PARACHEM 2013-18

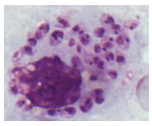
Team 4- Antiparasite Chemotherapy

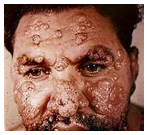
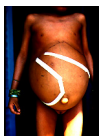
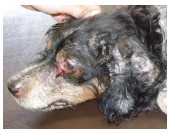
Presentation of the scientific activities and upcoming research projects

Antiparasite Chemotherapy

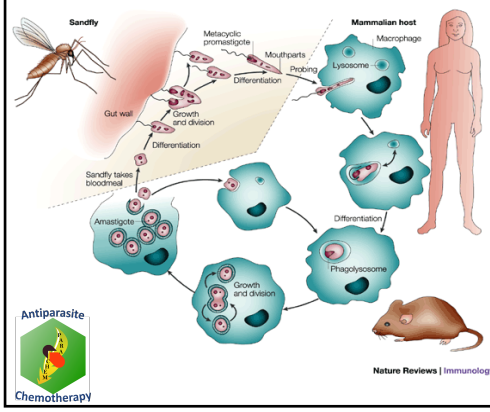
PARACHEM 2013-18: SCIENTIFIC TOPICS

Team 4- Antiparasite Chemotherapy

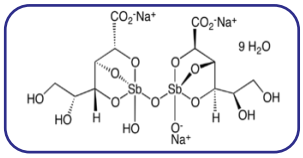
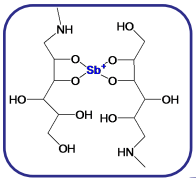
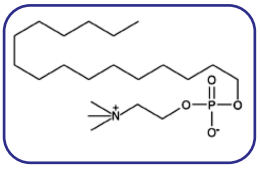
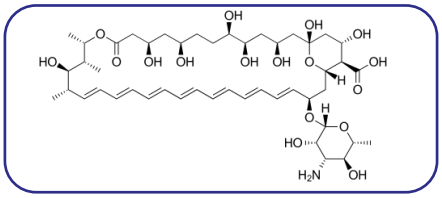
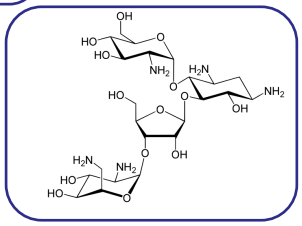



Life cycle of *Leishmania sp.*



Current treatments and their limitations

Toxicity and drug resistance → Need for new chemical series

PARACHEM 2013-18: Workforce

Team 4- Antiparasite Chemotherapy

6 PERMANENT STAFFS

26 NON PERMANENT STAFFS

11 PhD students

6 Postdoctoral scientists including 1 Visitor (Chaire d'Alembert) and 1 ATER

9 Master 2 students

5 Biologists

21 Biologists

Chemistry-Biology Interface

New treatments against leishmaniasis

1 Chemist

5 Chemists/particle technologists

PARACHEM 2013-18: Workforce

Team 4- Antiparasite Chemotherapy

6 PERMANENT STAFFS

26 NON PERMANENT STAFFS

11 PhD students

6 Postdoctoral scientists including 1 Visitor (Chaire d'Alembert) and 1 ATER

9 Master 2 students

FUNDING SOURCES – POSTDOC

20% → Industry
80% → Public

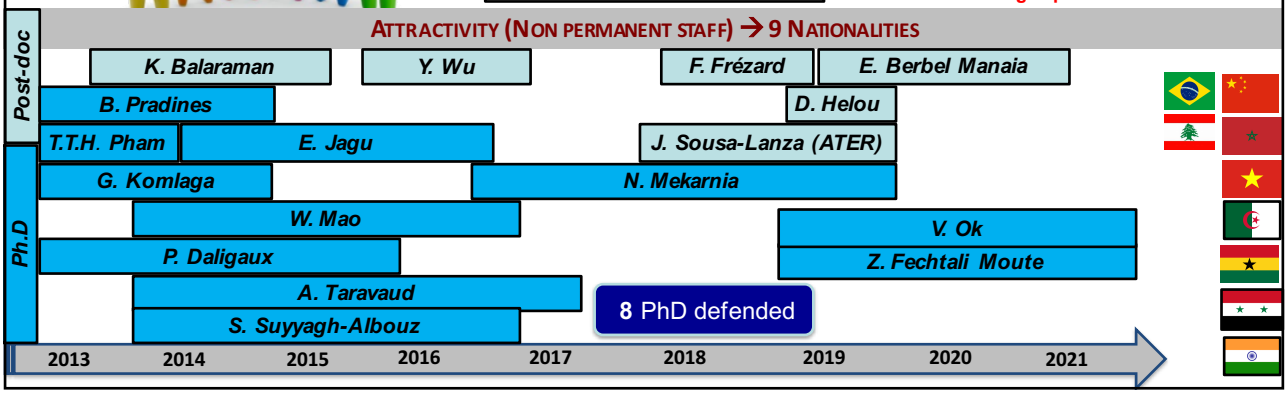
FUNDING SOURCES – PhD

28% → Private sector
72% → Public sector

CURRENT SITUATION - PhD DEFENDED

100% → working in public sector

ATTRACTIVITY (NON PERMANENT STAFF) → 9 NATIONALITIES



PARACHEM 2013-18: Workforce

Team 4- Antiparasite Chemotherapy

THE FUTURE OF PHD AND POST-DOC SCIENTISTS

Post-doc

- *K. Balaraman* → Post-doc in Chemistry in Washington (US)
- *Y. Wu* → Post-doc Cell Biology in CEA-Saclay (FR)

