JOURNAL OF NATURAL PRODUCTS

Volume 65, Number 10 October 2002

Full Papers

Antiplasmodial Activity of Alkaloids from Various *Strychnos* Species

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Received February 28, 2002

The in vitro antiplasmodial activities of 69 alkaloids from various *Strychnos* species were evaluated against chloroquine-resistant and chloroquine-sensitive lines of *Plasmodium falciparum*. The compounds, comprising mainly indolomonoterpenoid alkaloids, exhibited a wide range of biological potencies in the antiplasmodial assays. The most active alkaloids were also tested for cytotoxicity against HCT-116 colon cancer cells to determine their antiplasmodial selectivity. As a result of these studies, structure−activity relationships for these alkaloids have begun to emerge. Alkaloids presenting four types of bisindole skeleton exhibited potent and selective activities against *Plasmodium*. They were sungucine-type (IC_{50} values ranging from 80 nM to 10 μM), longicaudatine-type (IC_{50} values ranging from 0.5 to 10 μM), matopensine-type (IC_{50} values ranging from 150 nM to 10 μM), and usambarine-type alkaloids. Within the last structural type, isostrychnopentamine (**49**) and ochrolifuanine A (**46**) were found to be active against chloroquine-sensitive and -resistant strains (IC_{50} values of 100−150 and 100−500 nM, respectively), and dihydrousambarensine (**51**) exhibited a 30-fold higher activity against the chloroquine-resistant strain (IC_{50} = 32 nM) than against the chloroquine-sensitive one.

Malaria is the major parasitic infection in many tropical and subtropical regions, leading to more than one million deaths (principally among young African children) out of 400 million cases each year.[1] More than half of the world's population lives in areas where they remain at risk of malaria infection. During recent years, the situation has worsened in many ways, mainly due to malarial parasites becoming increasingly resistant to several antimalarial drugs.[2] This resistance concerns numerous drugs, but is thought to be most serious with chloroquine, the cheapest and most widely used drug to treat malaria. Furthermore, the control of malaria is becoming more complicated by the parallel spread of resistance of the mosquito vector to currently available insecticides. Urgent efforts are therefore necessary to identify new classes of antimalarial drugs.[3]

The in vitro antiplasmodial, antiamebic, and cytotoxic activities of several indole alkaloids, particularly those isolated from various *Strychnos* species, have been previously investigated.[4−9] To complement these studies it was considered to be of interest to test the antiplasmodial activity of other *Strychnos* alkaloids available in our laboratories. In total, 69 alkaloids with various structures have been tested against three lines of *P. falciparum*, thus allowing us to determine preliminary structure−activity relationships.

Results and Discussion

Among a total of 69 alkaloids examined, compounds **1**−**4** were of the monoterpenoid type, compounds **5**−**45** were monoindole alkaloids, and compounds **46**−**69** were bisindole alkaloids. Compounds exhibiting IC_{50} values higher than 10 μM, between 1 and 10 μM, and lower than 1μM

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10.1021/np020070e CCC: $22.00
Published on Web 08/16/2002

