Malaria is one of the major public health challenges undermining development in the poorest countries of the world. The disease kills over one million people, and sickens between 350 - 500 million individuals in more than 90 countries. Global climate change has resulted in the expansion of areas suitable for malaria transmission, particularly at the borders of areas where malaria is endemic, and at higher altitudes of these malarious regions. The disease also poses a risk to travelers with imported cases increasing in non-endemic areas. Traditional mechanisms for drug development have provided few affordable drugs to treat diseases of the developing world. Moreover, disease control is hampered by the lack of an efficacious vaccine, and the occurrence of multi-drug-
resistant strains of *Plasmodium falciparum* to most agents in the current therapeutic armamentarium against malaria. Malaria endemic regions are now faced with an unprecedented situation in which the only affordable treatment options are rapidly losing therapeutic efficacy. There is a pressing need for the discovery of new pharmacophores and novel molecular targets in light of the drug resistance crisis. Throughout history, natural products have played a significant role in the discovery of lead compounds for the treatment of malaria. Past success portends that new chemotypes may continue to emerge from natural product sources, and this optimism fuels continued drug development in this area. Numerous natural products with antimalarial activity have been reported in the literature. This focused review aims to summarize the role of various phytochemical classes of natural products, from both terrestrial and marine sources, as potential lead molecules for drug development against malaria. Only representative compounds that possess chemotherapeutically relevant activity will be highlighted. Where information is available, the molecular target and the mechanism of action of these novel agents will also be discussed.

1. Introduction

Malaria is a mosquito-borne, life-threatening disease caused by unicellular protozoan parasites of the genus *Plasmodium*. *Plasmodium* belongs to the phylum Apicomplexa, a large group of eukaryotic microorganisms possessing a unique organelle called the apicoplast, and a complex structure termed the apical complex that is involved in host cell invasion. Malaria is transmitted only by the female *Anopheles* mosquito. Five species of *Plasmodium* are pathogenic to humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. *P. falciparum* and *P. vivax* are the most prevalent, occurring in countries where malaria is endemic. However, *P. falciparum* is the most virulent causing the most severe cases of malaria, and malaria-associated complications and deaths.

Malaria is the most prevalent disease in the world, killing 1–2 million people each year. Approximately, 3.3 billion people, i.e., half of the world’s population, are at risk of contracting malaria, with 300–500 million new clinical cases being reported annually. The biggest burden of the disease is experienced in sub-Saharan Africa, where about 90% of cases and deaths occur mostly among African children. Pregnant women are also at high risk of dying from the complications of malaria. Tropical and subtropical parts of the world such as Central and South America, South and Southeast Asia, the Middle East, the Caribbean, the South Pacific Islands and parts of Europe are also affected. While malaria is commonly associated with poverty, it is also a direct cause of poverty in addition to debilitation, ultimately contributing to economic deterioration in the poorest of countries.

2. Life Cycle of *Plasmodium*

The life cycle of the *Plasmodium* (Figure 1) incorporates both a vertebrate and an invertebrate host. An understanding of the life cycle of the malaria parasite is fundamental to the appreciation of the methods of prevention, treatment, and ongoing research endeavors geared towards the treatment and eradication of the disease. The vertebrate portion of the life cycle begins with the bite of an infected Anopheles.
mosquito and the introduction of the parasite into the bloodstream of the human host. The parasite, initially in the form of a haploid sporozoite, immediately moves to the liver and invades a liver cell (or hepatocyte) where it reproduces by mitosis. The sporozoite transforms into a schizont. Each schizont contains thousands of haploid cells called merozoites. In less than a week after the infectious bite, the schizonts rupture, killing the hepatocyte and releasing millions of merozoites into the bloodstream. This replicative stage is referred to as asexual exo-erythrocytic schizogony.

This liver stage of the Plasmodium life cycle is also associated with dormant forms of the parasite called hypnozoites. Considerable mystery still surrounds the hypnozoites, even though it has been established that they are the cause of relapses in P. vivax and P. ovale malaria in humans. A relapse is characterized by an asymptomatic latency period measured in months or years. In contrast, recrudescence is a recurrence of malaria within days or weeks of an apparent cure, without new infection, and is caused by inadequate clearing of parasites from the bloodstream. The trigger(s) for the reactivation of hypnozoites is/are yet unknown.

The merozoites that are released from the ruptured hepatocyte into the bloodstream quickly invade erythrocytes initiating the erythrocytic stage of malaria. The merozoites undergo a period of rapid mitotic amplification during erythrocytic schizogony. The early ring forms of the parasite matures into trophozoites that feed on the hemoglobin found in erythrocytes.

