

Pharmacodynamics of Antimalarial Agents

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Abstract

Antimalarials were among the first, and today are among the most widely used, anti-infective agents. The fundamental pharmacodynamic endpoint for antimalarials is quite simple: elimination of this eukaryotic protozoal pathogen from its host; numerous surrogates for this have been developed. Antimalarial therapy is confounded by several key factors including the coexistence of multiple pharmacologically distinct *Plasmodium* life cycle forms in the human host; limited resources for discovery, development, and deployment of new drugs; and a high requirement for safety due to the enormous patient population and use for chemoprophylaxis of healthy travelers. Further, for any particular drug, myriad influences impact the pharmacological endpoint, including rapidity of the onset of action, potency, 'static vs. 'cidal activity, susceptibility to parasite resistance, immune status of the host, and the suitability of prevailing pharmacokinetics. Classic and recently described pharmacodynamic endpoints in preclinical models are presented, as are new insights into the pharmacokinetic drivers of antimalarial pharmacodynamics. The efficacy and safety of existing drugs are surveyed, and some novel experimental agents are discussed.

Key words Malaria, *Plasmodium*, Antimalarial, Pharmacokinetics, Pharmacodynamics, PK/PD, Parasite reduction ratio, Resistance, Combination therapy

1 Malaria

Malaria is a mosquito-borne infectious disease that afflicts hundreds of millions and kills nearly a million children every year in Africa. Recent efforts have brought encouraging progress toward eliminating this major public health challenge, via a combination of vector control, use of insecticide-impregnated bednets, improved diagnostics, and chemotherapy campaigns. Nevertheless, the lack of a vaccine and widespread drug resistance make the need for effective and safe new antimalarial agents compelling. Of the *Plasmodium* species pathogenic to humans, *falciparum* is most aggressive, causing life-threatening infection and having the greatest propensity for drug resistance [1, 2].

Malaria parasites have a complex life cycle (Fig. 1). From a therapeutic perspective, by far the most important forms are asexually dividing parasites that dwell within erythrocytes. They alone

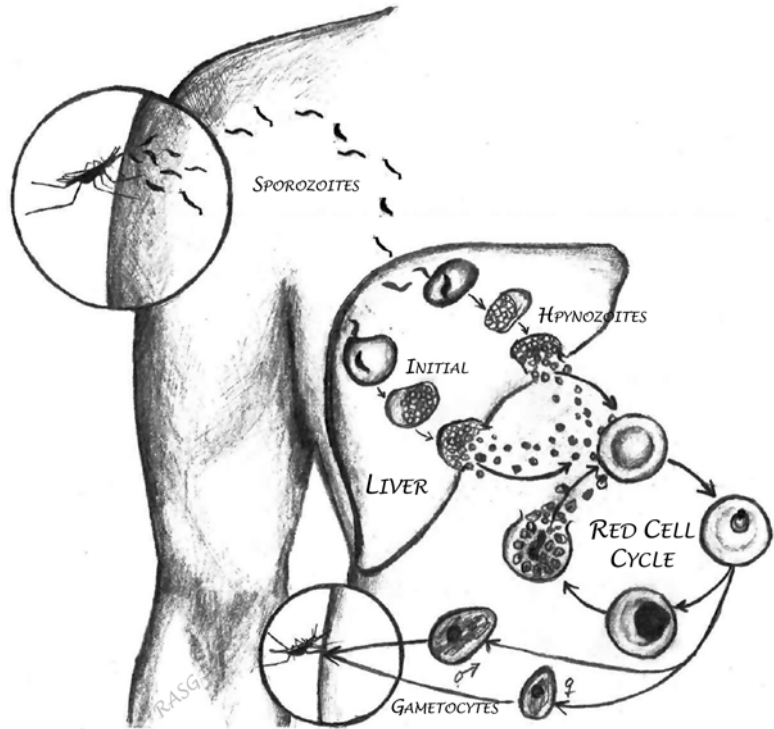


Fig. 1 Life cycle of malaria parasites. Sporozoites inoculated by a mosquito rapidly make their way to the liver and infect hepatocytes. Parasites replicate within the hepatocyte, rupture the cell, and enter the blood stream as merozoites. These merozoites invade erythrocytes, initiating the blood stage of the infection. Erythrocytic parasites amplify 8–32-fold over 48–72 h, rupture the red blood cell, invade new erythrocytes, and restart the cycle. All parasite proliferation within the human is asexual. Some erythrocytic forms differentiate into gametocytes that will infect a biting mosquito, reproduce sexually, and eventually generate sporozoites that complete the life cycle. Some liver stage parasites of *Plasmodium vivax* and *Plasmodium ovale* form latent hypnozoites that may remain quiescent for years, before activating and establishing symptomatic disease

are responsible for the morbidity and mortality of malaria, and in severe infection may number hundreds of millions per milliliter of blood. It is the cyclical release of naked parasites into the bloodstream that gives rise to the classic periodic agues of malaria. *P. falciparum*'s unique lethality stems from adherence of infected erythrocytes to blood vessel walls, which causes a cytokine response and functional vascular obstruction. In severe cases this results in cerebral malaria, extensive hemolysis, and multiple end-organ failures. The remaining life cycle stages (all of which may coexist simultaneously within the same patient) are responsible for transmission of infection to the mosquito and may cause late reactivation of symptomatic disease. Unfortunately, each life cycle form has a different profile of drug susceptibilities (Fig. 2) [3]; hence

multiple drugs may be required for complete eradication of the infection from a patient. In addition to their obvious necessity for the treatment of established infection, antimalarial drugs will also remain essential for the chemoprophylaxis of travelers to malarious regions until a suitable vaccine becomes available.

The discovery and development of antimalarial drugs has proven challenging. *Plasmodium* parasites are host-specific, such that species pathogenic for humans rarely propagate in other animals, and they have proven equally difficult to study in vitro. Obligate intracellular pathogens with an ~80 % AT genome [4], long term in vitro cultivation was not accomplished until 1976, when the preference of *P. falciparum* for microaerophilic conditions was realized [5]. *P. vivax* still cannot be maintained in continuous culture. Over time, however, animal models and in vitro assay systems have matured to the point that important parameters such as potency, pharmacokinetic–pharmacodynamic linkage, and proof-of-principle in vivo efficacy can be measured. Though eukaryotic like its human host, *Plasmodium* has numerous unique

| | | Liver stages | | Blood stages | |
|------------------|---------------|--------------|---------|--------------|-------------|
| | | Latent | Primary | Asexual | Gametocytes |
| Group I | | | | | |
| | Chloroquine | - | - | + | ± |
| | Quinine | - | - | + | ± |
| | Mefloquine | - | - | + | - |
| | Artemisinin | - | - | + | ± |
| FANSIDAR { | Pyrimethamine | - | ± | + | - |
| | Sulfadoxine | - | ± | + | - |
| | Tetracyclines | - | - | ± | - |
| Group II | | | | | |
| MALARONE { | Atovaquone | - | + | + | - |
| | Proguanil | - | + | + | - |
| Group III | | | | | |
| | Primaquine | + | + | - | ± |

