Review

Expanding the Antimalarial Drug Arsenal—Now, But How?

Brian T. Grimberg * and Rajeev K. Mehlotra *

Center for Global Health and Diseases, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA; E-Mails: brian.grimberg@case.edu (B.T.G.); rajeev.mehlotra@case.edu (R.K.M.); Tel.: +1-216-368-6328 or +1-216-368-6172, Fax: +1-216-368-4825.

Abstract: The number of available and effective antimalarial drugs is quickly dwindling. This is mainly because a number of drug resistance-associated mutations in malaria parasite genes, such as *crt*, *mdr1*, *dhfr*/*dhps*, and others, have led to widespread resistance to all known classes of antimalarial compounds. Unfortunately, malaria parasites have started to exhibit some level of resistance in Southeast Asia even to the most recently introduced class of drugs, artemisinins. While there is much need, the antimalarial drug development pipeline remains woefully thin, with little chemical diversity, and there is currently no alternative to the precious artemisinins. It is difficult to predict where the next generation of antimalarial drugs will come from; however, there are six major approaches: (i) re-optimizing the use of existing antimalarials by either replacement/rotation or combination approach; (ii) repurposing drugs that are currently used to treat other infections or diseases; (iii) chemically modifying existing antimalarial compounds; (iv) exploring natural sources; (v) large-scale screening of diverse chemical libraries; and (vi) through parasite genome-based ("targeted") discoveries. When any newly discovered effective antimalarial treatment is used by the populus, we must maintain constant vigilance for both parasite-specific and human-related factors that are likely to hamper its success. This article is neither comprehensive nor conclusive. Our purpose is to provide an overview of antimalarial drug resistance, associated parasite genetic factors (1. Introduction; 2. Emergence of artemisinin resistance in *P. falciparum*), and the antimalarial drug development pipeline (3. Overview of the global pipeline of antimalarial drugs), and highlight some examples of the aforementioned approaches to future antimalarial treatment. These approaches can be categorized into "short term" (4. Feasible options for now) and "long term" (5. Next generation of antimalarial treatment—Approaches and candidates). However, these two categories are interrelated, and the

approaches in both should be implemented in parallel with focus on developing a successful, long-lasting antimalarial chemotherapy.

Keywords: malaria; falciparum; artemisinin resistance; natural products; drug discovery; kinases; HDAC; DHODH

1. Introduction

1.1. Malaria

More than 40% of the world's population, much of it socioeconomically and politically challenged, live in areas where malaria, alone or together with HIV/AIDS and tuberculosis, is a significant health risk [1,2]. According to the World Health Organization (WHO), approximately 250 million clinical cases of malaria occur every year [3]. Malaria is estimated to kill nearly one million people annually, with most of the deaths occurring in children under 5 years of age in sub-Saharan Africa [4]. If children survive multiple infections, such exposure leads to a natural immunity that limits the severity of the disease later in life. However, this immunity wanes in the absence of continued exposure to malaria infections. Additionally, pregnant women and newborns have reduced immunity, and therefore are vulnerable to severe complications of malaria infection and disease [5,6].

Malaria is primarily caused by four species of the protozoan parasite *Plasmodium*: *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*, which are transmitted by over 70 species of *Anopheles* mosquitoes [7,8]. These parasite species occur sympatrically both in human populations and within infected individuals, with *P. falciparum* and *P. vivax* being the predominant species [9-11]. Approximately 80% of all malaria cases and 90% of malaria-attributed deaths occur in Africa, and are caused by *P. falciparum* [3]. Outside of Africa, *P. vivax* is the most widespread species, occurring largely in Asia, including the Middle East and the Western Pacific, and in Central and South America [12]. This parasite species causes a relatively less lethal form of the disease compared with *P. falciparum* [12,13].

1.2. Overview of Antimalarial Drugs

Today when many mosquito vectors are resistant to insecticides [14,15], and an effective vaccine is not yet available [14,16], chemoprophylaxis/chemotherapy remains the principal means of combating malaria. Over the past 60 to 70 years, since the introduction of synthetic antimalarials, only a small number of compounds, belonging to three broad classes, have been found suitable for clinical usage [17,18]. These classes are described below.

1.2.1. Quinine and related drugs

Quinine, originally extracted from cinchona bark in the early 1800s, along with its dextroisomer quinidine, is still one of the most important drugs for the treatment of uncomplicated malaria, and often the drug of last resort for the treatment of severe malaria. Chloroquine (CQ), a 4-aminoquinoline derivative of quinine, has been the most successful, inexpensive, and therefore the most widely used

antimalarial drug since the 1940s. However, its usefulness has rapidly declined in those parts of the world where CQ-resistant strains of *P. falciparum* and *P. vivax* have emerged and are now widespread. Amodiaquine, an analogue of CQ, is a pro-drug that relies on its active metabolite monodesethylamodiaquine, and is still effective in areas of Africa, but not in regions of South America. Other quinine-related, commonly used drugs include mefloquine, a 4-quinoline-methanol derivative of quinine, and the 8-aminoquinoline derivative, primaquine; the latter is specifically used for eliminating relapse causing, latent hepatic forms (hypnozoites) of *P. vivax*. Halofantrine and lumefantrine, both structurally related to quinine, were found to be effective against multidrug-resistant falciparum malaria [17,19,20]. While lumefantrine, in combination with an artemisinin derivative, artemether (Coartem), is recommended by the WHO for treating uncomplicated falciparum malaria, halofantrine generally is not recommended because of serious side effects and extensive cross-resistance with mefloquine [21].

1.2.2. Antifolate combination drugs

Antifolate drugs include various combinations of dihydrofolate reductase enzyme (DHFR) inhibitors, such as pyrimethamine, proguanil, and chlorproguanil, and dihydropteroate synthase enzyme (DHPS) inhibitors, such as sulfadoxine and dapsone. With rapidly growing sulfadoxine-pyrimethamine (SP) resistance, a new combination drug, Lapdap (chlorproguanil-dapsone), was tested in Africa in the early 2000s, but was withdrawn in 2008 because of hemolytic anemia in patients with glucose-6-phosphate dehydrogenase enzyme (G6PD) deficiency [22].

1.2.3. Artemisinin and its derivatives

Artemisinin drugs, which originated from the Chinese herb qing hao (*Artemisia annua*), belong to a unique class of compounds, the sesquiterpene lactone endoperoxides [18]. The parent compound of this class is artemisinin (quinghaosu), whereas dihydroartemisinin (DHA), artesunate, artemether, and β-arteether are the most common derivatives of artemisinin; DHA is the main bioactive metabolite of all artemisinin derivatives (artesunate, artemether, β-arteether, *etc.*), and is also available as a drug itself [17,18,20].

Since 2001, the WHO has recommended the use of artemisinin-based combination therapies (ACTs) for treating falciparum malaria in all countries where resistance to monotherapies or non-artemisinin combination therapies (e.g., SP) is prevalent [23]. The rationale for the use of ACTs is based on the facts that artemisinin derivatives are highly potent and fast acting, and that the partner drug in ACT has a long half-life, which allows killing the parasites that may have escaped the artemisinin inhibition. Thus, it is thought that ACTs will delay the onset of resistance by acting as a "double-edged sword" [18,24,25]. ACTs, such as artemether-lumefantrine, artesunate-amodiaquine, and artesunate-mefloquine are being used in China, Southeast Asia, many parts of Africa, and some parts of South America [17,18,20]. Introduction of ACTs has initiated noticeable reduction in malaria prevalence in these endemic regions of the world [18]. Although the mechanism(s) of action is poorly understood (described below), the current high level of interest in artemisinin drugs is due to their well-recognized pharmacological advantages: These drugs act rapidly upon asexual blood stages of CQ-sensitive as well as CQ-resistant strains of both *P. falciparum* and *P. vivax*, and reduce the parasite

biomass very quickly, by about 4-logs for each asexual cycle. In addition, these drugs are gametocytocidal. Thus, through rapid killing of asexual blood stages and developing gametocytes, artemisinin drugs significantly limit the transmission potential of the treated infections. These drugs have large therapeutic windows, and based on extensive human use, they appear to be safe, even in children and mid/late pregnant women. Furthermore, there is no reported "added toxicity" when these drugs are used in combination with other types of antimalarial compounds.

In addition to these three main classes of compounds, the antibiotic tetracycline and its derivatives, such as doxycycline, are consistently active against all species of malaria, and in combination with quinine, are particularly useful for the treatment of severe falciparum malaria [17]. Until recently, a combination of atovaquone, a hydroxynaphthoquinone, and proguanil (Malarone) was considered to be effective against CQ- and multidrug-resistant falciparum malaria; atovaquone resistance has recently been noticed in Africa [26]. Piperaquine, another member of the 4-aminoquinoline group, in combination with DHA (Artekin), holds the promise of being successful in CQ-resistant endemic areas of Southeast Asia [17,18,20].

1.3. Overview of Antimalarial Drug Resistance

This limited antimalarial armament is now severely compromised because of the parasite's remarkable ability to develop resistance to these compounds [19,27]. In many different malaria-endemic areas, low to high-level resistance in the predominant malaria parasites, *P. falciparum* and *P. vivax*, has been observed for CQ, amodiaquine, mefloquine, primaquine, and SP. *Plasmodium falciparum* has developed resistance to nearly all antimalarial drugs in current use, although the geographic distribution of resistance to any one particular drug varies greatly. In particular, Southeast Asia has a highly variable distribution of falciparum drug resistance; some areas have a high prevalence of complete resistance to multiple drugs, while elsewhere there is a spectrum of sensitivity to various drugs [19]. Until 2009, no noticeable clinical resistance to artemisinin drugs was reported. However, as described below, a number of recent studies have raised concerns about the efficacy of ACTs, particularly in Southeast Asia.

1.4. Overview of Genetic Basis for Antimalarial Drug Resistance

It is believed that the selection of parasites harboring polymorphisms, particularly point mutations, associated with reduced drug sensitivity, is the primary basis for drug resistance in malaria parasites [28,29]. Drug-resistant parasites are more likely to be selected if parasite populations are exposed to sub-therapeutic drug concentrations through (a) unregulated drug use; (b) the use of inadequate drug regimens; and/or (c) the use of long half-life drugs singly or in non-artemisinin combination therapies. In recent years, significant progress has been made to understand the genetic/molecular mechanisms underlying drug resistance in malaria parasites [30,31]. Chloroquine resistance (CQR) in *P. falciparum* is now linked to point mutations in the chloroquine resistance transporter (PfCRT [encoded by *pfcrt*, located on chromosome 7]). *Pfcrt*-K76T mutation confers resistance *in vitro*, and is the most reliable molecular marker for CQR. Polymorphisms, including copy number variation and point mutations, in another parasite transporter, multidrug resistance (PfMDR1 or Pgh1 [encoded by *pfmdr1*, located on chromosome 5]), contribute to the parasite's susceptibility to

a variety of antimalarial drugs. Point mutations in *pfmdr1* play a modulatory role in CQR, which appears to be a parasite strain-dependent phenomenon [32].

Point mutations in the *P. falciparum* DHPS enzyme (encoded by *pf-dhps*, located on chromosome 8) are involved in the mechanism of resistance to the sulfa class of antimalarials, and accumulation of mutations in the *P. falciparum* DHFR domain (encoded by *pf-dhfr*, located on chromosome 4) defines the major mechanism of high-level pyrimethamine resistance. In field studies, a *pf-dhps* double mutant (437G with either 540E or 581G), combined with the *pf-dhfr* triple mutant (108N_51I_59R), was found to be frequently associated with SP treatment failure [28,29]. Orthologues of *pfcrt* (*pvcrt-o*), *pfmdr1* (*pvmdr1*), *pf-dhps* (*pv-dhps*), and *pf-dhfr* (*pv-dhfr*) in *P. vivax* have been identified, and found to be polymorphic. However, associations of the mutant alleles of *pvcrt-o*/*pvmdr1* and *pv-dhps*/*pv-dhfr* with clinical resistance to CQ and SP, respectively, are unclear [33].

2. Emergence of Artemisinin Resistance in *P. falciparum*

A major advance in the search for effective treatment for drug-resistant malaria came with the discovery of artemisinin and its derivatives. These compounds show very rapid parasite clearance times and faster fever resolution than any other currently licensed antimalarial drug [18]. Given the recent significant impact of ACTs on malaria morbidity and mortality, it is worrisome that higher recrudescence rates of *P. falciparum* malaria after artemisinin treatment are seen in some areas. Recrudescence, the reappearance of an infection after a period of quiescence, occurs in up to 30% of patients on artemisinin monotherapy, and in up to 10% of patients on ACTs [18,34]. The underlying mechanism of recrudescence after artemisinin treatment is unclear. As illustrated by recent *in vitro* studies, the occurrence of parasite dormancy, where parasites enter a temporary growth-arrested state, may provide a plausible explanation for this phenomenon [35,36].

Furthermore, there are recent concerns that the efficacy of ACTs has declined near the Thai-Cambodia border, a historical "hot spot" for emergence and evolution of multidrug-resistant malaria parasites [37]. In this region, artemisinin resistance is characterized by significantly slower parasite clearance *in vivo*, with or without corresponding reductions on conventional *in vitro* susceptibility testing [38,39]. A recent heritability study found that the observed artemisinin-resistant phenotype of the parasites has a genetic basis [40]. The study showed that genetically identical parasite strains, defined by microsatellite typing, strongly clustered in patients with slow *versus* faster parasite clearance rates. It was suggested that the artemisinin-resistant phenotype is expected to spread within parasite populations that live where artemisinins are deployed unless associated fitness costs of the putative resistance mutation(s) outweigh selective benefits. The genetic basis for artemisinin resistance is not known at present. The main reason for this lack of knowledge is that the molecular mechanism(s) of action of artemisinin drugs remains uncertain and debatable, although a number of potential targets have been proposed [41,42]. Investigations into potential protein targets of artemisinin drugs have included PfATP6, a *P. falciparum* SERCA-type calcium-dependent ATPase in the endoplasmic reticulum [43]; PfCRT; PfMDR1; *P. falciparum* multidrug resistance-associated protein 1 (PfMRP1), residing on the parasite plasma membrane; a putative deubiquitinating protease termed UBP-1; and mitochondrial proteins [41,42]. A recent study, conducted on artemisinin-resistant *P. falciparum*

strains from western Cambodia, did not find any correlation between artemisinin-resistant phenotype and *pfmdr1* mutations/amplification, as well as mutations in *pfatp6* (or *pfserca*), *pfcrt*, *ubp-1*, or mtDNA [44].

