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Article

Antimalarial Activity of Piperidine Alkaloids from *Senna spectabilis* and Semisynthetic Derivatives

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Dando continuidade as pesquisas de identificação de metabólitos secundários com propriedades anti-infecciosas potenciais a partir de espécies de plantas dos biomas brasileiros, dois alcaloides piperidínicos (–)-cassina e (–)-espectalina foram isolados das flores de *Senna spectabilis* (sin. *Cassia spectabilis*). As estruturas destes compostos foram elucidadas a partir de dados espectroscópicos e espectrométricos. Adicionalmente, esses alcaloides foram acetilados, resultando nos derivados (–)-3-*O*-acetilcassina e (–)-3-*O*-acetilespectalina. Todas as substâncias foram submetidas ao bioensaio empregando culturas de eritrócitos, infectadas com *Plasmodium falciparum*, um teste específico para avaliação antimalárica. Dentre as substâncias avaliadas, os dois primeiros alcaloides (IC_{50} 1,82 μM e IC_{50} 2,76 μM) foram mais potentes que os derivados (IC_{50} 24,47 μM e IC_{50} 25,14 μM) em comparação com a cloroquina (IC_{50} 0,30 μM), utilizada como padrão. Estes dados mostram que os alcaloides piperidínicos constituem uma classe de produtos naturais que apresenta amplo espectro de atividades biológicas, sendo portanto, importantes modelos para o planejamento de fármacos, incluindo os antimaláricos.

In our continuing work looking for new anti-infective lead compounds from Brazilian biomes, the two known piperidine alkaloids (–)-cassine and (–)-spectaline were isolated from the flowers of *Senna spectabilis* (syn. *Cassia spectabilis*). Their structures were elucidated using a combination of spectroscopic and spectrometric data analysis. Further, these compounds were acetylated yielding the derivatives (–)-3-*O*-acetylcassine and (–)-3-*O*-acetylspectaline. All compounds were screened against *P. falciparum*-infected red blood cells (RBC) in culture, aiming to identify antimalarial prototypes. Among all compounds screened, the first two alkaloids (IC_{50} 1.82 μM and IC_{50} 2.76 μM) were more effective than the derivatives (IC_{50} 24.47 μM and IC_{50} 25.14 μM) in comparison to the standard compound chloroquine (IC_{50} 0.30 μM). These data show that piperidine alkaloids constitute a class of natural products that feature a broad spectrum of biological activities, and are, therefore, important templates for drug design, including antimalarial.

Keywords: piperidine alkaloids, *Senna spectabilis*, semisynthetic derivatives, antimalarial activity, *Plasmodium falciparum*

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Introduction

Malaria, a life-threatening disease caused by *Plasmodium* parasites, infects and destroys red blood cells, leading to fever, severe anemia, and cerebral malaria and if untreated may cause death. It is estimated that 3.3 billion of the world's population living in 109 countries are at risk of contracting this serious and often life-threatening disease. In recent statistics, malaria accounts for ca. 250 million clinical cases and nearly 1 million deaths each year, the great majority of which occur in children younger than 5 years of age and in young, pregnant women, which are more frequently attacked because of the lower immunological protection.[1] The global malaria map has been shrinking over the past 50 years and this disease, affecting societies, by interfering with educational accomplishments, and causing serious economic problems, remains a devastating disease largely because of widespread drug resistance.[2] Without regular monitoring and reporting of antimalarial drug resistance, the disease burden and the economic costs of malaria will rise dramatically.[1] Therefore, new drugs and a better understanding of the mechanisms of drug action and resistance are essential for fulfilling the promise of eradicating malaria. Chloroquine is the most widely used antimalarial drug, but the emergence of drug-resistant parasites is rapidly reducing its effectiveness as a single agent. It is now most effective as part of an artemisinin-based drug combination. In this case, the parasite evolved a way to pump out chloroquine before the drug could accumulate to levels that would interfere with a process known as heme polymerization (which is needed to prevent the buildup of the toxic by-products of hemoglobin metabolism).[3] Chloroquine remains effective only in Central America, where clinical studies in Honduras and Nicaragua have confirmed its 100% efficacy.[1] In an analysis based on published studies conducted in 30 countries, the median treatment failure rates were high to extremely high (19.8-100%) in all the countries except Honduras, Malawi and Nicaragua (0-1.3%). In India, monitoring of chloroquine continued until 2008 despite consistently high failure rates.[4] Quinine-resistant *P. falciparum* was first reported in 1910 in Brazil. Today this parasite is resistant in most endemic areas to the widely used blood schizonticide, chloroquine. Many strains are resistant also to antifols (e.g., pyrimethamine, proguanil) and some are also no longer eliminated by quinine. Therefore, it is essential to search for radically new compounds, for drugs that reverse chloroquine resistance and for new strategies to impede the progress of this problem.

