Potential antimalarial activity of indole alkaloids

Michel Frederich *, Monique Tits, Luc Angenot

Université de Liège, Centre Interfacultaire de Recherche du Médicament (CIRM), Département de Pharmacie, CHU – Tour 4 – Bâtiment B36, Avenue de l’Hôpital, 1 – B4000 Liége, Belgium

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1. Introduction

Malaria is the major parasitic infection in many tropical and subtropical regions, leading to more than one million deaths out of 400–500 million cases each year (WHO, 2005). Discovering new drugs in this field is therefore a health priority. The challenge in malaria chemotherapy is to find safe and selective agents with potency that will not be compromised by plasmodial resistance. In this context, the search for antiprotozoal compounds from terrestrial plants and marine organisms could provide new leads to antimalarial drugs. The natural active principles are detected either after bioguided isolation from species with a reputation for use in traditional medicine or after a screening campaign involving in vitro or in vivo bioassay procedures. The topic of new antimalarial drugs from traditional medicines has been largely reviewed in recent years (Bourdy et al., 2007; Willcox and Bodeker, 2004).

In this review, we will focus on indole alkaloids that show antiplasmodial properties. The alkaloids are a phytochemical class known to possess antiprotozoal properties, the most famous being quinine, which is a quinoleic alkaloid but has a biosynthetic pathway common to indolomonoterpeneoid alkaloids (the most important class of indole alkaloids).

In order to compare all compounds, we will use the 50% inhibitory concentration (IC50) and, when available, the selectivity index (SI), which is defined as the ratio of cytotoxicity over antiprotozoal activity, each one expressed with IC50. Table 1 summarizes the IC50 and SI of the most important compounds described in this manuscript. Several
plasmodial strains were used to assess the activity of the compounds described below. We have chosen to classify them into 'chloroquine-sensitive' (CQS) and 'chloroquine-resistant' (CQR) strains.

### 2. Indole analogues from emetine

The first studies dealing with the antiplasmodial activity of indole alkaloids were initiated in England more than 20 years ago. The aim of those studies was to compare the amoebicide activity of emetine (1, Figure 1) with indolic natural analogues such as tubulosine (2, Figure 1). Some of these analogues were active, but at concentrations much higher than emetine and without selectivity compared to human cells (Keene et al., 1983, 1987). The in vitro screening was then extended to other indolic analogues of emetine, which have been previously isolated in the Laboratory of Pharmacognosy at the University of Liège (Belgium), and other protozoa were included in the screening, especially *Plasmodium falciparum*. This time, some indolic alkaloids, such as strychnopentamine (3, Figure 2) (IC$_{50}$ = 150 nM) and dihydrousambarembose (6, Figure 2) (IC$_{50}$ = 23 nM), were shown to be very active against *P. falciparum*. This last alkaloid was nevertheless seen to be inactive in vivo (at the dose of 30 mg/kg p.o. or s.c.) in mice infected with *P. berghei* (Wright et al., 1991). Other indole alkaloids from various plant species from the genus *Strychnos* were then evaluated (*S. usambarensis*, *S. variabilis* and *S. heningsii*). In this study, only strychnobilene from *S. variabilis* and dihydroflavopereirine from *S. usambarensis* presented a slight activity (IC$_{50}$ near 1 µM) (Wright et al., 1994). The most interesting alkaloids were still the usambarembose (5, Figure 2) derivatives from *S. usambarensis*. Investigations concerning these alkaloids were then continued in Liège and showed that dihydrousambarembose was much more active against CQR strains (IC$_{50}$ = 32 nM, SI = 375) than against CQS ones (IC$_{50}$ = 857 nM, SI = 14). This observation could explain the failure of in vivo experiments conducted on a CQS strain.
Potential antimalarial activity of indole alkaloids

strain of *P. berghei* (Frederich et al., 1999a). Afterwards, it was shown that strychnopentamine (3, Figure 2) and isostrychnopentamine (4, Figure 2) were active with the same IC\textsubscript{50} against all strains of *P. falciparum* (IC\textsubscript{50} = 120nM and SI = 60) (Frederich et al., 1999a) and at all stages of development, including rings (Frederich et al., 2004b). Isostrychnopentamine (4, Figure 2) was also active in vivo, with a median effective dose (ED\textsubscript{50}) of 30mg/kg (i.p., *P. berghei* and *P. vinckei petteri*) (Frederich et al., 2004b).

