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DRUG RESISTANCE AND RESISTANCE REVERSAL STRATEGIES IN MALARIA PARASITE

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ABSTRACT

The public health care system is currently facing a major problem with malaria. Globally, malarial deaths have decreased by an estimated 40% in the past two decades because of the clinically effective drugs (artemisinin-based combination therapies) against *Plasmodium falciparum*. In recent years, *P falciparum* develop resistance against all antimalarial drugs and then becomes developed multidrug resistance that a major challenge. Even though drug discovery programs have made substantial progress in the past decade, the potential for new drugs/combinations to improve the effectiveness of current malaria control strategies. Beyond, we have compiled a comprehensive review of clinically approved anti-malarial drugs with resistance mechanisms and a novel drug-resistance reversal approach in one place to meet this demand. The review aimed to provide detailed information on drug resistance, its regulatory molecular mechanisms responsible for resistance, and the novel available treatment of malaria. In this review, the article will help in developing effective interventions, potential approaches, and strategies to handle antimalarial resistance. This will prevent life-threatening infections.

Keywords: Antimalarial drug, drug-resistant, reversal strategies

INTRODUCTION

Malaria parasite infections are still one of the leading causes of death in third-world countries. Malaria parasites are protozoa that belong to genus Plasmodium. In 2021, an estimated 229 million new cases, with 409,000 people had died of malaria. The five species of malaria parasites are responsible for human malaria (*Plasmodium falciparum*, *P.vivax*, *P .ovale*, *P. malariae*, and *P. knowlesi)* **(Phillips et al. 2017)**. Among these species, *P. falciparum* and *P. vivax* cause most malaria cases (95%) in the world **(Kotepui et al. 2020)**. In malaria control, antimalarial drugs are the only option for treating malaria and protecting at-risk populations; safe and effective treatments prevent malaria patients from developing severe diseases and death. For over four decades, chloroquine (CQ) was the first-line treatment of malaria, and molecular markers for its resistance were first observed in 2001**(Djimdé et al. 2001)**. Artemisinin-based combination therapies (ACTs) have been introduced after widespread resistance to CQ **(Valecha et al. 2009)**. ACTs have been recommended as a first-line treatment by the World Health Organization (WHO) between 2001 and 2004, ACT has been launched in 20 African countries recommendation by WHO. Six ACTs are currently for treating malaria worldwide **(Rasmussen et al. 2022)**. ACT is currently used worldwide, which has significant control malaria. Despite the World Health Organization's strict rules and surveillance of malaria treatment, the emergence and dissemination of ART-resistant *P. falciparum*, with the first cases in the Greater Mekong Subregion (GMS) and now in East Africa, poses a major challenge to global malaria elimination efforts **(Kamau et al. 2015; Ngwa et al. 2022)**. Furthermore, eleven years later, in February 2018, Southeast Asia experienced artemisinin resistance, notably resistance to dihydroartemisinin-piperaquine combination therapy **(Win et al. 2022)**. The development of resistance to the partner drugs used in ACTs continues to be a problem in malaria treatment. However, parasites with diminished susceptibility to ACTs have decreased susceptibility to ART as well as ART combination drug resistance **(Kamau et al. 2015)**. Since resistant malaria parasite strains have therapeutic options are limited. Taking problems with multi-drug resistance, several approaches are applied for new drugs for malaria treatment **(Kamau et al. 2015)**, including optimization of combination therapy with existing drugs**(van der Pluijm et al. 2020)**, drug resistance-reversal agents **(Watt et al. 1990; Van Schalkwyk et al. 2001)**, combination therapies with new drugs **(Pandey et al. 2023)**, an analog of drugs that were previously clinically used, natural products **(Bhatt et al. 2022)**, drug repurposing **(Van Schalkwyk et al. 2001)**, hybrid drug development **(Dola et al. 2017)**, and new drug targets against resistant parasites. Thus, this review will focus on drug resistance and resistance reversal strategies in malaria parasites.

Clinically approved Antimalarial Drugs

Malaria infection can be treated and prevented by antimalarial drugs. Antimalarial drugs are most effective at treating the erythrocytic stage of malaria infection, which causes symptoms. Based on their chemical structure and activities against parasites, antimalarial drugs are classified according to their effectiveness in combating malaria. Antimalarial drugs can be categorized according to their stages specifically anti-plasmodial activity. They are categorized into gametocidal prophylaxis, blood schizonticides, and tissue schizonticides**(Azad et al. 2017)**.

Table 1 Classification and mechanism of action of the various drugs evaluated in malaria parasites

Class of drugs	Names of drugs	Mode of actions	References
Tissue schizonticides	Primaquine, chloroquine, and tafenoquine	Acts on the liver dormant stage of plasmodium	**(Cabrera and Cui 2015; Ebstie et al. 2016; St Jean et al. 2020)**
Blood schizonticides	Quinoline derivatives include chloroquine, amodiaquine, quinine, quinidine, mefloquine, primaquine, lumefantrine, and halofantrine. Antifolates include sulfadoxine-pyrimethamine and atovaquone-proguanil. Antimicrobials include tetracycline, doxycycline, clindamycin, and ACT (Artemisinin combination therapy)	Acts on blood stages of plasmodium and cures diseases	**(Cabrera and Cui 2015; Parhizgar and Tahghighi 2017; Alven and Aderibigbe 2019)**
Gametocidal prophylaxis	Primaquine, artemisinin and derivatives, chloroquine, quinine, and methylene Blue	Acts as a sexual form of the parasite and finally stop transmissions of parasites	**(Vennerstrom et al. 1999)**

To prevent malaria transmission to mosquitos, tissue schizonticides drugs destroy the parasite's dormant hypnozoites in the hepatocytes, while gametocytocidal drugs kill the parasite's sexual erythrocytic forms in the circulation **(Mancio-Silva et al. 2022; Ruberto et al. 2022; Vantaux et al. 2022)**. Most antimalarial treatments (blood schizonticidal drugs) target the parasite's asexual erythrocytic stages **(Parhizgar and Tahghighi 2017)**. The two types of antimalarials are fast-acting and slow-acting. There are three major types of antimalarial drugs. i) Quinine derivatives. ii) Antifolate drugs, and iii) artemisinin derivatives. The class of antimalarial drugs is highlighted in **Table 1**, and stage-specific targets are in Figure 1. Malaria can only be transmitted by Anopheles mosquitoes to uninfected people. During malaria's blood stage, gametocytocidal destroy the parasite's female and male gametocytes. ART and derivatives and CQ are examples of gametocytocidal. Travelers from nonendemic malaria countries who plan to visit malaria-endemic countries are given prophylactic antimalarial drugs to prevent malaria infections. Travelers with weakened immune systems are more likely to get malaria than healthy ones. In addition to pyrimethamine and proguanil, primaquine is also a prophylactic drug. Few antimalarial drugs have limited pre-erythrocytic (hepatic) activity **(Table 2).** The acute blood stage must be treated to treat malaria caused by all malaria species. A drug that inhibits hypnozoites (which remain dormant in the liver for months or even years after initial infection), is also required for infections due to *P. ovale* or *P. vivax* **(Sylvester et al. 2021; Mancio-Silva et al. 2022; Vantaux et al. 2022)**.