Ph.D

- *B. Pradines* → Clinical research assistant in Biology CHRU Lille (FR)
- *T.T.H. Pham* → Just State-certified in Pharmacy Paris-Saclay (FR) → Start-up in Viet-Nam
- *E. Jagu* → ATER in Chemistry Paris-Saclay (FR)
- *G. Komlaga* → Lecturer in Pharmacognosy (University of Kumasi Ghana)
- *W. Mao* → Research engineer in Biochemistry in INRA-Jouy (FR)
- *P. Daligaux* → Post-doc in Chemistry in University of Orléans (FR)
- *A. Taravaud* → Searching for a post-doc position in Microbiology
- *S. Suyyagh-Albouz* → Biologist practitioner in Créteil Hospital (FR)

2013 2014 2015 2016 2017 2018 2019 2020 2021

PARACHEM 2013-18: SKILLS & RESEARCH AXES

Team 4- Antiparasite Chemotherapy

SCIENTIFIC FOCUS

LEAD SELECTION

DRUG TARGETING

Focusing parasite

From a target to a lead compound

Focusing host cell

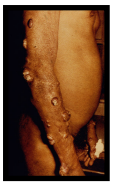
From vesicular trafficking to a drug candidate

Formulations → Cutaneous and visceral leishmaniasis

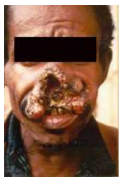
CUTANEOUS / MUCOCUTANEOUS FORMS

VISCERAL FORM

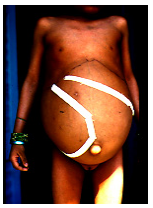
Localized cutaneous



Diffuse cutaneous



Muco-cutaneous



Antiparasite Chemotherapy

THERAPEUTIC INNOVATION

TECHNOLOGICAL TRANSFER

PARACHEM 2013-18: SKILLS & RESEARCH AXES

Team 4- Antiparasite Chemotherapy

SCIENTIFIC FOCUS

LEAD SELECTION

DRUG TARGETING

Skills

Cell/Molecular Biology/Biochemistry

From a target to a lead compound

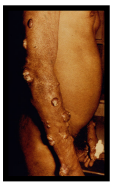
In vitro / in vivo Leishmania models

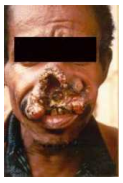
From vesicular trafficking to a drug candidate

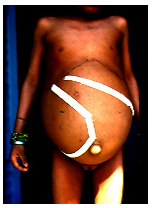
Formulations → Cutaneous and visceral leishmaniasis