With regard to the indole analogues of emetine, a study published in 1996 was devoted to *Pogonopus tubulosus* (Sauvain et al., 1996), which is a tree from South America used traditionally in Bolivia against malaria (decoction of stem bark) and called 'falsa quina'. A bioguided fractionation attributed the antiplasmodial properties to the alkaloidal fraction and mainly to tubulosine (2, Figure 1), already cited previously. Tubulosine is active against all strains of *P. falciparum* (IC\textsubscript{50} = 24nM) but has no selectivity compared to human cells. Active in vivo (ED\textsubscript{50} = 1mg/kg), there was also shown to be a high level of toxicity (all mice died following a dose of 2mg/kg).

3. Other bisindole alkaloids from *Strychnos* species

As some bisindole alkaloids from *Strychnos* species showed some promising activity, our team in Li`ege continued to screen several of these species and various alkaloids isolated from these plants (Frederich et al., 1999a, 1999b, 2002, 2004a; Philippe et al., 2005). Among the *Strychnos* species investigated, *S. icaja* was particularly interesting. The monoindole alkaloids known in this plant were deprived of any significant antiplasmodial activity. Bioguided fractionation led to the isolation of several oxygenated bisindole alkaloids, derivatives of sungucine (7, Figure 3), which were responsible for the activity of the plant (Frederich et al., 2000, 2001a). The most active compounds were isosungucine (8, Figure 3) (IC\textsubscript{50} = 168nM, CQR), 18-hydroxyisosungucine (9, Figure 3) (IC\textsubscript{50} = 85nM, CQR) and strychnogucine B (10, Figure 3) (IC\textsubscript{50} = 85nM, CQR) with, for the last one, an SI of 176 (W2, CQR). These compounds were clearly more active against CQR than against CQS strains. Iso sungucine, isolated in higher quantities than the two other compounds, was evaluated in vivo and showed an ED\textsubscript{50} of close to 30mg/kg [i.p., mice, *P. vinckei petteri* (Philippe et al., 2007)]. It was also shown that these bisindole alkaloids, structurally related to strychnine, were deprived of their strychnine-like convulsivant activity (Philippe et al., 2006). The underlining of the antimalarial activity of 'isosungucine' alkaloids could explain the traditional use of *S. icaja* by the Pygmies from Cameroon to treat malaria fevers. Nevertheless, further studies will be necessary to confirm the interest of these alkaloids for human medicine.

Thanks to collaboration with the pharmacognosy department from the University of Reims (France), other bisindole alkaloids were also evaluated. Of the more than 50 alkaloids tested, only three were of some interest: ochrolifuanine A (11, Figure 3), matopensine and longicaudatine (12, Figure 3), but all three were shown to be less active than isostrychnopentamine or strychnogucine B (Frederich et al., 2002).

![Figure 3](attachment:Figure_3.png)  
**Figure 3** Chemical structures of compounds 7—12. The circles indicate the isomery between sungucine and isosungucine.
4. Other bisindole alkaloids from seed plants

Several *Alstonia* species (Apocynaceae) are traditionally used in Africa for their antimalarial properties (Wright et al., 1993). Several species (especially *Alstonia scholaris*, *A. macrophylla* and *A. glaucescens*) were investigated and their antimalarial properties were attributed to bisindole alkaloids, notably villalstonine (13, Figure 4) and macrocarpamine (14, Figure 4), which possess IC_{50} of 270 and 360 nM, respectively, against a CQR strain of *P. falciparum* (Keawpradub et al., 1999).

