Chapter

Malaria: Introductory Concepts, Resistance Issues and Current Medicines

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Abstract

Malaria continues to be the main community health problem in numerous nations. Six species of *Plasmodium* are documented as the cause of human malaria infection. Among others, *Plasmodium falciparum* and *Plasmodium vivax* parasites produce an immense challenge in the public health. *Anopheles funestus* and *Anopheles gambiae* are the major transimmiter of the disease (malaria) from one person to another. The disease parasite has a complicated cycle of life that occurs in human and mosquitoes. In general, malaria diagnosis is divided into parasitological and clinical diagnosis. Internationally, the death rate of malaria becomes reduced although few records from Ethiopia describe the presence of raised prevalence of malaria in certain areas. Apart from reduction in incidence and prevalence, transmission of malaria is continued throughout the globe. Hence, its control needs a combined approach comprising treatment with effective antimalarial agents. A lot of novel compounds are under pre-clinical and clinical studies that are triggered by the occurrence of resistance among commonly used antimalarial drugs. In addition to the already known new compounds and targets for drug discovery, scientists from all corner of the world are in search of novel targets and chemical entities.

Keywords: Malaria, *Plasmodium*, antimalarial drugs, resistance, clinical trials, novel compounds

1. Introduction

Malaria itself or a disease looks like malaria has been distinguished before 4,000 years. The term *malaria* was derived from two Italian words "*mala aria*" meaning foul or bad air [1]. This name is originated from the observation that malaria cases were prevalent in areas where there is bad air associated with the accumulation of pools [2]. Malaria is caused by the genus *Plasmodium* (mosquito-borne apicomplexan parasite). At the time of bite by infected female *Anopheline* mosquitoes, this protozoal blood infection become conveying from one person to the next person [3, 4]. According to Miller *et al.* [5], malaria is expressed as a disease caused by repeated life cycle of the *Plasmodium* in the red blood cell. It is also defined as an illness brought by a parasite that lives some of the life in humans and some in mosquitoes [6].

The causative agents for malaria infection are among the genus *Plasmodium*, phyllum *Apicomplexa*, class sporozoa, family *Plasmodidae* and order Haemosporidia

[7]. *Plasmodium* is considered to be instigated from photosynthetic protozoa, which is known as dinoflagellate. Among more than 200 different *Plasmodium* species, around 14 species are pathogenic to humans [8, 9]. The remaining species affect animals, such as rodents, monkeys and reptiles [10]. Five of the human pathogens, *P. ovale* sub-species (*P. ovale curtisi* and *P. ovale wallikeri*), *P. falciparum, P. vivax* and *P. malariae* are well known etiologic agents for human malaria. Infrequently, we could be naturally or accidentally infected by many simian species including *P. knowlesi*, *P. cynomolgi, P. bastianelli, P. brasilianum*, *P. schwetzi* and *P. inui*. Disease with *P. knowlesi* happens in individuals if an *Anopheles* mosquito previously diseased by a monkey malaria parasite bites humans. Incubation period (the time between the bite of mosquito and developing malaria symptoms) for falciparum, vivax and ovale, and malariae is 12, 14 and 30 days, respectively. But infections by *P. malariae* can exist in the blood for a very long period, may be decades, without ever producing symptoms. A person with asymptomatic (no symptom) *malariae* infection, however, can infect others, either through blood donation or mosquito bites. Incubation period is different for different persons and depends on the amount of the parasite involved [11–13].

Malaria is widely distributed throughout tropical regions in Africa, Asia, Hispaniola (Dominican Republic and Haiti), Central and South America, the Middle East and Oceania. The global prevalence of malaria species differs. *Falciparum* and *vivax* malaria pose the greatest public health challenge. *Falciparum* is mainly prevalent on the African continent and in the World Health Organization (WHO) regions of South East Asia, the eastern Mediterranean and Western Pacific. It is responsible for most deaths from malaria. *Plasmodium* parasites are affected by temperature. The development of *Plasmodium* species become slows as the temperature drops. When the temperature drops below 60°F, *P. vivax* totally stops developing. *P. falciparum* can regrate to develop at a bit elevated temperatures. This effect elaborates why malaria parasites are present in temperate environments. *Vivax* has a wider geographic distribution since it can grow in its vector at lower temperatures, cooler climates and elevated altitudes. However, *vivax* is more common in the Indian subcontinent and Central America. Despite it occurs in over all Africa, the risk of *vivax* infection is relatively low there due to lack of Duffy gene in most people of Africa [6, 14]. But, there is a supporting facts that *vivax* can be transmitted to negative Duffy blood group residents in Africa including Ethiopia [15]. South America and South East Asia have both *falciparum* and *vivax* species. *P. ovale* has an unusual distribution (present in West Africa, New Guinea and Philippines). Although, *malariae* has been wiped out from temperate climates, it persists in African sub-region [16]. *P. knowlesi* occurs in South East Asia with cases widely distributed in Sabah and Sarawak in Malaysian Borneo, and peninsular Malaysia. Cases have been reported from a number of other countries in South East Asia, and in travelers [12].

Mosquitoes of the genera Culex, *Anopheles*, Mansonia and Aedes may act as malaria vectors [16]. Nonetheless, malaria is transmitted mainly via the bite of *Anopheles* mosquitoes, which comprise 537 known species and majority (87%) of them have been formally named [17]. Nearly, 70 of these species are able to transmit *Plasmodium* parasite to human hosts and 41 of 70 are considered to be dominant vector species [1]. *Anopheles gambiae* and *A. funestus* are the most efficient vectors of malaria in the world. They are also the primary vectors of malaria in Africa [18]. In Ethiopia, two primary vectors of Africa and *A. pharoensis* are recognized as the dominant malaria vectors [15].

While some species grow in temperate climates and even continue to exist in the Arctic summer, majority of *anopheline* mosquitoes survive in tropical and subtropical regions. It was believed that *anopheline* mosquitoes are not breed on altitudes

higher than 2,000 to 2,500 m. In this geographical boundary, there are a lot of malaria free places as its transmission is extremely reliant on the local environment and epidemiologic situations. *Anopheline* mosquitoes prefer comparatively clean water as their larval habitat (site for egg-laying and development of larvae) though species vary in the quantity of salinity and organic content and amount of sun exposure and temperature they prefer in their breeding sites. For example, city conditions can generate new spaces to mosquito larvae for development. Agricultural activities can also affect breeding site of mosquitoes. While the draining and drying of swamps removes the breeding areas of larvae, water-filled irrigation ditches could provide mosquitoes a new site for breeding. Egg, larva, pupa and adult (imago) are the four developmental phases of *anopheline* mosquitos. Adult males copulate to females in flight to provide adequate sperm for all subsequent egg-laying. To develop the first batch of their eggs, adult females require at least 2 blood meals but one blood meal is enough to develop each successive batch. As development of egg needs around 48 h, blood-seeking is recurring every two to three nights. Under most favorable conditions, the average lifetime of the female (adult) *anopheline* mosquito is equal to or more than three weeks. External factors including temperature, moisture and natural enemies could decrease its prolonged existence. Adult males, in contrast, generally live a few days. If the mean ambient temperature goes beyond 35°C or humidity drops below fifty percent, longevity is drastically decreased, directly affects malaria transmission. In most tropical regions, cases of malaria become increased at the time of rainy season as the rainfall expands breeding grounds. The adult male *anopheline* feeds on nectar, while the adult female feeds primarily upon blood of warm-blooded animals, predominantly mammals. Some female *anopheline* mosquitoes that have a preference toward humans are termed *anthropophagic* (*anthropophilic*). Others who choose animals, such as cattle, are expressed as *zoophagic* (*zoophilic*). The interval over which a mosquito is attracted to its favorite source of blood usually ranges 7–20 m. Many *Anopheles* mosquitoes are either nocturnal (active at night) or crepuscular (active at dusk or dawn). Some are endophagic (feed indoors) while others are exophagic (feed outdoors). After blood feeding, some of them wishs to rest indoors (endophilic) while others intended to rest outdoors (exophilic) [6, 19, 20].

Mentioned earlier, malaria is transmitted from one individual to the next individual via the bite of female *Anopheles* mosquito that has been acquiring the parasite from the first person. The female mosquito needs blood protein for her egg maturation. *Anopheles* mosquitoes are attracted to human by a number of factors (for example heat, odor and exhaled carbon dioxide) and usually bite us between sunset and sunrise [12]. Since *Plasmodum* resides in red blood cells, malaria is also transmitted via donation of blood, transplantation of organ and sharing of needles or syringes contaminated by infected blood. A new born child could also acquire congenital malaria from her/his mother before/during birth [11, 21]. Rarely, accidental nosocomial (hospital acquired) transmission of malaria may occur, for example, where there is a breach in infection control or as a result of a medical procedure [12, 22]. Moreover, transmission of malaria can largely be affected by global warming [23, 24].

Cases of malaria occur in non-endemic areas without an apparent travel history is known as **cryptic malaria**. If the conditions are appropriate for the transmission cycle of *Plasmodium* to be maintained, periodic (sporadic) outbreaks of locally acquired malaria may occur when an imported malaria case happens in a non-endemic district and is bitten by a malaria vector that can transmit parasite to another person. This is called **introduced malaria**. This is generally results in a small cluster of 1 or 2 cases even though larger outbreaks may sometimes occur. If the environmental (climatic) conditions allow, malaria may also occur if a person

is bitten with infected mosquito that has been imported to a non-endemic region. This can be occured around airports (**airport malaria**) or from a mosquito that has stowed away in hand luggage (**baggage** or **luggage malaria**) if aircraft have not been disinsected in a well manner [12].

Chills, high fever, malaise, headache, muscle aches and sweating are the most frequently reported symptoms of malaria infection. The current diagnostic methods used for identification of *Plasmodium* species from blood samples are light/fluorescence microscopy (gold standard method), immuno-chromatographic lateral flow assays (RDTs-rapid diagnostic tests), serology tests, and nucleic acid amplification techniques including PCR (polymerase chain reaction) and isothermal amplification [25]. Rolling circle enhanced enzyme activity detection (REEAD) and micromagnetic resonance reflaxometric (MMR) tests are recently developed parasitological methods appropraite for utilization in field detection of malaria infected individuals for population screenings [26].

Around 44% of world population is at risk from malaria [27]. The risk varies according to season, geographic location, activities, type of accommodation, and the use of malaria prevention drugs and bite avoidance measures. Approximately 229,000,000 cases of malaria, most (94%) from the WHO African Region, are taken place globally in 2019. The disease was caused 409,000 deaths worldwide and most (94%) of which are also from the African Region. Most cases of malaria in Africa are resulted from *P. falciparum*. In 2019, global case incidence and mortality rate of malaria was reduced by 57 and 10%, respectively. Malaria continues to strike hardest against children and pregnant women in Africa. Children aged <5 years are the most exposed group affected by malaria, accounted 67% of global malaria deaths in 2019 [28]. In the USA, roughly 1,500–2,000 cases of malaria in recent travelers are reported every year. Pregnant mothers have high vulnerability to *falciparum* malaria. *P. falciparum* malria contributes 8 to 14 percent low birth weight in malaria-endemic areas, which in turn minimize the likelihood of a baby's survival [19].

