New approaches in antimalarial drug discovery and development - A Review

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Malaria remains a major world health problem following the emergence and spread of Plasmodium falciparum that is resistant to the majority of antimalarial drugs. This problem has since been aggravated by a decreased sensitivity of Plasmodium vivax to chloroquine. This review discusses strategies for evaluating the antimalarial activity of new compounds in vitro and in animal models ranging from conventional tests to the latest high-throughput screening technologies. Antimalarial discovery approaches include the following: the discovery of antimalarials from natural sources, chemical modifications of existing antimalarials, the development of hybrid compounds, testing of commercially available drugs that have been approved for human use for other diseases and molecular modeling using virtual screening technology and docking. Using these approaches, thousands of new drugs with known molecular specificity and active against P. falciparum have been selected. The inhibition of haemozoin formation in vitro, an indirect test that does not require P. falciparum cultures, has been described and this test is believed to improve antimalarial drug discovery. Clinical trials conducted with new funds from international agencies and the participation of several industries committed to the eradication of malaria should accelerate the discovery of drugs that are as effective as artemisinin derivatives, thus providing new hope for the control of malaria.

Key words: human malaria - Plasmodium falciparum - antimalarials - drug screening - chloroquine - artemisinin - drug resistance

According to the World Health Organization (WHO), malaria remains a major health problem and affects more than 225 million individuals, causing approximately 700 thousand deaths each year. Plasmodium falciparum is the most common causative agent (WHO 2011). However, Plasmodium vivax malaria, which was previously considered benign, also causes life-threatening symptoms. P. vivax is the most prevalent species in Latin America, South-East Asia, the Eastern Mediterranean and the Western Pacific (WHO 2011). Plasmodium malariae and Plasmodium ovale are less prevalent and cause less severe disease in humans, whereas Plasmodium knowlesi, a parasite of Old World monkeys, causes human disease in Southeast Asia (Cox-Singh & Singh 2008). In addition, drug resistance has been reported in P. vivax malaria (Price et al. 2009).

Sporozoites, injected by Anopheles mosquitoes as they bite into the skin of mammalian hosts, rapidly enter the blood circulation to reach liver hepatocytes, where they mature in an entirely asymptomatic phase that lasts for approximately two weeks. Sporozoites of P. vivax and P. ovale remain dormant (hypnozoites) in the human hepatocyte, where they mature months to years later. These forms cause late malaria relapses under conditions that are not well understood and are related to host stress and low primaquine (PQ) doses (Townell et al. 2012); such relapses require new drug treatment. The ideal antimalarial should destroy sporozoites soon after they are inoculated into the vertebrate host by the mosquitoes. However, no effective prophylactic anti-sporozoite drug is currently in use. Medicinal plants that hamper sporozoite development in host cells have been reported and these plants appear to act as prophylactics, as further discussed below.

Merozoites are liberated as merosomes from liver cells and then bud off from the hepatocytes to invade and develop in red blood cells (RBCs) (Sturm et al. 2006). In the RBCs, the parasites undergo asexual multiplication by schizogony and release merozoites, which invade other RBCs, thereby reinitiating the blood-stage cycle (Greenwood et al. 2008). The infected RBCs (iRBCs) are responsible for the disease symptoms, i.e., high and periodic fever (paroxysms), headaches (common to all human malaria species) and anaemia. The symptoms of P. falciparum include cerebral malaria and respiratory distress (life-threatening manifestations that are related to iRBC cytoadherence on microvascular endothelial cells), blockage of deep capillaries with neurological symptoms and death. The pathogenesis of severe malaria is not completely understood, although proinflammatory cytokines are known to be involved.

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(Clark et al. 2006) and these cytokines contribute to the suppression of erythropoiesis, particularly in infected children (Perkins et al. 2011). Evidence supports the role of type 1 pro-inflammatory cytokines that increase the expression of adhesion molecules on vascular endothelium and iRBC sequestration (Schofield & Grau 2005). Experimental data demonstrate that E6446, a synthetic antagonist of nucleic acid-sensing tool-like receptors (TLRs), diminishes the activation of TLR9 and prevents the increased production of cytokines in response to Plasmodium infections, consequently preventing severe malaria symptoms (Franklin et al. 2011).

In previous reviews, the approaches used for antimalarial drug discovery and development have been discussed (Krettli 2009, Mazier et al. 2009, Wells et al. 2010, Burrows et al. 2011a, b, Muregi et al. 2012) and these methods have since been updated. One of the promising approaches currently being used in the discovery of new antimalarials aimed at drug-resistant parasites and the interruption of the transmission of malaria is the testing of commercially available drugs that are currently prescribed for other indications; if successful, this approach will rapidly accelerate the production of new antimalarials at a lower cost. High-throughput screening (HTS) and molecular modelling (MM) have been successfully used in collaborative projects to select three potential antimalarial candidates (Penna-Coutinho et al. 2011), as discussed below.

**Malaria treatment and drug-resistant parasites** - The discovery of the first antimalarial treatment almost 400 years ago resulted from observations that acutely ill patients were cured of malaria after treatment with infusions of bark obtained from plants growing in the Peruvian Amazon (Graham 1966). Such activity in Cinchona calisaya and Cinchona succirubra plants was later attributed to the alkaloid quinine (QN), which was characterized by French chemists in 1820. QN remains important for treating complicated *P. falciparum* malaria despite its toxicity when used for extended periods of time (WHO 2010a, b).

Several 4-aminoquinolines were later synthesised based on the QN ring (Table I). Among them, chloroquine (CQ) is the safest and least expensive drug and most frequently was used to treat malaria worldwide as an essential component of the Global Malaria Eradication Campaign. This campaign, launched in 1955, was based on the treatment of malaria patients using CQ in association with mosquito control measures. In the late 1960s, *P. falciparum* CQ-resistant strains appeared in Latin America and South East Asia and gradually spread to most endemic regions. This campaign was interrupted in the 1970s. Malaria had been eradicated in a few countries (Karunamoorthi 2011). Most importantly, multi-drug and cross-drug resistance among existing antimalarials, mainly the aminoquinolines, were reported. CQ is now used only in drug combinations against *P. falciparum* or as the schizonticide of choice to treat *P. vivax* and other human plasmodia species.

