REVIEW

The Past, Present & Future of Antimalarial Medicines

Edwin G Tse[1†], Marat Korsik[1†] and Matthew H Todd[1,2*]

Abstract

Great progress has been made in recent years to reduce the high level of suffering caused by malaria worldwide. Notably, the use of insecticide-treated mosquito nets for malaria prevention and the use of artemisinin-based combination therapies (ACTs) for malaria treatment have made a significant impact. Nevertheless, the development of resistance to our past and present antimalarial drugs highlights the need for continued research to stay one step ahead. New drugs are needed, particularly those with new mechanisms of action. Here we review the range of antimalarial medicines developed across the years, beginning with the discovery of quinine in the early 1800s, through to modern day ACTs. We finally outline a number of new potential antimalarial drugs currently in development.

Keywords: Malaria; *Plasmodium*; Mechanism of action; Drug discovery; Drug development

Background

In 2017, the World Health Organization (WHO) estimated that there were 219 million cases of malaria worldwide, an increase of 2 million from the previous year, and as a result there were 435 thousand deaths, or 1190 per day, mostly young children.[1] Encouragingly, since 2000, these figures have decreased by about 37% worldwide, but a number of recent reports have shown that this level is slowly plateauing, emphasising that we must not become complacent with the current treatment and prevention strategies.[2, 3] There are five strains of the *Plasmodium* parasite, with *P. falciparum* being the most prevalent in Africa and *P. vivax* being the most prevalent in countries outside Africa. Almost half the world's population is at risk of contracting malaria, with Africa having the biggest share of cases and deaths of any continent (∼90%).

Over the past few years, a number of reviews have been published which evaluate the potential future of antimalarial drugs. Of note: Triple-antimalarial drug combinations were examined in 2014;[4] a review on the numerous strategies currently used in antimalarial drug discovery was published in early 2017,[5] and an in-depth primer on all aspects of malaria was published in late 2017.[6] In early 2018, a review was published highlighting the discovery and development of a number of new antimalarial drug candidates.[7, 8]

This review aims to summarise the past, present and future of compounds used to treat malaria. There is a focus on projects supported by the Medicines for Malaria Venture (MMV), a non-governmental organisation that maintains a website[9] highlighting collaborations from the very early stages of drug discovery and lead optimisation (e.g. the Open Source Malaria (OSM) project with which we are involved[10]), to the progression of lead compounds through clinical trials (e.g. Cipargamin, which has been developed by Novartis[11]) and all the way to the final stages of bringing a drug to market (e.g. Artesunate for injection, developed by Guilin Pharmaceutical). After a brief survey of past medicines, and those that are currently being used, we will focus on compounds that are currently in development, and in particular the lead optimisation campaigns of each. Since it is such a crucial component of modern antimalarial drug discovery, we will end with a survey of the most promising mechanisms of action of those compounds in development.

Past

Since the isolation in 1820 of quinine, the first chemically purified effective treatment for malaria, a number of other natural and synthetic compounds have been developed (Figure 1). However, as time passed, strains of the parasite began to show signs of resistance towards these drugs, rendering them less effective. Ac-

*Correspondence: matthew.todd@ucl.ac.uk
[1]School of Chemistry, The University of Sydney, Sydney, Australia, NSW, 2006
[2]School of Pharmacy, University College London, London, United Kingdom, WC1N 1AX
Full list of author information is available at the end of the article
[†]Equal contributor

cordingly, their use has ceased or is restricted to particular situations.

Figure 1 Well known antimalarial medicines discovered between 1820 and the 1980s. Some are still used today while some have been rendered ineffective due to development of resistant strains or the emergence of undesirable side effects. Dates of first reported resistance are shown in brackets.

Quinine

First isolated from the bark of the cinchona tree in 1820, quinine has been used as one of the most effective treatments for malaria to date.[12] Resistance was first reported in the 1980s[13] and as of 2006, quinine is no longer used as a front-line treatment for malaria but is still on the WHO's Model List of Essential Medicines (MLEM)[14] for the treatment of severe malaria in cases where artemisinins are not available.

Mepacrine

Mepacrine (a.k.a quinacrine) was predominantly used throughout the Second World War as a prophylatic, sold under the trade name Atabrine.[15] It is no longer used today due to the high chance of undesirable side effects such as toxic psychosis.[16]

Chloroquine

During the 1940s, chloroquine (CQ)was used to treat all forms of malaria with few side effects.[17] Resistance to CQ was first reported in the 1950s and over the years many strains of malaria have developed resistance. Indeed, resistant strains (K1, 7GB, W2, Dd2, etc.) of the malaria parasite are now used in potency evaluation assays as a way of showing efficacy.[18] Chloroquine is on the MLEM for the treatment of *P. vivax* in regions where resistance has not developed.[14]

Mefloquine

Mefloquine was developed in the 1970s by the United States Army[19] and is still used today, also being one of the medicines on the MLEM. Originally introduced for the treatment of chloroquine-resistant malaria, it has been used as both a curative and a prophylactic drug. Resistance was first reported in 1986.[20]. It is thought that the structurally related quinoline drugs (such as quinine, mepacrine, chloroquine and mefloquine) act through the disruption of haemoglobin digestion in the blood stage of the parasite.[21] These drugs are commonly used in combination with a complementary drug (e.g. melfoquine and artesunate, sold as Artequin^{TM}) to reduce the chance of resistance development to the quinoline family of compounds. Mefloquine is commonly sold in its racemic form under the brand name Lariam®, however it is no longer widely used due to the perception of central nervous system toxicity that has been suggested to affect a large number of its users.[22]

Halofantrine

Developed between the 1960s and 1970s by the Walter Reed Army Institute of Research,[23] halofantrine was initially used for treatment against all forms of the *Plasmodium* parasite. Its use has diminished over time due to a number of undesirable side effects, such as the potential for high levels of cardiotoxicity. It is only used as a curative drug and not for prophylaxis due to the high toxicity risks and its unreliable pharmacological properties. Halofantrine is still used today, under the brand name Halfan^{TM}, but only in cases where patients are known to be free of heart disease and where infection is due to severe and resistant forms of malaria.[24]

Present

On the WHO Model List of Essential Medicines,[14] there are currently listed 14 medicines for curative treatment of malaria and 4 medicines for prophylactic treatment, with the treatments formulated either as single compounds or as combinations. Perhaps the most effective of these are the artemisinin-based combination therapies (ACTs) which use an artemisinin derivative (short-acting) in combination with one or more complementary compounds (long-acting and possessing different mechanisms of action).

Artemisinin & its derivatives

Artemisinin was first isolated in 1971 by Tu Youyou from the plant *Artemisia annua*, a herb that has commonly been used in Chinese traditional medicine.[25] Due to the great positive impact of artemisinin in combating malaria, Youyou was awarded the joint

Nobel Prize in Physiology or Medicine in 2015 for "*her discoveries concerning a novel therapy against malaria*".[26] Artemisinin has been shown to be efficacious against all multi-drug resistant forms of *P. falciparum*. The most common derivatives of artemisinin are artemether, artesunate and arteether. These semi-synthetic derivatives are prodrugs which are transformed to the active metabolite, dihydroartemisinin (Figure 2). The use of artemisinins has been integral in the fight against malaria with ACTs making up the majority of modern day treatments.[27] Although slow to develop, the first report of resistance to artemisinin was in western Cambodia in 2008.[28] Ten years later, in February of 2018, a report was published identifying more than 30 independent cases of artemisinin resistance in southeast Asia, specifically with resistance to the dihydroartemisinin–piperaquine combination therapy.[29]

Figure 2 Artemisinin and its semi-synthetic derivatives artemether, arteether and artesunate. Once in the body, these compounds are converted to the active metabolite dihydroartemisinin.

The mechanism of action (MoA) through which artemisinin acts has been widely debated.[30] The most accepted theory is that the molecule is activated by haem to generate free radicals, which in turn damage proteins required for parasite survival.[31, 32] Still, evidence for a number of other possible mechanisms have been found. In 2013, a computational approach was taken to determine the MoA based around previous studies which identified haem and *Pf*ATP6 (Ca^{2+} transporter) as potential MoAs.[33] More recently in 2015, artemisinin was shown to be associated with the up-regulation of the unfolded protein response (UPR) pathways which may be linked to decreased parasite development.[34] Another study showed that artemisinin was is a potent inhibitor of *P. falciparum* phosphatidylinositol-3-kinase (*Pf*PI3K).[35]

MMV have been involved in progressing eight new antimalarial treatments based on different formulations/combinations of approved drugs, as follows (Figure 3). All of these compounds are on the MLEM as combination therapies.[14]

Amodiaquine

Amodiaquine was first synthesised in 1948.[36] It is mainly used for the treatment of uncomplicated *P. falciparum* malaria when used in combination with artesunate and is commonly sold under the trade name Camoquine® or Coarsucam™.[37] Similar to chloroquine, amodiaquine's MoA is thought to involve complexation with haem and inhibition of haemozoin formation.[38]

Piperaquine

Piperaquine was developed in the 1960s as a part of the Chinese National Malaria Elimination Programme.[39] Initially used throughout China as a replacement for chloroquine, resistance led to its diminished use as a monotherapy. While the MoA of piperaquine is not completely understood, studies have suggested that it acts by accumulating in the digestive vacuole and inhibiting haem detoxification through the binding of haem-containing species.[27, 40] These days, piperaquine is used as a partner drug with dihydroartemisinin (commonly sold under the trade name Eurartesim®).

Lumefantrine

Lumefantrine (a.k.a. benflumetol) was first synthesised in 1976 as a part of the Chinese antimalarial research effort "Project 523" which also resulted in the discovery of artemisinin.[41] It is currently sold under the trade name Coartem®. The exact MoA of lumefantrine is unknown, however studies suggest that it inhibits nucleic acid and protein synthesis through the inhibition of β-haematin formation by complexation with haemin.[38] Lumefantrine is currently used only in combination with artemether.

Figure 3 New drug combinations/formulations that have been approved for use. Brand name of the drug (in bold), partnered company (in italics) and drug combination (colour-coded to the structures) are listed.

Figure 4 Snapshot of the projects supported by MMV at different stages of the drug discovery and development pipeline. (Adapted from the MMV-supported projects webpage)[42]

Proguanil & Atovaquone

Proguanil was first reported in 1945 as one of the first antifolate antimalarial drugs,[43]while atovaquone was first reported in 1991 for the treatment of protozoan infections.[44] The combination of these, commonly sold as Malarone^{TM}, has been marketed by GlaxoSmithKline (GSK) since the early 2000s, and has proven to be a very effective antimalarial due to the synergistic effect of the two components. This is, in large part, due to the different MoAs for each compound. Atovaquone acts as a cytochrome bc_1 complex inhibitor which blocks mitochondrial electron transport.[45] Proguanil (when used alone) acts as a dihydrofolate reductase (DHFR) inhibitor through its metabolite, cycloguanil (CG) which disrupts deoxythymidylate synthesis. When used in combination with atovaquone however, proguanil does not act as a DHFR inhibitor but has instead been shown to reduce the concentration of atovaquone required for treatment.[46] Generic atovaquone/proguanil is still available today for the treatment of chloroquine-resistant malaria.

Pyrimethamine & Sulfadoxine

Pyrimethamine (PYR) was developed in the early 1950s by Gertrude Elion and George Hitchings and is now sold under the trade name Daraprim^{TM}.[47] The development of pyrimethamine was a part of the efforts that won Elion, Hitchings and Black the joint Nobel Prize in Physiology or Medicine in 1988 for "*their discoveries of important principles for drug treatment*".[48] Sulfadoxine was developed in the early 1960s.[49] It is no longer used as a preventative drug due to high levels of resistance. The combination of pyrimethamine and sulfadoxine was approved for use for the treatment of malaria in 1981 and is now commonly sold under the trade name Fansidar®. Both drugs are known to target the parasite folate biosynthesis pathway.[50] Pyrimethamine inhibits dihydrofolate reductase, while sulfadoxine inhibits dihydropteroate synthetase.

Pyronaridine

Pyronaridine was first synthesised in the 1970s at the Institute of Chinese Parasitic Disease.[51, 52] It has been found to be efficacious against chloroquine resistant strains and has been in use for over 40 years, sold under the trade name Pyramax® (in combination with artesunate). Like lumefantrine, pyronaridine has been found to act through the inhibition of β-haematin formation.[53]

Tafenoquine

First discovered in 1978 at the Walter Reed Army Institute of Research, Tafenoquine (TQ) was recently approved by the United States Food and Drug Administration for use as the first new single-dose for the radical treatment of *P. vivax* malaria in over 60 years.[54] TQ is thought to be a prodrug which is metabolised to the active quinone TQ, however the MoA is not well known.[55] It is currently sold under the brand name Krintafel.

Future

Since its establishment in 1999, the Medicines for Malaria Venture has been frontlining the discovery and development of new medicines for the treatment of malaria. The potential for these compounds to act as new antimalarials is judged by a number of requirements: novel modes of action with no cross-resistance to current drugs; single-dose cures (artesunate and chloroquine are unable to do this); activity against both the asexual blood stages that cause disease and the gametocytes responsible for transmission; compounds that prevent infection (chemoprotective agents); and compounds that clear *Plasmodium vivax* hypnozoites from the liver (anti-relapse agents).[56, 57] MMV has been aiding the fight against malaria by partnering with universities and pharmaceutical companies around the world to bring new antimalarials to market (Figure 4).

Besides the traditional drug discovery and development methods for the identification of new antimalarials that will be described below, there are a number of other ways in which a new antimalarial drug may be discovered. One way, as previously mentioned, is through the exploration of new combinations and formulations of current antimalarial drugs. This may help overcome issues with resistance to a particular component or may assist in the delivery of the drug allowing it to be more effective. Alternatively, existing drugs used for other purposes may be found efficacious against malaria and subsequently be repurposed as a new antimalarial treatment. This can be advantageous since these compounds may have already shown good biological properties and may also reveal novel MoAs. Examples include those shown in Figure 5:

- Methylene blue, a drug for the treatment of methemoglobinemia. Last completed Phase II trials in 2017 (NCT02851108) as a combination with primaquine.
- Fosmidomycin, an antibiotic. Most recently in Phase II trials in 2015 (NCT02198807) as a combination with piperaquine.
- Rosiglitazone, an antidiabetic drug. Currently in clinical trials as an adjunctive therapy for severe malaria (NCT02694874).
- Imatinib, a cancer therapy drug. Currently in Phase II trials (NCT03697668) as a triple combination with dihydroartemisinin-piperaquine.

- Sevuparin, a drug for the treatment of sickle cell disease. Last in Phase I/II trials in 2014 (NCT01442168) as a combination with atovaquone-proguanil.

Figure 5 New antimalarial compounds in development as a result of drug repurposing. Original indications for the drugs are shown in parentheses. Many of these repurposed drugs are already in Phase II trials as new potential antimalarials.

The following seven compounds were discovered and developed with the hope of progressing into clinical trials as potential new antimalarial candidates (Figure 6). However, over the past two years, progress in the development of these compounds has slowed, making the fate of these drug candidates less clear.

