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Plant-derived antimalarial agents: new leads and efficient phythomedicines. Part I. Alkaloids

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ABSTRACT

Malaria remains one of the most serious world health problem and the major cause of mortality and morbidity in t endemic regions. Brazil is among the 30 high-burden countries and most of the cases occur in the Legal Amazoni Region. New chemotherapeutical agents are needed for the treatment of malaria. Many plant species are used traditional medicines of malarious countries and a relatively few number of these have been investigated for evaluati of their antimalarial effect. Still lower is the number of those that have had the active natural compounds isolat and the toxicity determined. This area is, then, of great research interest. A discovery project of antimalarial natu products from plants traditionally used to treat malaria must include *in vitro* and *in vivo* assays as well as bioguid isolation of active compounds. The final products would be antimalarial chemical entities, potential new drugs templates for new drugs development, and/or standardized antimalarial extracts which are required for pre-clinical a clinical studies when the aim is the development of effective and safe phythomedicines. This review discusses the two approaches, presents briefly the screening methodologies for evaluation of antimalarial activity and focuses t activity of alkaloids belonging to different structural classes as well as its importance as new antimalarial drugs or lea and chemical markers for phytomedicines.

Key words: natural products, alkaloids, antimalarial activity, medicinal plants, phytomedicines.

INTRODUCTION

Malaria remains one of the most prevalent infectious disease in the world. In 2006, there were approximately 247 million cases of malaria and 3.3 billion people that were at risk of the disease. Nearly 1 million deaths, mostly of children under the age of 5, were caused by malaria. There are currently 109 malarious countries and territories, of which 45 are within the World Health

Although malaria is a curable and preventa ease, its prevalence increased in the 1980s and as the parasites developed resistance to the m quently used antimalarial drugs and the vectors b resistant to insecticides. During the 1990s, child caused by malaria increased by up to two-fold i parts of sub-Saharan Africa. The disease also re ged in several countries in Central Asia, Easte rope and South-East Asia. The majority of th

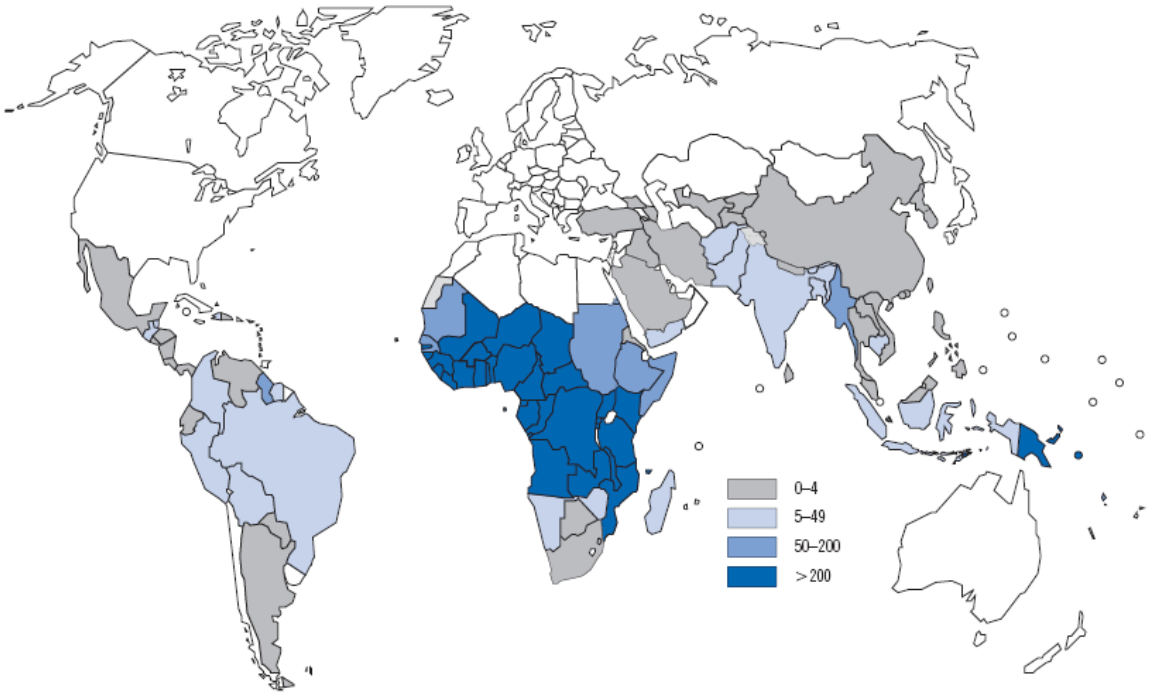


Fig. 1 – Estimated incidence of malaria *per* 1000 population, 2006 (WHO 2008).

In the Americas, Brazil reported the highest number of malaria cases (549,184 in 2007), and 1.4 million cases have been estimated. This estimate represents over half of the total number of cases for the WHO region of the Americas. More than 350,000 cases were reported annually over the period from 2001-2007, with a maximum of 603,532 cases occurring in 2005. Transmission occurred mainly in the Brazilian Legal Amazon Region, where 10–15% of the population is at risk. Almost all of the reported malaria cases in this region are confirmed. In 2007, 19% of these were caused by *P. falciparum*. Brazil is among the thirty high-burden countries (WHO 2008). Intense migration to agricultural and mining areas in the Legal Amazon Region, in conjunction with inappropriate living conditions and inadequate health care, limits the effectiveness of interventions that are designed to control the disease. Insufficient human and other resources and technical and managerial weaknesses at the local level are also to blame (Chaves and

in 1998 by the WHO in a partnership with the United Nations Children's Fund (UNICEF), the United Nations Development Programme (UNDP) and the World Bank. The aim of this partnership was to bring together the major stakeholders in a fight against malaria. Participants included the governments of malaria-endemic countries, international organizations, private foundations, non-governmental organizations, and research and academic institutions. The principal goal was to reduce the rate of malaria-related mortality by 50% by 2010. In 2005, the World Health Assembly resolved to "ensure a reduction in the burden of malaria of at least 50%, by 2010, and by 75%, by 2015". This resolution has been interpreted as meaning a reduction in malaria-related morbidity as well as mortality. The reference year for measuring changes in morbidity and mortality was taken as 2000 (WHO 2008).

tions can include headache, periodically recurrent fever (every 48 to 72 h), chills, myalgia, sudoresis, hepato- and splenomegalia, prostration, and the presence of high anaemia in cases of severe malaria in children and pregnant women. Severe *P. falciparum* human malaria can include neurological symptoms, such as delirium and convulsions, metabolic acidosis, multi-organ system failure and, if not properly treated, it can lead to coma and death (Fidock et al. 2004, Brasil 2006).

The aetiological agents of malaria are protozoans that belong to the genus *Plasmodium*, phyllum Apicomplexa, and family Plasmodidae. Four species of malaria parasites are pathogenic to humans: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. *P. ovale* seems to be limited to sub-Saharan Africa and some islands of the western Pacific, whereas *P. falciparum* and *P. vivax* are prevalent in endemic malarial countries, such as Brazil. *P. falciparum* is the agent of severe and potentially fatal human malaria (Krettli et al. 2001, Brasil 2006, 2007).

The vectors of the malarial parasites are mosquitoes of the genus Anopheles (family Culicidae). There are approximately 430 known species of Anopheles, but only 30–50 of them transmit malaria. Transmission to humans occurs via a bite of the infected female mosquito. In humans, the parasites grow and multiply, first in the liver cells, and then in red blood cells. In the liver cells, there is an initial round of replication (exo-erythrocytic schizogony), after which they undergo asexual multiplication in erythrocytes (erythrocytic schizogony). This process results in the destruction of erythrocytes and the release of daughter parasites (merozoites). The blood stage parasites are responsible for the clinical manifestations of the disease, and are the source of infection to mosquitoes (CDC 2008). Non-treated or inadequately treated individuals can be sources of infection to mosquitoes for a period of 2–3 years; the mosquitoes themselves remain infectious until death (Brasil 2006).

DRUGS CURRENTLY USED FOR THE TREATMENT OF MALARIA

The treatment of human malaria aims to interrupt the

is probably the most cost-effective method of
control. Oral treatment prevents progression t
vere state of the disease and the resultant complic
If the drugs are administered effectively, a decr
overall malaria-related morbidity and mortality
achieved. However, most people living in endemi
have little or no access to diagnosis and treatmer
thermore, the treatment is commonly inadequate
the lack of availability of quality-assured, effectiv
(Guérin et al. 2002, Fidock et al. 2004).

Most of the antimalarial drugs that are curre
use belong to the classes of aminoquinolines (
quine, amodiaquine, primaquine), quimolinom
derivatives (quinine, mefloquine, halofantrine),
nopyrimidines (pyrimethamine), sulfonamides
doxine, sulfadiazine), biguanides (proguanil and
atives), antibiotics (tetracyclines, doxycyclin,
mycin), sesquiterpenes (artemisinin, dihydroarten
arteether, artemether, artesunate) and naphtoqu
(atovaquone) (Chart 1) (Guérin et al. 2002).

The available drugs exert their effects as
schizonticides (artemisinin and derivatives, quin
folate inhibitors, atovaquone). Few drugs that
to the classes of 8-aminoquinolines and folate
tors are as effective as liver schizonticides (asexu
schizogony) (Guérin et al. 2002). Primaquine
aminoquinoline, is the only commercially availab
that destroys hypnozoites (a latent form of paras
is observed in infections by *P. vivax*; it remains
liver for variable periods of time, and causes r
of the disease). This drug is also active against g
cytes, a human parasitic form that initiates the n
cycle upon its ingestion by mosquitoes, (Brasil 2

The most widely used antimalarial drugs ar
roquine (CQ) and the combination of sulfadoxin
doxamine (SP). In most regions of endemic mala
effectiveness of these drugs is declining at an eve
erating rate, with consequent increases in malaria-
morbidity and mortality (Guérin et al. 2002, Fi
al. 2004, WHO 2005).