Artemisinin (ART), a sesquiterpene endoperoxide, is a natural product that has been isolated from *Artemisia annua* (Asteraceae), a medicinal plant that has been used for over a thousand years in China. ART derivatives are used as antimalarials and have been recommended by the WHO for use in drug combinations [ART-based combination therapies (ACTs)] to treat uncomplicated *P. falciparum* malaria and *P. vivax* in areas of CQ resistance (WHO 2010a, b). Drugs that complement the use of ACT include lumefantrine, amodiaquine, mefloquine (MQ), sulfadoxine-pyrimethamine and antibiotics (Table II). However, reduced susceptibility to ART derivatives has been described in *P. falciparum* (Dondorp et al. 2009).

The radical cure of *P. vivax* and *P. ovale* requires PQ, an 8-aminoquinoline that prevents late relapses (Wells et al. 2010). PQ also targets the primary liver exo-erythrocytic forms (EEFs), iRBCs and gametocytes; for example, PQ is used to block malarial transmission in Brazil (WHO 2010b) (Table III). However, PQ metabolites cause severe haemolytic anaemia and methemoglobinemia in patients who are genetically deficient in glucose-6-phosphate dehydrogenase (G6PD) (Carmona-Fonseca et al. 2009). This side effect imposes a pre-screening requirement for G6PD deficiency in *P. vivax* malaria patients and limits PQ use. Tafenoquine, a new drug that targets the liver forms (EEFs and hypnozoites) is currently in clinical trials (Wells et al. 2010) (Table IV).

The control of malaria has become gradually more complex due to the spread of drug-resistant *P. falciparum* strains and drugs that block the transmission of malaria are recommended by the WHO, especially in areas of high *P. falciparum* transmission. The use of PQ to control the transmission of malaria in endemic areas requires medical supervision due to the haemolytic toxicity of the drug (WHO 2010a). Interventions to reduce mosquito density, human-vector contact and the vectorial capacity of *Anopheles* species are not simple (Karunamoorthi 2011). Some methods that are available to reduce the transmission of malaria include indoor spraying with insecticides, ultra-low volume space spraying, the chemical or biological control of larvae and personal protection using repellents or insecticide-treated nets. These methods are hampered by insecticide resistance and the high maintenance costs required to sustain the measures. The development of genetically modified mosquitoes (GMMs) that are resistant to parasite infection (which aims to reduce and/or block *Plasmodium* transmission) is being undertaken (James et al. 1999, Rodrigues et al. 2008, Isaacs et al. 2011), although the impact of GMMs in the field must be carefully evaluated prior to widespread use.

Currently, no effective vaccine is available to fight human malaria; however, various antigen formulations are undergoing field trials. In particular, RTS,S/AS01E, a vaccine based on the *P. falciparum* circumsporozoite protein and blood stage parasite proteins, has demonstrated promising results (Cohen et al. 2010, Aghanjii et al. 2011, Schwartz et al. 2012). Vaccines based on the anti-merozoite surface protein of blood stages (McCarthy et al. 2011) and a transmission-blocking vaccine (Herrera et al. 2007, Arevalo-Herrera 2010, Gregory et al. 2012) are being tested.
TABLE I
Chemical structure and classification of drugs used for human malaria treatment, mostly combined with artemisine derivatives, monotherapy is no longer recommended.

<table>
<thead>
<tr>
<th>Antimalarial</th>
<th>Structure</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amodiaquine</td>
<td><img src="image1" alt="Structure" /></td>
<td>4-aminoquinoline</td>
</tr>
<tr>
<td>Artemether</td>
<td><img src="image2" alt="Structure" /></td>
<td>Semi-synthetic endoperoxide</td>
</tr>
<tr>
<td>Artesunate</td>
<td><img src="image3" alt="Structure" /></td>
<td>Semi-synthetic endoperoxide</td>
</tr>
<tr>
<td>Bulaquine&lt;sup&gt;a&lt;/sup&gt;</td>
<td><img src="image4" alt="Structure" /></td>
<td>8-aminoquinoline</td>
</tr>
<tr>
<td>Dihydroartemisinin</td>
<td><img src="image5" alt="Structure" /></td>
<td>Semi-synthetic endoperoxide</td>
</tr>
<tr>
<td>Lumefantrine</td>
<td><img src="image6" alt="Structure" /></td>
<td>Amino alcohol</td>
</tr>
<tr>
<td>Mefloquine</td>
<td><img src="image7" alt="Structure" /></td>
<td>Amino alcohol</td>
</tr>
<tr>
<td>Piperaquine</td>
<td><img src="image8" alt="Structure" /></td>
<td>Bisquinoline</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td><img src="image9" alt="Structure" /></td>
<td>Diaminopyrimidine</td>
</tr>
<tr>
<td>Primaquine</td>
<td><img src="image10" alt="Structure" /></td>
<td>8-aminoquinoline</td>
</tr>
<tr>
<td>Quinine</td>
<td><img src="image11" alt="Structure" /></td>
<td>Amino alcohol</td>
</tr>
<tr>
<td>Sulfadoxine</td>
<td><img src="image12" alt="Structure" /></td>
<td>Antibiotic</td>
</tr>
<tr>
<td>Tafenoquine&lt;sup&gt;a&lt;/sup&gt;</td>
<td><img src="image13" alt="Structure" /></td>
<td>8-aminoquinoline</td>
</tr>
</tbody>
</table>

<sup>a</sup>: bulaquine and tafenoquine are undergoing clinical screening and target the hepatic forms.
In vitro assays of new antimalarials - Most programs aiming to discover new antimalarials are based on microtests against *Plasmodium falciparum* in blood cultures and animal models (Kretti 2009). New methods have replaced traditional assays and various stages of the malaria parasites are currently used. Large-scale drug screening and techniques based on the computer analysis of drug banks are available and being used, as discussed below.

**Tests of blood schizonticides** - The evaluation of *P. falciparum* drug susceptibility in vitro, formerly based on microscopy, has been replaced by the incorporation of radioactive [3H] hypoxanthine into live parasite DNA (Desjardins et al. 1979); however, the need to store radioactive solid wastes for decades, expensive equipment requirements and the need for well-trained technicians restrict the use of this method.

Two enzyme-linked immunosorbent assays are available for drug testing; these assays use monoclonal antibodies that are specific for the parasite enzyme lactate dehydrogenase (PfLDH) (Druilhe et al. 2001) or a parasite histidine-rich protein (HRP) II (Noedl et al. 2002). The assessment of parasite growth is effective in both of these tests. However, the existence of genetic diversity amongst *Plasmodium* strains interferes with the performance of PfHRP2 tests (Baker et al. 2005).

