Chapter

Recent Advances in Antimalarial Drug Discovery: Challenges and Opportunities

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Abstract

Malaria is a global health problem that needs attention from drug discovery scientists to investigate novel compounds with high drug efficacy, safety and low cost to encounter the malaria parasites that are resistant to existing drug molecules. Antimalarial drug development follows several approaches, ranging from modifications of existing agents to the design of novel agents that act against novel targets. Most of market and clinical drugs act on blood schizonticide are in current therapy for malaria reduction. This chapter will intend to highlight the currently available drugs including various novel agents. In addition, emphasis has been given on the prospective pharmacophores that are likely to emerge as effective clinical candidates in the treatment of malaria. Besides all aspects, some alternative approaches will also be highlight.

Keywords: Antimalarial, drug resistance, current drug, Plasmodium, chemotherapeutic target

1. Introduction

Malaria is a prevalent infectious disease, affecting about 150 million people globally and responsible for around 4, 45,000 deaths annually [1]. Geographically, it is prevalent in 106 countries of the tropical and semitropical world. Africa accounts for 80% of total malaria cases and 90% global death. Malaria is caused by the apicomplexan protozoa *Plasmodium* genus which is transmitted from one to another through bitting by female *Anopheles mosquito* [2]. Five species are known to cause malaria fever in human *i.e. P. vivax*, *P. falciparum*, *P.ovale, P. malariae* and *P. knowelsi* [3, 4].

Among all plasmodium species, *P. vivax* is prevalent in central and South America, Asia [5]. *P. ovale* infections are rare and occur only in Africa i.e. <0.5% [6] *P. malariae* is present at the globe irregularly. *P. falciparum* is most fatal, because it produces large progeny in a short time and has the ability to cause cerebral malaria which is a severe complication and leads to death of the patient. Malaria has been a long-term health issue in world. In earlier 1960s to 1980s incidence of malaria prevalence have been highest, but now, there are several effort and projects handle by the international government to reverse malaria burden. Chemotherapy against the malaria parasite had been a vital component. However, resistance to existing

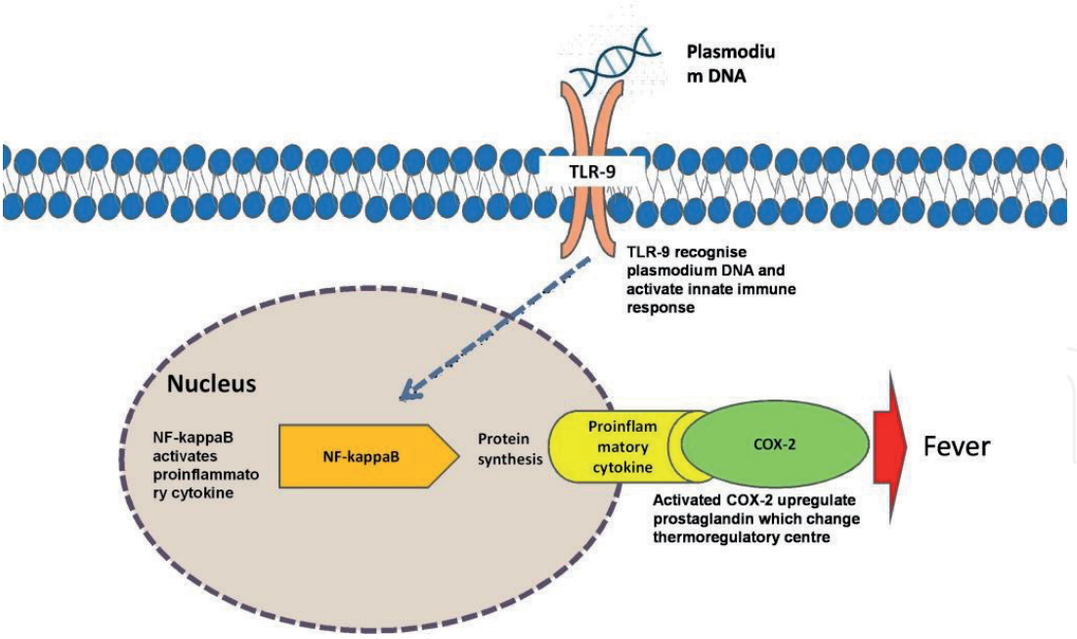

Figure 1.
Schematic representation of malaria pathogenesis in the host cell.

