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Chronology of Drug Development for Malaria

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1.1 Introduction

The deadly malaria parasite is transmitted by mosquitoes and has been documented since earlier times. Malaria still remains an important cause of illness and death in children and adults in countries in which it is endemic. World Health Organization defines malaria as a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes, and it is preventable and curable [1]. The name malaria comes from the Italian word *mal'aria*, meaning "bad air." This was due to the reason that people thought the disease was transmitted via contaminated air (miasmas). Human malaria is caused by four different species of *Plasmodium* namely *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*. Of the four, *P. falciparum* and *P. vivax* pose the greatest threat. Scientists succeeded in sequencing the *P. falciparum* genome in 2002, which has permitted researchers to make great progress in comprehending better ways to target it [2].

The history of malaria depicts many attempts to defeat it. Quinine, a substance derived from the bark of the cinchona tree, has been known to be effective against malaria since the 1600s [3]. After discovering the role of mosquitoes in malaria transmission, scientists focused on vector control. They presumed that by killing the vector, they could halt the cycle of infection. As a result, DDT and other insecticides came into vogue in the mid-1900s and have been used ever since. Bed nets to protect sleeping people from mosquito bites are another form of vector control that is not only effective, but also extremely cost-effective [4]. Subsequently, the development of different anti-malaria drugs has changed the way travelers view malaria-endemic countries and the risk associated with traveling. In fact, and likely due to all of the above measures, estimated deaths from malaria fell 13%, from 755 000 in 2000 to 655 000 in 2010 [5]. Cases of the disease fell as well, although less dramatically, from 223 million in 2000 to about 216 million in 2010.

With all of these developments, why does malaria remain a problem? The emergence of resistance to drugs and insecticides is a major concern. Research in recent decades has shed light on many aspects of *Plasmodium* biology, broadening

Drug Development for Malaria: Novel Approaches for Prevention and Treatment, First Edition.
Edited by Pravin Kendrekar.

understanding of how parasites interact with the human immune system, cause human disease, and are transmitted by mosquitoes [6]. The malaria parasite has survived for more than 50 000 years, and natural selection favors strains of the organism with mutations that help them evade threats. Today we are seeing more and more drug-resistant parasites and insecticide-resistant mosquitoes. Global efforts are underway in the next era of malaria prevention: the development of malaria vaccines that have the potential to save countless lives and that could ultimately help eradicate this historic plight.

The World Malaria Report 2019 by WHO pays attention to the burden of malaria in two high-risk groups – children and pregnant women. In 2018, an estimated 228 million cases occurred worldwide and 405 000 deaths were reported. Twenty-seven countries reported less than 100 cases of malaria in 2018 compared with 17 countries in 2010. Inadequate funding being a barrier to future progress renewed R&D agenda is one of the major priorities to achieve a malaria-free world.

The report details on regional and global trends in burden of malaria cases and deaths, maternal, infant, and child health consequences of malaria, high burden to high-impact approach, investments in malaria programs and research, preventing malaria, diagnostic testing and treatment, world malaria report 2019 malaria surveillance systems and responding to biological threats to the fight against malaria [7].

The global technical strategy for malaria 2016–2030 adopted by the World Health Assembly in May 2015 provides a comprehensive framework to guide countries in their efforts to accelerate progress toward malaria elimination. The strategic framework comprising of three major pillars and two supporting elements sets the target of reducing global malaria incidence and mortality rates by at least 90% by 2030. The three major pillars being: (i) ensure universal access to malaria prevention, diagnosis, and treatment; (ii) accelerate efforts toward elimination and attainment of malaria-free status; and (iii) transform malaria surveillance into a core intervention. The two supporting elements are (i) innovation and research and (ii) a strong enabling environment. The program aims at achieving the vision – a world free of malaria [8].

There are many review articles emphasizing on the approaches available for prevention and treatment of malaria. This chapter aims at summarizing the information available on the erstwhile, current, and promising future in the drug development for malaria (Figure 1.1).

1.1.1 Life Cycle of Malaria (Adapted from CDC)

The malaria parasite life cycle involves two hosts [9].