Methods based on DNA dyes are useful for drug screening and take advantage of the lack of DNA and RNA in erythrocytes. The following nucleic acid dyes are used in *P. falciparum* tests: (i) SYBR Green I is the most widely used in vitro (Izumiyama et al. 2009) and in vivo (Somsak et al. 2012) and costs less than other dyes, (ii) YOYO-1 is the most sensitive dye and has the advantage of being able to detect low levels of parasitaemia, (iii) the SYTO series of dyes has increased applicability due to its binding affinities (Jiménez-Díaz et al. 2009), (iv) propidium iodide, which is used with other stains, has a broad emission spectra that limits the range of dye combinations that can be used, (v) acridine orange, a relatively toxic molecule previously used for the diagnosis of human malaria, is laborious to use and has low reproducibility (Grimberg et al. 2008) and (vi) the Hoechst dye series, although selective, is of limited use particularly because of its high cost (Grimberg et al. 2008) and (vii) PicoGreen provides reliable results, but is limited by the quenching of its fluorescence by haemoglobin, which confounds the detection of low levels of parasitaemia (Quashie et al. 2006).

Transfected parasites expressing nucleic acid dyes are detected by flow cytometry or fluorescence microscopy and appear to be useful models for drug screening; green fluorescent protein (GFP)-recombinant *Plasmodium berghei* and *P. falciparum* parasites have been successfully used (Sanchez et al. 2004, 2007, Wilson et al. 2010).

The inhibition of haemoglobin (Hb) (β-haematin) formation in vitro is an indirect test that does not require *P. falciparum* blood cultures and has been reported to be useful for antimalarial screening because the majority of 4-aminoquinoline antimalarials test positive, i.e., they inhibit the formation of Hb crystals. The results, which are measured using enzyme-independent reactions (Dorn et al. 1995, Parapini et al. 2000, Ncokazi & Egan 2005), are rapidly obtained (a 60-min incubation is used) and were shown to agree across four different laboratories (Ncokazi & Egan 2005). We have successfully used this test after many attempts to establish a protocol to evaluate the activity of two CQ analogues [monoquinoline (MAQ) and bisquinoline (BAQ)]; both analogues inhibited Hb formation in vitro, a result that

### TABLE II

<table>
<thead>
<tr>
<th>Treatment/route</th>
<th>Malaria type</th>
<th>Resistance reported</th>
<th>Countries where treatment is adopted(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL/oral</td>
<td>Uncomplicated <em>falciparum</em></td>
<td>Yes</td>
<td>South America, Africa, Asia (56 countries)</td>
</tr>
<tr>
<td>AS + AQ/oral</td>
<td>Uncomplicated <em>falciparum</em></td>
<td>Yes</td>
<td>27 countries, 25 in Africa</td>
</tr>
<tr>
<td>AS + MQ/oral</td>
<td>Uncomplicated <em>falciparum</em></td>
<td>Yes</td>
<td>South America, Asia, in eight different countries</td>
</tr>
<tr>
<td>AS + SP/oral</td>
<td>Uncomplicated <em>falciparum</em></td>
<td>Yes</td>
<td>11 countries, mainly Asia</td>
</tr>
<tr>
<td>DHA + PPQ/oral</td>
<td>Uncomplicated <em>falciparum</em></td>
<td>Yes</td>
<td>China and South East Asia</td>
</tr>
<tr>
<td>AS/i.v. or i.m. + ACT/()oral</td>
<td>Severe <em>falciparum</em></td>
<td>No</td>
<td>Worldwide</td>
</tr>
<tr>
<td>QN/i.v. + ACT/()oral</td>
<td>Severe <em>falciparum</em></td>
<td>No</td>
<td>Worldwide</td>
</tr>
<tr>
<td>ACT/()oral + PQ/()oral</td>
<td>CQ resistant <em>P. vivax</em></td>
<td>No</td>
<td>Where ACT(b) has been adopted for <em>falciparum</em> malaria treatment(c)</td>
</tr>
<tr>
<td>Artemisinin derivatives + PQ/oral</td>
<td>Severe <em>vivax</em></td>
<td>No</td>
<td>Worldwide</td>
</tr>
</tbody>
</table>

\(a\): schedule used as first or second-line treatment; \(b\): artesether + lumefantrine (AL), artesunate (AS) + amodiaquine (AQ), AS + mefloquine (MQ), AS + sulfadoxine/pyrimethamine (SP), dihydroartemisinin (DHA) + piperaquine (PPQ); \(c\): in *P. vivax*, the use of primaquine (PQ) aims radical cure of the late relapses; \(d\): Solomon Islands, Vanuatu, Papua New Guinea, Brazil and Indonesia (Bassat et al. 2011); i.m.: intramuscular injection; i.v.: intravenous injection; QN: quinine. Adapted from WHO (2010a, b, 2011).
was further supported by docking tests; the interactions with dimeric haematin and the inhibition of haeme polymerisation were dose-dependent (Aguiar et al. 2012).

Several in vivo tests that use animal models are available; all require the approval of protocols regarding the ethical issues for animal use (Krettli et al. 2009). In the 4-day suppressive test, drug activity is measured based on the inhibition of P. berghei parasitaemia in comparison with untreated control mice, as described by Peters (1965).

**Drugs that act on liver stages and block the transmission of malaria -** The primary asexual development of *Plasmodium yoelii* or *P. berghei* sporozoites in mice or *P. falciparum* in monkeys has also been reproduced in cultured hepatoma cells; however, few laboratories produce these sporozoite stages in *Anopheles mosquitoes*. When available, the tests are useful tools to investigate drug activity against pre-erythrocytic stages of the parasites (Krettli et al. 2009, Mazier et al. 2009). The use of GFP sporozoites would be useful in protocols because this approach would allow the parasite route to be followed from the site of inoculation to the liver cells (Gueirard et al. 2010), thus elucidating where the parasite is targeted by the antimalarials. The anti-sporozoite activity of two medicinal plants, *Ampelozyziphus amazonicus* and *Strychnopsis thouarsii*, has been demonstrated; these drugs were used in malaria-endemic areas of Brazil (Andrade-Neto et al. 2008, Krettli et al. 2001) and Madagascar (Carraz et al. 2006), as discussed below.