At present there is no concrete evidence of artemisinin resistance occurring elsewhere. Preliminary reports of emerging artemisinin resistance in South America and some African countries are as yet unconfirmed. For now, the situation appears to be confined to the Thai-Cambodia border, and is most likely a result of distinctive features of malaria treatment in this geographic area. These include unregulated artemisinin monotherapy for more than 30 years, creating massive drug pressure, and counterfeited or substandard tablets that contain less active ingredients than stated [45]. However, if the problem is not urgently and appropriately addressed, it is feared that artemisinin resistance could spread worldwide. Given the pivotal role that artemisinin drugs now play, this would be a major blow for malaria treatment and control, since there are currently no alternative drugs to replace them. Even the development pipeline of new drugs consists predominantly of ACTs.

3. Overview of the Global Pipeline of Antimalarial Drugs

Olliaro and Wells [46] reviewed the global pipeline of new antimalarial combinations and chemical entities, in various stages of development till February 2009. Overall, the pipeline consists of 22 projects, either completed (artemether-lumefantrine and artesunate-amodiaquine), in various clinical stages (phase III/registration stage, n = 4; phase II, n = 8; phase I, n = 5), or in preclinical translational stage (n = 3). Chemical novelty is relatively low among the products in clinical stages of development; the prevailing families are of artemisinin-type and quinoline compounds, mostly in combination with each other. Among the non-artemisinin and/or non-quinoline combinations and single compounds are: Azithromycin-CQ (phase III), fosmidomycin-clindamycin (phase II), methylene blue-amodiaquine (phase II), SAR 97276 (a choline uptake antagonist, phase II), and tinidazole (an anti-parasitic nitroimidazole compound, phase II).

It is clear from the size of the antimalarial drug development pipeline that the pipeline has not reached critical mass, which is of concern particularly when we consider the recent emergence of artemisinin resistance, and the apparent decrease in time to resistance to each new drug/drug combination. The challenges for now and for the future are: (i) how to ensure that we have compounds to combat emerging and future antimalarial drug resistance; and (ii) how to initiate and expedite the development of compounds that are as innovative as possible with respect to their chemical scaffold and molecular target.

4. Feasible Options for Now

4.1. Re-Optimizing Existing Antimalarials

4.1.1. Replacing/rotating drugs—Examples of CQ and SP

Recently, a drug replacement/rotation approach has been suggested to stem the tide of resistance by changing the "playing field" for malaria parasites [47,48]. This approach is based on the understanding that the drug-resistant mutant forms are likely to be less fit than their wild-type strains in the absence of drug selection [49]. It has been shown that when CQ was withdrawn from Malawi, where a high

level of CQR prevailed, CQ sensitivity returned [50]. The return of CQ-sensitive falciparum malaria in Malawi took almost 10 years, and was associated with a re-expansion of *pfcrt*-76K allele-carrying diverse, sensitive parasites [51,52]. Similar association between cessation of CQ use and decrease in *pfcrt*-76T allele-carrying resistant parasites has been observed in Kenya [53] as well as in China [54,55]. However, it is estimated that in these areas, sensitivity to CQ may take many more years to return to a level where the drug could be used effectively again [53,54].

Regarding SP, it has been shown that in the Amazon region of Peru, the frequencies of the mutant *pf-dhfr* and *pf-dhps* alleles, conferring SP resistance, declined significantly 5 years after the drug was replaced [48]. This, however, was not the case in Venezuela [56] and Cambodia [57], where the SP resistance-conferring alleles have remained at a high frequency 8 years and 2 decades, respectively, after the replacement of SP. It appears that the outcome of the drug replacement/rotation approach is not universal but malaria-endemic region dependent; a number of diverse ecological and epidemiological factors are likely to play a role in determining the outcome of this approach. In addition, the logistics, including the cost component, of this approach may pose challenges for some countries.

4.1.2. Combining drugs—Examples of CQ-CQR reversers and multiple-drug therapy

Improving the use of existing antimalarial drugs by designing new combinations is another potential approach to combat resistance. CQ entered widespread usage in the 1940s [19]. In spite of widespread resistance, this drug is still widely available because it is inexpensive and relatively non-toxic. In a thought-provoking article, Ginsburg [58] suggested that differential use of CQ in holoendemic areas that precludes children and pregnant women could still confer an efficacious protection for semi-immune adults, and more efficient treatment protocols could be devised to treat even patients infected with CQ-resistant parasite strains. According to the author [58], since the antimalarial activity of CQ is pleiotropic, drug resistance may be due to different mechanisms, each amenable to reversal by drug combination. Since the pivotal study by Martin and colleagues [59], which showed that CQR in *P. falciparum* was partly reversible by verapamil, it has been envisioned that the usefulness of CQ can be rescued by attempting to reverse the resistance—By co-administering CQ with another drug that chemosensitizes the parasite to its effect [60-64]. Among more than 40 such compounds, only one, chlorpheniramine, a histamine H1 receptor antagonist, in combination with CQ has been tested in patients with some success [58,60]. Of further interest, primaquine and antiretroviral protease inhibitors (PIs) saquinavir, ritonavir, and indinavir, may act as CQR reversers [60]. Fluoxetine, an antidepressant of the selective serotonin reuptake inhibitor class, has been shown to reverse both CQ and mefloquine resistance, while ketoconazole, a synthetic antifungal drug of the imidazole class, has been shown to reverse mefloquine resistance in a monkey model [60]. In addition, novel compounds that are more potent and less toxic than the archetypal CQR reverser verapamil are under investigations [60]. Thus, the reports of new resistance reversers over the last few years have generated considerable interest, and may lead to an effective stopgap therapy for drug-resistant malaria.

Use of combination therapy is currently mandated by the WHO [23]. With widespread high-level CQR, SP, as a 2-drug combination, was a successful first-line treatment in Africa for almost two decades. Currently, our hope rests mainly upon ACTs and atovaquone-proguanil (Malarone). As mentioned before, it is of concern that some of these combinations have started showing signs of

failure. A further approach to minimize/delay the development of drug resistance in malaria is to apply combinations of three or more drugs ("multiple-drug therapy"), as is practiced for treating HIV/AIDS and tuberculosis. Attempts at combining existing drugs with CQ (e.g., CQ-SP) have proved less successful, whereas amodiaquine-SP has shown encouraging clinical activity [65]. Recently, a triple combination of artesunate-Lapdap, despite the side effects associated with Lapdap, is under evaluation for the treatment of uncomplicated falciparum malaria in Africa [66]. A limitation of this approach is the increased possibility of drug-drug interactions or the need for a reformulation of the drugs, both of which can prove costly and time-consuming to resolve. Furthermore, given that only a handful of effective antimalarial drugs are now left, this approach seems less promising.

4.1.3. (Re) Considering drugs with limitations—Example of tafenoquine

With any drug, one of the major limitations is side effects. Mefloquine was first synthesized in 1969 primarily for the purpose of chemoprophylaxis in the U.S. military following the then recently discovered threat of CQ-resistant falciparum malaria. The drug has been in use as monotherapy and as combination therapy with either SP or artesunate. However, due to increasing incidences of resistance, particularly in the areas of multidrug resistance [67,68], newer information about toxicity [69,70], and the potential for severe neuropsychiatric adverse events among US military personnel [71], doxycycline is considered as a replacement for mefloquine chemoprophylaxis [72]. Although doxycycline has scanty side effects, a major limitation is that in prophylactic therapy, it must be taken daily leading to high non-compliance. Therefore, a new investigational drug, tafenoquine, an analogue of primaquine, related to mefloquine, was introduced in 1990s [73,74]. Although like primaquine, tafenoquine causes hemolysis in patients with G6PD deficiency, the drug has the potential advantage of a shorter treatment course, and therefore increased patient compliance, and higher therapeutic index than primaquine [73-75]. Thus, the interest in tafenoquine has been continued and efforts are directed at ameliorating side effects, so that this new efficacious compound can be used for malaria prevention as well as treatment [76-78].

4.2. Repurposing Drugs Used for Other Infections or Diseases

One of the approaches for rapidly identifying and bringing new drugs to the market for the treatment of malaria is to repurpose existing drugs that are currently approved for treatment of other infections or diseases.

4.2.1. Drugs used for other infections—Anti-HIV/AIDS drugs

It is known that some antimalarial drugs have moderate anti-HIV activities [79,80]. However, recent studies have also demonstrated that certain antiretroviral agents can inhibit malaria parasite growth. Skinner-Adams *et al.* [81] showed that antiretroviral PIs saquinavir, ritonavir, and indinavir were effective against *P. falciparum*. Subsequently, other antiretroviral PIs were also shown to exhibit potent antimalarial activity [82,83]. The current working hypothesis for the antimalarial activity of antiretroviral PIs is that these compounds inhibit one or more of the parasite's aspartic proteases (termed plasmepsins), located on the food/digestive vacuole or non-digestive vacuole [84-86].

Recently, we found that a non-nucleoside reverse transcriptase inhibitor, TMC-125, which had been shown to be highly active against HIV [87], demonstrated potent activity against *P. falciparum* [88]. It is theorized that this compound targets the parasite enzyme PfTERT, a catalytic reverse transcriptase component of telomerase, expressed in asexual blood-stage parasites that have begun DNA synthesis [89]. There appears to be only one gene for TERT in *P. falciparum* [89], *P. vivax* [90], and *P. knowlesi* [89], containing some highly conserved regions across species [89,90]. Since telomerase activity is likely to be necessary during blood-stage parasite proliferation, it is hoped that screening other reverse transcriptase inhibitors, or designing specific anti-telomerase compounds may lead to novel antimalarial drugs with potent activity against multiple-species infections.

The potential for crossover between malaria and HIV treatments is undoubtedly intriguing, as most of the malaria-endemic areas also bear the brunt of the HIV pandemic. However, at a time when access to antiretroviral drugs is increasing, and new combinations of antimalarial drugs, particularly ACTs, are being evaluated, it is important that potential interactions between therapies for these 2 infections are also understood [79,80,91]. In this regard, it is important to mention that human drug-metabolizing enzymes CYP2B6 (phase I metabolism) and UGT2B7 (phase II metabolism) play important roles in the metabolism of antiretroviral drugs efavirenz, nevirapine, and zidovudine, as well as antimalarial artemisinin drugs, including their active metabolite DHA [92-94]. Functional polymorphisms in *CYP2B6* and *UGT2B7* are highly prevalent in the regions where HIV/AIDS and malaria co-occur [93-95]. Phenotypic consequences of polymorphisms in these enzyme genes on the pharmacokinetics and effectiveness of artemisinin drugs are yet to be determined, and comprehensive studies evaluating the potential interactions between these antiretroviral and antimalarial drugs in individuals with or without polymorphisms are needed.

4.2.2. Drugs used for other diseases—Anti-cancer drugs

A new area in antimalarial drug discovery is the exploration of anti-cancer drugs that target cellular programs such as cell proliferation, cell differentiation, and cell death [88,96,97]. The interesting revelation that artesunate is effective against certain types of cancer, and may target one or more of these cellular programs [98-100], also lends credence to this path of investigation. Our recent study has shown that two anti-cancer compounds, SU-11274 and Bay 43-9006, exhibit potent activities (IC_{50} values <1 μM) against *P. falciparum* [88]. In humans, SU-11274 acts as a specific inhibitor of the MET receptor tyrosine kinase activity [101]. Bay 43-9006, a dual-action inhibitor, targets the RAF/MEK/ERK signaling pathway in tumor cells, and receptor tyrosine kinases VEGFR/PDGFR in tumor vasculature [102]. The specific targets for SU-11274 and Bay 43-9006 activities against *P. falciparum* are not clear yet, but with the analysis of *Plasmodium* spp. genomes, plasmodial protein kinases ("*Plasmodium* kinome") are emerging as highly promising targets for such compounds [103,104]. In addition, inhibition of protein kinases of the human host is being viewed as another major parasiticidal strategy [97,103,105]. Recently, Doerig and colleagues [97] showed that the host PAK-MEK signaling pathway was selectively activated in *P. falciparum*-infected red blood cells. Selective inhibitors of PAK (IPA-3, IC_{50} value 2 μM) and MEK (U0126 [IC_{50} value 3 μM], PD184352 [IC_{50} value 7 μM]) inhibited the parasite proliferation. Moreover, the MEK inhibitors showed *in vitro*

parasiticidal effects on hepatocyte and erythrocyte stages of the rodent malaria parasite *P. berghei*, indicating conservation of this subversive strategy in plasmodia [97].

As also discussed by Doerig and colleagues [97], several kinase-inhibiting drugs are now used clinically, mostly in a variety of cancers, with many more in different stages of clinical trials. Evaluating these inhibitors for antimalarial properties would considerably reduce the overall discovery/development cost and accelerate the process. A potential problem associated with most of these inhibitors is toxicity, as they tend to have small therapeutic indices. Most fatalities in malaria occur in children under 5, which emphasizes the need for not only highly efficacious but also very safe drugs. Although the kinase-inhibiting anti-cancer drugs have toxic effects, using them in malaria chemotherapy would require much shorter treatment periods, hopefully limiting the problem of toxicity. It would also be of interest to see whether these drugs can be used in combination with artemisinin drugs, which show no "added toxicity", and whether such a combination helps in reducing the effective parasiticidal concentration of these drugs, thus reducing their toxicity.

