



## Current Antimalarial Therapies and Advances in the Development of Semi-Synthetic Artemisinin Derivatives

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### ABSTRACT

According to the World Health Organization, malaria remains one of the biggest public health problems in the world. The development of resistance is a current concern, mainly because the number of safe drugs for this disease is limited. Artemisinin-based combination therapy is recommended by the World Health Organization to prevent or delay the onset of resistance. Thus, the need to obtain new drugs makes artemisinin the most widely used scaffold to obtain synthetic compounds. This review describes the drugs based on artemisinin and its derivatives, including hybrid derivatives and dimers, trimers and tetramers that contain an endoperoxide bridge. This class of compounds is of extreme importance for the discovery of new drugs to treat malaria.

**Key words:** malaria, *Plasmodium falciparum*, artemisinin, hybrid.

#### Abbreviations:

amodiaquine - AQ

artemisinin - ART

artemisinin-based combination therapy - ACT

chloroquine - CQ

chloroquine-resistant - CQR

dihydroartemisinin - DHA

dihydrofolate reductase - DHFR

dihydropteroate synthase - DHPS

Drugs Initiative for Neglected Diseases - DNDi

European Medicines Agency - EMA

fixed-dose combination - FDC

Medicines for Malaria Venture - MMV

mefloquine - MQ

*p*-aminobenzoic acid - PABA

pharmaceutical fixed-dose combination - FDC

*Plasmodium falciparum* - *P. falciparum*

*Plasmodium knowlesi* - *P. knowlesi*

*Plasmodium malariae* - *P. malariae*,

*Plasmodium ovale* - *P. ovale*

*Plasmodium vivax* - *P. vivax*,

primaquine - PQ

pyronaridine - PYR

quinine - QN

World Health Organization - WHO

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### INTRODUCTION

Malaria is one of the most important public health problems worldwide, with almost half of the global population exposed to the risk of contamination. This disease is present in 91 countries, mostly in tropical and subtropical regions of the planet. However, due to globalization, the incidence of malaria has been alarming in virtually all countries, given that malaria cases are increasing in first-world countries. In 2016, it was estimated using data from the World Health Organization (WHO) that there were 212 million malaria cases in the world, leading to 429,000 deaths, mainly in African

countries, among children under 5 years of age (WHO 2016).

Malaria is caused by single-celled protozoa of the genus *Plasmodium* and is considered a severe, infectious, parasitic disease. This disease is transmitted by the bite of the female mosquito of the genus *Anopheles* infected with the protozoa, of which five species are responsible for infecting humans: *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi* (Garcia 2010).

The life cycle of *Plasmodium* species (Figure 1) is divided into different phases and between two hosts: a vertebrate and the mosquito. The onset of infection occurs with the bite of an infected female

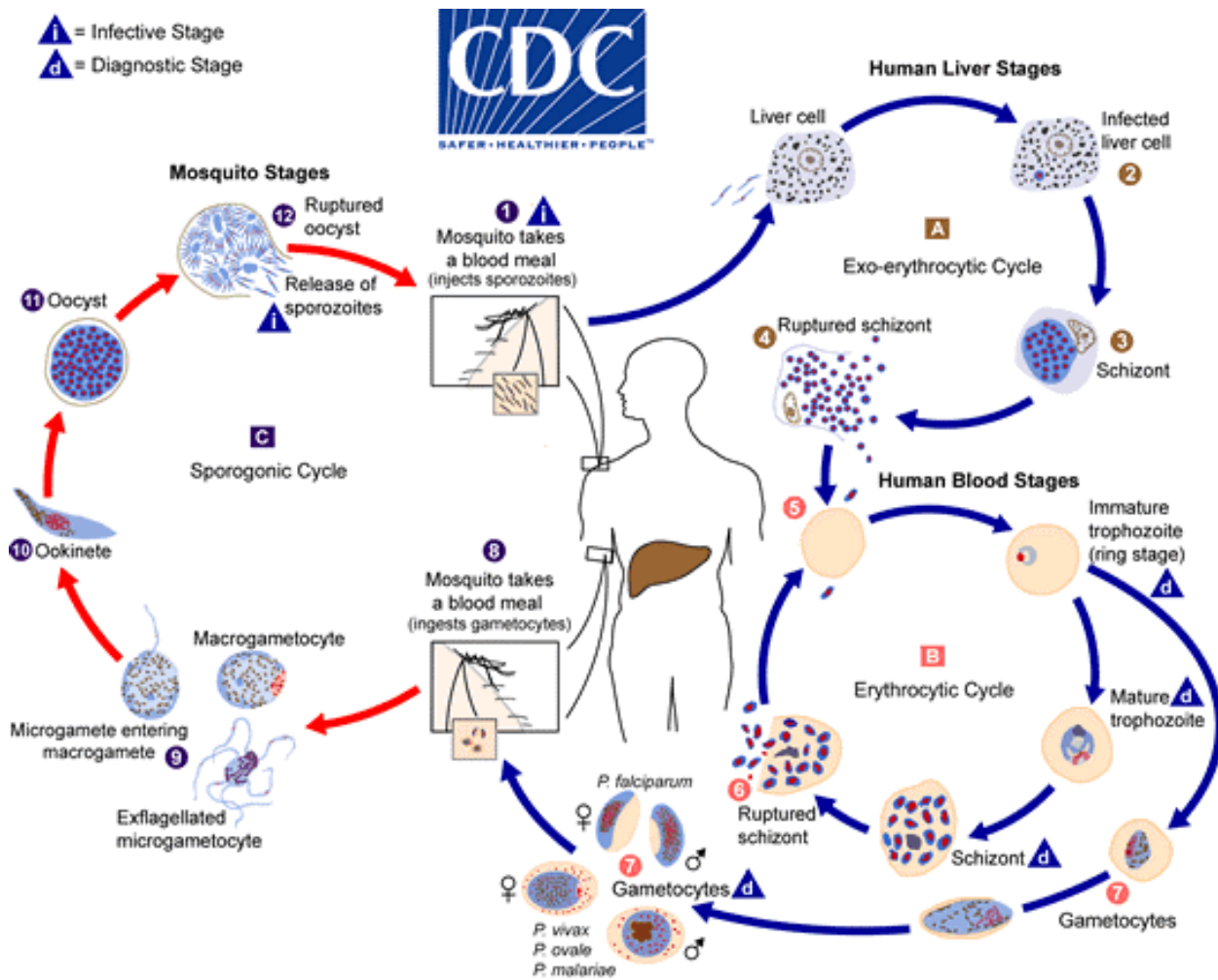


Figure 1 - *Plasmodium falciparum* life cycle (CDC 2017).

*Anopheles* mosquito. As the mosquito performs blood repast, the salivary glands release sporozoites on the skin, and after reaching the bloodstream the sporozoites rapidly invade liver cells, starting the infection. In the liver cells, the sporozoites differentiate and multiply asexually into thousands of merozoites, which invade the erythrocytes and proceed to multiply. In this phase, begin the symptoms of malaria. Based on the species, the duration of the erythrocytic stage will be different: 48 h for *P. falciparum*, *P. vivax* and *P. ovale* and 72 h for *P. malariae*.

Some merozoites will not reproduce asexually; these develop into sexual forms called gametocytes, which are ingested by the female *Anopheles* mosquito during hematophagy, beginning the sexual cycle in the stomach of the mosquito. The new sporozoites move to the salivary glands of the mosquito, and their inoculation into a new human host restarts the life cycle of *Plasmodium*. Some merozoites evolve into a latent form of the parasite (hypnozoites), whose reactivation is responsible for the recurrence of malaria (in cases of infection caused by *P. vivax* and *P. ovale*) (Schlitzer 2008, Midha et al. 2015, MMV 2017a, CDC 2017).

#### CURRENT CHEMOTHERAPY FOR MALARIA

The main means used to eliminate or reduce the number of cases of malaria are the development of vaccines, vector control, chemoprophylaxis and chemotherapy employing antimalarial drugs (Rappuoli and Aderem 2011).