An indirect assay that measures the activity of new compounds that target hypnozoites was described many years ago based on the rationale that gametocytes and hypnozoites share certain characteristics. Both have a long period of latency, do not proliferate or differentiate and are killed by PQ and other 8-aminoquinolines (Gwadz et al. 1983). The result was further confirmed in our work using *Plasmodium gallinaceum* sporogony in mosquitoes that were blood-fed on chickens with low parasitaemia (Carvalho et al. 1992). Several new naphthoquinone analogues were tested in parallel with PQ and crude extracts of *A. amazonicus* (a plant often used against malaria in the Amazon); however, only PQ inhibited oocyst formation. Several new PQ derivatives (Neuenfeldt et al. 2011) were tested using the same protocol. Three among 15 compounds tested inhibited sporogony in the mosquito vector and all 15 tested were active against *P. falciparum*-iRBCs (AU Krettli et al., unpublished observations).

An assay that employs a GFP chimera of the early sexual *P. falciparum* blood stage protein (Pfs16), as a marker for commitment to gametocytogenesis, was used to assess the activity of various compounds against gametocytes. The use of a transgenic parasite (3D7-GFP16B) in cultures allowed for the identification of asexual parasites that will differentiate into gametocytes (Dixon et al. 2009) in cultures containing conditioned media, i.e., those harvested from *P. falciparum* cultures (Williams 1999). The obtained gametocytes were sorted based on the expression of the GFP reporter gene and used for the assay; gametocytes were exposed to test or control drugs (24-48 h) and the results were measured using the ATP-based bioluminescent assay to detect and quantify the viable gametocytes (Peatey et al. 2012).

### TABLE III

Specificity of antimalarial drugs against the malaria parasite stages and drug resistance

<table>
<thead>
<tr>
<th>Antimalarial</th>
<th>Parasite targeted stage</th>
<th>GM</th>
<th>Drug resistance reported in <em>Plasmodium falciparum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Amodiaquine</td>
<td>EEF, HP, iRBC</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Artemisins</td>
<td></td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Atovaquone</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Chloroquine</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Clindamycin</td>
<td></td>
<td></td>
<td>No</td>
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<tr>
<td>Doxycycline</td>
<td></td>
<td></td>
<td>No</td>
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<tr>
<td>Lumefantrine</td>
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<td></td>
<td>No</td>
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<tr>
<td>Mefloquine</td>
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<td>Yes</td>
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<tr>
<td>Primaquine</td>
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<td>Yes</td>
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<tr>
<td>Proguanil</td>
<td></td>
<td></td>
<td>Yes</td>
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<tr>
<td>Pyrimethamine</td>
<td></td>
<td></td>
<td>Yes</td>
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<tr>
<td>Pyronaridine</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Quinine</td>
<td></td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Sulfadoxine</td>
<td></td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Tetracycline</td>
<td></td>
<td></td>
<td>No</td>
</tr>
</tbody>
</table>

EEF: exoerythrocytic forms from the primary sporozoite development hepatocytes; GM: sexual stages; HP: hypnozoites; iRBC: the erythrocytic forms responsible for the disease. Adapted from Mazier et al. (2009).
**MM in the discovery of antimalarials** - MM techniques, including virtual library screening (VLS) and docking studies, have been employed in theoretical medicinal chemistry to discover antimalarials (Luzhkov et al. 2007, Mugnaini et al. 2007). VLS is used to identify chemical structures in the parasite that are likely to bind to the candidate drug, thereby significantly reducing the number of compounds for experimental validation using enzymatic and/or cell-based assays (Kortagere et al. 2010). The results of chemical database querying are refined using docking programs with ligand-receptor algorithms that are able to rapidly process a number of compounds. The programs Gold® (Luzhkov et al. 2007), FlexX (DeLisle et al. 2001) and Molegro Virtual Docker® (MVD) (Morris et al. 1998) assess the best conformations of each candidate in terms of the energy and positioning. These programs optimise interactions in the active site and allow for the discovery of novel pharmacophoric groups. New drugs are designed based on previous leads and the stabilities of the ligands inside the binding pockets are further verified using molecular dynamics simulations. A large variety of new antimalarial candidates has been identified using MM among new and well-established drug targets, such as enzymes involved in the folate cycle (SHMT and DHFR-TS) (França et al. 2004, 2005, 2006, da Silva et al. 2010), LDH (Penna-Coutinho et al. 2011), enoyl-acyl carrier protein reductase (ENR) (Nicola et al. 2007), protein kinases (Keenan et al. 2005), cysteine proteases (Shah et al. 2011), topoisomerases (Roy et al. 2011) and spermidine synthase (SpdSyn) (Jacobsson et al. 2008).

Hundreds of new potential inhibitors of PfLDH were selected using VLS and 15 were submitted to docking studies using MVD®. Those compounds showing the lowest binding energies, as well as the commercially available compounds itraconazole, atorvastatin and posaconazole, were tested in vitro against *P. falciparum* and found to be active in CQ-resistant parasites (Penna-Coutinho et al. 2011).

Abagyan et al. (1994) used the Internal Coordinate Mechanics program to screen the ChemBridge Express database (chembridge.com/screening_libraries/) for chemical compounds that fit into the binding pocket of PfENR. This search yielded 750 compounds with scores better than -50. A further theoretical absorption, distribution, metabolism and excretion (ADME) prediction yielded 169 compounds, which were experimentally tested using a PfENR enzyme inhibition assay; 16 compounds showed greater than 45% inhibition of *P. falciparum* growth at 500 μM (Nicola et al. 2007).

Novel binders to the active site of *P. falciparum* SpdSyn (PfSpdSyn), an enzyme involved in the polyamine pathway, were selected using VLS and experimentally tested for parasite binding. Among 2.8 million structures that were tested, representing approximately three million commercially available compounds, 28 were selected for nuclear magnetic resonance testing for binding to PfSpdSyn. The compounds were predicted to target the amino substrate binding pocket and thus exhibited stronger binding upon the addition of methylthioadenosine (Wiesner et al. 2002).