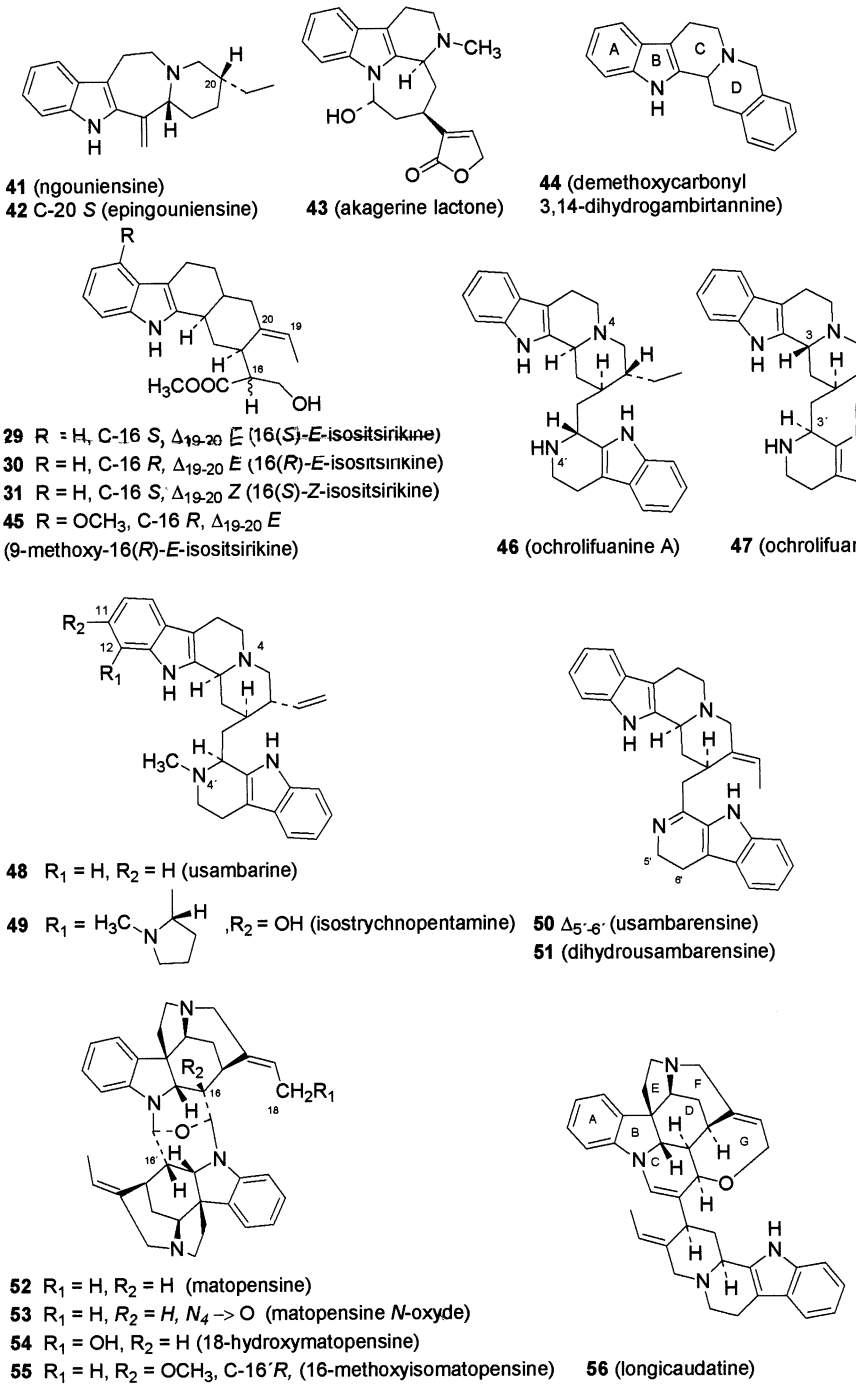

Figure 1. Structures of alkaloids **29**−**31** and **41**−**56**.

were respectively considered as weak, moderately active, and potent antiplasmodial agents. Compounds **1**−**40** were inactive or showed less than 30% growth inhibition at a concentration of 50 μM (Supporting Information). The results obtained for compounds **5**−**40** were largely confirmatory of previous studies where other monoindole alkaloids (akagerine, retuline, strychnine, icajine, vomicine, novacine derivatives, diaboline, holstiine) were shown to be devoid of any antiplasmodial activity.[7−9] However, five monoindole alkaloids evaluated in the present study showed a weak activity, with antiplasmodial IC_{50} values of 10−30 μM: ngouniensine (**41**), epingouniensine (**42**), akagerine lactone (**43**), demethoxycarbonyl 3,14-dihydrogambirtanine (**44**), and 9-methoxy 16(*R*)-*E*-isositsirikine (**45**) (Table 1). Since the compounds possess highly varied structures, no structure−activity relationships could be deduced from these data. The most active of the monomeric compounds was **45**, with an IC_{50} value of approximately 10 μM against all *P. falciparum* strains tested. This compound contains an additional methoxy substituent on the aromatic ring, compared to its inactive analogues **29**−**31**.