The chiral 8-aminoquinoline derivative, **NPC1161B** was developed at the University of Mississippi and was still in preclinical studies in 2014.[58–61] **MK4815** was developed in 2012 at Merck but is still in preclinical studies due to safety issues.[62] **CDRI 97/78** is a fast-acting novel trioxane antimalarial first synthesised in 2001 by a team at the Council of Scientific and Industrial Research in India.[63] Having passed all preclinical studies, it was last seen to have completed first-in-human Phase I trials in 2014.[64] Developed at the Liverpool School of Tropical Medecine in 2009, ***N-tert* butyl isoquine/GSK369796** was designed as an alternative to amodiaquine.[65, 66]. It completed preclinical studies,[67] and was last in Phase I trials in 2008 (NCT00675064). **Artemisone** is a second-generation semi-synthetic artemisinin derivative developed at the Hong Kong University of Science and Technology, that has previously been shown to be as efficacious as artesunate, with no toxicity and a comparably low cost of production.[68] It was withdrawn from Phase II/III trials in 2010 (NCT00936767). The bisthiazolium salt, **SAR97276**, was discovered and developed by Sanofi in 2005,[69] however further investigation was terminated in 2012 after Phase II trials (NCT01445938). **AQ-13** is a chloroquine derivative that was first described in 1946.[70] While only differing to CQ in the amine side-chain, this difference has been linked to its increased efficacy against CQ-resistant strains.[71] It has a MoA and pharmacokinetic properties similar to that of CQ.[72, 73] **AQ-13** last completed Phase II trial at the end of 2017 (NCT01614964),[74] however there has been no mention of any following active trials so the ongoing status of this compound is unclear.

Figure 6 New antimalarial drug candidates that have reported no significant progress in the last two years. These compounds may have encountered issues during preclinical or early clinical studies.

The compounds in the remainder of this section are currently still actively being pursued. For ease of visualisation throughout in this section, a graphical summary of each compound structure and its code will be displayed, along with key physical and biological properties identified during the hit to lead campaigns.

Preclinical

Once a lead compound has been identified, optimisation of the structure can begin. This largely involves investigation into the structure activity relationship (SAR) of the drug, optimising for properties such as potency (*both in vitro* and *in vivo*), solubility and metabolic stability. The candidate must also be assessed for any possible toxicity (e.g. dosing, cytotoxicity/genotoxicity levels, etc.).

M5717 (Figure 7) was developed in 2015 by a team led by the Drug Discovery Unit (DDU) in Dundee and

was shown to have potent activity against multiple stages of the *Plasmodium* parasite *via* a novel mechanism of action.[75, 76]

Figure 7 Key biological and physical properties of M5717.

Initial phenotypic screening of the Dundee protein kinase scaffold library against the 3D7 multi-drug-resistant *P. falciparum* strain identified a compound (**M1**, Figure 8) that possessed high potency against the parasite, albeit with poor physicochemical properties. Optimisation of this structure (via **M2** and **M3**) led to improvements across the board (**M5717**).

Figure 8 Key stages in the hit to lead pathway of M5717. Initial replacement of Br for F, replacement of pyridine with ethylpyrrolidine, and addition of a morpholine fragment led to the optimised compound **M5717**.

In addition to the nanomolar activity against the 3D7 strain, **M5717** has shown almost equal potency against a number of other drug-resistant strains (K1, W2, 7G8, TM89C2A, D6 and V1/S) as well as similar potencies across multiple life cycle stages (liver schizonts, gametocytes and ookinetes).

M5717 was found to be as effective as current antimalarial drugs (chloroquine, mefloquine, artemether, dihydroartemisinin and artesunate) when evaluated in the *in vivo P. berghei* mouse model for single-dose efficacy showing >99% reduction in parasitemia at doses of 4 × 30 mg/kg *p.o. q.d.* and an ED_{90} of 0.1-0.3 mg/kg.

Due to its novel MoA (*Pf*eEF2 inhibition, *vide infra*) and its ability to clear blood-stage parasites completely, **M5717** satisfies the requirements to be a long duration partner and could be used as part of a combination therapy with a fast-acting compound.[77] Additionally, the compound has shown the ability to act as a transmission-blocking drug (stage IV–V) and also to be effective for chemoprotection.

In late 2017, **M5717** was cleared for progression from development to phase I clinical trials for volunteers in Australia (NCT03261401).

Identified by AstraZeneca in 2015, **MMV253** (Figure 9) is a novel triaminopyrimidine (TAP) that has shown good *in vitro* potency and *in vivo* efficacy, and acts through another novel MoA.[78]

Figure 9 Key biological and physical properties of MMV253. logD and *in vivo* ED90 kindly provided by V. Sambandamurthy, S. Hameed P. and S. Kavanagh, personal communication, 2018.

High-throughput screening of 500,000 compounds from AstraZeneca's library against blood stage *P. falciparum* resulted in the identification of a promising series of TAPs. The initial hit (**M'1**, Figure 10) suffered from hERG inhibition and poor solubility which, through lead optimisation, was improved upon to give a compound that possessed high potency and desirable pharmacokinetic properties (**MMV253**).

When screened against numerous mutant resistant strains with various mechanisms of resistance, **MMV253** showed no spontaneous reduction in potency which can be attributed to its novel MoA (*Pf*ATP4 inhibition, *vide infra*). Good *in vitro-in vivo* correlation (IVIVC) was shown with a predicted human half-life of ∼36 hours (which is long compared to another fast-killing drug, artemisinin, which has a human half-life of 1 hour).

Figure 10 Key stages in the hit to lead pathway of MMV253. Initial replacement of ethylbenzene on **M'1** with 2-methylpyridine resulted in lower hERG affinity and improved solubility. Substitution of the pyrrolidine in **M'2** with an imidazole containing an amine spacer further improved solubility and greatly improved the potency. Addition of an *N*-methyl group and a cyclopropane moiety led to the optimised compound **MMV253**.

As of late 2016, the pharmaceutical company Cadila Healthcare owns the license for the compound series and is now doing further lead development in order to progress the drug through preclinical trials.[79]

UCT943 (Figure 11) was identified in 2016 by a team at the University of Cape Town, South Africa and is a key compound in a novel class of 2-aminopyrazine antimalarials that has shown single dose curing ability *in vivo* and potential as a clinical candidate.[80]

Figure 11 Key biological and physical properties of UCT943.

The original 3,5-diaryl-2-aminopyridine series was identified from a high-throughput screen of 36608 compounds from the commercially available SoftFocus kinase library.[81] The initial hit (**U1**, Figure 12) showed promising *in vitro* activity against the drug-sensitive NF54 strain (IC_{50} = 49 nM) but suffered from poor solubility and high metabolic clearance. To address the poor measured *in vivo* stability, the labile hydroxy and methoxy groups were replaced by a single trifluoromethyl group (**U2**), but this change resulted in a significant loss of solubility. Significant improvements in solubility and potency were obtained by first replacing the mesyl group with a piperazine carboxamide group (**U3**) and subsequently introducing another nitrogen atom into the pyridine ring (**UCT943**).[82, 83]

Figure 12 Key stages in the hit to lead pathway of UCT943. Initial change the phenyl substituents with a single trifluoromethyl group led to greater *in vivo* stability. Introduction of piperazine amide instead of methylsulfonyl and a pyrazine instead of a pyridine led to the improved solubility and potency of the optimised compound. Surprisingly, introduction of a nitrogen in the red circle resulted in complete inactivity *in vivo*.

In the *P. berghei* mouse model, **UCT943** has shown the ability to cure malaria at doses of 4 × 10 mg/kg and has an ED_{90} of 1 mg/kg. Interestingly, another closely related molecule (with a nitrogen instead of a carbon in the red circle) which was evaluated during the SAR study, displayed similar *in vitro* antimalarial activity (IC_{50} = 9.1 nM) and solubility (198 μM) but showed a complete lack of *in vivo* activity with a <40% reduction in parasitemia using a comparable dosage.

UCT943 is potent across multiple parasite life stages of both *P. falciparum* and *P. vivax*. Its target is *Plasmodium* phosphatidylinositol-4-OH kinase (*Pf*PI4K), which has also been implicated as the target for MMV048 (*vide infra*).[84] **UCT943** was in originally in place as a back-up to MMV048, however, due to preclinical toxicity, this candidate has been withdrawn.

From a discovery process by Anacor Pharmaceuticals that began in 2010 with a novel class of benzoxaborole antimalarial compounds,[85] **AN13762** (Fig-

ure 13) emerged in 2017 as the lead compound, showing excellent activity *in vitro* and *in vivo*, multi-strain efficacy and the ability to perform as a rapid-acting drug.[86]

Figure 13 Key biological and physical properties of AN13762 Solubility kindly provided by Y.-K. Zhang, personal communication, 2018.

By screening a library of boron-containing compounds (previously shown to have selective activity against fungi, bacteria, parasites and inflammation) in a whole cell assay against *P. falciparum*, the initial hit compound (**AN3661**, Figure 14) was identified to have potent *in vitro* activity against the 3D7 strain (IC_{50} = 26 nM). Further SAR and optimisation studies led to the development of **A1** in which the alkylcarboxylic acid chain was moved and replaced by a substituted pyrazine ether. This compound showed greater potency but still suffered from high metabolic clearance.[87] Replacement of the ester group with an amide group (**A2**) led to improved metabolic stability and bioavailability, with a significant decrease in potency. Modification of the primary amide to a cyclic tertiary amide, and introduction of a methyl group on the benzoxaborole gave the lead compound **AN13762** which possessed improved potency and metabolic stability.[86]

AN13762 has been shown to be equally potent across a wide range of drug resistant strains. The drug has displayed similar *in vivo* clearance rates when compared to artesunate. There is no inherent genotoxicity (as shown in Ames assays and *in vivo* rat micronucleus studies), and no cytotoxicity at concentrations up to 100 μM in human cell lines.[86]

The precise mechanism of action for **AN13762** remains unknown, though initial MoA studies on hit compound **AN3661** identified the *Pf* cleavage and polyadenylation specificity factor (*Pf*CPSF3) as a potential target.[88] **AN13762** has proceeded into the preclinical phase, with first-in-human studies planned for 2019.[42]

Developed in 2017 by a team at Heidelberg University, **SC83288** (Figure 15) is an amicarbalide derivative that possesses a novel chemotype for current antimalarials, may have a potentially new MoA and has shown the ability to be a fast-acting drug for the intravenous treatment of severe malaria.[89]

Figure 14 Key stages in the hit to lead pathway of AN13762. Initial replacement of the carboxylic acid chain with a pyrazine, and subsequent switch of the ester to a substituted amide helped to improve *in vivo* stability and bioavailability leading to the optimised compound.

Figure 15 Key biological and physical properties of SC83288. *in vivo* ED90 kindly provided by M. Lanzer, personal communication, 2018.

An *in silico* screen of a library of small molecule compounds for their ability to dock into *P. falciparum* lactate dehydrogenase led to the identification of amicarbalide (**S1**, Figure 16), which was found to be highly potent (IC_{50} = 10 nM) against the Dd2 strain.[90] In order to overcome a potential DNA binding effect, an amidino group was replaced with a sulfonamide linker leading to **S2** which possessed better solubility and improved metabolic stability. Further modification of the other amidine group with a substituted piperazine ring (**S3**) led to improved potency and water solubility, but the compound suffered from poor permeability. Ultimately, replacement of the butyl chain with an

acetyl group led to the highly potent lead compound **SC83288**.

Figure 16 Key compounds in the discovery of SC83288. Initial modification of one amidino group with a sulfonamide linker (**S2**) resulted in improved solubility. Further modification of the remaining amidine group with substituted piperazine moieties ultimately led to the optimised compound with good solubility, permeability and high potency.

SC83288 has been shown to be potent against a number of drug-resistant strains at IC_{50} values of <20 nM and is also efficacious against early stage (I-III) gametocytes (IC_{50} = 199 nM) but not against late stage gametocytes (IV and V). It has an excellent safety profile, with no cytotoxicity, genotoxicity or hERG binding. In the *P. vinckei* rodent malaria model, **SC83288** was able to fully cure the infection at a dose of 4 × 20 mg/kg i.p. *q.d.* with no resurgence of infection. It is however, completely inactive against *P. berghei*.

While the exact MoA of SC83288 is unknown, the generation of resistant clones has identified *Pf*ATP6 as a possibly relevant target. However, it has been shown that SC83288 does not directly inhibit this target suggesting *Pf*ATP6 may have a less direct role in the mechanism of **SC83288**. *Pf*MDR2 has been identified as another possible mechanism of resistance, facilitating the clearance of the drug from the parasite. **SC83288** has been evaluated against artemisinins, showing no cross resistance and highlighting its potential as an alternative to artesunate for the treatment of severe malaria when combined with a slow-acting partner drug.[91]

Discovered in 2010 by a team at Portland State University and further developed by DesignMedix, **DM1157** (Figure 17) is part of a class of compounds known as "reversed chloroquines" (RCQs), designed to overcome chloroquine-resistant and chloroquine-sensitive strains of the malaria parasite. The compound has been shown to be efficacious *in vitro* and *in vivo*.[92]

Figure 17 Key biological and physical properties of DM1157. CLint HLM and *in vivo* ED90 kindly provided by D. Peyton, personal communication, 2018.

CQ resistance is known to be linked to the *P. falciparum* chloroquine resistance transporter (*Pf*CRT): mutations to this target facilitate the expulsion of CQ from the parasite. A class of molecules has been identified, so-called "reversal agents", that can inhibit *Pf*CRT, thus slowing the exportation of CQ from the parasite. By combining the chloroquinoline core of CQ (**D1**, Figure 18) with the known reversal agent, imipramine, the first RCQ (**D2**) was designed, but this molecule suffered from poor bioavailability and metabolic stability.[93] Subsequent SAR studies resulted in the substitution of the imipramine motif with a 1-(2,2-diphenylethyl)piperazine moiety which led to a compound (**D3**) that was more stable to metabolic cleavage, but suffered from a high cLogP. In order to overcome this, the two phenyl groups were replaced with pyridines and the piperazine replaced with an aminopiperadine resulting in the lead compound, **DM1157** that possessed a lower cLogP value (3.6) while still maintaining high potency against both CQ-resistant (Dd2 = 1.6 nM) and CQ sensitive strains (D6 = 0.9 nM) when compared to CQ (cLogP = 5.1, Dd2 = 102 nM, D6 = 6.9 nM).

In a *P. berghei* rodent model, **DM1157** showed good efficacy both orally and subcutaneously. Most notably, a >99.9% reduction in parasitemia was seen at an oral dose of 4 × 30 mg/kg with 2/3 mice cured 30 days post infection. **DM1157** has also been evaluated against *P. falciparum* and *P. vivax* multi-drug resistant field isolates in Indonesia and was found to be 3-fold more potent than CQ in both species.[94]

CQ is known to bind to heme and inhibit β-hemozoin formation. **DM1157** (and other RCQ compounds) have been shown also to act in this manner, but with much higher levels of inhibition of β-hemozoin both

Figure 18 Key compounds in the discovery of DM1157. Initial combination of the reversal agent, imipramine, with the CQ core resulted in the potent RCQ compound **D2**. Subsequent replacement of the reversal agent with 1-(2,2-diphenylethyl)piperazine (**D3**), and further modification with pyridine rings led to improved potency and cLogP values for the optimised compound.

in vitro and *in vivo*. **DM1157** is currently in Phase I trials to evaluate its safety and pharmacokinetics in humans (NCT03490162).