Fig. 2 Simplified pharmacodynamic specificities of antimalarial drugs. Depicted are the classes of antimalarial drugs, in the context of their major activities against distinct forms of *Plasmodium* in a human host. Disease-initiating sporozoites are not listed since no drug has meaningful activity against this stage. Group I drugs primarily target the disease-causing asexual blood stages, and form the bulwark of antimalarial therapy. The Group II synergistic combination of atovaquone plus proguanil has reliable action against pathogenic red cell stages as well as the initial liver stage of *P. falciparum*; it is useful for prophylaxis as well as treatment. Group III primaquine targets regular and latent liver stages and gametocytes, but has no useful activity against asexual blood stages. Interspecies differences and unknown or unreliable activities are not shown

and distinguishing features exploitable for drug targeting. These include, for example, a chloroplast-like organelle whose self-contained genome and proteins have clinically useful susceptibility to several conventional antibacterials (e.g., tetracyclines, clindamycin) [6]; efficient hemoglobin digestion and corollary brisk detoxification of byproduct heme by chloroquine-sensitive crystallization [7]; and absolute dependence on pyrimidine biosynthesis disrupted by atovaquone-mediated blockade of mitochondrial electron transport [8]. In recent years availability of the complete genome sequence [4] and use of high throughput screens against libraries of millions of compounds [9–11] have provided much-needed promising new therapeutic leads (Sect. 4.7).

2 Pharmacodynamic Endpoints for Antimalarial Drugs

2.1 *In Vivo*

For most animal models of malaria neither the host nor the parasite is directly pertinent to human infection, and the time course and characteristics of human disease are not faithfully recapitulated. However, different animal model systems mimic some aspects of the host–parasite interaction and allow for biological and pharmacological investigations. In addition to humans, three major animal models have been utilized for studying antimalarial agents.

2.1.1 *Avian*

Avian malaria was the initial model of choice for chemotherapeutic development [12]. *P. gallinaceum* was particularly practical since both the mosquito vector and avian host (ducks, chickens) are easily infected. Although avian red cells are nucleated and the life cycle of *P. gallinaceum* differs significantly from that of human parasites, the erythrocytic stage is usefully mimicked. Mid-twentieth century development of major drugs chloroquine, primaquine, proguanil, and some antifolates was accomplished using *P. gallinaceum*-avian malaria. Subsequent discovery of rodent malaria, a system relatively easy to manipulate and with greater similarity to human physiology, resulted in a shift away from avian models.

2.1.2 *Rodent*

Rodent malaria parasites *P. berghei*, *P. yoelii*, and *P. chabaudi* have proven invaluable for pharmacodynamic screening of candidate compounds [13] and rodent models are now standard in antimalarial drug development [14]. However, no rodent model fully recapitulates the disease profile seen in human malaria, rodent parasite behavior in vivo can differ significantly from that of human-tropic *Plasmodium* species, and rodents may differ substantially in their handling of antimalarials. Controversy surrounds the use of the *P. berghei* ANKA-mouse model of cerebral malaria that reproduces some, but not all, pathophysiological aspects of human cerebral malaria [15]. Recent development of a mouse model in which human erythrocytes are maintained by immunosuppression enables

pharmacodynamic evaluations against the parasites of greatest interest (asexual blood stages of *P. falciparum*), but in a mouse background [16].

2.1.3 Nonhuman Primate

Multiple *Plasmodium* species naturally infect nonhuman primates and mimic important features of human infection, arguably making them the best surrogates for human disease. However, the expense, limited availability, and ethical concerns surrounding use of primates severely limit their practical utility. Notable models include the *P. cynomolgi*-macaque that mimics human *P. vivax* infection, and the *P. coatneyi*- and *P. fragile*-macaque models that in many aspects resemble human *P. falciparum* infections [17]. In fact, the *P. cynomolgi*-macaque model proved critical in the development of primaquine, the only agent known to target latent liver forms (hypnozoites), thus preventing *P. vivax* and *P. ovale* relapses. The demonstration that *P. knowlesi*, normally infective to Old World Monkeys, can cause significant, sometimes lethal, zoonotic disease in humans has made this pathogen a subject of considerable study [18]. While monkey-to-human transmission is more frequent than previously estimated, human-to-human transmission has not been demonstrated. There have been efforts to infect nonhuman primates with human parasites. Some success has been obtained using the *Aotus* monkey host for *P. falciparum*, *P. vivax*, or *P. malariae*; however, results from these artificial self-curing infections need to be interpreted with caution.

2.1.4 Humans

Experimental malaria in humans has a long and checkered history [19], but thoughtful and safe studies conducted during the World War II era provided invaluable new knowledge on the complex biology (Fig. 1) and pathophysiology of *falciparum* and *vivax* malaria, and made possible the rapid development and deployment of amodiaquine, chloroquine, and proguanil to troops fighting in malarious areas of Africa, Europe, and the Pacific. Even today, studying some aspects of antimalarial pharmacodynamics depends heavily on experimental human infections. Prior to large and difficult to control field trials of prophylactic efficacy, small, tightly controlled studies in which well-informed and consenting healthy volunteers are challenged by the bite of malaria-infected mosquitoes or by the inoculation of malaria-infected blood, are standard in assessing drug [20] or vaccine candidates [21] for malaria prophylaxis.

Classical pharmacodynamic endpoints for antimalarial development and use have been clinical—both symptomatic (e.g., time to fever reduction) and microbiological (e.g., time to parasite clearance, 28 day cure rate). In recent years, new metrics have been devised for use in clinical trials. They take cognizance of the fact that unlike most other infections, the pathogenic forms of malaria are confined to erythrocytes in the bloodstream. In severe illness infected cells can number up to 10^{12} , and clinical success of a drug

depends on reducing this burden rapidly and completely. From this realization have come clinical measures of rate of killing, rate of recrudescence and parasite reduction ratio (Sect. 2.2.3). In some cases these endpoints are now also being applied to animal and in vitro studies. Demonstrating and counting parasites in a blood smear by simple light microscopy remains the gold standard for measuring antimalarial pharmacodynamic activity. PCR- and antibody-based assays are now also available but logistical and resource limitations restrict their widespread field-deployment.

2.2 *In Vitro*

For much of the twentieth century, malaria research was restricted to in vivo models since human parasites could not be cultured in vitro. Trager and Jensen's breakthrough report of the continuous culture of *P. falciparum* erythrocytic stages in vitro enabled a veritable explosion in malaria research [5]. Unfortunately, this success has not translated to *P. vivax*, which preferentially infects immature red cells, or to liver stages of the *Plasmodium* life cycle. Nonetheless, *P. falciparum* blood stages maintained in vitro form the basis for numerous and diverse pharmacodynamic assays. The following pharmacodynamic endpoints all focus on the asexual erythrocytic parasites, which are responsible for symptomatic disease.

2.2.1 *Growth Inhibition*

As developed in a micro-titer format, this assay allows high throughput screens and facile measurement of dose–response relationships. Maximum sensitivity is provided by measuring the incorporation of [³H]hypoxanthine into parasite nucleic acid polymers [22]; however, the use of dyes [23] and flow cytometry [24], though less sensitive, avoids the logistical restraints of radioisotopes. Growth inhibition assays do not discriminate between the 'static or 'cidal nature of growth inhibition (that is, whether or not the parasite proliferates once drug pressure is lifted). In addition, they provide limited information about the speed of effect—an important parameter for in vivo consideration. Nevertheless, this is a rapid, simple and important first step in screening for antimalarial activity.

