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REVIEW ARTICLE

To quest new targets of *Plasmodium* parasite and their potential inhibitors to combat antimalarial drug resistance

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Abstract

Malaria remains a global health challenge with significant mortality and morbidity annually, with resistant parasite strains complicating treatment efforts. There is an acute need for novel antimalarial drugs that can put a stop to the future public health crisis caused by the multi-drug resistance strains of the *Plasmodium* parasite. However, the discovery of these new components is very challenging in the context of the generation of multi-drug resistance properties of malaria. The novel drugs also need to have several properties involving enhanced therapeutic prospects, successful treatment capabilities, and novel mechanisms of action that will forestall the resistance. To successfully achieve this aim researchers are trying to focus on exploring promising malaria targets. Various approaches have been made for the development of drugs for malaria including the remodelling of existing drugs and the development of novel inhibitors which acts on new targets. Advancement in the study provides more information on the biology of parasites and the new targets which help in the development of novel drugs. The present review focuses on the study of novel targets of malaria parasites and subsequent inhibitors of those particular targets. Some of these targets include malarial protease, various transporter proteins, enzymes involved in the synthesis of DNA, and nucleic acids like dihydroorotate dehydrogenase, dihydrofolate reductase, apicoplast and dihydropteroate synthase. Other potential targets are also included in this review such as isoprenoid biosynthesis, farnesyl transferase of parasite, *P. falciparum* translational elongation factor 2, and phosphatidyl inositol 4 kinase. These promising targets have also been summed up along with their corresponding inhibitors for combating multi-drug resistance malaria.

Keywords Malaria · Multi-drug resistance · *Plasmodium* parasite · Dihydrofolate reductase · Phosphatidyl inositol 4 kinase

Introduction

Malaria: major health concern

Malaria is a female *Anopheles* mosquito-borne parasitic disease. It is a major global public health problem, causing a significant number of deaths and infections each year. Malaria has a great impact on global morbidity and mortality rates, but it is neglected and it has given little importance. Over 400,000 deaths and 160 million infections annually are consistent with the estimation provided by various health organizations. *Plasmodium falciparum* and *Plasmodium vivax* are deadly species of the malaria parasite and are responsible for the majority of malaria-related deaths and severe cases. However, other species like *Plasmodium ovalecurtisi*, *Plasmodium ovalewallikeri*, and *Plasmodium malariae* can also cause malaria and contribute to the overall burden of the disease. In rare cases, zoonotic infections caused by species like *Plasmodium knowlesi*, *Plasmodium*

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cynomolgi, and *Plasmodium simium* can occur in specific geographical areas.

Malaria parasite (disease-causing), through vectors, can arrive in the human body cells but remain inactive for a long time. Initially, these parasites mature and then proliferate inside the hepatic cells and RBCs of the host body which causes the symptoms of malaria in humans. Common symptoms of malaria are severe fever along with nausea, vomiting, headache, abdominal pain, diarrhea, anaemia, convulsions, coma, and bloody stool in order of progression from moderate to severe disease (Nandi et al. 2022).

Morbidity and mortality rate

As per WHO's latest update, there are 85 malaria-endemic countries in 2020. According to the world malaria report, 247 million malaria cases were found in 2021, and 245 million malaria cases in 2020 globally and 5% increase in cases in 2020 as compared to 2019. Number of malaria deaths are 619,000 in 2021, 625,000 in 2020 and 558,000 in 2019. The mortality increased 12% in 2020 as compared to 2019. The COVID-19 pandemic caused an abnormal rise in malaria cases and deaths in 2020 as compared to 2019 (WHO 2022).

Virulence of the malaria parasite

In malaria, the sporozoite forms are the infective stage of *Plasmodium* sp. The disease is transmitted by the bite of the female *Anopheles* mosquito and the sporozoite stage is inoculated from the salivary glands of the mosquito into the subcutaneous tissues and finally blood-stream. The needle-shaped sporozoite (10–12 µm in length) circulates through blood shortly and then traverses into sinusoidal Kupffer cells and hepatocytes in the liver with the help of sporozoite microneme protein which is essential for cell traversal (SPECT) or perforin like protein 1 (PLP1). The thrombospondin domains on the circumsporozoite protein (CSP) and the thrombospondin-related adhesive protein (TRAP) help the sporozoite in binding with heparan sulfate proteoglycans on the surface of hepatocytes. It multiplies rapidly over 4–10 days and transforms into schizont containing approx. The hepatic schizonts (hypnozoites) burst and release merozoites that invade RBCs to produce erythrocyticschizogony which destroys red blood cells (RBC), relapsing daughter merozoites. 30,000 merozoites that rupture hepatocytes are released into the blood-stream in the form of membrane-enclosed structures called merosomes to avoid host cell immune responses (Roberts et al. 2019). In some cases, *P. vivax* and *P. ovale* sporozoites do not form schizonts and transform into dormant hypnozoites which can live in the liver for several months or years (Walker et al. 2010).

Merozoites then attack RBCs. RBC membranes are invigilated to form parasitophorous vacuole. After entering into RBC, it develops the signet ring stage and then continuously feeds on hemoglobin to form trophozoite stage. It undergoes several rounds of mitosis within infected RBC at about 30 h to form erythrocyticschizont with 6–32 merozoites which rupture erythrocyticschizont and are released into the blood at about 48 h (Nureye, et al. 2020). Black pigment hemozoin, the RBC digestion product, with merozoites is released into the blood and causes host paroxysms of fever. Some merozoites continue multiplication by attacking new RBCs and the erythrocytic cycle occurs again and again to produce a huge number of merzoites.

Some merozoites transform into male or female gametocytes with dispersed pigment in nucleus and circulate in peripheral blood for 5 days to form mature crescent-shaped gametocytes which are ingested by healthy *Anopheles* mosquito and arrive midgut of mosquito. Female gametocyte develops into one macrogamete and male gametocyte undergoes repeated karyokinesis to form motile microgametes through exflagellation. Microgamete reaches macrogamete and fertilizes it to produce zygote. It transforms into ookinete which migrates through the midgut epithelial wall and rests on the surface of gut (Roberts et al. 2019). It transforms into a round oocyst which undergoes repeated karyokinesis to produce 10,000 sporozoites. Mature sporozoites rupture oocyst and are released into the hemocoel. Then sporozoites migrate to the salivary gland of mosquito and wait until the mosquito bites another healthy human host (Fig. 1) (Nureye, et al. 2020).

Sporozoite membrane coreceptors such as thrombospondin (TSP) domains of circumsporozoite proteins (CSPs) bind to glycoaminoglycan chains of the heparan sulfate proteoglycans (HSP) which is present on both hepatocytes and Kupffer cells of the liver. After penetrating the hepatocyte membrane, sporozoite migrates through several hepatocytes, and in the final invasion stage, it forms parasitophorous vacuolar membrane (PVM) which is ruptured by sevteral proteases secreted by *Plasmodium*. Then merozoites are released into the blood-stream (Nureye et al. 2020).

P. vivax and *P. knowlesi* merozoites use Duffy antigens (blood group antigen present on RBC membrane) as host ligands during erythrocytic cycle, while *P. falciparum* uses sialic acid of glycophorin A on RBC. Parasite receptors that bind with these specific host ligands during RBC invasion belong to erythrocyte binding protein (EBP) family which includes the Duffy binding protein (DBP) of *P. vivax* and *P. knowlesi* and erythrocyte binding antigen (EBA) of *P. falciparum* (Roberts et al. 2019).

Glycosylphosphatidylinositol (GPI) anchored proteins such as merozoite surface protein-1 (MSP-1) on parasite

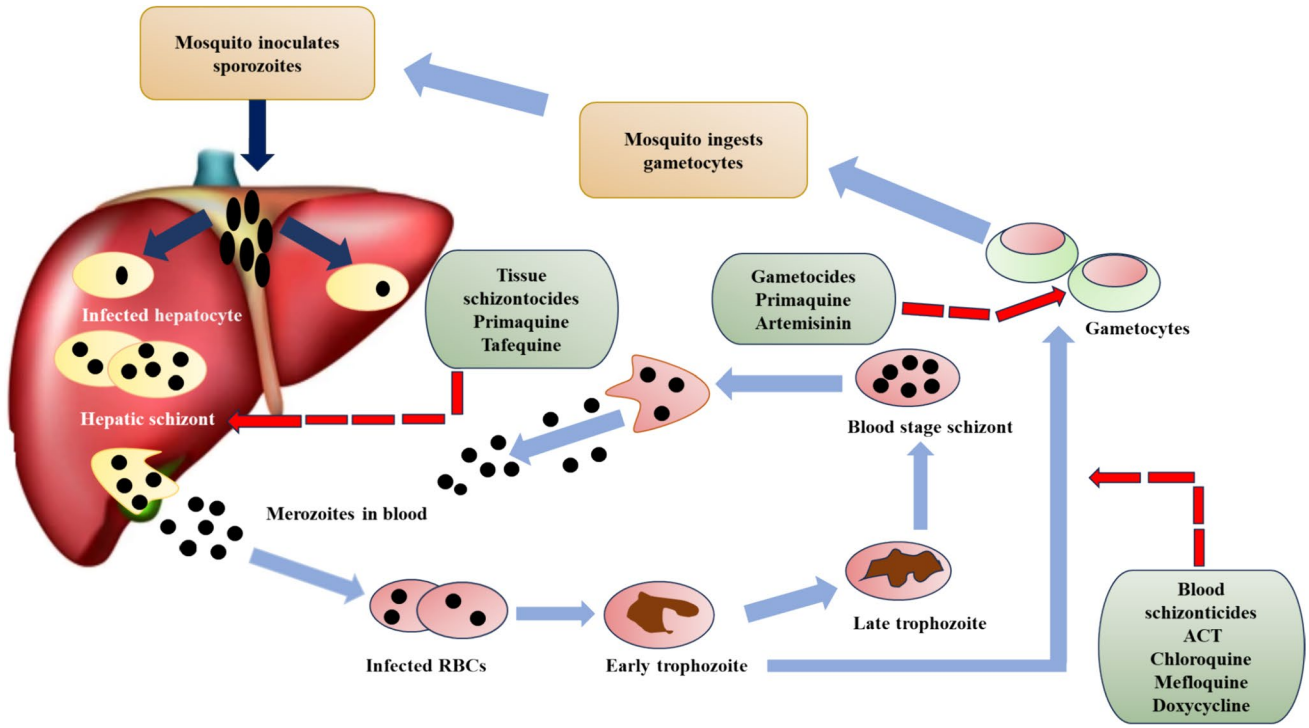


Fig. 1 Life cycle of Malaria parasite and molecular mechanism of actions of drugs

membrane bind with the surface protein of RBCs such as CD55. Then reorientation of merozoites occurs by the action of apical membrane antigen 1 (AMA-1) which is helpful for the interaction between the apical end of parasites and the RBC membrane. RON2 proteins in the neck of rhoptry which represents the secretory organelles present in the invasive stages of most apicomplexan parasites. They are incorporated in the RBC membrane and then attached to AMA-1 to make a tight junction. In the contact region between RBC and merozoite, any RBC membrane protein is absent. So, the serine protease enzyme of merozoite destroys the submembrane cytoskeleton and the arrangement and structure of lipids. This junctional area helps to release the rhoptry bulb which facilitates to production of PVM by supplying proteins and lipids. Then the junction looks like a ring or annulus through which merozoites enter into the parasitophorous vacuole. After that, the PVM and RBC cell membrane are closed.

Malaria parasites glide through this junction and it continuously releases micronemes. Apicomplexan myosin is attached to the inner membrane complex (IMC) below the cell membrane and the IMC-myosin complex interacts with actin filament to form a glidesome which helps the parasite to move inside the host cell (Nureye, et al. 2020).

Antimalarial chemotherapeutics

There have been a number of existing synthetic antimalarial chemotherapeutics. Primaquine is a tissue schizontocidal and gametocidal drug, whereas 4-aminoquinolines (chloroquine, hydroxychloroquine, and amodiaquine), quinoline-methanol (mefloquine), and natural cinchona sourced quinine chemotherapeutics are blood schizontocides and can inhibit the ferri/ferroprotoporphyrin IX and hemozoin as well as DNA sequestration of the parasite by inhibiting the glutathione and FP-binding proteins (Nandi et al. 2023).

Chloroquine has been used in the treatment and prevention of malaria caused by *Plasmodium vivax*, *P. ovale*, and *P. malariae*. It is generally not used for *Plasmodium falciparum* as there are widespread resistance issues. The hydroxychloroquine is active against the erythrocytic forms of chloroquine-sensitive strains of *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium vivax* but is not active against the gametocytes and exoerythrocytic forms including the hypnozoite stage (*P. vivax* and *P. ovale*) of the *Plasmodium* parasites. Amodiaquine is an important component of the artemisinin-based combination therapy (ACT) for uncomplicated *P. falciparum* malaria. It has been found to work against chloroquine-resistant *P. falciparum* strains of malaria. It produces severe hepatoxicity,

therefore, it is restricted in use. Mefloquine is a quinine-derived arylamino alcohols and a synthetic antimalarial drug to inhibit *Plasmodium falciparum* 80 s ribosome (Pf80s) responsible for the cytoplasmic translation. It is now used solely in combination with artesunate for the prevention of resistant strains of *P. falciparum* (usually combined despite being effective against *P. vivax*, *P. ovale* and *P. marlariae* whereas the *c*hloroquine/proguanil or sulfa drug-pyrimethamine combinations can be used in all other plasmodia infections) (Fig. 1) (Plowe 2005; Staedke et al. 2001).

The existing chemotherapeutics may produce resistance if being delayed in detection. The malaria parasite generates more potential in late diagnosis. Various targets are hidden to attack the host. Therefore, these potential targets have been explored.

Major drawbacks of antimalarial drug discovery

Generation of drug-resistance and cross-resistance against several antimalarial compounds are the major drawbacks of antimalarial drug developments. Spontaneous mutations in the parasite's target genes are mainly responsible for several drug resistance. Chloroquine, which is the most used drug is resistant to malarial parasites because of the spontaneous mutations of the gene of PfCRT and PfMDR1.These mutations are also responsible for the cross-resistance of other drugs of the quinine family. Resistance against Atovaquone is generated by a single point mutation in the cytochrome B gene and mutations in the gene that encodes dihydrofolate reductase are responsible for Pyrimethamine resistance (Pandey et al. 2023). Artemisin, which is one of the major antimalarial compounds becomes resistant to malarial parasites due to the point mutation in the Kelch protein gene of *P. falciparum* on chromosome 13 in the propeller region. It is also known as Kelch 13 or K13 (Hanboonkunupakarn and White 2016). Immunity, on the other hand plays a major role in drug resistance. People who are nonimmune to malaria, develop specific immunity against malaria parasite and malaria parasite gradually evades this immunity by remodeling its antigenic variation in its epitopes (Pandey et al. 2023). In addition to resistance, some adverse effects of antimalarial compounds are also documented. In the pregnancy period, Artemisin and its derivatives are not safe for 1st trimester. The uncertainties of the safety of the use of Mefloquine in the pregnancy period are also documented (Arrow et al. 2004).

The majority of the medicines now in use within the malaria parasite have an unclear mechanism of action. Furthermore, for the majority of medications, the mechanism of resistance is poorly understood (Kumar et al. 2018). The majority of the antimalarial drugs have activity against the sexual, and asexual stages of the parasite. However, there is very little effort to develop drugs against the hypnozoite stage, thus more investments are required to consider these stages as drug targets (Diagana 2015). Another barrier in the eradication of malaria is poor medicine quality which is often ignored in the control of disease, but it is one of the massive problems. Even though malaria has a reasonable global profile, in Asia and America it is mostly a disease of the poor or marginalized, it has low priorities for national health (Hanboonkunupakarn and White 2022). The methods for finding novel antimalarial drugs are imprecise, it is one of the disadvantages in the development of novel drugs (Diagana 2015).

Quest of potential targets

These potential targets include malaria protease, various transporter proteins and enzymes involved in the DNA and nucleic acid synthesis of *Plasmodium* like dihydroorotate dehydrogenase, dihydrofolate reductase, apicoplast, and dihydropteroate synthase. Virulence of other potential targets such as isoprenoid biosynthesis, farnesyl transferase of the parasite, *P. falciparum* translational elongation factor 2, and phosphatidyl inositol 4 kinase are also discussed.

Proteases: breakdown of haemoglobin

The matured *Plasmodium* parasite schizonts release merozoites which can egress and invade the host RBC with the help of serine protease (Alam 2014). The merozoites enter into RBC and break down the host haemoglobin utilizing proteases. Hemoglobin digestion is essential for the growth and development of trophozoites stage of *Plasmodium* during erythrocytic phase of the life cycle. Some amount of RBC cytoplasm is ingested by trophozoites and transported to the food vacuole. In this vacuole, hemoglobin is converted into heme and globin (small peptides) by cysteine proteases falcipain1-3 and aspartic proteases plasmepsin I-IV (Nasamu et al. 2020; Rosenthal 2004).

On oxidation, the heme is being further modified into hematin which polymerises to produce hemozoin (characteristic pigment). The aminopeptidases of *Plasmodium* trophozoites cause hydrolysis of globin (small peptides) into smaller units by cysteine proteases and the smaller units are broken into free amino acids with the help of cytosolic aminopeptidases which are not found in food vacuoles. The heme and free amino acids are necessary for parasite protein synthesis.

The food vacuoles are very similar to lysosomes in terms of their acidic environment and hemoglobin-degrading proteases. Generally, serine proteases, cysteine proteases falcipain1–3, and aspartic proteases plasmepsin I–IV are responsible for the breakdown of host hemoglobin (Fig. 2) (Rosenthal 2002).

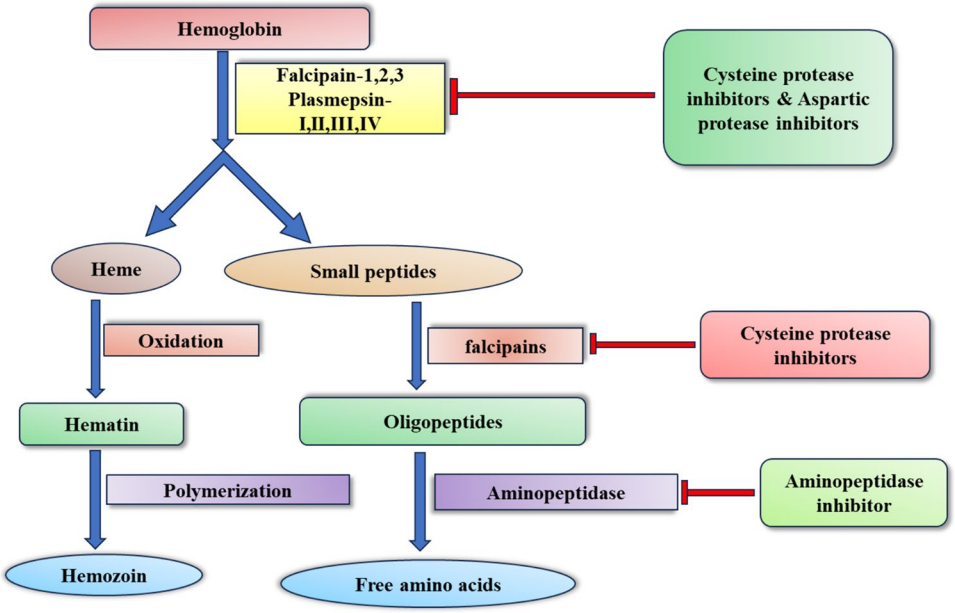


Fig. 2 Mechanisms of haemoglobin breakdown caused by malaria parasitic proteases and the different sites of action of the inhibitors of parasitic proteases

PbSERA4 is identified as a member of the papain-like cysteine protease family required for the egress of *Plasmodium* parasites from the liver. The process involves proteolytic processing of PbSERA4 during both liver and blood stages of infection. This protease activity is essential for the efficient initiation of blood-stage infection. Proteases recognize specific amino acid sequences in proteins and cleave the peptide bonds at those sites. In the case of PbSERA4, its cysteine protease activity likely targets particular substrates involved in the egress process. This precise cleavage allows the parasite to exit infected cells, completing the liver-stage development and initiating the blood-stage infection (Putrianti, et al. 2020).

SUB1 and SERA6 play crucial roles in a coordinated proteolytic cascade leading to the sequential rupture of the parasitophorous vacuole membrane (PVM) and red blood cell membrane (RBCM) during malaria parasite egress. SUB1, a serine protease, is discharged into the parasitophorous vacuole minutes before egress. It cleaves multiple substrates, including SERA6. In parasites lacking SUB1, morphological transformations preceding egress do not occur, and PVM rupture fails. SERA6 is an enzyme that, when processed by SUB1, functions in the cascade. In SERA6-null parasites, PVM rupture and RBCM poration occur normally, but RBCM rupture is impaired. Complementation studies indicate that SERA6 requires processing by SUB1 to function. RBCM rupture is associated with SERA6-dependent proteolytic cleavage within the actin-binding domain of β-spectrin, a major RBC cytoskeletal protein. This suggests that SUB1 and SERA6 work together in a coordinated manner: SUB1 activates SERA6 through processing, and SERA6, in turn, plays a vital role in disassembling the RBC cytoskeleton, leading to precise RBCM disruption (Thomas et al. 2018).

Transporter for nutritional uptake

Plasmodium parasites rapidly germinate into the human host both in the erythrocytic and extraerythrocytic stage which occurs inside the hepatic cells. The asexual stage of this parasite relies on the nutrients of the host body and uptakes different nutrients and excretes the metabolic end products to the host system. For this purpose, the malaria parasite utilizes various transporters that help in the uptake of nutritional elements essential for their silent survival.

