Phytomedicine 20 (2012) 71–76

Contents lists available at SciVerse ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Comparative *in vitro* and *in vivo* antimalarial activity of the indole alkaloids ellipticine, olivacine, cryptolepine and a synthetic cryptolepine analog

L.F. Rocha e Silva[a,b,c], A. Montoia[a,b], R.C.N. Amorim[a], M.R. Melo[a], M.C. Henrique[a,b], S.M. Nunomura[a], M.R.F. Costa[d], V.F. Andrade Neto[e], D.S. Costa[a,c], G. Dantas[e], J. Lavrado[f], R. Moreira[f], A. Paulo[f], A.C. Pinto[g], W.P. Tadei[a], R.S. Zacardi[h], M.N. Eberlin[h], A.M. Pohlit[a,*]

[a] *National Institute for Amazonian Research, Av. André Araújo, 2936, Aleixo, 69060-001 Manaus, Amazonas, Brazil*
[b] *Federal University of Amazonas, Av. General Rodrigo Otávio, 3000, Coroado, 69077-000 Manaus, Amazonas, Brazil*
[c] *University Center of the North, R. Dez de Julho, 873, Centro, 69010-060 Manaus, Amazonas, Brazil*
[d] *Heitor Vieira Dourado Tropical Medicine Foundation, Av. Pedro Teixeira, 25, Dom Pedro, 69040-000 Manaus, Amazonas, Brazil*
[e] *Federal University of Rio Grande do Norte, C.P. 1524, Campus Universitário Lagoa Nova, 59078-970 Natal, Rio Grande do Norte, Brazil*
[f] *University of Lisbon, Alameda da Universidade, 1649-004 Lisbon, Portugal*
[g] *Federal University of Rio de Janeiro, Av. Athos da Silveira Ramos, 149, Cidade Universitária, 21941-909 Rio de Janeiro, Brazil*
[h] *State University of Campinas, Campus Universitário Zeferino Vaz SN, Cidade Universitaria Campinas, 13083-970 Campinas, São Paulo, Brazil*

ARTICLE INFO

Keywords:
Plasmodium falciparum
Plasmodium berghei
Murine macrophages
Antiplasmodial activity
Indole alkaloids

ABSTRACT

Indole alkaloids ellipticine (**1**), cryptolepine triflate (**2a**), rationally designed 11-(4-piperidinamino)cryptolepine hydrogen dichloride (**2b**) and olivacine (**3**) (an isomer of **1**) were evaluated *in vitro* against *Plasmodium falciparum* and *in vivo* in *Plasmodium berghei*-infected mice. **1**–**3** inhibited *P. falciparum* ($IC_{50} \leq 1.4$ μM, order of activity: **2b** > **1** > **2a** > **3**). *In vitro* toxicity to murine macrophages was evaluated and revealed selectivity indices (SI) of 10–12 for **2a** and SI > 2.8×10^2 for **1**, **2b** and **3**. **1** administered orally at 50 mg/kg/day was highly active against *P. berghei* (*in vivo* inhibition compared to untreated control (IVI) = 100%, mean survival time (MST) > 40 days, comparable activity to chloroquine control). **1** administered orally and subcutaneously was active at 10 mg/kg/day (IVI = 70–77%; MST = 27–29 days). **3** exhibited high oral activity at ≥50 mg/kg/day (IVI = 90–97%, MST = 23–27 days). Cryptolepine (**2a**) administered orally and subcutaneously exhibited moderate activity at 50 mg/kg/day (IVI = 43–63%, MST = 24–25 days). At 50 mg/kg/day, **2b** administered subcutaneously was lethal to infected mice (MST = 3 days) and moderately active when administered orally (IVI = 45–55%, MST = 25 days). **1** and **3** are promising compounds for development of antimalarials.

Introduction

Despite great effort and resources for the eradication of malaria this disease still remains a grave public health problem involving hundreds of thousands of deaths annually (WHO 2010). While research on vaccines is at an advanced stage, drug therapy is still the principle tool for the control and eradication of the disease. The emergence of strains of *Plasmodium falciparum* and *P. vivax* which are resistant to first and second line antimalarials (multidrug resistant or MDR) have motivated the search for new drugs representing new and distinct chemical classes and mechanisms of action than those of the antimalarial drugs currently in use. Chemical compounds of novel structure and of natural origin represent a major source for the discovery and development of new drugs for diseases, especially malaria (Kaur et al. 2009; Schmidt et al. 2012a,b).

