VECTOR-BORNE DISEASES

Antimalarial drug discovery — approaches and progress towards new medicines

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Abstract | Malaria elimination has recently been reinstated as a global health priority but current therapies seem to be insufficient for the task. Elimination efforts require new drug classes that alleviate symptoms, prevent transmission and provide a radical cure. To develop these next-generation medicines, public–private partnerships are funding innovative approaches to identify compounds that target multiple parasite species at multiple stages of the parasite life cycle. In this Review, we discuss the cell-, chemistry- and target-based approaches used to discover new drug candidates that are currently in clinical trials or undergoing preclinical testing.

Elimination
An absence of the local transmission of malaria.

Eradication
The complete removal of malaria parasites so that there is no transmission worldwide.

Malaria is a devastating infectious disease that is characterized by intermittent high fevers and, in the case of cerebral malaria, neurological complications, such as brain injury and coma. It affects pregnant women and children disproportionately, with 85% of deaths occurring in children under the age of 5 years. It is caused by protozoan parasites of the genus *Plasmodium*, which are transmitted to humans by the bites of female *Anopheles* spp. mosquitoes. There are four main *Plasmodium* species that cause disease in humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. A simian parasite, *Plasmodium knowlesi*, also occasionally infects humans[1]. *P. falciparum* causes the most deaths, whereas *P. vivax* is the most widespread species. There is no sterilizing immunity against malaria and the disease can be fatal, although symptoms in repeatedly infected individuals tend to decrease over time. Efforts to develop an effective vaccine have been unsuccessful but drugs are able to cure infections. Malaria imposes a heavy social burden that has delayed economic development in regions where it is endemic. It also causes hundreds of thousands of deaths worldwide each year: estimates range from 660,000 to 1,238,000 deaths in 2010 (REFS 2,3) and the highest mortality occurred in Africa.

Malaria was once found throughout many regions of the world, including North America and Europe. It was eliminated from North America, Europe and parts of Asia and South America during the 1950s and 1960s following a global campaign that heavily relied on the new synthetic insecticide, dichlorodiphenyltrichloroethane (DDT), and powerful new synthetic drugs, such as chloroquine and sulphadoxine–pyrimethamine. When the parasites became resistant to these drugs and DDT use was restricted because of environmental and health hazards, malaria returned to many areas and the number of deaths peaked at 1.8 million in 2004 (REF. 3). Nevertheless, because of novel, more effective medicines (BOX 1), improved vector control, increased funding and increased public awareness, the mortality rate has recently declined by ~30%, which suggests that it is time to consider new malaria elimination or even eradication campaigns. However, malaria is a complex disease and might be more difficult to eradicate than viral diseases, such as smallpox, which have been successfully eradicated mainly as a result of effective vaccines.

All species of the *Plasmodium* parasite have a complex life cycle that involves molecular interactions with both the vertebrate and the invertebrate host (BOX 2). Although the parasite transitions between several developmental forms in the human host, all disease symptoms are caused by the repeated lysis and invasion of erythrocytes by the asexual blood-stage parasites. Therefore, nearly all past and current therapies target the blood-stage parasite.

Although effective antimalarial drugs (BOX 1) that could be used in eradication campaigns are currently available, there are two major problems. The first problem is the potential emergence of resistance to artemisinin and its derivatives, which are the most effective drugs that are currently available. Artemisinins (for example, artemether and artesunate) constitute the only

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Box 1 | **Current drug regimens**

The first-line treatment for *Plasmodium falciparum* infections in regions where chloroquine-resistant parasites are present is a combination therapy of artemisinin derivatives with partner drugs that ideally have longer half-lives than the artemisinin-derivatives. Artemether–lumefantrine (Coartem; Novartis) and amodiaquine–artesunate (Coarsucam; Sanofi-Aventis) are the most widely used, whereas dihydroartemisinin–piperaquine (Euartesim; Pfizer) and artesunate–pyronaridine (Pyramax; Shin Poong Pharamaceuticals) are the most recently approved[77]. Several reformulations with specific doses for children and pregnant women are in clinical trials[77]. Artemisinin derivatives are hypothesized to interact with Fe(II) species in the food vacuole of the parasite, and early blood-stage parasites combat this by slowing down haemoglobin digestion[55]. Artemisinins are fast-acting, very potent against blood-stage parasites and show activity against the early sexual stages of the parasite, which is important to block transmission. Their major limitation is that they have a short half-life, which is why they are partnered with longer-lasting drugs.

Chloroquine, which is a 4-aminoquinoline, is still the recommended treatment for *Plasmodium vivax* infections because resistance has not fully developed in this parasite, in contrast to *P. falciparum*[110]. In areas where chloroquine-resistant *P. vivax* is endemic, artemisinin combination therapies are recommended for first-line treatment (except the artesunate–sulfadoxine–pyrimethamine combination, which is ineffective in many areas). The best are those with partner drugs that have long half-lives (for example, dihydroartemisinin–piperaquine, and amodiaquine–artesunate). In all cases, a 15-day course of primaquine is required to prevent relapse and to provide a radical cure.

For travellers who are visiting areas with endemic malaria transmission, the Centers for Disease Control and Prevention (CDC), USA, recommend atovaquone–proguanil (Malarone; GlaxoSmithKline), choloroquine, doxycycline or mefloquine; specific recommendations depend on individual and regional factors.

known drug class that effectively functions against multi-drug-resistant parasites, although reports of prolonged parasite clearance times in artemisinin-treated patients (BOX 3) have raised concerns that the advances of the past few years might be lost. The second problem is that only one drug, primaquine, can completely eliminate *P. vivax* and *P. ovale* and thus provide a radical cure. *P. vivax* and *P. ovale* infections are challenging to treat because they form dormant liver stages (which are known as hypnozoites) that are refractory to most drugs. Primaquine — an 8-aminoquinoline — requires repeated dosing (for up to 15 days) and is toxic to individuals with glucose-6-phosphate dehydrogenase deficiency[4], which is a common condition in malaria-endemic regions. This limits the use of primaquine in more than 2.85 billion people who are at risk of *P. vivax* infection in Central and Southeast Asia, and in Central and South America[5]. Therefore, new drugs that are active against all stages of the parasite life cycle and drugs that have new mechanisms of action are needed to help to achieve the ultimate goal of elimination[6,7].

Concerns about artemisinin resistance have led universities and research institutes, funding agencies, governments, non-governmental organizations, the military and public–private partnerships to work together to find possible replacement medicines. Although several drugs are active against blood-stage parasites, the ultimate goal is to develop a new compound that blocks all stages of the parasite life cycle, including the transmission and liver stages. In addition, because patient compliance is an issue, the ideal drug would be potent enough to work in a single, curative dose — described as a single exposure, radical cure and prophylaxis (SERCaP) treatment[8,9] — and would be inexpensive to manufacture (US$0.15 per dose), especially as many antimalarial treatments are paid for by non-profit organizations and governments. This target product profile has provided a framework for drug discovery campaigns.

In this Review, we discuss the cell-, chemistry- and target-based approaches that are currently leading the way to the discovery of the next generation of antimalarial drugs. We describe compounds that are in the drug development pipeline (TABLE 1) and the methods available to discover novel chemically validated targets and novel chemical drug classes that have broad activity against multiple stages of the parasite life cycle.

