*Perspective*

**Antimalarial Chemotherapy: Natural Product Inspired Development of Pre-clinical and Clinical Candidates with Diverse Mechanisms of Action**

**Elena Fernández-Álvaro,† W. David Hong,‡ Gemma L Nixon, ‡ Paul M O’Neill ‡ and Félix Calderón†,\***

**†** Diseases of the Developing World- Tres Cantos Medicines Development Campus. GlaxoSmithKline. c/ Severo Ochoa, 2. 28760, Tres Cantos, Madrid, Spain

**‡** Robert Robinson Laboratories, Department of Chemistry, University of Liverpool, Crown St, Liverpool L69 7ZD, UK.

**Abstract:** Natural products have played a pivotal role in malaria chemotherapy progressing from quinine and artemisinin through to ozonide-based compounds. Many of these natural products have served as template for the design and development of antimalarial drugs currently in the clinic or in the development phase. In this perspective article, we will detail those privileged scaffolds which have guided medicinal chemistry efforts yielding molecules that have reached the clinic.

**Keywords:** antimalarial, natural products, drug discovery, *Plasmodium*

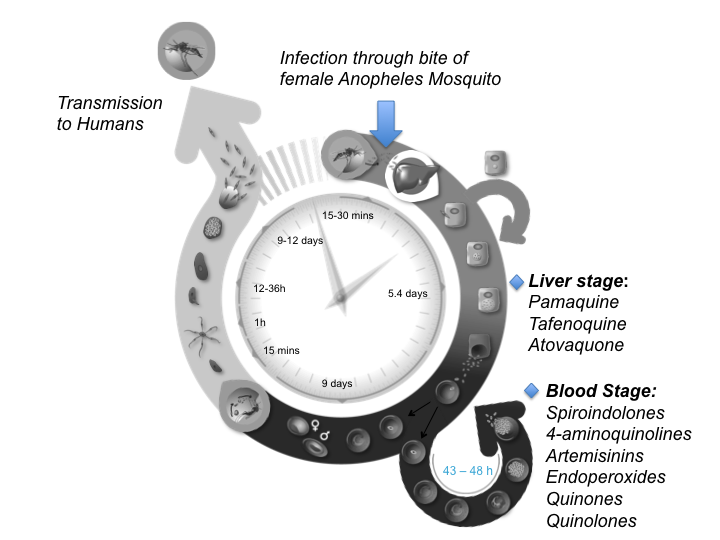
1. **Introduction**
   1. **Malaria burden**

Despite efforts to eradicate Malaria in the last century, the disease remains a major global health problem. The 2014 global Malaria report published by the WHO estimated 128 million cases of Malaria which caused 584,000 deaths in 2013.[1](#_ENREF_1) Malaria is particularly devastating in Africa where 90% of reported deaths are children under the age of 5. Malaria has a severe socio-economic impact in countries where it is endemic because of the persistent and disabling symptoms of the disease. Approximately 25% of the endemic countries incomes are devoted to treating and minimizing the impact of this disease. In the African continent alone the economic burden is estimated at $12 billion annually.[2](#_ENREF_2)

Several strategies are currently being deployed in parallel to fight this disease. These strategies include the use of vaccines, novel drugs and vector control methods. Currently, the most advance Malaria vaccine, RTS,S/AS01, is in clinical development. Results from a Phase III clinical trial have shown that RTS,S/AS01 is able to prevent many cases, particularly amongst children in high impact areas.[3](#_ENREF_3)However, strategies to control the mosquito (including insecticide-treated mosquito nets and indoor residual spraying) are at risk due to increase resistance to the insecticides used (mainly pyrethroids)[4](#_ENREF_4). Similarly, first-line drug based treatments including Artemisin combination therapies (ACTs) are at risk due to the emergence of resistance. [5](#_ENREF_5)

* 1. **Malaria life cycle**

Malaria is a mosquito-borne protozoal infection caused by parasites of the genus *Plasmodium* with five species currently known to cause the disease in humans (*P. falciparum, P. ovale, P. malariae, P. vivax and P. knowlesi*). Disease in humans is initiated when an infected female *Anopheles* mosquito takes a blood meal during which infective forms of the parasite (named sporozoites) are injected into the blood stream (Figure 1).[6](#_ENREF_6) Within 15-30 minutes sporozoites reach the liver and infect hepatocytes where they develop into liver schizonts that undergo mitosis to produce thousands of infective merozoites. These merozoites are released into the blood stream and invade red blood cells where they undergo several cycles of asexual replication. Merozoites inside erythrocytes evolve to ring-stage parasites, that become trophozoites and then multinucleated schizonts. Schizonts produce another wave of infective merozoites that invade new erythrocytes and the cycle starts again. It is this asexual erythrocytic phase of the parasite lifecycle that causes the clinical symptoms of fever, fatigue and chills that can lead to coma and even death. At a given time point of infection and for reasons unknown, part of the asexual merozoite population will evolve into sexual gametocytes which are transferred to another mosquito in the next blood meal. Once in the mosquito, gametocytes will develop into gametes that reproduce sexually to form the zygote that evolves to ookinete and then to oocyst. The oocyst liberates thousands of sporozoites that migrate to mosquito salivary glands and are inoculated in the next blood meal enabling infection of the next host hereby completing the parasite lifecycle. In the case of *P. vivax* and *P.* *ovale* some of the sporozoites develop in the liver and persist as dormant forms called hypnozoites. After weeks or even months, hypnozoites can reactivate to produce infective merozoites causing malaria relapse. Insights into how quiescent hepatic forms resume normal growth are becoming clearer but the exact mechanism of reactivation is still unknown.[7](#_ENREF_7)



**Figure 1.** Malaria life cycle and Site of Action of Antimalarials Covered in this Review [8](#_ENREF_8)

* 1. **The natural antimalarial arsenal**

Akin to many other infectious diseases, malaria drug discovery has greatly benefited from nature and its products. The current gold-standard treatment, Artemisinin, is a well-known historical antipyretic treatment used in Traditional Chinese Medicine. Since 2009 there have been reported cases of slower parasite clearance on treatment with ACTs in the region around the Thai-Cambodia border.[9](#_ENREF_9) This evidence, along with widespread resistance to other historical antimalarials, highlights the need to identify new chemical diversity, ideally with novel antimalarial modes of action. This will lessen the likelihood of resistance appearing in next-generation antimalarials particularly if they are used in affordable combination therapies.

From a drug-development perspective, natural products can be troublesome as starting points for optimization programs as they are commonly associated with complex synthetic routes, high cost of goods (CoGs) and poor drug like properties due to their low ligand efficiency and high molecular weight.[10](#_ENREF_10) However, positive outcomes for malaria drug discovery based on natural products are supported by the following:

1. Malaria chemotherapy has always been successfully influenced by natural products.
2. Latest technological advances are now addressing issues associated with natural products, especially challenges associated with identifying active components and difficulties with their synthesis.
3. The functional-group diversity and architectural platforms engineered into natural products during biosynthesis continue to provide biologically active mimics and selective ligands for cellular targets.

In recent years, thousands of new antimalarial hits have been released into the public domain.[11](#_ENREF_11) These hits come from phenotypic screens of several corporate collections against intraerythrocytic stages of the human parasite using diverse high throughput formats. But, the history of antimalarial chemotherapy is based on the use of natural products so nature should not be discounted as being a source of new scaffolds in the future. In fact, there is now evidence that novel scaffolds derived from plants are playing a role in the development of new antimalarial drugs.[12](#_ENREF_14)

This article will discuss how natural products have served as the structural basis for antimalarial drug discovery programs which have subsequently afforded clinical candidates. This is not intended to be a review of the antimalarial chemical diversity that can be found in nature, this information can be found in several recent excellent reviews.[13](#_ENREF_15)

1. **Natural product scaffolds as antimalarials**
   1. **Quinolines**

One of the most prominent antimalarial scaffolds derived from nature is the quinolone template.[14](#_ENREF_19) Quechua Indians of Peru and Bolivia realized that the bark of *Cinchona sp.* was able to reduce fever. The powdered bark of the “fever tree” was widely distributed in Europe by the Jesuits during the 17th century but it was not until 1820 when quinine (**1**), the major alkaloid of *Cinchona* plant was isolated and found to be responsible for the antipyretic effect. Subsequently, the historical development of quinine and its derivatives as antimalarials is littered with both success and failure. The template has inspired the development of most marketed antimalarials, however it is also associated with well-defined toxicological issues. Until very recently quinine has been the backbone of the standard of care defined by WHO in the treatment of cerebral malaria.

Quinine has diverse pharmacological portfolio. In additional to its antimalarial activity it has been found to possess anticholinergic,[15](#_ENREF_20) antihypertensive,[16](#_ENREF_21) hypoglycemic,[17](#_ENREF_22) potassium channel blocker,[18](#_ENREF_23) and skeletal muscle relaxant effects[19](#_ENREF_24). It is also used worldwide in tonic water as a flavoring.[20](#_ENREF_25)

Chemically, quinine is quinolone methanol derivative with a low molecular weight as well as having good physicochemical properties.[21](#_ENREF_26) As is the case with many other natural products, isolation of quinine from its natural source in quantities required to satisfy an ever increasing demand proved challenging. Subsequently chemistry groups initiated work on its total synthesis. In fact, it proved to be one of the remarkable achievements in the art of synthetic chemistry.[22](#_ENREF_27) The debate started in 1945 when Woodward and Doering published the “Total Synthesis of Quinine”.[23](#_ENREF_28) What Woodward and Doering actually achieved was the synthesis of *d*-quinotoxine whose transformation to quinine was initially reported by Rabe and Kindler.[22](#_ENREF_27), [24](#_ENREF_29) It was not until 2001 that Stork published the first stereoselective synthesis of quinine.[25](#_ENREF_30)

In the quest for a synthetic antimalarial which could satisfy the existing demand, an alternative to quinine, methylene-blue (**2**, MB), was discovered by Paul Ehrlich at the end of the 19th century.[26](#_ENREF_31) This compound was cheap and could easily be manufactured on a large scale. At that point in time, quinine could only be extracted from the Cinchona tree, which limited its supply. MB exerts its antimalarial activity by specifically inhibiting the *P. falciparum* glutathione reductase and so preventing the polymerization of haem into haemozoin. The use of MB was minimized after the discovery of the synthetic 4-aminoquinolines which are still today a class of compounds under discussion as a potential tool to reverse resistance to current first line treatments.[27](#_ENREF_32)

The discovery of the antimalarial properties of MB led to the development of the first synthetic quinoline. In the 1920s German scientist developed the 8-aminoquinoline, Pamaquine (**3**). Although less effective than MB against *P. falciparum*, Pamaquine was effective at preventing *P. vivax* relapse.[28](#_ENREF_33) The alkyl chain of Pamaquine was used for another quinoline-like compound that was developed as a potential replacement for quinine: Aminoacridine, named quinacrine (**4**, Atebrine® and Mepacrine®). This new derivative was developed by Bayer and approved in 1930 having displayed good efficacy in avian models of malaria. Although quinacrine did not present huge advantages over quinine in terms of safety and physicochemical profile, there were no supply limitations as this compound is synthetically accessible.[26](#_ENREF_31) Bayer replaced the acridine ring of quinacrine with the quinoline ring whilst maintaining the alkyl chain; this effort yielded one of the most important quinoline-based antimalarials in history, chloroquine (CQ, **5**), latterly registered as Resochin. Although very effective, the new synthetic antimalarial was ignored for ten years as it was thought to be toxic in humans (this issue is known as the resochin error). Resochin and its methylated version Sontochin (**6**) were patented in 1939 but it was not until the mid-1940’s when the potential of Resochin was elucidated and formally named chloroquine.



