthan, India
Supervisor: Professor Shyam Sundar

Abstract: Development of MicroRNA based Therapeutics for Visceral Leishmaniasis Infection

## Adeniyi Yahaya Tijani

Country of Birth: Nigeria
National Institute for Pharmaceutical Research and Development, Nigeria
Supervisor: Professor Karyinus Shingu Gamaniel

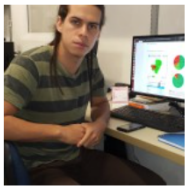
Abstract: Pharmacological effects of the crude extract and alkaloidal fraction obtained from the roots of Nauclea latifolia in laboratory animals

## Adriana Corrêa da Silva

Country of Birth: Brazil
Federal University of Rio Grande do Sul, Brazil
Supervisor: Marilene Henning Vainstein

Abstract: CHARACTERIZATION OF THE MECHANISMS OF ACTION OF PLUMIERIDE AND PLUMIERIDINE COMPOUNDS AGAINST CRYPTOCOCCUS GATTII

## Christian Bustamante Toro



Country of Birth: Colombia
Universidad de Antioquia, Colombia
Supervisor: Carlos Muskus

Abstract: Repositioning of approved drugs as anti-,leishmanial agents based on pharmacological modelling

## Dereje Nigussie Woldemichael

Country of Birth: Ethiopia
Addis Ababa University, Ethiopia
Supervisor: Prof. Eyasu Makonnen, Dr Asfaw Debella, Mr. Geremew Tasew

Abstract: Repositioning of approved drugs as anti-,leishmanial agents based on pharmacological modelling

## Farnaz Zahedifard

Country of Birth: IRAN
Pasteur Institute of IRAN, Iran
Supervisor: Professor Sima Rafati

Abstract: COMPARISON OF DIFFERENT SYNTHETIC PEPTIDE CANDIDATES AGAINST L. MAJOR AND L. TROPICA INFECTION IN BALB/C MICE

## Julián Ernesto Nicolás Gulin

Country of Birth: Argentina
Service of Parasitology and Chagas Disease – Children's Hospital "Dr. Ricardo Gutiérrez", Argentina
Supervisor: Facundo García-Bournissen

Abstract: In vitro and in vivo evaluation of new drugs and combination therapies for Chagas Disease

## Junya de Lacorte Singulani

Country of Birth: Brazil
Universidade Estadual Paulista, Brazil
Supervisor: Maria José Soares Mendes-Giannini

Abstract: Evaluation of new drugs in paracoccidioidomycosis: antifungal activity of alkyl gallates in alternative models Galleria mellonella and Caenorhabditis elegans and in mice

## Kaio Moraes de Farias

Country of Birth: Brazil
Universidade Federal do Ceará, Brazil
Supervisor: Claudia do Ó Pessoa

Abstract: USING PROTEOMIC APPROACH FOR MOLECULAR CHARACTERIZING FROM LEISHMANIA CHAGASI CULTURE TREATED WITH NOR-ß–LAPACHONE

## Lizzi Maelis Herrera Sánchez

Country of Birth: Panama
Acharya Nagarjuna University, India
Supervisor: Patricia Llanes & Ricardo Lleonart

Abstract: Development and Characterization of a Murine Model of Infection by Leishmania (Viannia) panamensis

## Mounir Tilaoui

Country of Birth: Morocco
Sultan Moulay Slimane University, Morocco
Supervisor: Patricia Llanes & Ricardo Lleonart

Abstract: The anti-malarial artemisinin drug is also active against cancer

## Rafael Ferreira Dantas

Country of Birth: Brazil
Oswaldo Cruz Foundation (IOC/FIOCRUZ), Brazil
Abstract: Evaluation of antichagasic activity of 1,2,3-triazoles having as target active enzymes on carbohydrates (CAZy's) of Trypanosoma cruzi

## Adriana Campos

Country of Birth: Brazil
Universidade do Vale do Itajaí, Brazil
Supervisor: Valdir Cechinel Filho

Abstract: Antiproliferative and antifungal effect of pyranonaphthoquinones obtained from Cipura paludosa bulbs

## Aline Alves dos Santos Naujorks

Country of Birth: Brazil
Federal University of Mato Grosso do Sul, Brazil
Supervisor: Dênis Pires de Lima

Abstract: NOVEL NAPHTHOQUINONE DERIVATIVES AND EVALUATION OF THEIR TRYPANOCIDAL AND LEISHMANICIDAL ACTIVITIES

## Celso Oliveira Rezende Junior

Country of Birth: Brazil
State University of Campinas, Brazil
Supervisor: Luiz Carlos Dias

Abstract: SYNTHESIS, BIOLOGICAL EVALUATION, AND STRUCTURE-ACTIVITY RELATIONSHIPS OF CARBAMOYLIMIDAZOLE DERIVATIVES AS CRUZAIN INHIBITORS AND ANTI-TRYPANOSOMA CRUZI AGENTS

## Chiara Borsari

Country of Birth: Italy
University of Modena and Reggio Emilia, Italy
Supervisor: Prof Maria Paola Costi

Abstract: Flavonoids and flavonoid-like compounds as candidates to face neglected tropical diseases

## Colin Rylott Wilson

Country of Birth: South Africa
University of Cape Town, South Africa
Supervisor: Kelly Chibale

Abstract: Aminopyrazolo[1,5-a]pyrimidines as Potential inhibitors of Mycobacterium tuberculosis: Structure activity relationships and ADMET characterization

## Eder Lorenzato Junior

Country of Birth: Brazil
University of São Paulo, Brazil
Supervisor: Maria Cristina Nonato

Abstract: DIHYDROOROTATE DEHYDROGENASE FROM LEISHAMIA VIANNIA BRAZILIENSIS: A NEW TARGET IN THE FIGHT AGAINST LEISHMANIASIS CUTANEOUS AND MUCOCUTANEOUS

## Fanny Palace-Berl

Country of Birth: Brazil
University of São Paulo, Brazil
Supervisor: Prof. Tit. Leoberto Costa Tavares

Abstract: Study of N'-[(5-nitrofuran-2-yl) methylene] substituted hydrazides against Trypanosoma cruzi strains more prevalent in Chagas disease patients

## Federica Prati

Country of Birth: Italy
University of Bologna, Italy
Supervisor: Maria Laura Bolognesi

Abstract: 2-PHENOXY-1,4-NAPHTHOQUINONE-ANACARDIC ACID HYDRIDS AS MULTITARGET LIGANDS FOR TRYPANOSOMATID INFECTIONS

## Fernanda Rosa Andre

Country of Birth: Brazil
Universidade Federal de São Paulo, Brazil
Supervisor: Daniela Gonçales Rando

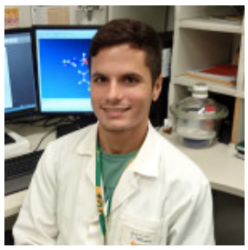
Abstract: PARALLEL SYNTHESIS AND STUDIES OF STRUCTURE ACTIVITY RELATIONSHIPS OF POTENTIALLY ANTILEISHMANIAL N-ACYLHYDRAZONES

## Fernando Fumagalli

Country of Birth: Brazil
University of São Paulo, Brazil
Supervisor: Flavio da Silva Emery

Abstract: Development of carbazole library targeting antileishmanial compounds

## Frederico Silva Castelo Branco



Country of Birth: Brazil
Federal University of Rio de Janeiro, Brazil
Supervisor: Angelo da Cunha Pinto, PhD and Núbia Boechat, PhD

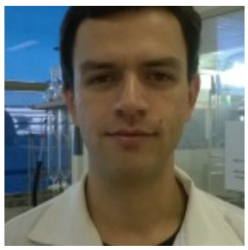
Abstract: Development of Low Cost And Pharmacokinetic-Optimized Triazoles As Potent Inhibitors of Trypanosoma Cruzi

## Giovanni Pupo

Country of Birth: Italy
Sapienza Università di Roma, Italy
Supervisor: Roberto Di santo

Abstract: HETEROCYCLIC DERIVATIVES TARGETED TWO CENTRAL METABOLIC PATHWAYS UNIQUE TO TRYPANOSOMATIDS: ERGOSTEROL AND TRYPANOTHIONE PATHWAYS

## Ismael Raitz



Country of Birth: Brazil
University of Campinas, Brazil
Supervisor: Dr. Ronaldo Aloise Pilli

Abstract: “Synthesis of goniothalamin analogue bearing a fluorescent group for cell-imaging experiments”

## Jaime Franco

Country of Birth: URUGUAY
UNIVERSIDAD DE LA REPUBLICA, URUGUAY
Supervisor: MARCELO COMINI & LAURA SCARONE

Abstract: ELUCIDATING THE MODE OF ACTION OF OLIGOTHIAZOLES AS ANTI TRYPANOSOMA BRUCEI AGENTS

## Lucia Fargnoli

Country of Birth: Argentina
Rosario National University, Argentina
Supervisor: Guillermo R. Labadie

Abstract: TARGETING PROLINE TRANSPORT FOR CHAGAS´ DISEASE DRUG DEVELOPMENT

## Luis Miguel Zaravia Argomedo

Country of Birth: Peru
University of Sao Paulo – USP, Brazil
Supervisor: Hélio Alexandre Stefani

Abstract: Síntese de Clusteres Luminescentes de Lantanídeos: Potenciais Bio-Marcadores

## Marina Amaral Alves

Country of Birth: Brazil
Federal University of Rio de Janeiro, Brazil
Supervisor: Lídia Moreira Lima end Luzineide W. Tinoco

Abstract: DISCOVERY OF NEW LEISHMANIA DONOVANI NUCLEOSIDE HIDROLASE INHIBITORS BY MOLECULAR FRAGMENTS USING STD NMR

## Michele Panciera

Country of Birth: Brazil
Unicamp, Brazil
Supervisor: Carlos Roque Duarte Correia

Abstract: Synthesis of natural marinoquinolines and unnatural analogues with potential biological application against neglected diseases

## Rodrigo Lucarini


Country of Birth: Brazil
Universidade de Franca, Brazil
Supervisor: Prof. Dr. Wilson R. Cunha

Abstract: Leishymanicidal acvity of Gochnatia pulchra

## Ronan Batista

Country of Birth: Brazil
UNIVERSITY OF DUNDEE, Scotland, UK
Supervisor: ALAN H. FAIRLAMB

Abstract: DESIGN, SYNTHESIS AND EVALUATION OF INHIBITORS OF QUINONOID DIHYDROPTERIDINE REDUCTASE (qDPR) AS TOOLS FOR LEISHMANIA DONOVANI
TARGET VALIDATION

## Salman Zafar

Country of Birth: PAKISTAN
UNIVERSITY OF PESHAWAR, PAKISTAN

Abstract: PROTECTING SKIN LESIONS FROM B ACTERIAL SUPERINFECTION: ANTI-BACTERIAL POTENTIAL OF LEAVES OF ACACIA MODESTA

## Samuel Silva da Rocha Pita

Country of Birth: Brazil
Universidade Federal da Bahia, Brazil

Abstract: VIRTUAL SCREENING APPLIED IN THE SEARCH OF Trypanosoma cruzi TRYPANOTHIONE REDUCTASE (TCTR) INHIBITORS OF THE NATURAL PRODUCTS DATABASE FROM STATE OF BAHIA

## Vida Terzic

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Institut de Chimie des Substances Naturelles,
National Center for Scientific Research, France
Supervisor: Dr. Joëlle DUBOIS

Abstract: CHEMISTRY APPLICATION TO VISUALIZE AND QUANTIFY DRUG ENTRY INTO PROTOZOAN PARASITES


São Paulo School of Advanced Sciences on
Neglected Diseases Drug Discovery
Focus on Kinetoplastids
CNPEM

# *Abstracts*

## IN VITRO ANTI LEISHMANIAL ACTIVITY OF SOME SELECTED LOCAL MEDICINAL PLANTS IN GHANA

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Leishmaniasis is a disease of public health concern worldwide, caused by various parasites that belong to the genus Leishmania. The pentavalent antimonials developed in 1945 are still first line treatment drugs for both Cutaneous and Visceral leishmaniasis while Amphotericin B and Pentamidine are second line treatment drugs. The poor conventional treatment, toxic side effects at effective doses and the lack of vaccine demand the urgent need for new anti leishmanial agents. This study aimed at investigating plants used traditionally to treat leishmaniasis. Plant parts were dried, powdered and extracted. Different concentrations of the extracts ranging from 15.1 to 500µg/mL in 0.1% DMSO with M199 and a positive control of Amphotericin B were prepared in triplicates in 96-well plates that contained 75,000parasites/well. The plates were incubated at 25°C and promastigotes counted on 6, 12, 24 and 48 hours. Phytochemical screening revealed the presence of steroids, triterpenoids, tannins, saponins, alkaloids, flavonoids and glycosides. The IC50 for Anthostema aubrynum, Coelocaryon oxycarpum, Erythrophloem ivorense and Amphotericin B were 25.04, 136.5, 18.95 and 2.4µg/mL respectively after 24hours. The activities of the rest of the plants on Leishmania sp. promasigotes are yet to be performed.
Keywords: Amphotericin B, Pentamidine, leishmanicidal, promasigotes, Cutaneous Leishmaniasis,Visceral Leishmaniasis.

**Aline Araujo Zuma**

## INHIBITION OF Trypanosoma cruzi HISTONE ETHYLTRANSFERASES AFFECTS PARASITE PROLIFERATION, CELL CYCLE AND ULTRASTRUCTURE

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Histone methyltransferases (HMT) control chromatin remodeling and are essential for DNA replication, transcription, repair and in gene expression. Thereby, HMT inhibitors have been used as chemotherapeutic agents. The aim of this study is to evaluate the role of Chaetocin (HMT inhibitor) in T. cruzi proliferation, ultrastructure and cell cycle. Epimastigote forms were treated with different concentrations of the drug and samples were collected after each 24 hours for counting on Neubauer's chamber, to MTS/PMS viability method, for processing to transmission (TEM) and scanning electron microscopy (SEM), and to flow cytometry. Our data showed that Chaetocin inhibited parasite proliferation and reduced cell viability, resulting in IC50 of 2 μM. Flow cytometry analyses revealed that this compound led to cell cycle arrest after treatment with 5 μM for 72h. Furthermore, TEM analysis revealed that Chaetocin promoted an intense unpacking of nuclear heterochromatin, nucleolar fragmentation and cytoplasmatic disorganization. SEM analysis also showed that with 50 μM for 72h, parasites were rounded and flattened in the posterior end of the cell body. Taking together, these data showed that the inhibition of HMT alters different aspects of parasite cell biology, reinforcing the idea that such enzymes constitute potential targets against T. cruzi.
Supported by CNPq and FAPERJ

## A proteomic based approach to gain insight into reprogramming of THP-1 cells exposed to Leishmania donovani over an early temporal window

**Singh AK1**, Pandey RK2, Jair Lage Siqueira-Neto3, Yong-Jun Kwon4, Lucio H. Freitas-Junior5, Shaha C2 and Madhubala R1
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2Cell Death and Differentiation Research Laboratory, National Institute of Immunology, New Delhi, India
3Center for Discovery and Innovation in Parasitic Diseases and Department of Pathology, University of California, San Francisco, San Francisco, California, USA
4Samsung Medical Center, Seoul, South Korea
5Laboratório Nacional de Biociéncias (LNBio), Centro Nacional de Pesquisas em Energias e Materiais (CNPEM), Campinas, São Paulo, Brazil.

In order to investigate, how intracellular parasite Leishmania donovani manipulates the host cell environment, we undertook a quantitative proteomic study of human monocyte derived macrophages (THP-1) following infection with L. donovani. We Used isobaric tags for relative and absolute quantification method (iTRAQ) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to compare the proteome profile in non-infected and L. donovani-infected THP-1 cells. We detected modification of protein expression in key metabolic pathway, including glycolysis and fatty acid oxidation, suggesting a global reprogramming of cell metabolism by the parasite. An increased abundance of proteins involved in gene transcription, RNA splicing (heterogeneous nuclear ribonucleoproteins (hnRNPs), histones, DNA repair and replication was observed at 24 h post infection. Proteins involved in cell survival and signal transduction were more abundant at 24 h post infection. Selected proteomics results were validated by real-time PCR and immunoblot analyses. Similar changes were observed in L. donovani-infected human monocyte-derived primary macrophages. The effect of RNA interference (RNAi) mediated gene knock-down of proteins validated the relevance of the host quantitative proteomic screen and open the avenue of identification of druggable host factors. Our findings indicate that the host cell proteome is modulated after L. donovani infection and provides evidence for global reprogramming of cell metabolism and demonstrates the complex relations between host-parasite at molecular level.