**Strategies used to discover new antimalarial drugs** - A search based on medicinal plants and biodiversity

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Table IV

**Antimalarials under clinical trials and their parasite stage targets**

<table>
<thead>
<tr>
<th>Parasite stage target</th>
<th>Antimalarial</th>
<th>Phase of clinical trial</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>iRBC</td>
<td>Azithromycin + chloroquine</td>
<td>III</td>
<td>MMV</td>
</tr>
<tr>
<td></td>
<td>Ferroquine + artesunate (methalocenic)</td>
<td>II</td>
<td>Clinicaltrials.gov&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Fosmidomycin + clindamycin (antibiotics)</td>
<td>II</td>
<td>Clinicaltrials.gov&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Ozonide-OZ439 (synthetic peroxide)</td>
<td>II</td>
<td>Charman et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Piperaquine</td>
<td>IV</td>
<td>Warhurst et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Spiroindolone-NITD609 (natural product)</td>
<td>I</td>
<td>Rottmann et al. (2010)</td>
</tr>
<tr>
<td>iRBC + GM</td>
<td>Artesunate-amodiaquine</td>
<td>IV</td>
<td>MMV</td>
</tr>
<tr>
<td></td>
<td>Artesunate (i.v.)</td>
<td>IV</td>
<td>MMV</td>
</tr>
<tr>
<td></td>
<td>Coartem&lt;sup&gt;a&lt;/sup&gt; (artemether + lumefantrine)</td>
<td>IV</td>
<td>MMV</td>
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<td></td>
<td>Eurartesim&lt;sup&gt;b&lt;/sup&gt; (dihydroartemisin + piperaquine)</td>
<td>IV</td>
<td>MMV</td>
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<td></td>
<td>Pyramax pediatric&lt;sup&gt;c&lt;/sup&gt; (pyronaridine + artesunate)</td>
<td>III</td>
<td>MMV</td>
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<tr>
<td></td>
<td>Pyramax&lt;sup&gt;d&lt;/sup&gt; (pyronaridine + artesunate)</td>
<td>III</td>
<td>MMV</td>
</tr>
<tr>
<td>iRBC + LS + GM</td>
<td>Imidazolopiperazine (antifungal + anti-helminthic)</td>
<td>I</td>
<td>Wu et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Timidazole (anti-helminthic)</td>
<td>II</td>
<td>Clinicaltrials.gov&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>LS</td>
<td>Tafenoquine (8-aminooquinolone)</td>
<td>III</td>
<td>MMV</td>
</tr>
<tr>
<td></td>
<td>Bulaquine (8-aminooquinolone)</td>
<td>III</td>
<td>Valecha et al. (2001)</td>
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<td></td>
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<td>Krudsood et al. (2006)</td>
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<sup>a</sup> clinicaltrials.gov/ct2/show/NCT00988507; <sup>b</sup> clinicaltrials.gov/ct2/show/NCT01361269 and clinicaltrials.gov/ct2/show/NCT00214643; <sup>c</sup> clinicaltrials.gov/ct2/show/NCT00811096; GM: gametocytes; i.v.: intravenous injection; iRBC: infected red blood cells; LS: liver stages; MMV: Medicines for Malaria Venture (mmv.org/research-development/science-portfolio).
In Africa, herbal products are widely used to treat malaria (Adebayo & Krettli 2011), although they are not used as extensively in Latin America and other parts of the world. Our group has participated in the search for the active anti-malarial principles in medicinal plants of Brazil, mainly inspired by the success of two long-established drugs: (i) QN obtained from plants of Latin American origin (Cinchona sp. from the Peruvian Amazon) and (ii) ART derivatives isolated from *A. annua*, a medicinal plant that has been used for millennia in China for the treatment of malaria and fever (Carvalho & Krettli 1991, Krettli 2009, Krettli et al. 2009).

We have confirmed the antimalarial activity of extracts and fractions from *Cecropia pachystachya* (Cecropiaceae) (Supplementary data), a plant species largely used to treat fever (including that caused by malaria parasites) and as food by native indigenous populations in the Amazon Region of Latin America (Uchoa et al. 2010). Crude extracts of the plant roots were found to be active against *P. falciparum* in vitro and against *P. berghei* blood-induced infections in mice. Beta-sitosterol and tormentine acid have been purified from the active fractions and both compounds showed intense in vitro activity. All *Cecropia* plant extracts and fractions had low, if any, toxicity; thus, a high therapeutic index was observed with some fractions. Therefore, this species may be useful as a phytotherapeutic compound against human malaria.

Extracts of *Bidens pilosa*, another medicinal plant that has been used against fevers in general, are active against malaria parasites in vivo and in vitro; flavonoids and polyacetylenes present in this and several other *Bidens* spp have proven to be active (Brandão et al. 1997, Andrade-Neto et al. 2004). Interestingly, *B. pilosa* was validated for human use by the Ministry of Health in Brazil (portal.saude.gov.br/portal/arquivos/pdf/RENISUS_2010.pdf). *Physalis angulata* extracts were shown to exhibit intense antimalarial activity and potent immunomodulatory and anti-inflammatorv activities (McKerrow et al. 1993, Ridley 2002, Murata & Goldberg 2003, Newman & Cragg 2007, Sá et al. 2011, Willcox 2011).

Approximately 50% of other species of medicinal plants that are commonly used in Brazil have a demonstrated activity against malaria parasites (Carvalho et al. 1991, Brandão et al. 1997, Andrade-Neto et al. 2007). These results affected our research on plants. Recently, species that have been used elsewhere against malaria, such as in Nigeria and other African countries, have been reviewed (Adébayo & Krettli 2011); however, the only species that we tested, *Coccus nucreia*, had little, if any, activity (Adébayo et al. 2012).

In attempts to find an ideal alkaloid to replace QN, we have tested dozens of crude extracts from *Cinchona*-like medicinal plants (*falsas-quinias* in Portuguese) for use against malaria and fever and as body stimulants; however, most were found to be inactive in experimental models (unpublished observations). The characteristic bitter taste is taken as an indication of the therapeutic activ of *falsas-quinias* and healers sell these plants as dried samples in open-air markets and natural products stores. In all cases, plant identification is difficult (Fig. 1A) and buyers must rely on the seller’s recommendations.

The ethanolic extract from *Remijia ferruginea*, a *falsa-quina* that is endemic in Brazil, was found to be active against malaria parasites, among three species of malaria parasites tested (Andrade-Neto et al. 2003). *R. ferruginea* bark infusions were widely used to treat human malaria in the Amazon before CQ was available as a surrogate for the Peruvian *Cinchona* species, which is not found in Brazil. Twenty-six dried plant samples were acquired in markets as *falsas-quinias* in Minas Gerais (MG) (Estrada Real) and ethanolic extracts were prepared and tested in mice with *P. berghei*. Of these extracts, one reduced parasitaemia by > 40% and was also active against *P. falciparum* in vitro (IO Freitas & AU Krettli, unpublished observations).