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 schizogonic 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, in other words, to destroy the

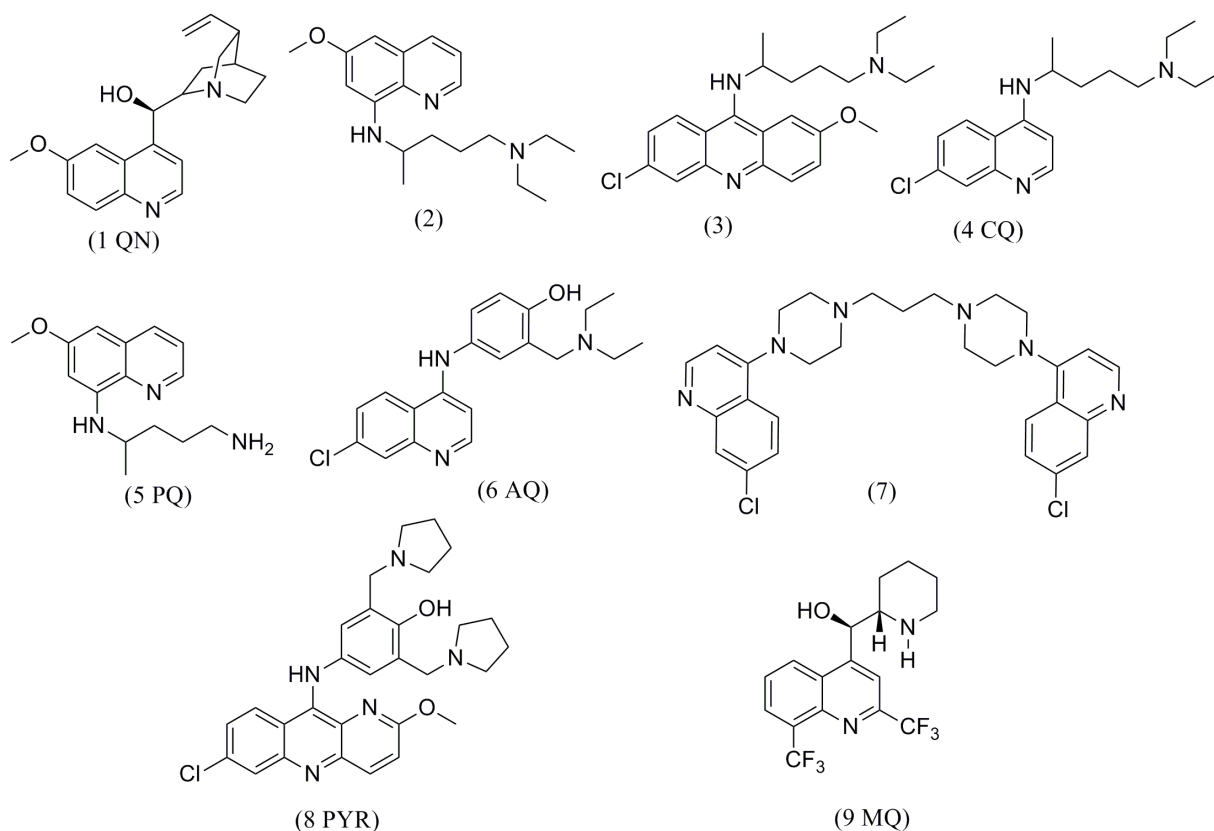
parasite in the tissue cycle of the species *P. vivax* and *P. ovale*, interrupting the transmission of the parasite and avoiding relapses. Various drugs are available for achieving these goals, where each acts in a specific way to inhibit the development of the parasite in the host (Teixeira et al. 2014, Biamonte et al. 2013).

Antimalarial chemotherapy has been based on an endless search for the next agent to combat *Plasmodium* parasites when they are able to prevent the effect of current drugs. 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 (Seder et al. 2013, Raj et al. 2014, Teixeira et al. 2014).

Antimalarial chemotherapy is based on natural products, semi-synthetic and synthetic compounds developed since the 1940s. Safe drugs used in the treatment of the disease are divided into three main classes: quinoline derivatives (Figure 2), antifolates (Figure 3) and artemisinin derivatives (Figure 4) (Staines and Krishna 2012, Leite et al. 2013).

#### QUINOLINE DERIVATIVES

Historically, quinolines are among the most widely used drugs for the treatment of malaria. Quinine (QN 1) (Figure 2), an alkaloid isolated from the bark of *Cinchona* trees, 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 (Achan et al. 2011). The emergence of resistant strains of *P. falciparum*, in addition to the high toxicity, has contributed to the limitation of the use of QN. However, this drug still used against malaria in the clinic, most often combined with a second agent to shorten the duration of therapy and thus minimize the adverse effects (Achan et al. 2011, Giao and Vries 2001).



**Figure 2** - Quinoline derivatives used in the treatment of malaria.

At the beginning of the 20th century, research conducted by Bayer gave rise to the first synthetic antimalarial drug, an 8-aminoquinoline, pamaquine (2) (Figure 2), whose clinical use was abandoned due to its high toxicity and limited activity (Tekwani and Walker 2006). After this discovery came mepacrine, also known as quinacrine (3) (Figure 2), an acridine derivative that was widely used in the 1930s during World War II. During this time, Bayer discovered chloroquine (CQ 4) (Figure 2), a 4-aminoquinoline that has become the most important antimalarial drug due to its high efficacy, low cost, and tolerable adverse effects (Krafts et al. 2012, O'Neill et al. 2012, Teixeira et al. 2014). Being a weak base, CQ accumulates in the acidic food vacuole of the parasite and exerts its activity by binding free heme and inhibiting hemozoin biocrystallization (hemozoin formation). CQ was the first choice for antimalarial treatment

for a long time, but its uncontrolled use soon led to the emergence of chloroquine-resistant (CQR) *P. falciparum* strains, only 15 years after the introduction of CQ as first-line antimalarial chemotherapy (Cunico et al. 2008, Kaur et al. 2010).

Primaquine (PQ 5) (Figure 2), an 8-aminoquinoline clinically used since 1950, is still the only drug used worldwide for the treatment of relapsing *P. vivax* malaria caused by hypnozoites, and it inhibits the formation of gametocytes (Waters and Edstein 2012).

This fact intensified the need to obtain new drugs with anti-*P. vivax* activity. New quinoline derivatives with side chain modifications were synthesized, giving rise to new drugs such as amodiaquine (AQ 6) (Figure 2), a 4-phenylaminoquinoline (Teixeira et al. 2014).

Piperaquine (7) (Figure 2) is a bis-4-aminoquinoline discovered in 1960s that showed activity against CQR strains. It is a bisquinoline, and although its precise mechanism of action is unknown, it is thought to be similar to that of CQ, which is structurally similar. The bulky bisquinoline structure of piperaquine (7) is postulated to inhibit transporter-mediated drug efflux, protecting the drug against CQR. However, high rates of resistance to 7 have been reported in areas where it has been widely used as a monotherapy (EMA 2017b, Keating 2012).

In the 1970s, pyronaridine (PYR 8) (Figure 2) was synthesized; a 10-phenyl aminobenzo[*b*][1,5] naphthyridine derivative that was active against drug-resistant *P. falciparum* strains (Kaur et al. 2010). After the emergence of parasite resistance to CQ, AQ gained prominence, as this phenyl-substituted analogue of CQ was found to have an excellent activity/toxicity profile while possibly sharing its mechanism of antimalarial action with CQ (Teixeira 2014, Barnett and Guy 2014).

PYR was synthesized in 1970 in China and has been used there for over 30 years for the treatment of malaria. It has high potency against *P. falciparum*, including CQR strains. PYR inhibits the formation of heme, thus preventing the malarial parasite from neutralizing heme, which is toxic to the parasite. Additionally, by forming a drug-hematin complex, PYR inhibits the glutathione-dependent degradation of hematin and enhances hematin-induced lysis of red blood cells. Both these actions lead to parasite death. The activity of PYR against erythrocytic *P. falciparum* is greatest in the ring-

form stage, followed by the schizonts, and then the trophozoites. It was more active against all of these stages than CQ (Croft et al. 2012).

Mefloquine (MQ 9) (Figure 2), developed by the United States Army, came into use in the mid-1980s and is currently recommended for chemoprophylaxis of malaria caused by all species. Mefloquine has been used in the fight against CQR strains; however, its use is associated with several side effects, and resistance to this drug has been reported. MQ is an antimalarial agent with high schizonticidal, but not gametocidal, activity. The exact mechanism of action of MQ is not clear. It does, however, have a high affinity for erythrocyte membranes, with activity presumably related to its interference with the polymerization of heme units (Nosten et al. 2012, Palmer 1993, WHO 2015).