5. Next Generation of Antimalarial Treatment—Approaches and Candidates

5.1. Development of New Drugs by Modifying Existing Compounds

5.1.1. Derivatization

One method to synthesize new antimalarial drugs is to start with the chemical framework of existing antimalarial drugs; the 4- and 8-aminoquinolines and artemisinin drugs are the two common examples of this approach [106]. Recently, a variety of 4-aminoquinoline derivatives (thiourea, triazine, and bisquinoline) have been found to be highly active against *P. falciparum in vitro*, and against *P. yoelii* or *P. berghei* in mice [107-109]. Synthesis/screening of 4-position modified quinoline-methanol compounds is anticipated to yield an efficacious and less toxic replacement for mefloquine [110-112]. In addition to 4-aminoquinoline analogs, extensive derivatization approaches followed by better understanding of structure-activity relationships and biotransformation mechanisms of toxicity have also provided 8-aminoquinoline analogs with better pharmacologic and reduced toxicologic profiles [113].

Derivatives of artemisinin, particularly the water-soluble and efficacious artesunate, are another obvious choice for the synthesis of new compounds [20,114]. Although highly potent and fast acting, the currently available semisynthetic artemisinin derivatives are prone to hydrolysis, resulting in a short biological half-life and the generation of potentially toxic DHA [115,116]. Therefore, extensive efforts have been made to synthesize new derivatives of artemisinin and chemically-related compounds, which are more stable and exhibit potent *in vitro* [117,118] and *in vivo* [119-122] antimalarial activities. The *in vitro* activity of these new derivatives did not indicate any apparent cytotoxicity [118].

Thus, it appears that derivatization is one possible approach to prolong the clinical usefulness of aminoquinoline and artemisinin classes of compounds. However, as a potential limitation of this approach, toxicity and cross-resistance with the parent compound may still occur in some of the derivatives [123].

5.1.2. Hybridization

Burgess *et al.* reported a highly innovative hybrid molecule that combines the pharmacophore of an active 4-amino-7-chloroquinoline antimalarial with a resistance-reversing group based on imipramine [124]. The compound was found to be highly active against both CQ-sensitive and CQ-resistant strains of *P. falciparum in vitro*, and against *P. chabaudi* in mice, with no obvious signs of toxicity. The authors named this class "reversed chloroquine (RCQ)". Further studies showed that linking any of several reversal-agent-like moieties to a 4-amino-7-chloroquinoline yielded good parasiticidal activity [125]. Structure-activity relationship investigation indicated that RCQ molecules inhibit hemozoin formation in the parasite's digestive vacuole in a manner similar to that of CQ [126].

Recently, in a deliberate rational design of antimalarials acting specifically on multiple targets, several hybrid molecules have been developed in what has been termed "covalent bitherapy" [127]. One interesting report described the synthesis of a novel artemisinin-quinine hybrid with potent antimalarial activity [128]. Artemisinin was reduced to DHA and coupled to a carboxylic acid derivative of quinine via an ester linkage. This novel hybrid molecule had potent activity against CQ-sensitive and CQ-resistant strains of *P. falciparum in vitro*. The activity was superior to that of artemisinin alone, quinine alone, or a 1:1 mixture of artemisinin and quinine [128]. Another example is a trioxane motif of sesquiterpene lactone artemisinin covalently linked to a quinoline entity of an aminoquinoline (e.g., CQ), to form new modular molecules referred to as trioxaquines [127]. The trioxaquines had more improved antimalarial activity than their individual components, indicating a potential additive/synergistic effect of the hybrids, as well as good oral bioavailability and low toxicity [127]. Such hybrid molecules have the potential to delay or circumvent the development of resistance and reduce the risk of drug-drug adverse interactions, the latter often seen with multiple-drug therapies. The antimalarial activities, combined with a good drug profile (preliminary absorption, metabolism, and safety parameters) seem favorable for the selection of hybrid molecules for development as drug candidates [129].

5.2. Finding New Antimalarial Compounds by Exploring Natural Products

Historically, drug discovery and development has greatly benefited from sourcing compounds from nature. In fact, between 1981 and 2002, 61% of new chemical entities brought to the market can be traced back to, or were inspired by, natural sources [130]. Malaria drug discovery is no exception. The isolation of the antimalarial drug quinine from cinchona bark was accomplished in 1820. The bark had long been used by indigenous peoples in the Amazon region for the treatment of fevers. The Chinese herb qing hao (*Artemisia annua*) was also used as a treatment for fevers in China for more than 2,000 years, but it was not until 1972 that the active compound artemisinin was extracted, and later identified as a potent antimalarial drug. The 1990s saw a demise in research into natural products for drug discovery, due in part to the emergence of high-throughput screening and combinatorial chemistry. Today, however, the current demand for novel compounds to tackle emerging antimalarial resistance has stimulated new interest in their potential [131]. Several screening projects utilizing different natural sources, from the rainforest to the deep sea and from exotic microorganisms to plants, have been carried out, resulting in several interesting antimalarial lead compounds with remarkable

chemical diversity [132-136]. Some such projects at the Medicines for Malaria Venture (MMV) are in the early-stage discovery pipeline [46].

The mode of action of compounds originating from natural products is mostly unknown, and, in order to understand the basis for their pharmacological effects, research focused on their synergistic or antagonistic interactions is needed [137]. It is also clear that the much desired success of this approach faces several challenges, such as species selection criteria, screening procedures, pharmacological models and fractionation processes, as well as prediction of clinical safety and efficacy [138,139]. Nevertheless, it is hoped that once the activity of natural medicinal products in humans is characterized, it can be used to identify new molecular scaffolds which will form the basis of the next generation of antimalarial therapies [140].

5.3. Finding New Antimalarial Drugs by Screening Diverse Chemical Libraries

5.3.1. High-throughput screening

One of the major thrust areas for antimalarial drug discovery is to screen diverse chemical libraries using high-throughput assays. In recent years, impressive efforts by several drug-screening groups at pharmaceutical companies and academic institutions have generated a plethora of compounds, which may serve as new starting points for potential antimalarial drugs with novel mechanisms. Here, we summarize five such studies (Table 1).

Table 1. Recent antimalarial drug screening efforts.

Group	Number of compounds screened	Number of hits (effective against malaria parasites)	Number of new leads (with no reported toxicity to humans)	Pre-IND	Reference
Plouffe *et al.*	1,700,000	6,000	530	*	[141]
Gamo *et al.*	2,000,000	13,533	51	*	[142]
Guiguemde *et al.*	309,474	1,300	172	34	[143]
Rottmann *et al.*	12,000	275	17	1	[144]
Rush *et al.*	16,000	17	*	*	[145]
Grimberg *et al.*	33	28	6	3	[88]

* = Ongoing investigations, outcome pending.

(i) From a screen of ~1.7 million compounds, Plouffe *et al.* [141] identified a diverse collection of ~6,000 small molecules comprised of >530 distinct scaffolds, all of which showed potent antimalarial activity (<1.25 μM). Most known antimalarials were identified in this screen, thus validating their approach. In addition, the authors identified many novel chemical scaffolds, which likely act through both known and novel pathways.

(ii) Gamo *et al.* [142] screened nearly two million compounds in GlaxoSmithKline's chemical library for inhibitors of *P. falciparum*, of which 13,533 were confirmed to inhibit parasite growth by at least 80% at 2 μM concentration. More than 8,000 also showed potent activity against the multidrug

resistant strain Dd2. Further analyses suggested several novel mechanisms of their antimalarial action, such as inhibition of protein kinases and host-pathogen interaction related targets. All of these proven plasmodial inhibitors, of which ~11,000 (82%) were previously proprietary and thus unknown to the general research community, were made public to accelerate the pace of drug development for malaria.

(iii) Guiguemde *et al.* [143] used a phenotypic forward chemical genetic approach to assay 309,474 chemicals to discover new antimalarial chemotypes. The primary screen resulted in ~1,300 hits with >80% activity against *P. falciparum* at 7 µM. Of these, antimalarial potencies of 172 compounds were re-confirmed to within tenfold by three laboratories using distinct methods providing the cross-validated hit set. A reverse chemical genetic study of this set identified 19 new inhibitors of four validated drug targets, and 15 novel binders among 61 malarial proteins. One exemplar compound (SJ000025081) at 100 $mg\,kg^{-1}$ b.i.d. $\times$ 3 days resulted in a 90% suppression of *P. yoelii* parasitemia in mice.

(iv) From a library of ~ 12,000 pure natural products and synthetic compounds with structural features found in natural products, Rottmann *et al.* [144] identified 275 primary hits with sub-micromolar activity against *P. falciparum*. After a series of analyses, a synthetic compound, related to the spiroazepineindole class, was selected for a medicinal chemistry lead optimization effort. Synthesis and evaluation of about 200 derivatives yielded the spirotetrahydro-β-carboline (or spiroindolone) compound NITD609. This compound displayed potent activity against a panel of *P. falciparum* strains (average IC_{50} range 0.5 to 1.4 nM), with no evidence of diminished potency against drug-resistant strains. The IC_{50} values against a clone of multidrug resistant strain Dd2, however, increased 7- to 24-fold (attaining a mean of 3 to 11 nM) after 3 to 4 months of constant drug pressure. Subsequent passaging of drug-selected parasites in drug-free media for 4 months showed no evidence of revertants, indicating that the resistance was stable. The resistance was associated with point mutations in the gene encoding the P-type cation-transporter ATPase4 (*pfatp4*, PFL0590c). Further safety and pharmacological preclinical evaluation of NITD609 is currently ongoing to support the initiation of human clinical trials.

(v) Using a high-throughput colorimetric assay for the detection of heme crystallization inhibitors, Rush *et al.* [145] identified 17 hit compounds out of 16,000 small molecules belonging to diverse structural classes. The IC_{50} values of these 17 hit compounds in the *P. falciparum* growth inhibition assay ranged from 0.2 µM to 19 µM, and the compounds belonged to structurally related pyrimidine and 1,3-benzoxathiol-2-one classes. These hit compounds are being tested against multidrug-resistant strains of *P. falciparum* to further confirm that they do not show cross-resistance with currently deployed heme crystallization inhibitors.

5.4. Virtual Screening

Virtual screening involves the rapid *in silico* assessment of large libraries of chemical structures in order to identify those which are most likely to bind to a drug target, typically a protein receptor or enzyme. This approach either alone or combined with high-throughput screening is gaining popularity in the antimalarial drug discovery area. Plouffe *et al.* [141] showed that in some cases the mechanism of action of novel antimalarial compounds, identified by high-throughput cell-based screen, can be determined by *in silico* compound activity profiling. Using clustering by historical activities and the

guilt-by-association principle, the authors found that the compounds may segregate based on their mechanism of action (e.g., clusters of protein synthesis inhibitors or folic acid antagonists). Using an *in silico* approach, Woynarowski *et al.* [146] found that the targets for AT-specific DNA-reactive antitumor drugs adozelesin and bizelesin, which inhibited the growth of *P. falciparum* at picomolar concentrations, were "super-AT island" regions of the parasite genome. Furthermore, investigation of the essentiality of a reaction in the metabolic network of *P. falciparum* by deleting (knocking-out) such a reaction *in silico* [147], and comparison of reconstructed metabolic network models from the parasite and its human host [148] has revealed essential enzymes from the parasite alone, representing new potential drug targets. As molecular databases of compounds and target structures are becoming larger, more and more computational screening approaches are becoming available, providing new and better insights into *in silico* antimalarial drug discovery [149,150]. Using such computational screening approaches, novel and potent inhibitors of *P. falciparum* plasmepsins [151,152], glutathione-*S*-transferase [153], and DHFR [153] have been identified.

Thus, it is anticipated that with the aid of these new screening technologies, ongoing and new projects, including those at the MMV, will keep the malaria drug discovery and development pipeline full with the next generation of potential malaria chemotherapeutics [46].

5.5. Genome-Based ("Targeted") Drug Discovery

The complete genomes of the predominant malaria parasites *P. falciparum* and *P. vivax*, and the human host are now known. The *P. falciparum* genome sequence comprises of 14 chromosomes containing 5,300 identified genes [154]. Undoubtedly, the completion of these *Plasmodium* spp. genomes, and their comparison with the human genome, provides the opportunity to discover parasite-specific, novel molecular targets for malaria therapy and prevention [155]. The information regarding gene expression and key regulatory components of metabolism in *P. falciparum* is being assembled into various databases, to generate and visualize network models of various cellular pathways and processes [156,157]. Efforts are also underway to characterize the importance of many of these pathways and to predict their potential for pharmacological interventions [158-160]. Furthermore, linking this increase in advancing new genetic information with the expression of drug resistance will greatly assist in identifying sites of drug resistance as well as strategies to improve the treatment [161].

A wealth of information on these themes has been presented in various recent articles [162-168]. The MMV currently has 30 projects in the discovery phase [46]. Out of these, 14 discovery projects are on molecularly defined targets, with defined mechanisms of action, and 16 are on whole-cell activity, and therefore have unknown mechanisms of action. In addition to this, the MMV has a target database that currently contains more than 23 new targets at different stages of screening and validation. The molecular targets in ongoing 14 discovery projects at the MMV include *P. falciparum* cysteine proteases, termed falcipains [169-172], heat shock protein 90 (Hsp90) [173-175], DHFR with new series of inhibitors, a variety of kinases, histone deacetylases (HDAC), and dihydroorotate dehydrogenase (DHODH). Here, we present a summary of the recent key findings related to parasite kinases, HDAC, and DHODH as drug targets. As described below, since these enzymes seem essential for the parasite, they may be highly conserved among *Plasmodium* spp., and mutations in them are

likely to be lethal. Therefore, the probability of the parasite becoming resistant to compounds directed against these enzymes should be minimal to none. In addition to these enzyme targets, we summarize key findings related to parasite sequestration receptors/anti-sequestration compounds as another interesting possible drug target.