Other natural products such as fungi are used as sources of new drugs, from which secondary metabolites that have anti-malarial activity can be isolated. Pycnidione, isolated from the marine fungus *Phoma* sp., has been shown to exhibit significant activity against *P. falciparum*. Pycnidione is structurally similar to atovaquone, an ingredient of the antimalarial medication Malarone®, which is comprised of atovaquone and proguanil hydrochloride (Wright & Lang-Unnasch 2005). Molecules isolated from the marine sponge *Plakortis simplex*, such as polyketide cycloperoxides, and from the sponge *Xestospongia* sp., such as xestocinoline, have antiplasmodial activity (Botté et al. 2012), as do compounds taken from fertile soils, which exhibit sesquiterpene-related antimalarial activity (Koeni 2003).

Compounds with anti-sporozoite and/or anti-primary liver stage activity have been found in extracts of the medicinal plant *A. amazonicus* (“Indian bear”), which is used in the Brazilian Amazon (Fig. 1B) (Krettli et al. 2001). *A. amazonicus* was the most cited plant species in recent ethnobotanical surveys in the state of Amazonas (AM) (de Oliveira et al. 2011). We found no activity in crude extracts of this plant and/or in their fractions against *P. falciparum* in cultures or against rodent malaria caused by *P. berghei* infected erythrocytes in mice. However, ethanolic plant fractions were active against *P. berghei* sporozoite-induced infections in mice in a dose-dependent manner (Fig. 2). The ethanolic plant extracts also inhibited the exo-erythrocytic development of *P. berghei* sporozoites in vitro (Andrade-Neto et al. 2008). The chemical compound responsible for the anti-sporozoite activity remains to be identified to develop a new antimalarial that is useful for malaria prophylaxis.

Another medicinal plant, *Strychnos throuasi*, which is used in malaria-endemic areas of Madagascar, has been studied and found to be highly efficient against sporozoite-induced malaria in mice, although this plant was inactive against plasmodia blood stages (Carraz et al. 2006). This plant species also appears to be ideal for malaria prophylaxis and a morphinan-like alkaloid, tazosmine, has been isolated and found to be responsible for the anti-sporozoite activity against *P. falciparum* in hepatoma cell cultures and against *P. berghei* sporozoite infections in mice. Thus, tazosmine may represent a good candidate for human malaria prophylaxis.

**Drug combinations and hybrids used in antimalarial chemotherapy** - The use of combinations of existing an-
timalarials with known pharmacokinetic and pharmacodynamic profiles optimises the antimalarial therapeutic efficacy and decreases or delays the risk of selecting resistant parasites. Drugs used in combination must have complementary mechanisms of action, synergistic activity and no cross-chemical interactions and must be free from side effects and affordable.

Atovaquone, a naphthoquinone that is highly active in vitro against blood forms of *P. falciparum* and in animal models, resulted in a low cure rate in human malaria trials and selected parasite mutants with reduced drug susceptibility if used alone. However, when combined with proguanil hydrochloride (Malarone®), atovaquone is recommended by the WHO for uncomplicated malaria in travellers returning to non-endemic countries (WHO 2010).

Combinations based on ART (WHO 2010b) are listed in Table II. Combinations of MQ and artesunic acid with ciprofloxacin, which are experimentally active in vitro against *P. falciparum*-iRBCs, appear promising (Andrade et al. 2007). The derivatisation of the fluoroquinolone ciprofloxacin enhanced in the in vitro activity and yielded compounds that were 10-100-fold more active than ciprofloxacin (Dubar et al. 2009); chalcones combined with ART (Bhatattacharya et al. 2009) and pyromarine combined with artesunate (AS) (Tshefu et al. 2010) and dihydroartemisinin-piperaquine (Menan et al. 2011) showed intense activity, with the latter exhibiting advantages over artemether-lumefantrine in human malaria.

The design of antimalarials based on the covalent linking of drugs into a single hybrid molecule is a relatively new approach and the resulting molecule tends to be more effective than the isolated components. A series of hybrids containing drugs with different biological functions, distinct pharmacophores, reduced toxicity and improved activity (as compared to the isolated compounds) are being developed.

The ART hybrid, 1,2,4,5-tetraoxane (RKA 182), is considered to be an outstanding antimalarial candidate with the advantages of enhanced stability, lower toxicity and improved biopharmaceutical properties (ADME) compared to the semisynthetic antimalarial ART (O’Neill et al. 2010).

The hybrid molecule MEFAS, which is derived from MQ and AS, is more effective and less toxic than the combination of MQ and AS, as shown using an experimental in vitro test with *P. falciparum* and in animal models (de Pilla Varotti et al. 2008). The hybrid compound comprises dual functions and, as in acridone hybrid molecules, the drug is based on the aminoquinoline ring. The acridone T3.5 (3-chloro-6-(2-diethylamino-ethoxy)-10-(2-diethylamino-ethyl)-acridone) targets drug-resistant strains at nanomolar concentrations and represents an innovative strategy for developing antimalarial drugs (Kelly et al. 2009).

**Compounds that are chemically related to known antimalarials** - An important strategy in drug design is the chemical modification of known antimalarials, such as 4-aminoquinolines (Hocart et al. 2011). The alkylamine sidechains allow for structural modification of the 4-aminoquinolines, resulting in compounds that have different lipophilicity and drug-resistance profiles (Kouznetsov & Gómez-Barrio 2009). Substitutions on the distal amino sidechain of CQ derivatives increase the activity and decrease the cross-resistance to CQ (Stocks et al. 2002).

Among the CQ analogues that have been synthesised by linking 4,7-dichloroquinoline with monoalkynes...
and dialkynes, 12 compounds have demonstrated activity against P. berghei in mice, especially those with methylene groups in the sidechain (de Souza et al. 2011). Two new CQ analogues, MAQ and BAQ, in which the 4-aminoquinoline pharmacophore group and proton-accepting sites are maintained to increase the bioavailability of the compound in the digestive vacuole of the parasite, were active in vitro against P. falciparum at nanomolar concentrations and exhibited a therapeutic index similar to that of CQ (Aguiar et al. 2012).

