Chapter

Molecular Approaches for Malaria Therapy

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Abstract

Malaria is a potentially fatal blood disease spread by mosquitos. Malaria is preventable, but it is more prevalent in developing countries where prevention is difficult and prophylaxis is often inaccessible. Malaria remains one of the world's most serious public health problems, according to the World Health Organisation (WHO). The development of resistance is a current problem that poses a danger to the environment. Resistance is a current problem that could jeopardise the use of well-established and cost-effective antimalarials. The World Health Organisation recommends an artemisinin-based drug combination (ACT) to avoid or postpone the development of resistance. This book's chapter discusses current medicines as well as potential and rational possibilities for finding new drugs to treat malady. There were also WHO recommendations for both complicated and non-complicated malaria. Other preventive measures such as ITN and IPT are listed in the manuscript in addition to routine care. While a brief overview of the vaccine tested so far has been included, there is currently no vaccine available to treat malaria.

Keywords: Malaria, *Plasmodium falciparum*, artemisinin, drug repurposing, drug resistant malaria

1. Introduction

Malaria is a life-threatening disease spread by mosquito bites from infected female Anopheles mosquitos ("malaria vectors"). The Plasmodium parasite is borne by infected mosquitos. When an individual is bitten by this mosquito, the parasite is released into the bloodstream. Malaria is caused by a parasitic protozoan of the genus Plasmodium. *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae,* and *Plasmodium falciparum* are the four types of malaria parasites that can infect humans. *Plasmodium falciparum* and *Plasmodium vivax* are the two most deadly species, responsible for more than 95 percent of all malaria cases worldwide. Each year, approximately 125 million pregnant women are at risk of infection; maternal malaria is linked to up to 200,000 reported child deaths in Sub-Saharan Africa. [1, 2] Maternal malaria is often linked to a number of negative outcomes for the mother, the foetus, and the infant.

The sporozoite form of the protozoan is preserved in the salivary glands of the mosquito. When a person is bitten by an infected female Anopheles mosquito, sporozoites are injected into the bloodstream and easily move into the human liver.

The sporozoites replicate asexually in the liver cells for the next 7 to 10 days, causing no symptoms. The parasites are released in the form of merozoites from the liver cells and settle in the capillaries of the lungs. Merozoites are released from lung capillaries in the blood phase (also known as pathologic blood stages) of their growth, invading red blood cells (erythrocytes) and multiplying until the cells burst. This cycle continues forever, invading younger red blood cells. These infections in the blood can last for months. Some infected blood cells break the asexual multiplication cycle. Merozoites evolve into sexual forms of the parasite called gametocytes (precursors of male and female gametes) in these cells, which then circulate in the bloodstream. Malaria parasites will now abandon their human hosts and complete their life cycle in an insect vector. When a fertilised mosquito bites an infected individual, the gametocytes in the blood are swallowed by the mosquito, and the gametocytes mature in the mosquito gut. An ookinete is a fertilised, motile zygote produced when male and female gametocytes fuse. Ookinetes grow into new sporozoites, which migrate to the salivary glands of the bug, ready to infect a new host and restart the human infection cycle (**Figure 1**) [3].

Only certain species of mosquitoes of the Anopheles genus—and only females of those species—can transmit malaria. Chills, high fever, profuse sweating, headache, muscle pains, malaise, diarrhoea, and vomiting are all common symptoms of malaria. Malaria, on the other hand, is marked by occasional paroxysmal febrile episodes (i.e., a sudden recurrence or intensification of fever), and untreated infection results in spleen enlargement. *P. falciparum* can affect the lungs, liver, and kidneys, as well as cause extreme anaemia and coma in cerebral malaria, which sometimes leads to death. P. malariae infection can cause kidney damage, which can lead to nephrotic syndrome, which can be fatal. Malaria infections are highly debilitating and can render a person vulnerable to other diseases [4].

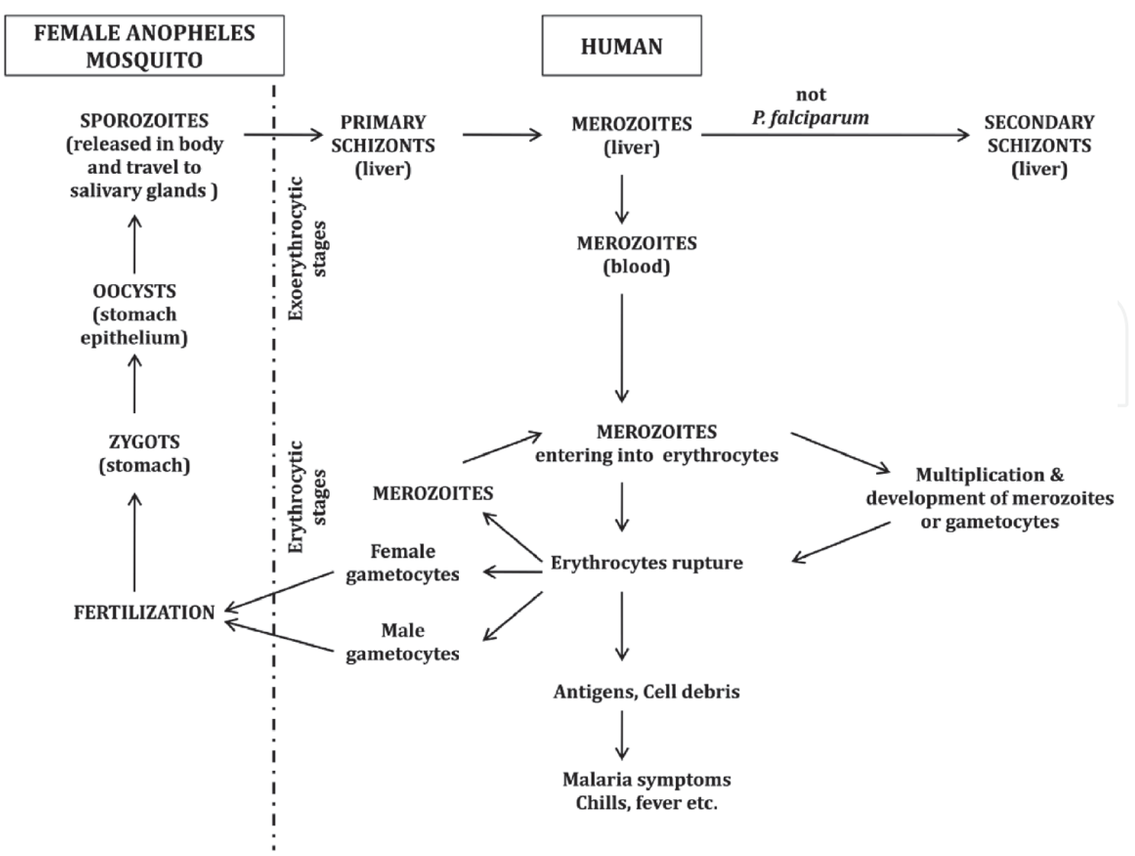

Figure 1.
Malaria parasite life cycle; outlines the steps of the parasite as it is injected into the human victim.

2. Prevention

The primary method for preventing and reducing malaria transmission is vector control. All people at risk of malaria should be covered with successful malaria vector control, according to the WHO. Insecticide-treated mosquito nets and indoor residual spraying are two types of vector control that are effective in a variety of situations [1].

2.1 Insecticide-treated mosquito nets

An insecticide-treated net (ITN) is a mosquito-blocking bed net that has been treated with a protected, residual insecticide for the purpose of killing and/or repelling mosquitoes. By providing both a physical barrier and an insecticidal effect, sleeping under an ITN may minimise mosquito-human interaction. Nets treated with insecticide have been shown to minimise infant mortality. Uncomplicated *P. falciparum* and *P. vixax* malaria episodes are also reduced by ITN [1, 2].

2.2 Indoor residual spraying (IRS)

Indoor residual spraying (IRS) consists of spraying a residual insecticide on the walls and other housing structures once or twice a year [1]. Mosquitoes and other insects that come into contact with these surfaces can be destroyed by the insecticide. Several pesticides have been used for IRS in the past, with DDT being the first and most well-known. Pyrethroids are also used in IRS, where they are sprayed on indoor surfaces.

3. Vaccines against malaria

Since there is currently no effective way to eradicate malaria, developing safe, effective, and cost-effective vaccines against the disease remains a top priority. The malaria vaccine RTS,S/AS01E (RTS,S; (brand name MosquirixTM) is the first and only vaccine to demonstrate that it can substantially reduce malaria in young African children, including life-threatening extreme malaria. RTS,S/AS01E is a candidate for a pre-erythrocytic *P. falciparum* vaccine. Over a four-year period, the vaccine prevented approximately 4 in 10 cases of malaria among children who received four doses in large-scale clinical trials [3]. A vaccine targeting the whole body, a live attenuated vaccine, a genetically modified vaccine, and a subunit vaccine are among the four alternatives to a malaria vaccine that have been studied. Preclinical and clinical studies have recently revealed that Pf sporozoite (SPZ) vaccines show great promise for human safety, but larger sample sizes are required to confirm their protective effects [3, 4].

4. Treatment

Malaria must be identified quickly in order to minimise infection spread in the population and avoid deaths. Before beginning care, the WHO suggests that all reported cases of malaria be confirmed using microscopy or a rapid diagnostic test.

4.1 Antimalarial drugs

Malaria-endemic areas are visited by an estimated 50 million tourists per year. Chemoprophylaxis, which suppresses the blood stage of malaria infections, may be used to avoid malaria in such travellers. WHO recommends at least three doses of sulfadoxine-pyrimethamin intermittent preventive treatment for pregnant women, particularly those living in moderate-to-high transmission areas. Similarly, for infants living in high-transmission areas of Africa, three doses of sulfadoxine-pyrimethamine intermittent preventive care are recommended. Seasonal malaria chemoprevention has been advised by the World Health Organisation (WHO) since 2012. It entails giving all children under the age of 5 months monthly courses of amodiaquine plus sulfadoxine-pyrimethamine [5].