#### ANTIFOLATE DERIVATIVES

Antifolates are an important class of antimalarials. However, many of these compounds are toxic to humans and have low oral tolerance. These drugs, besides acting as schizonticides in the blood in the treatment of malaria, can also be used to combat various other diseases, such as cancer. Antifolates are divided into two classes (I and II) according to their mechanisms of action. Sulfadoxines (10) (Figure 3) have structures similar to *p*-aminobenzoic acid (PABA) and belong to the type I class of antifolates. They block the production of dihydrofolic acid by inhibiting dihydropteroate synthase (DHPS), an essential enzyme for the synthesis of nucleic acids. Formerly, tests were conducted to use DHPS inhibitors alone as antimalarial agents, but this

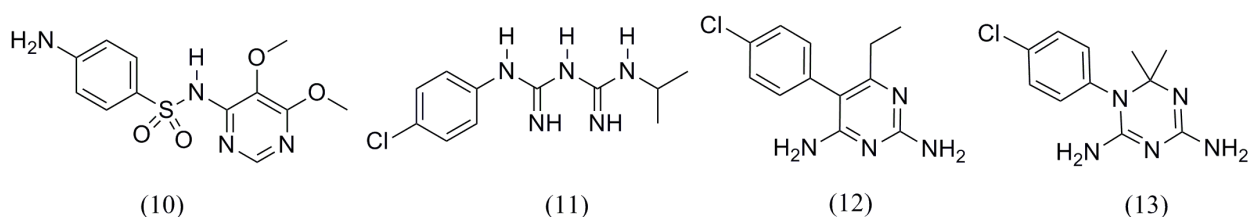


Figure 3 - Antifolates used in the treatment of malaria.

method was abandoned due to problems of toxicity and low efficacy (Nzila 2012).

Proguanil (**11**) and pyrimethamine (**12**) (Figure 3) correspond to the type II class of antifolates and are powerful schizonticidal agents that act on asexual forms of the parasite. These drugs inhibit the dihydrofolate reductase (DHFR) in the parasite, suppressing the reduction of dihydrofolate to tetrahydrofolate, which is necessary for the synthesis of amino acids and nucleic acids (Nzila 2012).

Proguanil (**11**) is a prodrug that is metabolized to cycloguanil (**13**) (Figure 3); it was the first antifolate produced for the treatment of malaria. Its low toxicity is indispensable in prophylaxis because it acts by destroying parasites before they invade red blood cells during their passage into the bloodstream. Pyrimethamine (**12**) has been the most frequently employed antifolate administered in combination with other faster-acting drugs. Although it was initially developed as an anticancer drug, it was later recognized as an antimalarial because of its structural similarity to Proguanil (**11**). However, the use of this class has been decreased due to the development of resistance in parasites to these drugs (Nzila 2012, Barnett and Guy 2014).

#### ARTEMISININ DERIVATIVES

Artemisinin (ART **14**) (Figure 4) was discovered by a Chinese scientist, Youyou Tu, who in 2015 shared the Nobel Prize for Medicine for her discovery, which lowered the mortality rates of people with

malaria. In 1972, ART was isolated for the first time from a Chinese medicinal plant, *Artemisia annua* (an herbaceous plant of the family *Asteraceae*), and is a sesquiterpene lactone natural product. Since the discovery of the antimalarial activity of ART and its semi-synthetic derivatives, they have been used in the treatment of malaria as first-line drugs. In addition, ART is largely used in the treatment of some types of fever and is recommended in cases of severe malaria (Misra et al. 2014, França et al. 2008, The Nobel Foundation 2015).

In 1979, the use of ART was implemented in the rest of the world due to its unusual chemical structure and potent antimalarial activity. The semisynthesis of ART from artemisinic acid and a total synthesis in 1983 were results of a great interest in exploring strategies of chemical synthesis. ART has gained considerable attention as a chemotherapy against malaria, but activity against other parasites such as *Schistosoma*, *Leishmania* and *Toxoplasma* has also been described. In addition, it has been considered an imminent candidate to decrease coccidial infection in chickens. This drug and its semisynthetic derivatives have other biological effects, including antiviral and anticancer activities (Li and Zhou 2010, Karunajeewa 2012).

The chemical synthesis of ART is known to be expensive, and its main commercial sources are the field-grown leaves and flowering tops of *A. annua*. Therefore, the search for increased productivity follows two strands: the application of new strategies for genetic improvement and the

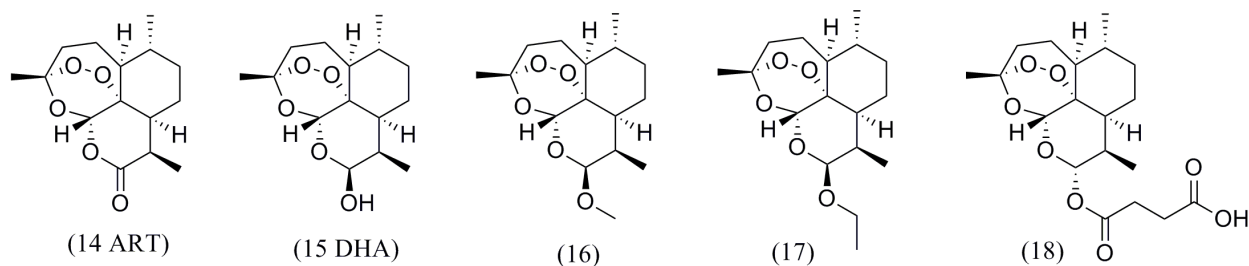


Figure 4 - Artemisinin derivatives used in the treatment of malaria.

search for new *Artemisia* species (Misra et al. 2014, Wright et al. 2010).

ART and its derivatives, dihydroartemisinin (DHA **15**), artemether (**16**) arteether (**17**) and artesunate (**18**) (Figure 4) are drugs that act by killing the parasites at an early phase of their development, quickly decreasing their numbers. Other characteristics of these drugs are low bioavailability and short half-life, which, in addition to previous reports of resistance, make them inefficient as monotherapies for the treatment of malaria. Therefore, these drugs are recommended in artemisinin-based combination therapy (ACT) (Araujo et al. 2009, WHO 2015).

Artemether (**16**) has several proposed mechanisms of action, including interfering with plasmodial transport proteins and with mitochondrial electron transport and producing free radicals to reduce blood antioxidants and glutathione. When used as a monotherapy, artemether has a relatively high recrudescence rate and is rapidly absorbed after oral dosing, reaching a maximum concentration in adults after approximately 2 hours. Once in systemic circulation, artemether (**16**) is hydrolyzed in the gut and liver to DHA. The bioavailability of **16** increases 2-fold when given in the presence of food (Karunajeewa 2012, WHO 2015).

Artesunate (**18**) is the most important semisynthetic derivative due to its rapid antimalarial action, lack of considerable clinical resistance and significantly greater solubility in water than ART, DHA or **16**, which is favorable for the preparation of formulations (Haynes 2006, WHO 2015).

These drugs act on the gametocytes and are blood schizonticides, preventing the transmission to other hosts and reducing the propagation of resistant forms. Literature reports have shown that, in the presence of the ferrous ions from the heme group of hemoglobin, the endoperoxide bridge of these substances can undergo a reductive cleavage, generating free radicals that can alkylate or modify the proteins of the parasite, causing its death. O'Neill

and co-workers described in detail the nature of the proposed radical and the mechanistic pathways bioactivation of artemisinin, the reductive scission model and the open peroxide model. (O'Neill et al. 2010, Na-Bangchang and Karbwang 2009).

ART is a sensitive molecule for large-scale derivatization. The carbonyl group can be easily reduced to a hydroxyl group, preserving the crucial endoperoxide moiety, using sodium borohydride to obtain DHA (**15**). This led to the preparation of a series of semisynthetic first-generation analogues: artemether (**16**); arteether (**17**); and artesunic acid, which is commercially known as artesunate (**18**) (Figure 4). These analogues share the same basic structure of ART with different substituents. The carbonyl exchange exerts influence on the solubility of each ART derivative and on some of their pharmacokinetic properties. ART itself is poorly soluble in water but is soluble in many aprotic organic solvents.

To increase antimalarial potency, researchers have become interested in synthesizing ART dimers, trimers and tetramers. Many of them have shown more promising antimalarial activity than ART and its first-generation analogs (Chaturvedi et al. 2010, Karunajeewa 2012).

ART derivatives, which are effective against chloroquine- and mefloquine-resistant strains, not only are active against the mature ring stage of *P. falciparum*, when the parasites are most metabolically active, but also target the young ring stages of the parasites. Another potential benefit is that they are active against the gametocytes transmitted from humans to mosquitoes and are capable of killing >99.9% of parasites per asexual cycle. Their use results in a significant reduction in gametocytemia and subsequent decrease in the transmission to mosquitoes compared to the use of previous first-line non-artemisinin antimalarial drugs. However, these compounds are not active against the pre-erythrocytic stages or the dormant hypnozoite stages of *P. vivax* and *P. ovale* in the liver

(Okell et al. 2008). Despite that, the concentration of ARTs needs to be at a parasitocidal level for at least 6 days (corresponding to three asexual life cycles for *P. falciparum*) to remove all parasites from the blood. Therefore, when administered alone, ARTs must be given as 7-day regimens to maximize their cure rates. However, compliance to 7-day treatment courses is poor, particularly when the clinical symptoms of malaria disappear within a couple of days of treatment initiation. When used in combination with partner drugs with longer elimination half-lives, 3-day treatment courses are sufficient (Nyunt and Plowe 2007).

