CHEMICAL REVIEWS

REVIEW

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Plasmodium falciparum Apicoplast Drugs: Targets or Off-Targets?

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1. INTRODUCTION

The phylum Apicomplexa includes obligate intracellular parasites that cause both benign and serious diseases. Human infection by *Toxoplasma gondii* can be asymptomatic but can provoke serious or even fatal effects on immunocompromised patients or on fetuses.[1] *Cryptosporidium* spp. affect the intestines of mammals; clinical manifestations include acute, persistent, or chronic diarrhea. In healthy individuals, cryptosporidiosis can be cured easily, but therapy remains a challenge in immunocompromised patients.[2] *Babesia bovis* and *Theileria parva* cause babesiosis and theileriosis in cattle, respectively.[3] Constraints in livestock production imposed by these two diseases have considerable economic importance. The most devastating impact on humans is malaria. *Plasmodium* parasites are the causative agents of malaria, which affects an estimated 225 million humans worldwide and results in up to 1 million deaths, predominantly children in sub-Saharan Africa and Southeastern Asia.[4] Malaria is transmitted via the bite of an infected mosquito vector (i.e., a female *Anopheles*). The sexual cycle of *Plasmodium* occurs in the gut of the mosquito. In the mammalian host, *Plasmodium* undergoes cycles of asexual divisions, known as schizogony, in the liver cells initially and then in the erythrocytes, which cause the deadly consequences of the disease. Importantly, there is no vaccine and the parasite is increasingly resistant to several existing drugs. There is therefore an urgent need for novel strategies to fight against *Plasmodium*.

Apicomplexan parasites are unicellular eukaryotes, protists, named for their apical complex, an intracellular structure required for their invasion into host cells and composed of cytoskeletal elements combined with unique secretory organelles. All apicomplexan parasites, with the noticeable exception of *Cryptosporidium* spp. and perhaps the gregarines, harbor a unique nonphotosynthetic plastid referred to as the apicoplast (apicomplexan plastid).[5,6] The apicoplast was acquired by the secondary endosymbiosis of a plastid-containing red alga, as confirmed by the recent discovery of the photosynthetic apicomplexan *Chromera velia*.[7,8] As a consequence, the apicoplast is surrounded by four membranes: two innermost membranes thought to correspond to the initial plastid envelope, a periplastid membrane corresponding to the plasma membrane of the engulfed alga, and an outermost membrane, which is most likely the initial phagotrophic membrane.[9,10] The *P. falciparum* apicoplast (Figure 1) possesses a circular 35 kb genome, which is highly A−T-rich and completely lacking any genes related to photosynthesis.[11] The relatively small size of the apicoplast genome, the second smallest

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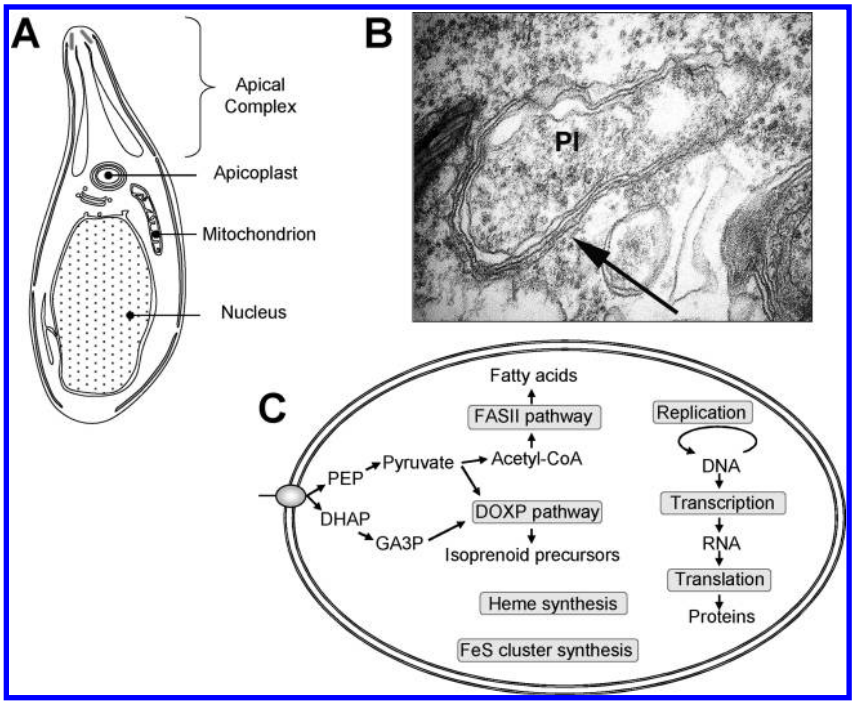

Figure 1. Apicoplast of *P. falciparum*: a relict nonphotosynthetic plastid involved in essential metabolic functions. (A) *P. falciparum*, a unicellular eukaryote belonging to the Apicomplexa phylum, harbors an essential nonphotosynthetic plastid: the apicoplast. The scheme shows the apical complex that gave its name to the phylum and the three DNA-containing organelles, i.e., the nucleus, the mitochondrion, and the apicoplast. (B) Electron micrograph of the apicoplast in *P. falciparum*. The apicoplast is basically a plastid (Pl) acquired by the secondary endosymbiosis of a red alga and is therefore surrounded by four membranes (arrow). (C) Biological functions of the apicoplast explored for the development of novel drugs in the past decade. The apicoplast possesses a 35 kilobase genomic DNA which is replicated, transcribed into RNA, and translated into proteins via its own machineries of prokaryotic origin. The apicoplast hosts four remarkable metabolic pathways: a type II fatty acid synthesis pathway (FASII), a nonmevalonate pathway or 1-deoxy-D-xylulose 5-phosphate (DOXP) pathway, which catalyzes the synthesis of isoprenoid precursors, a heme synthesis pathway, and an iron/sulfur (FeS) cluster synthesis pathway. The FASII and DOXP pathways both rely on the import of triose phosphates, i.e., phosphoenolpyruvate (PEP) and dihydroxyacetonephosphate (DHAP), generated in the parasite's cytosol and converted into pyruvate, acetylCoA, and glyceraldehyde-3-phosphate (GA3P) in the apicoplast. Reprinted with permission from ref 16. Copyright 2003 Elsevier.

in plastids,[8] is due to a massive lateral gene transfer of the initial cyanobacterial/plastid genome to the nucleus. Consequently, most proteins that function in the apicoplast are nuclear-encoded and thus need to be imported to the organelle. Briefly, proteins are imported via recognition of a bipartite N-terminal peptide that directs the protein into the endoplasmic reticulum (ER) and then through the apicoplast membrane(s).[12−16] During the *Plasmodium* life cycle and division processes, the single apicoplast undergoes drastic morphological changes before being transmitted to daughter cells: from a single round structure in early intraerythrocytic rings to elongated and highly branched in trophozoites, midliver stage, and oocyst in the mosquito.[17,18]

Since its initial discovery in 1996, the apicoplast attracted a particular attention, mostly because of its plant/algal origin, making it a potentially exciting new target. Vulnerability of the apicoplast was confirmed when treatment with antibiotics directed against housekeeping functions of the organelle such as DNA replication, transcription, and translation were shown to be parasiticidal (detailed in this review, ref 19). A challenging question is that of the role of the apicoplast and what essential pathways could potentially be chemically impaired. These issues are not yet completely resolved, but important advances have been made in understanding the apicoplast metabolic functions, which has also led to identification of some possible targets for intervention. Soon after the discovery of the apicoplast, a prokaryotic-type fatty acid synthetic pathway was identified and localized in the plastid of *T. gondii* and *P. falciparum*, which challenged the dogma that Apicomplexa were unable to synthesize fatty acids de novo.[12] The following year, Jomaa et al.[20] showed that the apicoplast was able to synthesize isopentenyl diphosphate, an essential precursor for the isoprenoid synthesis, via a pathway restricted to bacteria and plastids. Products of the apicoplast isoprenoid pathway are likely to be used for a wide range of important products within the parasite: here we discuss their potential cellular fate and use for possible drug intervention. The apicoplast was also found to be the site for the biogenesis of Fe−S clusters[21−23] and the synthesis of heme, and biosynthesis of the latter appears to be distributed between the apicoplast and the mitochondrion.[17,24] Interestingly, the apicoplast is almost always observed in tight association with the single mitochondrion during asexual life stages.[12,24−26] This interaction may reflect important metabolite exchanges for the heme synthesis pathway. A map of the apicoplast metabolic pathways has been assembled by genome data mining.[27,28] The apicoplast is therefore indispensable to the parasite and metabolically very similar to plant and algal plastids except for the lack of photosynthesis.