As part of our ongoing research project on *Senna* and *Cassia* species prospecting for new bioactive piperidine alkaloids,[5-10] a further investigation on the flowers of *Senna spectabilis* (D.C.) H.S. Irwin and R.C. Barneby (Fabaceae, Caesalpinioideae) was carried out, in the hope of finding new unusual piperidine alkaloids in an EtOH extract of these flowers and to evaluate their action as antimalarial agents. To this end, this extract was subjected to successive liquid-liquid partitioning, which yielded a chloroformic alkaloidal fraction. This fraction was chromatographed using a combination of flash chromatography followed by high-performance liquid chromatography (HPLC) separations and afforded the two piperidine alkaloids ((–)-cassine (**1**) and (–)-spectaline (**2**), Figure 1). Further, the acetyl derivatives (–)-3-*O*-acetylcassine (**3**) and (–)-3-*O*-acetylspectaline (**4**) were prepared from **1** and **2**, respectively (Figure 1), in greater amounts since on previous works they were identified as a mixture of two homologous piperidine alkaloids and **4** was isolated in very low yield from *S. spectabilis*.[6,7] These compounds were then assessed for their antimalarial inhibitory activity by means of flow cytometry screening assay.[11]

Experimental

General experimental procedures

Optical rotations were measured with a 341 LC polarimeter (PerkinElmer) at 28 °C. Melting points were recorded on a differential scanning calorimeter (DSC) TA Instruments DSC-Q10 apparatus and are uncorrected. Infrared (IR) spectra were registered on a Nicolet iS10 FT-IR spectrometer coupled with an attenuated total reflectance (ATR) accessory (the samples were pressed against a crystal of Ge). The 1D (^{1}H, ^{13}C, and distortionless enhancement by polarization transfer (DEPT)) and 2D (^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear

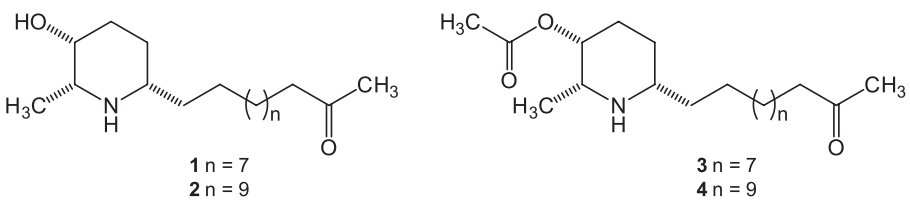

Figure 1. Piperidine alkaloids (**1** and **2**) isolated from *S. spectabilis* and (**3** and **4**) semisynthetic derivatives.

multiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC)) nuclear magnetic resonance (NMR) experiments were accomplished on a Varian INOVA 500 spectrometer (11.7 T) at 500 MHz (^{1}H) and 125 MHz (^{13}C), at 30 °C, using tetramethylsilane (TMS, δ_{TMS} 0.00 ppm) as internal standard or residual solvent resonances of methanol-d_4 at δ 3.30 and 49.0 ppm or chloroform-*d* at δ 7.26 and 77.0 ppm, as reference for ^{1}H and ^{13}C, respectively. High-resolution mass spectra (HRMS) with electrospray ionization (ESI) were measured on an ultrOTOF$_Q$ (Bruker Daltonics) apparatus operating in the positive mode. The samples were infused into the ESI source at a flow rate of 5 μL min^{-1} and methanol/water (4:1) was used as solvent system.