#### CURRENT COMBINATION CHEMOTHERAPY IN MALARIA

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 (WHO 2015).

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. Several new pharmaceutical fixed-dose combination (FDC) formulations have been produced to treat this disease because of the high effectiveness and potency of ACT (WHO 2015).

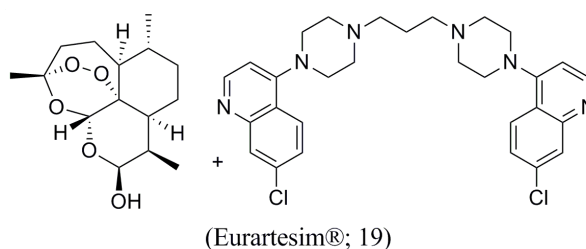
Mixed malaria infections are frequent in endemic areas. These infections are characterized by contamination by more than one species of *Plasmodium*. People infected with serious *P. vivax* can also have *P. falciparum* infections, for example.

In addition, acute *P. falciparum* infections can be succeeded by a presumed relapse of *P. vivax* malaria. The treatment of choice for mixed malaria infections is ACTs because this therapy is efficient against all types of malaria (WHO 2015).

The ACTs for the treatment of malaria are as follows (Figures 5 - 11):

- Dihydroartemisinin (**15**) + piperazine (**7**) (Eurartesim<sup>®</sup>; **19**)
- Artemether (**16**) + lumefantrine (**20**) (Coartem<sup>®</sup>; **21**)
- Artesunate (**18**) + mefloquine (**9**) (ASMQ; **22**)
- Artesunate (**18**) + amodiaquine (**6**) (Winthrop<sup>®</sup> or Coarsucam<sup>™</sup>; **23**)
- Artesunate (**18**) + sulfadoxine (**10**) + pyrimethamine (**7**) (**24**)
- Pyronaridine (**8**) + artesunate (**18**) (Pyramax<sup>®</sup>; **25**), in development.
- ARCO<sup>®</sup> (combination **26** (artemisinin + naphthoquine (**27**)))

Eurartesim<sup>®</sup> (dihydroartemisinin + piperazine - combination **19**) (Figure 5) was developed by Sigma-Tau in partnership with the Medicines for Malaria Venture (MMV). In October 2011, the European Medicines Agency (EMA) granted marketing authorization for Eurartesim<sup>®</sup> for the treatment of uncomplicated *P. falciparum* malaria in adults. It is administered once a day for 3 days, making it easier for patients to comply with the dosing. In 2015, Sigma-Tau entered into an exclusive licensing agreement with Pierre Fabre



**Figure 5** - Structure of drugs used in Eurartesim<sup>®</sup> combination **19**.



to expand its ability to support country registration requirements and national adoption in 32 African countries. A pediatric formulation of Eurartesim<sup>®</sup> for children over 5 kg is in phase III clinical trials. In October 2015, Eurartesim<sup>®</sup> was prequalified by the WHO, and a dossier for a hydrodispersible child-friendly formulation of the drug was submitted to the EMA. Through this prequalification process, the WHO assesses and approves the quality, safety and efficacy of the medicinal product. DHA, the main active metabolite in Eurartesim<sup>®</sup>, achieves high concentrations in red blood cells infected with *P. falciparum* (Valecha et al. 2010, Bassat et al. 2009, Nguyen et al. 2009, Schrader et al. 2012, Anthony et al. 2012, EMA 2017a, MMV 2017b).

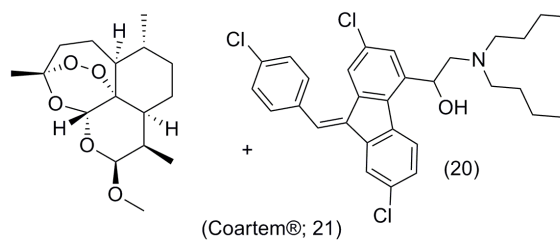
Coartem<sup>®</sup> (artemether + lumefantrine (**20**) - combination **21**) (Figure 6) is used in over 50 endemic countries to treat adults and children over 5 kg with uncomplicated *P. falciparum* infection, including mixed infection. Coartem<sup>®</sup> Dispersible, developed by Novartis and MMV, was approved in 2009 by Swissmedic and more recently by the EMA and the US Food and Drug Administration. It was the first prequalified child-friendly ACT as a sweet-tasting cherry-flavored tablet that disperses in a small amount of water. Moreover, antimalarial tablets for adults are bitter and need to be broken up or crushed, making it difficult to give the correct dosing (Anthony et al. 2012, Bassat et al. 2009, Sirima et al. 2016, MMV 2017c).

ASMQ (artesunate + mefloquine - combination **22**) (Figure 7) is a formulation developed by a Brazilian government-owned pharmaceutical company, Farmanguinhos/Fiocruz, in partnership with the Drugs Initiative for Neglected Diseases (DNDi). ASMQ was transferred to the Indian pharmaceutical company Cipla and was prequalified by the WHO in 2012. The ASMQ combination therapy has been one of the WHO-recommended forms of ACT for first-line treatment of uncomplicated malaria in adults and children over 5 kg in several countries. The fixed dose containing

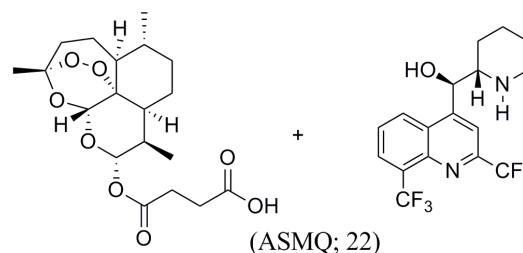
100 mg of artesunate and 220 mg of mefloquine hydrochloride has demonstrated efficacy and safety. This combination is less commonly used in Africa because of the availability of other affordable and already registered ACTs (Anthony et al. 2012, Valecha et al. 2010, Wells et al. 2013).

Studies have shown that ASMQ had the highest cure rate, the lowest rate of gametocyte carriage, and the most effective suppression of *P. vivax* malaria. In addition, a large phase IV trial in Brazil confirmed its effectiveness for treating uncomplicated *P. falciparum* infection (Sirima et al. 2016, MMV 2017d).

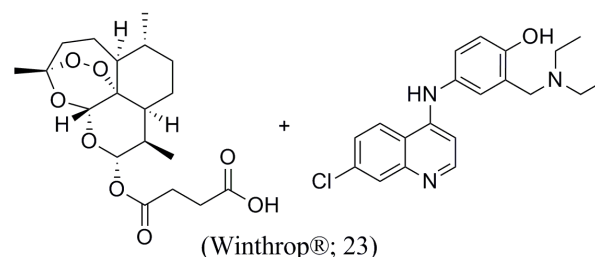
Winthrop<sup>®</sup> or Coarsucam<sup>™</sup> (artesunate + amodiaquine - combination **23** (ASAQ)) (Figure 8) was developed by Sanofi and the DNDi and



**Figure 6** - Structure of drugs used in Coartem<sup>®</sup> combination **21**.



**Figure 7** - Structure of drugs used in ASMQ combination **22**.



**Figure 8** - Structure of drugs used in Winthrop<sup>®</sup> combination **23**.

was prequalified by the WHO in 2008. Winthrop<sup>®</sup> has been approved in 33 countries and has the lowest price of the fixed-dose ACTs. Winthrop<sup>®</sup> should be provided as a FDC to improve the severe adverse effects noted in the past associated with amodiaquine when administered as a monotherapy at high doses for treatment or prophylaxis.

As amodiaquine is effective against several CQR strains, this drug remains an important component of current antimalarial combination therapies, including being used with artesunate. The Winthrop<sup>®</sup> fixed-dose combination is effective in clearing *P. falciparum* parasites from infected individuals.

Winthrop<sup>®</sup> is rapidly absorbed and undergoes rapid and extensive metabolism to DHA and desethylamodiaquine (Anthony et al. 2012, Navaratnam et al. 2009, Adjei et al. 2010, MMV 2017e).

The combination **24** (artesunate (**18**) + sulfadoxine (**10**) + pyrimethamine (**12**)) (Figure 9) is used for the treatment of acute uncomplicated malaria but is not available as a FDC yet. It is available in a blister pack containing 50 mg of artesunate (**18**) and fixed-dose combination tablets comprising 500 mg of sulfadoxine (**10**) and 25 mg of pyrimethamine (**12**). A target dose of artesunate (**18**) is given once a day for 3 days, and a single administration of a single dose of sulfadoxine-pyrimethamine is given on the first day. This combination is the first-line therapy for uncomplicated *P. falciparum* malaria in Sudan and globally (WHO 2015, Matar et al. 2014).