## STAGE-SPECIFIC REPORTER GENE EXPRESSION IN TRYPANOSOMA CRUZI

1,2 - Fesser, A.F.; 1,2 - Schmidt, R.S.; 1,2 - Mäser, P.; 1,2 - Cal, M.; 1,2 - Kaiser, M.
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2 – University of Basel, Switzerland

About 7 million people globally are affected by Chagas' disease caused by Trypanosoma cruzi. After decades of chronic infection, 30 % of the patients develop cardiac and/or digestive symptoms. The current standard drugs (benznidazole and nifurtimox) are not satisfying in regard to their efficacy and safety. Recently, two CYP51 inhibitors (posaconazole and ravuconazole) have been tested in drug trials. Although in vitro results had been promising, both drugs showed a low sustained efficacy, which rendered them unusable for treatment. Therefore, new methods in the drug discovery process are needed. Stage-specific assays are one in vitro tool to increase the predictability of preclinical data. T. cruzi has four morphologically distinct stages. The relevant stages for assay purposes are the two forms occurring in humans: the intracellular, proliferative amastigotes and the extracellular, infectious trypomastigotes. Depending on the action of a drug, its effects on parasite survival may be different on the two stages. In order to quantify these effects, the project aims at establishing an assay using a T. cruzi parasite that expresses reporter genes specific to the amastigote and trypomastigote stages. In order to create the transgenic parasite, we are combining comparative transcriptomics with reverse genetics in T. cruzi.

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

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1- Discovery Biology group, The Eskitis Institute for Drug Discovery- Griffith University, Australia

Leishmaniasis is characterized as a parasitic disease caused by the trypanosomatid protozoan termed Leishmania. Leishmania is endemic in 88 countries around the globe with increased cases of morbidity and mortality emerging each day. Although leishmaniasis is treatable, it faces challenges largely due to emerging resistance and extensive toxicity for current drugs. Therapeutic efficacy varies depending upon the species, symptoms and geographical regions of the Leishmania parasite. There is considerable need for assays which are cost effective, robust, automated for ideal therapeutic candidate selection. This project focuses on the development of a panel of assays suitable for the identification and characterization of novel molecules against leishmaniasis. The development, optimization and execution of one of these, a resazurin based high throughput assay to identify new chemical scaffolds for different species of Leishmania is discussed. For this purpose a promastigote stage resazurin viability assay and a complimentary THP-1 (host cell) resazurin based cytotoxicity assay have been established in 384-well format for high throughput phenotypic screening of Leishmania donovani DD8 parasites. Assay development and primary screening results will be presented.

## ACTIVITY OF SOME COMPOUNDS ISOLATED FROM COMBRETUM LEPROSUM IN THE INFECTIVITY AND INTRACELLULAR DEVELOPMENT OF L. AMAZONENSIS IN MURINE MACROPHAGES

1 - TELES, C.B.G., 1 - MOREIRA-DILL, L. S.; 2 - SILVA, A. A.; 2 - FACUNDO, V. A.; 1 - SILVA, L. H. P; 1 - STÁBELI, R. G., 3 - SILVA-JARDIM, I.
1 - Oswaldo Cruz Foundation (Fiocruz Rondônia). Porto Velho-RO, Brazil.
2 - Federal University of Rondônia,Porto Velho-RO, Brazil;
3 – State University of Santa Cruz (UESC), Ilheús - BA, Brazil,

Although considerable advances have been made in recent years, the chemotherapy of leishmaniasis still requires long-term treatment and sometimes is followed by considerable toxicity. In this paper, we tested the in-vitro activity of the ethanolic extract (EE), obtained from the fruits of Combretum leprosum and the triterpen 3beta,6beta,16beta-triidroxilup-20(29)-eno (1) and its derivates (1-3) on Leishmania amazonensis promastigotes and amastigotes and on the funcionality of murine macrophage. Our results demonstrated that the EE displayed leishmanicidal activity and the IC50 was 24.8 µg/mL. Triterpen (1) is very potent in inhibiting promastigotes growth with IC50 3,3µg/mL. Among the synthetic derivatives, only (2) and (3) were active against promastigotes (IC50 = 3.48 µg/mL and 5.8 µg/mL, respectively). Derivate (1) did not display any effect against the parasite. The action of this triterpens was not reverted after treatment, since the growth of the parasite remained reduced even after the suspension of the drug usage. There was also researched citotoxicity against mammalian cells for all the drugs, but none of them showed cytotoxicity. Of all the triterpenes, the triterpen (1) showed the highst leishmanicidal activity, reducing the number of amastigotes in about 84% after 96h of treatment. In this way, our results contribute for the advance in the research for new anti-protozooarian drugs.

## PHARMACOLOGICAL SCREENING OF TRYPANOSOMA CRUZI AND LEISHMANIA SPP.

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1- Centro para el Desarrollo de la Investigación Científica (CEDIC)/Paraguay; 2- Departamento de Química Orgánica / Facultad de Química - UDeLaR / Universidad de la República / Uruguay 3- CEDIC/FIOCRUZ/FOCEM/COF 0311

Chagas disease and leishmaniasis are diseases caused by parasitic protozoa Trypanosoma cruzi and Leishmania spp, respectively. These are diseases that have no effective, safe and affordable treatment, so it continues to seek new and better drugs that can be used in the treatment of both diseases.
Based on this problem our research focuses on the primary pharmacological screening of new compounds, chemical or natural origin, on these parasites and thereby identify potential candidates that can be used as new drugs; these assays include determination of the activity, trypanocidal and leishmanicidal, of compounds, chemical or natural origin, in the extracellular and intracellular parasitic forms, cytotoxicity of phagocytic cells and non-phagocytic mammalian toxicity in mice, in vivo testing of the active compounds in different experimental murine models and monitoring of antiparasitic activity by molecular and biochemical techniques. Cribado farmacológico sobre Trypanosoma cruzi y Leishmania spp. La Enfermedad de Chagas y la Leishmaniosis son enfermedades producidas por los parásitos protozoos, Trypanosoma cruzi y Leishmania spp, respectivamente. Son enfermedades que no poseen un tratamiento eficaz, seguro y accesible, por lo que se continúa en la búsqueda de nuevos y mejores fármacos que puedan utilizarse en el tratamiento de ambas enfermedades. Basados en esta problemática nuestra línea de investigación se centra en el cribado farmacológico primario de nuevos compuestos de origen químico o natural sobre estos parásitos y de esta forma seleccionar posibles candidatos que puedan ser utilizados como nuevos fármacos; estos ensayos incluyen la determinación de la actividad tripanocida y leishmanicida de compuestos de origen químico o natural en la forma parasitaria extra e intracelular, citotoxicidad en células de mamíferos fagocíticos y no fagocíticos, toxicidad en ratones, ensayos in vivo de los compuestos activos en diferentes modelos experimentales murinos, y monitoreo de la actividad antiparasitaria por técnicas moleculares y bioquímicas.

## EXPRESSION, REFOLDING AND PURIFICATION OF A PUTATIVE CYCLOOXYGENASE LIKE-ENZYME FROM TRYPANOSOMA BRUCEI FOR STRUCTURAL STUDIES

1- Mudogo C. N. and Duszenko M.
1- Interfaculty Institute of Biochemistry, University of Tübingen

Human African trypanosomiasis is caused by infection with Trypanosoma brucei gambiense or Trypanosoma brucei rhodesiense. These parasites are transmitted to the human host by tsetse flies. In the past, it has been shown that parasites of the kinetoplastid group (Trypanosoma and Leishmania) produce PGD2, PGE2, and PGF2a from exogenous arachidonic acid. A PGF2a synthase was detected in T. brucei lysate, identified, structurally solved and enzymatically characterized. It had been demonstrated that PGD2 induces a form of caspase-independent apoptosis in stumpy parasites for cell density regulation and sustain the infection within the host. In this study a putative cyclooxygenase (TbCOX) was detected by Western blotting in trypanosome lysates, the upstream enzyme upon which the entire arachidonic acid cascade in these parasites depends and which is the rate-limiting step in the production of prostaglandins (PGs). We hypothesized that Trypanosoma brucei may well possess a unique TbCOX obtained by co-evolution processes that is responsible for PGs production.
Hereby we reported the cloning, expression, refolding and purification of TbCOX protein using BL21 (DE3) and SF9 insect cells prior to in vitro or in vivo protein crystallization. Analysis of the crystal structure of TbCOX will be valuable in elucidating the function of this protein and design of specific inhibitors that may serve as trypanosome-alternative drugs.

## ESTABLISHMENT OF MURINE MODEL FOR MODULATION OF TOXICITY OF BENZNIDAZOLE DURING TREATMENT OF CHAGAS DISEASE (TRYPANOSOMA CRUZI).

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After a century of discovery, Chagas disease caused by the protozoan Trypanosoma cruzi is a major neglected tropical diseases in several Latin American countries. Benznidazole (BNZ) is the drug used for the treatment of the disease,However this drug shows a variable therapeutic efficacy in the acute and chronic phases of the disease. Moreover, the problem of finding a substance capable of eradicating the parasite may be directly related with heterogeneity of different populations of T. cruzi, which differ in morphology, in virulence and pathogenicity. Moreover, the unfavorable pharmacokinetic properties, the low aqueous solubility and bioavailability of weightless BNZ could be the reason for the difference in antiparasitic efficacy. BERENICE project is a European Commission's project whose the main objective is to obtain a more effective, better tolerated and low-cost formulation of a drug with trypanocidal activity to cure Chagas disease in endemic and non endemic countries.This study is based on the determination of the in vitro susceptibility to BNZ of different strains of T. cruzi belonging to groups TcI, TcII and TcIII and determining the toxicity of BNZ, the excipients and the respective nano formulations BNZ model murine under the.

## IN VITRO AND IN VIVO ACTIVITY OF THE CHLOROARYL-SUBSTITUTED IMIDAZOLE VINICONAZOLE AGAINST TRYPANOSOMA CRUZI.

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Chagas disease (CD) is caused by the intracellular protozoan parasite Trypanosoma cruzi and affects more than 10 million people in poor areas of Latin America. There is an urgent need for alternative drugs with better safety, broader efficacy, lower costs and shorter time of administration. Thus the biological activity of viniconazole, a chloroaryl-substituted imidazole was investigated using in vitro and in vivo screening models of T. cruzi infection. Ultrastructural findings demonstrated that the most frequent cellular damage was associated with plasma membrane (blebs and shedding events), Golgi (swelling aspects) and the appearance of large numbers of vacuoles suggesting an autophagic process. Our data demonstrated that although this compound is effective against bloodstream and intracellular forms (16 and 24 μ m, respectively) in vitro, it does not present in vivo efficacy. Due to the urgent need for novel agents against T. cruzi, the screening of natural and synthetic products must be further supported with the aim of finding more selective and affordable drugs for CD.

## INVESTIGATING THE DOWNSTREAM EFFECTORS OF CAMP SIGNALLING AS POTENTIAL DRUGGABLE TARGETS IN TRYPANOSOMA BRUCEI

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2-Institute of Infection, Immunity and Inflammation, University of Glasgow
3-Biocenter, Genetics, Ludwig-Maximillians-University of Munich, Martinsried, Germany

Current drug treatment options in trypanosomiasis are old, toxic and highly ineffective. Signal transduction pathways have been shown to have essential cellular functions and have therefore often been exploited as pharmacological targets. The metabolism of cyclic adenosine monophosphate (cAMP) has recently been validated as a drug target in T. brucei. The phosphodiesterase inhibitor CpdA, used in the validation, was fatal to bloodstream forms. However, the downstream effector proteins of cAMP activity in trypanosomes are unknown. We use reverse genetics, genomics and proteomics to elucidate potential pathways sensitive to changes in cellular cAMP levels. An RNAi library screen identified novel cAMP Response Proteins (CARPs), which are mainly unique to kinetoplastids. Deletion of any of the allele of the CARP genes increases resistance to CpdA. Extensive characterization shows all the CARPs are involved in the cAMP response individually or in a complex. Particularly, CARP3 seems to be an important determinant of cAMP activity, as its overexpression limits growth, and significantly increased both internal cAMP levels and efflux of cAMP from the cells. Deep sequencing (RIT-Seq) of T. brucei expressing an RNAi library (RIT-Seq), after exposure to CpdA, has delineated several new potential CARPs, including protein phosphatases and adenylyl cyclases. CARP3 is trypanosome-specific thus providing a potentially unique therapeutic target for drug development as well as elucidating the biochemistry of cAMP signalling in trypanosomes.

## DISSECTING THE KINOME OF T. BRUCEI: RIT-SEQ OF CELL CYCLE SORTED T. BRUCEI IDENTIFIES KINASES INVOLVED IN THE REGULATION OF NUCLEAR DNA REPLICATION

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The coordinated replication and segregation of the genome to daughter cells is an integral cellular process. Several protein kinases (PKs) have been documented to regulate multiple steps in nuclear DNA replication in yeast and other eukaryotes. However, though information is emerging about the machinery and coordination of T. brucei nuclear replication, nothing is known about the putative PK regulation of the reaction. Identification of how nuclear replication is regulated would provide new avenues for the therapeutic intervention of the diseases caused by T. brucei and other kinetoplasts. To address this, we pooled all bloodstream form T. brucei cell lines that individually target every PK (183 in total) by inducible RNAi. The pool was then sorted, with and without RNAi induction, according to their cell cycle stage based on DNA content (G1, S-phase & G2/M) and relative read depth mapped over time and per cell cycle stage. This screen revealed PKs already known to be involved in cell cycle progression, as well as several novel PKs. We are characterising several of the PKs in detail by examining cellular localization and the effect of their RNAi on DNA synthesis and replication factor localisation. Once the absence of these PKs is confirmed to be detrimental for DNA replication and survival we want to carry out target-based and phenotype-directed high content cell screens with PK-focused chemical libraries.

## ASSESSMENT OF PRIMARY AND SECONDARY METABOLITES FROM FUNGI AND VEGETALS AS LEISHMANICIDAL DRUGS

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Leishmaniasis is a zoonotic disease transmitted by protozoa of the genus Leishmania. In Brazil, the viceral form is found in 19 of 27 states, especially in the Northeastern region. The action of primary or secondary metabolites of fungi and plants can kill the parasite. The Caryocar brasiliense Camb. products the basis for the treatment of coughs, bronchitis and has shown activity against sarcoma 180, molluscicidal action and leishmanicide. Ricinus communis Linn. is considered the basis for the treatment of arthritis, asthma, cholera, convulsions, and can be used as a bactericide and effective antifungal. Endophytic fungi are organisms found within plants without causing any apparent damage. These organisms are essential for the biological control through the production of secondary metabolites, having a great potential for exploitation. The primary metabolites, as amino acids, fatty acids and others, are essential for survival. Secondary metabolites, on the other hand, are apparently not essential for the survival. Among such compounds has flavonoids, quinones and phenols. The exploitation of active ingredients of various species of fungi and plants can lead to identification of priceless metabolites that can act as drugs or lead to the development of new therapeutic substances. Thus, a study on these metabolites can contribute to the determination of intervention methodologies.
Keywords: Leishmania. Natural product, enzyme, lipase, leishmanicide.

## MARINE NATURAL PRODUCTS AS CANDIDATES TO TREAT CHAGAS' DISEASE: A HIGH-THROUGHPUT SCREENING APPROACH

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Chagas'disease (CD), a parasitic disease caused by the protozoan Trypanosomacruzi, is still a public health issue in Latin America with more than 7 million people currently infected. Thirty percent of these patients will develop deadly cardiac myopathy or digestive syndromes and the available treatment options are toxic and inefficient.New drugs are urgently needed and marine products represent a new chemical space to be explored.Important to mention, about 2/3 of the drugs used to treat human diseases are derived or related to natural products.A library of marine derived bacteria wasassembled and pre-filtered based on toxicity screening and polarity to improve drug-like properties.The library, named Global Health Library, was screened against T. cruzi infecting mice myocytes using an image-based high-content screening approach.Initial "hits" were fractioned and individual compounds were tested in dose-response for anti-parasitic activity confirmation, potencydetermination (EC50)and host cell toxicity assessment (CC50).After screening 3200 pre-fractions, 102 "hits" were identified followed up by 33 isolated compounds with confirmed and specific anti-parasitic activity. In the dose-response assays, one compound showed submicromolar EC50 against intracellular parasites, no cytotoxicity up to 20 μM and is now being evaluated for in vivo efficacy in Chagas mouse model.

## COPPER (II)–FLUOROQUINOLONE COMPLEXES: BIOLOGICAL ACTIVITY AGAINST TRYPANOSOMA CRUZI

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Quinolones, and their subset fluoroquinolones are chemotherapic agents, which represent an important class of synthetic broad-spectrum antibiotics exhibiting activity in vivo and in vitro, against bacterial, virus and protozoa. Trypansoma cruzi is the etiological agent of the Chagas disease that still remains a public health problem without an effective therapy. In this context, our aim was synthesize new Cu(II) complexes with sparfloxacin (SPAR), containing or not 1,10-phenanthroline (phen), in order to verify their anti-T.cruzi activity. The effect of the activity against bloodstream trypomastigote forms of T. cruzi (Y strain) of SPAR exerted a low trypanocidal, exhibiting EC50 values of 114±20μM. The association of the Cu(II) ion with SPAR led to an increase of the trypanocidal activity. [CuCl2(H2O)(SPAR)] was more active than the correspondent free ligands and CuCl2.2H2O salt (EC50=45±30μM). The elimination of the two water molecules from the previous complex by addition of phen showed greater activity than the precursor [CuCl2(phen)] (4.7±0.1μM vs 7.2±5 μM). The increment of the activity of [CuCl2(phen)(SPAR)] when compared with the free drug (SPAR) may be explained by increased compound ability to penetrate into the parasites and/or different cellular targets (mitochondrial–kDNA or nuclear DNA). So, this association proved to be a good strategy of activity improvement.