Plasmodium sugar transporter

The malaria parasite *Plasmodium* uses glucose as a primary energy source. It depends on the anaerobic glycolysis pathway and consumes glucose from the human hosts (Nerlich et al. 2021). Due to the increased utilization of glucose by parasites, the erythrocytes that are infected increase the uptake of glucose at about 100 fold than normal erythrocytes. For the transportation of glucose into the parasite, at first, the glucose molecules have to travel through the plasma membrane of erythrocytes. Glucose enters through the plasma membrane of erythrocytes via a specialized glucose transporter protein known as GLUT-1. Then the glucose molecules pass through a parasitophorous vacuole membrane (PVM) which has big pores and does not have any barriers for glucose transportation. After that, the glucose molecules enter into the parasite by crossing the parasite's

plasma membrane which has specialized hexose transporter proteins known as PfHt1 (*Plasmodium falciparum* Hexose transporter 1) in the case of *Plasmodium falciparum* (Jiang 2022). It is the product of *PF3D7_0204700* which is the hexose transport gene of *Plasmodium* genome. PfHt1 is a member of the Major Facilitator Superfamily (MFS), showing 30% similarity of sequences with human GLUT1 proteins (Jiang et al. 2020). This transporter moves the glucose across the lipid bilayer of the parasitic membrane which is usually impermeable to the sugars. Quershi et al. found the binding site of sugar in the transporter molecules where it has been found to shield the glucose molecules from the exterior aqueous environment. The substrate-binding site is surrounded by a bundle of α helix (Patel et al. 2008). From the study of heterologous expression of *PfHt1* mRNA in *Xenopus lavies* oocyte, it is observed that fructose is also transported by PfHt1. This study also suggests that single amino acid residue (Q169N) mutation which is present in helix 5 stops the uptake of fructose but does not interfere with the capacity and affinity of glucose transportations. So, it can be concluded that the selection of substrate is specifically determined by helix 5. PfHt1 shows stereospecificity for D-glucose as the excess presence of L-glucose in the uptake assays medium did not obstruct the uptake of D-glucose (Jiang 2022).

Plasmodium lactate transporter

For the rapid growth and proliferation, *P. falciparum* depends on the process of glycolysis. It consumes glucose from the host body and generates ATP by the process of glycolysis which also leads to the formation of lactates and protons as by-products (Nerlich et al. 2021). *Plasmodium* generates two moles of ATP and two moles of L-lactate and proton from one mole of D-glucose as a byproduct (Nerlich et al. 2021). The parasites promptly excrete lactate through the lactate transporter to prevent acidification in cytosol (Nerlich et al. 2021). At first, the L-lactate and protons are excreted out to the extra parasitic space through the *P. falciparum* formate-nitrite transporter(PfFNT) and then to the extracellular space with the help of human monocarboxylate transporter 1 (hMCT1)(Peng et al. 2021). The gene of parasite *pf3D7_0316600* is responsible for the production of formate-nitrite transporter which acts as an important component for energy supply and maintenance of homeostasis in parasites (Peng et al. 2021). PfFNT consists of 5 protomers which give it the shape of a pentagon-like homopentamer. It has a width of approximately 80 angstroms and a height of approximately 70 Å (Peng et al. 2021). Every protomer functions as an individual transport unit which is bidirectional (Nerlich et al. 2021). The central hydrophobic tunnel is enclosed by the 5 protomers but the functional characterization of family members of both PfFNT and FNT shows that one tunnel of every protomer acts as a translocation path for the substrate instead of a central tunnel. Every protomer of PfFNT is composed of 6 transmembrane segments where a typical 3 + 3 conserved inverted repeat is present all over the family members of FNT (Peng et al. 2021). The N and C terminals of transmembrane helices are situated at the cytoplasmic side (Nerlich et al. 2021). Two discontinuous helices TM2 and TM5 enclose the central pore of every protomer. These two helices help in the formation of constriction sites in the extracellular as well as intracellular side of the membrane (Peng et al. 2021). These are two lipophilic helices that separate central histidine residue that is highly conserved, from the broader vestibule of both sites of entrance. The surface of FNT protein is marked by a positive electrostatic potential that attracts the lactate anion and passes the anion through the vestibules. The lipophilic environment in the vestibule causes protonation of following lactate anions, resulting in neutral lactic acid with the potential to pass through lipophilic constriction points (Nerlich, et al. 2021).

Plasmodium p-type Na^+ ATPase transporter (pfATP4)

In the erythrocytic stage *P. falciparum* remains within the parasitophorus vacuole which is formed in the infected erythrocytes (Rosling et al. 2018). Like other normal cells, erythrocytes normally keep their cytoplasmic [Na^+] concentration at a lower level (Rosling et al. 2018). This is achieved by the P-type Na^+/K^+ ATPase transporter which is ouabain sensitive, resulting in efflux of [Na^+] from cytosol to extracellular fluid (Spillman and Kirk 2015). So when the parasitic merozoite stage enters into the host cytoplasm, it meets a sudden change in the ionic surroundings because they migrate from the high [Na^+]/low [K^+] condition to low [Na^+]/high [K^+] condition (Spillman and Kirk 2015). But when the parasite undergoes maturation into the trophozoite stage in the RBC, it raises the permeability of the plasma membrane of RBC, allowing the increased influx of [Na^+] towards the cytoplasm of erythrocytes. The [Na^+] enters into infected erythrocytes through the "New Permeability Pathway" which is induced by the parasites (Spillman and Kirk 2015). This results in an elevation in the intracellular fluid with [Na^+] ion concentration and gradually becomes equal to the concentration of [Na^+] in the extracellular medium (Rosling et al. 2018). The parasitophorous vacuole is easily permeable to the solute which has low molecular weight because of the presence of high-conductance broad selectivity channels (Spillman and Kirk 2015). For this reason, although the parasite remains in the intracellular region, the mature stage of trophozoite meets an unusual environment that has a high [Na^+] concentration. So it needs to efflux [Na^+] ions through its plasma membrane to maintain low [Na^+] concentration in the cytoplasm (Rosling et al. 2018). A transporter protein which is known as PfATP4 functions

primarily as an efflux pump for [Na^+]. The functional evidence also shows that during the time of [Na^+] efflux, it also imports H^+ ions into the cytoplasm of the parasite, thus increasing the pH of cytosol of parasite (Rosling et al. 2018). So the acid load which is arised by the inflow of [H^+] ion is similar to the V-type H^+-ATPase. Thus PfATP4 is thought to be responsible for (at least too some extent) "Abundant proton pumping" (it means the existence of powerful H^+ ATPase in the plasma membrane though it has no clear need for metabolical activities) (Spillman and Kirk 2015).

Plasmodium v-type H^+-ATPase

It was observed that infection with malaria parasite results in a decrease in pH level of RBC and causes massive acidosis in the extracellular region of parasites. A v-type H^+ ATPase pump is found in numerous endomembrane organelles and the plasma membrane of parasites which pumps out protons (H^+) from intracellular to extracellular region with the help of ATP hydrolyzing activity. The previous studies suggested that the v-type H^+ ATPases are only present in the digestive vacuole membrane (DVM) of parasites where it maintains an acidic environment in the vacuole and neutral pH in the cytosolic region for functioning in hemoglobin hydrolysing process. However, the recent data shows that besides the presence in the digestive vacuole, this transporter is also present in small clear vesicles and the plasma membrane of parasites. It has been found that a crescent-like weakly acidic location (pH 6.9 ± 0.03) around the parasite is located in the infected erythrocytes. This region is created by a v-type H^+ ATPase pump in the plasma membrane which might help in the accession of nutrients and maintenance of ion homeostasis and pushing out the antimalarial drugs from parasites. The mechanism of action of small clear vesicles is unknown however it was hypothesized that it may act in endocytosis/exocytosis process of parasites with the help of an acidic environment (Hayashi et al. 2000).Like the eukaryotic v-type H^+ ATPase pump which is a heteromultimeric transmembrane protein the malaria parasite also has a multimeric v-type H^+ ATPase pump which translocates H^+ ions to the outside of their body with the help of ATP hydrolyzing process. The V1 domain of the pump which is associated with ATP hydrolysis resides in the cytoplasmic region and the V0 domain helps in the transportation of protons across the membrane against the concentration gradient. There are eight subunits present in V1 in which catalytic hexameric region is present where A and B function in ATP hydrolysis, and rotate the central stalk which is composed of D and F. There also peripheral stalks are found which three in number, consisting of E and G which helps in holding the catalytic hexamer. The V0 domain consists of five subunits of which subunit d is associated with the rotator stalk of V1. The genome of the parasite codes for all proteins which is required for v-type H^+ ATPase assembly including two isoforms of subunit c (c' and c''). According to the immune electron microscopic analysis, the catalytic hexamer is present from DVM to endomembrane organelle to the periphery of parasites. Immunofluorescence study suggests that there is an exportation of subunit B to the cytoplasm and parasitic plasma membrane (Alder et al. 2023).

Aquaporin-3

Aquaporins are a group of highly-conserved proteins that have multiple membrane-spanning regions and help in maintaining homeostasis in eukaryotic organisms (Bietz et al. 2009). The human aquaporin family consists of 13 members which are divided into 2 types. One is orthodox aquaporin which is only permeable to water and helps to transport water across the plasma membrane and the other is aquaglycoporin. The aquaglycoporin transports small-sized solutes such as glycerol including the water. Aquaporin 3 is an aquaglycoporin which transports glycerol along with water in the mammalian system. It has been observed that the host aquaporin protein is highly expressed in hepatocytic cells and erythrocytic cells when they are infected with *Plasmodium berghei* and *Plasmodium falciparum* respectively (Posfai et al. 2018). It has been shown that the infected hepatocytes contain parasitophorous vacuole which encircles the parasites into the host's cells. The parasitophorous vacuole membrane (PVM) contains lipids and proteins of the plasma membrane of the host (Vijayan et al. 2021).The parasitophorous vacuole membrane (PVM) is formed due to the invagination of the host cell plasma membrane at the time of penetration of parasites. The molecular studies suggested that the host aquaporin-3 is actively directed to the PVM after the invasion of parasites. The immunofluorescence studies with the help of antibodies against human AQP3 reveal the intracellular location of AQP-3 protein in the infected host cells. The increased expression of host aquaporin into the PVM helps the parasites to transport more nutrients mainly glycerol which induces replication and rapid growth of parasites (Posfai et al. 2018).

Choline transport

The phospholipid contents of erythrocytes are increased about fivefold at the infected condition *by Plasmodium* parasites. The parasitic growth and proliferation in the RBC depend on the phospholipid contents mainly phosphatidylcholine and phosphatidylethanolamine of the erythrocytes of the host. It has been shown that there are approximately 45% of phospholipids are phosphatidylcholine in the parasitic cell membrane (Lehane et al. 2004). Usually the human RBC takes up choline which is a monovalent, quaternary ammonium cation through a carrier in the membrane that

is saturated. The in vitro and in vivo study of malaria infection shows that an additional carrier component of choline transfer is present which is non-saturated known as 'New Permeation Pathways'(NPP) at the infected RBC membrane, produced by the parasite itself. With the help of two types of transport pathways, the uptake of choline is greatly increased in infected RBC. After crossing the host cell membrane, the choline molecules enter the parasitophorus vacuoles of parasites through the pores with high-capacity which facilitates permeability of solute with low molecular weight in the infected erythrocytes. Then the parasites take up the choline and trap it in the cytosol of parasites by phosphorylating it with the help of the choline kinase enzyme. It is the first enzyme of the Kennedy pathway. The molecular study reveals that there is a transporter that transports choline across the membrane of parasites and it is observed that the transport of choline into the parasite is independent of sodium and mainly proton-pump of plasma membrane gives energy to it (Lehane et al. 2004; Biagini et al. 2004). The transport of choline from the extracellular region of parasites for the de novo synthesis of phosphatidylcholine is a rate-limiting step. The choline transporter and the Kennedy pathway of phosphatidylcholine biosynthesis is a major antimalarial drug target as the survival and growth of parasites in RBC are highly dependent on the choline precursor of host cells and the de novo phosphatidylcholine synthesis helps in membrane production and other enzymatic activity of parasite (Biagini et al. 2004).

Formation of *Plasmodium* DNA and nucleic acid

An essential step for the germination and multiplication of malaria parasites in humans is the rapid and extensive replication of the DNA of parasites which depends on the presence of essential metabolites for example purines and pyrimidines.

As *Plasmodium* sp. is capable of its entry into red blood cells, they tend to replicate rapidly and at the time of symptomatic condition of disease, these parasites tend to exponential replication at the rate of $> 10^{12}$ per patient. For achieving this rapid replication and growth rate sustained amounts pf nucleotides are required for DNA and RNA synthesis. As a result, many of the antimalarial drugs render pyrimidine synthesis as promising route in the prevention of malaria. (Phillips et al. 2017a, b).

P. falciparum salvages host cell purine for the synthesis of nucleic acid and cofactors. Hypoxanthine is the main precursor of all purine synthesis in *Plasmodium.* The main source of hypoxanthine in vivo is from erythrocytic purine pool. Hypoxanthine is therefore converted to inosine monophosphate (IMP) by HGXPRT (hypoxanthine–guanine-xanthine phosphoribosyl transferase). IMP then acts as a metabolic precursor of all deoxynucleotides and purines required for the synthesis of nucleic acid (Krungkrai and Krungkrai 2016).

Pyrimidine nucleotides are required for the synthesis of sugar nucleotides, DNA, and RNA. *Plasmodium* lacks pyrimidine salvage enzymes and de novo pathway acts as the only source of pyrimidines required for cell growth. Thus, by inhibiting de novo pyrimidine synthesis in *Plasmodium* cellular growth can be prevented. (Phillips et al. 2015). Six enzymes are required in *Plasmodium* species to synthesize UMP, which then is used to synthesize UTP, dTMP, CTP, and some additional metabolites that are essential. These include (GAT/CPS) glutamine amidotransferase/carbamoylphosphatesynthetase, (ACT) aspartate carbamoyltransferase, (DHOtase) dihydroorotase, (DHODH) dihydroorotate dehydrogenase, (OPRT) orotatephosphoribosyltransferase and (OMPDC) orotidine 5'-monophosphate decarboxylase. (Cheviet et al. 2019).

At first, L-glutamine produces ammonium and bicarbonate ions through GAT/CPS action. This enzyme then leads to the production of carbamoyl phosphate (CP) that along with L-aspartic acid produces carbamoyl aspartate (CA). Dihydroorotase converts CA into dihydroorotate which is in turn converted to orotate by PfDHODH. Then this orotate is sequentially transformed to OMP and UMP by OPRT and OMPDC enzymes respectively. Then ultimately dUMP and dTMP are produced that are utilized to synthesize RNA and DNA in *Plasmodium* (Fig. 3).

Dihydroorotate dehydrogenase (DHODH)

Dihydroorotate dehydrogenase (DHODH), localized to the inner mitochondrial membrane, catalyzes the fourth step of the de novo pyrimidine synthesis pathway. It is a flavin mononucleotide-dependent mitochondrial enzyme that catalyzes the main step of oxidation of dihydroorotate to orotic acid, which is the precursor for the biosynthesis of pyrimidine bases. (Kokkonda et al. 2020) This FMN-depending oxidation reaction facilitated by DHODH is divided into two half-reactions; at first, the dihydroorotate is oxidized through the reduction of FMN and second, FMNH2 is reoxidized to regenerate the active enzyme. Coenzyme Q is employed as an oxidant in this reaction (Khairy et al. 2022). So, this enzyme performs in de novo biosynthesis of uridylate, which is an essential precursor of all pyrimidine nucleotides.

Thus, the inhibition of DHODH reduces the pool of essential pyrimidines, thus slowing down the rate of replication in *Plasmodium*. DHODH is present in both humans and *Plasmodium* but in humans, pyrimidine synthesis occurs through a salvage pathway. Thus, DHODH inhibition renders no effect on essential functions in humans and can act as a remarkable therapeutic target (Alzain et al. 2022).

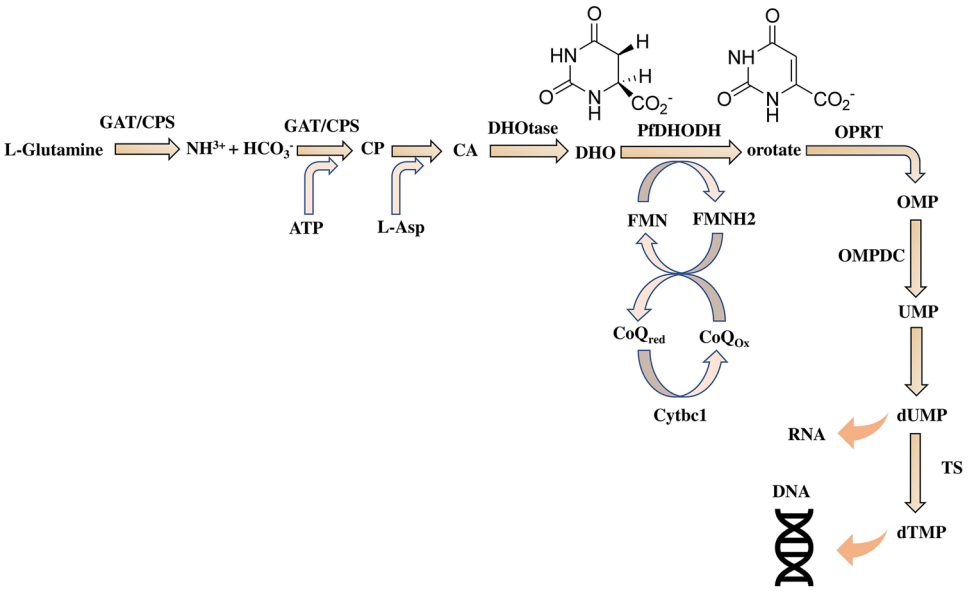


Fig. 3 de novo pyrimidine biosynthetic pathway in *Plasmodium*

Dihydrofolate reductase (DHFR)

Dihydrofolate reductase (DHFR) is the enzyme that mediates NADPH—dependent dihydrofolate (FH2) reduction to tetrahydrofolate (FH4) in malaria parasites. This reduction reaction is very much essential for the synthesis of purines, and other amino acids and de novo synthesis of pyrimidines. DHFR inhibition thus, results in an imbalance in the thymidylate synthesizing pathways. As a result, DNA replication is disrupted leading to cell death. (Wróbel et al. 2020). In *Plasmodium*, DHFR and Thymidylate synthase (TS) act as separate enzymes. DHFR leads to the catalytic reduction of dihydrofolic acid to tetrahydrofolic acid. Along with this, tetrahydrofolic acid is changed to N5, N10-methylenetetrahydrofolate. Then thymidylate synthase mediates the formation of 2-deoxythymidine-5-monophosphate from 2-deoxyuridine-5-monophosphate through utilizing N5, N10-methylenetetrahydrofolate as a methyl donor and reducing agent. (Ibrahim et al. 2022).

Thereby, inhibiting DHFR or the junctional region between the DHFR and thymidylate synthase is regarded as the promising route to prevent *Plasmodium* growth (Fig. 4). It has also been discovered that a critical length of at least 44 amino acids in this region is essential for catalytic activity of thymidylate synthase. (Luth et al. 2018).

Apicoplast

Apicoplast is regarded to be derived by a secondary endosymbiotic event. Most genes in apicoplast are essential for transcription, and translation. (Low et al. 2018). It is regarded as a plastid-derived organelle that lacks the ability of photosynthesis. The apicoplast of *Plasmodium* has a circular genome of 35 Kb that mediates the encoding of several transcription, translation-related genes of proteins along with tRNAs, rRNAs, and other genes of several functions. The apicoplast genome of *Plasmodium* has a high AT content. (Arisue et al. 2019). Apicoplast is algal chloroplast reminiscent and the independent genome of apicoplast is maintained by apPol which is the apicoplast DNA polymerase. (Nieto et al. 2022). As apicoplast can undergo its replication, transcription, and translation it has proved to be a valuable target for antimalarial drugs.

Dihydropteroate synthase (DHPS)

In *Plasmodium falciparum* dihydropteroate synthase is encoded in the form of a bifunctional protein in conjugation with 7,8-dihydro-6-hydroxymethylpterin pyrophosphokinase (HPPK). This enzyme mediates the generation of Dihydropteroate (DHP) by linking DHPP (6-hydroxymethyl-7,8-dihydropterin pyrophosphate) with PABA (para-aminobenzoic acid). Then the DHP conjugates with glutamate to form dihydrofolate, which is required by malarial parasites in de novo synthesis of pyrimidines. (Stratton et al. 2015). Along with DHFR, DHPS is essential for *Plasmodium* nucleic acid synthesis. In mammals DHPS is absent so it can be utilized as a remarkable target by antimalarial drugs (Fig. 5) (Hafiz et al. 2020).