5. Cinchona alkaloids

The medicinal use of the bark of the cinchona tree, which is native to South America, is the starting point for the discovery and synthesis of quinine and synthetic quinoline-containing antimalarial drugs. The quinoline derivatives quinine, quinidine, cinchonidine, and cinchonine are the four most abundant biologically active alkaloids found in the bark (**Figure 2**). The bark also includes quinoline derivatives such as quinicine (also known as quinotoxine) and indole-containing alkaloids including cinchonamine [6].

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5.1 Quinine and quinidine

Quinine is the first example of a pure chemotherapeutic agent to be produced on an industrial scale and it was the only drug available for the treatment of malaria

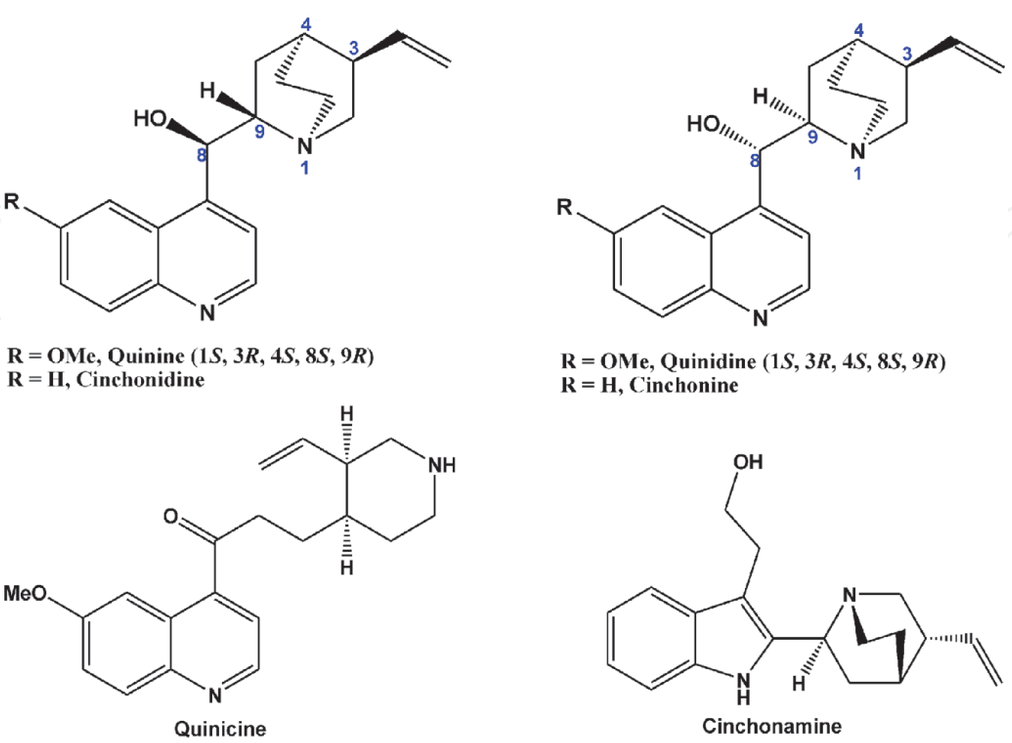

Figure 2.
Structures of the four major and two minor quinoline containing alkaloids obtained from the bark of the cinchona tree.

until the 1930s. Quinine has been used for "fevers" in South America since the 1600s. It is one of the major alkaloids was first used to treat malaria as early as the beginning of the 17th century, and became the standard therapy for malaria from the mid-19th century to the 1940s. The extraction of QN is still more economically viable than its synthetic production [6]. The pure alkaloids, quinine, and cinchonine were isolated in 1820. The stereoisomer, quinidine, is a more potent antimalarial, but it is also more toxic (less selectively toxic). Quinine is lethal for all *Plasmodium* schizonts and the gametocites from *P. vivax* and *P. malariae*, but not for *P. falciparum*. Today, quinine's spectrum of activity is considered too narrow for prophylactic use relative to the synthetic agents [7].

The emergence of resistant strains of *P. falciparum* was first reported in the 1980s [8] and as of 2006, quinine is no longer used as a front-line treatment for malaria but is still on the WHO's Model List of Essential Medicines (MLEM) [9] for the treatment of severe malaria in cases where artemisinins are not available. The mechanism of resistance to quinine is poorly understood and varies with the susceptibility of the parasite to other aminoquinoline antimalarial drugs. Quinine is the only treatment recommended for pregnant women in the first trimester3 and, until recently, it was the only clinical option for the treatment of severe malaria because it can be formulated for safe intravenous administration. However, intravenous artesunate is now preferred when available [10].

QN in clinical uses most often combined with a second agent to shorten the duration of therapy and thus minimise the adverse effects [11]. A toxic syndrome is referred to as cinchonism. Symptoms start with tinnitus, headache, nausea, and disturbed vision. If administration is not stopped, cinchonism can proceed to involvement of the gastrointestinal tract, nervous and cardiovascular system, and the skin. The stereoisomer, quinidine, is a schizonticide, but its primary indication is cardiac arrhythmias. It is a good example where stereochemistry is important because it provides a significantly different pharmacological spectrum [12].

6. 4-Aminoquinolines

4-aminoquinolines (4-AQ), 4-anilinoquinolines, 9-aminoacridines, and azaacridines are all members of this class. These groups are thought to have similar mechanisms of action, are successful on the same stage of the parasite, and may share similar mechanisms of resistance, in addition to their structural similarity (**Figures 3** and **4**).

4-Aminoquinoline is a type of aminoquinoline in which the amino group is located at the quinoline's 4-position. Antimalarial agents derived from 4-aminoquinoline can be used to treat erythrocytic plasmodial infections. Amodiaquine, chloroquine, and hydroxychloroquine are some examples [13].

Malaria is treated with 4-aminoquinolines (4-AQs) during the blood stage of the disease (the merozoites). The unprotonated form of these drugs is a weak base that can cross the food vacuolar membrane of parasites; however, once within the vacuole, both the quinoline nitrogen and the amino group of the side chain of 4-AQs become protonated species that is impermeable to the vacuolar membrane, causing ion-trapping inside the vacuole [14].

6.1 Chloroquine and hydroxychloroquine

Chloroquine, a 4-aminoquinoline, prevents ferriprotoporphyrin IX polymerisation, causing oxidative membrane damage and parasite death in infected erythrocytes. Because of widespread resistance, it's normally only used to treat malaria

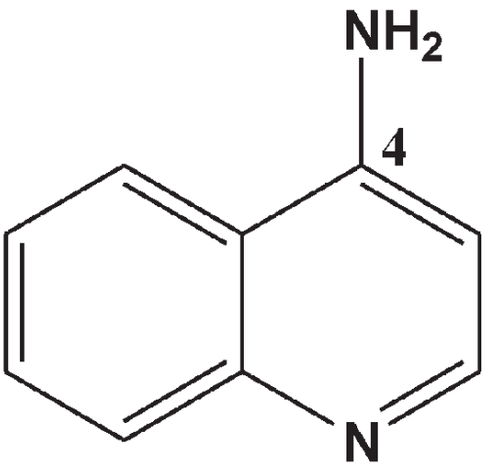

Figure 3.
General chemical structure of 4-aminoquinoline.

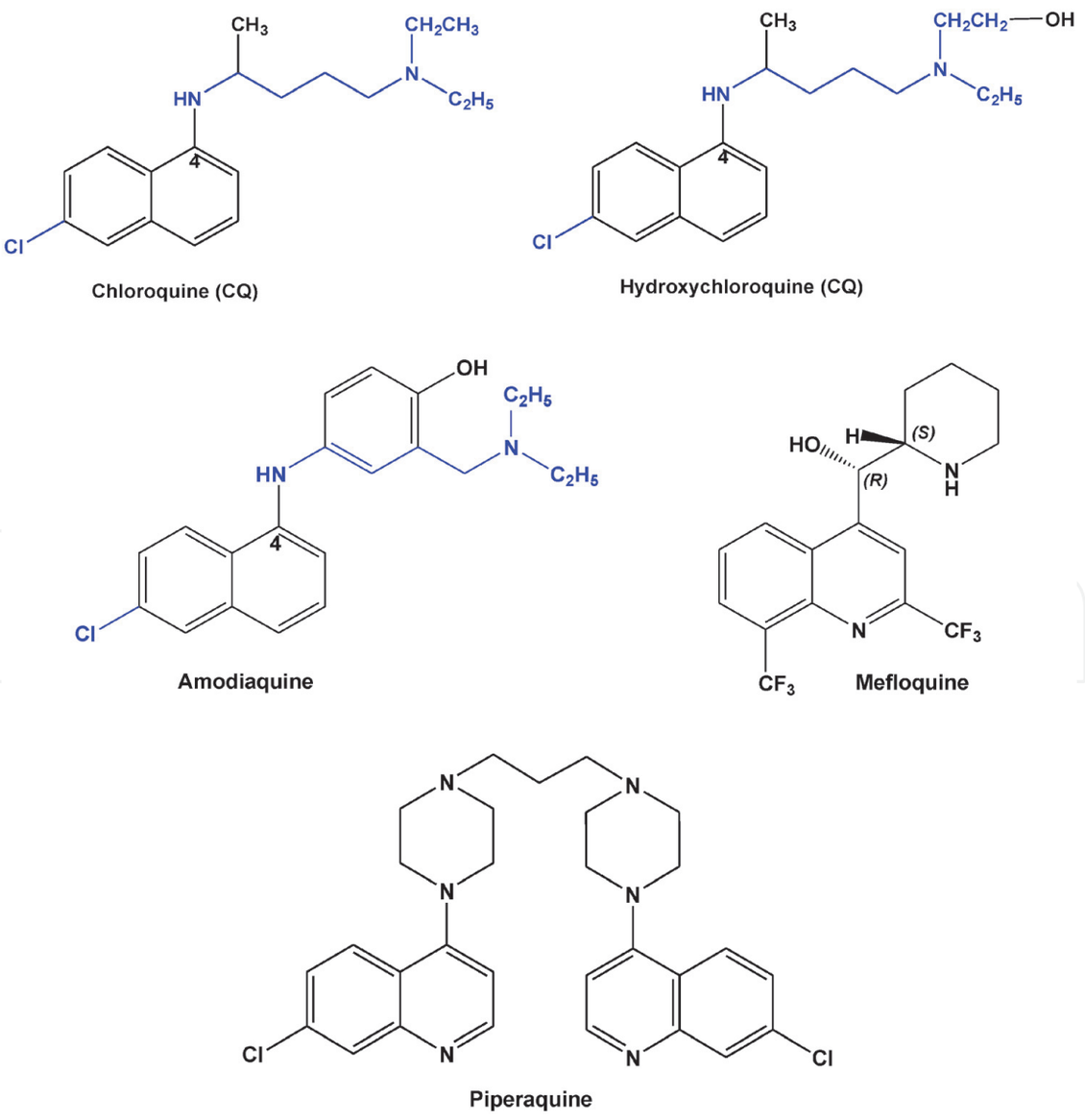

Figure 4.
Structures of some representative 4-aminoquinolines.

patients in situations where chloroquine susceptibility can be guaranteed. Malaria caused by *P. falciparum* is almost always immune to choloroquine, with the exception of cases acquired in Haiti.