All the other active alkaloids were bisindole derivatives (Table 1). Twelve compounds (**48**, **53**, **54**, **57**, **59**, **63**, **64**, **65**, **66**, **67**, **68**, **69**) exhibited moderate to weak activity, with antiplasmodial IC_{50} values of 2−20 μM in at least one of the *Plasmodium* lines. The remaining 12 compounds (**46**, **47**, **49**, **50**, **51**, **52**, **55**, **56**, **58**, **60**, **61**, **62**) showed IC_{50} values of <2 μM against all *Plasmodium* lines tested. Of these, the two compounds isostrychnopentamine A (**49**) and ochrolifuanine A (**46**) demonstrated very potent activity of

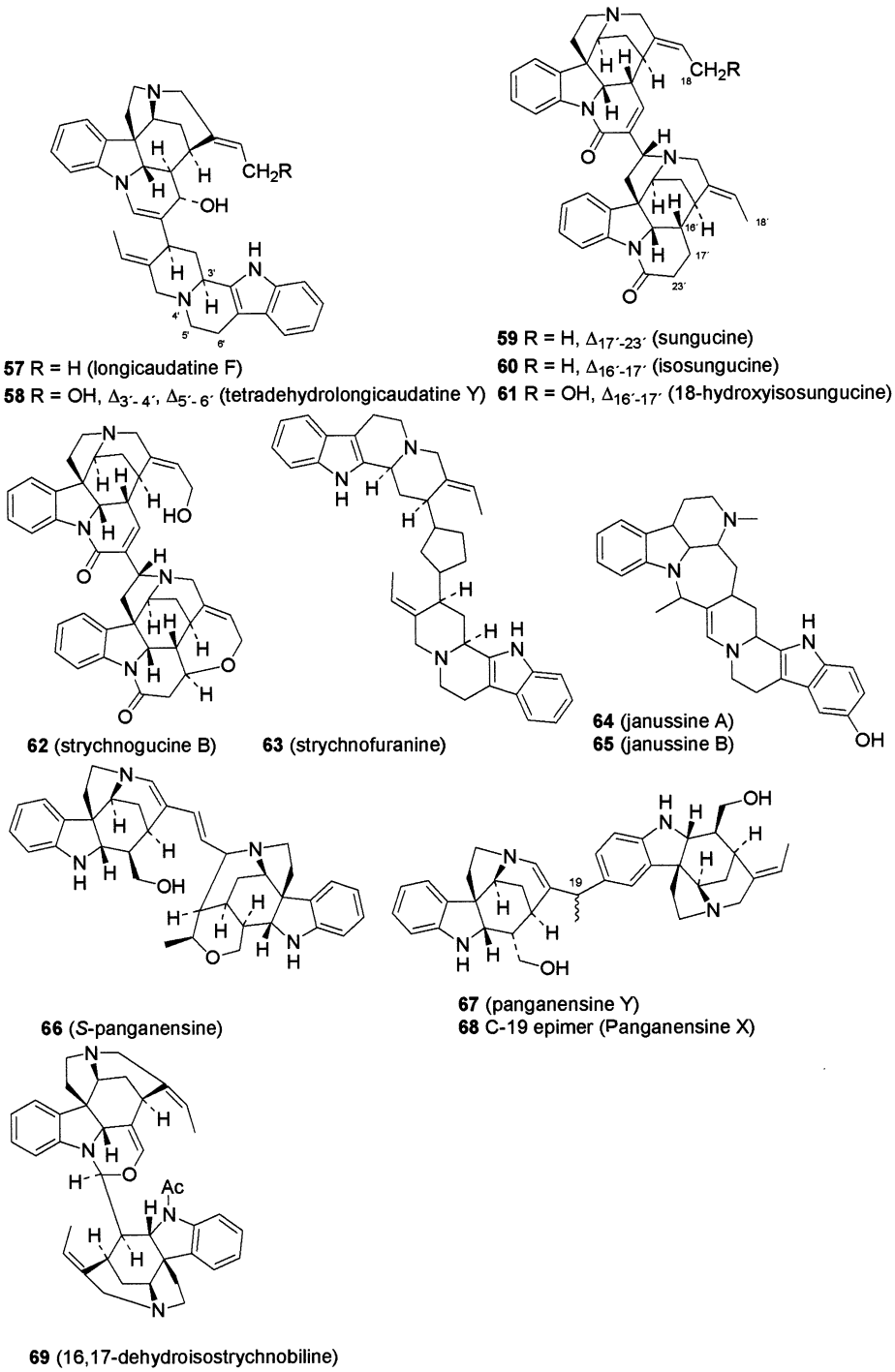

Figure 2. Structures of alkaloids **57**−**69**.

<500 nM against all lines tested, and one compound, dihydrousambarensine (**51**), was selectively highly active (39 nM) against the chloroquine-resistant strain W2. Among these 24 bisindole compounds it was possible to distinguish four principal structural classes: usambarine-type or quasi-dimeric-type (i.e., made of two tryptamine units with a single iridoid unit) (**46**−**51**), matopensine-type (**52**−**55**), longicaudatine-type (**56**−**58**), and sungucine-type (**59**−**62**) alkaloids.