Finding new drug candidates

Diverse strategies are available for the development of novel antimalarial drugs. Although efforts have been made to improve molecules that are currently available (for example, by modifying a scaffold to work against parasites that have acquired resistance to the parent scaffold), most new classes of antimalarial compounds have been discovered using high-throughput screens. In this approach, a large compound library is screened to identify compounds that are active against the parasite in what is called a 'phenotypic' or 'whole-cell' assay (FIG. 1). Alternatively, the library can be screened for activity against 'targets' — typically proteins that are crucial for parasite survival. Such screens often use biochemical assays (for example, to detect ATP hydrolysis in a kinase activity assay). After screening, the most promising scaffolds are identified by cheminformatic analysis. Further selection is based on potency, cost, ease of synthesis, toxicity and novelty (FIG. 1). Once scaffolds are selected, additional derivatives are often synthesized and tested against the whole parasite or against the specific protein target. These tests can reveal structure–activity relationships (SARs), which can be used to predict the effect of chemical modifications on the properties of the molecule (for example, its bioavailabilty and solubility). Several rounds of iterative SAR analyses can lead to the identification of candidates for efficacy testing and eventually to lead compound identification. The type of assay and the number of compounds that are screened is typically balanced by the cost. Malaria drug discovery has, until recently, focused on finding replacements for compounds that are active against blood-stage *P. falciparum,* such as artemisinin. With the development of cellular screens that can identify compounds that are active against all stages of the parasite life cycle[10–15], as well as the development of assays to test the stage-specificity of candidate compounds (FIG. 2), the focus of the field has now shifted.

Whole cell-based approaches

Cellular screening is the traditional approach to identify compounds with antimicrobial activity[16]. This involves exposing the microorganism (which is, in this case, a culture of the parasite) to the test compound (which is a pure chemical compound or a natural product extract) and, after a short incubation period, examining the culture to determine if the compound is capable of killing

Radical cure
A treatment that eliminates the hypnozoite form of the parasite and thus prevents relapse from *Plasmodium vivax* or *Plasmodium ovale* infections.

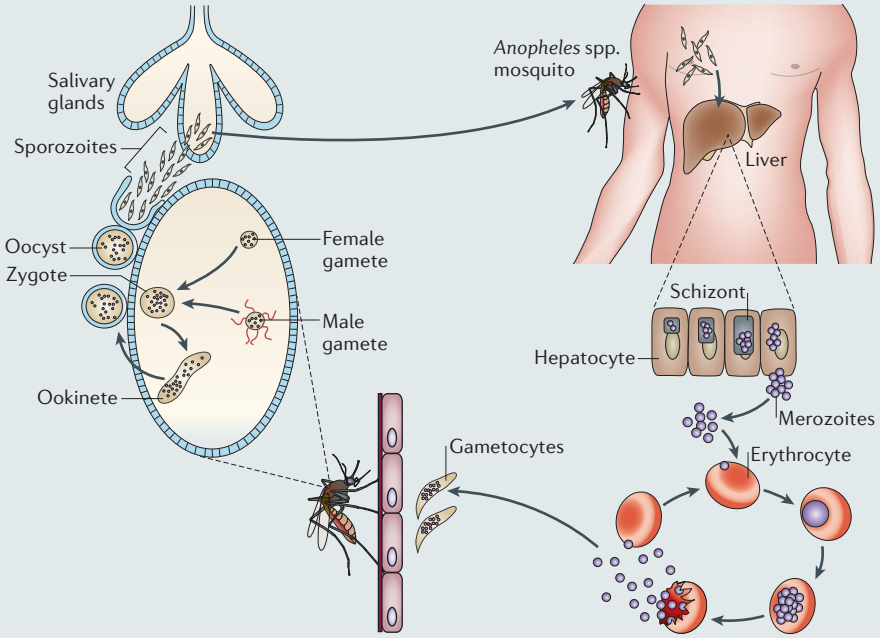
Hypnozoites
The dormant liver-stage forms of the parasite that develop when some *Plasmodium vivax* and *Plasmodium ovale* sporozoites invade hepatocytes. These hypnozoites do not replicate but can become activated weeks, months or years later, resulting in a blood-stage infection.

Single exposure, radical cure and prophylaxis
(SERCaP). A treatment that would only need to be administered in one dose and that would eliminate blood-stage parasites (alleviating the symptoms of malaria) and kill hypnozoites (preventing a new infection from developing).

Box 2 | **The** Plasmodium **spp. life cycle — multiple opportunities for intervention**

Plasmodium spp. have a complex life cycle (see the figure) that is not well characterized at the molecular level. Human infection begins after transmission of sporozoites into the bloodstream following the bite of an infected female *Anopheles* spp. mosquito. Motile sporozoites migrate to the liver and invade hepatocytes. For all *Plasmodium* spp., parasites incubate and multiply to form liver schizonts, which eventually burst, releasing thousands of merozoites into the bloodstream 2–16 days after the initial infection[112]. *Plasmodium vivax* and *Plasmodium ovale* can remain dormant in the liver as hypnozoites, but can re-emerge and begin a blood-stage infection months or years after the initial infection (which is known as relapsing malaria). Once they reach the bloodstream, merozoites attach to and invade red blood cells and, as a result of DNA replication and schizogony, produce 8–32 new merozoites per red blood cell. This 48-hour growth cycle is followed by red blood cell rupture and merozoite re-invasion, which further increases the infection and causes the symptoms of the disease. This cycle of asexual reproduction can persist indefinitely in the absence of treatment.

In a process that is not completely understood, a small fraction of the haploid asexual parasites differentiate into male and female gametocytes within the red blood cell[113]. It is possible that secreted parasite factors induce this differentiation[114,115]. These asymptomatic, non-replicating forms can persist for weeks and are responsible for malaria transmission. After the ingestion of gametocytes by a mosquito during a blood meal, they differentiate into male and female gametes that fuse to form a zygote in the midgut of the mosquito. Meiosis occurs in the zygote, which then develops into a motile ookinete that migrates through the gut wall and eventually forms an oocyst. In the oocyst, multiple rounds of DNA replication take place, which produces thousands of sporozoites[116]. These sporozoites migrate to the salivary glands and are transmitted to the next human host during a blood meal. Remarkably, few of the molecular regulators that control this intricate cycle are known — indeed, even the factors that determine if a parasite becomes male or female are unknown.



Target product profile
A set of guidelines that describes the ideal product. For antimalarial drugs, it might include pharmacokinetic and pharmocodynamic parameters, oral bioavailability, cost, potency and activity against different life cycle stages.

Scaffold
The fixed part of a lead molecule on which chemical functional groups are substituted or exchanged.

Cheminformatic analysis
Computational analysis using systematic or common chemical names; used to group scaffold families and to discover known activities, toxicities and sometimes targets.

Structure–activity relationships
(SARs). The relationships between the chemical structures of molecules and their biological activities. The analysis of SARs allows scientists to identify the chemical groups that are responsible for a compound's biological activity.

Lead compound
This is a chemical compound often discovered in a screen that has pharmacological or biological activity. Its chemical structure is used as a starting point for chemical modifications that improve potency, selectivity or pharmacokinetic parameters.

Merozoites
The infectious parasites that are released when blood-stage schizonts rupture. The merozoites can bind to and invade erythrocytes in a matter of seconds.

the microorganism. Although it might seem straightforward, the challenge is to carry out the assay reproducibly and cost-effectively for a very large number (typically millions) of compounds in a very small test volume. Technological advances in liquid handling, image analysis, assembly of pure chemical libraries and high-throughput automation have made possible the screening of millions of individual compounds in 384- or 1536-well plates in a way that would have been unimaginable 20 years ago[17]. In the last 5 years, high-throughput, cell-based screens have identified hundreds of previously unknown chemical compounds that have the potential to treat malaria.

Blood-stage screens. The first large-scale cellular screens began in 2008 and were carried out by groups that had the capacity for high-throughput screening: the Genomics Institute of the Novartis Research Foundation (GNF, San Diego, California, USA); GlaxoSmithKline (GSK, Tres Cantos, Spain) and St. Jude Children's Research Hospital, Memphis, USA[18–20]. These screens identified compounds that inhibit the proliferation of *P. falciparum* blood stages using a 384- or 1536-well format (FIG. 2). As parasites grow in anucleated human erythrocytes, increases in the nucleic acid content in the well over a 72-hour period can be used as a readout of parasite growth. In this method, fluorescent DNA-binding dyes are used to identify wells containing compounds that inhibit parasite growth[19–21]. Alternatively, parasite viability can be measured on the basis of the activity of parasite lactate dehydrogenase[18]. Using these approaches, over 4 million chemicals have been screened in both academic and commercial settings and between 0.4% and 1.0% of the compounds that were screened showed activity against *P. falciparum* blood stages[18–20].

