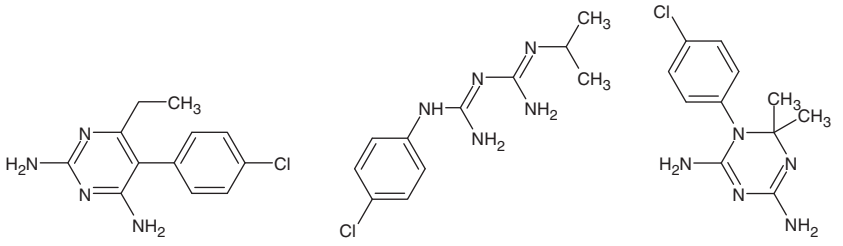

Figure 1.8 Structure of antimalarial drugs belonging to class antifolates.

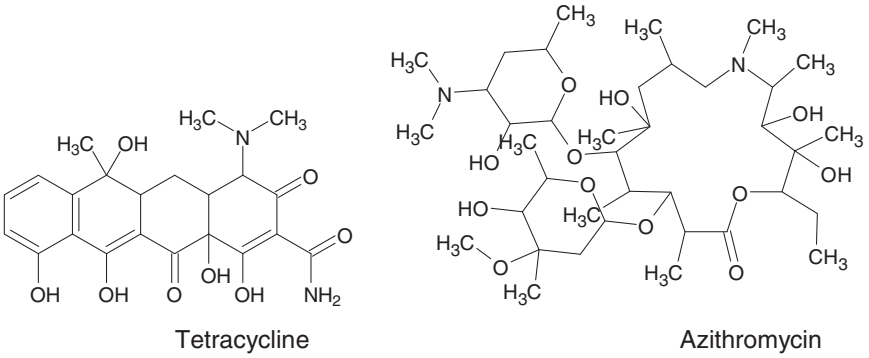

Figure 1.9 Structure of antimalarial drugs belonging to class antibiotics.

The ACTs for the treatment of malaria are as follows:

- Dihydroartemisinin + piperaquine (Eurartesim®)
- Artemether + lumefantrine (Coartem®)
- Artesunate + mefloquine (ASMQ)
- Artesunate + amodiaquine (Winthrop® or Coarsucam™)
- Artesunate + sulfadoxine + pyrimethamine
- ARCO® (artemisinin + naphthoquine)

1.4 Drug Resistance of Antimalarial Drugs

Resistance to antimalarial drugs has been described for two of the four species of malaria parasites that naturally infect humans; *P. falciparum* and *P. vivax*. *P. falciparum* have developed resistance to nearly all antimalarials in current use.

Antimalarial drug resistance has been defined as the "ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject." This definition was later modified to specify that the drug in question must "gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action" [21].

Most researchers interpret this as referring only to persistence of parasites after treatment doses of an antimalarial rather than prophylaxis failure, although the latter is a useful tool for early warning of the presence of drug resistance [22].

This definition of resistance holds true if the patient has been administered the required treatment dose and also demonstrates adequate blood drug and metabolite concentrations using laboratory methods such as high-performance liquid chromatography or *in vitro* tests.

A differentiation must be made between a failure to clear malarial parasitemia and true antimalarial drug resistance. While drug resistance can cause treatment failure, not all treatment failures are due to drug resistance. Factors that contribute to treatment failure include incorrect dosing, noncompliance with duration of dosing regimen, poor drug quality, drug interactions, poor or erratic absorption, and misdiagnosis.

Mechanisms of antimalarial resistance – In general, resistance appears to occur through spontaneous mutations that confer reduced sensitivity to a given drug or class of drugs. For some drugs, only a single point mutation is required to confer resistance, while for other drugs, multiple mutations appear to be required. Provided the mutations are not deleterious to the survival or reproduction of the parasite, drug pressure will remove susceptible parasites while resistant parasites survive. Single malaria isolates have been found to be made up of heterogeneous populations of parasites that can have widely varying drug response characteristics, from highly resistant to completely sensitive [23].

The biochemical mechanism of resistance has been well described for chloroquine, the antifolate combination drugs, and atovaquone.

