

**Potential antimalarial activity of indole alkaloids<sup>+</sup>**

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## ABSTRACT

New antimalarial treatments are now urgently required, following the emergence of resistance to the most used drugs. Natural products contribute greatly to the therapeutic arsenal in this area, including artemisinin, quinine (and atovaquone, semi-synthetic). 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  $\mu\text{M}$  range and with a good selectivity index. This review covers the indole alkaloids with high antiplasmodial activity (in vitro, in vivo) isolated from natural sources and is organized according to the different chemical structures.

## KEYWORDS

*Plasmodium falciparum*, *Plasmodium berghei*, antiplasmodial, malaria, indole alkaloid, natural products.

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 whose potency 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 these last years (Willcox and Bodeker, 2004; Bourdy et al., 2007).

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

In order to compare all compounds, we will use the 50% inhibitory concentration ( $IC_{50}$ ) and, when available, the selectivity index (SI), which is defined as the ratio of cytotoxicity over antiplasmodial activity, each one expressed with  $IC_{50}$ . Table 1 summarizes the  $IC_{50}$  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.

### 1. 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) with indolic natural analogues such as tubulosine (2) (Figure 1). Some of these analogues were active, but at concentrations much higher than emetine (1) and without selectivity compared to human cells (Keene et al., 1983; Keene et al., 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 were shown to be very active against *P. falciparum*, such as strychnopentamine (3) ( $IC_{50}$  = 150 nM) and dihydrousambarensine (6) ( $IC_{50}$  = 23 nM) (Figure 2). This last alkaloid was nevertheless seen to be inactive in vivo (at the dose of 30 mg/kg *per os* or subcutaneously) in mice infected with *Plasmodium 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. henningsii*). In this study, only strychnobiline from *S. variabilis* and dihydroflavopereirine from *S. usambarensis* presented a slight activity ( $IC_{50}$  near 1  $\mu$ M) (Wright et al., 1994). The most interesting alkaloids were still the usambarensine (5) derivatives from *S. usambarensis* (Figure 2). Investigations concerning these alkaloids were then continued in Liège and showed that dihydrousambarensine was much more active against chloroquine-resistant strains ( $IC_{50}$  = 32 nM and SI of 375) than against chloroquine-sensitive ones ( $IC_{50}$  = 857 nM and SI of 14). This observation could explain the failure of in vivo experiments conducted on a chloroquine sensitive strain of *P. berghei* (Frederich et al., 1999a). Afterwards, it was shown that strychnopentamine (3) and isostrychnopentamine (4) were active with the same  $IC_{50}$  against all strains of *P. falciparum* ( $IC_{50}$  = 120 nM and SI = 60) (Frederich et al., 1999a) and at all stages of development, including rings (Frederich et al.,

2004b). Isostrychnopentamine (4) was also active in vivo with an ED<sub>50</sub> of 30 mg/kg (i.p., *P. berghei* and *P. vinckei petteri*) (Frederich et al., 2004b).

In the continuation of this paragraph dedicated to the indole analogues of emetine, we will cite a study published in 1996 and devoted to *Pogonopus tubulosus* (Sauvain et al., 1996). This tree from South-America is used traditionally in Bolivia against malaria (decoction of stem bark) and is called "*falsa quina*". A bioguided fractionation attributed the antiplasmodial properties to the alkaloidal fraction and mainly to tubulosine (2), already cited previously. Tubulosine is active against all strains of *P. falciparum* (IC<sub>50</sub> = 24 nM) but has no selectivity compared to human cells. Active in vivo (ED<sub>50</sub> = 1 mg/kg), there was also shown to be a high level of toxicity (all mice died following a dose of 2 mg/kg).

## 2. Other bisindole alkaloids from *Strychnos* species

As some bisindole alkaloids from *Strychnos* species showed some promising activity, our team in Liège continued to screen several of these species and various alkaloids isolated from these plants (Frederich et al., 1999b; Frederich et al., 1999a; Frederich et al., 2002; Frederich et al., 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), which were responsible for the activity of the plant (Frederich et al., 2000; Frederich et al., 2001a) (Figure 3). The most active compounds were isosungucine (8) (IC<sub>50</sub> = 168 nM - CQR), 18-hydroxy-isosungucine (9) (IC<sub>50</sub> = 85 nM - CQR) and strychnogucine B (10) (IC<sub>50</sub> = 85 nM -CQR) with, for the last one, an SI of 176 (W2-CQR). These compounds were clearly more active against CQR than against CQS strains. Isosungucine, isolated in higher quantities than the two other compounds was evaluated in vivo and showed an ED<sub>50</sub> of close to 30 mg/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 *Strychnos 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), matopensine and longicaudatine (12), but all three were shown to be less active than isostrychnopentamine or strychnogucine B (Figure 3) (Frederich et al., 2002).