Box 3 | **Artemisinin resistance**

The first signs of artemisinin resistance came from a study that was conducted in 2008, in which parasite clearance times after initial artesunate monotherapy were slower in patients from the Eastern Thailand–Cambodian border (who had a clearance time of 84 hours) than in patients from the Thailand–Myanmar border in the West (who had a clearance time of 48 hours)[31]. Furthermore, the proportion of slow-clearing infections (defined as a parasite half-life of ≥6.2 hours) on the Thailand–Myanmar border increased from 0.6% in 2001 to 20% in 2010, approaching the rate of 42% that was observed in Cambodia between 2007 and 2010 (REF. 32). This suggests that resistance has spread and is now also present in Western Thailand. At present, the genes that confer this delayed clearance are unknown, although studies have suggested that a region of chromosome 13 is involved[33,34]. Further work indicated that four single-nucleotide substitutions on chromosomes 10, 13 and 14 strongly correlate with delayed parasite clearance times[35]. These parasite loci seem to be under positive selection in these regions and could thus be responsible for resistance, although further studies will be needed to determine the exact genes involved. It has been proposed that parasite resistance is caused by an allele that downregulates metabolism in the early stages of the erythrocytic life cycle and increases it during the later stages, which would allow parasites to circumvent the drug and survive for long enough for the short-lived artemisinins to be degraded[36]. Although this is disturbing, there has been no clear increase in mortality in regions where reduced parasite clearance rates are observed. The spread of artemisinin resistance from the Thailand–Cambodia border (as was seen with choloroquine and sulphadoxine–pyrimethamine) would be devastating for malaria treatment worldwide.

As many interested researchers might not have access to sophisticated screening equipment, such as ultra-accurate liquid dispensing and robotic compound plate storage, the results from many of the large-scale, blood-stage screens, including the specific structures of the effective chemicals and their inhibition constants, have been made available online (chEMBL — Neglected Tropical Disease archive)[22]; this includes data from a collection of 13,000 compounds that are designated the Tres Cantos Antimalarial Set (TCAMS)[18]. On the basis of these public screening results, a library of 400 unique compounds with blood-stage antimalarial activity was created. These compounds have been resynthesized and this 'malaria box' of potential chemical starting points can be obtained from the Medicines for Malaria Venture (MMV). The malaria box concept enables biologists who cannot resynthesize compounds to participate in the drug discovery process and to help to identify how the compounds function. The MMV requests that the results of tests that use the malaria box are made public and encourages collaboration between groups.

Drug candidates from blood-stage screens. One of the first novel drug classes to be identified using modern cellular screening methods was the spiroindolones. The lead for this class, a racaemic spiroazepineindole[23] (FIG. 3), was identified in a screen of ~10,000 natural compounds that were analysed for their activity against proliferating blood-stage *P. falciparum*. Several hundred derivatives, including various enantiomers, were synthesized and further evaluated in blood-stage parasite proliferation tests, in pharmacokinetic tests and in animal models of malaria. The most promising molecule, KAE609 (also known as NITD609)[24], is potent enough to cure *Plasmodium berghei*-infected mice with a single oral 100 mg per kg dose and to prevent transmission[25], as indicated by the reduced oocyst numbers measured in a standard membrane-feeding assay (FIG. 2). The spiroindolone class has a novel mechanism of action, which was identified using *in vitro* evolution and whole-genome scanning. This drug is believed to target the outer membrane transporter, P-type ATPase 4 (PfAtp4)[24], which was initially identified as a calcium ion pump but is now reported to be important for maintaining sodium homeostasis in the parasite[26]. Inhibition of this enzyme by KAE609 presumably increases the intracellular sodium concentration, which causes the parasite to swell up and die. KAE609 is the first candidate compound in 20 years with a novel mechanism of action to enter into clinical trials. As a result of the combined efforts of a global consortium (known as the NGBS consortium, which involves the Novartis Institute for Tropical Diseases, the GNF, the Biomedical Primate Research Center, and the Swiss Tropical and Public Health Institute), and with the help of industry and private–public partnerships (specifically the Wellcome Trust and MMV), KAE609 has very quickly progressed from the screening stage, which began in 2007, to Phase II clinical trials in 2013 (REF. 27) (see MMV research and development for the latest information on preclinical and clinical trials).

GSK also identified chemical classes to be developed further by structural clustering of the lead compounds from the TCAMS. By filtering for properties such as high bioavailability and parasite potency[28], 47 scaffolds were identified, five of which were chosen for SAR analysis and lead compound identification, and the rest were made publicly available with an invitation for collaboration[28]. One of these scaffolds (which is represented by TCMDC-139046 in FIG. 3) contains an indoline core[29] and is a known inhibitor of the human serotonin receptor, 5-hydroxytryptamine 2C, which limits its potential usefulness as an antimalarial drug because of probable adverse side effects. However, using divergent SAR analysis, the team was able to create additional compounds with selective and potent activity against *P. falciparum* and with reduced activity against the human serotonin receptor, although the *in vivo* efficacy of these compounds still needs to be improved. A second class of scaffolds, known as the cyclopropyl carboxamides[30], have potent *in vitro* activity against *P. falciparum* and at least one (which is known as GSK1057714) has *in vivo* activity in the *P. falciparum* severe combined immunodeficient (SCID) mouse model[31] (FIG. 2). However, *in vitro* resistance to these compounds rapidly emerged, which has raised concerns about the development of resistance if these compounds are to be used in patients. Therefore, further optimization will be needed if these compounds are to be developed.

Other leads from blood-stage screens. Since the first high-throughput screens that used blood-stage parasites, several other screens have been implemented that focus on specialized compound libraries or parasite lines. One example of such a focused approach was a screen of a commercially available kinase library[32] that used a parasite proliferation assay[33] to identify 3,5-diaryl-2-aminopyridines as a new class of antimalarial compounds.

Enantiomers
Molecules that are structurally equivalent but are mirror images of one another and therefore not superimposable. It is common for one enantiomer of a drug to have more activity than the other.

Standard membrane-feeding assay
An assay used to determine if a blood culture contains gametocytes that are infectious to mosquitoes. In the assay, mosquitoes feed on human blood that is infected with *Plasmodium falciparum* parasites and covered with parafilm.

Table 1 | **Novel antimalarial compounds in preclinical and clinical development***

Compound (product name)	Chemical class	Presumed target or mechanism of action	Therapeutic activity
Preclinical			
DSM265 (REF. 83)	Triazolopyrimidine	DHOD	Chemotherapeutic
21A092	Pyrazole	Unknown	Chemotherapeutic
MMV390048 (REF. 34)	Aminopyridine	Unknown	Chemotherapeutic
NPC-1161B (REF. 64)	8-aminoquinoline	Unknown	Chemotherapeutic; transmission blocking; and radical cure
ELQ-300 (REF. 68)	Quinolone-3-diarylether	Cytochrome *bc1*	Chemotherapeutic; prophylactic; and transmission blocking
BCX4945 (REF. 130)	Immucillin G	PNP	Chemotherapeutic
RKA182 (REF. 61)	1,2,4,5-tetraoxane	Haemoglobin digestion	Chemotherapeutic
P218 (REF. 76)	Diaminopyridine	DHFR	Chemotherapeutic; and prophylactic
Phase I			
DSM265 (REF. 83)	Triazolopyrimidine	DHOD	Chemotherapeutic
Phase IIa			
KAF156 (also known as GNF156)[42]	Imidazolopiperazine	PfCarl	Chemotherapeutic; prophylactic; and transmission blocking
NITD609 (also known as KAE609)[24]	Spiroindolone	PfATP4	Chemotherapeutic; and transmission blocking
OZ439 (REF. 58)	1,2,4-trioxolane	Haemoglobin digestion	Chemotherapeutic; prophylactic; and transmission blocking
Ferroquine[72]	4-aminoquinoline	Haemozoin formation	Chemotherapeutic
Phase IIb or III			
Tafenoquine[131]	8-aminoquinoline	Unknown	Chemotherapeutic; prophylactic; and radical cure
Nauclea pobeguinii[97,132]	Natural product extract	Unknown	Chemotherapeutic
Argemone mexicana[99]	Natural product extract	Unknown	Chemotherapeutic
OZ277 (also known as RBx11160)[54]	1,2,4-trioxolane	Haemoglobin digestion	Chemotherapeutic

DHFR, dihydrofolate reductase; DHOD, dihydroorotate dehydrogenase; PfAtp4, P-type ATPase 4; PfCarl, cyclic amine resistance locus protein; PNP, purine nucleoside phosphorylase. *Data from clinicaltrials.gov and MMV research and development.

