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Effects of Antimalarial Molecules on the Gametocyte Stage of *Plasmodium falciparum*: The Debate

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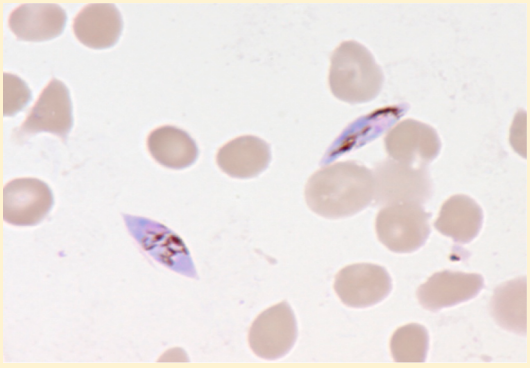
ABSTRACT: Although the illness malaria is caused by the asexual blood stages, the presence of gametocytes is directly responsible for the infection of the vector *Anopheles*, thus perpetuating the plasmodial cycle. Fight against malaria is more than ever a current problem, and the solution will probably go through the development of efficient molecules against gametocytes. Knowledge of the pharmacological properties of antiplasmodials is helpful in term of using relevant molecules to treat malaria and to eradicate this dramatic public health problem. The effects of the major antiplasmodial drugs including artemisinin-based combination therapies on gametocyte load are thus reviewed herein, making the difference whenever possible between the effects on gametocytogenesis and the gametocytocidal activity. Current status on the portfolio of the most promising anti-gametocytes compounds is also presented. A close analysis of the relationship between chemical structure and antiplasmodial activity should help the design of novel antimalarial drugs targeting *Plasmodium* sexual stages.



1. INTRODUCTION

Plasmodium falciparum malaria remains a major public health problem mostly in tropical and subtropical areas of the world's poorest countries.[1,2] The life cycle of *P. falciparum* malaria comprises an exogeneous sexual phase with multiplication in certain mosquitoes corresponding to the vector of the disease and an endogeneous asexual phase with multiplication in the human host. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host. After several asexual cycles in the blood following the hepatic cycle, some merozoites can differentiate into sexual forms known as macro (female) or micro (male) gametocytes. This production of gametocytes from asexual parasites is named gametocytogenesis. Ingestion of these gametocytes by the invertebrate host allows for the following of the gametocytogenesis process and the continuation of the parasite cycle.

Since no malaria vaccine is yet available, antimalarial drug treatments and prophylaxis are the most efficient strategies to control falciparum malaria infections. Although the malaria illness is caused by the asexual blood stages, the presence of gametocytes is directly responsible for the infection of the anopheline vector, thus perpetuating the plasmodial cycle. The fight against these transmissible parasite forms is thus an essential strategy, as recommended by the research agenda consultative group for malaria eradication.[3] However, antimalarial drugs are often only tested against the erythrocytic asexual stages because it is these that are responsible for the pathology, whereas the gametocytes stages are asymptomatic.

In the fight against malaria, gametocytes have a dual importance. On one hand, gametocytes should be a target for antimalarials to limit transmission. As this stage is crucial in the spread of malaria, targeting gametocytes would be an obligatory step for all the drugs used against *Plasmodium* in addition to their action against asexual erythrocytic stages. On the other hand, evaluation of the gametocytes' response to chemotherapy is warranted because some antimalarials act against symptomatic asexual erythrocytic parasites but also indirectly on gametocytes by inducing an increase in gametocytogenesis and thus an increase in transmission. This consequently increases the total number of new hosts infected. Indeed, it has been demonstrated that gametocytogenesis is linked to environmental changes and antiplasmodial treatments.[4−6] Moreover, treatments of malaria do not always result in complete parasite clearance and surviving parasites can produce compensatory responses by increasing their rate of gametocyte production leading to increased transmission. It was demonstrated that the number and infectivity of *P. falciparum* gametocytes are different according to the drug used and its efficacy. *P. falciparum* gametocytes are independent of the density of the asexual stages,[7] whereas in in vitro cultures their number is correlated with the concentration of asexual stages.[8] Risk factors influencing the gametocyte load in *P. falciparum* infected patients are linked both to the state of the host such as anemia,

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splenomegaly, a prolonged preadmission history of fever, and also to parasite characteristics like recrudescent infection, history of illness longer than 2 days, and slow cure.[9] This shows that gametocytogenesis is profoundly linked directly or indirectly to the parasite response to the antiplasmodials.

In this context, knowledge of the properties of antiplasmodials is helpful in terms of using relevant molecules with the objective of treating malaria and eradicating the transmission. Therefore, drug performance needs also to be evaluated for the ability to limit transmission.[10]

Here we review the effects of antimalarial molecules on gametocytogenesis (the effect of molecules on the proportion of asexual parasites that produce gametocytes) and gametocytocidal activity (action directly against gametocytes). The antiplasmodial properties of the most promising drug candidates active against gametocytes are also reported.

2. METHODOLOGY USED FOR GAMETOCYTOCIDAL AND GAMETOCYTOGENESIS ASSAYS

2.1. Laboratory Assays To Evaluate the Effect of Different Molecules on the Gametocyte Stage. *2.1.1. In Vitro Tests.* In vitro and in vivo, the production of mature gametocytes takes around 8−15 days. Stage I is morphologically indistinguishable from asexual parasites at the ring stage in Giemsa-stained blood smears. Changes in cellular architecture are first observed during stage II, and the morphology of the later stages (III and IV) progressively lengthens to take the characteristic elongated and curved appearance like a scythe.[11] The mature stage V male and female gametocytes can remain at this stage for over several weeks, waiting for the mosquito blood meal before they differentiate into microgametocytes and macrogametocytes, respectively.

The development of continuous culture techniques for *P. falciparum*[12] made it possible to study the erythrocyte stages of development of the parasite outside the human host. Since the publication of the *P. falciparum* gametocyte culture in 1981 by Ifediba and Vanderberg,[13] in vitro assays of drug activity against gametocytes remain relatively few and little developed because of the difficulties inherent in the gametocyte culture. Indeed, the culture of gametocytes is long and time consuming (14 days are necessary to obtain mature gametocytes) and requires skills and know-how for *Plasmodium* culture (low gametocyte numbers in culture, lack of stability, and reproducibility). Moreover, obtaining gametocytes in culture is not systematic, since clones progressively lose their ability to product gametocytes in continuous culture conditions, certainly by the loss of genes necessary for sexual development.[6]

In recent years, a better understanding of drugs effective against gametocytes appeared to many laboratories to be a promising avenue in the fight against malaria. Thus, several in vitro studies have assessed the gametocytogenesis and gametocytocidal properties of different antimalarial molecules.[14−18] The goal of the in vitro gametocytocidal and gametocytogenesis assays is to evaluate the antiplasmodial activity of treatments directly against gametocytes and on gametocyte production, respectively.

For the in vitro gametocytocidal test,[19] young (7-days-old) or old (13-days-old) gametocyte cultures of *P. falciparum* are transferred to a microplate and treated with dilutions of each drug to be tested and compared with control wells of untreated gametocyte cultures. After 48 h of incubation, the numbers of gametocytes per erythrocyte are counted.

For the in vitro gametocytogenesis test,[15,20] several identical subcultures (in flasks or on microplates) of the young parasite forms are established. Some subcultures are treated with the molecule to be evaluated for its ability to induce gametocytes production for around 17−48 h according to the protocols used, whereas the control subcultures are not treated (corresponding to the basal level of the gametocytes production of this strain under the same conditions). Blood smears of the cultures are then regularly taken until mature gametocytes in untreated control subcultures are obtained and the number of sexual parasites per erythrocyte is counted.

The counting of sexual parasites per erythrocyte is usually done manually on thin blood films stained with Giemsa, which is labor intensive, slow, and subjective. Contrary to what is done with asexual parasites, the gametocyte stage cannot be monitored using tritiated hypoxanthine, since DNA synthesis is arrested during gametocyte development. However, an assay was recently proposed based on the incorporation of radioactive hypoxanthine into newly synthesized DNA of microgametes to successfully monitor gametogenesis until the exflagellation stage.[21]

Alternative methods of gametocytemia determination have been proposed to increase the rate of screening: flow cytometry on cultures stained with hydroethidine[22] or fluorescence measurements using the oxidoreduction indicator alamarBlue as a marker of the metabolic activity[23] or an ATP-based bioluminescent assay.[24] Notably, these simple and economical methods, once optimized to reduce false positives, should allow gametocytemia to be determined on any parasite lines including isolates.