All travelers visiting malaria endemic regions are at risk of acquiring malaria. Certain travelers including pregnant women, children, older travelers, immunosuppressed individuals, those with an absent or dysfunctional spleen, and those with complex co-morbidities are at high risk for severe disease if they have malaria. As they are peculiarly attractive to mosquitoes and have high risk of developing severe infection with increased risk of death compared to non-pregnant mothers, pregnant women should be advised to stay away from (not travel to) malarious areas. Travelers who lost their spleen or travelers who have severe impairement of spleen are at particular risk of severe malaria and are advised to avoid travel to malarious areas. If travel is essential, antimalarial drugs are advised in both high and low risk areas, together with rigorous bite avoidance and awareness of the need for prompt medical attention if symptoms develop [12].

Malaria endemic regions are classified into stable and unstable malaria transmission areas. In stable regions, for example in most of sub-Saharan African countries, transmission of malaria is year-round with high infection rates. The population, predominantly adults, may therefore develop a degree of immunity with the majority of clinical cases occurring in infants and children. In unstable regions such as India, malaria transmission has a tendency to be seasonal with short epidemics of varying intensity. Transmission of malaria in these unstable regions is less sustained, hence the communities have weak immunity and all age categories may be affected [12]. In addition to health related impacts, there is a severe burden on economic setors in terms of lost days of labor due to the disease. In fact, malaria is considered to take off 1.3 percent from the economic growth and 40 percent from public health costs of some African countries. It also affects developing nations in most aspects including determent of tourism [29].

Malaria is one of the major infectious diseases in Ethiopia [30, 31]. *Falciparum* and *vivax* are the main two species found in Ethiopia, accounting for 60% and 40% of malaria cases, respectively [32]. *Falciparum* has been the major cause of epidemics, and of most malaria deaths [33]. In Ethiopia, the epidemiological pattern of malaria transmission is generally unstable and seasonal; the level of transmission varies from place to place because of differences in altitude and rainfall patterns [32]. Depending on these rainfall patterns, transmission tends to be highly heterogenous geo-spatially within each year as well as between years [34]. Changes have been observed in the epidemiology of malaria through time. Global warming (changes in climate) are likely to lengthen the transmission seasons of important vector-borne diseases like malaria and to alter their geographic range [35]. Previously, malaria was known to occur in areas below 2000 m but currently it has been documented to occur indigenously even in areas above 2400 m, such as Addis Ababa [32]. Months from September to December and June to August are high malaria transmission seasons in Ethiopia. About 30,485,416 Ethiopians are living at high risk places for malaria infection. In 2019, 213 deaths and 904,496 confirmed cases due to malaria were reported by Ethiopian Federal Ministry of Health (FMoH) [25, 28]. Despite decreased malaria occurrence rate and death rate in Ethiopia since 2010 [28], high prevalence was observed in some areas in contrast to high household coverage of control interventions [36, 37]. This increment may be associated with individuals having poor socio-economic status [38]. Ethiopia has achieved only half of the millennium development reduction target of malaria. For this reason, the country must strengthen its malaria control and treatment approaches to attain the sustainable development goals [39].

2. The parasite life cycle

All types of malaria parasite have a similar and complex life cycle (**Figure 1**). The main part of the complexity related with the life cycle of *Plasmodium* is due to the parasite‘s capability to (a) modifiy its cellular and molecular make up, which is under control by a genome with more than 5,000 genes, and (b) developed in intra-cellular and extra-cellular niches in both mosquito and mammalian host [42]. The life cycle of every *Plasmodium* species infecting humans is distinguished by an exogenous sexual phase (sporogony), in which replication takes place in many *Anopheles* mosquito species, and an endogenous asexual phase (schizogony), which occurs in the vertebrate hosts. The sexual cycle is taken place in the gut and abdominal wall of some species of female mosquito, whereas the asexual cycle that causes the disease symptoms is taken place in the liver and RBCs of the humans [16]. The life cycle within the mosquito takes approximately 8 to 35 days, after which the parasite becomes infective. When the mosquito bites the skin, the sporozoite (motile infectious form of the parasite) will be injected in to human’s dermis and then searchs a blood vessel to feed from it. The insect discharges different vasodilators to raise the possibility of finding a vessel. It also salivates into our blood to avoid blood clotting. The destiny of these sporozoites is not clearly illustrated; however they can take one to two hour to exit from the dermis. The trap-like protein of the sporozoites plays a role to exit the dermis (using gliding motility) and enters to the blood-stream. Those sporozoites remained in the skin could be killed and drained by the lymphatics, where a host immune response is activated. After 30 to 60 minutes of the injection, the thread-like shaped sporozoites will be transported to the liver through the vascular system. One single sporozoite in one hepatocyte multiplies into tens of thousands of exoerythrocytic merozoites [6, 43].

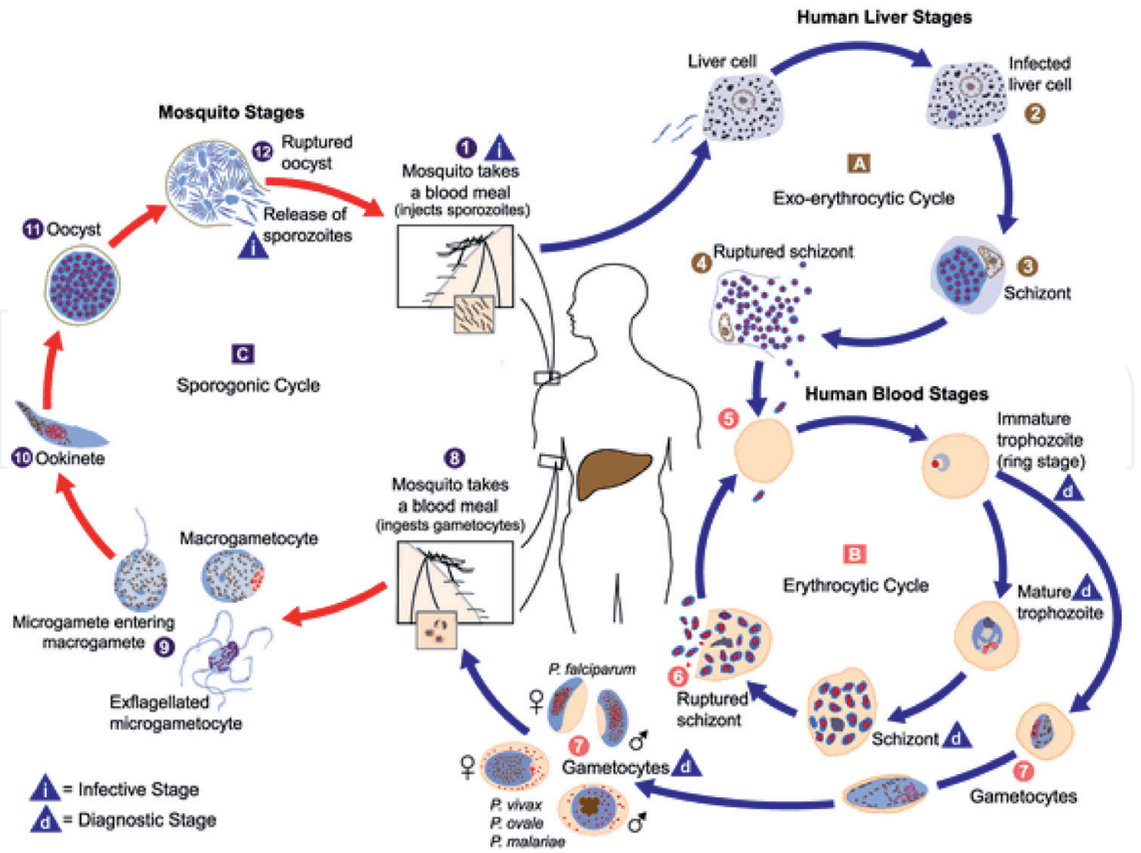

Figure 1.
Life cycle of malaria parasites [40]. The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female Anopheles mosquito inoculates sporozoites into the human host 1. Sporozoites infect liver cells 2 and mature into schizonts 3, which rupture and release merozoites 4. After this initial replication in the liver (exo-erythrocytic schizogony A), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony B). Merozoites infect red blood cells 5. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes) 7. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an Anopheles mosquito during a blood meal 8. The parasites' multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes 9. The zygotes in turn become motile and elongated (ookinetes) 10 which invade the midgut wall of the mosquito where they develop into oocysts 11. The oocysts grow, rupture, and release sporozoites 12, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites 1 into a new human host perpetuates the malaria life cycle [41].

Within 7 to 12 days, the sporozoites develop into schizonts and then grow up to thirty thousand merozoites, which burst the liver cells [44]. Alternatively, some of the sporozoite of *vivax* and *ovale* species turn into hypnozoites (dormant form) in the liver for months/years and can cause relapsed malaria [4, 45]. Unusually, the reappearance of *falciparum* malaria was observed in patient's years after departure of an endemic area. This indicates that *falciparum* has a dormant stage although occurs occassionally [46–49]. Then, the asexual erythrocytic cycle begins and the merozoites start invading red blood cell to consume hemoglobin for their growth. The parasites then multiply 10 times every 2 days, destroying RBCs and infecting new cells throughout the body. Inside the host red blood cell, the *Plasmodium* continues its maturity from the early ring stage to late trophozoite. Then, following mitotic divisions, the trophozoite undergoes to the schizont stage, which consists 6–32 merozoites depending on the *Plasmodium* species [50, 51].

The period from acquiring infection through mosquito bite and the first appearance of the trophozoites in RBCs is called "prepatent period". This constant time is the characteristic of every species. It lasts 9 days in falciparum, 11 up to 13 days in vivax, 10 up to 14 days in ovale, 15 days in malariae and 9 up to 12 days in knowlesi.

When the blood schizont bursts, the discharged merozoites maintain the life cycle through invading the neighbor red blood cells until it is brought under control. The rupture of schizonts is accompanied by the manifestation of the malaria febrile paroxysm typically lasting 8–12 h ("Golgi cycle") and characterized by 3 stages. The first stage (cold stage) is manifested by the quick rise of the temperature together with chills (sensation of the extreme cold). The patient desires to cover with the blankets. The second stage (hot stage) is with the temperature peak (may rises to 41°C), skin vasodilatation, myalgia and very severe headache. Patients feel too burning hot and cast their clothes. During the third stage (sweating stage), the patients have profuse sweating and their fever become drops. Then after, the patients may go to sleep due to tiredness. The typical (classical) symptoms which are stated above may not be appeared in some patients [40, 52]. Cyclical fevers are classically occurs soon before or during lysis of RBC (schizonts rupture). This happens every 48 h in tertian malaria (*vivax*, *ovale* and *falciparum*), and every 72 h in *malariae* infection (quartan *malaria*). At the time of this repetitive cycle, some merozoites differentiate into male and female sexual stages, which are called erythrocytic gametocytes (the only stages transmitted to the mosquito vector) with one nucleus and then cleared by drugs or the immune system, or awaiting the arrival of a blood-seeking *Anopheles* mosquito [6, 50].