Historically, plants used in traditional medicine as antimalarials and febrifuges have provided substances which have proved to be useful as antimalarials or have served chemists as structural models for the development of semi-synthetic drugs or purely synthetic analogs. This is true of the most important antimalarial natural products revealed to date: quinine (isolated from the bark of *Cinchona* spp.) and artemisinin (isolated from *Artemisia annua* leaves). The therapeutic efficacy and complex molecular structure of quinine lead to the development of purely synthetic analogs chloroquine, primaquine, mefloquine, among others in the last century. More recently, semi-synthetic derivatives (*e.g.* sodium artesunate, artemether, arteether and dihydroartemisinin) prepared in one or more steps from isolated artemisinin have become key pharmaceutical components in formulations used in what is commonly called artemisinin combination therapy (ACT) for the treatment of resistant and MDR *Plasmodium falciparum* infections

* Corresponding author. Tel.: +55 92 3643 3177; fax: +55 92 3643 3079.
E-mail addresses: ampohlit@inpa.gov.br, ampohlit@gmail.com (A.M. Pohlit).

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http://dx.doi.org/10.1016/j.phymed.2012.09.008

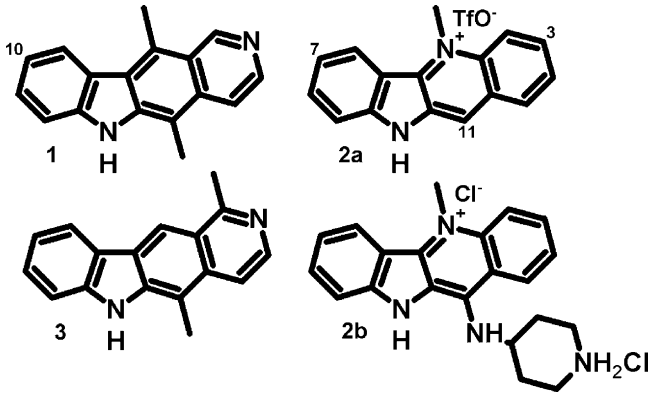

Fig. 1. Structures of antimalarial compounds ellipticine (**1**), cryptolepine triflate (**2a** triflate), 11-(4-piperidinamino)cryptolepine hydrogen dichloride (**2b**) and olivacine (**3**).

(Plowe 2009; Willcox 2011). The extracts of a large number of plant species including many that are used in traditional medicine have been evaluated for *in vitro* antiplasmodial activities and some have also been tested in *in vivo* models, usually in mice infected with *Plasmodium berghei*, *P. yoelii* or *P. chabaudi*. In some cases, the constituent(s) responsible for their activities have been isolated but relatively few have been studied further to assess their potential as lead compounds for the development of new antimalarial drugs (Wright 2005).

In recent years, the monoterpene indole alkaloid ellipticine (**1**, Fig. 1) has been the subject of a number of pharmacological studies and its derivatives have been studied in clinical trials against different forms of cancer. Ellipticine has been isolated from the alkaline ethanol extract of the bark of the Amazonian tree *Aspidosperma vargasii* (Apocynaceae) (Andrade-Neto et al. 2007; Henrique et al. 2010) which is used in traditional medicine as an antimalarial (Oliveira et al. 2003). *In vitro* antiplasmodial activity of **1** was first reported by Andrade-Neto et al. (2007). Recently, the antimalarial activity of **1** was independently confirmed and the comparable or superior activity of four derivatives of **1** against *P. falciparum in vitro* was described (Passemar et al. 2011; Pohlit et al. 2012).