**Figure 2.** Key compounds in the race for a quinine (**1**) replacement: methylene blue (**2**), Pamaquine (**3**), Quinacrine (**4**), Chloroquine (**5**) and Sontochin (**6**).

In 1947 chloroquine entered clinical practice and was the most widely used antimalarial up until the 1970’s despite a poor therapeutic index. Ten years later the first CQ-resistant *P. falciparum* isolates were identified and today CQ is no longer recommended for *P. falciparum* malaria treatment,[29](#_ENREF_34) although it is still recommended for treatment of other human malaria parasites like *P. vivax* for which CQ- resistance has only been detected in Indonesia, Irian Jaya and several countries in the Pacific region.[30](#_ENREF_35) Several medicinal chemistry approaches to overcome resistance to CQ have been described and have afford promising compounds: (Figure 3) modification of the alkyl chain by shortening it (i.e. AQ13 (**7**) or F2Bu (**8**))[31](#_ENREF_36) or by addition of an aromatic moiety (i.e. amodiaquine (AQ, **9**), isoquine (**10**), fluoroamodiaquine (**11**), *tert*-butylisoquine (**12**), tebuqine (**13**), naphthoquine (**14**) ferroquine (**15**)), dimer formation (**16** and Piperaquine **17**)[32](#_ENREF_37) etc. Only one of these analogues has reached the market: piperaquine, which is currently marketed in combination with dihydroartemisinin and branded as Euartesim®. Euartesim is being developed by Medicines for Malaria Venture (MMV) in partnership with Sigma-TAU.[33](#_ENREF_38) AQ13 and ferroquine are being evaluated in Phase II clinical trials.[34](#_ENREF_41)



**Figure 3.** Key exemplars obtained after medicinal chemistry efforts focused on overcoming CQ resistance.

The emergence of CQ resistance in the 1970s reinvigorated research efforts. In a screening campaign at the Walter Reed Army Institute for Research (WRAIR) a promising quinoline-containing hit was identified: Mefloquine (MF, **18** Lariam®), and because of need, was marketed even before Phase III clinical trials.[35](#_ENREF_42) Although MF treatment is associated with some safety concerns (central nervous system and gastro intestinal side effects),[36](#_ENREF_43) it is still used in practice with only a low level of resistance being reported thus far. In 2013 the FDA approved label change for MF and now a box warning that neurological and psychiatric side effects may persist or become permanent is included.[37](#_ENREF_45)

After the Second World War there was a renewed interest for 8-aminoquinolines. pamaquine demonstrated that this scaffold has the added value of being active against liver hypnozoites and therefore can be used to tackle malaria relapse.[28](#_ENREF_33) Extensive research resulted in the development of the 8-aminoquinoline, primaquine (PQ, **19**). Currently PQ is the only marketed antimalarial with activity against hypnozoites[38](#_ENREF_46" \o "Wells, 2010 #71) although another 8-aminoquinoline, tafenoquine (TFQ, **20**) is now in Phase III clinical trials as a radical cure for *P. vivax* relapsing malaria.[39](#_ENREF_48)



**Figure 4.** Mefloquine and other 8-aminoquinolines

Whilst the use of the quinoline class of anti-malarials is limited by resistance and the potential toxicity exhibited by some of the currently available drugs within this class, the importance of quinoline anti-malarials and the potential for further development should not be overlooked. Quinolines have the lowest cost of goods and the richest clinical data available of all clinically used antimalarials derived from natural products. It is also the only class of antimalarial that has proven to be efficacious for against liver hypnozites and *P. vivax*.

In spite of the rich history of quinolines, especially 4-aminoquinolines as antimalarials, there is still some mystery surrounding this class of compounds. It is accepted that the hemoglobin breakdown product heme is the target for the 4-aminoquinolines and a recent study by Egan provided direct evidence for the effect of chloroquine on hemozoin formation in the intact malaria cell. 39 It is suggested that free heme diffuses out of the digestive vacuole in it protonated form to accumulate in the cytosolic compartment where it can interact with membranes. However, the ultimate mechanism by which the “free heme” induces parasite death is still not clear.[40](#_ENREF_49)

Clarifying the mechanism of action of the 8-aminoquinolines along with the mechanism of toxicity is essential to enable medicinal chemistry approaches to designing safer alternatives to primaquine and tafenoquine. On the pathway to malaria eradication, it is foreseeable that these aspects of quinoline antimalarials are becoming more and more significant.

* 1. **Artemisinins and Endoperoxides**

Artemisinin (qinghaosu, ART, **21**) is a sesquiterpene lactone and endoperoxide-containing natural product isolated from leaves of the Chinese plant *Artemisia annua* (qinghao or sweet wormwood). In traditional Chinese medicine the qinghao plant is used as an antipyretic remedy, although artemisinin was not discovered to be the active ingredient until 1971. A research program initiated by the Chinese Army designed to find improvements for malaria treatment identified Artemisinin as the active ingredient, which was successfully isolated by low temperature ethyl ether extraction from *A. annua* leaves. Subsequently, the molecule was further characterized and derivatives were synthesized. Even though this was first published in 1979, Artemisinin combination therapies are still the recommended standard of care today to treat malaria in endemic countries.[41](#_ENREF_50)

Furthermore, Artemisinins are being investigated as anti-cancer agents because they inhibit angiogenesis and cell growth in several neoplastic cell models.[42](#_ENREF_51) These compounds have demonstrated efficacy in the treatment of schistosomiasis and fasciolasis and in animal models of *Clonorchis* infection.[43](#_ENREF_52)

Although the intracellular molecular target and antimalarial mode of action is unknown, artemisinin has a labile peroxide bond in a 1,2,4-trioxane heterocycle, which is thought to be reductively activated by haem, released from haemoglobin digestion during *Plasmodium* intraerythrocytic growth. This irreversible redox reaction would produce carbon-centered free radicals that cause alkylation of haem and proteins leading to parasite death.[44](#_ENREF_54)An alternative co-factor model has recently been put forward for endoperoxides such as artemisinin and this model may well explain different activities seen between different classes of endoperoxides undergoing development.[45](#_ENREF_55)

Artemisinins are fast-killing agents and active against all erythrocytic stages of the parasite. These compounds kill early ring-stage parasites and therefore provoke a very fast clearance of parasitemia in patients. In addition, rapid action on the ring stages reduces the number of parasites that mature to be sequestered in and block capillaries. This attribute leads to a fast clinical response and provides life-saving benefits in severe malaria.[46](#_ENREF_57) In addition, its effects on gametocytes can contribute to blocking transmission of the disease.[47](#_ENREF_58) Unwanted side-effects of this chemical family are temporary suppression of erythropoiesis and hypersensitivity reactions. Artemisinins are not recommended in the first trimester of pregnancy (except in the case of severe malaria) because temporary suppression of fetal erythropoiesis has, in some cases resulted in fetal resorption in animal studies.[48](#_ENREF_59) There are also concerns of potential neurotoxicity, which has been observed in animal studies.[48a](#_ENREF_59)

After the discovery of Artemisinin, several medicinal chemistry initiatives were initiated and different promising semi-synthetic Artemisinin derivatives (**22-26**) were obtained. Artemether (**23**) is the most prescribed derivative while Artesunate (**25**) is the drug of choice for intravenous administration in cerebral malaria cases (where the coma state of patients makes oral administration not practicable) as the free carboxylate enhances water solubility.[49](#_ENREF_61)



**Scheme 1.** Artemisinin and other semi-synthetic ARTs, fully synthetic endoperoxides have completed or in the process of (pre)clinical trials: Artemisinin (**21**), dihydroartemisinin (**22**), artemether (**23**), arteether (**24**), artesunate (**25**), artelinate (**26**), RKA182 (**27**), OZ277 (**28**) and OZ439 (**29**).

Artemisinins have a very short half-life in humans and are recommended to be used in combination in the clinic. The use of Artemisinin Combination Therapies (ACTs) for treatment of uncomplicated malaria has enabled a reduction in length of treatment from 7 days for artemisinins when used as monotherapy, to 3 days in combination yet still avoiding recrudescence. The use of combinations will hopefully hinder the development of resistance to artemisinins as a class. The partner drug for combination therapy is usually an antimalarial agent with longer half-life that can complete parasite clearance after the fast parasite reduction by artemisinin. The most frequently used combinations are: artemether-lumefantrine (Coartem®), artesunate-amodiaquine, artesunate-mefloquine and artesunate – sulphadoxine-pyrimethamine.[50](#_ENREF_62)

Efforts to find more metabolically stable derivatives suitable for providing efficacy with shorter treatment duration are ongoing and have highlighted a series based on the 10-(alkylamino)-artemisinins (Figure 5), exemplified by artemisone (**30**)[51](#_ENREF_63) and artemiside (**31**).[52](#_ENREF_64) Alternative strategies yielded azaartemisinins (i.e. **32** and **33**),[53](#_ENREF_65) which aim to improve the oral bioavailability of this scaffold.



**Figure 5.** Key exemplars obtained after medicinal chemistry efforts focused on finding antimlarial peroxides with improved pharmacokinetics.

Apart from the DMPK-associated liabilities, a major handicap of the artemisinin family (frequently affecting other drugs derived from natural products) is the problematic worldwide distribution because of a higher cost of goods and poor reliability of supply of a plant-derived product. Significant efforts have been described in the literature to develop synthetic and semi-synthetic biology approaches to artemisinin synthesis.[54](#_ENREF_66) One of the most noticeable examples is a viable industrial process for the production of semi-synthetic artemisinin published in 2013 by Paddon and coworkers (Scheme 2).[54b](#_ENREF_67) The combined efforts of several groups led to advances in yeast strain engineering, fermentation and artemisinic acid chemistry to produce artemisinin from simple carbon sources. First, novel enzymes relevant in the biosynthetic production of artemisinic acid were discovered and cloned into *Saccharomyces cerevisiae*. Furthermore, the fermentation process to produce artemisinic acid (**34**) in this organism was carefully optimized to yield up to 25 g/L compound. Last, an efficient synthetic process to obtain artemisinin from artemisinic acid that does not require specialized photochemical equipment was developed.



**Scheme 2.** Biotechnological process for artemisinin production developed by Paddon et al.[54b](#_ENREF_67)

However, these newly developed (semi)synthetic approaches to artemisinin are still undergoing development. Currently the only practical means of obtaining the compound is by extraction of the active component from the plant source, which contributes to the variability of harvesting and higher extraction costs. Therefore, significant efforts have been focused on identification of fully-synthetic artemisinin-like peroxides with a simpler and cheaper synthesis.