The time required for the maturation of gametocytes (do not cause disease) are prominently different among different *Plasmodium* species. *P. falciparum* gametocytes require 8 up to 10 days for development into 5 morphologically different phases or stages (I–V) but *vivax* gametocytes take 48 h for maturity and disappear from blood within three days of sexual phase. In *falciparum*, the first identifiable stages of gametocytes are round compact forms having hemozoin. This stage (stage I) and the subsequent growth steps (stage 2–4) are principally absent from the vascular system, but sequestered in deep tissue in which they grow into mature sausage-shaped stage 5 gametocytes and reappeared in the blood and infective for mosquitoes. In diffrent to *falciparum*, matured *vivax* gametocytes are large and round, filling up almost the whole stippled red blood cell with a prominent nucleus. Because of their rapid maturation than *falciparum*, *vivax* gametocytes become exist in vascular system within a week subsequent to inoculation by mosquito and prior to parasite detection by light microscopy. This creates a major challenge in strategies of *vivax* elimination, as infected persons may be infectious prior to parasite detection using microscopy [53].

When a mosquito takes up erythrocytic gametocytes at the time of blood meal, the gametocytes migrate to the mosquito gut. At the midgut of mosquito, matured gametocytes egress from the host cell and differentiate into male and female gametes. The triggering factors for this differentiation are a fall in temperature, raise in pH and increase in xanthurenic acid concentration. Afterward, undergo fertilization (gametogenesis) - the flagellated forms of microgametes/male gametocytes formed by exflagellation penetrate/fertilize the macrogametes/female gametocytes to form a diploid zygote. The zygote develops into motile ookinetes, which penetrate the mosquito midgut and develop into round oocysts. The oocyst development is the longest developmental phase (takes three up to thirty days) and the only extracellular portion of the *Plasmodium* life cycle. The *falciparum* oocysts mature over a period of 11 to 16 days before releasing the infectious sporozoites (**Figure 1**). The sporozoites vigorously get away from the oocyst and only twenty five percent of those released from oocyst travel via the hemocoelomic fluid to the acinal cells of salivary glands, where following residence for a day, they turn into highly infective stage. They are permanently programmed for their trip in the vertebrate host because they totally lost their capability to invade salivary glands again. The chance of a mosquito for acquiring an infection at the time of blood meal is depend on

various human, *Plasmodium* and mosquito factors. The maturity of gametocytes in human host is fundamental to the continuation of malaria transmission and represents a potential bottleneck in the life cycle of malaria parasites. Knowing the biology of gametocyte maturity and the human infectious reservoir at both the individual and population level is therefore essential to ablate disease transmission nonetheless, it is remained ambiguous [52, 53].

3. Conventional medicines

Malaria is a serious and potentially life threatening disease. It can lead to fatal outcomes in only few days, thus treatment should be started as soon as possible. According to their chemical structure and activity, the available antimalarial agents are grouped into 5 classes as shown in **Table 1** [54, 55]. The key targets of modern antimalarial agents are asexual blood stages of *Plasmodium* species (**Figure 2**), responsible for the malaria symptoms [56]. The *4- aminoquinolines* are blood schizonticidal agents and their mechanism of action is ascribed to their ability to form drug-heme adducts and accumulation of free heme, which is toxic for the parasite [57]. It is also belived that their mode of action is attributed by their inhibition of hemoglobin endocytosis and digestion or disruption of normal vesicle trafficking [58].

Chloroquine, the prototype anti-malarial drug, is the drug of choice for both treatment and chemoprophylaxis of all malaria parasites except for chloroquine-resistant *Plasmodium* strains. In addition to schizonticidal activity, it is also moderately effective against gametocytes of *vivax, ovale,* and *malariae* but not against those of *falciparum* gametocytes. Chloroquine does not eliminate dormant liver forms of *vivax* and *ovale,* for that reason primaquine must be added for the radical cure of these species. Although almost all strains of *malariae* are susceptible, *falciparum*, *vivax* and even some *ovale* strains have been reported as resistant to chloroquine. It is no longer recommended for prophylaxis against *falciparum* [59]. Although chloroquine is the first-line therapy for *vivax* malaria in majority of endemic countries, resistance is the core problem facing this drug in different parts of the world. In Africa and South America, its resistance to *falciparum* first appeared in 1978 and 1996, respectively [60, 61]. In Ethiopia, chloroquine treatment failure against *falciparum* and *vivax* malaria was reported for the fist time from Debre Zeit in 1995. Then after, chloroquine resistance has abeen detected

Major Classes	Groups	Specific agents
Quinolines	4-aminoquinolines	Chloroquine, amodiaquine and piperaquine
	8-aminoquinolines	Primaquine, and tafenoquine
Arylaminoalcohols	-	Quinine, mefloquine, halofantrine, and lumefantrine
Antifolate compounds	-	Pyrimethamine, proguanil, Dapsone, and sulfadoxine
Artemisinin and its derivatives	First generation	Dihdyroartemisinin, artesunate, arteether, and artemether
	Second generation	Artemisone
Hydroxynapthoquinone	-	Atovaquone

Table 1.
Classification of antimalarial drugs.

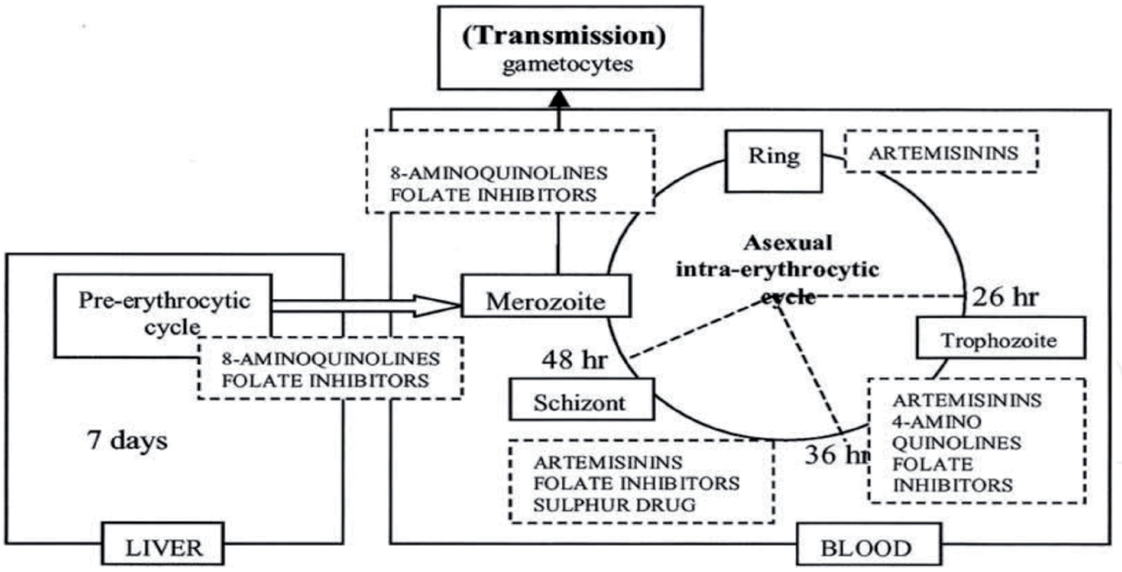

Figure 2.
Plasmodium life cycle with phases targeted by antimalarial drugs. 4-Aminoquinolines target the parasite at the stage where hemoglobin is degraded by parasite protease enzymes. 4-aminoquinolines such as chloroquine and amodiaquine have no effect on the pre-eythrocytic liver stages of parasite development.

in Ethiopia [25, 62]. However, drug resistance to chloroquine can be reversed by certain agents, including verapamil, desipramine, and chlorpheniramine, but the clinical value of resistance-reversing drugs is not established [59].

Amodiaquine is closely related to chloroquine and it probably shares mechanisms of action and resistance with chloroquine. Amodiaquine has been widely used to treat malaria (10 mg base/kg/day for 3 days) because of its low cost, limited toxicity, and, in some areas, effectiveness against chloroquine-resistant strains of *falciparum*. The most important current use is in combination therapy with: (i) sulfadoxine-pyrimethamine (SP) for prophylaxis, and (ii) artesunate [artemisinin-based combination therapy (ACT)] for treatment [13, 59]. **Piperaquine** is a potent and well-tolerated bisquinoline compound thought to act like chloroquine. This lipophilic drug is rapidly absorbed and has an excellent activity on chloroquine-resistant species. Currently, piperaquine combined with dihydroartemisinin (DHA) in co-formulated tablets has shown remarkable efficacy and safety in treating *falciparum* malaria, without visible drug resistance. Piperaquine has a larger half-life (28 days) than amodiaquine (14 days), mefloquine (14 days), and lumefantrine (4 days), leading to a prolonged duration of post-treatment prophylaxis with DHA-piperaquine than with other ACTs; this characteristic is advantageous especially in high transmission areas. DHA-piperaquine (one of the ACTs) is used to treat uncomplicated malaria [59, 63].

Eight-aminoquinolines, tissue schizonticidal agents, are belongs to the only class proven to be effective against the hypnozoites (exoerythrocytic forms) of *vivax* and *ovale* (**Figure 2**). In addition to hypnozoites activity, 8-aminoquinolines can kill gametocytes (the sexual stages of malaria parasites) and consequently block the malaria transmission. Although *falciparum* gametocyte clearance takes days, gametocytes are sterilized within hours; therefore, its effect on oocyst and sporozoite formation (and thus onward transmission of treated infection) precedes its effect on gametocytes carriage. Due to this effect some literatures classify primaquine as sporontocide. The addition of primaquine single dose to ACT is, therefore, recommended by the WHO to reduce gametocyte burden and thus transmission. It has weak activity against the asexual blood stage of *vivax* malaria but with negligible activity against *falciparum* malaria [13].

Primaquine (the prototype drug in 8-aminoquinolines) is indicated for radical cure of *vivax* or *ovale* malaria; for presumptive anti-relapse therapy (terminal prophylaxis- use after the completion of travel to an endemic area to markedly diminish the hypnozoite stages) in population widely exposed to *vivax* or *ovale;* to decrease onward *falciparum* malaria transmission in *falciparum* malaria elimination programmes and in areas threatened by *falciparum* resistance to artemisinins; and as an option for primary (causal and suppressive) prophylaxis against all *Plasmodium* species. Except its use in primary prophylaxis (prevent establishment of infection in the liver by inhibiting the pre-erythrocytic schizogony), primaquine is used in conjunction with an effective blood schizonticide (either ACT or chloroquine) to eradicate erythrocytic stages of *vivax* or *ovale* malaria, and to reduce the possibility of emerging drug resistance [13]. Its mechanism of action is unknown but it is thought to interfere with the cellular respiration of the parasite by means of generating oxygen-free radicals and deregulating the electron transport [64].