The roots of the West African climbing shrub *Cryptolepis sanguinolenta* (Lindl.) Schltr. (Apocynaceae) are a traditionally used herbal for malaria treatment. Dry aqueous root extracts of *C. sanguinolenta* have proven efficacy according to clinical trials (Willcox 2011). Cryptolepine (**2a**, Fig. 1) is the major alkaloid constituent in the roots of this plant and **2a** sulfate exhibits *in vitro* activity (IC_{50} = 0.44 μM) against multidrug-resistant K1 strain of *P. falciparum* (Wright et al. 2001). However, **2a** sulfate failed to cure malaria in mice when given orally and is toxic at a dose of 20 mg/kg to *P. berghei*-infected mice when administered intraperitoneally (i.p.) (Cimanga et al. 1997; Wright et al. 2001). Also, **2a** has *in vivo* chronic effects causing necrosis of rodent liver cells at a dose of 30 mg/kg and also damages the DNA of lymphocytes *in vitro*. These and other experimental results do not support the use of the indole alkaloid cryptolepine (**2a**) as an antimalarial (Gopalan et al. 2011; Willcox 2011).

Derivatives of cryptolepine (**2a**) have been introduced having greater antiplasmodial activity and better toxicity profiles. One of the most promising of these derivatives is 2,7-dibromocryptolepine which has potent *in vitro* activity (IC_{50} = 50 nM) against K1 strain of *P. falciparum* and exhibits significant suppression (89–91%) of *P. berghei* growth in infected mice at doses of 20–25 mg/kg/day over 4 days. While 2,7-dibromocryptolepine does *not* apparently intercalate DNA bases as does **2a**, both these compounds damage lymphocyte DNA based on results of the comet assay (Wright et al. 2001; Gopalan et al. 2011). Recently, cryptolepine triflate (**2a** triflate) and ten synthetic analogs of **2a** containing aminoalkyl side chains at C-11 were synthesized and screened for *in vitro* antiplasmodial activity and cytotoxicity. One of the most promising of these compounds was 11-(4-piperidinamino)cryptolepine hydrogen dichloride (**2b**) which exhibited potent inhibitory activity (IC_{50} = 44 nM) against *P. falciparum* W2 strain and was the least toxic of all the compounds tested, including **2a** triflate and had the largest cytotoxicity to antiplasmodial inhibition ratio (46.4:1) (Lavrado et al. 2008, 2011).

Olivacine (**3**) is a rare alkaloid which is isolated from *Aspidosperma olivaceum*. The antitumor activity of **3** has been the subject of studies for decades. Compound **3** and analogs have also been synthesized (Besselièvre & Husson 1981; Chevallier-Multon et al. 1990; Guillonneau et al. 2005).

Mechanistic studies demonstrate that cryptolepine (and it analogs) and ellipticine (**1**) may have important inhibitory effects on the formation of hemozoin in *P. falciparum*. Hemozoin formation is a fundamental process related to the survival of this parasite within the red blood cell. Heme is toxic to *Plasmodium* spp. and is a by-product of digestion of hemoglobin by proteolytic enzymes in the parasite's digestive vacuole. Crystallization of heme to hemozoin is a detoxifying process that occurs naturally within the *Plasmodium* digestive vacuole and is necessary for the proliferation of these parasites within the red blood cell. Inhibition of hemozoin formation is associated with death of parasites within the red blood cell due to osmotic imbalances and other effects. Early mechanistic studies involving chemical assays showed that ellipticine (**1**) can inhibit heme crystal growth (that is, hemozoin formation) in the lab (Chong and Sullivan 2003). In other work, it was presumed that cryptolepine (**2a**) interacts directly with heme molecules in the process of inhibiting hemozoin formation (Kumar et al. 2007; Lavrado et al. 2011).

Previous studies point to the fact that large or small structural differences among analogous indole alkaloids, such as cryptolepine analogs and β-carbolines (harmane analogs), can lead to large differences in *in vitro* and *in vivo* antimalarial activity and cytotoxicity of these compounds. Thus, small structural differences probably modulate and ultimately define the primary mechanisms of action of these compounds (DNA intercalation, inhibition of heme polymerization, inhibition of protein synthesis, among other mechanisms yet to be revealed) (Arzel et al. 2001; Ancolio et al. 2002; Van Baelen et al. 2009).