Translational (Human volunteers)

Upon the completion of preclinical trials, the drug will have either passed or failed the required safety standards and pharmacokinetic profiling. For a compound that has passed these requirements, trials may then be conducted in human volunteers in order to show its efficacy as a potential treatment.

P218 (Figure 19), discovered by BIOTEC Thailand in 2012, is an antifolate antimalarial drug bearing resemblance to the 2,4-diaminopyrimidine core structure of PYR, and is highly selective for the *P. falciparum* dihydrofolate reductase (*Pf*DHFR).[95]

Figure 19 Key biological and physical properties of P218.

Unlike the typical high-throughput screening that is used for the identification of hit compounds in medicinal chemistry, **P218** was identified through careful examination of the cocrystal structures of known *Pf*DHFR inhibitors and their substrates. The initial observation that 2,4-diaminopyrimidines acted as antagonists to folic acid led to the discovery and development of methotrexate (**MTX**) as an antitumor drug (Figure 20). By examining the binding interactions with DHFR, the structure of **P65** was identified. A total of over 200 compounds (not described in the original report) were designed and synthesised after further examination of potential interactions with amino acid residues, with the optimised structure of **P218** being identified as the best compound.

Figure 20 Key compounds in the discovery of P218. The key 2,4-diaminopyrimidine core highlighted in red can be found in a number of DHFR inhibitors.

The 2,4-diaminopyrimidine scaffold of **P218** has been found to bind deep in the active site of *Pf*DHFR in both wild-type and mutant strains. This, along with the hydrogen bonding interaction of the carboxylate group with an Arg residue at the opposite end of the active site results in tighter binding and a longer residence time when compared to **PYR**. Since **P218** is contained almost entirely within the dihydrofolate binding site, the strength of the binding should be strong enough to overcome any amino acid mutations, thus minimising the chance of drug-resistant mutations to arise. The novel two-step mechanism of action for binding to *Pf*DHFR (*vide infra*) allows **P218** to overcome resistance that has emerged from the use of pyrimethamine. P218 has also shown high selectivity to the binding of malarial over human DHFR, which translates into reduced toxicity.

In vivo studies have shown **P218** to be highly efficacious against *P. falciparum* and *P. chabaudi* in mice with ED_{90} values of 1 mg/kg and 0.75 mg/kg respectively. In the *in vitro* and *in vivo* potency assays that were run, **P218** was found to be more potent than PYR in all cases.

Along with its high potency and good safety profile, **P218** has the potential to be a replacement for

PYR combination with **CG** in areas where *Pf*DHFR resistance has emerged. P218 has currently completed phase I trials (NCT02885506).

Discovered by a partnership between St Jude Children's Research Hospital and Rutgers University in 2010, **(+)-SJ733** (Figure 21) is a novel tetrahydroisoquinolone carboxanilide that possesses excellent antimalarial activity *in vivo*.[96]

Figure 21 Key biological and physical properties of (+)-SJ733. Sexual stage potency and logD kindly provided by K. Guy, personal communication, 2018.

High-throughput phenotypic screening of >300,000 compounds against the 3D7 strain of *P. falciparum* discovered a number of bioactive scaffold types, in which the lead compound (**S'1**, Figure 22), belonging to the tetrahydroisoquinolone carboxanilide class, was found to have potent *in vitro* activity (EC_{50} = 53 nM). Metabolic studies using mouse liver microsomes identified the susceptibility of the methoxy group to demethylation. In order to overcome this issue, the aniline substituents were replaced with fluoro and cyano groups (**S'2**), which had an added effect of improving the potency more than twofold. The thiophene group was suspected to be a metabolic hot spot, which was addressed by its replacement with a pyridyl group (**S'3**) resulting also in improved solubility, while maintaining potency. The final metabolically labile group (isobutyl) was replaced with a trifluoromethyl moiety, further improving solubility and maintaining potency to give the lead compound **(+)-SJ733**. The (+)-(3*S*,4*S*) isomer was found to be significantly more potent (EC_{50} = 36 nM) than its (−)-(3*R*,4*R*) enantiomer (EC_{50} = 587 nM).[97]

In the *P. berghei* mouse model, **(+)-SJ733** was found able to cure malaria at doses of 4 × 100 mg/kg and has an ED_{90} of 1.9 mg/kg. It has shown transmission blocking activity in infected mice with an ED_{50} of 5 mg/kg. It possesses a good safety profile with no cytotoxicity and was found to be more potent *in vivo* when compared to existing antimalarials such as artesunate, chloroquine, and pyrimethamine.[98].

Figure 22 Key stages in the hit to lead pathway of (+)-SJ733. Poor metabolic stability of the hit compound was addressed by replacement of the chloro and methoxy groups with cyano and fluoro groups respectively. Further *in vivo* stability and solubility improvements were made by changing the thiophene to a pyridine. Finally, the *gem*-dimethyl group was substituted with a trifluoromethyl group to eliminate possible metabolic oxidation.

The molecular target of **(+)-SJ733** has been identified as the P-type Na^+–ATPase transporter (*Pf*ATP4, *vide infra*), which has been implicated as a target for a number of other structurally diverse compounds.[99] The compound is currently in the recruiting stage of first-in-human study trials (NCT02661373).

ACT-451840 (Figure 23) is a phenylalanine-based compound that was developed in 2016 through a collaboration between Actelion Pharmaceuticals and the Swiss Tropical and Public Health Institute (STPHI). It has the potential to be a fast-acting drug with a long half-life and has shown efficacy against multiple stages of the *P. falciparum* parasite.[100]

Figure 23 Key properties of ACT-451840

Initial erythrocyte-based phenotypic screening of ∼5000 compounds identified **A'1** (Figure 24) as a highly potent compound against the chloroquine-resistant K1 strain of *P. falciparum* (IC_{50} = 3.8 nM). The SAR studies in this project were unique in that the antimalarial activity was measured in parallel in two different media: 10% bovine serum albumin and 50% human serum, with the latter used to help identify any potential problems with protein binding at an early stage of the optimisation. The stereogenic centre of the amino acid residue proved to be important for the activity, with the (*S*)-isomer showing more than 10-fold higher activity compared to the non-natural (*R*)-isomer. Modification of the *n*-pentyl chain to an acylpiperazine group (**A'2**) resulted in an improvement in the physical and chemical properties of the compound. Replacement of the trifluoromethyl group with a *tert*-butyl group (**A'3**) led to improved antimalarial activity, especially in the presence of human serum proteins. Final installation of a 4-cyano moiety on the southern phenyl ring gave the highly potent (IC_{50}=0.4 nM) lead candidate **ACT-451840**.

Figure 24 Key stages in the hit to lead pathway of ACT-451840. Initial change of the *n*-pentyl group to an acylpiperazine (**A'2**) helped to improve the physicochemical properties. Subsequent introduction of a *tert*-butyl in place of the trifluoromethyl (**A'3**) and a cyano group on the southern phenyl ring resulted in the optimised compound.

Interestingly, all compounds were found to be significantly less active against *P. berghei* than the human parasite. This is notable since most new antimalarial drugs in development have shown similar potency against both rodent and human parasites. As a result, for *in vivo* studies it was crucial to use a humanised *P. falciparum* severe combined immunodeficiency (SCID) mouse model. In the *P. berghei* mouse model, **ACT-451840** showed the ability to cure malaria at doses of 3 × 300 mg/kg with an ED_{90} of 13 mg/kg. In the *P. falciparum* SCID mouse model it had an ED_{90} of 3.7 mg/kg. The importance of the delivery system for **ACT-451840** was shown through *in vivo* experiments: a 60 mg dose in corn oil was as effective as a 100 mg dose in a mixture of Tween-EtOH/water = 10:90.

ACT-451840 has shown activity against multiple parasite life cycle stages of both *P. falciparum* and *P. vivax*.[101] The MoA is suspected to be novel but is currently unknown. **ACT-451840** last completed first-in-human studies in 2014 (NCT02223871)[102] and is currently awaiting a decision to proceed.

Product Development (Patient exploratory)

Discovered in 2011 by a partnership between Monash University, the University of Nebraska and the Swiss Tropical Institute, **OZ439** (Figure 25) is a synthetic trioxolane that possesses fast-acting curative and transmission-blocking ability, and is active against artemisinin-resistant parasites.[103]

Figure 25 Key biological and physical properties of OZ439. *in vivo* ED90 kindly provided by S. Wittlin, personal communication, 2018.

The discovery of **OZ439** (a.k.a. Artefenomel) stems from the lead optimisation of the pre-existing trioxolane **OZ277** (a.k.a. Arterolane), which was discovered in 2010.[104] As previous studies have shown that the adamantane-peroxide moiety is essential for antimalarial activity, studies were focused on the eastern portion of the molecule.[105–107]. Notably, analogues with variation in this area were found to have largely similar *in vitro* activities. In order to improve upon the original lead compound **OZ277** (mean survival 11 days, 0 from 5 mouses cured), replacement of the amide linker with a phenyl ether linker (**O1**) led to improved exposure and single-dose curative efficacy (mean survival >30 days, 5 from 5 mice cured when

applied in single dose of 30 mg/kg 1 day after infection). An attempt to further improve the exposure saw replacement of the alkylamine chain with a terminal piperazine unit (**O2**), however this led to a significant reduction in curative efficacy (mean survival 17 days, 0 from 5 mice cured). Encouragingly, using morpholine (**OZ439**) resulted in a mean survival of >30 days, with 5 from 5 mice cured.[108] While **OZ439** has shown excellent curative efficacy, no clear correlations were found between *in vitro* and *in vivo* activity or physiochemical properties and exposure. As a result, this new lead compound possess significantly lower solubility and slightly lower potency than **OZ277**.

Figure 26 Key stages in the hit to lead pathway of OZ439. Initial replacement of the amide linker with a phenyl ether linker resulted in improved exposure while maintaining potency (**O1**). The exposure was further improved by changing the alkylamine chain to a piperazine ring (**O2**). Final replacement of the piperazine ring with a morpholine unit led to the optimised compound **OZ439**, which possessed better curative efficacy *in vivo*.

Unlike other peroxide-containing antimalarials (e.g. artemisinin), **OZ439** was found to be curative at a single dose of 20 mg/kg in the *P. berghei* mouse model and possessed prophylactic activity superior to mefloquine when a 30 mg/kg dose was applied 24 hours prior to malaria infection. **OZ439** also showed full antimalarial protection when administered 96 hours before infection at a dose of 100 mg/kg. Only minor signs of toxicity were observed in a rat model when five consecutive doses of 300 mg/kg were administered with 3 days interval. Conversely, **O1** resulted in the death of 9 from 12 animals when used at the same dose.[108] **OZ439** has shown a significantly longer half-life in humans (about 46-62 hours) when compared to the hit compound **OZ277** (3 hours).[109–111]

Much like the current peroxide-containing antimalarials, the precise MoA for **OZ439** has yet to be discovered but it is believed that oxidative stress plays a major role.[112, 113] First-in-human results for **OZ439** were published in 2013[114] and since then the molecule has progressed into phase IIb clinical trials with planned completion in 2019 (NCT02497612).[115]

KAF156 (Figure 27) was identified in 2008 by Novartis and The Scripps Research Institute and is part of the second-generation of imidazolopiperazine antimalarials that potentially possesses a novel MoA and performs as a rapid-acting drug.[116]

Figure 27 Key biological and physical properties of KAF156. Solubility and logD kindly provided by T. Diagana, personal communication, 2018.

A high-throughput screen of 1.7 million compounds in a *P. falciparum* proliferation assay led to the identification of lead compound **K1** (Figure 28). In order to protect the metabolically vulnerable positions in the lead compound and to optimise the potency, the benzodioxole and phenyl fragments were replaced with 4-fluorobenzene groups (**K2**). Installation of a dimethyl group in the piperazine ring led to the twofold more potent compound **KAF156**.[117, 118] Notably, removal of the glycine residue from the piperazine ring was shown to increase the *in vitro* potency further (EC_{50} = 5 nM), but this was accompanied by a lower parasitemia reduction, indicating the importance of the amino acid side chain for *in vivo* efficacy.[117]

In the causal prophylactic rodent malaria model, a single oral dose of 10 mg/kg administered 2 hours before intravenous infection with *P. berghei* sporozoites was shown to be fully protective. **KAF156** has also shown transmission blocking ability in the *P. berghei* model.

The MoA for **KAF156** remains unknown. Through the culturing of resistant strains, mutations have been identified in three genes: *P. falciparum* Cyclic Amine Resistance Locus (*Pf*CARL), UDP-galactose and Acetyl-CoA transporters.[42, 119] **KAF156** is currently in phase IIb clinical trials, administered in combination with Lumefantrine (NCT03167242).

KAE609/Cipargamin/NITD609 (Figure 29) was discovered by a partnership between Novartis, the STPHI and the Wellcome Trust.[120, 121]

Figure 28 Key stages in the hit to lead pathway of KAF156. Potential metabolic stability issues were addressed through fluorine bioisosteres giving **K2**. Introduction of a dimethyl group on the piperazine ring resulted in increased potency and led to the optimised compound.

Figure 29 Key biological and physical properties of KAE609. *significant inhibition at 50 and 500 nM doses.

A high-throughput *P. falciparum* proliferation assay identified a racemic spiroazepineindole compound (**K'1**, Figure 30) which possessed moderate potency against K1 and NF54 strains, as well as potent *in vivo* efficacy. Confirmation of the hit result by resynthesis of **K'1** resulted in the isolation of a ∼9:1 diastereomeric ratio favouring the (1*R*,3*S*) and (1*S*,3*R*) pair of enantiomers. Chiral separation of this major pair identified the (1*R*,3*S*) enantiomer **K'2** as being >250-fold more potent than the (1*S*,3*R*) enantiomer. Reduction of the ring size (**K'3**) facilitated a diastereoselective Pictet–Spengler reaction, which ultimately led to the production of highly potent spiroindolone **KAE609**.

KAE609 is equipotent against drug-resistant strains and was found to be as effective as artesunate against *P. falciparum* and *P. vivax* isolates.[122] It shows a good safety profile, with low cytotoxicity, cardiotoxicity and mutagenic activity, and is able to clear parasitemia rapidly in adults with uncomplicated *P. falciparum* or *P. vivax* malaria at a dose of 30 mg/day for 3 days. **KAE609** displays low clearance from the body, has a long half-life and excellent bioavailability.

Figure 30 Key stages in the hit to lead pathway of KAE609. Resolution of the initial racemic hit (**K'1**) gave the significantly more potent stereoisomer (**K'2**). Reducing the ring size further increased the potency and final halogenation of the indole ring led to the optimised compound **KAE609**.