CUTANEOUS / MUCOCUTANEOUS FORMS

VISCERAL FORM







Localized cutaneous

Diffuse cutaneous

Muco-cutaneous

Antiparasite Chemotherapy

THERAPEUTIC INNOVATION

TECHNOLOGICAL TRANSFER

PARACHEM 2013-18: SKILLS & RESEARCH AXES

Team 4- Antiparasite Chemotherapy

SCIENTIFIC FOCUS

LEAD SELECTION

DRUG TARGETING

From a target to a lead compound

In vitro / in vivo Leishmania models

From vesicular trafficking to a drug candidate

Formulations → Cutaneous and visceral leishmaniasis

Antiparasite Chemotherapy

THERAPEUTIC INNOVATION

TECHNOLOGICAL TRANSFER

SCIENTIFIC ENVIRONMENT

ICSN

UNIVERSITY OF OXFORD

I2BC

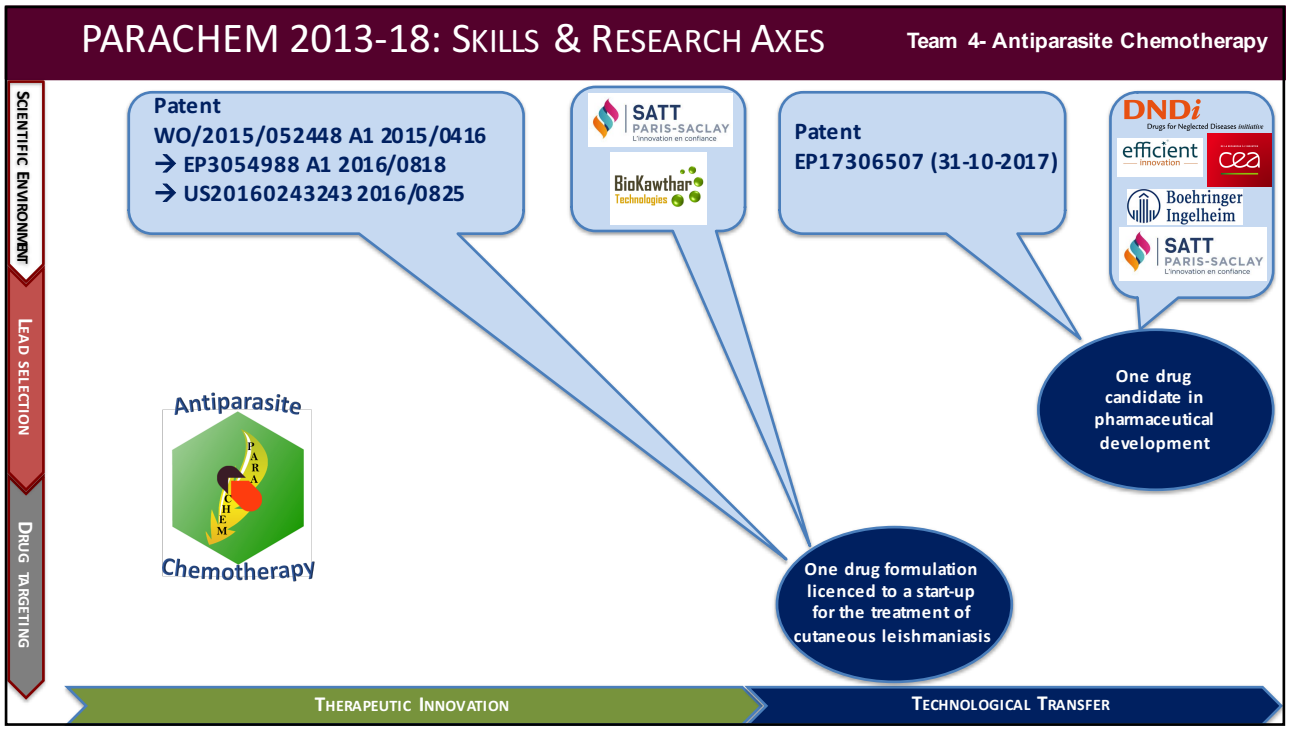
gsk GlaxoSmithKline

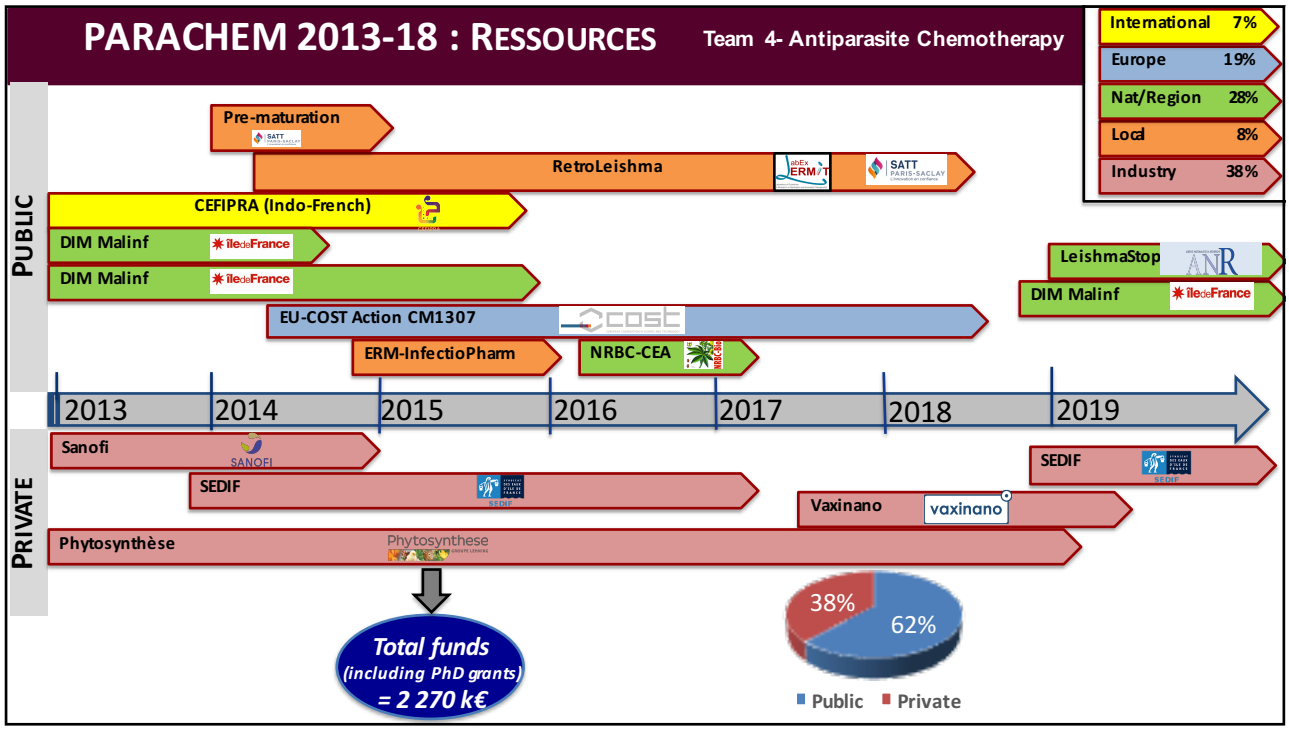
DRUGABILIS from molecules to drug candidates

