EVALUATION AND ANALYSIS OF THE MECHANISM OF NEW ENDOPEROXIDES FOR THE TREATMENT OF LEISHMANIASES AND MALARIA

Institution **Université Paris-Saclay**
Doctoral school **Innovation thérapeutique : du fondamental à l'appliqué**
Pole of doctoral School **Microbiologie et Thérapeutique anti-Infectieuses**
Speciality **Microbiologie**
Research Unit **Biomolécules : Conception, Isolement et Synthèse**
Supervision of the thesis Sébastien POMEL et Isabelle FLORENT
Beginning of the thesis le **1st october 2023**
Deadline for application **31st july 2023**

Keywords

Endoperoxides, *Leishmania*, *Plasmodium*, mechanisms of activation and action

Supervision modalities

70% Sébastien POMEL (Université Paris-Saclay, Faculté de Pharmacie, UMR CNRS 8076, BioCIS, Orsay, France) et 30% Isabelle FLORENT (Muséum National d'Histoire Naturelle, UMR CNRS 7245, MCAM, Paris, France)

Summary of the thesis project*

Leishmaniases are neglected tropical disease transmitted by the protozoan parasite *Leishmania.* Only few antileishmanial treatments are available, and have all issues of high toxicity and drug resistance. In this context, there is a crucial need for the development of new antileishmanial series. Derivatives of the iconic antimalarial natural product artemisinin, belonging to endoperoxide family, constitute a singularly interesting class of molecules for the treatment of leishmaniasis as the molecules have previously shown to be active against several pathogenic organisms including *Leishmania*, besides *Plasmodium* the agent of malaria. In the present thesis project, novel endoperoxides synthesized by our collaborators in the ANR consortium will be evaluated *in vitro* on both *L. donovani* and *L. major*, responsible of visceral and cutaneous leishmaniasis, respectively, as well as on *P. falciparum*. The cytotoxicity of the compounds will also be evaluated to determine their selectivity indexes. The best compounds will be further evaluated *in vivo* on murine models of visceral and cutaneous leishmaniases, and of malaria. The mechanisms of compounds activation and of action within the parasite will also be investigated in confocal microscopy and eventually by transcriptomics/proteomics with the most active compounds.

Context *

Leishmaniases are neglected tropical diseases of human and veterinary importance causing thousands of human and canine deaths per year (Burza et al., 2018). Among the leishmaniases, visceral leishmaniasis caused by *L. infantum* is present in tropical regions, as well as in the Mediterranean areas, including the south of France, whereas *L. major*, responsible of cutaneous leishmaniasis, is mainly present in Africa and Middle East. There is a high need to develop novel therapies due to the lack of human

vaccines, the poor efficacy of canine vaccine, the toxicity of current antileishmanial drugs and drug resistance that is well documented or at risk (Roatt et al., 2020). In this context, endoperoxides constitute a singularly interesting class of molecules, offering promising perspectives to fight against *Leishmania* parasites. Indeed, derivatives of the iconic antimalarial natural product artemisinin have been shown to be active against several pathogenic organisms including *Leishmania sp.*, besides *Plasmodium* species, the agent of malaria (Ho et al., 2014; Cabral et a. 2020). However, this trail remains largely under-explored, notably because of the low diversity of molecules currently available that are being tested. Regarding malaria, after a decline in the number of cases over the past ~20 years, rate of recoil has been slowed down or reversed. A rise of cases and mortality has recently been observed, justifying the development of new compounds despite the introduction of the first vaccine, especially in a context where resistance to currently available drugs has been spread to African region, where more than 90% of fatal cases are located (World Malaria Report 2021). In the current project, we seek novel specific, potent and cheap compounds that could be used to fight leishmaniases and/or malaria. A new method of endoperoxide synthesis, developed by our collaborators chemists of the ANR project in the Ecole Polytechnique and the Ecole Normale Supérieure, will allow to open a new diversity panel of endoperoxide compounds. The PhD student will then evaluate their antileishmanial and antimalarial activities and analyze the mechanisms of activation and action of the best compounds.

Objectives*

The thesis project has the following objectives:

1) Evaluate the *in vitro* antileishmanial activities of the novel endoperoxides on axenic and intramacrophage amastigotes of *L. donovani* and *L. major*.
   In a first step, the PhD student will evaluate the *in vitro* antileishmanial activities of the endoperoxide compounds on both *Leishmania donovani* and *Leishmania major*, responsible of visceral and cutaneous leishmaniasis, respectively. The evaluation of the antileishmanial activities will be performed on axenic and intramacrophage amastigotes, the infectant form of the parasite found in mammalian. Antileishmanial assays will be performed at the Université Paris-Saclay.
2) Evaluate the *in vitro* antimalarial activity of the novel endoperoxides on *Plasmodium falciparum*.
   In vitro assays will be performed against asynchronous and synchronous cultures of chloroquine-resistant (FcB1) and chloroquine-sensitive (3D7) strains of *P. falciparum*, at the erythrocyte stage. Antimalarial assays will be performed at the Museum National d'Histoire Naturelle.
3) Evaluate the cytotoxicity of the compounds
   The cytotoxicity values will allow to determine the selectivity indexes of the compounds.
4) Evaluate the *in vivo* antileishmanial activity of the best compounds on murine models of visceral and cutaneous leishmaniases. If the *in vitro* results are judged sufficiently interesting, complementary in vivo studies could also be performed on murine models of malaria. Antileishmanial assays will be performed at the Université Paris-Saclay and antimalarial assays will be performed at the Museum National d'Histoire Naturelle.
5) Determine the mechanism of activation of the best compounds