Column chromatography (CC) was carried out on silica gel (70-230 mesh, Acros) and neutral alumina (70-290 mesh, Sigma). Thin-layer chromatography (TLC) was performed on silica gel F_{254} plates (0.20 mm, Fluka), and spots were visualized under UV light (254 and 366 nm) and spraying with iodochloroplatinate reagent (Merck) or anisaldehyde-H_2SO_4, followed by charring for 5 min, prepared as described in the literature.[12] Analytical and preparative HPLC separations were accomplished on a Shimadzu CLASS-VP instrument equipped with a binary pump model LC-8A, a UV-Vis detector model SPD-10A*vp*, an evaporative light scattering detector model ELSD-LT, a fraction collector model FRC-10A, and an automatic sample injector model SIL-10AF and controlled with the aid of an LC workstation CLASS-VP version 6.14 SP2 software. The columns used were a Phenomenex Gemini C_{18} (250 × 4.60 mm, 5 μm) and a preparative Phenomenex Synergi Hydro C_{18} 80 Å Axia Packed (100 × 21.20 mm, 4 μm) protected with the corresponding guard columns. All solvents utilized in the experimental procedures were HPLC-grade or had been previously distilled. Water was purified immediately prior to use with a Millipore Milli Q plus system.

Plant material

Leaves of *S. spectabilis* were collected by M. Pivatto in Araraquara (São Paulo, Brazil), in July 2010. The plant was identified by Inês Cordeiro from Instituto de Botânica in São Paulo-SP, Brazil. A voucher specimen (SP 384109) has been deposited in the herbarium of this institute.

Extraction and isolation

The dried and powdered flowers (950.0 g) were extracted with aqueous 95% ethanol (9 L × 5) for seven days at room temperature. The solvent was removed under reduced pressure in a rotary evaporator to yield a thick syrup (184.0 g). The crude ethanol extract (100.0 g) was reconstituted in MeOH-H_2O (4:1, 500 mL), filtered, and successively partitioned with *n*-hexane (250 mL × 5, 5.5 g), CH_2Cl_2 (250 mL × 5, 25.8 g), and EtOAc (250 mL × 5, 10.3 g). After removal of solvent, the CH_2Cl_2 extract (8.0 g) was subjected to CC over neutral alumina, eluting with a gradient of increasing MeOH in $CHCl_3$ (10-100%), to produce twenty fractions (F_1-F_{20}, Supplementary Information Figure S1) on the basis of TLC analysis. Fraction F_{1-4} (1.76 g) was further fractionated via silica gel CC, eluting with $CHCl_3$-MeOH-NH_4OH (9:1:0.25), to yield two pure compounds, **1** (F_{16-17}, R_f 0.58, *m/z* 298, 54.8 mg) and **2** (F_{3-4}, R_f 0.58, *m/z* 326, 44.9 mg), and a mixture of **1** and **2** (F_{5-15}, R_f 0.58, *m/z* 298 and 326, 575.9 mg) on the basis of TLC and MS analysis (Figure S2). The mixture containing **1** and **2** was submitted to semipreparative HPLC on RP-C_{18} and eluted with a gradient of increasing MeOH in 0.1% HOAc aqueous solution (35-100, flow rate 8.0 mL min^{-1}, 25 min), which furnished the pure alkaloids **1** (t_R 11.6 min, 120.0 mg) and **2** (t_R 15.2 min, 15.0 mg) (Figures S4-S7).

General procedure for the synthesis of compounds **3** and **4**

To a solution of **1** or **2** in chloroform was bubbled a stream of hydrogen chloride gas generated in a modified Kipp's apparatus (30 min) to prepare (–)-cassine hydrochloride and (–)-spectaline hydrochloride (Figure S61).