cea

UFMG

SANOFI


PARACHEM 2013-18: SKILLS & RESEARCH AXES
Team 4- Antiparasite Chemotherapy
SCIENTIFIC ENVIRONMENT
LEAD SELECTION
DRUG TARGETING
Patent
WO/2015/052448 A1 2015/0416
→ EP3054988 A1 2016/0818
→ US20160243243 2016/0825
SATT PARIS-SACLAY
BioKawthar Technologies
Patent
EP17306507 (31-10-2017)
DNDi
efficient innovation
cea
Boehringer Ingelheim
SATT PARIS-SACLAY
One drug candidate in pharmaceutical development
Antiparasite Chemotherapy
One drug formulation licenced to a start-up for the treatment of cutaneous leishmaniasis
THERAPEUTIC INNOVATION
TECHNOLOGICAL TRANSFER


PARACHEM 2013-18 : RESSOURCES
Team 4- Antiparasite Chemotherapy
International 7%
Europe 19%
Nat/Region 28%
Local 8%
Industry 38%
PUBLIC
Pre-maturation
RetroLeishma
CEFIPRA (Indo-French)
DIM Malinf
DIM Malinf
EU-COST Action CM1307
ERM-InfectioPharm
NRBC-CEA
LeishmaStop
DIM Malinf
2013
2014
2015
2016
2017
2018
2019
PRIVATE
Sanofi
SEDIF
SEDIF
Vaxinano
Phytosynthèse
Total funds (including PhD grants) = 2 270 k€
38%
62%
Public
Private

PARACHEM 2013-18 : QUANTITATIVE DATA

Team 4- Antiparasite Chemotherapy

	Articles	Patents	Chapters	IF	FTE
Current contract	69 (+18)	2 (+1)	2 (+1)	4.25	5.5
Previous contract	51	1	1	3.94	5.5

Sci. Prod. + 35%

Average number of publications/Ph.D
3

Average number of publications/Post-doc
1.5

- ☛ *14 contracts signed*
- ☛ *22 seminars & invited lectures*

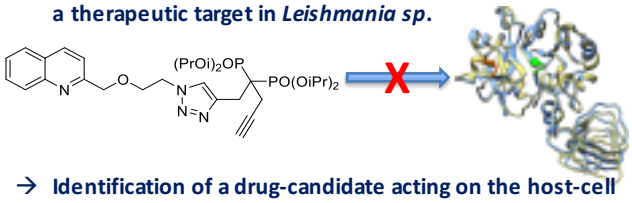
- ▪ *2 Diploma : Two PARACHEM permanent staffs have obtained their HDR (2017 and 2018)*
- ▪ *3 Distinctions: 2 Knights of Academic Palms, Academy of Pharmacy*

PARACHEM 2013-18 : HIGHLIGHTS

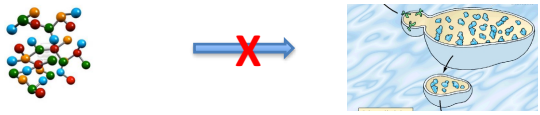
Team 4- Antiparasite Chemotherapy

Major scientific achievements

→ Identification of a promising inhibitor of GDP-MP, a therapeutic target in *Leishmania sp*.



→ Identification of a drug-candidate acting on the host-cell vesicular trafficking



→ Optimisation of a formulation for the treatment of cutaneous leishmaniasis and another one for visceral leishmaniasis

Scientific recognition

International

→ Coordination of an European consortium gathering 27 countries dedicated to antiparasite chemotherapy on the period 2014-2018
→ EU-COST Action CM1307

National

→ National Council of Universities
→ Academy of Pharmacy

Regional

→ Scientific Council of DIM1HEALTH

PARACHEM 2020-24 : PROJECT

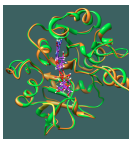
Team 4- Antiparasite Chemotherapy

	Current contract	Next contract
DRUG TARGETS	Getting a GDP-MP inhibitor	Diversifying scaffolds/ Docking and pharmacomodulations → drug candidate
DRUG TARGETS	Getting a drug candidate based on an inhibition of the host-cell vesicular trafficking	Deciphering the molecular mechanism of action of the drug candidate
DRUG RESISTANCE	No study in current contract (Old experience about drug resistance)	Study of drug resistance through a « One Health approach »
DRUG TARGETING	Getting formulations active *in vivo* against visceral and cutaneous leishmaniasis	Developing new formulations with drug candidates for the treatment of both visceral and cutaneous leishmaniasis