## OPTIMIZATION OF PAULLONE SCAFFOLD AS ANTI-TRYPANOSOMATID AGENT BY TARGET-BASED AND PHENOTYPIC DRUG DISCOVERY APPROCHES.

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Trypanosomatids are endowed with a unique redox system that relies on the low molecular mass thiol trypanothione. It is synthesized by the ATP-dependent enzyme trypanothione synthetase (TryS), absent in mammals. Here we showed some genetic and chemical approaches demonstrating that TryS is indispensable for trypanosomes. We performed a drug discovery campaign of a chemically diverse library (>450 compounds) against TryS from 3 pathogenic trypanosomatids (T. brucei, T. cruzi and L. infantum). In parallel we investigated the biological activity towards T. brucei and Leishmania. The screening revealed differences in inhibitory activity towards species-specific TryS and identified paullones as nM-range inhibitors of LiTryS. SAR analysis revealed that substitution at the N5 position confers anti-LiTryS activity. Performing enzymatic and biological evaluations, a structural optimization of this N5-substituted paullones serie was performed. We archived one candidate (with low μM potency against amastigotes L. braziliensis and selectivity index, SI > 50) for the study of the mode of inhibition against LiTryS. 9- and 11-substituted 4-azapaullones were sub-μM potent inhibitors of the infective form of T. brucei, without activity against host kinases and good SI. This study proposes paullones as promising drug candidates to selectively target LiTryS activity and the proliferation of bloodstream T. brucei.

## EFFECT OF A DRUG CANDIDATE FORMULATED INTO NANOPARTICLES IN LEISHMANIA MEXICANA.

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New compounds and strategies to treat Leishmaniasis are needed due to drug resistance and toxicity of current drugs. In these series of experiments a small polyene was formulated into nanoparticles and tested against L.-mexicana promastigotes and amastigotes. Natamycin alone (NMC) and formulated into nanoparticles (NMC/PLGA) showed activity against both stages of L.-mexicana. The inhibitory concentration (IC50) for NMC was 3.9 μM and 2.2 μM against promastigotes and axenic amastigotes, respectively. Amphotericin B (Amp B) at a concentration ≤10 μM was more active than NMC alone and encapsulated into PLGA, against both forms of the parasite. PLGA nanoparticles with and without drug were stable at different physiological pH and temperature conditions. In a macrophage-murine infection model, NMC/PLGA showed higher inhibiton of intracellular amastigotes at concentrations ≥50 μM and no toxicity against host cells was observed, compared with Amp B and to a lessen extent with NMC alone. Although NMC formulations showed properties as leishmanicidal, further studies and experimental approaches are needed to understand their exact mechanism of action which is related to ergosterol affinity.

## OPTIMIZATION AND BIOLOGICAL EVALUATION OF A CLASS OF COMPETITIVE INHIBITORS OF THE PARASITIC ENZYMES CRUZAIN AND RHODESAIN

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Cruzain in T. cruzi and rhodesain in T. b. rhodesiense are target enzymes involved in parasite survival. Ferreira et al had investigated a library of 197,861 substances against by quantitative HTS and virtual screening. Substance VIII stood out for presenting Ki 6μM, molar mass of 387g/mol and ClogP of 2.93. Whereas the tests reported in the literature to VIII were carried out with a commercially available mixture of stereoisomers, we are synthesizing the 4 possible stereoisomers to identify the most potent isomer, as well as others analogues. We selected 12 comercial analogues of substance VIII from a search in the ZINC database, using as criterion the Tanimoto coefficient, that quantifies the 2D similarity between molecules. This allow us to explore the relationship structure activity in this series. These compounds are being evaluated against the target enzymes based on enzyme kinetics experiments, employing the fluorogenic substrate Z-FR-AMC. After a initial screening at of 100μM, if more than 80% of enzyme inhibition is observed the IC50 value and the mechanism of enzyme inhibition are determined. We also aim to obtain crystal structures of complexes between the enzymes and the most potent inhibitors, to explain the SAR through molecular docking studies, to determine the inhibitory activity in vitro against the parasites and to evaluate their cytotoxicity.

## MOLECULAR MODELING STUDY AND PHARMACOPHORE MAPPING OF CYP51 ENZYME FOR DESIGN NEW LEISHMANICIDE AGENTS

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Leishmaniasis is a disease caused by protozoa parasites of the genus Leishmania, which are mainly transmitted to humans by the bite of infected sandflies, and have diverse clinical manifestations, from cutaneous lesions to the visceral disease. The chemotherapy is costly, have limited effectiveness, and cause severe adverse effects, which leads to patient withdrawing from treatment and the increase of parasitic resistance. This scenario makes urgent the development of new drugs affordable for the marginalized populations. Sterol 14a-demethylase (CYP51) is an excellent target to design new antiparasitic drugs because it is essential for sterol biosynthesis, one of the main parasite's membrane sterols. Therefore, the main goal of this work is to apply Computer-Assisted Drug Design (CADD) approaches to find new and selective CYP51 inhibitors, which could represent new potential leishmanicide agents. We used Ligand- and Structure-Based Drug Design (LBDD and SBDD, respectively) to generate and validate pharmacophore models of CYP51. The shape-based model showed the best internal and external evaluation metrics (Top 1% hits, AUC = 0.867; BEDROC = 0.635; Se = 0.608; 1-Sp = 0.409) when compered with structure-based models such as Docking-based and LigandScout. Therefore, the best pharmacophore model will be used to virtual screen of ChemBridge database to select and buy compounds to the experimental validation.

## MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF ALDOSE 1-EPIMERASE AND GLUCOSE 6-PHOSPHATE 1-EPIMERASE FROM TRYPANOSOMA CRUZI.

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In Trypanosoma cruzi, the 6 or 7 first glycolysis enzymes and the pentose phosphate pathway (PPP) are compartmentalized on the glycosomes. The Glycolisis aren't regulated on the classical checkpoints (HK and PFK). Glycosome enzymes have special features compared with the homologous in humans (unique kinetic properties, activity-regulation mechanisms and peroxisome-targeting signal). These are the basis for development of the specifics inhibitors. Glucose is the principal source of energy and carbons, but is unknown how its regulation is about to the a and b anomers. The Aldose 1-Epimerase and Glucose 6-phosphate 1-Epimerase are the enzymes that interconvert a and b anomers of the glucose and glucose 6-phosphate (G6P) respectively. We propose that these enzymes have the important role in the fate of a and b anomers of glucose and G6P (Glycolysis or PPP). For this purpose, it is proposed: will be cloned the genes for both enzymes, overexpressed and purified the recombinant enzymes, determined their kinetic properties (Km, Vmax, Kcat) and resolved their tridimensional structure through protein crystallography. With the native enzymes, will be determined the subcellular localization through: differential and isopicnic centrifugation, membrane permeabilization by digitonin and immunofluorescence. Further will be determined if the expression depends of the parasite form and the energy and carbon source.

## RUTHENIUM-CLOTRIMAZOLE COMPLEXES AS POTENT CHEMOTHERAPEUTIC AGENTS AGAINST LEISHMANIA MAJOR.

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Cutaneous leishmaniasis caused by the protozoan parasite Leishmania major (L. major) affects millions of people worldwide. Drug resistance parasite strains have emerged against current drugs available, and toxicity to the host and high cost of this drugs limits their wider application and use. Thus, there is an urgent need for the development of new chemotherapies against leishmaniasis. We reported a novel series of organometallic compounds, RuII complexed with clotrimazole (AM160 and AM162) displaying potent anti-leishmanial activity for both extracellular (LD50 14.6 nM and 400 nM respectively), and intracellular forms of L. major propagated in macrophages (IC70= 29.25 nM and IC40= 1 μM respectively) with imperceptible toxicity toward normal mammalian cells. Current in vivo experiments with AM162 showed that BALB/c mice infected with L. major, and treated with 6 mg/kg/day, decreased the footpad lesion by a 62% compared with vehicle control, with no significant toxicity. Furthermore, in this study we investigated the mode of action of AM162 in L. major promastigotes. We demonstrate that AM162 is able to induce a mitochondrial dependent apoptotic-like death in the parasite based on in situ labeling of DNA fragments and oligonucleosomal DNA fragmentation, mitochondrial depolarization, and plasma membrane phospholipid externalization. Therefore, this compound represents an excellent lead for the development of new chemotherapeutic agents to treat leishmaniasis.

## BACTERIAL CELLULOSE BIO-CURATIVES CONTAINING DETC FOR THE TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY LEISHMANIA BRAZILIENSIS

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Leishmaniasis remains a worldwide public health problem. The limited therapeutic options, drug toxicity and reports of resistance reinforce the need for the development of new treatment options. Herein, we tested a topical formulation of bacterial cellulose (BC) membranes containing Diethyldithiocarbamate (DETC), a superoxide dismutase 1 inhibitor. Exposure of leishmania-infected murine macrophages to BC-DETC resulted in a dose-dependent killing of intracellular parasites, without pronounced toxic effects to host cells. Parasite killing was associated with decreased SOD1 activity paralleled by the increased production of superoxide and pro-inflammatory mediators. Topical application of BC-DETC to dermal lesions significantly decreased ear thickness and parasite load at the infection site. Additionally, expression of IFN-y and TNF-a, was down modulated in situ as well as in recall responses employing draining lymph node cells. BC-DETC also decreased parasite load following exposure to human macrophages infected with L. braziliensis, an effect reversed in the presence of anti-oxidants. These results highlight the feasibility of using BC-DETC as a topical formulation for chemotherapy of cutaneous leishmaniasis caused by L. braziliensis. Key words: Cutaneous leishmaniasis, Ditiocarb, bacterial cellulose

## EFFECTS OF DISUBSTITUTED UREA COMPOUNDS IN TRYPANOSOMA CRUZI

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Certain disubstituted urea compounds activate mammalian heme-regulated inhibitor (HRI) kinase, inhibiting translation initiation due to the phosphorylation of the eukaryotic translation initiation factor alpha (eIF2a). Three compounds BTdCPU, I-m6, I-17 were used, as well as an inactive diarylurea (NCdCPU). The epimastigotes dose-response curves indicated an IC50 of 3 μM for I-17, 5 μM for BTdCPU and 10 μM for I-m6. We then tested the effect of these compounds on epimastigotes over-expressing wild-type eIF2a, or versions containing each one or both of the two possible phosphorylation sites replaced by alanine. The parasites expressing double mutant were more resistant to I-17, revealing the importance of eIF2a phosphorylation for the activity of this compound. We evaluated the morphology of eIF2a overexpressing parasites treated with I-17. While wild type eIF2a expressing cells presented a higher globular morphology (over 30%), parasites with double mutated eIF2a have no morphological change. We also observed that I-17 can interfere with cell cycle resulting in an increased G1 phase and in intracellular multiplication of amastigotes at 1-3μM with its cytotoxicity in mammalian cells being 10 times lower. Therefore, we concluded that HRI-activators could be used to inhibit growth of T. cruzi via eIF2a phosphorylation. The target eIF2a kinase remains to be elucidated. Research support: FAPESP and FAPERJ.

## PRODUCTION OF STATINS AND ANTIPARASITIC MOLECULES BY FUNGI ISOLATED FROM SUGAR CANE PLANTS OF BRAZIL USING SOLID STATE FERMENTATION

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Statins are the most effective cholesterol lowering agents for prevention of cardiovascular disease and, some of these can be produced through biological process including growth of fungi using solid state fermentation. We discovered that other molecules are produced in addition to statins and, our results have demonstrated that these have antiparasitc activity. The aim of this study was to investigate co-production of statins and antiparasitics by five strains of fungi isolated from sugar cane plants of Brazil. Solvent extracts of the fungi cultures were analyzed using HPLC and NMR, confirming statin production. These extracts were tested against Trypanosoma cruzi, which is the causal agent of the Chagas' disease. Two of the extracts produced an interesting effect against this parasite. The next steps in our research will be to purify the active compound(s) and to determinate biological effects in different stages of the life cycle of T. cruzi. Citotoxicity against mammalian cells will also be investigated. There is a lack of options to treat neglected disease, therefore it is important developing new avenues for lead compound discovery.

## IDENTIFICATION OF MOLECULES WITH SELECTIVE INHIBITORY ACTION AGAINST HSP90 OF PROTOZOA PARASITES: A THERMODYNAMIC AND COMPARATIVE APPROACH.

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Hsp90 chaperones are proteins involved in the folding, stabilization and activation of different client proteins. They are also involved in the regulation of gene expression and signaling events. Co-chaperones proteins assist Hsp90 in the folding process by directing its structural transitions and promoting their interaction with the client protein. In the case of intracellular parasites the role of Hsp90 and co-chaperones goes beyond maintaining the protein homeostasis, once they are also involved in processes related to the development and pathogenesis of these organisms. The ability of Hsp90 to affect important cellular transformations is very exploited by Leishmania and Plasmodium parasites. These organisms use the Hsp90 to trigger transitions among its various life stages. Therefore the Hsp90 are potential targets for drug development against these parasites, since its inhibition affects different protozoa signaling pathways. Despite this, the high similarity between Hsp90 belonging to parasites and to human, arises as a point of attention in this approach. Unlike Hsp90, many co-chaperones share low sequence homology with the corresponding homologues in humans, appearing as suitable targets for the Hsp90 modulation. Thus, the aim of this study is to identify molecules capable of modulating the activity of Hsp90 from Leishmania and Plasmodium, without affecting the activity of the human protein.

## A SERIES OF TETRADENTATE POLYAMINES HAVE POTENTIAL ACTIVITY IN VITRO AND IN VIVO AS INHIBITORS OF SEVERAL SPECIES OF TRYPANOSOMATIDS

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A series of tetraamine-based compounds was prepared and their trypanocidal effects against T. cruzi, T.b. brucei and Leishmania sp. were evaluated. Cytotoxicity was also determined against mammalian cells. High-selectivity indexes observed in vitro were the basis of promoting the tested compounds to in vivo assays (in BALB/c mice) in the case of T.cruzi, in which the parasitaemia levels were quantified by fresh blood examination; the assignment of a cure was determined by PCR and reactivation of blood parasitaemia levels after immunosuppression. In vitro screening showed that Comp 2 has an IC50 of 40 nM, 1.2 μM, and 1.3 μM against T.b.brucei, T.cruzi and L. infantum, respectively; with selectivity index of 2430, 65 and 75, respectively. While Comp 3 showed IC50 1.3 μM against L. donovani; with selectivity index of 105. The tests on the murine model for the acute phase of Chagas disease showed that Comp 2 and 3 induced a remarkable decrease in the reactivation of parasitaemia after immunosuppression and curative rates of 33 and 50%, respectively. The mechanisms of action were elucidated at metabolic, ultra-structural, and antioxidant levels. Comp 3 turned out to be a great inhibitor of Fe-SOD and TR. The high anti-parasitic activity and low toxicity render allow us to point these tetraamines as interesting molecules for the development of affordable anti-kinetoplastids agents.

## LEISHMANIA PROMASTIGOTES INDUCE CYTOKINE SECRETION IN MACROPHAGES THROUGH THE DEGRADATION OF SYNAPTOTAGMIN XI

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Leishmania causes debilitating diseases found worldwide. After inoculation with promastigotes, macrophages ingest promastigotes and these form parasitophorous vacuoles (PV). The GP63 protease is a pathogenicity factor that enables promastigotes to subvert macrophage biology. Moreover, lipophosphoglycan (LPG) promotes parasite survival in PVs. In macrophages, Synaptotagmins (Syts) regulate exocytosis and phagocytosis. We discovered that Syt XI dampens TNF and IL-6 secretion. In this research, we show that Syt XI is directly degraded by GP63 and excluded from PVs via LPG. Furthermore, infected macrophages released TNF and IL-6 in a GP63-dependent fashion. To demonstrate that this release depended on Syt XI degradation, siRNA knockdown of Syt XI before infection revealed that the effects of siRNA knockdown and GP63 action were not cumulative. Interestingly, injection of GP63-expressing parasites into mice also led to increased TNF and IL-6 secretion and to augmented influx of neutrophils and inflammatory monocytes to the infection site. In sum, our data show that Leishmania induces cytokine release via GP63-mediated degradation of Syt XI. Increased neutrophil and monocyte infiltration may help Leishmania propagate, since both cell types are infection targets. The trafficking of GP63 and LPG is being investigated. This work will improve current understanding of how Leishmania deregulates phagocyte biology.