Pyramax<sup>®</sup> (pyronaridine + artesunate-combination **25**) (Figure 10), co-developed by Shin Poong Pharmaceutical and MMV, is the first ACT for which the EMA has adopted a positive scientific opinion and is the first and only ACT to be approved for the blood-stage treatment of the main strains of malaria, namely, *P. falciparum* and *P. vivax*. It is also the first Korean product included in the WHO list of prequalified medicines for malaria. Pyramax<sup>®</sup> is recommended for the treatment of uncomplicated malaria in adults and children over 20 kg (tablets) and in children between 5 and 20 kg (granules). Resistance to PYR appears slowly and is further retarded when used in combination with other antimalarials, particularly artesunate (Anthony et al. 2012, Schrader et al. 2012, MMV 2017f, Croft et al. 2012).

ARCO<sup>®</sup> (combination **26** (artemisinin + naphthoquine (**27**)) (Figure 11), recommended for the treatment of uncomplicated *P. falciparum* malaria, was developed in China to counter antimalarial drug resistance. Both artemisinin and naphthoquine drugs have proven to be efficacious, safe and well tolerated as monotherapies. ARCO<sup>®</sup> offers a novel advantage over existing ACTs: it can be administered as a single oral dose (or a 1-day treatment). Several therapeutic studies conducted recently indicate that a single oral dose administration of the artemisinin-naphthoquine combination is equally effective and safe as the 3-day treatment with the artemether-lumefantrine combination and other existing ACTs (Anthony et al. 2012, Biamonte et al. 2013).

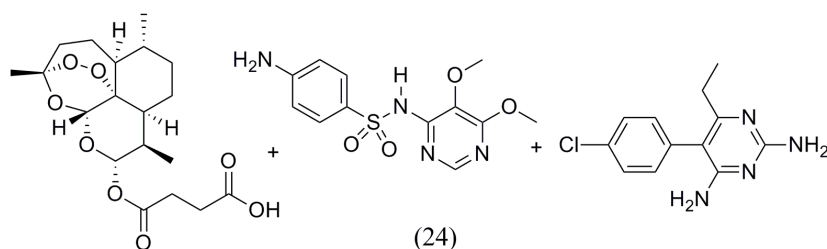
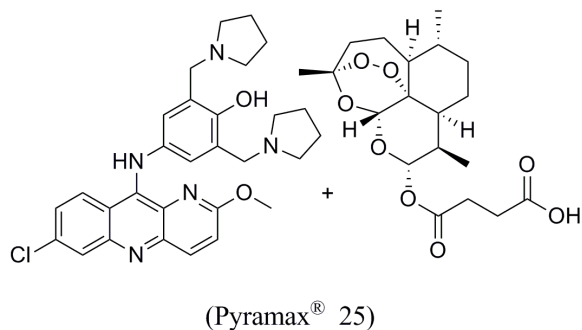
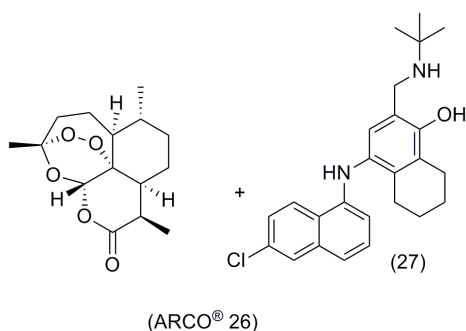


Figure 9 - Structure of drugs used in combination 24.



**Figure 10** - Structure of drugs used in Pyramax<sup>®</sup> combination 25.



**Figure 11** - Structure of drugs used in ARCO<sup>®</sup> combination 26.

Other combinations without the presence of ART, such as sulfadoxine (**10**) + pyrimethamine (**7**) + amodiaquine (**6**), have been evaluated. This combination was more efficient than the substances that were employed singly but not as effective as ACTs (WHO 2015, MMV 2017g).

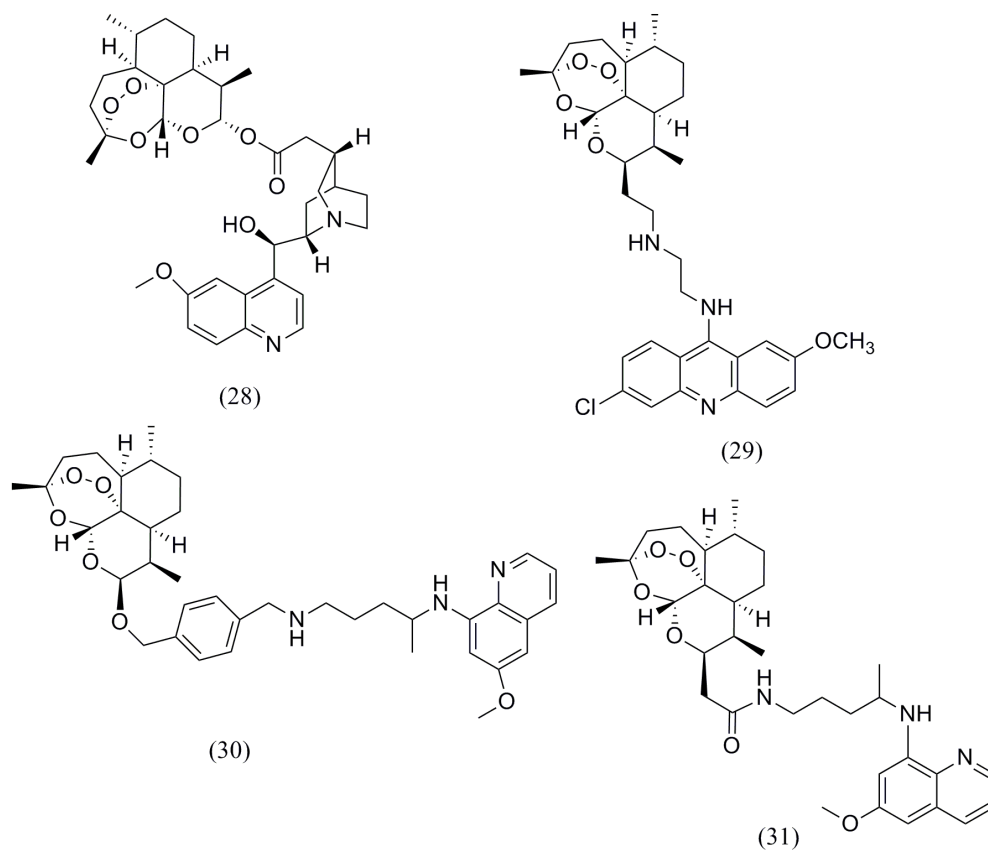
#### ARTEMISININ HYBRIDS

The combination of pharmacological fragments from two or more molecules into a single hybrid molecule as a strategy employed in medicinal chemistry for discovering new drugs is called molecular hybridization. There are several advantages of employing hybrid molecules instead of using multicomponent therapy. A hybrid is a single compound that can have double action; in other words, they can act by two distinct mechanisms or by a third, unique one, as these compounds are new pharmaceutical entities. A hybrid has

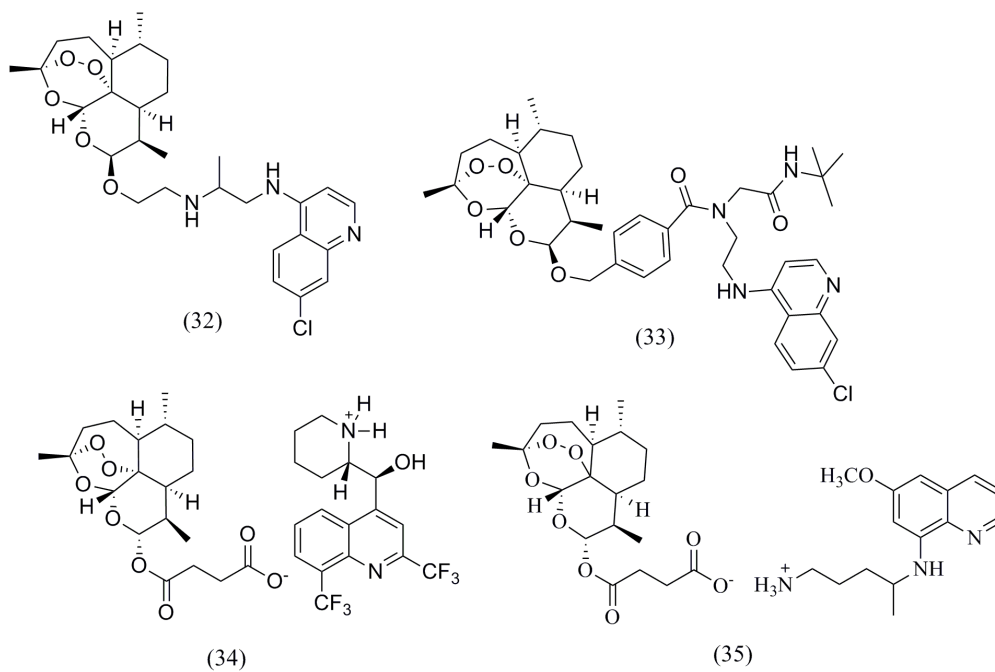
the potential to increase safety, enhance efficacy, improve cost-effectiveness and reduce resistance to the original drugs. Another advantage would be a lower risk of drug interactions compared to a FDC (Vandekerckhove and D'hooghe 2015, Morphy and Rankovic 2005, Meunier 2008, Muregi and Ishih 2010).