Each trophozoite matures into a schizont containing newly formed merozoites. The schizont, along with the erythrocyte, rupture after 2-3 days releasing the merozoites, which quickly invade uninfected erythrocytes. The classical fever paroxysms (periodic cycle of fever and chills) observed with malaria are due to the synchronous lysis of infected erythrocytes releasing merozoites and toxins into the blood.

A fraction of the erythrocytic merozoites are committed to becoming gametocytes (gamete producing cells). This paves the way for the invertebrate (mosquito) stage of the life cycle which begins when the Anopheles receives a blood meal containing gametocytes. In a matter of minutes, the gametocytes transform into male and female haploid gametes within the mosquito midgut. The male gamete fertilizes the female gamete to produce a diploid zygote. The zygote undergoes meiotic division to become an ookinete.

The motile ookinete migrates toward the mosquito gut wall and invades the semi-permeable membrane called the peritrophic matrix. Once it traverses the gut wall, the ookinete transforms into an oocyst in the intercellular space between the gut wall and the basal lamina.

The oocyst breaks and releases sporozoites into the hemocoel cavity. The sporozoites proceed to invade and establish residence in the salivary glands of the mosquito. The sporozoites are released from the salivary glands into the vertebrate host during the next blood meal, thus completing the life cycle. Chemotherapeutic strategies to combat malaria aim to disrupt this complex life cycle that embody many different intermediate forms of the parasite with drastically different biology.
3. Established Natural Product Antimalarial Drugs

Quinine (Figure 2), a 4-methanolquinoline alkaloid isolated from the bark of Cinchona species (Rubiaceae) in 1820 by Pelletier and Caventou, is one of the oldest and most important antimalarial drugs still in use today. Cinchona sp. was traditionally used as an antimalarial remedy by the Incas in Peru. Quinine was first used to treat human falciparum malaria in the 17th century. Subsequently, this alkaloid constitutes the sole active principle effective against \textit{P. falciparum} for much of the next three centuries. It is considered the prototype for the development of the synthetic 4- and 8-aminoquinoline classes of antimalarial drugs, as exemplified by chloroquine (CQ) and primaquine, respectively. Before the advent of artemisinin (ART), CQ was the only effective and affordable drug used for the treatment of malaria.

Quinine is a stage-specific blood schizonticide since it acts principally on the mature trophozoite stage of parasite development. The mechanism of action of quinine and quinoline compounds in general has not been fully resolved, but a widely held hypothesis involves the inhibition of parasite heme detoxification. During the intraerythrocytic stage, \textit{P. falciparum} proteolytically degrades hemoglobin as a source of essential amino acids. An enormous amount of free heme (ferroprotoporphyrin IX) is released during the degradation of hemoglobin. Free heme is toxic to both erythrocyte and the parasite by inducing the formation of reactive oxygen species. Heme is oxidized to \(\alpha\)-hematin (ferriprotoporphyrin IX) and subsequently polymerized to hemozoin.
(nontoxic malaria pigment) in the parasite food vacuole via a process called “biocrystallization”. Hemozoin is essentially an ordered arrangement of dimeric heme units (β-hematin). Quinine, a monoprotic base, is thought to accumulate within the acidic food vacuole by an ion trapping mechanism. The protonatable quinoline and terminal nitrogen atoms are important for both uptake and accumulation of the drug in the food vacuole. The protonated compound binds to hematin (and/or hemozoin) within the food vacuole, thus inhibiting the spontaneous process of crystal formation. Similarly, the 4-aminoquinoline chloroquine (CQ) accumulates in the vacuole and forms a stable complex with β-hematin. The CQ-hematin complex incorporates into the growing polymer to terminate chain extension, blocking further sequestration of toxic heme.

Artemisinin (ART) is also known as qinghaosu. It is an endoperoxide sesquiterpene lactone isolated in 1972 from the leaves of *Artemisia annua* L. (Asteraceae) (sweet wormwood), a plant species that has been used traditionally in China for the treatment of fever for several millennia.

The use of the parent compound ART has been superseded by the more potent semisynthetic dihydroartemisinin and its derivatives, artemether, artemotil and artesunate. At the time of this writing (June, 2010), combination therapies that include ART are the preferred treatment for malaria since these are both effective and well tolerated in patients. The endoperoxide linkage is an essential molecular feature for the potent antimalarial activity of ART and its derivatives. It is widely accepted that the endoperoxide moiety interacts with the iron (II) center of free heme released during the digestion of hemoglobin in the parasite food vacuole.