Causal prophylaxis
The prevention of a blood-stage infection by a therapeutic compound that prevents sporozoites from invading or developing within the liver.

Ookinetes
The motile parasite forms that develop from zygotes. Ookinetes are tetraploid as a result of meiosis in the zygote and develop into oocysts on the midgut wall.

High-content imaging
Automated microscopy that collects images of cellular monolayers stained with antibodies. Computer algorithms are then used to automatically identify features such as number of cells, number of cells in mitosis, size of cells or aberrant shape.

One member of this class, MMV390048 (which was previously named compound 15 (REF. 34)), cures 100% of *P. berghei*-infected mice (FIG. 2) after a single 30 mg per kg dose. Although the cellular target remains unpublished, MMV390048 (FIG. 3) has progressed to preclinical trials because of its high antiparasitic potency (with a half-maximal inhibitory concentration (IC_{50}) of 25 nM), its 7–8 hour half-life and its good oral bioavailability. In a second example of a focused approach, genetically modified parasites were used to identify compounds that are active against PfAbcg2, which is one of the parasite's ABC transporters[35]. A library of 2,816 approved drugs was screened against wild-type *P. falciparum* and against a recombinant strain that lacks PfAbcg2. This screen identified the antihistamine ketotifen as a potential antimalarial drug. The IC_{50} for the mutant strain was higher than the IC_{50} for the wild-type strain, which suggests that PfAbcg2 is involved in the antimalarial activity of ketotifen. Previous studies using ketotifen showed that it has some anti-relapse activity in *Plasmodium cynomolgi*-infected monkeys[36] and that it results in causal prophylaxis in *Plasmodium yoelii*-infected mice[37] (FIG. 2). Furthermore, it reduces oocyst formation in both *P. falciparum* and *P. yoelii* mouse models[35]. The ketotifen metabolite norketotifen (which has improved pharmacokinetic properties) was recently shown to be active against both blood and early-liver stages[38]. Thus, further lead optimization of ketotifen might help to identify new clinical candidates.

Liver-stage screens. Recent progress has been made in the development of high-throughput screens to identify compounds that are active against the sexual and liver stages of the parasite life cycle[39]. It can be argued that these are the most important life cycle stages with regard to eradication because of the population 'bottleneck' that occurs during these stages[15]. Specifically, there may be billions of parasites in a human blood-stage infection, but only a limited number of ookinetes, hypnozoites or early-liver forms (<50) exist during transmission and reinfection, which means that resistance is less likely to emerge. Since the first report of a liver-stage screen[40], several groups have established liver-stage assays (FIG. 2); for example, high-content imaging has been used to

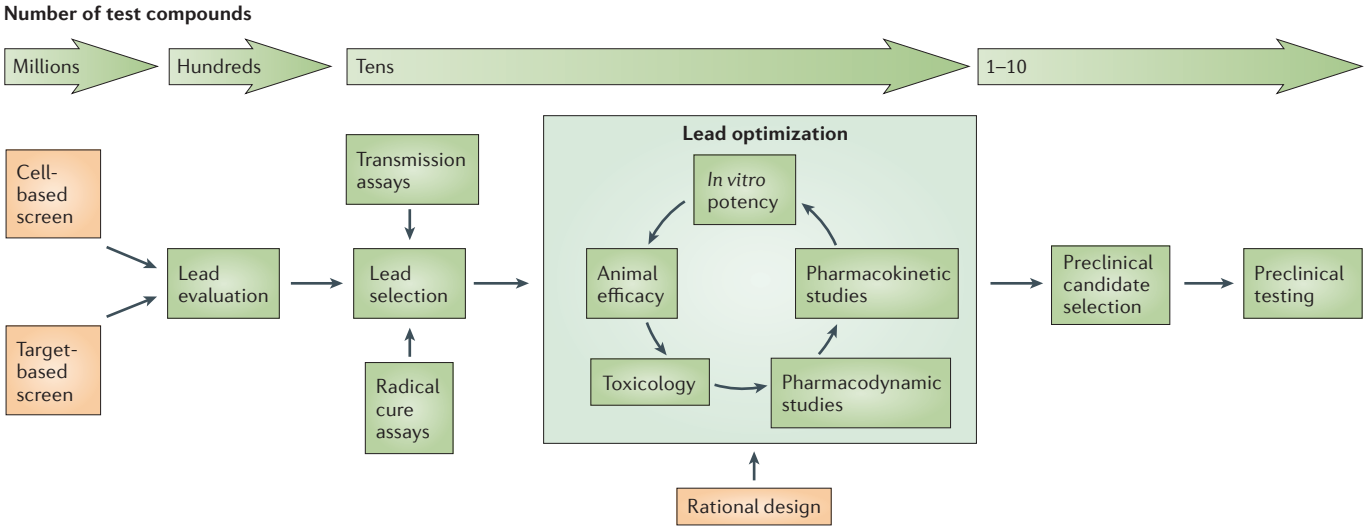


Figure 1 | **Drug development strategy for novel antimalarial drugs.** At the screening stage, millions of compounds can be screened (1,000 –2,000,000 compounds per screening campaign). When hits are identified (on average, a hit rate of 1.0% is observed), they are ranked on the basis of several criteria (such as potency, ease of synthesis, known limitations to their use and novelty) to determine a possible lead compound. These compounds are tested in transmission and radical cure assays, which are low-throughput, time-consuming and expensive and are therefore only applied to a small number of compounds. Following lead compound selection, multiple chemical derivatives of the lead compound are synthesized with the goal of maximizing potency and bioavailability in addition to reducing cross-reactivity with possible human targets (this is known as lead optimization). The best candidate is selected for preclinical testing, which is an expensive and time-consuming process that involves assessing safety and finding the optimal doses that can be used in Phase I human trials.

determine the efficacy of drugs that have known blood-stage activity in *P. yoelii*-infected hepatocytes[41]. One candidate compound that was identified in this screen was GNF179, which is a member of the imidazolopiperazine class. The function of this compound is believed to involve the cyclic amine resistance locus protein (PfCarl), which was identified by *in vitro* evolution and whole-genome scanning. PfCarl contains several transmembrane domains, but its function and whether it is the actual target of GNF179 remain unknown. Further lead optimization of the imidazolopiperazine class led to the identification of KAF156 (also known as GNF156) (FIG. 3) as a clinical candidate that is currently in Phase II clinical trials[42,43]. KAF156 is slightly less potent than KAE609 but it provides prophylactic protection in animal models and has activity against gametocytes. Similar liver-stage screens using other libraries have been carried out[44,45] but have not yet yielded new preclinical candidate drugs.

Radical cure assays. During *P. vivax* and *P. ovale* infections, a subset of parasites can remain undetected in the liver in a dormant state, known as hypnozoites, for months or years before reactivation occurs, which results in a blood-stage infection. Thus, this developmental stage is an important reservoir of *P. vivax* and *P. ovale* parasites in endemic countries. In previous eradication campaigns, *P. vivax* persisted at low levels in regions where *P. falciparum* had been eliminated[46], presumably as a result of the hypnozoite reservoir seeding new infections. This highlights that, in terms of a radical cure, it is crucial that the drug is capable of eliminating this reservoir.