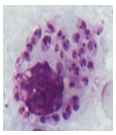
PARACHEM 2020-24 : PROJECT

Team 4- Antiparasite Chemotherapy

Main topic



Antileishmanial chemotherapy



DRUG TARGETS

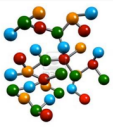
1- Identification and valorization of therapeutic targets to identify new antileishmanial drug candidates

DRUG RESISTANCE

2- Studying drug resistance through OneHealth approaches to understand resistance transmission and identify markers

DRUG TARGETING

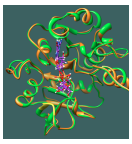
3- Drug targeting for an optimal drug delivery to reduce toxicity and prevent drug resistance emergence



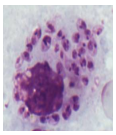
PARACHEM 2020-24 : PROJECT

Team 4- Antiparasite Chemotherapy

Main topic



Antileishmanial chemotherapy



DRUG TARGETS

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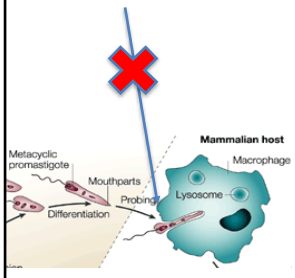
PARACHEM 2020-24 : PROJECT

Team 4- Antiparasite Chemotherapy

1- Identification and valorization of therapeutic targets to identify new antileishmanial drug candidates

Global objective → Finding antileishmanial drugs involved in host-parasite interactions

Virulence ↘



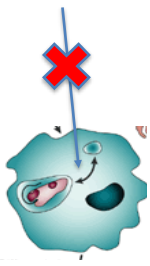
Targeting the parasite

To prevent host-cell recognition

Targeting the host cell

To prevent the parasite development inside

Development ↘



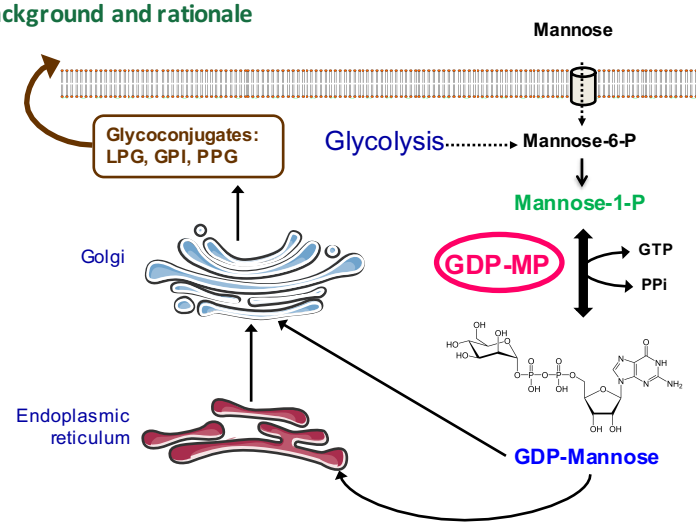
PARACHEM 2020-24 : PROJECT

Team 4- Antiparasite Chemotherapy

1- Identification and valorization of therapeutic targets to identify new antileishmanial drug candidates

1-1- Strategy focused on a parasite target → GDP-MP (Guanosine-Diphospho-Mannose-Pyrophosphorylase)

Background and rationale



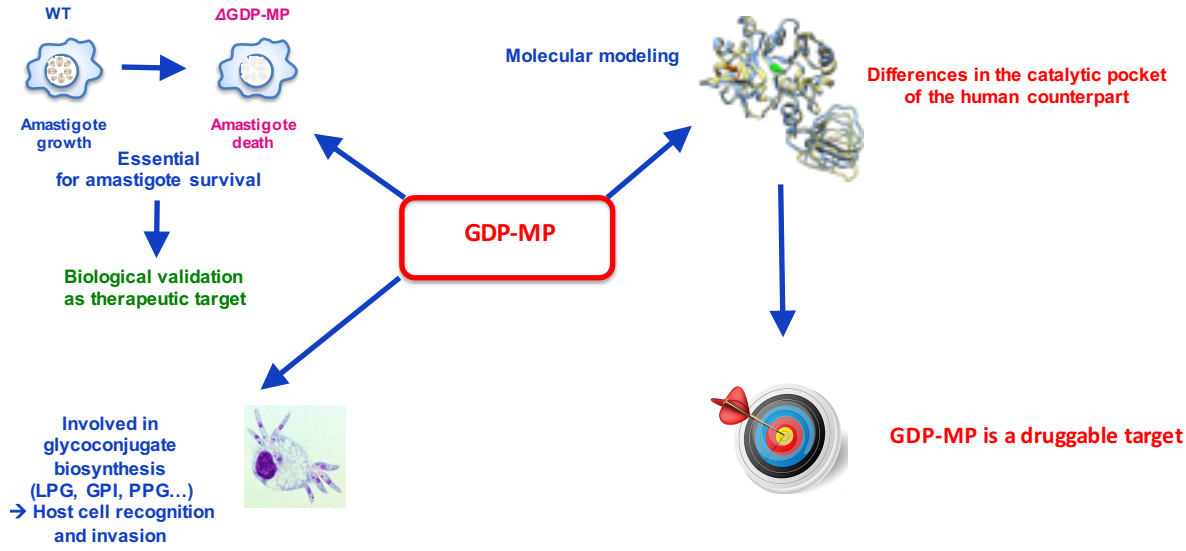
GDP-MP =
Guanosine Di-Phospho-D-Mannose Pyrophosphorylase