## 3. 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), which possess, respectively, IC<sub>50</sub>s of 270 nM and 360 nM against a CQR strain of *P. falciparum* (Keawpradub et al., 1999).

*Tabernaemontana fuchsiaefolia* A. DC. (synonym: *Peschiera fuchsiaefolia* (DC) Miers) is again 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 again to bisindole alkaloids, the principal one being voacamine (15, Figure 4) ( $IC_{50} = 411$  nM, SI = 47 on CQR strain) (Federici et al., 2000). Voacamine is active in vivo (43% of reduction of parasitaemia at 10 mg/kg p.o. in the 4-day suppressive test of Peters) and presents some specificity for trophozoites and schizonts (Ramanitrahasimbola et al., 2001).

A phytotherapeutic preparation containing *Peschiera fuchsiaefolia* extracts is used in 13 African countries and in the Dominican Republic under the registered names MMH Malarex<sup>®</sup> and MMH 18<sup>®</sup>. The company Millenia Hope claims to have carried out clinical trials, but the absence of scientific publications aroused questions from the scientific community about the efficacy and the exact composition of this preparation (Martindale, 2002; Orellana, 2003).

#### 4. Semi-synthetic bisindole and trisindole

To the best of our knowledge, the only indole semi-synthetic antiparasmodial 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 antiparasmodial 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) ( $IC_{50} = 540$  nM, SI = 47, CQR) and a trimeric derivative of festuclavine (16) ( $IC_{50} = 540$  nM, SI = 185, CQR). Nevertheless, these compounds, administered to mice, induced behavioural modifications, inviting caution (Jenett-Siems et al., 2004).

#### 5. Indoloquinolines

It is impossible to write about indolic alkaloids and malaria without mentioning cryptolepine, isolated from *Cryptolepis sanguinolenta*. Cryptolepine (18, Figure 6) is an indole alkaloid, but not from indolomonoterpenic biosynthetic pathways. This alkaloid, initially known as synthetic (synthesis in 1906 (Fichter & Boehringer, 1906)), was then isolated from roots of *C. sanguinolenta*, previously known as *C. triangularis*, (*Asclepiadaceae*) in 1929 (Clinquart, 1929). Cryptolepine 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, antidiarrhoeal and febrifuge remedy in Central and Western Africa. Between 1995 and 1997, three independent teams evidenced the antiparasmodial properties of cryptolepine in vitro ( $IC_{50} = 114$  nM, SI = 9, CQR) and in vivo (mice,  $ED_{50} < 50$  mg/kg p.o. and  $DE_{50} = 10$  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 have been synthesized. The most interesting are:

- 2,7-dibromocryptolepine ( $IC_{50} = 49$  nM, CQR), active against *Plasmodium berghei* in mice (90% of suppression of parasitaemia at 12.5 mg/kg i.p.) (Onyeibor et al., 2005; Wright et al., 2001);
- 1-methyl- $\delta$ -carboline (21), anhydronium base with an  $IC_{50}$  of 1.5  $\mu$ M and an SI of higher than 100 (Arzel et al., 2001);
- 2-bromoneocryptolepine, less active than cryptolepine ( $IC_{50} = 4$   $\mu$ M), but which also presents less affinity towards DNA. Neocryptolepine (19) is a natural alkaloid from the genus *Cryptolepis* (Jonckers et al., 2002; Van Miert et al., 2004);
- Isonocryptolepine (20), synthetic, ( $IC_{50} = 40$  nM) and N-methyl-isocryptolepinium iodide (23) ( $CI_{50} = 17$  nM), these two compounds presenting a much smaller cytotoxicity than cryptolepine. Nevertheless, they 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 high level of cytotoxic, genotoxic, DNA intercalating, topo II inhibition properties (Ansah et al., 2005; Ansah & Gooderham, 2002; Bonjean et al., 1998; Lisgarten et al., 2002). Further studies are therefore needed prior to the development of an antimalarial drug from this family.