The radical cure activity of primaquine, which is the only currently approved drug that is capable of eliminating hypnozoites, was identified in the 1950s by dosing hundreds of *P. cynomolgi*- (a hypnozoite-producing simian strain) infected monkeys with various aminoquinoline derivatives[47] and determining which animals no longer relapsed. Primaquine is a prodrug that must be metabolized to become active. As it is unclear which of the many metabolic products work against hypnozoites, its mechanism of action remains unknown[48]. Reports of primaquine resistance have been made[49,50], which could help to identify the primaquine target. However, the level of primaquine resistance remains controversial because it is difficult to rule out incomplete patient adherence to the 15-day dosing schedule or patient metabolic deficiencies as a cause of low drug potency. As there are no validated targets for radical cure compounds, target-based screens cannot be initiated.

The development of cellular screens that detect activity against hypnozoites is difficult and has been hindered by the fact that *P. vivax* cannot be maintained in cell culture. A low-throughput *in vitro* assay (FIG. 2) was recently developed using the *P. cynomolgi* monkey model, in which *P. cynomolgi* sporozoites from mosquitoes that have fed on infected monkeys are used to infect primary monkey hepatocytes and image analysis is used to determine infection levels[11]. This assay can distinguish compounds that are active against 'small-form' parasites (which are believed to be the dormant hypnozoites) from compounds that only prevent infection or parasite proliferation in the liver. However, validation of this model is difficult: *P. cynomolgi* sporozoites do not

Gametocytes
The sexual stages of parasites that develop from asexual parasites and that differentiate into gametes in the mosquito. They are thus the parasite forms responsible for transmission.

Sporozoites
The motile infectious forms of the parasite that are transmitted from the mosquito to the human, where they migrate from the dermis to a blood vessel and eventually invade a liver cell.

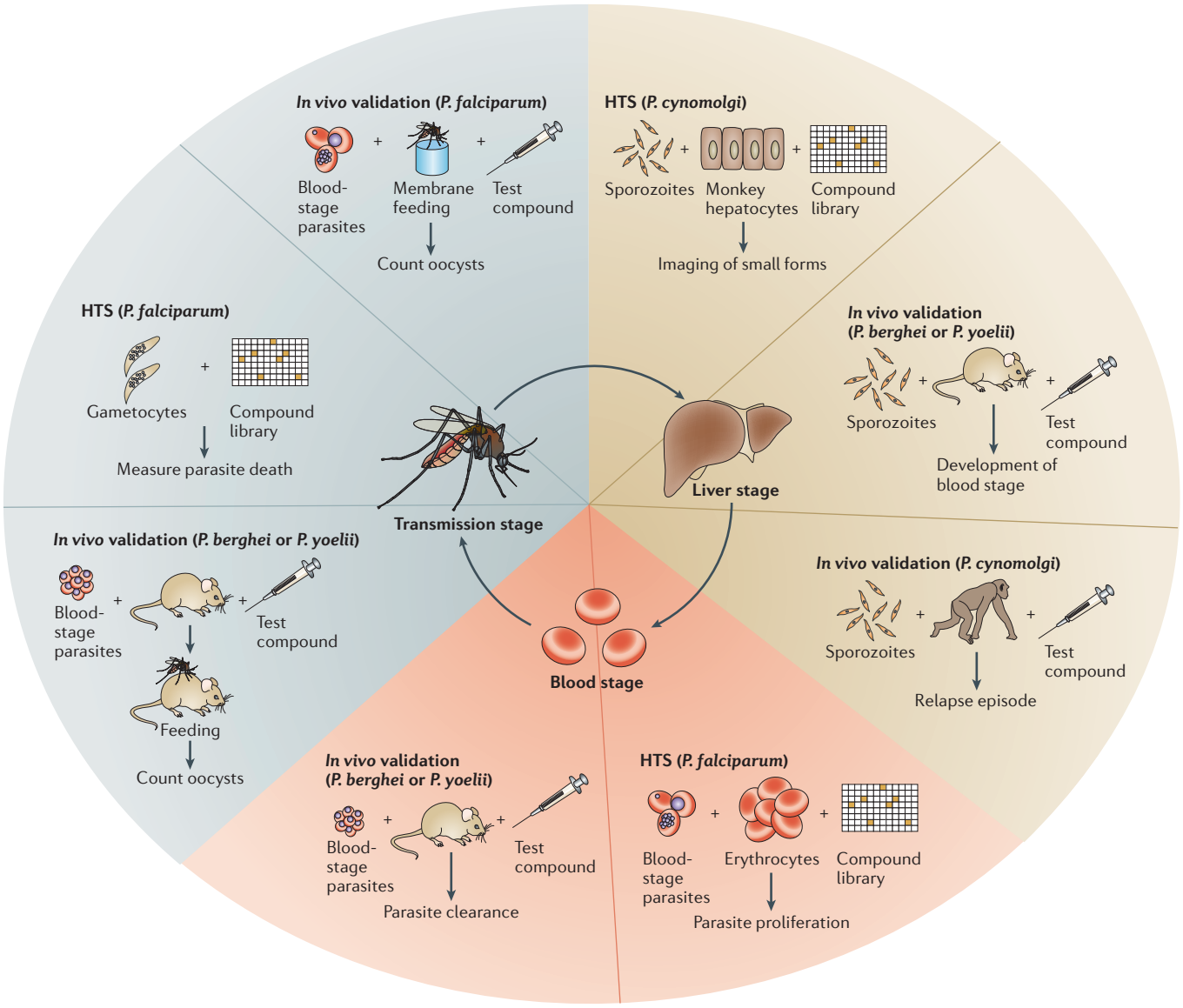


Figure 2 | **Assays available to determine the potency and stage of activity of potential antimalarial compounds.** To detect probable prophylactic activity, *Plasmodium berghei* or *Plasmodium yoelii* sporozoites are seeded onto human hepatoma cells (not shown) and then the infection rate is imaged[41] or detected enzymatically[122]. To detect prophylactic activity *in vivo*, rodent malaria sporozoites are injected into a mouse shortly before or after the mouse has been treated with the compound. The infection can be visualized using luciferase (when genetically modified parasites are used) or by measuring the reduction in blood-stage parasitaemia and/or improved survival. The radical cure potential of a compound is tested using the hypnozoite-forming monkey model, *Plasmodium cynomolgi*[11]. In the *in vitro* assay, *P. cynomolgi* sporozoites are seeded onto primary monkey hepatocytes and imaged to determine the ratio between large, rapidly developing liver-stage schizonts and dormant 'small forms' (thought to be hypnozoites). Radical cure agents eliminate all parasites including hypnozoites, whereas prophylactic compounds only affect growing schizonts. In the *in vivo* model, monkeys are infected with *P. cynomolgi* sporozoites followed by treatment with a compound that eliminates all blood stage parasites (for example, chloroquine) and then with a potential radical cure compound[123]. The monkeys are then monitored over several months to measure reductions in the frequency of hypnozoite-caused relapses. The half-maximal inhibitory concentration (IC_{50}) of *P. falciparum* blood stages is typically measured in a parasite proliferation assay[20]. *Plasmodium vivax* blood-stage sensitivity is determined using a schizont maturation assay using parasites that have been directly taken from infected patients[124] (not shown). The *in vivo* efficacy of blood-stage compounds is typically measured in mice infected with *P. berghei* or *P. yoelii*, although severe combined immunodeficient (SCID) mice can be infected with *P. falciparum*[125]. Transmission-blocking activity can be assayed *in vitro* by looking at either the viability[126] or the development[127] of purified *P. falciparum* gametocytes or ookinetes[52]. The ability of gametocytes to infect mosquitoes is measured using a standard membrane feeding assay (with *P. falciparum*)[128,129] or by direct feeding from infected mice (with *P. berghei* or *P. yoelii*)[53]. After feeding, the number of oocysts per mosquito midgut is counted to determine drug efficacy. HTS, high-throughput screen.