**Happyness Jeremia**

## CURRENT EPIDEMIOLOGY OF BACROFTIAN FILARIASIS AND CLINICAL DISEASE PRESENTATION IN RURAL AND URBAN SETTINGS DURING ELIMINATION PROCESS IN TANZANIA

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Lymphatic filariasis is a disabling and disfiguring disease caused by the filarial nematode Wuchereria bancrofti and Brugia. Transmission is facilitated through anopheline and culicine mosquitoes. The disease is a wide spread and a major public health problem in the tropics, where it's almost neglected. It is estimated to affect about 120 million people yet only 7% develop lymphoedema and 50% develop hydrocele causing severe morbidity. Despite several studies showing some immunological and environmental risk factors for developing the disease (lymphoedema and hydrocele) still there is no clarity on the pathogenesis of the disease and whether, parasite genetic variability and immune genetical aspect has a role in the clinical epidemiological presentation of the pathology. On the other hand, of recent, two observations provided potential important clues; first; Single Nuclear Polymorphisms in the genes associated with lymphatic filariasis pathologies. Some are associated with more than one disease state. Second; Signs and symptoms of lymphatic filariasis such as swelling of limbs, breast and the genitals, are due to lymphatic filariasis pathologies targeting the lymphatic vessels whose development is controlled by angiogenic factors. Broadly therefore we are aiming at testing whether hydrocele and lymphoedema are associated with SNPs in the Matrix metalloprotease 2 and CAECAM-1 genes in persons infected with filarial worms (2) Vascular Endothelial Growth Factors that target the lymphatic vessels whose development is controlled by angiogenic factors (3) Parasite genetic variability in human filarial disease could have implications in the epidemiology and control. These findings may be useful in utilizing a candidate gene approach to identify the cause of different disease manifestations in lymphatic filariasis particularly in patient with pathology.

## THE NOVEL MOLECULE 3-CHLORO-6-METHOXY-2-(METHYLSULFONYL) QUINOXALINE KILLS TRYPANOSOMES BY A MIXED CELL DEATH PATHWAY

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Tropical Neglected Diseases (TNDs) are a group of severe disabling conditions characterized by their social and economic impacts. Despite their important morbidity and mortality rates, historically TNDs have not been subject of appropriate studies in the search and development of new drugs. Among the TNDs, the parasitic diseases caused by protozoa belonging to order Kinetoplastida are known by affecting millions of people all over the world. Based on that, our research group has worked on the search of new drugs for the treatment of Trypanosomiasis. After in vitro screenings of libraries of compounds, we found a quinoxaline derivative (3-chloro-6-methoxy-2-(methylsulfonyl) quinoxaline) selectively active against parasitical forms of Trypanosoma cruzi (IC50: 1.1 μM) and T. brucei (IC50: 1.6 μM). The molecule is also promising because of its safety against erythrocytes and kidney cells at tested concentrations, and the synergistic inhibitory effect against T. cruzi when used together with Benznidazole. Regarding the mechanism of action of the molecule, the ultrasctructural and biochemical alterations indicated a mixed profile of apoptosis and autophagy, including DNA fragmentation, mitochondrial depolarization, phosphatidylserine exposure and increase in autophagic vacuoles. Further, we plan to go deeper into the mechanism of action and investigate the in vivo activity against T. cruzi infected mices.

## THE SPLENOCYTE CELLULAR IMMUNE RESPONSES FROM CHRONIC MICE AFTER STIMULATION WITH TRYPANOSOMA CRUZI VESICLES

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Trypanosoma cruzi, the etiologic agent of Chagas disease, releases vesicles containing a wide range of surface molecules including GPI-anchored glycoconjugates and are enriched with trans-sialidase (TS)/ gp85 glycoproteins and other alpha-galactosyl (∂-Gal)-containing glycoconjugates, like mucins. Trocoli Torrecilhas et al. (2009) demonstrated that vesicles released by trypomastigote forms modulated innate immune responses in murine macrophages (TNF-∂, IL-12 and NO) and increase amastigote nests (heart tissue). Our group demonstrated that T. cruzi vesicles are potent agonists of TLR2 in murine macrophages having an important role during the initial steps of infection especially in the strains Yu-Yu and CL-14. Also, in vitro experiments showed that YuYu strain of T. cruzi release more vesicles than the Y strain. The aim of this study was evaluate the impact of two T. cruzi strain vesicles on the intracellular cytokine profiles during of acute and chronic phase. In conclusion, vesicles differ in their pattern of liberation and composition and increase infection in the host cell.

## AFRICAN TRYPANOSOMES VARY THEIR MOTILITY BEHAVIOUR DEPENDING ON THE CONDITIONS OF CULTURE ENVIRONMENT

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African trypanosomes are able to survive in harsh conditions in the vertebrate host, despite constant attacks by host antibodies. These extracellular hemoprotozoa evade immune response through antigenic variation and incessant motility. Their motility patterns are highly variable in different environments. Findings of this study proved that variations in swimming patterns, speeds and behaviour of trypanosomes depended on their swimming environment. High-speed video microscopy was used to quantify motility in Trypanosoma congolense, rodent-adapted T. vivax, T. brucei brucei and T. evansi cultivated in mouse, rat, rabbit and sheep. The proportions of persistent, intermediate, and tumbling swimming patterns varied within and between trypanosome species and in different hosts. In mouse blood, only T. evansi displayed continuous backward swimming and this helped in maneuvering through the obstacles such as blood cells. In addition, T. vivax swam faster than T. congolense in mouse and sheep blood. We hypothesize that differences in swimming speed influences parasite survival in the host and that T. vivax (the fastest swimmer) experiences greater hydrodynamic drag forces thus should clear the host-derived surface antibodies more efficiently than T. congolense. The average swimming speed of T. congolense was higher in mouse than sheep blood, unlike in T. vivax whose average speeds were similar in both hosts ($p=0.5874$). Notably, these variations in motility behaviour exhibited by the same isolate in different vertebrate hosts suggest adaptation to survival in different mammalian hosts.

## TC95, A DRUG CANDIDATE AGAINST LEISHMANIA SP.: ANTIPROLIFERATIVE EFFECTS AND MECHANISMS OF ACTION.

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The parasites of the Leishmania genus cause leishmaniasis, a disease with a large spectrum of clinical manifestations. .The current chemotherapy is based on antimonials, amphotericin B and pentamidine. However, there is an urgent need for new therapeutic regimens that are safer, accessible and more efficacious. An interesting approach in drug development is the combination of different inhibitors with known activity against the parasites. Thus, the aim of this work was to study the effects of TC95, a hybrid molecule between trifluralin and miltefosine, in different species of Leishmania. The antiproliferative effects showed that TC95 has a high leishmanicidal activity against L. amazonensis, L. mexicana, L. guyanenesis, L. brasiliensis, L. donovani and L. infantum, with just 24h of treatment. Studies of mechanisms of action in L. amazonensis demonstrated that TC95 induced an increase in ROS production, loss of mitochondrial membrane potential, and reduction in ATP production. Transmission electron microscopy confirmed these effects showing dramatic lesions in the mitochondrial ultrastructure. In axenic amastigotes isolated from murine lesions, TC95 also presented a potent effect with IC50 value of 200nM. Taken together, these results indicate that TC95 affect important cell targets during the treatment and is a potential compound against Leishmania sp. Keyword: chemotherapy, Leishmania, hybrid molecule

## MOLECULAR CHARACTERIZATION OF NATURAL POPULATIONS OF TRYPANOSOMA CRUZI IN CHAGAS DISEASE PATIENTS ENROLLED IN CLINICAL TRIALS WITH ANTIPARASITIC DRUGS

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The natural populations of T. cruzi are composed of multiple clones from six discrete typing units (DTUs). The role that this genetic diversity plays in clinical, epidemiologic and therapeutic scenarios remains unknown. Our research project aims to analyze the genetic polymorphism of parasite populations in Chagas disease patients enrolled in clinical trials with antiparasitic drugs. We have initiated this study in the context of the E1224 (a pro-drug of ravuconazole) clinical trial by means of Satellite DNA sequencing and PCR-RFLP analysis of kinetoplastid DNA. At baseline, 31% of samples showed SatDNA type II sequences (present in DTUs II/III) and 69% type I/II (present in DTUs V/VI); whereas after treatment, 12.8% of samples showed SatDNA type II and 87.2% type I/II sequences. The mean of Jaccard distances of kDNA signatures for placebo and the E1224 branches were around 0.45, whereas it was 0.82 for the single refractory case of the group treated with benznidazole. These findings indicate that the genetic variability of T. cruzi bloodstream populations during follow-up reflects the natural fluctuation of multiclonal parasite populations during chronic Chagas disease, except for the refractory case to benznidazole which could be due to drug selective pressure and/or acquired resistance. Further analysis is currently ongoing with samples from CHAGASAZOL and TRAENA trials to deepen insight on this matter.

## EVALUATION OF THE IN VITRO LEISHMANICIDAL ACTIVITY OF PLANT EXTRACTS AGAINST THE PARASITE LEISHMANIA AMAZONENSIS

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Leishmaniasis is a parasitic disease potentially fatal caused by distinct species of the protozoan Leishmania sp. Due to high toxicity of drugs used in treatment there is a increasable intensification in the research for antiparasitic products from natural resources, especially from plants. Thus, the goal of this work is to evaluate leishmanicidal activity of thirtheen hexane vegetable extracts against the parasite Leishmania amazonensis. Of the thirtheen extracts evaluated against promastigotes, Bidens sulphurea (1) and Plectranthus neochilus (2) presented activity, showed an IC50 (Inhibitory Concentration of 50% of parasites) 84,81 and 43,66 µg/mL at 24 hours and 42,19 and 38,86 µg/mL in 48 hours, respectively. Regarding the cytotoxic activity against peritoneal macrophages, 1 showed CC50 (Cytotoxic Concentration of 50% of the cells) values of 41,57 and 104 µg/mL at 24 and 48 hours, respectively, and 2 values of 46,32 and 26,18 µg/mL at 24 and 48 hours respectively. The HC50 (Hemolisys Concentration of 50%) presented values of 1 and 2 of 246 and 239 µg/mL, respectively. Scanning electron microscopy showed that the extract 1 not cause morphological changes in promastigotes and 2 caused reduced cell body in the concentrations of IC50. Thus, studies using in vivo model for toxicity testing and for the treatment of leishmaniasis may be performed to verify the effectiveness of the above plant extracts.

## 2D LC SYSTEM FOR ON-FLOW LIGANDS SCREENING BY ZONA BIOCHROMATOGRAPHY APPROACH FOR NUCLEOSIDE DIPHOSPHATE KINASE B FROM LEISHMANIA MAJOR

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Nucleoside Diphosphate Kinase b (NDKb) modulate levels of NTPs and deoxynucleotides in cells, the NDK utilizes extracellular ATP as its principal substrate, which by binding to purinergic receptors to trigger the death of immune cells this enzyme underlies the virulence of parasites like Leishmania, and suppresses the host immune response. Furthermore Leishmania are auxotrophic for purines and the NDK can be used as pathway salvage of purines. Therefore, NDK is a potential biological target for selective ligands screening, and it is requires the tool development for identification of these molecules. In particular, the affinity chromatography on HPLC-immobilized capillary enzyme reactor (ICERs) have been successfully applied in interaction ligands-target studies, for identification of inhibitors as potential drug, and also employed in the mechanism studies. Herein we describe of the a two-dimensional, where an ICER of LmNDKb - prepared by the Schiff method approach with glutaraldehyde as spacer - was used in the first dimension of a 2D LC system, with UV as the detector. The developed method allowed the characterization of the ICER-LmNDKb. The Kmap value for ATP was 792,2 μM comparable with values for free enzyme (500 μM) while for GDP the Kmap value was of 500.37 μM, and the KI value for GDP excess was 1.54 mM. This innovative approach is an automated screening for identifying antileishmanial hits ligands.

## ROLE OF REDOX-REGULATED INTRINSICALLY DISORDERED CHAPERONE HSP33 IN THE OXIDATIVE STRESS DEFENSE SYSTEM OF TRYPANOSOMA BRUCEI

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Protection against oxidative stress is highly interrelated with the function of the ancient cellular defense system, the network of molecular chaperones.
Hsp33 is a conserved redox regulated molecule which belongs to a recently discovered group of intrinsically disordered molecular chaperones that in bacteria serve as the first line of defense and specifically protects against oxidative insults.
Since bloodstream parasites need to cope with extreme temperature shifts and high levels of oxidants produced by the innate immune system, parasite- specific antioxidant chaperones seem like a highly promising key proteins in parasite defense and life cycle regulation. RNAi preliminary experiments show that silencing of T. brucei-Hsp33 causes both a heat shock and a peroxide-sensitive phenotype. Moreover, luciferase thermally-induced aggregation test confirmed its powerful chaperone activity, induced by oxidation. These results strongly suggests that the T. brucei-Hsp33 is a redox- regulated chaperone, which has crucial antioxidant importance in the trypanosomes.
Based on these results, we would like to assess the impact of T. brucei-Hsp33 on redox homeostasis and oxidative stress defense of the parasite. The ultimate goal is better understanding of the mechanisms of parasite survival in host and translating this knowledge into possibility to exploit T. brucei-Hsp33 as a drug target.

## NOVEL COMPOUNDS ISOLATED FROM CVP005B CRUDE EXTRACT SHOW STRONG ANTI-TRYPANOSOMAL ACTIVITY IN VITRO

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Human African Trypanosomiasis is a devastating disease in Africa. Due to toxicity and parasite resistance issues, development of novel chemotherapy is urgently needed. Africa has a long history on the use of medicinal plants but the scientific basis of activity as well as active compounds within these plants remains unknown. Our study therefore aims at screening selected Ghanaian medicinal plants for trypanocidal activity and identification of active compounds.
High-throughput screening (HTS) of 113 Ghanaian medicinal plants led to the isolation of 3 novel compounds, “ML-2-2”, “ML-2-3” and “ML-F52” from one of the plant extracts, CVP005B. Nexin assay with HT FACS revealed ML-2-3 and ML-F52 to induce apoptosis in T. b. brucei (GUTat3.1 strain), whereas ML-2-2 did not. Cell cycle assay with FACS revealed an alteration in the G2/M phase in ML-2-3-treated parasites. Immunohistochemical analysis of compound-treated trypanosomes showed nuclei fragmentation in ML-F52- and ML-2-3-treated parasites, a feature of apoptosis. ML-2-3 and ML-F52, as shown by western blot analysis, suppressed flagella protein, PFR-a, in the parasites, while ML-2-2 did not. Time course experiments show PFR-a suppression and cell cycle arrest to precede apoptosis induction, which suggests that PFR-a suppression could lead to apoptosis via cell cycle arrest. Further mechanistic and in-vivo efficacy studies of compounds are ongoing.

## ANTILEISHMANIAL AND CYTOTOXICITY ACTIVITIES OF THREE ANNONACEAE PLANTS FROM CAMEROON

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Leishmaniasisis among the deadliest neglected infectious diseases in the world. Leishmania parasites are increasingly resistance to available drugs, highlighting the urgent need for new drugs with improved efficacy. Within this scope, we have investigated extracts from three plants of the Annonaceae family (A. muricata, A. reticulata and A. senegalensis) for activity against promastigotes and intracellular amastigotes forms of L. major and L. donovani in culture. Cytotoxicity of extracts was assessed against J774.2 macrophages. Extracts and fractions were prepared using organic solvents, and screened at serially diluted concentrations in vitro for activity. Results showed antileishmanial activity for all extracts with IC50 values ranging from 0.12µg/ml to 272.32µg/ml. L. donovani showed to be more sensitive than L. major. Extracts showed cytotoxic activity with CC50 values ranging from 0.1 to 40.88µg/ml. Overall, plants extracts potently inhibited the parasites, but also showed significant cytotoxicity against J774.2 macrophages. However, the observed activities indicated that the investigated extracts contain secondary metabolites with potential interest. Future fractionation of active extracts coupled with biological screening will enable us to shed light into the likely progression of the purified active ingredients.
Keywords: Leishmania, Annonaceae, plants extracts, activity, cytotoxicity

## RANKING OPTIMIZATION IN VIRTUAL SCREENING USING PHYSICAL-CHEMICAL AND STRUCTURAL DESCRIPTORS OF THE NANO-ENVIRONMENT FOR PROTEIN-LIGAND INTERACTIONS

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During the last two decades, virtual screening (VS) has become an important tool for rational design of drugs and agrochemicals. However, despite of many successful cases VS approaches still have some limitations, especially regarding the ranking of potential ligands according to their binding affinity. In order to create models that can improve the ranking phase in VS campaigns, we propose an in silico approach to identify features that are important and essential to the molecular recognition process. Relying on nonparametric machine learning methods, the proposed approach involves analyzing the protein-ligand interaction nano-environment (characterized by physical-chemical and structural descriptors from both ligand and binding pocket residues) of experimentally determined structures. More precisely, we use a characterization of the protein-ligand interaction nano-environment space based upon descriptors provided mainly by the STING_DB and STING_RDB databases (developed by the Embrapa's Computational Biology Research Group). Given the success of similar approaches designed by our research group for other biological problems (protein-protein interface prediction, identification of catalytic site residues), and also by considering our preliminary results, we hope that it will be possible to derive models that can reliably and efficiently predict the affinity of small chemical compounds for protein targets.