## 6. 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 lyolytica* cultures (Schindler & Zahner, 1971), tryptanthrin has been subsequently isolated from several higher plants: *Couroupita guianensis* from Amazonia, *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 thryptanthrin, firstly against *Leishmania* sp., then against *Plasmodium falciparum*. Tryptanthrin and several analogues were tested and showed very low  $IC_{50}$  values: 69 ng/ml for thryptanthrin and 0.43 to 10 ng/ml for some of the analogues. These compounds were particularly active against atovaquone, chloroquine or mefloquine resistant strains. Novel tryptanthrin compounds, possessing increased solubility, have been recently prepared 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).

## 7. Marine sources

Several 1-aminopolycyclic  $\beta$ -carbolin alkaloids isolated from marine sources possess high in vitro and in vivo antimalarial properties (Ang et al., 2000; Rao et al., 2003; Rao et al., 2004). The most active alkaloids are manzamine A ( $IC_{50} = 4.5 - 8$  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% (versus 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 days and manzamine A has also been shown to be active via the oral route. The development of these compounds was supported by "Medicine 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 5 times higher than the concentration inducing a parasitaemia decrease.

## 8. 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: rasped stems of *Strychnos myrtoides*, *S. diplotricha* and *S. mostueoides* were used in decoctions associated to a sub-therapeutic dose of chloroquine (100-200 mg) (Rasoanaivo et al., 1996b; Rasoanaivo et al., 2002; Rasoanaivo et al., 2005). Phytochemical investigations of these species led to the isolation of two bioactive alkaloids: strychnobrasiline (26, Figure 8) and malagashanine (27) (Caira & Rasoanaivo, 1995; Rasoanaivo et al., 1994; Rasoanaivo et al., 1996a).

These two alkaloids are very weak antiplasmodial compounds ( $IC_{50}$  around 100  $\mu$ M), but, associated to 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*, but there, 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 quinine, mefloquine, halofantrine, quinacrine and pyronaridine (Rafatro et al., 2000a). A very small clinical study has also been conducted in Antananarivo (20 patients, chloroquine + 500 mg stem bark *S. myrtoides*) and showed promising results (Ramialiharisoa et al., 1994). Nevertheless, other studies following WHO guideline 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 (Ramanitrahasimbola et al., 2006). Metabolization of malagashanine has also been studied, on human microsomes and rats (Rafatro et al., 2000b; Rafatro et al., 2000c) and toxicity studies have been conducted on guinea pigs: malagashanine appears as not cytotoxic and without cardiac toxicity (either alone or associated to 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 (Figure 8, 28) and isoretuline (29), 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 potentiating effect was also demonstrated for N-formyl-aspidospermidine (30) and aspidospermine (31) (Mitaine-Offier et al., 2002).

## 9. 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  $\mu\text{M}$  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 of higher than 400 and a quite important steric crowding:

- indole "analogues" of emetine (usambarensine, ochrolifuanine, strychnopentamine),
- other bisindole alkaloids such as voacamine, 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 groups 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 contains insaturated monomeric heterocycles. These are chemically much more attainable. There are two main models that have been developed:

- derivatives of cryptolepine (the most interesting compound being 2,7-dibromocryptolepine);
- 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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Table 1: Antiplasmodial IC<sub>50</sub> of various indole alkaloids and selectivity index (SI), if available. CQR = chloroquine resistant strain, MQR = mefloquine resistant strain.

Alkaloid	IC <sub>50</sub> (μM)	SI	Strain	Resistance
quinine	0.413	ND	W2	CQR
dihydrousambarensine	0.032	375	W2	CQR
isostrychnopentamine	0.152	49	W2	CQR
strychnogucine B	0.085	176	W2	CQR
tubulosine	0.024	0.9	INDO	CQR
voacamine	0.411	47	W2	CQR
villalstonine	0.270	18	K1	CQR
macrocarpamine	0.360	13	K1	CQR
cryptolepine	0.114	9	K1	CQR
neocryptolepine	2.610	1.2	K1	CQR
isoneocryptolepine	0.230	19	K1	CQR
N-methyl-isocryptolepinium	0.017	747	K1	CQR
2-bromoneocryptolepine	4.0	> 8	W2	CQR
2,7-dibromocryptolepine	0.049	122	K1	CQR
1-methyl-delta-carboline	1.5	> 100	K1	CQR
thryptantrine	0.278	-	W2	CQR
manzamine A	0.956	-	W2	CQR
dimeric derivative of terguride ("p-xylene linked")	0.54	46	Dd2	CQR - MQR
trimeric derivative of festuclavine	0.54	>185	Dd2	CQR - MQR

## FIGURES LEGEND

FIGURE 1: Chemical structures of compounds 1-2.