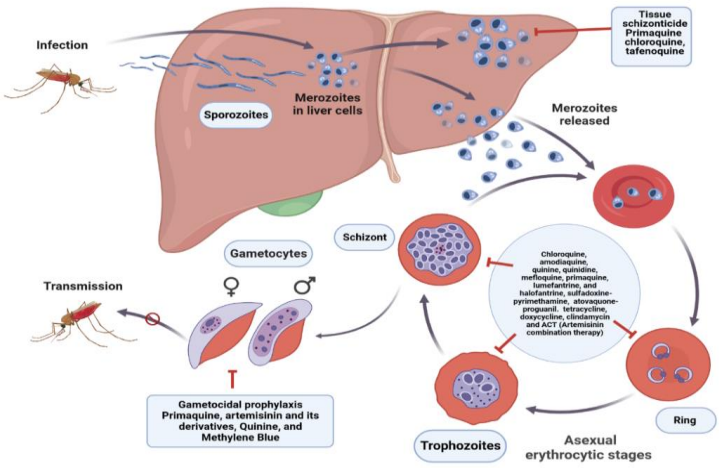
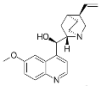
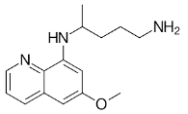
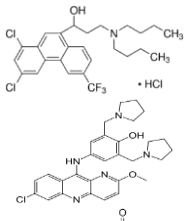
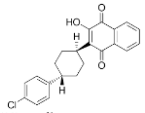
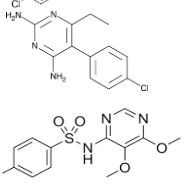
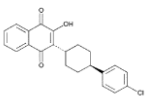
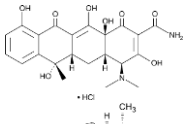
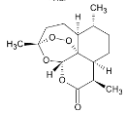


Figure 1 Overview of the stage-specific drug targets for malaria

Table 2 Common clinically used antimalarial drugs with structure and their mechanism of action

Drug	Chemical structure	Inhibition of stage	Mode of action	Reference
Quinine		Schizont	Inhibit heme Fe (II) FPIX Polymerase	(Desjardins et al. 1979; Varela et al. 2014)
Chloroquine		Rings, Trophozoite, Gametocyte	Inhibit heme Fe (II) FPIX Polymerase	(Ginsburg et al. 1998)
Mefloquine		Trophozoite, Schizont	Formation of a toxic substance, Swelling of the food vacuole, Oxidative stress	(Gunjan et al. 2016)
Primaquine		Gametocytocide, Trophozoite	Generation of toxic metabolites, Oxygen radicals in Plasmodial Mitochondria	(Divo et al. 1985; Lalève et al. 2016; Murithi et al. 2020)
Halofantrine/ Pyronaridine		Schizont	Inhibit heam polymerase In vacuole degradation	(Ringwald et al. 1999)
Atovaquone		Erythrocytic stage	Inhibit mitochondrial electron Transport	(Khositnithikul et al. 2008; Delves et al. 2012; Tinto et al. 2014)
Pyrimethamine/ Sulfadoxine		Schizont	Inhibitor of DHPS/DHFR enzyme, thereby inhibiting parasitic DNA	(Sadler et al.)
Proguanil		Schizont	Inhibit DHFR and stops pyrimidine Biosynthesis	(Adebayo et al. 2020)
Tetracycline		Erythrocytic stage	Inhibit mitochondrial protein synthesis, block nucleic acid synthesis	(Khositnithikul et al. 2008)
Artemisinin and its derivatives		Erythrocytic stage	Formation of iron-catalyzed free radical, alkylation of heme, membrane damage by free radical	(van der Pluijm et al. 2020)

During the 20th century, four major classes of drugs were developed for treating malaria. Since drug-resistant Plasmodium strains are on the rise, finding new antimalarials with novel targets is particularly important. The following sections discuss the modes of action of the major antimalarial drug classes used for treatment and curative options.

Inhibitors of heme polymerization

These drugs accumulate in *Plasmodium* species' digestive vacuoles and work by binding to heme, preventing it from polymerizing into hemozoin and enabling toxic heme aggregation (Figure2)**.** As a result, these drugs interfere with parasite hemozoin formation **(Monti et al. 1999; Oliveira et al. 2004; Solomon et al. 2013; Dow and Smith 2017)**. These drugs are Amino alcohols, 4-

aminoquinolines, 8-aminoquinolines, 4-amino benzo-naphthyridine, and 4-aminoacridine.

Amino alcohols

Quinine, mefloquine, halofantrine, and lumefantrine are examples of primary amino alcohols. Quinine is still effective and often treats severe *P. falciparum*, especially when CQ and sulfadoxine-pyrimethamine are resistant. It is less effective and toxic than CQ as a schizonticidal blood agent. *P. falciparum* is resistant to several drugs, although mefloquine is effective against it. It's a highly effective blood schizonticide with a long half-life. In CQ-resistant strains, its combination of artesunate-mefloquine is currently routinely employed. The phenanthrene methanol halofantrine is a blood schizonticide that acts against all Plasmodium species, despite its potency against drug-resistant parasites. Currently, lumefantrine is used commonly with ART derivatives against CQ resistance parasites**(Kamugisha et al. 2012)**.

4-Aminoquinolines

CQ is the most extensively utilized because it is the cheapest and safest antimalarial drug potential against all *Plasmodium* parasites. Schizonts and gametocytes are susceptible to CQ. It's also advised for use in areas where *P. vivax* and CQ - sensitive *P. falciparum* strains are present. Except for mild itching, no adverse effects of CQ have been recorded. It is also thought to be highly safe to use when pregnant. Drug-resistant parasite strains have dramatically reduced their effectiveness. However, because it also has a considerable antimalarial effect, it is still the medicine of choice in most of Sub-Saharan Africa.
Amodiaquine (AQ) is a 4-aminoquinoline used in places where CQ is resistant, and certain patients prefer it since it causes less scratching than CQ. However, it is one of the WHO-recommended drugs combined with artesunate (AS-AQ) (WHO guidelines, 2010). It is also considered to be highly safe to use when pregnant. This class of drugs such as Piperaquine, Ferroquine, Naphthoquinone, AQ-13, and Tert-butyl iso-quine, or GSK 369796, are in various phases of clinical development **(O'Neill et al. 2011; Rajapakse et al. 2015)**.

8-Aminoquinolines

Primaquine (PQ) is the most widely used 8-aminoquinoline antimalarial drug **(Nothdurft and Kain 2017; Schlagenhauf et al. 2018)**. Drugs in this class are unique in having activity against asexual, sexual, and liver stages of the parasite. Because they target the hypnozoites of *P. vivax* and *P. ovale*. 8-aminoquinolines are used to treat liver-stage malaria, it is no longer used in clinical practice. In *P. vivax* and *P. ovale* infections, primaquine is most effective against gametocytes, blood schizonticides, and the dormant stage of parasites. It is well-known medicine for treating both recurrent and acute malaria infections. WHO and India's National Antimalarial Programmed (NAMP) recommend for *P. falciparum*, single dose of primaquine 45 mg. Bulaquine (CDRI 80/53) drug developed by CDRI-India and is metabolized to PQ and varies from PQ only in the eight locations- 2, 4 dihydrofuran group connects to the quinoline nucleus(Pandey et al. 1990; Noel et al. 2008). Bulaquine is currently used in India for the radical cure of *vivax* malaria dosed at 25 mg/day for five days, but not as a gametocytocidal agent in *Plasmodium falciparum*.