In chloroquine resistance, as the malaria parasite digests hemoglobin, large amounts of toxic by-product are formed. The parasite polymerizes this by-product in its food vacuole, producing nontoxic hemozoin (malaria pigment). It is believed that resistance of *P. falciparum* to chloroquine is related to an increased capacity for the parasite to expel chloroquine at a rate that does not allow chloroquine to reach levels required for inhibition of heme polymerization [15]. This chloroquine efflux occurs at a rate of 40–50 times faster among resistant parasites than sensitive ones [24]. Further evidence supporting this mechanism is provided by the fact that chloroquine resistance can be reversed by drugs, which interfere with this efflux system [25]. It is unclear whether parasite resistance to other quinoline antimalarials (amodiaquine, mefloquine, halofantrine, and quinine) occurs via similar mechanisms [15].

Combination drugs, such as sulfadoxine + pyrimethamine, act through sequential and synergistic blockade of two key enzymes involved with folate synthesis. Pyrimethamine and related compounds inhibit the step mediated by DHFR while sulfones and sulfonamides inhibit the step mediated by dihydropteroate synthase (DHPS) [21]. Specific gene mutations encoding for resistance to both DHPS and DHFR have been identified. Specific combinations of these mutations have been associated with varying degrees of resistance to antifolate combination drugs [26].

Atovaquone acts through inhibition of electron transport at the cytochrome bc1 complex [27]. Although resistance to atovaquone develops very rapidly when used

Table 1.4 Resistance to antimalarial drugs [36].

Antimalarial drug	Introduction date	First reported resistance
Quinine	1632	1910
Chloroquine	1945	1957
Proguanil	1948	1949
Sulfadoxine + Pyrimethamine	1967	1967
Mefloquine	1977	1982
Halofantrine	1988	1993
Atovaquone	1996	1996
Artemisinin	1971	1980

alone, when combined with a second drug, such as proguanil (the combination used in Malarone™) or tetracycline, resistance develops more slowly [28]. Resistance is conferred by single point mutations in the cytochrome-b gene.

In one study, patients experiencing chloroquine treatment failure had recrudescent infections that tended to be less severe or even asymptomatic [29]. Schizont maturation may also be more efficient among resistant parasites [30, 31]. There is some evidence that certain combinations of drug-resistant parasites and vector species enhance transmission of drug resistance, while other combinations inhibit transmission of resistant parasites.

Many antimalarial drugs in current usage are closely related chemically, and development of resistance to one can facilitate development of resistance to others. Chloroquine and amodiaquine are both 4-aminoquinolines, and cross-resistance between these two drugs is well known [32, 33]. Development of resistance to mefloquine may also lead to resistance to halofantrine and quinine. Antifolate combination drugs have similar action, and widespread use of sulfadoxine/pyrimethamine (SP) for the treatment of malaria may lead to increased parasitological resistance to other antifolate combination drugs [34]. Development of high levels of SP resistance through continued accumulation of DHFR mutations may compromise the useful life span of newer antifolate combination drugs such as chlorproguanil/dapsone (LapDap) even before they are brought into use. This increased risk of resistance due to sulfadoxine–pyrimethamine (SP) use may even affect non-malarial pathogens; use of SP for treatment of malaria increased resistance to trimethoprim/sulfamethoxazole among respiratory pathogens [35] (Table 1.4).

1.4.1 Detection of Drug Resistance

In vivo test, *in vitro* test, animal models, molecular characterization, and additional methods such as case reports and case series are used for detection of drug resistance in malaria.

In an *in vivo* test, the symptomatic and parasitemia individuals are administered a known dose of drug and monitored over time to check for clinical response. In spite of *in vivo* tests offering reliable information on efficacy of antimalarial treatment, they do not necessarily reflect the actual level of antimalarial drug resistance. The *in vivo* test requires follow-up for a long period, but it has been modified to shorter period of 7–14 days as recurrence is more likely than reinfection. Hematological recovery can be checked as anemia that is a major effect of malaria [37].

The *in vitro* tests consist of collecting the parasite from a blood prick of an infected individual and exposing it to known quantities of drugs (microtiter) under suitable experimental conditions and checking for inhibition of maturation into schizonts. Several drugs can be assessed, but its correlation to actual clinical response in infected individuals was not found to be consistent. Prodrugs cannot be tested, and non-falciparum erythrocytic parasites cannot be generally evaluated by *in vitro* methods [38].

Animal model studies are a type of *in vivo* test but carried out on human models. This test allows testing of drugs not yet approved for human use, but only parasites adaptable to non-human primates can be tested.

Therefore, ACT, which is effective against chloroquine- and mefloquine-resistant strains, not only is active against the mature ring stage of *P. falciparum*, when the parasites are most metabolically active, but also targets the young ring stages of the parasites.

