Chapter 3

Terpenes as Potential Antimalarial Drugs

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Abstract

A fact which favors the increase in morbidity and mortality of malaria cases in the world is the resistance to chemotherapeutic agents that the parasite presents. Therefore, it is necessary to identify new potential targets specific to the parasite in order to be able to perform a rational planning. One target for the evaluation of potential antimalarial compounds is isoprenoid synthesis, which occurs via the 2-*C*-methyl-*d*-erythritol-4-phosphate pathway in *Plasmodium falciparum*. Several intermediaries and final products of this pathway were identified in the parasite and lead us to the conclusion that it is different from the vertebrate host. In this chapter, we describe the effect of some monoterpenes and sesquiterpenes on *Plasmodium falciparum* and *Plasmodium berghei* as potential antimalarial drugs.

Keywords: terpenes, malaria, *Plasmodium falciparum*, *Plasmodium berghei*, isoprenoid

1. Introduction

Malaria is one of the major threats to human health, affecting an estimated number of 216 million peoples in 2016 all over the world, leading to 445,000 deaths, mainly in the African continent [1]. The human malaria is caused by six different species of the genus *Plasmodium*, which are *P. falciparum, P. vivax, P. ovale, P. malariae, Plasmodium knowlesi* and *P. simium*, where the last one is exclusive to the Brazilian Atlantic Forest [2].

Plasmodium was first described in 1880 by Laveran, who observed it in human erythrocytes. In human *Plasmodium* genus, the life cycle is very similar between species, being characterized by a sexual phase in vector *Anopheles* and an asexual phase in the human host that can be divided into liver phase and intraerythrocytic phase [3] (**Figure 1**).

The cycle begins with the injection of infective sporozoites of salivary glands of *Anopheles* into the human host bloodstream. Once there, the sporozoites make their way into the liver, where they infect hepatocytes to start the first massive replication, an asexual process, known as exoerythrocytic schizogony. After 9–16 days, those new-formed merozoites are then released into the bloodstream in hepatocyte-derived vesicles [4], called merosomes, to avoid its capture by Kupffer cell. This liver endures this phase differently across different species which can last an average of 6 days (*P. falciparum*), 10 days (*P. vivax*), or 15 days (*P. ovale* and *P. malariae*) [5].

In the bloodstream, the merosomes are then disrupted liberating the merozoites, each merozoite infecting a single red blood cell (RBC). The process of invasion is complex, relying on diverse cell machineries, which permit the parasite to attach, reorientate, and invade, forming the parasitophorous vacuole [6]. Once in the erythrocyte, the parasite starts its asexual division, passing through different stages. The early trophozoite, called "ring stage", starts to develop, enlarging to a mature trophozoite that has a high metabolic index. In the late stage, multiple nuclear divisions