Quinine is one of the four antimalarial cinchona alkaloids and has rapid schizonticidal activity against intraerythrocytic malaria parasites. Quinine kills large ring and tropozoite asexual parasites and is gametocidal against *vivax, ovale* and *malariae* but not *falciparum* malaria [65]. Its mechanism of action has not been completely elucidated. The most widely accepted hypothesis is that the drug can inhibit hemozoin crystallization interfering with the heme detoxification process inside the food vacuole (membrane enclosed cell vacuole with a digestive function) [66]. The antimalarial and resistance mechanism of quinine is thought to share similarities to chloroquine.

Mefloquine was first used to treat chloroquine-resistant *falciparum* malaria in Thailand. However, the slow elimination of mefloquine fostered the emergence of drug-resistant parasites [63]. This drug is structurally related to quinine and has two racemic forms, *erythro-* and *threo-*, each composed of a pair of enantiomers, of which the racemic mixture of the *erythro-* enantiomers is the most active against Plasmodia [13]. Mefloquine is a blood schizonticide, active against the erythrocytic stages (15 mg/kg in a single dose) of all malaria parasites. It has more or less the same stage specificity of action as quinine, killing mainly the large ring and trophozoite asexual parasites. It has no significant pre-erythrocytic activity. In combination with artesunate, it can be used to treat uncomplicated malaria [13, 66]. The drug is especially useful as a chemoprophylactic agent for travelers spending weeks, months, or years in areas where *falciparum* and *vivax* infections are endemic due to its slow elimination (delayed half-life), except in clearly defined Thai border regions associated with MDR strains. The mechanism of action is still unknown, probably being different from 4-aminoquinolines. Activity on the parasite seems to be related to the ability of mefloquine to interfere with the transport of hemoglobin from the erythrocyte to the food vacuole. It is also proposed that it inhibits endocytosis of the cytosol by the parasite [13, 63].

Halofantrine hydrochloride is a phenanthrene methanol structurally related to quinine. It is effective against erythrocytic (but not other) stages of all four human malaria species. This synthetic anti-malarial is effective against MDR (including mefloquine resistant) *falciparum* malaria, but its use is limited by irregular absorption and cardiac toxicity. It should not be used for chemoprophylaxis. The mechanism of action by halofantrine is mysterious. It may be similar to that of chloroquine, quinine, and mefloquine; through forming toxic complexes with ferritoporphyrin IX that damage the membrane of the parasite [59, 67].

Antifolates are drugs that target two important enzymes of the folate pathway, namely the Dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS). Proguanil and Pyrimethamine target DHFR, whereas sulfadoxine and dapsone act on DHPS. Pyrimethamine and proguanil are active against susceptible

strains of all four human malaria species. In *falciparum* malaria sensitive antimalarial drugs, proguanil exhibit activity against both the primary hepatic stages and the asexual blood stages, thus sufficiently controlling the acute attack and usually eradicating the infection. Chloroguanide (proguanil) is also effective in treating acute *vivax* malaria, but relapses may arise after the drug is withdrawn because the latent tissue stages are not affected by this drug. Proguanil therapy does not obliterate gametocytes, but acts as a sporontocide (oocytes in the mosquito gut fail to develop normally) and thus ablate the transmission. It is not actually used alone as resistance to proguanil develops very quickly. Proguanil accentuates the mitochondrial membrane-potential-collapsing action of atovaquone against *falciparum* but displays no such activity by itself. The combination of proguanil and atovaquone is known as Malarone® and it is used as chemoprophylaxis in adults and children ≥11 kg. Atovaquone-proguanil may be considered for the treatment of uncomplicated malaria in travelers (adults and children ≥5 kg) outside malaria-endemic areas. It is highly effective and safe in a 3-day regimen for treating mild-to-moderate attacks of chloroquine- or SP-resistant *falciparum* malaria. The combination of chloroquine (500 mg weekly) and proguanil (200 mg daily) was previously widely used, but with increasing resistance to both agents it is no longer recommended [59, 63].

Pyrimethamine (2, 4-diaminopyrimidines) is a slow-acting blood schizontocide with antimalarial effect ssimilar to proguanil. However, pyrimethamine has greater antimalarial potency. The effectiveness of pyrimethamine against liver stages of *falciparum* is less than that of proguanil, and at therapeutic concentrations, pyrimethamine fails to eradicate hypnozoites of *vivax* and gametocytes of any malaria species. It raises the number of circulating mature infecting gametocytes of *falciparum*, likely leading to increased transmission to mosquitoes during treatment period. Pyrimethamine is typically administered with either a sulfonamide such as sulfadoxine or sulfone such as dapsone to enhance its antifolate activity. Sulfonamides and sulfones are weakly active against erythrocytic schizonts but not against liver stages or gametocytes. They are not used alone as antimalarials but are effective in combination with other agents. Although, no longer recommended due to drug resistance, pyrimethamine was used in synergistic combination with sulfadoxine (Fansidar®) or sulfalene (Metakelfin®) for treatment of uncomplicated malaria and with dapsone for prophylaxis. SP is active predominantly against later development stages of asexual parasites. In the few areas in which it remains effective, SP can be used with artesunate for the treatment of acute uncomplicated malaria. Its resistance is caused by point mutations in DHPS and DHFR [13, 63].

Atovaquone, a highly lipophilic analogue of ubiquinone, is active against all *Plasmodium* species, *Pneumocystis jiroveci* and *Toxoplasma gondii*. It is highly active against asexual blood stage (erythrocytic schizonts) of *falciparum* malaria. This drug (only administered orally) is also effective against liver stages (tissue schizonts) of *falciparum* (allowing prophylaxis to be discontinued only one week after the end of exposure) but not against *vivax* hypnozoites. Since atovaquone is tissue schizonticidal, malarone has an advantage over mefloquine and doxycycline in requiring shorter periods of treatment before and after the period at risk for malaria transmission, but it is more expensive than the other agents. Atovaquone selectively inhibits the parasite mitochondrial electron transport chain at the cytochrome bc1 complex. Selectivity is due to structural differences between the cytochrome b encoded by the parasite mitochondrial DNA and that encoded by the host mitochondrial DNA. Regeneration of ubiquinone (electron acceptor for *Plasmodium* dihydroorotate dehydrogenase [DHODH] enzyme, essential for pyrimidine biosynthesis) is the primary function of mitochondrial electron transport in *falciparum* species. Synergism activity between proguanil and atovaquone is resulted from the

capability of non-metabolized proguanil to enhance the mitochondrial toxicity by atovaquone [59, 63, 68].

Artemisinins (endoperoxide sesquiterpene lactone) is a potent and fast acting blood schizonticidal killing all parasite stages, inducing more rapid parasite clearance and fever resolution than any other currently licensed antimalarial drug. Artemisinins have no effect on hepatic stages. They have been reported to reduce gametocytogenesis (young *falciparum* gametocytes), thus reducing transmission of malaria (preventing the spread of resistant strains). However, artemisinin has some pharmacokinetic limitations such as low solubility, poor bioavailability, and short half-life. To overcome some of these problems, semi-synthetic derivatives have been developed. First generation derivatives include the oil-soluble methyl ether, artemether (artemotil [arteether] is a closely related compound); the water soluble hemi-succinate derivative, artesunate; and DHA. Moreover, all active compounds possess a distinctive 1,2,4-trioxane pharmacophore, which is essential for the antimalarial activity since the corresponding acyclic compounds lacking the endoperoxide are biologically inactive [67, 69]. The precise mechanism of action of artemisinin is unclear and still controversial [70]. It has been suggested that the endoperoxide bond undergoes reductive activation by iron^{2+} or iron^{3+}-heme. This redox reaction produces carbon-centered radicals that alkylate target molecules leading to parasite's death [71]. Alternative views suggest that artemisinin inhibits *P. falciparum* encoded sarcoendoplasmic reticulum Ca^{2+}-ATPase (*PfATP6*) [72]. Another proposed mechanism is that artemisinins act as oxidant drugs through oxidation of flavin adenine dinucleotide (FADH2) and parasite flavoenzymes [73].

The standard treatment of malaria employs ACTs to increase treatment efficacy and reduce selection pressure for the emergence of drug resistance. Artemisinins cause a significant reduction of the parasite burden. As such, only 6–8 days of treatment are required to remove the parasites from the blood. Artemisinins do not display significant clinical cross-resistance with other drugs. Artemisinins should not be used for chemoprophylaxis because of their short half-life, which translates into high recrudescence rates. ACTs have lower toxicity and are considered safe to use in children and non-pregnant mothers. However, the widespread distribution of counterfeit (clinically sub-standard) agents that contain small quantities of artemisinin derivative threatens the effective administration of ACTs. The artemisinins and its derivatives generally are not used alone because of their limited ability to eradicate infection completely or its short plasma $t_{1/2}$ translates into substantial treatment failure rates. ACT consists of an artemisinin derivative combined with a long-acting antimalarial drug. To promote patient adherence to treatment by reducing course of therapy from 6 to 8 days to 3 days and to avoid the use of artemisinins as monotherapies due to their brief duration of action, fixed-dose combination formulations into a single tablet are available for all recommended ACTs (artemether + lumefantrine, artesunate + amodiaquine, artesunate + mefloquine, dihydroartemisinin + piperaquine, pyronaridine + artesunate, and artesunate + SP), except for artesunate plus SP. Artesunate-SP is not recommended in many areas owing to unacceptable levels of resistance to sulfadoxine-pyrimethamine. **Lumefantrine** is a fluorene derivative belongs to the group of quinine, halofantrine and mefloquine. This drug is belived to act similar to other members of the group (prevent haem detoxification within the food vacuole of the parasite, thus causing accumulation of the toxic haem complex). Lumefantrine (benflumetol) is formulated with artemether (COARTEM) [13, 63].

The WHO recommends ACT for the treatment of uncomplicated malaria caused by *falciparum* parasite or by chloroquine resistant *vivax*, *ovale, malariae* and *knowlsi*. Quinine plus clindamycin is used for uncomplicated malaria treatment in the first trimester of pregnancy [13]. In Ethiopia, Coartem (artemether-lumefantrine) is

suggested as the first-line drug for uncomplicated *falciparum* malaria and chloroquine for other species (*vivax, malariae, ovale* and *knowlesi*) but oral quinine is considered as a second option [62]. Given their rapid and potent activity against even MDR parasites, injectable artesunate becomes the drug of choice for severe malaria globally in infants, children, lactating women and pregnant mothers of all trimester. After one day, the course of therapy should be completed using oral ACT [13]. Quinidine plus tetracycline, doxycycline, or clindamycin is the treatment of choice for severe malaria in the USA [74]. In Ethiopia, injectable artesunate is the drug of choice and intramuscular artemether is an alternative agent. When these 2 drugs are not available, injectable quinine is used to treat severe malaria [62].