The follow-up of the induction of gametocyte production can also be carried out by quantitative molecular biology with the monitoring of genes of interest such as *Pfs16* or *Pfg27* from *P. falciparum* gametocytes, enabling the proportion of gametocyte induction to be evaluated according to the doses of the test molecules.[25]

Finally the use of transgenic parasites has recently been proposed to facilitate gametocytemia determination. Chimeric green fluorescent protein (GFP) parasites were constructed that can easily and accurately be analyzed by fluorescence-activated cell sorting (FACS).[26−28] This assay allows the gametocytogenesis to be seen very quickly by quantification of early gametocytes even before they are morphologically distinguishable from asexual stage parasites. Analogously, recombinant *P. falciparum* parasites expressing GFP-luciferase were also developed and analyzed both via a luciferase-assay and by fluorescence microscopy, which enables the assessment of dose- and time-dependent drug action on gametocyte maturation and transmission to be achieved.[29]

2.1.2. In Vivo Tests. To evaluate the host load of transmission-stage malaria parasites (i.e., the gametocyte number) in a murine malaria model, mice are infected by a murine *Plasmodium* strain (for example, *P. chabaudi* or *P. vinckei* or mainly *P. berghei*) known for its ability to produce gametocytes.

Tropism of *P. berghei* parasites for reticulocytes and the fixed percentage of parasites that differentiate into sexual forms appear as *P. berghei* phenotypic properties that differ from those of *P. falciparum*. Moreover, the development of *P. berghei* gametocytes takes 26−30 h, largely shorter than the developmental time of 8−14 days for *P. falciparum* gametocytes.[30] However, a recent comparison of *P. falciparum* and *P. berghei* gametocyte transcriptomes showed that more

than half the genes found in the transcriptome of *P. berghei* gametocytes possessed *P. falciparum* orthologues. This comparison allowed the detection of 64 genes that were common to both species, including well-characterized sexual stage genes whose role in sexual development is major.[31] As with all models, *P. berghei* thus shows some limits but can be considered as a valid model for gametocytes studies.

The mice are treated with the molecules of interest and compared with a negative control group of untreated mice corresponding to the basal level of gametocytes and sometimes also compared with a positive control group of mice treated with a known molecule. Thin blood smears obtained daily from the tail vein are Giemsa stained and microscopically analyzed to determine the proportion of erythrocytes parasitized with asexual parasites and with gametocytes.

Some molecules can act only on the infectivity of the treated gametocytes. In this case, the number of the gametocytes can be the same with or without drug but the only difference will be that the gametocytes cannot become gametes in the vector mosquito or are unable to give oocysts. To evaluate the effect of molecules on the gametocytes infectivity, the standard assay for measuring factors affecting *Plasmodium* transmission to the mosquito (gametocyte to oocyst transition) is to feed groups of mosquitoes on parasite-infected hosts like mice (infected and treated by the molecule to be evaluated)[32] or on infected blood in artificial membrane feeders (molecule to be tested is mixed with freshly collected murine *Plasmodium*-infected mouse blood containing exflagellating gametocytes).[33] Then oocysts that develop in the mosquito midgut are counted after dissection to ascertain the prevalence of the parasite.[34] This test, widely used in the evaluation of potential transmission-blocking vaccines, was recently semiautomated to considerably increase the throughput of oocyst counting using GFP-expressing parasites.[35]

Just before human clinical trials, monkeys can also be used as in vivo models to evaluate the gametocytocidal action of a molecule. In this case, monkeys are infected with *P. cynomolgi* sporozoites, harvested from infected *Anopheles* mosquitoes.[36] The major interest of this model is that monkeys can also be infected with the human malaria species *P. falciparum* by sporozoites harvested from infected *Anopheles* mosquitoes during a blood meal.[37] The infected monkeys are then treated with the molecule of interest, and their blood is collected by mosquitoes during blood meals. Analysis of the presence of oocysts and of viable sporozoites in mosquitoes some days postinfective blood meal enables the infectivity rate and the gametocytocidal effect of the drug tested to be calculated.

2.2. Clinical Evaluation of Drug Effects. The evaluation on gametocytogenesis in vivo is relatively difficult because of confusion with gametocytemia, since the presence of gametocytes can be due to an increase of gametocytes production (gametocytogenesis) or to an inability of the drugs to kill gametocytes already produced (gametocytocidy) whereas in vitro tests can clearly distinguish these two phenomena. Even if the impact of treatment on gametocytes was underestimated for a long time, gametocyte prevalence is now quasi-systematic data analyzed during clinical trials evaluating antimalarial drugs according to World Health Organization (WHO) guidelines.[38−40]

The drugs are therefore studied both to determine their ability to kill gametocytes and to influence the gametocytogenesis. A person who has been successfully treated with antimalarial drugs may thus be healthy but infective for several weeks until the gametocytes disappear. In a clinical trial, the parasitological (blood parasite concentration) and clinical (symptoms and biology) evaluations of patients treated by the antimalarial therapy under test (single antiplasmodial drug, drug combinations, or several drugs used sequentially) are done in comparison to a standard and already approved antiplasmodial drug. The parasite study is evaluated by blood smears and PCR to differentiate new infestations and resistance, and the percentage of asexual and sexual parasites in the blood of patients is determined.

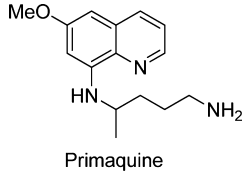
However, it is always difficult to reach conclusions about the efficacy of drugs against gametocytes and to determine if the drug has a real action directly against gametocytes or acts by clearing asexual parasites. Although the question of drug-induced gametocytogenesis has been investigated in a number of clinical studies,[41] drawing firm conclusions from these studies has been difficult. Indeed, the hypothesis elaborated in the murine model to explain the increase in the gametocyte concentration after exposure to antimalarial drugs due to increased gametocyte conversion does not appear to be applicable in the case of the human malaria *P. falciparum*, since the peak of gametocyte prevalence[41−43] generally occurs relatively quickly and in a shorter time than is necessary for the development of new gametocytes. This means that the gametocytes were already present when the drug was administered.[43] Certain drugs do not seem to cause the production of new gametocytes, but they could induce the release or the redistribution of gametocytes into the peripheral blood.

3. REFERENCE ANTIPLASMODIAL DRUGS

Most antimalarial drugs have a schizonticide action but no effect against gametocytes. So while the patients are cured, the drugs do not stop the disease transmission to others in the population. The propagation of the infection can occur a long time after clearance of asexual parasites, since mature gametocytes may still be present and infective for several weeks in the blood. Some drugs have even been reported to increase gametocyte numbers and transmission to mosquitoes. Furthermore there is evidence of enhanced rates of transmission of drug resistant parasites from drug treated individuals.[44]

Here we report the anti-gametocytes properties of the most important antiplasmodial drugs used. As already known, primaquine appears as the major drug against gametocytes but promising results obtained in vitro and/or in vivo and for some of the drugs in clinical trials suggest new therapeutic solutions.

3.1. Primaquine. Primaquine (PQ) (Figure 1) is an 8-aminoquinoline active against liver stages of *P. falciparum* that can also eliminate latent liver forms of *P. vivax* and *P. ovale*. PQ is used for prophylaxis against *P. falciparum* and for the prevention of relapses of *P. vivax* too. PQ acts on the parasites'



Primaquine

Figure 1.

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mitochondria, the number of which is increased in gametocytes, certainly by selective generation of oxidative stress in the parasitized erythrocytes (for review, see ref 45). It is also gametocytocidal against all malaria species.