During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host. Sporozoites infect liver cells and mature into schizonts, which rupture and release merozoites. (Of note, in *P. vivax* and *P. ovale*, a dormant stage [hypnozoites] can persist in the liver [if untreated] and cause relapses by invading the bloodstream weeks or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony). Merozoites infect

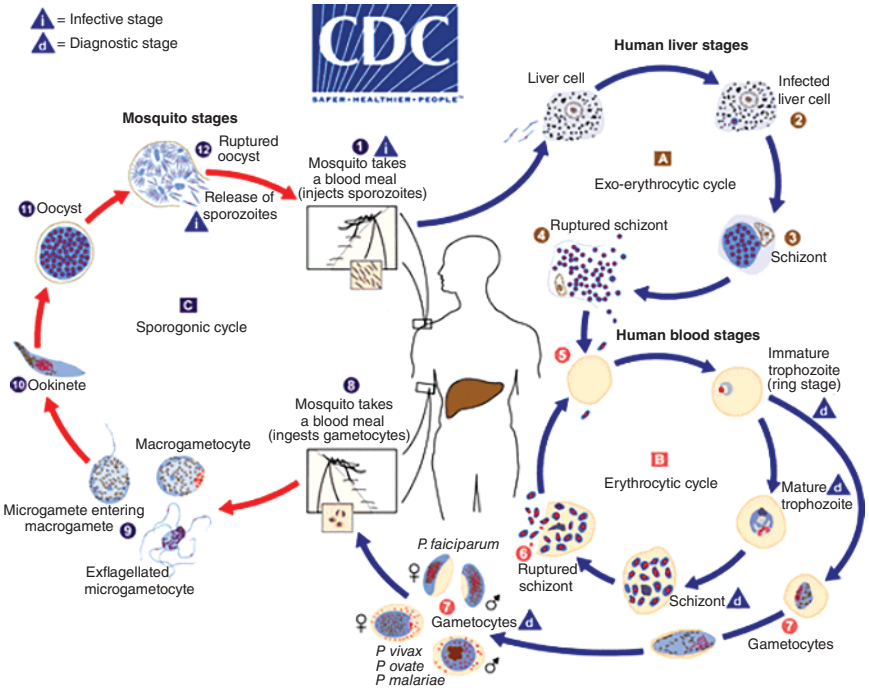


Figure 1.1 Life cycle of malaria adapted from CDC.

red blood cells. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male and female, are ingested by an *Anopheles* mosquito during a blood meal. The parasites' multiplication in the mosquito is known as the sporogonic cycle. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes), which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle.

1.2 Malaria – Erstwhile Memories

1.2.1 Progress Fighting Malaria

Since the dawn of modern microbiology research, beginning in the nineteenth century, scientists have known that the disease is actually caused by a microscopic parasite called Plasmodium, which is spread by mosquitoes common to wet, marshy places. Two of the earliest Nobel Prizes went to the scientists who made these basic discoveries, and at the dawn of the twentieth century, the situation had never seemed

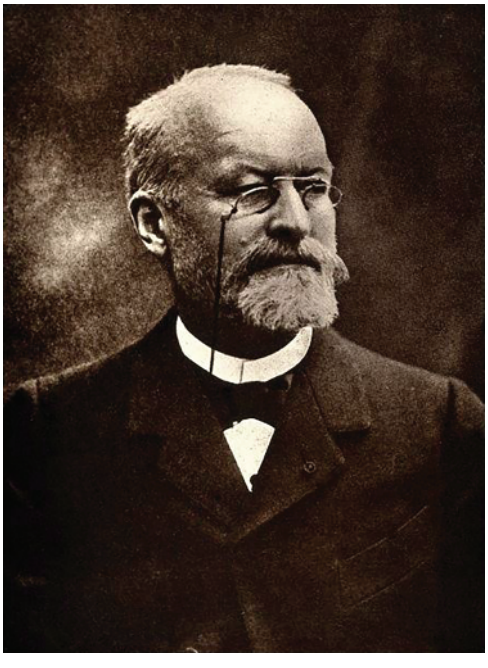


Figure 1.2 Charles Louis Alphonse Laveran first identifies the malaria parasite. Credit: Photo Researchers/ Science History Images/Alamy Stock Photo.

brighter. The possibility that malaria would be eliminated or eradicated was exciting and real then. History proved otherwise (Figures 1.2 and 1.3, Table 1.1).