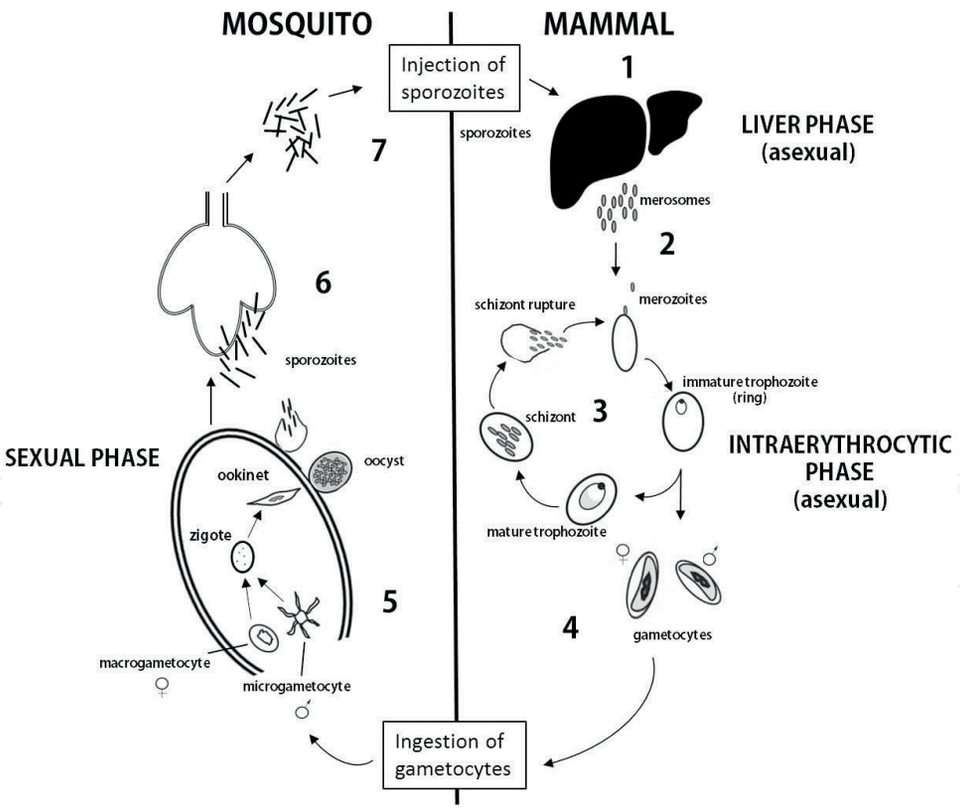

Figure 1. Malaria parasite life cycle. Schematic life cycle of *P. falciparum* in the invertebrate (left) and vertebrate hosts (right). 1. Hepatocytes invasion and exoerythrocytic schizogony to merozoites formation. 2. Release of merosomes in the blood stream. 3. Intraerythrocytic phase. 4. Parasite differentiation to gametocytes which ones could be ingested by invertebrate host. 5. Sexual phase in the midgut of invertebrate host. 6. Migration of sporozoites into salivary glands. 7. Injection of sporozoites during the blood meal.

are triggered without cytokinesis, forming schizonts. Each schizont holds an average of 32 merozoites (10 merozoites in average for *P. knowlesi* [7]) that are unleashed upon the RBC lysis. The whole process can take about 36–48 h in *P. falciparum*, 48 h in *P. vivax* and can reach even 72 h in *P. malariae*, but in *P. knowlesi* the cycle is 24 h, which is one factor that leads to its high virulence in humans [8]. Cell lysis coincides with fever symptoms, a response of immune system to the liberation of hemozoin and other parasite products into the bloodstream [9].

Within the red blood cells, the parasite can follow another path of development, differentiating into gametocytes. During a blood meal in an infected individual, the *Anopheles* female ingests those gametocytes. In the female of the *Anopheles* mosquito the parasite undergoes a meiotic division. Inside the mosquito gut, the gametocytes mature to form male and female gametes. The gametes undergo fertilization, forming the zygote, which transforms into an ookinete. The ookinete then penetrates the midgut and installs itself developing into oocyst. Under multiple cellular divisions, thousands of sporozoites are formed, which migrate to the salivary glands of the mosquito, to get expelled with anticoagulant factor contained in saliva during the next blood meal, restarting the cycle [10] (**Figure 1**).

Although *P. vivax* is the most prevalent parasite in the world, *P. falciparum* is responsible for most cases of severe malaria, being the most prevalent malarial parasite in the African continent, which accounts for 80% of the global disease burden. The groups with higher risk of malaria disease includes pregnant women, patients with HIV/AIDS, infants and children under 5 years old, whereas *P. falciparum* is responsible for about 70% of the malaria-related deaths. Although a lot of efforts have been made aiming to eradicate malaria, the World Health Organization (WHO) strives to reduce the mortality rates and malaria cases in 90% up to the year of 2030 [11]. The acquired drug resistance of the parasite continues to be a struggle in the fight against the disease, which led to rising of malaria-related death.

The resistance to antimalarial drugs is due to the indiscriminate use of the drugs and its incorrect use in treatment of malaria cases, such as wrong dosage, drug quality problems, erroneous diagnosis, not sticking to treatment, and others. These are characterized as treatment failure but can lead to a strong selective pressure in parasites, resulting in drug resistance. In recent years, with the emergence of artemisinin derivatives, resistance has allowed the number of cases to grow fast, especially in East Asia. Artemisinin, a sesquiterpene lactone, and its derivatives were adopted in the early 2000s as a first-line treatment in combined therapy for *P. falciparum* [12]. Artemisinin and its derivate can clear early trophozoites (ring stage), but the drug has a short span in vertebrate organisms, making it necessary to combined it with other drugs. In countries where *P. vivax* is the main malaria transmitter, the first-line treatment remains using chloroquine and primaquine, although WHO suggests that changes must be made to artemisinin-based combined therapy (ACT) when the rate of chloroquine resistance have reached more than 10%. Some strategies have been adapted to control malaria, such as vector control; insecticide-treated bed nets, indoor residual spraying, preventive treatment for pregnant women, and rapid diagnosis and treatment of infected individuals [1, 12, 13]. But considering the fast acquirement of resistance by parasites and vectors to drugs and insecticides, respectively, the development of an effective vaccine turns out to be an important issue. However *Plasmodium* species, especially *P. falciparum*, has a highly variant antigen pool, responsible for the adhesion

of infected red blood cells (RBCs) to small vessels, which causes aggregation that leads to severe stage of the disease, making difficult advances in this area of interest [9]. The pathogenesis of *P. falciparum* relies on a complex interaction of RBC alterations, microcirculatory anomalies, and immune response. The infected RBCs start to agglomerate in small vessels by action of adhesins expressed by the parasite on the surface of infected RBCs. Those adhesins are capable of interacting with endothelial cells of small vessels, to avoid the clearance of infected RBCs by the spleen, leading to a sequestration in diverse organs, such as brain, lungs, and placenta. This, together with other factors, causes the severe forms of malaria [9].