Whatever the methodology used to quantify its gametocytocidal activity in vitro, PQ was largely less effective in vitro (with IC_{50} values between 0.7 and 15 μM)[19,22,27,46] compared with the expected activity obtained from well-documented clinical studies. The lack of in vitro activity could be explained by the hypothesis that it is the metabolites of PQ that are responsible for its gametocytocidal activity in vivo.[27]

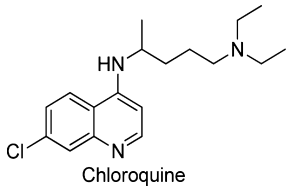
The gametocytocidal activity of PQ assessed in vivo by treating *P. berghei* infected mice with a single intraperitoneal injection gave ambiguous results: PQ completely inhibited the production of oocysts at a dose of 50 μmol/kg (23 μg mL^{-1} kg^{-1})[47] but not totally at the same dose in another study.[45] Moreover, even if PQ at a dose of 25 mg/kg of mouse body weight did not effectively clear erythrocyte parasites within 6 days of drug administration (a significant number of gametocytes were present), at this same dose, there was a total elimination of ookinetes, oocysts, and sporozoites in the mosquitoes' midgut and salivary glands.[48]

The gametocytogenesis effects of PQ on *Plasmodium* asexual stages are poorly documented. A study conducted in vitro showed surprisingly that PQ used at the value corresponding to IC_{10} (concentration inhibiting 10% of the parasite growth in the Desjardins' assay) led to a statistically significant increase in gametocyte production.[27]

In clinical trials, PQ has no effect on asexual stages of *P. falciparum*, but a single dose of PQ was effective on the *P. falciparum* gametocytes by accelerating clearance in all the endemic areas and whatever the chemosensitivity of the asexual parasites treated.[49−54] Very recently, a comparison of the efficacy of several drug regimens against *P. falciparum* gametocytes with or without PQ showed a marked decline in gametocytemia on days 4 and 8 in patients treated with PQ.[55] However, complementary clinical trials are still required to optimize PQ use such as optimal time of administration, treatment duration, dose, etc.[55] Therefore, for the moment, PQ is the only recommended molecule really active against *P. falciparum* gametocytes. Unfortunately, this drug is dangerous during pregnancy and for patients with glucose 6-phosphate dehydrogenase (G6PD) deficiency (for review, see ref 56). The general use of PQ is therefore limited, since severe deficiency of G6PD is highly prevalent in malaria endemic areas. The dramatic side effects of PQ treatment in individuals with G6PD deficiency led to hemolytic anemia[45] by producing significant oxidative stress.[57]

The question currently asked concerning PQ use is the balance between the benefit to the community by reducing transmission and the risk to the individual due to the PQ side effects.[58] That is why, despite its adverse side effects known for many decades, WHO recommends the use of PQ for the elimination of *P. falciparum* gametocytes. The WHO recommendations for the treatment of malaria in endemic areas are clear: "A single dose of primaquine is recommended in addition to artemisinin-based combination therapies (ACTs) as an anti-gametocyte medicine in treatment of *P. falciparum* malaria, particularly as a component of a pre-elimination or an elimination program, provided the risks for hemolysis in G6PD-deficient patients have been considered."[40]

3.2. Chloroquine. Chloroquine (CQ) (Figure 2), a cheap and nontoxic 4-aminoquinoline (possible to administer to young children and also during pregnancy), became the mainstay of therapy and prevention because of its marked and rapid schizontocidal activity against blood forms of all *Plasmodium* sp. Even if resistance began by the end of the 1950s and reached all the endemic areas by the late 1970s, CQ has been the most common aminoquinoline compound used for the treatment of *P. falciparum* infection during many years later.



Chloroquine

Figure 2.

In vitro assays demonstrated that CQ had little or no gametocytocidal activity in the range of concentrations used for the drug tests on asexual parasites (against stage III).[19,59,60] Moreover, Buckling et al. showed that subcurative CQ treatment increased in vitro gametocytogenesis in *P. falciparum*.[15] When CQ was used at the doses corresponding to the IC_{10} but also the IC_{50} and IC_{90} values obtained on asexual forms, gametocyte formation was increased in *P. falciparum* cultures.[27] These observations may explain the relative ineffectiveness of CQ in reducing malaria transmission. Notably these data have been observed without measuring maturation and infectivity of the resulting gametocytes.

These results were confirmed in vivo. Mice with *P. chabaudi* infections treated with a subcurative dose of CQ (corresponding to the commonly recommended dose for treatment of people with *P. falciparum*) showed an earlier peak and increased gametocytogenesis compared to untreated mice.[61]

Some clinical trials such as those conducted in Mozambique,[62] in Gambia,[43] or in Cameroon[63] determined that CQ had no gametocytocidal activity with gametocytemia remaining relatively stable but no induction of gametocytogenesis during the course of CQ chemotherapy. However, most of the trials showed that the CQ regimen was often associated in *P. falciparum* with increased gametocyte levels with a peak of gametocytemia between day 7 and day 21 after CQ treatment.[64−66] CQ was also implicated in an increased gametocyte load after *P. vivax* infection.[67]

All studies show that the increase in parasites drug resistance is one of the main causes of the increase in gametocyte load maintaining the transmission of malaria resistant parasites, independent of the intrinsic effect of CQ on gametocytes.[68] As CQ resistance largely spread in all endemic areas, it led to high levels of gametocytes in CQ-treated patients. The increase in gametocytes appeared as a good indicator of the spread of antimalarial resistance even before treatment failure. These results showed that under CQ pressure, the main selective advantage of resistant parasites is their ability to achieve gametocytogenesis. Studies conducted in Senegal[44] (with a high prevalence of resistant infections) showed that gametocytemia peaks at day 7 after treatment with CQ, with the prevalence of gametocytes reaching 38.2% and 89.6% in the CQ-sensitive and CQ-resistant groups, respectively.[7] Similar results were found in Mozambique,[69] Colombia,[70] and Sudan.[42] These data were confirmed by the higher infectivity of the gametocytes from CQ-treated-patients carrying *P. falciparum* CQ-resistant parasites than from those carrying CQ-sensitive parasites, leading to 38 times more oocysts in *Anopheles*.[71]

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In conclusion, CQ does not seem to be active against gametocytes and worse CQ appears to favor gametocytogenesis with a dramatically increased prevalence of gametocytes linked to CQ resistance.

3.3. Mefloquine. Mefloquine (MQ) (Figure 3) is a 4-aminoquinoline methanol developed in the 1970s by the Walter

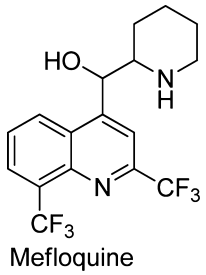

Figure 3.

Reed Army Institute of Research in the U.S. as a synthetic analogue of quinine. In vitro assays showed that MQ used at the IC_{10} values resulted in 8-fold increase in gametocyte formation,[27] but these experiments were carried out only with sublethal doses and without analysis of infectivity. In vivo assays on murine malaria showed no activity against gametocytes and with no significant change in the proportion of gametocytes following MQ therapy in *P. chabaudi* infected mice.[72]

A trial conducted in 1982 demonstrated that MQ had no effect against *P. falciparum* gametocytes.[73] Furthermore, clinical trials in Thailand showed a 3- to 6-fold increase in gametocyte load after a single dose of 15 or 25 mg/kg of MQ.[9,74] Thus, MQ is active against the asexual blood stages of all malaria parasites and has activity against the gametocytes of *P. vivax, P. ovale*, and *P. malariae*[75] but not against *P. falciparum* gametocytes. It can even increase *falciparum* gametocytogenesis.