Co-resistance of quinine with artesunate-amodiaquine (one of the most widely used ACTs) was fully verified both *in vivo* and *in vitro*. Given the widespread use of ACT worldwide, the suggestion that ART pressure might also favor quinine resistance is of major concern. Undeniably, the present dependence on artemisinins to manage both uncomplicated and complicated malaria, together with absence of possible therapeutic options, leaves decision-makers with very limited alternatives. This would have very bad consequences not only in the therapy of individual cases, but would cripple efforts to conquer malaria globally [75]. According to the current study, DHA-piperaquine is not treating malaria effectively across the eastern Greater Mekong subregion. A highly drug-resistant *falciparum* co-lineage is evolving, acquiring novel resistance mechanisms. So, resistance among artemisinin and its partner drug will continue to evolve, producing *Plasmodium* strains more capable of surviving treatment, which can subsequently spread across a wider geographical area. As a consequence, accelerated *falciparum* malaria elimination in this region is required urgently, to avert further spread and avoid a potential global health emergency. In the dearth of new antimalarial classes to replace the present first-line therapies, the use of existing treatments in the form of triple ACTs, in which an artemisinin is combined with 2 partner agents such as DHA-piperaquine and mefloquine, could be a viable alternative [76].

Despite decades of intense research, no licensed malaria vaccines are available until now [77]. A lot, but a better understanding is required on host immunity and the *Plasmodium* to improve vaccines. In Phase-3 clinical testing, the first proven antiparasite vaccine (a circumsporozoite protein vaccine [RTS, S/AS01]) reduced clinical malaria in children. Nonetheless, young infants do not respond well, and implementation studies with mortality endpoints are awaited. The irradiated *P. falciparum* sporozoites such as PfSPZ, which is closer to pivotal Phase-III trials, can be manufactured and have been shown to prevent infection in some African countries. Most recently, African trials of gamete protein vaccines started and placental malaria vaccines entered human testing. Blood-stage targets of protective antibodies remain unknown, but new proteins implicated in erythrocyte invasion and egress offer promise [78]. Limitations in efficacy, absence of standard predictive biomarkers of protective efficacy and the need to constantly update vaccine formulations due to antigenic polymorphism further underscore the current reliance on chemotherapy [79]. However, the occurrence of resistance (malaria parasites survive and/or multiply despite the proper administration and absorption of an antimalarial medicine in the dose normally recommended) [80] among commonly used drugs is a major problem. Resistance against antimalarial drug results in a global revival of malaria creating a major problem to malaria control. Indiscriminate and widespread utilization of antimalarial agents contributes to *Plasmodium* species to evolve and develop resistance mechanisms [81, 82]. As a result, old and novel chemicals are under per-clinical and clinical studies. Despite the widespread development of resistance and difficulties in poor areas to afford and access effective antimalarial drugs, currently used and potent drugs, such as

artemether, chloroquine and quinine, are obtained from plant sources. Hence, it is imperative to focus on traditionally used medicinal plants for the discovery of possible new innovative antimalarial sources for the future.

4. Genetic basis of drug resistance

Resistance to antimalarial compounds occurs because of the parasites selection with genetic mutations such as single nucleotide polymorphisms (SNP) or gene amplifications that confer decreased susceptibility [83]. A number of factors aid the emergence of current antimalarial drug resistance. Some of them, among others, are the mutation rate of *Plasmodium*, the overall parasite load, the strength of drug selected, the treatment compliance, and poor adherence to treatment guidelines. Inappropriate dose, poor pharmacokinetic profile, fake drugs lead to inadequate drug exposure on parasites [84, 85], and poor quality antimalarial (falsified antimalarial without active ingredients) drugs may aid and abet the occurnce of resistance by increasing the risk of hyperparasitaemia, recrudescence, and hypergametocyopaenia [86, 87].

The two malaria parasites (*falciparum* and *vivax*) that cause most of malaria cases of human beings have developed resistance to almost all current antimalarial drugs. The capability of these *Plasmodium* species to develop resistance is mainly due to the large numbers of parasites in the infected individual's bloodstream at the time of the asexual blood stage infection in conjunction with the mutability of their genomes [88]. Now a day, controlling MDR *falciparum* malaria is become a very challenging work for the reason that endogenous allelic exchanges occurred in *falciparum* species have increased the treatment failures and drastically increased the resistance level globally. Since evolution is a continuous process, how we stop the formation of drug resistant mutant alleles is a very concerning question. Usually, high mean parasitemia index is observed in *falciparum* infected persons but *vivax* infection generally exhibits low parasitemia index secondary to its preference to invade reticulocytes rather than erythrocytes [89, 90].

Resistance to chloroquine in *falciparum* is due to point mutations in the gene encoding *pfcrt* (*P. falciparum* chloroquine resistance transporter) and *pfmdr* (*P. falciparum* multidrug resistance protein [P-glycoprotein transporter proteins]), resulting in reduced drug accumulation in the food vacuole [91]. Chloroquine-resistant *vivax* was first reported from Papua New Guinea in 1989. High grade chloroquine-resistant *vivax* is prevalent in areas such as Indonesia and Oceania (considered as chloroquine resistance epicenters) [92]. It is more challenging to detect chloroquine resistance in *vivax* since parasitemia is generally low relative to *falciparum*. In addition, it is not easy to distinguish *vivax* recrudescence from relapses as a result of reactivation of dormant hepatic parasites in endemic settings. Moreover, there is no robust *in vitro* culture system for *vivax*, so confirmation with *in vitro* susceptibility testing is even more challenging for *vivax* than for *falciparum*. Although *pvcrt-o* (*P. vivax* chloroquine resistance transporter-o) is orthologous to *pfcrt*, there is no clear direct association between chloroquine resistance and mutations in *pvcrt-o.* One current study in patients with recurrent *vivax* infections in the Brazilian Amazon found that chloroquine resistance was associated with increased copies of gene encoding *pvcrt-o* [88].

Amodiaquine and its slowly eliminated active metabolite (desethylamodiaquine) are structurally related to chloroquine, this explains the cross resistance observed in the field, where parasites were reported to harbor mutations on *pfcrt* and *pfmdr1* after amodiaquine treatment failure [93]. Therefore, amodiaquine is used in combination therapy with SP for prophylaxis and artesunate for treatment.

DHA-piperaquine (co-formulated tablet) has shown excellent efficacy (without apparent drug resistance) and safety in treating *falciparum* malaria. But now, resistance has been reported from Western Cambodia to be associated with a point mutation of *pfcrt* and amplification of *plasmepsin* 2 and 3 genes in *falciparum* parasites. The *plasmepsin* genes encode aspartic proteases that function as hemoglobinases in the parasitie's digestive vacuole. The mechanism of resistance is not clearly known; however hypothesized that increased hemoglobin digestion due to the amplification decreases the reactive heme species concentrations that piperaquine binds, thereby overcoming the inhibition of heme detoxification by piperaquine [88].

Documents written regarding **quinine** resistance are rare, but isolated cases have been reported from Thailand, North India, East Africa and South America [93]. Resistance mechanisms to quinine appear to be more complex. *In vitro* cross resistance between quinine, other aryl aminoalcohols, and 4-aminoquinolines is observed, suggesting that there may be a common genetic mechanism of resistance among those drugs. Mutations in *pfmdr1* and *pfcrt* have been found to confer decreased susceptibility of the parasite to quinine. Yet, they are not sufficient to bring resistance, implying that there are additional genes involved. A quantitative trait loci analysis done to detect genes associated with quinine resistance in 71 *falciparum* isolates from diverse locations has been identified *pfmdr1*, *pfcrt*, and *pfnhe-1* (*P. falciparum Na*$^+$*/H*$^+$*exchanger-1*). *Pfnhe-1* encodes *falciparum* Na^+/H^+exchanger 1 and is on chromosome 13. M**efloquine** resistance by both *falciparum* and *vivax* was found to be primarily mediated by *mdr1* amplification (increased *mdr1* copy number), rather than through point mutations similar to chloroquine and antifolate drugs. Resistance to **primaquine** in *vivax* is difficult to verify as it is confounded by reinfections in malaria-endemic regions. A research that done whole genome sequencing of *vivax* from known relapses that occurred despite primaquine therapy found polymorphisms in many putative resistance genes. However, there are currently no known genetic markers of primaquine and tafenoquine resistance [88].

In contrast to chloroquine resistance, which took many years to develop, antifolates resistance developed much faster. The genetic mechanism of resistance for antifolates is more straightforward than chloroquine resistance. The reason for resistanc against antifolates is single point-mutations in the genes encoding either DHFR—*pfdhfr* in *falciparum* and *pvdhfr* in *vivax* malaria, or DHPS—*pfdhps* in *falciparum and pvdhps* in *vivax* malaria. *Dhfr* mutations reduce the overall efficacy of the enzyme and result in a fitness cost for *Plasmodium*. Following changes in first-line therapy of malaria from sulfa-drugs to ACTs, a decline in triple and quadruple *dhfr* mutants has been observed in certain regions. Nonetheless, in nations where SP is part of the ACT or SP is used as intermittent preventive therapy (IPT), these mutants remain prevalent. In addition, the persistence of the *Plasmodium* species carrying *dhfr* mutations may be attributed to the use of trimethroprim-sulfamethoxazole for prophylaxis or for treating opportunistic infections in HIV positive individuals. Interestingly, *falciparum* species in Southeast Asia are able to develop a compensatory mutation for the fitness cost incurred by the mutant *dhfr*. A genome scanning study of *falciparum* strains first identified an amplification surrounding GTP-cyclohydrolase 1 (*gch1*), which encodes an enzyme in the folate biosynthesis pathway that is upstream from DHFR and DHPS. The amplification reduces the cost of acquiring the drug-resistance mutations further downstream in the folate synthesis pathway [88]. **Atovaquone** acquires resistance related to a single mutation of cytochrome b gene of the parasite [68].

Artemisinin resistance in *falciparum* has currently been detected in five countries of Greater Mekong sub-region (Cambodia, Lao People's Democratic Republic, Myanmar, Thailand and Viet Nam). These resistant *Plasmodium* strains

have the ability of spreading into many world countries including Africa and then they become a global threat for malaria control and treatment [13, 94]. Though different studies associate artemisinin resistance with mutuation in *pfatp6* (*P. falciparum encoded sarcoendoplasmic reticulum* Ca^{2+}*-ATPase6*), *pfmdr1*, *pffd* (*P. falciparum ferredoxin*), *pfarps10* (*apicoplast ribosomal protein s10*), *pfmdr2* or *pfcrt* genes of falciparum, these mutations are thought to represent a background upon which the *kelch13* mutations are especially likely to occur. The genetic mediator(s) of *vivax* resistance against artemisinins is/are not reported till now. Lumefantrine resistance in field isolates has not yet been convincingly demonstrated. However, amplification of the *pfmdr1* gene in *falciparum* and *pvmdr1* in *vivax* has been associated with increased risk of treatment failure of coartem®. Antibacterials such as tetracycline, doxycycline, clindamycin and azithromycin also have antiplasmodial activity although in general their action is slow for malaria treatment. They are recommended only in combination with other antimalarials. Apicoplast ribosomal RNA (23S rRNA) mutation mediated *falciparum* resistance to clindamycin has been found in field isolates. There are no clear markers of doxycycline resistance that have been identified thus far [88, 93].