Simple, fully synthetic and easily scalable endoperoxides have been developed. An example of this is the analogs developed by Vennerstrom and coworkers at University of Nebraska Medical Center. These dispiro-1,2,4-trioxolanes, are one of the most successful chemical class of fully synthetic endoperoxides (Scheme 3).[55](#_ENREF_74) Initial efforts on the ozonide class were focused on understanding the structure-activity relationships, and the resulting data suggested that the antimalarial activity is linked to the accessibility of Fe(II) species to the peroxide bond of the molecule. While the relatively unhindered peroxide moiety of **35** presented antiplasmodial *in vitro* activity, the sterically hindered **36** was almost inactive butpresented improved pharmacokinetics. This knowledge led to the discovery of trioxolane **37**, a novel structure with antimalarial activity *in vivo.* This early version of the trioxolane analogue displayed a balance between accessibility to iron (II)-species and metabolic stability as the adamantine ring system protects the trioxolane from fast clearance. The focus was then to improve the oral bioavailablity by improving the physicochemical profile of **37**.The introduction of an amino amide group led to the discovery of **38**,OZ277, the first ozonide that reached clinical trials. A follow-up strategy facilitated the discovery of **39**, OZ439, an improved analog which had a biopharmaceutical profile with the potential to deliver a single dose malaria cure (half-life of 25-30 h in humans).[56](#_ENREF_76) Another interesting characteristic of OZ439 is its prophylactic activity, not observed with either Artesunate or OZ277. After demonstrating a good safety profile in Phase I clinical trials, OZ439 is currently in Phase II trials.[34](#_ENREF_41)



**Scheme 3**. Medicinal chemistry strategy followed to obtain OZ439 (**39**).[55a](#_ENREF_74), [56](#_ENREF_76)

An interesting step forward in the quest for alternative endoperoxide-based strategies was carried out by the O´Neill group at Liverpool University (Scheme 4). They maintained the crucial endoperoxide bridge but using a tetraoxane moiety instead of the trioxolane used by Vennerstrom. This change led to biological and chemical changes as the tetraoxane analog of **40** presented a high antiplasmodial potency as well an improved stability. An extensive SAR study of different tetraoxanes derivatives using the scaffold **41** was performed.[57](#_ENREF_77) The group identified compound RKA 182 (**42**) as an antimalarial drug development candidate with better solubility and bioavailability properties than their 1,2,4-trioxolane or 1,2,4-trioxane counterparts.[58](#_ENREF_78)



**Scheme 4**. Medicinal chemistry strategy followed to obtain RKA182 (**35)**

In order to characterise the potential mediators of the antimalarial activity of RKA182 performed mechanistic studies were performed with ferrous (II) bromide in THF in the presence of the spin-trapping agent 2,2,6,6-tetramethyl-1-piperidine-1-oxyl (TEMPO). From these studies, both the primary and secondary carbon centered radicals were intercepted to produce two TEMPO adducts A (**43**) and B (**44**) (Scheme 5).



**Scheme 5:** Mechanistic studies of tetraoxane RKA182

The behavior of the tetraoxanes reported was distinct from 1,2,4,-trioxolanes since only the secondary carbon centered radical species has been characterized from OZ277 and other 1,2,4-trioxolanes.[59](#_ENREF_79) Since heme alkylation is believed to play a vital role in the mechanism of action of endoperoxide antimalarials the reactivity of one tetraoxane analogue with ferrous heme was examined by LC-MS analysis which confirmed an m/z 782.3 Da for three adducts that result from the covalent bonding of the tetraoxane-derived secondary C-centered radical and the heme porphyrin. Although the exact mechanism of action for these compounds is still to be determined this process may play an important role.

Recently, hybrid compounds (Figure 6) of the tetraoxanes or artemisinins with primaquine, such as **45** or **46** have been prepared aimed at targeting simultaneously blood and dormant liver stages of the parasite and to block the transmission to mosquitoes.[60](#_ENREF_80) Some of these tetraoxane derivatives showed nanomolar potency in erythrocytic and extra-erythrocytic stages when assayed *in vitro* as well as transmission blocking potential *in vivo*. Therefore, the combination of an endoperoxide and an 8-aminoquinoline scaffold could be an interesting starting point to develop multi-stage specific antimalarial drugs.



**Figure 6**. Hybrid antimalarial compounds with activity against *Plasmodium* liver stages

* 1. **Quinones and Quinolones**

Naphthoquinones are the chemical series that produced the marketed antimalarial Atovaquone (AQ, **49**, Scheme 6). Atovaquone was discovered in the 1980s at the Wellcome Research Laboratories whilst exploiting the lateral chain of lapinone (**48**). Lapinone was identified when optimizing hits from a random screening of several hundreds of compounds against *P. lophurae* in ducks.[61](#_ENREF_82) The name was derived in acknowledgement of the research done by Hookers on lapachol (**47)**, a prenyl naphthoquinone present in families such as *Tabebuia avellanedae*, *Verbenaceae*, *Proteaceae*, *Leguminosae*, *Sapotaceae*, *Scrophulariaceae*,and *Malvaceae*. Lapachol was first isolated in 1882 from *Tabebuia avellanedae* and even being weakly active, was the compound that provided the clues for the latter development of naphthoquinones as antimalarials.[62](#_ENREF_85)

Atovaquone is a lipophilic hydroxy naphthoquinone[63](#_ENREF_87" \o "Wendel, 1946 #78) which has activity against *P. falciparum* and is effective against several other eukaryotic microbial parasites such as toxoplasma and *Pneumocystis*.[64](#_ENREF_90) Atovaquone exerts its antimalarial effects blocking the parasite’s mitochondrial electron transport chain (ETC) through inhibition of the cytochrome *bc*1 complex.[65](#_ENREF_91) *Plasmodium* cytochrome *bc*1 complex is critical for re-oxidizing ubiquinol to ubiquinone which is the final electron acceptor for the five parasitic mitochondrial dehydrogenases present in parasite mitochondria. One of these dehydrogenases, DHODH (dihydroorotate dehydrogenase), is a key enzyme of pyrimidine biosynthetic pathway and is essential for parasite survival. The inhibition of the ETC pathway as an antimalarial target has been well validated clinically with atovaquone, currently in use in combination with proguanil and marketed as Malarone®, which is now the recommended drug for travelers for malaria prophylaxis.[66](#_ENREF_93) Resistance to atovaquone caused by mutations in cytochrome *b* has been detected and toxicity associated with unselective inhibition of human cytochrome *b* has also been noticed.[67](#_ENREF_94) To circumvent the resistance and toxicity issues of Atovaquone, some of the more recent advanced molecules, such as ELQ300 (**50**) and SL-2-25 (**51**) which incorporate a quinolone core in replacement of the quinone core have been reported.[68](#_ENREF_95) ELQ300 is a 3-diarylether substituted quinolone that has shown excellent efficacy *in vivo* in comparison to Atovaquone, but no cross resistance to the Atovaquone resistant strain of *P. falciparum* TMC90 C2B and possesses much better selectivity for the parasite’s mitochondrial cytochrome *bc*1 complex over the human cytochrome *bc*1 complex . ELQ300 is in Phase I clinical trials at the moment.[34](#_ENREF_41) SL-2-25 is a 2-bisaryl substituted quinolone that targets two different enzymes, namely *Pf*NDH2 and *Pfbc*1 in the parasite’s mitochondrial ETC chain. The feature of dual target inhibitions has not only proved to be highly efficacious *in vivo* against *P. berghei* in animal models, but also had advantages in terms of lowering the likelyhood of parasite resistance. An improved analogue of SL-2-25 is in the process of the completing its preclinical evaluations.



**Scheme 6.** From Lapachol (**47**) to Atovaquone (**49**), ELQ300 (**50**) and SL-2-25 (**51**)

* 1. **Spiroindolones**

Coming into the 21st century, natural products continue to deliver good opportunities in the antimalarial drug discovery arena. A new chemical entity (NCE), NITD609 (**56**), based on a spirocyclic oxoindole scaffold has recently entered clinical trials. NITD609 is the most recent exemplar of a compound originating from a natural product inspired library that will likely be incorporated into the current antimalarial treatments. The starting point of this antimalarial class of compounds (known as spiroindolones) was identified by Novartis through the screening of a small library of 12,000 compounds. The collection contained natural products and fully synthetic drugs with structural features found in natural products (Scheme 9).[69](#_ENREF_97) The hit (**52**) had nanomolar potency against NF54 (sensitive) and K1 (cloroquine and pyrimithamine resistant) *P. falciparum* strains. During the medicinal chemistry optimization process the seven-membered ring in the original hit was replaced by a six-membered ring (**53**) and one of the four stereoisomers (1*R*, 3*S*, **54**) was identified and separated as the active conformation. Introduction of halogens into the C6 and C7 positions (**55**) improved the antimalarial *in vitro* potency and the pharmacokinetic profile. The derivative with a C6-fluorine and a C7-chlorine yielded compound NITD609 which was subsequently selected as a preclinical candidate.[70](#_ENREF_98)NITD609 is currently in Phase II clinical trials and has been renamed KAE609. Scientific evidence now suggests that spiroindolones affect parasite Na+ homeostais by inhibiting the cation ATPase *Pf*ATP4.[71](#_ENREF_99) This chemical series has been described to be active against asexual erythrocytic stages and also has demonstrated transmission blocking potential in the standard membrane feeding assay (SMFA).[72](#_ENREF_100)



**Scheme 7.** Medicinal chemistry strategy followed to obtain NITD609 **(56)**

During the development of the spiroindolone scaffold advances in organic synthetic chemistry and enzymatic engineering contributed significantly to reduce the cost of goods and provide sustainable supply of material for the potential demand of this class of antimalarial drugs. For example, very recently an elegant method was published to build the Spiro[pyrrolidin-3,2’-oxoindole] motif contained in NITD609 using synthesis mediated by an organocatalyst (Scheme 8).[73](#_ENREF_101)



**Scheme 8.** Organocatalytic synthesis of Spiro[pyrrolidin-3,2’-oxoindoles]

Alternatively, Novartis in collaboration with Codexis (company specialized in enzyme engineering and production for biocatalysis applications) has developed an efficient chemoenzymatic route to yield the active enantiomer of NITD609 (Scheme 9). The process combines traditional organic chemistry with transaminase-catalyzed introduction of a chiral amine-group. For the biocatalytic step, Codexis engineered the transaminase from *V. fluvialis* to obtain a mutant enzyme that displayed higher activity, stability and resistance to inhibition by the product amine.[74](#_ENREF_102)



**Scheme 9.** Chemoenzymatic synthesis of NITD609 developed by Novartis and Codexis

* 1. **Natural product derived antibiotics with antimalarial activity**

Several scaffolds of antibiotics derived from natural products have shown activity against *Plasmodiu*m parasites and can be potentially used for malaria treatment or prophylaxis. There are some very distinctive advantages and properties of using or further developing these antibiotics as antimalarials. Although most of these antibiotic scaffolds only exhibited modest antimalarial activity, usually in the µM range, *in vitro*, their mechanism of actions were well understood (protein synthesis inhibitors), their pharmacokinetics and toxicology in animal models and human are well defined, and more importantly they demonstrate reasonable efficacy *in vivo*. Also, as not many antibiotics have been widely used as antimalarials in the past, the parasite has to date not shown any significant resistance to these antibiotics. Finally, one of the most unique characteristics for some antibiotics against *Plasmodiu*m parasites is their delayed effects (except for fosmidomycin).[75](#_ENREF_103) All these characteristics make them good partner drugs in combination with other anti-malarials.

**Clindamycin** (**58**) is a semi-synthetic (7-chloro-7-deoxy) derivative of the natural antibiotic lincomycin (**57**), produced by *Streptomyces lincolnensis*.[76](#_ENREF_104) The change of hydroxyl- to chloro- group increases the lipophilicity of the molecule and translated into lower absorption times; in addition, clindamycin has a different antibacterial spectrum and it is effective and well tolerated for malaria treatment (Scheme 10).[77](#_ENREF_105) Clindamycin inhibits parasite protein biosynthesis and displays a delayed death phenotype (parasites are killed in the second lifecycle after exposure), therefore it is only recommended in combination with chloroquine or quinine. Clindamycin-quinine is the treatment of choice for children and pregnant women in chloroquine-resistance areas. One of the main drawbacks of the use of this antibiotic in malaria treatment is the identification of clindamycin-resistant *P. falciparum* clones isolated in patients in the Peruvian Amazon.[78](#_ENREF_106)



**Scheme 10**. Clyndamycin (**58**) from Lyncomycin (**57**) and the significant delayed effects of Clyndamycin against *P. falciparum in vitro*.