3.4. Antifolates. *3.4.1. Sulfadoxine−Pyrimethamine.* Sulfadoxine−pyrimethamine (SP) (Figure 4), commercialized

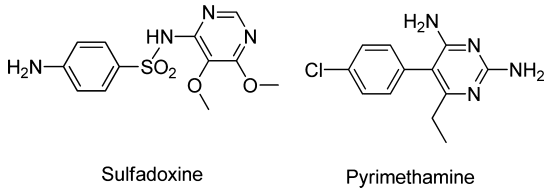

Figure 4.

under the name Fansidar, is a widely used combination of antifolate drugs. This combination acts synergistically to inhibit folic acid metabolism because of pyrimethamine, which is a dihydrofolate reductase (DHFR) inhibitor, and sulfadoxine, which is a dihydropteroate synthase (DHPS) inhibitor. In vitro assays have shown that pyrimethamine-treated gametocytes were more infective to the *Anopheles* vector than untreated control gametocytes.[59] It has been reported that there was an immediate increase in gametocytogenesis following administration of pyrimethamine to *P. chabaudi* infected mice.[72] Surprisingly, certain studies reported that in areas of high SP efficacy, the infectiousness of gametocytes to the vector is very low.[76] Moreover gametocytes present in peripheral venous blood post-SP treatment had reduced infectivity for *Anopheles* as well as a reduced oocyst density, and SP also decreased mosquito survival.[77] However, except in sporadic cases[62,65] reporting a reduction in gametocyte load (certainly due to the clearance of asexual parasitemia), SP is globally linked to enhanced gametocyte production in treated *P. falciparum* malaria patients. Treatment with SP has thus been associated with a greater prevalence and density of post-treatment gametocytemia, as shown in trials conducted in Congo-Brazzaville,[78] Kenya,[79] South Africa,[80] Zambia,[5] Gambia,[64,81] and Nigeria.[82] SP enhances transmission even more considerably than CQ.[5,64,66]

Like CQ,[15] SP resistance (in *dhfr* and *dhps* mutant parasites) is also associated with increased gametocytogenesis[83] and gametocyte load. However, different mechanisms are involved in the transmission of SP-resistant and CQ-resistant parasites. Indeed, under SP pressure, SP-sensitive parasites are able to achieve gametocytogenesis whereas under CQ pressure, only CQ-resistant parasites can achieve gametocytogenesis.[7] Noteworthy, since SP was introduced as first line therapy, this increase of gametocytemia among patients fuels the spread of resistance.[80]

In conclusion, the generation of gametocytes seems to be an inherent property of the antimalarial class of antifolates.[82] Use of SP alone as an alternative first line treatment results in very high post-treatment prevalence of gametocytes.[64,81]

3.4.2. Atovaquone−Proguanil. Atovaquone−proguanil (Figure 5), commercialized under the name Malarone, a

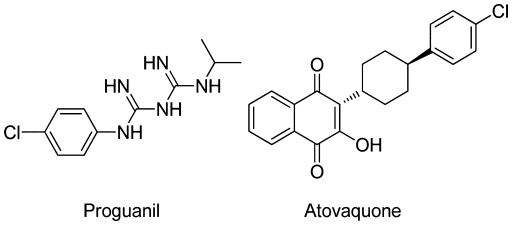

Figure 5.

combination of atovaquone, a hydroxy-1,4-naphthoquinone analogue of ubiquinone, and proguanil, a chlorguanide, is essentially used alone for prophylaxis or in association with ACTs for treatment.[84] Atovaquone−proguanil has fewer side effects but is more expensive than MQ. Resistance to atovaquone−proguanil was rapidly observed just after its introduction as a malaria treatment.[85]

Atovaquone, which is known to inhibit mitochondrial electron transport, has no gametocytocidal activity in vitro on II−III or IV−V stages.[19] Moreover, Fleck et al. reported a small increase of 15% of the gametocyte stage V by atovaquone in an in vitro study but a decrease in gametocyte stages II, III, and IV.[86] Exposure to the doses of atovaquone corresponding to the IC_{10}, IC_{50}, and IC_{90} values obtained against asexual parasites led to a 5-, 4-, and 10- fold increase in gametocyte production, respectively.[27] The little, if any, effect of atovaquone at nanomolar concentrations was recently confirmed whatever the gametocyte stage.[29] Sera collected from volunteers treated with atovaquone−proguanil strongly inhibited oocyst production in *P. berghei* until day 56 post-treatment.[87]

A clinical trial conducted in Mozambique suggested that atovaquone−proguanil caused a significant infectivity reduction with a decrease in gametocytes number of 68% by day 4 until 0% by day 21.[88] Another trial carried out in Thailand on patients with uncomplicated multidrug-resistant *falciparum* malaria showed that in the group of atovaquone−proguanil

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treated patients, gametocyte load decreased slightly from 9.1% at admission to 6.2% whereas adding artesunate reduced the subsequent gametocyte carriage 21-fold (0.29%).[84] The intrinsic action of atovaquone−proguanil on gametocytogenesis appears unclear.

In conclusion, it is our understanding that little is therefore known about the influence of atovaquone alone or in combination with proguanil on gametocyte load in patients, and further investigations are required.

3.5. Artemisinins and Artemisinin-Based Combination Therapies (ACTs). ACTs are the recommended first-line drugs for the treatment of uncomplicated malaria in several countries where resistance of the parasites to previous molecules has developed following WHO recommendations.[40,89] Five ACTs are currently used all over the world (Figure 6): artemether + lumefantrine (ATM + LUM, commercialized under the names Coartem or Riamet), artesunate + amodiaquine (ATS + AQ), artesunate + mefloquine (ATS + MQ), artesunate + sulfadoxine-pyrimethamine (ATS + SP), and dihydroartemisinin + piperaquine (DHA + Pip).[40] Artemisinins, the class of artemisinin derivatives, are highly effective against asexual erythrocytic *P. falciparum* parasites and well-tolerated. Several studies have also reported the very promising efficacy of ACTs against gametocytes.[90] In vitro, at the doses corresponding to the IC_{50} values obtained against asexual parasites, artemisinins have anti-gametocyte properties against stages I−III.[19,46] Several in vivo studies have also suggested that ACTs kill immature forms but not mature gametocytes.[24,91,92]

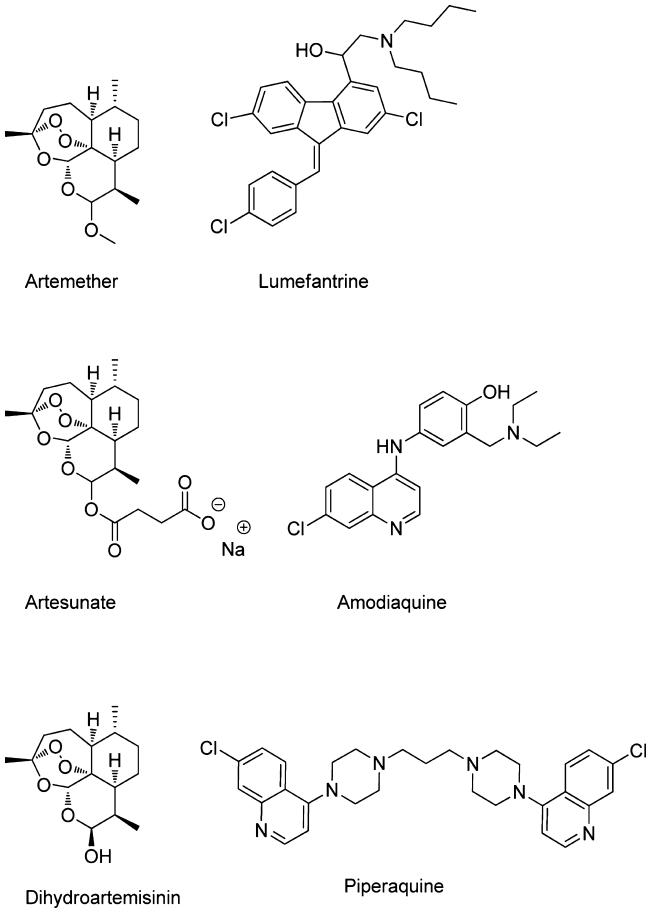

Figure 6.