5. Novel compounds in the pipeline

Mentioned above, the evolving of resistant strains and absence of newer drugs are the limiting aspects in the fight against malaria. These factors prompt the continuing need of studies to bring novel grups of antimalarial compounds, and a re-examination of the present ones. That's why; synthetic peroxides (ozonides) are approved to be viable substitutes of artemisinin. **OZ277** (the first generation ozonide discovered in 2004 and subsequently called **arterolane)** was developed through a collaborative effert between Ranbaxy and MMV (Medicines for Malaria Venture). After a limited phase-3 trials on the combination effects of arterolane and piperaquine, the combined drug has got approval under the trade name **Synriam** in India in 2013, followed by approval in 7 African nations in 2014 [95]. Many new combination treatments, including **azithromycin-chloroquine** [96], **pediatric pyronaridine-artesunate**, **pediatric DHA-piperaquine** [97] and **trimethoprim-sulfamethoxazole** [95], are in phase-3 trials.

A lot of novel chemicals are in phase-II clinical trials (**Table 2**). **Ferroquine (SR97193)** is new organometallic drug completed phase-2 trials in combination with artesunate [98]. Ferroquine retains *in vitro* activity against piperaquine- and chloroquine-resistant *Plasmodium* species. It has a long elimination half-life (16 days). Ferroquine is only moderately effective as single therapy but when combined to artesunate (daily dose of 4/6 mg/kg ferroquine plus 4 mg/kg artesunate for three days); the PCR corrected efficacy at 28 days in treating uncomplicated *falciparum* malaria was 99% [103]. **OZ439** (a synthetic trioxolane) possesses curative and transmission-blocking capacity, and is active against artemisinin-resistant malaria parasites. Much like the current peroxide containing antimalarial agents, the exact mechanism of action of OZ439 has yet to be revealed but it is believed that oxidative stress plays a major role as shown in **Figure 3**. OZ439 [discovered in 2011 by a partnership between Monash University, the University of Nebraska and the Swiss Tropical and Public Health Institute (STPHI)] possesses significantly lower solubility and slightly lower potency than OZ277 [100]. In contrast to other synthetic peroxides and artemisinin derivatives, OZ439 **(**artefenomel**)** totally cured mice infected with *P. berghei* at a single oral dose (20 mg/kg) and showed higher prophylactic effect compared to most antimalarial drugs. Next to reports on its safety and pharmacokinetic properties, a combination of artefenomel (fast- and

Class	Name	Activity (Target)	Development Partner	Remark	Ref.
Second-generation peroxide	OZ439 (artefenomel)	Active against blood stage of falciparum and vivax malaria	MMV and Sanofi	Being tested in Phase IIb combination trial with piperaquine, & with ferroquine	[95, 98]
4-aminoquinoline (Organometallic)	SR97193 (ferroquine)	Active against chloroquine-resistant strains	MMV	Currently in combination trial with artesunate	[98]
Synthetic spiroindolone analogue	KAE609 (Cipargamin)	Blood schizonticide for vivax and falciparum	MMV and Novartis	Currently in Phase IIb trials (NCT03334747)	[99, 100]
Second-generation imidazolopiperazine	KAF156	It acts at multiple stages of the parasite life cycle	MMV and Novartis	Now in phase IIb trial in combination with lumefantrine (NCT03167242)	[101, 102]
	DSM265	Long acting agent with blood and liver stage activity and also active against drug resistant parasites	Takeda, MMV and UT Southwestern	Completed human study in combination with OZ439 (NCT02389348).	[100, 101]
Phenothiazine derivative	Methylene blue	Has blood stage activity and also active against mature male and female falciparum gametocytes	University of Heidelberg	Completed Phase-II trial as a combination with primaquine (NCT02851108) in 2017	[103]
3,5-diaryl-2-aminopyridine	MMV048	Has prophylactic and transmission blocking activity	MMV	Currently in Phase IIa clinical trials in Ethiopia	[100]

Table 2.
Promising new antimalarial agents in phase II clinical development.

long-acting drug) with ferroquine was progressed into a phase-2 trials in 2015 to assess the efficacy of a single oral dose in adults and children aimed at replacing the current three doses of artemisinin derivatives. **Artefenomel–ferroquine** has an elimination half-life of 46–62 h. An advantage of this product is that neither of the constituent drugs has been deployed as monotherapy previously [79, 103].

Artemisone (second-generation semi-synthetic artemisinin derivative developed at the Hong Kong University of Science and Technology), a drug in phase-II study, provides a single dose cure in Aotus monkeys infected with *falciparum* malaria at 10 mg/kg when combined with mefloquine 5 mg/kg [104]. Artemisone

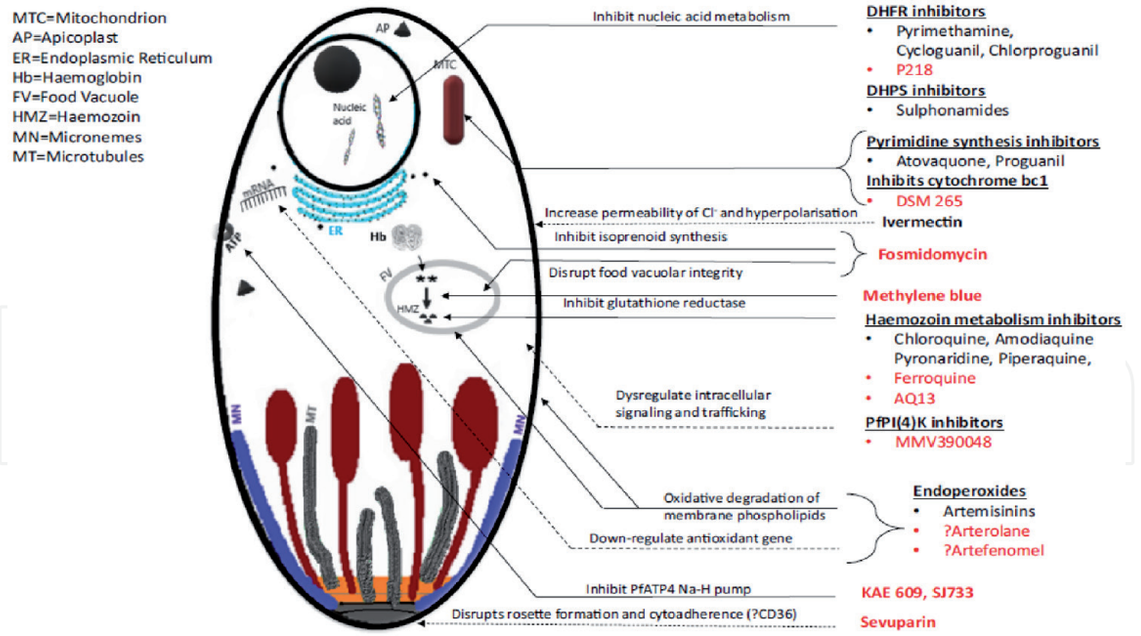

Figure 3.
Schematic representation of intra-erythrocytic trophozoite showing sites of action of newer antimalarials. Agents in red are still in development [100].

has shown to be efficacoiuse as artesunate and possess improved pharmacokinetic properties such as longer half-life and lower neuro- and cytotoxicity than the first generation artemisinins [105]. With the motivation of urgent requirement to develop new artemisinins in combination with new drugs that impart activities toward both intra-erythrocytic asexual and transmissible gametocyte stages, in particular, those of resistant parasites, amino-artemisinins (oxidant drug in which an amino group replaces the oxygen-bearing substituents attached to carbon number 10 of the present clinical artemisinin derivatives DHA, artemether and artesunate) including artemisone and artemiside exhibit potent *in vitro* activities against the asexual erythrocytic stages of *falciparum* malaria. Particularly, these compounds are active against late erythrocytic stage *falciparum* gametocytes, and are highly synergistic in combination with the redox active agent methylene blue. In order to strengthen the selection of best amino-artemisinins for development into novel triple combination treatments also active against artemisinin-resistant *P. falciparum* mutants, new amino-artemisinins were formulated based on the easily accessible and low-priced drug DHA-piperazine. DHA-piperazine was converted into alkyl sulfonamides, aryl sulfonamides, ureas and amides. These derivatives were screened together with the comparator drugs DHA and the amino-artemisinins (until now most active compounds against asexual and sexual erythrocytic stages of *falciparum* and hepatic stage *P. berghei* sporozoites) artemisone and artemiside. Many new amino-artemisinins that contain aryl-urea and -amide groups are found to be potently active against both asexual and late erythrocytic stage gametocytes. Although the activities are superior to those of artemiside and artemisone, the latter (aryl sulfonamide, the aryl urea, and the aryl amides) are more active against the liver stage *P. berghei* sporozoites. In addition, these compounds tend not to display reduced susceptibility against *Plasmodium* species bearing the *Pf* Kelch 13 propeller domain C580Y mutation characteristic of artemisinin-resistant *falciparum* malaria. Thus, the advent of the amino-artemisinins will enable the development of novel combination drugs that by virtue of the amino-artemisinin component itself will possess intrinsic transmission-blocking abilities and may be effective against artemisinin-resistant *falciparum* malaria [106, 107].

Novartis currently has 2 new antimalarial compounds (**KAE609 (Cipargamin)** and **KAF156)** in phase-II clinical testing (**Table 2**) [99]. The occurrence of resistance in artemisinin raises the concern of cross-resistance with arterolane and artefenomel due to chemical similarities between the two groups of compounds. By contrast, cipargamin and KAF156 are structurally unrelated to the artemisinin derivatives. KAE609 has inhibitory effect on *falciparum* cation channel/P-type ATPase-4 transporter (*PfATPase4*) resulting in a build up of Na^+ inside the parasite, leading to cell death. **Cipargamin** was discovered by a partnership between Novartis, the STPHI and the Wellcome Trust. It is equially potent against drug-resistant *Plasmodium* strains and as effective as artesunate against *falciparum* and *vivax* malaria. KAE609 displays a good safety with low cytotoxicity, cardiotoxicity and mutagenic activity. Cipargamin has the ablity to clear parasitaemia quickly in adult individuals (30 mg/day for 3 days) with uncomplicated *falciparum* or *vivax* malaria. This drug is also shows low body clearance, long half-life and excellent bioavailability [100].

KAF156 (identified in 2008 by Novartis and The Scripps Research Institute) is with potential to treat and prevent malaria, and has an elimination half-life of around 48 h. KAF156 is shown to have potent *in vitro* activity against both asexual and sexual blood stages and the pre-erythrocytic liver stages of *Plasmodium* species. In the causal prophylactic rodent malaria model, a single oral dose of 10 mg/kg was shown to be fully protective. KAF156 has also shown transmission blocking activity in the berghei model. A recent phase-2 trial among adults with acute *falciparum*/*vivax* malaria at five centers in Thailand and Vietnam has showed that KAF156 cleared parasites more rapidly than SP or malarone®, though this rate was slightly slower than artemisinin and DSM265. Additionally, therapeutic responses to treatment by KAF156 suggested effectiveness against *falciparum* and *vivax* infections resistant to each and every one of currently available antimalarials without evident safety concerns. The mode of action of KAF156 is still unclear although mutations have been identified in three genes (*P. falciparum* Cyclic Amine Resistance Locus [*PfCARL*], UDP-galactose and Acetyl-CoA transporters) through culturing of resistant strains [79, 100]. **DSM265** is another compound that complete phase-2a trials (**Table 2**) and inhibit DHODH both in *falciparum* and *vivax* species [104]. It was discovered through collaboration between the University of Texas (UT) Southwestern, the University of Washington, and Monash University. DSM265 has an excellent safety profile, a very low clearance rate and a long half-life in humans. *In vitro* studies suggest a relatively low barrier to resistance selection, so measures to protect this drug, such as matching with a partner with similar elimination kinetics and deploying only as part of a fixed-dose combination will be important [103].