Chloroquine is only effective against the parasite's erythrocytic stages; for the full cure of *P. vivax* and *P. ovale* infections, another agent (primaquine) is needed. Chloroquine has been used in conjunction with dehydroemetine to treat invasive amebiasis that has not responded to other treatments and to treat connective tissue autoimmune disorders that have not responded to other treatments.

Chloroquine is thought to get stuck in the parasite's food vacuole, where it prevents -hematin from crystallising. The acidic nature of the vacuole (pH 4.8–5.2) causes chloroquine to become 'trapped' in its membrane-impermeable doubly protonated shape, which is membrane-impermeable. Chloroquine then forms a complex with free heme, causing heme to accumulate and the parasite to die. *P. falciparum* resistant strains were discovered to have a mutation in the Pfcrt gene, which encodes the chloroquine resistance transporter (Pfcrt) protein [8, 9]. Because of changes in the membrane protein, this transporter protein induces reduced drug concentration inside the food vacuole, allowing chloroquine to disperse away from the vacuole. CQ-resistant parasite strains have a neutral threonine residue at position 76 of the Pfcrt protein in place of the positively charged lysine moiety, allowing chloroquine efflux from the digestive vacuole [15–17].

Within the parasite's food vacuole, the parasite catabolises the protein of the host cell haemoglobin, resulting in peptides, which are further degraded to produce amino acids, which are used by the parasite for survival and development. As a byproduct of haemoglobin degradation, free heme (iron (II) centred porphyrin) is produced, which quickly oxidises to hematin (iron (III) centred species). Heme and hematin are also extremely toxic to parasites. Heme may interfere with the parasite's organelles' various membranous structures, causing irreversible damage and disrupting transport processes and ion homeostasis. Multiple pathways result in parasite death. 25.19 Biomineralisation removes heme and hematin, resulting in hemozoin, a parasite-unfriendly substance. Hloroquine prevents or inhibits the parasite's ability to detoxify heme, resulting in parasite death [18–20].

Chloroquine tablets are bitter in taste and are available in the United States. Chloroquine suspensions are commonly available for paediatric use in other countries and are much more well tolerated. As a malaria chemoprophylactic, chloroquine may be used. Most people tolerate chloroquine well, even when used for long periods of time. Mild gastrointestinal symptoms (which are usually relieved if the medication is taken with food), intermittent headaches, blurred vision, dizziness, weakness, confusion, hair depigmentation, skin eruptions, corneal opacity, weight loss, and myalgias are all potential side effects. Antihistamines are commonly used to treat intense pruritus, which is a common problem among black Africans who take the medication. Patients with psoriasis, retinal disease, or porphyria should avoid chloroquine. In most respects, hydroxychloroquine is similar to chloroquine. The only structural difference is a hydroxy moiety on one of the N-ethyl groups. It stays in the body for over a month, much like chloroquine, and prophylactic dosing is once weekly. Children tolerate hydroxychloroquine better [21–24].

6.2 Amodiaquine

Amodiaquine was first documented to have antimalarial activity in 1946, but due to its toxicity, it was removed as a prescribed monotherapy in the early 1990s. It acts in a similar way to chloroquine and has some cross-resistance with CQ, but it does not have any advantages over other 4-aminoquinoline drugs [25] (**Figure 5**).

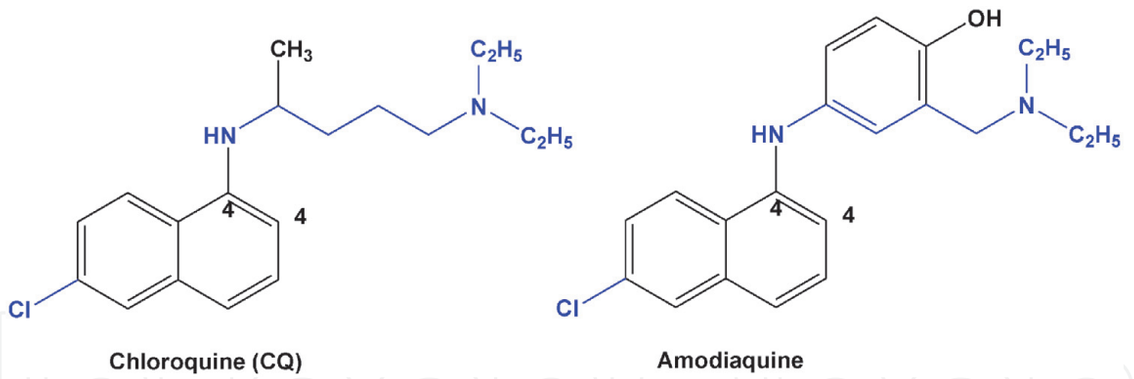

Figure 5.
The inter-nitrogen distance in the side chains of amodiaquine and CQ.

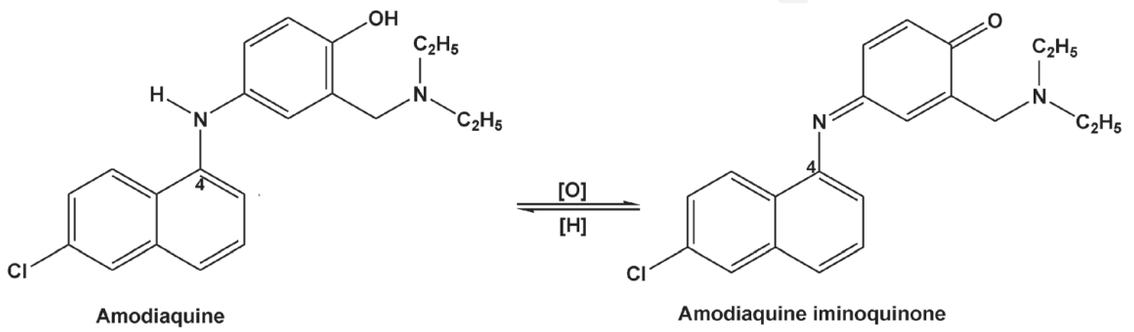

Figure 6.
Metabolic activation of amodiaquine to amodiaquine iminoquinone.

It had a higher rate of extreme hepatitis and agranulocytosis than chloroquine when used for malaria prophylaxis. The toxicity of amodiaquine is thought to be due to P450-mediated and/or autooxidation of the 1, 4-aminophenol group, which produces a quinone imine intermediate [26] (**Figure 6**).

Amodiaquine's efficacy against some chloroquine-resistant *Plasmodium falciparum* strains has led to a resurgence in its use, especially in combination therapy with artesunate. Although amodiaquine resistance in some parts of Africa can restrict the effectiveness of this combination in those areas, the artesunate–amodiaquine combination has proven to be very effective in areas where amodiaquine alone produces responses of more than 80% [27].

6.3 Mefloquine

The racemic form of mefloquine is the newest of the 4-aminoquinolines. The drug's optical isomers are all active in the same way. The US Army [28] built it in the 1970s. It was initially developed to treat chloroquine-resistant malaria, but it has since been used as a curative and prophylactic medication (travellers coming into regions of malaria) (**Figure 7**).

Mefloquine differs from other 4-aminoquinoline agents in that it has two trifluromethyl moieties at positions 2 and 8, and no electronegative substituents at positions 6 (quinine) or 7 (mefloquine) (chloroquine). Mefloquine is not schizonticidal, which distinguishes it from chloroquine and its analogues. Mefloquine is slowly metabolised to carboxymefloquine, its main inactive metabolite, through CYP3A4 oxidation. The majority of the parent compound is excreted in its natural state in the urine [29] (**Figure 8**).