ART-derivative hybrids that are active against *Plasmodium* strains are shown in Figures 12 and 13. Compound **28** is a conjugate hybrid between DHA (**15**) and QN (**1**) (Figure 12). The original vinyl functionality in QN was used to connect the molecules. The connection was obtained by coupling DHA to a carboxylic acid derivative of QN via a covalent ester linkage. Studies have shown that changes to other potential sites in QN had unfavorable effects on activity, supporting the change made in its vinyl functionality. The hydroxyl group and the quinoline ring are essential for activity, but the quinuclidine ring can be replaced by another one without loss of activity. The hybrid **28** was active *in vitro* against both sensitive (3D7;  $IC_{50} = 0.008 \mu\text{M}$ ) and resistant (FcB1;  $IC_{50} = 0.009 \mu\text{M}$ ) strains of *P. falciparum*. This shows that the hybrid has higher potency than ART (3D7, FcB1;  $IC_{50} = 0.049$  and  $0.050 \mu\text{M}$ , respectively) and QN (3D7, FcB1;  $IC_{50} = 0.149$  and  $0.096 \mu\text{M}$ , respectively) used as a control (Walsh et al. 2007, Teixeira et al. 2014).

The relevance of ART-based antimalarials in current clinical strategies against CQR malaria led to the exploration of several endoperoxides and derived hybrid constructs as potential antimalarial leads. Araujo (2009) developed the artemisinin/quinacrine hybrid (**29**) (Figure 12) by joining the quinacrine core with an ART derivative, that features a metabolically stable linkage. The hybrid **29** was evaluated against CQS (3D7;  $IC_{50} = 0.012\text{--}0.016 \mu\text{M}$ ) and CQR (K1;  $IC_{50} = 0.014\text{--}0.020 \mu\text{M}$ ) *P. falciparum* strains and was found to display micromolar activities against both strains, thus exhibiting no cross-resistance



**Figure 12** - Structures of ART hybrids **28-31**.



**Figure 13** - Structures of ART hybrids **32-35**.

with CQ. In fact, this hybrid was more active than ART (**14**) and was easily transformed into a water-soluble salt, making it suitable for oral and intravenous administration. Although this analogue is more potent than ART (3D7;  $IC_{50} = 0.011 \mu\text{M}$ ) (K1;  $IC_{50} = 0.009 \mu\text{M}$ ), it was not more potent than artemether (**16**) (3D7;  $IC_{50} = 0.003 \mu\text{M}$ ) (K1;  $IC_{50} = 0.001 \mu\text{M}$ ). This is surprising because it should accumulate within the acidic digestive vacuole much more efficiently than the parent drug, through an ion-trapping mechanism. This may indicate that other targets outside the food vacuole may be more important for this class of hybrid drug (Araujo et al. 2009, Kaur et al. 2010).

For DHA (**15**) / PQ (**5**) hybrids **30** and **31** (Figure 12), a double-drug approach was used by synthesizing molecular constructs where PQ (**5**) is covalently bound to the DHA core, another potent moiety with complementary antimalarial properties. Compound **30** was synthesized starting from artelinic acid, a 4-methylbenzoic acid derivative of ART (**14**) that has been shown to be the most metabolically stable of the ARTs. Compound **31** was designed by replacing the oxygen atom with a  $\text{CH}_2$  group. This modification was intended to produce a compound with greater hydrolytic stability, a longer half-life, and potentially lower toxicity. Both **30** and **31** displayed enhanced *in vitro* activities against liver-stage *P. berghei* compared to their parent drugs. They were also evaluated *in vitro* against *P. falciparum* W2 strains ( $IC_{50} = 0.0125$  and  $0.0091 \mu\text{M}$ , respectively), in which they were equipotent to ART (**14**) and superior to PQ (**5**) (Capela et al. 2011).

Hybrid **32**, a DHA (**15**) - 4-aminoquinoline hybrid (Figure 13), was developed by Lombard et al. (2011) by joining a DHA motif to 4-aminoquinoline via an ether/amine bond for the purpose of increasing the half-life of DHA. For solubility and stability reasons, this hybrid was treated with oxalic acid to obtain the oxalate salts.

Compound **32** exhibited higher potency against both CQS (D10;  $IC_{50} = 0.012 \mu\text{M}$ ) and CQR (Dd2;  $IC_{50} = 0.017 \mu\text{M}$ ) strains than CQ (**4**). One drawback of the compound was its lower activity than DHA irrespective of the *P. falciparum* strain (Lombard et al. 2011, 2013).

Artemisinin/chloroquinoline hybrid **33** (Figure 13) was synthesized by Feng et al. (2011) by coupling DHA (**15**) and a chloroquinoline moiety through an ether/amide bond. The compound displayed excellent *in vitro* antiplasmodial activities against sensitive (D10;  $IC_{50} = 0.027 \mu\text{M}$ ) and resistant (K1;  $IC_{50} = 0.019 \mu\text{M}$ ) *P. falciparum* strains compared to CQ (**4**). No cross-resistance with CQ was observed for this hybrid compound in CQR malaria parasites, even though it contains a CQ moiety. It was also found that this hybrid shares the same mechanism of action with both ART (**14**) and CQ, as it displayed potent activity against  $\beta$ -hematin formation and contributed to an increase in the accumulation of hemoglobin within the parasites (Feng et al. 2011, Vandekerckhove and D'Hooghe 2015).

MEFAS (**34**) (Figure 13) is a hybrid salt between artesunate (**18**) and MQ (**9**) that is under development by Boechat and co-workers (2014). MEFAS was active against *P. falciparum* CQS 3D7 and CQR W2, showing an  $IC_{50}$  value of  $0.001 \mu\text{M}$  for both strains. Studies have shown that MEFAS was at least 5-fold more potent than MQ alone, more potent than **18** against 3D7, as effective as **18** against W2, and more potent than mixtures of **18** with MQ. In *in vivo* tests in *P. berghei* infected mice, a cure was observed after treatment at a dose of  $10 \text{ mg/kg}$ , without recrudescence of parasitemia. Assessments of the *in vivo* cytotoxicity of MEFAS showed that its toxicity is 5-fold lower than that of MQ and 3-fold lower than the FDC ASMQ.

Drugs that are able to target both asexual parasites and gametocytes would improve malaria control. MEFAS has been shown to be an active blood schizonticidal drug. Its ability to block

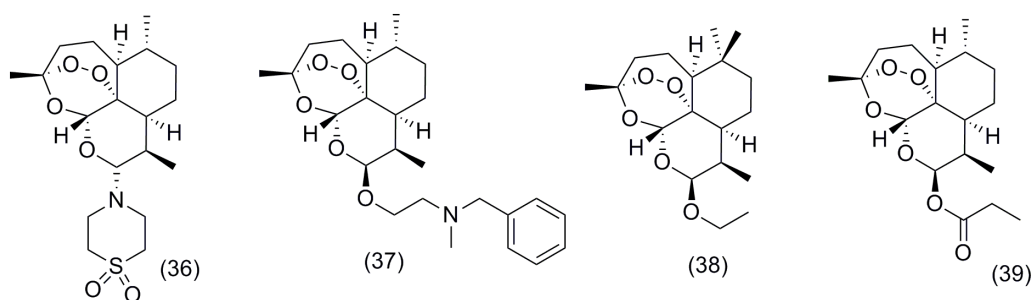


Figure 14 - Structures of ART derivatives 36-39.

the infectivity of *P. falciparum* gametocytes was evaluated, and it was found to be 280- and 15-fold more effective than MQ and **18** alone, respectively (Varotti et al. 2008, Penna-Coutinho et al. 2016)

PRIMAS (**35**) (Figure 13), which was designed using the same approach as MEFAS, is also a hybrid salt between artesunate (**18**) and PQ (**5**) under development by Boechat and co-workers (2014). PRIMAS was developed with the goal of minimizing the toxicity of PQ. The efficacy studies of the PRIMAS hybrid salt in *in vivo* and *in vitro* models showed that it is more active and less toxic than the isolated pattern drugs (Boechat et al. 2014).