2.2.2 *Parasitocidal vs. Parasitostatic Activity*

A logical follow-up to growth inhibition assay is to determine whether the effect is 'static, or 'cidal. In this method parasites are treated with drug, the drug is removed, and individual parasites are cloned out by limiting dilution. Survivors are detectable after a period of 3–6 weeks [25, 26]. The 48 h erythrocytic life cycle and frequent requirement for fresh medium and red cells make these assays cumbersome, time-consuming, costly, and subject to microbial contamination. Less rigorous approaches have been reported [27, 28] but not extensively validated by comparison with the classic method.

2.2.3 *Speed of Action and Parasite Reduction Ratio (PRR)*

The rapidity of drug action is important in clinical care, and may determine the utilization profile of a drug, particularly in the setting of patients with high parasitemia. Speed of action is measured via the Parasite Reduction Ratio (PRR), the number of parasites

present before drug treatment divided by those remaining after treatment. PRR assays are constrained to one life cycle of the parasite; 48 h for *P. falciparum*. Thus, the higher the PRR, the more rapid is a drug's effect. PRR studies are usually performed in vivo (in humans or in nonhuman models) wherein high parasitemia before dosing makes measurement of the decline both straightforward and sensitive. Measurement of PRR in vitro has been accomplished, with log PRR values ranging from >8 for artemisinin to <3 for atovaquone [25]. However, in vitro assays rely on limiting dilution cloning and outgrowth, thus taking weeks to yield a result. The standard growth inhibition assay has recently been modified to yield information about speed-of-action of antimalarial drugs [29]. Output of this assay is binary, with compounds being classified as fast-acting or non-fast-acting.

2.2.4 Pharmacokinetic/ Pharmacodynamic Linkage

Modifications of an existing hollow fiber cartridge apparatus have very recently made possible studies in which *P. falciparum* can be exposed in vitro to dynamically changing drug concentrations, akin to those that occur in vivo and distinctly different from the constant drug concentrations usually studied in vitro [30]. This system allows studies of the pharmacokinetics that drive many different antimalarial pharmacodynamics. For example, using known human pharmacokinetics, different dosing regimens can be tested to identify those that provide maximal parasite reduction and/or minimal emergence of resistance. Alternatively, the fundamental governance of drug action by either peak concentration or time of exposure can be discerned by applying a given dose of drug by two artificial (and extremely different) kinetic regimens (Sect. 3.4).

In summary, from a century ago when ducks and chickens were the major vehicle for antimalarial drug development, pharmacodynamic analysis has progressed to the stage where most microbiological endpoints can be assayed in vitro. Complexities of the parasite and the host give rise to a significant number of issues that must be addressed for successful antimalarial development and use, and no single model system or assay is sufficient to address all of them. Multiple approaches remain necessary.

3 Pharmacodynamic Issues

3.1 Life Cycle Stage Specificity

Antimalarial drugs can only be understood, and properly used, in the context of their activity against different forms of the parasite. *Plasmodium* has a multistage, complex, and dynamic life cycle, even just within the confines of the human host (Fig. 1). Human disease is initiated by the bite of an infected female Anopheline mosquito (the vector), which inoculates sporozoites in the course of taking a blood meal. This form circulates for just a few minutes before infecting hepatocytes, undergoing multiple rounds of asexual reproduction over several weeks, amplifying 10–30,000-fold,

rupturing the cell and spilling progeny into the bloodstream. The released merozoites invade erythrocytes, differentiate and replicate asexually 8–32-fold over 48–72 h, before lysing the cell and infecting new erythrocytes. Severely ill patients may harbor up to 10^{12} erythrocytic parasites. Some erythrocytic forms differentiate into gametocytes, responsible for infecting and undergoing sexual reproduction within the mosquito, eventually to generate salivary gland-resident sporozoites that complete the life cycle. The above biology is common to all pathogenic species of *Plasmodium*, but *vivax* and *ovale* have the additional feature that some liver stage parasites, termed hypnozoites, are latent and may remain quiescent for decades after the mosquito bite, before activating and establishing an erythrocytic cycle [1].

The various life cycle forms within a patient are morphologically, biochemically, and pharmacologically distinct (Fig. 2). The activity of drugs against the various life cycle stages can be used to classify antimalarials into pharmacologically convenient groups [3]. No drug works against sporozoites, and, unfortunately, no drug is active against all forms other than sporozoites. Group I drugs primarily target asexual blood stages. They alone cause morbidity and mortality, hence are the major target of drug therapy. The Group II synergistic combination of atovaquone and proguanil has additional activity against the initial liver stage of *P. falciparum*. Group III primaquine targets liver stages and gametocytes, but has no useful activity against asexual blood stages. Primaquine's activity against latent hypnozoites of *P. vivax* or *ovale* prevents the late reactivation of symptomatic erythrocytic parasites that characterizes these species. Killing gametocytes prevents transmission of infection to the mosquito and is hence of public health importance.

Choice of appropriate drug is driven, in large part, by the desired outcome. Treatment of, or prophylaxis against, symptomatic malaria is provided by Group I and II agents. Public health campaigns, or regimens striving for complete cure, may include primaquine for its reliable activity against hypnozoites and gametocytes. Prophylaxis can also be obtained by drugs that target liver stage parasites (Groups II and III). Life cycle stage specificity is also important in new drug discovery, the ideal agent being one that has reliable activity against all parasite forms.

3.2 Drug Resistance

The World Health Organization (WHO) defines malaria drug resistance as “the ability of a parasite strain to survive or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the tolerance of the subject” [31]. Acquisition of resistance, which may be rapid and at a high level, negates the clinical utility of a drug and may jeopardize its entire chemical class. Recognized resistance mechanisms include amplification of, or most commonly point mutation(s) in, a target protein sequence. In recent years experimentally induced

resistance to “lead” compounds in drug development has been exploited to obtain invaluable insight into molecular mechanism of action as well as long-term vulnerability to resistance. Interestingly, for leads that generate resistance in the lab, pharmacodynamic utility of the class may be preserved by screening for class members that retain activity against the primary resistance [32]. Additionally, emergence of resistance may be delayed if the molecular mechanism involves multiple molecular targets. As for other anti-infective classes, the most reliable route for precluding resistance in the field is to avoid monotherapy by use of drug combinations.

3.3 Drug Combinations

The pharmacological battle against malaria has laid bare the inadvisability of monotherapy, the end result of which has been emergence and dissemination of resistance to nearly every class of antimalarial drug that has been deployed. Laboratory, and in some cases field, data indicate that antimalarial drug resistance can be delayed, and perhaps avoided, by drug combinations. The earliest and best-studied antimalarial drug combinations stem from Nobel prize-winning studies by Hitchings and Elion on the antibacterial pairing of a sulfonamide inhibitor of dihydropteroate synthase plus a folate reductase inhibitor, both of which interfere with production of essential nutrient tetrahydrofolate [33]. Profound synergism is obtained against malaria parasites by this dual inhibition: when sulfadiazine and pyrimethamine are given in combination (as opposed to singly) the same efficacy is obtained by a 20-fold (or more) reduction in the dose of each drug [34]. (Interestingly, the first-ever inkling of sulfonamide/antifolate synergy against any organism came from Joseph Greenberg’s studies of *P. gallinaceum* in chicks [35].) The ability to use lesser doses for maximal efficacy reduces cost and the likelihood of host toxicity. Synergism and the well-matched pharmacokinetics of this pair were designed to minimize the emergence of resistance. Two other antimalarial fixed dose combinations have since been marketed. Atovaquone plus proguanil (Sects. 4.4 and 4.5.1) relies on synergistic collapse of the parasite’s transmembrane potential [36]. The more empirical pairing of artemether and lumefantrine targets different processes and their pharmacokinetic mismatch (2–3 h vs. 3–6 days) results in long-term persistence of lumefantrine alone, a concern for resistance.