The apicoplast thus constitutes an attractive target for medical intervention directed against numerous unique targets. Novel procedures have been designed to measure the antimalarial

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properties of apicoplast drug candidates. A delayed cell death was observed in a mutant of *T. gondi* impaired in the division of its apicoplast:[29] daughter cells with no apicoplast die after one division cycle. It is considered that a drug acting at the level of the apicoplast should therefore induce such delayed cell death.[9,29,30] The determination of the half-maximal in vitro inhibitory concentration (IC_{50}) for these drug candidates is thus estimated after the proliferation of *P. falciparum* during two division cycles, i.e., 96 h, rather than one 48 h cycle. After nearly 2 decades of drug discovery and development aimed at the apicoplast, some lessons can be drawn, particularly in relation to whether all the targeted steps are essential during the parasite phase being targeted and whether the leads are on or off target. Here we review the potential of several pathways and lead molecules explored in the last 15 years. We also discuss whether in the light of new insights the apicoplast is indeed the Achilles' heel of the malaria parasite it was initially thought to be.

2. IS THE APICOPLAST DRUGGABLE?

To reach the stroma of the apicoplast, a blood-circulating drug has to cross the plasma membrane of an infected erythrocyte, the parasitophorous vacuole membrane, the plasma membrane of *Plasmodium*, two membranes of the inner membrane complex or avoid this barrier in zones where it is interrupted, and eventually cross the four membranes that bound the organelle. Thus, at least seven and up to nine membranes have to be crossed. If one aims to target the apicoplast of liver-stage parasites, a further two membranes, i.e., those of the endothelial cells of capillary vessels, have to crossed. Apicoplast drugs should therefore have appropriate properties to partition in hydrophilic and lipophilic environments so as to make their way to their targets. Considering a drug must also be formulated in a bioavailable form, be administered orally, and pass all pharmacokinetic pitfalls, the search for efficacious apicoplast drugs appears challenging. At first glance, sophisticated strategies like the design of prodrugs reaching their targets as multistage rockets would appear as necessary solutions. Nevertheless, an antimalarial mitochondrial drug, atovaquone, is currently commercialized and widely used in combination with proguanil,[31] and atovaquone faces almost as many membrane obstacles as the mitochondrion is bounded by two membranes.

Can we predict whether or not a small molecule could cross so many biomembranes? The log of the ratio of the octanol/water partition of a small molecule, called log *P*, is a classical measure of lipophilicity in medicinal chemistry. A negative log *P* is expected for highly hydrophilic drugs, unlikely to diffuse in lipid bilayers. For example, doxycycline ($\log P \cong -1$ to -0.01) and fosmidomycin ($\log P \cong -2$ to -1) have surprisingly low lipophilic function, perhaps suggesting that they undergo transport via cellular channels or transporters. A positive log *P*, like that of ciprofloxacin ($\log P \cong 2.3$), rifampicin ($\log P \cong 1$ to 3), and thiolactomycin ($\log P \cong 3$) suggests an ability to diffuse across biomembranes, as long as the log *P* value does not exceed the Lipinski limit of 5, indicating that the drug might simply be insoluble and aggregate in the absence of a vehicle. Triclosan ($\log P \cong 5$) and atovaquone ($\log P \cong 4.7-5$) would thus be at the limit of some rules of the Lipinski guidelines. Thus, we are left with a paradox where some drugs appear not to have appropriate physical characteristics but are nevertheless efficacious, which demonstrates that the apicoplast is druggable, but that it may not be valid to invoke any kind of general rule for organelle-drug design. Apicoplast drug development programs have thus relied on long and empirical trials.

3. TARGETING THE APICOPLAST DNA REPLICATION, TRANSCRIPTION, AND RNA TRANSLATION

3.1. Two Prokaryotic Machineries to Replicate and Transcribe DNA and Translate RNA into Polypeptides in the Stroma of the Apicoplast and the Mitochondrion

The organellar machineries for the maintenance and replication of DNA, its transcription into tRNAs, rRNAs, and mRNAs, and translation of the latter into proteins, offers a complete series of targets that can be inhibited by previously characterized families of antibacterials. Many drugs, e.g., macrolides, ketolides, lincosamides, oxazolidinones, aminoglycosides, and tetracyclines, used in clinical medicine to treat infectious bacterial diseases interfere with protein synthesis by targeting the pathogen ribosomes,[32] and many of these drugs are contenders as antimalarials perturbing organelle housekeeping. Antimalarial antibiotics are now known to act at the level of the apicoplast.[33−35] Different groups have studied *Plasmodium* rRNAs as targets for antimalarial drugs, including thiostrepton, known to bind the apicoplast large ribosomal subunit rRNA,[36−38] clindamycin, acting on the large ribosomal subunit rRNA in *Toxoplasma*,[39] and tetracycline, whose antimalarial effect was suspected to be due to its binding to the mitochondrial and/or apicoplast small subunit rRNA.[40] Determining whether an antibiotic acts at the level of the apicoplast, the mitochondrion, or both is difficult to assess. In the case of novobiocin, an inhibitor of prokaryotic gyrase B, the effect was higher on the replication of apicoplast DNA compared to that of mitochondrion DNA.[41] For most other drugs impairing DNA metabolism of prokaryotic origin,[42] the specificity on the apicoplast machinery versus that of the mitochondrion remains an open question. Below we describe three examples of drugs acting at the levels of 35 kb DNA replication in the apicoplast (ciprofloxacin), transcription (rifampicin), and translation (doxycycline, a derivative of tetracycline) (Figure 2).

3.1.1. Ciprofloxacin Ciprofloxacin is a synthetic antibiotic of the fluoroquinolone drug class (Table 1). At the end of the 1980s, quinolones and fluoroquinolones were reported to be active against *P. falciparum*.[43] In vitro IC_{50} values based on the innoculum size method were in the 50 μM range. Their mode of action in parasites was studied in comparison to their known mode of action in (myco)bacteria, where these compounds affect bacterial DNA by targeting topoisomerases II and IV.[44,45] A decade before the discovery of the apicoplast, the initial hypothesis was an effect at the level of the mitochondrion. As an indirect test of the mitochondrial gyrase hypothesis, combinations of ciprofloxacin and tetracycline or novobiocin were first examined for synergy.[43] Because the tested agents could hypothetically act upon a rate-limiting site with the mitochondrion, the combinations tested represented potentially synergistic regimens. However, tested combinations were modestly additive.[43] The discovery of the apicoplast in *Toxoplasma* and thereafter in *Plasmodium* was the clue to elucidating the mode of action of ciprofloxacin.[5] Ciprofloxacin was proposed to target the plastid gyrase and to inhibit DNA replication leading to the formation of abnormal apicoplasts and a delayed death of treated parasites.[19,34] Other studies have shown that halogenated alkyl- and alkoxy-4(1*H*)- and 1-hydroxy-2-docecyl-4(1*H*)-quinolones were active in vitro against chloroquine-susceptible (D6) and chloroquine-resistant