*Tabernaemontana fuchsiaefolia* A. DC. (synonym: *Peschiera fuchsiaefolia* (DC) Miers) is also a tree from the family Apocynaceae, known in Brazil by the name ‘leiteira’ because of the presence of latex in the plant. This species is currently used in traditional medicine to treat malaria in Sao Paulo and Parana states (Zocoler et al., 2005). The activity was attributed to bisindole alkaloids, the principal one being voacamine (15, Figure 4) (IC_{50} = 411 nM, SI = 47, CQR) (Federici et al., 2000). Voacamine is active in vivo (43% of reduction of parasitaemia at 10 mg/kg p.o. in the 4 d suppressive test of Peters) and presents some specificity for trophozoites and schizonts (Ramanitrahasimbola et al., 2001).

5. Semi-synthetic bisindole and trisindole

To the best of our knowledge, the only indole semi-synthetic antiplasmodial compounds known to date are derivatives of ergolines, which are either natural compounds isolated from *Claviceps purpurea* (festuclavine...) or semi-synthetic compounds used in clinical routine (terguride...) (Figure 5). After the detection of a slight antiplasmodial activity for some monomeric ergolines, a German team tried to investigate semi-synthetic dimeric or oligomeric derivatives of these ergolines linked by a central benzenic bond. Only two derivatives were of interest: a dimeric derivative of terguride (17, Figure 5) (IC_{50} = 540 nM, SI = 47, CQR) and a trimeric derivative of festuclavine (16, Figure 5) (IC_{50} = 540 nM, SI = 185, CQR). Nevertheless, when administered to mice these compounds induced behavioural modifications, inviting caution (Jenett-Siems et al., 2004).

6. Indoloquinolines

It is impossible to write about indolic alkaloids and malaria without mentioning cryptoplepine, isolated from *Cryptolepis sanguinolenta*. Cryptoplepine (18, Figure 6) is an indole alkaloid, but not from indolomoterpenic biosynthetic pathways. This alkaloid, initially known as synthetic [synthesis in 1906 (Fichter and Boehringer, 1906)], was then isolated from roots of *C. sanguinolenta*, previously known as *C. triangularis* (Asclepiadaceae), in 1929 (Clinquart, 1929). Cryptoplepine was then isolated from *Sida acuta* (Malvaceae) and from *Microphilis guianensis* (Gunatilaka et al., 1980; Yang et al., 1999).
Cryptolepis sanguinolenta is a plant frequently used as an antimalarial, antidysentery and febrifuge remedy in Central and West Africa. Between 1995 and 1997, three independent teams evidenced the antiplasmodial properties of cryptolepine in vitro (IC₅₀ = 114 nM, SI = 9, CQR) and in vivo (mice, ED₅₀ = 50 mg/kg p.o. and ED₅₀ = 10 mg/kg i.p.) (Cimanga et al., 1997; Grellier et al., 1996; Kirby et al., 1995; Wright et al., 1996). Recently, it has been shown that cryptolepine and derivatives are able to inhibit hemozoin polymerization (Onyeibor et al., 2005).

Several semi-synthetic derivatives of cryptolepine have been synthesized. The most interesting of these are: (1) 2,7-dibromocryptolepine (IC₅₀ = 49 nM, CQR), active against *P. berghei* in mice (90% suppression of parasitaemia at 12.5 mg/kg i.p.) (Onyeibor et al., 2005; Wright et al., 2001); (2) 1-methyl-δ-carboline (21, Figure 6), anhydronium base with an IC₅₀ of 1.5 μM and an SI of higher than 100 (Arzel et al., 2001); (3) 2-bromoneocryptolepine, which is less active than cryptolepine (IC₅₀ = 4 μM) but also presents less affinity towards DNA; (4) neocryptolepine (19, Figure 6) is a natural alkaloid from the genus Cryptolepis (Jonckers et al., 2002; Van Miert et al., 2004); (5) isoneocryptolepine (20, Figure 6), synthetic (IC₅₀ = 40 nM), and N-methylisocryptolepinium iodide (23, Figure 6) (IC₅₀ = 17 nM) both present a much smaller cytotoxicity than cryptolepine but were found to be unusable in vivo (mice, s.c., 50 mg/kg, *P. berghei*), the quaternary compound being highly toxic, in spite of an impressive SI of >700 (toxicity probably due to curarizing activity) (Van Miert et al., 2005).