KAE609 is one of the key novel compounds that has been found to act through inhibition of *Pf*ATP4 (*vide infra*). Along with a number of other structurally distinct compounds that are also thought to inhibit *Pf*ATP4 as their MoA, **KAE609** has shown great promise as an new antimalarial candidate and is currently in phase IIb trials (NCT03334747).

DSM265 (Figure 31) was discovered through a collaboration between the University of Texas Southwestern, the University of Washington and Monash University.[123]

Figure 31 Key biological and physical properties of DSM265.

Identified in a high-throughput screen of *P. falciparum* dihydroorotate dehydrogenase (*Pf*DHODH)-based enzyme activity, the selective inhibitor **D'1** (Fig-

ure 32) was found. The compound showed high potency but was rapidly metabolised and was inactive *in vivo*. Substitution of the naphthyl ring with a trifluoromethylphenyl group led to a metabolically stable analogue (**D'2**). Examination of the bound crystal structures of **D'1** and **D'2** with *Pf*DHODH identified key residue interactions which eventually led to the installation of a difluoroethyl group (**D'3**) which significantly improved the potency and solubility. Final modification of the trifluoromethyl group with a pentafluorosulfur group led to the optimised compound **DSM265**.

Figure 32 Key stages in the hit to lead pathway of DSM265. Replacement of the second phenyl ring in the naphthyl group with a trifluoromethyl group improved solubility. Addition of a 1,1-difluoroethyl group significantly increased the potency and final replacement of the trifluoromethyl group in **D'2** with a pentafluorosulfur moiety led to the optimised compound **DSM265**.

DSM265 has been shown to be a highly selective inhibitor of malarial DHODH and is potent against both blood and liver stages of *P. falciparum* and also drug-resistant parasite isolates. It has an excellent safety profile (provides therapeutic concentrations after 8 days of a single oral dose, tolerated in repeat-doses, non-mutagenic, inactive against human enzymes/receptors) and has a very low clearance rate and a long half-life in humans.[124] **DSM265** has completed phase IIa trials in Peru in patients with *P. falciparum* or *P. vivax* (NCT02123290) and also completed a controlled human malaria infection study in combination with OZ439 (NCT02389348).

Identified in 2012 by a team at the University of Cape Town, South Africa in the same campaign as UCT943, **MMV048** (Figure 33) is another compound in the 3,5-diaryl-2-aminopyridine class that possesses good prophylactic activity against *P. cynomolgi in vivo* and has the potential to act as a transmission-blocking drug.[81]

Figure 33 Key biological and physical properties of MMV048.

The initial hit (**M"1**, Figure 34) showed potent *in vitro* activity against the NF54 (drug-sensitive) strain of *P. falciparum* (IC_{50} = 49 nM) but suffered from poor solubility and high metabolic clearance. The metabolically labile 3-methoxy-4-hydroxyphenyl moiety was replaced by a methoxypyridyl ring giving the more stable and equipotent compound **M"2**. Further SAR studies revealed that replacement of the methoxy group by a trifluoromethyl group led to the improved potency and stability of **MMV048**.

Figure 34 Key stages in the hit to lead pathway of MMV048. Initial replacement of the 3-methoxy-4-hydroxyphenyl moiety helped to improve *in vivo* stability and solubility. Further replacement of the methoxy group with a trifluoromethyl group improved potency and metabolic stability leading to the optimised compound.

MMV048 has shown 99.3% reduction in parasitemia in the *P. berghei* mouse model at a single dose of 30 mg/kg with no signs of parasites after 30 days

(ED_{90} = 1.7 mg/kg). This highlights the potential of **MMV048**, to act as a single dose treatment.

The target of **MMV048** is *Pf*PI4K, which was recently revealed as a new MoA for antimalarial drugs (*vide infra*).[84] **MMV048** is currently in phase IIa clinical trials in Ethiopia.[42]

Modes of Action

The continual emergence of drug resistant *Plasmodium* strains means that developing new drugs with novel MoAs is important.[125] Below, we summarise some newer MoAs that are currently being pursued and which are exemplified by the compounds described above.

Translational elongation factor 2 (*Pf*eEF2) inhibitor

The *P. falciparum* translational elongation factor 2 (*Pf*eEF2) is one of the essential factors for eukaryotic protein synthesis, catalysing the translocation of tRNA and mRNA.[126] The overall efficacy of drugs that target this elongation factor may be increased due to *Pf*eEF2 being expressed in multiple stages of the parasite life-cycle.[75]

M5717 has been shown to inhibit *Pf*eEF2 as its MoA, but a binding pocket for **M5717** could not be elucidated from the modelling studies.[75] This MoA was found by culturing blood stage parasites with five times the EC_{50} of **M5717**, leading to resistance in the 3D7, 7G8 and Dd2 strains. Through whole-genome sequencing of these resistance lines, a common mutation was found which identified *Pf*eEF2 as the target.

P-type Na^+–ATPase inhibitor (*Pf*ATP4) inhibitor

The malaria parasite must maintain a low intracellular Na^+ concentration to survive, especially in the presence of the high concentration of extracellular sodium ions. In this case, the parasite's influx of Na^+ is regulated by using a P-type ATPase transporter (*Pf*ATP4) that shuttles sodium ions out of the cell. Inhibition of this transporter results in a build up of Na^+ inside the parasite, ultimately leading to cell death.[99]

A number of structurally diverse compounds (including **(+)-SJ733** and **KAE609**) are thought to target *Pf*ATP4 as their MoA again suggested by the development of resistant mutants and sequencing.[120] The Kirk group have developed a fluorescence assay for this MoA,[127] which has been used by the OSM project to implicate *Pf*ATP4 as a MoA for the Series 4 triazolopyrazines.[128] Of note, however, is that this MoA is suggested for a very broad range of chemotypes,[129, 130] and there is no direct evidence for binding of these compounds to *Pf*ATP4, the structure of which remains unsolved.

V-type H^+–ATPase inhibitor

At the same time that the parasite is regulating its Na^+ concentration through the above mechanism, it also imports H^+ through the same pathway. To regulate this increasing H^+ concentration and maintain an intracellular pH of ∼7.3, the parasite uses a complementary V-type ATPase transporter to efflux H^+.[131]

MMV253 has been shown to inhibit the V-type H^+ ATPase as its MoA through mutant selection and whole-genome sequencing.[78]

Phosphatidylinositol 4-kinase (*Pf*PI4K) inhibitor

Phosphatidylinositol 4-kinase (PI4K) is a eukaryotic enzyme that phosphorylates lipids, allowing them to regulate intracellular signalling and trafficking.[84] Inhibition of the ATP-binding pocket of PI4K leads to disruption of the intracellular distribution of PI4-phosphate (PI4P), which in turn results in decreased late-stage parasite development.

By culturing resistant lines and identifying the genomic mutations, two compounds (**UCT943** and **MMV048**) have been found to inhibit the *Pf*PI4K enzyme.[84]

Dihydroorotate dehydrogenase (*Pf*DHODH) inhibitor

To be able to replicate in the human erythrocyte, the *Plasmodium* parasite requires pyrimidines, which are essential metabolites in all cells. As the parasite is unable to use endogenous pyrimidines, it must synthesise them *de novo*. A key step in the biosynthesis of pyrimidines is the oxidation of dihydroorotate to produce orotate, a reaction that is catalysed by the enzyme dihydroorotate dehydrogenase (DHODH). By inhibiting this enzyme, the malaria parasite can no longer obtain the required metabolites to survive, and is killed.[132]

The *P. falciparum* D10 strain (chloroquine sensitive) has been known to be less susceptible to bc1 complex inhibitors and *Pf*DHODH inhibitors. Addition of proguanil to this strain is known to restore the sensitivity to bc1 complex inhibitors but not to *Pf*DHODH inhibitors. This cell line was found to be resistant to **DSM265**, regardless of whether proguanil was present or not, which identifies inhibition of *Pf*DHODH as its MoA.[123, 133]

Dihydrofolate reductase (*Pf*DHFR) inhibitor

Similar to the above case, the malaria parasite also requires folates in order to maintain their high rate of replication. The parasite is able to scavange folates or synthesise them *de novo*. Dihydrofolate reductase (DHFR) is an enzyme that catalyses a step required for the recycling of folates, which in turn are used in the synthesis of thymidylate, purines and methionine.[134]

*Pf*DHFR is a known target of a number of well known antifolate antimalarial drugs including cycloguanil and pyrimethamine. Through examinations of the key interactions in the cocrystal structure of known inhibitors with the enzyme, **P218** was designed as a potent inhibitor.[95]

Conclusions

Although the rate of malaria related deaths has declined over the past few years, the progress is beginning to slow. With the recent emergence of resistance to our current front-line artemisinin-based combination therapies, the need for the discovery of new antimalarials that can act through novel mechanisms of action has been pushed firmly to the top of the development agenda.

Over the past few years, high-throughput screens have identified a number of novel chemotypes that have since been developed into highly promising antimalarial candidates. Crucially, many of these compounds have demonstrated novel MoAs which are essential if future drugs are to succeed. This is wonderful progress, and testament to the hard work of the groups involved.

The discovery of these novel MoAs will pave the way for the development of future antimalarials. The exploration of further novel MoAs has become a possibility through the use of *in vitro* evolution and whole-genome analysis (IVIEWGA), which uses genome-base target discovery methods on compounds identified from the more common phenotypic screens.[135] In all such future development, creativity and ingenuity in the hit-to-lead campaigns will continue to be needed if we are to remain able to combat this formidable disease.

Abbreviations

ACT: Artemisinin-based combination therapy; CG: cycloguanil; CL_{int}: intrinsic clearance; CQ: chloroquine; DDU: Drug Discovery Unit; EC_{50}: half maximal effective concentration; ED_{90}: 90% effective dose; GSK: GlaxoSmithKline; hERG: human Ether-à-go-go-Related Gene; HLM: human liver microsome; IC_{50}: half maximal inhibitory concentration; i.p.: intraperitoneally; IVIEWGA: *in vitro* evolution and whole-genome analysis; IVIVC: *in vitro-in vivo* correlation; MLEM: Model List of Essential Medicines; MLM: mouse liver microsome; MMV: Medicines for Malaria Venture; MoA: mode/mechanism of action; mRNA: messenger ribonucleic acid; MTX: methotrexate; OSM: Open Source Malaria; *P.*: *Plasmodium*; *p.o.*: (*per os*) orally; *Pb*: *Plasmodium berghei*; *Pc*: *Plasmodium* cynomolgi; *Pf*: *Plasmodium falciparum*; *Pf*ATP4: *Plasmodium falciparum* ATPase transporter; *Pf*CARL: *Plasmodium falciparum* cyclic amine resistance locus; *Pf*CPSF3: *Plasmodium falciparum* cleavage and polyadenylation specificity factor; *Pf*DHFR: *Plasmodium falciparum* dihydrofolate reductase; *Pf*DHODH: *Plasmodium falciparum* dihydroorotate dehydrogenase; *Pf*eEF2: *Plasmodium falciparum* translational elongation factor 2; *Pf*PI3K: *Plasmodium falciparum* phosphatidylinositol-3-kinase; *Pf*PI4K: *Plasmodium falciparum* phosphatidylinositol-4-OH kinase; *Py*: *Plasmodium yoelli*; PYR: pyrimethamine; *q.d.*: (*quaque die*) one a day; RCQ(s): reversed chloroquine(s); SAR: structure activity relationship; SCID: severe combined immunodeficiency; STPHI: Swiss Tropical and Public Health Institute; TAP: triaminopyrimidine; TQ: tafenoquine; tRNA: transfer ribonucleic acid; WHO: World Health Organization;

Competing interests

The authors declare that they have no competing interests.

Author's contributions

All authors contributed to the writing,editing and proofing of the manuscript.

Acknowledgements

The authors thank the Australian Research Council and the Medicines for Malaria Venture for funding (LP150101226)

Author details

[1]School of Chemistry, The University of Sydney, Sydney, Australia, NSW, 2006. [2]School of Pharmacy, University College London, London, United Kingdom, WC1N 1AX.

References

1. World Malaria Report 2018. Accessed 27 Nov 2018. https://www.who.int/malaria/publications/world-malaria-report-2018/en/
2. Cheney, C.: Fight Against Malaria Stalling and Could Reverse, Warns 2017 World Malaria Report. Accessed 24 Mar 2018. http://theconversation.com/global-malaria-report-reveals-africas-hits-and-misses-heres-what-to-do-88577
3. Mwangi, T.: Global Malaria Report Reveals Africa's Hits and Missed: Here's What to Do. Accessed 24 Mar 2018. https://www.devex.com/news/fight-against-malaria-stalling-and-could-reverse-warns-2017-world-malaria-report-91636
4. Shanks, G.D., Edstein, M.D., Jacobus, D.: Evolution from double to triple-antimalarial drug combinations. Transactions of the Royal Society of Tropical Medicine and Hygiene **109**(3), 182–188 (2014). doi:10.1093/trstmh/tru199
5. Mishra, M., Mishra, V.K., Kashaw, V., Iyer, A.K., Kashaw, S.K.: Comprehensive review on various strategies for antimalarial drug discovery. European Journal of Medicinal Chemistry **125**, 1300–1320 (2017). doi:10.1016/j.ejmech.2016.11.025
6. Phillips, M.A., Burrows, J.N., Manyando, C., van Huijsduijnen, R.H., Voorhis, W.C.V., Wells, T.N.C.: Malaria. Nature Reviews Disease Primers **3**, 17050 (2017). doi:10.1038/nrdp.2017.50
7. Okombo, J., Chibale, K.: Recent updates in the discovery and development of novel antimalarial drug candidates. MedChemComm **9**(3), 437–453 (2018). doi:10.1039/c7md00637c
8. Ashley, E.A., Phyo, A.P.: Drugs in development for malaria. Drugs **78**(9), 861–879 (2018). doi:10.1007/s40265-018-0911-9
9. Medicines for Malaria Venture. Accessed 24 Mar 2018. https://www.mmv.org/
10. Williamson, A.E., Ylioja, P.M., Robertson, M.N., Antonova-Koch, Y., Avery, V., Baell, J.B., Batchu, H., Batra, S., Burrows, J.N., Bhattacharyya, S., Calderon, F., Charman, S.A., Clark, J., Crespo, B., Dean, M., Debbert, S.L., Delves, M., Dennis, A.S.M., Deroose, F., Duffy, S., Fletcher, S., Giaever, G., Hallyburton, I., Gamo, F.-J., Gebbia, M., Guy, R.K., Hungerford, Z., Kirk, K., Lafuente-Monasterio, M.J., Lee, A., Meister, S., Nislow, C., Overington, J.P., Papadatos, G., Patiny, L., Pham, J., Ralph, S.A., Ruecker, A., Ryan, E., Southan, C., Srivastava, K., Swain, C., Tarnowski, M.J., Thomson, P., Turner, P., Wallace, I.M., Wells, T.N.C., White, K., White, L., Willis, P., Winzeler, E.A., Wittlin, S., Todd, M.H.: Open source drug discovery: Highly potent antimalarial compounds derived from the tres cantos arylpyrroles. ACS Central Science **2**(10), 687–701 (2016). doi:10.1021/acscentsci.6b00086
11. Leong, F.J., Li, R., Jain, J.P., Lefèvre, G., Magnusson, B., Diagana, T.T., Pertel, P.: A first-in-human randomized, double-blind, placebo-controlled, single- and multiple-ascending oral dose study of novel antimalarial spiroindolone KAE609 (Cipargamin) to assess its safety, tolerability, and pharmacokinetics in healthy adult volunteers. Antimicrobial Agents and Chemotherapy **58**(10), 6209–6214 (2014). doi:10.1128/aac.03393-14
12. Achan, J., Talisuna, A.O., Erhart, A., Yeka, A., Tibenderana, J.K., Baliraine, F.N., Rosenthal, P.J., D'Alessandro, U.: Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. Malaria Journal **10**(1), 144 (2011). doi:10.1186/1475-2875-10-144
13. Bunnag, D., Karbwang, J., Na-Bangchang, K., Thanavibul, A., Chittamas, S., Harinasuta, T.: Quinine-tetracycline for multidrug