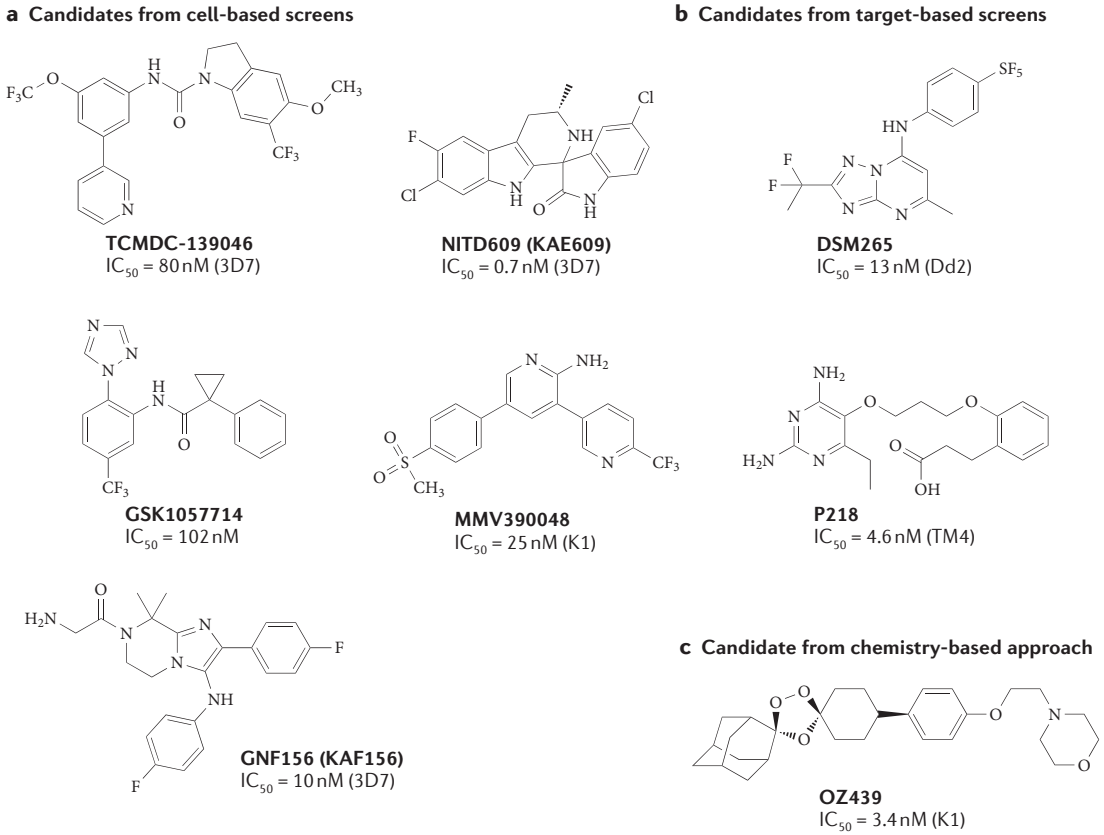


Figure 3 | **Chemical structures of new antimalarial candidates.** Lead compound chemical structures that have been developed by the optimization of chemical classes that were identified using cellular-based screens (TCMDC[29], GSK1057714 (REF. 31), GNF156 (REF. 42), KA609 (NITD609)[23,24], MMV390048 (REF. 34)) (part **a**), target-based screens (part **b**) or chemistry-based approaches (part **c**) are shown. For the cellular screen, the compound classes are novel and are all derived from lead compounds that have potent activity against parasite blood stages. DSM265 (REF. 83) and P218 (REF. 76) were derived from target-based screens against *Plasmodium falciparum* dihydroorotate dehydrogenase (DHOD) and *P. falciparum* dihydrofolate reductase (DHFR), respectively. OZ439 (REF. 58) was rationally designed to have the parasite killing activities of artemisinins, but with a longer half-life. Half-maximal inhibitory concentrations (IC_{50}) were determined in the strains indicated in brackets.

freeze well and obtaining fresh ones is challenging as a result of constraints on primate research. Furthermore, molecular markers that distinguish a hypnozoite from an early-liver schizont do not yet exist and it has not yet been shown that clearing small-form parasites *in vitro* predicts anti-hypnozoite activity. Nevertheless, this is a promising first step towards the development of an assay that examines compounds that target this important developmental stage.

Transmission screens. As gametocytes that are infectious to the mosquito can persist in the patient long after symptoms have resolved, a good lead compound should also kill gametocytes in order to disrupt transmission. If the gametocytes remain viable and mosquitoes are present, then the person can infect his neighbours as well as reinfecting himself. Several groups are developing high-throughput screens to identify compounds that are active against gametocytes (FIG. 2). These assays are particularly difficult to develop because the process of gametocytogenesis is not well understood. As gametocytes do not divide during the 12 days it takes them to mature from the early to the late stage (of which the late stage is transmissible) methods based on the detection of inhibited parasite proliferation cannot be used. Therefore, alternative high-throughput techniques to detect gametocyte death have been developed. Several of these assays use overall ATP hydrolysis to measure viability, with the loss of this activity used as an indicator for gametocyte death[10]. Alternatively, it is possible to selectively count parasites that express a gametocyte-stage-specific GFP tag using flow cytometry[12]. The first gametocyte screen was carried out using Alamar Blue[13], which is an oxidation–reduction indicator that both fluoresces and changes colour in response to the chemical reduction of the growth medium that occurs when gametocytes are metabolically active. Other screens have since been implemented that involve lactate dehydrogenase[51]. As these assays only attempt to predict whether or not a compound might block transmission by killing gametocytes, efforts have been devoted to finding more predictive assays that measure viability in later stages, such as in the ookinete[52]. The gold-standard test is to feed mosquitoes on infected, treated blood (known as

Schizont
A malaria parasite that has completed the process of DNA replication and syncytial nuclear division but that is still contained within a single red blood cell or hepatocyte.

the standard membrane feeding assay for *P. falciparum*), or on infected, treated mice (known as the direct feeding assay) (FIG. 2). These assays (reviewed in REF. 53) are difficult to automate. Interestingly, primaquine is the only drug known to block transmission in humans but, because it is a prodrug, it functions poorly in cellular assays and even in the standard membrane feeding assay.

Medicinal chemistry-based approaches

Directed, chemistry-based approaches use the known chemical structures of antimalarial drugs that have been successful in the clinic as a basis for new compounds. The compounds are modified to optimize their therapeutic properties and to reduce limitations to their use (for example, resistance and bioavailability) using SAR and whole-cell or biochemical assays. Although this approach has been successful (for example, in the case of the synthetic ozonides, which are based on artemisinin (see below)), the major limitation is that there is no model compound that functions during all stages of the parasite life cycle and thus a template for a SERCaP molecule is lacking.

Synthetic ozonides. One of the best examples of the chemistry-based approach is the development of the synthetic ozonides. Ozonides (which are synthetic peroxides) retain the endoperoxide bridge that gives artemisinin its potent blood-stage activity but they also contain a bulky amantadine ring[54], which increases their stability in the plasma. It is hypothesized that their activity results from the peroxide bond being reduced by ferrous iron and haem, which are liberated through the digestion of haemoglobin by the parasite[55]. This reduction produces carbon-centred radicals that alkylate haem and parasite proteins, which ultimately leads to parasite death. The first-generation synthetic ozonide, OZ277 (REF.54) (a 1,2,4-trioxolane), is as potent as artesunate *in vitro* and has increased activity in the *P. berghei* mouse model — it can completely cure mice after three 10 mg per kg oral doses. OZ277 was the first synthetic ozonide to be evaluated in the clinic but, after the Phase II results showed only 70% efficacy after 7 days of treatment, the level of priority of its development was reduced[56]. Nevertheless, OZ277 (or RBx11160, as it is now known) has been approved for use in India since April 2012 in Syrniam (Ranbaxy Laboratories)[57], which is a combination of OZ277 with piperaquine.