**Azythromycin** (**60**), an azalide is another antibiotic with antimalarial activity. It is a semi-synthetic macrolide derived from the polyketide natural product erythromycin (**59**) by introduction of a nitrogen atom into the macrolactone ring via Beckmann rearrangement (Scheme 11).[79](#_ENREF_107) The chemical modifications yielded a compound with a 10-fold increase of anti-malarial activity *in vitro* and was well tolerated with unusual pharmacokinetics characterized by high metabolic stability, and high tissue distribution.[80](#_ENREF_108) Azythromycin inhibits protein biosynthesis by targeting the *P. falciparum* 70S ribosomal subunit within apicoplast and mitochondria. It also displays a delayed death phenotype and is therefore needs to be administered in combination with a fast acting agent such as chloroquine.[81](#_ENREF_109)



**Scheme 11.** Synthesis of Azythromycin (**60**) from Erythromycin (**59**) and their activity against *P. falciparum in vitro*.

**Tetracyclines**, the antimalarial potential of tetracyclines was discovered in the 1960s when the rise of *Plasmodium* resistance to chloroquine motivated the search for new drugs.[82](#_ENREF_110) Tetracyclines also shown delayed effects against *P. falciparum in vitro*, but it is not as significant as Clyndamycin or Azythromycin. From this class of antibiotics, doxycycline (**61**) was the most widely used to prevent malaria, and it is also used for treatment in combination therapies. Except in case of severe malaria which is resistant to all other drugs, administration of tetracyclines is not recommended to children under the age of 8 and pregnant women because these drugs affect bone and teeth development.



**Figure 7.** Chemical structure ofdoxycycline (**61**) and its activity against CQ sensitive (3D7) and resistant (W2) strains of *P. falciparum in vitro*.[75](#_ENREF_103)

**Fosmidomycin** (**62**) and its acetyl derivative FR900098 (**63**) are another class of antibacterials with antimalarial activity.[83](#_ENREF_111) Isolated from bacteria of the genus *Streptomyces*,[84](#_ENREF_113) these compounds inhibit the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway for isoprenoid biosynthesis, which is essential in several microorganisms including *Plasmodium* and absent in humans. Fosmidomycin and FR900098 are structural analogues of MEP and inhibit the second step in the MEP biosynthetic pathway, catalyzed by the enzyme DXP reductoisomerase (called DXR or IspC). *Plasmodium* can develop resistance to drugs targeting this pathway by increasing the copy number of DXP reductoisomerase.[85](#_ENREF_114) Despite fosmydomycin and FR900098 not displaying a delayed death phenotype, they are not administered as single agents because of the propensity for recrudescent infection. Clinical trials with different antimalarials have been conducted. The most promising combination was with clindamycin, but efficacy in children under 3 was significantly reduced. However, its high solubility in aqueous media suggested that treatment for severe malaria (i.v. administration) should be feasible. However, an oral drug based on this scaffold is challenging as their oral bioavailability is around 30% due to its low lipophilicity mediated from the phosphono moiety. Several medicinal chemistry efforts focused on improving oral bioavailability have been followed (i.e. modifications of the phosphonate group (**64** and **65**), the hydroxamic acid or the linker (**66**)),[86](#_ENREF_115) but so far, none of these derivatives have progressed to clinical trials. Fosmidomycin and its analogues have not yet yielded any clinical approved drug for malaria treatment. However, it has proven that the MEP pathway and DXR (IspC) in *Plasmodium* are validated antimalarial drug targets.



**Scheme 12.**  Examples of medicinal chemistry efforts focused on improving the efficacy of fosmydomycin (**55**) and FR900098 (**56**)

* 1. **Further natural product scaffolds identified from traditional herbal medicines with antimalarial activity**

Thousands of alkaloids isolated from natural organisms have shown inhibitory activity against *P. falciparum* growth. However, most of these compounds display poor antimalarial potencies in the micromolar range, as well as cytotoxicity in human cell lines, therefore requiring intensive medicinal chemistry efforts. Today, the most widely used antimalarial alkaloids are not used as purified entities, but as part of plant extracts.

The plant *Argemona mexicana*, from the Papaveraceae family, has been recognized as a phytomedicine for home-based malaria treatment.[87](#_ENREF_118)The decoction extract of aerial parts of the plant has good antimalarial activity *in vitro* (IC50 = 5.89 µg/ml) and in clinical trials 89% of patients treated recovered clinically.[88](#_ENREF_119) The Argemona benzylisoquinoline alkaloids berberine (**67**), protopine (**68**) and allocryptopine (**69**) (Figure 8) were subsequently identified as the active ingredients. In the nineties there were some medicinal chemistry attempts to improve their overall activity but they were not successful and the efforts were rapidly abandoned.[89](#_ENREF_120)

*Nauclea pobeguinii* is a plant traditionally used in Africa for home management of malaria which has undergone clinical trials. Extracts from *Nauclea pobeguiini* are active in rodent malaria models and also have displayed efficacy in patients, although none of the alkaloids are active *in vitro*. The reason for this is probably that strictosamide (**70**), the major component[90](#_ENREF_122) and what is thought to be the active ingredient responsible of the antimalarial activity, needs to be first bioactivated in the gastrointestinal tract.[13c](#_ENREF_17)



**Figure 8.** Antimalarial alkaloids used in the clinic.

Yingzhaosu (**71**) is another natural product sesquiterpene peroxide that has been used as scaffold for antimalarials. Yingzhaosu is isolated from *Artabotrys uncinatus*, and shows efficacy in *Plasmodium berghei* infected mice comparable to that of mefloquine or quinine but is ten-fold less potent than artemisinin. Yingzhaosu was studied as a potentially more stable artemisinin-like antimalarial scaffold. As a result of a structure-activity study published in 1994 arteflene (**72**) was identified. (Scheme 13)[91](#_ENREF_123) Arteflene is a more potent synthetic derivative (comparable to artemisinin) generated by adding a keto group in the 2,3-dioxabicyclo[3.3.1][nonane](http://europepmc.org/abstract/MED/7899801/?whatizit_url_Chemicals=http://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI%3A32892" \t "_blank) moiety and replacing the di-hydroxy group by a lipophilic moiety. It was found to be negative in different mutagenicity tests and to be well tolerated in doses up to 400 mg/kg/day in rats. Arteflene reached clinical trials; however, when compared with mefloquine it did not present advantages in a single dose monotherapy treatment. In terms of physicochemical profile it shows a low aqueous solubility and an amenable methodology for synthetic production is challenging.[92](#_ENREF_124)



**Scheme 13.** Key SAR knowledge that allowed obtaining of Arteflene (**72**)

Febrifugine (**73**) was identified as the active components against malaria in the Chinese herb Chang Shan (*Dichroa febrifuga Lour*). Chang Shan has been employed as medicine against fevers caused by malaria parasites for a long time by the locals. An early report indicated that the extracts of Chang Shan is clinically efficacious for the treatment of malaria.[93](#_ENREF_126) However, adverse side effects, including strong liver toxicity and gastrointestinal irritation were also observed in human and various animal models. Since then various research groups have investigated this scaffold using a range of medicinal chemistry approaches to improve efficacy and reduce toxicity. Research activity from 1960s to 2000s lead to all three regions within febrifugine, including the 4-quinazolinone ring core, the piperidine ring containing side-chain and the linker between being subjected to medicinal chemistry modification. In 1960s, halofuginone (**77**) was synthesized by the U.S. Army Medical Research Command with the aim of identifying novel antimalarials with clinical profiles better than those of febrifugine. Halofuginone demonstrated better antimalarial activity and an improve safety window in comparison to febrifugine, but both febrifugine and halofuginone were found not to be curative for *P. berghei* infected mice *in vivo*.[94](#_ENREF_127) The lack of curative effect and toxicity of febrifugine can be explained by the easy isomerization of febrifugine to isobrifugine (**74**) which has lower antimalarial activity and the potential reactive intermediate **75** formation during the process (Scheme 14). To stop this isomerization process, removal of the methylene group between the carbonyl and the piperidine side-chain has proven to be successful, and the antimalarial activity was also maintained as demonstrated by compound **78**.[95](#_ENREF_128)



**Scheme 14:** Possible isomerization mechanism between febrifugine and isofebrifugine.

Subsequent research has shown that various lipophilic substitution on the 4-quinazolinone core could enhance the antimalarial activity and improve metabolic stability, but scaffold hopping excercises which tried to identify replacement for the 4-quinazolinone core have been proved to be less successful.[96](#_ENREF_129) Conversely, the piperidine ring side-chain is well tolerated for a range of modifications, such as ring size contraction to pyrrolidine ring and alkylation of the hydroxyl group to various ethers and in addition some of the modifications have resulted in analogues with enhanced anti-malarial activity and reduced undesired toxicity. (Scheme 15) More importantly, the combination of all these modifications have resulted in analogues, i.e. compound **79** which demonstrated excellent *in vivo* efficacy in animal models including *P. falciparum* infected *Aotus* monkeys.[97](#_ENREF_130) Recently, more research results were published to examine the detailed mechanism of action for febrifugine and its analogues. Using an integrated chemogenomics approach that combined drug resistance selection, whole-genome sequencing, and an orthogonal yeast model it was demonstrated that the cytoplasmic prolyl–tRNA (transfer RNA) synthetase (PfcPRS) of the malaria parasite *P. falciparum* is a biochemical and functional target of febrifugine and its synthetic derivative halofuginone.[98](#_ENREF_131)



**Scheme 15:** Medicinal chemistry strategy for optimization of febrifugine

**Table 1** A Summary of Clinically Used Antimalarial Drug Molecules Derived from Natural Products

|  |  |  |  |
| --- | --- | --- | --- |
| Natural product Structure | Molecules Derived | Mechanism of Action | References on Mechanism of Action Studies |
|  | Chloroquine  AQ13  Amodiaquine  Isoquine  Fluoroamodiaquine  Ferroquine  Piperaquine  Mefloquine  Pyronaridine (Azaacridine quinoline relative) | Inhibition of hemozoin formation | 40 |
| Pamaquine  Bulaquine  Primaquine  Tafenoquine | Mitochondrial damage potentially due to generation of redox active intermediates | 38(b) |
|  | Dihydroartemisinin  Artemether  Arteether  Artesunate  Artelinate  OZ277  OZ439 | Fe (II) catalysed free radical alkylation of haem and proteins | 44, 59 |
|  | Atovaquone  ELQ300  SL-2-25 | Inhibition of components within mitochondrial electron transport chain. | 65, 67, 68 |
|  | KAE609 (NITD609) | Inhibition of PfATP4 | 69-72 |
|  | Clyndamycin | Inhibition of protein biosynthesis by targeting 70S ribosome (50S subunit) | 75, 78 |
|  | Azythromycin | Inhibition of protein biosynthesis by targeting 70S ribosome (50S subunit) | 75, 79 |
|  | Doxycycline | Inhibition of protein biosynthesis by targeting 70S ribosome (30S subunit) | 75 |
|  | FR900098 | Inhibition of PfDXR (IspC) in the MEP pathway | 83a |
|  | Atreflene | Presumed to be similar to Artemisinin | 91 |
|  | Halofuginone | Targeting the cytoplasmic prolyl–tRNA (transfer RNA) synthetase (PfcPRS) | 98 |

1. **Outlook – The Natural Antimalarial Arsenal – making the most of it**

In the history of mankind’s battle against malaria parasite, natural products and their derivatives continue to play a prominent role in both prevention and treatment. Some of the most important antimalarials used in the past and current first line drugs, such as the quinolines, peroxides and quinones originated from or were inspired by natural products (Table 1). In addition, natural products continue to provide a rich source of novel scaffolds as inspiration for the development of the next generation of antimalarials, both fully synthetic and semi-synthetic drugs. Currently a number of clinical candidates of antimalarial drugs i.e. Phase II – AQ13, ferroquine, OZ439 and KAE606 (NITD609); Phase III – tafenoquine etc. in the development pipeline have their roots in natural products. Recent advances in organic synthesis, process chemistry and enzymatic engineering have enabled medicinal chemists to modify scaffolds originating from natural products with greater flexibility to provide a sustainable supply for drugs developed from natural product sources with acceptable costs. Other innovative approaches, such as “reverse pharmacology” approaches to discovery of active ingredients or the synergetic effects of chemicals from clinically efficacious herbal medicines will continue to provide novel starting points for discovery programmes. In this new era of combating malaria, eradication of the disease is no longer a dream, but new challenges such as the development of single dose cures, combating resistance development and treatment of liver-stage vivax malaria along with transmission blocking and chemoprotection need to be tackled along the way.[99](#_ENREF_132) There is no doubt that natural products will have an important role to play in this process.