5.5.1. Kinases

The life cycle of malaria parasites is very tightly regulated, since it passes through a succession of developmental stages that vary in terms of proliferation and differentiation. The successful completion of such a complex life cycle requires a strict control of the cellular machinery that carries out phosphorylation, transcriptional control, post-transcriptional control, and protein degradation. These mechanisms most likely involve fine interactions among various cell-cycle proteins (particularly kinases), which may represent strategic targets for pharmacological interventions [96,104,176].

There are a large number and variety of kinases ("*Plasmodium* kinome"), many of which are clearly distinct from human protein kinases, and thus are considered as targets of choice for antimalarial drug development [176,177]. A serine/threonine protein kinase, casein kinase 2α (PfCK2α), crucial for asexual blood-stage parasites, has been identified as a potential target for antimalarial chemotherapeutic intervention [178]. Among cyclin-dependent protein kinases, Pfmrk is the most attractive target for antimalarial drug development; a variety of inhibitors selectively inhibit Pfmrk at sub-micromolar concentrations [179-181]. Calcium-dependent protein kinase 1 (PfCDPK1), essential for parasite survival, can be inhibited by small-molecule compounds at nanomolar concentrations [182,183]. An orphan protein kinase PfPK7, significant for asexual stage development in humans and oocyst production in mosquitoes, has recently been identified as a lead target [184-187]. In addition, mitogen-activated protein kinase 2 (PfMAP-2), essential for completion of the asexual cycle 188, NIMA-related protein kinase Pfnek-2, predominantly expressed in, and required for transmission of, gametocytes [189], and cAMP-dependent protein kinase (PfPKA), essential for parasite growth and survival [190], hold the promise of being selective and effective targets for the development of new antimalarial drugs.

5.5.2. Histone deacetylases

Histone acetylation plays key roles in regulating gene transcription in both prokaryotes and eukaryotes, the acetylated form inducing gene expression while deacetylation silences genes. A number of HDAC inhibitors are currently in clinical trials, alone or in combination, for the treatment of a variety of cancers, parasitic/infectious diseases, and hemoglobinopathies [191,192]. *Plasmodium falciparum* has at least five putative HDACs, which play a major role in transcriptional regulation of the parasite life cycle [193]. Among these, PfHDAC1 in particular is considered as a promising new antimalarial drug target. A variety of PfHDAC1 inhibitors showed potent *in vitro* antimalarial activity (IC$_{50}$ values in nM range) against CQ-sensitive and CQ-resistant strains of the parasite, and several of the inhibitors were significantly more toxic to the parasites than to mammalian cells [194,195]. Furthermore, an HDAC inhibitor WR301801 (YC-2-88) exhibited cures in *P. berghei*-infected mice at a dose of 52 mg/kg/day orally, when combined with subcurative doses of CQ [196]. Recently, using a modified schizont maturation assay, certain HDAC inhibitors were found to exert potent activity (at nM concentrations) against multidrug-resistant clinical isolates of both *P. falciparum* (n = 24) and

P. vivax (n = 25) from Papua, Indonesia, suggesting that these inhibitors may be promising candidates for antimalarial therapy in geographical locations where both species are endemic [197].

5.5.3. Dihydroorotate dehydrogenase

Erythrocytic stages of *P. falciparum* seem to maintain an active mitochondrial electron transport chain to serve just one metabolic function: regeneration of ubiquinone required as the electron acceptor for DHODH, an essential enzyme for de novo pyrimidine biosynthesis [198]. For this very reason, PfDHODH is also receiving increasing attention as a new target for antimalarial drug discovery [199,200]. A variety of potent inhibitors of PfDHODH showing strong selectivity for the malarial enzyme over that from the human host have been designed and synthesized, and identified by high-throughput screening [201-204]. Potent activity against *Plasmodium* spp. parasites *in vitro* at nM concentrations, with good correlation between activity against the parasites and the enzyme from the parasites has been observed for a number of these inhibitors [201,205]. Lead optimization of a triazolopyrimidine-based series of inhibitors has identified Genz-667348, which is orally bioavailable, with prolonged plasma exposure, and cures *P. berghei* infection in mice at a total dose of 100 mg/kg/day [201]. Further refinement of the structure-activity relationship within this series is underway, with 2 recent analogs of Genz-667348 undergoing further testing to determine if they represent suitable candidates for preclinical development [201].

5.5.4. Anti-sequestration compounds—An interesting possibility

In addition, new compounds may participate in the combat against falciparum malaria not by directly killing but preventing the parasite from causing severe forms of the disease. One way to achieve this is to inhibit the parasite's ability to sequester. Sequestration occurs because parasite-derived adhesins, expressed on the surface of mature infected erythrocytes, bind to receptors on human cells (often referred to simply as cytoadherence or cytoadhesion). Organ-specific sequestration in brain and placenta play an important role in the pathogenesis of cerebral and placental malaria, respectively [5,206]. *Plasmodium falciparum*-infected erythrocytes have been shown to have the potential for binding to a diverse array of endothelial receptors; however, only two of these receptors, CD36 and intracellular adhesion molecule 1 (ICAM1), have been studied in detail [206]. Chondroitin sulfate A (CSA) has been consistently identified as the dominant placental adhesion receptor [5].

In recent years, some progress has been made in inhibiting cytoadherence that is mediated by these receptors. CD36-mediated cytoadherence can be inhibited *in vitro* by antiretroviral PIs ritonavir and saquinavir [207], and polysaccharides derived from seaweed (carrageenans) [208]. A randomized clinical trial of Thai patients with uncomplicated malaria showed that levamisole, an antihelminth drug, known to block cytoadherence to CD36, when used as an adjunctive therapy with quinine and doxycycline, resulted in increased numbers of early-mid trophozoites in the peripheral blood [209]. It was suggested that levamisole prevented the sequestration of these parasites as they matured from ring stages following treatment [209]. Using the crystal structure of ICAM1, Dormeyer and colleagues [210] found that (+)-epigalloylcatechin gallate, a naturally occurring polyphenol compound in green tea, inhibited cytoadherence to ICAM1 in a dose-dependent manner with two variant ICAM 1-binding parasite lines. Using CSA-expressing CHO-K1 cells and *ex vivo* human placental tissue, Andrews *et al.* [211]

showed that two carrageenans and a cellulose sulfate (CS10) were able to inhibit cytoadhesion to CHO-K1 cells as well as human placental tissue; the inhibitory effect on CHO-K1 cells was dose-dependent.

Inhibition of cell adhesion processes is becoming increasingly interesting in the discovery of novel therapeutics [212]. Although our understanding of the molecular mechanisms of parasite adhesion is still incomplete, and many questions remain unanswered [206], the discoveries outlined above open up the possibility of developing therapeutic interventions aimed at blocking or reversing parasite adhesion.

6. A Final Note

In these various approaches to: (i) efficient use of existing as well as forthcoming new antimalarial drugs and (ii) discovery of novel antimalarial drugs, one important aspect not discussed here is the pharmacology of antimalarial agents. Since malaria is a blood infection, the response to an antimalarial treatment is likely determined by the concentration of a drug and/or its active metabolite(s) in the blood. Although antimalarial drugs have been in use since the 1930s, until recently, no data were available on the absorption-distribution-metabolism-excretion parameters of these drugs in humans. Over the past 10–15 years, there have been many methodological developments in the area of pharmacology related to antimalarials. Through these advancements, pharmacokinetics and the major metabolic pathways of a number of antimalarial drugs have been elucidated [33,167,213]. However, little emphasis has been placed on understanding the basic mechanisms responsible for the pharmacokinetic and pharmacodynamic behaviors of the major classes of antimalarial drugs in various populations. Particularly, what role does human genetic diversity play in the outcomes of treatment, such as failure, resistance, and side effects, with antimalarial drugs? Since drug combinations are increasingly being considered to successfully treat malaria worldwide, such information is essential to optimize antimalarial drug regimens, particularly new regimens, in order to achieve high treatment effectiveness across all malaria-endemic regions.

7. Conclusions

In the battle against malaria, we are currently at a stalemate at best. If and when the resistance to artemisinin drugs further develops and spreads, we will be caught unarmed, without any effective weapon against this deadly parasite. Looking at the antimalarial drug development pipeline, new classes of agents, and, in the meantime, a re-optimization of existing drugs using new creative strategies are needed. Re-optimization of existing drugs through replacement/rotation and combination approaches may prolong their life spans for effective treatment and prophylaxis, until new treatments are found. The future of antimalarial drug discovery lies in innovative thinking and novel areas, some currently under exploration and some yet to be explored. As described in this article, there are a number of different promising approaches to the identification of new antimalarials. Among these approaches, repurposing drugs that are used for other infections or diseases, exploring natural products with medicinal significance (in malaria), and target-based drug discovery are of particular interest. In addition, evolving technologies, such as high-throughput cell-based assays and virtual screening, will certainly be useful in discovering new effective treatments for malaria relatively quickly. We do not have any choice but to explore many or all of these approaches in parallel, until existing drugs with

renewed potential, as well as entirely new series of compounds are available for deployment in the field. The discovery of new treatments for malaria, along with their proper execution in the field, will contribute to an important achievement of controlling, and eventually eradicating, this global infectious disease.

Acknowledgements

References

1. Bates, I.; Fenton, C.; Gruber, J.; Lalloo, D.; Medina Lara, A.; Squire, S.B.; Theobald, S.; Thomson, R.; Tolhurst, R. Vulnerability to malaria, tuberculosis, and HIV/AIDS infection and disease. Part 1: Determinants operating at individual and household level. *Lancet Infect. Dis.* **2004**, *4*, 267-277.
2. Stratton, L.; O'Neill, M.S.; Kruk, M.E.; Bell, M.L. The persistent problem of malaria: Addressing the fundamental causes of a global killer. *Soc. Sci. Med.* **2008**, *67*, 854-862.
3. WHO. World Malaria Report 2010. http://www.who.int/malaria/world_malaria_report_2010/en/index.html/ (accessed 26 December 2010).
4. Roca-Feltrer, A.; Carneiro, I.; Armstrong Schellenberg, J.R. Estimates of the burden of malaria morbidity in Africa in children under the age of 5 years. *Trop. Med. Int. Health* **2008**, *13*, 771-783.
5. Rogerson, S.J. Malaria in pregnancy and the newborn. *Adv. Exp. Med. Biol.* **2010**, *659*, 139-152.
6. Schantz-Dunn, J.; Nour, N.M. Malaria and pregnancy: A global health perspective. *Rev. Obstet. Gynecol.* **2009**, *2*, 186-192.
7. Krogstad, D.J. Malaria. In *Tropical Infectious Diseases-Principles, Pathogens, & Practice*, Guerrant, R.L., Walker, D.H., Weller, P.F., Eds.; Churchill Livingstone: Philadelphia, PA, USA, 1999; Volume 1, pp. 736-766.
8. Service, M.; Townson, H. The anopheles vector. In *Essential Malariology*; Gilles, H., Warrell, D.A., Eds.; Arnold: London, UK, 2002; pp. 59-84.
9. Gurarie, D.; Zimmerman, P.A.; King, C.H. Dynamic regulation of single- and mixed-species malaria infection: Insights to specific and non-specific mechanisms of control. *J. Theor. Biol.* **2006**, *240*, 185-199.
10. Mayxay, M.; Pukrittayakamee, S.; Newton, P.N.; White, N.J. Mixed-species malaria infections in humans. *Trends Parasitol.* **2004**, *20*, 233-240.
11. Zimmerman, P.A.; Mehlotra, R.K.; Kasehagen, L.J.; Kazura, J.W. Why do we need to know more about mixed *Plasmodium* species infections in humans? *Trends Parasitol.* **2004**, *20*, 440-447.
12. Baird, J.K. Neglect of *Plasmodium vivax* malaria. *Trends Parasitol.* **2007**, *23*, 533-539.

13. Price, R.N.; Tjitra, E.; Guerra, C.A.; Yeung, S.; White, N.J.; Anstey, N.M. Vivax malaria: Neglected and not benign. *Am. J. Trop. Med. Hyg.* **2007**, *77 (Suppl.6)*, 79-87.

14. Greenwood, B.M.; Fidock, D.A.; Kyle, D.E.; Kappe, S.H.; Alonso, P.L.; Collins, F.H.; Duffy, P.E. Malaria: Progress, perils, and prospects for eradication. *J. Clin. Invest.* **2008**, *118*, 1266-1276.

15. Hemingway, J.; Ranson, H. Insecticide resistance in insect vectors of human disease. *Annu. Rev. Entomol.* **2000**, *45*, 371-391.

16. Girard, M.P.; Reed, Z.H.; Friede, M.; Kieny, M.P. A review of human vaccine research and development: Malaria. *Vaccine* **2007**, *25*, 1567-1580.

17. Schlitzer, M. Antimalarial drugs—What is in use and what is in the pipeline. *Arch. Pharm. (Weinheim)* **2008**, *341*, 149-163.

18. White, N.J. Qinghaosu (artemisinin): The price of success. *Science* **2008**, *320*, 330-334.

19. Wernsdorfer, W.H.; Payne, D. The dynamics of drug resistance in *Plasmodium falciparum*. *Pharmacol. Ther.* **1991**, 50, 95-121.

20. Wiesner, J.; Ortmann, R.; Jomaa, H.; Schlitzer, M. New antimalarial drugs. *Angew. Chem. Int. Ed. Engl.* **2003**, *42*, 5274-5293.

21. Croft, A.M. A lesson learnt: The rise and fall of Lariam and Halfan. *JR Soc. Med.* **2007**, *100*, 170-174.

22. Luzzatto, L. The rise and fall of the antimalarial Lapdap: A lesson in pharmacogenetics. *Lancet* **2010**, *376*, 739-741.