resistant falciparum malaria. The Southeast Asian journal of tropical medicine and public health **27**, 15–18 (1996)
14. Model List of Essential Medicines. Accessed 24 Mar 2018. http://www.who.int/medicines/publications/essentialmedicines/en/
15. Green, R.: A report on fifty cases of malaria treated with atebrin. a new synthetic drug. The Lancet **219**(5668), 826–829 (1932). doi:10.1016/s0140-6736(00)56672-0
16. Weina, P.J.: From atabrine in world war II to mefloquine in Somalia: the role of education in preventive medicine. Military medicine **163**, 635–639 (1998)
17. Loeb, F.: Activity of a new antimalarial agent, chloroquine (SN 7618). Journal of the American Medical Association **130**(16), 1069 (1946). doi:10.1001/jama.1946.02870160015006
18. Mushtaque, M., Shahjahan: Reemergence of chloroquine (CQ) analogs as multi-targeting antimalarial agents: A review. European Journal of Medicinal Chemistry **90**, 280–295 (2015). doi:10.1016/j.ejmech.2014.11.022
19. Trenholme, C., Williams, R., Desjardins, R., Frischer, H., Carson, P., Rieckmann, K., Canfield, C.: Mefloquine (WR 142,490) in the treatment of human malaria. Science **190**(4216), 792–794 (1975). doi:10.1126/science.1105787
20. Brasseur, P., Druilhe, P., Kouamouo, J., Brandicourt, O., Danis, M., Moyou, S.R.: High level of sensitivity to chloroquine of 72 plasmodium falciparum isolates from southern Cameroon in January 1985. The American journal of tropical medicine and hygiene **35**, 711–716 (1986)
21. Foley, M., Tilley, L.: Quinoline antimalarials: Mechanisms of action and resistance. International Journal for Parasitology **27**(2), 231–240 (1997). doi:10.1016/s0020-7519(96)00152-x
22. Nevin, R.L., Croft, A.M.: Psychiatric effects of malaria and anti-malarial drugs: historical and modern perspectives. Malaria Journal **15**(1) (2016). doi:10.1186/s12936-016-1391-6
23. Cosgriff, T.M., Desjardins, R.E., Pamplin, C.L., Canfield, C.J., Doberstyn, E.B., Boudreau, E.F.: Evaluation of the antimalarial activity of the phenanthrenemethanol halofantrine (WR 171,669)*. The American Journal of Tropical Medicine and Hygiene **31**(6), 1075–1079 (1982). doi:10.4269/ajtmh.1982.31.1075
24. Croft, A.M.: A lesson learnt: the rise and fall of lariam and halfan. Journal of the Royal Society of Medicine **100**(4), 170–174 (2007). doi:10.1258/jrsm.100.4.170
25. Group, Q.A.C.R.: Antimalarial studies on qinghaosu. Chinese Medical Journal **92**(12), 811–816 (1979)
26. Nobelprize.org: The Nobel Prize in Physiology or Medicine 2015. Accessed 24 Mar 2018. https://www.nobelprize.org/nobel_prizes/medicine/laureates/2015/
27. Eastman, R.T., Fidock, D.A.: Artemisinin-based combination therapies: a vital tool in efforts to eliminate malaria. Nature Reviews Microbiology **7**(12), 864–874 (2009). doi:10.1038/nrmicro2239
28. Noedl, H., Se, Y., Schaecher, K., Smith, B.L., Socheat, D., Fukuda, M.M.: Evidence of artemisinin-resistant malaria in western cambodia. New England Journal of Medicine **359**(24), 2619–2620 (2008). doi:10.1056/nejmc0805011
29. Amato, R., Pearson, R.D., Almagro-Garcia, J., Amaratunga, C., Lim, P., Suon, S., Sreng, S., Drury, E., Stalker, J., Miotto, O., Fairhurst, R.M., Kwiatkowski, D.P.: Origins of the current outbreak of multidrug-resistant malaria in southeast Asia: a retrospective genetic study. The Lancet Infectious Diseases **18**(3), 337–345 (2018). doi:10.1016/s1473-3099(18)30068-9
30. O'Neill, P.M., Barton, V.E., Ward, S.A.: The molecular mechanism of action of artemisinin–the debate continues. Molecules **15**(3), 1705–1721 (2010). doi:10.3390/molecules15031705
31. Wang, J., Zhang, C.-J., Chia, W.N., Loh, C.C.Y., Li, Z., Lee, Y.M., He, Y., Yuan, L.-X., Lim, T.K., Liu, M., Liew, C.X., Lee, Y.Q., Zhang, J., Lu, N., Lim, C.T., Hua, Z.-C., Liu, B., Shen, H.-M., Tan, K.S.W., Lin, Q.: Haem-activated promiscuous targeting of artemisinin in plasmodium falciparum. Nature Communications **6**(1) (2015). doi:10.1038/ncomms10111
32. Tilley, L., Straimer, J., Gnädig, N.F., Ralph, S.A., Fidock, D.A.: Artemisinin action and resistance in *Plasmodium falciparum*. Trends in Parasitology **32**(9), 682–696 (2016). doi:10.1016/j.pt.2016.05.010
33. Shandilya, A., Chacko, S., Jayaram, B., Ghosh, I.: A plausible mechanism for the antimalarial activity of artemisinin: A computational approach. Scientific Reports **3**(1) (2013). doi:10.1038/srep02513
34. Mok, S., Ashley, E.A., Ferreira, P.E., Zhu, L., Lin, Z., Yeo, T., Chotivanich, K., Imwong, M., Pukrittayakamee, S., Dhorda, M., Nguon, C., Lim, P., Amaratunga, C., Suon, S., Hien, T.T., Htut, Y., Faiz, M.A., Onyamboko, M.A., Mayxay, M., Newton, P.N., Tripura, R., Woodrow, C.J., Miotto, O., Kwiatkowski, D.P., Nosten, F., Day, N.P.J., Preiser, P.R., White, N.J., Dondorp, A.M., Fairhurst, R.M., Bozdech, Z.: Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. Science **347**(6220), 431–435 (2014). doi:10.1126/science.1260403
35. Mbengue, A., Bhattacharjee, S., Pandharkar, T., Liu, H., Estiu, G., Stahelin, R.V., Rizk, S.S., Njimoh, D.L., Ryan, Y., Chotivanich, K., Nguon, C., Ghorbal, M., Lopez-Rubio, J.-J., Pfrender, M., Emrich, S., Mohandas, N., Dondorp, A.M., Wiest, O., Haldar, K.: A molecular mechanism of artemisinin resistance in plasmodium falciparum malaria. Nature **520**(7549), 683–687 (2015). doi:10.1038/nature14412
36. Berliner, R.W., Earle, D.P., Taggart, J.V., Zubrod, C.G., Welch, W.J., Conan, N.J., Bauman, E., Scudder, S.T., Shannon, J.A.: Studies on the chemotheraphy of the human malarias. VI. The physiological disposition, antimalarial activity, and toxicity of several derivatives of 4-aminoquinoline. Journal of Clinical Investigation **27**(3 Pt 2), 98–107 (1948). doi:10.1172/jci101980
37. Bompart, F., Kiechel, J.-R., Sebbag, R., Pecoul, B.: Innovative public-private partnerships to maximize the delivery of anti-malarial medicines: lessons learned from the ASAQ winthrop experience. Malaria Journal **10**(1), 143 (2011). doi:10.1186/1475-2875-10-143
38. Combrinck, J.M., Mabotha, T.E., Ncokazi, K.K., Ambele, M.A., Taylor, D., Smith, P.J., Hoppe, H.C., Egan, T.J.: Insights into the role of heme in the mechanism of action of antimalarials. ACS Chemical Biology **8**(1), 133–137 (2012). doi:10.1021/cb300454t
39. Chen, L., Qu, F.Y., Zhou, Y.C.: Field observations on the antimalarial piperaquine. Chinese medical journal **95**, 281–286 (1982)
40. Vennerstrom, J.L., Ellis, W.Y., Ager, A.L., Andersen, S.L., Gerena, L., Milhous, W.K.: Bisquinolines. 1. N,N-bis(7-chloroquinolin-4-yl)alkanediamines with potential against chloroquine-resistant malaria. Journal of Medicinal Chemistry **35**(11), 2129–2134 (1992). doi:10.1021/jm00089a025
41. Cui, L., Su, X.-z.: Discovery, mechanisms of action and combination therapy of artemisinin. Expert Review of Anti-infective Therapy **7**(8), 999–1013 (2009). doi:10.1586/eri.09.68
42. MMV-Supported Projects. Accessed 14 Jun 2018. https://www.mmv.org/research-development/mmv-supported-projects
43. Curd, F.H.S., Davey, D.G., Rose, F.L.: Studies on synthetic antimalarial drugs. Annals of Tropical Medicine & Parasitology **39**(3-4), 208–216 (1945). doi:10.1080/00034983.1945.11685237
44. Hudson, A.T., Randall, A.W.: Naphthoquinone Derivatives. US5053432A, 1991
45. Fry, M., Pudney, M.: Site of action of the antimalarial hydroxynaphthoquinone, 2-[trans-4-(4'-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone (566C80). Biochemical Pharmacology **43**(7), 1545–1553 (1992). doi:10.1016/0006-2952(92)90213-3
46. Srivastava, I.K., Vaidya, A.B.: A mechanism for the synergistic antimalarial action of atovaquone and proguanil. Antimicrobial agents and chemotherapy **43**, 1334–1339 (1999)
47. Russell, P.B., Hitchings, G.H.: 2,4-diaminopyrimidines as antimalarials. III. 5-aryl derivatives. Journal of the American Chemical Society **73**(8), 3763–3770 (1951). doi:10.1021/ja01152a060
48. Nobelprize.org: The Nobel Prize in Physiology or Medicine 1988. Accessed 19 June 2018. https://www.nobelprize.org/nobel_prizes/medicine/laureates/2015/
49. Laing, A.B.: Treatment of acute falciparum malaria with sulphorthodimethoxine (fansil). British medical journal **1**, 905–907 (1965)
50. Lumb, V., Das, M.K., Singh, N., Dev, V., Khan, W., Sharma, Y.D.: Multiple origins of plasmodium falciparum dihydropteroate synthetase

mutant alleles associated with sulfadoxine resistance in india. Antimicrobial Agents and Chemotherapy **55**(6), 2813–2817 (2011). doi:10.1128/aac.01151-10