Full malaria eradication was a major public health effort in the first half of the twentieth century and was intensively pursued after World War II. Since that effort was launched, 108 countries have eliminated malaria from within their borders, with another 39 countries en route to that goal. Despite those efforts, malaria remains a major cause of illness in many parts of the world. Today almost half the world's population lives in places where the disease is common.

According to the Centers for Disease Control and Prevention, about 1500 cases of malaria still occur in the United States each year, but most are imported when people travel abroad. The real problem exists in several Asian and sub-Saharan African countries, where malaria is both a major leading cause of death and a significant drain on the economy. The World Health Organization estimates that the disease eats up nearly half of all public health expenditures and measurably lowers the gross domestic product of countries where it is common.

Several new approaches to controlling malaria have become available in the last few decades, such as insecticide-treated bed nets, but there remains a dire need for new drugs and for effective vaccines to control it.

There are many references in early history indicating malaria afflicting mankind. In earlier times herbal remedies found importance in treatment of malaria [11]. The first efficacious treatment for malaria was quinine from the bark of the cinchona

Figure 1.3 Sir Ronald Ross first demonstrates mosquitoes transmit malaria.

tree. With the association of spread of malaria to mosquitoes, many control measures such as spraying of insecticides, covering of open water surfaces, insecticide treated nets were initiated.

With the global increase in malaria cases, need was felt to develop stronger measures in prevention and treatment of malaria. Following the isolation of quinine from the bark of cinchona tree in 1820, a number of other natural and synthetic drugs have been developed as shown in the table below (Table 1.2):

1.3 Current Chemotherapy Used to Treat Malaria

Antimalarials mostly act by mechanisms that seek to inhibit one or two stages of the parasite's life cycle. The treatment aims to act on the parasite in two different ways. One of them is to interrupt the schizogenic blood stage responsible for the symptoms of the disease, that is, to kill the parasite during the evolutionary cycle. The other is to employ drugs that prevent the development of gametocytes parasites in the tissue cycle of the species *P. vivax* and *P. ovale*, interrupting the transmission of the parasite and avoiding relapses. Various drugs are available for achieving these goals, where each acts in a specific way to inhibit the development of the parasite in the host (Table 1.3).

Table 1.1 Major events in discovery of antimalarials.

1820	Quinine first purified from tree bark. For many years prior, the ground bark had been used to treat malaria
1880	Charles Louis Alphonse Laveran first identifies the malaria parasite. He was awarded the 1907 Nobel Prize for the discovery
1898	Sir Ronald Ross demonstrates that mosquitoes transmit malaria. He wins the 1902 Nobel Prize for this work
1934	Hans Andersag in Germany discovers the anti-malarial drug Chloroquine, which is not widely used until after World War II
1939	Paul Hermann Muller in Switzerland tests the insecticide DDT. He won the Nobel Prize for this work in 1948
1952	Malaria is eliminated in the United States
1957	First documented case of resistance to Chloroquine is reported
1976	William Trager and JB Jensen grow parasites in culture for the first time, opening the way for drug discovery and vaccine research
1989	The U.S. Food and Drug Administration approves the use of the anti-malaria drug Mefloquine hydrochloride, registered as Lariam® by Hoffman-LaRoche
1992	Malaria vaccine candidate RTS, -S, developed by GlaxoSmithKline and the Walter Reed Army Institute of Research, enters clinical trials
2000	The U.N. General Assembly adopts the Millennium Development Goals, setting a target to halt and begin reversing malaria incidence by 2015
2001	WHO prequalifies first fixed-dose Artemisinin combination therapy (ACT), sold by Novartis as Coartem® and recommends ACT as first-line malaria treatment
2002	Genome sequencing of *Anopheles gambiae* (mosquito) and *Plasmodium falciparum* (parasite) completed
2005	The World Health Assembly adopted a target of 80% worldwide coverage of insecticide nets and ACTs by 2010
2007	UCSF study shows combination malaria therapy effective in treating African children
2007	World Malaria Forum convenes in Seattle, hosted by Bill and Melinda Gates Foundation
2008	The Global Health Group at UCSF comes forward with the first high-level strategy for the eventual achievement of malaria eradication. This strategy has since been widely adopted
2008	The United Nations adopted April 25 as World Malaria Day

Source: Data from Ref. [10].