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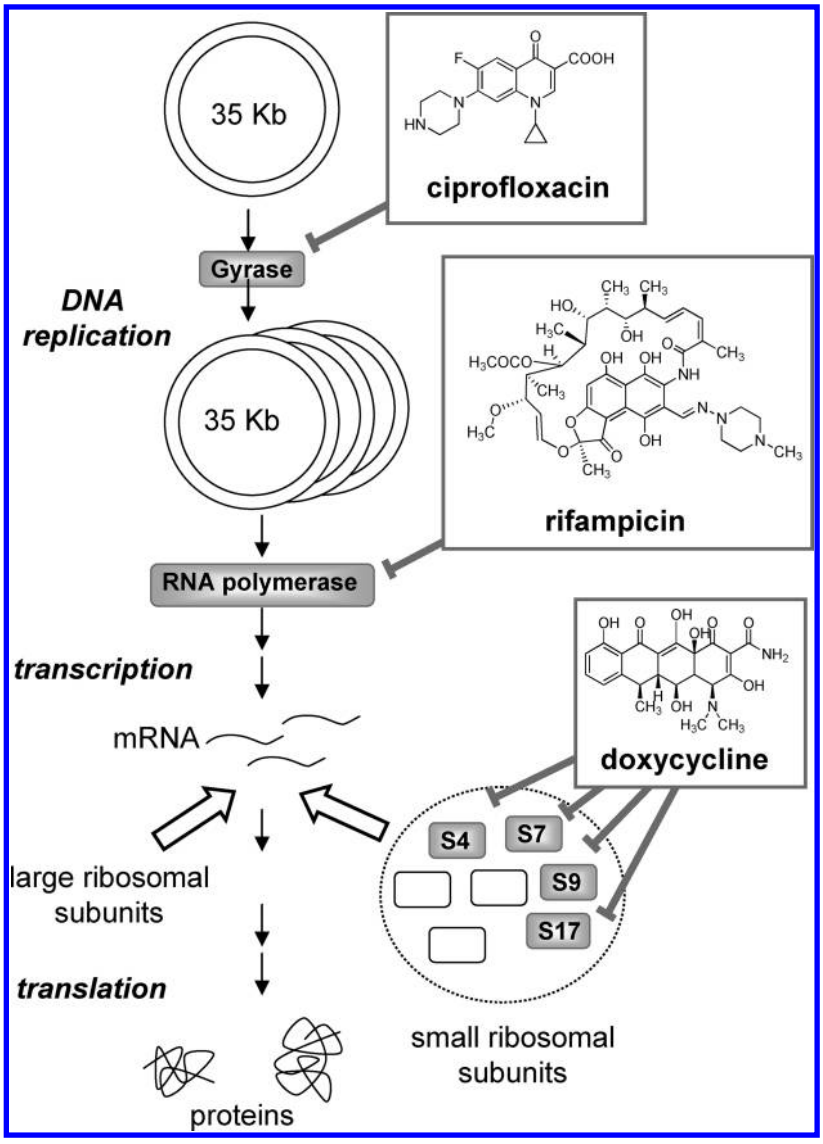

Figure 2. Antibiotics targeting the prokaryotic DNA and RNA machineries. DNA metabolism: in all living cells, most important housekeeping functions are DNA replication (equals the process used to copy DNA), transcription (equals the process of building RNA copy of a DNA sequence), and translation (equals the conversion of the mRNA sequence into amino acids). Numerous antibiotics previously known to impair DNA maintenance and replication, transcription, and RNA translation have proven to be efficient on apicoplast (and mitochondria) machineries (see text).

(Dd2) *P. falciparum* strains.[46] These quinolone derivatives were shown to target the cytochrome bc_1 complex and NADH dehydrogenase, respectively.[47] More recently, lipophilic prodrugs derived from ciprofloxacin were found to be 10−100-fold more active than ciprofloxacin against *P. falciparum* chloroquine-susceptible (3D7) and chloroquine-resistant (W2) strains.[48] The ethyl ester derivatives killed parasites more rapidly than did ciprofloxacin, with a promising therapeutic index.[49]

3.1.2. Rifampicin. Rifampicin or rifampin is a bactericidal antibiotic drug of the rifamycin group (Table 1). Rifampicin is a semisynthetic compound derived from *Amycolatopsis rifamycinica* (formerly known as *Amycolatopsis mediterranei* and *Streptomyces mediterranei*). Rifampicin is used to treat *Mycobacterium* infections such as leprosy and tuberculosis. In bacteria, the action of rifampicin is due to the inhibition of the RNA polymerase (RNAP).[50] A similar mechanism of action is supposed to explain the antimalarial activity of rifampicin by targeting the transcription functions in the apicoplast (Figure 2). The immediate killing or the delayed-death effect is still a matter of debate.[51,52] The in vitro activity against intraerythrocytic stages of multidrug-resistant *P. falciparum* and their efficacy in vivo favor the use of rifampicin as an antimalarial drug.[53] Extension of the "piggy-back" strategy[54] led to the evaluation of the antimalarial activity of antituberculosis drugs rifampicin, isoniazide, and ethambutol in combination in a murine model.[55] This study showed that only the combination of the three drugs is safe and efficacious for treating malaria in *P. berghei*-infected mice.[55] Each drug alone failed to clear parasites.[55] When tested in humans, a parasitological failure rate was higher for cotrifazid (a fixed combination including cotrimoxazole, rifampicin, and isoniazid) than for mefloquine or quinine and sulfadoxine−pyrimethamine. Despite a very good safety profile, the authors concluded against cotrifazid as a suitable antimalarial drug.[56]

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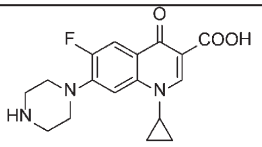
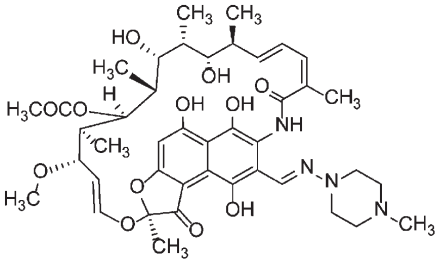
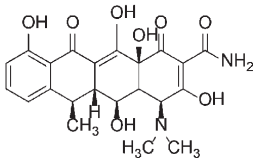
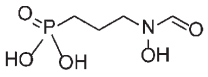
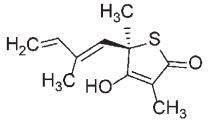
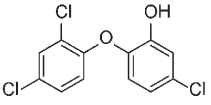
Table 1. Examples of Apicoplast-Targeting Drugs

Drug	Formula	Targeted pathway	Antimalarial effect
ciprofloxacin		DNA replication	immediate effect (?)
rifampicin		transcription	delayed-effect (?)
doxycycline		translation	delayed-effect
fosmidomycin		isoprenoid biosynthesis	immediate effect
thiolactomycin		FAS II biosynthesis and unknown off-target(s)	immediate effect
triclosan		FAS II biosynthesis and unknow off-target(s)	immediate effect

3.1.3. Doxycycline. The tetracyclines, first described in 1948,[57] are a well-known and widely used family of antibiotics acting as broad-spectrum protein synthesis inhibitors. Tetracyclines bind to the 30S subunit of microbial ribosomes and block the attachment of charged aminoacyl-tRNA. Doxycycline is a semisynthetic tetracycline clinically developed in the early 1960s (Table 1). When first examined for its potential antimalarial property, doxycycline was much more active than tetracycline, still exhibiting a rather high IC_{50} value measured on the in vitro proliferation of various isolates of *P. falciparum*, with an average value in the 10 μM range, highest efficacies in the <1 μM range, and lowest in the >100 μM range.[58] Despite side effects such as photosensitivity skin reactions, doxycycline is a chemoprophylaxic drug recommended for travelers in Africa, Southeast Asia, and South America.[59,60] Doxycycline is recommended in the second-line treatment of uncomplicated *P. falciparum* malaria and in the treatment of severe malaria in combination with artesunate or quinine for seven days.[61,62] As yet, no clinical resistance to doxycycline has been reported.[63] The mechanism of action of doxycycline was first linked with the inhibition of nucleotides and deoxynucleotides synthesis.[64] Nevertheless, more recent data suggest a specific action by doxycycline on the apicoplast and the mitochondria.[30,65] In the apicoplast, doxycycline presumably targets the translation functions (Figure 2),

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resulting in the delayed-death drug response. In bacteria, doxycycline is known to target binding the S4, S7, S9, and S17 proteins of the small 30S ribosomal subunit and various ribonucleic acids of the 16S rRNA.[66] In both the apicoplast and the mitochondrion, S4, S7, S9, and S17 proteins were identified in the complete inventory of ribosomal subunits.[67] The molecular mechanisms involved are not well-understood but presumably involve the arrest of apicoplast translation, a subsequent defect of the apicoplast biogenesis and functioning, and a delayed cell death.[66] Despite unresolved questions on the mode of action, doxycycline development has been supported by Medicines for Malaria Venture[65] and used for malaria treatment.

3.1.4. Will Apicoplast Housekeeping Antibiotics Trigger Bacterial Resistance? Treatments with antibiotics targeting apicoplast DNA replication, transcription, and RNA translation raise some concern that such usage could trigger resistance in pathogenic bacteria. Ciprofloxacin is thus currently used for its efficiency on multiresistant pathogenic Gram-positive and Gram-negative bacteria and is considered one of the most important treatments for various community acquired and nosocomial infections, e.g., respiratory tract, urinary tract, and skin infections and sexually transmitted diseases.[68] Rifampicin is a major drug in the anti-TB arsenal, and the treatment of patients with both malaria and tuberculosis could simply trigger resistance of one or both pathogens to this antibiotic.[69] Doxycycline has also broad antibacterial properties but was also shown to have various anticancer properties, especially cytotoxic and antiproliferative activities, due to its interference with mitochondrial-mediated apoptosis in various tissues.[70] Thus, all the antibiotics discussed here have other concurrent applications. Future drug development programs should keep this risk in mind and perhaps seek specificity against the parasites, or even restrict use of such drugs to malaria only if that is feasible. Specific inhibitors against *P. falciparum* proteins of prokaryotic origin might be designed based on differences between bacterial and malarial proteins. In particular, large amino acid insertions (often predicted to be disordered stretches) are often detected in the sequences of malaria proteins.[71−73] Molecular modeling of *Plasmodium* DNA gyrase B has thus revealed extensive fold conservation with *Escherichia coli* counterpart but also large disordered insertions in close proximity to the sites of action.[41] Future progress might benefit of the presence of such domains to design antibiotic analogues binding specifically to *P. falciparum* protein targets; however, accurate de novo or ab initio techniques for structure prediction will be needed to predict the fold assumed to be the one adopted by these low-complexity amino acid sequences.