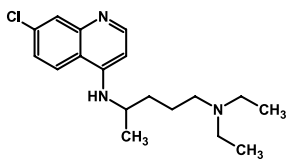
For several decades, the gold standard for tre
of malaria was CQ; this is a 4-aminoquinoline

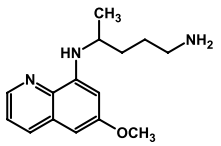
1. Blood schizonticides, acting on intraerythrocytic (asexual and partly also in sexual parasites)
1.1 Quinoline based drugs
1.2 Sesquiterpenes: artemisinin and derivatives
1.3 Nucleic acid inhibitors: antifolates (sulphonamides, sulphones, pirymethamine, biguanides, triazine metabolites, quinazolines)
1.4 Atovaquone (1,4-naphthoquinone)

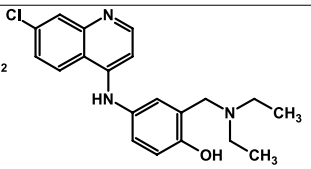
2. Tissue schizonticides, acting on liver stages
2.1 Primaquine (quinoline)

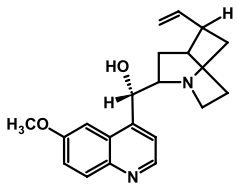
Chemical structures

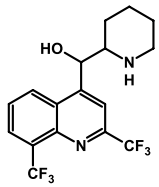
1.1 Antimalarial quinolines


Chloroquine

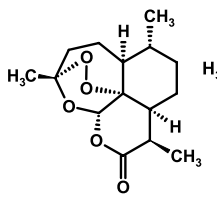

Primaquine

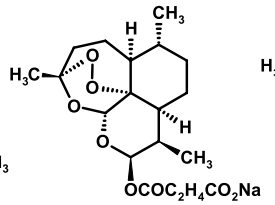

Amodiaquine

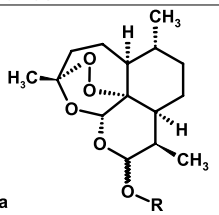

Quinine


Mefloquine

1.2 Sesquiterpenes: artemisinin and derivatives


Artemisinine


Sodium artesunate


Artemether R = CH_3
Arteether R = CH_2CH_3

1,4–Naphthoquinone

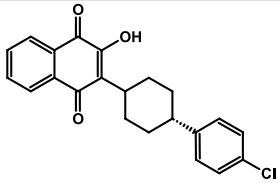

Atovaquone

Chart 1 – Classes of antimalarial drugs in clinical use (Guérin et al. 2002).

resistant strains of *P. falciparum*. Nowadays, CQ resistance has spread to the majority of malaria endemic areas, and this drug has become increasingly ineffective (Fidock et al. 2004).

artemisinin and its derivatives with existing antimalarial drugs. Several artemisinin-based pharmaceutical variants, including artesunate-amodiaquine, artemether-lumefantrine (Coartem®), artesunate-mefloquine, and

malaria (the most lethal form of the disease). The drug has passed extensive efficacy and safety trials and is recommended as a first- or second-line treatment for uncomplicated falciparum malaria. Atovaquone-proguanil (Malarone®) is one of the non-artemisinin combination therapies (WHO 2005, 2008).

Questions regarding the affordability and accessibility of these high cost artemisinin and atovaquone combinations for communities in poor countries (Koech 2006) are being addressed by the Roll Back Malaria (RBM) Partnership. RBM is working to make the drugs available for use in the public sector of these countries. By the end of 2006, ACT was the first-line treatment for *P. falciparum* infections in a total of 66 countries, and in almost all countries in the African, South-East Asia and Western Pacific regions. By June 2008, only four countries and territories worldwide had not yet adopted ACT as the first-line treatment for *P. falciparum* malaria. Free treatment with ACT is more widely available in the South-East Asia and Western Pacific regions than in the African Region (WHO 2008). In Brazil, the combination artemether-lumefantrine (Coartem®) has been used for malaria falciparum since October 2006. Fortunately, the prevalent infections in Brazil are caused by *P. vivax* and *P. malariae* (Brasil 2006). The lower number of infections by *P. falciparum* is certainly an important factor that explains the affordability of this treatment in Brazil.

New antimalarial drugs are urgently needed. Not only should these drugs be efficacious against resistant *P. falciparum* strains, but, to ensure good compliance, they should provide a cure within a reasonable length of time (3 days or less), they should be safe and of low cost, and they should be available in an appropriate formulation for oral use (Wright 2005a, Fidock et al. 2004).

PLANT-DERIVED ANTIMALARIAL AGENTS

The first antimalarial drug was quinine (1). In 1820, it was isolated by the French scientists Pelletier and Caventou from the bark of the *Cinchona spp.* (Rubiaceae) tree that was used by Peruvian Indians. Plant materials were taken to Europe by Jesuits in the XVII century. The struc-

means is both complex and costly. Quinine is st
today, and it is currently derived by extraction fr
Cinchona spp., which grow wild in South Amer
are cultivated in Java (Boulos et al. 1997).

Quinine was used as a template for the synth
CQ in 1940. Despite the prevalence of CQ-resi
falciparum strains, it was, until recently, the on
available for malaria chemotherapy. Over the
years, the situation worsened and the increasing
lence of resistant strains of *P. falciparum* was the
factor responsible for the increase in mortality t
curred mainly in Africa (WHO 2005).

Despite intensive efforts to produce new sy
antimalarial drugs, the most significant recent co
tions came from research on medicinal plants th
tained artemisinin. In 1972, this compound w
lated from *Artemisia annua*; this plant species h
many traditional uses in China for several mil
Artemisinin represents a new structure of antim
pharmacophore that comprises an endoperoxide
terpene lactone. Semi-synthetic derivatives, inc
arteether, artemether and sodium artesunate, are u
creasingly more often (Wright 2005b). Althoug
are effective against CQ-resistant *P. falciparum*
gle therapeutic agents, to minimize the risks of r
scence and the development of resistance, a co
tion treatment with a second antimalarial drug is
mended (WHO 2005).

The newest antimalarial drug is atovaquone
rone®). This synthetic 2-alkyl-3-hydroxynaph
none compound is an analogue of lapachol (a pr
phthoquinone from the *Tabebuia* species (Big
ceae). The discovery of this drug provided a nov
for antimalarials that resulted in the developm
atovaquone. When used in combination with pr
(Malarone®) (Looareesuwan et al. 1999), this com
is effective for the treatment of malaria; howev
high cost of this drug precludes its wide-scale
many malaria endemic countries (Fidock et al. 2

APPROACHES TO ANTIMALARIAL DRUG DISCO

There are several different approaches to antim

of the drug itself. Several important ongoing efforts include the following:

- optimization of therapy with available drugs, including the use of combined therapy;
- development of analogues of existing agents;
- discovery of natural antimalarial products;
- investigation of compounds that were originally developed to treat other diseases;
- evaluation of drug resistance reversers; and
- chemotherapeutic exploitation of novel molecular targets (Rosenthal 2001, 2003).

As the last category benefits from recent advances in both malaria research and genomics, it is most likely to result in the identification of new classes of drugs. Transgenic malaria parasites that express green fluorescent proteins have been produced. This advancement has led to the development of new high-throughput assays (HTS). As a number of new antimalarial therapies will likely be needed in the coming years, it is important to pursue multiple strategies for drug discovery (Rosenthal 2001, 2003).

Antimalarial drug development is constrained by the same factors as any drug development program; new agents must demonstrate efficacy, be safe and have additional properties that are required for the specific disease indication. In the case of malaria, the most pressing need is for a drug that can be used for widespread treatment of the disease in developing countries. Considering resource limitations in this setting, it is generally agreed that new antimalarials should be administered orally, that they should be effective with single-daily dosing, and that curative regimens should be short (ideally 1–3 days in length). Therefore, the prime consideration in antimalarial drug development is economic in nature. First, antimalarial drugs that are to be widely used in endemic areas must be very inexpensive. Indeed, considering the severe poverty that exists in most of the malarious countries and the inexpensive cost of the currently available drugs (especially chloroquine, which costs less than U$0.1 *per* treatment), even U$1 investment in antimalarial drug discovery and development has been insubstantial and highly dependent on support from outside of the large pharmaceutical companies. Such support includes grants that are issued to academic and industry groups by research agencies and new public-private partnerships; however, this imbalance remains large (Rosenthal 2003).

ANTIMALARIAL DRUG DISCOVERY: *IN VITRO* ASSAYS

Most of the antimalarial drugs currently in use were not developed on the basis of rationally selected targets, but by investigation of traditional medicinal plants (quinine and artemisinin), synthesis of analogues (CQ, mefloquine, primaquine, atovaquone), chemical modification of an active natural product (arteether, artemether, artesunate), or by assaying drugs that were used against other infectious pathogens (antifolates, antibiotics) (Fidock et al. 2004).

In vitro screens for compound activity require the ability to culture *P. falciparum in vitro* in human erythrocytes (Trager and Jensen 1976). Typically, parasites are propagated in leukocyte-free erythrocytes with 2–5% haematocrit at 37°C under a reduced oxygen atmosphere (typically 3–5% O_2, 5% CO_2, 90–92% N_2), in tissue culture (RPMI 1640) media that contains either human serum or Albumax (a lipid-rich bovine serum albumin) (Fidock et al. 2004).

A traditional and low-cost assay for testing small numbers of samples relies on the microscopic scoring of parasitized and uninfected erythrocytes. Briefly, parasites are incubated with test samples for 48 and 72 hours, and then the parasitaemias of treated and control groups are determined by microscopically counting Giemsa-stained smears (Trager and Jensen 1976). Although this methodology is extremely labour-intensive and time consuming, it is accurate and has the advantage of permitting observation of the effect of test samples on different intraerythrocytic stages of the parasite (Fidock et al. 2004, Krettli et al. 2009).

The standardized radioisotopic protocol consists of the measurement of the uptake of [^{3}H]-hypoxanthine

are cultured in the presence of different concentrations of test compounds in media that contains reduced concentrations of hypoxanthine. Afterwards, [^{3}H]-hypoxanthine is added for an additional period of incubation before cell harvesting and measurement of the radioactive counts. IC_{50} values can be determined by linear regression analyses of the linear segments of the dose-response curves (Desjardins et al. 1979). [^{3}H]-hypoxanthine incorporation is the *in vitro* methodology that is most commonly used to assay antimalarial activity. However, as a result of utilization of a radioactive compound, it is a somewhat costly and complex technique, which limits its utility for resource-poor institutions and high-throughput screening (Fidock et al. 2004, Krettli et al. 2009).