Among alkaloids of the usambarine-type (**46**−**51**), most of the compounds evaluated exhibited potent antiplasmodial activity (IC_{50} below 1 μM). The most interesting compound was isostrychnopentamine (**49**), which possessed an IC_{50} value of about 100 nM against all plasmodial lines tested. The absence of the hydroxyl substituent at C-11 and of the pyrrolidine ring at C-12 in its analogue usambarine (**50**) led to a 20-fold reduction in activity. As previously reported,[7] the 11-hydroxy derivative of usambarine was also 3 times more active than usambarine. Ochrolifuanine A (**46**) also exhibited potent activity against the three strains tested (100−500 nM). This compound was also of interest, as its total synthesis has been recently described.[10] Ochrolifuanine E (**47**), exhibiting the H-3β and H-3′α configuration, was clearly less potent than ochrolifuanine A (**46**) (H-3′β, H-3α and an ethyl side chain). Furthermore, within this group, two compounds (**50**, **51**), presenting a fully or partially aromatized lower portion of the molecule, were clearly more potent against the chloroquine-resistant strain, particularly dihydrousambarensine (**51**), which showed a 30-fold lower IC_{50} value for the chloroquine-

Table 1. In Vitro Activities of Some *Strychnos* Alkaloids Against Three Lines of *P. falciparum*