**Acknowledgments**

We want to thank our colleagues Dr. Javier Gamo and Dr. John Haselden for reviewing this manuscript.

**5. AUTHOR INFORMATION**

**Corresponding Author**

E-mail: [felix.r.calderon-romo@gsk.com](mailto:felix.r.calderon-romo@gsk.com)

**Notes**

The authors declare no competing financial interest.

**Biographies**

**Elena Fernández** is agraduated pharmacyst (Complutense University, Spain) and PhD in Biotechnology (Greifswald University, Germany). She worked in AkzoNobel (The Netherlands) in green chemistry processes before joining GSK in 2010. Currently she works in malaria drug discovery aiming to deliver new drugs to combat malaria. Main interests include drug discovery, neglected diseases and collaborative initiatives to maximize efficiency of scientific research.

**W. David Hong** got his BSc degree in Chemistry from Fudan University (Shanghai, China) and PhD in synthetic organic chemistry at the University of Liverpool. Since then he started to work as a medicinal chemist in the areas of infectious diseases drug discovery, including antimalarials, anti-TBs and anthelmintics, and insecticide development. He is currently holding a role as the chemistry scientific team leader in an anti-Wolbachia macrofilaricidal drug discovery programme.

**Gemma L. Nixon** completed herMChem in Chemistry with Pharmacology and PhD in Organic Chemistry at the University of Liverpool. Since then she has work as a medicinal chemist both in industry (Peakdale Molecular) and academia (Liverpool School of Tropical Medicine, University of Liverpool) in area of infectious diseases drug discovery focusing on malaria, tuberculosis, filariasis and fungal infections. She joined the Department of Chemistry, University of Liverpool as a Lecturer in Medicinal Chemistry in April 2015.

**Paul M. O'Neill** graduated in Chemistry and Pharmacology at the University of Liverpool in 1990 followed by a PhD in medicinal chemistry in 1995 with Professor Kevin Park and Dr Richard Storr. In 1997, he carried out postdoctoral research with Professor Gary H. Posner at the Johns Hopkins University, Baltimore, USA. In 1998 Paul was appointed to a lectureship between the Departments of Chemistry and Pharmacology at Liverpool and he became Senior Lecturer in 2003, Reader in 2005 and Professor in 2006. He is currently the Head of Medicinal Chemistry at the University of Liverpool and in 2011 was awarded the RSC Malcolm Campbell Prize for excellence in biological chemistry. The O’Neill group research has led to a drug candidate (Isoquine) entering clinical trials in 2008 and his group have also recently produced two additional candidate antimalarials (E209 and RKA 182) selected for full preclinical testing on route to Phase 1 clinical trials in humans. He has published 135 papers and 15 patents and his research covers a range of medicinal chemistry and chemical biology areas focused on malaria, TB, HIV and filariasis.

**Félix Calderón** started to work in Malaria since he joined GSK in 2007. Prior to GSK his background include training in synthetic chemistry at the University of Turin (Italy) and the Spanish National Council for Scientific research (CSIC) and computational chemistry at the University of California Los Angeles (UCLA). He received his Ph. D. degree from the CSIC in 2006 for his work in the synthesis of azasugars through organocatalytic reactions. His research interest includes Malaria, medicinal and computational chemistry.

1. **References**

1. World Malaria report. 2014.

2. Roll Back Malaria.

3. Agnandji, S. T.; Lell, B.; Fernandes, J. F.; Abossolo, B. P.; Kabwende, A. L.; Adegnika, A. A.; Mordmueller, B.; Issifou, S.; Kremsner, P. G.; Loembe, M. M.; Sacarlal, J.; Aide, P.; Madrid, L.; Lanaspa, M.; Mandjate, S.; Aponte, J. J.; Bulo, H.; Nhama, A.; Macete, E.; Alonso, P.; Abdulla, S.; Salim, N.; Mtoro, A. T.; Mutani, P.; Tanner, M.; Mavere, C.; Mwangoka, G.; Lweno, O.; Juma, O. A.; Shekalaghe, S.; Tinto, H.; D'Alessandro, U.; Sorgho, H.; Valea, I.; Ouedraogo, J. B.; Lompo, P.; Diallo, S.; Traore, O.; Bassole, A.; Dao, E.; Hamel, M. J.; Kariuki, S.; Oneko, M.; Odero, C.; Otieno, K.; Awino, N.; Muturi-Kioi, V.; Omoto, J.; Laserson, K. F.; Slutsker, L.; Otieno, W.; Otieno, L.; Otsyula, N.; Gondi, S.; Otieno, A.; Ogutu, B.; Ochola, J.; Onyango, I.; Oyieko, J.; Njuguna, P.; Chilengi, R.; Akoo, P.; Kerubo, C.; Maingi, C.; Olotu, A.; Bejon, P.; Marsh, K.; Mwabingu, G.; Gitaka, J.; Owusu-Agyei, S.; Asante, K. P.; Boahen, O.; Dosoo, D.; Adjei, G.; Adeniji, E.; Yawson, A. K.; Kayan, K.; Chandramohan, D.; Greenwood, B.; Lusingu, J.; Gesase, S.; Malabeja, A.; Abdul, O.; Mahende, C.; Liheluka, E.; Lemnge, M.; Theander, T. G.; Drakeley, C.; Mbwana, J.; Ansong, D.; Agbenyega, T.; Adjei, S.; Boateng, H. O.; Rettig, T.; Bawa, J.; Sylverken, J.; Sambian, D.; Sarfo, A.; Agyekum, A.; Martinson, F.; Hoffman, I.; Mvalo, T.; Kamthunzi, P.; Nkomo, R.; Tembo, T.; Tsidya, G. T. M.; Kilembe, J.; Chawinga, C.; Ballou, W. R.; Cohen, J.; Guerra, Y.; Jongert, E.; Lapierre, D.; Leach, A.; Lievens, M.; Ofori-Anyinam, O.; Olivier, A.; Vekemans, J.; Kaslow, D.; Leboulleux, D.; Savarese, B.; Schellenberg, D.; Partnership, R. S. C. T., Efficacy and Safety of the RTS,S/AS01 Malaria Vaccine during 18 Months after Vaccination: A Phase 3 Randomized, Controlled Trial in Children and Young Infants at 11 African Sites. *Plos Medicine* **2014,** *11* (7).

4. N'Guessan, R.; Corbel, V.; Akogbeto, M.; Rowland, M., Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerging infectious diseases* **2007,** *13* (2), 199-206.

5. Lubell, Y.; Dondorp, A.; Guerin, P. J.; Drake, T.; Meek, S.; Ashley, E.; Day, N. P. J.; White, N. J.; White, L. J., Artemisinin resistance - modelling the potential human and economic costs. *Malaria journal* **2014,** *13*.

6. White, N. J.; Pukrittayakamee, S.; Hien, T. T.; Faiz, M. A.; Mokuolu, O. A.; Dondorp, A. M., Malaria. *Lancet* **2014,** *383* (9918), 723-735.

7. Dembele, L.; Franetich, J.-F.; Lorthiois, A.; Gego, A.; Zeeman, A.-M.; Kocken, C. H. M.; Le Grand, R.; Dereuddre-Bosquet, N.; van Gemert, G.-J.; Sauerwein, R.; Vaillant, J.-C.; Hannoun, L.; Fuchter, M. J.; Diagana, T. T.; Malmquist, N. A.; Scherf, A.; Snounou, G.; Mazier, D., Persistence and activation of malaria hypnozoites in long-term primary hepatocyte cultures. *Nature Medicine* **2014,** *20* (3), 307-312.

8. Belen Jimenez-Diaz, M.; Viera, S.; Fernandez-Alvaro, E.; Angulo-Barturen, I., Animal models of efficacy to accelerate drug discovery in malaria. *Parasitology* **2014,** *141* (1), 93-103.

9. Uhlemann, A.-C.; Fidock, D. A., Loss of malarial susceptibility to artemisinin in Thailand. *Lancet* **2012,** *379* (9830), 1928-1930.

10. Leeson, P. D.; Springthorpe, B., The influence of drug-like concepts on decision-making in medicinal chemistry. *Nature reviews. Drug discovery* **2007,** *6* (11), 881-90.

11. (a) 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.; Jimenez-Diaz, M. B.; Martinez, M. S.; Wilson, E. B.; Tripathi, A. K.; Gut, J.; Sharlow, E. R.; Bathurst, I.; El Mazouni, F.; Fowble, J. W.; Forquer, I.; McGinley, P. L.; Castro, S.; Angulo-Barturen, I.; Ferrer, S.; Rosenthal, P. J.; DeRisi, J. L.; Sullivan, D. J., Jr.; Lazo, J. S.; Roos, D. S.; Riscoe, M. K.; Phillips, M. A.; Rathod, P. K.; Van Voorhis, W. C.; Avery, V. M.; Guy, R. K., Chemical genetics of Plasmodium falciparum. *Nature* **2010,** *465* (7296), 311-315; (b) Gamo, F.-J.; Sanz, L. M.; Vidal, J.; de Cozar, C.; Alvarez, E.; Lavandera, J.-L.; Vanderwall, D. E.; Green, D. V. S.; Kumar, V.; Hasan, S.; Brown, J. R.; Peishoff, C. E.; Cardon, L. R.; Garcia-Bustos, J. F., Thousands of chemical starting points for antimalarial lead identification. *Nature* **2010,** *465* (7296), 305-U56; (c) Plouffe, D.; Brinker, A.; McNamara, C.; Henson, K.; Kato, N.; Kuhen, K.; Nagle, A.; Adrian, 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 of the United States of America* **2008,** *105* (26), 9059-9064.

12. Bero, J.; Frederich, M.; Quetin-Leclercq, J., Antimalarial compounds isolated from plants used in traditional medicine. *Journal of Pharmacy and Pharmacology* **2009,** *61* (11), 1401-1433.

13. (a) Fotie, J., Key Natural products in Malaria Chemotherapy: From Quinine to Artemisinin and beyond. In *Bioactive Natural Products*, 2012; pp 223-272; (b) Xu, Y.-J.; Pieters, L., Recent Developments in Antimalarial Natural Products Isolated from Medicinal Plants. *Mini Reviews in Medicinal Chemistry* **2013,** *13* (7), 1056-1072; (c) Wells, T. N. C., Natural products as starting points for future anti-malarial therapies: going back to our roots? *Malaria journal* **2011,** *10*; (d) Guantai, E.; Chibale, K., How can natural products serve as a viable source of lead compounds for the development of new/novel anti-malarials? *Malaria journal* **2011,** *10*.

14. Singh, S. K. S., S. , A Brief History of Quinoline as Antimalarial Agents. *Int. J. Pharm. Sci. Rev. Res.* **2014,** *25*, 295-302.

15. Mirro, M. J.; Manalan, A. S.; Bailey, J. C.; Watanabe, A. M., Anticholinergic effects of disopyramide and quinidine on guinea pig myocardium. Mediation by direct muscarinic receptor blockade. *Circulation research* **1980,** *47* (6), 855-65.

16. Collins, L. L.; Papacostas, C. A.; Elkin, S., New derivatives of quinuclidine as potential antihypertensive agents. *Journal of pharmaceutical sciences* **1967,** *56* (12), 1668-70.