A newer generation synthetic ozonide, known as OZ439 (FIG. 3), has now been developed; OZ439 is potent and fast-acting, as well as being pharmacologically active for longer and having improved bioavailability compared to the current artemisinin derivatives and OZ277 (REF. 58). Importantly, OZ439 is able to cure and to prevent *P. berghei* blood-stage mouse infections with a single 30 mg per kg dose and blocks mosquito infection (that is, transmission) in the *in vitro* membrane feeding assay[59]. Encouraging results from Phase I clinical trials confirm this single-dose potency and this, combined with its longer half-life, make this synthetic compound class very attractive[60]. However, as with artemisinins, ozonides are only active against blood stages and, because their activity results from the endoperoxide bridge that is found in artemisinins, they might be less effective against artemisinin-resistant parasites, although whether parasites are truly resistant or not is a matter of debate (BOX 3).

Another synthetic ozonide chemical class, the 1,2,4,5-tetraoxanes, which also contain the endoperoxide bridge, are being developed[61]. The lead compound, known as RKA182, reduces *P. berghei* parasitaemia in mice to undetectable levels 24 hours after treatment. This compound is currently in preclinical trials and, although it has greater stability than OZ277 (REF. 61), its antimalarial activity is inferior to OZ439 (REF. 62).

Other improvements on known scaffolds. There are also efforts to design new versions of primaquine (the only 8-aminoquinoline in clinical use) that lack some of its undesirable toxic properties, such as inducing haemolytic anaemia in patients with glucose-6-phosphate deficiency[4]. Tafenoquine is a 3-phenoxy-substituted 8-aminoquinoline (which was previously named WR238605) that is 4–100 times more potent than primaquine and has a longer half-life[63]. It was identified in 1993 and it is in a dose range Phase II trial as a single dose anti-relapse agent. Similarly, NPC1161C[64] and its enantiomer NPC1161B, which are both 8-aminoquinolines, seem to be promising candidate compounds but it is unclear whether these closely related molecules will have similar levels of side effects as primaquine. Furthermore, assays to measure primaquine resistance are lacking and the genes that confer resistance are unknown[65], which might pose problems in the future for drugs that are designed using primaquine as a scaffold. Another disadvantage of this class of drugs is that some patients do not respond to primaquine therapy, possibly owing to differences in primaquine metabolism between patients[66].

Substantial effort has also been devoted to the development of novel antimalarial drugs that are based on the pyridone scaffold of endochins, the antimalarial activity of which was first recognized in the 1940s[67]. Endochins have been extensively optimized to improve their oral pharmacokinetic properties and a new preclinical candidate, the endochin ELQ-300 (REF. 68), has now been developed. On the basis of their structure (quinolone-3-diarylethers), it is probable that endochins target mitochondrially encoded cytochrome *bc1*, as is the case for atovaquone.

Hybrid molecules. Another rational chemistry-based approach is to design hybrid molecules combining several chemical groups that provide stability, solubility, potency or other attractive features[69]. Two molecules, each with their own antimalarial activity, can be covalently linked to produce a single hybrid molecule that has dual activity. The need for parasites to digest human haemoglobin in asexual blood stages is a vulnerability of the parasite that has been targeted by drug designers. Furthermore, the parasite must also convert the resulting reactive haem molecule to non-reactive haemozoin. Dual-function acridones contain a haem-targeting

acridone group that provides potent blood-stage activity and a chemosensitizing component that counteracts resistance to current aminoquinoline antimalarial drugs[70]. The lead compound, T3.5, shows synergy with chloroquine, piperaquine, quinine and amodiaquine *in vitro* when used to treat aminoquinoline-resistant parasites. In combination with primaquine, it also reduces parasitaemia in *P. yoelii*-infected mice[70]. Similarly, ferroquine is an organometallic compound composed of a lipid-targeting, ferrocenyl group that is covalently linked to a 4-aminoquinoline and a basic alkylamine[71]. Ferroquine is designed to simultaneously disrupt membranes and prevent haemozoin formation[72]. Although interesting for chemical biology and chemical synthesis, the antimalarial effect of hybrid molecules might be achieved using a combination therapy. Furthermore, some hybrids have the same liabilities as their component molecules; for example, the 4-aminoquinolines' liability that they activate the ERG channel[73] (also known as KCNH2) has, for some drugs, been implicated in cardiac arrhythmias[74].

Target-based approaches

Target-based drug discovery is another popular approach for lead compound identification, although its popularity seems to be waning owing to the disappointing results of this approach in the search for next-generation antibacterial drugs[75]. Targets are typically proteins with essential cellular functions, the inhibition of which results in cell death. Target molecules can be identified using chemical inhibition studies (resulting in a chemically validated target). Alternatively, a target can be chosen because it is expected to be essential for parasite viability, which is usually determined by genetic knockdown experiments (resulting in a genetically validated target) or by the fact that it is highly conserved and is therefore likely to have an essential function. Once a target has been selected, the recombinant protein is produced and a biochemical assay is developed that can be used to identify compounds that inhibit the target's function. The process can be further enhanced if crystal structures of the protein are available to guide the design of potential inhibitors and to increase selectivity and specificity against the parasite protein, relative to the host protein.

Key targets. Examples of chemically validated targets in *P. falciparum* include dihydrofolate reductase (DHFR) and cytochrome *bc1*, which are inhibited by the antifolates (pyrimethamine and proguanil) and by atovaquone, respectively. These drugs work well in the clinic and pyrimethamine is currently used in combination therapy with artesunate and sulphadoxine[2]. Inhibition of DHFR prevents folate synthesis, which is essential for DNA replication in the parasite. DHFR inhibitors show specificity for parasite DHFR over human DHFR. As Dhfr targeting has been successful in the past, an *in silico* method was developed to design new drugs that are capable of binding to DHFR enzymes that have mutations that confer resistance to pyrimethamine and proguanil[76]. Co-crystal structures of DHFR complexed with its substrate, with known inhibitors and with lead candidates, were used to guide the development of a lead compound that binds to both wild-type DHFR and the mutated form. This lead compound, known as P218 (FIG. 3), is specific for *Plasmodium* spp. DHFR and has an IC_{50} in the nanomolar range against *P. falciparum* that expresses wild-type or mutant DHFR. This diaminopyridine was designed so that it binds to DHFR in the same place as the natural substrate (dihydrofolic acid) in the hope that the resistance mutations that emerge would not be tolerated. P218 has now advanced to preclinical testing[77].

The mitochondrial enzyme, dihydroorotate dehydrogenase (DHOD) (FIG. 3), has long been recognized as a potential antimalarial target because it catalyses the fourth step in the essential *de novo* pyrimidine biosynthesis pathway[78,79]. The first high-throughput screen to directly measure activity against recombinant DHOD was conducted in 2005 (REF. 80), and, although this screen identified several potential DHOD inhibitors, they lacked adequate activity in parasite proliferation assays. A newly identified chemical class from the original screen, the triazolopyrimidines, was shown to have potent activity in whole-cell assays (IC_{50} of 79 nM in *P. falciparum*) and >5000-fold specificity for parasite DHOD over human DHOD; however, this class was inactive in the *P. berghei in vivo* model[81]. After a series of chemical modifications[81,82] and analysis of drug–enzyme co-crystal structures to further optimize binding, a potent lead compound was discovered that had an IC_{50} of 40–50 nM against drug-sensitive and drug-resistant *P. falciparum,* including *P. falciparum* with resistance to chloroquine, atovaquone and the antifolate, pyrimethamine[83]. The DHOD inhibitor DSM265 (FIG. 3) showed a similar potency to chloroquine in the humanized SCID mouse model. DMS265 is the first DHOD inhibitor to enter preclinical testing[83].