51. Zheng, X.Y., Xia, Y., Gao, F.H., Chen, C.: Synthesis of 7351, a new antimalarial drug. Acta Pharmaceutica Sinica **14**, 736–737 (1979)
52. Chang, C., Lin-Hua, T., Jantanavivat, C.: Studies on a new antimalarial compound: pyronaridine. Transactions of the Royal Society of Tropical Medicine and Hygiene **86**(1), 7–10 (1992). doi:10.1016/0035-9203(92)90414-8
53. Croft, S.L., Duparc, S., Arbe-Barnes, S.J., Craft, J., Shin, C.-S., Fleckenstein, L., Borghini-Fuhrer, I., Rim, H.-J.: Review of pyronaridine anti-malarial properties and product characteristics. Malaria Journal **11**(1), 270 (2012). doi:10.1186/1475-2875-11-270
54. US FDA Approves *Krintafel* (tafenoquine) for the Radical Cure of *P. Vivax* Malaria. Accessed 25 September 2018. https://www.mmv.org/newsroom/press-releases/us-fda-approves-krintafel-tafenoquine-radical-cure-p-vivax-malaria
55. Ebstie, Y.A., Abay, S.M., Tadesse, W.T., A.Ejigu, D.: Tafenoquine and its potential in the treatment and relapse prevention of *Plasmodium vivax* malaria: the evidence to date. Drug Design, Development and Therapy **Volume 10**, 2387–2399 (2016). doi:10.2147/dddt.s61443
56. Wells, T.N.C., Gutteridge, W.E.: Negleted Diseases and Drug Discovery. RSC, ??? (2012)
57. Burrows, J.N., Duparc, S., Gutteridge, W.E., van Huijsduijnen, R.H., Kaszubska, W., Macintyre, F., Mazzuri, S., Möhrle, J.J., Wells, T.N.C.: New developments in anti-malarial target candidate and product profiles. Malaria Journal **16**(1) (2017). doi:10.1186/s12936-016-1675-x
58. McChesney, J.D., Nanayakkara, D.N.P., Bartlett, M., Ager, A.L.: Preparation of 8-(aminoalkylamino)quinolines as Parasiticides. WO 9736590 A1, 1997
59. Tekwani, B.L., Walker, L.A.: 8-aminoquinolines: future role as antiprotozoal drugs. Current Opinion in Infectious Diseases **19**(6), 623–631 (2006). doi:10.1097/qco.0b013e328010b848
60. Nanayakkara, N.P.D., Ager, A.L., Bartlett, M.S., Yardley, V., Croft, S.L., Khan, I.A., McChesney, J.D., Walker, L.A.: Antiparasitic activities and toxicities of individual enantiomers of the 8-aminoquinoline 8-[(4-amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3,4-dichlorophenoxy]quinoline succinate. Antimicrobial Agents and Chemotherapy **52**(6), 2130–2137 (2008). doi:10.1128/aac.00645-07
61. Marcsisin, S.R., Sousa, J.C., Reichard, G.A., Caridha, D., Zeng, Q., Roncal, N., McNulty, R., Careagabarja, J., Sciotti, R.J., Bennett, J.W., Zottig, V.E., Deye, G., Li, Q., Read, L., Hickman, M., Nanayakkara, N.P., Walker, L.A., Smith, B., Melendez, V., Pybus, B.S.: Tafenoquine and NPC-1161b require CYP 2d metabolism for anti-malarial activity: implications for the 8-aminoquinoline class of anti-malarial compounds. Malaria Journal **13**(1), 2 (2014). doi:10.1186/1475-2875-13-2
62. Powles, M.A., Allocco, J., Yeung, L., Nare, B., Liberator, P., Schmatz, D.: MK-4815, a potential new oral agent for treatment of malaria. Antimicrobial Agents and Chemotherapy **56**(5), 2414–2419 (2012). doi:10.1128/aac.05326-11
63. Singh, C., Puri, S.K.: Asdf. US6316493 B1, 2001
64. Shafiq, N., Rajagopalan, S., Kushwaha, H.N., Mittal, N., Chandurkar, N., Bhalla, A., Kaur, S., Pandhi, P., Puri, G.D., Achuthan, S., Pareek, A., Singh, S.K., Srivastava, J.S., Gaur, S.P.S., Malhotra, S.: Single ascending dose safety and pharmacokinetics of CDRI-97/78: First-in-human study of a novel antimalarial drug. Malaria Research and Treatment **2014**, 1–10 (2014). doi:10.1155/2014/372521
65. O'Neill, P.M., Shone, A.E., Stanford, D., Nixon, G., Asadollahy, E., Park, B.K., Maggs, J.L., Roberts, P., Stocks, P.A., Biagini, G., Bray, P.G., Davies, J., Berry, N., Hall, C., Rimmer, K., Winstanley, P.A., Hindley, S., Bambal, R.B., Davis, C.B., Bates, M., Gresham, S.L., Brigandi, R.A., Gomez-de-las-Heras, F.M., Gargallo, D.V., Parapini, S., Vivas, L., Lander, H., Taramelli, D., Ward, S.A.: Synthesis, antimalarial activity, and preclinical pharmacology of a novel series of 4'-fluoro and 4'-chloro analogues of amodiaquine. identification of a suitable "back-up" compound for *N-tert*-butyl isoquine. Journal of Medicinal Chemistry **52**(7), 1828–1844 (2009). doi:10.1021/jm8012757
66. Bora, S., Chetia, D., Prakash, A.: Synthesis and antimalarial activity evaluation of some isoquine analogues. Medicinal Chemistry Research **20**(9), 1632–1637 (2010). doi:10.1007/s00044-010-9460-9
67. O'Neill, P.M., Park, B.K., Shone, A.E., Maggs, J.L., Roberts, P., Stocks, P.A., Biagini, G.A., Bray, P.G., Gibbons, P., Berry, N., Winstanley, P.A., Mukhtar, A., Bonar-Law, R., Hindley, S., Bambal, R.B., Davis, C.B., Bates, M., Hart, T.K., Gresham, S.L., Lawrence, R.M., Brigandi, R.A., Gomez-delas-Heras, F.M., Gargallo, D.V., Ward, S.A.: Candidate selection and preclinical evaluation of *N-tert*-butyl isoquine (GSK369796), an affordable and effective 4-aminoquinoline antimalarial for the 21st century. Journal of Medicinal Chemistry **52**(5), 1408–1415 (2009). doi:10.1021/jm8012618
68. Haynes, R.K., Fugmann, B., Stetter, J., Rieckmann, K., Heilmann, H.-D., Chan, H.-W., Cheung, M.-K., Lam, W.-L., Wong, H.-N., Croft, S.L., Vivas, L., Rattray, L., Stewart, L., Peters, W., Robinson, B.L., Edstein, M.D., Kotecka, B., Kyle, D.E., Beckermann, B., Gerisch, M., Radtke, M., Schmuck, G., Steinke, W., Wollborn, U., Schmeer, K., Römer, A.: Artemisone - a highly active antimalarial drug of the artemisinin class. Angewandte Chemie International Edition **45**(13), 2082–2088 (2006). doi:10.1002/anie.200503071
69. Nicolas, O., Margout, D., Taudon, N., Wein, S., Calas, M., Vial, H.J., Bressolle, F.M.M.: Pharmacological properties of a new antimalarial bisthiazolium salt, t3, and a corresponding prodrug, TE3. Antimicrobial Agents and Chemotherapy **49**(9), 3631–3639 (2005). doi:10.1128/aac.49.9.3631-3639.2005
70. Drake, N.L., Creech, H.J., Garman, J.A., Haywood, S.T., Peck, R.M., van Hook, J.O., Walton, E.: Synthetic antimalarials. the preparation of certain 4-aminoquinolines1. Journal of the American Chemical Society **68**(7), 1208–1213 (1946). doi:10.1021/ja01211a021
71. Ramanathan-Girish, S., Catz, P., Creek, M.R., Wu, B., Thomas, D., Krogstad, D.J., De, D., Mirsalis, J.C., Green, C.E.: Pharmacokinetics of the antimalarial drug, AQ-13, in rats and cynomolgus macaques. International Journal of Toxicology **23**(3), 179–189 (2004). doi:10.1080/10915810490471352
72. Sáenz, F.E., Mutka, T., Udenze, K., Oduola, A.M.J., Kyle, D.E.: Novel 4-aminoquinoline analogs highly active against the blood and sexual stages of *Plasmodium In Vivo* and *In Vitro*. Antimicrobial Agents and Chemotherapy **56**(9), 4685–4692 (2012). doi:10.1128/aac.01061-12
73. Mzayek, F., Deng, H., Mather, F.J., Wasilevich, E.C., Liu, H., Hadi, C.M., Chansolme, D.H., Murphy, H.A., Melek, B.H., Tenaglia, A.N., Mushatt, D.M., Dreisbach, A.W., Lertora, J.J.L., Krogstad, D.J.: Randomized dose-ranging controlled trial of AQ-13, a candidate antimalarial, and chloroquine in healthy volunteers. PLoS Clinical Trials **2**(1), 6 (2007). doi:10.1371/journal.pctr.0020006
74. Koita, O.A., Sangaré, L., Miller, H.D., Sissako, A., Coulibaly, M., Thompson, T.A., Fongoro, S., Diarra, Y., Ba, M., Maiga, A., Diallo, B., Mushatt, D.M., Mather, F.J., Shaffer, J.G., Anwar, A.H., Krogstad, D.J.: AQ-13, an investigational antimalarial, versus artemether plus lumefantrine for the treatment of uncomplicated plasmodium falciparum malaria: a randomised, phase 2, non-inferiority clinical trial. The Lancet Infectious Diseases **17**(12), 1266–1275 (2017). doi:10.1016/s1473-3099(17)30365-1
75. Baragaña, B., Hallyburton, I., Lee, M.C.S., Norcross, N.R., Grimaldi, R., Otto, T.D., Proto, W.R., Blagborough, A.M., Meister, S., Wirjanata, G., Ruecker, A., Upton, L.M., Abraham, T.S., Almeida, M.J., Pradhan, A., Porzelle, A., Martínez, M.S., Bolscher, J.M., Woodland, A., Luksch, T., Norval, S., Zuccotto, F., Thomas, J., Simeons, F., Stojanovski, L., Osuna-Cabello, M., Brock, P.M., Churcher, T.S., Sala, K.A., Zakutansky, S.E., Jiménez-Díaz, M.B., Sanz, L.M., Riley, J., Basak, R., Campbell, M., Avery, V.M., Sauerwein, R.W., Dechering, K.J., Noviyanti, R., Campo, B., Frearson, J.A., Angulo-Barturen, I., Ferrer-Bazaga, S., Gamo, F.J., Wyatt, P.G., Leroy, D., Siegl, P., Delves, M.J., Kyle, D.E., Wittlin, S., Marfurt, J., Price, R.N., Sinden, R.E., Winzeler, E.A., Charman, S.A., Bebrevska, L., Gray, D.W., Campbell, S., Fairlamb, A.H., Willis, P.A., Rayner, J.C., Fidock, D.A., Read, K.D., Gilbert, I.H.: A novel multiple-stage antimalarial agent that inhibits protein synthesis. Nature **522**(7556), 315–320 (2015). doi:10.1038/nature14451

76. Baragaña, B., Norcross, N.R., Wilson, C., Porzelle, A., Hallyburton, I., Grimaldi, R., Osuna-Cabello, M., Norval, S., Riley, J., Stojanovski, L., Simeons, F.R.C., Wyatt, P.G., Delves, M.J., Meister, S., Duffy, S., Avery, V.M., Winzeler, E.A., Sinden, R.E., Wittlin, S., Frearson, J.A., Gray, D.W., Fairlamb, A.H., Waterson, D., Campbell, S.F., Willis, P., Read, K.D., Gilbert, I.H.: Discovery of a quinoline-4-carboxamide derivative with a novel mechanism of action, multistage antimalarial activity, and potent *in Vivo* efficacy. Journal of Medicinal Chemistry **59**(21), 9672–9685 (2016). doi:10.1021/acs.jmedchem.6b00723
77. Burrows, J.N., van Huijsduijnen, R.H., Möhrle, J.J., Oeuvray, C., Wells, T.N.: Designing the next generation of medicines for malaria control and eradication. Malaria Journal **12**(1), 187 (2013). doi:10.1186/1475-2875-12-187
78. P., S.H., Solapure, S., Patil, V., Henrich, P.P., Magistrado, P.A., Bharath, S., Murugan, K., Viswanath, P., Puttur, J., Srivastava, A., Bellale, E., Panduga, V., Shanbag, G., Awasthy, D., Landge, S., Morayya, S., Koushik, K., Saralaya, R., Raichurkar, A., Rautela, N., Choudhury, N.R., Ambady, A., Nandishaiah, R., Reddy, J., Prabhakar, K.R., Menasinakai, S., Rudrapatna, S., Chatterji, M., Jiménez-Díaz, M.B., Martínez, M.S., Sanz, L.M., Coburn-Flynn, O., Fidock, D.A., Lukens, A.K., Wirth, D.F., Bandodkar, B., Mukherjee, K., McLaughlin, R.E., Waterson, D., Rosenbrier-Ribeiro, L., Hickling, K., Balasubramanian, V., Warner, P., Hosagrahara, V., Dudley, A., Iyer, P.S., Narayanan, S., Kavanagh, S., Sambandamurthy, V.K.: Triaminopyrimidine is a fast-killing and long-acting antimalarial clinical candidate. Nature Communications **6**(1) (2015). doi:10.1038/ncomms7715
79. MMV and Zydus Join Forces to Develop New Antimalarial. Accessed 17 June 2018. https://www.mmv.org/newsroom/press-releases/mmv-and-zydus-join-forces-develop-new-antimalarial
80. Manach, C.L., Nchinda, A.T., Paquet, T., Cabrera, D.G., Younis, Y., Han, Z., Bashyam, S., Zabiulla, M., Taylor, D., Lawrence, N., White, K.L., Charman, S.A., Waterson, D., Witty, M.J., Wittlin, S., Botha, M.E., Nondaba, S.H., Reader, J., Birkholtz, L.-M., Jiménez-Díaz, M.B., Martínez, M.S., Ferrer, S., Angulo-Barturen, I., Meister, S., Antonova-Koch, Y., Winzeler, E.A., Street, L.J., Chibale, K.: Identification of a potential antimalarial drug candidate from a series of 2-aminopyrazines by optimization of aqueous solubility and potency across the parasite life cycle. Journal of Medicinal Chemistry **59**(21), 9890–9905 (2016). doi:10.1021/acs.jmedchem.6b01265
81. Younis, Y., Douelle, F., Feng, T.-S., Cabrera, D.G., Manach, C.L., Nchinda, A.T., Duffy, S., White, K.L., Shackleford, D.M., Morizzi, J., Mannila, J., Katneni, K., Bhamidipati, R., Zabiulla, K.M., Joseph, J.T., Bashyam, S., Waterson, D., Witty, M.J., Hardick, D., Wittlin, S., Avery, V., Charman, S.A., Chibale, K.: 3,5-diaryl-2-aminopyridines as a novel class of orally active antimalarials demonstrating single dose cure in mice and clinical candidate potential. Journal of Medicinal Chemistry **55**(7), 3479–3487 (2012). doi:10.1021/jm3001373
82. Cabrera, D.G., Douelle, F., Younis, Y., Feng, T.-S., Manach, C.L., Nchinda, A.T., Street, L.J., Scheurer, C., Kamber, J., White, K.L., Montagnat, O.D., Ryan, E., Katneni, K., Zabiulla, K.M., Joseph, J.T., Bashyam, S., Waterson, D., Witty, M.J., Charman, S.A., Wittlin, S., Chibale, K.: Structure–activity relationship studies of orally active antimalarial 3,5-substituted 2-aminopyridines. Journal of Medicinal Chemistry **55**(24), 11022–11030 (2012). doi:10.1021/jm301476b
83. Younis, Y., Douelle, F., Cabrera, D.G., Manach, C.L., Nchinda, A.T., Paquet, T., Street, L.J., White, K.L., Zabiulla, K.M., Joseph, J.T., Bashyam, S., Waterson, D., Witty, M.J., Wittlin, S., Charman, S.A., Chibale, K.: Structure–activity-relationship studies around the 2-amino group and pyridine core of antimalarial 3,5-diarylaminopyridines lead to a novel series of pyrazine analogues with oral in vivo activity. Journal of Medicinal Chemistry **56**(21), 8860–8871 (2013). doi:10.1021/jm401278d
84. McNamara, C.W., Lee, M.C.S., Lim, C.S., Lim, S.H., Roland, J., Nagle, A., Simon, O., Yeung, B.K.S., Chatterjee, A.K., McCormack, S.L., Manary, M.J., Zeeman, A.-M., Dechering, K.J., Kumar, T.R.S., Henrich, P.P., Gagaring, K., Ibanez, M., Kato, N., Kuhen, K.L., Fischli, C., Rottmann, M., Plouffe, D.M., Bursulaya, B., Meister, S., Rameh, L., Trappe, J., Haasen, D., Timmerman, M., Sauerwein, R.W., Suwanarusk, R., Russell, B., Renia, L., Nosten, F., Tully, D.C., Kocken, C.H.M., Glynne, R.J., Bodenreider, C., Fidock, D.A., Diagana, T.T., Winzeler, E.A.: Targeting plasmodium PI(4)K to eliminate malaria. Nature **504**(7479), 248–253 (2013). doi:10.1038/nature12782
85. Zhang, Y.-K., Plattner, J.J., Freund, Y.R., Easom, E.E., Zhou, Y., Gut, J., Rosenthal, P.J., Waterson, D., Gamo, F.-J., Angulo-Barturen, I., Ge, M., Li, Z., Li, L., Jian, Y., Cui, H., Wang, H., Yang, J.: Synthesis and structure–activity relationships of novel benzoxaboroles as a new class of antimalarial agents. Bioorganic & Medicinal Chemistry Letters **21**(2), 644–651 (2011). doi:10.1016/j.bmcl.2010.12.034
86. Zhang, Y.-K., Plattner, J.J., Easom, E.E., Jacobs, R.T., Guo, D., Freund, Y.R., Berry, P., Ciaravino, V., Erve, J.C.L., Rosenthal, P.J., Campo, B., Gamo, F.-J., Sanz, L.M., Cao, J.: Benzoxaborole antimalarial agents. part 5. lead optimization of novel amide pyrazinyloxy benzoxaboroles and identification of a preclinical candidate. Journal of Medicinal Chemistry **60**(13), 5889–5908 (2017). doi:10.1021/acs.jmedchem.7b00621
87. Zhang, Y.-K., Plattner, J.J., Easom, E.E., Jacobs, R.T., Guo, D., Sanders, V., Freund, Y.R., Campo, B., Rosenthal, P.J., Bu, W., Gamo, F.-J., Sanz, L.M., Ge, M., Li, L., Ding, J., Yang, Y.: Benzoxaborole antimalarial agents. part 4. discovery of potent 6-(2-(alkoxycarbonyl)pyrazinyl-5-oxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaboroles. Journal of Medicinal Chemistry **58**(13), 5344–5354 (2015). doi:10.1021/acs.jmedchem.5b00678
88. Sonoiki, E., Ng, C.L., Lee, M.C.S., Guo, D., Zhang, Y.-K., Zhou, Y., Alley, M.R.K., Ahyong, V., Sanz, L.M., Lafuente-Monasterio, M.J., Dong, C., Schupp, P.G., Gut, J., Legac, J., Cooper, R.A., Gamo, F.-J., DeRisi, J., Freund, Y.R., Fidock, D.A., Rosenthal, P.J.: A potent antimalarial benzoxaborole targets a plasmodium falciparum cleavage and polyadenylation specificity factor homologue. Nature Communications **8**, 14574 (2017). doi:10.1038/ncomms14574
89. Pegoraro, S., Duffey, M., Otto, T.D., Wang, Y., Rösemann, R., Baumgartner, R., Fehler, S.K., Lucantoni, L., Avery, V.M., Moreno-Sabater, A., Mazier, D., Vial, H.J., Strobl, S., Sanchez, C.P., Lanzer, M.: SC83288 is a clinical development candidate for the treatment of severe malaria. Nature Communications **8**, 14193 (2017). doi:10.1038/ncomms14193
90. Leban, J., Pegoraro, S., Dormeyer, M., Lanzer, M., Aschenbrenner, A., Kramer, B.: Sulfonyl-phenyl-ureido benzamidines. Bioorganic & Medicinal Chemistry Letters **14**(8), 1979–1982 (2004). doi:10.1016/j.bmcl.2004.01.083
91. Duffey, M., Sanchez, C.P., Lanzer, M.: Profiling of the anti-malarial drug candidate SC83288 against artemisinins in plasmodium falciparum. Malaria Journal **17**(1) (2018). doi:10.1186/s12936-018-2279-4
92. 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. Journal of Medicinal Chemistry **53**(17), 6477–6489 (2010). doi:10.1021/jm1006484
93. 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. Journal of Medicinal Chemistry **49**(18), 5623–5625 (2006). doi:10.1021/jm060399n
94. Wirjanata, G., Sebayang, B.F., Chalfein, F., Prayoga, Handayuni, I., Noviyanti, R., Kenangalem, E., Poespoprodjo, J.R., Burgess, S.J., Peyton, D.H., Price, R.N., Marfurt, J.: Contrasting *Ex Vivo* efficacies of "reversed chloroquine" compounds in chloroquine-resistant plasmodium falciparum and *P. vivax* isolates. Antimicrobial Agents and Chemotherapy **59**(9), 5721–5726 (2015). doi:10.1128/aac.01048-15
95. Yuthavong, Y., Tarnchompoo, B., Vilaivan, T., Chitnumsub, P., Kamchonwongpaisan, S., Charman, S.A., McLennan, D.N., White, K.L., Vivas, L., Bongard, E., Thongphanchang, C., Taweechai, S., Vanichtananukul, J., Rattanajak, R., Arwon, U., Fantauzzi, P., Yuvaniyama, J., Charman, W.N., Matthews, D.: Malarial dihydrofolate reductase as a paradigm for drug development against a resistance-compromised target. Proceedings of the National Academy of Sciences **109**(42), 16823–16828 (2012).