A great challenge for malaria treatment in recent decades has been to overcome the parasite's ability to acquire resistance to antimalarials, requiring the development of more effective drugs.

(1) **Aminoquinoline** [13, 14]

In general they are the derivatives, i.e. quinolines substituted in any position by one or more amino groups. Depending upon the substitution, they are further classified to 4-aminoquinoline and 8-aminoquinoline. Drugs that belong to this class are chloroquine (CQ), amodiaquine, primaquine, bulaquine, etc.

Table 1.2 Origin of antimalarial drugs.

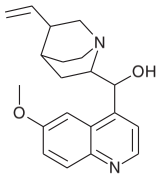
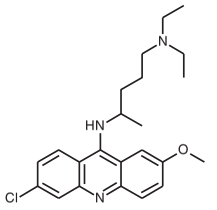
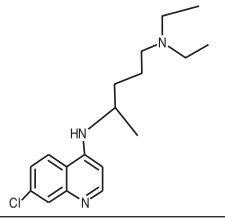
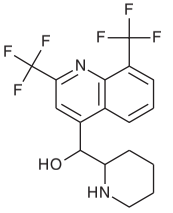
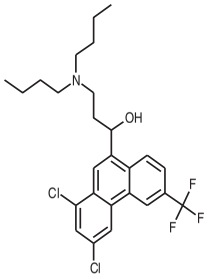
Name of the drug	Structure	Origin	Year	Current status
Quinine		Natural Alkaloid from bark of cinchona tree	1820	Resistance was reported in 1980 but still used in severe malaria cases when artemisinin was not available. Appears on WHO's Model List of Essential Medicines (MLEM)
Mepacrine (Quinacrine)		Synthetic Acridine derivative	1930	No longer used due to side effects of toxic psychosis
Chloroquine		Synthetic Quinoline derivative	1934	Resistance reported in 1950. Appears on MLEM and used where resistance is not developed and for treatment of *P. vivax*

Table 1.2 (Continued)

Name of the drug	Structure	Origin	Year	Current status
Mefloquine		Synthetic	1970	No longer used due to CNS toxicity. Appears on MLEM to be used in combination with artesunate
Halofantrine		Synthetic	1980	Still used in patients free of heart diseases. Used in severe and resistant forms of malaria

Source: Based on Tse et al. [12].

Table 1.3 Classification of the antimalarial drugs.

Past drugs	A. Aminoquinolines	Chloroquine, Hydroxychloroquine, Amodiaquine
	B. 8-Aminoquinolines	Primaquine, Tafenoquine, Bulaquine
	C. Arylamino alcohols	Quinine
Present drugs	D. Methanols i. 4-Quinoline methanol ii. 9-Phenanthrene methanol	Mefloquine Halofantrine, Lumefantrine
	E. Biguanides	Proguanil
	F. Diaminopyrimidines	Pyrimethamine
	G. Antimalarial endoperoxides i. First generation endoperoxides (Artemisinin derivatives) ii. Second generation endoperoxides a. Trioxanes b. Tetroxanes	Artesunate, Artemether, Arteether
	H. Hydroxynaphthoquinone	Atovaquone
	I. Benzonaphthyridine derivative	Pyronaridine
	J. Antibiotics	Sulfonamides, Tetracycline, Doxycycline, Clindamycin, Azithromycin

Mechanism of action: Generally not known, but it believes that it accumulates in acidic pH Vescuoles, thus forming drug – heme complex thus preventing the free heme toxic to be available for the parasite.

(2) **Methanols/aryl amino alcohol** [15]

Drugs that belong to this class include quinine, lumefantrine, mefloquine, halofantrine

Mechanism of action: Interfere with the digestion of hemoglobin in blood stage of malaria life cycle by accumulating in the acidic vacuole of the parasite. Higher concentration of drug leads to polymerization of heme.

(3) **Biguanides** (https://go.drugbank.com/drugs/DB01131, [16])

Popularly known as proguanil (chloroguanide). It is a biguanide derivative, which is converted to an active metabolite called cycloguanil pamoate.