The now-accepted requirement for combination therapy and emergence of several promising new antimalarial leads (Sect. 4.7) have given rise to spirited discussions of the most rational basis for choosing drug pairs [37, 38]. The relative importance of (1) similarities/differences in molecular mechanism of action and in mechanisms of resistance; (2) synergistic, additive or antagonistic interaction; (3) matching half-life; (4) ‘static/‘cidal activity; are all under consideration. While thoughtful consideration and various *in silico* models can usefully examine these various factors, only experimental work will identify the key determinants of success.

3.4 Pharmacokinetic/ Pharmacodynamic Linkage

Ironically, though malaria featured prominently in Paul Ehrlich's seminal studies toward rational drug discovery [39], and antimalarial drug concentrations were amongst the earliest to be measured in order to understand and guide therapy [40], today the status of pharmacokinetic/pharmacodynamic linkage is notably incomplete. Particularly lacking is an understanding of the fundamental PK governance of antimalarial activity. Extensive study of antibacterials has revealed that pharmacodynamic efficacy is usually driven by a specific pharmacokinetic parameter— C_{MAX} or T_{MIC} (Fig. 3) [41, 42]. Indeed, antibacterials are classified and clinically

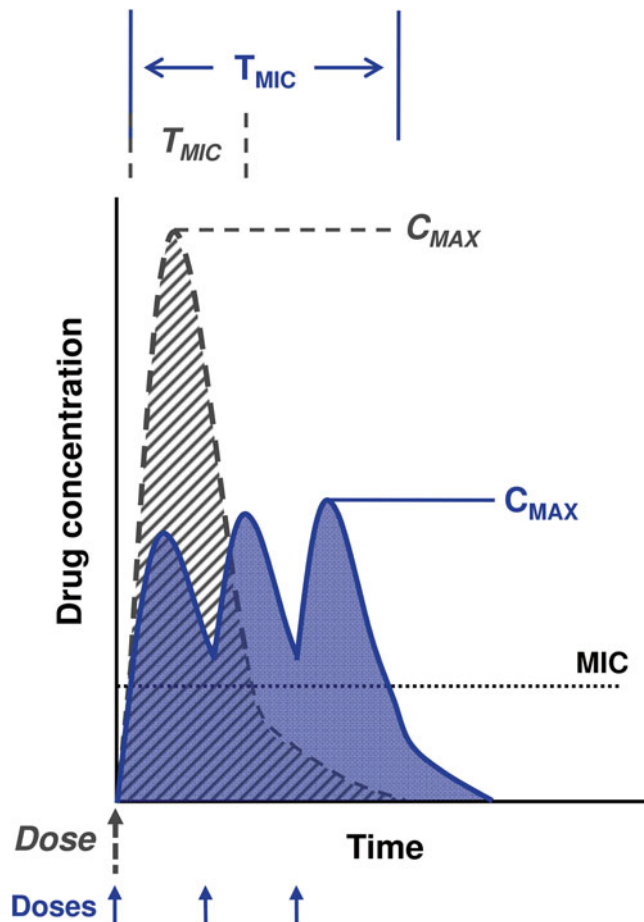


Fig. 3 Pharmacokinetic parameters of a drug in vivo. Following dosing, drug concentration in blood rises (*dashed line*) until reaching a peak (C_{MAX}), and then decays at a rate characteristic of the drug and the dosed organism, until all drug is cleared from the system. During this time, drug concentrations spend a certain interval (T_{MIC}) above a predetermined Minimal Inhibitory Concentration (MIC). The Area Under the concentration–time Curve (AUC, *diagonal black lines*) is an indicator of total drug exposure. The same amount of drug can be dosed via a different regimen using multiple smaller doses (*solid line*). This regimen yields lower C_{MAX} s but a longer T_{MIC} while maintaining the same AUC (*shaded area*)

dosed based this PK/PD link. Recent in vitro studies have demonstrated that such PK/PD governance also pertains for antimalarials. Initial proof of principle work indicates that antimalarials too can be classified as being driven by C_{MAX} or T_{MIC} , independent of their 'static or 'cidal action, and that this governance is class-wide [30]. This information may improve empirical dosing regimens [38], and, more importantly, provide new guidance in drug development. For example, an experimental compound that is C_{MAX} -driven may be fully efficacious in vivo, despite having a short-half life. However, short in vivo half-life for a compound that has been shown to be governed by time of exposure would suggest either a NOGO decision or efforts to modify its chemical structure so as to prolong plasma half-life.

3.5 Safety

Given the enormous number of people afflicted with malaria, the often limited health resources in malaria endemic areas, and the tens of millions of travelers to malarious countries every year who should take chemoprophylaxis (<http://www.cdc.gov/malaria/travelers/index.html>), it becomes immediately obvious that the requirement for safety is unusually high for antimalarial drugs. This imposing barrier of safety must be kept in mind when selecting candidates for development, and in designing studies to test potential drugs.

3.6 Considerations for New Drug Development

Rational drug development starts with the identification of a suitable molecular target. Screening small molecules in vitro for activity against the cell-free target is followed by testing and development of favored candidates in more complex whole cell assays and animal models. For *Plasmodium* this process presents several challenges. The eukaryotic nature of *Plasmodium*, and resultant similarity of basic biochemical mechanisms between the pathogen and its host, immediately narrows the list of unique molecular entities suitable for selective targeting. Intracellular residence of the parasite further complicates drug design. For a lead to be truly efficacious, its molecular target should be accessible and essential during all stages of the parasite life cycle, and preferably be present and required in all species of *Plasmodium* pathogenic to humans. Finally, there should be a high barrier to resistance, a facet influenced by both the function and redundancy of the target. Rational drug development schema can yield candidates that are tremendously effective in cell-free screens, but a great many of these prove ineffective against erythrocytic parasites in vitro or in vivo. An alternative strategy is to screen against whole cells or animal models; determination of molecular mechanism of action occurs later, if at all, in the process. Most successful antimalarials have been developed through this less-than-rational strategy, indeed chloroquine was discovered and developed using avian

and rodent models [43] and its molecular mechanism of action was not described until 50 years later [44]. Artemisinins were co-opted from ancient Chinese remedies [45] and details of their mechanism of efficacy are debated.