The increasing resistance of the parasite to practically all current medications, such as artemisinin in five countries in Asia, Southeast Asia and probably South America [1], calls for the use of combination drug therapy, as well as for the identification of new targets [12, 13]. Targets targeting the parasite for the development of new therapies for the treatment of malaria encompass both cellular functions, such as detoxification of heme or ferriprotoporphyrin IX (Fe (III) PPIX), and folate metabolism, already explored for drugs established as antimalarial, as well as other metabolic pathways, such as fatty acid synthesis, and isoprenoid biosynthesis, both of which are found in the apicoplast [14].

The apicoplast, an organelle originating from a secondary endosymbiotic origin of red algae, has lost its photosynthetic function in the course of evolution [15], and speculations have demonstrated its importance in the formation of essential components incorporated into the membrane of the parasitophorous vacuole [16]. Recently, it has been shown that isoprenoid biosynthesis is not only essential for the parasite but, in fact, is the only function of the apicoplast during blood stage growth [17] and sexual forms [18]. Parasites that lacked apicoplast can be chemically rescued by addition of isopentenyl pyrophosphate (IPP) to the growth media [17].

2. Isoprenoids in *Plasmodium* spp

All isoprenoids are derived from a common precursor, IPP and its dimethylallyl pyrophosphate isomer (DMAPP) [19] (**Figure 2**). The identification and characterization of farnesyl pyrophosphate (FPP) in *P. falciparum* [20], as well as the presence of proteins covalently modified by isoprenoids [21, 22] and dolichols [23], were the first evidence for the study of isoprenoid biosynthesis in *Plasmodium*. In the last decade, there has been a broad characterization of isoprenoid biosynthesis products in the parasite [22–27] resulting from the alternative route 2-C-methyl-*d*-erythritol-4-phosphate (MEP) [28, 29] (**Figure 2**). The essential and important step in the metabolism of the biosynthesis of all isoprenoids is the elongation of the isoprene chain by enzymes called prenyltransferases. These enzymes are classified according to the chain length of the final product and the stereochemistry of the double bond formed by condensations, with FPPS (farnesyl pyrophosphate synthase) and GGPPS (geranylgeranyl pyrophosphate synthase) being the most studied prenyltransferases [30]. FPPS catalyzes the condensation of IPP with DMAPP and geranyl pyrophosphate (GPP) to form the 15-carbon isoprenoid compound, farnesyl pyrophosphate (FPP). FPP is the substrate that catalyzes the first step in the biosynthesis of ubiquinone, carotenoids, dolichols, and protein prenylation. FPP can also be condensed with an additional molecule of IPP by the enzyme GGPPS to form the 20-carbon isoprenoid, geranylgeranyl pyrophosphate (GGPP), also essential in protein isoprenylation [30] (**Figure 2**).