Recently, a protocol for chemotherapy studies was established that uses a *P. falciparum* strain transformed with the green fluorescent protein (PfGFP) that can be quickly and specifically quantified by flow cytometry. In comparison to other methodologies, the PfGFP assay showed similar results to those obtained with the standard radioisotopic method. *In vivo* assays that employed the rodent-parasite *P. berghei* transformed with GFP to screen for the blood schizonticidal effect have been previously reported by the same group of Brazilian researchers (Sanchez et al. 2007).

Novel DNA-based fluorimetric methods that used PicoGreen® (Invitrogen – Molecular Probes™) to assess the susceptibility of parasites to antiplasmodial compounds were recently reported. PicoGreen® is a fluorochrome that selectively intercalates into double-stranded DNA (dsDNA), which results in a marked increase in fluorescence emission. A positive correlation was observed between the amount of PicoGreen® fluorescence and the percentile of parasitaemia between 0.1% and 15%. This method yielded IC_{50} values for CQ and pyrimethamin that were statistically similar to those obtained by use of the [^{3}H]-hypoxanthine assays of *P. falciparum* lines 3d7(CQS) and K1(CQR). Moreover, as this method is not time-consuming, it may soon replace the traditional *in vitro* drug sensitivity assays (Quashie et al. 2006).

a cytosolic pathway used for energy production i
modia. *P. falciparum* LDH (pLDH) has been st
ally characterized and found to differ from huma
at both the structural and immunological levels.
modia species are dependent on LDH for the m
ism of carbohydrates. pLDH is used for the con
of lactate into pyruvate, which is the last step in
ysis; however, only pLDH can use coenzyme 3-
pyridine adenine dinucleotide (APAD). In the pr
of APAD, the detection of LDH is specific for t
asite enzyme. Its determination is carried out
presence of nitro blue tetrazolium (NBT) whicl
duced to a formazan derivative that is detected at
(Wright 2005b, Fidock et al. 2004, Deharo et al.

More recently, with advances in our knowl
the biochemistry of malarial parasites, the mech
of action of older drugs have been elucidated, and
tial targets for new drugs have been identified (
et al. 2004). Targets that are shared between th
site and the human host offer opportunities for
therapy when structural differences can be exp
About 20 potential molecular targets with kno
hibitors have been identified for *P. falciparum*. A
parasite molecular targets are validated by genomi
grammes, the use of HTS technology and othe
ern approaches is expected to increase (Pink et al
Itokawa et al. 2008, Queiroz et al. 2009).

For example, the antimalarial activity of
hydrofolate inhibitors pyrimethamine and progu
in part due to their relative selectivity for the p
enzyme. Other potential targets in this group a
teine proteases and farnesyl transferases. Altern
targets can be selected from enzymes or biosy
pathways that are present in the malaria parasi
absent in humans. Potential selective targets fo
malarial drug discovery that have recently been
fied are components of type II fatty acid biosy
and mevalonate-independent isoprenoid synthesi
ways; this last one is now identified as the 2C-m
D-erythritol-4-phosphate (MEP) pathway. Both
targets are present on the apicoplast – an intrac
compartment in the intra-erythrocytic *P. falc*

been developed (Rathore et al. 2006). Malaria parasites contain acidic food vacuoles in which erythrocyte haemoglobin is hydrolyzed. These vacuoles appear to be the site of action of a number of existing antimalarial drugs. In the oxygen-rich lysozome-like food vacuoles, several parasite proteases (for example, plamepsins, falcipain 2, metallopeptidases) participate in the hydrolysis of haemoglobin, leading to the production of Fe(ii) haem, which is rapidly oxidized to Fe(iii) haematin, before being sequestered as an inert pigment called haemozoin (also known as β-haematin or malaria pigment). Free haem is extremely toxic to the parasite as it affects cellular metabolism by inhibiting enzymes, peroxidizing membranes and producing free radicals. Therefore, detoxification of haem is absolutely necessary for uninterrupted growth and proliferation of the parasite. The detoxification of haem occurs rapidly, via polymerization into the insoluble nontoxic crystalline hemozoin that can be synthesized in the laboratory. Although haem detoxification is a unique drug target, this process is not yet fully understood (Rhatore et al. 2006, Sullivan 2002, Deharo et al. 2002).

Several antimalarials have been shown to exert their effect by interacting with haem (Rhatore et al. 2006). Among the proposed theories for the inhibition of haem detoxification, numerous studies have shown that quinolines, such as chloroquine, quinine and amodiaquine, bind noncovalently to iron protoporphyrin IX (FPIX) and prevent its conversion to non-toxic hemozoin (Rathore et al. 2006, Leed et al. 2002, Warhurst 1981).

Spectroscopic studies and conformational analyses that aimed to investigate the correlation between structural aspects of the quinolines and antimalarial activity have been described (*apud* Silva et al. 2005). Based on quantitative molecular modelling studies, a pharmacophore that supports binding of the quinuclidine sp3 nitrogen to the iron atom and π-π interactions of the aromatic quinoline moiety with the FP IX porphyrin ring was proposed (Silva et al. 2005, 2001, 1997).

Most of these assay methodologies can be applied to pure natural or synthetic compounds, as well as to haem-targeted assays are being explored (Wright et al. 2001, Steele et al. 2002, Ajaiyeoba et al. 2005).

Compounds that exhibit good *in vitro* activity (for example, $IC_{50} \leq 1\mu$M for pure compounds) can be tested against a range of geographically distinct *P. falciparum* lines with different drug-resistance profiles. This approach will allow for determination of whether or not resistance to the existing antimalarial drugs reduces the sensitivity of the parasite to the compounds under evaluation. Crude extracts that display an $IC_{50} \leq 50\mu$g/ml against *P. falciparum* can be submitted to *in vitro* bioguided isolation-purification processes to pursue the development of pure active natural products. Promising compounds, crude extracts and semi-purified extracts that display promising *in vitro* activity can be submitted to *in vivo* assays for testing of their antimalarial effects.

ANTIMALARIAL DRUG DISCOVERY: *IN VIVO ASSAYS*

In vivo evaluations of antimalarial activity begin with the use of the rodent malaria parasites *P. berghei*, *P. yoelli*, *P. chabaudi* and *P. vinckei*. The most widely used model for initial drug evaluation is the *P. berghei*-infected mouse model. These evaluations involve a four-day suppressive test, in which the efficacy of four daily doses is measured by comparisons of blood parasitaemia (on day four after infection) and survival times of treated and untreated mice. Test samples can be administered by intraperitoneal, intravenous, subcutaneous or oral routes. CQ is often used as a positive control. Active compounds identified in the four-day *in vivo* assays can subsequently be further examined through the use of several secondary tests in mice. These tests include assays that define the optimal dose, type of affected parasitic activity and potential to induce resistance (Fidock et al. 2004).

Primate models have also had an important role in preclinical development by providing a model for the final evaluation of a drug candidate prior to human studies. As primates (particularly *Aotus infulatus*) are susceptible to *P. falciparum* infection, they can be used

have been well characterized in both *Aotus* and *Saimiri* species, which has provided a clearer prediction of human efficacy and pharmacokinetics than rodent models. Therefore, these models present a logical transition to clinical studies (Fidock et al. 2004).

Considering the requisites for an antimalarial drug candidate (particularly with respect to low cost), a natural product can only be used if it is abundant, easily isolated and is produced in widely growing plant species or in a plant that can be cultivated. Alternatively, a less ubiquitous natural product may be produced by cultured plant tissues or may serve as a template for the synthesis of related compounds. Moreover, if an antiplasmodial natural product is derived from traditionally used plants, attention must be drawn to the rights of local peoples as set out by the Convention on Biological Diversity (CBD) that was held in Rio de Janeiro, Brazil, in 1992 and has been ratified by 170 nations.

As manufactured drugs are generally costly, exploitation of the well recognized potential of natural products is not a guarantee of low prices. This limitation is exemplified by artemisinin and its derivatives. Without the support of governmental programs, these drugs would be economically unviable and/or unaffordable to many people who live in malarious endemic areas. However, it is important to draw attention to the fact that plant-derived antimalarials have made and continue to make a great contribution to malaria chemotherapy. Not only has this approach led to the production of newer drugs, but it has also led to the discovery of new lead molecules (Kirby 1996, Wright 2005a, b).

Most of the currently used antimalarial drugs have been developed from knowledge and investigation of medicinal plants. This relationship is especially applicable to those with a reputation for use of traditional (popular, indigenous, folkloric) medicines. Extracts of a large number of plant species, including many that are used in traditional medicines, have been evaluated for *in vitro* antiplasmodial activity and some have been tested *in vivo* (usually in mice infected with *P. berghei* or *P. yoelli*; for reviews, see Tagboto and Townson 2001, Schwikkard and van Heerden 2002, Carvalho et

but relatively few have been further studied to
their potential as lead compounds for the develo
of new antimalarial drugs (Wright 2005a).

In the following section, potent, natural an
modial products (with a particular emphasis o
loids) will be discussed. Other classes of activ
constituents will be the subject of a forthcoming
cation. Finally, remarks on the use of traditiona
cines and/or phytomedicines for the treatment o
aria will be made.

ALKALOIDS: NEW LEADS FOR ANTIMALARIAL D

Alkaloids are one of the major classes of natura
ucts that exhibit antimalarial activity. Indeed, q
the first antimalarial drug, belongs to this class
100 alkaloids from higher plants were reported
monstrate significant antimalarial activity in studi
lished from 1990 to 2000; some of these were m
tent than chloroquine (Saxena et al. 2003). Herein
of the active reported alkaloids are grouped acc
to their structural classes.