Ferroquine, a methalocenic CQ analogue, that is considered to be the most advanced organometallic antimalarial (Delhaes et al. 2002), is in clinical trials for the treatment of uncomplicated malaria (clinicaltrials.gov/ct2/show/NCT00811096) (Table IV).

**Drugs available to treat other human diseases** - The testing drugs that are indicated against other diseases provides the opportunity to find new clinical uses for licensed drugs that are approved for human use and for which safety and pharmacokinetic information is previously available. The purposes of this approach are to lower the costs and shorten the timeframe of product development. Good examples of this strategy include the existing anti-human immunodeficiency virus (HIV) protease inhibitor drugs, among which lopinavir has proven to be potent and promising against malaria parasites (Nsanzabana & Rosenthal 2011). The mechanism of action of HIV protease inhibitors against malaria is attributed to the inhibition of the food vacuole protease, plasmspsin II (Parikh et al. 2006). Tipranavir, although less active against malaria, demonstrates gametocytocidal activity and thus should have a significant effect on the transmission and control of malaria (Peatey et al. 2010).

Agents such as posaconazole (an inhibitor of ergosterol biosynthesis), itraconazole (an antifungal agent) and atorvastatin (widely used to reduce cholesterol levels) have proven to be active against P. falciparum blood stage parasites in vitro and against P. berghei-infected mice (Penna-Coutinho et al. 2011). These compounds are candidates for clinical trials in combination with other antimalarials.

The antifungal and anti-helminthic compound imidazolopiperazine, which is active against liver stages of Plasmodium, may prevent clinical disease by providing prophylactic protection. The drug has also presented significant in vivo antimalarial activity against blood-stage parasites (Meister et al. 2011).

Several drugs that were originally developed as antibiotics are useful for the treatment of human malaria in combination with antimalarials. Sulfadoxine (S), a synthetic antimicrobial agent, interferes in the folate synthesis pathway (which is essential for DNA and RNA synthesis) of malaria parasites when combined with pyrimethamine (P). However, with the emergence of resistant P. falciparum strains, SP is no longer recommended against malaria (WHO 2010b). Clindamycin, which is usually prescribed to treat anaerobic bacterial infections, is also active as an antimalarial and has been used in clinical studies either alone or in combination with QN and CQ (Lell & Kremsner 2002). As a monotherapy, however, the use of clindamycin is limited due to its slow onset of action; studies conducted to evaluate its effectiveness in combination with QN were not conclusive (Obonyo & Juma 2012). Clindamycin is recommended in combination with AS as a second-line antimalarial treatment and in combination with other antibiotics, such as tetracyclines and doxycycline (WHO 2010a).

Fosmidomycin, a natural antibacterial agent, has successfully been used in clinical studies against uncomplicated P. falciparum; however, recrudescence infections were observed in treated subjects (Wiesner et al. 2003). Fosmidomycin inhibits 1-deoxy-D-xylulose 5-phosphate reductoisomerase, an enzyme involved in the synthesis of isoprenoids, which is essential for parasite survival but not for the human host (which synthesises isoprenoids through the mevalonate pathway) (Wiesner et al. 2003). Azithromycin, a macrolide antibiotic, affects P. falciparum growth by targeting the apicoplast 50S ribosomal subunit and inhibits protein synthesis in this organelle (Sidhu et al. 2007). Geldanamycin, a benzoxquinone ansamycin antibiotic, also shows antimalarial activity by inhibiting the heat shock protein, HSP90, a molecular chaperone that is important for the maturation of proteins that promote the survival and the growth of dividing cells (Mout et al. 2012).

In vitro HTS - Drug discovery by HTS allow the large-scale testing of potentially active products (“hits”) by optimising and accelerating the identification of “lead” molecules for further development. Various automated HTS bioassay systems are used to screen synthetic and natural products to an extent not possible using conventional technologies. Further evaluation of drug activities selected using HTS can be assessed against Plasmodium parasites in culture or by biochemically screening targets.

Several methods are available to screen for parasite viability and are suitable for HTS technology. The most often used methods are based on the measurement of DNA content in recombinant strains of malaria parasites using SYBR green (Smilkstein et al. 2004), GFP (Wilson et al. 2010) and 4′,6′-diamidino-2-phenylindole (Duffy & Avery 2012), or in a stably expressed cyttoplasmic firefly luciferase parasite strain (3D7-luc) (Lucumi et al. 2010, Che et al. 2012).

The biochemical approach based on structure-based drug design holds promise for the search for lead-like antimalarials. This approach is based on a defined target such as a protein and/or enzyme that is necessary for parasite survival, as identified in binding assays that screen for inhibitors. The integration of HTS with a computational methodology (namely, virtual HTS) can be applied to in silico drug selection from compound libraries. This method is based on the binding affinity of the target receptor for the test compounds (i.e., molecular docking).

New antimalarial drugs undergoing clinical trials - Several new chemical groups with promising antimalarial activity are now undergoing clinical trials. Certain imidazolopiperazines that are experimentally active against the blood, liver and gametocyte stages of P. falciparum are active in malaria models and have a
safe profile were selected for clinical trials (Wu et al. 2011). The spiroindolone compound NITD609, a potent inhibitor of \textit{P. falciparum} selected in a phenotypic HTS of natural products, is also undergoing Phase I trials (Rottmann et al. 2010). The ozoneide OZ439, a synthetic peroxide antimalarial that is designed to provide a single oral dose to cure human malaria, is currently undergoing Phase II trials (Charman et al. 2011).

New medicines in Phases III/IV clinical trials include the following: (i) a fixed-dose combination of azithromycin and CQ for intermittent preventive treatment in pregnant women, (ii) tafenoquine, an 8-aminoquinoline with demonstrated activity against dormant liver forms of \textit{P. vivax} (hypnozoites) in vitro and blood stages in the acute disease, (iii) Pyramax paediatric, a child-friendly granule formulation of the ACT Pyramax, is in Phase IV trials, (iv) Coartem dispersible (artemether-lumefantrine), ASAQ (Artesunate-Amodiaquine, Winthrop), Artesunate for injection and Eurartesim (dihydroartemisinin-piperaquine) (Medicines for Malaria Venture) and (iv) piperaquine, a 4-aminoquinoline that acts similar to CQ (Warhurst et al. 2007). Although bulaquine, an 8-aminoquinoline, has been approved to treat \textit{P. vivax} malaria in India, its safety has not been assured using large clinical trials (Valecha et al. 2001, Kudsood et al. 2006). Drugs derived from medications against other diseases are undergoing clinical trials as antimalarials (Table IV).