4. TARGETING APICOPLAST ISOPRENOID PRECURSOR SYNTHESIS

4.1. Synthesis of Isoprenoid Precursors Is an Essential Metabolic Function of the Apicoplast, but Why?

Isoprenoids are an important class of lipid components that are essential for the synthesis of sterols, chlorophylls, and quinones in both animal and plant cells. They are also required for protein anchoring either by protein prenylation or as part of GPI anchors and can serve as prosthetic group of tRNAs in mitochondria and plastids. Isoprenoids are made of repeated units of isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which can be synthesized via two different pathways: (i) the canonical mevalonate pathway mainly found in animal cells and the cytosol of plants and (ii) the nonmevanolate pathway also referred to as the MEP/DOXP (2-*C*-methyl-D-erythritol4-phosphate/1-deoxy-D-xylulose-5-phosphate) pathway found in bacteria and plastids.[74] Evidence of isoprenoid precursor synthesis in the apicoplast was first detected in *P. falciparum*, whose genome contains homologues of algal and bacterial genes from the DOXP pathway (i.e., DOXP reductoisomerase/IspC and DOXP synthase), and *T. gondii*, where DOXP reductoisomerase is apicoplast-localized.[20] The canonical mevanolate pathway is apparently absent from malaria parasites, making the apicoplast the sole site for isoprenoid precursor synthesis.[28,75]

A complete DOXP pathway is inferred from in silico analyses in three Apicomplexa: *P. falciparum*, *T. gondii*, and *Eimeria tenella*.[28,76] The seven enzymes of the pathway, DOXP synthase, IspC, IspD, IspE, IspF, IspG, and IspH (Figure 3), are all predicted to be nuclear-encoded genes whose products are targeted into the apicoplast.[28] Initial characterization of the apicoplast DOXP pathway demonstrated that *P. falciparum* intraerythrocytic stages were killed by fosmidomycin, an inhibitor of the DOXP reductoisomerase/IspC, suggesting that the pathway was essential for the parasite, as discussed below.[20] The DOXP pathway is initiated by the condensation of glyceraldehyde-3-phosphate and pyruvate, catalyzed by the DOXP synthase (Figure 3). Interestingly, clomazone, an herbicide known to inhibit the DOXP synthase, was shown to affect the growth of *T. gondii* and *E. tenella*.[76] It would be interesting to know whether this herbicide is a also potential drug against *Plasmodium*. Inhibitors of IspF belonging to the thiazolopyrimidine family have recently been identified via an in vitro screening performed on an *A. thaliana* homologue.[77] These inhibitors and related chemical derivatives inhibit *P. falciparum* IspF in vitro and kill intraerythrocytic stages in the low-micromolar range.[77]

The apicoplast isoprenoid pathway therefore seems to be one of the more promising targets for novel antimalarials. However, one essential question remains: what does the apicoplast DOXP pathway provide to the parasite? Several metabolites have been proposed as recipients of apicoplast-synthesized isoprenoid precursors, but direct evidence for the role of the apicoplast in their production is still missing. An obvious possible role is the production of prenyl groups, repetitive units of IPPs: geranylpyrophosphate groups (geranylPP, 10 carbons), farnesylpyrophosphate groups (farnesylPP, 15 carbons), and geranylgeranylpyrophosphate groups (geranylgeranylPP, 20 carbons) (Figure 3), for anchoring proteins to membranes via post-translational prenylation. Although inhibitors of prenyl transferases, which catalyze the transfer of prenyl groups to a target protein(s), were effective against *P. falciparum* and *T. gondii*,[78−80] no protein has yet been shown to be directly prenylated via the apicoplast isoprenoid pathway. Synthesis of prenyl groups via the DOXP pathway usually takes place in plastids, but so far, no experimental data has confirmed the apicoplast as the site for their synthesis.

tRNA isopentenylation involves transfer of DMAPP to the anticodon loop, which is an essential modification for the correct binding to the ribosome-mRNA as well as the suppression of stop codons and frameshift mutations during translation in mitochondria and plastids. This process is predicted to allow the correct translation of the apicoplast genome since a homologue of the plant isoprenyltransferase catalyzing this reaction was found in *P. falciparum* genome.[28] Again, no experimental data verifying apicoplast isoprenoid synthesis as the source of the DMAPP

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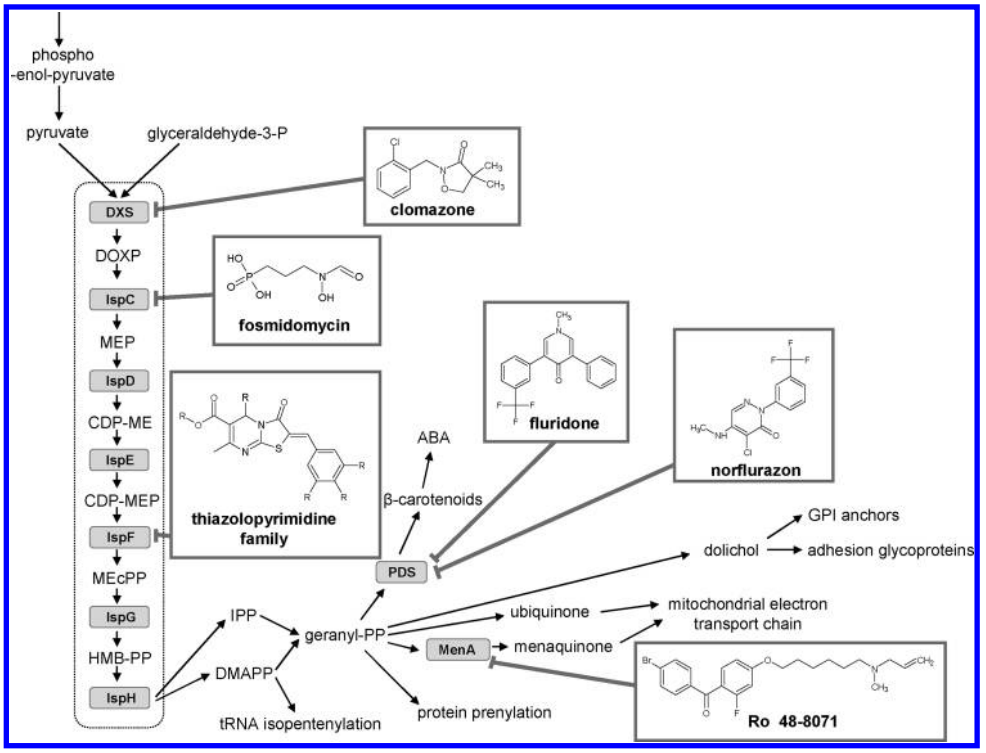

Figure 3. Isoprenoid pathway drug candidates. Isoprenoids are synthesized from isopentenyl pyrophosphate (IPP) and dimethyallyl pyrophosphate (DMAPP) precursors via the apicoplast DOXP (1-deoxy-D-xylulose-5-phosphate) pathway (dashed frame), which is formed of seven enzymes. DOXP is synthesized through the condensation of pyruvate and glyceraldehyde-3-phosphate (glyceraldehyde-3-P) via the action of a DOXP synthase (DXS). DOXP is then converted into methylerythritol-4-phosphate (MEP) by a DOXP reductase (IspC). MEP is converted into IPP and DMAPP via five consecutive reactions catalyzed by a CDP-MEP synthase (IspD), a CDP-ME kinase (IspE), an MEcyclodiphosphate synthase (IspF), a hydroxymethylbutenyl-PP synthase (IspG), and an HMP-PP reductase (IspH). IPP and DMAPP can be condensed to form geranyl-PP, which serves as a substrate for the synthesis of carotenoids and abscisic acid (ABA). Their synthesis is catalyzed by a series of enzymatic reactions, one of them being performed by a phytoene desaturase (PDS). Geranyl-PP is also a precursor for menaquinone synthesis through the action of a dihydroxynaphtoate prenyltransferase (MenA). Clomazone inhibits DXS; fosmidomycins inhibit IspC; the thiazolopyrimidine family inhibits IspF; fluridone and norflurazon inhibit PDS; Ro 48-8071 inhibits MenA.

required for tRNA isopentenylation has been obtained yet (Figure 3).