23. WHO. The use of antimalarial drugs: Report of an informal consultation (WHO/CDS/RBM/2001.33). http://rbm.who.int/cmc_upload/0/000/014/923/am_toc.htm/ (accessed 23 November 2010).

24. Bosman, A.; Mendis, K.N. A major transition in malaria treatment: The adoption and deployment of artemisinin-based combination therapies. *Am. J. Trop. Med. Hyg.* **2007**, *77 (Suppl.6)*, 193-197.

25. Hastings, I. How artemisinin-containing combination therapies slow the spread of antimalarial drug resistance. *Trends Parasitol.* **2010**, doi:10.1016/j.pt.2010.09.005.

26. Musset, L.; Bouchaud, O.; Matheron, S.; Massias, L.; Le Bras, J. Clinical atovaquone-proguanil resistance of *Plasmodium falciparum* associated with cytochrome b codon 268 mutations. *Microbes Infect.* **2006**, *8*, 2599-2604.

27. Hyde, J.E. Drug-resistant malaria. *Trends Parasitol.* **2005**, *21*, 494-498.

28. Hayton, K.; Su, X.Z. Genetic and biochemical aspects of drug resistance in malaria parasites. *Curr. Drug Targets Infect. Disord.* **2004**, *4*, 1-10.

29. Hyde, J.E. Drug-resistant malaria—An insight. *FEBS J.* **2007**, *274*, 4688-4698.

30. Ekland, E.H.; Fidock, D.A. Advances in understanding the genetic basis of antimalarial drug resistance. *Curr. Opin Microbiol.* **2007**, *10*, 363-370.

31. Kidgell, C.; Winzeler, E.A. Using the genome to dissect the molecular basis of drug resistance. *Future Microbiol.* **2006**, *1*, 185-199.

32. Valderramos, S.G.; Fidock, D.A. Transporters involved in resistance to antimalarial drugs. *Trends Pharmacol. Sci.* **2006**, *27*, 594-601.

33. Mehlotra, R.K.; Henry-Halldin, C.N.; Zimmerman, P.A. Application of pharmacogenomics to malaria: A holistic approach for successful chemotherapy. *Pharmacogenomics* **2009**, *10*, 435-449.

34. Meshnick, S.R.; Taylor, T.E.; Kamchonwongpaisan, S. Artemisinin and the antimalarial endoperoxides: From herbal remedy to targeted chemotherapy. *Microbiol. Rev.* **1996**, *60*, 301-315.

35. Teuscher, F.; Gatton, M.L.; Chen, N.; Peters, J.; Kyle, D.E.; Cheng, Q. Artemisinin-induced dormancy in *Plasmodium falciparum*: Duration, recovery rates, and implications in treatment failure. *J. Infect. Dis.* **2010**, *202*, 1362-1368.

36. Witkowski, B.; Lelievre, J.; Barragan, M.J.; Laurent, V.; Su, X.Z.; Berry, A.; Benoit-Vical, F. Increased tolerance to artemisinin in *Plasmodium falciparum* is mediated by a quiescence mechanism. *Antimicrob. Agents Chemother.* **2010**, *54*, 1872-1877.

37. Enserink, M. Malaria's drug miracle in danger. *Science* **2010**, *328*, 844-846.

38. Dondorp, A.M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A.P.; Tarning, J.; Lwin, K.M.; Ariey, F.; Hanpithakpong, W.; Lee, S.J.; *et al.* Artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* **2009**, *361*, 455-467.

39. Noedl, H.; Se, Y.; Schaecher, K.; Smith, B.L.; Socheat, D.; Fukuda, M.M. Evidence of artemisinin-resistant malaria in western Cambodia. *N. Engl. J. Med.* **2008**, *359*, 2619-2620.

40. Anderson, T.J.; Nair, S.; Nkhoma, S.; Williams, J.T.; Imwong, M.; Yi, P.; Socheat, D.; Das, D.; Chotivanich, K.; Day, N.P.; *et al.* High heritability of malaria parasite clearance rate indicates a genetic basis for artemisinin resistance in western Cambodia. *J. Infect. Dis.* **2010**, *201*, 1326-1330.

41. Cui, L.; Su, X.Z. Discovery, mechanisms of action and combination therapy of artemisinin. *Expert Rev. Anti-Infect. Ther.* **2009**, *7*, 999-1013.

42. O'Neill, P.M.; Barton, V.E.; Ward, S.A. The molecular mechanism of action of artemisinin—The debate continues. *Molecules* **2010**, *15*, 1705-1721.

43. Eckstein-Ludwig, U.; Webb, R.J.; Van Goethem, I.D.; East, J.M.; Lee, A.G.; Kimura, M.; O'Neill, P.M.; Bray, P.G.; Ward, S.A.; Krishna, S. Artemisinins target the SERCA of *Plasmodium falciparum*. *Nature* **2003**, *424*, 957-961.

44. Imwong, M.; Dondorp, A.M.; Nosten, F.; Yi, P.; Mungthin, M.; Hanchana, S.; Das, D.; Phyo, A.P.; Lwin, K.M.; Pukrittayakamee, S.; *et al.* Exploring the contribution of candidate genes to artemisinin resistance in *Plasmodium falciparum*. *Antimicrob. Agents Chemother.* **2010**, *54*, 2886-2892.

45. Dondorp, A.M.; Yeung, S.; White, L.; Nguon, C.; Day, N.P.; Socheat, D.; von Seidlein, L. Artemisinin resistance: Current status and scenarios for containment. *Nat. Rev. Microbiol.* **2010**, *8*, 272-280.

46. Olliaro, P.; Wells, T.N. The global portfolio of new antimalarial medicines under development. *Clin. Pharmacol. Ther.* **2009**, *85*, 584-595.

47. Laufer, M.K.; Thesing, P.C.; Eddington, N.D.; Masonga, R.; Dzinjalamala, F.K.; Takala, S.L.; Taylor, T.E.; Plowe, C.V. Return of chloroquine antimalarial efficacy in Malawi. *N. Engl. J. Med.* **2006**, *355*, 1959-1966.

48. Zhou, Z.; Griffing, S.M.; de Oliveira, A.M.; McCollum, A.M.; Quezada, W.M.; Arrospide, N.; Escalante, A.A.; Udhayakumar, V. Decline in sulfadoxine-pyrimethamine-resistant alleles after change in drug policy in the Amazon region of Peru. *Antimicrob. Agents Chemother.* **2008**, *52*, 739-741.

49. Walliker, D.; Hunt, P.; Babiker, H. Fitness of drug-resistant malaria parasites. *Acta Trop.* **2005**, *94*, 251-259.

50. Kublin, J.G.; Cortese, J.F.; Njunju, E.M.; Mukadam, R.A.; Wirima, J.J.; Kazembe, P.N.; Djimde, A.A.; Kouriba, B.; Taylor, T.E.; Plowe, C.V. Reemergence of chloroquine-sensitive *Plasmodium falciparum* malaria after cessation of chloroquine use in Malawi. *J. Infect. Dis.* **2003**, *187*, 1870-1875.

51. Laufer, M.K.; Takala-Harrison, S.; Dzinjalamala, F.K.; Stine, O.C.; Taylor, T.E.; Plowe, C.V. Return of chloroquine-susceptible falciparum malaria in Malawi was a reexpansion of diverse susceptible parasites. *J. Infect. Dis.* **2010**, *202*, 801-808.

52. Mita, T.; Kaneko, A.; Lum, J.K.; Zungu, I.L.; Tsukahara, T.; Eto, H.; Kobayakawa, T.; Bjorkman, A.; Tanabe, K. Expansion of wild type allele rather than back mutation in *pfcrt* explains the recent recovery of chloroquine sensitivity of *Plasmodium falciparum* in Malawi. *Mol. Biochem. Parasitol.* **2004**, *135*, 159-163.

53. Mwai, L.; Ochong, E.; Abdirahman, A.; Kiara, S.M.; Ward, S.; Kokwaro, G.; Sasi, P.; Marsh, K.; Borrmann, S.; Mackinnon, M.; *et al.* Chloroquine resistance before and after its withdrawal in Kenya. *Malar. J.* **2009**, *8*, 106.

54. Chen, N.; Gao, Q.; Wang, S.; Wang, G.; Gatton, M.; Cheng, Q. No genetic bottleneck in *Plasmodium falciparum* wild-type *Pfcrt* alleles reemerging in Hainan Island, China, following high-level chloroquine resistance. *Antimicrob. Agents Chemother.* **2008**, *52*, 345-347.

55. Wang, X.; Mu, J.; Li, G.; Chen, P.; Guo, X.; Fu, L.; Chen, L.; Su, X.; Wellems, T.E. Decreased prevalence of the *Plasmodium falciparum* chloroquine resistance transporter 76T marker associated with cessation of chloroquine use against *P. falciparum* malaria in Hainan, People's Republic of China. *Am. J. Trop. Med. Hyg.* **2005**, *72*, 410-414.

56. McCollum, A.M.; Mueller, K.; Villegas, L.; Udhayakumar, V.; Escalante, A.A. Common origin and fixation of *Plasmodium falciparum dhfr* and *dhps* mutations associated with sulfadoxine-pyrimethamine resistance in a low-transmission area in South America. *Antimicrob. Agents Chemother.* **2007**, *51*, 2085-2091.

57. Khim, N.; Bouchier, C.; Ekala, M.T.; Incardona, S.; Lim, P.; Legrand, E.; Jambou, R.; Doung, S.; Puijalon, O.M.; Fandeur, T. Countrywide survey shows very high prevalence of *Plasmodium falciparum* multilocus resistance genotypes in Cambodia. *Antimicrob. Agents Chemother.* **2005**, *49*, 3147-3152.

58. Ginsburg, H. Should chloroquine be laid to rest? *Acta Trop.* **2005**, *96*, 16-23.

59. Martin, S.K.; Oduola, A.M.; Milhous, W.K. Reversal of chloroquine resistance in *Plasmodium falciparum* by verapamil. *Science* **1987**, *235*, 899-901.

60. Egan, T.J.; Kaschula, C.H. Strategies to reverse drug resistance in malaria. *Curr. Opin. Infect. Dis.* **2007**, *20*, 598-604.

61. Guantai, E.; Chibale, K. Chloroquine resistance: Proposed mechanisms and countermeasures. *Curr. Drug Deliv.* **2010**, *7*, 312-323.

62. Henry, M.; Alibert, S.; Orlandi-Pradines, E.; Bogreau, H.; Fusai, T.; Rogier, C.; Barbe, J.; Pradines, B. Chloroquine resistance reversal agents as promising antimalarial drugs. *Curr. Drug Targets* **2006**, *7*, 935-948.

63. van Schalkwyk, D.A.; Egan, T.J. Quinoline-resistance reversing agents for the malaria parasite *Plasmodium falciparum. Drug Resist. Updat.* **2006**, *9*, 211-226.

64. Ward, S.A.; Bray, P.G. Is reversal of chloroquine resistance ready for the clinic? *Lancet* **2001**, *357*, 904.

65. Sendagire, H.; Kaddumukasa, M.; Ndagire, D.; Aguttu, C.; Nassejje, M.; Pettersson, M.; Swedberg, G.; Kironde, F. Rapid increase in resistance of *Plasmodium falciparum* to chloroquine-Fansidar in Uganda and the potential of amodiaquine-Fansidar as a better alternative. *Acta Trop.* **2005**, *95*, 172-182.

66. Wootton, D.G.; Opara, H.; Biagini, G.A.; Kanjala, M.K.; Duparc, S.; Kirby, P.L.; Woessner, M.; Neate, C.; Nyirenda, M.; Blencowe, H.; *et al.* Open-label comparative clinical study of chlorproguanil-dapsone fixed dose combination (Lapdap) alone or with three different doses of artesunate for uncomplicated *Plasmodium falciparum* malaria. *PLoS One* **2008**, *3*, e1779.

67. Price, R.N.; Dorsey, G.; Ashley, E.A.; Barnes, K.I.; Baird, J.K.; d'Alessandro, U.; Guerin, P.J.; Laufer, M.K.; Naidoo, I.; Nosten, F.; *et al.* World Antimalarial Resistance Network I: Clinical efficacy of antimalarial drugs. *Malar. J.* **2007**, *6*, 119.

68. Schlagenhauf, P.; Adamcova, M.; Regep, L.; Schaerer, M.T.; Rhein, H.G. The position of mefloquine as a 21st century malaria chemoprophylaxis. *Malar. J.* **2010**, *9*, 357.

69. Dow, G.; Bauman, R.; Caridha, D.; Cabezas, M.; Du, F.; Gomez-Lobo, R.; Park, M.; Smith, K.; Cannard, K. Mefloquine induces dose-related neurological effects in a rat model. *Antimicrob. Agents Chemother.* **2006**, *50*, 1045-1053.

70. Dow, G.S.; Caridha, D.; Goldberg, M.; Wolf, L.; Koenig, M.L.; Yourick, D.L.; Wang, Z. Transcriptional profiling of mefloquine-induced disruption of calcium homeostasis in neurons *in vitro*. *Genomics* **2005**, *86*, 539-550.

71. Nevin, R.L.; Pietrusiak, P.P.; Caci, J.B. Prevalence of contraindications to mefloquine use among USA military personnel deployed to Afghanistan. *Malar. J.* **2008**, *7*, 30.

72. DoD. Policy memorandum on the use of mefloquine (Lariam[R]) in malaria prophylaxis. http://www.lariaminfo.org/pdfs/policy-memo-secy-defense%20malaria-prophylaxis.pdf (accessed 1 April 2011).

73. Shanks, G.D.; Oloo, A.J.; Aleman, G.M.; Ohrt, C.; Klotz, F.W.; Braitman, D.; Horton, J.; Bruekner, R. A new primaquine analogue, tafenoquine (WR 238605), for prophylaxis against *Plasmodium falciparum* malaria. *Clin. Infect. Dis.* **2001**, *33*, 1968-1974.