medicines is always a risk. Consequently, all treatments must be combinations of two or more active ingredients such that no compound is exposed as a monotherapy to high levels of parasites for a significant period of time wherever possible [7]. Artemisinin-based and nonartemisinin based combination with partner drug has been approved. This chapter will provides an overview of malaria, focusing on progress in drug discovery efforts, clinical development and the future highlight of malaria eradication agenda.

1.1 Malaria pathogenesis

Symptoms of malaria arose from hemozoin that released after rupture of infected RBCs. Hemozoin cause inflammation and take part in the immunogenic action in the blood which activates pro-inflammatory and anti-inflammatory cytokines [8]. Some studies have shown that IL-1B, IL-6, IL-8, and TNF-alpha increased in late-onset severe disease [9, 10]. Pathogenic phase of malaria is outcome of secreted cytokines tumor necrosis factor (TNF-α), interferon-γ (INF-γ), IL-6, IL-8, from macrophage and endothelial, and elevated level of as superoxide and nitric oxide (NO) [11]. These increased factor play role in dys-erythropoietic anemia, TNF-α may contribute to cerebral malaria through up-regulation of intracellular adhesion molecule-1 (ICAM-1) in cerebral blood vessel endothelium [12]. Pro-inflammatory cytokine induce cytokinemia and fever through interacting intracellularly with Toll-like receptor-9 (**Figure 1**), which lead to release of proinflammatory cytokines that can induce COX-2 up regulating prostaglandins [13, 14].

2. Diagnosis

Diagnosis of malaria infection in a patient is of critical importance since symptoms of complicated malaria may develop suddenly, causing the death of the patient. Clinical diagnosis based on the patient's symptoms and on physical findings at examination

- Microscopic diagnosis parasite can be identified by examining under the microscope a drop of the patient's blood, spread out as a "blood smear" on a microscope slide stained with Giemsa stain [15].

- A rapid diagnostic test based on antigen detection, this type of immunologic test most often uses an antigen-coated dipstick which gives results in 2–15 minutes. These "Rapid Diagnostic Tests" (RDTs) offer less time taking and more accurate results over microscopy [16, 17].

- Diagnosis of nuclei of the parasite through PCR (polymerase chain reaction) which is a quicker method [18].

- In serum-based diagnosis, malaria parasite responding antibodies generated in the human body can be detected indirectly by immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA). Serology can detect past exposure but unable to detect current infection [19].

3. Life cycle

Asexual Phase (Human) stage completed in humans after the invading of sporozoite to the liver which further infected other RBCs after parasitemia establishment. **Sexual Phase (Mosquito)** completed in the gut of mosquito [20]. When parasite bite infected humans, gametocytes occur in female and male form reach in their salivary gland and enter into the gut of the mosquito. The male and female gametes are fused in the gut of the mosquito to form zygotes [21]. After fertilization of gametes, ookinetes form which penetrates the gut epithelial cells and finally converts into an oocyst. The oocyst multiplies by asexual replication and produces sporozoites. Rupture of the mature oocyst releases the sporozoites into the hemocoel (body cavity) of the mosquito, from where they travel to the mosquito salivary glands [22]. When an infected mosquito has a blood meal, it injects saliva containing the parasite (sporozoites) into the human bloodstream, causing malaria infection in the new human host. Now the sporozoites travel through the circulatory system to the liver and invade hepatocytes, where the sporozoites multiply and grow through asexual replication known as exoerythrocytic schizogony. Each sporozoite develops into a schizont containing thousands of merozoites, which are released into the bloodstream. In the case of *P. vivax* and *P. ovale,* they live in a latent form in the liver-cell which can be dormant up to months or years. These hypnozoites relapse when a new primary infection reaches to liver cells [23]. The erythrocytic life cycle begins when free merozoites invade through erythrocytes. The erythrocytic cycle is responsible for all clinical manifestations of the disease. Merozoites invade erythrocytes by multiple receptor-ligands interactions within a few seconds. The early trophozoite is often called 'ring form' because of its morphology. Ring stage is developed into the trophozoite stage by metabolizing host cytoplasm and degradation of hemoglobin into globin and amino acids. The trophozoite is developed to schizont by multiple rounds of nuclear division without cytokinesis and produces 8–32 daughter merozoites [24, 25]. Mature merozoites came outside the red blood cell and released merozoites invade new RBCs and this cycle repeats. This blood stage cycle is responsible for the pathology related to malaria. Asexual stage converted into sexual stages i.e. male, female gametocyte [26, 27]. Which helps in the transmission of the infection to others through the female Anopheles mosquitoes, wherein they continue the sexual phase of the parasite's life cycle.