Numerous clinical trials have been carried out with artemisinins alone or in combination to evaluate the effect of ACTs on gametocytes and to inhibit gametocytogenesis (for review, see ref 93). These studies showed that in *P. falciparum* infections the gametocyte load was significantly higher after treatment with whatever antiplasmodial drug was used (MQ, quinine, SP, etc) than after treatment with artemisinins alone or in ACTs.[74] ACTs thus appeared as a new safe anti-gametocytes alternative that could stop transmission of drug resistant parasites, in the context of the toxicity of PQ as well as the emergence of drug resistance against *P. falciparum*. However, the effects of ACTs on malaria transmission are not so clear-cut. Indeed, artemisinins are well-known for reducing the development of gametocytes and consequently the load of gametocytes in the peripheral blood,[94] but the choice of the partner drug appears to be essential depending also on the level of transmission in the area studied.[95] All the data concerning ACTs efficacy against gametocyte load therefore need to be confirmed for each ACT used. Moreover, it is interesting to distinguish between the direct anti-gametocyte activity of ACTs and the indirect action via the reduction of the asexual parasite population, which is the source of new gametocytes. The latter consequently leads to the reduction of transmission intensity as well as a reduction in the spread of resistant parasites.[71,92] The reduction in gametocyte charge would be the result from the failure of preceding asexual parasites to mature and release invasive merozoites. This confirms the assumption that the eradication of asexual forms of *P. falciparum* by effective antimalarial treatment is one of the most effective means of preventing subsequent gametocytemia.

Finally, the impact of ACTs on malaria transmission needs to be moderated, since a mathematical model predicted that in higher transmission settings an efficacious antimalarial regimen with no gametocytocidal properties but a long prophylactic time is more effective at reducing transmission than a short-acting ACT, independent of the risk of the development of resistance.[95]

3.5.1. Artemether + Lumefantrine (ATM + LUM). An analysis of six clinical trials conducted to evaluate the efficacy of a six-dose regimen of ATM + LUM showed that in many different patient populations from Asia, Africa, and South America, the gametocyte load was significantly reduced and more rapidly with this first fixed ACT dose proposed by WHO, when compared with other antimalarial regimens.[96] This was also demonstrated with Gambian children under an ATM + LUM regimen, with gametocytes at significant lower densities, for shorter periods and less infective to mosquitoes than the CQ-SP-treated group.[92]

3.5.2. Artesunate + Amodiaquine (ATS + AQ). The gametocyte load found in children in Kenya, Senegal, and Gabon was significantly more rapidly decreased after ATS + AQ treatment than after amodiaquine alone from day 0 to day 21 but comparable for both regimens on day 28.[97] Another study on Nigerian children showed that gametocyte carriage rates decreased after ATS + AQ treatment, explained by the accelerated clearance of the asexual forms by artesunate.[98]

3.5.3. Artesunate + Mefloquine (ATS + MQ). The addition of ATS to the MQ regimen produced a reduction of gametocyte emergence and of gametocyte density, faster gametocyte clearance, and significant gametocyte sex ratio modifications.[99] However, after the ATS + MQ standard regimen, patients from Thailand were negative for asexual parasites by day 7, whereas gametocytemia persisted in some

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patients. ATS + MQ thus appeared to be ineffective as a gametocytocidal drug to effectively stop gametocyte transmission.[100]

3.5.4. Artesunate + Sulfadoxine–Pyrimethamine (ATS + SP). The addition of ATS to SP resulted in a decrease of gametocytemia in Gambian children on day 7 from 56% (SP alone) to 11.4% (SP + 1 dose of ATS) and to 8.7% (SP + 3 doses of ATS).[64] The impact of the ATS with SP regimen on gametocytemia reduction was also reported in another study[43] and was also confirmed in Kenyan children.[101]

3.5.5. Dihydroartemisinin + Piperaquine (DHA + Pip). At the IC_{50} value, artemisinin and piperaquine each caused a statistically significant increase in the number of gametocytes.[27] In a clinical trial, the combination DHA + Pip appears as a good ACT regimen for asexual *falciparum* parasites. Many studies reported an action of this combination against gametocytes.[102−106] Some treatment failures seemed to be linked with cross-resistance between piperaquine and chloroquine[107] and could explain that DHA + Pip sometimes showed a poorer clearance of gametocytes.[108]

In conclusion, ACTs led to a lower gametocyte load in most clinical trials, whatever the geographic area.[74,92] ACTs activity certainly corresponds to an indirect effect principally due to a rapid clearance of asexual parasites that stops gametocytogenesis and reduces malaria transmission.[49] Indeed, all antimalarial drugs that kill asexual stages also kill the early stages of *P. falciparum* gametocytes.[109] ACTs are thus associated with lower infectiousness, but transmission is not totally stopped by these regimens.[110] A study carried out on Kenyan children showed more precisely that the proportion of infected mosquitoes was reduced whereas the proportion of infectious children was not significantly reduced after ACT treatment.[111] This moderate effect of ACTs directly against gametocytes could also be due to the short half-life of artemisinin derivatives. ACTs therefore seem to act essentially on gametocytogenesis, whereas PQ really acts directly against gametocytes.[49,53,112]

4. ANTIMALARIAL DRUG ASSOCIATIONS

In this context, drug combinations are strongly needed that will act both against asexual parasites to stop the pathology and against sexual parasites to also stop transmission. According to the results to date, PQ remains the anti-gametocyte drug par excellence. That is why WHO currently requests that one dose of PQ be added to each treatment.[40]

PQ has thus been tested in association with molecules targeting the asexual *P. falciparum* parasites stages such as quinolines and more recently with ACTs. A study conducted in Thai patients with uncomplicated *falciparum* malaria found no evidence of synergy of PQ with quinine or PQ with artesunate against asexual stages of *Plasmodium*; however, the combinations including PQ accelerated gametocyte clearance in *P. falciparum* malaria.[49] Another study in Indonesia on patients treated with CQ ± PQ versus CQ + SP ± PQ confirmed the absence of a PQ effect on asexual stages but significantly accelerated clearance of gametocytes.[52] Surprisingly, this is in contrast to the findings of Suputtamongkol[113] and El-Sayed et al.[114] showing that PQ was not very effective in the eradication of gametocytes.

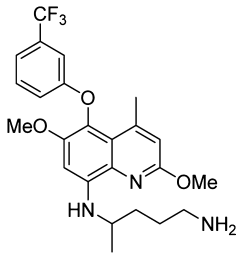
More recently, a clinical trial carried out in Myanmar showed that whatever the ACT regimen used (ATS + MQ, ATS + AQ, DHA + Pip, ATM + LUM) and whatever its efficacy on asexual parasites, patients who received a single dose of PQ in addition to the ACT had a 12-fold reduction of their gametocyte carriage in comparison to patients treated without PQ.[115] Optimally timing the PQ treatment is also studied by mathematical model to lead to a total interruption of *falciparum* malaria transmission.[116] Therefore, a gametocytocidal drug such as PQ appears globally to be useful in combination with an antiplasmodial compound regimen to clear gametocytes and to block transmission.[109] Unfortunately, the use of PQ is limited because of the risk of hemolysis in patients with G6PD deficiency and hemolytic anemia production. Notably, it was recently proposed that the activity and the toxicity of PQ were not mediated through the same metabolic pathways, and hence, a partner drug that increases the activity but not the toxicity of PQ could theoretically be found.[117]

5. NEW COMPOUNDS: RESEARCH AGAINST SEXUAL STAGES

Because of the dramatic risk of PQ for patient safety, new molecules with equivalent anti-gametocytes properties as PQ but with fewer side effects are needed to be used in association with antiplasmodial drugs active against asexual *Plasmodium* stages. The first approach to search for such drug candidates may be the synthesis of PQ derivatives.

5.1. Primaquine Derivatives. Primaquine is the only available transmission-blocking antimalarial displaying a marked activity against gametocytes from all species of parasite causing human malaria. However, the use of PQ is limited by both its short plasma half-life and its toxic effects, among them methemoglobinemia. In the search for a less toxic alternative to PQ, numerous derivatives have been prepared through pharmacomodulation of the PQ skeleton (for review, see ref 45). Two different strategies were envisioned: (1) the introduction of substituents on the quinoline ring and (2) the modification of the terminal primary amino group.