To a solution of the hydrochloride of **1** (20.0 mg, 0.07 mmol) or **2** (20.0 mg, 0.06 mmol) in chloroform (5 mL) was added acetyl chloride (0.25 mL), and the mixture was refluxed for 4 h under nitrogen atmosphere, followed by quenching with saturated $NaHCO_3$ aqueous solution and extracted with $CHCl_3$ (10 mL × 3). The organic layer was dried over anhydrous magnesium sulfate. The dried solution was filtered and the filtrate was concentrated under reduced pressure to afford the desired products **3** (22.0 mg, 97.5% yield) and **4** (21.4 mg, 96.1% yield) as white solids.

(–)-Cassine (**1**): white solid; $[\alpha]_D^{25}$ –0.62 (*c* 8.0, EtOH); m.p. 54.3 ºC (97.0% purity); IR (film) ν_{max}/cm^{-1} 3203, 2918, 2852, 1709, 1541, 1392, 1159, 1024, 957, 721, 650; ^{1}H and ^{13}C NMR data, see Table 1; HRMS *m/z* 298.2748 $[M + H]^+$ (calcd. for $C_{18}H_{36}NO_2$: 298.2746); TLC R_f 0.64 (9:1:0.25 $CHCl_3$-MeOH-NH_4OH).[13]

(–)-Spectaline (**2**): white solid; $[\alpha]_D^{25}$ –3.35 (*c* 1.0, $CHCl_3$); m.p. 67.7 ºC (93.3% purity); IR (film) ν_{max}/cm^{-1} 3089, 2916, 2848, 1707, 1471, 1425, 1356, 1157, 1074,

993, 912, 856, 787, 719, 698, 596; ^{1}H and ^{13}C NMR data, see Table 1; HRMS *m/z* 326.3056 $[M + H]^+$ (calcd. for $C_{20}H_{40}NO_2$: 326.3054); TLC R_f 0.64 (9:1:0.25 $CHCl_3$-MeOH-NH_4OH).[6,13]

(–)-3-*O*-Acetylcassine (**3**): pale yellow oil; HRMS *m/z* 340.2852 $[M + H]^+$ (calcd. for $C_{20}H_{38}NO_3$: 340.2852); TLC R_f 0.79 (9:1:0.25 $CHCl_3$-MeOH-NH_4OH).[7]

(–)-3-*O*-Acetylspectaline (**4**): pale yellow oil; HRMS *m/z* 368.3159 $[M + H]^+$ (calcd. for $C_{22}H_{42}NO_3$: 368.3165); TLC R_f 0.79 (9:1:0.25 $CHCl_3$-MeOH-NH_4OH).[6,7]

In vitro culture of *Plasmodium falciparum* (3D7)

Parasites were cultured and synchronized as described previously.[14] In brief, parasites were maintained at 1-10% parasitemia and 2% hematocrit in Roswell Park Memorial Institute (RPMI) 1640 culture medium supplemented with erythrocytes, 10% human serum, 0.16% glucose, 0.2 mM hypoxanthine, 2.1 mM *L*-glutamine and 22 mg mL^{-1} gentamycin. Cultures were incubated at 37 ºC, 3% O_2, 3% CO_2 and 94% N_2. Synchronization of parasites in culture to ring stages was carried out by repetitive treatment with 5% (m/v) sorbitol. Parasite growth and parasitemia were monitored by assessing Giemsa-stained blood smears under the microscope.

Drug treatment

Drug treatment experiments were conducted in 96-well plates with different concentrations of each drug in triplicate for each concentration. For this assay, 1% parasitemia and 2% hematocrit were set for each well and 200 µL of RPMI with 10% human serum and drug were added. The parasites were exposed to the drug and the 96-well plates were incubated for 48 h.

Flow cytometry analysis

Sample analysis was done according to an already published report.[11] In brief, after 48 h incubation, the plates were centrifuged at 3000 rpm for 5 min and the RPMI medium was removed. Any traces of drug were washed with phosphate buffered saline (PBS) solution (pH 7.2-7.4). The sample was incubated with 2% formaldehyde in PBS for 24 h to fix the parasite. After fixation, the samples were washed with PBS again. Permeabilization and staining was done with 0.1% Triton-X100 and 5 nM YOYO-1 dye (Molecular Probes).[15] This was done by incubating the sample reaction mixture at 37 ºC for 30 min. Parasitemia and proportions of parasites at each concentration of all drugs and control samples (without drug treatment) were determined from dot plots [side scatter (SSC) *vs.* fluorescence] of 10^5 cells acquired on a FACS Calibur flow cytometer using CELLQUEST software (Becton & Dickinson). YOYO-1 was excited with a 488 nm argon laser and fluorescence emission was collected at 520-530 nm. Parameters subject to adjustment of the FACS Calibur flow cytometer were forward scatter (FSC) (log scale, E-1), SSC (log scale, 269), FL-1 (log scale, 530), and compensation parameters were FL1 – 0.8% FL2 and FL2 – 23.6% FL1.[11]