4. Treatment of malaria

This classification of candidate drugs is strictly based on the stage in which they exhibit their potency within the parasite life cycle. In this classification, drugs are divided into five major categories (**Table 1**) [28].

- **Blood schizontocides**: These drugs act on the blood stages of parasites and therefore prevent spreading out of malaria. The common members of this class include artemisinin (ART) and its derivatives, chloroquine (CQ), quinine (QN), mefloquine (MQ), halofantrine (HF), pyrimethamine and sulfadoxine.

- **Tissue schizontocides for causal prophylaxis**: These drugs act on the hypnozoites (liver forms of Plasmodium) occurring prior to the erythrocytic stage. These drugs are very important since they could prevent the onset and development of clinical infection at the early stage of the disease, e.g. Primaquine (PQ) and pyrimethamine.

- **Tissue schizonticides for relapse prevention**: These drugs act on the sporozoites of *P.vivax* and *P.ovale* in host liver cells, which is responsible for the relapse of malaria symptoms and regeneration of the disease e.g. primaquine.

- **Gametocytocidal**: These drugs target the gametocyte of the parasite in the blood and also prevent the transmission parasitic stages to the mosquito. CQ and QN have gametocytocidal activity against *P. vivax* and *P. malariae* but not against *P. falciparum*, but ART has activity against *P. falciparum*. Primaquine found to suppress *P. vivax* as well as *P. falciparum*.

5. Drug resistance in *P. falciparum*

Drug resistance is the reduction in the effectiveness of a medication such as an antimicrobial or an antineoplastic in curing a disease or condition [29]. The term is used in the context of pathogen that acquired survival potential in presence of drug. When an organism is resistant to more than one drug, it is said to be multidrug resistant.

P. falciparum resistance to chloroquine and sulphadoxine–pyrimethamine first developed on the in Southeast Asia and South America in the late 1950s and 1960s, respectively. The spread of resistant parasite strains elsewhere, including Africa, have been well documented retrospectively with molecular markers of the resistance to each drug. Chloroquine has been replaced by the combination of sulphadoxine and pyrimethamine (SP) in 1973. SP is widely used antimalarial worldwide and also used as the first line of treatment for malaria alone or in combination with other antimalarial drugs. SP resistance also became a big challenge to malaria control programs. For tackling this problem, SP was replaced by mefloquine but resistance to this new drug developed very rapidly. Mefloquine resistance was first observed in the late 1980s again in the same endemic area near the Thai-Cambodian border which spread out to Southeast Asia, South America and some pockets in Africa [30]. Resistance to SP was first described from the Thai-Cambodian border [31]. After chloroquine and sulphadoxine–pyrimethamine failures, Thailand introduced mefloquine in place of SP. For tackling this problem, Thailand imposed strict controls on its use but mefloquine resistance was first observed in the late 1980s and early 1990s again in the same endemic area near the Thai-Cambodian border. The increasing morbidity