GPI anchors, mitochondrial dolichols, and abscisic acid are other putative end uses of the DOXP pathway products (Figure 3) that would appear vital for the parasite. Here we will describe how fosmidomycin has shed light on GPI anchor and dolichol synthesis. We will also describe how the synthesis of abscisic acid, a typical plant hormone, may constitute a novel pathway of interest for medical intervention.[81−84]

4.1.1. Fosmidomycin, a Codrug? Fosmidomycin is an antibacterial agent with a hydroxamic acid function and a phosphonic acid group (Table 1). The demonstration of its potential as an active drug candidate acting on *P. falciparum* is one of the first examples of a fruitful cooperation between plant scientists and malaria experts.[20] Fosmidomycin acts as a specific inhibitor of the DOXP reductoisomerase (DXR also designated IspC) (Figure 3), an essential enzyme of the isoprenoid biosynthetic DOXP pathway. Disappointingly, the use of fosmidomycin monotherapy failed to completely cure patients, and recrudescence was observed.[85] Fosmidomycin was nevertheless efficient in association with other antimalarial drugs.[86−89] A phase II clinical trial was conducted in Gabon to allow safety and efficacy data to be collected with the association fosmidomycin−clindamycin in children with acute, uncomplicated *P. falciparum* malaria.[88] The efficacy of fosmidomycin and clindamycin/artesunate, when coadministered to adults with acute uncomplicated *P. falciparum* malaria, is also currently being tested in Thaïland (ClinicalTrials ID: NCT01002183).

Interestingly, the use of fosmidomycin has helped understanding the fate of some precursors of the apicoplast DOXP pathway and could therefore allow the identification of downstream targets for new drug development. Dolichol is a polyprenyl compound, the synthesis of which is inhibited by fosmidomycin particularly during ring and schizont stages in *P. falciparum*.[90] Dolichol served as polysaccharide transporting unit during glycoprotein synthesis in the ER. Furthermore, dolichol is believed to be required for the synthesis of GPI anchors and adhesion glycoproteins, which are essential for parasite invasion of host cells.[91,92]

Ubiquinone is a lipid-soluble component of the mitochondrial electron transport chain that has to be polyprenylated to be functional. Ubiquinone synthesis is highly affected by fosmidomycin treatment in *P. falciparum*.[90] Moreover, molecular disruption of the plastidial phosphate transporter TPT, which provides precursors for apicoplast isoprenoid synthesis,[28] resulted in a drastic loss of the mitochondrial membrane potential (energetic state) in *T. gondii*,[93] which is consistent with ubiquinone depletion. Very recently Tonhosolo et al.[94] reported that *P. falciparum* could synthesize menaquinone as an alternative mitochondrial electron transporter to ubiquinone in anaerobic conditions. Correspondingly, the mycobacterial inhibitor of menaquinone synthesis, Ro 48-8071, blocked menaquinone synthesis

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and affected parasite growth in vitro with an IC_{50} in the low-micromolar range.[94] In conjunction with fosmidomycin treatment, these data suggest that the apicoplast DOXP pathway is essential to fuel the mitochondrial ATP production via the synthesis of essential electron transporters such as ubiquinone and menaquinone. We believe that the use of fosmidomycin in combination with specific inhibitors of dolichol and/or ubiquinone synthesis may yield efficient treatments against *Plasmodium*.

4.1.2. Drugs Acting on Abscisic Acid and Carotenoid Biosyntheses in a Nonphotosynthetic Parasite? Very recently abscisic acid[95] or ABA, a well-characterized phytohormone, was detected in *T. gondii* and shown to play an essential role in calcium-dependent mechanisms.[84] In plants, ABA is synthesized by the plastid DOXP pathway via carotenoid intermediates (Figure 3). Importantly, fluridone, a herbicide specifically blocking plastidial ABA synthesis (i.e., phytoene desaturase), disrupted parasite growth (EC_{50} = 15 μM) and protected mice against toxoplasmosis.[84] It will be important to determine whether this compound can be used against *Plasmodium*. However, the route of ABA synthesis in parasites remains unclear since there has been no clear identification of the genes responsible for ABA synthesis despite the presence of ABA-response genes in both *T. gondii* and *P. falciparum*.[84] Inconsistency between biochemical or pharmacological evidence of plant enzymes and the apparent lack of homologous genes is an essential issue in the validation of targets for herbicide-drugs.[96−98] ABA is likely to be synthesized in the apicoplast in a similar manner to the plant pathway, but further investigation is required to confirm the pathway's nature and localization. Carotenoids were reported to be neosynthesized by *P. falciparum* intraerythrocytic stages, mainly at the schizont stage.[83] Their synthesis was specifically inhibited by treatment with norflurazon, an inhibitor of phytoene desaturase, which also affected parasite growth (IC_{50} = 25 μM). A phytoene synthase was identified in the *P. falciparum* genome, characterized, and proposed as part of the putative carotenoid synthesis machinery of the parasite.[83] These data indicate that both carotenoid and ABA synthesis are required for the parasite development, further implicating these pathways as possible novel targets for medical intervention.

5. TRICLOSAN AND THIOLACTOMYCIN: DEBATES OVER FATTY ACID SYNTHASE II DRUGS

5.1. Is FASII a Valid Target?

The apicoplast contains a bacterial, type II fatty acid synthase machinery (FAS II). Although the erythrocytic stage of *P. falciparum* was known to be capable of scavenging fatty acids from the host,[99,100] the discovery of a an apicoplast FAS II system in the late 1990s was hypothesized to contribute de novo synthesized fatty acids to the huge demand of membrane glycerolipids required in the proliferative cycle of the parasite.[12,13] Apicoplast FAS II soon became a focus of attention, and antibiotics and herbicides known to impair FAS activity in bacteria and plants were tested on malaria parasites as potential novel drugs. Substantial efforts were made to develop novel drug candidates, but ambiguous and nonreproducible results in in vitro and in vivo trials cast doubts on the validity of the target and the specificity of the developed molecules. The debate is currently most intense for a lead compound known as triclosan.

The FAS II machinery is attractive as a drug target because the apicoplast FAS II diverges markedly from the cytosolic FAS I of human cells. Moreover, FAS II is a multicatalytic system composed of several proteins and potentially targetable by multiple drug candidates. The biosynthesis of fatty acids is an iterative process that requires carbon substrates thio-esterified to coenzyme-A, i.e., acetyl-CoA as an initiator and malonyl-CoA as a 2C-donor, for elongation of the acyl chain. The latter is generated by the activity of an acetyl-CoA carboxylase (ACCase, Figure 4) which, through its biotin prosthetic group, binds to and transfers a molecule of CO_2 to acetyl-CoA to form malonyl-CoA. Molecules of malonyl-CoA are sequentially added to acetyl-CoA through the action of FAS of two types: type I FAS (FASI), an enzyme complex whose subunits/catalytic sites are each responsible for one step of the complete elongation reactions and FAS of type II (FASII), which consists of a group of proteins that can be separated and purified (Figure 4). FASII is often referred to as a "dissociated" machinery. FASI is specific for the cytosol of animal cells, fungi, and some prokaryotes that have secondarily acquired this system by lateral gene transfer; FAS II is found in all bacteria and in the plastid of plants, algae, and apicomplexan parasites.[101,102]

Fatty acid synthesis requires the attachment of a malonyl group to acyl carrier protein (ACP), a reaction catalyzed by a malonyl-CoA:ACP malonyltransferase (MAT) also known as FabD in the dissociated FASII (Figure 4, FabD). The sequential addition of two carbons begins at this point. Initially, a keto-acyl-ACP synthase (KS) activity catalyzes the transfer of 2C from malonyl-ACP to acetyl forming an acetoacetyl-ACP. This specific KS enzyme is termed FabH (Figure 4, FabH). The ketone group is reduced to alcohol by an NADPH-dependent β-keto acyl-ACP reduction (KR) catalyzed by a FabB/F type of enzyme (Figure 4, FabB/F). The enoyl-ACP hydrase (DH) (Figure 4, FabZ) then catalyzes the formation of a *cis*-2,3-enoyl, which is reduced by enoyl-ACP reductase (ER) (Figure 4, FabI). Three cycles are required for the synthesis of octanoate (C8:0), six for myristate (C14:0), seven for palmitate (C16:0), and eight for stearate (C18:0). The saturated acyl chain eventually formed is thio-esterified to ACP and serves for acyl-lipid syntheses, including membrane lipids and triacylglycerol droplets.