Statistical analysis

GraphPad Prism (GraphPad Software) software was used for statistical analysis to calculate IC_{50} values. At least three independent experiments were performed for each experimental condition (Tables S1-S5).

Results and Discussion

(–)-Cassine **1** was isolated as an optically active, white solid, with m.p. 54.3 °C. Its molecular weight was measured by HRMS, and the molecular formula was established as $C_{18}H_{35}NO_2$, which inferred two degrees of unsaturation. The observed protonated molecular ion at *m/z* 298.2748 $[M + H]^+$ was close to the value calculated for $C_{18}H_{36}NO_2$, 298.2746. The IR absorption bands were assigned to hydroxy (3203 cm^{-1}) and carbonyl (1709 cm^{-1}) functionalities. Examination of the ^{1}H, ^{13}C, and DEPT NMR spectra recorded in chloroform-*d* (Table 1) showed the presence of a 2,3,6-trisubstituted piperidine ring. It was possible to observe a double quadruplet at δ 2.82 (1H, $J_{2,7}$ 6.5 and $J_{2,3}$ 1.0 Hz, H-2, δ_C 56.20), a broad singlet at δ 3.59 (1H, H-3, δ_C 68.04), two methylenes at δ 32.11 (2H, δ_H 1.91, H-4a and δ_H 1.51, H-4b), and δ 25.78 (2H, δ_H 1.50, H-5a and δ_H 1.40, H-5b), a multiplet at 2.59 (1H, H-4a, δ_C 57.60), and a methyl group at δ 1.16 (d, $J_{2,7}$ 6.5 Hz, H-7, δ_C 18.52). Additionally, ^{13}C NMR data evidenced ten methylenes at δ 36.71 (C-1', δ_H 1.50, H-1'a and δ_H 1.40, H-1'b), 25.99 (C-2', δ_H 1.30), 29.72 (C-3', δ_H 1.26), 29.64 (C-4', δ_H 1.26), 29.76 (C-5', δ_H 1.26), 29.60 (C-6', δ_H 1.26), 29.39 (C-7', δ_H 1.26), 29.95 (C-8', δ_H 1.26), 24.09 (C-9', δ_H 1.56), 44.04 (C-10', δ_H 2.41, t, *J* 7.0 Hz), a carbonyl at δ 209.63 (C-11'), and a methyl group at δ 30.07 (C-12, δ_H 2.13, s), which suggested the presence of a long linear side-chain, typical of the structural feature of piperidine alkaloids, as assembled for **1**. Furthermore, the side-chain (C-1'–C-12') attached at C-6 of the piperidine nucleus was also confirmed by an HMBC NMR experiment. Three-bond correlations were observed from H-5 to C-1', H-1' to C-5, and H-6 to C-1' and C-2' (Figure 2), supporting

a 2-methyl-3-hydroxy-6-*n*-alkyl piperidine substitution pattern. The methyl-ketone at the end of the straight side-chain (C-11') and the methyl group at C-2, were also defined by HMBC data analysis, for which the main correlations are represented in Figure 2. The relative configuration of C-2, C-3 and C-6, were established by spectroscopy, particularly from their hydrogen-hydrogen coupling constants and nuclear Overhauser effect spectroscopy (NOESY) 1D correlations observed when H-2 (δ 2.82) was irradiated to show correlations with H-3 (δ 3.59), H-4b (δ 1.51) and H-6 (δ 2.59) (Figure S34). Furthermore, polarimetric analysis ($[\alpha]_D^{25}$ –0.62) confirm the absolute stereochemistry which was established by Rice and Coke.[13] The overall data analysis confirms the structure of the piperidine alkaloid **1** as (2*R*,3*R*,6*S*)-2-methyl-6-(11-oxododecyl)piperidin-3-ol, named (–)-cassine.