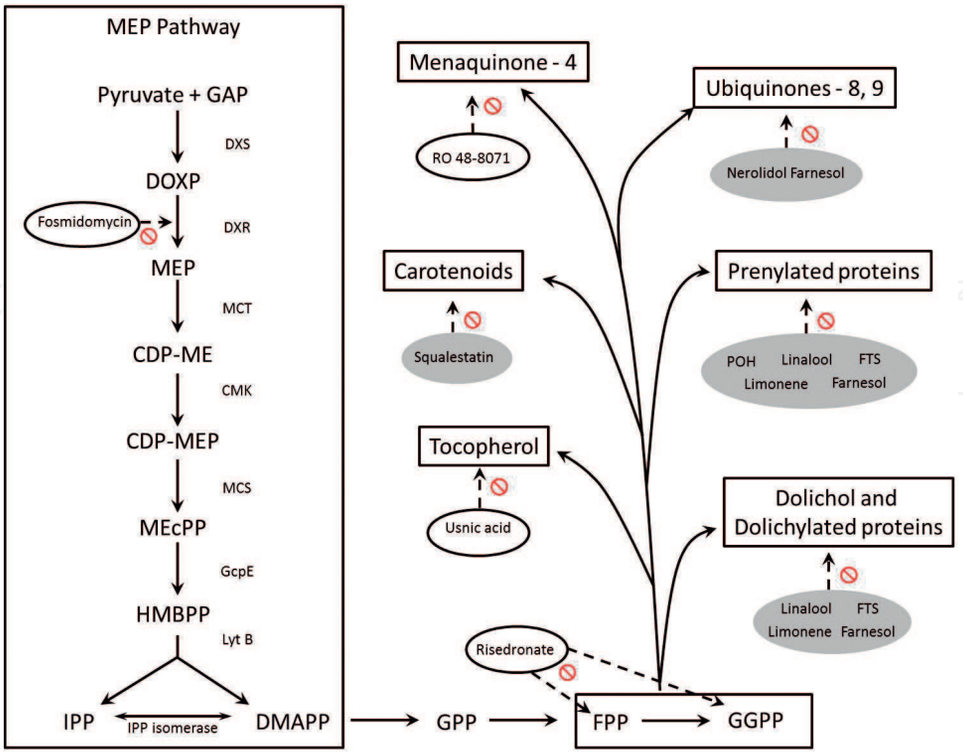

Figure 2. MEP pathway and final products in *P. falciparum*. In the left box, the MEP pathway to the formation of isoprenic units (IPP and DMAPP). In the right, final isoprenic products biosynthetized by *P. falciparum*. The circles are representing inhibitors tested against the parasite and the gray circles correspond to terpenes which its inhibitory effect on final isoprenic products biosynthesis was demonstrated. Abbreviations: *Precursors*: GAP, glyceraldehyde 3-phosphate; DOXP, 1-deoxy-*d*-xylulose 5-phosphate; MEP, 2C-methyl-*d*-erythritol 4-phosphate; CDP-ME, 4-(cytidine-5′-diphospho)-2C-methyl-*d*-erythritol; CDP-MEP, 4-(cytidine-5′-diphospho)-2C-methyl-*d*-erythritol 2-phosphate; MEcPP, 2C-methyl-*d*-erythritol 2,4-cyclodiphosphate; HMBPP, 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate; IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate; GPP, geranyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate. *Enzymes*: DXS, 1-deoxy-*d*-xylulose 5-phosphate synthase; DXR, 1-deoxy-*d*-xylulose 5-phosphate reductoisomerase; MCT, 2C-methyl-*d*-erythritol 4-phosphate cytidine transferase; CMK, 4-(cytidine-5′-diphosph)-2C-methyl-*d*-erythritol kinase; MCS, 2C-methyl-*d*-erythritol 2,4-cyclodiphosphate synthase; GcpE, hydromethylbutenyl pyrophosphate synthase; Lyt B, hydromethylbutenyl pyrophosphate reductase; IPP isomerase, isopentenyl pyrophosphate isomerase. *Inhibitors*: FTS, s-farnesylthiosalicylic acid; POH, perillyl alcohol.

For several decades, the mevalonate pathway, present in animals and plants, was considered the only route for synthesis of the isoprene units in isoprenoid biosynthesis. The existence of a second pathway for the biosynthesis of isoprene units was discovered in 1988 by Flesch and Rohmer when they were studying the hopanoids biosynthesis (pentacyclic triterpenic steroids) in bacteria [31]. Originally called the Rohmer's pathway or mevalonate-independent pathway, its name was changed after the identification of the first step in the pathway (pyruvate/glyceraldehyde-3-phosphate GAP pathway) or the first intermediate, 1-deoxy-*d*-phosphate (DOXP pathway). However, the most accepted name is MEP [32], since this compound is the first exclusive pathway precursor. One particularly intriguing target from the MEP pathway is 1-deoxy-D-xylulose-5-phosphate synthase (DXS). DXS catalyzes the first and rate-determining step of MEP and the condensation of pyruvate and *d*-glyceraldehyde-3-phosphate to 1-deoxy-*d*-xylulose-5-phosphate (DXP) and CO_2, this reaction being the first and rate-determining step of MEP [33]. The reactions catalyzed by DXS and DXR (1-deoxy-*d*-xylulose-5-phosphate reductoisomerase) practically share the full control of the flux through MEP pathway. It was shown

that reducing the flux through the pathway by inhibiting enzymes (DXS & DXR inhibition) is an important mechanism of action of some drugs leading to killing of the malarial parasite [34].