Mechanism of action: By inhibiting the enzyme parasitic dihydrofolate reductase (DHFR) thus inhibiting bacterial growth. It has both suppressive and prophylactic attack against *P. falciparum* and also effective against vivax malaria.

(4) **Diaminopyrimidines** [17]

Like biguanides, they also inhibit enzyme DHFR, which interfere in the synthesis of tetrahydro folic acid, a cofactor needed for bacteria to make the nucleotide bases thymine, guanine, uracil, and adenine.

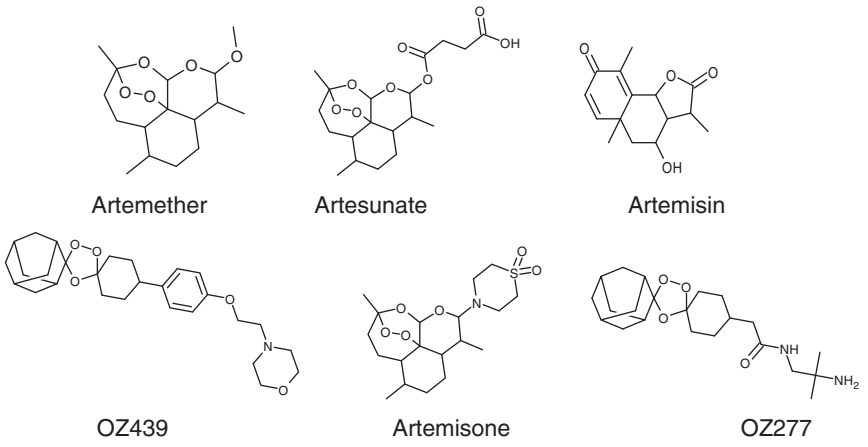


Figure 1.4 Structure of antimalarial drugs belonging to class endoperoxide.

(5) **Antimalarial endoperoxides** [18]

Drugs belonging to this class include artemisinin (ART) and its derivatives, first- and second-generation endoperoxides. This class of antimalarial drugs has several advantages over other antimalarial drugs

- Little or no cross resistance
- Clear the peripheral blood parasite
- Short half life
- Sustained effect

ART and its derivatives also act as gametocytocidal and effective against specific stages in the malaria life cycle. Trophozoites and parasites are more susceptible while the liver stage of *P. vivax* and *P. falciparum* is not affected.

Mechanism of action: It acts as free radical scavengers, thus high oxygen tension is increased and. Drugs in this class produce oxygen free radicals such as superoxide, which damage the malarial cell. Other effects can also be seen as hemolysis, decreased erythrocytes deformability.

It acts differently from other drugs; instead of reacting with oxygen, it produces large quantities of oxygen-free radicals such as superoxide and OH^-.

(6) **Hydroxynaphthoquinone** [19, 20]

Drugs belonging to this class, i.e. atovaquone, are used for both prevention and treatment of malaria. Usually atovaquone is given in a fixed-dose combination with proguanil. Its primary mode of action is it acts on mitochondrial functions. And with proguanil, it gives asynergistic effect and interferes with mitochondrial membrane potential.

Mechanism of action: It inhibits cytochrome c reductase activity in *P. falciparum* by binding with cytochrome bc1 complex of the parasite mitochondrial electron transport chain. Thus, disposes of electrons generated by dihydroorotate dehydrogenase during the synthesis of pyrimidines and inhibition of this process kills parasites (Figures 1.4–1.9).

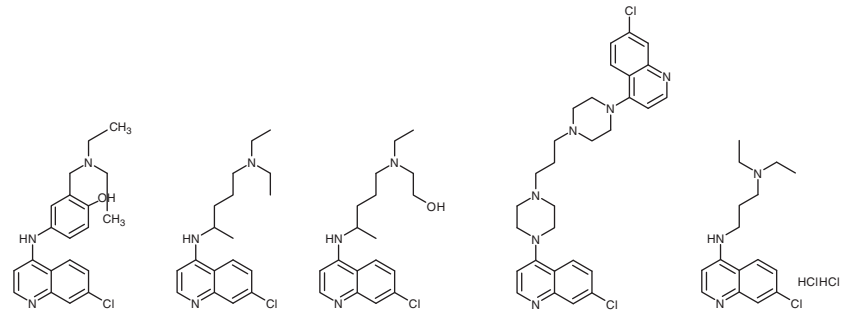


Figure 1.5 Structure of antimalarial drugs belonging to class 4-aminoquinolines.