The malaria research community has coalesced around the SERCaP—Single Exposure Radical Cure and Prophylaxis—as the ideal for new antimalarial drug development [46]. This sets a very high bar. SERCaP dictates that the treatment regimen be single dose, rapidly efficacious, target all forms of the parasite including latent stages, and have a long-lasting pharmacodynamic effect so as to prevent reinfection. Prudent drug development also demands that the agent be inexpensive, orally bioavailable, and provide a high barrier to resistance. It is improbable that any single molecule will satisfy all of these requirements. Instead, future malaria therapies will likely be based on the combination of multiple agents, each providing a unique spectrum of action. In subsequent sections, we discuss clinically used antimalarial drugs and some experimental agents in development, in context of the aspects described above.

4 Pharmacodynamics of Antimalarial Drugs

4.1 4-Substituted Quinolines

Agents in this class share structural similarities (Fig. 4) and a common molecular mechanism of action; however, resistance is mediated by multiple different mechanisms, not all of which have been characterized.

4.1.1 Chloroquine

Synthesized by the Germans as Resochin in 1934, and rediscovered by the Allies as SN7618 in 1944, chloroquine (Fig. 4, 1) was for decades the mainstay of antimalarial chemotherapy [43]. Christened chloroquine by E. K. Marshall in 1945, the drug inhibits the essential parasite process of heme detoxification [44]. *Plasmodium* satisfies most of its amino acid requirements by digesting host cell hemoglobin, releasing free heme in the process. Heme-induced oxidative damage is avoided by nonenzymatic crystallization into the inert polymer hemozoin [7]. Chloroquine concentrates in parasites and inhibits heme crystallization, leading to oxidative damage and death.

While chloroquine-resistant parasites are not readily generated *in vitro*, the eventual emergence and global dissemination of resistance has rendered chloroquine useless in all but a few locales. Elegant parasite cross-breeding studies pinpointed resistance in *P. falciparum* to point mutations in PfCRT (*P. falciparum* chloroquine resistance transporter) [47]. Presence of resistance mutations decreases the accumulation, hence the cytotoxicity, of chloroquine. Curiously, although other 4-substituted quinolines also inhibit heme polymerization [48], and *P. vivax* may be

chloroquine-resistant [31], PfCRT appears not to mediate these resistances [49].

Chloroquine's tremendous clinical success prior to resistance can be explained by its ability to satisfy many of the pharmacodynamic requirements for an ideal antimalarial. It targets a pathway not present in the human host, is parasiticidal, fast acting, and has a relatively high PRR. Furthermore, the pharmacokinetics of chloroquine are favorable. It persists with a plasma half-life of weeks to months [50]. This long half-life permits the convenient weekly dosing of chloroquine for antimalarial prophylaxis.

Chloroquine toxicity is both dose- and age-related; doses must be substantially reduced for safe use in children. At doses and regimens required to treat malaria, toxicity in adults is negligible [51]. Rare adverse events include diplopia and dizziness. Cumulative high doses of chloroquine used in anticancer [52] and immunosuppressive therapy [53] may lead to neurotoxicity, cardiotoxicity, and irreversible retinopathy.

4.1.2 Quinine

Quinine (Fig. 4, 2), an alkaloid extracted from the bark of the cinchona tree, was used as an antipyretic by the Quechua in South America. It was transported to Rome by the Jesuits in the early seventeenth century and, in a vivid indication of today's great need for new antimalarials, this antique natural product remains a drug of choice for treating patients with severe falciparum malaria [54].

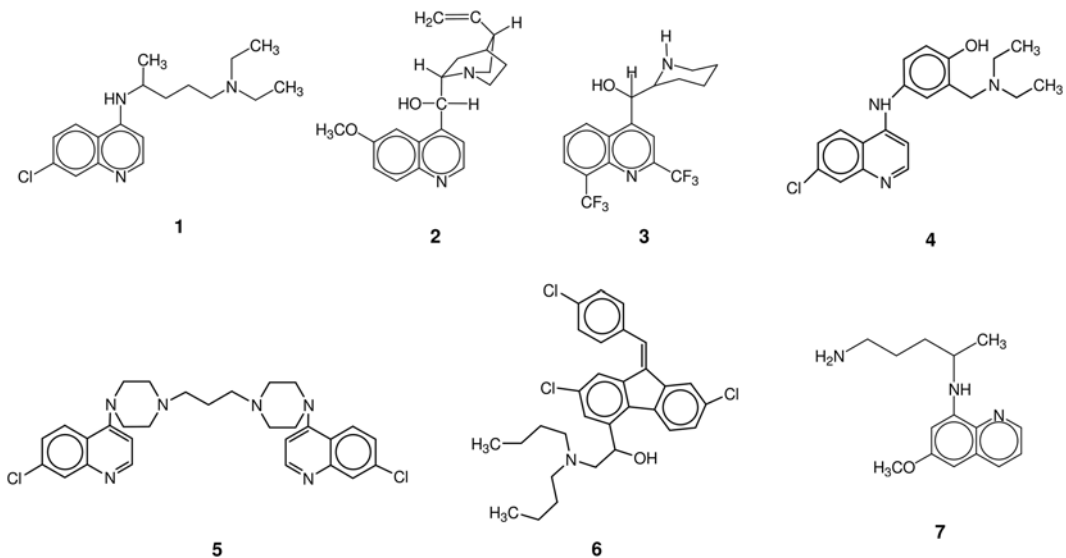


Fig. 4 The substituted quinolines and structurally related compounds. Drugs with substitutions at the 4-position include chloroquine (1), quinine (2), mefloquine (3), and amodiaquine (4). Piperaquine (5) is a bisquinoline while lumefantrine (6) is structurally similar to substituted quinolines. All have potent activity against asexual erythrocytic parasites. Primaquine (7), an 8-substituted quinoline, targets liver stages and gametocytes

Quinine differs from chloroquine in having an ~8 h plasma half-life [50]. This necessitates more frequent dosing, and in severely ill patients, a loading dose [55]. Quinine resistance has been observed in the field, with genetic studies indicating a multifactorial phenotype [56]. Quinine has significant toxicity [51]. Symptoms include the classic cinchonism (disturbances of vision and hearing, headache, nausea), as well as hypoglycemia and hypotension that may be life threatening. This toxicity profile and its relatively short half-life make quinine unsuitable as a prophylaxis agent. Quinidine, an efficacious antiarrhythmic, is a stereoisomer of quinine with potent antimalarial activity. In cases where quinine is unavailable, intravenous quinidine is an acceptable substitute for temporary management of severe malaria [55].

4.1.3 Mefloquine

Mefloquine (Fig. 4, 3) was discovered in a whole-cell screen by the Walter Reed Institute of Medical Research [57], and became an immediate agent of choice for its high activity against drug-resistant *P. falciparum* [58]. PfCRT does not confer mefloquine-resistance [59]. This fact, combined with a weeks-long half-life [60], enables the use of mefloquine for prophylaxis against chloroquine-resistant malaria. Unfortunately, now-widespread resistance limits mefloquine's utility. Resistance appears to be multifactorial, and is usually associated with increased expression of multidrug-transporter proteins [59]. Mefloquine toxicity is dose-related, and usually mild at doses used for short-term prophylaxis [51]. The adverse event spectrum expands at higher doses, to include CNS toxicity and neuropsychiatric effects. For this reason, it is not utilized in long-term prophylaxis regimens.