The subsequent cleavage of the peroxide bridge leads to the formation of oxygen-centered radicals which, after an intramolecular rearrangement, convert into free C-centered radicals that have the ability to alkylate macromolecular targets. The *P. falciparum*-specific Ca$^{2+}$-dependent ATPase (PfATP6) has been suggested as a potential target for the arteminins.

Lapachol, a prenylated naphthoquinone from *Tabebuia* sp. (Bignoniaceae) provided the new pharmacophore that lead ultimately to the development of atovaquone, a synthetic 2-alkyl-3-hydroxy-1,4-naphthoquinone. Atovaquone is a highly lipophilic drug and an analog of ubiquinone (coenzyme Q), the lipid soluble mobile carrier that receives electrons from Complex I (NADH dehydrogenase or NADH-Q oxidoreductase) and Complex II (succinate dehydrogenase or succinate-Q oxidoreductase) in the electron transport chain and conveys them to Complex III (cytochrome bc1 or coenzyme Q-cytochrome c oxidoreductase).

In *Plasmodium*, the site of action of atovaquone appears to be the cytochrome bc1 complex (Complex III). In essence, the drug acts by the irreversible and selective inhibition of mitochondrial electron transport and parallel processes such as ATP synthesis. In addition, obstruction of the electron transport chain ultimately inhibits de novo pyrimidine biosynthesis since dihydroorotate dehydrogenase, a key enzyme in pyrimidine biosynthesis, is unable to transfer electrons to ubiquinone.
4. Antimalarial Drug Resistance

The development of parasite resistance to frontline antimalarial drugs such as CQ, antifolates and recently ART, has underscored the importance of developing new drugs and drug targets to treat the disease. The continuous appearance of drug-resistant *P. falciparum* strains has made the chemotherapeutic management of malaria increasingly problematic in virtually all malarious regions of the world. The gravity of the situation has been compounded by the absence of a vaccine for protection, and the availability of ART and its derivatives as the only viable - if not cheap - option for the treatment of drug resistant malaria. ART is a key ingredient in combination drug therapies recommended by the World Health Organization (WHO) for the treatment of multi-drug resistant strains of falciparum malaria. Intense efforts are ongoing to develop ART-based combination therapies (ACTs) to extend the life of existing drugs, while major initiatives are underway to discover new antimalarials.

The number of stereogenic centers present in the ART molecule renders unfeasible the synthesis of a large number of analogs for drug optimization. Nevertheless, the rationale for ACTs is to provide an inexpensive, short course treatment regimen with a high cure rate and good tolerability that will reduce transmission and protect against the development of parasite resistance. ART is fast acting albeit with a short duration of action. A large number of fixed-dose ACTs are now available containing an ART derivative and a partner drug that has a long half-life and a complementary mechanism of action, such as mefloquine, lumefantrine, amodiaquine, piperaquine, atovaquone and antifolates (e.g. proguanil). Fixed-dose combinations are preferred as this guarantees that the partner drug is present to eradicate residual parasites while the ART component
removes the majority of the organisms at the start of the treatment. Effective ACT preparations in wide use include artemesunate in combination with atovaquone and proguanil (the latter two-drug combination being called Malarone®), and artemether in combination with lumefantrine (Coartem®).

5. The Promise of Natural Products

Since the recognition of malaria, a great number of plant species have been identified by various cultures as having antimalarial properties, and current antimalarial therapy consists substantially of natural products and related derivatives (quinine, CQ, mefloquine, ART, arteether, artemether and artesunate). Based on this historically high success rate among natural products, the diversity of chemicals found in nature continues to be an important source of molecular templates in the search for new and novel antimalarial drugs. Numerous natural products with antimalarial activity have been described in the literature. This chapter aims to summarize the role of natural products in the treatment and prevention of malaria to date, and to highlight specific classes of compounds that possess a requisite level of activity that would be considered worthy of further scrutiny as potential drug candidates. Barring synthetic nonfeasibility, analogs of these compounds may be generated to explore the extent and limits of structural variation permitted toward the improvement of potency and the elimination of toxicity.