## SEARCH FOR MOLECULAR TARGETS FOR THERAPEUTIC CONTROL OF INFECTION BY TRYPANOSOMATIDS

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Diseases caused by trypanosomatids as leishmaniasis, Chagas disease and sleeping sickness affects more than 1 billion people worldwide. Treatments for these diseases are perfomed by a limited drug set and with unsatisfactory characteristics due to potential toxicity, high cost, variable efficacy, and frequent occurrence of resistant strains. The synthetic route of trypanothione has potential candidates for therapeutic targets, due to specificity and the essentiality for trypanosomatids. Furthermore, this is key molecule to infection, pathogenesis and disease development. It was shown that trypanothione synthesis involves two distinct enzymes: glutathionylspermidine synthetase and trypanothione synthetase. This study aimed to identify conserved amino acids in the catalytic sites from these enzymes among trypanosomatid species and perform virtual screening of potential inhibitors . Initially, comparative protein sequences and structures will be performed through sequence and structure alignments using ClustalX and PyMOL programs, respectively. Computational screening by docking analysis will allow identification of compounds binding to conserved amino acids and the best molecules will be tested to block recombinant proteins. Experimental validation of therapeutic potential of compounds will also be carried out to control in vitro and in vivo infection by T. cruzi and Leishmania.

## MODULATION OF TRYPANOSOMA BRUCEI ORC1/CDC6 BY ATP BINDING AND HYDROLYSIS

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As in other eukaryotes, DNA replication in T. brucei starts with the licensing of a pre-replication machinery (pre-RC) in specific regions of DNA called replication origin. The dynamics and the proteins involved in pre-RC assembly are not well established in these parasites. Our group has demonstrated that the protein TbOrc1/Cdc6, which is responsible for the recognition and activation of replication origins in T. brucei, binds and hydrolyzes ATP in vitro. Our purpose is to evaluate the importance of these in the assembly and stability of pre-RC. To check this, we analyzed the primary sequence of T. brucei Orc1/Cdc6 and replaced (by PCR overlap) one aminoacid at domain important for ATP binding (TbOrc1/Cdc66K79T) and two aminoacids at region essential for ATPase activity (TbOrc1/Cdc6R251,252E). Plasmids containing these mutated genes were transfected into T. brucei procyclic cells and, as expected, cells expressing TbOrc1/Cdc6R251,252E reduced the ATPase activity as well as TbOrc1/Cdc66K79T lost their ability to interact with ATP. Furthermore, these cells showed problems in cell proliferation resulting in an inefficient loading of MCM onto DNA and alteration in the cell cycle progression. These data suggest that TbOrc1/Cdc6 needs to hydrolyze ATP to recruit MCM. This knowledge may assist future antiparasitary strategies, since DNA replication is related directly to chromosome maintenance and cell viability.

## UNLOCKING THE ANTILEISHMANIAL POTENTIAL OF HALOPHYTE PLANTS FROM THE ALGARVE COAST, PORTUGAL

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Human leishmaniasis (HL) caused by Leishmania infantum is a severe public health problem in the Mediterranean region, where dogs are the primary reservoirs and humans act as accidental hosts of the disease. In the last decades, plants have been pointed out as a promising alternative to current available drugs for HL treatment, as sources of natural antileishmanial compounds. Halophytes biosynthesize several bioactive compounds in response to the biotic and abiotic stresses characteristic of their habitat (e.g. salt marshes). They also display important bioactivities, such as antioxidant and anti-tumoural. Their potential as sources of natural products with antileishmanial activity, however, remains unknown. In this study, dichloromethane (DCM) and acetone extracts were prepared from the leaves of 26 species of halophytes common in the Algarve area, and tested in vitro against L. infantum promastigotes and THP-1 derived macrophages. DCM extracts from Inula chritmoides and Spergularia rubra were the most active against L. infantum (IC50, = 59.5 and 70.5 µg/ml), with moderate toxicity against macrophages (IC50, 20.3 and 49.3 µg/ml). The activity of these extracts on intracellular amastigotes of L. infantum, and their chemical characterization are in course.

## ACTIONS OF FLAVONOIDS TRANS CHALCONE, HESPERIDIN METHYL-CHALCONE AND QUERCETIN IN EXPERIMENTAL LEISHMANIASIS

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The fact that drugs currently used in the treatment of Leishmania spp. are highly toxic and associated with acquired resistance has promoted the search for new therapies for treating American tegumentary leishmaniasis. A number of studies have already demonstrated the effect of flavonoids in experimental leishmaniasis through the direct action on crucial targets for the parasite survival, modulating the immune response and the subsequent tissue damage. Based on experimental evidences, we have been evaluating the leishmanicidal and /or anti-inflammatory activity of trans-chalcone, hesperidin methyl-chalcone and quercetin in experimental leishmaniasis. Our in vitro results showed that L. braziliensis-infected macrophages treated with quercetin did not modulate the cytokine or nitric oxide profile, however quercetin exerted its antileishmanial effects by its capacity to activate antioxidant responses, followed by modulation the labile iron pool, culminating in depletion of available iron for L. braziliensis replication. Hesperidin methyl-chalcone and trans-chalcone in vitro results also showed direct effect against L. amazonensis promastigote forms. Preliminary results of in vivo experiments demonstrated that L. amazonensis-infected BALB/c mice treated with quercetin microencapsulated (100mg/kg, per oral) during the initial stage of infection (10 days) were able to delay the leishmaniotic lesion appearance.

## PHYTOCHEMICAL, ANTILEISHMANIAL AND CYTOTOXIC EVALUATION OF ARTEMISIA SCOPARIA AND FRAXINUS XANTHOXYLOIDES, COLLECTED FROM DISTRICT ISLAMABAD

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In this research project, we investigated phytochemical, antileishmanial and cytotoxic activity of Artemisia scoparia whole plant and Fraxinus xanthoxyloides leaves crude methanolic extracts and derived fractions. To identify the presence of various phytochemical classes, qualitative tests were performed. Antileishmnaial activity was performed against Leishmanai tropica promastigote stage parasite and cytotoxicity was assessed against brine shrimps. Our results showed the presence of terpenoids, flavonoids, coumarins, tannins, quinones and saponins in A. scoparia and terpenoids, coumarins, flavonoids, tannins, quinones in F. xanthoxyloides in crude methanol extracts. Variations in phytochemicals were observed in derived fractions with change of polarity. In antileishmanial activity, chloroform fractions of both tested plants exhibited high activity with IC50 values for A. scoparia =18.42±1.6 and F. xanthoxyloides =15.23±0.9 µg/mL against Leishmania tropica promastigotes. As far as cytotoxic activity is concerned, potent activity was observed in the chloroform fraction of A. scoparia with LD50 value of 4.315±0.8 µg/mL, when compared to other plant. On the basis of above mentioned results we can conclude that, chloroform fractions of A. scoparia and F. xanthoxyloides are potential source antileishmanial and cytotoxic activities.

## NATURAL PLANT BASED-EXTRACTS, A POTENTIAL SOURCE TO FIGHT CUTANEOUS LEISHMANIASIS

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Leishmaniases are neglected protozoan diseases that are worldwide, including Tunisia, They constitute major public health problems; novel drugs, less toxic and costly treatments remain research priorities. Medicinal plants have evolved to overcome competitive disadvantage by producing diverse secondary metabolites that are valuable for screening biological activities. In this context, we have conducted a comparative biochemical study on secondary metabolites of nine medicinal plants growing in Tunisia. We evaluated the antioxidant and antileishmanial activities of their aqueous and ethanol extracts.
The total polyphenols, flavonoids and condensed tannins contents were determined as well the antioxidant capacities. Thereafter, the antileishmanial activity was determined on cultured Leishmania major promastigotes. Our results showed that secondary metabolites rates vary between species and extracts. The polyphenols in the ethanol extracts were found to be significantly higher than in the aqueous extracts. Furthermore, many extracts exhibited potent antioxidant activities. Among 16 tested extracts, only two extracts are proven strongly active against L.major promastigotes. These active extracts and those ineffective against promastigotes will be further screened against the intracellular amastigotes. Along with above findings, we suggest that the examined extracts could carry potential compounds for the development of novel drugs against cutaneous Leishmaniasis.

## ROLE OF BCL-2 FAMILY PROTEINS DURING LEISHMANIA DONOVANI INFECTION

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Immunity to parasites requires induction and maintenance of robust innate and adaptive immune responses by the host. Leishmania donovani, the causative agent of Human Visceral Leishmaniasis evades many of such defense mechanisms to survive and persist in the host. Here we show a novel mechanism through which L. donovani insures not only enhanced viability of the macrophages it infects, but also results into attenuation of host mechanisms targeted at killing this parasite. L. donovani brings about significant changes in the expression profile of the Bcl-2 family member proteins in infected macrophages. These changes were partially mediated through TLR-2 and PI3K signal transduction pathways. Among Bcl-2 family members we propose a special role for Bcl-2 protein during early stages of infection and demonstrate the ways in which the parasite utilizes this molecule to its own advantage. Gene-specific knockdown and inhibitor-specific approaches further identify this molecule as a potential therapeutic target.

## NEW USE OF OLD DRUG: IMIPRAMINE AND LIPOSOMAL IMIPRAMINE IS AN EFFECTIVE DRUG AGAINST BOTH ANTIMONY SENSITIVE AND RESISTANT LEISHMANIA DONOVANI CLINICAL ISOLATES IN EXPERIMENTAL INFECTION

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In an endeavor to find an orally active & affordable antileishmanial drug against antimony resistant (SbR) Leishmania donovani (LD), we tested the efficacy of a cationic amphiphilic drug, imipramine (IMI), commonly used for the treatment of depression in humans. IMI is highly active against antimony sensitive (SbS) & SbR LD in both promastigotes & intracellular amastigotes & in LD infected hamster as well as mouse model. The drug decreases the mitochondrial transmembrane potential of LD promastigotes & purified amastigotes & induces apoptosis faster compared to the known drug miltefosine. The drug restores defective antigen presenting ability of the parasitized macrophages. The status of the host protective factors TNF-∂, IFN-ß & iNOS activity was increased & decrease in IL-10 & TGF-ß level in IMI treated SbRLD infected hamsters & mouse with the evolution of matured sterile hepatic granuloma. The 10-day therapeutic window shows about 90% clearance of organ parasites regardless of their Sb sensitivity. The liposomal formulation of IMI with squalene & phosphatidylcholine is more potent antileishmanial candidate than IMI alone. We observed a drastic decrease of IMI dose in this new formulation to clear ~100% organ parasite load in smaller treatment regimen compared to the oral delivery. This clearance is associated with skewing of Th2 immune response in infected state to Th1 after treatment.

## IN VITRO ANTILEISHMANIAL DRUG SUSCEPTIBILITY OF CLINICAL ISOLATES FROM PATIENTS WITH INDIAN VISCERAL LEISHMANIASIS STATUS OF NEWLY INTRODUCED DRUGS

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Regional variations in susceptibility of Leishmania donovani clinical isolates have been reported to antimonials but not to other antileishmanial drugs. Therefore, evaluation of the susceptibility of other antileishmanial drugs in clinical use is necessary in clinical isolates. In this study, 28 clinical isolates from endemic and non-endemic regions were evaluated in the J774A.1 macrophage cell line. Effective dose for 90% killing (ED90) values were significantly higher for miltefosine (P = 0.005) and paromomycin (P = 0.02) in isolates from the high endemic region, although there were no significant differences between ED50 values for paromomycin, miltefosine, and amphotericin B in the non-endemic versus endemic region isolates. This report shows higher ED90 values for miltefosine and paromomycin for the first time indicating susceptibility difference between regions for these antileishmanial drugs by the parasite, and stress on the fact that their use should be carefully monitored through directly observed therapy or multidrug treatment to preserve their efficacy for longer periods.

## RATIONAL DISCOVERY OF DRUGS FOR THE TREATMENT OF LEISHMANIASIS & CHAGAS' DISEASE

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Chagas' disease and leishmaniasis are two neglected diseases of high prevalence and impact on public health. The search for new drugs is a need as prevention and control tools remain limited. The aim of this project is searching for new active compounds against Trypanosoma cruzi and Leishmania. A sequential screening comprising computer-aided modeling, in vitro and in vivo assays was followed. Two data bases resulting from HTS against L. major promastigotes and T. cruzi trypomastigotes were modeled using molecular descriptors implemented in DRAGON and ISIDA softwares. Accuracy rates about 90% and false-positive rates under 10% were achieved in external data sets. Virtual search for potential active molecules is being focused on licensed drugs for other indications. Near 40 compounds with high classification scores have also been previously reported as leishmanicides, a fact that supports the predictability of the models. From a sample of the best classified molecules, five resulted parasiticide against L. amazonensis promastigotes at concentrations ≤5 μg/mL, and two of them inhibited lesion growth and reduced parasite loads in mice infected with L. amazonensis. In vitro and in vivo assays for T. cruzi are pending, but a list of potential inhibitors has already been proposed. Cross-activity of new molecules will also be explored.

## IN VITRO AND IN VIVO ACTIVITY OF A PALLADIUM COMPOUND ON LEISHMANIA INFANTUM

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Leishmaniasis is a widespread disease that affects 12 million people around the world with about 1-2 million estimated new cases occurring every year1. The clinical manifestations range from simple cutaneous lesion to progressive disseminated visceral, which can be fatal if left untreated2. There are small number of effective drugs available for treatment of leishmaniasis, and all of them present several problems including high toxicity and low efficacy due to resistant parasites3, 4. Thus, searching for new drugs with high and specific antileishmanial activity is very important, especially in developing countries, where these parasitic diseases constitute a serious public health problem. From this perspective, the objective of our work is to evaluate in vitro and in vivo the antileishmanial potential of a series of palladium derivatives against Leishmania infantum, and investigate the mechanism of action of the most promising compound through proteomic analysis. Preliminary results showed that compound 2 presented antileishmanial activity with the IC50= 4.0 μM for promastigotes forms and low cytotoxicity with a selectivity index of 126.1. For intracellular amastigotes, the compound presents IC50= 5.3 μM with a selectivity index value of 95.4. We are currently carrying out in vivo treatment to determine the efficacy of this compound using hamster as animal model.

## AMITRIPTYLINE AND CYCLOBENZAPRINE ARE EFFECTIVE IN VITRO ANTILESHMANIALS AND SUPPRESS PRO-INFLAMMATORY CYTOKINES

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Based in the drug repurposing approach, two chemically related drugs, amitriptyline (AMT) and cyclobenzaprine (CBP) have been clinically used in the treatment of depression and as a skeletal muscle relaxant, respectively. With the aim to identify new therapeutic alternatives for Visceral Leishmaniasis (VL), this study was carried out to investigate the anti-L. infantum activity and their possible activation of the cellular immune response. Both drugs exerted a leishmanicidal effect, with IC50 values of 21 (CBP) and 8,7 μM (AMT) against promastigotes and 112 (CBP) and 38 μM (AMT) against amastigotes. No toxicity was observed for NCTC cells. By using flow cytometry, AMT incubated with infected macrophages induced suppression of TNF, IFN-y, MCP-1, IL-10, IL-6, but when co-cultivated with lymphocytes, a high production of IFN-y was observed. CPB demonstrated a similar effect, but when co-cultivated with lymphocytes did not elicit production of IFN-y. In addition, AMP and CBP induced no NO production. Considering the structural differences between AMT and CBP, it is clear that the presence of a double bound in the aromatic ring of CBP conferred a reduce effectiveness. The leishmanicidal effect promoted by both drugs was independent of NO production, also high levels of IL-6 and IL-10 are correlated with an elevated parasite burden. So, our results suggest that AMT reduced the parasite burden by suppressing IL-6 and IL-10, with an increase of IFN-y. Minor structural differences between AMT and CBP could be responsible for the differences in the activity. AMT could be a candidate for future preclinical studies. Supported by FAPESP 2013/07275-5.

## MOLECULAR EPIDEMIOLOGY OF TRYPANOSOMA CRUZI AND ITS CORRELATION WITH IMMUNOLOGICAL RESPONSE (OR INFLAMMATORY MARKERS) OF CHRONIC CHAGASIC PATIENTS IN PARANÁ STATE

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Molecular epidemiology of T. cruzi have tried to correlate parasites genotype with epidemiology and clinical manifestations of Chagas Disease (CD) and to demonstrated the effect of genetic variation on host immune response. Thereby, Pentraxin 3 (PTX3) a multifunctional soluble pattern recognition molecule of innate immune response is considered a new inflammatory marker. We aimed to investigate the molecular epidemiology of T. cruzi and its possible association between PTX3 plasma levels and other molecule of immune response, with clinical manifestations in chronic CD patients. PTX3 plasma levels were determined by ELISA in 128 chronic CD patients from southern Brazil (41 indeterminate, 52 cardiac, 19 digestive and 16 cardiodigestive) and 87 controls. DTUs from these patients are been characterized. Clinical and laboratory parameters were analyzed with adjustment for age, sex, and ancestry using logistic regression analysis. PTX3 levels were significantly lower in chronic CD patients than in controls. These results suggest an immunoregulatory role associated with cardioprotective feature for PTX3 in chronic CD. Molecular epidemiology in association with immunogenetic studies are needed to understand what lead different clinical manifestations in infected chronic patients and thus find molecular markers that could indicate disease progression in order to treat it before manifestation in chronic patients.