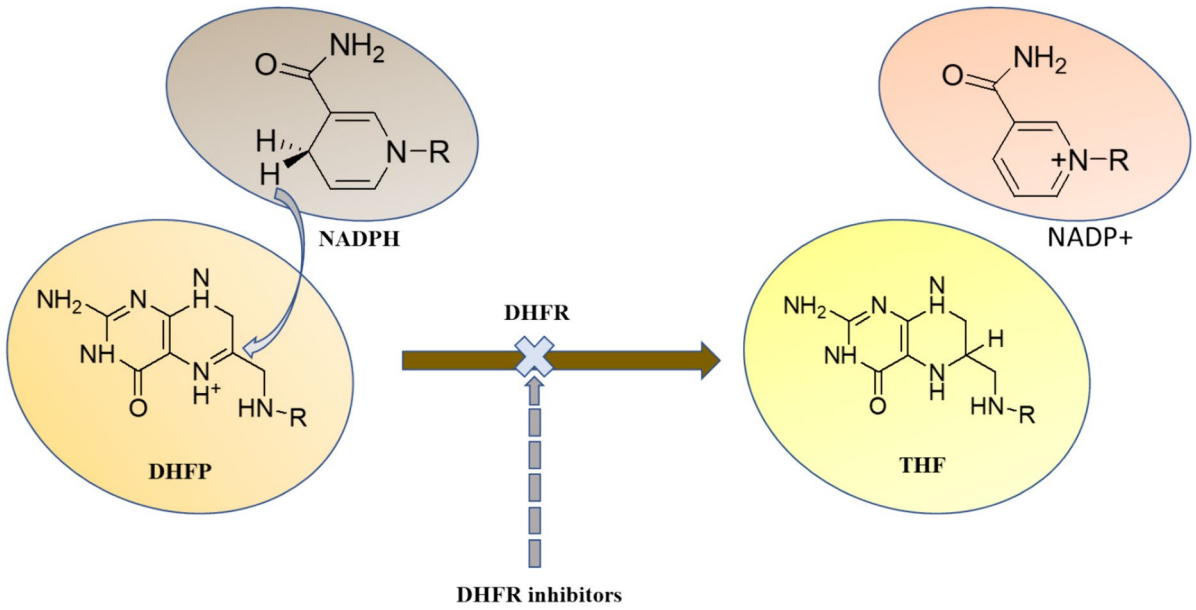


Fig. 4 General mechanism of action of DHFR inhibitors

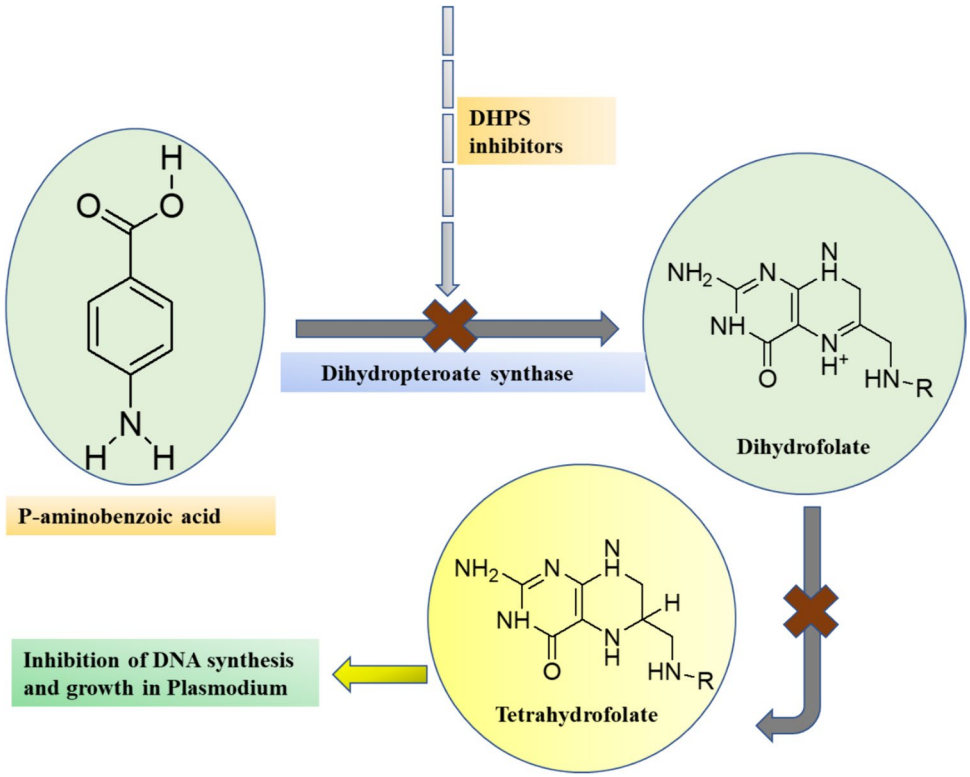


Fig. 5 General mechanism of action of DHPS inhibitors

Other potential targets

Isoprenoid biosynthesis

Plasmodium is a member of the Apicomplexa phylum which has a plastid-like unusual structure known as apicoplast, surrounded by four-membranes (Saggu et al. 2016; Price et al. 2016). This organelle is the location where major metabolic pathways of *Plasmodium* take place and becomes essential for the survival of the parasites. The isoprenoid biosynthesis pathway is carried out through 2C-methyl-d-erythritol-4-phosphate/1-deoxy-d-xylulose-5-phosphate (MEP/DOXP) pathway in the body of *Plasmodium* (Saggu et al. 2016). Isoprenoid a macromolecules, is formed by the isomers of 5-carbon, known as Isopentenyl pyrophosphate (IPP) and Dimethylallyl pyrophosphate (DMAPP) through MEP/DOXP pathway (Price et al. 2016). The DOXP reductoisomerase, encoded by *IspC*, *IspD*, *IspF, IspE,* and *IspG* plays a key role in this pathway. The initial substrate of the isoprenoid biosynthesis is pyruvate and glyceraldehyde-3-Phosphate which is transferred into 1-deoxy-D-xylose-5-phosphate (DOXP/DXP) by the enzyme DXS and it is the major rate-limiting step. Then, IspC which is the first enzyme associated with the MEP pathway, converts DOXP to 2-C-methyl-D-erythritol-4-phosphate, and again it is a rate-limiting step. Then the other major seven steps which are essential for isoprenoid biosynthesis occur with the help of IspD, IspE, IspF, and IspG. The MEP pathway of *Plasmodium* is very essential for the survival of parasites in the intraerythrocytic and hepatic stages of their lifecycle (Saggu et al. 2016). IPP and DMAPP are necessary for various cellular mechanisms like the tRNA isopentenylation, trafficking of vesicles, prenylation modification of proteins, and protein glycosylation by Dolichol in the asexual stage of parasites and become a major target for antimalarial drugs (Price, et al. 2016; Saggu et al. 2016).

Farnesyl transferase

Though normal erythrocytes do not have any membrane-bound organelle, the infected erythrocytes are highly marked by the presence of various endomembrane systems created by *Plasmodium* parasites. Protein prenylation which is a post-translational modification of proteins plays an important role in this membrane formation. The modified proteins function in the formation of food vacuole and parasitophorus vacuole membrane (Gisselberg et al. 2017). It has been shown that inhibition of the activity of prenyl transferase stops the growth of parasites (Suazo et al. 2016) Mainly 3 types of Prenyltransferases are found which are Farnesyltransferase, Geranylgeranyltransferase, and Rabgeranylgeranyltransferase.

Farnesyltransferase modifies proteins by adding a 15-carbon chain at their specific site which is marked by 4-amino acid Cysteine–aliphatic-aliphatic-terminal residue (CaaX) motif present at the C-terminus tail. Targeting Farnesyl transferase (FT) for the treatment of malaria is highly effective because human farnesyltransferase shows a great divergence from the parasitic one so the mistargeting of host protein may be prevented. As a lack of modification of the targeted proteins causes them to mislocalize into the cytosol, inhibition of Farnesyl transferase activity results in the inhibition of parasitic growth in host cells (Gisselberg et al. 2017).

P. falciparum translational elongation factor 2

P. falciparum eEF2 mediates the translocation of ribosome which is a GTP-dependent process along the messenger RNA. This step is very crucial for protein synthesis. (Baragaña et al. 2015). Recently an inhibitor targeting PfeEF2 was identified resulting in the inhibition of the protein synthesis process in *P. falciparum*. This target is therefore treated as valuable in the prevention of malaria (Fontinha, et al. 2022).

P. falciparum phosphatidylinositol 4-kinase (PI4K)

The type III beta phosphatidylinositol 4-kinase in *Plasmodium* was recently identified as a crucial drug target in malaria and it is the only validated clinical target to date (Fienberg, et al. 2020).This novel target is discovered by in vitro evolution and whole genome analysis (IVIEWGA). This crucial drug target is indispensable in all stages of the *Plasmodium* life cycle and sets off the transformation of phosphatidylinositol (PI) into phosphatidylinositol-4-phosphate (PI4P) is essential for cytokinesis, signal transduction, and membrane trafficking at the time of merozoite development. In *Plasmodium* the lipid binding effector proteins that are recruited by PI4P are used by Rab11A during merozoite development for later-stage membrane entrance or access. (Luth, et al. 2018).

Inhibitor section

Cysteine protease inhibitors

Malarial cysteine protease inhibitors are also called falcipain inhibitors which can be broadly divided into peptidic, non-peptidic and peptidomimetic inhibitors. The peptide-based inhibitors include peptidyl fluoromethyl ketones, peptidyl vinyl sulfones, peptidyl aldehydes and α-ketoamide

Table 1 Malarial cysteine protease inhibitors

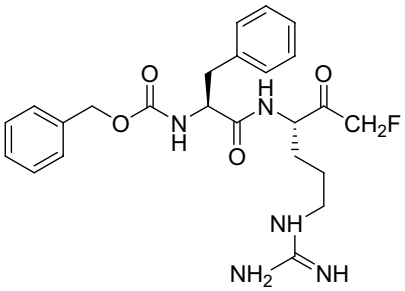
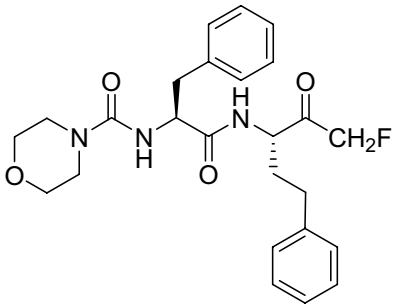
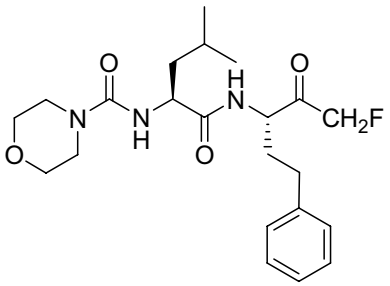
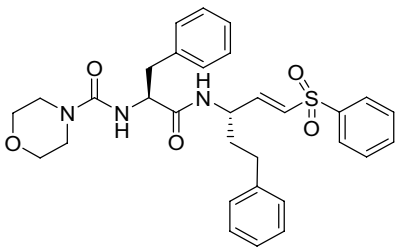
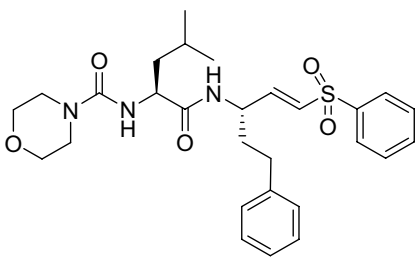
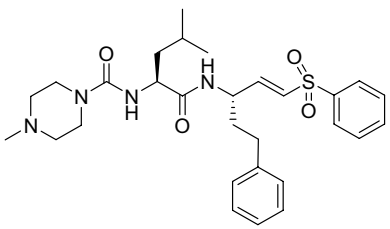
Sl No	Name	Structure	IC_{50}	References
1	(Z)-Phe-Arg-CH2F		0.10 µM	Ettari et al. (2021)
2	Mu-Phe-HomoPhe-CH2F		3 nM	Ettari et al. (2021)
3	Mu-Leu-HomoPhe-CH2F		0.42 nM	Ettari et al. (2021)
4	Mu-Phe-hPhe-VSPh (K11002)		0.08 µM	Ettari et al. (2021)
5	Mu-Leu-hPhe-VSPh (K11017)		0.003 µM	Ettari et al. (2021)
6	Vinyl sulfone		–	Ettari et al. (2010)

Table 1 (continued)

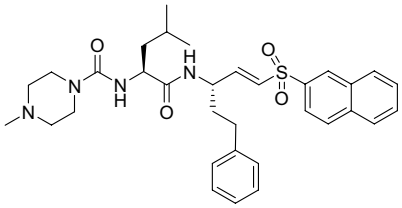
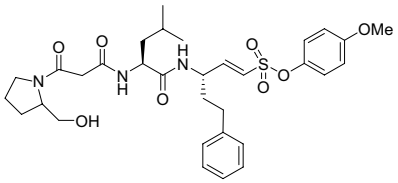
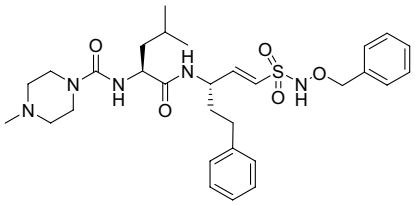
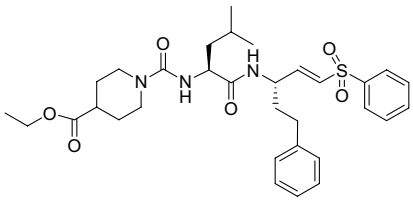
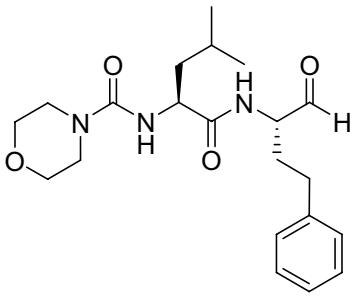
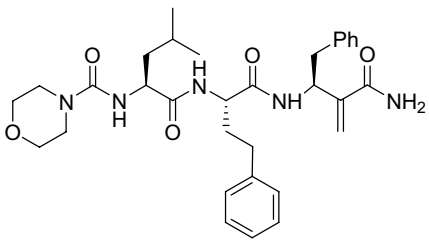
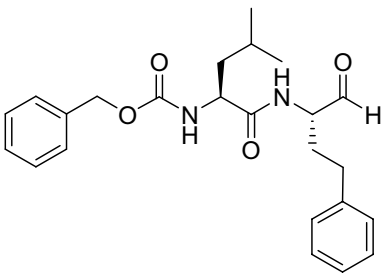
Sl No	Name	Structure	IC_{50}	References
7	Vinyl sulfone		–	Ettari et al. (2010)
8	Vinyl sulfonate ester		0.9 nM	Ettari et al. (2010)
9	Vinyl sulfonamide		2.3 nM	Ettari et al. (2010)
10	phenyl vinyl sulfone		6.9 nM	Ettari et al. (2010)
11	Mu-Leu-hPhe-al		1 nM	Ettari et al. (2021)
12	Mu-Leu-hPhe-Phe-(CO)-NH2		1 nM	Ettari et al. (2021)
13	Z-Leu-hPhe-al		2 nM	Ettari et al. (2021)

Table 1 (continued)

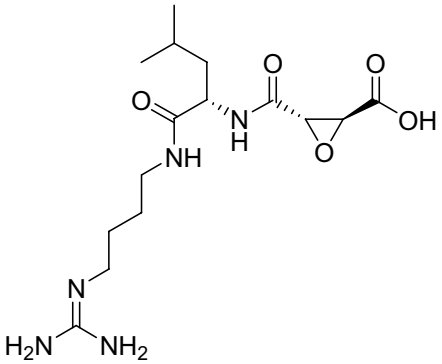
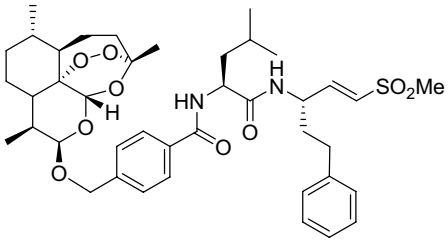
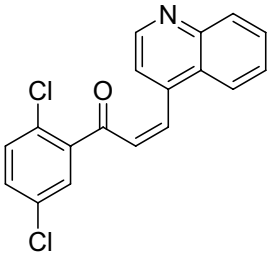
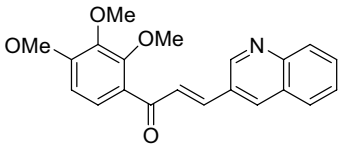
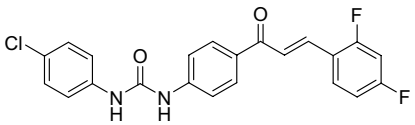
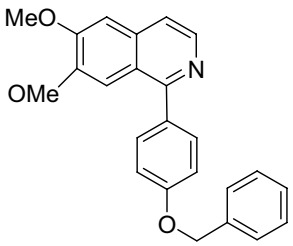
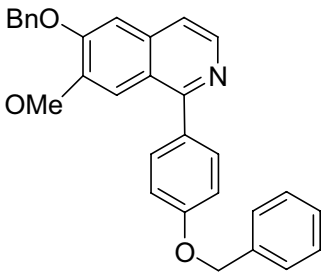
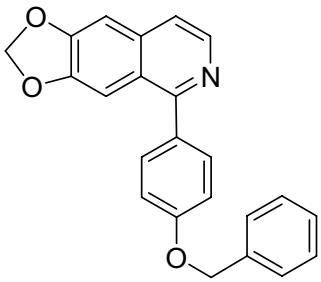
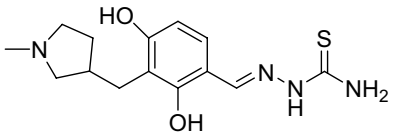
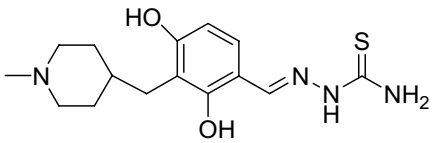
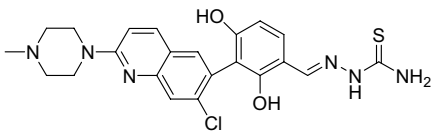
Sl No	Name	Structure	IC_{50}	References
14	E64		0.015 and 0.075 µM against falcipain-2 and falcipain-3, respectively	Ettari et al. (2010)
15	Peptidyl aziridine		0.21 nM	Bekono et al. (2018)
16	1-(2,5 dichlorophenyl)-3(4-quinolinyl)-2-propen-1-one		200 nM	Ettari et al. (2010)
17	1-(2',3',4'-trimethoxy-phenyl)-3-(3-quinolinyl)-2-propan-1-one		2 µM	Ettari et al. (2010)
18	1-[4'-N-(N'-p-chlorophenylurenyl)-phenyl]-3-(3,4,5 trimethoxyphenyl)-2-propen-1-one		1.8 µM	Ettari et al. (2010)
19	Isoquinolines		3 µM	Ettari et al. (2010)

Table 1 (continued)

Sl No	Name	Structure	IC_{50}	References
20	Isoquinolines		3 µM	Ettari et al. (2010)
21	5-(4-(benzyloxy)phenyl)-[1,3]dioxolo[4,5-g]isoquinoline		3 µM	Ettari et al. (2010)
22	(E)-2-(2,4-dihydroxy-3-((1-methylpyrrolidin-3-yl)methyl)benzylidene)hydrazine-1-carbothioamide		5.8 µM	Ettari et al. (2010)
23	(E)-2-(2,4-dihydroxy-3-((1-methylpiperidin-4-yl)methyl)benzylidene)hydrazine-1-carbothioamide		3.8 µM	Ettari et al. (2010)
24	(E)-2-(3-(7-chloro-2-(4-methylpiperazin-1-yl)quinolin-6-yl)-2,4-dihydroxybenzylidene)hydrazine-1-carbothioamide		2.25 µM	Ettari et al. (2010)

derivatives, epoxysuccinyl derivatives and peptidyl azirines. The α-ketoamide and peptidyl aldehydes inhibit falcipain reversibly whereas other group of inhibitors inhibits irreversibly. Although these inhibitors act in the nanomolar range their functionality is limited by less absorption via cell membranes and protease degradation susceptibility (Table 1) (Bekono et al. 2018).

Peptidyl fluoromethyl ketones

The peptidyl fluoromethyl ketones are benzyloxycarbonyl (Z)-Phe-Arg-CH2F, Mu-Phe-HomoPhe-CH2F and Mu-Leu-HomoPhe-CH2F (Sl. No. 1–3). The most potent is benzyloxycarbonyl(Z)-Phe-Arg-CH2F with an IC_{50} value of 0.36 nM. It blocks the degradation of hemoglobin with an IC_{50} of 0.10 µM. This inhibitor is effective against Itg2, W2, D6, FCR3 strains of *P. falciparum.* It acts by inhibiting the falcipains. Mu-Phe-HomoPhe-CH2F and Mu-Leu-HomoPhe-CH2F show IC_{50} values of 3 and 0.42 nM respectively. These fluromethyl ketones have morpholine urea (Mu) group at the P3 position and are very effective inhibitors against falcipain-2. Mu-Phe-HomoPhe-CH2F is the most effective inhibitor of *P. vinckei* cysteine protease (IC_{50}-5.1 nM). When this inhibitor is administered in *P. vinckei* infected mice, it cures 80% of treated mice and also induces long-lasting immunity. They act as promising inhibitors of cysteine protease (Ettari et al. 2021). The main limitation of these inhibitors is their frequent administration due to susceptibility of protease degradation in serum, tissues and they possess short half-life.

Peptidyl vinyl sulfones

These are covalent inhibitors of falcipain-2 and they function irreversibly by the addition of thiol group in Cys42 active site to vinyl sulfone electrophilic moiety that acts as Michael acceptor.

Vinyl sulfones Mu-Phe-hPhe-VSPh (K11002) and Mu-Leu-hPhe-VSPh (K11017) (Sl. No. 4–5) were tested against FP-2 showing IC_{50} values of 0.08 µM and IC_{50} of 0.003 µM, respectively. The compound Mu-Leu-hPhe-VSPh has better effectiveness against FP-2 because of the presence of Leu residue at P2 position, towards which the preference of FP-2 is very high. These inhibitors also block hemoglobin degradation and parasite development (Ettari et al. 2021).

The in vivo bioavailability and aqueous solubility were improved by replacing the Morpholine urea (Mu) group with N-methyl piperazine urea nucleus (Sl. No. 6) and a naphthalene nucleus was introduced at the P1 site to produce a potent compound (Sl. No. 7) which are orally active and reduces the murine malaria progression showing 40% cure of the treated animals. (Ettari, et al. 2010).

Shenai et al. synthesized and tested 39 new compounds that have vinyl sulfones, vinyl sulfonate esters and vinyl sulfonamide groups. Inhibitors that possess the Leu group in P2 position and homophenylalanine (HPhe) group in the P1 position have a remarkable inhibitory effects against falcipain-2, falcipain-3, and cultured *P. falciparum*. Many vinyl sulfonate esters were synthesized and tested. The vinyl sulfonate compounds such as (Sl. No. 8) showed good inhibitory activity with IC_{50} of 0.9 nM against falcipain-2, followed by vinyl sulfonamides (Sl. No. 9) with IC_{50} of 2.3 nM and phenyl vinyl sulfones (Sl. No. 10) with $IC_{50} = 6.9$ nM against Falcipain-2. They are in preclinical stage of development (Ettari et al. 2010).

The cocrystallization of Mu-Leu-hphe-VsPh (K11017) (Sl. No. 5) with falcipain-3 displays that the inhibitor binds to the S1 and S3 subsites to generate a covalent, irreversible bond with the sulfur present in active site cysteine thiol in falcipain-3. Falcipain-3 favours Leu at P2 of Mu-Leu-Hph-VsPh. This inhibitor engages the S3, S2, S1 and S1' subsites of the enzyme. According to the hydrophobic properties of P2 and P1 substituents in Mu-leu-Hph-VsPh, the active site of falcipain-3 contains several residues that are capable of forming nonpolar interactions with their respective inhibitor. This inhibitor engages the S3, S2, S1 and S1' subsites of enzyme (Pandey and Dixit 2012).

Peptidyl aldehydes and α-ketoamides

The α-ketoamide derivatives such as Peptidyl aldehyde Mu-Leu-hPhe-al (Sl. No. 11) and Mu-Leu-hPhe-Phe-(CO)-NH2 (Sl. No. 12) inhibit falcipain-2 with an IC_{50} value of 1 nM for each. Another active peptidyl aldehyde is Z-Leu-hPhe-al, (Sl. No. 13) which shows an IC_{50} value of 2 nM. These compounds are very effective in inhibiting falcipain-2 at the nanomolar range. These are promising inhibitors of cysteine protease (Ettari et al. 2021).