Other teams have also shown that cryptolepine presents a high level of cytotoxic, genotoxic, DNA intercalating, topo II inhibition properties (Ansah and Gooderham, 2002; Ansah et al., 2005; Bonjean et al., 1998; Lisgarten et al., 2002). Further studies are therefore needed before the development of an antimalarial drug from this family of compounds.

7. Tryptanthrin derivatives

Tryptanthrin (24, Figure 7) is an indoloquinazolin well known to possess antimicrobial properties against several bacterial strains, including *Mycobacterium tuberculosis*. Firstly isolated from tryptophane-enriched *Candida lipolytica* cultures (Schindler and Zahner, 1971), tryptanthrin has been isolated subsequently from several higher plants: *Couroupita guianensis*, *Isatis tinctoria*, *Polygonum tinctorium*, *Strobilanthes cusia* and *Wrightia tinctoria* (George et al., 1996; Hamburger, 2002; Ho et al., 2003; Honda et al., 1980; Iwaki et al., 2005; Muruganandam et al., 2000). It was the Walter Reed Research Institute from the USA that tested tryptanthrin, firstly against *Leishmania* spp., then against *P. falciparum*. Tryptanthrin and several analogues were tested and showed very low IC₅₀ values: 69 ng/ml for tryptanthrin and 0.43 to 10 ng/ml for some of...
Figure 7 Chemical structures of compounds 24—25.

the analogues. These compounds were particularly active against atovaquone-, chloroquine- or mefloquine-resistant strains. Novel tryptanthrin compounds, possessing increased solubility, have been prepared recently in order to carry out in vivo testing (Bhattacharjee et al., 2004; Nichols et al., 2003). These results are impatiently awaited. It is also interesting to note that tryptanthrin also possesses immunostimulating properties, which could be useful in the fight against malaria (Valiante, 2004).

8. Marine sources

Several 1-aminopolycyclic β-carbolin alkaloids isolated from marine sources possess high in vitro and in vivo antimalarial properties (Ang et al., 2000; Rao et al., 2003, 2004). The most active alkaloids are manzamine A (IC₅₀ = 4.5—8 ng/ml, CQS and CQR) (25, Figure 7) and manzamine B, initially discovered in sponges from Manzamo (Okinawa, Japan). But, in fact, manzamines appear to be synthesized by actinomycetes (Micromonospora), symbiotic with these sponges (Hill et al., 2006).

Administered to mice at a dose of 55 mg/kg, i.p., manzamine A induces a reduction of parasitaemia of 96% (vs. 99% for chloroquine and 57% for artemisinin). The relative inefficacy of artemisinin in this assay could be explained by its short half-life. An effective treatment with artemisinin requires several daily administrations. The survival of mice is also increased by up to 60 d and manzamine A has also been shown to be active via the oral route. The development of these compounds was supported by the Medicines for Malaria Venture (http://www.mmv.org), but the support was stopped in 2005 following serious worries concerning the toxicological profile of the compound. Effectively, in vivo toxicity appears at concentrations of five times higher than the concentration inducing a parasitaemia decrease.