FIGURE 2: Chemical structures of compounds 3-6. The circle indicate the isomery in isostrychnopentamine.

FIGURE 3: Chemical structures of compounds 7-12. The circles indicate the isomery between sungucine and isosungucine.

FIGURE 4: Chemical structures of compounds 13-15.

FIGURE 5: Chemical structures of compounds 16-17.

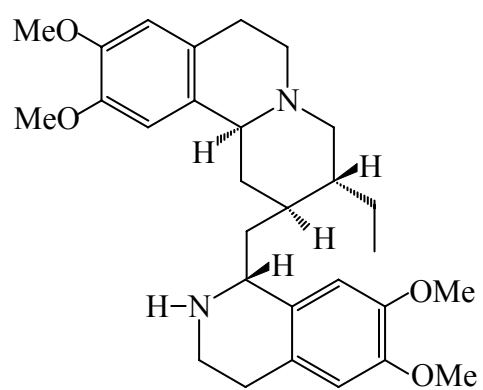
FIGURE 6: Chemical structures of compounds 18-23.

FIGURE 7: Chemical structures of compounds 24-25.

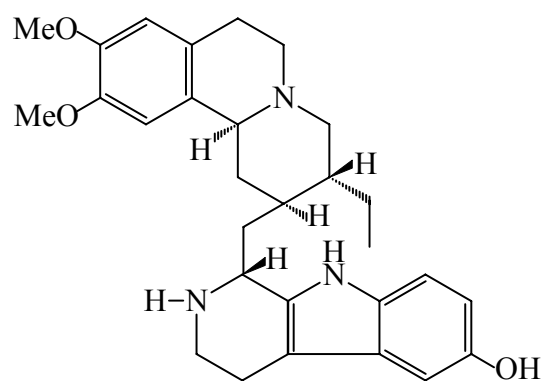
FIGURE 8: Chemical structures of compounds 26-31.



Fig. 1

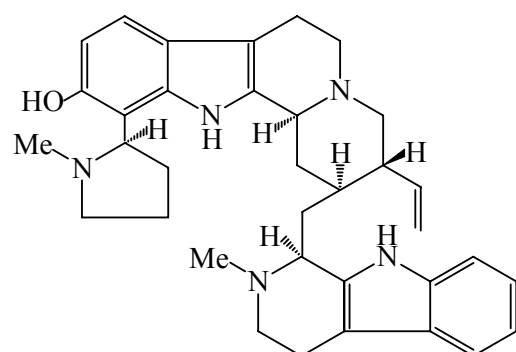


Emetine (1)

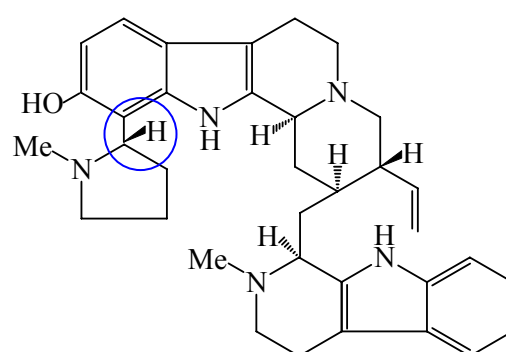


Tubulosine (2)