Bisbenzylisoquinolines are a large and
group of alkaloids that occur in many plant speci
ticularly in members of the Menispermaceae, B
daceae, Ranunculaceae, Annonaceae and Monim
Many of the plants that contain these compound
reputations as medicinals in the folklore of vario
tures. In an effort to discover new antimalarial
from natural sources, Angerhofer and co-workers
53 bisbenzylisoquinoline alkaloids that were i
via phytochemical studies and bioassay-directe
tionation. The cytotoxicity of the isolates was a
against KB cells (human oral epidermoid carci
and their selectivity for inhibiting the growth o
erythrocytic malaria parasites was evaluated.
vitro Selectivity Index (SI) for each compou
been defined as the ratio ED_{50} (KB cells) / IC_{50}
ciparum) (Angerhofer et al. 1999).

Alkaloids from *Cyclea barbata*, *C. atjehens*
phania pierrei, *S. erecta*, *Pachygone dasycarpa*
rea candicans, *Albertisia papuana* (Menisperm
Hernandia peltata (Hernandiaceae), and *Berbe*

man KB cells. More than half of the tested compounds showed selective antiplasmodial activity, with an SI of $>$ 100-fold greater toxicity towards one or both of the *P. falciparum* clones (D6 and W2), relative to KB cells. Three alkaloids that contained only one diaryl ether bridge were considered as promising compounds. These alkaloids combined low KB cell cytotoxicity with high antiplasmodial activity ($IC_{50} < 200$ nM) and SI > 100: the biscoclaurine analogues (+)-neothalibrine (D6 IC_{50} 47 nM; SI 215; W2 IC_{50} 135 nM; SI 75) and (+)-temuconine (D6 IC_{50} 213 nM, SI > 150; W2 IC_{50} 227, SI > 140) and the bisreticuline derivative, (+)-malekulatine (D6 IC_{50} 61 nM, SI > 490; W2 IC_{50} 164, SI > 180) (Chart 2). These results show that the antiplasmodial and cytotoxic effects of bisbenzylisoquinoline alkaloids are influenced by the configuration at chiral centres and by substituents on the aromatic rings. However, it was observed that a decrease in lipophilicity, as in quaternarized or N-oxide derivatives, resulted in the loss of both toxicity and antiplasmodial activity. This loss was probably a consequence of altered membrane permeability (Angerhofer et al. 1999).

Newly identified phenolic **aporphine-benzylisoquinoline** alkaloids isolated from the roots of *Thalictrum faberi* Ulbr.(Ramunculaceae) were shown to be more active against CQR *P. falciparum* clones (W2; $IC_{50} < 25$ ng/ml) than against CQS clones (D6; $IC_{50} > 100$ ng/ml). Selectivity indexes ranging from 9.4 to 65.7 were observed for 3-hydroxy-6'-desmethylthalifaboramine (A), 3-hydroxythalifaboramine (B) and 6'-desmethylthalyfaboramine (C) (Chart 2), whereas these indexes were > 540 and $> 1,800$ for quinine against the *P. falciparum* clones W2 and D6, respectively (Lin et al. 1999).

A **morphinan** alkaloid, which was biogenetically derived from benzylisoquinolines via aporphines, was recently isolated from *Strychnopsis thouarssi* (a Menispermaceae plant species that is endemic to Madagascar) and it was named tazopsine (tazo = malaria) (Carraz et al. 2006) (Chart 2). This plant is the only ingredient in a widely used remedy that is reputed to provide specific protection against malaria. Stem bark decoction has shown weak activity against the FcB1 strain of liver stages of *P. yoelli* and *P. falciparum*. Bioassays of plant decoction in cultured mouse primary hepatocytes infected with *P. yoelli* sporozoites produced an IC_{50} of $8.5 \pm 0.7\mu$g/ml with hepatic forms that were completely eliminated at concentrations of 20μg/ml or higher. Bioguided isolation of active compounds led to the identification of tazopsine, which is the major constituent of the plant material (0.56% w/w). However, it has been shown to be cytotoxic in mice and in cultured human cells, which has motivated the synthesis of a series of derivatives that are active against cultured *P. yoelli* ($IC_{50} < 50\mu$M) and of which NCP-tazopsine (N-cyclopentyltazopsine) (Chart 2), with the lowest IC_{50} value ($3.3 \pm 0.05\mu$M), is the most promising. Dose-dependent inhibition of *P. falciparum* hepatic stages was also obtained with NCP-tazopsine, whereas no detectable effect on the multiplication of *in vitro* cultured erythrocytic stages (3D7 and FCR3 lines) was observed. The IC_{50} values and the therapeutic indices for NCP-tazopsine do not differ substantially from those of licensed prImaquine. This is a novel class of antimalarial drugs with outstanding inhibitory activity against *Plasmodium* hepatic stages (Carraz et al. 2006).

Naphtylisoquinolines, comprising $>$ 70 natural alkaloids and $>$ 150 derivatives, are a new class of structurally unique acetate biogenetically-derived alkaloids that have been isolated from tropical lianas belonging to the families Dioncophyllaceae and Ancistrodaceae (Bringmann and Feineis 2001). Plant species of these families are widely used in the traditional medicine of West African countries, and Southern and Southeast Asia to treat malaria and other diseases, such as dysentery, leprosy, fever, and measles. Good correlations between *in vitro* (CQR *P. falciparum* NF54 strain) and *in vivo* (*P. berghei*, Anka strain) antimalarial activities were observed for representatives of this group of alkaloids. Dioncophylline C, dioncophylline B and dioncopeltine A caused complete clearance of parasites after oral administration to *P. berghei*-infected mice, without noticeable toxic effects (Bringmann et al. 2003, François et al. 1997). Korupensamine A, from Ancistrocladus korupensis, was highly active against CQR and CQS

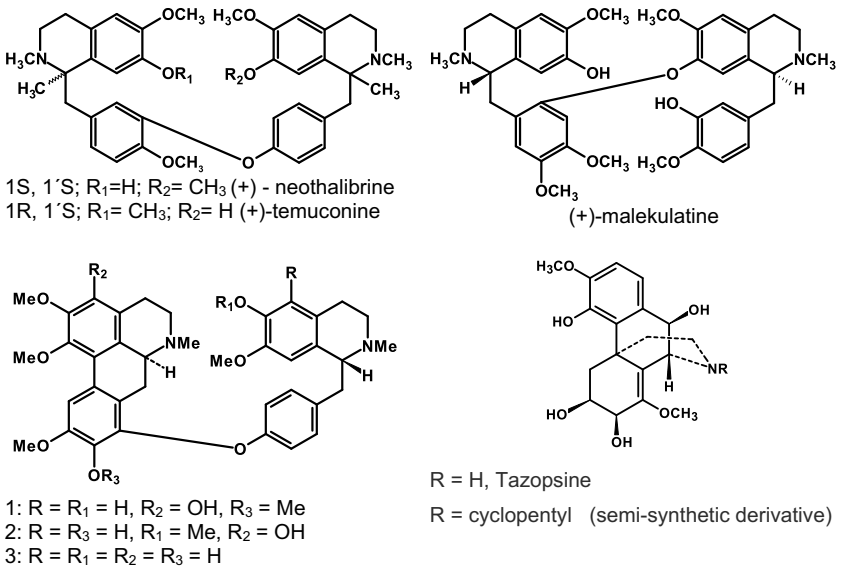


Chart 2 – Structures of biogenetically derived benzylisoquinoline alkaloids with selective antiplasmodial activity (Angerhofer et al. 1999, Carraz et al. 2006).

thollonii, exists as two configurations of semi-stable atropo-diastereomers. This compound exhibited good antiplasmodial activity against both CQS (NF4 IC_{50} 22 ng/ml) and CQR (K1 IC_{50} 21 ng/ml) *P. falciparum* strains (Chart 3). Indeed, it was only approximately 5–10 fold weaker than the standards artemisinine (2.8 and 1.1 ng/ml) and chloroquine (4.4 and 65 ng/ml) (Bringmann et al. 2002). Habropetaline A, from *Tryphyophyllum peltatum* (Ancistrocladaceae), displayed strong antiplasmodial activity against *P. falciparum* (K1 strain IC_{50} 1.2 ng/ml; NF4 strain: IC_{50} 1.2 ng/ml), as was expected by its close structural similarity to dioncopeltine A (Bringmann et al. 2003).

In addition to monomeric structures, the naphtylisoquinoline group of alkaloids includes a few dimeric natural derivatives. Examples of the latter include michellamines, such as michellamine B (Chart 3), which is a highly effective inhibitor of the replication of human immunodeficiency virus (HIV-1 and HIV-2). Monomeric alkaloids have been shown to be useful as building blocks or intermediates for the synthesis of novel dimeric arylisoquinoline compounds. The isolation of monomeric and dimeric arylisoquinolie alkaloids, their

ment of malaria infections are the subjects of patents (Bringmann et al. 2000).

Cryptolepine, an **indoloquinoline** alkaloid 4), is the major constituent (this alkaloid constitut 1% of its weight) and the most potent antiplas compound derived from *Cryptolepis sanguinole* decoction of the roots of this climbing shrub in West Africa for the treatment of malaria. F more, its major constituent, cryptolepine, has effects against both CQS (D6 IC_{50} 27.0 $\pm$ 0.3 and CQR *P. falciparum* strains *in vitro* (K1 IC $\pm$ 0.1 ng/ml, W2 IC_{50} 41.0 $\pm$ 0.5 ng/ml); ho cytotoxic effects have been observed. It has be monstrated that cryptolepine intercalates with DN stabilizes the topoisomerase II-DNA covalent co thus, the scission of DNA by topoisomerase is lated (Cimanga et al. 1997, Wright et al. 2001).