When it became obvious that the apicoplast was an invaluable mine of potential antimalarial targets a decade ago, the recycling of known antibiotics and herbicides acting on FASII was attempted by several research groups. These drug development programs jumped ahead of the usual rigorous target validation studies requiring long and difficult genetic knock outs and measures of FASII activity across the parasitic life cycle. Inhibitors were sourced and tested against parasites and assumed to be acting specifically against the presumed targets identified by genome mining. Seven genes coding for FASII components are known in *P. falciparum*: ACP (PFB0385w), ACCase (PF10_0409), FabD (PF13_0066), FabH (PFB0505c), FabF (PFF1275c), FabG (PFI1125c), FabZ (PF13_0128), and FabI (PFF0730c). FadD and FabH have been characterized;[103] FabH has been shown to catalyze the conversion of acetyl-CoA into acetyl-ACP, although this activity was very low compared to the acetoacetyl-ACP synthesis. That PFF1275c is a FabF rather than a FabB orthologue was recently deduced from complementation studies of *E. coli* mutants.[104]

The validity of the system as a target requires that it is active and essential in a life stage that is relevant for a chemotherapeutic treatment, i.e., during the erythrocytic stage. The activity of FASII has been studied using *T. gondii* as a working model, although this model did not fully reflect the situation in *P. falciparum* as *T. gondii* contains an apicoplast FASII and a cytosolic FASI, whereas the malaria parasite only contains a plastid FASII. Bisanz et al.[105]

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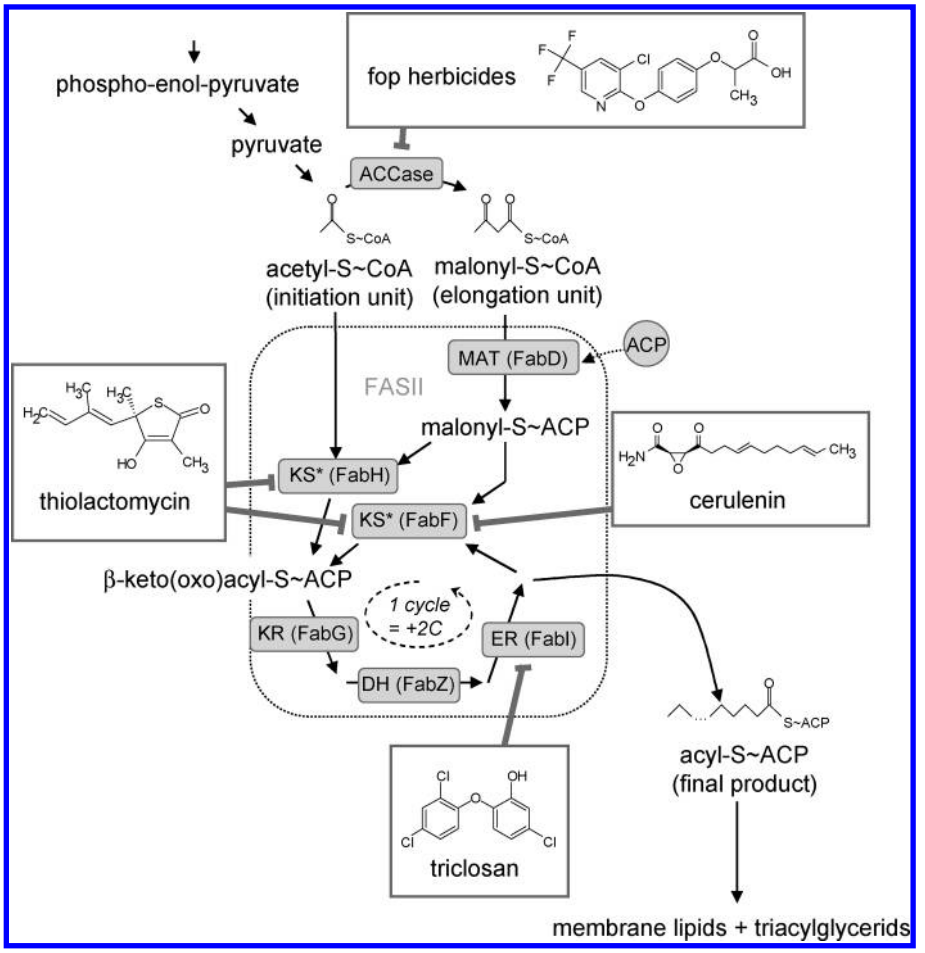

Figure 4. FASII drug candidates: fop herbicides, thiolactomycin, and triclosan. FASII is a multienzymatic machinery. Acetyl-CoA is converted into malonyl-CoA by an acetyl-CoA carboxylase (ACCase) and then into malonyl-ACP by a malonyl-CoA:ACP transacylase (MAT or FabD). Malonyl-ACP is condensed with another molecule of acetyl-CoA under the action of a ketoacyl synthase (see note below) (or β-oxoacyl-ACP synthase III, KS, or FabH) to form a first β-keto(oxo)acyl-ACP, i.e., acetoacetyl-ACP. It is then a substrate for a series of reactions catalyzed by a ketoacyl reductase (or β-oxoacyl-ACP reductase, KR, or FabG), a dehydratase (or β-hydroxyacyl ACP dehydratase, DH, or FabZ), an enoyl-ACP reductase (ER or FabI) to form butyryl-ACP. This product re-enters the iterative process and undergoes a two-carbon elongation. The enzymes involved in elongation correspond to ketoacyl synthases (KS or FabB/FabF) differing to that involved in the first elongation of acetyl. Note: KS activity can be catalyzed by FabH in the initial step, and by FabB/F in successive iterations of elongation. Fop herbicides (here the structure of haloxyfop) inhibit ACCase; thiolactomycin inhibits all KS enzymes (FabH, FabB, FabF) and is specific of FASII; cerulenin inhibits FabB/F of FASII but is nonspecific since it also inhibits KS activity of FASI. Eventually triclosan was studied as a potential antimalarial as it was reported to act as an inhibitor of FabI, but this activity has been recently questioned (see text).

first analyzed the labeling of acyl-lipids containing C16 and C18 acyls, after incubation of *T. gondii* with [^{14}C]acetate. Incorporation was completely blocked by haloxifop, a known inhibitor of plant plastid ACCase[106] (Figure 4). This work suggested that FASII could supply palmitate (C16:0) and stearate (C18:0) for the syntheses of most classes of glycerolipids in the parasites. By contrast, Mazumdar et al.[107] did not support the bulk fatty acid synthesis (as measured by [^{14}C]acetate incorporation) as the main function of the apicoplast FASII in *T. gondii*. Labeling with [^{14}C]acetate failed to be conclusive in short labeling courses, probably because the enzymes required for acetate activation are not present in the apicoplast, and because of possible interference with cytosolic ACCase of *T. gondii*. Indeed, acetyl-CoA derives from the pyruvate metabolism (Figure 4); labeling with [^{14}C]acetate follows therefore indirect routes in the parasite metabolism prior to being incorporated into the apicoplast acetyl- and malonyl-precursors. Mazumdar et al.[107] showed that *T. gondii* FASII was at least required for the lipoylation (i.e., the covalent attachment of lipids or fatty acids to proteins) of pyruvate dehydrogenase, supporting a role in octanoate (C8:0) production. Transfers of precursors from the mitochondria to supply carbon to the apicoplast FASII, and of octanoate metabolite(s) from the apicoplast to the mitochondria, were thus suspected to occur and explain the close physical relation bridging together these two organelles within the cell. Nevertheless, both studies[105,107] indicated that FASII was at least essential in apicoplast maintenance and biogenesis, likely because of a requirement for the synthesis of lipids for the growth and division of plastid membranes.[108,109] Together these studies established that the apicoplast FASII pathway was essential for *T. gondii* survival in vitro and in vivo and represented a viable target for drugs targeting this parasite.

In *Plasmodium*, which lacks a cytosolic FASI, and which lives within a host cell (erythrocytes) that also lack a FASI, metabolic labeling experiments had shown that plasma lipids, including C16:0 and C18:1 fatty acids, could be easily scavenged.[110] This

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situation left a hypothetical role for FASII in the production of octanoate for lipoic acid production. However, Allary et al.[111] reported that *P. falciparum* could scavenge lipoic acid during the blood stage, eventually leaving no indispensable role to FASII during the blood stage.