In the present study, the antiplasmodial activity of structurally related indole alkaloids ellipticine (**1**), cryptolepine derivative **2b** and olivacine (**3**) is investigated for the first time in *P. berghei*-infected mice and the data are compared to those for cryptolepine triflate (**2a** triflate). The *in vitro* antiplasmodial activity against chloroquine-resistant and chloroquine sensitive strains of *P. falciparum* and cytotoxicity of these compounds was evaluated *in vitro* against murine macrophages as a means to comparatively evaluate selectivity of the antimalarial effect. The overall aim of this work was to provide comparative *in vitro* and especially *in vivo* antimalarial data for all four compounds which might lead to insights into the relative importance of cryptolepine and ellipticine ring systems/skeletons for the further development of antimalarials.

Materials and methods

Chemicals

Ellipticine (**1**) used in this work was obtained from two sources. Synthetic **1** was purchased from Sigma–Aldrich (Steinheim, Germany). Also, **1** was isolated from the bark of *Aspidosperma vargasii* from INPA's Ducke Reserve in Amazonas State, Brazil through an alkaline extraction sequence followed by column

chromatography as described previously (Andrade-Neto et al. 2007; Henrique et al. 2010). Cryptolepine triflate (**2a** triflate) and cryptolepine analog **2b** were obtained by synthesis as described previously (Lavrado et al. 2008). Olivacine (**3**) was isolated from *Aspidosperma olivaceum* from Minas Gerais State, Brazil, by acid–base extraction. The purity of these compounds was checked by TLC, UPLC–MS and NMR and was >98%.

Culture and test for in vitro inhibition of P. falciparum parasites

Strains of P. falciparum used in this study were the antimalarial drug-susceptible 3D7 clone of the NF54 isolate and the chloroquine-resistant, pyrimethamine-resistant and cycloguanil-resistant K1 strain. Parasites were cultured according to the method of Trager and Jensen (1976) as modified by Andrade-Neto et al. (2007). The parasite culture was carried out at 37 °C with a hematocrit of 3–5% and in an atmosphere of 5% CO_2. The parasites were maintained *in vitro* in A+ human red blood cells. The culture medium was RPMI 1640 (Sigma–Aldrich) supplemented with 10% human serum and containing 25 mM HEPES and 2 mM L-glutamine. The micro-test was performed using the method of Rieckmann et al. (1978) with modifications which were described in Andrade-Neto et al. (2007). Stock solutions of indole alkaloids ellipticine (**1**), cryptolepine triflate (**2a** triflate), olivacine (**3**) and 11-substituted cryptolepine hydrogen dichloride analog **2c** were prepared in DMSO at a concentration of 5.0 mg/ml. Seven dilutions were performed in culture medium (RPMI 1640) of each stock sample solution resulting in final test concentrations (well concentrations) of 50–3.2×10^{-3} μg/ml. Each diluted sample was tested in duplicate in 96-well test plates containing a suspension of parasitized red blood cells at a hematocrit of 3% and initial parasitemia of 1% of synchronized young trophozoites (ring forms). The final volume of each well was 200 μl. Reference antimalarial compounds chloroquine and quinine were used as positive controls at concentrations recommended by WHO (2001). The test plate was incubated for 48 h at 37 °C under the same low oxygen gas mixture used for parasite culture. After the incubation period, thin smears were prepared from the contents of each well and evaluated using a microscope. The half maximal inhibitory (IC_{50}) responses compared with the drug-free controls were estimated by interpolation using Microcal Origin® software.

Test for in vivo suppression of Plasmodium berghei

In vivo antimalarial activity was evaluated using *P. berghei* NK65 strain (drug-sensitive). This strain was maintained by successive passages of blood forms from mouse to mouse. The test protocol is based on the 4-day suppressive test as described by Peters (1965). Female Webster Swiss mice weighing 26 ± 2 g were used in this study. Animals were infected intraperitoneally with 0.2 ml of infected blood suspension containing 1×10^5 parasitized erythrocytes and randomly divided into groups of three individuals. Test groups were treated orally and subcutaneously at doses ranging from 100 to 1 mg/kg/day. Positive control groups received a dose of 10 mg chloroquine/kg/day orally or subcutaneously and negative control groups received 0.2 ml of 2% DMSO or saline. The animals were treated for 4 days starting 24 h after inoculation with *P. berghei*. On days 5 and 7 after inoculation with parasites, blood smears were prepared from all mice, fixed with methanol, stained with Giemsa dye, then microscopically examined (1000× magnification). Parasitemia was determined in coded blood smears by randomly counting 2000–4000 erythrocytes in the case of low parasitemias (≤10%); or up to 1000 erythrocytes in the case of higher parasitemias. Overall mortality was monitored daily in all groups during a period of 4 weeks following inoculation. The difference between the average parasitemia of control groups (100%) and test groups was calculated as a percentage of parasite growth suppression (PGS) according to the equation: $PGS = 100 \times (A - B)/A$, where A is the average parasitemia of the negative control group and B corresponds to the parasitemia of the test group.