## STUDY OF THE EFFECTS OF PRMT7-CATALYZED METHYLATION ON THE FUNCTION AND EXPRESSION OF THE RNA-BINDING PROTEIN ALBA20 IN LEISHMANIA MAJOR

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Arginine methylation is a widely distributed post-translational modification. Protein arginine methyltransferase (PRMT) enzymes have been described in parasitic protozoa. Recently, our research group showed the expression profile of the PRMT7 enzyme from Leishmania major (LmjPRMT7) during promastigote development and its implication in parasite virulence. We observed that LmjPRMT7 associates to nine different RNA-binding proteins, including LmjAlba20, which is not arginine methylated in LmjPRMT7 knockout parasites. Furthermore, the knockout of this gene led to a dramatic alteration in LmjAlba20 expression pattern in stationary phase promastigotes. In this project, we will investigate a putative regulatory mechanism promoted by arginine methylation on LmjAlba20 affinity for transcripts and on the levels of this protein during L. major development. Initially, we will assess LmjPRMT7 methylation activity on LmjAlba20 in vitro using recombinant protein. In vivo, we will compare samples from wild-type L. major and null mutant Δlmjprmt7. The transcripts associated to FLAG-HA-LmjAlba20 will be purified and identified by RNA sequencing (RNA-seq). The expression profile of methylated LmjAlba20 mimetic mutants will be evaluated in the different parasite stages. The proposed study will investigate regulatory mechanisms yet unexplored in trypanosomatids.

Financial support: FAPESP

## DEVELOPMENT OF MICRORNA BASED THERAPEUTICS FOR VISCERAL LEISHMANIASIS INFECTION

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The protective immune response to leishmania intracellular parasites is mediated by proliferation and differentiation of IFNy secreting CD4+ T helper (Th1) cells. Bioinformatics analyses have shown that several microRNAs such as miR-17, miR-19b, miR-340 and Let-7e can activate differentiation of CD4+ T helper (Th1) type of immune response and lead to secretion of INFy and IL-12 to generate leishmania specific protective response. Furthermore, seed region present in the microRNAs (miR-29-b, miR-29a, miR-146a) have the putative binding site in the 3'-UTR region of T-bet/TBX21 transcription factor of CD4+ T helper (Th1), which may suppress the Th1 specific protective immune response. Development of Th2 type specific immune response can be suppressed by binding of miR-135b and miR-340 microRNAs over the 3'-UTR region of GATA-3 transcription factor of Th2 specific CD4+ T helper cells. Interestingly, miR-21 can inhibit the Th1 immune response and simultaneously activate the Th2 immune response by stimulating T helper cell proliferation and differentiation. We are indicating that miR-27b, miR-155 and miR-128 are important players for protective gene regulation as they suppress Th2 specific immune response. Thus, in future it can be used as therapeutic agent to activate Th1 specific CD4+ T helper cells for generating IFNy specific protective immune response during VL infection.

## PHARMACOLOGICAL EFFECTS OF THE CRUDE EXTRACT AND ALKALOIDAL FRACTION OBTAINED FROM THE ROOTS OF NAUCLEA LATIFOLIA IN LABORATORY ANIMALS

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The National Institute for Pharmaceutical Research and Development has developed NIPRIMAL® (an anti-malarial phytomedicine) from Nauclea latifolia Smith (Rubiacea) freeze –dried aqueous root extract. NIPRIMAL® Phase II clinical trial was supported by the World Health Organization.
The aim of this study was to determine the pharmacological effects of Nauclea latifolia freeze-dried aqueous root extract on the central nervous system and its possible mechanisms of action as well as assess the microbial quality and formulation properties of the extract.
Nauclea latifolia root powder was extracted by cold maceration in distilled water for 24h and freeze-dried. The alkaloidal rich fraction of Nauclea latifolia root was extracted using standard procedures (WHO,1998 ). Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) profiles of the extract were determined. Microbiological assessment of the extract was carried out to monitor quality, whilepreliminary pre-formulation and formulation studies were determined to predict appropriate dosage form. The effect of the extract and its alkaloidal fraction were assessed against PTZ-induced anxiety in mice, spatial memory of mice in Y-maze, copper sulphate –induced emesis in a day old chicks and apomorphine –induced pecking in pigeon. To investigate mechanism of pharmacological effects of Nauclea latifolia extract and alkaloid, interactive studies involving flumazenil, extract and alkaloid were carried out. The effect of extract and alkaloid on cholinesterase activities were evaluated in vitro and in vivo.

The extract was shown to have a total aerobic bacteria and yeast/mould counts of 6.0x102 cfu/g and 1.0 x 100 cfu/g respectively. There was absence of pathogenic microorganism. The extract (12.5 mg/kg body weight p.o.), its alkaloidal fraction (2.5 mg/kg body weight, p.o.) and diazepam (1 mg/kg body weight, p.o) antagonized PTZ-induced anxiety in mice. Flumazenil (0.2 mg/kg body weight, i.p.) antagonized anxiolytic effect produced by extract, alkaloid and diazepam. The extract and the alkaloid obtained from Nauclea latifolia root enhanced memory acquisition and retrieval on elevated plus maze. The extract and the alkaloid significantly decreased the cholinesterase activity in vivo and in vitro. The extract and its alkaloid significantly ($p<0.05$) and dose-dependently reduced copper sulphate –induced retches in day old chicks, an effect comparable to metoclopramide (5 mg/kg body weight, p.o.). The antagonism of apomorphine-induced pecking by the extract and the alkaloid in pigeons compare favourably, with the activity of haloperidol (2 mg/kg body weight, i.p.). Granules and tablets produced from the extract had very good micromeritic and excellent mechanical properties suitable for pilot scale up. However, the tablets had very poor disintegration properties (Table 1).

Nuclea latifolia root extract met the standards for microbial limits as specified in the World Health Organization guidelines for assessing quality of herbal medicines (ref). Nuclea latifolia root extract and its alkaloid possess anxiolytic, anti-emetic and memory enhancing effects mediated possibly via facilitations of GABA-ergic, cholinergic and antagonism of dopaminergic neurotransmission pathways. While tablets of suitable physical properties can be produced from the extract, a disintegrant would need to be included in the formulation to ensure adequate drug release.

Key words: Nauclea latifolia; finger printing, pharmacology; cholinesterase, anxiety, memory, emesis, microbiology, formulation,

TABLE 1. Some Micromeritic and Mechanical Properties of the freeze-dried extract of *N. latifolia*

| Tapped density (g/ml) | Flow Rate (g/s) | Angle of repose (degree) | Carr's index (%) | Hausnerinde x | Porosity (%) | True density (g/ml) | Hardness (KgF) | Disintegration (min) | Friability (%) |
|---|---|---|---|---|---|---|---|---|---|
| 0.56 (0.00) | 15.00 (0.00) | 24.62 (0.00) | 10.00 (0.00) | 1.11 (0.00) | 10.00 (0.00) | 1.39 (0.00) | 4.45 (0.71) | 101.00 (1.00) | 0.00 (0.00) |

***Values in parenthesis are standard deviations***

## CHARACTERIZATION OF THE MECHANISMS OF ACTION OF PLUMIERIDE AND PLUMIERIDINE COMPOUNDS AGAINST CRYPTOCOCCUS GATTII

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Cryptococcus neoformans and Cryptococcus gattii are fungal pathogens of humans that cause cryptococcosis. Disease characterized by meningoencephalitis and lung injury. This species cause an estimated one million cases of cryptococcal meningitis per year among people with HIV/AIDS, resulting in nearly 625,000 deaths. Many authors suggest that cryptococcosis must be considered a neglected disease. Most cryptococcosis deaths is due to lack of proper diagnosis and treatment. The most common drug treatment is high-dose amphotericin B and 5-flucytoseine or fluconazole monotherapy, but have some limitations: high cost, toxicity and resistance. Which justify the search for new drugs. Our group identified antifungal activity in two iridoids - plumieride and plumieridine - isolated from Allamanda polyantha (Apocynaceae), a brazilian native plant. In order to elucidate the molecular mechanisms of plumieride and plumieridine, a C. gattii mutant library with 8,000 mutants is being screened to find sensitive and resistant mutants. At the moment, was selected 4 strains in the library. The interrupted locus in these mutants will be determined by inverse PCR and sequencing to elucidate the cellular functions affected by compounds. Our results may contribute for description and development of new antifungal drugs for cryptococcosis treatment.

## REPOSITIONING OF APPROVED DRUGS AS ANTI-LEISHMANIAL AGENTS BASED ON PHARMACOLOGICAL MODELLING

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Leishmaniasis is a neglected tropical disease caused by Leishmania spp. parasites and transmitted by the bite of infected sandflies. An estimated of 1.3 million new cases and 20.000 to 30.000 deaths occur annually. Currently, the most commonly used drugs for leishmaniasis treatment comprises: pentavalent antimonials, amphotericin B, miltefosine, pentamidine, paromomycin and sitamaquine. Nevertheless, these drugs are expensive, some report contraindications and are associated with mild to severe side effects. In addition, the current treatments occasionally fail to eliminate the parasite and are prone to chemoresistance. In silico methods have proved to be a useful tool in drug discovery, saving time and money by improving the likelihood of success during experimental validations. In this regard, our work aims to identify new purposes (anti-Leishmania activity) of existing drugs through pharmacological modeling, which implies the analysis of population-based pharmacokinetics simulations. The models will be fitted based on data stored in public databases. In the end, the models outputs will serve to establish ranges of concentrations suitable to filter a list of potential drugs obtained by bioinformatics approaches. The final set of chosen drugs will be assayed in vitro in order to validate its anti-leishmanial activity. Promising compounds might be feasible for in vivo assays.

## EX-VIVO INVESTIGATION OF FRACTIONATED EXTRACTS OF ALBIZIA GUMMIFERA SEED AGAINST LEISHMANIA AMASTIGOTE STAGE.

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Leishmainasis is a group of diseases caused by protozoan parasites of the genus Leishemania and transmitted by female sandy fly. Recently it is reported to be endemic in about 98 countries, mainly among the under-developed and developing countries including some countries in Europe. The disease causes significant mortality and morbidity in different countries. Globally about 350 million people are at risk of infection, and an estimated 1.5 million to 2 million new cases occur annually. Current treatment depends solely upon chemotherapy as no efficient vaccine is available so far. However, there are few drugs for the treatment of the disease and unfortunately they are toxic, expensive and share a tendency to generate resistance and require long-term treatments, which would make the chemotherapy complicated. As a result, the disease contributes significantly to the propagation of poverty in developing countries because of the above mentioned problems. Herbal medicines are source of different leads to anti-leishmanial drugs which can offer potential of therapeutic switch chemotherapy and becoming widely accepted by many authorities including the World Health Organization (WHO) as a viable treatment for various diseases. The in vitro study of medicinal plant against Promastigote stages of Leishmania result from a research group of Ethiopian Public Health Institute (EPHI) and Addis Ababa University (AAU) indicated that Albezia gummifera seed; extract has got an anti-promastigote activity with low toxicity nearly compared to a control drug, amphotericin B. However the promastigote stages are not the ideal stage for the discovery of drug as compared to an amstigotes, the intercellular stage responsible for causing pathological diseases. Thus this medicinal plant extract should further be tested using amastigotes stages of the parasite for the discovery of lead molecule. Thus, this study will attempt to investigate the ex-vivo activity of Albizia gummifera seed fractionated extract for its anti-amastigote activity. Determining the activity of the extract will be done by culturing the amastigote in experimentally isolated mouse macrophages in 8 wells chamber slides. The results will be expressed in terms of infection rate (IR) and the multiplication index (MI) and compared with the reference drug.

**Farnaz Zahedifard**

## COMPARISON OF DIFFERENT SYNTHETIC PEPTIDE CANDIDATES AGAINST L. MAJOR AND L. TROPICA INFECTION IN BALB/C MICE

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One of the major health problems in IRAN, is cutaneous leishmaniasis. This disease is highly endemic and affects many people in urban and suburb areas annually. Antimonies, in Iran, are the first line of treatment as many developing countries but drug resistance, unresponsiveness and severe hepatic side effects, are making medical services looking for new effective agents. From ancient times, people conventionally have been using a great spectrum of natural resources to treat the wounds and infections. Different investigations revealed that herbal and animal substances may act as antimicrobial peptide or a strong immunomodulator. In recent years, Global resistance to conventional antibiotics, especially in hospitalized and immunocompromised patients, arises the tendency to find better and more effective substitutions. In these cases, antimicrobial peptides would be promising candidates for treatment of bacterial, fungal or parasitic disease. In our current study, we are evaluating a collection of different antimicrobial peptides or Immunomodulators single or together to find the most promising one for testing in Balb/c mice model againstLeishmania infection. In vitro studies consist of Cytotoxicity, hemolysis assay and survival test in the macrophages and efficacy of wound healing or prevention in Balb/c mice will be performed through monitoring the wound size, parasite burden and cytokine production.

## IN VITRO AND IN VIVO EVALUATION OF NEW DRUGS AND COMBINATION THERAPIES FOR CHAGAS DISEASE

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Current research project focuses on systematic in vitro and in vivo evaluation of drugs and drug combinations potentially actives against T. cruzi which could be repurposed for Chagas disease treatment. Eligible drug candidates must meet certain criteria, such as previous approval for human use, pharmacokinetics studies in adults and, desirably, in children, good absorption by PO route, low rate of adverse effects reported, among others. Reported activity against related protozoan parasites is an ideal condition but not limited. For in vitro assays, VERO cells are infected with trypomastigotes for 24 hs and treated with different concentrations of drug candidates. If IC50 is similar or higher than Benznidazole (Bz) IC50 (positive control), the compound continues to a stringent evaluation in an acute murine model. BALB/c mice are infected with VD strain trypomastigotes and treatment with compound (or combination) starts at parasitaemia onset, for 20 consecutive days. Surviving animals are submitted to immunosuppression cycle. Finally, parasitological response is determined with qPCR from blood and target organs. At present, several drugs have been evaluated, including voriconazole (monotherapy and in combination with Bz), lumefantrine (alone and in combinations) and dihydroartemisinin. Most drugs tested so far proved to be inferior to Bz alone, but more potent drugs are currently being studied.

## EVALUATION OF NEW DRUGS IN PARACOCCIDIOIDOMYCOSIS: ANTIFUNGAL ACTIVITY OF ALKYL GALLATES IN ALTERNATIVE MODELS GALLERIA MELLONELLA AND CAENORHABDITIS ELEGANS AND IN MICE

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The search for new drugs is needed in fungal diseases such as paracoccidioidomycosis (PCM). The PCM is a neglected disease caused by genus Paracoccidioides and appears endemic in Central and South America. Despite the good efficacy of antifungals against P. brasiliensis and P. lutzii, some of them such as amphotericin B can be toxic to the host cells. Furthermore, it was described the occurrence of P. brasiliensis isolates resistant to ketoconazole. In this context, the search for natural products with improved antifungal efficacy and safety have increased. Additionally, alternative models as Galleria mellonella and Caenorhabditis elegans have been used to study the fungus-host interaction and for search of drugs in a simple way and on large scale. In this work, G. mellonella and C. elegans has been infected with Paracoccidioides spp. and efficacy and toxicology of amphotericin B, itraconazole and esters of gallic acid derivatives has been evaluated. The compound with best activity in alternative models will be evaluated for efficacy, toxicology and pharmacokinetics in mice.

## USING PROTEOMIC APPROACH FOR MOLECULAR CHARACTERIZING FROM LEISHMANIA CHAGASI CULTURE TREATED WITH NOR-ß–LAPACHONE

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Leishmania spp. is a trypanosomatid protozoan responsible for diverse clinical forms of neglected diseases known as leishmaniases. There are no vaccines available for this disease and current treatments suffer from several limitations. The drugs of choice for the treatment of leishmaniasis are drugs administered by parenteral route, which have disagreeable and serious side effects. Naphthoquinones are a class of chemical compounds exhibiting a variety of anti-carcinogenic, immunomodulatory and antimicrobial activities and several have shown activity against the protozoan parasite Leishmania. This project aims to evaluate the set of expressed proteins, their structures and interactions in Leishmania cells treated with nor-ß-lapachone, detailing processes involved in the cytotoxicity activity during treatment. Also, allows revealing cellular elements, candidate proteins and their interactions with each other as strong components during the promastigote cycle. This study also purpose to identify target molecules and reactions between molecules, such as important pathways in the disease development, as elements for new agents with potential leishmanicidal activity. The ability to identify the components/processes drug-cell interactions and model them by different techniques such proteomics and in silico simulations provides a possible elucidation on the effectiveness of the studied naphthoquinones compounds.
Keywords: Kinetoplastidae; drug discovery; naphthoquinones

## DEVELOPMENT AND CHARACTERIZATION OF A MURINE MODEL OF INFECTION BY LEISHMANIA (VIANNIA) PANAMENSIS

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New world cutaneous leishmaniasis is an endemic disease in many regions of Latin America. Leishmania panamensis is the main causing agent specie of the cases in Panama. The development of new drugs against this disease is limited by the high variability observed in the immune response produced by infections of the same Leishmania specie. The development and characterization of an animal model of L. panamensis, able to reproduce the disease observed in humans, will allow the understanding of the immunopathogenesis of the host-parasite interactions. In this study we will characterize the response of Balb/c mice to L. panamensis infection. Animals were inoculated subcutaneously in the left hind footpad and intradermal in the ear pinnae of Balb/c mice. The experiment was monitored with a caliper to estimate the inflammation level for 24 weeks. Additionally, macrophages derived from Balb/c mice were infected in vitro with L. panamensis and was evaluated the response of this cells to the infection. Our preliminary results show that Balb/c mice develop lesions in the ear but not in the footpad that are evident after 8 weeks of infection with L. panamensis. We also showed that Balb/c macrophages produce TNF and IL-10 in response to the infection and the pre-incubation of the cells with LPS increase their killing capacity.