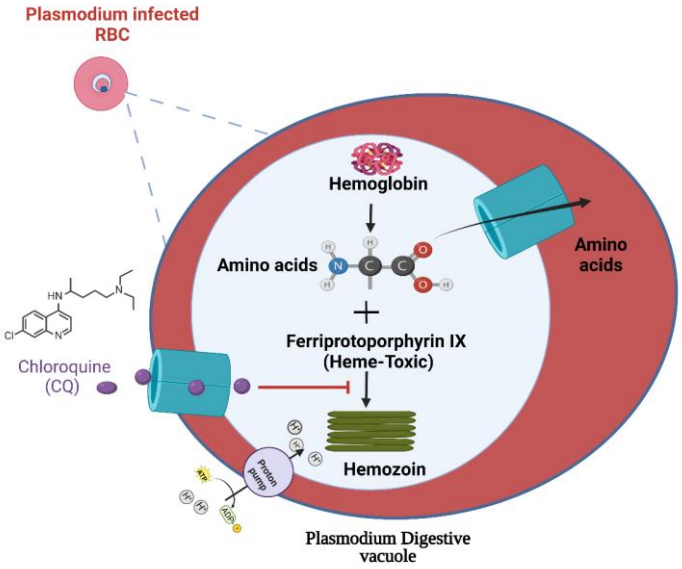


Figure 2 Overview of the digestive vacuoles as drug targets

Inhibitors of folate biosynthesis (antifolates)

Antifolates are used as anti-malarial by inhibiting the folate metabolism of the parasite. The most important antifolate drugs used against malaria are pyrimethamine-proguanil, which transforms to the active form cycloguanil, as well as sulphonamide, sulfadoxine and sulfone, and dapsone. They primarily target dihydrofolate reductase (DHFR) or dihydropteroate synthase (DHPS) enzymes and limiting purine and pyrimidine pathways hinders parasite DNA synthesis, cell division, and proliferation **(Sridaran et al. 2010a)**.

Inhibitors of Dihydrofolate reductase (DHFR)

Drugs from two types of inhibitors are used: biguanides and 2, 4-diaminopyrimidines. Proguanil, a biguanide, is a synthetic pyrimidine derivative converted to the active metabolite cycloguanil *in vivo*. Because it lacks schizonticide efficacy, it is not indicated for treating acute infections. Nevertheless, it helps prevent malaria when coupled with atovaquone or CQ. Proguanil and chlorproguanil are almost similar. Cycloguanil, a 2,4-diaminopyrimidines, is a proguanil *in vivo* metabolite. It's a promising DHFR inhibitor for the malaria parasite. Another drug in this class, pyrimethamine, cures uncomplicated malaria by targeting erythrocytic schizonts **(Sridaran et al. 2010a)**. In cases of CQ resistance, it is only used in combination with a sulfonamide, such as sulfadoxine.

Dihydropteroate synthetase (DHPS) Inhibitors

The enzyme dihydropteroate synthetase (DHPS) in the tetrahydrofolate production pathway of malaria parasites is inhibited by sulphonamides and sulfamethoxypyridazine. They are structural analogs of *p-aminobenzoic acid (*PABA) that compete with PABA to prevent dihydrofolic acid conversion. Blood schizont stages are inhibited by sulphonamides. Sulphonamides are ineffective in the treatment of malaria when used alone. Nonetheless, combining them with pyrimethamine, most typically in the form of fixed-dose sulfadoxine-pyrimethamine, synergistic interaction between them to cure sensitive malaria strains. Sulphonamides include sulfadoxine and dapsone **(Wang et al. 1997, 1999; Carter et al. 2012; Mita et al. 2014)**. Sulfamethoxazole, also known as sulfamethoxypyridazine, is a drug candidate now in a clinical trial.

Inhibitors of mitochondrial electron transport

Atovaquone (hydroxy-quinone), a member of this class of inhibitors, is routinely used to prevent and cure falciparum malaria in industrialized countries. It's only used in combination with Proguanil **(Khositnithikul et al. 2008)**. The cytochrome bc1 complex (Complex III) appears to be its target, leading to electron transport inhibition. GSK 932121 is a 4-pyridone that acts similarly to atovaquone. It is also undergoing clinical trials.

Alkylation of cellular proteins

A covalent interaction between artemisinin and a 25 kDa homolog of the translationally controlled tumor protein has been found (TCTP) (figure3). This protein is also thought to be involved in the formation of hemozoin. Artemisinin has also been shown to alkylate cysteine proteases, such as Falcipain, in *P. falciparum*, impairing their function in the heme-dependent pathway **(Dondorp et al. 2009; Jourdan et al. 2019; Ribbiso et al. 2021)**.

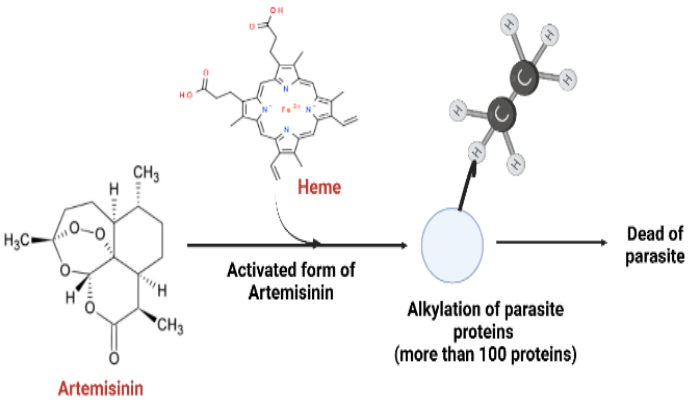


Figure 3 Artemisinin by covalently attaching to over 100 malaria parasite targets, artemisinin kills malaria parasites

Inhibition of PfATP6

PfATP6, also known as Sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), has been shown to block ART and its derivatives, disrupting intracellular calcium (Ca^{2+}) homeostasis. It's present in the endoplasmic reticulum membrane and helps

transport Ca^{2+} ions from the cytoplasm to the endoplasmic reticulum, where they're deposited **(Dondorp et al. 2009; Ribbiso et al. 2021)**. Heme is required for artemisinin to alkylate proteins.

Generation of reactive oxygen species

The production of reactive oxygen species (ROS) like hydroxyl, superoxide, alkoxyl, and peroxyl radicals has been connected to the mechanism of action of artemisinin. In a Fe^{2+}-dependent reaction, they are produced by the Fenton reaction. The parasite's antioxidant species become depleted as ROS levels rise. Initially, it was thought that the conversion of artemisinin to a carbon-centred radical was necessary for ROS formation. It acts primarily on the trophozoite stage, halting parasite progression. ART is thought to have a two-step mechanism of action. Intra-parasitic heme-iron catalyzes the cleavage of this endoperoxide, which activates ART. The parasite may then be killed by a free radical intermediate that alkylates and poisons one or more critical malarial proteins(s). ART has been demonstrated to interfere with hemozoin formation by producing potentially lethal heme-adducts due to a chemically unusual peroxide bridge coupling. Still, it has been linked to the alkylation and oxidation of proteins and probably lipids via reactive intermediates formed by iron/heme-mediated endoperoxide bridge activation. Studies with radiolabelled ART have revealed many covalently changed proteins, suggesting that their alkylation and deactivation may account for parasite mortality. The primary target is thought to be the inhibition of PfATP6, a *P. falciparum* SERCA-type calcium-dependent ATPase in the endoplasmic reticulum (figure 4). However, few research groups have revealed that PfATP6 is unlikely to play a role. ART is a sesquiterpene lactone molecule with an endoperoxide bridge. For simple resistant *P. falciparum*, arteether is an ethyl ether derivative of dihydroartemisinin. ART and other artemisinin derivatives have the active metabolite dihydroartemisinin. It's the most potent form of ART **(Ribbiso et al. 2021)**. It inhibits gametocyte development and has a robust blood schizonticide effect. The active metabolite dihydroartemisinin is transformed into artesunate, a highly soluble hemi-succinate derivative. It's combined with other drugs.