Less than 2 years ago, Yu et al.[112] and Vaughan et al.[113] showed that a knockout mutation of *P. falciparum* FabI had no effect on the phenotype of the parasite during blood stage, proving the dispensability of apicoplast fatty acid synthesis. The knockout immediately cast severe doubt on FASII as a target of choice, for erythrocyte stages at least. In addition to FASII, a cytosolic fatty acid elongase (FAE) was pointed out as an alternative system to produce fatty acids in *Plasmodium*.[91,114] The current understanding of apicoplast fatty acid synthesis, based on FAS gene knockouts in *P. yoelii*, is that FASII is nonessential in mosquito and erythrocytic stages but has a specific and vital role during the liver stage.[113]

If undertaken 10 years earlier, these genetic experiments would have simply ruled out FASII as a target of interest. Nevertheless, we would probably have foregone opportunities to resolve the protein structures of FASII components such as ACP,[115] FabG,[116] FabZ,[117] and FabI,[118−122] which have enriched our understanding of this delicate multicatalytic complex. Last but not least, we would have certainly missed some of the most intriguing and fascinating results in modern time drug development. Unaware of the FASII apparent dispensability in *P. falciparum* blood stage, FASII drugs have been tested and surprisingly "improved" by chemical diversification of initial scaffolds, focusing on those targeting ketoacyl synthases FabH and FabF, in particular thiolactomycin and chemical analogues, and enoyl-ACP reductase FabI, with triclosan and its derivatives.

5.2. Thiolactomycin: FabH and FabF Targets in the Dissociated FASII System and the Unresolved Question of Off-Target(s)

Thiolactomycin is an antibiotic with the structure (4*S*)-(2*E*,5*E*)-2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene (Table 1), and its mode of action on bacterial FASII has been functionally dissected by Nishida et al.[123] Thiolactomycin interferes with two activities of *E. coli* FASII, i.e., an inhibition of the ACP acetyltransferase, competitive with respect to ACP and noncompetitive with respect to acetyl-CoA, and inhibition of the keto-acyl-ACP synthase activities, competitive with respect to malonyl-ACP and noncompetitive with respect to acetyl-ACP (Figure 4). A very low level of ACP acetyltransferase activity could be measured in in vitro assays of *Plasmodium* FabH, compared to the acetoacetyl-ACP synthase activity harbored by this enzyme.[103] Thiolactomycin had little activity on the *Plasmodium* enzymes tested,[103] but some analogues exhibited inhibiting effects in the micromolar range. Thiolactomycin was shown to inhibit the growth of *P. falciparum* with a relatively high IC_{50} value of 50 μM,[12] which is nevertheless in the range of some antifolate drugs like proguanil that proved to be useful in combination therapies.

Thiolactomycin analogues have been evaluated to improve the initial efficacy, and greater than two logs could be gained.[103,124−127] Thiolactomycin analogues have therefore fascinating antimalarial properties, although the targets they are supposed to kill have now been demonstrated to be nonessential for the parasite during blood stage. Thiolactomycin analogues also kill other apicomplexans such as *T. gondii*,[128] as well as nonapicomplexan pathogens of medical importance. In the *T. gondii* model, metabolic labeling of extracellular parasites was carried out following the method introduced by Bisanz et al.,[105] using [^{3}H]acetate, showing that these drugs affected acylglycerol synthesis. As discussed above, [^{3}H]acetate is not the appropriate substrate to assess FASII activity, and the measured decrease in acylglycerol might indeed result from a failure of FASII and/or downstream reactions, or result from defaults in the metabolic route of labeled carbon upstream FASII and the pyruvate metabolism. The rapid reduction of parasite load suggested that thiolactomycin analogues had selective cytotoxic effects against *T. gondii*.

What is the mode of action of thiolactomycin and its derivatives? Thiolactomycin is known to act on FabH and FabF in a competitive manner with ACP and/or malonyl-ACP. It is therefore reasonable to hypothesize that the thiolactomycin analogues could interfere with other ACP-related processes, including the acyltransferase activities requiring acyl-ACP substrates, like those occurring in the apicoplast.

Undoubtedly thiolactomycin drug development would not have been pursued if FASII had been set aside as a target earlier. Future studies will be in the assessment of off-targets, in particular at the level of the complete acyl-lipid metabolism.[129]

5.3. Triclosan, FASII FabI Target, and the Debate over Drug Specificity and in Vivo Efficacy

Triclosan is a biocide with the structure 5-chloro-2-(2,4-dichlorophenoxy)phenol, inhibiting FabI in bacteria[130,131] (Figure 4). The triclosan case is a much more complex puzzle as compared to thiolactomycin. The story began when McLeod et al.[132] and Surolia and Surolia[133] independently reported that the growth of *P. falciparum* was inhibited by triclosan with IC_{50} values in the micromolar range. The in vitro and in vivo effect on fatty acid synthesis was based on metabolic labeling experiments with [^{14}C]acetate and [^{14}C]malonyl-CoA, and the demonstration of an in vivo efficacy was based on the curative effect of mice infected by *P. berghei*, treated with daily doses of ∼40 mg/kg.[133] Immense efforts were subsequently channelled into the synthesis of chemical libraries to explore the chemical diversity of the triclosan active structure (a nonexhaustive list includes works by refs 134−138). The genetic knockout of FabI in *P. falciparum*, which unequivocally showed that blood-stage parasites were not affected by the lack of the triclosan target,[112] raised concerns about the rational for such drug development programs. Ben Mamoun et al.[102] deduce from the vast number of publications relating improvements of antimalarial properties after chemical derivation of triclosan that the efficacy gained was most likely due to an enrichment in off-target inhibitors.

The question of triclosan off-targets is not the only concern. Very recently, Bashong et al.[139] published a comparative study of in vivo trials achieved with triclosan on rodent malaria models, showing that significant curative effects could not be reproduced with *P. berghei*, as initially reported by Surolia and Surolia,[133] or with another rodent malaria *P. chabaudi*. In vivo trials were carried out with doses as high as ∼130 to ∼510 mg/kg. Bashong et al.[139] suggest that the initial trials might have been misleading because of impurities in the triclosan batch, unexpected effects of the drug vehicle, or specific physiological status of the treated mice. They draw the conclusion that drug development programs should be carried out only when a target, here FASII, has been previously fully validated, to avoid wasting time and resources. Surolia and Surolia[140] replied that, indeed, triclosan had shown some promising properties in numerous independent studies, and that FASII activity might be critical at some specific stages. Given the indispensability of FASII in livers stages, it is now obvious that putative inhibitors should be screened on

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liver-stage parasites.[102] As a final caution, we note that the nutritional regimen of mice (and humans) might also impact the indispensability of FASII. The parasite's access to carbohydrate and lipids will obviously vary depending on the nutritional status of the host.[141] For instance, *Plasmodium* might require an active FASII during the erythrocyte phase if the host is lipid-depleted.

Thus, although the principle of only screening for drugs when a target is unequivocally validated is sound, one should not exclude drugs acting on multiple sites, or acting on specific stages, or eventually having no known targets but being very efficient at the cell phenotype level. One should never forget that drug development is long, risky, and to a large extent unpredictable. These unorthodox drug development programs might lead to the discovery of promising compounds, as long as they are not toxic for humans, and it would be probably unwise to throw out the baby with the bathwater.

5.4. Is the Quest for FASII Drugs Over?

The search for FASII drugs has thus motivated and inspired the design of thiolactomycin and triclosan analogues as possible antimalarial drugs, and led the scientific community to scrutinize a very complex side of metabolism, i.e., that of acyl-lipids. Other FASII drug programs have been achieved and reviewed by refs 102 and 142. Interestingly, the improvement of the cerulenin scaffold (Figure 4), initially unspecific for FASI/FASII, in order to increase its specificity on FASII was not undertaken. Concurrent FASII drug candidates also include fop herbicides, like clodinafop or haloxyfop, known to inhibit plastid ACCases, with IC_{50} values in the 100−200 μM range, when measured on the in vitro proliferation of *P. falciparum* (review: ref 142). Some of these herbicides had some promising activities on *T. gondii* ACCase,[143] but it now seems unlikely that improvements of the fop chemotypes against *Plasmodium* ACCase will be pursued. Another question that now arises is whether it is worthwhile to employ direct chemical genetic approaches[144,145] to "fish" for the off-targets of thiolactomycin and triclosan analogues that proved to be efficient in in vivo trials.