The mechanism of activation of the most active compounds will be further investigated *in vitro* using inhibitors of different factors previously characterized to be essential for artemisinin activation (*e.g.* free radicals, iron, …).

6) Determine the mechanism of action of the best compounds
   The mechanism of action of the most active compounds will be further investigated *in vitro* using inhibitors of factors previously characterized to be essential for the mechanism of action of artemisinin (*e.g.* heme oxygenase …). The type of cell death induced by the compounds will also be investigated and compared with artemisinin, known to induce apoptosis in *Leishmania* (Sen et al., 2007).
7) Determine drug resistance characteristics for the best compounds in both *L. donovani* and *L. major*: kinetics of appearance, maximal concentration, reversibility, cross-resistance with antileishmanial reference drugs?

Methods

- Determination of *in vitro* antileishmanial activities on axenic and intramacrophage amastigotes of *L. donovani* and *L. major*
- Determination of *in vitro* antimalarial activities.
- Determination of cytotoxicities on RAW264.7 macrophages and on AB943 fibroblasts
- Determination of the *in vivo* activities on murine models of visceral and cutaneous leishmaniases
- Determination of the *in vivo* activities on a murine model of malaria.
- Analysis in confocal microscopy of the mechanism of activation and the mechanism of action of the compounds using inhibitors of different factors (free radicals, iron, heme oxygenase,…) previously shown to be essential for artemisinin

Expected results

Novel endoperoxides with antileishmanial and/or antimalarial activity(ies) are expected. The most active compounds will be evaluated *in vivo* in murine models of visceral and cutaneous leishmaniasis, and of malaria. If a compound shows interesting *in vivo* activities, the compound will be patented, without delaying publication process, and will be proposed for further development for the treatment of leishmaniasis and/or malaria. All the scientific data obtained within the project will be published in journals with international readership and communicated in national and international congresses.

References

Burza S., Croft S.M. and Boelaert M. (2018). Leishmaniasis. *Lancet* 392(10151): 951-970.

Cabral L.I.L., Pomel S., Cojean S., Amado P.S.M., Loiseau P.M. and Cristiano M.L.S. (2020). Synthesis and antileishmanial activity of 1,2,4,5-tetraoxanes against *Leishmania donovani*. *Molecules*, 25(3): 465.

Ho W.E., Peh H.Y., Chan T.K. and Wong W.S.F. (2014). Artemisinins: pharmacological actions beyond antimalarials. *Pharmacol. Ther.*, 142(1): 126-139.

Roatt B.M., de Oliveira Cardoso J.M., De Brito R.C.F., Coura-Vital W., de Oliveira Aguiar-Soares R.D. and Reis A.B. (2020). Recent advances and new strategies on leishmaniasis treatment. *Appl. Microbiol. Biotechnol.*, 104(21): 8965-8977.

Sen R., Bandyopadhyay S;, Dutta A., Mandal G., Ganguly S., Saha P. and Chatterjee M. (2007). Artemisinin triggers induction of cell-cycle arrest and apoptosis in *Leishmania donovani* promastigotes. *J. Med. Microbiol.* 56(Pt9): 1213-1218.

World Malaria Report 2021 published by the World Health Organization: https://www.who.int/publications/i/item/9789240040496

Scientific material and financial conditions

Material conditions: Laboratory Safety Level 3 (L3) in the Université Paris-Saclay allowing culture and experimentations on *L. major* and *L. donovani*, and Laboratory Safety Level 2/3 (L2/3) in the Muséum National d'Histoire Naturelle allowing culture and experimentations of *P. falciparum*.

Financial conditions: ANR ARDIROX

Type of funding

ANR

Beginning of the thesis project

01/10/2023

End of the thesis project

30/09/2026

Employer

Université Paris-Saclay

Profile and skills required*

Confirmed skills in *in vitro* cell culture and, if possible, animal experimentations. Skills in cell biology and biochemistry would be appreciated. Skills in parasitology are not pre-required.

Contacts

Sébastien Pomel: sebastien.pomel@universite-paris-saclay.fr
Isabelle Florent: isabelle.florent@mnhn.fr