74. Walsh, D.S.; Looareesuwan, S.; Wilairatana, P.; Heppner, D.G., Jr.; Tang, D.B.; Brewer, T.G.; Chokejindachai, W.; Viriyavejakul, P.; Kyle, D.E.; Milhous, W.K.; *et al.* Randomized dose-ranging study of the safety and efficacy of WR 238605 (Tafenoquine) in the prevention of relapse of *Plasmodium vivax* malaria in Thailand. *J. Infect. Dis.* **1999**, *180*, 1282-1287.

75. Dutta, G.P.; Puri, S.K. New antimalarial drug development in india: radical curative agents CDRI 80/53 (Elubaquine) and WR 238605 (Tafenoquine). *Proc. Indian Nat. Sci. Acad.* **2003**, *B69*, 871-882.

76. Crockett, M.; Kain, K.C. Tafenoquine: A promising new antimalarial agent. *Exp. Opin. Invest. Drugs* **2007**, *16*, 705-715.

77. Elmes, N.J.; Nasveld, P.E.; Kitchener, S.J.; Kocisko, D.A.; Edstein, M.D. The efficacy and tolerability of three different regimens of tafenoquine *versus* primaquine for post-exposure prophylaxis of *Plasmodium vivax* malaria in the Southwest Pacific. *Trans. R Soc. Trop. Med. Hyg.* **2008**, *102*, 1095-1101.

78. Nasveld, P.E.; Edstein, M.D.; Reid, M.; Brennan, L.; Harris, I.E.; Kitchener, S.J.; Leggat, P.A.; Pickford, P.; Kerr, C.; Ohrt, C.; *et al.* Randomized, double-blind study of the safety, tolerability, and efficacy of tafenoquine *versus* mefloquine for malaria prophylaxis in nonimmune subjects. *Antimicrob. Agents Chemother.* **2010**, *54*, 792-798.

79. Khoo, S.; Back, D.; Winstanley, P. The potential for interactions between antimalarial and antiretroviral drugs. *Aids* **2005**, *19*, 995-1005.

80. Skinner-Adams, T.S.; McCarthy, J.S.; Gardiner, D.L.; Andrews, K.T. HIV and malaria co-infection: Interactions and consequences of chemotherapy. *Trends Parasitol.* **2008**, *24*, 264-271.

81. Skinner-Adams, T.S.; McCarthy, J.S.; Gardiner, D.L.; Hilton, P.M.; Andrews, K.T. Antiretrovirals as antimalarial agents. *J. Infect. Dis.* **2004**, *190*, 1998-2000.

82. Parikh, S.; Gut, J.; Istvan, E.; Goldberg, D.E.; Havlir, D.V.; Rosenthal, P.J. Antimalarial activity of human immunodeficiency virus type 1 protease inhibitors. *Antimicrob. Agents Chemother.* **2005**, *49*, 2983-2985.

83. Peatey, C.L.; Andrews, K.T.; Eickel, N.; MacDonald, T.; Butterworth, A.S.; Trenholme, K.R.; Gardiner, D.L.; McCarthy, J.S.; Skinner-Adams, T.S. Antimalarial asexual stage-specific and gametocytocidal activities of HIV protease inhibitors. *Antimicrob. Agents Chemother.* **2010**, *54*, 1334-1337.

84. Andrews, K.T.; Fairlie, D.P.; Madala, P.K.; Ray, J.; Wyatt, D.M.; Hilton, P.M.; Melville, L.A.; Beattie, L.; Gardiner, D.L.; Reid, R.C.; *et al.* Potencies of human immunodeficiency virus protease inhibitors *in vitro* against *Plasmodium falciparum* and *in vivo* against murine malaria. *Antimicrob. Agents Chemother.* **2006**, *50*, 639-648.

85. Parikh, S.; Liu, J.; Sijwali, P.; Gut, J.; Goldberg, D.E.; Rosenthal, P.J. Antimalarial effects of human immunodeficiency virus type 1 protease inhibitors differ from those of the aspartic protease inhibitor pepstatin. *Antimicrob. Agents Chemother.* **2006**, *50*, 2207-2209.

86. Skinner-Adams, T.S.; Andrews, K.T.; Melville, L.; McCarthy, J.; Gardiner, D.L. Synergistic interactions of the antiretroviral protease inhibitors saquinavir and ritonavir with chloroquine and mefloquine against *Plasmodium falciparum in vitro*. *Antimicrob. Agents Chemother.* **2007**, *51*, 759-762.

87. Janssen, P.A.; Lewi, P.J.; Arnold, E.; Daeyaert, F.; de Jonge, M.; Heeres, J.; Koymans, L.; Vinkers, M.; Guillemont, J.; Pasquier, E.; *et al.* In search of a novel anti-HIV drug: multidisciplinary coordination in the discovery of 4-[[4-[[4-[(1E)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile (R278474, rilpivirine). *J. Med. Chem.* **2005**, *48*, 1901-1909.

88. Grimberg, B.T.; Jaworska, M.M.; Hough, L.B.; Zimmerman, P.A.; Phillips, J.G. Addressing the malaria drug resistance challenge using flow cytometry to discover new antimalarials. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5452-5457.

89. Figueiredo, L.M.; Rocha, E.P.; Mancio-Silva, L.; Prevost, C.; Hernandez-Verdun, D.; Scherf, A. The unusually large *Plasmodium* telomerase reverse-transcriptase localizes in a discrete compartment associated with the nucleolus. *Nucl. Acid. Res.* **2005**, *33*, 1111-1122.

90. Durand, P.M.; Oelofse, A.J.; Coetzer, T.L. An analysis of mobile genetic elements in three *Plasmodium* species and their potential impact on the nucleotide composition of the *P. falciparum* genome. *BMC Genomics* **2006**, *7*, 282.

91. Dooley, K.E.; Flexner, C.; Andrade, A.S. Drug interactions involving combination antiretroviral therapy and other anti-infective agents: Repercussions for resource-limited countries. *J. Infect. Dis.* **2008**, *198*, 948-961.

92. Belanger, A.S.; Caron, P.; Harvey, M.; Zimmerman, P.A.; Mehlotra, R.K.; Guillemette, C. Glucuronidation of the antiretroviral drug efavirenz by UGT2B7 and an *in vitro* investigation of drug-drug interaction with zidovudine. *Drug Metab. Dispos.* **2009**, *37*, 1793-1796.

93. Mehlotra, R.K.; Bockarie, M.J.; Zimmerman, P.A. Prevalence of *UGT1A9* and *UGT2B7* nonsynonymous single nucleotide polymorphisms in West African, Papua New Guinean, and North American populations. *Eur. J. Clin. Pharmacol.* **2007**, *63*, 1-8.

94. Mehlotra, R.K.; Ziats, M.N.; Bockarie, M.J.; Zimmerman, P.A. Prevalence of *CYP2B6* alleles in malaria-endemic populations of West Africa and Papua New Guinea. *Eur. J. Clin. Pharmacol.* **2006**, *62*, 267-275.

95. Mehlotra, R.K.; Bockarie, M.J.; Zimmerman, P.A. *CYP2B6* 983T>C polymorphism is prevalent in West Africa but absent in Papua New Guinea: Implications for HIV/AIDS treatment. *Br. J. Clin. Pharmacol.* **2007**, *64*, 391-395.

96. Kozlov, S.; Waters, N.C.; Chavchich, M. Leveraging cell cycle analysis in anticancer drug discovery to identify novel plasmodial drug targets. *Infect. Disord. Drug Targets* **2010**, *10*, 165-190.

97. Sicard, A.; Semblat, J.P.; Doerig, C.; Hamelin, R.; Moniatte, M.; Dorin-Semblat, D.; Spicer, J.A.; Srivastava, A.; Retzlaff, S.; Heussler, V.; *et al*. Activation of a PAK-MEK signalling pathway in malaria parasite-infected erythrocytes. *Cell. Microbiol.* **2010**, doi: 10.1111/j.1462-5822.2011.01582.x.

98. Firestone, G.L.; Sundar, S.N. Anticancer activities of artemisinin and its bioactive derivatives. *Expert Rev. Mol. Med.* **2009**, *11*, e32.

99. Ghantous, A.; Gali-Muhtasib, H.; Vuorela, H.; Saliba, N.A.; Darwiche, N. What made sesquiterpene lactones reach cancer clinical trials? *Drug Discov. Today* **2010**, *15*, 668-678.

100. Li-Weber, M. Targeting apoptosis pathways in cancer by Chinese medicine. *Cancer Lett.* **2010**, Epub ahead of print.

101. Sattler, M.; Pride, Y.B.; Ma, P.; Gramlich, J.L.; Chu, S.C.; Quinnan, L.A.; Shirazian, S.; Liang, C.; Podar, K.; Christensen, J.G.; *et al*. A novel small molecule met inhibitor induces apoptosis in cells transformed by the oncogenic TPR-MET tyrosine kinase. *Cancer Res.* **2003**, *63*, 5462-5469.

102. Adnane, L.; Trail, P.A.; Taylor, I.; Wilhelm, S.M. Sorafenib (BAY 43-9006, Nexavar), a dual-action inhibitor that targets RAF/MEK/ERK pathway in tumor cells and tyrosine kinases VEGFR/PDGFR in tumor vasculature. *Methods Enzymol.* **2006**, *407*, 597-612.

103. Doerig, C.; Abdi, A.; Bland, N.; Eschenlauer, S.; Dorin-Semblat, D.; Fennell, C.; Halbert, J.; Holland, Z.; Nivez, M.P.; Semblat, J.P.; *et al*. Malaria: Targeting parasite and host cell kinomes. *Biochim. Biophys. Acta* **2010**, *1804*, 604-612.

104. Jirage, D.; Keenan, S.M.; Waters, N.C. Exploring novel targets for antimalarial drug discovery: Plasmodial protein kinases. *Infect. Disord. Drug Targets* **2010**, *10*, 134-146.

105. Leiriao, P.; Albuquerque, S.S.; Corso, S.; van Gemert, G.J.; Sauerwein, R.W.; Rodriguez, A.; Giordano, S.; Mota, M.M. HGF/MET signalling protects *Plasmodium*-infected host cells from apoptosis. *Cell. Microbiol.* **2005**, *7*, 603-609.

106. Vangapandu, S.; Jain, M.; Kaur, K.; Patil, P.; Patel, S.R.; Jain, R. Recent advances in antimalarial drug development. *Med. Res. Rev.* **2007**, *27*, 65-107.

107. Khan, M.O.; Levi, M.S.; Tekwani, B.L.; Khan, S.I.; Kimura, E.; Borne, R.F. Synthesis and antimalarial activities of cyclen 4-aminoquinoline analogs. *Antimicrob. Agents Chemother.* **2009**, *53*, 1320-1324.

108. Sunduru, N.; Sharma, M.; Srivastava, K.; Rajakumar, S.; Puri, S.K.; Saxena, J.K.; Chauhan, P.M. Synthesis of oxalamide and triazine derivatives as a novel class of hybrid 4-aminoquinoline with potent antiplasmodial activity. *Bioorg. Med. Chem.* **2009**, *17*, 6451-6462.

109. Sunduru, N.; Srivastava, K.; Rajakumar, S.; Puri, S.K.; Saxena, J.K.; Chauhan, P.M. Synthesis of novel thiourea, thiazolidinedione and thioparabanic acid derivatives of 4-aminoquinoline as potent antimalarials. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2570-2573.

110. Dow, G.S.; Milner, E.; Caridha, D.; Gardner, S.; Lanteri, C.; Kozar, M.; Mannila, A.; McCalmont, W.; Melendez, V.; Moon, J.; *et al.* Central Nervous System (CNS) exposure of next generation quinoline methanols is reduced relative to mefloquine after intravenous (IV) dosing in mice. *Am. J. Trop. Med. Hyg.* **2010**, *83*, 213.

111. Milner, E.; McCalmont, W.; Bhonsle, J.; Caridha, D.; Carroll, D.; Gardner, S.; Gerena, L.; Gettayacamin, M.; Lanteri, C.; Luong, T.; *et al.* Structure-activity relationships amongst 4-position quinoline methanol antimalarials that inhibit the growth of drug sensitive and resistant strains of *Plasmodium falciparum. Bioorg. Med. Chem. Lett.* **2010**, *20*, 1347-1351.

112. Milner, E.; McCalmont, W.; Bhonsle, J.; Caridha, D.; Cobar, J.; Gardner, S.; Gerena, L.; Goodine, D.; Lanteri, C.; Melendez, V.; *et al.* Anti-malarial activity of a non-piperidine library of next-generation quinoline methanols. *Malar. J.* **2010**, *9*, 51.

113. Tekwani, B.L.; Walker, L.A. 8-Aminoquinolines: Future role as antiprotozoal drugs. *Curr. Opin. Infect. Dis.* **2006**, *19*, 623-631.

114. Jefford, C.W. New developments in synthetic peroxidic drugs as artemisinin mimics. *Drug Discov. Today* **2007**, *12*, 487-495.

115. Efferth, T.; Kaina, B. Toxicity of the antimalarial artemisinin and its dervatives. *Crit. Rev. Toxicol.* **2010**, *40*, 405-421.

116. Kongpatanakul, S.; Chatsiricharoenkul, S.; Khuhapinant, A.; Atipas, S.; Kaewkungwal, J. Comparative study of dihydroartemisinin and artesunate safety in healthy Thai volunteers. *Int. J. Clin. Pharmacol. Ther.* **2009**, *47*, 579-586.

117. Held, J.; Soomro, S.A.; Kremsner, P.G.; Jansen, F.H.; Mordmuller, B. *In vitro* activity of new artemisinin derivatives against *Plasmodium falciparum* clinical isolates from Gabon. *Int. J. Antimicrob. Agents* **2011**, submitted.