4.1.4 Other 4-Substituted Quinolines and Structural Relatives

Amodiaquine (Fig. 4, 4), an old antimalarial with structural and mechanistic features of chloroquine, has significant activity against chloroquine-resistant *Plasmodium* [61]. However, its use is disfavored due to an association with hepatotoxicity and agranulocytosis [51]. Piperaquine (Fig. 4, 5) is also active against chloroquine-resistant malaria; its molecular mechanism of action is unclear [62]. It is clinically utilized in combination with dihydroartemisinin. Lumefantrine (Fig. 4, 6), a molecule structurally similar to substituted quinolines, acts against asexual erythrocytic forms of *P. falciparum* by an unknown molecular mechanism of action [63]. Lumefantrine is FDA-approved and marketed as a fixed-dose combination with artemether (Sect. 4.3) for use against both drug-sensitive and drug-resistant malaria.

4.2 8-Aminoquinolines

8-aminoquinoline primaquine (Fig. 4, 7) is the only clinically used antimalarial with reliable activity against initial and latent liver stages and gametocytes (Fig. 2) [64]. Conversely, primaquine has no useful effect on blood stage asexual forms of *Plasmodium*, and hence it has no place in the acute treatment of symptomatic malaria.

Primaquine is the only agent known to eliminate hypnozoites of *P. vivax* and *P. ovale*, thus preventing late relapses of these infections. The molecular mechanism of action of primaquine is unclear, but appears to be mediated largely by its metabolites. Primaquine's vivid toxicities suggest that it acts by generating oxidative species and interfering with redox balance in the pathogen. Primaquine is associated with frequent gastrointestinal intolerance and at high doses causes methemoglobinemia in most people (primaquine use reviewed in [65]). Patients with glucose-6-phosphate dehydrogenase deficiency are particularly susceptible, even at therapeutic doses, to acute, sometimes life threatening, hemolysis and hemolytic anemia [66]. Indeed, primaquine-induced hemolysis led to the discovery of glucose-6-phosphate dehydrogenase deficiency, the first genetic abnormality associated with an enzyme [67].

4.3 The Artemisinins

Artemisinin (Fig. 5, 8) or qinghaosu is the active moiety in *Artemisia annua*, a plant utilized by Chinese herbalists for over 2000 years [45]. A sesquiterpene lactone endoperoxide, the artemisinins currently form the last line of defense against multidrug-resistant *P. falciparum*. Their mechanism of action has been the subject of much study. It is generally accepted that the endoperoxide is the active pharmacophore. Iron- or heme-catalyzed cleavage of the oxygen-oxygen bond likely leads to subsequent formation of a carbon-centered radical [68] that in turn alkylates parasite macromolecules. Semisynthetic derivatives artemether (Fig. 5, 9), arteether (Fig. 5, 10), and artesunate (Fig. 5, 11) are more soluble than parent artemisinin, but act in a similar fashion. While these compounds themselves have antimalarial activity, they also act as prodrugs in vivo. All derivatives (8–11) are rapidly converted in vivo to dihydroartemisinin (Fig. 5, 12), itself an antimalarial [69]. The artemisinins have potent and rapid activity against asexual erythrocytic stages of *P. falciparum* and *P. vivax*, making them particularly useful in severely ill patients with high parasite burden.

A distinguishing characteristic of the artemisinins is their extremely short half-life [70]. Cleared from plasma within minutes, their great activity against a parasite with a complex 48 h life cycle has always been puzzling. However, in vitro PK/PD studies

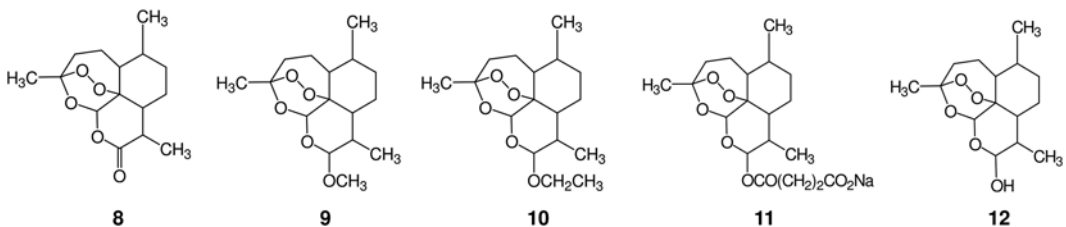


Fig. 5 The artemisinins. Members of this family include natural product artemisinin (8), and its semisynthetic derivatives artemether (9), arteether (10), artesunate (11), and dihydroartemisinin (12). These compounds are an obligate component of most clinically efficacious antimalarial drug regimens

have demonstrated that artemisinin efficacy is C_{MAX} -driven, a mechanism that aligns ideally with its short half-life, providing a satisfactory explanation for the clinical success of such remarkably short-lived drugs [30]. Interestingly, daily dose artemisinin monotherapy, even for 7 days, invariably leads to recrudescence of parasitemia [71]. Explanations for this phenomenon include differential effects on life-cycle stages and/or induction of post-treatment “quiescence” in surviving parasites [72]. Long-lived endoperoxides (Sect. 4.7.4) have been developed in an effort to remedy this problem [73]. In any case, artemisinins are now almost always used in combination regimens.

Efficacy of the artemisinins has recently been threatened by the discovery of a resistance-like phenomenon. While not meeting WHO’s definition of leading to treatment failure, the effect manifests pharmacodynamically as a slower rate of parasite clearance and longer persistence of parasites in vivo [74]. Genetic studies suggest that parasite genotype accounts only partially for the observed clinical phenotype, implying the existence of as-yet-undefined contributions from the human host [75]. Although geographically still restricted, this “resistance” phenotype has begun a slow march out of its initial focus in Southeast Asia. Recent genome-wide association studies on “resistant” parasites [76], as well as attempts to recapitulate resistance in vitro, have focused on polymorphisms in a region of chromosome 13 (encoding a protein containing a kelch 13 propeller domain) that appear to correlate with the phenotype [77]. Recent in vitro work has demonstrated that these kelch 13 polymorphisms are necessary and sufficient to enhance the survival of the parasite ring stage in the face of artemisinin pressure [78]. While the exact molecular mechanism is unclear, transcriptomic analysis suggests that this ‘resistance’ is mediated via an upregulation of the parasite’s unfolded protein response [79]. Artemisinins currently form the last line of defense against drug-resistant malaria and the threat to their efficacy has, appropriately, spurred greater urgency in new antimalarial drug development.

Artemisinins are considered relatively safe, having been used for decades in millions of humans. Recognized toxicities include hemolysis and hypersensitivity reactions. Animal toxicology studies indicate that brain, liver, bone marrow, and fetus may be affected. This adverse event profile has not been unambiguously demonstrated in humans treated with therapeutic doses [51].