To assess its potential as a lead compound timalarial drug development, cryptolepine ha further investigated. The synthesis of crypt from isatin was carried on by a three-step proces straightforward process allowed for easy synth cryptolepine analogues and supporting subst

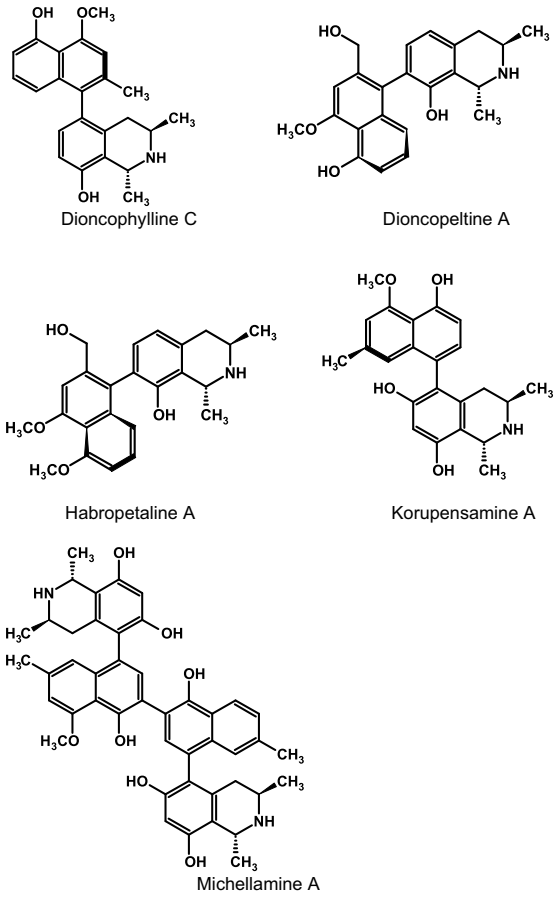


Chart 3 – Naphtyisoquinoline alkaloids: antimalarial (monomeric) and anti-HIV (dimeric) naphtylisoquinoline (François et al. 1997, Bringmann and Feineis 2001).

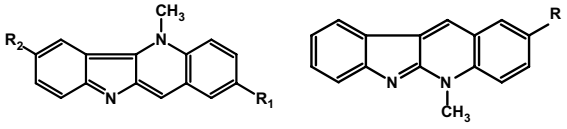


Chart 4 – Antimalarial cryptolepine, neocryptolepine and syntetic bromo derivatives (Wright 2001a, b, Jonckers et al. 2002, Wright 2005).

Plasmodium falciparum (K1). After being injected i.p. at a dose of 25 mg/kg/day into mice that were infected with *Plasmodium berghei*, parasitaemia was reduced by 90% and there was no apparent toxicity. Cytotoxicity (Wright et al. 2001, Wright 2005a). Neocryptolepine, a minor alkaloid that is isolated together with cryptolepine from the roots of *C. sanguinolenta*, has also demonstrated antimalarial activity. It has a lower potency than cryptolepine, but it is also less cytotoxic. A series of synthetic neocryptolepine derivatives was screened and 2-bromoneocryptolepine was identified as a favourable lead compound that exhibited both good antimalarial activity (IC_{50} 4.0μM, *P. falciparum* W2 strain) and very low cytotoxicity (MRC-5 cells, $IC_{50} > 32\mu$M) (Jonckers et al. 2002).

Mono- and bis-indole alkaloids have been isolated from several plants that are traditionally used to treat malaria in different continents. The most active compounds are those that originate from plants that belong to the genera *Strychnos* (Loganiacae) and *Alstonia* (Apocynaceae). A review covering the indole alkaloids that have high antiplasmodial activities *in vitro* and *in vivo*, and favourable selectivity indices ($SI=CC_{50}/IC_{50}$) was published recently (Frederich et al. 2008).

The antiplasmodial activity of 69 **indolomonoterpenoid** alkaloids (Chart 5) from various *Strychnos* species (Loganiaceae) have been evaluated against CQR and CQS lines of *P. falciparum in vitro* (Frederich et al. 2002, 2003). The most active alkaloids were also tested for cytotoxicity against HCT-1116 (colon cancer cells) and their antiplasmodial SIs were calculated. Of the assayed compounds, 40 were of the mono-indole type and 24 were bis-indole alkaloids. A wide range of antiplasmodial potencies were observed (IC_{50} ranged from 32 to 500 nM). Twelve out of the 24 bisindole alkaloids showed IC_{50} values $< 2\mu$M against all *Plasmodium* lines assayed. Most of the 12 *Strychnos* alkaloids tested for cytotoxicity exhibited between 5 and 400-fold higher potency against *P. falciparum* than against the evaluated cancer cell lines. This difference is indicative of the variability of antiplasmodial selectivity. The most selective compounds were isostrychnopentamine from *S. usambarensis* (with IC_{50} of 100–150 nM against all *Plasmodium* lines), and ochrolifuamine A from *S. potatorum* (with IC_{50} of 100–500 nM). The SIs for these alkaloids had ranges of 50–70 and 30–

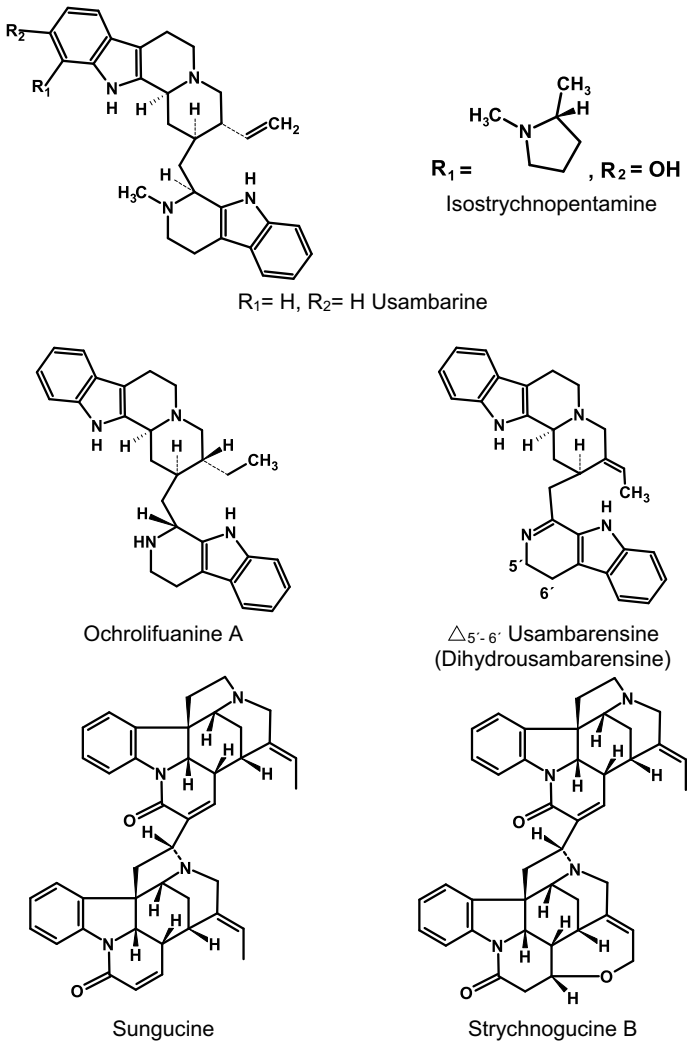


Chart 5 – Structures of indolomonoterpenoid alkaloids from *Strychnos spp.* with selective antiplasmodial activity (Frederich et al. 2002, 2003).

bis-indole alkaloids (Frederich et al. 2003). However, it was inactive in *P. berghei*-infected mice at a dose of 30 mg/kg/day. Differences in the biology of CQR *P. falciparum* strains and the CQS *P. berghei* strain used in that study were considered to explain the negative *in vivo* result, since it had been shown that this alkaloid was essentially active against CQR strains of *P. falciparum*, and a CQS *P. berghei* strain was used in that

indole alkaloids have not shown significant antip
dial activity. The most active were derivatives
bis-indole sungucine, of which strychnogucine
an IC_{50} of 85 nM (W2, CQR), has shown a fav
SI (176, W2) (Philippe et al. 2007) (Chart 5).

Alstonia species (Apocynaceae) are tradit
used in Africa and South-East Asia for the treatm
malaria. The investigation of several *Alstonia* s

villalstonine and macrocarpamine were the most active against the CQR K1 strain *in vitro* (IC_{50} 270 and 360 nM, respectively) (Wright et al. 1993, Keawpradub et al. 1999). Voacamine, isolated from *Tabenamontana fuchsiaefolia* A. DC. (synonym *Peschiera fuchsiaefolia* (DC) Miers), has been traditionally used to treat malaria in Brazil, Africa and the Dominican Republic. This compound was effective against the CQR W2 strain both *in vitro* and *in vivo*, and it had a good SI ($IC_{50} = 411$ nM, SI = 47) (Frederici et al. 2000) (Chart 6).

Antiplasmodial **indole alkaloids** have recently been isolated from *Geissospermum* and *Aspidosperma* species (family Apocynaceae) that occur in tropical and sub-tropical regions of the Americas. *Geisospermum* is a small genus of trees (including *G. laeve*, *G. sericeum*, *G. vellosii*, and *G. argenteum*) that are found in Brazil and French Guiana, and are traditionally used for the treatment of malaria (Brandão et al. 1992, Milliken and Albert 1996, 1997, Bertani et al. 2005). These plants are traditionally consumed as bark decoctions.

The antiplasmodial activity of a hydromethanol extract of *G. sericeum* bark that was collected in the state of Roraima, in Brazil, showed an IC_{50} of 1.78μg/ml, against a CQR *P. falciparum* strain (K1). From alkaloidal fractions, four alkaloids were isolated: geissoschizoline and flavopereirine, which were previously isolated from *G. vellosii* and *G. laeve*, and geissoschizoline N^4-oxide and 1,2-dehydrogeissoschizoline, which are novel natural indolomonoterpenoid derivatives (Steele et al. 2002; Chart 7). Flavopereirine, a β-carboline alkaloid, was the most active of the four compounds that were assayed against K1 (CQR) and T9-96 (CQS) strains of *P. falciparum* ($IC_{50} = 11.53$ and 1.83μM, respectively), but it also showed moderate cytotoxicity against KB cells ($IC_{50} = 10.7\mu$M), with essentially no selectivity against the K1 strain. Geissoschizoline and its N^4-oxide lacked both cytotoxicity and antiplasmodial activity at 40μM. The 1,2-dehydro derivative showed moderate antiplasmodial activity against both K1 and T9-96 strains (IC_{50} of 27.26 and 35.37μM, respectively) and some selectivity was indicated by the finding that less than 50% inhibition of KB cells was observed at 40μM.

loids. In addition, even the alkaloidal fractions obtained by sequential extractions with Et_2O, $CHCl_3$ and EtOAc were less active (with IC_{50} values of 10.15, 2.21 and 2.47μg/ml, respectively). This result suggests that compounds other than alkaloids might be responsible for the antiplasmodial activity of *G. sericeum* (Steele et al. 2002). The antiplasmodial activity displayed *in vitro* by the crude extract of *G. sericeum* and some of its alkaloidal constituents seem to confirm earlier reports describing the traditional use of this plant for the treatment of malaria by populations from the Amazonia region (Milliken and Albert 1997, Brandão et al. 1992).