118. Sun, L.; Shah, F.; Helal, M.A.; Wu, Y.; Pedduri, Y.; Chittiboyina, A.G.; Gut, J.; Rosenthal, P.J.; Avery, M.A. Design, synthesis, and development of novel guaianolide-endoperoxides as potential antimalarial agents. *J. Med. Chem.* **2010**, *53*, 7864-7868.

119. Posner, G.H.; Chang, W.; Hess, L.; Woodard, L.; Sinishtaj, S.; Usera, A.R.; Maio, W.; Rosenthal, A.S.; Kalinda, A.S.; D'Angelo, J.G.; *et al.* Malaria-infected mice are cured by oral administration of new artemisinin derivatives. *J. Med. Chem.* **2008**, *51*, 1035-1042.

120. Singh, A.S.; Verma, V.P.; Hassam, M.; Krishna, N.N.; Puri, S.K.; Singh, C. Amino- and hydroxy-functionalized 11-azaartemisinins and their derivatives. *Org. Lett.* **2008**, *10*, 5461-5464.

121. Singh, C.; Chaudhary, S.; Kanchan, R.; Puri, S.K. Conversion of antimalarial drug artemisinin to a new series of tricyclic 1,2,4-trioxanes1. *Org. Lett.* **2007**, *9*, 4327-4329.

122. Singh, C.; Chaudhary, S.; Puri, S.K. New orally active derivatives of artemisinin with high efficacy against multidrug-resistant malaria in mice. *J. Med. Chem.* **2006**, *49*, 7227-7233.

123. Dow, G.S.; Koenig, M.L.; Wolf, L.; Gerena, L.; Lopez-Sanchez, M.; Hudson, T.H.; Bhattacharjee, A.K. The antimalarial potential of 4-quinolinecarbinolamines may be limited due to neurotoxicity and cross-resistance in mefloquine-resistant *Plasmodium falciparum* strains. *Antimicrob. Agents Chemother.* **2004**, *48*, 2624-2632.

124. Burgess, S.J.; Selzer, A.; Kelly, J.X.; Smilkstein, M.J.; Riscoe, M.K.; Peyton, D.H. A chloroquine-like molecule designed to reverse resistance in *Plasmodium falciparum. J. Med. Chem.* **2006**, *49*, 5623-5625.

125. Andrews, S.; Burgess, S.J.; Skaalrud, D.; Kelly, J.X.; Peyton, D.H. Reversal agent and linker variants of reversed chloroquines: Activities against *Plasmodium falciparum. J. Med. Chem.* **2010**, *53*, 916-919.

126. Burgess, S.J.; Kelly, J.X.; Shomloo, S.; Wittlin, S.; Brun, R.; Liebmann, K.; Peyton, D.H. Synthesis, structure-activity relationship, and mode-of-action studies of antimalarial reversed chloroquine compounds. *J. Med. Chem.* **2010**, *53*, 6477-6489.

127. Muregi, F.W.; Ishih, A. Next-generation antimalarial drugs: Hybrid molecules as a new strategy in drug design. *Drug Dev. Res.* **2010**, *71*, 20-32.

128. Walsh, J.J.; Coughlan, D.; Heneghan, N.; Gaynor, C.; Bell, A. A novel artemisinin-quinine hybrid with potent antimalarial activity. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3599-3602.

129. Cosledan, F.; Fraisse, L.; Pellet, A.; Guillou, F.; Mordmuller, B.; Kremsner, P.G.; Moreno, A.; Mazier, D.; Maffrand, J.P.; Meunier, B. Selection of a trioxaquine as an antimalarial drug candidate. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17579-17584.

130. Newman, D.J.; Cragg, G.M.; Snader, K.M. Natural products as sources of new drugs over the period 1981-2002. *J. Nat. Prod.* **2003**, *66*, 1022-1037.

131. Ginsburg, H.; Deharo, E. A call for using natural compounds in the development of new antimalarial treatments—An introduction. *Malar. J.* **2011**, *10 (Suppl.1)*, S1.

132. Fernandez, L.S.; Sykes, M.L.; Andrews, K.T.; Avery, V.M. Antiparasitic activity of alkaloids from plant species of Papua New Guinea and Australia. *Int. J. Antimicrob. Agents* **2010**, *36*, 275-279.

133. Hnawia, E.; Hassani, L.; Deharo, E.; Maurel, S.; Waikedre, J.; Cabalion, P.; Bourdy, G.; Valentin, A.; Jullian, V.; Fogliani, B. Antiplasmodial activity of New Caledonia and Vanuatu traditional medicines. *Pharm. Biol.* **2011**, *49*, 369-376.

134. Kaur, K.; Jain, M.; Kaur, T.; Jain, R. Antimalarials from nature. *Bioorg. Med. Chem.* **2009**, *17*, 3229-3256.

135. Turschner, S.; Efferth, T. Drug resistance in *Plasmodium*: Natural products in the fight against malaria. *Mini Rev. Med. Chem.* **2009**, *9*, 206-214.

136. Wright, C.W. Recent developments in research on terrestrial plants used for the treatment of malaria. *Nat. Prod. Rep.* **2010**, *27*, 961-968.

137. Efferth, T.; Koch, E. Complex interactions between phytochemicals. The multi-target therapeutic concept of phytotherapy. *Curr. Drug Targets* **2011**, *12*, 122-132.

138. Bourdy, G.; Willcox, M.L.; Ginsburg, H.; Rasoanaivo, P.; Graz, B.; Deharo, E. Ethnopharmacology and malaria: New hypothetical leads or old efficient antimalarials? *Int. J. Parasitol.* **2008**, *38*, 33-41.

139. Willcox, M.; Benoit-Vical, F.; Fowler, D.; Bourdy, G.; Burford, G.; Giani, S.; Graziose, R.; Houghton, P.; Randrianarivelojosia, M.; Rasoanaivo, P. Do ethnobotanical and laboratory data predict clinical safety and efficacy of anti-malarial plants? *Malar. J.* **2011**, *10 (Suppl.1)*, S7.

140. Wells, T.N. Natural products as starting points for future anti-malarial therapies: Going back to our roots? *Malar. J.* **2011**, *10 (Suppl.1)*, S3.

141. Plouffe, D.; Brinker, A.; McNamara, C.; Henson, K.; Kato, N.; Kuhen, K.; Nagle, A.; Adrian, F.; Matzen, J.T.; Anderson, P.; *et al. In silico* activity profiling reveals the mechanism of action of antimalarials discovered in a high-throughput screen. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 9059-9064.

142. Gamo, F.J.; Sanz, L.M.; Vidal, J.; de Cozar, C.; Alvarez, E.; Lavandera, J.L.; Vanderwall, D.E.; Green, D.V.; Kumar, V.; Hasan, S.; *et al.* Thousands of chemical starting points for antimalarial lead identification. *Nature* **2010**, *465*, 305-310.

143. Guiguemde, W.A.; Shelat, A.A.; Bouck, D.; Duffy, S.; Crowther, G.J.; Davis, P.H.; Smithson, D.C.; Connelly, M.; Clark, J.; Zhu, F.; *et al.* Chemical genetics of *Plasmodium falciparum. Nature* **2010**, *465*, 311-315.

144. Rottmann, M.; McNamara, C.; Yeung, B.K.; Lee, M.C.; Zou, B.; Russell, B.; Seitz, P.; Plouffe, D.M.; Dharia, N.V.; Tan, J.; *et al.* Spiroindolones, a potent compound class for the treatment of malaria. *Science* **2010**, *329*, 1175-1180.

145. Rush, M.A.; Baniecki, M.L.; Mazitschek, R.; Cortese, J.F.; Wiegand, R.; Clardy, J.; Wirth, D.F. Colorimetric high-throughput screen for detection of heme crystallization inhibitors. *Antimicrob. Agents Chemother.* **2009**, *53*, 2564-2568.

146. Woynarowski, J.M.; Krugliak, M.; Ginsburg, H. Pharmacogenomic analyses of targeting the AT-rich malaria parasite genome with AT-specific alkylating drugs. *Mol. Biochem. Parasitol.* **2007**, *154*, 70-81.

147. Fatumo, S.; Plaimas, K.; Mallm, J.P.; Schramm, G.; Adebiyi, E.; Oswald, M.; Eils, R.; Konig, R. Estimating novel potential drug targets of *Plasmodium falciparum* by analysing the metabolic network of knock-out strains *in silico. Infect. Genet. Evol.* **2009**, *9*, 351-358.

148. Fatumo, S.; Plaimas, K.; Adebiyi, E.; Konig, R. Comparing metabolic network models based on genomic and automatically inferred enzyme information from *Plasmodium* and its human host to define drug targets *in silico. Infect. Genet. Evol.* **2011**, *11*, 201-208.

149. Ekenna, C.; Fatuma, S.; Adebiyi, E. *In-silico* evaluation of malaria drug targets. *Int. J. Engineer. Technol.* **2010**, *2*, 132-135.

150. Wolf, A.; Shahid, M.; Kasam, V.; Ziegler, W.; Hofmann-Apitius, M. *In silico* drug discovery approaches on grid computing infrastructures. *Curr. Clin. Pharmacol.* **2010**, *5*, 37-46.

151. Degliesposti, G.; Kasam, V.; Da Costa, A.; Kang, H.K.; Kim, N.; Kim, D.W.; Breton, V.; Kim, D.; Rastelli, G. Design and discovery of plasmepsin II inhibitors using an automated workflow on large-scale grids. *ChemMedChem* **2009**, *4*, 1164-1173.

152. Kasam, V.; Zimmermann, M.; Maass, A.; Schwichtenberg, H.; Wolf, A.; Jacq, N.; Breton, V.; Hofmann-Apitius, M. Design of new plasmepsin inhibitors: A virtual high throuphut screening approach on the EGEE grid. *J. Chem. Inf .Model.* **2007**, *47*, 1818-1828.

153. Kasam, V.; Salzemann, J.; Botha, M.; Dacosta, A.; Degliesposti, G.; Isea, R.; Kim, D.; Maass, A.; Kenyon, C.; Rastelli, G.; Hofmann-Apitius, M.; *et al.* WISDOM-II: Screening against multiple targets implicated in malaria using computational grid infrastructures. *Malar. J.* **2009**, *8*, 88.

154. Gardner, M.J.; Hall, N.; Fung, E.; White, O.; Berriman, M.; Hyman, R.W.; Carlton, J.M.; Pain, A.; Nelson, K.E.; Bowman, S.; *et al.* Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* **2002**, *419*, 498-511.

155. Ntoumi, F.; Kwiatkowski, D.P.; Diakite, M.; Mutabingwa, T.K.; Duffy, P.E. New interventions for malaria: mining the human and parasite genomes. *Am. J. Trop. Med. Hyg.* **2007**, *77 (Suppl.6)*, 270-275.

156. Ginsburg, H. Progress in *in silico* functional genomics: The malaria Metabolic Pathways database. *Trends Parasitol.* **2006**, *22*, 238-240.

157. Rao, A.; Yeleswarapu, S.J.; Raghavendra, G.; Srinivasan, R.; Bulusu, G. PlasmoID: A *P. falciparum* protein information discovery tool. *In Silico Biol.* **2009**, *9*, 195-202.

158. Ben Mamoun, C.; Prigge, S.T.; Vial, H. Targeting the lipid metabolic pathways for the treatment of malaria. *Drug Dev. Res.* **2010**, *71*, 44-55.

159. Huthmacher, C.; Hoppe, A.; Bulik, S.; Holzhutter, H.G. Antimalarial drug targets in *Plasmodium falciparum* predicted by stage-specific metabolic network analysis. *BMC Syst. Biol.* **2010**, *4*, 120.

160. Oyelade, J.; Ewejobi, I.; Brors, B.; Eils, R.; Adebiyi, E. Computational identification of signalling pathways in *Plasmodium falciparum*. *Infect. Genet. Evol.* **2010**, doi:10.1016/j.meegid.2010.11.006.

161. Mu, J.; Seydel, K.B.; Bates, A.; Su, X.Z. Recent progress in functional genomic research in *Plasmodium falciparum*. *Curr. Genomics* **2010**, *11*, 279-286.

162. Choi, S.R.; Mukherjee, P.; Avery, M.A. The fight against drug-resistant malaria: novel plasmodial targets and antimalarial drugs. *Curr. Med. Chem.* **2008**, *15*, 161-171.

163. Dharia, N.V.; Chatterjee, A.; Winzeler, E.A. Genomics and systems biology in malaria drug discovery. *Curr. Opin. Investig. Drugs* **2010**, *11*, 131-138.

164. Gardiner, D.L.; Skinner-Adams, T.S.; Brown, C.L.; Andrews, K.T.; Stack, C.M.; McCarthy, J.S.; Dalton, J.P.; Trenholme, K.R. *Plasmodium falciparum*: New molecular targets with potential for antimalarial drug development. *Expert Rev. Anti-Infect. Ther.* **2009**, *7*, 1087-1098.

165. Jana, S.; Paliwal, J. Novel molecular targets for antimalarial chemotherapy. *Int. J. Antimicrob. Agents* **2007**, *30*, 4-10.

166. Muregi, F.W.; Kirira, P.G.; Ishih, A. Novel rational drug design strategies with potential to revolutionize malaria chemotherapy. *Curr. Med. Chem.* **2011**, *18*, 113-143.

167. Na-Bangchang, K.; Karbwang, J. Current status of malaria chemotherapy and the role of pharmacology in antimalarial drug research and development. *Fundam Clin. Pharmacol.* **2009**, *23*, 387-409.

168. Wells, T.N.; Alonso, P.L.; Gutteridge, W.E. New medicines to improve control and contribute to the eradication of malaria. *Nat. Rev. Drug Discov.* **2009**, *8*, 879-891.

169. Coteron, J.M.; Catterick, D.; Castro, J.; Chaparro, M.J.; Diaz, B.; Fernandez, E.; Ferrer, S.; Gamo, F.J.; Gordo, M.; Gut, J.; *et al.* Falcipain inhibitors: optimization studies of the 2-pyrimidinecarbonitrile lead series. *J. Med. Chem.* **2010**, *53*, 6129-6152.