## THE ANTI-MALARIAL ARTEMISININ DRUG IS ALSO ACTIVE AGAINST CANCER

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Artemisinin is the active principle of the Chinese herb Artemisia annua L. In addition to its anti-malarial activity, artemisinin and its derivatives have been shown to exert profound anti-cancer activity. The endoperoxide moiety in the chemical structure of artemisinin is thought to be responsible for the bioactivity. The present study aims at defining the differential cytotoxicity effect of artemisinin toward P815 (murin mastocytoma) and BSR (kidney adenocarcinoma of hamster) cell lines. Cytotoxicity was measured by the growth inhibition using MTT assay. These in vitro cytotoxicity studies were complemented by the determination of apoptotic DNA fragmentation and Annexin V- Streptavidin-FITC assay. Furthermore, we examined the in vitro synergism between artemisinin and the chemotherapeutic drug, vincristin. The in vivo study was investigated using the DBA2/P815 (H2d) mouse model. While artemisinin acted on both tumor cell lines, P815 was much more sensitive to this drug than BSR cells, as revealed by the respective IC50 values (12 µM for P815 and 52 µM for BSR cells). On another hand, and interestingly, apoptosis was induced in P815 but not induced in BSR. In vivo, our results clearly showed that the oral administration of artemisinin inhibited solid tumor development.

## EVALUATION OF ANTICHAGASIC ACTIVITY OF 1,2,3-TRIAZOLES HAVING AS TARGET ACTIVE ENZYMES ON CARBOHYDRATES (CAZY'S) OF TRYPANOSOMA CRUZI

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Carbohydrates Active Enzymes (CAZy's), such as glycosidases, are involved in different metabolic pathways, which has stimulated their use as molecular targets for the treatment of various types of diseases, including parasitosis. The parasite Trypanosoma cruzi has a cell surface made up of highly glycosylated proteins that are important in a number of biological functions, such as infectivity and immune evasion. Thus, the study of CAZy's from T. cruzi can provide a new and interesting target for the development of antichagasic drugs. We are investigating the effect of new 1,2,3-triazole compounds (putative transition-state mimic inhibitors of glycosidases) on T. cruzi CAZy´s activities as well as on parasites' viability, development and infectivity in human cells and in the triatomine vector. To achieve this goal, several computational (e.g.: molecular modeling and virtual screening) and experimental (e.g.: enzymatic assays and High Content Analysis - HCA) approaches are being applied. Our preliminary results demonstrated that the series of 1,2,3-triazoles tested so far do not strongly inhibit a panel of glycosidase activities from the insect vector nor showed any toxic effect on C2C12 skeletal myoblasts. The same compounds are now being tested on trypomastigotes and infected myoblasts to evaluate their effect on T. cruzi infectivity and amastigote viability, respectively.

## ANTIPROLIFERATIVE AND ANTIFUNGAL EFFECT OF PYRANONAPHTHOQUINONES OBTAINED FROM CIPURA PALUDOSA BULBS

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Cipura paludosa (Iridaceae) is popularly used to treat several disorders, such as inflammation, infections and pain. The aim of this study was to evaluate the antiproliferative, antifungal and antileishmanial activity of pyranonaphthoquinones isolated from C. paludosa bulbs. Phytochemical analysis was carried out by conventional chromatographic techniques, and the resulting compounds were identified by NMR. The antiproliferative activity was analyzed using the sulforhodamine B assay and the antifungal activity was tested against Candida albicans, Candida tropicalis, Saccharomyces cerevisiae and Cryptococcus neoformans. Three pyranonaphthoquinones (eleutherine, isoeleutherine and eleutherol) were isolated from C. paludosa bulbs and all exhibited promising antiproliferative effect against several human carcinoma cell lines, especially the two main compounds, eleutherine and isoeleutherine, against glioma and breast cancer cell lines. These compounds also presented significant antifungal activity, with MIC values between 7.8 and 250 µg/mL. Studies are needed to evaluate the possible antileishmanial effects of these compounds. In conclusion, the results demonstrate that C. paludosa bulbs produce active principles with relevant antiproliferative and antifungal potential, contributing, at least in part, with the antimicrobial effect evidenced for this plant and justifying its popular use against infections.

## NOVEL NAPHTHOQUINONE DERIVATIVES AND EVALUATION OF THEIR TRYPANOCIDAL AND LEISHMANICIDAL ACTIVITIES

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Chagas disease is a potentially fatal disease caused by the protozoan Trypanosoma cruzi and one of the most important widespread ones. On the other hand, the Leishmaniases are diseases associated with several clinical syndromes caused by protozoan parasites from Leishmania species that are transmitted to humans and other vertebrates by the bite of the infected female phlebotomine sandflies. Our research mainly focuses on the development of new drug candidates to treat against neglected diseases, especially Chagas as well as Leishmaniases. Considering that the class of naphthoquinones is also reported to show antiprotozoal activity, we have synthesized a series of naphthoquinones derivatives and tested in vitro as trypanocidal and leishmanicidal compounds. In this, two derivatives compounds were active against the amastigote stage of T. cruzi and they exhibited low cytotoxic effects to fibroblast LLCMK2. These compounds are promising candidates as trypanocidal agents. Remarkably, in the assays of leishmanicidal activity, all the compounds were inactive against L. chagasi and L. braziliensis.

## SYNTHESIS, BIOLOGICAL EVALUATION, AND STRUCTURE-ACTIVITY RELATIONSHIPS OF CARBAMOYLIMIDAZOLE DERIVATIVES AS CRUZAIN INHIBITORS AND ANTI-TRYPANOSOMA CRUZI AGENTS

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Cruzain is an essential enzyme of Trypanosoma cruzi. The development of cruzain inhibitors is driven by the urgent need to develop novel and more effective drugs for the treatment of Chagas's disease. Most cruzain inhibitors were developed based on knowledge of substrate specificity and are irreversible peptidic inhibitors that bind covalently to the enzyme. Therefore, new classes of inhibitors were investigated by a variety of techniques, such as identification of quantitative structure-activity relationships, chemical modification of hits identified in substrate library screening, and optimization of compounds discovered by high-throughput screening and virtual screening.
Based on virtual screening results, a lead compound was selected and then planning and molecular optimizations were performed. This lead is an imidazole derivative showing reversible competitive inhibition of cruzain. Analogs of the lead compound were designed seeking new interactions between the compounds and the enzyme subsites through molecular modeling and the use of bioisosteres.
To date, twenty one derivatives of the lead compound were synthesized showing IC50 values against cruzain in low micromolar and nanomolar concentrations. The studies related to the anti-Trypanosoma cruzi assays and the mechanism of action are underway.

## FLAVONOIDS AND FLAVONOID-LIKE COMPOUNDS AS CANDIDATES TO FACE NEGLECTED TROPICAL DISEASES

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Parasites of the family of Trypanosomatidae are agents of serious human diseases, including African sleeping sickness, Chagas disease and Leishmaniasis. Since drugs currently in use have limitations, there is an urgent requirement for new effective drugs.
The folate pathway is a successful target for the treatment of bacterial infections and some parasitic diseases (e.g. malaria). However, the classical inhibitors of dihydrofolate reductase (DHFR) are ineffective against Leishmania and Trypanosoma because Pteridin Reductase 1 (PTR1), a trypanosomatidic enzyme, overlaps DHFR activity. Therefore, PTR1 is a promising target for the development of improved therapies. Computational studies were performed to screen a library of 90 natural compounds from plants. Flavonoids turned out to be an interesting class to be explored as PTR1 inhibitors. We have synthesized 76 compounds: chalcones, flavanon- and flavonol-like compounds. X-ray crystallographic structures of some of the synthesized compounds were solved. According to enzymatic inhibition assays, many molecules showed IC50 values lower than 30 μM, with 4.3 μM being the best one. The compounds were assessed also for ADME and in vitro toxicity. Two compounds turned out to have IC50 towards Trypanosoma brucei lower than 8 μM. These molecules were selected for pharmacokinetic studies and will be loaded in drug delivery systems for in vivo tests.

## AMINOPYRAZOLO[1,5-A]PYRIMIDINES AS POTENTIAL INHIBITORS OF MYCOBACTERIUM TUBERCULOSIS: STRUCTURE ACTIVITY RELATIONSHIPS AND ADMET CHARACTERIZATION

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A 35,000 compound SoftFocusTM library was obtained from Biofocus and screened against virulent Mtb H37Rv. High-throughput screening was carried out in liquid culture (7H9 medium) under aerobic conditions, using glucose as a carbon source. Whole-cell active compounds were selected from the screens using several criteria, including potency, intellectual property overlap, and chemical-structural properties. One of the libraries was designated SFK29 (SoftFocus Kinase) based on a 7-aminomethylaryl pyrazolo[1,5-a]pyrimidine series. The whole-cell screen confirmed that, of the 248 7-aminomethylaryl pyrazolo[1,5-a]pyrimidines screened, five compounds showed minimum inhibitory concentrations (MIC99, representing 99% growth inhibition) less than 10 μM. A medicinal chemistry program was then initiated to explore this series as potential anti-tubercular chemotherapeutic agents. After numerous iterative rounds of optimization the whole cell anti-tubercular in vitro potency (< 2.5 μM) was improved, however, it was discovered that the series suffered from poor aqueous solubility (<5 μM for > 90% of compounds synthesized) and persistent in vitro cytotoxicity against Chinese Hamster Ovary Cells (CHO). The apparent inherent insolubility and cytotoxicity are the primary concerns in this series and need to be addressed if this series was to be considered for further development.

## DIHYDROOROTATE DEHYDROGENASE FROM LEISHAMIA VIANNIA BRASILIENSIS: A NEW TARGET IN THE FIGHT AGAINST MUCOSAL LEISHMANIASIS

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Neglected tropical diseases represent a group of diseases that cause significant morbidity and mortality but have until recently received limited investment in the development of effective therapies. As our contribution for the search of new targets for innovative drug development, our laboratory has been working in the characterization and evaluation of the role of the enzyme dihydroorotate dehydrogenase from Leishmania Viannia braziliensis (LbDHODH), the species responsible for the mucosal form of Leishmaniasis. DHODH plays a key role in the synthesis of pyrimidine nucleotides, and it is considered an important target in the development antiproliferative and antiparasitic compounds. In this context we have developed a reprodutible protocol for heterologous expression of LbDHODH and determined its crystal structure at 2.12 Å resolution. In the search for inhibitors, two commercial molecules, LCPSP3027 and LCPVL898, were identified as LbDHODH inhibitors that display IC50 values of 4.43 ± 0.32 μM and 5.42 ± 0.09 μM, respectively. These ligands showed leishmanicidal activity in vitro cultures, proved not be cytotoxic to macrophages and amastigotes studies showed a significant reduction of infectivity. Development of a synthetic route for both molecules and analogues are currently under way. Formulation and pre-clinical trials is also currently in progress.

## STUDY OF N'-[(5-NITROFURAN-2-YL) METHYLENE] SUBSTITUTED HYDRAZIDES AGAINST TRYPANOSOMA CRUZI STRAINS MORE PREVALENT IN CHAGAS DISEASE PATIENTS

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The lack of therapeutic options for the treatment of Chagas disease has been driving the design of more active compounds. Nitroheterocyclic moiety has antiparasitic activity, and is present in the chemical structure of drugs as Benznidazole and Nifurtimox. Herein, Nifuroxazide (NF) was used as lead to design a set of twenty one compounds in order to investigate the anti-Trypanosoma cruzi activity. The set of N'-[(5-nitrofuran-2-yl) methylene] substituted hydrazides was assayed against three T. cruzi strains, which represent the discrete typing units more prevalent in human patients: Y (TcII), Silvio X10 cl1 (TcI), and Bug 2149 cl10 (TcV). Nineteen derivatives have showed trypanocidal activity enhanced against the three strains in comparison to BZD. For the Y strain, the compounds (62 %) were more active than NFX. The most active compound was N'-((5-nitrofuran-2-yl)methylene)biphenyl-4-carbohydrazide. It presented IC50 values of 1.17 (± 0.12) μM; 3.17 (± 0.32) μM; and 1.81 (± 0.18) μM, for Y, Silvio X10 cl1, and Bug 2149 cl10 strains, respectively. Cytotoxicity assays (LLC-MK2 cells) have demonstrated high selectivity indices. Regarding the exploratory data analysis, topological, steric/geometric, and electronic properties have influenced more the discrimination of the investigated compounds. Taking all together, the findings can be helpful to drive the designing of new drugs against Chagas disease.

## 2-PHENOXY-1,4-NAPHTHOQUINONE-ANACARDIC ACID HYDRIDS AS MULTITARGET LIGANDS FOR TRYPANOSOMATID INFECTIONS

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Multitarget-directed ligands (MTDLs), that is single small molecules able to modulate multiple targets in the parasite's pathway, have emerged as useful tools against trypanosomatid infections.
In a search for new MTDLs, we considered the previously identified 2-phenoxy-1,4-naphthoquinone (B6) and anacardic acid (AA) as suitable starting points. Indeed, both B6 and AA were able to inhibit trypanosomal glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a validated antitrypanosomatid target. Furthermore, thanks to the presence of the naphthoquinone, B6 was shown to generate reactive oxygen species (ROS), a mechanism that may further contribute to its trypanocidal activity. In fact, B6 exhibited high potency against T. brucei rhodesiense (Tbr) (IC50 = 80 nM) and a promising selectivity index of 74. On this basis, following a merging design approach, the molecular features of B6 and AA have been combined to give a small set of hybrid compounds, possibly endowed with a MTDL profile. In this respect, they will be evaluated for their ability to generate ROS, inhibit GAPDH, and arrest parasites' growth. Notably, being GAPDH located in the glycosome organelle, the long chain of AA may improve the membrane permeation. As another peculiar value, we envisage that such hybrid compounds, obtained from renewable material, might be promising and cost-effective molecules from an antitrypanosomatid drug discovery viewpoint.

## PARALLEL SYNTHESIS AND STUDIES OF STRUCTURE ACTIVITY RELATIONSHIPS OF POTENTIALLY ANTILEISHMANIAL N-ACYLHYDRAZONES.

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Leishmaniasis is a neglected disease which is responsible for 350 million cases in 88 countries. Due to a limited and inappropriate therapeutics, it has become urgent the need for new antileishmanial drugs. Cysteine proteases are enzymes related with several parasite vital process, therefore, it has become potential targets to be explored. Nitro compounds have their antiprotozoal activities recently associated with inhibition of parasites cysteine proteases, in addition to their well-known free radical production mechanism through nitro group reduction. This work aims the search for new antileishmanial compounds by applying parallel syntheses to obtain a library of N-acylhydrazones compounds, analogs of nitroderivatives. Parallel synthesis proved to be an appropriate methodology for obtaining our structurally related compounds, with good molecular diversity, yield and purity. The initial screening results show that substitution of thiophene by its isostere benzene decreased the antileishmanial action, indicating the importance of sulfur heteroatom to this activity. Nitro group seems to be important but not essential to the activity. The best derivatives, however, were those presenting the nitro group and thiophene system. Qualitative structure-activity relationship studies have already been started aiming to explain the behavior of the series.

## DEVELOPMENT OF CARBAZOLE LIBRARY TARGETING ANTILEISHMANIAL COMPOUNDS

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Carbazoles compounds are of great interest to medicinal chemistry because of their useful biological activity. Among of them, leishmanicidial proprieties was recently related for the natural carbazole Lansine and some analogs of it. Clauraila A is other natural carbazole with structure related to Lansine, therefore attracting our interest in the development of new antileishmanial compounds. Besides, both natural alkaloids have interesting cytotoxicity activity reported. Considering the potential of carbazole core to develop antileishmanial compounds, we develop a library of carbazoles based in the structure of the formylcarbazole Clauraila A. It was used de cyclodehydrogenation of diarylamine to obtain these carbazoles. The vanillin and several anilines was used as start material in a three-step synthesis strategy. First, it was obtained the vanillin triflate in good yields (84%). After, we optimized the Buchwald-Hartwig amination (synthesis of diarylamine) and Åkermark-Knölker cyclization (synthesis of carbazole core) to the synthesis of Clauraila A. Using the optimized conditions was obtained 7 carbazoles analogs of Clauraila A, including other two natural products, Clausenal and 6-methoxymurrayanine. The overall yield after three steps varied from 6% to 35%, being compatible with datas in the literature. The activity of carbazole compounds against the leishmania parasite is being evaluated.

