Characterization of the *Plasmodium falciparum* replication licensing factor, Pfmcm6, a substrate of two *Plasmodium falciparum* cyclin-dependent kinases, Pfpk6 and Pfmrk

Fredrick Lunyagi Eyase

A thesis submitted in partial fulfillment for the degree of Doctor of Phylosophy in Molecular Medicine in the Jomo Kenyatta University of Agriculture and Technology

2012

ABSTRACT

Malaria remains a big challenge the world-over especially in Sub-sahara Africa. Since there is no malaria vaccine currently, chemotherapy is the only curative intervention option available. At present, there are few known drug targets in the parasite. Worse still, most of these targets have mutated leading to widespread resistance to current drugs. There is therefore an urgent need for discovery and development of new antimalarial chemotherapeutic interventions and targets. An ñAchilles' heelò in the parasite biology that could be exploited for chemotherapeutic intervention is the parasite cell cycle mechanisms. Thus, recent malaria drug discovery efforts have focused on targeting parasite-derived cyclin-dependent kinase proteins as potential new drug targets. The aim of this study was therefore to to establish the functional relationships between two of these kinases, PfMRK and PfPK6, with a putative *P. falciparum* replication licensing factor (PfRLF) with a view of establishing their potential as drug targets.

A non-radiactive kinase assay was used to assess phosphorylation capacities of PfMRK and PfPK6 on PfRLF. Bioinformatic tools were also used to characterize PfRLF. Kinase inhibition assays using locally sourced natural products as inhibitors to PfMRK and PfPK6 were carried out to unravel the drug target potential of these kinanses. Bioinformatic analyses revealed that the putative PfRLF is actually the *P.falciparum* Minichromosome Maintenance 6 (PfMCM6) protein involved in replication. The kinase assays established that both PfPK6 and PfMRK phosphorylate PfMCM6 *in vitro* and that PfMAT1 enhances PfMRK activity on PfMCM6. Enhancement of PfMRK activity by PfMAT1 confirms previous observations that

PfMRK is the plasmodial CDK7 equivalent. The Kinase inhibition assays showed that the Abyssinone class of flavonoids actively inhibits the activity of PfMRK and PfPK6 on PfMCM6.

This study has confirmed the potential of PfMRK and PfPK6 as drug targets for malaria treatment. Flavonoids, especially prenylated abyssinones are lead compounds for antimalarials targeting these two kinases. Modification and further characterization of these lead compounds may lead to therapeutic agents with higher efficacy and specificity.