170. Gibbons, P.; Verissimo, E.; Araujo, N.C.; Barton, V.; Nixon, G.L.; Amewu, R.K.; Chadwick, J.; Stocks, P.A.; Biagini, G.A.; Srivastava, A.; *et al.* Endoperoxide carbonyl falcipain 2/3 inhibitor hybrids: Toward combination chemotherapy of malaria through a single chemical entity. *J. Med. Chem.* **2010**, *53*, 8202-8206.

171. Shah, F.; Mukherjee, P.; Gut, J.; Legac, J.; Rosenthal, P.J.; Tekwani, B.L.; Avery, M.A. Identification of novel malarial cysteine protease inhibitors using structure-based virtual screening of a focused cysteine protease inhibitor library. *J. Chem. Inf. Model.* **2011**, submitted.

172. Teixeira, C.; Gomes, J.R.; Gomes, P. Falcipains, *Plasmodium falciparum* cysteine proteases as Key drug targets against malaria. *Curr. Med. Chem.* **2011**, submitted.

173. Pallavi, R.; Roy, N.; Nageshan, R.K.; Talukdar, P.; Pavithra, S.R.; Reddy, R.; Venketesh, S.; Kumar, R.; Gupta, A.K.; Singh, R.K.; *et al.* Heat shock protein 90 as a drug target against protozoan infections: biochemical characterization of HSP90 from *Plasmodium falciparum* and *Trypanosoma evansi* and evaluation of its inhibitor as a candidate drug. *J. Biol. Chem.* **2010**, *285*, 37964-37975.

174. Pesce, E.R.; Cockburn, I.L.; Goble, J.L.; Stephens, L.L.; Blatch, G.L. Malaria heat shock proteins: Drug targets that chaperone other drug targets. *Infect. Disord. Drug Targets* **2010**, 10, 147-157.

175. Shonhai, A. Plasmodial heat shock proteins: Targets for chemotherapy. *FEMS Immunol. Med. Microbiol.* **2010**, *58*, 61-74.

176. Doerig, C.; Meijer, L. Antimalarial drug discovery: Targeting protein kinases. *Expert Opin. Ther. Targets* **2007**, *11*, 279-290.

177. Doerig, C.; Billker, O.; Haystead, T.; Sharma, P.; Tobin, A.B.; Waters, N.C. Protein kinases of malaria parasites: An update. *Trends Parasitol.* **2008**, *24*, 570-577.

178. Holland, Z.; Prudent, R.; Reiser, J.B.; Cochet, C.; Doerig, C. Functional analysis of protein kinase CK2 of the human malaria parasite *Plasmodium falciparum*. *Eukaryot. Cell* **2009**, *8*, 388-397.

179. Caridha, D.; Kathcart, A.K.; Jirage, D.; Waters, N.C. Activity of substituted thiophene sulfonamides against malarial and mammalian cyclin dependent protein kinases. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3863-3867.

180. Geyer, J.A.; Keenan, S.M.; Woodard, C.L.; Thompson, P.A.; Gerena, L.; Nichols, D.A.; Gutteridge, C.E.; Waters, N.C. Selective inhibition of Pfmrk, a *Plasmodium falciparum* CDK, by antimalarial 1,3-diaryl-2-propenones. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1982-1985.

181. Geyer, J.A.; Prigge, S.T.; Waters, N.C. Targeting malaria with specific CDK inhibitors. *Biochim. Biophys. Acta* **2005**, *1754*, 160-170.

182. Kato, N.; Sakata, T.; Breton, G.; Le Roch, K.G.; Nagle, A.; Andersen, C.; Bursulaya, B.; Henson, K.; Johnson, J.; Kumar, K.A.; *et al.* Gene expression signatures and small-molecule compounds link a protein kinase to *Plasmodium falciparum* motility. *Nat. Chem. Biol.* **2008**, *4*, 347-356.

183. Lemercier, G.; Fernandez-Montalvan, A.; Shaw, J.P.; Kugelstadt, D.; Bomke, J.; Domostoj, M.; Schwarz, M.K.; Scheer, A.; Kappes, B.; Leroy, D. Identification and characterization of novel small molecules as potent inhibitors of the plasmodial calcium-dependent protein kinase 1. *Biochemistry* **2009**, *48*, 6379-6389.

184. Bouloc, N.; Large, J.M.; Smiljanic, E.; Whalley, D.; Ansell, K.H.; Edlin, C.D.; Bryans, J.S. Synthesis and *in vitro* evaluation of imidazopyridazines as novel inhibitors of the malarial kinase PfPK7. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5294-5298.

185. Dorin-Semblat, D.; Sicard, A.; Doerig, C.; Ranford-Cartwright, L.; Doerig, C. Disruption of the PfPK7 gene impairs schizogony and sporogony in the human malaria parasite *Plasmodium falciparum*. *Eukaryot. Cell* **2008**, *7*, 279-285.

186. Klein, M.; Diner, P.; Dorin-Semblat, D.; Doerig, C.; Grotli, M. Synthesis of 3-(1,2,3-triazol-1-yl)- and 3-(1,2,3-triazol-4-yl)-substituted pyrazolo[3,4-d]pyrimidin-4-amines via click chemistry: potential inhibitors of the *Plasmodium falciparum* PfPK7 protein kinase. *Org. Biomol. Chem.* **2009**, *7*, 3421-3429.

187. Merckx, A.; Echalier, A.; Langford, K.; Sicard, A.; Langsley, G.; Joore, J.; Doerig, C.; Noble, M.; Endicott, J. Structures of *P. falciparum* protein kinase 7 identify an activation motif and leads for inhibitor design. *Structure* **2008**, *16*, 228-238.

188. Dorin-Semblat, D.; Quashie, N.; Halbert, J.; Sicard, A.; Doerig, C.; Peat, E.; Ranford-Cartwright, L.; Doerig, C. Functional characterization of both MAP kinases of the human malaria parasite *Plasmodium falciparum* by reverse genetics. *Mol. Microbiol.* **2007**, *65*, 1170-1180.

189. Reininger, L.; Tewari, R.; Fennell, C.; Holland, Z.; Goldring, D.; Ranford-Cartwright, L.; Billker, O.; Doerig, C. An essential role for the *Plasmodium* Nek-2 Nima-related protein kinase in the sexual development of malaria parasites. *J. Biol. Chem.* **2009**, *284*, 20858-20868.

190. Wurtz, N.; Chapus, C.; Desplans, J.; Parzy, D. cAMP-dependent protein kinase from *Plasmodium falciparum*: An update. *Parasitology* **2011**, *138*, 1-25.

191. Andrews, K.T.; Tran, T.N.; Wheatley, N.C.; Fairlie, D.P. Targeting histone deacetylase inhibitors for anti-malarial therapy. *Curr. Top. Med. Chem.* **2009**, *9*, 292-308.

192. Rotili, D.; Simonetti, G.; Savarino, A.; Palamara, A.T.; Migliaccio, A.R.; Mai, A. Non-cancer uses of histone deacetylase inhibitors: Effects on infectious diseases and beta-hemoglobinopathies. *Curr. Top. Med. Chem.* **2009**, *9*, 272-291.

193. Chaal, B.K.; Gupta, A.P.; Wastuwidyaningtyas, B.D.; Luah, Y.H.; Bozdech, Z. Histone deacetylases play a major role in the transcriptional regulation of the *Plasmodium falciparum* life cycle. *PLoS Pathog.* **2010**, *6*, e1000737.

194. Andrews, K.T.; Tran, T.N.; Lucke, A.J.; Kahnberg, P.; Le, G.T.; Boyle, G.M.; Gardiner, D.L.; Skinner-Adams, T.S.; Fairlie, D.P. Potent antimalarial activity of histone deacetylase inhibitor analogues. *Antimicrob. Agents Chemother.* **2008**, *52*, 1454-1461.

195. Wheatley, N.C.; Andrews, K.T.; Tran, T.L.; Lucke, A.J.; Reid, R.C.; Fairlie, D.P. Antimalarial histone deacetylase inhibitors containing cinnamate or NSAID components. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7080-7084.

196. Dow, G.S.; Chen, Y.; Andrews, K.T.; Caridha, D.; Gerena, L.; Gettayacamin, M.; Johnson, J.; Li, Q.; Melendez, V.; Obaldia, N., 3rd.; *et al.* Antimalarial activity of phenylthiazolyl-bearing hydroxamate-based histone deacetylase inhibitors. *Antimicrob. Agents Chemother.* **2008**, *52*, 3467-3477.

197. Marfurt, J.; Chalfein, F.; Prayoga, P.; Wabiser, F.; Kenangalem, E.; Piera, K.A.; Fairlie, D.P.; Tjitra, E.; Anstey, N.M.; Andrews, K.T.; *et al. Ex vivo* activity of histone deacetylase inhibitors against multidrug-resistant clinical isolates of *Plasmodium falciparum* and *P. vivax. Antimicrob. Agents Chemother.* **2011**, *55*, 961-966.

198. Painter, H.J.; Morrisey, J.M.; Mather, M.W.; Vaidya, A.B. Specific role of mitochondrial electron transport in blood-stage *Plasmodium falciparum. Nature* **2007**, *446*, 88-91.

199. Phillips, M.A.; Rathod, P.K. *Plasmodium* dihydroorotate dehydrogenase: A promising target for novel anti-malarial chemotherapy. *Infect. Disord. Drug Targets* **2010**, *10*, 226-239.

200. Rodrigues, T.; Lopes, F.; Moreira, R. Inhibitors of the mitochondrial electron transport chain and de novo pyrimidine biosynthesis as antimalarials: The present status. *Curr. Med. Chem.* **2010**, *17*, 929-956.

201. Booker, M.L.; Bastos, C.M.; Kramer, M.L.; Barker, R.H., Jr.; Skerlj, R.; Sidhu, A.B.; Deng, X.; Celatka, C.; Cortese, J.F.; Guerrero Bravo, J.E.; *et al.* Novel inhibitors of *Plasmodium falciparum* dihydroorotate dehydrogenase with anti-malarial activity in the mouse model. *J. Biol. Chem.* **2010**, *285*, 33054-33064.

202. Davies, M.; Heikkila, T.; McConkey, G.A.; Fishwick, C.W.; Parsons, M.R.; Johnson, A.P. Structure-based design, synthesis, and characterization of inhibitors of human and *Plasmodium falciparum* dihydroorotate dehydrogenases. *J. Med. Chem.* **2009**, *52*, 2683-2693.

203. Heikkila, T.; Ramsey, C.; Davies, M.; Galtier, C.; Stead, A.M.; Johnson, A.P.; Fishwick, C.W.; Boa, A.N.; McConkey, G.A. Design and synthesis of potent inhibitors of the malaria parasite dihydroorotate dehydrogenase. *J. Med. Chem.* **2007**, *50*, 186-191.

204. Ojha, P.K.; Roy, K. Chemometric modeling, docking and *in silico* design of triazolopyrimidine-based dihydroorotate dehydrogenase inhibitors as antimalarials. *Eur. J. Med. Chem.* **2010**, *45*, 4645-4656.

205. Patel, V.; Booker, M.; Kramer, M.; Ross, L.; Celatka, C.A.; Kennedy, L.M.; Dvorin, J.D.; Duraisingh, M.T.; Sliz, P.; Wirth, D.F.; *et al.* Identification and characterization of small molecule inhibitors of *Plasmodium falciparum* dihydroorotate dehydrogenase. *J. Biol. Chem.* **2008**, *283*, 35078-35085.

206. Rowe, J.A.; Claessens, A.; Corrigan, R.A.; Arman, M. Adhesion of *Plasmodium falciparum*-infected erythrocytes to human cells: molecular mechanisms and therapeutic implications. *Expert Rev. Mol. Med.* **2009**, *11*, e16.

207. Nathoo, S.; Serghides, L.; Kain, K.C. Effect of HIV-1 antiretroviral drugs on cytoadherence and phagocytic clearance of *Plasmodium falciparum*-parasitised erythrocytes. *Lancet* **2003**, *362*, 1039-1041.

208. Adams, Y.; Smith, S.L.; Schwartz-Albiez, R.; Andrews, K.T. Carrageenans inhibit the *in vitro* growth of *Plasmodium falciparum* and cytoadhesion to CD36. *Parasitol. Res.* **2005**, *97*, 290-294.

209. Dondorp, A.M.; Silamut, K.; Charunwatthana, P.; Chuasuwanchai, S.; Ruangveerayut, R.; Krintratun, S.; White, N.J.; Ho, M.; Day, N.P. Levamisole inhibits sequestration of infected red blood cells in patients with falciparum malaria. *J. Infect. Dis.* **2007**, *196*, 460-466.

210. Dormeyer, M.; Adams, Y.; Kramer, B.; Chakravorty, S.; Tse, M.T.; Pegoraro, S.; Whittaker, L.; Lanzer, M.; Craig, A. Rational design of anticytoadherence inhibitors for *Plasmodium falciparum* based on the crystal structure of human intercellular adhesion molecule 1. *Antimicrob. Agents Chemother.* **2006**, *50*, 724-730.

211. Andrews, K.T.; Klatt, N.; Adams, Y.; Mischnick, P.; Schwartz-Albiez, R. Inhibition of chondroitin-4-sulfate-specific adhesion of *Plasmodium falciparum*-infected erythrocytes by sulfated polysaccharides. *Infect. Immun.* **2005**, *73*, 4288-4294.

212. Simmons, D.L. Anti-adhesion therapies. *Curr. Opin. Pharmacol.* **2005**, *5*, 398-404.

213. Kerb, R.; Fux, R.; Morike, K.; Kremsner, P.G.; Gil, J.P.; Gleiter, C.H.; Schwab, M. Pharmacogenetics of antimalarial drugs: Effect on metabolism and transport. *Lancet Infect. Dis.* **2009**, *9*, 760-774.