1.5 Newer Drugs Approved for Malaria Treatment

Besides the traditional drug discovery and development methods for the identification of new antimalarials that will be described below, there are a number of other ways in which a new antimalarial drug may be discovered. One way, as previously mentioned, is through the exploration of new combinations and formulations of current antimalarial drugs. This may help overcome issues with resistance to a particular component or may assist in the delivery of the drug allowing it to be more effective.

The new drug, Krintafel (tafenoquine), prevents relapse of malaria caused by *P. vivax*, one of several parasites that causes the disease.

Other Drugs Which are Under Clinical Trial

(1) **Tulane University researchers developed a new drug called AQ-13 and were able to clear the parasite responsible for disease within a week** [39]
It is a chloroquine (CQ) analogue with short side chain. It has a remarkable property of retrieving its activity against CQ resistant *P. falciparum*, and has a synonym Ro47-0543. CQ targets blood stage schizonts and interferes with heme detoxification within the food vacuole. CQ resistance is mediated by mutations in *P. falciparum* CQ resistance transporter gene (pfcrt) that leads to augmented efflux of CQ and restores hematin crystallization.

(2) **Methylene Blue** [40, 41]
Water-soluble dye, well absorbed from the GI tract. It was the first synthetic antimalarial to be used, which occurred in a German hospital some 120 years ago. Like 4-aminoquinolines, it also interacts with the polymerization of heme to hemozoin.

(3) **Fosmiomycin** [42]
A phosphoric acid derivative, which blocks a key enzyme 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase, thus interferes in the DOXP pathway thereby inhibiting the growth of multi-drug-resistant strain.

(4) **Imtainib** [43]
Like all tyrosine-kinase inhibitors, it works by preventing a tyrosine kinase enzyme. It inhibits the phosphorylation of erythrocyte membrane band 3 by an erythrocyte tyrosine kinase. Since tyrosine phosphorylation of band 3 causes a destabilization of the erythrocyte membrane required for parasite to move out of erythrocytes, inhibition of the erythrocyte tyrosine kinase leads to parasite entrapment and termination of the malaria infection.

(5) **Sevuparin** [44]
A heparin analogue having low anticoagulant activity thus has anti-adhesive properties. It inhibits sequestration of late-stage infected erythrocyte to uninfected erythrocyte. Sevaparin is developed by Dilaforette AB, a Swedish pharmaceutical company founded by researchers of the Karolinska Institute. It is under Phase II clinical study, and probable further trials will show if this adds to therapy of severe malaria.

1.6 Current Approaches to Developing a Malaria Vaccine

Although there have been many efforts and substantial progress to control malaria, combination therapy is available to treat the resistance against the *P. falciparum*, but the disease is still a critical problem in endemic areas, affecting millions of children and adults. Vaccines aimed at different stages in the *Plasmodium* life cycle are in development, and in the future, successful candidates could be combined to achieve the greatest activity. One candidate, the RTS, -S vaccine that targets pre-erythrocytic stage parasites, has been implemented in three African countries.

1.6.1 Hope for Vaccine Lies in the Parasite Itself

The *Plasmodium* parasite leads a strange and complicated life, crisscrossing between two "host" species – humans and mosquitoes. Within the short span of just a few weeks, the organism cycles through a half dozen radically different sizes and shapes and alternatively makes its home in the human liver, a person's bloodstream, the insect stomach, and a mosquito's spit [45].

For years scientists knew that the most fruitful way to fight the parasite would be to target the form in which it exists in the bloodstream, since that is where the

majority of clinical symptoms occur. Existing drugs, such as quinine and ART, both target the parasite in the blood.

About 15 years ago, scientists discovered a potential new source of drug targets in a tiny, factory-like enveloped organelle called an apicoplast that exists within the parasite. It was unlike anything found normally in the human body, which suggested that drugs designed to interfere with it might kill the parasite while essentially leaving people unharmed.

In the last decade, the evolutionary history of this strange organelle has unfolded. The apicoplast is the strange remnant of collisions between competing cells far back in evolutionary history. Scientists reason that through the course of evolution, the apicoplast arose from its origin as a stand-alone bacterium into its current form through a series of at least two endosymbiotic events, in which one cell engulfs and permanently acquires genetic material and cellular machinery of another for its own benefit.