9. Chloroquine potentialization

The development of chloroquine resistance in Madagascar during the 1980s led to the widespread use of traditional medicines, and a very particular use of some plants appeared: rapsed stems of Strychnos myrtoides, S. diplotricha and S. mostueoides were used in decoctions associated with a sub-therapeutic dose of chloroquine (100—200 mg) (Rasoanaivo et al., 1996b, 2002, 2005). Phytochemical investigations of these species led to the isolation of two bioactive alkaloids: strychnobrasiline (26, Figure 8) and malagashanine (27, Figure 8) (Caira and Rasoanaivo, 1995; Rasoanaivo et al., 1994, 1996a).

These two alkaloids are very weak antiplasmodial compounds (IC₅₀ ~ 100 μM), but, associated with chloroquine, they are able to reverse the in vitro resistance of P. falciparum to this drug (Rasoanaivo et al., 1994). Malagashanine is also active in vivo in mice contaminated by P. yoelii, whereas strychnobrasiline is completely inactive. Authors attribute this discrepancy to a difference in solubility, malagashanine being less hydrophilic. Malagashanine is also able to reverse the resistance to mefloquine, halofantrine, quinacrine and pyronaridine (Rafatro et al., 2000a). A very small clinical study has also been conducted in Antananarivo, Madagascar (20 patients, chloroquine + 500 mg stem bark S. myrtoides) and showed promising results (Ramialiharisoa et al., 1994). Nevertheless, other studies following WHO guidelines are required in order to confirm the interest of this association.

The mode of action of malagashanine has been recently described as a stimulation of the chloroquine influx towards resistant Plasmodium and a diminution of the efflux from the inside (Ramanltrahasimbola et al., 2006). Metabolization of malagashanine has also been studied in human microsomes and rats (Rafatro et al., 2000b, 2000c), and toxicity studies have been conducted on guinea pigs: malagashanine appears not to have cytotoxicity or cardiac toxicity (either alone or associated with chloroquine). Some simplified analogues of malagashanine have also been recently synthesized and are active in vitro (Chouteau et al., 2005).

At the university of Liège, icajine (28, Figure 8) and isoretuline (29, Figure 8) alkaloids presenting some analogies to strychnobrasiline and malagashanine have
also been shown to be chloroquine- and mefloquine-resistance reversal agents (Frederich et al., 2001b). A chloroquine-potentiation effect was also demonstrated for N-formyl-aspidospermidine (30, Figure 8) and aspidospermine (31, Figure 8) (Mitaine-Offer et al., 2002).

10. Conclusions

Among the natural products, indole alkaloids represent an interesting class of compounds. Screening carried out to date has revealed several substances active in vitro under the micromolar range and with a good selectivity index. Nevertheless, in vivo activity has been confirmed only in a small number of cases, and there is a need to undertake research focused on the mode of action of these compounds.

Antiplasmodial indole alkaloids can be separated into three main categories.

The first category contains the alkaloids with a molecular weight higher than 400 and an important steric crowding: (1) indole ‘analogues’ of emetine (usambarensine, ochrolifua-nine, strychnopentamine); (2) and other bisindole alkaloids such as voacamine and ergoline derivatives, matopensines and isosungucines. Several of these alkaloids have been shown to be much more active against chloroquine-resistant strains. This phenomenon deserves to be elucidated. Considering the complexity of this group of compounds, very few attempts have been made to modify them chemically, as has been the case (in cancer therapy) for Vinca alkaloids. This approach could nevertheless be very interesting.

The second category of antiplasmodial indole alkaloids contains unsaturated monomeric heterocycles. These are chemically much more attainable. There are two main models that have been developed: (1) derivatives of cryptolepine (the most interesting compound being 2,7-dibromocryptolepine); (2) derivatives of tryptanthrin. Considering this last group, there is still a lot of investigation needed concerning their in vivo potentiality.

Finally, the last group of interesting indolic compounds includes monoindole alkaloids able to reverse the resistance to chloroquine. Among these, the most interesting compound seems to be malagashanine, found in Strychnos myrtoides from Madagascar. This compound is active in vivo, but further clinical assays will be necessary to confirm the interest of this unconventional approach.

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