E-64

N-(L-3-Trans-carboxyoxiran-2-carbonyl)-L-leucyl-amido (4-guanido) butane is the first epoxysuccinyl peptide derivative. E-64 (Sl. No. 14) is an irreversible inhibitor of papain-like cysteine proteases (Clan CA, family C1). E-64 blocks cysteine protease activity through S-alkylation in active site cysteine, which mediates the opening of epoxide ring. The leucine side chain of E64 mimics substrate P2 amino acid thus occupying the S2 binding pocket of the target. Peptidyl epoxysuccinates can block enzyme activity by the formation of a thioether bond through nucleophilic attack at C-3 or C-2 in the epoxide ring. This inhibitor produced IC_{50} values of 0.015 and 0.075 µM against falcipain-2 and falcipain-3 strains respectively. This acts as an effective inhibitor of cysteine proteases (Ettari et al. 2010).

The in silico docking and molecular dynamic simulations showed that E64 can either interact with D170, Q171, C168, G169, A151, and G230 at the "recruiter group A" (RA) or by first interacting with K76, N77, and N81 for the "recruiter group B" (RB) while binding to the Falcipain-2 (FP2) of *Plasmodium falciparum* with a binding energy of -12.2 ± 1.1 kJ/mol. (Salawu 2018).

Peptidyl aziridine derivatives

These are selective, irreversible inhibitors of cysteine protease. They are aza analogues of epoxides. Schulz et al. tested the falcipain-2 and falcipain-3 inhibitory activities of aziridines, aziridine-2-carboxylic acid derivatives, N-acylated aziridine-2,3-dicarboxylic acid derivatives and aziridine-2-carboxylates having a lysine residue.

In this series N-((S)-4-methyl-1-(((S,E)-1-(methylsulfonyl)-5-phenylpent-1-en-3-yl)amino)-1-oxopentan-2-yl)-4-((((3S,5aR,6S,9S,10R,12S,12aS)-3,6,9-trimethyldecahydro-12H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromen-10-yl)oxy)methyl)benzamide (Sl. No. 15) exhibited potent inhibitory activity against falcipain-2 with an IC_{50} of 0.21 nM. In addition, this inhibitor has better activity against multiple malaria strains and is in the preclinical stage of development. (Bekono et al. 2018).

Chalcones

Chalcones are biosynthetic precursors of flavonoids. Licochalcone A is a natural chalcone that is isolated from Chinese

liquorice roots. It is the first compound of this family that has in vivo and in vitro activity against malaria.

First studies in the field of synthetic chalcones were performed by Li and co-workers and the most effective chalcone compound is 1-(2,5 dichlorophenyl)-3(4-quinolinyl)-2-propen-1-one (Sl. No. 16) having an IC_{50} value of 200 nM against both CQ-resistant W2 strain and CQ-sensitive D6 strain of *P. falciparum*. The C2–C3 double bond in this chalcone is essential for inhibitory activities, as it extends the molecular conformation results in a better interaction with the active site.

A series of alkoxylated and hydroxylated chalcones were synthesized by Liu and co-workers and the most active compound is 1-(2',3',4'-trimethoxy-phenyl)-3-(3-quinolinyl)-2-propan-1-one (Sl. No. 17) having an in vitro IC_{50} value of 2 µM against K1 strain of CQ-resistant *P. falciparum*.

Recently, Dominguez et al. reported that phenylurenyl chalcones have good inhibitory properties against falcipain-2. The most effective inhibitor of this series is 1-[4'-*N*-(*N*'-p-chlorophenylurenyl)-phenyl]-3-(3,4,5 trimethoxyphenyl)-2-propen-1-one (Sl. No. 18) having an IC_{50} value of 1.8 µM and also causes inhibition of cultured malaria parasite development (IC_{50} = 3 µM). These are promising inhibitors of cysteine proteases and are currently in the preclinical stage of development (Ettari et al. 2010).

Isoquinolines

Isoquinoline derivatives were designed as falcipain-2 inhibitors through homology modeling studies and validation was carried out through docking of known peptidyl vinyl sulfone inhibitors. In these experiments, the interaction of 1-(4-hydroxy-phenyl) group with the Asp234 residue in the S2 pocket of falcipain-2 is considered essential. Isoquinoline compounds (Sl. No. 19–21) with p-benzyloxyphenyl group are docked into the active pocket of the homology-modeled falcipain-2. They are potent in comparison to corresponding hydroxyl analogs. It suggests that the S2 pocket of falcipain-2 prefers to accommodate hydrophobic groups. They have an IC_{50} value of 3 µM. (Ettari, et al. 2010).

Thiosemicarbazones

Recently, some thiosemicarbazones having phenolic Mannich base were synthesized and administered against falcipain-2 and CQ-resistant *P. falciparum* (W2). In this series of thiosemicarbazones, three compounds (E)-2-(2,4-dihydroxy-3-((1-methylpyrrolidin-3-yl)methyl) benzylidene)hydrazine-1-carbothioamide, (Sl. No. 22), (E)-2-(2,4-dihydroxy-3-((1-methylpiperidin-4-yl)methyl) benzylidene)hydrazine-1-carbothioamide (Sl. No. 23) and (E)-2-(3-(7-chloro-2-(4-methylpiperazin-1-yl)quinolin-6-yl) 2,4-dihydroxybenzylidene)hydrazine-1-carbothioamide (Sl. No. 24) showed better inhibitory activity against falcipain-2 with IC_{50} values of 5.8, 3.8 and 2.25 µM respectively. So, it is suggested that the thiosemicarbazone moiety has an essential role in falcipain-2 inhibition. Compounds without this Mannich base do not inhibit falcipain-2 and parasites. (Ettari, et al. 2010).

Aspartic protease inhibitors

In the developmental stage from the tiny ring stage to the big trophozoite active stage inside the erythrocytes, *Plasmodium* parasite acquires free amino acids for their nutrition by degrading hemoglobin. It produces various proteolytic enzymes through which they degrade the host's hemoglobin of RBC. Aspartic protease, which is also known as Plasmepsin (Plm), is one of the major enzymes that helps in the degradation of Hb. By inhibiting the activity of this enzyme, the growth and spread of parasites can be reduced (Table 2).

Aryltetrahydropyridines

It is mainly a renin inhibitor but it also functions in inhibiting Plm II of parasites (Sl No. 25) .19 Plm inhibitors were identified in this group, which was divided into two classes. Class A has the IC_{50} value of < 100 nM and for Class B $100\ nM < IC_{50} < 10$ µM. They interact with the aspartic protease active site and inhibit its action. It is a promising drug candidate against malaria (Bobrovs et al. 2019).

2-aminoquinazolin-4(3H)-one

It is the powerful and selective inhibitor of Plms but the mode of binding is still unknown (Sl. No. 26). Rasina et al. identified 10 compounds of inhibitory activity by nuclear magnetic resonance (NMR) based fragment screening against Plm II. 2-aminoquinazolin-4(3H)-one showed the most favorable activity among those 10 compounds in the enzymatic assay. Open-flap and closed-flap models have given a possible mode of binding. It has been shown that in the open flap conformation, there is a hydrogen bond between the N1 and 2-amino groups of this inhibitor and the catalytic dyad of Asp34-Asp214 (Bobrovs et al. 2019). The compound of this group has the lowest IC_{50} value of 0.28 ± 0.02 µM which is determined by the study of triplicate experiments of enzymatic FRET assay (Rasina et al. 2018). It inhibits the growth of *P. falciparum* in the in vitro culture of the cell-based assay with the IC50 value of 1 µM (Rasina,

Table 2 Malarial aspartic protease inhibitors

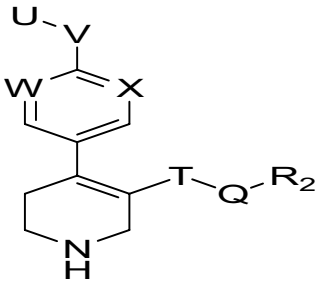
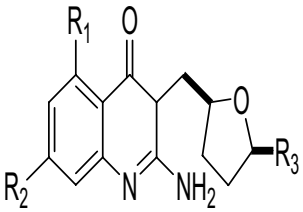
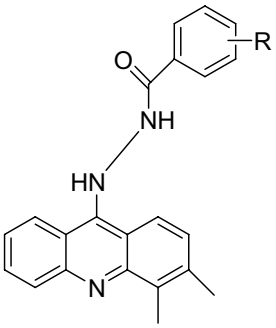
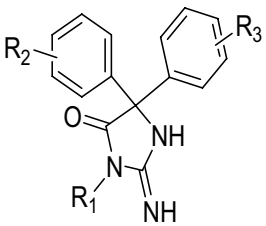
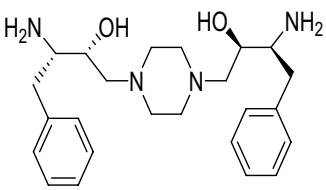
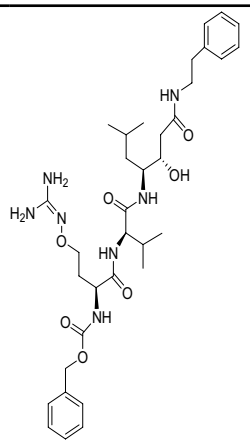
Sl No	Name	Structure	IC_{50}	References
25	Aryltetrahydropyridines		For class A $IC_{50} < 100$ nm and for class B 100 nM $< IC_{50} < 10$ µM	Bobrovs et al. (2019)
26	2-aminoquinazolin-4(3H)-one		0.28 ± 0.02 µM	Rasina et al. (2018) Bobrovs et al. (2019)
27	Hydrazide based inhibitor		> 10 nM	Bobrovs et al. (2019)
28	Aminohydantoin based inhibitor		12 nM for Plm II and 1 µM for Plm V	Bobrovs et al. (2019)
29	Hydroxyethylamine core scaffold		IC_{50} of 1SR = 29 nM against Plm IV	Mishra et al. (2019)

Table 2 (continued)

Sl No	Name	Structure	IC_{50}	References
30	WEHI-842		0.2 nM	Mishra et al. (2019)

et al. 2015). Currently, it is in the preclinical stage and is a promising compound for anti-malarial targets.

Hydrazide-based inhibitors

The screening of synthetic compounds of the in-house library by Azim et al. has led to the discovery of 9-hydrazinylacridine derivatives which are strong inhibitors of Plms (Sl. No. 27). Two inhibitors of IC_{50} value of > 10 nM have been identified with the models of Plm II and cat D crystal structure by using HTVS. In vitro experiments with enzyme inhibition assay show similar results of inhibitory action of acridinyl compounds as the docking prediction. It is in preclinical condition. Investigation shows that the core of hydrazine functions as mimetics of the transition state and interacts with the aspartic dyad by charged hydrogen bonds (Bobrovs et al. 2019).

Aminohydantoin-based inhibitors

The GlaxoSmithKline screening campaign indicates the inhibitory function of aminohydantoin compounds against *P. falciparum* 3D7 strain. It acts as a highly potent inhibitor against Plm II with an IC_{50} value of 12 nM and also produces an optimal IC_{50} value of 1 µM against Plm V (Sl. No. 28). The inhibitors interact with the semi-open structure of Plms where two phenyl groups bind with S2'and S1/S3 pockets (Bobrovs et al. 2019).

Hydroxyethylamine core compounds

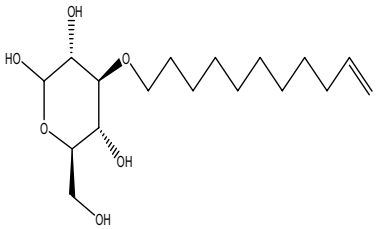
The peptidomimetic compounds bearing hydroxyethylamine scaffold act as strong inhibitors against Plasmepsin (Plms I, II, IV). One of the compounds (1 SR) which is a potent inhibitor of Plms IV showed an IC_{50} value of 29 nM (Sl. No. 29). But it has no selectivity against parasitic Plms. Thus, it can also act against Cathepsin D which is the aspartic protease of humans.

C2 symmetric hydroxyethylamine analog is also found as a strong inhibitor of parasitic aspartic protease (Plm II and IV) and shows potent growth inhibitory properties against *P. falciparum* in vitro culture (Mishra et al. 2019). Currently, they are in the preclinical stage and could be promising candidates for anti-malarial drug development.

WEHI-842

This small molecule can inhibit Plms V of parasites and stops the protein exportation and growth of parasites (Sl. No. 30). This inhibitor acts against *P. falciparum* with the IC_{50} value of 0.2 nM. It acts as a mimetics of transition state which causes proteolysis of amide-bond for the substrate of PEXEL. Thus, it inhibits the exportation of proteins and stops parasitic growth (Mishra, et al. 2019). According to the findings of Ji, et al. WEHI-842 showed binding interaction inside the active pockets of aspartic protease plasmepsin V. The inhibitor molecule interacted with the protein at S1, S2 and S3 subpockets. Asp365, Thr369, and Ser368 created an H-bond connection with

Table 3 Malarial glucose transporter inhibitors

Sl No	Name	Structure	IC_{50}	References
31	3-O-[undecyl-10-en]-1-yl-D-glucose (C3361)		33.1 ± 2 µM	Jiang 2022 Jiang et al. (2020)

the active chemical at the S2 subpocket, while Glu179 and Gly367 formed H-bond interactions at the S3 pocket. Val227, Tyr177, and Ile116 formed hydrophobic interactions with the molecule (Ji, et al. 2022).

Plasmodium transporters inhibitors

Plasmodium malaria parasite takes essential nutrient molecules like sugar, lactate, Na^+, H^+ and choline from the host, utilizing various transporters which have already been discussed. These nutrients are responsible for their survival. Therefore, inhibition of these transporters to stop the supply of these specific nutrients aims to kill the dreadful malarial parasite.

Inhibitors of glucose transporter

Small derivatives of glucose molecules have been found to inhibit the function of glucose transporter in the parasite in a culture of *P. falciparum* (Table 3).

C3361 C3361, chemically known as 3-O-[undecyl-10-en]-1-yl-D-glucose, is an O3 hexose derivative that targets more actively against PfHT1 than GLUT1 of the host (Sl. No. 31). It was shown that it can target hexose transporter of *P. vivax, P. knowlesi and P. yoelii* at the same time which indicates that it is an inhibitor of hexose-transporter acts on the broad-spectrum range. The in vivo study of the life cycle of *P. berghei* in mice model indicates that C3361 blocks the hepatic stage of parasitic growth and reduces parasitemia. The IC_{50} value of C3361 is 33.1 ± 2 µM and it is scientifically proven to be a potential antimalarial compound. Currently, it is in the preclinical stage of drug development. The total structure of C3361 binds with PfHT1 suggesting that it blocks the tunnel of substrate transportation both on the extracellular and intracellular sides. According to the "Lollipop model", ball of lollipop which is the glucose moiety of C3361 occupies the site in PfHT1 where sugar binds and the stick of lollipop which is the aliphatic chain interacts with the lipid bilayer. Thus, it inhibits glucose uptake antagonistically and stops the growth of parasites (Fig. 6) (Jiang 2022). Jiang et al. explored the X-ray crystal structure of PfHT1-bound co-crystal inhibitor of C3361 at 3.7 Å. The

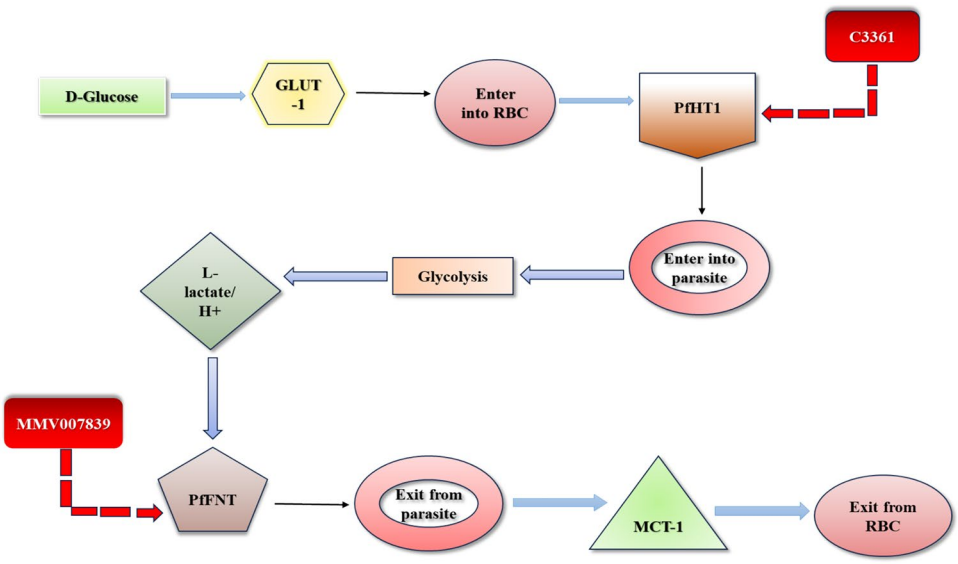
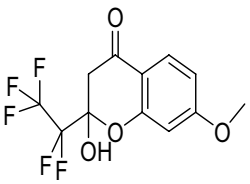
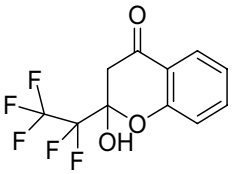


Fig. 6 Inhibitors of glucose transporter and lactate transporter of *Plasmodium*

Table 4 Malarial lactate transporter inhibitors

Sl No	Name	Structure	IC_{50}	References
32	Pentafluoro-3-hydroxy-pent-2-en-1-ones (MMV007839)		0.14 ± 0.01 µM for *P. falciparum* 3D7 strain	Golldack et al. (2017) Nerlich et al. (2021) Peng et al. (2021)
33	MMV000972		1.7 ± 0.1 µM for *P. falciparum* 3D7 strain	Golldack et al. (2017)

computational study indicated that, unlike the binding of glucose, an additional pocket is formed when C3361 binds with PfHT1. Due to a shift in transmembrane 1e (TM1e), TM2 and TM7b, C3361's aliphatic tail is now bordered by a variety of hydrophobic residues on the extracellular region of TM1, TM2, TM7b and TM11. The detailed rearrangements of TM1, TM2, and TM7b upon C3361 binding showed crucial interactions with residues such as L47, F85, L81, V44, V312, S315, N38 and K51 respectively (Jiang et al. 2020).

Inhibitors of lactate tranporter

Two fluoroalkyl vinylogous malaria box compounds such as MMV007839 and MMV000972 act as potent inhibitors of lactate transporter of *Plasmodium.* These compounds interact with the channel of the lactate transporter (Table 4).

MMV007839 It is chemically known as Pentafluoro-3-hydroxy-pent-2-en-1-ones. This compound has an IC_{50} value of 0.14 ± 0.01 µM for *P. falciparum* 3D7 strain when it is incubated in the in vitro conditions (Sl. No. 32). It has a cyclic hemiketal structure with which fluroalkyl chain is attached with a phenone component. This hemiketal form switches reversible from lipophilic form to vinylogous linear form which is polar and vice-versa. The hemiketal lipophilic form allows the compounds to cross the infected erythrocytic plasma membrane. The linear form of MMV007839 is suspected the active inhibitory form which mimics the lactate substrate present in two successive forms. The vinylogous acid section which is deprotonated acts like the anionic lactate form and the fluoroalkyl chain acts like neutral lactic acid form and blocks the transfer of lactate (Golldack et al. 2017). Specifically, three polar residues of the central pore, Thr106, Gly107, and His230, establish a hydrogen bond network with the vinylogous acid moiety of MMV007839, whereas the rest of the inhibitor contacts the hydrophobic section of the central pore via Van der Waals interactions and blocks the pathway for lactate transfer (Fig. 6). According to the docking study of Peng, et al. the binding energy of wild type PfFNT and MMV007839 is −5.53 kcal/mol whereas the binding of MMV007839 with G107S mutant of PfFNT shows binding energy of −3.44 kcal/mol. The phenol ring plugs the intracellular constriction site, allowing it to open towards the cytosolic side and bind to PfFNT mediated by both hydrophilic and hydrophobic interactions. The computational study indicates that the intracellular constriction region of PfFNT is formed by two conserved amino acids which are Leu104 and Val196 and a variable residue of Phe94. At the inhibitor-bound form of PfFNT, there is a notable shift of the side chain of Phe94 and Ile98. The phenyl group of Phe94 with Leu104 and Val196 moves away from its first location to circumvent the collision with the fluroalkyl group of MMV007839 and keeps the intracellular gate open. The change in the position of the side chain of Ile98 reduces steric hindrance of methoxy group which is attached at the end of MMV007839. It is proven scientifically as a lead compound that kills *Plasmodium* parasite at a submicromolar level. (Peng, et al. 2021).

MMV000972 It has the same structural scaffold but differs only in the presence of an aromatic methoxy group. It acts in the same fashion as MMV007839 and blocks the pathway of lactate transport (Nerlich et al. 2021). It has the IC_{50} value of 1.7 ± 0.1 µM for the 3D7 strain of *P. falciparum* (Sl. No. 33).