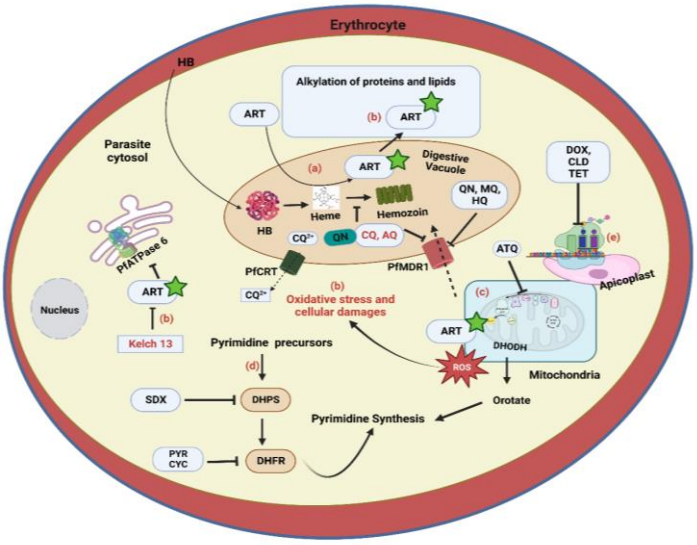


Figure 4 Overview of erythrocytic stage drug targets for malaria

Malaria-related deaths have decreased markedly in recent years due to ACTs targeting *P. falciparum*. P falciparum develops resistance to all antimalarial drugs become develops multidrug resistance **(Morita et al. 2012; Rout and Mahapatra 2019; Hodoameda et al. 2022; Yao et al. 2022)**. ACTs in Southeast Asia fail due to resistance to frontline artemisinin and partner drugs. The following sections provide an overview of our current understanding of the markers and mechanisms of drug resistance to the most used antimalarial drugs.

Antimalarial drug resistance is defined as "a parasite's ability to survive and proliferate despite receiving the prescribed doses. They are some factors responsible for drug resistance like clinically not proper diagnosis and inappropriate treatment of malaria, non-compliance and adherence, poor medication quality, interactions with other drugs, and poor absorption. Resistance is generally caused by spontaneous mutations that reduce drug binding with the target and efflux of drugs that reduced drug susceptibility. Within a parasite population, resistance may evolve. Antimalarial drug resistance is caused by two mechanisms: (1) changes in the parasite genome that cause the drug to be flushed from the digesting vacuole, or (2) loss of binding capacity between the drug and its target. Additionally, antimalarial drug resistance is also associated with increased copy number in the pfmdr1 gene. According to molecular, genetic, and biochemical studies, resistant strains have reduced CQ uptake by the parasite digestive vacuole, which is connected to mutation, and changed the copy number in the *Pfmdr1*, *Pfcg2*, and *Pfcrt* genes **(Fidock et al. 2000; Duraisingh and Cowman 2005; Ariey et al. 2014a; Patgiri et al. 2019)** (ii). Mutations cause resistance in enzyme dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) that are involved in the Folate pathway, (iii) ART and derivatives therapy for K13-propeller mutations frequently lead to resistance to new conversions. According to epidemiological studies, CQ- resistance varies among isolated parasite populations, although antifolate resistance is ubiquitous in most malaria-endemic countries. Multi-drug resistance in Southeast Asian and South American forests is most likely due to established and intense drug pressure and poor antiparasitic immunity. Due to extensive malaria transmission in Africa, *Plasmodium* genetic recombinations are prevalent. The prevalence of *falciparum* CQ-resistant malaria appears to be stable at the same level as CQ-sensitive malaria.

Chloroquine resistance mechanism

CQ became one of the most effective antimalarial drugs for first-line therapy shortly after World War II. In the late 1950s, CQ resistance was discovered in *P. falciparum*. It then spread over the world, requiring the development of new drugs that finally led to today's more expensive artemisinin-based combination therapies (ACTs). The *P. falciparum* CQ resistance transporter gene (*pfcrt*) locus was previously thought crucial for CQ resistance development in classical genetic studies. This gene was later found, and reverse genetic techniques were used to establish its function. Despite being the first-line treatment, *P. vivax* has developed resistance to CQ **(Fidock et al. 2000)**.

CQ is a diprotic base that accumulates in the digestive vacuole of the parasite by diffusion and protonation. In sensitive strains, CQ accumulated into the digestive vacuole, inhibited the heme detoxification pathway, and promoted the parasite's death. CQ-resistance transporter **(**PfCRT) is present in the digestive vacuole membrane, enhancing CQ export and reducing heme polymerization. Single nucleotide polymorphisms (SNPs) in PfCRT in field isolates are sensitive markers for treatment failure in patients because they are like a resistance phenotype *in vitro* study. Because other factors, such as previous malaria exposure, can affect a patient's treatment response, these molecular indicators aren't always reliable. At position 76, there is one polymorphism (K76T). By eliminating a positive charge from a predicted substrate-binding location in PfCRT, protonated CQ can escape the digestive vacuole. This residue is modified further (K76I and K76N) **(Bertin et al. 2005; Sanchez et al. 2007a; Ecker et al. 2012; Afoakwah et al. 2014; Sondo et al. 2015; Asare et al. 2017)**, allowing parasites to survive and emphasizing the importance of this residue. It is hypothesized to aid in hemoglobin degradation by promoting the transport of peptides/amino acids generated from hemoglobin from the digestive vacuole.

CQ resistances were connected to mutations in the *P. falciparum* CQ-resistance transporter which encodes the digestive vacuole membrane protein (PfCRT). It does not affect the heme target, CQ resistance in *Plasmodium spp*. CQ enhances the toxic by-product by inhibiting GSH-mediated heme breakdown. Glutathione is essential in this defence system because it keeps the cells healthy. CQ-resistance transporter (PfCRT) transports peptides from the parasite's digestive vacuole lumen to the cytosol, supplying amino acids for parasite metabolism and alleviating osmotic stress. The malaria parasite's CQ-resistant mechanism was linked to the mutant version of the protein PfCRT. PfCRT is a 424-amino-acid protein found in the parasite's digestive vacuole membrane with ten putative transmembrane domains. A gene called *pfcrt*, which codes for a 45-kDa protein with ten putative transmembrane domains, was discovered in a chromosome 7 region, comparing *P. falciparum* (strain HB3) with *P. falciparum* (Dd2). Eight codon differences were found using PfCRT. By analyzing numerous geographically diverse CQ-sensitive and CQ-resistant clones, position 76 transforming lysine to threonine was the most reliable molecular marker of resistance.

Anti-folates resistance

Plasmodium has evolved resistance to antifolate drugs, notably a sulfadoxine-pyrimethamine combination (SP). Antifolates are antimalarial drugs that block folate metabolism, a vital pathway for malaria parasite survival. This drug class contains potent causal, preventative, and therapeutic chemicals that function in tandem when taken together. Unfortunately, the malaria parasite has developed resistance to antifolates. Point mutations cause antifolate resistance in the dihydrofolate reductase and dihydropteroate synthase, two key enzymes in the folate production pathway **(Plowe et al. 1997; Urdaneta et al. 1999; Bwijo et al. 2003; Sridaran et al. 2010b)**. The genetic basis of antifolate resistance is probably the best understood of all the identified drug-resistance mechanisms. Mutations in the DHFR gene, notably at codons 108, 59, 51, and 164, induce high-level pyrimethamine resistance. Allelic variation leads in Ser108Asn, Cys59Arg, Asn51Ile, and Ile164Leu. Sulphadoxine resistance levels in cultivated parasite lines are roughly correlated with mutations in DHPS at codons 436, 437, 581, and 613.