Biosynthesis of GDP-mannose in *Leishmania sp.*

PARACHEM 2020-24 : PROJECT

Team 4- Antiparasite Chemotherapy

1-1- Strategy focused on a parasite target → GDP-MP (guanosine-diphospho-mannose-pyrophosphorylase)



Pomel *et al.*, 2012, *Parasite*, 19: 63-70; Daligaux *et al.*, 2016, *Eur. J. Med. Chem.*, 107: 109-118

PARACHEM 2020-24 : PROJECT

Team 4- Antiparasite Chemotherapy

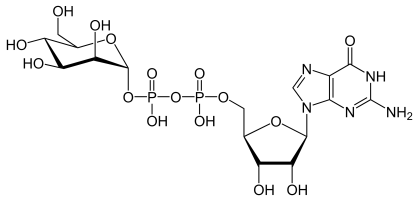
1- Identification and valorization of therapeutic targets to identify new antileishmanial drug candidates

1-1- Strategy focused on a parasite target

→ GDP-MP (guanosine-diphospho-mannose-pyrophosphorylase)

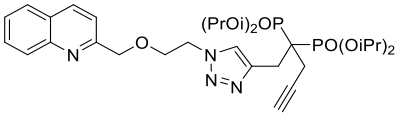
Present situation

From the substrate GDP-mannose

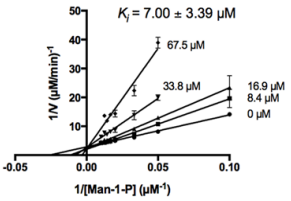


- Modelling
- Docking

-Synthesis of substrate analogs
- Biological evaluation



Compound 99



→ Competitive GDP-MP inhibitor

→ *In vitro*

→ IC_{50} = 0.6 µM on *L. donovani* intramacrophage amastigotes

(Mao *et al.*, 2017; *Sci. Rep.*)

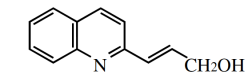
PARACHEM 2020-24 : PROJECT

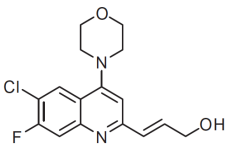
Team 4- Antiparasite Chemotherapy

1-1- Strategy focused on a parasite target → GDP-MP (guanosine-diphospho-mannose-pyrophosphorylase)

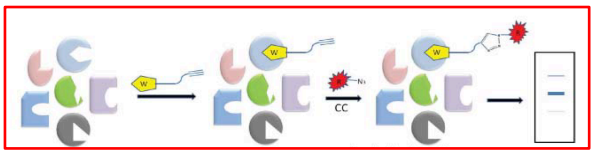
Project

Pharmacomodulations

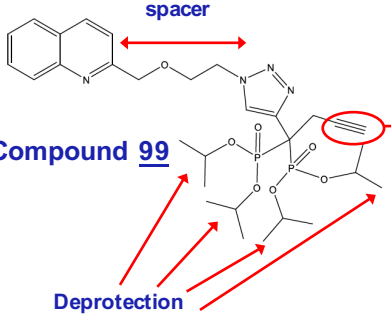




2-substituted quinolines



Modulation of the spacer



Compound 99

Deprotection

Target visualisation by « click chemistry »

PARACHEM 2020-24 : PROJECT

Team 4- Antiparasite Chemotherapy

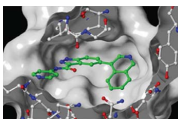
1-1- Strategy focused on a parasite target → GDP-MP (guanosine-diphospho-mannose-pyrophosphorylase) Project

***In vitro* and *in vivo* biological evaluation**

→ **HTS on human and leishmanial recombinant GDP-MPs**

→ Chemical libraries (BioCIS, ICSN, CNE, GSK and WIPO Re:Search BVGH) → New scaffolds

→ ***In vitro* and *in vivo* evaluation against *Leishmania donovani***



→ Hit identification

→ **Docking analysis of identified hits**

→ Pharmacomodulations → to optimize affinity and specificity for the target, as well as antileishmanial activity → Lead

→ **GDP-MP functional analysis**

→ To be studied in several *Leishmania* species

to determine its biochemical importance in the main *Leishmania* species

→ Functional analysis → knockout (CRISPR-Cas9) → analysing the phenotype of the GDP-MP KO