doi:10.1073/pnas.1204556109
96. 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., Jiménez-Díaz, M.B., Martinez, M.S., Wilson, E.B., Tripathi, A.K., Gut, J., Sharlow, E.R., Bathurst, I., Mazouni, F.E., Fowble, J.W., Forquer, I., McGinley, P.L., Castro, S., Angulo-Barturen, I., Ferrer, S., Rosenthal, P.J., DeRisi, J.L., Sullivan, D.J., Lazo, J.S., Roos, D.S., Riscoe, M.K., Phillips, M.A., Rathod, P.K., Voorhis, W.C.V., Avery, V.M., Guy, R.K.: Chemical genetics of plasmodium falciparum. Nature **465**(7296), 311–315 (2010). doi:10.1038/nature09099
97. Floyd, D.M., Stein, P., Wang, Z., Liu, J., Castro, S., Clark, J.A., Connelly, M., Zhu, F., Holbrook, G., Matheny, A., Sigal, M.S., Min, J., Dhinakaran, R., Krishnan, S., Bashyum, S., Knapp, S., Guy, R.K.: Hit-to-lead studies for the antimalarial tetrahydroisoquinolone carboxanilides. Journal of Medicinal Chemistry **59**(17), 7950–7962 (2016). doi:10.1021/acs.jmedchem.6b00752
98. Jiménez-Díaz, M.B., Ebert, D., Salinas, Y., Pradhan, A., Lehane, A.M., Myrand-Lapierre, M.-E., O'Loughlin, K.G., Shackleford, D.M., de Almeida, M.J., Carrillo, A.K., Clark, J.A., Dennis, A.S.M., Diep, J., Deng, X., Duffy, S., Endsley, A.N., Fedewa, G., Guiguemde, W.A., Gómez, M.G., Holbrook, G., Horst, J., Kim, C.C., Liu, J., Lee, M.C.S., Matheny, A., Martínez, M.S., Miller, G., Rodríguez-Alejandre, A., Sanz, L., Sigal, M., Spillman, N.J., Stein, P.D., Wang, Z., Zhu, F., Waterson, D., Knapp, S., Shelat, A., Avery, V.M., Fidock, D.A., Gamo, F.-J., Charman, S.A., Mirsalis, J.C., Ma, H., Ferrer, S., Kirk, K., Angulo-Barturen, I., Kyle, D.E., DeRisi, J.L., Floyd, D.M., Guy, R.K.: (+)-SJ733, a clinical candidate for malaria that acts through ATP4 to induce rapid host-mediated clearance of plasmodium. Proceedings of the National Academy of Sciences **111**(50), 5455–5462 (2014). doi:10.1073/pnas.1414221111
99. Spillman, N.J., Kirk, K.: The malaria parasite cation ATPase PfATP4 and its role in the mechanism of action of a new arsenal of antimalarial drugs. International Journal for Parasitology: Drugs and Drug Resistance **5**(3), 149–162 (2015). doi:10.1016/j.ijpddr.2015.07.001
100. Boss, C., Aissaoui, H., Amaral, N., Bauer, A., Bazire, S., Binkert, C., Brun, R., Bürki, C., Ciana, C.-L., Corminboeuf, O., Delahaye, S., Dollinger, C., Fischli, C., Fischli, W., Flock, A., Frantz, M.-C., Girault, M., Grisostomi, C., Friedli, A., Heidmann, B., Hinder, C., Jacob, G., Le Bihan, A., Malrieu, S., Mamzed, S., Merot, A., Meyer, S., Peixoto, S., Petit, N., Siegrist, R., Trollux, J., Weller, T., Wittlin, S.: Discovery and characterization of ACT-451840: an antimalarial drug with a novel mechanism of action. ChemMedChem **11**(18), 1995–2014 (2016). doi:10.1002/cmdc.201600298
101. Bihan, A.L., de Kanter, R., Angulo-Barturen, I., Binkert, C., Boss, C., Brun, R., Brunner, R., Buchmann, S., Burrows, J., Dechering, K.J., Delves, M., Ewerling, S., Ferrer, S., Fischli, C., Gamo–Benito, F.J., Gnädig, N.F., Heidmann, B., Jiménez-Díaz, M.B., Leroy, D., Martínez, M.S., Meyer, S., Moehrle, J.J., Ng, C.L., Noviyanti, R., Ruecker, A., Sanz, L.M., Sauerwein, R.W., Scheurer, C., Schleiferboeck, S., Sinden, R., Snyder, C., Straimer, J., Wirjanata, G., Marfurt, J., Price, R.N., Weller, T., Fischli, W., Fidock, D.A., Clozel, M., Wittlin, S.: Characterization of novel antimalarial compound ACT-451840: Preclinical assessment of activity and dose-efficacy modeling. PLOS Medicine **13**(10), 1002138 (2016). doi:10.1371/journal.pmed.1002138
102. Krause, A., Dingemanse, J., Mathis, A., Marquart, L., Möhrle, J.J., McCarthy, J.S.: Pharmacokinetic/pharmacodynamic modelling of the antimalarial effect of actelion-451840 in an induced blood stage malaria study in healthy subjects. British Journal of Clinical Pharmacology **82**(2), 412–421 (2016). doi:10.1111/bcp.12962
103. Charman, S.A., Arbe-Barnes, S., Bathurst, I.C., Brun, R., Campbell, M., Charman, W.N., Chiu, F.C.K., Chollet, J., Craft, J.C., Creek, D.J., Dong, Y., Matile, H., Maurer, M., Morizzi, J., Nguyen, T., Papastogiannidis, P., Scheurer, C., Shackleford, D.M., Sriraghavan, K., Stingelin, L., Tang, Y., Urwyler, H., Wang, X., White, K.L., Wittlin, S., Zhou, L., Vennerstrom, J.L.: Synthetic ozonide drug candidate OZ439 offers new hope for a single-dose cure of uncomplicated malaria. Proceedings of the National Academy of Sciences **108**(11), 4400–4405 (2011). doi:10.1073/pnas.1015762108
104. Dong, Y., Wittlin, S., Sriraghavan, K., Chollet, J., Charman, S.A., Charman, W.N., Scheurer, C., Urwyler, H., Tomas, J.S., Snyder, C., Creek, D.J., Morizzi, J., Koltun, M., Matile, H., Wang, X., Padmanilayam, M., Tang, Y., Dorn, A., Brun, R., Vennerstrom, J.L.: The structure-activity relationship of the antimalarial ozonide arterolane (OZ277). Journal of Medicinal Chemistry **53**(1), 481–491 (2010). doi:10.1021/jm901473s
105. Dong, Y., Chollet, J., Matile, H., Charman, S.A., Chiu, F.C.K., Charman, W.N., Scorneaux, B., Urwyler, H., Tomas, J.S., Scheurer, C., Snyder, C., Dorn, A., Wang, X., Karle, J.M., Tang, Y., Wittlin, S., Brun, R., Vennerstrom, J.L.: Spiro and dispiro-1,2,4-trioxolanes as antimalarial peroxides: Charting a workable structure-activity relationship using simple prototypes. Journal of Medicinal Chemistry **48**(15), 4953–4961 (2005). doi:10.1021/jm049040u
106. Dong, Y., Tang, Y., Chollet, J., Matile, H., Wittlin, S., A.Charman, S., N.Charman, W., Tomas, J.S., Scheurer, C., Snyder, C., Scorneaux, B., Bajpai, S., Alexander, S.A., Wang, X., Padmanilayam, M., Cheruku, S.R., Brun, R., Vennerstrom, J.L.: Effect of functional group polarity on the antimalarial activity of spiro and dispiro-1,2,4-trioxolanes. Bioorganic & Medicinal Chemistry **14**(18), 6368–6382 (2006). doi:10.1016/j.bmc.2006.05.041
107. Kaiser, M., Wittlin, S., Nehrbass-Stuedli, A., Dong, Y., Wang, X., Hemphill, A., Matile, H., Brun, R., Vennerstrom, J.L.: Peroxide bond-dependent antiplasmodial specificity of artemisinin and OZ277 (RBx11160). Antimicrobial Agents and Chemotherapy **51**(8), 2991–2993 (2007). doi:10.1128/aac.00225-07
108. Dong, Y., Wang, X., Kamaraj, S., Bulbule, V.J., Chiu, F.C.K., Chollet, J., Dhanasekaran, M., Hein, C.D., Papastogiannidis, P., Morizzi, J., Shackleford, D.M., Barker, H., Ryan, E., Scheurer, C., Tang, Y., Zhao, Q., Zhou, L., White, K.L., Urwyler, H., Charman, W.N., Matile, H., Wittlin, S., Charman, S.A., Vennerstrom, J.L.: Structure–activity relationship of the antimalarial ozonide artefenomel (OZ439). Journal of Medicinal Chemistry **60**(7), 2654–2668 (2017). doi:10.1021/acs.jmedchem.6b01586
109. Phyo, A.P., Jittamala, P., Nosten, F.H., Pukrittayakamee, S., Imwong, M., White, N.J., Duparc, S., Macintyre, F., Baker, M., Möhrle, J.J.: Antimalarial activity of artefenomel (OZ439), a novel synthetic antimalarial endoperoxide, in patients with plasmodium falciparum and plasmodium vivax malaria: an open-label phase 2 trial. The Lancet Infectious Diseases **16**(1), 61–69 (2016). doi:10.1016/s1473-3099(15)00320-5
110. Gautam, A., Ahmed, T., Sharma, P., Varshney, B., Kothari, M., Saha, N., Roy, A., Moehrle, J.J., Paliwal, J.: Pharmacokinetics and pharmacodynamics of arterolane maleate following multiple oral doses in adult patients with *P. falciparum* malaria. The Journal of Clinical Pharmacology **51**(11), 1519–1528 (2011). doi:10.1177/0091270010385578
111. Valecha, N., Krudsood, S., Tangpukdee, N., Mohanty, S., Sharma, S.K., Tyagi, P.K., Anvikar, A., Mohanty, R., Rao, B.S., Jha, A.C., Shahi, B., Singh, J.P.N., Roy, A., Kaur, P., Kothari, M., Mehta, S., Gautam, A., Paliwal, J.K., Arora, S., Saha, N.: Arterolane maleate plus piperaquine phosphate for treatment of uncomplicated plasmodium falciparum malaria: A comparative, multicenter, randomized clinical trial. Clinical Infectious Diseases **55**(5), 663–671 (2012). doi:10.1093/cid/cis475
112. Jourdan, J., Matile, H., Reift, E., Biehlmaier, O., Dong, Y., Wang, X., Mäser, P., Vennerstrom, J.L., Wittlin, S.: Monoclonal antibodies that recognize the alkylation signature of antimalarial ozonides OZ277 (Arterolane) and OZ439 (Artefenomel). ACS Infectious Diseases **2**(1), 54–61 (2015). doi:10.1021/acsinfecdis.5b00090
113. Allman, E.L., Painter, H.J., Samra, J., Carrasquilla, M., Llinás, M.: Metabolomic profiling of the malaria box reveals antimalarial target pathways. Antimicrobial Agents and Chemotherapy **60**(11), 6635–6649 (2016). doi:10.1128/aac.01224-16
114. Moehrle, J.J., Duparc, S., Siethoff, C., van Giersbergen, P.L.M., Craft, J.C., Arbe-Barnes, S., Charman, S.A., Gutierrez, M., Wittlin, S., Vennerstrom, J.L.: First-in-man safety and pharmacokinetics of synthetic ozonide OZ439 demonstrates an improved exposure profile relative to other peroxide antimalarials. British Journal of Clinical Pharmacology **75**(2), 535–548 (2013). doi:10.1111/j.1365-2125.2012.04368.x
115. McCarthy, J.S., Baker, M., O'Rourke, P., Marquart, L., Griffin, P.,

van Huijsduijnen, R.H., Möhrle, J.J.: Efficacy of OZ439 (artefenomel) against early *Plasmodium falciparum* blood-stage malaria infection in healthy volunteers. Journal of Antimicrobial Chemotherapy **71**(9), 2620–2627 (2016). doi:10.1093/jac/dkw174