4.4 Atovaquone

Atovaquone (Fig. 6, 13) is an analog of coenzyme Q that specifically targets the cytochrome bc_1 complex of the mitochondrial respiratory chain. It interferes with mitochondrial functions, including pyrimidine biosynthesis, by inhibiting electron transport and collapsing the mitochondrial transmembrane potential [80]. Potent activity against asexual erythrocytic forms of *P. falciparum* and *P. vivax*, and the ability to eliminate *P. falciparum* liver stage

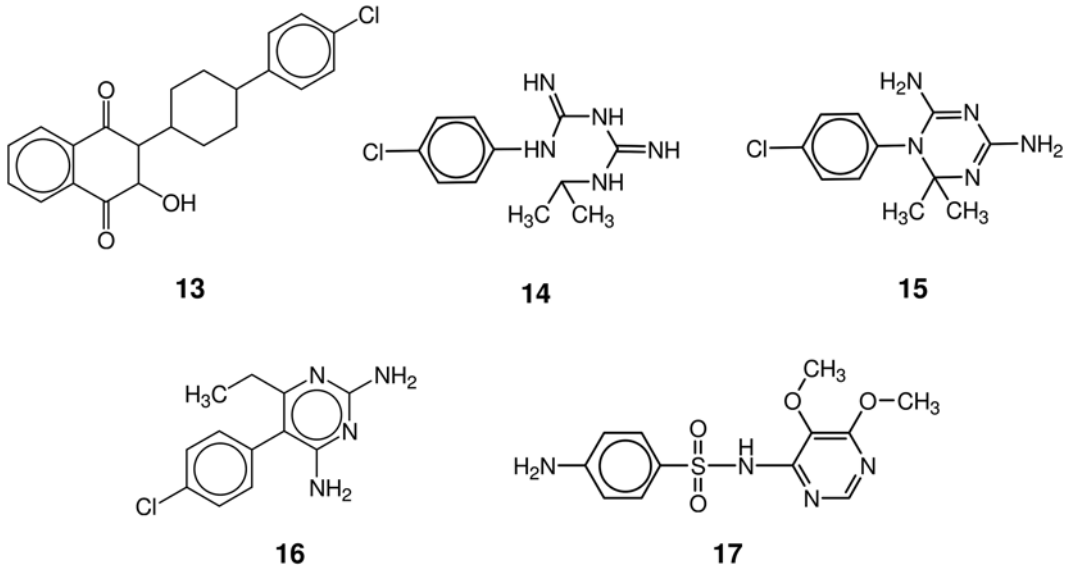


Fig. 6 Atovaquone, antifolates, and sulfonamides. Atovaquone (**13**) is a cytochrome bc_1 inhibitor that is effective on its own, synergizes with the biguanide proguanil (**14**), and is clinically always used in combination. Proguanil is a prodrug metabolized in vivo to cycloguanil (**15**), a dihydrofolate reductase inhibitor. Pyrimethamine (**16**) also targets dihydrofolate reductase and is used in synergistic combination with sulfadoxine (**17**), a dihydropteroate synthase-targeting sulfonamide

parasites makes this agent especially useful for prophylaxis. Atovaquone is relatively safe—common adverse events include headache, rash, abdominal pain, vomiting, and diarrhea.

In its very first clinical trials atovaquone failed spectacularly, and quite unexpectedly, thanks to the rapid emergence of drug-resistant *P. falciparum*, mediated by point mutations in target cytochrome b (atovaquone reviewed in [36]). Utility of this potent, safe, relatively long-lived candidate was saved by its synergistic combination, demonstrable both in lab and clinic, with veteran antimalarial proguanil (Sect. 4.5.1).

4.5 Antifolates and Sulfonamides

Sulfonamides and antifolates were among the earliest synthetic antimalarial agents, and were developed primarily using avian models of malaria.

4.5.1 Proguanil and Cycloguanil

The biguanide proguanil (Fig. 6, **14**) targets *Plasmodium* in multiple ways. Best known as a prodrug, in vivo it is metabolized by CYP2C19 to cycloguanil (Fig. 6, **15**) [81], which inhibits *P. falciparum* dihydrofolate reductase [82]. However, proguanil is also active in vitro (where it is not converted to cycloguanil) suggesting other direct (and unknown) targets [83]. Pharmacogenetic differences in CYP2C19 can affect the conversion to cycloguanil, and patients with CYP2C19*2–CYP2C19*8 alleles (poor metabolizers) may not fully convert proguanil to cycloguanil [81]. Clinical

relevance of the poor metabolizer phenotype with respect to malaria is the subject of debate. Proguanil is remarkably safe at therapeutic doses. Resistance against cycloguanil is conferred by point mutations in *Plasmodium* dihydrofolate reductase [84], and these arose and spread rapidly in the era of proguanil monotherapy. Clinical use of proguanil is now largely confined to its synergistic combination with atovaquone (Sect. 4.4). While proguanil itself displays no effect on the mitochondrial membrane potential, it greatly enhances atovaquone's effect [85].

4.5.2 Pyrimethamine

Pyrimethamine (Fig. 6, 16) is a diaminopyrimidine that targets *Plasmodium* dihydrofolate reductase [82]. The effects of inhibiting folate metabolism manifest late in the replication cycle of asexual erythrocytic forms, making pyrimethamine a slow-acting drug. Pharmacodynamic efficacy of pyrimethamine can be augmented by host immunity, and significantly inhibited by dietary *p*-aminobenzoic acid or folate. Point mutations in *Plasmodium* dihydrofolate reductase confer resistance to this drug [86]. Therapeutic doses of pyrimethamine are safe; excessive doses can recapitulate symptoms of folate deficiency [51]. Pyrimethamine is usually dosed in combination with a sulfonamide or sulfone to create a synergistic effect. However, the utility of this combination is limited by widespread drug resistance, as well as by intrinsic toxicity of the sulfonamides.

4.5.3 Sulfonamides and Sulfones

Sulfonamides and sulfones inhibit *Plasmodium* dihydropteroate synthase [87], an enzyme involved in folate biosynthesis that has no counterpart in humans. These agents are slow acting and readily generate mutations in target protein dihydropteroate synthase [88], which confers class-wide resistance. Clinical utility is confined to coadministration with an antifolate partner drug. The combination of sulfadoxine (Fig. 6, 17) with pyrimethamine is particularly effective since multiple steps of the same biosynthetic pathway are affected. This particular combination was designed to include partners with matched in vivo pharmacokinetics (both drugs are long-lived), thus avoiding functional monotherapy with either agent towards the end of the dosing interval. The use of sulfonamides and sulfonamide-containing combinations is limited by toxicity, including Stevens–Johnson syndrome and exfoliative dermatitis [51]. Additionally, widespread resistance of *Plasmodium* to both partner drugs has compromised the utility of this drug class and its combinations.

4.6 Antibacterials

Tetracycline (Fig. 7, 18) and doxycycline (Fig. 7, 19) are slow acting agents [61] that target the apicoplast, a chloroplast-like organelle in malaria parasites. Interference with apicoplast function results in delayed death of the parasite: effects of the drug are not manifest until the next replication cycle [89]. This delayed effect makes

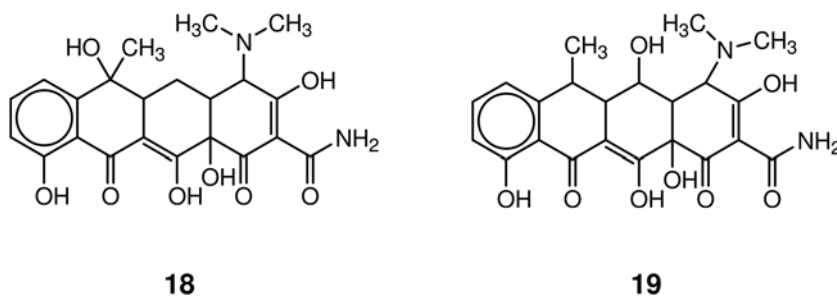


Fig. 7 Antibacterials. Tetracycline (**18**) and doxycycline (**19**) are slow-acting antimalarials useful for adjunctive therapy and short-term prophylaxis

the tetracyclines unsuitable for treatment of established severe infections; however, they are useful as adjunctive therapy and for short-term prophylaxis. Adverse effects that limit their use include discoloring depositions in bones and teeth, and a tendency to cause photosensitivity [90].