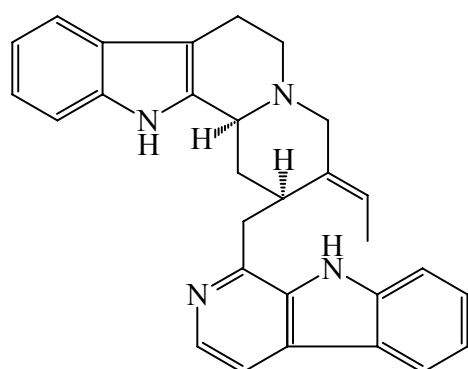
Fig. 2



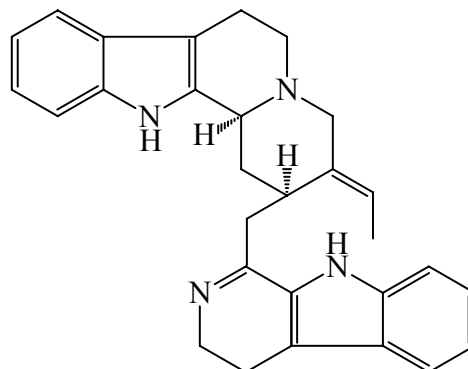
Strychnopentamine (3)



Isostrychnopentamine (4)

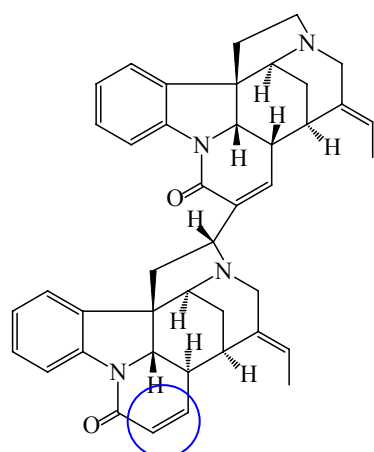


Usambarensine (5)

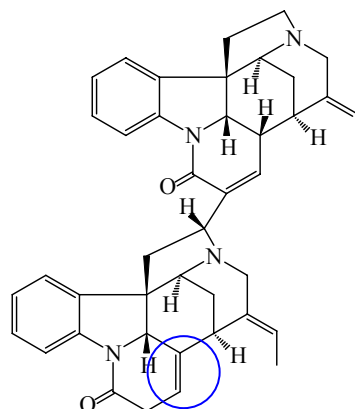


Dihydrousambarensine (6)

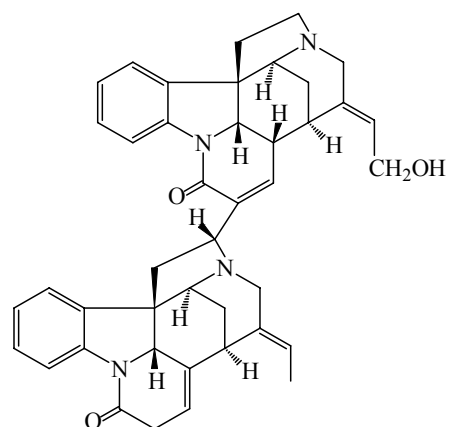
Fig. 3



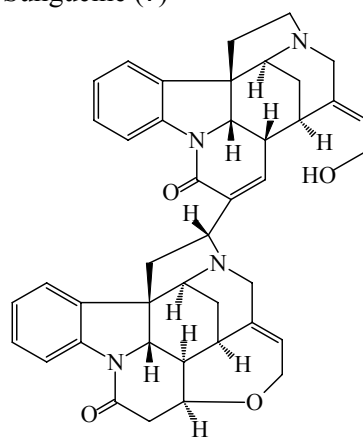
Sungucine (7)



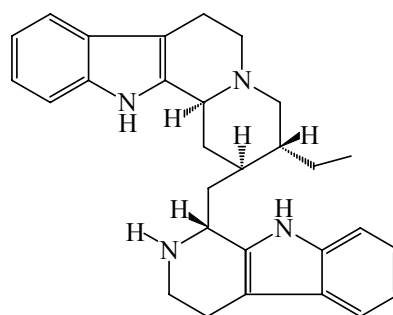
Isosungucine (8)



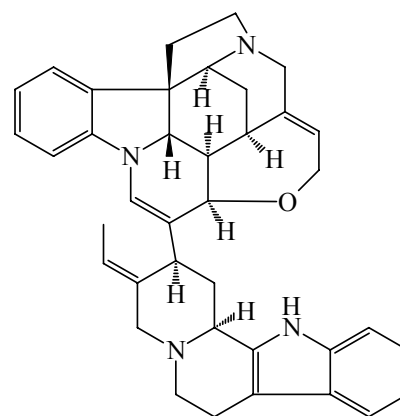
18-Hydroxyisosungucine (9)