Fosmidomycin is a natural product antibiotic with activity against a number of important pathogens (**Figure 2**). It is a phosphoric acid that is a substrate mimic and direct inhibitor of the first dedicated MEP pathway enzyme, in which DXP is converted to MEP by DXR (also called IspC) [35, 36]. The use of fosmidomycin as a single-drug treatment for *P. falciparum* malaria has been hampered by low bioavailability, recrudescent, and rapid clearance from the parasite, although the compound has been used more successfully in combination with clindamycin [37]. Many efforts, with great results, have been made to improve the efficacy of fosmidomycin as modifications to the phosphonate group, extensions to the hydroxamic acid group [38, 39], and substitution of the α-position [40, 41] or, more recently, β-position [42]. The inhibition of downstream enzyme IspD (2-C-methyl-*d*-erythritol 4-phosphate cytidylyltransferase) which catalyzes the cytidylation of MEP to cytidine diphosphate methylerythritol (CDP-ME) is also metabolically apparent in fosmidomycin-treated cells. Although IspD homologs are not directly inhibited by fosmidomycin in vitro, this enzyme has been shown as a promising target in isoprenoid pathway for some new antimalarial drugs studied [43, 44].

The first evidence for the study of isoprenoid biosynthesis in *Plasmodium* were the identification and characterization of farnesyl pyrophosphate (FPP) [45], dolichols [23], and prenylated proteins in *P. falciparum* [21, 22]. Other products were functionally characterized, shown to be essential for the survival of the parasite, such as vitamin K2 (menaquinone-4) [24] and vitamin E (tocopherol) [25], for example. It was demonstrated that the vitamin K2 biosynthesized by the parasite acts as electron transporter in the respiratory chain in microaerophilic conditions and that this biosynthesis can be inhibited treating parasite culture with RO 48-8071, a specific inhibitor of manequinone-4 biosynthesis [24]. The biosynthesis of tocopherol (vitamin E) was characterized in the parasite and has been shown essential for antioxidant that protects against environmental stress, including maintaining ROS levels [46]. The parasite lives in a pro-oxidant environment that contains oxygen and iron and therefore have evolved extensive detoxifying and protective mechanisms, which both limit the production of and potential damage by ROS [27, 46]. Further, tocopherol has been showing excellent results in antimalarial drug studies, presented excellent results with usnic acid, a drug that inhibits hydroxyphenylpyruvate dioxygenase, the enzyme that catalyzes the conversion of p-hydroxyphenylpyruvic acid to homogentisic acid, a precursor of vitamin E biosynthesis [27] (**Figure 2**).

The enzymes are important tool for study the parasite physiology and an important target for antimalarial drugs, due to its specificity. The essential step in all isoprenoids biosynthesis is the elongation of the isoprene chain by enzymes called prenyltransferases. These enzymes are classified according to the chain length of the final product and the stereochemistry of the double bond formed by condensations [47]. The most studied of these prenyltransferases has been FPPS and GGPPS in *P. falciparum* [20]. The gene coding for FPPS and GGPPS have already been identified and characterized in several species such as *Saccharomyces cerevisiae* [48], *Trypanosoma cruzi* [49], *T. brucei* [50], *Toxoplasma gondii* [51], *P. vivax* [52] *and P. falciparum* [20]. Both *P. falciparum* and *T. gondii* enzymes are bifunctional (FPPS/GGPPS), being able to catalyze the biosynthesis of the isoprene compounds FPP and GGPP [20, 51], the main precursors

of all secondary products from isoprenoids pathways. Transcriptional analyses of the FPPS/GGPPS gene have shown a high variability in alternative splicing of this *P. falciparum* gene [53]. It has been proven that the importance of this enzyme for the MEP pathway, due to the complexity of the gene regulation, is necessary for the formation of the main precursors. Therefore, FPPS/GGPPS is a potential and promising target for new antimalarial drugs.

Risedronate, a bisphosphonate containing nitrogen (N-BP), showed potent activity against the blood phases of *P. falciparum* "*in vitro*" which inhibits FPPS/GGPPS activity by competitive inhibitor toward GPP and FPP [20] (**Figure 2**). Bisphosphonates are inhibitors of bone resorption and are in clinical use for the treatment and prevention of osteoporosis [54]. The activity of the risedronate was confirmed because only farnesyl-PP and geranylgeranyl-PP restored the growth intraerythrocytic stages of *P. falciparum*, after treatment with risedronate [55]. This drug also showed a significant inhibitory effect against murine blood stage malaria, without showing toxicity effects to the animals [55] and showed great results in synergism experiments with other drugs in *P. falciparum* [56]. Also, crystallography assays of the *P. vivax* GGPP enzyme inferred that GGPP could be a major target for the lipophilic bisphosphonates [57].