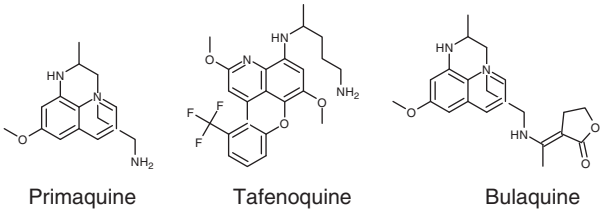


Figure 1.6 Structure of antimalarial drugs belonging to class 8-aminoquinolines.

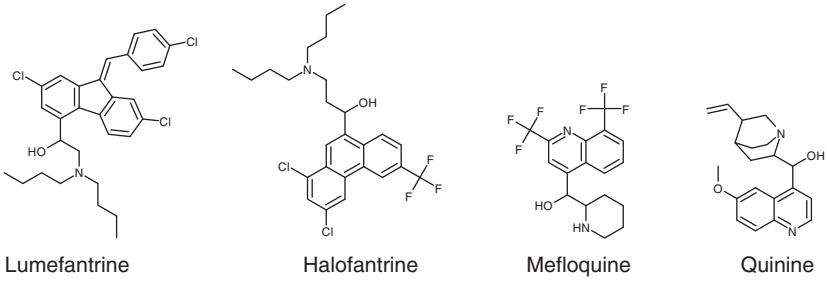


Figure 1.7 Structure of antimalarial drugs belonging to class quinoline derivatives.

1.3.1 Current Combination Therapy

The combination of a fast-acting ART derivative and a long-acting antimalarial with different mechanisms of action is called artemisinin-based combination therapy (ACT). The use of ACTs with at least two drugs is recommended by the WHO to prevent or reduce the development of antimalarial resistance. These combined therapies may act concomitantly through several mechanisms of action and in different biochemical targets of the parasite, achieving better results than monotherapy. Replacement of monotherapy by ACT has started in all countries where *P. falciparum* is endemic. ART derivatives are considered the basis for the treatment of *P. falciparum* malaria and are used to combat uncomplicated malaria.

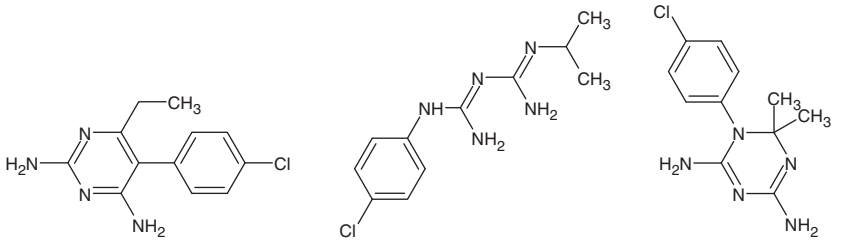


Figure 1.8 Structure of antimalarial drugs belonging to class antifolates.

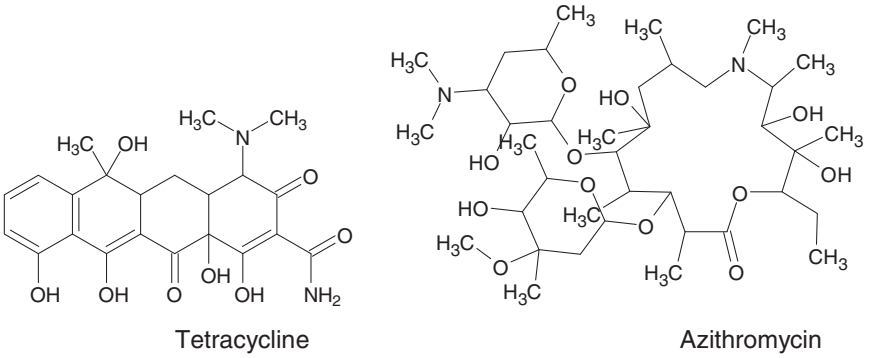


Figure 1.9 Structure of antimalarial drugs belonging to class antibiotics.

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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