	FCA 20/ Ghana (chloroquine-sensitive line)			FCB1-R/Colombia (moderately chloroquine-resistant line)			W2/Indochina (chloroquine-resistant line)		
compound	IC_{50} μM ±SD[a]	IC_{90} μM	n[b]	IC_{50} μM ±SD[a]	IC_{90} μM	n[b]	IC_{50} μM ±SD[a]	IC_{90} μM	n[b]
monoindole alkaloids									
ngouniensine (**41**)	26.8	71.0	1	17.8	62.8	2	20.2	70.1	1
epingouniensine (**42**)	7.8	26.9	1	16.3	49.7	1	15.8	52.8	1
akagerine lactone (**43**)	17.0	60.7	1	27.5	55.2	1	25.9	53.3	1
demethoxycarbonyl 3,14-dihydrogambirtannine (**44**)	11.3	42.2	1	12.9	47.4	2	18.3	89.5	1
9-methoxy 16(*R*) *E*-iso-sitsirikine (**45**)	13.6 ± 5.9	37.8	3	12.5	36.3	2	9.9	34.6	1
usambarine-type bisindole alkaloids									
ochrolifuanine A (**46**)	0.118 ± 0.024	0.495	3	0.266 ± 0.185	1.02	3	0.492 ± 0.145	1.38	3
ochrolifuanine E (**47**)	0.276	1.49	1	0.702 ± 0.431	2.42	6	1.90	6.76	1
usambarine (**48**)	2.50[c]	9.21	1	ND			2.36[c]	7.29	2
isostrychnopentamine (**49**)	0.120[c]	0.450	2	0.104 ± 0.036	0.386	3	0.152[c]	0.628	2
usambarensine (**50**)	1.52 ± 0.031[c]	4.41	6	ND			0.594 ± 0.052[c]	4.83	3
dihydrousambarensine (**51**)	0.857 ± 0.061[c]	2.49	5	ND			0.032 ± 0.002[c]	4.68	4
matopensine-type bisindole alkaloids									
matopensine (**52**)	1.25	6.63	1	0.362 ± 0.035	4.11	3	0.243	7.74	1
matopensine-*N*-oxide (**53**)	13.2	73.3	1	4.67	24.8	2	8.66	86.7	1
18-hydroxymatopensine (**54**)	5.11	12.3	2	2.43 ± 0.37	11.8	4	0.121	2.26	2
16-methoxyisomatopensine (**55**)	0.54	2.53	2	1.54 ± 0.21	9.52	3	0.122 ± 0.076	2.84	4
longicaudatine-type bisindole alkaloids									
longicaudatine (**56**)	0.986	3.87	2	0.560	3.64	2	0.569 ± 0.228	4.83	3
longicaudatine F (**57**)	7.70	26.7	1	11.7	33.4	2	9.58	28.1	2
tetradehydrolongicaudatine Y (**58**)	1.236[c]	7.970	1	ND			0.958[c]	12.087	2
sungucine-type bisindole alkaloids									
sungucine (**59**)	7.82 ± 1.14[d]	26.3	3	ND			10.1 [d]	33.2	2
isosungucine (**60**)	1.32 ± 0.25[d]	7.06	3	ND			0.265[d]	1.72	2
18-hydroxyisosungucine (**61**)	0.847 ± 0.141[d]	4.35	4	0.207 ± 0.126	1.47	3	0.140[d]	1.35	2
strychnogucine B (**62**)	0.617[d]	3.79	2	0.529 ± 0.038	2.24	3	0.085[d]	0.358	2
various bisindole alkaloids									
strychnofuranine (**63**)	4.11	15.0		10.8	33.5	2	2.80	16.0	2
janussine A (**64**)	9.55	30.9	2	15.9	28.3	2	12.7	27.6	2
janussine B (**65**)	12.6	34.9	2	17.0	33.1	2	4.75	27.8	2
S-panganensine (**66**)	9.77	17.7	1	5.27	12.7	2	> 20		1
panganensine Y (**67**)	10.4	68.2	1	13.5	43.7	2	17.4	78.7	1
panganensine X (**68**)	14.9	34.8	1	12.9	26.1	2	17.0	85.8	1
16,17-dehydroisostrych-nobiline (**69**)	4.58	18.7	1	2.12	7.13	2	3.31	18.5	1
reference compounds									
chloroquine (**70**)	0.011 ± 0.005	0.071	6	0.032 ± 0.019	0.084	3	0.284 ± 0.017	1.75	5
quinine (**71**)	0.269 ± 0.006	1.91	3	0.200 ± 0.033	2.74	4	0.413 ± 0.011	1.72	3
artemisinin (**72**)	ND			0.005	0.013	2	0.002	0.017	1

[a] Values are expressed as mean ± standard deviation (for $n > 2$). ND = not determined. [b] n = number of independent experiments. All tests were realized in duplicate. [c] Data from ref 7. [d] Data from ref 5.

resistant line W2 (32 nM). Its IC_{90} value, however, was similar to that obtained with the chloroquine-sensitive line, FCA20.

For alkaloids of the matopensine-type (**52**−**55**), the *N*-oxide derivative (**53**) was clearly less potent than the other compounds in this class. This may be attributed to the ionic nature of the *N*-oxide, since quaternary alkaloids have consistently been inactive in our assays (data not published). The three alkaloids **52**, **54**, and **55** were slightly more potent against the chloroquine-resistant strain, but no clear structure−activity relationships could be deduced for these three compounds.