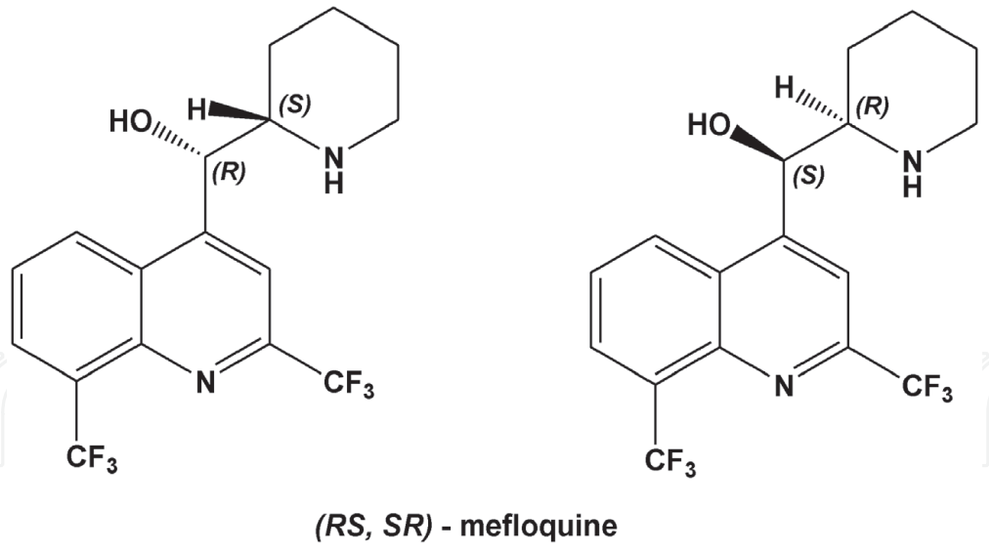

Figure 7.
*Racemic forms (*RS *&* SR*) of mefloquine.*

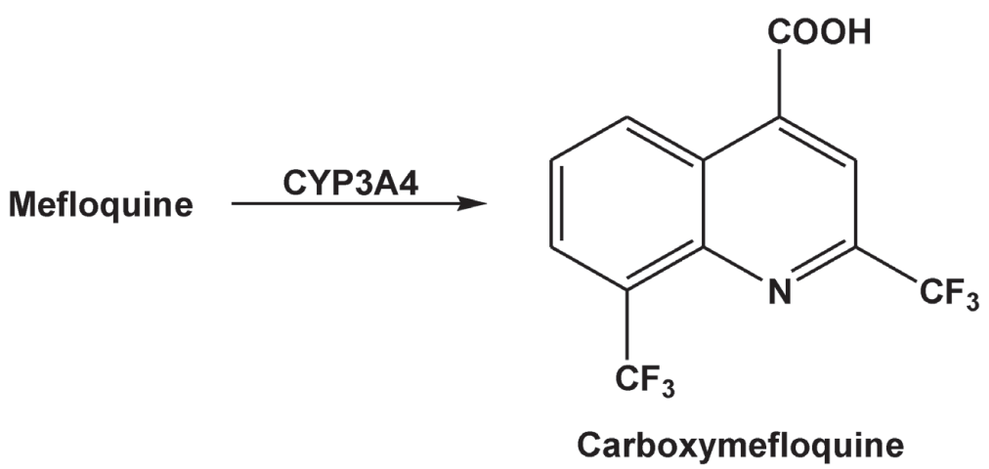

Figure 8.
Metabolic activation of mefloquine to carboxymefloquine.

P. falciparum mefloquine-resistant strains were first identified in 1986 [23]. In rats, rodents, and rabbits, mefloquine causes teratogenicity. This medication comes with an FDA-mandated warning that it can worsen mental illnesses, and the neuropsychiatric effects can be severe (e.g., suicidal impulses or seizures) or mild (e.g., headaches) (e.g., dizziness, vertigo, ataxia, and headaches). Bradycardia, arrhythmias, and extrasystoles are all possible cardiovascular side effects [30].

7. 8-Aminoquinolines

Another big class of antimalarial drugs based on the cinchona alkaloid quinoline moiety is substituted 8-aminoquinolines (**Figure 9**). The 8-aminoquinolines were the first synthetic antimalarial drugs approved by the FDA. The German researchers introduced pamaquine as the first compound in this sequence. Due to its high toxicity and restricted activity, pamaquine was no longer used in clinical trials [31]. In 1950, primaquine was introduced as a pamaquine analogue. It was the only approved treatment for removing the Plasmodium parasite from the liver and preventing malaria relapses caused by *P. ovale* and *P. vivax* until July 2018 [32].

In patients with erythrocytic glucose-6-phosphate dehydrogenase deficiency, all of the 8-aminoquinolines have been reported to cause hemolytic anaemia. This is a common genetic trait in people who live in malaria-endemic areas [33]. Since 4-AQs are active during the blood stages of the parasite life cycle, and the blood stages of *P. falciparum* can be cultured and thus studied relatively easily, the mechanism of action for 8-aminoquinolines (8-AQs) is less well known than for 4-aminoquinolines (4-AQs) [34, 35].

The mechanism of action for the 8-AQs cannot be inhibition of hemozoin formation because liver cells do not produce haemoglobin. Primaquine suggested an autoxidation of the 8-amino group to produce ROS. Augusto et al. [36] suggested the creation of a radical anion at the 8-amino group. Cell-destructive oxidants like hydrogen peroxide, superoxide, and the hydroxyl radical can form as a result, causing oxidative damage to essential cellular components.

The structure–activity relationships in this series display very little variance. The four agents in **Figure 9**, all have the same 6-methoxy moiety as quinine, but the substituent on quinoline are at position 8 rather than carbon-4, as they are on cinchona alkaloids. Between the two nitrogens, all of the agents in this sequence have a four to five carbon alkyl linkage or bridge. The other three 8-aminoquinolines, with the exception of pentaquine, all have one asymmetric carbon. Although there are some variations in the metabolism of each stereoisomer and the form of adverse reaction, there are little differences in antimalarial activity based on the stereochemistry of the compounds. In patients with a glucose-6-phosphate dehydrogenase deficiency, all of the 8-aminoquinolines can cause hemolytic anaemia (G6PD). This is a common genetic trait found in people who live in malaria-prone areas. The key clinical problems associated with primaquine are a short half-life (4–6 hours), which means it must be taken everyday for 14 days to be successful, and hemolysis [37–40].

7.1 Primaquine

Primaquine (PQ), an 8-aminoquinoline that has been clinically used since 1950, is still the only drug used worldwide to treat relapsing *P. vivax* malaria caused by

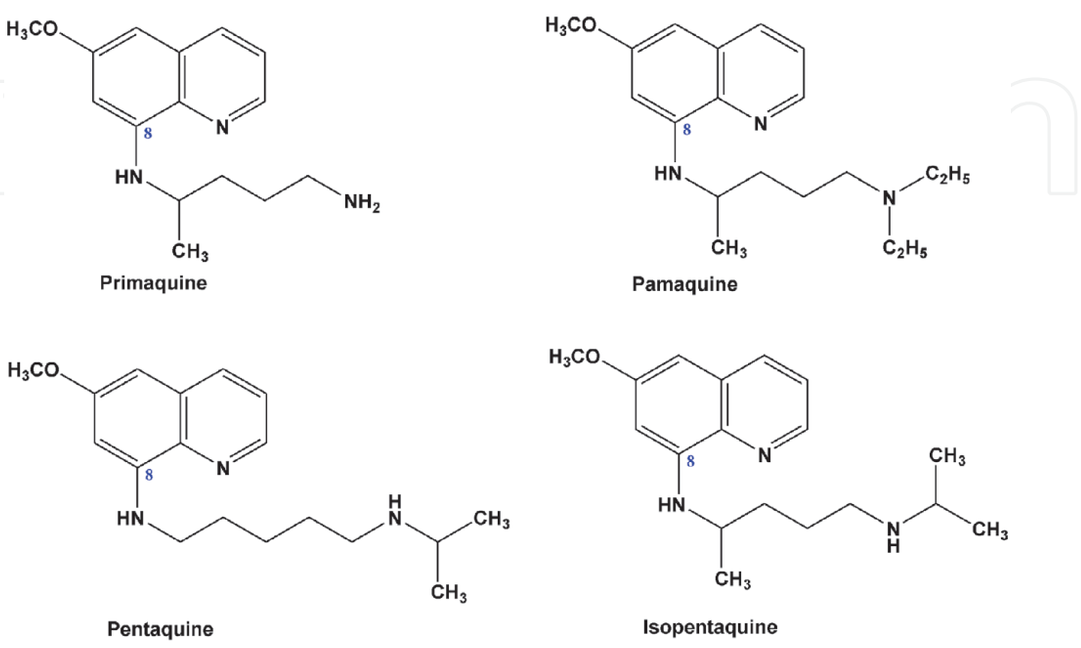

Figure 9.
Structures of some representative 8-aminoquinolines.

hypnozoites, and it inhibits gametocyte formation. It is not used in the prevention of disease. The only drug available for treating *P. vivax* and *P. ovale* latent hepatic life cycle forms is primaquine [41, 42]. Following the initiation of chloroquine treatment for the erythrocytic stages of infection, primaquine is recommended for the radical cure of these infections. It has one of the narrowest spectrums of activity of any currently used antimalarial drug, as it is only indicated for exoerythrocytic *P. vivax* malaria. Chloroquine or a drug prescribed for chloroquine-resistant *P. vivax* is combined with primaquine to treat endoerythrocytic *P. vivax*. It is also active against the exoerythrocytic stages of *P. ovale* and the main exoerythrocytic stages of *P. falciparum* [43], in addition to its accepted indication.

Primaquine is readily absorbed, widely spread, and cleared mainly by non-renal removal. Carboxyprimaquine is the primary metabolite. Primaquine is successful when administered once daily or even once weekly [44], despite the fact that the medication is quickly removed from the plasma. Except in people with G6PD deficiency, where administration can cause brisk hemolysis, primaquine is typically well tolerated. Prior to starting primaquine therapy, patients should be tested for G6PD deficiency. Regardless of G6PD status, primaquine should never be given to a pregnant woman. Neutropenia, gastrointestinal disturbances, and methemoglobinemia are rare side effects. *P. vivax* relapses after primaquine therapy have been treated with chloroquine and higher doses or longer courses of primaquine [45].

Primaquine is the most effective currently available prophylactic for *P. vivax* malaria and equivalent to such regimens as doxycycline, mefloquine, and atovaquone-proguanil for the prevention of *P. falciparum* malaria, according to a recent meta-analysis and systematic review [46, 47]. NPC1161B, a chiral 8-aminoquinoline derivative developed at the University of Mississippi, was still undergoing preclinical testing in 2014 [48–52].

8. Artemisinin and its derivatives

Artemisinin (ART), a sesquiterpene lactone[1], was discovered in 1971 by Tu Youyou, a Chinese scientist, in the plant *Artemisia annua* (a herbaceous plant in the Asteraceae family), which is widely used in Chinese traditional medicine [55]. Youyou shared the Nobel Prize in Physiology or Medicine in 2015 for "her findings concerning a novel therapy against malaria" [56], owing to the significant positive effect of ART in the fight against malaria.