Atovaquone Resistance

The hydroxynaphthoquinone atovaquone inhibits the cytochrome bc1 complex's mitochondrial electron transport chain in malaria parasites. This process increases

the antiparasitic activity of proguanil and its metabolite, cycloguanil. By sensitizing mitochondria to atovaquone, proguanil enhances atovaquone's ability to collapse the membrane potential of malaria parasites. Cycloguanil is converted to proguanil by the hepatic CYP2C19 system, which prevents parasite dihydrofolate reductase (DHFR) from generating folate and replicating. In *P. falciparum*, cytochrome b (*PfCYTb*) is a mitochondrial membrane protein of the CYTb family with 376 amino acids and a molecular mass of 43.37 kDa. There are ten helical transmembrane domains in the mitochondrial membrane of the parasite **(Wang et al. 1997; Painter et al. 2007; Fisher et al. 2012)**. Atovaquone is resistant to Y268S/C/N. Atovaquone must be taken in combination with other antimalarial drugs. A single point mutation in the gene coding for cytochrome-b causes atovaquone resistance when used alone.

Artemisinin Resistance

Based on studies on the antimalarial properties of medical plants described in ancient texts, Chinese researchers discovered the antimalarial properties of ART in 1972, motivated by a rise in resistance to the most widely used antimalarial drugs. The advantages and disadvantages of ART and its derivatives (such as artesunate) have been known for some time now. Despite the adverse effects, the drugs were well tolerated, fast-acting, and greatly reduced the number of parasites in the bloodstream. However, adequate drug concentration levels were only maintained in the plasma for a relatively short time after administration, and concise oral treatment courses led to high rates of recrudescence. Using ART or derivative as a monotherapy, 7 days of treatment was needed to prevent recurrent parasitemia. An ACT that includes an artemisinin derivative and a longer half-life partner drug takes advantage of artemisinin derivatives' fast action **(Apinjoh et al. 2017)**.

In contrast, the partner drug helps prevent recrudescence even after a short three-day treatment. Artemisinin-based combination drugs are the first-line treatment in most malaria-endemic countries. An artemisinin-resistant variant of *P. falciparum* malaria has emerged near the Cambodia-Thailand border. ACT has replaced CQ and sulphadoxine-pyrimethamine medications for uncomplicated falciparum malaria, as recommended by the WHO **(WHO, 2006)**. Parenteral artesunate recovers quinine levels in severe malaria patients.

On the other hand, these substitutions have lowered malaria-related morbidity and mortality. There have been indications that the efficacy of ACTs and artesunate monotherapy has decreased in western Cambodia (near the Thai-Cambodian border), which has historically been a hotbed of antimalarial drug resistance. Slow parasite elimination *in vivo* characterizes the resistance. Resistance is increasing in Southeast Asia. A mutation in the locus-producing multidrug-resistance protein 1 (PfMDR1) and CQ-resistance transporter can affect ART susceptibility, according to investigations employing transgenic *P. falciparum* asexual blood-stage parasites cultivated *in vitro* (PfCRT). Point mutations and duplications in the Pfmdr1 gene influence parasite sensitivity to antimalarial. The amount of these medications accumulating in a digestive vacuole, where heme detoxification occurs, can affect their effectiveness. According to a recent study, the K13-propeller polymorphism has been identified as a useful molecular marker for large-scale surveillance efforts to propagate *P. falciparum* parasites with low ART sensitivity.

In clinical parasite isolates and laboratory lines, several K13-propeller mutations cause artemisinin resistance, although the molecular mechanisms underlying these resistances are unknown. The K13 protein comprises 726 amino acids and a molecular mass of 83.66 kDa, found on chromosome 13. In the C-terminal part of the K13 protein, six kelch motifs fold into a propeller domain, and changes in this area were predicted to disrupt the domain scaffold and impair its function. Kelch proteins, among other things, coordinate and interact with other proteins. Furthermore, the functions of the K13 protein, the impact of various mutations on the protein, and the pharmacological action mechanism connected with the protein have all been extensively researched. *P. falciparum's* propeller region has recently been discovered to be a powerful predictor of artemisinin resistance. In the kelch repeat region of the K13 propeller domain, nonsynonymous SNPs at Y493H, R539T, I543T, and C580Y have been linked to improved artemisinin resistance **(Wootton et al. 2002; de Laurent et al. 2018; Dafalla et al. 2020)**. More study is needed to connect these findings to previous discoveries that ART-resistant parasites have altered intraerythrocytic growth patterns and enhanced cellular stress responses. ART targets *P. falciparum* phosphatidyl inositol-3-kinase (PI3K), which is K13's binding partner. According to their notion of ART mechanism of action, the interaction between wild-type K13 and PI3K targets the latter for proteasomal degradation. Because they contain low basal levels of PI3-phosphate (PI3P), the result of PI3K activity, these parasites are vulnerable to ART-induced PI3K inhibition. Without a functioning PI3K, parasites cannot create the high levels of PI3P essential for growth. PI3P plays a role in membrane formation and fusion, and its levels rise as parasites progress from rings to schizonts.) According to their artemisinin resistance theory, PI3K accumulates and produces high baseline levels of PI3P because mutant K13 does not bind it **(Roper et al. 2004)**. The increased basal levels of PI3P in ART-resistant parasites allowed them to maintain PI3P-dependent development while recovering from PI3K inhibition. Dihydroartemisinin-piperaquine resistance in Cambodia and high prevalence of K13 C580Y mutation associated with artemisinin resistance, new *pfcrt* mutations (H97Y, M343L, and G353V) were revealed to induce *in vitro* piperaquine resistance. The common antimalarial drug function and their gene polymorphism are highlighted in **Table 3**.

Table 3 Common antimalarial drugs and gene polymorphism associated with the resistance

Protein	Function	Location	Principal drugs affected	Polymorphisms	References
PfATP6 (SERCA)	Membrane Ca^{2+}-transporting ATPase	Endoplasmatic reticulum	ART	L263E	(Valderramos et al. 2010)
PfCRT	Transporting CQ Into cytosol	Digestive vacuole membrane	CQ, AQ MQ, HF, LUM, ART, QN, PQ	C72S, M74I, N75E, K76T, S163R, N326S/D, I356T/L,	(Sanchez et al. 2007b; Martin et al. 2009; Lehane and Kirk 2010)
NHE1	Transporter	Cytoplasm and Digestive vacuole membrane	QN	Copy the number of repeat motifs	(Henry et al. 2009; Cheruiyot et al. 2014)
PfMDR1	Transporter	Digestive vacuole membrane	MQ, HF, LUM, QN, CQ, AQ, ART	N86Y, Y184F, S1034C, N1042D D1246Y, CNV	(Cheruiyot et al. 2014)
PfMRP1	Transporter	Cytoplasm membrane	CQ, QN, LUM	H191Y, S436A, I876V, R1466K	(Cheruiyot et al. 2014)
PfCYT b	Electrons transfer the respiratory chain	Mitochondria	ATV	Y268S/N/C	(Wichmann et al. 2004)
DHPS	Folate pathway	Cytoplasm	SDX, dapsone	S436A/F, A437GK540E, A581G	(Wang et al. 1997; Carter et al. 2012; Mita et al. 2014)
DHFR	Folate pathway	Cytoplasm	Proguanil, PYR, PG,	A16V, C50R N51I, C59R, S108N/T, I164L, A613S/T	(Wang et al. 1997; Carter et al. 2012; Mita et al. 2014)
Kelch-13	Oxidative stress	Cytoplasm	ART	Y493H, R539T, I543T, and C580Y	(Dafalla et al. 2020)
Plasmepsin 2/3	Heme metabolism	Digestive vacuole	DHA-Piperaquine	Copy number amplification	(Ansbro et al. 2020)