For alkaloids of the longicaudatine-type, only three compounds were available (**56**−**58**). The most interesting was longicaudatine itself (**56**), which exhibited an IC_{50} of 0.5−1 μM. Longicaudatine F (**57**), whose structure differs from that of **56** only by the opening of ring G in the strychnine part of the molecule, was less active. Compound **58**, which possessed a β-carboline structure in the lower part of the dimer (as in **50**), demonstrated enhanced activity against the chloroquine-resistant strain. It would be interesting to test additional analogues of longicaudatine.

Among alkaloids of the sungucine-type, compounds **60**−**62** possessed potent activities against the different lines of *Plasmodium falciparum*. Isosungucine (**60**), a compound possessing a C-16′/C-17′ double bond in the lower part of the dimer, was more potent than its isomer sungucine (**59**). The presence, in 18-hydroxyisosungucine (**61**), of an additional hydroxyl substituent on the 18−19 ethylidene side chain resulted in a small increase in activity. Finally, cyclization of ring G in the lower portion of the molecule

Table 2. Cytotoxic Activity Against the Human Colon Cancer Cell Line HCT-116 and Antiprotozoal Selectivity Index[a] of the More Potent Antiplasmodial Alkaloids

compound	IC_{50}, μM[b]	FCA SI	FCB1 SI	W2 SI
ochrolifuanine A (**46**)	16.1	136	60.7	32.8
ochrolifuanine E (**47**)	5.70	20.7	8.12	3.0
isostrychnopentamine (**49**)	7.47	62.3	71.8	49.1
dihydrousambarensine[c] (**51**)	12.0	14	ND	375
matopensine (**52**)	12.0	9.59	33.1	49.5
18-hydroxymatopensine (**54**)	42.5	8.31	17.5	351
16-methoxyisomatopensine (**55**)	24.5	45.3	15.9	200
longicaudatine (**56**)	4.93	4.59	8.81	7.96
sungucine[c] (**59**)	6.2	0.79	ND	0.611
18-hydroxyisosungucine[c] (**61**)	16.2	19.1	78.3	115
strychnogucine B[c] (**62**)	15	24.3	28.3	176.4
chloroquine (**70**)	33.7	3063	1053	118.6

[a] Selectivity index (SI) is defined as the ratio of 50% cytotoxicity over 50% antiplasmodial activity. [b] Number of independent experiments = 2. Each experiment was realized in triplicate. [c] Data on KB cells from refs 5 and 7.

and the presence of a hydroxyl substituent on the C-18 in the upper portion led to strychnogucine B (**62**), the most potent derivative in this group of alkaloids. In contrast, strychnogucine A, presenting a C-16′/C-17′ double bond, with a G cyclization on the upper part of the dimer, was more active than sungucine but markedly less active than **60**−**62** (IC_{50} near 3 μM for all strains).[4] Thus, strychnogucine B (**62**) and 18-hydroxyisosungucine (**61**) were found to be the most active compounds in this series, with 12−120 times higher activity than sungucine (**59**) against the three plasmodial lines. Furthermore, the activities were several-fold higher against the chloroquine-resistant lines W2 and FCB1-R than against the chloroquine-sensitive strain FCA of *Plasmodium falciparum*.

To further assess the clinical potential of these antiplasmodial bisindolomonoterpene alkaloids, the most active compounds were evaluated for cytotoxicity with a human colon cancer cell line (HCT-116), to distinguish general cellular toxicity from specific antiplasmodial activity (Table 2). Many cytotoxic compounds also possess antiplasmodial properties under conditions of in vitro testing. Hence, the selectivity index (SI) of a compound was used, defined as IC_{50}(in HCT-116 cells)/IC_{50}(in *P. falciparum*). This selectivity index is an attempt to estimate the potential of tested compounds to inhibit the growth of the intracellular malaria parasite without cellular toxicity. The HCT-116 cell line was selected because it demonstrated no resistance to current anticancer drugs.[11] In addition, this cell line was easily cultured and exhibited comparable sensitivity to noncancer cell lines (such as WI38 fibroblasts).