Artemisinins are successful not only against multi-resistant strains of *Plasmodium falciparum*, but they also have strong stage specificity against the Plasmodium life cycle, including activity during the asexual blood stages [57] as well as the sexual gametocyte stages, which may help to minimise disease spread in low-transmission areas [58]. Artemisinin resistance was first recorded in western Cambodia in 2008 [59]. ART and its derivatives have been used as first-line drugs in the treatment of malaria since their antimalarial activity was discovered.

Since chemical synthesis of ART is considered to be costly, the key commercial sources of ART are field-grown leaves and flowering tops of *A. annua*. Since mature plants will lack the active drug, the plant must be grown from seed every year. To

[1] Sesquiterpene lactones (SLs) are a type of sesquiterpene with a lactone ring; a sesquiterpene has three isoprene (2-methyl-1,3-butadiene) units. Lactones are cyclic carboxylic esters with a 1-oxacycloalkan-2-one structure (C(=O)O); sesquiterpene lactones (SLs) are present primarily in Asteraceae plants (daisies, asters). Umbelliferae (celery, parsley, carrots) and Magnoliaceae (magnolias) are two other plant families with SLs [53, 54].

maximise artemisinin yield, the increasing conditions must be perfect. Plants grown in North Vietnam, China's Chongqing province, and Tanzania have recorded the highest yields so far [60].

As compared to other compounds historically and currently used, the artemisinin sequence is structurally distinct. The endoperoxide (C–O–O–C) and dioxepin oxygens tend to form a "trioxane," which appears to be the most significant structural element. Artemisinine, a sesquiterpene trioxane lactone with an endoperoxide bridge that is necessary for antimalarial activity, does indeed represent a new chemical class of antimalarial agents. It distinguishes artemisinins from other antimalarial drugs by limiting cross-resistance. Artemisinin derivatives such as artemether, artesunate, and arteether are the most common. These semisynthetic derivatives are prodrugs that are converted to dihydroartemisinin, the active metabolite. Unlike quinine, artemisinin derivatives destroy young circulating parasites until they sequester in the deep microvasculature [61–65] (**Figure 10**).

Following oral administration, the artemisinins are rapidly absorbed, with maximum plasma concentrations occurring in 2 to 3 hours for artemisinin and artemether, and less than 1 hour for artesunate [66, 67]. Artemisinin is transformed to inactive metabolites in the liver, such as deoxyartemisinin, deoxydihydroartemisinin, and others, where the endoperoxide group is lost and the metabolites become ineffective. CYP2B6 is the enzyme that catalyses the reaction. Different artemisinin derivatives are metabolised. They're converted to dihydroartemisinin first (DHA). DHA is a potent antimalarial molecule that lasts for two to three hours in the bloodstream. Artesunate's antimalarial operation is mediated exclusively by DHA. (Direct antimalarials include artemisinin, arteether, artemether, and others.) Within a minute of absorption, artesunate is converted to DHA. DHA is converted to inactive metabolites in the liver by the cytochrome P450 enzyme system (which includes CYP2A6, CYP3A4, and CYP3A5). Both metabolites are glucuronidated before being excreted in the urine or faeces. Artemisinins are relatively safe drugs due to their quick metabolism [68–70].

Artesunate is a water-soluble semisynthetic form of artemisinin that can be taken orally or injected intravenously or intramuscularly. Artesunate is superior to artemisinin and other oil-based derivatives [71] due to its chemical property and pharmacokinetic profile, as it is almost instantly converted into dihydroartemisinin after ingestion, which accounts for the antimalarial activity. The endoperoxide pharmacophore alone has stimulated the production of many different groups of totally synthetic endoperoxides, including the trioxolane OZ277 [72] and the tetraoxane 3 [73], despite the fact that the exact mechanism of action is still highly debated [72] (**Figure 11**).

Although the exact mechanism of artemisinin is unknown, it is thought to be triggered by haem, which produces free radicals, which damage parasite survival proteins [74, 75]. The initial formation of highly reactive oxygen-centered radicals

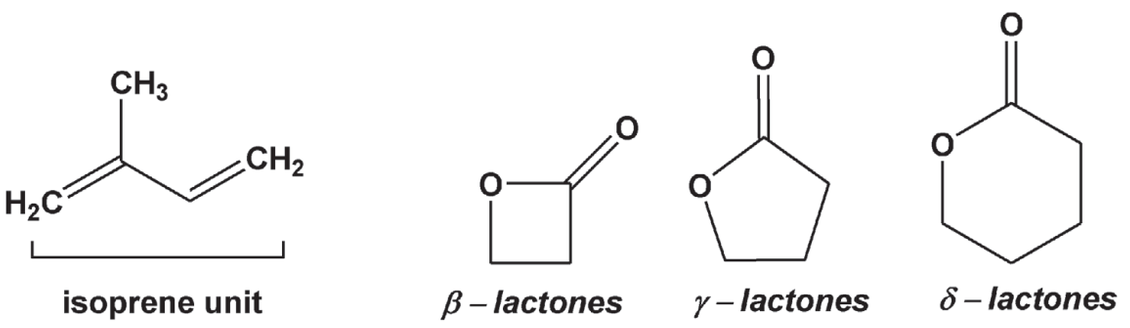

Figure 10.
Structural units of artemisinine family of compounds.

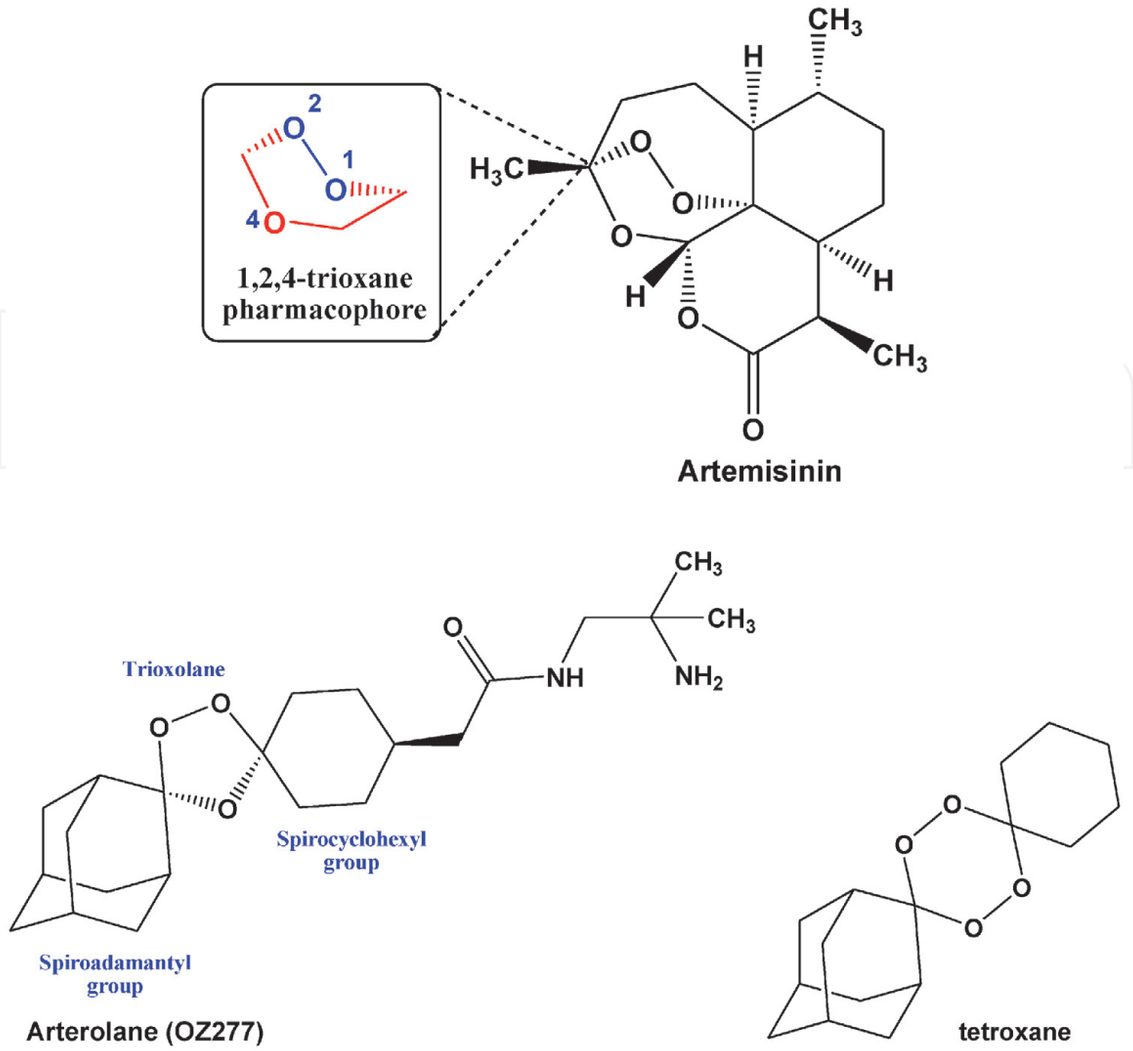

Figure 11.
Pharmacophoric structures of ART derivatives.

by iron(II)-catalysed homolytic cleavage of the peroxide bond is thought to result in rapid intramolecular rearrangement to give cytotoxic carbon-centered radical organisms, which then cause widespread damage to parasite biomolecules by alkylation or by initiating peroxidation (**Figure 12**).

9. Polycyclic antimalarial drugs

Three antimalarial drugs have polycyclic ring structures in common (**Figure 13**). Doxycycline, a popular tetracycline antibiotic, is the first. The second is halofantrine, and the third is quinacrine [76], a discontinued agent that was used in the South Pacific.