Many studies of novel antimalarial drugs have been performed using enzymes as the primary target of action [58–60], although in isoprenoid biosynthesis of *P. falciparum,* few enzymes are still known and studied. The enzyme OPP/PSY (octaprenyl pyrophosphate synthase/phytoene synthase) was characterized in the parasite as a bifunctional [25, 61] responsible for form two important secondary products of the isoprenoid biosynthesis— carotenoids [25], important for antioxidant protection, and ubiquinone [24], essential for electron transfer in respiratory chain among other functions. Squalestatin, a carboxylic acid inhibitor of squalene synthase, the enzyme responsible for the first step of sterol biosynthesis, presented promising results as an antimalarial drug, specifically inhibiting OPP/PSY [62]. This results have proven then that the first carotenoid phytoene is essential for parasite development during the intraerythrocytic cycle [62], probably due its antioxidant protection function [25]. Also, through studies *in silico* characterized the secondary and tertiary structures of this enzyme, OPPS/PSY presented an unconserved unique loop in *P. falciparum* then be exploited for structure based drug designing against malaria parasite [63].

Since it was shown that the MEP pathway provides IPP precursors for the biosynthesis of higher isoprenic compounds, one of the strategies to identify secondary products of the MEP pathway was the metabolic labeling using a radioactive precursor and a posterior analysis by an appropriate method. In this context, it was identified in *P. falciparum* prenylated proteins [22], dolichols [23], ubiquinones [24], carotenoids [25], vitamin K2 [26], and vitamin E [27].

Different types of terpenes that exert antifungal, antibacterial, and antimalarial activity can be easily found in literature [19, 64, 65]. However, not all authors described a clear explanation about their mechanism of action. Recently, Silva et al. [66] listed 114 terpenes and their semisynthetic derivate with antimalarial activity, but only three have their mechanism of action elucidated. For several years, studies have been made of the large diversity of prenylated compounds biosynthesized by *P. falciparum*. That is why some research groups investigate the possibility of developing new antimalarial drugs that could interfere with the biosynthesis of isoprenoid compounds [19]. This interference in biosynthesis of isoprenoid compounds could

be produced by inhibiting the enzymes as isoprenyl diphosphate synthases and isoprenyl transferases. Thus, research in the area can benefit from obtaining better knowledge about the antimalarial activity of natural terpenes. Due to their structural similarity, it was suggested that terpenes might interfere with the parasite's polyisoprenoid biosynthesis through inhibition of the isoprenyl diphosphate synthases which condense molecules of IPP among other isoprenic substrates to form isoprene chains [67]. Terpenes could also establish a competition with several enzymes which use isoprenic compounds as substrates. In fact, several terpenes, such as farnesol or linalool, have already demonstrated a capacity to inhibit at least one point of isoprenoid biosynthesis including isoprenic chain of ubiquinone and dolichol biosynthesis and protein isoprenylation [67]. These results suggest a widespread inhibitory action. Studies on antimalarial activity, drug combination, and mechanisms of action of different compounds are commonly performed *in vitro* (**Figure 2**).

It is known that IPP, FPP, and GGPP are substrates of the enzymes prenyltransferases involved in the biosynthesis of dolichol, the isoprenic side chain of ubiquinones, and the isoprenic chains attached to proteins, among other plasmodia prenylated compounds [68]. In order to determine if different drugs produce biosynthesis inhibitory effects on specific isoprenic compounds, treated cultures (using drugs concentration under the IC_{50} value), or untreated cultures can be radiolabeled by isoprenic precursors such as [1-(*n*)-^{3}H]geranylgeranyl pyrophosphate triammonium salt ([^{3}H]GGPP), 1-(*n*)-^{3}H]farnesyl pyrophosphate triammonium salt ([^{3}H]FPP), or [1-^{14}C] isopentenyl pyrophosphate triammonium salt ([^{14}C]IPP). Using these precursors, it is possible to label most isoprenic compounds without performing prenyl diphosphate pool substrate depletion, as it is necessary in other cell systems [69]. This fact suggests that the *Plasmodium* may have a different kind of isoprenic precursor uptake from the extracellular medium in comparison to other organisms [24, 67, 69]. After purifying infected red blood cells containing parasites at different stages, prenylated compounds can be isolated by diverse methods, once their radioactivity incorporation is evaluated [19]. For apolar compounds, such as dolichol and ubiquinones, the apolar extracts are commonly obtained and chromatographed by different TLC or HPLC methods, while isoprenylated proteins can easily be isolated in a polyacrylamide electrophoresis gel. Specific proteins such as Ras or Rap proteins can be easily immunoprecipitated [67, 70].