Artemisone (**36**) (Figure 14) is a second-generation semi-synthetic ART (**14**) with the introduction of a polar heterocycle. This modification improved pharmacokinetic properties and eliminated the neurotoxicity. It inhibits *Pf*ATPase6 with a  $K_i$  of 1.7 nM and was approximately two to five times more efficient than artesunate (**18**) in animal experiments. Artemisone (**36**) had been in clinical studies, but further development was not reported (Anthony et al. 2012, Schrader et al. 2012, Barnett and Guy 2014).

A series of 10-aminoethylether derivatives of artemisinin were synthesized, and their antimalarial activity against *P. falciparum* was determined. The derivative **37** (Figure 14), containing only one nitrogen atom, showed the highest overall activity against a CQS strain (D10) ( $IC_{50} = 1.44$  nM), while long-chain polyamine derivatives were found to have the lowest activity (Cloete et al. 2012).

The biotransformation of ART using *R. stolonifer* resulted in 10b-hydroxyartemisinin, a promising metabolite that was a precursor for the series of synthetic derivatives. Compound 10b-hydroxy-12b-artether **38** (Figure 14) has significant anti-malarial activity ( $IC_{50} = 18.29$  nM) compared to the natural drug ART (Gaur et al. 2014).

A series of esters were synthesized in a one-step reaction by derivatization on carbon C-10 of DHA (**15**). The *in vitro* antiplasmodial activity was measured against *P. falciparum* 3D7 and K1 strains. The 10a-n-propyl ester (**39**) (Figure 14) exhibited the greatest activity against the 3D7 and K1 strains (0.0031 and 0.0003 mM, respectively), in comparison with artesunate (**18**) (Cloete et al. 2012).

A series of artesunate-polyamines were evaluated for antimalarial activity towards the K1 and NF54 strains of *P. falciparum*. (Bis)-Boc-(bis)-artesunate-polyamine and (tetra)-artesunate polyamine conjugates exhibited potent *in vitro* activity towards both strains, with  $IC_{50}$  values in the range of 0.3-1.1 nM, comparable to the parent artesunate (**18**). The *in vivo* evaluation of compound **40** (Figure 14) demonstrated 99.8% reduction in parasitemia with maximal 30-day survival rates. Oral testing of **40** proved less efficacious, with 95.7% activity and inconsistent survival rates of 16-30 days (Pearce et al. 2017).

A series of artemisinin-vinyl sulfone hybrids was shown to display potent antiplasmodial

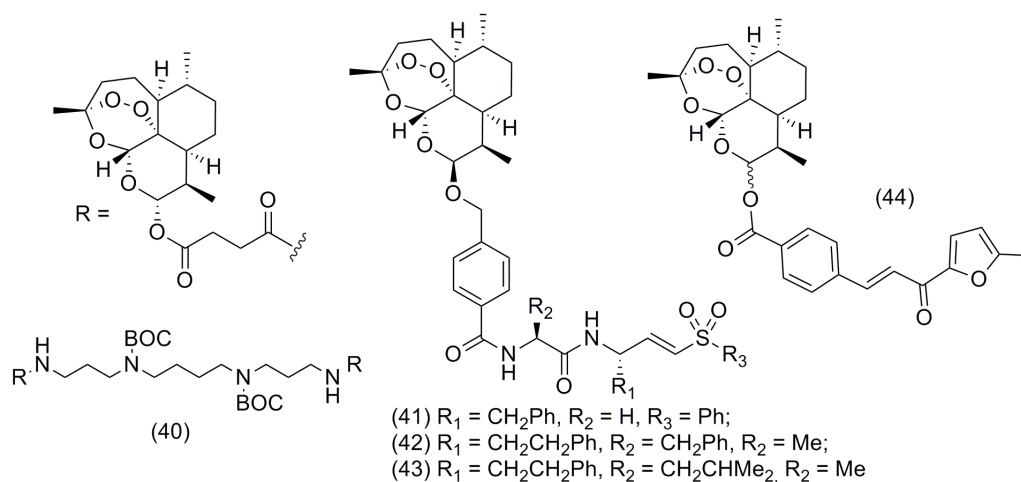


Figure 15 - Structures of ART derivatives 40-44.

activity against *P. falciparum* chloroquine-sensitive and multidrug-resistant strains W2 (chloroquine-resistant), FCR3 (atovaquone-resistant), 3D7 (chloroquine-sensitive), V1/S (chloroquine- and pyrimethamine-resistant) and D6 (chloroquine-sensitive and mefloquine-resistant). The  $\text{IC}_{50}$  values ranging from 0.002 to 0.005 mM show the superior activity of compounds **41-43** (Figure 15) compared to CQ (**4**) and ART (**14**) against all strains (Capela et al. 2009, Muregi and Ishih 2010).

A series of dihydroartemisinin-chalcone esters were screened against 3D7 and W2 strains of *P. falciparum* parasites. The esters featuring oxygenated aryl as ring B in the chalcone were found to be equipotent to DHA (**15**) but were two to three times more active than artesunate (**18**) and had more than fortyfold higher activity than CQ (**4**) against the W2 strain. Compound **44** (Figure 15) was identified as having the best activity, showing  $\text{IC}_{50} = 0.0019$  mM and 0.0014 mM against 3D7 and W2, respectively (Smit et al. 2015).

#### DIMERIC ARTEMISININ DERIVATIVES

It was thought that the extent of antimalarial activity depends upon the number of peroxide units, which can be increased by including an additional artemisinin moiety.

Thus, dimers, trimers and tetramers of artemisinin with various lengths, stereochemistries and flexibilities have been synthesized. ART trimer and tetramer derivatives without acetal groups have also been reported (Chaturvedi 2011).

Dimer **45** (Figure 16) had antimalarial activities *in vitro* against CQS *P. falciparum* parasites and was considerably more potent as an antimalarial ( $\text{IC}_{50} = 0.00077$   $\mu\text{M}$ ) than ART (**14**) (Paik et al. 2006).

A series of ART (**14**) derivatives trioxane dimer esters were tested for antimalarial efficacy in malaria-infected mice at a single oral dose combined with MQ (**9**) (6 mg/kg and 18 mg/kg, respectively). The most efficacious dimer **46** (Figure 16) prolonged mouse survival past day 30 of infection, with three of the four mice in this group having no detectable parasitemia and appearing and acting healthy on day 30. The dimer esters outperformed the antimalarial drug artemether (**16**) (Conyers et al. 2015).

A series of ART (**14**) hybrids were synthesized and evaluated for their *in vitro* potential as antimalarial, antileukemia, and antiviral agents. Regarding the activity against *P. falciparum* 3D7 parasites. The dimer derivatives **47** and **48** (Figure

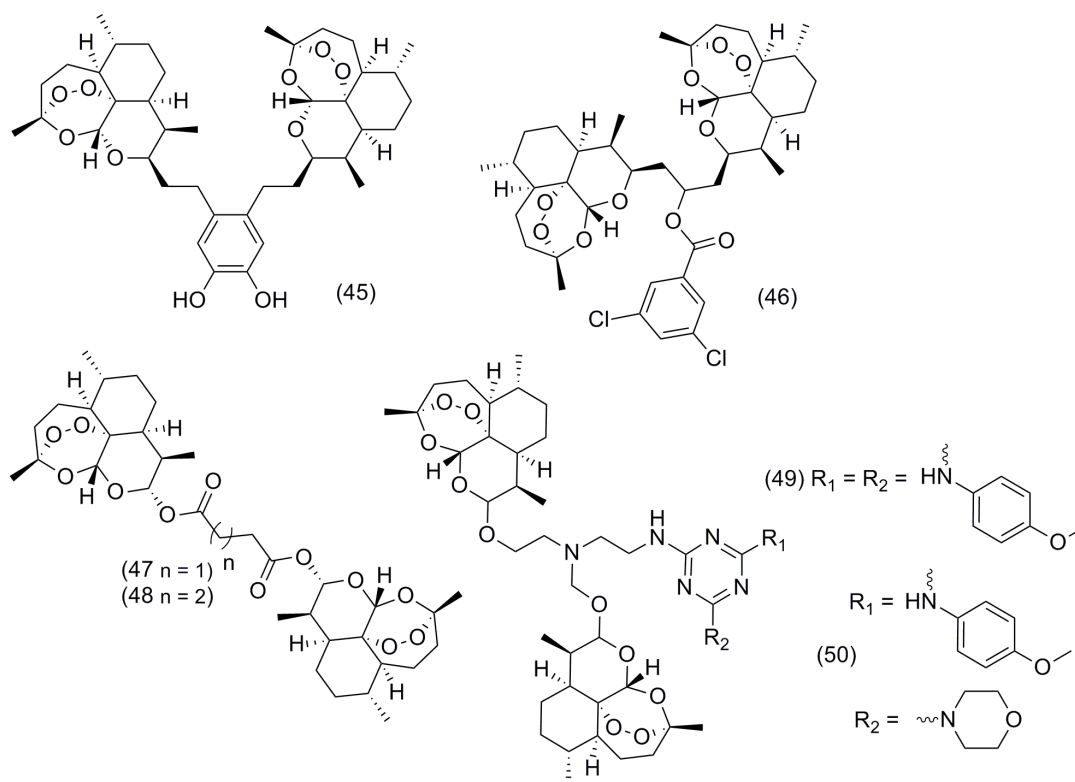


Figure 16 - Structures of ART dimer 45-50.