5.1.1. Modification of the Quinoline Ring. Tafenoquine (WR238605)[118] (Figure 7), a 5-phenoxyl derivative of PQ, is



Tafenoquine

Figure 7.

currently in a clinical trial (phase IIb/III) for the treatment and prevention of malaria. Tafenoquine is also being developed in combination with standard doses of CQ for the radical cure of acute *P. vivax* malaria. This compound is more potent than PQ against asexual stages both in vitro on *P. falciparum* isolates coming from various endemic areas[119] and in vivo on *P. cynomolgi* and *P. fragile* in rhesus monkeys.[120] Moreover, tafenoquine has a longer human plasma half-life than PQ (2 weeks versus 5−6 h)[121] and it was recently shown to be safe and well-tolerated in patients following 6 months of prophylaxis.[122] Nevertheless it is also hemolytic in G6PD-deficient patients, although to a lesser extent than PQ. Studies are still being carried out to further separate the antimalarial

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activity of tafenoquine from the hemolytic side effect,[123] yet this goal will be more easily achieved when a validated G6PD-deficient model is developed.

Tafenoquine showed moderate in vitro activity against *P. falciparum* sexual stages with gametocytocidal activity only against early stages, as reported for PQ.[29] In vivo studies on mice infected with *P. berghei* also showed the efficacy of tafenoquine on sexual parasites.[48] At a dose of 100 mg/kg, tafenoquine significantly reduced both oocyst and sporozoite production whereas PQ had no such effect.[124,125] Finally, no clinical trial results on the tafenoquine gametocytocidal activity have yet been published.

More recently, another optimization of the substitution pattern of the quinoline nucleus led to (−)-8-[(4-amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3,4-dichlorophenoxy]quinoline succinate **1** (NPC1161B)[126] (Figure 8). **1** showed moderate activity in vitro against mature gametocytes with an IC_{50} of 3.8 μM[24] but totally inhibited exflagellation at 10 μM.[127]

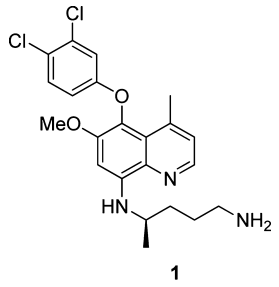

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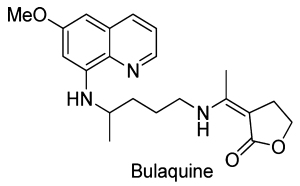
Figure 8.

5.1.2. Modification of the Terminal Primary Amino Group. Alternatively, the Central Drug Research Institute, Lucknow, India, developed bulaquine (CDRI 80/53 or elubaquine)[118] (Figure 9), a PQ prodrug in which the primary amino terminal group was replaced by an enamine moiety. Bulaquine showed significantly reduced metHb toxicity in dogs in comparison to PQ.[128]



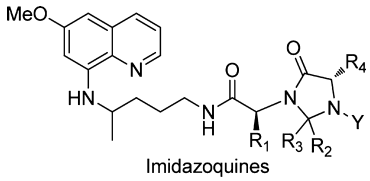
Bulaquine

Figure 9.

Bulaquine was initially shown to have antirelapse activity against established sporozoite-induced infections with *P. cynomolgi* in rhesus monkeys.[129] The gametocytocidal activity of bulaquine was then evaluated using the *P. cynomolgi*−rhesus monkey−*Anopheles stephensi* model and showed a faster action than PQ:[36] a complete loss of oocyst development and mosquito infectivity was observed within 5 h after administration of a single dose of bulaquine, whereas similar activity was observed within 24 h for an equivalent dose of PQ.

Bulaquine was also tested against *P. falciparum* in humans. A preliminary limited clinical study employing a single dose of bulaquine (75 mg) at day 4 or PQ (45 mg) with quinine (30 mg kg^{-1} day^{-1} for 7 days) + doxycycline (100 mg/day) was conducted in India. At day 8, 7-fold more PQ-treated patients than bulaquine-treated patients had gametocytes. Bulaquine thus showed a better clearance of gametocytes than PQ.[130] This was followed by a randomized study in a larger population with *P. falciparum* malaria successfully treated with blood shizonticides that confirmed the superior efficacy of bulaquine to clear gametocytes than PQ.[51] In parallel, bulaquine was shown to be as efficient as PQ in the antirelapse treatment of *P. vivax* malaria in Thailand.[131] Moreover, bulaquine did not cause clinically significant hemolysis in G6PD-deficient patients. In spite of the lack of pharmacokinetics data, bulaquine appears to be a safe and promising alternative to PQ as a *P. falciparum* gametocytocidal agent.

Following this idea of the modification of the terminal primary amino group to prevent or reduce the oxidative deamination metabolic pathway, alternative chemical structures have been proposed including peptidomimetic imidazolidin-4-one derivatives named imidazoquines[47,45,132−135] (Figure 10).



Imidazoquines

Figure 10.

Promising in vivo results were obtained for the imidazolidin-4-one derived from Ala-Ala-PQ and acetone (R_1, R_2, R_3, R_4 = Me and Y = H in Figure 10) administered by intraperitoneal route using the *P. berghei*−*Anopheles stephensi* model. This new imidazoquine compound reduced the number of oocysts in the midguts of infected mosquitoes more efficiently than PQ.[133] Further in vivo studies and then clinical studies should be established to validate the potential advantages of imidazoquines over PQ.

Analogously, a trioxane derivative was added via reductive amination of the primary amino group of PQ leading to **2** (DU2302)[136] (Figure 11), a new kind of trioxaquine, the synthetic hybrid molecules containing a trioxane motif linked to an aminoquinoline entity, developed by Meunier et al.[137−139] In vitro activities of **2** against young and old gametocytes of the *P. falciparum* strain W2 were evaluated and compared to reference antimalarial drugs (PQ, CQ, ATS, atovaquone).[19] **2** was very potent against both asexual stages and gametocytes, whatever the parasite stage targeted, showing slightly greater activity than artesunate.[19] Nevertheless the analogous trioxaquine **3** (DU1302)[136] built on a 4-aminoquinoline nucleus instead of the 8-aminoquinoline one of **2** showed similar good in vitro activity on sexual stages, indicating that the activity is certainly attributable to the trioxane pharmacophore. Indeed, all the endoperoxides-derived compounds tested in this work presented good gametocytocidal activities compared with other compounds such as quinolines.[19]

Analogously, PQ-artemisinins were recently designed to act on both erythrocytic and liver stages,[140] but their gametocytocidal activity was not evaluated.

5.2. New Molecules. The search for new transmission-blocking compounds to replace PQ may also involve the synthesis or the discovery of completely new compounds to enlarge the structural diversity of the therapeutic tools to delay the resistance phenomenon. As is usually the case in medicinal

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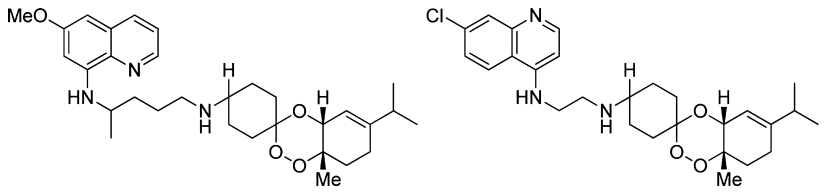
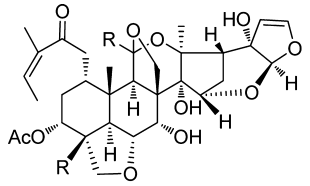


Figure 11.

chemistry, three different strategies may be envisioned: (1) the screening of the natural products, (2) the evaluation of drugs indicated in other therapies, and (3) the complete drug-design strategy using the recently identified therapeutic targets that are specific to *Plasmodium* sexual stages, such as heme detoxification pathway or certain protein kinases. Another way to find new drugs can be phenotypic screening which has been the major success in antimalarial drug discovery in the past 5 years.[141]

5.2.1. Natural Products. Historically, natural products have led the way in malaria therapy, quinine and artemisinin being important as such examples. Various medicinal plants are traditionally used to treat malaria in endemic areas and thus represent a possible source for the discovery of antimalarial compounds. One of the most advanced searches for gametocytocidal natural compounds concerns the extracts of the neem tree, traditionally used in India to treat malaria and other diseases,[142] that have been reported to possess in vitro inhibitory activity against both asexual and sexual stages of *P. falciparum*.[143−145] Several studies led to the identification of azadirachtin (Figure 12) as the most promising compound with



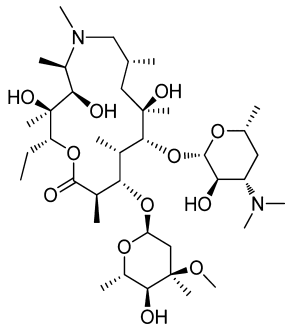
Azadirachtin (R = CO_2Me)

Figure 12.

transmission blocking activity. This triterpenoid, present in neem leaves and particularly abundant in neem seeds, was reported to completely block *P. berghei* development in the *Anopheles stephensi* vector at a dose of 50 mg/kg mouse body weight administered intraperitoneally.[146] Moreover, a study of azadirachtin's impact on gametocyte formation showed that this molecule disrupts the formation of organized microtubule arrays during microgametogenesis in *P. berghei*.[147] No clinical studies have been reported yet to confirm the potential transmission-blocking activity of azadirachtin.