Cytotoxicity test

For this test, macrophages from Swiss mice were used which were described in Mota et al. (2012), with modifications. The macrophages were obtained at the time of use by collection with cold, sterile phosphate saline solution (PSB) from the exudates of the peritoneal cavity of mice. After centrifuging the peritoneal exudate solutions, the supernatant was discarded and pellet was re-suspended in 5 ml of RPMI medium without FBS for counting the macrophages in a Neubauer chamber. 1×10^5 cells were added to each well. The plate was incubated in a CO_2 incubator at 37 °C for 24 h. The cytotoxicity of the samples was determined using the methylthiazoletetrazolium (MTT) colorimetric assay (Mosmann 1983). For the assays, the cells were trypsinized, washed, suspended in DMEM, and distributed into 72 wells per plate (5×10^3 cells per well) then incubated for 18 h at 37 °C. The samples were separately diluted in DMSO and tested in triplicate at the following concentrations: 1.5, 3.1, 6.3, 12.5, 25, 50, and 100 μg/ml. In parallel, we evaluated a control group consisting of RPMI 1640 without FBS, a control group consisting of 1% DMSO (vehicle) and a positive control (chloroquine, BS Pharma, Belo Horizonte, MG, Brazil) at the same concentrations used for substances **1–3**. After 24 and 48 h of incubation at 37 °C, 100 μl of MTT (5 mg/ml in RPMI 1640 without FBS and without phenol red) was added to each well. After 3 h in a CO_2 incubator at 37 °C, the supernatant was removed and added to 100 μl DMSO in each well. The absorbance of each well was obtained from a spectrophotometric reading at 562 nm. The minimum lethal doses that inhibited 50% of cell growth were obtained from the drug concentration response curves. Results are expressed in mean ± standard deviation.

Selectivity index

The relative cytotoxicity to antiplasmodial activity for a given compound was evaluated as a selectivity index (SI), where $SI = IC_{50(\text{murine macrophages})}/IC_{50(P.\ falciparum)}$.

Animals and ethical approval

Adult Webster Swiss albino mice (26 ± 2 g weight) were used for the antimalarial and toxicity tests and received water and food *ad libitum*. *In vivo* tests were performed using Guidelines for Ethical Conduct in The Care and Use of Animals of Federal University of Rio Grande do Norte (CEUA 043/2010).

Results

Indole alkaloids ellipticine (**1**), cryptolepine triflate (**2a** triflate) and olivacine (**3**) and synthetic analog 11-(4-piperidinamino)cryptolepine hydrogen dichloride (**2b**) were assayed for *in vitro* activity against *Plasmodium falciparum* K1 e 3D7 strains and cytotoxic activity against murine macrophages. From the IC_{50} values for each substance against murine macrophages and malaria parasite strains it was possible to determine selectivity indices. The *in vitro* results are presented in Table 1. Olivacine (**3**) is a previously known indole alkaloid for which antimalarial activity has not been previously described. It was the least active compound *in vitro*, however, it did significantly inhibit *P. falciparum* growth (IC_{50} = 1.2 μM against K1 strain). The potent *in vitro* activity of ellipticine (**1**) reported previously was confirmed herein (IC_{50} values of 0.81 and 0.35 μM against *P. falciparum* K1 and 3D7 strains,

Table 1
Median inhibition concentrations (IC_{50}) and selectivity indices (SI) for indole alkaloids **1**–**3** *in vitro* against *Plasmodium falciparum* K1 and 3D7 strains and murine macrophages.