4.7 Experimental Agents

Today's global development of new antimalarial drugs is largely coordinated by the Medicines for Malaria Venture (MMV), a non-profit public-private foundation. MMV coordinates industrial and academic antimalarial efforts and facilitates progress of drug candidates through the long and complex development pathway [91]. A current snapshot of the global antimalarial development portfolio can be found on the foundation's website (www.mmv.org). There are multiple new drugs or drug combinations in various stages of development [92], ranging from the discovery phase to post-approval management. The following are agents currently in human trials, with a focus on novel pharmacophores, molecular targets, or pharmacokinetics.

4.7.1 DSM265

Developed by a multinational academic collaboration, DSM265 (Fig. 8, **20**) targets dihydroorotate dehydrogenase, a mitochondrial enzyme in *P. falciparum* essential for pyrimidine biosynthesis and parasite survival [93]. The compound achieves several important benchmarks in that it is potent, selective, active against chloroquine-resistant parasites, bioavailable and metabolically stable. It will likely be deployed in combination therapy with a suitable partner.

4.7.2 KAE609

KAE609 (Fig. 8, **21**) is a spiroindolone that targets a P-type ATPase Na(+) channel of *P. falciparum* [94]. Developed from a natural product screen in an industry-academia collaboration, KAE609 is a novel pharmacophore targeting a subsequently described and hitherto unexplored *Plasmodium* function, and has successfully completed Phase IIA clinical trials. The relative ease of generating resistance in vitro is somewhat worrisome; however, this efficacious compound will provide much-needed diversity to the antimalarial armamentarium.

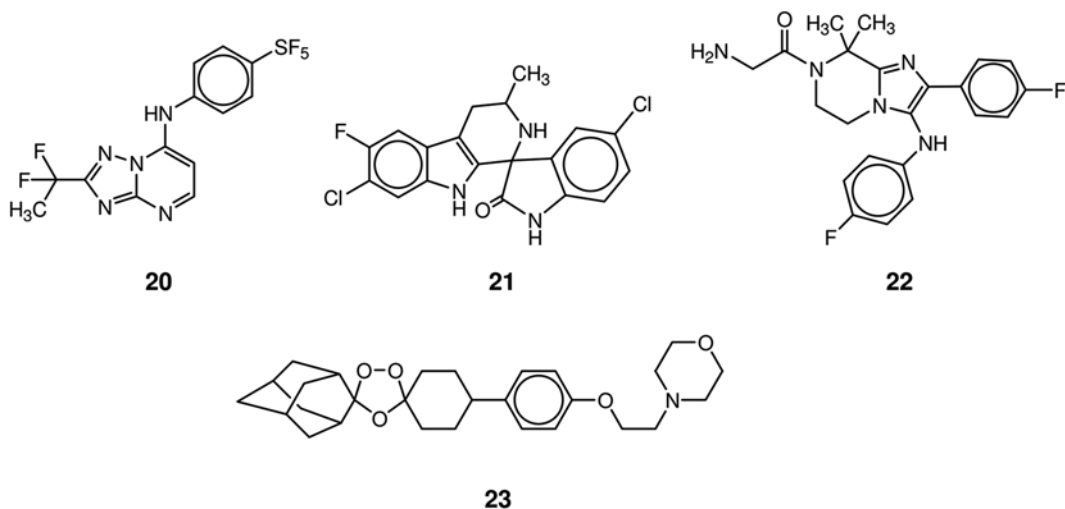


Fig. 8 Experimental antimalarials. Triazolopyrimidine DSM265 (**20**) inhibits dihydroorotate dehydrogenase, an essential malarial enzyme. KAE609 (**21**) targets the *Pf*ATP4 (Na⁺) channel and KAF156 (**22**) is an imidazopyrazine active against both blood and liver stages of the parasite life cycle. OZ439 (**23**) contains the endoperoxide pharmacophore of the artemisinins, but is designed to persist in the blood

4.7.3 KAF 156

An imidazopyrazine derivative discovered and developed through a whole cell screening approach, KAF156 (Fig. 8, **22**) targets both liver and blood stages of *Plasmodium* [95]. This multistage activity, coupled with acceptable pharmacokinetics, suggests that KAF156 has potential to provide both the Radical Cure and the Protection demanded by the SERCaP model.

4.7.4 OZ439

OZ439 (Fig. 8, **23**) is member of the synthetic ozonides [96]. Its endoperoxide motif mimics that of the artemisinins, and their mechanism of action is likely in common. The unique feature of OZ439 is its metabolic stability: a terminal half-life of 25–30 h compared to half-lives of just minutes for the artemisinins [73]. Recent in vitro research on artemisinin resistance has yielded the intriguing suggestion that the resistance phenotype may be mediated by a quiescence or dormancy mechanism that simply allows the parasite to outlast the short-lived drug [72]. If so, long- $t_{1/2}$ endoperoxide OZ439 will be crucial for combating and perhaps reversing artemisinin resistance in the field.

5 Summary

Malaria is a public health problem of immense proportions. The complex biology of malaria makes drug development and use particularly challenging, a situation exacerbated by drug resistance. The parasite is a eukaryote, which limits the availability of

targets unique to the pathogen. Plasmodium species pathogenic to humans will not infect other animals, rendering animal models deficient. Finally, human parasites are difficult, and in some cases impossible, to culture in vitro, limiting development of laboratory assays.

The asexual erythrocytic stages that cause symptomatic illness are the primary targets of treatment. However, prevention and public health strategies necessitate the targeting of liver stages and gametocytes, both of which are pharmacologically distinct from the asexual blood stages.

Initial pharmacodynamic endpoints for antimalarial development were clinical and required human studies. Surrogate animal models and in vitro assays have matured to the point where a rational combination of assays yields adequate information to aid drug development, and these in conjunction with contemporary screening methods have yielded a number of highly promising experimental drug candidates.

Successful antimalarial drugs must fulfil certain criteria. The drug must be potent; provide a single-dose cure; act rapidly; and target all stages of the parasite life cycle. Drug pharmacokinetics in vivo must align with the essential pharmacokinetic–pharmacodynamic linkage. The molecule must possess a high barrier to resistance. And ultimately, the drug must be extremely safe, given expected administration to millions of patients and to healthy uninfected travelers. No single drug currently fulfils all these criteria. Indeed, it is unlikely any agent ever will. Successful antimalarial regimens will require combinations of drugs, rationally selected to provide best activity.

Existing antimalarials have proven successful because they fulfill some of the requirements listed above. New drug development efforts have formalized these benchmarks and progress along the development pathway is now governed by these criteria. While there are several new pharmacophores targeting novel targets, and some old ones aiming for different processes, antimalarial drug development is likely to remain a challenging endeavor for the foreseeable future.

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