9.1 Halofantrine

The Walter Reed Army Institute of Research [77] developed halofantrine in the 1960s and 1970s. It is a phenanthrene-type compound that is structurally distinct from all other antimalarial drugs. The trifluromethyl moiety [78] is an excellent example of drug design that integrates bioisosteric concepts. Halofantrine is a synthetic antimalarial that functions as a blood schizonticide but has no effect on

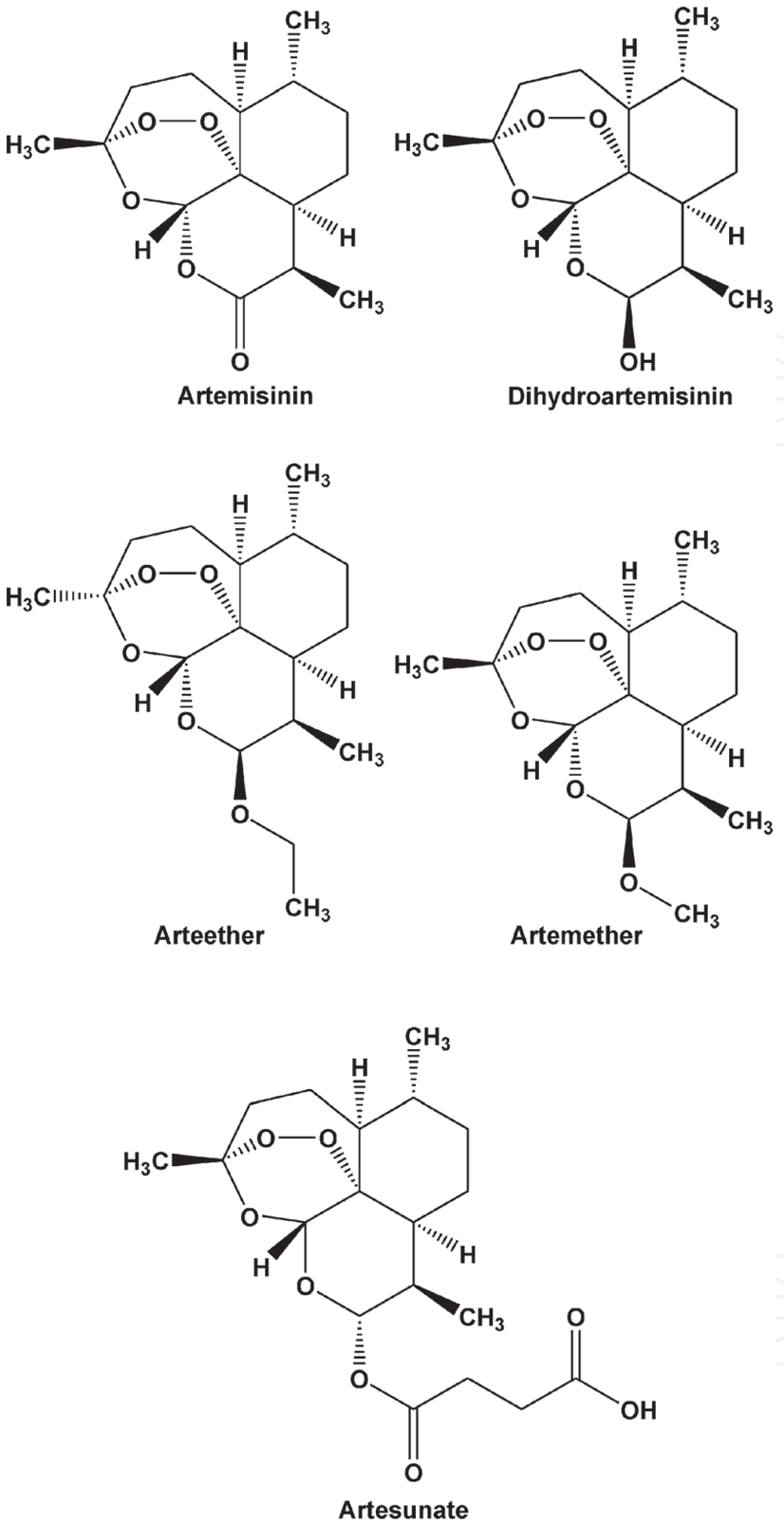

Figure 12.
Structures of ART derivatives.

the sporozoite, gametocyte, or hepatic stages of the parasite. It works against *P. falciparum* malaria that is immune to several drugs (including mefloquine).

The mechanism of action of halfantrine against the parasite is unknown. It tends to prevent heme molecules from polymerising (by the parasite enzyme "heme polymerase"), allowing the parasite to become infected by its own waste. CYP3A4 converts halofantrine to N-desbutyl-halofantrine, which is excreted primarily in the

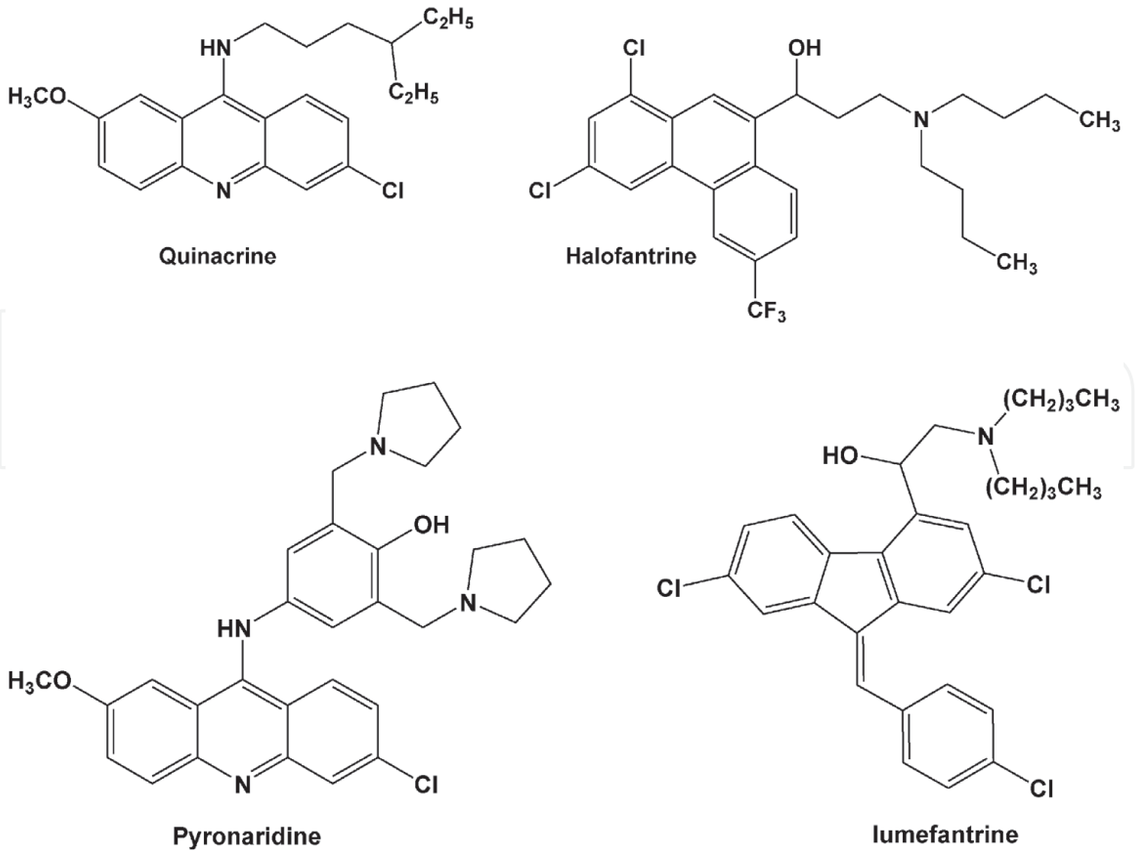

Figure 13.
Structures of some representative polycyclic antimalarial compounds.

faeces; both the parent compound and the N-desbutyl metabolite are involved in vitro.

Halofantrine is usually well tolerated, but consistent absorption requires that it be taken with a fatty meal. Halofantrine has been shown to block open and inactivated HERG channels preferentially, disrupting nerve conduction in cardiac tissue and inducing cardiotoxicity. Cardiac arrhythmias are the most dangerous side effect: halofantrine induces severe QT prolongation [79], which is seen even at normal doses.

9.2 Doxycycline

Doxycycline is a tetracycline derived from oxytetracycline that inhibits pathogen protein synthesis by reversibly inhibiting the 30S ribosomal subunit, preventing the 50s and 30s units from bonding. Since the ribosomal subunits of bacteria and Plasmodium differ significantly from mammalian ribosomes, this class of antibiotics does not readily bind to mammalian ribosomes and therefore has good selective toxicity [80].

Antimalarial doxycycline is mainly used for prophylaxis against *P. falciparum* strains immune to chloroquine and sulfadoxine–pyrimethamine. During malaria exposure, 100 mg (adult dose) of doxycycline should be given every day as prophylactic therapy. It can also be used in conjunction with quinine to treat resistant *P. falciparum* infections, although it has a slow onset of action in acute malaria and should not be used alone [81]. When treating acute cases, 100 mg of doxycycline per day for seven days should be given in accordance with quinine.

Doxycycline is well absorbed when taken orally (90–100% when fasting; decreased by 20% when taken with food or milk) and excreted partially in the faeces and partially in the urine. Since tetracyclines chelate calcium, they can

interfere with the growth of permanent teeth in children. Doxycycline should only be used in children for a limited period. Tetracycline also increased photosensitivity, which is important because areas where malaria is endemic also have the most sunlight [82].

9.3 Pyronaridine

Pyronaridine was developed in 1970 at the Institute of Chinese Parasitic Disease [83, 84], and has been used in clinical trials in China since the 1980s. It is one of the components of the artemisinin combination therapy pyronaridine/artesunate and has been shown to be effective against chloroquine-resistant strains (Pyramax). It's also being investigated as a possible anticancer medication and Ebola treatment. Pyonaridine, like lumefantrine, tends to interfere with haematin, but it does not seem to share resistance mechanisms with chloroquine [85]. Pyronaridine is a form of pyronaridine that is The most common side effects of pyronaridine are headache, vomiting, stomach pain, bradycardia, and hypoglycemia.