Inhibitors of P-type Na^+ ATPase

Spiroindolone compounds which are the major inhibitors of P-type Na^+ ATPase can stop the growth of the trophozoits stage of *P. falciparum* and the ring stage of *P. vivax* (Rottmann et al. 2010). Various spiroindolone derivatives have

Table 5 Malarial P-type Na^+ ATPase inhibitors

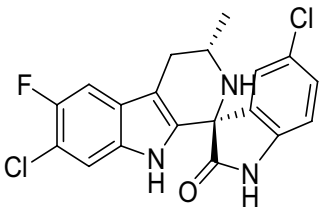
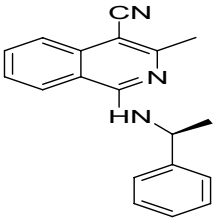
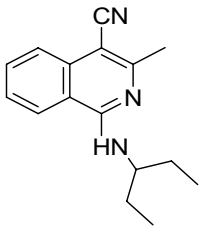
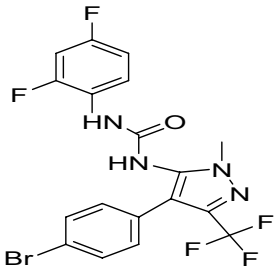
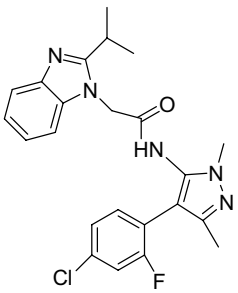
SL No	Name	Structure	IC50	References
34	Spiroindolone Cipargamin (NITD609)		0.5–1.4 nM	Rottmann et al. (2010) Gilson et al. (2019) Spillman and Kirk (2015)
35	4-cyano-3-methylisoquinoline (MB14, MB10)	 MB10  MB14	MB14 = 52 ± 16 nM in 3D7 parasite MB10 = 62 ± 9 nM in 3D7parasite	Gilson et al. (2019)
36	Aminopyrazoles (GNF-Pf4492) (N-[4-(4-bromophenyl)-1-methyl-3-(trifluoromethyl)-1H-pyrazol-5-yl]-N'-(2,4-difluorophenyl)urea)		184.1 nM in the asexual stage *of P. falciparum* strain which is multi drug resistance, known as Dd2	Spillman and Kirk (2015)
37	Pyrazolamide (PA21A092)		EC_{50} value-12.9 ± 2.0 nM in Dd2 strain of *P. falciparum*	Spillman and Kirk (2015)

Table 5 (continued)

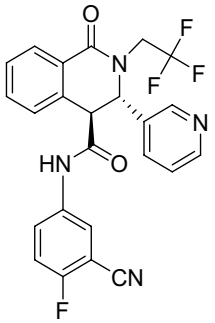
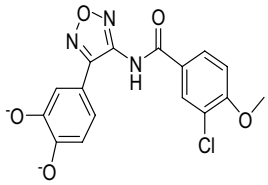
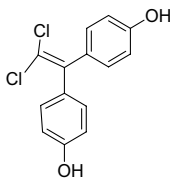
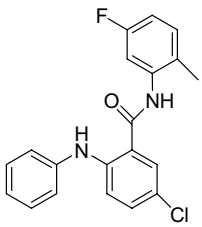
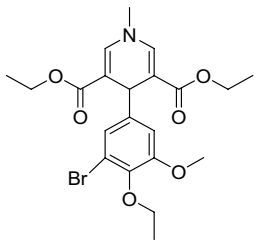
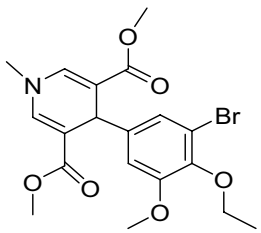
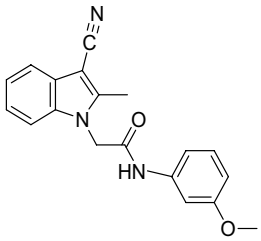
SL No	Name	Structure	IC50	References
38	Dihydroisoquinolones (+)-SJ733		EC_{50} value-20 nM for Dd2 strain 30 nM for 3D7 strain	Spillman and Kirk (2015)
39	Compounds of Malaria Box			Lehane et al. 2014
A	MMV011567		0.33 ± 0.03 µM in Dd2 strain	
B	MMV665949		1.12 ± 0.13 µM in Dd2 strain	
C	MMV007275		0.22 ± 0.02 µM in Dd2 strain	
D	MMV006656		0.15 ± 0.01 µM in Dd2 strain	
E	MMV006764		0.45 ± 0.05 µM in Dd2 strain	

Table 5 (continued)

SL No	Name	Structure	IC50	References
F	MMV665826		0.28 ± 0.04 μM in Dd2 strain	

been found and NITD609 (Cipargamin) is the most potent inhibitor of P-type Na^+ ATPase pump (Table 5).

Cipargamin Cipargamin, also known as NITD609, shows no cytotoxic effect when it is applied to a mouse model. It is in the clinical phase and is considered the first molecule that successfully crosses the Phase II clinical trial (Sl. No. 34) (Spillman and Kirk 2015). It displayed an IC_{50} value of 0.5–1.4 nM for the *P. falciparum* in in vitro condition (Rottmann et al. 2010). Cipargamin when applied, directly blocks the P-type Na^+ ATPase pump of parasites and destroys the homeostasis of the internal environment of parasites as it does not allow to excrete Na^+ ions from the cytosol of parasites. Along with it, NITD246 causes an increase in the pH of cytosol by increasing H^+ transmembrane gradients. Due to the accumulation of extra Na^+ ions in the cytosol of parasites, the infected erythrocytes undergo lysis due to osmotic swelling (Spillman and Kirk 2015). Fatoki et al. studied the molecular docking interaction between PfATP4 and Cipargamin and found a binding energy of − 12.40 kcal/mol. The amino acids residues of PfATP4 which interact with cipargamin are Ile378, Pro384, Asn1082, Glu1084, Arg1113 and Asp1116 (Fatoki et al. 2022).

4-cyano-3-methylisoquinoline There are two compounds of 4-cyano-3-methylisoquinoline which are MB14 and MB10 (Sl. No. 35). MB14 and MB10 function similarly to spiroindolone as they target directly the Na^+ ATPase and block the pathway of Na^+ efflux. It causes swelling of infected RBC due to the accumulation of excess Na^+ and lysis of it. MB14 shows an IC_{50} value of 52 ± 16 nM and MB10 shows an IC_{50} value of 62 ± 9 nM for the 3D7 strain of *the P. falciparum* membrane. Now it is in the preclinical stage of drug development (Gilson et al. 2019).

GNF-pf4492 It is a compound of pyrazole-urea that stops the growth of the asexual stage of *P. falciparum* (Sl. No. 36). It produced an IC_{50} value of 184.1 nM for *P. falciparum* Dd2 strain which is multi-drug resistance. Adding GNF-Pf4492 into the isolated parasites causes an increase in the concentration of Na^+ in the cytoplasm of parasites. However, the increase in Na^+ concentration is more or less slower than other inhibitors. It is under the preclinical stage of drug development (Spillman and Kirk 2015).

PA21A092 It is a compound of Pyrazolamide. It produced an EC_{50} value of 12.9 ± 2.0 nM for *P. falciparum* (Sl. No. 37). It increases the cytoplasmic Na^+ concentration rapidly in saponin isolated parasites. It also increases the cytosolic pH of the parasite. An increase in the Na^+ concentration of cytoplasm causes swelling of parasites due to consistent uptake of water followed by increased Na^+ concentration. It is scientifically tested and currently is in the preclinical stage (Spillman and Kirk 2015).

(+)-SJ733 It is the derivative of dihydroisoquinolones (Sl. No. 38). It produced an EC_{50} value of 20 nM for the Dd2 strain and 30 nM for the 3D7 strain of *P. falciparum*. The addition of this inhibitor consistently inhibits the PfATP4 and increases the concentration of Na^+ of the cytoplasm of the parasite as well as increases the pH of cytoplasm. Therapy with (+)-SJ733 causes phosphatidylserine to expose rapidly at the outer side of the plasma membrane of parasites and gives the signal for erythrophagocytosis. In in vivo conditions, the osmotic swelling of infected RBC is observed which causes premature stage eryptosis and quick clearance of infected RBC (Spillman and Kirk 2015).

Malaria-box compounds: From the 400 compounds of Malaria-box venture, many 28 compounds target Na^+ ATPase pump similarly to spiroindolone which can increase the pH of parasitic cytoplasm. The in vitro experiments have screened six potential compounds (39 A-F) showing IC_{50} values at a micromolar range of 0.15–1.12 μM in Dd2 strain (Lehane et al. 2014).

Inhibitors of v-type H^+ ATPase pump

The Bafilomycin A1 and the Concanamycin are anti-cancer antibiotics that targets the V-type H^+ATPase pump

and inhibit the transportation of H^+ ions. In the in vitro conditions these two drugs show antimalarial activity from the range of low micromole to sub nanomole but it is still not investigated whether these drugs block the parasitic H^+ATPase pump or not. Therefore, further design of potential compounds was carried. Bafilomycin A1 and Concanamycin are scientifically tested as antimalarial agents.

Mainly 30 indole derivatives have been investigated in in vitro conditions (Sl. No. 40A-40E). From the indole derivatives, indolepentediene amides which have two members, 11383 shows an IC_{50} value of 0.49 ± 0.03 µM in 7G8 strain and 0.54 ± 0.04 µM in K1 strain of *P. falciparum* and 50010 displayed 0.59 ± 0.01 µM of IC_{50} value for K1 strain. Indole Benzamides (12192) shows IC_{50} value of 0.41 ± 0.08 µM in 7G8 strain and 0.39 ± 0.02 µM in K1 strain. Benzoimidazole benzamides has two members from which 12194 shows IC_{50} value of 0.38 ± 0.10 µM in 7G8 strain and 0.34 ± 0.01 µM in K1 strain and 22434 show IC_{50} value of 4.8 ± 1.3 µM in 7G8 strain and 3.9 ± 0.7 µM in K1 strain. Benzoimidazole acetamides (11384) shows IC_{50} value of > 10 µM in 7G8 and K1 strain. 3-Phenylindoles (14121) shows IC_{50} value of 3.8 ± 0.4 µM in 7G8 strain and 5.6 ± 0.5 µM in K1 strain. They show rapid acidifications of the parasitic cytoplasm and inhibition of parasitic growth. They show no synergistic or antagonistic activity when they were targeted with other antimalarial compounds (Table 6) (van Schalkwyk et al. 2010).

Inhibitors of aquaporin-3

From the investigation, it has been shown that AQP-3 deletion shows no harmful effect in mice. So this host protein is targeted for antimalarial drug discovery.

Auphen is a known inhibitor of AQP-3 (Sl. No. 41). It has an EC_{50} value of 0.77 ± 0.08 µM when it is injected into liver stage *P. berghei* and EC_{50} value of 0.81 ± 0.10 µM when it is injected to erythrocytic *P. falciparum* Dd2 strain. It blocks the AQP-3 channel of hepatocytes and erythrocytes and reduces the parasitic load in these two locations. It is a gold-based particle where the gold particle [Au(III)] interacts with the cysteine amino acid which is conserved in the selectivity filter of aquaporin 3. This interaction blocks the AQP-3 function specifically as orthodox aquaporin does not contain this region (Table 7). The antimalarial effect of Auphen is scientifically tested (Posfai et al. 2018). Martins, et al. carried out a preliminary docking calculation for the binding of Au(III) with AQP-3. This docking study showed that the non-covalent interaction between Au(III) and AQP-3 generally occurs in the periplasmic pocket of AQP-3. According to the docking poses, the periplasmic pocket of AQP-3 is the most accessible sterically with the interacted Au(III). The SF domain amino acids residue of AQP-3 which are Phe63, Tyr212 and Arg218 together with Cys40 side chain interact with auphen. It is also noted that the thiol group of Cys40 of AQP-3 is in close proximity which is 9-10Å and oriented efficiently for binding with a metal ion (Martins et al. 2012).

Inhibitors of choline transport

Various analogs of choline have been designed to block the transport and metabolism of choline into the body of parasites. Among the analogs bis-thiazolium salts show high antimalarial activity in the in vitro study with *P. falciparum* and in vivo study with *P. berghei*. Albitiazolium, a T3 compound crosses the Phase II level of the trial which shows potent antimalarial activity in the body of infected adults (Penarete-Vargas et al. 2014). Apart from bis-thiazolium salts bis quaternary ammonium derivatives of choline analogs also show a great extent of antimalarial activity (Table 8).

Albitiazolium It is a compound of bis-thiazolium salts named T3/SAR97276 (Sl. No. 42). It has an IC_{50} value of 2.3 nM against *P. falciparum*. It acts as a competitive inhibitor of Choline transport into the parasites and blocks the activity of the enzymes involved in phosphatidylcholine biosynthesis in de-novo pathway (Penarete-Vargas et al. 2014). It specifically accumulates into the *Plasmodium*-infected RBC irreversibly and mainly targets the digestive vacuole of parasites where it interacts with heme group of hemoglobin. This interaction suggests its contribution towards potent antimalarial activity (Peyrottes et al. 2012). Currently, it is in Phase II clinical trial to treat severe malaria (Wein et al. 2012).

G25 It is a bis quarternary ammonium derivative which accumulates irreversibly about 100 fold into the *Plasmodium*-infected RBC (Sl. No. 43). It has an IC_{50} value of 0.65 nM against *P. falciparum*. It increases in the concentration as the parasite matures and inhibits the influx of choline into the parasites and also prevents the incorporation of choline into phosphatidylcholine. The action of this compound is scientifically tested and in the preclinical stage (Peyrottes et al. 2012).

Plasmodium DNA and nucleic acid synthesis inhibitors

Malaria parasite multiplication is an essential step for germination in the human host cells. One parasite is germinated into multiple on the synthesis of DNA and nucleic acid. *Plasmodium* multiplication is carried out by utilizing many enzymes such as dihydroorotate dehydrogenase,

Table 6 Malarial V-type H^+ ATPase inhibitors

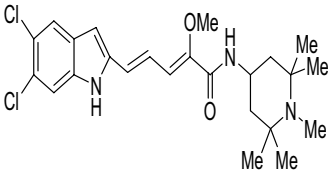
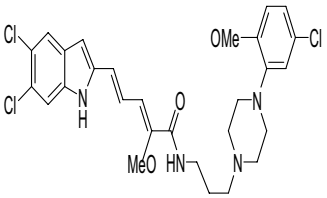
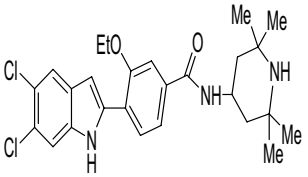
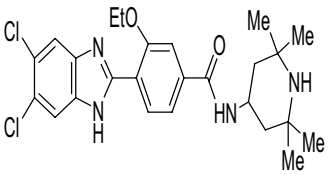
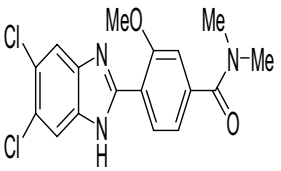
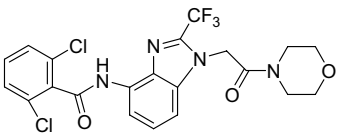
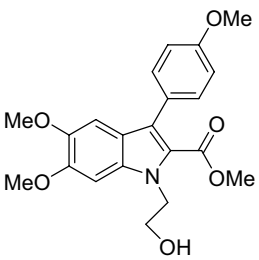
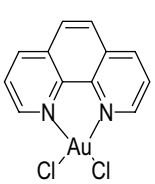
Sl No	Name	Structure	IC_{50}	References
40	Indole derivatives			van Schalkwyk et al. (2010)
A	Indolepentadiene amides			
	11383		0.49 ± 0.03 µM in 7G8 strain and 0.54 ± 0.04 µM in K1 strain	
	50010		0.59 ± 0.01 µM in K1 strain	
B	Indole Benzamides (12192)		0.41 ± 0.08 µM in 7G8 strain and 0.39 ± 0.02 µM in K1 strain	
C	Benzoimidazolebenzamides			
	12194		0.38 ± 0.10 µM in 7G8 strain and 0.34 ± 0.01 µM in K1 strain	
	22434		4.8 ± 1.3 µM in 7G8 strain and 3.9 ± 0.7 µM in K1 strain	
D	Benzoimidazoleacetamides (11384)		> 10 µM in 7G8 and K1 strain	
E	3-Phenylindoles (14121)		3.8 ± 0.4 µM in 7G8 strain and 5.6 ± 0.5 µM in K1 strain	

Table 7 Aquaporin-3 inhibitors

Sl No	Name	Structure	IC_{50}	References
41	Auphen		EC_{50} value of 0.77 ± 0.08 μM when it is injected to liver stage *P. berghei* and EC_{50} value of 0.81 ± 0.10 μM when it is injected to erythrocytic *P. falciparum* Dd2 strain	Posfai et al. (2018)

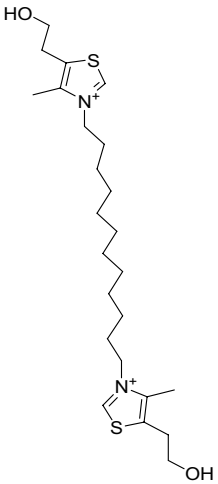
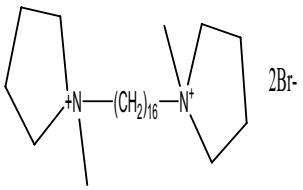
dihydrofolate reductase, apicoplast and dihydropteroate synthase. Therefore, the quest for potential inhibitors that can inhibit these enzymes is very urgent.

Inhibitors of dihydroorotate dehydrogenase (DHODH)

DSM1 It contains aminonapthyl moiety substituted in the triazolopyrimidine core. High throughput screening (HTS) of 220,000 compound library of "druglike" compounds utilizing a colorimetric enzyme test yields DSM1 (Sl. No. 44). The IC_{50} value of DSM1 is 0.047 ± 0.022 μM and it has > 5000 fold selectivity in comparison with human DHODH. DSM1 is currently in the preclinical stage of development. In whole-cell assays this inhibitor inhibits the *P. falciparum* parasite proliferation with a similar powerfulness. This inhibitor also displays high activity against *P. falciparum* drug-resistant Dd2 strain. There is a corresponding linear increase in the IC_{50} of DSM1 with an increased CoQD concentration that is demonstrated by steady-state kinetic analysis and is predicted for competitive and tight-binding inhibitors. It forbids the electron transfer from FMNH2 to CoQ by oxidative half-reaction (kox) inhibition. But on the other hand, it does not have any effect on reductive half-reaction (kred) which is dependent on dihydroorotate (DHO) (Phillips et al. 2008).

In silico docking study revealed that the intermolecular interaction between *Pf*DHODH-DSM1 involves five main amino acid residues- Phe188, Phe227, Val532, Leu172 and Arg265. In case of *Pf*DHODH-DSM1 complex, variations in energy values occurred within radii 2.0–2.5 Å, among them, Leu172, Leu189, Arg265, and Val532 were shown to be more significant in promoting the largest energy discrepancy. The binding energy is − 50.79 kcal/mol with a dielectric constant value of 10 (Lima Costa et al. 2023).

Table 8 Malarial choline transporter inhibitors

Sl No	Name	Structure	IC_{50}	References
42	Albitiazolium		2.3 nM against *P. falciparum*	Wein et al. (2014)
43	G25		0.65 nM in *P. falciparum*	Peyrottes et al. (2012)

DSM265 The amidino moiety is substituted in the triazolopyrimidine core to produce a potent compound DSM265 (Sl. No. 45) which blocks the synthesis of nucleotides that are essential for DNA and RNA synthesis by inhibition of *Pf*DHODH with an IC_{50} value of 20 nM and forbids the production of parasites' liver and blood stages with similar vigour, arresting their development before the synthesis of schizont stage that is multinucleated. (Phillips et al. 2017a, b) DSM265 also has moderate activity against oocyst (a multinucleated stage in insect) demonstrating the fact that it may have some transmission-blocking potency. The growth-arresting phenomenon of parasites treated with DSM265 results from depletion in the amount of RNA synthesis. DSM265 has a longer predicted human half-life (> 100 h) for this reason it will be useful in malaria treatment. DSM265 also has potent in vivo activity in SCID mice affected with *P. falciparum* [ED_{90}-3 mg/kg/day]. DSM265 has concluded its Phase IIa trials in patients with *P. falciparum* or *P. vivax*. (Phillips, et al. 2016).

The DSM265 inhibitor-binding pocket is situated between FMN and the N-terminal α-helix. It is also known to be the binding place for CoQ. This pocket is predominantly hydrophobic and in silico study shows that DSM265 forms only two hydrogen bonds with DHODH via Arg-265 and His-185 with the binding energy of -5.42 ± 0.26 kcal/mol. (Phillips et al. 2015; Agoni, et al.2019).

DSM421 The SF5-aniline residue on DSM265 is replaced by CF_3 pyridinyls involving the maintenance of triazolopyrimidine core, resulting in the formation of DSM421 (Sl. No. 46) compound. This replacement improves the solubility, lowers the intrinsic clearance and increases the plasma exposure following oral dosing in DSM421 in comparison to DSM265. DSM421 inhibits several laboratory strains of *P. falciparum* involving an EC_{50} from 0.010 to 0.080 µM. DSM421 has both liver and blood stage activities involving prolonged half-life and very good oral exposure suggesting this compound can be used as an effective antimalarial therapeutics. It can inhibit the resistant strains of *Pf*DHODH and *Pv*DHODH with IC_{50} values of 0.053 and 0.094 µM. DSM421 is progressed as a preclinical development candidate for malaria. (Phillips et al. 2016).

The CF3-pyridinyl group of DSM421 is incorporated in π alkyl interaction with Leu 197 and halogen interaction with Phe198 amino acid residue, which is revealed at 20 ns of molecular dynamics simulation. At 60 ns of MD simulation, interactions with leu197 and Phe198 are maintained. However, By the end of the 100 ns MD simulation, the total number of interactions generated by CF3-pyrinidyl group increased, resulting in two halogen bond connections with Phe188 and Ser236. Ile237, a fluorine atom, also formed a π–π stacking interaction. The firm connections maintained by CF3-pyrinidyl group during the simulation may have increased the stability of DSM421 with the inhibitor binding pocket. (Agoni et al.2019).

DSM74 Substitution of trifluoromethyl phenyl replacements was done to assess the metabolic stability and were being examined in human liver microsomes. By this way the compound DSM74 (Sl. No. 47) is identified. It has a long exposure level in plasma after several oral doses and it inhibits the parasitemia in 4 day activity test utilizing *P. berghei* model in mice. This compound has an EC_{50} value of 0.34 µM in Pf 3D7 cells and the IC_{50} value is 0.28 µM for *Pf*DHODH. So the in vivo efficacy of DSM74 is also studied, suggesting that it would be a potent antimalarial compound. DSM74 is currently in the preclinical stage of development (Phillips et al. 2010).

The triazolopyrimidine ring of DSM74 binds to a large hydrophobic pocket generated by Val532, Leu176, Leu172, Gly181 and Cys184. The nonhydrophobic interactions include ion pair H-bonds between the bridging nitrogen N-1 and His185 and between pyridine nitrogen N-5 and Arg265. The phenyl-trifluoromethyl groups of DSM74 bind to a completely hydrophobic pocket generated by amino acid residues Leu189, Ile237, Met536, Leu197, Phe188 and Phe227. In the case of DSM74, ion pairs/H–bonds between Arg265 and His185 and the inhibitor provides much more energy to binding interaction than Phe188 amino acid residue. (Deng et al.2009).

BRD7539 This inhibitor is an azetidine-2-carbonitrile compound (Sl. No. 48) that is more selective towards *P. falciparum* DHODH than human DHODH and potently inhibits the asexual liver and blood stages of the parasite. This inhibitor is discovered through in vitro evolution and whole genome analysis. (Luth et al.2018) The IC_{50} against *Pf*DHODH is 0.033 µM. This inhibitor is presently in the preclinical stage. (Maetani et al. 2017).