6. CONCLUSION

This review briefly covered decades of drug developments attempting to target the malaria parasite at the level of its apicoplast Achilles' heel. Reaching this organelle, which is locked inside as many as 10 membrane barriers, appears challenging at first, but empirical testing of old antibiotics or herbicides, screening for novel hits, and syntheses of chemical analogues have allowed the discovery and improvement of active compounds capable of inhibiting numerous enzymatic activities occurring in the apicoplast. The question of the specificity of these drugs, and the actual validity of the apicoplast targets, remains to be unequivocally established in many instances still, triclosan being a case in point. It serves as a reminder that useful substances were not always purposefully designed or discovered. Acetylsalicylic acid (marketed as aspirin in 1899), turned out to be active on numerous targets.[146,147] Indeed, the Protein Data Bank contains 16 structures of proteins cocrystallized with aspirin, corresponding to 5−6 nonredundant targets possibly involved in different human diseases. Single-target drugs are not the rule, although they are the aim of any development program; multitarget drugs, or drug analogues hitting off-targets should not be disregarded. Nanomolar-range drugs are not the rule, as micromolar drugs appear useful in multiple therapies. Lessons learned from recent searches for apicoplast drugs counsel more caution in validating targets before extensive development, but we should avoid dogmatic rules that would possibly prevent major discoveries. Chemoinformatic models are still insufficient to predict the physicochemical including lipophilicity criteria an apicoplast drug should comply with, to predict the full pharmacokinetic behavior or the potential side effects. Recent works have shown that some specific targets for drugs may be specific for simian and human malaria parasites and absent form the rodent species commonly used for in vivo demonstrations,[148] requiring even more caution when validating the in vivo efficacy and curative effect. It seems that the exploration of previously known antibiotics and herbicides is tapering off, and future programs will now rely on screening libraries of novel molecules, with more empirical data and unexpected results, the elucidation of which will then help better understand the sophisticated biology of one of the most fascinating human parasites. The race is not over, since a new antimicrobial drug is always a "chronicle of a death foretold" and, in the absence of efficient vaccines, the need will be always be there for the following one.

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Cyrille Y. Botté is currently a Marie Curie IOF Research Fellow for a joint project at the Plant Cell Physiology laboratory, iRTSV, Grenoble, France and the McFadden laboratory, School of Botany, University of Melbourne, Australia. He received his B.Sc. in plant molecular biology at the University of California San Diego and M.S. degree in cellular biology and physiology at Université Joseph Fourier (Grenoble, France) in 2003. He completed a Ph.D. (2007) at Université Joseph Fourier. His Ph.D. project focused on the synthesis of galactolipids as a potential target for herbicide with anti-Apicomplexa parasite properties. He then set up a collaborative postdoctoral project supported by the European Union (FP7, Marie Curie Actions, Apicolipid project) to study the role of the apicoplast in the lipid

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metabolism of Apicomplexa parasites and characterize potential drug target(s). He undertook the initial part of this project within the team of Professor McFadden in Melbourne (2008−2010) and is currently in a second part of the project within Dr. Maréchal's team. His research project thus focuses on deciphering how the apicoplast provides essential metabolites to the parasitic cell via novel cellular biology and lipidomics approaches. Cyrille Y. Botté has published seven articles in international peer-reviewed journals, one book chapter, and has coauthored five patents. He has been awarded a Marie Curie Outgoing International fellowship, an Endeavour Research fellowship, and the Trainee Award for the 18th ISEP meeting in Kanazawa (Japan, 2010). He is a fellow of the International Society of Protistologists, the International Society for Evolutionary Protistology, and the Endeavour Alumni.

Faustine Dubar received her Master's degree in organic chemistry from the University of Lille 1 in 2009. Since October 2009, she is a Ph.D. student under the supervision of Dr. Christophe Biot in the area of chemical biology. She investigates the mechanism of action of antimalarial drugs and the synthesis of new antiparasitic compounds. Her current research involves the localization of antimalarials using biophysical techniques such as synchrotron X-ray fluorescence imaging. Faustine Dubar is the recipient of a French Post Graduate Research fellowship, MENRT fellowship (financial support granted by the French Ministry of Research). She was laureate of the "L'Oréal-Unesco France" award for Women in Science in 2011.

Dr. McFadden received his Ph.D. in cell biology in 1984 from the University of Melbourne. He conducted postdoctoral research from 1984 to 1986 at the University of Münster in Germany. In 1986 he returned to the University of Melbourne, first as a Queen Elizabeth II Fellow and later as an ARC Senior Research Fellow. From 1995 to 1996, he was guest scientist at the Institute for Marine BioSciences, National Research Council in Halifax, Canada. He is the recipient of the 2009 Royal Society of Victoria Research Medal for Scientific Research in Biological Sciences. Geoff McFadden now holds the Australian Research Council's premier post of Federation Fellow and is back in the School of Botany, University of Melbourne. Geoff McFadden identified the relict chloroplast in malaria parasites and is developing herbicides as antimalarial drugs. He has published more than 180 papers, many in high-profile journals such as *Nature*, *Science*, *EMBO Journal*, and *Proceedings of the National Academy of Sciences of the United States of America*. Geoff McFadden has been awarded the Goldacre Medal, the Australian Academy of Science's Frederick White Prize, two Howard Hughes Medical Institute Scholar's awards, the David Syme Medal, the Woodward Medal for excellence in Science and Technology, the Julian Wells Medal, the Miescher−Ishida Prize, the Royal Society of Victoria Research Medal, the Ramaciotti Medal, and is a member of the Australian Academy of Sciences.

Eric Maréchal (1967) is currently head of the Membrane Lipidomics team in the Plant Cell Physiology laboratory at iRTSV, Grenoble, France. He was appointed an Agrégation Professorship in Life Science (1990) from the Ministry of National Education, received an M.S. (1991) in cell biology at Ecole Normale Supérieure de Lyon and a Ph.D. (1994) in molecular and cell biology at University of Grenoble, France. From 1994 to 1997 he joined the Nam-Hai Chua laboratory at the Rockefeller University, New York, U.S.A., as a Human Frontier postdoctoral fellow in plant biochemistry. After a short period at Rhône-Poulenc Industrialisation, Lyon, he joined the CNRS in 1998. An important part of his research activities has focused on the plant features of apicomplexan parasites, including the malaria parasite, particularly related to lipid metabolism, taking this opportunity to start target and drug discovery projects in collaboration with the Cerep and Gene-IT Companies. He coauthored more than 55 research and review articles and 15 book chapters in lipidomics, biochemistry, cell biology, plant physiology, toxoplasmosis and malaria research, biomathematics, and bioinformatics. He coauthored seven patents and was directing editor of two books on chemogenomics. He contributed to the setting up of an automated HTS platform in iRTSV to screen libraries of compounds on biological targets. His scientific interests include chemogenomics, from pharmacological HTS to

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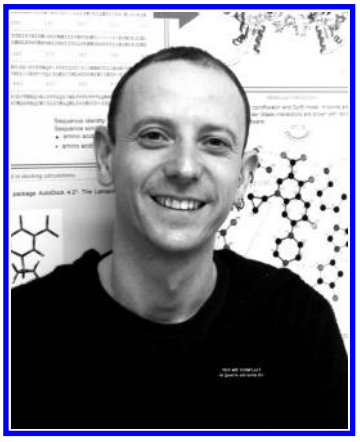
drug candidate development, comparative genomics of plant and apicomplexans, and membrane lipidomics.



Christophe Biot (1971) studied at the University of Lille 1 and obtained his Ph.D. degree in organic chemistry in 1998. He made a first postdoctoral fellow at the Institut Pasteur of Lille with Dr. E. Davioud Charvet working on the dual-drug concept applied to antimalarial therapy. He then pursued his scientific career as a postdoctoral fellow at the "Ecole Polytechnique" of the "Université Libre de Bruxelles" with Professor M. Rooman where he was trained in bioinformatics. In 2004, he was appointed Maître de Conférences at the University of Lille 1, and he received his Habilitation in 2006. In 2009, he joined the team of Dr. Y. Guerardel at the Unit of Structural and Functional Glycobiology. During his Ph.D., he discovered a new ferrocene–quinoline antimalarial drug named ferroquine (SSR97193). This project has received financial support from Pierre Fabre Medicament and later from Sanofi Aventis. Having a drug candidate selected by a major pharmaceutical company was a landmark event in his scientific career. This research provides insight into the mode of action and the resistance mechanism of antimalarial drugs and will shed light on the molecular basis of the resistance and trigger the development of new antimalarial drugs in the future. He has published more than 60 articles, coauthored two book chapters, and given many lectures throughout the country and abroad.

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