Compound		IC_{50} (μM)			SI[a]
Name	No.	*P. falciparum*		Murine macrophages	
		K1	3D7		
Ellipticine	**1**	0.81	0.35	$>4.1 \times 10^2$	$>5.0 \times 10^2/>1.2 \times 10^3$
Cryptolepine triflate	**2a**	0.80	0.91	9.1	11/10
Cryptolepine analog	**2b**	0.10	0.087	34	$3.3 \times 10^2/3.9 \times 10^2$
Olivacine	**3**	1.4	1.2	$>4.1 \times 10^2$	$>2.9 \times 10^2/>3.4 \times 10^2$
Chloroquine diphosphate		0.13	0.058	1.4×10^2	$1.1 \times 10^3/2.4 \times 10^3$
Quinine sulfate		0.16	0.11	n.t.	–
Artemisinin		0.0021	0.0011	n.t.	–

Data values are expressed as two (2) significant figures as per the precision of the methods used. n.t.: not tested; –: not calculable.
[a] $SI = IC_{50(\text{"macrophages"})}/IC_{50(P.\ falciparum)}$ and is reported for K1/3D7 strains.

respectively). Ellipticine (**1**) and its structural isomer olivacine (**3**) were the least cytotoxic compounds in this study ($IC_{50} > 0.41$ mM, highest concentration tested). This low cytotoxicity contributed greatly to the high selectivity indices obtained for **1** and **3** against *P. falciparum* 3D7 ($>1.2 \times 10^3$ and $>3.4 \times 10^2$, respectively). The compound with the most *in vitro* activity against *P. falciparum* was rationally designed 11-(4-piperidinamino)cryptolepine hydrogen dichloride (**2b**) ($IC_{50} = 0.10$ and 0.087 μM, against *P. falciparum* K1 and 3D7 strains, respectively) which had been selected from among a number of synthetic cryptolepine analogs reported earlier based on its favorable *in vitro* antimalarial and cytotoxic properties reported in that earlier work (Lavrado et al. 2008). In the present work, cryptolepine analog **2b** exhibited an IC_{50} value against murine macrophages of 34 μM thus making it the second most cytotoxic compound after **2a** triflate. Relatively high cytotoxicity against murine macrophages lead to **2a** triflate and **2b** exhibiting the lowest SI values.

The indole alkaloids **1**–**3** were evaluated *in vivo* in *P. berghei*-infected mice in the 4-day suppressive test and the result is presented in Table 2. Ellipticine (**1**) was highly active at an oral dose of 50 mg/kg/day (100% inhibition versus controls on days 5 and 7). At this same dose, the mean survival time (MST) of the animals was >40 days (limit of the observation period and identical to the MST of the control substance chloroquine). Also, **1** had good oral activity on day 5 and good activity via subcutaneous injection on day 7 at 10 mg/kg/day (77 and 70% inhibition, respectively; MST = 27–29 days) and moderate oral activity at 1 mg/kg/day (61–67% inhibition, MST = 22–23 days). **3** exhibited high oral activity at ≥50 mg/kg/day (90–97% inhibition, MST = 23–27 days) and low to moderate oral and subcutaneous activity at 1 and 10 mg/kg/day (7–64% inhibition, MST = 24–27 days). Cryptolepine triflate (**2a** triflate) exhibited only moderate oral and subcutaneous activity at 50 mg/kg/day (43–63% inhibition, MST = 24–25 days). At a dose of 50 mg/kg/day, subcutaneously injected cryptolepine derivative **2b** was lethal to infected mice (MST = 3 days) and oral activity at this dose was moderate (45–55% inhibition, MST = 25 days). At 10 mg/kg/day, **2b** administered orally and subcutaneously exhibited low to moderate activity (25–60% inhibition, MST = 24 days).

Discussion

Indole alkaloids **1**–**3** were active against chloroquine-resistant K1 and chloroquine-sensitive 3D7 strains of *P. falciparum*. Thus, **1**–**3** do not exhibit cross-resistance to chloroquine, though, as discussed below, like chloroquine, they inhibit hemozoin formation.

The *in vitro* activity of **1** against *P. falciparum* was described for the first time by Andrade-Neto (2007) ($IC_{50} = 73$ nM, K1 strain) and was recently independently confirmed against the chloroquine-resistant FcM29-Cameroon strain of *P. falciparum* ($IC_{50} = 1.13$ μM) (Passemar et al. 2011; see also Pohlit et al. 2012). Presented herein are the first data on the antimalarial activity of olivacine (**3**) which exhibited important *in vitro* antimalarial activity and low cytotoxicity.