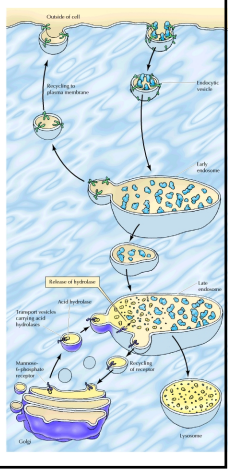
→ Optimizing the strategy of specific inhibitor development

PARACHEM 2020-24 : PROJECT

Team 4- Antiparasite Chemotherapy

1- Identification and valorization of therapeutic targets to identify new antileishmanial drug candidates

1-2- Identification of target(s) of a drug-candidate affecting the vesicular trafficking of the host cell preventing the parasitophorous vacuole formation



Background and rationale

Identifying a drug-candidate for the treatment of visceral leishmaniasis having the following characteristics:

→ **An original mechanism of action that interferes with vesicle trafficking in host-cell impairing the development of the vacuole in which the parasite proliferates**

→ **No direct and intrinsic antiparasitic activity on the parasite itself in order to reduce the risk of drug resistance**

→ **No toxicity on the host-cell**

→ **A suitable druggability for oral or intravenous administration**

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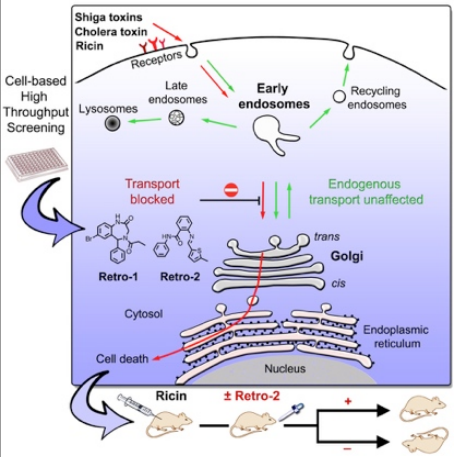
Team 4- Antiparasite Chemotherapy

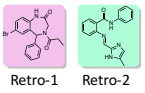
1-2- Identification of target(s) of a drug-candidate affecting the vesicular trafficking of the host cell preventing the parasitophorous vacuole formation

Background and rationale

Identification by HTS of compounds selectively blocking retrograde transport of toxins

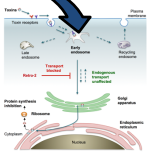


Screening of 20,000 compounds

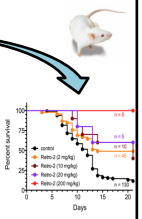


Retro-1 Retro-2

2 active molecules



Stechmann *et al.*, **Cell** 2010

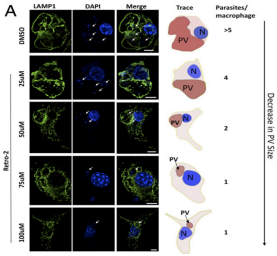


POC in mice

Compounds inhibiting the vesicular trafficking were able to

→ **impair the formation of the parasitophorous vacuole**

→ **inhibit the development of the parasite inside**



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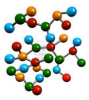
Team 4- Antiparasite Chemotherapy

1-2- Identification of target(s) of a drug-candidate affecting the vesicular trafficking of the host cell preventing the parasitophorous vacuole formation

Present situation



Getting an antileishmanial drug-candidate (compound ABMA-2) and one back-up (compound ABMA-3) from a library of 300 compounds selected as affecting vesicular trafficking

Compound ABMA-2

→ ***In vitro*** **activity**
- → IC_{50} = 40 nM on intramacrophage amastigotes
- → Selectivity Index = CC_{50}/IC_{50} = 5625
- → RetroLeishma Index= IC_{50} amas axenic/IC_{50} intramacrophage amastigotes = 240

→ Active ***in vivo*** on the *L. infantum*/BALB/c mice model at 10 mg/kg/day x 5 days by oral and iv routes (60% reduction of parasite burden)

→ No ***in vivo*** toxicity at 100 mg/kg

→ Microsomal stability (human): 46% at 45 min

→ PK
- → Per os at 10 mg/kg: C_{max}=80 ng/ml in 15 min
- → Iv: at 1 mg/kg: Bioavailability 1%
- → $T_{1/2}$ = 4h

PARACHEM 2020-24 : PROJECT

Team 4- Antiparasite Chemotherapy

1-2- Identification of target(s) of a drug-candidate affecting the vesicular trafficking of the host cell preventing the parasitophorous vacuole formation

Post-doc fellow recruited on December 2018

ANR LeishmaStop beginning on January 2019

Project

The goals of the study consist in:

- **Understanding the basis for cellular protection against *Leishmania* given by ABMA-2 and ABMA-3 by confocal microscopy and videomicroscopy**
  → Effect on the parasitophorous vacuole development using GFP expressing parasites and markers of different steps of the endo-lysosomal pathway (such as EEA1, Rab7, Lamp1, etc.)