BRD9185 This inhibitor contains 3,4-trifluoromethylbiphenyl moiety (Sl. No. 49) having good activity in vitro against parasites' liver and blood stages and can eliminate the infection in mice models in only three doses. IC_{50} value of this inhibitor-*Pf* DHODH- 0.012 µM. It acts as a promising inhibitor of DHODH and is currently in preclinical stage. (Luth et al.2018; Maetani et al. 2017).

7N-alkyl-5-(1H-benzimidazol-1-yl) thiophene-2-carboxamides

A high throughput screening method discovers a series of N-alkyl-5-(1H-benzimidazol-1-yl) thiophene-2-carboxamides (Sl. No. 50) that has in vitro less nanomolar activity against *P. vivax*, *P. berghei* and *P. falciparum* DHODH

respectively. Compounds from this series display good potency in vitro against Dd2 and 3D7 strains of *P. falciparum* with IC_{50} values of 0.022, 0.044 and 0.050 µM also showing fine oral exposure and tolerability in mouse. The compounds of this series are specific to the parasite PfDHODH. One of the analogs Genz-667348 (50a) is a potent compound and the x-ray study of enzyme-inhibitor complex shows that the conformational flexibility of this compound directs this to bind to the hydrophobic pocket of DHODH that is different from triazolopyrimidines resulting in effective binding. Other two analogs Genz-669178 (50c) and Genz-668857 (50b) produced very potent activities by hERG and cytochrome P450 in comparison with Genz-667348 (50a). After testing all doses (up to 200 mg/kg/day) it is identified that tolerability of this compound is excellent. Genz-669178 and Genz-668857 are under further procedures of testing to identify if they are suitable for undergoing development preclinically. (Booker et al. 2010).

Genz-669178 (50c) forms H-bond with His185, Arg265 at a distance of 2.44 and 2.10 Å respectively. Other interactions include H_2O mediated H–bond with Met536 and π-stacking interaction with Phe188 and Phe171 with a glide score of − 11.189. Genz-667348 (50a) forms H-bond with His185, Arg265 at a distance of 2.46 and 2.09 Å respectively. Other interactions include π-stacking interaction with Phe188 and Phe171 with a glide score of − 10.082. (Rawat and Verma 2020).

Malaria parasitic dihydroorotate dehydrogenase inhibitors have been given in Table 9.

Inhibitors of dihydrofolate reductase (DHFR)

Pyrimethamine and proguanil Pyrimethamine (Sl. No. 51) and proguanil (Sl. No. 52) are potent inhibitors of dihydrofolate reductase. (Sirawaraporn 1998).

Both of these drugs can inhibit *P. falciparum* DHFR in the same orientation. The inhibitors contain a 4-amino group that forms a hydrogen bond with the carbonyl oxygen of the Ile164 backbone of DHFR.

The proguanil is metabolized into cycloguanil. Cycloguanil is a cyclic dihydrotriazine that resembles greatly Pyrimethamine. The drug closely resembles the natural substrate of DHFR that is dihydrofolate and binds to DHFR very tightly leading to the inhibition of DHFR. (Sirawaraporn 1998).

The mean IC_{50} values of Pyrimethamine and cycloguanil were shown as 15.4 and 11.1 nM respectively. (Basco, et al. 1994).

Pyrimethamine was previously utilized as an antimalarial drug but now due to resistance this drug is no longer recommended. Cycloguanil is currently not utilized as an antimalarial drug but research is ongoing to use cycloguanil in combination with other medication, as resistance to existing antimalarial medications continues to emerge.

Pyr binds to the PvDHFR active site via H-bonds and van der Waals interactions, with its pyrimidine ring buried in the deep cleft. 2-amino nitrogen and N-1 atoms of Pyrimethamine are hydrogen bonded to carboxylate oxygen atoms of Asp-53 (distances are 2.89 and 2.57 Å respectively), and 4-amino nitrogen atom is hydrogen bonded to the main-chain carbonyl oxygen atoms of Ile-173 and Ile-13 (distances are 2.92 and 2.96 Å, respectively). The pyrimidine ring forms van der Waals interaction with Ile-13, Phe-57 and Leu-45. The p-chlorophenyl ring forms an interaction with the nicotinamide residue of the NADPH cofactor. The p-Cl atom of Pyrimethamine lies ≈3.36 Å from the Ser-120 hydroxyl group with an angle C–Cl···(H)O of 141.8° which can be regarded as a hydrogen bond between the weak acceptor and a strong donor, respectively. The binding energy is − 10.60 kcal/mol. (Choowongkomon et al.2010; Kongsaeree et al.2005).

In-silico docking study revealed that cycloguanil forms interactions with PvDHFR active site that involves Ile13, Leu45, Asp53, Phe57, Ser120, Ile173 and Thr194 amino acid residues with the binding energy of − 10.63 kcal/mol. (Choowongkomon et al.2010).

PS-15 PS-15 (Sl. No. 53 a) represents a new antifolate class of drugs that is also referred to as oxyguanils or hydroxylamine-derived biguanides. This compound displays intrinsic antimalarial activity and also is metabolized in vivo to WR99210, an extremely active triazine inhibitor of DHFR. It is also not cross-resistant with other DHFR inhibitors. (Canfield et al. 1993).

WR99210 Due to the mutations that lead to the development of resistance against Pyrimethamine novel attempts are made to develop new antifolates that selectively bind to the active site of quadruple mutant of *P. falciparum* TS-DHFR. (Dasgupta et al. 2009) WR99210 (Sl. No. 53 b) is a triazine derivative containing a trichlorophenoxypropyloxy side chain which is very promising to PYR-resistant parasites. (Sirichaiwat et al. 2004) It is also very effective against the quadruple mutant. The reason behind it is that it lacks the p-chlorophenyl moiety that is present in PYR and a flexible linker that enables this inhibitor to avoid steric hindrance with Asn 108. (Dasgupta et al. 2009) IC_{50} for wild-type *Plasmodium vivax* is 0.38 µM (Hastings and Sibley 2002). Even at low dosages, preclinical and clinical trials for WR99210 showed adverse outcomes, including significant gastrointestinal side effects in humans and nonhuman primates, which eventually lowered the drug's once-exciting potential as an antimalarial treatment candidate. (Remcho et al. 2020).

Table 9 Malarial dihydroorotate dehydrogenase inhibitors

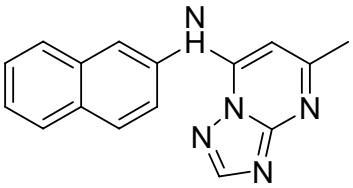
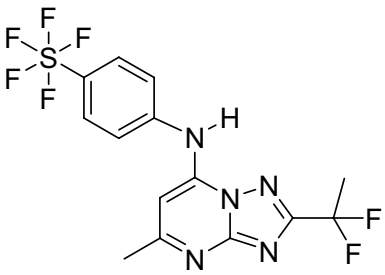
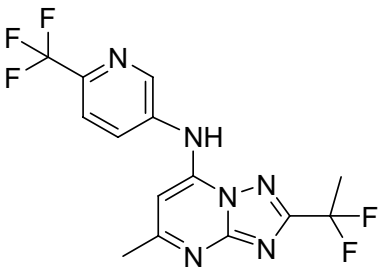
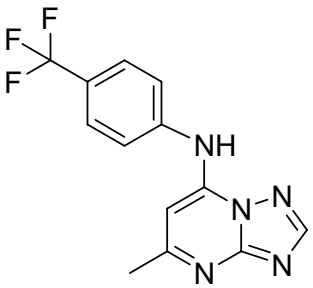
Sl No	Name	Chemical structure	IC_{50} value	References
44	DSM1		0.047 ± 0.022 µM	Phillips et al. (2008)
45	DSM265		*Pf*DHODH-20 nM	Phillips et al. (2008)
46	DSM421		*Pf*DHODH-0.053 µM *Pv*DHODH-0.094 µM	Phillips et al. (2016)
47	DSM74		*Pf*DHODH-0.28 µM	Phillips and Rathod (2010)

Table 9 (continued)

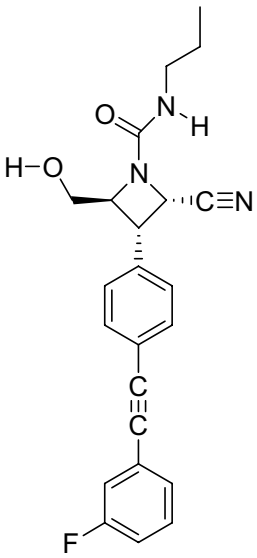
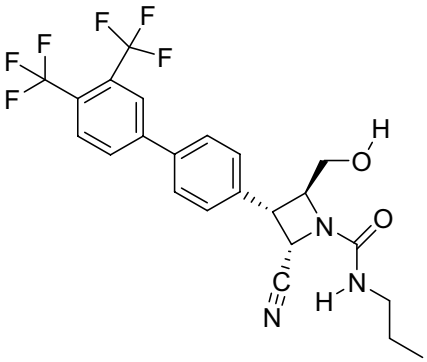
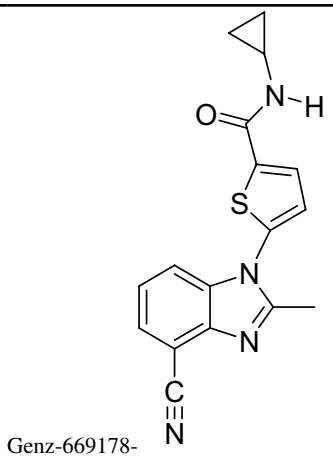
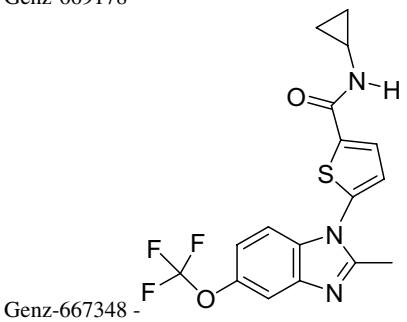
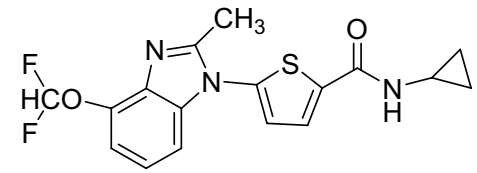
Sl No	Name	Chemical structure	IC_{50} value	References
48	BRD7539		*Pf* DHODH-0.033 µM	Maetani et al. (2017)
49	BRD9185		*Pf* DHODH-0.012 µM	Maetani et al. (2017)

Table 9 (continued)

Sl No	Name	Chemical structure	IC_{50} value	References
50	a.Genz-667348 b.Genz-668857 c.Genz-669178	 Genz-669178-  Genz-667348 - Genz-668857-  R1- OCHF2, R2-H	Genz-667348-IC50 PfDHODH: 0.022 µM Genz-668857- IC50 PfDHODH: 0.044 µM Genz669178- IC50 PfDHODH: 0.050 µM	Booker et al. (2010)

In silico docking study revealed that WR99210 forms hydrogen bonds and hydrophobic interactions with *P. falciparum* DHFR active site involving Ile 14, Cys 15, Asp 54, Met 55, Phe 58, Ile 112, Pro 113, Leu 164 amino acid residues. (Dasgupta et al. 2009).

Trimethoprim (TMP) Trimethoprim (Sl. No. 54) possesses a flexible trimethoxy benzyl side chain and the hydrophobic interaction between the side chain of inhibitor and the active site of DHFR enzyme around position 108 is enhanced by the introduction of longer and more hydrophobic side chain of TMP's 5-Benzyl moiety. The aromatic and other hydrophobic substitutes on 5-Benzyl moiety increase the binding affinity, resulting in enhanced inhibition and parasite killing. (Sirichaiwat et al. 2004). Although trimethoprim was previously used as an antimalarial agent now it is used as an antibacterial agent as it acts as an inhibitor of parasitic and bacterial dihydrofolate reductase. (Scholar 2007).

In silico docking, study revealed that trimethoprim forms interactions with PvDHFR active site that involves Ile13, Leu45, Asp53, Phe57, Ser120, Ile173 and Thr194 amino acid residues with the binding energy of − 10.87 kcal/mol. (Choowongkomon et al.2010).

Triclosan Triclosan (Sl. No. 55) is an antimicrobial agent that is a strong inhibitor of *P. falciparum* enoyl reductase. So, this inhibitor is capable of inhibiting blood-stage *Plasmodium* via its action against DHFR and the liver stage of the parasite via the inhibition of a key enzyme in fatty acid synthesis with an IC_{50} PvDHFR inhibitory value of

775 ± 384 nM. (Bilsland, et al. 2018) It was also shown to potentially inhibit fatty acid synthesis in the apicoplast with an IC_{50} value of 0.7 µM. (Surolia and Surolia 2001).

In silico docking and molecular dynamics, simulation shows that triclosan forms interaction with *Pv*DHFR active site via Phe57 and Ile173 amino acid residues with the binding energy of − 7.235 kcal/mol. (Bilsland et al. 2018).

RJF compounds The three compounds namely RJF 001302, RJF 00670, and RJF 00719 (56c) (Sl. No. 56) with IC_{50} around 20 µM has been identified. Both RJF 00670 (56b) and RJF 01302 (56a) form hydrogen bonds with the carboxyl side chain of Asp 54 and the backbone of Ile 164. So these compounds avoid steric clash as WR99210 and suggest a mechanism for overcoming antifolate resistance in malaria and these are the novel inhibitors of drug-resistant *P. falciparum* malaria. It acts as a promising inhibitor of DHODH and is currently in the preclinical stage. (Dasgupta et al. 2009).

In silico docking study revealed that RJF 00670 (56b) forms hydrogen bond and hydrophobic interactions with *P. falciparum* DHFR active site involving Ile 14, Cys 15, Ala 16, Asp 54, Phe 58, Leu 46, Met 104, Ile 112, Leu 119, Leu 164, Tyr 170 amino acid residues. In silico docking study revealed that RJF 01302 (56a) forms hydrogen bonds and hydrophobic interactions with *P. falciparum* DHFR active site involving Asp 54, Met 55, Phe 58, Ile 164 amino acid residues. (Dasgupta et al. 2009).

Methotrexate (MTX) The antimalarial potential of MTX (Sl. No. 57) has been established for about 40 years. Two relatively small clinical trials, involving seven patients, have demonstrated that doses as low as 2.5 mg per day for 3–5 days were effectively treating malaria infection in humans (*Plasmodium falciparum* and/or *Plasmodium vivax*). However, MTX has not come into widespread use because of concerns over toxicity. It inhibits *Plasmodium* DNA synthesis by inhibiting DHFR. It produced an IC_{50} value of < 85 nM. (Nzila et al. 2010) After the phase I evaluation of methotrexate over a small group of healthy volunteers it was found that this drug cannot effectively clear malaria infection in-vivo. So proper studies are essential to ensure its suitability as an anti-malarial agent (Chilengi et al. 2011).

Trimetrexate (TMX) TMX (Sl. No. 58) has been used primarily for the treatment of solid tumours. Evidence is also available that TMX has good activity against *P. falciparum*, and the addition of the folate derivative 5-methyl tetrahydrofolate (5-Me-THF) does not reduce TMX activity. Thus, this form of folate could be used as an adjuvant, in combination with TMX, to increase its safety margin. Doses of < 10–20 mg of TMX would be effective in treating malaria suggested by clinical trials. It produced IC_{50} of < 50 nM. (Nzila et al. 2010).

P65 The 2,4-diaminopyrimidine anchor of P65 (Sl. No. 59) provides the minimum functionality necessary to achieve good binding deep in the DHFR active site. When combined with a flexible five-atom linker the conformational rigidity that would occur with the 2,4-diaminopteridine, quinazoline, or pyridopyrimidine scaffolds is avoided. (Yuthavong et al. 2012) It is a flexible diaminopyrimidine that is more effective than pyrimethamine. (Tarnchompoo et al. 2018) IC_{50} value for this promising compound is 229 ± 68 nM against wild-type *P. falciparum*. Preclinical trials are ongoing to see the effectiveness of this inhibitor (Yuthavong et al. 2012).

The X-ray cocrystal structure of P65 with quadruple mutant *Pf*DHFR indicated that the substitution at the 3′-hydrogen or at the 2′-chloro of the trichlorophenyl group by the alkyloxy or alkyl group containing a terminal carboxylate may favour the interaction with Arg122. (Yuthavong et al. 2012).

P218 This inhibitor (Sl. No. 60) has a flexible pyrimidine side chain and carboxylate group that facilitates its binding to the active site of PfDHFR. It binds to mutant and wild-type PfDHFR in a tight binding conformation that favors its long residence time. So, it exhibits potent activity not only for wild-type malaria but also for pyrimethamine-resistant malaria with an IC_{50} of 4.6 ± 1.9 (wild type) and 56 ± 20 (quadruple mutant) type of *P. falciparum*. (Yuthavong et al. 2012).

For assessing the safety of this drug or whether it has any effect on cardiac activity it is tested in healthy male human individuals. From this study, it is determined that P218 has no significant effect on cardiac function with the ascending dose of 10, 30, 100, 250, 500, 750 and 1000 mg. (Täubel et al. 2022) P218 has currently concluded its Phase I clinical trial.

The S108N mutation of dihydrofolate reductase (DHFR) renders *Plasmodium falciparum* malaria parasites resistant to pyrimethamine through steric clash with the rigid side chain of the inhibitor. Inhibitors with flexible side chains can avoid this clash and retain effectiveness against the mutant. However, other mutations such as N108S reversion confer resistance to flexible inhibitors. So, the generation of hybrid inhibitors are important that will be effective against wild-type and mutant *P. falciparum*. Furthermore, the hybrid inhibitors can forestall the emergence of new resistant mutants.

The carboxylate of P218 that is bound to quadruple mutant PfDHFR results in the generation of two charge mediated hydrogen bonds with Arg122, displayed by in silico docking studies. (Yuthavong et al. 2012).

BT1 and BT2 The rigid part of Pyrimethamine derivative is joined with a flexible diaminopyrimidine moiety of P65 and in this way, the hybrid inhibitors are synthesized. This mechanism furnishes the desired hybrid compounds BT1 and BT2, (Sl. No. 61a- b) bearing two DHFR binding sites on opposite ends of the molecule. BT1 consists of the rigid end linked at the m-phenyl substituent to the flexible end, whereas BT2 has the rigid end linked at the p-phenyl substituent to the flexible end.

The rigid end of BT1 with a m-phenyl substituent is expected to bind preferentially to the active site pocket containing S108 of the wild-type Pf DHFR with no steric hindrance. However, it was possible that the bulky p-phenyl substituent of BT2 might encounter steric clash even with S108, and BT2 might preferentially bind to the wild-type active site with the flexible end. BT1 shows good IC_{50} (< 5 μM) against both wild-type and Pyr-resistant mutant *P. falciparum*. Both of these inhibitors remarkably inhibit DHFR. Both of these inhibitors are presently in the preclinical stage.

BT1 binds to wild-type enzyme with the rigid end present in the active site, by the pyrimidine moiety that interacts with D54 and in the vicinity of S108 the rigid phenyl moiety is present. The nicotinamide residue of NADPH is not affected by the binding of BT1 and the rigid end of BT1 have pyrimidine core that forms π−π interaction with F116. (Tarnchompoo et al. 2018).

In silico docking study reveals the interactions between BT2 and wild-type *Pf*DHFR involving the amino acid residues I164, Y170, I14, D54 and F116. (Tarnchompoo, et al. 2018).

BT3 This inhibitor (Sl. No. 62) is furnished by linking the rigid part of pyrimethamine derivative with a flexible five-atom linker on both sides. These rigid moieties facilitate its high affinity binding with wild type as well as quadruple mutant *P. falciparum* in the PfDHFR quadruple mutant, BT3 binding causes a significant displacement of the nicotinamide moiety, resulting from a severe steric clash. So, the enhanced inhibition of BT3 in PfDHFR quadruple mutant occurs through displacement of NADPH cofactor. Therefore, the displacement of the nicotinamide ring of NADPH is important for the inhibition of the PfDHFR quadruple mutant by BT3. It is also in the preclinical stage of development.

Due to the presence of rigid moieties on both ends, this inhibitor has good binding properties. The interactions with wild type PfDHFR include the amino acid residues I164, Y170, I14, D54 and F116. (Tarnchompoo et al. 2018).

BT2S and BT3S These compounds BT2S and BT3S, (Sl. No. 63 a, b) are hydrochloride salt forms of BT2 and BT3 respectively, having higher solubility. The bi-rigid inhibitor BT3S shows good IC_{50} against both wild-type and mutant parasites with better selectivity than BT1 and BT2S. BT2S IC_{50} for wild-type *P. falciparum* is 0.063 ± 0.014 μM and BT3S IC_{50} is given as 0.035 ± 0.0033 μM against the same starin (Tarnchompoo et al. 2018).

Chlorproguanil It is a biguanide class of inhibitor (Sl. No. 64) that has inhibitory activity against *Plasmodium* DHFR. After entering the body, it is metabolized into the triazine active compound which is chlorcycloguanil. This inhibitor is more effective than pyrimethamine. In vitro, against a pyrimethamine-resistant strain of *P. falciparum*, the IC_{50} of chlorcycloguanil is 4.8 nM. (Winstanley, et al. 1997). It is an antimalarial drug that was used earlier in combination to dapsone to treat uncomplicated malaria. But its use is prohibited because it causes haemolytic anaemia in people with glucose-6-phosphate dehydrogenase (G6PD) deficiency (Winstanley, 2001).

Malaria parasitic dihydrofolate reductase inhibitors have been given in Table 10.

Apicoplast inhibitors

Ciprofloxacin Ciprofloxacin (Sl. No. 65) is a fluoroquinolone that was found to inhibit the DNA replication in apicoplast (i.e. prokaryotic DNA replication). The IC_{50} value against *pf* is 50 μM. (Ralph et al. 2001) Ciprofloxacin mainly targets DNA gyrase or topoisomerase IV essential for replication in *Plasmodium*. There is shreds of evidence that *Plasmodium* possesses bacterial-type DNA gyrases. (Dahl and Rosenthal 2008) It was also shown that *P. falciparum* has gyrA and gyrB-like sequences. (Ralph et al. 2001).

At first, ciprofloxacin hinders the process of transcription or replication since gyrase activity is needed to unwind DNA when the progression of polymerases along the strand takes place. It can also block the function of topoisomerase IV which is essential for separate concatemerized DNA following replication. Gyrase inhibition that is required for replication and transcription might be fatal for the first cycle on the other hand blocking topoisomerase IV might result in a delayed death phenomenon by hindering the segregation of the replicated apicoplast genome. (Dahl and Rosenthal 2008). This inhibitor has good potency to be used as an antimalarial agent and currently, it is in preclinical trial to visualize its efficacy as an antimalarial agent.