Most compounds evaluated (Table 2) exhibited a 5- to 400-fold higher activity against *Plasmodium* than against the human cancer cells used, thus indicating some antiplasmodial selectivity (except **59**). The most generally selective compounds were isostrychnopentamine (**49**) and ochrolifuanine A (**46**), which showed selectivity indices of 50−70 and 30−140 times, respectively. Dihydrousambarensine (**51**), 18-hydroxymatopensine (**54**), and 16-methoxyisomatopensine (**55**) yielded a favorable SI only for the W2 strain (SI = 400, 350, and 200, respectively). Even though sungucine (**59**) exhibited no selectivity against *Plasmodium*, 18-hydroxysungucine (**61**) and strychnogucine B (**62**) exhibited 20−180-fold higher activity against the *P. falciparum* lines than against the human cancer cell line, thus indicating a satisfactorily high selectivity.

The results of the present study confirmed the previously reported antiplasmodial activities of *Strychnos* bisindole alkaloids[8] and more particularly of four alkaloid subtypes. All active compounds were tertiary dimers. A certain degree of basicity seems to be necessary for the antiplasmodial activity of this family of compounds. For example, usambarine (**48**) (possessing a methyl substituent at $N_{4'}$) was less active than the ochrolifuanines (**46** and **47**) ($N_{4'}$−H being more basic) or isostrychnopentamine (**49**) (which possesses a third basic nitrogen in the pyrrolidine ring). Also, *N*-oxide or quaternary derivatives were also less active than the respective tertiary compounds. These observations were consistent with the ability of basic compounds to accumulate to higher levels in the acidic food vacuole of the parasite, as has been hypothesized for chloroquine. Chloroquine is thought to be selectively accumulated (to at least a 1000-fold level) in the parasite food vacuole, where digestion of hemoglobin takes place. The weakly basic properties of chloroquine explain its accumulation in the food vacuole: at neutral pH, chloroquine has the ability to diffuse freely through membranes, but at the acidic pH of the food vacuole, the compound is protonated and is, therefore, sequestered.[12,13]

In the case of the sungucine-type alkaloids, it was apparent that the antiplasmodial selectivity was clearly associated with the presence of a 16′−17′ double bond or a 17′−18′ ether link. Isostrychnopentamine (**49**) exhibited potent and selective activities against all three lines of *Plasmodium* used in this study. The activity of this compound seemed to be directly linked to the presence of the basic methylpyrrolidine substituent. It would be useful to test semisynthetic and closely related compounds possessing these kinds of structures.

Dihydrousambarensine (**51**), has been previously tested in vivo against *Plasmodium berghei* but was inactive at a dose of 30 mg/kg/day.[8] Nevertheless, this compound was essentially active against chloroquine-resistant strains of *P. falciparum* and the *P. berghei* strain used in this study was chloroquine-sensitive. This could explain its inactivity in vivo, in addition to the differences between the biology of the two species. It is now desirable to examine if other lead alkaloids are able to inhibit *Plasmodium* growth in animal models and if they possess original modes of action (in particular, if they act or not on the heme polymerization process).

Experimental Section

Chemicals. All alkaloids tested (**1**−**69**) were isolated and purified in our laboratories from various *Strychnos* species as previously described. Compounds **1**, **3**, and **29** were isolated from *Strychnos pungens* Solered.;[14,15] **2**, **26**, and **31** from *S. angolensis* Gilg;[16] **4**, **11**, **20**, **56**, and **57** from *S. longicaudata* Gilg;[17−19] **5**, **6**, **12**, **18**, **19**, **41**, and **42** from *S. ngouniensis* Pellegr.;[18,20,21] **7**, **8**, **27**, and **28** from *S. potatorum* L.;[22,23] **9**, **10**, **53**, and **69** from *S. kasengaensis* De Wild;[24] **13** from *S. xantha* Leeuwenberg;[25] **14**, **15**, **16**, **17**, **21**, **22**, **43**, **44**, **64**, and **65** from *S. johnsonii* Hutch. et M.B. Moss;[26,27] **23**, **24**, **25**, **32**, **33**, **34**, and **37** from *S. henningsii* Gilg;[28] **30**, **52**, **54**, **55**, and **63** from *S. matopensis* S. Moore;[29,30] **39** from *S. staudtii* Gilg;[31] **45** from *S. lucens* Bak.;[32] **46** and **47** from *S. potatorum* L.;[22] **48**, **49**, **50**, **51**, and **58** from *S. usambarensis* Gilg;[33−35] **59**, **60**, **61**, and **62** from *Strychnos icaja* Baill.; [4,5] and **66**, **67**, and **68** from *S. panganensis* Gilg.[36] The purity (>95%) of the compounds has been determined by TLC and spectroscopic (UV, IR, MS, NMR) comparison with authentic samples. Chloroquine diphosphate (Sigma, Bornem, Belgium), artemisinin (Sigma, Bornem,