9.4 Lumefantrine

Lumefantrine was first synthesised in China in 1976 and is now only used in conjunction with artemether. This mixture is often referred to as a "co-artemether" [86]. Lumefantrine is very lipophilic and has a much longer half-life than artemether, so it's thought to remove any lingering parasites after a combined injection. [No. 29] Lumefantrine has an uncertain molecular weight, but studies indicate that it inhibits the development of -hematin by forming a complex with hemin [87].

9.5 Quinacrine

Quinacrine (also known as mepacrine) was commonly used as a prophylactic during WWII, and was marketed under the brand name Atabrine [88]. Quinacrine is a derivative of methylene blue, a different anti-malarial discovered in 1891 [89, 90]. Quinacrine is no longer used because it has a high risk of harmful side effects, such as toxic psychosis [88]. It is one of the most dangerous antimalarial medications, despite the fact that it was once widely used (**Table 1**).

Intercalation of DNA strands, succinic dehydrogenase and mitochondrial electron transport, and cholinesterase are all sites where it functions within the cell. It

Drug	Original indications	Recent status as new potential anti-malarials
Methylene blue	In treatment of Methaemoglobinemia	Completed Phase II trials in 2017 (NCT02851108) as a combination with primaquine
Fosmidomycin	Antibiotic	Phase II trials in 2015 (NCT02198807) as a combination with piperaquine
Rosiglitazone	Antidiabetic drug	In clinical trials as an adjunctive therapy for severe malaria (NCT02694874)
Imatinib	In cancer treatment	In Phase II trials (NCT03697668) as a triple combination with dihydroartemisinin-piperaquine
Sevuparin	In treatment of sickle cell disease	Last in Phase I/II trials in 2014 (NCT01442168) as a combination with atovaquone-proguanil.

Table 1.
List of drugs repurposed in malaria treatment.

has been used as a sclerosing agent and may be tumorgenic and mutagenic. Quinacrine can cause yellow discoloration of the skin and urine because it is an acridine dye.

10. Fixed combinations

Resistance is a common concern in malaria prophylaxis and treatment; combination therapy seeks to minimise resistance through synergism and, when combined with longer-acting medications, improved therapy. Combination therapies have been developed that employ two distinct mechanisms.

10.1 Sulfadoxine and pyrimethamine

A sulfonamide antibacterial drug and a pyrimidinediamine similar to trimethoprim are used in this combination. Sulfonamides can be used in conjunction with pyrimethamine in a variety of ways. A sulfonamide with similar pharmacokinetic properties to the dihydrofolate reductase inhibitor is usually used (**Figure 14**).

Sulfadoxine, a sulfonamide with a structure identical to p-aminobenzoic acid (PABA), inhibits the parasite's ability to synthesise folic acid, whereas pyrimethamine, a pyrimidinediamine, prevents folic acid from being reduced to its active tetrahydrofolate coenzyme type. Sulfonamides avoid the production of dihydropteroic acid by preventing the incorporation of p-aminobenzoic acid (PABA). Since humans do not need to synthesise folic acid, sulfonamides have excellent selective toxicity. Nonetheless, sulfadoxine has been linked to serious to fatal cases of erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, and serum sickness syndromes. Pyrimethamine, first formulated in the 1950s, prevents the conversion of folic acid and dihydrofolic acid to the active tetrahydrofolate coenzyme form, which is required for amino acid and nucleic acid synthesis [91]. This combination is recommended for the prevention and treatment of *P. falciparum* chloroquine resistance and can be used in conjunction with quinine. Despite the fact that the combination is only suggested for *P. falciparum*, it is successful against all asexual erythocytic forms. It does not have any impact on the sexual gametocyte form [92].

10.2 Atovaquone and proguanil

Two separate and unrelated mechanisms of action against the parasite serve as the foundation for this combination. Atovaquone is a dihydrofolate reductase inhibitor, and cycloguanil is a selective inhibitor of the Plasmodium mitochondrial electron transport system. In a ratio of 2.5 atovaquone to 1 proguanil HCl calculated

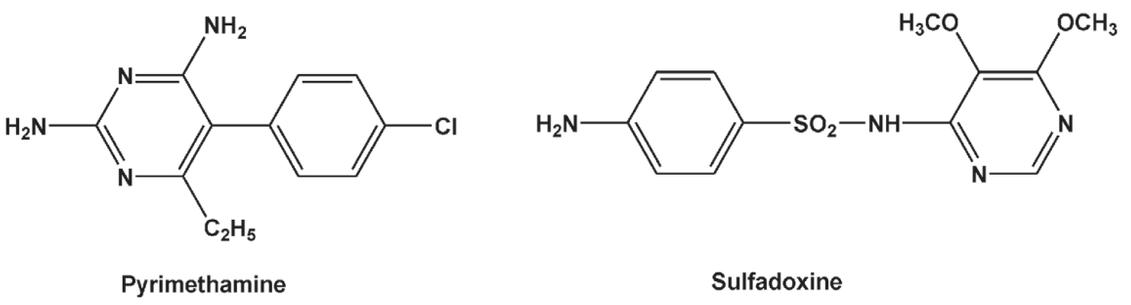

Figure 14.
Chemical structure of pyrimethamine and sulfadoxine in combination.

in mg (not mmoles), atovaquone and proguanil are given together [93]. The sporozoite stage is the primary focus of this mixture. Proguanil decreases the effective concentration of atovaquone needed to damage the mitochondrial membrane, while atovaquone increases the effectiveness of proguanil but not its active metabolite (Malarone) (**Figure 15**) [94–96].

Atovaquone was first formulated as an antimalarial, however due to its high failure rate (30%), it is no longer prescribed as a single chemical entity. However, atovaquone has been combined with proquanil to produce an effective prophylactic and therapeutic antimalarial [98]. Proguanil is an early example of a prodrug, having been produced in 1945. CYP2C19 is the enzyme that converts it to cycloguanil (**Figure 16**).

The chemistry of atovaquone is based on the fact that it is a naphthoquinone that participates in oxidation–reduction reactions as part of its quinonehydroquinone mechanism. The drug targets mitochondrial electron transport, specifically at the cytochrome bc1 site of the parasite. This deprives the cell of required ATP, potentially contributing to anaerobic conditions. A mutation in the parasite's cytochrome causes resistance to this drug, and a single-point mutation appears to be adequate [99]. The pharmacokinetics of atovaquone, when used as a monotherapy, are thought to be related to resistance. Since atovaquone is lipophilic and has a slow absorption rate, the pathogen is exposed to low concentrations of the medication for a prolonged period of time, which promotes resistance growth.

Cycloguanil (Proguanil) inhibits dihydrofolate reductase, which prevents deoxythymidylate synthesis. Amino acid shifts near the dihydrofolate reductase binding site are linked to resistance to proguanil/cycloguanil. Malaria immune to chloroquine, halofantrine, mefloquine, and amodiaquine is treated with this drug combination. To date, there has been no evidence of resistance to the combination [100].

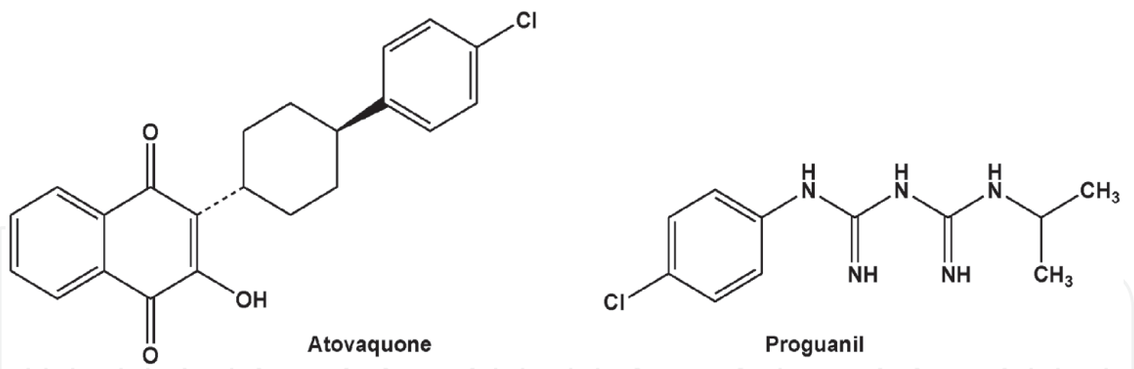

Figure 15.
Chemical structure of atovaquone and proguanil in combination. Sulfadoxine–pyrimethamine are among the other reportedly used combinations [97].

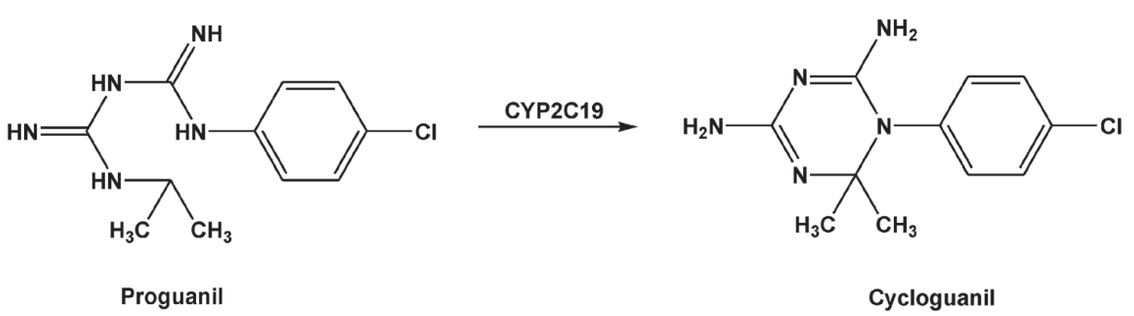

Figure 16.
Metabolic activation of proguanil to cycloguanil.