Rifampin Rifampin (Sl. No. 66) acts as the inhibitor of RNA transcription in apicoplast with an IC_{50} value of 3 μM. The genome in apicoplast encodes the β' and β subunits of polymerase (rpoC2, rpoC1 and rpoB genes). The putative target of rifampin is the RNA polymerase β subunit (rpoB). (Ralph et al. 2001) This leads to the inhibition of transcription and most importantly the antimalarial effect of rifampin

is to hinder the development and growth of parasites instead of killing them directly. The in-vitro and in-vivo antimalarial effectivity of rifampin is studied but this is yet to be supported by human data. (Strath et al.1993).

Thiostrepton The IC_{50} value is 1.8 µM. Thiostrepton (Sl. No. 67) inhibits the growth of parasites by inhibiting of protein synthesis process in *Plasmodium* apicoplast. It is a thiopeptide and the putative target of thiostrepton is the large subunit (LSU) rRNA that is encoded by the apicoplast genome. The 35 Kb genome of apicoplast in *P. falciparum* contains A in the GTPase portion of LSUrRNA corresponding positions to the *E. coli* LSU rRNA. These are the sites of interaction with thiostrepton in LSU rRNA. (McConkey et al. 1997) By binding with the GTPase domain of LSU rRNA thiostrepton blocks the apicoplast translation thereby parasite growth. Several preclinical trials are performed to observe *in-vitro* antimalarial activity of thiostrepton (Ralph et al. 2001).

Micrococcin The IC_{50} value of micrococcin(Sl. No. 68) is 3.5×10^{-8} M. Micrococcin inhibits the growth of plasmodium by blocking the protein synthesis in apicoplast. Like thiostrepton it is also a thiopeptide and the target of this inhibitor is the large subunit rRNA. In the presence of EF-G micrococcin stimulates GTP hydrolysis in a ribosome-dependent manner. (Rogers et al. 1998).

Clindamycin It produced an IC_{50} value of 20 nM. Clindamycin (Sl. No. 69) is also an inhibitor of protein translation in apicoplast but the mode of action is different. (Ralph et al. 2001) Clindamycin blocks the peptidyl tRNAs translocation and by interaction with peptidyl transferase domain of 23 s rRNA it hinders protein synthesis. (Dahl and Rosenthal 2008) This drug is used in combination with quinine to treat uncomplicated falciparum malaria. (Belete 2020).

Thiolactomycin (Sl. No. 70) Thiolactomycin (Sl. No. 70) inhibits the β-ketoacyl-ACP synthases I, II, and III (fabB, fabF, fabH respectively) in the type II fatty acid synthesis pathway in apicoplast. This results in the blocking of parasite growth with an IC_{50} value is 49 ± 8 µM. In-vitro assays were performed to check the antimalarial activity of thiolactomycin and it act as a promising inhibitor. (Waller et al. 2003).

Cerulenin Cerulenin (Sl. No. 71) acts as the inhibitor of fabB and fabF in the fatty acid synthesis pathway showing an IC_{5o} value of 11 ± 4 µM. As found in *E. coli* cerulenin has a long hydrophobic side chain that sits in the hydrophobic groove of enzyme and the interaction sites with cerulenin in *E. coli* enzyme (Phe 392, Cys 163, His 333, His 298) are all conserved in *P. falciparum* fabB/F. So, this is likely that this enzyme is inhibited by cerulenin. Like thiolactomycin cerulenin act as a promising inhibitor (Waller et al. 2003).

Doxycycline It is a tetracycline group of antibiotics. The putative target of doxycycline (Sl. No. 72) in *P. falciparum* is 70S ribosomes that are partially encoded by apicoplast. This compound causes delayed death phenomenon in *P. falciparum*. This means the apicoplast that is formed and spread into *P. falciparum* progeny after the treatment of the antibiotic, the parasites died as they tried to induce cell division. The reason behind this is the parasites that were treated with antibiotic lacks proteins encoded by apicoplasts that are needed for nuclear protein processing and importing. (Dahl and Rosenthal 2008) IC_{50} range was found as 0.49–65.1 µM. (Gaillard et al. 2013).

The sole FDA-approved use of doxycycline for malaria prevention involves short-term travellers (less than four months) to regions where strains of *Plasmodium falciparum* are resistant to pyrimethamine-sulfadoxine and/or chloroquine. (Tan et al. 2011).

Azithromycin The azithromycin (Sl. No. 73) is a macrolide antibiotic that inhibits *P. falciparum* 70S ribosomes that are partially encoded by apicoplast. (Dahl and Rosenthal 2008) The IC_{50} value was found as 8.4 ± 1.2 µM. (Nakornchai and Konthiang 2006) The antimalarial efficacy of azithromycin is lower than other drugs so, it may not be considered for chemoprevention of malaria. (Rosenthal 2016).

Novobiocin Novobiocin (Sl. No. 74) inhibits DNA replication in apicoplast of *P. falciparum* by inhibiting the DNA gyrase activity. The ATPase activity of PfGyrB was inhibited by novobiocin which also caused parasite death in culture. The reduction of apicoplast/nuclear DNA ratio in the presence of novobiocin indicated that the drug targets apicoplast DNA replication. Novobiocin acts as a promising inhibitor but further research is ongoing to see its effectiveness. (Raghu Ram et al. 2007) The IC_{50} values against *P. falciparum* FCC1 and VNS strains include 0.28 and 0.21 M respectively. (Divo et al. 1988).

Malarial apicoplast inhibitors have been tabulated in Table 11

Inhibitors of dihydropteroate synthase

The sulfone/sulfonamides are an important group of antimalarial compounds that act either to directly inhibit the *P. falciparum* enzyme DHPS or are converted by this enzyme to a toxic sulfa analog that inhibits this enzyme in the folate biosynthetic pathway. (Triglia and Cowman 1994).

The Table 12 summarizes promising inhibitors of dihydropteroate synthase.

Sulfadoxine One of the compound that inhibits DHPS is sulfadoxine (Sl. No. 75). It inhibits the uptake of para amino benzoic acid by *Plasmodium* and so it can not generate dihydrofolate. By this way this inhibitor inhibits the nucleic acid synthesis in *Plasmodium* sp. showing an IC_{50} of 400 µM. (Zhang and Meshnik 1991).

Presently there is the generation of sulfadoxine resistant in *Plasmodium* that is due to mutation in certain amino acids within the DHPS gene. The first mutation that seems to be responsible for amino acid change in DHPS gene is Ala 437 to Gly 437. It is found in all sulfadoxine-resistant *Plasmodium* strains. (Triglia et al. 1997) But *Plasmodium* can utilize exogenous folate, this fact reduces the inhibitory activity of sulfadoxine. The Use of sulfadoxine is restricted to the treatment of uncomplicated Chloroquine-resistant falciparum malaria (Belete 2020).

Dapsone Dapsone (Sl. No. 76) acts as a competitive inhibitor of *Plasmodium* DHPS activity. The malaria parasites require para amino benzoic acid and this compound acts by inhibiting the uptake of PABA by *Plasmodium* thereby DHPS can't convert it to dihydrofolate and the growth of the parasite is inhibited with an IC_{50} value of 33 µM. (Zhang and Meshnik 1991) Dapsone can be used in combination with chlorproguanil in case of uncomplicated malaria.

Pyrimethamine-sulfadoxine The combination of pyrimethamine-sulfadoxine is a synergistic as well as antifolate amalgamation. Pyrimethamine and sulfadoxine inhibit DHFR and DHPS respectively. This synergistic combination can be used in chloroquine-resistant malaria. Single dosing of this combination is presently utilized in several East African parts as a first-line treatment of malaria.

(Mutabingwa et al. 2001).

In this synergistic combination, the inhibition of tetrahydrofolate generation occurs. There is also depletion of the crucial cofactor, which thereby prevents the formation of dTMP, methionine, mediating the killing of parasites. (Sibley et al. 2001).

Pyrimethamine-sulfadoxine has long residence stay property in the body. It mediates the speedy elimination of parasites present in the body and also affirms the infection-free interval in the body of the host. This facilitates the physiological recovery in the host. (Watkins and Mosobo 1993).

Chlorproguanil-dapsone This novel antifolate combination is more effective against strains of the parasite in Africa that have borderline sensitiveness to pyrimethamine-sulfadoxine combination and it can also act as second-line drug combination.

In the case of quadruple mutant dhfr (S108-N511-C59R-I164L), the combination of pyrimethamine-sulfadoxine becomes entirely ineffective as it is unable to attain the inhibitory concentration required to inhibit this genotype. The action of CCG (Chlorcycloguanil) which is the active triazine metabolite of chlorproguanil-dapsone tends to act synergistically and is affected by I164L mutation to a lesser extent. So, it can be anticipated that this combination can have borderline efficacy. (Mutabingwa et al. 2001) (one daily dose for 3 d of 1.2 and 2.4 mg/kg respectively).

Trimethoprim-sulfamethoxazole This drug combination can be utilized and can be effective when there is decreased resistance to antifolates. But the half-life of this combinatorial drug is much less, only 10–12 h. For this reason, these drugs require multiple doses than a single dose of pyrimethamine-sulfadoxine. So that adequate drug levels can be maintained for at least three replication cycles of parasites. (Gregson and Plowe 2005).

Inhibitors of isoprenoid biosynthesis

There are 6 major enzymes which play crucial role in 2C-methyl-d-erythritol-4-phosphate/ 1-deoxy-d-xylulose-5-phosphate (MEP/DOXP) pathway in the body of *Plasmodium*. Various molecules have been discovered which targets different specific enzyme of this pathway and inhibit their activities (Table 13).

Inhibitors of DXS enzyme

The β-fluoropyruvate which is the pyruvate analog (Sl. No. 77) and the Methylacetylphosphonate (Sl. No. 78) competitively inhibit the DXS enzyme in *P. vivax* and *P. falciparum*. β-fluoropyruvate has IC_{50} value of 35 ± 1.7 µM *for P. vivax* and 43 ± 3.8 µM for *P. falciparum*. Methylacetylphosphonate inhibits DXS enzyme with the IC_{50} value of 80 µM for *P. vivax* and 46 ± 3.8 µM for *P. falciparum.* These compounds are in the preclinical stage of drug development (Saggu et al. 2016).

Inhibitors of IspC enzymes

Fosmidomycin (Sl. No. 79) is an important drug that qualified in the Phase 2 clinical trial when it is combined with Clindamycin. Now it is in the clinical stage of drug development. It acts as a substrate-analogue for DOXP and competes with it for the binding site in IspC enzyme. It has been shown that Fos is mainly effective in the erythrocytic stage of *Plasmodium* for the formation of new permeability pathways in the infective erythrocytes. It has no or less effect in the hepatic stages of *P. berghei.* It has IC_{50} value of 350 ± 170 nM in *P. falciparum.* However treatment with fosmidomycin has numerous drawbacks such as minimum bioavailability, fast clearance from the parasites, low half-life and show toxicity when it is applied in higher doses.

Table 10 Malarial dihydrofolate reductase inhibitors

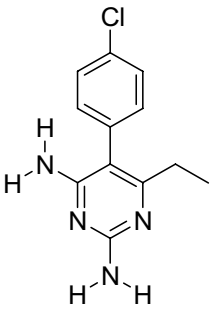
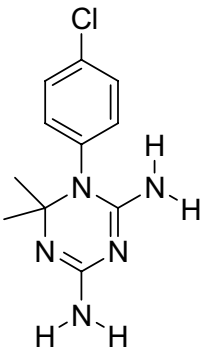
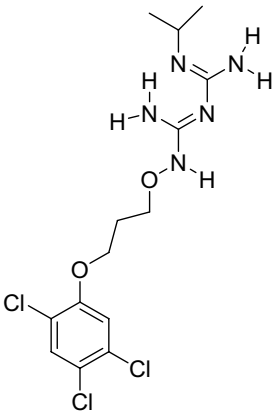
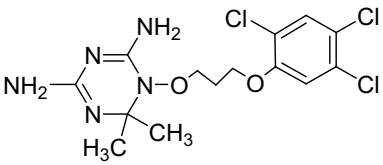
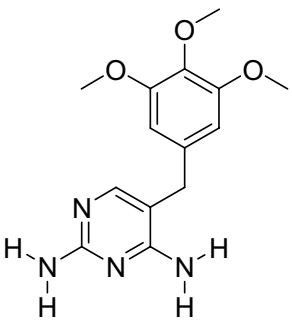
Sl No	Name	Chemical structure	IC_{50} value	References
51	Pyrimethamine		mean IC_{50} 15.4 nM	Basco et al. (1994)
52	Cycloguanil		mean IC_{50} 11.1 nM	Basco et al. (1994)
53. a	PS-15		–	Canfield et al. (1993)
53. b	WR99210		IC_{50} for wild type *Plasmodium vivax*- 0.38 µM	Hastings and Sibley 2002)
54	Trimethoprim		–	Sirichaiwat et al. (2004)

Table 10 (continued)

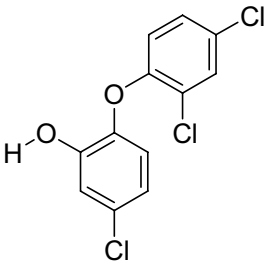
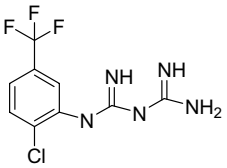
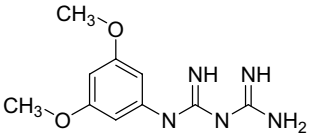
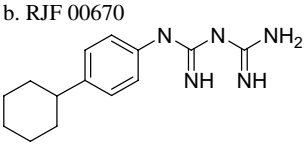
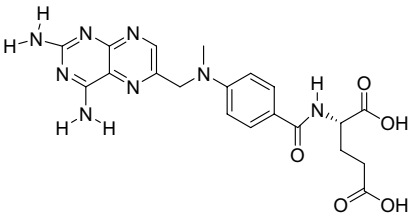
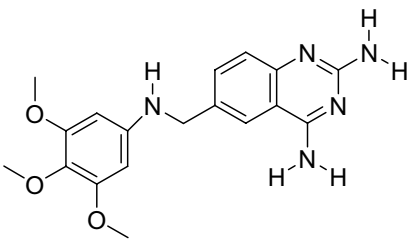
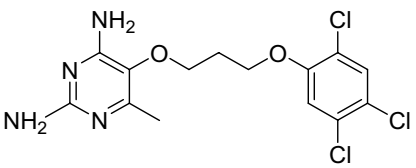
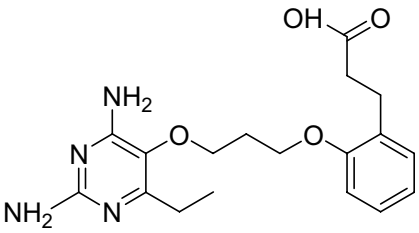
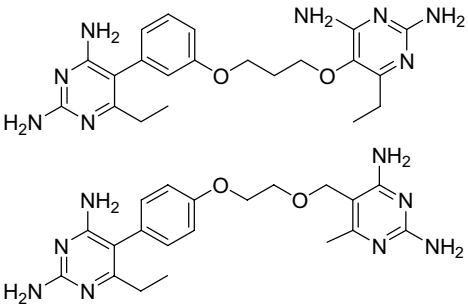
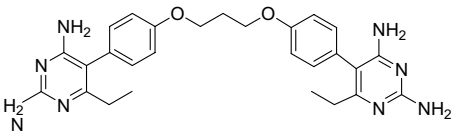
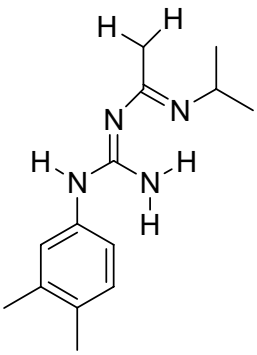
Sl No	Name	Chemical structure	IC_{50} value	References
55	Triclosan		*Pv* DHFR IC_{50}-775 ± 384 nM	Bilsland et al. (2018)
56	RJF compounds a. RJF 01302 b. RJF 00670 c. RJF 00719	 a. RJF 01302  b. RJF 00670  c. RJF 00719	~20 µM	Dasgupta et al. (2009)
57	Methotrexate		IC_{50} < 85 nM	Nzila et al. (2010)
58	Trimetrexate		IC_{50} < 50 nM	Nzila et al. (2010)
59	P65		IC_{50} wild-type *P. falciparum*-229 ± 68 nM	Yuthavong et al. (2012)

Table 10 (continued)

Sl No	Name	Chemical structure	IC_{50} value	References
60	P218		IC_{50}- wild type *P. falciparum* 4.6 ± 1.9 IC_{50}- quadruple mutant *P. falciparum* 56 ± 20	Yuthavong et al. (2012)
61	a. BT1 b. BT2		IC_{50} < 5 µM	Tarnchompoo et al. (2018)
62	BT3		–	Tarnchompoo et al. (2018)
63	BT2S and BT3S	Hydrochloride salts of BT2 and BT3	BT2S IC_{50} for wild type *P. falciparum*- 0.063 ± 0.014 µM. BT3S IC_{50}-0.035 ± 0.0033 µM	Tarnchompoo et al. (2018)
64	Chlorproguanil		pyrimethamine-resistant strain of *P. falciparum*, the IC_{50} of chlorcycloguanil is 4.8 nM	Winstanley et al. (1997)

So numerous derivatives of fosmidomycin have been identified which show anti-malarial activity (Saggu, et al. 2016). Steinbacher, et al. studied the docking interaction of fosmidomycin and IspC of *Plasmodium* which showed that the phosphonate moiety of Fos is anchored by hydrogen bond network with Ser186, Ser 222, Asn227 and Lys228 and the N-formyl oxygen of (N-formyl-N-hydroxy) amino head group of Fos remains in trans position with Glu231 and N-hydroxyl oxygen is found in trans position with Asp150 (Steinbacher et al. 2003).

FR900098 (Sl. No. 80) is the natural derivative of fosmidomycin which has IC_{50} value of 170 ± 100 nM for *P. falciparum* and competitively inhibits IspC enzyme. When the formyl-hydrogen of Fosmidomycin is replaced by methyl group, it forms FR900098 which is an inhibitor of IspC with increased affinity. According to the docking study of Steinbacher, et al. the methyl group of FR900098 makes contact with side chain of Trp212 which is present at the catalytic loop (Steinbacher et al. 2003).

[1-(3,4-Difluorophenyl)-4-(hydroxylamino)-4-oxobutyl] phosphonic acid (Sl. No. 81) is the reverse derivatives of Fosmidomycin which show IC_{50} value of 3 nM for *P. falciparum* and competitively inhibits IspC enzyme.

The most active Fos derivative is ((3,4-Difluorophenyl(2-(hydroxy(methyl)amino)-2-oxoethoxy)methyl)phosphonic acid (Sl. No. 82) which shows IC_{50} value of 12 nM in *P. falciparum* and acts as a potent anti-malarial drug in in vitro condition.

4-[Hydroxy(methyl)amino]-1-(4-methoxyphenyl)-4-oxobutylphosphonic acid (Sl. No. 83) is the reverse derivative of Fosmidomycin and competitively inhibits IspC with the IC_{50} value of 20 nM for *P. falciparum* (Saggu, et al. 2016).

Inhibitors of IspD enzymes

6-Amino-7-(1H-benzo[d]imidazol-2-yl)-5-[5-(diethylamino)-1-methylbut-1-yl)-5H-pyrrolo[32-b]pyrazine-2,3-dicarbonitrile (Sl. No. 84) is a pyrrolopyrazine stop the growth of *Plasmodium* in cell-based assay. It has an EC_{50} value of 50 nM for *P. falciparum* and acts as a competitive inhibitor of IspD.

MMV008138 (Sl. No. 85) is also found to act as antimalarial component which competitively inhibits IspD with the CTP substrate. It has no activity against the hepatic phase of *P. yoelli* and the sexual stages of *P. falciparum*. It becomes effective when *P. vivax* contain low level of CTP substrate concentration. So, it requires more investigations to use as a common drug for both *P. falciparum* and *P. vivax* (Saggu, et al. 2016).

Inhibitors of IspF enzyme

Thiazolopyrimidines (Sl. No. 86) are non cytidine-like and the inhibit IspF most successfully to date. It is highly active against *P. falciparum* with an IC_{50} value of 9.6 µM.

Aryl bis sulphonamide (Sl. No. 87) is another inhibitor of IspF of *P. falciparum* competitively inhibits IspF with the IC_{50} value of 1.4 µM (Saggu et al. 2016).

Inhibitors of farnesyl transferase

Farnesyl transferase is a novel target for combating drug-resistant malaria parasites. The FT inhibitors strongly disrupt the growth of malaria parasites in the blood stage. Moreover, they have no or low effect on the growth of mammalian cells at the IC_{50} doses of parasitic inhibition. Tetrahydroquinoline series (Table 14) act as potent inhibitors of FT and act as an antimalarial compound (Gisselberg et al. 2017).

BMS-386914 (Sl. No. 88)

It is a compound of Tetrahydroquinoline series and inhibits the prenylation of protein in parasitic cells which further disrupts the localization of proteins in the body of parasites. It has the IC_{50} value of 0.9 nM for *P. falciparum.* It is a scientifically tested antimalarial compound (Gisselberg et al. 2017).

L-745631(Sl. No. 89)

It is a peptidomimetic compound that shows effective inhibition of FT when applied at an IC_{50} dose of 3–4 nM in the in vitro culture of *P. falciparum* (Chakrabarti et al. 2002).

FTI-276 (Sl. No. 90)

It is the best inhibitor of FT to date which has the power lightly more than the L-745631. It has IC_{50} value of 0.9 nM for *P. falciparum* (Chakrabarti et al. 2002).

Inhibitors of *P. falciparum* translational elongation factor 2(PfEF2)

M5717 (DDD107498) (Sl. No. 91) is a novel antimalarial compound discovered through a phenotypic screen and the molecular target of this compound is the translational elongation factor 2. This compound tends to inhibit this elongation factor and thereby the translational process in *P. falciparum.*(Baragaña et al. 2015) M5717 also has high selectivity for *Plasmodium* eEF2 and activity against multiple *Plasmodium* life cycle stages including the liver stage and both sexual and asexual blood stage parasite. (McCarthy et al. 2021) The IC_{50} value for WT Dd2 strain is 0.14 nM (Table 15). (Baragaña et al. 2015) Phase I trial of this inhibitor is completed.