Belgium), and quinine base (Aldrich 14590-4) were used as antimalarial references. [^{3}H]Hypoxanthine was from NEN Life Science Products (Zaventem, Belgium).

***Plasmodium falciparum* Lines.** Three *P. falciparum* lines were used in this study: the W-2 chloroquine-resistant line from Indochina, the FCB1-R line from Columbia, and the chloroquine-sensitive FCA 20 from Ghana. These lines were provided by Prof. P. Grellier (Laboratoire de Biologie Parasitaire et Chimiothérapie, Muséum d'Histoire Naturelle, Paris), Prof. J. Le Bras (Hôpital Bichat-Claude Bernard, Laboratoire de Parasitologie, Centre National de Référence de la Chimiosensibilité du Paludisme, Paris), and Prof M. Wéry (Tropical Medicine Institute, Antwerp, Belgium), respectively. The FCB1-R line was described as moderately chloroquine-resistant, but IC_{50} values near 30 nM were obtained (the chloroquine resistance threshold was set at 100 nM).

In Vitro Antiplasmodial Testing. Continuous in vitro cultures of asexual erythrocytic stages of the three *P. falciparum* strains were maintained following the procedure of Trager and Jensen[37] and as described previously.[5] Each test sample was applied in a series of eight 4-fold dilutions (final concentrations ranging from 20 to 0.0012 μg/mL) and was tested in duplicate. Parasite growth was estimated by the determination of [^{3}H]hypoxanthine incorporation as described by Desjardins et al.[38] and modified by Mirovsky et al.[39] The Student *t*-test was used to test the significance of differences between results obtained for different samples. Statistical significance was set at $p \leq 0.05$.

Evaluation of Cytotoxic Potential. The HCT-116 colon cancer cell line was cultured as described previously.[40] Compounds were tested in 96-well microplates using the tetrazolium salt WST-1 (Boehringer) colorimetric assay based on the cleavage of the reagent by mitochondrial succinate-tetrazolium reductase in living cells. Altogether, 5000 cells were seeded per well in 200 μL of medium supplemented with adequate concentrations of tested drugs. Corresponding controls with analogous concentrations of DMSO were included in parallel. At least two experiments were performed per cell line. After a 72 h incubation, 20 μL of WST-1 was added to the well. After 30 min at 37 °C, plates were shaken and absorbance values were recorded as described in the commercial assay, against a background control as blank (medium plus WST-1 without cells). Relative absorbance values were expressed as percent of the respective controls (100% in ordinate). Means ± SEM were calculated. IC_{50} values were calculated from graphs.

Acknowledgment. The authors wish to thank Prof. J. Boniver (Anatomie et Cytologie Pathologique, Université de Liège) for liquid scintillation measurements, V. Bours and M. P. Merville (CTCM, ULg) for HCT-116 cell lines and access to their laboratory, and M. Bentires (CTCM, ULg) for his technical assistance. This research was supported by the Belgian National Fund for Scientific Research (FNRS) (Grant No. 3453201 and fellowship for M.F.).

Supporting Information Available: Table of alkaloids exhibiting less than 30% growth inhibition at a concentration of 50 μM against *P. falciparum* FCA-20 Ghana line. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP020070E