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PALUKILL: Toward a novel mode of action-acting antimalarial drug candidate targeting multi-stages of *Plasmodium falciparum*

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Abstract

We reported the synthesis of original 2-aminothienopyrimidinones, among them, the lead-compound M1 showed excellent *in vitro* activities toward both the erythrocytic and the hepatic stages of *P. falciparum* and *P. yoelii*. Furthermore, a strong effect on gametocytes reduced the number of oocysts and sporozoites in the Anopheles vector, demonstrating a high potential as a transmission blocker. M1 exerts its antiplasmodial activity by a different mechanism of action than marketed/known antimalarials. Its *in vivo* activity presently suffers from low water-solubility and short microsomal stability. The chemical scaffold will be redesigned to improve the pharmacokinetic parameters. To elucidate the mechanism of action, a chemo-proteomic (affinity chromatography) and a phosphoproteomic approaches will be undertaken to allow further rational designing. This integrative chemical biology project aims to access to a new multi-stage acting antimalarial drug candidate with a novel mechanism of action.

Positioning versus the state of the art

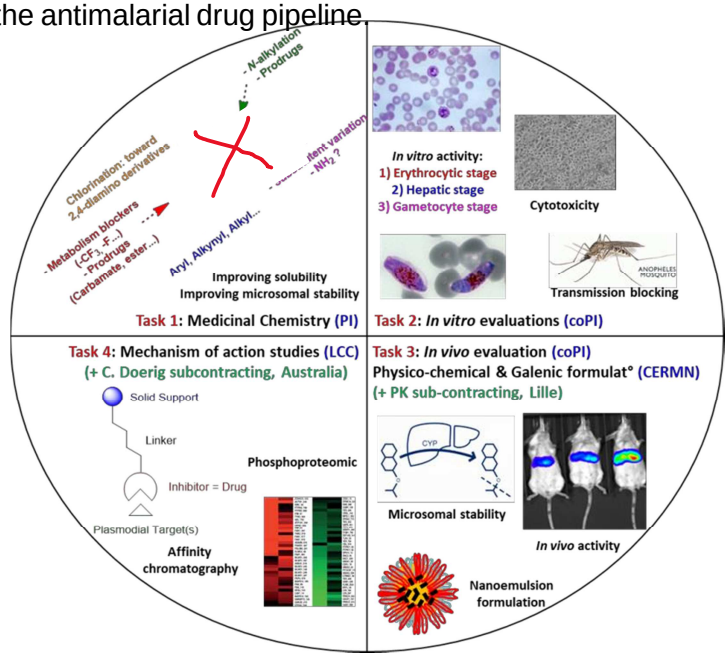
To date, there is no commercial antimalarial drug active on the sexual stage of *Plasmodium*. The current antimalarial pipeline of Medicines for Malaria Venture (international consortium fighting against malaria), contains only 7 multi-stage molecules with a novel mechanism of action in 2018: 3 in pre-clinical studies and 4 in clinical studies [1]. As an example, out of the 4 molecules in clinical studies with a novel multi-stage acting mechanism of action, MMV048 is an aminopyridine derivative active against artemisinin-resistant strains and all stages of the parasite (including gametocytes) that originally inhibits the parasite's phosphatidylinositol 4-kinase [2]. Regarding antimalarial hit-compounds with new mechanisms of action, a 2010 publication reported the identification by GSK of thousands of antiplasmodial hit-molecules probably targeting plasmodial kinases, including thienopyrimidinone derivatives [3]. Two recent publications also highlighted 2,4-diaminothienopyrimidine derivatives as promising antimalarial lead-compounds without information regarding their mechanism of action [4-5]. In this context, studying and developing original thienopyrimidinone derivatives as potential kinase inhibitors with multi-stage acting properties against *Plasmodium* appears of great interest in order to strengthen the antimalarial drug pipeline.

a) Research project for the period of requested support - specific aims

PALUKILL is a drug design project which ambitions to:

1) evelop a novel antimalarial drug candidate starting from molecule M1 which has an excellent *in vitro* profile with multi-stage activity against *Plasmodium*, by improving the metabolic stability and increasing the aqueous solubility of the scaffold to obtain a drug-like molecule suitable for preclinical evaluation (*in vivo* activity, non-toxic, non-mutagenic, good pharmacokinetic and physicochemical properties)

2) Discover its plasmodial target(s) by 2 complementary approaches: Phosphoproteomic and chemoproteomic (affinity chromatography on the immobilized inhibitor M1)



- significance, importance, novelty in the field

Malaria is the first cause of death due to a parasitic infection. The worldwide spreading of the artemisinin-resistant strains would turn into a major public health concern as there is no therapeutic option today [6-7]. To bypass parasitic resistance, the first criterion for a novel antimalarial drug is to present an original mechanism of action and be active on the artemisinin-resistant strains [8-9]. Moreover, considering that malaria is a transmissible disease depending on the sexual multiplication of the parasite in the *Anopheles* mosquito, it is also crucial to develop molecules which target a) the sexual stage of the parasite (gametocytes), to block transmission, and b) the asexual stages of the parasite: hepatic stage to prevent infection and erythrocytic stage to cure the patients [8-10]. To date, there is no commercial antimalarial drug

active on the sexual stage of *Plasmodium*. The current antimalarial pipeline of Medicines for Malaria Venture, contains only 7 multi-stage molecules with a novel mechanism of action in 2018: 3 in pre-clinical studies and 4 in clinical studies [1]. In this context, PALUKILL project aims at studying and developing original thienopyrimidinone derivatives as potential plasmodial kinase inhibitors with multi-stage acting properties against *Plasmodium*. Significant preliminary results [11-14] strongly suggest that this project could lead to a novel antimalarial preclinical drug candidate in order to strengthen the antimalarial drug pipeline.

The candidate will be mainly in charge of the studies concerning the efficacy at the pre-erythrocytic level.

Hepatic stages *in vitro* assays will be performed using primary murine (*P. yoelii,* 17XNL strain expressing GFP-Luciferase [13,15] or human hepatocytes (*P. falciparum* [16] & *P. vivax* [17]). Human hepatocytes will be isolated from liver segments taken after oral informed consent from adult patients undergoing partial hepatectomy as part of their medical treatment (Service de Chirurgie Digestive, Hépato-Bilio- Pancréatique et Transplantation Hépatique, Hôpital Pitié-Salpêtrière, Paris, France). [18]

Depending on the *in vitro* results, molecules of interest will be tested in mice infected with *P. yoelii* [14] then in humanized TK Nog mice infected with *P. falciparum* or *P. vivax*, a model established in CIMI laboratory [19] in order to identify a drug candidate for preclinical development [20].

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