- **Identifying drug targets of ABMA-2 and ABMA-3 by proteomic analysis and siRNA libraries**

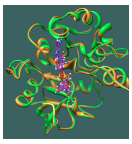
- **Identifying the intracellular localization of the targets by confocal microscopy**
  - in infected macrophages during cell invasion and intracellular parasite development
    → If altered localization after treatment → confirmation of involvement in the mechanism of action of the compounds

- **Functional analyses of the identified target genes**
  → Knockout using CRISPR-Cas9, or by knockdown using si/shRNA specific of the target genes
  → Phenotype analysis of these cell lines and as well as their susceptibility to ABMA-2/ABMA-3

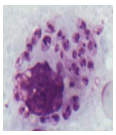
PARACHEM 2020-24 : PROJECT

Team 4- Antiparasite Chemotherapy

Main topic



Antileishmanial chemotherapy



DRUG TARGETS

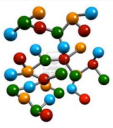
1- Identification and valorization of therapeutic targets to identify new antileishmanial drug candidates

DRUG RESISTANCE

2- Studying drug resistance through OneHealth approaches to understand resistance transmission and identify markers

DRUG TARGETING

3- Drug targeting for an optimal drug delivery to reduce toxicity and prevent drug resistance emergence



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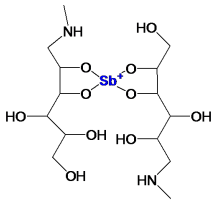
Team 4- Antiparasite Chemotherapy

2- Studying drug resistance through OneHealth approaches to understand resistance transmission and identify markers



Background, rationale and objectives

→ Development of drug resistance in the field

→ Resistance to antimonials

→ Main molecular mechanisms described from isolates/clones

→ What about the influence of the successive hosts (mammal and insect) in the transmission of drug-resistant parasites ?

Project

→ Developing a OneHealth concept for studying drug resistance parameters by modelling a natural life cycle of cutaneous leishmaniasis

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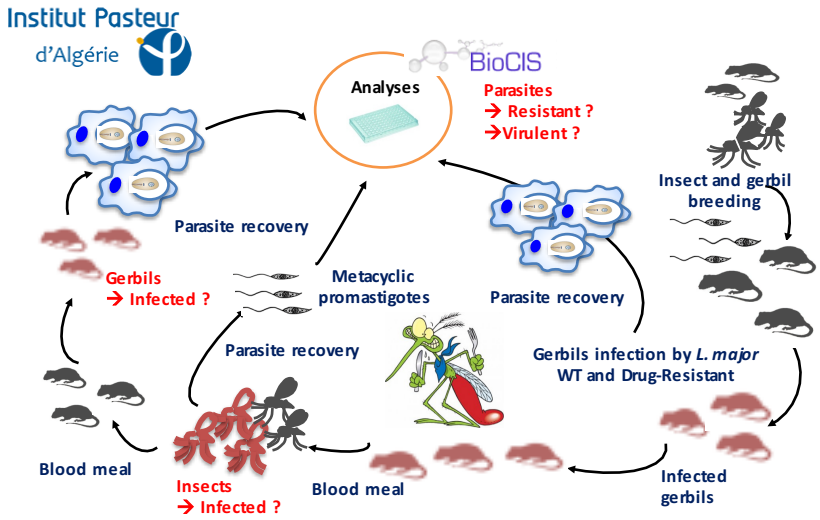
Team 4- Antiparasite Chemotherapy

➢ Setting up of a natural life cycle with *Leishmania major*, its host *Meriones shawii* and the insect vector *Phlebotomus papatasi* collected in gerbil holes in Algeria (Collaboration with Algeria Pasteur Institute, Dr. Z. Harrat)



➢ Phenotype analysis of selective pressions from host change on parasite biological parameters:

- ➢ *In vitro* et *in vivo* fitness
- ➢ Virulence
- ➢ Variation of resistance intensity
- ➢ Stability/reversibility of resistance

➢ Molecular analysis of levels of amplification and expression of some molecular markers:

- ➢ MRPA (Multidrug Related Protein A)
- ➢ MDR1 (Multidrug Resistance 1)
- ➢ TP (Tryparedoxine Peroxidase)

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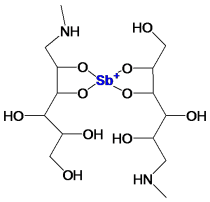
Team 4- Antiparasite Chemotherapy

2- Studying drug resistance through OneHealth approaches to understand resistance transmission and identify markers

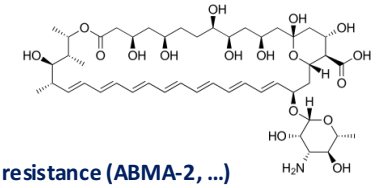
The natural life cycle of *L. major* → Proposed as a predictive model to measure the resistance parameters for any drug candidate in development

→ First application to:
  → Meglumine antimoniate



→ Next applications

  → Amphotericin B



  → Any drug candidate to assess the risk of drug resistance (ABMA-2, ...)

PARACHEM 2020-24 : PROJECT

Team 4- Antiparasite Chemotherapy

Conclusion

→ Main PARACHEM objectives

  → Reinforcing relevant international collaborations

  → Participating to the development of new antileishmanial drug candidates /formulations