Drug resistance surveillance for antimalarial drugs

Several methods can be employed *in vivo* to study antimalarial drug resistance, including chemotherapy efficacy studies, cultured malaria parasite studies, and molecular studies using markers known to indicate resistance. A therapeutic efficacy study's outcome is directly related to clinical outcomes, so it is considered a gold standard for determining antimalarial drug resistance and changing drug regimens. Malaria-endemic countries use **(Ariey et al. 2014b; Duru et al. 2015; Huang et al. 2021; Nsanzabana 2021)**WHO protocols at sentinel sites to assess the efficacy of antimalarial drugs. After 28- or 42-days starting treatment, parasitemia is typically absent, indicating a successful treatment response. The World Health Organization recommends switching to another first-line drug if a first-line medicine fails to cure the condition in 10% of cases.

Strategies to overcome drug- resistance

A new anti-malarial agent is discovered through a variety of traditional drug discovery approaches. Various approaches were being used to overcome the drug resistance of *P. falciparum*. Optimization of combinational therapy with existing drugs, drug resistance-reversal agents, combination therapy with new drugs, analogs of previously available drugs, natural products, drug repurposing, hybrid drug development, new drug targets against the resistant parasite, and vaccine development has been considered **(Mina et al. 2021; Pandey et al. 2023)**.

Antimalarial drug resistance is on the rise, prompting the discovery of a new antimalarial molecule. To overcome this challenge, researchers must develop a unique drug target and a thorough understanding of the parasite's biochemical and metabolic processes.

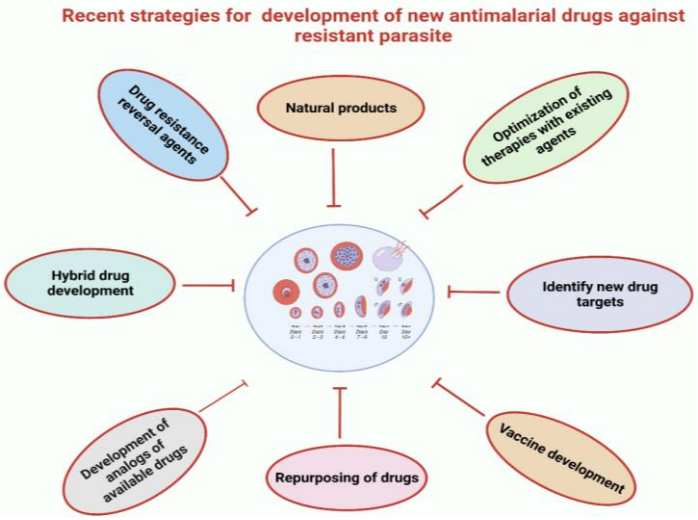


Figure 5: Recent strategies of drug development for malaria

Optimization of combinational therapy with existing drugs

Although most antimalarial drugs have developed drug resistance, WHO bans monotherapy for malaria to limit the potential of drug resistance **(Mina et al. 2021)**. The idea behind the combination is that if a pair of medicines with different modes of action and hence separate resistance mechanisms are used together, the risk of developing resistance to both drugs during the same cell division is the result of their probabilities. Combination therapy with antimalarial medications involves the simultaneous administration of two or more blood schizonticide agents with independent mechanisms of action. Because the combinations target many biological targets, they can improve therapeutic efficacy while delaying the development of resistance to the individual components. Artemisinin-based combinations (ACTs) are used as initial treatments for uncomplicated falciparum malaria.

These compounds are effective against various stages of the malaria parasite. Because artemisinin derivatives have a lower half-life, it is advised that the other ACT partner drugs have a longer half-life. Because the artemisinin derivatives are fast eliminated whereas the companion drugs are slowly eliminated, the artemisinin derivatives are completely protected. Furthermore, resistance/delayed parasite clearance against widely used antimalarial combinations, including ACTs, worsens the problem and threatens the development of novel medicines. WHO recommended **(WHO Recommendations for Treating Malaria, 2010)** for uncomplicated falciparum malaria. Artemisinin derivatives (artesunate, artemether, and dihydroartemisinin) are combined with a partner drug in ACTs. Its role is to reduce parasite biomass during the first three days of treatment (reduction of parasite numbers and biomass), while the partner drug is to eliminate parasites that are left behind (cure). A total of 6 ACTs are currently recommended by the WHO. For severe malaria, artesunate and artemether should be administered as two injectable treatments, followed by oral antimalarial treatment. Over the last fifteen years, the global malaria burden has been reduced significantly as ACTs have been made more widely available in malaria-endemic countries. ACT treatment courses were sold worldwide by manufacturers between 2010 and 2021 for almost 3.8 billion dollars. Public sector procurements accounted for approximately 68% of these purchases in malaria-endemic regions. ACTs used to treat uncomplicated malaria include artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, artesunate-sulphadoxine-pyrimethamine, and dihydroartemisinin-piperaquine (Table 4). Specifically, in the effort to eliminate falciparum malaria, evidence suggests that PQ may reduce malaria transmissibility. Combination therapies that include a gametocytocidal agent may further decrease the transmission of parasites, both those that are sensitive and those that are drug resistant. As a gametocytocidal, primaquine 0.25 mg base/kg has enough safety and efficacy evidence to support wide-scale use. It is important to conduct prospective studies to determine whether a single dose of primaquine 0.25 mg base/kg combined with ACT is safe for individuals with G6PD deficiency, as well as the dose-response relationship of transmission-blocking activity in different geographical areas, particularly in the context of ART-resistant falciparum malaria. According to WHO guidelines, this is the most realistic choice for general use for uncomplicated cases. Artemisinin-based combination therapy (ACT) is the initial treatment for uncomplicated falciparum malaria. ACT involves administering an artemisinin derivative along with a longer-acting partner drug. Artemisinin and its partners pose a major threat to malaria control activities since *P. falciparum* is resistant to both. There is a need for novel strategies to prevent the spread of these resistant parasites and reverse their spread. Recently, TACT (triple artemisinin combination therapy) is one of these strategies. TACT has the potential to decrease the chance of the emergence of a de novo resistance, as well as rescue a regimen in which one of the ACT components is already failing. Two antimalarial clinical trials are underway to determine the efficacy of TACT for uncomplicated falciparum malaria: Artemether-Lumefantrine plus Amodiaquine and Dihydroartemisinin-Piperaquine plus Mefloquine. These are being compared to the standard ACTs alone.

Triple ACTs are currently being considered in Southeast Asia, where additional partner drugs are added to ACT to combat resistant parasites. The Tracking Resistance to Artemisinin Collaboration (TRAC) II Trial is currently underway and the first results are reassuring. If successfully developed and deployed, triple ACTs will play an important role in halting and potentially reducing the rate of spread of drugs resistant parasites.