Up to 1983, 46 species of *Aspidosperma* had been chemically investigated (Bolzani et al. 1987). This number increased to 55 by 1996 (Pereira et al. 2007). Hundreds of indolomonoterpenoid alkaloids have been isolated and their structures have been determined mainly by spectrometric methods. These compounds represent a valuable library of rich structural diversity and great interest for bioprospection. In fact, an antineoplasic drug, Elliptinium (Celiptium®) (9-hydroxy-2-methylellipticinium acetate) is a semi-synthetic derivative of the cytotoxic alkaloid ellipticine (Chart 8); it was initially isolated from the *Ochrosia* species (Apocynaceae) but it also occurs in the *Aspidosperma* species (Pereira et al. 2007). Elliptinium is marketed in France for the treatment of breast cancer (Cragg and Newman 2005).

Aspidosperma spp., which is found from Mexico to Argentina (Marcondes-Ferreira Neto 1988), is another genus of the Apocynaceae family that includes some species that have been traditionally used for the treatment of malaria. These species include *A. nitidum* (Brandão et al. 1992), *A. desmanthum* (Milliken and Albert 1997), *A. auriculatum* (Barbosa et al. 2003), and *A. megalocarpon* (Weniger et al. 2001). Further investigation of the *Aspidosperma* species, as part of the quest for bioactive alkaloids, has been a task of phytochemists for the last few years (Weniger et al. 2001, Mitaine-Offer et al. 2002, Jácome et al. 2004, Andrade-Neto et al. 2007).

Eleven known aspidospermane alkaloids have been isolated from *A. pyrifolium* and *A. megalocarpon* (both

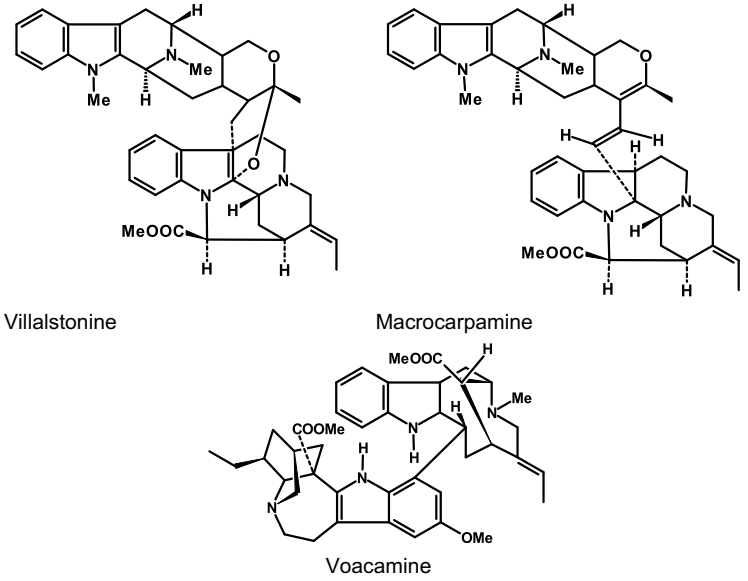


Chart 6 – Structures of indolomonoterpenoid alkaloids from *Alstonia spp.* with selective antiplasmodial activity (Frederici et al. 2000).

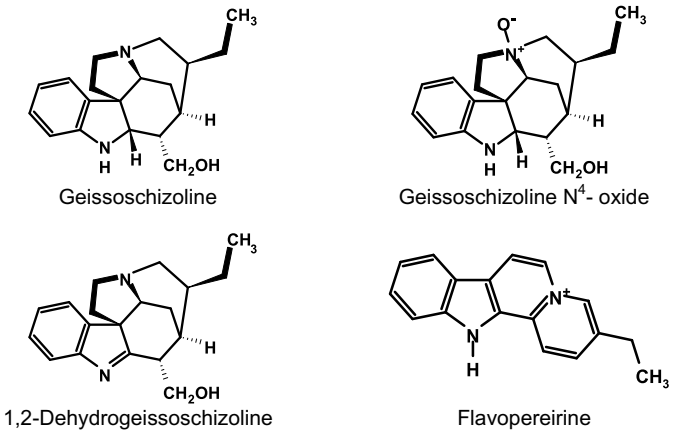
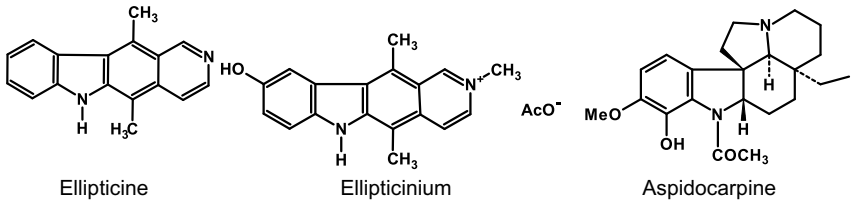
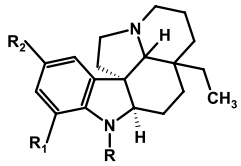
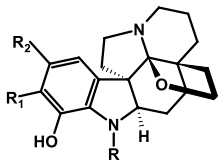


Chart 7 – Structures of indole and β-carboline alkaloids from *Geissospermum sericeum* (Steele et al. 2002).







R = H, R_1= H, R_2= H apisdospermine

R = H, R_1= H, R_2= OCH_3 10-methoxy-aspidospermidine

R = HCO, R_1= H, R_2= H N-formyl-aspidospermidine

R = HCO, R_1= OCH_3, R_2= H vallesine

R = CH_3CO, R_1= OCH_3, R_2= H aspidospermine

R = CH_3CO, R_1= OH, R_2= H demethyl-aspidospermine*

R = CH_3CO, R_1= H, R_2= H demethoxy-aspidospermine

R = C_2H_5CO, R_1= OCH_3, R_2 = H palosine

*semi-synthetic compound

R = C_2H_5CO, R_1= H, R_2= H haplocine

R = C_2H_5CO, R_1= OCH_3, R_2= H fendlerine

R = C_2H_5CO, R_1= OCH_3, R_2= OCH_3 aspidoalbine

R = CH_3CO, R_1= OCH_3, R_2= H aspidolimidine

Chart 9 – Antiplasmodial aspidospermane alkaloids from *Aspidosperma pyrifolium* and *A. megalocarpon* (Mitaine-Offer et al. 2002).

(Mitaine-Offer et al. 2002; Chart 9). CQR (FcM29 – Cameroon) and CQS (Nigeria) isolates of *P. falciparum* were used for evaluation after 24 h and 72 h. Cytotoxicity against the human fibroblast cell line (NIH 3T3) was determined after 24 h and 72 h, which allowed for estimation of the SIs for antiplasmodial activity. Most of the alkaloids that have been assayed have demonstrated better antiplasmodial activity after incubation for 72 h. According to the IC_{50} values, the compounds were ranked into one of two groups: the most active (eight alkaloids), with IC_{50} values between 3.2 and 15.4μM, and the less active ones (four alkaloids), with IC_{50} values between 22.6 and 52.6μM. The first group of compounds is structurally characterized by the presence of a free ethyl group, whereas the corresponding carbons are involved in a tetrahydrofuran ring in the less active alkaloids. The three most active antiplasmodial compounds, 10-methoxy-aspidospermidine, N-formyl-aspidospermine, Once again, the antiplasmodial activity of constituents from plants that have been traditionally used to treat malaria was experimentally confirmed. Indeed, the antiplasmodial activity of a crude extract of *A. megalocarpon* that was collected in Bolivia and assayed against F32 and D2 strains of *P. falciparum* (IC_{50} of 25 and 8 μg/ml, respectively) has been previously reported (Deharo et al. 2001).

Recently, ellipticine and aspidocarpine (Chart 8) were isolated from the trunk bark of *Aspidosperma* vargasii and *A. desmanthum*, respectively. Both of these were collected from the Ducke Reserve, in Manaus, state of Amazonas, Brazil, and have shown a remarkable *in vitro* activity against the multi-drug resistant K1 strain of *P. falciparum* (with IC_{50} values of 73 and 19 nM, respectively) (Andrade-Neto et al. 2007). Ellipticine is highly cytotoxic and was used as a template for the development of Elliptinium (Celliptium®), an anti-

toxicity and, thus, a favourable antimalarial selectivity is expected (Andrade-Neto et al. 2007).

Plants belonging to the Rutaceae family are a source of different classes of alkaloids, such as benzophenantridines, quinolines, furoquinolines, 2-alkylquinolines and acridines (Michael 2003, Waterman 1999). Representatives of these classes have been found in species that have folkloric antimalarial reputations and their antiplasmodial activities have been evaluated (summarized in the following section).

Benzofenantridine alkaloids (Chart 10) were isolated by bioassay-guided fractionation of the trunk bark of *Zanthoxylum rhoifolium*; this species was traditionally used in French Guiana to treat and prevent malaria. The antiplasmodial activity was concentrated in the alkaloid fraction, which comprised seven benzophenantridine alkaloids, of which nitidine was the most potent against *P. falciparum* ($IC_{50} < 0.27\mu M$). The investigation of a trunk bark decoction that was employed as a traditional remedy revealed the presence of alkaloids, including nitidine; therefore the traditional use of *Z. rhoifolium* for the treatment of malaria was justified (Julian et al. 2006). *Zanthoxylum* species are frequently used to treat malaria in Madagascar. *Z. tsihamimposa* is used either alone or in combination with other plants to relieve malarial symptoms, such as tiredness and muscular aches. Five alkaloids that were isolated from the stem bark were assayed *in vitro* for antiplasmodial activity against *P. falciparum* (FCM 29); IC_{50} values in the range of 459.1 to 87.7μM were obtained. The most potent alkaloid was the quinolone γ-fagarine, which had an IC_{50} of 98.4μM (Randrianarivelojosia et al. 2003; Chart 10). Nitidine was also isolated by biossay-guided fractionation of extracts from *Toddalia asiatica*, a Rutaceae used by the Pokot tribe of Kenya as the major antimalarial component. Fractions containing nitidine showed IC_{50} values against *P. falciparum* in the range of 9 to 108μg/ml. Moreover, no cross-resistance was observed between chloroquine and nitidine (Gakunju et al. 1995).