116. Plouffe, D., Brinker, A., McNamara, C., Henson, K., Kato, N., Kuhen, K., Nagle, A., Adrián, F., Matzen, J.T., Anderson, P., Nam, T.-g., Gray, N.S., Chatterjee, A., Janes, J., Yan, S.F., Trager, R., Caldwell, J.S., Schultz, P.G., Zhou, Y., Winzeler, E.A.: In silico activity profiling reveals the mechanism of action of antimalarials discovered in a high-throughput screen. Proceedings of the National Academy of Sciences **105**(26), 9059–9064 (2008). doi:10.1073/pnas.0802982105
117. Nagle, A., Wu, T., Kuhen, K., Gagaring, K., Borboa, R., Francek, C., Chen, Z., Plouffe, D., Lin, X., Caldwell, C., Ek, J., Skolnik, S., Liu, F., Wang, J., Chang, J., Li, C., Liu, B., Hollenbeck, T., Tuntland, T., Isbell, J., Chuan, T., Alper, P.B., Fischli, C., Brun, R., Lakshminarayana, S.B., Rottmann, M., Diagana, T.T., Winzeler, E.A., Glynne, R., Tully, D.C., Chatterjee, A.K.: Imidazolopiperazines: Lead optimization of the second-generation antimalarial agents. Journal of Medicinal Chemistry **55**(9), 4244–4273 (2012). doi:10.1021/jm300041e
118. Wu, T., Nagle, A., Kuhen, K., Gagaring, K., Borboa, R., Francek, C., Chen, Z., Plouffe, D., Goh, A., Lakshminarayana, S.B., Wu, J., Ang, H.Q., Zeng, P., Kang, M.L., Tan, W., Tan, M., Ye, N., Lin, X., Caldwell, C., Ek, J., Skolnik, S., Liu, F., Wang, J., Chang, J., Li, C., Hollenbeck, T., Tuntland, T., Isbell, J., Fischli, C., Brun, R., Rottmann, M., Dartois, V., Keller, T., Diagana, T., Winzeler, E., Glynne, R., Tully, D.C., Chatterjee, A.K.: Imidazolopiperazines: Hit to lead optimization of new antimalarial agents. Journal of Medicinal Chemistry **54**(14), 5116–5130 (2011). doi:10.1021/jm2003359
119. Kuhen, K.L., Chatterjee, A.K., Rottmann, M., Gagaring, K., Borboa, R., Buenviaje, J., Chen, Z., Francek, C., Wu, T., Nagle, A., Barnes, S.W., Plouffe, D., Lee, M.C.S., Fidock, D.A., Graumans, W., van de Vegte-Bolmer, M., van Gemert, G.J., Wirjanata, G., Sebayang, B., Marfurt, J., Russell, B., Suwanarusk, R., Price, R.N., Nosten, F., Tungtaeng, A., Gettayacamin, M., Sattabongkot, J., Taylor, J., Walker, J.R., Tully, D., Patra, K.P., Flannery, E.L., Vinetz, J.M., Renia, L., Sauerwein, R.W., Winzeler, E.A., Glynne, R.J., Diagana, T.T.: KAF156 is an antimalarial clinical candidate with potential for use in prophylaxis, treatment, and prevention of disease transmission. Antimicrobial Agents and Chemotherapy **58**(9), 5060–5067 (2014). doi:10.1128/aac.02727-13
120. Rottmann, M., McNamara, C., Yeung, B.K.S., Lee, M.C.S., Zou, B., Russell, B., Seitz, P., Plouffe, D.M., Dharia, N.V., Tan, J., Cohen, S.B., Spencer, K.R., Gonzalez-Paez, G.E., Lakshminarayana, S.B., Goh, A., Suwanarusk, R., Jegla, T., Schmitt, E.K., Beck, H.-P., Brun, R., Nosten, F., Renia, L., Dartois, V., Keller, T.H., Fidock, D.A., Winzeler, E.A., Diagana, T.T.: Spiroindolones, a potent compound class for the treatment of malaria. Science **329**(5996), 1175–1180 (2010). doi:10.1126/science.1193225
121. Yeung, B.K.S., Zou, B., Rottmann, M., Lakshminarayana, S.B., Ang, S.H., Leong, S.Y., Tan, J., Wong, J., Keller-Maerki, S., Fischli, C., Goh, A., Schmitt, E.K., Krastel, P., Francotte, E., Kuhen, K., Plouffe, D., Henson, K., Wagner, T., Winzeler, E.A., Petersen, F., Brun, R., Dartois, V., Diagana, T.T., Keller, T.H.: Spirotetrahydro β-carbolines (spiroindolones): A new class of potent and orally efficacious compounds for the treatment of malaria. Journal of Medicinal Chemistry **53**(14), 5155–5164 (2010). doi:10.1021/jm100410f
122. White, N.J., Pukrittayakamee, S., Phyo, A.P., Rueangweerayut, R., Nosten, F., Jittamala, P., Jeeyapant, A., Jain, J.P., Lefèvre, G., Li, R., Magnusson, B., Diagana, T.T., Leong, F.J.: Spiroindolone KAE609 for falciparum and vivax malaria. New England Journal of Medicine **371**(5), 403–410 (2014). doi:10.1056/nejmoa1315860
123. Coteron, J.M., Marco, M., Esquivias, J., Deng, X., White, K.L., White, J., Koltun, M., Mazouni, F.E., Kokkonda, S., Katneni, K., Bhamidipati, R., Shackleford, D.M., Angulo-Barturen, I., Ferrer, S.B., Jiménez-Díaz, M.B., Gamo, F.-J., Goldsmith, E.J., Charman, W.N., Bathurst, I., Floyd, D., Matthews, D., Burrows, J.N., Rathod, P.K., Charman, S.A., Phillips, M.A.: Structure-guided lead optimization of triazolopyrimidine-ring substituents identifies potent plasmodium falciparum dihydroorotate dehydrogenase inhibitors with clinical candidate potential. Journal of Medicinal Chemistry **54**(15), 5540–5561 (2011). doi:10.1021/jm200592f
124. McCarthy, J.S., Lotharius, J., Rückle, T., Chalon, S., Phillips, M.A., Elliott, S., Sekuloski, S., Griffin, P., Ng, C.L., Fidock, D.A., Marquart, L., Williams, N.S., Gobeau, N., Bebrevska, L., Rosario, M., Marsh, K., Möhrle, J.J.: Safety, tolerability, pharmacokinetics, and activity of the novel long-acting antimalarial DSM265: a two-part first-in-human phase 1a/1b randomised study. The Lancet Infectious Diseases **17**(6), 626–635 (2017). doi:10.1016/s1473-3099(17)30171-8
125. Nigussie, D., Beyene, T.: New targets in malaria parasite chemotherapy: A review. Malaria Control & Elimination **s1** (2015). doi:10.4172/2470-6965.1000s1-007
126. Jorgensen, R., Merrill, A.R., Andersen, G.R.: The life and death of translation elongation factor 2. Biochemical Society Transactions **34**(1), 1–6 (2006). doi:10.1042/bst0340001
127. Spillman, N.J., Allen, R.J.W., McNamara, C.W., Yeung, B.K.S., Winzeler, E.A., Diagana, T.T., Kirk, K.: Na^+ regulation in the malaria parasite plasmodium falciparum involves the cation ATPase PfATP4 and is a target of the spiroindolone antimalarials. Cell Host & Microbe **13**(2), 227–237 (2013). doi:10.1016/j.chom.2012.12.006
128. Open Source Malaria Series 4 GitHub Repository. Accessed 17 June 2018. `https://github.com/OpenSourceMalaria/Series4`
129. Voorhis, W.C.V., Adams, J.H., Adelfio, R., Ahyong, V., Akabas, M.H., Alano, P., Alday, A., Resto, Y.A., Alsibaee, A., Alzualde, A., Andrews, K.T., Avery, S.V., Avery, V.M., Ayong, L., Baker, M., Baker, S., Mamoun, C.B., Bhatia, S., Bickle, Q., Bounaadja, L., Bowling, T., Bosch, J., Boucher, L.E., Boyom, F.F., Brea, J., Brennan, M., Burton, A., Caffrey, C.R., Camarda, G., Carrasquilla, M., Carter, D., Cassera, M.B., Cheng, K.C.-C., Chindaudomsate, W., Chubb, A., Colon, B.L., Colón-López, D.D., Corbett, Y., Crowther, G.J., Cowan, N., D'Alessandro, S., Dang, N.L., Delves, M., DeRisi, J.L., Du, A.Y., Duffy, S., El-Sayed, S.A.E.-S., Ferdig, M.T., Robledo, J.A.F., Fidock, D.A., Florent, I., Fokou, P.V.T., Galstian, A., Gamo, F.J., Gokool, S., Gold, B., Golub, T., Goldgof, G.M., Guha, R., Guiguemde, W.A., Gural, N., Guy, R.K., Hansen, M.A.E., Hanson, K.K., Hemphill, A., van Huijsduijnen, R.H., Horii, T., Horrocks, P., Hughes, T.B., Huston, C., Igarashi, I., Ingram-Sieber, K., Itoe, M.A., Jadhav, A., Jensen, A.N., Jensen, L.T., Jiang, R.H.Y., Kaiser, A., Keiser, J., Ketas, T., Kicka, S., Kim, S., Kirk, K., Kumar, V.P., Kyle, D.E., Lafuente, M.J., Landfear, S., Lee, N., Lee, S., Lehane, A.M., Li, F., Little, D., Liu, L., Llinás, M., Loza, M.I., Lubar, A., Lucantoni, L., Lucet, I., Maes, L., Mancama, D., Mansour, N.R., March, S., McGowan, S., Vera, I.M., Meister, S., Mercer, L., Mestres, J., Mfopa, A.N., Misra, R.N., Moon, S., Moore, J.P., da Costa, F.M.R., Müller, J., Muriana, A., Hewitt, S.N., Nare, B., Nathan, C., Narraidoo, N., Nawaratna, S., Ojo, K.K., Ortiz, D., Panic, G., Papadatos, G., Parapini, S., Patra, K., Pham, N., Prats, S., Plouffe, D.M., Poulsen, S.-A., Pradhan, A., Quevedo, C., Quinn, R.J., Rice, C.A., Rizk, M.A., Ruecker, A., Onge, R.S., Ferreira, R.S., Samra, J., Robinett, N.G., Schlecht, U., Schmitt, M., Villela, F.S., Silvestrini, F., Sinden, R., Smith, D.A., Soldati, T., Spitzmüller, A., Stamm, S.M., Sullivan, D.J., Sullivan, W., Suresh, S., Suzuki, B.M., Suzuki, Y., Swamidass, S.J., Taramelli, D., Tchokouaha, L.R.Y., Theron, A., Thomas, D., Tonissen, K.F., Townson, S., Tripathi, A.K., Trofimov, V., Udenze, K.O., Ullah, I., Vallieres, C., Vigil, E., Vinetz, J.M., Vinh, P.V., Vu, H., Watanabe, N.-a., Weatherby, K., White, P.M., Wilks, A.F., Winzeler, E.A., Wojcik, E., Wree, M., Wu, W., Yokoyama, N., Zollo, P.H.A., Abla, N., Blasco, B., Burrows, J., Laleu, B., Leroy, D., Spangenberg, T., Wells, T., Willis, P.A.: Open source drug discovery with the malaria box compound collection for neglected diseases and beyond. PLOS Pathogens **12**(7), 1005763 (2016). doi:10.1371/journal.ppat.1005763
130. Dennis, A.S.M., Rosling, J.E.O., Lehane, A.M., Kirk, K.: Diverse antimalarials from whole-cell phenotypic screens disrupt malaria parasite ion and volume homeostasis. Scientific Reports **8**(1) (2018). doi:10.1038/s41598-018-26819-1
131. Saliba, K.J., Kirk, K.: pH regulation in the intracellular malaria parasite,plasmodium falciparum. Journal of Biological Chemistry **274**(47), 33213–33219 (1999). doi:10.1074/jbc.274.47.33213
132. Baldwin, J., Farajallah, A.M., Malmquist, N.A., Rathod, P.K., Phillips, M.A.: Malarial dihydroorotate dehydrogenase. Journal of Biological Chemistry **277**(44), 41827–41834 (2002).

doi:10.1074/jbc.m206854200
133. Phillips, M.A., Lotharius, J., Marsh, K., White, J., Dayan, A., White, K.L., Njoroge, J.W., Mazouni, F.E., Lao, Y., Kokkonda, S., Tomchick, D.R., Deng, X., Laird, T., Bhatia, S.N., March, S., Ng, C.L., Fidock, D.A., Wittlin, S., Lafuente-Monasterio, M., Benito, F.J.G., Alonso, L.M.S., Martinez, M.S., Jimenez-Diaz, M.B., Bazaga, S.F., Angulo-Barturen, I., Haselden, J.N., Louttit, J., Cui, Y., Sridhar, A., Zeeman, A.-M., Kocken, C., Sauerwein, R., Dechering, K., Avery, V.M., Duffy, S., Delves, M., Sinden, R., Ruecker, A., Wickham, K.S., Rochford, R., Gahagen, J., Iyer, L., Riccio, E., Mirsalis, J., Bathhurst, I., Rueckle, T., Ding, X., Campo, B., Leroy, D., Rogers, M.J., Rathod, P.K., Burrows, J.N., Charman, S.A.: A long-duration dihydroorotate dehydrogenase inhibitor (DSM265) for prevention and treatment of malaria. Science Translational Medicine **7**(296), 296–111296111 (2015). doi:10.1126/scitranslmed.aaa6645
134. Yuthavong, Y., Yubaniyama, J., Chitnumsub, P., Vanichtanankul, J., Chusacultanachai, S., Tarnchompoo, B., Vilaivan, T., Kamchonwongpaisan, S.: Malarial (plasmodium falciparum) dihydrofolate reductase-thymidylate synthase: structural basis for antifolate resistance and development of effective inhibitors. Parasitology **130**(3), 249–259 (2005). doi:10.1017/s003118200400664x
135. Luth, M.R., Gupta, P., Ottilie, S., Winzeler, E.A.: Using in vitro evolution and whole genome analysis to discover next generation targets for antimalarial drug discovery. ACS Infectious Diseases **4**(3), 301–314 (2018). doi:10.1021/acsinfecdis.7b00276

Figures

Additional Files

MMV pipeline compounds reference list
List of SciFinder reference dois for each compound described in this review.

Biological data for MMV pipeline compounds
Tabulation of biological potencies and pharmacokinetic properties for each compound described in this review.