(–)-Spectaline **2** was also isolated as an optically active, white solid, with m.p. 67.7 °C. Its molecular weight was measured by HRMS, and the molecular formula was established as $C_{20}H_{39}NO_2$, which implied two degrees of unsaturation. The observed protonated molecular ion at *m/z* 326.3056 $[M + H]^+$ was close to the value calculated for $C_{20}H_{40}NO_2$, 326.3054. The IR absorption bands were assigned to hydroxy (3089 cm^{-1}), and carbonyl (1707 cm^{-1})

Table 1. NMR Spectroscopic data for **1** and **2** (δ in ppm)[a]

Position	**1** (chloroform-*d*)		**2** (methanol-d_4)	
	δ_C (mult.)	δ_H[b] (mult., *J* / Hz)	δ_C (mult.)	δ_H[b] (mult., *J* / Hz)
2	56.20 (CH)	2.82 dq (6.5; 1.0)	56.35 (CH)	2.78 dq (7.0; 1.5)
3	68.04 (CH)	3.59 br s	67.97 (CH)	3.59 br s
4a	32.11 (CH_2)	1.91 m	32.57 (CH_2)	1.90 m
4b		1.51 m		1.63 m
5a	25.78 (CH_2)	1.50 m	26.01 (CH_2)	1.52 m
5b		1.40 m		1.39 m
6	57.60 (CH)	2.59 m	57.79 (CH)	2.58 m
7	18.52 (CH_3)	1.16 d (6.5)	18.16 (CH_3)	1.12 d (7.0)
1'a	36.71 (CH_2)	1.50 m	37.34 (CH_2)	1.49 m
1'b		1.40 m		1.35 m
2'	25.99 (CH_2)	1.30 m	26.79 (CH_2)	1.36 m
3'	29.72[c] (CH_2)	1.26 br s	30.51[c] (CH_2)	1.29 br s
4'	29.64[c] (CH_2)	1.26 br s	30.61[c] (CH_2)	1.29 br s
5'	29.76[c] (CH_2)	1.26 br s	30.70[c] (CH_2)	1.29 br s
6'	29.60[c] (CH_2)	1.26 br s	30.67[c] (CH_2)	1.29 br s
7'	29.39[c] (CH_2)	1.26 br s	30.67[c] (CH_2)	1.29 br s
8'	29.95[c] (CH_2)	1.26 br s	30.58[c] (CH_2)	1.29 br s
9'	24.09 (CH_2)	1.56 m	30.24[c] (CH_2)	1.29 br s
10'	44.04 (CH_2)	2.41 t (7.0)	30.86[c] (CH_2)	1.29 br s
11'	209.63 (C)	–	24.89 (CH_2)	1.55 m
12'	30.07 (CH_3)	2.13 s	44.32 (CH_2)	2.46 t (7.0)
13'	–	–	212.21 (C)	–
14'	–	–	29.76 (CH_3)	2.12 s

[a]Recorded at 500 and 125 MHz for ^1H and ^{13}C NMR, respectively; [b]multiplicity of signals is given as follows: s, singlet; br, broad; d, doublet; t, triplet; dq, double quadruplet; m, multiplet; [c]the assignments were based on calculated δ_C (ChemDraw Ultra 10.0) and may be interchanged in the column.