Prenylated proteins are post-translational modified by farnesyl transferase and geranylgeranyl transferase by attaching isoprenic chains to C-terminal cysteine groups. Protein prenylation had already been characterized in several parasites such as *Giardia lamblia*, [71] *Trypanosoma brucei* [72] and *Schistosoma mansoni* [73]. *P. falciparum* expresses different classes of lipidic-membrane-associated prenylated proteins such as GTPases Ras, Rho, and Rab including endosomal vesicles Rab7 protein [74] farnesylated PfPRL tyrosine phosphatase, SNARE protein Ykt6.1 [75, 76], and some putative plasmodial isoprenylated proteins such as Rab2 and Rab11a [77]. In fact, proteins of 21 to 24 kDa and 50 kDa are identified using specific prenylated protein antibodies [22, 67, 78]. Some terpenes and prenyltransferase inhibitors have already shown interesting anti-cancer and anti-plasmodial activities [21, 79]. Limonene, linalool, and perillyl alcohol (POH) are some examples of these antineoplastic and antimalarial activities, and the mechanisms of action of most of them seem to be related to protein isoprenylation interferences [67, 80–82]. Several terpenes and protein isoprenylation inhibitors were studied for its antimalarial activity. Limonene, nerolidol, farnesol, perillyl alcohol, linalool and an terpene modified as s-farnesylthiosalicylic acid (FTS) are just a few examples of compounds tested in

P. falciparum and for the IC_{50} value in 3D7 *P. falciparum* isolate was calculated [67, 82]. All these compounds have demonstrated to produce a dose–response inhibition. The lowest IC_{50} values were found for nerolidol (IC_{50}~ 760 nM), perillyl alcohol (IC_{50}~ 489 μM), FTS (IC_{50}~ 14 μM) and farnesol (IC_{50}~ 64 μM), whereas limonene and linalool showed IC_{50} values in the millimolar scale [67, 82]. All this compounds showed effects on protein isoprenylation which vary in function of the parasitic stage and the given isoprenic radiolabeled precursor. Different drug effects in function of the parasitic stage and the given isoprenic radiolabeled precursor allow us to understand compound mechanism of action at the different intraerythrocytic stages of the parasite. Treatments on protein isoprenylation seem to be specific, since there was no significant effect on L-[^{35}S] methionine-labeled proteins [22, 67].

Anti-p21*ras* or anti-p21*rap* antibodies were used for immunoprecipitation with [^{3}H]FPP or [^{3}H]GGPP labeled proteins of untreated and treated parasites at different stages. Results indicate that schizont p21*rap* radioactive incorporation is reduced after treatment with nerolidol, farnesol, linalool, limonene, and FTS. Farnesol inhibits isoprenylation of Ras and Rap proteins in all plasmodium intraerythrocytic stages whereas linalool inhibits Ras isoprenylation in ring and schizont stages. Also in the schizont stages, this effect on Ras proteins was observed as well as after FTS and limonene treatments. At ring stages, the limonene reduced the radioactive incorporation into Rap and Ras proteins, and linalool reduced incorporation into 21 kDa band. In trophozoite stages, these effects in Rap radioactive incorporation were observed after limonene and nerolidol treatments [67]. FTS is a prenylated compound that also restrains the development of some tumors by inhibiting specifically Ras post-translational modifications [83]. As mentioned, the inhibition of Ras post-translational modifications in schizont stage and the hindrance of the parasite at this stage were also demonstrated for *P. falciparum*.

Carotenoids, dolichol, and coenzyme Q are examples of prenylated compounds biosynthesized by *P. falciparum* isolated by diverse methods. Coenzyme Q has a role in mitochondrial respiration, in pyrimidine biosynthesis, and in preventing membrane lipoperoxidation, among other functions [84]. Carotenoids are believed to avoid oxidative stress; and dolichol and its phosphorylated derivatives can participate in protein prenylation [85] or act as lipids' carriers, being used for several glycoconjugates [86] including N-linked glycoproteins. Those play an important role in the parasite's intraerythrocytic stages differentiation [87]. The *P. falciparum* bifunctional enzyme OPPS/PSY is involved in the biosynthesis of carotenoids and isoprenic side chains attached to benzoquinone rings derived from *p*-hydroxybenzoic acid. The parasite is able to synthesize several carotenoids and at least two homologs of coenzyme Q (Q_8 and Q_9) that differ on the isoprenic units of the lateral chain [24, 25, 61]. Some authors suggested that the homologs might act against different kinds of oxidative stress conditions [24]. Several terpenes also demonstrated effects on dolichol and ubiquinone biosynthesis in different organisms. In hepatoma and Neuro2A cells, the monoterpenes limonene and linalool interfered in the biosynthesis of dolichol and the isoprenic side chains of benzoquinones [81]. Similarly, in *P. falciparum,* both monoterpenes and also farnesol and nerolidol interfered in the biosynthesis of dolichol and ubiquinones when the parasite was [^{14}C]IPP labeled. Only the monoterpenes limonene and linalool interfered in geraniol and farnesol radioactive incorporation. That is why some authors suggested that limonene and linalool could be interfering in the condensation between IPP and DMAPP, while farnesol or nerolidol could be interfering in the isoprenic chains' elongation [67]. In order to demonstrate terpenes, specifically effects on

P. falciparum isoprenoid-related enzymes, nerolidol was used to inhibit the OPPS/PSY activity using [^{3}H]FPP and [^{14}C]IPP as substrates. OPPS/PSY activity was competitively inhibited by nerolidol (K_i 15 nM) [61].