Fosmidomycin, a natural antibacterial drug that inhibit 1-deoxy-D-xylulose 5-phosphate reductoisomerase (an enzyme involved in the synthesis of isoprenoids), is under combination therapy trial with piperaquine (NCT02198807) in phase 2 in order to destroy blood schizonts of uncomplicated *falciparum* malaria [97, 108]. **AQ-13**, a modified chloroquine (differ to chloroquine only in the amine side-chain), last completed phase-II trial (NCT01614964) at the end of 2017 [100], retains activity against chloroquine-resistant strains [109]. The result showed that there are no serious adverse events and the asexual parasites were cleared by day 7 in both groups [79].

Methylene blue, a drug used to treat methaemoglobinemia, acts by inhibiting *falciparum* glutathione reductase and as a result prevents haem polymerization. It is being developed in combination (phase II) with artesunate–amodiaquine as a strategy to protect against emergence of artemisinin resistance secondary to its *falciparum* schizonticidal effect and reduce transmission owing to gametocytocidal

activity [110]. **Rosiglitazone**, an anti-diabetic drug, is currently in clinical trials (NCT02694874) as an adjunctive therapy for severe malaria. **Imatinib**, a cancer therapy, is now in phase-2 trials (NCT03697668) as a triple combination with DHA-piperaquine [100]. Polysaccharide heparin analogue **Sevuparin (DF02),** which is taken as an adjunctive therapy, retains the anti-adhesive effects of heparin without the antithrombin properties and has been shown to block merozoite invasion, cytoadherence and rosetting [111]. Sevuparin, a drug treating sickle cell disease, was completed its phase-1/2 trials (NCT01442168) in 2014 as a combination with atovaquone-proguanil [100]. **MMV390048** is an aminopyridine currently in phase-2a trials (NCT02880241) and its target was identified to be lipid *P. falciparum* phosphatidylinositol 4-kinase (*PfPI4K*). This blood schizonticidal drug has destructive activity on multiple stage of the *Plasmodium* with possible efforts for chemoprevention as it inhibits gametocytogenesis and oocyst formation [102, 112]. **Albitiazolium (SAR97276) or bisthiazolium salt**, discovered and developed by Sanofi in 2005, has also reached phase-2 clinical tests (NCT01445938), however further study was terminated in 2012 [100]. It acts mainly by deterring the transport of choline into the parasite [113]. Descoverd in 2012 by a team at the Cape Town University, South Africa, **MMV048** has shown 99.3% reduction in parasitaemia in the *P. berghei* mouse model at a single dose of 30 mg/kg with no signs of parasites after 30 days. This highlights the potential of this compound to act as a single dose therapy. Its target is *PfPI4K*, eukaryotic enzyme that phosphorylates lipids to allow them to regulate intracellular signaling and trafficking. Inhibiting the ATP-binding pocket of *PI4K* (recently revealed as a novel mechanism of action for antimalarial agents) causes disruption in the intracellular distribution of PI4-phosphate (*PI4P*), which inturn results in decreased late-stage development of the parasite. MMV048 is now in phase-2 clinical studies [100].

Quinoline-4-carboxamide **DDD107498 (**previously known as **M5717**) is additional treatment panorama that was developed in 2015 by the Drug Discovery Unit (DDU) in Dundee. It is an inhibitor of *P. falciparum* translational elongation factor 2 (*PfeEF2*) with activity against pre-erythrocytic and blood stages as well as mature male and female gametocytes. Henece, it can act as curative and transmission blocking drug. *PfeEF2* is responsible for catalyzing the translocation of mRNA and tRNA. The overall efficacy of drugs that target this elongation factor may be increased due to the expression of *PfeEF2* in multiple stages of the *Plasmodium* life cycle [77, 114]. DDD107498 has shown excellent activity against a number of drug-resistant strains of *Plasmodium* species, and exhibited superior potency than artesunate against *falciparum* and *vivax* in *ex vivo* assays. It has been also demonstrated magnificent pharmacokinetic profiles including better oral bioavailability and long plasma half-life (critical for chemoprevention and single dose therapy) in pre-clinical species. Owing to its *PfeEF2* inhibition and its ability to clear blood stage parasites completely, DDD107498 satisfies the requirements to be a long duration partner and could be used as part of a combination therapy with a fast-acting compounds. In late 2017, DDD107498 was cleared for progression from development to phase-1 clinical tests for volunteers in Australia (NCT03261401) [79, 100].

A dihydroisoquinolone compound **(+)-SJ733**, which inhibits gametocytogenesis and blood schizonts in *falciparum* and *vivax*, is now in human trial. The pre-clinical trials showed that SJ733 (inhibitor of *PfATP4*) worked against *Plasmodium* species that are resistant to current frontline agents. It binds to a malaria parasite protein that serves as a sodium pump to interfere with the protein or to disrupt the malaria parasite's capability to remove excess Na+ from RBCs [115, 116]. When sodium builds up, infected cells become develop rigidity (less flexible) and as a result distroyed by our immune system or get caught in small blood vessels. Currently, around 38 healthy volunteers were recruited as part of the phase-Ia trial in Memphis

and phase-Ib test in Brisbane, Australia. In Memphis, about 23 healthy volunteers received increasing doses of the new compound to understand dosing, absorption, safety profile and metabolism. Based on those results, the 15 Australian volunteers received SJ733 after being infected with malaria to understand the antimalarial effectiveness of this novel molecule. No significant SJ733 treatment related side effects were notified in any of the volunteers [117].

Additionally, **CDRI97/78** (fast-acting trioxane first synthesized in 2001 by a team at the Council of Scientific and Industrial Research in India), **ACT-451840** (phenylalanine-based compound developed in 2016 through collaboration between Actelion Pharmaceuticals and the STPHI, **P218** (2,4-diaminopyridine analog and *PfDHFR* inhibitor discovered by BIOTEC Thailand in 2012) and **GSK369796** (N-tert-butyl isoquine developed at the Liverpool School of Tropical Medicine in 2009) are also among componds under/completed phase-1 trials [95, 102]. CDRI97/78 (blood schizonticidal molecule) was well-tolerated in healthy adult volunteers with a half-life of around 12 h. It has shown few and not severe adverse effects. ACT-451840 has the potential to be a fast-acting drug with a long half-life. This agent has shown efficacy against multiple life cycle (asexual and sexual) stages of both *falciparum* and *vivax* malaria, and also harbor additional gametocytocidal activity and, thereby, transmission-blocking properties. The new two step mechanism of action for binding to *PfDHFR* allows P218 to conquer resistance that has emerged after clinical use of pyrimethamine. P218 showed high selectivity to bind malarial than human DHFR, which translates into reduced toxicity. P218 is highly efficacious against *falciparum* and *chabaudi* in mice with ED90 of 1 mg/kg and 0.75 mg/kg, respectively. Along with its high potency and good safety profile, P218 has the potential to be a replacement for pyrimethamine combination with cycloguanil in areas where *PfDHFR* resistance has emerged. P218 has currently completed phase-I trials (NCT02885506). GSK369796 was designed as an alternative to amodiaquine. It completed pre-clinical experments, and was last in phase-I trials in 2008 (NCT00675064) [100].

DM1157, part of a class of compounds known as "reversed chloroquines", was designed to overcome chloroquine-resistant (the parasites expel the drug before it can affect them) strains of *falciparum* malria. Like chloroquine, DM1157 (discovered in 2010 by a research team in Portland State University and further developed by DesignMedix) interferes with the parasite's metabolism, but it also inhibits the parasite's ability to expel the drug. It is currently in Phase I trials (NCT03490162) to evaluate its safety and pharmacokinetics in humans, which is sponsored by the National Institute of Allergy and Infectious Diseases (NIAID). Results of earlier tests in animals suggest that DM1157 could have the same safety and efficacy as chloroquine [100, 118]. Human trials of innovative antimalarial compounds are in the pipeline following Kenyan scientists fruitfully used a derivative from bacteria to kill *Plasmodium* that causes malaria. According to the Kenya Medical Research Institute and its global health partners, the breakthrough could potentially lead to the discovery of new approach for tackling malaria. The promise of a new treatment comes after trials in Burkina Faso found that ivermectin, a conventional drug used for non-malaria parasitic diseases, reduced the transmission rate of malaria. The drug is acted by making the blood of repeatedly treated people lethal to mosquitoes. The experment also revealed that ivermectin can kill *P. falciparum* in mosquitoes that fed on humans who took the drug. As they are more vulnerable, the study is more focused on pregnant women and children and the researchers are getting very encouraging lead compunds. In the near future, latest antimalarial drugs could be in the market if the recent research findings are going ahead. The same bacteria known to kill dangerous pathogens including scabies and river blindness can also be applied in malaria [119].

After identification of a lead compound, optimization of the chemical structure can be started. This step mainly involves examination of the structural activity relationships (SARs) of the compound and optimization of properties such as potency (*in vitro* and *in vivo*), solubility and metabolic stability. The new candidate must also be evaluated for any possible toxicity including cytotoxicity and genotoxicity in pre-clinical trials. **NPC1161B** (the chiral 8-aminoquinoline derivative), developed at the University of Mississippi, was in late preclinical studies for relapse prevention. This compound has a multi-stage activity and there is a development plan to see whether this single enantiomer drug has a more favorable hematological toxicity profile than tafenoquine in Phase-I. **AN13762** (blood schizonticidal), a novel class of benzoxaborole anti-malarial compounds, is emerged in 2017 as the lead compound, showing excellent activity in *in vitro* and *in vivo* (pre-clinical) studies. It has multi-strain efficacy and the ability to act rapidly. It has been shown to be equally potent across a wide range of drug resistant strains. AN13762 has exhibit similar *in vivo* clearance rate when compared to artesunate. The precise mechanism of action for AN13762 remains unknown, though initial studies on hit compound (AN3661) identified the P. *falciparum* cleavage and polyadenylation specificity factor 3 (*PfCPSF3*) as a potential target [100, 103, 120].