Strychnogucine B (10)

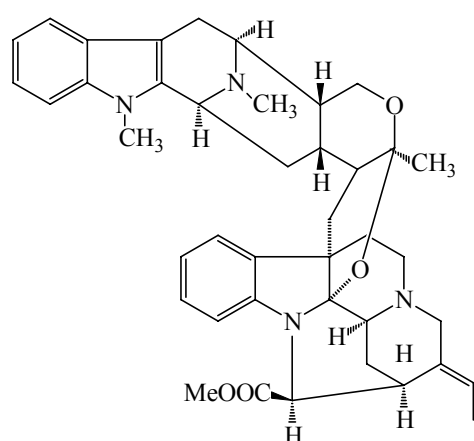


Ochrolifuanine A (11)

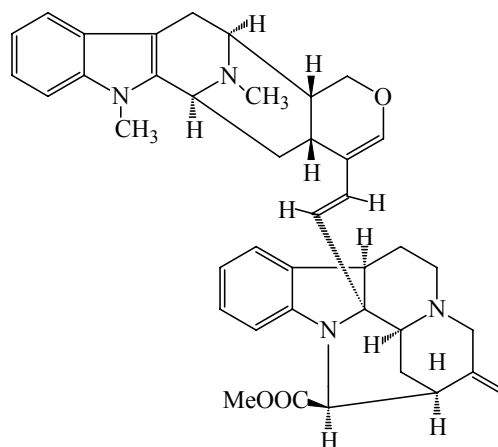


Longicaudatine (12)

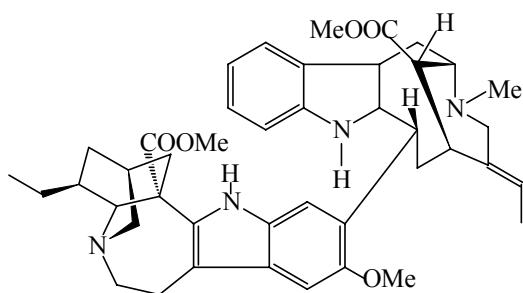
Fig. 4



Villalstonine (13)

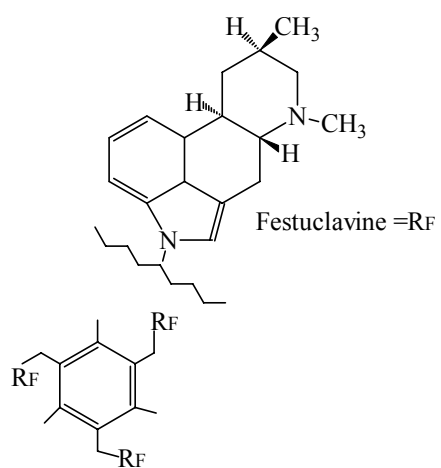


Macrocarpamine (14)

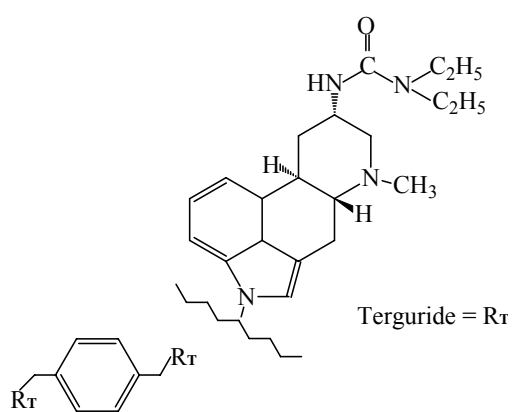


Voacamine (15)

Fig. 5

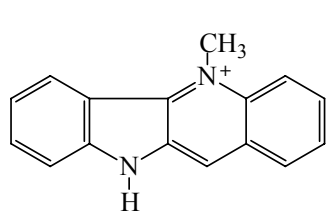


Trimeric derivative of Festuclavine (**16**)

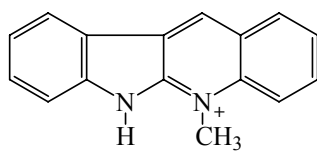


Dimeric derivative of Terguride (**17**)