Drug class	Drug	Target of action	Mode of Action	Clinical use
4-Aminoquanoline	Chloroquine	Blood-stage schizonticides	Direct heme binding, Inhibit heme Fe(II) FPIX Polymerase	Treatment and chemoprophylaxis of sensitive parasites
Cinchona alkaloid	Quinine	Erythrocyte schizonticides	Same as CQ	Treatment of CQ-resistant *P. falciparum*
Quinoline-methanol	Mefloquine	Blood-stage schizonticides	Formation of a toxic substance, Swelling of the food vacuole	Chemoprophylaxis and treatment of *P. falciparum*
8-Aminoquanoline	Primaquine	Tissue-stage schizonticides & gametocytocidales	Generation of toxic metabolites, Oxygen radicals in Plasmodial mitochondria	Radical cure and terminal prophylaxis of *P. vivax & P. ovale*
Amino alcohol	Halofantrine/ Pyronaridine	Erythrocyticschizonticides	Inhibit heme polymerase, inhibit vacuolar degradation	Treatment of CQ-resistant *P. falciparum*
Naphthoquinone	Atovaquone	Blood-stage schizonticides	Inhibit mitochondrial electron transport	Treatment and chemoprophyla xis of *P. falciparum*, in combination with Proguanil
Diaminopyrimidine/ Sulfonamide	Pyrimetham ine/ Sulfadoxine	Blood-stage schizonticides	Inhibitor of dhfr-ts/dhps, thereby, inhibit parasitic DNA	A headache. SJS, Skin rash Treatment of CQ-resistant *P. falciparum* (in combination as SP)
Biguanide	Proguanil	Erythrocyticschizonticides	Inhibit dhfr and stops pyrimidine biosynthesis	Chemoprophyla xis (with CQ)
Tetracyclines	Tetracycline/ Doxycyclin e	Blood-stage schizonticides	Inhibit mitochondrial protein synthesis, block nucleic acid synthesis	Treatment and chemoprophyla xis of *P. falciparum*
Sesquiterpene lactone	Artemisinin and its derivatives	Erythrocyticschizonticides & gametocytocidales	Formation of iron catalyzed free radical, alkylation of heme, membrane damage by free radical	Treatment of multidrug-resistant *P. falciparum*

Table 1.
Common antimalarial drugs and their mechanism of action.

rate was reversed with the introduction of artemisinin. The introduction of the artemisinin saved millions of lives around the world [32]. Artemisinin leads to a high rate of recrudescence (reinfection of parasites) other drugs are required to clear the body of all parasites and prevent recurrence hence several more potent derivatives were synthesized *viz*., artesunate, arte-ether, arte-mether and dihydroartemisinin [33]. In 1995, Thailand replaced mefloquine with artesunate–mefloquine. The same combination was the first-line therapy in Cambodia from 2000 to 2012.

5.1 Resistance to artemisinin-based combination therapies

To linger off artemisinin resistance, treatment for malaria is given as artemisinin based combination therapy (ACTs) in place of artemisinin alone or its derivative to treat uncomplicated malaria. ACT must include 1 artemisinin or its derivative another is other antimalarial drug or compound as prescribed by WHO 2001. ACTs are more efficient medicine today that is available, as it has great potential; it replaced antifolates and quinoline drug class which was used as the first-line treatment for *P. falciparum*.