Table 4 Clinically used ART combination against malaria

Treatments with ACTS	Route	Used against	Resistance status	Used where	Reference
Artemether + Lumefantrine	oral	Uncomplicated *P. falciparum*	Presence	56 countries, mainly South America, Africa, Asia, India	(WHO 2015; Peto et al. 2022)
Artesunate and Sulfadoxine/Pyrimethamine	Oral	Uncomplicated *P. falciparum*	Presence	11 countries, mainly Asia	(Peto et al. 2022)
Artesunate and Amodiaquine	Oral	Uncomplicated *P. falciparum*	Presence	27 countries, mainly Africa	(Peto et al. 2022)
Artesunate + Mefloquine	Oral	Uncomplicated *P. falciparum*	Presence	8 Countries, mainly South America, and Asia	(Eziefula 2016; Sirima et al. 2016)
Dihydroartemisinin + Piperaquine	Oral	Uncomplicated *P. falciparum*	Presence	China and Southeast Asia	(Sirima et al. 2016; Chan et al. 2018)
Artesunate* + ACT#	*I. V or I.M, # Oral	Severe *P. falciparum*	Absence	Worldwide	Tripura et al. 2018)
Primaquine + ACT#	Oral	Chloroquine resistant *P. vivax*	Absence	Where ACT used against *P. falciparum*	
Artemisinin derivatives + Primaquine	Oral	Complicated *P. vivax*	Absence	Worldwide	(Abay 2013; Zorc et al. 2019)

The development of antimalarial drug resistance is becoming more prevalent due to several factors, including drug abuse, mutations in transporter genes, and mutations in identifiable target genes. In addition to their high cost and strict regulations, lead optimization and clinical trials are time-consuming processes. To reintroduce existing antimalarials into the mainstream, it is essential to optimize existing antimalarials. A lack of action mechanisms and toxicity limit the identification of resistance-reversal agents as well. The researchers have identified several effective agents to reverse resistance to clinically using drugs such as CQ. CQ-resistant parasites accumulate less CQ in their digesting vacuoles. CQ efflux from the digestive vacuole. The PfCRT mutation has been linked to CQ, halofantrine, and quinine. Drug resistance can be reversed by designing a target against a crucial protein (PfCRT), verapamil, desipramine, chlorpromazine, promethazine, chlorpheniramine, citalopram, trifluoperazine, and 9, 10-dihydroethanoanthracene derivatives (BG920, BG932, BG958, and BG996) act on this target protein and block CQ efflux from the digestive vacuole **(Millet et al. 2004)**. Some research groups were focused on the relationship between CQ resistance of *P. falciparum* and the GSH metabolic pathway because GSH-medicated heme detoxification is inhibited by CQ. In the resistant strain of *P. falciparum*, raising GSH levels and lowering CQ impact by blocking GSH-mediated heme breakdown. Glutathione levels were regulated by glutathione synthesis, glutathione reduction, and glutathione efflux in *Plasmodium*. As a result, researchers investigated the impact of CQ on this redox balancing system to determine if there were any differences in how CQ-sensitive and CQ-resistant *P. falciparum* strains handled the glutathione redox balancing system. 4-

Chlorothymol was able to decrease the reduced GSH level in CQ-resistant, which showed synergistic interaction between 4-Chlorothymol and CQ **(Kumar et al. 2021)**. Drug resistance can be overcome by targeting a crucial enzyme that regulates glutathione availability in the parasite. These reversal agents may help reduce drug resistance when used with the regularly used CQ. In addition to decreasing efficacy and resistance to drugs, drug metabolizing enzymes also play a crucial role. Mefloquine, quinine, and quinidine may lose bioavailability due to excessive metabolism, resulting in diminished efficacy and increased drug resistance. Drugs might remain effective if certain inhibitors inhibit these drug-metabolizing enzymes. Nevertheless, these combinations might present a major concern regarding toxicity. Researchers have successfully reversed resistance using this approach. The combination of clarithromycin with mefloquine/quinine/quinidine against multidrug-resistant malaria has been reported to be advantageous due to the resistance-reversal action of ketoconazole for mefloquine.

Analogs of existing drugs

In a new class of antimalarial drugs, a few drugs have been identified and are currently in clinical use, including amino alcohols (mefloquine, halofantrine, and lumefantrine), sesquiterpene trioxanes (artemisinin derivatives), and naphthoquinones (atovaquone) **(Tibon et al.; Tse et al. 2019)**. A new chemical series with the potential to be a potent antimalarial need to be identified and led to optimization at several levels, including financial, social, and scientific. Among them are concerned about toxicity, cost, and ethics. Meanwhile, new chemotherapeutic interventions can be designed easily using analogs of existing antimalarial drugs, which can address the above problems more positively.

Hybrid drug development

The development of hybrid molecules with dual functionality as well as multitherapeutic strategies, using new chemical entities with two (or more) different heterocyclic skeletons (pharmacophores), represents a valid and rational strategy for developing novel antimalarials. By reducing the risk of drug-drug interactions, these drugs can eliminate the rapid development of resistance. Although traditional drugs like CQ have been rendered ineffective due to resistance, the strategy can restore their efficacy, and these drugs have the advantage of being affordable, effective to synthesize, limited in toxicity to the host, and short course. Researchers are currently working on full-synthetic antimalarials and hybrid molecules that are yielding promising results in the laboratory, and some are now entering clinical trials. Since fully synthetic peroxides have superior purity, cost, and biopharmaceutical properties to semi-synthetic artemisinin, they may overtake their limitations. The development of OZ277 (RBx-1160), a fully synthetic peroxidic antimalarial, is underway. It is being tested in Phase III clinical trials in India in combination with piperaquine **(Zwang et al. 2009; Toure et al. 2015, 2017)**. Trials are currently being conducted on CDRI97/78, a trioxolane. A new generation ozonide compound called OZ439 started Phase I clinical trials in April 2009. A collaboration between Monash University and the University of Nebraska is developing through the Medicines for Malaria Venture (MMV).

Natural products and derivatives

Natural products have contributed to modern medicine through their use as traditional medicine for thousands of years. Most studies of plant extract and isolates for novel antimalarials have been conducted *in vitro*, although many studies have also been conducted in animal models. Moreover, the isolation of active compounds from plants has been a recent addition to the use of plants as medicines, starting with the isolation of morphine from opium in the early nineteenth century, followed by the discovery of quinine from *Cinchona officinalis* and artemisinin from *Artemisia anua*. Plant-derived antimalarials had alkaloidal compounds with anti-plasmodial and antimalarial properties, including terpenes, limonoids, flavonoids, chromones, xanthones, anthraquinones, and miscellaneous compounds. Several powerful natural compounds have been identified in plants that have antimalarial activity *in vitro*. These include *Glycyrrhiza glabra, Boswellia sarrata, Putranjiva roxburghii,* and *Alnus nepalensis*. As well as pure phytomolecules isolated from *Glycyrrhiza glabra* and *Boswellia sarrata* **(Gupta et al. 2021; Bhatt et al. 2022; Kapkoti et al. 2023; Mishra et al. 2023; Kumar et al. 2023)**. There is no synthetic equivalent of atovaquone, artemisinin (and its semi-synthetic derivatives), clindamycin (a derivative of the natural product lincomycin), erythromycin, and tetracycline that is extremely effective against malaria. Developing antimalarial drugs from natural products is challenging due to several hurdles, including moderate activity, toxicity, and characterizing physicochemical and biological properties, and when a single drug candidate is isolated from crude, it usually loses its antimalarial potential. Nevertheless, clinical trials are being conducted on some antimalarial drugs developed from natural products worldwide. The development of Argemone mexicana as a potent antimalarial is the best recent example of this. Plumbagin, bergenin, pellitorine, thymol **(Kumar et al. 2021)**, roxburghonol, rutin **(Bhatt et al. 2022)**, and silymarin which has been reported for anti-plasmodial activity **(Bhardwaj et al. 2015; Dola et al. 2017)**. The development of resistance towards the current therapies warrants for development of new antimalarials.