In most cases compounds have only been evaluated in vitro against the asexual blood stage of the parasite. Antiplasmodial assays reported in the literature have utilized various strains/isolates of Plasmodium, which are CQ-sensitive (e.g., D6, NF54, 3D7, F32, D10, HB3, FCA 20, T996, FCH-5 and 2087), CQ-resistant (e.g., INDO, W2, FcB1, FCB, FGB1, FeM29, Dd2, ENT30, and FCR3) or multidrug resistant (e.g., K1 and TM91C235). Thus far, researchers in the field have neither adopted a standard assay nor an IC₅₀ threshold as a benchmark and universal criterion for in vitro antimalarial activity. In order to facilitate the selection of compounds for inclusion in this chapter, an IC₅₀ of 5 µM will be utilized as a loose cut-off for antimalarial activity. This threshold is based on the authors’ years of experience in the pharmacological screening of natural products as antimalarial agents. Furthermore, compounds featured in this encyclopedia chapter will preferably possess little or no reported cytotoxicity (where data is available) and a high selectivity index (SI) value for Plasmodium. A combination of these carefully chosen criteria is deemed appropriate to discriminate compounds that constitute promising candidates for further drug development from entities with moderate- to low- probability of ever garnering enough interest for further pre-clinical development as single chemical entities. In addition, the structures and chemical classes of these potent antimalarial compounds and their sources, whether terrestrial or marine, will be presented.

Fifty percent (median) inhibitory concentration (IC₅₀) and selectivity index (SI) values (where available) will be used to assess the antimalarial activity of compounds published in the literature against the thresholds defined in this review. CQ or ART have often been evaluated in assays as the standard or control antimalarial agent for quality control purposes. In general, in vitro antiplasmodial IC₅₀ values of CQ against various CQ-sensitive and CQ-resistant strains of P. falciparum are reported to be in the

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range of 10 - 50 nM and 50 – 500 nM, respectively. Similarly, ART is generally associated with IC50 values of 5 – 15 nM against both CQ-sensitive and CQ-resistant strains of *P. falciparum*.

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Biographical Sketches

Fred Musoke Sebisubi, was born in Kampala, Uganda. He received his B.Sc. degree in chemistry from Makerere University, a Masters Degree in Pharmacy from Pyatigorsk Pharmaceutical Institute, Russia, and a Ph.D. in Medicinal/Pharmaceutical Chemistry from Makerere University, Kampala. As part of his Ph.D. program, and through a collaborative arrangement with the University of Illinois at Chicago (UIC), he joined the UIC-based International Collaborative Biodiversity Groups (ICBG) consortium as a key investigator in the malaria drug discovery project. Subsequent to his 2-year training in the United States, he resumed active appointment at the Department of Pharmacy at Makerere University, where he has been instrumental in the teaching of young pharmacists since 1991. He has mentored over 400 pharmacists in Uganda. He later joined the Ministry of Health, Division of Pharmaceutical Services, Uganda, in 2004 where he rose to the rank of Principal Pharmacist. He has worked collaboratively with the malaria control program and other disease control programs to ensure the constant availability of safe and efficacious medicines in the country. He has participated in many national and international meetings advocating the availability of essential medicines, especially for malaria, tuberculosis, HIV and cancer, in least developed countries. Currently, he serves as Research Associate at the University of Hawaii at Hilo, where he continues his quest for lead molecules active against the malaria parasite. Dr. Sebisubi has immense interest in natural products research with a bias towards anti-malarial compounds, and has collaboratively published in international journals.

Ghee Tan received her B.Sc. (Hons.) in Pharmacy from the National University of Singapore, and her Ph.D. in molecular pharmacology at the University of Illinois at Chicago (UIC), where her studies focused on the biochemistry and natural product inhibitors of the human immunodeficiency virus (HIV) reverse transcriptase. After postdoctoral training in cell and molecular biology, and a 2 year appointment as research scientist in the HIV vaccine development arena at the Public Health Research Institute of New York/New York University, she returned to UIC as a research faculty. The next decade was devoted to natural products drug discovery research in the context of HIV/AIDS, malaria, tuberculosis and cancer. These largely National Institutes of Health (NIH)-funded endeavors resulted in the publication of numerous research articles on biologically active natural products. She served as Program Leader for the drug discovery arm of the 10-year NIH-funded UIC-based International Cooperative Drug Discovery
Groups (ICBG) consortium that explored the biodiversity of Vietnam and Laos as sources of lead molecules for potential development as chemotherapeutic agents against malaria, tuberculosis, AIDS and cancer. She has authored review articles including book chapters on natural products relevant to HIV, malaria and cancer. Over the years, she has also mentored students both at the graduate and undergraduate levels. She is currently Assistant Professor, and an inaugural Faculty member of the Department of Pharmaceutical Sciences, College of Pharmacy, University of Hawaii at Hilo, where she seeks to establish a drug discovery program that addresses the major diseases that impact global health.