The discovery of this strange organelle in modern *Plasmodium* immediately suggested that there might be ways to target it with new drugs. However, even after extensive research revealed the genes of this apicoplast, efforts to raise new drugs against it were mostly fruitless – largely because nobody knew what the organelle actually did while the parasite was inside the human bloodstream.

Now DeRisi and Yeh have shown that the sole essential function of the apicoplast while the parasite is in the blood to produce a single chemical known as isopentenyl pyrophosphate (IPP), a necessary building block the parasite uses to construct a variety of other molecules.

They discovered this by growing samples of *P. falciparum* within red blood cells in the test tube. If they treated the parasite with antibiotic drugs that kill the apicoplast, the parasites would all die. If they fed the parasites IPP at the same time, they lived – even though the parasites lost the organelle completely over time.

The work provides a new tool for probing the basic biology of the *Plasmodium* parasite, and it also suggests a new way of discovering promising new drugs to fight malaria. While many previous drug-screening efforts have identified multitudes of compounds that appear to inhibit growth of the parasites, most are without a known target within the parasites. Knowing the target of a drug greatly enables the necessary process of medicinal chemistry, in which the compound is optimized with respect to the target. Now, DeRisi and Yeh's discovery has provided a simple tool to determine whether any particular drug candidate targets the apicoplast [46].

The attenuated form of the parasite also provides an intriguing hypothetical vaccine candidate – and one that would be relatively cheap to produce, DeRisi said. However, he cautioned, the history of malaria control is filled with failed efforts, and several past vaccines have fallen short. Only time and clinical trials will tell if this is a viable solution to the problem. "This parasite has clearly evolved to be an immune system escape artist," DeRisi said. "It's no surprise that the simple approaches have not worked." (Table 1.5).

The idea of a pre-erythrocytic vaccine took shape with the landmark observation by Ruth Nussenzweig that vaccination of mice with irradiated sporozoites resulted in protection [47] and, further, that protection could be achieved by immunization

Table 1.5 Vaccine candidates that target each stage.

1. Pre-erythrocytic stages (Sporozoites)	**Anti-infection vaccines** Subunit vaccines: RTS, -S/AS01E, R21, full-length CSP Whole sporozite vaccines: PfSPZ vaccine, chemoprophylaxis vaccination, genetically attenuated sporozoites
2. Asexual blood stages (Merozoites)	**Blood stage vaccines** Merozoite antigens: AMA1, MSP1, MSP3, EBA-175, PfRh5, AMA1-RON2 complex Other antigens: PfSEA1, PfGARP, chemically attenuated parasites Placental malaria: VARCSA
3. Mosquito sexual stages (Gametocytes)	**Transmission blocking vaccines** Pfs25, Pfs230, Pfs48/45, Pvs230

with the CS protein (CSP) alone [48]. Development of human pre-erythrocytic vaccines began with the cloning of the *P. falciparum* CSP and the entry of SmithKline with the Walter Reed Army Institute of Research (WRAIR) into vaccine development in 1985. This research led to the development of the RTS, -S vaccine, which consists of hepatitis B surface antigen (HBsAg) particles with 25% of the HBsAg fused to the central repeat and thrombospondin domain of the CSP formulated in the adjuvant AS01 [48, 49].

1.7 Conclusion: The Path Forward

Vaccines for malaria are an important measure for prevention of the disease and minimize malaria transmission. Intense efforts by many groups in this field have happened over the last few decades. In spite of this, currently there is no malaria vaccine available. Many approaches have been worked upon to develop a malaria vaccine. The task of development of malaria vaccine is difficult due to the complexity of the malaria parasite.

About 275 000 children have received their first dose of the RTS, -S malaria vaccine through the pilot implementation program initiated in Ghana, Kenya, and Malawi in 2019. The malaria vaccine is the first vaccine that can significantly reduce malaria in children – the group at highest risk of dying from malaria.

1.7.1 RTS, -S Vaccine: A New Tool with Potential for Africa

Since 2000, the scale-up of proven interventions, such as insecticide-treated bed nets, has driven down malaria illness and death, but progress has slowed, particularly in Africa. Efforts are underway to increase the use of the tools we have, but even with scale-up of existing tools, there is a critical need for new tools to further drive down malaria illness and deaths – the RTS, -S malaria vaccine may be one of those tools. The malaria vaccine can reduce malaria in children, including cases of severe malaria, related hospital admissions, and the need for blood transfusions. The current pilot program will help guide and optimize the vaccine's future use.

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