**Concluding remarks -** Malaria remains a major health problem, especially now that \textit{P. falciparum} and \textit{P. vivax} have also become resistant to CQ and evidence of \textit{P. falciparum} resistance to ART has been reported. Commitment to the eradication of malaria, together with substantial financial support from national and international agencies, has furthered the selection of various molecules, some of which will hopefully help to interrupt disease transmission. Among the new drugs undergoing clinical trials, ferroquine, tafenoquine and bulaquine have shown promising results.

The overall goal regarding the control of malaria is to expand the drug arsenal with a new generation of molecules that target the various life stages of the parasites, i.e., asexual drug-resistant parasites, gametocytes and liver forms, as well as hypnozoites, which cause late relapses of \textit{P. vivax}. Parallel projects intended to diminish poverty among less-privileged populations need to be concentrated in areas where the transmission of malaria is endemic. However, the global economic crisis, political conflicts experienced by African countries where malaria is highly endemic and inadequate health care are likely to affect funding for the further support of drug discovery and poverty alleviation.

Different methodologies are available to aid in the search for new antimalarials. The most frequently used technology is in vitro drug screening with blood-stage \textit{P. falciparum} parasites maintained in continuous cultures. Large-scale drug testing uses $[\text{H}]$ hypoxanthine incorporation, which is a relatively simple assay; however, this assay requires radioactive reagents, thus imposing the need for surrogate methods. Two tests were developed that use specific monoclonal antibodies, either to parasite enzymes or a HRP, and both have proven useful and reproducible. Other tests use genetically modified parasites and parasite nucleic acid stains for DNA (e.g., SYBR Green I, the most cost-effective choice, YOYO-1, the most sensitive dye, and the SYTO series, with high binding affinities) have been developed.

Hz formation is a critical step for the survival of the malaria parasite and the basis for in vitro Hz inhibition, which is a simple and inexpensive indirect assay used for drug screening. It is hoped that once this assay is well standardised, it could also provide clues regarding the mechanism of the action of drugs.

HTS are especially useful in drug discovery and allow for the rapid analysis of potentially biologically active compounds. Millions of new molecules targeting blood stages of \textit{P. falciparum} have been selected in recent years and are available in drug banks; improved VSLs, which benefit from computer analysis using refined docking programs, are also helpful. However, the confirmation of the usefulness of such molecules in the treatment of human malaria depends on their direct validation against malaria parasites. Commercially available drugs for human use, such as itraconazole, atorvastatin and posaconazole, which have already been proven to inhibit \textit{P. falciparum} growth in cultures, represent an important source of new drug combinations that can be used to treat drug-resistant parasites. The chemical modification of available antimalarial drugs to enhance their pharmacodynamics and pharmacokinetic properties (e.g., absorption, metabolism, bioavailability, lipophilicity and drug distribution) is also a powerful tool, as is the hybridization of highly active antimalarial drugs. Imidazolopiperazine and timidazole, which have passed toxicological and pharmacological tests and have been found to be effective in humans against other pathologies, are currently under clinical trials after their activity against the liver forms and blood stages of malaria parasites were demonstrated.

Tafenoquine and bulaquine are in initial clinical trials using \textit{P. vivax} malaria; these drugs target the primary liver stages of sporozoites and the late developing hypnozoite forms. The large-scale screenings of other drugs that are less toxic than PQ are not trivial to accomplish; thus, this technique remains an ongoing challenge that is restricted by the limited availability of in vivo models and/or sporozoite cultures that reproduce hypnozoites. Sporozoite production is difficult, expensive and complex, thus limiting their use to a few laboratories worldwide.

Recently, important funds have become available through international and national agencies, as well as the pharmaceutical industry and communities committed to anti-malaria programs. Through projects supported by Brazilian agencies [The National Council for Scientific and Technological Development, The Minas Gerais State Research Foundation and Oswaldo Cruz Institute (Fiocruz), in particular], we have recently worked in close collaboration with senior scientists (mainly chemists) and built a network within research institutions (Fig. 3). In the area in which malaria is endemic, two collaborating groups are participating in the search for new antimalarials; one group, led by Maria Meneses Pereira (Federal University of Amazonas), is...
located in AM, and the other group, led by Cláudio Nahum Alves (Federal University of Pará), is in the state of Pará. In the Northeast Region, our network collaborators include A Euzébio Santana, Marília FO Goulart and Mario Meneguetti (Federal University of Alagoas), as well as Valter Andrade-Neto (Federal University of Rio Grande do Norte) and Milena Soares (Fiocruz-Bahia). The groups in the Southeast include the following: Lúcia Maria X Lopes (São Paulo State University-state of São Paulo), Carlos Zani and Tânia Alves (Fiocruz-MG), Núbia Boechat and J Mendonça [Fiocruz-state of Rio de Janeiro (RJ)] and Tanos CC França (Military Institute of Engineering-RJ). The groups in the South include Arildo Bráz Oliveira (State University of Maringá-state of Paraná) and Wilson J Cunico (Federal University of Pelotas-state of Rio Grande do Sul). The latter group has synthesized new PQ analogues that are quite potent and we hope to find a new drug among these analogues that is active against the liver forms of malaria.

The resilience and ingenuity of the malaria parasite in preserving itself, whatever the threat, requires the constant evaluation of control programs and searching for new antimalarials. An ideal antimalarial drug combination to replace ACT and halt the spread of resistant parasites is urgently needed. The ozonide OZ439, a synthetic peroxide, is considered promising because it is active in curing uncomplicated malaria in a single dose and is currently undergoing clinical trials. Whether OZ439 will control drug-resistant *P. falciparum* parasites in regions where ACT is less effective remains to be demonstrated.

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assay study of novel inhibitors for dengue virus ns2a ns3a (nucleoside-2'-o)-methyltransferase. Bioorg Med Chem 15: 7795-7802.


Cecropia sp., a medicinal plant popularly known as *imbaíba* or *embaíba*, is used against human malaria in endemic areas of the Amazon and in other regions of Latin America. A strong activity against malaria parasites have been demonstrated.