5.2.2. Drugs Used in Other Diseases. Another strategy in the search of a safe alternative for PQ consists of evaluating the gametocytocidal activity of drugs used for other pathologies and thus already safety-approved.

Azithromycin (Figure 13), a licensed antibiotic broadly used for the treatment of bacterial infections, is an interesting antimalarial drug because of its safety in children and the extensive experience with its use during pregnancy. Given its good safety profile and its activity on liver and blood stages, azithromycin seems to be a safe antimalarial, ideal for mass drug



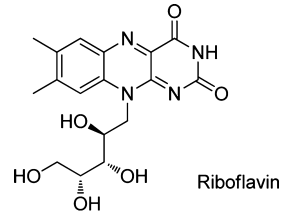
Azithromycin

Figure 13.

administration. Azithromycin inhibits the parasite development in the mosquito stage: both *P. berghei* gametocyte−ookinete transformation in the midgut and sporozoite production in the oocyst.[148] However, very recently, azithromycin treatment was reported to have no significant effect in vitro on the development of stage II gametocytes.[149] The combination azithromycin + CQ is currently in phase III clinical trials.[150]

Antibiotics used in new malaria regimens seem to have moderate activity against gametocytes. Cotrimoxazole, a combination of sulfamethoxazole and trimethoprim,[98,151,152] did not show interesting gametocyte activity in patients with acute or uncomplicated *P. falciparum* malaria. Analogously, fosmidomycin−clindamycin, which is a new antimalarial therapy currently in phase III trials[150] with promising antiplasmodial activity and a new mechanism of action blocking the synthesis of isoprenoids pathway,[153] showed a very weak ability to stop the development of sexual stage parasites.[153,154]

Riboflavin (Figure 14) is commonly used to treat inborn errors of metabolism and neonatal jaundice and in the



Riboflavin

Figure 14.

prophylaxis of migraine.[155] Its safety is thus well-established. Riboflavin was shown to be effective at 100 μM in vitro against *P. falciparum* gametocytes at all stages. Additionally, it was found to have a synergistic and additive effect with MQ, pyrimethamine, quinine, and artemisinin but not CQ. This suggests that riboflavin might be a good combination partner against both asexual and sexual *P. falciparum* parasites.[14] In vivo

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studies should be instigated to confirm these interesting in vitro results.

Considerably better results were obtained with methylene blue (MB) (Figure 15) that can be seen as structurally

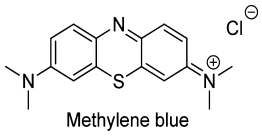

Figure 15.

analogous to riboflavin, since both compounds may be involved in the same kind of redox-based mechanisms of action.[156,157] MB provides the most important example of reuse of an ancient drug.[158] It has been proposed for many indications including malaria and has recently been re-examined for its activity on sexual stages of *P. falciparum*.[29] Given its safety in a population[159] even in G6PD-deficient patients in Burkina Faso,[160] the efficacy of MB-based combination therapy against gametocytes was evaluated through a randomized controlled phase II study in Burkina Faso.[161] All the MB-based treatments tested were more effective than the classical ATS-AQ treatment, and they were associated with a pronounced gametocytocidal activity against both existing and developing *P. falciparum* gametocytes.[161] MB could thus be considered as a possible safe alternative to PQ. The combination MB + CQ was taken through to phase II studies[162] but did not meet the WHO criterion of 95% efficacy, so new combinations with AQ or ATS are currently under study.[150] The high activity of MB observed in clinical tests was recently confirmed in an in vitro quantitative test using *P. falciparum* lines expressing gametocyte-specific GFP-luciferase reporters. The IC_{50} value on mature gametocytes was 12 nM, which is the best in vitro activity reported for 44 compounds provided by the MMV foundation (Medicines for Malaria Venture).[24] MB was also reported to be both the most effective gametocytocidal agent against gametocyte stages I–V and a highly potent transmission-blocking agent.[29] MB should thus be considered as the most promising safe and affordable transmission-blocking drug to be incorporated in combination therapies.

5.2.3. Design of Novel Antimalarial Drugs. Another strategy consists of the identification of new targets and the design of novel effective antimalarials toward these targets. This strategy can reasonably be envisioned, since the complete genome sequences of *Plasmodium*[163] are now available. In particular, the identification of the genes that are differentially regulated during gametocytogenesis[6,164] may lead to the identification of promising specific targets for the search for a transmission-blocking compound.

The biological evaluation of proteasome inhibitors as new gametocytocidal compounds has recently been proposed, since genes predicted to code for the *P. falciparum* proteasome are expressed throughout gametocytogenesis.[165]

Epoxomicin (Figure 16), a natural peptide isolated from an actinomycete strain, is among the most potent proteasome inhibitors known.[166] This compound was shown to be highly active in vitro against both asexual *P. falciparum* laboratory strains and field isolates from Gabon (IC_{50} in the nanomolar range).[167] Moreover, epoxomicin was reported to kill gametocytes (stages III–V) in culture with an IC_{50} of around 2 nM[23] and to block oocyst production in the midgut of *Anopheles stephensi* mosquitoes.[168] Epoxomicin is thus a

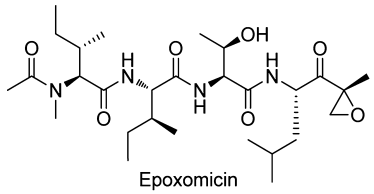

Figure 16.

benchmark in terms of potency for the development of highly active specific proteasome inhibitors. Further studies should be initiated to evaluate if its overall properties are druglike.

In the search of proteasome inhibitors exhibiting higher therapeutic indices than epoxomicin, the activity of the thiopeptide thiostrepton (Figure 17) was evaluated on *P.*

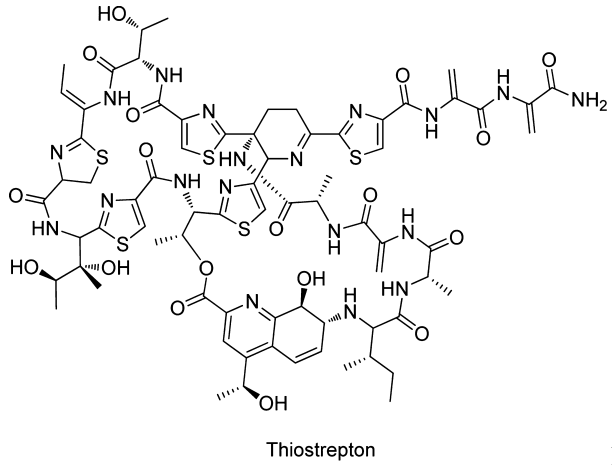

Figure 17.

falciparum.[149] Although having weak activity against asexual parasites (IC_{50} = 8.9 μM), thiostrepton showed a moderate activity in vitro against both young[149] and mature gametocytes (IC_{50} < 10 μM)[24] but a high inhibition of ookinete formation.[127] Notably, thiostrepton seems to inhibit several independent targets, among them the parasite proteasome, the apicoplast ribosome,[149] and probably also the mitochondrial ribosome,[169] which makes it an interesting compound for malaria therapy, provided that further optimization of its in vitro activity can be achieved.