17. Taylor, T. E.; Molyneux, M. E.; Wirima, J. J.; Fletcher, K. A.; Morris, K., Blood glucose levels in Malawian children before and during the administration of intravenous quinine for severe falciparum malaria. *The New England journal of medicine* **1988,** *319* (16), 1040-7.

18. Cook, N. S.; Haylett, D. G., Effects of apamin, quinine and neuromuscular blockers on calcium-activated potassium channels in guinea-pig hepatocytes. *The Journal of physiology* **1985,** *358*, 373-94.

19. Waldman, H. J., Centrally acting skeletal muscle relaxants and associated drugs. *Journal of pain and symptom management* **1994,** *9* (7), 434-41.

20. Bernhard, H. O. T., K. Process for preparing bitter beverages. US4133903 A, 1979.

21. Machatha, S. G.; Yalkowsky, S. H., Comparison of the octanol/water partition coefficients calculated by ClogP, ACDlogP and KowWin to experimentally determined values. *International journal of pharmaceutics* **2005,** *294* (1-2), 185-92.

22. Seeman, J. I., The Woodward-Doering/Rabe-Kindler total synthesis of quinine: setting the record straight. *Angew Chem Int Ed Engl* **2007,** *46* (9), 1378-413.

23. Woodward, R. B.; Doering, W. E., THE TOTAL SYNTHESIS OF QUININE. *Journal of the American Chemical Society* **1945,** *67* (5), 860-874.

24. Rabe, P. K., K. Ber., Über die partielle Synthese des Chinins. Zur Kenntnie der China-Alkaloide XIX. *Ber. Dtsch. Chem. Ges.* **1918,** *51*, 466-467.

25. Stork, G.; Niu, D.; Fujimoto, R. A.; Koft, E. R.; Balkovec, J. M.; Tata, J. R.; Dake, G. R., The first stereoselective total synthesis of quinine. *Journal of the American Chemical Society* **2001,** *123* (14), 3239-42.

26. Krafts, K.; Hempelmann, E.; Skorska-Stania, A., From methylene blue to chloroquine: a brief review of the development of an antimalarial therapy. *Parasitology research* **2012,** *111* (1), 1-6.

27. Meissner, P. E.; Mandi, G.; Coulibaly, B.; Witte, S.; Tapsoba, T.; Mansmann, U.; Rengelshausen, J.; Schiek, W.; Jahn, A.; Walter-Sack, I.; Mikus, G.; Burhenne, J.; Riedel, K. D.; Schirmer, R. H.; Kouyate, B.; Muller, O., Methylene blue for malaria in Africa: results from a dose-finding study in combination with chloroquine. *Malaria journal* **2006,** *5*, 84.

28. Brueckner, R. P. O., C.; Baird, J. K; Milhous, W. K., In *8-Aminoquinolines*, 2001; pp 123-151.

29. Slater, A. F., Chloroquine: mechanism of drug action and resistance in Plasmodium falciparum. *Pharmacology & therapeutics* **1993,** *57* (2-3), 203-35.

30. Fidock, D. A.; Eastman, R. T.; Ward, S. A.; Meshnick, S. R., Recent highlights in antimalarial drug resistance and chemotherapy research. *Trends in parasitology* **2008,** *24* (12), 537-44.

31. De, D.; Krogstad, F. M.; Cogswell, F. B.; Krogstad, D. J., Aminoquinolines that circumvent resistance in Plasmodium falciparum in vitro. *The American journal of tropical medicine and hygiene* **1996,** *55* (6), 579-83.

32. Davis, T. M.; Hung, T. Y.; Sim, I. K.; Karunajeewa, H. A.; Ilett, K. F., Piperaquine: a resurgent antimalarial drug. *Drugs* **2005,** *65* (1), 75-87.

33. (a) Rijken, M. J.; McGready, R.; Phyo, A. P.; Lindegardh, N.; Tarning, J.; Laochan, N.; Than, H. H.; Mu, O.; Win, A. K.; Singhasivanon, P.; White, N.; Nosten, F., Pharmacokinetics of dihydroartemisinin and piperaquine in pregnant and nonpregnant women with uncomplicated falciparum malaria. *Antimicrob Agents Chemother* **2011,** *55* (12), 5500-6; (b) Valecha, N.; Phyo, A. P.; Mayxay, M.; Newton, P. N.; Krudsood, S.; Keomany, S.; Khanthavong, M.; Pongvongsa, T.; Ruangveerayuth, R.; Uthaisil, C.; Ubben, D.; Duparc, S.; Bacchieri, A.; Corsi, M.; Rao, B. H.; Bhattacharya, P. C.; Dubhashi, N.; Ghosh, S. K.; Dev, V.; Kumar, A.; Pukrittayakamee, S., An open-label, randomised study of dihydroartemisinin-piperaquine versus artesunate-mefloquine for falciparum malaria in Asia. *PloS one* **2010,** *5* (7), e11880; (c) Bassat, Q.; Mulenga, M.; Tinto, H.; Piola, P.; Borrmann, S.; Menendez, C.; Nambozi, M.; Valea, I.; Nabasumba, C.; Sasi, P.; Bacchieri, A.; Corsi, M.; Ubben, D.; Talisuna, A.; D'Alessandro, U., Dihydroartemisinin-piperaquine and artemether-lumefantrine for treating uncomplicated malaria in African children: a randomised, non-inferiority trial. *PloS one* **2009,** *4* (11), e7871.

34. Wells, T. N.; Hooft van Huijsduijnen, R.; Van Voorhis, W. C., Malaria medicines: a glass half full? *Nature reviews. Drug discovery* **2015,** *14* (6), 424-42.

35. Croft, A. M., A lesson learnt: the rise and fall of Lariam and Halfan. *Journal of the Royal Society of Medicine* **2007,** *100* (4), 170-4.

36. (a) Croft, A. M.; Whitehouse, D. P.; Cook, G. C.; Beer, M. D., Safety evaluation of the drugs available to prevent malaria. *Expert opinion on drug safety* **2002,** *1* (1), 19-27; (b) Tansley, R.; Lotharius, J.; Priestley, A.; Bull, F.; Duparc, S.; Mohrle, J., A randomized, double-blind, placebo-controlled study to investigate the safety, tolerability, and pharmacokinetics of single enantiomer (+)-mefloquine compared with racemic mefloquine in healthy persons. *The American journal of tropical medicine and hygiene* **2010,** *83* (6), 1195-201.

37. FDA Drug Safety Communocations: FDA approves label changes for antimalarial drug mefloquine hydrochloride due to risk of serious psychiatric and nerve side effects.

38. (a) Wells, T. N. C.; Burrows, J. N.; Baird, J. K., Targeting the hypnozoite reservoir of Plasmodium vivax: the hidden obstacle to malaria elimination. *Trends in parasitology* **2010,** *26* (3), 145-151; (b) Vale, N.; Moreira, R.; Gomes, P., Primaquine revisited six decades after its discovery. *European journal of medicinal chemistry* **2009,** *44* (3), 937-53.

39. Shanks, G. D.; Oloo, A. J.; Aleman, G. M.; Ohrt, C.; Klotz, F. W.; Braitman, D.; Horton, J.; Brueckner, R., A new primaquine analogue, tafenoquine (WR 238605), for prophylaxis against Plasmodium falciparum malaria. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2001,** *33* (12), 1968-74.

40. 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* **2013,** *8* (1), 133-137.

41. Hsu, E., The history of qing hao in the Chinese materia medica. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2006,** *100* (6), 505-8.

42. Lai, H. C.; Singh, N. P.; Sasaki, T., Development of artemisinin compounds for cancer treatment. *Investigational new drugs* **2013,** *31* (1), 230-46.

43. (a) Utzinger, J.; N'Goran, E. K.; N'Dri, A.; Lengeler, C.; Xiao, S.; Tanner, M., Oral artemether for prevention of Schistosoma mansoni infection: randomised controlled trial. *Lancet* **2000,** *355* (9212), 1320-5; (b) Keiser, J.; Shu-Hua, X.; Jian, X.; Zhen-San, C.; Odermatt, P.; Tesana, S.; Tanner, M.; Utzinger, J., Effect of artesunate and artemether against Clonorchis sinensis and Opisthorchis viverrini in rodent models. *International journal of antimicrobial agents* **2006,** *28* (4), 370-3.

44. Klonis, N.; Creek, D. J.; Tilley, L., Iron and heme metabolism in Plasmodium falciparum and the mechanism of action of artemisinins. *Current opinion in microbiology* **2013,** *16* (6), 722-7.

45. (a) Haynes, R. K.; Chan, W. C.; Wong, H. N.; Li, K. Y.; Wu, W. K.; Fan, K. M.; Sung, H. H.; Williams, I. D.; Prosperi, D.; Melato, S.; Coghi, P.; Monti, D., Facile oxidation of leucomethylene blue and dihydroflavins by artemisinins: relationship with flavoenzyme function and antimalarial mechanism of action. *ChemMedChem* **2010,** *5* (8), 1282-99; (b) Haynes, R. K.; Cheu, K. W.; Tang, M. M.; Chen, M. J.; Guo, Z. F.; Guo, Z. H.; Coghi, P.; Monti, D., Reactions of antimalarial peroxides with each of leucomethylene blue and dihydroflavins: flavin reductase and the cofactor model exemplified. *ChemMedChem* **2011,** *6* (2), 279-91.

46. Udomsangpetch, R.; Pipitaporn, B.; Krishna, S.; Angus, B.; Pukrittayakamee, S.; Bates, I.; Suputtamongkol, Y.; Kyle, D. E.; White, N. J., Antimalarial drugs reduce cytoadherence and rosetting Plasmodium falciparum. *The Journal of infectious diseases* **1996,** *173* (3), 691-8.

47. Lelievre, J.; Almela, M. J.; Lozano, S.; Miguel, C.; Franco, V.; Leroy, D.; Herreros, E., Activity of clinically relevant antimalarial drugs on Plasmodium falciparum mature gametocytes in an ATP bioluminescence "transmission blocking" assay. *PloS one* **2012,** *7* (4), e35019.

48. (a) Brewer, T. G.; Peggins, J. O.; Grate, S. J.; Petras, J. M.; Levine, B. S.; Weina, P. J.; Swearengen, J.; Heiffer, M. H.; Schuster, B. G., Neurotoxicity in animals due to arteether and artemether. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1994,** *88 Suppl 1*, S33-6; (b) White, N. J., Qinghaosu (artemisinin): the price of success. *Science* **2008,** *320* (5874), 330-4.

49. Calderon, F.; Wilson, D. M.; Gamo, F. J., Antimalarial drug discovery: recent progress and future directions. *Progress in medicinal chemistry* **2013,** *52*, 97-151.

50. Banek, K.; Lalani, M.; Staedke, S. G.; Chandramohan, D., Adherence to artemisinin-based combination therapy for the treatment of malaria: a systematic review of the evidence. *Malaria journal* **2014,** *13*, 7.

51. Nagelschmitz, J.; Voith, B.; Wensing, G.; Roemer, A.; Fugmann, B.; Haynes, R. K.; Kotecka, B. M.; Rieckmann, K. H.; Edstein, M. D., First assessment in humans of the safety, tolerability, pharmacokinetics, and ex vivo pharmacodynamic antimalarial activity of the new artemisinin derivative artemisone. *Antimicrob Agents Chemother* **2008,** *52* (9), 3085-91.

52. Guo, J.; Guiguemde, A. W.; Bentura-Marciano, A.; Clark, J.; Haynes, R. K.; Chan, W. C.; Wong, H. N.; Hunt, N. H.; Guy, R. K.; Golenser, J., Synthesis of artemiside and its effects in combination with conventional drugs against severe murine malaria. *Antimicrob Agents Chemother* **2012,** *56* (1), 163-73.