A similar discovery effort identified the carboxamide chemical class as DHOD inhibitors. Recombinant DHOD from *P. falciparum*, *P. berghei* and *P. vivax* were screened against the Genzyme library of 208,000 compounds (Genzyme, Massachusetts, USA) and hits were validated in cell proliferation assays against *P. falciparum*[84]. Further optimization resulted in a lead compound (known as Genz667348) that is effective in the *P. berghei* blood-stage model and that is undergoing further optimization for preclinical trial selection[85]. Although the triazolopyrimidines and the carboxamides both target DHOD, structural analysis of the drug-binding pocket suggests that these two classes bind to overlapping but distinct sites on DHOD[83].

These former approaches used biochemical assays, whereas an *in silico* molecular docking approach was used to identify potential inhibitors that disrupt the interaction between the carboxy-terminal tail of myosin A and the myosin A tail domain-interacting protein (MTIP) of the parasite. This interaction is required for erythrocyte invasion[86] and is thus essential for the parasite life cycle. Potential inhibitors were identified using an algorithm that computationally docked 300,000 possible inhibitors to the crystal structure of MTIP. After ranking, 15 promising molecules with the appropriate

drug-like properties were procured and subsequently tested for their ability to block parasite proliferation. Further optimization of a urea–pyrazole scaffold has yielded a molecule, known as 21A092, that has advanced to preclinical studies (MMV research and development). However, given the high rate of positive results that random compounds show in cellular proliferation screens, it is possible that the parasiticidal activity of this class is a result of it binding to another cellular target.

New target discovery. It is probable that several new chemically validated targets will be available for target-based screening studies in the future. These are the proteins that were determined as targets of the promising compounds identified in the aforementioned cellular screens. Although discovering how a compound functions in the cell has traditionally been very challenging in malaria parasites, targets can now be identified by creating parasites that are resistant to a compound, and then comparing the genomes of resistant progeny clones to the genomes of their sensitive parent clones[41,87]. This method has led to the identification of several novel[24,88,89] targets and the confirmation of previously known targets[90,91]. Interestingly, the protein biosynthetic pathway seems to be a rich source of novel, chemically validated targets: three tRNA synthetases have been identified as targets of antimalarial compounds[88,89,92], and some of these compounds (for example, the natural products cladosporin and halofuginone) have been shown to be active against multiple stages of the parasite life cycle[88,93]. It is interesting to note that several of the targets that have been discovered using this approach (for example, PfAtp4, PfCarl and lysyl tRNA synthetase) were not on the lists of the most desirable targets predicted by structure-based druggability and essentiality[94], and that few proteins that were hypothesized to be good targets have yielded compounds that have progressed into development, with the exception of the well-known antimalarial targets DHOD, DHFR and cytochrome *bc1* (see above). Although many hypothetical targets seem to be attractive from a structural biology perspective, it is possible that some of these proteins are simply too abundant in the cell and thus the inhibition of all cellular copies is difficult to achieve at physiologically relevant inhibitor concentrations.

Other approaches to new medicines

Two of the most effective antimalarial drugs, artemisinin and quinine, are natural products derived from traditional herbal remedies that are used to treat fevers. A third antimalarial drug, atovaquone, is a synthetic version of the natural product lapichol[95]. There are other fever-reducing folk remedies that could yield a next-generation antimalarial drug; for example, the bark of the plant *Nauclea pobeguinii*, from the Democratic Republic of the Congo, can substantially reduce parasitaemia in mice that are infected with rodent malaria[96] and has been shown to be effective in Phase IIb clinical trials[97]. It is interesting to note that the chemical structure of a possible active ingredient, strictosamide, is similar to that of the spiroindolones[98]. A second natural product from the *Argemone mexicana* plant was also shown to be effective in a Phase II clinical trial[99].

The formulation of a drug, for example, in a pill or as a liquid suspension, can affect the usefulness of the drug. Thus, efforts have been made to reformulate traditional antimalarial compounds, including antibiotics; for example, a combined artemether and lumefantrine tablet works well in adults but may be rejected by children because of the bitter taste. Thus, sweet-tasting liquid formulations need to be designed for children[100]. In addition, antimalarial drugs cannot ethically be given as monotherapies because of the risk of resistance emerging, so the choice of partner drugs must be carefully considered and is often determined by how long and when a compound is active and whether parasite resistance is likely to develop. More effective medicines can thus be created by the combination of new antimalarial compounds or by the novel combination of traditional approved drugs. Multiple novel combination therapies, which include pyronaridine–artesunate[101], azithromycin–chloroquine[102], dihydroartemisinin–piperaquine[103] and sulphadoxine–pyrimethamine with amodiaquine (SP+AQ)[104], are in clinical trials. It is possible that a SERCaP medicine could be created by combining three different molecules that are independently active against blood, liver and transmission stages.

Future perspectives

Malaria elimination will ultimately require an integrated strategy that includes new and old drugs, vaccines, vector control and public health measures. Although the task seems daunting, new scientific discoveries could rapidly change the outlook; for example, vector control strategies have traditionally depended on pesticides (for example, indoor residual spraying and insecticide-treated nets) and, although these interventions decrease the number of malaria infections, they are insufficient to eliminate the disease in endemic regions[105]. New vector control strategies, such as genetically modified mosquitoes and, more recently, the colonization of mosquitoes with *Wolbachia* spp. (which renders them refractory to *Plasmodium* spp. infection[106]) could help with elimination. An effective vaccine would also be very helpful; however, there is currently no vaccine available, and the most developed vaccine (RTS,S) showed disappointing preliminary results in Phase III trials, which reported a 16.8% vaccine efficacy that declined to 0% over 4 years[107,108]. Although these trials provided a proof-of-principle for blood-stage vaccines, second generation vaccines that have greater efficacy are urgently needed. Pre-erythrocytic vaccines, attenuated parasites and multisubunit vaccines are all being investigated[24].

Even though elimination could potentially be accomplished with the range of drugs that are currently available, it might be less costly if more effective drugs that interrupt transmission were available. Although target-based drug discovery could be a useful approach to find a SERCaP drug, it should be noted that currently known targets fall short of the SERCaP requirements. Furthermore, cellular screens that could lead to the identification of small molecules with SERCaP activity are

difficult to implement and are not available to the average researcher, nor can they be routinely used during lead optimization steps. One source of problems is that the organisms (such as *P. vivax* sporozoites) or the specialized assays that are used for testing are often located thousands of miles away from the chemical libraries and sophisticated screening equipment. Nevertheless, the recent progress suggests that a SERCaP drug could realistically be developed, especially now that funding agencies have made this a priority. Although distinct challenges might be associated with the elimination of metabolically quiescent hypnozoites and very late-stage gametocytes, targets that are central to all stages of the life cycle could nevertheless exist.

Considering the high mortality and morbidity caused by malaria, there is no question that new drugs are needed. It is an exciting time for malaria drug discovery; the combination of new and innovative screens to identify compounds with broad-range activity is hoped to yield new insights into proteins that are essential in all parasite stages. With the support of various funding agencies the time is optimal to take advantage of these opportunities and to discover drugs that will lead the way to the global eradication of malaria.

Note added in proof

Recently, it was shown that the parasite's enzyme phosphoethanolamine methyltransferase is necessary for gametocyte development and subsequent transmission, which makes it a potential target for further drug development[133]. Furthermore, torins, which affect protein trafficking and thus have a novel mechanism of antimalarial action, are active against both blood- and liver-stage parasites[134].

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Competing interests statement
The authors declare competing interests: see Web version for details.

DATABASES
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Medicines for Malaria Venture research and development: http://www.mmv.org/research-development/rd-portfolio
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ERRATUM

Antimalarial drug discovery — approaches and progress towards new medicines

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In the above article, the structure of DSM265 in figure 3 was missing a methyl group. This has now been corrected in the PDF and online. We apologize to readers for any confusion caused.