During intraerythrocytic infection, *P. falciparum* parasites crystallize toxic heme released during hemoglobin catabolism resulting in hemozoin formation. In mechanistic studies, **1** (ICG, $IC_{50} = 7.9$ μM) exhibited greater *in vitro* inhibition of hemozoin crystal growth (ICG) than quinine (ICG, $IC_{50} = 17.1$ μM). ICG by **1** may occur by surface binding or a substrate sequestration mechanism (Chong and Sullivan 2003).

Alternatively, **1** may inhibit *Plasmodium* spp. by interaction with DNA. Highly planar, **1** intercalates DNA bases *in vitro* with high affinity. Also, **1** strongly inhibits DNA topoisomerase II *in vitro* (Moody et al. 2007). Recently, formation of covalent DNA adducts mediated by ellipticine oxidation with cytochrome P450 and peroxidases was proposed as a mode of action (Kotrbova et al. 2011).

Cryptolepine triflate (**2a** triflate) exhibited IC_{50} values of 0.80 and 0.91 μM against the K1 and 3D7 strains of *P. falciparum*, respectively, in agreement with earlier reports for **2a** against K1 (Cimanga et al. 1997; Wright et al. 2001) and 3D7 strains (Lavrado et al. 2011). Herein, **2a** triflate was the most toxic compound to murine macrophages ($IC_{50} = 9.4$ μM) and its selectivity indices (SI) were the lowest of all compounds studied (SI = 10–12).

Lavrado et al. (2008) synthesized analogs of **2a** containing diamino-alkane side chains at C-11. The basis for this approach was the observation that a basic amino side chain is a requirement for chloroquine accumulation in the acidic digestive vacuole of the parasite. These analogs of **2a** were potent inhibitors ($IC_{50} = 20$–455 nM) of *P. falciparum* strains having different drug resistance phenotypes (Lavrado et al. 2008, 2011). Herein, the rationally designed 11-(4-piperidinamino)cryptolepine **2b** which has optimal *in vitro* antimalarial and SI (Lavrado et al. 2008) was the most active compound against *P. falciparum in vitro*.

Cryptolepine analog **2b** was less toxic ($IC_{50} = 34$ μM) to macrophages than **2a** and exhibited good SI. Similarly, **2a** triflate and **2b** exhibited low toxicity to HUVEC cells ($IC_{50} = 1.18$ and 2.04 μM, respectively) (Lavrado et al. 2008, 2011). In previous work, **2a** exhibited high cytotoxic, genotoxic, DNA intercalating and topoisomerase II inhibitory properties (Bonjean et al. 1998). **2a** iodide, its derivatives and analogs (*e.g.* 2,7-dibromocryptolepine) cause DNA damage in lymphocytes, but do not affect human sperm. Due to *in vitro* DNA damage by **2a**, 2,7-dibromocryptolepine and related compounds, these substances may not be suitable for preclinical development as antimalarials (Gopalan et al. 2011).

Ellipticine (**1**), olivacine (**3**) and related ellipticine-like compounds have received attention due to their high toxicity to tumor cells and low number of side effects. Thus, derivatives of these compounds are excellent target compounds for clinical studies (Sizum et al. 1988; Jasztold-Howorko et al. 2004). In tumor cells, there is evidence that the mechanism of action involves DNA intercalation

Table 2
In vivo suppression of *Plasmodium berghei* in infected mice and average mouse survival time after oral and subcutaneous treatments with compounds **1**–**3**.

Dose (mg/kg/day)	% Parasite inhibition				Average survival time ± SD (day)	
	Oral		Subcutaneous		Oral	Subcutaneous
	Day 5	Day 7	Day 5	Day 7		
Ellipticine (**1**)						
50	100	100	NT	NT	>40	NT
10	77	42	33	70	27 ± 2	29 ± 3
1	67	61	0	44	22 ± 4	23 ± 5
Cryptolepine (**2a**)						
50	43	50	63	61	24 ± 5	25 ± 4
10	38	28	21	46	18 ± 3	21 ± 3
1	3	5	16	31	17 ± 3	22 ± 2
Cryptolepine analog (**2b**)						
50	55	44	D	D	25 ± 4	3 ± 1
10	47	25	60	30	24 ± 4	24 ± 4
Olivacine (**3**)						
100	97	90	14	55	26 ± 6	27 ± 2
50	91	90	NT	NT	23 ± 2	NT
10	48	64	40	44	27 ± 2	26 ± 2
1	25	42	7	45	24 ± 3	25 ± 1
Chloroquine						
10	99	98	98	99	>40	>40
Control	0	0	0	0	20 ± 5	22 ± 3