53. Singh, C.; Verma, V. P.; Hassam, M.; Singh, A. S.; Naikade, N. K.; Puri, S. K., New orally active amino- and hydroxy-functionalized 11-azaartemisinins and their derivatives with high order of antimalarial activity against multidrug-resistant Plasmodium yoelii in Swiss mice. *J Med Chem* **2014,** *57* (6), 2489-97.

54. (a) Zhu, C.; Cook, S. P., A concise synthesis of (+)-artemisinin. *Journal of the American Chemical Society* **2012,** *134* (33), 13577-9; (b) Paddon, C. J.; Westfall, P. J.; Pitera, D. J.; Benjamin, K.; Fisher, K.; McPhee, D.; Leavell, M. D.; Tai, A.; Main, A.; Eng, D.; Polichuk, D. R.; Teoh, K. H.; Reed, D. W.; Treynor, T.; Lenihan, J.; Fleck, M.; Bajad, S.; Dang, G.; Dengrove, D.; Diola, D.; Dorin, G.; Ellens, K. W.; Fickes, S.; Galazzo, J.; Gaucher, S. P.; Geistlinger, T.; Henry, R.; Hepp, M.; Horning, T.; Iqbal, T.; Jiang, H.; Kizer, L.; Lieu, B.; Melis, D.; Moss, N.; Regentin, R.; Secrest, S.; Tsuruta, H.; Vazquez, R.; Westblade, L. F.; Xu, L.; Yu, M.; Zhang, Y.; Zhao, L.; Lievense, J.; Covello, P. S.; Keasling, J. D.; Reiling, K. K.; Renninger, N. S.; Newman, J. D., High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature* **2013,** *496* (7446), 528-32; (c) Kopetzki, D.; Levesque, F.; Seeberger, P. H., A continuous-flow process for the synthesis of artemisinin. *Chemistry* **2013,** *19* (17), 5450-6; (d) Acton, N.; Roth, R. J., ON THE CONVERSION OF DIHYDROARTEMISINIC ACID INTO ARTEMISININ. *Journal of Organic Chemistry* **1992,** *57* (13), 3610-3614; (e) Haynes, R. K. a. V., S. C. Cyclic peroxyacetal lactone, lactol and ether compounds. U. S. Patent 5310946, 1994; (f) Ro, D. K.; Paradise, E. M.; Ouellet, M.; Fisher, K. J.; Newman, K. L.; Ndungu, J. M.; Ho, K. A.; Eachus, R. A.; Ham, T. S.; Kirby, J.; Chang, M. C.; Withers, S. T.; Shiba, Y.; Sarpong, R.; Keasling, J. D., Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature* **2006,** *440* (7086), 940-3; (g) Westfall, P. J.; Pitera, D. J.; Lenihan, J. R.; Eng, D.; Woolard, F. X.; Regentin, R.; Horning, T.; Tsuruta, H.; Melis, D. J.; Owens, A.; Fickes, S.; Diola, D.; Benjamin, K. R.; Keasling, J. D.; Leavell, M. D.; McPhee, D. J.; Renninger, N. S.; Newman, J. D.; Paddon, C. J., Production of amorphadiene in yeast, and its conversion to dihydroartemisinic acid, precursor to the antimalarial agent artemisinin. *Proc Natl Acad Sci U S A* **2012,** *109* (3), E111-8; (h) Dietrich, J. A.; Yoshikuni, Y.; Fisher, K. J.; Woolard, F. X.; Ockey, D.; McPhee, D. J.; Renninger, N. S.; Chang, M. C.; Baker, D.; Keasling, J. D., A novel semi-biosynthetic route for artemisinin production using engineered substrate-promiscuous P450(BM3). *ACS chemical biology* **2009,** *4* (4), 261-7.

55. (a) Dong, Y.; Chollet, J.; Matile, H.; Charman, S. A.; Chiu, F. C.; Charman, W. N.; Scorneaux, B.; Urwyler, H.; Santo Tomas, J.; 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. *J Med Chem* **2005,** *48* (15), 4953-61; (b) Vennerstrom, J. L.; Arbe-Barnes, S.; Brun, R.; Charman, S. A.; Chiu, F. C.; Chollet, J.; Dong, Y.; Dorn, A.; Hunziker, D.; Matile, H.; McIntosh, K.; Padmanilayam, M.; Santo Tomas, J.; Scheurer, C.; Scorneaux, B.; Tang, Y.; Urwyler, H.; Wittlin, S.; Charman, W. N., Identification of an antimalarial synthetic trioxolane drug development candidate. *Nature* **2004,** *430* (7002), 900-4.

56. Charman, S. A.; Arbe-Barnes, S.; Bathurst, I. C.; Brun, R.; Campbell, M.; Charman, W. N.; Chiu, F. C.; 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. *Proc Natl Acad Sci U S A* **2011,** *108* (11), 4400-5.

57. O'Neill, P. M.; Amewu, R. K.; Nixon, G. L.; Bousejra ElGarah, F.; Mungthin, M.; Chadwick, J.; Shone, A. E.; Vivas, L.; Lander, H.; Barton, V.; Muangnoicharoen, S.; Bray, P. G.; Davies, J.; Park, B. K.; Wittlin, S.; Brun, R.; Preschel, M.; Zhang, K.; Ward, S. A., Identification of a 1,2,4,5-tetraoxane antimalarial drug-development candidate (RKA 182) with superior properties to the semisynthetic artemisinins. *Angew Chem Int Ed Engl* **2010,** *49* (33), 5693-7.

58. Marti, F.; Chadwick, J.; Amewu, R. K.; Burrell-Saward, H.; Srivastava, A.; Ward, S. A.; Sharma, R.; Berry, N.; O'Neill, P. M., Second generation analogues of RKA182: synthetic tetraoxanes with outstanding in vitro and in vivoantimalarial activities. *MedChemComm* **2011,** *2* (7), 661-665.

59. O'Neill, P. M.; Posner, G. H., A medicinal chemistry perspective on artemisinin and related endoperoxides. *J Med Chem* **2004,** *47* (12), 2945-64.

60. (a) Capela, R.; Cabal, G. G.; Rosenthal, P. J.; Gut, J.; Mota, M. M.; Moreira, R.; Lopes, F.; Prudencio, M., Design and evaluation of primaquine-artemisinin hybrids as a multistage antimalarial strategy. *Antimicrob Agents Chemother* **2011,** *55* (10), 4698-706; (b) Miranda, D.; Capela, R.; Albuquerque, I. S.; Meireles, P.; Paiva, I.; Nogueira, F.; Amewu, R.; Gut, J.; Rosenthal, P. J.; Oliveira, R.; Mota, M. M.; Moreira, R.; Marti, F.; Prudencio, M.; O'Neill, P. M.; Lopes, F., Novel endoperoxide-based transmission-blocking antimalarials with liver- and blood-schizontocidal activities. *ACS medicinal chemistry letters* **2014,** *5* (2), 108-12.

61. (a) FIESER, L. F.; HEYMANN, H.; SELIGMAN, A. M., NAPHTHOQUINONE ANTIMALARIALS: XX. Metabolic Degradation. *Journal of Pharmacology and Experimental Therapeutics* **1948,** *94* (2), 112-124; (b) Fawaz, G.; Fieser, L. F., NAPHTHOQUINONE ANTIMALARIALS .24. A NEW SYNTHESIS OF LAPINONE. *Journal of the American Chemical Society* **1950,** *72* (2), 996-1006; (c) Fieser, L. F.; Berliner, E.; et al., Naphthoquinone antimalarials; general survey. *Journal of the American Chemical Society* **1948,** *70* (10), 3151-5.

62. (a) Hussain, H.; Krohn, K.; Ahmad, V. U.; Miana, G. A.; Green, I. R., Lapachol: an overview. *Arkivoc* **2007**, 145-171; (b) Eyong, K. O.; Puppala, M.; Kumar, P. S.; Lamshoft, M.; Folefoc, G. N.; Spiteller, M.; Baskaran, S., A mechanistic study on the Hooker oxidation: synthesis of novel indane carboxylic acid derivatives from lapachol. *Organic & biomolecular chemistry* **2013,** *11* (3), 459-68.

63. (a) Wendel, W. B., The influence of napthoquinones upon the respiratory and carbohydrate metabolism of malarial parasites. *Federation proceedings* **1946,** *5*, 406; (b) Nixon, G. L.; Moss, D. M.; Shone, A. E.; Lalloo, D. G.; Fisher, N.; O'Neill, P. M.; Ward, S. A.; Biagini, G. A., Antimalarial pharmacology and therapeutics of atovaquone. *The Journal of antimicrobial chemotherapy* **2013,** *68* (5), 977-85; (c) Srivastava, I. K.; Rottenberg, H.; Vaidya, A. B., Atovaquone, a broad spectrum antiparasitic drug, collapses mitochondrial membrane potential in a malarial parasite. *The Journal of biological chemistry* **1997,** *272* (7), 3961-6.

64. Hughes, W. T.; Gray, V. L.; Gutteridge, W. E.; Latter, V. S.; Pudney, M., Efficacy of a hydroxynaphthoquinone, 566C80, in experimental Pneumocystis carinii pneumonitis. *Antimicrob Agents Chemother* **1990,** *34* (2), 225-8.

65. (a) Painter, H. J.; Morrisey, J. M.; Mather, M. W.; Vaidya, A. B., Specific role of mitochondrial electron transport in blood-stage Plasmodium falciparum. *Nature* **2007,** *446* (7131), 88-91; (b) Vaidya, A. B.; Mather, M. W., Mitochondrial evolution and functions in malaria parasites. *Annual review of microbiology* **2009,** *63*, 249-67.

66. Nakato, H.; Vivancos, R.; Hunter, P. R., A systematic review and meta-analysis of the effectiveness and safety of atovaquone proguanil (Malarone) for chemoprophylaxis against malaria. *The Journal of antimicrobial chemotherapy* **2007,** *60* (5), 929-36.

67. Sutherland, C. J.; Laundy, M.; Price, N.; Burke, M.; Fivelman, Q. L.; Pasvol, G.; Klein, J. L.; Chiodini, P. L., Mutations in the Plasmodium falciparum cytochrome b gene are associated with delayed parasite recrudescence in malaria patients treated with atovaquone-proguanil. *Malaria journal* **2008,** *7*, 240.

68. (a) Nilsen, A.; LaCrue, A. N.; White, K. L.; Forquer, I. P.; Cross, R. M.; Marfurt, J.; Mather, M. W.; Delves, M. J.; Shackleford, D. M.; Saenz, F. E.; Morrisey, J. M.; Steuten, J.; Mutka, T.; Li, Y.; Wirjanata, G.; Ryan, E.; Duffy, S.; Kelly, J. X.; Sebayang, B. F.; Zeeman, A. M.; Noviyanti, R.; Sinden, R. E.; Kocken, C. H.; Price, R. N.; Avery, V. M.; Angulo-Barturen, I.; Jimenez-Diaz, M. B.; Ferrer, S.; Herreros, E.; Sanz, L. M.; Gamo, F. J.; Bathurst, I.; Burrows, J. N.; Siegl, P.; Guy, R. K.; Winter, R. W.; Vaidya, A. B.; Charman, S. A.; Kyle, D. E.; Manetsch, R.; Riscoe, M. K., Quinolone-3-diarylethers: a new class of antimalarial drug. *Science translational medicine* **2013,** *5* (177), 177ra37; (b) Biagini, G. A.; Fisher, N.; Shone, A. E.; Mubaraki, M. A.; Srivastava, A.; Hill, A.; Antoine, T.; Warman, A. J.; Davies, J.; Pidathala, C.; Amewu, R. K.; Leung, S. C.; Sharma, R.; Gibbons, P.; Hong, D. W.; Pacorel, B.; Lawrenson, A. S.; Charoensutthivarakul, S.; Taylor, L.; Berger, O.; Mbekeani, A.; Stocks, P. A.; Nixon, G. L.; Chadwick, J.; Hemingway, J.; Delves, M. J.; Sinden, R. E.; Zeeman, A.-M.; Kocken, C. H. M.; Berry, N. G.; O'Neill, P. M.; Ward, S. A., Generation of quinolone antimalarials targeting the Plasmodium falciparum mitochondrial respiratory chain for the treatment and prophylaxis of malaria. *Proceedings of the National Academy of Sciences of the United States of America* **2012,** *109* (21), 8298-8303.