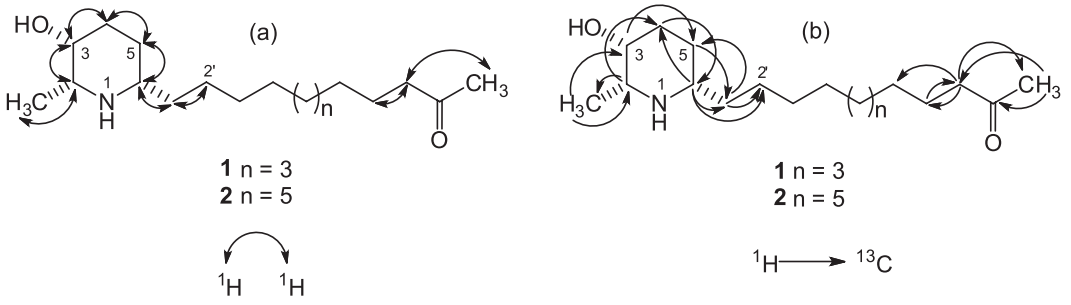

Figure 2. Key COSY (a) and HMBC (b) correlations for compounds **1** and **2**.

functional groups. Analysis of the 1D and 2D NMR data collected for **2** (Table 1; Figure 2) confirmed the same substitution pattern for **1**, except for two more methylene groups on the straight side-chain at C-6. Therefore, it had the same stereochemistry and structural features of **1** which was (2*R*,3*R*,6*S*)-2-methyl-6-(13-oxotetradecyl)piperidin-3-ol (Figure S57).

The two natural products and semisynthetic derivatives were assessed for their antimalarial inhibitory activity using flow cytometry test. Among them, **1** (IC_{50} 1.82 μM) and **2** (IC_{50} 2.76 μM) were more effective (IC_{50} < 5.00 μM) than **3** (IC_{50} 24.47 μM) and **4** (IC_{50} 25.14 μM) in comparison with the standard compound chloroquine (IC_{50} 0.30 μM) (Figures 3-7), which led us to deduce a moderate activity against *P. falciparum* 3D7 strain for the tested alkaloids (**1** and **2**). Some evidence on the structural features of these compounds, as the size of the straight side-chain combined with the values found for inhibitory concentration showed that the compounds with shorter side-chain (**1** and **3**) were more effective against *P. falciparum*, in comparison with the homologous (**2** and **4**). Additionally, the acetyl group in the parent structures at 3-*O* positions (**3** and **4**) reduced the antimalarial effectivity of the alkaloids. These findings were in agreement with a biological criterion established by Nwaka *et al*.[16] to screening for hit-to-lead compounds as antimalarial agents. According to the author, all compounds with an in vitro IC_{50} ranging from 1 to 10 μM can be classified as a 'hit'. These findings suggest **1** and **2** as examples of hits, and thus are potential candidates for further investigations aiming natural antimalarial drugs. To reinforce this statement a recent work reported piperidine alkaloids as antimalarial agents.[17] Furthermore, the

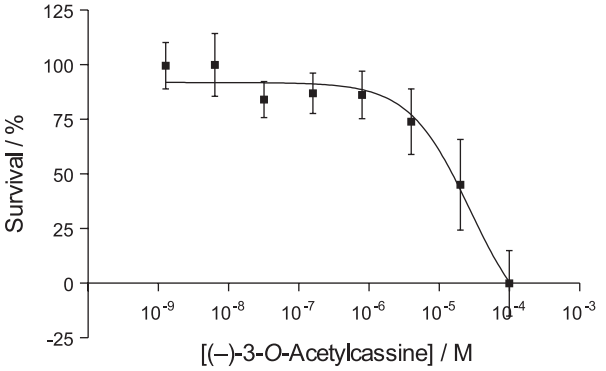

Figure 5. IC_{50} value of (–)-3-*O*-acetylcassine (**3**) against *P. falciparum* was calculated to be 24.47 μM.

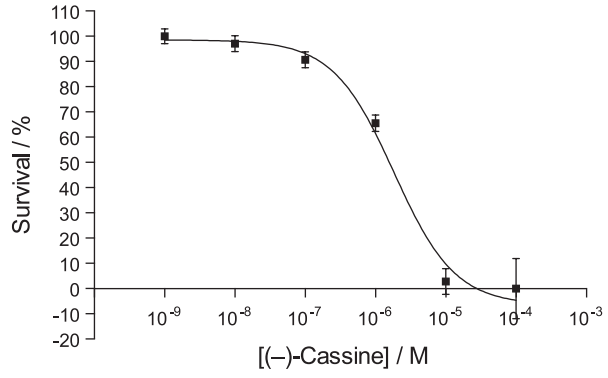

Figure 3. IC_{50} value of (–)-cassine (**1**) against *P. falciparum* was calculated to be 1.82 μM.