As we have seen, some MEP pathway and isoprenoid pathway inhibitors show good antimalarial activity and produce important metabolic alterations in *P. falciparum.* Different authors asked if using terpenes individually or in combination with other drugs could be a good strategy to treat malaria. It is well established that fosmidomycin and clindamycin, when combined, produce a synergistic activity *in vitro* and *in vivo* [88], and several drugs which act at different points of the MEP/isoprenoid showed supra-additive *in vitro* effects when combined [56]. These kinds of drug combination studies are useful for a better understanding of the interactions between the different intermediates of the MEP and isoprenoid pathways and to evaluate its antimalarial potential. Fosmidomycin, risedronate, nerolidol and squalestatin (supposed to be a phytoene synthase inhibitor) are drugs which are believed to act in different cellular compartments, and all of them seem to inhibit at least one point related to isoprenoid metabolism [56]. Except nerolidol-risedronate, most binary combinations between fosmidomycin, risedronate, nerolidol and squalestatin showed a supra-additive effect [56]. Probably it is because they target different enzymes in the same biosynthetic pathway. On the other hand, nerolidol and risedronate when combined showed a sub-additive effect. It was suggested that this fact could be explained because both compounds affect the same target of isoprenoid pathways [56]. Nerolidol affects the synthesis of several isoprenoid compounds, including protein isoprenylation as well as risedronate [55, 67].

Due to the terpenes effectiveness to inhibit the *P. falciparum* growth *in vitro*, several studies have been focused on studying these effects on experimental models of malaria by *P. berghei* infection. Nerolidol for example, being administered on Balb/c mice at a dose 2000 mg/kg/day by oral and intranasal via, had an inhibitory effect on the *P. berghei* ANKA growth, since the parasitemia were reduced and the survival rates were increased significantly with the nerolidol treatment [89]. Also, some derivatives of 4-nerolidylcatechol, at oral doses of 50 mg/kg/day, had suppressed *P. berghei* NK65 in infected BALB/c mice by 44%, showing marked improvement over the parasite's growth [90].

On the other hand, some metabolites derived from limonene, such as perillyl alcohol, have also been shown to be effective against the severe conditions development caused by *P. berghei* infection. C57BL/6 mice infected with *P. berghei* ANKA and treated with 500 mg/kg/day intranasal via had a significant increase in survival rates, showing a preventive effect against the experimental cerebral malaria development [82].

Furthermore, plant extracts that contain several terpenes have also been tested on experimental models. The antimalarial activity of ethanolic bark extract of *A. lebbeck* was determined. Phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, saponins, terpenes, and phytosterols [91].

Limonoids isolated from the residual seed biomass from *Carapa guianensis* were tested against *P. berghei* and 6α-acetoxy-gedunin was more active than 7-deacetoxy-7-oxogedunin. At oral doses of 50 and 100 mg/kg/day, 6α-acetoxy-gedunin suppressed parasitemia versus untreated controls by 40 and 66%, respectively, evidencing a clear dose response [92].

In other studies, methanol extracts of *Carpesium rosulatum* were found to have potential antimalarial activity *in vivo* when tested against *P. berghei* in mice. A dose of 2, 5, 10 mg/kg/day exhibited a significant blood schizonticidal activity in four-day early infection with a significant mean survival time comparable to that of the standard drug, chloroquine (5 mg/kg/day) [93].

Some others triterpenes isolated from the African medicinal plant, *Momordica balsamina* L. such as, balsaminoside B, karavilagenin C, and the karavoates B and D were synthesized by diacylation from these extracted terpenes. Derivatives exhibited sub-micromolar IC_{50} *in vitro* against *P. falciparum* strains and exhibited greater *in vivo* antimalarial activity. Orally and subcutaneously administered karavoate B exhibited the greatest *in vivo* antimalarial activity (55.2–58.1% maximal suppression of parasitemia at doses of 50 mg/kg/day) [94].

3. Conclusion

Plasmodium spp. has an organelle, the apicoplast, which is essential for the development of the parasite because it is linked to two metabolic pathways, one of which is the isoprenoid biosynthesis that is different in several steps from the isoprenoid pathway in the vertebrate host. The biosynthesis pathway is an important target for evaluating new antimalarial drugs and the terpenes for being derived from the isoprenoid pathway and having a similar structure can interfere in the synthesis of isoprenoids and should be evaluated as antimalarial potentials.

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