From *Esenbeckia febrifuga*, a Rutaceae plant species popularly used in Brazil to treat malaria, γ-fagarine

75.3 $\pm$ 2.7μM; 19.5 $\pm$ 0.7μg/ml), whereas γ-f
was more active against the 3d7 strain (IC_{50} = 1
18.3μM; 25.0 $\pm$ 4.2μg/ml). However, an etha
tract from the stems was more active (IC_{50} =
0.7μg/ml) than either of these alkaloids, whic
cates the existence of more active, non-isolate
pounds or synergy between the various cons
(Dolabela et al. 2008).

Seven alkaloids were isolated from *Teclea*
carpa (synon. *Toddalia trichocarpa*) from Ken
these alkaloids, two (normelicopicine and arbo
displayed limited *in vitro* activity against *P. falc*
strains (HB3 and K1). Normelicopicine was also
to be active against *P. berghei*-infected mice (32
pression of parasitaemia at a dose of 25 mg/kg/
addition to having low *in vitro* KB cell cytot
($IC_{50} > 328\mu M$) (Mauriithi et al. 2002; Chart 1

Acridone alkaloids derived from species t
long to the genera *Citrus* (*Glycosmis* and *Severim*
are members of the family Rutaceae were tested
timalarial activity *in vitro* against *P. yoelli* and
against *P. berghei*- and *P. vinckei*-infected mice
concentration of 10μg/ml *in vitro*, seven out of
tested alkaloids inhibited 90% or more of the p
growth. Against *P. yoelli*, they were shown to b
equally or more effective than chloroquine *in vitr*
$\pm$ 4 growth inhibition). Of the seven or more activ
loids, atalaphillinine was the only one to be teste
vivo activity. A daily dose of 50 mg/kg of this a
was injected i.p. into mice for a period of thre
Marked prophylactic activity against *P. berghei*-
vinckei-infected mice was observed by days 4 a
ter infection. Very few intraerythrocytic parasite
were seen in blood smears and they had complet
appeared by day 9 or 10. No sign of recrudescen
observed on day 30. Moreover, no obvious acu
city was observed in mice for 30 days after adm
tion, whereas all control mice died between days
No acute toxic effect was observed after injectio
of a single dose of 150 mg/kg into mice (Fujiok
1989; Chart 10).

Furoquinoline and **acridine** alkaloids hav

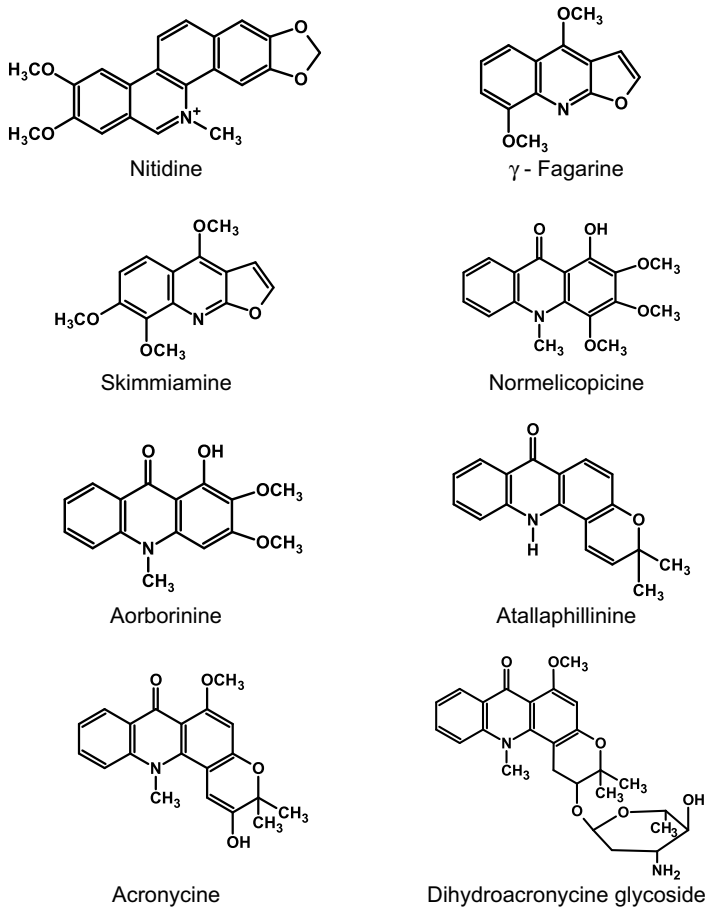
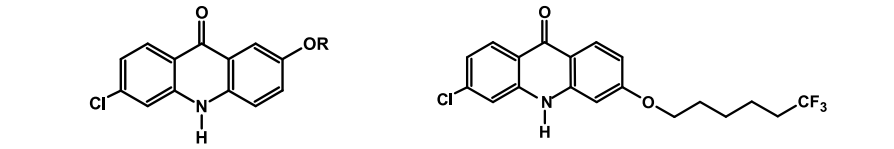


Chart 10 – Antiplasmodial alkaloids from *Rutaceous* plant species.

fect of 23 furoquinoline and acridone alkaloids against CQR (W2) and CQS (HB3) clones of *P. falciparum* have been reported (Basco et al. 1994). The assayed alkaloids included isolates from three New Caledonian plants (*Geijera balansae*, *Sarcomelicope glauca* and *Sarcomelicope dogniensis*) and derivatives that were obtained by chemical modifications and the dimerization of acronycine. Fourteen alkaloids had $IC_{50} < 10\mu$g/ml against the W2 strain. Most of the active alkaloids were more than twice as active against the resistant clone than they were against the susceptible one. The most active alkaloid was an O-pyranoglycoside derivative of acronycine (Chart 10), which had an IC_{50} of ical efficacy and tolerance were evaluated in a phase II study. However, acronycine was only moderately active against *P. falciparum* (IC_{50} of 7.03 and 1.44μg/ml in HB3 and W2 strains, respectively) (Basco et al. 1994).

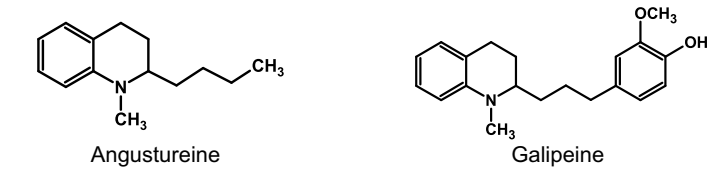
Natural and synthetic acridones are of great biomedical interest for their potential as anticancer, antimicrobial, antiviral and antiparasitic agents (Winter et al. 2006). However, only moderate antimalarial activity has been reported for acridones (Basco et al. 1994, Fujioka et al. 1989), which has motivated a recent re-investigation of commercially available compounds by Winter and collaborators (2006). The surprisingly high activity of 2-methoxy-6-chloroacridone (which displayed an



R = CH_3 , 2-methoxy-6-choroacridone
R = H, 2-hydroxy-6-chloroacridone

3-(6, 6, 6)-Trifluorohexyloxy-6-chloroacridone

Chart 11 – Antimalarial synthetic haloalkoxy-acridones (Winter et al. 2006).



Angustureine

Galipeine

Cuspareine

Galipinine

Chart 12 – Antiplasmodial tetrahydroquinoline alkaloids from *Galipea officinalis* (Jacquemond-Collet et al. 2002)

dinary potential for development as therapeutic agents to treat or prevent malaria in humans. The influence of substituents at positions 2, 3 and 6 of the tricyclic acridone skeleton was investigated, and led to the development of over 30 synthetic derivatives. The *in vitro* activities of these derivatives were evaluated against D6 (CQR) and Dd2 (multidrug-resistant) strains of *P. falciparum*. The results clearly point to the influence of an ether group at position 2, with the corresponding phenolic derivatives displaying significantly decreased antimalarial activity. This effect is exemplified by 2-hydroxy-6-chloroacridone, which showed IC_{50} values of 190 and 260 nM on D6 and Dd2 strains, respectively. A similar profile was observed for substitutions at position 3. The most potent synthetic acridones supported O-alkyl chains that terminated in trifluoromethyl groups at positions 2 or 3 of the tricyclic system. These compounds exhibited *in vitro* antimalarial IC_{50} values in the nanomolar and picomolar ranges, and were not cytotoxic to cultured murine splenic lymphocytes at con-

laborators) had an IC_{50} value of approximately
It was proposed that the haloalkoxyacridones exe
effects through inhibition of a *P. falciparum* mit
drial component – the cytochrome bc1 complex (
et al. 2006; Chart 11).

The genus *Galipea* (family Rutaceae) c
approximately 14 species, which occur across
America (Costa Rica, Panama, Guatemala, Nica
in Southern Brazil and Bolivia, and in other p
South America (Pirani 2004).

Tetrahydroquinoline alkaloids were isolate
the trunk bark of *G. officinalis* (Chart 12). Thi
tional medicinal plant from Venezuela, con
named angostura bark, is reputed to be the sour
tonic and stimulant that is used against fever (J
mond-Collet et al. 1999). Hexane, chloroform e
and pure alkaloids were tested for their *in vitro*
against *P. falciparum* strains. The IC_{50} values
from 1.8 to 40.0μg/ml against a CQS strain (Ni
and 0.09 to 38.0μg/ml against CQR strains (Fc

assessed on a HeLa cell line. IC_{50} values ranged from 5.8 to $> 50\mu$g/ml, and the SI for galipinine varied from 10 (Nigerian strain) to > 100 (FcM29 strain, 24 h = 91.5; 72 h > 5000) (Jacquemond-Collet et al. 2002).