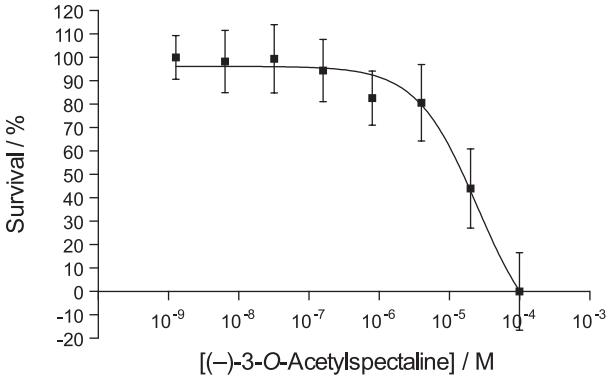

Figure 6. IC_{50} value of (–)-3-*O*-acetylspectaline (**4**) against *P. falciparum* was calculated to be 25.14 μM.

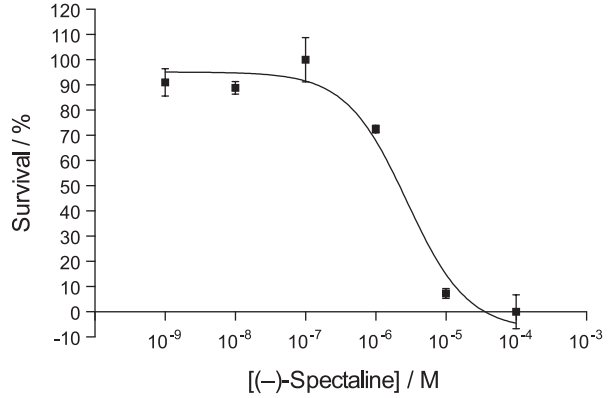

Figure 4. IC_{50} value of (–)-spectaline (**2**) against *P. falciparum* was calculated to be 2.76 μM.

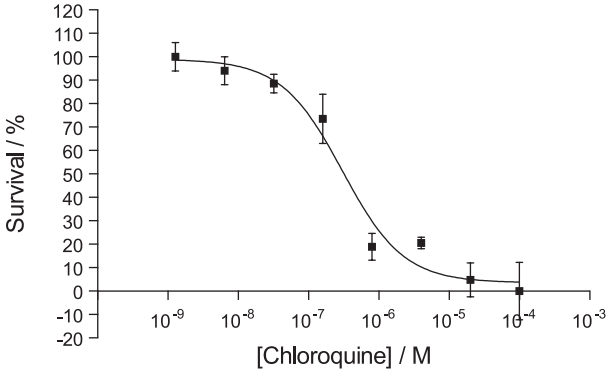

Figure 7. IC_{50} value of chloroquine (positive control) against *P. falciparum* was calculated to be 0.30 μM.

alkaloids described here show a novel structural scaffold when compared to other natural products clinically used, like quinine or artemisinin and their derivatives, oppening a new window for further studies on drug discovery and lead optimization for innovative antimalarial medicines.

Conclusions

Nowadays, malaria remains one of the most widespread infectious diseases causing thousands of deaths around the world and one of the major problems is the growing parasite resistance to current antimalarial drugs. This study reports the antimalarial activity of two piperidine alkaloids (**1** and **2**) isolated from *Senna spectabilis* and two semisynthetic derivatives (**3** and **4**). The natural compound with shorter side chain (**1**) is more effective against *P. falciparum*. Addition of acetyl group to the parent structures at 3-*O* positions (**3** and **4**) reduced the antimalarial effectivity of alkaloids. According to these data, the alkaloids were chemical hits and potential candidates for further investigations.

Supplementary Information

Supplementary data (Figures S1-S61, Tables S1-S5), including TLC plates as well as DSC analysis, IR, high-resolution mass, ^{1}H and ^{13}C NMR and selected 2D spectra for compounds **1** to **4**, are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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