Drug repurposing

Drug repurposing for the treatment of life-threatening diseases such as cancer, malaria, tuberculosis, and diabetes is an emerging trend at present. Currently, most of the clinical, pharmacological, pharmacokinetic, and pharmacodynamic information about these drugs is available, which makes it safe, economical, and faster to reach the clinical stage. Many molecules have been identified with this approach, particularly those for the treatment of malaria, after several successful efforts in this field. Drug repurposing, also known as drug repositioning, reprofiling, redirection, etc., is the process of finding new medical uses for existing drugs. This has been a very successful strategy, with approximately 25% of the pharmaceutical industry's annual revenue coming from recycled medicines. When selecting drugs to be reused, we consider compounds that were effective against the target disease in the clinical development program, compounds that were not effective, and drugs that were withdrawn from the market due to strategic reasons such as low profitability. may occur. This approach reduces the risk of failure, as repurposed drugs have already been shown to be safe for human use. It also takes less time and requires less investment as most preclinical studies have already been completed. These advantages translate into less investment in 'De Novo drug discovery than for diseases that require rapid detection and neglected tropical diseases. diseases) are particularly attractive. Several antibiotics (clindamycin, doxycycline) have been previously tested against malaria parasites by various expert groups **(Chinh et al. 2011; Gaillard et al. 2016)**. There are several notable examples of these agents, including itraconazole (an antifungal compound), atorvastatin (a cholesterol-lowering drug), lopinavir and tipranavir (HIV protease inhibitors), and imidazolopiperazine. Different stages and specific targets of the malaria parasite are targeted by these molecules with potent antimalarial activity. In addition, several antibiotics are being used in clinical trials to treat malaria, including doxycycline, azithromycin, clindamycin, tetracycline, and fosmidomycin. In combination with pyrimethamine, sulfadoxine is one of the most effective antimicrobial agents against malaria. This combination has been adopted by several countries as part of their antimalarial policies.

New drug targets against a resistant parasite

Multidrug-resistant malaria poses a major challenge to the identification of new drug targets **(Dey et al. 2009; Alam et al. 2011, 2012; Iqbal et al. 2016)**. As a result of drug resistance, closely related drugs become ineffective in treating malaria, a phenomenon called "cross-resistance," reducing management options. To achieve this goal, some new vital targets need to be developed as well as new pharmacophores. Malaria's new target has been identified by whole-genome sequencing. Some several key pathways and targets are involved, including histone deacetylation, adenylosuccinate synthetase, choline kinase, deoxyuridine triphosphate nucleotide hydrolase, glutamate dehydrogenase, guanylate kinase, N-myristoyl transferase, orotidine 5-monophosphate decarboxylase, farnesyl pyrophosphate synthase, and S-adenosylhomocysteine hydrolase, shikimate pathway, enzymes of the folate pathway, and many more from different vital pathways of the malaria parasite are being explored to identify a safe, novel target and its validation, as well as to develop some new and safe therapies against these identified targets. The metabolic pathways involved in parasite and host genes were found to differ significantly, leading to the identification of existing antimalarial drugs **(Dey et al. 2009; Alam et al. 2011, 2012; Goyal et al. 2012)**.

Vaccines development

Malaria vaccine development has been ongoing since the 1960s, with significant advancements achieved in the previous decade. As a result of the WHO recommendation for widespread use of the RTS, S/AS01 (RTS, S) malaria vaccine among children in Sub-Saharan Africa and other areas with moderate to high malaria transmissions, October 6, 2021, is going to be remembered as an important turning point in malaria vaccine development. Currently, there is no effective way to eradicate malaria, so developing safe, effective, and cost-effective vaccines against it remains a top priority. RTS, S/AS01E (RTS, S; trademark Mosquirix ™) is the only vaccine that has been shown to significantly reduce malaria in young African children, including life-threatening extreme malaria. The RTS, S/AS01E vaccine candidate is suitable for use as a pre-erythrocytic vaccine against *P. falciparum* **(Kester et al. 2009; Sacarlal et al. 2009; Olotu et al. 2010; Kazmin et al. 2017)**. According to large-scale clinical trials, the vaccine prevented approximately four in 10 malaria cases in children who received four doses. There have been four alternative malaria vaccines studied, including a whole-body vaccine, a live attenuated vaccine, a genetically modified vaccine, and a subunit vaccine. Recently, preclinical, and clinical studies have shown the safety of *P. falciparum* sporozoite (SPZ) vaccines, but larger sample sizes are needed to verify their efficacy. The malaria parasite *P. falciparum* sporozoite, which is spread by mosquitoes, has been employed to make the PfSPZ vaccine. In challenge studies where volunteers are infected with malaria (in a highly controlled way), the

vaccine, which was developed by Sanaria Inc. in Rockville, Maryland, has been demonstrated to provide at least 90% protection. These studies were conducted by both CVD researchers in the United States and Tanzania.

Concluding remarks and future perspectives

Malaria is a severe public health issue, with unusual parasite strains resistant to treatment and ineffective vector management. The complicated life cycle of the malaria parasite and the evolution of drug-resistant species have hindered numerous therapeutic efforts. To combat parasite resistance, scientists are searching for a new therapy that is both efficient and acts in an approach that is distinct from other drugs. The stakes have been elevated by mutations in *P. falciparum* genome that confer antimalarial resistance, especially *Pfcrt*, *Pfmdr1*, *Pfdhps*, *Pfdhfr*, *Pfcyt b*, and *PfKelch13*. In retaliation, scientists have developed novel combination drugs and discovered genetic markers of drug resistance. The use of molecular markers to identify emergence from different loci may then be monitored to ensure that the proper therapies are being applied in a particular area. Understanding drug resistance will open new avenues for efficient treatment and stop the spread of this frequently fatal illness. Over the years, several strategies have been used to improve existing antimalarial drugs. Combination therapy with existing drugs, and analogs of previously available drugs to presently utilized drugs are only some possibilities. Combination drugs have been discontinued due to resistance or poor sensitivity reports. Drug resistance-reversal agents, combination therapy with new drugs, natural products, drug repurposing, hybrid drug development, and new drug targets against the resistant parasite are expected to yield new drugs that are more effective than artesunate and have novel mechanisms of action, which can be used sequentially to combat the emergence of multidrug-resistant strains over time.

CONCLUSION

In conclusion, we concluded that several therapeutic targets should be discovered and developed in the future for the discovery and development of novel antimalarial agents. Antimalarial drugs with unknown mechanisms of action must be designed to combat the emergence of resistance to antimalarial drugs. *P. falciparum* resistance is highly prevalent in Southeast Asia, Africa, and South America. Even though malaria parasites have a complex life cycle that makes the development of new antimalarial drugs complex, the discovery of novel biochemical pathways holds much promise for introducing new antimalarial drugs. Developing therapeutic agents aimed at existing targets and discovering novel cellular targets are crucial to combating drug-resistant malaria.

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