SD: standard deviation; NT: not tested; –: not calculable.

and interference with the activity of topoisomerase II with consequent cytotoxic effects which are related to size, shape and flatness of **1** and **3** (Carvalho and Laks 2001; Braga et al. 2004). Importantly, we observed low cytotoxicity for **1** and **3** against mouse macrophages whereas **2a** triflate and cryptolepine derivative **2b** exhibited relatively high toxicity to macrophages.

As seen above, compounds **1**–**3** inhibit *P. falciparum in vitro*. Only cryptolepine has been studied previously using *in vivo* antimalarial models. So, the effects of **1**–**3** in *P. berghei*-infected mice were explored. Ellipticine (**1**) was the most active compound *in vivo* suppressing parasitemia by 100% and providing MST of >40 days at an oral dose of 50 mg/kg/day. Remarkably, this was the same result obtained for control compound chloroquine at 10 mg/kg/day for all animals. Also, up to 77% inhibition of parasitemia was observed for **1** at doses of 10 mg/kg/day. Few compounds (*e.g.* chloroquine) significantly reduce parasitemia in mice infected with *P. berghei*. At the highest dose of **1** (50 mg/kg/day), no mortality or other signs of intoxication were observed. The *in vivo* antimalarial activity of **3** was lower than that of its structural isomer **1**. No toxic effects were observed in mice which were administered **3** at up to 100 mg/kg/day.

The *in vivo* antimalarial activity of **2a** triflate was low to moderate. Thus, **2a** triflate administered subcutaneously (50 mg/kg/day) inhibited *P. berghei* by 63% (day 5). Similarly, Kirby et al. (1995) observed that parasitemia in *P. berghei*-infected mice was not reduced by subcutaneously administered cryptolepine (113 mg/kg/day).

Cryptolepine iodide (**2a** iodide) was toxic to *P. berghei*-infected mice by intraperitoneal injection at 12.5 mg/kg/day (Wright et al. 2001). Toxicity is related to the intraperitoneal route of administration. However, no deaths were reported when **2a** iodide was administered subcutaneously (at 113 mg/kg/day) or orally (50 mg/kg/day) (Wright et al. 2001). Herein, **2a** triflate exhibited no toxic effects.

Cryptolepine analog **2b** exhibited moderate activity *in vivo* and acute toxicity. Orally at 50 mg/kg/day, **2b** inhibited the growth of *P. berghei* by 55%. However, subcutaneously at 50 mg/kg/day, **2b** killed all mice after the second dose. Subcutaneously, at 10 mg/kg/day, **2b** caused ulceration at the place of injection and inhibited *P. berghei* only moderately (60%).

Cryptolepine triflate (**2a** triflate) and derivatives (*e.g.* 2,7-dibromocryptolepine and **2b**) exhibit limited efficacy in *P. berghei*-infected mice. Also, cytotoxicity, acute toxicity and genotoxicity are potential drawbacks to their use as drugs. Importantly, structural isomers **1** and **3** exhibited good *in vitro* SI and **1** provided *P. berghei*-infected mice with mean survival times greater than 40 days. These data reveal the potential of ellipticine (**1**) and olivacine (**3**) as antimalarial leads.

Acknowledgements

This work was supported by grants from the Brazilian Malaria Network (CNPq 555665/2009-7 and CNPq 555669/2009-2), Bionorte CNPq 554317/2010-9, MCT/INPA/INPA, PRONEX/FINEP 023/2009 and The Science and Technology Foundation of Portugal (PTDC/SAU-FAR/114864/2009 and PEst-OE/SAU/UI4013/2011). LFRS, AMP, AM, RCNA, GD and DCS thank FAPEAM and/or CNPq for individual fellowships received.

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