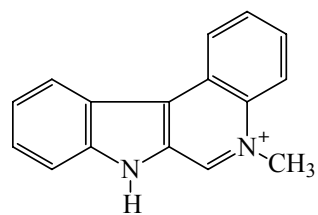
Fig. 6



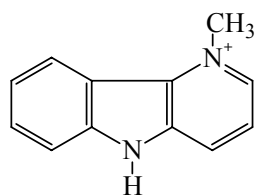
Cryptolepine (**18**)



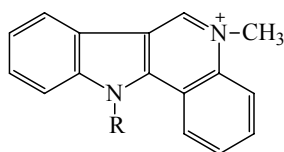
Neocryptolepine (**19**)



Isonocryptolepine (**20**)



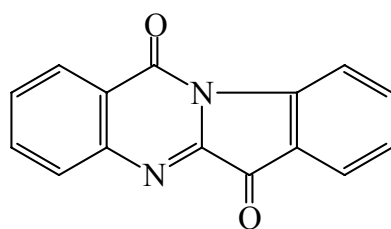
N1-Methyl- $\delta$ -carboline (**21**)



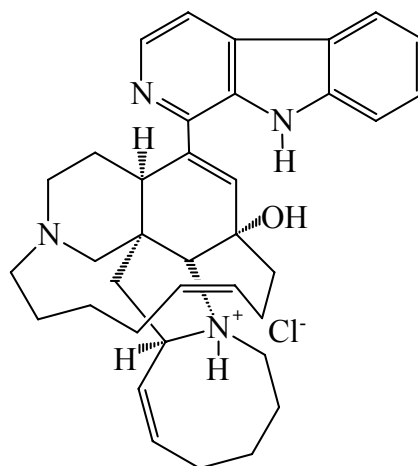
R=H Isocryptolepine (**22**)

R=CH<sub>3</sub> N-Methylisocryptolepinium (**23**)

Fig. 7

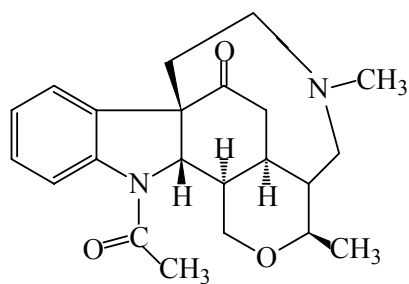


Tryptanthrine (24)

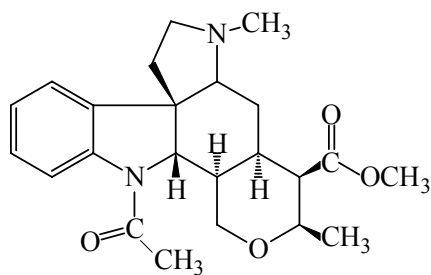


Manzamine A (25)

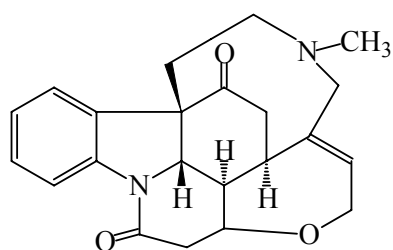
Fig. 8



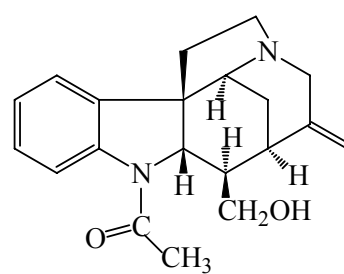
Strychnobrasiline (26)



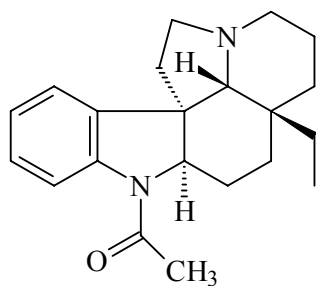
Malagashanine (27)



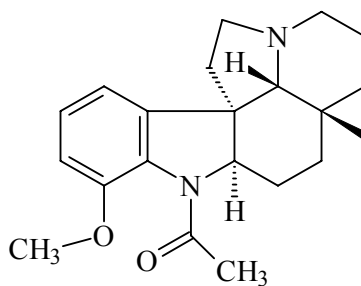
Icajine (28)



Isoretuline (29)



N-formyl-aspidospermidine (30)



Aspidospermine (31)