The search for new gametocytocidal compounds targeting DNA is also ongoing. Given the specific A/T richness of the *Plasmodium* genome, many A/T-specific alkylating agents have been evaluated for their antimalarial activity, among which centanamycin (Figure 18) showed promising results in transmission-blocking activity.[170] Centanamycin showed potent antiplasmodial activity against *P. falciparum* in vitro (IC_{50} = 1.8 nM) and against *P. chabaudi* rodent malaria in vivo (ED_{50} = 1.5

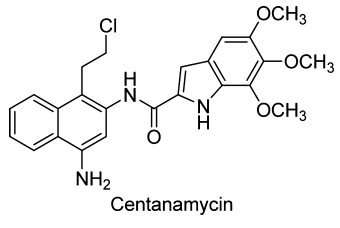

Figure 18.

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mg/kg). Notably, centanamycin had no immediate effect on gametocytes in mice but induced a 83% reduction in the number of oocysts in mosquitoes and a 99% reduction in sporozoites, indicating that oocysts failed to develop into the sporozoite stage.[170] Moreover, treatment of *P. berghei* sporozoites with centanamycin impaired parasite functions with inhibition of hepatocyte infection by sporozoites and arrest of the liver stage development.[171] Further studies should be carried out to gain insight into its particular mechanism of action. Centanamycin seems therefore to be a real transmission-blocking compound, compared to PQ which has no effect on either oocyst or sporozoite development in *P. falciparum* or *P. berghei*, even at doses as high as 100 mg/kg.[125] Nevertheless given its probable toxic profile, the use of alkylating agent will require close clinical monitoring which can be highly difficult in malaria endemic areas.

9-Anilinoacridines should also be mentioned as potential DNA-targeted gametocytocidal compounds. These potent inhibitors of mammalian DNA topoisomerase II, initially developed as potential anticancer agents, have been tested in vitro for their gametocytocidal activity.[172] The anilinoacridine **4** depicted in Figure 19 was the most potent gametocytocidal

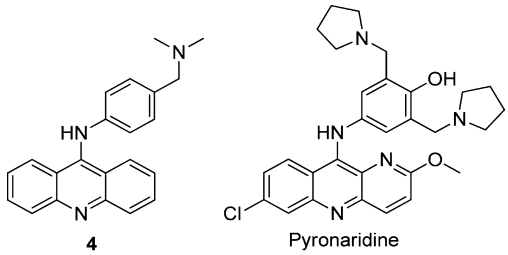

Figure 19.

compound with an IC_{50} of 0.6 μM on gametocytes from both KT1 and KT3 strains, two *P. falciparum* gametocyte-producing isolates, which is more active than PQ (IC_{50} of 0.8 and 2.1 μM on KT1 and KT3, respectively). The antimalarial pyronaridine, a *P. falciparum* DNA topoisomerase II inhibitor[172] also described as targeting hematin,[173] was recently developed in an ACT with artesunate.[150,174] Notably, pyronaridine bears an analogous anilinoacridine nucleus in its chemical structure that confers a similar gametocytocidal activity in vitro with IC_{50} of 0.006 and 0.02 μM on KT1 and KT3 strains, respectively,[17] or an IC_{50} 0.023 μM on the transgenic parasite line 3D7GFP16B.[24] Others studies have also shown inhibition of oocyst production[29] and a potent inhibition of *P. berghei* ookinete formation.[127] However, the in vitro culture of the transgenic chimeric Pfs16-GFP parasites with 1 nM pyronaridine led to a significant increase in the gametocyte number,[27] and a preliminary study on mature gametocytes from patients treated with pyronaridine indicated an absence of gametocytocidal activity.[63] Further clinical studies and complementary analyses on the transmission-blocking activity may indicate the real significance of this newly proposed ACT.

Finally, significant advances have been made since the establishment of *Plasmodium* genome and researchers have now identified several genes that play a key role in gametocytogenesis[175] or in the transition from gametocytes to sporozoites (for review, see ref 176). Several potential targets for the design of a gametocytocidal or transmission-blocking drugs have been highlighted such as PKG, a cGMP-dependent protein kinase,[6] Pfs16, a membrane protein located in the parasitophorous vacuole membrane,[25] and the RNA-binding phosphoprotein Pfg27.[177] Compounds capable of interacting with or inhibiting these targets may soon be designed, opening the way to novel chemical entities in the antimalarial drug research.

6. CONCLUSION AND PERSPECTIVES

Effective treatments and the reduction of malaria transmission would have a considerable impact on the fight against malaria. Chemotherapy is one of the most important intervention strategies for reducing the burden of *P. falciparum* malaria. However, it is vital that antimalarials also reduce malaria transmission. Unfortunately, the systematic appearance of resistance of *P. falciparum* to the commonly antimalarials used is one of the limitations of this strategy. Additionally, most of the current antimalarials have an effect on asexual stages of the parasite, and their partial activity may increase the gametocyte load by reducing asexual parasite and by stressing the surviving asexual population.[109] Primaquine is the most documented drug available and currently recommended by WHO against sexual stages of *P. falciparum*. However, it is used with limitations because of major side effects and the fact that it acts only against the sexual and not the asexual stages of *P. falciparum* parasites. With the exception of primaquine, non-artemisinin combinations are in general poorly effective against sexual stages, or worse, they increase the gametocytes carriage. ACTs are the recommended first-line drugs for the treatment of uncomplicated malaria and have been shown to be highly effective against asexual stages of *P. falciparum* but also indirectly against sexual forms principally because of their action on previous stages. However, alarming signs of emerging resistance to artemisinin derivatives along the Thai−Cambodian border are of major concern[178] both for patient treatment and for malaria-transmission blocking. The development of new antimalarial treatments acting against both sexual and asexual stages of the *P. falciparum* life cycle is thus encouraged in order to treat infection and also to reduce the malaria transmission. The research into compounds aimed specifically at gametocytes is thus a major avenue. Several end points in sexual malaria life cycle can be envisaged: targeting asexual parasites will avoid gametocytogenesis; targeting early gametocytes stages will avoid development of infective gametocytes; targeting mature gametocytes will avoid infections of *Anopheles* vector or avoid development of the gametes in the vector (as exflagellation inhibition). For that, many molecules are currently in preclinical and clinical studies. However, the targeted gametocytes stage, pharmacokinetics−pharmacodynamics relationships, and rate of gametocytes killing needed for optimal activity are still unclear. It is evident that the evaluation of all new antiplasmodial drug candidates against the gametocyte stage is an essential road to go down.

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Notes

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Odile Dechy-Cabaret obtained her Ph.D. in 2001 from Université Paul Sabatier in Toulouse, France, working on the synthesis of

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Françoise Benoit-Vical completed her Ph.D. in 1997 in malaria pharmacology from the University of Montpellier, France. After postdoctoral positions (1998−2001) in Pasteur Institute, France, and in the French National Center of Scientific Research (CNRS), France, she joined as INSERM researcher (French National Institute of Health and Medical Research) the group of Dr. Bernard Meunier in Toulouse, France. Since 2009, she is the head of the group "New Antiplasmodial Drugs and Pharmacological Approaches" in CNRS. Her research interests, conjointly led in the Department of Parasitology of Toulouse Hospital, France, are in the field of malaria, parasite resistances, and medicinal chemistry including the discovery and pharmacomodulations of novel antimalarial leads, with particular focus on mechanisms of action of endoperoxides.

■ ABBREVIATIONS USED

ACT, artemisinin-based combination therapy; AQ, amodiaquine; ATM, artemether; ATS, artesunate; CQ, chloroquine; DHA, dihydroartemisinin; DHFR, dihydrofolate reductase; DHPS, dihydropteroate synthase; FACS, fluorescence-activated cell sorting; G6PD, glucose 6-phosphate dehydrogenase; LUM, lumefantrine; MB, methylene blue; metHb, methemoglobin; MMV, Medicines for Malaria Venture; MQ, mefloquine; Pip, piperaquine; PQ, primaquine; SP, sulfadoxine−pyrimethamine; WHO, World Health Organization

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