Presently, artemisinin resistance is only prevalent in to the Cambodia, Thailand, Lao people's Democratic Republic [34], Viet Nam, Myanmar, and the Myanmar-China-India border area. In 2006, the declined efficacy of ASMQ (artesunate/mefloquine) was suspected for the first time on the Cambodia-Thailand border [35]. Thereafter, ASMQ clinical failures were reported on the Thailand-Myanmar border in correlation with delayed parasite clearance time [36]. Reason for resistance toward artemisinin derivatives because it promotes selection for partner-drug resistance mainly due to mismatches in the pharmacokinetics of the two drugs, causing frequent treatment failure of ACTs [37], amplification of pfmdr1 gene copy numbers. Clinical failures after dihydroartemisinin-piperaquine (DHA/PPQ) treatment have been reported, first in Cambodia in 2013 [38] and later in Vietnam in 2017 [39, 40] five and twelve years, respectively, after DHA/PPQ treatment introduction. DHA/PPQ resistance was confirmed by several reports and correlated with pfk13polymorphism, plasmepsin 2–3 gene amplification and single copies of the pfmdr1 gene [41]. Clinical failure rates greater than 10% have now been reported for the 5 ACTs in Cambodia, for 2 ACTs in Thailand and Lao PDR and for 1 ACT in Viet Nam, Myanmar, and in the Chinese and Indian border regions with Myanmar. It has been demonstrated that plasmepsin 2–3 gene amplification in DHA/PPQ resistant parasites is associated with pfmdr1 gene single copies, so these resistant parasites are sensitive to mefloquine [42]. In contrast, ASMQ-resistant parasites with pfmdr1 gene amplification are sensitive to piperaquine [37]. Based on the amplification of pfmdr1 gene copy numbers of ACT-resistant parasites, the alternating use of ASMQ and DHA/PPQ is under consideration.

5.2 Potential chemotherapeutic target

Developing resistance toward antimalarial drug has tinted requirement of new compound with antimalarial activity. To overcome this problem new validated drug target needed with detailed study of biochemical and metabolic processes of the parasite [43]. One way is to search for new drug(s) which inhibit parasite growth and cure malaria, secondly to find ways to reverse drug resistance mechanism. Research over the years have identified a number of potential drug targets mainly proteins in the parasite that can be utilized as drug targets.

6. Drug development research in during 2010–2019

There is continuous efforts has been given after resistance toward existing drug chloroquine, mefloquine, piperaquine, sulphadoxine-pyrimethamine, artemisinin derivatives, in Southeast Asia. Resistance to the partner drug, not artemisinin, is the primary driver for failure of ACT. Hence along with combination therapy of artemisinin second alternative drug is needed. Medicine of malaria venture is a non governmental organization which support collaborations with a library of antimalarial leads drug discovery (www.mmv.org.in). Clinically used antimalarial combination dose described in (**Table 2**).

A study of literature peformed to find out the new leads and their clinical stage along with survey on www.mmv.org, www.mpmp.huji, and ClinicalTrials.gov website (https://www.clinicaltrials.gov/). Major new drugs focus the blood schizonticide stage of uncomplicated *P. falciparum*. These potential inhibitor of plasmodium cycle must be single dose with minimum exposure and minimized toxicity in pregnant women and children with quite affordability to common people of minimum income.

There are at least 13 agents in clinical development (**Table 3**). Krintafel (tafenoquine) developed by Glaxosmith in collaboration with MMV has the potential to clear hypnozoites is approved for a single dose by regulatory authorities as a treatment for *Plasmodium vivax* relapse prevention. This represents an advance over standard 14-day primaquine regimens; however, the risk of acute haemolytic anemia in patients with glucose-6-phosphate dehydrogenase deficiency remains. Cipargamin (KAE609), developed by Novartis in collaboration with MMV. Cipargamin targets a cell membrane channel in the parasite, which is the new molecular target for malaria in more than 20 years. 75 mg for over 8 days require killing parasite in blood, and also having malaria transmission blocking. Intravenous formulation for severe malaria is also planned for 2020. One of the leading pipeline combinations are artefenomel (OZ439)–ferroquine and lumefantrine-KAF156, both in Phase 2b. Artefenomel is nonartemisinin based drug which has been designed by joint sanofi and mmv effort for children and to allow for once-daily. The combination is currently in a phase IIb trial, which is completed in the 2018. A novel trioxane 97/78, contains 1,2,4-trioxane nucleus similar to artemisinin developed by Central Drug Research Institute (CDRI), India, has shown promising antimalarial activity and is currently in clinical trials phase I. This 97/78 target, plasmodial phospholipid metabolism responsible for their pharmacological activity. Firstly 97/63 was synthesized but, due to its poor bioavailability, it was resynthesized as a hemisuccinate derivative and coded as 97/78. Upon administration of 97/78 it gets converted into its active *in vivo* metabolite 97/63. The concentrations of 97/63 and 97/78 can be measured by validated LC–MS/MS method [44].