69. 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* **2010,** *329* (5996), 1175-1180.

70. 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 beta-Carbolines (Spiroindolones): A New Class of Potent and Orally Efficacious Compounds for the Treatment of Malaria. *Journal of Medicinal Chemistry* **2010,** *53* (14), 5155-5164.

71. 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* **2013,** *13* (2), 227-237.

72. van Pelt-Koops, J. C.; Pett, H. E.; Graumans, W.; van der Vegte-Bolmer, M.; van Gemert, G. J.; Rottmann, M.; Yeung, B. K. S.; Diagana, T. T.; Sauerwein, R. W., The Spiroindolone Drug Candidate NITD609 Potently Inhibits Gametocytogenesis and Blocks Plasmodium falciparum Transmission to Anopheles Mosquito Vector. *Antimicrobial Agents and Chemotherapy* **2012,** *56* (7), 3544-3548.

73. Chen, X.; Chen, H.; Ji, X.; Jiang, H.; Yao, Z. J.; Liu, H., Asymmetric one-pot sequential Mannich/hydroamination reaction by organo- and gold catalysts: synthesis of spiro[pyrrolidin-3,2'-oxindole] derivatives. *Organic letters* **2013,** *15* (8), 1846-9.

74. Crowe, M. F., M.; Francese, G.; Grimler, D.; Kuesters, E.; Laumen, K.; Li, Y.; Lin, C.; Nazor, J.; Ruch, T.; Smith, D.; Song, S. and Teng, S. Chemical process for preparing spiroindolones and intermediates there of. 2013.

75. Dahl, E. L.; Rosenthal, P. J., Multiple antibiotics exert delayed effects against the Plasmodium falciparum anicoplast. *Antimicrobial Agents and Chemotherapy* **2007,** *51* (10), 3485-3490.

76. Lell, B.; Kremsner, P. G., Clindamycin as an antimalarial drug: review of clinical trials. *Antimicrob Agents Chemother* **2002,** *46* (8), 2315-20.

77. Obonyo, C. O.; Juma, E. A., Clindamycin plus quinine for treating uncomplicated falciparum malaria: a systematic review and meta-analysis. *Malaria journal* **2012,** *11*, 2.

78. Dharia, N. V.; Plouffe, D.; Bopp, S. E.; Gonzalez-Paez, G. E.; Lucas, C.; Salas, C.; Soberon, V.; Bursulaya, B.; Kochel, T. J.; Bacon, D. J.; Winzeler, E. A., Genome scanning of Amazonian Plasmodium falciparum shows subtelomeric instability and clindamycin-resistant parasites. *Genome research* **2010,** *20* (11), 1534-44.

79. Mutak, S., Azalides from azithromycin to new azalide derivatives. *The Journal of antibiotics* **2007,** *60* (2), 85-122.

80. Gingras, B. A.; Jensen, J. B., Activity of azithromycin (CP-62,993) and erythromycin against chloroquine-sensitive and chloroquine-resistant strains of Plasmodium falciparum in vitro. *The American journal of tropical medicine and hygiene* **1992,** *47* (3), 378-82.

81. Miller, R. S.; Wongsrichanalai, C.; Buathong, N.; McDaniel, P.; Walsh, D. S.; Knirsch, C.; Ohrt, C., Effective treatment of uncomplicated Plasmodium falciparum malaria with azithromycin-quinine combinations: a randomized, dose-ranging study. *The American journal of tropical medicine and hygiene* **2006,** *74* (3), 401-6.

82. Tan, K. R.; Magill, A. J.; Parise, M. E.; Arguin, P. M., Doxycycline for malaria chemoprophylaxis and treatment: report from the CDC expert meeting on malaria chemoprophylaxis. *The American journal of tropical medicine and hygiene* **2011,** *84* (4), 517-31.

83. (a) Jomaa, H.; Wiesner, J.; Sanderbrand, S.; Altincicek, B.; Weidemeyer, C.; Hintz, M.; Turbachova, I.; Eberl, M.; Zeidler, J.; Lichtenthaler, H. K.; Soldati, D.; Beck, E., Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs. *Science* **1999,** *285* (5433), 1573-6; (b) Wiesner, J.; Borrmann, S.; Jomaa, H., Fosmidomycin for the treatment of malaria. *Parasitology research* **2003,** *90 Suppl 2*, S71-6.

84. Wiesner, J.; Reichenberg, A.; Hintz, M.; Ortmann, R.; Schlitzer, M.; Van Calenbergh, S.; Borrmann, S.; Lell, B.; Kremsner, P.; Hutchinson, D.; Jomaa, H., Fosmidomycin as an Antimalarial Agent. In *Isoprenoid Synthesis in Plants and Microorganisms*, Bach, T. J.; Rohmer, M., Eds. Springer New York: 2013; pp 119-137.

85. Dharia, N. V.; Sidhu, A. B.; Cassera, M. B.; Westenberger, S. J.; Bopp, S. E.; Eastman, R. T.; Plouffe, D.; Batalov, S.; Park, D. J.; Volkman, S. K.; Wirth, D. F.; Zhou, Y.; Fidock, D. A.; Winzeler, E. A., Use of high-density tiling microarrays to identify mutations globally and elucidate mechanisms of drug resistance in Plasmodium falciparum. *Genome biology* **2009,** *10* (2), R21.

86. (a) Behrendt, C. T.; Kunfermann, A.; Illarionova, V.; Matheeussen, A.; Pein, M. K.; Grawert, T.; Kaiser, J.; Bacher, A.; Eisenreich, W.; Illarionov, B.; Fischer, M.; Maes, L.; Groll, M.; Kurz, T., Reverse fosmidomycin derivatives against the antimalarial drug target IspC (Dxr). *J Med Chem* **2011,** *54* (19), 6796-802; (b) Schluter, K.; Walter, R. D.; Bergmann, B.; Kurz, T., Arylmethyl substituted derivatives of Fosmidomycin: synthesis and antimalarial activity. *European journal of medicinal chemistry* **2006,** *41* (12), 1385-97; (c) Ortmann, R.; Wiesner, J.; Reichenberg, A.; Henschker, D.; Beck, E.; Jomaa, H.; Schlitzer, M., Acyloxyalkyl ester prodrugs of FR900098 with improved in vivo anti-malarial activity. *Bioorganic & medicinal chemistry letters* **2003,** *13* (13), 2163-6.

87. Rubio-Pina, J.; Vazquez-Flota, F., Pharmaceutical applications of the benzylisoquinoline alkaloids from Argemone mexicana L. *Current topics in medicinal chemistry* **2013,** *13* (17), 2200-7.

88. Willcox, M. L.; Graz, B.; Falquet, J.; Diakite, C.; Giani, S.; Diallo, D., A "reverse pharmacology" approach for developing an anti-malarial phytomedicine. *Malaria journal* **2011,** *10 Suppl 1*, S8.

89. (a) Vennerstrom, J. L.; Klayman, D. L., Protoberberine alkaloids as antimalarials. *J Med Chem* **1988,** *31* (6), 1084-7; (b) Iwasa, K.; Kim, H. S.; Wataya, Y.; Lee, D. U., Antimalarial activity and structure-activity relationships of protoberberine alkaloids. *European journal of medicinal chemistry* **1998,** *33* (1), 65-69.

90. Xu, Y.-J.; Foubert, K.; Dhooghe, L.; Lemiere, F.; Cimanga, K.; Mesia, K.; Apers, S.; Pieters, L., Chromatographic profiling and identification of two new iridoid- indole alkaloids by UPLC-MS and HPLC-SPE-NMR analysis of an antimalarial extract from Nauclea pobeguinii. *Phytochemistry Letters* **2012,** *5* (2), 316-319.

91. Hofheinz, W.; Burgin, H.; Gocke, E.; Jaquet, C.; Masciadri, R.; Schmid, G.; Stohler, H.; Urwyler, H., Ro 42-1611 (arteflene), a new effective antimalarial: chemical structure and biological activity. *Trop Med Parasitol* **1994,** *45* (3), 261-5.

92. (a) Turschner, S.; Efferth, T., Drug resistance in Plasmodium: natural products in the fight against malaria. *Mini Rev Med Chem* **2009,** *9* (2), 206-2124; (b) Radloff, P. D.; Philipps, J.; Nkeyi, M.; Sturchler, D.; Mittelholzer, M. L.; Kremsner, P. G., Arteflene compared with mefloquine for treating Plasmodium falciparum malaria in children. *The American journal of tropical medicine and hygiene* **1996,** *55* (3), 259-62.

93. Jang, C. S.; Fu, F. Y.; Wang, C. Y.; Huang, K. C.; Lu, G.; Chou, T. C., Ch'ang Shan, a Chinese Antimalarial Herb. *Science* **1946,** *103* (2663), 59.

94. Jiang, S. P.; Zeng, Q.; Gettayacamin, M.; Tungtaeng, A.; Wannaying, S.; Lim, A.; Hansukjariya, P.; Okunji, C. O.; Zhu, S. R.; Fang, D. H., Antimalarial activities and therapeutic properties of febrifugine analogs. *Antimicrobial Agents and Chemotherapy* **2005,** *49* (3), 1169-1176.

95. Zhu, S.; Wang, J.; Chandrashekar, G.; Smith, E.; Liu, X.; Zhang, Y., Synthesis and evaluation of 4-quinazolinone compounds as potential antimalarial agents. *European journal of medicinal chemistry* **2010,** *45* (9), 3864-3869.

96. Kikuchi, H.; Yamamoto, K.; Horoiwa, S.; Hirai, S.; Kasahara, R.; Hariguchi, N.; Matsumoto, M.; Oshima, Y., Exploration of a new type of antimalarial compounds based on febrifugine. *Journal of Medicinal Chemistry* **2006,** *49* (15), 4698-4706.

97. Zhu, S.; Chandrashekar, G.; Meng, L.; Robinson, K.; Chatterji, D., Febrifugine analogue compounds: Synthesis and antimalarial evaluation. *Bioorganic & Medicinal Chemistry* **2012,** *20* (2), 927-932.

98. Herman, J. D.; Pepper, L. R.; Cortese, J. F.; Estiu, G.; Galinsky, K.; Zuzarte-Luis, V.; Derbyshire, E. R.; Ribacke, U.; Lukens, A. K.; Santos, S. A.; Patel, V.; Clish, C. B.; Sullivan, W. J., Jr.; Zhou, H.; Bopp, S. E.; Schimmel, P.; Lindquist, S.; Clardy, J.; Mota, M. M.; Keller, T. L.; Whitman, M.; Wiest, O.; Wirth, D. F.; Mazitschek, R., The cytoplasmic prolyl-tRNA synthetase of the malaria parasite is a dual-stage target of febrifugine and its analogs. *Science translational medicine* **2015,** *7* (288).

99. Burrows, J. N.; Burlot, E.; Campo, B.; Cherbuin, S.; Jeanneret, S.; Leroy, D.; Spangenberg, T.; Waterson, D.; Wells, T. N.; Willis, P., Antimalarial drug discovery - the path towards eradication. *Parasitology* **2014,** *141* (1), 128-39.