Triaminopyrimidine **MMV253** (identified by AstraZeneca in 2015) and an aminomethylphenol **JPC-3210** (active against multidrug resistant falciparum *in vitro*) are long-acting blood schizonticidal agents present in early preclinical experiments [121, 122]. MMV253 (previously AZ13721412) has shown good *in vitro* potency and *in vivo* efficacy. When screened against several mutant resistant strains with different mechanisms of resistance, MMV253 displayed no spontaneous decline in potency which can be attributed to its new mode of action (inhibition of *PfATP4*). Good *in vitro* and *in vivo* correlation was shown with a forecasted human half-life of ~36 h, which is long compared to another fast killing agent (artemisinin, human half-life of 1 h). As of late 2016, Cadila Healthcare pharmaceutical company owns the license for the compound series and is now making further lead development in order to progress the chemical through pre-clinical trials. At the same time that the *Plasmodium* is regulating its Na^+ concentration using *PfATP4*, it also brings in H^+ via the same pathway. To control this increasing concentration of H^+ and maintain an intracellular pH of about 7.3, the *Plasmodium* uses a complementary V-type ATPase transporter to pump out H^+ ion. It was shown that MMV253 has the ability to inhibit the V-type H^+ ATPase as its mechanism of action. **UCT943** (identified in 2016 by a team at the Cape Town University, South Africa in the same campaign as MMV048) is a key compound in a novel class of 2-aminopyrazine antimalarials that has shown single dose curing capability *in vivo* and potential as a clinical candidate. UCT943 (target *PfPI4K*) is potent across multiple life stages of both *falciparum* and *vivax* malaria. UCT943 was in originally in place as a back-up to MMV048, however, due to pre-clinical toxicity, this candidate has been withdrawn [100]. A Mannich base compound, **MK-4815** (2-aminomethyl-3, 5-di-tert-butylphenol), showed potent *in vitro* activity against *falciparum* and hundred percent survival was seen in mice orally treated with 25/12.5/6.25 mg/kg once on the day of infection and then twice daily for an additional 4 days. While comparable volume of distribution at steady state was seen in mice and rhesus monkey, the compound exhibited lower clearance and long plasma half-life in monkeys, indicating the drug possess better pharmacokinetic parameters in the higher species. Although the mechanism of action is still remains unclear, evidences indicate the involvement of the mitochondrial electron transport chain of the *Plasmodium*. Owing to its structural simplicity, effectiveness against MDR *falciparum* strains, good pharmacokinetic profiles and capability to cure acute *P. berghei* infection at a single dose of 50 mg/kg, MK-4815 has a potential

as an antiplasmodial agent and of course, is now under additional assessment by MMV as a pre-clinical candidate [79].

In an attempt to identify antiplasmodial agents with new mechanism of action, Kato and his colleagues found a lead compound coded as **BRD7929**. It was shown to target the cytosolic *falciparum* phenylalanyl-tRNA synthetase. This enzyme serves to enable transfer-RNAs deliver the amino acid phenylalanine to nascent proteins during RNA translation and protein synthesis. This bicyclic azetidine showed *in vivo* against *falciparum* and *berghei* infected mice at a single low doses. This molecule was also very potent against the hepatocytic and asexual stages of *falciparum* and exihibited transmission-blocking effect at concentrations that achieved single dose cures of asexual erythrocytic stage infections. Even if BRD7929 showed good (80%) oral bioavailability, improved aqueous solubility and longer half-life in mice (32 h), moderate cytotoxicity was seen thus presenting possible setbacks, which would have to be addressed during further optimization. Nonetheless, the capability of this lead molecule to eliminate blood stage (asexual and sexual) and liver stage parasites suggests that this compound has the potential to cure the disease, provide prophylaxis and block transmission. Currently, a tetraoxane-based antiplasmodial drug candidate, **E209** that can overcome *PfK13* Cys-580-Tyr dependent artemisinin resistance was identified. Further evaluation revealed retention of *in vitro* potency against sensitive and MDR *falciparum* isolates, with no observable cross-resistance with artemisinin. Compound E209 also exhibited equipotent *ex vivo* activity against *vivax* and *falciparum* Indonesian clinical isolates while screening for gametocytocidality showed a transmission reducing profile consistent with the endoperoxides. Equally important *in vivo* studies in *P. berghei* infected mice showed complete parasite clearance with an estimated oral ED_{50} of 4 mg per kg after 3 doses and a 66 percent cure rate following a 30 mg/kg single oral dose. Therefore, this chemical has the potential to use in a superior combination therapies with a partner drug devoid of *in vivo* resistance liabilities hence offers a substantial improvement on the current ACTs and provides an urgently needed alternative agent for malaria treatment and elimination. Moreover, its efficacy against *vivax* and gametocytes indicates the potential of E209 to prevent relapse and block transmission, respectively [79].

SC83288, an amicarbalide derivative developed in 2017 by a team at Heidelberg University, is the only agents in pre-clinical study that are going to treat sever malaria [123]. This new mlecule was shown to be fast-acting and cured *falciparum* infection in a humanized mouse model, with pre-clinical pharmacokinetic and toxicological studies revealing no apparent shortcomings. While the precise mode of action is uknown, *PfATP6* was identified as a putative determinant of resistance to SC83288. However, it has been shown that SC83288 does not directly inhibit this target suggesting *PfATP6* may have a less direct role in its mechanism of action. SC83288 has been evaluated against artemisinins, showing no cross resistance. *Pfmdr*2 has been identified as another possible mechanism of resistance, facilitating the clearance of the drug from the parasite. Its distinct chemotype, ability to rapidly kill parasites, potentially new mechanism of activity and good safety indices than artesunate and quinine support the clinical development of SC83288 as an IV application for the treatment of severe malaria when combined with a slow-acting partner drug. Presently, Heidelberg University Hospital and the German Centre for Infection Research are collaboratively in the process of conducting the regulatory preclinical procedures with the hope of initiating clinical trials in due course [79, 100]. More recently, Miguel-Blanco and his co-workers identified a compound coded as **DDD01034957**. This new antiplasmodial molecule is fast-acting and potent against resistant strains *in vitro*, *in vivo*, and possesses a resistance mechanism linked to the membrane transporter *P. falciparum* ATP-binding cassette-I3

(*PfABCI3*). These finding support further medicinal chemistry lead-optimization of DDD01034957 as a new antimalarial chemical class and provide latest insights to further reduce *in vivo* metabolic clearance [124].

A 4(1*H*)-quinolone derivative **ELQ-300**, structurally engineered from pyridone analogue by Oregon Health and Science University, was potently inhibited blood stages of *falciparum* and *vivax* malaria in clinical field isolates as well as liver stages and transmissible stages of the parasite. ELQ-300 is proved to be highly selective against plasmodial cytochrome bc_1 complexes like atovaquone, suggesting minimized possibility of causing side effects by inhibiting the host enzyme. Similar to atovaquone, it is a slow acting molecule with a delayed parasite reduction ratio, and exhibited strong synergy with proguanil. Mutant selection studies failed to achieve variants, signifying a significantly low susceptibility for resistance. ELQ-300 was extremely potent in *berghei* infected mice with an ED_{50} of 0.016 mg/kg/day and cures the infection by doses as low as 0.1 mg/kg/day, thus owing the capacity to be a combination partner aimed of single dose cure. Further safety assessment indicated that there are no remarkable off target pharmacological activities by this compound. The main obstacle in the clinical development of ELQ-300 is its relatively poor water solubility, which limits the absorption to the extent that only low blood concentrations can be achieved with oral doses. Even though these low blood levels are adequate for treatment, the concentrations remain too low to establish an acceptable safety margin necessary for clinical development. The way forward intended to design bioreversible alkoxycarbonate ester pro-drugs has currently been effectively explored to overcome the physicochemical problems of ELQ-300 and attain bloodstream levels adequate for safety and toxicological studies, as well as getting single dose cures [79]. It is also possible to list **Genz-668764**, **ML238**, **ACT-213615, SAR121** and **TDR84420** within the new chemical entity group [77, 103].

Besides, a **pyrazoleamide 21A092**, which targets sodium channel (ATPase4) like KAE609 and SJ733, is in preclinical discovery phase [125]. **Dantrolene** was identified as a novel inhibitor of plasmodial surface anion channel (PSAC) and it may be a lead compound for antimalarial drug development [126]. **Acridinones** such as **WR249685** and **T3.5**, new class of selective malaria parasite mitochondrial *bc*1 inhibitors, had a great potential to become novel antimalarial drugs [127, 128]. Some antibiotics that have shown potential effects on malaria parasite have been recently studied *in vitro* or *in vivo* intensively. **Macrolide antibiotics** were identified for the first time that they inhibit *in vitro* RBC invasion by merozoite of Plasmodium species. This result directs the development of safe and effective macrolide antibiotics with dual modalities to combat malaria and reduce the parasite's options for resistance. Other antibiotics, such as **quinolones**, **tigecycline**, **co-trimoxazole** or **fusidic acid**, could be used to prevent malaria in the future. Antiadhesion adjunctive therapies, including **levamisole**, are under research in the laboratory [129, 130]. Both *in vitro* and *in vivo* experments showed that an antibacterial and anticancer drug **acriflavine** impairs DNA replication foci formation in *P. berghei* malaria and affects the enzymatic activities of apicoplast specific Gyrase protein. This attention-grabbing work tells us the potential of this old compound to become future antimalarial agent [131]. In another pre-clinical studies, the receptor protein *PfATP6* has been recognized as the common target of curcumin and artemisinin. This reseach was initiated to evaluate the anti-malarial activity of 6 **derivatives of curcumin** based on their binding affinities and correlating the *in silico* docking outcome with the *in vitro* anti-malarial screening results. The *in vitro* results superimpose the results obtained from the *in silico* study thereby encouraging development of promising curcumin leads in the battle against malaria [132]. One approach to discover new biologically active compounds is to combine a steroid skeleton with structural elements endowed with appropriate biological activities.

Recently, Krieg and his co-workers reported on low molecular weight **arylmethylamino steroids** with varying constitutions of the basic gonane core and exhibiting excellent antimalarial activity [79]. Moreover, researchers' team has recently discovered thioredoxin enzymes, which are different from the human enzyme but critical for the survival of malaria parasite by balancing the redox state inside the *Plasmodium*. So that, a team is doing experments in collaboration to industry partners to develope novel drugs, which will successfully target this enzyme and kill the parasite without affecting the human host [133]. Although many drugs are in the pipeline, most of them are not able to kill both gametocytes and hypnozoites.

6. Conclusion

Malaria is one of the ancient human diseases and remains an important cause of illness and death among adults as well as children in the world. However, an increasing resistance toward currently available antimalarial drugs is a big obstacle in the fight against malaria. The past instances indicate that resistance to the conventional antimalarial medicines will spread to Africa including Ethiopia. As a result, we are in an urgent need of novel, safe, and effective drugs. Some of the newer compounds possess multi-stage activity and are highly potent in inhibiting the parasite multiplication. Those novel agents that have different structure and new mechanism of action than older drugs could be the game changer in combating malaria. The current breakthroughs will still require long-term financial investments, political will, and scientific endeavor to ensure sustainability and translate to more reduction in global burden of malaria.

List of abbrivations and acronyms

ACT	Artemisinin-based combination therapy
CDC	Center for Disease Prevention and Control
DHA	Dihydroartemisinin
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthase
DHODH	Dihydroorotate dehydrogenase
FMoH	Federal Ministry of Health
MDR	Multi-Drug Resistant
MMV	Medicines for Malaria Venture
NIAID	National Institute of Allergy and Infectious Diseases
RBC	Red Blood Cell
SP	Sulfadoxine-pyrimethamine
UNICEF	United Nations International Children's Emergency Fund
USA	United States of America
WHO	World Health Organization

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