Inhibitors of phosphatidylinositol 4 kinase(PfPI4K)

In blood stages of *Plasmodium* imidazopyrazines inhibit the later stage of the development of the parasite by interrupting the ingression of plasma membrane that occurs surrounding the daughter merozoites during their time of development. The cell-based screening against asexual blood stages of *P. falciparum* helps in the discovery of these inhibitors such as KDU691, KAI407 with imidazopyrazine core (Table 16).

KDU691

In vivo, it is seen that when KDU691 (Sl. No. 92) is administered orally during the period of infection it protects the mice prophylactically from transgenic *P. berghei* sporozoites colonization. KDU691 inhibits liver stages of several species

Table 11 Malarial apicoplast inhibitors

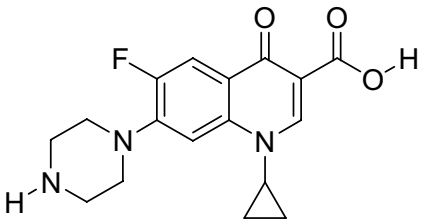
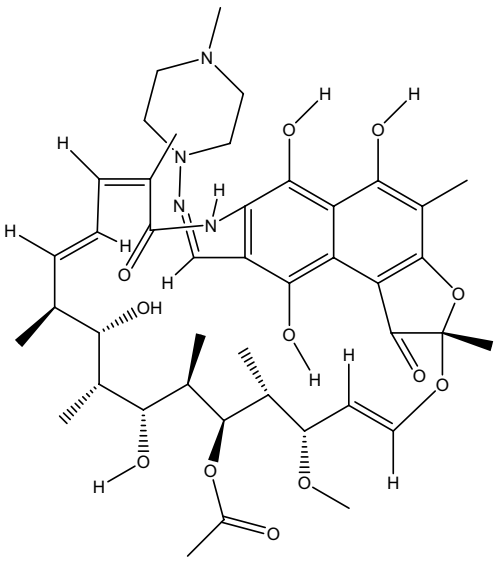
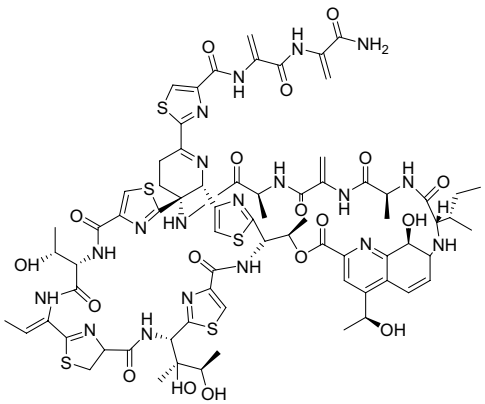
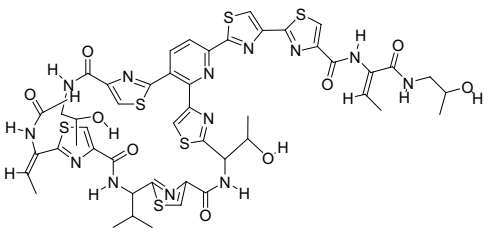
Sl No	Name	Chemical structure	IC_{50} value	References
65	Ciprofloxacin		*P. falciparum*-50 µM	Ralph et al. (2001)
66	Rifampin		*P. falciparum*-3 µM	Ralph et al. (2001)
67	Thiostrepton		*P. falciparum*-1.8 µM	McConkey et al. (1997)
68	Micrococcin		3.5×10^{-8} M	Rogers et al. (1998)

Table 11 (continued)

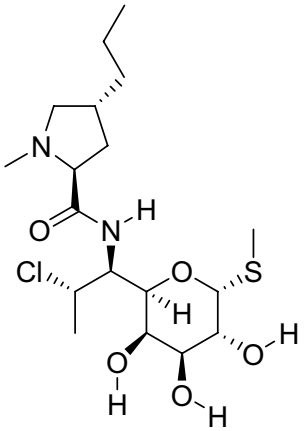
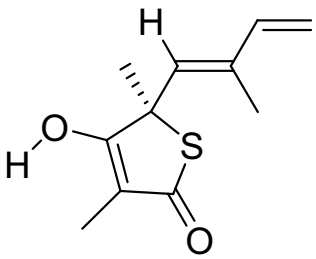
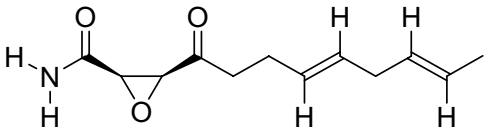
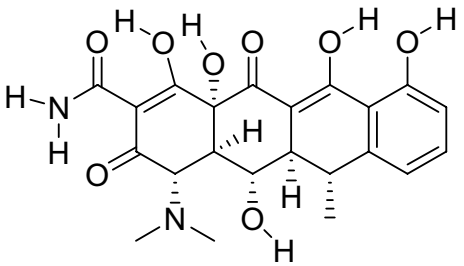
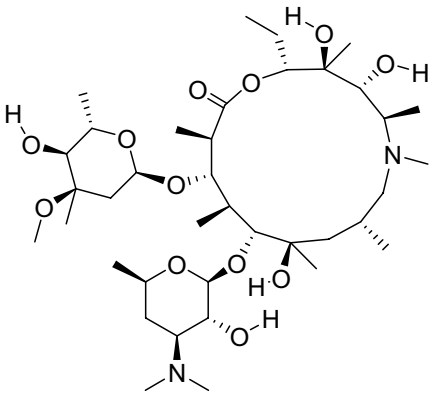
Sl No	Name	Chemical structure	IC_{50} value	References
69	Clindamycin		*P. falciparum*-20 nM	Ralph et al. (2001)
70	Thiolactomycin		49 ± 8 µM	Waller et al. (2003)
71	Cerulenin		11 ± 4 µM	Waller et al. (2003)
72	Doxycycline		0.49-65.1 µM	Gaillard et al. (2013)
73	Azithromycin		8.4 ± 1.2 µM	Nakornchai and Konthiang (2006)

Table 11 (continued)

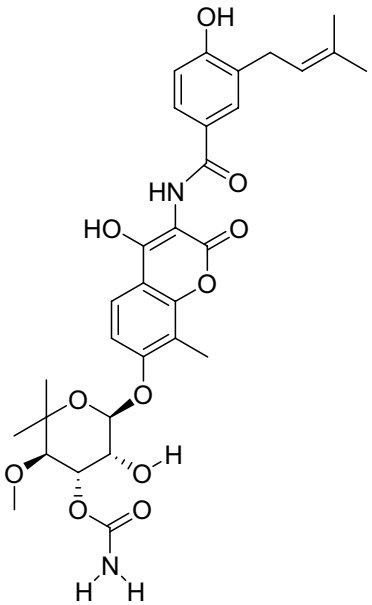
Sl No	Name	Chemical structure	IC_{50} value	References
74	Novobiocin		The IC_{50} values against *P. falciparum* FCC1 and VNS strains includes 0.28 and 0.21 M respectively	Raghu Ram et al. (2007) Divo et al. (1988)

Table 12 Malarial dihydropteroate synthase inhibitors

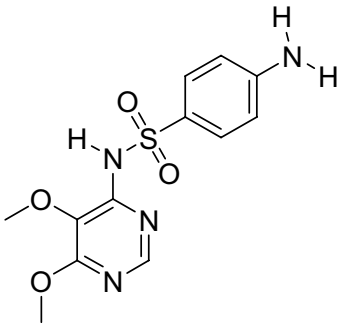
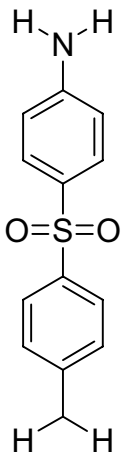
Sl No	Name	Chemical structure	IC_{50} value	References
75	Sulfadoxine		400 µM	Zhang and Meshnick (1991)
76	Dapsone		33 µM	Zhang and Meshnick (1991)

Table 13 Malarial isoprenoid biosynthesis inhibitors

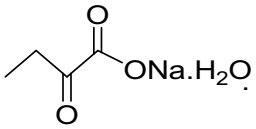
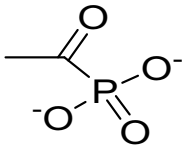
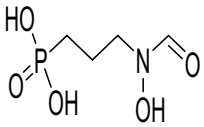
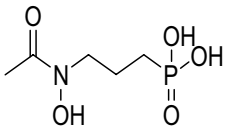
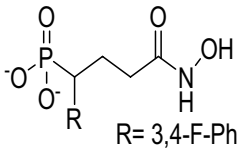
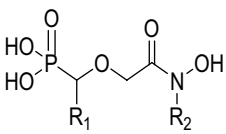
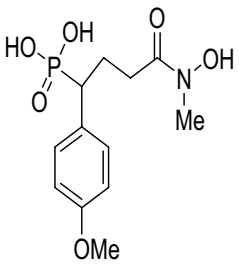
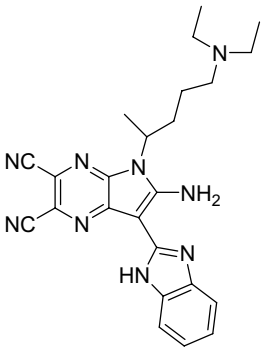
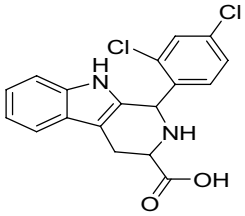
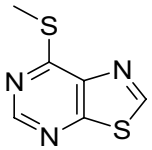
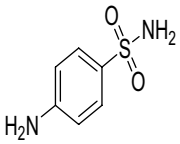
Sl No	Name	Structure	IC_{50}	References
77	β-fluoropyruvate		For *P. vivax* = 35 ± 1.7 µM, For *P. falciparum* = 43 ± 3.8 µM	Saggu et al. (2016)
78	Methylacetylphosphonate		For *P. vivax* = 80 µM, For *P. falciparum* = 46 ± 3.8 µM	Saggu et al. (2016)
79	Fosmidomycin		*P. falciparum* = 350 ± 170 nM	Saggu et al. (2016)
80	FR900098		*P. falciparum* = 170 ± 100 nM	Saggu et al. (2016)
81	[1-(3,4-Difluorophenyl)-4-(hydroxylamino)-4-oxobutyl]phosphonic acid	 R= 3,4-F-Ph	*P. falciparum* = 3 nM	Saggu et al. (2016)
82	((3,4-Difluorophenyl(2-(hydroxy(methyl)amino)-2-oxoethoxy)methyl) phosphonic acid	 R1=Ph,naphth-1-yl, 4-F-Ph, 2,4-F-Ph, 3,4-F-Ph, 3,4-Cl-Ph, 4-CH3-Ph R2= CH3	*P. falciparum* = 12 nM	Saggu et al. (2016)
83	4-[Hydroxy(methyl)amino]-1-(4-methoxyphenyl)-4-oxobutylphosphonic acid		*P. falciparum* = 20 nM	Saggu et al. (2016)
84	6-Amino-7-(1H-benzo[d] imidazol-2-yl)-5-[5-(diethylamino)-1-methylbut-1-yl)-5H-pyrrolo[32-b] pyrazine-2,3-dicarbonitrile		*P. falciparum* EC_{50} = 50 nM	Saggu et al. (2016)

Table 13 (continued)

Sl No	Name	Structure	IC_{50}	References
85	MMV008138		*P. falciparum* = 47 nM, *P. vivax* = 310 nM	Saggu et al. (2016)
86	Thiazolopyrimidine		*P. falciparum* = 9.6 µM	Saggu et al. (2016)
87	Aryl bissulphonamide		*P. falciparum* = 1.4 µM	Saggu et al. (2016)

of *Plasmodium* also involving hypnozoites connected with relapse of malaria. The mean IC_{50} of KDU691 in *P. vivax* and *P. falciparum* are ~ 69 and ~ 118 nM. The gametocyte population that was treated with KDU691 also resulted in reduced viability of these gametocytes and a 200 nM dose of KDU691 resulted in 60% reduction in gamete formation. So, this indicates that KDU691 has transmission-blocking, preventive and therapeutic roles in malaria by alteration of PI4P levels including membrane trafficking. This inhibitor is currently in its preclinical trial.

KDU691 forms a hydrogen bond between the hinge of *Pf*PI4K and its core. The hinge with chloro-phenyl moiety of this inhibitor occupies the ribose pocket of *Pf*PI4K without a hydrogen bond interaction. The terminal amide group of KDU691 generates H-bonds with both the catalytic E1316 and K1308 in the catalytic site. (Fienberg et al. 2020).

KAI407

The reinvasion of parasites is inhibited dramatically as KAI407 (Sl. No. 93) is added at a later stage of the intraerythrocytic cycle till 44 h post-invasion. The KAI407 treated parasites display the disorganized and incomplete type of segmentation inadequate merozoites indicating impairment of ingression of the plasma membrane. PfATP4-GFP was utilized as a plasma membrane marker to observe the ingression of plasma membrane around the daughter merozoites and the nuclei were stained by Hoechst 33342. In this experiment, it was seen that the parasites that were treated with 500 nM KAI407 for up to 4 h displayed a disorganized structure of the membrane. [PfATP4-*P. falciparum* P-type cation transporter ATPase4 that is localized in the plasma membrane of the parasite] (McNamara et al. 2013). The IC_{50} value against *P. falciparum* 3D7 strain is 50 ± 16 nM. This inhibitor is currently in its preclinical trial.

KAI407 is docked into *Pf*PI4K which shows that a N-hetero-atom receives a backbone hydrogen bond from the hinge. The phenyl-CF3 moiety resides in the lipophilic affinity pocket and the cyano-phenyl moiety resides in the ribose pocket, with nitrile N proximal to S1362. The amino acid residues include V1357, Y1356. (Fienberg et al. 2020).

BRD73842

*Pv*PI4K IC_{50} = 0.021 µM, *Pf* Dd2 EC_{50} 0.069 µM. This inhibitor selectively inhibits *P. falciparum* PI4K. This inhibitor is functional against asexual blood stage and liver stage of parasites. This inhibitor is discovered by screening through diversity-oriented synthesis (DOS). BRD73842 (Sl. No. 94) requires stereochemistry for its activity that is shown by its structure (R stereoisomer). To determine whether this inhibitor inhibits PI4K, an assay is done against *P. vivax* purified and recombinant PI4K protein. In this experiment, the compound inhibits *Pv* PI4K not the PI4K of humans. Preclinical trials are ongoing with this inhibitor (Kato et al. 2016).

Table 14 Malarial farnesyl transferase inhibitors

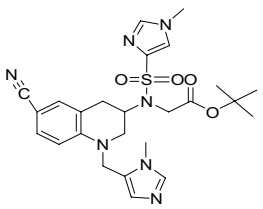
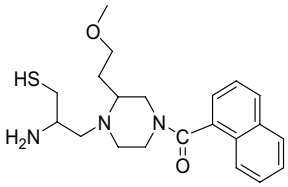
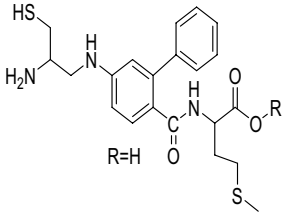
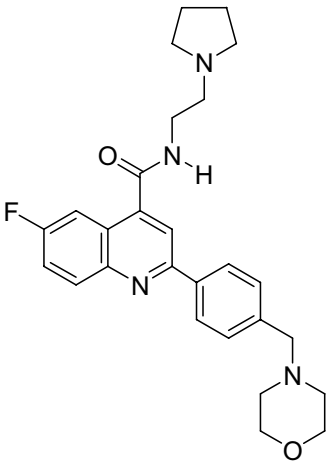
Sl No	Name	Structure	IC_{50}	References
88	BMS-386914		*P. falciparum* = 0.9 nM	Gisselberg et al. (2017)
89	L-745631		*P. falciparum* = 3–4 nM	Chakrabarti et al. (2002)
90	FTI-276		*P. falciparum* = 0.9 nM	Chakrabarti et al. (2002)

Table 15 *P. falciparum* translational elongation factor 2 inhibitors

Sl No	Name	Chemical structure	IC_{50} value	References
91	DDD107498		WT Dd2 IC_{50}-0.14 nM	Baragaña et al. (2015)

MMV390048

High-throughput screening along with phenotypic-based optimization identifies this compound MMV390048 (Sl. No. 95) which belongs to the class of 2-aminopyridine molecules. The in vitro functionality of this compound against *P. falciparum* intraerythrocytic stages shows IC_{90} and IC_{50} values of 40 and 28 nM respectively. This compound has also a similar blood stage specificity to pyrimethamine and shows the highest action against the young stage of schizont. Several in vitro assays on the parasite's sexual stages of life cycle show that MMV390048 also inhibits the erythrocytic gametocytes (late-stage) viability. These gametocytes were destroyed 2.5 times more rapidly than gametocytes of the early stage. So, the potentiality of MMV390048 as an antimalarial compound

Table 16 *P. falciparum* phosphatidylinositol 4-kinase (PI4K) inhibitors

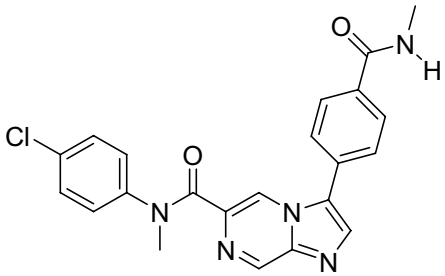
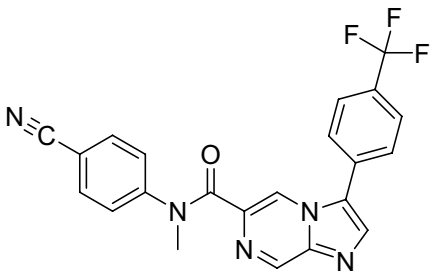
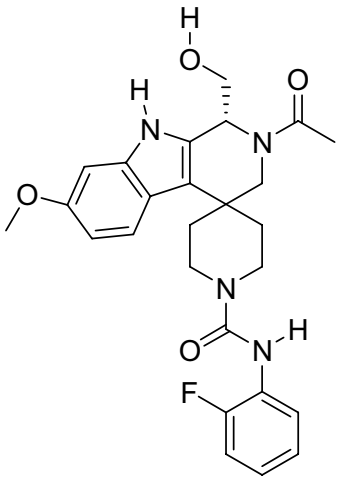
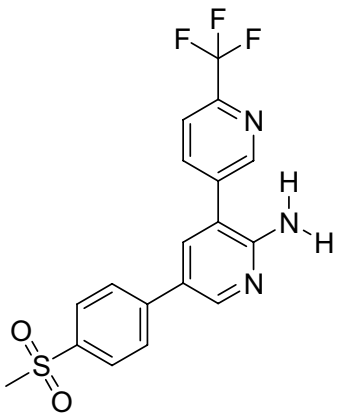
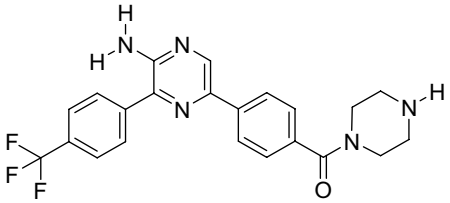
Sl No	Name	Chemical structure	IC_{50} value	References
92	KDU691		*Pv* PI4K- ~ 69 nM *Pf* PI4K- ~ 118 nM	McNamara et al. (2013)
93	KAI407		*P. falciparum* 3D7 strain-50 ± 16 nM	McNamara et al. (2013)
94	BRD73842		*Pv* PI4K-0.021 µM *Pf* PI4K-0.069 µM	Kato et al. (2016)
95	MMV390048		*Pf* PI4K-28 nM	Paquet et al. (2017)

Table 16 (continued)

Sl No	Name	Chemical structure	IC_{50} value	References
96	UCT943		*Pf* K1 strain-4.7 nM	Brunschwig et al. (2018)

needs to be further investigated. (Paquet et al. 2017) Phase II trial of this inhibitor is terminated.

In silico docking reveals that MMV390048 forms two hydrogen bond interactions with the hinge. The phenyl sulfone moiety of this inhibitor generates a hydrogen bond with catalytic K1308 from the affinity pocket. The trifluoromethyl pyridyl group of this inhibitor forms no permanent interaction, it only sits in the ribose pocket. The amino acid residues that are involved in this interaction include S1365, and V1357. (Fienberg et al. 2020).

UCT943 (Sl. No. 96)

The MMV390048 which belongs to the 2-aminopyridine class having an IC_{50} value of 4.7 nM against the K1 strain of *P. falciparum* is now under clinical development. Further efforts are conducted to determine next-generation inhibitors of PI4K, by optimizing the series to enhance properties for example antiplasmodialpotency and solubility throughout the life cycle of *Plasmodium*. The conversion of 2-aminopyridine to 2-aminopyrazine core is achieved by the introduction of a piperazinylamide moiety at the 5th position on the phenyl ring of 2-aminopyrazine scaffold leads to the compound UCT943.

This conversion leads to several improvements in activities against the asexual stages and liver stages of the parasite. UCT943 shows robust activities against gametocytes [both early and late stages] and it is capable of inhibiting both female and male gametes. This information is found in DGFA [dual gamete formation assay]. (Brunschwig et al. 2018) Due to pre-clinical toxicity, this inhibitor has been withdrawn.

Conclusion

The ways of combating malaria have advanced in various directions but the global burden of malaria remains massive due to the generation of multi-drug resistance strains of *Plasmodium* which hinder the activity of traditional drugs. Moreover, the early-stage infection of *Plasmodium* occurs in liver cells and can be cured by using traditional drugs but at the later stage, the parasites infect the erythrocyte cells and hijack the immune system of hosts. Researches are ongoing to generate novel inhibitors of DNA and nucleic acid synthesis in *Plasmodium*, so that the growth of the parasite can be blocked. Clinical reports have shown complicacy in the different pathological parameters of the infected human host on late diagnoses. Therefore, there might be more dreadful hidden malaria parasitic targets that are yet to be explored. These targets comprise increased potentiality and efficacy in terms of eradication of malaria. So, in this review, we have discussed various novel targets which are important for the survival of parasites to be used in the formation of novel treatments for malaria. We have also discussed different types of inhibitors associated with different target proteins responsible for conventional drug resistance. The present study will help the researchers to design, generate and screen chemically modified drugs against malaria.

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Declarations

Conflict of interest No conflict of interest to declare. This manuscript and its subheadings are related to the journal.

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