TRADITIONAL ANTIMALARIAL PLANTS

The traditional use of plants for the treatment of human malaria and fevers all over the world has been widely documented. The number of investigations into their effects *in vitro* and *in vivo* is increasing; however, little is known about their efficacy and safety. The validation of plants that are traditional treatments for malaria is currently stimulating the interest of researchers across the world. A significant development in this area was the founding of RITAM (**R**esearch **I**nitiative on **T**raditional **A**ntimalarial **M**ethods). The aim of this global network, which was initiated by Dr. G. Bodeker, Oxford, UK, is to further research on the traditional medicines for malaria and to make a significant contribution to global malaria control through the use of plant-based antimalarials, insect repellents and vector control. RITAM has established international partnerships with over 30 countries, including African countries (Willcox and Bodeker 2004).

A review by Willcox and Bodeker (2004) on traditional herbal medicines for malaria in three continents revealed that 1277 plant species from 160 families have been classified according to their importance for the treatment of malaria. However, only eight clinically-controlled trials have thus far been reported; these have involved falciparum and vivax malaria. It would be worth mentioning the results that have been obtained with *Cryptolepis sanguinolenta*, *Artemisia annua* and *Dichroa febrifuga*. *C. sanguinolenta* (aqueous extract) has been revealed to be a promising treatment for malaria falciparum; not only was the time taken for parasite clearance (3.3 days) only one day longer than that observed with CQ (2.2 days), but the clearance of the fever was achieved in a shorter time than with CQ (36 and 48 hours, respectively). A trial that compared quinine with infusions of *A. annua* for the treatment of malaria falciparum also demonstrated good parasite clearance with studies (in 1947 in China) had reported good results, but undesirable side effects with *Dichroa febrifuga* (*apud* Willcox and Bodeker 2004).

In South America, the RAVREDA (**R**ede **A**mazônica de **V**igilância da **R**esistência a **D**rogas **A**ntimaláricas – Amazonian Network for Vigilance of Antimalarial Drug Resistance), which was created in 2001, is a project whose goal is to furnish technical support to governmental actions that are aimed at controlling malaria in the Legal Amazon region (PAHO 2007). This project is supported by USAID (**U**nited **S**tates **A**gency for **I**nternational **D**evelopment), and the regional South American net is under the coordination of the Pan American Health Organization – PAHO (Organização Pan-Americana da Saúde – OPAS). The participating countries are Brazil, Bolivia, Equator, Guiana, Peru, Suriname and Venezuela (Brasil 2007). However, unlike RITAM, the RAVREDA project does not include research on antimalarial plants.

CONCLUDING REMARKS

The alkaloid quinine, which is derived from the South American *Cinchona* species, was traditionally used as an antimalarial remedy by the Incas in Peru. It was the first drug to be introduced for malaria chemotherapy and served as a template for the synthesis of chloroquine (a quinoline that has been used since the 1940's). The potential of alkaloids as antimalarials has been widely documented and concisely presented in this paper. As some of the active plants that produce alkaloids carry a reputation for traditional usage, further investigations that include both pre-clinical and clinical assays are encouraged. Moreover, the pharmacokinetic properties of crude and semi-purified plant extracts can be improved by appropriate formulation using pharmaceutical technology.

In this regard, current research on new antimalarial agents faces two distinct avenues: the search for new chemical entities (NCE) of natural or synthetic origin, and the development of phytomedicines.

The naturally-occurring, antiplasmodial/antimalarial alkaloids that have been described in this short review can be divided into two groups: the first group con-

pounds with moderate to low activity that possess relatively simple structures. The synthesis of compounds and/or their analogues from the latter group could be undertaken. Plant species that produce alkaloids of the first group are potential candidates for the development of phytomedicines, whereas alkaloids from the second group could represent templates for the production of synthetic drugs.

Will the research on traditional plants contribute to the discovery of new antimalarial drugs? Of this, there is no doubt. Atovaquone, artemisinin and its semisynthetic derivatives are remarkable examples of the diverse contribution of natural products to the development of effective antimalarial drugs. These drugs are particularly valuable for the treatment of chloroquine-resistant parasites.

Although several potent antiplasmodial alkaloids have been described in this review, most of them have only been evaluated using *in vitro* assays. Few of them have been evaluated for cytotoxicity, and even smaller is the number of those that have been assayed *in vivo*. As many of these compounds are found in low concentrations in various plant species and usually as part of complex mixtures, their isolation and purification are highly expensive. In these situations, the benefits to be obtained from the development of phytomedicines (with the known active compounds being useful as chemical or biological markers to guarantee product quality) are evident.

The validation of traditional plant remedies has limitations, such as the prioritization of plant species for research, a lack of information on the ethnobotany of these plants (location and abundance, parts used, form of use, duration of treatment), and the definition of dosages due to variations in the concentrations of active ingredients in a plant species (Willcox and Bodeker 2004, Bourdy et al. 2007). For each of these questions, there are scientific and technical solutions. At the present time, the more serious questions concern biodiversity, property rights, equitable distribution of benefits from the use of traditional medicines, and their sustainable use and conservation.

tion includes identification and quantification of
cal and/or biological markers to ensure the develo
of efficient and safe phytomedicines in a short
of time and at a low cost. It is well known th
the qualitative and quantitative contents of sec
metabolites in a plant are susceptible to marked
tions; these contents are influenced by intrinsic
(ontogeny and phenology), abiotic factors (light
ture, nutrient availability), and biotic factors (d
physiological and growth stages; Harborne 2001
result, standardization is obligatory.

Finally, we present a streamlined process a
from Fidock and collaborators (2004) emphasiz
role of traditional medicinal plants in the resear
development of antimalarial phytopharmaceutica
drugs and/or phytomedicines) (Fig. 2).

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suggestions.

RESUMO

A malária ainda é um dos mais sérios problemas d
pública e a principal causa de mortalidade e morbid
regiões endêmicas. O Brasil está entre os 30 países co
incidência de malária e a maior parte dos casos oc
Amazônia Legal. Novos agentes terapêuticos são r
rios para o tratamento da malária. Muitas espécies
são utilizadas na medicina tradicional de vários país
micos mas é relativamente reduzido o número daquela
foram investigadas quanto à sua atividade antimalárica
ainda é o número de espécies das quais foram isolad
tâncias ativas e tiveram sua toxidade determinada. E
de pesquisa é, portanto, de alta relevância. Um pr
descoberta de produtos naturais antimaláricos a partir
tas de uso tradicional deve incluir ensaios *in vitro* e *in v*

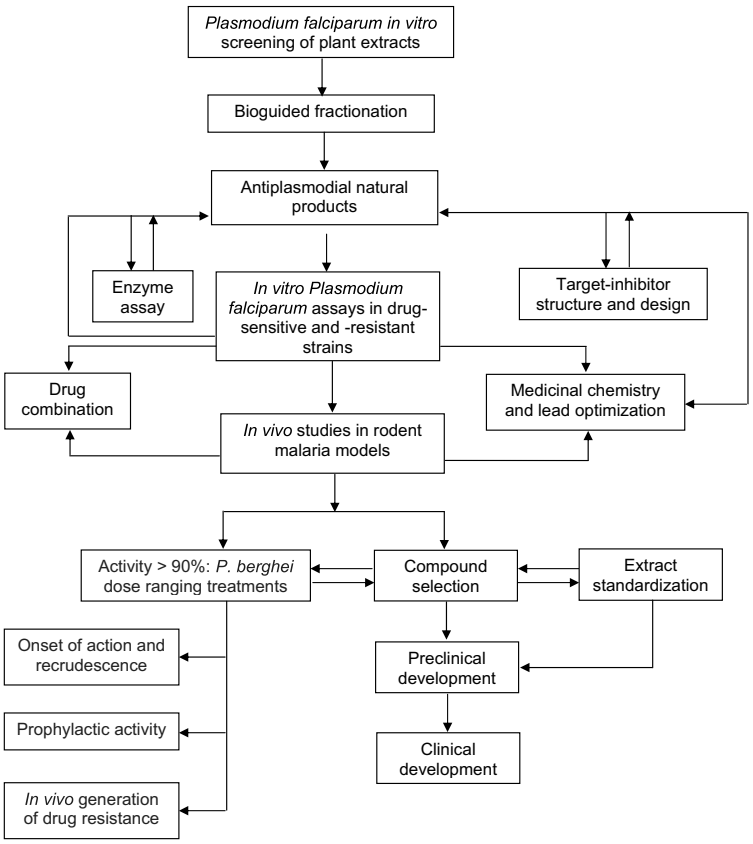

Fig. 2 – General approach for antimalarial drug discovery and phytomedicines development (adapted from Fidock et al. 2004). Discovery starts with *Plasmodium falciparum in vitro* screening of plant extracts, followed by bioguided fractionation of active extracts, resulting in the isolation of antiplasmodial compounds. At this stage, active target enzymes can be employed in biochemical screens, which may well support molecular modeling and design of potential active compounds, based on target-inhibitor interactions. *In vitro* assays comprise determination of IC_{50} against sensitive and resistant strains of *Plasmodium falciparum* and such results may allow selecting templates for chemical optimization. *In vivo* assays include tests for suppression of parasitaemia in rodents. In combination, *in vitro* and *in vivo* studies are directed towards template selection. Compounds showing activity above 90% are subjected to further studies aiming at evaluating recrudescence, prophylactic efficacy and resistance development. Alternatively, standardized extracts (phytomedicines) could be developed in a shorter and less expensive pipeline.

novos fármacos, e/ou extratos padronizados, com atividade antimalárica, os quais são necessários para estudos pré-clínicos e clínicos quando o objetivo é o desenvolvimento de fitoterápicos (fitomedicamentos) eficazes e seguros. A presente revisão discute estas duas abordagens, apresenta resumidamente as metodologias de bioensaios para avaliação de atividade antimalárica e focaliza a atividade de alcalóides pertencentes a diferentes classes estruturais bem como sua importância como fármacos ou protótipos e como marcadores químicos

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