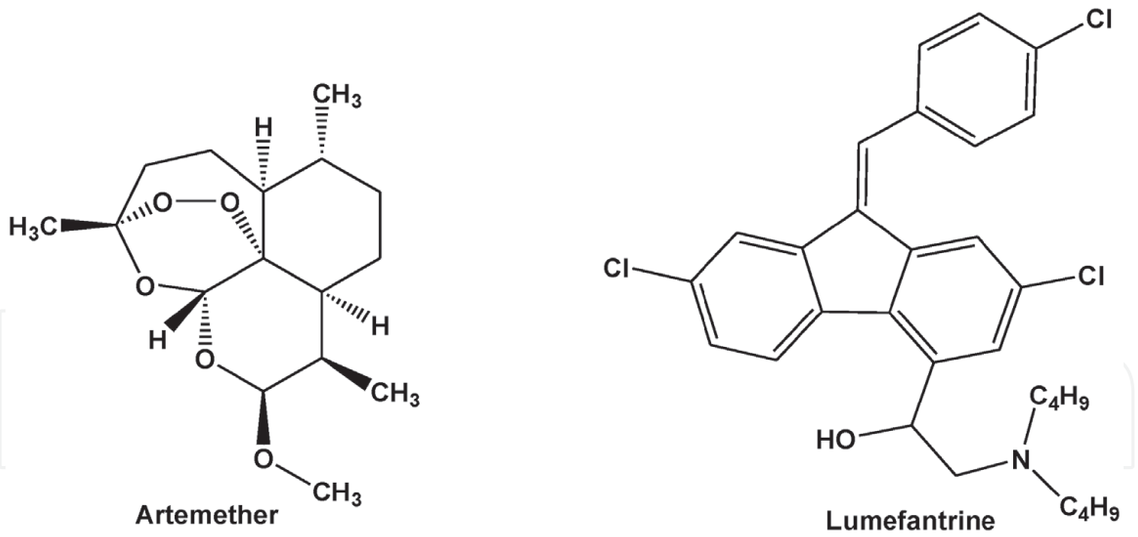

Figure 17.
Chemical structure of artemether and lumefantrine in combination.

10.3 Artemether and lumefantrine

Artemether–lumefantrine (Coartem) is one of the artemisinin-based combination therapy's fixed-dose formulations (ACT). The cure rates for these ACTs have been stated to be greater than 90%. Artesunate–fosmidomycin, amodiaquine–artesunate, chloroquine–artemisinin, and artesunate.

Artemether–lumefantrine interferes with heme metabolism, preventing development of parasite in erythrocyte states. Artemether acts oxidatively due to its endoperoxide, and lumefantrine can form a complex with hemin (**Figure 17**).

11. Future prospects of antimalarial drugs

With the recent emergence of resistance to existing frontline artemisinin-based combination therapy, the antimalarial drug pipeline is in dire need of newer lead molecules. The need for new anti-malarials that function through novel mechanisms of action has been moved to the forefront of the development agenda (**Figure 18**).

A variety of criteria are used to determine the ability of newer compounds to function as new anti-malarials: single-dose cures (artesunate and chloroquine are unable to do this); activity against both the asexual blood stages that trigger disease and the gametocytes that transmit the disease; compounds that avoid infection (chemoprotective agents); and compounds that clear *P. vivax* hypnozoites from the liver (anti-relapse agents) [101, 102].

Researchers can experiment with new combinations and formulations of currently available anti-malarial drugs. This may aid in the delivery of the drug, allowing it to be more successful, or it may help resolve issues with resistance to a specific component. The following are some recent methods used in the detection of new antimalarial agents:

1. Use of available antimalarial drugs to improve antimalarial therapy

2. Development of analogs of currently available medications.

3. Covalent bitherapy

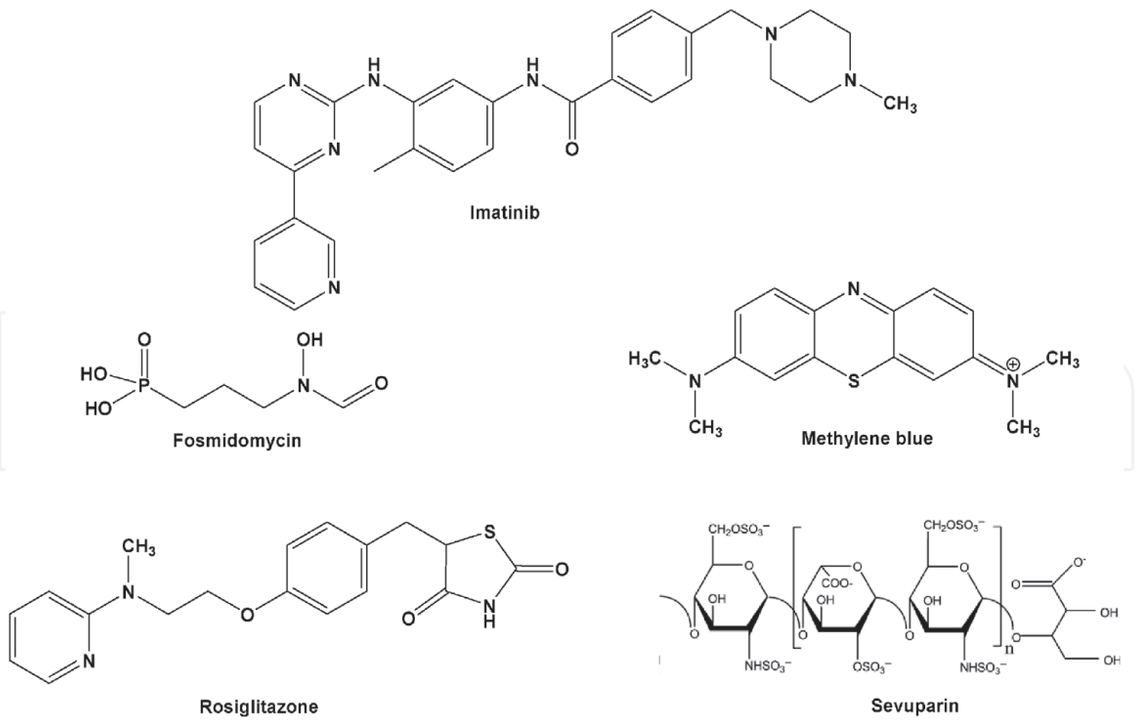

Figure 18.
Structures of some drugs repurposed for malaria therapy and are under various phases of clinical trials.

4. Exploitation of natural products and their derivatives.

5. Use of compounds that are effective against other diseases

6. Development of compounds that are active against novel targets.

7. Drug resistance reversers

8. Development of a malaria vaccine

11.1 Optimization of antimalarial therapy with available drugs

This technique involves mixing existing medications with new dosing regimens or formulations, as well as optimising treatment with currently available drugs. Other non-artemisinin-based combination therapies, in addition to the already proven and recommended artemisinin-based combination therapy, are intended to prevent the development of resistance, improve effectiveness, and tolerability. The idea is to combine two or more drugs with different mechanisms of action, resulting in different molecular targets in the parasite. In an area where *P. falciparum* transmission is endemic and Chloroquine resistance is high, chloroquine combined with sulfadoxine-pyrimethamine demonstrated a large therapeutic return over sulfadoxine-pyrimethamine alone.

11.2 Development of analogs of available drugs

This technique includes altering existing compounds chemically in order to produce newer compounds that are more potent, stronger, and cost-effective. The benefit of this strategy is that the biological target or mechanism of action of the lead compound is already known. Chemical modifications of quinine, for example, led to the discovery of chloroquine, halofantrine, and primaquine.

11.3 Covalent bitherapy

Covalent bitherapy entails the covalent bonding of two chemical moieties that act on different/same biological targets through different mechanisms of action [103–105]. Trioxaquine (SAR116242) is a synthetic hybrid compound with 1,2,4-trioxane and 4-aminoquinoline pharmacophores covalently related [106–108]. Since covalently linked pharmacophore is designed to target the parasite by: alkylation of heme with the trioxane moiety and binding of heme with the aminoquinoline moiety to inhibit hemozoin formation [109, 110], trioxaquine has been found to work on both asexual and sexual stages of malaria parasites.

11.4 Exploitation of natural products and their derivatives

Natural ingredients have long been a mainstay in drug production. The study of natural products, mostly by reverse pharmacology, yields useful knowledge about molecular models for the creation of new drugs. Natural molecular scaffolds including quinine, artemisinin, febrifugine, spiroindolone, and lapachol, for example, were discovered in herbal medicinal products [111].

11.5 Exploitation of compounds active against other diseases

This technique, also known as drug repurposing, could be used to combat malaria. Existing medicines that were previously used for other purposes could be found to be effective against malaria and repurposed as a new anti-malarial medication. N. M. Pazhayam et al. [112] looked at a variety of FDA-approved drugs that could be repurposed as antimalarials.

11.6 Development of compounds active against novel targets

The sixth strategy is the most clinically tested and appealing modern chemotherapy strategy. It requires knowledge of genomics and proteomics methods that have the potential to speed up the detection of new drug targets and the subsequent discovery of molecules that function on these targets. The identification and characterisation of putative targets in a particular biochemical pathway for parasite growth is an urgent need to combat the disease, given the rapid emergence of drug resistance to traditional therapeutics. Our fast advancement in the characterisation of the genomes of malaria parasites suggests that this strategy is plausible to identify important new antimalarial compounds in the future. Because of our rapid progress in sequencing the genomes of malaria parasites, we conclude that this approach will be useful in discovering essential new antimalarial compounds in the future.

11.7 Drug resistance reversers

The discovery and production of drug resistance reversers not only revitalises the use of chloroquine and other antimalarial drugs, but also offers a new way to keep existing drugs effective [113]. For example calcium channel blockers [114] like verapamil and diltiazem have been shown to reverse chloroquine resistance.

11.8 Development of a malaria vaccine

Vaccination of infectious diseases has a long history of success in curing disease. Similarly, malaria vaccination is being introduced to protect the vaccinated individual while also preventing malaria transmission in the environment. Malaria

vaccines can prove to be a dynamic approach to malaria control and eradication. Malaria vaccines target three stages of the parasite's life cycle: (i) pre-erythrocytic (sporozoite/hepatic), (ii) erythrocytic (asexual), and (iii) reproductive (transmission blocking).

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