## DEVELOPMENT OF LOW COST AND PHARMACOKINETIC-OPTIMIZED TRIAZOLES AS POTENT INHIBITORS OF TRYPANOSOMA CRUZI

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Chagas disease is caused by the parasite Trypanosoma cruzi (Tc). Nowadays, it is a global public health problem, affecting about 6-7 million people and threatening another 100 million inhabitants of risk areas. Nifurtimox and benznidazole, discovered in the 60´s, are the only two drugs used for the treatment of this disease. However, they present severe adverse effects and poor activity in the later chronic phase. The research on ergosterol biosynthesis inhibitors (EBI) stands out in the search for new active drugs against Tc, as some azoles of this class, i.e. posaconazole, were able to cure mice in the experimental acute and chronic phases. However, these substances have a high synthetic cost and pharmacokinetics issues, such as low oral bioavalability. Thus, the aim of this work is the design, synthesis and biological evaluation of new triazolic derivatives bearing the pharmacophore of active EBIs, with optimized pharmacokinetic parameters and relative low synthetic cost. These derivatives were obtained by a simple four-step synthetic route with overall good yields. So far, all the evaluated derivatives showed excellent anti-Tc activity, one of which being more than 200-fold more potent than fluconazole (an EBI with anti-Tc activity) and more than 17-fold more potent than benznidazole, presenting a selectivity index greater than 1500. The best compounds will be subjected to in vivo evaluation.

## HETEROCYCLIC DERIVATIVES TARGETED TWO CENTRAL METABOLIC PATHWAYS UNIQUE TO TRYPANOSOMATIDS: ERGOSTEROL AND TRYPANOTHIONE PATHWAYS

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Leishmania spp. is widespread in 22 countries in the New World and in 66 nations in the Old World. 1,3 million new cases are considered to occur annually, with an estimated 12 million people presently infected worldwide. Trypanosoma cruzi (TC) affects about 8 million people in Latin America. Current therapeutic approaches against trypanosomatids rely on highly toxic drugs. Thus our aim is to discover new drugs to treat leishmaniasis and Chagas disease, endowed with reduced side effects. The rationale is to target two central metabolic pathways unique to trypanosomatids:
i) Ergosterol metabolism by inhibition of C14∂-demethylase;
ii) Trypanothione metabolism by inhibition of trypanothione reductase (TR).
In early experiments, we identified 3 hit compounds:
• RDS 416 that showed high potency (nanomolar concentration) in in vitro assays against TC amastigotes. This compound was demonstrated to target the C14∂-demethylase.
• RDS 1413 that showed good potency (submicromolar concentration) in in vitro assays against L. infantum.
• RDS 777 that showed activity (decimicromolar concentration) in in vitro assays against L. infantum. This compound was proven to inhibit TR and cocrystallized with this enzyme.

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## SYNTHESIS OF GONIOTHALAMIN ANALOGUE BEARING A FLUORESCENT GROUP FOR CELL-IMAGING EXPERIMENTS.

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Goniothalamin is a styryl ∂, ß-unsaturated O-lactone found in plants of the genus Goniothalamus displaying in vitro antiproliferative effects against different human cancer cell lines and in vivo inhibition of Erlich solid tumor without signs of toxicity. Our research group has shown the ∂, ß-unsaturated O-lactone to be the pharmacophore, and that modifications in the aromatic ring could improve its potency. In order to probe the location of goniothalamin at the cellular level, a derivative bearing a fluorescent moiety – 2,1,3-benzothiadiazole (BTD) ring – was synthetized in 7 steps from a commercially available precursor and its fluorescence spectrum was measured in dichloromethane (¥ exc = 400 nm; ¥ emis. = 535 nm). Studies are now underway regarding its use as a probe for the site of interaction of goniothalamin in human cancer cells.

## ELUCIDATING THE MODE OF ACTION OF OLIGOTHIAZOLES AS ANTI TRYPANOSOMA BRUCEI AGENTS.

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Human and animal African trypanosomiasis is an infectious disease caused by parasites from the Trypanosoma brucei clade. Novel drugs with a safer, effective and known mechanism of action are urgently needed to overcome the drawbacks of the current therapy.[1-2]
Our work aims at the synthesis and bioprofilling (mechanism of action) of novel bis- and tris-thiazoles, analogues to the oligoamides described by Lang (2014) [3] that have potency on the nm range against T. brucei. Using Hanztch methodologies, the thiazoles are synthezised, modified and coupled with HBTU to obtain bis and tris thiazoles [4]. Modifications of the amino and carboxylic group allowed us to obtain novel thiazoles with different physicochemical properties. A phenotype-based screening revealed that one of compounds act as a potent trypanocidal agent against bloodstream T. brucei. Time-lapse analysis of membrane integrity (propidium iodide) by flow cytometry suggests that this compound triggers a rapid (< 2 h) disruption of cell membranes even at concentrations below its EC50. The possible inhibition of the Trypanothione synthetase (TryS), a kinase responsible for the major thiol cofactor of trypanosomatids synthesis: trypanothion was discharded [5].Ongoing experiments with a redox reporter (Grx-roGFP2) cell line of infective T. brucei aim at determining whether this thiazole interferes with parasite redox homeostasis.

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## TARGETING PROLINE TRANSPORT FOR CHAGAS´ DISEASE DRUG DEVELOPMENT

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L-Proline (L-Pro) is an important amino acid for protozoan parasite of the genus Trypanosoma and Leishmania, which include the causative agents of Chagas´s disease, African sleeping sickness and leishmaniasis. In Trypanosoma cruzi, the etiological agent of Chagas' disease, this amino acid is involved in energy metabolism, differentiation processes and resistance to osmotic stress. In this study, we pretended to explore the scope of L-Pro uptake as a chemotherapeutic target for T. cruzi. The designed inhibitors contained L-Pro as recognizable motif, a linker and a variable region to be able to block the transporter. The final products were a series of 1,2,3-triazolyl-proline derivatives prepared starting from L-Pro through alkylation and copper(I)-catalyzed azide-alkyne cycloaddition (click chemistry) and were tested against T. cruzi epimastigotes. L-Pro uptake inhibition of the most active compounds was assayed to validate the mechanism of action showing a decrease in the aminoacid uptake. The analogues antiparasitic activity seems to be related to long aliphatic chains substitution over the triazole. In order to determine the therapeutic index of the hits found the cytotoxicity on Vero cells is being evaluated. In addition, a series of fluorescent tagged analogs are being prepared to study the internalization and location within the parasite by fluorescent microscopy.

## SYNTHESIS OF LUMINESCENT LANTHANIDE CLUSTERS: POTENTIAL BIOMARKERS

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Luminescent compounds are being increasingly used as signaling molecules in biological processes and are used as cell markers, the real-time tracking of cells and drugs, event visualization in living animals in real time, coupling probes in various functions and structures. However, what has been used as luminescent compounds are organic chromophores, and these in turn have the disadvantage of high toxicity and low quantum yields, leading to a short time of luminescence. Clusters coordinated lanthanide ions are an alternative to these organic chromophores having advantages such as minimal toxicity, low cost and easy preparation, leading to high quantum yields, therefore long time luminescence, enabling its application as bioluminescent markers in cells, bio-imaging, in vivo applications, and events in real time through electronic and nuclear magnetic resonance microscopy. The synthesis of the ligands; is made from the amino acid (L)-threonine, which allows the formation of the oxazoline and subsequent connection with lanthanide ions to form the cluster.
Keywords: Lanthanides, Luminescence, Cluster, Oxazoline, Biomarkers.

## DISCOVERY OF NEW LEISHMANIA DONOVANI NUCLEOSIDE HIDROLASE INHIBITORS BY MOLECULAR FRAGMENTS USING STD NMR

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Nucleoside hydrolase (NH) enzyme is a strategic target for the development of drugs to leishmaniasis treatment. NH is as part of purines and pyrimidines uptake pathway, essential for DNA synthesis of parasites. Leishmania donovani nucleoside hydrolase (LdNH) has a high degree of homology between various trypanosomatid and is not found in mammals.1
To identify new substances for LdNH inhibition we are using approaches based on the use of STD NMR technique2: the molecular fragment based drug discovery. STD can be used as a original approach for the study of bioactive products.3 Unlike the biological tests, STD does not require prior knowledge of protein function or any specific set-up the target to its use in pharmaceutical research.
A molecular fragments library was created according to the rule of three4 and based on the structural similarity of the fragments with NH substrates. STD5 was applied for the screening of molecular fragments to identify the presence of LdNH ligands. 129 molecular fragments were tested using STD and 12 fragments showed LdNH interaction. Some fragments inhibit the LdNH activity in mM range. The selected fragments, after being connected via organic synthesis, could be able to act as antileishmanicidal. We demonstrate in this work that the use of STD with molecular fragments is an effective and powerful tool for the discovery new ligands to an original target as LdNH.

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# SYNTHESIS OF NATURAL MARINOQUINOLINES AND UNNATURAL ANALOGUES WITH POTENTIAL BIOLOGICAL APPLICATION AGAINST NEGLECTED DISEASES

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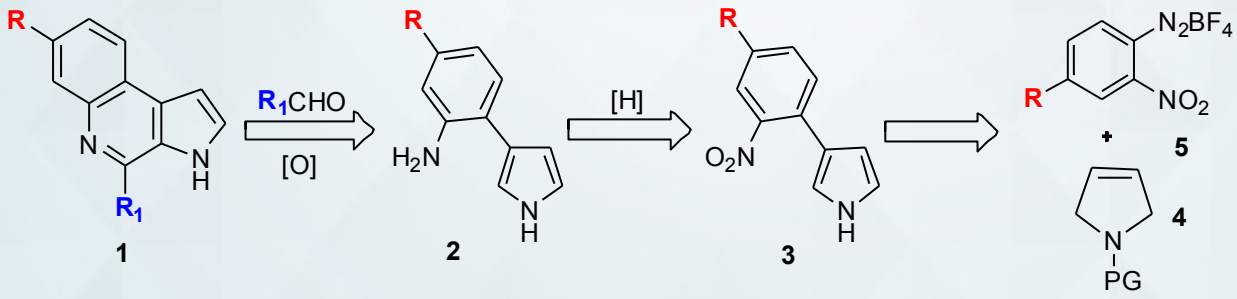
Nitrogen containing heteroaromatic compounds have been playing a crucial role in the treatment of many types of diseases. A less known class of heteroaromatic compounds displays as its main core structure the uncommon tricyclic system 3H-pyrrolo[2,3-c]quinoline. Isolated from extracts of the Rapidithrix thailandica bacteria the marinoquinoline A was the first reported natural product having this skeleton. Five other natural analogues were discovered later, namely the marinoquinolines B-F, which were extracted from Ohtaekwangia kribensis bacteria. These compounds have demonstrated moderate antiprotozoal activity against Plasmodium falciparum K1 lineage resistant to chloroquine (IC50 between 1.7 and 15 μM) and Trypanosoma cruzi (IC50 between 21.8 and 53.1 μM), as well as cytotoxic activity against tumor cell lineages L929, MCF-7 and KB-3-1. The group I work with at Unicamp has developed a concise and flexible methodology for the synthesis of many natural and unnatural marinoquinolines in high yields (Scheme 1). Preliminary In vitro biological studies have demonstrated that the novel unnatural analogues display moderate to good activity against Plasmodium falciparum.1




**Scheme 1**: Retrosynthetic scheme of synthesis unnatural analogues of marinoquinolines.

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## LEISHMANICIDAL ACVITY OF GOCHNATIA PULCHRA

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Plants are potential alternative sources for the research of new and selective therapeutic agents for the treatment of leishmaniasis. In this context, this report aims to investigate the leishmanicidal activity of the crude extract and hexane, diclchloromethane, ethyl acetate and water fractions of plant species Gochnatia pulchra against Leishmania amazonensis, seeking to identify the correlation between the major compounds and leishmanicidal activity. Our results showed that the fraction FH, where triterpenes appear in high proportion (59.4% and 50.2%), showed better anti-promastigote activity. On the other hand, the other fractions did not show promising activity when compared with hexane fraction. Due to increased activity of anti-promastigote FH phytochemical study was performed to identify which were the majority in this fraction compounds and correlate with data from the scientific literature of these compounds against leishmanicidal activity. Our results indicate that the FH exerts a strong anti-promastigote activity at concentrations of 400 and 200µg/mL, chemical profile with potential for future development of therapy against leishmaniasis.

## DESIGN, SYNTHESIS AND EVALUATION OF INHIBITORS OF QUINONOID DIHYDROPTERIDINE REDUCTASE (QDPR) AS TOOLS FOR LEISHMANIA DONOVANI TARGET VALIDATION

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Leishmaniasis is a neglected disease caused by protozoan parasites belonging to the genus Leishmania and transmitted by the bite of certain species of sand fly. Visceral Leishmaniasis (VL) is caused by L. donovani and corresponds to the most serious form of this disease. Pentavalent antimony plus paromomycin (Africa) and liposomal amphotericin B (India) constitute the front line treatments for VL, but limitations of these drugs require the development of novel, more active and safer alternatives. Previous work on pterin and folate metabolic pathways in L. major have suggested that the enzyme quinonoid dihydropteridine reductase (qDPR) might be an attractive drug target for VL. Due to the multicopy genomic arrangement of qDPR in Leishmania, evaluation of its essentiality via genetic approaches is extremely challenging. Thus, a chemical approach was chosen to establish whether qDPR constitutes a valid drug target for VL. A fragment screen against LmqDPR identified both one pyridazine and one cinnoline units as promising fragment hits. Thus, the aim of this current project is to design and synthesise cinnoline analogues as potential inhibitors of LdqDPR. To date, 26 compounds including 10 cinnoline analogues have already been synthesized and fully characterized by usual NMR (1H, 13C, 2D) and MS spectrometric methods. All synthesized cinnoline and related compounds will be assessed against LdqDPR.

## PROTECTING SKIN LESIONS FROM BACTERIAL SUPERINFECTION: ANTI-BACTERIAL POTENTIAL OF LEAVES OF ACACIA MODESTA

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Leishmaniasis is one of the neglected health problems in various countries of the world. Bacterial superinfection is believed to play a role not only in the appearance of lesions and affecting the healing process, but also influencing the size, shape and severity of skin lesions. Natural products play a key role in drug discovery. Acacia modesta is used as a remedy for skin diseases by the local “Hakeems”. Phytochemical investigation of the plant followed by evaluation of anti-bacterial potential revealed the efficacy of various plant fractions against different bacterial strains, including those involved in affecting skin lesions. All fractions showed positive anti-bacterial activity except ethyl acetate fraction. However the aqueous layer showed better activity than the standard antibiotics (Streptomycin and Ampicillin) used in this study. Bioassay guided isolation of the active compounds from aqueous fraction may lead to potential anti-bacterial metabolites from Acacia modesta. The synergic effects of the anti-bacterial potential and efficiency of the plant against skin diseases may prove extremely vital in the fight against leishmania.

## VIRTUAL SCREENING APPLIED IN THE SEARCH OF TRYPANOSOMA CRUZI TRYPANOTHIONE REDUCTASE (TCTR) INHIBITORS OF THE NATURAL PRODUCTS DATABASE FROM STATE OF BAHIA

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Chagas disease, caused by Trypanosoma cruzi, affects 21 countries in the Latin America, and is considered a serious public health issue. There is not currently treatment for this disease, especially in its chronic phase. Thus, the search for inhibitors of Trypanothione Reductase Trypanosoma cruzi (TcTR) is crucial. In this way, we applied the Virtual Screening in Natural Products Database of Bahia (http://natprodb.uefs.br/) employing the Autodock4.2 program aiming to search for new scaffolds.
In our results, we first selected the TcTR-ligand complexes according to the best interaction energy values (TCA≈CGA>>REA>CLA>EAA). Secondly, the automatic conformation analysis of this screening using a self-organizing map (AuPosSOM) was made and it allowed us to compare the interactions at TcTR active site. The TCA interacts with all the residues of y-Glu site and site Z of TcTR which reinforces its best binding energy. CLA interacts with all sites, except Phe396, showing an intermediate activity. REA and EAA interacted with the site same, except with Phe396 and Glu466, indicating a lower potency of these compounds. Then we could note that Virtual Screening based on the target structure allowed us to evaluate the most prevalent interactions with NatProDB molecules and TcTR. Finally, we noted the interactions that are useful for the development of most potent inhibitors of TcTR.

## CHEMISTRY APPLICATION TO VISUALIZE AND QUANTIFY DRUG ENTRY INTO PROTOZOAN PARASITES

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Our project is to create a fluorescent tool that will allow visualizing and quantifying the penetration of active principles in protozoan parasites, especially those responsible for malaria and HAT.
It is based on bioconjugation involving a specific coupling reaction between alkyne and azide functions, more precisely: a « click » reaction without copper, the usual catalyst for this type of reaction but nevertheless toxic for cells. New fluorescent probes with specific photophysical properties, though biologically compatible have been designed and synthetized. After checking their chemical reactivity in the « click » reaction with an antiparasitic molecule previously synthetized in our laboratory, their behavior in Trypanosoma brucei and Plasmodium falciparum parasites has been evaluated. Today, some fluorescent probes have been obtained and photophysically described. After cytotoxicity measurements, a preliminary visualization by fluorescence microscopy showed that they indeed penetrated in both parasites. The synthesis of these compounds as well as their chemical reactivity will be presented and discussed. Moreover, some microscopy images will be given to illustrate their specific penetration in parasites.