16) were more active ( $IC_{50}$  value of 2.6 nM for both) than the trimers (Reiter et al. 2015a).

A series of ART (**14**) and triazine hybrids were active against the CQS and CQR strains of *P. falciparum*. The dimer derivatives demonstrated higher activity than their monomeric counterparts. Compound **49** showed  $IC_{50}$  values of 0.0055 and 0.010 mM, and the compound **50** showed  $IC_{50}$  values of 0.0079 and 0.0102 mM for NF54 and Dd2, respectively, possessing potencies comparable to those of artesunate (**18**) and DHA (**15**) against the NF54 strain while being slightly less active than both against the CQS strain (Figure 16) (Cloete et al. 2014).

Posner and co-workers described the administration of a single oral dose of only 5 mg/kg of dimer secondary alcohol **51a** or **51b** plus 15 mg/kg of MQ (**9**) hydrochloride, which prolonged the lives of *P. berghei*-infected mice to an average of 25 days after infection. The result

of this ACT combination is of high medicinal significance because the antimalarial efficacy of the drug artemether (**16**) plus MQ (**9**) under the same conditions was significantly lower. The carbamate derivatives **52-54** also significantly outperformed artemether (**16**) in prolonging the survival times (25–27 days) of malaria infected mice (Figure 17) (Conyers et al. 2014).

In another study, Posner and co-workers demonstrated that the partially curative carbonate and thiocarbonate derivatives all had average survival times (26–30 days) that were considerably higher than the average survival times of their parent dimer secondary alcohol **51a** (19.5 days) (Mazzone et al. 2014).

The 1,2,4-trioxane-ferrocene hybrid **55** (Figure 17) was active against *P. falciparum* 3D7 strain, with a slightly higher  $IC_{50}$  value (7.2 nM) than that of its parent compound DHA (**15**) (Reiter et al 2015b).



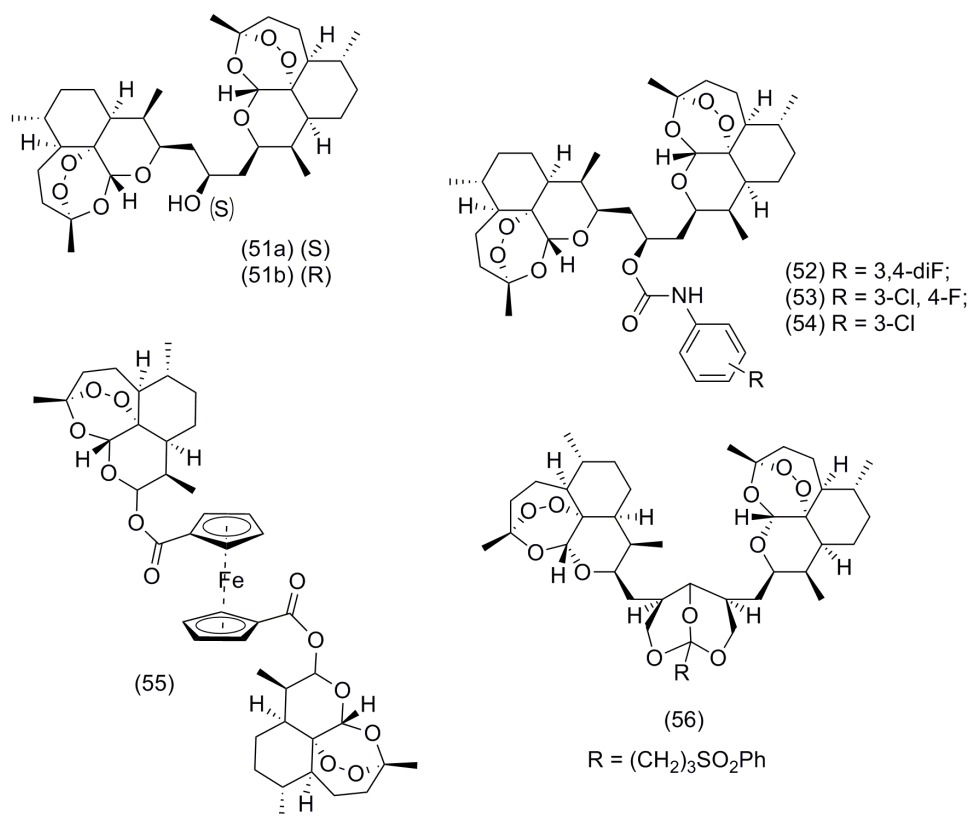


Figure 17 - Structures of ART dimer 51-56.

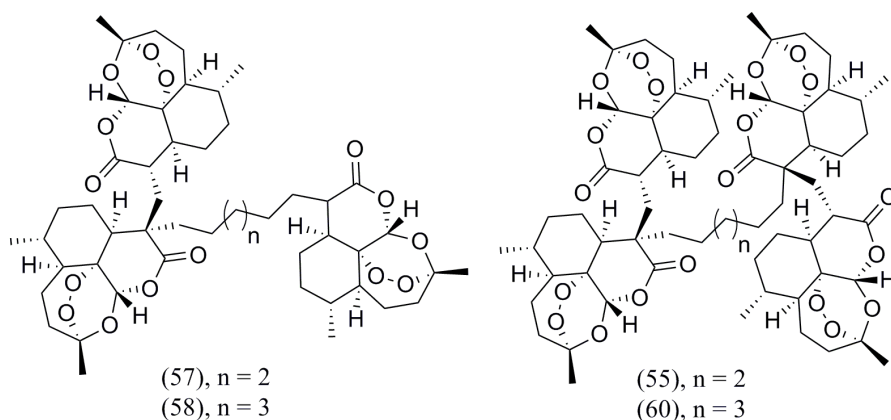


Figure 18 - Structures of ART trimer 57 and 58 and tetramer 59 and 60.

The trioxane dimer orthoester sulfone 56 (Figure 17) was administered orally as a single oral dose (6 mg/kg) combined with mefloquine hydrochloride (18 mg/kg) in *P. berghei*-infected mice. The combination safely cured the mice after 30 days, and the efficacy was superior to that of artemether (16).

#### TRIMERIC AND TETRAMERIC ARTEMISININ DERIVATIVES

Trimers 57 and 58 and tetramers 59 and 60 (Figure 18) were synthesized and evaluated in relation to their antimalarial activities. The obtained results for trimers 57 ( $EC_{50} = 0.0024 \mu\text{M}$ ) and 58 ( $EC_{50} =$

0.0031  $\mu\text{M}$ ) and tetramers **59** ( $\text{EC}_{50} = 0.0058 \mu\text{M}$ ) and **60** ( $\text{EC}_{50} = 0.02 \mu\text{M}$ ) were quite impressive and superior (except **60**) to those for ART ( $\text{EC}_{50} = 0.012 \mu\text{M}$ ) (Chaturvedi et al. 2010).

### CONCLUSIONS

This review describes the drugs based on artemisinin and its derivatives that are recommended by the WHO for the treatment of malaria. Semisynthetic artemisinin derivatives, including dimers, trimers and tetramers, were developed as prototypes of the most important class of compound used in the fight against this disease. ACT is a combination of artemisinin derivatives that acts rapidly clears parasites from the blood while the partner drug acts more slowly cleaning the remaining parasites and provides protection against development of resistance to the artemisinin derivatives. New artemisinin derivatives can be the key to discover new drugs to be used in combination with other antimalarials or as monotherapies.

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