7. Conclusion

In last ten years of discovery and development of new anti-malarial medicines showed an explosion in new molecules in the malaria pipeline. These current leads are result of a dramatic increase in the number and diversity of new molecules presently in pre-clinical and early clinical development. MMV itself and along with collaboration make this discovery possible. When malaria remains a challenge because of drug failure resist toward current line therapies time to time in parallele a successful drug discovery programmes also been run that provide satisfactory results with no reason to worry. The malaria drug development pipeline, at

Combinations	Dosing schedule and summary	Trade name/associated organization	Year of Launching
Artemether-lumefantrine	• Dosing twice/day for three days • Qualified WHO prequalification in Feb 2009 • Approved from the US-FDA • Several generic version of this have been produced.	(Coartem®/Novartis, MMV)	2008
Artesunate-amodiaquine	• Dosing once/day for three days. • Approved in 31 countries including 25 in Africa • Prequalified in 2008 by WHO.	(Carsucam®; (Sanofi/DNDi/MMV)	2008
Artesunate-Mefloquine	• It given once/day over three days. • Prequalified by WHO in September 2012 • Registered in India.	(Cephalon/DNDi/ Cipla/MMV)	2008
Dihydroartemisinin-piperaquine	• It given once/day for three days. Approved by EMA in 2011. • Prequalified by WHO • Included in the malaria treatment guidelines of WHO in 2011.	(Eurartesim®/Artekin®/sigma-Tau/MMV/Pfizer)	2010
Artesunate-Pyronaridine	• It given once/day for three days • Approved by the KFDA in 2011 and by the EMA in 2012. • Prequalified by WHO.	(Pyramax®/ShingPoong/MMV)	2011

Table 2.
Antimalarial combination along with prescribed dose.

Company	Supporter of fund		project	Clinical Phase
AbbVie	MMV	DSM265, MMV390048, DSM421	PK studies, formulation evaluation, PD and metabolite sample analysis, pathology peer review, technical consulting	I, II, preclinical
Eisai	Fiocruz	E6446	TLR9 antagonist for cerebral Malaria	Preclinical
	St. Jude, MMV, GHIT -	SJ733	Inhibitor of Plasmodium ATP4	Phase I
GlaxoSmithKline	MMV	Tafenoquine	(radical cure of *P. vivax*)	Approved
Novartis	Company	Coartem 80/480	developing a new formulation with 75% reduced pill burden for patients with body weight 35 kg+	Phase IV
	Wellcome, MMV, BPRC, Swiss TPH	Imidazolopiperazines (KAF156):	developing an NCE for patients with artemisinin-resistant strains of malaria	Phase II
		Spiroindolone (KAE609):	developing an NCE for patients with artemisinin-resistant strains of malaria	Phase II
	MMV	Coartem®	Dispersible: developing a new formulation for younger children	Phase IV
Sanofi	MMV	Oz 439/Ferroquine		Phase IIb
		Ferroquine (SSR97193)		Phase II
		MMV533		Phase-I
Takeda	MMV, GHIT	DSM265		Phase II

Company	Supporter of fund	project	Clinical Phase
		DSM421 Preclinical (plans to go into Phase I in 2017)	-pta kro
Zydus Cadila (AstraZeneca)		MMV253	Phase-I
CDRI		CDRI97/78	Phase-II

Table 3.
Antimalarial pipeline drugs.

present, is in a state where we still have leads in final stage to be released at market level. Currently Tafenoquine is permit to use for *P.vivax* malaria. With the current scenario of drug development against malaria we are able to control the situation and in next year we will be on the path of control malaria eradication. In many countries encouraging progress toward malaria elimination achieved e.g. Sri Lanka, China. With new clinically approved agents (arterolane, cipargamin, KAF156) on the horizon show potential to replace failing artemisinin combination therapies as part of novel combinations. Malaria drug discovery studies are in the successive direction where we can stay with malaria free country till of 2030 aim of malaria controlled region.

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