# ABSTRACT OF PH.D. THESIS

**Title of Ph.D. Thesis**: "*Plasmodium falciparum* food vacuole protein as a novel drug target"

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The present thesis comprises of TWO CHAPTERS. The aim of the first chapter is to design and develop antimalaria inhibits against falcipain-2, a papain families of cysteine protease. In second chapter we study about the characterization of ankyrin repeats containing protein Pf10b antigen.

Malaria remains one of the leading causes of morbidity and mortality in the world leading to over a million deaths every year. The clinical symptoms of malaria are attributed to the blood stage of the parasite. During the blood stage, the parasite resides in the parasitophorous vacuole in the infected RBC. The two most prevalent species of *Plasmodium* that cause malaria in humans are *P.falciparum* and *P.vivax*. Severe disease and resistance to antimalarials has been documented for both species and efforts to controls malaria have become more challenging in recent years due to widespread dug resistance. To date, drug resistance to artemisinins, the key compounds in artemisinin combination therapies (ACTs), has been detected in at least 4 countries in Southeast Asia. Overall, drug resistance to nearly all existing antimalarial agents is of great concern; development of novel antimalarial agents is of wide interest. There is also a need to identify novel molecular targets for the development of clinical agents yet.

Falcipain-2 is a papain family cysteine protease and an emerging antimalarial drug target. A pseudotripeptide scaffold I was designed using in silico screening tools and the three-dimensional structures of falcipain-2, falcipain-3, and papain. This scaffold was investigated at four positions, T1, T2, T3, and T03, with various targeted substitutions to understand the structure–activity relationships. Inhibitor synthesis was accomplished by first obtaining the appropriate dipeptide precursors with common structural components. The pyrrolidine moiety introduced interesting rotamers in a number of synthesized molecules, which was confirmed using high-temperature $^{1}H$ NMR spectroscopy. Among the synthesized compounds, 61, 62, and 66 inhibited falcipain-2 activity with inhibition constants (Ki) of 1.8 ± 1.1, 0.2 ± 0.1 and 7.0 ± 2.3 μM, respectively. A

group of molecules with a pyrrolidine moiety at the T2 position (68, 70, 71, 72, and 73) also potently inhibited falcipain-2 activity (Ki = 0.4 ± 0.1, 2.5 ± 0.5, 3.3 ± 1.1, 7.5 ± 1.9, and 4.6 ± 0.7 μM, respectively). Overall, compound 74 exhibited potent anti-parasitic activity (IC50 = 0.9 ± 0.1 μM), corresponding with its inhibitory activity against falcipain-2, with a Ki of 1.1 ± 0.1 μM. Compounds 62 and 67 inhibited the growth of the drug resistant parasite Dd2 with better efficacy, and compound 74 exhibited a 7- to 12-fold higher potency against Dd2 and MCamp isolates, than the laboratory strain (3D7). These data suggest that this novel series of compounds should be further investigated as potential antimalarial agents.

A 33-residue ankyrin repeat (ANK) motif is one of the most widely existing repeat motifs in eukaryotes including parasitic organisms and microbes. Here, we characterize one of the *Plasmodium* ANK protein referred as Pf10b antigen (PF3D7_1021900) that possess two ANK repeat domains (Pf10bANK) and a **P**HAX_**R**NA **b**inding domain (Pf10bPRB). Immunostaining of infected erythrocytes using anti-Pf10b antibody revealed that Pf10b antigen localizes within the parasite as well as in infected RBC membrane. Immunoprecipitation analysis of asexual blood stage parasite extract using anti-Pf10b antigen antibodies revealed that Pf10b antigen is associated with the components of PfRhopH–Clag9 complex that includes Pf14-3-3I, PfRhopH3, PfClag9, PfRhopH2 proteins as well and this complex is associated with RBC membrane proteins. Protein-protein interaction tools includes ELISA based binding analysis and far-western analysis confirmed the interaction between Pf10b antigen and Pf14-3-3I or PfClag9 proteins. Overall, the results describe the existence and diversity of the ankyrin repeat-containing proteins in *Plasmodium* and also provide evidence to show that Pf10b antigen, an ANK protein is a component of PfRhopH-Clag9 complex.